

Write your name here

Surname

Other names

Pearson Edexcel
Level 3 GCE

Centre Number

--	--	--	--	--

Candidate Number

--	--	--	--	--

Biology A (Salters-Nuffield)

Advanced

Unit 3: General and Practical Applications in Biology

Monday 26 June 2017 – Morning

Time: 2 hours

Paper Reference

9BN0/03

Candidates must have:

Calculator, HB pencil, ruler and a copy of the scientific article adapted from *The Energy of Life* (enclosed)

Total Marks

--

Instructions

- Use **black** ink or ball-point pen.
- **Fill in the boxes** at the top of this page with your name, centre number and candidate number.
- Answer **all** questions.
- Show your working in any calculation questions and include units in your answer where appropriate.
- Answer the questions in the spaces provided – *there may be more space than you need.*
- You may use a scientific calculator.
- In questions marked with an **asterisk (*)**, marks will be awarded for your ability to structure your answer logically, showing how the points that you make are related or follow on from each other where appropriate.

Information

- The total mark for this paper is 100.
- The marks for **each** question are shown in brackets – *use this as a guide as to how much time to spend on each question.*

Advice

- Read each question carefully before you start to answer it.
- Try to answer every question.
- Check your answers if you have time at the end.

Turn over ►

P52220RA

©2017 Pearson Education Ltd.

2/2/1/1/1/



P 5 2 2 2 0 R A 0 1 2 8



Pearson

Answer ALL questions.

Write your answers in the spaces provided.

1 Plant fibres and oil-based plastics have both been used to make ropes.

(a) Describe one advantage and one disadvantage of using ropes made from plant fibres rather than ropes made from oil-based plastics.

(2)

.....

.....

.....

.....

.....

.....

.....

(b) Ropes used in climbing need to have high tensile strength.

State what is meant by the term **tensile strength**.

(1)

.....

.....

.....

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

(d) Tissues that contain lignin provide strength to plant fibres.

Describe the positions in the stem of those tissues that contain lignin.

(2)

.....

.....

.....

.....

.....

.....

.....

.....

(Total for Question 1 = 9 marks)



DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

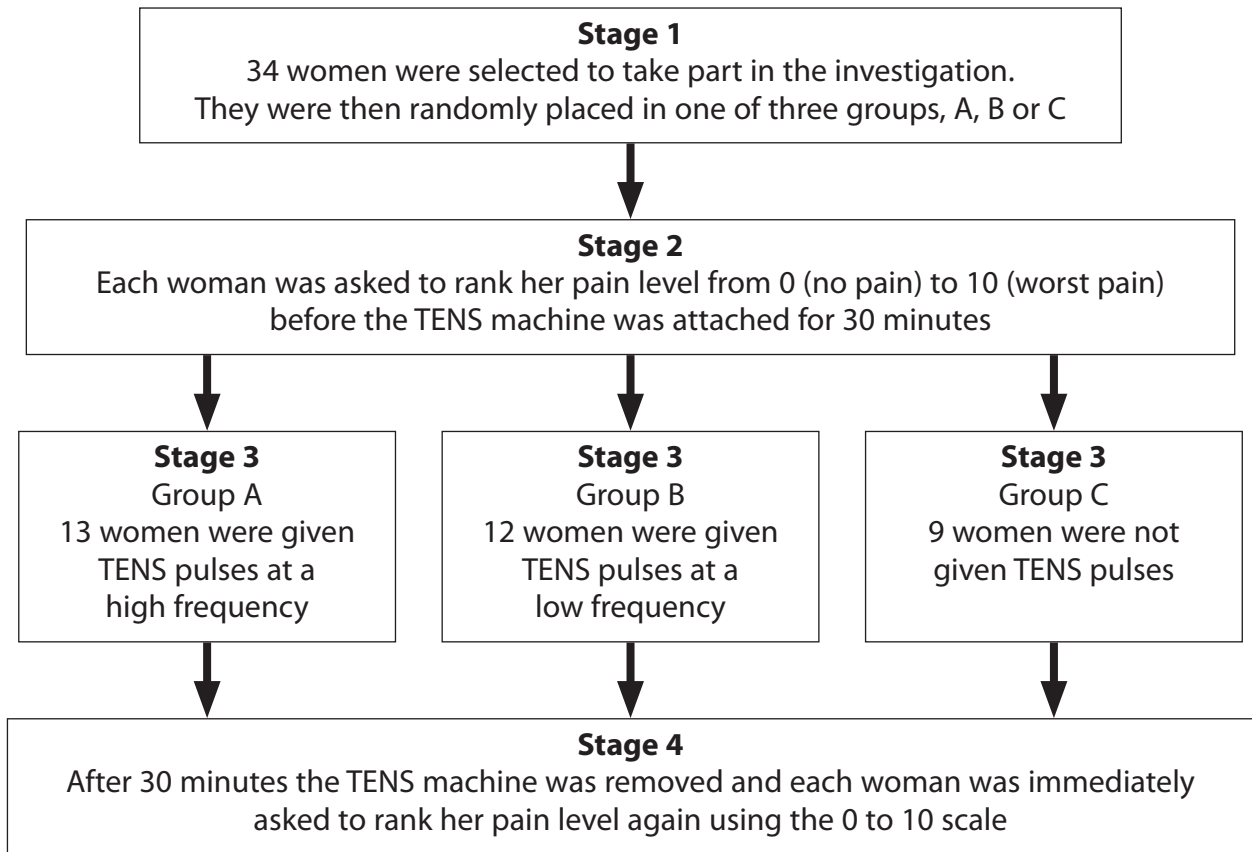
DO NOT WRITE IN THIS AREA

2 Some women need to have surgery to aid childbirth. This can lead to pain after surgery.

A TENS (transcutaneous electrical nerve stimulation) machine releases regular pulses of electricity onto the skin surface and can be used in pain relief.

An investigation was carried out to study whether the frequency of the pulses from a TENS machine could help these women with their pain relief.

The diagram shows how the investigation was carried out.



(a) Describe how the 34 women were selected in stage 1 to ensure that this investigation was valid.

(2)

.....

.....

.....

.....

.....

.....

.....

.....



(b) Explain why group C was included in this investigation.

(2)

.....

.....

.....

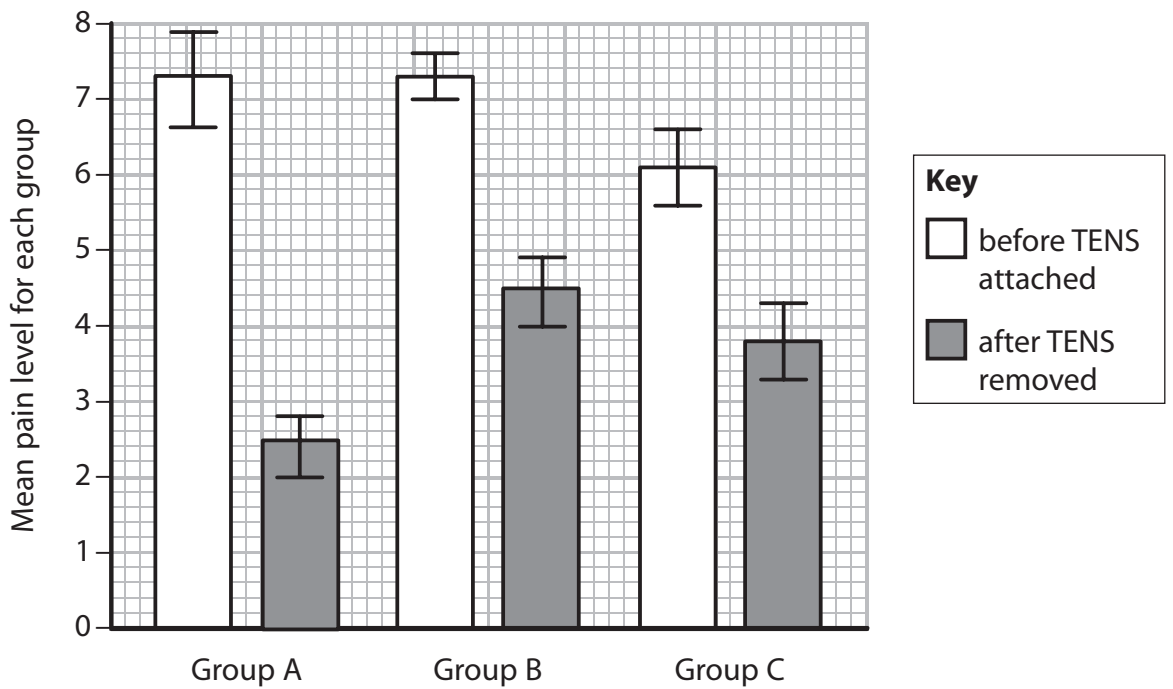
.....

.....

.....

.....

(c) The results and range of data for the investigation are shown in the graph.



DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



Determine the effectiveness of the use of a TENS machine in relieving pain following surgery.

(4)

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

(d) This investigation used only one 30-minute session of TENS pulses. This was done to reduce the risk of habituation.

Describe the process that occurs at a synapse that leads to habituation.

(4)

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

(Total for Question 2 = 12 marks)



P 5 2 2 2 0 R A 0 7 2 8

(ii) Give two reasons why there were different numbers of people in each resting heart rate group.

(2)

(iii) Give two reasons why the number of people in each resting heart rate group did not affect the validity of this investigation.

(2)

(b) Cancer can be due to body cells continuing to undergo mitosis and cell division. Prophase is one stage of mitosis.

Describe the events that occur during prophase in an animal cell.

