

# **Non-Invasive Surgery Techniques in Fish Research: A Review on Esophageal Intubation, Dorsal Aorta Cannulation, and Urinary Catheterization in Sturgeon**

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## **Abstract:**

The combined technique consisting of esophageal intubation, dorsal aortal cannulation and urinary catheterization has been shown to be an effective technique for the oral administration of a compound, repeated blood sampling, and continuous urinary collection while minimizing the stress response associated with handling and sampling. The successful use of the combined technique warrants further research for refinement and expansion of the technique to include additional sampling routes to better account for clearance pathways not currently accounted for. The combined technique can be adapted for use in studies of nutritional, toxicological, physiological, or therapeutic agents of importance.

## **Introduction**

Esophageal intubation, dorsal aorta cannulation, and urinary catheterization techniques have been used singly or in combination to study the biology of many species of fish. Different aspects of surgical techniques such as anesthetics, cannulation, confinement, post-operational recovery, etc. have been reviewed by Summerfelt and Smith (1990), Houston (1990), Iwama and Ackerman (1994), Axelsson and Fritsche (1994), and Horsberg (1994). The objective of the present paper is not to review extensively or exhaustively any single technique, but to focus on the combination of the above techniques and possible inclusion of other techniques in future research using sturgeon as a model species.

Fitting of an indwelling tube into the mouth (Holeton and Randall 1967, Wood and Randall 1973), esophagus (Carrick and Balment 1983, Glover and Hogstrand 2002), or intestine (Youson et al. 1988) has been used to deliver various compounds into the gastrointestinal tract (GIT) of different species of fish. The intubation method allows quantitative delivery of specific compounds at multiple doses and at different time intervals with minimum regurgitation, disturbance, and stress to the fish.

Dorsal aorta cannulation was developed by Conte et al. (1963) and Smith and Bell (1964), and modified and improved by Houston (1971) and Soivio et al. (1972, 1975). The technique has been modified, adapted, and used to monitor blood/plasma parameters in Atlantic salmon, common carp (Kayama and Iijima 1976), tuna (Jones et al. 1986), channel catfish (Mazik, 1994), striped bass (Cech et al. 1996), tilapia (Ron et al. 1995), charr (Haug and Hals 2000), and grouper (Lo et al. 2003) with minimum stress. Other vascular cannulation techniques including ventral aorta (Kirsch 1972), caudal arterial and venous (Watters and Smith 1973), ventricle (Thorpe and Ince 1974), afferent and efferent vessels of gill arches (Wells et al. 1984), sinus venous (Ishimatsu et al. 1988), hepatic portal vein (McLean and Ash 1988), and pneumogastric artery and vein (Hyde and Perry 1989) cannulations have been developed for

different species of fish. These techniques have also been used singularly or in combinations in different species of fish.

Urinary bladder catheterization has been used to study the kidney and urinary bladder functions in fishes (Wood and Patrick 1994) including lungfish (Sawyer 1966), rainbow trout (Holmes and Stainer 1966), American eel (Butler 1969), southern flounder (Hickman 1968), and common carp (Kakuta et al. 1986). The esophageal intubation, vascular cannulations, and urinary catheterization have been used individually and in combination to study the dynamics of specific compounds in fishes under different treatment and environmental conditions. Data obtained with the combined techniques are especially suited for estimating kinetic parameters in models describing a particular biological process or metabolic dynamics of a specific compound (Schultz and Hayton 1991. and Kleinow 1991).

### **Validation and Uses of Combined Technique in White Sturgeon**

Force-feeding different carbohydrates in gelatin capsules were used as an oral challenge test in white sturgeon (Hung 1991). Various vascular cannulations were used to study the blood/plasma responses of Adriatic (Randall et al. 1992, Di Marco et al. 1999), white (McEnroe and Cech 1985, Crocker and Cech 2000, Crocker et al. 1998), and green (Belanger et al. 2001) sturgeon to different treatments and environmental conditions. Urinary catheterization of white sturgeon was used to monitor urinary free amino acid after an oral dose of a free amino acid mixture (Ng et al. 1996).

The first simultaneous use of dorsal aorta cannulation, urinary catheterization, and esophageal intubation in the white sturgeon was described by Deng et al. (2000). This study was carried out in order to assess the effectiveness of the combined technique to quantitatively deliver nutrients, sample blood repeatedly, and continuously collect urine while minimizing the handling stress associated with force-feeding and blood sampling. The quantitative delivery of nutrients through the esophageal intubation tube was confirmed by the complete recovery ( $105\pm 5\%$ ) of an intubated dose of  $\text{Cr}_2\text{O}_3$ , an inert unabsorbable marker, in the GIT. Repeated blood sampling and collection of urine was also achieved with the absence of a stress response during sampling of the experimental fish.

