Sinus Venosus Catheterization for Repeated Vascular Access in the Hybrid Striped Bass

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Abstract.—Placement of vascular cannulae for repeated venous blood sampling has proven to be a useful technique in many fish species. The anatomy and size of the hybrid striped bass (striped bass *Morone saxatilis* ♀ × *M. chrysops* ♂) makes this procedure challenging in this species. The sinus venosus was determined to be the best site for catheter placement based on size, accessibility, and ability to stabilize the catheter within it. Catheterization of the sinus venosus with a 20-gauge × 3.8-cm flexible Teflon catheter was unsuccessful because the catheter folded on itself, occluding the lumen. Catheterization with an 18-gauge × 3.8-cm hypodermic needle was also unsuccessful because the sinus wall occluded the needle lumen when negative pressure was applied. However, 18-gauge × 3.8-cm intravascular needles remained patent in free-swimming fish for 2 weeks without major complications. An external fixation device to prevent migration of the catheter out of the sinus venosus and laceration of the sinus wall during normal swimming movements improved the technique.

Catheterization of a major blood vessel to allow for repeated venous blood sampling or drug administration is a necessary part of many fish studies. It is especially useful in nutritional and pharmacokinetic experiments. Reducing handling

stress and the associated physiological effects during repeated sampling is critical to obtaining data more comparable to the free-swimming fish.

A variety of intracoelomic veins have been cannulated, including the gonadal mesenteric and coeliac (Axelsson and Fritzsche 1994). This allows for extremely specific sampling but is technically challenging and requires opening of the coelom, which could affect the results in many studies. Additionally, the use of the small-diameter cannulae that would be required to accomplish this task in market-sized hybrid striped bass (striped bass *Morone saxatilis* ♀ × white bass *M. chrysops* ♂) would prolong sampling times and withdrawal pressures, which can damage blood cells.

Cannulation of the afferent or efferent branchial vein has been successfully carried out in several species, including Atlantic cod *Gadus morhua* (Wahlquist and Nilsson 1977), shorthorn sculpin *Myoxocephalus scorpius*, the eelpout *Zoarces viviparus*, and fivebearded rockling *Ciliata mustela* (Fritzsche 1990), for collecting blood pressure data. While it is possible to place a cannula in this location in hybrid striped bass or smaller fish species, it is extremely difficult to stabilize the device for long-term use in free-swimming fish. This technique also requires the use of an extremely small-diameter catheter, resulting in difficulty drawing samples efficiently.

Ventral aortic cannulation has been achieved in a tuna *Euthynnus affinis* (Jones et al. 1986) and in spotted dogfish *Scyliorhinus canicula* and rainbow

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FIGURE 1.—Schematic representation of the catheter stabilization device. The depression shown with a horizontal mattress suture traversing it is filled with epoxy resin after placement of the catheter through the device.

trout *Onchorynchus mykiss* (Axelsson and Fritsche 1994). It is a straightforward method of percutaneous cannulation of a major vessel. Unfortunately the ventral aorta in hybrid striped bass is very short, which makes maintaining the catheter for prolonged periods in a free-swimming fish very difficult.

Catheterization of the caudal artery and the caudal vein were attempted in hybrid striped bass in our laboratory. While vascular access could be obtained, patency of the catheter was lost after recovery of the fish from anesthesia. The movement of the musculature in the region dislodged the very-small-diameter catheters from the vessel.

Attempts to use the dorsal aorta catheterization techniques developed for pink salmon *Onchorynchus gorbuscha* (Smith and Bell 1964; Pye-MacSwain et al. 1994), rainbow trout (Beyenbach and Kirschner 1975), and channel catfish *Ictalurus punctatus* (Kitzman et al. 1988) in hybrid striped bass were not satisfactory in our laboratory. The dorsal aorta of the hybrid striped bass is small in diameter relative to similarly sized salmonids or catfish. The vessel also curves sharply dorsad immediately posterior to the pharynx, making placement and stabilization of the catheter problematic. These anatomic differences make the classic dorsal aorta catheterization technique extremely difficult in this species. Because of the difficulties associated with these techniques the development of a new, safer technique for achieving repeated vascular access in hybrid striped bass is highly desirable.

