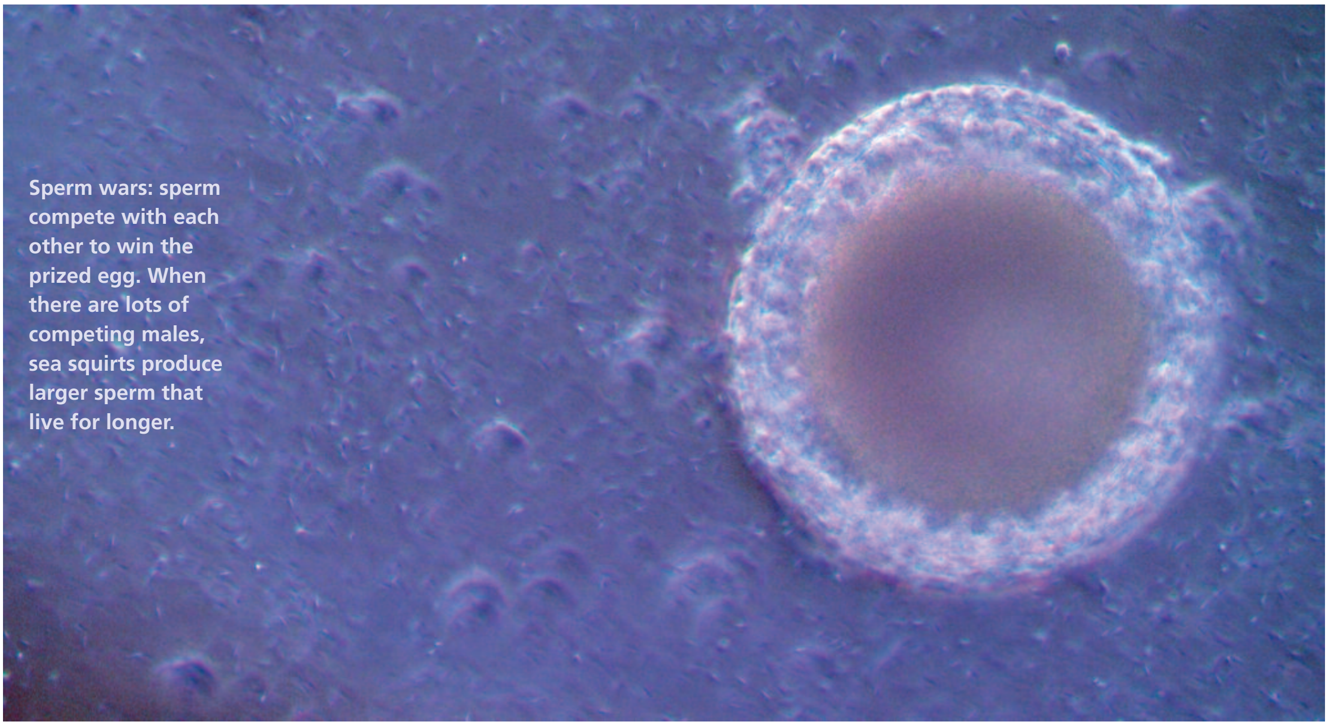


Sperm wars: sperm compete with each other to win the prized egg. When there are lots of competing males, sea squirts produce larger sperm that live for longer.

SEA SQUIRT SEX

Surprising Sophistication behind the Simplicity

By Angela Crean and Dustin Marshall

Although sea squirts appear to have a relatively simple sex life, these primitive animals show remarkable sophistication by adjusting the quality of their gametes according to their surroundings.

If you spend a minute observing behaviour at your next party, it is easy to grasp the basics of sexual selection theory – females are choosy and males compete. But how do you choose or compete for the best partner when you are permanently stuck to a rock?

Most marine organisms reproduce by releasing their eggs and sperm into the ocean. This mode of reproduction is called broadcast spawning. You've probably seen an example of broadcast spawning on nature documentaries: the coral spawning on the Great Barrier Reef every October/November is one of the best-known examples.

Because fertilisation in broadcast spawners occurs externally, competition among males and mate choice by females occurs at the gamete level. Therefore we decided to test whether broadcast spawners change their gametes to suit local conditions.

Before we can answer this question we must understand a little more about what types of problems a broadcast spawner faces when trying to mate. First let us examine mothers. Assuming there is a fixed energy budget for reproduction, mothers can either invest a lot of energy in a few large eggs or less energy in many small eggs. The advantage of producing small eggs is that you can make lots of them. However, the advantage of producing large eggs is that these offspring are more likely to survive, particularly in poor conditions. Therefore, the first reproductive challenge facing any mother is what size offspring to make.

