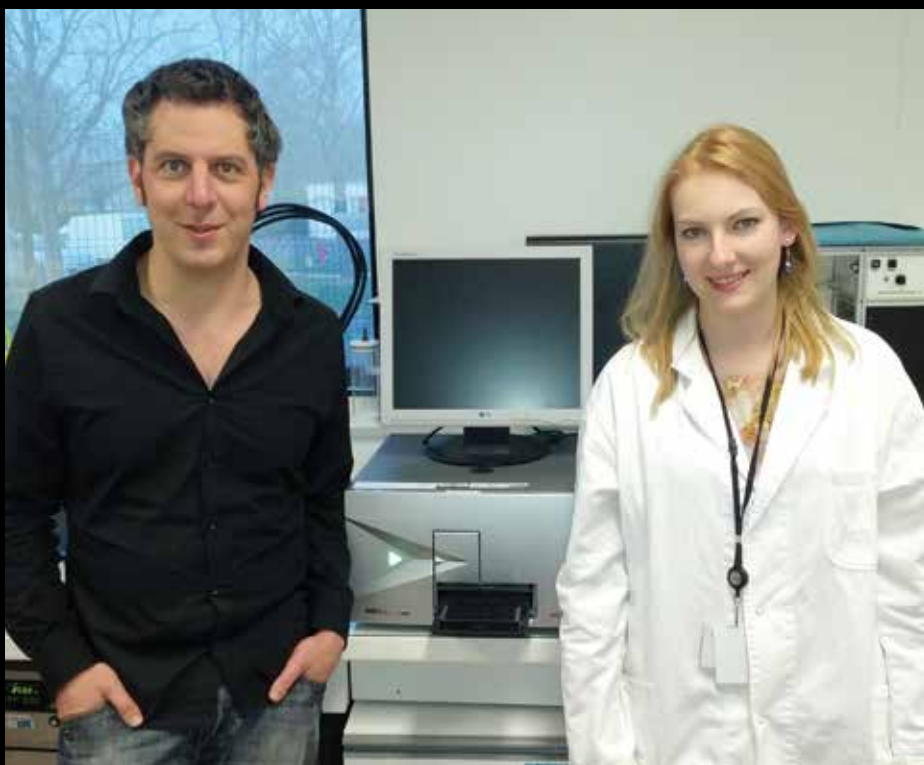


Looking out for the environment

Monitoring the effects of environmental pollutants on live organisms is now much faster thanks to a technique developed by researchers in France, who are using an Infinite® F200 PRO microplate reader to screen aquatic model organisms.



Andrew Tindall and Petra Spirhanzlova with WatchFrog's Infinite F200 PRO

WatchFrog, a small biotech company based in Paris, is a spin-off from the comparative physiology research department at the French Museum of Natural History. The Company develops, manufactures and markets tests to detect a wide range of potential pollutants, including pesticides, plastics, cosmetics, food additives and pharmaceutical residues. WatchFrog's technology is based on physiological changes that occur within aquatic model vertebrate organisms, amphibian larvae or fish fry, when exposed to these contaminants. These models primarily reveal changes in endocrine signaling, where abnormal hormone levels can disrupt normal sexual function, brain development and metabolism, and can be followed using fluorescence-based genetic reporter mechanisms.

Robotized imagery is the traditional method of reading experiments of this kind; photographing each larva over time and quantifying the images. However, WatchFrog has developed a microplate reader protocol which is considerably faster, easier and less expensive and, importantly, can be standardized between different laboratories. Andrew Tindall, a researcher in WatchFrog's research and development team, explained: "The underlying principle behind our tests involves exposing small, intact, translucent, vertebrate larvae to the contaminants in question. These larvae have endocrine systems that are physiologically close to humans and they harbor genetic constructs, typically containing green fluorescent protein (GFP), which cause them to fluoresce when the biological pathway

of interest is activated. Using early stage larvae rather than adult organisms means we are effectively combining the advantages of seeing what is really happening in an *in vivo* model with the simplicity, sensitivity, automation, low costs and ethical advantages of an *in vitro* approach. We can rapidly screen a large number of molecules far more cost effectively and on a much smaller scale, reducing the need for aquariums or dedicated laboratories. This approach also significantly cuts the time required for tests, typically to 24, 48 or 72 hours compared to 21 days for adult organisms."

