Inside JEB highlights the key developments in *The Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

CORALLINE ALGA STANDS THE TEST OF TIME ON SHORELINE

No one likes getting bashed about, but with waves constantly crashing into the shoreline, seaweeds have little choice; they just have to go with the flow, and bend over to become streamlined. It is therefore surprising to find a group of seaweeds, the coralline algae, which have calcified most of their cells and essentially turned themselves into living rocks. However, Mark Denny, from Hopkins Marine Station of Stanford University, USA, explains that despite their rocky appearances, the presence of specialised joints, made up of decalcified cells, actually makes them very flexible. Nonetheless, Denny points out: 'Whether it's a bit of rubber or one of these seaweeds, you can't make a flawless material, and if you pull on them in one direction it stretches that flaw and creates a crack. Every time you pull on the crack it can grow a little bit, and the more it grows, the weaker the material gets and it will eventually break – it fatigues.' With the bending, and thus most of force, occurring at the small joints (they make up just 15% of the alga), Denny wondered how resistant coralline algae are to fatigue (p. 3772).

Denny admits that at the onset of his experiments, he suspected that the alga had specialised joints that would minimise cracks spreading from cell to cell. He explains that under the microscope, the joint cells don't seem to be attached to each other. Instead, they look like individual cables, which are attached at each end to the fragments of calcified alga either side of the joint, but not to the neighbouring cable. 'The best way we could show that was by a torsional, twisting test', says Denny. 'If they were hooked [attached] to each other, the joints would be pretty stiff.' So, after collecting some algae from the shorelines near the marine station, he twisted the algal joints and found they weren't very stiff in torsion – a sure sign that the individual cells weren't attached to each other.

Despite the algae's fatigue-resistant design, which would prevent a crack in one cell spreading to its neighbour, Denny still didn't know how resistant they were, so he moved on to his second experiment: 'This test was blissfully simple – we just put them into an apparatus that pulls on a segment of the seaweed at a set force per area. So, you're always applying the same amount of stress to the material and you keep doing it and doing it until the thing breaks and you count how many cycles it takes to break it.' As a force of 25.9 MPa is enough to tear the alga in one single blow, Denny chose to reduce that by 50% for his fatigue test: 58 days and 51 million cycles later, to his surprise, the first sample still hadn't broken.

It was time to change tactics, recalls Denny: 'I was thinking: well, I've got to have 25 or 30 more of these samples to get a good sample size to write a paper, and if it's taking a month or two per sample, I'm going to be here forever! So, I ended up loading most of them to 70–80% of their breaking stress, and still they would take a week to break.' Even using weaker forces of up to 20.1 MPa, the seaweed stood up to at least a million cycles. However, Denny points out that even these levels of force are very rare on the shoreline, and it's likely that only once or twice a year a wave will come along with that level of force. So, it seems that these seaweeds are indefatigable, at least when it comes to withstanding waves.

10.1242/jeb.095489

Denny, M., Mach, K., Tepler, S. and Martone, P. (2013). Indefatigable: an erect coralline alga is highly resistant to fatigue. *J. Exp. Biol.* **216**, 3772-3780.

Nicola Stead

CRAB SPIDERS' TRICKS FOR YELLOW CAMOUFLAGE

Chameleons and octopuses may be famed for their extraordinary camouflage skills, but this ability is not unique and many animals, big and small, can match their surroundings. Perhaps one of the smallest is the tiny crab spider, *Thomisus onustus*, which measure between 1 and 10 mm. These tiny arachnids match the vibrant yellows, pinks and whites of their flowery hosts, where they lie in wait for their pollinator prey. Little is known about the reason behind the crab spiders' camouflage (pollinators visit flowers regardless of a spider's colour and these spiders have few predators themselves) or even what controls the reversible colour changes. During her PhD, Ana L. Llandres studied the benefits of camouflage; however, when it came to her post-doc at the IRIB, France, she wanted to turn her attention to the regulation of camouflage: 'What are the factors that makes spiders change colour?' With the help of her post-doctoral advisor,

Jérôme Casas, and lab members Florent Figon, Jean-Philippe Christides and Nicole Mandon, she set out to investigate (p. 3886).

In early April 2012, Llandres travelled to the Extremadura region of Spain, and over the course of 4 days captured 160 female spiders hiding out in bright yellow corn marigolds. Llandres then allowed her spiders to adapt to their new white holding tanks over the course of 23 days, watching them gradually turn a whitish shade. She then wanted to find out whether simply changing the background colour was enough to get the spiders to change colour. So she placed 32 spiders in yellow containers and 18 remained in white containers in a sunny outdoor garden. Measuring light reflectance to precisely distinguish the spiders' colour, she found that, after 15 days, the initially whitish spiders matched their coloured abodes, becoming even whiter in the white containers and yellow in the yellow containers.

