Diffusion: Calculating Water Vapor Conductance of Avian Eggs

Diffusion is the spontaneous movement of particles from higher chemical potential to lower chemical potential (chemical potential can in most cases of diffusion be represented by a change in concentration). The rate of diffusion (i.e. the quantity of a solute diffusing per unit time) is described by the Fick diffusion equation:

$$\frac{dQ_s}{dt} = D_s A \frac{dC_s}{dx} \qquad (1)$$

in which dQ_s/dt is the rate of diffusion of a solute s, D_s is the diffusion coefficient of s (which varies with the nature and molecular weight of s) , A is the cross-sectional area through which s is diffusing, and dC_s/dx is the concentration gradient of s (change of concentration with distance).

The Fick diffusion equation can be used in biological systems to measure the amount of solutes that passes through a unit area of membrane every second in one direction. For gases, the Fick equation (1) can rise to the formula:

$$\text{Rate of diffusion } (M) = (D/RT)\ (A/L)\ (P_2 - P_1) \qquad (2)$$

It states that the rate of diffusion of a gas across a membrane is (i) constant for a given gas at a given temperature by an experimentally determined factor D/RT (where D is the diffusion coefficient, R is the gas universal constant and T is the absolute temperature); (ii) proportional to the surface area over which diffusion is taking place, A; (iii) proportional to the difference in partial pressures of the gas across the membrane, P_2-P_1; and (iv) inversely proportional to the distance over which diffusion must take place, or in other words the thickness of the membrane L.

The Avian egg

The avian egg is a self-contained womb in which the embryo is protected and provided with food and nutrients it needs to develop and hatch. But the egg is not a completely closed system. Embryos are warm-blooded, with all the metabolic activity that homoeothermy demands. The developing chick within the egg must be able to respire, to exchange gases with outside the world. This is done by diffusion because the internal and external concentration of exchanging gases is different. The bird egg must allow an efficient exchange of gases to support the growing chicks. Thus, the eggshell and its internal membranes are not a smooth closed barrier, but instead its surface is disrupted by hundreds of microscopic pores, which allows the diffusion of O_2 into the embryo and CO_2 and H_2O out of the egg. Avian eggs lose between 10-23% of the initial mass during incubation due to water vapor loss. The factors (D/RT)•(A/L) in equation 2 are often combined into the term "G" (mg•day^{-1}•mmHg^{-1}) which describes the conductance of the eggshell to gases. In this particular case A is the total functional pore area and L is the thickness of the eggshell. Therefore, Equation 2 becomes:

$$M = G \bullet \Delta P \qquad (3)$$

G is a convenient term to use since it is considerably easier to measure accurately than A and L. Although conductance of the egg to O_2, CO_2 and H_2O can all be calculated G_{H2O} is measured because it is the easiest method. Thus, to measure the water vapor conductance G_{H2O} of avian eggs we use the formula:

$$G_{H2O} = M_{H2O} / \Delta P_{H2O} \qquad (4)$$

where G_{H2O} = water vapor conductance (mg•day^{-1}•mmHg^{-1}); M_{H2O} = the rate of weight loss (mg•day^{-1}); ΔP_{H2O} = water vapor pressure difference across the shell (mmHg).

The calculation of G_{H2O} has important ecological and physiological implications for the adaptation of birds to different climate and conditions. G_{H2O} depends on the pore area and eggshell thickness (G_{H2O}=(D/RT)•(A/L), see above). Thus, for a bird living in a hot and dry environment, a reduction in pore area and/or an increase in eggshell thickness may be adaptive to reduce G_{H2O}, and consequently avoid egg desiccation. Likewise, there is an allometric relationship between G_{H2O} and egg mass for several avian species

$$G_{H2O} = 0.432 \bullet M^{0.780} \ldots\ldots\ldots (5)$$

The exponent of this relationship is less than one, i.e. G_{H2O} does not increase in direct proportion to egg mass. Ultimately, this means that large eggs can save more water than small eggs.

In summary, the water vapor conductance measures the rate of egg weight loss under a water vapor pressure gradient across the shell. Under this gradient, the water will diffuse (according to the Fick's law) at different rates depending upon the eggshell thickness and egg pore size.

Objectives

1) To understand that gas interchange of avian eggs is the result of passive diffusion through the eggshell pores.
2) To use the Fick's law of diffusion to determine the water vapor conductance G_{H2O} of two species of avian eggs of different sizes: quail and domestic chicken.
3) To contrast our measurement of G_{H2O} for each species with that expected from the allometric relationship $G_{H2O} = 0.432 \bullet M^{0.780}$
4) Estimate the functional pore size of eggs for each species based on the equation $G_{H2O}=(D/RT)\bullet(A/L)$.