Stress levels, determined by the levels of cortisol and glucose in the plasma up to 72 h post-operation, returned to basal levels within 48 h post operation and remained at basal levels through 72 h post-operation (Deng et al. 2000). The basal cortisol levels determined by Deng et al. (2000) were in agreement with those reported in Rainbow trout (Brown et al. 1986), channel catfish (Mazik et al. 1994), Siberian sturgeon (Maxime et al. 1995), and Adriatic sturgeon (Di Marco et al. 1999). The return of cortisol and glucose levels to basal levels 48 h post-operation and maintenance at basal levels during blood sampling between 48-72 h post-operation permit the study of a wide variety of physiological processes and metabolic dynamics of any compound present at any desired concentration. For example, the physiological dynamics associated with the temporal stress response of sturgeon caused by the manipulation of environmental variables (Crocker and Cech 1998), or exogenous injections of stress-related hormones (Belanger et al. 2001) can be explored using the combined technique.

Subsequent to the development and validation of the combined technique, the technique has been used to study carbohydrate utilization when present in different forms (Deng et al. 2001), and in different concentrations (Gisbert et al. 2003). Tashjian (unpublished data) has recently extended the usefulness of the combined technique by successfully applying the

technique to study the kinetics of selenium absorption, distribution and excretion of L-selenomethionine, one of the most prevalent forms of selenium present in the natural food sources of the white sturgeon in the San Francisco Bay-Delta (Fan et al. 2002). The same study has provided insight into the mechanisms of acute toxicity of organic forms of selenium by demonstrating a significant decrease in urinary flow within 6 hours post-intubation, suggesting kidney failure may be the primary cause of acute toxicity and death in sturgeon intubated with elevated doses of L-selenomethionine (Tashjian, unpublished data). Additional studies investigating the kinetics of selenium absorption, distribution and excretion of L-selenomethionine when intubated at different dosage levels are in progress.

## **Technical Considerations**

Although the use of the combined technique has help gain considerable insight into carbohydrate and selenium absorption, distribution, and excretion, there are a number of technical aspects regarding the individual components of the combined technique that must be dealt with carefully and/or improved to further refine the combined technique.

### ***Esophageal Intubation***

Many factors such as the type of carrier used, amount of material intubated, and consistency of material intubated must be carefully considered when delivering a dose of a desired compound via esophageal intubation. The type of carrier used to deliver a desired compound can greatly influence the absorption dynamics by influencing the rate of movement of the bolus through the GIT, binding with the compound being intubated (decreasing bioavailability), and competing with the intubated compound at absorption sites in the intestine, pyloric caeca, and spiral valve of sturgeon. A recent study has demonstrated a very rapid passage time through the intestine (<3 h) and spiral valve (6-12 h) when sturgeon were intubated with a starch gel solution via esophageal intubation (Tashjian, unpublished data). Deng et al. (2000) also reported a very short passage time of a gelatin gel bolus (3-6 h) when intubated into the esophagus. In contrast, the passage time of a commercial diet through the GIT of sturgeon may be as long as 48 h (Tashjian, personal observation). Because the passage time through the GIT can have a strong influence on the absorption dynamics of a compound, the type of carrier to be used should be dependent on the objectives of the study. Simple carriers may be used to study the absorption of a compound without the interactive effects of other dietary components. More complex carriers such as commercial or purified diets (Hung et al. 1987), however, may be used as carriers to integrate the complex interactions among dietary components. The use of complex carriers may be beneficial when a more realistic understanding of the absorption and metabolic dynamics of a compound are desired (i.e. when a compound is incorporated into fish feed). The use of complex carriers may be useful to simulate a more ecologically relevant oral exposure to a compound.

The amount of intubated material in a single dose, and the diameter of the tube used, must be carefully considered in order to avoid regurgitation of an intubated bolus. Deng et al. (2000) demonstrated that sturgeon (1-2 kg) intubated with 2 g gelatin gel kg<sup>-1</sup> body weight (bw) did not regurgitate the bolus. An examination of the swim bladders (connected to the GIT in the forestomach) also demonstrated that no intubated material entered the swim bladders. However, regurgitation did occur when sturgeon were intubated with ≥4 g gelatin gel kg<sup>-1</sup> bw using larger

diameter tubing (O.D. 4.0 mm) during preliminary experimentation. The regurgitation could be caused by either too much intubated material or by using tubing with an outer diameter larger than the tubing used by Deng et al. (2000) (O.D. 3.2 mm). Preliminary trials with the selected intubation volume and tube diameter should be conducted before initiation of the experiments. Additional information on a variety of compound administration techniques is available in Perry and Reid (1994) and information on the bolus-injection of radiolabels for study of steady state glucose metabolism is available in West (1994).

