Fish Physiol Biochem (2012) 38:201–218
DOI 10.1007/s10695-011-9565-1

Anaesthesia of farmed fish: implications for welfare

Inger Hilde Zahl · Ole Samuelsen · Anders Kiessling

Received: 30 December 2010 / Accepted: 8 October 2011 / Published online: 9 December 2011

Abstract During their life cycle as farmed animals, there are several situations in which fish are subjected to handling and confinement. Netting, weighing, sorting, vaccination, transport and, at the end, slaughter are frequent events under farming conditions. As research subjects, fish may also undergo surgical procedures that range from tagging, sampling and small incisions to invasive procedures. In these situations, treatment with anaesthetic agents may be necessary in order to ensure the welfare of the fish. The main objective of this paper is to review our knowledge of the effects of anaesthetic agents in farmed fish and their possible implications for welfare. As wide variations in response to anaesthesia have been observed both between and within species, special attention has been paid to the importance of secondary factors such as body weight, water temperature and acute stress. In this review, we have limited ourselves to the anaesthetic agents such as benzocaine, metacaine (MS-222), metomidate hydrochloride, isoeugenol, 2-phenoxyethanol and quinaldine. Anaesthetic protocols of fish usually refer to one single agent, whereas protocols of human and veterinary medicine cover combinations of several drugs, each contributing to the effects needed in the anaesthesia. As stress prior to anaesthesia may result in abnormal reactions, pre-anaesthetic sedation is regularly used in order to reduce or avoid stress and is an integral part of the veterinary protocols of higher vertebrates. Furthermore, the anaesthetic agents that are used in order to obtain general anaesthesia are combined with analgesic agents that target nociception. The increased use of such combinations in fish is therefore included as a special section. Anaesthetic agents are widely used to avoid stress during various farming procedures. While several studies report that anaesthetics are effective in reducing the stress associated with confinement and handling, there are indications that anaesthesia may in itself induce a stress response, measured by elevated levels of cortisol. MS-222 has been reported to elicit high cortisol release rates immediately following exposure, while benzocaine causes a bimodal response. Metomidate has an inhibitory effect on cortisol in fish and seems to induce the lowest release of cortisol of the agents reported in the literature. Compared to what is observed following severe stressors such as handling and confinement, the amount of cortisol released in response to anaesthesia appears to be low but may represent an extra load under otherwise stressful

I. H. Zahl · O. Samuelsen
Institute of Marine Research, P.O. Box 1870 Nordnes, 5817 Bergen, Norway
e-mail: inger.hilde.zahl@gmail.com

A. Kiessling (✉)
Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden
e-mail: Anders.kiessling@slu.se

A. Kiessling
Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Aas, Norway

circumstances. Furthermore, anaesthetics may cause secondary adverse reactions such as acidosis and osmotic stress due to respiratory arrest and insufficient exchange of gas and ions between the blood and the water. All in all, anaesthetics may reduce stress and thereby improve welfare but can also have unwanted side effects that reduce the welfare of the fish and should therefore always be used with caution. Finally, on the basis of the data reported in the literature and our own experience, we recommend that anaesthetic protocols should always be tested on a few fish under prevailing conditions in order to ensure an adequate depth of anaesthesia. This recommendation applies whether a single agent or a combination of agents is used, although it appears that protocols comprising combinations of agents provide wider safety margins. The analgesic effects of currently used agents, in spite of their proven local effects, are currently being debated as the agents are administrated to fish via inhalation rather than locally at the target site. We therefore recommend that all protocols of procedures requiring general anaesthesia should be complemented by administration of agents with analgesic effect at the site of tissue trauma.

Keywords Welfare · Anaesthesia · Sedation · Fish · Teleost

Introduction

Anaesthetic agents have been used for fish since the beginning of the last century (McFarland 1959; McFarland and Klontz 1969; Schoettger and Julin 1967). They derive partly from human and veterinary medicine and partly from other sources and were introduced to fish through trial and error. They were initially employed in order to make handling easier but improved knowledge of fish physiology and a better understanding of the importance of anaesthetic treatment to maintain welfare have led to an increased use.

Anaesthesia (from Greek: an- 'without' and aisthesis 'sensation') comprises several components, including sedation, immobilisation, unconsciousness (narcosis), amnesia (loss of memory) and analgesia (pain relief). Sedation is a reduction in sensitivity, which results in tranquillity and calmness. Narcosis (general anaesthesia) causes a state of unconsciousness and amnesia and also includes immobilisation and pain relief (analgesia). The components of anaesthesia can be obtained by various anaesthetic agents, each of which gives rise to one or several of them. In this review, the agents such as benzocaine, metacaine (MS-222), metomidate hydrochloride, isoeugenol, 2-phenoxyethanol and quinaldine are discussed as anaesthetic agents for fish. Traditionally, these agents have been used and are still used in fish to obtain different components of anaesthesia irrespective of the actual effect of the specific agent. Agents with known analgesic effect, used in human and veterinary medicine as local analgesics, such as benzocaine, are administrated to fish for the purpose of sedation, immobilisation, analgesia as well as general anaesthesia (narcosis). Agents are also applied indiscriminately of species. Anaesthetic protocols of new species that are introduced to research or cultivation are generally based on drugs and dosages developed for the more established species. For example, in Norway, the anaesthetic protocols of Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*), which were introduced to fish farming in the 1980s, have thus been based on the protocols used for salmonids, which have been farmed since the 1960s and are the most important species in Norwegian fish farming.

Anaesthetic protocols of human and veterinary medicine comprise combinations of several drugs, each contributing to one or more of the effects needed in the anaesthesia. Different drugs are applied for induction and maintenance, and analgesic treatment is used both under and following surgical procedures. The drugs are selected on the basis of their properties, tailored both to the surgical intervention and to the physiological state of the patient or the animal. Pre-anaesthetic sedation used in order to avoid stress prior to anaesthesia is an integral part of veterinary protocols. By combining drugs of different properties, a more complete anaesthesia is obtained than what is possible with one single substance alone. Further, synergistic effects between different drugs may also permit a reduction in the dosage of each drug compared to individual administration. This may result in smoother induction and recovery and reduce the incidence of adverse effects (Rang et al. 2003).

Anaesthesia of fish

Anaesthetic agents are now used widely, ranging from light sedation that aims to reduce stress during

handling and non-invasive procedures to full anaesthesia to avoid inflicting pain during surgery and larger interventions (Ackerman et al. 2005; Neiffer and Stamper 2009; Ross and Ross 2008; Summerfelt and Smith 1990). The most common route of administration is via inhalation. The anaesthetic agent is dispersed in the water and is absorbed across the gills. The effect is usually assessed by induction and recovery time, reflex reactions to external stimuli and responsiveness to handling.

The progress of induction and depth of anaesthesia are generally divided into distinct stages and 'planes' (Table 1). Common clinical indicators evaluated in order to determine stages and planes during anaesthesia of animals include behaviour, activity, corneal reflexes and pupil size, muscle tone, reflexes, respiratory rate, heart rate and blood pressure. As several of these indicators are difficult to assess in fish, it may be difficult to distinguish one stage or plane from the other, especially in situations where induction is rapid. In fish, the stages are therefore usually described by changes in swimming activity, balance, respiratory frequency and reactions to external stimuli (Table 1).

In order to obtain efficient surgical anaesthesia through inhalation using the agents described in this review, respiration will stop in spite of maintained heart rate and blood pressure (Kiessling et al. unpublished results). However, injecting a local anaesthetic at the site of incision will provide sufficient analgesic effect and allow surgical procedures at an anaesthetic depth where spontaneous respiration is maintained, corresponding to stage III, plane 2 (Table 1; Karlsson et al. 2011a; Kiessling et al. 2003, 2009).

Nociception in fish

Studies on rainbow trout and goldfish have demonstrated that fish possess the basic neural system necessary for nociception, i.e. perception of painful stimuli (Dunlop and Laming 2005; Sneddon 2002, 2003; Sneddon et al. 2003). Thus, to ensure the welfare of fish subjected to procedures that might inflict pain, anaesthetic agents with the ability to block nociceptive pathways are necessary. Of the agents assessed in this review, benzocaine and MS-222 possess this ability, as does isoeugenol. However, the route of administration may be of importance for the effect of this class of agents (local anaesthetics). Anaesthetic effect is produced locally at the site of administration, e.g. topically. Administration systemically has, on the other hand, been found inadequate for blocking peripheral nociceptive pathways in higher vertebrates (mice, rat, cat and man; Boas et al. 1982; Haegerstam 1979; Rigon and Takahashi 1996; Wiesenfeld-Hallin and Lindblom 1985; Woolf and Wiesenfeld-Hallin 1985). Haegerstam (1979) concluded that it was unlikely that peripheral pain pathways could be blocked by systemic i.v. injections

Table 1 Stages of anaesthesia in fish

Stage	Plane	Description stage/plane	Appearance	Swimming activity	Equilibrium	Responsiveness[a]	Muscle tone	Respiration	Heart rate
0		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
I		Light sedation	Disoriented	Reduced	Normal	Slightly reduced	Normal	Normal	Normal
II		Excitatory stage	Excited	Increased	Struggles to maintain balance	Normal or exaggerated	Normal	Irregular or increased	Irregular, may increase
III	1	Light anaesthesia	Anaesthetised	Stopped	Lost	Reacts to strong tactile stimuli	Decreased	Normal or decreased	Regular
	2	Surgical anaesthesia	Anaesthetised	Stopped	Lost	None	Relaxed	Shallow	Depressed
	3	Deep narcosis	Anaesthetised	Stopped	Lost	None	None	Nearly absent	Depressed
IV		Impending death	Moribund	Stopped	Lost	None	None	Stopped	Cardiac arrest

[a] Responsiveness refers to reaction to external stimuli. The stimulus may be visual or tactile

