Fish Sedation, Anesthesia, Analgesia, and Euthanasia: Considerations, Methods, and Types of Drugs

Donald L. Neiffer and M. Andrew Stamper

Abstract

Fish display robust neuroendocrine and physiologic stress responses to noxious stimuli. Many anesthetic, sedative, or analgesic drugs used in other vertebrates reduce stress in fish, decrease handling trauma, minimize movement and physiologic changes in response to nociceptive stimuli, and can be used for euthanasia. But extrapolating from limited published anesthetic and sedative data to all fish species is potentially harmful because of marked anatomic, physiologic, and behavioral variations; instead, a stepwise approach to anesthetizing or sedating unfamiliar species or using unproven drugs for familiar species is advisable. Additionally, knowledge of how water quality influences anesthesia or sedation helps limit complications. The most common method of drug administration is through immersion, a technique analogous to gaseous inhalant anesthesia in terrestrial animals, but the use of injectable anesthetic and sedative agents (primarily intramuscularly, but also intravenously) is increasing. Regardless of the route of administration, routine preprocedural preparation is appropriate, to stage both the animals and the supplies for induction, maintenance, and recovery. Anesthetic and sedation monitoring and resuscitation are similar to those for other vertebrates. Euthanasia is most commonly performed using an overdose of an immersion drug but injectable agents are also effective. Analgesia is an area in need of significant research as only a few studies exist and they provide some contrasting results. However, fish have μ and κ opiate receptors throughout the brain, making it reasonable to expect some effect of at least opioid treatments in fish experiencing noxious stimuli.

Key Words: analgesia; anesthesia; chemical restraint; drugs; elasmobranch; euthanasia; fish; sedation; teleost

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Introduction

Whether or not fish feel pain is an ongoing debate. Although both sides of the argument set the same requirements for pain perception, opinions differ on where the criteria appear phylogenetically and whether or not parallel systems have evolved in different classes and species (Chandroo et al. 2004; Huntingford et al. 2006; Rose 2002; Sneddon 2003; Sneddon et al. 2003; Volpato 2009). Each publication builds on or refutes earlier works, and a chronological evaluation of the literature is recommended (Neiffer 2007).

Many anesthetic or analgesic drugs used in other vertebrates have been used in fish. In this review we focus on the practical considerations for these uses.

Chemical Restraint in Fish

Indications for the Use of Chemical Restraint in Fish

The use of chemical restraint increases safety for both the fish and the handler during minor procedures and allows them to be performed out of the water with decreased stress for the fish (Harms and Bakal 1995); during major or surgical procedures it minimizes movement and physiologic changes in response to nociception (Harms and Bakal 1995; Myszkowski et al. 2003; Ross 2001). Chemical restraint also reduces excitement and hyperactivity-related trauma that can occur during routine handling and thus directly reduces mortality and morbidity (Cooke et al. 2004; Harms 1999; Kumlu and Yanar 1999; Myszkowski et al. 2003; Ross 2001). The decrease in movement minimizes integument damage, associated osmoregulatory disturbances, and increased susceptibility to pathogens (Kumlu and Yanar 1999; Ross 2001), and it reduces metabolism, resulting in decreased oxygen demand and the production of less waste (i.e., CO_2 and ammonia) (Cooke et al. 2004; Crosby et al. 2006; Guo et al. 1995; Hoskonen and Pirhonen 2004; Ross and Ross 1984).

Chemical Restraint, Stress, and Immunosuppression

Physical restraint of fish activates the hypothalamo-pituitary-interrenal (HPI) axis, resulting in a cortisol release that causes secondary stress responses (Bressler and Ron 2004;

Myszkowski et al. 2003; Ross and Ross 1984; Small 2003, 2005). Chemical restraint minimizes handling stress, but it is stressful (Bressler and Ron 2004) and is a strong potentiator of catecholamine release (Rothwell et al. 2005). Although unbuffered immersion drugs induce a stress response (Ross and Ross 1984), anesthesia-associated catecholamine release is likely due to hypoxemia rather than direct drug effects or acidemia (Ross and Ross 1984; Rothwell et al. 2005). Hypoxemia in fish is usually due to drug-induced hypoventilation (decreased buccal movement). In anesthetized air-breathing fish, it can be exacerbated by preventing access to the water's surface (Rantin et al. 1993, 1998). Hypoventilation, and the resulting decreased water flow in the buccal cavity, usually leads to reflex bradycardia and dorsal aortic hypotension, producing a progressive hypoxemia (Ross 2001). Notwithstanding these negative effects, chemical sedation or anesthesia produces a lower stress response than drug-free handling and transport, based on a comparison of circulating cortisol levels as well as secondary indicators such as blood glucose, hematocrit (HCT), hemoglobin (Hgb), lactate, and osmolarity (Bressler and Ron 2004; Crosby et al. 2006; Hseu et al. 1996; Small 2005).

Some researchers have argued that the stress-induced corticosteroid response is not harmful and is in fact essential for recovery from severe acute or prolonged stressors, and that a transient, relatively small elevation of cortisol does not necessarily reduce immunocompetency but may instead bolster it (Bressler and Ron 2004; Davis and Griffin 2004; Small 2003; Thomas and Robertson 1991). Based on this argument, drugs that suppress the HPI (e.g., metomidate, the only commonly used drug that consistently and significantly blocks HPI activation in a broad range of species) are contraindicated in fish (Davis and Griffin 2004; Iversen et al. 2003; Olsen et al. 1995; Small 2003; Thomas and Robertson 1991). However, the contrary argument is that typical husbandry and handling procedures do not result in high stress levels associated with chronic immunosuppression (Bressler and Ron 2004; Davis and Griffin 2004; Small 2003; Thomas and Robertson 1991). For anesthetics that do not block the HPI, the intensity and duration of the stress response depend on either the duration of exposure or the drug, dosage, and species (Bressler and Ron 2004; Gomes et al. 2001; Thomas and Robertson 1991).

In addition to affecting the stress response in some fish species, certain anesthetic/sedative drugs result in immunosuppression, either through direct interaction with immune components or indirectly through the nervous system (Bressler and Ron 2004). For example, benzocaine significantly depresses both humoral and cellular immune responses in gilthead seabream (Bressler and Ron 2004). Metomidate, through its associated blockade of the HPI, is thought to prevent immunosuppression (Davis and Griffin 2004).

Considerations of Fish Taxonomy, Anatomy, and Physiology

Of the nearly 30,000 species of fish,[1] teleosts constitute approximately 96%, so most anesthesia studies involve these and, to a lesser extent, elasmobranchs (sharks, rays, and skates) and other fish. But given the anatomic, physiologic, and behavioral variation among fish, extrapolation of the limited published data to all species is potentially harmful. Development of anesthetic regimens for unfamiliar species-drug combinations will benefit from an understanding both of fish natural history and of the taxonomic relationship between the species of concern and more studied species.

Respiration

Species-Related Respiratory Methods

All fish have gills, although reliance on these structures for respiration varies by species. Most force water over the gills through rhythmic movements of the lower jaw and opercula, but some use ram ventilation with minimal opercular movement. Among this latter group, some (e.g., tuna) are obligate ram ventilators—species that meet their respiratory demand by ensuring an adequate flow of water across the gills through constant forward motion with minimal or no opercular movement. For these species, failure to perfuse the gills during anesthesia causes suffocation (Brill and Bushnell 2001; Bushnell and Jones 1994).[2]

In response to hypoxic environments or other selective pressures, many species have evolved anatomical, physiologic, and behavioral adaptations for breathing (Graham 1997). Such adaptations include modifications of gill design for improved oxygen extraction as well as the evolution of accessory respiratory organs, which for some species enable the use of atmospheric air. In many species, increased aerial gas exchange surface exists in portions of the anterior alimentary canal (i.e., the buccal and pharyngeal cavities) either as a direct proliferation of the respiratory surface in the lumen or as a single pouch or pair of pouches extending from it (Ishimatsu and Itazawa 1993). Alternatively, branchial diverticulae exist, as in anabantoids (e.g., gouramis, bettas) (Graham 1997).

Many species take in atmospheric air through aquatic surface respiration (ASR), positioning their mouths to skim the air/water interface, which is richer in oxygen. Some species (e.g., pacu, *Piaractus mesopotamicus*) respond to hypoxic conditions by developing temporary dermal swellings of the lower jaw to facilitate ASR (Rantin et al. 1993, 1998). Others (e.g., snakeheads, anabantoids) employ alternate filling of an air-breathing chamber (labyrinth organ in anabantoids) with air and water during aerial ventilation (Ishimatsu and Itazawa 1993).

[1] For comparison, there are some 10,000 bird species and only about 50 rat and fewer than 40 mouse species.

[2] In addition, some ram ventilating species rely on constant forward speed to produce lift from their pectoral fins for hydrostatic equilibrium (Brill and Bushnell 2001). During recovery it is necessary to manually move these animals through water in a forward motion or hold them in sufficient flow until adequate voluntary forward motion returns.

Lungfish and polypterids (birchirs) possess true lungs (not gas or "swim" bladders), with pneumatic duct openings in the alimentary canal (Graham 1997). Of these, the African (*Propterus* sp.) and South American (*Lepidosiren* sp.) lungfish are obligate air breathers (Bassi et al. 2005; Graham 1997).

Responses to Hypoxia

At the onset of a hypoxic event or upon entering a hypoxic environment, the fish's physiological goal is to continue to provide energy and oxygen to the brain. Freshwater cyprinids (carps, goldfish) respond by increasing blood glucose; this supports the production of glycolytic adenosine triphosphate (ATP), which in turn increases cerebral blood flow through its vasodilation effects. An increase in hematocrit (which increases the blood's oxygen-carrying capacity; Routley et al. 2002; Söderström et al. 1999) and the inhibitory neurotransmitter gamma-aminobutyric acid (GABA, which contributes to neuroprotection; Mulvey and Renshaw 2009) also occurs. In contrast, the epaulette shark (*Hemiscyllium ocellatum*) shows no increase in glucose, hematocrit, or GABA levels (Mulvey and Renshaw 2009; Routley et al. 2002; Söderström et al. 1999); instead, vasodilation occurs, as indicated by a drop in both blood pressure (up to 50%, but it is not adenosine mediated) and heart rate. The result is the maintenance of cerebral blood flow, but it is not increased as is the case with teleosts (Söderström et al. 1999). Although blood glucose remains low in the dogfish (*Scyliorhinus canicula*) during hypoxia in the laboratory (Butler et al. 1979) it is not clear whether the response of dogfish or other elasmobranchs to hypoxia is otherwise similar to that of the epaulette shark.

