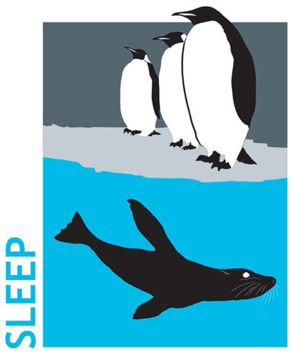
OUTSIDE JEB

Penguins prefer power naps



If you ask a student, parent or working professional whether they get enough sleep each night, many will laugh at the question. Sufficient rest may seem an impossible goal in the hustle and bustle of modern life, but what if you could achieve the prized 8 hours of sleep without ever sleeping at all? This is, of course, impossible for humans. However, busy penguins never seem to sleep. Chinstrap penguins nest as a colony and in every penguin pair, one parent stays home with their young while the other parent forages to feed the family. As any human parent knows, full-time childcare is tiring work, as is providing for the family. Factor on top of these the necessity for chinstrap penguins to be vigilant against predators and it is obvious that both nesting and foraging penguin parents will need a lot of rest. However, sleeping for hours would put them, and their chicks, at great risk. A new study shows that chinstrap penguins mitigate this trade-off by utilizing a strategy known as microsleeping.

Paul-Antoine Libourel (Neuroscience Research Centre of Lyon, France) and colleagues from France, the Republic of Korea and Germany investigated how nesting chinstrap penguins are able to obtain enough rest without sleeping for extended periods of time. The researchers monitored the activity of nesting penguins by filming them and outfitting individuals with wearable motion sensors. The sensors tracked the animal's body posture and sleep stages to identify when the tired penguins nodded off, similar to a human fitness tracker. The research team was particularly interested in slow wave sleep picked up by the sensor, which indicates deep and restorative rest. The team also investigated how sleep quality compares between individuals nesting on the colony's border, who are more vulnerable to predators, and individuals in the center of the colony, who are protected from predation.

Libourel and colleagues found that nesting chinstrap penguins attained an incredible total of ∼15 hours of slow wave sleep per day despite never appearing to take a nap. Astonishingly, these hours came from the accumulation of thousands of daily naps that lasted only 4 seconds. Not only that, but most of these naps were not really naps at all. Rather, the penguins were taking microsleeps, where they put one hemisphere of their brain to sleep and close only the associated eye, while concurrently the other hemisphere of the brain, and the other eye, remain wide awake. Both the right and left hemispheres of the brain received a luxurious 11–12 hours of sleep per day. Moreover, contrary to expectation, the team discovered that penguins on the edge of the colony enjoyed longer, deeper and less fragmented sleep than penguins at the center of the colony, suggesting that aggression from penguin peers is more stressful than the risk posed by predators.

Microsleeping is an incredible testament to the strategies that animals can employ to balance their physiological and ecological needs. In the case of chinstrap penguins, taking microsleeps allows them to restore their physiological systems while remaining vigilant for threats from predators and peers. The success of the species suggests that, although fragmented, this sleep pattern provides the same large-scale restorative functions as uninterrupted sleep. However, further research is warranted into how the full restorative value of microsleeping compares to that of typical sleep.

Although it has long been known that birds experience slow wave sleep in shorter bouts than mammals, Libourel and colleagues' discovery that chinstrap penguins accumulate 15 hours of sleep daily over thousands of microsleeps that last only 4 seconds is unprecedented. If only human systems could operate this way and allow us to benefit from the many times we've nodded off during boring lectures.

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Libourel, P.-A., Lee, W. Y., Achin, I., Chung, H., Kim, J., Massot, B. and Rattenborg, N. C. (2023). Nesting chinstrap penguins accrue large quantities of sleep through seconds-long microsleeps. *Science* **382**, 1026-1031 doi:10.1126/science.adh0771

Sarah Young-Veenstra (0009-0007-8863-1323)
Saint Mary's University, Halifax, Canada
sarah.young@smu.ca

Brain estrogen encourages egg ejection

Reproduction is a complicated affair, requiring the well-timed coordination of hormone levels, brain signals and behavior. The humble zebrafish makes it look easy – their frequent reproduction and abundant offspring contribute to the popularity of this species in research. Estrogens are among the most important steroid hormones involved in reproduction. Fish have a unique quirk in their estrogen production system due to a gene duplication: they have two specialized versions of the estrogen-producing enzyme whereas most mammals and birds have just

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one. One version is primarily found in the gonads of fish, including the ovaries, where estrogen production is critical for proper development and function. The other version is primarily found in the brain, but its role there is not so clear. Could this tissue-specific production of estrogen in the brain of fish impact their reproduction? Katherine Shaw (University of Ottawa, Canada) and colleagues from the same institution and Sun Yat-Sen University, China, investigated whether disruption of estrogen production in the fish brain can really throw a wrench into the gears of reproduction.

