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| Using elemental data to combat illegal wildlife trade |



*Image source: https://taronga.org.au/news/2018-07-11/echidnas-look-sharp-love*

**The following link provides access to the data spreadsheet required to complete the activities:**

[**Data sets | ANSTO**](https://www.ansto.gov.au/education/school-resources/data-sets) **under the heading ‘Using elemental data to combat illegal wildlife trade’.**

This document enables students to interpret and analyse authentic research data, presented in the accompanying **Echidna Quills Student Data** **MS Excel** **spreadsheet**. The source datasets were generated during a collaborative project between UNSW, UTS, ANSTO and Taronga Zoo Sydney, which is detailed in the scientific paper Brandis KJ, Meagher PJ, Tong L, Shaw M, Mazumder D, Gadd P, and Ramp D (2018) ‘Novel detection of provenance in the illegal wildlife trade using elemental data’. The project examines the effectiveness of high-resolution x-ray fluorescence as a tool to combat the international **illegal wildlife trade (IWT)** of short beaked echidnas, which are being removed from the wild and claimed as captive-bred.

These activities are suitable for Senior Biology students as well as students in Years 9 and 10.

**Students will:-**

* examine the adaptations of short beaked echidnas that increase their ability to survive in their environment
* explore the use of technology in contributing to the study and conservation of biodiversity
* construct simple graphs of the provided data using MS Excel, and interpret and analyse these graphs
* investigate how scientific knowledge interacts with social, economic, cultural and ethical issues.

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| **Australian Curriculum Content Descriptions and Skills**The activities provided address the following Australian Curriculum Science Understanding from**Biology Unit 1: Biodiversity and the interconnectedness of life**Science as a Human Endeavour * Scientific knowledge can be used to develop and evaluate projected economic, social and environmental impacts and to design action for sustainability (ACSBL014)

Science Understanding**Describing biodiversity** International biodiversity protection* Within the international scientific community, methods and findings related to biodiversity monitoring and analysis are shared through peer reviewed articles in international journals (ACSBL014).

**Ecosystem dynamics**Keystone species and conservation* Human activities (for example, over-exploitation, habitat destruction, monocultures, pollution) can reduce biodiversity and can impact on the magnitude, duration and speed of ecosystem change (ACSBL028)

The activities provided address the following Australian Curriculum Science Inquiry Skills (Biology Unit 1)Science Inquiry Skills * Represent data in meaningful and useful ways; organise and analyse data to identify trends, patterns and relationships; qualitatively describe sources of measurement error, and uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions (ACSBL004)
* Interpret a range of scientific and media texts, and evaluate processes, claims and conclusions by considering the quality of available evidence; and use reasoning to construct scientific arguments (ACSBL005)
* Select, construct and use appropriate representations, including classification keys, food webs and biomass pyramids, to communicate conceptual understanding, solve problems and make predictions (ACSBL006)
* Communicate to specific audiences and for specific purposes using appropriate language, nomenclature, genres and modes, including scientific reports (ACSBL007)
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**Combating the illegal trade of short-beaked echidnas**

**An Australian icon**

 The short-beaked Echidna (Tachyglossus aculeatus) is one of Australia’s most well-known animals and is featured on the 5c coin. It is a monotreme, an egg-laying mammal, like the platypus, and is found throughout Australia in almost all habitats, from snow-covered mountains to deserts. The short-beaked echidna is easily recognised by the quills (or spines) covering its back, which are actually modified hairs and can be up to 50 mm long. Hair is present between the quills, with echidnas from cold areas having more dense hair than the echidnas from warmer areas.



 Echidnas have a highly specific diet, feeding mainly on termites and ants, as well as larvae, worms, and other invertebrates found within their range, and they are well adapted for foraging for this food. They have an acute sense of smell which they rely on to detect their food, and large, sharp, flattened claws on their front feet, as well as a hairless snout or beak between 7 and 8 cm long, which they use to tear open rotten logs and break into ant and termite nests. They also have a long, sticky tongue, that is around 15 cm long and enables them to capture insects located in deep, narrow crevices. Echidnas have no teeth and grind their food between the tongue and roof of their mouth. Echidnas can eat up to two kilograms of insects in one meal!

