NATIONAL ENTRANCE EXAMINATION 2022

Examination Date: Wednesday 24 August 2022

Total Time allowed: 2 hours.

This examination consists of 60 multi-choice and simple calculation questions delivered on Education Perfect. It may be sat at any time on the 24th between 8am and 8pm.

Students are advised to allocate equal time to each question (**2 minutes per question**). Marks will not be deducted for incorrect answers.

1. Open Google Chrome (note this technology is not supported by safari so you must not use this browser).
2. Login to Education Perfect using the username and password sent to the email you registered under.
3. Click on the exam and begin. Once you begin you will have exactly 2 hours to complete all 60 questions.

The exam must be sat under exam conditions and is invigilated by a teacher in your school. It also uses remote monitoring, notifying NZIBO if a student is off task or having connection issues.

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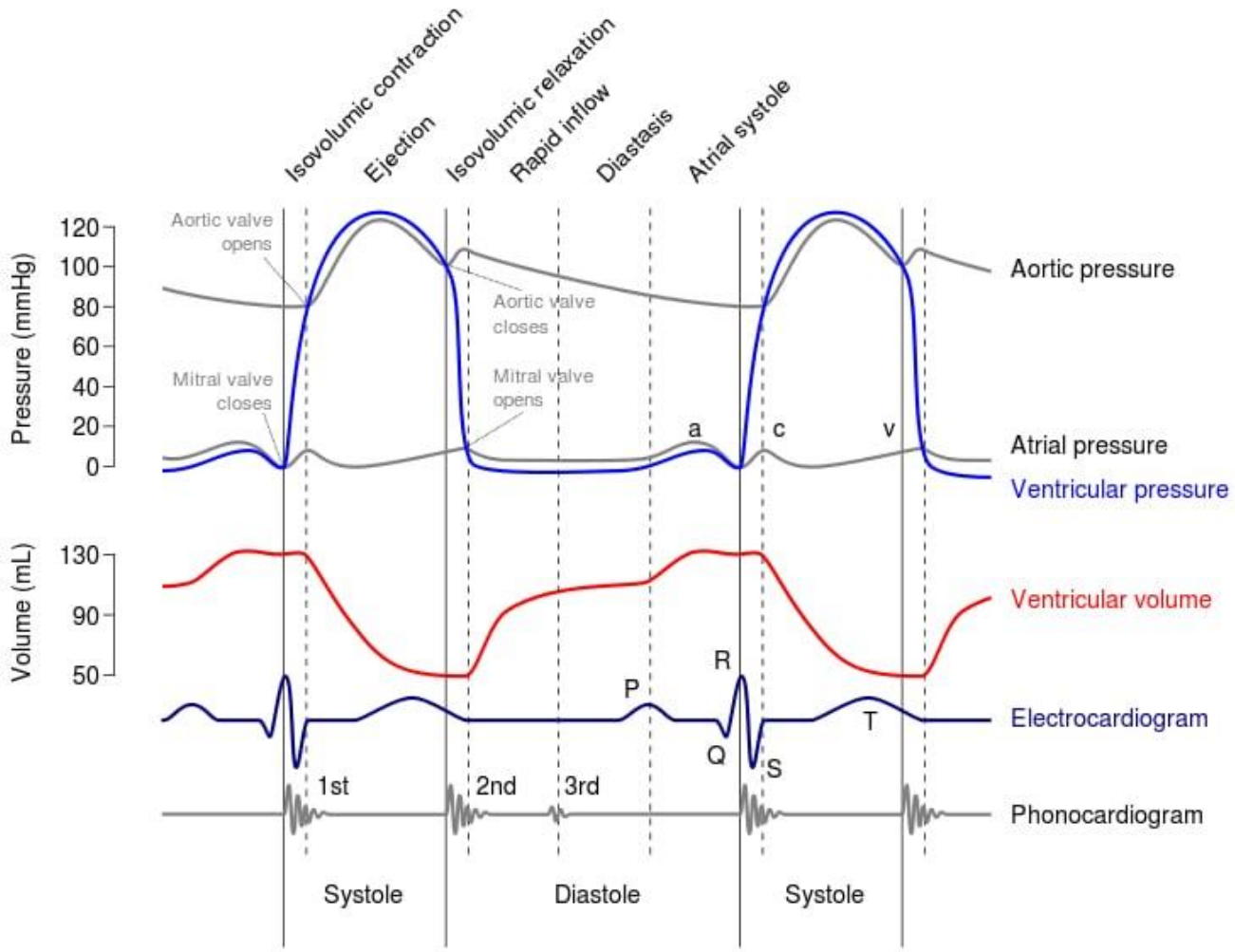
Section A

Question 1
The diagram below shows the changes in the volume and pressure in the heart as it contracts and relaxes.



Source: https://litfl.com/cardiovascular-physiology-overview/

What is the ventricular volume during the isovolumic relaxation phase of the heart cycle?
Enter your answer here _____

Question 2

The amoeba sisters are two sisters from Texas that create science YouTube videos, GIFs, comics, and resources to help us understand science.

Check out their website after the exam https://www.amoebasisters.com/

In the cartoon at left "Bacteria and verses from the song Grace Kelly" they teach us a few facts about bacteria set to "Grace Kelly" by MIKA.

Escherichia coli, also known as *E. coli* is a Gram-negative coliform bacterium of the class Bacillus. They are commonly found in the lower intestine of warm-blooded organisms. Some can cause serious food poisoning and are transmitted by fecal contamination.

What shape are E. coli?

A. Spiral
B. Rod
C. Paired rods
D. Spherical
E. Chain of spheres

Question 3

In ants, bees and wasps, fertilised eggs develop into females and unfertilised eggs develop into males. As a result, males are haploid (have a single set of chromosomes); their sperm are produced by mitotic division and are thus identical.

In the honeybee, all members of a colony are descended from a single female, the queen. Depending on their diet, her daughters either develop into sterile workers or fertile queens. Her sons become drones, whose function is to fertilise a young queen.

After fertilisation, a young queen stores the sperm, which survive in her reproductive tract for her entire reproductive life. All her female offspring therefore receive identical genetic contribution from their male parent.

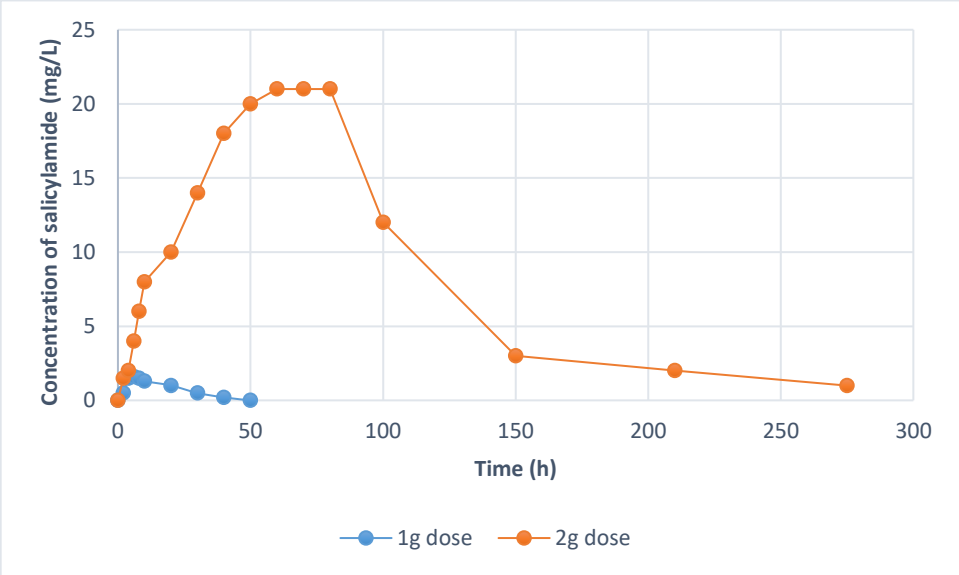
Which of the following alternatives indicates the proportion of genes shared between workers and others in the colony?

	Proportion of genes shared between workers and		
	Mother	Sisters	Brothers
A.	¾	¼	½
B.	¾	½	½
C.	½	½	¼
D.	½	¾	½
E.	½	¾	¼

Questions 4 & 5

Salicylamide is a non-prescription drug used for pain relief and lowering fever. It is closely related to aspirin. The graph below shows its absorption into the blood serum after a 1 g dose (blue dots) and after a 2 g dose (orange dots). Peak blood serum concentrations are usually achieved in 4–6 hours after therapeutic doses.

2–30% of salicylamide is excreted unchanged in the urine. Serum concentrations will also decline as the salicylamide is metabolized. The major metabolic pathways of biotransformation are capacity-limited which leads to accumulation and slower elimination as salicylate in the body increases. This dose dependent, prolonged excretion increases a person's risk of serious toxicity. Toxic doses of this drug result in depression of the central nervous system.



Source: modified from Barr, W.H. 1969 Factors involved in the assessment of systemic or biologic availability of drug products, Drug Inform. Bull., 3, 27-45

Question 4

What is the peak blood serum concentration for a therapeutic dose of salicylamide.

A. 1.8 mg/L
B. 8 mg/L
C. 10 mg/L
D. 21 mg/L
E. None of the above

Question 5

Using all the data above, which of the statements below (1-5) about the toxicity of salicylamide are correct?

I. A 2g dose of salicylamide is toxic to humans.
II. A toxic dose of salicylamide prolongs metabolism and excretion leading to higher blood serum concentrations of salicylamide.
III. Toxicity is higher at higher doses of salicylamide because the blood serum concentration exceeds the therapeutic dose.
IV. Symptoms of toxic doses of salicylamide are likely to include confusion and tiredness.
V. Patients with renal (kidney) failure are more likely to exhibit toxic effects from salicylamide.

A. All statements above (i. – v.) are correct.
B. Statements i., ii., and iv. are the only correct statements.
C. Statements i., ii., and v. are the only correct statements.
D. Statements ii., iv., and v. are the only correct statements.

E. Only statements iv. and v. are correct.

Question 6
Waimarie was asked to paint apartment numbers on the door of each apartment in her father's apartment block. The block has 105 apartments. How many times will she have to paint the number eight?

A. 10
B. 15
C. 19
D. 20
E. 25

Question 7
In children with vomiting and diarrhoea managing fluid balance is critical to prevent dehydration which is a major cause of morbidity and mortality in infants and young children worldwide.

Normal urine output in a healthy adult individual should be between 0.5-1.5 mL/kg/hour, and patients should generally be urinating at least every 6 hours. Oliguria is a decreased urine output of < 500mL per day in adults, < 0.5 mL/kg/hour in children, and < 1.0 mL/kg/hour in infants. Anuria is when there is no or minimal urine output. Usually < 100 mL/day in adults or less than half of normal output in children and infants. To calculate the rate of urine output you divide the volume of urine produced by the number of hours expressed per kilo of weight for a child or infant.

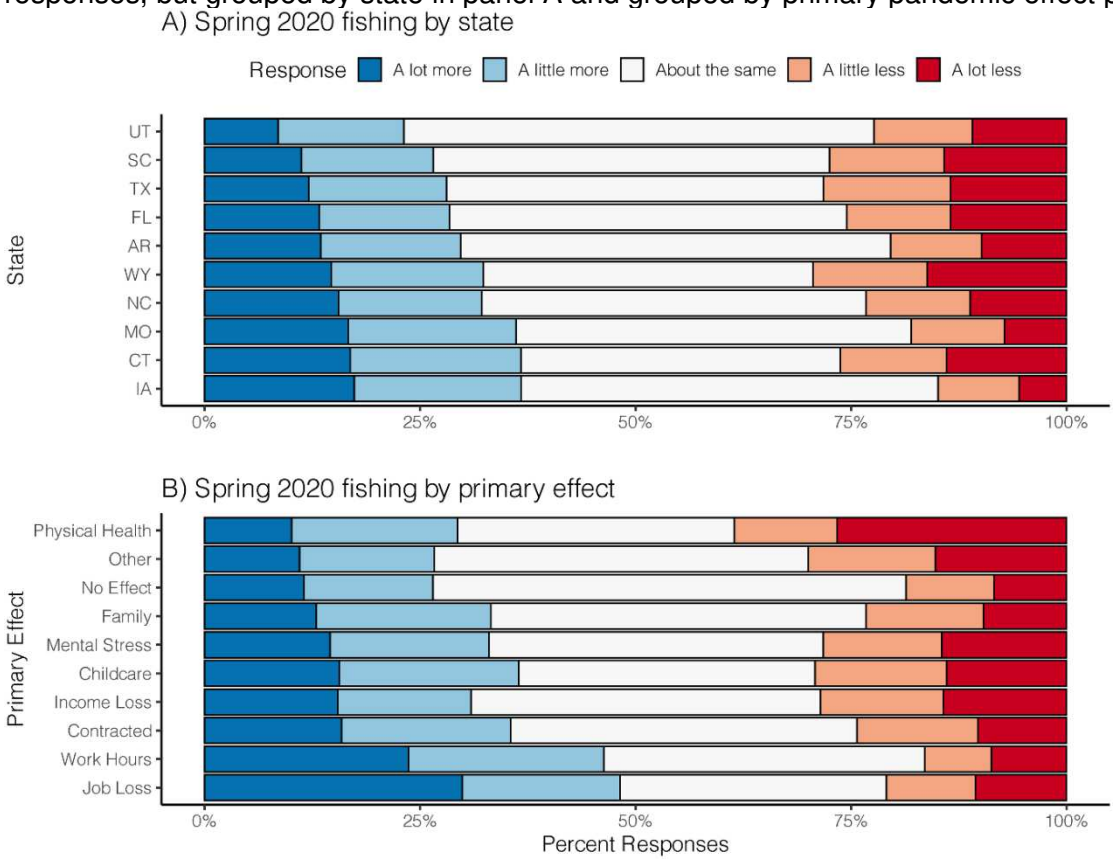
A 1-month-old infant was hospitalised to manage dehydration after catching a nasty stomach bug. The infant weighs 4.8kg. After a day of IV fluids the nurses recording the infant's fluid balance note that the baby had only produced 8 mL of urine over the last 4 hours. Calculate the infant's urine output and decide whether the infant has now recovered from dehydration, has oliguria, or anuria.

Enter the infant's urine output here _____

Has the infant recovered, has oliguria or has anuria (Choose one).

Question 8

The diagram below shows the response of almost 18000 people in America who responded to a survey comparing their fishing trips before and after the outbreak of COVID 19. Note that the data are the same responses, but grouped by state in panel A and grouped by primary pandemic effect panel B



Breakdown of self-reported fishing in spring 2020 (during pandemic) compared to a typical spring.
Source: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0254652#pone-0254652-g004

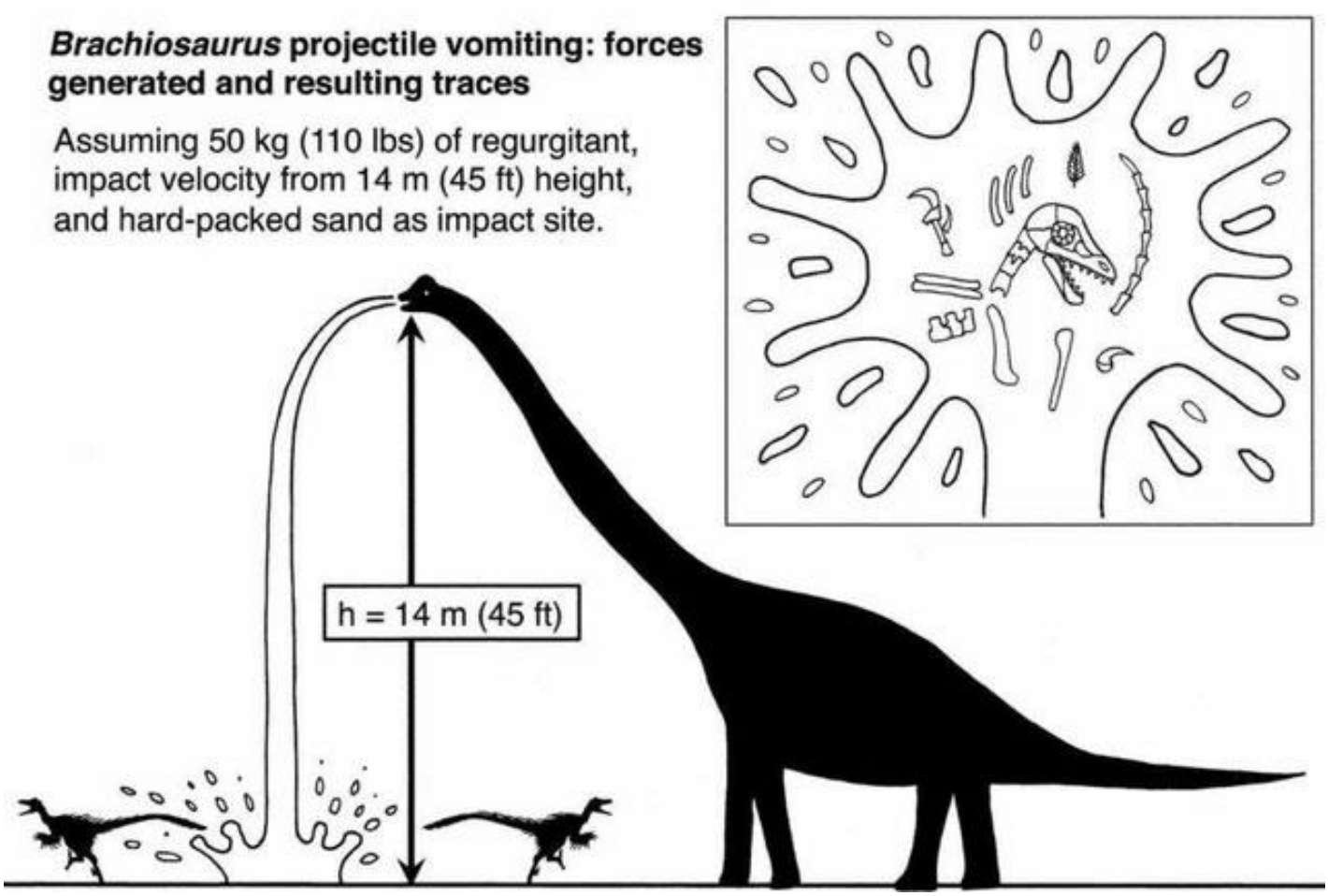
What is the best conclusion that can be made from these data?

