

INSIDE JEB

Juvenile macaques lose vocal communication as they age



Rhesus macaque, Red Fort, Agra, India. Photo credit: By Yann (own work) [CC BY-SA 4.0-3.0-2.5-2.0-1.0], via Wikimedia Commons.

Ceaselessly chattering, humans are a garrulous species that have developed thousands of languages for communication over the course of evolution. Yet, it can be hard to appreciate just how far human verbal communication has come as we converse continually. ‘Basically, speech is the ability to produce arbitrary acoustic signals to communicate’, says Steffen Hage from the University of Tübingen, Germany, adding that some of our more distant relatives may have had some of the essential aptitudes that eventually allowed our ancient ancestors to evolve the rudiments of speech. Explaining that humans diverged from our macaque monkey ancestors about 25 million years ago, Hage was curious to find out whether modern macaques might have the ability to respond vocally to an abstract event that they would not experience in everyday life. ‘If these precursors of speech also exist in rhesus macaques, it would imply that they already existed in the last common ancestor’, says Hage, who teamed up with Andreas Nieder and Natalja Gavrilov to test the monkeys’ abilities.

Working with two male rhesus macaques, Caruso and Torkel, Hage and Gavrilov trained the animals to initiate an experiment by gripping a bar to make a white square appear on a computer screen and then to utter a call when the square changed colour. ‘This took us quite a while’, says Hage, remembering months of training before

the animals mastered the task. And once the animals had grasped the concept at 4.9 years of age, Hage and Gavrilov periodically retested the animals’ ability to make a call in response to changes on the computer screen over several years.

At first, all went well. The two monkeys enthusiastically initiated hundreds of experimental runs in return for a juice reward and produced a call showing that they were capable of communicating vocally in response to an event. However, as the years progressed and the monkeys aged, something seemed to go wrong. ‘The performance decreased’, says Hage, explaining that the animals continued initiating the test, but they began taking longer to respond when the square changed appearance and made fewer and fewer calls over time. Eventually, by the age of 8, neither monkey made a vocal sound as the square changed colour. ‘This is something that we wouldn’t have expected’, says Hage, adding, ‘Most animals that are trained in other tasks get better and better over the years’.

Perplexed, the team trained the monkeys to perform another – more complex – operation, where they had to grasp a bar and release it after they had been shown two matching boxes. Both animals picked up the new activity and performed it with relish: they had not lost the ability to learn. Hage and Gavrilov also monitored the animals’ interactions with other members of the troop, and Caruso and Torkel continued calling and communicating with them.

Having ruled out the possibility that the animals had lost the ability to learn or to communicate vocally, Hage and Nieder realised that the monkeys began to lose the ability to respond vocally to the changing square as they matured into adults. ‘We said, “OK, this is probably when something is changing in the connections in the brain”’, says Hage. He suspects that juvenile macaques have some of the prerequisite aptitudes that are necessary for the development of speech, but that these are lost as they develop into adults – which also ties in with the theory that an extended period of juvenile

development was, and still is, crucial for the evolution of human speech.

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Hage, S. R., Gavrilov, N. and Nieder, A. (2016). Developmental changes of cognitive vocal control in monkeys. *J. Exp. Biol.* **219**, 1744-1749.

Kathryn Knight

Clam mitochondria respond better to hypoxia than scallop mitochondria



A mitochondrion (watercolour and tempera). Image credit: Inna Sokolova.

Few terrestrial animals ever experience hypoxia (when oxygen is scarce), but spells of low oxygen availability are routine for creatures clinging to life on the seashore: ‘Diurnal cycles of respiration and photosynthesis in the coastal zones commonly lead to O₂ swings from near-anoxia during the night to hyperoxia during the day’, says Inna Sokolova from the University of North Carolina at Charlotte, USA. Yet, many of these creatures can withstand periods of oxygen deprivation lasting weeks and even months. Sokolova explains that several of the mechanisms that underpin the coast dwellers’ survival are already known, including the use of alternative mechanisms to generate ATP and the animals’ ability to drop their metabolic rate to conserve energy. However, Sokolova explains that little was known about how these resilient species protected the essential mitochondria – which consume oxygen to generate ATP – from the damaging effects of oxygen deprivation.

