

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives

Editors

Mark Smith
Doug Warmolts
Dennis Thoney
Robert Hueter



Published by
Ohio Biological Survey, Inc.

Ohio Biological Survey

Special Publication

ISBN-13: 978-0-86727-152-3

ISBN-10: 0-86727-152-3

Library of Congress Number: 2004115835

Publication Director

Brian J. Armitage

Editorial Committee

Barbara K. Andreas, Ph. D., Cuyahoga Community College & Kent State University
Brian J. Armitage, Ph. D., Ohio Biological Survey
Benjamin A. Foote, Ph. D., Kent State University (Emeritus)
Jane L. Forsyth, Ph. D., Bowling Green State University (Emeritus)
Eric H. Metzler, B.S., The Ohio Lepidopterists
Scott M. Moody, Ph. D., Ohio University
David H. Stansbery, Ph. D., The Ohio State University (Emeritus)
Ronald L. Stuckey, Ph. D., The Ohio State University (Emeritus)
Elliot J. Tramer, Ph. D., The University of Toledo

Literature Citation

Smith, M., D. Warmolts, D. Thoney, and R. Hueter (editors). 2004. The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives. Special Publication of the Ohio Biological Survey. xv + 589 p.

Cover and Title Page

Illustration by Rolf Williams, The National Marine Aquarium, Rope Walk, Coxside, Plymouth, PL4 0LF United Kingdom

Distributor

Ohio Biological Survey, P.O. Box 21370, Columbus, Ohio 43221-0370 U.S.A.

Copyright

© 2004 by the Ohio Biological Survey

All rights reserved. No part of this publication may be reproduced, stored in a computerized system, or published in any form or in any manner, including electronic, mechanical, reprographic, or photographic, without prior written permission from the publishers, Ohio Biological Survey, P.O. Box 21370, Columbus, Ohio 43221-0370 U.S.A.

Layout and Design: Brian J. Armitage, Ohio Biological Survey
Printing: The Ohio State University, Printing Services, Columbus, Ohio

Ohio Biological Survey
P.O. Box 21370
Columbus, OH 43221-0370
<ohiobiosurvey@sbcglobal.net>
www.ohiobiologicalsurvey.org

11-2004—1.5M

Chapter 21

Immobilization of Elasmobranchs

M. ANDREW STAMPER

The Living Seas
Walt Disney World Resorts
2020 North Avenue of the Stars
Lake Buena Vista, FL 32830, USA.
E-mail: andy.stamper@disney.com

Abstract: Anesthesia is a procedure that may be required during the husbandry and clinical care of elasmobranchs. Elasmobranchs may be anesthetized using tonic immobility or a suitable immersion or injectable anesthetic. Combinations of anesthetic drugs can yield a smoother anesthesia, and reversal agents can ease the process of recovery or prematurely end anesthesia if required. Variability of reaction to an anesthetic depends on many factors including body temperature, liver function, kidney function, reaction with non-target tissues, drug binding sites, injection site, specimen size and weight, seasonal variation in specimen body composition, gill area to body weight ratio, specimen age, nutritional status, lipid content, stress, and disease status. Monitoring a specimen before, during, and after anesthesia is critical to success. In particular, ventilation rate, heart rate, and, if possible, blood gas and lactate values should be monitored and recorded. The pharmacology of anesthetics used in elasmobranchs is a developing area of study and merits a great deal more research.

There is much debate about the question of whether fish experience “pain” or not. Recent philosophical and physiological endeavors have determined that fish do not have the required neural structures (or alternative neural systems) for producing the “pain” experience (Rose, 2002). Yet fish display physiological stress responses to noxious stimuli. For this reason alone, appropriate anesthetic regimes should be given when handling fish in stressful situations or performing invasive sampling or surgical procedures. Much of the information for safe and effective anesthetic procedures in many aquatic species, including elasmobranchs, is still anecdotal. Furthermore, with so many species of elasmobranchs, each with its own physiological niche, potentially wide variations in environmental conditions, as well as changes in physiological status, the outcome of an anesthetic procedure can be difficult to manage. The relatively recent alliance of veterinary science and fish biology can greatly widen the current state of elasmobranch medicine, with the veterinary community providing

a wider breadth of regulated anesthetics and clinical skills. This chapter highlights issues to consider when anesthetizing elasmobranchs and identifies areas to be investigated in the realm of aquatic animal anesthesia.

VARIABILITY OF DRUG INTERACTIONS

Variability of reaction to a drug depends on many factors:

1. The body temperature of most elasmobranchs is dependent on ambient temperatures (poikilothermic), but some species do have the ability to elicit an endothermic (internal heat generation) response (e.g., the porbeagle shark, *Lamna nasus*, the mako shark, *Isurus oxyrinchus*, and the white shark, *Carcharodon carcharias*) (Muñoz-Chápuli and Satchell, 1999). This endothermic response could theoretically affect enzyme activity and may affect biochemical reactions and drug interactions.

2. The liver metabolizes many drugs to either active or inactive forms. The weight and composition of the liver varies between elasmobranch species, but can account for up to 23% body weight of which as much as 80% may be fat (Holmgren and Nilsson, 1999). The elasmobranch liver contains a greater percentage of oils than other vertebrate species. The amount of oil in elasmobranchs will affect chemical reactions since the pharmacodynamics of drugs depends on their lipophilic ("fat-loving") and lipophobic ("fat-avoiding") solubility.
3. The kidneys of sharks are different to those of mammals, having a higher filtration rate and differing selectivity (Lacy and Reale, 1999), influencing the elimination rate of drugs. Drug elimination is dependant on blood flow. The renal-portal system is an anatomical adaptation that allows blood from the caudal half of the animal to drain directly into the kidneys. This arrangement may have direct consequences for drugs that are nephrotoxic or are eliminated directly by the kidneys. Although a concern in reptiles, recent studies indicate that this risk may not be as critical as at first thought (Beck et al., 1995) and may not pose a threat to elasmobranchs. Regardless, it would be prudent to err on the side of caution when using potentially nephrotoxic drugs.
4. In any species of animal, drugs can react with blood proteins or bind with non-target tissue, thus altering the amount of drug available and, therefore, the effect on target tissue.
5. Drug binding sites are the molecular locations on target tissue that bind the drug to produce the desired effect. Sharks are different biochemically from other vertebrates, and drugs developed for mammals may result in several possible outcomes in elasmobranchs: (a) they may bind to the same active binding site and produce the desired effect; (b) the binding site may be slightly different or there may be a lower number of binding sites, resulting in a weakened response; (c) there may be no binding sites at all, resulting in no effect; or (d) there may be a chemically similar binding site but one that triggers a different physiological function, resulting in a completely different response.
6. Injection site may result in a variable reaction to drugs. Not only is there the concern of

getting the drug into the muscle or vasculature, but some species have regional heterothermy (Bernal and Graham, 2001; Totland et al., 1981) and thus a variable density of vasculature which may result in unpredictable drug uptake.

