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Anaesthesia of Atlantic cod (*Gadus morhua*) — Effect of pre-anaesthetic sedation, and importance of body weight, temperature and stress

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ABSTRACT

The efficacy of the anaesthetic agents benzocaine, metacaine (MS-222), metomidate and 2-phenoxyethanol was studied in Atlantic cod (*Gadus morhua*) with average body weights of 10 ± 4 g, 99 ± 33 g and 1022 ± 274 g at water temperatures of 8 °C and 16 °C. The agents were tested individually and as combination anaesthesia comprising pre-anaesthetic sedation with a low dosage of metomidate or 2-phenoxyethanol followed by anaesthesia with benzocaine or MS-222. All agents were administered through bath immersion with an exposure time of 5 min. The different treatments resulted in average induction and recovery times ranging from 52 ± 6 s to 182 ± 16 s and 77 ± 26 s to 659 ± 46 s respectively. Induction and recovery times varied in relation to water temperature and were generally shorter at 16 °C for all weight groups and treatments compared to 8 °C. For benzocaine and MS-222 induction and recovery times were found to increase with increasing body weight. For metomidate the recovery time increased with increasing weight whereas there were no weight related differences in induction time. No differences in either induction or recovery times associated to body weight were found for 2-phenoxyethanol. Acute stress prior to anaesthesia with MS-222 resulted in significantly shorter induction time and prolonged recovery time, as well as deeper anaesthetised fish. The dosage of MS-222 had to be reduced in order to avoid mortality in fish subjected to acute stress. Combination anaesthesia allowed a reduction of the dosages used for inducing anaesthesia and produced markedly reduced recovery times compared to agents administered individually.

1. Introduction

A variety of agents with different properties have been used to anaesthetise fish in order to reduce stress and mechanical damage, and to facilitate handling (Gilderhus and Marking, 1987; McFarland, 1959; McFarland and Klontz, 1969; Ross and Ross, 2008; Summerfelt and Smith, 1990). In Norway the two most used anaesthetics are metacaine (MS-222) and benzocaine (Anon, 2007). These are local anaesthetics that exert their effect by blocking voltage-sensitive sodium channels (Frazier and Narahashi, 1975; Neumcke et al., 1981). In fish they produce general anaesthesia and inhibit neural signal transmission ranging from the periphery to higher parts of the nervous system. However, their mechanism at CNS level is not fully understood (Hara and Sata, 2007; Ueta et al., 2007). Other anaesthetics used in fish are 2-phenoxyethanol, which have been shown to inhibit the activity of excitatory N-methyl-D-aspartate (NMDA) receptors (Musshoff et al., 1999), and metomidate hydrochloride, a non-barbiturate hypnotic that stimulates the activity of inhibitory gamma-aminobutyric acid type A ($GABA_A$) receptors, and which thus affect higher regions of the nervous system (Ashton and Wauquier, 1985; Yang and Uchida, 1996).

The efficacy of anaesthetic agents varies both within and between species. Fish of different age, size and sex may respond differently to a particular dosage, and the response is also influenced by environmental factors such as salinity, pH, oxygen level and water temperature (McFarland, 1959; Sylvester and Holland, 1982). Increased temperature has been reported to shorten induction and recovery times for a number of agents in several teleost species (Hikasa et al., 1986; Hoskonen and Pirhonen, 2004; Houston and Woods, 1976; Mylonas et al., 2005; Stehly and Gingerich, 1999). The importance of body size on the response to anaesthesia is, on the other hand, less clear. Some studies show no relationship between body size and induction and recovery times whereas others indicate that a relationship exists (Gilderhus and Marking, 1987; Houston et al., 1976; Olsen et al., 1995; Stehly and Gingerich, 1999; Tsantilas et al., 2006). Hoskonen and Pirhonen (2004) observed a decrease in induction time with increasing body size in whitefish (*Coregonus lavaretus*), whereas

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Table 1
Anaesthetic agents and concentrations administered to Atlantic cod of different body weight and at different temperatures.

Weight (g)	Temperature (°C)	Benzocaine (mgl^{-1})	MS-222 (mgl^{-1})	Metomidate (mgl^{-1})	2-phenoxyethanol (mll^{-1})
10	8	25[A]	60[B]	4	0.25
100	8	25	60	4	0.25
100	16	25	60[C]	6	0.25
1000	8	25–32[F]	55	3	0.25[E]
1000	16	35	60[D]	4	0.25

($n=18$ if not otherwise noted).
A: $n=28$, B: $n=24$, C: $n=20$, D: $n=22$, E: $n=15$, F: no suitable dosage was found.

they found the opposite relationship in rainbow trout (*Oncorhynchus mykiss*), and found no size related variations in Atlantic salmon (*Salmo salar*) or brown trout (*Salmo trutta*).

The physiology and the physiological state of an animal are important during anaesthesia. Stressed animals display abnormal reactions to anaesthetics and may require increased dosages both for induction and maintenance (Hall et al., 2001). Pre-anaesthetic sedation administered in order to reduce acute stress is therefore an integrated part of the anaesthetic protocols in veterinary medicine. These protocols comprise combinations of drugs, each one contributing with effects needed in anaesthesia; relaxation, immobilisation, unconsciousness, amnesia, and analgesia. By combining drugs of different properties a more complete anaesthesia can be achieved than what is possible with one single substance. Drug combinations may also generate a synergy where the drugs potentiate each other thereby allowing a reduction of the dosages required compared to drugs administered individually (Rang et al., 2003). This may reduce the incidence of adverse effects and lead to a smoother recovery.

