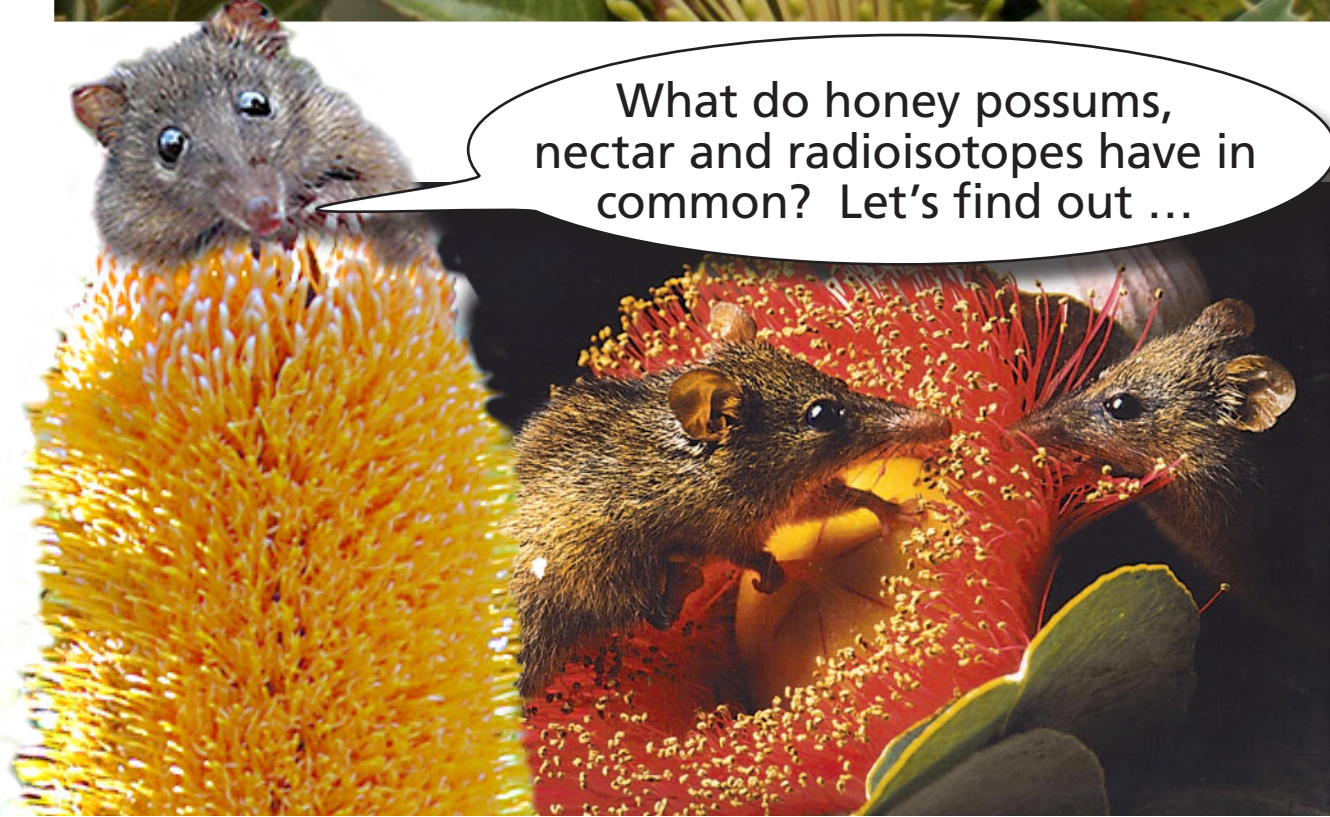




## Investigating energy intake

What do honey possums, nectar and radioisotopes have in common? Let's find out ...



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# Protecting the honey possum means protecting its habitat.

Scientists can estimate the area of habitat required to sustain a healthy population of honey possums by establishing their daily energy requirements.

Finding out how much food a honey possum needs is no mean feat – scientists conduct research in the field using some very special techniques.

Traditionally, scientists have measured the **basal metabolic rate** (BMR) of an animal. This is performed in a laboratory and provides information about an animal's energy expenditure when at rest. Subjecting animals such as the honey possum to laboratory

procedures can cause extreme stress and even death. This means data gathered may be scientifically invalid. Studying animals in their natural habitat avoids these problems. It also provides information about their energy requirements during normal daily activity.

