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# Anaesthesia of Atlantic halibut (*Hippoglossus hippoglossus*) – Effect of pre-anaesthetic sedation, and importance of body weight and water temperature

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## Abstract

The efficacy of the anaesthetic agents benzocaine, metacaine (MS-222), metomidate, 2-phenoxyethanol, quinaldine and isoeugenol was studied in Atlantic halibut (*Hippoglossus hippoglossus*). Fish with an average body weight of 33 g were anaesthetized at 8 °C and fish with an average body weight of 1243 g were anaesthetized at 8 and 15 °C. Agents were tested individually and as combination anaesthesia comprising pre-anaesthetic sedation, followed by anaesthesia. Induction and recovery times varied in relation to the body weight and water temperature. Large fish had longer induction times and shorter recovery times, and displayed reduced responsiveness to handling compared with small fish. A higher temperature resulted in shorter induction times, longer recovery times and increased responsiveness to handling. Lower dosages were used for all agents in combination anaesthesia. In small fish, this had no effect on the induction times but resulted in shorter recovery times and reduced responsiveness to handling. In large fish, combination anaesthesia resulted in shorter induction times whereas no uniform trend in recovery times and no differences in responsiveness to handling were observed. Neither individual agents nor combinations blocked all reflex reactions to external stimulation in all fish of any treatment group. MS-222 and benzocaine, used separately or in combination anaesthesia, were the most effective agents in reducing reflex reactions.

**Keywords:** Atlantic halibut, anaesthesia, pre-anaesthetic sedation, MS-222, benzocaine, metomidate, 2-phenoxyethanol, isoeugenol, quinaldine

## Introduction

Anaesthetics in commercial fish farming are mainly used for immobilization of fish in order to facilitate handling but are now also applied in various situations ranging from mild sedation during transport to full anaesthesia during more invasive procedures. A large selection of anaesthetic agents, some deriving from human and veterinary medicine, are being used. Among the most common compounds are metacaine (MS-222), benzocaine, quinaldine, 2-phenoxyethanol, metomidate and isoeugenol (Ackerman, Morgan & Iwama 2005; Ross & Ross 2008). Good anaesthetic effect, rapid induction and recovery times and good safety margins are important properties for fish anaesthetics (Marking & Meyer 1985; Bell 1987). Dosage regimes maintaining these properties vary between agents and between fish species, but are also influenced by biological factors such as body weight, age and sex, as well as environmental factors such as salinity, pH, oxygen level and water temperature (Ross & Ross 2008).

The basal metabolism of ectothermic animals and the physiological processes involved in the uptake and elimination of anaesthetics are strongly dependent on temperature. Reduced induction and recov-

ery times with increased water temperature have been reported during anaesthesia in a number of fish species (Houston & Woods 1976; Hikasa, Takase, Ogasawara & Ogasawara 1986; Stehly & Gingerich 1999; Hoskonen & Pirhonen 2004; Mylonas, Cardinaletti, Sigelaki & Polzonetti-Magni 2005). Metabolism and physiological processes are also affected by body size, with an inverse correlation between size and the basal metabolic rate. The relationship between body size and uptake and elimination of anaesthetics in fish, however, seems to be more complex (Gilderhus & Marking 1987; Hoskonen & Pirhonen 2004; Olsen, Einarsdottir & Nilssen 1995; Tsantilas, Galatos, Athanassopoulou, Prassinos & Kousoulaki 2006). Factors like body composition, sexual maturity and gill surface to body weight ratio, which may all vary with species, size and age, may be responsible for this variation.

Anaesthesia consists of several components, including muscle relaxation, pain relief (analgesia) and loss of consciousness. In both human and veterinary medicine, these are obtained with a combination of drugs rather than with one single drug. In addition to producing multiple effects, a combination of different anaesthetic agents may also generate a synergy where the drugs potentiate each other, allowing a reduction in dosages compared with each drug administered individually (Rang, Dale, Ritter & Moore 2003). This may minimize adverse effects and provide a faster and safer induction and recovery. Although existing protocols for fish do not typically include administration of combinations of anaesthetics, there are reports describing combination anaesthesia in connection to surgical procedures. MS-222 and benzocaine are currently being used in combination with pre-anaesthetic sedation with metomidate and local intramuscular (i.m.) injections of lidocaine to anaesthetize Atlantic salmon (*Salmo salar*) undergoing surgery for insertion of dorsal aorta cannulae (Kiessling, Dosanjh, Higgs, Deacon & Rowshandeli 1995; Kiessling, Olsen & Buttle 2003) and hepatic portal vein cannulae (Eliason, Kiessling, Karlsson, Djordjevic & Farrell 2007), and Atlantic cod (*Gadus morhua*) undergoing surgery for the insertion of caudal aorta cannulae (A. Karlsson, B. O. Rosseland, J. C. Massabuau & A. Kiessling, pers. comm.). MS-222 has also been used in combination with butorphanol and ketoprofen during surgery of koi carp (*Cyprinus carpio*) (Harms, Lewbart, Swanson, Kishimori & Boylan 2005).