Few marine species have evolved adaptations for hypoxic environments other than surface piping or migrating from the area. These species may therefore be less tolerant of hypoxic conditions during anesthesia than the freshwater species discussed above (Rothwell et al. 2005). Notable exceptions do exist, however, particularly among species that inhabit tidal zones. Among teleosts, the tarpon (*Megalops atlanticus*) is able to cruise in low-oxygen waters by gulping surface air, while the oyster toadfish (*Opsanus tau*) can tolerate 20 hours of anoxia at 22°C in the laboratory (Ultsch et al. 1981). Where elasmobranchs are concerned, studies have shown that the torpedo ray (*Torpedo marmorata*; Hughes 1978), Pacific spiny dogfish (*Squalus acanthias*; Sandblom et al. 2009), and epaulette shark (Mulvey and Renshaw 2009; Routley et al. 2002; Söderström et al. 1999) all tolerate hypoxic conditions in the laboratory. The epaulette shark responds to hypoxia (e.g., on shallow reef platforms during low tide) by entering a phase of metabolic and ventilatory depression (Mulvey and Renshaw 2009; Routley et al. 2002; Söderström et al. 1999).

Understanding the range of respiration in fish species is important for two reasons. First, immersion drug uptake and induction rate are linked to oxygen demand. For fish that depend primarily or entirely on dissolved oxygen (DO^3) in water, induction rates are shorter compared to those of air-breathing species (Hseu et al. 1997). Responding to confinement or hypoxic anesthetic baths, the latter pull air from the water surface and reduce or temporarily stop opercular movement, and the decreased branchial contact with the water results in a slower rate of anesthetic uptake (Hseu et al. 1997). For this reason injectable anesthetics are useful in species capable of aerial respiration (Bruecker and Graham 1993). Second, although many fish that use an accessory respiratory organ retain gills for aquatic gas exchange, effective gill tissue is so minimal in some species (e.g., channids, lepidosirenid lungfish, and clariids or walking catfish) that they will "drown" if denied access to atmospheric air (Ishimatsu and Itazawa 1993; Peters et al. 2001). Thus, regardless of anesthetic route, these species must remain in very shallow water, or on a moist substrate, until sufficiently recovered to reach the water/air interface under their own power.

Integument

Although most fish have scales, there is marked variation in their distribution and structure, and this variety has implications for the hand injection of drugs, especially for species with large or hard scales or protective denticles.

For many fish the skin is a respiratory organ, responsible for up to 30% of oxygen uptake in some species (Bruecker and Graham 1993); most marine species in particular have well-vascularized skin capable of significant gas exchange (Ishimatsu and Itazawa 1993). Younger fish, regardless of species, tend to have thinner and less scaled skin, which permits greater oxygen uptake (Myskowski et al. 2003). And fish species that start out as larvae lacking gills require skin respiration until differentiation of gill lamellae is complete (Oikawa et al. 1994).

Skin is also a route for immersion drug uptake (and presumably drug excretion) and in some species may actually be more efficient than other respiratory organs—in the electric eel (*Electrophorus electricus*), for example, quinaldine uptake across the skin was higher than through the gills (Brown et al. 1972).

Metabolism

Most fish are ectothermic, and lower temperatures are usually associated with prolonged induction and recovery times, with the converse true at higher temperatures (Detar and Mattingly 2004; Gelwicks and Zafft 1998; Gomes et al. 2001; Peters et al. 2001; Stehly and Gingerich 1999). During immersion anesthesia this relationship is primarily related to an altered respiratory rate. And because the blood acid-base

[3]Abbreviations used in this article: DO, dissolved oxygen; MS-222, tricaine methanesulfonate

status of fish is temperature dependent, increased temperature leads to acidemia and hypercapnia that stimulate hyperventilation, which in turn decreases induction and recovery times for drugs taken up or eliminated through the gills (Aguiar et al. 2002; Stehly and Gingerich 1999).

Some fish (e.g., tuna and other members of the Scombridae family; mako shark, *Isurus oxyrinchus*; porbeagle shark, *Lamna nasus*) have evolved various degrees of endothermy and have the capacity to conserve metabolic heat and elevate tissue temperature above the ambient temperature (Blank et al. 2004; Brill and Bushnell 2001; Bushnell and Jones 1994; Cooper et al. 1994; Muñoz-Chápuli and Satchell 1999). In general, endothermic species exhibit an increased rate of anesthetic uptake and metabolism compared to similarly sized ectothermic species.

Most fish have an anatomic separation between aerobic, slow-oxidative muscles and anaerobic, fast-twitch, glycolytic muscles. In many species regional heterothermy exists, with a distinct pattern of highly oxygenated slow-oxidative muscle running along the midline of the body (Bernal and Graham 2001; Totland et al. 1981; Williams et al. 2004). Given the increased capillarization, injection of anesthetic agents into this region may result in more rapid induction times compared to injections administered elsewhere.

Many drugs undergo hepatic biotransformation. Compared to other fish, elasmobranchs have proportionally large fatty livers (up to 23% body weight with as much as 80% lipid content) (Holmgren and Nilsson 1999); the increased hepatic cellular exposure and high lipid content may result in marked differences in drug pharmacodynamics between elasmobranchs and other fish.

Environmental and Other Factors

Temperature

In addition to its effects on metabolism, temperature influences DO concentrations. At higher temperatures, DO in the water decreases (Harms 2003), an effect that can exacerbate anesthetic-induced hypoxia.

pH

The pH of immersion anesthetic solutions influences their efficacy; specifically, a drop in the pH generally decreases efficacy because the increased ionization interferes with absorption (Ross and Ross 1984). Immersion anesthetics that are acidic in solution require buffering agents to neutralize the pH and thus both promote efficacy (Ferreira et al. 1984) and prevent metabolic acidemia, a condition precipitated by anesthetic-induced hypoxemia and anaerobic metabolism. Saltwater, with its higher pH and greater natural buffering capacity compared to freshwater, may not require the addition of buffering agents (Harms and Bakal 1995; Harms 1999; Oikawa et al. 1994; Ross 2001; Ross and Ross 1984) depending on the dose of immersion agent, size and activity of the fish, and number of fish being anesthetized in the same container. Erring on the side of caution, it is advisable to at least check the pH of the anesthetic container after the addition of the drug to determine whether buffers are indicated.

Nitrogenous Compounds

Nitrogenous compounds (e.g., ammonia and nitrite) can damage or alter gill morphology and these changes can have several impacts. They may affect uptake and clearance of inhalant anesthetics. They may compromise oxygen uptake and thus affect metabolism of the anesthetic agents and lead to acidemia. Last, marked elevation of nitrite may result in methemoglobinemia, which reduces the oxygen-carrying capacity of blood.

Drug Concentration/Dosage

Regardless of water temperature, a higher drug concentration (for immersion agents) or dosage usually (but not always) decreases induction and increases recovery times (Hseu et al. 1997, 1998). Furthermore, dosing requires careful attention as some immersion drugs (e.g., MS-222; more on this drug below) continue to increase in the brain and muscle despite blood equilibration (Ross 2001). Thus an initially satisfactory drug dosage can produce progressively deeper anesthesia and even respiratory arrest, even when the fish is recovering in anesthetic-free water (Ross 2001).

Drug Administration Methods

General Suggestions

Before anesthetizing an unfamiliar species, we recommend performing a literature review using terms such as "physiology," "anatomy," "site fidelity," "fecundity," along with the scientific name of the species to identify studies that required chemical restraint. The use in adult fish of dosages and regimens from the literature requires careful consideration as many studies use young or small fish. The anesthetic regimen for an unfamiliar species should be tested through recovery using one or a small group of fish before applying it to a larger number of fish (Harms and Bakal 1995; Ross 2001; Stetter 2001).

Preanesthetic Preparation

1. When possible, baseline behavioral parameters (i.e., ventilation, caudal fin stroke rate, and overall activity level) should be recorded.
2. Withholding the animals' food for 12 to 24 hours limits regurgitation (which clogs gill rakers) and decreases nitrogenous waste production (Harms and Bakal 1995; Stetter 2001).

3. Containers with adequate water for transportation, induction, maintenance, recovery, and possible required water changes should be readily available (Harms 1999; Stetter 2001).
4. The physical (e.g., temperature) and chemical (e.g., pH, salinity) variables of water used should match those of the fish's source water. Dissolved oxygen should be maintained at >5 ppm (mg/l); the ideal is 6-10 ppm (Harms 1999; Stetter 2001).
5. Out-of-water procedures must include a plan for preventing drying of the skin, fins, and eyes. Arrangements may include coverage with clear plastic drapes and regular rinsing of tissues with water from a bulb syringe or a small portable atomizer (Harms 1999; Ross 2001).
6. Personal protective gear must be available. Use of a respiratory mask may be appropriate when measuring powdered anesthetics, and the use of gloves limits systemic absorption and reduces the potential for zoonotic disease transmission.

Immersion Anesthesia

Immersion anesthesia in fish is analogous to gaseous inhalant anesthesia in terrestrial animals (and is referred to as inhalant anesthesia in some of the literature). The fish ventilates the anesthetic in solution, which enters the bloodstream through the gills and/or accessory respiratory organs and/or the skin and then passes rapidly to the central nervous system (CNS). Both gill tissue and skin contain large amounts of lipid, so the efficiency of uptake of immersion agents across these surfaces is directly related to the drug's lipid solubility (that of MS-222, for example, is relatively high). Skin thickness and scalation also affect uptake, with thinner skin and loosely scaled or scaleless surfaces favoring drug uptake compared to thicker skin or densely packed scaled surfaces (Ferreira et al. 1984).

When the fish is placed in drug-free water, it excretes drugs or their metabolites via the gills and presumably accessory respiratory organs; some elimination also occurs through the kidneys and skin (Ross 2001; Walsh and Pease 2002). It is important to note that excreted water-soluble drugs or metabolites may be reabsorbed through the body surface and gills while being simultaneously metabolized and eliminated (Oikawa et al. 1994).

Immersion drugs must be water soluble or use a water-soluble solvent as a vehicle. It is preferable to use water from the fish's tank to make the anesthetic solution. For simple, short procedures the drug concentration is either prepared in an aerated container to which the fish are transferred or added directly to the water containing the fish. The latter approach requires the addition of a buffer before drug administration to minimize acute drops in pH (Harms 1999; Ross 2001). Ideally, spontaneous ventilation is maintained during short procedures. When immersion is impractical or dangerous to handlers (e.g., for large fish), drugs in solution may be applied directly to the gills.

The use of an artificial ventilation system is necessary for procedures that last more than 10 minutes, those that involve debilitated fish or slow-recovering species, and all but the shortest out-of-water procedures. All of these conditions require holding the fish in shallow water or placing it on a fenestrated surface (in lateral recumbency or upright/upside down in a foam holder), with a bifurcated pipe or mouthpiece placed in the buccal cavity to deliver aerated anesthetic solution across the gills (Figure 1).

