SURVIVAL RIVALS

EXPERIMENTS INSPIRED BY DARWIN

wellcome trust

BRINE DATE

TEACHER, TECHNICIAN AND STUDENT NOTES

WWW.SURVIVALRIVALS.ORG

Before you read this manual,
have you put your
Algal culture
in the fridge?

Sub-culture the algae as soon as possible so that there will be enough food for the larvae when they hatch.

Please refer to page 17 for full instructions to set up this experiment

CONTENTS

WELCOME TO BRINE DATE!

This kit contains everything you need to carry out the 'Brine Date' protocol with your class, as well as this manual which will help you to get the most out of this resource. The 99% Ape book will provide you with a comprehensive guide to evolution and the latest thinking on the subject.

Brine Date is part of the Survival Rivals series of experiments. There are three kits in this series:

I'm a Worm, Get Me Out of Here for 11-14 year olds
Brine Date for 14-16 year olds
The X-Bacteria for 16-19 year olds

Every secondary state school within the UK is entitled to one of each of these kits for free. You can find out more and order at www.survivalrivals.org.

Survival Rivals is designed to allow secondary schools to celebrate the 200th anniversary of Charles Darwin and the 150th anniversary of the publication of On the Origin of Species by Means of Natural Selection. The three kits enable young people of different ages to carry out practical investigations and to explore Darwin's ideas of evolution and selection.

In Brine Date sexual selection is investigated by observing brine shrimps as they form mating pairs, measuring the relative sizes of males and females and recording the evidence that sexual selection is occurring.

The Survival Rivals website (www.survivalrivals.org) is packed with additional resources and information to support you in delivering these experiments, including online games and videos filled with tips about carrying out the experiments.

For further support about the Survival Rivals experiments and about delivering evolution in the classroom, please see the Science Learning Centres website at www.slcs.ac.uk/darwin200 for details of their courses.

Survival Rivals is funded by the Wellcome Trust, who are also running a number of other Darwin-related initiatives. Find out more about the Great Plant Hunt for primary schools, an animated version of the Tree of Life and various other projects at www.wellcome.ac.uk/darwin200.

This manual contains Teacher notes (pages 3 to 14), Technician notes (pages 15 to 18) and Student notes (pages 19 to 22). We recommend that teachers read all sections and photocopy and distribute accordingly.

NOW GET STARTED WITH BRINE DATE...

Brine Date Teacher Notes

Equipment and materials required

The following items are provided in the Survival Rivals: Brine Date kit:

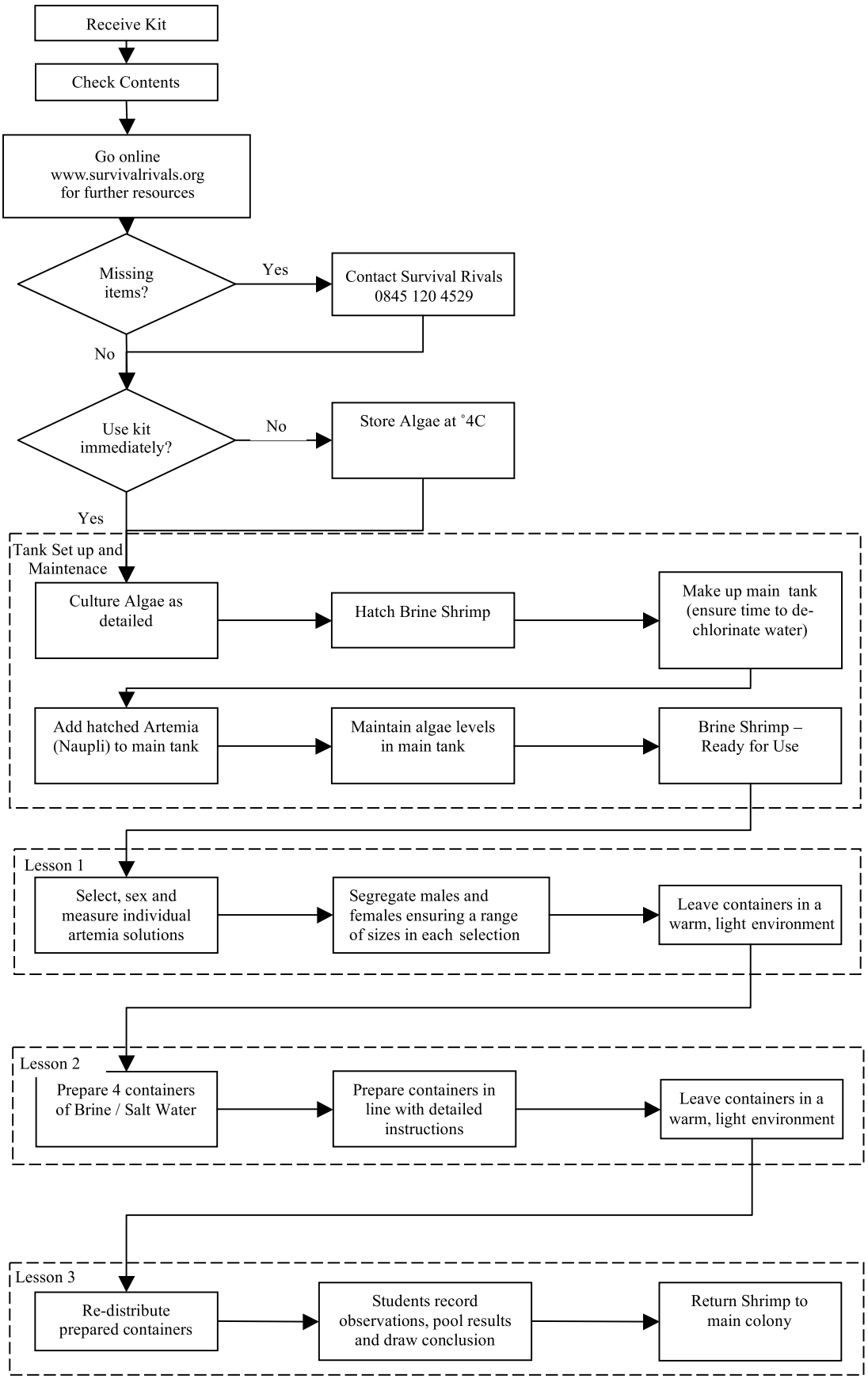
Item No	✓	Description	Quantity
1	☐	*Artemia* Eggs	20mL
2	☐	*Artemia* Food - Liquizell	50mL
3	☐	Liquid Fertiliser	175mL
4	☐	Sea Salt	1kg
5	☐	Pastettes 5mL	30
6	☐	Book - '99% Ape'	1
7	☐	Tank - Plastic box	1
8	☐	Algae	30mL
9	☐	Grit	100g
10	☐	Acetate grid	15

If you have any items missing from your kit, please call **0845 120 4529**

What's not in the kit, but which you may also need:

- Larger Tank
- Light bank or additional light source
- Aquarium heater (if your lab conditions are below 20-25°C)
- Washed sand (see below)

