EVALUATING METHODS TO ANESTHETIZE GOPHER ROCKFISH (*SEBASTES CARNATUS*) FOR IMMEDIATE RELEASE IN THE FIELD: INDUCTION, RECOVERY, AND CORTISOL STRESS RESPONSE TO SURGICAL ANESTHESIA

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ABSTRACT

EVALUATING METHODS TO ANESTHETIZE GOPHER ROCKFISH (*SEBASTES CARNATUS*) FOR IMMEDIATE RELEASE IN THE FIELD: INDUCTION, RECOVERY, AND CORTISOL STRESS RESPONSE TO SURGICAL ANESTHESIA

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Chemical anesthetics requiring a mandatory withdrawal period to allow for dissipation of drug residues pose severe limitations to acoustic research conducted at sea where captured fish undergo surgical implantation of transmitters and are released shortly after treatment. The efficacy and safety of three unrestricted approaches to anesthesia were evaluated in Gopher Rockfish *Sebastes carnatus*: carbon dioxide (CO_2), sodium bicarbonate ($NaHCO_3$), and pulsed direct current (pDC) electroanesthesia. These immediate-release methods were used to assess anesthetic induction and recovery times, plasma cortisol concentrations, and survival rates following surgery compared to those obtained from the widely used chemical anesthetic, tricaine methanesulfonate (MS-222). All anesthetics were effective at the concentrations tested. However, the times required to achieve stage IV anesthesia differed significantly, being shortest for electroanesthesia (nearly instantaneous) and longest for CO_2 (3.56 ± 0.21 min [mean ± SE]). Recovery times were significantly longer for $NaHCO_3$ (7.21 ± 1.17 min) and CO_2 (7.78 ± 0.93 min) compared to pulsed DC electroanesthesia (3.76 ± 0.21 min) and MS-222 (3.65 ± 0.38 min). Plasma cortisol levels differed among treatments but tended to peak around 0.5 h post-anesthesia and decline within 2 h. Given the prolonged recovery times of $NaHCO_3$ and CO_2, electroanesthesia is the most preferable method for rapid induction, recovery, and immediate release of Gopher Rockfish following surgery at sea.

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I had the honor of being in the last cohort of students taken under the wings of the illustrious Gregor Cailliet, Professor Emeritus of the Ichthyology Lab. Gregor is a wealth of knowledge and was always very encouraging as I navigated through my experiments. His keen memory came in handy countless times and he always provided me with suggestions that helped me to look at my research in different ways. Gregor's ability to instantly track down information, such as a decades-old tide pool collection paper, in his impressive bank of file cabinets never ceased to amaze me. Similarly, his uncanny knack of citing specifics from generations of student research showed me how much pride he takes in the work of Moss Landing graduates. Thank you for all of your positivity Gregor, you can officially retire with the knowledge that you inspired a new generation of marine scientists.

I am so thankful to have had the support of Larry Young from the Department of Veterinary Science and Institutional Animal Care and Use Committee (IACUC) Coordinator at San José State University. My thesis research was conducted with the approval of SJSU's IACUC (protocol number 963). Larry's vast experience from the regulatory side of the research world and tremendous attention to detail served to compliment my thesis committee tremendously and keep us all grounded. From the very beginning of this project, Larry had a keen interest in anesthetic research on fishes. I would like to thank him for his unending enthusiasm and support, coming out to work long hours with us on experiment days, providing experiment supplies, and generally providing great insight every step of the way. Thank you so much Larry, I have really enjoyed working with you and getting to know you over the years.

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I give honor to the Gopher Rockfish that unwittingly served as experimental subjects in my research. At various points throughout this project I couldn't help but feel just like the Catholic father played by the incomparable Michael Palin:

> Wait! I've got something to tell the whole family. The mill's closed! There's no more work. We're destitute. Come in, my little loves…I've got no option but to sell you all for scientific experiments. Children, I know you're trying to help, but believe me I've given this long and careful thought and it has to be medical experiments for the lot of you!
>
> [From Monty Python's *The Meaning of Life* 1983]

The Gopher Rockfish utilized for this experiment were unfortunate alien abductees taken from the blue sea, never to return, and I must express my gratitude for their sacrifice. Although unwitting subjects, it is my hope that their role in my project serves to advance acoustic studies and provide alternative methods for fish anesthesia in the future research.

Finally, I would like to dedicate this thesis to my family who has always supported my endeavors and had faith that I would succeed in whatever I do. Thanks for the love and for believing in me!

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INTRODUCTION

Anesthetics have long been used in fisheries research and aquaculture to reduce handling stress, minimize incidental damage during transport, and perform surgical procedures in a humane manner. Increasingly, researchers are conducting acoustic studies *in situ* to tag and track fish to learn more about their habitat use and movements. Historically, the chemical compound tricaine methanesulfonate (MS-222) has been widely used to anesthetize fish prior to the surgical implantation of acoustic tags. In the United States, it is currently the only drug approved for provisional use by the Food and Drug Administration (FDA) as an anesthetic in wild fish that may subsequently be caught and consumed upon release. However, to ensure human food safety, the FDA stipulates that treated fish must undergo a 21-day holding period prior to release to allow for full depuration of the drug (USFDA 2014). Furthermore, this approval is restricted for use in ictalurids, salmonids, esocids, and percids in water temperatures greater than 10°C (50°F). Though MS-222 is highly effective and has been rigorously field-tested, the mandatory regulations governing its use pose severe limitations to field research that requires the immediate release of wild fishes.

When conducting experimental or routine field procedures on animals that may cause more than momentary or slight pain, the appropriate use of sedation, analgesia, or anesthesia is required (US Public Health Service 1986). Institutional Animal Care and Use Committees (IACUCs) are responsible by law to uphold this principle while overseeing and evaluating all aspects of an institution's animal research protocols. Prior

to conducting a field or laboratory study involving animals, researchers must obtain a scientific collecting permit from their state and/or federal regulatory agency. Additionally, they are required to submit their research proposal to an IACUC for review and approval as well. The guidelines governing the protocols for IACUC permit issuance are federally mandated and require that the criteria set by many agencies, including the U.S. FDA, be upheld.

Current research using MS-222 as an anesthetic on food fish continues as many state agencies and IACUCs are either unaware of the FDA regulations or simply issue permits regardless of the rules. However, given the FDA's increased concern with regard to the use of pharmaceutical drugs in food animals and its regulations governing withdrawal times, it is highly probable that this current state of affairs will not be allowed to continue (R. Starr, Moss Landing Marine Laboratories, personal communication). As state regulatory agencies and IACUCs have begun to understand and adhere to FDA regulations, permits have been more difficult to obtain for researchers wanting to use MS-222 on wild fish that will immediately be released. Given the lack of alternative anesthetics, this scenario could effectively prohibit many research projects, including acoustic tagging studies of fishes, which require the use of an anesthetic before surgery. Therefore, there is a compelling need for the development of approved zero-withdrawal anesthetic approaches and immediate-release sedatives for use in wild fish research.

Although many studies have been conducted comparing the use of various anesthetics in fishes and assessing the related physiological stress response, few studies

have focused on the use of zero-withdrawal anesthetics for the purpose of conducting surgeries in a marine species. No studies have been published that evaluate the effects of anesthesia on Pacific rockfishes (*Sebastes* spp.), a group of more than 60 species that are commonly harvested by commercial and recreational fishers. Therefore, very little is known about the sub-lethal effects and short- to long-term survival rates following induction of surgical anesthesia in field applications. Of the many studies that have been published examining the effects of electrofishing and its effects on commercially important freshwater salmonids, relatively few have evaluated the use of electroanesthesia in the context of management practices or field applications.

I conducted a study to compare the efficacy of three immediate-release anesthetics: carbon dioxide gas (CO_2), sodium bicarbonate ($NaHCO_3$), and pulsed direct current (pDC) electroanesthesia for anesthetizing Gopher Rockfish *Sebastes carnatus* to the level necessary to perform invasive surgical procedures. To assess the different methods, CO_2, $NaHCO_3$, and pDC electroanesthesia were evaluated in side-by-side comparisons with MS-222. Two of these anesthetics, CO_2 and $NaHCO_3$, though not currently FDA approved fish anesthetics, are both classified as 'low regulatory priority drugs,' indicating that regulations are unlikely to be enforced provided that they are administered in compliance with the FDA specifications (USFDA 2011). Both CO_2 and $NaHCO_3$ have been declared 'generally recognized as safe' by the FDA as general-purpose food additives (Schnick et al. 1986). The third method, electroanesthesia, is currently unregulated and induces an anesthetic effect not through chemical means, but by physical means via electric current. Carbon dioxide, sodium bicarbonate, and

electroanesthesia are promising immediate-release anesthetics for conducting research on wild fishes, requiring no withdrawal time as they leave no harmful tissue residues. Additionally, these three methods have no adverse effects on the handler or the environment when used properly.

Research Questions

The limited number of immediate-release anesthetics for use on wild fishes and lack of data with regard to how marine fishes may respond to these techniques prompted the need for this research. Although the following experiments were conducted in a laboratory setting using clinical-based protocols, the ultimate goal was to compare the efficacy of immediate-release anesthetics and assess their applicability to field research conducted at sea aboard a research or fishing vessel. My study is composed of three experiments that evaluate the stage (depth) of anesthesia reached, induction and recovery times, the cortisol stress response, and overall survival rates of study fish.

The following questions were addressed:

1) How long does it take to achieve stage IV anesthesia for a range of concentrations of CO_2, $NaHCO_3$, and MS-222 in Gopher Rockfish?
2) What range of voltages, frequencies, and durations of exposure to pDC electroshock are sufficient to safely achieve stage IV anesthesia in Gopher Rockfish?
3) Do spinal injuries (e.g., vertebral compression, vertebral fracture, spinal-column fracture) or broken bones occur in Gopher Rockfish as a result of pDC electroshock? If so, at what point (i.e., at what levels) do they occur?
4) Do the sub-lethal effects of anesthesia (e.g., recovery times, healing rates, feeding rates) differ among the four treatments?

5) Are there significant differences in the immediate, short-term, and long-term survival rates associated with the different techniques?

6) Does the stress response of Gopher Rockfish, as measured by plasma cortisol concentration, vary among the four treatments?

Definition of Terms

The tendency to use the terms 'sedation' and 'anesthesia' somewhat ambiguously with reference to fishes is fairly prevalent. Given the casual overlap in the use of these terms in fish research, each will be defined here for clarity. Ross and Ross (2008) define sedation as a light state of anesthesia that provides a calming effect without gross loss of sensory perception or of equilibrium. Sedation is characterized by drowsiness, dulled sensory perception, reduced response to stimuli, and perhaps some analgesia or insensitivity to pain (Ross and Ross 2008).

The term 'anesthesia' has a Greek derivation meaning 'without sensation.' Anesthesia is defined as "a reversible, generalized loss of sensory perception accompanied by a sleeplike state induced by drugs or by physical means" (Heavner 1981). Another definition states that anesthesia is a "biological state induced by an external agent, which results in the partial or complete loss of sensation or loss of voluntary neuromotor control through chemical or non-chemical means" (Summerfelt and Smith 1990). General anesthesia affects the entire body and is characterized by general depression of the central nervous system that may result in "analgesia, suppression of reflex activity, and relaxation of voluntary muscle" (Green 1979). Since sedation and full general anesthesia occur along a continuum, it can be difficult to

pinpoint the exact processes that are occurring at a specific moment in time. The terms 'anesthesia' and 'electroanesthesia' will be used throughout for consistency and to reflect the stage along the continuum that is of greatest import to the present work.

Historical Use of Anesthetics in Fishes

Anesthetizing agents have long been essential tools for wild fish collection and aquaculture as well as clinical and field research on fishes. Common marking and biological collection procedures such as external tagging, fin clips, scale samples, and length/weight measurements can typically be achieved without the use of anesthetics. However, during routine handling practices, sedation helps to minimize stress and physical damage that can occur as a result of capture, crowded conditions, and transport by depressing the central and peripheral nervous system (Summerfelt and Smith 1990). Partial to total immobilization is necessary for more invasive procedures such as blood sampling and surgery. In addition, lengthy surgical procedures can necessitate the induction and maintenance of anesthesia over a longer time period.

Surgery provides a means to better understand fish physiology, conduct endocrinology studies, collect genetic samples, implant acoustic devices, and determine the overall health of fishes (Summerfelt and Smith 1990). Anesthesia is an important component of invasive surgical procedures, as improper immobilization could result in injuries to the fishes and/or their handlers. Anesthetic drugs can serve to lessen physiological changes associated with handling and surgery in some species and may provide a level of analgesia in some cases (Ross and Ross 2008). The only exception

which allows surgical procedures to be conducted without an anesthetic are specific research scenarios that the National Institutes of Health condone when a state of anesthesia would ultimately defeat the purpose of the experiment (NIH 1985).

For surgery, it is desirable to achieve a complete state of relaxation in which experimental fish show absolutely no rigidity in their musculature and fail to respond to external stimuli (Hudson et al. 2011). Surgical requirements may be facilitated by flushing a low concentration of anesthetic solution over the gills or by applying a low level of continuous DC electricity throughout the procedure. Anesthetization serves to protect both the fish and the human handler who will be performing these procedures. Depending on the dosage, a given anesthetic can produce a full continuum of effects from calming and immobilization to sedation and anesthesia, and ultimately can result in euthanasia. Factors to consider when designing an appropriate anesthetic protocol for fisheries research include efficacy, cost, withdrawal period (if any), legal status, ease of use, safety to handler and fish, and disposal considerations (Marking and Meyer 1985). Suitability for the species being studied, on-site conditions, and available resources may play a role as well.

Studies comparing anesthetics in fishes have shown that there are significant differences in response to anesthetics depending on the species being tested (Ferreira et al. 1984, Jennings and Looney 1998, Peake 1998, Taylor and Roberts 1999, Pramod et al. 2010). Differences in the biological factors affecting anesthesia can often be related to the "gill area to body weight ratio, which can vary considerably among fish species" (Coyle et al. 2004). Additionally, metabolic rates, which vary greatly between cold-water

and warm-water species, affect the rate at which chemicals are absorbed and therefore the rate at which anesthetic induction occurs (Coyle et al. 2004). Neiffer and Stamper (2009) caution that "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."

A new fish anesthetic, AQUI-S® E, is being used in New Zealand as an anesthetic for use on food fish with no withdrawal period. The active ingredient in AQUI-S® E, eugenol (2-methoxy-4-[2-propenyl] phenol), is one of the major components of clove oil and used in perfumes, culinary flavorings, essential oils, and in medicine as a local antiseptic and anesthetic. Although not yet approved in the United States, Benzoak (20% benzocaine, Frontier Scientific Laboratories, Logan, UT), AQUI-S® E (50% eugenol), and AQUI-S® 20E (10% eugenol; AQUI-S New Zealand, Lower Hutt, New Zealand) can be legally used under the U.S. Fish and Wildlife Service (USFWS) Investigational New Animal Drug (INAD) exemption for the purpose of generating clinical efficacy trial data to support their future approval and use (Trushenski et al. 2012b).

INAD exemptions allow for the collection of scientific data necessary to establish the effectiveness of an anesthetic in a variety of fish species under a variety of environmental conditions (e.g., temperature, water hardness, pH, turbidity, etc.). This provides an opportunity for fish culturists and fisheries managers to legally use unapproved anesthetics for the period of time necessary to collect efficacy, safety, and residue data, all of which are required for a New Animal Drug Application (NADA) in

fishes (USFWS 2009). Although INAD investigations to show the efficacy and safety of Benzoak, AQUI-S® E, and AQUI-S® 20E are currently underway, a three day withdrawal period still remains following the use of these compounds before treated fish can be released into the food chain.