- **A.** People were generally fishing more during the pandemic.
- **B.** People were generally fishing less during the pandemic.
- **C.** There was no significant change in fishing trips before and during the pandemic.
- **D.** People were generally working longer hours during the pandemic.
- **E.** There is no valid conclusion that can be made.

Question 9

Brachiosaurus was an herbivorous four-legged dinosaur. The body of the Brachiosaurus was about 26 m long, and it was 12-16 m tall and weighed about 33-88 tons.

The size of brachiosaurus likely provided protection from predators, and it may also have used its tail or legs to defend itself by hitting predators. However, recent work suggests this is unlikely because the tail of this species is short and small compared to the whole body generating a relatively small force and the length of its neck means it would be difficult to lift its front legs off the ground to stomp a predator. Researchers have suggested that they may have vomited on attackers as a defence mechanism. Could this even be possible? Would the vomit have sufficient impact force to injure a predator?



Source: Dinosaurs without bones, Tony Martin

Calculate the impact velocity in ms^{-1} of the brachiosaurus vomit using the formula

$$V = \sqrt{2gh}$$

Calculate the kinetic energy before impact in Joules. The kinetic energy formula defines the relationship between the mass of an object and its velocity. It is given by the equation

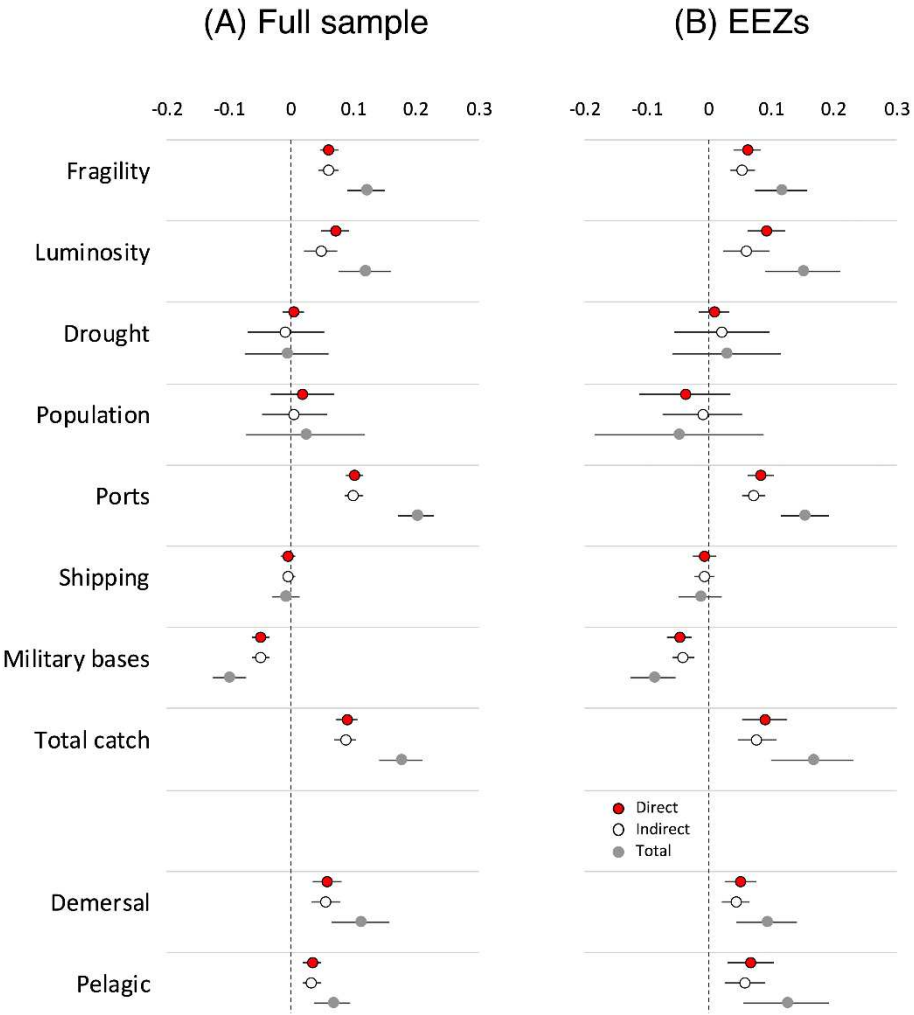
$$\mathrm{KE} = \frac{1}{2}mv^2$$

Remembering that the acceleration due to gravity on Earth is a constant 9.8 $ms^{-1}s^{-1}$, calculate the average impact force in Newtons.

Enter your answer here _____

Question 10

The figure below shows an analysis of a range of factors and their correlation with piracy (e.g. attacking a ship for ransom). There are two sets of data displayed – that from all piracy events and those that occurred in the Exclusive Economic Zone (EEZ) of a country. The 'total catch' refers to illegal, unreported, or unregulated fisheries. This is an estimate of the level of destructive fishing that occurs in an area. This category is then further split into fish found on the ocean floor (demersal) and fish found higher in the water column (pelagic).



Which is the best conclusion that can be made from these data?

- **A.** The bigger a country's population, the more piracy.
- **B.** There is more piracy in an area where there is more destructive fishing.
- **C.** There is less piracy in an area where there is more destructive fishing.
- **D.** The more shipping in an area, the more piracy.
- **E.** There is more piracy in the Exclusive Economic Zone of countries that outside of it.

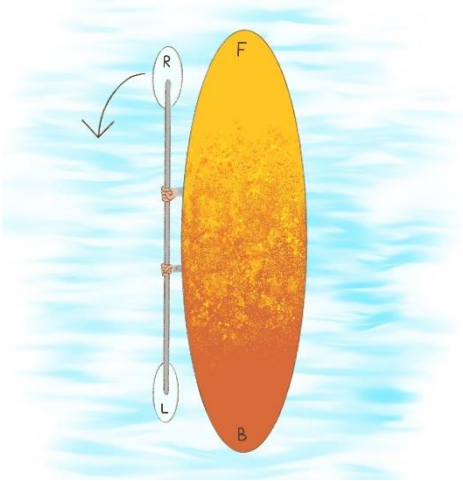
Question 11

When white-water kayaking, it is important to learn how to roll up your kayak if you flip over. When upside-down, you must move your paddle into the setup position, push the blade along the surface of the water and flip the boat over with your hips, finishing in the finish position above water.

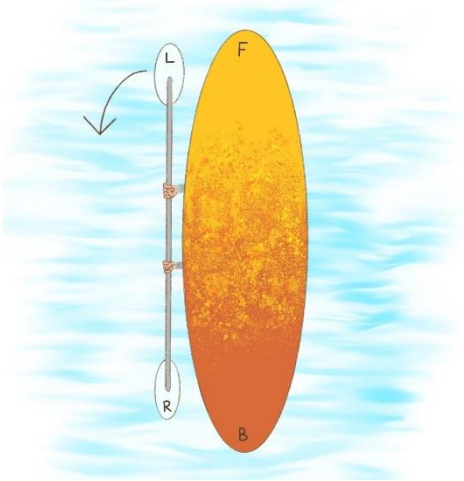
In the first two images we see a student practicing the setup position above water and practicing the finish position too. The kayak is labelled F and B for front and back and the paddle is labelled L and R for left and right.

What would the setup position look like when the boat is actually upside-down? All images are viewed from above the water. **A.**, **B.**, **C.**, **D.**, or **E.** None of the diagrams is correct.

A.



B.



C.

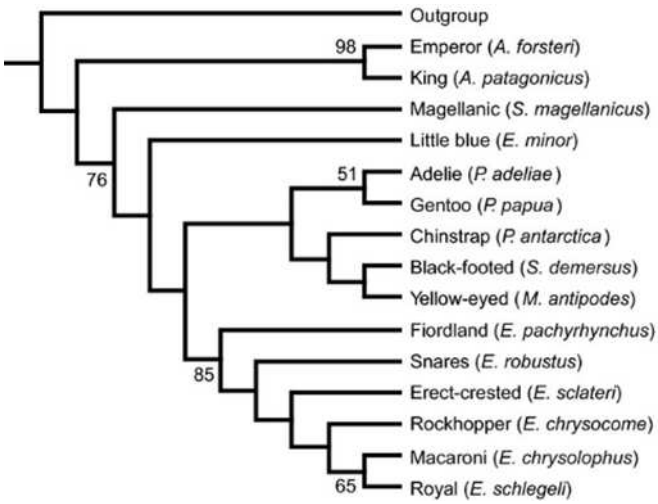
D.

Question 12

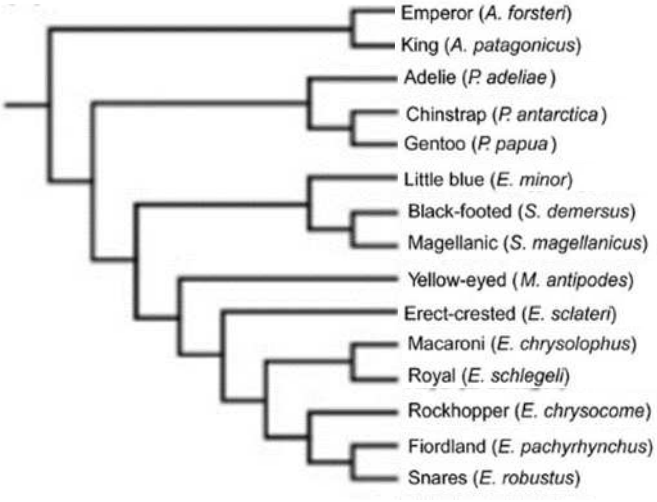
Lorenz (1958) and Tinbergen (1959) claimed that behavioural characters could be used to infer evolutionary relationships but similarities among behavioural traits are not always indicative of homology (a similarity due to shared ancestry between a pair of structures or genes in different taxa). In phylogenetics, a sister group comprises the closest relative(s) of another given unit in an evolutionary tree. When trees from independent datasets are congruent (in agreement), it is assumed that the congruence is caused by a common phylogenetic signal contained within each.

In the Figure below the behaviour and life history (BLH) (**A.**) and molecular (**B.**) traits of 15 penguin species were used to generate phylogenetic trees that allow us to evaluate hypotheses about penguin evolutionary relationships and the use of BLH traits to infer phylogeny. The phylogeny based in molecular data provided in **B.** is congruent with most other phylogenies published for penguins.

A.



B.



Source: https://onlinelibrary.wiley.com/doi/10.1111/cla.12040

Considering the information provided in **BOTH** trees which of the following statements is/are correct?

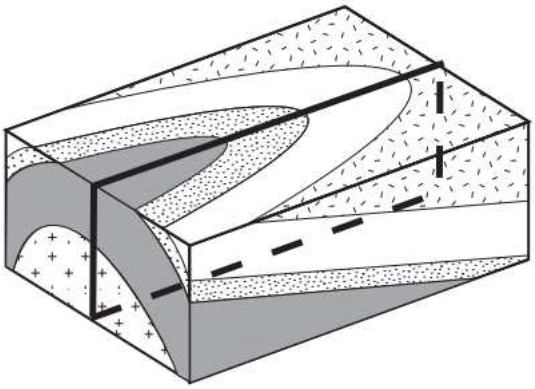
i. The Rockhopper is a sister group to the Macaroni and Royal penguins.
ii. The Little Blue is a sister group to the Black-footed and Magellanic penguins.
iii. The King and Emperor penguins are closely related.
iv. Adelie and Gentoo penguins share a recent common ancestor.
v. The BLH and molecular trees differ significantly therefore the structure found in the BLH data is not necessarily generated by phylogeny.

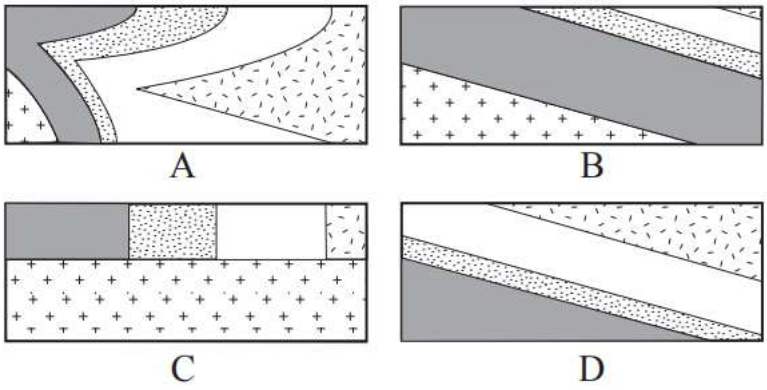
A. All statements, i. – v. are correct.
B. Only i., ii., iii., and iv. are correct.
C. Only i., iii., iv., and v. are current.
D. Only iii., iv. and v. are correct.
E. Only iii. and v. are correct.

Question 13

Study the geologic structure that is displayed in the 3-D block diagram below left. Visualize what the cross-section of that geologic structure would look like on the surface of the vertical plane intersecting the block.

Which answer, A – D that MOST LIKELY illustrates the structure along that plane.

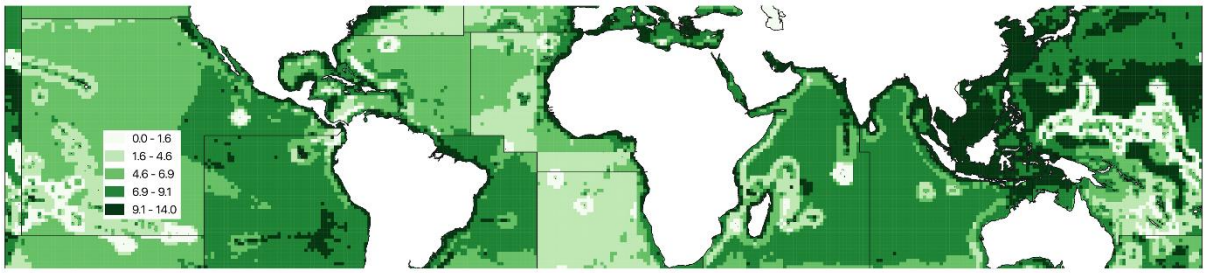




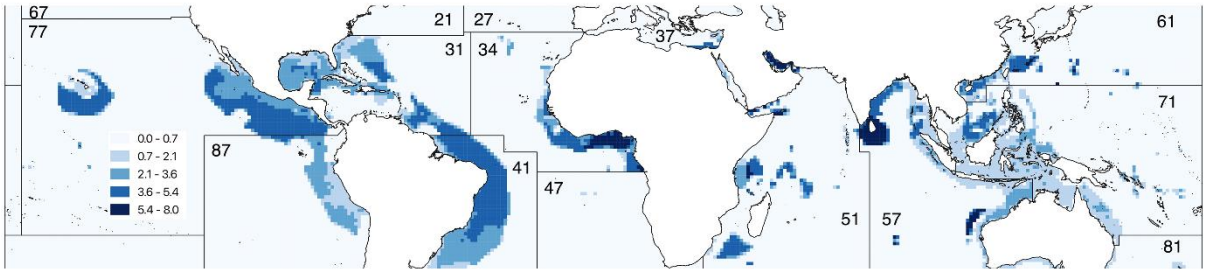
Question 14

The figure below shows estimated total fish catch using destructive methods of fishing (e.g. high levels of bycatch and/or habitat destruction for A) bottom dwelling ('demersal') fish and B) upper water column fish ('pelagic'). The numbers given are in metric tonnes.