Methods

Animals.—Twenty-five market-sized (400–800 g) hybrid striped bass were conditioned for 1 month in a 1,200-L, freshwater, flow-through water system with supplemental aeration. Water temperatures ranged between 16°C and 18°C and pH ranged between 6.8 and 7.0. The fish were fed number 3 trout pellets (Zeigler Brothers, Gardners, Pennsylvania) at a rate of 3% body weight once daily.

Anesthesia.—Induction of anesthesia was achieved by placing the fish in a 20-L container containing 10 L of a 200-mg/L solution of tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, Washington). Induction time was approximately 3 min. The fish was then transferred to an anesthesia machine that recycled 10 L of an 80-mg/L solution of MS-222 past the gills at a rate of 1.3 L/min, and the fish was periodically bathed with water to prevent drying. Buffering capacity of well water maintained stable pH upon addition of MS-222. The fish was placed in a foam trough that maintained the fish in ventral recumbency. After catheterization, fish recovered in individual 600-L freshwater flow-through tanks with supplemental aeration.

Anatomical studies.—A latex cast of the vasculature was made in three anesthetized fish by injecting 3 mL of latex (Injection Medium, Red, Carolina Biological Supply Co., Burlington, North Carolina) through the opercular membrane into the lumen of the ventricle as each fish died. After ex-

FIGURE 2.—Placement of the catheter within the sinus venosus of the hybrid striped bass: sinus venosus (A), atrium (B), ventricle (C), and bulbus arteriosus (D). The fish has been dissected to the level of the heart, and the liver was removed for better visualization. Scale = 1 cm.

posing the peritoneal cavity with a ventral midline incision from the vent to the pectoral girdle, each fish was placed in 10% neutral-buffered formalin for 7 d to allow the tissues to fix and the latex to cure before dissection. Criteria to determine suitable catheterization sites included (1) a structure with adequate diameter and length to allow placement and stabilization of a catheter no smaller than 22 g, (2) percutaneous accessibility without passing through vital organs, and (3) the potential to stabilize movement of the catheter relative to the structure during normal swimming movements of the fish.

The sinus venosus is large enough to allow catheter placement without compromising blood flow and is accessible from a dorsolateral approach. The needle passes through the cranial epaxial muscles where there is relatively little movement during normal swimming.

Catheter placement and stabilization.—Initial efforts to catheterize the sinus venosus with a 20-gauge $\times$ 3.8-cm over-the-needle, Teflon catheter (Critikon, Tampa, Florida) were unsuccessful. Pa-

tency of the catheters was lost in four of four fish immediately after the needles were removed from the catheters. Euthanasia and dissection of these fish indicated movement of the catheters was a major cause of failure for this technique.

An external fixation device was designed to prevent the catheter from migrating out of the sinus venosus during swimming or when the fish hit the catheter on the side of the tank. This fixation device was created by removing the tabs of a 13 $\times$ 20-mm gray butyl-rubber stopper from a serum vial. Then a 5 $\times$ 8 $\times$ 3-mm section of rubber was removed from the center of the resulting disk and a loose 3-0 nylon horizontal mattress suture was placed across the resulting depression (Figure 1).

