The influence of size and abiotic factors on cutaneous water loss

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INTRODUCTION

Objectives and Overview

Examining the influence of abiotic factors on organisms can be challenging in a biology teaching laboratory due to difficulty in obtaining regulatory approval, effort required to maintain living organisms, and student hesitation in subjecting animals to potential harm. This laboratory examines water balance through the lens of cutaneous evaporation using gelatin frog models in place of live animals. Gelatin frogs of different sizes were used to assess the effect of surface area and volume on water loss. Additionally, gelatin frogs of a uniform size were placed in various environmental conditions to determine the effect of various abiotic factors (heat, cold, wind, and still air) on water loss.

Background

The evolution of terrestrial life allowed animals easy access to oxygen but also exposed them to the threat of dehydration. Avenues of water loss can vary between species; however, all animals lose water cutaneously across their surface. Rate of cutaneous water loss is strongly influenced by water content and permeability of the animal’s surface, as well as physical factors, such as air temperature, movement of air currents over the animal’s surface, and moisture level of the air (3). To survive, animals evolved physiological mechanisms, such as enhancing their waterproofing cutaneous barrier, to limit water loss (3, 8). In addition, animals also rely on behavior to minimize exposure to dehydrating conditions, especially those most prone to dehydration, such as small and/or moist-skinned animals.

Even though maintaining water is central to life, examining the effect of abiotic factors on water loss in a teaching laboratory can be challenging. Examining water balance in a research laboratory setting can frequently cause harm to or require long-term care of animals (8). Thus using vertebrates to demonstrate aspects of water balance in a teaching laboratory would require extensive paperwork and may be morally objectionable when other alternatives are available. Using invertebrates, like insects, may require specialized equipment due to their small size or extended periods of dehydration to accurately measure water loss (12). Estimating animal water loss outside of the controlled laboratory environment can also prove challenging due to microclimate variability and differential access to free water (3, 8). However, researchers have estimated water loss of amphibians in nature by using agar and plaster models (7, 11). Here we take a similar approach by using a gelatin-based model to examine parameters of cutaneous water loss in a teaching setting.

Cutaneous Water Loss

Water loss in terrestrial animals can occur through many avenues, such as urine formation, defecation, lactation, and across the body surface. Water loss across the body surface can be further broken down into cutaneous (across the integument) or respiratory (across respiratory organs). Which factor contributes most to overall water loss across the body surface can vary greatly between animals based on the size of the animal, effectiveness of the water proofing layers of the integument, size of the respiratory organs, and need for gas exchange (i.e., metabolism). All things being equal, small and or ectothermic animals may lose most of their water cutaneously (13). The abiotic factors that influence cutaneous water loss are the focus of this exercise.

Water Activity, Relative Humidity, and Water Loss

The driving force of terrestrial cutaneous water loss is the vapor pressure deficit between water in the animal and water in the environment, and the potential for water loss will increase with greater vapor pressure deficits. Even though almost all terrestrial animals will have a vapor pressure deficit in any environmental condition, the magnitude of deficit can greatly
increase due to higher temperature and or lower relative humidity (RH). These general ideas are sufficient to understand and make hypotheses regarding water loss when the gelatin frogs are exposed to different environmental conditions in this experiment. However, the paragraphs below examine the underlying parameters dictating vapor pressure gradient and could be incorporated into advanced courses or used to further the instructor’s understanding of the science behind the lesson.

Vapor pressure deficit can be calculated with the following equation:

\[ \Delta P = \left[ \frac{55.5}{55.5 + \text{Osm}} - \frac{\text{RH}}{100} \right] P_w. \]

The first portion of the equation estimates activity of water in an animal by calculating the molar fraction of water that can be lost to the environment; 55.5 is the molar concentration of pure water; and Osm represents the solute concentration of the body fluids (4). Relative humidity (RH) represents the water activity of the atmosphere, and \( P_w \) is the temperature-dependent vapor pressure of pure water and can be calculated with the Antoine equation as follows:

\[ P_w = 10^{\frac{A-B}{C+T}}. \]

\( P_w \) is vapor pressure in mmHg, and \( T \) is ambient temperature in the Celsius scale. \( A, B, \) and \( C \) are component-specific constants, and for water they are 8.07131, 1730.63, and 223.45, respectively (4).