(A) Demersal



(B) Pelagic



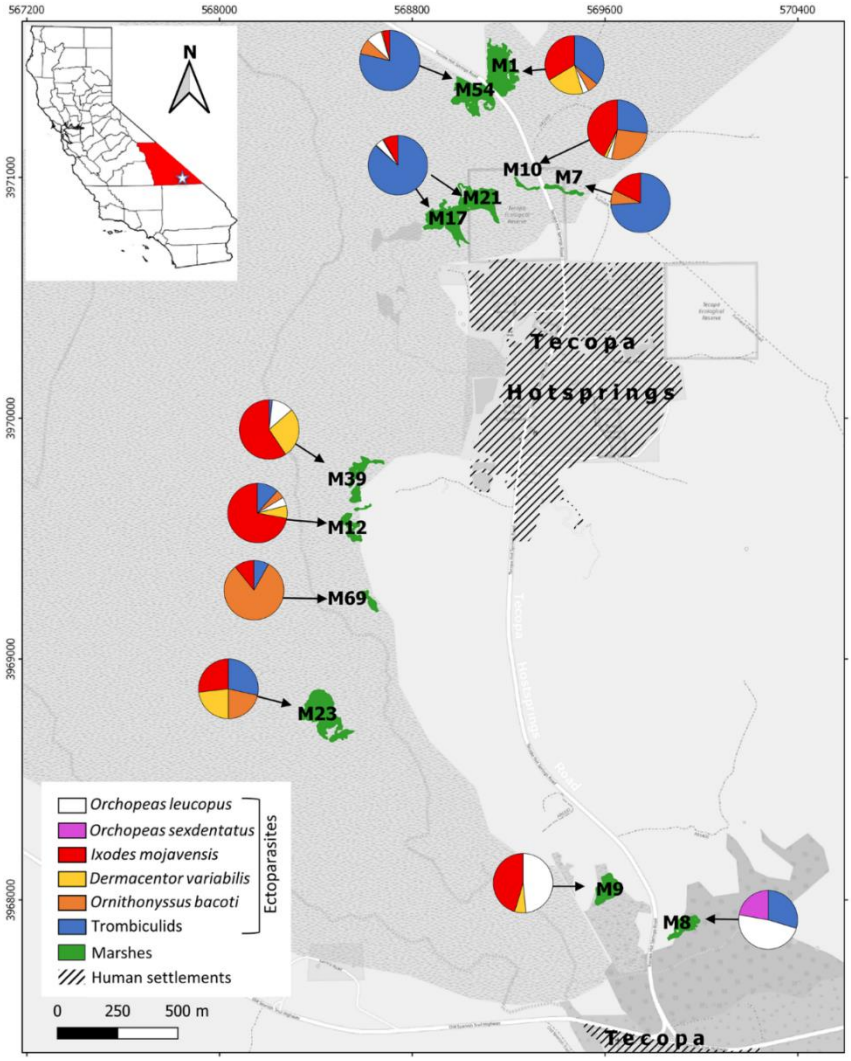
Source: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246835#pone-0246835-g003

What is the best conclusion that can be made from these data?

A. New Zealand does not have destructive fishing practices.
B. There is equal destructive fishing pressure on demersal and pelagic species.
C. Every country in the world displays destructive fishing practices.
D. Destructive fishing practices are more strongly correlated with coastal regions.
E. Fish populations are shrinking worldwide.

Question 15

Below is a Map of Tecopa, California in the Mojave Desert showing pie charts of community composition of ectoparasites on rodents for eleven marsh habitat patches surveyed. A total of 1,571 ectoparasites in Mesostigmata (mites), Trombidiformes (chiggers), Siphonaptera (fleas) and Ixodida (ticks) were collected from 341 rodents at eleven marshes. Trombiculids accounted for 82.5% of mites, followed by the mesostigmatid *Ornithonyssus bacoti* (17.5%), with chiggers predominating on voles and harvest mice. There were at least three genera of chiggers (*Eutrombicula alfreddugesi, Euschoengastia* sp. novel, and *Blankaartia* sp. novel). Fleas included *Orchopeas leucopus* (90.3% of all fleas) and *O. sexdentatus* (9.7%), and ticks were the novel endemic *Ixodes mojavensis* (82.1% of ticks) and *Dermacentor similis* (17.9%).



Source: PLoS ONE 17(6): e0269160. https://doi.org/10.1371/journal.pone.0269160

Which of the statement below are support by the data contained in the map?

i. Dissimilarities in ectoparasite community structure were driven mainly by chiggers, *I. mojavensis* and *O. leucopus*.
ii. Northern marshes were dominated by chiggers.
iii. Central marshes were dominated by *I. mojavensis* at all sites.
iv. Southern marshes were dominated by *O. leucopus*.

A. Only i., ii., and iii. are supported by the data.
B. Only i., and ii. are supported by the data.
C. Only ii. and iii. are supported by the data.
D. Only ii. iii. and iv. are supported by the data.
E. All statements i. – iv. are supported by the data.

Question 16 & 17

Carbon is a key element in all organisms. Carbon is brought into the organism's cells from the environment in the form of either carbon dioxide or carbon-based food molecules such as glucose; then used to build biologically important molecules such as sugars, proteins, fats, and nucleic acids. Therefore, all organisms from a single-celled bacteria to the largest of the dinosaurs leave behind carbon-based remains.

Carbon dating is based upon the decay of 14C, a radioactive isotope of carbon with a relatively long half-life (approximately 5700 years). While 12C is the most abundant carbon isotope, there is a close to constant ratio of 12C to 14C in the environment, and hence in the molecules, cells, and tissues of living organisms. This constant ratio is maintained until the death of an organism, when 14C stops being replenished. At this point, the overall amount of 14C in the organism begins to decay exponentially. This allows us to determine how long ago an organism died by examining the departure of the observed 12C to 14C ratio from the expected ratio for a living organism.

Given the half life of 14C we can derive an equation for modelling the decay of 14C:

$$N_{(t)} \cong N_{(0)}e^{-0.0001216t}$$

Question 16

Given the half-life of carbon-14, if a fossil that has 1/16 of the normal proportion of carbon-14 to carbon-12 approximately how old is the fossil?

Enter your answer here. _____

Question 17

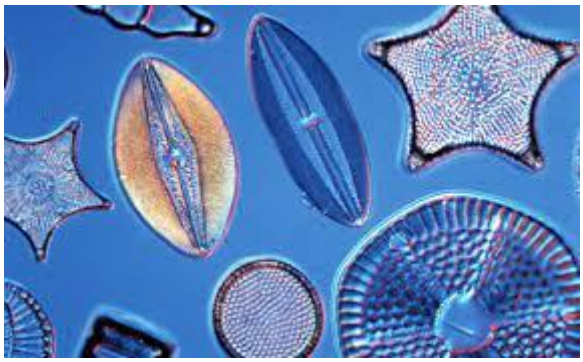
Which of the following statements are correct

I. The 14C isotope is only useful for dating fossils up to about 100,000 years old.
II. After 50,000 years fossils may have undetectable amounts of 14C
III. Isotopes with a longer half-life should be used for fossils older than 50,000.

A. Only statement I. is correct.
B. Only statement II. is correct.
C. Only statement III. is correct.
D. I. and II. are both correct.
E. II. and III. are both correct.

Question 18

Diatoms are single-celled algae encased in in petri-plate-like cases (valves) made of translucent hydrated silica whose thickness can be varied. The material used to store excess calories can also be varied. At certain times, diatoms store excess calories in the form of the liquid polysaccharide, laminarin, and at other times as oil. The table above gives information about the density (specific gravity) of various components of diatoms, and of their environment.



Source: https://diatoms.myspecies.info/

Material	Specific Gravity (kg/m^3)
Pure water	1000
Seawater	1026
Hydrated silica	2250
Liquid laminarin	1500
Diatom oil	910

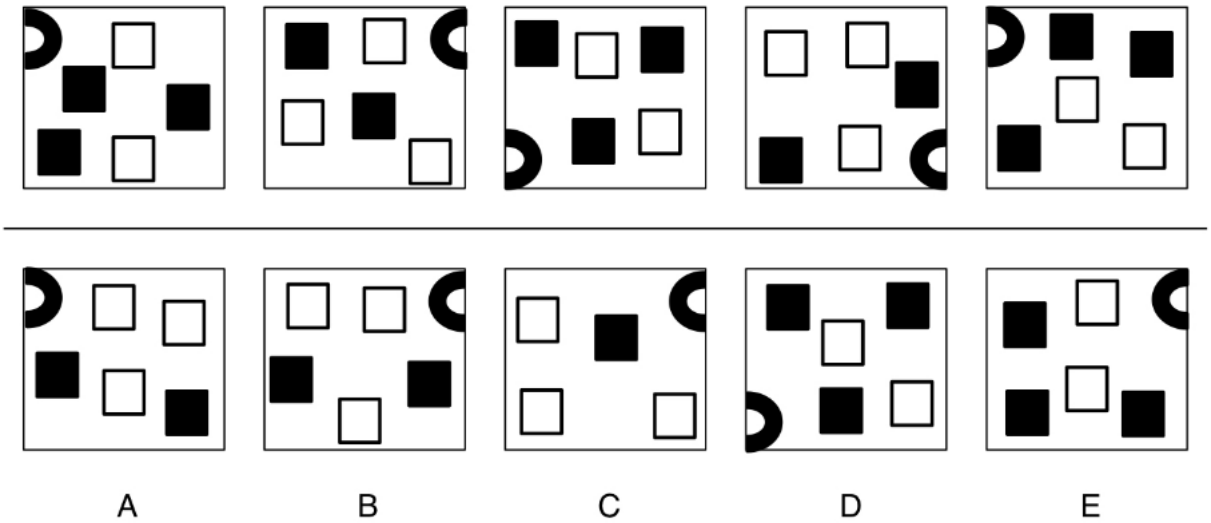
Source: Modified from Pearson's MasteringBiology https://mlm.pearson.com/northamerica/masteringbiology

Water's density and, consequently, its buoyancy, decrease at warmer temperatures. Based on this consideration, and using data from the table above, at which time of year would you expect diatoms to be storing excess calories mostly as oil?

A. Early spring
B. Late summer
C. Late autumn
D. Early winter
E. Mid-winter

Question 19

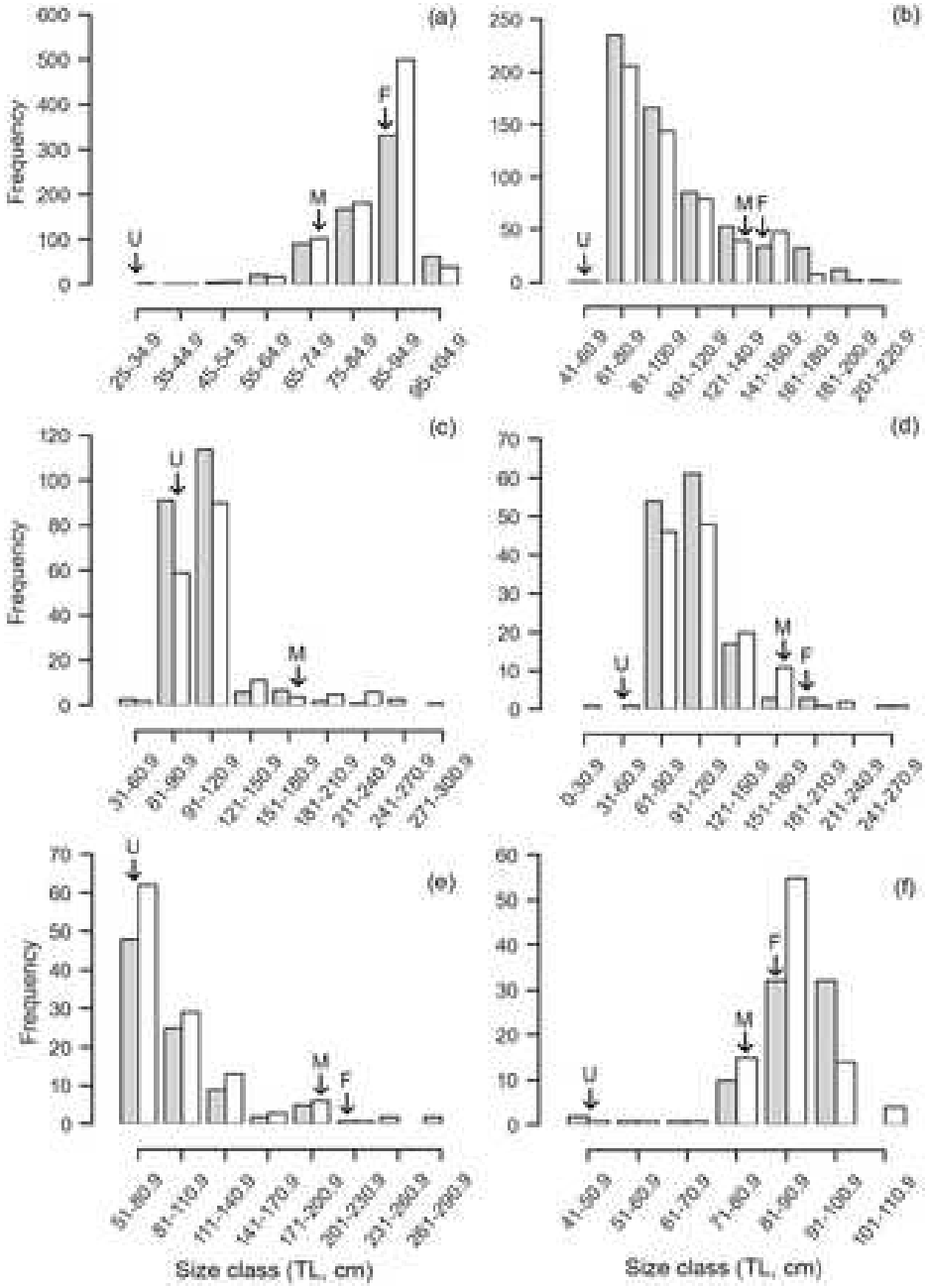
Which of the boxes comes next in the sequence, A – E?



Source: https://www.practiceaptitudetests.com/test/spatial-reasoning-test

Question 20

A 2017-2018 study measured the size distribution of 6 common commercially fished shark species in the South Andamans in the Indian Ocean. The arrows on the graphs represent the smallest individual representing young of year with the presence of an umbilical scar 'U', 'F' the smallest gravid females (carrying eggs or young) recorded, and 'M' the smallest recorded mature males. The shark species are (a) *L. macrorhinus*, (b) *C. amblyrhynchos*, (c) *S. lewini*, (d) *C. albimarginatus*, (e) *C. brevipinna*, and (f) *P. randalli*. The grey bars represent females and the white bars represent males.