(3)

(Total for Question 3 = 12 marks)

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



P 5 2 2 2 0 R A 0 9 2 8

- 4 A student investigated the effect of salt concentration on the growth of one species of brine shrimp.

The student placed 100 shrimp eggs in a beaker containing 1dm^3 of 3% salt solution. Three days after the eggs hatched, 10 shrimps were collected and their lengths measured. Seven days after hatching, another 10 shrimps were collected and their lengths measured.

The procedure was repeated using a 5% salt solution. All other variables were kept constant.

The results are shown in the table.

Specimen number	Length of specimen in 3% salt solution / mm		Length of specimen in 5% salt solution / mm	
	3 days after hatching	7 days after hatching	3 days after hatching	7 days after hatching
1	0.75	1.00	0.75	0.98
2	0.78	1.25	0.73	0.95
3	0.66	1.10	0.61	0.93
4	0.73	1.03	0.63	0.83
5	0.85	1.15	0.53	0.98
6	0.78	1.08	0.60	1.08
7	0.90	1.13	0.52	0.95
8	0.90	1.05	0.81	1.03
9	0.80	1.18	0.58	0.88
10	0.85	1.05	0.68	0.88
Mean	0.800	1.102		0.949

- (a) (i) Calculate the mean length of shrimp three days after hatching in the 5% salt solution. Give your answer to an appropriate number of significant figures.

(1)

Answer mm



(ii) Explain how these data can be used to show the effect of salt concentration on the rate of growth of brine shrimps.

(2)

.....

.....

.....

.....

.....

.....

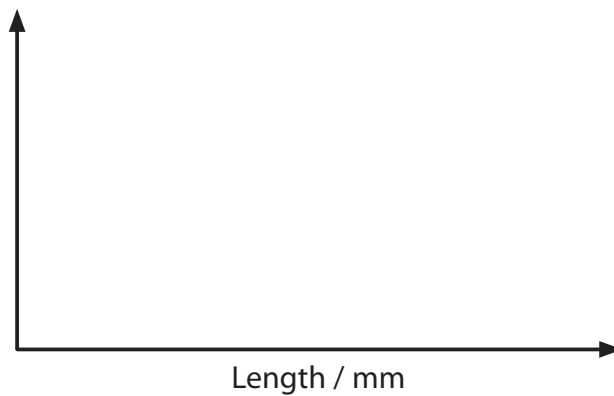
.....

(b) The student carried out a statistical test to see if there was a significant difference between the mean lengths of brine shrimps in these two salt solutions. The student used the 7 days after hatching data.

The student selected the *t*-test because the data are normally distributed.

(i) Draw a line on the graph to show a normal distribution for a population of brine shrimp.

(1)



DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



(ii) Complete the table by filling in the missing value for specimen 7 and then calculate the value for Σx_1^2 .

(1)

Specimen number (n)	3% salt solution		5% salt solution	
	Length of specimen (x_1)	Square of length of specimen (x_1^2)	Length of specimen (x_2)	Square of length of specimen (x_2^2)
1	1.00	1.000	0.98	0.960
2	1.25	1.563	0.95	0.903
3	1.10	1.210	0.93	0.865
4	1.03	1.061	0.83	0.689
5	1.15	1.323	0.98	0.960
6	1.08	1.166	1.08	1.166
7	1.13	0.95	0.903
8	1.05	1.103	1.03	1.061
9	1.18	1.392	0.88	0.774
10	1.05	1.103	0.88	0.774
Sum (Σ)	$\Sigma x_1 = 11.020$	$\Sigma x_1^2 =$	$\Sigma x_2 = 9.490$	$\Sigma x_2^2 = 9.055$
Mean	$\bar{x}_1 = 1.102$		$\bar{x}_2 = 0.949$	

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



(iii) The variances are used in the calculation of a t -value.

The variance for the 3% salt solution $S_1^2 = 0.0059$.

Calculate the variance for the 5% salt solution (S_2^2) using the formula

$$S_2^2 = \frac{\sum x_2^2 - \frac{(\sum x_2)^2}{n}}{n - 1}$$

n = the number of specimens

(2)

S_2^2

(iv) Calculate the t -value using the formula.

Give your answer to an appropriate number of significant figures.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

(3)

$t =$

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



(v) The calculated t -value is greater than the critical value at $p = 0.05$.

Describe what this result indicates about the effect of salt concentration on the length of brine shrimp.

(2)

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

(Total for Question 4 = 12 marks)

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



(c) Scientists have investigated the effect of UV-B on both chlorophyll and flavonoid content.

A group of plants was exposed to UV-B for 20 minutes per day for one month. The chlorophyll and flavonoid content of each plant were then determined and the means calculated.

This was repeated for three more groups of plants. Each group was exposed to UV-B for different lengths of time. All other variables were kept constant.

The results are shown in the table.