Anesthetic Techniques for Surgical or Invasive Procedures

Immersion Anesthetics. Immersion anesthesia is the most widely used technique to sedate aquatic animals. Anesthetics administered to fish through bath immersions work systemically in a manner analogous to the use of gaseous inhalants in human and veterinary medicine (Neiffer and Stamper 2009). Gill diffusion is the main route for absorption and excretion of immersion anesthetics (Hunn and Allen 1974). Diffusion rates affect induction and recovery times and can be influenced by respiration, gill blood flow and permeability, and the physiochemical properties of the agents themselves (Zahl et al. 2009). As the anesthetic solution is ventilated by the fish, molecules rapidly diffuse into the blood surrounding the secondary lamellae, draining into the efferent arterial blood and moving directly towards the central nervous system (Ross and Ross 2008). Upon return to fresh seawater, the fish will begin to regain its sense of equilibrium as the drugs and their metabolites are excreted. While the excretion occurs primarily through gill diffusion, anesthetics can also be excreted, to a lesser extent, through the skin and kidney (Ross and Ross 2008).

In this study, three of the four approaches being compared are considered immersion anesthetics and were applied by submerging experimental fish in a bath

containing a dilute concentration of each anesthetic. Both CO_2 and $NaHCO_3$ are considered gaseous immersion anesthetics, inducing anesthesia to the subjects through release of carbon dioxide into the water. Conversely, MS-222 is a synthetic drug produced in the form of a crystalline powder that is highly soluble in water or can be pre-dissolved into a stock solution.

Carbon Dioxide. First described as an anesthetic method by Fish (1943), carbon dioxide gas has been used for over 70 years, primarily as a sedative for transportation and to reduce handling stress in hatcheries. As a non-pharmaceutical anesthetic, it is useful because it is safe, effective, extremely soluble in water, easily obtained, inexpensive, and nontoxic (Post 1979). Considered a viable immediate-release alternative, it leaves no toxic residues in fish tissue and does not have adverse effects on the handler or the environment if handled properly. Gaseous CO_2 is typically diffused into a solution using a pressurized cylinder attached to a regulator and administered via air stone to create an anesthetic bath in which the fish are placed.

"The sedative mode of action for CO_2 is based on the ability of high environmental concentrations to slow or reverse excretion at the gill, causing CO_2 build-up within the central nervous system and other tissues. Gradually, widespread central nervous system depression occurs, resulting in the loss of consciousness and voluntary motor function" (Trushenski et al. 2012b). In previous studies, it has been difficult to achieve complete surgical anesthesia with carbon dioxide alone (Bernier and Randall 1998), unless experimental fish underwent prolonged induction times (Yoshikawa et al.

1988). In general, CO_2 has been found to be less efficient than other anesthetics in achieving the deeper levels of anesthesia needed to perform surgical procedures.

Fishes undergoing CO_2 anesthesia typically display a brief but acute level of hyperactivity (Bernier and Randall 1998), struggling violently upon immersion in CO_2 saturated water (Yoshikawa et al. 1988). Specific problems associated with the use of CO_2 when compared to other anesthetics may include higher levels of stress indicators, such as blood adrenaline and cortisol (Iwama et al. 1989), hyperventilation, hypoxemic disturbances, and acidosis in Rainbow Trout *Oncorhynchus mykiss* (Bernier and Randall 1998). The difficulties with this technique are thought to arise due either to the disruption of the acid-base balance or the inability to reach dissolved concentrations high enough to achieve the desired level of anesthesia (Bell 1987). The addition of a buffering agent, usually sodium bicarbonate, helps solve these problems by maintaining a stable acid-base equilibrium in the anesthetic solution.

Sodium Bicarbonate. Fisheries researchers began to test sodium bicarbonate as a fish anesthetic as early as 1978 (Booke et al. 1978). Aside from being a controlled known source of carbon dioxide, it was unregulated, readily available, easily transportable, and extremely inexpensive compared to MS-222. Booke et al. (1978) found a concentration of 642 mg/L of $NaHCO_3$ effective in inducing hatchery-reared juvenile Rainbow Trout (15–16 cm TL), Brook Trout *Salvelinus fontinalis* (6–7 cm TL), and Common Carp *Cyprinus carpio* (4.6–7.5 cm TL) to stage II anesthesia, based on the criteria of Schoettger and Julin (1967). Stage II anesthesia is characterized by cessation of locomotion and slowed opercular movement with a retained reflex response to

pressure on the caudal fin. However, the highest concentration tested in the study (2142 mg/L) resulted in the death of all experimental fishes (Booke et al. 1978).

When sodium bicarbonate is used as an anesthetic agent itself, it is simply dissolved in water to release CO_2. However, building on the work of Booke et al. (1978), Prince et al. (1995) described a technique using sodium bicarbonate activated by glacial acetic acid to anesthetize adult Sockeye Salmon *Oncorhynchus nerka* (1.5–3.5 kg, 50–66 cm fork length [FL]) to stage IV (surgical) anesthesia based on the criteria of Yoshikawa et al. (1988) for implantation of radio transmitters. The addition of acetic acid served to speed and enhance the liberation of carbon dioxide by lowering the pH of the solution and maintain a more stable acid-base equilibrium. Once CO_2 has been released from the $NaHCO_3$ molecule, it works in a manner similar to the direct diffusion of gaseous CO_2 to create an anesthetic bath.

MS-222. Classified as an ester-type local anesthetic, MS-222 is one of the most commonly used anesthetic drugs in fishes. Developed by Sandoz pharmaceuticals in an attempt to find a cocaine substitute, MS-222 has been used as a local anesthetic in both human and veterinary medicine since its first production in 1920 (Ross and Ross 2008). As its use has been investigated for a number of ectotherms, the literature on the physiological effects of MS-222 is extensive. "A formidable list of physiological consequences of MS-222 use have been documented, including elevated hematocrit, erythrocyte swelling, hypoxia, hypercapnia, hyperglycemia, changes in blood electrolytes, hormones, cholesterol, urea, lactate and inter-renal ascorbic acid" (Ross and Ross 2008). Both biological factors (e.g., species and size of fish) and abiotic factors

such as water temperature, pH, and salinity can have a great impact on the efficacy of MS-222 (Treves-Brown 2000). Although the negative effects of MS-222 appear to be numerous, it is important to note that its long use has allowed for many investigations on its effects to be conducted.

Electroanesthesia. Electroanesthesia induces an anesthetic effect not by chemical means, but by physical means via electric current. More specifically, electroanesthesia "immobilizes fish by interfering with neurotransmission and causing electronarcosis (stunning) or electrotetany (tetanic muscle contraction)" (Trushenski et al. 2012b). The three main pulse types of electrofishing and electroanesthesia current are alternating current (AC), continuous direct current (cDC), and pulsed direct current (pDC).

Currently, neither FDA nor any other regulatory body guidelines exist governing the use of electroanesthesia to immobilize, sedate, or anesthetize fishes. Preliminary research has been conducted to demonstrate its efficacy and safety (Mitton and McDonald 1994; Barton and Dwyer 1997; Holliman and Reynolds 2002; Holliman et al. 2003a, 2003b; Chiba et al. 2006; Bowzer et al. 2012; Trushenski and Bowker 2012; Trushenski et al. 2012a, 2012b, 2012c), but like any other anesthetic approach its application should be applied in a stepwise fashion (Neiffer and Stamper 2009). Pulsed DC, which immobilizes fish by electrotetany is the pulse type that I evaluated. Current was applied with the head of the study fish at the anode, its body parallel to the direction of electron flow, until immobilization was reached. Generally, once pulsed DC was

applied, fish were immobilized in a matter of seconds and remained unconscious for a matter of minutes, during which surgical procedures were conducted.

Stages of Anesthetic Induction and Recovery

External cues such as opercular movement, swimming motion, and sense of equilibrium provide the criteria to determine which stage of anesthesia the fish are undergoing. I followed the progressive stages of anesthesia described by Yoshikawa et al. (1988) in a study examining changes in the depth of anesthesia of Common Carp anesthetized with a constant level of gaseous CO_2 (Table 1). In the broadest sense, recovery begins to occur when the anesthetic is withdrawn and fish return to a normal state. Notable benchmarks observed during the recovery process include eye movement/tracking, pectoral finning, caudal finning, tactile response, partial equilibrium, and finally full equilibrium. For the purposes of my study, recovery was defined as the point when fish regained and were able to maintain full equilibrium.

Table 1. Criteria used to determine progressive stages of anesthesia. (Source: Yoshikawa et al. 1988)

Stage of anesthesia	Opercular movement	Swimming motion	Sense of equilibrium
0	Normal	Normal	Normal
I	Normal	Normal	Partial loss
II	Normal	Normal	Total loss
III	Weak	Partial loss	Total loss
IV	Very Weak	Total loss	Total loss
V	Stop	Total loss	Total loss

Cortisol Stress Response

Like higher vertebrates, fishes show a wide range of external & internal signs of stress (Wendelaar Bonga 1997). The preliminary reaction to a stressor activates the neuroendocrine system called the hypothalamus-pituitary-interrenal axis (HPI axis), which leads to a massive release of two major classes of stress hormones: corticosteroids & catecholamines (Donaldson 1981; Mazeaud and Mazeaud 1981). Under stressful conditions, the corticosteroid cascade triggers the synthesis of cortisol, which is secreted by the interrenal tissue in the anterior kidney of a fish (Mommsen et al. 1999). Plasma cortisol has been used widely as an index of the stress response in fish, however, given the requisite of hormonal triggers to activate the synthesis of cortisol prior to release, the cortisol response the relatively slower component of the stress response in fishes (Barton and Iwama 1991).

MATERIALS AND METHODS

Study Species

Gopher Rockfish were chosen to evaluate the efficacy of the four anesthetic approaches being compared in this study. They are a nearshore species endemic to the Pacific coast of North America, ranging from Cape Blanco, Oregon to San Roque, Baja California Sur. Gopher Rockfish most commonly occur from Sonoma County to Santa Monica Bay, California, in close association with kelp beds and rocky reefs (Love et al. 2002). They occur from the intertidal to around 80 m, most commonly in depths between

9 and 37 m (Love et al. 2002). The species name, *carnatus*, is derived from the Latin word meaning 'flesh-colored,' describing the coloration of Gopher Rockfish, which is reddish-brown to olive-brown with large pink to whitish blotches (Love et al. 2002) (Figure 1).

Figure 1. Gopher Rockfish captured during a California Collaborative Fisheries Research Program (CCFRP) tag and release study. Photo credit: Sabrina Brennan.

Gopher Rockfish were chosen for study due to their high abundance in the collection areas, marked tolerance to aquarium conditions, and relative ease of capture. Populations north of Point Conception, California are considered healthy and were

estimated to be above the precautionary threshold based on the first stock assessment conducted for this species by Key et al. (2005). Recent recreational and commercial landings of Gopher Rockfish have been well below the Allowable Biological Catch (ABC) levels set by the Pacific Fisheries Management Council (PFMC). Given the population estimates for this species and the recently reported landings, Gopher Rockfish are considered to be abundant along the Pacific coast of California and not deemed a species of concern.

Gopher Rockfish are a major component of the live-fish commercial fishery in California that began in the mid-1980s to satisfy the demand for premium live fishes in Asian and specialty markets (Lucas 2006). In recent years, Gopher Rockfish have been the second most profitable (price per pound) and the third most commonly landed live (by weight) rockfish species in the northern California commercial fishery (Lucas 2006). Within the industry, it is common practice to transport live fishes to markets and restaurants where they are kept alive in aquariums until purchased for consumption by customers.

Gopher Rockfish have good survival rates and a high tolerance for aquarium conditions, making them prime species for the experimental design of this study, which required holding individuals for an extended duration of time to assess long-term effects. In addition to their high abundance, Gopher Rockfish were relatively easy to capture in the study area. In an ongoing tag and release study conducted along the central coast by the California Collaborative Fisheries Research Program (CCFRP), Gopher Rockfish

were one of the most abundant species caught with hook-and-line gear during from 2007–2009, representing 31% of the total catches (Starr et al. 2010). In concurrent surveys conducted using trap gear in 2008 and 2009, Gopher Rockfish comprised 55% of the total catches (Starr et al. 2010).

Fish Acquisition

Gopher Rockfish ranging from 21 to 32 cm total length (TL) were targeted for use in this study. Study fish were captured in open fishing areas in central California using a combination of hook-and-line and trap gear. To minimize collection time and effort, most fish were obtained during various CCFRP sampling seasons as well as aboard Commercial Passenger Fishing Vessel (CPFV), or party boat, fishing trips. On research and CPFV trips, only standard recreational fishing gear approved by the California Department of Fish and Wildlife (CDFW) were employed as methods of take. Baited shrimp fly lures with two hooks on one line were the most commonly used gear type and, when possible, barbs were crimped down to minimize hook damage to fish.

Following capture, individual Gopher Rockfish were marked for ease of identification during subsequent experiments with an external spaghetti tag (Hallprint®, South Australia, Australia) imprinted with a unique number code. The needle of a standard tagging gun was sterilized in isopropyl alcohol and inserted approximately 1.25 cm into the musculature of the fish below the dorsal fin between the base of the third and fourth spines where the T-bar of the tag would anchor. All study fish were vented to relieve barotrauma by releasing expanded air from the swim bladder, which allowed them

to maintain neutral buoyancy in the live wells. The abdominal wall and underlying swim bladder were punctured with a sterile 16-gauge hypodermic needle to achieve venting.

Tom Hafer, a commercial fisherman from Half Moon Bay, California, designed the fish traps employed in this study. They were intended to function similarly to traps commonly used by the live fin-fish commercial fishery in the local region. Traps were baited with approximately 16 ounces of frozen market squid *Doryteuthis* (formerly *Loligo*) *opalescens* and deployed to the bottom of nearshore rocky reefs. Intervals between the deployment and retrieval (i.e., soak times) of trap sets were kept as short as possible within a timeframe allowing for adequate numbers of specimens to be captured. Based on the catch rates of previous studies using this method, 90 minutes was a standard estimate of sufficient soak time (Starr et al. 2010). Traps were intentionally set in shallow water (< 24 m) to reduce the occurrence of barotrauma in the study fish.

All non-target species of fish or invertebrates caught incidentally using hook-and-line or trapping methods were released immediately at the site of capture. When deemed necessary, non-target fishes possessing swim bladders that appeared to be suffering barotrauma were vented, allowing them to swim back to the bottom without the aid of a descending device. The target sample number needed to successfully complete the experiments in this study was approximately 247 Gopher Rockfish (Table 2).

Table 2. Experimental design showing sample size of Gopher Rockfish per treatment. Only 10 fish for $NaHCO_3$ treatments (pilot study and EXP 2) were used for data analyses.

Experiment	CO_2	$NaHCO_3$	MS-222	pDC	Control
Pilot: Immersion Anesthetics	10	11	10	–	–
Pilot: pDC Electroanesthesia	–	–	–	13	–
EXP 1: pDC Recovery & Injuries	–	–	–	60	–
EXP 1: pDC Size Effects	–	–	–	12	–
EXP 2: Post-surgical Effects	10	11	10	10	10
EXP 3: Cortisol Stress Response	16	16	16	16	16
Treatment Totals	36	38	36	111	26

Collection Locations

Collection locations included open fishing areas near the cities of Half Moon Bay and Pescadero in San Mateo County and Monterey and Carmel in Monterey County, California (Figure 2). These areas have large numbers of Gopher Rockfish and were within close enough proximity to aquarium holding facilities to ensure that transport times and fish mortality were minimized. Upon capture, fish were held onboard the fishing vessels in live wells with recirculating ocean water until the end of the fishing day.

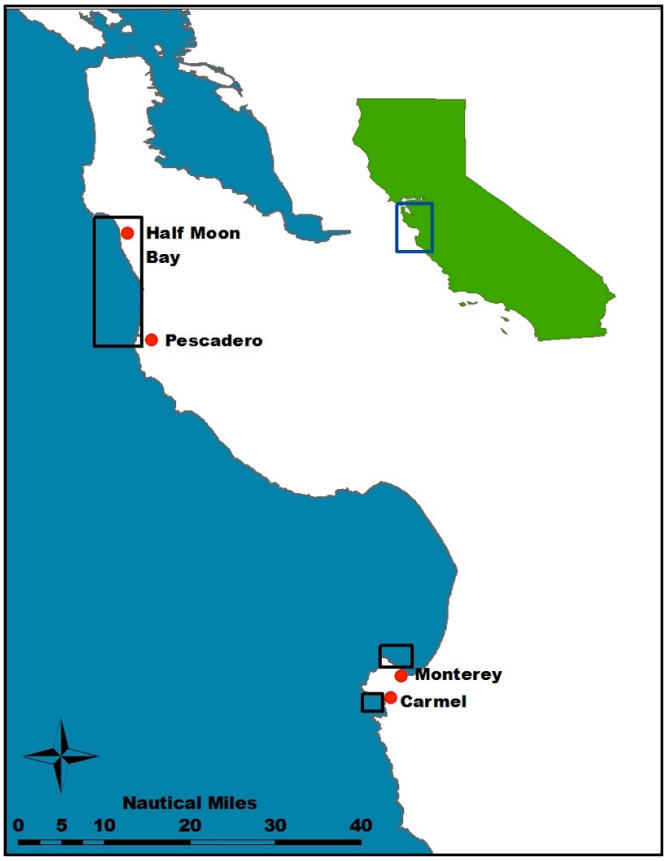

Figure 2. Map of collection locations. Areas outlined in black denote locations where study fish were collected excluding State Marine Reserves (SMR) within these areas.