Detailed lesson-by-lesson instructions



Introduction

Sexual selection

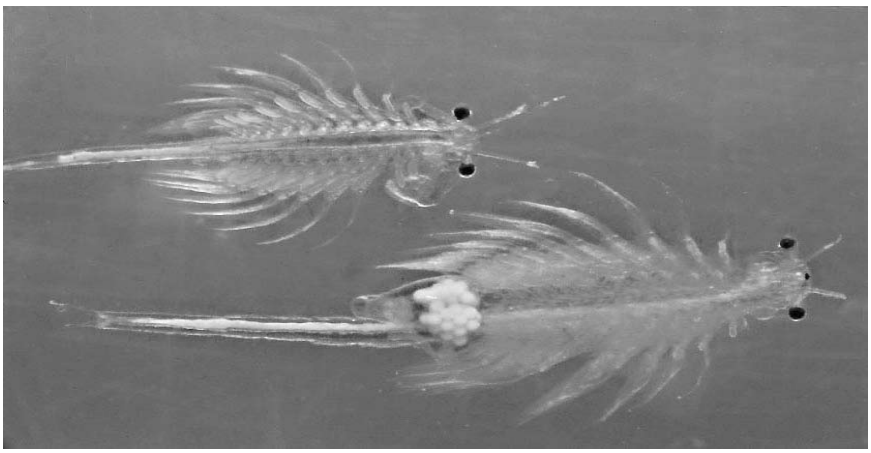
Charles Darwin proposed two principal ideas to account for the diversity of life on earth. While 'The Origin of Species' was mostly concerned with natural selection, Darwin noted briefly in that book that sexual selection by mates was also a force for evolutionary change. He wrote:

'and this leads me to say a few words on what I call Sexual Selection. This depends, not on the struggle for existence, but on a struggle between the males for the possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring.' [1]

By 1871 Darwin had expanded those few words to take up the greater part of his second book on evolution: The Descent of Man, and Selection in Relation to Sex. Sexual selection, he suggested, was largely responsible for human diversity — a conclusion with which many of today's modern evolutionary biologists would agree.

Can the phenomenon of sexual selection be seen and investigated further by children in the classroom? Brine shrimps, *Artemia fransiscana*, kept in a brightly-illuminated aquarium provide an easily-observed and sustainable ecosystem for classroom-based ecological and behavioural studies by students at Key Stages 3 and 4 or Scottish Stages S1–S4. [2]

When students observe brine shrimps they will see the animals swimming singly or together. They will quickly distinguish between the sexes, as mature animals will swim together, apparently in 'mating' pairs.



Male and female brine shrimps.

The female (with eggs) can readily be identified. Males have large 'claspers' (modified second antennae) which are used for holding females securely; the females have no claspers.

These mate-guarding males with the females to which they are clasped are either yet to mate with each other or will have already mated, The females are being guarded by the males to prevent other matings. The females may also equally choose the males they are paired with*. Students will find this social arrangement inherently interesting to investigate.

In the classroom therefore, the preamble to this investigation is initially observation see 'Student Notes' (pages 19 to 22). This is what Darwin the naturalist would have done. Then, through a teacher-led discussion, students may suggest ways of investigating this phenomenon of mate-guarding. The proposal here is that this discussion will generate hypotheses that these students may test. For example, one of the hypotheses may be that larger females pair with larger males. This may be tested experimentally by students; (a) setting up pair-choice experiments; and (b) measuring the relative sizes of paired individuals.

References

[1] On the Origin of Species, First edition, 1859, Chapter IV.
[2] Dockery and Tomkins, 2000.

Further links with Darwin

Coincidentally, Darwin had observed brine shrimps while in South America. In 'The Voyage of HMS Beagle' he had written:

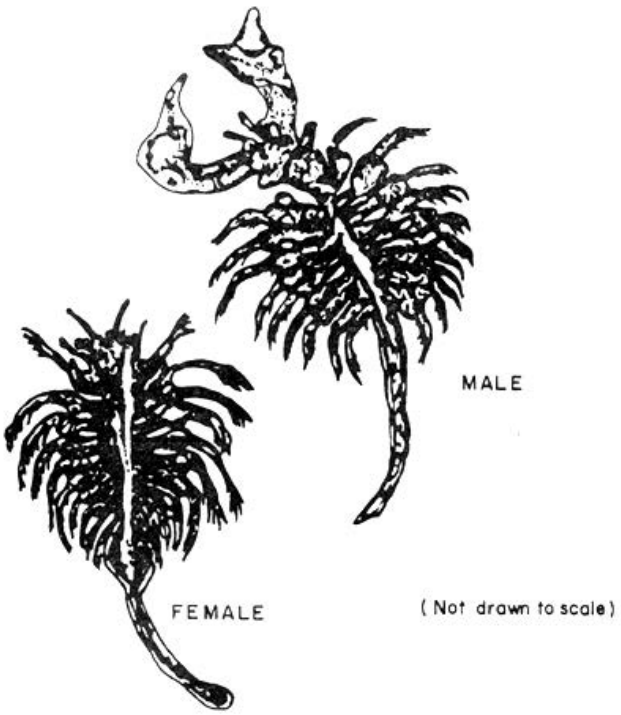
Flamingos in considerable numbers inhabit this lake, and breed here; throughout Patagonia, in Northern Chile, and at the Galapagos Islands, I met with these birds wherever there were lakes of brine. I saw them here wading about in search of food — probably for the worms which burrow in the mud; and these latter probably feed on infusoria [protozoa] or confervae [algae]. Thus we have a little living world within itself adapted to these inland lakes of brine. A minute crustaceous animal (Cancer salinus)** is said to live in countless numbers in the brine-pans at Lymington***: but only in those in which the fluid has attained, from evaporation, considerable strength — namely, about a quarter of a pound of salt to a pint of water. Well may we affirm, that every part of the world is habitable! Whether lakes of brine, or those subterranean ones hidden beneath volcanic mountains — warm mineral springs — the wide expanse and depths of the ocean — the upper regions of the atmosphere, and even the surface of perpetual snow — all support organic beings.
[The Voyage of HMS Beagle, Third edition, 1860, Chapter IV]

Notes

* In later editions of The Origin of Species, Darwin suggested that females may also play a role in mate selection. The following quotation is from the sixth edition of 1882:
'This form of selection depends, not on a struggle for existence in relation to other organic beings or to external conditions, but on a struggle between the individuals of one sex, generally the males, for the possession of the other sex.'

** Darwin's nomenclature was out-of-date. In 1758 Linnaeus had named brine shrimps Cancer salinus, but they were reclassified as *Artemia salina* by Leach in 1819. A. salina is found in southern Europe.

*** The first scientific description of brine shrimps was written by Schlösser in 1755–6, who observed them in salt pans at Lymington in Hampshire. Brine shrimps are no longer found in the UK.



Brine shrimps by Schlösser, 1755–6. Left: female on which the eggs can be seen; right: male, showing the 'claspers' used during courtship and mating.