Adapted from Bell (1987), Burka et al. (1997), McFarland (1959), McFarland and Klontz (1969) and Summerfelt and Smith (1990)

of local anaesthetics. On the basis of these data, it is unclear whether a sufficient level of anaesthesia can be obtained peripherally when anaesthetic agents are administered to fish via inhalation. As the whole body surface of the fish is exposed to anaesthetics during bath immersions, i.e. topical administration in addition to inhalation, the agents will also have a peripheral effect. The absorption of small amounts of anaesthetic through the skin of the fish has been observed (Ferreira et al. 1984), but whether such amounts produce the peripheral analgesia needed for surgical procedures is not known. It is therefore important to determine whether a lack of response to nociceptive stimuli is due to a peripheral blockage of nociceptive afferent pathways or whether it is due to a general inhibition of the CNS. The latter may have severe welfare consequences as an inadequate blockage of nociceptive pathways may result in sensitisation and thus a reduction in threshold and an increased response to noxious stimuli. Work by Zahl et al. (2009, 2011) indicates that neither of the agents covered in this review administered via inhalation either individually or in combination blocks peripheral nociceptive pathways in fish. Kiessling et al. (1995, 2003) show that this is obtained when lidocaine is administered locally during combination anaesthesia with metomidate and MS-222 administered via inhalation. In the studies by Zahl et al. (2009, 2011), lower dosages in combination anaesthesia resulted in similar induction time as individually administrated agents. However, the fish displayed increased reactions to nociceptive stimulation although agents blocking nociception were used. This is possibly due to an enhanced central effect without a parallel increase in effect peripherally. Since all surgical procedures are associated with nociception, our conclusion must therefore be that any such procedure should never be conducted without local injections of anaesthetic agents with analgesic effect.

Anaesthetic agents

A wide range of anaesthetic agents is currently used for fish. Among the most common are MS-222, benzocaine, isoeugenol, metomidate, 2-phenoxyethanol and quinaldine (Ackerman et al. 2005; Neiffer and Stamper 2009; Ross and Ross 2008; Summerfelt and Smith 1990). Dosage recommendations are listed in Table 2. The sites of action in the nervous system, i.e. central or peripheral, for these drugs are not well known in fish, and most knowledge is based on that of higher vertebrates. There are many reasons for applying this knowledge in fish with caution. First, there are more than 30,000 different species of fish (Froese and Pauly 2011) that inhabit highly diverse environments, which vary in factors like salinity, temperature, chemistry and depth. Secondly, and possibly the most important and most often overlooked fact, the route of administration may differ both between different species and between different agents, *i.m.* for local anaesthetics vis-à-vis *i.v.* or inhalation for general anaesthetics. Finally, fish and higher vertebrates, particularly mammals, differ in the development of the central nervous system, while their peripheral nervous systems seem to be surprisingly similar (see e.g. Sneddon et al. 2003).

Metacaine and benzocaine

Metacaine (ethyl 3-aminobenzoate, tricaine methanesulphonate and MS-222) and benzocaine (ethyl 4-aminobenzoate) are the two most common anaesthetics for fish used in Norway (Anon 2007). They are approved for use in food fish production with a withdrawal period of 21 days. MS-222 and benzocaine are local anaesthetics that act by blocking voltage-sensitive sodium channels (Frazier and Narahashi 1975; Neumcke et al. 1981), thereby preventing the voltage-dependent increase in sodium conductance. This inhibits the initiation and propagation of action potentials in excitable cells; thus, local anaesthetics block most neurons and muscle cells and may cause paralysis in addition to blocking nociception. This class of drugs is used in human and veterinary medicine for local analgesia. When administrated to fish via bath immersion, they enter the circulation and produce general anaesthesia by inhibiting neural signal transmission ranging from the periphery to higher parts of the nervous system. The precise mechanism of action in the central nervous system is not fully understood (Hara and Sata 2007; Hedrick and Winmill 2003; Ueta et al. 2007). Many of the adverse effects caused by this class of drugs in man are related to effects on the CNS and the cardiovascular system (Rang et al. 2003).

A range of adverse effects of MS-222 and benzocaine have been reported in fish. The initial response to these agents is characterised by a period of raised heart

Table 2 Anaesthetic agents—dosage and exposure time recommendations by manufacturers/distributors

Anaesthetic agent	Sedation		Anaesthesia, light		Anaesthesia		Species
	Dosage (mg l^{-1})	Exposure (min)	Dosage (mg l^{-1})	Exposure (min)	Dosage (mg l^{-1})	Exposure (min)	
MS-222[a]	10–30	>480	30–80	>30	80–180	>10	Trout species
MS-222[a]	7–30	>240	30–80	>10	80–100	>5	Salmon species
MS-222[b]					60	5	Atlantic cod
Benzocaine[c]					30–40	2–5	Salmon and trout
Isoeugenol[d]	<7.5				7.5–10	12–15	Salmon species
Metomidate[e]	0.25–1	>480			5–10	>60	Various species
2-phenoxyethanol[f]					0.1–0.5[g]	3	Various species
Quinaldine[f]					15–40	2–4	Salmonid species

[a] Pharmaq AS, Oslo, Norway

[b] ScanVacc AS, Aarnes, Norway

[c] A.C.D Pharmaceuticals AS, Leknes, Norway

[d] Aqui-S, New Zealand Ltd

[e] Syndel International Inc., Vancouver, Canada

[f] Ackerman et al. (2005)

[g] The dosage of 2-phenoxyethanol is ml l^{-1}

rate and respiration accompanied by elevated levels of blood glucose, followed by a depression of cardiovascular and respiratory function that eventually may come to a complete stop (Hill et al. 2002; Houston et al. 1971a; Lochowitz et al. 1974; Randall 1962; Ryan 1992). This results in hypoxaemia, observed as changes in the partial pressure of arterial O_2 and CO_2, accompanied by lower blood glucose levels and increased levels of lactate, hematocrit and haemoglobin, and erythrocyte swelling (Bourne 1984; Hill and Forster 2004; Holloway et al. 2004; Houston et al. 1971b; Iwama et al. 1989; Ortuno et al. 2002; Ryan 1992; Soivio et al. 1977; Thomas and Robertson 1991; Velisek et al. 2009). Elevated plasma catecholamine levels have also been observed (Gingerich and Drottar 1989; Iwama et al. 1989; Wedemeyer 1970) Benzocaine has also been found to have immunodepressant effects (Cuesta et al. 2004; Ortuno et al. 2002).

Metomidate

Metomidate hydrochloride (methyl 3-(1-phenylethyl) imidazole-4-carboxylate hydrochloride) is a methyl analogue of the imidazole derivate etomidate, which activates and modulates inhibitory gamma-aminobutyric acid type A ($GABA_A$) receptors, thus affecting higher regions of the nervous system (Ashton and Wauquier 1985; Yang and Uchida 1996). Activation of the $GABA_A$ receptor triggers opening of a chloride ion-selective pore. The increased chloride conductance drives the membrane potential towards the reversal potential of the Cl^- ion, thus inhibiting the firing of new action potentials. $GABA_A$ receptor activation produces sedation and hypnosis, but limited analgesia and immobilisation (Grasshoff et al. 2006).

Etomidate is commonly used in human and veterinary medicine as a sedative and for inducing anaesthesia (Ching and Baum 2009; Darrouj et al. 2009; Falk and Zed 2004; Sams et al. 2008). One of the side effects of the drug in addition to respiratory and cardiovascular depression is suppression of adrenal steroidogenesis, thus leading to an inhibition of cortisol synthesis (Vanden Bossche et al. 1984; Wagner et al. 1984; man, cow and rat). Metomidate, in parity with etomidate in mammals, has also been found to have an inhibitory effect on cortisol in fish (Davis and Griffin 2004; Olsen et al. 1995; Small 2004; Thomas and Robertson 1991) but the efficiency and dose dependence of this inhibition are still unclear (Eliason et al. 2007; Olsen et al. 1995; Sandodden et al. 2001). However, a number of reports indicate cortisol release in spite of metomidate treatment (Kiessling, et al. 2009; Zahl et al. 2010). Other adverse effects of metomidate in fish are related to

depressant effects on respiration and circulation that subsequently lead to hypoxaemia, observed as a decrease in partial pressure of O_2, elevated partial pressure of CO_2 and reduced pH of the blood (Hill and Forster 2004; Hill et al. 2002; Iwama et al. 1989).

2-Phenoxyethanol

2-Phenoxyethanol is a compound with antibacterial properties used as a preservative in vaccines, in dermatological products and as fixative for perfumes. To the best of our knowledge, the exact mechanism of its anaesthetic effect in fish has not been reported, but it has been suggested that it involves an expansion of neuronal cell membranes (Burka et al. 1997). 2-Phenoxyethanol has been found to inhibit the activity of excitatory *N*-methyl-D-aspartate (NMDA) receptors in *Xenopus* oocytes (Musshoff et al. 1999), and its anaesthetic effect may thus be related to the suppression of neural activity in higher regions of the nervous system. Activation of the NMDA receptors increases neuron excitability by lowering the threshold for firing of action potentials due to opening of ion channels that cause influx of positively charged ions, especially Ca^{2+}, Na^{+} and K^{+}. NMDA receptor inhibition has been linked to an analgesic effect (Grasshoff et al. 2006).

Adverse effects of 2-phenoxyethanol include reduced ventilation, decreased heart rate and blood pressure, reduced blood partial pressure of O_2 accompanied by increased CO_2 and decreased blood pH and elevated plasma levels of adrenaline and glucose (Fredricks et al. 1993; Iwama et al. 1989; Lambooij et al. 2009; Ortuno et al. 2002). 2-Phenoxyethanol has also been found to have immunodepressant effects (Cuesta et al. 2004; Ortuno et al. 2002).