To our knowledge, there are no data in the literature regarding combination anaesthesia of Atlantic halibut (*Hippoglossus hippoglossus*). Of the range of substances used to anaesthetize fish, the characteristics of only two agents have been examined in Atlantic halibut. A practical evaluation of MS-222 and metomidate has been carried out on wild and farmed Atlantic halibut following bath administration (Malmstrom, Salte, Gjoen & Linseth 1993), and the pharmacokinetic and pharmacodynamic properties of metomidate have been studied in farmed Atlantic halibut after both intravenous (i.v.) and bath administration (Hansen, Nymoen & Horsberg 2003).

The main objective of the current investigation was to carry out a first evaluation of the importance of body weight and water temperature on the efficacy of anaesthetics in Atlantic halibut. A further aim was to investigate whether combinations of anaesthetics would produce synergy and thereby possibly contribute to an improved anaesthetic protocol.

## Materials and methods

### Atlantic halibut

Two groups of Atlantic halibut, bred and cultured at the Institute of Marine Research, Austevoll, Norway, were used in this study. The fish of group 1 had a body weight of 33 ± 14 g (mean ± STD) and were studied at a water temperature of 8 °C. Fish of group 2 had a body weight of 1243 ± 353 g (mean ± STD) and were studied at water temperatures of 8 and 15 °C. The fish were fed a commercial marine diet (Skretting, Stavanger, Norway) until 24 h before exposure to anaesthetic agents.

### Anaesthetic agents

The following anaesthetic agents were examined: benzocaine (Benzoak® Vet, A.C.D Pharmaceuticals, Leknes, Norway), metacaine (MS-222®, Pharmaq AS, Oslo, Norway), metomidate hydrochloride (Aquacalm®, Syndel International, Vancouver, Canada), 2-phenoxyethanol (Sigma-Aldrich AS, Oslo, Norway), quinaldine (Sigma-Aldrich AS) and isoeugenol (Aqui-S®, Scan Aqua AS, Aarnes, Norway).

### Experimental design

Anaesthetic agents were administered via bath immersions at the dosages presented in Table 1. In order to identify when the Atlantic halibut lost balance due to the anaesthesia, the circular tank for the anaes-

  

thesia bath was equipped with two immersion pumps (Eheim compact+, EheimGmbH, KG Deizisau, Germany) and outputs set at approximately 4.5 and 12 l min$^{-1}$ for fish of group 1 and group 2 respectively. The fish were caught using a dip net and placed in the anaesthetic bath. As anaesthesia was induced, the fish began to follow the current generated by the pumps. Induction time was set at the point where the fish became immobilized and started to drift passively along with the water current. After 5 min in the anaesthesia bath, the fish were carefully placed upside down in a recovery bath containing still water without oxygenation. Upon regaining consciousness, the fish tried to turn over and the recovery time was set at the moment where the fish managed to turn upright.

Balance, respiration and swimming activity were observed throughout the experiments, whereas sensory perception was assessed following the 5 min of exposure. Anaesthetized fish were carefully lifted out of the anaesthesia tank and weighed before being placed in the recovery tank. Completely anaesthetized fish that did not respond to being lifted out of the anaesthesia bath, weighed and transferred to the recovery bath were given the handling score of 0, whereas fish that reacted to this handling procedure or were impossible to handle (due to light anaesthesia) were given scores 1 and 2 respectively. To test reflex reactions, all fish were given a caudal peduncle pinch during weighing. Reflex reactions to the pinch were recorded as either present or absent.

**Table 1** Anaesthetic agents and concentrations administered to Atlantic halibut of body weight 33 and 1243 g at water temperatures 8 and 15 °C ($n = 18$)

| Anaesthetic | Dosage (mg L$^{-1}$)* |
|---|---|
| Benzocaine | 40 |
| MS-222 | 80 |
| Metomidate | 2.9 |
| 2-phenoxyethanol | 0.6 |
| Quinaldine | 10 |
| Isoeugenol | 40 |

*The dosage of 2-phenoxyethanol is mL L$^{-1}$.

After testing each anaesthetic individually, some agents were selected for further testing in combination anaesthesia, comprising pre-anaesthetic sedation and anaesthesia. The experimental design was based on a study of combination anaesthesia in Atlantic cod (Zahl, Kiessling, Samuelsen & Hansen 2009) and on protocols of combination anaesthesia applied during surgery of Atlantic cod (A. Karlsson, B. O. Rosseland, J. C. Massabuau & A. Kiessling, pers. comm.) and Atlantic salmon (Kiessling *et al.* 1995; Kiessling, Olsen & Buttle 2003; Eliason *et al.* 2007). A pilot study where the dosages were tested on a small number of fish ($n = 5$) was conducted before full series of experiments were performed. The dosages are presented in Table 2. In the examination of combination anaesthesia, the fish were given a sedative treatment for 5 min in a bath containing a low dosage of either metomidate or 2-phenoxyethanol, and were then transferred to a second bath for 5 min of anaesthesia by benzocaine, MS-222 or quinaldine. Following the anaesthesia, the fish were carefully placed upside down in the recovery bath. The tanks containing baths for pre-anaesthetic sedation and anaesthesia were equipped with immersion pumps as described above, and the fish recovered in clean still water without extra oxygenation.

