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Effects of water temperature and body weight on anaesthetic efficiency in marbled rabbitfish (*Siganus rivulatus*)

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Abstract

The effects of four anaesthetic agents, tricaine methanesulphonate (MS-222) (112.5 mg L^{-1}), 2-phenoxyethanol (400 μL L^{-1}), clove oil (70 mg L^{-1}) and benzocaine (65 mg L^{-1}) on juvenile marbled spinefoot (*Siganus rivulatus*) of three mean body weights (7.3 g, 19.1 g, 55.5 g) and at three temperatures (20, 25, 30°C) were evaluated. In addition, the relationship between body lipid content and efficacy of the four anaesthetic agents was evaluated in juvenile *S. rivulatus*. Times necessary for induction and recovery were recorded. Significant effects of temperature on induction and recovery times were observed. Induction and recovery times decreased with increasing water temperature. No uniform relationship between body weight of juvenile marbled spinefoot and anaesthetic efficacy was observed. Body fat content was positively correlated with induction time only when MS-222 was used but did not affect induction times of fish exposed to 2-phenoxyethanol, clove oil or benzocaine. Recovery times were generally longer for all fish containing more body fat. Results of the study show that anaesthetic efficiency increases with increasing water temperature but is not strongly affected by body weight for juvenile marbled spinefoot. In addition, body fat in fish affected the efficacy of the various anaesthetic agents tested in this study, generally slowing down recovery.

Keywords: *Siganus rivulatus*, rabbitfish, anaesthesia, MS-222, 2-phenoxyethanol, clove oil, benzocaine

Introduction

Anaesthetic agents are widely used in fisheries research and aquaculture to reduce physiological stress and/or injuries during various routine procedures such as capture, handling, transportation, tagging, grading, measurements, disease and parasite treatments and during more invasive procedures such as blood sampling, surgery, vaccination and spawning (Summerfelt & Smith 1990; Mylonas, Cardinaletti, Sigelaki & Polzonetti-Magni 2005; Ross & Ross 2008; Zahl, Samuelsen & Kiessling 2012; Ghanawi, Monzer & Saoud 2013). Among a wide selection of anaesthetic agents, the most commonly used are tricaine methanesulphonate (MS-222), benzocaine, 2-phenoxyethanol, clove oil, metomidate and quinaldine (see Ross & Ross 2008; Sneddon 2012; Zahl *et al.* 2012). There are several criteria used to select an optimal anaesthetic for aquatic species including availability and ease of use, cost, efficacy in reducing stress and possible toxicity to fish, humans and the environment (Summerfelt & Smith 1990; Burka, Hammell, Horsberg, Johnson, Rainnie & Speare 1997; Mylonas *et al.* 2005; Ross & Ross 2008; Zahl *et al.* 2012). A good anaesthetic agent should induce anaesthesia within 3 min or less, allow recovery within 5 min or less and allow for good safety margins (Marking & Meyer 1985). Presumably, various environmental factors (e.g. salinity, pH, oxygen levels, water temperature, hardness) and biological factors (e.g. body condition and weight, developmental stage, physiological status, lipid content) can affect the efficacy of an anaesthetic agent on fish (Oikawa,

Takeda & Itazawa 1994; Burka *et al.* 1997; Ross & Ross 2008; Zahl, Kiessling, Samuelsen & Hansen 2011; Sneddon 2012; Zahl *et al.* 2012), but these factors vary among fish (Sneddon 2012).

Tricaine methanesulphonate (TMS) (or also known as MS-222, ethyl 3-aminobenzoate methanesulphonic acid, tricaine mesilate, Tricaine-S™) and benzocaine (ethyl-4-aminobenzoate) are routinely used as topical analgesics (Sneddon 2012). Benzocaine is similar in pharmacology to MS- 222; however, it is much less acidic and water soluble (Burka *et al.* 1997; Ross & Ross 2008). Both anaesthetics inhibit the initiation and propagation of action potentials by blocking voltage-sensitive sodium channels and thus prevent the voltage-dependent increase in sodium conductance inhibiting the initiation and propagation of action potentials in excitable cells (reviewed by Zahl *et al.* 2012). However, the precise mode of action is not well understood (Sneddon 2012; Zahl *et al.* 2012). Both anaesthetic agents are relatively safe to handle. Benzocaine and TMS are approved for use in aquaculture (Zahl *et al.* 2012). However, MS-222 is expensive and a 21-day withdrawal period following anaesthesia treatment is necessary before the fish can be safely consumed or released into natural habitats (Pirhonen & Schreck 2003; Yamamoto, Woody, Shoji & Ueda 2008).