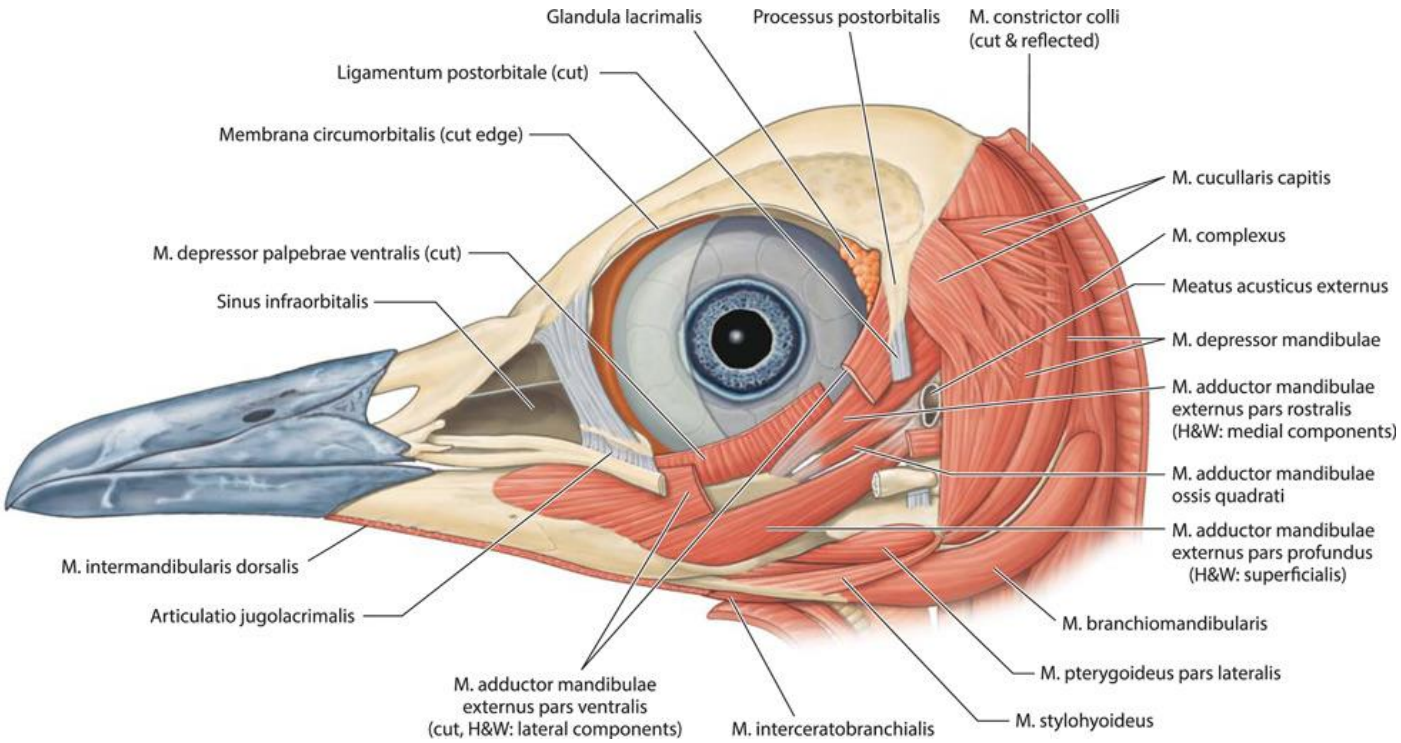
Source: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0231069#pone-0231069-g001

Which species of shark are the most at risk of population collapse related to the fishing practices shown here?

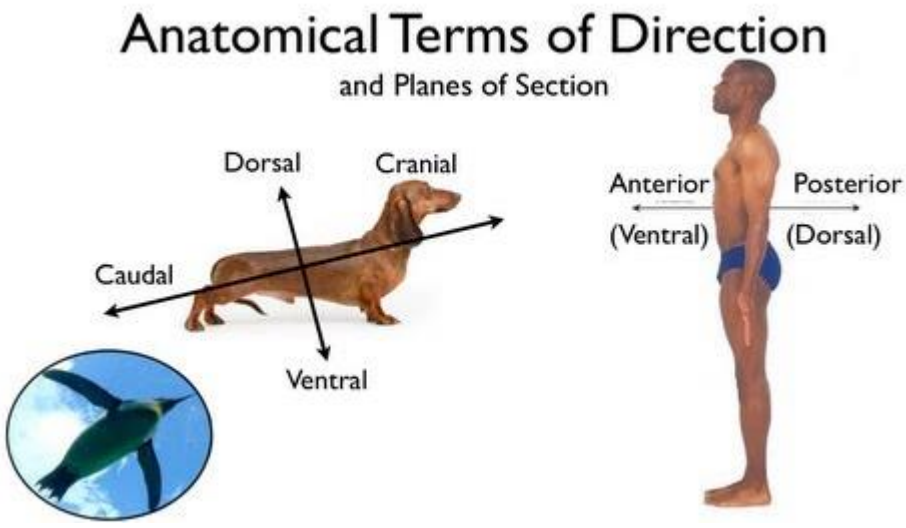
- **A.** Species a
- **B.** Species b
- **C.** Species c
- **D.** Species d and f
- **E.** Species b, d and e

Question 21

Scientific illustration is an important tool to help describe and understand anatomy. Hadden et al., 2022 recently published a paper on the head anatomy of penguins (Aves: Sphenisciformes: Spheniscidae). The diagram below shows a left lateral view of the deeper muscles of the head in *Eudyptula minor*.



Source: https://onlinelibrary.wiley.com/doi/full/10.1002/jmor.21476



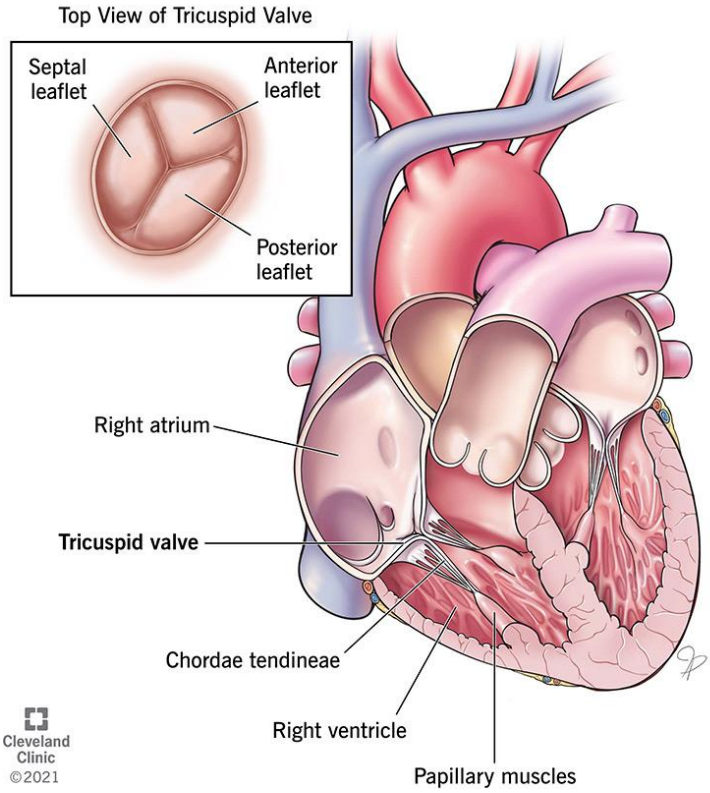
Given the diagram of the head muscles of the penguin and the anatomical terms of direction above, which of the statements below is **CORRECT**?

Musculus pterygoides pars lateralis is:

- **A.** Ventral to M. branchiomandibularis.
- **B.** Anterior to M. depressor mandibulae.
- **C.** Caudal to M. cucullaris capitis.
- **D.** Ventral to M. depressor mandibulae.
- **E.** Dorsal to M. stylohyoideus.

Question 22

The tricuspid valve is in the right side of the heart and separates the right ventricle, which pumps blood to the lungs, and the right atrium, which receives blood from the rest of the body.



Source: https://my.clevelandclinic.org/health/body/21851-tricuspid-valve

The ***reason*** why the tricuspid valve closes is

A. To stop the blood flowing backwards.
B. It stops oxygenated blood mixing with deoxygenated blood.
C. There is a difference in pressure on either side of it.
D. It gives the blood time to enter the atrium.
E. The chordae tendineae contract.

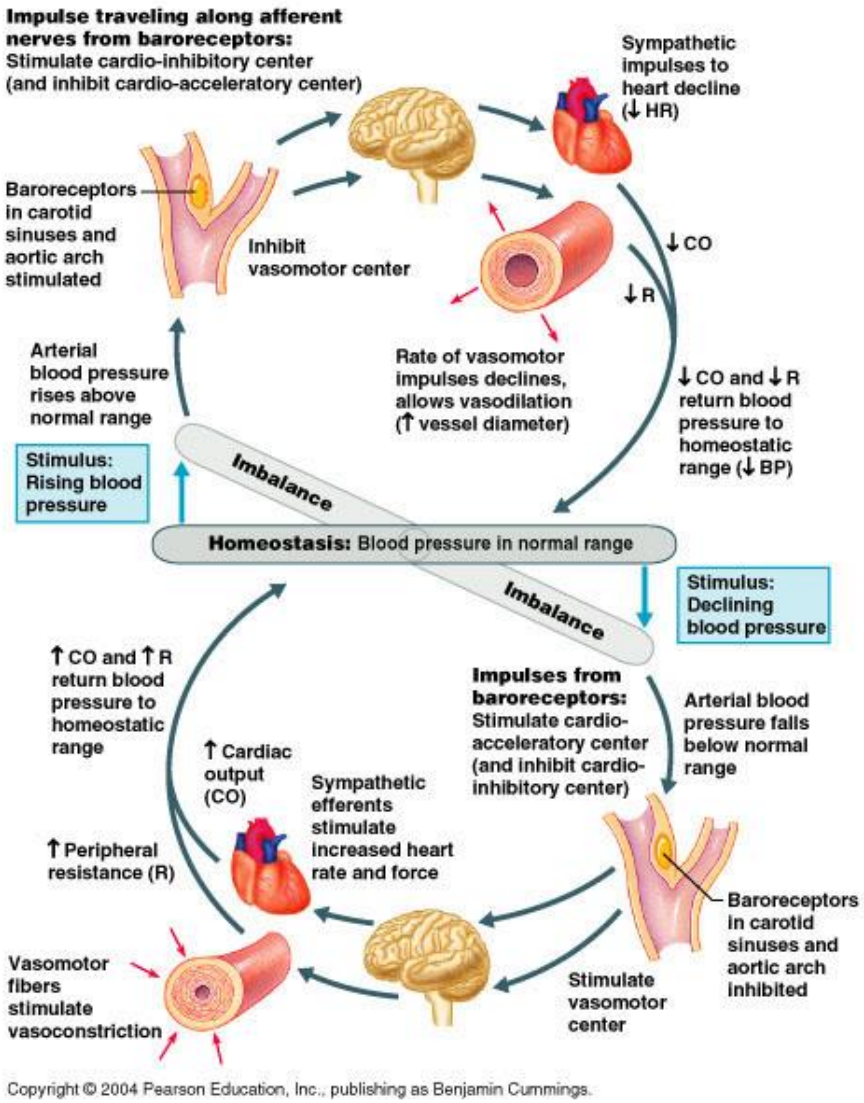
Question 23

Blood pressure is a measure of the force that your heart uses to pump blood around your body ensuring that every part of the body receives the oxygen and nutrients needed. The equation for blood pressure is described by the equation:

BP = HR*SV*TPR

Where HR = Heart Rate, SV = Stroke Volume, and TPR = Total Peripheral Resistance (controlled mainly by the diameter of the blood vessels).

One of the important feedback loops that controls blood pressure involves controlling heart rate.



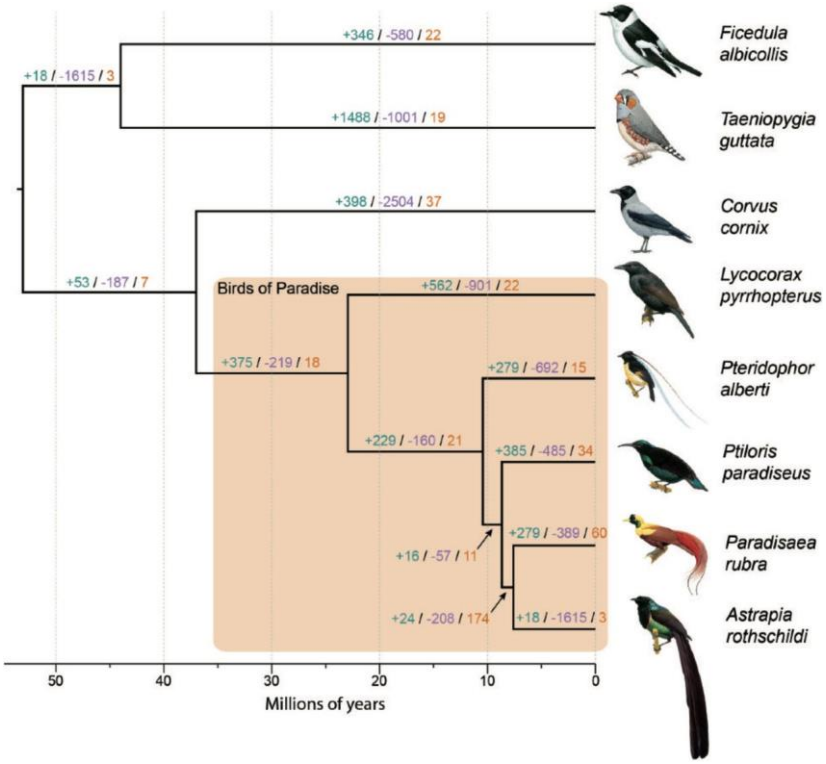
Source: https://lobsterweight.com/en/article/why-does-my-heart-rate-increase-after-deep-freediving/

Rising blood pressure after a free dive will result in, amongst other changes …..

- **A.** Stimulation of the cardio-inhibitory and vasomotor centres with a decline in heart rate and increase in vessel diameter.
- **B.** Stimulation of the cardio-inhibitory centre and inhibition of the cardio-acceleratory and vasomotor centres with a decline in heart rate and an increase in vessel diameter.
- **C.** A decrease in the resistance of the blood vessels through vasodilation and an increase in heart rate decreasing carbon monoxide in the blood.
- **D.** An increase in the resistance of the blood vessels together with a decrease in heart rate and increasing carbon monoxide in the blood.
- **E.** A decrease in the resistance of the blood vessels through vasodilation and an increase in carbon monoxide in the blood.

Question 24

A phylogenetic tree is a diagram used to represent a relationship (a phylogeny) between groups of organisms, showing how they are related, and their most recent common ancestors. The tree below shows the phylogeny of birds-of-paradise using data from 4,656 genes, scaled using the divergence times between crow (*Corvus spp.)* and the birds-of-paradise, and zebra finch (*Taeniopygia* spp.) and flycatcher (*Ficedula* spp) (obtained from Timetree.org) as calibration points. Branches are labelled as: # gene family expansions (green) / # gene family contractions (purple) / # rapidly evolving gene families (orange).



Source: https://www.biorxiv.org/content/10.1101/287086v1.full

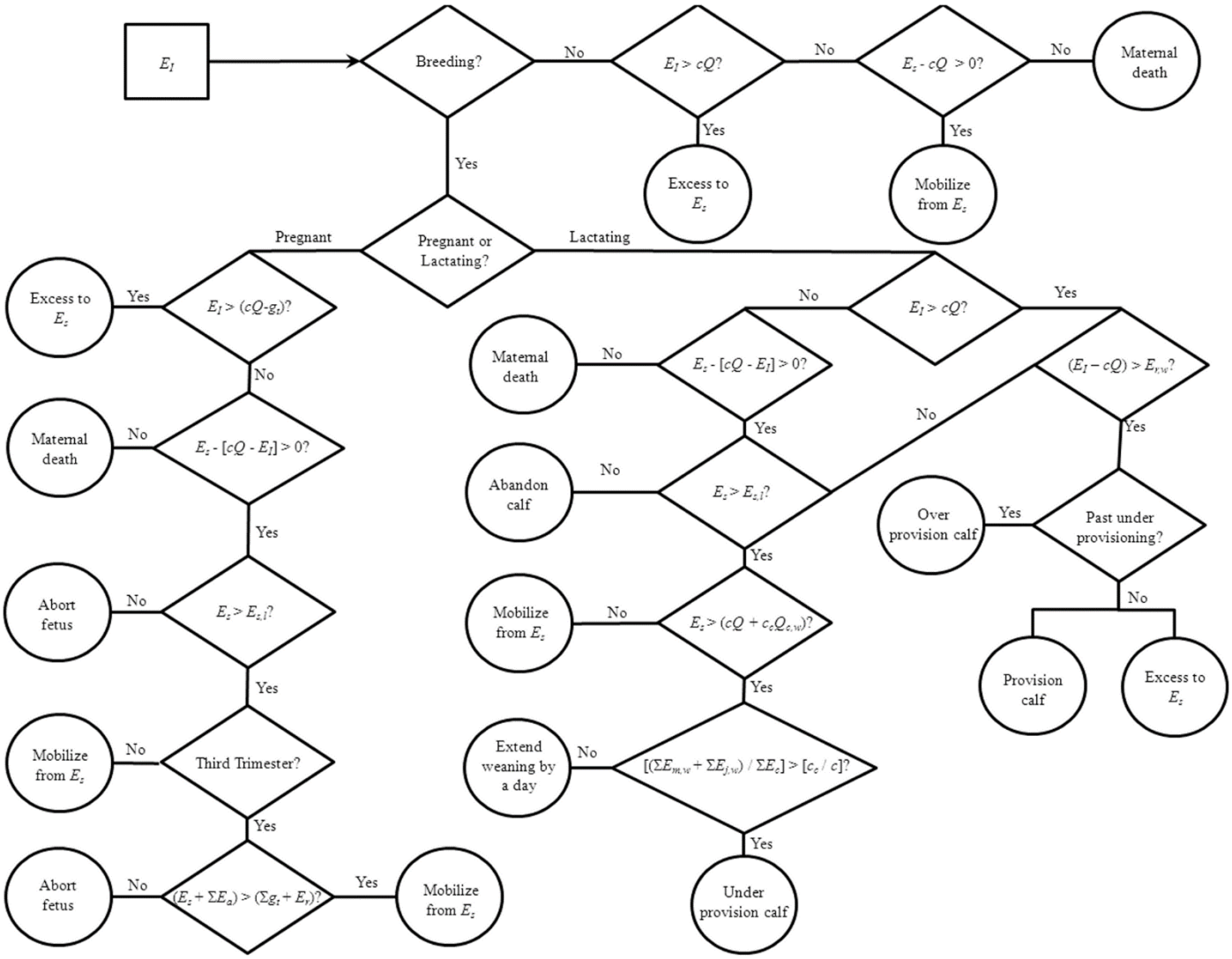
Based on the tree above, which statement is **NOT** correct?

