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Anesthetic Effects and Physiological Responses of Lidocaine-HCl in Siberian Sturgeon, *Acipenser baerii*

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ABSTRACT

Anesthetic Effects and Physiological Responses of Lidocaine-HCl in Siberian Sturgeon, *Acipenser baerii*

by

In Bon GOO

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Department of Marine Bioscience and Environment

Graduate School of

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(Supervised by In-Seok PARK, Ph. D.)

The objective of this study is to determine the optimal dose of lidocaine-HCl for anesthetizing Siberian sturgeon, *Acipenser baerii*, investigate the relationship between anesthetic effectiveness and fish size, and to analyze re-anesthetic effects and stress responses to lidocaine-HCl use. The concentration of the anesthetic and fish body size significantly affected the anesthesia and recovery times. Anesthesia

time was markedly decreased as both the lidocaine-HCl concentration and body size increased (P<0.05), while recovery time decreased as the lidocaine-HCl concentration increased (P<0.05). Anesthesia time and recovery time were decreased significantly as the lidocaine-HCl concentration and water temperature increased (P<0.05). Plasma cortisol, plasma glucose, and lactic acid concentrations were indicative of stress reactions. At 1-, 2-, and 3-day intervals, the anesthesia and recovery times increased significantly as the number of anesthesia treatments increased (P<0.05) but were not substbatilaly different between duplicate and triplicate treatments (P>0.05). In 4-day interval groups, anesthesia and recovery times were hardly different (P>0.05) among the initial, duplicate, and triplicate treatments. The second anesthesia treatment (P<0.05) increased anesthesia and recovery times. As the number of anesthesia treatments increased (P<0.05) anesthesia time decreased significantly, but recovery times hardly differed significantly with the increase in number of anesthesia treatments (P>0.05). Lidocaine-HCl concentrations of 50 and 200 ppm in the larval and juvenile groups, respectively, showed an optimal anesthesia time of approximately 1 minute. The optimal anesthesia interval of lidocaine-HCl was 4 days, and frequent anesthesia resulted in negative effects by inhibiting sensitivity.

Key words: Anesthetic effect, Lidocaine-HCl, Siberian sturgeon (*Acipenser baerii*), Stress response

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KOREAN ABSTRACT

(국문요약)

염산리도카인에 대한 시베리안 철갑상어, *Acipenser Baerii*의 마취효과 및 생리반응

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본 연구는 시베리안 철갑상어, *Acipenser baerii*를 마취하기 위한 염산리도카인(lidocaine-HCl)의 적정 농도를 구명하고, 어체 크기와 마취효과 사이의 상관관계, 재마취 효과 및 스트레스 반응을 조사하였다. 마취 및 회복 시간은 마취농도와 어체 크기에 따라 유의한 영향을 받았다. 마취시간은 염산리도카인의 농도 및 어체의 크기가 클수록 유의하게 감소하였으며($P<0.05$), 회복시간은 염산리도카인의 농도가 증가할수록 감소하였다($P<0.05$). 마취시간 및 회복시간은 염산리도카인의 농도 및 수온이 증가함에 따라 유의하게 감소하였다($P<0.05$). 혈장

코티솔(plasma cortisol), 혈장 글루코스(plasma glucose) 및 젖산(lactic acid)의 농도를 스트레스 반응의 지표로 활용하였다. 1, 2 및 3 일 간격으로 재마취 시, 마취 및 회복시간은 마취 횟수가 증가함에 따라 유의하게 증가 하였으나(P<0.05), 2 반복 및 3 반복 실험군에서는 유의한 차이가 나타나지 않았다(P>0.05). 4 일 간격의 실험군에서, 마취 및 회복시간은 초기, 2 반복 및 3 반복 처리군과는 유의한 차이가 없었다(P>0.05). 마취 및 회복시간은 재마취시 유의하게 증가하였다(P<0.05). 마취 시간은 마취 횟수가 증가함에 따라 유의하게 감소하였으나(P<0.05), 회복 시간은 마취 횟수의 증가와 유의한 차이가 없었다(P>0.05). 염산리도카인 농도 50 및 200 ppm 에 대한 자어 및 치어의 최적 염산리도카인의 마취 시간은 약 1 분으로 나타났다. 염산리도카인의 최적 마취 간격은 4 일 이었으며, 빈번한 마취는 마취 감수성을 억제함으로서 마취시 부정적인 영향을 초래하였다.

주제어: 마취효과, 스트레스 반응, 시베리안 철갑상어(*Acipenser baerii*), 염산리도카인

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I. General information

1. Sturgeon

1.1. Summary

Sturgeon is the common name for the 27 species of fish belonging to the family Acipenseridae (Choudhury & Dick, 2001). Their evolution dates back to the Triassic some 245 to 208 million years ago (Birstein et al., 1997). The family is grouped into four genera: *Acipenser, Huso, Scaphirhynchus* and *Pseudoscaphirhynchus*. Four species may now be extinct (Chadwick et al., 2010). Two closely related species, American paddlefish, *Polyodon spathula* and Chinese paddlefish, *Psephurus gladius* are of the same order, Acipenseriformes, but are in the family Polyodontidae and are not considered to be true sturgeons. Both sturgeons and paddlefish have been referred to as "primitive fishes" because their morphological characteristics have remained relatively unchanged since the earliest fossil record (Chesapeake Bay Field Office, 2011). Sturgeons are native to subtropical, temperate and sub-Arctic rivers, lakes and coastlines of Eurasia and North America (Birstein et al., 1997).

Sturgeons are long-lived, late-maturing fishes with distinctive characteristics, such as a heterocercal caudal fin similar to that of sharks, and an elongated spindle-like body that is smooth-skinned, scaleless and armored with 5 lateral rows of bony plates called scutes (Sturgeon web, 2000). Several species can grow quite large, typically ranging 2 – 3.5 m in length. The

largest sturgeon on record was a Beluga female captured in the Volga estuary in 1827, weighing 1,571 kg and 7.2 m in length (Sturgeon web, 2000). Most sturgeons are anadromous bottom-feeders which migrate upstream to spawn but spend most of their lives feeding in river deltas and estuaries (Sturgeon web, 2000). Some species inhabit freshwater environments exclusively while others primarily inhabit marine environments near coastal areas, and are known to venture into open ocean (Berg, 1962). Several species of sturgeon are harvested for their roe which is processed into the luxury food caviar. This has led to serious overexploitation, which combined with other conservation threats that brought most of the species to critically endangered status, at the edge of extinction (Nelson, 1994; WSCS, 2019).

1.2. Physical characteristics

Sturgeons retain several primitive characters among the bony fishes. Along with other members of the subclass Chondrostei, they are unique among bony fishes because the skeleton is almost entirely cartilaginous (Choudhury & Dick, 2001). Notably, however, the cartilagineous skeleton is not a primitive character, but a derived one: sturgeon ancestors had bony skeletons (Caleb, 1994; McPhail, 2007; Gene et al., 2009). They also lack vertebral centra, and are partially covered with 5 lateral rows of bony plates called scutes rather than scales (Gene et al., 2009). They also have four barbels—sensory organs that precede their wide, toothless mouths (Zhang et al., 2012). They navigate their riverine habitats traveling just off the bottom

with their barbels dragging along gravel, or murky substrate (Zhang et al., 2012). Sturgeons are recognizable for their flattened rostra, elongated bodies, distinctive scutes and barbels, and elongated upper tail lobes (Zhang et al., 2012). The skeletal support for the paired fins of ray-finned fish is inside the body wall, although the ray-like structures in the webbing of the fins can be seen externally (Zhang et al., 2012).

Sturgeons are among the largest fish: some beluga, *Huso huso* in the Caspian Sea reportedly attain over 5.5 m and 2,000 kg (Frimodt, 1995) while for kaluga, *H. dauricus* in the Amur River, similar lengths and over 1,000 kg weights have been reported (Krykhtin & Svirskii, 1997). They are also among the longest-lived of the fishes, some living well over 100 years and attaining sexual maturity at 20 years or more (Berg, 1962). The combination of slow growth and reproductive rates and the extremely high value placed on mature, egg-bearing females make sturgeon particularly vulnerable to overfishing (IUCN, 2010).

1.3. Life cycle

Sturgeons are long-lived, late maturing fishes. Their average lifespan is 50 to 60 years, and their first spawn does not occur until they are around 15 to 20 years old (Fontana et al., 2001). Sturgeons are broadcast spawners, and do not spawn every year because they require specific conditions. Those requirements may or may not be met every year due to varying environmental conditions, such as the proper photoperiod in Spring, clear

water with shallow rock or gravel substrate where the eggs can adhere, and proper water temperature and flow for oxygenation of the eggs (Sturgeon web, 2000). A single female may release 100,000 to 3,000,000 eggs but not all will be fertilized. The fertilized eggs become sticky and will adhere to the bottom substrate upon contact (Sturgeon web, 2000). It takes 8–15 days for the embryos to mature into larval fish. During that time, they are dependent on their yolk sac for nourishment (HSBS, 2015). River currents carry the larvae downstream into backwater areas, such as oxbows and sloughs where the free-swimming fry will spend their first year feeding on insect larvae and crustacea (Howkins, 1980). During their first year of growth, they will reach 18 to 20 cm in length and migrate back into the swift-flowing currents in the main stem river (Howkins, 1980).

1.4. Range and habitat

Sturgeon range is from subtropical to subarctic waters in North America and Eurasia. In North America, they range along the Atlantic Coast from the Gulf of Mexico to Newfoundland, including the Great Lakes and the St. Lawrence, Missouri, and Mississippi Rivers, as well as along the West Coast in major rivers from California and Idaho to British Columbia (Sturgeon web, 2000). They occur along the European Atlantic coast, including the Mediterranean basin, especially in the Adriatic Sea and the rivers of North Italy; in the rivers that flow into the Black, Azov, and Caspian Seas (Danube, Dnepr, Volga, Ural and Don); the north-flowing rivers of Russia that feed the

Arctic Ocean (Ob, Yenisei, Lena and Kolyma); in the rivers of Central Asia (Amu Darya and Syr Darya) and Lake Baikal (Nelson, 1994). In the Pacific Ocean, they are found in the Amur River along the Russian-Chinese border, on Sakhalin Island, and some rivers in northeast China (Berg, 1962; Nelson, 1994).

Throughout this extensive range, almost all species are highly threatened or vulnerable to extinction due to a combination of habitat destruction, overfishing, and pollution (Nelson, 1994). No species are known to naturally occur south of the equator, though attempts at sturgeon aquaculture are being made in Uruguay, South Africa, and other places (Burtzev, 1999).

Most species are at least partially anadromous, spawning in fresh water and feeding in nutrient-rich, brackish waters of estuaries or undergoing significant migrations along coastlines (Nelson, 1994). However, some species have evolved into purely freshwater existences, such as the lake sturgeon, *Acipenser fulvescens* and the Baikal sturgeon *A. baerii baicalensis*, or have been forced into them by anthropogenic or natural impoundment of their native rivers, as in the case of some subpopulations of white sturgeon, *A. transmontanus* in the Columbia River (Duke et al., 1999) and Siberian sturgeon, *A. baerii* in the Ob basin (Ruban, 1999).

1.5. Behavior

Sturgeons are primarily benthic feeders, with a diet of shells, crustaceans and small fish. Exceptionally, both *Huso* species, the white sturgeon, and the

pallid sturgeon feed primarily on other fish as adults (Nelson, 1994). They feed by extending their syphon-like mouths to suck food from the benthos. Having no teeth, they are unable to seize prey, though larger individuals and more predatory species can swallow very large prey items, including whole salmon (Zolotukhin & Kaplanova, 2007). Sturgeons feed non-visually. They are believed to use a combination of sensors, including olfactory, tactile and chemosensory cues detected by the four barbels, and electroreception using their ampullae of Lorenzini (Zhang et al., 2012).

The sturgeons' electroreceptors are located on the head and are sensitive to weak electric fields generated by other animals or geoelectric sources (Herzog, 2011). The electroreceptors are thought to be used in various behaviors such as feeding, mating and migration (Zhang et al., 2012).

Many sturgeons leap completely out of the water, usually making a loud splash which can be heard half a mile away on the surface and probably further under water (Sulak et al., 2002). It is not known why they do this, but suggested functions include group communication to maintain group cohesion, catching airborne prey, courtship display, or to help shed eggs during spawning (Sulak et al., 2002). Other plausible explanations include escape from predators, shedding parasites, or to gulping or expelling air (Sulak et al., 2002). Another explanation is that it "simply feels good" (Waldman, 2001). There have been some incidents of leaping sturgeon landing in boats, and causing injuries to humans (Wilson et al., 2009).

1.6. Fossil history

Acipenseriform fishes appeared in the fossil record some 245 to 208 million years ago near the end of the Triassic, making them among the most ancient of still-living actinopterygian fishes **(**Gardiner, 1984). True sturgeons appear in the fossil record during the Upper Cretaceous. In that time, sturgeons have undergone remarkably little morphological change, indicating that their evolution has been exceptionally slow and earning them informal status as living fossils (Gardiner, 1984; Krieger & Fuerst, 2002). This can be explained in part by the long generation interval, tolerance for wide ranges of temperature and salinity, lack of predators due to size and bony plated armor, or scutes, and the abundance of prey items in the benthic environment **(**Gardiner, 1984). Although their evolution has been remarkably slow, they are a highly evolved living fossil, and do not closely resemble their ancestral chondrosteans **(**Gardiner, 1984). They do however still share several primitive characteristics, such as heterocercal tail, reduced squamation, more fin rays than supporting bony elements, and unique jaw suspension (Gene et al., 2009).