A moderately water-soluble oily liquid 2-Phenoxyethanol (2-PE) (1-hydroxy-2-phenoxyethane) is used as a topical anaesthetic, particularly for fish transportation (Hseu, Yeh, Chu & Ting 1996, 1997, 1998; Hamackova, Lepicova, Kozak, Stupka, Kouril & Lepic 2004; Neiffer & Stamper 2009).

Mechanism of action in fish is not clear; there is evidence that it inhibits the activity of excitatory N-methyl-D-aspartate (NMDA) receptors (Musshoff, Madeja, Binding, Witting & Speckmann 1999) and thus higher regions of the nervous system. The inhibition of NMDA receptors has been associated with an analgesic effect (Grasshoff, Drexler, Rudolph & Antkowiak 2006; Zahl *et al.* 2012). Suitability of 2-phenoxyethanol as an anaesthetic agent for many fish species lies in its easy preparation, low cost, rapid induction and rapid uneventful recovery and bactericidal and fungicidal properties (see Hseu *et al.*1998; Tsantilas, Galatos, Athanassopoulou, Prassinos & Kousoulaki 2006). However, 2-phenoxyethanol may pose potential hazard to the handler (Hseu *et al.* 1998; Tsantilas *et al.* 2006).

Clove oil is a natural substance derived from leaves, buds and stems of the clove tree (*Eugenia caryophyllata*) containing 70–90% (by weight) eugenol (2-methoxy-4-(2-propenyl) phenol) (Soto & Burhanuddin 1995). Clove oil and eugenol are incompletely water soluble (Neiffer & Stamper 2009). Clove oil is widely used for a number of beneficial properties as an additive for the food industry, antioxidant, antifungal and antibacterial agent and as a potent analgesic in dentistry (Anderson, McKinley & Colavecchia 1997; Keene, Noakes, Moccia & Soto 1998; Ross & Ross 2008). Eugenol impedes sodium, potassium and calcium channels, inhibits N-Methyl D-aspartate (NMDA) receptors and potentiates $GABA_A$ receptors (see Sneddon 2012; Zahl *et al.* 2012). Interest in clove oil has increased because of its low price, availability and safety while handling (Anderson *et al.* 1997; Sladky, Swanson, Stoskopf, Loomis & Lewbart 2001).

Ambient water temperature regulates the rate of physiological processes and basal metabolism in aquatic animals (Brett 1979; Jobling 1996; Clarke & Johnston 1999) as well as processes involved in the uptake and elimination of anaesthetics (see Zahl *et al.* 2012). Typically, a 10°C rise in water temperature (Q10) doubles the basal metabolic rate of teleost fish resulting in increased respiration and oxygen demand, increased cardiac output and increased blood flow through the gills (Graham & Farrell 1989; Nilsson & Sundin 1998; Webber, Boutilier & Kerr 1998; Clarke & Johnston 1999). A number of authors report reduced induction and recovery times with increase in temperature in various fish species during anaesthesia (Hoskonen & Pirhonen 2004; Mylonas *et al.* 2005; Zahl *et al.* 2011, 2012). The rate of physiological processes and basal metabolism in all aquatic organisms is also dependent on body size (Clarke & Johnston 1999). However, studies regarding the relationship between uptake and elimination of anaesthetics and body size show a less consistent relationship (Gilderhus & Marking 1987; Hoskonen & Pirhonen 2004; Tsantilas *et al.* 2006; Weber, Peleteiro, García Martín & Aldegunde 2009; Zahl, Kiessling, Samuelsen & Hansen 2009; Zahl *et al.* 2012). Variation among studies could be caused by factors such as species, strain of fish, body composition, age, sexual maturity, growth rate and gill surface to body weight ratio (see Zahl *et al.* 2012).

