NONINVASIVE CARDIAC FUNCTION IN FISH: BLOOD VELOCITY SPECTROGRAMS FROM THE ATRIUM TO VENTRICLE, AND VENTRICLE TO BULBUS

Various fish species are used to study cardiac development and tissue regeneration, including adult Zebrafish (Danio rerio) and the Giant Danio (Devario aequipinnatus). Unlike the adult mammalian heart, some species of fish are able to regenerate cardiomyocytes following injury induced by either tissue resection, genetic modification, or thermal ablation of myocardial cells within the ventricle. Such injury results in necrotic cell death and significant scar formation in adult mammals. In fish, the damaged heart tissue is repaired with new cardiomyocytes over the course of several months.

Conventional analysis typically involves histological evaluation of the tissue at various time points following injury. This analysis requires that the animal be euthanized and the tissue removed for processing. While regeneration of the ventricular myocardium can be easily documented, functional characterization of the regenerated myocardium can be challenging. Methodological approaches to study the progression and functional restoration of the recovering heart in fish are still limited.

The fish heart (Figure 1) is composed of three chambers: the atrium, ventricle and bulbus. Blood flows into the atrium and through the atrioventricular valve to the ventricle. Contraction of the ventricle then forces the blood through the ventricular outflow tract (VOT) into the bulbus arteriosus.

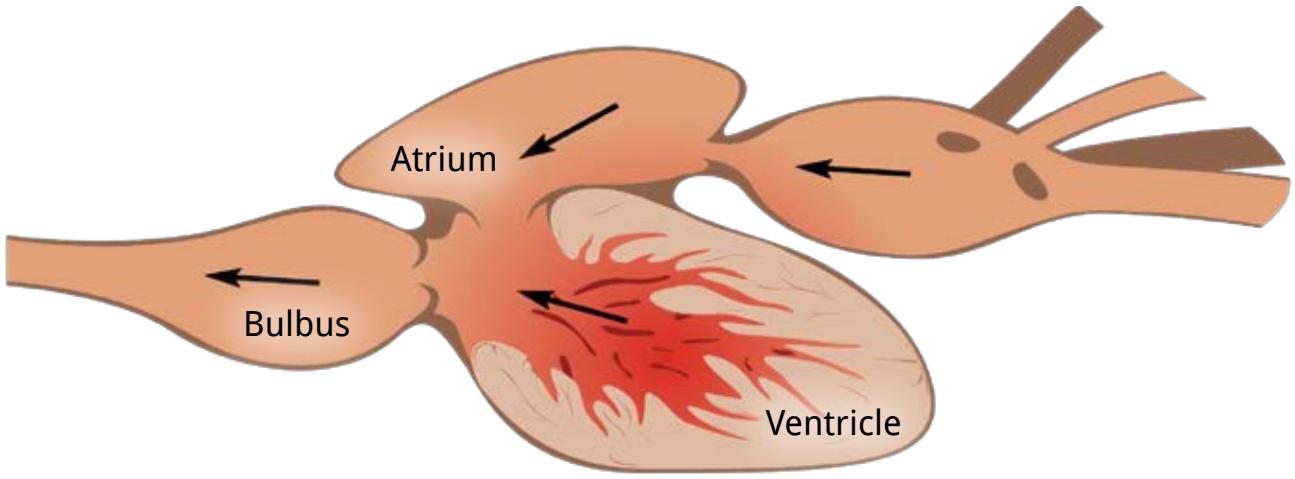

Figure 1 – The fish heart has 3 chambers: the atrium, ventricle and bulbus. Blood flows into and through the heart as shown by the black arrows. *Adapted illustration from Ahnode, "Two Chamber Heart", CC BY 3.0, Wikimedia Commons*

The Doppler Flow Velocity System (DFVS) from Indus Instruments was recently used to detect and quantify blood flow from both the atrium-to-ventricle (AV flow), and through the ventricular outflow tract into the bulbus (VOT flow) in the Zebrafish heart (Figure 2a – d). The DFVS uses single-crystal 20MHz ultrasound probes with a small footprint (2mm outer diameter), to measure flow velocities from various locations within the heart.

