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Physiological Response of Elasmobrachs During Propofol Immersion

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PHYSIOLOGICAL RESPONSE OF ELASMOBRANCHS DURING PROPOFOL IMMERSION

by

MATTHEW LEVENDOSKY

(Under the Direction of Christine Bedore)

ABSTRACT

Sensory experiments require anesthesia so the animal is immobilized, however fish anesthetics have shown to depress sensory responses. Newer anesthetics may offer similar anesthetic relief, but differ in means of action so sensory responses may be unaffected. Propofol has been used intravenously on small elasmobranchs but may provide prolonged effects if used as an immersion anesthetic. Objectives of this study were 1. Determine appropriate concentration of anesthetic to minimize induction and recovery for animals anesthetized at a surgical plane of anesthesia and 2. Measure physiological response of the pupil to light stimuli during anesthetic immersion. To address these objectives, I used the coral catshark (*Atelomycterus marmoratus*) and the Atlantic stingray (*Hypanus sabinus*). Ventilation rate and reflex responses were recorded to measure induction and recovery in increasing concentrations of tricaine and propofol. Appropriate concentrations of anesthetics are approximately 160 and 1.4, and 140 and 0.7 mg $L^{-1}$of tricaine and propofol in *A. marmoratus* and *H. sabinus*, respectively. After 1.5 hours of dark adaptation in anesthetic (50, 100, or 150 mg L^{-1} tricaine or 0.5, 1, or 1.5 mg L^{-1} propofol) or no anesthesia (control), tricaine 100 mg L^{-1} trials show reduction in percent pupil constriction ($p<0.05$; ANOVA) in both species as well as tricaine 150 mg L^{-1} trials in Atlantic stingrays ($p<0.05$; ANOVA). In both species, rate of constriction increased when using 1.5 mg L^{-1} of propofol ($p<0.05$; ANOVA) and the dark-adapted eye of coral catshark was dilated less than when anesthetized using 1.5 mg $L^{-1}$of propofol ($p<0.05$; ANOVA).

INDEX WORDS: Elasmobranch, Anesthesia, Propofol, Tricaine methanesulfonate, Pupil constriction

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by

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CHAPTER 1

INTRODUCTION

The use of anesthetics is common practice in fish biology as a means to sedate, immobilize and/or produce analgesia in fish during stressful or invasive procedures (Burka et al. 1997; Carter et al. 2011; Popovic et al. 2012; Mylniczenko et al. 2014). Anesthesia is an artificially induced state of altered consciousness (Brown et al. 2010; Hudetz 2012), during which physiological responses – including unconsciousness, amnesia, and analgesia – allow invasive procedures to be performed on patients (Brown et al. 2010; Hudetz 2012).

Tricaine is the most commonly used anesthetic in fish biology and is the only anesthetic approved for use in food fish in the USA, Canada, and UK (Burka et al 1997; Palmer and Mensinger 2004; Carter et al. 2011; Popovic et al. 2012). Immersion in tricaine is characterized by quick induction to and recovery from the desired anesthetic plane (Burka et al. 1997; Stamper 2004; Carter et al. 2011; Popovic et al. 2012; Mylniczenko et al. 2014). Additionally, because of its widespread use, many published studies exist describing its induction/recovery properties in a variety of species making it a rather predictable and safe anesthetic when properly utilized (Massee et al. 1995; Roubach et al. 2001; Sladky et al. 2001; Stamper 2004; Carter et al. 2011; Popovic et al. 2012; Mylniczenko et al. 2014). Although species specific variations do occur, the margin of safety seen in tricaine is wider than other fish anesthetics (Sladky et al. 2001; Mylniczenko et al. 2014). As with all anesthetics, however, tricaine has drawbacks associated with its use. Unbuffered tricaine in freshwater reduces the pH of anesthetic baths potentially irritating or harming fish (Burka et al. 1997; Carter et al. 2011; Popovic et al. 2012). Additionally, tricaine has been labeled as both a potential carcinogen and retinotoxin (Bernstein et al. 1997; Popovic et al. 2012), possibly affecting aquaculture workers that have prolonged exposure to the drug.

Propofol is a common general anesthetic used in medical and veterinary procedures of mammals and some species of bird (Short and Bufalari 1999; Trapani et al. 2000; Hudetz 2012; Lamont and Grimm 2014; Berry 2015). Most often, propofol is administered through intravenous injection and yields quick

induction and recovery (Short and Bufalari 1999; Trapani et al. 2000; Hudetz 2012; Lamont and Grimm 2014). However, because the drug is rapidly metabolized when administered through injection it produces only short periods of anesthesia, and therefore continuous doses of propofol or pairing propofol with another anesthetic to maintain longer periods of anesthesia is required (Short and Bufalari; Berry 2015). In fishes, longer duration of anesthesia may be provided by immersion in propofol whereby the drug slowly enters the bloodstream at the gills (Carter et al. 2011). Propofol immersion has already shown to safely induce anesthesia in several species of teleost and chondrostean fishes, such as; the silver catfish (*Rhamdia quelen*, Gressler et al. 2012), koi (*Cyprinus carpio*, Oda et al. 2014) goldfish (*Carassius auratus*, Balko et al. 2017), rainbow trout (*Oncorhynchus mykiss*, Gomulka et al. 2015; Prieto et al 2017), and the Gulf of Mexico (*Acipenser oxyrinchus de soti*, Fleming et al. 2003), Siberian (*Acipenser baerii*, Gomulka et al. 2015), and Persian sturgeon (*Acipenser persicus*, Adel et al. 2016). However, propofol has only been administered to elasmobranch fishes through injection (Miller et al. 2005; Mylniczenko et al. 2014), leaving the anesthetic effects of immersion unknown in this group.

Anesthesia is induced by impairment of neural function which, in fishes, is accompanied by physiological responses that can be used to determine the anesthetic plane reached (Burka et al. 1997; Stamper 2004; Carter et al. 2011; Mylniczenko et al. 2014; Table 1). The means by which neural function is disrupted depends on the drug, however, and may influence its use for various procedures. Under tricaine induced anesthesia, tricaine molecules prevent sodium ions from entering neurons. In this state, cell excitability is reduced, which in turn reduces the frequency of action potentials generated, preventing transmission of sensory information (Carter et al. 2011; Popovic et al. 2012). Propofol, however, is thought to produce anesthesia through increased affinity of gamma aminobutyric acid (GABA) to $GABA_A$ receptors (Short and Bufalari 1999; Trapani et al 2000). GABA is an inhibitory neurotransmitter in vertebrates, involved in many pathways throughout the central nervous system (Trapani et al. 2000). During propofol anesthesia, cerebral metabolic rate, blood flow, and functional connectivity of synaptic pathways is reduced (Hudetz 2012). The disruption of communication in these pathways is thought to

induce unconsciousness and reduce integration of sensory information to processing areas, such as the cortex (Mhuircheartaigh et al. 2010; Hudetz 2006; Schrouff et al. 2011; Tu et al. 2011; Hudetz 2012).

Depending on the species, several factors may play a role in the efficacy of immersion anesthetic agents in fishes, including metabolism and mass/lipid content of the fish, temperature of the bath, and the lipophilic properties of the drug (Zahl et al. 2009; Carter et al. 2011; Sneddon 2012). Bath temperature exerts influence on induction/recovery because of its effects on ventilation rate, metabolism, and the diffusion/clearance rate of the anesthetic (increasing all with higher temperatures; Neiffer and Stamper 2009; Carter et al. 2011; Sneddon 2012). Metabolism and lipid content of the fish, as well as the lipophilic properties of the drug must be considered because they determine the amount of drug that is taken up into the bloodstream, the rate drugs are distributed to the central nervous system, redistributed to other tissues, and broken down and excreted from the body (Short and Bufalari 1999; Carter et al. 2011; Sneddon 2012).

