

INTERNAL SONIC TRANSMITTER IMPLANTATION IN STURGEON (*ACIPENSERIDAE*):
EVALUATION OF SURGICAL TECHNIQUES AND THE EFFECTS OF SHORT- AND
LONG- TERM TRANSMITTER PRESENCE.

by

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(Under the direction of Sonia M. Hernandez)

ABSTRACT

Surgical methods used for transmitter implantation should be carefully evaluated to determine their effect on fish. One-hundred twenty Siberian sturgeon (*Acipenser baerii*) were assigned to one of four surgical groups: Monocryl-Plus (triclosan-coated poliglecaprone 25), Monocryl (poliglecaprone 25), Vicryl (polyglactin 910), or Prolene (polypropylene). Fifteen fish in each surgical group received an intracoelomic sonic transmitter. Thirty fish were assigned to the control group. Post-operative assessments evaluated surgical healing, transmitter retention, and physiological and pathological effects of intracoelomic transmitters. Incisions closed with Vicryl or Prolene were more likely to exhibit erythema or incomplete healing compared to Monocryl or Monocryl-Plus. Long-term transmitter presence did not increase plasma cortisol levels or affect growth. Organ damage was not evident when adhesions were present. Results indicated that overall transmitter loss was 36.2%. Based on the results, I recommend using either Monocryl or Monocryl-Plus when performing a coeliotomy with intracoelomic transmitter implantation in sturgeon.

INDEX WORDS: Sturgeon, Surgery, Telemetry, Suture Materials, Plasma Cortisol

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

TELEMETRY

Transmitter implantation relies on the assumption that transmitters do not adversely affect the behavior or physiology of the animal (Schreck 1990; White and Garrott 1990), or cause any pathology over the long-term (Guynn et al. 1987; Jepsen 2003). As with most species, telemetry is used to survey aspects of fish ecology such as identifying spawning and rearing habitats, migratory patterns, and interactions with environmental variables that are not typically revealed by standard sampling practices (Bridger and Booth 2003; Neely et al. 2009). In general, when choosing a type of transmitter, one should consider study aspects such as, the duration of monitoring needed, the range of transmitter, the battery life, transmitter size, animal size, and choose a type of transmitter that will have the least affect on the overall health of the species in question (Winter 1983). The goal should be to minimize any potential effects on the animal to obtain the most accurate data that will most accurately reflect the animals' natural behavior (Bridger and Booth 2003; Mech and Barber 2002). Some researchers suggest that data from transmitters should not be considered reliable until the animal has had at least one week of acclimation to the transmitter (White and Garrott 1990). Radio transmitters may cause ill effects on the animal from the radio signals themselves; however, the effective power radiated (ERP) is so low that this possibility appears very unlikely (Mech and Barber 2002). In general, if the animal maintains its weight, mates successfully and/or produces offspring, establishes and defends a territory, and appears to look and act normal, then researchers can consider the effects

of the transmitter to have a minimal effect on the animal (Mech and Barber 2002). In addition to adverse transmitter effects that may lead to loss of data, the loss of the transmitter itself from inappropriate attachment procedures may also lead to a loss of data. For example, external transmitters damaged by extreme weather or mechanical wear may fail prematurely. This may result in a loss of data if the transmitter is unable to be located or if the data were only stored onboard the transmitter (Koehler et al 1987; Mech and Barber 2002).

Radio and acoustic (or sonic) transmitters have been used both externally and internally with varying results depending on the fish species. Acoustic transmitters do not have an antenna, whereas radio transmitters have an antenna that trails from the fish or is coiled within the embedding material of the transmitter. Acoustic transmitters produce sound waves that are sent to receivers, and radio transmitters produce radio waves that are received by antenna that are then converted to audible sounds (Winter 1983). Radio transmitters are well suited for fish that inhabit shallow, turbulent, or low conductivity fresh water because signals may be reduced as water depth and conductivity increases (Winter 1983). Acoustic transmitters are better suited for saline water, high conductivity water, and deep water because these habitats cause minimal reduction in signal strength (Winter 1983). Three types of transmitter attachments are used for fish telemetry; external attachment, gastric implantation, or surgical implantation.

Transmitters: Comparison of Three Attachment Techniques

External transmitters, such as back-mounted harnesses, leggings, radio collars, tail-mounts, and transmitters attached with glue and suture have been used in many vertebrates, including birds, fish, reptiles, amphibians, and mammals; however, their effects to the animal and utility vary depending on the type of transmitter and the species on which they are used (Bridger and Booth 2003; Goodman et al. 2009; Koehler et al. 1987; Perry 1981; Rowley and

Alford 2007; Wheeler 1991). Externally mounted transmitters may cause persistent adverse effects such as infection, entanglement, increased drag, predation, or chronic weight loss (Bridger and Booth 2003; Perry 1981). For example, Perry (1981) noted that canvasback ducks (*Aythya valisineria*) equipped with back-mounted transmitters exhibited decreases in feeding and increases in feather wear, swimming, preening, and wing-flapping behavior which may have led to emaciation, compared to canvasbacks without transmitters. Transmitter collars or harnesses used for small mammals have resulted neck irritation and restricted movements through burrows (Koehler et al. 1987).

External transmitters have been used in fish and are considered less invasive than surgically implanted transmitters (Bridger and Booth 2003). Using external transmitters may avoid the need for invasive surgery, but external transmitters have been documented to affect the fish's center of gravity, thus affecting behavior and increasing metabolic costs (Weimer et al. 2006). They have caused abrasions or entanglement, led to infection at the transmitter site, caused muscle atrophy, or been prematurely lost (Jadot 2003; Mitamura et al. 2006). Collins et al. (2002) concluded that external transmitter attachment at the dorsal fin base in shortnose sturgeon (*Acipenser brevirostrum*) should only be used for short-term studies, because the transmitters were lost between 2 and 41 days due to excessive rubbing against the rocks leading to the attachment wire breaking. Although external transmitter attachment does not require invasive surgical procedures, this method should only be used when the duration of the research is short term.

Transmitters that are inserted through the oral cavity into the stomach are termed intragastric transmitters. The method of intragastric insertion is conducted using a tube to push the transmitter through the oral cavity into the esophagus and then into the stomach or by feeding the

fish food containing the transmitter (Winter 1983). Intra-gastric transmitters are used for short-term studies where handling time is a concern, and when researchers wish to completely eliminate the possibility of adverse effects typically associated with surgery (Neely et al. 2009). This method may also be warranted when temperatures are below 6° C or above 20° C. At these temperature extremes, surgical complications such as slow healing or increased mortality rates and infection are more likely (Winter 1996). Intra-gastric implants have been used in adult salmonids, but are more commonly used with juveniles to decrease complications associated with anesthesia and handling. Intra-gastric transmitter implantation can be quick and performed with minimal anesthesia (Bridger and Booth 2003). Disadvantages of intra-gastric transmitters include esophageal tears, transmitter regurgitation, or alterations in feeding behavior (Adams et al. 1998; Martinelli et al. 1998). Although intra-gastric transmitter implantation may be rapid, the high expulsion rate may limit its use to short-term studies.

Although transmitters are typically attached to mammals by external collars, some species have a life-style or body shape that makes external attachment impractical (Vuren 1989). Thus, intraperitoneal transmitters may be the best alternative for telemetry studies. However, intraperitoneal surgery involves the risk of surgery and anesthesia, post-operative surgical site infection, or wound dehiscence (Vuren 1989). Internally implanted transmitters may have either external trailing antennae that exit the body cavity through a subcutaneous tunnel, or an internally coiled antenna (Bridger and Booth 2003).

External trailing antennae may interfere with the normal behavior of some animals. For example, Latty et al (2010) suggested that researchers use caution as they found that captive common eiders (*Somateria mollissima*) implanted with transmitters with external antennae had slower vertical speeds and longer dive durations than before implantation occurred. However,

issues with external antennae may be overcome when using intraperitoneal transplants with an internally-coiled antenna. In a review of telemetry attachment methods in West Indian rock iguanas, Goodman et al (2009) found that based on longevity and range, intraperitoneal transmitters appeared to be the most successful method for securing transmitters. This method also appeared to minimize behavioral effects from external transmitters, but is more invasive and costly compared to external or gastric attachment procedures. Horning et al (2008) and Guynn et al (1987) also agreed that intraperitoneal transmitters were well-suited for long-term telemetry studies in otterids and beavers (*Castor canadensis*), with minimal adverse effects.

One specific disadvantage associated with the use of external transmitters in fish is the potential to interfere with buoyancy and hydrodynamics (Bridger and Booth 2003). Implanting the transmitter within the coelom is assumed to minimize these problems because the transmitter remains close to the center of gravity (Bridger and Booth 2003). In fish, antennae that exit through the body wall and trail externally have been associated with greater transmitter expulsion, increased inflammatory reactions at the exit site, and impaired swimming ability when compared to internal transmitters without external trailing antennae (Adams et al. 1998; Bauer and Loupal 2007; Collins et al. 2002; Jadot 2003; Murchie et al. 2004).

Recent technological advances have helped overcome characteristics such as short battery life and large sizes that are often associated with internal transmitters with an internal antenna (Hall et al. 2009). Internally implanted transmitters without trailing antennae, eliminate problems such as entanglement with debris and increased drag (Bridger and Booth 2003). Adverse effects, such as infection, expulsion, pressure necrosis, decreased growth rates, and anesthetic risks are also noted with internal transmitters without trailing antennae, but at much lower rates (Bauer and Loupal 2007; Fabrizio and Pessutti 2007; Jadot 2003; Jepsen et al. 2001; Jepsen et al. 2008;

Martinelli et al. 1998; Murray 2002). Collins et al. 2002 compared different transmitter attachment methods in shortnose sturgeon (*Acipenser brevirostrum*) over a 93-day period. They found that fish implanted with intracoelomic transmitters with an internally coiled antenna had a high survival rate and a 100 percent retention rate. Fish implanted with intracoelomic transmitters with an externally trailing antenna had low retention rates, and blood and pus surrounded the opening where the antenna exited. Fish equipped with external transmitters with a trailing antenna exhibited a rapid loss of transmitters because they were rubbed against rocks (Collins et al. 2002). Although external and intragastric transmitters may be appropriate for very short-term studies, intracoelomic transmitters should be used for long-term studies as complications are minimal.

Just as transmitter attachment may be responsible for causing adverse effects, the weight of the transmitter should also be taken into consideration. A general rule for birds and mammals is that transmitter weight should not exceed 3-5% of the body weight (Cochran 1980; White and Garrott 1990). Species-specific differences must also be considered. The literature is fraught with contradictions regarding these guidelines. For example, Aldridge and Brigham (1988) found that transmitters weighing more than 5% of the body weight of bats caused a reduction in maneuverability and foraging efficiency, and increased energy costs. Reproductively active male rats with radio-collared transmitters between 4.7 and 7.1% of their body weight increased mortality compared to rats with transmitters that weighed between 2.1 and 4.0% of their body weight (Theuerkauf et al. 2007). On the other hand, Koehler et al (1987) evaluated four species of small mammals and noted that intraperitoneal transmitters weighing more than 10 % of their body weight did not cause adverse effects to locomotion or behavior.

Although some studies do not indicate effect on the growth or behavior of the species evaluated, others have found an increase in morbidity and mortality associated with heavier transmitters. For example, transmitters that weighed 2% of body weight and were attached with glue to adult European Golden-Plovers (*Pluvialis apricaria*) did not affect behavior or feeding when compared to control birds (Whittingham 1996), whereas backpack radiotransmitters (1.8 % bodyweight) placed on Takahes (*Porphyrio hochstetteri*) increased daily energy requirements by 8.5% (Godfrey et al. 2003). In comparison, Vuren (1989) surgically implanted transmitters weighing less than 4% of the yellow-bellied marmots (*Marmota flaviventris*) weight and did not note differences in growth rates, compared to controls, after 30 days.

Mulcahy (2003) suggested that the smallest and lightest transmitter that will provide the desired signal strength, and battery life needed should be used. Typically, the battery makes up the majority of the transmitter's weight, and often limits the length of the study; however, with recent technological advances, batteries are now lighter and last longer (Mulcahy 2003). Generally, transmitters weighing less than 2% of the fish's body weight should not be used for telemetry studies (Winter 1983). The 2% rule in fish is based on the assumption that transmitters above this ratio may affect the fish's behavior, or impede activities, such as swimming or escaping predators (Brown et al. 1999; Winter 1983). Larger internal transmitters may also place higher relative pressure on the incision and can lead to pressure necrosis at the surgical site, which contributes to transmitter expulsion (Brown et al. 1999). Although Winter (1983) is commonly cited for recommending transmitters weighing 2% or less of the fish's body weight, the author did not provide any data upon which that this recommendation was based.

The 2% rule appears to hold true in shovelnose sturgeon (*Scaphirhynchus platorhynchus*), and the South American catfish (*Rhamdia quelen*) (Neely et al. 2009; Schulz

2003). However, other studies indicate that slightly higher transmitter weight may be acceptable. For example, Hall et al. (2009) recommended using a transmitter less than 5.8% in juvenile Chinook salmon (*Oncorhynchus tshawytscha*), Collins et al. (2002) found that transmitters less than 3.6% were appropriate for shortnose sturgeon (*Acipenser brevirostrum*), Jadot (2003) recommended using a transmitter less than 3% in Salema porgy (*Sarpa salpa*), and Fabrizio and Pessutti (2007) concluded transmitters less than 2.5% in black sea bass (*Centropristis striata*) and summer flounder (*Paralichthys dentatus*) was appropriate. Lacroix et al. (2004) recommended that the transmitter weight not exceed 8% of the body weight in juvenile Atlantic salmon (*Salmo salar*). This recommendation was based on the evaluation of swimming performance and survival in juvenile salmonids implanted with radio transmitters at 6-12% of body weight; this transmitter to body weight percentage did not show negative effects (Anglea et al. 2004; Brown et al. 1999; Brown et al. 2006). The 2% rule may have been meant as a “rule of thumb” for transmitters used for fish (Jepsen et al. 2003), although many researchers have abided by this recommendation. However, adhering to this rule may be beneficial in some species until more research can provide evidence that transmitters heavier than the 2% of the fish’s body weight will not adversely affect the fish.

Most research evaluating transmitter placement effects on fish has focused on short-term effects (<54 days) assessed by observing feeding behavior thus far. Studies that assess the long-term effects (>300 days) are lacking and are important because decreased growth could potentially affect migratory preparation and fecundity, whereas abnormal behavior may increase predation. Adams et al. (1998) and Martinelli et al. (1998) found that the initial growth rate for fish with surgically implanted transmitters was slightly slower than the control fish that did not undergo surgery, but was similar towards the end of the 54-day study or 21-d study, respectively,

illustrating the importance of longer assessment periods. Lucas (1989) also did not find significant differences in mortality or growth in implanted rainbow trout over a seven month period. In addition, Caputo et al. (2009) indicated that there were no long-term (335 to 1402 days) physiological effects from transmitters implanted into largemouth bass as defined by calcium, magnesium, phosphorus, total protein, triglycerides and cholesterol blood levels. Although this studied evaluated long-term physiological effects, it did not specifically measure overall growth during the study.

Physiologically, a transmitter is foreign material that has the potential to cause irritation. The typical reaction against a foreign body is inflammation, followed by fibroplasia, and ultimately leading to adhesions surrounding the transmitter implant. Adhesions are strands of fibrous tissue (scars) that form from abnormal healing between injured tissues and the peritoneum. Adhesions typically occur at the surgical (trauma) site, and are a result of the body's normal healing process. For the most part, adhesions do not affect the animal. However, abnormal healing may occur in tissues surrounding a foreign body where an ongoing inflammatory reaction is present. This constant irritation continues to incite inflammation, thus causing continual adhesion formation (Jones et al 1997).

Few studies have evaluated adhesion formation in non-human animals. Thus, knowing exactly what factors lead to adhesion formation or how soon after the surgery adhesions form is difficult. When striped skunks (*Mephitis mephitis*) were surgically implanted with miniature temperature data-loggers, pathology was not noted after five months (Ten Hwang et al. 2009). However, another studied found that adhesions formed around the internal transmitter and adjacent intestines in beavers (*Castor canadensis*) causing an obstruction leading to the death of one animal six weeks after the surgical implantation (Guynn et al. 1987). In another case,

adhesions formed around the transmitter about 15 months after implantation and caused an abdominal torsion that resulted in the death of an American badger (*Taxidea taxus*) (Quinn et al. 2010). Transmitters may incite chronic inflammation in the surrounding tissue and lead to adhesion formation (Lacroix et al. 2004; Lucas 1989). For example, in a one year study of common carp, granulation tissue encapsulated the externally trailing antenna transmitter, and adhesions formed to the liver and intestines; however, organ damage was unknown (Bauer and Loupal 2007). During a seven month evaluation period, Lucas (1989) also found that surgically implanted transmitters induced chronic inflammation in rainbow trout and then became encapsulated in fibrous tissue which adhered to surrounding organs. Currently, surgically implanted transmitters are known to incite chronic inflammation and become encapsulated with fibrous tissue; however, the extent of organ damage from the adhesions is unknown (Bauer and Loupal 2007; Collins et al. 2002; Lacroix et al. 2004; Lucas 1989; Martinelli et al. 1998).