During ground transportation fish were held in large (141.95 L) coolers equipped with air bubblers and seawater was chilled with marine ice or frozen water bottles. Lowered temperatures have a tranquilizing effect on fish and reduce their metabolic rates, thereby decreasing ammonia and solid waste production (Ross and Ross 2008). Ultimately, fish were transported to holding aquaria at the Southwest Fisheries Science Center (Fisheries Ecology and Research Division of the National Oceanic and

Atmospheric Association's [NOAA] National Marine Fisheries Service [NMFS]) and Long Marine Laboratory (University of California, Santa Cruz) both located in Santa Cruz, California.

Holding Aquaria, Care, and Final Disposition of Study Fish

All fish used in this study were held in aquarium tanks located at the Southwest Fisheries Science Center and the Long Marine Lab facility for the duration of the experiments. Aquaria consisted of large polypropylene tanks (2.44 m diameter) holding a volume of approximately 4.86 m^3 (1284 gallons), connected to the facility's flow-through seawater system. Study fish were acclimated for a minimum of two weeks prior to the outset of experiments, ensuring that they had sufficiently recovered from possible capture-related or transport stress. Fish were fed to satiation twice a week with previously frozen Surf Smelt *Hypomesus pretiosus*, market squid, Capelin *Mallotus villosus,* or other commercially available Rainbow Smelt *Osmerus mordax mordax* that were chopped and thawed prior to feeding. Tank water quality parameters such as temperature, salinity, pH, turnover, and filtration were monitored throughout the duration of the study. Fish were held for a period of one week to two months following the experiments to assess short-term and long-term mortality.

At the conclusion of post-experimental observations, efforts were made to find the remaining study fish permanent homes in nearby aquariums. As required by CDFW, all remaining study fish were euthanized using approved techniques to prevent potential disease transmission to wild stocks. Euthanasia was achieved by placing fish in a tub of

seawater with an overdose of MS-222 (> 250 mg/L) as recommended in the American Veterinary Medical Association's (AVMA) Guidelines on Euthanasia (2007). Fish remained immersed in the solution for at least 10 minutes after cessation of opercular movement to ensure that they had expired. Some study fish were retained for radiographic imaging or dissected to determine if electroanesthesia caused internal injuries. Preserved specimens were donated upon request to the University of California, Santa Cruz and Moss Landing Marine Laboratories to be used as dissection fish for ichthyology classes, thereby reducing collection pressure on wild populations. Carcasses of study fish were then frozen until transported to Salinas Tallow Company for final disposal.

Justification of Anesthetic Concentrations Used

After an extensive review of the primary literature, concentration ranges for each anesthetic were chosen based on those successfully applied in the previous studies described here. In some cases, freshwater experiments provided the only references available for particular anesthetics so most dosage levels were adapted from studies conducted on unrelated species. As research regarding the effects of anesthetics on marine fish species is limited, thorough pilot studies were necessary to determine appropriate dosage levels for effective anesthesia in Gopher Rockfish.

Carbon dioxide concentrations in water are relatively difficult to control or measure with any accuracy in laboratory settings, let alone in the field. Previous laboratory experiments evaluating CO_2 as an anesthetic have been conducted in isolated

bubbling chambers where thermoregulators were used to maintain constant temperatures and flowmeters, or rotameters, were used to deliver gas at measurable rates (Yoshikawa et al. 1988). Studies conducted in laboratories or in the field without isolated chambers typically diffuse CO_2 from a compressed gas cylinder into an anesthetic bath and control flow with a regulator (Sanderson and Hubert 2007).

Sanderson and Hubert (2007) successfully implanted radio transmitters into Cutthroat Trout *Oncorhynchus clarkii* (400 mm mean TL) after effective anesthesia via diffusion of CO_2 into a water bath at a rate of 3L/min for 1 min before placing the fish in the anesthetic bath. Similarly, the flow rate of compressed CO_2 in my experiments was controlled by a calibrated rotameter attached to the tank regulator and diffused into the anesthetic bath via an aquarium airstone. Efficacies of various flow rates between 1L/min – 5/L min were evaluated during the pilot study.

The range of sodium bicarbonate concentrations tested was chosen based on a study comparing $NaHCO_3$ to clove oil as an anesthetic for nonsalmonid fishes (Peake 1998). Peake used three concentrations of $NaHCO_3$ (1.33, 2.66, and 4.00 g/L) activated with the addition of acetic acid on 36 Walleyes *Stizostedion vitreum* (38–67 cm FL) and found that 2.66 g/L was the optimal concentration to achieve stage IV anesthesia. Peake (1998) administered this concentration on 24 individuals of three other species: Small Mouth Bass *Micropterus dolomieu* (26 cm [mean FL]), Northern Pike *Esox lucius* (30 cm) and Lake Sturgeon *Acipenser fulvescens* (27 cm). Using this concentration, each species achieved stage IV anesthesia with induction times ≤ 5.4 min and recovery times ≤

4.8 min. Given that 2.66 g/L $NaHCO_3$ was used successfully on a variety of species with reasonable induction and recovery times, it was chosen for evaluation in the pilot study in addition to a range of three nearby concentrations: 2.00, 3.33, and 4.00 g/L.

Appropriate concentrations of MS-222 are more widely documented on members of the genus *Sebastes* given that MS-222 is the most commonly used fish anesthetic. MacFarlane and Bowers (1995) used 200 mg/L MS-222 to surgically anesthetize Yellowtail Rockfish *Sebastes flavidus* (816–1034 g) in 2.5–3.0 min for the administration of a radiolabel. Fish were maintained under surgical anesthesia with a continuous irrigation of 55 mg/L MS-222 solution over their gills. Jorgensen et al. (2006) used a 10% solution (150 mg/L) of MS-222 to implant acoustic tags into Blue Rockfish *Sebastes mystinus* (35.6 ± 0.5 cm TL [mean ± SE]) to monitor their movements in Northern California. Green and Starr (2011) used a solution of 100 mg/L MS-222 to anesthetize Black Rockfish *Sebastes melanops* (25.0–41.0 cm) prior to surgery.

Low concentrations of 50 mg/L MS-222 are generally used to produce light sedation for routine handling procedures in rockfishes (MacFarlane and Bowers 1995; Sabrina Beyer, NOAA Fisheries, personal communication) so pilot study concentrations above this level were chosen. Since concentrations of MS-222 greater than 250 mg/L are recommended for fish euthanasia, pilot study concentrations were set ≤ 200 mg/L. The following four concentrations were tested: 100, 150, 175 and 200 mg/L MS-222.

I used the Portable Electroanesthesia System (PES™) manufactured and sold by Smith-Root, Inc., to apply electroanesthesia to the study fish. The unit (Figure 3) consists

of a modified Coleman cooler serving as an anesthesia tank in which an electrical current at user-set levels is applied to anesthetize fish. Smith-Root debuted their product in 2009 at the Aquaculture America conference in Seattle, Washington.

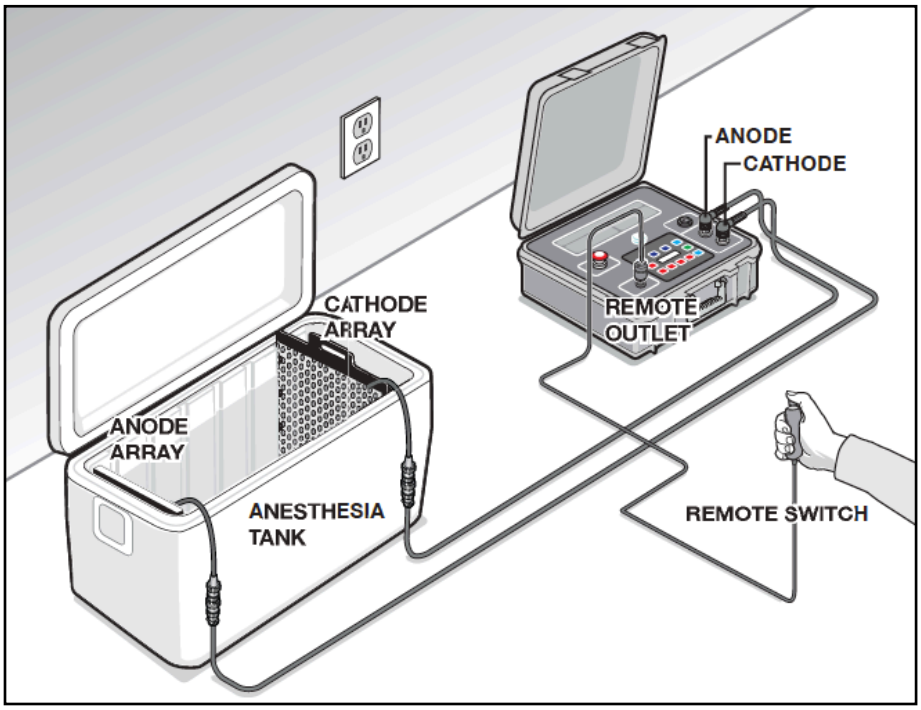

Figure 3. Diagram of a Smith-Root Portable Electroanesthesia System (PES™) with labeled major components of anesthesia tank and control unit labeled. (Source: Smith-Root, Inc. PES™ User's Manual 2009)

The PES™ unit is capable of delivering both pulsed and continuous DC electroshock with settings fully adjustable up to 400 watts. Induction time is nearly instantaneous using this technique, as can be viewed in the product video on the Smith-Root website: http://www.smith-root.com/electroanesthesia/pes/. Human safety can

easily be assured during this type of procedure by electrically isolating the operator via the donning of rubber gloves and boots and through the use of an insulated net or net handle.

Waveform parameter settings on the PES^{TM} are fully adjustable, and are programmed by the user on the control unit to determine the electroanesthetic dose that will be delivered. Specific doses are defined by the following electrical parameters: pulse type, exposure time, volts, frequency, and duty cycle. The pulse type denotes the type of electric current output available using the PES™: ramped DC, standard pulsed DC, or burst of pulses (DC). Exposure time refers to the pulse duration, or length of time, study fish will be exposed to electroshock. When the pulse type chosen is standard pulsed DC, output immediately puts out maximum voltage, stays constant for the entire pulse duration, and drops back to zero (Smith-Root PES™ User's Manual 2009). Standard pulsed DC is a type of pulse referred to as 'rectangular.' Voltage, or volts (V) refers to the amplitude of the output, which is variable on the PES™ between 20 and 400 V, in 5 V increments. Frequency is defined as the number of occurrences of a repeating event per unit time. Frequency in hertz (Hz) means the number of cycles per second or, in this circumstance, the number of pulses of electroshock per second. Pulse frequency on the PES™ can be varied between 5 Hz and 100 Hz, in increments of 5 Hz, and then at set increments of 250 Hz, 500 Hz, or 1000 Hz (Smith-Root PES™ manual 2009). Duty cycle is the percent of the wave cycle where the output is at the set voltage or calculated 'on-time.'

Preliminary tests were conducted using various levels of field strength (voltage), frequency (hertz), and exposure time to determine the optimal range needed to reach stage IV anesthesia in Gopher Rockfish. In this study, the anesthesia tank was filled with fresh water rather than seawater to ensure that the anesthetic bath was less conductive than the body of the fish. Using freshwater is necessary to achieve effective electroanesthesia. The short duration of exposure (< 10 s) was deemed brief enough to be negligible in terms of stress to the study fish. Freshwater 'dips' of 5 to 15 min are a common technique used by aquarists to combat external parasites on marine fishes in captivity (P. Macht, Seymour Marine Discovery Center, personal communication).

Overview of Experimental Design

All of the experimental procedures detailed herein were conducted under the guidance and approval of San José State University's Institutional Animal Care and Use Committee (IACUC; protocol 963). Between June 18, 2011 and September 18, 2012 a total of 247 Gopher Rockfish were obtained using the methods previously described. One pilot study (n = 30) was performed to reduce the total number of study fish by predetermining the proper dosages of the three immersion anesthetics: CO_2, $NaHCO_3$, and Finquel (MS-222; 100% tricaine methanesulfonate; Argent Laboratories, Redmond,Washington). A second pilot study (n = 13) was conducted to determine the appropriate strength and duration of pulsed DC electroanesthesia prior to the main experiments.

In order to reduce observer variability, the same observer monitored progression through the stages of anesthesia and recovery throughout all of the experiments, as the criteria for various stages are somewhat subjective. The first experiment consisted of trials to establish induction and recovery times for pulsed DC electroanesthesia and to determine if perivertebral hemorrhages or spinal injuries occurred following exposure. Potential injuries were assessed through radiographic imaging and dissection of the study fish following euthanasia. Additionally, a small subset of this experiment served to assess the effect of size (fish total length) on recovery times following exposure electroanesthesia.

During a second experiment, mock acoustic transmitters were surgically implanted into fish following anesthesia by one of the four methods. Ideal concentrations of the four anesthetics were used to compare induction and recovery times and assess healing and survival rates post-surgery. A third experiment focused on the physiological stress response of study fish following anesthetic treatments by examining plasma cortisol levels isolated from blood samples. For the general purposes of this study, six criteria with the following thresholds were used to define a suitable field anesthetic for use when surgically implanting transmitters into fish: (1) zero withdrawal period, (2) stage IV level of anesthesia achieved, (3) induction time to stage IV anesthesia < 7 min, (4) recovery time from stage IV anesthesia < 10 min, (5) postsurgical survival rate > 90%, and (6) delayed (2 weeks to 2 months post-surgery) mortality rates < 5%.

Pilot Study: Determination of Effective Concentrations for Immersion Anesthetics

Prior to the primary experiments, a pilot study was conducted using 30 Gopher Rockfish of adult size (509.1 ± 31.8 g [mean weight ± SE]; 28.0 ± 0.6 cm [TL]) to establish appropriate concentrations for the three immersion anesthetics: CO_2, $NaHCO_3$, and MS-222. Three fish per concentration of each immersion anesthetic were evaluated. Feed was withheld for 24 h before starting the experiment. Individual fish were transferred from holding tanks into an anesthetic bath (37.85 L glass aquarium) containing 19 L of seawater and specified concentrations of each anesthetic.

Each anesthetic bath was individually prepared immediately before use, water was replaced between each fish treated, and the tank was rinsed before preparation of the next concentration. Temperature and pH (Multi-Parameter PCTestr™ 35; Oakton Instruments, Vernon Hills, Illinois) were measured prior to the introduction of a new fish into the anesthetic bath. Immersion anesthetic baths were prepared with aerated culture water from the holding system as follows:

1. CO_2: delivered from a pressurized cylinder (regulator reading 20 psi) at a rate of 3, 4, or 5 L/min through a calibrated rotameter via airstone for 5 min prior to introduction of the fish
2. $NaHCO_3$: 2.00, 2.66, and 3.33 g/L solutions activated with 14.25, 18.95, or 23.73 mL glacial acetic acid, respectively
3. MS-222: 100, 150, 175, and 200 mg/L unbuffered solutions of Finquel

In order to saturate seawater with enough CO_2 to achieve an anesthetic effect, gas was bubbled for approximately 5 min prior to the immersion of study fish into the tanks. During this time pH was taken every 30 s with a waterproof handheld meter. Regardless of flow rate used, once 5 min had elapsed the pH of a CO_2 saturated anesthetic bath had decreased substantially to levels between pH 5.1 to 5.3. Therefore, it was necessary to add 65 to 76 g of sodium bicarbonate to each 19L volume of CO_2 saturated seawater to function as a buffer and increase pH levels to between 6.2 and 7.0, insuring that pH levels were not drastically lower than other treatments. Once the sodium bicarbonate was added, the tank water was stirred by hand until it dissolved, and pH readings were recorded again directly before the introduction of the study fish into the anesthetic bath.