Siganids (rabbitfish) are a small family of economically important herbivorous fish widely

distributed in the Indo-West Pacific Region (Woodland 1983). Marbled spinefoot is a commercially important algaevorous, euryhaline and eurythermal marine teleost (Saoud, Kreydiyyeh, Chalfoun & Fakih 2007; Saoud, Mohanna & Ghanawi 2008). The opening of the Suez Canal in 1869 linked the Red Sea to the Mediterranean and resulted in the invasion of the eastern Mediterranean Sea by various siganid species including marbled spinefoot (*Siganus rivulatus*) (Forsskål 1775) (Galil 2000; Quignard & Tomasini 2000). Marbled spinefoot was first recorded in the Levant basin in 1927 (Tortonese 1970) and has since established large populations in its new environment. It is considered among the most successful of Lessepsian fish (Ben-Tuvia 1985; Papaconstantinou 1990).

The aim of this study was to assess the effect of temperature and fish size on induction and recovery time of marbled spinefoot subjected to various anaesthetics. Moreover, as some anaesthetics are lipid soluble and work through diffusion into adipose tissue and fat cells of the fish, we investigated if the amount of lipid in a fish affects anaesthetic efficiency.

Materials and methods

Fish acquisition and holding

Juvenile marbled spinefoot (*S. rivulatus*) were caught in traps off the Beirut beach and transported live to the aquatic research laboratory at the American University of Beirut (AUB) and maintained for 4 months in an outdoor recirculating system consisting of six 1 m^3 circular tanks. For the trials, fish ($N = 200$) were transferred to an indoor system consisting of four identical recirculating systems, each composed of four 180 L square fibreglass tanks (60 × 60 × 50 cm; L × W × H), connected to a biological filter and settling tank. Aeration was provided by a regenerative blower and submerged diffusers. During acclimation, the fish were offered a 35% protein commercial diet (Rangen EXTR 350; Rangen, Buhl, ID, USA) *ad libitum* twice daily. The fish were fasted for 24 h prior to start of all experiments.

Anaesthetic agents

The anaesthetic agents tested were as follows: tricaine methanesulphonate (MS-222) (Tricaine-S™, Western Chemical, Ferndale, WA, USA), 2-phenoxyethanol (Sigma-Aldrich, St. Louis, MO, USA), clove oil (82–87% eugenol, Carolina Biological Supply, Burlington, NC, USA), and benzocaine (Sigma-Aldrich). The concentrations used for the study were as follows: 112.5 mg L^{-1}, 400 μL L^{-1}, 70 mg L^{-1}, 65 mg L^{-1} for MS-222, 2-phenoxyethanol, clove oil and benzocaine respectively. All concentrations were chosen based on results reported by Ghanawi *et al.* (2013). All stock solutions were prepared immediately before the start of each experiment.

Effect of temperature and size on anaesthetic efficacy

Juvenile marbled spinefoot were sorted by size into three groups: (1) Large (L) 55.5 ± 2.7 g; (mean ± SE); (2) Medium (M) (19.1 ± 0.5 g); (3) Small (S) (7.3 ± 0.4 g). One third of the fish in each size class were acclimated and maintained at one of three water temperatures (20.3 ± 0.21°C (mean ± SD), 25.1 ± 0.37°C, and 29.7 ± 0.16°C) for 2 weeks. Water quality parameters during the experiment were assessed daily and maintained as follows: pH: 8.06 ± 0.07 (mean ± SD), salinity: 35.4 ± 0.17 g L^{-1}, dissolved oxygen: 6.4 ±0.60 mg L^{-1}; ammonia-N: 0.12 ± 0.1 mg L^{-1}, nitrite-N: 0.06 ± 0.02 mg L^{-1}.

To assess anaesthetic effects at various temperatures and fish sizes, fish from each size class and temperature were individually transferred from a stock tank to a 15 L glass container containing 10 L of aerated anaesthetic solution. The water in the glass container was taken from the sump tank of the fish culture system and maintained at the proper temperature using submersible water heaters. Induction time I [time required to reach stage I anaesthesia; partial loss of equilibrium, little body movements, reduced reaction to external stimuli (visual and tactile)], induction time II (time to reach stage II anaesthesia; total loss of equilibrium, no body movements, no reaction to external stimuli, low frequency of opercular movements) and recovery time (recovery of equilibrium, increase in body movements, increase in opercular movements, response to external stimuli) for individual fish were determined using an electronic stopwatch (method modified from Schoettger & Julin 1969; Weber *et al.* 2009). An external stimulus means to lightly prod the anaesthetized fish with a long glass pipette. Individual weight (g)

and length (cm) of each fish were recorded right after induction time II, immediately prior to transfer to a recovery tank containing 10 L of clean, filtered sea water at the test temperature. After anaesthetic assessments, fish from each treatment were placed in a large tank and monitored for 24 h before feeding was resumed.

