Ichthyol Res (2017) 64:84–92
DOI 10.1007/s10228-016-0545-4

FULL PAPER

Recovery of blood gases and haematological parameters upon anaesthesia with benzocaine, MS-222 or Aqui-S in the air-breathing catfish *Pangasianodon hypophthalmus*

Le My Phuong[1,3] · Christian Damsgaard[3] · Do Thi Thanh Huong[1] · Atsushi Ishimatsu[2] · Tobias Wang[3] · Mark Bayley[3]

Received: 7 April 2016 / Revised: 13 July 2016 / Accepted: 28 July 2016 / Published online: 17 August 2016

Abstract Fish anaesthesia is used to minimize handling stress and damage during harvesting, transportation, and surgical procedures. Through depression of cardiovascular and respiratory functions, it causes significant changes in blood gases and pH. Here, we present the effects of benzocaine (100 mg l^{-1}), MS-222 (100 mg l^{-1}), and Aqui-S (30 mg l^{-1}) on blood gases and haematological parameters of commercial-sized ($\approx$1 kg) striped catfish (*Pangasianodon hypophthalmus*) and the time course of recovery. Blood was taken through a dorsal aorta catheter immediately after catheterization, and regularly during the following 72 h recovery in aerated water. All anaesthetics caused increases in PCO_2 and lactate resulting in a decrease in pH_e, closely mirrored by RBC pH_i, as well as a marked rise in Hct, associated with elevated [cortisol] and [glucose] and increased RBC counts but no change in RBC volume, as confirmed by the lack of an adrenergic response of RBC *in vitro*. All anaesthetics showed similar efficacy and blood parameters were normalized within 24 to 48 h.

Keywords *Pangasianodon hypophthalmus* · Anaesthesia induction · Haematology · Catheterization

✉ Le My Phuong
lemyphuong00@gmail.com

[1] College of Aquaculture and Fisheries, Can Tho University, Can Tho City, Vietnam

[2] Institute for East China Sea Research, Nagasaki University, Taira-machi, Nagasaki 851-2213, Japan

[3] Zoophysiology Section, Department of Biological Sciences, Aarhus University, Aarhus, Denmark

Introduction

Anaesthetics are widely used in aquaculture to diminish stress and physical injuries during harvesting or transport, and proper anaesthesia is absolutely essential to alleviate pain during surgeries and health examinations (Coyle et al. 2004; Kiessling et al. 2009; Abdolazizi et al. 2011). For aquatic organisms, soluble anaesthetics can be added directly to the water and are readily absorbed across the gills, and transported by the blood to the nervous system; loss of equilibrium and mobility follows rapidly (Coyle et al. 2004; Popovic et al. 2012). However, the anaesthesia also disturbs the respiratory and cardiac function causing hypoxaemia (Soivio et al. 1977; Fredricks et al. 1993; Andersen and Wang 2002). Thus, disturbances of blood gases and haematological parameters occur within seconds of administration, due to splenic contraction and adrenergic activation of the RBC sodium-proton exchanger and hence extracellular acidosis and red cell swelling (Jensen 2004). These respiratory, haematological and metabolic disturbances during anaesthesia have been studied extensively in water-breathing fish, such as Atlantic salmon, rainbow trout, kelp grouper, red drum and perch (Soivio et al. 1977; Iwama et al. 1989; Thomas and Robertson 1991; Iversen et al. 2003; Park et al. 2008; Velíšek et al. 2009), but little is known about such responses in air-breathing fish, which often thrive at higher temperatures than water breathers (Lefevre et al. 2014). Furthermore, it has been suggested that air breathers, with their reduced branchial ion and respiratory gas exchange, are less able to regulate pH_e than water breathers (Shartau and Brauner 2014) and, therefore, the restoration of acid–base status and blood gases may be different from those of water breathers.

Striped catfish *Pangasianodon hypophthalmus* is a tropical facultative air-breathing fish, which is effective at

both air and water breathing (Lefevre et al. 2011). *Pangasianodon hypophthalmus* is intensively cultured in South East Asia and, while various anaesthetics are widely used for transport, there is no information on the rate of recovery or physiological effects. In aquaculture, MS-222 (tricaine methanesulfonate), benzocaine (ethyl para-aminobenzoate), and Aqui-S (50 % isoeugenol) are the most commonly utilised anaesthetics (Coyle et al. 2004; Kiessling et al. 2009; Weber et al. 2009; Abdolazizi et al. 2011). Both benzocaine and MS-222 are local anaesthetics that provide general anaesthesia in fish by blocking voltage-gated sodium channels and hence inhibit neural transmission within the central and peripheral nervous systems (Attili and Hughes 2014). Eugenol is a widely used local analgesic agent to alleviate tooth pain that shares several pharmacological actions with local anaesthetics, including inhibition of voltage-gated sodium channel as well as activation of transient receptor potential vanilloid subtype 1 (TRPV1) (Park et al. 2009). Here, we evaluate the disturbance caused by these three anaesthetics on blood gases and haematological parameters of *P. hypophthalmus* and the time course for normalisation when allowed to recover in clean normoxic and normocapnic water. In addition, since adrenergic swelling responses have been found in erythrocytes of numerous water-breathing fish (Nikinmaa and Huestis 1984), leading to an increase in cell volume and changes in haematocrit, and since we expected pH and haematocrit changes, we also investigate the existence of this β-adrenergic response in the present study.

Materials and methods

Abbreviations used in this study are shown in Table 1.