Sokolova and a team of colleagues investigated the function of mitochondria in two shoreline species: the hypoxia-

tolerant hard clam (*Mercenaria mercenaria*), which can survive for 2 weeks with no oxygen at all, and the less robust bay scallop (*Argopecten irradians*), which can only survive a few hours in anoxia. Keeping the bivalves in deoxygenated water (less than 0.1% oxygen) for 18 h, the team then collected samples from the gills, hepatopancreas tissue and adductor muscles to find out how the mitochondria had responded to oxygen deprivation. In addition, the team collected tissues from molluscs that had not experienced hypoxia, and from molluscs that had been transferred back into water with normal oxygen concentrations after hypoxia to find out how they responded as damaging oxygen flooded back into their bodies.

Analysing the animals' responses, the team saw that when the oxygen returned after an extended period of hypoxia, the scallops suffered similar experiences to those of other hypoxia-sensitive animals: the mitochondria were impaired, reducing their oxidation and phosphorylation capacities as they partially depolarized and the potential difference across the membrane reduced. In parallel, the vulnerable scallops ramped up production of heat shock proteins – which mop up proteins that have been damaged by the oxygen influx – in order to reduce the damage incurred by the mitochondria during reoxygenation. In contrast, the robust clams increased the oxidative capacity of their mitochondria during hypoxia and this also continued rising when the oxygen returned. Meanwhile, both of the molluscs reduced phosphorylation activity (which is normally responsible for ATP production via the F₀F₁-ATPase) during the extended period of hypoxia, 'Likely to prevent ATP wastage by the reverse action of the ATPase', say Sokolova and colleagues, although the clams regained this activity during the 1 h period of reoxygenation, when the scallops' phosphorylation activity failed to improve. And when the team tested the bivalves' ability to conserve energy by downregulating non-essential metabolic processes, it was clear that the clam was better able to reduce its energy expenditure than the more vulnerable scallop, although neither animal was severely energy deprived.

So, a period of hypoxia dramatically affects both the hypoxia-tolerant hard

clam and the more susceptible bay scallop, but alterations in the physiology of the clam leave it better prepared to tackle extended periods of hypoxia than its scallop shore-mate.

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Ivanina, A. V., Nesmelova, I., Leamy, L., Sokolov, E. P. and Sokolova, I. M. (2016). Intermittent hypoxia leads to functional reorganization of mitochondria and affects cellular bioenergetics in marine molluscs. *J. Exp. Biol.* **219**, 1659–1674.

Kathryn Knight

How high temperatures affect smelt in extreme California drought



Two delta smelt. Photo credit: Ken Jeffries.

With the current drought in California considered to be the worst in more than a millennium and with the state desperately trying to reduce water consumption, conservationists are becoming increasingly concerned about the impact of the extreme conditions on local ecosystems. 'Many regions of freshwater habitats have been showing an increase in water temperatures', says Ken Jeffries from the University of California (UC), Davis, USA, and the longfin smelt and delta smelt, which reside in the inland California Delta, are at particular risk. 'Their abundance has reached record lows', says Jeffries, warning, 'It has been feared that the current drought will be the final "nail in the coffin" for these species in Northern California.' Knowing that delta smelt are vulnerable to high temperatures, Jeffries says, 'However, we really did not know whether the temperatures have reached levels that may be detrimental to longfin smelt'. So, Jeffries teamed up with colleagues from UC Davis and the California Department of Water Resources – Richard Connon, Ted Sommer and Nann Fangue – to design a series of experiments to find out

how the larvae of both species fare as the temperatures rise.

As populations of longfin and delta smelt have crashed over recent years, Jeffries, Brittany Davis and Lisa Komoroske investigated the resilience of smelt larvae bred at the UC Davis fish culture facility instead of collecting animals from the wild. Jeffries also admits that working with the longfin smelt was challenging. 'They are extremely sensitive to handling,' he explains.

Having learned how to work with the delicate creatures, the trio placed individual longfin smelt in small tanks and gently warmed the water until the fish toppled over to find the maximum temperature that they could tolerate. Recording that the longfins succumbed at 24.8°C, Jeffries explains that the delta smelt were already known to remain upright until 27.6°C, which corresponds well with the highest temperatures that the fish appear to bear in the wild. Jeffries warns that the longfin smelt are more vulnerable to high temperature than the delta smelt as the temperatures where the longfin smelt live in the delta are already approaching their thermal limit. And when Davis, Jeffries and Anne Todgham measured the metabolic rate of both species at 14 and 20°C, the metabolic rate of the longfin smelt changed little as the temperature rose from 14 to 20°C (29.0–21.9 μmol O₂ h⁻¹ g⁻¹), although the metabolic rate of the delta smelt increased dramatically from 47.9 to 65.5 μmol O₂ h⁻¹ g⁻¹ as the temperature increased.