Although the result of reduced perception of external stimuli is the desired effect for most surgical procedures in fishes (Burka et al. 1997; Sneddon 2012), experiments aimed at measuring the physiological response of sensory neurons can be affected by the use of anesthesia (Hensel et al. 1975; Spath and Schweickert 1977; Palmer and Mensinger 2004; Yamamoto et al. 2008). Experiments investigating responses of the electrosensory system (Hensel et al. 1975), lateral line system (Hensel et al. 1975; Spath and Schweickert 1977; Palmer and Mensinger 2004), and olfactory nerve (Yamamoto et al. 2008) of various fishes demonstrate reduced firing rates from both spontaneous and evoked potentials. Since elasmobranchs have not been included in a majority of previous works and their physiology differs from teleost fishes, the aim of this study was to assess physiological responses under both tricaine and propofol induced anesthesia in elasmobranchs. These physiological responses included those typically used to define anesthetic depth in fishes (Table 1), as well as the pupillary light response (PLR). The PLR in vertebrates is controlled by the autonomic nervous system (a division of the peripheral nervous system). When photons strike the retina, photoreceptors absorb the photons and become hyperpolarized, which passes the signal from the retina through several nuclei in the midbrain, and then to ganglia behind

the eye that innervate circular muscles in the iris and cause the pupil to constrict (Moller 2003; Douglas 2017). Conversely, when dark adapted photoreceptors are depolarized and the radial muscles of the iris constrict to cause dilation (Moller 2003). Comparing changes in pupil physiology under both drugs can inform us about potential effects on various levels of the nervous system in this pathway.

To understand how anesthesia affects the PLR pathway in elasmobranchs, I first measured physiological responses to immersion in both drugs. Using the data from these responses, pupil constriction during induction to a surgical plane of anesthesia was compared in two species of elasmobranch, the coral catshark (*Atelomycterus marmoratus*) and the Atlantic stingray (*Hypanus sabinus*). Both species are relatively small elasmobranchs which facilitate handling during anesthetic procedures. These two species differ in several aspects of their ecology and morphology that may be reflected in both physiological response to anesthesia and changes to the PLR. Coral catsharks inhabit crevices of shallow inshore reefs of the Indo-West Pacific ocean (White 2003), whereas the Atlantic stingray is commonly found in sandy bottom coastal and freshwater environments of the western Atlantic (Piercy et al. 2016; Ramsden et al. 2017). The photic environment of these species differs considerably. While both are found in relatively shallow waters, coastal environments contain higher amounts of dissolved organic matter than inshore reefs (Lythgoe 1980). This reduces the range of available wavelengths of light in the water column, making coastal environments spectrally narrower compared to clear reefs (Lythgoe 1980). Past research has shown that the photic environment impacts visual capabilities such as spectral sensitivity, temporal resolution, and pupil constriction (Levine and MacNichol 1978; Lythgoe 1980; Lisney et al. 2012). Additionally, these species exhibit differences in pupil morphology. The pupil of the coral catshark is slit shaped, allowing it to constrict to a higher degree than round pupils (Lisney et al. 2012). When constricted, the pupil opening exists as two pinhole apertures on either end of the pupil. Atlantic stingrays possess pupil operculae, thin flaps of skin that extend over the pupil during constriction, which further reduce the amount of light entering the eye (Lisney et al. 2012). Studying both species will provide physiological responses that may be unique to species depending on differences in photic environment or pupil morphology.

Knowledge on the species-specific effects of anesthesia in elasmobranchs is lacking and most published data regarding safe immersion concentrations are from personal communication. Further, the effects of anesthesia on sensory physiology of elasmobranchs is poorly understood. This study seeks to address these gaps and provide information for fish handlers to select appropriate drug-concentration combinations for a range of anesthetic procedures in elasmobranchs.

Table 1: Anesthetic plane descriptions and corresponding changes in behavior. Adapted from Stamper 2004 and Carter et al. 2011.

Plane	Description	Behavioral Response
0	Normal	Swimming, response to stimulus, muscle tone, and equilibrium normal
1	Light sedation	Swimming, muscle tone, and equilibrium normal; slight reduction in response to stimulus
2	Deep sedation	Voluntary swimming, response to stimulus ceases; slight decrease in ventilation rate and muscle tone; equilibrium normal
3	Light narcosis	Excitement phase; uncoordinated swimming; exaggerated response to painful stimuli; erratic respiration.
4	Deep narcosis	No response to positional changes; total loss of equilibrium; respiration rate returns to a normal rhythm
5	Light anesthesia	Total loss of muscle tone; further decrease in respiration rate; appropriate for minor surgical procedures
6 – 9	Surgical anesthesia	Respiration rate significantly reduced (<1 breath/minute); heart rate reduced; necessary for major surgical procedures
10	Medullary collapse	Respiration completely ceases; cardiac arrest possible if anesthetic regiment is not changed

CHAPTER 2

MATERIALS AND METHODS

Animals

Coral catsharks (*Atelomycterus marmoratus*) were obtained from an aquarium distributor (n=6; Sea Dwelling Creatures LLC, Los Angeles, CA 90045) and Atlantic stingrays were either obtained from an aquarium distributor (n=3; Gulf Specimen Marine Laboratory, Panacea, FL 32346) or collected during routine sampling efforts with the GA DNR (n=3; Brunswick, GA United States of America). Animals were kept in the onsite animal facility at Georgia Southern University under a 12:12 light: dark cycle in 70 gallon tanks. Tanks were equipped with recirculating seawater filtration systems (biological, mechanical, and chemical filtration; Marineland Multi-Stage C530 Aquarium Canister Filter; Marineland, Spectrum Brand Pet, LLC, Blacksburg, VA 24060) and maintained at 21-24 °C and 30-35 ppt. Water parameters (nitrite, ammonia, salinity, dissolved oxygen and pH) were measured three times per week, and adjusted as necessary. All procedures were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) of Georgia Southern University protocol #I18022.

Concentration-response measurements

Before each trial began, resting criteria were established by measuring the ventilation rate (gill slit beat per minute in sharks and spiracle beat per minute in rays), and response to stimuli by performing response tests (Table 2) in the animal's holding tank. Afterwards, an individual fish was placed into a 10L anesthetic bath containing a randomly selected drug-concentration combination of tricaine (MS-222, Snydel Washington, USA) or propofol (Propoflo 28, Zoetis Michigan, USA; Table 3). Ventilation rate and response to stimuli were recorded every two minutes until induction was achieved or until 30 minutes elapsed. Induction to surgical anesthesia was defined as the point at which ventilation rate reached less than one breath per minute and all responses scored a zero. After induction fish were removed from the anesthetic bath and placed in a recovery tank and artificially ventilated using a pump to pass aerated water over the gills, until unassisted ventilation resumed. Ventilation rate and reflex responses were recorded every two minutes while fish were in the recovery tank. Recovery was defined as the point at which

ventilation rate returned to 10% of the resting ventilation rate and the fish scored a three on all response tests. After recovery was reached, the trial ended and the fish was placed back in its holding tank. Time to induction and recovery were recorded (min) for each trial. If induction did not occur within 30 minutes, the fish was returned to its holding tank and times of 30 and zero minutes were recorded for induction and recovery times, respectively. A drug was considered to safely induce anesthesia if the fish was able to recover and survived 48 hours after immersion.

Pupil constriction measurements

Acrylic tanks (45.72 x 20.32 x 15.24 cm for coral catsharks and 78.74 x 38.1 x 15.24 cm for Atlantic stingrays) were equipped with an aerated seawater recirculating system and treated with a randomly selected drug-concentration combination (Table 3). Coral catsharks were placed in anesthetic baths in a light-tight room and allowed to dark adapt for 90 minutes before recordings. Under propofol anesthesia, the dark adapted pupil did not appear to dilate completely in the coral catshark. To avoid this in the Atlantic stingray, rays were dark adapted prior to being placed in anesthetic baths, however also exposed to the anesthetic for 90 minutes before recording. Fish were secured in a plastic cage and confined to reduce movement and maintain calibration with the camera throughout the duration of each trial. During high concentration trials where ventilation ceased, fish were artificially ventilated by inserting a hose in the mouth and passing aerated seawater over the gills (.6-.7 L min^{-1}).

After 90 minutes, an LED lamp suspended next to the tank (60W Clamp Lamp; Wood Enterprises, Cove, AR 71937, United States of America) was used to illuminate the eye. Pupil constriction in response to light was video recorded using a Canon ® G12 digital camera (Canon U.S.A., One Canon Park, Melville, NY 11747) for 15 minutes at 24 frames per second. Still images were taken from the video recording every 30 seconds for the first three minutes and every 60 seconds for the subsequent 12 minutes. Eye measurements (Figures 1 and 2) were recorded using ImageJ image analysis software (ImageJ 1.48v, Wayne Rasband, National Institutes of Health, United States of America). Eye diameter and pupil diameter along the same axis was measured for each image. Pupil size was measured as a percent of eye diameter:

$$\text{Pupil diameter/Eye diameter} * 100\%$$

where *Eye diameter* is the diameter of the eye (cm) along the longest axis, and *Pupil diameter* is the diameter of the pupil (cm) along the same axis. Pupil constriction was measured as a percent change in pupil diameter from the initial image:

$$\text{Pupil diameter} - \text{Pupil diameter}_1 / \text{Pupil diameter}_1 * 100\%$$

where *Pupil diameter* is the diameter of the pupil (cm) in a given image and $Pupil\ diameter_1$ is the pupil diameter (cm) in the initial image.