Fish. Striped catfish *Pangasianodon hypophthalmus* weighing 700-1000 g were transferred from local farms to tanks with aerated water at the Department of Aquaculture, Can Tho University (Vietnam) several weeks before the study commenced. During this period, they were fed to satiation with commercial pellets on a daily basis, but fasted for 24 h pre-instrumentation.

Anaesthetics preparation. Benzocaine was pre-dissolved in 3 ml ethanol 70 % and mixed with water (100 mg l^{-1}, Florindo et al. 2006). Aqui-S was dissolved directly in the water at 30 mg l^{-1} (Iversen et al. 2003). MS-222 was dissolved in 100 mg l^{-1} tank water with 100 mg l^{-1} $NaHCO_3$ used as a buffer (Iwama et al. 1989).

Experimental procedures. Each fish was kept in the induction chamber until total loss of equilibrium and reaction to touch (Iwama et al. 1989; Coyle et al. 2004). When anaesthetized, the fish was catheterized into the dorsal aorta using polyethylene tubing (I.D. 0.58 mm, O.D. 0.96 mm) containing heparinized saline (50 IE ml^{-1}) (Soivio et al. 1975), whilst the fish gills were constantly irrigated with aerated water containing one-third of the initial dose of anaesthesia. Times required for induction time and catheterization were recorded for each fish. An arterial blood sample was collected from the catheter immediately after catheterization (0 h) and subsequently at 3, 6, 24, 48, and 72 h of recovery, whilst the fish were maintained in a 200 l tank containing aerated water. Great care was taken not to disturb the fish during recovery. Water temperatures during recoveries were 21.6 ± 0.2, 24.2 ± 0.1, and 25.1 ± 0.2 °C in benzocaine, Aqui-S, and MS-222, respectively.

Table 1 Abbreviations used in this study

$[Cl^-]_e$	plasma Cl^- concentration
$[Cl^-]_i$	red blood cell intracellular Cl^- concentration
[cortisol]	plasma cortisol concentration
[glucose]	plasma glucose concentration
[lactate]	plasma lactate concentration
$[HCO_3^-]$	bicarbonate concentration
[Hb]	haemoglobin concentration
$[HbO_2]$	concentration of haemoglobin-bound oxygen
Hct	Haematocrit
MCHC	mean cell haemoglobin concentration
$[O_2]_{total}$	total concentration of oxygen in blood
PCO_2	partial pressure of carbon dioxide
pH_e	plasma pH
pH_i	red blood cell intracellular pH
pK	acid dissociation exponent for carbon dioxide in plasma
PO_2	partial pressure of oxygen
RBC	red blood cell
α_{CO2}	solubility of carbon dioxide in plasma
α_{O2}	solubility of oxygen in plasma

Blood collection and treatment. Each blood sample (0.3 ml) was collected using a 1 ml syringe, carefully avoiding air bubbles, for measurements of PCO_2, pH_e, and [lactate] using an iSTAT blood gas analyser (Abbott Laboratories, Abbott Park, Illinois, USA); 0.7 ml of blood was transferred to a 1.5 ml Eppendorf tube and kept on ice for immediate determination of other haematological parameters, such as Hct, [Hb], and RBC counts. The remaining blood was centrifuged at 6000 rpm for 6 min, to separate plasma and RBC, and stored at -80 °C for subsequent analysis of [glucose], [cortisol], $[Cl^-]_e$, and $[Cl^-]_i$.

Measurement of the haematological and biochemical parameters. Hct was determined by centrifugation in a standard microhaematocrit centrifuge. Hb concentration was determined by the Drabkin's method; spectrophotometrically at 540 nm using an extinction coefficient of

10.99 $mmol^{-1}$ cm^{-1} (Zilstra et al. 1983). Plasma glucose concentration was determined according to Huggett and Nixon (1957), and [cortisol] was determined using a DRG Salivary Cortisol ELISA commercial Kit (USA). RBC counts were determined by counting the number of RBC in a Neubauer chamber under a microscope after diluting 200 times of 0.5 µl blood sample with Natt & Herrick's stain solution. $[Cl^-]_e$ was measured using a chloride titrator (Sherwood model 926S MK II chloride analyser). For $[Cl^-]_i$, a known mass of RBC pellet was transferred to a known volume of distilled water to induce cell lysis, and Cl^- was measured in the haemolysate. The RBC water content was measured gravimetrically in another RBC aliquot from the same centrifuged cell pellet before and after drying at 60 °C for 16 h.

In vitro assessment of red cell adrenergic response. Four fish were catheterized under anaesthesia with benzocaine and allowed to recover for at least 48 h before a 3 ml blood sample was taken. Blood was placed in an Eschweiler tonometer (Kiel, Germany) and equilibrated with humidified gas mixtures supplied from two serially linked Wösthoff gas mixing pumps (Bochum, Germany). Initially, blood was equilibrated with 30 % O_2 (PO_2 = 216 mmHg) and 3 % CO_2 (PCO_2 = 21.6 mmHg) to determine blood O_2-carrying capacity and then reduced to a PO_2 of 15.1 mmHg (approximately 10 % air) at 3 % CO_2, resulting in HbO_2 saturations of 15-30 %. At this PCO_2, pH_e is expected to be 7.35 (Damsgaard et al. 2015). The beta-adrenergic agonist isoprenaline was added to the blood to a final concentration of 10^{-5} mol l^{-1} (Brauner et al. 2002; Koldkjær et al. 2002). At both steps, the concentration of Hb-bound O_2 ([Hb-O_2]), Hct, and [Hb] were determined.

