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For the Wellcome Trust

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Sexual selection in brine shrimps

Practical investigations using *Artemia franciscana*

Aim

To investigate mate selection in the brine shrimp, *Artemia franciscana*.

Introduction

Charles Darwin proposed two principal ideas to account for the diversity of life on Earth. While *On 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.

On the Origin of Species, First edition, 1859
Chapter IV

By 1871 Darwin had expanded those few words to take up the greater part of his second major 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.

The phenomenon of sexual selection can be seen and investigated in the classroom. *Artemia franciscana* (brine shrimps) kept in a brightly-illuminated aquarium provide an easily-observed and sustainable ecosystem for classroom-based ecological and behavioural studies by students. When students observe brine shrimps they will see the animals swimming singly or together. They will quickly distinguish between the sexes, for mature animals will swim together, apparently in 'mating' pairs.

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.

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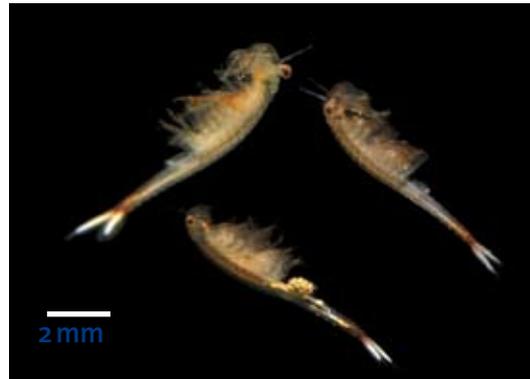


Figure 1. Adult brine shrimps, which swim with their limbs uppermost. The female (with eggs) can readily be identified. The two males have large 'claspers' (modified second antennae) which are used for holding females securely; the females have no claspers

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. 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) by setting up pair-choice experiments; and b) by measuring the sizes of paired individuals.

Darwin, brine shrimps and natural selection

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

*Flamingoes 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!*

The Voyage of HMS Beagle, Third edition, 1860, Chapter IV

Later, Darwin wrote about sexual selection:

The males of many... crustaceans, when adult, have their legs and antennae modified in an extraordinary manner for the prehension of the female...

If the chief service rendered to the male by his prehensile organs is to prevent the escape of the female before the arrival of other males... these organs will have been perfected through sexual selection... in such cases the males have acquired their present structure, not from being better fitted to survive in the struggle for existence, but from having gained an advantage over other males.

On mate selection by females, Darwin wrote:

But in very many cases the males which conquer their rivals, do not obtain permission of the females [to mate with them]...

The females are most excited by, or prefer pairing with, the more ornamented males... but it is obviously probable that they would at the same time prefer the more vigorous and lively males... Thus the more vigorous females, which are the first to breed, will have the choice of many males.

The Descent of Man, Second edition, 1877
Chapter VIII

Notes:

1. In later editions of *On the Origin of Species* Darwin suggested that females may also play a rôle 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.
2. 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.
3. 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.

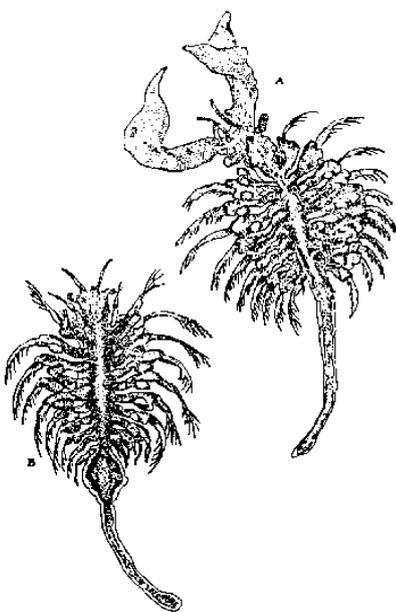


Figure 2. Drawings of male and female brine shrimps by Schlösser, 1755–6. Left: female on which the eggs can be seen; right: male, showing the 'claspers' used during courtship and mating.

The brine shrimp, *Artemia franciscana*

This background information is adapted from Dockery and Tomkins (2000).