Nonrecirculating or recirculating systems are available. A nonrecirculating system, in its simplest form, uses an IV fluid bag with a drip set value (Figure 2). Aeration of the anesthetic water with an air stone in the nonsealed IV bag ensures near-saturation of oxygen and removal of dissolved CO_2. Used water is collected but not recycled (Harms 1999; Ross 2001; Stetter 2001). This system is suitable for small to medium-sized fish depending on the reservoir volume, drip set tube diameter, and rate of fluid delivery.

Recirculating systems enable both the delivery of anesthetic water from a reservoir to the gills and the recycling of the effluent (Figure 3), either manually or with a submersible pump. A valve on the tube leading to the mouth controls the flow rate. Minimum effective flow rate during fish anesthesia has not been determined; 1 to 3 liters/min/kg is recommended. Low delivery rates fail to keep the gills wet and reduce gas exchange; high flow rates result in alimentary anesthetic delivery and gastric dilation. An air stone provides oxygenation. Recirculating systems are appropriate for large fish, where cost and wastewater concerns require conservation of anesthetics and water (Harms 1999; Ross 2001; Stetter 2001).

During immersion anesthesia, adjustment of the drug concentration in response to anesthetic depth is difficult. One option is to prepare measured volumes of anesthetic-free water and concentrations of anesthetic solution in separate bags or reservoirs for use in either recirculating or nonrecirculating systems. Alternatively, a bulb or other syringe enables rapid delivery of small amounts of anesthetic fluid directly to the gills without disconnecting the fish from the system

Figure 1 Goliath grouper (*Epinephelus itajara*) positioned on bubble wrap in a foam holder during tricaine methanesulfonate (MS-222) anesthesia. The bifurcated mouthpiece placed in the buccal cavity delivers oxygenated anesthetic water to both sets of gill arches.

Figure 2 A simple nonrecirculating system using an IV bag and drip set. Gravity-dependent flow rate is controlled by the drip set valve. Used water is collected (in the tank below) but not recycled. This system is suitable for small to medium-sized fish depending on the volume of the reservoir, the drip set tube diameter, and the rate of fluid delivery.

(Harms 1999). The flow is usually normograde to achieve optimal gas and anesthetic exchange. Though retrograde flow is sometimes necessary (e.g., in oral surgery or because of species anatomical variations), it nullifies the normal countercurrent exchange mechanism and may damage the gills.

Parenteral Anesthesia

In addition to immersion methods, anesthetics can be delivered orally, intravenously (IV), intracoelomically (ICo), and intramuscularly (IM). With the exception of metomidate (see below), there have been few studies on oral anesthetics (e.g., Hansen et al. 2003; Harms and Bakal 1995; Steers and Sherrill 2001) and this route likely has limited use because precise dosing is difficult and the rate and degree of absorption are uncertain (Harms 2003).

IV injection results in rapid induction and usually a short duration of effect, but it has the disadvantage of requiring either manual restraint or prior administration of drugs by another route to allow IV access (Fleming et al. 2003; Hansen et al. 2003).

ICo delivery, although effective, has two disadvantages: an increased risk of visceral damage (although this can be minimized by inserting the needle at an acute angle directed anteriorly) and inconsistent induction times, because the drugs must pass through the serosal surface.

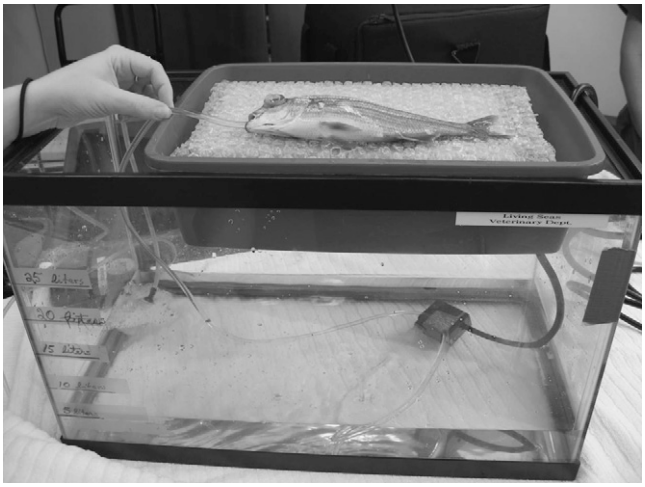

Figure 3 A grunt (*Haemulon* sp.) anesthetized with tricaine methanesulfonate (MS-222). A simple recirculating system maintains anesthesia by delivering anesthetic water from a reservoir to the gills and recycles the effluent back to the fish through the use of a submersible pump.

The most common parenteral route is IM injection by hand syringe, pole syringe, or a darting system (Harms 1999; Harvey et al. 1988; Williams et al. 1988). When possible, the recommended site for IM administration is the dorsal saddle, an area that surrounds the dorsal fin and extends laterally to just above the lateral line, from the operculum or posterior gill slit caudal to a point approximately one-third the distance between the dorsal fin and the caudal peduncle. IM injections often result in leakage as the needle is withdrawn and the surrounding muscles contract (Harms and Bakal 1995; Peters et al. 2001); drug loss is preventable by holding the fish in hand, placing the needle directly on the dorsal midline between the epaxial muscles, and then laterally inserting it into a muscle bundle (Harms and Bakal 1995). It is important to note that (1) skeletomuscular movement helps blood and lymph circulate (Gruber and Keyes 1981), so anesthetic induction time may be delayed in sedentary species, and (2) injection volumes are often large and may contribute to sterile abscess formation in the musculature (Tyler and Hawkins 1981).

Regardless of route, parenteral anesthetics often do not provide adequate sedation or anesthesia and so require supplemental immersion anesthesia. In addition, ventilatory support is necessary with parenteral anesthetics, particularly if recovery is prolonged (Harms 1999).

Monitoring

Anesthetic Depth

Investigators have used various schemes to assess anesthetic depth in fish (Detar and Mattingly 2004; Myszkowski et al.

2003; Oikawa et al. 1994); criteria include activity, reactivity to stimuli, equilibrium (righting reflex), jaw tone, muscle tone, and respiratory and heart rates. Broad stages include sedation, narcosis or loss of equilibrium, and anesthesia, with each stage subdivided into light and deep planes (Harms 2003; Stetter 2001). But depending on the species, drug, and dosage, some stage components are not available (Harms 1999; Stetter 2001). Conversely, some signs attributed to drug effect are instead responses to stress. For example, Gulf of Mexico sturgeon (*Acipenser oxyrinchus de soti*) often turn to a ventral/dorsal position when stressed (Fleming et al. 2003), so loss of the righting reflex is not appropriate to determine anesthetic stage in this species.

With proper dosing, induction with immersion drugs usually occurs within 5 to 10 minutes, but may take longer via other routes. Induction is marked by decreases in caudal fin strokes, swimming, respiratory rate, and reaction to stimuli; the drop in caudal fin stroke activity is usually the first sign, followed by loss of equilibrium and response to stimuli. At surgical anesthesia there is total loss of muscle tone and a further decrease in respiratory rate. A firm squeeze at the base of the tail can be an effective way to determine response to stimuli: if the animal does not respond, general anesthesia has taken effect (Harms 2003; Stetter 2001).

Some species go through a short excitement phase during immersion induction and may traumatize themselves (Harms 1999; Stetter 2001). And fish exposed to high drug concentrations may exhibit rapid and repeated flaring of the opercula, with a "coughing" reflex to flush the chemical irritant from the branchial cavity.

Cardiopulmonary Activity

Respiratory and cardiac rates usually decrease with increased anesthetic dosage and duration. But in some species (e.g., tuna) opercular movement is minimal or even nonexistent due to their mode of ventilation (ram ventilation). These fish maintain adequate ventilation, at least in the short term, through the passage of oxygenated water over their gills (respiratory arrest in fish can precede cardiac arrest by an extended period of time; Harms 1999).

In species with thin pliable body walls and proportionally large hearts, heart beats are directly observable (Harms and Bakal 1995; Harms 1999). For most species, however, monitoring of the heart rate requires the use of cardiac ultrasonography, Doppler flow probes, or electrocardiography (ECG) (Harms and Bakal 1995; Harms 1999; Ross 2001). Ultrasonography and Doppler probes are placed either into the opercular slit (for medium to large fish) or directly over the heart (for smaller specimens and small-scaled/scaleless species; Figures 4 and 5). ECG entails lightly clamping electrodes to the surface of the fins (the pectoral and anal fins are a good combination) (Ross 2001); alternatively, subcutaneous placement of needle electrodes minimizes skin trauma, reduces the chance of grounding out the ECG signal by contact with a wet external surface, and improves signal quality

Figure 4 Ultrasonography is useful for monitoring heart rate in fish. In specimens of sufficient size, the probe can be placed in the opercular slit as in this rainbow parrotfish (*Scarus quacamaia*). In smaller specimens or small-scaled/scaleless species, the probe is placed directly over the heart.

(Harms 1999). Studies have not shown that pulse oximetry is effective for measuring hemoglobin saturation in fish (Harms 1999; Ross 2001).

Although a fish may appear to be ventilating well, bradycardia and an increased resistance to gill capillary flow (as erythrocytes accumulate in the capillary bed and become swollen) may cause hypoxemia (Tyler and Hawkins 1981). Useful variables in detecting hypoxemia include gill color (pallor indicates hypoxemia, hypotension, hypovolemia, or asystole) and fin margin color (pallor indicates hypoxemia, hypotension, or peripheral vasoconstriction) (Harms 2003; Stetter 2001). In large fish, an effective way to monitor respiratory efficiency is periodic blood gas sampling to determine

Figure 5 Doppler flow is useful for monitoring heart rate in fish, with probes placed in the opercular slit (for large and medium-sized fish) or directly over the heart (for smaller specimens and small-scaled/scaleless species). This figure demonstrates placement of the probe in the opercular slit of an African cichlid (*Protomelas insignis*).

oxygenation, carbon dioxide, and pH. Venous samples are easier to collect and provide useful information about physiological trends. Other blood variables used to assess metabolic status include hematocrit, glucose, and lactate.

Water Quality

It is critical to monitor water quality in order to reduce anesthetic morbidity and mortality. Assuming aeration, DO, pH, and temperature are appropriate, the greatest concern is ammonia concentrations. These rise—and buffering capacity decreases—during prolonged procedures and when fish are sequentially anesthetized in a recirculating anesthetic system. Ammonia toxicity is greater in more alkaline water (e.g., marine systems). Ammonia is easy to measure with tankside kits; or the anesthetist may use other indicators of deteriorating water quality such as surface foam formation due to increased protein from fish slime (Harms 1999). For species that are facultatively ureotelic and ureogenic (e.g., Gulf toadfish, *Opsanus beta*), excreting most nitrogenous waste as urea in response to stressors (e.g., anesthesia) (Gilmour et al. 1998), the measurement of ammonia concentrations as a guide to water quality is inaccurate and the handler must rely on the other parameters.