Length of time exposed to UV-B / minutes	Mean chlorophyll content / arbitrary units	Mean flavonoid content / arbitrary units
0	32	25
20	30	24
40	27	39
60	24	40

(i) Compare and contrast the effect of length of exposure to UV-B on the chlorophyll and flavonoid content of these plants.

(3)

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....



DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

BLANK PAGE



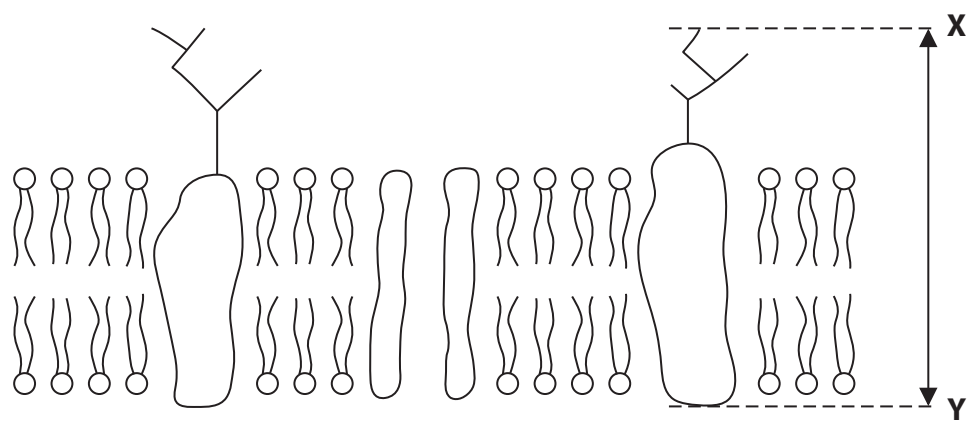
DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

6 The structure of the cell surface membrane can be explained using the fluid mosaic model. This model suggests that there are a variety of different proteins and glycoproteins present in a phospholipid bilayer.

(a) The drawing shows the cell surface membrane of a liver cell. The drawing has a magnification of 5×10^6 .



Calculate the actual length of the glycoprotein between points X and Y. Give your answer with an appropriate unit.

(2)

Answer



*(b) The ratio of lipid to protein in a cell surface membrane is approximately 1:1.

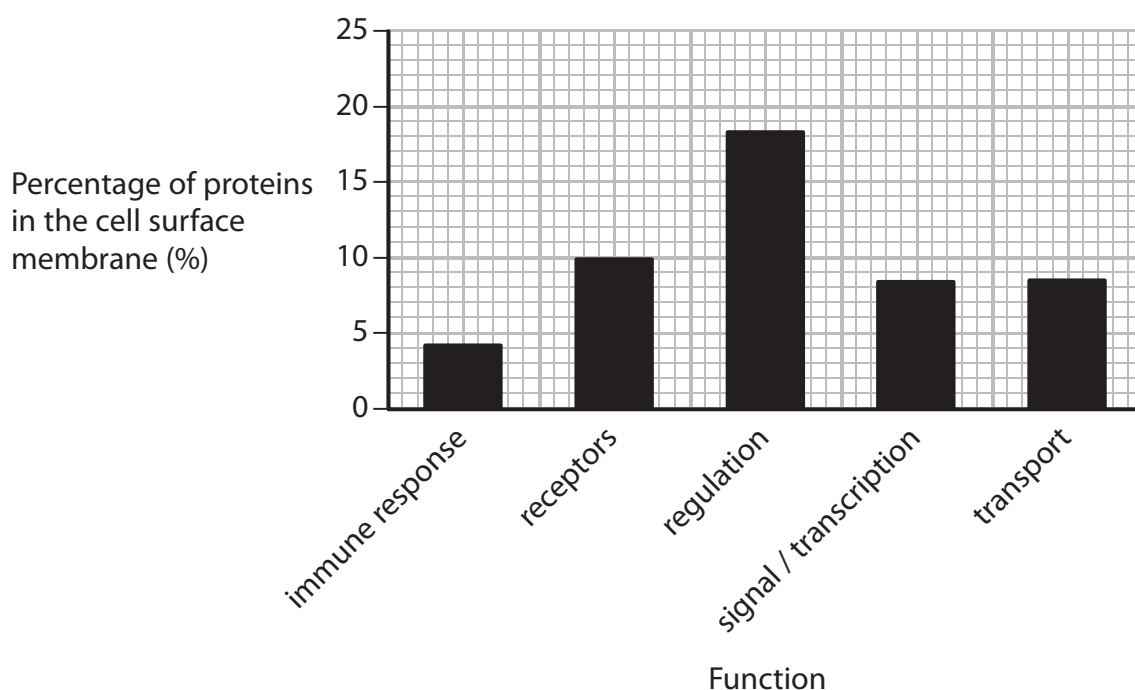
Scientists have studied the proteins present within human cells. Proteins in a cell are found in several locations.