Ecology

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

As their common name implies, brine shrimps 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 — in comparison, sea water contains only 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 b-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.

Figure 3. The Great Salt Lake, Utah. A railway separates the north and south sections of the lake, which have different salinities. A phytoplankton community dominated by blue-green or green algae tint the water south of the railway causeway a greenish colour. North of the causeway, the lake is dominated by *Dunaliella salina* (which releases b-carotene) and bacteria-like Haloarchaea. These organisms give the water an unusual reddish or purplish colour. Because of the high salinity, brine shrimps are the only aquatic animals able to live in the lake. They are restricted mainly to the lake's southern arm. In this image, taken from the International Space Station in August 2003, the water level of the lake is very low: note how the northeastern arm of the lake has disappeared, and how Antelope Island [A] is no longer separated from the mainland.



IMAGE COURTESY NATIONAL AERONAUTICS AND SPACE ADMINISTRATION (NASA)

Life cycle

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 worldwide.

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 that are resistant to severe drought and in dried yeast.

In Spring, the overwintering 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.

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: 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. These claspers can be seen in Schlösser's original drawings.

Apart from the claspers of mature males, adult brine shrimps differ in other ways. Adult males are 8–14 mm long when fully-grown. Mature females are on average 1–2 mm longer than males. The males have a translucent body, which sometimes accumulates a blue-green pigment. 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.