Correction of declining water quality during anesthesia requires a partial water change, by adding either water containing a known anesthetic concentration or, when reducing anesthetic depth, a known volume of clean water. One of the authors (DLN) routinely changes out up to 50% of water in the anesthetic chamber at a time without negative effects. When using a recirculating system or the same water for multiple fish, the frequency of water changes needed will depend on the size of the fish (in proportion to water volume) and waste production. For example, one author (DLN) changes out 100% of the anesthetic bath after 3 to 4 adult southern stingrays (*Dasyatis americana*) have been anesthetized in a 200 liter container (as long as visible waste material is not significant), but will anesthetize 6 to 12 grunts (15-30 cm) in an 80 liter cooler before changing the water. It is particularly useful to look for the buildup of bubbles on the surface of the water, as these indicate increasing protein levels from waste production and the need for at least a partial water change.

Recovery

With immersion anesthesia, recovery occurs when the fish is placed in anesthetic-free water (Ross 2001), and for injectable α_2-agonists reversal drugs are available (Harms 1999; Horsberg et al. 1999; Snyder et al. 1998; Williams et al. 2004). Although recovery occurs by different mechanisms, transfer to a new water container is advisable after any route of anesthesia as some of these drugs and their metabolites may be excreted into the water and reabsorbed through the gills and/or skin. The recovery water is aerated and the fish's mouth oriented toward water flow. In an artificial ventilation system, anesthetic-free water is passed over the gills until spontaneous ventilation resumes; alternatively, the fish is pulled forward through the water with its mouth open. Dragging the animal backward is inadvisable as retrograde flow provides inefficient oxygen and anesthetic exchange and may cause gill damage.

Most fish fully recover from inhalant anesthetics within 5 minutes; recoveries extending more than 10 minutes indicate an excessive anesthetic dosage or a compromised animal (Ross 2001; Stetter 2001). In comparison, recovery from oral and parenteral agents is more variable. As the fish recovers, respiration increases, muscle tone returns, fin movements resume, and the fish swims with progressively less ataxia until it regains full equilibrium.

Some fish go through an excitement phase (sometimes quite severe) during recovery and should be prevented from escaping from the tank (Harms 1999) with the use of lids, nets, foam pads, or other barriers. Individual fish may even require manual restraint during this period to prevent damage to their eyes and body. In either case, the handler should take precautions to avoid injury from the fin rays and teeth of certain bony fish and from the stingers (rays), teeth, and skin of some elasmobranchs.

Resuscitation

Marked hypoventilation (bradypnea) is usually not a cause for alarm. If cardiac output is steady and oxygenated water is flowing appropriately over the gills, tissue gas exchange is probably adequate, but decreasing the anesthetic concentration or moving the fish to anesthetic-free water may be appropriate (Stetter 2001). In either case, the anesthetist should take advantage of the buccal flow/heart rate reflex, which causes the heart rate to accelerate in response to an increase in water flow through the buccal cavity. The resulting increase in gill blood flow hastens both elimination of the inhalant drugs and recovery. These effects occur naturally after the gentle movement of the fish forward through the water until spontaneous ventilation returns (Ross 2001). This method is also effective with bradycardia.

Unresponsive fish may require the administration of fluids, doxapram, and/or forced ventilation. One author (DLN) has observed a positive response to doxapram administration (5 mg/kg IV or ICo) in several cases of respiratory arrest. However, doxapram can be a marked stimulant in elasmobranchs, causing them to become extremely excited and dangerous (Stoskopf 1986). Other traditional mammalian emergency drugs (e.g., epinephrine, corticosteroids) may be given in the case of physiological collapse, although the effects are not well understood.

Anesthetic Drugs

Table 1 provides a summary of commonly used sedative, analgesic, and anesthetic agents used in fish. We describe the applications, impacts, and limitations of each in the following sections.

Table 1 Summary of selected anesthetic agents used in fish, from anecdotal and published sources (see discussion and citations in text). These doses are not suitable for all species or under all conditions. When working with unfamiliar species or agents, start with low doses and low numbers of fish. See text for notes on dosing and on researching appropriate agents and doses. The authors are not responsible for the doses and protocols in this formulary.

Drug[a]	Route	Fish taxa	Dose	Comments
Tricaine methane-sulfonate (MS-222)	IN	General (nonelasmobranch)	75-200 mg/l 50-150 mg/l	Anesthesia induction Anesthesia maintenance
	IN	Sturgeon	100-125 mg/l	Mild sedation to light anesthesia
	IN	Freshwater eels	75 mg/l	Sedation
	IN	Characins (pacus, tetras) and cyprinids (carp, koi, goldfish, roach, dace, minnows)	60-300 mg/l	Dose-dependent anesthesia
	IN	Livebearers (exc. mollies, platys)	30 mg/l	Light sedation
	IN	Sunfish/bass (centrarchids)	50 mg/l for induction; 25 mg/l for maintenance	Sedation
	IN	Cichlids	150 mg/l for induction; 60 mg/l for maintenance	Anesthesia
	IN	Anabantoids (exc. gouramis)	200-400 mg/l for induction; 80 mg/l for maintenance 60 mg/l	Deep anesthesia Light sedation
	IN	Salmonids and catfish	50-100 mg/l	Light to deep anesthesia
	IN	Most marine reef teleosts, puffers, balloonfish, lionfish, frogfish, scorpionfish, moray eels	45-100 mg/l	Dose-dependent sedation to anesthesia
	IN	Seahorses and pipefish	25-75 mg/l	Variable sedation
	IN	Snappers, cod, grunts, groupers, jacks, tripletail, sea bass, snook, porgies, seabream, drums, tuna, mackerel	50-125 mg/l	Dose-dependent sedation to deep anesthesia
	IN	Striped bass and relatives	20-25 mg/l	Sedation
	IN	Toadfish	670 mg/l	Induced fish wrapped in anesthetic solution-soaked paper towels for 30 min surgical procedure
	IN		50-250 mg/l	Dose-dependent sedation to deep anesthesia
	IN	Flounder/flatfish	75 mg/l 250 mg/l	Sedation Anesthesia
	IN	Sharks	50-125 mg/l	Anesthesia
	IN	Rays	80-100 mg/l 45-55 mg/l	Anesthesia Moderate to deep sedation
Benzocaine	IN	General (nonelasmobranch)	25-200 mg/l	Dose-dependent anesthesia
	IN	Lungfish	1 g/l for induction; 0.25 g/l for maintenance	Anesthesia
	IN	Freshwater eels	60-80 mg/l	Anesthesia
	IN	Pacus and other characins	50-150 mg/l	Light to deep anesthesia
	IN	Catfish	100 mg/l	Anesthesia
	IN	Silver perch	20 mg/l	Sedation
	IN	Salmonids, cod, porgies, seabream	40-50 mg/l 20-35 mg/l	Anesthesia Light to heavy sedation

Table 1 (Continued)

Drug	Route	Fish taxa	Dose	Comments
	IN	Striped bass and relatives	55-80 mg/l	Anesthesia
	IN	Epaulette sharks	60-75 mg/l	Anesthesia
Clove oil	IN	General (nonelasmobranch)	20-100 mg/l	Anesthesia
	IN	Sturgeon	50-100 mg/l	Anesthesia
		Freshwater eels	100 mg/l	Anesthesia
	IN	Cyprinids (carp, koi, goldfish, dace, sharks, roach)	25-100 mg/l 4 mg/l	Anesthesia Sedation
	IN	Sunfish/bass (centrarchids)	5-9 mg/l 15-20 mg/l	Deep sedation Moderate anesthesia
	IN	Redcap triplefin	40 mg/l	Anesthesia
	IN	Perch	6 mg/l	Sedation
	IN	Silver perch	15-50 mg/l	Light to deep anesthesia
	IN	Catfish	100-150 mg/l	Anesthesia
	IN	Salmonids	20-60 mg/l 2-8 mg/l	Dose-dependent anesthesia Dose-dependent sedation
	IN	Most reef teleosts, filefish, convict tang, drummers, gobies, mullet, porgies, seabream	40 mg/l	Sedation to anesthesia
	IN	Jacks	10 mg/l	Sedation
	IN	Rabbitfish	100 mg/l	Anesthesia
	IN	Sea bass, snook	6-9 mg/l	Anesthesia
	IN	Striped bass and relatives	8 µl/l	Light sedation
Eugenol	IN	General (nonelasmobranch)	100-120 mg/l 40 mg/l	Anesthesia induction Anesthesia maintenance
	IN	Pacus and other characins	50-200 mg/l	Anesthesia
	IN	Rays	50 mg/l 20-25 mg/l	Anesthesia Sedation
Isoeugenol	IN	General (nonelasmobranch)	6-17 mg/l	Anesthesia
	IN	Sunfish/bass (centrarchids), perch, silver perch, catfish	15-66 mg/l	Variable sedation to anesthesia
	IN	Salmonids	120 mg/l 20-60 mg/l	Surgical anesthesia Dose-dependent sedation
	IN	Striped bass and relatives	3.6 mg/l	Light sedation
	IN	Porgies and seabream	120 mg/l for induction; 60 mg/l for maintenance	Surgical anesthesia
	IN	Rays	15 mg/l	Deep sedation to light anesthesia
Metomidate	IN	General (nonelasmobranch)	5-10 mg/l 2.5-5 mg/l 0.06-0.2 mg/l	Anesthesia Heavy sedation Light sedation
	IN	Freshwater tropical fish (livebearers, gouramis)	0.8-1 mg/l	Light sedation
	IN	Salmonids	2-10 mg/l	Light sedation
	IM		42 mg/kg	Anesthesia
	IN	Catfish	6 mg/L	Anesthesia
	IM	Snappers	50 mg/kg	Anesthesia
	IM	Jacks	80-100 mg/kg	Sedation
	IN	Cod	5 mg/l	Anesthesia
	IM	Sablefish	62 mg/kg	Light sedation
	IM	Rockfish	100 mg/kg	Anesthesia
	IN	Drums	7 mg/l	Anesthesia

Table 1 (Continued)

