

OUTSIDE JEB

The orb-weaver's step-by-step guide to homebuilding



It's hard to believe that the intricate patterns of fine silk we call cobwebs are each constructed by a single spider. They diligently design and build homes strong enough to stop an insect in its tracks. A team of researchers at Johns Hopkins University, USA, led by Andrew Gordus, set out to characterize the way orb-weavers, *Uloborus diversus*, construct such exquisite webs by closely analyzing the spiders' movements while they build.

To observe the spiders' skills up close, the researchers outfitted a chamber with a video camera looking down onto a plexiglass box. They then annotated a portion of video, frame by frame, labelling the body, legs and joints so that they could adapt algorithms to predict where the legs and body would be in the rest of the videos. Using this approach, the researchers characterized each spider's movements as they spun their webs.

It turns out that web assembly is made up of four previously defined construction phases: proto-web, radii, auxiliary and capture. In the initial stage of a web the spider builds a proto-web, which serves as scaffolding and looks like a messy tangle. The spiders paused most often while building the proto-web, suggesting that

they use this time to assess the integrity of their composition. During the radii stage the spiders removed much of the proto-web and anchored silk between the edges and the center hub. Following the radii stage, the spiders built an auxiliary web, which is a single spiral that sweeps out from the hub to the edge of the web. And finally, the spiders removed the auxiliary spiral during the final capture stage, when the spider spun silk around the radii spiralling in tightly toward the center.

By tracking the spiders' limbs, the research team distinguished behaviors such as walking, moving right or left legs, and pulling silk quickly or slowly. When they organized these behaviors by construction phase, they saw that particular behaviors are more likely to occur at some stages than others, such as leg sweeps during capture and increased pausing while building the proto-web. By tracking the position of the spider, they predicted when the spider was building radii by the straight walks from the center to the edge and back again. They then analyzed transitions between behaviors by calculating the probability that one behavior will follow another. They found that although each stage is made up of similar sets of behaviors, the probability that one will follow another is different for each stage. For example, during the capture stage, a fast silk pull is likely to follow multiple different behaviors, whereas during the radii stage, slow silk pulls are more common.

Finally, the team used a mathematical model to test if the spiders' motions were distinct enough to predict the stage based on the probability of movements and transitions between movements. They found that their model successfully predicted different stages except for the proto and radii stage, which the model did not differentiate well. The researchers interpreted this outcome to mean that spiders move similarly while building the proto-web and the radii but the different patterns of web construction result from where the spider is on the web.

A close and careful investigation of orb-weavers honing their craft shows that even a complex masterpiece like a spiderweb is ultimately made by an individual's flexible execution of a limited set of repeated movements.

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Birds of a feather cope with challenges together



Global climate change and the transformation of habitats due to the unstoppable human invasion of pristine territories are bad for life on Earth. However, recent research has shown that many organisms can tweak their physiology in order to cope well with change. A current challenge for wildlife ecologists and managers is to connect physiological measurements with subsequent changes in the health status of populations and species. This is exactly what interested the team led by Ashlee Mikkelsen, Oregon State University and USDA Forest Service (Oregon, USA). Focusing on a vulnerable species that is currently considered at risk, the northern spotted owl (*Strix occidentalis caurina*),

they explored the connections between physiological stress responses with environmental factors that might predict the population declines of this species.

Corticosterone is a main hormonal regulator of energy allocation in birds and generally increases in response to life challenges. Over 15 consecutive years (2001–2017), Mikkelsen and her team quantified the concentration of the hormone in 4720 feathers from 1056 juvenile spotted owls across 7 different study areas, in order to measure the stress responses of the youngsters as they developed. To minimise harm to the owls, most of the feathers were collected when the owls were banded and weighed as part of the annual historical monitoring of the population. Additionally, the team knew how big each owl territory was at the time of feather collection, how much cover – for breeding and foraging – each patch of forest provided and how good the parents were at caring for their chicks. They also gathered information on the local weather when the feather collections took place, to find out the conditions that the youngsters were facing.