Isoeugenol

Isoeugenol (2-methoxy-4-prop-1-enyl-phenol) is an oily liquid found in ylang–ylang and other essential oils. It is structurally similar to eugenol, a widely used analgesic in dentistry that inhibits sodium, potassium and calcium channels, inhibits NMDA receptors and potentiates $GABA_A$ receptors (Aoshima and Hamamoto 1999; Lee et al. 2005; Li et al. 2007; Park et al. 2006; Wie et al. 1997). Like eugenol, isoeugenol is one of the constituents of clove oil, another widely used fish anaesthetic. Isoeugenol is the active ingredient in Aqui-S®, which has approval for use in food fish production in New Zealand, Australia and Chile, Costa Rica and Republic of Korea with no withdrawal period. In Norway, Aqui-S® is used for brood stock and in research.

Adverse effects of isoeugenol (Aqui-S®) in fish include reduced ventilation and depression of the cardiovascular system, which result in slower heart rate and decreased cardiac output, in addition to reduced blood pressure and vascular tone (Hill and Forster 2004; Hill et al. 2002; Rothwell and Forster 2005). Elevated plasma levels of catecholamines have also been observed, as has increased haematocrit (Hill and Forster 2004). Eugenol works synergistically with d-tubocurarine; it is therefore believed to block $nicotine_2$ receptors at the motor end plate in muscle (Brodin and Røed 1984). $Nicotine_2$ receptor blockade causes paralysis without analgesic or hypnotic effect. This effect has since been confirmed for isoeugenol by Ingvast-Larsson et al. (2003), who used a rat phrenic nerve–diaphragm muscle preparation.

Quinaldine

Quinaldine (2-methylquinoline) is a colourless oily liquid that has been used to anaesthetise fish since early in the last century. To the best of our knowledge, its precise mechanism of action has not been reported.

Quinoline family compounds possess antiseptic and antipyretic properties. They are widely used as parent compounds to make drugs, especially anti-malarial medicines, and are also used in fungicides, biocides, alkaloids, dyes, rubber chemicals and flavouring agents.

Fish anaesthetised with quinaldine respond by increasing their heart rate, followed by bradycardia and impaired respiratory function (Lochowitz et al. 1974). Elevated concentrations of plasma cortisol and glucose have also been observed, as have increased levels of serum IgM (Cuesta et al. 2004; Davis and Griffin 2004; Ortuno et al. 2002).

Combination anaesthesia

Current protocols of fish do not typically include administration of combinations of anaesthetics. Schoettger and Steucke (1970) examined the possible synergistic effects of combination anaesthesia consisting of a mixture of MS-222 and quinaldine in rainbow trout (*Oncorhynchus mykiss*) and northern

pike (*Esox lucius*) and found that the mixture resulted in safer and more effective anaesthesia. The same mixture tested in 14 freshwater species showed that the dosages needed when administered in combination were considerably lower than when each drug was used alone (Gilderhus et al. 1973). However, it has also been found that not only efficacy but also toxicity increased when these drugs were combined (Dawson and Marking 1973). Synergistic effects have also been found in combination anaesthesia using a mixture of quinaldine sulphate and the benzodiazepine diazepam (Kumlu and Yanar 1999; Yanar and Kumlu 2001). Administered in combination with diazepam, quinaldine sulphate could be used in lower dosages in anaesthesia of both gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Hyperactivity and excitement, unwanted side effects of quinaldine sulphate, were reduced, and there were no mortalities (Kumlu and Yanar 1999; Yanar and Kumlu 2001). Combinations consisting of MS-222 anaesthesia and injections of local analgesics during surgery have been studied in koi carp (*Cyprinus carpio*; Harms et al. 2005). Injections of the opiate butorphanol during surgery reduced post-surgery behavioural changes, while the non-steroidal anti-inflammatory drug (NSAID) ketoprofen reduced muscle tissue damage. Protocols that include both pre-anaesthetic sedation and local analgesic treatment in addition to general anaesthesia have been successfully employed in anaesthesia of salmonids undergoing surgery for insertion of dorsal aorta and portal vein cannulae (Eliason et al. 2007; Karlsson et al. 2006; Kiessling et al. 1995, 2003). Pre-anaesthetic sedation with metomidate followed by full anaesthesia with MS-222 combined with injections of lidocaine at the site of incision improved recovery in comparison with MS-222 used individually, and fish quickly resumed their normal behaviour (Eliason et al. 2007; Karlsson et al. 2006; Kiessling et al. 1995, 2003). The response of Atlantic cod subjected to caudal artery cannulation using pre-anaesthetic sedation with metomidate has been found to be markedly different from that of salmonids, in that the former display a delay rather than a reduction in post-surgery cortisol release, and a prolonged recovery, measured as blood glucose, lasting from 3 to 5 days (Karlsson et al. 2011b). Zahl et al. (2009) found that pre-anaesthesia sedation of Atlantic cod with metomidate permitted a significant reduction in the dosage of anaesthetic to be made, with no change in induction or recovery time. However, the same authors report that Atlantic cod, subjected to pre-anaesthetic sedation with metomidate and the reduced dose of anaesthetic, displayed an increased response to external stimulation in the form of a caudal peduncle pinch. They also report that exposing Atlantic cod to pre-anaesthetic stress instead of pre-anaesthetic sedation resulted in the same induction time in spite of a lower dose of anaesthetic. These results clearly underline the fact that the effect of an anaesthetic agent varies with pre-anaesthetic stress and show that the use of an anaesthetic protocol that includes a combination of pre-sedation and a reduced anaesthetic dosage may produce an acceptable hypnotic effect without a sufficient analgesic effect.

Variations in response

There are substantial variations between fish species in response to anaesthetic agents, as well as large individual differences within each species. Variations may be the result of pharmacokinetic differences, usually described as what the body does to the drug, and by pharmacodynamic differences, usually described as what the drug does to the body. Both pharmacokinetic and pharmacodynamic differences in fish may be influenced by biological factors such as age, sex, life stage, body weight, growth rate, body composition, physiological condition and health status, as well as environmental factors such as water temperature, salinity, pH and oxygen level.

In the majority of assessments of anaesthesia in fish, the fish have been anaesthetised to a predetermined stage, often corresponding to loss of equilibrium, no response to external stimuli and reduced or even stopped respiration (Table 1, stage III, plane 3 to stage IV) and thereafter directly moved to clean water for recovery (Hoskonen and Pirhonen 2004; Mattson and Riple 1989; Mylonas et al. 2005; Stehly and Gingerich 1999; Sylvester and Holland 1982). As the induction time may differ between individual fish, exposure time has varied accordingly. Another approach is to impose an identical exposure time for all fish. When comparing the results of different studies, it is important to distinguish between these two approaches. The duration of the exposure should always be kept within an adequate margin of safety, as practical situations often involve anaesthesia of a large

number of fish in one tank, with exposure time usually exceeding the induction time for individual fish.

Pharmacodynamics

The pharmacodynamic characteristics of a drug describe the consequences of the interaction between the drug and the receptor or other primary sites of action and include all effects and adverse effects. As described earlier, anaesthetic agents may act by binding to receptors or by interacting with ion channels. As several types of tissue may contain the target molecules of a drug, the primary responses may induce secondary responses or side effects that subsequently affect the primary response. Pharmacodynamic effects may also influence the pharmacokinetic processes. Thus, an effect on respiration and circulation will also be of importance for the absorption, distribution and elimination of a drug.

Pharmacodynamic properties studied in Atlantic cod (Zahl et al. 2009) and Atlantic halibut (Zahl et al. 2011) include wide differences within and between these two species as well as between the agents. Induction time, defined as time of loss of equilibrium, occurred within 3 min for all agents tested, thus easily meeting one criterion of suitability for fish anaesthetic agents (Bell 1987; Marking and Meyer 1985). The recovery time differed widely between the agents. Metomidate produced the longest recovery time in Atlantic cod and was together with isoeugenol and 2-phenoxyethanol one of the agents that gave rise to the longest recovery time in Atlantic halibut. This agrees with pharmacokinetic data that indicate that a slower elimination and a prolonged recovery might be the expected outcome in agents of higher lipid solubility (Guenette et al. 2007; Hansen et al. 2003; Kiessling et al. 2009; Kildea et al. 2004). The recovery time in Atlantic cod reported by Zahl et al. (2009, 2011) was within the range defined for suitable anaesthetics, i.e. 5 min, (Bell 1987; Marking and Meyer 1985) for all agents except for metomidate, but exceeded this in Atlantic halibut. However, as the parameter 'recovery time' was recorded differently in Atlantic halibut and in Atlantic cod, values for recovery time need to be compared with caution. In pelagic fish such as Atlantic cod, recovery time is normally defined as time of recovery of equilibrium, whereas in Atlantic halibut, a bottom dwelling flatfish, recovery time is more difficult to assess. In the work by Zahl et al. (2011), recovery time was set at when the halibut managed to right itself after having been placed upside down in a recovery tank.

The agents studied by Zahl et al. (2009, 2011) differed greatly in their capacity to depress responses to external stimulation, assessed during handling of the anaesthetised fish and by a caudal peduncle pinch. Reflex reactions to the pinch and responsiveness to handling were most effectively reduced by two local anaesthetics MS-222 and benzocaine. This is probably related to the mode of action, as these substances suppress signal transmission in both central and peripheral nervous system. The result also tallies well with findings in higher vertebrates, in which systemic injections of local anaesthetics did not block peripheral nociceptive pathways, but merely increased the response threshold. Suppression of nerve signals at central level may have adverse effects. After being inhaled by the fish, the agents enter the circulation, where they may be distributed throughout the body. By passing the blood–brain barrier, these agents affect the brain and the centres that control respiration and circulation. A depression of respiration and circulation would produce hypoxaemia, metabolic acidosis and changes in blood electrolytes and lead to extended recovery time, thus enhancing the effects of the anaesthetic.