**Field metabolic rate** (FMR) is the amount of energy a free-living animal expends during normal daily activity. If an animal's FMR is calculated it is possible to estimate daily food intake. Measuring an animal's FMR also presents challenges. Using traditional laboratory equipment in the field

isn't practical, so FMR is measured indirectly. This involves measuring metabolic by-products, or gas output, by sampling the blood of free-living animals, such as the honey possum. One such field technique is the doubly-labelled water method. This has been successfully used with honey possums.

So how does measuring the metabolic by-products of honey possums help determine daily energy expenditure?

Let's start by looking at what's going on in the cell.

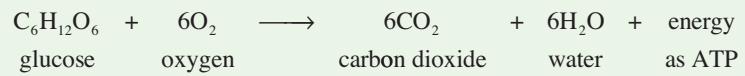
# What is cellular respiration?



Energy is required by all organisms for growth, repair and reproduction. This energy is produced during the process of cellular respiration. Cellular respiration occurs within the mitochondria of each and every cell. It is an oxidative process, which means it requires oxygen.

Glucose molecules are oxidised to carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O). Energy is released and subsequently stored within ATP (adenosine triphosphate) molecules for later use.

The equation for respiration is:



CO<sub>2</sub> and H<sub>2</sub>O are by-products of this process. These molecules leave the cell and diffuse into the bloodstream. The end point of this journey occurs when CO<sub>2</sub> diffuses from the bloodstream into the air spaces of the lungs. CO<sub>2</sub> (and some H<sub>2</sub>O, as vapour) is then expired into the external environment.

Measuring gas inputs and outputs provides an indirect measure of metabolism.

## Calculating energy expenditure



Energy expenditure can be determined by measuring the carbon dioxide produced (litres per day) from blood samples of free-living animals. The amount of CO<sub>2</sub> produced by an animal depends on its diet. An animal that metabolises protein produces more CO<sub>2</sub> than one that metabolises carbohydrates. Nectar, the main food source of honey possums, is pure carbohydrate.

Scientists know that for every litre (L) of CO<sub>2</sub> produced during the metabolism of carbohydrate, 20.8 kilojoules (kJ) of energy is expended. This is a standard value for the metabolism of carbohydrates in all animals.

With this information, it is possible to calculate the daily energy expenditure, or field metabolic rate (FMR), of the honey possum using the following formula:

$$\text{FMR (kJ day}^{-1}\text{)} = \text{CO}_2 \text{ produced (L)} \times 20.8$$





Question 1: What is the FMR of a honey possum that produces 0.73 L of CO<sub>2</sub> per day?

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Question 2: If the amount of CO<sub>2</sub> produced varies with the type of food metabolised, what else may vary?

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Research scientists have identified plants from which honey possums source nectar (see 'Nectar as fuel' on page 4). The energy content (in kJ) of nectar from each plant has also been determined. With this information, and the calculated FMR, it is possible to estimate the number of flowers/inflorescences a possum visits daily.

Question 3: A captured honey possum is found to feed exclusively on *Banksia coccinea*. If its FMR is 15.2 kJ day<sup>-1</sup>, how many inflorescences did it visit?

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Question 4: A researcher determines the average FMR of a honey possum is 2.7 times greater than its BMR. Suggest reasons for this.

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Question 5: What biotic and abiotic factors might affect the FMR of a honey possum?

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Field research has revealed the average honey possum consumes 6 -7 mL of nectar each day. For a 9 g possum, this amounts to almost 70 % of its own body mass. That's a huge amount of food!

Question 6: What might be implications of such large food requirements for honey possum survival?

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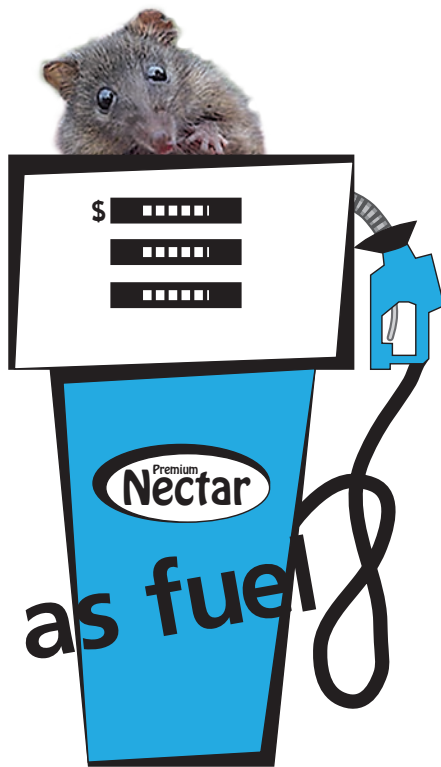
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*Banksia baxteri*  
small tree  
1.7–4 m  
0.08 kJ  
per inflorescence