But what was controlling this colour change? During the spiders' first 23 days in the lab, Llandres recalls: 'I saw that spiders that moulted tended to change to a white colour at a slower speed compared to spiders that did not moult. We know from previous studies that when spiders moult they show a peak of ecdysone [hormone] just prior to the moulting event, which made us think that ecdysone hormone could also be linked to the process of yellowing in this species'. To test this, the team decided to inject the spiders with the synthetic version of the hormone, hydroxyecdysone, and keep them in a yellow container in the lab. Sure enough, in the 3 days following the injection the spiders had adopted a yellower colour. However, by day 6, they had reverted to their white colour again. Llandres explains that when hormones are injected, spiders quickly break them down. Without the hormone, and in spite of the yellow backdrop, these spiders just couldn't maintain their yellow colour. In contrast to the earlier experiment, Llandres points out: 'Natural illumination is much stronger than the illumination present in the laboratory and this may be an important factor that may affect a spider's colour change.'

In conclusion, the study has highlighted that both environmental and hormonal factors control the colour switching. What's more, the results suggest that spiders can actively choose to camouflage themselves based on their background, and won't always just choose a flower that matches their colour. However, the question still remains why are they camouflaging themselves in the first place? By eventually developing a way to actively manipulate the direction of colour change, the group hope they will be in a unique position to answer this century-old question.

10.1242/jeb.095422

Llandres, A. L., Figon, F., Christides, J.-P., Mandon, N. and Casas, J. (2013). Environmental and hormonal factors controlling reversible colour change in crab spiders. *J. Exp. Biol.* **216**, 3886-3895.

Nicola Stead

$GABA_B$'S ROLE IN STAYING ASLEEP

It's easy to take sleep for granted; after all, for most of us it's something we do every night with relative ease. However, our ability to fall, and stay, asleep is tightly regulated. In mammals, it is controlled by at least three different centres in our brain and so many researchers have turned to the simpler fruit fly instead: 'In flies there is at least a hint that perhaps it's much simpler and that the three centres are just located in a small subset of neurons, called the large ventrolateral neurons', explains Charlotte Helfrich-Förster, a researcher at the University of Würzburg, Germany. Helfrich-Förster goes on to explain that these small insects have already highlighted the role the neurotransmitter GABA plays during sleep: 'If you down regulate GABA in the fly, you find that they have difficulties in falling asleep and finally, after they have fallen asleep, they also wake up earlier.' However, when researchers blocked the $GABA_A$ receptor subtype, they found that flies had no problem maintaining sleep, just difficulties in drifting off. So, how was GABA helping flies stay in their state of slumber if not through $GABA_A$? Helfrich-Förster decided to investigate (p. 3837).

Helfrich-Förster and her team knew of a second type of GABA receptor, called $GABA_B$ receptors, which act *via* a slower mechanism than $GABA_A$ receptors, and wondered whether they might be responsible for maintaining GABA-induced sleep. However, first the team needed to see whether $GABA_B$ receptors were also found in the same ventrolateral ($l\text{-}LN_V$) region as the $GABA_A$ receptors. Sure enough, using an antibody targeting $GABA_B$ receptors, the team were able to show that the receptors were present in the region of the fly brain that controls sleep.

Next, the team needed to deplete these $l\text{-}LN_V$ neurons of $GABA_B$ receptors. Luckily, Dick Nässel, from Stockholm University, Sweden, already had fly lines where they could reduce expression of the $GABA_B$ gene specifically in the $l\text{-}LN_V$ region. To test what affect this reduction of $GABA_B$ was having on the flies, the team then needed to monitor their sleeping patterns: 'Of course, it is not as easy as in mammals because we cannot record an EEG [electroencephalogram measuring electrical activity]', says Helfrich-Förster. 'So, instead, we monitored the activity of the flies using an infrared light beam; when they interrupt the beam it was recorded as being active and when the flies are inactive for more than 10 min it was recorded as sleeping.' During the first 12 daylight hours all the flies followed the same routine, including a mid-day snooze, and when it came to lights-out all the flies dozed off just as quickly as each other. However, during the second half of the night, half an hour before most flies were waking up, flies with reduced $GABA_B$ levels became more active.

To double check that the reduction of $GABA_B$ wasn't due to a change in the circadian clock, the team kept the flies in constant darkness for 24 h a day for 10 days and found there was no difference between flies with or without $GABA_B$ in the $l\text{-}LN_V$ region. Helfrich-Förster's research has finally answered the question of how GABA keeps flies asleep and it's quite possible the same is true for us. So, if you're having difficulties staying asleep perhaps it's time to blame your $GABA_B$ receptors.

10.1242/jeb.094623

Gmeiner, F., Kołodziejczyk, A., Yoshii, T., Rieger, D., Nässel, D. R. and Helfrich-Förster, C. (2013). $GABA_B$ receptors play an essential role in maintaining sleep during the second half of the night in *Drosophila melanogaster*. *J. Exp. Biol.* **216**, 3837-3843.

