

INSIDE JEB

Sociable crayfish get drunk more easily than loners



Inebriated crayfish. Photo credit: Alexis Exum and Jens Herberholz (University of Maryland, College Park).

The effects of alcohol can be unpredictable; while some consumers become amiable and affectionate, others turn into brutish thugs, and Jens Herberholz, from the University of Maryland, USA, explains that the cellular mechanisms that underpin the consequences of intoxication remain elusive. ‘Alcohol is a complicated drug’, he says, because it affects a wide range of cellular systems, making it difficult to unravel which factors contribute to alcohol sensitivity. However, humans are not the only animals that can suffer the consequences of over-indulgence; inebriated crayfish tail-flip animatedly while under the influence and become heavily intoxicated after lengthy exposures. Having studied the cellular mechanisms that underlie decision-making and aggression in these crustaceans, Herberholz was curious to learn how previous social experience might impact the effect of alcohol on crayfish.

‘How past social experience might shape the neurobehavioural effects of acute alcohol exposure is significantly understudied’, says Herberholz, who teamed up with his students Matthew Swierzbinski and Andrew Lazarchik to find out how inebriated crayfish behave. Intoxicating individual crayfish – which had previously been housed together – in tanks of dilute alcohol ranging from 0.1 to 1 mol l⁻¹, members of the lab filmed the animals as they initially began walking aggressively on stiff straight legs, before switching to tail-flipping as they became more intoxicated, and finally losing

control as they rolled on their backs like incapacitated humans. And the effects took hold much faster at the highest concentrations, with the intoxicated animals enthusiastically tail-flipping after 20 min in the strongest alcohol, while the animals that were bathed in the most dilute alcohol took almost 2 h to feel the effects. However, when the trio tested the effects of the most concentrated alcohol on crayfish that had been held in isolation for a week before their drinking spree, the animals were far less sensitive to the alcohol, taking 28 min to become inebriated and begin tail-flipping.

But how were the effects of intoxication manifested in the neurons that control the crayfish’s drunken behaviour? Inserting fine silver wires near the sensory nerves that excite the lateral giant interneuron – which controls the tail-flipping behaviour – Lazarchik recorded that the neural circuit became more sensitive in both the isolated and gregarious crayfish when the crustaceans were inebriated. However, the effects of alcohol became apparent more swiftly in the sociable crayfish’s lateral giant interneuron, mirroring the animals’ behavioural sensitivity. Swierzbinski was even able to use intracellular electrodes to measure a difference in the effects of alcohol in individual neurons in the isolated and communal crayfish. Paying tribute to Swierzbinski, Herberholz says, ‘It takes talent and patience to collect data from enough animals’.

As the inhibitions of the drunk socialised crayfish were loosened more than those of the drunken loners, Herberholz suspects that the alcohol has more of an impact on the GABA neurotransmitter, which inhibits behaviour, in the gregarious crayfish. He also speculates that isolation could make humans less sensitive to the effects of alcohol, leading them to consume more. Herberholz says, ‘Our study shows that social experience can change the sensitivity to acute alcohol’. He adds, ‘Inebriated people...could potentially have different responses to alcohol depending on their prior social experience’. And, although we are still a long way from confirming that social experience produces similar effects in the brains of inebriated mammals

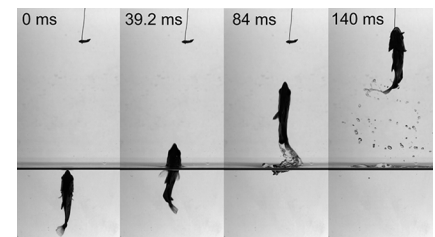
(including humans), Herberholz is optimistic that, one day, drunken crayfish could help us to develop better treatments and preventative measures to support humans suffering from alcohol abuse.

10.1242/jeb.159822

Swierzbinski, M. E., Lazarchik, A. R. and Herberholz, J. (2017). Prior social experience affects the behavioral and neural responses to acute alcohol in juvenile crayfish. *J. Exp. Biol.* **220**, 1516-1523.