The protective nature of shark denticles and epidermis requires the use of heavy needles (16-18 gauge) to penetrate the tough integument. Additional problems may occur because the skin of the shark does not have a great degree of contractibility and muscle is at a positive resting potential, resulting in possible leakage of the drug from the injection site. The author recently designed a device to collect fluids leaking from injection sites and noted considerable drug loss during initial trials. Three relatively tranquil sand tiger sharks (*Carcharias taurus*) lost 3-16% of a drug (0.9% saline) when injected with 5.0 ml, at a depth of 25 mm, using an 18 gauge needle. These losses could account, at least in part, for observed variation in reactions to drugs within a species. To minimize drug leakage, it may be possible to angle the needle either anteriorly or posteriorly, depositing the drug away from the injection site to reduce leakage. Recently the author has used a Teleinject dart (Teleinject USA Inc., Aqua Dulce, California, USA) and left the dart in the animal until it has become sedated. This technique appears to reduce leakage of the drug.

Other factors that may influence an effective anesthetic protocol in elasmobranchs include: size and weight (body condition), seasonal variation (body composition), gill area to body weight ratio, age (sexual maturity and variation in body composition), nutritional status, lipid content, stress, and disease status. These factors should all be considered when attempting to determine safe and effective anesthetic drugs for elasmobranchs.

STAGES OF ANESTHESIA

The different stages of anesthesia have been summarized in Table 21.1. It should be noted that different animals do not exhibit anesthetic stages equally, nor do all drugs elicit all of the indicated stages in an equivalent manner.

MONITORING

Before a drug is administered, several parameters should be monitored for reference purposes. A

Table 21.1. The different stages of anesthesia. Please note that all animals will not necessarily exhibit stages equally, nor will drugs elicit all of the stages as described. Modified from Soma (1971) and Stoskopf (1993).

Stage	Plane	Description	Corresponding Behavioural response
Stage 0		Normal	Swimming actively, reactive to external stimuli, muscle tone and equilibrium normal.
Stage 1			Subjective in nature. Disorientation.
	Plane 1	Light sedation	Voluntary swimming continues; slight loss of reactivity to visual and tactile stimuli; respiratory rate, equilibrium, and muscle tone normal.
	Plane 2	Deep sedation	Voluntary swimming stopped; total loss of reactivity to visual and tactile stimuli; slight decrease in respiratory rate; equilibrium normal; muscle tone slightly decreased; still responds to positional changes.
Stage 2	Plane 1	Light narcosis	This stage is also known as the excitement phase. There is a loss of consciousness and subsequent excitement (uninhibited action, uncoordinated movements, struggling, exaggerated response to painful stimuli, and spinal reflexes). Efforts to right self, muscle tone decreased, and still weakly responds to positional changes. Respirations can be irregular.
	Plane 2	Deep narcosis	Ceases to respond to positional changes; decrease in respiratory rate to approximately normal; total loss of equilibrium; no efforts to right self; muscle tone decreased; some reactivity to strong tactile and vibration stimuli; suitable for external sampling (e.g., gill biopsy).
Stage 3			Four planes with increasing depression of respiration, circulation, protective reflexes, and muscle tone.
	Plane 1	Light anesthesia	Total loss of muscle tone; responds to deep pressure; further decrease in respiratory rate; suitable for minor surgical procedures.
	Plane 2-4	Surgical anesthesia	Respiratory rate very low; heart rate slow.
Stage 4		Medullary collapse	Represents complete respiratory arrest. Cardiac arrest will ensue unless anesthetic regime is not modified.

safe and quiet environment should be available to the animal since other sharks in the tank may disturb or prey on immobilized animals. Ventilation rates should be taken if the animal is actively pumping water through the gills and not ram-ventilating (i.e., using forward motion to force water through the gills). Caudal fin strokes can be measured to gauge the initial effects of the drug, as this activity is usually the first to be reduced when drugs take effect. After the animal becomes recumbent (i.e., lying down), respiration rates and righting reflex should be closely monitored. If respiration ceases, heart rate should be monitored using an ultrasound or Doppler unit placed over the region of the heart. Monitoring can be done with the animal in or out of the water.

If the animal is stable, respiratory and cardiac parameters should be monitored every 2-5 minutes and trends such as changes in rhythm or speed noted. Although animals may be ventilating well, a lowered heart rate and increased resistance to circulatory flow through the gill capillaries, as erythrocytes accumulate within the capillary bed and become swollen, can cause hypoxia (Tyler and Hawkins, 1981). A better way to monitor respiratory efficiency is to obtain periodic blood gas samples. These samples enable the worker to determine blood O_2 , CO_2 , and pH, as well as lactic acid levels. Blood gas analyses are usually based on arterial samples, but these are difficult to obtain from sharks. Venous samples are not true indicators of the

animal's current physiological status, but they do give useful information about trends. Handheld analyzers are expensive but offer the most rapid monitoring system. Blood gas units are frequently built to accommodate mammalian body temperatures, but many units allow the observer to calibrate for lower body temperatures (e.g., ambient water temperature). Lactic acid is often a good parameter to determine an animal's physiological state; upward trends are of concern, but further investigations need to be performed to determine what levels indicate that an animal is at risk.

VARIOUS TECHNIQUES OF IMMOBILIZATION

Physical methods

The following have been described as methods of anesthesia used for non-invasive procedures. The first two methods are not recommended due to possible subclinical pathophysiological (clinically unnoticeable but physiologically damaging) effects.

Electronarcosis

Electronarcosis or galvanonarcosis is performed by the application of an uninterrupted direct electric current that induces immobility of the animal (Harthoorn, 1976).

Hypothermia

Submerging animals in cold water can induce immobility. This process has been done alone, or in combination with chemical anesthesia (Schoettger, 1967).

Tonic Immobility (TI)

Tonic immobility, also known as hypnosis, is immobility induced by turning an animal upside-down for a period of time. Both batoids and shark species such as leopard sharks (*Triakis semifasciata*), whitetip reef sharks (*Triaenodon obesus*), blacktip reef sharks (*Carcharhinus melanopterus*), Caribbean reef sharks (*Carcharhinus perezi*), swellsharks (*Cephaloscyllium ventriosum*), shovelnose guitarfish (*Rhinobatos lentiginosus*), clearnose skates (*Raja eglanteria*), cownose rays (*Rhinoptera bonasus*), and southern stingrays (*Dasyatis americana*) have been restrained using

tonic immobility, though variation is great between species. For a list of potential variations refer to Henningsen (1994).

Chemical methods

Immersion anesthesia

Immersion or inhalation anesthesia has the advantage of being safe to deliver. The drugs can be modified through addition or dilution. However, a major disadvantage, especially with large sharks, is the large amount of drug needed to accomplish the task. Furthermore, the use of immersion drugs in large bodies of water is usually not practical or economically feasible.

Injectable anesthesia

Injectable anesthesia can have several advantages over immersion anesthesia. If performed carefully, injection anesthesia can allow animals to be captured in large exhibits without the expense of excessive employee time and capital. Delivery can be achieved by hand injection, pole syringe, Hawaiian sling, or remotely, through an underwater dart gun (Harvey et al., 1988). Several injectable drugs have been investigated in sharks, but use is currently experimental. Unfortunately, at the time of writing, no injectable anesthesia on batoids has been reported.