Taken together, we can manipulate each variable to determine its effect on vapor pressure deficit. For instance, a typical terrestrial animal with a body water solute concentration of 0.300 mosmol/kgH\(_2\)O, exposed to 25°C and 90% RH, which would be representative of a tropical rainforest in the rainy season, would have a vapor pressure deficit between its body water and the environment of 9.5 mmHg. If the same animal was in a desert at 25°C and 4% RH, the vapor pressure deficit, or pull of water out of the animal, would greatly increase to 29.8 mmHg. If the same animal was placed under a heat lamp at 34°C and 50% RH, the vapor pressure deficit would be 31.8 mmHg. Moving the animal to a refrigerator at 4°C and 50% RH would greatly reduce the vapor pressure deficit to 4.8 mmHg.

Water Loss and Convective Currents

All terrestrial animals are at risk of dehydration; however, abiotic conditions can greatly influence that risk. As seen above, environmental temperature and RH can increase or decrease vapor pressure deficit between the animal and environment. In addition, other factors, such as wind currents, can increase water loss. As water is lost from the animal, the RH immediately surrounding the animal increases from the surrounding water vapor. When RH is higher, the vapor pressure gradient or pull of water out of the animal is reduced (5). But wind currents can prevent lost body water from accumulating around the animal and prevent local increases in RH. Thus animals exposed to convective currents will lose body water at a faster rate (5).

Water Loss Based on Animal Size

Body size is a key trait of any species and one that will directly affect aspects of their ecology and physiology. One aspect that body size greatly influences is an animal’s surface area and volume. Surface area, or the space occupied by the outer face of an object, is two dimensional (i.e., cm\(^2\)) and represents the available area to lose water across the skin. By contrast, volume is three dimensional (cm\(^3\)) and generally indicates the amount of water in an animal. Because it is a cubed term, volume will change more than surface area if an object is increased or decreased in size. Specifically, linear regression analysis of a volume-to-surface area plot of various sized cubes results in a slope of 0.67 (Fig. 1), indicating volume changes at a faster rate than surface area when a cube size increases or decreases. Thus larger cubes will have more absolute surface area and volume than smaller cubes, but the ratio of surface area to volume is much lower in large cubes (Fig. 1). Ultimately, this means large cubes have a smaller surface area per unit of volume over small cubes.

Even though animals are irregular in shape and their surface areas and volumes are more difficult to directly measure, the same principles apply. Small animals have reduced amounts of body water (i.e., lower body volume) but relative greater risk of water loss across their cuticle (i.e., greater surface area-to-volume ratio) compared with larger ones. Thus animals like insects are at extreme risk of dehydration, even in humid environments, and relatively minor disruptions in their waterproofing cutaneous layers can result in rapid water loss and death (12). Conversely, larger animals have greater volumes (i.e., potential body water content) and reduced relative surface area. Even though large animals can lose more water in a size-independent manner to the environment (i.e., mass of water loss per unit time), they typically have a lower risk of dehydration from cutaneous water loss because of their reduced surface area-to-volume ratio. Therefore, large animals can lose more water overall to the environment.

Learning Objectives

After completing this activity, the student will be able to:

- Define cutaneous water loss. Explain the importance of vapor pressure to cutaneous water loss.
- Explain how animal surface area and volume vary and how this impacts cutaneous water loss.

![SA vs. Volume](http://advan.physiology.org)

**Fig. 1.** Scatterplot of surface area (SA) to volume (V) for a simple cube. As cube side size increases, both SA and V increase. However, SA-to-V ratio decreases (dark gray line), as cubes get larger. [Modified from Willmer et al. (13), with permission.]
• Identify how abiotic factors may contribute to water loss.
• Interpret figures based on water loss data and make conclusions from collected data.

Activity Level

This activity can be modified to be suitable at primary, secondary, and higher education levels. Procedures in this laboratory are straightforward, allowing students with less intensive science training to actively participate. The concept of surface area-to-volume ratio and its effects on water loss is challenging and may test more advanced students. The required equipment is common in a science classroom, and materials can be easily found in homes and are relatively inexpensive.

Prerequisite Student Knowledge or Skills

Before this activity, students should understand basic ideas of water loss. Students should be familiar with the role abiotic factors may play in creating a dehydrating environment. Also, students should have a basic understanding of surface area and volume and how those parameters change with body size. In addition, students should understand the difference between size-dependent water loss (i.e., mass of water lost per wet mass per unit time) and size-independent water loss (i.e., mass of water lost per unit time). Students should be familiar with Microsoft Excel or similar programs.

Students need to be aware that the proposed experiments are designed to directly examine how abiotic factors and animal size influence animal water balance. The experiments will not explore specific adaptations a species or population of animal may have evolved to limit water loss when faced with large vapor pressure deficits. Thus evolutionary pressures are the focus, not how life has evolved to limit water loss when faced with dehydrating conditions.