1.7. Phylogeny and taxonomy

Despite the existence of a fossil record, full classification and phylogeny of the sturgeon species has been difficult to determine, in part due to the high individual and ontogenic variation, including geographical clines in certain features, such as rostrum shape, number of scutes and body length (Gene et

al., 2009). A further confounding factor is the peculiar ability of sturgeons to produce reproductively viable hybrids, even between species assigned to different genera (Gene et al., 2009). While ray-finned fishes (*Actinopterygii*) have a long evolutionary history culminating in our most familiar fishes, past adaptive evolutionary radiations have left only a few survivors, like sturgeons and garfish (Gene et al., 2009).

The wide ranges of the acipenserids and their endangered status have made collection of systematic materials difficult. These factors have led researchers in the past to identify over 40 additional species that were rejected by later scientists (Bemis et al., 1997). It is still unclear whether the species in the *Acipenser* and *Huso* genera are monophyletic (descended from one ancestor) or paraphyletic (descended from many ancestors)—though it is clear that the morphologically motivated division between these two genera is not supported by the genetic evidence (Krieger & Fuerst, 2002). There is an ongoing effort to resolve the taxonomic confusion using a continuing synthesis of systematic data and molecular techniques (Fontana et al., 2001; Krieger & Fuerst, 2002).

The phylogeny of the Acipenseridae, as on the cladogram, shows that they have evolved from among the bony fishes (Laurin & Reisz, 1995; Near et al., 2012; Ricardo et al., 2013).

In currently accepted taxonomy, the class Actinopterygii and the order Acipenseriformes are both clades. The family Acipenseridae is subdivided into two subfamilies, the Acipenserinae including the genera *Acipenser* and

Huso, and Scaphirhynchinae, including the genera *Scaphirhynchus* and *Pseudoscaphirhynchus* (Nelson, 1994).

1.8. Caviar

Globally, sturgeon fisheries are of great value, primarily as not only a source for caviar, but also for flesh (Profita, 2015). Several species of sturgeons are harvested for their roe which is processed into caviar—a luxury food and the reason why caviar-producing sturgeons are among the most valuable and endangered of all wildlife resources (Convention on International Trade in Endangered Species of Wild Fauna and Flora, 2019).

During the 19th century, the US was the global leader in caviar production, having cornered 90% of the world's caviar trade (Mizerek, 2013). Atlantic sturgeon once thrived along the east coast from Canada down to Florida. They were in such abundance in the Hudson River and they were called "Albany beef" and sturgeon eggs were given away at local bars as an accompaniment to 5 cents beer (Kleiman, 1990). White sturgeon populations along the US west coast declined simultaneously under the pressure of commercial fishing and human encroachment (Mizerek, 2013). Within the course of a century, the once abundant sturgeon fisheries in the US and Canada had drastically declined, and in some areas had been extirpated under the pressure of commercial overharvesting, pollution, human encroachment, habitat loss, and the damming of rivers that blocked their ancestral migration to spawning grounds (Mizerek, 2013; Fox et al., 2018).

By the turn of the century, commercial production of sturgeon caviar in the US and Canada had come to an end. Regulatory protections and conservation efforts were put in place by state and federal resource agencies in the US and Canada, such as the 1998 federal moratorium that closed all commercial fishing for Atlantic sturgeon (Fox et al., 2018). It was during the 20th century that Russia grew to become the global leader as the largest producer and exporter of caviar (Mizerek, 2013). As with the decline in sturgeon populations in the US and Canada, the same occurred with sturgeon populations in the Caspian Sea (Convention on International Trade in Endangered Species of Wild Fauna and Flora, 2010).

Beginning with the 1979 US embargo on Iran, poaching and smuggling sturgeon caviar was big business but an illegal and dangerous one. Officers with the Washington Department of Fish and Wildlife (WDFW) busted a poaching ring that was based in Vancouver. The poachers had harvested 1.65 tons of caviar from nearly 2,000 white sturgeons that were poached from the Columbia River. The caviar was estimated to be worth around 2 million dollars. WDFW busted another ring in 2003, and conducted an undercover sting operation in 2006-2007 that resulted in 17 successful attempts out of a total of 19 (NPR, 2015).

In response to concerns over the future of sturgeons and associated commercial products, international trade for all species of sturgeons has been regulated under CITES since 1998 (Convention on International Trade in Endangered Species of Wild Fauna and Flora, 2019).

1.9. Conservation

Sturgeons are long-lived, late maturing fishes with reproductive cycles that include long migrations, and require specific environmental conditions (Earthwave Society, 2011). They are an ancient species that have survived for millions of years (Choudhury & Dick, 2001) but their future is threatened due in part to their inherent ancestral characteristics and reproductive specificities. The negative impacts of overfishing, poaching, habitat destructions, and the construction of dams that have altered or blocked their annual migration to ancestral spawning grounds have taken a serious toll (Clover, 2004; IUCN, 2019). Some species of sturgeon are extinct and several are on the verge of extinction, including the Chinese sturgeon, (Griggs, 2014) the highly prized beluga sturgeon (University of Miami Rosenstiel School of Marine & Atmospheric Science, 2008), and North American pallid sturgeon and Alabama sturgeon (Pallid Sturgeon Recovery Program, 2013). Many species are classified as threatened or endangered with noticeable declines in sturgeon populations as the demand for caviar increases. IUCN data indicates that over 85% of sturgeon species are at risk of extinction, making them more critically endangered than any other group of animal species (IUCN, 2010).

In addition to global restocking efforts, the monitoring of populations and habitat, and various other conservation efforts by national and state resource agencies as applicable to their respective countries, several conservation organizations have been formed to assist in the preservation of sturgeons

around the world. On a global scale, one such organization is the World Sturgeon Conservation Society (WSCS) whose primary objectives include fostering the "conservation of sturgeon species and restoration of sturgeon stocks world-wide", and supporting the "information exchange among all persons interested in sturgeons."(WSCS, 2019) WSCS has been instrumental in organizing global conferences where scientists and researchers can exchange information and address the various conservation challenges that threaten the future of sturgeons.

2. Anesthesia of fish

2.1. Introduction

The use of general anesthetics is a common practice, especially during artificial propagation (Kim & Nam, 2018). Anesthetics are also used during sorting, tagging, surgery, and other stress-inducing procedures. Among its principal uses, anesthesia facilitates the following operations: weighing and measuring, marking and tagging, studying fish physiology and behavior, performing surgery, collecting fish in tidal pools and with scuba, photography, preparing fish for live shipment and transport, manual spawning, injecting vaccines and antibiotics, and collecting blood and other tissues (Park et al., 1998, 2011). The use of adequate anesthetic protocols in fish research is advocated from an ethical perspective in promoting animal welfare and scientific perspectives (Nordgreen et al., 2014). However, efficacy of anesthetic agents should potentially be influenced by a number of biotic factors, including species, size, age, sex, and maturity, as well as abiotic factors such as temperature, water parameter, and salinity (Zahl et al., 2012; Skar et al., 2017). Since no anesthetic agent is effective in all scenarios, the choice of anesthetic and dosage regimes should be determined based on experiment with each target species (Martins et al., 2018). Anesthesia can decrease the stress levels in fish subjected to blood sampling, immobilization, handling, injection of vaccines and antibacterial substances, medical treatment for diseases, artificial spawning, transport, and sorting (Park et al., 2011).

Considerations of toxicity (to users and fish), efficacy, price, regulations for use, and the purpose of using anesthesia influence the choice of the ideal anesthetic. This ideal anesthetic should have the following characteristics: (1) an anesthesia time within 3 min and a recovery time within 5 min, (2) non-toxicity to the fish, (3) ease of use and non-toxicity to the user, (4) absence of effects on the physiology and movement of the experimental fish, (5) excretion of the anesthetic from the body so that no withdrawal period is required, (6) absence of accumulation effects from repeated administrations of anesthesia and absence of side effects, and (7) cost-effectiveness (Park et al., 1998, 2003). Traditionally, chemicals (e.g., urethane, ether, and chloroform) have been used to anesthetize fish. However, these substances are now restricted because they are carcinogenic (Hasler & Meyer, 1942).

In aquaculture, many anesthetics have been used to immobilize fish. The most commonly used are tricaine methanesulphonate (MS-222), benzocaine, carbon dioxide, clove oil, AQUI-S, quinaldine, quinaldine sulphate, 2-phenoxyethanol, metomidate, and etomidate (Marking & Meyer, 1985; Ross & Ross, 2008).

2.2. Anesthesia preperation

If possible, fish should be starved for 12-24 hrs prior to anesthesia, as regurgitated food will decrease water quality and can become lodged in the gills. Baseline behavioural parameters should be obtained, such as ventilation, caudal fin stroke rate and overall activity rate (Park et al., 1998, 2011). The

anesthetic and recovery water tanks should contain water from the aquatic system the fish is used to. All water parameters should be in an acceptable range and the temperature should be constant. The water tanks can be aerated using an air stone or air diffuser. If the procedure is going to be completed out of the water, arrangements must be in place to prevent drying out of the skin, fins and eyes. This may include coverage with clear plastic drapes and regular wetting of tissues with a syringe or an atomiser. Gloves should be worn when handling fish to prevent damage to the skin and transmission of zoonotic diseases (Nordgreen et al., 2014).

2.3. Methods of anesthesia

2.3.1. Chemical anesthesia

Immersion

The most common anesthetic technique in fish is to add the anesthetic agent in the water. It is similar to inhalation anesthesia in terrestrial animals (Neiffer & Stamper, 2009). An artificial ventilation system is necessary in procedures of more than 10 min or in all but the shortest out-of-water anesthetics (Gilderhus & Marking, 1987). Nonrecirculating and recirculating systems are available. Adjustement of the drug concentration in response to depth of anesthesia is difficult with immersion anesthesia (Son et al., 2001; Park et al., 2003). Different concentrations of anesthetic solutions can be prepared in different tubes that can be exchanged if necessary. Small amounts of anesthetic fluid can be delivered directly to the gills via a bulb syringe

without disconnecting the fish from the system (Sneddon, 2012).

Common products used for immersion anesthesia include: Benzocaine, Clove oil, Halothane, Isoflurane, Lignocaine, MS-222 and Phenoxyethol animals (Neiffer & Stamper, 2009).

Parenteral anesthesia

Anaesthetics can also be administered orally, intramuscularly, intravenously or intracoelomically. Intramuscular injection is the preferred route, usually in the dorsal saddle area (Neiffer & Stamper, 2009). Supplementary immersion anaesthesia is often required, and ventilatory support is essential, particularly if recovery is prolonged.

Parenteral products include: Ketamine, Ketamine and medetomidine (Neiffer & Stamper, 2009).

2.3.2. Non-chemical anesthesia

Electroanethesia

Electrofishing is a common method for capturing juvenile and adult fish in fisheries management (Reynolds, 1996). Electroanesthesia has primarily been used to immobilize adult fish for tagging or hatchery broodstock (Ackerman et al., 2006). Three types of electric current have been used to immobilize fish: alternating current (AC), direct current (DC), and pulsating forms of AC and DC. Direct current can cause anodotaxis (movement to the anode pole), electronarcosis (stunning) and electrotetany (tetanic muscle

contractions), whereas alternating current causes only electronarcosis and tetany (Ackerman et al., 2006). The purpose of electroanesthesia is to induce electronarcosis, and avoid severe muscle tetany which can result in spinal injuries (Sterritt et al., 1994). The response of the fish to electricity depends on the intensity of the electric field and the duration of the shock (Redman et al., 1998). Others factors such as water conductivity, temperature, fish size and species can also affect the efficacy of electroanesthesia (Ackerman et al., 2006).

When used appropriately, there appear to be few long term deleterious effects on fish; however, there are acute physiological perturbations and some evidence of increased susceptibility to predation after recovery from electrofishing (Schreck et al., 1976). It has been found that electroshocking induces immediate elevation in plasma corticoid and lactate concentrations in rainbow trout, with persistent increases in plasma glucose and corticoids for at least 6 hours following capture, and cardiovascular changes including rythm changes (Schreck et al., 1976). These responses were attributed to trauma, oxygen debt, and general adaptation syndrome, hence the use of electroanesthesia should be regarded as an invasive and stressful procedure (Ackerman et al., 2006).

Great care must be taken when using electricity in water, and proper protective equipment should be worn when handling fish. Only properly trained individuals should operate electroanesthesia units, operators should never work alone, and first aid should be readily available in case of an

accident (Ackerman et al., 2006).

Hypothermia

Hypothermia is accomplished by lowering the ambient temperature of the fish with ice or cold water. The only potential danger to the handler is the risk of exposure to high concentrations of CO_2 from the use of dry ice as the coolant (Ackerman et al., 2006). The use of dry ice could result in hypercapnic (high CO_2) and acidic conditions in the water, if it is placed in the water. Fish acclimated to higher temperatures may experience stress as a result of cold shock (Ackerman et al., 2006).