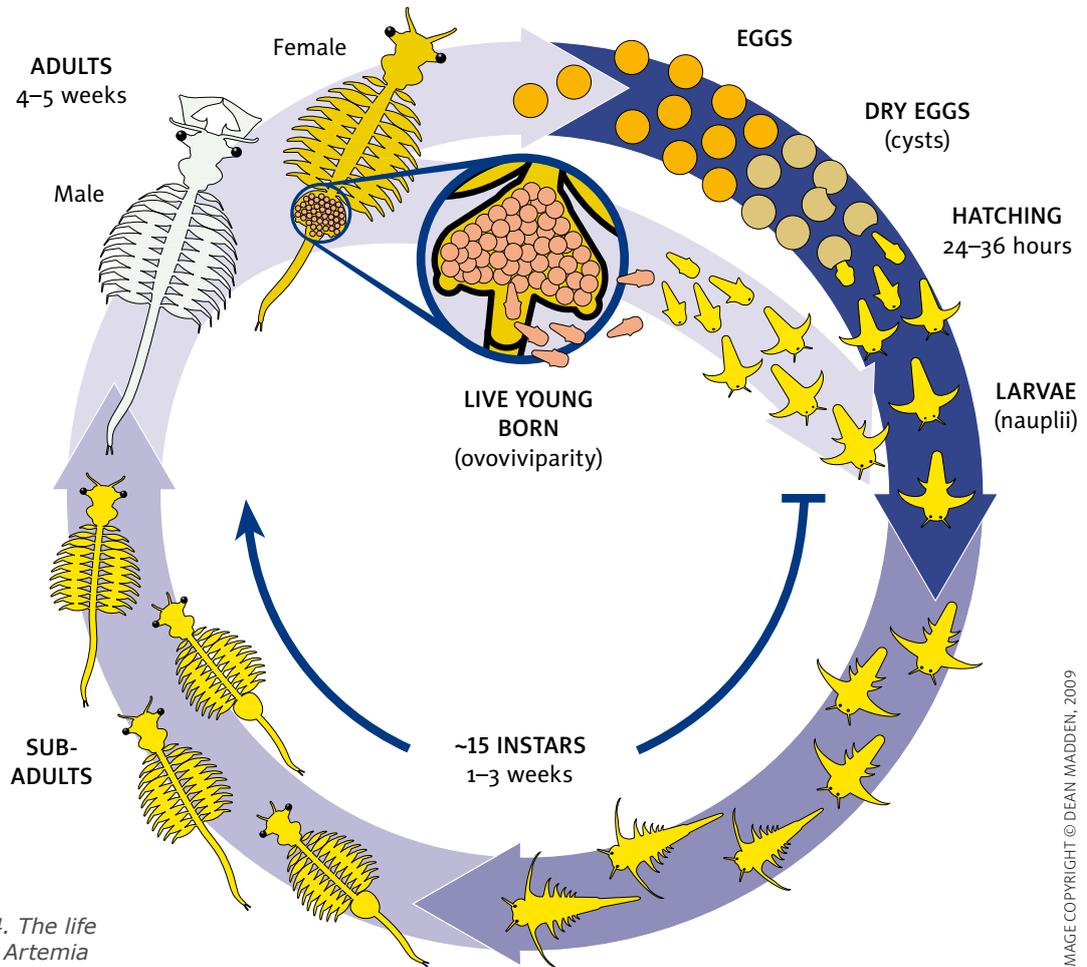


Figure 4. The life cycle of *Artemia franciscana*.

IMAGE COPYRIGHT © DEAN MADDEN, 2009

Figure 5. Mate-guarding in *Artemia*: female above, male below. This process precedes and follows mating.



IMAGE COPYRIGHT © EL VIEJO, 2006

Mate selection

The pre-copulation or guarding phase is initiated by the male, who grasps the female with his claspers between the uterus and the last pair of limbs. In this 'riding position' the two animals can swim around for many hours, even days. Brine shrimps certainly exercise mate choice. Large males may be seen to encounter more females as they swim than smaller males.

There may be male-male competition for females. The very large claspers, in addition to being important in securing females, may also be sexual ornaments that females notice. 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.

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.

Reproduction

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.

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 often develop immediately into free-swimming nauplii when released by the female. This is termed ovoviviparous reproduction.

In the Great Salt Lake this occurs in May or June. 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.

Some species of *Artemia* consist of parthenogenetic females only and reproduce asexually. They are therefore not suitable for the investigation described on the following pages.

Growth rates and physiology

The growth rate of brine shrimps is affected by temperature, salinity 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 water containing more than 20% salt but at 18% their growth rate is half of what it would be 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 it does in a more saline 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.



CAUTION! Do not place hot lamps too near to the culture.

Please refer to the Safety guidelines also.

Figures 6 and 7. Top: Unstressed *Dunaliella salina*. Bottom: When stressed (e.g., by high salinity or light intensity) *D. salina* produces large quantities of *b*-carotene. This pigment accounts for the orange colour of adult female brine shrimps.



Equipment and materials

Required by each class:

Equipment:

- Large plastic tank, e.g., 15 L clear plastic storage box with a removable lid (for the brine shrimps)
- Smaller plastic tank, e.g., 8 L clear plastic storage box with a removable lid (for cultivating the algae)
- 1 L beaker (for hatching the brine shrimp cysts)
- Rulers or graph paper with millimetre gradations (one for each working group of students)
- Wide-mouthed, plastic, 5 mL pipettes ('Pastettes'), 30
- Thermometer, 0–50 °C (e.g., liquid crystal type for side of tank)
- OPTIONAL: Additional light source e.g., 18 W energy-saving bulbs (equivalent to 100 W), 2
- OPTIONAL: Aquarium heater (if your room is cooler than 20–25 °C)

Materials

- Brine shrimp cysts
- Sea salt, 1 kg
- Algal culture (any of the following three species of halophytic algae are suggested: *Dunaliella salina*, *Platymonas* spp. or *Tetraselmis suecica*)
- Crushed oyster shell (grit), 100 g
- Sand, 2 kg
- Plant fertiliser e.g., *Baby Bio*® (for the algae)
- OPTIONAL: *Hobby*® *Liquizell*, 50 mL, (food for the newly-hatched *Artemia* larvae if algae are not immediately available)

Cultivating the algae

Before you can begin growing and observing brine shrimps, you will need to culture the algae so that there will be enough food for the larvae when they hatch and to feed the adult brine shrimps.