Each fish was individually monitored from the time of immersion in the anesthetic bath to determine the time required to reach each stage of anesthesia up to stage IV as described by Yoshikawa et al. (1988). Stage IV is characterized by a very weak opercular rate, total loss of swimming motion, and total loss of equilibrium. Additional behaviors cited by other researchers during the progressive stages of anesthetic induction were also taken into account to insure that fish were not venturing too deep into anesthesia or approaching stage V. Most notably, after fish had lost equilibrium (i.e., were no longer to able to maintain an upright posture) and were exhibiting signs of slowed swimming movements, they were regularly challenged with tactile stimuli in the form of moderate pressure applied to the caudal peduncle. Summerfelt and Smith (1990) characterized fish in stage III of anesthesia as reactive only to strong tactile stimuli, whereas fish in stage IV have complete loss of spinal reflexes.

For the purposes of this study, once equilibrium was lost, swimming movements ceased, and there was no reaction to caudal peduncle stimulation, stage IV anesthesia was considered reached.

Induction and recovery times were measured with a stopwatch and recorded to the nearest second. Following induction into stage IV each fish was quickly weighed (to the nearest 0.1 g), measured to determine TL (to the nearest 0.1 cm), and transferred to a recovery tub that had been filled with aerated seawater at the same time the anesthetic baths were prepared. In the recovery tub the fish were monitored continuously to determine time to full equilibrium. Recovered fish were promptly returned to their holding system and monitored for survival at 24 and 72 h post-experiment.

Pilot Study: Determination of Strength and Duration of Electroanesthesia

Preliminary tests were conducted to determine the appropriate waveform parameters (pulse type, voltage, frequency, exposure time, and duty cycle) of electroshock capable of achieving stage IV anesthesia in Gopher Rockfish (n = 13), 29.4 ± 0.5 cm TL. A range of combinations of various voltages (50, 75, 100, or 150 V), frequencies (30 or 60 Hz), and exposure times (3, 5, or 10 s) were tested with one fish being exposed to each setting. The anode and cathode array of the PES™ were set to a distance of 62.5 cm apart and the anesthesia tank was filled with municipal fresh water. Conductivity (µs) and temperature (Oakton® Multi-Parameter PCTestr™ 35; Oakton Instruments, Vernon Hills, Illinois) were measured prior to the introduction of the fish into the PES™ unit. Feed was withheld for 24 h before starting the experiment.

Individual fish were transferred from holding tanks into the unit via a fishing net with an insulated handle and electricity was applied once the researcher had let go of the handle. Water for anesthetic baths was exchanged after 6 to 9 fish had been treated, depending on the level of the conductivity reading.

Induction into stage IV was considered instantaneous following exposure to electroanesthesia. Fish typically displayed a high degree of body rigidity including opercular flaring and fin extension, underwent visible tremors for a brief period following exposure, and were generally unresponsive. Upon removal from the PES™ unit each fish was quickly weighed, measured, and transferred to a recovery tub with aerated seawater.

In the recovery tub the fish were monitored extensively to determine time to full equilibrium. Additionally, other characteristic signifiers of recovery were noted. When tremors were present following electroanesthesia, the time they ceased was recorded. If opercular movement was absent following electroanesthesia, the time movement resumed was noted and the number of opercular beats during a 10 s period was recorded. Times were recorded for other benchmarks of the recovery process as well as time required for fish to reach full equilibrium (recovery). Recovered fish were promptly returned to their holding system and monitored for survival at 24 and 72 h post-experiment.

Experiment 1: Determining Recovery Times, Size Effects, and Potential Injuries of Electroanesthesia

Effective dosages identified in the pilot study were used in the primary investigation of electroanesthesia, testing a larger group of fish in equivalent replicates (*n*

= 60) to compare efficacies among the doses and establish a single ideal dose to use for the remainder of the experiments. Additionally, an effective dose was applied to a small group of fish (n = 12) whose total lengths spanned a range from 23 to 31 cm to assess whether length (as a proxy of surface area exposed to electroshock) had a noticeable effect on recovery rates. Following treatment, fish were humanely euthanized using an overdose of MS-222 and frozen at -20°C until which time they could be processed for assessment of potential injuries.

Dr. Dave Casper, UCSC attending veterinarian and marine specialist, conducted radiograph analyses on the study fish. Following euthanasia, Dr. Casper took digital x-rays (MinXray 100kV/30mA High Frequency Portable X-Ray System, Pacific Northwest X-Ray Inc., Gresham, Oregon) of individual study fish and conducted lateral radiograph analysis (IDEXX I-Vision CR® System powered by IDEXX-PACS™ Imaging Software, IDEXX Laboratories, Westbrook, Maine) to assess whether vertebral compressions, spinal fractures, or broken bones had occurred as a result of severe muscle tetany during electroshock.

Lastly, these fish were dissected to examine the possibility of internal hemorrhaging or bruising associated with the vertebral column undetectable by radiograph. The presence of perivertebral hemorrhages were assessed by dissection involving a fillet cut beginning immediately posterior to the head and continuing along the length of the fish to the caudal peduncle. This was essential not only in determining

the effective range of electroshock for use in subsequent experiments comparing anesthetic methods, but also to ensure that this technique did not injure study fish.

It should be noted that these types of injuries were not a predicted outcome of the pDC electroanesthesia being tested in this study. Electrically induced injury and mortality rates are a function of the type and strength of the waveform used, as well as the fish involved (Snyder 2003). In general, short duration exposure to low-intensity, pulsed DC waveforms is considered less risky than longer duration exposure to high intensity, AC waveforms (Bowzer et al. 2012).

Although electroanesthesia appeared to cause no injury in Walleye (Vandergoot et al. 2011), some species have incurred vertebral and internal injuries resulting from exposure to pulsed DC electrosedation, including Lake Trout *Salvelinus namaycush* (Gaikowski et al. 2001), Chinook Salmon *Oncorhynchus tshawytscha* (Zydlewski et al. 2008), and American Eels *Anguilla rostrata* (Reynolds and Holliman 2004). Incidences where vertebral injuries resulted from the application of pDC electroshock have generally occurred in large, strong-swimming, body-undulating fishes such as eels and salmonids. In a study of injury in American Eels captured by electrofishing, Reynolds and Holliman (2004) reported that 60% of the fish suffered spinal damage as a result of pulsed DC electroshock (30 Hz; peak voltage of 336V; peak current of 4 – 5 amperes [A]). The authors hypothesized that the high rates of injury were most likely due to the large size (> 90 cm) and the high vertebral count (> 100) of the eels. Unlike eels, Gopher Rockfish are fairly small, compact, and have a much lower vertebral count (< 30).

Experiment 2: Comparison of Anesthetics on Post-surgical Effects

The optimal level or dosage of each type of anesthetic, as determined by pilot studies, was applied to treatment groups comprised of 10 individual Gopher Rockfish. Experimental fish were parsed by tank and binned by size (based on TL) into roughly equivalent numbers for each treatment group and feed was withheld for 24 h before the start of the experiment. Pulsed DC was applied in the PES^{TM} cooler using municipal freshwater. Immersion anesthetic baths were prepared in 37.85 L glass aquarium tanks using aerated seawater taken from the holding system as follows:

1. CO_2: delivered from a pressurized cylinder (regulator reading 20 psi) at 5 L/min through a calibrated rotameter via airstone for 5 min prior to introduction of the fish
2. $NaHCO_3$: 2.66 g/L solutions activated with 18.95 mL glacial acetic acid
3. MS-222: 175 mg/L unbuffered solution of Finquel
4. pDC: 150 V, 60 Hz, 12% duty cycle, 3 s exposure in municipal freshwater
5. Control: immersion in untreated seawater in both treatment tank and recovery tub for mean induction and recovery times

A control group (n = 10) was included in this experiment to assess the effects of netting and handling on subsequent weight gain or loss of the study fish. Control fish were netted and handled in the same way as the anesthetic treatment groups and placed into aquarium tanks and recovery tubs filled with plain untreated seawater. They were held in the anesthetic bath for 2 min 52 s and in recovery tubs for 5 min 22 s, the

combined average stage IV induction and recovery times of the immersion anesthetics tested in the pilot study. As in previous experiments, the times required to reach each stage of anesthesia were recorded. Once fish reached stage IV anesthesia, a surgical procedure was conducted to implant a mock acoustic tag into the peritoneal cavity.

Stage IV anesthetized fish were placed dorsal side down on a V-shaped surgical cradle. Scales were carefully removed with the edge of a sterilized scalpel from a small area (2.0 cm long x 1.0 cm wide) along the ventral midline between the pelvic fins and the anus of the fish. In this descaled area, a short incision (~ 1.5 cm) was made along the midline using a scalpel sterilized with isopropyl alcohol. A compact, sterile mock transmitter was then inserted into the peritoneal cavity of the fish through the incision. The mock transmitters were cylindrical in shape and cut to size (25.5 mm long x 9.5 mm wide) from Delrin® Acetal Rod (DuPont Engineering Polymers, Wilmington, Delaware), a stable thermoplastic polymer highly resistant to moisture. The weight of the mock transmitter was light enough to avoid transmitter to body weight ratios that could cause undue stress to the fish, and the edges were sanded round to reduce internal irritation. Incisions were closed with 2 – 4 stainless steel staples (6.5 mm × 4.7 mm closed) depending on fish size, using a standard surgical skin stapler. Staples were selected for use rather than sutures in order to reduce surgery time and infection rates (Sanderson and Hubert 2007).

After surgery was conducted, fish were returned to clean seawater and the time to full recovery of equilibrium was recorded. After the experiments, fish were held in aquaria for two months to monitor healing times of incisions, infection rates, subsequent

weight gain or loss, and to monitor any delayed mortality (i.e., assess short- and long-term survival rates). Visual observations, weights, and photographs were taken for individual fish every day for the first 72 h following surgery and weekly to bimonthly thereafter. Special attention was paid to inflammation or redness along the incision and staple sites and the presence of incision characteristics were recorded. Descriptors characterizing the overall appearance of the incision, whether it was raised, slightly raised, overlapping, open, or slightly open under staples were noted. Additionally, qualities of the incision site that might indicate irritation (redness, swelling, staples were protruding into the incision) or healing status (infection, closure, presence black dots surrounding it) were noted. During post-surgery observations percent closure of the incision was estimated for each fish.

Experiment 3: Effect of Anesthetic on the Cortisol Stress Response

Following anesthesia, blood samples were collected to measure plasma cortisol levels, a parameter of physiological stress response. Optimal dosages of each anesthetic were applied to treatment groups of 16 Gopher Rockfish (n = 80 including controls). Fish were quickly netted and placed into an anesthetic bath or given electroanesthesia. Time-series sampling was conducted at t = 0, 0.5, 1, 2, and 4 h after stage IV anesthesia had been reached to establish basal levels and capture the signature rise time, peak, and fall of cortisol within Gopher Rockfish. Baseline cortisol levels were established for each treatment group by drawing blood immediately after induction to stage IV (t = 0 h).

To monitor the rise, plateau (if any), and fall of cortisol over time, treatment groups were anesthetized, allowed to recover, and euthanized at a specific time period (0.5, 1, and 2 h) at which time blood was taken (four fish per treatment per time point). In addition to referencing cortisol rise times for other fish species from the literature, these time points were determined prior to the experiment by testing a subset of individuals to estimate the general curve of the rise time of cortisol in Gopher Rockfish. These preliminary tests determined the overall rise time of cortisol and, in the interest of minimizing the number of study fish necessary for the experiment, allowed an additional time point (t = 4 h) to safely be removed from the sampling design.

Once the desired time point had been reached, all blood samples were collected in 5 min or less following recapture to minimize the compounding stress response resulting from a repeated handling episode and venipuncture. Blood (1 mL) was drawn from the caudal vasculature using a syringe with 21 or 22 gauge hypodermic needle. Blood samples were transferred to blood collection tubes coated with lithium heparin (BD Diagnostics, Franklin Lakes, New Jersey) and inverted at least 10X to insure anticoagulation before being centrifuged for 4 min to separate the plasma fraction from the blood sample.

Recovered plasma was pipetted *in situ*, transferred to matrix tubes, and kept in a cooler on ice for the duration of the experiment day (< 10 h). Samples were stored at -80°C until they could be analyzed. Plasma cortisol levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit for cortisol

(EIA 1887, DRG International, Mountainside, New Jersey) according to manufacturer's instructions. Optical densities of the samples were analyzed using a microtiter plate reader (Synergy HT^{TM}, Bio-Tek Instruments, Winooski, Vermont) at a wavelength of 410 nm and KC4^{TM} software (Bio-Tek Instruments, Winooski, Vermont).

Due to largely differing results between electroanesthesia and other treatment groups following preliminary cortisol analysis it was determined that an additional lower pulsed DC dosage (100 V, 60 Hz, 3 s) should be tested at a later date to see if cortisol levels would mirror those in the main experiment. Using similar methods, 4 fish per time point were exposed to this lower dosage and plasma samples were obtained and analyzed in the manner described here. Although blood draws for this treatment group of fish occurred at a later date, all plasma samples for all treatments were run on the same ELISA plate to avoid encountering potential between-plate differences.

Data Analyses

All data were analyzed using the SPSS statistical package for Macintosh, Version 21.0 (IBM Corporation, Armonk, New York). Results from experiments 1, 2, and 3 were analyzed separately. All statistics were assessed using a level of significance of $\alpha < 0.05$. For experiment 1, mean recovery rates were determined following exposure to the various pulsed DC electroanesthesia settings being evaluated. Rate data were tested for normality with the Shapiro-Wilkes test. Differences in mean recovery time between electroanesthesia settings were evaluated using one-way analysis of variance (ANOVA). The correlation of fish size and recovery time from electroanesthesia was analyzed using

a linear regression. For experiment 2, mean induction and recovery times were recorded and, following surgery, mean weight changes and percentage of the incisions healed over time were determined for each of the four treatments. Data were screened for normality with the Shapiro-Wilkes test. Differences in mean induction and recovery times and the sub-lethal effects of each anesthetic were evaluated using ANOVA. For experiment 3, the arithmetic means of plasma cortisol concentrations were determined and compared by one-way ANOVA.

RESULTS

Pilot Study: Determination of Effective Concentrations for Immersion Anesthetics

Induction times to stage IV anesthesia ranged from 1.67 ± 0.19 min (mean ± SE) to 6.11 ± 1.13 min for the three concentrations of $NaHCO_3$ and CO_2 and four concentrations of MS-222 that were tested (Table 3). Of the concentrations tested, stage IV anesthesia was reached most rapidly in fish treated with 200 mg/L MS-222 (1.67 ± 0.19 min), followed by CO_2 bubbled at 4 L/min (2.53 ± 0.21 min), then 2.66 g/L $NaHCO_3$ (3.17 ± 0.54 min). Among the three concentrations of $NaHCO_3$ tested, the 2.66 g/L concentration achieved the quickest mean induction times and ranged from 1.06 ± 0.15 min (stage I) to 3.17 ± 0.54 min (stage IV). At a concentration of 200 mg/L of MS-222 the mean induction times for the stages of anesthesia ranged from 0.79 ± 0.10 min (stage I) to 1.67 ± 0.19 min (stage IV). CO_2 bubbled at a rate of 4 L/min yielded mean induction times that ranged from 1.08 ± 0.24 min (stage I) to 2.53 ± 0.21 min (stage IV).

Table 3. Induction and recovery times for Gopher Rockfish anesthetized with various concentrations of immersion anesthetics. Data are mean times in decimal minutes ± SE in parentheses. $NaHCO_3$, sodium bicarbonate plus glacial acetic acid; MS-222, tricaine methanesulphonate; CO_2, carbon dioxide buffered with baking soda.