Background information on brine shrimps (*Artemia franciscana*) and their ecology

For those unfamiliar with brine shrimps, this section, taken from Dockery and Tomkins (2000), provides background information to the species under study.

Brine shrimps are examples of invertebrates with jointed legs (arthropods) and are classified in the sub-Phylum Crustacea. There are many species of brine shrimp, most being in the genus *Artemia*, with a global distribution.

As their common name implies they are found in salt lakes and brine ponds. These environments often dry up completely in the hottest season. The ecological conditions in which these populations occur are often extreme (for example, the salinity of the water can exceed 280g salts per litre — sea water is 35g per litre), and thus only a small variety of algae (such as *Dunaliella*) and bacteria (such as *Halobacterium*) can survive. As a consequence, blooms of algal species occur and the more usually green water may occasionally appear red because of the formation of ß-carotene in the cells. Very few invertebrates can tolerate these conditions but *Artemia* has successfully adapted to such extreme environments. As a consequence, and because there are no fish predators, their numbers are often very high. The natural predators of the brine shrimp are birds like flamingos and avocets that fly in when the shrimps are abundant.

At the end of the year in the Great Salt Lake, Utah, USA, the salt water takes on a brownish colour, due to very small brown particles appearing at the water surface. These small particles are the inactive dry egg cysts of *Artemia franciscana*. These egg cysts drift on the wind and in the waves to the shore in huge numbers. They are collected from the lake shore commercially to provide the dried brine shrimp eggs that are sold in aquarists and pet shops in Britain and elsewhere.

The egg cysts remain dormant as long as they are dry. They contain a protective polysaccharide called trehalose which preserves life in a desiccated state. Trehalose is also found in the seeds and tissues of desert plants resistant to severe drought.

In Spring, the over-wintering egg cysts hatch at the first rains (April). The cyst hydrates and the shrimp embryo becomes active. Some hours later the cyst bursts and the embryo emerges, surrounded by only the hatching membrane. At this stage the single eye of the nauplius larva is visible. Within a few hours the antennae and mandibles start moving and then the nauplius begins swimming. This first stage larva is orange-brown in colour.

The larva goes through about 15 moults and as it does so the trunk and abdomen lengthen. At first, the antennae collect food particles and at this stage lateral compound eyes first develop. Soon the middle instars begin feeding with their paired legs. Shrimps, from this stage on, swim on their backs with their legs on the uppermost side.

The eleven pairs of legs are used for three purposes, namely, as filters, for locomotion and as gills. From the tenth instar on, significant sexual changes occur. The most marked of these is that the second pair of male antennae develop into large, hooked claspers which will be used to seize and hold on to the female during the mate-guarding phase of reproductive behaviour.

The adult animals are 8–14mm long when fully-grown. The males have a translucent body, large claspers and a paired penis may be seen in the posterior part of the trunk region. The females are brown/red in colour and have a brood-pouch (or uterus) which receives ripe oocytes from the ovaries via two oviducts. The sexes differ in colour, older females are darker brown whilst older males accumulate a blue-green pigment.

The pre-copulation or guarding phase is initiated by the male which grasps the female with his claspers between the uterus and the last pair of thoracopods. In this 'riding position' the two animals can swim around for many hours, even days. When copulation occurs it is a fast reflex. The male abdomen is bent forward and one of the pair of penises is inserted into the aperture of the uterus. The fertilised eggs develop in two possible ways. The first generation of eggs (in the Great Salt Lake this is in May or June) often develop immediately into free-swimming nauplii when released by the female. This is termed ovoviviparous reproduction.

The Great Salt Lake in Utah.

This May/June population goes on to produce a larger proportion of much browner eggs that do not hatch immediately. These brown egg cysts have a thick shell and are dormant until stimulated to continue development by a change in environmental conditions. Ovoviviparity is common in booming populations, whilst a population in which nutrient levels or other necessary conditions are declining will produce more of the dormant egg cysts. There may be up to five generations in one year in the Great Salt Lake. Population densities in the wild may exceed 10 shrimps to the litre, levels that may easily be exceeded in laboratory culture.

Naupilar larvae which hatch from egg cysts in 48 hours and become mature adults in two to three weeks.

Growth rates in the wild are affected by temperature and nutrition. At 25°C with optimal nutrients, adults are sexually mature in 14 days and achieve full size in 26 days. As salinity increases, the growth rate and final size decrease. Brine shrimps will live in more than 20% salt but at 18% their growth rate is half that at 3.5%. This is due in part to the energy costs of salt secretion (see below). At low salinities, < 3.5% salt, *Artemia* grows well but competes less well with other more freshwater species and does not thrive as well as in a more salty environment.

Physiologically, *Artemia* is a hypo-osmotic regulator in saline solutions. This means that the animal's blood is hypotonic to the medium outside (having less dissolved solutes) and that water is therefore lost by osmosis through the outer integument. To prevent desiccation the continual flow of ingested water through the gut is believed to be the source of water uptake but as this water is also salty, powerful salt secretion from the gill surfaces occurs at the same time to compensate for the salt inadvertently gained. The pumping of ions across membranes is energy demanding and hence although the saline environment is one where growth can be rapid there is a cost to living there if the medium becomes too salty.

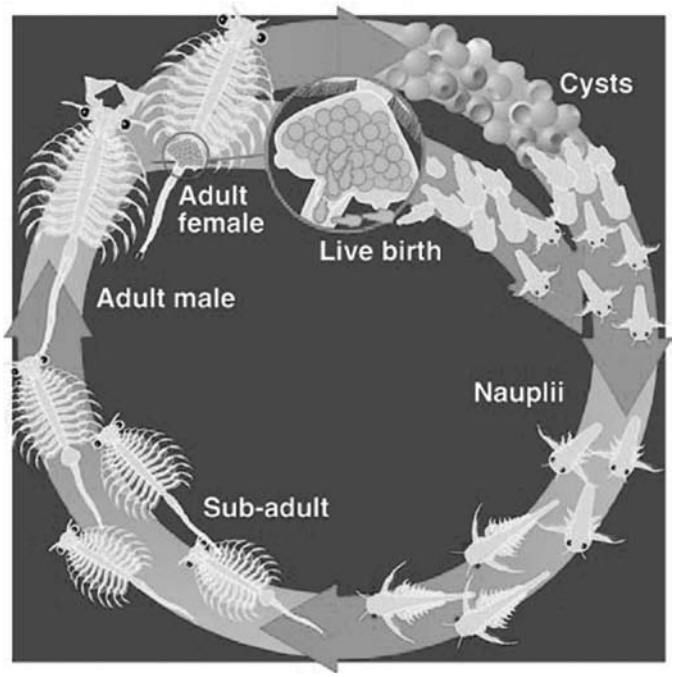


Diagram shwoing *Artemia* lifecycle, from US Geological Survey

About ten species of brine shrimp are recognised. *A. franciscana* is the San Francisco brine shrimp, which is found in the Great Salt Lake of Utah, which is a major commercial source of brine shrimp egg cysts. The other main commercial producers are in China and Russia.

