Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

SCHOOL RANK

FINDING YOUR PLACE IN SCHOOL

In a fish school, position means a lot. Animals at the front will have first access to food; animals in the center are well protected from predators, and those pulling up the rear get to save a little metabolic energy as they draft off those in front of them. But who ends up where in a school, and why? This is precisely the question that Shaun Killen of the University of Montpellier and his colleagues Stefano Marras, John Steffensen and David McKenzie recently set out to answer.

Given that it is physically, and thus physiologically, more demanding to swim at the front of a school than in the middle or at the back, the researchers hypothesized that an animal's aerobic scope – the difference between its resting and maximal metabolic rate – was probably important in determining its position. To test this idea, Killen and colleagues used wild-caught, juvenile grey mullet to quantify schooling behavior of individual animals in a flume. First, a focal fish was introduced into the swim tunnel with seven companion fish and the group was allowed to acclimate to slow-speed swimming for 5h. After the acclimation phase, the team recorded the fish schools from above using a digital video camera for 20min at each of three speeds. For each 20min block they analyzed the videos in 5s increments to determine the typical position of the focal fish and how much it varied within the small school. Repeating this procedure for 20 focal fish, the authors also measured the tailbeat frequencies of the focal fish and used respirometry to measure their standard and maximal metabolic rates.

Analyzing the results, the authors realized that while there were no significant relationships between standard metabolic rate and mean position within a school, there were significant relationships between an animal's aerobic scope and its mean position. Specifically, animals with a higher aerobic scope were more likely to be found near the front of a school at higher speeds. Moreover, although most fish maintained similar positions as the school's speed varied, some did not, and those that tended to move toward the back of a school when speeds increased were fish with a lower aerobic scope. The researchers also demonstrated that fish near the back of a school were able to get away with lower tailbeat frequencies at intermediate and high speeds than fish at the front of the school.

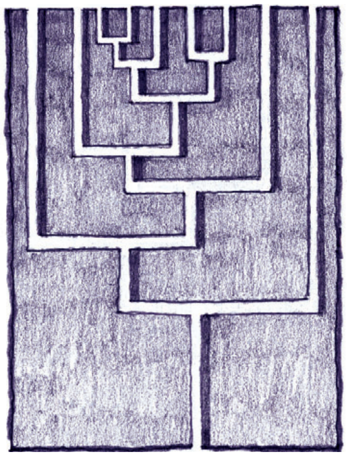
So aerobic capacity appears to influence where in a school a fish ends up, especially if the school is swimming at a relatively high speed. Killen and his colleagues raise the important question of whether their results apply to larger schools, and even more intriguingly suggest that aerobic ability could influence what school a fish might join. Studying aerobic capacity, and its variability among different schools, would be an exciting next step.

10.1242/jeb.049973

Killen, S. S., Marras, S., Steffensen, J. F. and McKenzie, D. J. (2011). Aerobic capacity influences the spatial position of individuals within fish schools. *Proc. R. Soc. Lond. B.* doi:10.1098/rspb.2011.1006.

Gary B. Gillis
Mount Holyoke College
ggillis@mtholyoke.edu

METABOLIC RATE



DINOSAURS WERE NO LAZY BONES!

Were dinosaurs lethargic, cold-blooded creepers or lively, warm-blooded creatures? This controversy has plagued paleobiologists for decades and prompted Roger Seymour at the University of Adelaide and collaborators in Australia, Canada and Germany to employ a novel measurement to investigate the matter: one in our very bones! The nutrient foramen of a bone is an opening through which blood vessels enter, supplying blood to the bone cells inside. These active cells require the oxygen and nutrients in blood to dissolve older bone and to deposit new bone during growth and maintenance (remodelling) throughout life. Seymour wondered whether the size of these entryways might indicate the amount of blood required to sustain the bone. Highly active animals might require more bone maintenance (because of a higher incidence of tiny fractures in bone caused by locomotion) and consequently a greater blood supply.

The team measured the nutrient foramen, volume and mass of the femur (the large leg bone crucial for support and locomotion) in 59 mammalian species and 40 reptilian species, ranging in size from mice to elephants and geckos to crocodiles. They then set about assessing how these data relate to body mass and metabolic rate values obtained from previous experiments. The researchers discovered that larger animals have larger bones and larger bone holes, but the holes in mammals are much larger than those of most reptiles of the same body mass.