In all experiments, fish of group 1 were anaesthetized two by two and fish of group 2 were anaesthetized one by one. The baths containing anaesthetic agents, 6 L for fish of group 1 and 70 L for fish of group 2, were renewed after every 4th fish tested. The recovery baths contained 20 L for fish of group 1 and 350 L for fish of group 2 and water was renewed after every 2nd fish tested. Each treatment, individual anaesthetics and combinations of anaesthetics, was tested on 18 individuals.

**Table 2** Combinations and concentrations of anaesthetic agents for pre-anaesthetic sedation and anaesthesia administered to Atlantic halibut of body weight 33 and 1243 g at a water temperature of 8 °C ($n = 18$)

| Pre-sedative | Dosage (mg L$^{-1}$)* | Anaesthetic | Dosage (mg L$^{-1}$) | Body weight (g) |
|---|---|---|---|---|
| Metomidate | 0.4 | Benzocaine | 15 | 33 |
| 2-Phenoxyethanol | 0.1 | | 20 | |
| Metomidate | 0.6 | Benzocaine | 25 | 1243 |
| Metomidate | 0.4 | MS-222 | 40 | 33 |
| Metomidate | 0.4 | MS-222 | 40 | 1243 |
| Metomidate | 1 | Quinaldine | 5 | 33 |

*The dosage of 2-phenoxyethanol is mL L$^{-1}$.

### Statistical analysis

The data were analysed using the main factorial model [general linear model, STATISTICAL ANALYSIS SYSTEM (SAS) for PC (ver. 8.2), ANOVA for unbalanced data]. Included in the model as the main factors were anaesthetics and anaesthetic combinations (categorical), and temperature (categorical). Body weight was either included in the model as the predetermined size group (categorical) or as a covariate (continuous) using the individual body weights. Groups were compared by the *ad hoc* variance test (*F*-test) using the least-squares means procedure when significant effects were found in the main model. The level of statistical significance was set at $P < 0.05$. All data were tested for normality by a normal probability plot (PROC UNIVARIATE, SAS, ver. 8.2). Differences between treatments in handling and pinching scores were compared by $\chi^2$ (in PROC FREQ. procedure, SAS, ver. 8.2).

## Results and discussion

During the progress of induction and recovery, the fish pass through several stages of anaesthesia, where some are further divided into planes of depth, characterized by alterations in physiology and behaviour (McFarland 1959; Bell 1987). Activity level, swimming behaviour, balance, opercular movements and responses to specific external stimuli and handling are common parameters that are assessed in order to identify these stages and planes. Induction and recovery times are often set at loss and regain of equilibrium, which are easily assessed in free-swimming fish. In order to determine these parameters in the Atlantic halibut, a sedate, bottom-dwelling flat fish, the tanks of the anaesthesia baths were equipped with water pumps generating a current strong enough to identify when the fish lost equilibrium. In the recovery bath, the fish were placed upside down, and recovery was set when normal posture was resumed. Consequently, the values for induction and recovery time, i.e. loss and regain of equilibrium found here for Atlantic halibut should be used cautiously when comparing with data reported for free-swimming fish species. The efficacy of specific anaesthetics and combinations may be compared, however, as well as factors important for efficacy such as water temperature and body weight.

The dosages of the agents used in the current study are within the range of those reported as optimal for various fish species (see Ackerman, Morgan & Iwama (2005) and Ross & Ross (2008) for details) but are much lower than the dosages used both by Malmstrom *et al.* (1993) in the evaluation of metomidate and MS-222 as anaesthetics for Atlantic halibut and by Hansen, Nymoen & Horsberg (2003) in the pharmacokinetic and pharmacodynamic study of metomidate in Atlantic halibut and turbot (*Scophthalmus maximus*). In the evaluation by Malmstrom *et al.* (1993), the exposure time was matched for each individual fish, as opposed to the current study, where all fish received the same exposure time, i.e. five min. The uniform exposure time was chosen in order to ensure a sufficient margin of safety as the normal practice during field conditions involves anaesthesia of large numbers of fish simultaneously, and the exposure time may thus exceed the induction time for individual fish, which could possibly lead to overdosing.