Anesthetic Dose	*n*	Induction Time to Anesthesia Stage				Recovery Time
		I	II	III	IV	
$NaHCO_3$						
2.00 g/L	3	1.94 (0.16)	2.64 (0.07)	3.33 (0.05)	5.61 (0.40)	9.26 (0.96)
2.66 g/L	3	1.06 (0.15)	1.88 (0.40)	1.95 (0.40)	3.17 (0.54)	7.21 (1.17)
3.33 g/L	3	0.91 (0.12)	1.72 (0.36)	2.84 (0.52)	4.46 (0.85)	11.87 (1.86)
MS-222						
100 mg/L	3	1.64 (0.43)	2.67 (0.72)	4.41 (0.89)	6.11 (1.13)	5.49 (0.69)
150 mg/L	3	0.97 (0.08)	1.68 (0.19)	2.10 (0.07)	3.08 (0.39)	5.30 (0.91)
175 mg/L	3	1.08 (0.33)	1.69 (0.49)	1.96 (0.40)	2.56 (0.32)	3.65 (0.38)
200 mg/L	3	0.79 (0.10)	1.19 (0.18)	1.44 (0.17)	1.67 (0.19)	3.11 (0.03)
CO_2						
3 L/min	3	1.58 (0.43)	2.86 (0.18)	4.28 (0.37)	4.81 (0.15)	12.89 (1.95)
4 L/min	3	1.08 (0.24)	1.88 (0.26)	2.28 (0.23)	2.53 (0.21)	11.75 (0.76)
5 L/min	3	1.06 (0.46)	1.50 (0.38)	2.30 (0.51)	3.48 (0.84)	7.78 (0.93)

The clear pattern for MS-222 was that higher concentrations yielded more rapid induction times (Table 3). The pattern of decreasing induction times at higher concentrations held true for $NaHCO_3$ until stages III and IV where the middle concentration tested (2.66 g/L) gave more rapid induction than the highest concentration (3.33 g/L). Likewise, CO_2 followed this pattern until stage IV when the middle concentration (4 L/min) induced fish to stage IV more quickly than the highest concentration (5 L/min).

Overall, MS-222 had the most rapid mean recovery times and CO_2 had the longest (Table 3). Of the concentrations of each anesthetic tested, the most rapid recovery times occurred in fish treated with concentrations of 2.66 g/L $NaHCO_3$ (7.21 ± 1.17 min), 200

mg/L MS-222 (3.11 ± 0.03 min), and 5 L/min CO_2 (7.78 ± 0.93 min). The difference between the most rapid recovery time (200 mg/L MS-222) and the slowest (3 L/min CO_2) was 9.78 min. Recovery times among anesthetic types and concentrations within each of the anesthetics varied a good deal more than the induction times. The clear effect of increasing concentrations of MS-222 and CO_2, however, was a decrease in recovery times. This pattern did not hold true for increasing concentrations of $NaHCO_3$ where the highest concentration resulted in the longest recovery times and the intermediate concentration resulted in the quickest.

Based on the induction and recovery times resulting from this pilot study, one concentration of each immersion anesthetic was chosen for further testing in the remainder of the experiments. Of the six criteria proposed earlier to define a suitable field anesthetic for use when surgically implanting transmitters into fish, only the first four could be considered based on pilot study results: (1) zero withdrawal period, (2) stage IV level of anesthesia achieved, (3) induction time to stage IV anesthesia < 7 min, and (4) recovery times from stage IV anesthesia < 10 min. Only $NaHCO_3$ and CO_2 are considered zero-withdrawal anesthetics suitable for immediate release of fishes following anesthesia. MS-222 was included in this study as an industry standard for comparison purposes only.

All concentrations of the anesthetics tested successfully induced Gopher Rockfish to stage IV anesthesia in under 7 min (Table 3). Three of the concentrations examined failed to meet the third criterion of recovery times under 10 min: 3.33 g/L $NaHCO_3$, and CO_2 bubbled at 3 L/min and 4 L/min. In the case of CO_2, the only concentration that was

found suitable based on the proposed criteria was 5 L/min, which was also the maximum flow rate allowable on the rotameter. For $NaHCO_3$, the 2.66 g/L concentration was chosen because the only other viable concentration (2.00 g/L) had a mean recovery time approaching the threshold of 10 min.

With the exception of classifying as a zero-withdrawal anesthetic, MS-222 met all remaining criteria at all concentrations tested. The concentration of 175 mg/L was chosen for further use in subsequent experiments despite the fact the 200 mg/L concentration had both shorter induction and recovery times. Generally, it is desirable to use the lowest concentration possible that can achieve significantly shorter times while still being regarded as a safe dosage. In the case of MS-222, the 200 mg/L concentration was purposefully avoided because its strength was approaching that of the recommended dose for euthanasia (> 250 mg/L).

To establish effective levels of CO_2 saturation, the gas was allowed to bubble for 5 min prior to the immersion of experimental fish. As a result of CO_2 saturation, pH levels decreased appreciably during this time (Figure 4). With the addition of 65 to 76 g of sodium bicarbonate to buffer the CO_2 saturated water, pH was brought up to a mean level of 6.50 ± 0.10. Observed mean pH levels for MS-222 and $NaHCO_3$ baths were 6.97 ± 0.11 and 6.36 ± 0.04, respectively.

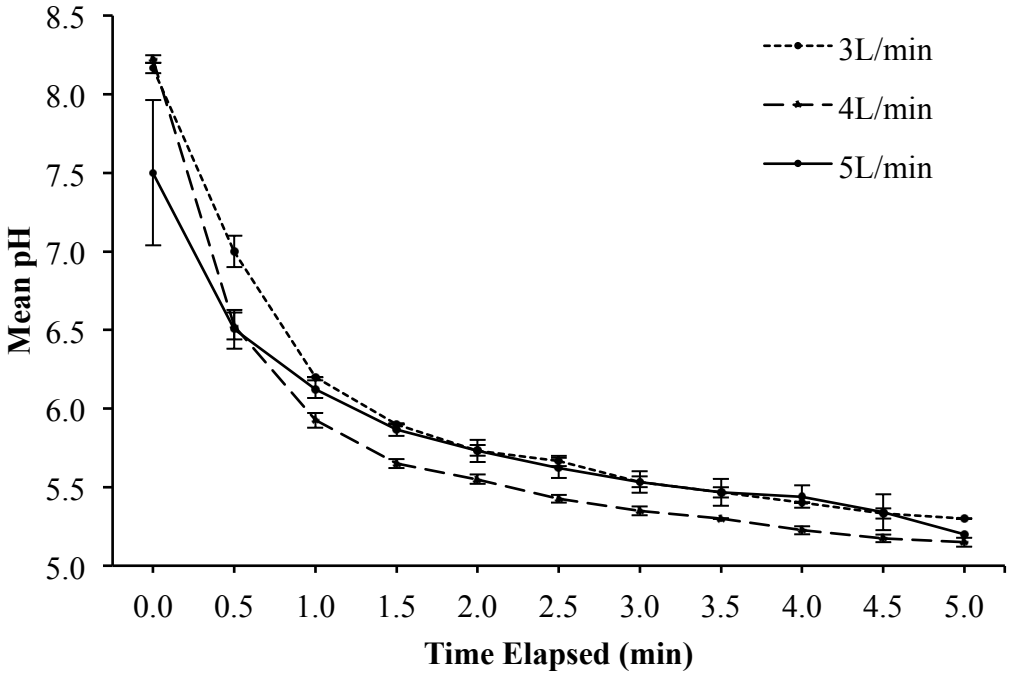

Figure 4. Plot of decreasing pH of CO_2 anesthetic baths during saturation and prior to buffering (n = 3). Error bars represent SE.

Pilot Study: Determination of Strength and Duration of Electroanesthesia

Induction times to stage IV anesthesia were nearly instantaneous at all doses tested with the exception of the fish exposed to 50 and 75 V, which were not effectively anesthetized (Table 4). The lowest voltage used (50 V) was not adequate to sedate the study fish to the level that would be necessary to handle and perform a surgical procedure given the nearly nonexistent recovery times. When 50 V was paired with 60 Hz and 3 s exposure there was no apparent induction and when paired with 30 Hz and 10 s exposure, the fish was behaving normally after 2 s. The next lowest voltage (75 V) was applied

with a frequency of 30 Hz for 5 s to three fish, two of which recovered in less than 10 s and the third of which recovered in 1 min 16 s.

Table 4. Recovery times for Gopher Rockfish undergoing various strengths and exposure times of pulsed DC electroanesthesia. Distance between electrode plates (62.5 cm) remained constant. Note that pulse width is a function of frequency.

Fish TL (mm)	Voltage (V)	Frequency (Hz)	Exposure Time (s)	Duty Cycle	Pulse Width (ms)	Recovery Time (decimal minutes)
295	50	60	3	12%	2	0.00
310	50	30	10	12%	4	0.03
310	75	30	5	12%	4	1.27
260	75	30	5	12%	4	0.17
270	75	30	5	12%	4	0.08
295	100	30	3	12%	4	2.78
320	100	60	3	12%	2	6.42
310	100	30	5	12%	4	4.00
265	100	30	10	12%	4	7.25
295	100	60	5	12%	2	4.82
275	150	30	3	12%	4	4.00
290	150	30	3	12%	4	0.83
295	150	30	5	12%	4	6.00

Recovery times varied widely across the electroanesthetic doses applied (Table 4). The results of the pilot study generally indicate that increasing voltage (V), frequency (Hz), or exposure time correlate to increasing recovery times in study fish. The fish with the longest recovery time was exposed to 100 V, 30 Hz, for a 10 s exposure time, the longest exposure time applied. However, when other parameters were held equal, results

indicate that both voltage and frequency can play a large role in recovery times. Based on this indication, care was taken to select twelve doses with various waveform parameters for further testing in Experiment 1 (Table 5).

Table 5. Waveform specifications for doses of pulsed DC electroanesthesia chosen for further testing.

Dose	Voltage (V)	Frequency (Hz)	Exposure Time (s)
1	100	30	3
2	150	30	3
3	200	30	3
4	100	60	3
5	150	60	3
6	200	60	3
7	100	30	5
8	150	30	5
9	200	30	5
10	100	60	5
11	150	60	5
12	200	60	5

Experiment 1: Determining Recovery Times, Size Effects, and Potential Injuries of Electroanesthesia

Having excluded those doses identified as ineffectual by the pilot study, every dose of electroanesthesia tested was strong enough to bring all fish successfully into stage IV anesthesia (Table 6). Once again, induction was nearly instantaneous. Conductivity measurements taken in the PES^{TM} cooler just prior to delivery of electroshock ranged

from 452 to 705 µs. Water temperatures remained between 14.2 and 15.5 °C and the pH readings were steady between 7.9 and 8.5 for the duration of the experiment. Mean recovery times ranged from 1.33 ± 0.63 min to 14.87 ± 3.54 min.

Table 6. Mean recovery times of Gopher Rockfish following exposure to 12 doses of pulsed DC electroanesthesia. Data are given in decimal minutes with ± SE in parentheses. Total *n* for experiment = 60 fish. Distance between electrode plates (62.5 cm) and duty cycle (12%) remained constant. Pulse width varied as a function of frequency where 30 Hz and 60 Hz had pulse widths of 4 and 2 ms respectively.

Dose	*n*	100 V	150 V	200V
30 Hz 3 s	5	1.33 (0.63)	2.19 (0.82)	3.36 (1.06)
30 Hz 5 s	5	1.47 (0.58)	6.36 (1.03)	7.91 (1.59)
60 Hz 3 s	5	3.11 (1.01)	3.76 (0.21)	9.76 (1.83)
60 Hz 5 s	5	3.36 (1.90)	10.85 (3.64)	14.87 (2.54)

As observed in the pilot study, the general trend was that increasing voltages, frequencies, and exposure times to electroanesthesia each resulted in longer recovery times when all other parameters were held equal (Figure 5). At the lowest voltage (100 V) and highest voltage (200 V) tested, recovery times increased with increasing frequencies and exposure times such that recovery times for 30 Hz 3 s < 30 Hz 5 s < 60 Hz 3 s < 60 Hz 5 s. The intermediate voltage (150 V) was unlike the other voltages tested in that fish exposed to 60 Hz 3 s dose had more rapid mean recovery times than those subjected to the 30 Hz 5 s dose.

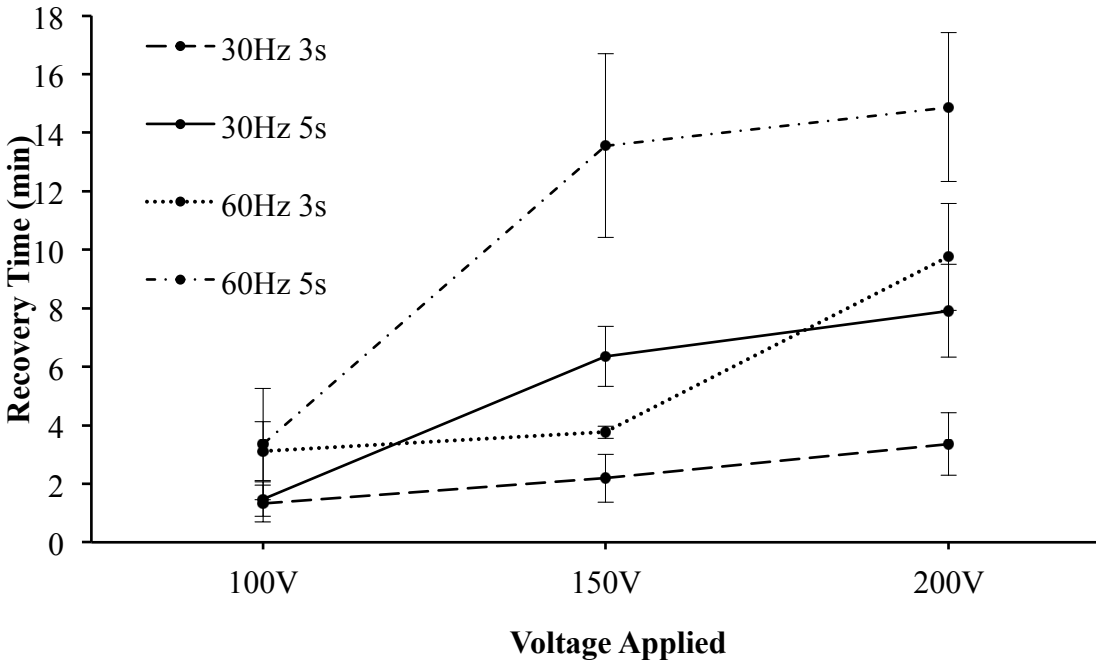

Figure 5. Plot of mean recovery times of Gopher Rockfish anesthetized to stage IV with a range of voltages, frequencies, and exposure times to pulsed direct current (n = 10). Error bars represent SE.

When determining if fish size (TL) influences recovery, a positive linear correlation (p = 0.03, r^2 = 0.57) was found between increasing length and longer recovery times (Figure 6). An effective dose (150 V, 30 Hz, 5 s) was applied to a group of fish (n = 17) with total lengths spanning from 228 to 311 mm. However, during the course of the experiment it was determined that fish orientation with respect to the electrode plates played an important role on the amount of electroshock received. Fish that were oriented parallel to the plates were noticeably unphased by the exposure to the electroshock and indeed seemed to undergo no anesthetic effect at all.