- **A.** The birds-of-paradise shared a common ancestor with crows 43 million years ago.
- **B.** *Paradisaea rubra* belongs to a clade with many rapidly evolving gene families.
- **C.** *Lycocorax pyrrhopterus* diverged from other birds-of-paradise clades around 23 million years ago.
- **D.** *Paradisaea rubra* and *Astrapia rothschildi* diverged most recently.
- **E.** Zebra finch are not closely related to the birds-of-paradise.

Question 25

The diagram below shows a decision tree representing one day (a time step) in a model for the energy budget of an adult female beaked whale. All individuals ingest energy (EI) at the start of the time step but how that energy is used and partitioned depends on the female's reproductive status and her energy stores (Es). Beaked whales accumulate lipid stores prior to and between reproductive events, as well as taking in energy throughout the breeding cycle. Therefore, a proportion of the energy mobilized for reproduction is derived from lipid stores (Es, kCal). Accepting that energy transfer from one state to another is imperfect, the largest proportion of energy will go to growth, activity and maintenance, then pregnancy. Any "surplus" energy, energy not used for the basal metabolic rate (cQ in kCal day−1) or gestation (G) will either go to lactation (Er) or storage (E_2).

The square is the starting point of the decision process, diamonds represent decision points and circles are a possible outcome for the time step, given the previous decisions.



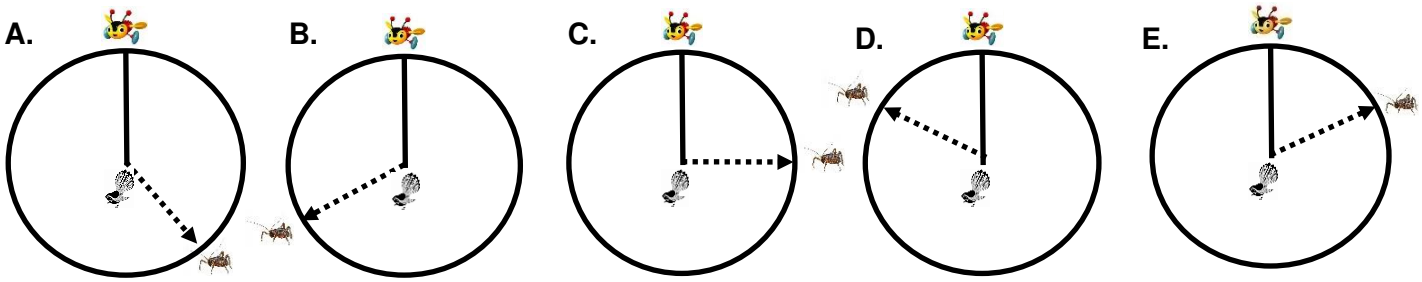
Source: https://doi.org/10.1371/journal.pone.0068725.g002

What will be the outcome in a breeding female that is lactating when the ingestion of energy and the energy needed for the basal metabolic rate subtracted from the energy stores is greater than zero. Further, that the available energy stores are less than the energy intake and energy stores available for mobilistation?

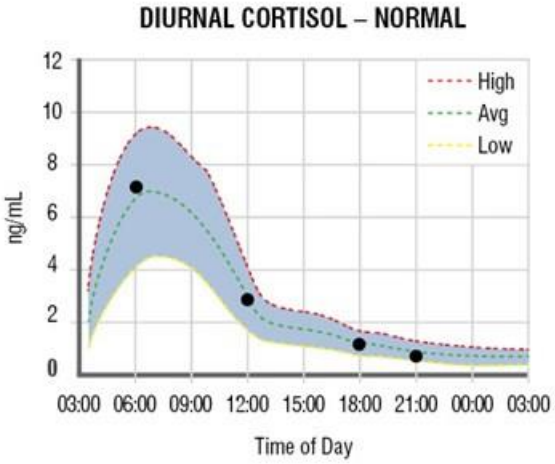
- **A.** Maternal death
- **B.** Abandon calf
- **C.** Mobilise energy from the energy stores
- **D.** Extend weaning
- **E.** Provision the calf

Question 26
Imagine you are standing at the piwakawaka (fantail) and looking towards the buzzy bee.

Which diagram below (A-E) shows the direction you need to point to point at the weta?



Question 27
Cortisol is a stress hormone that is released by the adrenal gland when the body is experiencing stress. The levels of cortisol were measured over the course of a day and are recorded in the graph below.



Source: http://www.zrtlab.com/media/1476/adrenal_curve_normal.jpg?width=359&height=299

Which statement correctly describes the daily rhythm of cortisol?

- **A.** It rises in the morning, peaking when the person wakes and then decreases throughout the day.
- **B.** It rises throughout the night then decreases throughout the day.
- **C.** It rises during the day and is lowest at night.
- **D.** It is lowest at night and highest during the day.
- **E.** It is highest in the early morning and lowest in the evening.

Questions 28 & 29
As our ocean warm and acidify, species with calcified exoskeletons like sea urchins may find it difficult to produce their skeleton because ocean acidification decreases calcium carbonate saturation and accompanying hypercapnia suppresses metabolism. In 2010, a study examined the effect of ocean warming and acidification on the larval development and calcification in the Sea Urchin, *Tripneustes gratilla*. Figure 1 shows the morphology of larvae reared for 5 days in 3 pH and 2 temperature treatments.

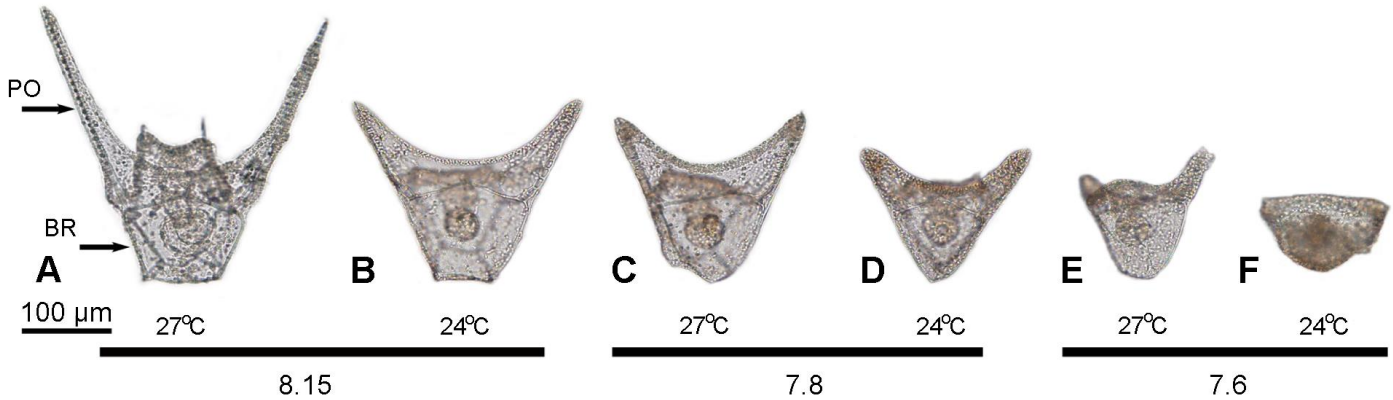


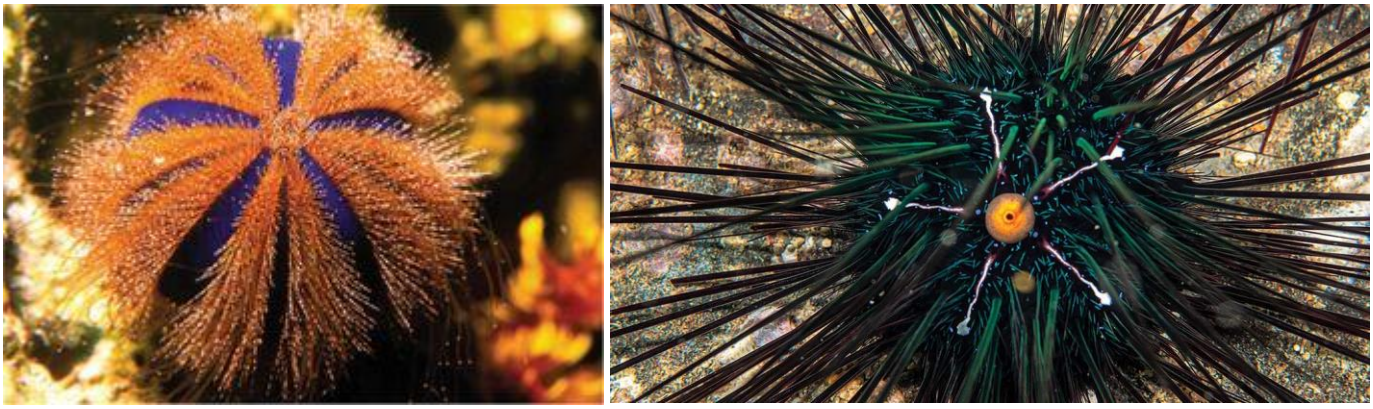
Figure 1: A-B. Control pH 8.15, largest larvae were from +3°C (27°C) treatments. PO, post oral arms; BR, body rod. C-D. pH 7.8. E-F. pH 7.6.
Source: doi: https://doi.org/10.1371/journal.pone.0011372.g001

Question 28
Approximately what length is the post oral arm of larvae raised at 27°C and a pH of 8.15?
Enter your answer here. _____

Question 29
Which of the statements below can be correctly concluded from the data in Figure 1?

- **A.** With increased acidity larval size decreased and there was an increase in abnormal development.
- **B.** With decreased acidity larval size decreased and there was an increase in abnormal development.
- **C.** There was no effect of temperature on larval development.
- **D.** Increased pH resulted in abnormal development of the post oral arms of the sea urchin larvae.
- **E.** Increased temperature increased the negative effects on sea urchin larval development.

Question 30
Adult sea urchins show stunning diversity, but all share a common body plan.



Using the photos above, what is the best description of sea urchin symmetry in the adult?

- **A.** None
- **B.** Spherical
- **C.** Radial
- **D.** Pentaradial
- **E.** Bilateral

Section B

THE BIODIVERSITY OF AOTEAROA NEW ZEALAND

The questions in Section B are designed to get you thinking about current biological research. Hopefully, you will be interested, inspired, and learn something new as you sit this exam. This year's exam highlights the biodiversity crisis facing New Zealand and the importance of mātauranga Māori and traditional science research in addressing this crisis. How do communities, researchers, and government contribute to the collective action needed to protect our unique native plants and wildlife.

Te Mana o te Taiao, the Aotearoa New Zealand Biodiversity Strategy 2020 outlines a coordinated approach to protecting our environment and biodiversity and is the first time key conservation activities across all government departments and regional councils have been identified in a plan of action to protect our native plants and wildlife. It also puts biodiversity protection at the heart of economic activity and brings together other programmes across government, such as the One Billion Trees programme, Predator Free 2050, and Jobs for Nature. Perhaps this exam will inspire you to become part of this collective action. What will you choose to do?

All of this work is underpinned by mātauranga Māori knowledge and biological research so let's learn about some of the amazing research happening in Aotearoa New Zealand.

PORAKA - THE LITTLE PEOPLE OF THE FOREST
Our native frogs and the deadly Chytrid fungus

Some say the frog is the ecological "canary in the coal mine" the biological indicator of an ecosystem in decline. It is estimated that a third of the world's 6000 or so amphibian species have been wiped out over the last 15 years. If frogs continue to be lost at the current rate, the earth will see the single largest extinction since the disappearance of dinosaurs.

New Zealand is home to four species of endemic frog, Hochstetter's frog *(Leiopelma hochstetteri),* Maud Island Frog *(Leiopelma pakeka)* Archey's frog *(Leiopelma archeyi)* and Hamilton's frog (*Leiopelma hamiltoni).* New Zealand also has three species of introduced frogs, *Littoralis ewingii,* the whistling tree frog, and the golden bell frogs, *Litoria aurea* and *Litoria raniformis*,

Our native frogs belong to an ancient and primitive family that is now extinct in the rest of the world. They are taonga, unique to Aotearoa and represent the most endangered amphibian genus in the world.

Interestingly, our New Zealand endemic frogs are very "unfrog-like". They don't use their tongues to catch insects, the pupils of their eyes are round not slit shaped, and they do not croak or sing like other frogs. Their feet are not webbed, and they do not even have tadpoles. The embryo develops inside the egg and hatches as an almost fully-formed frog.

Like all amphibians they do need to keep their skin moist to breathe – but that doesn't mean they live in ponds. Some live on misty ridges, far from wetlands and streams. Only Hochstetter's frogs live near water and they're our only native frog with partial webbing between their hind toes. The other species keep damp in moist, shady places in the forest or amongst rocks. They're nocturnal and during the day they hide under logs, stones and bushes, making them very hard for researchers to find.

Populations of Archey's frog have crashed in recent years declining by 88% from 1996 to 2001. The discovery of Archey's frogs with chytridiomycosis (caused by infection with the fungus *Batrachochytrium dendrobatidis Bd*) suggested that disease was the cause of the decline as this infection has been linked to the drastic decline of many anuran populations around the world.

B. dendrobatidis appears to have evolved in Africa and entered NZ via the pet trade of *Xenopus* frogs. The fungus attacks keratin in the frog's skin. Since frogs use their skin in respiration, this makes it difficult for the frog to breathe. The fungus also damages the nervous system, affecting the frog's behaviour with infected frogs becoming sluggish and sitting out in the open. In severe infections the fungus may spread throughout the frog's body including onto its bones resulting in the death of the frog, but not before they have spread the fungal spores to other areas.

Once a pond has become infected with chytrid fungus, the fungus may stay in the water forever.

Question 31
Frog extinction is of concern because?

- **A.** Frog populations can be considered an indicator of the health of an ecosystem.
- **B.** Introduced frogs are outcompeting our native taonga species.
- **C.** In 6000 years or so all amphibian species will have been wiped out.
- **D.** Our native frogs are already extinct in the rest of the world.
- **E.** Our *Leiopelma* species are the most endangered genus in the world.

Question 32
The frogs of Aotearoa New Zealand

- **A.** Are a mixture of species of the *Leiopelma* species.
- **B.** Evolved in Africa and entered New Zealand via the pet trade.
- **C.** Include endemic and exotic species.
- **D.** Are members of the *Littoria* genus, the most endangered group in the world.
- **E.** Are all quite "unfrog-like".

Question 33
The Chytrid fungus is?

- **A.** A pathogen.
- **B.** A fungal disease.
- **C.** Specific to amphibians.
- **D.** A microbial infection of the skin that spreads into bones.
- **E.** All the statements above (**A. – D.**) are correct.

