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Short Communication

Carbon dioxide is an effective anesthetic for multiple marine fish species

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ABSTRACT

Fisheries research involving surgery is aided by, and sometimes requires, anesthesia, but health and safety regulations limit the anesthetic methods that can be used on species considered food fish. Carbon dioxide is one anesthetic that the United States Food and Drug Administration (FDA) tolerates when certain guidelines are met, and it complies with Institutional Animal Care and Use Committee protocols. But, there is very little published work that characterizes this compound's utility on marine fishes and no studies have compared its effectiveness across species or sizes. We used acetic acid and sodium carbonate to create a carbon dioxide rich sea water bath to induce anesthesia and measured induction time and recovery time for five species and several sizes of marine fishes. We found that carbon dioxide quickly and effectively anesthetized these marine fishes to stage-4 anesthesia, a level acceptable for minor surgery. Induction time was positively related to body size (total length or wet mass), but recovery time was independent of size. Using red drum, we also found differences between rested and fatigued individuals. These results provide needed documentation of the effectiveness of carbon dioxide on marine fishes and are useful for planning field studies that involve minor surgery on marine food fish.

1. Introduction

The use of anesthetics has facilitated a variety of important procedures in fisheries research. These procedures include making measurements, administering tags, extracting tissue samples, and minor surgery. When properly applied, anesthesia calms the fish, reduces its movement, and minimizes stress and pain. These attributes increase researchers' ability to handle fish without injuring themselves or the fish. Furthermore, anesthesia is required by Institutional Animal Care and Use Committees (IACUC) following guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and regulations provided by the Animal Welfare Act (Garber et al., 2011).

Many different methods of anesthetization have been tested on fishes, but technical or legal reasons limit applications in the field. When tagging or surgery is involved, stage-4 anesthesia should be induced, which is characterized by complete loss of equilibrium, loss of swimming motion, and weakened opercular movements (Summerfelt and Smith, 1990). Some methods of inducing anesthesia are ineffective or inconsistent, impractical, or do not meet regulatory requirements. A review of 16 anesthetization methods for a freshwater fish, rainbow trout (*Oncorhynchus mykiss*), found that effectiveness can vary widely among methods (Gilderhus and Marking, 1987). Two products, tricaine methanesulfonate (MS-222) and clove oil (eugenol, AQUI-S®20E), are commonly used because they induce high stages of anesthetization rapidly (<3 min) and have quick recovery times (<10 min) (Gilderhus and Marking, 1987, reviewed by Neiffer and Stamper, 2009; Javahery et al., 2012). Electroanesthesia can be used in fresh water, but it requires a significant initial investment for equipment, which could be prohibitive for many fisheries professionals (Trushenski et al., 2012a).

A limited number of anesthetic drugs have been approved for use on food fish, and special precautions have been prescribed for the approved drugs (Bowker and Trushenski, 2012; Trushenski et al., 2013). The United States Food and Drug Administration (FDA) authorized tricaine methanesulfonate for use on fishes in the families Ictaluridae, Salmonidae, Esocidae, and Percidae with a 21-day withdrawal period (Kelsch and Shields, 1996; Bowker and Trushenski, 2012). Eugenol- and benzocaine-based anesthetic drugs, AQUI-S®20E and BENZOAK, are "Investigational New Animal Drugs" for freshwater finfish (Trushenski et al., 2013). Since these policies exclude all non-salmonid marine fishes, field researchers

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who study marine food fishes and wish to release the fish alive immediately after handling must use an anesthetic technique that does not violate FDA regulations (Trushenski et al., 2013).

Carbon dioxide is the only alternative anesthetic that the FDA has explicitly stated it is unlikely to oppose, provided that conditions regarding appropriate and safe use are met (USFDA, 2011). As carbon dioxide is a 'generally recognized as safe' (GRAS) food ingredient, this form of anesthesia poses no risk to humans if an animal is released immediately after recovery then recaptured and consumed. Finally, carbon dioxide complies with IACUC requirements at our university and is likely to be approved for use elsewhere.

Carbon dioxide concentrations in water can be increased by bubbling carbon dioxide gas directly into a holding tank, but this requires carrying inconvenient and potentially dangerous gas cylinders. Carbon dioxide can also be created by reacting sodium bicarbonate ($NaHCO_3$) or sodium carbonate (Na_2CO_3) with an acid (e.g., sulfuric or acetic) to form carbonic acid (H_2CO_3), which then releases carbon dioxide into the water (Gelwicks et al., 1998). Total carbon dioxide in sea water is normally around 95 mg L^{-1}, whereas the solubility of carbon dioxide is estimated to be 1365 mg L^{-1} in 35 ppt aqueous NaCl at 24 °C (Duan and Sun, 2003; Keeling et al., 2005). Elevated concentrations of carbon dioxide in water (for example, 750 mg L^{-1} total carbon dioxide; Trushenski et al., 2012b), induce hypercapnia (elevated levels of carbon dioxide in the blood) and decrease blood pH (Post, 1979), which reduces oxygen transport to the brain and results in anesthetization. When a fish is returned to water with a normal carbon dioxide concentration, carbon dioxide diffuses out of the fish, blood pH increases, and equilibrium and swimming return (Gelwicks et al., 1998).