Calculations. pH_i was calculated from the Donnan-like equilibrium using the ratio of $[Cl^-]_e$, $[Cl^-]_i$, and pH_e (Jensen 2004)

$$pH_i = log_{10}\left(\frac{[Cl^-]_e}{[Cl^-]_i} \cdot 10^{-pH_e}\right).$$

Based on our previous validation of the iStat for *P. hypophthalmus* (Damsgaard et al. 2015), the iStat PCO_2 was corrected according to the equation:

$$PCO_2(\text{true})\,[\text{mmHg}] = -2.17 + 1.20P_aCO_2\ (\text{iStat}).$$

Plasma $[HCO_3^-]$ was calculated from the Henderson Hasselbach equation:

$$\left[HCO_3^-\right] = \alpha_{CO_2} \cdot PCO_2 \cdot 10^{pH_e - pK},$$

where α_{CO2} is the temperature-compensated CO_2 solubility in trout plasma (Boutilier et al. 1985) and pK is the pH_e-corrected dissociation exponent for CO_2 in *P. hypophthalmus* plasma (Damsgaard et al. 2015).

[Hb-O_2] was determined by measuring $[O_2]_{total}$ and subtracting physically dissolved O_2:

$$[Hb - O_2] = [O_2]_{total} - \alpha_{O_2} \cdot PO_2,$$

where $[O_2]_{total}$ was determined according to Tucker (1967); α_{O2} is the temperature-compensated solubility of O_2 (Dejours 1981) and PO_2 the partial pressure of O_2 in the gas mixture.

O_2 saturation of Hb (HbO_2 sat) during equilibration with 10% air was calculated as

$$\text{Saturation} = \frac{[Hb - O_2]_{10\%air}}{[Hb - O_2]_{30\%O_2}}.$$

MCHC was calculated as

$$\text{MCHC} = \frac{[Hb]}{Hct}.$$

Statistics. All data were presented as means ± standard error of the mean. One-way repeated measures ANOVA was applied to determine significant differences amongst the sampling times for each haematological parameter. One sample t test was used to test whether isoprenaline exerted significant effects on the red cells *in vitro*. A probability (P) value at the 0.05 level was considered as significant.

Results

Time required for induction of anaesthesia, surgery, and recovery stage. A surgical plane of anaesthesia was 3.1 ± 0.2 min for benzocaine, whereas it took 9.3 ± 0.9 and 8.1 ± 0.7 min for MS-222 and Aqui-S, respectively (Fig. 1). Regardless of the anaesthetic, the catheter was inserted and secured within the dorsal aorta in less than

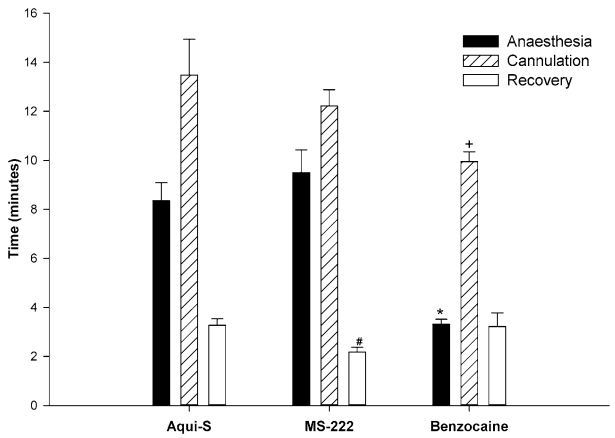

Fig. 1 Time recorded for duration of anaesthesia to reach the surgical plane, cannulation, and post-operative recovery of *Pangasianodon hypophthalmus* with the three anaesthetics. Time values are presented as means ± S.E.M (n = 7). Significant differences between anaesthesia treatments, cannulation, and post-operative recovery are indicated with *, #, and +, respectively

15 min and the fish regained equilibrium within 2-4 min (Fig. 1).

Haematological and biochemical parameters. Following full anaesthesia, Hct, RBC counts, and [Hb] changed in a similar manner, with maximal disruption immediately after catheterization, followed by a rapid decrease within 6 h of recovery (Fig. 2a–c). MCHC was stable around 25 mmol l^{-1} throughout the entire recovery period except with MS-222 (Fig. 2d).

All three anaesthetics caused high [glucose] and [cortisol] immediately after surgery. The plasma glucose was high initially within 0-3 h, then decreased gradually during recovery, and normalized at approximately 3 mmol l^{-1} at 72 h (Fig. 2e). Cortisol concentrations fell by more than a factor of 5 during recovery and stabilized at approximately 30 mmol l^{-1} within 24 h (benzocaine and MS-222) to 48 h (Aqui-S) (Fig. 2f).

Acid–base status and chloride ions. Immediately after surgery, there was a significant plasma acidosis, which rapidly returned to normal values over the subsequent 6-24 h (Fig. 3a); pHi followed a similar pattern, with a difference between pHe and pHi of around 0.2-0.3 units throughout the experiment for all three anaesthetics (Fig. 3b). Arterial PCO_2 was elevated during this time and this elevation was most pronounced in benzocaine (up to 6 mmHg). During recovery from anaesthesia with all three compounds, PCO_2 decreased to approximately 2.5 mmHg (Fig. 3c). Similarly, all three anaesthetics caused elevated [lactate], which remained high at 6-8 mmol l^{-1} immediately after catheterization. In line with PCO_2, lactate also recovered almost completely to approximately 0.3 mmol l^{-1} at 24 h post-operation (Fig. 3d). Immediately after surgery with Aqui-S and MS-222, [HCO_3^-] was depressed, but stabilized within 6 h of recovery at approximately 9 mmol l^{-1} (Fig. 3e). No significant changes were observed in $[Cl^-]_e$ and $[Cl^-]_i$ after anaesthesia, with the concentrations being approximately 100 and 60 mmol l^{-1}, respectively (Figs. 3g, h). Davenport diagrams (Figs. 4a, b, c) show the respiratory status of *Pangasianodon hypophthalmus* during and after anaesthesia with the three anaesthetics and show that the low pH_e immediately after surgery can be largely ascribed to a metabolic acidosis with a minor respiratory component.