Materials

- Glass desiccators
- Dessicant: Anhydrous calcium sulfate (Drierite)
- Electronic Hygrometer/ thermometers
- Electronic balance with an accuracy of ± 0.05 mg.
- Fresh eggs of domestic chicken (*Gallus gallus*)
- Fresh eggs of Japanese quails (*Coturnix coturnix*)
- Scientific calculators.
- 1 micrometer calipers

Methods

Eggs will be placed in desiccators with the drierite at a constant room temperature (approximately 18^{o}C) and weighed daily for seven consecutive days. Relative humidity and temperature inside the desiccator will be recorded by an electronic hygrometer and thermometer.

1) Preparing the desiccators.

Approximately 1 kg of fresh dehydrated (baked in open trays at 190^{o}C for 4 hours) drierite will be used on each glass desiccator. Eggs will be placed directly on a dish on top of drierite. Remember that the role of drierite is to absorb all the water vapor inside the desiccator. The experiments will be carried out at a constant temperature of 18-20^{o}C.

2) Egg weighing

Each egg will be marked with a small number using a pencil. The eggs will be weighed in an electronic balance to the nearest 0.05 mg. Once weighed, the eggs will be placed in the desiccator and the desiccator will be closed and sealed with vacuum silicone. The eggs will be weighed at the same time each day during seven consecutive days. It is very important to handle the eggs gently to avoid any damage or breakage of the eggshell.

3) Procedures

3.1) Groups of 3 - 5 students will be formed and each will be assigned to one desiccator containing 3 eggs of chickens and 3 eggs of quails.
3.2) Label each egg (letters or numbers) using a pencil.
3.3) Weigh each egg and keep your records in Table 1.
3.4) Prepare the glass desiccators.
3.5) Turn on the hygrometer/thermometer and put it inside the desiccator.
3.6) Place the eggs inside the desiccator.
3.7) Seal the glass desiccator.
3.8) Wait for 30 minutes to check that the relative humidity decreases near to zero. If the hygrometer still record relative humidity above 10%, you will need to check the desiccator and seal it again.
3.9) Repeat steps 3-7 each day at the same time. When sealing the desiccator, be sure to stir the silica gel such that the layer of silica gel at the surface will be replaced by silica gel below it.
3.10) Record relative humidity and temperature before opening the desiccator. Although these measurements will not be used in our calculations, they will be used to check that the relative humidity and temperature inside the desiccator are close to 0% and 18-20^{o}C, respectively.

Calculations

Water vapor conductance is calculated by rearranging equation 4. Thus, we need to determine first two variables: the rate of weight loss (M_{H2O}) and the difference in water vapor pressure between the interior of the egg and the environment (ΔP_{H2O}). An important assumption for this calculation is that all mass loss is due to the loss of water vapor.

Calculating M_{H2O}

The first variable can be estimated by weighing the eggs daily during 7 consecutive days. The relationship between egg weight at day *i* (*i* = 0 represents the day of first weighing, *i*=1 the day of the second weighing and so on) and weighing day *i* is linear with a slope representing the rate of weight loss (mg•day^{-1}).

1) Plot the data from Table 1 for each egg (day in the x-axis and egg weight in the y-axis) on in Excel or SigmaPlot (graphing programs).
2) The points will generate a straight line for each egg (Figure 1) with a negative slope. This means that the egg weight decreased in a constant rate from the beginning to the end of the experiment.
3) Calculate the slope of each line using Regression functions in graphing program. The instructor will guide you on these calculations.
4) Record this value in Table 2. This value represents the rate of water vapor loss M_{H_2O} expressed in $\text{mg}\bullet\text{day}^{-1}$

Calculating ΔP_{H_2O}

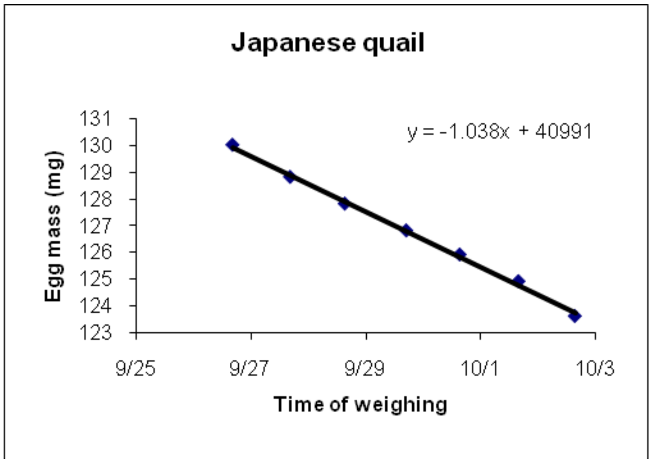
The difference of pressure across the eggshell ($P_{ins} - P_{out}$) provides the driving force for continuous loss of water from the egg during the experiment. The inside of the egg is saturated with water vapor, and therefore P_{ins} can be easily estimated from standard saturated water vapor tables. To obtain this value, we need to know first the average temperature during the experiment. Calculate the average temperature from Table 1. Use this average value to find the water vapor pressure in Table 3. P_{out} is zero because the water vapor inside the sealed chamber is completely absorbed by the desiccant.