Image source: [Short-beaked Echidna - The Australian Museum](https://australian.museum/learn/animals/mammals/short-beaked-echidna/)

Animals that prey on echidnas include feral cats, foxes, domestic dogs, dingos and goannas. When under threat, an echidna will roll up into a ball to protect its underside and tuck its snout and legs beneath itself, or dig into the ground until only the spiny back is exposed. In fact, these animals are very active diggers and help keep ecosystems healthy by turning over the soil. Echidnas are well-suited for digging and their backwards-pointing rear feet help to push soil away when they are burrowing.

Short-beaked echidnas breed only once a year, with the mating season being from June to September. Many males may follow one female until she is ready to mate, forming a line known as an ‘echidna train’, trailing along behind the female over long distances for many weeks.

After mating, the female lays a single egg, that has a soft leathery shell and is about the size of a grape. The egg is kept in her pouch and hatches after about 10 days. The emerging baby echidna, called a puggle, is blind and hairless, and around the size of a jellybean. The puggle clings to hairs inside the mother’s pouch and laps up milk that is secreted from special glands in the pouch. When the puggle develops quills, the female removes it from the pouch, and will leave the puggle in a nursery burrow that she has built while she forages for food, returning to the burrow to feed the puggle every few days. The puggle continues to suckle until it is about 7 months old.

**It’s all about the quills!**

In Australia, short-beaked echidnas are a protected species, making it illegal to capture or trade them. However, short-beaked echidnas are one of the few species of mammals that lay eggs, so they are highly prized as zoo animals around the world and are highly desired in the international pet trade. The mating practice of these echidnas is extremely difficult to replicate in captivity, and only four Australian zoos have successfully bred echidnas. Within the last ten years, fewer than 30 puggles have been born in captivity and successfully raised in Australian zoos, but as many as 50 short-beaked echidnas are sold around the world as ‘captive-bred’ each year! This very low captive breeding success rate at highly reputable Australian zoos and wildlife parks suggests most of the echidnas sold around the world must have been illegally taken from the wild and are being falsely claimed as ‘captive-bred’.

The illegal trade in protected wildlife is a serious and widespread crime that leads to increased biodiversity loss, as well as the potential spread of zoonotic and agricultural diseases. To combat this illegal trade, the origin of a traded short-beaked echidna needs to be accurately determined to identify if it has been raised in captivity or has been illegally taken from the wild. Researcher Dr Kate Brandis from UNSW, working in conjunction with ANSTO scientists Patricia Gadd and Dr Debashish Mazumder, as well as wildlife experts from the Taronga Wildlife Hospital, have developed a non-invasive, non-destructive and cost-effective way to tackle this problem. Their method involves elemental analysis using **high-resolution x-ray fluorescence** **(XRF)** - and it is all to do with the echidna quills!

Echidna quills are made of keratin, like hair, nails, feathers and scales of animals and, when they are growing, they become a record of the diet that the animal has been eating at that time. In other words, the quills are like a “time capsule” for what the echidna ate as it was growing. Because the quill grows over time, the base of the quill will be a record of the echidna’s recent diet and the tip of the quill reflects the animal’s diet further back in time.

The project involved **12 short-beaked echidna quills from captive** animals at Taronga Park Zoo in Sydney, and **11 quills from wild-caught** individuals that had been brought to either the Taronga Zoo Wildlife hospital or the Wildlife Veterinary Department of Mogo Zoo on the south coast of NSW. These wild echidnas came from a range of locations within 290 km of Sydney. All the captive echidnas had been at the zoo for between 22 months and 20 years, and they were all fed the same diet. This consisted of a powdered mixture of meat meal, corn, soy protein, potato starch and roughage from plant-derived material, with added vitamins and minerals, that was reconstituted with water to form a thick paste - quite different from the diet for wild echidnas which primarily consume termites and ants found within their home range.