The team also found that the scaling of foramen size is closely related to maximum whole-body metabolic rate during exercise in both mammals and reptiles, though less closely related to basal metabolic rate. This suggests that blood supply through the holes relates to bone maintenance requirements following activity, and that blood flow to bone is about 10 times higher in mammals than in reptiles. This fits with the fact that mammals have much higher metabolic rates during exercise than reptiles, requiring more oxygen to fuel their muscles and other tissues, including the cells responsible for bone remodelling. In addition to a larger nutrient foramen, mammals also benefit from higher blood pressures and blood that can carry more oxygen, all of which contribute to a much larger oxygen supply for mammalian tissues. Interestingly, varanid reptiles, including active hunters like the Komodo dragon and monitor lizards, had a foramen size similar to that of mammals. Varanids are capable of increasing their metabolic rates far above those of other reptiles, filling a niche akin to mammalian predators. Varanids also remodel their long bones, but other living reptiles apparently do not.

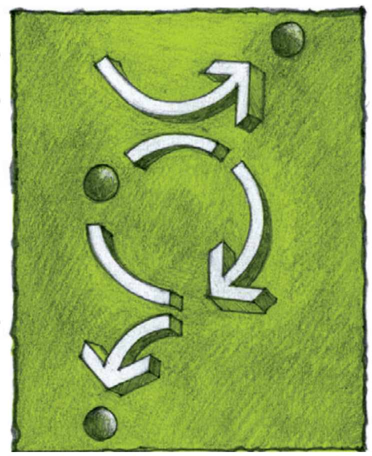
Next, the researchers took their analysis one step further to fossils from 10 species of dinosaur. They found that dinosaurs have even larger bone holes than mammals (even after correcting the results for body mass differences). This suggests that dinosaurs were highly active and lively, perhaps even more so than mammals! Make no bones about it, this implies that the notion of dinosaurs as sluggish and sloth-like may soon be as extinct as the charismatic creatures themselves. Novel applications of these parameters could also provide insight into activity levels of other living and extinct vertebrates.

10.1242/jeb.049981

Seymour, R. S., Smith, S. L., White, C. R., Henderson, D. M. and Schwarz-Wings, D. (2011). Blood flow to long bones indicates activity metabolism in mammals, reptiles, and dinosaurs. *Proc. R. Soc. Lond. B.* Published online before print, doi:10.1098/rspb.2011.0968

Jessica U. Meir
University of British Columbia
meir@zoology.ubc.ca

PARASITE ADHESION



PARASITES' GRAPPLING HOOK FOR HOST CELL BOARDING

Apicomplexan parasites are a group of intracellular parasites that can live in various animal hosts, including humans, where they can cause serious diseases such as malaria and toxoplasmosis. However, entering a host cell is not an easy undertaking for the parasite and resembles boarding a well-fortified galley. For this purpose they are equipped with a unique grappling device known as a 'moving junction', a ring-like structure, which allows the parasite to penetrate the host cell, folding the host's plasma membrane around itself for disguise in the hosts' cytoplasm. Some of the constituents of the moving junction have been identified in recent years and researchers have started to elucidate their roles during invasion. In a recent study published in *Science*, a French/Canadian team of researchers led by Maryse Lebrun and Martin Boulanger provide detailed insight into the core structure of the moving junction and explain how it can resist the strong forces that occur during invasion.

After the parasite has become attached to its host cell with the help of adhesion molecules that recognize carbohydrates on the host's surface, it injects a set of proteins that initiate intrusion by establishing an intimate contact zone between the plasma membranes of the host and parasite. For this purpose, the parasite produces receptor proteins and the appropriate ligands, which are secreted by specialized organelles called micronemes and rhoptries, respectively. The rhoptry proteins are injected into the host cell's cytoplasm where they form the rhoptry neck (RON) complex, which is anchored to the hosts' plasma membrane by RON2, a transmembrane protein whose extracellular part functions as a ligand for parasite docking. The micronemes secrete apical membrane antigen 1 (AMA1), which is the receptor for RON2, on the surface of the parasite. The assembled AMA1–RON complexes are an essential part of the moving junction ring, which is finally pulled backwards during intrusion driven by the parasite's actin–myosin motor.

To examine the precise nature of the AMA1–RON2 interaction in the *Toxoplasma gondii* parasite, the scientists mapped and characterized the AMA1 binding site of RON2. They were successful in narrowing down the binding site of RON2 to a region of 37 amino acids, which is stabilized by a disulphide bridge between two cysteines and forms a U-shaped loop. Next, they crystallized the AMA1 receptor in complex with a synthetic version of the U-shaped RON2 peptide to reveal fascinating details of the binding site. Most strikingly, they found that the loop of the RON2 peptide functions like a grappling hook. It is inserted deeply into a hydrophobic groove on the surface of AMA1, allowing the junction to withstand the strong mechanical forces that occur while pulling back the moving junction ring. Comparing this structure with another one obtained previously for the AMA1 receptor without the ligand suggests that major conformational changes occur upon RON2 binding, which optimize shape and charge complementarity between AMA1 and the RON2 peptide for a perfect fit.