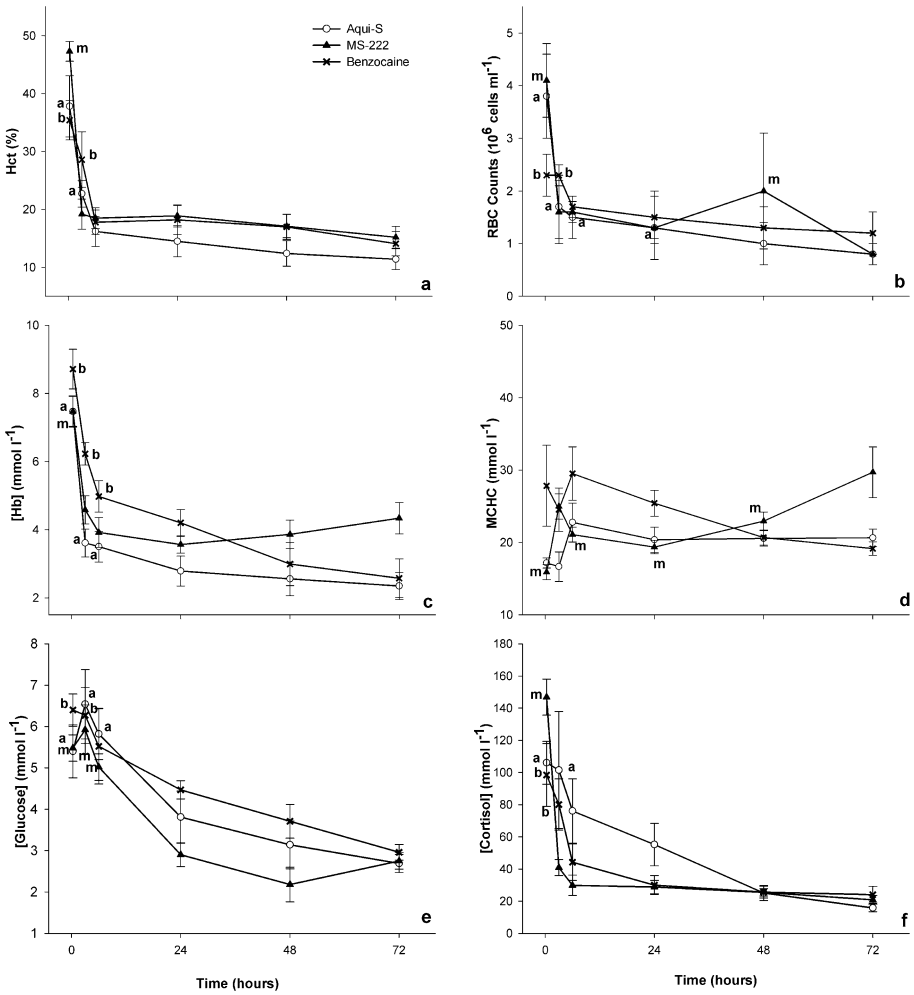

Fig. 2 Haematological and biochemical parameters of arterial blood following anaesthesia in *Pangasianodon hypophthalmus* with Aqui-S (circles), MS-222 (triangles), and benzocaine (crosses) with (**a**) Hct, (**b**) RBC counts, (**c**) [Hb], (**d**) MCHC, (**e**) [glucose], (**f**) [cortisol]. Letters ^{a,m,b} indicate significant difference ($P < 0.05$) from 72 h, for Aqui-S, MS-222, and benzocaine, respectively (one way RM ANOVA). Values are presented as means ± S.E.M ($n = 7$)