1. Prepare 5 L of dechlorinated tap water by leaving the water to stand overnight loosely covered, in the small tank (chlorine in the water will evaporate).
2. Dissolve 175 g of sea salt in the dechlorinated water, making a 3.5% salt solution. Add the algal culture.
3. Place the tank near a good source of light (lamps or a brightly-lit window sill in the summer). Cover the tank to prevent excessive evaporation. Keep the culture at (20–25 °C).
4. Every week, add a drop of plant fertiliser per litre of water.

5. In about a week you should have a green suspension of algae. Check the culture by placing a drop on a microscope slide and viewing under medium power magnification. You should see lots of small, motile algae (see upper photo, right).
6. Maintain this high-density culture and take algae from it to feed the adult and young brine shrimps, topping up with salt water as you remove liquid.

Growing brine shrimps in schools

Brine shrimps are relatively easy to grow and maintain as long as their basic requirements are met. *Artemia franciscana* comes from the Great Salt Lake, Utah, USA. The 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 and salt water. Brine shrimps can tolerate salinities from 0.01% to saturation, although growth is restricted at higher salinities. Above 0.1% salt, no predators or food competitors can survive which, in the wild, results in a simple ecosystem of shrimps and algae. *Artemia* will become sexually mature in 12 days at 28°C and 18–21 days at 20°C. Consequently the system must be set up well in advance (see *Preparation and timing*, below).

A tank of adult, reproductive brine shrimps and algae, if kept warm and well lit, can be maintained as a simple ecosystem. The algae will grow in the tank, supplying food for the brine shrimps. Adult shrimps will regularly and naturally die in the container after some weeks. Their remains will quickly rot and be decomposed by bacteria. Nutrients will be returned to the ecosystem through plant cell uptake in fresh algal growth. To maintain this system you need only to add a small amount of plant fertiliser each week to sustain the algae. Stirring the tank every few days helps to recycle minerals and encourage shrimp growth. Add dechlorinated tap water as necessary to make up for volumes lost by evaporation.

In this way, it is possible to maintain a permanent brine shrimp culture in school. If you wish to run a culture down to a dormant state (e.g., over the school holidays), merely allow the water to evaporate fully. Egg cysts are virtually immortal. At the beginning of the school term, add dechlorinated water to the tank to the correct level to start the culture again.

The brine shrimp tank

To be successful, the tank should be set up where it is warm (20–28 °C) and well lit by lamps or by sunlight from a south-facing window. One 15 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 can be used as long as it is well washed (see below). Brine shrimps graze the sand for algae. Oyster grit is added to provide extra calcium for the brine shrimps.

Preparing the tank:

1. Wash about two kilogrammes of sand, along with the oyster grit, in a few changes of tap water to clean it and to remove fine particles.
2. Put all the sand and grit into the large tank you are going to use for the brine shrimps. It should cover the floor of the tank fully.
3. Make up some salt water (3.5% sea salt), enough to half fill the tank. Mark the level of the water on the side of the tank, so that the water can be topped up as it evaporates.
4. Once the sand has settled, the tank is ready for the algae and brine shrimp larvae (nauplii).

Hatching the brine shrimps

The best temperature to hatch and grow brine shrimps is 25–28 °C.

1. To a one litre beaker half full with salt water add a spatula tip of the brine shrimp eggs (cysts).
2. Place the beaker near a lamp and check that the temperature is 25–28 °C. *Light is essential for cysts 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.*
3. In 1–2 days the eggs will hatch. Add a few drops of the Liquizell feed. Although the larvae will feed on this in the first stages of their development, their preferred food is single-celled algae, so after 3–4 days add about 200 mL of algal suspension to the beaker. Add another 200 mL of algal suspension 2–3 days later.
4. After a week the nauplii will be about 1–2 mm long and clearly visible. Gently pour the nauplii (with the algae) into the larger, main tank. *Brine shrimps will grow to maturity in 3–4 weeks, when they will be observed to be either male or female and starting to pair up.*

Feeding the brine shrimps

It is a good idea to maintain a separate high-density culture of algae in a small tank which can be taken from to feed the brine shrimps. You can judge whether the brine shrimps need feeding by the colour of the water in the tank. Almost clear — not enough algae; light green — just right; very green — you have an algal bloom.