ROUTES USED FOR INJECTABLE ANESTHETICS

Intravenous (IV)

Intravenous injection is the fastest and most reliable method of anesthetic delivery, producing a rapid induction and often short duration of anesthesia. The disadvantage of IV application is that the animal has to be appropriately restrained in order to deliver the drug. One of the most suitable blood vessels for IV injection in sharks and rays is the vein that lies along the midline, just ventral to the vertebral column. Locating this vein can be done by placing the needle just posterior to the trailing edge of the first or second anal fins and holding it at an angle of 30-90° relative to the length of the shark, directed anteriorly (refer to Figure 29.1 in Chapter 29). When the needle is inserted ~4 cm, for a 10 kg shark, it should penetrate the vein. This

technique is suitable for catheterization and drug administration (Stoskopf et al., 1984).

Catheterization refers to the introduction of a catheter into a vessel, allowing direct access to the vasculature and thus permitting medications to be given in a direct and consistent manner, and possibly over a long period of time, if the catheter is secured in place. This process can be accomplished through the use of a Tuohy needle.

In larger sharks, needles have the potential to become plugged with cartilage since they have to penetrate a cartilage wall protecting the vessel. In this case, a spinal needle (with a removable stylet protecting the needle aperture) can be used. Intravenous injections can be given in the lateral portal vessel, the dorsal lymph vessel, and even the heart itself by direct cardiac puncture (Tyler and Hawkins, 1981).

A study by Walker (1972) using indigo cyanine green found a circulation time of 1-2 minutes when the marker was injected into the caudal vein of the tail. Slower circulation has been further reflected in cases where intravenous drug onset times were correspondingly slow.

Intraperitoneal (IP)

Intraperitoneal (into the body cavity) injection is another avenue for drug administration. In this case, the sedative must pass through the serosal membrane that lines all the organs of the coelomic cavity, making anesthetic induction time erratic. Inserting the needle at an acute angle directed anteriorly to the pelvic girdle, on the right side of the specimen, minimizes the possibility of puncturing any internal organs and causing unnecessary damage to the specimen (refer to Figure 29.1 in Chapter 29).

Intramuscular (IM)

Intramuscular injection is possible to deliver by hand, to slow-swimming sharks, or by remote-injection devices. Regardless of the avenue of IM administration, injection time is minimal, reducing handling times. The bulk of muscle tissue in sharks has a poor blood supply, limiting the number of effective injection sites. The best region for IM administration is the dorsal saddle, an area of musculature surrounding the first dorsal fin, extending laterally to just above the lateral line, and longitudinally from the posterior gill slit to a point

halfway between the first and second dorsal fins (Stoskopf et al., 1984) (refer to Figure 29.1 in Chapter 29).

Skeletal-muscular movement helps circulate blood and lymph (Gruber and Keyes, 1981). This movement has a direct impact on drugs that are delivered IM, since they may not be circulated to the appropriate tissues if the animal is sedentary. Consequently, anesthetic induction time may be erratic or delayed and the injection of large volumes of drugs may form a sterile abscess in the musculature (Tyler and Hawkins 1981).

ANESTHETIC AGENTS

The following drug information is a composite of documented anesthetic protocols in peer-reviewed venues. Table 21.2 provides additional anecdotal drug information for various species of elasmobranchs. The author does not take responsibility for doses or protocols presented in this formulary. Drug dose experiences will be added periodically to the web-based version of this manual and such contributions, which should conform to the format established within the manual, are encouraged.

Immersion anesthetics

Benzocaine

Most work with benzocaine has been performed on teleosts. Benzocaine is similar in action to MS-222 (see below), but is much less soluble in water unless first dissolved in acetone or ethanol. Advantages include its high potency, quick onset of effect, relatively high margin of safety, and its relatively low cost (Larid and Oswald, 1975; Tyler and Hawkins, 1981). As with MS-222, Tyler and Hawkins (1981) report some subsequent physiological changes, most likely due to hypoxia resulting from anesthetic-induced respiratory suppression, decreased cardiac rate, increased resistance to circulatory flow through gill capillaries, and a swelling of erythrocytes resulting in their accumulation within the gill capillaries.

Etomidate or metomidate

Etomidate and metomidate (Aquacalm®, Syndel International, Inc., Canada) provide a more rapid induction and recovery time than MS-222. In sandbar sharks (*Carcharhinus plumbeus*), 10 mg

Table 21.2. Elasmobranch anesthetic drug formulary showing anecdotal information for various species of elasmobranch.

Drug name	Species name	Common name	Body weight (kg)	Body length (cm)	Water temperature (°C)	Dose
Tricaine methanesulfonate	<i>Carcharhinus melanopterus</i>	blacktip reef shark	-	-	24.0-25.5	50-125 mg l ⁻¹ buffered
	<i>Carcharhinus plumbeus</i>	sandbar shark	-	-	24.0-25.5	50-125 mg l ⁻¹ buffered
	<i>Cephaloscyllium ventriosum</i>	swellshark	-	-	14.0	50-125 mg l ⁻¹ buffered
	<i>Heterodontus francisci</i>	horn shark	-	-	14.0	50-125 mg l ⁻¹ buffered
	<i>Pteroplatytrygon violacea</i>	pelagic stingray	50.0 kg	100 cm DW	20.0	80-100 mg l ⁻¹
	<i>Squatina californica</i>	Pacific angelshark	-	-	10-14	80-100 mg l ⁻¹
	<i>Stegostoma fasciatum</i>	zebra shark	-	-	24.0-25.5	50-125 mg l ⁻¹ buffered
	<i>Torpedo californica</i>	Pacific electric ray	0.5 kg	50 cm DW	10-14	80-100 mg l ⁻¹
	<i>Triaenodon obesus</i>	whitetip reef shark	-	-	24.0-25.5	50-125 mg l ⁻¹ buffered
	<i>Triakis semifasciata</i>	leopard shark	-	-	14.0	50-125 mg l ⁻¹ buffered
Diazepam	<i>Carcharhinus plumbeus</i>	sandbar shark	-	-	-	1.2-1.6 mg kg ⁻¹
	<i>Carcharias taurus</i>	sand tiger shark	-	-	-	0.1 mg kg ⁻¹
Butorphanol	<i>Taeniura lymma</i>	bluespotted ribbontail ray	-	-	-	0.5 mg kg ⁻¹
Detomidine + Ketamine	<i>Carcharhinus leucas</i>	bull shark	-	-	-	0.2 mg kg ⁻¹
			85.7 kg	-	23.0	128 µg kg ⁻¹

of either of these drugs provides stage 2 induction in approximately 2-4 minutes. Increasing to 20 mg reduces the induction time to less than a minute but anesthetic depth is much more difficult to control. Etomidate is considerably more potent than metomidate in freshwater teleosts, but no noticeable differences were observed in sandbar sharks. Recovery from these drugs is as rapid as the induction. Recovery from stage 2 plane 2 is approximately 3-5 minutes for metomidate. Recovery from deeper planes can be considerably

prolonged (e.g., >1 hour) which might be due to decreased cardiac output (Stoskopf, 1986).

Halothane-oxygen-nitrous oxide

Anesthesia is achieved by using a medical vaporizer to mix the three gasses and subsequently introduce them into the water via an aeration bubbler. Dunn and Koester (1990) have reported an initial administration of 1.5% halothane, 100-200 ml

Please note that the author does not take responsibility for doses or protocols presented in this formulary.