Handling of fish is associated with acute stress. Several studies report that anaesthetic agents are effective in preventing stress associated with handling in several fish species (Davis and Griffin, 2004; Kreiberg and Powell, 1991; Olsen et al., 1995; Small and Chatakondi, 2005; Thomas and Robertson, 1991). Protocols that include pre-anaesthetic sedation with metomidate have also been successfully applied prior to anaesthesia of fish. Atlantic salmon undergoing surgery for insertion of dorsal aorta cannulae are given both preoperative sedation with metomidate and intraoperative local analgesic treatment with lidocaine in combination with MS-222 anaesthesia (Kiessling et al., 2003; Kiessling et al., 1995). A combination of intraoperative injections of analgesics with MS-222 anaesthesia has also shown a reduction in behavioural changes and muscle tissue damage following surgery in koi carp (*Cyprinus carpio*) (Harms et al., 2005).

Data concerning anaesthesia of Atlantic cod (*Gadus morhua*) are limited, and documentation on combination anaesthesia nonexistent. Anaesthetic protocols are based on drugs and dosages recommended for other fish species (primarily salmonids) and experienced technical staff and scientists at the Institute of Marine Research, Bergen, Norway (Hari Rudra, pers. comm.) have reported recurring incidents of mortalities when anaesthetising cod, due to narrow margins of safety. Mattson and Riple (1989) concluded that from the five anaesthetic agents examined in their study, metomidate is the only safe agent for anaesthetising Atlantic cod, and recommended benzocaine and MS-222 to be possible although less safe alternatives. However, since metomidate is reported to have no or very limited analgesic effect, its use should be restricted to non noxious procedures.

The purpose of this investigation was to examine the importance of water temperature, body weight and acute stress on the efficacy of anaesthetics in Atlantic cod. A further aim was to search for combinations of anaesthetics that would produce synergy and thus allow a reduction of the dosages, thereby improving the anaesthetic protocol.

2. Materials and methods

2.1. Atlantic cod

Atlantic cod of three weight groups, 10, 100 and 1000 g, bred and cultured at the Institute of Marine Research, Austevoll, Norway, were used in this study. The fish of these groups had average body weights of 10 ± 4 g, 99 ± 33 g and 1022 ± 274 g (mean ± STD) respectively. The fish of the 10 g group were held indoors in a 500 l circular tank with a constant flow of aerated sea water with a temperature of 8 °C. Experiments were conducted at 8 °C. Fish of the 100 and 1000 g groups were held in sea cages and were thus subjected to seasonal variations in temperature, salinity and daylight. The experiments were conducted at ambient temperatures of 8 ± 2 °C and 16 ± 2 °C. The fish were fed a commercial marine diet (Skretting, Stavanger, Norway) until 24 h prior to exposure to anaesthetics.

2.2. Anaesthetic agents

The following anaesthetic agents were tested: Benzocaine (Benzoak® Vet, A.C.D Pharmaceuticals, Leknes, Norway), metacaine (MS-222®, Pharmaq AS, Oslo, Norway), metomidate hydrochloride (Aquacalm®, Syndel International Inc., Vancouver, Canada) and 2-phenoxyethanol (Sigma-Aldrich AS, Oslo, Norway). Stock solutions of MS-222 (30 mgl^{-1}) and metomidate (1 mgl^{-1}) dissolved in ionised water were made each day of experimentation while benzocaine (as Benzoak®, 200 $mgml^{-1}$ benzocaine) and 2-phenoxyethanol were used directly. 2-phenoxyethanol was shaken with a small quantity of water prior to being added to the anaesthesia bath. The anaesthesia baths were prepared by carefully adding the anaesthetic while stirring the water in order to ensure adequate mixing. Protective gloves were used at all times. Due to the buffering capacity of the sea water MS-222 induced only a minor change in pH from 7.9 to 7.5 whereas for the other agents no change in pH was detected.

2.3. Experimental design

The anaesthetic agents were tested both individually and as combination anaesthesia, the latter comprising of a low dosage pre-anaesthetic sedation followed by anaesthesia. An overview of the agents and dosages is provided in Table 1 (individual agents) and Table 2 (combination anaesthesia). Experimental design, anaesthetics and dosages used were based on a protocol established through a pilot study of anaesthesia of Atlantic cod (M.K. Hansen et al., unpublished results). All anaesthetics were administered via bath immersions in

Table 2
Combinations and concentrations of agents for pre-anaesthetic sedation and anaesthesia administered to Atlantic cod of different body weight and at different temperatures.

Pre-sedative	Dosage (mgl^{-1})[A]	Anaesthetic	Dosage (mgl^{-1})	Weight (g)	Temperature (°C)	(n)
Metomidate	1.0	Benzocaine	15	10	8	24
2-phenoxyethanol	0.1		15			18
Metomidate	1.0	Benzocaine	20	100	8	18
2-phenoxyethanol	0.1		15			18
Metomidate	1.2	Benzocaine	20	1000	8	18
2-phenoxyethanol	0.2		20			18
Metomidate	3.0	Benzocaine	22	100	16	4
2-phenoxyethanol	0.15		20			18
Metomidate	3.0	Benzocaine	22	1000	16	18
2-phenoxyethanol	0.15		20			18
Metomidate	0.5	MS-222	40	10	8	30
Metomidate	1.0	MS-222	40	100	8	18
Metomidate	1.0	MS-222	42	1000	8	18
Metomidate	2.0	MS-222	45	100	16	18
Metomidate	1.5	MS-222	37.5	1000	16	22

[A] The dosage of 2-phenoxyethanol is mll^{-1}.