 The echidna quills were taken from the animals with minimal handling and were cleaned of any surface contaminants. They were scanned at ANSTO using an **ITRAX micro** **X-ray fluorescence core scanner**, which measured the abundance of 24 different elements, including calcium, nickel, zinc and chromium, in samples taken every 200 μm (0.2 mm) along each quill. Many samples were taken along the quill (the actual number taken depended on the length of the quill) to take into account variations in diet due to time of year, and changes in uptake of nutrients as each quill developed. Machine learning, a type of artificial intelligence that enabled a computer algorithm to learn from the elemental data and more accurately make predictions about the quills, was able to correctly classify the quills as either wild-caught or captive-bred 100% of the time!

Dr Kate Brandis (right) and ANSTO Scientist Patricia Gadd with the ANSTO **ITRAX micro** **X-ray fluorescence** **core scanner** used to provide elemental scans of Echidna quills.

(*Supplied: UNSW and ANSTO*)

The XRF method has great potential for use *in-situ* at locations where trade in animals occurs, including at markets, breeding farms and ports of entry, as hand-held multi-elemental XRF scanners are currently available. This will enable enforcement officers on the ground to immediately determine whether an animal has been taken from the wild or legally bred, providing a less expensive, accurate, versatile and accessible alternative to sending samples for possible expensive laboratory testing techniques such as genetic testing and stable isotopes analysis.

XRF can also be used on other keratin tissue that records the diet as the animal grows, such as feathers, scales, and nails, to detect illegal trade of particular birds, reptiles and mammals. This study provides compelling support for elemental analysis using x-ray fluorescence as a new tool in combating illegal trade of wildlife to protect threatened species and wild populations.

**Further resources for students**

The following lists further resources that students may like to view as part of these activities:

* [There’s no way these cute, spiny creatures are all sold legally | National Geographic](https://www.nationalgeographic.co.uk/animals/2019/09/theres-no-way-these-cute-spiny-creatures-are-all-sold-legally)
* [New research cracks illegal wildlife trade | UNSW Newsroom](https://newsroom.unsw.edu.au/news/science-tech/new-research-cracks-illegal-wildlife-trade)
* [Dr Phoebe Meagher and Dr Lydia Tong, Taronga Zoo - YouTube](https://www.youtube.com/watch?v=Ffcd9DIlna4)

This video presents a discussion by researchers Dr Phoebe Meagher and Dr Lydia Tong who work at the Taronga Zoo Wildlife hospital, two of the authors of the paper on which this data set is based, about using elemental signatures to trace the origins of illegally traded wildlife. The authors discuss the use of scientific method, limitations of the study, the importance of working in a team, and the need for effective science communication.

* [What is X-ray fluorescence (XRF)? | XRF explained - YouTube](https://www.youtube.com/watch?v=cAKcOyrt5Vc&t=39s)

This video explains what X-ray fluorescence (XRF) is, and how it works. It also outlines the advantages of XRF over other techniques.

**Reference**

This resource is based on a study that is reported in the following scientific paper:

Brandis KJ, Meagher PJ, Tong L, Shaw M, Mazumder D, Gadd P, and Ramp D (2018) ‘Novel detection of provenance in the illegal wildlife trade using elemental data’. *Scientific Reports* 8:15380.

The paper can be accessed via this link [https://www.nature.com/articles/s41598-018-33786-0](https://aus01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.nature.com%2Farticles%2Fs41598-018-33786-0&data=05%7C01%7Cjuliem%40ansto.gov.au%7C01343edc51b14d8d036908dbf087b8ad%7C4cbf9a84567a44d09db11dbfbf8393f0%7C0%7C0%7C638368237958255216%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=W%2FRnTidxktbybzkJGaCfx7D%2BdAn135nwAz%2BKxgaF1Gg%3D&reserved=0)

ANSTO would like to acknowledge all who assisted in this study.