Key information on brine shrimp reproduction

The brine shrimp sexes differ in size, form and colour. Mature females are on average 1–2mm longer than males. Mature males have huge claspers (2nd antennae) — these can be seen in Schlösser's drawings. Older females are darker brown whilst older males are sometimes a blue-green.

Brine shrimps certainly exercise mate choice. Large males may be seen to encounter more females as they swim than smaller males. There may be an element of male-male competition for females. This is indicated by the very large claspers, which are perhaps important in securing females or, additionally, they may be sexual ornaments.

There is also, however, good evidence for female mate-choice operating as well, as large females seem reluctant to accept pairing with smaller males. This choice results in positive assortative mating, that is, larger females pair with larger males by choice. Larger female brine shrimps produce more eggs than smaller females.

The pre-copulation or guarding-phase is initiated by the male who grasps the female with his claspers between the uterus opening and the last pair of limbs. In this 'riding position' the two animals can swim around for many hours, even days. When copulation occurs it is a fast reflex. The female sexual cycle is about six days, from one ovulation to the next. Pairs of shrimps swim measurably faster than single female shrimps. Larger pairs swim faster than smaller pairs.

Mate-guarding in *Artemia*: female above, male below. This process precedes and follows mating.

One may postulate that it is to the advantage of a male to mate with a larger female brine shrimp as she will produce more offspring for which he is the father, and therefore pass on his DNA to the next generation. It is to the advantage of a female to be mated by a larger male as he may add to the propulsion of the pair and the capacity of the female to provision her eggs with more food.

The cultivation of brine shrimps in schools

We strongly recommend that teachers read the Technician's Guide (pages 15 to 18)**.**

It is possible to maintain permanent brine shrimp cultures in schools. This is recommended for long term culture. If you want to run a culture down to a dormant state (e.g. over the summer holidays), merely allow the water to evaporate fully from July onwards. Egg-cysts are virtually immortal. Adult shrimps will regularly and naturally die in the container after some weeks. In this case their remains will quickly rot, and be further decomposed by bacteria. Nutrients are returned to the ecosystem through plant cell uptake in fresh algal growth. It is a living ecosystem. At the beginning of the Autumn term, add water to the tank to start it off again. Equally it could be allowed to dry up through the winter. It will revive again in the spring if the right amount of water is added.

Notes:
Light is essential for *Artemia* to hatch. A light-sensitive enzyme converts trehalose in the cyst to glycerol. Glycerol is hygroscopic, so water enters the cyst, bursting the membrane and releasing the larva.

Artemia tolerate salinities from 10 ppt to saturation, although growth is restricted at higher salinities. Above 100 ppt no predators or food competitors can survive, which results in a simple ecosystem of *Artemia* and algae.

Brine shrimps will become sexually mature in 12 days at 28°C and 18–21 days at 20°C. The lower lethal temperature for brine shrimps is 0°C; the upper lethal temperature is 38°C.

The practical investigations, see also student notes (pages 19 to 22)

Initial observation of brine shrimps by pupils is important. Rear the brine shrimps in the classroom for the investigations that are to be done later.

Two investigations beyond the important initial student observation are suggested:

(1) setting up a mating-pair choice experiment,
(2) measuring the relative sizes of mate-guarding pairs.

These require similar apparatus. All students should make themselves familiar with the animals. Teachers should then induct students into the capture and safe transfer of animals. It is suggested that some days before the mate choice experiment the class learn to identify male and female shrimps. In the first experiment, the class could then set up two small tanks with segregated sexes of up to 100 females and 100 males of all sizes. It would be best to do this some 2–4 days before the experiment. This will test students' sexing skills! Handling of brine shrimps is done best by scooping from the large tank, sieving of shrimps (using a kitchen sieve) or by individual capture using a wide-mouthed pipette.

Initial discussion questions

Discussion with pupils approaching this practical activity might be some audio-visual input on animal courtship behaviour. This might centre round (a) how male deer contest with each other for females (intra-sexual competition) and (b) how peahens choose those peacocks with the most ornate displays (inter-sexual selection). In brine shrimps there is ample evidence that both males and females are able to express a mate choice.

Some learning outcomes for students

The outcomes from this exercise will be two-fold. Firstly, students will learn some of the practical skills of scientific inquiry and the ways in which scientists use information. Secondly, the investigation will support a scientific understanding of Darwin's ideas and support students' knowledge of and interest in living things.

Scientific enquiry

Students will be expected to listen carefully to the challenge before they plan their investigation. In (1) they will need to think about experimental design and practise fine manipulative skills (using a pipette). In (2) they will need to measure accurately, then record their measurements on a spreadsheet, process the data by generating a scatter plot and interpret the data by inferring a relationship from the results. They will be expected to communicate their conclusions to the class and their teacher and finally evaluate their method and findings. Pupils will need to act responsibly while handling living organisms.

Scientific understanding

Pupils will need to understand something of fair testing. They will need to know that they are working with an ecosystem in which the crustacea are herbivores feeding on algae and bacteria. This introduces the essentially Darwinian outcome of the adaptation of organisms. The salt-lake ecosystem is unusual in that there are no fish predators, but they will learn that birds, like avocets and flamingos do feed on the shrimps. This understanding requires a concept of energy flow and material transfer between trophic levels.

They will need to understand from observation that brine shrimps exhibit sexual dimorphism and pair, male with female, for mating. They will realise that natural selection operates in this ecosystem as brine shrimps are competing with each other for food. A special case of sexual selection also operates. Males are competing with each other for larger females that produce more offspring. Females may be selecting male partners that are larger and stronger and help them to swim faster and so gather more food for more eggs. Natural selection should thus favour larger shrimps. However, larger shrimps will be captured more easily from the water by greater flamingos. Different selection pressures operate on a living organism in its environment. Near perfect adaptation is the result of selection pressures.

Additional sources of information

- Captain's Universe video clips, photographs, culture details etc www.captain.at/artemia
- *Artemia* reference centre, University of Ghent www.aquaculture.ugent.be
- Brine shrimp ecology by Michael Dockery and Stephen Tomkins (2000)
- British Ecological Society. ISBN: 1 900579 10 3. A handbook of investigations for schools, available from the British Ecological Society's web site: www.britishecologicalsociety.org

Health and safety

There are no health and safety issues associated with this work other than a possible allergy to shellfish. However, schools will need to do their own risk assessment. Advice for this may be had from;

- CLEAPSS, The Gardiner Building, Brunel Science Park, Kingston Lane, Uxbridge UB8 3PQ
  T 01895 251496 - Email science@cleapss.org.uk – Web www.cleapss.org.uk
- SSERC, 2, Pitreavie Court, South Pitreavie Business Park, Dunfermline KY11 8UB
  T 01383 626070 - Email sts@sserc.org.uk - Web www.sserc.org.uk

Other issues

Animal welfare

Artemia are living organisms and they should be handled responsibly by students and teachers alike. This activity presents teachers with an opportunity to discuss the issues associated with the use of animals in scientific research.