Dose of combination drug	Route	Times used	Comments	Reference
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l ⁻¹ for 10-60 minutes (<i>Carcharhinus melanopterus</i> : 45 minutes). Recovery: good. Observations: with some ram ventilators, start with high dose (usually 50-100 mg l ⁻¹) in a round transport tank until they go down, then switch to a manageable regime for physical examinations, work ups, etc. Make sure oxygenated water is running through the gills. 50% of blacktip sharks will stop breathing and are prone to capture myopathy, so method of capture critical. Animals suffering from capture myopathy may recover slowly and exhibit increased LDHs and CPKs.	Mylniczzenko, pers. com.
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l ⁻¹ for 10-60 minutes (<i>Carcharhinus plumbeus</i> : 45 minutes). Recovery: good.	Mylniczzenko, pers. com.
-	Immersion	n = ~50	Time to handling: 15 minutes. Induction: 15 minutes. Maintenance: good if kept at 50-75 mg l ⁻¹ for 10-60 minutes. Recovery: good, 15-20 minutes.	Mylniczzenko, pers. com.
-	Immersion	n = ~50	Time to handling: 15 minutes. Induction: 15 minutes. Maintenance: good if kept at 50-75 mg l ⁻¹ for 10-60 minutes. Recovery: good, 15-20 minutes.	Mylniczzenko, pers. com.
-	Immersion	n = 20	Time to handling: up to 10-15 minutes at 100 mg l ⁻¹ (most other species succumb quicker). Maintenance: good, preferable to maintain anesthesia for <5 minutes to avoid problems.	Ezcurra, pers. com.
-	Immersion	-		Ezcurra, pers. com.
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l ⁻¹ for 10-60 minutes (<i>Stegostoma fasciatum</i> : 1 hour). Recovery: good.	Mylniczzenko, pers. com.
-	Immersion	-		Ezcurra, pers. com.
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l ⁻¹ for 10-60 minutes (<i>Triaenodon obesus</i> : 1 hour). Recovery: good.	Mylniczzenko, pers. com.
-	Immersion	n = ~50	Time to handling: 15 minutes. Induction: 15 minutes. Maintenance: Good if kept at 50-75 mg l ⁻¹ for 10-60 minutes. Recovery: good, 15-20 minutes.	Mylniczzenko, pers. com.
See comments	PO	-	Supplementation: Initial dose followed by MS-222 staged anesthesia. Observations: oxygenated water should be pumped over the gills regardless of species.	Mylniczzenko, pers. com.
-	IM	n = 20	Time to handling: 20 minutes. Maintenance: good for 5 hours. Observation: each animal had a different reaction and therefore required dosage. Assess level of activity, following the initial dose, by manipulating the caudal and dorsal fin after 20 minutes of post-dosage swimming. If activity is low then basic husbandry procedures may be performed. If activity is still too high after 30 minutes post-dosage, supplement the induction dose with an additional 20% of the anesthetic.	McEwan, pers. com.
-	IM	n = 2	Observations: bradycardia and cessation of respiration. Reversed: Naloxone IV at 0.01 ml kg ⁻¹ .	Mylniczzenko, pers. com.
-	IM	-		Mylniczzenko, pers. com.
5 mg kg ⁻¹	IM	n = 1	Time to handling: no affect. Induction: poor. Maintenance: poor. Recovery: no affect. Observations: injected while free-swimming with pole syringe. Injection believed to be complete. Reversal: Yohimbine given despite no anesthesia observed.	Walsh, pers. com.; Stamper, personal observation.

minute⁻¹ nitrous oxide, and 200-300 ml minute⁻¹ oxygen. Maintenance levels were reduced to 0.5-0.8% halothane, 100-200 ml minute⁻¹ nitrous oxide, and 200-300 ml minute⁻¹ oxygen. Reportedly, easy control over anesthetic depth, shorter recovery times, and a high survival rate are some of the advantages of this regime. A disadvantage is that the vapors will contaminate the room, so precautions must be taken to protect personnel.

Oxygen

Oxygen has a sedative effect on some species. Oxygenated water is flushed across the gills of the elasmobranch by bubbling 100% oxygen in front of a water current directed into the mouth of the animal. Elevated dissolved oxygen levels (concentration dependent on temperature and height above sea level) will usually have a sedative effect. Caution must be exercised as prolonged exposure to elevated oxygen can result

Table 21.2 (continued). Elasmobranch anesthetic drug formulary showing anecdotal information for various species

Drug name	Species name	Common name	Body weight (kg)	Body length (cm)	Water temperature (°C)	Dose	
Medetomidine + Ketamine	<i>Carcharhinus acronotus</i>	blacknose shark	-	-	25.5	59.2-70.4 µg kg ⁻¹	
	<i>Carcharhinus leucas</i>	bull shark	85.7 kg	-	23.0	90 µg kg ⁻¹	
	<i>Carcharhinus plumbeus</i>	sandbar shark	-	-	24.5	87 µg kg ⁻¹	
	<i>Carcharias taurus</i>	sand tiger shark	-	-	24.0	70.0-80.0 µg kg ⁻¹	
				-	-	21.0-22.0	60.0-80.0 µg kg ⁻¹
	<i>Chiloscyllium plagiosum</i>	whitespotted bambooshark	-	-	23.4	60 µg kg ⁻¹	
	<i>Ginglymostoma cirratum</i>	nurse shark	20.0 kg	-	-	75 µg kg ⁻¹	
			68.0 kg	-	-	90 µg kg ⁻¹	
				-	-	70-100 µg kg ⁻¹	
	<i>Negaprion brevirostris</i>	lemon shark	-	-	26.0	90 µg kg ⁻¹	
<i>Triaenodon obesus</i>	whitetip reef shark	-	-	27.0	90 µg kg ⁻¹		

in toxicity through depressed ventilation and an associated rise in blood CO₂ concentrations. If left unchecked, acidosis will ensue resulting in potentially life-threatening acid-base imbalances (Spotte, 1992). Signs of oxygen narcosis include depressed respiratory effort, behavioral changes, loss of equilibrium, and eventually death if the animal is not carefully monitored and the regime regulated.

Quinaldine

Quinaldine (e.g., Quinaldine, Synergy, India) has been successfully used in the past (Gruber and Keyes, 1981), although specifics have not been given.

Tricaine Methane Sulfonate (MS-222)

MS-222 (Finquel®, Argent Laboratories, USA) is a water-soluble narcotic that is a derivative to p-aminobenzoic acid. Both sharks and batoids can be anesthetized using 75-95 mg l⁻¹ of MS-222. MS-222 may be added slowly to evaluate effect. If greater doses are used, then the solution should be buffered with sodium bicarbonate, especially when inducing animals in open systems with a hand pump, as water containing high concentrations of MS-222 can become extremely acidic. Gilbert and Kritzler (1960) found that 1.0 g l⁻¹ of MS-222 delivered via a hand sprayer could be used to anesthetize large sharks and rays. Gilbert and Wood (1957) describe a technique of first

of elasmobranch. Please note that the author does not take responsibility for doses or protocols presented in this formulary.