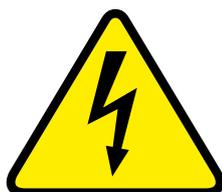
Once a week, gently scrape the walls of the main tank to remove any algal film — brine shrimps will have difficulty feeding on this. Brine shrimps will lay their dormant eggs in the water, where they float. Eggs adhere to the walls of the tank, so scraping refloats the dried eggs on the water.

A mature brine shrimp ecosystem will contain animals at all stages of their life cycle. It may therefore be necessary to add a few drops of *Liquizell* to the tank from time to time for nauplii to feed on.

Safety guidelines

Brine shrimps

Some students may be allergic to crustacea ('shellfish').



Cultivating the algae

Care should be taken to ensure that lights used to illuminate the culture cannot come into contact with liquid should it spill or leak from the culture vessel. It is therefore a wise precaution to place the culture vessel in a deep tray with sufficient capacity to hold all the liquid in the event of a leak. Any electrical equipment should then be placed outside this tray.

Lamps

When lamps are used to illuminate the algae, care should be taken to ensure that they are not used in such a manner that there is a risk of electrical shock or overheating that may cause burns or fire.

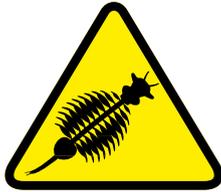
Preparing and timing

The cultures of algae and brine shrimps need to be started at least four weeks before students carry out the practical work.

Here is a suggested timetable:

Week 1	Start to grow the algae in salt water.
Week 2	Hatch the brine shrimp cysts and make up the tank ready to receive the brine shrimp nauplii and algae.

Week 3	Transfer the brine shrimp nauplii and algae to the tank.
Week 4	Feed algae to the brine shrimps.
Week 5	Brine shrimps ready for class use.



Animal welfare

Brine shrimps are living organisms and they should be handled carefully and 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.

Other sources of information

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 to download free-of-charge from the BES web site: http://www.britishecologicalsociety.org/educational/brine_shrimp/index.php

Ward-Booth, K. and Reiss, M. (1988) *Artemia salina*: an easily-cultured invertebrate ideally suited for ecological studies *Journal of Biological Education* 22 (4) 247–251. Suggests investigations for schools.

Artemia: Basic and applied biology (Biology of aquatic organisms) by Th.J. Abatzopoulos, J.A. Beardmore, J.S. Clegg and Patrick Sorgeloos [Eds] (2002) Kluwer Academic Publishers. ISBN: 978 1402007460. A costly but authoritative academic reference.

The Wellcome Trust

The Wellcome Trust's Survival Rivals web site includes additional resources such as an animated game and video clips to accompany this protocol. www.survivalrivals.org
Captain's Universe video clips, photographs, culture details etc

www.captain.at/artemia

Artemia reference centre, University of Ghent

www.aquaculture.ugent.be

Suppliers

Brine shrimp ecology kit

Blade's Biological supply a kit to accompany the *BES* Brine shrimp ecology book by Dockery and Tomkins. This kit is not designed to be used for the current exercise although it provides many of the materials required. The kit contains a copy of the book, 60 mL *Artemia franciscana* cysts, inoculum, liquid fertiliser, 65 mL *Dunaliella* culture, 120 g sea salt and 250 g coral sand. Blade's also supply 60 mL quantities of *Artemia franciscana* cysts. Blade's Biological Limited, Cowden, Edenbridge, Kent TN8 7DX UK.

T: + 44 (0) 1342 850 242.

F: + 44 (0) 1342 850 924.

E: info@blades-bio.co.uk



Any of the following three species of halophytic algae can be used: *Dunaliella salina*, *Platymonas* spp. or *Tetraselmis suecica*.

Halophytic algae

Cultures of halophytic algae are available from Sciento, 61 Bury Old Road, Whitefield, Manchester M45 6TB UK. T/F: + 44 (0) 161 773 6338. E: sales@sciento.co.uk.

