
Sleeping glassfrogs

Science Watch – Hiding in Plain Sight

"If it wasn't for that green skin on their back, you would probably be able to read a newspaper through them." – J. Delia

Transparent animals are readily found in the open ocean where it's hard to find something to hide behind. Many jellyfish, octopuses and squid are transparent. Transparency in vertebrates is more difficult to achieve because their red blood cells (RBCs) are full of brightly colored hemoglobin. Larval eels solved this problem by eliminating RBCs entirely, getting their oxygen by simple diffusion. Transparent land vertebrates are even harder to find. Among those few is the glassfrog (*Hyalinobatrachium flieschmanni*) that lives in rainforests throughout Central America.

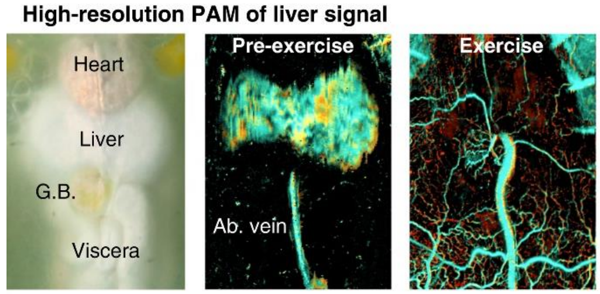
Viewed from above, its muscles are transparent, and its skin is semi-transparent, but for a green smudge. That, and its highly transparent ventral skin, makes it all but invisible when sleeping during the day on a green leaf (see backlit frogs above). In 2020 researchers showed that glassfrogs exhibit "dynamic transparency." Awake they are more visible, but as they fall asleep and are more vulnerable to predation, they become more transparent. But what happens to their RBCs?

Carlos Taboada and Jesse Delia, Duke University, Durham, NC, and American Museum Natural History, New York, NY, respectively, wanted to answer that question. "When they are awake, the circulatory system is red. When they are asleep, it's not. Where are the red cells going?" said Delia. They and their team published the answer in the December 23, 2022 issue of *Science*.

Taboada *et al*. discovered that glassfrogs have few circulating RBCs when asleep. Upon awakening the number rises sharply. But they couldn't use ordinary light microscopy to see where the RBCs hide because many of the frog's organs have a mirror-like surface that helps it be less visible. In addition, the vanishing process is readily disrupted when the frogs are active, stressed or anesthetized. So, the team resorted to photoacoustic microscopy (PAM), a technique that is non-invasive and requires no special dyes in order to follow changes in red blood cell distribution. PAM uses ultrasound to penetrate and excite molecules within the opaque organs to release ultrasonic waves which can be imaged.

The researchers found that resting frogs increase their transparency two-to-three-fold by sequestering almost 90% of their RBCs in their mirrored liver. "All the signal was coming from the liver," said Taboada. PAM images (see figures below) of 13 sleeping or resting frogs clearly showed RBCs densely packed in the liver when the frogs were at rest. "The heart stopped pumping red, which is the normal color of blood, and only pumped a bluish liquid," said Taboada. Just a few seconds after the frogs started moving, red blood cells began spreading throughout the body, making them more visible.

Using ultrasound, the team showed that the liver volume of glassfrogs increases by 40% when they are resting. Liver sections of resting frogs revealed many swollen sinuses filled with tightly packed RBCs.



During the transparent state only 3.4% of the frogs' total hemoglobin is bound to oxygen, indicating they are in a condition of reduced metabolic activity. Perhaps they undergo transient hibernation? No one yet knows.

The unique finding that glassfrogs can densely pack RBCs into their liver and then unpack them to circulate throughout the body raises an important question. How do they avoid strokes? As tightly packed RBCs rub against each other they form clots, which cause strokes if they occur in blood vessels feeding the brain. Yet glassfrogs somehow circumvent this life-threatening event.

Glassfrogs can control when their blood clots. When wounded, a clot forms and becomes a scab. Yet when asleep with their RBCs squeezed into their liver, no clots form. Blood clots kill as many as 100,000 Americans each year. Understanding how glassfrogs limit clotting could lead to treatments that reduce strokes and deaths from clots in humans.

Almost 60 years ago, then Senator William Proxmire issued his first "Golden Fleece Award," for "wasteful spending" of government grants by scientists. The movement he spawned pressured many scientists seeking grants to justify their research by concocting "practical applications." This study demonstrates that so-called "blue skies research," with no "real-world" application in mind, can potentially lead to practical discoveries.

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