To tackle the question, Shaw and colleagues genetically modified zebrafish to disrupt the gene that codes for this estrogen-producing enzyme in the brain. This approach allowed them to specifically inhibit estrogen production in the brain, while leaving ovarian estrogen production intact. Accordingly, they measured lower levels of estradiol (the primary estrogen in fish) in the brain of these mutated female fish. They also observed that these female fish were about four times slower to start releasing eggs when paired with a normal male during their morning mating session. Without this enzyme supplying estrogen in their brain, the normal mating behavior of the female zebrafish was thrown off. But this result led to another question – how does a drop in brain estradiol levels bring about the delay in egg release?

This is where an important duo of signaling molecules – arginine vasopressin and oxytocin – join the story. These two molecules are produced in the brain of fish and can contribute to signaling pathways that control reproductive behavior and gamete release. Since egg release was delayed in the mutated females, the researchers suspected that arginine vasopressin and/or oxytocin might link the estrogen hormone in the brain to the timing of egg release. They measured these signaling molecules in the brain of the mutated female fish and found that both were reduced. Was this the link between reduced brain estradiol and the delay in releasing eggs? Could they perhaps restore the normal timing of egg release during mating by increasing levels of arginine vasopressin or oxytocin in the fish's body?

To find out, the team injected mutated female fish (with reduced brain estradiol) with either arginine vasopressin or oxytocin and observed them during mating. They found that the arginine vasopressin-injected fish now released their eggs within a more typical timeframe. The restoration of this behavior showed that arginine vasopressin is a key signal linking estrogen production in the brain to egg release during mating. When the researchers fluorescently labeled arginine vasopressin and the enzyme for estrogen production in the zebrafish brain, they observed that the two molecules were produced in different cell types; however, the cells were in close proximity, potentially allowing brain-derived estrogens to stimulate nearby production of arginine vasopressin.

What have we learned from all this? Fish have a gene that encodes brain-specific estrogen production, which is, with the help of arginine vasopressin, critical in the prompt timing of egg release during zebrafish mating. This has laid a foundation to further explore the roles of brain-specific estrogen production in zebrafish as well as in other fishes, so researchers can spawn new ideas about how sex steroids influence the brain and behavior.

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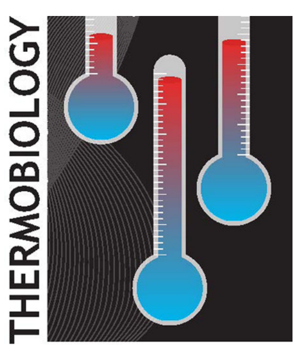
Shaw, K., Lu, C., Liu, X. and Trudeau, V. L. (2023). Arginine vasopressin injection rescues delayed oviposition in *cyp19a1b*-/- mutant female zebrafish. *Front. Endocrinol.* **14**, 1308675. doi:10.3389/fendo.2023.1308675

Carol Best (0000-0003-4769-4241)
University of Guelph, Canada
cbest03@uoguelph.ca

Lovebird cooling before, after and on the fly



In the wild, many animals generate body heat when they move which they need to get rid of to avoid overheating. Out of all the various ways that animals move, flight requires the greatest amount of energy, leading to one of the highest rates of body heat production. To lower their body temperatures, birds get rid of heat though their head, feet and the top of their wings. Losing the heat that they produce while flying is especially important for birds when considering the rapidly warming global temperatures. But how important each body area is for dissipating heat before, during and after flight has not yet been determined for any bird species. This question led Agnès Lewden and colleagues from the University of Leeds, UK and Institut Universitaire Europèen de la Mer, France to determine how yellow-collared lovebirds (*Agapornis personatus*) dissipate their body heat before, during and after flight.

To test which body areas were important for getting rid of the excess heat the lovebirds generate while flying, the team studied seven lovebirds that were trained to fly in a wind tunnel located at the University of Leeds. The researchers randomly flew each lovebird 11 times and used a thermal camera to continuously record the lovebirds. Each recording started with the birds standing on a wooden perch before the birds flew, during flying and ended when the birds were resting again on the perch after landing. Following all flight trials, the researchers analysed the thermal videos and measured the surface temperature of the birds' head (including the eyes and bill), the legs (including the feet), the body (including the front, back and the top of the wing), and – while the lovebirds flew – the underside of the wings (which was visible only during flight). The team then used mathematical modelling to calculate the different forms of heat loss (i.e. convection – heat loss due to air moving past the bird; radiation – heat lost to the air through infrared rays; and conduction – heat loss through physical contact) for each part of the birds before, during and after flying.

Lewden and colleagues found that the birds' heat dissipation while flying was 12-times higher than before their flight and 19-times higher than after they landed again, likely resulting from the greater amount of body heat produced during flight and their need to get rid of it. When the researchers looked at each body area separately, the team uncovered differences in how and when they dissipate heat. Specifically, before flying, body heat was mainly dissipated by the head and body but during the flight, the birds dissipated the majority of their body heat (85.9%) through the underside of the wings, despite this body area only making up 26% of the birds' surface area. This shows the importance of the underside of the wings in keeping birds cool. The researchers also discovered that the lovebirds dissipated heat differently depending on whether they were perched or flying. The birds lost heat mainly through infrared rays (radiation) before and after their flights. However, heat loss during flight was primarily through air rushing past the underside of the wings (convection), likely reflecting the change in the lovebird's body posture during flight.