Time Required

Time required includes gathering materials, preparing for the laboratory by the instructor, and conducting the laboratory exercises by the students. Refer to Table 1 for the specific breakdown. Instructor preparation can take between 1 and 1.5 h. The lesson can take between 50 min to 2 h, based on class size and how the experiments are performed.

Table 1. Activity and typical time associated with each activity

| Activity                              | Approximate Time Required, min |
|---------------------------------------|-------------------------------|
| Obtaining materials                   | Variable                      |
| Gelatin frog making*                  | 30                            |
| Instructor prelaboratory lecture      | 10–15                         |
| Activity: body-size experiment†       | 20–30                         |
| Activity: abiotic conditions experiment‡ | 10–20                      |
| Creation of graphs                    | 10–20                         |
| Students answering questions          | 5–8                            |
| Instructor postlaboratory discussion  | 5                             |

Table 1. Activity and typical time associated with each activity

Time for experiments are variable, but a general range is given. Measurable differences in mass loss between treatment groups can occur in as little as 10 min, although longer periods of exposure may emphasize differences. *Need to wait overnight to have gelatin solidify, in addition to the time it takes to make the gelatin. †Can be done simultaneously with the other experiment.

METHODS

Equipment and Supplies

The following materials are necessary to perform the laboratory exercise:

• Candy molds to create the gelatin frogs. We used small molds from CybrTrayd (Amazon.com ID no.: B000EBOYM0, item model no.: A002), medium molds from CybrTrayd (Amazon.com ID no.: B000EBMUNU, item model no.: A126), and large molds from Calif Molds (item model no.: PI A288; Fig. 2A).
• Dry gelatin mix purchased from a local grocery store
• Heat lamps
• Fan to generate convective currents
• Refrigerator, ice box, or cooler
• Sensitive balance (e.g., to the nearest hundredth gram)
• Timing device

Human and Animal Subjects

The following laboratory activity uses no human or animal subjects. The gelatin frogs are used to simulate water loss in a real animal without any of the complications involved.

Instructions

Experimental preparation. Gelatin frogs should be made the 1 or 2 days before their use in the laboratory. Follow the instructions on the box of gelatin to make the mixture; however, use one-half the amount of water called for on the package. Less water firms the gelatin and allows for easier removal from the mold. Pour the gelatin mixture into the molds that have been lightly wiped with cooking spray and have them set for several hours or overnight. Remove the models and place them in an airtight plastic container until use. Removing fully intact gelatin models can be problematic and may require placing the molds briefly in warm water to loosen the frogs.

Experiment 1: The effect of size on cutaneous water loss. The steps for experiment 1 are as follows:

1. Obtain five small (average mass: 3.48 g, average snout-vent length (SVL): 3.1 cm, average width: 2.9 cm), five medium (average mass: 22.73 g, average SVL: 6.1 cm, average width:
4.3 cm), and five large frogs (average mass: 118.38 g, average SVL: 9.9 cm, average width: 9.9 cm; Fig. 2). SVL is the standard measure of body length for amphibians, measured from the tip of the nose to the opening of the cloaca. We estimated the location of the cloaca by using the end of the trunk between the two hindlimbs.

2. Record the initial or “wet” mass of each frog using the balance.
3. Once all masses are recorded, subject all frogs to room temperature for a set period (e.g., 20–30 min or more). Frogs can be placed under heat lamps to speed water loss if there are time constraints.
4. After the allotted time has passed, remove frogs from the counter and record the final or dehydrated mass of each frog.
5. The measures of wet mass, dehydrated mass, and time between recording masses will be used to calculate rates of water loss. Those data can be analyzed differently to highlight size-independent or size-dependent rates of water loss (Fig. 3).

Experiment 2: The effect of abiotic conditions on cutaneous water loss. The steps for experiment 2 are as follows:

1. Obtain frog models of the same size and record the initial or wet mass of each. We found the medium frogs (average mass: 22.98 g, average SVL: 6.1 cm, average width: 4.3 cm) worked best for these experiments because they were easier to handle yet only used a moderate amount of gelatin to produce.
2. Place at least five frogs into each abiotic condition you choose to examine. For demonstration purposes, we subjected models to control conditions, elevated temperature, reduced temperature, or elevated convective air currents for 10–20 min (Fig. 4). Control conditions were achieved by simply placing the models on the benchtop in the teaching laboratory at 23°C with minimal convective air currents. Elevated temperature conditions were created with a 250-W heat lamp placed ~0.5 m above the benchtop, which created a surface temperature of 34°C in the models. Water loss rates were also measured in models that were placed in a refrigerator maintained at 4°C, representing the low-temperature exposure. Although it is not necessary, chilling or warming the frogs before measuring their initial wet mass would best represent the effect temperature has on water loss. This would be especially helpful if fewer numbers of frogs are used or have shorter temperature exposure periods. Placing the frogs in a prechilled box while in the refrigerator would help eliminate convection that may occur in some appliances. Lastly, a small fan set on “high” and placed ~0.25 m away from the gelatin frogs was used to determine the effect of convective air currents on water loss.
3. Remove all frogs from the respective abiotic conditions. Measure and record the dehydrated or final mass of each frog.