Carbon dioxide

Carbon dioxide (CO_2) is a colorless, odorless, non-flammable gas with a water solubility of 1.71 L/L water at 0℃ and 760 mmHg (Bell, 1987). CO_2 is safe to use, but a level of 10% or more in the air will cause anesthesia or even death to the operator; therefore, ample ventilation is necessary (Bell, 1987). The hydration of CO_2 will acidify water, and therefore, should be buffered to reduce this potential stress to the fish.

2.4. Anesthetic agents

A variety of anesthetic agents are commonly applied to fish via immersion (Neiffer & Stamper, 2009). Correct dosing can result in effective anesthesia for acute procedures as well as loss of consciousness for surgical

interventions. Dose and anesthetic agent vary between species of fish and are further confounded by a variety of physiological parameters (Sneddon, 2012). The major anesthetic agents and estimates for optimum doses, as well as induction and recovery times, for various fish shows in Table 1.

Table 1. List of selected anesthetics and estimates for optimum doses, as well as induction and recovery times, for various fish*

Anesthetic agents	Dose	Anesthesia time	Recovery time	Test fish	References
MS-222	25-100 mg/L	<3 min	<10 min	Salmonids, Carp, Minnows	Bell & Blackburn, 1984; Gilderhus & Marking, 1987; McFarland & Klontz, 1969; Schoettger & Julin, 1967; Sylvester & Holland, 1982; Yesaki, 1988
	250-480 mg/L	<5 min	< 10 min	Atlantic halibut	Malmstroem et al., 1993
	150 mg/L	<3 min	<10 min	Striped bass	Lemm, 1993
	75 mg/L	Rapid	3.7-7.1 min	Cod	Mattson & Riple, 1989
	80-100 mg/L	2.6-6.8 min	2.5-1.2min	Tilapia	Ferriera et al., 1979; Ross & Ross, 1984
Benzocaine hydrochloride	40 mg/L			Cod	Ross & Ross, 1984
	25-50 mg/L	3 min	4.3-6.32 min	Salmonids	Yesaki, 1988
	55-85 mg/L	3 min	<10 min	Bass	Gilderhus, 1989; Gilderhus, 1990; Gilderhus & Marking, 1987; Gilderhus et al, 1991;
	50-100 mg/L	1.2-3.9 1.6-6.5 min	3.1-2.2 2.9-2.2 min	Carp Tilapia	Ferriera et al., 1979

Table 1. Continued

Anesthetic agents	Dose	Anesthesia time	Recovery time	Test fish	References
Lidocaine plus 1g/L $NaHCO_3$	350 mg/L	53 sec	13 min	Carp	Carrasco et al., 1984
	250 mg/L	88 sec	12.6 min	Catfish	Carrasco et al., 1984
	350 mg/L	89 sec	10.2 min	Tilapia	Carrasco et al., 1984
Metomidate	5-20 mg/L	Rapid	8.2-19.2 min	Cod	Mattson & Riple, 1989
	7.5-10 mg/L	< 3 min	< 10 min	Striped Bass	Lemm, 1993
	10-60 mg/L	< 5 min	< 20 min	Atlantic halibut	Malmstroem et al., 1993
	5 mg/L	2.7 min	18 min	Rainbow trout	Gilderhus & Marking, 1987
Etomidate	1-7 mg/L	~ 3 min	< 20 min	Salmonids	Bell, 1987; Gilderhus &Marking, 1987
	2-7 mg/L	90 sec	40 min	Tropicals	Amend et al., 1982
	1.35-2.2 mg/L	3-4 min	5-20 min	Catfish	Limsuwan et al., 1983b
	0.5-2.3 mg/L	5-18 min	~30 min	Golden shiners	Lisumwan et al., 1983a
	1.0 mg/L	5 min		Striped Bass	Plumb et al., 1983
Propoxate	1-4 mg/L	< 10 min			Ross & Ross, 1984
Ketamine hydrochloride	30 mg/kg	10-300 sec	1-2 hrs	Salmonids	Graham & Iwama, 1990

Table 1. Continued

Anesthetic agents	Dose	Anesthesia time	Recovery time	Test fish	References
Quinaldine sulfate	15-40 mg/L	2-4 min	1-20 min	Salmonids	Bell, 1987; Gilderhus & Marking, 1987; McFarland & Klontz, 1969
	30-70 mg/L	2 min	1-24 min	Catfish	Schoettger & Julin, 1969
	10-30 mg/L	2 min	2-60 min	Bluegill	Schoettger & Julin, 1969
	25-55 mg/L	< 3 min	< 10 min	Striped Bass	Lemm, 1993
	15-70 mg/L	2 min	1-60 min	Largemouth Bass	Schoettger & Julin, 1969
Propanidid	1.5-3 mL/L	1-4 min	4-10 min	Salmonids	Siwicki, 1984
Clove oil & AQUI-S	40 mg/L	2.5-4 min	3 min	Rainbow trout (FW, 11℃)	Anderson et al., 1997
	40-60 mg/L	3-4 min	12-14 min	Rainbow trout (FW, 9℃)	Keene et al., 1998
	100 mg/L	1-2 min	0.5-2.5 min	Rabbitfish (SW, 28℃)	Soto & Burhanuddin, 1995
	100 mg/L (for field use)	11 sec	2 min	Damselfish (SW, 29℃)	Munday & Wilson, 1997
	20 mg/L (AQUI-S)	5 min	5-10 min	Chinook salmon (FW)	AQUI-S New Zealand Ltd., 2004

Table 1. Continued

Anesthetic agents	Dose	Anesthesia time	Recovery time	Test fish	References
2-Phenoxyethanol	200-500 mL/L	3 min	2-10 min	Salmonids	Barton & Helfrich, 1981; Bell & Blackburn, 1984; Sehdev et al., 1963; Yesaki, 1988
	100-500 mL/L	3 min	< 4 min	Various species	Mattson & Riple, 1989; McFarland & Klontz, 1969
Hypothermia	Instant drop of 6℃			Tilapia	Ross & Ross, 1984
	Immersion in ice water			Various species	McFarland, 1959
Carbon Dioxide	200-1500 mg/L (50%CO_2:50%O_2)	< 3 min	8.14 min	Salmonids	Barton et al., 1986; Bell, 1987; Bell & Blackburn, 1984; Britton, 1983; Gilderhus & Marking, 1987; Iwama et al., 1989; Turvey & Genoe, 1984
	290-460 mL/min	20 min	30 min	Carp	Itzawa & Takeda, 1982
	1-1.78 L/min @50%CO_2	30 min	20-30 min	Carp	Mitsuda et al., 1988; Mitsuda et al., 1980; Mitsuda et al., 1982

Table 1. Continued

Anesthetic agents	Dose	Anesthesia time	Recovery time	Test fish	References
Carbon Dioxide	100-250 mmHg CO_2	~ 30 min	< 40 min	Carp	Yoshikawa et al., 1988; Yoshikawa et al., 1991
Sodium bicarbonate	pH 6.5 + 642mg/L $NaHCO_3$	5 min	10 min	Trout/Carp	Booke et al., 1978
	900 mg/L	5 min	12.1 min	Adult salmon	Gilderhus & Marking, 1987
Carbonic acid	150-600 mg/L H_2CO_3				Post, 1979
Electro-anesthesia	12 V DC	Rapid	Immediate	Chinook salmon (17 ‰)	Gunstrom & Bethers, 1985
	12 V DC 150 mA	Rapid	Immediate	Chinook salmon (SW)	Orsi & Short, 1987
	12 V DC 1-3 A	Rapid	30 sec	Coho salmon (FW)	Sterritt et al., 1994
	12 V DC 30 mA	8 sec	9 sec	Striped bass (5‰, 21℃)	Jennings & Looney, 1998

Table 1. continued

Anesthetic agents	Dose	Anesthesia time	Recovery time	Test fish	References
Electro-anesthesia	60 V pulsed DC 50 Hz, 7.3 ms pulse width pulsed for 10-15 sec	Rapid	2-3 min	Brown trout (FW, 12℃) Northern pike (FW, 11℃)	Redman et al., 1998 Walker et al., 1994

* From Ackerman et al. (2006)

MS-222

MS-222 (3-aminobenzoic acid ethyl ester methane sulfonate) is the most widely used fish anesthetic, and it is exceedingly effective for rapid induction of deep anesthesia. It is a white crystalline powder that is easily dissolved in water, with a solubility of 1.25 g/mL water, at 20°C. MS-222 is generally safe to handle, but contact with eyes and mucous membranes should be avoided (Porter, 2018), as irritation can result.

Benzocaine

Benzocaine (*p*-aminobenzoic acid ethyl ester) has two forms: a crystalline salt with a water solubility of 0.4 g/L, or a freebase form which must be dissolved in ethyl alcohol first at 0.2 g/mL (Porter, 2018). Benzocaine hydrochloride is generally harmless to humans and is commonly used as a local anesthetic in cough drops, sprays, sunburn creams, and haemorrhoid preparations (McErlean & Kennedy, 1968). However, the powder is a respiratory irritant and reasonable care should be exercised. It is also used as a topical and local anesthetic for veterinary purposes (Porter, 2018).

Lidocaine

Lidocaine [2-(diethylamino)-N-(2,6-dimethylphenyl) acetimide], in freebase form, is insoluble in water, but freely soluble in acetone or alcohol. It is generally used in the hydrochloride salt form which is freely soluble in water (Porter, 2018). It is a cardiac depressant which is used by veterinarians

topically or injected as a nerve block (Porter, 2018).

Metomidate and Etomidate

Metomidate [1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid methyl ester] is a watersoluble powder which has the properties of a hypnotic, or sleep-inducing, drug. Etomidate [1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid ethyl ester] is a colorless, odorless crystalline analogue of metomidate and propoxate (Porter, 2018). It has been used on humans as a hypnotic drug, but it is very expensive and difficult to obtain (Bell, 1987). A side effect of anesthesia with metomidate is muscle twitching which can make blood sampling difficult (Gilderhus & Marking, 1987). This effect has not been reported for etomidate. Metomidate has been demonstrated to be ineffective for use on larval fishes causing high mortalities (Massee et al., 1995).

Propoxate

Propoxate [propyl-DL-1-(phenylethyl) imidazole-5-carboxylate hydrochloride] is a crystalline powder which resembles metomidate and etomidate structurally, and is freely soluble in both fresh water and salt water (Porter, 2018). It is stable in solution for long periods and is 100 times more soluble than MS-222 (Thienpont & Niemegeers, 1965).

Ketamine hydrochloride

Ketamine hydrochloride [2-(0-chlorophenyl)-2-(methyl-amino) cyclohexanone hydrochloride]is a white crystalline powder, which has a water solubility of 200 g/L at 20℃ (Porter, 2018). It has been widely used as an anesthetic both in human and veterinary medicine, and is safe for the handler (Porter, 2018).

Quinaldine sulfate

Quinaldine sulfate (2-methylquinoline sulfate) is a light yellow crystalline powder which has a water solubility of 1.041 g/L (Porter, 2018). It is one of the most widely used anesthetics by marine biologists to collect tidepool and coral reef fishes (Munday & Wilson, 1997). Extended exposure of fish to quinaldine sulfate has been shown to be toxic (Amend et al., 1982), and is therefore only useful as a short-term anesthetic.

Propanidid

Propanidid (4-[2-(diethylamino)-2-oxoethoxy]-3-methoxybenzeneacetic acid propyl ester) is a pale yellow liquid which is insoluble in water, but soluble in alcohol (Porter, 2018).

Clove oil and derivatives

Clove oil has recently been suggested as an alternative fish anesthetic (Park et al., 2011). Clove oil is a pale yellow liquid derived from the leaves,

buds and stem of the clove tree (*Eugenia* sp.). Its active ingredients are eugenol (4-allyl-2-methoxyphenol) and iso-eugenol (4-propenyl-2-methoxyphenol), which can comprise 90-95% of clove oil by weight (Porter, 2018). Clove oil has been used for many years as a food additive and a topical analgesic in dentistry, and is recognized as a GRAS (Generally Recognized As Safe) substance by the US FDA for use in humans. AQUI-S is a pharmaceutical derivative that contains 50% active ingredient and is registered for use with food fish in New Zealand and Australia with a nil withdrawal period (AQUI-S New Zealand Ltd., 2004). Both substances are safe to handle, but as with all chemical anesthetics, contact with eyes and mucous membranes should be avoided.

2-phenoxyethanol

2-Phenoxyethanol (1-hydroxy-2-phenoxyethane) is a colorless, oily, aromatic liquid with a burning taste, and has a solubility in water of 27 g/L at 20℃ (Porter, 2018). It is often used as a topical anesthetic (Porter, 2018). 2-Phenoxyethanol is a mild toxin and may cause some irritation to the skin, therefore any contact with the eyes should be avoided (Bell, 1987). Based on human toxicology data, it may also cause liver and kidney damage (Summerfelt & Smith, 1990).

Methylpentynol

Methylpentynol (3-methyl-1-pentyn-3-ol) is a liquid with a noxious odor

and a burning taste, and has a solubility in water of 128 g/L at 25℃ (Porter, 2018). It is a hypnotic sedative which, like 2-phenoxyethanol, varies in effectiveness with size and species of fish, as well as with water temperature. Other water quality parameters such as pH do not seem to have significant effects on the efficacy of anaesthetization (Ackerman et al., 2006).