Overall, Lewden and colleagues showed how lovebirds dissipate body heat through different body areas before, during and after flight. Their research is especially important when considering the rapidly warming global temperatures and suggests how other bird species may lose excess heat in hotter temperatures. Further research is needed to determine if other species cool themselves like lovebirds do before, after and on the fly.

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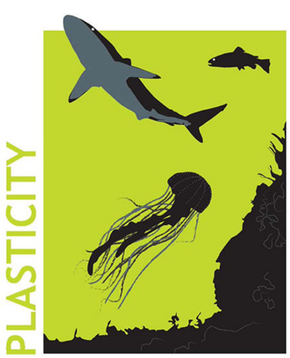
Lewden, A., Bishop, C. M. and Askew, G. N. (2023). How birds dissipate heat before, during and after flight. *J. R. Soc. Interface.* **20**, 20230442. doi:10.1098/rsif.2023.0442

Kristina A. Muise (0000-0002-0497-4086)
Royal Veterinary College, London, UK
kristinamuise@gmail.com

Fish hum, grunt and growl with their swim bladder



Animals experience interesting seasonal transformations, such as the thick fur that animals in the Arctic grow in the winter. This phenomenon can also extend to vocal systems. The plainfin midshipman fish, found along the North American coast of the Pacific Ocean during summer, undergo seasonal changes in their swim bladder. Swim bladders are gas-filled organs that most fish use for buoyancy; however, plainfin midshipman vibrate the organs to produce distinctive sounds for communication. The male fish hum to the females during the breeding season and these calls are supported by the muscles around the bladder that develop before the summer. In addition, the swim bladder helps them to hear the calls of other chatty fish in the vicinity by transmitting sounds that they then pick up with their ears. But how much does the structure of the swim bladder change in the months leading up to, and during, the mating season and how do these changes affect the fish's hearing and the sounds they produce? A recent study by Joseph Sisneros and colleagues at the University of Washington and Seattle Children's Research Institute, USA, addresses these questions.

In their investigation, Sisneros and colleagues gathered fish from Puget Sound, USA, during February 2022 when the fish were not breeding and again between May and June, during the fish's breeding season. They then used X-ray-based CT scans to closely examine changes in the fish's swim bladder over the months. Sure enough, the swim bladder changed size and shape with the season. In February, the swim bladders of the non-reproductive males had horn-like extensions that brought the swim bladder closer to the fish's inner ear, enhancing their sensitivity to low-frequency (less than 800 Hz) sounds. In contrast, during the mating season in May and June, the males had three times more muscle mass around the bladder, enabling them to sing and call to females for longer. However, this increase in muscle mass also altered the shape of the swim bladder – increasing its width, while shortening the length – increasing the distance to the inner ear, which could reduce the sensitivity of the fish's hearing in the summer.

In a follow-up experiment, the researchers checked the hearing of the fish to find out how the organ contributes to their hearing over the seasons. They found that the hearing of the non-breeding males in February is enhanced (12.5 times more sensitive than the hearing of non-breeding males without a swim bladder) and tuned to sounds in the environment. In contrast, when they removed the swim bladder from breeding males and compared their hearing with that of intact breeding males, their hearing was unaffected. The breeding fish were not using their swim bladders to help them pick up sound, probably to protect their hearing from their own droning 2-hour-long serenades while courting females.

Lastly, Sisneros and the team tried to mimic how the swim bladder vibrates, to understand how well the swim bladder is tuned to produce sounds in one season while contributing to hearing in another. The researchers did this by building a model of the breeding and the non-breeding fish's swim bladders in a computer simulation and then calculating how the structures would vibrate. This showed that the swim bladder of the breeding male midshipman fish is tuned to produce the low frequency social calls – hums, grunts and growls – that they use during courtship. On the flip side, non-breeding males in February have swim bladders that are geared towards higher frequency sounds, tuning their hearing to the ambient sounds in the ocean.

It seems that the male plainfin midshipman swim bladder adapts to the seasons: fine-tuning the fish's hearing to listen out for sounds in the surrounding water out of the breeding season and building up to produce amorous courtship serenades during the breeding season, while also protecting their own hearing from their droning vocal performance. So male plainfin midshipman have expanded the swim bladder's repertoire from simple buoyancy aid to hearing aid and vocal box by modifying the organ through the seasons.

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Rogers, L. S., Lozier, N. R., Sapozhnikova, Y. P., Diamond, K. M., Davis, J. L. and Sisneros, J. A. (2023). Functional plasticity of the swim bladder as an acoustic organ for communication in a vocal fish. *Proc. Royal Soc. B* **290**, 20231839. doi:10.1098/rspb.2023.1839

Madhuri Srinivasan (0009-0002-3386-5812)
National Centre for Biological Sciences, Bangalore, India
smadhuri@ncbs.res.in