*Banksia baueri*  
bushy shrub  
0.5–2 m  
0.08 kJ  
per inflorescence



*Banksia coccinea*  
small tree  
1–8 m  
0.096 kJ  
per inflorescence



*Banksia attenuata*  
small tree  
0.4–10 m  
0.078 kJ  
per inflorescence



*Banksia nutans*  
shrub  
0.3–1.3 m  
0.733 kJ  
per inflorescence



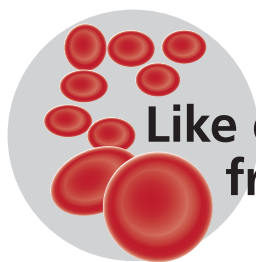
*Adenanthos cuneatus*  
spreading shrub  
0.3–4 m  
0.029 kJ  
per inflorescence

Different flowers produce nectar in different volumes. The energy content (kJ) of this nectar is highly variable.

So do animals favour one type of flower over another? And if so, how do we find out?

Scientists find out which plants the honey possum prefers by taking samples of pollen stuck to their noses and whiskers. The pollen is classified to reveal its source.

The honey possum's favourite meals, and the amount of energy (kJ) available from nectar per inflorescence (a group of flowers on a single stem or branch) is shown above.



## Like getting blood from a possum

Research scientists use a special technique called infraorbital sinus bleeding to take blood samples from the tiny and sensitive honey possum.

Blood is taken from a sinus behind the eye! This sounds gruesome but actually causes little or no distress to the animal due to the lack of nerve endings in this area. A 25  $\mu$ L microcapillary tube is inserted gently between the eyeball and eye socket, into the sinus. The tube fills through capillary action and is then removed. The pressure of the eyeball returning into place stops any further blood flow. This technique is widely used with other animals, including reptiles.

## Talking about torpor



Torpor, or heterothermy, is a behavioural and physiological state of inactivity and reduced metabolic rate. It is triggered by environmental stimuli including temperature, food or water. Physiological changes occur in body temperature, metabolism and water balance. Torpor might last for a few hours or even several days.

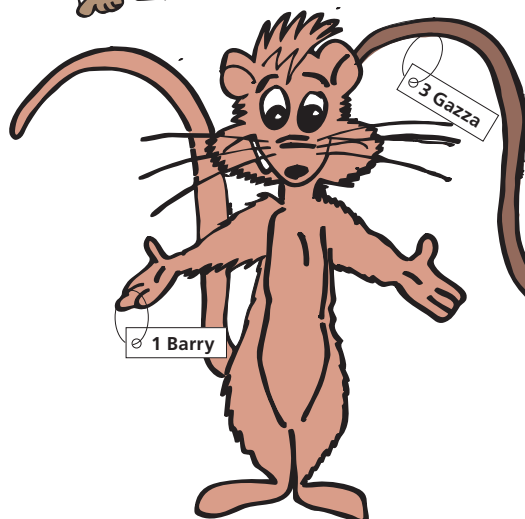
The honey possum displays shallow daily torpor. During these periods, body temperature can fall from 35 °C to as low as 5 °C, and metabolic rates can fall to 10–60% of normal values. Researchers often find honey possums in torpor after a lengthy stay in a pitfall trap.





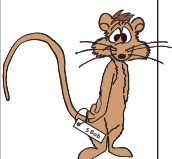




## A tale of five possums

During a field trip, research scientists collected data (including blood samples) from five honey possums, from various study sites. By analysing these blood samples they can calculate CO<sub>2</sub> production. These results, and other data regarding the possums, are recorded in the table below.



	Subject	Sex	Mass (g)	Length (cm)	Food source	Estimated time in trap (hrs)	CO <sub>2</sub> /L produced per day	FMR
1		M	10.2	8.3	<i>Banksia nutans</i>	2	1.58	
2		M	8.6	7.9	<i>Adenanthos cuneatus</i>	0.5	1.76	
3		M	8.4	7.6	<i>Banksia baxteri</i>	1	1.62	
4		M	6.8	6.6	<i>Banksia baueri</i>	4	1.41	
5		M	9.1	8.1	<i>Banksia coccinea</i>	8	0.73	

Question 7: Calculate the FMR in kilojoules per day ( $\text{kJ day}^{-1}$ ) for each honey possum using the information provided. (Remember the standard conversion factor for metabolism of carbohydrate is  $20.8 \text{ kJ}$ )

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Question 8: Calculate how many inflorescences each honey possum visited to meet its energy needs. Round values to the nearest whole number.