Drug	Route	Fish taxa	Dose	Comments
	IN	Striped bass and relatives	1.5 mg/l	Light sedation
	IN	Flounder/flatfish	9-30 mg/l	Variable anesthesia
2-PE	IN	General (nonelasmobranch)	0.1-0.5 ml/l	Anesthesia
	IN	Cyprinids (carp, koi, goldfish)	0.1-0.5 ml/l	Dose-dependent sedation to light anesthesia
	IN	Livebearers (exc. mollies, platys)	600 mg/l 220 mg/l	Anesthesia Light sedation
	IN	Milk fish	400 mg/l	Anesthesia
	IN	Cichlids	600 mg/l	Anesthesia
	IN	Porgies, seabream, mullet, snappers	400 mg/l	Sedation to anesthesia
Quinaldine	IN	Cyprinids	10-50 mg/l	Anesthesia
Quinaldine sulphate	IN	General (nonelasmobranch)	50-100 mg/l 15-60 mg/l	Anesthesia induction Anesthesia maintenance
	IN	Freshwater tropicals (exc. livebearers, gouramis)	5-10 mg/l	Light sedation
	IN	Drums, porgies, seabream	20 mg/l	Anesthesia
	IN	Moronidae (striped bass and relatives)	8.3 mg/l	Light sedation
Ketamine	IM	General (nonelasmobranch)	66-88 mg/kg	Anesthesia
	IM	Sturgeon	77-88 mg/kg	Anesthesia
	IV	Cichlids and salmonids	30 mg/kg	Anesthesia following sedation in 100-125 mg/l MS-222
	IM	Salmonids	50-150 mg/kg	Anesthesia
	IM	Elasmobranchs	12-20 mg/kg	Anesthesia
Medetomidine + ketamine	IM	General (nonelasmobranch)	0.05-0.10 mg/kg 1-2 mg/kg	Anesthesia; atipamezole IM for reversal at 0.2 mg/kg
	IM	Lungfish	0.053 mg/kg 5.26 mg/kg	Mild sedation, but physical restraint necessary for performing diagnostics
	IM	Sturgeon	0.06 mg/kg 6 mg/kg	Light anesthesia for minor diagnostic procedures; atipamezole IM for reversal at 0.30 mg/kg
	IM	Snappers	1.1-1.7 mg/kg 27-42 mg/kg	Mild to moderate sedation
	IM	Cobia	0.122-0.240 mg/kg 6-13.5 mg/kg	Mild sedation; atipamezole IM for reversal at 5X medetomidine dose
	IM	Tuna	0.4 mg/kg 4 mg/kg	Anesthesia; atipamezole IM for reversal at 2 mg/kg
	IM	Mackerel	0.6-4.2 mg/kg 53-228 mg/kg	Anesthesia; atipamezole IM for reversal at 5X medetomidine dose
	IM	Sharks	0.09-0.10 mg/kg 4-5 mg/kg	Anesthesia; atipamezole IM for reversal at 5X medetomidine dose
Xylazine + ketamine	IM	Sharks	6 mg/kg 12-20 mg/kg	Anesthesia
Propofol	IV	Sturgeon	6.5 mg/kg	Light anesthesia
	IV	Small sharks	2.5 mg/kg	Anesthesia

[a]IM, intramuscular; IN, inhalant; IV, intravenous; MS-222, tricaine methanesulfonate; 2-PE, 2-phenoxyethanol

Immersion (Waterborne or Inhalant) Drugs

Tricaine Methanesulfonate (MS-222)

Tricaine methanesulfonate (MS-222[3]) is a benzocaine derivative that is absorbed across the gills (and the skin in some species), biotransformed in the liver and probably kidney, and cleared primarily through the gills, with additional metabolites eliminated in urine and bile (Harms 1999; Maren et al. 1968). Powder is directly mixed into the anesthetic chamber or administered as a stock solution of 10 g/l (10,000 mg/l). Both powder and liquid are unstable in light and require storage in a dark container. Oily residues or a change in color of buffered stock solutions indicate the presence of a desulfonation product and decreased potency.

MS-222 is more effective and safe in its neutralized form, which is achieved by buffering the solution to the pH of the fish's holding water. Sodium bicarbonate is primarily used, but other buffers are also effective (e.g., imidazole, sodium hydrogen phosphate, sodium hydroxide, and calcium carbonate). Exact amounts of sodium bicarbonate are measured or the powdered buffer is incrementally mixed into the anesthesia chamber until the solid no longer dissolves, indicating saturation (Harms 1999; Oikawa et al. 1994; Roubach et al. 2001; Stetter 2001).

MS-222 is not efficacious for all fish. For example, in Gulf of Mexico sturgeon it is minimally effective at providing immobilization even at 400 mg/l (4 to 10 times the dosage for most species) (Fleming et al. 2003).

MS-222 can be associated with hypoxemia resulting from drug-induced hypoventilation (Davis and Griffin 2004; Harms and Bakal 1995; Oikawa et al. 1994), and the safety margin is narrower for young fish in warm, soft water (Harms 1999; Roubach et al. 2001). MS-222 may also cause reversible retinal deficits in fish and humans (Bernstein et al. 1997; Sladky et al. 2001).

Benzocaine

Benzocaine, the parent compound of MS-222, is similar in pharmacology but less acidic and much less water soluble, features that may account for its usefulness in species sensitive to MS-222 (e.g., striped bass, *Morone saxatilis*) (Hseu et al. 1998). The compound requires advance preparation of a stock solution in ethanol or acetone (100 g in 1 liter) or propylene glycol, with storage of the solution in a dark bottle at room temperature (Harms and Bakal 1995; Iversen et al. 2003).

Other advantages of benzocaine include low toxicity for humans (at the concentrations used for fish) and its removal from facility effluents using activated carbon filtration; but even without filtration, breakdown in water occurs in approximately 4 hours, making environmental contamination less likely. Exposure also does not impair fish growth or reproductive capacity (Gomes et al. 2001; Hseu et al. 1998; Tyler and Hawkins 1981). As with all drugs, efficacy and sensitivity to benzocaine vary with the species and dosage (Gomes et al. 2001). One major concern is the drug's fat solubility, which may result in prolonged recovery in older and gravid fish (Iversen et al. 2003).

Clove Oil, Eugenol, Isoeugenol, and Aqui-S

Clove oil is a mixture of compounds: phenolic eugenol (which accounts for 85-95% of the active ingredients), isoeugenol, and methyleugenol. Commercially available clove oil has approximately 84% eugenol, but it is possible to purchase 100% eugenol. Aqui-S™ contains primarily isoeugenol (Iversen et al. 2003; Kildea et al. 2004) and is not available in the United States. Clove oil and eugenol are incompletely water soluble, particularly at cold temperatures. A 1:10 mixture of either in 95% ethanol yields a 100 mg/ml stock solution (Harms 2003).

Clove oil results in more rapid induction times and consistent anesthesia compared to other anesthetics (Bressler and Ron 2004; Detar and Mattingly 2004; Sladky et al. 2001), but longer recoveries (Detar and Mattingly 2004; Sladky et al. 2001). Other reported advantages include efficacy at a range of temperatures, availability, low cost, and handler safety (Bressler and Ron 2004; Detar and Mattingly 2004), and one study claimed increased safety for fish compared to other immersion drugs (Detar and Mattingly 2004). However, another study reported a narrow safety margin (compared with MS-222) in the red pacu (*Piaractus brachypomus*; Sladky et al. 2001), and one author (DLN) has seen increased sensitivity to eugenol and Aqui-S in preliminary studies with southern stingrays. Possible explanations for ventilatory failure and medullary collapse observed in the red pacu include neurotoxic or hepatotoxic effects similar to those in mammals (Sladky et al. 2001), or the fact that eugenol, an oil, coats gill epithelia and may block gaseous diffusion (Sladky et al. 2001). In addition, researchers have described mild gill necrosis from repeated exposure to low doses of eugenol (Afifi et al. 2001).

Although clinical studies have demonstrated analgesic effects of eugenol in humans (Keene et al. 1998), there is no proof of such effects in fish (Sladky et al. 2001). Red pacus anesthetized with eugenol were more likely to react to a hypodermic needle puncture than fish anesthetized with MS-222, raising the question of the appropriateness of the clove oil complex for invasive or otherwise noxious procedures.

Metomidate

Metomidate is a nonbarbiturate imidazole available for investigational use in the United States (Harms 1999) and sold in Canada under the trade name Marini™. It is readily water soluble and requires storage in tight light-protected containers.

Metomidate suppresses the cortisol response to anesthesia by blocking adrenocorticotropic hormone (ACTH) stimulation of steroidogenesis, even after ICo injection of exogenous ACTH. The suppression occurs by a direct effect on the

interrenal gland and the mitochondrial cytochrome P_{450}-dependent enzymes that catalyze cortisol synthesis (Davis and Griffin 2003; Iversen et al. 2003; Small 2005). Cortisol synthesis blockade may cause the fish to transiently darken, possibly because of reduced cortisol production terminating the negative feedback loop on ACTH synthesis. As ACTH synthesis is linked to melanocyte-stimulating hormone production, both compounds increase with the associated color change (Harms 1999; Harms and Bakal 1995).

Metomidate is useful for sedation and anesthesia for minor procedures as well as limiting transport trauma, as dosages that suppress the cortisol stress response still allow maintenance of equilibrium (Davis and Griffin 2004; Harms 1999). However, it is a hypnotic and does not induce general anesthesia, as is evident in the maintenance of opercular respiration for twice as long as with other inhalants at the effective concentration (Mattson and Riple 1989) and in muscle fasciculations (indicating incomplete relaxation) at low doses (Harms 1999). Furthermore, metomidate is probably a poor analgesic and should not be used alone for major surgical or noxious procedures (Hansen et al. 2003; Harms 1999). Gouramis (Osphronemidae) are very sensitive to metomidate, and its use in cichlids (Cichlidae) in water of pH < 5 is contraindicated (Harms 1999).

2-Phenoxyethanol

2-Phenoxyethanol (2-PE) is a moderately water-soluble clear or straw-colored oily liquid widely used for sedation (particularly in fish transportation) and anesthesia (Hseu et al. 1996, 1997; Ross 2001). Advantages are low cost and the fact that no pH change occurs with the addition of 2-PE to seawater (Hseu et al. 1998). However, 2-PE produces hypoventilation and provides poor analgesia (Oswald 1978). Also, sustained and regular exposure to 2-PE solution causes a neuropsychological syndrome in some handlers (Hseu et al. 1998).

Quinaldine and Quinaldine Sulphate

Quinaldine, a quinoline derivative, is a yellowish oily liquid that must be dissolved in acetone or alcohol prior to mixing in water. Quinaldine sulfate (QS) is a strongly acidic, highly water-soluble powder that must be buffered and administered as a stock solution (10 g/l). Due to high lipid solubility, quinaldine tends to accumulate in the brain more than QS (Harms 1999; Hseu et al. 1998; Ross 2001) and may result in deeper anesthesia compared with QS, factors that may make it less safe than QS. Like MS-222 and most inhalants, quinaldine and QS depress the CNS sensory centers. Unlike MS-222, neither drug is metabolized by fish and both are excreted entirely unchanged (Harms 1999; Hseu et al. 1998).