Fig. 3. Mean ± SE of size-independent rates of water loss (A) and size-dependent rates of water loss (B) for gelatin frogs of different sizes (n = 5 per bar). Each model was dehydrated for ~10 min while under a heat lamp at 34°C to speed water loss. *a,b*Means not sharing a letter within a given panel were statistically different when analyzed using a Kruskal-Wallis one-way ANOVA on ranks and Dunn’s post hoc test (P < 0.05).

4.3 cm), and five large frogs (average mass: 118.38 g, average SVL: 9.9 cm, average width: 9.9 cm; Fig. 2B). SVL is the standard measure of body length for amphibians, measured from the tip of the nose to the opening of the cloaca. We estimated the location of the cloaca by using the end of the trunk between the two hindlimbs.

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Fig. 4. Abiotic conditions used in this protocol were as follows: control conditions at room temperature (23°C; A), elevated temperature under a 250-W heat lamp yielding a model surface temperature of ~34°C (B), placement in a standard refrigerator maintained at 4°C (C), and elevated convective currents using a small fan placed on the highest setting (D). Fan was positioned closer than specified in METHODS so that it could be easily seen in the photograph.
4. Calculate rates of water loss as above. The frog models used in this experiment should all be similar in size, making it unnecessary to calculate the rates of water loss in both a size-independent and size-dependent manner (see similar data trends between Fig. 5, A and B).

5. As with the previous exercise, figures created from the averaged data can include measures of variation and statistical analysis.

Troubleshooting

Several issues that could affect the data or pose problems for data collection are as follows:

1. The gelatin can lose substantial amounts of water between making the models and using them in the experiment. To limit the amount of water loss, the frogs should be made within 24 h and stored in an airtight container before their use. The models should also be blotted dry before taking their wet mass.

2. Tools used to perform the experiment, such as the balance, fan, refrigerator, heat lamp, etc., may differ greatly from the ones used here. Thus preliminary data would be necessary to ensure appropriate mass losses occur within the time frame of given experiment or meeting period(s).

Safety Considerations

The described exercises utilize no hazardous chemicals, sharp instruments, or dangerous equipment that could cause injury, although one should take care to prevent burns if a heat source is used. The gelatin models should not be consumed, because they could be handled by multiple people and potentially exposed to laboratory chemicals.

RESULTS

Expected Results

Size experiment. The largest frogs, with the greatest volume (water mass) and greatest total surface area should lose the most water in terms of milligrams of water loss per minute of exposure to dehydrating conditions. This pattern was seen when using as few as five animals per size and dehydrating the animals for only ~10 min under a heat lamp (Fig. 3A). The largest animals lost 690 ± 141 mg/min (mean ± SE) compared with the intermediate and small frogs, which averaged loss rates of 175 ± 35 mg/min. Interestingly, even though medium frogs tended to lose water at a faster rate than the small frogs, the means were similar when analyzed with a Kruskal-Wallis one-way ANOVA (P > 0.05). The likelihood of obtaining a significant difference could be enhanced if more frogs were tested or the desiccation exposure was longer.

When analyzed in a size-dependent manner, which factors in the surface area per volume ratio, the smaller frogs should lose water at a much faster rate. As expected, the small frogs had high rates of mass-dependent water loss, losing an average of 2.4 ± 0.4 mg mass-g wet mass⁻¹-min⁻¹ (mean ± SE), compared with the large frogs, which averaged rates of only 0.6 ± 0.1 mg mass-g wet mass⁻¹-min⁻¹ (Fig. 3B).

Differences in cutaneous water loss based on environmental exposure. When using models of the same size, the gelatin frogs should have the greatest cutaneous water loss at higher temperatures and when exposed to high convective currents. As expected, animals subjected to those conditions lost the most body water (averaging 440 ± 70 mg/min) compared with those in the control and low-temperature treatments (averaging 206 ± 30 mg/min; Fig. 5A). When using the same size frog, the data for water loss to different abiotic conditions should have the same trends, regardless if it is examined in a size-independent (Fig. 5A) or size-dependent manner (Fig. 5B). Thus either method of data presentation would be sufficient.