STRESS-INDUCED CORTISOL ELEVATIONS

Stress is defined as a physiologic response to a short- or long-term event that may be potentially harmful to the well-being of the animal (Barton 2002). Just as in most mammals, cortisol has been described as being the most abundant corticosteroid in fish, and an elevation of blood cortisol has been correlated to a stress response (Van Der Boon et al. 1991; Wedemeyer et al. 1990). During acute stress, cortisol, an endogenous glucocorticoid released by the adrenal gland, actually protects the animal through various mechanisms, such as stimulation of glucose release, which provides energy to escape the stressful situation. Cortisol serves to maintain homeostasis by maintaining osmoregulation and energy metabolism under normal circumstances. Cortisol production is under the control of the hypothalamic-pituitary axis (HPA) in mammals or the hypothalamic-pituitary-interrenal axis (HPI) in fish. The HPA and HPI are major parts of the

neuroendocrine system that regulates many body processes and reactions to stimuli, and are controlled by negative feedback. The hypothalamus controls many of the endocrine functions of the pituitary gland, which in turn controls other endocrine tissues within the body, such as the interrenal cells of the pronephric kidney. The hypothalamus synthesizes and secretes corticotrophin-releasing hormone (CRH), which stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, thus controlling cortisol release. As plasma levels of cortisol increase, negative feedback is exerted on the CRH and ACTH secretions, thus maintaining homeostasis. ACTH controls the production and release of cortisol from the interrenal cells of the pronephric kidney in fish and the adrenal glands in mammalian species. The secretion of ACTH is affected by cortisol metabolism and stress, which in turn decreases the negative feedback and releases CRH. If negative feedback is diminished, ACTH stimulates an overproduction of cortisol (Barton 2002). Persistently elevated cortisol levels can potentially affect growth, fecundity, behavior, and suppress the immune system. These effects occur because energy that would normally be used for these roles are being redirected to coping with stress (Moberg 1993; Mommsen et al. 1999; Wedemeyer et al. 1990).

Although plasma cortisol measurements may help determine if an animal is stressed, there are limitations to the results. An animal may become stressed during capture and translocation (Morton et al. 1995), overcrowding (Semenkova et al. 1999), or while exposed to poor water quality (Barton 2002), all of which may result in elevated cortisol levels. Barton (2002) states that interpreting changes to physiological variables may be problematic because there are many factors that can affect the magnitude and duration of the stress response; thus without knowing the extent of these factors, interpreting the biological significance of the response may be difficult.

In fish, chronic elevations of cortisol are correlated with an increased risk to disease by suppressing the immune system, decreased survival, and decreased reproductive abilities (Moberg 1993). Several studies have assessed the effects of chronic cortisol elevation on feeding behavior, growth, and swimming ability of fish and found that elevated cortisol may be correlated with poor growth rates, but not on swimming ability (De Boeck et al. 2001; Gregory and Wood 1999). Fewer studies have been conducted on long-term cortisol response in fish with surgically implanted transmitters. Gregory and Wood (1999) postulated that because cortisol mobilizes tissue glucose and increases gluconeogenesis, appetite was suppressed in juvenile rainbow trout implanted with cortisol implants leading to a slower growth rate. Davis et al. (1985) and Barton et al. (1987) found similar findings. Jepsen et al. (2001) suggested that radiotransmitters did not cause chronic stress over a 14 day period to Chinook salmon smolts because plasma levels of cortisol and glucose returned to normal within a few days after gastric or surgical insertion of radiotransmitters. Caputo et al. (2009) recaptured wild fish both with and without transmitters and showed that implants were not correlated with any long-term change in physiological parameters, such as glucose. Results from previous studies indicate that chronically elevated cortisol levels may have some adverse effects on fish physiology; however, more studies should be conducted to determine if long-term surgically implanted transmitters are associated with elevated cortisol levels.

SURGICAL METHODS AND MATERIALS

Sterilization can be defined as a complete elimination in all forms of life, including bacteria, viruses, or spores on an object such as instruments, drapes, or needles. Disinfection is the destruction of most pathogenic organisms on non-living objects. Antisepsis is the destruction of most pathogenic organism on living objects, and is used to kill organisms during patient skin

preparation and surgical scrubbing. Due to the inherent sterile nature of the internal organs within the coelomic cavity, any equipment or supplies (such as transmitters) that may come into contact with these tissues must also be sterile (Fossum 2002). There are several types of sterilization and disinfecting techniques, and each must be used appropriately based on the type of instruments and animals involved. Moreover, the goal of aseptic/sterile techniques is to minimize the introduction of microorganisms to the patient during procedures (e.g., surgery).

Aseptic technique is the standard practice in mammalian surgery and has also been suggested when performing surgery on fish (Summerfelt and Smith 1990; Stoskopf 1993). Although the majority of surgical methods have remained unchanged in fish research, there has been minimal empirical testing evaluating the effectiveness of sterile or non-sterile surgical techniques (Wagner and Cook 2005; Wagner et al. 1999). However, seventy-three percent of researchers believe it is important to maintain a sterile environment and equipment between fish surgeries in order to preserve fish health (Wagner and Cooke 2005). On the other hand, there is evidence suggesting that sterile techniques do not improve wound healing. For example, Wagner et al (1999) investigated the use of the topical antiseptic povidone-iodine when performing surgery on rainbow trout *Oncorhynchus mykiss*, and found that the application of this antiseptic did not improve wound healing or alter the healing rate compared to incisions prepared without an antiseptic. Although there is not conclusive experimental evidence for fish regarding the use of sterile techniques that is commonly used in other vertebrates, the minimization of pathogens may be an important consideration when performing surgery on fish..

According to the American Fisheries Society Guidelines for the Use of Fishes in Research (AFS, AIRAB, ASIH 2004), care should be exercised to prevent the introduction of infective agents during surgery and to minimize the stress of such procedures. The minimization

or prevention of bacteria or other foreign materials into the incision and coelomic cavity can only be accomplished by using standard surgical techniques (Mulcahy 2003; Summerfelt and Smith 1990; Wagner and Cooke 2005). The use of sterile techniques for fish surgery includes using sterile instruments or transmitters that will enter the coelom, sterile gloves, and applying an antiseptic to the surgical site before the incision is made (Summerfelt and Smith 1990). Most of the common antiseptics used in mammalian species may not be acceptable in fish. The antiseptics may damage the fish's skin and disrupt the mucous layer, which is the fish's first line of protection from the external environment (Mulcahy 2003). However, some form of pre-surgical site preparation should be conducted to minimize the number of skin bacteria that enter the coelom (Murray 2002; Summerfelt and Smith 1990). This preparation should minimally disrupt the protective mucous layer (Murray 2002; Stoskopf 1993). For example, a cotton tip applicator moistened with sterile saline can be used to reduce the surface contamination of the proposed incision (Murray 2002). Although the sterile saline applicator will not sterilize the skin, it will serve to reduce the surface contamination. According to Stoskopf (1993), surgical site infections do not seem to be a serious problem when skin preparation is conducted in this manner, as long as subsequently, the proper sterile techniques are employed. Thus, utilizing surgical techniques that minimize an inflammatory reaction and risk of infection may be important when performing surgery on fish. The use of these techniques is likely to result in decreased incision dehiscence and prevent a loss of skin integrity (Hurty et al. 2002).

The use of proper surgical closure materials is also an important aspect of minimizing the introduction of pathogens to the incision or coelomic cavity. Surgical transmitter implantation studies often include the evaluation of different materials, such as sutures for the closure of skin at the surgical incision (Cooke et al. 2003; Deters et al. 2010; Sutton et al. 2004; Wagner et al.

2000). Other studies have evaluated surgical wound healing in fish using staples (Swanberg et al. 1999), or cyanoacrylate adhesive (Nemetz and MacMillan 1988; Petering and Johnson 1991). Wagner and Cooke (2005) undertook a survey to determine the most common materials used for surgical closure of the piscine coelom and found that the majority of researchers used suture to close surgical wounds; however, some respondents utilized staples, while none of the respondents used glue as primary closure material. However, each closure material has a unique set of characteristics that should be considered when choosing a material for surgical incision closure.

Cyanoacrylate adhesives are commonly used in mammals for some procedures such as a feline onychectomy (declawing), or for closure of minor skin wounds; however, it is not typically used for primary closure of larger surgical incisions (Fossum 2002). Cyanoacrylate adhesives have been evaluated in two studies when closing surgical incisions in some fish species (Nemetz and MacMillan 1988; Petering and Johnson 1991). Nemetz and MacMillan (1988) indicated that adhesives may be used effectively in the closure of short incisions (<1.5cm) in channel catfish, but transmitters were not used in their study. Nemetz and MacMillan (1988) and Petering and Johnson (1991) found that visceral adhesions near the incision formed in 60% of fish with incisions closed with cyanoacrylate adhesive. Both studies agreed that cyanoacrylate caused minor inflammation and decreased the surgical time. However, due to its poor strength, high incidence of adhesion formation, and its associated high incidence of wound dehiscence, surgical glue may have limited use for telemetry studies.

Surgical staples are routinely used for skin closure in mammals with good results, as they offer a faster method for surgical closure than suture (Waldron 1994). However, surgical wounds in mammals are closed by suturing several layers separately and staples are only used for the

last, cutaneous layer (Fossum 2002). Surgical staples have been used in a few studies of fish. According to Wagner and Cooke's (2005) survey, eight percent of fish surgeons use surgical staples as a means of primary surgical closure. Although some studies indicate staples allow for fast placement, decreased surgical time, and decreased inflammation compared to sutures (Mulford 1984; Swanberg et al. 1999), other studies found that the use of staples increased mortality and transmitter loss due to staple loss or other undetermined causes (Haeseker et al. 1996; Starr et al. 2000). Typically, all layers are not closed separately in fish, and therefore it is not surprising that staples are not used successfully as a primary closure method.

Given the disadvantages discussed about other materials, suture is often the preferred material for wound closure in telemetry studies. Each suture material has different characteristics and should be chosen based on the species and tissues that are involved in the surgery. Suture materials are classified by the characteristics of the thread and their behavior in tissue (Table 1)(Fossum 2002). Suture characteristics include strand diameter, flexibility (stiffness of the material), capillarity (the ability to wick fluid), knot tensile strength (measured by the force that the suture can withstand before it breaks at the knot), and tensile strength (which is the strength required to break an untied piece of suture) (Fossum 2002). Multifilament sutures are generally more flexible than monofilament sutures, and monofilament sutures have much less capillarity than multifilament sutures. Suture can be further categorized according to its behavior in tissue: absorbable or non-absorbable; its structure: monofilament or multifilament, or origin: organic or synthetic (Fossum 2002). Sound surgical principles guide suture choice (Fossum 2002). These principles include selecting a suture based on the time it will be required to help strengthen the wound, minimize capillarity to reduce the risk of infection at the surgical site, and choosing a material with the appropriate diameter and strength for the specific tissue closure needed

(Fossum 2002). A suture should be chosen based on the amount of time that a particular tissue needs reinforcement to heal. Typically, the smaller the suture size, the less tensile strength it has in reinforcing a tissue while it heals. The largest possible suture available should not be used either because as suture size increases, the potential trauma caused by the suture passing through the tissue increases, as well as the amount of foreign material present in the wound. Instead, the surgeon should use a suture that matches the tissue's natural strength.

Surgical principles also dictate that certain suture characteristics not be used for skin closure in certain vertebrates. For example, multifilament sutures should not be used for skin closure in mammals due to the potential risk of bacterial wicking into the incision and the coelom (Fossum 2002). Both an absorbable or nonabsorbable monofilament may be appropriate for skin closure in mammals. However, nonabsorbable monofilament sutures should not be used for skin closure unless the sutures will be removed after healing is complete (Fossum 2002). If nonabsorbable sutures remain in the skin, there is a higher likelihood that the sutures will induce a chronic inflammatory reaction (Fossum 2002).

According to Wagner and Cooke's (2005) survey, most fish surgeons prefer a monofilament for skin closure, with 47% of fish surgeons using an absorbable filament, while 27% use a non-absorbable filament. However, there was no mention of suture removal when a nonabsorbable suture was used. Based on their survey, it appears that there is not a consensus on the appropriate suture material used for skin closure in fish.

Deters et al. (2010) reported that the absorbable monofilament Monocryl (poliglecaprone 25) and the nonabsorbable monofilament Ethilon (nylon) exhibited higher suture retention and a lower incidence of inflammation and ulceration compared to nonabsorbable multifilament Nurolon (nylon), the absorbable multifilament Vicryl (polyglactin 910),

absorbable multifilament Vicryl-Plus (antibacterial polyglactin 910), and nonabsorbable multifilament Perma-hand (silk). This study concluded that the absorbable monofilament Monocryl-Plus (antibacterial poliglecaprone 25) was preferred for surgical closure in subyearling Chinook salmon (*Oncorhynchus tshawytscha*). Wagner et al. (2000) also reported that either an absorbable or non-absorbable monofilament was preferred over silk for surgical closure in rainbow trout because those materials produced less inflammation, particularly in cold water. Conversely, another study that evaluated five suture materials deemed Maxon (polyglyconate), an absorbable monofilament, the most appropriate suture material to close the body wall, muscle, and skin for koi because it resulted in less tissue reaction compared to silk, Dermalon, Vicryl, and chromic gut (Hurty et al 2002).

Contrary to established surgical principles, which consider multifilament suture inappropriate for skin closure in mammals (Fossum 2002), some researchers use it routinely for wound closure in fish. For example, Jepsen et al. (2008) showed that Vicryl, a multifilament absorbable suture, provided better wound healing and fewer transmitter expulsions compared to the monofilament polyamide in wild brown trout (*Salmo trutta*) with surgically implanted transmitters (Jepsen et al. 2008). Chapman and Park (2005) and Jepsen et al. (2008) noted that Vicryl was more easily manipulated when compared to other absorbable monofilament materials, which is likely the reason it is preferred over other suture materials.

There is at least some evidence that Vicryl is not appropriate for skin closure in fish. Using five different suture materials for a histologic comparison in tissue reactions in the skin of koi (*Cyprinus carpio*), Hurty et al. (2002) concluded that Vicryl may be limited in fish surgery due to the risk of surgical site contamination from multifilament properties of this suture that may allow wicking of bacteria into the tissues. Furthermore, fish are constantly immersed in

water containing potential pathogens, and multifilament suture is more likely to provide a route of pathogen introduction into the tissues of the fish. Hurty et al (2002) reported that Vicryl may have limited use for fish surgery; however, this study did not evaluate the effect of an intracoelomic transmitter when performing skin closure. Therefore, it seems necessary to evaluate the skin reactions and transmitter retention rates in fish when skin incisions are closed with the absorbable multifilament Vicryl.

Most suture materials, as they are foreign objects, stimulate an inflammatory reaction. Inflammation is associated with discomfort and delayed healing in mammals and other invertebrates. In addition, mammalian tissue that is inflamed is more susceptible to infection, which is a particular concern for skin that is colonized by bacteria with the potential to become pathogenic (Fossum 2002). Some suture materials may also increase the probability for infection by wicking fluid into the incision site of mammals (Fossum 2002) and fish (Hurty et al. 2002). One possible solution that may minimize the inflammatory reaction or risk of infection is to use an antibacterial suture. Although there is evidence in humans and animals that antibacterial suture will inhibit bacterial growth and thereby prevent post-surgical infection (Mangram et al. 1999; Rothenburger et al. 2002; Storch et al. 2004), there is minimal evidence in fish (Deters et al. 2010). Only one study has evaluated the use of an antibiotic-impregnated suture material for wound closure in Chinook salmon that were surgically implanted with acoustic transmitters. Deters et al. (2010) found that Vicryl-Plus had significantly lower suture retention compared to Monocryl at day 14, had significantly lower tag retention compared to Monocryl, and Vicryl at day 7, and had a more pronounced inflammatory reaction at day 14 compared to monofilament Ethilon, Monocryl, or multifilament Nurolon. Although this study evaluated an antibacterial suture, the antibacterial suture material was a multifilament. The use of a multifilament suture for

skin closure in fish may have caused the incisions to exhibit a lower suture retention rate and a higher inflammatory rate compared to the non-antibacterial monofilaments used in the study. Therefore, it is important to evaluate an antibacterial monofilament suture for the skin closure in fish to determine if a lower inflammatory or infection rate is seen at the incision.

POST-OPERATIVE CARE

Analgesia

While the benefits of analgesia are well documented in the mammalian species, the question of analgesia in fish is still controversial. Fish, like mammals, have an intricate nervous system with nociceptors. Nociceptors are the receptors that detect noxious stimuli, thus suggesting that fish have the ability to sense the adverse effects that are associated with pain; however, the extent of these responses that are processed is uncertain (Noga 2010). Currently, there are no definitive recommendations for providing appropriate postoperative analgesia in fish (Harms 2005). However, several studies have documented the behavior-sparing effects of opioids and non-steroidal anti-inflammatory drugs. For example, Harms et al. (2005) found that fish given butorphanol just before recovery from a surgery appeared not to have a reduction in activity, tail fin beat, vertical water column position, or feeding intensity compared to fish without analgesics. Also, the results of the study indicated that ketoprofen lowered the increase of creatine kinase post-operatively possibly by reducing muscle damage through its anti-inflammatory properties. Intramuscular injections of butorphanol (0.4mg/kg) and ketoprofen (2mg/kg) have been used to help provide analgesia with no adverse side effects observed in fish (Davis et al. 2006; Harms et al. 2005; Lewbart 2001). In addition, precautions should be taken during the placement of intracoelomic transmitters to minimize the effects from noxious stimuli such as handling, restraint, and surgery (Jepsen et al. 2008). Attachment methods also should not

impede the return to normal behavior afterwards (Angela et al 2004; Bridger and Booth 2003). Therefore, if the administration of analgesics does not interfere with the objectives of the study, analgesics should be given as there is evidence that morphine-like drugs and anti-inflammatory drugs have behavior-sparing effects and help decrease inflammation and muscle damage that is potentially associated with invasive surgical procedures.

OBJECTIVES

In the present study, I evaluated the effects of different suture materials for coelomic closure in fish, and evaluated the pathological and physiological effects in Siberian sturgeon (*Acipenser baerii*) from intracoelomic transmitter implantation in an attempt to meet the following objectives: (1) Measure and evaluate macroscopic and microscopic tissue reactions in fish to three suture materials (Monocryl, Vicryl, and Prolene) that are currently used in telemetry research and one newer suture material (Monocryl-Plus) that is currently used in human and veterinary medicine, and (2) Determine if implanting sonic transmitters into the coelomic cavity of Siberian sturgeon will result in adverse physiologic or pathologic effects during a 12 month period.