Chlorobutanol

Chlorobutanol (1,1,1-trichloro-2-methyl-2-propanol) is a crystalline powder with a camphor odor. It has a high solubility 1 g/mL in alcohol (Porter, 2018), although it can also be dissolved in water (McFarland & Klontz, 1969). Stock solutions can be prepared well in advance of use and stored for long periods of time at 4℃. In humans, chlorobutanol is used as a dental analgesic (Porter, 2018). Chlorobutanol has limited use in aquaculture as it is toxic to small fish, and the fish response to this anesthetic is highly variable (McFarland & Klontz, 1969; Mattson & Riple, 1989).

Halothane

Halothane (2-bromo-2-chloro-1,1,1-trifluoro-ethane) is a non-flammable, highly volatile liquid with a sweetish smell. It is used as an inhalant anesthetic in humans (Porter, 2018), but it is very light sensitive and has been shown to become toxic within 10 minutes of exposure to the effective concentration (Gilderhus & Marking, 1987).

Urethane

Urethane (carbamic acid ethyl ester) is a crystalline powder with a water solubility of 2 g/mL (Porter, 2018). Until it was shown to be a carcinogen for humans, it was a popular fish anesthetic as it has a wide margin of safety between lethal and effective dosages, and there seemed to be no ill effects to the fish with repeated exposures (McFarland & Klontz, 1969).

Diethyl ether

Diethyl ether (1,1'-oxybesethane) is a very volatile, highly flammable liquid which, when exposed to light and air, will form explosive peroxides. It has a sweet pungent odor and a burning taste. It is slightly soluble in water, with saturation occurring at 8.43%, weight/weight (w/w), at 15℃ (Porter, 2018). It is a skin irritant to humans and inhalation can lead to narcosis and unconsciousness, with death occurring due to respiratory paralysis (Porter, 2018). Although reports of its use with fishes date in the 1940s and 1950s, the irritation to users has discouraged its common use (McFarland & Klontz, 1969).

Chloral hydrate

Chloral hydrate (2,2,2-trichloro-1,1-ethanediol) is an aromatic, acrid smelling powder with a bitter taste. Its solubility in water is temperature dependent: 2.4 g/mL at 0℃; 5 g/mL at 10℃; 8.3 g/mL at 25℃; and 14.3 g/mL at 40℃ (Porter, 2018). Chloral hydrate can irritate the skin, and is a

potentially addictive drug which has sedative, narcotic, hypnotic, as well as depressant qualities (Porter, 2018). Anesthesia is not deep, however, and chloral hydrate is more useful where sedation rather than deep anesthesia is required (McFarland & Klontz, 1969), such as in transport or various research applications.

2.5. Monitoring

Anesthesia stage can be determined by assessing activity, reactivity to stimuli, equilibrium, respiratory and heart rates. Induction usually takes 5-10 minutes and is marked by a decrease in caudal fin strokes, swimming, respiratory rate and reaction to stimuli (Gilderhus & Marking, 1987; Son et al., 2001; Park et al., 2003). There is also a loss of equilibrium and the righting reflex is poor. Opercular movements usually show that respiration is occurring, but in deep anesthesia these movements may cease (Summerfelt & Smith, 1990). There may be a short excitement phase during immersion induction and some fish may traumatise themselves. The heart rate can be monitored by directly observing cardiac movement, or via ultrasonography, Doppler flow probes or ECG (Ackerman et al., 2006). Gill color may be a useful indicator of oxygenation (Neiffer & Stamper, 2009). Venous blood gas samples can be obtained from large fish to periodically monitor trends in oxygenation, ventilation and pH. Water quality should be monitored to prevent anesthetic mortality (Sneddon, 2012). Partial water replaces may be necessary.

2.6. Recovery

With immersion anesthesia, recovery involves placing the fish in an anesthetic-free tank. Reversal agents are available for some injectable anesthetic agents (Sneddon, 2012). Fresh water can also be administered directly to the fish. The recovery water should be aerated and the fish's mouth should be directed towards water flow. The fish can also be pulled forward through the water with its mouth open. Recovery is usually complete within 5 minutes when immersion anesthesia was used (Gilderhus & Marking, 1987; Son et al., 2001; Park et al., 2003). Respiration rate increases, ataxia disappears. An excitement phase may occur and fish should be prevented from escaping from the tank and from injuring themselves and others.

2.7. Conclusion

A various anesthetic agents have been investigated for their properties to effectively provide anesthesia to fish. However, given the significant diversity of fish species and their associated environmental and physiological requirements, it would be prudent to apply caution when selecting an anesthetic agent (Sneddon, 2012). Tentative exploration of the correct dose is vital because many factors influence anesthetic action including physiological status, body size and water temperature.

II. Anesthetic Effects and Physiological Responses of Lidocaine-HCl in Siberian Sturgeon, *Acipenser baerii*

1. Introduction

1.1. Siberian sturgeon

The Siberian sturgeon, *Acipenser baerii* belongs to the Acipenseriformes (chondrostei) order and subclass, considered “living fossils” (Bemis et al., 1997). Their primitive characteristics, such as a heterocercal tail and cartilaginous skeleton, have been maintained over approximately 100–200 million years despite major environmental changes (Gomulka et al., 2008). The Siberian sturgeon has undergone multiple genome duplications during their evolution, which may account for their resistance to deleterious mutations, since there are probably several functional copies of every gene (Blacklidge & Bidwell, 1993). Their primitive characteristics make sturgeons intriguing animals for study, since their biochemical hematological profile may differ substantially from that of teleost fishes. All sturgeon species worldwide are covered under the provisions of the convention on international trade in endangered species (WSCS, 2019). Several species are considered to be threatened with extinction as a result of over-fishing, poaching, water pollution, damming, and destruction of natural water courses

and habitats (Gomulka et al., 2008). The culture of sturgeons is a growing aquaculture field in Europe and Northeastern Asia due to the need to actively protect natural populations and the high demand for caviar. Usually, their large size and sharp bony shields on the body surface make handling sturgeon spawners difficult and dangerous for operating personnel (Gomulka et al., 2008).

1.2. Lidocaine-HCl

The human anesthetic compound lidocaine-HCl [2-(diethylamino)-N-(2, 6-dimethylphenyl) acetimide hydrochloride], a white, water-soluble powder, is safe, inexpensive, non-toxic in the environment, and does not require a withdrawal period compared with other anesthetic chemicals. Aqueous solutions of lidocaine-HCl are used in human medicine to control ventricular arrhythmia in acute myocardial infarction; it is also used as an anesthetic of the amide type for production of local or regional anesthesia, and as one of several ingredients in anesthetics for surface applications. It was first administered to fish by Carrasco et al. (1984). Lidocaine-HCl, which has been safely used in dentistry, has been proven to be a safe anesthetic for some freshwater and marine fish in Korea (Park et al., 1998). A number of studies have investigated its effectiveness, economic viability, reusability, toxicity, and sideeffects to ascertain its appropriateness as a fish anesthetic (Summerfelt & Smith, 1990). Despite the common use of anesthetics in fish, there is little information about the influence of lidocaine-HCl on sturgeon.

The objective of this study is to determine the optimal dose of lidocaine-HCl for anesthesia in Siberian sturgeon, one of the most widely cultured sturgeon species around the world, including in Korea (Park et al., 2013a, 2013b) to investigate the relationship between anesthetic effects and fish size, and to analyze the re-anesthetic effects and stress responses to lidocaine-HCl.

2. Materials and Methods

Described experiment was approved by the Current Laws of Korea (Ordinance of Agriculture, Food and Fisheries, No. 1-the Law Regarding Experimental Animals, No. 9982) and the Ethical Guidelines of Korea Maritime & Ocean University (KMOU), Korea.

2.1 Experimental fish

Two year-olds Siberian sturgeon, *Acipenser baerii* and fertilized eggs were obtained from Pukyung National University, Korea. While 7 years, 2 year-olds sturgeons were reared and bred, and fertilized eggs were hatched and bred in the Fishery Genetics and Breeding Sciences Laboratory (FGBS lab) of the KMOU, Korea. On July 30 2017, fertilized eggs were collected from matured sturgeon (8 year-olds samples), and were hatched and reared in FGBS lab of KMOU. On July 14 2018, fertilized eggs were collected from matured samples (9 year-olds), and were hatched and reared for 3 weeks. On August 4 2018, small, middle and large samples were selected for the study at 3 weeks, 1 year, and 9 years after hatching, respectively. 3 week-olds (small size; fertilized on July 2018), 1 year-olds (middle size; fertilized on July 2017), and 9 year-olds (large size; obtained on June 2011) samples used in the study were weighed using an electronic balance (Shimadzu, Japan) and measured using Vernier calipers (Mitutoyo, Japan). Hereafter, I refer to the small, middle, and large size groups as larval, juvenile, and adult groups, respectively. The average body lengths in the larval, juvenile, and adult

groups were 10.2±0.84 cm (*n*=50), 40.8±3.52 cm (*n*=50), and 84.1±6.91 cm (*n*=50), respectively. The average body weights of the larval, juvenile, and adults groups were 4.6±0.45 g (*n*=50), 334.9±60.04 g (*n*=50), and 2351±534.4 g (*n*=50), respectively. Physico-chemical parameters of water uesd for anesthesia and recovery were maintained during the experimental period Table 2, which began on August 10, 2018 and ended on December 21, 2018.

Table 2. Quality parameters of water used for anesthetic/recovery experiments in this study

Test parameter*	Condition
pH	7.1 ± 0.65
DO (dissolved oxygen; ppm; Saturated concentration in 26℃)	7.6
Ammonia (ppm)	0.01
Nitrite (ppm)	1.8 ± 0.14
Nitrate (ppm)	0.01
Conductivity (μs/cm)	238

*Test parameter was analyzed at 1 hr before experiment. Dissolved oxygen, pH and salinity were measured using an oxygen measurement electrode and a multi-data logger system (Oxyguard, Denmark). Ammonia nitrogen, nitrite, nitrate, and conductivity were measured using spectrophotometer (DR2800, HACH, Loveland, Colorado, USA). Mean±SD was based on triplicate groups.

2.2. Anesthetic effect of lidocaine-HCl

Twenty specimens from each size group were randomly selected to investigate the anesthetic effects of lidocaine-HCl (Hongsung Chemical, Korea) In order to neutralize the anesthetic solution and to amplify its effect (Carrasco et al., 1984; Park et al., 1998), 1000 ppm $NaHCO_3$ (Sigma, USA) was prepared as the total concentration.. Lidocaine-HCl concentrations of 50, 100, 150, 200, 250, and 300 ppm were used. Aquarium and anesthetic waters were maintained at 20℃ for the duration of the experiment. All fish were starved for 24 hrs before the study began. The study methods followed those of Park et al. (2011). Briefly, one specimen was selected randomly from the breeding water tank using a net. The fish was then anesthetized in a 10 L rectangular parallelepiped plastic water tank controlled by an aeration system. Once the fish was anesthetized, it was moved immediately to the recovery water tank. The anesthesia levels and recovery times of the fish were measured in seconds using a stopwatch. All experiments were completed in triplicate.

As shown in Table 3, the anesthetizing and recovery protocols followed the decision-based anesthetic effect table developed by Park et al. (2011). Anesthesia was divided into 6 stages (A1~A7): A1, Normal swimming, opercular movement and normal general movement; A2, Swimming speed slowed, rolling from side to side; A3, Partial loss of equilibrium, swimming erratic; A4, Complete loss of equilibrium, swimming perfectly inside out, pectoral fin, pelvic fin and dorsal fin movement stop; A5, Little sedation, anal

Table 3. Stages of anesthesia induction and recovery in lidocaine-HCl efficacy tests performed in Siberian sturgeon, *Acipenser baerii*[*]

	Anesthesia
Stage	Characteristic behavior
A1	Normal swimming, opercular movement and normal general movement
A2	Swimming speed slowed, rolling from side to side
A3	Partial loss of equilibrium, swimming erratic
A4	Complete loss of equilibrium, swimming perfectly inside out, pectoral fin, pelvic fin and dorsal fin movement stop
A5	Little sedation, anal fin and tail fin movement stop
A6	Perfect sedation, only opercular movement, when flip the sample, no reaction observed
A7	Opercular movement ceased
	Recovery
Stage	Characteristic behavior
R1	Resume opercular movement
R2	Preferential movement of pectoral fin and tail fin
R3	Dorsal fin, pelvic fin and anal fin movement
R4	Swimming perfectly inside out
R5	Swimming erratic, recovery of balance, when flip the sample, observed to flip themselves
R6	Normal swimming, responsiveness to visual stimuli

[*]**Modified from Park et al. (2011).**

fin and tail fin movement stop; A6, Perfect sedation, only opercular movement, when flip the sample, no reaction observed; A7, Opercular movement ceased (Park et al., 2011). Recovery was divided into 6 stages (R1~R6): R1, Resume opercular movement; R2, Preferential movement of pectoral fin and tail fin; R3, Dorsal fin, pelvic fin and anal fin movement; R4, Swimming perfectly inside out; R5, Swimming erratic, recovery of balance, when flip the sample, observed to flip themselves; R6, Normal swimming, responsiveness to visual stimuli (Park et al., 2011). Briefly, anesthetizing Siberian sturgeon involved several stages, from slowed swimming speed and side-to-side rolling (stage A2) to only opercular movement (stage A6). At stage A6, individuals were transferred to a recovery tank. Recovery time was established as the point at which erratic swimming began. Recovery time included redressing balance (stage R5) and normal swimming, as well as responsiveness to visual stimuli (stage R6). This study used stages A6 and R6 as endpoints for anesthesia and recovery, respectively.