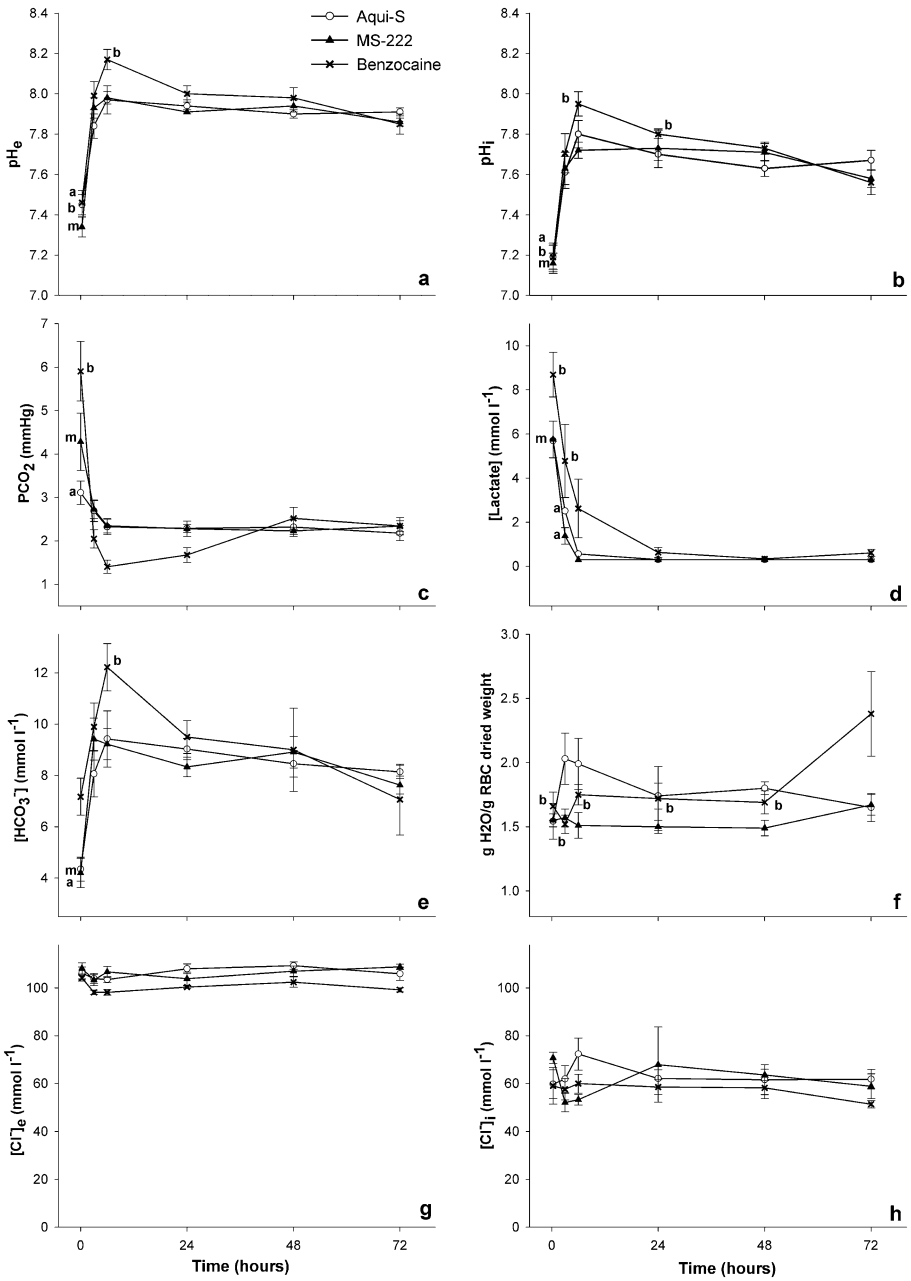

Fig. 3 Acid–base status and chloride ions of arterial blood following anaesthesia in *Pangasianodon hypophthalmus* with Aqui-S (circles), MS-222 (triangles), and benzocaine (crosses) with (**a**) pH_e, (**b**) pH_i, (**c**) PCO_2, (**d**) [lactate], (**e**) $[HCO_3^-]$, (**f**) gH_2O/g RBC dried weight, (**g**) $[Cl^-]_e$, and (**h**) $[Cl^-]_i$. Letters ^{a,m,b} indicate significant difference ($P < 0.05$) from 72 h for Aqui-S, MS-222, and benzocaine, respectively (one-way RM ANOVA). Values are presented as means ± S.E.M ($n = 7$)

Effects of ß-adrenergic stimulation on red cells in vitro. Addition of isoprenaline to whole blood *in vitro* caused only a minor, albeit statistically significant, rise in blood O_2 saturation of 2.8 % (one sample t test, $P < 0.01$). However, neither Hct nor MCHC was affected (one sample t test, $P = 0.1817$ and $P = 0.1884$, respectively) (Table 2).

Discussion

Anaesthesia induction and recovery. The times required to induce anaesthesia and the duration of the subsequent recovery depend on the type, concentration of the anaesthetic, and fish species (da Cunha et al. 2010; Maricchiolo and Genovese 2011). In *Pangasianodon hypophthalmus*, anaesthesia was reached more rapidly with benzocaine than with Aqui-S and MS-222, whereas recovery was prolonged upon benzocaine anaesthesia compared to the other two anaesthetics. With MS-222 (100-200 mg l^{-1}), silver catfish *Rhamdia quelen* can be anaesthetized within 1-2.4 min and can recover within 0.25-1.45 min (da Cunha et al. 2010); and with 20–50 mg l^{-1} Aqui-S, channel catfish *Ictalurus punctatus* can be anaesthetized within 2–5 min (Stehly and Gingerich 1999). Akbulut et al. (2011) found statistically significant correlations between anaesthetic concentration and