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Table 1: Classification of the suture materials and their characteristics that are commonly used in veterinary medicine.

Trade Name	Generic Name	Structure Characteristics and Suture Behavior	Reduction in Tensile Strength	Days to Complete Absorption	Relative Knot Security	Tissue Reaction
Monocryl-Plus (Antibacterial)	Poliglecaprone 25 with Triclosan	Monofilament Absorbable	40-50% at 7 days 70-80% at 14 days	90-120	Good (70-85%)	Mild
Monocryl	Poliglecaprone 25	Monofilament Absorbable	40-50% at 7 days 70-80% at 14 days	90-120	Good (70-85%)	Mild
Vicryl	Polyglactin 910	Multifilament Absorbable	35% at 14 days 60% at 21 days	60	Good (70-85%)	Mild
Vicryl-Plus (Antibacterial)	Polyglactin 910 with Triclosan	Multifilament Absorbable	35% at 14 days 60% at 21 days	60	Good (70-85%)	Mild
Prolene	Polypropylene	Monofilament Non-Absorbable	N/A	N/A	Excellent (>85%)	Minimal to None
PDS II	Polydioxanone	Monofilament Absorbable	14% at 14 days 31% at 42 days	180	Good (70-85%)	Mild
Perma-Hand	Silk	Multifilament Non-Absorbable	30% at 14 days 50% at 1 year	>2 years	Poor (<60%)	Severe

CHAPTER 2

EVALUATION OF FOUR SUTURE MATERIALS FOR SURGICAL INCISION CLOSURE IN SIBERIAN STURGEON (*ACIPENSER BAERII*)¹

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ABSTRACT

The macroscopic and microscopic tissue reactions to the absorbable monofilament Monocryl (poliglecaprone 25), absorbable monofilament triclosan-coated Monocryl-Plus (triclosan-coated poliglecaprone 25), absorbable multifilament Vicryl (polyglactin 910), and non-absorbable monofilament Prolene (polypropylene) were evaluated for their use of surgical closure in Siberian sturgeon *Acipenser baerii*. Post-operative assessments were conducted at 1, 2, 8, 12, and 26 weeks to visually evaluate the surgical incision for suture retention, incision healing, erythema, and swelling. Incisions were also assessed microscopically at one, two, and eight weeks for necrosis, inflammation, hemorrhage, and fibroplasia. The results indicated that incisions closed with either Vicryl or Prolene suture materials were more likely to exhibit more erythema or incomplete healing when compared to Monocryl or Monocryl-Plus. The surgical implantation of a transmitter did not significantly affect the response variables among the four suture materials. Based on the current results and the characteristics of Vicryl and Prolene sutures, we recommend using either Monocryl or Monocryl-Plus when closing surgical incisions in Siberian sturgeon or closely related species of sturgeon. Furthermore, Monocryl or Monocryl-Plus may decrease the risk of transmitter expulsion through the incision, as surgical wounds appear to heal faster and exhibit less erythema compared to Vicryl.

INTRODUCTION

Suture materials are commonly used in biotelemetry research, biomedical research, and aquaculture. When performing surgery on a fish, appropriate wound closure is an important element in order to minimize morbidity associated with the procedure (Mulcahy 2003; Stoskopf 1993; Wagner and Cooke 2005). Many types of suture materials (e.g., absorbable, non-absorbable, monofilament, or multifilament) are used for surgical skin closure in fish; although, the preferred suture material is debatable (Chapman and Park 2005; Cooke et al. 2003; Deters et al. 2010; Hurty et al. 2002; Swanberg et al. 1999; Wagner et al. 2000). According to a survey conducted by Wagner and Cooke (2005), fish surgeons prefer a monofilament for skin closure. Forty seven percent of fish surgeons use an absorbable filament and 27% use a non-absorbable filament. Eight percent of the respondents preferred surgical staples for wound closure, while none of the respondents used glue as primary closure material.

Each suture material has different thread characteristics such strand diameter, flexibility (stiffness of the material), capillarity (the ability to wick fluid), and knot tensile strength (force needed to withstand breakage when knotted) (Fossum 2002). Suture material is also categorized according to its behavior in tissue: absorbable or non-absorbable; its structure: monofilament or multifilament, or origin: organic or synthetics (Fossum 2002). Suture choice should be guided by sound surgical principles that include selecting a suture based on the time it will be required to help strengthen the wound, capillarity minimization in order to reduce the risk of infection at the surgical site, and choosing a material with the appropriate diameter and strength for the specific tissue closure needed when healing (Fossum 2002).

Foreign material such as suture materials stimulates an inflammatory reaction. Tissue that is inflamed is more susceptible to infection than non-inflamed tissue, and an infection is a

particular concern for skin that is colonized by bacteria with the potential to become pathogenic (Fossum 2002). Multifilament suture materials may also increase the probability for infection by wicking fluid into the incision site of mammals (Fossum 2002) and fish (Hurty et al. 2002). Utilizing materials that minimize the inflammatory reaction and risk of infection are important when closing an incision because this will result in decreased incision dehiscence and prevent a loss of skin integrity (Hurty et al. 2002).

Based on surgical principles when performing surgery on mammals, a monofilament suture should be used when closing skin wounds to prevent wicking of bacteria to deeper tissues that may lead to an infection. Monofilament sutures reduce bacterial wicking compared to a multifilament, as multifilament sutures allow bacteria to be carried down the many filamentous strands. Furthermore, choosing the smallest suture size needed to reinforce the wound will minimize the amount of tissue to suture contact, thereby reducing inflammation while the tissue mends. Sutures should be removed as soon as possible after the tissue has healed to minimize ongoing inflammatory reactions or possible infections in mammals (Fossum 2002). However, a non-absorbable monofilament may be inappropriate for skin closure in fish if the telemetry study involves releasing the fish without plans of recapture because retained sutures may elicit a chronic inflammatory response.

Contrary to established surgical principles for other vertebrates that consider multifilament suture inappropriate for skin closure (Fossum 2002), some researchers use it routinely for wound closure in fish. For example, Jepsen et al. (2008) showed that the absorbable multifilament Vicryl (polyglactin 910) suture provided better wound healing and fewer transmitter expulsions compared to the monofilament polyamide in wild brown trout *Salmo trutta* with surgically implanted transmitters (Jepsen et al. 2008). Chapman and Park (2005) also

concluded that Vicryl is most appropriate for gonadal biopsy wound closure in Gulf of Mexico sturgeons *Acipenser oxyrinchus de sotoi* when compared to the monofilament absorbable PDS (polydioxanone) and the multifilament absorbable Panacryl because it did not require removal, reduced prolonged skin irritation, was easily manipulated, and absorbed within 30 days of the surgery.

In addition, there is evidence in humans and mammals that antibacterial suture will minimize inflammatory reactions, inhibit bacterial growth, and thereby prevent post-surgical infection (Edmiston et al. 2006; Mangram et al. 1999; Ming et al. 2007; Rothenburger et al. 2002; Storch et al. 2004); however, there is minimal evidence for these same benefits in fish (Deters et al. 2010). Deters et al. (2010) found that the triclosan-coated Vicryl-Plus had significantly lower suture retention compared to Monocryl at day 14, and had a more pronounced inflammatory reaction at day 14 compared to monofilament Ethilon, Monocryl, or multifilament Nurolon. Although this study evaluated an antibacterial multifilament suture, the evaluation of an antibacterial monofilament is necessary to determine the effects of a monofilament suture material on wound healing.

To investigate the effects of different suture materials for coelomic closure in fish implanted with transmitters, the authors investigated four different types of suture material. The objective of the present study was to measure and evaluate macroscopic and microscopic tissue reactions to absorbable monofilament Monocryl (poliglecaprone 25), absorbable triclosan-coated monofilament Monocryl-Plus (triclosan-coated poliglecaprone 25), absorbable multifilament Vicryl (polyglactin 910), and non-absorbable monofilament Prolene (polypropylene) when used for coelomic closure in the Siberian sturgeon *Acipenser baerii*. The Siberian sturgeon was

chosen as a model for other sturgeon species because it was a commercially and readily available aquaculture species at the University of Georgia.

MATERIAL AND METHODS

Hatchery-reared Siberian sturgeon were obtained from the Cohutta Fisheries Center of the Warnell School of Forestry and Natural Resources (WSFNR) of the University of Georgia. The sturgeon were transported to the Whitehall Fisheries Lab of the WSFNR at the University of Georgia in November 2009. All procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (AUP # A2009-10079). A total of one-hundred fifty sturgeon were randomly assigned to one of three 3785-L rectangular semi-recirculating tank systems, each with its own biological and mechanical filters. Fish were allowed to acclimate for six weeks before experiments began. The sturgeon were fed 0.3% of their body weight of a 6mm pellet (Silver Cup Fish Feed, Murray, Utah) on a daily basis. Total ammonia-nitrogen and nitrite-nitrogen concentrations were monitored daily during the first 10 weeks and twice weekly afterwards. Dissolved oxygen, pH, and temperature were monitored twice weekly throughout the entire experiment. Total ammonia-nitrogen, nitrite-nitrogen, dissolved oxygen, and pH were monitored with the LaMotte Smart 2 colorimeter system and reagents (LaMotte Company, Chestertown, MD). Temperature was monitored with a glass mercury thermometer (Miller and Weber, Inc., Ridgewood, Queens NY). During the experiment, total ammonia nitrogen (range: 0.0-0.6 ppm), un-ionized ammonia-nitrogen (range: 0.000-0.005 ppm), nitrite-nitrogen (range: 0.000-0.097 ppm), dissolved oxygen (range: 5.5-9.3 mg/L), pH (range: 6.2-7.4), and temperature (10-20 °C) were maintained within levels previously proven successful for rearing sturgeon (Hochleithner and Gessner 1999).

All surgical procedures were performed between January 5, 2010 and January 8, 2010 by the same surgeon (Boone). The sturgeon were fasted between 72 and 120 hours before surgery to minimize regurgitation and reduce the chance of gill damage from nitrogenous waste. One-hundred twenty sturgeon (mean fork length =57.2 cm [range: 46.0-67.0 cm]; mean wet weight = 945.0 g [range: 480.0-1525.0 g]) were randomly assigned to one of four suture treatment groups: absorbable monofilament poliglecaprone 25 (2-0 Monocryl, FS-1 needle; Ethicon Inc., Somerville, NJ), absorbable tricolsan-coated monofilament poliglecaprone 25 (2-0 Monocryl-Plus, FS-1 needle; Ethicon Inc., Somerville, NJ), absorbable multifilament polyglactin 910 (2-0 Vicryl, FS-1 needle; Ethicon Inc., Somerville, NJ), and non-absorbable monofilament polypropylene (2-0 Prolene, FS needle; Ethicon Inc., Somerville, NJ). Fifteen fish from each of the four suture treatment groups underwent intracoelomic transmitter implantation. A total of 30 sturgeon (mean fork length =57.4 cm [range: 48.5-65.0 cm]; mean wet weight = 947.8 g [range: 550.0-1480.0 g]) underwent the same handling and anesthetic procedures as the suture treatment groups, but did not undergo surgery. These 30 fish served as the control treatment.

Anesthesia was induced by placing each fish in an aerated 114-L plastic container that contained a 150 mg/L solution of tricaine methanesulphonate (MS-222) (Finquel, Argent Chemical Laboratories, Redmond, WA) buffered with 300 mg/L sodium bicarbonate. Once the fish reached a total loss of equilibrium (known as stage II of anesthesia) (Stoskopf 1993), each fish was transferred to the surgical table and placed in dorsal recumbancy between two V-shaped foam pads lined with a disposable plastic lining. The re-circulating anesthesia delivery system consisted of a rubber tube placed in the oral cavity to wash 80 mg/L of MS-222 buffered with 160mg/L of sodium bicarbonate over the gills. Immediately before surgery, 0.4 mg/ml of butorphanol tartrate (Fort Dodge Animal Health, Overland Park, KS) was administered

intramuscularly for its known behavioral-sparing effects (Harms et al 2005). To accurately measure the inflammatory and tissue responses to the four suture materials, we used surgical techniques during this experiment to minimize inflammatory reactions or wound contamination that may be associated with surgery.

Surgical instruments were exposed to a high-level disinfectant by immersing them in a 0.55% *ortho-phthalaldehyde* (CIDEX OPA) solution (Advanced Sterilization Products, Irvine, CA) for 12 minutes, followed by rinsing in sterile water before each use. To prepare the surgical site, a sterile swab moistened with 0.9% sterile saline was swiped along the ventral midline. Once the fish reached a total loss of reactivity with a very low respiratory and heart rate (known as stage II of anesthesia; Stoskopf 1993), an approximate four to five centimeter incision was made with a number 15 sterile surgical blade through the ventral midline skin, linea alba, and into the coelomic cavity. Fifteen fish from each suture treatment group underwent intracoelomic transmitter implantation with a 16mm x 68mm dummy transmitter (model V16 VEMCO Division, Halifax, Nova Scotia, Canada). Each transmitter weighed 11 g in water and 25 g in air. The transmitter weight ranged from 0.7% to 2.0% of fish's body weight. Each dummy transmitter was cold sterilized and rinsed as previously described before intracoelomic placement. Each incision was sutured with a single-layer closure by passing a reverse-cutting needle through the muscle and skin layers. Four to six simple interrupted sutures were evenly placed approximately two to three millimeters away from the skin edge. Each interrupted suture was tied with one surgeon's knot followed by two square knots. All suture material was sterile and prepackaged, and a new package was used for each fish. After surgery, a color-coated and numbered T-bar anchor tag (Floy Tag, Inc., Seattle, Washington) was placed through both sides of the base of the dorsal fin, and a sterile passive integrated transponder (PIT) tag (Biomark,

Inc., Boise, Idaho) was inserted into the caudal epaxial muscles. The control treatment fish were anesthetized, and then placed on a surgical/anesthesia table for a total of five minutes based on observations from the initial surgery times of the suture treatment fish. The suture and control treatment fish were allowed to recover in a large aerated plastic container with tank water until righting reflexes were regained. After recovery, each fish was returned into its respective rectangular holding tank, and fasted for 24-48 hours after surgery to permit a full anesthetic recovery.

During the post-surgical evaluations, fish were gently captured with a net and briefly anesthetized at 1, 2, 8, and 26 weeks to visually evaluate the surgical incisions. Because each tank contained fish that underwent surgery on different days, the fish were sorted according to the appropriate date of surgery. Visual examination entailed using a subjective scoring system modified from Wagner et al. (2000). Examination included inspecting the incision site for suture retention, evidence of incision healing (Table 2.1; Figure 2.1), erythema (Table 2.2, Figure 2.2), and swelling (Table 2.2). At 12 weeks, the fish were gently captured with a net and visually evaluated without anesthesia. During the evaluations, fish were weighed, and surgical wounds were evaluated, before being permitted to recover as previously described. Five fish from each suture group and the control group were euthanized using a 300 mg/L buffered MS-222 solution at one, two, and eight weeks. A complete gross necropsy was performed on each fish. The surgical incision site was removed in a 3cm by 8cm long block and fixed in 10 % buffered prior to histologic processing.

Tissues were processed in a standard fashion for microscopic examination (Jones et al 1997). A thin section of the fixed tissue that included a representative portion of the surgical incision was trimmed, embedded in paraffin, sectioned with a microtome, and stained with

hematoxylin and eosin (H&E). Histologic sections were subjectively evaluated by a veterinary pathologist (Camus) who was not aware of the treatment group to which each fish belonged. Criteria similar to those used by Hurty et al. (2002) and Anderson et al. (2010) was applied to the histological sections in the current study. Cross sections of three suture sites were processed on a single slide from each incision and randomized prior to evaluation by the pathologist. A scale of 0-3 was applied to each of four criteria and included assessment of tissue inflammation, necrosis, hemorrhage and fibrin deposition, and fibrosis (Table 2.3). The four histology variables were summed to create a total histology score from 0 – 12 for each individual, and then an average score was calculated for each test group and each of the three sample periods.

Statistical Analyses

Each rectangular tank served as a statistical block and had an equal number of treatment groups for this experimental design. The variables of interest for the visual surgical site evaluations were surgical site healing, redness/erythema, and swelling. Initially, cumulative logistic regression was used to evaluate all variables for weeks 1, 2, 8, and 12. However, there was a lack of variability of the data, violation of the proportional odds assumption, and quasi-complete separation of the data. These issues occurred when evaluating the surgical site healing during weeks 1, 2, and 12, and when evaluating redness for week 12. Quasi-complete separation of the data most likely occurred because of a response level only appearing in a particular group of fish, the control group. Based on these issues, the data were believed to be inappropriate for cumulative logistic regression when the control group was included in the data. Therefore, a Chi-square test was used to evaluate the variables surgical site healing during weeks 1, 2, and 12 and redness during week 12. The control group was compared to the combination of all the other fish during the time periods for each variable. In some cases, responses were combined when there

were few fish with a particular response to meet the assumptions of the Chi-square test. The variables of interest for the histological evaluations were necrosis, inflammation, hemorrhage, and fibroplasia. The four histological variables were summed to create a total microscopic score on a scale from 0 to 12 because the variability among the groups was minimal for each variable. Using the total microscopic score, cumulative logistic regression was used to test the variables for weeks 1, 2, and 8. Transmitter retention rates among the suture treatment groups were analyzed using a Chi-square test of independence. For all comparisons, $p < 0.05$ was considered significant.