Data analysis

All statistical analyses were conducted using R Statistical Software. Stage of anesthesia was identified for each drug-concentration combination in either species using previously outlined criteria (Table 1). Differences in time to induction and recovery from surgical anesthesia in different concentrations were determined using mixed effects ANOVA assigning the concentration as a fixed effect and individual as the random effect. Induction and recovery concentration response curves were generated for each drug-species combination using the drm() function in the R Statistical software package *drc* (Ritz et al. 2015). Data were fit with five-parameter log-logistic curves (fct = LL.5() in source code). Time and concentrations at which induction and recovery curves intersect were recorded, and induction/recovery times at concentrations immediately following these intersections were compared using two tailed t-tests or non-parametric Mann Whitney-U tests. From concentration-response curves, 50% effective dose values (ED50) were extracted, representing the median dose that induces surgical anesthesia in either species. Differences in tricaine and propofol ED50 were investigated between species using one way ANOVAs. Relationships between resting ventilation rate/mass and induction/recovery were investigated using linear regression.

Constriction (%) and time (s) data were fit with nonlinear curves for each species' drug-concentration trials using the nls() function in the R Statistical software core package *stats*. Concentration rate (percent change/second) was calculated as the slope of the constriction curve at the point that constriction reached 50% total constriction for that trial. Differences in pupil constriction and constriction

rate between drug-concentration combinations and control trials for either species were investigated using mixed effect ANOVAs where the concentration was assigned as a fixed effect and the individual was assigned as the random effect.

Table 2. Definition of and score criteria for response tests used during concentration-response measurements.

Test	Definition	Score
Escape Response	Degree of an attempt to avoid being handled	0- No attempt 1- Weak attempt 2- Moderate effort, but unsuccessful 3- Strong attempt and/or successful escape
Righting Reflex	Ability of an individual to right itself when turned on its back	0- No attempt 1- Weak attempt 2- Moderate effort, but unsuccessful 3- Strong attempt and/or successful righting
Noxious Stimuli	Degree of response to a tail pinch with a pair of hemostats	0- No response 1- Weak response by tail only 2- Moderate response, mostly tail 3- Strong response by whole body (e.g. attempt to flee)

Table 3. Drug-concentration combinations used during concentration-response and pupil constriction experiments

Drug	Concentration-response ($mg\ L^{-1}$)	Pupil Constriction ($mg\ L^{-1}$)
Tricaine	0	0
	25	50
	50	100
	100	150
	150	-
	200	-
	250	-
Propofol	0	0
	0.5	0.5
	1	1
	1.5	1.5
	2	-
	2.5	-
	3	-

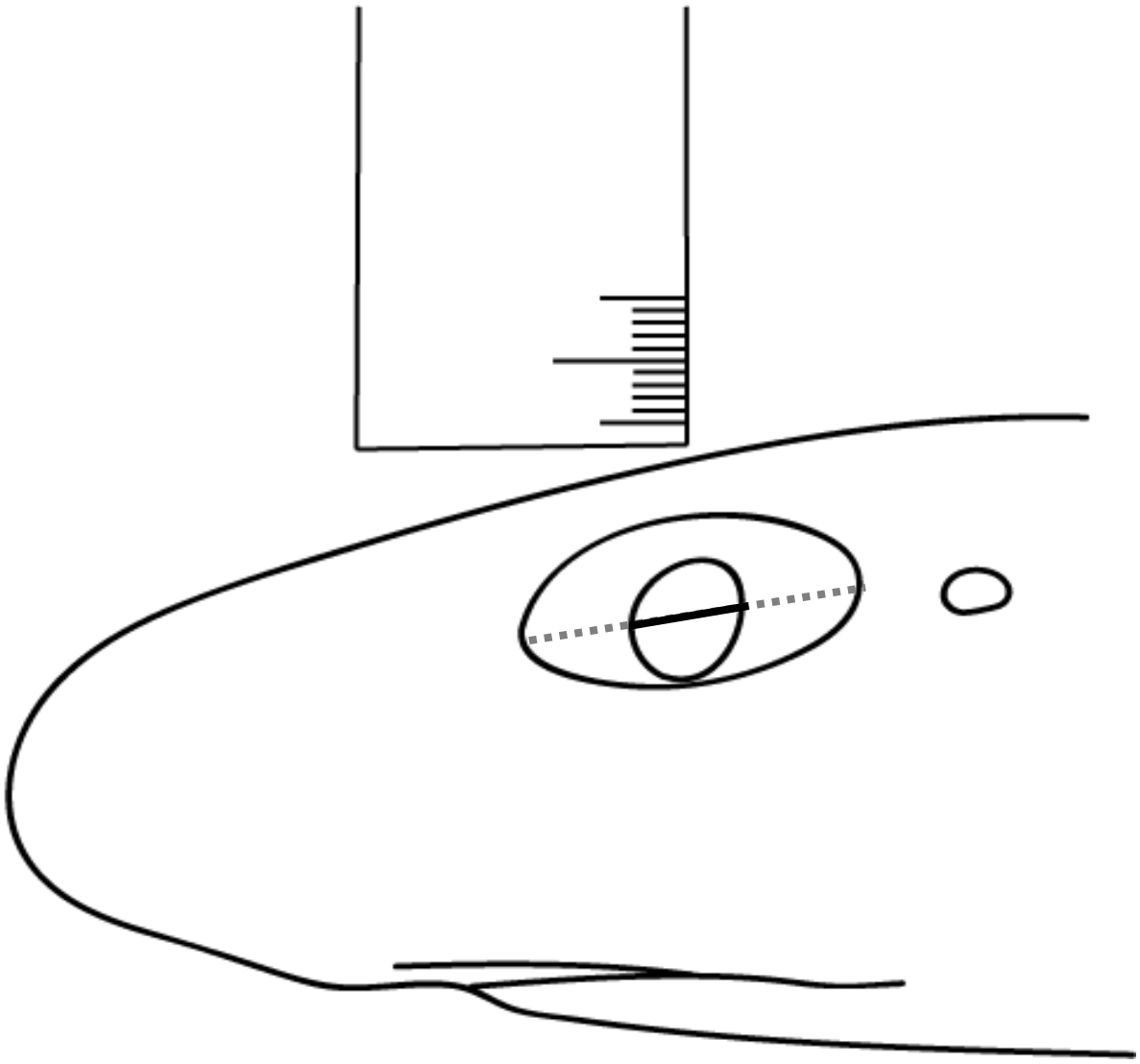

Figure 1: Eye measurements (cm) of *Atelomycterus marmoratus* recorded from still images, and ruler placement for calibration. Eye diameter (grey, dashed) and pupil diameter (black, solid) were measured along the longest axis of the eye in each frame.

Figure 2. Eye measurements (cm) of *Hypanus sabinus* recorded from still images, and ruler placement for calibration. Eye diameter (grey, dashed) and pupil diameter (black, solid) were measured along the vertical axis of the eye in each frame.

CHAPTER 3

RESULTS

Both tricaine and propofol safely induced a surgical plane of anesthesia in the coral catshark and Atlantic stingray in a concentration-dependent manner (Table 4). Comparisons of drugs between the coral catshark and Atlantic stingray, as well as between induction/recovery and ventilation rate and mass were made using concentrations immediately following intersections of concentration-response curves (200 and 150 mg L^{-1} tricaine, and 1.5 and 1 mg L^{-1} propofol in the coral catshark and Atlantic stingray, respectively; Figures 3 and 4). There were no differences in the time to induction or recovery from surgical anesthesia between species when anesthesia was induced using either tricaine or propofol (tricaine, t-test, $p>0.05$; propofol t-test, $p>0.05$). The effective dose (ED50) of tricaine and propofol induction curves differed between coral catsharks and Atlantic stingrays (tricaine, ANOVA, $F_{1,8} = 22.08$, $p = 0.00154$; propofol, ANOVA, $F_{1,8} = 21.3$, $p = 0.00172$, Figure 5). The ED50 of recovery curves did not differ between species (ANOVA, $p>0.05$)

Concentration-response

In the coral catshark, the lowest concentrations that induced a surgical plane of anesthesia were 150 and 1.5 mg L^{-1} when tricaine and propofol were used, respectively (Table 4). Under tricaine-induced anesthesia, the intersection of induction and recovery curves occurred at 160 mg L^{-1}, estimating a minimized time to induction and recovery of seven minutes (Figure 3a). Recovery times were longer when higher concentrations of tricaine were used (mixed effects ANOVA, $F_{2,15} = 21.221$, $p<0.001$), however induction time did not differ (mixed effects ANOVA, $p>0.05$, Tukey HSD). Under propofol induced anesthesia, the intersection of the induction and recovery curves occurred at 1.4 mg L^{-1}, estimating a minimized time to induction and recovery of 22 minutes (Figure 4a). Recovery time did not differ among propofol concentrations that induced a surgical plane of anesthesia (mixed effects ANOVA, $p>0.05$), but induction occurred faster at the highest concentration than at lowest concentration that induced a surgical plane (mixed effects ANOVA, $F_{3,20} = 8.4172$, $p<0.001$). Induction was reached faster

(t-test; t = 3.7498, df = 6.5784, p = 0.008) and recovery was shorter (Mann Whitney-U; W = 25, p = 0.01) under tricaine induced anesthesia than under propofol induced anesthesia (Figures 6a and 7a).