The table shows the percentage of protein found in some locations in a cell.

Location within a cell	Percentage of total protein present (%)
cell surface membrane	68.2
cytoplasm	1.4
endoplasmic reticulum / Golgi apparatus	1.4
nucleus	14.5
other	14.5

The proteins within the cell surface membrane were further analysed for their function.

The graph shows some of the results of this analysis.



7 The scientific article you have studied is adapted from an article from *'The Scientist'*.

Use the information from the scientific article and your own knowledge to answer the following questions.

(a) Explain why the 'anaerobic oxidation of methane' could be a 'key climate regulator' (paragraph 2).

(3)

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

(b) Give reasons why 'humble microbes', such as bacteria, are considered less complex than eukaryotes (paragraph 4).

(2)

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....



(e) Explain how the unfolding of the enzymes in *E. coli* makes them 'become ineffective at 60°C' (paragraph 12).

(3)

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

(f) State what is meant by the genome of a thermophile (paragraph 14).

(1)

.....

.....

.....

.....

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA



(g) Explain how cyanobacteria evolved to 'handle high temperatures' (paragraphs 14 and 15).
(5)

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

Handwriting practice area consisting of 20 horizontal dotted lines.



DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

(k) *Bacillus subtilis* 'initiate a hibernation protocol' (paragraph 25).

Mycobacterium tuberculosis can also become dormant for long periods of time.

Compare and contrast the dormancy of these two species of bacteria.

(4)

Area with horizontal dotted lines for writing the answer.

(Total for Question 7 = 30 marks)

TOTAL FOR PAPER = 100 MARKS



DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

DO NOT WRITE IN THIS AREA

BLANK PAGE

Every effort has been made to contact copyright holders to obtain their permission for the use of copyright material. Pearson Education Ltd. will, if notified, be happy to rectify any errors or omissions and include any such rectifications in future editions.



Pearson Edexcel Level 3 GCE

Biology A (Salters-Nuffield)

Advanced

Unit 3: General and Practical Applications in Biology

June 2017

Scientific Article for use with Question 7

Paper Reference

9BN0/03

Do not return the insert with the question paper.

Turn over ►

P52220RA

©2017 Pearson Education Ltd.

2/2/11/11/



Pearson

Scientific article for use with Question 7

The Energy of Life



© ISTOCK.COM/LORCEL

1. The images displayed on the wall of screens in front of me (Marlow) show a shadowy, alien world, with bizarre life forms moving in and out of frame like apparitions. Slow-moving crabs dance precariously on submerged cliff sides, bug-eyed pink rockfish hug the crevasses, and serpentine fish slither across the sediment in search of food. It's September 2011, and my fellow scientists and I are bobbing comfortably aboard the research vessel *Atlantis* on the surface of the eastern Pacific Ocean. Seven hundred meters below, the robotic craft *Jason* dangles beneath the ship, exploring a marine methane seep known as Hydrate Ridge while skirting calcium carbonate mounds that rise hundreds of meters above the seafloor.
2. At Hydrate Ridge and many similar sites around the world, methane derived from deeply buried hydrocarbons or subseafloor microbial communities is squeezed upward through cracks in the Earth's crust. This narrow zone of methane-rich waters near the seafloor is home to one of the most extreme and confounding biological processes known to science: the anaerobic oxidation of methane, likely a key climate regulator and a phenomenon that could hold important clues to finding life elsewhere in the universe.
3. We look on with nervous excitement as *Jason* collects an intact plug of seafloor sediment, stows it in its sample basket, and begins the long trip back to the surface.
4. The ocean is well stocked with mysterious creatures, and while the tentacled and the sharp-toothed may be the Gorey-esque stuff of nightmares, humble microbes also deserve a nod as some of the most biologically exotic denizens of the deep sea. These single-celled organisms have found ways to "breathe" metals, survive without oxygen, and fix carbon into biomass in several distinct ways, allowing them to handle nearly the full range of physical and chemical extremes that our planet has to offer.

5. The marine archaeon *Geogemma barossii*, for example, can survive at a blistering 121°C, while other microbes have retained metabolic activity at temperatures as low as –20°C in Siberian permafrost. The bacterium *Deinococcus radiodurans* remains viable after exposure to 1,000 times the fatal human radiation dose, and the aquatic archaeon *Ferroplasma acidarmanus* can withstand extremely acidic water, with pH values as low as 0. Such flashy feats have earned these and other microbes the title “extremophiles”—lovers of extreme conditions. But as remarkable as their metabolic capabilities are, calling them “extreme” is a bit human-centric. Because of our own requirement for oxygen and narrow acceptable ranges of temperature, salinity, pressure, pH, and radiation, the survival of other organisms in a wide range of environments seems extreme to us. But for a microbe that has come to depend on the abundant hydrogen ions of acidic hot springs, an air-conditioned suite at the Ritz is a threatening proposition. The wide variety of biochemical modes of existence reflect billions of years of evolution, adaptation, and niche differentiation rather than a standardized characterization of biological fortitude.
6. For the title of “extremophile” to be broadly meaningful, it must refer to a more objective measure of extremeness—an advantageous capability enacted in response to a common challenge. One such challenge, something that all living organisms must face, is the acquisition of chemical energy to drive cellular reactions. Perhaps the ways in which organisms handle this task could separate the truly industrious from the merely viable.