RESULTS

Gross Evaluations

All surgical and anesthetic procedures were performed without any major complications. In all groups, fish gained weight following the surgical procedures. There were significant differences in the degree of incomplete healing among groups (Figure 2.3). Incomplete healing is directly related to healing times. The use of Vicryl resulted in a higher degree of incomplete healing during weeks one (non-transmitter: 2.07 ± 0.26 ; transmitter: 2.33 ± 0.49 ; $p=0.0001$), two (non-transmitter: 2.00 ± 0.47 ; transmitter: 2.53 ± 0.64 ; $p=0.0001$), and 12 (transmitter: 0.27 ± 0.46 ; $p=0.0004$) compared to the other groups during weeks one (non-transmitter: 2.00 ± 0.00 ; transmitter: 2.00 ± 0.00), two (non-transmitter: 2.00 ± 0.00 ; transmitter: 2.00 ± 0.00), and 12 (non-transmitter: 0.00 ± 0.00 ; transmitter: 0.00 ± 0.00). Closure with Vicryl (non-transmitter: 0.60 ± 0.89 ; transmitter: 0.53 ± 0.52 ; $p=0.0038$) and Prolene (non-transmitter: 0.40 ± 0.89 ; transmitter: 0.40 ± 0.83 ; $p=0.0038$) resulted in a higher degree of incomplete healing during the week eight evaluations when compared to Monocryl-Plus (non-transmitter: 0.00 ± 0.00 ; transmitter: 0.13 ± 0.52 , and Monocryl (non-transmitter: 0.00 ± 0.00 ; transmitter: 0.07 ± 0.27).

There was a significant difference in the degree of redness between groups for week 12 (Figure 2.4). Incisions closed with Prolene exhibited a significantly higher degree of redness during week 12 (transmitter: 0.47 ± 0.52 ; $p=0.0004$) compared to Monocryl-Plus (transmitter: 0.00 ± 0.00), Monocryl (transmitter: 0.07 ± 0.27), and Vicryl (transmitter: 0.13 ± 0.35). There were not significant differences in the degree of swelling at the incision site at weeks one, two, eight, and 12 among the suture groups (Figure 2.5).

There were not significant differences among groups when a transmitter was surgically implanted into the coelom for the degree of incomplete healing, redness, or swelling during weeks one, two, and eight after surgery. Interestingly, the absorbable monofilament antibiotic-impregnated Monocryl-Plus group retained 81.8%, the non-absorbable monofilament group retained 75%, the absorbable monofilament Monocryl group retained 72.7%, and the absorbable multifilament Vicryl group retained 38.5% of the surgically implanted transmitters. The Chi-Square distribution test revealed no statistical significance for transmitter expulsion among the suture treatment groups. The 26-week evaluations revealed that none of the fish remaining retained any sutures, the incision sites were completely healed, and no further transmitter expulsions had occurred.

Histological Evaluations

Incisions from all suture groups were subjectively and microscopically evaluated for necrosis, inflammation, hemorrhage, and fibrosis. With the combined sums of the variables, the analysis did not reveal significant differences among the four suture materials for weeks one, two, and eight (Figure 2.6).

One week after surgery, the majority of incisions were typified by marked inversion of incision margins, forming a large wedge shaped defect with its wide base directed toward the

coelomic cavity and its apex in the superficial dermis (Figure 2.7). The epithelium at the incision margin was characterized by minimal to mild focal hyperplasia (e.g., tissue enlargement from an increase in number of cells) accompanied by a similar degree of predominantly intercellular edema recognized by the increased prominence of intercellular bridges. The surgical defect was filled by a fibrinohemorrhagic (mixture of scar tissue and blood) clot, occasionally infiltrated by minimal numbers of neutrophils along its junction with the hypodermis. A thin layer of epithelial cells had migrated across the exposed dermis, often to the level of the dermal-hypodermal junction. Minimal hemorrhage was present in the hypodermis of some sections. Necrosis and mesothelial responses were generally not present.

Two weeks after surgery, the majority of incisions exhibited evidence of epithelial hyperplasia, edema (swelling), and lymphocytic infiltration. The inner lining (peritoneum) of the coelomic cavity at the surgical defect remained mostly sealed by fibrinohemorrhagic clots that often reflected a short distance onto the peritoneal surface. A layer of edematous epithelium, often approximating the thickness of normal epidermis, extended along incision margins to cover fibrin clots and similarly reflected onto the peritoneum (Figure 2.8). Hypertrophy (e.g., tissue enlargement due to increase in cell size) and migration of limited numbers of fibroblasts onto the margins of the fibrin clot was evident in some sections. Mild to moderate hyperplasia of mesothelial cells was present in most samples. Congestion, minimal hemorrhage, and small numbers of predominantly perivascular lymphocytes were present in subperitoneal tissues of some sections, particularly when overlaid by fibrin clot.

Eight weeks after surgery, the surface epithelium was contiguous in all incision samples. Incisions were filled by extensive variable mixtures of fibrosis, fibroplasia, and neovascularization that in many samples formed proliferative masses that extended into the

coelomic cavity (Figure 2.9). In many samples, there was significant collagen deposition and wound contraction, with partial remodeling of collagen bundles toward a normal dermal architecture (Figure 2.10). Adjacent to the hypodermis, healing was predominated by fibroplasia and neovascularization with variable degrees of collagen deposition and production of small villonodular proliferations.

DISCUSSION

Evaluation of the macroscopic and microscopic tissue reactions of four different suture materials when used for coelomic closure in Siberian sturgeon was successful. All sturgeon tolerated surgery and anesthesia without any major complications. Surgery did not affect growth rates, as the suture groups exhibited the same rate of weight gain as the control fish. Surgical incisions closed with Monocryl and Monocryl-Plus exhibited significantly less erythema, suture breakdown, and transmitter expulsions compared to Vicryl and Prolene.

Surgical wounds closed with Vicryl had a significantly longer healing time throughout the 12-week experiment compared to other suture materials. The surgical incisions of several fish within the Vicryl group remained more than 50% open for approximately four to six weeks after surgery. This was in contrast to one experiment concluding that Vicryl was superior to nylon because it provided better incision healing and was easier to manipulate when used for wound closure in wild brown trout *Salmo trutta* implanted with transmitters (Jepsen et al. 2008). Another experiment demonstrated that Vicryl was the most appropriate suture material for surgical wound closure as it did not require removal, reduced prolonged skin irritation, and was easier to handle when compared to surgical wounds closed with non-absorbable polyester or with absorbable Panacryl (Chapman and Park 2005). Both Jepsen et al. (2008) and Chapman and Park (2005) agree that Vicryl was easier to manipulate compared to the more rigid monofilament

sutures. The manipulability of Vicryl is directly related to the composition and multifilament nature of the suture. Although Chapman and Park (2005) reported that surgical wounds closed with Vicryl exhibited reduced skin irritation compared to the other sutures, all sutures were broken down by day 30 after surgery in their experiment. In the current study, sutures were not removed, which allowed additional time for evaluation of skin irritation. In addition, our results differed from the Chapman and Park (2005) because we made longer incisions for transmitter implantation, whereas they made shorter incisions because they were only performing gonadal biopsies. Longer incisions require more suture material for surgical closure, thus increasing the possibility for skin irritation and knot failure.

Furthermore, surgical wounds closed with Vicryl in the current study exhibited a higher transmitter expulsion rate compared to the other suture materials. Transmitters may have been expelled through the open incision. The results suggest that Vicryl broke down prior to complete healing, and the transmitters may have been expelled through the open incision. Suture breakdown, if premature, may lead to incision dehiscence, organ evisceration, and transmitter loss. Interestingly, transmitter retention rates differed among the suture groups. Results from past studies indicated that Vicryl sutures tend to break down faster and did not remain long enough to promote wound closure compared to polydioxanone (PDS) in captive yellow fin tuna *Thunnus albacores* (Block et al. 1998), possibly contributing to transmitter loss. Similarly, Deters et al. (2010) recommended using monofilament sutures because it has a higher suture retention time, results in lower dehiscence, and a higher tag retention compared to multifilament sutures in juvenile Chinook salmon. The multifilament nature of Vicryl may provide a route for infections at the incision, which will lead to wound dehiscence. Although there was no conclusive evidence that transmitters were expelled more frequently from the surgical site, incisions closed with

Vicryl appeared more likely to exhibit dehiscence compared to the other three suture materials. The longer healing times noted in fish sutured with Vicryl in this study was likely related to the fast breakdown of suture material at the surgical site compared to the other groups. The lack of appropriate retention time before the incision heals, the risk for bacteria wicking at the surgical site, and decreased transmitter retention may limit the use of Vicryl for surgical wound closure in fish.

Tissues sutured with Prolene also displayed a significant increase in healing time during the eight week evaluation and a higher degree of erythema during the 12-week evaluations compared to the other suture materials. An important aspect of performing surgery is using a suture material that will remain at the surgical site long enough for adequate healing and reinforcement, but does not remain for an extended time because any foreign material will eventually elicit an inflammatory response. Surgical incisions closed with Prolene exhibited higher suture retention as expected because Prolene is made from a non-absorbable material. The increased suture retention time most likely caused chronic tissue irritation. Chronic irritation at the incision site due to the non-absorbable nature of polypropylene explained the increased degree of erythema noted at the 12-week evaluation when compared to the other absorbable suture materials. Interestingly, Deters et al. (2010) reported that the absorbable monofilament Monocryl (poliglecaprone 25) and the nonabsorbable monofilament Ethilon (nylon) exhibited higher suture retention and a lower incidence of inflammation and ulceration compared to nonabsorbable multifilament Nurolon (nylon), the absorbable multifilament Vicryl (polyglactin 910), absorbable triclosan-coated multifilament Vicryl-Plus (triclosan-coated polyglactin 910), and nonabsorbable multifilament Perma-hand (silk). Deters et al. (2010) recommended Monocryl for surgical closure in juvenile Chinook salmon *Oncorhynchus tshawytscha* citing that

other researchers have seen undesirable effects from the long-term retention of non-absorbable sutures. Although incisions sutured with Prolene exhibited high transmitter retention rates, polypropylene stayed at the surgical site after the incision was healed which may have caused an unnecessary inflammatory response. The use of a non-absorbable suture material such as Prolene, without removal, may lead to a chronic inflammatory reaction. Furthermore, inflamed tissue is more susceptible to a bacterial infection that may result in wound dehiscence and lead to organ evisceration or transmitter loss through the incision. Based on the results from our study and previous studies, the non-absorbable suture, Prolene, may not be appropriate for surgical wound closure in fish unless sutures are to be removed once healing has occurred.

The inversion of the skin at the surgical incision, which most likely contributed to the slow healing noted in all surgical groups, was an interesting microscopic observation. Inversion of the surgical site was noted during the first week in most fish, and the epithelium had completely grown over the apposed edges of the incision and reflected onto the peritoneum by the second week. The inversion of the incision seen in this species may be a result of the thickness and lack of elasticity of the skin. Indeed, Chapman and Park (2005) observed that most species of sturgeon have thick, poorly vascularized, and inelastic skin. Their recommendation was to use a horizontal mattress (everting) pattern when closing skin incisions made for gonadal biopsies in Gulf sturgeon *Acipenser oxyrinchus de sotoi*.

Skin inversion has been documented in other species of fish. Wagner et al (2000) compared three suture materials and two different suture patterns including a vertical mattress pattern. The vertical mattress pattern is an everting pattern similar to the horizontal mattress pattern, but differs in the placement of the suture material in relation to the incision. Wagner et al. (2000) found that a vertical mattress pattern increased surgical time, did not provide better

apposition of the skin, and resulted in more inflammation compared to a simple interrupted pattern in rainbow trout *Oncorhynchus mykiss* (Wagner et al. 2000). The increased inflammation noted by Wagner et al. (2000) was likely caused by the nature of the vertical mattress pattern, as the suture was passed through the incision twice in a parallel fashion. Everting suture patterns are used in areas of tension, but should be placed in a manner to oppose the incision rather than evert the incision (Fossum 2002). In reptiles, the skin has a tendency to invert immediately after being incised; thus, the recommendation is to use everting suture patterns for closure (Hernandez-Divers 2004). However, the skin of the fish in this study was not grossly noted to invert after the incision was made, and therefore inversion most likely occurred during the days to weeks after surgery. Although skin inversion was noted, all incisions were completely healed by 26 weeks after surgery suggesting that inversion did not cause long-term healing effects to the fish. Given that placement of everting suture patterns may be time consuming and that all incisions completely healed during the study, the need for an everting suture pattern may not be necessary.

Temperature plays an important role in surgical wound healing in ectotherms (Deters et al 2011; Knights and Lasee 1996). In the current study, visual evidence of healing was noted during the eighth week evaluations, and was complete by 12 weeks at a mean temperature of 12.5°C. Knights and Lasee (1996) concluded that cold temperatures (6°C) may result in lower mortality and slower healing, while warm temperatures (20°C) facilitated healing but increased mortality in adult bluegills *Lepomis macrochirus* over eight weeks. Deters et al. (2010) also found that juvenile Chinook salmon *Oncorhynchus tshawytscha* held in 12°C water exhibited less incision openness, wound inflammation, and ulceration at 7 and 14 day post-surgery compared to fish held at 17°C. Walsh et al. (2000) found that when surgery was performed on

hybrid striped bass *Morone saxatilis* x *Morone chrysops* and maintained in 22-29°C water, the incision exhibited more irritation than fish held at 12-18°C water during the initial 7 days. However, Walsh et al. (2000) also noted that fish in the cooler water exhibited more skin irritation at 60 days post-surgery which suggested that higher suture retention at the lower water temperature may have caused this skin irritation. The slow healing of surgical wounds in this study appears to be consistent with past studies indicating that cold temperatures may decrease mortality, but delay healing.

Histologic examination of the surgical incision did not reveal significant differences in inflammation, fibrosis, necrosis, or hemorrhage among the suture groups and control group. However, microscopic evaluations of the progression of healing closely resembled the gross findings. Although histology is the gold standard for documenting the host response to disease and inflammation, our results indicated that visual evaluations also may allow for interpretation of healing of surgical wounds. Furthermore, visual examinations may be more biologically relevant than histological evaluations when assessing overall healing, incision dehiscence, suture loss, and transmitter retention.

The current study demonstrated that using the appropriate suture material is important for surgical closure in fish. Although there is currently no ideal suture, surgeons should choose a suture material based on proven surgical factors to minimize tissue reactions. The minimization of an inflammatory reaction and risk of infection are important elements of wound closure because this will result in decreased incision dehiscence and skin integrity, and may decrease transmitter loss through the incision. Surgical wounds closed with the absorbable triclosan-coated monofilament Monocryl-Plus and absorbable monofilament Monocryl exhibited less erythema, dehiscence, and transmitter loss than the absorbable multifilament Vicryl. The

absorbable monofilament triclosan-coated Monocryl-Plus and absorbable monofilament Monocryl also exhibited less erythema than the non-absorbable monofilament Prolene. The current data suggested that Vicryl and Prolene are not appropriate for skin closure in sturgeon. Based on the results, there was no difference between Monocryl and Monocryl-Plus when used for skin closure. If funding is limited, then one may consider using Monocryl as opposed to Monocryl-Plus because Monocryl is about 10% less per package of suture. However, researchers should at least consider using a suture material that minimizes unnecessary bacterial colonization at the incision when performing surgery. Although the current study was conducted in a laboratory setting, most telemetry studies are conducted in waters containing more potential pathogens. Therefore, Monocryl-Plus may be the more appropriate suture material to use for coelomic closure when fish are released back into the wild. The use of Monocryl-Plus may be further supported given that there is evidence in the human and veterinary literature that using a triclosan-coated suture material inhibits bacterial growth and prevents post-surgical infections. Furthermore, we believe that an absorbable monofilament such as Monocryl or triclosan-coated Monocryl-Plus, may decrease the risk of transmitter expulsion through the incision as surgical wounds appear to heal faster and exhibit less erythema compared to Vicryl or Prolene.

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Table 2.1 Rating score and criteria used to describe macroscopic healing of the surgical sites for Siberian sturgeon during the weekly assessments. Data were collected from January 2010 to January 2011.

Rating Score	Rating Criteria
0	Incision completely healed.
1	Incision is abnormally healed, with scar tissue present along the incision.
2	Incision held in proximity, but incompletely healed.
3	Incision partially open at one end or the middle.
4	Incision is 25 to 75% open.
5	Incision is completely open, with evisceration.

Table 2.2 Rating score and criteria used to describe macroscopic redness and swelling of the surgical sites for Siberian sturgeon during the weekly assessments. Data were collected from January 2010 to January 2011.

Rating Score	Rating Criteria
0	No redness or swelling present.
1	Mild amount of redness or swelling present.
2	Moderate amount of redness or swelling present.
3	Severe amount of redness or swelling present.

Table 2.3 Rating score and criteria used to describe the degree of microscopic necrosis, inflammation, hemorrhage, and fibroplasias at the incision for Siberian sturgeon during the weekly assessments. Data were collected from January 2010 to January 2011.

Rating Score	Rating Criteria
0	No apparent changes from normal skin, underlying muscle and peritoneum
1	Changes limited to scattered inflammatory cells, with minimal to mild necrosis, hemorrhage and fibrosis immediately adjacent to the suture tract.
2	Widespread mixed inflammatory infiltrates associated with suture tracts. Necrosis of individual muscle fibers and small foci of interstitial inflammation were occasionally present in muscle bundles of the abdominal wall, as well as mild perivascular inflammation in the subcutis.
3	Moderate to large numbers of inflammatory cells, with more pronounced necrosis and edema, and broader multifocal involvement of skeletal muscle and connective tissues.



Figure 2.1 Photographic representations of the rating score and rating criteria used for macroscopic healing of the surgical sites for Siberian sturgeon during the weekly assessments. Data were collected from January 2010 to January 2011. A representation of grade 5 is not available.



Figure 2.2 Photographic representations of the rating score and rating criteria used for macroscopic redness and swelling of the surgical sites for Siberian sturgeon during the weekly assessments. Data were collected from January 2010 to January 2011.