Thanks to the laborious work of Lebrun, Boulanger and their colleagues, we now have deep structural insight into the moving junction's core structure, much of which can be applied to help us understand the function of the moving junction in the malaria parasite as it is highly conserved in Apicomplexian protozoans. Knowing the parasites' trick with the grappling hook may turn out to be extremely helpful for developing therapeutic antibodies, peptides and drugs that block the binding site and hence prevent parasite invasion.

10.1242/jeb.049999

Tonkin, M. L., Roques, M., Lamarque, M. H., Pugnière, M., Douguet, D., Crawford, J., Lebrun, M. and Boulanger, M. J. (2011). Host cell invasion by apicomplexan parasites: insights from the co-structure of AMA1 with a RON2 peptide. *Science* **333**, 463-467.

Hans Merzendorfer
University of Osnabrueck
merzendorfer@biologie.uni-osnabrueck.de

IT'S A GLASS HALF EMPTY FOR STRESSED HONEYBEES

When depressed or stressed we tend to have a pessimistic outlook on life. Everything seems dark and gloomy and it feels like life is conspiring against us. However, when things are going well, we feel optimistic and hopeful. Although very familiar to us, these emotional states are not unique to humans. Other vertebrates like dogs, sheep, rats and some birds, exhibit optimistic behaviours when housed in large enriched habitats and pessimistic behaviours when stressed or anxious. But when did these emotional states arise? Do invertebrate animals also become pessimistic when stressed? These questions prompted Melissa Bateson, Suzanne Desire, Sarah Gartside and Geraldine A. Wright, from Newcastle University, UK, to investigate whether stressed honeybees show evidence of pessimistic behaviour.

First, the team had to find a way of stressing the bees, so they decided to simulate a predatory attack by vigorously shaking the bees for 1 min. Then, they tested to see whether the attack had affected the insects' physiology. Knowing that the levels of key biogeneic amines, such as octopamine, dopamine and serotonin, change in stressed bees, the team tested their levels in the bees' haemolymph and found that they had decreased. The simulated attack had worked.

Next, the team tested how this stress affected honeybees' responses to odours that they associated with pleasant and unpleasant tastes. They wanted to find out whether a change in mood affected the bees' attitudes towards these odours. To do this, they collected bees from an outdoor colony at the university and trained each individual to associate an odour with a substance. One odour was always paired with a food reward (1 or $2\,\mathrm{mol\,l^{-1}}$ sucrose) while the other odour was paired with a punishment or a poor quality reward ($0.01\,\mathrm{mol\,l^{-1}}$ quinine solution or $0.3\,\mathrm{mol\,l^{-1}}$ sucrose). Pretty soon, the bees learned to associate the odours with either the reward or the punishment, extending their mouthparts to the reward odour but withholding their mouthparts when presented with the punishment odour. Once the bees were trained, the scientists simulated a predatory attack on half of the bees by shaking them in the same way as before.

Five minutes after the simulated attack, the team tested the trained bees' reactions to five different substances: the reward odour, the punishment odour and three new scents, where the reward and punishment odours were blended together. Bateson and her team wanted to know whether shaken bees would be pessimistic about the odours and withhold their mouth parts more than their unstressed counterparts. Indeed, the bees that had gone through the simulated attack were more likely to behave pessimistically when they experienced the punishment odour and its most similar novel scent. These results show that when stressed, honeybees increase their expectations of a negative outcome.

Although invertebrates are not normally regarded as animals that can exhibit emotions, the combination of physiological changes and behaviour, in addition to an increased expectation of punishment, indicates a negative emotional state in agitated honeybees. This study by Bateson and her colleagues is the first to show that stressful conditions induce pessimistic behaviour in an invertebrate and the presence of 'human-like' emotions. Moreover, a 'glass half empty' attitude might be a good marker for negative emotions across species, from insects to mammals. Finally, the researchers suggest that a 'glass half empty' attitude in response to a stressful situation may have evolved as a way of protection from harmful conditions.

10.1242/jeb.050005

Bateson, M., Desire, S., Gartside, S. E. and Wright, G. A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Curr. Biol.* **21**, 1070-1073.

Viviana Cadena
Brock University
viviana.cadena@brocku.ca