Advantages of the use of QS in fish are low cost, effectiveness at very low concentrations, and short recovery time. But despite the drug's purported low toxicity and wide safety margin, mortality occurs in some species and quinaldine can be an integumental and ocular irritant (Harms and Bakal 1995; Kumlu and Yanar 1999; Ross and Ross 1984). Several studies demonstrate that fish exposed to quinaldine or QS retain a strong reflex response to being touched, even when they have lost equilibrium and are "anesthetized." This potential lack of analgesia makes both drugs inappropriate for surgical or similarly noxious procedures (Cullen 1996; Harms 1999; Kumlu and Yanar 1999).

To reduce toxicity and increase efficacy and analgesia, some researches have combined diazepam in a bath with QS, so that fish enter a deeper anesthetic plane at lower QS dosages (Kumlu and Yanar 1999).

Azaperone

Azaperone reduces an animal's response to environmental stimuli without motor impairment or sedation. Preliminary studies in piked dogfish (*Squalus acanthias*) have shown that the most efficacious application of azaperone is directly over the gills (IM injection produces no effect) (Latas 1987). The advantages of using azaperone in fish include uninterrupted swimming patterns, normal gill ventilation, and normal cardiovascular function. Azaperone may also be useful for animals that are prone to panic, aggression, and self-induced trauma (Latas 1987).

Isoflurane and Halothane

There are few published studies evaluating chlorofluorocarbon-based anesthetics in fish (Dunn and Koester 1990; Stetter 2001). Their use requires care as anesthetic depth is difficult to control and the drug's insolubility in water results in localized higher concentrations that may cause overdosage. In addition, volatilization and difficulty in scavenging waste gas are hazardous to personnel (Harms 1999; Ross 2001). Given the disadvantages associated with the use of isoflurane and similar drugs, a number of researchers and clinicians recommend them only as a last resort for anesthesia, for euthanasia, or not at all (e.g., Harms 1999; Ross 2001).

Oxygen

Oxygen is a sedative for some elasmobranch species. Oxygenated water is flushed across the gills by bubbling 100% oxygen in the flow of water directed into the animal's mouth. But prolonged exposure to elevated oxygen depresses ventilation and produces hypercapnia and potentially life-threatening acidemia (Spotte 1992).

Injectable Anesthetics

Ketamine Hydrochloride

Ketamine, used alone or in combination with α_2-agonists (Harms 1999), is effective for a number of species (Williams

et al. 1988) and for short procedures (Bruecker and Graham 1993; Oswald 1978). The most common injection route, IM, requires high doses when used alone (Bruecker and Graham 1993; Fleming et al. 2003; Williams et al. 1988); IV administration entails dosages of one-third to one-half those for IM delivery and reduces oxygen demand during handling in several species (Bruecker and Graham 1993; Oswald 1978).

Ketamine alone has a species-specific effect in fish often characterized by incomplete anesthesia, periods of apnea, and prolonged recovery with excitement (Bruecker and Graham 1993; Graham and Iwama 1990; Oswald 1978). Elasmobranchs are more sensitive than teleosts and occasionally have seizure-like muscle spasms (Stoskopf 1993). To reduce dosage-related apnea and muscle spasms and to improve anesthesia, ketamine is often combined with the reversible α_2-adrenergic agonist medetomidine (see next section). This combination provides safe and effective anesthesia in some species but causes respiratory depression, bradycardia, and incomplete immobilization in others (Fleming et al. 2003; Snyder et al. 1998; Williams et al. 2004). For these reasons, ketamine or ketamine/medetomidine (K/M) is more appropriate as an aid to restraint or capture than as a substitute for inhalant anesthesia during major procedures.

Medetomidine

Medetomidine is effective as an immersion drug in rainbow trout to produce sedation (but not analgesia), after which atipamezole is added to the recovery water at six times the medetomidine concentration (Horsberg et al. 1999). Ordinarily, however, medetomidine is combined with ketamine and administered IM, again with the use of atipamezole to reverse the effects in both teleosts and elasmobranchs (Snyder et al. 1998; Williams et al. 2004). One of the authors (MAS, unpublished observations) has investigated the use of medetomidine with ketamine in several shark species and found that there appears to be great variation between species in their responses to similar dosages under the same conditions.

Xylazine

The use of xylazine with ketamine has provided safe and effective anesthesia in sharks (Andrews and Jones 1990; Stoskopf 1986, 1993), although xylazine variably alleviates the muscle spasms that can occur with ketamine use alone (Stoskopf 1993). However, xylazine can produce apnea (Oswald 1978) and it induces convulsant activity that occurs during teleost induction and recovery, making it difficult to ensure artificial ventilation because the convulsions frequently dislodge the water supply. In addition, gross ECG disturbances have been detected. For all these reasons, and given the proven usefulness of medetomidine in teleost species, xylazine is not a preferred α_2-agonist for bony fish and is not recommended in salmonids (Oswald 1978).

Propofol

Propofol use in spotted bamboo sharks (*Chiloscyllium plagiosum*) resulted in surgical anesthesia with stable respiration and heart rates throughout the anesthetic period (Miller et al. 2005). In Gulf of Mexico sturgeon propofol IV rapidly induced anesthesia but caused significant respiratory depression, which was addressed by passing oxygenated water across the gills (Fleming et al. 2003).

Alfaxalone/Alfadolone

A combination steroid, alfaxalone/alfadolone (A/A) mainly depresses CNS activity while leaving the integumentary sensory system operational. This makes it very valuable for research in sensory physiology (e.g., with mechanoreceptors; Peters et al. 2001). Another advantage of A/A is its cardiac chronotropic and inotropic stimulatory effect with vasodilation of the gill capillaries, which seems to ensure adequate oxygenation of the blood compared to many other anesthetics (Oswald 1978). As with other drugs, researchers have documented species differences (Harvey et al. 1988). For example, in several catfish species A/A provided stable surgical anesthesia lasting several hours (Peters et al. 2001), whereas in two trout species it was difficult to find an A/A dose that simultaneously abolished locomotion and preserved ventilation (Oswald 1978).

Analgesia

Limited information is available about the use of analgesics in fish; further study is needed, particularly given the increased interest in the use of analgesics for fish in the laboratory and clinical setting.

Drugs listed as an anesthetic for fish are often assumed to provide analgesia if they result in complete immobilization of the fish. And because fish have μ and κ opiate receptors throughout the brain, it is reasonable to expect some effect of opioid treatments in fish experiencing noxious stimuli (Harms et al. 2005). However, the distinction between anesthetic agent (with analgesic properties) and immobilizing drug is not always clear and depends on the properties of a particular drug; it is never appropriate to assume analgesia with all "anesthetics." Certainly, it is possible to determine which drugs provide better immobilization for a particular species by comparing the responses of individual fish to noxious stimuli (e.g., needle stick, tail squeeze, handling) when exposed to different "anesthetic" agents. This approach may be appropriate for an investigator who plans to use a particular species regularly in laboratory research.

In one study, the lips of juvenile rainbow trout were injected with 0.1 ml acetic acid, which resulted in an increased opercular rate compared to controls and anomalous behaviors such as rocking and lip rubbing against the substrate and tank sides (Sneddon 2003). Administration of high morphine dosages significantly reduced the opercular rate and the

anomalous behaviors, suggesting that morphine acts as an analgesic or at least antinociceptive in this and presumably other teleost fish (Sneddon 2003). In another study koi carp (*Cyprinus carpio*) that underwent exploratory celiotomy received butorphanol, ketoprofen, or saline; only the koi injected with butorphanol exhibited no significant differences between pre- and postsurgery caudal fin beat frequency and vertical position in the water column, a result that suggests a mild behavioral sparing effect compared with the ketoprofen-treated and saline control groups (Harms et al. 2005).

As is the case with mammals, fish can become tolerant of morphine, and naloxone reduces its effects (and presumably that of butorphanol) (Rose 2002). In a study involving an elasmobranch (chain dogfish, *Scyliorhinus retifer*), neither butorphanol nor ketoprofen provided appreciable analgesia in animals that received a noxious stimulus (needle stick in epaxial muscle). It is not clear whether these results represent a fundamental difference between elasmobranch and other fish physiology or an insufficient dose or frequency (Davis et al. 2006).

Aside from two studies in which ketoprofen was not effective (Davis et al. 2006; Harms et al. 2005), there are no published studies on the effectiveness of nonsteroidal anti-inflammatory drugs (NSAIDs).

Euthanasia

When necessary, overdosage of immobilization drugs is an acceptable means of euthanasia (AVMA 2007). The most frequent choice is the use of immersion drugs (and particularly MS-222) at five to ten times the anesthetic concentration for a particular species, although injectable agents are also effective (Ross and Ross 1984). For fish that are too large for a bath, the immersion drug is poured directly over the gills (Harms and Bakal 1995). Maintaining the fish in the anesthetic solution for 5 to 10 minutes after cessation of opercular movement (except in some ram ventilating species) usually, but not always, ensures that a fish has expired. Cardiac asystole typically lags behind brain death since fish myocardial cells use local glycogen stores for energy and do not need blood glucose (Stetter 2001). The use of Doppler flow probes, ultrasonography, or electrocardiography is recommended to confirm asystole and, to be certain, IV administration (in the heart or caudal vein) of an additional anesthetic drug or pentobarbitone should be performed (Ross 2001). Alternatively, cranial concussion, spinal transection, or exsanguination are effective methods in a deeply anesthetized fish (Harms 1999).

Although carbon dioxide is often reported as being useful for immobilization, its use has many disadvantages, including difficulty in controlling concentrations in water, the need for maintaining high oxygen levels, and the occurrence of markedly altered blood gases and acid-base balance (Gelwicks and Zafft 1998; Harms 1999; Prince et al. 1995). Following immersion in water with a high CO_2 concentration, there is a rapid decrease in blood pH that results in less efficient oxygen transport to tissues, including the brain. The resulting cerebral hypoxia causes an overall inhibition of spontaneous activity of the CNS and the observed "anesthetic" effect (Gelwicks and Zafft 1998; Harms and Bakal 1995). Although fish appear tolerant of the blood changes and cerebral hypoxia (Gelwicks and Zafft 1998), several studies point to CO_2's questionable analgesia and appropriateness for invasive procedures (Prince et al. 1995; Ross 2001). Many handlers feel that CO_2 should be used only for euthanasia or as a last resort immobilization chemical when noxious stimuli are involved (Harms 1999).

We encourage the reader to refer to the American Veterinary Medical Association Guidelines on Euthanasia (AVMA 2007; formerly the Report of the AVMA Panel on Euthanasia) for further information about approved and nonapproved methods for euthanasia of fish.