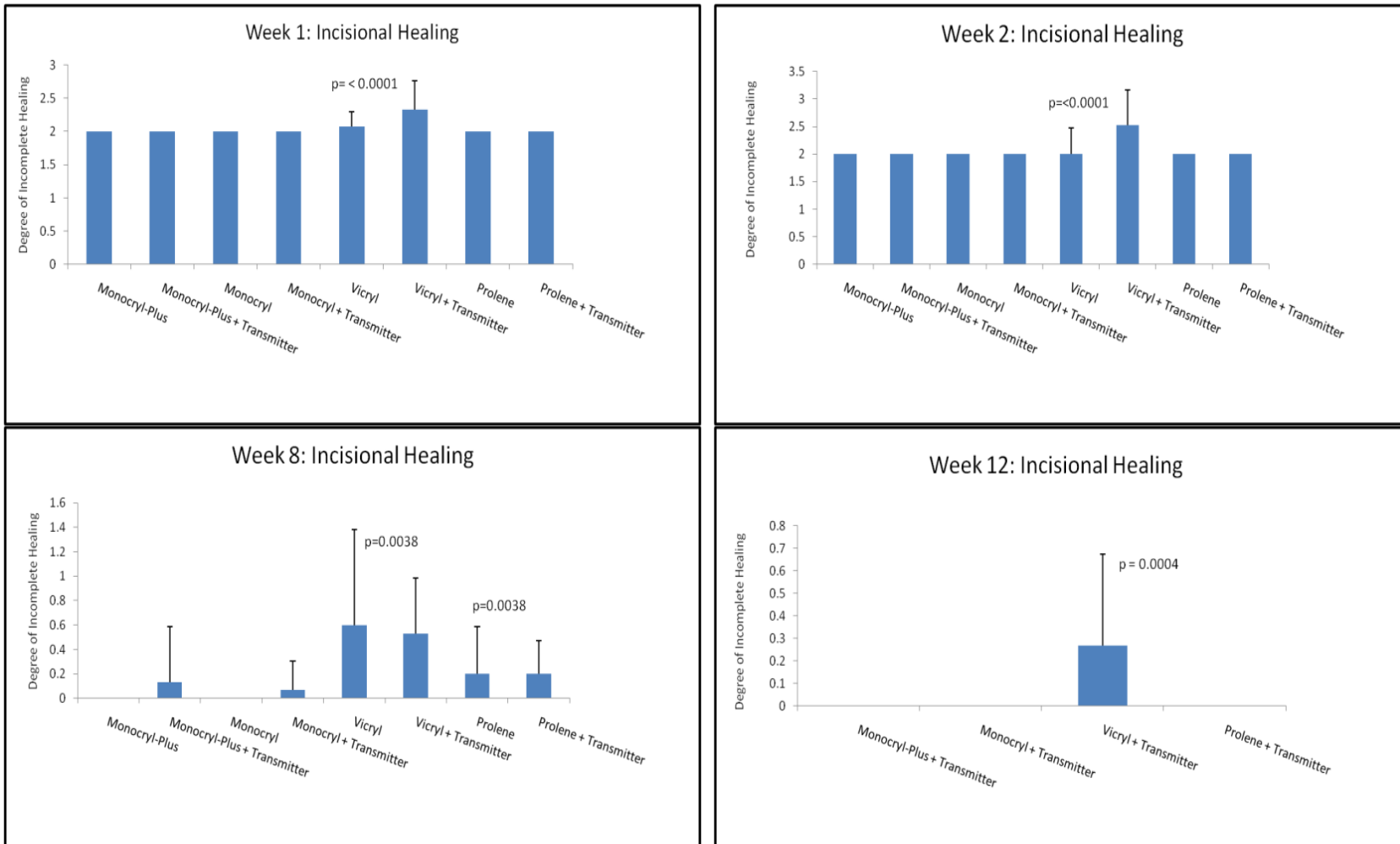


Figure 2.3 The degree of incomplete incisional healing for each surgical group during visual evaluations for weeks 1, 2, 8, and 12 after surgery in Siberian sturgeon. Each bar represents the mean + 95% confidence interval for incisional healing for each treatment group. If significant differences occurred among suture groups, a p -value is indicated. Data were collected from January 2010 to January 2011.

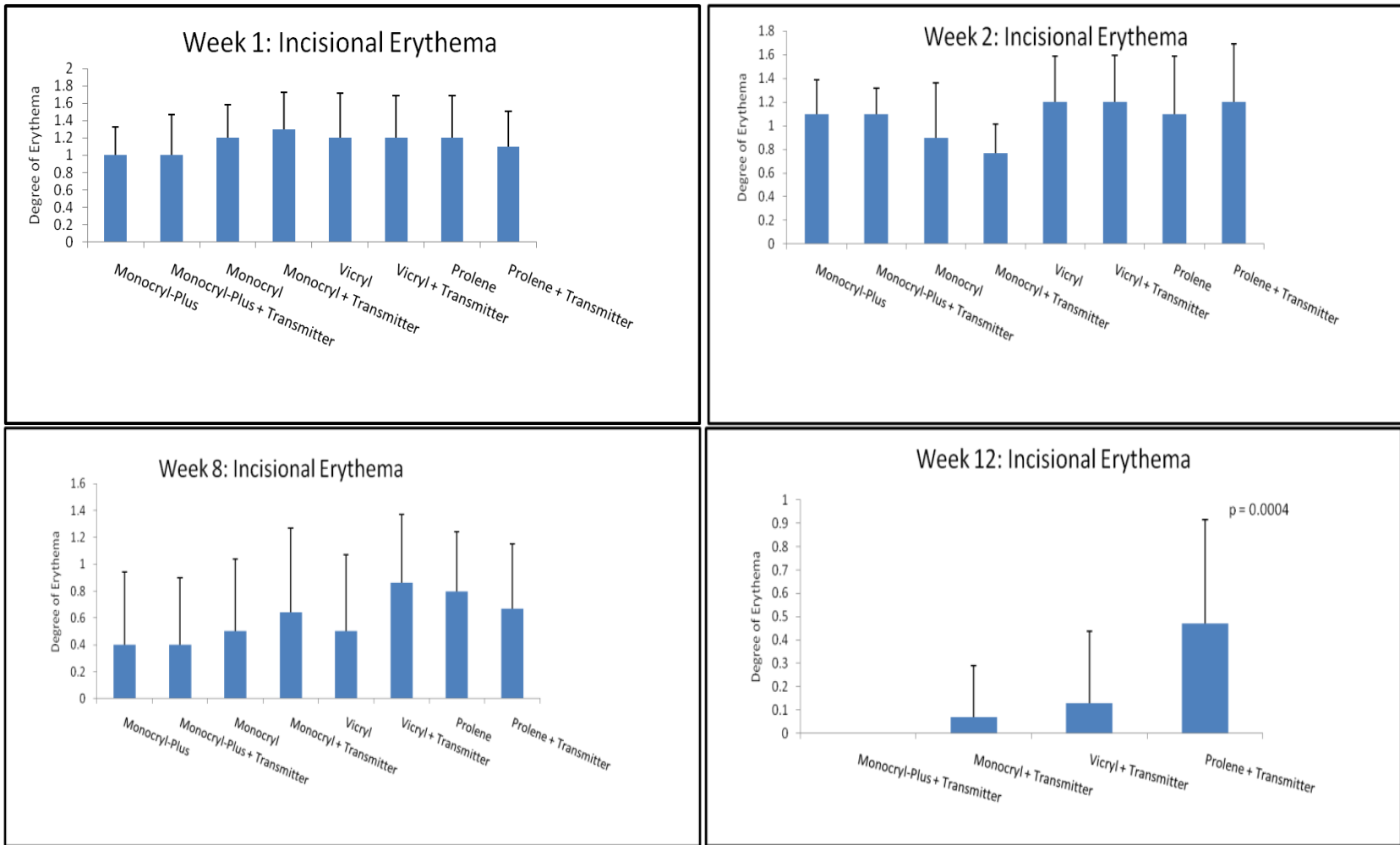


Figure 2.4 The degree of incisional erythema for each surgical group during visual evaluations for weeks 1, 2, 8, and 12 after surgery in Siberian sturgeon. Each bar represents the mean + 95% confidence interval for incisional erythema for each treatment group. If significant differences occurred among suture groups, a *p*-value is indicated. Data were collected from January 2010 to January 2011.

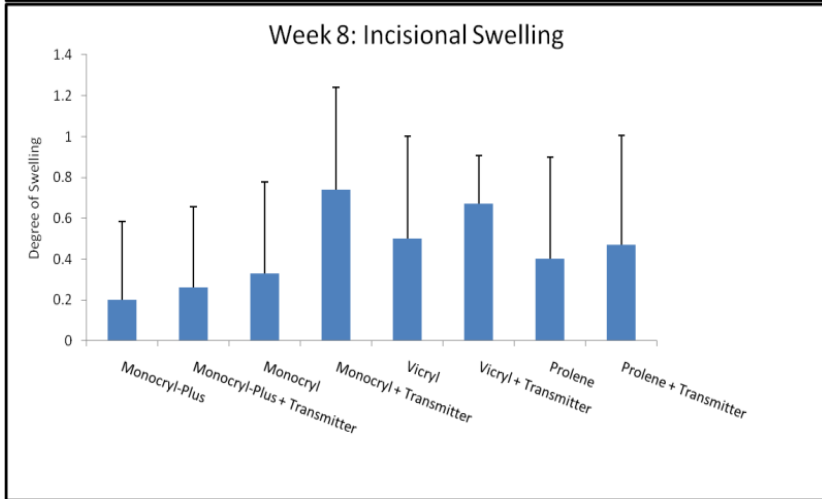
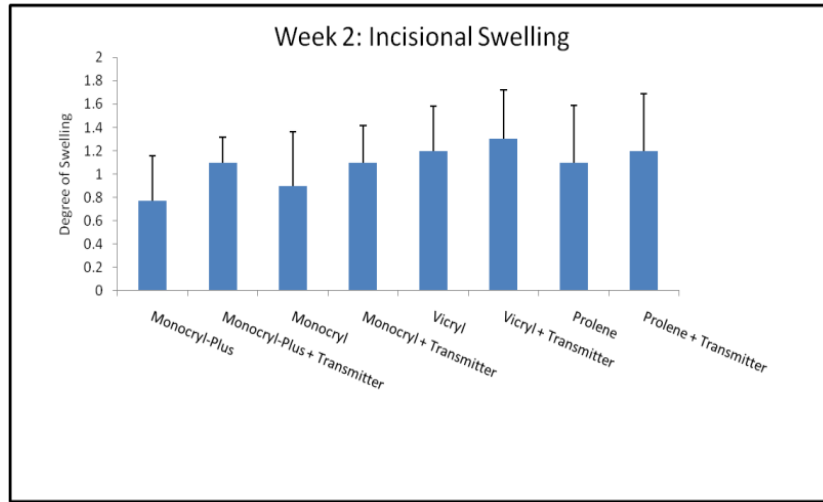
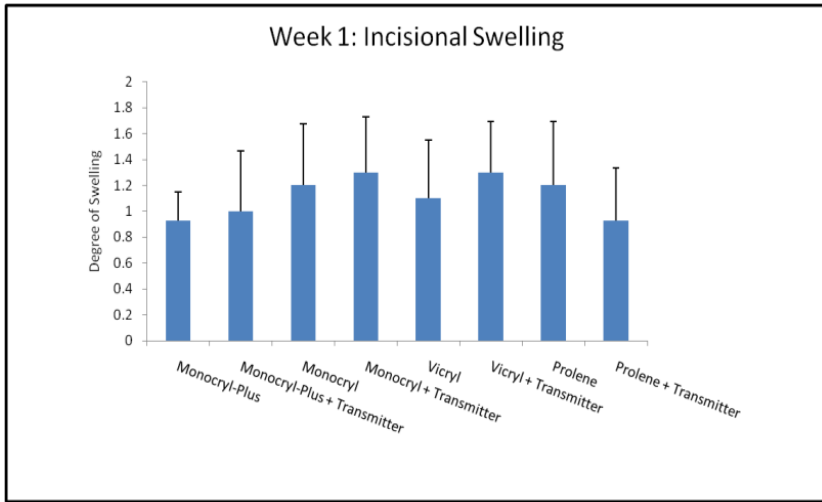


Figure 2.5: The degree of incisional swelling for each surgical group during visual evaluations for weeks 1, 2, and 8, after surgery in Siberian sturgeon. Each bar represents the mean + 95% confidence interval for incisional swelling for each treatment group. If significant differences occurred among suture groups, a p -value is indicated. Data were collected from January 2010 to January 2011.

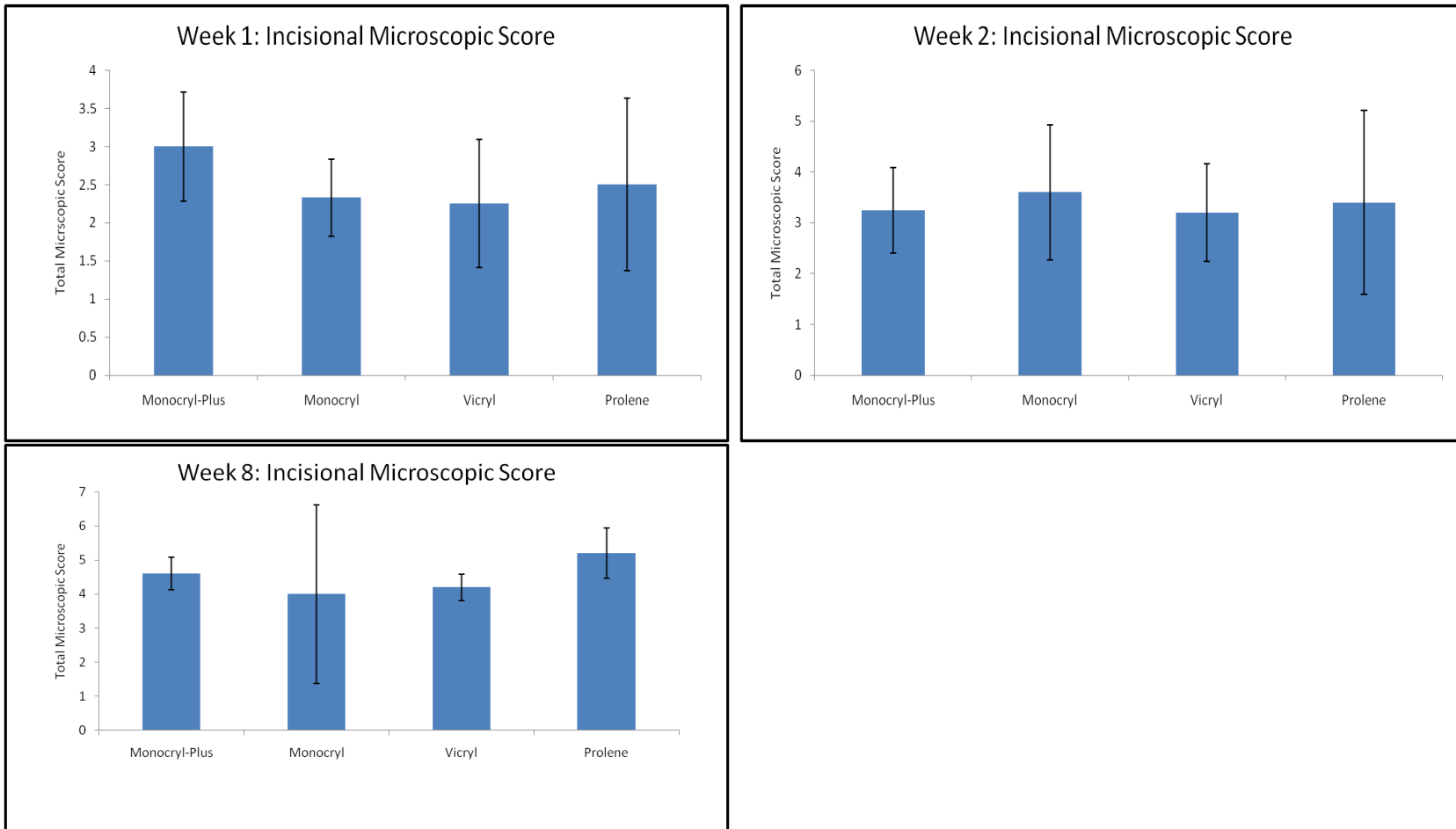


Figure 2.6: The total microscopic incisional scores for each surgical group for weeks 1, 2, and 8 after surgery in Siberian sturgeon. Each bar represents the mean + 95% confidence interval for the total microscopic score for each treatment group. If significant differences occurred among suture groups, a p -value is indicated. Data were collected from January 2010 to January 2011.

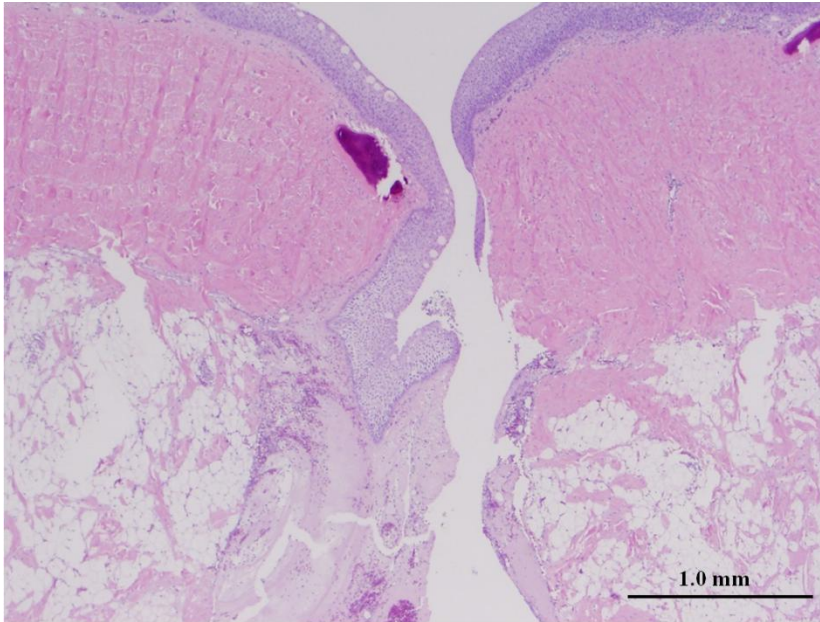


Figure 2.7 One week post-operative microphotograph of a surgical incision that is incompletely healed in a Siberian sturgeon. Data were collected from January 2010 to January 2011.