The induction times found in the current investigation did not vary much between the different agents and groups of fish (Table 3). The recovery times, on the other hand, varied noticeably, with metomidate and isoeugenol being among the agents resulting in the longest recovery times in both small and large fish (Table 4). This is in agreement with Hansen, Nymoen & Horsberg (2003), who reported long recovery times following metomidate anaesthesia for both Atlantic halibut and turbot, and also with observations in other species anaesthetized with these agents (Gilderhus & Marking 1987; Mattson & Riple 1989; Kiessling, Johansson, Zahl & Samuelsen 2009).

While short induction and recovery times are preferred in most situations of anaesthesia, the need for depressed reactions to handling and abolished reflexes will depend on the kind of procedure the fish are being subjected to. During practices such as confinement, grading and transport, light sedation might be sufficient in order to reduce alertness, responsiveness and activity, and to prevent stress. Invasive procedures such as surgery require deep anaesthesia without any responses or reflex reactions to the procedure, and will also require analgesic treatment.

The presence of responses to handling, in the current study in the form of weighing, is presented in Table 5. There were marked differences in the responses between the two weight groups, with small fish generally being more responsive. MS-222 and 2-phenoxyethanol as well as the combinations of anaesthetics were most effective in reducing responsiveness. However, the majority of fish anaesthetized

**Table 3** Induction time in Atlantic halibut anaesthetized with the following anaesthetic agents administered individually: benzocaine, MS-222, metomidate, 2-phenoxyethanol, quinaldine and isoeugenol, and with the following agents administered subsequent to 5 min of pre-anaesthetic sedation with metomidate or 2-phenoxyethanol: benzocaine, MS-222 and quinaldine

| **Temperature** | **8 °C** | | | | **15 °C** | | ***P*-value** | ***n*** |
|---|---|---|---|---|---|---|---|---|
| **Body weight** | **33 g** | **SE** | **1243 g** | **SE.** | **1243 g** | **SE** | | |
| **Treatment** | **Time (s)** | | **Time (s)** | | **Time (s)** | | **Temperature + body weight** | |
| Benzocaine | 143 | ± 12 a | 163 | ± 12 b AB | 130 | ± 12 a | 0.003 | 53 |
| MS-222 | 114 | ± 12 a | 193 | ± 12 b AB | 141 | ± 12 c | <0.0001 | 53 |
| Metomidate | 145 | ± 13 | 150 | ± 12 A | – | – | NS | 34 |
| 2-phenoxyethanol | 151 | ± 13 | 163 | ± 13 AB | – | – | NS | 32 |
| Quinaldine | 130 | ± 14 a | 190 | ± 12 b B | – | – | 0.0002 | 36 |
| Isoeugenol | 138 | ± 12 a | 195 | ± 12 b B | – | – | <0.0001 | 36 |
| Metomidate+benzocaine | 148 | ± 12 a | 94 | ± 12 b C | – | – | 0.05 | 35 |
| Metomidate+MS-222 | 120 | ± 13 | 108 | ± 12 C | – | – | NS | 34 |
| Metomidate+quinaldine | 128 | ± 13 | – | – | – | – | – | 16 |
| 2-phenoxyethanol+benzocaine | 161 | ± 12 | – | – | – | – | – | 18 |
| $p_{Treat.}$ | NS | | <0.0001 | | NS | | | |
| $p_{Bw}$* | NS | | NS | | NS | | | |
| *N* | 165 | | 141 | | 36 | | | |

Induction time is recorded in seconds, and is defined as the time from submersion in the anaesthesia bath to the total loss of equilibrium. The average body weight of the fish was 33 and 1243 g, the exposure time was 5 min and the water temperature was 8 or 15 °C.
*Body weight included as a covariate (continuous variable) in the statistical model
The data are given as mean ± standard error of mean (SE). Different upper case letters indicate a significant difference at $P<0.05$ level between the different anaesthetic treatments, while lower case letters are indicative of the same between temperature and body weight. NS = $P>0.15$.

**Table 4** Recovery time in Atlantic halibut anaesthetized with the following anaesthetic agents administered individually: benzocaine, MS-222, metomidate, 2-phenoxyethanol, quinaldine and isoeugenol, and with the following agents administered subsequent to 5 min of pre-anaesthetic sedation with metomidate or 2-phenoxyethanol: benzocaine, MS-222 and quinaldine