Concluding Thoughts

For the researcher, clinician, or technician who handles fish, an increasing body of information about the chemical restraint of fish is available in journal articles and secondary texts as well as agricultural extension agency pamphlets, instructional course handouts, and similar types of publications. Factors that may be driving this growing knowledge base include increases in three related areas: aquaculture activities, which often require sedation or anesthesia of large numbers of fish for transport and handling; requirements or interest in the use of fish in research over other vertebrate classes; and trends in the public zoological and aquarium industry to provide improved and advanced veterinary care to their fish. In research and display activities, interest in both fish welfare and environmental impacts is a motivating factor—collection from the wild is still a major means of marine specimen acquisition, and increased fish longevity decreases the need for such collecting. Efforts to ensure fish welfare are in aquaculture's best interest as improved welfare tends to correlate with decreased stress and, in turn, better product yield and quality.

As discussed, there is a need for basic information about the effects of the multitude of drugs across the many orders and families of fish. It is likely impossible to perform studies on a significant number of species, so the research emphasis should be on species from groups for which there is a dearth of knowledge, to improve extrapolation exercises that will forever be a part of fish medicine given the huge number of fish species. In particular, information about analgesia and drugs that may be appropriate is noticeably lacking (techniques and drugs used for fish euthanasia appear to be adequate and appropriate).

Beyond basic studies, investigations should be directed at bringing the practice of fish anesthesia to the level of familiarity and competence evident with other vertebrate classes. It is not uncommon for veterinarians, researchers, and similar personnel to be particularly pleased with themselves when they anesthetize a fish for the first time for a

short procedure and it recovers. While this indication of an increased interest in fish is a positive trend, the profession should not be satisfied with this level of performance. If fish medicine (and especially surgery) is to advance, it is essential to ensure that fish routinely survive medium- to long-term procedures. While lengthy procedures and survival are routine for a few species (e.g., carp), the literature is sparse concerning successful, prolonged surgical procedures in other fish species. In our experience, many fish that require advanced or lengthy surgical techniques often make it through the surgery only to decompensate or develop a metabolic crisis during recovery or shortly thereafter.

We have demonstrated that monitoring of metabolic status and vital signs is possible, but we have not explained how to monitor these trends over the long term or, more importantly, how to respond to signs of health problems. A movement away from immersion agents to injectable agents, which can be dosed more accurately based on individual weight, may help negate some of the variation that can occur with immersion agents, which are more affected by the water environmental status as well as the anatomy, physiology, and behavior of the fish species involved. The use of injectable agents does not, however, obviate the need for monitoring water quality. Alternatively, it may be worth considering immersion agents for induction (thus eliminating the need for handling awake fish), followed by administration of an injectable agent and postinduction removal of the fish to drug-free water.

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References

Afifi SH, Al-Thobaiti S, Rasem BM. 2001. Multiple exposure of Asian sea bass (*Lates calcarifer*, Centropomidae) to clove oil: A histological study. J Aqua Trop 16:131-138.

Aguiar LH, Kalinin AL, Rantin FT. 2002. The effects of temperature on the cardio-respiratory function of the neotropical fish *Piaractus mesopotamicus*. J Therm Biol 27:299-308.

Andrews JC, Jones RT. 1990. A method for the transport of sharks for captivity. J Aquaricult Aquat Sci 5:70-72.

AVMA [American Veterinary Medical Association]. 2007. Guidelines on Euthanasia (formerly the Report of the AVMA Panel on Euthanasia). Available online (http://www.avma.org/issues/animal_welfare/euthanasia.pdf), accessed March 11, 2009.

Bassi M, Klein W, Fernandes MN, Perry SF, Glass ML. 2005. Pulmonary oxygen diffusing capacity of the South American lungfish *Lepidosiren paradoxa*: Physiological values by the Bohr method. Physiolog Biochem Zool 78:560-569.

Bernal SD, Graham JB. 2001. Water-tunnel studies of heat balance in swimming mako sharks. J Exp Biol 204:4043-4054.

Bernstein PS, Digre KB, Creel DJ. 1997. Retinal toxicity associated with occupational exposure to the fish anesthetic MS-222 (ethyl-m-aminobenzoic acid methanesulfonate). Am J Ophthal 124:843-844.

Blank JM, Morrissette JM, Landeira-Fernandez AM, Blackwell SB, Williams TD, Block BA. 2004. In situ cardiac performance of Pacific bluefin tuna hearts in response to acute temperature change. J Exp Biol 207:881-890.

Bressler K, Ron B. 2004. Effect of anesthetics on stress and the innate immune system of gilthead bream (*Sparus aurata*). Israeli J Aquacult 56:5-13.

Brill RW, Bushnell PG. 2001. The cardiovascular system of tunas. In: Block BA, Stevens ED, eds. Tuna: Physiology, Ecology, and Evolution. New York: Academic Press. p 79-119.

Brown EAB, Franklin JE, Pratt E, Trams EG. 1972. Contributions to the pharmcology of quinaldine uptake and distribution in the shark and comparative studies. Comp Biochem Physiol 42A:223-231.

Bruecker P, Graham M. 1993. The effects of the anesthetic ketamine hydrochloride on oxygen consumption rates and behaviour in the fish *Heros* (*Cichlasoma*) *citrinellum* (Günther 1864). Comp Biochem Physiol 104C:57-59.

Bushnell PG, Jones DR. 1994. Cardiovascular and respiratory physiology of tuna: Adaptations for support of exceptionally high metabolic rates. Environ Biol Fishes 40:303-318.

Butler PJ, Taylor EW, Davison W. 1979. The effect of long-term, moderate hypoxia on acid base balance, plasma catecholamines and possible anaerobic end products in the unrestrained dogfish *Scyliorhinus canicula*. J Comp Physiol Part B 132:297-303.

Chandroo KP, Duncan IJH, Moccia RD. 2004. Can fish suffer? Perspectives on sentience, pain, fear, and stress. Appl Anim Behav Sci 86:225-250.

Cooke SJ, Suski CD, Ostrand KG, Tufts BL, Wahl DH. 2004. Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting largemouth bass (*Micropterus salmoides*). Aquacult 239:509-529.

Cooper R, Krum H, Tzinas G, Sylvia P, Belle S, Kaufman L. 1994. A preliminary study of clinical techniques utilized with bluefin tuna (*Thunnus thynnus Linnaeus*): A comparison of some captive and wild caught blood parameters. Proc Intl Assoc Aquat Anim Med 25:26-36.

Crosby TC, Hill JE, Watson CA, Yanong RPE. 2006. Effects of tricaine methanesulfonate, Hypno, metomidate, quinaldine, and salt on plasma cortisol levels following acute stress in threespot gourami *Trichogaster trichopterus*. J Aqua Anim Hlth 18:58-63.

Cullen LK. 1996. Muscle relaxants and neuromuscular block. In: Thurmon JC, Tranquilli WJ, Benson GJ, eds. Lumb and Jones' Veterinary Anesthesia, 3rd ed. Baltimore: Williams and Wilkins. p 337-364.

Davis KB, Griffin BR. 2004. Physiological responses of hybrid striped bass under sedation by several anesthetics. Aquacult 233:531-548.

Davis MR, Mylniczenko N, Storms T, Raymond F, Dunn JL. 2006. Evaluation of intramuscular ketoprofen and butorphanol as analgesics in chain dogfish (*Scyliorhinus retifer*). Zoo Biol 25:491-500.

Detar JE, Mattingly HT. 2004. Response of southern redbelly dace to clove oil and MS-222: Effects of anesthetic concentration and water temperature. Proc Ann Con Southeastern Assoc Fish Wildl Agen 58:219-227.

Dunn RF, Koester DM. 1990. Anesthetics in elasmobranchs: A review with emphasis on halothane-oxygen-nitrous oxide. J Aquaricult Aquat Sci 5:44-52.

Ferreira JT, Schoonbee HJ, Smit GL. 1984. The uptake of the anesthetic benzocaine hydrochloride by the gills and the skin of three freshwater fish species. J Fish Biol 25:35-41.

Fleming GJ, Heard DJ, Floyd RF, Riggs A. 2003. Evaluation of propofol and medetomidine-ketamine for short-term immobilization of Gulf of Mexico sturgeon (*Acipenser oxyrinchus de soti*). J Zoo Wildl Med 34:153-158.

Gelwicks KR, Zafft DJ. 1998. Efficacy of carbonic acid as an anesthetic for rainbow trout. N Am J Fish Mgmt 18:432-438.

Gilmour KM, Perry SF, Wood CM, Henry RP, Laurent P, Pärt P, Walsh PJ. 1998. Nitrogen excretion and the cardiorespiratory physiology of the gulf toadfish, *Opsanus beta*. Physiolog Zool 71:492-505.

Gomes LC, Chippari-Gomes AR, Lopes NP, Roubach R, Araujo-Lima CARM. 2001. Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. J World Aquacult Soc 32:426-431.

Graham JB. 1997. Air-Breathing Fishes: Evolution, Diversity, and Adaptation. San Diego: Academic Press.

Graham MS, Iwama GK. 1990. The physiologic effects of the anesthetic ketamine hydrochloride on two Salmonid species. Aquacult 90:323-332.

Gruber SH, Keyes RS. 1981. Keeping sharks for research. In: Hawkins AD, ed. Aquarium Systems. London: Academic Press, Harcourt Brace Jovanovich. p 373-402.

Guo FC, Teo LH, Chen TW. 1995. Effects of anaesthetics on the water parameters in a simulated transport experiment of platyfish, *Xiphophorus maculates* (Günther). Aquacult Res 26:265-271.

Hansen MK, Nymoen U, Horsberg TE. 2003. Pharmokinetic and pharmacodynamic properties of metomidate in turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*). J Vet Pharmacol Therap 26: 95-103.

Harms CA. 1999. Anesthesia in fish. In: Fowler ME, Miller RE, eds. Zoo and Wild Animal Medicine, Current Therapy 4. Philadelphia: WB Saunders. p 158-163.

Harms CA. 2003. Fish. In: Fowler ME, Miller RE, eds. Zoo and Wild Animal Medicine, 5th ed. St. Louis: Saunders. p 2-20.

Harms CA, Bakal RS. 1995. Techniques in fish anesthesia. J Sm Exot Anim Med 3:19-25.

Harms CA, Lewbart GA, Swanson CR, Kishimori JM, Boylan SM. 2005. Behavioral and clinical pathology changes in koi carp (*Cyprinus carpio*) subjected to anesthesia and surgery with and without intra-operative analgesics. Comp Med 55:221-226.

Harvey B, Denny C, Kaiser S, Young J. 1988. Remote intramuscular injection of immobilizing drugs into fish using a laser-aimed underwater dart gun. Vet Rec 122:174-177.

Holmgren S, Nilsson S. 1999. Digestive system. In: Hamlett WC, ed. Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes. Baltimore: Johns Hopkins University Press. p 144-173.

Horsberg TE, Burka JF, Tasker RAR. 1999. Actions and pharmacokinetic properties of the α_2-adrenergic agents, medetomidine and atipamezole, in rainbow trout (*Oncorhyunchus mykiss*). J Vet Anaesth 26:18-22.