Questions for students to answer after completion of the size and abiotic factors experiment are as follows:

1. Which frog lost the most water during the experimental condition? Why? (The largest frogs lost the most water. Larger frogs have greater total surface area and thus should lose the most total water if all other conditions are similar.)

2. When analyzed in a size-dependent manner, will the largest frogs lose more body water? (No, analyzing the data in units of mass lost per wet mass per minute, would incorporate surface area-to-volume ratio into estimates of water loss. Thus large models with the smallest surface area-to-volume ratio should lose the least relative amount of water.)

3. Which measure of water loss, size independent or size dependent, is most ecologically relevant? (Size-dependent water loss is most ecologically relevant, as animals can only lose a certain amount of water relative to their wet mass before irreversible physiological damage and/or death occurs. Examining size-dependent water loss across small, medium, and large sizes illustrates how...
much more susceptible small organisms are to water loss over large organisms.)

4. This laboratory exercise used wet mass to standardize for body size across models. Other ways to standardize for body size would be to use surface area of the animal rather than initial wet mass (i.e., units of mass lost per surface area2 per minute). What are the strengths and weaknesses of using each approach? (Using wet mass to standardize for size is much easier to measure accurately than estimating the surface area of an animal in three dimensions. Using surface area as a metric would be a better method to determine cutaneous adaptations, and animal may have to resist water loss.)

5. Which abiotic factors promoted notable water loss in our gelatin frogs? [Factors that promoted notable water loss were the heat condition (heat lamp) and wind condition (fan).]

6. Organisms living in arid environments (e.g., desert) are likely subjected to large vapor pressure deficits and large water vapor activity, promoting a relatively fast rate of water loss. To survive in arid environments, organisms have altered their anatomy and physiology, as well as their behavior to minimize water loss. What are some behavioral responses that would alter abiotic factors to potentially minimize water loss? (While animals have physiological adaptations to survive in arid environments, animals will resort to changing behavior first. Animals can change their periods of activity. They can be active during cooler portions of the day, such as dawn or dusk, or be nocturnally active to minimize the vapor pressure deficits. Also, animals may exploit buffered habitats during the warmest and most dehydrating portions of the day.)

7. Could environmental alterations from climate change impact water balance in terrestrial animals? Explain. (Yes. Climate change may cause reductions in precipitation and warmer average temperatures in certain areas or regions. Thus animals within these areas may lose more water to the environment and have reduced ability to take in water from the environment.)

Evaluation of student work. Students can determine their experimental procedures and collect data in groups. Data can be analyzed within a group or compiled as a class to have a larger data set. Individual students can be in charge of making their own graphs, allowing them to visualize trends in size-dependent and abiotic influenced cutaneous water loss. Students can write up their results as a report or other written work, addressing the questions posed in the Expected Results section, illustrating their mastery of course content. Additionally, the instructor can have a class discussion with the students to assess their knowledge of concepts and address shortcomings.

Inquiry Applications

The application of these exercises is highly adaptable and can vary greatly, depending on the experience level of the students and objective of the instructor. For students in primary and secondary school, the instructor can utilize either the methods level or facilitated inquiry approaches. The instructor can fully design desired experiments, as students make hypothesizes, carry out the protocol, collect data, and analyze and potentially present the results in graphical, written, or oral formats.

For advanced students, the instructor can utilize the guided inquiry approach. The instructor can pose questions about how to test water loss in organisms of different sizes and in different environments. Students can then select an environment in which to place frogs (heat lamp, fridge, fan) for a size comparison. To explore the effect of abiotic conditions on water balance, students can select a size of frog, if options exist, and the abiotic conditions to subject their models.

The proposed exercises can also be utilized in open inquiry fashion. Students can create their own questions and hypotheses, in particular, regarding abiotic factors that may or may not influence cutaneous water loss. Students could utilize a multitude of factors other than what was covered here. For instance, students could expose animals to different RHs created with saturated salt solutions (2) and how substrate may influence water loss to elevated temperature or convective currents, among many others.

Additional Resources

For additional information on this topic, see Danks (1), Navas and Araujo (6), and Titon and Gomes (10).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.M.H., H.-B.G., R.W., and J.B.W. conceived and designed research; J.M.H., H.-B.G., and R.W. performed experiments; J.M.H., H.-B.G., and R.W. analyzed data; J.M.H., H.-B.G., R.W., and J.B.W. interpreted results of experiments; J.M.H., H.-B.G., and J.B.W. prepared figures; J.M.H., H.-B.G., and J.B.W. drafted manuscript; J.M.H., H.-B.G., and J.B.W. edited and revised manuscript; J.M.H., H.-B.G., and J.B.W. approved final version of manuscript.

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