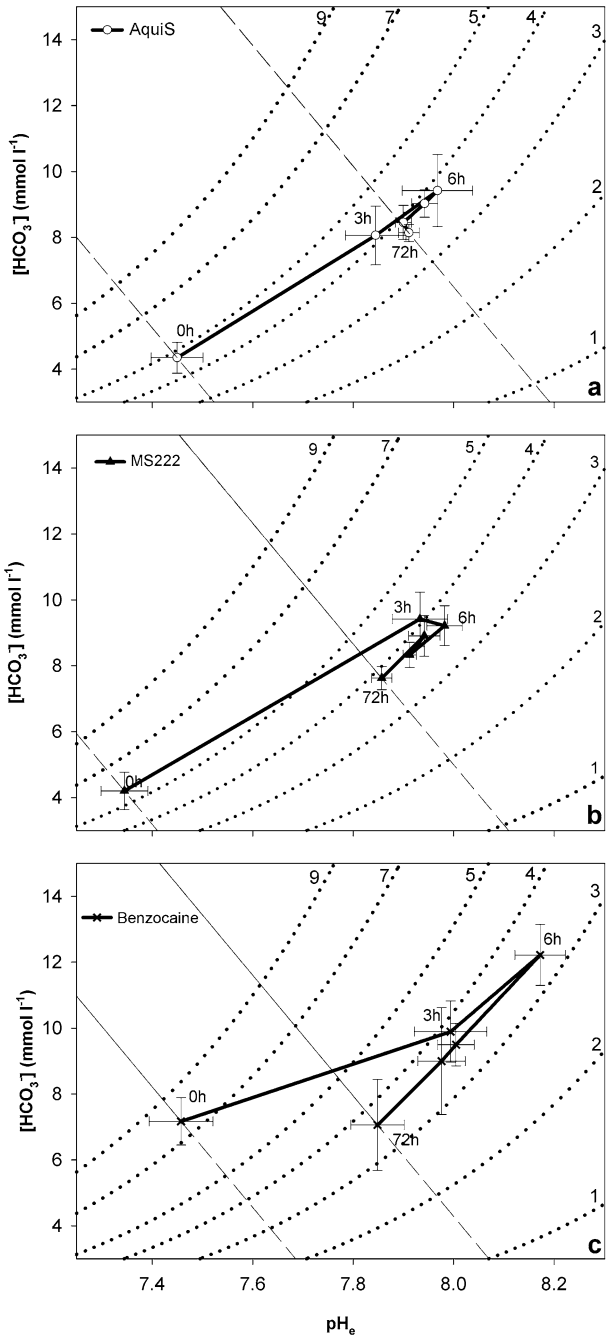

Fig. 4 Davenport diagrams of (**a**) Aqui-S, (**b**) MS222, and (**c**) benzocaine with the curved dotted lines indicating PCO_2-isopleths and dashed lines indicating *in vitro* buffer lines taken from Damsgaard et al. (2015). Values are presented as means ± S.E.M ($n = 7$)

recovery time, as well as duration of exposure and recovery time. In general, increased temperature is associated with faster clearance of the anaesthetics, but there was no indication that *P. hypophthalmus* recovered faster from anaesthesia than water-breathing teleosts studied previously at lower temperatures (Lefevre et al. 2014).

Evaluation of haematological and biochemical parameters. As reported for other species (e.g. Iwama et al. 1989; Iversen et al. 2003; Gholipour et al. 2011), we found that all three anaesthetics caused significant changes in haematological and biochemical parameters of *P. hypophthalmus*. Most notably, we observed a very pronounced rise in Hct during anaesthesia, and similar albeit typically smaller increases in Hct have been reported in various other species during anaesthesia with MS-222 (Reinitz and Rix 1977; Soivio et al. 1977; Iwama et al. 1989; Molinero and Gonzalez 1995). A rise in Hct can be caused by release of RBCs from the spleen, as a result of plasma loss from the vascular system typically in response to elevated blood pressure, or RBC swelling in response to adrenergic stimulation of the Na^+/H^+ exchanger (Ferreira et al. 1981; Nikinmaa and Huestis 1984; Wells and Weber 1990; Jensen 2004). In *P. hypophthalmus*, the rise in Hct was accompanied by proportional increases in [Hb] and RBC counts, such that MCHC did not change. This indicates that swelling of the RBCs did not occur and the present in *vitro* study showed that the RBCs from *P. hypophthalmus* do not respond to adrenergic stimulation. The lack of ß-adrenergic activation of the erythrocytes is further substantiated by the similarly low pH_i immediately after anaesthesia (Table 2). The present study cannot, however, reveal to what extent the increase of RBC counts is due to haemoconcentration by plasma loss or by splenic release of RBCs. It has been suggested that increasing [Hb] is an adaptation to increase blood oxygen transport capacity during stress (Pereira et al. 2013).

The high [cortisol] and [glucose] observed in *P. hypophthalmus* in the present study are similar to those reported for water-breathing fish species (Swift 1981; Tomasso et al. 1981; Barton and Peter 1982; Iwama et al. 1989; Ortuño et al. 2002; Park et al. 2008; Velisek et al. 2009; 2011). The mechanisms by which anaesthetics affect cortisol secretion in teleosts are unclear (Iwama et al. 1989; Thomas and Robertson 1991). Molinero and Gonzalez

Table 2 Effect of isoprenaline (10^{-5} mol l^{-1}) on blood O_2 saturation, Hct, and MCHC

	Before	After	Change (%)
Blood O_2 saturation (%)	18.6 ± 1.5	21.4 ± 1.7	2.8 ± 0.3*
Hct (%)	23.0 ± 2.8	23.5 ± 2.8	0.5 ± 0.3
MCHC (mmol l^{-1})	19.3 ± 1.6	18.4 ± 1.1	-0.9 ± 0.5

Blood samples were equilibrated to a PO_2 of 15.1 mmHg and a PCO_2 of 21.6 mmHg

Before values before addition of isoprenaline; *After* values after addition of isoprenaline to a final concentration of 10^{-5} mol l^{-1}; *Change* changes in blood parameters after the addition of isoprenaline

Data are presented as means ± S.E.M ($n = 4$)

* Significantly different from zero (one-sample *t* test, $P < 0.01$)

(1995) suggested that anaesthetics act on the hypothalamic–pituitary interrenal (HPI) axis stimulating cortisol secretion. They have also showed cortisol and glucose increases, but such responses were only found at high and intermediate dosages (25 and 30 mg l^{-1}), whereas the lower dose (15 mg l^{-1}) had no significant effect. It can be noted that the fivefold increase in [cortisol] during anaesthesia in the present study agrees with Thomas and Robertson (1991), who saw a similar cortisol increase after restraint and 2 minute air-exposure of red drum *Sciaenops ocellatus*. Previous studies have suggested that clove oil (isoeugenol) and Aqui-S (50 % isoeugenol) gave rise to lesser elevations in plasma cortisol during light anaesthesia than benzocaine in Atlantic salmon (Iversen et al. 2003) or MS222 in channel catfish (Small 2003). Similarly, isoeugenol had little effect on plasma cortisol in rainbow trout (Wagner et al. 2003).