After confirming that the threatened longfin smelt was more vulnerable to high temperatures than the already endangered delta smelt, the team warmed the two species to 20°C and collected samples of their RNA to find out which genes had been activated. Analysing the change in the gene transcript patterns with Monica Britton, Jeffries saw that the longfin smelt activated their cellular heat shock response for protection against the threatening high temperatures, in addition to expressing genes associated with growth and development. However, the delta smelt increased the expression of genes associated with metabolic

processes, reflecting their increased metabolic rate.

Having identified cellular markers that can be used in the field to identify when both species are suffering the effects of temperature, Jeffries says, 'Resource managers [in California] now have an

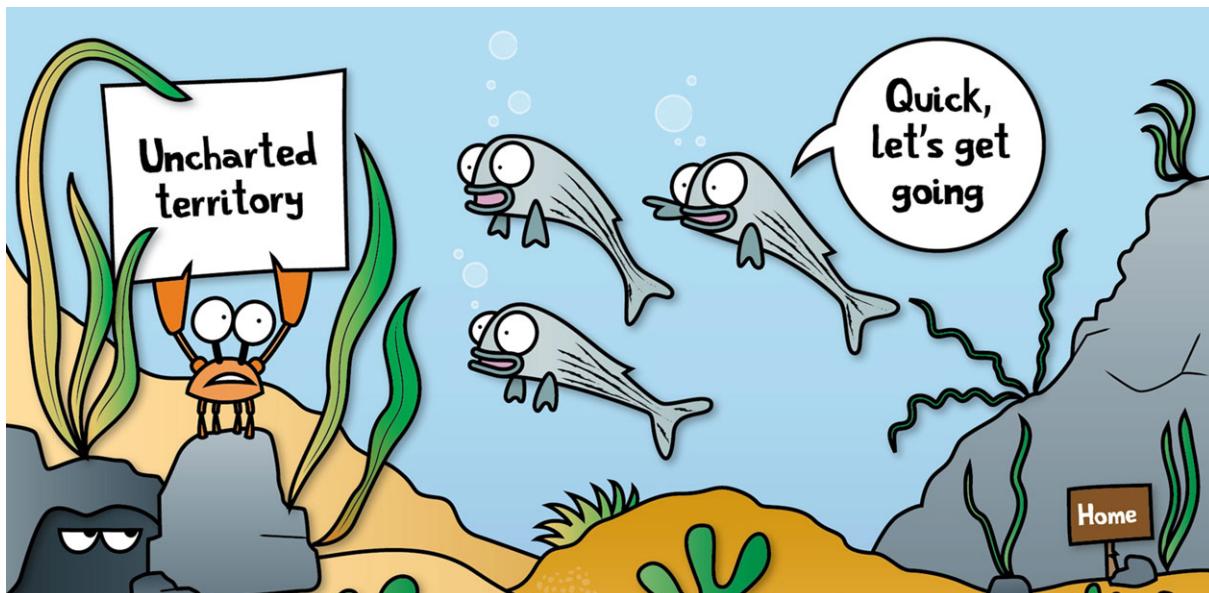
idea of when temperatures become stressful and unsustainable for longfin smelt', and adds that it is essential that we provide refuges at temperatures below 20°C for the freshwater life stage of the longfin smelt if they are to survive this and future droughts that may grip California.

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Kathryn Knight

Exploration more costly than staying put



Animals move continually, interacting with others, foraging for food and searching for new territory, but what determines the speed at which they go about their everyday business? 'Often, animals do not reach their maximal locomotor capacity when moving under undisturbed conditions', say Frank Seebacher and colleagues from the University of Sydney, Australia. Having discussed potential mechanisms that may determine the voluntary speed at which animals move, including the role of muscle efficiency and the impact of temperature on muscle contraction, Seebacher and his team monitored the movements of fish swimming at 18, 24 and 30°C in a shallow featureless tank

before analysing their movements during the first and tenth minute to determine how their voluntary speed varied with their familiarity with a location. In addition, the team measured the fish's top swimming speed and their metabolic rate in a bid to identify the factors that determine the fish's voluntary speed.

The team found that the fish increased their voluntary speed when exploring novel environments regardless of the increased metabolic cost that this incurred. However, when they were familiar with their surroundings and at the temperature that they normally inhabit, their energy expenditure did not increase. 'The implications of these data

are that the energetic costs of exploring and dispersal into novel environments is relatively greater than movement within familiar home ranges', says the team, who suspect that the urge to explore novel settings overrides the fish's determination to conserve energy, making exploration a costly prospect for fish that fail to locate new resources or encounter predators.

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Kathryn Knight
kathryn.knight@biologists.com