Curriculum Links

The National Curriculum for England, Wales and Northern Ireland now has relatively little specified biology content. Consequently this work can be used to augment existing studies with relative freedom. There is a requirement for data-handling and good potential for the effective and appropriate use of ICT.

Almost all of the statements in the 'How science works' section of the National Curriculum for Key Stage 4 Science are relevant. The National Curriculum also includes the following relevant statements under 'Organisms and health':

- organisms are interdependent and adapted to their environments
- variation within species can lead to evolutionary changes and similarities and differences between species can be measured and classified

Specific syllabus links:

England

AQA

AQA GCSE Biology unit 11.5, GCSE Science A Unit 1B, GCSE Science B
Animals often compete with each other for food, mates and territory.
Organisms have features (adaptations) which enable them to survive in the conditions in which they normally live.
Animals and plants may be adapted for survival in the conditions where they normally live e.g. deserts, the Arctic.

AQA GCSE Biology unit 12.5, Additional Science
In a stable community, the processes which remove materials are balanced by processes which return materials. The materials are constantly cycled.

EDEXCEL

Edexcel GCSE Science unit B1a
Students will be assessed on their ability to:
describe how organisms in an ecosystem compete with each other for resources.
demonstrate an understanding of the principles of natural selection, to include:
how individuals within a species can have characteristics that promote more successful reproduction (survival of the fittest).

Edexcel GCSE Additional Science unit B2
Students will be assessed on their ability to:
explore the principles of interdependence, competition and predation and explain how these factors influence the distribution and population sizes of organisms in a given terrestrial or aquatic environment.

Edexcel GCSE Biology unit B3
Reproductive behaviours:
sexual reproduction requires the finding and selection of a suitable mate, and some can involve courting behaviour.

OCR

OCR GCSE Gateway Science Suite Biology Module B, Science Module B
Research species that do not appear to have evolved but have stayed as they are for millions of years, so called 'living fossils', e.g. coelacanth, crocodiles, sharks, and suggest why they do not appear to have changed.Explain that animals and plants that are better adapted to their environment are more likely to survive; this is called natural selection.

OCR 21ST GCSE CENTURY SCIENCE BIOLOGY A MODULE B3

Understand the process of natural selection in terms of variation, competition, increased chance of survival and reproduction.
When provided with information about alternative views on the origin of life on Earth, or the evolutionary process:
can identify statements which are data and statements which are (all or part of) an explanation.
Can recognise data or observations that are accounted for by, (or conflict with), an explanation.
Can identify imagination and creativity in the development of an explanation.
Can justify accepting or rejecting a proposed explanation on the grounds that it:
accounts for observations.

Wales

WJEC

GCSE Science Biology1
Variation
Candidates should:
examine the variation in height/length in individuals of the same species by collecting and analysing data.

Scotland

CURRICULUM FOR EXCELLENCE

The entire Scottish 3-18 curriculum is being reviewed at time of print.
Please see http://www.ltscotland.org.uk/curriculumforexcellence/outcomes/science/index.asp for information. The Survival Rivals website will be updated to show the specific links once the new Scottish curriculum is published. The cover paper from the new draft states that "The new draft experiences and outcomes are designed to allow teachers to 'raise the bar', permitting greater depth and challenging young people to be ambitious in their learning, whilst ensuring that learning is enjoyable. They are designed to encourage a range of learning and teaching styles, whilst at the same time actively encouraging participation and the development of a range of skills - particularly important given the recognition given to schools' contribution to skills development in the Scottish Government's recently published Skills Strategy." (http://www.ltscotland.org.uk/Images/overarching_cover_paper_tcm4-442673.pdf). Survival Rivals will provide teachers with resources to enable them to achieve this.

Northern Ireland

CCEA GCSE BIOLOGY UNIT 3.2 ENVIRONMENT

Learn that living organisms are adapted to survive in the environment, for example, adaptations to life on land, and in water.

CCEA GCSE BIOLOGY UNIT3.3 VARIATION

CCEA GCSE Double Award Science Unit 3.2 Environment, Reproduction and Genetics
Find out that variation can be measured in living organisms.
Understand how variation and selection may lead to evolution or extinction, including:
natural selection as variation within phenotypes and competition for resources leading to differential survival;
the implications of natural selection for the concept of evolution as a continuing process

CCEA GCSE APPLIED SCIENCE

You need to be able to:
present data in tables, bar charts, histograms, pictograms, pie charts, graphs and other visual images, as appropriate;
carry out simple numerical calculations;
analyse and interpret your results;
evaluate your investigation and suggest improvements.

Post-2009 legacy

Because this work is relatively inexpensive and the resources required are readily available, it has the potential to be repeated in schools following the Darwin anniversary year. The legacy will be a novel protocol covering aspects of animal behaviour and evolution that has not hitherto formed part of the secondary school practical science repertoire. You can buy many of the items supplied in this kit from Philip Harris at www.philipharris.co.uk.

Technicians Notes

NB (Place your algal culture in the refrigerator at 4ºC on opening this package)

Equipment and materials required

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If you have any items missing from your kit, please call **0845 120 4529**

What's not in the kit, but which you may also need:

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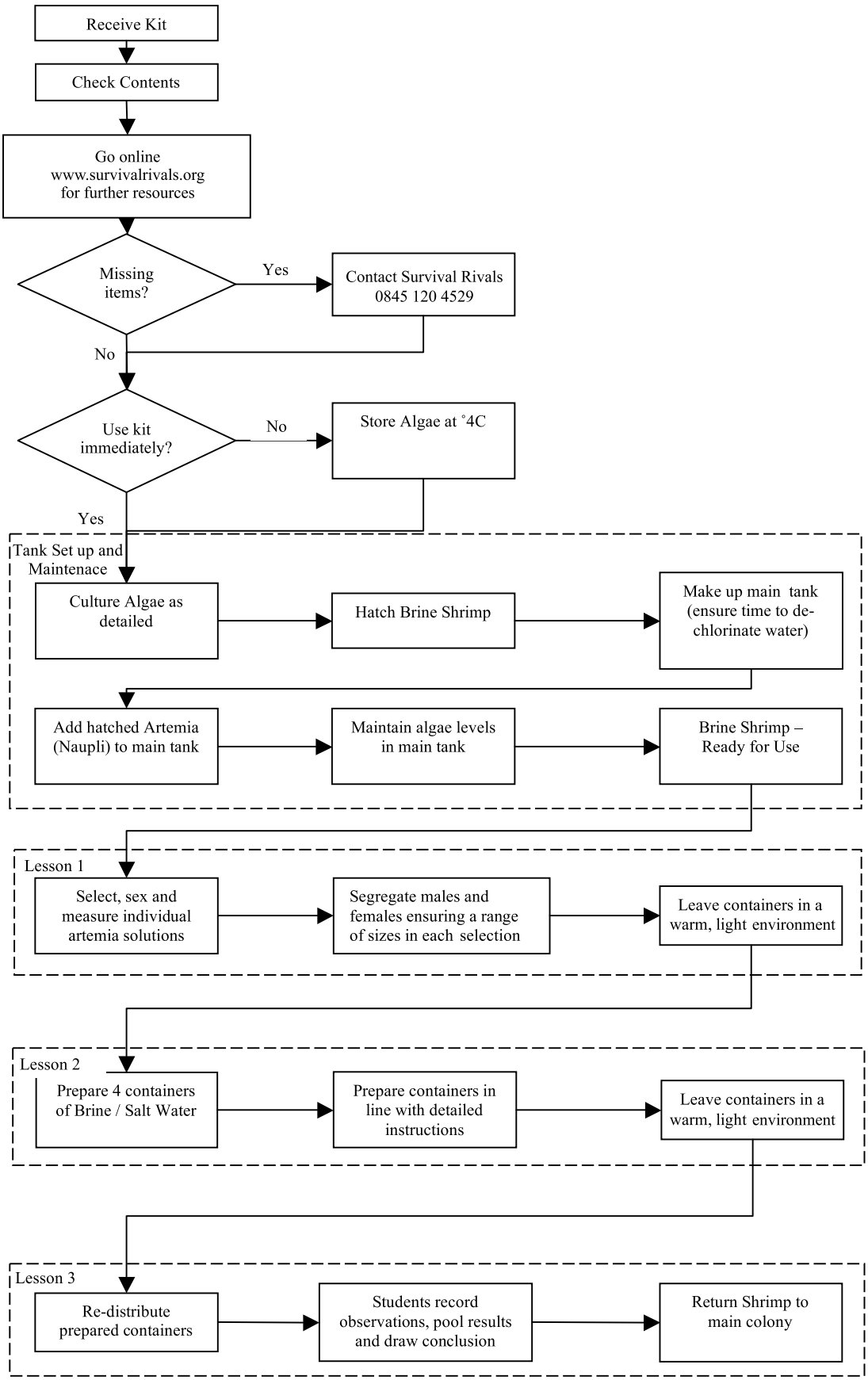
- Light bank or additional light source
- Aquarium heater (if your lab conditions are below 20-25°C)
- Washed sand (see below)

Detailed lesson-by-lesson instructions



Introduction

Growing brine shrimps

Brine shrimps are relatively easy to grow and maintain as long as their basic ecological requirements are met. The brine shrimp eggs in this kit (*Artemia franciscana*) have been collected from the Great Salt Lake, Utah, USA. Brine shrimps feed on unicellular algae and microbial matter suspended in the salt water. For the shrimps to survive they need suspended food, light (for algal photosynthesis), warmth (20-28ºC) and salt water (3.5% sea salt). The minimum time from hatching to adulthood is 3-4 weeks, so the system must be set up well in advance (see the suggested time plan below).

Setting up

The Algal Culture

As soon as you receive the kit, you will need to culture the algae* so there will be enough food for the larvae when they hatch. (* On opening this package, place your algal culture in the refrigerator at 4ºC, until you need it).

1. Make up 5 litres of 3.5% salt water using the sea salt and de-chlorinated tap water (leave the water exposed to the air overnight so the chlorine evaporates)
2. Pour this into the tank provided to about ¾ full. Add the algal culture.
3. Place the tank next to a good source of light (light bank, bench lamp, even the window sill in the summer time) and keep as warm as possible (20 - 25°C)
4. Add a drop of plant fertiliser per litre.
5. In about a week you should have a green suspension of algae. Check your culture by placing a drop on a microscope slide and view under medium power magnification. You should see lots of small motile algae.
6. Use this culture to feed your young brine shrimps and to provide algae for the main tank.

Should you require some more algae for your brine shrimp colony, please contact Survival Rivals on **0845 120 4529**.

The Brine Shrimp tank

A successful tank should be set up where it is warm (20-25ºC) and well lit by lamps, by a 'light bank' or by sunlight from a south-facing window. One fifteen litre tank will supply more than 50 brine shrimp pairs – enough for one class. If students are careful, the brine shrimps may be returned to the tank after a lesson for future use and to maintain the culture. Sand of any kind (which is not in the kit) will do as long as it is well washed (see below). Brine shrimps graze the sand for algae and also lay their eggs in it. Oyster grit (in the kit) is added to provide extra calcium for the brine shrimps. To calculate tank volume (in litres) multiply the length x width x depth (in cm) and divide total by 1000.

1. Wash about 2kg of sand, along with the oyster grit from the kit, in a few changes of tap water to clean it and to remove fine particles, and then put it all into the tank you are going to use for the brine shrimps. It should fully cover the floor of the tank.
2. Make up some more salt water (3.5% sea salt), enough to half fill the tank.
3. Add one drop of Liquizell per 10 litres of water.

Once the sand has settled, the tank is ready for the algae and brine shrimp larvae (nauplii).

Hatching the brine shrimps

The optimum temperature to hatch, and grow brine shrimps, is 25-28ºC. To a one litre beaker half full with salt water add a spatula tip of the Brine Shrimp eggs. Place it near a lamp and check that the temperature is 25-28ºC. In 1-2 days the eggs will hatch. Add a few drops of the Liquizell feed. Although the larvae will grow on this for the first stages of their development, their preferred food is algae, so add about 200ml of your algal suspension every few days. When the nauplii are clearly visible (about 1-2mm in length), pour this culture gently into your brine main tank along with your algae. Brine shrimps will grow to maturity in about three weeks, when they will be observed to be either male or female (see pupil notes) and starting to pair up.

Timing

Week 1: grow algae in salt water.
Week 2: hatch eggs and make up main tank ready to receive nauplii and algae.
Week 3: place nauplii and algae into main tank.
Week 4: onwards: add algae to growing brine shrimps in tank.
Week 5: brine shrimps ready for class use

Maintaining the tank

It is important to understand that a Brine Shrimp tank (kept warm and well lit) can be kept as a self-sustaining ecosystem. The algae will grow in the tank. They will supply food for the brine shrimps as they grow. You only need to add a small amount of plant fertiliser to sustain the algae. Stirring the tank every few days helps to recycle minerals and encourage shrimp growth. Add de-chlorinated tap water to make up for volumes lost by evaporation. Once there are adult brine shrimps producing eggs you will have an sustainable population.

Further information

Detailed information on growing and keeping brine shrimps can be found at www.britishecologicalsociety.org. Brine shrimp ecology – A classroom based introduction to Ecology p96 – 99. There are also very useful A4 diagrams of male and female brine shrimps.

Notes

The algae must be kept in the fridge before culturing.
When de-chlorinating tap water, use a conical flask and place kitchen roll in the neck to prevent spores entering.
Instead of adding all your algal culture to the main tank, you may wish to keep some in reserve, in culture, to feed your brine shrimps. You can judge how healthy your brine shrimp/algae community is by the colour of the suspension. Almost clear - not enough algae: light green – just right; very green – you have an algal bloom!
Gently scrape the walls of your main tank once to week. Brine shrimps lay their eggs at the surface as well as in the sand, so this removes them. Also algae adheres to the sides and brine shrimps have difficulty feeding on this.