The energy of life

7. Energy is the currency of biology. By harvesting electrons from a stunning range of starting materials, Earth’s organisms produce adenosine triphosphate (ATP), which powers biological reactions. In the case of mammals and most eukaryotes, sugars and other organic molecules are common electron sources, the oxidation of which drives ATP production. Bacteria and archaea can use a range of other chemicals, from sulfide to iron to ammonium.



Courtesy of Jeffrey Marlow

Oceanic extremes

This experimental setup on Hydrate Ridge off the coast of Oregon samples microbial metabolism in deep-sea methyl seeps, which host a variety of seemingly strange creatures, including some truly extreme archaea and bacteria that cannot survive without each other.

8. Cells take up these electron-rich molecules and capture their electrons, which jump down an electron transport chain in the mitochondrial or cell membrane. As electrons move along the membrane toward a final electron acceptor, protons are pumped from the cell’s interior to the exterior, setting up a chemical gradient. Finally, protons stream back into the cell, releasing the chemical pressure and generating ATP. With each energy-requiring reaction, from flagella construction to cell division and growth, cells draw upon their ATP bank.

9. This elegant, multistep process is a pervasive feature of life as we know it, but energetic challenges are ever-present. If the electrical potentials of electron donor and acceptor are too closely aligned, for example, it won't be possible to squeeze much energy from their coupling. The concentrations of the reactants and the speed at which enzymes can mobilize them are also key factors. These two components—the magnitude of energy available from a particular pairing and the rate of such reactions—determine how much energy a cell can produce.
10. The other half of the equation—the cost of living, as it were—is often harder to evaluate. Cataloging the biochemical parts list of a particular cell is one challenge. Individual biosynthetic pathways—the production of lipids from glycerol derivatives, for example—are relatively well characterized under “standard” conditions, but a cell's ever-changing chemical environment can render baseline calculations inaccurate. Scaled over millions of such reactions, the margin of error may be a substantial proportion of the available energy. And this is just considering the biosynthesis of new cellular material. In most environments, microbes must always be vigilant against biochemical breakdown resulting from environmental stresses, calling on energy reserves to restore old enzymes or patch holes in cell walls. Competition among residents may also demand additional energy expenditure, such as powering flagella to swim around in search of food or producing antibiotic molecules to keep predatory neighbors at bay.
11. If, however, we are able to estimate how much energy is required for survival, and compare that to how much energy is available to be extracted from the environment, we can begin to consider “extreme” organisms in a more objective fashion. Some of the most “exotic” environments actually offer luxurious energetic balances; it's the microbes with low net energy availability that are the real extremophiles, whether they live an expensive existence in a high-energy environment, or an ascetic life in an energetic desert.

Easy living

High energy availability, low energy requirements

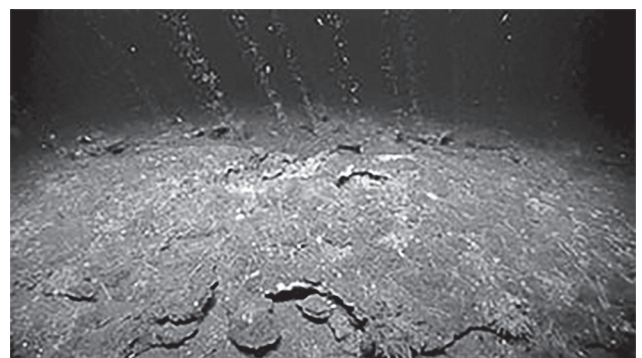
12. The hot springs of Yellowstone National Park are uniquely beautiful palettes: concentric rings transition from blue in the pools' centers to green, then yellow, orange, and red at the water's periphery. The mesmerizing visuals contrast sharply with the damp, sulfurous odors wafting across your nostrils and the stern warnings from signs and rangers to keep your distance. Against this otherworldly backdrop, the 1966 discovery of viable cells living in the ultrahot waters came as a surprise that forced a reconsideration of microbial limits. After all, water temperatures frequently topped out well above the tolerance range of most known organisms. Nearly all of *E. coli*'s enzymes, for example, unfold and become ineffective at 60°C.
13. Hot-springs microbes have traditionally been labeled “extremophiles,” yet their energetic bank account is typically well in the black. Like their moderate-temperature relatives that ply the planet's oceans, thermophilic cyanobacteria gain energy from light-driven reactions that mobilize electrons from water. Along the outer edges of thermal springs, energy-generating light is abundant, and cyanobacteria flourish. Indeed, the vibrant colors we see are the plentiful microbial pigments that coat the limestone surfaces.