The acid–base status during anaesthesia and recovery. While a large body of literature exists on acid–base and ion regulation in a variety of water-breathing fish species, little is known concerning these regulatory processes in bimodal breathers (Shartau and Brauner 2014). Acid–base disturbance in fish blood, resulting in increased plasma PCO_2 and a reduction in pH_e, can be induced by environmental challenges including as hypoxia and hypercapnia, as well as by exhaustive exercise (Baker et al. 2009; Shartau and Brauner 2014). Breathing air is associated with elevated PCO_2 as a result of the large difference in the solubility of CO_2 in air and water (Dejours 1981). Further air breathers are often poor at pHe regulation, possibly because of their reduced gills and reduced branchial irrigation leading to the suggestion that there is a compromise between oxygen uptake and ion and pH regulation in air-breathing fish (Ishimatsu and Itazawa 1983; Shartau and Brauner 2014). The present study with *P. hypophthalmus* anaesthesia reveals rapid pHe regulation, which is thus very unusual in air-breathing fish.

The cessation of ventilation during anaesthesia results in significant hypoxaemia and/or hypercapnia and is associated with respiratory acidosis (Iwama et al. 1989; Cooper and Morris 1998; Andersen and Wang 2002). This was also consistent with our findings in *P. hypophthalmus,* where a significant increase of PCO_2 immediately after catheterization contributed to the acidosis. The rise in PCO_2 is indicative of impaired gas exchange during anaesthesia despite the gills being constantly irrigated with aerated water during surgery. The marked reduction in pH_e was, however, primarily due to the production of lactic acid, *i.e.* metabolic in origin, presumably in response to severe hypoxaemia in various tissues, caused by a probable reduction in blood flows. The almost 20-fold rise of lactate subsided within the first few hours during recovery and was attended by normalization of pH_e and plasma [HCO_3^-] as well as reduction in PCO_2, presumably as normal ventilation of both gills and swim bladder were re-established. The increase of [lactate] in *P. hypophthalmus* is consistent with other studies on the effects of anaesthesia in water-breathing fish such as Atlantic salmon *Salmo salar*, brook trout *Salvelinus fontinalis*, and rainbow trout *Salmo gairdneri* (Houston et al. 1971; Soivio et al. 1977; Olsen et al. 1995; Iversen et al. 2003). Such increases in [lactate] are normally seen in the acid–base disturbance in fish after anaerobic exercise or following exposure to hypoxic conditions (Wood et al. 1977).

Post-anaesthesia recovery of blood gases has been well studied in water-breathing fish (Soivio et al. 1977; Molinero and Gonzalez 1995; Cooper and Morris 1998); whereas very little is known about recovery in these parameters in air-breathing fish. It is known that gills play a central role in acid–base regulation in water-breathing fish, which accounts for approximately 90 % of total ion transport during regulation from an acid–base disturbance (Claiborne et al. 2002; Evans et al. 2005). However, gill surface areas of bimodal air breathers in general are reduced (Hughes and Morgan 1973), which may prolong the acid–base recovery time. It has been suggested that *P. hypophthalmus* is unusual among air-breathing fish in having very large and well-developed gills at the same time as an air-breathing organ and that it can cover its entire oxygen requirements through the water phase in normoxic water (Lefevre et al. 2011). The present study supports this finding in that the rapid regulation of the acidosis induced by anaesthesia is regulated with rapidity, reminiscent of an active water breather such as rainbow trout.

Conclusions

All three anaesthetics effectively immobilized the fish and reduced responses to tactile stimulation to a level where transport or minor surgical procedures could be performed, but they also caused significant changes with similar patterns on gas and haematological parameters which were generally normalized within 24 h. The increase in Hct and [Hb] of *Pangasianodon hypophthalmus* caused by the anaesthetics resulted probably from increased RBC numbers and there was no indication of RBC swelling, which is different from active water-breathing fish such as rainbow trout. In addition, *P. hypophthalmus* is unusual among air-breathing fish with its strong capacity for acid–base regulation.

Acknowledgements This project was funded by The Danish International Development Agency (DANIDA), Danish Ministry of Foreign Affairs.

References

Abdolazizi S, Ghaderi E, Naghdi N, Kamangar BB (2011) Effects of Clove Oil as an anaesthetic on some hematological parameters of *Carassius auratus*. J Aquac Res Dev 02:108. doi:10.4172/2155-9546.1000108

Akbulut B, Çakmak E, Aksungur N, Çavdar Y (2011) Effect of exposure duration on time to recovery from anaesthesia of clove oil in juvenile of Russian sturgeon. Turk J Fish Aquat Sci 11:463–467

Andersen JB, Wang T (2002) Effects of anaesthesia on blood gases, acid-base status and ions in the toad *Bufo marinus*. Comp Biochem Physiol Part A 131:639–646

Attili S, Hughes SM (2014) Anaesthetic tricaine acts preferentially on Neural voltage-gated sodium channels and fails to block directly evoked muscle contraction. PLoS One 9:e103751. doi 10.1371/journal.pone.0103751