Pharmacokinetics

In order to induce a biological response, a drug must be present in a sufficient concentration at the receptor site for a certain length of time. The concentration achieved at the receptor site depends on the pharmacokinetic properties of the drug, i.e. how it is absorbed, distributed, metabolised and eliminated. In fish, drug administration via bath immersion is equivalent to inhalation anaesthesia in human and veterinary medicine. The processes of absorption and elimination are determined by the diffusion rate through the gill epithelium, which mainly depends on the lipid solubility, ionisation and polarity of the drug. Following absorption, a fraction of the drug binds to plasma protein, mainly albumin, while the rest remains unbound and pharmacologically active. The unbound drug is then distributed to various parts of the body, commonly separated into compartments, where it exerts its effect. The main compartments are plasma, interstitial fluid, intracellular fluid and adipose tissue.

Lipid solubility and ionisation are important factors in determining the distribution of the drug. High lipid solubility results in rapid diffusion through the cell membranes and the possible accumulation of the drug within hydrophobic compartments such as the adipose tissue and the lipid bilayers of cell membranes, while ionised molecules and molecules with high polarity are less lipid-soluble and do not easily pass through biological membranes.

The ionisation of weak acids and bases is determined by the dissociation constant p*K*a, defined as the pH at which 50% of the acid or base is in ionic form (p*K*a, Table 3). When a compound is allowed to distribute itself between equal volumes of two immiscible liquids, the ratio of the concentration of the solute in two phases at equilibrium is called the partition coefficient (logP). For drugs, two phases used are octanol and an aquatic buffer with pH of 7.4 (Table 3). As the logP value describes the partition of neutral uncharged molecules, it is not an exact measure of the distribution of ionisable compounds such as many anaesthetic agents. Anaesthetic agents are usually associated with high lipid solubility, which may lead to accumulation in adipose tissue both during long exposures and following repeated exposures (Rang et al. 2003).

In fish, anaesthetics are mainly eliminated via the gills in unchanged form (Hayton et al. 1996; Hunn and Allen 1974; Hunn et al. 1968; Meinertz et al. 1991; Stenger and Maren 1974). Some may also undergo metabolism before elimination (Hayton et al. 1996; Meinertz et al. 1991; Ryan 1992; Stenger and Maren 1974).

Models are used to describe the pharmacokinetic processes. Plasma concentration–time curves are employed to estimate the pharmacokinetic parameters and perform compartmental analyses to describe drug distribution. In the simplest compartmental pharmacokinetic model, the body is regarded as a single compartment throughout which the drug is distributed evenly following absorption. This model, called the one-compartment open model with first-order elimination, is a simplification used to illustrate basic pharmacokinetic principles. A two-compartment open model includes a peripheral compartment representing the tissue besides the central compartment, which represents the plasma, thereby introducing a distribution process into the model. From the central compartment, the drug can be removed both by distribution to the peripheral compartment and by elimination. The process of distribution is described by the initial rapid decrease in plasma concentration of the drug and is called the α–phase. When the distribution is complete, the elimination of the drug is determined by the slower β-phase.

The distribution of a drug between the plasma and the rest of the body is described by the pharmacokinetic parameter volume of distribution (Vd). The Vd indicates how a drug relocates into body compartments relative to the blood, and is a non-physiological volume that corresponds to the volume that would be necessary to dilute the drug to produce the concentration present in the blood. Lipophilic drugs that have a tendency to accumulate in body fat thus have high Vd values, while drugs with a high degree of plasma protein binding have low Vd values. The rate at which the drug is removed from the blood is described by the parameter clearance (C), which is defined as the volume of blood or plasma that is cleared per unit time. Together with clearance, the volume of distribution determines the plasma half-life ($t_{1/2}$) of a drug. A study by Kiessling et al. (2009) revealed large differences in the pharmacokinetics of various anaesthetics. MS-222, the most hydrophilic of the agents tested (Table 3), was distributed rapidly, followed by rapid clearance and elimination. The plasma data were best described by a one-compartment open model with first-order elimination, which indicates that distribution was homogenous and elimination proportional to plasma concentration. The plasma data of benzocaine and isoeugenol were best described by a two-compartment open model, indicating a rapid transfer of drug from plasma to tissues followed by the slower process of elimination from the plasma. Both benzocaine and isoeugenol were found by Kiessling et al. (2009) to be rapidly distributed, but their rates of clearance and elimination were much slower than for MS-222. An initial phase of rapid distribution followed by a period of slow elimination has also been found in rainbow trout anaesthetised with benzocaine (Meinertz et al. 1996; Stehly et al. 1998). The pharmacokinetic properties of benzocaine in rainbow trout were best described by a two-compartment model when benzocaine was administered through bath immersion, while a three-compartment model best described the properties of the distribution and clearance process following intra-arterial injection (Meinertz et al. 1996; Stehly et al. 1998). Guenette

Table 3 log *P* (octanol:water), p*K*a and water solubility of anaesthetic agents

Anaesthetic	log *P*	p*K*a	Water solubility (mg l^{-1})
MS-222	1.8	3.8[b]	1.0×10^5
Benzocaine	1.9	2.5	1.3×10^3
Metomidate hydrochloride[a]	3.1	4.5[c]	6.3×10
2-phenoxyethanol	1.2	15.1	2.7×10^4
Isoeugenol	3.0	9.9	3.6×10^2
Quinaldine	2.6	5.7	5.0×10^2

Source: ChemIDplus, U.S. National Library of Medicine

[a] The values indicated for log *P*, p*K*a and water solubility are applicable to etomidate

[b] Source: Stenger and Maren (1974)

[c] Source: Levron and Assoune (1990)

et al. (2007) found that eugenol, which is similar to isolegenol, was well absorbed and eliminated by rainbow trout. The plasma concentration versus time curve was biphasic, which indicates two compartments. A biphasic plasma elimination curve was also found in the elasmobranch dogfish (*Squalus acanthias*) following arterial injection of MS-222 (Stenger and Maren 1974). MS-222 was excreted rapidly across the gills, mainly in unchanged form. Kiessling et al. (2009) found that artificial ventilation had no influence on the compartment analysis. However, elimination rates were faster in the groups of fish that received artificial ventilation, demonstrating the importance of the gills as a pathway for drug elimination. This is in agreement with the previous reports in other species (Hayton et al. 1996; Hunn and Allen 1974; Meinertz et al. 1991) and emphasises the importance of respiratory function, i.e. exchange of water over the gills, during recovery (Table 4).

Water temperature

Temperature is an important factor in determining the rate of physiological processes in ectothermic animals such as fish and thus plays an important role in the processes related to the uptake and elimination of drugs. A 10°C rise in water temperature (Q_{10}) typically doubles the basal metabolic rate of teleost fish (Clarke and Johnston 1999). This leads to a higher oxygen demand that is met by enhanced respiration, increased cardiac output and increased blood flow through the gills (Graham and Farrell 1989; Nilsson and Sundin 1998; Webber et al. 1998). Poorer oxygen solubility due to rising water temperature leads to an additional need to enhance respiration and blood flow.

Temperature increases have been reported to shorten induction and recovery time for a number of anaesthetic agents in several teleost species, including benzocaine in striped bass (*Morone saxatilis*; Gilderhus et al. 1991), 2-phenoxyethanol and clove oil in European sea bass and gilthead sea bream (Mylonas et al. 2005), isoeugenol in rainbow trout (Stehly and Gingerich 1999), clove oil in rainbow trout, brown trout (*Salmo trutta*), Atlantic salmon, whitefish (*Coregonus lavaretus*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) (Hoskonen and Pirhonen 2004; Woolsey et al. 2004), and benzocaine, MS-222, metomidate and 2-phenoxyethanol in Atlantic cod (Zahl et al. 2009). Reduced induction time at higher water temperatures has also been demonstrated in common carp (*Cyprinus carpio*), rainbow trout, fathead minnows (*Pimephales promelas*) and Atlantic halibut anaesthetised with MS-222 and in Atlantic halibut anaesthetised with benzocaine (Hikasa et al. 1986; Houston and Woods 1976; Sylvester and Holland 1982; Zahl et al. 2011).

The rapid induction time seen at higher water temperature may be related to higher basal metabolic rate at higher temperatures (Clarke and Johnston 1999; Saunders 1963; Schurmann and Steffensen 1997) and the corresponding increase in oxygen demand leading to increased respiration and circulation. This facilitates a rapid absorption and distribution of anaesthetic as well as faster clearance rates but may also lead to a greater amount being absorbed. While faster clearance rates of both benzocaine and isoeugenol at higher temperature were found in rainbow trout and silver perch (*Bidyanus bidyanus*) (Kildea et al. 2004; Stehly et al. 1998), significantly higher concentrations of isoeugenol were found in rainbow trout fillets after exposure at 17°C than at 7°C (Meinertz et al. 2006). Zahl et al. (2009) report that Atlantic cod made a quicker recovery at higher water temperature and had a wider therapeutic window, indicating a more rapid clearance and elimination. Atlantic halibut, on the other hand, seem to recover more slowly at higher temperatures, but in spite of longer recovery time the fish displayed stronger reactions to handling, indicating a lack of anaesthetic effect (Zahl et al. 2011). In large Atlantic cod (1,000 g) anaesthetised at a low water temperature (8°C), the dosage of both