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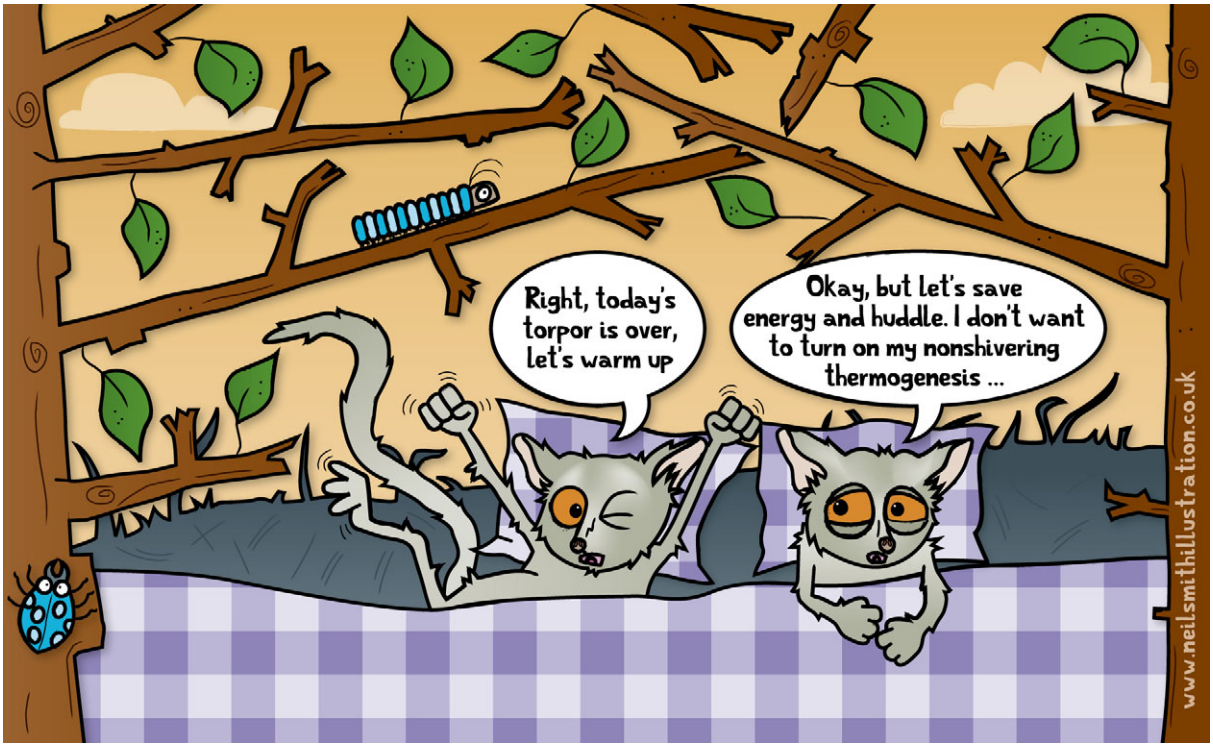
AROUSAL FROM TORPOR: A BUSHBABY'S METHODS



Dropping your body temperature and going into torpor is a sensible choice if you are an animal facing cooler temperatures and/or food scarcity. But when more favourable conditions arrive, you also need to be able to raise your body temperature again. Animals from arctic or temperate regions often stock up on their brown fat cells prior to winter, which, when stimulated with noradrenaline, burn off the fat as heat by a process called nonshivering thermogenesis (NST). However, do animals in warmer regions, such as Africa, also use NST or can they rely on passive heating from the environment? It's an area of much debate, explains Julia Nowack, from the University of Hamburg, Germany. With the help of her advisors, Kathrin Dausmann, also from the University of Hamburg, and Nomakwezi Mzilikazi, from Nelson Mandela Metropolitan University, South Africa, Nowack decided to investigate whether African lesser bushbabies, which sometimes have unusual difficulties rewarming after torpor, have the ability to use NST (p. 3811).

Nowack travelled to South Africa, where she trapped bushbabies by tempting them with banana, honey and peanut butter treats. She then kept them in outdoor enclosures, where the daily temperatures averaged 30°C in summer, but dipped as low as –5°C in winter. To test whether they could use NST, Nowack injected her bushbabies with noradrenaline and saw that they increased their oxygen consumption as well as raised their skin temperature by 1°C – good signs that they were burning off fat. Although there was no overall specific increase in NST during winter months, Nowack did find that during short colder snaps they did increase NST capacity to warm up. What's more, when she dissected two bushbabies that had died of natural causes, she clearly saw deposits of brown fat cells throughout their bodies.

It's clear that at least African lesser bushbabies can use NST. However, as there is no overall increase of NST during winter months it's possible that they rely more on passive heating or huddling together to warm up. Alternatively, bushbabies might use torpor throughout the year and as such don't go through a stocking up phase like their arctic friends, to increase NST.

10.1242/jeb.094615

Nowack, J., Dausmann, K. H. and Mzilikazi, N. (2013). Nonshivering themorgensis in the African lesser bushbaby *Galago moholi*. *J. Exp. Biol.* **216**, 3811-3817.

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