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Inside JEB

SOME LIKE IT HOT: LAMPREY'S HEATED SEXUAL TRAIT



'Is it hot in here or is it just you?' Clichéd chat-up lines may serve some humans well, but other animals prefer more imaginative ways to captivate and attract potential suitors. Take, for example, the male sea lamprey, which will coax ovulating females into its nest by releasing enticing pheromones. Once comfortably in the nest, the male will then perform an interesting dance routine, rubbing the female's belly with a small bump of tissue on his back. Should the female be happy with what she sees and feels, the two will then spawn their gametes simultaneously. This unusual courtship routine is well characterised but no one is quite sure what role this bump, called rope tissue, plays in the proceedings. 'We thought it's just a structure that was used for some kind of mechanical stimulation that they needed to trigger the female to lay eggs', says Yu-Wen Chung-Davidson, from Michigan State University, USA, who has been studying lampreys for 10 years. However, she wasn't sure if this was the case, and so with help from her colleagues she decided to investigate (p. 2702).

Chung-Davidson began by looking at the rope tissue under the microscope, and what she saw surprised her: 'It looked opaque, and it looked like fat to me.' Explaining her next step, she says, 'I happened to have tissues from various life stages of these lampreys and so I compared them and it's very interesting. When they are in the immature state, the male and females look more or less the same. But when I looked in the mature males and females, they were very different. So there's very obvious sexual dimorphism in their morphology and this part of their body', says Chung-Davidson.

When Chung-Davidson delved deeper, looking at the slides of the rope tissue under a transmission electron microscope, she was again surprised. The cells weren't just normal white fat cells. She explains that white fats cells have a characteristic giant oil droplet whereas these cells clearly had several smaller droplets and were packed full of mitochondria (powerhouse organelles that produce energy). In fact, these fats cells looked remarkably similar to another, rare type of fat – brown fat cells. To further characterise this fat, Chung-Davidson and her colleagues analysed what types of fatty

acids and proteins were present in the cells. While the fatty acid profile looked remarkably similar to that of mammalian brown fat cells, the pattern of proteins varied a little.

All in all, however, the fat looked very similar to brown fat but it remained to be seen whether it had brown fat's defining trait – the ability to produce heat. Chung-Davidson explains that this type of fat is usually found in mammals that need to maintain their own body temperature (unlike lampreys, whose body temperature varies with the environment). First the team looked for UCP-1, a protein that allows mitochondria to use fat to generate heat instead of energy – they found that lampreys didn't express it but they did express UCP-2, a related protein. To directly test the thermogenic abilities of the rope tissue, Chung-Davidson implanted tiny probes into the rope tissue of sexually mature male lampreys. The team found that the rope temperature immediately rose by up to 0.3°C when male lampreys encountered a female, with some getting hotter than others when encountering certain females. Whether they get hotter when more attracted and indeed what the role of this hot tissue is remain unknown – regardless, perhaps lampreys could use the altered chat-up line: 'Is it hot in here or is it just me?'

10.1242/jeb.089771

Chung-Davidson, Y.-W., Priess, M. C., Yeh, C.-Y., Brant, C. O., Johnson, N. S., Li, K., Nanlohy, K. G., Bryan, M. B., Brown, C. T., Choi, J. and Li, W. (2013). A thermogenic secondary sexual character in male sea lamprey. *J. Exp. Biol.* **216, 2702-2712.**

Nicola Stead

UNDER PRESSURE: STIFF ARTERIES KEY WHEN DIVING

Diving is not for the faint-hearted; the deeper you go the more crushing the pressure bearing down on you becomes. However, intrepid fin whales will regularly dive down 100–200 m in search of their next meal, and consequently face pressures of 1000–2000 kPa. But these immense pressures present very real physiological challenges; for a start, how do they maintain their transmural blood pressure? 'Transmural blood pressure is the pressure inside the artery minus the pressure outside and is produced by the heart when it contracts', explains Margo Lillie, from the University of British Columbia, Canada. 'Whatever the pressure is inside the thorax, where the heart is, the heart just bumps it up – it creates an overpressure. So, [in a terrestrial mammal] if the pressure in the thorax is 100 kPa the heart will bump it up to about 113 kPa.' On land, where pressure outside the arteries is also at equilibrium with atmospheric pressure



(100 kPa), overall transmural blood pressure remains at around 13 kPa. But what happens if the environmental pressure outside suddenly increases to 1000 kPa? How can the thorax pressure equilibrate to maintain constant transmural arterial blood pressure? Lillie and her colleagues decided to investigate (p. 2548).