Table 4 Effects of different anaesthetic agents in fish

Anaesthetic agent	Mechanism of action	Effects
Metacaine and Benzocaine	Blocks Na^+ channels	Blocks most neurons, glands and muscle cells (both striated, cardiac and smooth). May, in addition to nocicpetion, cause paralysis and respiratory depression. Adverse effects in fish include depression of cardiovascular and respiratory function, increased lactate and elevated catecholamine levels. Local analgesic effects is questionable when administered via inhalation
Metomidate	Activates $GABA_A$ receptors	Produces sedation and hypnosis. No nociception blockage. Adverse effects in fish include respiratory and cardiovascular depression and inhibition of cortisol synthesis
Isoeugenol	Inhibits Na^+, K^+ and Ca^{2+} channels Potentiates $GABA_A$ receptors Inhibits NMDA receptors	Produces analgesia and sedation. Adverse effects in fish include reduced ventilation, heart rate and cardiac output, blood pressure and vascular tone
2-phenoxyethanol	Not reported	Adverse effects in fish include reduced ventilation, heart rate and blood pressure. Efficiency is indicated as low in some species
Quinaldine	Not reported	Adverse effects in fish include reduced ventilation and heart rate. Efficiency is indicated as low in some species

metomidate and MS-222 had to be reduced, while for benzocaine no suitable dosage was found (Zahl et al. 2009). This was due to the fact that after five minutes of exposure to benzocaine the fish reacted both to handling and to the caudal peduncle pinch, but suffered respiratory arrest and died in the recovery bath. The findings in Atlantic cod agree with those in brown trout and goldfish, in which higher sensitivity was seen at lower water temperature during anaesthesia with benzocaine and 2-phenoxyethanol, respectively (Dawson and Gilderhus 1979; Weyl et al. 1996). However, the findings contradict with observations in striped bass, where higher dosages of benzocaine were used at lower water temperature for fish of large body weight (Gilderhus et al. 1991). In an evaluation of Aqui-S® in rainbow trout, reduced toxicity was found at higher temperature (Stehly and Gingerich 1999).

In the study on Atlantic halibut (Zahl et al. 2011), only MS-222 and benzocaine were tested at higher water temperature (15°C), but even so, the data indicate that in this species the central and peripheral effects of the anaesthetics are differently influenced by the temperature increase in comparison with Atlantic cod (Zahl et al. 2009). This might be explained in part by central effects in Atlantic halibut being more pronounced at higher water temperature, while the peripheral effects are less so, resulting in greater responsiveness and fish that regain equilibrium more slowly. Zahl et al. (2011) report that this effect is most prominent with benzocaine, which may reflect higher lipid solubility of the drug.

Anaesthetic agents are associated with high lipid solubility and a tendency to accumulate in tissues with a high content of fat (Rang et al. 2003). This might be a problem following repeated or long-duration exposures, for instance during larger surgical procedures.

Uptake of fat from the diet, storage in adipose tissue and the incorporation of lipids into membranes all vary with temperature. There is greater incorporation of unsaturated fatty acids and increased aggregation of hydrophobic substances in membranes as temperature falls (Berge et al. 1980; Cossins 1983; Ruyter et al. 2006; Sellner and Hazel 1982). Substances with higher lipid solubility such as benzocaine, isoeugenol and metomidate may therefore penetrate and possibly also accumulate in the lipid bilayer of cell membranes more readily at lower temperatures than more hydrophilic substances such as MS-222. Any accumulation would then alter the characteristics of the membranes and affect normal cell function by influencing transport mechanisms, stabilising the membranes and possibly inhibiting nervous signal propagation. If so, exposure time would influence this effect of membrane lipid changes and could explain some of the contradictory effects of temperature found in the literature. Anaesthetic depth has been associated with

the concentration of anaesthetic agent in the brain. This has been reported for snapper (*Pagrus auratus)* and several freshwater species anaesthetised with MS-222, as well as sting ray (*Dasyatis sabina*) and lemon shark (*Negaprion brevirostris*) anaesthetised with quinaldine (Brown et al. 1972; Hunn 1970; Ryan 1992). The brain receives a large supply of oxygen-rich blood from the gills, and the blood–brain barrier is permeable to anaesthetic agents. Accumulation in the brain probably leads to an enhanced central effect, with depression of brain centres controlling respiratory and cardiovascular function as a consequence.

Body weight

The relationship between body weight and sensitivity to anaesthetics may be influenced by several characteristics related to body weight, such as age, body composition, growth rate and sexual maturity, as all of these characteristics influence the physiology of the fish. Since the major route of both uptake and elimination is through the gills, the rate of oxygen consumption, the ratio of body volume to gill surface area and the rate of gill perfusion are of great importance. These factors vary within and between species and with life stage and life style as well as with body size. The gill surface area decreases in relation to increased body weight (Muir 1969; Oikawa and Itazawa 1985; Oikawa et al. 1999). The basal metabolic rate falls relative to increasing body size, and larger animals thus have lower oxygen consumption relative to body size than smaller animals (Clarke and Johnston 1999; Schmidt-Nielsen 1984). The scaling relationship between metabolic rate and body weight is usually described by the equation $\mathrm{Vo}_2 = a\, M_\mathrm{b}^{0.75}$, where the metabolic rate (Vo_2) scales as the 0.75 power of body mass (M_b) and the constant a indicates the level of the resting metabolic rate (Schmidt-Nielsen 1984). The constant a may differ between species and varies in relation to lifestyle, and in ectotherm animals such as fish, it is highly dependent on ambient temperature. For teleost fish, a scaling exponent of 0.8 has been found (Clarke and Johnston 1999). Sedate and less active fish species have a lower resting metabolic rate than more active species (Morris and North 1984). Clarke and Johnston (1999) found that the resting metabolic rate of fish varied between taxonomic groups, gadoids having higher resting metabolic rates than pleuronectiformes and salmoniformes, although there was a large degree of overlap.

Reports regarding the importance of body weight for the effect of anaesthetic agents in fish are contradictory. Some studies show no relationship between body size and induction and recovery time (Houston and Woods 1972; Stehly and Gingerich 1999), while others indicate that such a relationship exists (Gilderhus and Marking 1987; Houston et al. 1976; Olsen et al. 1995). Hoskonen and Pirhonen (2004) observed a shortening of induction time with increasing body size in whitefish, but observed the opposite relationship in rainbow trout, and found no size-related differences in Atlantic salmon or brown trout. Weber et al. (2009) found that induction time increased with increasing body weight in Senegalese sole (*Solea senegalensis*) anaesthetised with 2-phenoxyethanol, metomidate and clove oil, while this was not found for MS-222. The dynamics of the recovery time were more complex and were only weight-related for MS-222 (Weber et al. 2009).

Zahl et al. (2009, 2011) found no uniform relationship between the effects of anaesthetic agents and the body weight of the fish in either Atlantic cod or Atlantic halibut. For Atlantic cod, the results suggest that the body weight of the fish, or factors related to body weight, is less important for the differences in effect than the properties of the anaesthetic agents themselves.

Stress

When fish are subjected to handling, such as crowding or dip-netting, they respond by attempting to escape, which increases oxygen consumption. Paralleling the alterations in behaviour, a cascade of physiological reactions makes up the physiological stress response, first termed the General Adaptation Syndrome (see e.g. Selye 1985). The physiological stress response in fish is similar to that described in other vertebrates and is characterised by three phases (see Wendelaar Bonga 1997, for details). Large species differences in the magnitude of the cortisol response to stress have been found (Barton 2002; Barton and Iwama 1991), and high and low responders within species have also been identified (Fevolden et al. 1999; Mommsen et al. 1999; Pottinger and Carrick 1999; Pottinger et al. 1992, 1994; Sumpter et al. 1986; Tanck et al. 2001; Trenzado et al. 2003).

Stressed animals display abnormal reactions to anaesthesia and may require larger doses for both induction and maintenance (Hall et al. 2001). In fish, increased physical activity while attempting to escape handling, followed by the cascade of physiological alterations of the stress response, contributes to facilitating anaesthetic uptake. The elevated levels of catecholamines and corticosteroids further stimulate ventilation and enhance cardiac output, in turn increasing gill blood flow (Farrell 1984; Graham and Farrell 1989; Nilsson and Sundin 1998). The perfusion of the gill lamellae increases and lamellae that in a normal resting state are barely or not perfused at all are recruited, expanding the respiratory surface area and facilitating diffusion, and thereby affecting the uptake of substances across the gill.

Several reports show that stress associated with handling of fish can be effectively reduced by anaesthetic agents. Metomidate has been found to reduce handling stress in Atlantic salmon, Chinook salmon (*Oncorhynchus tshawytscha*), hybrid striped bass (*Morone chrysops* x *Morone saxatilis*), channel catfish (*Ictalurus punctatus*) and red drum (*Sciaenops ocellatus*), but this may be related to the cortisol blocking side effects of this drug. Isoeugenol has been shown to be effective in reducing stress in Atlantic salmon and channel catfish and MS-222 inhibits the stress response to handling in rainbow trout and channel catfish (Davis and Griffin 2004; Iversen et al. 2003; King et al. 2005; Kreiberg and Powell 1991; Olsen et al. 1995; Small 2003, 2004; Small and Chatakondi 2005; Thomas and Robertson 1991). However, it has also been found that exposure to anaesthetics may itself induce a stress response. Of the agents discussed in this review, MS-222 has been found to induce stress in Atlantic salmon, rainbow trout, Atlantic cod, Atlantic halibut, channel catfish, striped bass, hybrid striped bass, gilthead sea bream and red drum, while isoeugenol and eugenol induce stress in Atlantic salmon, rainbow trout and hybrid striped bass, and benzocaine and metomidate have a similar effect in Atlantic salmon, Atlantic cod and Atlantic halibut (Barton and Peter 1982; Davidson et al. 2000; Davis and Griffin 2004; Davis et al. 1982; Iversen et al. 2003; Kiessling et al. 2009; Molinero and Gonzalez 1995; Olsen et al. 1995; Small 2003; Thomas and Robertson 1991, Zahl et al. 2010). However, confinement and relocation in connection with anaesthesia influence the responses to anaesthesia and may also mask the stress-inducing effects of the anaesthetic agents themselves (Hill and Forster 2004; Molinero and Gonzalez 1995; Thomas and Robertson 1991).