**Activity 1: Investigating short beaked echidnas**

1. Echidnas are monotremes. What does this mean? Which other Australian animal is a monotreme?

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1. There are three types of adaptations:

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| Structural: | a feature of an organism's body that helps it to survive |
| Behavioural: | response made by an organism that helps it to survive |
| Physiological: | an internal and/or cellular process that helps an organism to survive |

1. Annotate the diagram of the echidna to show the **structural adaptations** it has for obtaining its food.



Diagram source: [factsheet3Echidnas.pdf](file:///J%3A%5CEchidnas%20DATA%20SET%5Cfactsheet3Echidnas.pdf)

1. Describe a **behavioural adaptation** of the echidna.

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1. Construct a **flow diagram** to show the steps in the natural breeding process of short-beaked echidnas.

1. What problem were the scientists wanting to solve through their project? Why is this important?

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1. Why is it thought that most of the echidnas sold around the world have been illegally taken from the wild and are being falsely declared as ‘captive-bred’? Why is this of concern?

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1. Explain why the elemental analysis of the quills of a short-beaked echidna could be used to show whether the animal is wild-caught or captive-bred.

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1. A team of scientists, wildlife experts and researchers worked on this project. The video, [Dr Phoebe Meagher and Dr Lydia Tong, Taronga Zoo - YouTube](https://www.youtube.com/watch?v=Ffcd9DIlna4), discusses the importance of teamwork in scientific research. Why do you think teamwork is important in scientific research?

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**Activity 2: Elemental analysis of echidna quills**

23 echidna quills were scanned at ANSTO using an **ITRAX micro** **X-ray fluorescence (XRF)** **core scanner**. The XRF samples were spaced every 200μm (0.2 mm) along the quill, and the abundance of 24 different elements was determined in each XRF sample. As quill lengths varied this meant that the number of XRF samples per quill ranged from 94 to 342. So, overall, the scientists had over 100 000 data samples to process!



*ANSTO scientists scanned each quill at 200µm intervals using micro X-ray fluorescence (XRF)*

While the scientists in this study used complex statistics to analyse their data, you will be analysing these data using simple graphs. Therefore, we have condensed this data into an average element count of the total number of XRF samples taken for each individual quill. The **‘Quill Element Averages’** sheet in the **Echidna Quills Student Data** **MS Excel** spreadsheet shows the average count for each of the 24 elements for all 23 quills examined. To aid the construction of the graphs, we have also inserted a column giving a numerical identifier for each of the short-beaked echidna groups, where captive is represented by 1, and wild is represented by 2.

Use the data provided in the **‘Quill Element Averages’** sheet of the accompanying MS Excel spreadsheet **Echidna Quills Student Data** to complete the activities and to assist you in answering the following questions:

a (i) Using MS Excel tools, construct a scatter graph of the average elemental count of **calcium** for each of the echidna quills shown in the MS Excel spreadsheet. Your graph should also indicate which echidna quills come from captive-bred echidnas and which come from wild-caught echidnas.

**HINT:** To construct your graph you can follow the instructions below:

1. Select the **captive echidnas** by highlighting the cells containing 1 in column C by clicking on cell C3 and dragging the cursor to cell C14.
2. Holding down the “Command” or “Control” key, click on cell K3 and drag the cursor to cell K14 to highlight the **average calcium count** for each captive echidna quill.
3. Click the **insert** tab, click **scatter** from the Charts shown, then click on the first scattergraph in the dialog box

(Insert -> Chart -> scatter)