Artemia cysts

Two sorts of dried cysts are available: whole cysts and non-viable decapsulated cysts. The latter are sold for direct use as fish food and are obviously not suitable for this investigation. There are parthenogenetic species of brine shrimps in Europe and Asia that have no males at all. Therefore for this protocol it is absolutely essential to use only the North American species, *Artemia franciscana*.

Artemia cysts, food, hatching kits, sea salt, etc.

These are usually sold in pet shops and aquarists for the hobby trade. There are two major suppliers in Europe.

NT Laboratories supplies resources for those wishing to cultivate brine shrimps as food for fish. Their products are well-supported by technical literature and include live adult brine shrimps (packaged in polythene tubes) and decapsulated brine shrimp cysts. The latter have a high viability that can be stored for many years at room temperature (these are sold as '*Artemia* Revolution'). Because these specially-decapsulated cysts have no shell, they sink, clumping together so that only a few cysts hatch. Therefore such cysts require a special hatcher through which air is bubbled to keep the cysts in suspension. NT Laboratories Limited, Unit B, Manor Farm, Watlington, Kent ME18 5PP UK.

T: + 44 (0) 1622 817 692.

F: + 44 (0) 1622 817 271.

E: info@ntlabs.co.uk

W: www.ntlabs.co.uk

Dohse Aquaristik (trading as *Hobby*®) provides a wide range of products, enabling aquarists to raise *Artemia* as fish food. The products include *Artemia* cysts and *Liquizell* food for *Artemia* larvae. Dohse Aquaristik KG, Otto-Hahn-Str. 9, D-53501 Graftschaf-Gelsdorf, Germany.

T: + 49 (0) 22 25 94 15 0.

F: + 49 (0) 22 25 94 64 94.

E: info@dohse-aquaristik.de

W: www.dohse-aquaristik.com

Sea salt and coral sand

Several brands of synthetic sea salt are available e.g., *Seachem*, *Insant Ocean*. Such products are used widely in both commercial and domestic aquaria. Similarly, coral sand of various grades and colours is available in bulk from aquarists.

Hobby aquarists' magazines list suppliers. For example, the magazine *Practical fishkeeping* produces an annual directory listing thousands of UK suppliers. Its web site is also a useful source of information: www.practicalfishkeeping.co.uk

Broad-mouthed plastic pipettes

Alpha Laboratories sells a wide range of large volume disposable plastic pipettes ('Pastettes') some of which are wide-bored and are therefore suitable for isolating brine shrimps.

Alpha Laboratories Limited, 40 Parham Drive, Eastleigh, Hampshire SO50 4NU UK

T: + 44 (0) 800 387 732.

F: + 44 (0) 800 614 249.

E: info@alphalabs.co.uk.

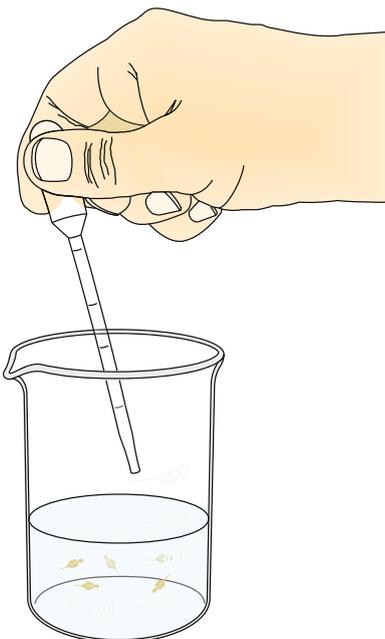
W: www.alphalabs.co.uk

Acknowledgements

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Packo Lamers of the Bioprocess Engineering Group at Wageningen University kindly provided the photographs of algae on page six. Special thanks are also due to Leighton Dann for arranging the *Survival Rivals* pilot experiments and to the following schools for taking part in these:

- Oundle School, Peterborough
- King Ecgbert School, Sheffield
- St Clement Danes School, Chorleywood
- Samuel Ward School, Haverhill



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Students' worksheets

The following pages are the students' worksheets for this activity.

How do brine shrimps choose their mating partners?