### ***Dorsal Aorta Cannulation***

Although increased sampling frequency is desirable to obtain a more accurate understanding of the metabolic or physiological processes being studied, sampling protocols through the cannula for individual fish must be carefully chosen to minimize a number of complications. One such reported complication is the decrease in hematocrit through the sampling period in the study conducted by Deng et al. (2000). The decrease in hematocrit may be due to red blood cell mobilization and acidosis generated from anaerobic glucose catabolism, which is in turn stimulated by stress hormones (Deng et al. 2000, Soivio et al. 1972, 1975). Another explanation may be hemodilution caused by the repeated sampling of blood. Although 10% of the blood volume has been suggested as the acceptable amount that can be removed during experiment (Schultz and Hayton 1991), the amount of blood removed by Deng et al. (2000) was only 3-5% of the total blood volume. Remediations to this problem include sampling smaller volumes of blood, replacing sampled volume with an equivalent amount of blood (Axelsson and Fritsche 1994), or re-injecting the red blood cells after each sampling.

Although protocols for dorsal aorta cannulation (Deng et al. 2000) and caudal vein cannulation (Belanger et al. 2001) in white sturgeon have been developed individually, the simultaneous use of dorsal aorta and caudal vein cannulation has not been explored. Simultaneous use of both cannulation techniques may be useful when both intravascular injection and sampling is desired. The use of separate cannula for injection and sampling will prevent contamination of sampled blood. The use of a double lumen cannula may also be used to prevent sample contamination when the use of only one cannula is possible (West 1994). The choice of sampling and injection sites may influence the kinetic parameters of certain metabolic processes (West 1994, Katz 1992, Norwich 1992, Wolfe 1984), and thus the sampling and injection sites must be chosen with caution. The dynamics of compounds with a high renal extraction ratio may be especially sensitive to the chosen sampling protocol, due to a first-pass effect in the kidney (Horsberg 1994). There are also many other types of arterial and venous cannulations as described above which might also be employed with the dorsal aorta and caudal vein cannulation to expand the scope of our investigation on the biology of white sturgeon. For a detailed discussion on issues relevant to the use of cannulation techniques such as choice of cannula material and anticoagulants, physical properties of cannula, preparation of cannula, and insertion techniques, see Axelsson and Fritsche (1994).

### ***Urinary catheterization***

Urinary catheterization of white sturgeon has been the most troublesome component of the combined technique, reflected by the large variance of urinary flow rates among different studies and large variance in metabolite excretion among individuals within studies (Ng et al.

1996, Deng et al. 2000 & 2001, Gisbert et al. 2003, Tashjian unpublished data). A number of factors can be the cause of variation among and within studies including; differences in experimental conditions, physiological variation among individuals, preparation of catheters, and construction of catheters.

The 20-70% difference in urinary flow rates between the studies of Ng et al. (1996) and Deng et al. (2000) are puzzling. Although variation in sturgeon body size (nearly 2-fold difference in body size) and water temperature (7°C difference in water temperature) between the two studies may be the cause of the differences in urinary excretion rates, the difference in the urinary catheter preparation between the two studies may have been the major cause. Both investigators inserted the catheters 10 cm into the urinary ducts, but the fish used by Deng et al. (2000) were twice as big, possibly allowing water to seep into the catheters due to the shallow insertion depth of the catheters into the urinary ducts. Moreover, only 2 cm of the urinary catheters were perforated by Ng et al. (1996), while 4 cm were perforated by Deng et al. (2000), which may have caused incomplete collection of urine through the catheters in the case of Ng et al. (1996) or allowed water to be siphoned into the catheters in the case of Deng et al. (2000).

Recent experimentation on the optimum insertion depth of the urinary catheters showed that insertion of catheters between the 4<sup>th</sup> and 5<sup>th</sup> scutes (ca. 13 cm) anterior to the urinary duct opening resulted in blood to be present in the urine and an abnormally low volume of urine collected, possibly due to the puncturing of the urinary ducts. Insertion of the urinary catheters between the 3<sup>rd</sup> and 4<sup>th</sup> scutes anterior to the urinary duct opening (ca. 10.5 cm) resulted in the collection of a similar volume of urine as collected by Deng et al (2000) with no blood present. Insertion of the urinary ducts between the 2<sup>nd</sup> and 3<sup>rd</sup> scutes (ca. 9.5 cm) resulted in a high volume of urine collected, approximately twice of the volume collected by Deng et al. (2000, 2001). Determining the insertion depth of the urine catheters by referring to the ventral scutes rather than using a predetermined depth as used by Ng et al. (1996) and Deng et al. (2000) provides an approximate normalization of catheter insertion depth to body length.

