As air breathers, we probably don’t appreciate how difficult it is for aquatic creatures to breathe. ‘The oxygen concentration in water is only about 3% that in air and the oxygen diffusion rate in water is $3 \times 10^5$ times lower’, says David Funk from the Stroud Water Research Center, USA, explaining that aquatic animals have to work $\sim 15$ times harder than terrestrial creatures to breathe. And the situation for cold-blooded (exothermic) creatures is even more perilous when the temperatures rise and their metabolism begins running faster. ‘The temperature-dependent performance of animals is believed to be shaped by the capacity for oxygen delivery in relation to oxygen demand’, Funk explains, adding that oxygen availability was thought to be the factor that sets the highest temperatures at which fish and aquatic insects can survive. Knowing that adult mayflies that develop in warm conditions tend also to be smaller, possibly as a consequence of running low on oxygen, Funk, Bernard Sweeney and John Jackson, also from Stroud Water Research, decided to find out how oxygen availability affects the development and survival of mayflies over a range of temperatures.

But the team didn’t stop at investigating one species of mayfly: Neocloeon triangulifer nymphs only thrive in well-oxygenated streams over a narrow temperature window of $8^\circ$C, while two robust populations of Cloeon dipterum are capable of surviving in relatively stagnant water in their comfort zone of $14–30^\circ$C. Allocating 50 larvae from each species to individual 1.8 l mini-aquaria, Funk created environments with high (45%) oxygen, down to normal ($\sim 20\%$ oxygen), while the temperature ranged from $20^\circ$C up to $32^\circ$C – the temperatures at which the females are unable to produce offspring. ‘We raised over 4400 hatchlings through their entire lives in 90 separate vessels housed in four giant water baths’, Funk says with a smile.

Monitoring the hottest youngsters with access to 45% oxygen, it was clear that the souped-up oxygen supply didn’t compensate for the challenge of developing in hot water: the youngsters failed to thrive. If oxygen was limiting the nymphs’ ability to survive in hot water, then those with access to unprecedented amounts of oxygen (45%) should have flourished. In short, the availability of oxygen does not limit the mayflies’ ability to survive and grow in sizzling conditions. Some other factor is responsible for their high mortality when the mercury rises.

Then Funk monitored how a lack or abundance of oxygen affected the youngsters’ development, paying close attention to their gills, eyes and leg length. ‘The most technically difficult thing was measuring the gill size’, says Funk, recalling how he had to be on hand as the youngsters transformed into adults, so that he could retrieve the discarded skins to measure the size of all seven pairs of gills. Comparing the nymphs’ growth at high and low temperatures ($25$ and $20^\circ$C), they found the hottest nymphs were always smallest, even in 45% oxygen. Oxygen availability wasn’t restricting the nymph’s growth either. However, the team was astonished by the impact that low oxygen had on the youngsters’ gills. ‘I noticed that mayflies reared in hypoxic conditions responded by greatly increasing the size of their gills. We had no idea they could do this, but quickly realised that gill size could be a valuable indicator of oxygen stress’, says Funk.

So, oxygen availability doesn’t seem to be the factor that restricts mayfly nymph growth and survival when the temperature soars, but the aquatic insects do respond to low oxygen by increasing the size of their gills to make the most of the meagre levels.

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