Humans are selective about who they like. Are other animals the same? Charles Darwin wondered whether the different appearance of males and females of the same species was something to do with which partners the animals themselves chose to mate with. Are peacocks with larger, magnificent tails preferred (and therefore selected for) by female peahens? This was one of Darwin's questions.

We now know, for example, that female deer find noisy male deer with large antlers more attractive than quiet ones with small antlers. The male deer 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.

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.



Figure 8. Male brine shrimps (top) are distinguished by their 'claspers'. These are enlarged antennae close to the head, enabling them to hold on to the female (below). Females do not have claspers. Mature females have brood pouches containing eggs immediately behind their last pair of antennae.

Observing Brine shrimps

Equipment and materials

Each working group requires a large tank of brine shrimps of all sizes and ages.

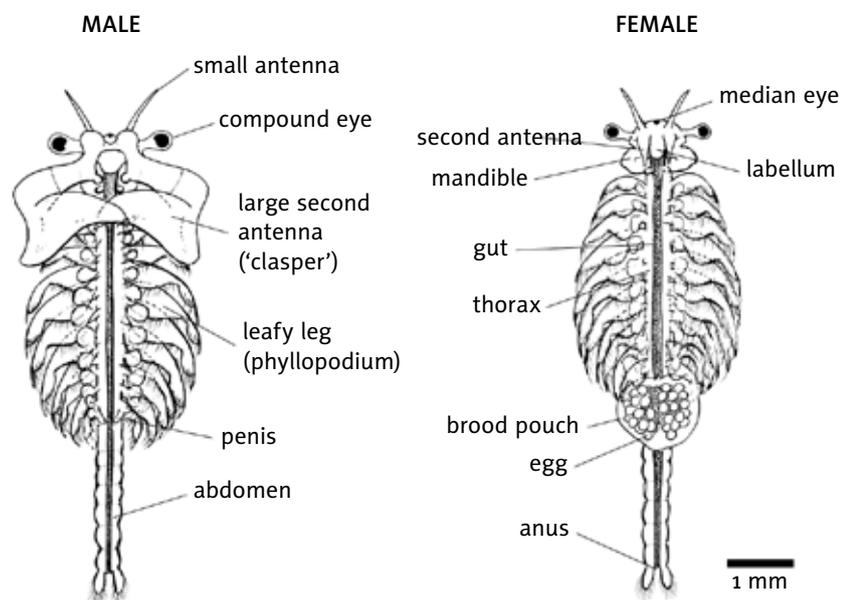
Procedure

Brine shrimps live in salt water lakes where they filter feed algae from the water. Begin by observing brine shrimps 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 alone or are some in pairs? When shrimps swim in pairs, this is called mate-guarding.
3. How can you distinguish males from females?
4. In a mate-guarding pair, which sex swims in front and which one behind?
5. Females have egg sacs half-way 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 antennae are greatly enlarged and are called 'claspers'. What is their function?
7. Suggest why the males and females are clasped together for about three days around the time of mating. (They don't simply mate for this length of time!)

Now that you have made some initial observations, there are two experimental investigations that you could undertake. Discuss with your teacher which of the two approaches suggested below would be the best one to help you to investigate brine shrimp mate choice.

Figure 9.
Anatomy of brine shrimps (From Dockery and Tomkins, 2000)



APPROACH 1: Mate pair choice experiment

One 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 choice of mate random? And if there is sexual selection, do the males or the females (or both) make the choice?

Before testing this hypothesis, you will need to separate male and female brine shrimps and keep them apart for a few days so that they are eager to pair up when brought back together.

Lesson 1: Separating the sexes

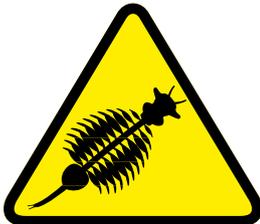
Equipment and materials

Required by each working group:

- Large tank of brine shrimps of all sizes
- Smaller beakers or containers of sea salt solution and algae, one labelled 'males', the other 'females', 2
- Wide-mouthed, plastic, 5 mL pipette ('Pastette').
Note: you may need to cut the end off the pipette so that shrimps can be collected easily and without harming them.
- Magnifying lens
- Petri dish

Procedure

With care, brine shrimps may be transferred between containers of salt water using a wide-mouthed pipette. Squeeze the bulb of the pipette and then as you chase the animal, release the bulb to capture it. Check that the shrimp has been sucked up properly, then release the animal carefully by gently squeezing the pipette bulb again.