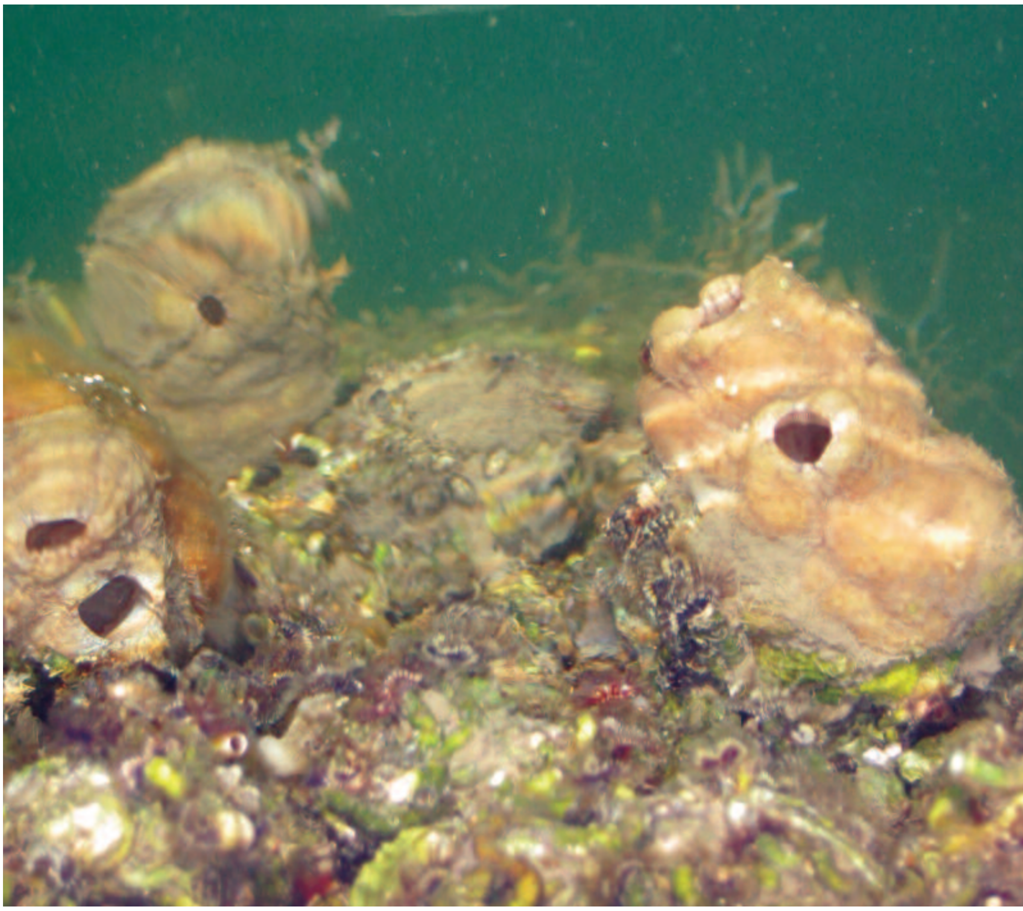
The unique challenge facing broadcast-spawning mothers is that the size of her eggs also represents the "target" size for searching sperm. Because broadcast spawners release their gametes into the water column, sperm have to find the rapidly dispersing eggs. This means that if the animals are sparsely distributed, sperm may not reach the eggs, and females may actually suffer from sperm limitation (some of her eggs will remain unfertilised).

At the other end of the spectrum you can have too much of a "good" thing. When adults are crowded together, too many sperm may swamp the female and eggs may become polyspermic (where multiple sperm penetrate the egg, which can be fatal).

Larger eggs provide a larger target for sperm. Therefore, large eggs are more likely to be successfully fertilised at low sperm concentrations but fatally fertilised by multiple sperm at high sperm concentrations. In other words, large eggs are more likely to be fertilised in low density populations but small eggs are more likely to be successfully fertilised in high density populations.

But that is not quite the end of the story. Many marine invertebrate eggs have accessory structures such as jelly coats or follicle cells that increase the overall target size of eggs without contributing any energy to embryonic development. Energy for embryonic development is provided by the ovicell (which is similar to the yolk of a chicken egg), but the follicle cells are mostly made of water and are comparatively cheap to make.

Hence broadcast-spawning mothers have two different properties of their eggs that they can potentially adapt to increase their reproductive success. Accessory structures provide a cheap way of increasing the target size of the egg (with consequences for fertilisation success), while the ovicell represents the main energetic investment in the egg (with consequences for offspring number and survival).

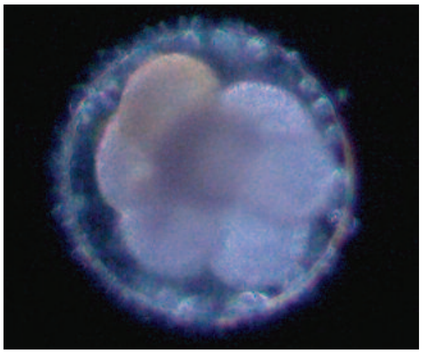


Sedentary sex: sea squirts live life stuck to the substrate, so they reproduce by squirting their eggs and sperm out into the ocean.

So the question remains: are broadcast-spawning mothers actually capable of changing the properties of their eggs depending on the local environment? To test this we studied the solitary sea squirt, *Styela plicata*.