| **Temperature** | **8 °C** | | | | **15 °C** | | ***P*-value** | ***n*** |
|---|---|---|---|---|---|---|---|---|
| **Body weight** | **33 g** | **SE** | **1243 g** | **SE** | **1243 g** | **SE** | | |
| **Treatment** | **Time (s)** | | **Time (s)** | | **Time (s)** | | **Temperature + body weight** | |
| Benzocaine | 1156 | ± 104 a A | 455 | ± 104 b AE | 945 | ± 104 a | 0.0035 | 54 |
| MS-222 | 746 | ± 107 B | 587 | ± 104 A | 922 | ± 104 | 0.09 | 53 |
| Metomidate | 1553 | ± 114 a C | 854 | ± 104 b B | – | – | 0.0003 | 33 |
| 2-phenoxyethanol | 925 | ± 107 AB | 1110 | ± 107 C | – | – | NS | 34 |
| Quinaldine | 740 | ± 122 a B | 185 | ± 104 b D | – | – | 0.0005 | 36 |
| Isoeugenol | 1572 | ± 104 a C | 888 | ± 101 b BC | – | – | 0.0002 | 37 |
| Metomidate+benzocaine | 307 | ± 107 a D | 666 | ± 104 b AB | – | – | 0.0085 | 35 |
| Metomidate+MS-222 | 334 | ± 104 D | 301 | ± 104 DE | – | – | NS | 36 |
| Metomidate+quinaldine | 411 | ± 104 D | – | – | – | – | – | 18 |
| 2-phenoxyethanol+benzocaine | 294 | ± 104 D | – | – | – | – | – | 18 |
| $p_{Treat.}$ | <0.0001 | | <0.0001 | | NS | | | |
| $p_{Bw}$* | NS | | NS | | NS | | | |
| *n* | 169 | | 144 | | 36 | | | |

Recovery time is recorded in seconds, and is defined as time from transfer to recovery bath to regaining of equilibrium. The average body weight of the fish was 33and 1243 g, the exposure time was 5 min and the water temperature was 8 or 15 °C.
*Body weight included as a covariate (continuous variable) in the statistical model.
The data are given as mean ± standard error of mean (SE). Different upper case letters indicate a significant difference at $P<0.05$ level between the different anaesthetic treatments, while lower case letters are indicative of the same between temperature and body weight. NS = $P>0.15$.

**Table 5** Responsiveness to handling in Atlantic halibut anaesthetized with the following agents administered individually: benzocaine, MS-222, metomidate, 2-phenoxyethanol, quinaldine and isoeugenol, and with the following agents administered subsequent to 5 min of pre-anaesthetic sedation with metomidate or 2-phenoxyethanol: benzocaine, MS-222 and quinaldine

| Treatment | Body weight (g) | Temperature (°C) | No response (%) | Response (%) | Impossible to handle (%) | $\chi^2$ 1 | $\chi^2$ 2 |
|---|---|---|---|---|---|---|---|
| Benzocaine | 33 | 8 | 44 | 33 | 22 | 0.02 | a |
| | 1243 | 8 | 83 | 17 | 0 | | A |
| | 1243 | 15 | 76 | 24 | 0 | | 1 |
| MS-222 | 33 | 8 | 82 | 18 | 0 | NS | b |
| | 1243 | 8 | 100 | 0 | 0 | | A |
| | 1243 | 15 | 89 | 11 | 0 | | 1 |
| Metomidate | 33 | 8 | 25 | 57 | 19 | 0.0002 | a |
| | 1243 | 8 | 94 | 6 | 0 | | A |
| 2-phenoxyethanol | 33 | 8 | 83 | 17 | 0 | NS | b |
| | 1243 | 8 | 89 | 11 | 0 | | A |
| Quinaldine | 33 | 8 | 0 | 20 | 80 | 0.0001 | c |
| | 1243 | 8 | 83 | 17 | 0 | | A |
| Isoeugenol | 33 | 8 | 6 | 29 | 65 | 0.0001 | c |
| | 1243 | 8 | 63 | 32 | 5 | | A |
| Metomidate+benzocaine | 33 | 8 | 72 | 22 | 5 | NS | b |
| | 1243 | 8 | 78 | 11 | 11 | | A |
| Metomidate+MS-222 | 33 | 8 | 100 | 0 | 0 | NS | b |
| | 1243 | 8 | 88 | 12 | 0 | | A |
| Metomidate+quinaldine | 33 | 8 | 94 | 6 | 0 | – | b |
| 2-phenoxyehtanol+benzocaine | 33 | 8 | 100 | 0 | 0 | – | b |

The average body weight of the fish was 33 and 1243 g, the exposure time was 5 min and the water temperature was 8 or 15 °C. The data are given as frequency distribution in per cent per treatment. $\chi^2$ 1 gives the *P*-value for analysis of variance within an anaesthetic. Different lower case letter ($\chi^2$ 2) indicate a significant difference at $P < 0.05$ level between the different anaesthetic treatments in fish of body weight 33 g and temperature 8 °C, while upper case letters are indicative of the same in fish of body weight 1243 g and temperature 8 °C, and numbers in fish of body weight 1243 g and temperature 15 °C (PROC FREQ., SAS, ver. 8.2) ($n = 18$).

with combinations displayed reflex reactions to the caudal peduncle pinch (Table 6). In small fish, reflex reactions were also present in two combination treatments where no responses to handling were observed. Also, in large fish, combination anaesthesia resulted in a noticeable increase in the presence of reflex reactions. MS-222 and benzocaine used individually were the most effective agents in eliminating reflex reactions, although there was a markedly increased presence of reflexes in fish anaesthetized at the high water temperature. No individual agent or combination blocked the reflex reactions in all fish of any group.