1. Right click in the plot area of the chart and choose ‘**select data’**.
2. In the dialog box that opens, select **edit,** and in the second dialog box that opens type in the series name “**Captive**”. Click OK.
3. Select **Add** in the dialog box.
4. In the second dialog box that opens type in the series name “**Wild”**.
5. Click in the **series X values**, then highlight column C cells containing 2 by clicking on cell C15 and dragging the cursor to cell C25. This selects the **wild echidnas**.
6. Click in the **series Y values**, delete ={1} and highlight cells K15 to K25 being the average calcium count for the wild echidna quills . Click OK, then click OK.
7. Click on **Chart Design** from the top row tools, then click **Add Chart Element** (Chart Design -> Add Chart Element). Scroll down to highlight **Chart Title**, then click **Above Chart.** Type an appropriate title for your graph then press **enter**.
8. Click on **Add Chart Element**, scroll down to highlight **Axis Titles**, then click **Primary Horizontal** and type an appropriate label. Repeat for the **Primary Vertical** axis title.
9. Click on **Add Chart Element**, scroll down to **Legend** and choose a position for your legend.
10. To have only the numbers 1 and 2 for the two groups of echidna shown on the X axis, right click on the X axis values and choose **format axis** from the dialog box that opens. A **Format Axis** box will show on the right-hand side. Under Axis Options-Bounds set Minimum at 0.0 and Maximum at 2.0, and under Units set Major at 1.0.

Insert your graph here.

1. Compare the range of values for the average calcium count per quill for captive-bred echidnas with the range for wild-caught echidnas.

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1. An echidna quill has a calcium count of 375. Predict whether the echidna is wild-caught or captive-bred and justify your answer.

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b (i) Construct a scatter graph of the average elemental count of **chromium** for each of the echidna quills given in column N of the MS Excel spreadsheet using the instructions provided for question a. Your graph should also indicate which echidna quills come from captive-bred echidnas and which come from wild-caught echidnas.

Insert your graph here.

1. Compare the range of values for the average chromium count per quill for captive-bred echidnas with the range for wild-caught echidnas.

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1. An echidna quill has an average chromium count of 220. Predict whether the echidna is wild-caught or captive-bred, and justify your answer.

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(c) From their elemental abundance data, researchers used machine learning to produce a ranked importance of the 24 different elements analysed to predict captive-bred versus wild-caught.

The table below shows the top ranked elements for distinguishing captive-bred from wild- caught echidnas, and the counts that were indicative of captive quills for these elements.

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| **Element** | **Counts that are indicative of captive quills** |
| nickel | Above 600 |
| calcium | Above 200 |
| chromium | Above 300 |
| Zinc | Above 250 |

The **Echidna Quills Student Data** MS Excel spreadsheet provides high resolution x-ray fluorescence scan data for three quills that were taken from three individual short-beaked echidnas, A, B and C (see sheet labelled **Echidna quills A, B, C**). The data shows the abundance of nickel, calcium, chromium and zinc in a 1 cm section of each echidna quill. 10 samples were taken at intervals of 1000 μm (1 mm) along the section of quill.

1. For each of the quill scans, use the information in the table above to predict whether each individual short-beaked echidna (A, B and C) is captive-bred or wild-caught, and justify your answers.

**HINT:** Use **conditional formatting** to assist you in determining whether the count is indicative of captive quills or not. To do this follow the instructions below:

1. Highlight all the data for calcium in column C by clicking on cell C3 and dragging the cursor to cell C32.
2. In the tool bar click **home**, then click **conditional formatting**. Choose ‘highlight cell rules’ then select ‘greater than’
3. In the dialog box that opens type in 200 (above this count is indicative of captive quills for calcium as shown in the table)
4. Click OK. This will highlight in red all values in the Calcium column that are greater than 200.
5. Repeat steps 1 to 4 for each of the elements shown in the spreadsheet, typing the correct number for that element in the dialog box from the **Counts that are indicative of captive quills** column in the table.

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1. Suggest TWO ways in which you could increase the precision and reliability of this data for the short-beaked echidna quills A, B and C.

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1. Why do you think it is necessary to consider the elemental abundance of more than one element to classify an echidna as wild-caught or captive-bred?