Effect of body lipid on anaesthetic efficacy

Two iso-nitrogenous diets (one containing 2% lipid and the other 7% lipid) were manufactured at the aquatic research laboratory at AUB. Dry ingredients were mixed thoroughly then boiling water was added, mixed for another 5 min and the mesh extruded in a meat grinder with a 3 mm die. The diets were dried in a forced air oven at 40°C to moisture content of 8% and stored at −20°C until used.

Juvenile marbled spinefoot [23.9 ± 0.8 g (mean ± SE)] were stocked into eight tanks connected to biological filters. Four tanks were offered the low lipid diet (2% lipid; 38.7% protein; 337.2 kJ kg^{-1}) while the other four were offered the high lipid diet (7% lipid; 38.7% protein; 415.6 kJ kg^{-1}) for 2 weeks then both groups were offered the commercial diet for 5 days. Water quality parameters were maintained at: temperature: 26.9 ± 0.2°C (mean ± SD), salinity: 35.5 ± 0.4 g L^{-1}, pH: 8.0 ± 0.2. The photoperiod was 12 h light: 12 h dark.

To test for effect of body lipid on anaesthetic efficacy, 20 fish from each lipid group were individually anaesthetized in baths of MS-222 (112.5 mg L^{-1}), 2-phenoxyethanol (400 μL L^{-1}), clove oil (70 mg L^{-1}), benzocaine (65 mg L^{-1}) and time to reach stage I and II induction and recovery time were recorded. Six fish from each group were euthanized and their total body lipids determined using a reflux extractor (Ankom^{xt10} Extractor; Ankom Technology Corporation, Macedon, NY, USA) and a mixture of 20% diethyl ether, 80% petroleum ether, as solvent.

Statistical analysis

All statistical analyses were performed using SPSS statistical software (V.18 for Windows, SPSS, Chicago, IL, USA). Differences among means were analysed using one-way ANOVA and Student Newman–Keuls (SNK) multiple-range test. In experiment 1, data were set in a 2 × 2 factorial model and effects of temperature, size and temperature-size interactions compared. In experiment 2, induction times and recovery time for fish from the two treatments were compared for each anaesthetic used. Differences were considered significant when $P < 0.05$.

Results

Effect of temperature and body weight on anaesthetic efficacy

Overall, induction times decreased significantly with increase in temperature in any of the four tested anaesthetic agents (Tables 1 and 2). Induction times for clove oil and 2-phenoxyethanol were relatively shorter as temperature increases compared to induction times for MS-222 and benzocaine. Recovery times showed similar patterns for all four anaesthetics; as temperature increased recovery time decreased. Recovery times for MS-222 and 2-phenoxyethanol were shorter as temperature increased as compared to longer recovery times for clove oil and benzocaine. All data from Table 1 were pooled to analyse the effects of body weight on anaesthetic efficacy and possible body weight and temperature interactions (Table 2). Body weight had no significant effects on induction times of MS-222, clove oil and benzocaine. Body weight had an effect on induction times of 2-phenoxyethanol. Recovery times for MS-222 and clove oil were not affected by body weight whereas recovery times for 2-phenoxyethanol and benzocaine were affected. No obvious interaction between body weight and temperature was noted.

Effect of body lipid on anaesthetic efficacy

Body fat content of the fish offered the diet with 7% lipids was 35.8 ± 2.73% (mean ± SE), significantly greater than body fat content (27.6 ± 1.90%) of fish offered the 2% lipid diet. Induction times of fatty fish exposed to 2-phenoxyethanol, clove oil and benzocaine did not differ from induction times of less fatty fish exposed to the same anaesthetics (Table 3). However, body fat did affect induction time of fish exposed to MS-222, where an increase in fish fattiness speeded up induction of anaesthesia. An increase in body fat prolonged recovery time for all anaesthetics used.