Detailed studies on the effect of body-size and temperature on the urinary flow rate are necessary in order to correctly scale for body size and temperature effects on urinary flow rate. The influence of catheter diameter, depth of catheter insertion into urinary ducts, and degree of perforation on urinary flow rate must be determined to further optimize the catheterization technique. Further refinement of the urinary catheterization technique should also be explored to reduce variance in urine collection. A possible refinement to the technique that should be explored further includes sealing the urinary duct opening with tissue cement, which would prevent water seepage into the catheters and prevent leakage of urine not collected by the urinary catheters. Careful attention must be paid to the possible causes of variation outlined (**Table 1** at end of this article) in order to reduce error variation in urinary flow rate between the two catheters in each fish and among individual fish. Although difficult to confirm, accurate measurements on the urinary flow rates of undisturbed sturgeon are needed to provide a reference point to determine whether modifications to the catheterization technique improve the collection of uncontaminated urine. For a more detailed discussion on methods for assessing kidney and urinary bladder function in fish refer to Wood and Patrick (1994).

Table 1. Troubleshooting guide when experiencing difficulties with urinary catheterization of white sturgeon.

Problem	Causes	Remediations (Troubleshooting should proceed by moving from the top to bottom of the remediation procedures)
Low urinary flow rate or no urine flow	Catheters not filled with water or air bubbles in urinary catheters	Check catheters for air bubbles. If air bubbles found, use a syringe to inject water through urinary catheters. Wait at least 24 hrs before sampling urine to allow injected water to drain out of urinary ducts.
	Height of urine collection tubes is too high, preventing a sufficient siphon of urine	Place the open tip of the catheter 2-8cm below the water-level to establish sufficient siphon (Wood and Patrick 1994).
	Height of urine collection tubes is too low causing a siphon strong enough to such urinary duct wall against catheter tip.	Place the open tip of the catheter 2-8cm below the water-level to establish sufficient siphon (Wood and Patrick 1994).
	Perforation holes in urinary catheter are blocked	Inject water into catheters with a syringe to clear blockage. Wait 24 hours before collected urine samples.
	Both urinary catheters inserted into only one urinary duct	Fish must be removed from chamber and re-operated. To prevent this problem, inject water into one urinary catheter after fish have been implanted with the urinary catheters on operating table. If water flows out of second catheter then both catheters are in the same urinary duct. <sup>1</sup>
	Catheterization of incorrect ducts (i.e. catheters inserted into the anus).	Remove incorrectly inserted catheterization tubes and insert into urinary ducts. <sup>1</sup>
	Catheters inserted too far into urinary ducts, possibly leading to puncture of catheters.	If blood is apparent in urine, remove fish from experiment. If above remediations do not solve the problem, remove fish from experiment.
High urinary flow rate	The height of urine collection tubes is too low causing a siphoning of tank water into catheters.	Place the open tip of the catheter 2-8cm below the water-level to establish sufficient siphon (Wood and Patrick 1994).
	Insertion depth of catheters is too shallow causing a siphoning of tank water into catheters	Remove fish from chambers and adjust catheter insertion depth. <sup>1</sup>
	High stress level in fish	Examine condition of holding chamber and fish for visible signs of stress.

<sup>1</sup> Because anesthesia and stress may induce a “laboratory diuresis,” any manipulation of urinary catheters during remediation procedures should be followed by a recovery period of at least 24h (Wood and Patrick 1994).

## Additions to the Combined Technique

A number of additions to the combined technique should be explored to more accurately determine the absorption, distribution, metabolism, and excretion of a compound. A more accurate mass balance of a compound under study would be achieved if the excretion of the compound through the bile, feces, and gills are monitored by the combined technique. Bile duct catheterization, as established for salmon (Klonz and Smith 1968), may be attempted in the white sturgeon. Catheterization of the GIT from the anus may also be useful to recover all feces and avoid contamination of tank water so that excretion via gills may be determined. Many methods have been proposed to account for gill excretion. The use of metabolic chamber system to separate anterior and posterior parts of the body combined with the use of urinary catheterization has been developed (Smith 1929). An elevated stress response due to confinement and the potential for water contamination among chambers, however, must be explored before using this technique with white sturgeon (Wood and Patrick 1994).

An alternate method without the use of a metabolic chamber system exists if the GIT and urinary ducts are fully catheterized and regurgitation of the intubated material does not occur. The alternate method is to determine gill excretion by direct water sampling (Horsberg 1994). A static system must be utilized for this type of study, and the measured compound must be present in the sampled water at detectable levels. In this type of static water study, experimental fish should be transferred through a series of static water tanks at predetermined time intervals and the water from the tanks should be sampled at the end of each time interval. If static water baths are to be used, ammonia accumulation and dissolved oxygen levels must be carefully monitored and dealt with. One complication of such studies would be the volatilization of highly volatile compounds, resulting in an underestimation of excretion rate through the gills. All additions to the combined technique must be validated to determine if results may be extrapolated to aquacultural or natural conditions.

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