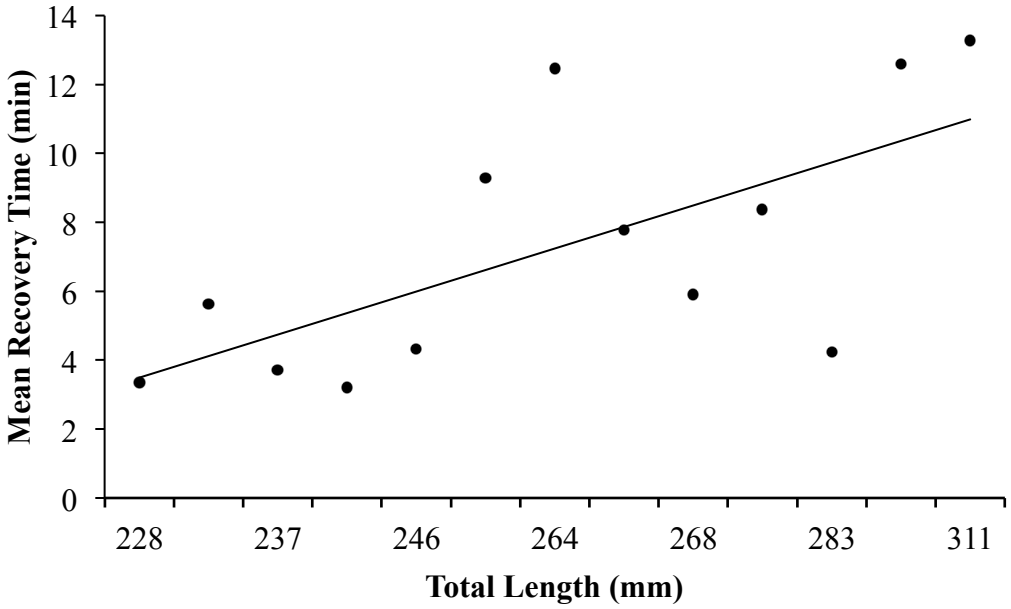

Figure 6. Linear regression of recovery times of Gopher Rockfish anesthetized to stage IV using pulsed direct current (150 V, 30 Hz, 5 s exposure) vs. size (total length). Each point represents an individual fish ($p = 0.03$, $r^2 = 0.57$).

After this phenomenon was noticed, care was taken to orient all fish perpendicular to the electrode plates before applying electroshock. For this reason, only 13 of the 17 fish exposed were analyzed for size effects on recovery times. Based on this finding, replicate fish for treatment groups in Experiment 1 were binned by size and parsed equally among treatments to normalize the effect that size had on recovery times.

Following euthanasia, Dr. Dave Casper took digital x-rays to determine if any vertebral injuries occurred as a result of electroshock. The radiographs revealed no vertebral fractures or broken bones occurring as a result of severe muscle tetany during electroshock (Figure 7).

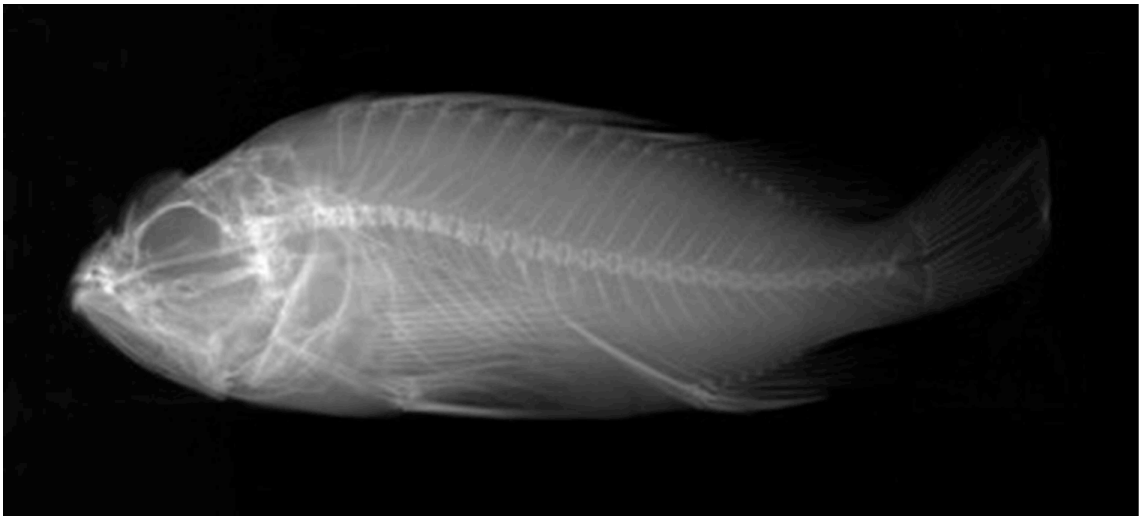

Figure 7. Lateral-aspect radiographic image of a Gopher Rockfish exposed to pDC electroanesthesia showing no vertebral damage or fracturing present. Image courtesy of Dr. Dave Casper.

Experiment 2: Comparison of Post-surgical Effects of Anesthetics

Based on pilot study induction and recovery times, an optimal dosage for each type of anesthetic was chosen for comparison on treatment groups comprised of 10 Gopher Rockfish per anesthetic plus a control group to monitor the effects of netting and handling ($n = 50$). For each anesthetic, induction times for each stage of anesthesia are plotted on Figure 8, with the exception of pDC electroanesthesia as its induction times were considered instantaneous. Relative induction times to stage IV anesthesia for this experiment mirrored the results obtained in the pilot studies with pDC electroanesthesia being the most rapid followed by MS-222 (2.26 ± 0.17 min), $NaHCO_3$ (3.21 ± 0.26 min), and CO_2 (3.56 ± 0.21 min). Mean induction times obtained during the pilot study and during Experiment 2 for the chosen concentrations did not differ significantly ($p > 0.05$).

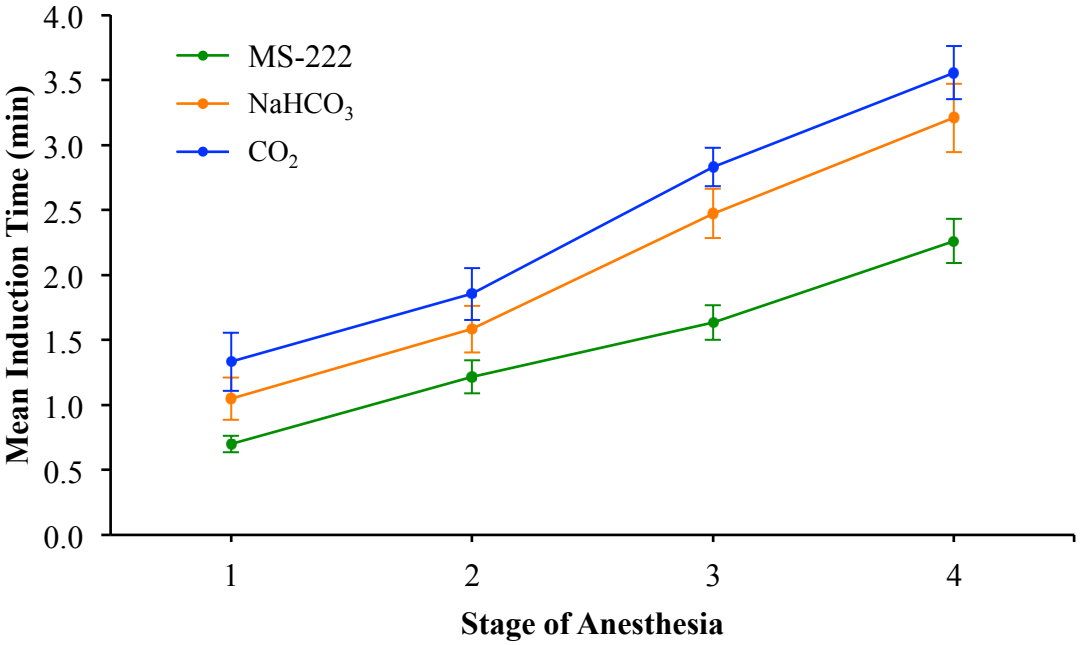

Figure 8. Plot of mean induction times into the four stages of anesthesia for Gopher Rockfish exposed to immersion anesthetics (n = 10). Points represent means ± SE.

Following anesthesia, a surgical procedure was conducted to implant a mock acoustic tag into the peritoneal cavity of the fish. Overall surgery times ranged from 1.05 to 3.57 min, with a mean surgery time of 2.06 ± 0.09 min. Mean surgery times for treatment groups were as follows: $NaHCO_3$ (1.90 ± 0.07 min), CO_2 (2.46 ± 0.24 min), MS-222 (2.03 ± 0.16 min), and electroanesthesia (1.86 ± 0.12 min). One fish exposed to $NaHCO_3$ that was presumed to have achieved stage IV anesthesia perished during surgery when it kicked its tail violently as the incision was being made and incurred a cut too deep to recover from. This mortality represented < 0.5% of the fish handled during the course of these experiments and was not caused by application of an anesthetic.

In general, comparative recovery times for fish that did not undergo surgery (pilot study fish) were shorter than recovery times for fish following surgery except in the case of MS-222 treatments in which fish displayed mean post-surgery recovery times that were quicker than those fish that did not experience surgery (Figure 9). Recovery times following surgery were most rapid for MS-222 (2.84 ± 0.24 min) < Electroanesthesia (4.78 ± 0.73 min) < $NaHCO_3$ (8.97 ± 0.54 min) < CO_2 (12.89 ± 1.73 min). When recovery times were plotted for study fish with respect to total length and body weight, no significant differences were detected ($p > 0.05$).

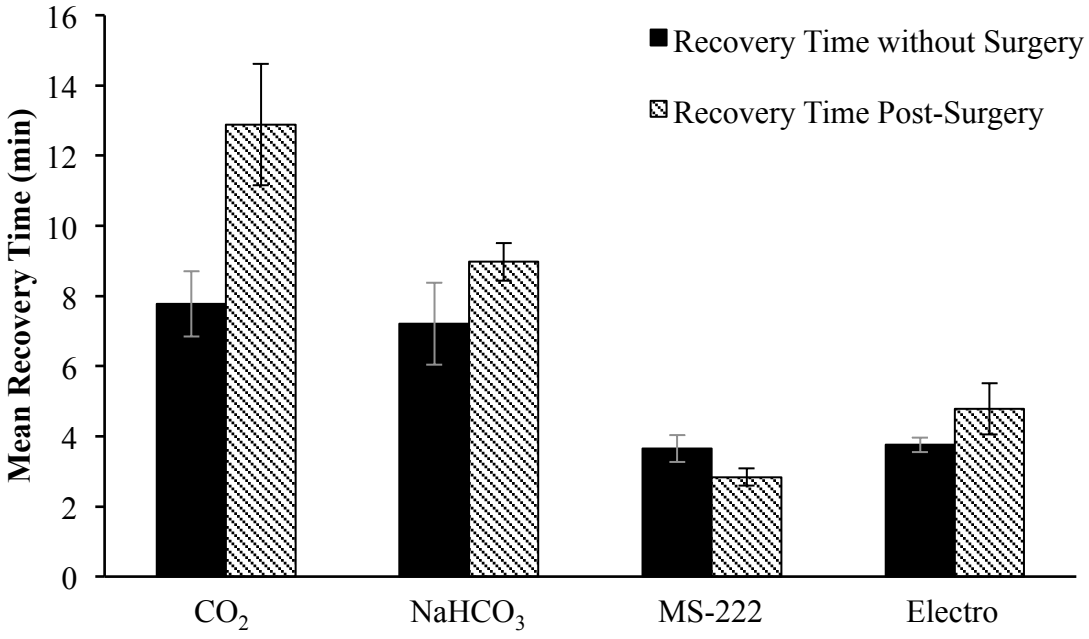

Figure 9. Comparison of mean recovery times (±SE) of Gopher Rockfish from stage IV anesthesia without surgery ($n = 3$) vs. following surgery ($n = 10$).

Fish weights were taken routinely during monitoring at one, two, four, and six weeks post-surgery and all treatment groups showed a steady weight increase relative to their initial weights (Figure 10). During the post-surgery observations at six weeks, the occurrence of high winds at the outdoor tank farm where fish were being housed prohibited accurate readings from the digital scale, so these data have been omitted.

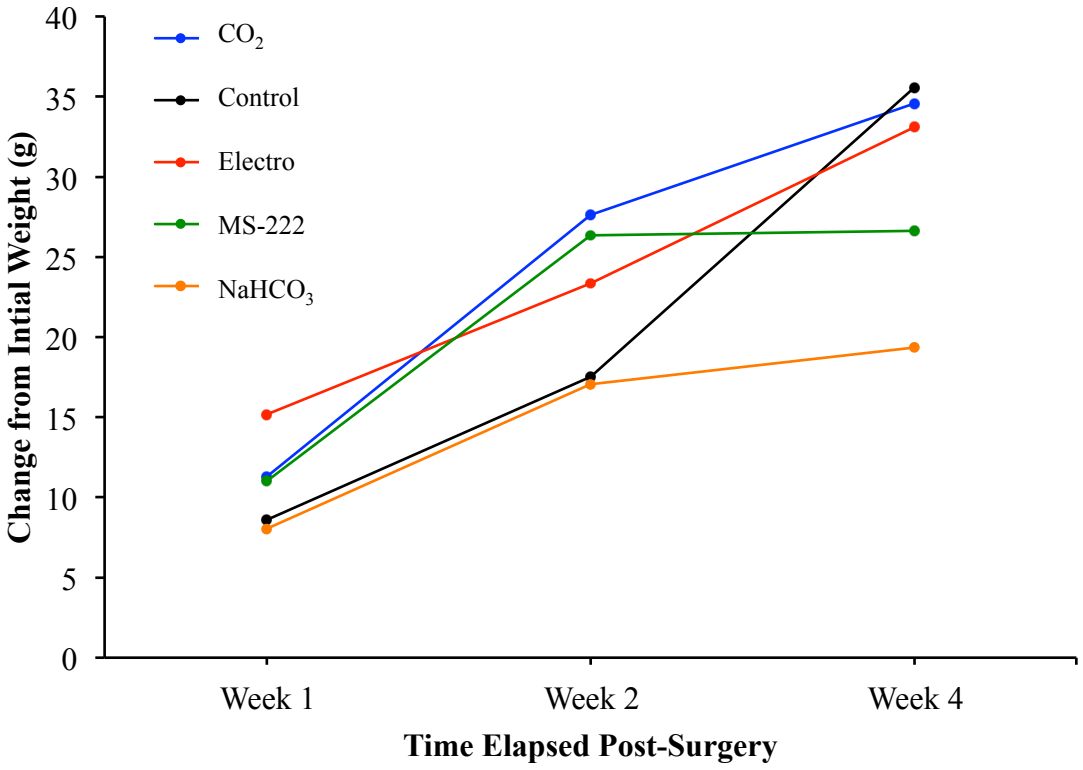

Figure 10. Plot of weight changes relative to initial weight on experiment date of Gopher Rockfish in the weeks following anesthesia and the surgical implantation of a mock acoustic tag (n = 10). Error bars depicting SE are omitted here for clarity, however, no significant ($p > 0.05$) differences of weight changes between treatment groups were found.

For six weeks following the surgeries, experimental fish were examined and photographed to estimate of percent closure of the wound. By four weeks after the surgery (Obs. 5, Dec 7th) incisions in all treatment groups had nearly achieved or surpassed a mean 50% wound closure (Figure 11). At the final observation period, roughly six weeks post-surgery, the mean percentage of incision healing ranged from 67.0% ± 8.9% for the electroanesthesia group to 78.3% ± 6.7% for the group anesthetized with CO_2.