Kathryn Knight

Archer fish jump as well as shoot



An archer fish take-off. Photo credit: Anna Shih.

While spitting is taboo in some human cultures, a few species have honed it to a fine art, from cobras that eject venom into the eyes of their victims to archer fish that down their prey with a precisely aimed jet of water. In addition, archer fish have implemented another weapon in their hunting arsenal – they jump out of the water to snap up prey – and Alexandra Techet, from the Massachusetts Institute of Technology, USA, explains that this alternative method of attack is more likely to be successful than spitting when other hungry archer fish are gathered round. ‘It’s a competitive world out there. When they spit there is no guarantee that they’ll actually get to eat the prey, but when they jump it’s almost guaranteed that they will’, says Techet. With a passion for designing aquatic robots, Techet turned to the agile archer fish for inspiration.

‘We trained them by hanging bait above the tank’, says Techet, who removed the bait whenever the fish tried to spit. Once the fish were vaulting reliably, Techet and Anna Shih filmed the animals with a high-speed camera as they attempted to reach *Gammarus* shrimp suspended as high as

2.5 times the fish's body length above the surface of the water. Analysing the fish's tactics during the countdown, Leah Mendelson observed that once the fish had spotted the tasty treat, they hovered below it weaving their pectoral fins to and fro until ready for the launch. 'This part of the process is the same as when they hunt by spitting', says Techet. But as soon as the take-off was initiated, the fish began beating their tails hard from side to side while extending their pectoral fins as they surged upward and broke through the surface. Sometimes the tail continued flapping to and fro even when they were sailing through the air. Correlating the number of propulsive tail beats with the height reached, Techet was surprised to see that instead of flapping harder, the fish increased the number of tail beats to reach the highest bait, landing an impressive 70% of their catches.

In addition to determining the fish's launch technique, Techet and Shih visualised the swirling patterns generated in the water as the fish powered upward to learn more about the forces that propel the fish into the air. Although convincing the fish to jump in a plane of laser light while filming was technically challenging, Mendelson eventually discovered that the fish used their tails in combination with the anal, pectoral and dorsal fins to generate enough thrust to become airborne. And when she calculated the energetic cost of a fish launch and compared it with the amount of energy consumed during the frantic dash to capture prey after a successful squirt, the two came out about equal – ranging from 2.5 to 47 mJ – suggesting that the jump strategy may be as efficient as pursuing a victim downed by a well-aimed jet of water.

Having revealed how the fish jump out of the water from a stationary position, Techet and Mendelson are now keen to learn more about the contribution of the tail and other fins to the lift-off, and Techet adds, 'This work serves as the foundation for our ultimate goal', which is to produce a 3D model of the physics of a launch to design archer-fish-inspired robots that can take off smoothly from water.

10.1242/jeb.159806

Shih, A. M., Mendelson, L. and Techet, A. H. (2017). Archer fish jumping prey capture: kinematics and hydrodynamics. *J. Exp. Biol.* **220**, 1411-1422.

Kathryn Knight

Spinner dolphin SCUBA tanks develop no faster



Spinner dolphins. Photo credit: US Fish and Wildlife Service Headquarters (uploaded by Dolovis) [CC BY 2.0, via Wikimedia Commons].

Just because dolphins are born in water doesn't necessarily mean that their in-built SCUBA system is fully prepared for action at birth; it can take between 1 and 3 years for the oxygen carrying capacity of whales and dolphins to mature sufficiently. Shawn Noren, from the University of California, Santa Cruz, USA, explains that the muscles of fully developed diving species – including dolphins, whales, birds and seals – contain more of the oxygen carrying protein, myoglobin, than land-based animals and are better prepared to neutralise lactic acid produced in the muscles when divers switch to anaerobic respiration after exhausting their oxygen toward the end of a dive. 'We wondered if pelagic (offshore) living promotes rapid postnatal maturation of muscle biochemistry', says Noren. In other words, might deep-diving ocean-going whales and dolphins develop large reserves of myoglobin and the ability to buffer muscle against acid earlier in life than species that remain in shallow coastal waters?