Following poor results with another microplate reader, and encouraged by good reports from collaborators using Tecan equipment, WatchFrog chose an Infinite F200 PRO reader in 2012. Andrew continued: "We knew from experience that Tecan equipment is robust and well built, and the Infinite F200 offered some particular features that we consider vital for biological screening, most notably, reliable temperature control; there is no danger of overheating the reader chamber even after multiple measures of each well on a series of plates. Bottom reading is also very important, because it allows us to measure the free swimming models in 96-well plates, taking multiple readings on the same larvae over time without the need for anesthesia, which could potentially interfere with the results."

"One larva 2-3 mm long is placed in a small amount of water in each well of a clear bottom plate, where it swims freely without any further preparation. The plate is then placed in the reader at a temperature appropriate to the organism, and the instrument is programmed to read at given times. The excitation light illuminates the well, causing the GFP produced by the larvae to emit green fluorescent light. The fluorescence in each well is then quantified

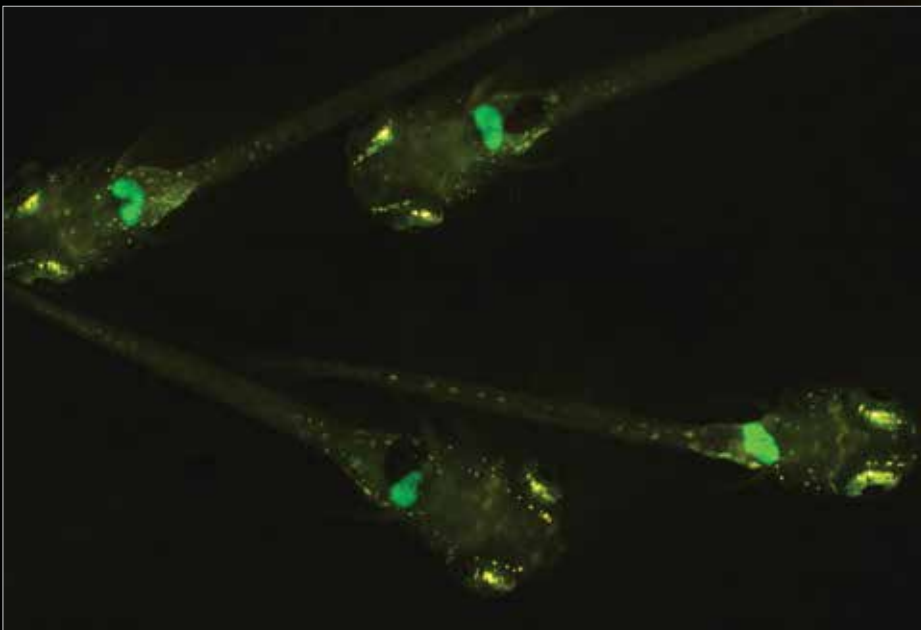
by the Infinite F200 PRO, with the results interpreted by staff to determine the sample's biological activity. We can then conclude whether the tested sample has an effect on the hormonal axis of interest, helping us to make informed decisions on the risk of the test substance to the environment or the public."

Andrew concluded: "Now that the technique is well established, we are discovering many more advantages to the Infinite F200 PRO; it is very robust, ideal for long read times and very easy to use. It also allows hands-free operation, releasing staff for other tasks and reliably running unattended, even overnight. WatchFrog is a partner of the EDA-EMERGE publicly-funded project to train young scientists to meet the major challenges in the monitoring, assessment and management of toxicants in European surface and drinking water. As part of this project, my colleague Petra Spirhanzlova successfully reduced the time taken to scan one 96-well plate from 40 minutes to only seven, improving our throughput considerably. We now have a reliable method that can be reproducibly used by our partner laboratories all over Europe, which is a condition for the validation

of our tests by the Organisation for Economic Co-operation and Development, and a great advantage to monitoring pollutants in this context."

To find out about the Infinite F200 PRO reader from Tecan, visit www.tecan.com/infinitezoopro

For more information on WatchFrog, visit www.watchfrog.fr



Fluorescence from medaka fry is monitored using the Infinite reader