## Body weight

The induction and recovery times obtained by the different anaesthetic treatments in the two weight groups of Atlantic halibut are shown in Tables 3 and 4. The main trends were longer induction times and shorter recovery times for fish of higher body weight. This is in agreement with the findings in white sea bream (*Diplodus sargus*) anaesthetized with 2-phenoxyethanol (Tsantilas *et al.* 2006). Increased induction times with increased body weight have also been found in rainbow trout (*Oncorhynchus mykiss*) anaesthetized with clove oil, and in Atlantic cod anaesthetized with benzocaine and MS-222 (Hoskonen & Pirhonen 2004; Zahl , Kiessling, Samuelsen & Hansen 2009). However, several agents have been examined and diverging results regarding the importance of body weight for induction and recovery times have been reported for various fish species (Houston, Corlett & Woods 1976; Gilderhus & Marking 1987; Olsen, Einarsdottir & Nilssen 1995; Stehly & Gingerich 1999; Hoskonen & Pirhonen 2004; Tsantilas *et al.* 2006).

Because anaesthetics administered through bath immersions are absorbed over the gills (Hunn & Allen 1974), factors affecting the respiration rate, gill blood flow and gill permeability will all influence the uptake rate. Large fish have a smaller gill surface area in relation to body weight than small fish (Oikawa & Itazawa 1985), and thus have a smaller area for drug diffusion in relation to weight. Furthermore, in large

  

**Table 6** Reflex reactions to caudal peduncle pinching in Atlantic halibut anaesthetized with the following agents administered individually: benzocaine, MS-222, metomidate, 2-phenoxyethanol, quinaldine or isoeugenol, and with the following agents subsequent to 5 min of pre-anaesthetic sedation with either metomidate or 2-phenoxyethanol: benzocaine, MS-222 and quinaldine

| Treatment | Body weight (g) | Temperature (°C) | Reflex absent (%) | Reflex present (%) | $\chi^2$ 1 | $\chi^2$ 2 |
|---|---|---|---|---|---|---|
| Benzocaine | 1243 | 8 | 78 | 22 | 0.007 | A |
| | 1243 | 15 | 33 | 67 | | 1 |
| MS-222 | 1243 | 8 | 94 | 6 | <0.0001 | A |
| | 1243 | 15 | 28 | 72 | | 1 |
| Metomidate | 1243 | 8 | 44 | 56 | – | C |
| 2-phenoxyethanol | 1243 | 8 | 67 | 33 | – | A |
| Quinaldine | 1243 | 8 | 39 | 61 | – | C |
| Isoeugenol | 1243 | 8 | 0 | 100 | – | D |
| Metomidate+benzocaine | 33 | 8 | 6 | 94 | 0.07 | a |
| | 1243 | 8 | 28 | 72 | | C |
| Metomidate+MS-222 | 33 | 8 | 33 | 67 | 0.009 | b |
| | 1243 | 8 | 0 | 100 | | D |
| Metomidate+quinaldine | 33 | 8 | 0 | 100 | – | a |
| 2-phenoxyethanol+benzocaine | 33 | 8 | 0 | 100 | – | a |

The average body weight of the fish was 33 and 1243 g, the exposure time was 5 min and the water temperature was 8 or 15 °C. The data are given as frequency distribution in per cent per treatment. $\chi^2$ 1 gives the *P*-value for analysis of variance within an anaesthetic treatment. Different lower case letters ($\chi^2$ 2) indicate a significant difference at $P<0.05$ level between the different anaesthetic treatments in fish of body weight 33 g and temperature 8 °C, while upper case letters are indicative of the same in fish of body weight 1243 g and temperature 8 °C, and numbers in fish of body weight 1243 g and temperature 15 °C (PROC FREQ., SAS, ver. 8.2) ($n=18$).

fish, the oxygen consumption rate in relation to weight is lower due to lower basal metabolism (Clarke & Johnston 1999).

One factor that may be important in explaining the variations in the recovery times observed between the two weight groups is differences in the body composition, which may result in differences in the redistribution of anaesthetic agents from the brain to the body. Other characteristics related to body weight such as age, growth and sexual maturity are also important for the physiology of the fish. Physiological differences, both within and between species, may result in altered pharmacokinetic and pharmacodynamic properties and thereby in different responses to anaesthetic agents. This may be important for some of the variations in the response found in various investigations.