In the Atlantic stingray, the lowest concentration that induced a surgical plane of anesthesia were 100 and 1.0 mg L^{-1} when tricaine and propofol were used, respectively (Table 4). Under tricaine induced anesthesia, the intersection of induction and recovery curves occurred at 140 mg L^{-1}, estimating a minimized time to induction and recovery of nine minutes (Figure 3b). Recovery time did not differ amongst concentrations of tricaine that induced a surgical plane of anesthesia (mixed effects ANOVA, $p>0.05$), however induction time decreased as tricaine concentration increased (mixed-effects ANOVA, $F_{3,12} = 16.746$, $p<0.001$, Tukey HSD). Under propofol induced anesthesia, the intersection of the induction and recovery curves occurred at 0.75 mg L^{-1}, estimating a minimized time to induction and recovery of 24 minutes (Figure 4b). Induction time decreased (mixed effects ANOVA, $F_{4,16} = 8.336$, $p<0.001$, Tukey HSD) and recovery time increased (mixed effects ANOVA, $F_{4,16} = 3.2958$, $p = 0.03767$, Tukey HSD) at the highest concentration of propofol tested. Induction was reached faster (t-test; t = 5.9448, df = 8, p = 0.0003) and recovery was shorter (t-test; t = 5.8033, df = 4.3856, p = 0.003) under tricaine induced anesthesia than under propofol induced anesthesia (Figures 6b and 7b).

Metabolic Rate

One shark did not reach surgical anesthesia under propofol at 1.5 mg L^{-1} and was removed from further statistical analyses. Under tricaine, there was no relationship between ventilation rate (breaths per minute) and time to induction or recovery from surgical anesthesia (min) in either species (linear regression; $R2 < 0.08$, $p > 0.05$). When propofol was used to induce surgical anesthesia, there was no relationship between induction for either species or for recovery in the Atlantic stingray (linear regression; $R2<0.2$, $p > 0.05$). A significant negative relationship (linear regression; $F_{1,3} = 15.22$, $R2 = 0.84$, $p= 0.029$) between ventilation rate and recovery time was observed in the coral catshark when anesthetized using propofol.

Mass

There were no significant relationships between mass and time to induction or recovery when using either tricaine (linear regression; $R2 < 0.09$, $p > 0.05$) or propofol (linear regression; $R2 = 0.3$, $p > 0.05$) in the coral catshark. In the Atlantic stingray, there were no significant relationships between mass and induction using propofol (linear regression; $R2 = 0.74$, $p > 0.05$) or mass and recovery when using either drug (linear regression; $R2 < 0.01$, $p > 0.05$). Time to induction significantly increased in the Atlantic stingray as mass increased when anesthesia was induced using tricaine (linear regression; $F_{1,3} = 35.39$, $R2 = 0.92$, $p = 0.0095$).

Pupil constriction

Propofol did not significantly affect the magnitude (%) of pupil constriction after 90 minutes of exposure for either species (mixed effects ANOVA, $p>0.05$, Figures 8a and 8b). Within the first 60 seconds of exposure to light, 50% total constriction was reached in both species under each drug-concentration combination and in control trials (Figure 9a-d).

In 100 mg L^{-1} tricaine trials, the magnitude of constriction was reduced in the coral catshark (mixed effects ANOVA, $p<0.01$, $F_{3, 15}= 6.47$, Tukey HSD, Figure 8a). Although the magnitude of constriction under propofol anesthesia was not different from control trials, constriction occurred faster under 1.5mg L^{-1} of propofol than the control (mixed effect ANOVA, $p<0.05$, $F_{3, 15}= 4.6935$, Tukey HSD, Figure 10a). Compared to the control, dilation was reduced in dark adapted coral catshark eyes when anesthetized using 1.5 mg L^{-1} propofol only (mixed effects ANOVA, $p<0.01$, $F_{3, 15}= 7.6337$, Tukey HSD, Figure 11).

In 100 and 150 mg L^{-1} tricaine trials, the magnitude of constriction was reduced in the Atlantic stingray (mixed effects ANOVA, $p<0.001$, $F_{3, 12} = 23.394$, Tukey HSD, Figure 8b). Pupils constricted the least in 100 mg L^{-1} trials. Although propofol had no effect on the magnitude of constriction, rate of constriction also occurred faster in rays anesthetized using 1.5 mg L^{-1} of propofol (mixed effects ANOVA, $p<0.05$, $F_{3, 12} = 9.671$, Tukey HSD, Figure 10b).

Table 4: Plane reached and time to induction to and recovery from surgical anesthesia when coral catsharks (*A. marmoratus*) and Atlantic stingray (*H. sabinus*) were anesthetized using tricaine or propofol. When using either drug, a higher concentration was needed to produce surgical anesthesia in the coral catshark than Atlantic stingray.

Species	Drug	Concentration (mg L^{-1})	Plane Reached (Mode)	Time to Induction (min)	Time to Recovery (min)
A. marmoratus	Tricaine	0	0	30	0
		25	0	30	0
		50	2	30	0
		100	4	28.8 ± 1.17	1.33 ± 1.33
		150	6	8.50 ± 1.80	9.33 ± 0.843
		200	6	6.33 ± 1.14	6.17 ± 0.477
		250	6	5.33 ± 0.421	12.0 ± 0.516
	Propofol	0	0	30	0
		0.5	4	30	0
		1.0	5	30	0
		1.5	6	17.8 ± 6.97	36.2 ± 7.64
		2.0	6	16.7 ± 5.24	44.2 ± 4.87
		2.5	6	12.0 ± 4.69	50.2 ± 6.86
		3.0	6	7.67 ± 1.37	42.2 ± 5.99
H. sabinus	Tricaine	0	0	30	0
		25	1	30	0
		50	4	30	0
		100	6	14.4 ± 2.39	6.80 ± 1.78
		150	6	7.20 ± 1.56	8.40 ± 1.60
		200	6	5.40 ± 0.758	9.20 ± 1.52
		250	6	3.60 ± 0.274	10.2 ± 1.24
	Propofol	0	0	30	0
		0.5	4	30	0
		1.0	6	19.0 ± 1.58	47.2 ± 7.30
		1.5	6	19.4 ± 1.98	57.6 ± 8.11
		2.0	6	15.0 ± 2.50	57.6 ± 9.36
		2.5	6	13.0 ± 2.73	68.6 ± 6.67
		3.0	6	11.80 ± 1.75	72.4 ± 6.02