2.3. Effect of water temperature on anesthesia

Twenty specimens of the juvenile group were randomly selected to investigate the anesthetic effects of lidocaine-HCl under water temperature. Experimental fish were adapted to 400-L glass water tanks, which were maintained at the same temperatures as the experimental water temperatures (15, 20, and 25℃). After being anesthetized, fish were transferred to recovery water tanks of equivalent water temperatures. All fish were starved

for 24 hrs prior to the experiment. The methods and stages of anesthesia and recovery followed those of Park et al. (2011).

2.4. Effect of anesthesia on stress response

To analyze the stress response to lidocaine-HCl, blood physiological responses of the control group (no anesthesia) and experimental group (200 ppm lidocaine-HCl anesthesia) were measured. Blood samples from each group were extracted from five randomly selected fish 0 (pre), 1, 6, 12, 24, 48, 72, and 96 hrs after anesthesia. Fish used in this experiment were not involved in the experiments assessing anesthetic effects. Blood was collected from the caudal vasculature using a disposable syringe (3 mL, Sung Shim Medical Co., Ltd, Bucheon, Korea) and heparin sodium (Shin Poong Pharm Co., Ltd, Ansan, Korea). Blood was extracted within 1 min to minimize the handling stress imposed on the fish and allowed to sit for 10 min at room temperature prior to centrifugation for 10 min at 20,000 g (Centrifuge Micro 17R, Hanil Science Industrial Co., Ltd, Incheon, Korea). The collected plasma was transferred to another 1.5 mL microtube and stored at -70℃ in a super low-temperature freezer (CLN-50UW Nihon Freezer, Nihon Co., Japan) prior to analysis.

Plasma cortisol concentrations were measured after the antigen-antibody response was derived using the 1470 WIZARD Automatic Gamma Counter (Cobra, Packard Co., Ramsey, MN, USA) and the Coat-A-count TKCO Cortisol RIA Kit (DPC, Los Angeles, CA, USA), following Donaldson

(1981). Plasma glucose concentrations were analyzed following Raabo and Terkildsen (1960; Kit 510, Sigma, St Louis, MO, USA), and production of H_2O_2 by glucose oxidase in the presence of *o*-dianisidine was measured as an absorbance peak at 450 nm. Lactic acid concentrations were analyzed using a blood automatic analysis (Boehringer Mannhein Reflotron, Germany).

2.5. Anesthetic sensitivity of lidocaine-HCl

Fifty specimens from the juvenile group were randomly selected to investigate anesthetic sensitivity to lidocaine-HCl and the effect of re-anesthesia. To analyze the anesthetic sensitivity to lidocaine-HCl, anesthesia treatments were performed three times (initial, duplicate, and triplicate). Anesthesia time intervals were 1, 2, 3, 4, 5, 6, and 7 days.

2.6. Re-anesthetic effect of lidocaine-HCl

To investigate the re-anesthetic effect of lidocaine-HCl, re-anesthesia was conducted seven times at 1-day intervals. The methods and stages of anesthesia and recovery used followed those of Park et al. (2011).

3. Results

No fish used in this experiment died due to the stress induced by lidocaine-HCl anesthesia.

3.1. Anesthetic effect of lidocaine-HCl

Table 4 presents the parameters associated with the anesthesia and recovery times of lidocaine-HCl at each anesthetic concentration and body size of Siberian sturgeon, *Acipenser baerii*. Anesthesia time and recovery time were affected significantly by the concentration of anesthesia and body size of the fish. Anesthesia time and recovery time were visualized in Fig. 1 and Fig. 2, respectively. Anesthesia time decreased significantly as both the lidocaine-HCl concentration and body size of Siberian sturgeon increased ($P<0.05$; Fig. 1), and recovery time decreased significantly as the lidocaine-HCl concentration increased ($P<0.05$; Fig. 2). The adult group had a slower anesthesia time than that of the other groups at equivalent lidocaine-HCl concentrations, while the fastest recovery time was seen in the juvenile group. Lidocaine-HCl concentrations of 50 and 200 ppm in the larval and juvenile groups, respectively, showed the optimal anesthesia time of approximately 1 min. The concentrations of lidocaine-HCl that displayed the optimal anesthesia time of approximately 1 min were not determined for adult fish.

Table 4. Effects of lidocaine-HCl dose and body size on anesthesia among Siberian sturgeon, *Acipenser baerii*[*]

Dose (ppm)	Anesthesia time (sec)			Recovery time (sec)		
	Larval	Juvenile	Adult	Larval	Juvenile	Adult
50	63 ± 2.2[a]	119 ± 21.5[a]	181 ± 21.4[a]	180 ± 23.8[a]	133 ± 13.2[a]	186 ± 13.8[a]
100	43 ± 1.6[b]	77 ± 13.3[b]	154 ± 14.7[b]	179 ± 30.5[a]	134 ± 14.2[a]	174 ± 14.8[b]
150	37 ± 2.1[c]	66 ± 4.5[c]	140 ± 13.2[c]	183 ± 23.6[a]	129 ± 9.4[ab]	167 ± 10.1[c]
200	33 ± 2.5[c]	60 ± 6.1[c]	135 ± 12.1[c]	178 ± 31.4[a]	129 ± 13.6[ab]	168 ± 9.1[c]
250	32 ± 1.9[d]	50 ± 4.2[d]	121 ± 17.1[d]	182 ± 35.1[a]	125 ± 10.3[ab]	169 ± 8.4[c]
300	32 ± 1.7[d]	43 ± 3.5[e]	110 ± 14.5[e]	179 ± 38.9[a]	124 ± 12.2[b]	165 ± 8.9[c]

Two-way ANOVA

	DF	Anova SS	Mean Square	F-value	P-value	DF	Anova SS	Mean square	F-value	P-value
Body size	2	185841.0	68594.629	550.483	< 0.0001	2	116492.0	21558.4	911.23	< 0.0001
Dose	5	389425.1	61573.945	538.561	< 0.0001	5	10618.6	3588.1	159.89	< 0.0001
Interaction	10	81269.4	21135.358	193.145	< 0.0001	10	9145.1	811.5	31.23	< 0.0001

[*]Water temperature of each size were 20℃ in each concentration of lidocaine-HCl. Each value is mean ± SD (n=50) of triplicate groups. Values in the same column not sharing common superscripts are significantly different among each concentration (P<0.05).

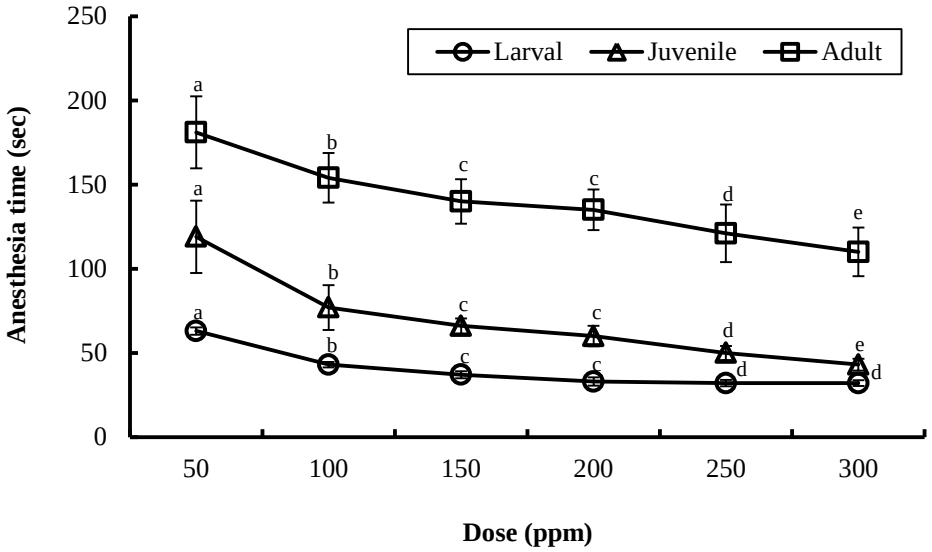

Fig. 1. Variations of anesthesia time on lidocaine-HCl dose and body size among Siberian sturgeon, *Acipenser baerii*. Water temperature was 20℃. Vertical bars are means ± SE (n=50). Different letters on error bars are significantly different among each concentration (P<0.05).

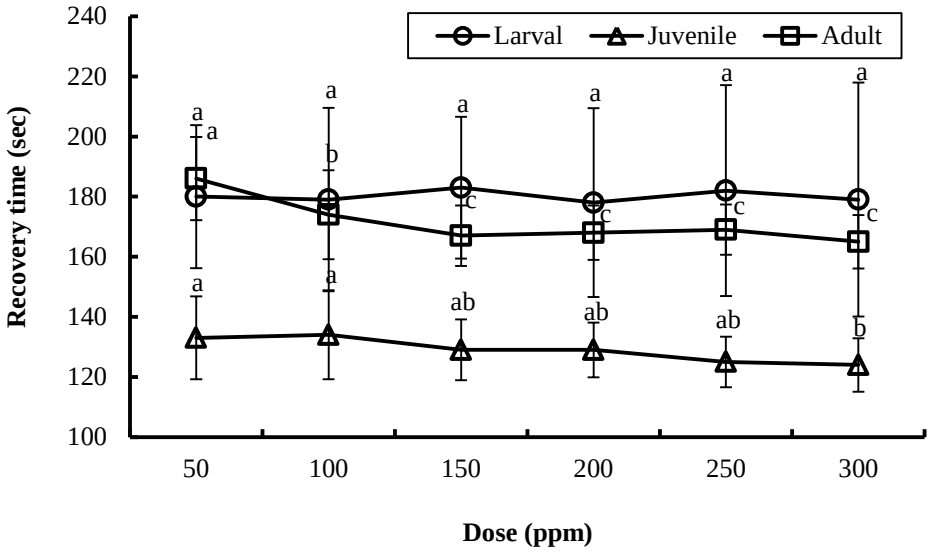

Fig. 2. Variations of recovery time on lidocaine-HCl dose and body size among Siberian sturgeon, *Acipenser baerii*. Water temperature was 20℃. Vertical bars are means ± SE (n=50). Different letters on error bars are significantly different among each concentration (P<0.05).

3.2. Effect of water temperature on anesthesia

Table 5 shows the parameters associated with the effects of lidocaine-HCl at each concentration and water temperature in the juvenile group. Anesthesia recovery times were affected significantly by the anesthetic's concentration and water temperature. Anesthesia time and recovery time were visualized in Fig. 3 and Fig. 4, respectively. Anesthesia time decreased significantly as the lidocaine-HCl concentration and water temperature increased ($P<0.05$; Fig. 3). As the concentration of lidocaine-HCl increased, the anesthesia time decreased significantly ($P<0.05$) at each temperature. At each lidocaine-HCl concentration, the anesthesia time also decreased significantly ($P<0.05$) as water temperature increased. Recovery time decreased significantly as the lidocaine-HCl concentration and water temperature increased ($P<0.05$). The recovery time of lidocaine-HCl decreased significantly as the lidocaine-HCl concentration and water temperature increased ($P<0.05$; Fig. 4), with the exception of the 200 and 250 ppm anesthesia concentrations at 15℃, the 150, 200, and 250 ppm concentrations at 20℃, and the 200, 250, and 300 ppm concentrations at 25℃. Lidocaine-HCl concentrations of 250 ppm at 15 and 20℃ and 100 ppm at 25℃ represented the optimal anesthetic time of approximately 1 min.

Table 5. Effects of lidocaine-HCl dose and water temperature on anesthesia among juvenile group of Siberian sturgeon, *Acipenser baerii*

Dose (ppm)	Anesthesia time (sec)*			Recovery time (sec)*		
	15℃	20℃	25℃	15℃	20℃	25℃
50	173 ± 26.9^{a}	119 ± 21.5^{a}	85 ± 19.9^{a}	190 ± 26.2^{a}	133 ± 13.2^{a}	126 ± 11.9^{a}
100	103 ± 6.7^{b}	77 ± 13.3^{b}	57 ± 3.4^{b}	179 ± 10.1^{bc}	134 ± 14.2^{a}	122 ± 5.5^{a}
150	80 ± 11.3^{c}	66 ± 4.5^{c}	42 ± 6.5^{c}	183 ± 10.1^{b}	129 ± 9.4^{ab}	119 ± 8.0^{ab}
200	72 ± 8.7^{c}	60 ± 6.1^{c}	40 ± 2.7^{c}	173 ± 8.7^{cd}	129 ± 13.6^{ab}	113 ± 9.5^{b}
250	57 ± 6.2^{d}	50 ± 4.2^{d}	32 ± 2.0^{d}	170 ± 10.3^{cd}	125 ± 10.3^{ab}	114 ± 9.5^{b}
300	52 ± 9.1^{d}	43 ± 3.5^{e}	28 ± 3.2^{d}	167 ± 5.6^{d}	124 ± 12.2^{b}	114 ± 9.7^{b}

Two-way ANOVA

	DF	Anova SS	Mean square	F-value	P-value	DF	Anova SS	Mean square	F-value	P-value
Temperature	2	74451.0	37225.525	229.337	< 0.0001	2	282359.0	141179.8	1011.23	< 0.0001
Dose	5	344669.7	68933.945	554.311	< 0.0001	5	1896.9	379.8	2.72	< 0.0001
Interaction	10	41357.9	4135.795	33.275	< 0.0001	10	8707.9	870.7	6.23	< 0.0001

*Each value is mean ± SD (n=20). Values in the same column not sharing common superscripts are significantly different among each concentration (P<0.05).