Sea squirts (ascidians) are our very ancient ancestors – although they don't appear to have any similarities with humans, they belong to the same phylum (Chordata). Sea squirts get their name from their feeding structures: they have an inhalant and exhalent siphon for filtering food, and these squirt water when they're exposed to air.

Styela plicata is an introduced pest in eastern Australia, and is commonly found stuck to pier pilings in a range of densities – from isolated individuals to large clumps. We were therefore able to manipulate their densities by placing either a single individual or 15 individuals in a cage, and monitoring them in the field for 1 month.



We have a winner! A successfully fertilised egg undergoing cleavage. Females also play the game, changing their egg size according to how many males are competing for their eggs.

This simple experiment produced striking results. We found that not only did sea squirt mothers adapt the size of their eggs to local conditions, but they changed the different properties of their eggs in opposite directions. Mothers put in high density conditions had a smaller conditions ranging from sperm limitation to sperm competition.

Using this technique we were able to show that not only did males in high density environments make sperm that lived for longer, but they actually made sperm that were more successful at fertilising eggs at higher sperm concentrations. This suggests that males who are surrounded by lots of competitors (and are therefore likely to face high sperm concentration conditions) are producing sperm that induce lower levels of polyspermy. Therefore, sea squirts are not only adapting the properties of their sperm, they are adapting their sperm in a way that increases their fertilisation success!

This research gives us an indication as to what selection pressures led to the evolution of sex in the first place. Because sea squirts reproduce by the ancestral mode of reproduction, they can provide insights into how competition between males led to alternative mating strategies. Specifically, it tells us a little about why sperm are so tiny and males make millions of them, whereas females make far fewer large eggs.

In our study, males living in isolation actually produced smaller sperm. This suggests that sperm limitation may play a larger role in selection on sperm size than previously thought.

Overall, it is clear that the sex lives of sea squirts are not as simple as they appear. These animals can sense changes in their local environment and adaptively adjust the properties of both their eggs and sperm, affecting both their fertilisation success and the fitness of their offspring.

It will be exciting to see if other broadcast spawners, such as corals, are similarly capable of adapting their eggs and sperm to local conditions. You never know, the humble sea squirt may even change the way you view the scene at your next party!

The Perils of Polyspermy

To prevent polyspermy, eggs have a block that is activated after the first sperm enters them. In internal fertilisers this block is very rapid, and therefore the early sperm gets the egg (regardless of the sperm concentration). Sperm competition in these species can therefore be thought of as a raffle – with the chances of winning increased through numbers and speed. However, polyspermy blocks of many broadcast spawners are slower, which means that polyspermy can significantly reduce fertilisation success, particularly at high sperm concentrations.

Recent Australian research shows that due to the perils of polyspermy, increasing the numbers of sperm may not always be the best competitive strategy in broadcast spawners. When playing the numbers game, fertilisation success in broadcast spawners follows a bell-shaped curve that is reduced at either end by sperm limitation and polyspermy.

As sex evolved in the sea, and broadcast spawning is believed to be the ancestral mode of reproduction, these discoveries provide new insights into the selection pressures that may have led to the evolution of anisogamy (separate eggs and sperm).

total egg size (and hence target size) than the eggs of mothers from low density conditions. However, these mothers also increased the ovicell size (and hence energetic investment) in their eggs. In other words, mothers in high density environments produce small eggs that are less likely to suffer polyspermy, but with a large energy reserve so their offspring are bigger and better competitors!

So, mums can adapt their eggs to local conditions, but what about dads? Although sperm are numerous and tiny, they are not limitless. Therefore, similar to the trade-off between egg size and number, males may either release small amounts of sperm often or lots of sperm rarely.

Classic sperm competition theory predicts that as the threat of sperm competition increases, a male should increase the number of sperm he releases. However, in broadcast spawners, increasing the number of sperm actually reduces fertilisation success due to polyspermy. So if you can't win the race with numbers, what do you do?

Well, sperm can also differ in traits such as size, motility and velocity. While it was traditionally thought that all sperm were created equal, recent research has shown that sperm differ in quality. For example, experiments changing the social status of male roosters have shown that males are actually capable of changing the quality of their sperm depending on the likely levels of sperm competition they will face.

However, one of the problems encountered when working with internal fertilisers is that you cannot observe what actually happens at fertilisation. So once again the sea squirt presented us with a unique opportunity to not only test if males can change the properties of their sperm, but also to test what effects these changes have on fertilisation success.

Using the same experimental methods as before, we manipulated the density of *Styela plicata* for 1 month in the field and studied the properties of their sperm. Surprisingly, we discovered that these seemingly simple animals showed quite sophisticated behaviour, adapting their sperm to suit predicted levels of local sperm competition. Males in high density environments produced sperm that were larger and more motile than males in low density environments. What consequences does this have for fertilisation success?

As sea squirts reproduce via external fertilisation, we were able to mix eggs and sperm together with sea water in the laboratory and observe fertilisation. Furthermore, by mixing eggs with varying concentrations of sperm, we can replicate

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