Lillie started by inflating different types of arteries with pressurized liquid. ‘With a terrestrial mammal’s artery, as you increase the pressure the artery increases in diameter up to a point and then it mostly stops stretching – that point is around the physiological pressure [13 kPa]’, explains Lillie. However, most of the tested arteries stopped increasing in diameter almost immediately, reaching their maximum diameters at very low pressures of just 1–2 kPa. ‘This was entirely unexpected’, recalls Lillie. When she looked at a cross-section of the arteries, Lillie found that they had an unusually thick layer of collagen, the material responsible for stiffening arteries.

Intrigued, Lillie wondered what would happen if she exposed the arteries to negative transmural pressures, and found that they were unusually resistant, withstanding negative pressures of up to –50 kPa. Again, collagen was responsible and Lillie calculated that the collagen would prevent the artery from ever fully collapsing.

Lillie was perplexed. All her data suggested that whales’ arteries, with their collagen-induced stiffness, were built for withstanding a range of pressures including negative pressure. Lillie wondered whether her previous assumptions had been wrong. Maybe, during descents, the pressure inside the thorax couldn’t equilibrate quickly enough to ambient pressure. As the internal blood pressure is set inside the thorax, Lillie realised that lower-than-ambient pressures in the thorax could create low or even negative pressure in parts of the body equilibrated to the higher, ambient pressure. Lillie explains that while most organs readily pressurize, the air-filled lungs have a tougher time pressurizing. The air needs to be compressed to equilibrate, but with stiff ribs that resist the pressure, something else needs to push down on the air in the lungs. In human divers, blood will move from equilibrated areas into thoracic vessels, filling up space and compressing the air. Many scientists

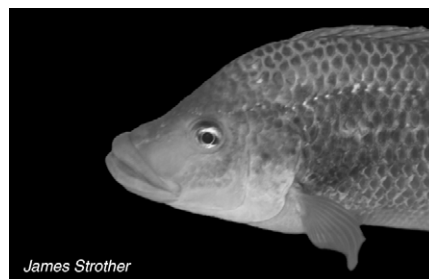
assumed that the same would happen in whales; however, when Lillie and her colleagues modelled it, she found this was not the case: ‘I started to realise, thinking about the volume of blood that has to shift, that what is important is the rate at which a whale is going down and how fast the tissues [blood] can respond, and I decided that they can’t respond fast enough.’ So depending on an artery’s location inside or outside the thorax, it may experience different transmural pressures. For humans this would be disastrous, but with their extra thick collagen coating, it’s no problem for a fin whale’s artery!

10.1242/jeb.090076

Lillie, M. A., Piscitelli, M. A., Vogl, A. W., Gosline, J. M. and Shadwick, R. E. (2013). Cardiovascular design in fin whales: high-stiffness arteries protect against adverse pressure gradients at depth. *J. Exp. Biol.* **216**, 2548–2563.

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INVESTIGATING GILLS’ HYDRODYNAMIC SECRETS



Gulping like a fish is not very attractive, but for a fish it’s an essential part of everyday life. Fish ‘breathe’ by swallowing water and pushing it through the respiratory chamber and over the gills. The gills themselves are made up of a large number of filaments that fill the respiratory chamber, and house a complex network of blood vessels that allow efficient uptake of oxygen. The question is, how does water weave its way through the gills, and are the gills strong enough to resist the resulting pressure? In the early 1960s and 1970s a lot of work went into understanding how much hydrodynamic resistance gills put up as water flowed over them. However, often the experimental and the predicted values didn’t add up, explains James Strother, currently a post-doc from the Janelia Farm Research Campus, USA, ‘You have these two, what should be, trusted sources of information in conflict, so I thought there’s a reason to suspect there’s more to the story, an interesting phenomenon that we haven’t observed before.’ So during his PhD in Matthew McHenry’s lab at the University of California, Irvine, USA, he decided to investigate further (p. 2595).