Table 1 *P*-values for the effect of temperature, body weight and their interaction on anaesthetic efficacy in marbled spinefoot (*Siganus rivulatus*)

Anaesthetic agent	Time to stage I (min)	Time to stage II (min)	Recovery time (min)
MS-222 (112.5 mg L^{-1})			
Temperature	0.0068	0.0061	<.0001
Body weight	0.5759	0.4202	0.4677
Temperature*body weight	0.0731	0.5475	0.6265
2-Phenoxyethanol (400 μL L^{-1})			
Temperature	<.0001	<.0001	<.0001
Body weight	0.0059	0.0389	<.0001
Temperature*body weight	0.8928	0.4454	0.2513
Clove Oil (70 mg L^{-1})			
Temperature	<.0001	<.0001	<.0001
Body weight	0.7872	0.7387	0.1145
Temperature*body weight	0.8072	0.5870	0.4752
Benzocaine (65 mg L^{-1})			
Temperature	<.0001	<.0001	0.0082
Body weight	0.4488	0.8325	0.0002
Temperature*body weight	0.0619	0.3032	0.1834

Table 2 Time needed to reach stages I and II and recovery for three sizes [large (L), medium (M), small (S)] of marbled spinefoot (*Siganus rivulatus*) anaesthetized with four anaesthetic agents at three temperatures (20, 25, 30°C). Means in the same column with different letters are significantly different from each other ($P < 0.05$)

Anaesthetic agent	Temperature (°C)/body size	Time to Stage I (min)			Time to Stage II (min)			Recovery time (min)		
		L	M	S	L	M	S	L	M	S
MS-222 (112.5 mg L^{-1})	20	0.75a	0.63a	0.57a	2.19a	2.05a	1.88a	2.38a	2.01a	2.41a
	25	0.45b	0.55a	0.54a	1.51a	1.65a	1.57ab	1.43b	1.33b	1.41b
	30	0.33b	0.52a	0.49a	1.75a	1.64a	1.42b	1.35b	1.24b	1.34b
	PSE	0.04	0.04	0.04	0.19	0.11	0.10	0.18	0.07	0.12
2-Phenoxyethanol (400 μL L^{-1})	20	0.48a	0.56a	0.74a	2.54a	1.95a	2.49a	3.93a	3.46a	3.11a
	25	0.38b	0.42b	0.48b	1.44b	1.29b	1.39b	2.72b	2.62b	2.46b
	30	0.28c	0.30c	0.33c	1.02c	0.90c	0.98c	2.03c	1.52c	1.15c
	PSE	0.02	0.03	0.03	0.12	0.12	0.11	0.20	0.19	0.14
Clove Oil (70 mg L^{-1})	20	0.47a	0.49a	0.47a	1.60a	1.70a	1.43a	5.83a	5.31a	5.44a
	25	0.33b	0.34b	0.36b	1.03b	1.07b	1.11b	4.19b	4.28b	4.22b
	30	0.26b	0.28b	0.29b	0.78b	0.81b	0.86c	4.09b	3.37c	3.68c
	PSE	0.03	0.02	0.02	0.13	0.09	0.07	0.24	0.19	0.14
Benzocaine (65 mg L^{-1})	20	0.70a	0.79a	0.69a	2.07a	2.53a	2.40a	3.91a	4.22a	3.81a
	25	0.55b	0.48b	0.49b	1.99a	1.94b	1.87b	3.89a	3.74a	3.03b
	30	0.34c	0.42b	0.44b	1.79a	1.42c	1.54b	3.98a	4.02a	3.26b
	PSE	0.03	0.03	0.04	0.17	0.12	0.14	0.20	0.20	0.15

PSE, pooled standard error.

Discussion

The efficacy of the MS-222, 2-phenoxyethanol, clove oil and benzocaine was tested in a previous study (Ghanawi *et al.* 2013) and the lowest effective doses that produced induction and recovery times in 3 min or less and 5 min or less, respectively, were utilized in this study. Although all four anaesthetic agents were found suitable in anaesthetizing juvenile marbled spinefoot, MS-222, 2-phenoxyethanol, and clove oil were the most effective anaesthetics agents (Ghanawi *et al.*

Table 3 Time needed to reach induction stages I and II and recovery for marbled spinefoot (*Siganus rivulatus*) containing high body lipids (H; 35.8% ± 2.73 (mean ± SE)) or low body lipids (L; 27.6% ± 1.90) and anaesthetized with one of four anaesthetizing agents