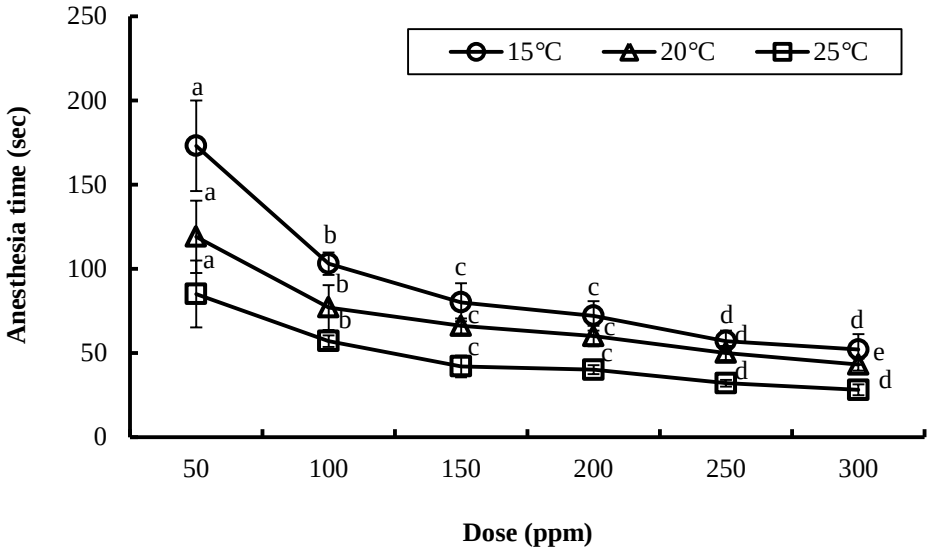

Fig. 3. Variations of anesthesia time on lidocaine-HCl dose and water temperature among juvenile group of Siberian sturgeon, *Acipenser baerii*. Vertical bars are means ± SE (n=50) of triplicate groups. Different letters on error bars are significantly different among each concentration (P<0.05).

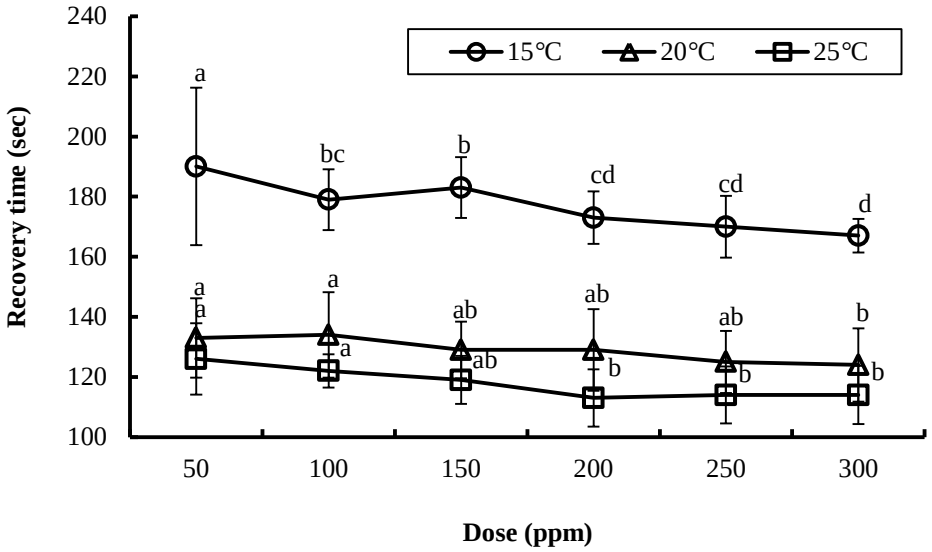

Fig. 4. Variations of recovery time on lidocaine-HCl dose and water temperature among juvenile group of Siberian sturgeon, *Acipenser baerii*. Vertical bars are means ± SE (n=50) of triplicate groups. Values in the same column not sharing common superscripts are significantly different among each concentration (P<0.05).

3.3. Effect of anesthesia on stress response

3.3.1. Plasma cortisol

Figure 5 shows the average plasma cortisol concentrations in the control (no anesthesia) and experimental (200 ppm lidocaine-HCl) groups over 96 hrs. The plasma cortisol concentration at each time point was affected significantly by anesthesia and was significantly different after 72 hrs. The mean plasma cortisol concentrations of the control were 1.0±0.15 μg/dL before the experiment, 15.4±1.51 μg/dL 1 hr after anesthesia, and 38.1±1.43 μg/dL 6 hrs after anesthesia (P<0.05; Fig. 5). The plasma cortisol concentration of the control recovered to 1.1±0.56 μg/dL after 96 hrs and was significantly higher than that before the experiment (P<0.05). The plasma cortisol concentrations of the experimental group were 0.9±0.20 μg/dL before the experiment (P<0.05; Fig. 5), 10.1±1.44 μg/dL 1 hr after anesthesia, and 29.4±1.58 μg/dL 12 hrs after anesthesia (P<0.05), and the concentration recovered to 1.0±0.61 μg/dL after 96 hrs.

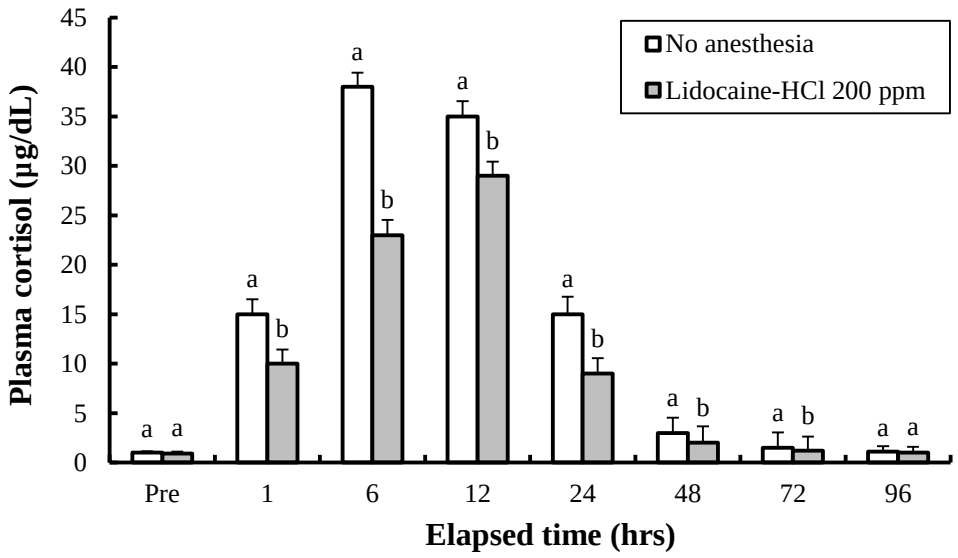

Fig. 5. Variations of the plasma cortisol concentrations in juvenile group of Siberian sturgeon, *Acipenser baerii*, during 96 hrs. Anesthetic dose of lidocaine-HCl and water temperature were 200 ppm and 20℃. Pre means control group before anesthesia. Vertical bars are means ± SE (n=50). Different letters on error bars are significantly different between no anesthesia and lidocaine-HCl anesthesia groups (P<0.05).

3.3.2. Plasma glucose

Figure 6 shows the average plasma glucose concentrations in the control (no anesthesia) and experimental (200 ppm lidocaine-HCl) groups over 96 hrs. Plasma glucose concentrations in the experimental group were significantly different from those of the control at each time point from 6 to 48 hrs after anesthesia. The plasma glucose concentrations of the control and experimental groups before the experiment were 30±2.0 mg/dL and 31±2.2 mg/dL, respectively Fig. 6 and increased from 60±14.4 mg/dL and 50±15.6 mg/dL at 1 hr to 380±14.5 mg/dL and 220±18.4 mg/dL at 12 hrs after anesthesia, respectively (P<0.05). Plasma glucose concentrations of the control and experimental groups recovered to 30±17.5 mg/dL and 30±15.0 mg/dL, respectively, by 96 hrs and were similar to the levels before the experiment (P<0.05).

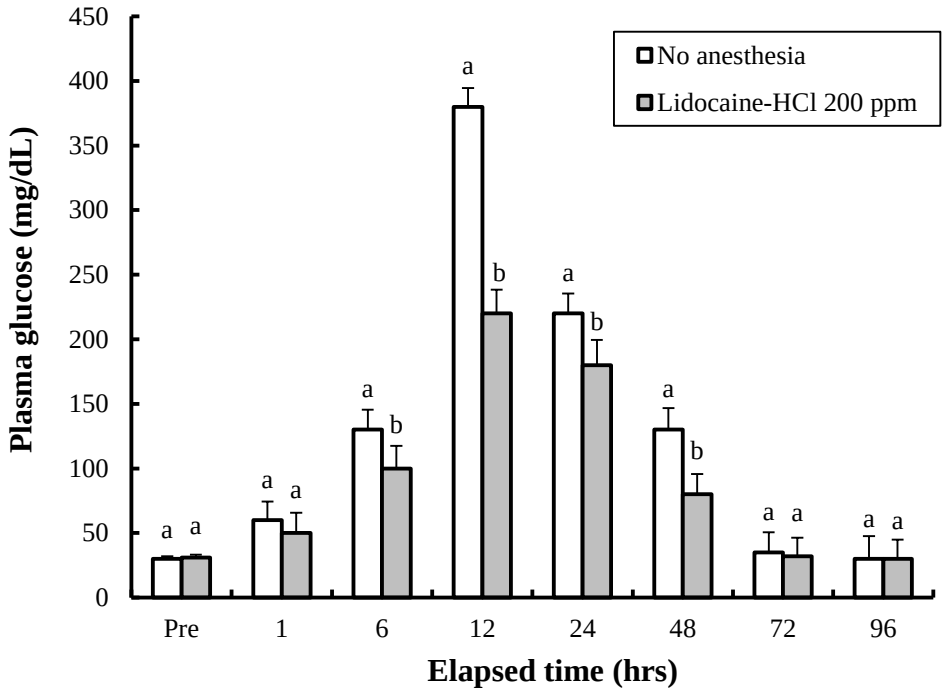

Fig. 6. Variations of the plasma glucose concentrations in juvenile group of Siberian sturgeon, *Acipenser baerii*, during 96 hrs. Anesthetic dose of lidocaine-HCl and water temperature were 200 ppm and 20℃. Pre means control group before anesthesia. Vertical bars are means ± SE (n=50). Different letters on error bars are significantly different between no anesthesia and lidocaine-HCl anesthesia groups (P<0.05).

3.3.3. Lactic acid

Figure 7 shows the average lactic acid concentrations in the control (no anesthesia) and experimental (200 ppm lidocaine-HCl) groups after 96 hrs. Lactic acid concentrations in the experimental group at each time point from 1 to 48 hrs after anesthesia were significantly different from those in the control group. Lactic acid concentrations of the control and experimental groups were 0.9±0.21 mmol/L and 0.9±0.22 mmol/L, respectively, before the experiment, peaking at 24 and 48 hrs in the control group and at 48 hrs after anesthesia in the experimental group Fig. 7. The concentration increased more rapidly in the control than in the experimental groups. The lactic acid concentrations in the control and experimental groups recovered to 1.3±0.49 mmol/L and 1.4±0.39 mmol/L, respectively, by 96 hrs and were higher than levels before the experiment ($P<0.05$).