When the team crunched all the data together to assess the connection between feather corticosterone levels and individual and landscape conditions, the analysis revealed three interesting patterns. First, feather corticosterone decreased as the body mass of the owl increased; the largest chicks were the least stressed. As heavier juvenile spotted owls are fitter and have greater chances of survival, this result confirms feather corticosterone as a reliable indicator of the overall health of free-living birds. Second, the levels of corticosterone in the spotted owl feathers were higher when it was warm and rainy, rather than when it was cold and dry, suggesting that the youngsters may have experienced higher energy demands possibly to avoid, or endure, higher temperatures or getting drenched. And third, the smallest owls had the highest corticosterone levels when there was heavy rain, so the owls' size had an impact on how stressful the youngsters found wet conditions. In addition, the owls experienced the most stress during the fledging period, when they were gradually leaving their nests to begin facing the daily demands and dangers of an independent life.

Mikkelsen's study is one of the first to provide long-term data in a wild bird, showing that feather corticosterone can be a reliable indicator of individual stress responses to life challenges that they experienced in the past. But most importantly, these results show that feather corticosterone can help wildlife ecologists to predict how stress responses related to climate changes and global warming might affect bird populations, marking a significant step forward in the field.

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Bats' brains encode single neurons for each of their buddies



Whether we're talking about a murder of crows or a crowd of people, social group dynamics can get incredibly complex. In each interaction, individuals within a group must juggle who is who, what is happening, whether they've met before and what happened in those past interactions. Keeping track of such details allows you to distinguish your best friend from your mortal enemy. In their new paper published in *Science*, Maimon Rose and colleagues from University of California Berkeley, USA, looked to unravel how complex ideas such as

identity and context in social communication are encoded in the brain.

To address this question, the team studied a long-lived and highly social species, the Egyptian fruit bat (*Rousettus aegyptiacus*). These animals typically live up to 25 years in colonies with thousands of other bats. Throughout their lifetime, they form long-lasting social ties and only chat with neighbours in close proximity, making them ideal to study the neural mechanisms of group social communication.

To study the bat brains, Rose strapped tiny wireless hats onto groups of 4–5 bats. These hats simultaneously recorded brainwaves from each bat and allowed the scientists to identify which bats were conversing at any one time. From scanning 1153 single neurons from 7 different bats, the researchers found that certain neurons fired in response to the bat's own calls while other neurons fired when it listened to calls from other bats. Moreover, calls from specific individuals caused specific neurons ('identity neurons') to fire in response, suggesting that the bat brain encodes a single neuron for each buddy.

When scientists compared brainwaves from each member of a group of bats, they found that all group members were on the same wavelength, meaning their brain activity patterns were remarkably synchronized whether a bat was calling or listening to a call. This neural coordination during communication among friends remained stable over at least 2 weeks. Thus, these long-lived, highly social bats can form 'best friendships' that are stable over time and reliably encoded in their neural repertoire.

To study the bat behaviours, Rose and Boaz Styr then built an LED light-based bat-tracking system to trace which individuals like to spend more or less time close to the rest of the group. The authors found that the identity neurons of anti-social bats, which spend less time close to the rest of the group, fired less reliably in response to their respective bat buddies. Moreover, their brainwaves were less synchronized with that of others in the group.

To show that these patterns of neural activity are specific to social interactions, the researchers trained bats to call in a

non-social context, this time in response to a reward rather than spontaneously. They found that in this context, much like in anti-social bats, identity neurons were less reliable indicators of identity and brain activity patterns of members in the group were less coordinated.

Many animals, including humans, are social creatures. Although communication often occurs in a group setting, prior studies had only looked at either one brain at a time or one side of communication at a time. This study provides novel insights into the neuronal process by which the complexities of group social communication are handled. Studies such as this one bring us one step closer to understanding what controls our ability to communicate gracefully in a group.

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A deep dive into the ecology of fear



The largest migration on Earth occurs on a daily basis in the ocean as creatures move up near the surface during the night to feed and descend to the relative safety of the dark depths during the day. This phenomenon is thought to reflect feeding opportunities and predator avoidance according to light levels, but the dynamics of this have rarely been studied. Samuel Urmy and Kelly Benoit-Bird from the

Monterey Bay Aquarium Research Institute, USA, inspired by the originally terrestrial concept of ‘landscapes of fear’, sought to investigate if the fear of predation shapes the ecological process of daily migrations.

Urmy and Benoit-Bird used an upwards-facing echosounder, which produces sonar pulses and records the returning echoes to detect and visualize schools of fish, squid and plankton, to provide information about their daily vertical movement patterns. Additionally, an underwater microphone next to the echosounder listened for the hunting sounds made by nearby toothed whale predators, such as Risso’s dolphins (*Grampus griseus*) and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). Recording the echolocation clicks gave the researchers an indication of the presence of a predator, allowing them to investigate the interactions between the predators and the prey in their vicinity. Successfully collecting data beneath almost 1 km of seawater for more than a year is no easy feat, so the team sent down an unmanned robot to survey the area with video to visually confirm the identities of the different species that they were picking up with the echosounder.