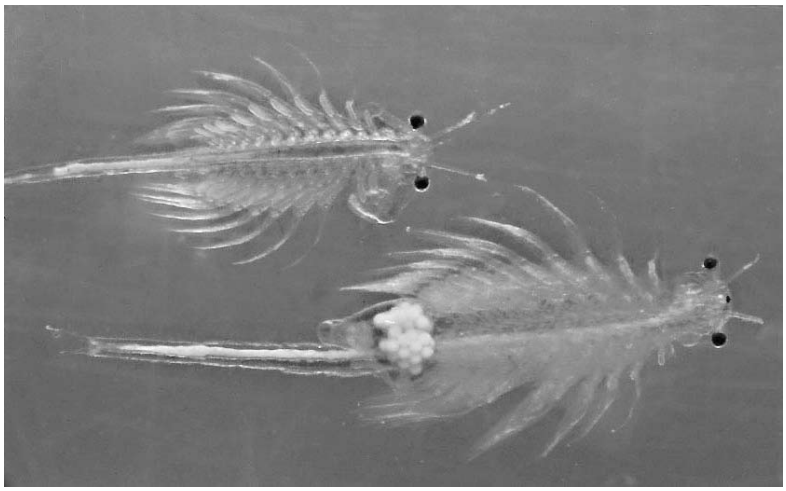
Student Notes

Introduction

Humans are quite choosey about who they fancy. Are other animals any different?

Charles Darwin wondered whether the different appearances of males and females was something to do with which partners the animals themselves chose to mate with. We now know, for example, that female deer are attracted to males with loud bellows and large antlers. The males also compete with each other in contests by fighting and bellowing. Females will be more likely to accept males to mate with that have won a male-male contest. Darwin suggested that such 'sexual selection' was important in exaggerating some of the characteristic differences between males and females and in ensuring greater survival chances for their offspring. Is the magnificent tail of a peacock selected for by their females? This was one of Darwin's suggestions.

This investigation with brine shrimps seeks to discover whether these animals make choices between their partners. Brine shrimps have clear differences between their sexes and it is easy to tell which ones mate with which as they form semi-permanent pairs.



Males are distinguished by their 'claspers'. These are enlarged antennae close to the head, enabling them to hold on to the female. Females do not have these. Mature females have brood pouches containing eggs immediately below their last antennae.

Brine shrimps live in salt water lakes where they filter feed algae from the water. Begin your investigation by observing brine shrimps in your classroom for long enough to answer the following questions and then think about what they mean.

1. Are all the brine shrimps the same size?
2. Do they all swim singly or are some in pairs (this behaviour is described as mate-guarding)?
3. Can you distinguish males from females?
4. In a mate-guarding pair which sex is in front and which behind?
5. Females are distinguished by egg sacs halfway down the body. Are all females egg sacs the same size? If not why might this be?
6. What are the characteristics of males? Their second pair of antennae is greatly enlarged and are called 'claspers'. What is their function?
7. Make a hypothesis as to why the males and females are clasped together for about three days around the time of mating. It is not only that they mate during this time!

Now that you have made your initial observations there are two further experimental investigations that you can make. Discuss with your teacher which would be the best one to help you discover about brine shrimp mate choice.

Approach 1. Mating pair choice experiment

One possible hypothesis that you could test is that brine shrimps choose their mates by size.

Does the size of a shrimp matter (to another brine shrimp) or is their mate choice random?

If there is sexual selection, is it the males or the females which make that choice?

Handling shrimps carefully

Brine shrimps may be lifted from one water container to another with a wide-mouthed pipette. Squeeze the bulb and then as you chase the animal release the bulb again to capture it. When it is sucked up, check that it is there, then release it gently by squeezing the bulb again.

First Lesson

Equipment and materials required (for each working group)

- A large container of brine shrimps of all sizes
- Two smaller beakers or containers, marked 'males' and 'females' each containing salt water with algae
- A wide-mouthed pipette
- A magnifier
- A petri dish

Method
1. From your large container capture single brine shrimps (not pairs) and place them in your petri dish.
2. Carefully examine the single brine shrimps with the magnifier and segregate them into two containers by gender, male brine shrimps into one container and females into another.
3. Ensure that you have a range of sizes of males and females in your two containers. Place these where they can be kept warm and well lit until you can continue your investigation.
4. Ideally, you should leave the males and females separate for at least two days, so females become more receptive to males.

Second lesson

Equipment and materials required (for each working group)
- Your two beakers or containers, marked 'males' and 'females'
- A wide-mouthed pipette
- Four containers (beakers, cups or bottles) each containing salt solution with algae
- A marker pen

Method
1. First check your males and females. Have any of them paired up? If so, did you sex them correctly?!
2. Prepare four containers of salt water and algae. Label them 1, 2, 3 and 4 and with your group name.
3. Study the tables below. What results might they show?
4. Select brine shrimps for your experiments on the basis of size and gender and gently put them into the four containers. Make sure that there is a good difference in size between the animals so that you can recognize each of them again.
5. Make a note as to whether a female has an egg sac or not when you put her in a container. Record her size and colour.
6. If you have more containers and brine shrimps the more replicates of this experiment you can set up the better. Place all your experimental containers together, where they are well lit and warm.

Experiment ONE	Male	Female	Female egg sac size and colour
Larger animal	1	1	
Smaller Animal	1		

Experiment TWO	Male	Female	Female egg sac size and colour
Larger animal	1		
Smaller Animal	1	1	

Experiment THREE	Male	Female	Female egg sac size and colour
Larger animal	1	1	
Smaller Animal		1	

Experiment FOUR	Male	Female	Female egg sac size and colour
Larger animal		1	
Smaller Animal	1	1	

Third Lesson (2-4 days after setting up)

Equipment and materials required (for each working group)

Your four experimental containers each containing three shrimps.

Method

1. Record what choices if any were made by your shrimps.
2. Pool the results from your class and then discuss them together.

Discussion of the results

These experiments raise at least the following questions:-

1. Do all the experiments result in shrimps pairing up?
2. If not, why not?
3. Do the larger males more easily obtain a partner than smaller males?
4. Do smaller males more easily obtain smaller female partners than larger males do?
5. Do larger females pair up with larger males more readily than the smaller females do?
6. Do larger females pair up with smaller males more readily than the smaller females do?
7. If a female is carrying a large egg sac does it affect her pairing or not?
8. Does the colour of the female's egg sac affect whether she pairs or not?

Further things to think about:-

1. Given that larger females produce more eggs, what would be the advantage to a male of pairing with a larger female?
2. Brine shrimps feed on algae and bacteria in the water. What is this method of feeding called?
3. Given that a larger male propels a female along faster and further in the water what advantage might a larger male be to a female?
4. If brine shrimps do pair by size, which pairs will be most successful in rearing more offspring?
5. Why do females with large egg sacs sometimes not seem to mate?