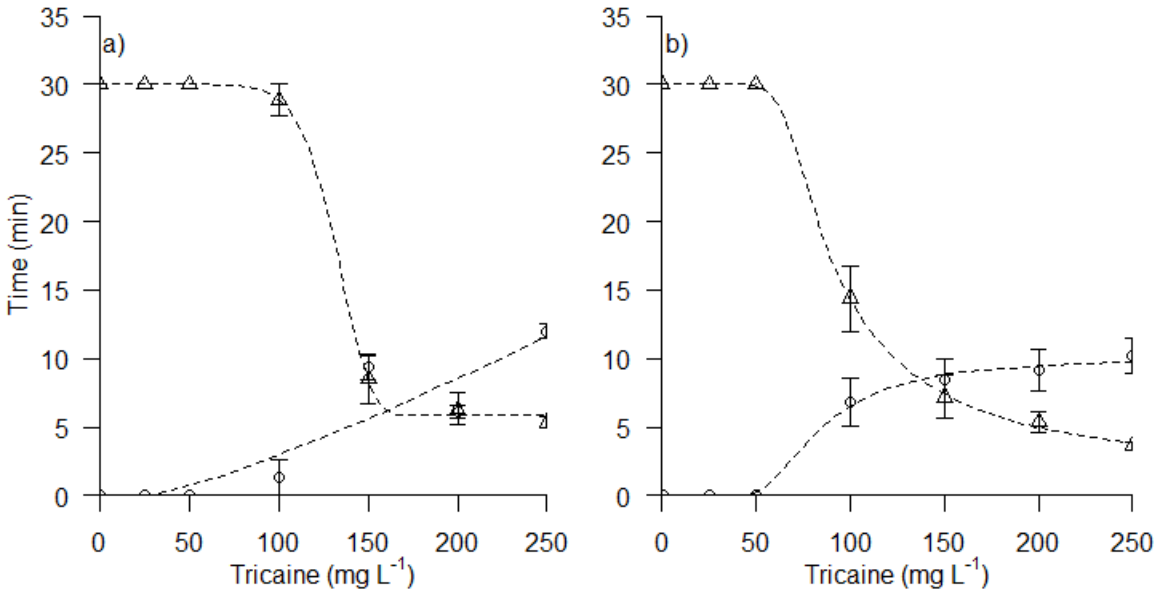

Figure 3: Concentration response curves of coral catsharks (n=6) (a) and Atlantic stingrays (n=5) (b) anesthetized using tricaine. Data points indicate the time to induction (triangle) and recovery from (circle) surgical anesthesia. As concentration increased, time to induction decreased and recovery increased in both species indicating tricaine affects both species in a concentration dependent manner. Intersection of induction and recovery curves occur at approximately 160 mg L^{-1} in the coral catshark and 140 mg L^{-1} in the Atlantic stingray. There were no differences in induction or recovery times between the coral catshark and the Atlantic stingray at concentrations immediately following curve intersections (200 and 150 mg L^{-1}, respectively). Data are presented as mean ± SE, and fit with log-logistic curves. One coral catshark reached surgical anesthesia during 100 mg L^{-1} tricaine trials.

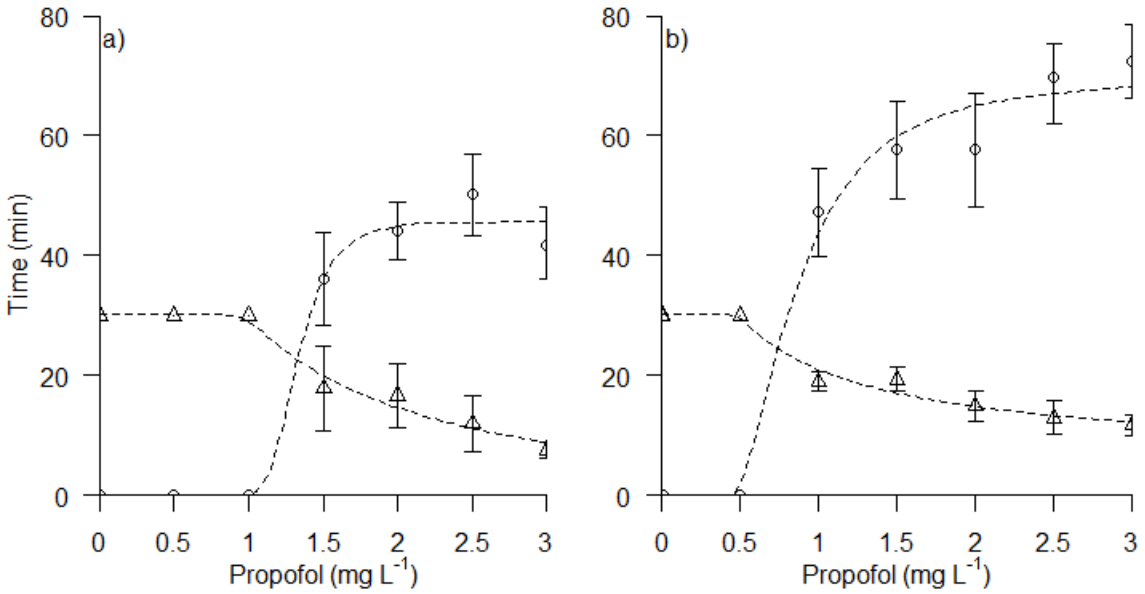

Figure 4: Concentration response curves of coral catsharks (n=6) (a) and Atlantic stingrays (n=5) (b) anesthetized using propofol. Data points indicate the time to induction (triangle) and recovery from (circle) surgical anesthesia. As concentration increased, time to induction decreased indicating propofol affects both species in a concentration dependent manner. Intersection of induction and recovery curves occur at approximately 1.4 mg L^{-1} in the coral catshark and 0.7 mg L^{-1} in the Atlantic stingray. There were no differences in induction or recovery times between the coral catshark and the Atlantic stingray at concentrations immediately following curve intersections (1.5 and 1 mg L^{-1}, respectively). Data are presented as mean ± SE, and fit with log-logistic curves.

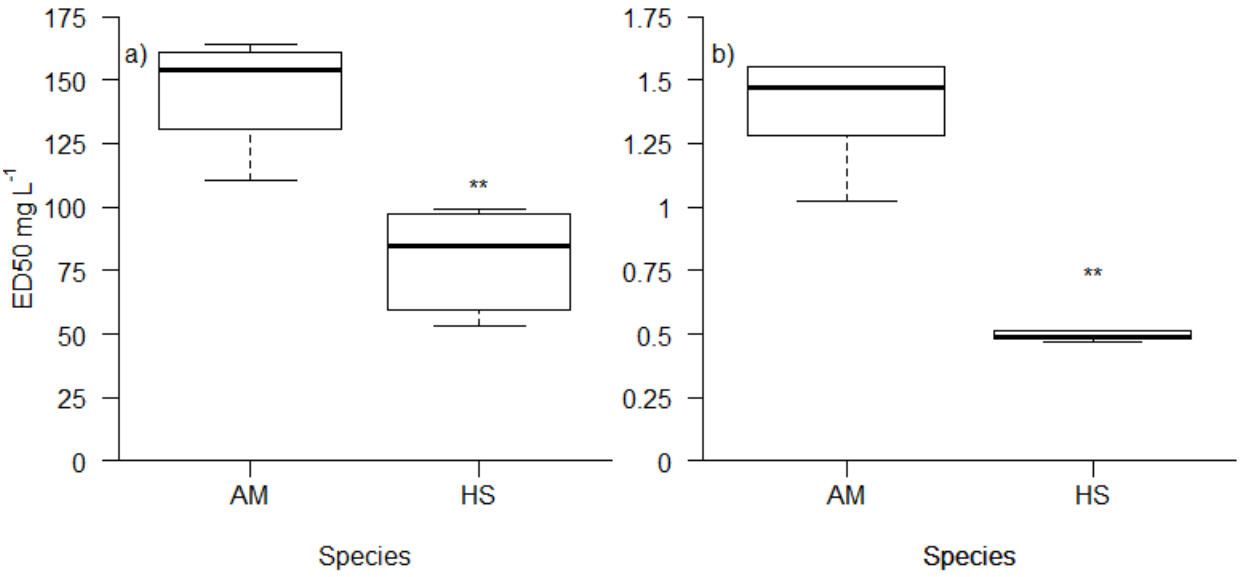

Figure 5: ED50 calculated from coral catshark and Atlantic stingray induction concentration-response curves using tricaine (a) and propofol (b) anesthetic. Under both drugs ED50 was higher in coral catsharks than ED50 of Atlantic stingray. Statistical differences are indicated using *.