Hoskonen P, Pirhonen J. 2004. The effect of clove oil sedation on oxygen consumption of six temperate-zone fish species. Aquacult Res 35: 1002-1005.

Hseu J-R, Yeh S-L, Chu Y-T, Ting Y-Y. 1996. Effects of anesthesia with 2-phenoxyethanol on the hematological parameters of four species of marine teleosts. J Fish Soc Taiwan 23:43-48.

Hseu J-R, Yeh S-L, Chu Y-T, Ting Y-Y. 1997. Different anesthetic effects of 2-phenoxyethanol on four species of teleost. J Fish Soc Taiwan 24:185-191.

Hseu J-R, Yeh S-L, Chu Y-T, Ting Y-Y. 1998. Comparison of efficacy of five anesthetics in goldlined seabream, *Sparus sarba*. Acta Zool Taiwan 9:11-18.

Huntingford FA, Adams C, Braithwaite VA, Kadri S, Pottinger TG, Sandøe P, Turnbull JF. 2006. Current issues in fish welfare. J Fish Biol 68:332-372.

Hughes GM. 1978. On the respiration of *Torpedo marmorata*. J Exp Biol 73:85-105.

Ishimatsu A, Itazawa Y. 1993. Anatomy and physiology of the cardiorespiratory system in air-breathing fish, *Channa argus*. In: Singh BR, ed. Advances in Fish Research. Delhi: Narendra Publishing House. p 55-70.

Iversen M, Finstad B, McKinley RS, Eliassen RA. 2003. The efficacy of metomidate, clove oil, Aqui-S™ and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquacult 221:549-566.

Keene JL, Noakes DLG, Moccia RD, Soto CG. 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquacult Res 29:89-101.

Kildea MA, Allan GL, Kearney RE. 2004. Accumulation and clearance of the anaesthetics clove oil and Aqui-S™ from the edible tissue of silver perch (*Bidyanus bidyanus*). Aquacult 232:265-277.

Kumlu M, Yanar M. 1999. Effects of the anesthetic quinaldine sulphate and muscle relaxant diazepam on sea bream juveniles (*Sparus aurata*). Israeli J Aquacult 51:143-147.

Latas PJ. 1987. The use of azaperone in the spiny dogfish (*Squalus acanthias*). In: International Association for Aquatic Animal Medicine Annual Proceedings. May 10-14, Monterey California. O'Fallon IL: Veterinary Software Publishing. p 157-165.

Maren TH, Embry R, Broder LE. 1968. The excretion of drugs across the gill of the dogfish, *Squalus acanthias*. Comp Biochem Physiol 26:853-864.

Mattson NS, Riple TH. 1989. Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol, and phenoxyethanol. Aquacult 83:89-94.

Miller SM, Mitchell MA, Heatley JJ, Wolf T, Lapuz F, Smith JA. 2005. Clinical and cardiorespiratory effects of propofol in the spotted bamboo shark (*Chiloscyllium plagiosum*). J Zoo Wild Anim Med 36:673-676.

Mulvey JM, Renshaw GMC. 2009. GABA is not elevated during neuroprotective neuronal depression in the hypoxic epaulette shark (*Hemiscyllium ocellatum*). Comp Biochem Physiol Part A 152:273-277.

Muñoz-Chápuli R, Satchell GH. 1999. Circulatory system: Anatomy of the peripheral circulatory system. In: Hamlett WC, ed. Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes. Baltimore: Johns Hopkins University Press. p 198-218.

Myszkowski L, Kamiński R, Wolnicki J. 2003. Response of juvenile tench *Tinca tinca* (L.) to the anaesthetic 2-phenoxyethanol. J Appl Ichthyol 19:142-145.

Neiffer DL. 2007. Boney fish (lungfish, sturgeon, and teleosts). In: West G, Heard D, Caulkett N, eds. Zoo Animal and Wildlife Immobilization and Anesthesia. Ames IA: Blackwell Publishing. p 159-196.

Oikawa S, Takeda T, Itazawa Y. 1994. Scale effects of MS-222 on a marine teleost, porgy *Pagrus major*. Aquacult 121:369-379.

Olsen YA, Einarsdottir IE, Nilssen KJ. 1995. Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. Aquacult 134:155-168.

Oswald RL. 1978. Injection anesthesia for experimental studies in fish. Comp Biochem Physiol 60:19-26.

Peters RC, Van Den Hoek B, Bretschneider F, Struik ML. 2001. Saffan®: A review and some examples of its use in fishes (Pisces: Teleostei). Nether J Zool 51:421-437.

Prince AMJ, Low SE, Lissimore TJ. 1995. Sodium bicarbonate and acetic acid: An effective anesthetic for field use. N Am J Fish Mgmt 15:170-172.

Rantin FT, Glass ML, Kalinin AL, Verzola RMM, Fernandes MN. 1993. Cardio-respiratory responses in two ecologically distinct erythrinids (*Hoplias malabaricus* and *Hoplias lacerdae*) exposed to graded environmental hypoxia. Env Biol Fish 36:93-97.

Rantin FT, Guerra CDR, Kalinin AL, Glass ML. 1998. The influence of aquatic surface respiration (ASR) on cardio-respiratory function of the serrasalmid fish *Piaractus mesopotamicus*. Comp Biochem Physiol 119:991-997.

Rose JD. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Rev Fisher Sci 10:1-38.

Ross LG. 2001. Restraint, anaesthesia, and euthanasia. In: Wildgoose WH, ed. BSAVA Manual of Ornamental Fish, 2nd ed. Gloucester: BSAVA. p 75-83.

Ross LG, Ross B. 1984. Anaesthetic and SedativeTechniques for Fish. Glasgow: Nautical Press.

Rothwell SE, Black SE, Jerrett AR, Forster ME. 2005. Cardiovascular changes and catecholamine release following anaesthesia in Chinook salmon (*Oncorhynchus tshawytscha*) and snapper (*Pagrus auratus*). Comp Biochem Physiol 140:289-298.

Roubach R, Gomes LC, Val AL. 2001. Safest level of tricaine methanesulfonate (MS-222) to induce anesthesia in juveniles of matrinxã, *Brycon cephalus*. Acta Amazonica 31:159-163.

Routley MH, Nilsson GE, Renshaw GMC. 2002. Exposure to hypoxia primes the respiratory and metabolic responses of the epaulette shark to progressive hypoxia. Comp Biochem Physiol Part A 131:313-321.

Sandblom E, Cox GK, Perry SF, Farrell AP. 2009. The role of venous capacitance, circulating catecholamines and heart rate in the hemodynamic response to increased temperature and hypoxia in the dogfish. Am J Physiol Regul Integr Comp Physiol 296:R1547-R1556.

Sladky KK, Swanson CR, Stoskopf MK, Loomis MR, Lewbart GA. 2001. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). Am J Vet Res 62: 337-342.

Small BC. 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. Aquacult 218:177-185.

Small BC. 2005. Routine measures of stress are reduced in mature channel catfish during and after Aqui-S anesthesia and recovery. N Am J Aquacult 67:72-78.

Sneddon LU, Braithwaite VA, Gentle MJ. 2003. Do fishes have nociceptors: Evidence for the evolution of the vertebrate sensory system. Proc Royal Soc Lond B 270:1115-1121.

Sneddon LU. 2003. The evidence for pain in fish: The use of morphine as an analgesic. Appl Anim Beh Sci 83:153-162.

Snyder SB, Richard MJ, Berzins IK, Stamper MA. 1998. Immobilization of sandtiger sharks (*Odontaspis taurus*). Proc Internat Assoc Aquat Anim Med 29:120-121**.** May 2-6, San Diego.

Söderström V, Renshaw GMC, Nilsson GE. 1999. Brain blood flow and blood pressure during hypoxia in the epaulette shark *Hemiscyllium ocellatum*, a hyopoxia-tolerant elasmobranch. J Exp Biol 202:829-835.

Spotte S. 1992. Captive Seawater Fishes. New York: John Wiley & Sons.

Stamper MA. 2007. Elasmobranchs (sharks, rays, and skates). In: West G, Heard D, Caulkett N, eds. Zoo Animal and Wildlife Immobilization and Anesthesia. Ames IA: Blackwell Publishing. p 197-203.

Steers JE, Sherrill J. 2001. Use of oral tiletamine-zolazepam for sedation and translocation of captive yellowtail jacks (*Seriola lalandi*). Proc Intl Assoc Aqua Anim Med. p 168-170.

Stehly GR, Gingerich WH. 1999. Evaluation of AQUI-S™ (efficacy and minimum toxic concentration) as a fish anaesthetic/sedative for public aquaculture in the United States. Aquacult Res 30:365-372.

Stetter MD. 2001. Fish and amphibian anesthesia. In: Heard DJ, ed. Veterinary Clinics of North America: Exotic Animal Practice. Philadelphia: WB Saunders. p 69-82.

Stoskopf MK. 1986. Preliminary notes on the immobilization and anesthesia of captive sharks. Erkrankungen der Zootiere. Akademie-Verlag Berlin 28:145-151.

Stoskopf MK. 1993. Shark pharmacology and toxicology. In: Stoskopf MK, ed. Fish Medicine. Philadelphia: WB Saunders. p 809-816.

Thomas P, Robertson L. 1991. Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulfate, and metomidate. Aquacult 96:69-86.

Totland GK, Kryvi H, Bone Q, Flood PR. 1981. Vascularization of the lateral muscle of some elamobranchiomorph fishes. J Fish Biol 18:223-234.

Tyler P, Hawkins AD. 1981. Vivisections, anaesthetics and minor surgery. In: Hawkins AD, ed. Aquarium Systems. London: Academic Press Inc., Harcourt Brace Jovanovich. p 248-278.

Ultsch GR, Jackson DC, Moalli R. 1981. Metabolic oxygen conformity in lower vertebrates: The toadfish revisited. J Comp Physiol 142:439-443.

Walsh CT, Pease BC. 2002. The use of clove oil as an anaesthetic for the longfinned eel, *Anquilla reinhardtii* (Steindachner). Aquacult Res 33:627-635.

Williams TD, Christiansen J, Nygren S. 1988. Intramuscular anesthesia of teleosts and elasmobranchs using ketamine hydrochloride. Proc Ann Conf Am Assoc Zool Parks Aquaria. p 132-135.

Williams TD, Rollins M, Block BA. 2004. Intramuscular anesthesia of bonito and Pacific mackerel with ketamine and medetomidine and reversal of anesthesia with atipamezole. JAVMA 225:417-421.

Yanar M, Kumlu M. 2001. The anaesthetic effects of quinaldine sulphate and/or diazepam on sea bass (*Dicentrarchus labrax*) juveniles. Turk J Vet Anim Sci 25:185-189.