## Water temperature

Increased water temperature resulted in shorter induction times and longer recovery times (Table 3 and 4). Reduced induction times with increased water temperature have been reported for a number of anaesthetic agents in several fish species (Hikasa *et al.* 1986; Stehly & Gingerich 1999; Hoskonen & Pirhonen 2004; Mylonas *et al.* 2005), and are associated with the temperature-dependent rate of physiological processes involved in the uptake and elimination of anaesthetics. At higher ambient temperature, fish have a higher basal metabolism and hence a higher oxygen demand (Clarke & Johnston 1999; Imsland, Jonassen, Stefansson, Kadowaki & Berntssen 2000). This increase in oxygen demand is met by an enhanced respiration rate and cardiac output (Barron, Tarr & Hayton 1987), hence leading to an increased blood flow through the gills. Enhanced respiration rate and blood flow facilitate the absorption of anaesthetic agents administered through bath immersion, which may result in shorter induction times and may also lead to absorption of larger amounts of anaesthetics. A significantly higher concentration of isoeugenol has been found in the fillet of rainbow trout following exposure at 17 °C compared with 7 °C (Meinertz, Greseth, Schreier, Bernardy & Gingerich 2006).

The shorter induction times associated with higher water temperature have been reported to be accompanied by shorter recovery times (Hikasa *et al.* 1986; Stehly & Gingerich 1999; Hoskonen & Pirhonen 2004; Mylonas *et al.* 2005). However, in the current study, it was found that the Atlantic halibut recovered more slowly at a higher water temperature. This may indicate a stronger effect by the temperature increase on the absorption rate of anaesthetic agents, as

shown by Meinertz *et al.* (2006), combined with a smaller effect on the elimination rates (half-lives). However, in order to reveal such effects, pharmacokinetic studies are needed. In addition to longer recovery times, the stronger reactions to handling displayed by the fish anaesthetized at the higher water temperature indicate that the fish were under lighter anaesthesia (Table 5 and 6). Reduced anaesthetic depth in spite of shorter induction and prolonged recovery times seems to be contradictory, but may possibly be explained if the central and peripheral parts of the nervous system are affected differently by anaesthesia at higher temperatures. If the brain centres that control balance are more affected by a faster uptake of anaesthetic than the peripheral nerves that control reflex responses to handling, the balance and thus loss of equilibrium might be affected at an earlier stage than that of the reflex arcs.

### Combination anaesthesia

Combination anaesthesia, comprising pre-anaesthetic sedation and anaesthesia, allowed a reduction in the anaesthetic dosages to almost half of the dosages used when the anaesthetics were administered individually (Table 1 and 2). The induction times following combination anaesthesia (Table 3) were shorter (large fish) or similar (small fish) in comparison with agents administered individually, hence indicating a synergetic effect between the pre-sedatives and the anaesthetics.

All handling of fish is associated with stress, and although anaesthetics are being used to reduce the stress response to handling, the procedure of anaesthesia and the anaesthetic agents themselves have been found to induce stress, observed as increased levels of cortisol (Tort, Puigcerver, Crespo & Padrós 2002; Zahl, Kiessling, Samuelsen & Olsen 2010). However, the cortisol released in response to anaesthesia is minor when compared with the response following handling (Tort *et al.* 2002; Ellis, James, Stewart & Scott 2004; Zahl, Kiessling, Samuelsen & Olsen 2010). One of the aims of the pre-anaesthetic sedation was to prevent stress from handling, and the subsequent stress induced rapid and excessive uptake of anaesthetic. As both the escape reaction associated with being caught in a dip net and the elevated level of stress hormones following netting will lead to enhanced respiration and circulation, the Atlantic halibut should ideally have been sedated before netting in order to avoid an uncontrolled uptake of anaesthetic. However, in a study of Atlantic cod, it was shown that the induction time during MS-222 anaesthesia without any stress from netting or handling resembled the induction time in fish sedated before netting, indicating that the pre-sedative treatment would reduce the acute stress caused by the netting (Zahl , Kiessling, Samuelsen & Hansen 2009).

In small fish, the recovery times following combination anaesthesia were significantly shorter in comparison with the recovery times obtained by individual agents. No uniform trend was observed in large fish (Table 4). Increased responsiveness to handling and the presence of reflexes indicate that the fish anaesthetized with combinations were under lighter anaesthesia (Table 5 and 6). Handling or experimental procedures that require deeper anaesthetized fish will require higher dosages of anaesthetic agents, but this was not studied in the current investigation.

Anaesthesia produced by a combination of agents that completely blocks the reflex arcs has been found to significantly reduce the recovery time following invasive procedures (Kiessling, Olsen & Buttle 2003; Karlsson, Eliason, Mydland, Farrell & Kiessling 2006; Eliason *et al.* 2007). Combination anaesthesia obviously offers an opportunity to optimize the anaesthetic protocol, which might be further improved if combined with analgesic agents administrated locally.