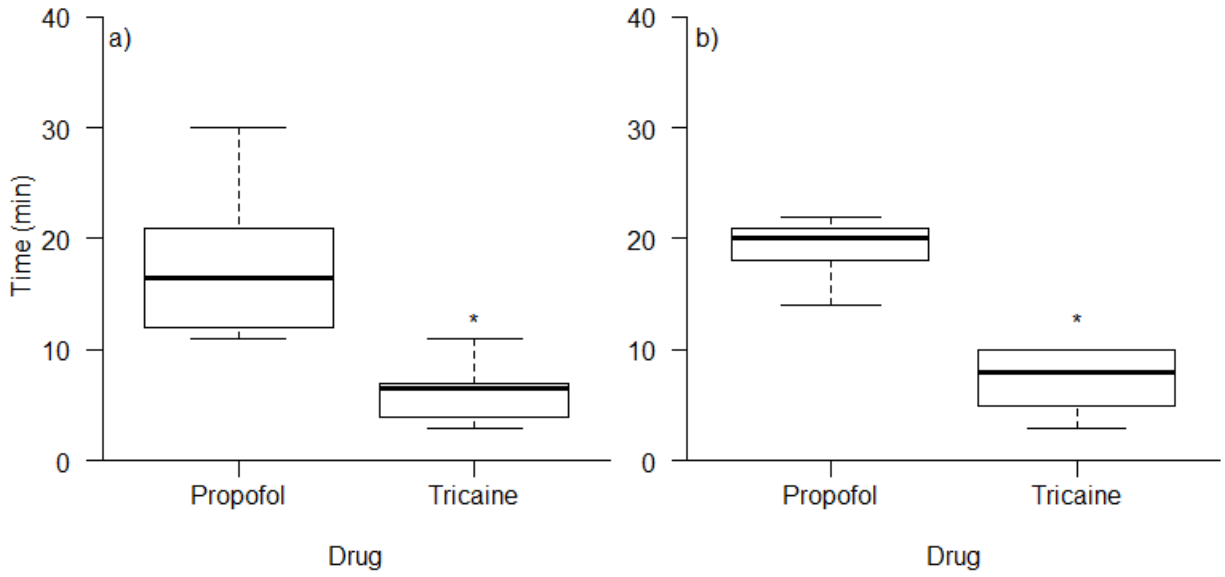

Figure 6: Time to induction in the coral catshark (a) and Atlantic stingray (b) at concentrations immediately following curve intersections using tricaine (200 and 150 mg L^{-1}, respectively) and propofol (1.5 and 1.0 mg L^{-1}, respectively) anesthetic. In either species, induction to a surgical plane of anesthesia took longer when propofol anesthesia was used. Statistical differences are indicated using *.

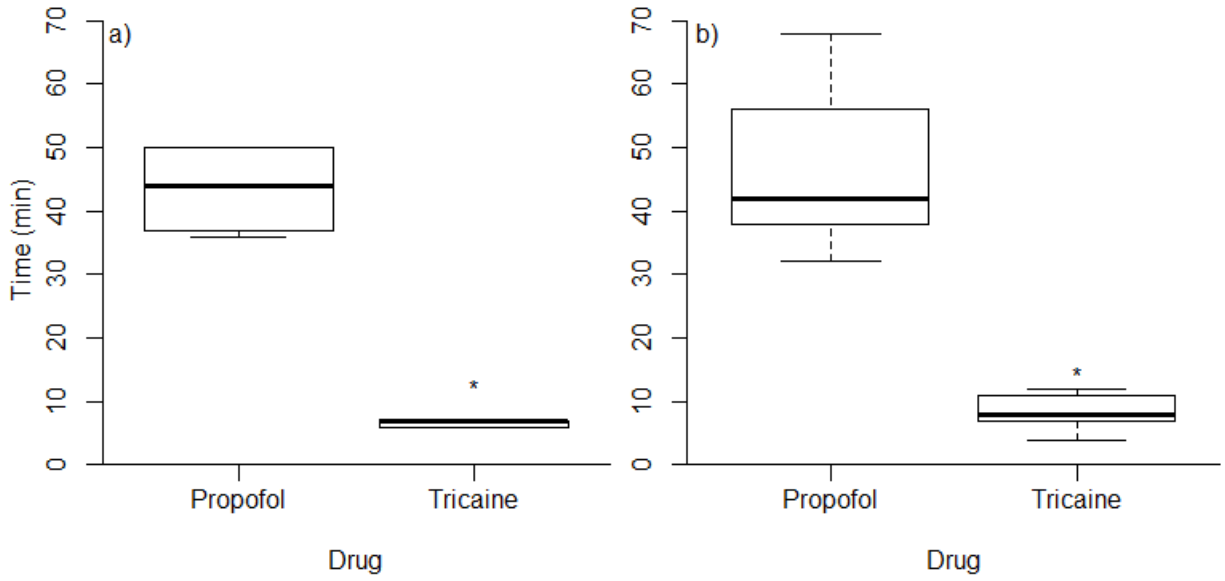

Figure 7: Time to recovery in the coral catshark (a) and Atlantic stingray (b) at concentrations immediately following curve intersections using tricaine (200 and 150 mg L^{-1}, respectively) and propofol (1.5 and 1.0 mg L^{-1}, respectively) anesthetic. In either species, recovery from a surgical plane of anesthesia took longer when propofol anesthesia was used. Statistical differences are indicated using *.

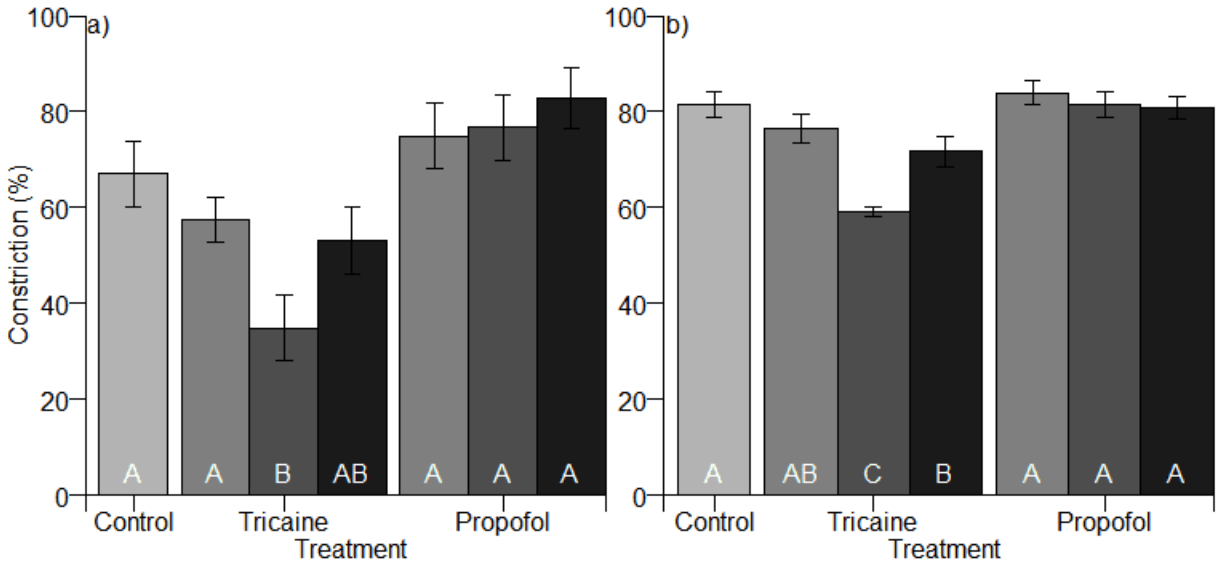

Figure 8: Percent constriction of the pupil diameter over 15 minutes of light exposure in the coral catshark (n=6) (a) and Atlantic stingray (n=5) (b) when using increasing concentrations of tricaine and propofol. In both species, pupil constriction (%) was not affected by propofol at any concentration. When using tricaine, however, constriction (%) was lower in both species when using 100 mg L^{-1} (dark grey) and at 150 mg L^{-1} (black) in the Atlantic stingray. Data are presented as mean ± SE, and ordered in trials of increasing concentration (control, grey; 50/0.5 mg L^{-1} tricaine/propofol, medium grey; 100/1.0 mg L^{-1} tricaine/propofol, dark grey; 150/1.5 mg L^{-1} tricaine/propofol, black). Bars not connected by the same letters are significantly different.