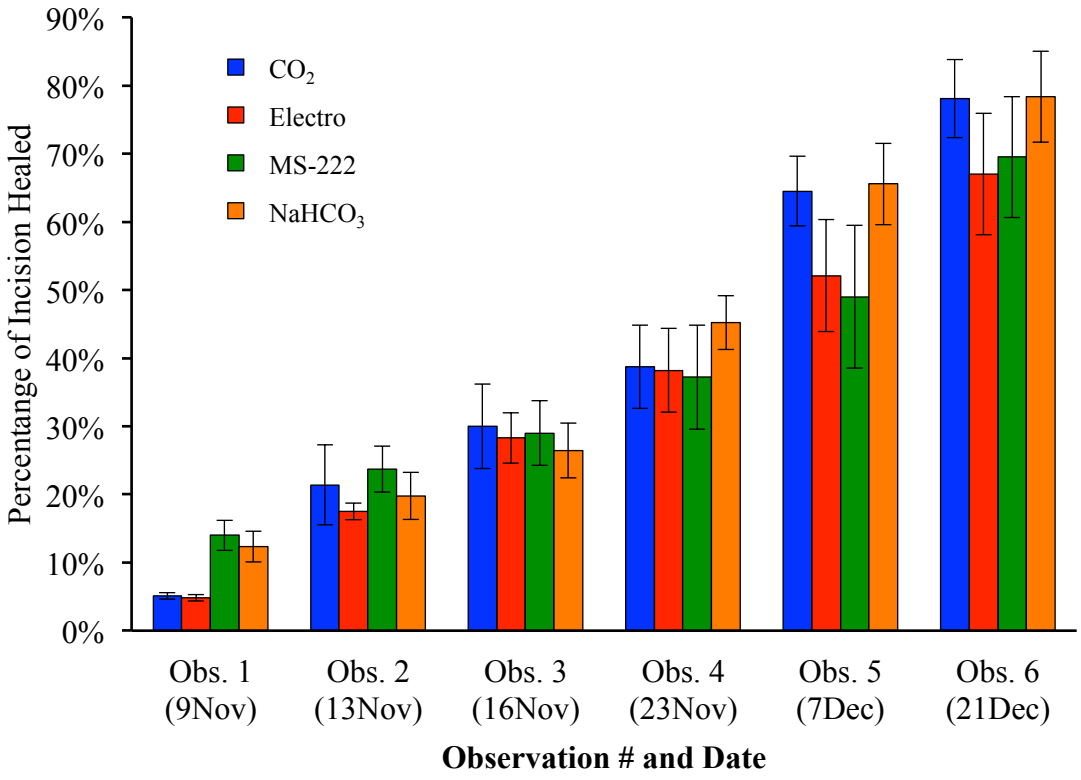

Figure 11. Depiction of wound healing rates (percentage of incision healed) of Gopher Rockfish in the weeks following anesthesia by one of four methods and the surgical implantation of a mock acoustic tag (n = 10). Error bars represent SE.

Relative to one another, healing rates for $NaHCO_3$ > CO_2 > MS-222 > Electroanesthesia by the last observation period. However, there were no significant differences ($p > 0.05$) among treatment groups. At no point during the 2-month period following surgery were any mortalities observed, indicating 100% short- and long-term survival. Study fish appeared to resume their normal swimming and feeding behaviors within 24 h after surgery.

Experiment 3: Effect of Anesthetic on the Cortisol Stress Response

While determining the effect of the anesthetic on the stress response, induction and recovery times were recorded as usual and are listed for comparison along with the times for all anesthetics tested across all experiments in Table 7. Stage IV induction times obtained during experiment 3 are in line with those obtained in the previous experiments, however, recovery times for $NaHCO_3$, MS-222, and electroanesthesia treatment groups were generally longer.

Table 7. Stage IV induction and recovery times for anesthetized Gopher Rockfish across all experiments. Data are mean times in decimal minutes +/- SE in parentheses. Concentrations are as follows: 2.66 g/L $NaHCO_3$ plus 19mL glacial acetic acid; 175 mg/L MS-222; CO_2 bubbled at 5L/min buffered with $NaHCO_3$; and pDC electroanesthesia (150 V, 60 Hz, 3 s exposure).

Experiment	Anesthetic	*n* (Induction)	Stage IV Induction	*n* (Recovery)	Recovery Time
Pilot	$NaHCO_3$	3	3.17 (0.54)	3	7.21 (1.17)
EXP 2	$NaHCO_3$	10	3.21 (0.26)	10	8.97 (0.54)
EXP 3	$NaHCO_3$	21	3.45 (0.17)	13	13.67 (1.95)
Pilot	MS-222	3	2.56 (0.32)	3	3.65 (0.38)
EXP 2	MS-222	10	2.26 (0.17)	10	2.84 (0.24)
EXP 3	MS-222	20	2.76 (0.15)	15	5.13 (0.47)
Pilot	CO_2	3	3.48 (0.84)	3	7.78 (0.93)
EXP 2	CO_2	10	3.56 (0.21)	10	12.89 (1.73)
EXP 3	CO_2	23	2.96 (0.23)	18	12.36 (0.92)
EXP 1	Electro	5	NA	5	3.76 (0.21)
EXP 2	Electro	10	NA	10	4.78 (0.73)
EXP 3	Electro	16	NA	12	6.43 (0.74)

Regardless of treatment, after 0.5 h post-anesthesia, all fish had significantly greater cortisol levels compared with those sampled to establish basal levels ($t = 0$) (Figure 12). In all treatment groups, mean plasma cortisol levels showed a dramatic increase that tended to peak around 0.5 h post-exposure. Following this peak, cortisol concentrations had decreased towards basal levels at 2 h post-exposure, with the exception of fish exposed to pDC electroanesthesia, which still had elevated levels (410 ng/mL) at 2 h.

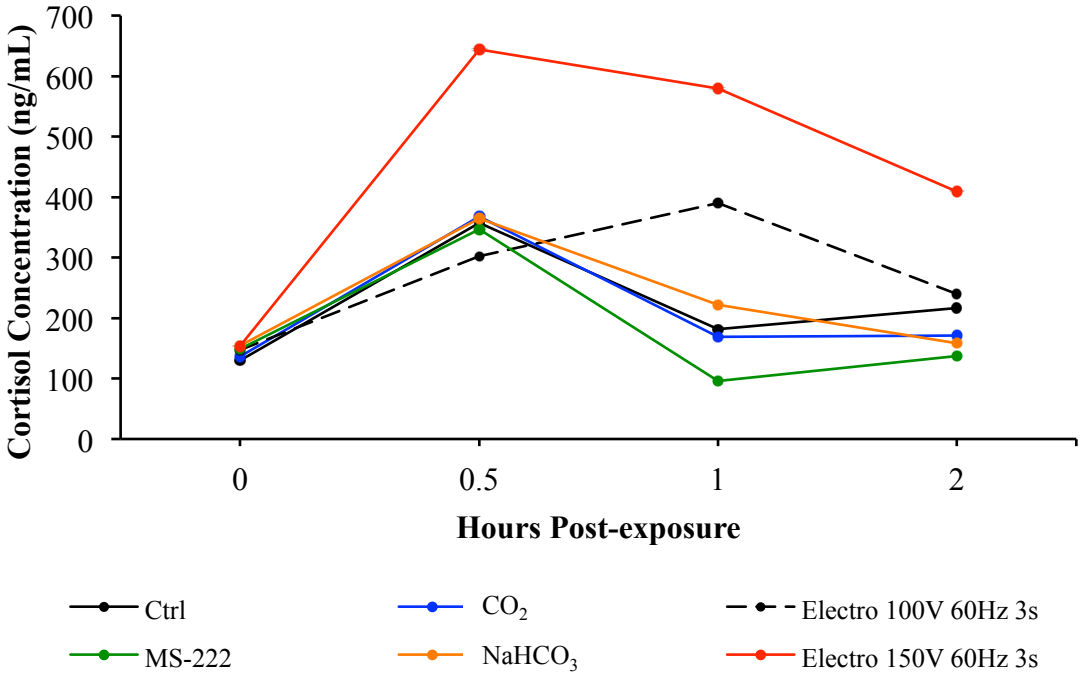

Figure 12. Time course of plasma cortisol fluctuations observed in Gopher Rockfish following anesthesia by one of four methods or a netting/handling event (control) (n = 4). Anesthetic concentrations were as follows: CO_2 bubbled at 5 L/min buffered with $NaHCO_3$, 175 g/mL MS-222, 2.66 g/L $NaHCO_3$ plus 19mL glacial acetic acid, and pDC electroanesthesia (Electro) 150 V, 60 Hz, 3 s exposure and 100 V, 60 Hz, 3 s exposure. Control groups were netted and put in a seawater bath for 2 min 52 s followed by 5 min 22 s in a recovery tub. Standard error bars have been omitted for clarity.

With the exception of electroanesthesia, the cortisol response appeared to be rapid and relatively transient, peaking (347 – 368 ng/mL) at 0.5 h post-anesthesia before decreasing steadily over the time series (137 – 216 ng/mL) to approach basal levels (130 – 154 ng/mL [t = 0 concentration range]) at 2 h post-exposure. In the 72 h period following the experiment, no mortalities were observed, indicating 100% short-term survival.

A comparison of overall cortisol concentrations with the inclusion of the secondary, lower pDC dosage (100 V, 60 Hz, 3 s) that was tested show that these concentrations more closely resemble the other treatments than the original pDC treatment (150 V, 60 Hz, 3 s) that was applied (Figure 12). In comparison to the original set of treatment groups, which showed mean plasma cortisol concentrations peaking at 0.5 h post-exposure, the lower electroanesthesia (100 V, 60 Hz, 3 s) treatment fish were an exception as their cortisol levels continued to rise after 0.5 h and peaked at the 1 h time point. When compared to the original electroanesthesia dosage (150 V, 60 Hz, 3 s) tested, the lower dose resulted in lower mean cortisol concentrations at every time point blood was drawn. Although the peak for the lower dose occurred at a later time point, mean cortisol concentrations more closely matched the concentrations observed for the other anesthetics and were returning to basal levels after 2 h post-exposure (130 – 154 ng/mL [t = 0 concentration range]).

DISCUSSION

In all but a very limited number of cases, anesthetics are deemed necessary for surgical procedures involving fishes. Due to differences in physiology, species may show marked variability in their response to the same anesthetic, necessitating a thorough screening of dosages prior to any experimental or routine surgical procedures. Based on the data generated from the present study, Gopher Rockfish respond well to a moderate range of concentrations of the anesthetics applied. The approaches tested are safe with

respect to post-exposure and post-surgical survival rates, as well as the overall physiological status of these fish.

All of the anesthetic approaches and concentrations investigated in Gopher Rockfish (21 – 32 cm TL) yielded relatively consistent results in terms of successful induction to stage IV anesthesia. In addition, specific concentrations of each anesthetic were found to be most effective for conducting a surgery to implant a mock acoustic tag into the peritoneal cavity of Gopher Rockfish (23 – 32 cm TL). However, the concentrations tested and the anesthetics used greatly influenced induction and recovery times and these differences were more pronounced following surgery. Recovery times were less consistent than induction times over the range of conditions tested. Overall, inducing fish to stage IV anesthesia with CO_2 appeared to result in the longest recovery times.

The results of this study are summarized in Table 8, which presents a checklist delineating whether or not the proposed criteria to define a suitable field anesthetic were met during each experiment. Given the data obtained from the range of experiments conducted, electroanesthesia was the only anesthetic tested across multiple scenarios that achieved the thresholds deemed appropriate for surgically implanting transmitters into fish. Based on these, $NaHCO_3$ and CO_2 failed to meet the threshold of recovery times < 10 min in one out of three and two out of three experiments, respectively. MS-222, though not a zero-withdrawal anesthetic, is shown here for comparison purposes, but otherwise met the established criteria.

Table 8. Criteria checklist for a suitable field anesthetic to surgically implant transmitters into fish. The ✔ symbol indicates yes, or that criterion was met, the ✗ symbol indicates no, or that criterion was not met.

Experiment	Surgery?	Anesthetic	Stage IV Achieved	Stage IV Induction < 7 min	Recovery Times < 10 min	Post-surgical Survival Rates > 90%	Delayed Mortality Rates < 5%
Pilot		$NaHCO_3$	✔	✔	✔	✔	✔
EXP 2	✔	$NaHCO_3$	✔	✔	✔	✔	✔
EXP 3		$NaHCO_3$	✔	✔	✗	✔	✔
Pilot		MS-222	✔	✔	✔	✔	✔
EXP 2	✔	MS-222	✔	✔	✔	✔	✔
EXP 3		MS-222	✔	✔	✔	✔	✔
Pilot		CO_2	✔	✔	✔	✔	✔
EXP 2	✔	CO_2	✔	✔	✗	✔	✔
EXP 3		CO_2	✔	✔	✗	✔	✔
EXP 1		Electro	✔	✔	✔	✔	✔
EXP 2	✔	Electro	✔	✔	✔	✔	✔
EXP 3		Electro	✔	✔	✔	✔	✔

Overall, the anesthetics tested met the majority of the criteria and it should be noted that all of the anesthetics could be considered viable immediate-release alternatives in terms of achieving stage IV induction, acceptable induction times, high post-surgical survival rates, and low overall mortality rates. The consistency of these results in terms of fish safety and high survival rates is particularly encouraging for the use of these anesthetics by fisheries professionals who wish to surgically implant acoustic transmitters for telemetry studies.

In terms of induction times, the responses of Gopher Rockfish to carbon dioxide, sodium bicarbonate, MS-222, and electroanesthesia fell well within the range of responses of other fish species to the same anesthetics despite broad taxonomic, biological, and physiological differences. In general, the recovery times of Gopher Rockfish anesthetized with CO_2, $NaHCO_3$, MS-222, and pDC electroanesthesia tended to be longer relative to the ranges described in the literature for other species. It is unclear whether observed intertaxa differences are influenced by variable metabolic rates, anesthetic excretion, or some other combination of biological or physiological factors. Yet, it is important to note that, with the exception of MS-222, none of the anesthetics tested have been applied to member of the rockfish genus *Sebastes*, therefore, the response of Gopher Rockfish in the present study may represent a completely normal response.

Stage IV induction times of Gopher Rockfish anesthetized with CO_2 were very similar to those found for comparatively sized Largemouth Bass *Micropterus salmoides* (Trushenski et al. 2012b) and Cobia *Rachycentron canadum* (Trushenski et al. 2012c), however, recovery times of Gopher Rockfish were longer. Even so, recovery times were not nearly as excessive as those described for Common Carp (Yoshikawa et al. 1988), Rainbow Trout (Bernier and Randall 1998), and Grass Carp *Ctenopharyngodon idella* (Gause et al. 2012). Prolonged induction and recovery times (Bell 1987) and the inability to achieve deep anesthesia (Gilderhus and Marking 1987) are just a few of the cited problems associated with the use of CO_2 anesthetic compared to other anesthetics.

In comprehensive experiments conducted on Common Carp, Yoshikawa et al. (1988) found that the constant partial pressures of CO_2 (bubbled in equal mixture with O_2) required to bring 100% of study fish into any stage of anesthesia represented an extremely narrow range between P_{CO_2} = 125 – 175 mm Hg. At these high partial pressures of CO_2, the mortality rate was between 10 and 70% (Yoshikawa et al. 1988). Such specific partial pressures of gas would be nearly impossible to achieve or measure in the field without very sophisticated equipment and the resulting mortality rates would be unacceptable for an acoustic tagging study. Bernier and Randall (1998) were unable to achieve complete anesthesia (Yoshikawa's stage V characterized by lack of opercular movements) in cannulated Rainbow Trout after 20 min of exposure to CO_2. Additionally, in the highest treatment used in their study, recovery times were prolonged and 33% of the fish died. Indeed, many of the fish in the present study that achieved stage IV anesthesia using CO_2 nonetheless displayed spasmodic and intermittent twitching, an indication that full anesthesia may not have been reached.

Stage IV induction times for Gopher Rockfish using $NaHCO_3$ as an anesthetic were very similar to those observed in Small Mouth Bass (21 – 33 cm FL), but were about 50% less than times observed in Walleye (38 – 64 cm FL), Northern Pike (27 – 34 cm FL), and Lake Sturgeon (26 – 29 cm FL) using the same concentration and similar protocols (Peake 1998). Recovery times in Gopher Rockfish were at least 50% longer than for the species examined by Peake (1998) but were comparable to recovery times found in Brooke Trout *Salvelinus fontinalis* (Booke et al. 1978), Common Carp (Booke et al. 1978), and Sockeye Salmon (Prince et al. 1995).

Based on anecdotal observations, $NaHCO_3$ + acetic acid appeared to be a strong irritant. Most fish blanched instantaneously when exposed to $NaHCO_3$ anesthetic, demonstrating an extreme color change on contact with the solution. This phenomenon may have been caused by the low pH of the solution, or perhaps the anesthetic bath acted as a skin irritant. Aside from this dramatic pallor, most struggled quite violently before the anesthetic effect commenced. Peake (1998) found that Walleyes would thrash violently for 20 – 30 s when placed in sodium bicarbonate solutions before relaxing and losing their equilibrium (induction to stage I). The single mortality observed during the present study occurred when a fish anesthetized with $NaHCO_3$ to stage IV anesthesia began to struggle during a surgery. Walleyes anesthetized to stage IV anesthesia with sodium bicarbonate (2.66 g/L) were reported to struggle during post-test FL measurements and routinely flinched during the first 30– 60 s on the surgical tray (Peake 1998). Upon placement in the recovery bath Gopher Rockfish often displayed a marked spurt of energy, followed by a regression into a deep stage of anesthesia.