In a practical situation, stress, accompanied by the cascade of the physiological stress response, facilitates the uptake of anaesthetic by stimulating respiration and circulation and increasing the area of the blood/water interface. A doubling of oxygen consumption has been observed in coho salmon (*Oncorhynchus kisutch*), rainbow trout and Atlantic cod following handling, returning to basal levels within 1 h in coho salmon and 3 to 5 h in Atlantic cod (Barton and Schreck 1987; Davis and Schreck 1997; Saunders 1963). This probably results in an immediate and excessive uptake of anaesthetic, leading to faster induction and possibly also more deeply anaesthetised fish. In the study by Zahl et al. (2009), in which Atlantic cod were subjected to stress in the form of 30 s in air in a dip-net prior to anaesthesia, the fish displayed shorter induction time, became more deeply anaesthetised and recovered more slowly than unstressed fish. Moreover, the dosage of MS-222 had to be reduced in order to avoid mortality in the stressed group, but was too low to provide sufficient anaesthetic effect in the unstressed group. Both stress and sedation prior to anaesthesia may have marked practical consequences. This was well demonstrated by Kiessling and co-workers in a series of studies on feeding and growth after vaccination in salmon smolts anaesthetised with benzocaine with and without pre-anaesthetic metomidate sedation (Kiessling et al. 2001; Oppedal et al. 2000). They found that fish vaccinated using combination anaesthesia resumed normal feeding behaviour within a week, while fish subjected to the ordinary procedure with netting directly into the benzocaine bath without pre-sedation needed more than 3 weeks to resume normal feeding behaviour. At that time, the weights of the fish in the pre-sedated group were 22 per cent higher than the fish in the non-pre-sedated group.

References

Ackerman PA, Morgan JD, Iwama GK (2005) Anesthetics. CCAC guidelines on: the care and use of fish in research, teaching and testing. http://www.ccac.ca/en/CCAC_

Programs/Guidelines_Policies/GDLINES/Fish/Fish Anesthetics—ENG.pdf. Canadian Council on Animal Care, Ottawa CA (last accessed December 2010)
Anon (2007) Pharmaceutical use in Norwegian fish farming in 2001–2007, Wholesale-based drug statistics. Norwegian Institute of Public Health
Aoshima H, Hamamoto K (1999) Potentiation of $GABA_A$ receptors expressed in *Xenopus* oocytes by perfume and phytoncid. Biosci Biotechnol Biochem 63:743–748
Ashton D, Wauquier A (1985) Modulation of a GABA-ergic inhibitory circuit in the in vitro hippocampus by etomidate isomers. Anesth Analg 64:975–980
Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrat Comparat Biol 42:517–525
Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann Rev Fish Dis 1:3–26
Barton BA, Peter RE (1982) Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia, and cold shock. J Fish Biol 20:39–51
Barton BA, Schreck CB (1987) Metabolic cost of acute physical stress in juvenile steelhead. Trans Am Fish Soc 116: 257–263
Bell G (1987) An outline of anesthetics and anesthesia for salmonids: a guide for fish culturists in British Columbia. Canadian technical report of fisheries aquatic sciences no. 1534
Berge RK, Slinde E, Farstad M (1980) Discontinuities in Arrhenius plots due to formation of mixed micelles and change in enzyme substrate availability. FEBS Lett 109: 194–196
Boas RA, Covino BG, Shahnarian A (1982) Analgesic responses to i.v. lignocaine. Br J Anaesth 54:501–505
Bourne PK (1984) The use of MS-222 (tricaine methanesulphonate) as an anaesthetic for routine blood sampling in three species of marine teleosts. Aquaculture 36:313–321
Brodin P, Røed A (1984) Effects of eugenol on rat phrenic nerve and phrenic nerve-diaphragm preparations. Arc Oral Biol 29:611–615
Brown EAB, Franklin JE, Pratt E, Trams EG (1972) Contributions to the pharmacology of quinaldine (uptake and distribution in the shark and comparative studies). Comp Biochem Physiol 42A:223–231
Burka JF, Hammell KL, Horsberg TE, Johnson GR, Rainnie DJ, Speare DJ (1997) Drugs in salmonid aquaculture—a review. J Vet Pharmacol Ther 20:333–349
Ching KY, Baum CR (2009) Newer agents for rapid sequence intubation: etomidate and rocuronium. Pediatr Emerg Care 25:200–207
Clarke A, Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. J Anim Ecol 68:893–905
Cossins AR (1983) Adaptive responses of fish membranes to altered environmental temperature. Biochem Soc Trans 11:332–333
Cuesta A, Meseguer J, Esteban MA (2004) Total serum immunoglobulin M levels are affected by immunomodulators in seabream (*Sparus aurata* L.). Vet Immunol Immunopathol 101:203–210
Darrouj J, Karma L, Arora R (2009) Cardiovascular manifestations of sedatives and analgesics in the critical care unit. Am J Therapeut 16:339–353
Davidson GW, Davie PS, Young G, Fowler RT (2000) Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-S (TM). J World Aqua Soc 31:105–114
Davis KB, Griffin BR (2004) Physiological responses of hybrid striped bass under sedation by several anesthetics. Aquaculture 233:531–548
Davis LE, Schreck CB (1997) The energetic response to handling stress in juvenile coho salmon. Trans Am Fish Soc 126:248–258
Davis KB, Parker NC, Suttle MA (1982) Plasma corticosteroids and chlorides in striped bass exposed to tricaine methanesulfonate, quinaldine, etomidate, and salt. Progress Fish Cultur 44:205–207
Dawson VK, Gilderhus PA (1979) Ethyl-*p*-aminobenzoate (Benzocaine): efficacy as an anesthetic for five species of freshwater fish, vol 87. Investigations in Fish Control, United States Department of the Interior
Dawson VK, Marking LL (1973) Toxicity of mixtures of quinaldine sulfate and MS-222 to fish, vol 53. Investigations in Fish Control, United States Department of the Interior
Dunlop R, Laming P (2005) Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*). J Pain 6:561–568
Eliason EJ, Kiessling A, Karlsson A, Djordjevic B, Farrell AP (2007) Validation of the hepatic portal vein cannulation technique using Atlantic salmon *Salmo salar* L. J Fish Biol 71:290–297
Falk J, Zed PJ (2004) Etomidate for procedural sedation in the emergency department. Ann Pharmacother 38:1272–1277
Farrell AP (1984) A review of cardiac performance in the teleost heart: intrinsic and humoral regulation. Can J Zool 62: 523–536
Ferreira JT, Schoonbee HJ, Smit GL (1984) The uptake of the anesthetic benzocaine hydrochloride by the gills and the skin of three freshwater fish species. J Fish Biol 25:35–41
Fevolden SE, Røed KH, Fjalestad KT, Stien J (1999) Post-stress levels of lysozyme and cortisol in adult rainbow trout: heritabilities and genetic correlations. J Fish Biol 54: 900–910
Frazier DT, Narahashi T (1975) Tricaine (MS-222): Effects on ionic conductances of squid axon membranes. Eur J Pharmacol 33:313–317
Fredricks KT, Gingerich WH, Fater DC (1993) Comparative cardiovascular effects of four fishery anesthetics in spinally transected rainbow trout, *Oncorhynchus mykiss*. Compar Biochem Physiol C Pharmacol Toxicol Endocrinol 104: 477–483
Froese R, Pauly D (2011) FishBase, http://www.fishbase.org, version 10/2011
Gilderhus PA, Marking LL (1987) Comparative efficacy of 16 anesthetic chemicals on rainbow trout. North Am J Fish Manag 7:288–292

Gilderhus PA, Berger BL, Sills JB, Harman PD (1973) The efficacy of quinaldine sulfate: MS-222 mixtures for the anesthetization of freshwater fish, vol 54. Investigations in Fish Control, United States Department of the Interior

Gilderhus PA, Lemm CA, Woods LC (1991) Benzocaine as an anesthetic for striped bass. Progress Fish Cultur 53: 105–107

Gingerich WH, Drottar KR (1989) Plasma-catecholamine concentrations in rainbow trout (*Salmo gairdneri*) at rest and after anesthesia and surgery. Gen Comp Endocrinol 73: 390–397

Graham MS, Farrell AP (1989) The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. Physiol Zool 62:38–61

Grasshoff C, Drexler B, Rudolph U, Antkowiak B (2006) Anaesthetic drugs: linking molecular actions to clinical effects. Curr Pharm Des 12:3665–3679

Guenette SA, Uhland FC, Helie P, Beaudry F, Vachon P (2007) Pharmacokinetics of eugenol in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 266:262–265

Haegerstam G (1979) Effect of i.v. administration of lignocaine and tetrodotoxin on sensory units in the tooth of the cat. Br J Anaesth 51:487–491

Hall LW, Clarke KW, Trim CM (2001) Veterinary anaesthesia. W. B. Saunders, London

Hansen MK, Nymoen U, Horsberg TE (2003) Pharmacokinetic and pharmacodynamic properties of metomidate in turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*). J Vet Pharmacol Ther 26:95–103

Hara K, Sata T (2007) The effects of the local anesthetics lidocaine and procaine on glycine and gamma-aminobutyric acid receptors expressed in xenopus oocytes. Anesth Analg 104:1434–1439

Harms CA, Lewbart GA, Swanson CR, Kishimori JM, Boylan SM (2005) Behavioral and clinical pathology changes in koi carp (*Cyprinus carpio*) subjected to anesthesia and surgery with and without intra-operative analgesics. Compar Med 55:221–226

Hayton WL, Szoke A, Kemmenoe BH, Vick AM (1996) Disposition of benzocaine in channel catfish. Aqua Toxicol 36:99–113