Anaesthetic agent	Lipid content	Wt (g) (mean ± SE)	Time to stage I (min)	Time to stage II (min)	Recovery time (min)
MS-222 (112.5 mg L^{-1})	L	24.0 ± 1.75	0.59 ± 0.05	1.85 ± 0.15	1.47 ± 0.04
	H	27.5 ± 1.82	0.38 ± 0.04	1.40 ± 0.13	1.64 ± 0.06
	P-value	0.1889	0.0030	0.0403	0.0189
2-Phenoxyethanol (400 μL L^{-1})	L	24.3 ± 1.75	0.43 ± 0.05	1.29 ± 0.15	2.09 ± 0.06
	H	27.5 ± 1.89	0.42 ± 0.04	1.23 ± 0.11	2.30 ± 0.13
	P-value	0.2270	0.8360	0.7670	0.1439
Clove oil (70 mg L^{-1})	L	24.6 ± 1.44	0.36 ± 0.03	1.04 ± 0.04	3.77 ± 0.09
	H	25.8 ± 2.14	0.36 ± 0.02	0.96 ± 0.02	4.18 ± 0.15
	P-value	0.6605	0.9191	0.1346	0.0313
Benzocaine (65 mg L^{-1})	L	27.1 ± 1.75	0.45 ± 0.02	1.39 ± 0.06	3.28 ± 0.16
	H	25.5 ± 1.92	0.53 ± 0.03	1.46 ± 0.08	3.84 ± 0.15
	P-value	0.5356	0.0400	0.5230	0.0201

2013). This study expanded on the previous study and tested for the effects of temperature and body size on the efficacy of the four aforementioned anaesthetic agents.

Water temperature seemed to affect anaesthetic efficiency, but body weight generally did not have a strong effect on anaesthesia of juvenile marbled spinefoot. Variation in fish response to anaesthetic agents is affected by various factors such as species, age, size, gill surface area to body weight ratio, body lipid content, gender, sexual maturity, physical condition, health state and stocking density, as well as water quality parameters (e.g. temperature, pH, salinity and oxygen and mineral content of the water) (see Hoskonen & Pirhonen 2004; Tsantilas *et al.* 2006 and references therein; Zahl *et al.* 2012). Zahl *et al.* (2009, 2011, 2012) suggest that physiological differences within and between species may result in altered pharmacokinetic and pharmacodynamic properties and thus in different responses to anaesthetic agents. This study was performed to assess anaesthetic efficacy on sizes of marbled rabbitfish that would be used for research or transportation and at temperatures that would possibly be encountered in normal situations when working with the fish.

Water temperature

Water temperature had a much more obvious effect on anaesthetic efficacy than body size of juvenile marbled spinefoot. Both induction time and recovery time decreased with increase in water temperature for all anaesthetics used. Similar results were observed in juvenile European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) exposed to 2-phenoxyethanol and clove oil (Mylonas *et al.* 2005), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), whitefish (*Coregonus lavaretus*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) exposed to clove oil (Hoskonen & Pirhonen 2004), and Atlantic cod (*Gadus morhua*) exposed to benzocaine, MS-222, metomidate and 2-phenoxyethanol (Zahl *et al.* 2009). This reduction in time of induction of anaesthesia and recovery as temperature increases is probably a result of increased metabolism caused by the increase in temperature. Increasing metabolic rate accelerates respiration, and increases cardiac output and blood flow through the gills. Thus, physiological processes involved in the absorption, distribution, elimination and clearance of anaesthetics are accelerated (Clarke & Johnston 1999; Hoskonen & Pirhonen 2004; Mylonas *et al.* 2005; Zahl *et al.* 2009, 2012), causing an increase in the effects of waterborne anaesthetics. However, differences in induction and recovery times among fish exposed to the various anaesthetics at similar temperatures probably result from the different pharmacokinetic and pharmacodynamic properties of the anaesthetic agents.

Body weight

The mass-specific basal metabolic rate – oxygen consumption divided by body mass – decreases with increase in body mass of an animal and thus larger animals have lower oxygen consumption relative to body size than do smaller animals (Oikawa *et al.* 1994; Clarke & Johnston 1999).

Because rate of oxygen consumption, ratio of body volume to gill surface area and rate of gill perfusion are important factors in uptake and elimination of anaesthetic agents (Zahl *et al.* 2012), and gill surface area decreases in relation to increased body weight (Oikawa & Itazawa 1985; Zahl *et al.* 2012), we would expect fish size to have an effect on anaesthetic efficiency. This only seems to be the case when 2-phenoxyethanol is used as an anaesthetic.