Dose of combination drug	Route	Times used	Comments	Reference
2.82 mg kg ⁻¹	IM	-	Time to handling: 10-11 minutes. Induction: excellent. Maintenance: excellent. Recovery: excellent, within 5 minutes. Observations: injected while manually restrained. Reversal: full dose IV.	Stamper, personal observation.
4.5 mg kg ⁻¹	IM	n = 1	Time to handling: 69 minutes. Induction: poor. Maintenance: good. Recovery: prolonged, 1 hour and 26 minutes. Observations: Injected with pole syringe. Specimen considered to be abnormal. Specimen became recumbent after handling; re-dosed with half supplemental dose. Reversal: 2x reversal dose (equivalent doses given IM and IV) at 54 minutes.	Walsh, pers. com.; Stamper, personal observation.
5.4 mg kg ⁻¹	IM	-	Time to handling: 20 minutes. Induction: excellent. Maintenance: good. Recovery: good. Observations: injected while free-swimming with pole syringe. Reversal: full dose IV.	Author's experience
4 mg kg ⁻¹	IM	n = 2	Time to handling: 20 minutes. Induction: excellent. Maintenance: excellent. Recovery: good, 20 minutes. Observations: injected while free-swimming with pole syringe. Reversal: full dose IV.	Author's experience
5.0-10.0 mg kg ⁻¹	IM	-	Time to handling: 4-18 minutes. Induction: excellent. Maintenance: excellent. Recovery: poor to good, 12-20 minutes. Observations: injected while manually restrained (a single specimen was injected while free-swimming). Specimens had scoliosis and lordosis. One animal was euthanized for medical reasons. Reversal: specimen 1 (anesthetized for 20 minutes) was given atipamezole IM and doxapram IV after 15 minutes; specimen 2 was given doxapram; specimen 3 was given atapamizole (50% IM and 50% IV) and swam away 18 minutes later with no noticeable side effects.	Stamper, personal observation.
3 mg kg ⁻¹	IM	-	Time to handling: 20 minutes. Induction: fair. Maintenance: poor. Recovery: good. Observations: injected while manually restrained. Reversal: full dose IV.	Author's experience
7.5 mg kg ⁻¹	IM	n = 1	Time to handling: 10 minutes. Induction: good. Maintenance: good, surgical, but some movement. Recovery: good. Observations: injected while manually restrained. Supplemented with MS-222	Mulican, pers. com.
9 mg kg ⁻¹		n = 1	Time to handling: 50 minutes. Induction: fair, still active. Maintenance: fair, still moving. Recovery: good, ~1 hour. Observations: injected with pole syringe.	Walsh, pers. com.; Stamper, personal observation.
5-7 mg kg ⁻¹	IM	-	Observations: poor to mild sedation. Medetomidine often repeated at 5 mg kg ⁻¹ and ketamine at 30 µg kg ⁻¹ .	Mylniczenko, pers. mom.
4.5 mg kg ⁻¹	IM	n = 2	Time to handling: 30 minutes. Induction: poor. Maintenance: undetermined. Recovery: prolonged, >24 hours. Observations: injected while manually restrained. Both specimens "blanched" after drug administration. Leakage noted from injection site (amount undetermined). Specimen became recumbent after handling. Reversal: full dose IV.	Walsh, pers. com.; Stamper, personal observation.
4.5 mg kg ⁻¹	IM	n = 1	Time to handling: 30 minutes. Induction: fair to good. Maintenance: fair to good. Recovery: good, 20 minutes. Observations: injected while manually restrained. Specimen had scoliosis and infection; considered to be abnormal. Specimen stopped gilling unless touched. Reversal: 2x reversal dose (equivalent doses given IM and IV).	Walsh, pers. com.; Stamper, personal observation.

bringing large sharks up to the surface of the water with a hook and line and then applying a high concentration of 1.0 g l⁻¹ MS-222 using a hand sprayer. Affects were noted within 10 seconds and the animals were anesthetized within a minute. It is recommended that the head of the patient remain out of water and the MS-222 should be buffered when applied directly to the gills. A direct linear relationship exists between the concentration of MS-222 and the time required to achieve muscular relaxation (Dunn and Koester, 1990). Dunn and Koester (1990) found that a large number of elasmobranch species can be anesthetized for surgery (i.e., stage 3) using 75-95 mg l⁻¹ MS-222, but that species-specific responses were common. Many sharks have been

anesthetized using a low dose of 50 mg l⁻¹ MS-222 as a "pre-anesthetic" dose, followed by doses of up to 85 mg l⁻¹ MS-222 (Davis, pers. com.). This "pre-anesthetic" dose appears to reduce the excitement phase and lower the overall maintenance level of MS-222.

MS-222 excretion in the spiny dogfish (*Squalus acanthias*) was primarily through the gills and excretion rate was a function of cardiac output (Maren, et al., 1968). Elimination of MS-222 into the water can result in a positive feedback of increasing anesthetic concentration, if the heart slows, resulting in a possible overdose if animals are not closely monitored.

Injectable anesthetics

Alfaxalone-alfadolone

Alfaxalone-alfadolone (Saffan[®], Pitman-Moore, Inc., USA) is a chemical that can be administered intramuscularly via dart gun. Alfaxalone-alfadolone has been administered to the spiny dogfish at 1.5 ml kg⁻¹ (stage 3 anesthesia; n=2), the brown ray (*Raja miraletus*) at 0.2-0.3 ml kg⁻¹ (stage 2 anesthesia; n=2), the skate (*Dipturus batis*) at 0.2 ml kg⁻¹ (stage 2 anesthesia; n=1), the black tip shark (*Carcharhinus limbatus*) at 0.4 ml kg⁻¹ (stage 2 anesthesia; n=1), and the spotted eagle ray (*Aetobatus narinari*) at 0.3 ml kg⁻¹ (stage 1 anesthesia; n=1) (Harvey et al., 1988), demonstrating the great variability of this drug between species.

Azaperone

Azaperone (Stresnil, Pitman-Moore, Inc., USA) is a butyrophenone tranquilizer that reduces response to the environment without motor impairment or sedation. Preliminary studies in spiny dogfish showed the most efficacious application of azaperone is directly over the gills rather than by injection. No effect was noted when animals were injected with the drug intramuscularly; however, when 4 mg kg⁻¹ of the drug were deposited on the gill filaments, and the animal held out of water for several seconds, an effect was observed (Latas, 1987). Following dosing with the drug or placebo, both exposed and control animals were left undisturbed for 4 hours. Thereafter, both groups were caught for blood sampling. Drugged animals showed no flight response when compared to control animals. Blood glucose levels were not depressed in animals exposed to azaperone and they fed the next day, compared to several days of anorexia in control animals. Drugged animals were capable of negotiating tank walls and returned to normal behavior within 24 hours. The advantages of using this drug include uninterrupted swimming patterns, normal gill ventilation, and normal cardiovascular function. Azaperone may be useful for animals that are prone to panic, aggression, and self-induced trauma (Latas, 1987).

Carfentanil citrate

Carfentanil citrate (Wildnil[®], Wildlife Pharmaceuticals, Inc., Canada) is a potent narcotic analogue of fentanyl, an agent commonly

used in veterinary medicine. Carfentanil citrate failed to achieve any effect when given at 0.25 mg kg⁻¹ to a nurse shark (*Ginglymostoma cirratum*) and a lemon shark (*Negaprion brevirostris*). Even when administered at massive doses no effect was observed (Stoskopf, 1986; Stoskopf, 1993).