Hedrick MS, Winmill RE (2003) Excitatory and inhibitory effects of tricaine (MS-222) on fictive breathing in isolated bullfrog brain stem. Am J Physiol Regul Integrat Compar Physiol 284:R405–R412

Hikasa Y, Takase K, Ogasawara T, Ogasawara S (1986) Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*. Jpn J Veterin Sci 48:341–351

Hill JV, Forster ME (2004) Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytscha*) during rapid anaesthetic induction and recovery. Compar Biochem Physiol C Toxicol Pharmacol 137:167–177

Hill JV, Davison W, Forster ME (2002) The effects of fish anaesthetics (MS222, metomidate and AQUI-S) on heart ventricle, the cardiac vagus and branchial vessels from Chinook salmon (*Oncorhynchus tshawytscha*). Fish Physiol Biochem 27:19–28

Holloway AC, Keene JL, Noakes DG, Moccia RD (2004) Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. Aquacult Res 35:1025–1030

Hoskonen P, Pirhonen J (2004) Temperature effects on anaesthesia with clove oil in six temperate-zone fishes. J Fish Biol 64:1136–1142

Houston AH, Woods RJ (1972) Blood concentrations of tricaine methane sulphonate in brook trout, *Salvelinus fontinalis*, during anesthetization, branchial irrigation, and recovery. J Fish Res Board Can 29:1344–1346

Houston AH, Woods RJ (1976) Influence of temperature upon tricaine methane sulphonate uptake and induction of anesthesia in rainbow trout (*Salmo gairdneri*). Compar Biochem Physiol C Pharmacol Toxicol Endocrinol 54:1–6

Houston AH, Madden JA, Woods RJ, Miles HM (1971a) Some physiological effects of handling and tricaine methanesulphonate anesthetization upon brook trout, *Salvelinus fontinalis*. J Fish Res Board Can 28:625–633

Houston AH, Madden JA, Woods RJ, Miles HM (1971b) Variations in blood and tissue chemistry of brook trout, *Salvelinus fontinalis*, subsequent to handling, anesthesia, and surgery. J Fish Res Board Can 28:635–642

Houston AH, Corlett JT, Woods RJ (1976) Specimen weight and MS-222. J Fish Res Board Can 33:1403–1407

Hunn JB (1970) Dynamics of MS-222 in the blood and brain of freshwater fishes during anesthesia, vol 42. Investigations in Fish Control, United States Department of the Interior

Hunn JB, Allen JL (1974) Movement of drugs across gills of fishes. Annu Rev Pharmacol Toxicol 14:47–55

Hunn JB, Schoettger RA, Willford WA (1968) Turnover and urinary excretion of free and acetylated MS 222 by rainbow trout, *Salmo gairdneri*. J Fish Res Board Can 25:25–31

Ingvast-Larsson JC, Axén VC, Kiessling A (2003) Effects of isoeugenol on rat phrenic nerve-diaphragm preparations. Am J Veter Res 64:690–693

Iversen M, Finstad B, McKinley RS, Eliassen RA (2003) The efficacy of metomidate, clove oil, Aqui-S (^{TM}) and Benzoak (^{R}) as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture 221:549–566

Iwama GK, McGeer JC, Pawluk MP (1989) The effects of five fish anaesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. Can J Zool Revue Canadienne de Zoologie 67:2065–2073

Karlsson A, Eliason EJ, Mydland LT, Farrell AP, Kiessling A (2006) Postprandial changes in plasma free amino acid levels obtained simultaneously from the hepatic portal vein and the dorsal aorta in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 209:4885–4894

Karlsson A, Rosseland B-O, Thorarensen H, Kiessling A (2011a) Changes in arterial oxygen tension and physiological status in resting and unrestrained Arctic charr *Salvelinus alpinus* (L.) exposed to mild hypoxia and hyperoxia. J Fish Biol 78:962–966

Karlsson A, Rosseland B-O, Massabuau JC, Kiessling A (2011b) Pre-anaesthetic metomidate sedation delays the stress response after caudal artery cannulation in Atlantic cod (*Gadus morhua*). Fish Physiol Biochem. doi: 10.1007/s10695-011-9516x

Kiessling A, Dosanjh B, Higgs D, Deacon G, Rowshandeli N (1995) Dorsal aorta cannulation: a method to monitor changes in blood levels of astaxanthin in voluntarily feeding Atlantic salmon, *Salmo salar* L. Aquac Nutr 1:43–50

Kiessling A, Johansson D, Axen C, Johansson B (2001) Anestesi och analgesi vid vaccinering av lax. In: Olsen RE, Hansen T (eds) Havbruksrapporten 2001, Fisken og havet, saernr.3-2001

Kiessling A, Olsen RE, Buttle L (2003) Given the same dietary carotenoid inclusion, Atlantic salmon, *Salmo salar* (L.) display higher blood levels of canthaxanthin than astaxanthin. Aquac Nutr 9:253–261

Kiessling A, Johansson D, Zahl IH, Samuelsen OB (2009) Pharmacokinetics, plasma cortisol and effectiveness of benzocaine, MS-222 and isoeugenol measured in individual dorsal aorta-cannulated Atlantic salmon (*Salmo salar*) following bath administration. Aquaculture 286:301–308

Kildea MA, Allan GL, Kearney RE (2004) Accumulation and clearance of the anaesthetics clove oil and AQUI-S (TM) from the edible tissue of silver perch (*Bidyanus bidyanus*). Aquaculture 232:265–277

King WV, Hooper B, Hillsgrove S, Benton C, Berlinsky DL (2005) The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). Aquacult Res 36:1442–1449

Kreiberg H, Powell J (1991) Metomidate sedation reduces handling stress in chinook salmon. World Aqua 22:58–59

Kumlu M, Yanar M (1999) Effects of the anesthetic quinaldine sulphate and muscle relaxant diazepam on sea bream juveniles (*Sparus aurata*). Israeli J Aqua Bamidgeh 51: 143–147

Lambooij B, Pilarczyk M, Bialowas H, Reimert H, Andre G, van de Vis H (2009) Anaesthetic properties of Propiscin (Etomidaat) and 2-phenoxyethanol in the common carp (*Cyprinus carpio* L.), neural and behavioural measures. Aquacult Res 40:1328–1333

Lee MH, Yeon KY, Park CK, Li HY, Fang Z, Kim MS, Choi SY, Lee SJ, Lee S, Park K, Lee JH, Kim JS, Oh SB (2005) Eugenol inhibits calcium currents in dental afferent neurons. J Dent Res 84:848–851

Levron JC, Assoune P (1990) Pharmacokinetics of etomidate. Annales Françaises d'Anesthésie Et de Réanimation 9:123–126

Li HY, Park CK, Jung SJ, Choi SY, Lee SJ, Park K, Kim JS, Oh SB (2007) Eugenol inhibits K^+ currents in trigeminal ganglion neurons. J Dent Res 86:898–902

Lochowitz RT, Miles HM, Hafemann DR (1974) Anesthetic-induced variations in cardiac rate of the teleost, *Salmo gairdneri*. Gen Pharmacol 5:217–224

Marking LL, Meyer FP (1985) Are better anesthetics needed in fisheries? Fisheries 10:2–5

Mattson NS, Riple TH (1989) Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol, and phenoxyethanol. Aquaculture 83:89–94

McFarland WN (1959) A study of the effects of anaesthetics on the behaviour and physiology of fishes. Publ Inst Mar Sci 6:22–55

McFarland WN, Klontz GW (1969) Anesthesia in fishes. Feder Proc 28:1535–1540

Meinertz JR, Gingerich WH, Allen JL (1991) Metabolism and elimination of benzocaine by rainbow trout (*Oncorhynchus mykiss)*. Xenobiotica 21:525–533

Meinertz JR, Stehly GR, Gingerich WH (1996) Pharmacokinetics of benzocaine in rainbow trout (*Oncorhynchus mykiss*) after intraarterial dosing. Aquaculture 148:39–48

Meinertz JR, Greseth SL, Schreier TM, Bernardy JA, Gingerich WH (2006) Isoeugenol concentrations in rainbow trout (*Oncorhynchus mykiss*) skin-on fillet tissue after exposure to AQUI-S $^{(TM)}$ at different temperatures, durations, and concentrations. Aquaculture 254:347–354

Molinero A, Gonzalez J (1995) Comparative effects of MS 222 and 2-phenoxyethanol on gilthead sea bream (*Sparus aurata* L) during confinement. Compar Biochem Physiol A Physiol 111:405–414

Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fisheries 9:211–268

Morris DJ, North AW (1984) Oxygen consumption of five species of fish from South Georgia. J Exp Mar Biol Ecol 78:75–86

Muir BS (1969) Gill dimensions as a function of fish size. J Fish Res Board Can 26:165–170

Musshoff U, Madeja M, Binding N, Witting U, Speckmann EJ (1999) Effects of 2-phenoxyethanol on N-methyl-$_D$-aspartate (NMDA) receptor-mediated ion currents. Arch Toxicol 73:55–59

Mylonas CC, Cardinaletti G, Sigelaki I, Polzonetti-Magni A (2005) Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. Aquaculture 246: 467–481

Neiffer DL, Stamper MA (2009) Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR J 50:343–360

Neumcke B, Schwarz W, Stampfli R (1981) Block of Na channels in the membrane of myelinated nerve by benzocaine. Pflügers Archiv Eur J Physiol 390:230–236

Nilsson S, Sundin L (1998) Gill blood flow control. Compar Biochemis Physiol Part A Mol Integrat Physiol 119: 137–147

Oikawa S, Itazawa Y (1985) Gill and body-surface areas of the carp in relation to body-mass, with special reference to the metabolism-size relationship. J Exp Biol 117:1–14