### Anaesthesia and nociception

It has been shown recently that fish possess the basic neural system necessary for nociception, i.e. perception of painful stimuli (Sneddon 2002, 2003; Sneddon, Braithwaite & Gentle 2003; Dunlop & Laming 2005), which means that agents with the ability to block the signals of nociceptive pathways should be used during procedures that can be expected to cause pain. The two local anaesthetics benzocaine and MS-222 possess such an effect, in addition to isoeugenol (see below for a detailed description of the effect). However, the route of administration may influence the effect of these agents. Systemic administration of local anaesthetics has been found to be inadequate for blocking nociceptive pathways in higher verterbrates (mice, rat and cat) (Haegerstam 1979; Wiesenfeld-Hallin & Lindblom 1985; Woolf & Wiesenfeld-Hallin 1985; Rigon & Takahashi 1996). Haegerstam (1979) concluded that it seemed unlikely

  

that peripheral pain pathways could be blocked by systemic i.v. injections of local anaesthetics. Neither of the agents tested in the current investigation, administered individually or in combination, resulted in the complete blockade of reflex reactions associated with the protocol used for anaesthesia of Atlantic salmon undergoing surgery for insertion of cannulae (Kiessling *et al.* 1995; Kiessling, Olsen & Buttle 2003; Eliason *et al.* 2007). The protocol used during surgery comprises pre anaesthetic sedation with metomidate, followed by anaesthesia with MS-222, and injections of the local anaesthetic lidocaine peripherally (Kiessling *et al.* 1995; Kiessling, Olsen & Buttle 2003; Eliason *et al.* 2007). In the current study, the decreased dosages used in combination anaesthesia resulted in similar induction times when compared with the individually administrated agents, but the fish displayed increased reactions to external stimuli. This is possibly due to an enhanced CNS effect without a parallel enhanced effect in the peripheral nociceptive pathways. Based on these data, it is unclear whether it is possible to obtain a sufficient analgesic effect in fish when anaesthetic agents are administered via inhalation, even though the agents display a proven local anaesthetic effect. Caution should therefore be exercised when inhaled substances are used during procedures that might involve nociception if no analgesic agent is administrated locally.

Both benzocaine and MS-222 inhibit neural signal transmission by blocking voltage-sensitive sodium channels (Frazier & Narahashi 1975; Neumcke, Schwarz & Stampfli 1981). Isoeugenol is closely related to eugenol, a widely used analgesic in dentistry that inhibits sodium, potassium and calcium channels, inhibits *N*-methyl-D-aspartate (NMDA) receptors and potentiates gamma-aminobutyric acid type A ($GABA_A$) receptors (Wie, Won, Lee, Shin, Lee, Suh, Song & Kim 1997; Aoshima & Hamamoto 1999; Lee, Yeon, Park, Li, Fang, Kim, Choi, Lee, Lee, Park, Lee, Kim & Oh 2005; Park, Li, Yeon, Jung, Choi, Lee, Lee, Park, Kim & Oh 2006; Li, Park, Jung, Choi, Lee, Park, Kim & Oh 2007). To our knowledge, the other agents examined in the present study do not block the signals of the peripheral nociceptive nerves. 2-phenoxyethanol inhibits the activity of excitatory NMDA receptors (Musshoff, Madeja, Binding, Witting & Speckmann 1999), and the non-barbiturate hypnotic metomidate hydrochloride stimulates the activity of inhibitory $GABA_A$ receptors (Ashton & Wauquier 1985; Yang & Uchida 1996), and both thus affect higher regions of the nervous system. These agents therefore should not be used as the only anaesthetic during potentially painful procedures. Data on the exact mode of action of quinaldine have not been found, but there are reports indicating that the anaesthetic effect is related to increased concentrations of the agent in the brain, thus indicating that quinaldine affects higher regions of the nervous system (Brown, Franklin, Pratt & Trams 1972; Hunn & Allen 1974).

## Conclusions

Both body weight and water temperature are important factors that affect the efficacy of the anaesthetic agents, visualized in the current investigation by induction and recovery times as well as responsiveness to external stimuli. In general, fish of large body weight have longer induction and shorter recovery times, whereas higher water temperature results in shorter induction and longer recovery times. Significant differences exist both between the anaesthetic agents and within each weight group of fish (33 and 1243 g) at both water temperatures tested (8 and 15 °C). Combination anaesthesia allows a reduction in the dosages for inducing anaesthesia and results in a reduced responsiveness to handling in small fish. Still, a vast majority of the fish of both weight groups maintain reflex reactions to the caudal peduncle pinch. As the dosages used in combination anaesthesia were almost halved compared with individually administered agents, there might be a possibility of increasing the dosages to obtain deeper anaesthetized fish and still meet the requirements of rapid induction and recovery. Whether one single agent or a combination of agents are used, it is always recommended that the anaesthetic protocol is tested on a few fish under prevailing conditions to ensure that the depth of anaesthesia is sufficient for the kind of handling or procedure the fish are being subjected to. In addition, analgesic treatment should always be used in relation to procedures that can be expected to cause pain.

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