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**Activity 3: stable isotope analysis of echidna quills**

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| Tissue of living organisms contains the elements carbon and nitrogen, which are obtained through what they eat. There are two stable (non-radioactive) isotopes (atoms of the same element, each having a different number of neutrons) of carbon in the environment – carbon-12 which makes up 98.9% of carbon, and the heavier carbon-13 isotope which makes up around 1% of carbon. Nitrogen exists in two stable isotopic forms, the most common is nitrogen-14 which makes up 99.6% of nitrogen in the environment, and the other is nitrogen-15 which is heavier and has an abundance of about 0.4%. food web illustrationThe nitrogen (δ15N) and carbon (δ13C) isotopic composition of tissues is a measure of an organism’s diet, and can be used to determine its relative position in the food chain (or food web)Image Credit: [USGS -- You are what you eat](https://wwwrcamnl.wr.usgs.gov/isoig/projects/fingernails/foodweb/isotopes.html)As animals eat, their tissues take on the isotopic composition of their food. Scientists can use the isotopic composition of an animal tissue as a measure of the diet of an organism, and use it to determine its relative position in the food chain (or food web) of its ecosystem. The scientists in this study carried out a stable isotope analysis for carbon (δ 13C) and nitrogen (δ15N) of each of the echidna quills to assess the accuracy of this method in separating captive-bred quills and wild-caught quills. They compared the accuracy of the stable isotope analysis with the accuracy of the high-resolution x-ray fluorescence elemental analysis in classifying the echidna quills as wild-caught or captive-bred. |

1. Food chains show the flow of energy from one living thing to another, using arrows that point in the direction in which the energy and nutrients flow through the organisms. They always begin with a primary producer or autotroph (an organism that can produce its own food, such as a plant).

Construct TWO food chains that include the short-beaked echidna.

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1. What are isotopes? State the two naturally-occurring, stable isotopes of carbon.

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1. The stable isotope analysis (δ 13C, δ15N) of echidna quills is a **destructive test**. The test required considerable sample preparation, involving grinding up the quills and weighing samples into tin caps, for analysis at the Bioanalytical Mass Spectrometry Facility at UNSW. A sample of the captive diet from Taronga Zoo was also analysed for comparison with captive quill results.

**Did you know?** 

When scientists carry out a stable isotopes analysis of a sample, they compare the relative amounts of a heavier isotope and a lighter isotope of that element to trace what the organism ate, and they report the results in a special notation, called "delta (δ) notation". δ notation is a way of relating the isotope composition of a sample to that of an international reference standard. The δ values are given in units of "per mil" **(‰)**, which is **parts per thousand** difference from the isotope ratio of the reference standard. Most living things have a smaller ratio of 13C to 12C isotopes than the carbon standard, so the δ 13C values are negative. Conversely, animals typically have a higher ratio of 15N to 14N isotopes than the nitrogen standard, so the δ15N values are positive.

The results of the stable isotopes analysis are shown in the **‘stable isotopes data’** sheet of the accompanying MS Excel spreadsheet **Echidna Quills Student Data**. One echidna quill is labelled Wild-Captive (W-C1), because the quill came from an echidna that was originally wild and was subsequently in captivity for 5 months.

 (i) Use the data provided in the **‘stable isotopes data’** sheet of the accompanying MS Excel spreadsheet **Echidna Quills Student Data** to construct a scatter graph of δN-15 versus δC-13 for each of the echidna quills shown in the MS Excel spreadsheet. Your graph should indicate which echidna quills come from captive-bred echidnas and which come from wild-caught echidnas, as well as show the data point for food and for the wild-captive echidna.

**HINT:** To construct your graph you can follow the instructions below:

1. Highlight the **δC-13 values** for all the captive echidna quills in column C by clicking on cell C2 and dragging the cursor to cell C13.
2. Holding down the “Command” or “Control” key, click on cell D2 and drag the cursor to cell D13 to highlight the **δN-15** **values**.