Oikawa S, Hirata M, Kita J, Itazawa Y (1999) Ontogeny of respiratory area of a marine teleost, porgy, *Pagrus major*. Ichthyol Res 46:233–244

Olsen YA, Einarsdottir IE, Nilssen KJ (1995) Metomidate anesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. Aquaculture 134: 155–168

Oppedal F, Johansson B, Kiesslin A (2000) Bedøvelse og vaksinering—økt appetitt ved bedre rutiner. 2000. Norsk Fiskeoppdrett no 9:24–26

Ortuno J, Esteban MA, Meseguer J (2002) Effects of four anaesthetics on the innate immune response of gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol 12:49–59

Park CK, Li HY, Yeon KY, Jung SJ, Choi SY, Lee SJ, Lee S, Park K, Kim JS, Oh SB (2006) Eugenol inhibits sodium currents in dental afferent neurons. J Dent Res 85:900–904

Pottinger TG, Carrick TR (1999) A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. Aquaculture 175:351–363

Pottinger TG, Pickering AD, Hurley MA (1992) Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 103: 275–289

Pottinger TG, Moran TA, Morgan JAW (1994) Primary and secondary indices of stress in the progeny of rainbow trout (*Oncorhynchus mykiss*) selected for high and low responsiveness to stress. J Fish Biol 44:149–163

Randall DJ (1962) Effect of an anaesthetic on the heart and respiration of teleost fish. Nature 195:506

Rang HP, Dale MM, Ritter JM, Moore PK (2003) Pharmacology. Churchill Livingstone, London

Rigon AR, Takahashi RN (1996) The effects of systemic procaine, lidocaine and dimethocaine on nociception in mice. Gen Pharmacol 27:647–650

Ross LG, Ross B (2008) Anaesthetic and sedative techniques for aquatic animals. Blackwell, Oxford

Rothwell SE, Forster ME (2005) Anaesthetic effects on the hepatic portal vein and on the vascular resistance of the tail of the Chinook salmon (*Oncorhynchus tshawytscha*). Fish Physiol Biochem 31:11–21

Ruyter B, Moya-Falcon C, Rosenlund G, Vegusdal A (2006) Fat content and morphology of liver and intestine of Atlantic salmon (*Salmo salar*): Effects of temperature and dietary soybean oil. Aquaculture 252:441–452

Ryan S (1992) The dynamics of MS-222 anaesthesia in a marine teleost (*Pagrus auratus*: Sparidae). Compar Biochemis Physiol C Pharmacol Toxicol Endocrinol 101:593–600

Sams L, Braun C, Allman D, Hofmeister E (2008) A comparison of the effects of propofol and etomidate on the induction of anesthesia and on cardiopulmonary parameters in dogs. Veterinary Anaesth Analg 35:488–494

Sandodden R, Finstad B, Iversen M (2001) Transport stress in Atlantic salmon (*Salmo salar* L.): anaesthesia and recovery. Aquacult Res 32:87–90

Saunders RL (1963) Respiration of the Atlantic Cod. J Fish Res Board Can 20:373–386

Schmidt-Nielsen K (1984) Scaling: why is animal size so important?. Cambridge University Press, New York

Schoettger RA, Julin AM (1967) Efficacy of MS-222 as an anesthetic on four salmonids, vol 13. Investigations in Fish Control, United States Department of the Interior

Schoettger RA, Steucke EW (1970) Synergic mixtures of MS-222 and quinaldine as anesthetics for rainbow trout and northern pike. Progress Fish Cultur 32:202–205

Schurmann H, Steffensen JF (1997) Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. J Fish Biol 50:1166–1180

Sellner PA, Hazel JR (1982) Incorporation of polyunsaturated fatty acids into lipids of rainbow trout hepatocytes. Am J Physiol 243:R223–R228

Selye H (1985) The nature of stress. Basal Facts 7:3–11

Small BC (2003) Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. Aquaculture 218:177–185

Small BC (2004) Effect of isoeugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. Aquaculture 238:469–481

Small BC, Chatakondi N (2005) Routine measures of stress are reduced in mature channel catfish during and after AQUI-S anesthesia and recovery. NA J Aqua 67:72–78

Sneddon LU (2002) Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss*. Neurosci Lett 319:167–171

Sneddon LU (2003) Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception. Brain Res 972:44–52

Sneddon LU, Braithwaite VA, Gentle MJ (2003) Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. Proc R Soc Lond Ser B Biol Sci 270:1115–1121

Soivio A, Nyholm K, Huhti M (1977) Effects of anesthesia with MS 222, neutralized MS 222 and benzocaine on blood constituents of rainbow trout, *Salmo gairdneri*. J Fish Biol 10:91–101

Stehly GR, Gingerich WH (1999) Evaluation of AQUI-S (TM) (efficacy and minimum toxic concentration) as a fish anaesthetic sedative for public aquaculture in the United States. Aquacult Res 30:365–372

Stehly GR, Meinertz JR, Gingerich WH (1998) Effect of temperature on the pharmacokinetics of benzocaine in rainbow trout (*Oncorhynchus mykiss*) after bath exposures. J Vet Pharmacol Ther 21:121–127

Stenger VG, Maren TH (1974) Pharmacology of MS 222 (ethyl-*m*-aminobenzoate) in *Squalus acanthias*. Gen Pharmacol 5:23–35

Summerfelt RC, Smith LS (1990) Anaesthesia and surgery and related techniques. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 213–272

Sumpter JP, Dye HM, Benfey TJ (1986) The effects of stress on plasma ACTH, α-MSH, and cortisol levels in salmonid fishes. Gen Comp Endocrinol 62:377–385

Sylvester JR, Holland LE (1982) Influence of temperature, water hardness, and stocking density on MS-222 response in three species of fish. Progress Fish Cultur 44:138–141

Tanck MWT, Vermeulen KJ, Bovenhuis H, Komen H (2001) Heredity of stress-related cortisol response in androgenetic common carp (*Cyprinus carpio* L.). Aquaculture 199: 283–294

Thomas P, Robertson L (1991) Plasma-cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow-water stressors and anesthesia with MS-222, quinaldine sulfate and metomidate. Aquaculture 96:69–86

Trenzado CE, Carrick TR, Pottinger TG (2003) Divergence of endocrine and metabolic responses to stress in two rainbow trout lines selected for differing cortisol responsiveness to stress. Gen Comp Endocrinol 133:332–340

Ueta K, Suzuki T, Sugimoto M, Uchida I, Mashimo T (2007) Local anesthetics have different mechanisms and sites of action at recombinant 5-HT_3 receptors. Reg Anesth Pain Med 32:462–470

Vanden Bossche H, Willemsens G, Cools W, Bellens D (1984) Effects of etomidate on steroid biosynthesis in subcellular fractions of bovine adrenals. Biochem Pharmacol 33: 3861–3868

Velisek J, Stejskal V, Kouril J, Svobodova Z (2009) Comparison of the effects of four anaesthetics on biochemical blood profiles of perch. Aquacult Res 40:354–361

Wagner RL, White PF, Kan PB, Rosenthal MH, Feldman D (1984) Inhibition of adrenal steroidogenesis by the anesthetic etomidate. N Engl J Med 310:1415–1421

Webber DM, Boutilier RG, Kerr SR (1998) Cardiac output as a predictor of metabolic rate in cod *Gadus morhua*. J Exp Biol 201:2779–2789

Weber RA, Peleteiro JB, Martin LOG, Aldegunde M (2009) The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858). Aquaculture 288:147–150

Wedemeyer GA (1970) Stress of anesthesia with MS-222 and benzocaine in rainbow trout (*Salmo gairdneri*). J Fish Res Board Can 27:909–914

Wendelaar Bonga SE (1997) The stress response in fish. Physiol Rev 77:591–625

Weyl O, Kaiser H, Hecht T (1996) On the efficacy and mode of action of 2-phenoxyethanol as an anaesthetic for goldfish, *Carassius auratus* (L.), at different temperatures and concentrations. Aquacult Res 27:757–764

Wie MB, Won MH, Lee KH, Shin JH, Lee JC, Suh HW, Song DK, Kim YH (1997) Eugenol protects neuronal cells from excitotoxic and oxidative injury in primary cortical cultures. Neurosci Lett 225:93–96

Wiesenfeld-Hallin Z, Lindblom U (1985) The effect of systemic tocainide, lidocaine and bupivacaine on nociception in the rat. Pain 23:357–360

Woolf CJ, Wiesenfeld-Hallin Z (1985) The systemic administration of local anaesthetics produces a selective depression of C-afferent fibre evoked activity in the spinal cord. Pain 23:361–374

Woolsey J, Holcomb M, Ingermann RL (2004) Effect of temperature on clove oil anesthesia in steelhead fry. NA J Aqua 66:35–41

Yanar M, Kumlu M (2001) The anaesthetics effects of quinaldine sulphate and/or diazepam on sea bass (*Dicentrarchus labrax*) juveniles. Turk J Veter Anim Sci 25:185–189

Yang J, Uchida I (1996) Mechanisms of etomidate potentiation of $GABA_A$ receptor-gated currents in cultured postnatal hippocampal neurons. Neuroscience 73:69–78

Zahl IH, Kiessling A, Samuelsen OB, Hansen MK (2009) Anaesthesia of Atlantic cod (*Gadus morhua*)—effect of pre-anaesthetic sedation and importance of body weight, temperature and acute stress. Aquaculture 295(1–2):52–59

Zahl IH, Kiessling A, Samuelsen OB, Olsen RE (2010) Anaesthesia induces stress in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus).* Fish Physiol. Biochemistry 36(3):719–730

Zahl IH, Kiessling A, Samuelsen OB, Hansen MK (2011) Anaesthesia of Atlantic Halibut (*Hippoglossus hippoglossus*)—effect of pre-anaesthetic sedation and importance of body weight and water temperature. Aquacult Res 42: 1235–1245