Detomidine hydrochloride

Detomidine hydrochloride (Dormosadan[®], Pfizer, Inc., USA) is an alpha-2 adrenergic and is of the same family of drugs as xylazine, although more potent. Detomidine can be used with ketamine (refer to injectable anesthetic combinations which follow), and is reversed with yohimbine and/or atipamezole.

Ethanol

Larger sharks have been injected intraperitoneally with 47.5% ethanol (Sudak, 1966). For animals weighing up to 113 kg, 1.1 ml kg⁻¹ were used, whereas larger animals received 0.55 ml kg⁻¹. Animals were visually unaffected for 50 minutes post-injection, but could be in dorsal recumbency for up to an hour (due to a lack of control animals, it is not clear whether this was a state of tonic immobility). Animals were reported to show effects of the alcohol after 3-4 hours, but were fine after 24 hours. The types of effects were not stated. Sudak's (1966) study indicated that a dusky shark (*Carcharhinus obscurus*) died during anesthesia. Having lacerations to its snout, the shark may have impacted an obstruction due to its decreased ability to maneuver.

Ketamine hydrochloride

Ketamine hydrochloride (Ketaset[®], Fort Dodge Animal Health, USA) is an analgesic and cataleptic cyclohexamine. Ketamine provides good peripheral analgesia (pain relief) in mammals through suppression of dorsal horn cell activity in the spinal cord, but provides little visceral analgesia. In addition, seizure-like muscle spasms due to spinal reflex firing are occasionally noted (Stoskopf, 1993). [Refer to injectable anesthetic combinations which follow.]

Medetomidine

Medetomidine (Dormitor[®], Pfizer Inc., USA) is an alpha-2 adrenergic of the same family of drugs

as xylazine and detomidine, but much more potent. Medetomidine has been used in combination with ketamine in several shark species (refer to injectable anesthetic combinations which follow).

Propofol

Propofol (Diprivan®, AstraZeneca Pharmaceuticals, USA) is a sedative-hypnotic that is a relatively new drug in exotic animal medicine. The advantage of using propofol is quick induction time and rapid metabolism, achieving surgical plane relatively quickly. Propofol is easily titrated (i.e., small incremental doses using a drip system), with non-cumulative effects, and recovery is swift once drug supply has been discontinued. The disadvantages of propofol is that it causes respiratory depression, it must be given intravenously, and it is expensive with a limited shelf life. Mitchell et al. (2001) gave 2.5 mg kg⁻¹ propofol to whitespotted bamboo sharks (*Chiloscyllium plagiosum*) over 30 seconds and the sharks achieved a surgical plane of anesthesia after 5 minutes. Righting response returned within 60 minutes in four of the sharks, and 75 minutes in the other two. No changes in respiration or cardiac effects were noted throughout the procedure.

Teletamine

Teletamine (Telazol®, Fort Dodge Animal Health, USA) is chemically related to ketamine and generally more potent in mammalian species when given in combination with zolazepam, a relative of diazepam (refer to injectable anesthetic combinations which follow).

Sodium pentobarbital

Sodium pentobarbital (Dibutal® (60 mg ml⁻¹) Diamond Laboratories, Des Moines, Iowa, USA) has been used for a satisfactory general surgical anesthesia in nurse sharks (n=9) when given as a rapid IV injection at 10 mg kg⁻¹ or less (Walker, 1972). Slow injections resulted in erratic responses. Intraperitoneal delivery was shown to be the slowest and most unreliable, while intramuscular injection resulted in only slightly improved responses unacceptable for sedation. Serum half-life is approximately 15 seconds with a second half-life of several days, due to an inability of the animals to excrete the drug through

their gills or kidneys. An intravenous dose of 10 mg kg⁻¹ resulted in a loss of gilling or ventilatory movements within a minute of injection. Gilling returned after 10 minutes and a weak righting response was observed at 3 hours. An intravenous dose of 20 mg kg⁻¹ resulted in a loss of gilling within a minute. Gilling returned after 3 hours and a weak righting response was observed after 5 hours. A high intravenous dose of 60 mg kg⁻¹ resulted in animal death. It appears that larger, more active sharks require smaller doses per kilogram than smaller, sedentary specimens. Specifically, sandbar sharks and bull sharks responded similarly to the nurse shark (at 10 mg kg⁻¹) when given 6 mg kg⁻¹ IV (Walker, 1972).

Xylazine

Xylazine (Rompun®, Bayer, Inc., Germany) is a thiazine derivative, distantly related to the phenothiazine tranquilizers. It is a convulsant in teleosts and causes major changes in the electrocardiogram (Oswald, 1978). [Refer to injectable anesthetic combinations which follow.]

Other injectable agents

Alternative, less well understood, barbiturates include pentobarbitone sodium (Nembutal®, Abbott Laboratories, Inc., USA), pentothal sodium (Pentothal®, Abbott Laboratories, Inc., USA), and tubcurare (Curare®, Abbott Laboratories, Inc., USA). Each of these drugs has been used to anesthetize elasmobranchs, although Gruber and Keyes (1981) claim that MS-222 resulted in a better overall anesthetic event.

Injectable anesthetic combinations

Several combinations of drugs have been or are currently being investigated. Xylazine has been used in combination with ketamine in several shark species to ameliorate the muscle spasms that can occur with ketamine alone, although individual and species variation has been noted (Stoskopf, 1993). Stoskopf (1986) found 12 mg kg⁻¹ ketamine and 6 mg kg⁻¹ xylazine to be an effective anesthetic combination. Andrews and Jones (1990) found 16.5 mg kg⁻¹ ketamine and 7.5 mg kg⁻¹ xylazine to be a safe regime for two male and five female adult sandbar sharks during a 4-hour transport. During an 8-hour simulated transport, an additional four mature female

sandbar sharks were immobilized safely using the same protocol. These animals reached a stage 1 plane 2 anesthesia.

Teletamine / zolazepam was tested unsuccessfully on a lemon shark, when dosed at 12 mg kg⁻¹, and a sand tiger shark, at an unstated dosage. The animals displayed irritability, rapid swimming, and unrestrained biting (Stoskopf, 1986; Stoskopf, 1993).

Medetomidine has been used in combination with ketamine in several shark species to ameliorate muscle spasms that can occur with ketamine alone (Snyder et al., 1998). However, there appears to be a great variation of reaction between species when given under similar conditions and doses (author's experience). For future investigations a starting dose of 0.09-0.10 mg kg⁻¹ medetomidine and 4-5 mg kg⁻¹ ketamine should be employed.

The efficacy of medetomidine in elasmobranchs is unknown. A combination of medetomidine and ketamine has been tried in a single bull shark (*Carcharhinus leucas*), on two separate occasions, with little to no effect (author's experience).

CANDIDATE ANESTHETIC AGENTS

Drugs that have not been documented in the scientific literature but should be investigated either alone or in combination with other anesthetics include the following:

Eugenol (clove oil)

Eugenol is an over-the-counter drug that has been used in teleosts, but is yet to be formally described in elasmobranchs (Sladky et al., 2001).

Diazepam

Diazepam (Valium®, F. Hoffmann-La Roche Ltd., Switzerland) is a benzodiazepine. Diazepam, having an injectable and oral form, causes sedation in many species of animals, and has been used as an anticonvulsant. Diazepam is often used with ketamine to prevent seizures and provides a synergistic response (i.e. less of each drug is required), however, there are anecdotal reports of erratic responses.