Approach 2: Measuring the relative sizes of mate-guarding pairs

Another way of testing whether brine shrimps pair up with respect to size is to measure the pairs that have chosen each other!

Are the larger males paired to the larger females? Are the smaller males paired to the smaller females? Or has size nothing to do with brine shrimp mate choice? If they do pair by size, the pattern (random or non-random) should be clear on a scattergraph of all the results.

Procedure

Your teacher will have provided the class with a selection of numbered containers, each with a mate-guarding pair of brine shrimps.

Equipment and materials required (for each working group)

- A wide-mouthed pipette
- A magnifier
- A small glass slide (CAUTION: beware of sharp edges)
- An acetate grid measuring in millimetres

Method (for each working group)

1. Place your glass slide over the acetate grid so that the length of the brine shrimps may be measured.
2. Collect one of the numbered containers and, with the wide-mouthed pipette, transfer the pair of brine shrimps on to the glass slide carefully, with a few drops of salt water. You may find they take a minute or two to settle down.
3. Quickly measure the length of the male (at the back of the pair) and the length of the female (at the front of the pair). Measure from the eyes (little dots) at the front to the very tip of the tail. This must be done with accuracy to the nearest millimetre. Do not worry if the brine shrimp pair separate during this procedure! Just measure each of them individually.
4. After measuring, return the brine shrimps to the container.
5. Record measurements for the male and female of a pair in a table.
6. Repeat with each pair of shrimps in turn.
7. Make a scattergraph of your results.

Discussion of results

1. Have you managed to record the length of males and females in each pair accurately to the nearest millimetre? How confident are you in your measurements?
2. Is one sex longer than the other on average? Look at the class results.
3. Have your results contributed to the scattergraph of the class results?
4. Is there a pattern in the scattergraph or are the pairs formed completely at random?
5. Discuss these results with your teacher.

Further things to think about:-

1. Given that larger females produce more eggs, what would be the advantage to a male of pairing with a larger female?
2. Brine shrimps feed from algae and bacteria in the water. What is this method of feeding called?
3. Given that a larger male propels a female along faster and further in the water what advantage might a larger male be to a female?
4. If brine shrimps do pair by size, which pairs will be most successful in rearing more offspring?
5. Why would you expect this 'selection' to operate in favour of bigger shrimps?
6. What are the natural predators of brine shrimps in a salt lake?
7. If these predators (like flamingos) filter brine shrimps from the water will they be the larger or smaller animals that get caught most easily?
8. Brine shrimps have not changed much in size or shape for millions of years. Does this mean that evolution does not happen?

Acknowledgements

Wellcome Trust

The Wellcome Trust is the most diverse biomedical research charity in the world. It spends £600 million every year in the UK and around the world to support and promote research that will improve the health of humans and animals. The Trust's funding has supported the work of scientists that has helped to expand our understanding of evolution in humans and other species. For example, research on the human genome has revealed patterns of human migration and ancestry. As part of Darwin 200, the Wellcome Trust is engaging with the public in educational, broadcast and cultural activities.

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Website: www.wellcome.ac.uk

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Philip Harris

Philip Harris has an outstanding heritage in education, dating back to 1817. They are the science education market leader both in the UK and internationally, with an unbeatable range. Their science resources cover Early Years right through to Further Education and are for teachers and technicians who want good value innovative resources, relevant to the changing demands of the science curriculum.

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Website: www.philipharris.co.uk

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Website:www.ncbe.reading.ac.uk

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Wellcome Trust Education Activities

The Wellcome Trust is committed to engaging with the public on science and has a strong interest in supporting science teachers through initiatives such as the National Science Learning Centre (http://www.sciencelearningcentres.org.uk). The Trust also produces a twice-yearly publication, the Big Picture, for science teachers and there is an edition of this all about evolution which is free to download or order at www.wellcome.ac.uk/bigpicture/evolution.

To celebrate 200 years since the birth of Charles Darwin, the Wellcome Trust is commissioning, developing and funding a unique and ambitious set of projects to engage the widest UK public with Darwin, his ideas, and his influence on contemporary science and culture.

The Trust is providing Darwin-inspired practical activity kits for every state school in the UK. Survival Rivals is the Wellcome Trust's offering for secondary schools. The Great Plant Hunt is the equivalent programme for primary schools.
See www.greatplanthunt.org for more information.

The Trust has developed the Tree of Life – an interactive fly-through of evolution on Earth, narrated by Sir David Attenborough- as well as Darwin-inspired visual arts, poetry and short film projects, placing Darwin and evolution into contemporary culture.
See www.wellcometreeoflife.org for more information.

Working with the BBC, the Wellcome Trust is highlighting a series of short visual clips, that help teachers to teach evolution related topics, available on BBC Learning Zone Broadband www.bbc.co.uk/learningzone.

DNA to Darwin is an education project funded by the Wellcome Trust and run by the NCBE (National Centre for Biotechnology Education). DNA to Darwin will allow 16-19 year old biology students to explore the molecular evidence for evolution by using computers to analyse DNA and protein sequence data. Each of the student activities centre around an engaging story from recent research in molecular genetics encompassing microbiology, plant and animal biology and human evolution.
See www.dnadarwin.org.

Find out more about all the Wellcome Trust's Darwin projects at www.wellcome.ac.uk/darwin200.

Other Links

Great Plant Hunt, Survival Rivals equivalent offering for primary schools, in partnership with Kew Gardens www.greatplanthunt.org

BBC Learning Zone includes clips on evolution www.bbc.co.uk/learningzone

Tree of Life An interactive fly-through of evolution on Earth, narrated by Sir David Attenborough. www.wellcometreeoflife.org

DNA to Darwin free resources for 16-19 year olds around molecular evidence of evolution www.dnadarwin.org

Wellcome Trust's Darwin projects www.wellcome.ac.uk/darwin200

Darwin200 is a national programme of events celebrating Charles Darwin's scientific ideas and their impact around his two hundredth birthday on 12 February 2009 www.darwin200.org

Evolution Megalab Did you know that thanks to a common little snail that you can find in your garden, in the park or under a hedge, you can see evolution in your own back yard? www.evolutionmegalab.org

CREST Awards to accredit young people's work in science and technology www.britishscienceassociation.org/crest

Royal Society for the Protection of Birds Information about birds, including education pages www.rspb.org.uk

Society for General Microbiology Information about microbiology, including educational resources www.sgm.ac.uk

Singtastic Featuring the Mr Darwin song www.singtastic.com

Teachers TV have produced a range of programmes for Darwin200 www.teachers.tv/evolution

Practical Biology Find lots of other investigations to carry out in school www.practicalbiology.org

Association for Science Education helping teachers teach science www.ase.org.uk

Your Genome Educational information from the Sanger Centre www.yourgenome.org

Inside DNA A genomic revolution www.insidedna.org.uk

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