As it is almost impossible to collect muscle samples from spinner dolphins in the open ocean, Noren depended on Kristi West, from Hawaii Pacific University, USA – who set up a dolphin stranding program in Hawaii 11 years ago and attends all strandings on the island – to collect the essential samples. Over 7 years, West collected small portions of the swimming muscle from 17 spinner dolphins that her team had been unable to rescue – ranging from a fetus that died during birth to newborns, adolescents and fully grown males and females. She then shipped the samples to Santa Cruz, where Noren painstakingly analysed the muscles' myoglobin content and how much sodium hydroxide she had to add to 0.5 g of minced muscle to raise the pH from 6 to 7 to measure the muscle's buffering capacity against

anaerobic acid production. Plotting the animals' body lengths (which correlate well with their ages) against their muscle myoglobin content, Noren could see that the dolphins' abilities to carry oxygen continued increasing as the animals aged. The ability of the muscle to buffer against pH changes also increased gradually; however, it reached the capacity of the mature dolphins and plateaued at an age around 1.6–2 years, when the dolphin youngsters are weaned, which is similar to the age at which the diving apparatus of some coastal species reaches maturity.

So ocean-going spinner dolphin calves do not develop the physical characteristics that are essential to sustain deep dives any faster than shallow-diving coastal species, such as bottlenose dolphins. However, the youngest spinner dolphins already had higher concentrations of muscle myoglobin than coastal bottlenose dolphins at the same ages, and the adult spinner dolphins' myoglobin concentrations (6–7.1 g Mb 100 g⁻¹ wet muscle mass) matched those that had been measured previously for other champion divers, including short-finned pilot whales and Gervais' beaked whales.

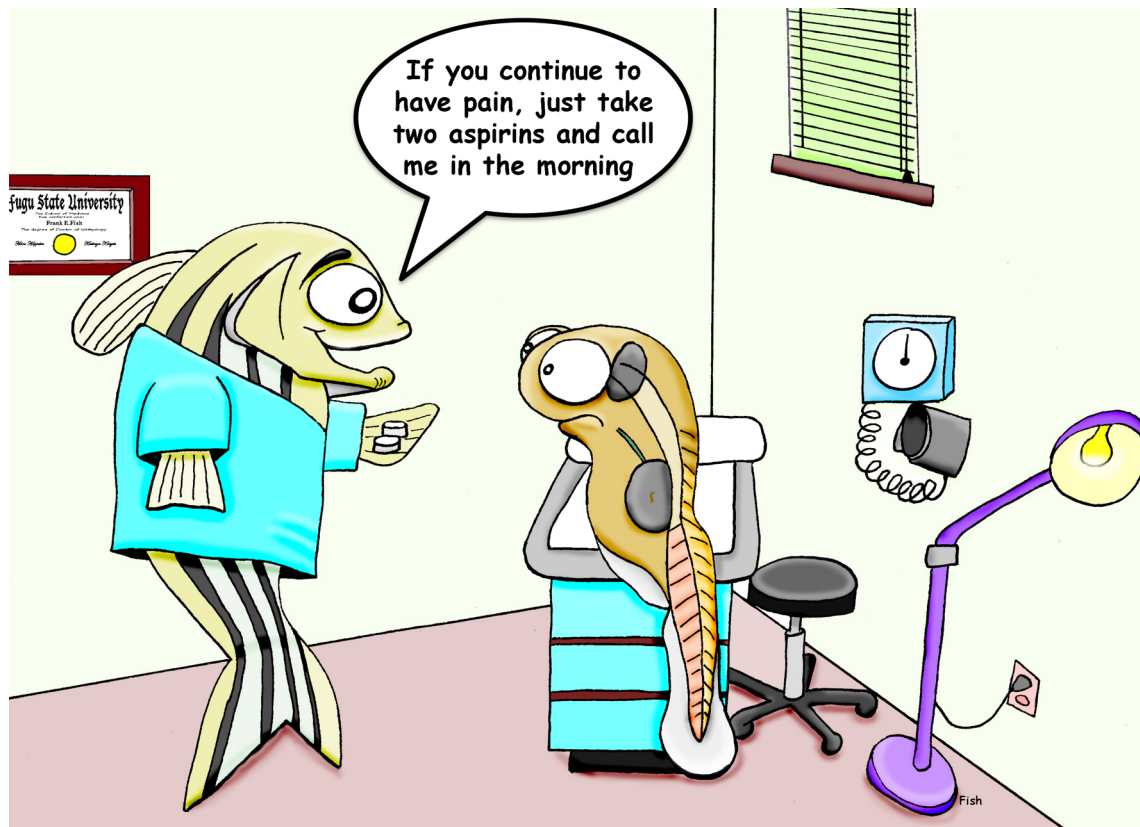
But what implications might the relatively slow development of their diving apparatus have for young spinner dolphins in the Eastern Tropical Pacific? Knowing that tuna purse-seine fisheries in this region specifically target dolphin pods – they pursue the animals to exhaustion before encircling them in enormous nets to capture the tuna shoals that reside beneath – Noren calculated that an immature calf that cannot keep up might be adrift of its mother by up to 15.4 km by the end of a 100 min pursuit. Noren says, 'The relatively underdeveloped muscle biochemistry of calves likely contributes to documented mother-calf separations for spinner dolphins chased by the tuna purse-seine fishery', and this could affect dolphin populations dramatically if our hunger for tuna continues to separate dolphin calves from their mothers.