Midazolam hydrochloride

Midazolam hydrochloride (Versed®, F. Hoffmann-La Roche Ltd., Switzerland) is a benzodiazepine only found in injectable form. In mammals, midazolam is shorter acting than diazepam and is reportedly amnesic (i.e., causes loss of memory) when given to humans (Connor, 2001).

REVERSAL AGENTS

Reversal agents are those that reverse or ameliorate the effects of anesthetic agents.

Atipemazole

Atipemazole (Antisedan®, Pfizer, Inc., USA) is a reversal agent for medetomidine and is given in equal volumes to medetomidine (equating to 5 times the microgram dose). The present recommendation for sharks is to give a full reversal dose intravenously and a full induction dose intramuscularly.

Doxapram hydrochloride

Doxapram hydrochloride (Dopram®, A. H. Robins Company, USA) has been touted as an anesthetic reversal agent; however, it has been noted to produce dramatic arousal in elasmobranchs (Stoskopf, 1986) and should be considered more as a stimulant. Doxapram does not competitively bind to the anesthetic's binding site, but rather causes stimulation of an unknown origin and should be used with caution because animals can be extremely excitatory and dangerous under the influence of this drug.

Yohimbine hydrochloride

Yohimbine hydrochloride (Watson Laboratories, Inc., USA) has been used to reverse alpha-2 adrenergics, predominantly xylazine. Yohimbine has been administered intravenously to a nurse shark and was noted to cause arousal after the animal had been previously sedated using a combination of ketamine and xylazine (Stoskopf, 1986).

Flumazenil

Flumazenil (Romazicon®, F. Hoffmann-La Roche Ltd., Switzerland) has been used to reverse the effects of the benzodiazepines such as diazepam

and midazolam. Flumazenil is most effective in mammals when given intravenously, but can be given intramuscularly.

SUPPORTIVE CARE AND EMERGENCY DRUGS

Sharks and rays under anesthesia should be carefully monitored. Animals exhibiting slowing respiration, and especially slowing heart rate, should be placed in fresh seawater or seawater with lower concentrations of anesthetic. If the animal is anesthetized with an injectable anesthetic, the reversal counterpart should be given in a partial or full dose depending on the deterioration of vital signs. Animals not responding to these tactics can be given fluids. When applied, fluid therapy needs to take into account the osmotic balance of the animal and the three major plasma components which account for osmoregulation in elasmobranchs: urea, NaCl, and trimethylamine oxide. An elasmobranch balanced salt solution can be made by adding 8.0 g l⁻¹ NaCl and 21.02 g l⁻¹ urea to phenol red-free Hank's balanced salt solution (Andrews and Jones, 1990). Freshwater given orally at 1-3% body weight can be beneficial.

If anesthetized animals continue to decline, doxapram can be given (see above). Elasmobranchs tend to be sensitive to doxapram and may respond with explosive excitement so caution should be exercised when giving this drug. Other traditional mammalian emergency drugs, such as epinephrine or corticosteroids, can be given in the case of physiological collapse, but the effects are not well understood (for more information about emergency drugs and shock therapies please refer to Chapter 29 of this manual).

FUTURE STUDIES

Drug anesthetic and pharmacokinetic studies are desperately needed in elasmobranch medicine. For nonprofit organizations it is often possible to co-publish a study by partnering with the anesthesia department of a university veterinary or medical school. Usually the aquarium can be responsible for providing the study animals and the collection of samples, whereas the university can provide expertise in regard to anesthetic protocols and laboratory analyses.

A model of a typical anesthetic study is outlined below. It is imperative that the reader recognize the following to be an example only. An

anesthesiologist should be contacted prior to any study to critique methodology. Depending on the institution, you may need an institutional review before experiments can proceed. Research institutions may need an animal research permit (e.g., USDA permit) as well.

Model of an anesthesia research project

A minimum of seven animals (animals can serve as their own controls) should be used for the pilot study, but more animals may have to be examined if variability is great. Sex and age should be considered since this may influence results. Prior to a drug trial each animal should be weighed (kg), examined visually, and have blood drawn for a complete blood count, using Natt-Herrick's stain techniques (Campbell, 1988), and serum chemistry (including lactate) analysis.

The elasmobranchs should be held individually in identical recirculating systems. Water parameters such as salinity, temperature, ammonia, nitrite, nitrate, calcium, etc., should be the same in all systems. Details of the recirculating system components should be documented, including flow rate, pump type, size of tanks and their configuration, and use of heating or cooling elements. Manufacturer addresses should be noted for all components.

Animals should be assigned blindly drawn numbers to randomly divide them into treatment groups of equal number. Each animal should receive a single dose of a known amount (mg kg⁻¹) of drug. Drug name, manufacturer name and address, route of administration (IM, IV, IP, or immersion), size of needles, size of syringes, and rate of delivery should all be noted. A description of how it was assured that the drug was properly administered should be included.

Prior to administering the anesthetic, the following need to be documented: date; environmental temperature; specimen weight; specimen health status; pre-dosage fasting time; initial ventilation (gilling) if the animals are not ram ventilating; activity level of the animal just prior to induction (calm, active, or excited); demeanor of the animal just prior to induction (depressed, alert, aggressive, or apprehensive); physical status (healthy or status of illness); the immobilizing conditions (single animal or school, etc.); environmental conditions (large enclosure or small enclosure); manual restraint or free swimming; and body condition (obese, good, fair, thin, poor, emaciated, etc.).

During the immobilization the following should be noted: drug and dose (amount in mg or percentage); route; time given; delivery success; effect of the drug (i.e. no effect, mild sedation, heavy sedation, light anesthesia, surgical anesthesia, excessively deep, or death); time until initial effect; time of animal recumbency; and the time from discontinuation or reversal (amount, placement, and time of drug administration).

Once the animal is in hand, the following should be monitored and documented at predetermined time intervals: ventilation rates (if it is not being ram ventilated); heart rate and rhythm (either through ultrasound or Doppler probe); and core temperature (note: take care not to damage the spiral colon).

Fresh blood may be analyzed for blood O₂, CO₂, and lactic acid levels with a hand-held blood gas unit. Ambient water temperature and supplemental O₂ (added to the water) need to be documented and considered when analyzing blood gases. Note whether intra-operative fluids have been given and at what rate. Finally, note whether the animal was kept in dorsal or ventral recumbency.

After anesthesia the following should be rated and recorded: induction time; muscle relaxation; anesthesia (e.g. poor, fair, good, excellent); complications (e.g. none, minor, major, fatal); recovery time; recovery process (e.g. normal, abnormal, prolonged, or stormy); and overall anesthesia success (e.g. complete, partial, or none).

ACKNOWLEDGEMENTS

The author would like to thank Mike Walsh for initiating this project, as well as access to his literature collection. I would like to thank Ilze Berzins for her editorial review.