Induction to stage IV anesthesia in Gopher Rockfish using MS-222 was fairly consistent across all experiments, averaging slightly longer than times reported for Hybrid Striped Bass (White Bass *Morone chrysops* × Striped Bass *M. saxatilis*) (Trushenski et al. 2012a), Cobia (Trushenski et al. 2012c), Large Mouth Bass (Trushenski et al. 2012b), and Grass Carp (Gause et al. 2012), but more rapid than values obtained for Atlantic Sturgeon *Acipenser oxyrinchus oxyrinchus* (Balazik et al. 2013). It should be noted that the concentration used in the present study was 175 mg/L MS-222 whereas the aforementioned fish were all sedated with 150 mg/L except the Atlantic

Sturgeon which was sedated with 100 mg/L MS-222 (+ 200 mg/L $NaHCO_3$). Recovery times of Gopher Rockfish were well within the range reported for other species. When conducting surgeries to implant acoustic transmitters or inject a radiolabel into rockfishes, researchers have used concentrations ranging from 100 mg/L (Green and Starr 2011) to 200 mg/L (MacFarlane and Bowers 1995) with no reported mortalities, although no induction or recovery times were given in these studies for comparison within the taxon.

Given that pulsed DC electroanesthesia yielded nearly instantaneous induction times, it brought on stage IV anesthesia faster than any of the chemical anesthetics evaluated. Immediately following electrical exposure study fish that experienced induction exhibited complete body rigidity, extreme opercular flaring, fin extension, and visible tremors throughout the length of their body. This reaction is a common observation in fish being exposed to electroanesthesia and in all cases the tremors abated shortly after exposure at which time fish returned to a relaxed, anesthetized state.

Other researchers have postulated that given that fish are not ventilating and unresponsive during this time, they may momentarily have lapsed into a deeper stage of anesthesia and induction is considered complete after the tremor ceases (Trushenski et al. 2012a). However, given the difficulty in gauging what is actually occurring during this tremor or what it truly represents, induction was judged to be instantaneous and the short period during which fish were experiencing tremors was considered an involuntary reaction to electrical exposure. Tremors may be related to tetany, which occurs when a

muscle has been stimulated by multiple impulses at a sufficiently high frequency and the twitches run together, resulting in tetanic contraction. When tetanized, the contracting tension in the muscle remains constant in a steady state representing the maximal possible contraction.

Recovery times following exposure to electroanesthesia were variable across experiments, yet overall this method achieved the second most rapid recoveries seen after MS-222 (Table 7). When compared across taxa, Gopher Rockfish displayed higher recovery times from pulsed DC electroanesthesia in relation to Hybrid Striped Bass (Trushenski and Bowker 2012; Trushenski et al. 2012a), Grass Carp (Gause et al. 2012), Cobia (Trushenski et al. 2012c), and Large Mouth Bass (Trushenski et al. 2012b), which had recovery times ranging from 1.0 ± 0.2 min for Grass Carp (100, 150, or 200V; 30 Hz; 5 or 10 s; 25% duty cycle) to 3.1 ± 0.3 min for Large Mouth Bass (100 V, 30 Hz, 3 s exposure, 25% duty cycle).

In some cases exposure to direct current electricity has been shown to cause internal injuries in fishes. Following euthanasia, lateral-aspect radiographs of Gopher Rockfish from experiment 1 exposed to a range of pulsed DC voltages, frequencies, and exposure durations revealed no spinal injuries (vertebral compression, vertebral compression fracture, or spinal-column fracture) or broken bones of study fish. Based on these data, it would seem that adult Gopher Rockfish are resilient to electroanesthesia at the range of voltage strengths, frequencies, and exposure times tested in this study and that electroanesthesia does not effect long-term survival.

Pulsed DC electroanesthesia serves to rapidly immobilize fishes and the results from the present study indicate that Gopher Rockfish treated using this form of electroanesthesia yielded desirable induction and recovery patterns for fisheries professionals to conduct surgery in the field. As with all anesthetics, a precautionary approach is recommended when applying electroanesthesia to unfamiliar taxa. A single combination of voltage, waveform, frequency, and exposure duration may result in variable recovery times across species and in different scenarios, therefore it is advisable to use the lowest voltage, frequency, and exposure time that yields the level of anesthesia required.

The transient changes in circulating cortisol observed in the present study indicate that Gopher Rockfish undergo an acute stress response following either a brief handling event or anesthetic exposure. Although the specific cortisol response varied depending on the anesthetic used, each treatment elicited cortisol increases consistent with the generalized stress response in fish. Regardless of the anesthetic used or concentrations tested, increases in circulating cortisol are commonly reported following exposure to anesthetics (Iwama et al. 1989; Wagner et al. 2002; King et al. 2005), suggesting that the anesthetics themselves may be considered a stressor (Zahl et al. 2010). However, Balazik et al. (2013) found that 1 h after conducting mock surgeries (mimicking tag implantation or laparoscopy) on Atlantic Sturgeon, blood cortisol levels with no anesthetic were significantly elevated over control, electroanesthesia, and MS-222 groups, indicating that anesthetics can serve to minimize stress in fish undergoing surgical procedures.

In Gopher Rockfish the peak levels of cortisol occurred at either 0.5 or 1 h post-exposure, depending on the anesthetic. The rapid rise of cortisol and gradual return to resting levels is consistent with reported observations in other taxa. However, both the basal levels ($t = 0$) and post-exposure plasma cortisol concentrations obtained from my study were markedly higher than any of those reported for other species. Relatively few fish were sampled at each time point, (i.e., four fish per treatment per time point) rendering the resultant power of the design somewhat limited. Given the magnitude of cortisol concentrations observed, further investigation using a greater sample size is warranted. Given the markedly lower induction times seen with electroanesthesia compared with the other anesthetics tested, the exaggerated cortisol response it seemed to elicit was unexpected. Early research conducted by Madden and Houston (1976) reported that cortisol concentrations returned to basal levels more rapidly in Rainbow Trout following electroanesthesia than they did in MS-222 anesthetized fish.

In general, one would expect a greater stress response associated with slow induction and prolonged recovery times. However, in the case of CO_2 and $NaHCO_3$, which had the lengthiest induction and recovery times recorded, such results were not observed with regard to cortisol levels. In studies aiming to characterize physiological effects in fishes following anesthesia, there are several other hematological variables, in addition to cortisol, that are regularly used to evaluate changes in the generalized stress response. These include, but are not limited to: blood glucose, hematocrit, osmolality, and lactate. Given the somewhat atypical results obtained in the cortisol portion of this study, it could be beneficial to examine a suite of blood parameters in future work.

Electroanesthesia was applied in freshwater and it is unclear whether exposure to freshwater contributed to the cortisol stress response observed. Given the brief exposure to freshwater (< 10 s), it seems highly unlikely, however a control treatment exposing fish to freshwater without electrical exposure would be necessary to determine if this provided an additional stressor beyond handling. Diurnal circulating cortisol levels may fluctuate naturally, so blood draw times of an experimental design may need to be taken into consideration. In a comparison of MS-222 and electroanesthesia on cortisol levels in juvenile Atlantic Sturgeon, Balazhik et al. (2013) processed two groups of four fish per day within a half hour of each other in order to minimize possible daily cyclic cortisol fluctuations.

Pulsed DC electroanesthesia (150 V, 60 Hz, 3 s) was the only dosage tested that resulted in significantly elevated cortisol levels following exposure that remained elevated at t = 2 h. Generally, the magnitude of the physiological stress response is considered indicative of stressor severity (Trushenski et al. 2012c). Therefore, the greater magnitude and duration of the cortisol pulses observed among Gopher Rockfish sedated with pulsed DC electroanesthesia (150 V, 60 Hz, 3 s) suggests that this anesthetic approach/dosage was the most stressful of those evaluated. However, when the voltage was decreased slightly (100 V, 60 Hz, 3 s), the resulting peak cortisol level (t = 1 h) was similar in magnitude to those of the other anesthetics being tested and cortisol levels were observed to return towards basal levels at t = 2 h.

Despite the seemingly high cortisol stress response observed, 100% short-term (72 h – 1 week) and long-term (2 month) survival rates were achieved throughout the course of this study, demonstrating that all of the anesthetics tested can be safely applied to rockfish without a substantial risk of mortality. This study was the first to evaluate electroanesthesia in Gopher Rockfish, however, and selection of the particular waveform tested was somewhat arbitrary. Additional research is needed to identify and fine-tune the optimal waveforms for use on Gopher Rockfish and other members of the genus *Sebastes,* as well as for unrelated species.

There were notable differences in induction times between anesthetics and particular dosages that were statistically significant. For other species, an appropriate induction time would most likely need to be predetermined depending on the desired sedation or anesthetic level for the procedure(s) being conducted. Some significant differences were detected among recovery times and specifically in the case of $NaHCO_3$ and CO_2, these recovery times would not be considered appropriate or acceptable for researchers conducting surgery to implant acoustic transmitters into fish. Though elevated, the transient endocrine response that was observed in Gopher Rockfish following electroanesthesia, followed the general pattern that was expected and seemed to be resolving rapidly. The stress response did not affect overall survival rates in the two-month period following the experiments and therefore, should not pose a concern for researchers with regard to survival of acoustically tagged fish or subsequent loss of costly acoustic transmitters.

The predominant distinctions seen among the anesthetics tested in terms of efficacy and applicability to field use are induction and recovery times, ease of use, and affordability. Studies have shown that total handling time, far more than any other factor, impacts the survival rate of captured fishes (Lowe and Kelley 2004). Given the rapid induction and relatively rapid recovery times associated with electroanesthesia, this approach would be useful for dealing with a high volume of fish while keeping total handling time to a minimum. When evaluating MS-222 as a surgical anesthetic for Atlantic Sturgeon, Matsche (2011) proposed that limiting fish handling time and manipulation might be more important in minimizing cardiovascular disturbance than the choice of anesthetic. In this sense, a portable electroanesthesia unit is the ultimate tool for quickly inducing fish for surgery in the field with zero withdrawal times.

Electroanesthesia and $NaHCO_3$ would both be effective zero-withdrawal anesthetics in the field as they are straightforward and easy to use. The Smith-Root PES^{TM} unit used in this study is relatively small (49.9 W × 39.4 H × 22.2 D cm), light enough to be portable (12.93 kg), and could easily be set-up and contained at sea using a minimal amount of deck space. Operator safety is easily achieved by the donning of rubber boots or rubber soled shoes and the use of an electrically isolated net handle. $NaHCO_3$ can readily be used in the field by fishery biologists by simply pre-measuring and preparing packets of sodium bicarbonate to be mixed with a known volume of seawater. The addition of acetic acid to activate the release of CO_2 in the anesthetic water could be accomplished in the field by premeasuring a volume of acid and storing it in tightly capped glass vials. Booke et al. (1978) did not recommend using an acid or

base to adjust pH for fieldwork in fresh waters of the United States, stating that $NaHCO_3$ should be an effective anesthetic in waters between pH 6.5 and 7.0. As acetic acid was used in all $NaHCO_3$ treatments in this study, it is unclear whether $NaHCO_3$ alone would provide acceptable anesthetic efficacy in seawater.

In contrast, the use of gaseous CO_2 delivered from a pressurized cylinder presents many difficulties that make it a poor candidate for zero-withdrawal field use. The cylinder is typically very heavy and must be transported very carefully. Depending on the equipment used, the regulator attachment and rotameter can be fairly expensive and delicate. On a rocking boat at sea, the entire apparatus would have to be heavily secured in order to avoid movement and breakage. To further complicate logistics, the creation, measurement, and maintenance of a specific CO_2 concentration in the field can be difficult, if not impossible to achieve (Gause et al. 2012).

Material cost is another important factor to consider when choosing the appropriate anesthetic for use in a study. The purchase of a commercially available electroanesthesia system like the Smith-Root PES™ unit used in the present study may represent a significant initial investment for some researchers. However, maintenance is simple and costs beyond the initial investment are negligible. Depending on usage patterns, such as numbers of fish to be anesthetized, frequency of use, and purpose of anesthesia, an electroanesthesia unit may be cost-effective in the long run.

If it is necessary to keep research costs to an absolute minimum, tutorials on the construction of a low-cost electroanesthesia unit using readily available materials are accessible in the literature. Jennings and Looney (1998) constructed a simple electrified

basket powered with a 12 V car battery controlled with a rheostat to subject adult Striped Bass *Morone saxatilis* (52 – 81 cm) to low doses (12 V, 30 mA) of continuous DC electricity before surgery. Balazik et al. (2013) applied electroanesthesia to Atlantic Sturgeon to conduct mock surgeries using a 0-60 V DC, 1.5 A (BK Precision; Model 1623A) power supply with positive and negative electrodes attached to 6.35 mm mesh-galvanized hardware cloth.

Hudson et al. (2011) provide detailed instructions for the assembly and operation of a portable electroanesthesia unit. This unit consists of a holding tank fitted with power supply components to provide continuous DC output for anesthetizing and implanting radio tags into two salmonids: Bull Trout *Salvelinus confluentus* and Coho Salmon *Oncorhynchus kisutch*. The total cost of materials for the full-sized unit (a converted 153-L marine grade cooler) was less than US$1000. The downsized unit contained in Rubbermaid ActionPacker (50.5 L × 35.8 W × 30.7 D cm) could be constructed for less than US$350.

This study evaluated the efficacy of three zero-withdrawal anesthetics and MS-222 for field procedures involving the marine species, *Sebastes carnatus*. Detailed information on the induction and recovery times, physiological responses, and survival of fish for all of the anesthetics was established. Results allowed us to determine the effectiveness of the various methods and provide a metric to gauge fish stress response to the anesthetics being tested. Given the lack of comparison studies of immediate-release anesthetics in marine fishes, these results provide an informative basis for applying these

techniques to other marine species and facilitating the implantation of acoustic transmitters in the field. Ultimately, the particulars of a research objective will dictate the most appropriate anesthetic to use.

Of the four anesthetics evaluated, only pulsed DC electroanesthesia met all of the criteria established for defining a suitable field anesthetic for the surgical implantation of transmitters into fish in all scenarios tested. Based on the effectiveness of $NaHCO_3$ in the majority of the experiments conducted, its use in exceptional circumstances (e.g., a distant or remote freshwater field location that would exclude the transport of an electroanesthesia unit to the site) could be warranted, but is not generally recommended. Although it is common practice to use bubbled CO_2 to momentary immobilize tide pool fishes for collection, surgical anesthesia achieved with gaseous CO_2 is too cumbersome to achieve and not dependable enough for most research applications. However, the immersion anesthetics tested may still be suitable for laboratory or hatchery situations requiring the sedation or anesthetization of small numbers of fish that do not require immediate release.

Although variations in the response of Gopher Rockfish exposed to pulsed DC electroanesthesia were observed in the present study, these differences can likely be minimized and tailored to more closely match desired induction and recovery times by making slight adjustments to wave form, voltage strength, frequency, or exposure duration as needed. It is generally recommended that preliminary tests on a small group of individuals be conducted to determine appropriate concentrations and administration protocols before embarking on an experiment with a larger sample size. The lowest

combination of voltage strength, frequency, and exposure duration that yields the level of sedation or anesthesia required should be chosen to accomplish procedures with minimal total handling time when possible. Ideally, this will serve to minimize stressful side effects, unseen potential injuries, and undesirable physiological responses as well.

The results generated in this study suggest that electroanesthesia is a highly suitable method for surgically anesthetizing Gopher Rockfish to implant acoustic transmitters. Survival rates of 100% were observed during the two months following surgery, indicating that the long-term safety of study fish was achieved. Pulsed DC electroanesthesia is a promising new alternative for marine fishes, providing the rapid induction and recovery times required for field procedures. This method, along with other forms of electroanesthesia, should be explored by researchers looking for a safe, effective, zero-withdrawal anesthetic for marine teleost fishes undergoing acoustic surgeries or other invasive procedures at sea.

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