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Question 9: Construct a bar graph that displays information regarding:  
a) FMR ( $\text{kJ day}^{-1}$ ) for each honey possum, and  
b) the number of inflorescences visited daily by each honey possum.

Question 10: What do you notice about the approximate number of inflorescences each honey possum visited?

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Question 11: Comment on similarities and differences in the data for the five honey possums.

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Question 12: Explain how the food source of each honey possum might influence energy expenditure.

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Question 13: Data obtained for individual 'five' are different from the other honey possums. Discuss.

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Question 14: All animals in this study are male. Using your background knowledge, explain how data might be affected by adding females to the sample.

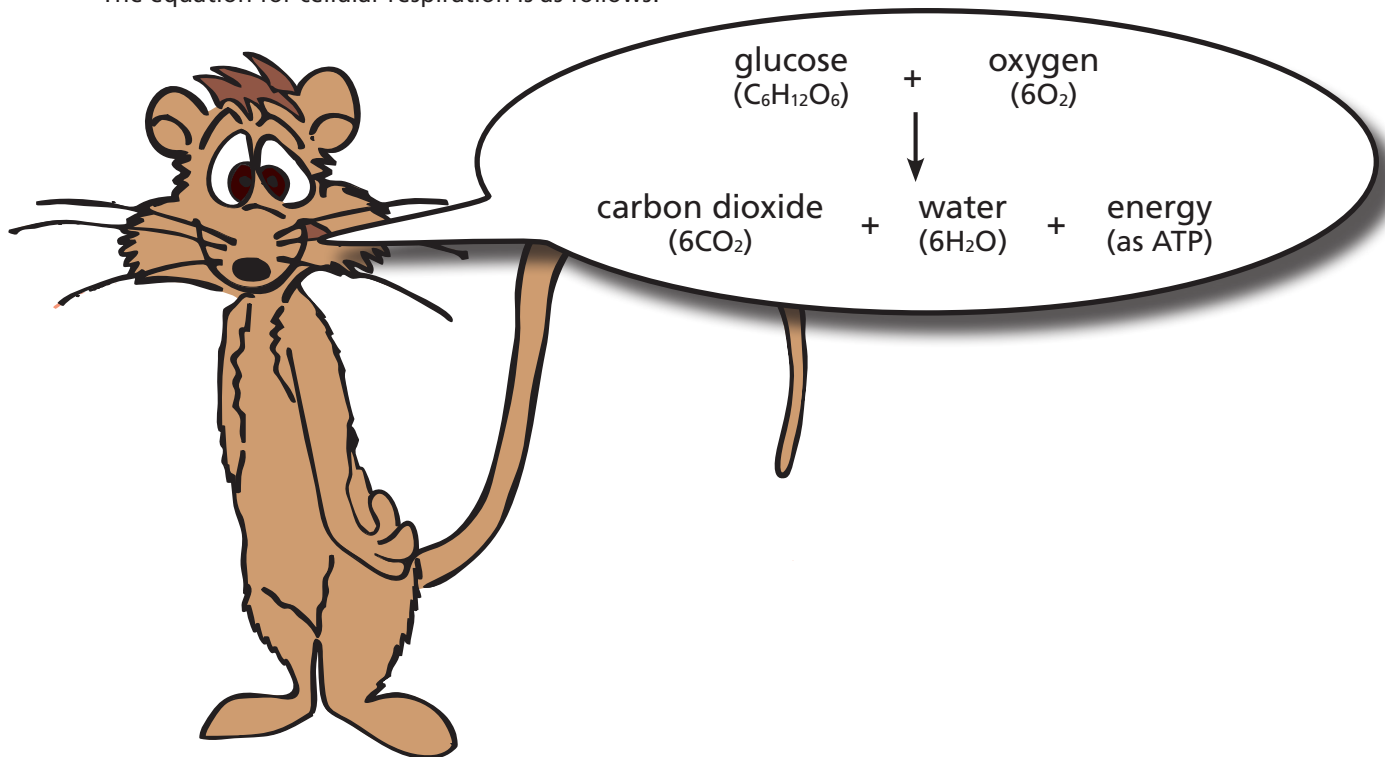
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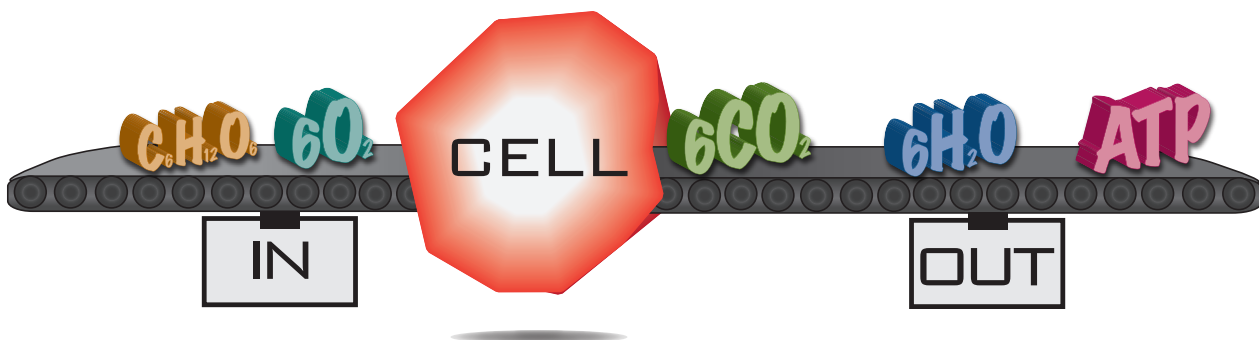
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The equation for cellular respiration is as follows:



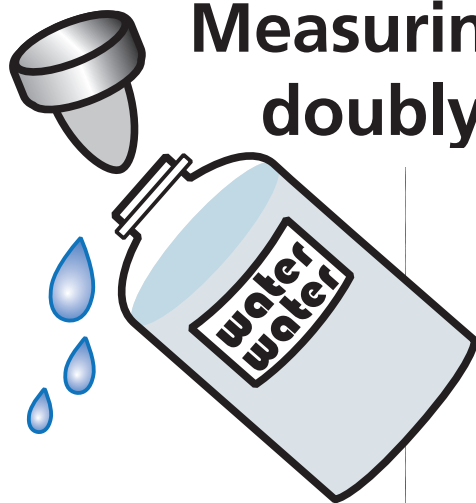
The cellular journey of these molecules is represented below:



Question 15: Using the honey possum as your reference, complete the following table:

Molecule	Where does it come from?	Where does it go?
glucose		
oxygen		
carbon dioxide		
water		





# Measuring respiration with doubly-labelled water

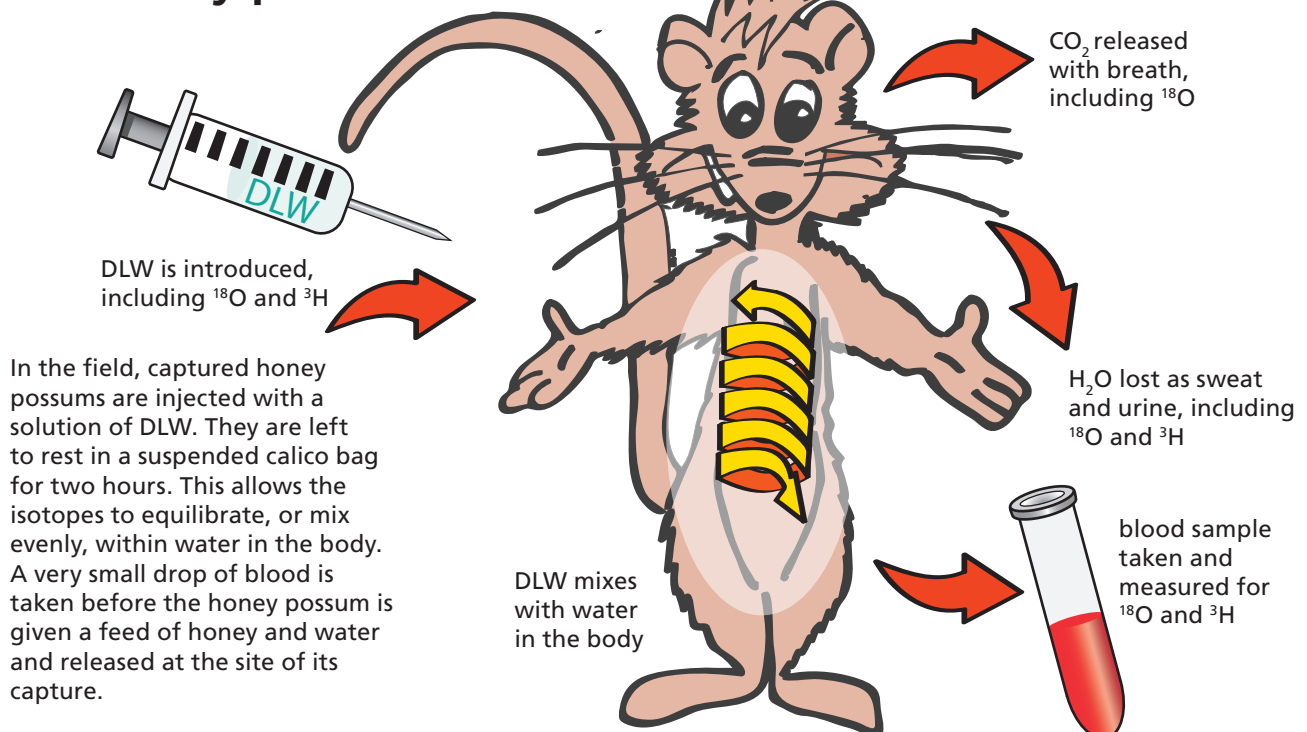


## What is doubly-labelled water?

Doubly-labelled water (DLW) is water that contains the radioisotopes heavy oxygen ( $^{18}\text{O}$ ) and tritium ( $^3\text{H}$ ). These isotopes act as molecular labels which allow scientists to measure changes in their concentration, over time. These isotopes occur naturally and have no adverse effects on subjects.  $^{18}\text{O}$  is stable and  $^3\text{H}$ , whilst radioactive, is used in such small amounts it is harmless. A solution of doubly-labelled water contains isotopes  $^3\text{H}_2\text{O}$  and  $\text{H}_2^{18}\text{O}$ .

The doubly-labelled water method is a field technique commonly used to measure the field metabolic rate of free-living animals such as mammals, birds, reptiles and even humans. Scientists find this method particularly effective with honey possums as they do not drink water. As no further dilution of the isotopes can occur within body water, the method can be used to provide an accurate measure of  $\text{CO}_2$  production.

## Doubly-labelled water and the honey possum



The tricky part comes next – recapturing the same honey possums. This is one of the most difficult aspects of field research because it relies mainly on luck! Recapture rates are low, around 4%, and recapture must occur quickly, within 36–48 hours.

This is because honey possums lose water rapidly, and after 48 hours the isotopes will be completely washed out of the body. Upon recapture, a second blood sample is taken. All collected blood samples are sent for analysis.

# What is being measured, and why?

The DLW method is effective because it is based on our understanding of biochemical processes that occur during cellular respiration.

