PREPARATION OF THE LONGHORN SCULPIN (*MYOXOCEPHALUS OCTODECIMSPINOSUS)* FOR SIMULTANEOUS CARDIOVASCULAR AND VIDEOMICROSCOPIC STUDIES

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Previous studies from our laboratory (e.g., *J. Exp. Zool.* 289: 273-284, 2001; *Physiol. Biochem. Zool.* 74: 120-126, 2001) have demonstrated that local effectors, such as endothelin, nitric oxide, and prostaglandins are vasoactive in a variety of fish species. In an effort to observe and quantify the effect of these putative agents on the perfusion of the fish gill, we have initiated a series of procedures to allow the simultaneous measurement of the cardiovascular and intra-gill perfusion effects of the agonists. To this end, we describe initial studies on the longhorn sculpin.

Longhorn sculpin (*Myoxocephalus octodecimspinosus*; 0.2 to 0.5 kg) were purchased from a local fisherman and maintained in running seawater (10-12°C) prior to experiments. Sculpin were anesthetized by submersion(< 5min) in a benzocaine (ethyl-*p*-aminobenzoate) in seawater solution (0.375g/L). Once the fish was anesthetized, the left operculum was cut along the ventral aspect and pinned to the paraffin substrate, in order to expose the gills, which were irrigated constantly with seawater. A (1/2 m) polyethylene cannula (PE-50, ID = 0.58mm, OD = 0.97mm) was tipped with a 1 cm, 23 g. stainless steel needle, filled with heparinized (1mg/ml) teleost Ringer's solution then inserted into the branchial artery of the 3rd left gill arch and secured with silk suture (5-0). The cannula was used to measure pre-branchial (= ventral aortic) blood pressure (BP), as well as for infusion of putative vasoactive substances. BP was monitored using a Biopac pressure transducer connected through an amplifier to a Macintosh G3 Powerbook using AcqKnowledge Software (Biopac Systems). Before each experiment, the pressure transducer was calibrated with a static column of water. To monitor cardiac rate and output (CO), a 3 cm ventromedial incision was made slightly anterior to the pectoral fins, and the overlying muscle was displaced using retractors. The ventral aorta was exposed through dissection and was fit with a flow probe (Carolina Medical, Models EP1035 or EP106). The pericardial sac and heart were not disrupted during surgery. The flow probe was connected to a Carolina Medical Square-Wave Electromagnetic Flowmeter and a ground wire was attached via alligator clamps to the sculpin's tail. Output from the flowmeter was digitized and recorded using the Biopac recording system as above. For videomicroscopy of the gills, the right operculum was cut along the ventral aspect, exposing the gills. Sculpin were placed ventral side up in a chamber (35cm x 18cm x 10cm) with flowing seawater. Gill blood flow observations were made as described previously (Evans, D. H. *Bull. MDIBL* 41: 97, 2002).

Fig. 1 demonstrates that we are able to record relatively consistent, ventral aortic blood pressure for periods approaching one hour. The average blood pressure was nearly identical to *in vivo* measurements reported previously for this species (43.1 cm H_2O; Claiborne, J. B. and Evans, D. H., Marine Biol. Lett. 2:123-130, 1981).

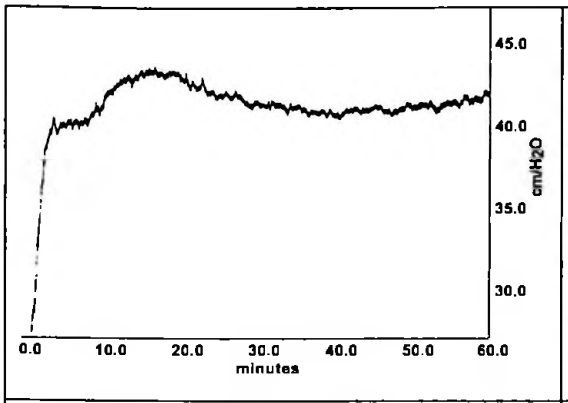

Fig. 1. Representative tracing of ventral aortic pressure measured in sculpin

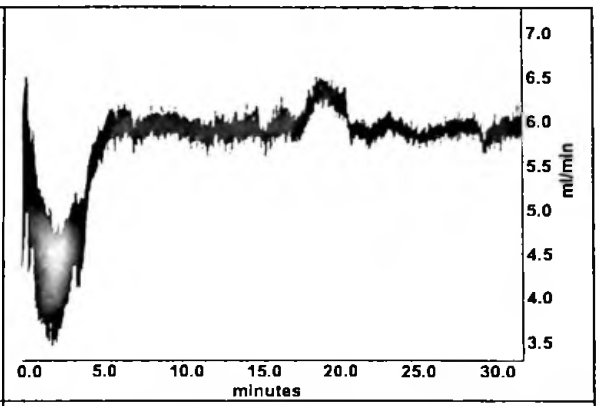

Fig. 2. Representative tracing of cardiac output measured in sculpin

Fig. 2 shows that CO can be monitored for at least 30 minutes. The CO (corrected for a ca. 300g fish) was slightly below that observed in rainbow trout (23 $ml.min^{-1}.kg^{-1}$; Conklin et al., *Ibid*), not unexpected considering the sedentary nature of the sculpin.

The video clip (http://www.zoo.ufl.edu/dhefish/Sculpingill.mov) shows a single filament with six lamellae at right angles. The filed of vision is approximately 400 microns and the clip is in real time. The flow of erythrocytes through the afferent filamental artery, prelamellar arterioles, and into the lamellar peripheral channel is obvious. Our concurrent studies (Hyndman and Evans, this volume) have demonstrated that endothelin receptors (ETB) can be localized on the afferent filamental artery and prelamellar arterioles, so this preparation will allow us to test the effect of ET, and the other paracrines of interest, on the dimensions of these vessels, while monitoring cardiovascular parameters. This preparation could also prove useful in studying the effects of hypoxia, xenobiotics, pH, and salinity on perfusion and cardiovascular parameters. (Supported by NSF IBN-0089943 to DHE)