14. This is not to say that life at high temperatures is easy. On the contrary, only through a range of sophisticated molecular adaptations, encoded by subtle edits in thermophiles' genomes, has this forbidding niche become habitable. Protein stability is perhaps the main challenge for life at high temperature. Higher thermal energy causes hyperactive atoms to vibrate with more kinetic energy, threatening the structural integrity of the molecules that perform biochemical reactions. If sulfur-containing cysteine amino acids are positioned strategically within protein structures, disulfide bridges can form interatomic support beams that resist unfolding. Some thermophilic enzymes also have larger hydrophobic cores, away from the proteins' exposed active sites, which act as additional glue to fight thermal destabilization. Other adaptations, such as simpler protein folds or fewer bound metal ions, further guard against molecular destabilization in the face of thermal stress.
15. Evolving the capability to handle high temperatures may not have been straightforward, and biosynthetic construction costs might have presented some hurdles, but the payoff does seem to have been worth it. By constructing heat-stable enzymes, the cyanobacteria inhabiting hot springs are able to photosynthesize in relative peace, away from the feeding frenzy of predatory microbes or larger creatures in habitats such as the ocean's surfaces. Thus, while Yellowstone's hot springs may seem like an extreme environment, not all of the microbes that inhabit them are struggling to survive.

An expensive lifestyle

High energy availability, high energy requirements

16. One needn't travel to the bottom of the ocean or into a scalding hot spring to find microbes living at the edge of energetic feasibility. Sometimes, the most remarkable habitats are in your own backyard, beneath well-manicured Kentucky bluegrass or a haphazard array of lawn furniture. "Generic" temperate soils are among the richest microbial milieus on the planet, with each pinch of dirt hosting up to a billion cells, and down there, it's all-out biochemical warfare.



NOAA Okeanos Explorer Program,
2013 ROV Shakedown and Field Trials

Methane munchers

Off the coast of Virginia, methane bubbles flowing out of the seafloor sediment support a variety of life, including some truly extreme microbes.

17. Among the more prominent denizens of this dense microbial metropolis are representatives of the bacterial genus *Streptomyces*: stringy, rod-shaped organisms that develop centimeter-scale networks branching through the soil. *Streptomyces* gain energy through heterotrophy, the consumption of organic molecules such as sugars, amino acids, or aromatic compounds. These are energetically juicy molecules, and they're abundant given the high density of plants in the vicinity, but it's far from a free lunch.

18. *Streptomyces* capture organic molecules largely by secreting enzymes into the soil to access and degrade energy-rich polymers before other competitors can get to them. It's a bold strategy susceptible to freeloader hijacking, with no guarantee that the processed material will find its way back to the same organism that went to the trouble of producing the enzyme. But at scale, the odds become more palatable, and the benefit from degraded organics that find their way to one *Streptomyces* or another outweighs the inefficiency of the strategy. Building a large network of interconnected cells is the only option that makes this spendthrift approach worthwhile.
19. Oxygen is the highest-potential electron acceptor on the market, and transferring electrons to O₂ provides the biggest payoff per electron-donating molecule. This makes the upper, oxygen-perfused layers of soil highly sought-after real estate, but it comes at a price. In a 2005 study, geomicrobiologists Tom McCollom of the University of Colorado, Boulder, and one of us (Amend, then at Washington University in St. Louis) calculated the energetic costs of synthesizing an extensive list of biomolecules, including amino acids, nucleotides, fatty acids, saccharides, and amines, from inorganic precursor molecules. We found that biosynthetic costs were actually higher—by more than an order of magnitude—in oxygen-infused conditions than in anaerobic settings. Part of this discrepancy is due to the fact that many precursors must be reduced from their oxidized state prior to biomolecular construction, but it suggests that the energetic windfall from using oxygen as an electron acceptor may be a necessary copay, not a bankable nest egg.
20. Perhaps *Streptomyces*'s most impressive adaptation is the genus's remarkable array of antibiotics, which can attack competitors' cell walls or protein-synthesis machinery. But these large, sophisticated weapons require a high flux of electron-rich intermediates and the repurposing of cellular supply chains. An analysis led by J. Stefan Rokem of the Hebrew University of Jerusalem showed that antibiotic production represents an enormous drain on biosynthetic pathways, frequently costing more than half of the stocked supply of precursor building blocks (such as pyruvate or acetyl-CoA) that would otherwise be used to construct biomass and generate new cells. But, while expensive, antibiotic synthesis is critical for survival in this crowded environment, minimizing competition for much-needed resources. From an energetic perspective, *Streptomyces* is an extreme organism, and it's hiding in plain sight.