Strother started his investigation by carefully removing a portion of the gills and placing it

in a flow-through chamber where he could control the water flow through the gills. By measuring the pressure on either side of the gills, Strother could then work out the pressure over the gills. At flow rates lower than 65 ml min^{–1} pressure across the gills increased linearly as expected – the faster the water flows, the more pressure there is acting on the filaments.

From these measurements Strother then calculated the resistance the filaments were providing and compared this with a value he had predicted using morphological measurements of the gill filaments. He was astonished – the values were still very different, with the measured resistance nearly four times greater than predicted. He explains his surprise: ‘the experiment was designed so that all of the complicating factors [such as active movement of the gills] that could obscure the relationship were removed. So this is a case where the measured and predicted value should match up very closely, but even in this situation there’s still this difference.’

However, this wasn’t the only interesting observation in store for Strother. At flow rates above 65 ml min^{–1} pressures across the gills started to plateau, indicating that the gills were providing less resistance. When he seeded the water with tiny diamond microparticles, which glinted when illuminated, he was able to film the flow patterns over the gill filaments. At low flow rates, all the water flowed uniformly over the gills, ensuring maximal gill–water contact. However, at flow rates above 60 ml min^{–1} things started to change, and by the time flows were ramped up to 80 ml min^{–1} flow patterns were significantly altered – the higher pressures caused the tips of the filaments to deform and allow large amounts of water to shunt right past them. Fish will increase their flow rates when they’re exercising or in hypoxic situations; unfortunately, however, water flowing through the gaps is wasted effort, as it never contacts the gills. Strother observed that this became even worse at the higher flow rates above 150 ml min^{–1}, where vortices developed, hindering the passage of further water.

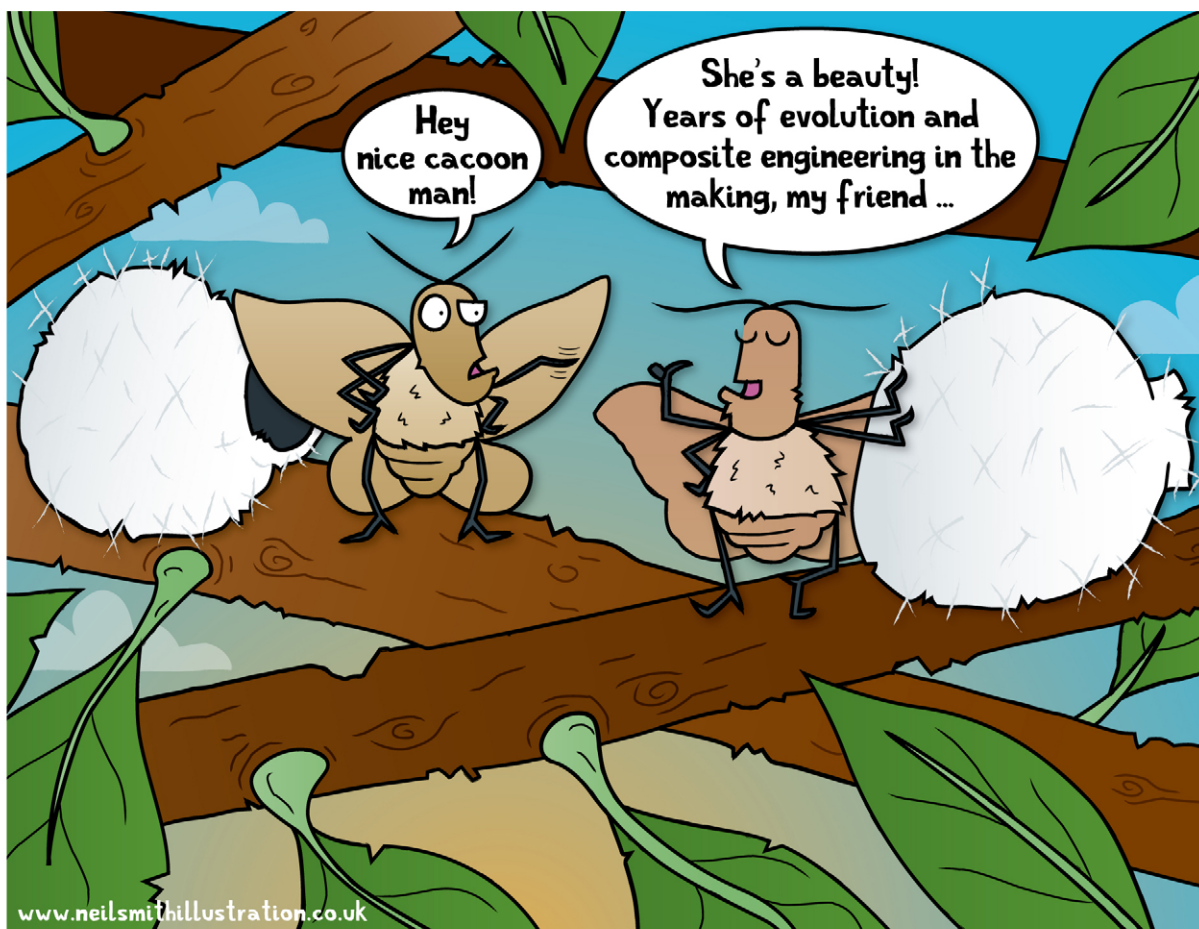
While Strother hasn’t quite yet figured out why predicted and measured values of hydrodynamic resistance still differ, his work has highlighted how biomechanics can affect how effective the gills are in taking up oxygen.