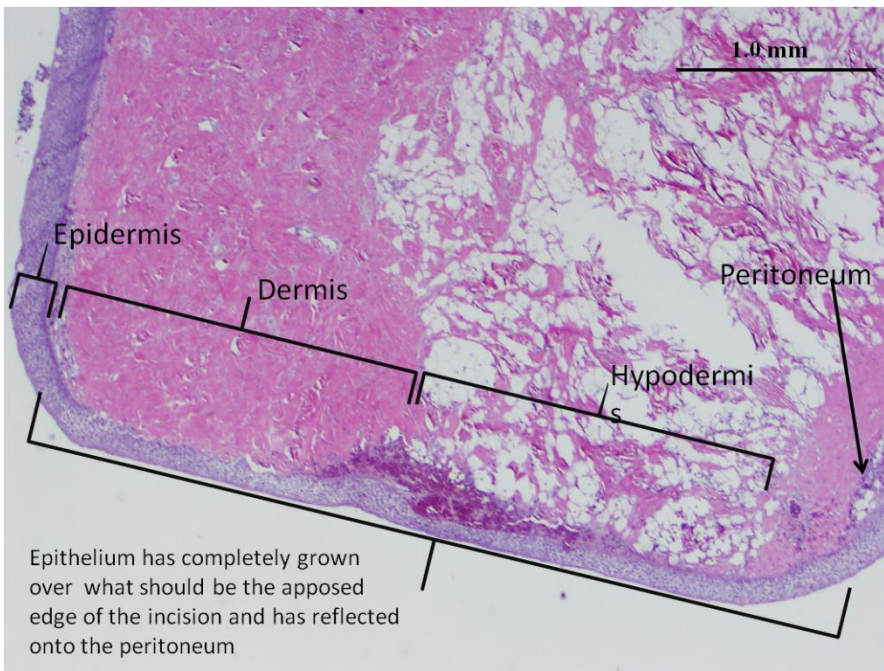


Figure 2.8 Two week post-operative microphotograph of a surgical incision in a Siberian sturgeon. The epithelium has completely grown over what should be the apposed edge of the incision and has reflected onto the peritoneum. Data were collected from January 2010 to January 2011.

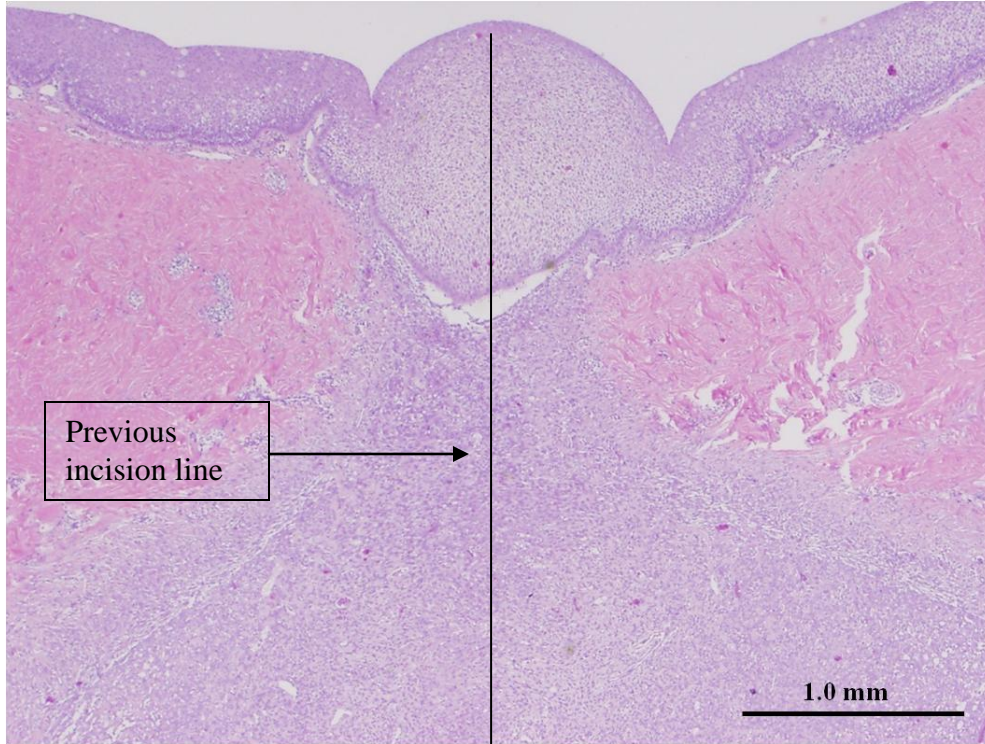


Figure 2.9 Eight week post-operative microphotograph of a surgical incision with extensive fibrosis at the incision in a Siberian sturgeon. Data were collected from January 2010 to January 2011.

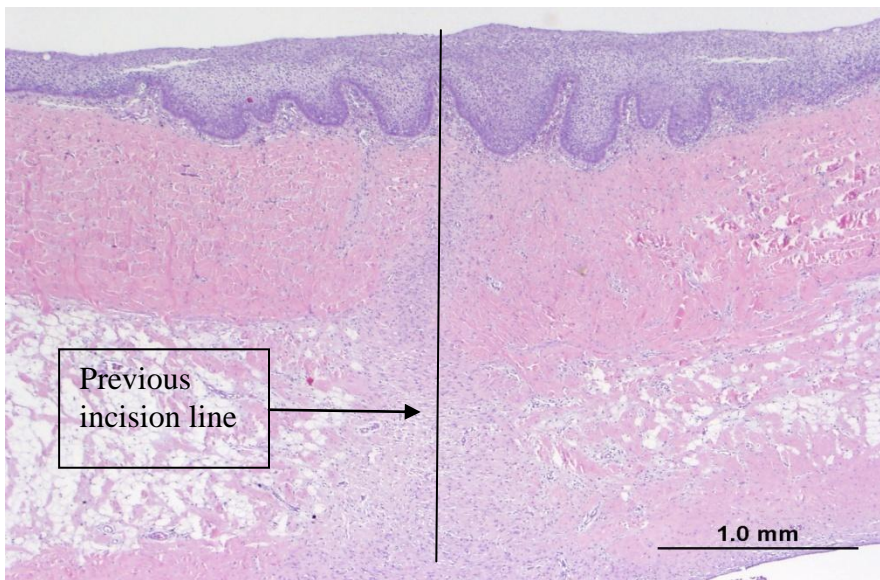


Figure 2.10 Eight week post-operative microphotograph of a surgical incision that has returned to a pre-surgery state in a Siberian sturgeon. Data were collected from January 2010 to January 2011.

CHAPTER 3

EVALUATION OF PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF INTRACOELOMIC TRANSMITTERS IN SIBERIAN STURGEON (*ACIPENSER BAERII*)¹

¹Boone, S. Shaun, Stephen J. Divers , Alvin C. Camus, Douglas L Peterson, Cecil A. Jennings, James L. Shelton, and Sonia M. Hernandez. To be submitted to *Transactions of the American Fisheries Society*.

ABSTRACT

Intracoelomic transmitters are commonly used to evaluate migratory patterns, distribution, and habitat use of many species of fish. Currently, transmitter implantation relies mostly on the assumption that transmitters do not cause any adverse physiological or pathological effects on the animal. To investigate these effects, we surgically implanted 60 sturgeon with transmitters that weighed less than 2% of their body weight. We additionally investigated whether surgical closure of the incision site with different suture materials was associated with transmitter loss. Post-operative assessments were conducted at one, two, eight, 12, 26, and 55 weeks to evaluate surgical healing and transmitter retention. Blood samples were collected before and after the 55-week study for plasma cortisol analysis. Results indicated that overall transmitter loss was 36.2%, and incisions closed with Vicryl exhibited the lowest transmitter retention rates. Minor to moderate adhesions were noted at necropsy, but did not appear to affect organ function. One fish was noted to have an intra-intestinal transmitter at necropsy, but the fish was in overall good health. Long-term transmitter presence does not appear to increase plasma cortisol levels, or affect overall growth when compared to non-transmitter fish. Although long-term telemetry studies can be undertaken with minimal concern for negative physiological or pathological effects from transmitters, researchers should be aware that transmitter loss rates may be higher than previously thought and may involve intra-intestinal capture and eventual expulsion as was seen in the current study.

INTRODUCTION

Telemetry has long been used for evaluating migration, distribution, and habitat selection in fish, and is especially useful when evaluating these parameters for threatened or endangered species (Caputo et al. 2009; Lucas 1989). To determine the type of transmitter that is best suited for the project at hand, one should consider the scope of the study and any potential complications that may lead to behavioral changes, morbidity, or mortality (Bridger and Booth 2003). Radio and sonic transmitters have been used both externally and internally with varying results (Bridger and Booth 2003). Transmitter implantation relies mostly on the assumption that the transmitters do not adversely affect the behavior or physiology on the animal (Schreck 1990; White and Garrott 1990), and that the transmitters do not cause any pathological effects over the long-term (Guynn et al. 1987; Jepsen 2003). Most fish studies use metrics such as short-term swimming performance, predator avoidance, or affects on feeding behavior to determine transmitter effects (Adams et al. 1998; Anglea et al. 2004; Jepsen et al. 2008; Martinelli et al. 1998), whereas fewer studies concentrate on the effects on organ function or growth rates over the long-term (Bridger and Booth 2003; Caputo et al. 2009).

Before surgically implanting a transmitter into a fish that will be released into the wild, experimental studies can be used to evaluate any long-term physiological effects (Lucas 1989). Physiological parameters may include overall health status and physiologic stress. Adams et al. (1998) and Martinelli et al. (1998) found that the initial growth rate of fish that underwent transmitter implantation was significantly slower than the control fish. However, the growth rates were comparable towards the end of the 54 or 21 day studies, respectively. Caputo et al. (2009) also indicated that there were not long-term physiologic effects from transmitter implantation in

wild largemouth bass *Micropterus salmoides* based on physiological measures of stress, tissue damage, and nutritional status.

Cortisol, an endogenous glucocorticoid, is released as part of the stress response in animals. During acute stress, cortisol actually protects the animal through various mechanisms such as mobilization of glucose stores, inhibition of additional glucose storage which increases energy into the muscle tissues, and prompts behavior changes for escape. Chronic cortisol elevations may negatively affect the animal by causing hyperglycemia, neuronal cell death, and the suppression of the immune and reproductive systems (Cyr and Michael Romero 2007). Elevations in plasma cortisol are a good indicator of acute stress in fish (Wedemeyer et al. 1990). Most studies conducted with fish evaluated the effects of chronic plasma cortisol elevation on feeding behavior, growth, and swimming ability (De Boeck et al. 2001; Gregory and Wood 1999). Gregory and Wood (1999) suggested that chronically elevated plasma cortisol levels may suppress fish appetite and result in reduced growth rate. Few studies have evaluated long-term plasma cortisol measurements in fish with intracoelomic transmitter implantation (Caputo et al. 2009; Jepsen et al. 2001; Wagner and Congleton 2004). For example, Jepsen et al. (2001) suggested that Chinook salmon *Oncorhynchus tshawytscha* smolts were not chronically stressed because plasma cortisol and glucose levels returned to normal within a few days of gastric insertion or surgical insertion of radiotransmitters. Chronically elevated plasma cortisol levels may increase the risk of disease by suppressing the immune system, decrease survival, and decrease reproductive abilities in fish (Moberg 1993).

Negative pathologic effects may be associated with intracoelomic transmitters in fish. For example, studies have shown that transmitter encapsulation within fibrous tissue may occur between 30 days and one year after implantation depending on the fish species. However,

encapsulation does not appear to cause any effects on the internal organs (Bauer and Loupal 2007; Chapman and Park 2005; Collins et al. 2002; Lacroix et al. 2004; Lucas 1989; Martinelli et al. 1998; Wagner and Cooke 2005). Intracoelomic transmitters may also act as a focus for adhesions involving adjacent organs and induce chronic inflammation in the surrounding tissue (Lacroix et al. 2004; Lucas 1989). Unfortunately, whether organ function is affected when fibrous tissue infiltrates internal organs, such as the liver, is mostly unknown (Bauer and Loupal 2007). Furthermore, any adverse pathologic effects associated with intracoelomic transmitter implantation are especially important when dealing with endangered or threatened species.

Using the commercially available Siberian sturgeon *Acipenser baerii* as a model for other sturgeon species, the aims of this study were to determine if implanting sonic transmitters into the coelomic cavity of Siberian sturgeon resulted in negative physiologic or pathologic effects during a 12 month period.

MATERIALS AND METHODS

Hatchery-reared Siberian sturgeon were obtained from the Cohutta Fisheries Center of the Warnell School of Forestry and Natural Resources (WSFNR) of the University of Georgia. The sturgeon were transported to the Whitehall Fisheries Lab of the WSFNR of the University of Georgia in November 2009. All procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (AUP # A2009-10079). Seventy-five sturgeon were randomly assigned to one of three 3785-L rectangular semi-recirculating tank systems, each with its own biological and mechanical filter (Figure 3.1). Fish were allowed to acclimate for six weeks before experiments began. Sturgeons were fed 0.3% of their body weight using a 6mm pellet (Silver Cup Fish Feed, Murray, Utah). Total ammonia-nitrogen and nitrite-nitrogen concentrations were monitored daily during the first 10 weeks and twice weekly afterwards.

Dissolved oxygen, pH, and temperature were monitored twice weekly throughout the entire experiment. Total ammonia-nitrogen, nitrite-nitrogen, dissolved oxygen, and pH were monitored with the LaMotte Smart 2 colorimeter system (LaMotte Company, Chestertown, MD).

Temperature was monitored with a mercury thermometer (Miller and Weber, Inc., Ridgewood, Queens NY). During this experiment, total ammonia nitrogen (range: 0.0-0.9 ppm), un-ionized ammonia-nitrogen (range: 0.00-0.02 ppm), nitrite-nitrogen (range: 0.00-0.26 ppm), dissolved oxygen (range: 5.5-9.5 mg/L), pH (range: 6.0-7.6), and temperature (10-27 °C) were maintained within the previously proven levels for rearing sturgeon (Hochleithner and Gessner 1999).

All surgical procedures were performed between January 5, 2010 and January 8, 2010 by the same surgeon (Boone). The sturgeon were fasted between 72 and 120 hours before surgery to minimize regurgitation and reduce the chance of gill damage from nitrogenous waste. A total of 60 sturgeon (mean fork length =57.4 cm [range: 52.0-61.0 cm]; mean wet weight = 954.0 g [range: 675.0-1480.0 g]) underwent intracoelomic transmitter implantation. The surgical incision was closed with one of four randomly assigned suture materials: absorbable monofilament poliglecaprone 25 (2-0 Monocryl, FS-1 needle; Ethicon Inc., Somerville, NJ), absorbable monofilament tricolsan-coated poliglecaprone 25 (2-0 Monocryl-Plus, FS-1 needle; Ethicon Inc., Somerville, NJ), absorbable multifilament polyglactin 910 (2-0Vicryl, FS-1 needle; Ethicon Inc., Somerville, NJ), and non-absorbable monofilament polypropylene (2-0 Prolene, FS needle; Ethicon Inc., Somerville, NJ). Additionally, 15 fish were assigned as treatment controls (mean fork length =58.0 cm [range: 50.0-63.0 cm]; mean wet weight = 954.7 g [range: 550.0-1480.0 g]). Although the control treatment group did not undergo surgery, this group underwent the same handling and anesthetic procedures as the suture treatment groups.

Anesthesia was induced by placing each fish in an aerated 114 L plastic container that contained a 150 mg/L solution of tricaine methanesulphonate (MS-222) (Finquel, Argent Chemical Laboratories, Redmond, WA) buffered with 300 mg/L sodium bicarbonate. Once the fish reached a total loss of equilibrium (stage II of anesthesia) as defined by Stoskopf (1993), the fish was transferred to the surgical table and placed in dorsal recumbancy between two V-shaped foam pads lined with a disposable plastic lining. The re-circulating anesthesia delivery system consisted of a rubber tube placed in the oral cavity to wash 80 mg/L of MS-222 buffered with 160mg/L of sodium bicarbonate over the gills (Figure 3.2). Following placement on the table, approximately 1-2 mL of blood was collected via the caudal tail vein, and placed in a plastic red top tube without silicone (BD Vacutainer, No additive. Becton, Dickinson, and Company, Franklin Lakes, NJ). The plasma was separated from the whole blood within 2 hours of collection and frozen at -80° C until it was analyzed by the University of Georgia, College of Veterinary Medicine Clinical Pathology laboratory. Immediately before surgery, 0.4 mg/ml of butorphanol tartrate (Fort Dodge Animal Health, Overland Park, KS) was administered intramuscularly for its known behavioral-sparing effects (Harms et al 2005). To accurately measure the pathological effects of the intracoelomic transmitters, we used standard sterile techniques during this experiment to minimize inflammatory reactions or wound contamination associated with surgery.