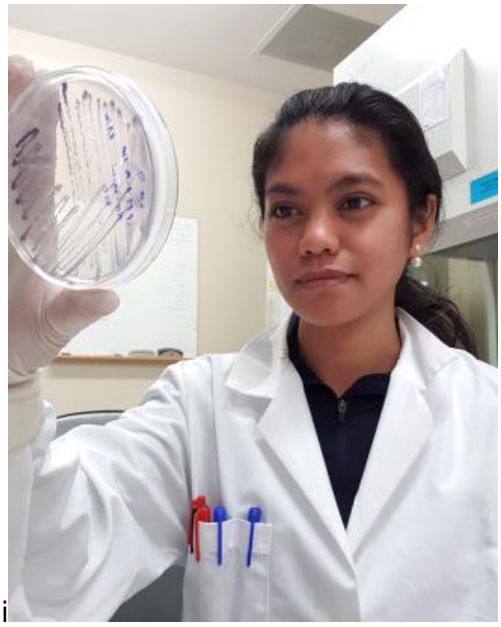
Question 34
Aotearoa New Zealand endemic frog species are likely so unique because?

- **A.** They have had to compete with three introduced species.
- **B.** They evolved in isolation after a split from Gondwanaland.
- **C.** Aotearoa New Zealand has only recently had Xenopus frogs enter the country carrying chytrid fungus.
- **D.** They do not use their tongues to catch insects, nor do they croak or sing, or have tadpoles.
- **E.** They are all tree frogs and do not live in ponds.

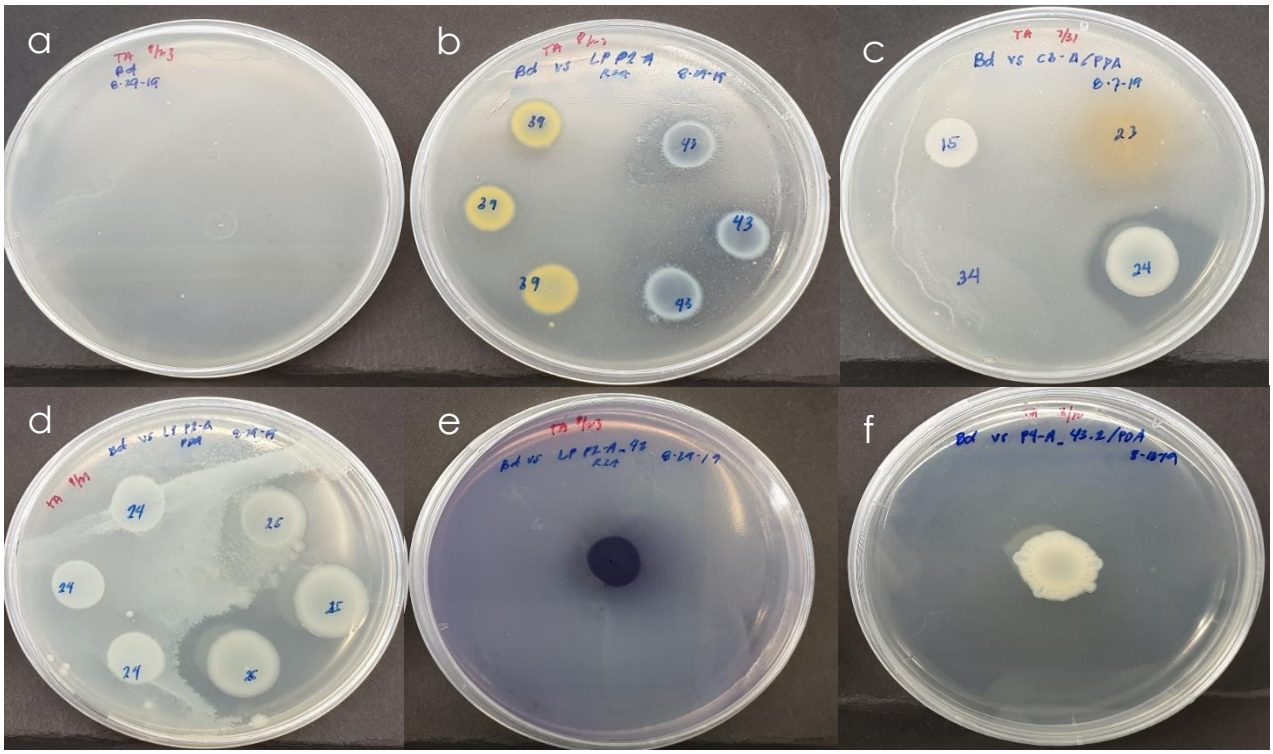
Archey's frog now faces extinction with the population decline tentatively linked to infection with the chytrid fungus and researchers like Adria Eda, a Master's student at the University of Otago, are racing to understand how to save these special little frogs.

Adria's research focuses on the presence of the inhibitory skin secretions in NZ's frogs. These antimicrobial peptides in the skin are thought to act as important components of the innate immune system that may protect some species from infectious diseases. Adria investigated the *in vitro* activity of the skin secretions of six frog species in New Zealand against *Bd* zoospore growth.

Adria had to culture the chytrid fungus (*Bd*) onto special plates (shown below in a). She then had to had to locate frogs in the wild and swab their skin to collect the skin secretions. A difficult job as our endemic frogs are cryptic and don't croak. She then assayed the activity of the skin secretions from the different species of frogs to see how well they inhibited Bd growth on the plates.



i



The figure above shows the results of an *in vitro Bd* inhibition assay. Skin isolates were placed onto the plates covered in the cultured *Bd*. If the skin secretions contained antimicrobial proteins, then they inhibit the growth of the *Bd* colony, with a clear area around the colony indicating the zone of inhibition. a. Control plate inoculated with *Bd* zoospores only, b-f. varying diameter of zones of inhibition from minimal (b-d) to complete (e-f) inhibition of *Bd* growth after 5-7 days of incubation at 17°C.

The activity of skin secretions produced by different species of frogs varied significantly, with those of *Leiopelma archeyi* being the most active with the greatest *Bd* zoospore inhibition. The skin secretions of native Leiopelmatid species showed greater *Bd* zoospore inhibition (31.0–71.9%) than the naturalized introduced species of frog *Litoria* species (17.4–18.2%). *L. archeyi* had the most active peptides, even though it is the only native species with known susceptibility to *Bd* infections.

Interestingly, there appears to be antimicrobial peptides in the skin of our endemic frogs which form part of the innate immune system and may protect some species from infection with *Bd*. Perhaps these ancient little taonga may hold the key to halt the extinction of other frog species around the world.

Question 35
Adria's results tell her that?

- **A.** The secretions from the skin of NZ frogs can reduce the growth of *Bd* fungal colonies.
- **B.** *Bd* zoospores make an excellent experimental control.
- ***C.*** The research challenge in carrying out this study was to make *Bd in vitro*
- **D.** *Bd* inhibition zones range from minimal to complete.
- **E.** Incubation at 17°C was the most successful conditions for culturing *Bd in vitro*.

Question 36
Adria's research has determined that *Leiopelma* spp;

- **A.** Are the most susceptible frogs in the world to the deadly chytrid fungus.
- **B.** Have an innate immunity to chytrid fungus infection.
- **C.** Have the most zoospore infestation in vitro.
- **D.** Carry bacteria in their skin that provide protection from chytrid fungus.
- **E.** Have keratin in their skin that inhibits their ability to use their skin to breathe.

Question 37
The anomaly in Adria's research that requires further research is?

- **A.** Why Aotearoa New Zealand's frogs are so "unfrog-like".
- **B.** How chytrid fungus entered Aotearoa New Zealand.
- **C.** Why the species with skin secretions resulting in greater *Bd* zoospore inhibition in the skin is the most susceptible to infection.
- **D.** Why the immunity is in the subcutaneous bacteria and not the frog skin itself.
- **E.** How frogs can breathe through their skin.

TOHEROA – SAVING THIS ICONIC SURF CLAM AND TOANGA SPECIES
Integrating mātauranga Māori and setting a Sustainable Seas Challenge / Ko ngā moana whakauka

Phil Ross and Taoho Patuawa, from the University of Waikato, have been working with iwi to review the scientific, local, and customary knowledge about the biology, ecology and history of toheroa (*Paphies ventricosa*). They have also begun a Sustainable Seas Challenge project in partnership with Te Roroa and Bluetide Aquaculture to investigate the establishment of community-based toheroa aquaculture in Te Taitokerau.

This iconic surf clam was once abundant on Orēti Beach in Southland and the west coast beaches of Te Taitokerau and they were a world-renowned kaimoana valued by both Māori and pākeha. Harvesting was intense during the early to mid-1900s with much of this harvest canned and shipped overseas. Toheroa populations collapsed in the 1960's and commercial canning was banned in 1969. Beaches were closed to recreational harvesters in a staggered way with the last legal recreational catch in 1979. Despite more than 50 years of extensive protection toheroa populations have largely failed to recover.

Clockwise from top left: Toheroa collected from Hokio Beach during the September open day in 1977 (EP/1077/3679/36-F. Alexander Turnbull Library (ATL)); Three women from Northland, photographed ca. 1910–1930s, shelling toheroa meat into tin cans for a toheroa cannery. Their kete (flax bags) are full of shellfish, and they are surrounded by empty shells (1/1-026522-G. ATL); Harvesting toheroa on a Northland beach, ca. 1920s–1930s (1/1-010575-G. ATL); Toheroa being dug from trenches on Muriwai Beach, 1962. (AAQT 6539, A70987. Archives New Zealand, The Department of Internal Affairs, Te Tari Taiwhenua).

Source: P. M. Ross, M. P. Beentjes, J. Cope, W. P. de Lange, B. G. McFadgen, P. Redfearn, B. Searle, M. Skerrett, H. Smith, S. Smith, J. Te Tuhi, J. Tamihana & J. R. Williams (2018) The biology, ecology and history of toheroa (*Paphies ventricosa*): a review of scientific, local and customary knowledge, New Zealand Journal of Marine and Freshwater Research, 52:2, 196-231, DOI: 10.1080/00288330.2017.1383279

Recreational harvesting regulations were first introduced in 1932 in response to dwindling numbers of toheroa coupled with mass mortality events at northern beaches. These regulations included a two-month closed season from October to November when toheroa spawn, a minimum allowable harvest size of 3 inches (76 mm), a ban on certain digging implements for non-commercial harvesters, and a quota for pakeha (50 toheroa per person per day), but not Māori until 1941. From as early as 1915 Māori had expressed concern about the declining numbers of toheroa, and they opposed commercial harvesting in traditional mahi kai areas. Māori at Ripiro lobbied for the establishment of 'Māori toheroa reserves but the Chief Inspector of Fisheries (1939) commented that toheroa were a 'national possession', belonging to everyone and Māori korero around the management of toheroa was largely ignored even though toheroa were a traditional and staple Māori food. We don't have data on the toheroa population size on these beaches at the start of the twentieth century, but it is likely that population sizes did fluctuate, as this is typical for many other surf clams.

The commercial fishery has traditionally received much of the blame for the decline of toheroa. Commercial harvesting practices varied between locations but at Ripiro Beach it was managed to protect the reproductive capacity of the beds. Harvest was restricted to lease areas from 1915 and individual toheroa beds were only partially harvested, and the harvest limited to mid-sized toheroa. Smaller toheroa were returned to the trenches from which they were dug and larger specimens either returned to the trenches or transplanted to the southern end of Pouto Peninsula, to act as a source of recruits to beds to the north. The recreational fishery was not managed spatially nor was there a maximum size limit. B. Searle stated to Ross that recreational harvesters following behind the commercial harvesters 'cleaning out' sections of toheroa beds purposefully left undug by the canneries. The pressure on the toheroa population from recreational harvesting is put into perspective by simple data on the number of recreational harvesters at Ripiro. In 1957 2000–3000 cars visited Ripiro over a weekend to harvest toheroa (Auckland Star 1957). In 1966 an estimated 12,000 cars and 50,000 people visited Ripiro Beach in one weekend, harvesting an estimated 1,000,000 toheroa.

Figure A, below left, shows the total commercial production of toheroa from 1928 to 1969 at 3 Northland beaches; Te Oneroa-a-Tōhē, Ripiro and Te Rangatira. At right below, are the population estimates for toheroa at 2 of these beaches and at Oreti and Bluecliffs beaches in Southland from 1930 – 2020. Both figures are from Ross et al. 2018 but Figure A is reproduced from Williams, Sim-Smith, et al. (2013) and Figure B includes figures reproduced from Williams, Ferguson, et al. (2013), Beentjes (2010a, 2010b) and Berkenbusch et al. (2015).

Figure A

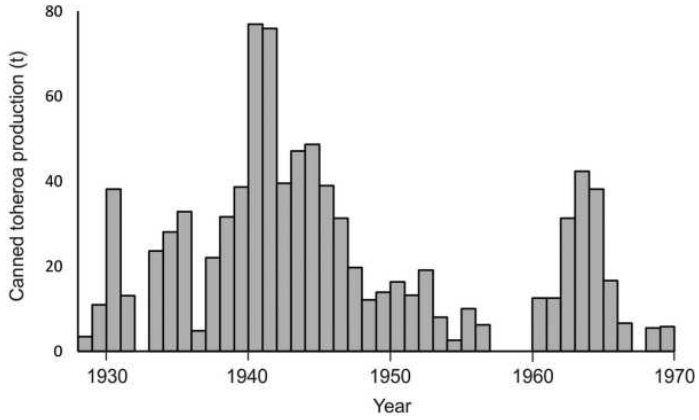
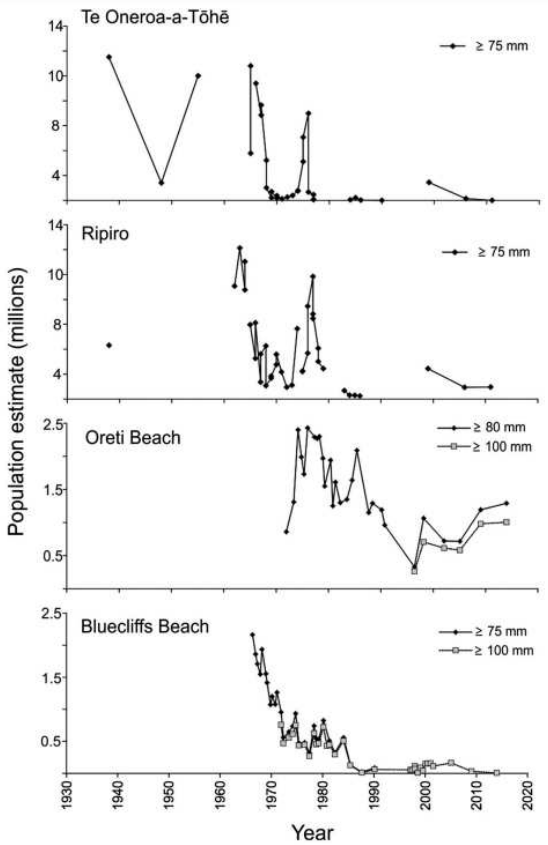


Figure B



Question 38
The data in Figure A clearly shows that?

- **A.** Recreational harvest of toheroa was greatest in the 1940's.
- **B.** Recreational harvest of toheroa was responsible for the crash in toheroa population in the 1950's.
- **C.** Commercial harvest of toheroa was greatest in the 1960's.
- **D.** Commercial harvest of toheroa peaked at almost 80 tonnes per annum in the 1940's.
- **E.** Both recreational and commercial harvest of toheroa were responsible for the declining populations in the 1970's.

Question 39
What is the reason for the lack of commercial harvest of toheroa in 1933?

- **A.** The toheroa population crashed.
- **B.** The introduction of recreational harvesting regulations in 1932.
- **C.** Mass mortality of toheroa at northern beaches.
- **D.** Difficulties in exporting the canned toheroa to Europe due to the increased geopolitical tensions.
- **E.** There is insufficient evidence in the information and data above to determine a specific reason.

Question 40
Which of the following statements is supported by the data in the graphs above?

- **A.** In the 1960's toheroa population sizes were similar at all 4 of the beaches in Figure B.
- **B.** Oreti Beach had the largest population of toheroa in the 1970's.
- **C.** Ripiro Beach had the largest population of toheroa in the early 1960's.
- **D.** The population of toheroa at Oreti Beach has recovered well and it now has more toheroa than any of the other three beaches.
- **E.** There are no toheroa at all left at Bluecliffs Beach.

Question 41
Considering the data on toheroa populations at the 4 beaches in Figure B; which statement is **NOT** true?

- **A.** Populations of toheroa naturally showed large variations unrelated to harvesting.
- **B.** The toheroa populations at Te Oneroa-A-Tōhē and Bluecliffs Beach have shown no recovery since the bans on harvesting.
- **C.** Toheroa populations at Oreti Beach have recovered somewhat since harvesting was banned.
- **D.** There is a small remnant population of toheroa at Ripiro Beach.
- **E.** The populations of toheroa in Northland have not recovered as well as those in Southland.