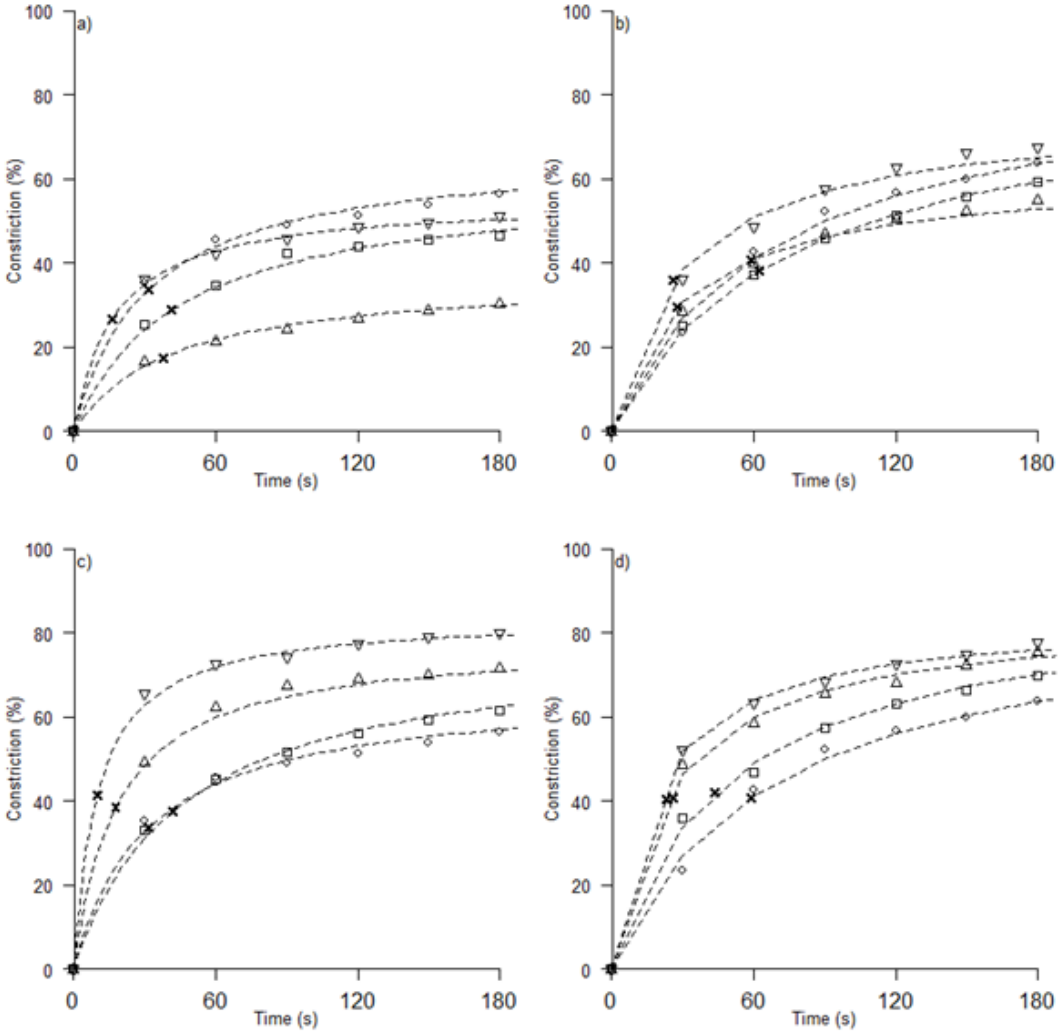

Figure 9: Constriction of the pupil in coral catsharks (n=6) and Atlantic stingrays (n=5) anesthetized using tricaine (a and b, respectively) and propofol (c and d, respectively). In both species, the pupil constricted to half maximum constriction (✖) within 60 seconds of light exposure at all concentrations of both tricaine and propofol. Data are presented as mean, and concentrations are represented by different shaped points (control, ○; 50/0.5 mg L^{-1} tricaine/propofol, □; 100/1.0 mg L^{-1} tricaine/propofol, △; 150/1.5 mg L^{-1} tricaine/propofol, △)

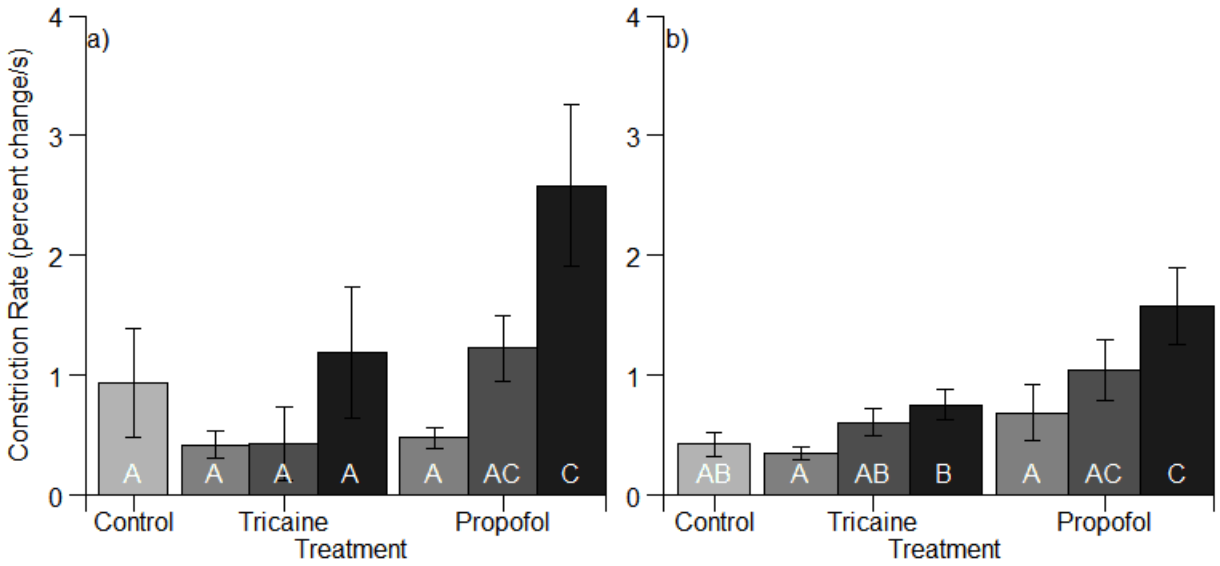

Figure 10: Constriction rate during pupil constriction after exposure to light in the coral catshark (n=6) (a) and Atlantic stingray (n=5) (b) when using increasing concentrations of tricaine and propofol. Rate of constriction was measured as the slope of dilation curves at half maximum constriction. In both species, rate of constriction was greater than no anesthetic trials when 1.5 mg L^{-1} (black) of propofol anesthetic was used. Data are presented as mean ± SE, and ordered in trials of increasing concentration (control, grey; 50/0.5 mg L^{-1} tricaine/propofol, medium grey; 100/1.0 mg L^{-1} tricaine/propofol, dark grey; 150/1.5 mg L^{-1} tricaine/propofol, black). Bars not connected by the same letters are significantly different.

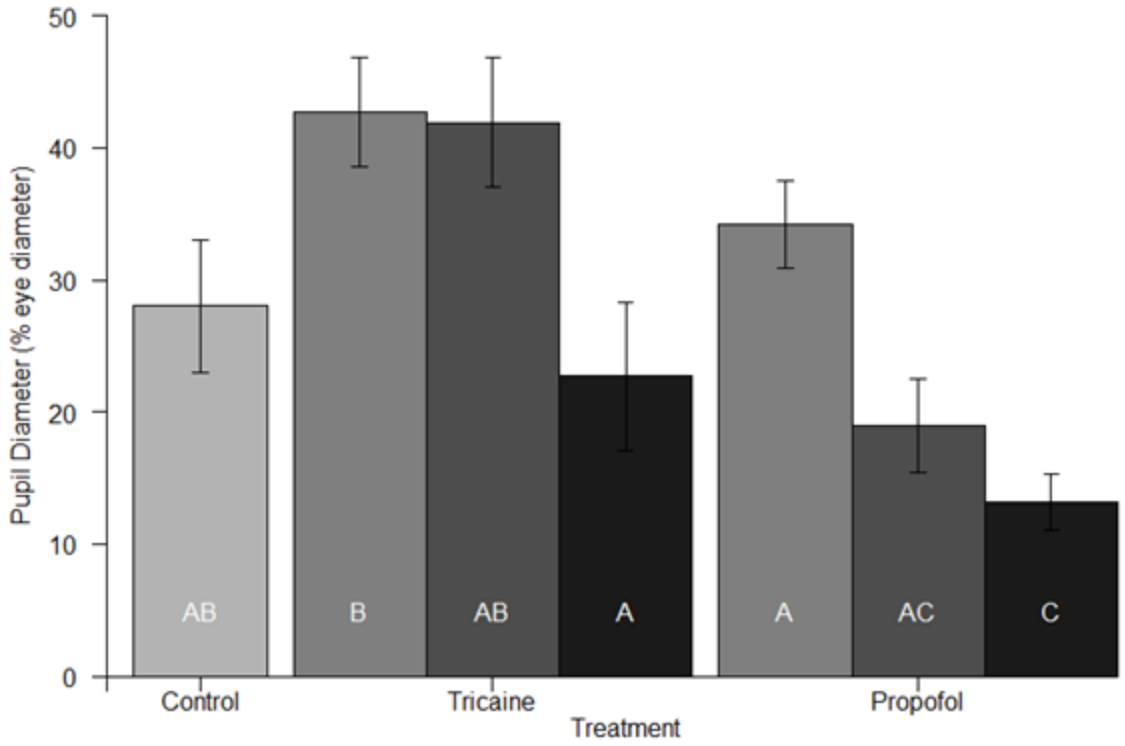

Figure 11: Starting pupil diameter expressed as percent of the eye diameter in coral catsharks (n=6) anesthetized using increasing concentrations of tricaine and propofol anesthetic. Dilation was reduced in sharks anesthetized using 1.5 mg L^{-1} of propofol. Data are presented as mean ± SE, and ordered in trials of increasing concentration (control, grey; 50/0.5 mg L^{-1} tricaine/propofol, medium grey; 100/1.0 mg L^{-1} tricaine/propofol, dark grey; 150/1.5 mg L^{-1} tricaine/propofol, black). Bars not connected by the same letters are significantly different.