10.1242/jeb.159798

Noren, S. R. and West, K. (2017). Muscle biochemistry of a pelagic delphinid (*Stenella longirostris longirostris*): insight into fishery-induced separation of mothers and calves. *J. Exp. Biol.* **220**, 1490-1496.

Kathryn Knight

Painkillers relieve zebrafish larvae discomfort



Lynne Sneddon is a myth buster. Having debunked the fisherman's legend that fish don't feel pain, Sneddon, from the University of Liverpool, UK, has become a leading figure in the movement to reduce, replace and refine the use of animals in scientific research.

Uncomfortable with the increasing use of adult fish in pain research, Sneddon and Javier Lopez-Luna decided to test whether tiny zebrafish larvae feel pain. 'Previous studies have identified multiple subtypes of nociceptors [pain receptors] in zebrafish...even as early as a few days post-fertilization', the team says. Could they replace the adult fish that are used in research with larvae that are a matter of days old? Only if they could prove that the fish respond to pain and any discomfort could be relieved.

Lopez-Luna and Sneddon exposed 5-day-post-fertilization zebrafish embryos to dilute concentrations of acetic acid and citric acid, both of which are known to irritate adult fish, and tracked the larvae's activity with software produced by Qussay Al-Jubouri and Waleed Al-Nuaimy. Analysing the minute fish's motion, Lopez-Luna and Sneddon noticed that the larvae became less active in the two most dilute concentrations of acetic acid (0.01 and 0.1%). However, the most concentrated acetic acid (0.25%) and all three concentrations of citric acid (0.1, 1 and 5%) stimulated the fish to swim harder and farther, possibly in a bid to escape the uncomfortable sensation. But when Lopez-Luna administered pain relief to the disturbed fish larvae – in the form of aspirin, morphine and lidocaine –

their discomfort appeared to be relieved and their behaviour returned to normal.

Having confirmed that larval fish are capable of experiencing pain and benefit from pain relief, Sneddon and Lopez-Luna recommend, 'Larval zebrafish can be used as a model for the study of pain and nociception', sparing many of the adult fish that are currently used in toxicity tests.

10.1242/jeb.159814

Lopez-Luna, J., Al-Jubouri, Q., Al-Nuaimy, W. and Sneddon, L. U. (2017). Reduction in activity by noxious chemical stimulation is ameliorated by immersion in analgesic drugs in zebrafish. *J. Exp. Biol.* **220**, 1451-1458.

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