Question 42
Which of the following statements can be concluded from the information and data provided above?

I. Commercial harvesting of toheroa was the main cause of the decline in abundance of this species in New Zealand.
II. It is likely that recreational harvesting was of at least equal importance in contributing to the decline of the toheroa as commercial harvesting.
III. Differences in harvesting practices may to some extent explain the persistence of adult toheroa beds at Ripiro and their almost complete disappearance at Te Oneroa-a-Tōhē.

All three statements above can be concluded.
Only statement I. can be concluded.
Only statement II. can be concluded.
Only Statement III. can be concluded.
Only statements II. and III. can be concluded.

Toheroa are linked to the Māori people through whakapapa (genealogy) and are a very important customary food. The giving of toheroa to visitors shows manaakitanga (the act of giving mana through the expression of hospitality and generosity) and is a tikanga (custom) that has clearly been compromised by the mismanagement of the fishery and its subsequent collapse.

Toheroa were thought to have been brought to Aotearoa by the high chief Mareao from Hawaiki and seeded on the west coast of the North Island. Live toheroa were almost certainly traded in pre-European times and evidence suggests that the translocation of toheroa to new locations and their cultivation may have been common practise for Māori. New Zealand government agencies were also involved in translocation of thousands of toheroa between North Island beaches in the 1920s and 1930s. Toheroa translocation appears to have been a common practice at least up until the 1970s when the toheroa fisheries closed. Despite widespread and repeated translocations, transplanted toheroa have failed to thrive or persist in most locations suggesting that they have very specific habitat requirements.

In Taitokerau, the story of toheroa is closely tied to that of the pingao (*Ficinia spiralis*), an endemic dune grass. The whakapapa of pingao is that she was put on the dunes by her father, Tangaroa (the god of the sea), to nurture her whānau (family), the toheroa. One version of toheroa reproduction in mātauranga Māori suggests that toheroa spat are born in the pingao (whose seeds are shaped like toheroa spat) and in another native dune grass spinifex (*Spinifex sericeus*). The other traditional understanding is that spat are carried to the dunes on the foaming surf at the highest of tides and are deposited on the sand dunes and into the pingao and spinifex where they are reared within the structures of these grasses. Transportation from the dune grasses to the juvenile beds in the upper intertidal occurs in spring. The spat held by the pingao are blown onto the upper beach, while the tumbleweed-like seed heads of the spinifex transport their toheroa pēpi (babies) along the beach through the action of cross-shore winds. The spat are then washed from the tumbling seed heads as they traverse the numerous streams and seeps running down the northern beaches. These streams are where the spat settle thus explaining the stream-associated distribution of toheroa in Taitokerau. During the times when the spat was being transferred along the beach children were not allowed to play their game of waiwatai, in which seed heads were chased as they were blown over the sand. This tikanga provided protection for both the nursery habitat and the vulnerable early life stages of toheroa.

Traditional Māori fisheries management practices were not restricted to translocation and nursery protection. The setting aside of reserves (rāhui), protection of spawning areas and a ban on harvesting during spawning, with spawning indicated by the annual flowering of kumarahou (*Pomaderris kumarahou*) and the full moon, was also practised by Māori. There is also some evidence that Māori may have preferentially harvested middle-sized toheroa, leaving larger individuals in place as broodstock to sustain the resource.

Western scientific research has shown that most northern toheroa reproduce after a year at a length of 32 mm. Southern toheroa reached reached sexual maturity by about 2 years and around 76 mm of length. Toheroa are broadcast-spawners, releasing their gametes into the seawater for external fertilisation. In northern toheroa spawning is in early spring with additional spawning events in summer and autumn when conditions are right. Southern toheroa, which experience much cooler water temperatures, have two main spawning periods, the first in spring and a second in the late summer. The release of eggs and sperm is synchronised, a behaviour likely to increase fertilisation success in a turbulent surf zone. Food abundance and changes in atmospheric and water temperature are thought to be the main factors influencing the onset and duration of spawning and peak spawning activity occurs during new and full moon phases. Adult females can release 15–20 million eggs during a single spawning event with fecundity of female toheroa increasing with size.

The larvae of toheroa are planktonic. For northern toheroa, the pelagic larval duration is about 3 weeks, for southern toheroa around 6 or 7 weeks/ The growth and development of larvae is temperature dependent with faster growth in warmer waters (20° vs. 12° or 16°C). Following a pelagic period, the larvae that can reach a suitable beach habitat settle out of the water column in the surf zone and metamorphose into juvenile toheroa (spat) of about 2 mm long. Waves carry the spat up the beach and in the interval of slack water just before each wave recedes from the beach, spat are able to dig themselves into the sand to a depth of 10–20 mm. This cycle of passive transport, settlement and resuspension over successive waves, tides and days gradually moves juveniles to the upper shore where they form a band just below the level of the high-water mark. Juveniles are located at the upper end of this range (nearer the top of the beach) with adults located lower down the shore and buried up to 15–20 cm beneath the beach surface.

Question 43
Considering the reproduction of toheroa, mātauranga Māori is **NOT** consistent with which of the following statements?

A. Toheroa spawning occurs in response to the highest tides.
B. Toheroa spat are produced throughout the summer.
C. Spat are transported down the beach to the upper shore just below high tide.
D. Toheroa spawning is strongly seasonal.
E. The highest abundance of toheroa is near freshwater streams and seeps.

Question 44
What scientific explanation could be given to support the tikanga of only harvesting the middle-sized toheroa?

A. The fecundity (number of eggs produced) increases as the size of the female toheroa increases.
B. Middle-sized toheroa are found just below the high tide mark and so are easier to harvest.
C. Toheroa are very slow growing so there are greater numbers of middle-sized toheroa in the population.
D. Middle-sized toheroa are more tender and taste better.
E. Middle-sized toheroa have not yet reached reproductive age.

Question 45
Which aspect of mātauranga Māori knowledge of the toheroa is consistent with the scientific research on the settlement of toheroa spat?

A. Toheroa spat are born in the pingao.
B. Spat are carried to the dunes on the foaming surf at the highest of tides.
C. Spat are deposited on the sand dunes and into the pingao and spinifex where they are reared within the structures of these grasses.
D. Transportation from the dune grasses to the juvenile beds in the upper intertidal occurs in spring.
E. The spat held by the pingao are blown onto the upper beach, while the tumbleweed-like seed heads of the spinifex transport their toheroa pēpi (babies) along the beach through the action of cross-shore winds.

Question 46
Given our knowledge of mātauranga Māori and the scientific research what management tools should be used to protect the toheroa in Aotearoa New Zealand?

I. Ongoing bans on commercial and recreational harvesting.
II. Closure of all toheroa harvesting; commercial, recreational and customary.
III. Management of customary harvesting for events such as tangihanga, including the issuing of permits and enforcement of both maximum and minimum size limits.
IV. Protection of the lower beach around the high tide mark from disturbance by cars and other uses during spring and summer.
V. Deliberate translocation of toheroa to areas where they were once plentiful.
VI. Toheroa aquaculture.
VII. Engagement with local iwi to manage the toheroa populations in partnership with government agencies.

A. All these strategies should be used.
B. I., III., IV., VI., and VII. should all be used.
C. II, III., IV., V., VI., and VII. should all be used.
D. Only I., III., and V. should be used.
E. Only II., IV., V., and VII should be used.

Question 47

Mātauranga Māori is important to help us manage toheroa populations because;

A. Māori have detailed customary knowledge of the ecology of the toheroa.
B. Traditional tikanga provides management techniques that could better protect toheroa populations.
C. The principles of the Treaty of Waitangi enshrine a partnership between the Crown and Māori and the Crown must actively protect Māori interests including their taonga.
D. Toheroa is a toanga species.
E. All 4 statements (A-D) are reasons why Mātauranga Māori is important to help us manage toheroa populations.

A 2018 study by Ross and his team published in Nature, assessed population genetic structure and levels of connectivity among toheroa across their entire distribution using the mitochondrial cytochrome c oxidase I (COI) gene. It was predicted, based on prior ecological knowledge, that North Island populations of toheroa would be well connected and for there to be isolation and genetic divergence between northern and southern toheroa.

The study found that twenty-three nucleotide positions were variable leading to the delineation of 20 haplotypes (a group of genomic variants that tend to be inherited together because they are found near each other on a chromosome). Note that a transition is a point mutation that changes a purine nucleotide to another purine (A ↔ G), or a pyrimidine nucleotide to another pyrimidine (C ↔ T). Transversions are point mutations in which a single (two ring) purine (A or G) is changed for a (one ring) pyrimidine (T or C), or vice versa.

Table 1 (below) summarises the results for toheroa populations around New Zealand including the number of COI sequences obtained (n), number of polymorphic sites (S) which are sites with two or more genetic variants, numbers of transitions (TS) and transversions (TV), numbers of haplotypes detected (HN), number of private haplotypes per location (HP), haplotype diversity (HD), nucleotide diversity (π), mean number of pairwise differences (k) and Tajima's D and Fu's Fs (with p-values). Values are presented both individually for Te Oneroa-a-Tōhē and Ripiro and with these two collection sites combined (Taitokerau).

Location	n	S	T_S	T_V	H_N	H_P	H_D (s.d.)	π (s.d.)	k	Tajima's D	p	Fu's Fs	p
Taitokerau	32	14	14	0	12	10	0.649 (0.096)	0.00380 (0.00095)	1.843				
Te Oneroa-a-Tōhē (NMB)	16	9	9	0	7	5	0.625 (0.139)	0.00404 (0.00140)	1.958	−1.02093	0.174	−1.780	0.097
Ripiro	16	10	10	0	7	5	0.629 (0.124)	0.00369 (0.00126)	1.792	−1.51227	0.053	−2.058	0.073
Waitarere	30	14	14	0	10	8	0.561 (0.109)	0.00359 (0.00098)	1.743	−1.69162	0.035	−3.815	0.017
Oreti	36	0	0	0	1	0	0	0	0	—	—	—	—
All locations	98	23	23	0	20	18	0.415 (0.064)	0.00240 (0.00051)	1.162	−2.18704	<0.001	−17.639	<0.001

Figure 1 (below) shows the Haplotype Network of the CO1 sequences from 98 toheroa. Each circle represents an individual haplotype with circle size indicating the number of individuals (n) sharing that haplotype (n(H1)=75, n(H2)=4 and n(H3)=2). For all other haplotypes n=1. Circle colour indicates the sampling location. Missing (unsampled) intermediate haplotypes are indicated by 'X's.

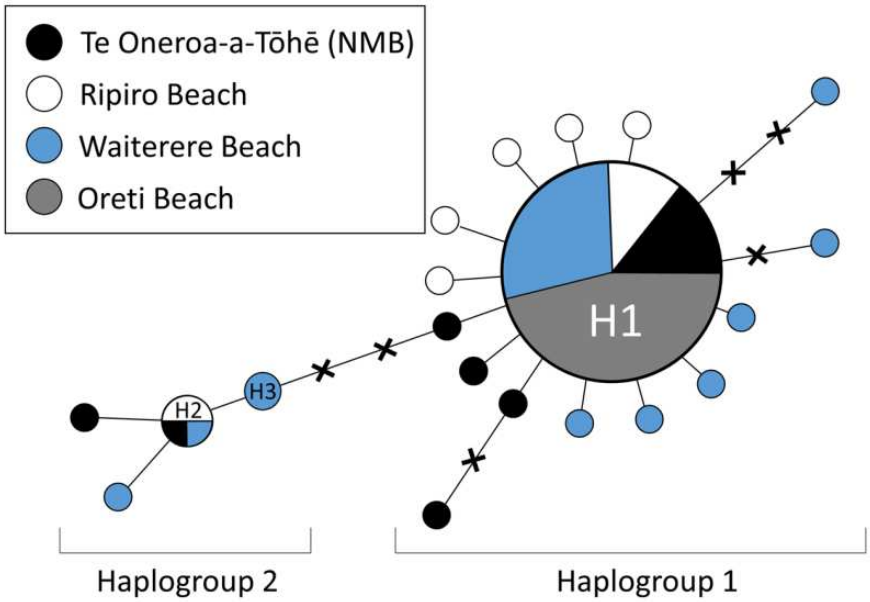
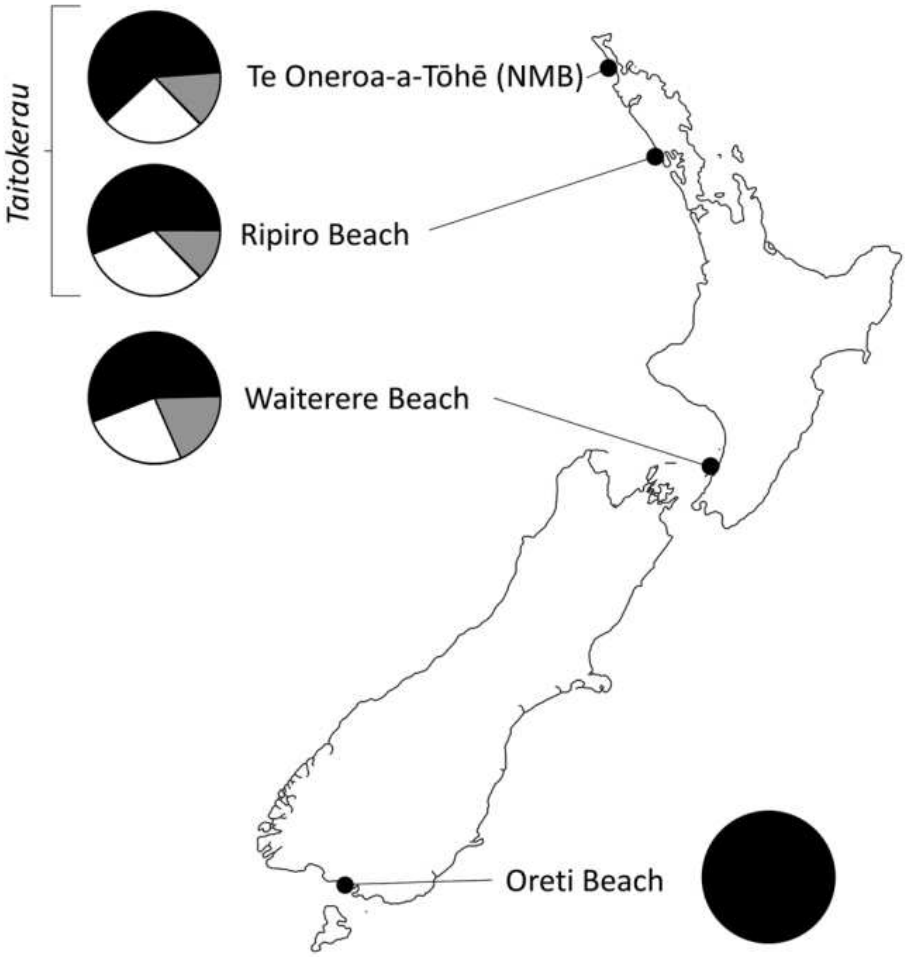


Figure 2 (below) is a map showing the geographic distribution of toheroa genetic diversity. Haplotype H1 is shown in black, other haplotypes from Haplogroup 1 (Fig. 1 above) are shown in white and haplotypes from Haplogroup 2 are shown in grey. Refer to Fig. 1 for the haplotype network and haplogroups.



Source for toheroa genetic data: https://www.nature.com/articles/s41598-018-35564-4.pdf

Question 48
There were 0 polymorphic sites in the toheroa population from Oreti Beach. Which of the statements below could explain this finding?

- **A.** There is no variability at any nucleotide position in the population of toheroa from Oretic Beach.
- **B.** Toheroa from Oreti Beach are all from the same parent.
- **C.** All toheroa from Oreti Beach share the same haplotype.
- **D.** The point mutation that differentiates the toheroa from Oreti Beach from other geographically separated toheroa populations changed a purine nucleotide to a pyrimidine.
- **E.** Toheroa from Oreti Beach have identical genetic diversity to those from beaches in the North Island.

Question 49
What haplotypes are found in toheroa from Ripiro Beach on the Kapiti Coast?

- **A.** Haplotype 1, 2, and 3.
- **B.** Haplotype 1 only.
- **C.** Haplotype 1 and 2 only.
- **D.** Haplotype 1, other haplotypes from Haplogroup 1 and Haplogroup 2.
- **E.** Haplotype 1 & other haplotypes from Haplogroup 1..