CHAPTER 4

DISCUSSION

This study shows that propofol immersion safely induces surgical anesthesia in two species of elasmobranch, the coral catshark (*Atelomycterus marmoratus*) and the Atlantic stingray (*Hypanus sabinus*), as demonstrated by a reduction in ventilation rate and lack of response to stimuli/reflex tests during immersion. During this study physiological effects of immersion in tricaine were also investigated in these species to compare the physiological effects of tricaine immersion with those observed in propofol to determine if propofol may be a suitable replacement for tricaine anesthesia in elasmobranch fishes.

Concentration-response

Immersion in both tricaine and propofol safely induced a surgical plane of anesthesia in the coral catshark and Atlantic stingray. During induction, the escape response was generally the first response to cease, followed by the righting reflex, then tail pinch. During recovery these reflexes returned in reversed order. Concentration-response curves were "S" shaped (Figures 3 and 4), except for the coral catshark tricaine recovery curve which was more linear than the others (Figure 3a). The "S" shape indicates that a physiological maximum response to immersion was reached in both species. The curve shape seen in coral catshark recovery under tricaine may have resulted from a longer recovery time at 150 than 200 mg L^{-1}. A higher concentration of both drugs was required to reach surgical anesthesia in the coral catshark than was needed in the Atlantic stingray. This was also reflected in the ED50 values calculated from induction concentration-response curves. Concentrations that elicited minimized induction/recovery times under tricaine for both species (Figure 3a and Figure 3b) were similar to ideal concentrations reported for teleost species (Mylniczenko et al. 2014) and other species of elasmobranch (Stamper 2004; Mylniczenko et al. 2014). However, concentrations of propofol that induced minimized induction/recovery times in both species (Figure 4a and 4b) are lower than concentrations reported for use in some teleost species (Gressler et al. 2012; Oda et al. 2014; Balko et al 2017). This difference may be explained by the highly lipophilic nature of propofol (Short and Bufalari 1999) and the high proportion of lipids in the

elasmobranch liver (Stamper 2004). These differences may also stem from differences in metabolic rates seen across species, such as differences between active and benthic species of fish (Bushnell et al. 1989). When compared to the intravenous injection of propofol in the whitespotted bamboo shark (*Chiloscyllium plagiosum*; Miller et al. 2005) induction via immersion took longer, however recovery times were similar. Longer induction times during immersion are typical as the drug is being delivered slower compared to intravenous injection (Carter et al 2011). The similarities seen in recovery may indicate that, compared to recovery from tricaine anesthesia, recovery from propofol anesthesia is long and variable in elasmobranchs despite the method of administration.

Metabolic rate

The metabolic rate of an organism influences the length of the anesthetic plane because it affects the rate at which anesthetic molecules are moved from the central nervous system to sites where they are metabolized (Short and Bufalari 1999). Human patients with slower metabolic rates from hypothyroidism metabolize opiate-based anesthetics slower than other patients (Lamb 1947). Additionally, mice injected with tricaine recover faster than frogs injected with an equivalent dose due to the mouse's higher liver metabolic rate (Wayson et al. 1976).

Although the metabolic rate of the individuals used in this study were not measured, metabolic rate and its effect on induction/recovery can be inferred by measuring the relationship resting ventilation rate has with induction/recovery times. Metabolic rate is the rate at which the body consumes oxygen, and is therefore intimately related to ventilation rate (Frisk et al. 2012). The only significant relationship I found between resting ventilation rate and induction or recovery time in either species was a negative relationship in the coral catshark's recovery time under propofol. This suggests that metabolic rate may influence the time it takes the coral catshark to recover from surgical anesthesia induced by propofol. This was not the case in the Atlantic stingray, however. Differences seen between species, including the higher concentration of drug needed for the coral catshark, may be explained by mass-specific metabolic rate. While larger species need to consume more oxygen than smaller species; smaller species consume more oxygen per gram of tissue per unit time (Chabot et al. 2016). The average mass of the coral catsharks used

in this study was 156 ± 41.06 g, whereas the average mass of the Atlantic stingrays was 605 ± 48.22 g. The coral catshark is a smaller species of elasmobranch and should therefore possess a higher metabolic rate per gram of tissue. This means that the anesthetic may clear from the central nervous system faster than in the Atlantic stingray, resulting in higher concentrations of drug needed for the coral catshark to reach a similar anesthetic plane.

Mass

The importance of mass (body weight) to immersion anesthesia of teleosts is divisive. Several studies claim that there is no effect of mass on induction or recovery during immersion (Stehly and Gingrich 1999), whereas others claim one or both may be affected (Zahl et al. 2009). The only significant relationship between mass and induction or recovery by immersion in this study was a positive relationship between the Atlantic stingray and time to induction when tricaine was used. This relationship was not observed in the coral catshark, however, or in the Atlantic stingray when propofol was used to induce surgical anesthesia.

Pupil Constriction

The effect of tricaine and propofol on sensory responses was also measured to determine propofol's potential use in such experiments. In all drug-concentration and control trials the pupil constricted quickly within the first minute of light exposure, after which the constriction rate slowed until the trial ended, resulting in asymptotic curves (Figure 9a-d).

Propofol did not affect the magnitude of constriction seen in either the coral catshark or Atlantic stingray. Reduced constriction was only observed at 100 mg L^{-1} concentration of tricaine in the coral catshark and 100 and 150 mg L^{-1} concentrations of tricaine in the Atlantic stingray. Differences in percent constriction may be explained by the different targets of the respective drugs. During tricaine immersion, tricaine molecules prevent sodium ions from entering neurons affecting cell excitability, preventing sensory information from reaching the brain (Palmer and Mensinger 2004; Carter et al. 2011). This may affect the PLR by preventing or reducing excitation of photoreceptors, which would prevent signals from reaching ciliary ganglia to innervate the sphincter muscles of the iris. While the means of propofol

anesthesia are not completely understood, the loss of consciousness is thought to be produced during disruption of communication between areas of the brain brought about by increased efficacy of the inhibitory neurotransmitter GABA (Short and Bufalari 1999; Trapani et al. 2000; Hudetz et al. 2006; Hudetz 2012). Since propofol acts primarily through breaking down communication of synaptic pathways in the brain (Trapani et al. 2000; Hudetz 2012), sensory cells may be unaffected during anesthesia, although there may be effects on output from the brain to ciliary ganglia that may explain differences observed under 1.5 mg L^{-1} propofol trials in both species.

The constriction rate in both species was faster when 1.5 mg L^{-1} of propofol was used to induce anesthesia. Additionally, coral catshark pupils did not fully dilate as much as in control trials at this concentration of propofol, but did in lower concentrations. Therefore, propofol may be acting on different fibers of the pupillary light reflex – dilation is controlled by the sympathetic nerve fibers whereas constriction by the parasympathetic. However the increased rate of constriction at this concentration may also be a result of a reduction of physiological responses from stress. During the stress response, the pupil dilates to allow more light to reach the retina (Bradley et al. 2008). This dilation would be in conflict with constriction from the light response. If the dilation effects of stress are removed, then the pupil may be allowed to constrict faster than it does under lower concentrations where physiological responses of the stress response are still active.

Conclusions

In this study, propofol induced a surgical plane of anesthesia in both species, suggesting it can be used as an immersion anesthetic in elasmobranchs. However, as previously noted, the effects of anesthetics are highly species specific (Burka et al. 1997; Carter et al. 2011; Mylniczenko et al. 2014), therefore the effects of propofol immersion on other species should be investigated before its widespread use is accepted. Additionally, this study did not look at other physiological responses, such as heart rate, metabolic rate, or stress hormone concentrations that can give further insights on the effects of propofol immersion on elasmobranchs. Propofol also had no effect on the magnitude of pupil constriction in either species, however changes to the constriction rate in both species and the dilated pupil in the coral catshark

at 1.5 mg L^{-1} were observed. Therefore further studies investigating propofol's effects on the different fibers and sensory cells of the visual system should be conducted to determine its appropriateness for use in such experiments.

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