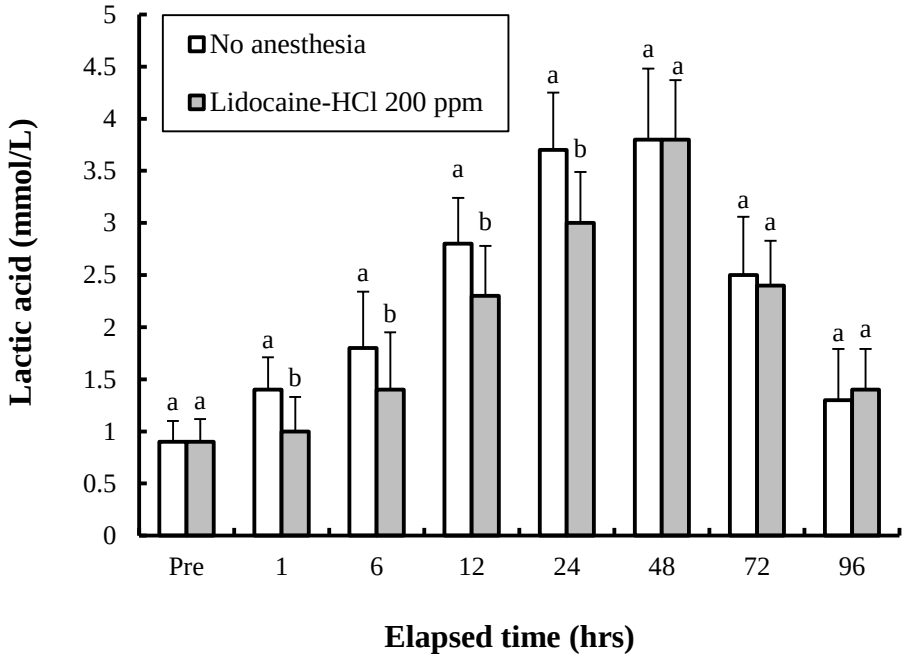

Fig. 7. Variations of the lactic acid concentrations in juvenile group of Siberian sturgeon, *Acipenser baerii*, during 96 hrs. Anesthetic dose of lidocaine-HCl and water temperature were 200 ppm and 20℃. Pre means control group before anesthesia. Vertical bars are means ± SE (n=50). Different letters on error bars are significantly different between no anesthesia and lidocaine-HCl anesthesia groups (P<0.05).

3.4. Anesthetic sensitivity of lidocaine-HCl

Table 6 shows the sensitivity of the juvenile group to lidocaine-HCl. Anesthesia times of the duplicate and triplicate anesthesia treatments decreased significantly as the anesthesia interval increased ($P<0.05$; Fig. 8). Recovery times after the duplicate and triplicate anesthesia treatments decreased as the anesthesia interval increased ($P<0.05$; Fig. 9), with the exception of the duplicate and triplicate treatments at the 4-, 5-, 6-, and 7-day intervals. At the 1-,2-, and 3-day intervals, the anesthesia and recovery times increased significantly as the number of anesthetic treatments increased ($P<0.05$) but were not significantly different between the duplicate and triplicate treatments ($P>0.05$). At the 4-, 5-, 6-, and 7-day intervals, the anesthesia and recovery time were not significantly different among the initial, duplicate and triplicate treatments ($P>0.05$).

Table 6. Anesthetic sensitivity of lidocaine-HCl in juvenile group of Siberian sturgeon, *Acipenser baerii*[*]

Anesthetic interval (Day)	Anesthesia time (sec)			Recovery time (sec)		
	Initial	Duplicate	Triplicate	Initial	Duplicate	Triplicate
1	60 ± 6.1[a]	81 ± 3.5[a]	75 ± 4.1[a]	129 ± 13.6[a]	146 ± 6.4[a]	145 ± 6.1[a]
2	60 ± 6.1[a]	75 ± 4.1[a]	74 ± 3.5[a]	129 ± 13.6[a]	141 ± 9.8[a]	146 ± 10.0[a]
3	60 ± 6.1[a]	66 ± 5.5[a]	66 ± 4.0[a]	129 ± 13.6[a]	134 ± 12.8[a]	134 ± 9.1[b]
4	60 ± 6.1[a]	61 ± 3.4[b]	60 ± 3.3[b]	129 ± 13.6[a]	128 ± 8.5[b]	130 ± 12.1[c]
5	60 ± 6.1[a]	60 ± 4.1[b]	61 ± 3.9[b]	129 ± 13.6[a]	126 ± 10.1[b]	129 ± 11.8[c]
6	60 ± 6.1[a]	61 ± 5.0[b]	59 ± 4.0[b]	129 ± 13.6[a]	127 ± 9.1[b]	128 ± 9.7[c]
7	60 ± 6.1[a]	59 ± 4.2[b]	60 ± 3.7[b]	129 ± 13.6[a]	130 ± 8.6[b]	129 ± 10.3[c]

[*]Anesthetic concentrations of lidocaine-HCl and water temperature were 200 ppm and 20℃ in each group. Each value is mean ± standard deviation (n=50) of triplicate groups. Values in the same column not sharing common superscripts are significantly different among each day (P<0.05).

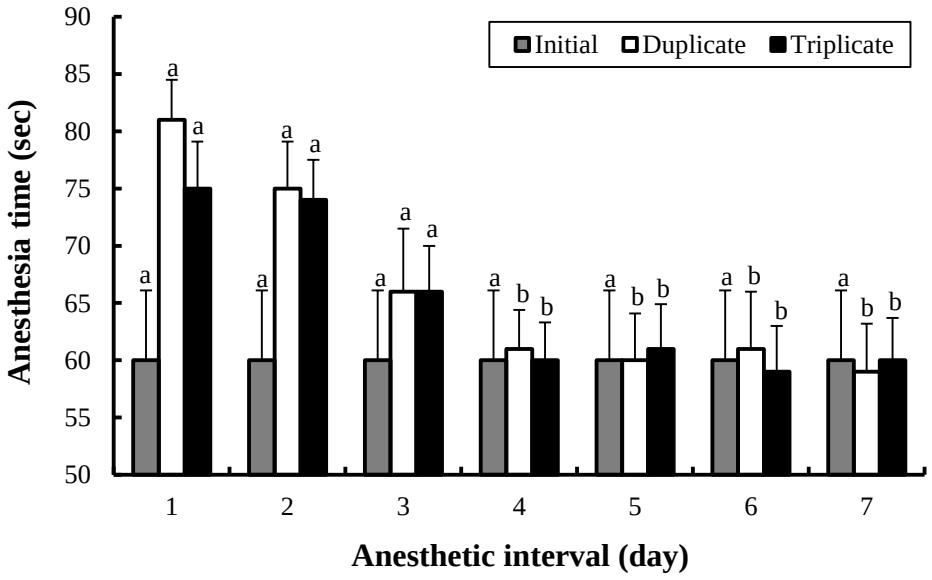

Fig. 8. Anesthesia time of lidocaine-HCl on anesthetic interval among juvenile group of Siberian sturgeon, *Acipenser baerii*. Vertical bars are means ± SE (n=50). Different letters on error bars are significantly different between anesthesia time and anesthetic interval groups (P<0.05).

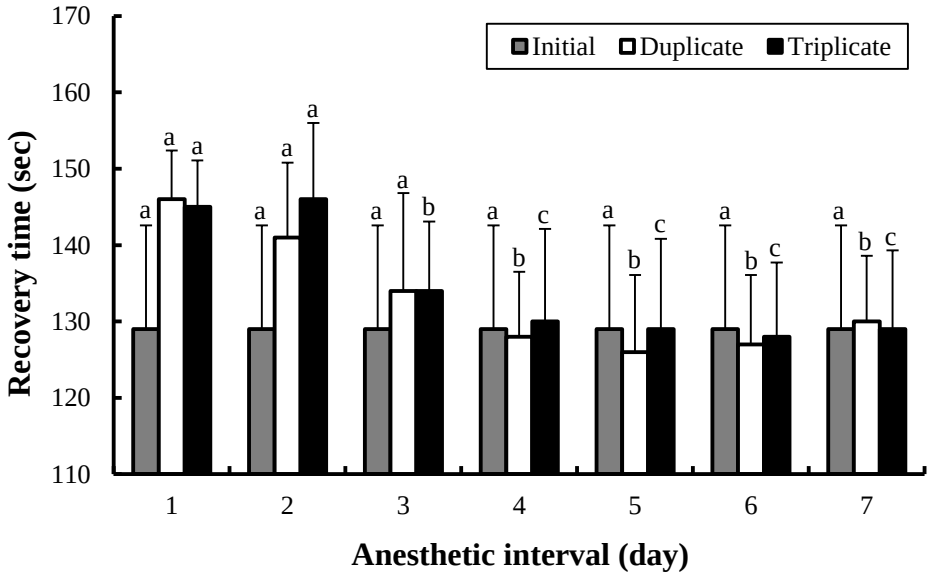

Fig. 9. Recovery time of lidocaine-HCl on anesthetic interval among juvenile group of Siberian sturgeon, *Acipenser baerii*. Vertical bars are means ± SE (n=50). Different letters on error bars are significantly different between anesthesia time and anesthetic interval groups (P<0.05).

3.5. Effect of re-anesthesia on lidocaine-HCl

Figure 10 shows the re-anesthesia effect of lidocaine-HCl on juvenile fish. Similar to the anesthetic sensitivity results, the anesthesia and recovery times increased from 60±6.1 s and 129±13.6 s at the first anesthesia treatment to 81±3.5 s and 146±6.4 s at the second treatment, respectively Fig. 10. Anesthesia time decreased significantly from 81±3.5 s at the second anesthesia treatment to 71±4.4 s at the seventh treatment (P<0.05). The anesthesia time of the seventh treatment was longer than that of the first (P<0.05). In other words, anesthesia and recovery times increased significantly after the second treatment (P<0.05). The anesthesia time decreased significantly as the number of treatments increased (P<0.05), but the recovery time did not differ significantly (P>0.05; Fig. 10).

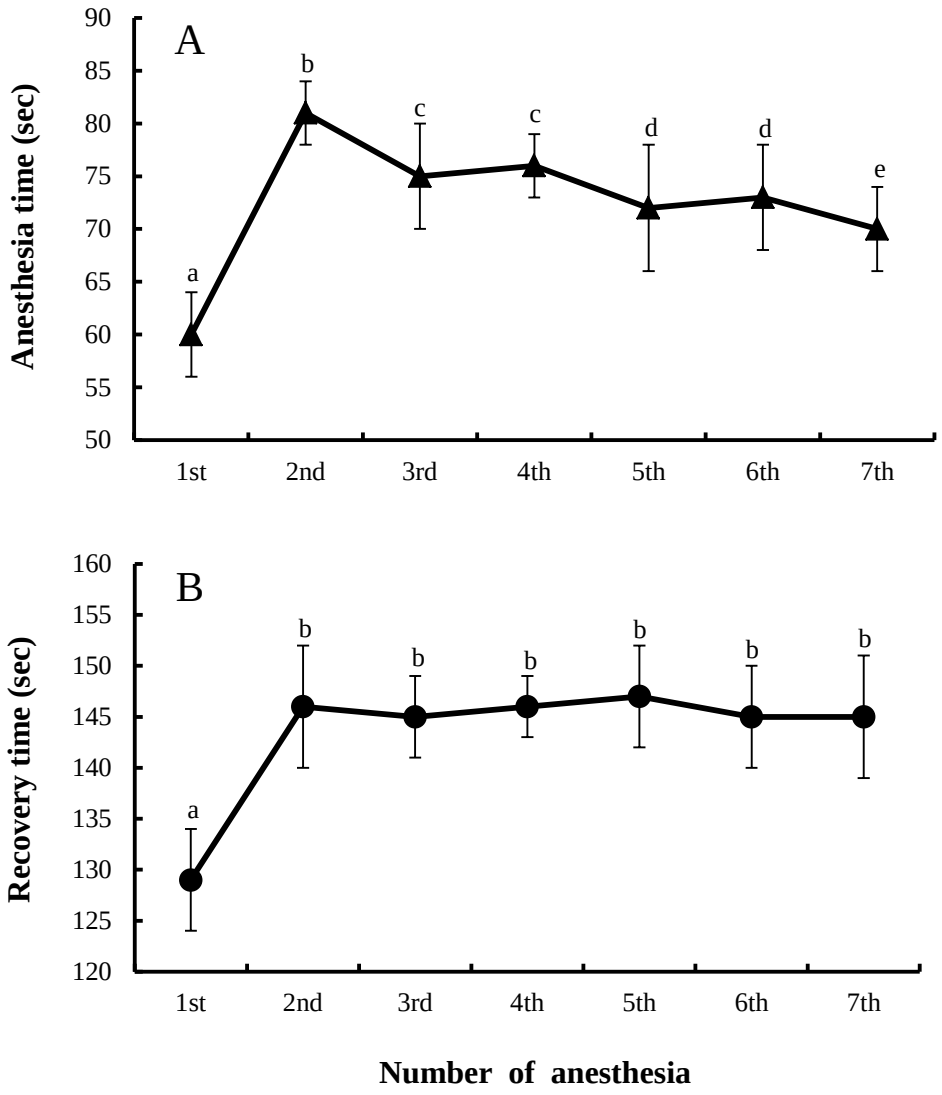

Fig. 10. Effect of re-anesthesia on juvenile group of Siberian sturgeon, *Acipenser baerii*, while 1 week. Re-anesthetic dose of lidocaine-HCl and water temperature were 200 ppm and 20℃ in every day. A: anesthesia time; B: recovery time. Vertical bars are means ± SE (n=50). Different letters on the error bars indicate statistical significance among each day (P<0.05).

4. Discussion

Anesthesia time is the time required to achieve the criteria for anesthesia for that species, and the recovery time is the time required for the animal to recover its vitality completely (Summerfelt & Smith, 1990). This study indicates that lidocaine-HCl is an effective anesthetic for Siberian sturgeon, *Acipenser baerii*. I assessed lidocaine-HCl concentrations of 50–300 ppm in larval and juvenile Siberian sturgeon to determine the concentration to meet the efficacy criteria for an anesthesia time of 3 min, a recovery time within 10 min, and no mortality (Gilderhus & Marking, 1987; Son et al., 2001; Park et al., 2003). However, the optimal lidocaine-HCl concentration for adult sturgeon was not determined in this study.