Catheterization of the sinus venosus with an 18-gauge $\times$ 3.8-cm, intravascular-point needle (Sherwood Medical, St. Louis, Missouri) and the external fixation device was performed in twelve fish. The external fixation device was sutured with the depression outward just dorsal to the operculum, using four simple interrupted 3-0 nylon skin sutures. An 18-gauge $\times$ 3.8-cm, intravascular-point

FIGURE 3.—A free-swimming hybrid striped bass after placement of the sinus venosus catheter: catheter stabilization device with catheter passing into the sinus venosus (A), extension set (B), Chinese finger trap suture (C), and heparin-filled syringe (D).

needle attached to a 3-mL syringe containing 1 mL of heparinized saline was inserted through the center of the fixation device and the body wall of the fish. The needle was then visually guided, as viewed through the opercular membrane, into the faintly red sinus venosus while slight negative pressure was applied with the syringe. Appropriate placement was determined by smooth blood flow into the syringe under gentle negative pressure (Figure 2). Once the needle was in place, the syringe was removed and a 45.7-cm extension set was filled with heparinized saline and attached to the needle. To stabilize the needle, the depression in the fixation device was filled with two-part epoxy resin (Duro, Lock-tite Corp., Rocky Hill, Connecticut). Epoxy resin was also applied to the point of connection between the needle and the extension set to ensure they did not separate after the fish was revived. Additional stabilization was provided by suturing the extension set to the fish just ventral to the dorsal fin with a Chinese finger trap suture of 3-0 nylon (Orton 1993). A three-way stopcock (Medex, Hillard, Ohio) was used to ter-

minate the open end of the extension set. A 3-mL syringe filled with air attached to the stopcock kept the extension set afloat. A second syringe containing heparinized saline was attached to the stopcock and was used to flush the catheter daily (Figure 3).

Ten percent povidone-iodine ointment was applied topically to the skin sutures of all fish immediately before recovery from anesthesia to prevent fungal infection. The time required to perform catheterization of the sinus venosus decreased as proficiency was gained with the procedure and ranged from 15 to 45 min per fish. Six additional attempts were made to perform this procedure with 18-gauge $\times$ 3.8-cm needles with standard hypodermic bevels (Sherwood Medical).

Euthanasia.—After the 14-d study period each catheterized fish was euthanatized by placing it in a 500-mg/L solution of MS-222 for 20 min, and a gross necropsy was performed.

Results and Discussion

The 20-gauge $\times$ 3.8-cm, over-the-needle Teflon catheters lost patency immediately after the needle

was removed from the catheter. Necropsy showed that in three of the four fish used, the catheters folded over themselves in the sinus venosus, and flow was occluded in the fourth fish by the sinus wall having been drawn into the lumen of the catheter.

The 18-gauge $\times$ 3.8-cm, hypodermic-point catheters required extensive manipulation of the syringe to draw blood, resulting in long sample acquisition times. On direct examination of the catheter during necropsy of the six fish that underwent this procedure, it was apparent the shallow angle (12°) and shape of the bevel of a standard hypodermic needle allowed the lumen to be completely occluded by the walls of the sinus venosus when gentle negative pressure was applied to the syringe.

The 18-gauge $\times$ 3.8-cm intravascular point catheters remained patent for the 2-week trials with daily flushing with heparinized saline. There were no major complications observed in any of the catheterized fish. Although there did not appear to be an accumulation of uneaten food in the fish holding tanks, the catheterized fish were never observed eating. At necropsy slight hyperemia of the muscle tissue was seen immediately surrounding the needle and in the areas of suture placement, as well as the presence of a small hematoma at the point of needle entry into the sinus venosus. No food was found within the gut of the fish at the time of necropsy. The angle of the bevel (18°) and shape of the opening of the lumen of the intravascular needle apparently prevented complete occlusion by the walls of the sinus venosus and allowed patency to be maintained.

The catheterization of the sinus venosus with an external stabilizer is a technique that provides a means of repeated blood collection from hybrid striped bass. This technique has several advantages over previously described cannulation techniques, including the ability to use a large-gauge catheter relative to the size of the fish, for rapid acquisition of intact blood samples and the ability to stabilize

the catheter for long-term use in free-swimming animals. This approach to catheterization may also have application in other fish species in which other vascular structures are difficult or impractical to catheterize.

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