Just getting by

Low energy availability, low energy requirements

21. As *Jason* is hauled back on deck, packed to the brim with samples from deep-sea methane seeps, *Atlantis* becomes a hive of activity. We rush the cores of seafloor sediment along with chunks of carbonate rocks to the onboard laboratory, where they're partitioned and allocated for experiments. Liquid is extracted for geochemical measurements, and a few grams of sediment are frozen for DNA and microscopic analyses. A separate aliquot is scooped into a shiny silver mylar bag, mixed with filtered seawater and isotopically labeled chemicals, and flushed with nitrogen and methane gas. The bag is heat-sealed and set aside, a time capsule to be opened several months later to determine how much of the isotope-labeled substrate has been taken up by the mysterious process of anaerobic methane oxidation.

22. What we ultimately find is confirmation of a bizarre biological partnership operating at the edge of what is energetically possible. As has been found at other methane seep sites around the world, certain types of archaea and bacteria aggregate together in multicellular clumps, with tens, hundreds, or occasionally thousands of microbes linked by mutual energetic necessity. The details of the association are still up for debate, but it appears that archaeal partners oxidize methane and transfer electrons to the bacteria to enable the reduction of sulfate to sulfide, generating energy to power cellular functions. Remarkably, when the energetics numbers are crunched, the archaea come out in the red—their half of the arrangement does not appear to produce enough energy for their own survival. This means that the sulfate-reducing reaction performed by their bacterial partners must supply power to both species. How this mutualism works, especially in an evolutionary framework, is far from certain.
23. The energetic demands for biosynthesis are relatively low given these organisms' very slow rates of growth, doubling only every few months. Nevertheless, given the difficulty of extracting and sharing energy in methane seep environments, anaerobic methane-oxidizing partnerships deserve the title of extremophiles, as characterized by the energetic framework. Hopefully, future studies will illuminate the nature of this symbiosis and provide insight into how the energetically improbable becomes possible, untangling the intricacies of these and other slow-growing extremophiles.

An impossible situation

Low energy availability, high energy requirements

24. The final permutation of energetic cost-benefit ratios seems like a non-starter: having higher energy demands than the rates of supply does not make for a sustainable situation. And while growth under such conditions seems impossible (with the notable exception of tightly-coupled metabolisms like those described above), an energy debt need not mean cell death.
25. When the going gets tough, some microbial species, such as the bacterium *Bacillus subtilis*, initiate a hibernation protocol, shutting down the furnace and turning off the lights before forming a life raft that will hopefully ferry them to greener pastures. The process is called sporulation, and it's a life-or-death decision not to be made lightly. *B. subtilis* is commonly found in soil environments susceptible to feast or famine swings in energy availability. When one of these cells senses nutrient stress, it draws on energy stores, activating flagella to search for food, flooding its surroundings with antibiotics to kill off competitors, or desperately importing foreign pieces of DNA in hopes that a novel capability will be the ticket out of a bad situation. If all else fails, it replicates its genetic material and partitions it into a protective capsule that can withstand extreme heat, radiation, chemical stress, desiccation, and energetically untenable conditions. Sensors located on the spore's outer surface probe the environment for friendlier surroundings and assess the possibility of returning to a more active way of life. Powering up is an extremely energetically demanding undertaking, permitting full resurrection only under ideal circumstances. Thus, while this behavior may be considered extreme in itself, spore formers dodge the true test of their extremophilic nature by waiting out the impossible in a state of metabolic hibernation.

Rethinking extremes

26. For millennia, microbes have searched for loopholes and experimented with novel molecular machines in an attempt to gain a foothold where others can't. From backyard soils to seafloor methane seeps, these extremophiles eke out a living, revealing adaptations to the energy equation that may point us toward other organisms awaiting discovery in the headlights of a future robotic spacecraft.
27. Many of the most promising astrobiological targets in our solar system may well possess the baseline requirements for life such as liquid water and key elements, but net energy availability is an unknown. The lakes of Titan, Saturn's largest moon, have plenty of organic fuel for the taking, but maintaining a viable microenvironment would likely be very difficult given the lack of an appropriate solvent at such frigid temperatures. The Martian subsurface, on the other hand, may lack easily obtainable energy sources, but with relatively few apparent hazards, a low-energy way of life could be feasible.
28. Researchers have yet to fully sample the diversity of bioenergetic regimes on Earth. With continued exploration of the seafloor or the planet's deep interior, we find novel geochemical cocktails that demand microbial innovation. And so, back on the Atlantis, we load *Jason* with cleaned collection tubes and send our robotic emissary on yet another journey into the unknown, in search of new microbial responses to the extreme conditions of the deep.

The Energy of Life by Jeffrey Marlow and Jan Amend, February 1, 2015. The Scientist Magazine.
<http://www.the-scientist.com/?articles.view/articleNo/41990/title/The-Energy-of-Life/>