1. Using a wide-mouthed pipette, capture single brine shrimps (not pairs) from the large container and place them in the Petri dish of salt water.
2. Examine the brine shrimps with the magnifier and segregate them into two containers by gender, placing male brine shrimps into one container and females into another.
3. Ensure that you have a range of sizes of males and females in the two containers. Place these where they can be kept warm and well lit until you can continue your investigation.
4. You should leave the males and females separate for *at least two days*, so that the females become more receptive to the males.

Lesson 2: Mate choice (two or more days later)

Equipment and materials

Required by each working group:

- Male and female brine shrimps (from previous lesson)
- Wide-mouthed, plastic, 5 mL pipette ('Pastette'). *Note: you may need to cut the end off the pipette so that shrimps can be collected easily and without harming them.*
- Four containers (beakers, cups or bottles) each containing sea salt solution with algae
- Waterproof marker pen

Procedure

1. Check the containers of 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 do you think 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 clear difference in size between the animals so that you can recognise each of them again.
5. Note whether each female has an egg sac or not when you put her in a container. Record its size and colour.
6. Place all the experimental containers together, where they are well lit and warm. *Note: if you have more containers and brine shrimps and can set up more replicates of this experiment, your results may be more reliable.*

EXPERIMENT 1	♂	♀	EGG SAC SIZE & COLOUR
Larger animal	1	1	
Smaller animal	1	–	–

EXPERIMENT 2	♂	♀	EGG SAC SIZE & COLOUR
Larger animal	1	–	
Smaller animal	1	1	–

EXPERIMENT 3	♂	♀	EGG SAC SIZE & COLOUR
Larger animal	1	1	
Smaller animal	–	1	–

EXPERIMENT 4	♂	♀	EGG SAC SIZE & COLOUR
Larger animal	–	1	
Smaller animal	1	1	–

Lesson 3: Results and their interpretation (2–4 days after pairing)

Equipment and materials

Required by each working group:

Containers from the previous lesson, each containing three shrimps

Procedure

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

Discussion of the results

These experiments raise at least the following questions:

1. Do all the experiments result in shrimps pairing up? If not, why not?
2. Do the larger males more easily obtain a partner than smaller males?
3. Do smaller males more easily obtain smaller female partners than do larger males?
4. Do larger females pair up with larger males more readily than do the smaller females?
5. Do larger females pair up with smaller males more readily than do the smaller females?
6. If a female is carrying a large egg sac does it affect her pairing or not?
7. 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: Mate size choice

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 with the larger females? Are the smaller males paired with 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.

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

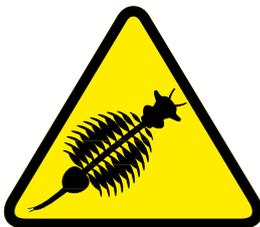
Required by each working group:

- Pairs of mate-guarding brine shrimps
- Wide-mouthed, plastic, 5 mL pipette ('Pastette'). *Note: you may need to cut the end off the pipette so that shrimps can be collected easily and without harming them.*
- Magnifying lens
- Microscope slide
- Graph paper marked in millimetres

Procedure

Please handle brine shrimps carefully!

With care, brine shrimps may be transferred between containers of salt water using a wide-mouthed pipette. Squeeze the bulb of the pipette and then as you chase the animal, release the bulb to capture it. Check that the shrimp has been sucked up properly, then release the animal carefully by gently squeezing the pipette bulb again.



1. Place the glass slide on the graph paper so that the length of the brine shrimps may be measured.
2. Collect one of the numbered containers and, with a wide-mouthed pipette, transfer the pair of brine shrimps 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. Measure each pair of shrimps.
7. Plot a scattergraph of your results.

Discussion of the 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 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?