1. Click the **insert** tab, click **scatter** from the Charts shown, then click on the first scattergraph in the dialog box (Insert -> Chart -> scatter)
2. Right click in the plot area of the chart and choose ‘**select data’**.
3. In the dialog box that opens, select **edit** and in the second dialog box that opens type in the series name “**Captive**”. Click OK.
4. Select **Add** in the dialog box.
5. In the second dialog box that opens type in the series name “**Wild”**.
6. Click in the ‘series X values’ box, then highlight column C δC-13 data from C14 to C24 for wild echidna samples W-1 to W-11.
7. Click in the series Y values, delete ={1} and highlight column D δN-15 data from D14 to D24 for the wild echidna samples. Click OK.
8. Select **Add** in the dialog box, and type in the series name “**Wild-Captive”**.
9. Click in the ‘series X values’ box, then highlight cell C25, then click in the series Y values, delete ={1} and highlight cell D25. Click OK.
10. Select **Add** in the dialog box, and type in the series name “**Food”**.
11. Click in the ‘series X values’ box, then highlight cell C26, then click in the series Y values, delete ={1} and highlight cell D26. Click OK to close the dialog box, then click OK.
12. Right click on the X axis values and choose **format axis** from the dialog box that opens.
13. Under Axis Options bounds, type -25.0 for minimum and -10.0 for maximum.
14. Click on Chart Design in the top tool bar. Click on **Add Chart Element** on the left-hand side.(Chart Design -> Add Chart Element). Scroll down to highlight **Chart Title**, then click **Above Chart.** Type an appropriate title for your graph then press **enter**.
15. Click on **Add Chart Element**, scroll down to highlight **Axis Titles**, then click **Primary Horizontal** and type an appropriate label. Repeat for the **Primary Vertical** axis.
16. Click on **Add Chart Element**, scroll down to **Legend** and choose a position for your legend.
17. To move the Y-axis values to the left-hand side of the graph, right click the Y-axis values and choose **Format Axis** in the dialog box that opens. This will open a box on the right-hand side.
18. Click **Labels,** and for label position select **Low**.

**OPTIONAL:**

You can **add labels to each of your data points**. Follow the instructions below to do this.

1. Right click on one of the ‘captive’ data points and select ‘Add data labels’ from the dialog box that opens. This will add the Y-values to each data point. To change this label, right click on one of the data labels and choose ‘format data labels’ at the bottom of the dialog box that opens.
2. In the format data labels box on the right-hand side, select ‘Value From Cells’. This opens another dialog box ‘Data Label Range’. Highlight cell A2 to cell A13, then click OK. This will also label the data points C-1 to C-12.
3. Click on ‘Y Value’ in box on right hand side to delete this label, so that only the C1 to C12 labels show.
4. Repeat for the ‘wild’ data points, and the ‘wild-captive’ and ‘food’ data points, highlighting the appropriate cells in column A.
5. You can change the position of the data labels by clicking on the labels and then moving the cursor over the label you want to move until the cross appears. Click and drag the label to the new position.

Insert your graph here.

1. Describe any patterns or trends that you observe from the graph.

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1. Suggest a reason for any patterns or trends you observed.

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1. Comment on any outliers in terms of quill classification.

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1. A Wild-Captive echidna quill (W-C1) was also subjected to δ 13C and δ15N stable isotope analysis. Comment on the position of this quill on the graph, and what this might mean in terms of detecting illegally traded short-beaked echidnas.

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1. What advantages does XRF elemental analysis have over stable isotope analysis as a tool for distinguishing between wild-caught and captive-bred short beaked echidnas?

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1. State any limitations of the study, and suggest further investigations that could be carried out to provide more evidence for the use of this technique in combating illegal laundering of these animals.

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1. The study provided evidence for the use of high-resolution x-ray fluorescence of echidna quills to identify captive-bred versus wild-caught short-beaked echidnas in order to detect illegal laundering of these animals as part of the international wildlife trade (IWT). The researchers also state that this method could also accurately determine the legal status of other animals and their parts, not just short-beaked echidnas.

Suggest a reason why high-resolution x-ray fluorescence could provide a much-needed solution for combating the illegal laundering of a variety of wildlife.

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