$^3\text{H}$  and  $^{18}\text{O}$  injected into a honey possum mix with body water, including blood.  $\text{CO}_2$  produced by cellular respiration is transported, through the bloodstream, to the lungs. During this process a series of reactions result in  $^{18}\text{O}$  mixing with  $\text{CO}_2$ . This is important because it means that any  $\text{CO}_2$  exhaled from the lungs will also have  $^{18}\text{O}$  mixed with it.

As the honey possum breathes out  $\text{CO}_2$ , the concentration of  $^{18}\text{O}$  in the blood steadily decreases. By measuring the concentration of  $^{18}\text{O}$  that remains in the blood, scientists can estimate the amount of  $\text{CO}_2$  produced. There's a complication though, because  $^{18}\text{O}$  isn't just lost through  $\text{CO}_2$  – it's also lost in water as urine, sweat and vapour.

That's where  $^3\text{H}$  comes in. This radioisotope is only lost through water – there's no hydrogen in  $\text{CO}_2$ . By measuring the decrease in concentration of  $^3\text{H}$  in blood, scientists can estimate the amount of water lost by the animal. Once that's known, it's possible to work out how much of the decrease in  $^{18}\text{O}$  is due to water-loss, and how much is due to  $\text{CO}_2$  production. Now we have the figure we need – the amount of  $\text{CO}_2$  produced by the animal between release and recapture.

Question 16: Why is it important to wait for DLW to mix with body water?

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Question 17: Why is the DLW method used?

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Question 18: The DLW method has been used in animals as small as insects and as large as seals. It is also used in humans. However, this method is not suitable for tropical animals. Why?

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Question 19: Why wouldn't the DLW method be used in amphibians?

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Question 20: The use of the DLW method has important implications for honey possum conservation. Discuss.

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