Scrutinizing a whole year of deep-sea data, the researchers observed many examples of prey taking evasive action to avoid predators. In one example, a bout of dolphin echolocation clicks triggered a school of fish to cluster together and dive for safety moments later. While such a diving response physically increases the distance between the fish and the predator, the fish’s vertical plunge also results in weaker echoes returning from the vulnerable fish, reducing the opportunity for the hunting predator to successfully home in on their fishy prey. In addition, the team could use the distinctive echo signatures produced by different species – such as larger Pacific hake (*Merluccius productus*) and smaller anchovies (*Engraulis mordax*) – to provide a best guess for which species were appearing in the echosounder traces when video data were not available. This was especially useful for identifying instances of predation that did not involve an echolocating species, such as an occasion when a school of predatory fish approached a cloud of zooplankton and the tiny creatures dived to avoid the hunters. The scientists also discovered

that it sometimes took clusters of zooplankton weeks to return to their usual depth after an encounter with a hungry predator. This shaping of a prey animal’s behaviour and movement, even in the absence of a predator, provides evidence that the concept of the ‘ecology of fear’ is indeed applicable in the 3D marine realm and complements recent research on marine ‘soundscapes of fear’, where prey fall silent to avoid detection by predators that could be nearby.

The vertical movement of animals in the ocean plays a major part in nutrient recycling, and Urmy and Benoit-Bird demonstrate that the lasting behavioural responses of prey to hunting predators potentially has a major influence on this flow of nutrients. The effect of predators on the behaviour of their prey can thus be more lingering than we had thought. Zooplankton layers in the open ocean are therefore not just governed by forces of nature or their particular lifestyles, but by the ecology of fear too.

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Broken teeth? Fish dentists hate this one simple trick



Many fishes like sharks are known for their ability to regrow sets of teeth. Regrowing teeth comes in handy if a tooth

breaks or is worn down over time. Fishes also often have a second set of jaws – pharyngeal jaws – which hide in their throats. These jaws and the teeth upon them are used to crush food, in contrast to oral jaws, which grab morsels. Previous tooth replacement studies have focused on fishes with tooth shapes specialized to the specific food they consume. But little was known about tooth replacement in fish that eat most anything, such as Pacific lingcod (*Ophiodon elongatus*) with simple cone-shaped teeth. What are the tooth replacement rates in either set of jaws? Is a new tooth fated to replace another of the same size? And does feeding influence tooth replacement rates? To study these questions, Emily Carr from the University of South Florida, USA, and colleagues from the University of Washington, USA, compared tooth replacement rates and positions in the oral and pharyngeal jaws of Pacific lingcod.

The scientists used special fluorescent staining techniques, called pulse–chase, to monitor new versus old teeth so that new teeth would fluoresce as green and old teeth would fluoresce as red.

First, the team separated the fish into two groups – one that was fed and a second that was fed nothing to determine whether feeding affects tooth replacement rates. The scientists then used a microscope and fluorescent lamp to monitor the replacement rates of the fishes’ teeth, as well as the size and location of new teeth, on both the oral and pharyngeal jaws.

It turns out that the size and locations of replacement teeth are fated. New teeth erupted as small cone-shaped structures next to the teeth that they were destined to replace, before growing to the size of the original teeth. In addition, small teeth were replaced more often than large teeth, as they broke more often. The team also found that the tooth replacement rate was mostly similar across the pharyngeal and oral jaws, except in the lower pharyngeal jaw, where the replacement rate was nearly twice as fast, possibly because food hits these teeth at many different angles thanks to the mobility of the jaw. Lastly, the team realised that whether a fish is actively feeding or not does not influence how often they replace their teeth.

So, it seems likely that all fishes with single simplified tooth shapes like the lingcod follow the replacement pattern that Carr and colleagues have found. Interestingly, neither wear nor the high cost of tooth replacement determines the replacement rate. Instead, the team suggests that replacement rates may be driven by a developmental network which controls when and where teeth are replaced. Future studies can investigate the signals that determine replacement tooth size and whether size is decided by the cells that surround the tooth. This work will help us to understand the diversity of tooth development and shape in fishes, which are the largest group of vertebrate animals on the planet.

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