10.1242/jeb.090233

Strother, J. A. (2013). Hydrodynamic resistance and flow patterns in the gills of a tilapia fish. *J. Exp. Biol.* **216**, 2595–2606.

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CRAFTING COMPOSITE COCOONS TO SUIT NEEDS



When the three little pigs were building their homes to protect themselves from the big bad wolf, none of them thought of using silk. It's understandable – we associate silk with fine clothes and not with its role in protective cocoons. Amazingly, silk is just one of two building materials that make up these cocoons, the other is a glue-like protein called sericin. While each component on its own is not very durable, put the two together and you have a resilient composite material. All cocoons are constructed to the same design, with layered sheets of entangled silk fibres held together by sericin, where the inner layers become more and more compact. However, by using different amounts of glue, cocoons with different properties can be made. David Porter and his colleagues from the University of Oxford, UK, decided to compare the properties of two very different cocoons from the moths

Antheraea pernyi and *Opodiphthera eucalypti* (p. 2648).

To begin, the team cut tiny squares from each cocoon and measured how well they coped with being stretched. They found that *O. eucalypti* cocoons, which use the most glue and are the least porous, were the strongest, withstanding more force. However, because of the glue, the multiple layers acted as one thick layer and when it failed, the stress penetrated all layers, causing a clean break. Although *A. pernyi* cocoons started failing under lower stress, the breakdown was more gradual, with the fibres gradually disentangling.

Next the team used a rod to mimic a predatory pecking beak and pressed down onto the side of each cocoon, measuring how each cocoon deformed. Using a mathematical model, the team worked out that stretching was greatest on the inside

convex surface of the indentation, hence why the innermost layer is the least porous and strongest. The team also found that by using less inter-layer glue, the individual layers could detach in *A. pernyi* cocoons, which the team thinks might help prevent stress transmitting between layers. Their results suggest that if your nemesis is a peckish bird, then less is more when it comes to gluing your cocoon together. While *O. eucalypti* cocoons would not stand up to a bird's beak, they may be more suited to different threats such as water or bacteria, where a strong barrier is required.

10.1242/jeb.090084

Chen, F., Hesselberg, T., Porter, D. and Vollrath, F. (2013). The impact behaviour of silk cocoons. *J. Exp. Biol.* **216**, 2648-2657.

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