Question 50
Which specific beach shows the most genetic diversity in toheroa CO1 sequences?

- **A.** Te Oneroa-a-Tōhē
- **B.** Ripiro
- **C.** Waitarere Beach
- **D.** Oreti Beach
- **E.** Taitokerau

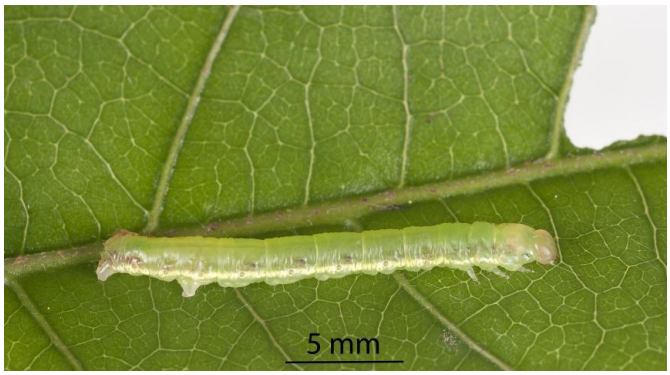
Question 51
Given the information and data from the study into the population genetic structure and levels of connectivity among toheroa across the entire distribution in Aotearoa New Zealand, what conclusion can be drawn?

- **A.** The population genetic structure of the toheroa is unique amongst surf clams in New Zealand.
- **B.** Limited gene flow is the most important factor influencing toheroa population dynamics.
- **C.** South Island toheroa are genetically isolated.
- **D.** Genetic diversity in northern toheroa is high proving that there is limited connectivity at smaller spatial scales.
- **E.** Human translocation of toheroa explains the disjointed current distribution and population dynamics of the toheroa.

KAWAKAWA & THE LOOPER MOTH CATERPILLAR
An arms race between herbivores and a plant that is utilised in Rongoā Māori

Kawakawa, *Piper excelsum*, is an endemic herbaceous shrub with large heart shaped leaves that are a dark green and slightly glossy, It is an important plant to Māori with many rongoā (medicinal) and cultural uses and its fruits and leaves are often consumed. Kawakawa leaves and roots contain several compounds that have been shown to be biologically active and which may underpin its traditional use in rongoā. Kawakawa tea was drunk to soothe the digestive system. A poultice was made from and applied to treat skin abrasions and ailments. The leaves were also chewed to soothe toothaches.

Kawakawa leaves are often full of holes (at left below), the result of the specialist herbivores *Cleora scriptaria*, the kawakawa looper moth (at right below). In response to herbivory the plant produces a cocktail of defensive chemicals in the leaves to deter the caterpillar from feeding on it.



In mātauranga Māori kawa (protocol & etiquette) suggests that kawakawa leaves with the most holes from looper moth herbivory should be preferentially harvested for rongoā because they are more potent.

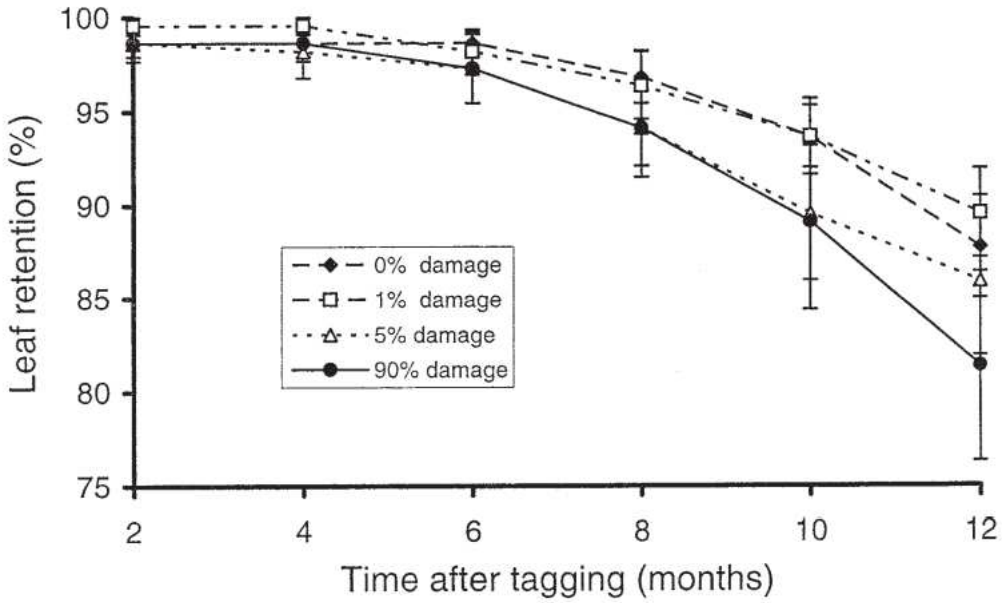
Question 52
In some species of plants there is a direct relationship between leaf abscission (leaf fall) and the extent of leaf damage from herbivores. Hodge, Keesing, and Wratten looked at whether this occurs in kawakawa in response to herbivory of the looper moth caterpillars in a paper published in the New Zealand Journal of Ecology in 2000. Undamaged kawakawa leaves of similar age (≅ 3 months) and initial size (5-7 cm midrib length) on 19 randomly selected trees on Banks Peninsula were used in the field experiment. Two leaves were covered with muslin bags, two were covered with bags containing two II / III instar *C. scriptaria* caterpillars (natural damage) and two were covered after being punctured five times (holes 4 mm diameter) using a mechanical hole punch (artificial damage; representing around 5% of leaf area removed).

What is the best explanation of the reason why the researchers covered two undamaged leaves with muslin bags.

A. To stop the leaves being damaged by other insects.
B. To stop the wind from causing leaf fall.
C. To prevent rain from washing away the caterpillars.
D. These leaves act as a control, allowing the researchers to minimize the effects of factors other than leaf damage on leaf retention.
E. These leaves act as a control, allowing the researchers to manipulate all other variables except leaf damage.

Question 53

The data in the graph below shows the pattern of leaf retention in kawakawa after different levels of simulated herbivory were made using a mechanical hole-punch. Vertical bars are two standard errors and significant overlap of bars suggests there is no difference between the values.



Source:
https://researcharchive.lincoln.ac.nz/bitstream/handle/10182/1293/NZJEcol24_1_87.pdf?sequence=1&isAllowed=y

What significant conclusion could the researchers draw from these data?

- **A.** Herbivory does not induce leaf fall in kawakawa
- **B.** Artificial damage or damage by the C. scriptaria larvae did not induce abscission of new leaves in kawakawa over this period.
- **C.** Artificial damage did not induce abscission of new leaves over this period.
- **D.** Damage to kawakawa leaves increased leaf retention.
- **E.** 90% leaf damage decreased leaf retention after a year.

Students in the first year Ecology & Evolution paper BIOSCI 109, at Auckland University collected and analysed data on whether leaves from different parts of the a kawakawa plant had more holes and might be expected to have a higher potency and greater efficacy when used for rongoā. Each student collected two kawakawa leaves from different kawakawa plants in the Kaipatiki Researve. One leaf was harvested from the tips of the branches (**A.**) and one from branches at the base closer to the main stem (**B.**). They measured leaf damage by scoring the % of leaf missing with a dot matrix. The data from 10 students is presented in the table below.

	1	2	3	4	5	6	7	8	9	10
% leaf missing in leaves harvested from **A.**	56.0	37.0	6.6	3.5	1.7	4.5	9.2	9.4	0	4.7
% leaf missing in leaves harvested from **B.**	5.0	14.3	5.3	0	9.4	73.0	9.0	23.7	9.0	11.6

Question 54

Calculate the sample mean for the % of leaf missing in leaves harvested from branches at the base of the plant.

Record your answer here to 1 decimal place _____

Questions 55& 56
The statistical data from all 30 students has been calculated to two decimal places and summarised below.

	Sample mean ($\bar{X}$)	Sample standard deviation (*s*)	Standard Error ($SE_{\bar{X}1-\bar{X}2}$)
% leaf missing in leaves harvested from **A.**	8.00	11.93	3.48
% leaf missing in leaves harvested from **B.**	14.57	14.83	

A t-test is a statistical test that is used to compare the means of two groups. It is often used in hypothesis testing to determine whether a process or treatment actually has an effect on the population of interest, Hypothesis tests start with a null hypothesis (Ho) which states there is no significant difference between the means of the two groups. An alternative hypothesis (Ha) states there is a statistically significant difference between the means. A t-value is calculated and then compared against the critical value in a t Table. If the t-value us greater than the critical value then the null hypothesis is rejected and there is a significant difference between the means of the two groups. Note that the t-value can be negative because we only care about the absolute value of the difference between the means, or the distance from 0. It doesn't matter which direction.

The degrees of freedom is related to your sample size, and shows how many 'free' data points are available in your test for making comparisons. A 95% confidence interval gives the range of numbers within which the true difference in means will be 95% of the time.

Question 55
What is the null hypothesis for this student investigation?

- **A.** There is no difference in caterpillar grazing intensity on kawakawa leaves.
- **B.** There is no difference in the % of leaf missing in leaves harvested from the tip of the of the branches or from the base of a kawakawa shrub.
- **C.** Caterpillar grazing on kawakawa occurs randomly.
- **D.** Leaves at the tips of the branches have more holes from caterpillar grazing.
- **E.** Caterpillars graze on leaves from all parts of the kawakawa plant.

Question 56
Calculate the t-statistic for the % of leaf missing from leaves from the two different parts of the kawakawa plant using this formula:

$$t = \frac{(\bar{X}_1 - \bar{X}_2)}{SE_{\bar{X}1-\bar{X}2}}$$

Note: the standard error has been calculated for you.

Enter your answer here. _____

Question 57
Assuming that the students wanted to be sure that they had estimated the true difference between the mean % of leaf missing from leaves from the tip and the base of the kawakawa shrub 90% of the time the t Table gives a critical value of 6.314. Based on this, what can you conclude about the data?

- **A.** There is no difference between the % of leaf grazed from leaves from the tip and the base of kawakawa plants.
- **B.** Caterpillars graze leaves from all parts of the kawakawa plant with equal intensity.
- **C.** The % of leaf grazed is higher on leaves from the tip of kawakawa branches.
- **D.** The % of leaf grazed is higher on leaves from the base of kawkawa branches.
- **E.** There is insufficient data to draw a conclusion.

In 2011, Chris Ryan, a student from Howick College, became interested in the use of kawakawa in rongoā. When he did a literature search he found there was no scientific evidence to back up the Māori practice of using kawakawa in rongoā where it is often used to treat inflammation. Chris realised that the way in which scientists tested kawakawa was quite different to how it is used in rongoā because the scientists were using organic solvents to extract the active compounds from kawakawa leaves but its traditional preparation in rongoā uses water. Chris carried out a series of investigations to test his hypothesis that kawakawa has anti-inflammatory properties. He studied the concentration of nitric oxide (NO) in kawakawa leaf extracts as NO is a signaling molecule that plays a key role in the pathogenesis of inflammation but it is a pro-inflammatory mediator that induces inflammation due to over production in abnormal situations. He also examined TNF-α and Il-6 in cells exposed to an aqueous kawakawa extract. A reduction in nitric oxide, TNF-α and Il-6 production would be indicative of anti-inflammatory activity.in the kawakawa extract.

The data are summarised in the figures below. Figure 1: Nitric oxide produced in a kawakawa extracts using water (aquous) and organic solvents (methanol/water). Figure 2: Il-6 production in cells exposed to the aqueous infusion extract: Figure 3: TNF-α production in cells exposed to the aqueous infusion extract: 0 µg/mL of Kawakawa extract was the control in all samples. Statistical significance was calculated using the t-test with significant results ($P < 0.05$). The vertical bars are the standard error about the mean.

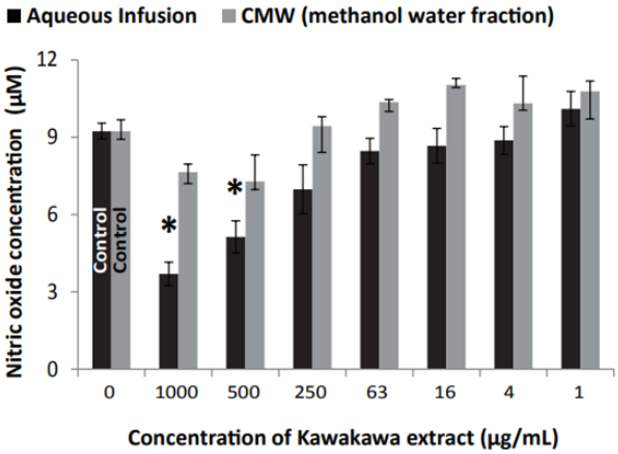


Figure 2

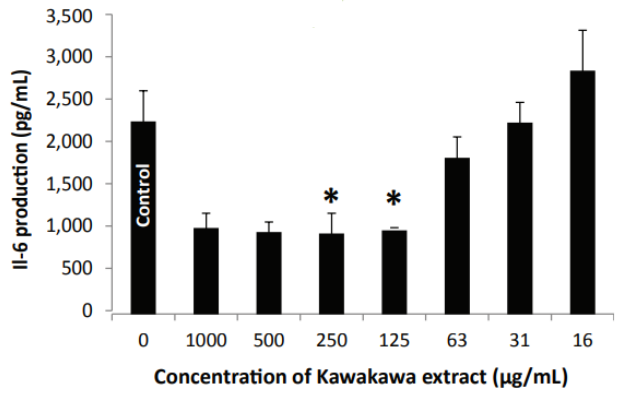
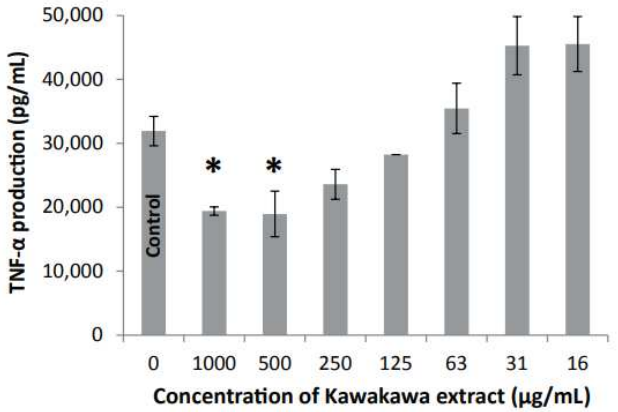


Figure 3



Sources: Science Learning Hub, *Connected* article: The Science of Rongoā.

Question 58
What conclusion/s can be drawn about the differences between kawakawa anti-inflammatory properties when extracted using organic solvents or in water?

I. Kawakawa extracts produced using organic solvents do not show anti-inflammatory actions.
II. A decrease in nitric acid concentration is seen only at higher concentrations of aqueous kawakawa extracts.
III. At higher concentrations, kawakawa extracts show anti-inflammatory actions only when an aqueous extraction method is used.
IV. Aqueous kawakawa extracts exert anti-inflammatory actions only at concentrations above 500 μg/mL.

A. Only I can be correctly concluded.
B. Only II can be correctly concluded.
C. I., II., and III can be correctly concluded.
D. I., II., and IV can be correctly concluded.
E. All of these conclusions (I. – IV.) can be drawn from the information provided above.

Question 59
The data and information provided above supports which of the following conclusions?

A. There is a dose dependant effect on the anti-inflammatory actions of all kawakawa extracts.
B. Kawakawa extracts have anti-inflammatory activity in aqueous solution.
C. Nitric oxide production was supressed only at the highest concentrations of 1000 μg/mL.
D. Inhibition of Il-6 production was greatest at aqueous extract concentrations of 16 μg/mL.
E. The inhibition of TNF-α production was lowest at aqueous extract concentrations of 250 μg/mL and125 μg/mL.

Question 60
The importance of this study is that”

A. It demonstrates the value of a holistic approach to scientific investigations, one that combines mātauranga Māori with traditional scientific methodology.
B. The results support traditional use of kawakawa extracts to treat inflammation.
C. Mātauranga Māori can inform traditional scientific methodology and *vice versa*.
D. Biological research starts with a noticing, interest, and a question.
E. All of the above (A. – D.) are true.

We all have an obligation to learn about our planet and to protect it from harm. NZIBO hopes you have enjoyed this exam and have learnt a little about the biological scientists working to understand and protect our wonderful planet here in New Zealand and on the global stage. We wish you well with your biology studies this year, regardless of whether you are selected for the tutorial programme. Thank you for taking the time to sit this exam.