In this study, larval Siberian sturgeon responded most sensitively to concentrations of lidocaine-HCl. Recovery times were much shorter in juvenile than in adults fish. This study demonstrated that larval Siberian sturgeon are more easily anesthetized and also recover from anesthesia more rapidly compared with adult fish. Similarly, the relationship between anesthesia time and fish size in sockeye salmon, *Oncorhynchus nerka* and marine medaka, *Oryzias dancena* anesthetized with clove oil followed a significant positive exponential curve (Woody et al., 2002; Park et al., 2011). Park et al. (2011) demonstrated that smaller-sized marine medaka were anesthetized easily and also recovered rapidly from anesthesia compared with larger-sized fish. Whereas This results indicate that both the anesthesia and recovery times increased with the anesthetic concentration, only the

anesthesia time was found to increase in sockeye salmon. However, the lengths of the sockeye salmon ranged from 400–550 mm, indicating that they were all adult fish. Woody et al. (2002) investigated the relationship between anesthesia concentration and the length of adult fish. Instead, I categorized the larval fish into the small size group and the adult fish into the large size group. Therefore, in addition to the simple comparison of anesthetic effects by body length, anesthetic effects among larval, juvenile, and adult fish were tested in this study. Up to date, no studies have investigated anesthetic effects on different growth stages of fish.

This study showed that increasing the concentration of anesthetic resulted in shorter anesthetic times. Anesthetic times in This study are similar to those reported elsewhere for greenling, *Hexagrammos otakii* and winter flounder, *Pleuronectes americanus* anesthetized with lidocaine-HCl (Park et al., 2003, 2004). The dose response of Siberian sturgeon to lidocaine-HCl followed a negative exponential curve, with increasing doses resulting in less time to stage A6 anesthesia. The relationship between water temperature and anesthesia time followed a negative exponential curve, with increasing water temperatures resulting in decreased anesthesia times. The relationship between anesthetic effects and water temperature was identical to that reported in many other species anesthetized by clove oil, lidocaine-HCl, and MS-222, including Atlantic sturgeon *Acipenser oxyrinchus*, European sea bass, *Dicentrarchus labrax*, gilthead sea bream, *Sparus aurata*, marine medaka, and Persian sturgeon, *A. persicus* (Constantinos et al., 2005;

Imanpoor et al., 2010; Matsche, 2011; Park et al., 2011). For these species, lower temperatures resulted in significantly longer anesthesia induction and recovery times (ANOVA, $P<0.001$; Constantinos et al., 2005). Also, studies on kelp grouper, *Epinephelus bruneus* and Siberian sturgeon anesthetized with clove oil and greenling anesthetized with lidocaine-HCl showed similar relationships (Park et al., 2003; Akbulut et al., 2012). Akbulut et al. (2012) anesthetized small Siberian sturgeon (standard length: 10.0±0.93 cm; body weight: 4.1±0.95 g) with 300 ppm clove oil and found an anesthesia time of 322±28.28 s, while those anesthetized with 300 ppm lidocaine-HCl had an anesthesia time of only 32±1.7 s. Lidocaine-HCl has also been found to immobilize marine medaka at lower doses more effectively compared with clove oil at the same anesthesia time.

The plasma cortisol and glucose levels observed in this experiment were indicative of stress responses. Plasma cortisol and glucose levels are recognized as useful indicators of stress in fish (Schreck, 1982; Park et al., 2008) and were reported to be elevated in red drum, *Sciaenops ocellatus* that were exposed to MS-222 and quinaldine simultaneously (Massee et al., 1995). Barton and Iwama (1991) stated that "Usually, phenomenon that plasma cortisol concentration of fishes rises by stress is first order reaction and phenomenon that plasma glucose concentration rises is result of second-order reaction by hormone rise reaction by stress."This trend has been reported in the gray mullet, *Mugil cephalus* and kelp grouper (Chang & Hur, 1999; Park et al., 2008). Das et al. (2004) suggested that increased glucose utilization

due to an increase in cell metabolism during early exposure may have inhibited the increase in blood glucose, even though glycogenolysis would have increased during this period (Martinez-Alvarez et al., 2002). However, reduced glucose use later during the exposure period (after 48 hrs) resulted in an increase in blood glucose levels because of dysfunctional cell metabolism. This results showed that plasma cortisol concentrations increased more quickly than did glucose concentrations, similar to the findings of Chang and Hur (1999) and Park et al (2008).

One of the more traditional stress indicators is blood lactic acid levels (Pickering & Pottinger, 1989). Experiments have shown that chronically stressed animals have higher lactic acid concentrations (Wedemeyer et al., 1990). The accumulation of lactic acid in muscle tissue or blood (hyperlactatemia) is a well-accepted indicator of anaerobic metabolism due to fright or severe exertion (Turner et al., 1983). However, others have challenged the view that lactic acidosis is the ultimate cause of death that can occur after extreme exertion (Wood et al., 1983).

I found that the optimal anesthesia interval of lidocaine-HCl was 4 days in Siberian sturgeon, and frequent anesthesia treatment caused negative effects by inhibiting sensitivity. Unfortunately, no previous studies have elucidated the sensitivity and re-anesthesia effects of lidocaine-HCl or other anesthetics in fish species. This study demonstrated that lidocaine-HCl is an effective anesthetic for Siberian sturgeon. The relationship between the anesthetic effect and size of Siberian sturgeon was also clarified. Lidocaine is

also a sodium channel blocker and local anesthetic that has previously been used for anesthesia in marine medaka with large margin of safety (Park et al., 2011). This chemical has been considered an alternative candidate to MS-222 based on its low cost and ease of preparation. The results of this experiment contribute to the safe laboratory handling of Siberian sturgeon by anaesthetic lidocaine-HCl, which is critical to research (e.g., morphometric experiments) and aquaculture. Other further investigations on Siberian sturgeon should focus on comparative physiological reactions induced by anesthetics. For example, the mechanism by which lidocaine-HCl shows anesthetic effect on Siberian sturgeon. It is necessary to confirm that there is no side effect of residual lidocaine-HCl. Furthermore, it may be necessary to further investigate anesthetic effect in other sturgeon species other than Siberian sturgeon.

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Anesthetic Effects and Physiological Responses of Lidocaine-HCl in Siberian Sturgeon, *Acipenser baerii*

(이학박사: 염산리도카인에 대한 시베리안 철갑상어, *Acipenser baerii*의 마취효과 및 생리반응)

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14. Hwang, J.-A., **I.B. Goo**, J.E. Kim, M.H. Kim, D.H. Kim, J.H. Im, H.-S. Choi & J.H. Lee. **2016**. Growth comparison of Israeli carp (*Cyprinus carpio*) to different breeding combination. ***Dev. Reprod.***, 20: 275~281.

15. **Goo, I.B.**, J.-A. Hwang, D.H. Kim, J.H. Lee, H.-S. Choi, S.G. Lim, H.K. Han, H.W. Gil & I.-S. Park. **2017**. Histological changes of Korean rose bitterling (*Rhodeus uyekii*) organs in the early growth period. ***Tur. J. Fish. Aquat. Sci.***, 17:581~588.

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17. Kim, J.E., **I.B. Goo**, J.-A. Hwang, H.S. Kim, H.-S. Choi & J.H. Lee. **2018**. Genetic variability comparison of cultured Israeli carp (*Cyprinus carpio*) from Korea using microsatellites. ***Genes & Genomics***., 40: 635~642.

18. **Goo, I.B.**, I.-S. Park, C.H. Park & Y.G. Nam. **2019**. Anesthetic effects

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ACADEMIC ACTIVITIES

1. Park, I.-S., H.J. Park, H.W. Gil & **I.B. Goo**. **2012**. Early growth and morphogenesis of the eye in larval dark banded rockfish, *Sebastes inermis*. ***Fish Barcode of Life World Conference***. *12-14 June, 2012.* Expo 2012 Yeosu, Yeosu, Korea.
2. **Goo, I.B.**, K.B. Seong, D.S. Kim & I.-S. Park. **2012**. Effect of starvation on the morphometric characteristics and histological changes in chum salmon, *Oncorhynchus keta* fries. ***Korea Society of Fisher and Aqua Science Spring Meeting***, *22 June, 2012.* Gyeongsang National Universit, Tongyoung, Korea.
3. Park, I.-S., H.W. Gil, **I.B. Goo**, J.W. Choi & D.S. Kim. **2012**. Sexual dimorphism and sexual behavior of marine medaka, *Oryzias dancena.* ***Korean Society of Developmental Biology 31th Annual Meeting***. *7 September, 2012.,* Seoul National University Hospital, Seoul, Korea.
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8. **Goo, I.B.**, H.W. Gil & I.-S. Park. **2013**. Stimulation of spermiation by human chorionic gonadotropin and carp pituitary extract in grass puffer, *Takifugu niphobles*. ***Korean Society of Developmental Biology 32th Annual Meeting***. 30 August, 2013, Seoul National University Hospital, Seoul, Korea.
9. Lim, S.G., H.K. Han, J.H. Kang, **I.B. Goo**, H.W. Gil & I.-S. Park. **2013**. Cytogenetic characteristics of Cyprinidae between diploid and spontaneous triploid in major river of Korea. ***Korean Society of***

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10. Park, I.-S., H.W. Gil & **I.B. Goo**. **2013**. Morphmetric characteristics of diploid and triploid far eastern catfish, *Silurus asotus*. ***Korean Society of Developmental Biology 32th Annual Meeting***. *30 August, 2013,* Seoul National University Hospital, Seoul, Korea.
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14. Im, S.Y., H.W. Gil, **I.B. Goo**, S.G. Lim, H.G. Han, H.J. Kong, B.S. Kim, C.M. Ahn & I.-S. Park. **2014**. Anesthetic effects and physiological responses of clove oil, lidocaine-HCl and tricaine methanesulphonate

on Korean seawater shellfishes. ***International Conference of Fishery Sciences Association***, 2 July, 2014. BEXCO, Busan, Korea.

15. **Goo, I.B.**, H.W. Gil & I.-S. Park. **2014**. Comparative analysis of histological changes in Ussurian bullhead, *Leiocassis ussuriensis*, and Korean bullhead, *Pseudobagrus fulvidraco*, in the early period of growth. ***Korean Society of Developmental Biology 33th Annual Meeting***. 12 September, 2014, Seoul National University Hospital, Seoul, Korea.
16. Gil, H.W., **I.B. Goo**, I.-S. Park, B.S. Kim, H.J. Kong, H.S. Kim, C.M. Ahn, S.G. Lim & C.H. Kim. **2014**. Cytogenetic analysis of diploid, triploid and tetraploid in Korean rose bitterling, *Rhodeus uyekii*. ***Korean Society of Developmental Biology 33th Annual Meeting***. 12 September, 2014, Seoul National University Hospital, Seoul, Korea.
17. Gil, H.W., **I.B. Goo**, I.-S. Park, B.S. Kim H.J. Kong, H.S. Kim, C.M. Ahn, S.G. Lim & C.H. Kim. **2014**. Cytogenetic analysis of diploid, triploid and tetraploid in Korean rose bitterling, *Rhodeus uyekii*. ***World Aquaculture 2015 Jeju***. 28 May, 2015, Jeju Jung Moon Convention Center, Jeju, Korea.
18. **Goo, I.B.**, H.W. Gil, C.H. Lee, J.H. Im, H.-S. Choi, J.H. Lee & I.-S. Park. **2015**. Histological changes of Korean rose bitterling (*Rhodeus uyekii*) organs in the early growth period. ***International Conference of Fishery Sciences Association***, 30 October, 2015. BEXCO, Busan, Korea.

19. Hwang, J.-A., **I.B. Goo**, J.E. Kim, B.M. Jeong, J.S. Park, D.H. Kim, H.S. Kim & J.H. Lee. **2017**. Susceptibility of koi x red common carp, and red common carp x koi to koi herpesvirus (KHV). ***Annual Meeting of The Korean Society of Marine Life Science***. 22 September, 2017. National institute of Fisheries Science, Busan, Korea.

20. Kim, J.E., B.M. Jeong, **I.B. Goo**, J.-A. Hwang, M.H. Kim, J.S. Kim, H.S. Kim, H.S. Choi & J.H. Lee. **2017**. Genetic variability comparison of cultured Israeli carp (*Cyprinus carpio*) using microsatellite. ***Annual Meeting of The Korean Society of Marine Life Science***. 22 September, 2017. National institute of Fisheries Science, Busan, Korea.

21. **Goo, I.B.**, B.M. Jeong, J.H. Im, H.S. Kim & J.H. Lee. 2017. Morphological research of Korean and Chinese strains in Israeli carp, *Cyprinus carpio*. ***Annual Meeting of The Korean Society of Marine Life Science***. 22 September, 2017. National institute of Fisheries Science, Busan, Korea.

AWARDS

1. ***Award of Excellent Presentation***: Fishery Sciences Association of Korea. Fall Meeting, No. 2013-30, BEXCO, 22 November, 2013.

2. ***Award of Excellent Poster Presentation***: International Conference of Fishery Sciences Association, No. 2015-28, BEXCO, 30 October,

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