Reports about effects of fish body weight on anaesthesia vary. Some authors report little or no relationship between body size and induction and recovery times in bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), lake trout (*Salvelinus namaycush*), rainbow trout (*Oncorhynchus mykiss*), walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*) exposed to AQUI-S™ (Stehly & Gingerich 1999), and long-finned eel (*Anguilla reinhardtii*) exposed to clove oil (Walsh & Pease 2002). In addition, no effects of size on induction time and no recovery time dependency were observed in goldfish (*Carassius auratus*) anaesthetized with 2-phenoxyethanol (Weyl, Kaiser & Hecht 1996). On the other hand, weight dependency of induction and recovery times was reported in white sea bream (*Diplodus sargus* L.) exposed to 2-phenoxyethanol (Tsantilas *et al.* 2006), and Weber *et al.* (2009) reported longer induction times with increase in body weight in Senegalese sole (*Solea senegalensis*) exposed to 2-phenoxyethanol, metomidate and clove oil but not when exposed to MS-222. Woody, Nelson and Ramstad (2002) found that induction time was related to size of migrating sockeye salmon (*Oncorhynchus nerka*) but not recovery time when anaesthetized with clove oil. Finally, Zahl *et al.* (2009) found no uniform relationship between body weight and induction and recovery times for Atlantic cod (*Gadus morhua*) exposed to benzocaine, MS-222, metomidate and 2-phenoxyethanol. The authors reported longer induction and recovery times with increase in body weight for fish exposed to benzocaine and MS-222, longer recovery times with increase in body weight of fish exposed to metomidate, and no weight-related differences in induction time and recovery times were associated with 2-phenoxyethanol (Zahl *et al.* 2009). Consequently, effects of anaesthetics on various weights of fish have to be determined for every species, as no direct relationship seems to exist for all fish.

Body lipid

The rate of passage of anaesthetic agents across biological membranes is largely influenced by lipid solubility, water solubility, degree of ionization, chemical stability and molecular weight (see Hunn & Allen 1974). Lipid solubility and ionization also influence the distribution of the anaesthetic agent within an organism. Highly lipophilic anaesthetic agents adhere on and penetrate through gill epithelia. Once in blood circulation, they are absorbed by body tissues such as fat and brain (Summerfelt & Smith 1990; Stoskopf 1993; Mylonas *et al.* 2005; Zahl *et al.* 2012). Accordingly, it is expected that more lipid-soluble anaesthetics will cause faster induction times and slower elimination from the body, thus causing prolonged recovery (see Zahl *et al.* 2012). Yet, the pharmacodynamic characteristics of the anaesthetic agents might negate this explanation. MS-222 is very hydrophilic and thus strongly repelled by lipids yet it is the only anaesthetic among those tested whose efficacy was affected by body lipid content. Possibly, being hydrophilic might have caused it to spread rapidly in the blood of the fish causing faster induction. A more detailed description of such a mechanism is given by Kiessling, Johansson, Zahl and Samuelsen (2009) and Zahl *et al.* (2012).

Increased body fat content may also influence cardiac performance and respiratory rate. MS-222 is absorbed and eliminated *via* gill membranes (reviewed by Popovic, Strunjak-Perovic, Coz-Rakovac, Barisic, Jadan, Persin Berakovic & Klobucar 2012). Greater respiratory rates may cause faster induction of anaesthesia. As the efficacy of anaesthetics is directly related to lipid solubility, fish with greater lipid content in nerves may experience faster nerve penetration and blockage of sodium channels and thus inhibit nerve conduction (Palmer & Mensinger 2004). MS-222 metabolism is hepatic, and utilizes cytochrome P-450 enzymes, yet Popovic *et al.* (2012) noted conflicting results in literature on the effects of MS-222 on spectral measurements of hepatic microsomal cytochrome P-450 and consequent implications on P-450 activity. Further studies on the pharmacokinetics and elimination kinetics are needed to understand the underlying mechanisms of the various anaesthetics in rabbitfish.

In conclusion, the anaesthetic efficiency of the four tested anaesthetic agents on juvenile marbled spinefoot increased with increasing water

temperature, but was not strongly affected by body weight. Rapid anaesthesia can be safely induced by using concentrations tested in this study. If fish are to be lightly anaesthetized for holding or transport, then lower doses are suggested by Ghanawi *et al.* (2013). In addition, body fat in fish affects the efficacy of various anaesthetic agents tested in this study. Apparently, body fat slows down recovery from anaesthetics.

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