Filled out the data of P_{ins} and P_{out} in Table 2.

Calculating G_{H_2O}

Determine the mean and standard deviation of the water vapor conductance for each bird egg in Table 2.

Figure 1



Assignments

1) Compare the G_{H2O} for each species with those expected from the allometric relationship $G_{H2O} = 0.432 \bullet M^{0.780}$ derived from 29 species of birds. M is the mean egg weight (mg). Are the measured and expected G_{H2O} similar or different?

Expected G_{H2O} chicken:
Measured G_{H2O} chicken:

Expected G_{H2O} quail:
Measured G_{H2O} quail:

2) Calculate the functional pore area. Break the eggs, discard the egg content and remove the inner membranes attached to the shells. Select 5 small fragments (1cm x 1cm) of the eggshell from different regions of the eggs (poles and equator) and measure the thickness of each fragment with the aid of the micrometer. Take the average of the five measurements (cm):

Mean eggshell thickness of chicken (cm):
Mean eggshell thickness of quail (cm):

Remember that $G_{H2O}=(D/RT)\bullet(A/L)$ where D is the diffusion coefficient of water vapor in air ($cm^2 \bullet sec^{-1}$), A is the total functional pore area (cm^2), L is the length of pores or shell thickness (cm). R and T are the gas universal constant and absolute temperature in ^{o}K, respectively. For this experiment, D/R = 6979.16 $^{o}K \bullet mg \bullet cm \bullet day^{-1} \bullet torr^{-1}$, and therefore:

$$G_{H2O}=(6979.16/T)\bullet(A/L) \text{ or}$$

$$A = G_{H2O}(L)(T)/6979.16$$

Thus, by knowing L (thickness of the eggshell) and T (absolute temperature in ^{o}K during the experiment) is possible to estimate the area of the eggshell pores. Calculate this value for each bird species:

Pore area of chicken (cm^2):
Pore Area of quail (cm^2):

What is the type of relationship between pore size and egg size?

3) This exercise was conducted for water vapor, but same principles would apply for respiratory gases like O2 and CO2. What are the functional consequences of increasing egg size in terms of gas exchange? How would a large egg cope with diffusive restraints?

4) What would be the physiological adaptations (in terms of egg pore size and eggshell thickness) of an egg that it is incubating in a dry desert? At high altitudes?

Table 1. Records of egg mass.

Chicken Eggs:

Day	Initials	Date	Time	Mass (mg) Egg 1	Mass (mg) Egg 2	Mass (mg) Egg 3	Relative humidity (%)	Temp. (^oC)
0								
1								
2								
3								
4								
5								
6								

Quail Eggs:

Day	Initials	Date	Time	Mass (mg) Egg 1	Mass (mg) Egg 2	Mass (mg) Egg 3	Relative humidity (%)	Temp. (^oC)
0								
1								
2								
3								
4								
5								
6								

Table 2. Physiological parameters for water vapor conductance estimations

Chicken Eggs:

Egg number	Rate of water loss (slope of the regression) M_{H2O} (mg•day^{-1})	Water vapor pressure inside the egg $P_{ins\ at\ T^oC}$ (mm Hg)	Water vapor pressure outside the egg (P_{out} assumed to be 0)	ΔP_{H2O} (mmHg)	Water vapor conductance $G_{H2O} = M_{H2O} / \Delta P_{H2O}$ (mg•day^{-1}•mmHg^{-1})
1					
2					
3					
Mean					
s.d.					

Quail:

Egg number	Rate of water loss (slope of the regression) M_{H2O} (mg•day^{-1})	Water vapor pressure inside the egg $P_{ins\ at\ T^oC}$ (mm Hg)	Water vapor pressure outside the egg (P_{out} assumed to be 0)	ΔP_{H2O} (mmHg)	Water vapor conductance $G_{H2O} = M_{H2O} / \Delta P_{H2O}$ (mg•day^{-1}•mmHg^{-1})
1					
2					
3					
Mean					
s.d.					

Saturated Vapor Pressure

Temp (°C)	Temp (°F)	Saturated Vapor Pressure (mmHg)	Temp (°C)	Temp (°F)	Saturated Vapor Pressure (mmHg)
-10	14	2.15	37	98.6	47.07
0	32	4.58	40	104	55.3
5	41	6.54	60	140	149.4
10	50	9.21	80	176	355.1
11	51.8	9.84	95	203	634
12	53.6	10.52	96	205	658
13	55.4	11.23	97	207	682
14	57.2	11.99	98	208	707
15	59	12.79	99	210	733
20	68	17.54	100	212	760
25	77	23.76	101	214	788
30	86	31.8	200	392	11659