DIRECT OBSERVATION OF BLOOD FLOW IN THE GILL OF THE EEL, *ANGUILLA ROSTRATA* BY VIDEOMICROSCOPY

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In the past few years, we have demonstrated that a variety of vasoactive substances (e.g., adenosine, natriuretic peptides, endothelin, nitric oxide, and prostaglandins) can alter tension in blood vessels from a variety of species of fishes, most notably the ventral aorta that carries blood from the heart to the gills (e.g., *J Comp Physiol [B]* 162: 179-83, 1992.; *J Exp Zool* 265: 84-7, 1993; *J Comp Physiol [B]* 165: 659-64, 1996; *Am J Physiol* 274: R1050-7, 1998.; *J. Exp. Zool.* 289: 273-284, 2001; *Physiol. Biochem. Zool.* 74: 120-126, 2001). In addition, more recent work has shown that at least ET, NO, and PGs also may control active transport across the marine fish gill (Evans, D.H. et al., *Bull. MDIBL* 39: 17; this volume). Since endothelin has also been shown to alter the pattern of blood flow through individual gill lamellae (Sundin, L., and G. E. Nilsson. *J. Comp. Physiol.* B 168: 619-623, 1998), we have initiated a research program to visualize and record the effects of these other vasoactive substances on blood flow through the eel gill filaments and lamellae *in vivo*. We report here the results of preliminary studies (on control eel gills) that demonstrate that videomicroscopy of eel gill blood flow is reasonably easy.

American eels, *A. rostrata*, were purchased from a local fisherman and maintained in running sea water at 16 °C. Fish were anesthetized in 0.1g/l MS222 in sea water and placed, ventral side up, in sufficient aerated sea water to cover the body (containing 0.025g/l MS222). The operculae were cut along the ventral aspect in order to expose the gills and to immobilize the fish (via pins to the underlying paraffin base). Gill blood flow was visualized through a 20X water-immersion lens on an Olympus BX30 reflected light microscope (with polarizing filters) and recorded by an Optronics DEI-750 video camera system, which output was connected via a Dazzle A/D converter to a Macintosh iBook G3 running iMovie. Representative video clips were exported to Quicktime, compressed, and saved as self-looping .jpg files. The clips may be viewed by cutting and pasting the URLs into a browser. If Quicktime is not installed on a specific computer, go to http://www.apple.com/quicktime/download/ for a free copy of Quicktime. The largest clip is 12 MB, which should download in less than one minute on an ISDN line; the others should take ca. 15 sec.

Fig. 1 (http://www.zoo.ufl.edu/dhefish/fishgill.jpg) (copyright by Benjamin Cummings). This diagram from Campbell's "Biology" text illustrates the basic structure of the teleost fish gill. Note that each gill arch contains a stack of gill filaments with numerous lamellae at right angles to the filament. Blood runs along the training edge of the filament in the afferent filamental artery, enters the lamella through the prelamellar arteriole, flows as a sheet through the lamellae (around pillar cells which separate the epithelial sheets of the lamella), exits the lamella via the postlamellar arteriole, and travels back to the gill arch via the efferent filamental artery. The lamellae are the site of gas exchange in fishes.

Clip 1. (http://www.zoo.ufl.edu/dhefish/Filament.1.mov) This shows the distal region of a single filament (on edge) running vertically and numerous lamellae at right angles (horizontal). The flow of blood through the afferent filamental artery is obvious (running upward in the

middle of the filament), as is the flow into each lamellae, through the prelamellar arterioles. Flow through individual lamellae is also visible, with somewhat greater flow (darker band of flowing erythrocytes) through the peripheral channel on the edge of the lamellae (especially visible on the first lamella on the left). It is obvious that blood is flowing even through the most distal lamellae of this filament. This total perfusion of all lamellae is in contrast to the conclusions of Booth (*J. Exp. Biol.* 83: 31-40, 1979) that suggested that only approximately 60% of the lamellae were perfused in the unstimulated trout filaments.

Clip 2. (http://www.zoo.ufl.edu/dhefish/Filament.2.mov) This clip shows a filament from the top, with several lamellae and afferent and efferent filamental arteries visible. As the focus changes, you should be able to see perfusion of a single lamella at the end of the filament.

Clip 3. (http://www.zoo.ufl.edu/dhefish/fil.moving.small.mov) This clip shows a single filament (on edge, from lower right to top left) with numerous lamellae to the right. Notice blood flow through the afferent filamental artery, the prelamellar arterioles, and in the peripheral channels of the many lamellae. At the start of the clip, flow through a postlamellar arteriole is seen at the bottom left of the frame. The filament is moving, presumably as the fish breathes. Note that the flow slows as the filament moves downward. We have no directly measurements, yet, of either ventilation or cardiac output, but we hypothesize that this alteration in perfusion rate may be associated with ventilation to match ventilation and perfusion.

Clip 4. (http://www.zoo.ufl.edu/dhefish/Lamella.mov) This is two lamellae, one in focus, showing the pattern of blood flow around the pillar cells, and through the peripheral channel, in particular in the second lamella, which is out of focus.

Clips 5 and 6. These both show an array of lamellae (mostly from the top) in a single filament from a fish 5 hrs (http://www.zoo.ufl.edu/dhefish/Lam.5hrs.mov) and 8 hrs (http://www.zoo.ufl.edu/dhefish/Lam.8hrs.mov) after the start of the experiment, demonstrating that the preparation is viable for prolonged periods.

Our data show that blood flow in the eel gill is easily visualized with this videomicroscopy system. Our next step is to monitor, and quantify, changes in these patterns elicited by infusion of the vasoactive signaling agents described above. In addition, this system could be utilized to study the effects of hypoxia, water-borne xenobiotics, or acidification on gill perfusion. (Supported by NSF IBN-0089943 to DHE)