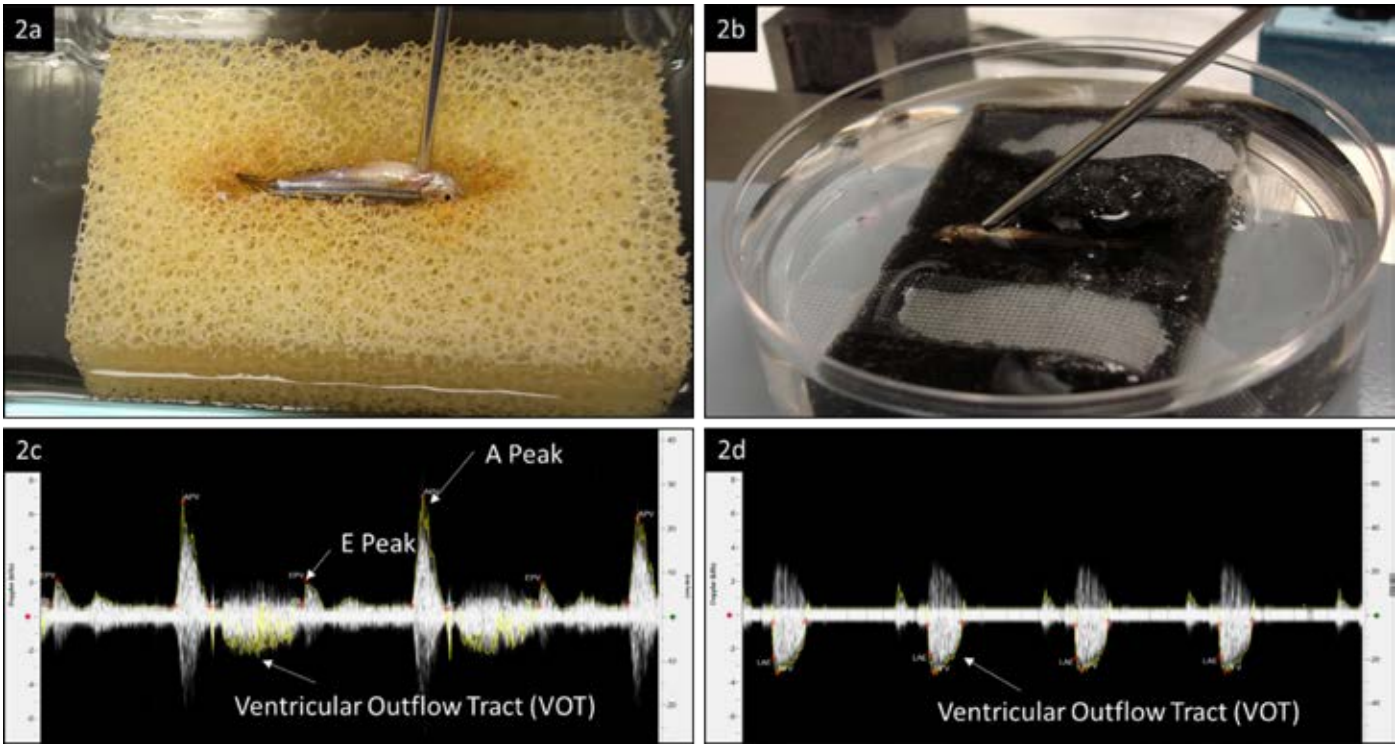

Figure 2 – (a) The 20MHz probe of the DFVS is positioned perpendicular to the ventral side of the fish to obtain the AV flow (Courtesy of Dr. Pascal J. Lafontant, DePauw University), or (b) at an angle to obtain the VOT flow (Courtesy of Dr. Zhen Zhuang, Yale Translational Research Imaging Center). The Doppler flow velocity spectrograms are shown for the AV flow (c) and the VOT flow (d) (Velocity spectrograms courtesy of Dr. Zhen Zhuang, Yale Translational Research Imaging Center).

The Doppler flow velocity spectrogram results shown in Figure 2 confirm the utility of this system to non-invasively assess cardiac function in fish; however, further refinement of these methods is necessary to fully assess cardiac function and hemodynamics in the fish heart.

Researchers may use the DFVS to follow the functional regeneration of the heart, or cardiac development in transgenic models, over the course of a longitudinal study - in the same animal - as the data acquisition is done non-invasively on anaesthetized animals. The fish is simply anaesthetized in water containing Tricaine (MS-222) and stabilized within a sponge. The measurements can be obtained quickly, limiting the time the fish must remain in the Tricaine solution. Full recovery of the fish is expected upon placement in fresh water. This allows the subject to act as its own control throughout the entire study, removing variability due to inter-animal variation caused by biological, genetic, or methodological differences.

The DFVS is easy to operate; new users, familiar with the animal's anatomy, become comfortable to continue practicing on their own after an initial on-site training (1 day). The system from Indus Instruments is a cost-effective and compact benchtop tool, which can easily be placed in the lab next to a fish colony or in the area where surgeries are performed.

To learn more about this exciting, novel approach, please contact Indus Instruments. We would be happy to connect interested researchers with our clients who are perfecting the methodology and application of doppler flow velocity measurements in fish.