Use of carbon dioxide as a fish anesthetic has had a long and controversial history in fisheries biology (Fish, 1943; Summerfelt and Smith, 1990). Although carbon dioxide anesthesia satisfied the criteria that define an effective anesthetic for small fishes, Gilderhus and Marking (1987) found that this method was slow to act on adult fishes and suggested it be used only in situations where a low level of anesthesia is acceptable. Others have found that carbon dioxide can completely anesthetize a fish, causing disequilibrium, loss of muscle control, and possibly an analgesic effect (Post, 1979; Summerfelt and Smith, 1990; Prince et al., 1995). Interestingly, a comparison of five different anesthetization techniques found that carbon dioxide caused the smallest decrease in blood oxygen concentrations during induction of deep stages of anesthesia (Iwama et al., 1989). Additionally, plasma cortisol levels (indicators of stress) were no higher in steelhead trout (*Oncorhynchus mykiss*) after anesthetization using carbon dioxide than when using MS-222 or clove oil (Pirhonen and Schreck, 2003), and plasma cortisol levels decreased to pre-handling concentrations sooner when using carbon dioxide compared with clove oil (Wagner et al., 2002). Carbon dioxide anesthesia has been found to be effective across a broad range of temperatures (Gelwicks et al., 1998). Through various delivery systems, carbon dioxide has been successfully used on multiple freshwater species, including yellow perch (*Perca flavescens*), burbot (*Lota lota*), carp (*Cyprinus carpio*), walleye (*Sander vitreus*), and salmon and trout (salmonids), where it was used while conducting minor surgical procedures, such as implanting telemetry transmitters (Yoshikawa et al., 1991; Prince et al., 1995; Erikson et al., 2006; Vandergoot et al., 2011). To the best of our knowledge, the only documented uses of carbon dioxide as an anesthesia for marine finfishes were to reduce stress during harvesting and prior to processing of aquacultured Atlantic salmon (*Salmo salar*; Erikson et al., 2006) and a comparison of anesthetization techniques on juvenile cobia (*Rachycentron canadum*; Trushenski et al., 2012b).

Despite concerns regarding the effects of carbon dioxide anesthesia, it is currently the only option for many fisheries professionals, particularly those working under the more restrictive regulations of academia. Therefore, the goal of this study was to assess the utility of carbon dioxide for inducing stage-4 anesthesia in a broad range of marine fishes by examining its effectiveness. An ideal anesthetic works quickly to minimize stress and injury and allows the fish to recover quickly and be returned to the wild immediately. Here, we assess effectiveness using induction time and recovery time. We evaluate variability in these measures related to species, size, and energetic state (fatigued vs. rested). Our successful experimentation with carbon dioxide anesthesia opens the door for field research that requires anesthesia for minor surgery on marine food fishes, such as tissue biopsy and implantation of electronic tags.

2. Methods

We performed anesthetization experiments on five marine fish species acquired from laboratory-maintained stocks or the wild. Because this study has direct relevance to field studies, where the targeted individuals are within a size range that commercial or recreational fishers are allowed to catch, we focused our efforts on evaluating whether carbon dioxide was an effective anesthetic for four harvestable fishes: red drum (*Sciaenops ocellatus*), southern flounder (*Paralichthys lethostigma*), common snook (*Centropomus undecimalis*), and Florida pompano (*Trachinotus carolinus*). We also tested larval red drum and young-of-the-year inland silversides (*Menidia beryllina*) to extend our results to smaller body sizes.

The goal of this study was to determine whether methods used on freshwater fishes were effective for marine species. Therefore, our experiment was designed to test the effectiveness of carbon dioxide as an anesthetic at one concentration using methods of Prince et al. (1995) and Trushenski et al. (2012b). We combined 1.33 g L^{-1} sodium carbonate and 0.75 ml L^{-1} glacial acetic acid to 30–55 L of sea water. Using a Corning 965 carbon dioxide analyzer (Ciba Corning Diagnostics Corporation, Medfield, MA), we determined that this method immediately elevated total carbon dioxide levels in the sea water to 669 ± 32 mg L^{-1} (mean ±SE) and decreased pH from 8.0 to 6.7. These levels remained constant throughout the use of a bath. If a species did not reach stage-4 anesthesia within 10 min, we doubled the concentrations of sodium carbonate and acetic acid. This higher concentration raised total carbon dioxide to 1248 ± 178 mg L^{-1}, which was likely saturation. Following Trushenski et al. (2012b), we aerated baths of water prior to the addition of sodium carbonate and acetic acid, but not after the chemicals were added and fish were being tested.

To test each fish, we carefully netted individual larval red drum, juvenile flounder, adult snook, adult flounder, or adult pompano from laboratory tanks and immediately placed the fish in a prepared anesthesia bath. We collected inland silversides and red drum in the wild by net or hand line and allowed each fish to recover in an oxygenated tank for at least 1 h prior to testing. To evaluate carbon dioxide anesthesia under conditions common during field sampling, we landed adult red drum using rod and reel (approximately 10–15 min) and immediately placed the fish in the anesthesia bath. These fish were referred to as "fatigued." While fish were in the bath, pH was between 6.7 and 7.2. For fish tested in the laboratory, we maintained water temperature at 24 °C. Fatigued red drum were tested at ambient water temperatures (27–29 °C). Due to the potential for loss of carbon dioxide from water-to-air exchange, a new bath was prepared if the bath was used for >30 min.

We conducted trials on most species in a covered clear glass container (91.5 cm × 30.5 cm × 40.5 cm) and monitored the fish continuously. Due to their large size, however, we tested adult