REFERENCES

- Andrews, J. C. and R. T. Jones. 1990. A method for the transport of sharks for captivity. *Journal of Aquaculture and Aquatic Sciences* 5: 70-72.
- Beck, K., M. Loomis, G. Lewbart, L. Spelman, and M. Papich. 1995. Preliminary comparison of plasma concentrations of gentamycin injected into the cranial and caudal limb musculature of the eastern box turtle (*Terrapene carolina carolina*). *Journal of Zoo and Wildlife Medicine* 26: 265-268.
- Bernal, S. D. C. and J. B. Graham. 2001. Water-tunnel studies of heat balance in swimming mako sharks. *Journal of Experimental Biology* 204: 4043-4054.
- Campbell, T. 1988. *Avian Hematology and Cytology*. Iowa State University Press, Ames, IA, USA. 104 p.
- Connor, E. P. (ed.). 2001. *Physicians' Desk Reference*. Medical Economics Company, Inc. Montvale, NJ, USA. 3506 p.
- Dunn, R. F. and D. M. Koester. 1990. Anesthetics in elasmobranchs: A review with emphasis on halothane-oxygen-nitrous oxide. *Journal of Aquaculture and Aquatic Sciences* 5(3): 44-52.
- Gilbert, P. W. and H. Kritzler. 1960. Experimental shark pens at the Lerner Marine Laboratory. *Science* 140: 424.
- Gilbert, P. W. and F. G. Wood. 1957. Method of anesthetizing large sharks and rays safely and rapidly. *Science* 126: 212-213.
- Gruber S. H. and R. S. Keyes. 1981. Keeping sharks for research. *In: Aquarium Systems*, p. 373-402. A. D. Hawkins (ed.). Academic Press, Harcourt Brace Jovanovich, London, United Kingdom. 452 p.
- Harthorn, A. M. 1976. *The chemical capture of animals. A guide to the chemical restraint of wild and captive animals*. Baillier Tindall, London, United Kingdom, 416 p.
- Harvey, D., C. Denny, S. Kaiser, and J. Young. 1988. Remote intramuscular injection of immobilizing drugs into fish using a laser-aimed underwater dart gun. *Veterinary Record* 122(8): 174-177.
- Henningsen, A. 1994. Tonic immobility in 12 elasmobranchs: Use as an aid in captive husbandry. *Zoo Biology* 13: 325-332.
- Holmgren, S. and S. Nilsson. 1999. Digestive System. *In: Sharks, Skates, and Rays. The Biology of Elasmobranch Fishes*, p. 144-173. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA. 515 p.
- Lacy, E. and E. Reale. 1999. Urinary System. *In: Sharks, Skates, and Rays. The Biology of Elasmobranch Fishes*, p. 353-397. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA.
- Larid, L. M. and R. L. Oswald. 1975. Benzocaine (ethyl p-aminobenzoate) as a fish anesthetic. *Fisheries Management* 64: 92-93.
- Latas, P. J. 1987. The use of azaperone in the spiny dogfish (*Squalus acanthias*). *In: International Association for Aquatic Animal Medicine Annual Proceedings*. p. 157-165. May 10-14, 1987, Monterey California. Veterinary Software Publishing Inc., O'Fallon, IL, USA.
- Maren, T. H., R. Embry, and L. E. Broder. 1968. The excretion of drugs across the gill of the dogfish, *Squalus acanthias*. *Comparative Biochemistry and Physiology* 26: 853-864.
- Mitchell, M. A., S. M. Miller, J. J. Heatley, T. Wolf, F. Lapuz, and J. A. Smith. 2001. Clinical and cardiorespiratory effects of propofol in the white spotted bamboo shark (*Chiloscyllium plagiosum*). *In: Proceedings of the 26th annual meeting of the American College of Veterinary Anesthesiologists*, October 11-12, New Orleans, Louisiana. *Veterinary Anaesthesia and Analgesia* Vol. 29(2) pp. 97-112. Blackwell Syngery Malden, MA, USA.
- Muñoz-Chápuli, R. and Satchell G. H. 1999. Circulatory System: Anatomy of the Peripheral Circulatory System. *In: Sharks, Skates, and Rays. The Biology of Elasmobranch Fishes*, p. 198-218. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA. 515 p.
- Oswald, R. L. 1978. Injection anesthesia for experimental studies in fish. *Comparative Biochemistry and Physiology*, 60C: 19-26.
- Rose, J. D. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science* 10(1): 1-38.
- Schoettger, R. A. 1967. MS-222 as an anesthetic for channel catfish; Its toxicity, efficacy, and muscle residues. Investigations in fish control. U.S. Dept. Interior, Bureau of Sport Fisheries and Wildlife, Technical Paper, pp 1-14.
- Sladky, K. K., C. R. Swanson, M. K. Stoskopf, M. R. Loomis, and G. A. Lewbart. 2001. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). *American Journal of Veterinary Research* 62(3): 337-342.

- Snyder, S. M. J. Richard, I. K. Berzins, and M. A. Stamper. 1998. Immobilization of sand tiger sharks (*Odontaspis taurus*). *In: Proceedings of the International Association for Aquatic Animal Medicine*, May 2-6, San Diego, CA. Volume 29: 120-121 p. Veterinary Software Publishing Inc., O'Fallon, IL, USA.
- Soma, L. R. (ed.). 1971. *Textbook of Veterinary Anesthesia*. Williams and Wilkins Company, Baltimore, MD, USA. 621 p.
- Spotte, S. 1992. *Captive Seawater Fishes*. John Wiley & Sons, New York, USA. 977 p.
- Stoskopf, M. K. 1986. Preliminary notes on the immobilization and anesthesia of captive sharks. *Erkrankungen Der Zootiere*. Akademie-Verlag, Berlin 28: 145-151.
- Stoskopf, M. K. 1993. Shark Pharmacology and Toxicology. *In: Fish Medicine*, p. 809-816. M. K. Stoskopf (ed.). W. B. Saunders, Inc., Philadelphia, Pennsylvania, USA. 882 p.
- Stoskopf, M. K., B. Smith, and G. Klay. 1984. Clinical note; Blood sampling of captive sharks. *Journal of Zoo and Wild Animal Medicine* 15: 116-117.
- Sudak, F. N. 1966. Immobilization of large sharks by means of ethanol. *Copeia* 3: 611-612.
- Totland, G. K., H. Kryvi, Q. Bone, and P. R. Flood. 1981. Vascularization of the lateral muscle of some elamobranchiomorph fishes. *Journal of Fish Biology* 18(2): 223-234.
- Tyler, P. and A. D. Hawkins. 1981. Vivisections, anaesthetics and minor surgery. *In: Aquarium Systems*, p. 248-278. A. D. Hawkins (ed.). Academic Press, Harcourt Brace Jovanovich, London, United Kingdom. 452 p.
- Walker, M. D. 1972. Physiologic and pharmacologic aspects of barbiturates in elasmobranchs. *Comparative Biochemistry and Physiology* 42(A): 213-221 pp.

PERSONAL COMMUNICATIONS

- Davis, R. 2002. Sea World, Orlando, FL 32821, USA.
- Ezcurra, M. 2001. Monterey Bay Aquarium, Monterey, CA 93940, USA.
- McEwan, T. 2002. The Scientific Centre, Salmiya, 22036, Kuwait.
- Mulican, T. 2002. The Newport Aquarium, Newport, KY 41071, USA.
- Mylniczzenko, N. D. 2002. The John G. Shedd Aquarium, Chicago, IL 60605, USA.
- Walsh, M. 2002. Sea World, Orlando, FL 32821, USA.