Surgical instruments were exposed to a high-level disinfectant by immersing them in a 0.55% *ortho-phthalaldehyde* (CIDEX OPA) solution (Advanced Sterilization Products, Irvine, CA) for 12 minutes. Afterwards, the instruments were rinsed in sterile water before each use. To prepare the surgical site, a sterile swab moistened with 0.9% sterile saline was swiped along the ventral midline. Once the fish reached stage III-2 of anesthesia defined as a total loss of

reactivity by Stoskopf (1993), an approximate four to five centimeter incision was made with a number 15 sterile surgical blade through the ventral midline skin, linea alba, and into the coelomic cavity (Figure 3.3). A dummy transmitter (model V16VEMCO Division, Halifax, Nova Scotia, Canada) was inserted into the coelomic cavity (Figure 3.4). The transmitter was 16mm by 68mm and had a volume of 13.67 cm³. Each transmitter weighed 11 g in water and 25 g in air. The transmitter weight to fish body weight ratio ranged from 0.7% to 2.0%. Each transmitter was prepared in the same manner as the surgical instruments before being inserted into the coelomic cavity. The transmitter was gently pushed caudally away from the surgical incision and allowed to free-float in the coelomic cavity. Each coeliotomy incision was sutured with a single-layer closure by passing the reverse-cutting needle through the muscle and skin layers. Four to six simple interrupted sutures were evenly placed approximately two to three millimeters away from the skin edge. Each interrupted suture was tied with one surgeon's knot followed by two square knots. All suture material was sterile and prepackaged, and a new package was used for each fish. A color-coated and numbered floy T-bar anchor tag (Floy Tag, Inc., Seattle, Washington) was placed through the base of the dorsal fin, and a sterile passive integrated transponders (PIT) tag (Biomark, Inc., Boise, Idaho) was inserted into the caudal epaxial muscles. Each fish was allowed to recover in a large aerated plastic container with tank water until its righting reflexes were regained. The control fish underwent handling and anesthesia in the same manner as the suture groups. These fish were induced and once anesthetized, placed on a surgical/anesthesia table for a total of five minutes based on observations from the initial surgery times of the suture fish. They were recovered in the same manner as the suture treatment groups. After recovery, each fish was returned into its respective rectangular holding tank and fasted for 24-48 hours after surgery to permit a full anesthetic recovery.

Plasma cortisol levels were determined with a fluorescent polarization immunoassay (Siemens Diagnostic Corp., Deerfield, IL) using an automated Immulite 1000 machine (Siemens Diagnostic Corp., Deerfield, IL). To preclude any variability, the pre- and post-surgery blood samples were analyzed using kits with the same lot number.

Fish were monitored on a daily basis for any signs of morbidity including behavioral distress, decreased appetite, and transmitter expulsion. At 1, 2, 8, 12, and 26 weeks after surgery, all fish were gently captured with a dip net and then anesthetized to visually evaluate signs of morbidity that may have resulted from surgical incision complications or transmitter expulsion. Each tank was fasted 24 to 48 hours before and 24 to 48 hours after assessment to minimize regurgitation. At week 55, all fish were anesthetized as previously described to obtain morphometric measurements, and a second blood sample was collected for cortisol analysis. The fish were then humanely euthanized by immersing them in a 300 mg/L buffered MS-222 solution. A complete gross necropsy was performed within two hours of euthanasia (Noga 2010). Any pathology associated with the surgical incision or the transmitter was recorded.

Adhesions were classified using the following subjective key: grade 1—no adhesions; grade 2—minor adhesions; grade 3—moderate adhesions; grade 4—severe adhesions. Transmitter encapsulation was graded using the following subjective key: grade 1—non-encapsulating or free-floating within the coelom; grade 2—encapsulated by fibrous tissue only, but not adhered to any surrounding tissue or organs; and grade 3—encapsulated by fibrous tissue and adhered to surrounding tissue or organs. If a fish was known to have been surgically implanted with a transmitter and it was not found, then the vent and intestine were suspended in 10 % neutral-buffered formalin to be fixed for microscopic evaluation. Any abnormal tissues were also fixed in formalin for later evaluation.

The tissue block containing the surgical incision site was processed in a standard fashion for microscopic examination (Jones et al 1997). A thin section of the fixed tissue that included a representative portion of the surgical incision was trimmed, embedded in paraffin, sectioned with a microtome, and stained with hematoxylin and eosin (H&E). Histologic sections were subjectively evaluated by a veterinary pathologist (Camus), who was not aware of the treatment group to which each fish belonged. Any pathology associated with the incision, organs, and transmitter was documented by quantifying and describing the degree of inflammation, fibrosis (scar formation), edema (swelling), and hypertrophy (tissue enlargement).

Statistical Analyses

Pre- and post-surgery plasma cortisol levels were analyzed using Analysis of Variance (ANOVA) to determine if there was a tank effect and any differences in cortisol among treatment groups for each time period. To determine if cortisol levels differed between pre- to post- surgical periods, the difference in plasma cortisol levels from each individual fish within the treatment groups during those two time periods were analyzed using ANOVA. For all comparisons, $p < 0.05$ was considered significant.

RESULTS

All surgical and anesthetic procedures were performed without any major complications. Seven days after surgery, one fish was euthanized because the transmitter was wedged between the posterior aspect of the large intestine and the vent (Figure 3.5). The position of this transmitter caused a tear of the vent, and the animal was considered to be septic and likely suffering. In contrast to other fish with a tear of the vent from suspected transmitter expulsion, this particular fish was noted to be swimming vertically and erratically, and gulping air. Approximately six months after the transmitter implantation, one fish was euthanized because of

severe spinal curvature and inability to right itself and feed. Necropsy revealed a severely curved spine, but the exact cause could not be determined. However, the cause did not seem to be related to the intracoelomic transmitter presence.

The sturgeon were evaluated for any signs of morbidity that may have been caused from transmitter expulsion. During the weekly visual evaluations, seven (11.7%) fish with transmitters exhibited inflamed and enlarged perianal areas, four (6.7%) exhibited vent tears, and four (6.7%) exhibited transmitter lodgment at the vent. Perianal inflammation or vent tears were not noted in the control fish during the weekly evaluations.

Gross necropsy examination of all transmitter fish at week 55 revealed that 52% of the incisions were completely healed and back to a pre-surgery state. However, grade 1 adhesions were noted in 31%, grade 2 adhesions were noted in 41%, and grade 3 adhesions were noted in 20% of the sturgeon (Figure 3.6). Grade 4 adhesions were not documented. These adhesions extended from the surgical site to the coelomic wall and/or liver, spleen, gonads, and small and large intestines. The adhesions did not appear to cause any intestinal obstructions. Including the unidentifiable fish, 94.6% of transmitters were found to be free-floating within the coelomic cavity (Grade 1), and 2.7% of transmitters were partially encapsulated by fibrous tissue and adhered to surrounding organs (Grade 3). One transmitter (2.7%) was found in the posterior lumen of the large intestine (Figure 3.7). Multilobular masses on the body wall and organs were noted in one fish with a transmitter. The masses were confirmed by histopathologic examination to be inflamed mesenteric adipose tissue (Figure 3.8). Inflamed and enlarged vents were noted in 12.1% of fish with intracoelomic transmitters. Transmitters were expelled from 36.2% of the sturgeon within the first 12 weeks after the implantation surgery.

Histopathological examination of the tissues from the surgical incision revealed that all incisions had healed and returned to pre-surgery state. Most incision sites retained residual proliferative mesothelial (lubricating) surfaces with evidence of inflammation that was most prominent where adhesions had formed. The most common pathological change was the formation of adhesions, but there were no signs of organ damage associated with the adhesions. Microscopically, the presence of the transmitter caused mesothelial proliferation and inflammation in the posterior coelomic cavity lateral to the vent. Microscopic evaluation of the extensive multilobular masses revealed numerous macrophages and proliferation of large, multinucleated giant cells (cells indicative of a foreign body). However, evidence of bacterial infection was not noted in this fish.

Statistical analyses revealed that the tank blocks did not have an effect on plasma cortisol levels; therefore, the tank blocks were not used in the final analysis. Plasma cortisol levels among the four suture groups during the pre- and post- surgical periods were not significantly different (Figure 3.9). When the pre-surgery and post-surgery differences were compared, the mean of the post-surgical plasma cortisol level of the Monocryl suture group was significantly higher than the pre-surgical level ($0.8 \pm 1.31 \mu\text{g/dL}$; $p=0.04$) compared to the mean plasma cortisol levels of the Monocryl-Plus group ($-1.04 \pm 1.08 \mu\text{g/dL}$), Vicryl group ($-0.85 \pm 1.04 \mu\text{g/dL}$), and the Prolene group ($-1.01 \pm 1.01 \mu\text{g/dL}$). However, the Monocryl suture group mean plasma cortisol levels were not significantly different from the control group ($-0.24 \pm 1.62 \mu\text{g/dL}$) (Figure 3.10).

All control and suture groups grew in length and gained weight over the 55-week period. Using the identified fish, the transmitter group grew in length an average of 5.8cm and had an average weight gain of 575.5g, while the control group grew an average length of 6.3cm and

gained an average of 575.1g. A significant difference between the control and suture groups was not noted.

DISCUSSION

Successful understanding of fish movement relies on the assumption that transmitters are retained for the duration of the study, and will not interfere with the fish's normal biology. The transmitter loss rate for Siberian sturgeon in this study was 36.2%. In other species, coelomic transmitter expulsion is not uncommon. For example, juvenile Atlantic salmon *Salmo salar* expelled 20% of intracoelomic acoustic tags through the body wall after five months of surgery (Moore et al. 1990), channel catfish *Ictalurus punctatus* expelled up to 89% of intraperitoneally implanted transmitters within 117 days (Summerfelt and Mosier 1984), and common carp *Cyprinus carpio* expelled 50% of dummy transmitters up to 362 days after surgery (Daniel et al. 2009). However, the loss rate noted in our study is much higher than the previously rate of 0% for similar species such as shortnose sturgeon *Acipenser brevirostrum* (Collins et al. 2002) and shovelnose sturgeon *Scaphirhynchus platorhynchus* (Neely and Steffensen 2009). Collins et al. (2002) used transmitters ranging from 1.2 to 3.6%, and Neely and Steffensen (2009) used transmitters less than 2% of the weight of the fish during the 93-day and 35-day studies, respectively. Using a transmitter that is 2% or less of the weight of the fish is important as heavier transmitters may affect the fish's behavior, or impede activities, such as swimming or escaping predators (Brown et al. 1999). Larger internal transmitters may place pressure on the incision leading to pressure necrosis at the surgical site and may contribute to eventual transmitter expulsion (Brown et al. 1999). The discrepancies between the results of the current study and past experiments may include the length of the studies, species of interest, suture materials used during coelomic closure, or surgical techniques. For example, Neely et al. (2009)

only evaluated intracoelomic transmitter implantation for 35 days and used braided silk for surgical closure of the incision. Collins et al. (2002) evaluated transmitter retention for approximately the same amount of time that the transmitters were lost in the current study. However, that study used an off-midline incision compared to a midline incision in the current study, and the study did not note any adhesions, encapsulation, or other tissue damage from intracoelomic transmitter implantation during necropsy.

Common reasons proposed for transmitter expulsion through the incision site include surgical site infections (Daniel et al. 2009), internal irritation (Lucas 1989), pressure necrosis (Knights and Lasee 1996; Lucas 1989), and water temperature that presumably interferes with the immune function of the fish (Daniel et al. 2009). Through surgical techniques, we took all potential precautions to avoid surgical site infections and indeed histopathologic analysis failed to detect any evidence of infection. Transmitter expulsion typically occurs when the transmitter becomes encapsulated within fibrous tissue and then migrates through the incision, abdominal body wall, or the intestines (Summerfelt and Mosier 1984). However, none of the transmitters were completely encapsulated with fibrous tissue; therefore if expulsion occurred, it may have occurred through to another mechanism.

Transmitters could have been lost one of two ways: through the incision as previously described in Boone et al. (2011) or through the mucosal junction of the large intestine and the vent. Multiple transmitters appeared to have been expelled through the mucosal junction of the large intestine and the vent (Figure 3.11). Migration of the transmitter caudally may have caused the transmitter to become lodged at this site, eventually tearing through the large intestine mucosa and skin epithelium. The tissues of the vent, large intestine, and caudal coelomic wall had mesothelial proliferation and inflammation, further supporting that transmitters were lodged

in this region and irritating these structures. The continual irritation may have exacerbated the inflammatory process and led to tissue necrosis. The tissue necrosis eventually may have led to perforation of the tissue and transmitter expulsion. Although determining previous sites of transmitter perforation and tissue necrosis was difficult, as most were healed when histologically evaluated, a high correlation of transmitter expulsion through the vent seemed likely based on necropsy findings and the position of the transmitter.

Interestingly, a transmitter was found within the large intestine in one fish.

Transintestinal expulsion is a rare occurrence that has been documented in rainbow trout *Oncorhynchus mykiss* and catfishes (Chisholm and Hubert 1985; Summerfelt and Mosier 1984), but has not been previously reported in sturgeon. Although the transmitter was not expelled at the time of the gross necropsy, it probably would have been expelled shortly thereafter. Even though a swollen and inflamed perianal area and vent has been associated with transintestinal expulsion of transmitters in catfish (Summerfelt and Mosier 1984), transmitter expulsion in the sturgeon in this study more likely occurred because of pressure necrosis at the large intestine-vent junction. Transmitter expulsion at this location was supported by the swollen and inflamed perianal area and vent noted in multiple fish during the weekly evaluations. Histologically, there was moderate to severe villus proliferation, edema, and inflammation associated with the serosa of the large intestine suggesting that the transmitter was acting as an irritant within the lumen of the large intestine.

Overall, the adverse effects of surgically implanted transmitters seemed to be mild and did not cause any organ damage as evidenced by microscopic evaluation of tissues. Fibrous encapsulation of transmitters was not noted. However, minor and moderate grades of adhesions were noted at necropsy. Adhesions are common surgical complications that may result from

local trauma and constant irritation from the transmitter (Gray et al. 2005). In addition, adhesions can potentially infiltrate organ tissue and impede normal movement of organs (Gray et al. 2005). Interestingly, 62% of fish with retained transmitters and 67% of fish that previously expelled the transmitter had adhesions at necropsy, respectively. These results may suggest that the transmitter-induced inflammatory reactions led to the adhesions during the 12 weeks after surgery. In one fish, multifocal, round, firm masses of giant cells were noted. Giant cell masses consist mostly of macrophages that are generated in response to the presence of a foreign body or infections, which can incite a chronic inflammatory reaction. Although why this particular fish developed such an extreme reaction to the transmitter is unclear, this study provides evidence that transmitters incite inflammatory reactions consistent with foreign bodies within the coelomic cavity. Although evidence of adhesions from the body wall to the internal organs in some fish with transmitters was noted, there was no evidence of organ damage, even when sturgeon retained the transmitter for the entire 55 weeks.

Cortisol is the most abundant corticosteroid in fish, and an elevation of blood cortisol has been related to a stress response (Van Der Boon et al. 1991). Although the pre- to post--surgery mean plasma cortisol levels for the Monocryl group were significantly increased compared to the other suture groups, there was no difference between the Monocryl group and the control group. When the control group was compared to the other three suture groups, differences did not exist either. The results suggest that there is not a true biological significance when the mean plasma cortisol differences were compared among the control and suture groups. The results further suggest that the long-term presence of intracoelomic transmitters does not appear to be chronically stressful to sturgeon. Plasma cortisol variations are known to be caused by genetic, developmental, environmental, and repeated stressor factors (Barton 2002). Normal variations of

plasma cortisol elevations have been described in multiple fish species when exposed to acute stressors (Barton 2002). In the current study, fish were handled eight times over the 55-week study. This repeated handling may have desensitized the fish and attenuated their neuroendocrine responses to this acute stressor. Another possibility for elevated post-surgery plasma cortisol levels may be normal temporal variations of this species. The results from this study with sturgeon are further supported by previous studies that conclude short- and long-term transmitter implantation is not stressful to fish. For example, Chinook salmon smolts and largemouth bass did not exhibit any long-term elevations of plasma cortisol with a gastrically-inserted transmitter or intracoelomic transmitter (Caputo et al. 2009; Jepsen et al. 2001).

Intracoelomic transmitter implantations did not negatively affect the sturgeon's growth or feeding behavior. Both control and transmitter fish grew and gained weight at a similar rate throughout the study period. These results are similar to results in other fish species. Lucas (1989) also did not find significant differences in mortality or growth in rainbow trout *Oncorhynchus mykiss* with intracoelomic transmitters over a seven month period.

Tagging allows for monitoring of growth, migration, and population-level parameters over time during mark-recapture studies. However, premature tag shedding may complicate these studies by decreasing sample size, and creating bias for density and mortality estimates (Buzby and Deegan 1999). The fundamental principles of a mark-recapture study assume mortality is equal for tagged and un-tagged fish, tagging does not influence growth or alter behavior, tags must be recognizable and readable, and tags are never lost nor shed. Passive integrated transponder (PIT) tags have been reported in multiple sturgeon species as having a retention rate between 51 and 100% (Clugston 1996; DeHann et al. 2008; Hamel et al. 2011; Musick and Hager 2007; Nelson et al. 2004; Smith et al. 1990). Sturgeon in the current study

retained 96% of PIT tags during the six months after surgery and 68% of the PIT tags from 6 to 12 months. The retention rate of PIT tags in our study was similar to the previously reported retention rates. To distinguish between failure and expulsion, all sturgeon with PIT tags that could not be read by the scanner were radiographed and rechecked with another scanner. Two PIT tags were found on radiographs which suggest the tags failed to respond to the automatic reader. The other 22 tags were not found, which suggest the tags were expelled. Most PIT tag failures occurred between the 26 and 55-week evaluation period and suggest that they were rejected in a similar manner as a foreign body. The tags may have been expelled sooner if they were initially improperly placed (Clugston 1996). In the current study, 79.3% of the T-bar anchor FLOY tags were retained during the six months after surgery, and 30.7% between 6 to 12 months. In multiple sturgeon species, 60-100% of T-anchor bar tags were typically retained during the study period. (Clugston 1996; Collins et al. 1994; Hamel et al. 2011). Possible reasons for a lower retention rate seen in this study include improper placement, injection of tag into the muscle instead of the interneural bones, or tag migration (Figure 3.12). Furthermore, the inability to properly identify the fish may have falsely altered the transmitter retention rates for the suture treatment groups.

The results of our study demonstrate that cylindrical transmitters with a transmitter to body weight ratio of 2% or less appeared to have a high expulsion rates in Siberian sturgeon when compared to other sturgeon species. This high rate of transmitter loss could affect the way researchers plan for telemetry studies, especially when dealing with endangered or threatened species. Given our observations, we believe that the transmitters were expelled at the junction of the large intestine and the vent. We believe that the cylindrical shape of the transmitter facilitated its caudal migration and perforation at this site and recommend that a different transmitter shape

or volume might prevent this complication. Furthermore, based on our findings and in keeping with general surgical guidelines, we believe multifilament sutures, such as Vicryl, should not be used for surgical closure because of the higher rate of wound dehiscence of surgical incisions.

Although adhesions were a common finding during the gross examinations, they did not appear to interfere with normal organ physiology or adversely affect organ function.

Transmitters did not appear to have long-term effects on plasma cortisol levels, feeding behavior, or growth rates. Although long-term telemetry studies can be undertaken with minimal concern for negative physiological or pathological effects from transmitters, researchers should be aware of the transmitter loss rates seen in the current study and plan accordingly.

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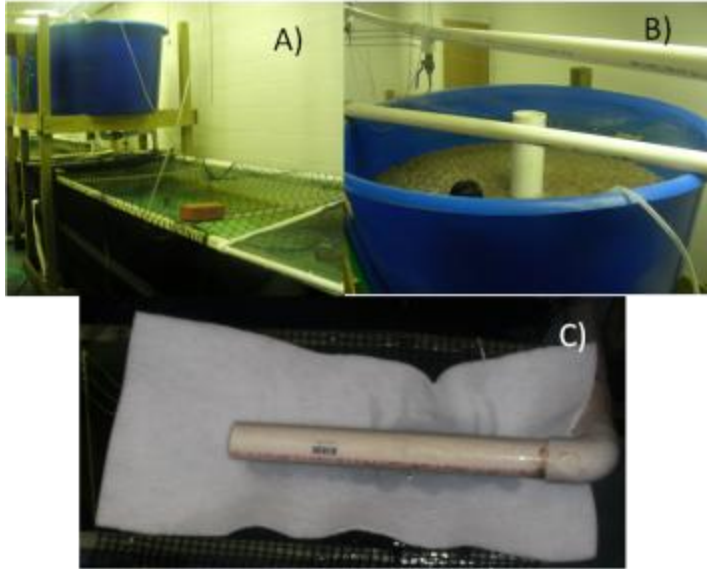


Figure 3.1 The semi-recirculating life support system used during the evaluation of physiological and pathological effects of intracoelomic transmitters in Siberian sturgeon. A) The rectangular tank used to hold the fish. B) The biological filter for the life support system. C) The mechanical filter used for the life support system. Data was collected from January 2010 to January 2011.



Figure 3.2 The re-circulating anesthesia delivery system used during surgery for the evaluation of physiological and pathological effects of intracoelomic transmitters in Siberian sturgeon. Data was collected in January 2010.

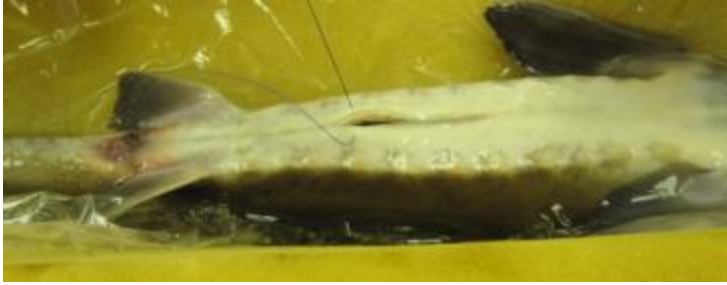


Figure 3.3 The 4-5cm incision made during surgery for the evaluation of physiological and pathological effects of intracoelomic transmitters in Siberian sturgeon. Data was collected in January 2010.



Figure 3.4 Intracoelomic transmitter implantation through the 4-5cm incision during surgery for the evaluation of physiological and pathological effects of intracoelomic transmitters in Siberian sturgeon. Data was collected in January 2010.



Figure 3.5 Wedging of a transmitter between the posterior aspect of the large intestine and the vent occurring 7 days after intracoelomic transmitter implantation. The wedging of the transmitter caused a tear in the tissues and caused the fish to become presumably septic. Data was collected in January 2010.

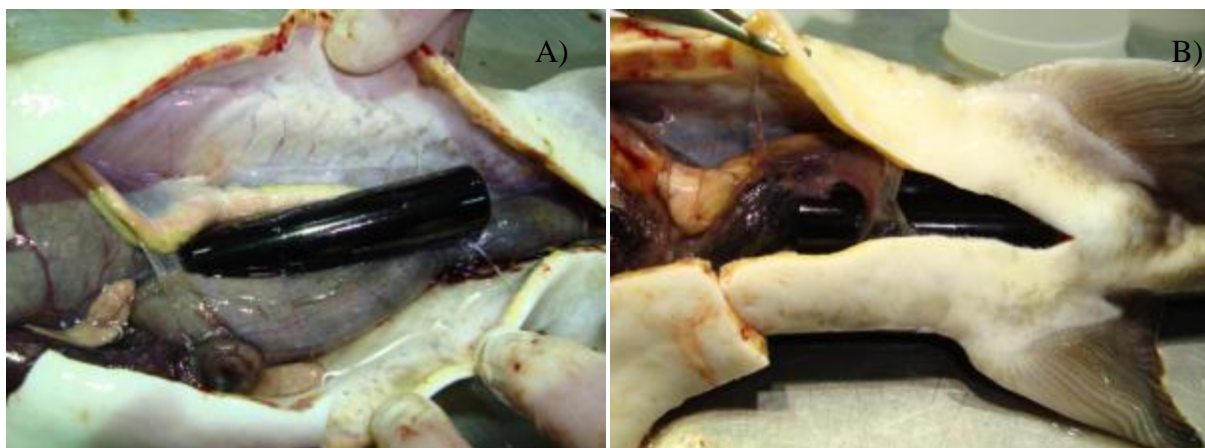


Figure 3.6 Adhesion classification used during the 55-week necropsy for the evaluation of pathological effects of intracoelomic transmitters in Siberian sturgeon. Data was collected in January 2011. A) Minor adhesions within the coelomic cavity at necropsy during the week-55 evaluations. B) Moderate adhesions within the coelomic cavity at necropsy during the week-55 evaluations.



Figure 3.7 Transmitter within the lumen of the large intestine during the 55-week necropsy for the evaluation of pathological effects of intracoelomic transmitters in Siberian sturgeon. Data was collected in January 2011.



Figure 3.8 Mesenteric fat necrosis within the coelomic cavity during the 55-week necropsy for the evaluation of pathological effects of intracoelomic transmitters in Siberian sturgeon. Data was collected in January 2011.

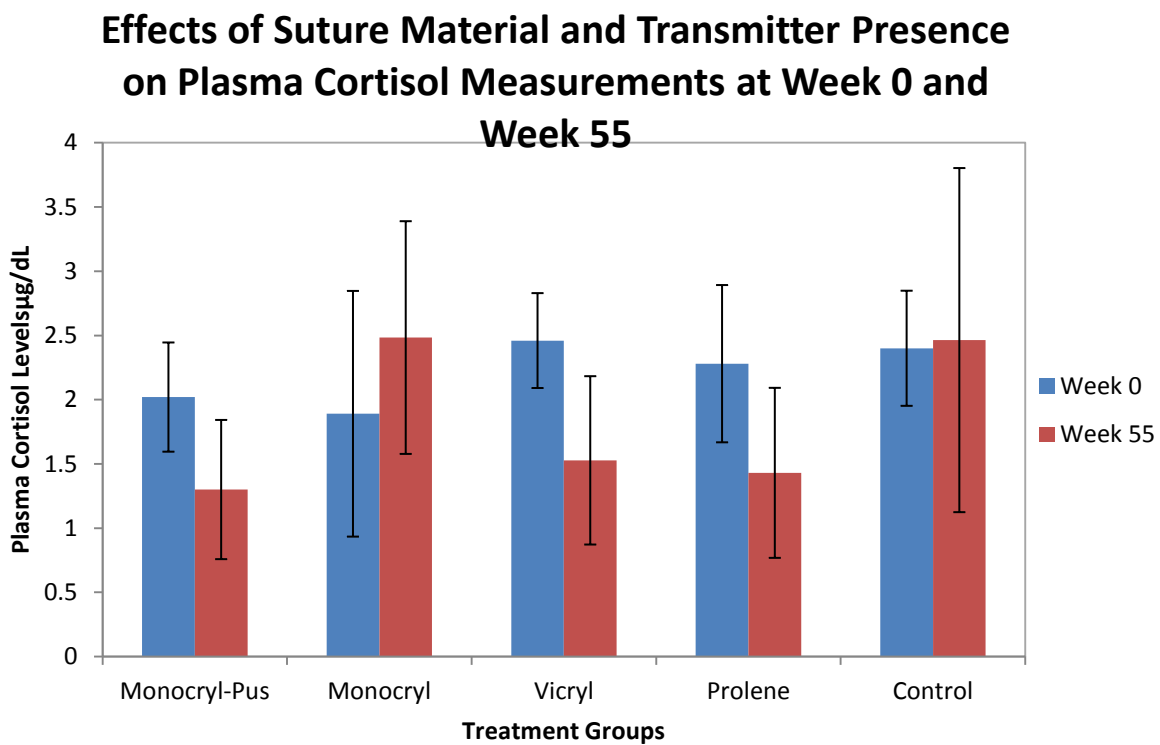


Figure 3.9 Plasma cortisol levels for all suture groups at the pre-surgery (week 0), and post-surgery (week55) evaluations during the evaluation of physiological effects of intracoelomic transmitters in Siberian sturgeon. Each bar represents the mean \pm 95% confidence interval for plasma cortisol levels during the week 0 or week 55 samples for each treatment group. If significant differences occurred between suture groups, a *p*-value is indicated. Data was collected from January 2010 to January 2011.

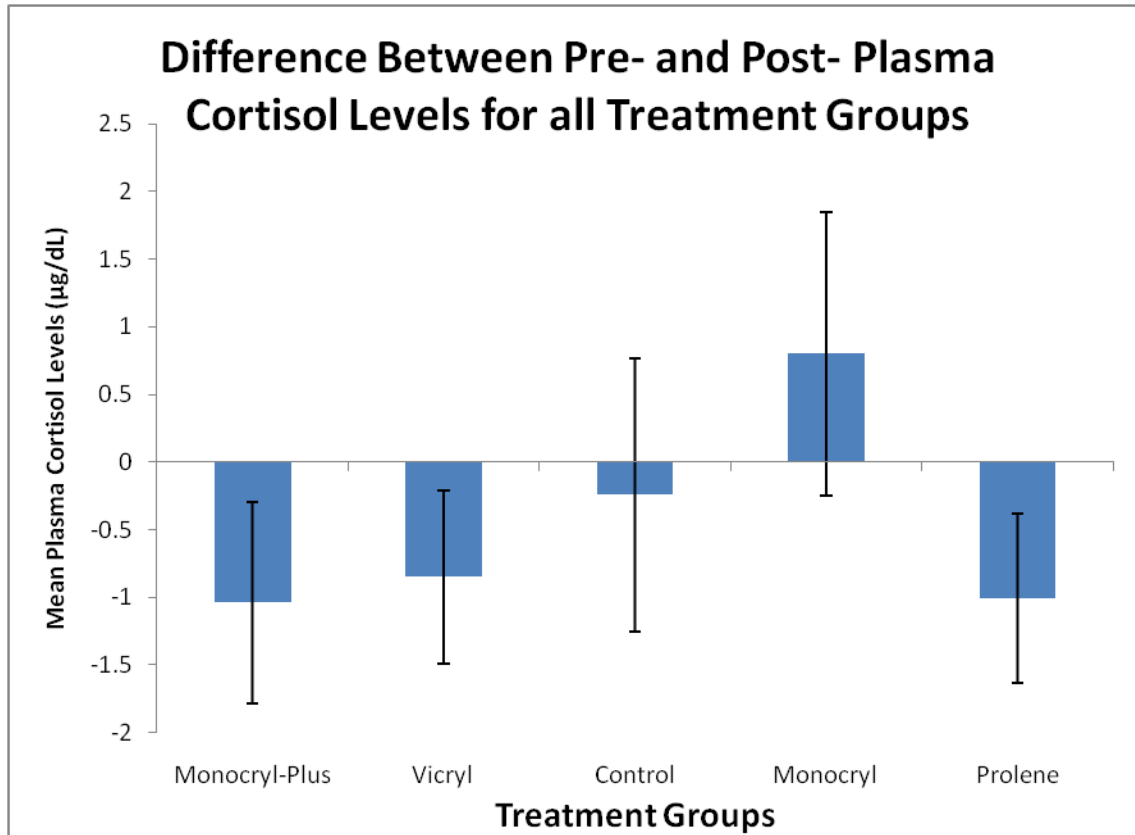


Figure 3.10 Mean plasma cortisol levels for all suture groups when the differences in the pre- and post- surgical cortisol levels were compared during the evaluation of physiological effects of intracoelomic transmitters in Siberian sturgeon. Each bar represents the mean \pm 95% confidence interval for plasma cortisol levels for each treatment group. If significant differences occurred between suture treatment groups, a *p*-value is indicated. Data was collected from January 2010 to January 2011.



Figure 3.11 Tear of the vent epithelium and large intestine mucosa seen during the initial 12 weeks after surgery when evaluating the pathological effects of intracoelomic transmitters in Siberian sturgeon. The black oval surrounds the tear in the vent at the large intestine-vent junction and is a proposed site for transmitter expulsion in Siberian sturgeon. Data was collected from January 2010 to January 2011.

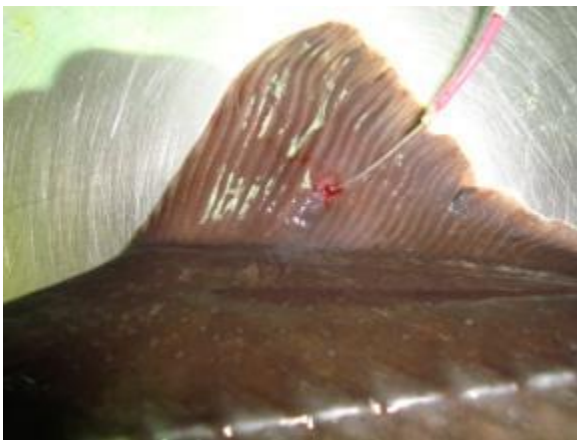


Figure 3.12 T-bar anchor tag within the dorsal fin of a Siberian sturgeon during the week-55 evaluations when evaluating the pathological and physiological effects of intracoelomic transmitters in Siberian sturgeon. The T-bar anchor tag appears to have migrated dorsally from the base of the dorsal fin where it was initially implanted. Data was collected from January 2010 to January 2011.

CHAPTER 4

CONCLUSIONS

Intracoelomic transmitter implantation is commonly used to evaluate migratory patterns, distribution, and habitat use of many species of fish. Currently, transmitter implantation relies mostly on the assumption that the transmitters do not cause any adverse physiological or pathological effects on the animal. The evaluation of different suture materials for surgical closure after intracoelomic transmitter implantation is also important, as these materials also play a critical role in long-term transmitter retention. However, there is not a clear consensus about the long-term physiological or pathological effects from intracoelomic transmitters, nor about the preferred suture material for coelomic closure after intracoelomic transmitter implantation.

To address the controversy regarding suture materials for coelomic closure in fish, four different types of suture materials were used for intracoelomic transmitter implantation in Siberian sturgeon *Acipenser baerii*. The first objective of this study was to measure and evaluate macroscopic and microscopic tissue reactions to an absorbable monofilament poliglecaprone 25 (Monocryl), antibiotic-impregnated absorbable monofilament poliglecaprone 25 (Monocryl-Plus), absorbable multifilament polyglactin 910 (Vicryl), and non-absorbable monofilament polypropylene (Prolene) when used for coelomic closure in sturgeon with and without sonic transmitter implantation. The second objective was to determine if surgically implanting sonic transmitters into the coelomic cavity of Siberian sturgeon resulted in negative overall health or pathologic effects during a 12 month period. The third objective was to evaluate transmitter retention rates over a 55-week period.

Surgical wounds closed with Vicryl or Prolene exhibited prolonged healing times or more incision erythema. Surgical wounds closed with Vicryl also exhibited decreased suture retention during the 12-week experiment. Inversion of the surgical site was noted during the first week of evaluations in most fish. The epithelium had completely grown over the apposed edges of the incision and reflected onto the peritoneum by the evaluations at the second week. The statistical analysis of the histological data did not reveal significant differences in inflammation, fibrosis, necrosis, or hemorrhage between the suture groups and control group. Microscopic evaluations closely resembled the gross findings regarding the progression of healing.

Transmitters were expelled in 36.2% of the sturgeon during the 55-week study. Multiple transmitters appeared to have been expelled through the mucosal junction of the large intestine and the vent. However, several fish exhibited severe wound dehiscence which may have also allowed transmitters to be expelled. The Vicryl suture group lost more transmitters compared to the other three suture groups over the course of the experiment. The most common pathological change was adhesion formation, but there were not any signs of organ damage associated with the adhesions. Intracoelomic transmitter implantation did not appear to have negative effects on growth or plasma cortisol levels.

The current study indicates that either the absorbable monofilament Monocryl or absorbable monofilament triclosan-coated Monocryl-Plus should be used for intracoelomic transmitter implantation in Siberian sturgeon or closely related species. Furthermore, we believe either Monocryl or Monocryl-Plus may decrease the risk of transmitter expulsion through the incision as surgical wounds appear to heal faster and exhibit less erythema compared to Vicryl or Prolene. Further work is needed to evaluate surgical patterns and suture materials that may limit

inversion of the incision, and to determine if volume or shape of a transmitter may decrease the risk of expulsion.