Baker DW, Matey V, Huynh KT, Wilson JM, Morgan JD, Brauner CJ (2009) Complete intracellular pH protection during extracellular pH depression is associated with hypercarbia tolerance in white sturgeon, *Acipenser transmontanus*. Am J Physiol Regul Integr Comp Physiol 296:1868–1880

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

Boutilier RG, Iwama GK, Heming TA, Randall DJ (1985) The apparent pK of carbonic acid in rainbow trout blood plasma between 5 and 15 °C. Respir Physiol 61:237–254

Brauner CJ, Wang T, Jensen FB (2002) Influence of hyperosmotic shrinkage and β-adrenergic stimulation on red blood cell volume regulation and oxygen binding properties in rainbow trout and carp. Comp Biochem Physiol Part B 172:251–262

Claiborne J, Edwards S, Morrison-Shetlar A (2002) Acid-base regulation in fishes: cellular and molecular mechanisms. J Exp Zool 293:302–319

Cooper AR, Morris S (1998) The blood respiratory, haematological, acid-base and ionic status of the Port Jackson shark, *Heterodontus portusjacksoni*, during recovery form anaesthesia and surgery: a comparison with sampling by direct caudal puncture. Comp Biochem Physiol Part A 119:895–903

Coyle SD, Durborow RM, Tidwell JH (2004) Anesthetics in aquaculture. Southern Regional Aquaculture Center Publication No. 3900

da Cunha MA, de Barros FMC, de Oliveira Garcia L, de Lima Veeck AP, Heinzmann BM, Loro VL, Emanuelli T, Baldisserotto B (2010) Essential oil of *Lippia alba*: A new anesthetic for silver catfish, *Rhamdia quelen*. Aquaculture 306:403–406

Damsgaard C, Gam LTH, Tuong DD, Thinh PV, Huong DTT, Wang T, Bayley M (2015) High capacity for extracellular acid/base regulation in the air-breathing fish *Pangasianodon hypophthalmus*. J Exp Biol 218:1290–1294

Dejours P (1981) Principles of comparative respiratory physiology. Elsevier/North-Holland Biomedical Press

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev 85:97–177

Ferreira JT, Smit GL, Schoonbee HJ (1981) Haematological evaluation of the anaesthetic benzocaine hydrochloride in the freshwater fish *Cyprinus carpio* L. J Fish Biol 18:291–297

Florindo LH, Leite CAC, Kalinin AL, Reid SG, Milsom WK, Rantin FT (2006) The role of branchial and orobranchial O_2 chemoreceptors in the control of aquatic surface respiration in the neotropical fish tambaqui (*Colossoma macropomum*): progressive responses to prolonged hypoxia. J Exp Biol 209:1709–1715

Fredricks KT, Gingerich WH, Fater DC (1993) Comparative cardiovascular effects of four fishery anaesthetics in spinally transected rainbow trout, *Oncorhynchus mykiss.* Comp Biochem Physiol Part C 104:477–483

Gholipour H, Mirzargar SS, Soltani M, Ahmadi M, Abrishamifar A, Bahonar A, Yousefi P (2011) Anesthetic effect of tricaine methanesulfonate, clove oil and electroanesthesia on lysozyme activity of *Oncorhynchus mykiss*. Iran J Fish Sci 10:393–402

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

Huggett AG, Nixon DA (1957) Enzymatic determination of blood glucose. Biochem J 66:12

Hughes GM, Morgan M (1973) The structure of fish gills in relation to their respiratory function. Biol Rev 48:419–475

Ishimatsu A, Itazawa Y (1983) Blood oxygen levels and acid-base status following air exposure in an air-breathing fish, *Channa argus*: the role of air ventilation. Comp Biochem Physiol A 74:787–793

Iversen M, Finstad B, McKinley RS, Eliassen RA (2003). The efficacy of metomidate, clove oil, Aqui-STM and Benzoak® 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 67:2065–2073

Jensen FB (2004) Red blood cell pH, the Bohr Effect, and other oxygenation-linked phenomena in blood O_2 and CO_2 transport. Acta Physiol Scand 182:215–227

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

Koldkjær P, Taylor E, Glass M, Wang T, Brahm J, McKenzie D, Jensen F (2002) Adrenergic receptors, Na+/H+ exchange and volume regulation in lungfish erythrocytes. Comp Biochem Physiol Part B 172:87–93

Lefevre S, Huong DTT, Wang T, Phuong NT, Bayley M (2011). Hypoxia tolerance and partitioning of bimodal respiration in the striped catfish (*Pangasianodon hypophthalmus*). Comp Biochem Physiol Part A 158:207–214

Lefevre S, Wang T, Jensen A, Cong NV, Huong DTT, Phuong NT, Bayley M (2014) Air-breathing fishes in aquaculture. What can we learn from physiology? J Fish Biol 84:705–731

Maricchiolo G, Genovese L (2011) Some contributions to knowledge of stress response in innovative species with particular focus on the use of the anaesthetics. TOMBJ 5:24–33

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

Nikinmaa M, Huestis WH (1984) Adrenergic swelling of nucleated erythrocytes: cellular mechanisms in a bird, domestic goose, and two teleosts, striped bass and rainbow trout. J Exp Biol 113:215–224

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

Ortuño J, Esteban MA, Meseguer J (2002) Effects of phenoxyethanol on the innate immune system of gilthead seabream (*Sparus aurata* L.) exposed to crowding stress. Vet Immunol Immunopathol 89:29–36

Park CK, Kim K, Jung SJ, Kim MJ, Ahn DK, Hong SD, Kim JS, Oh SB (2009) Molecular mechanism for local anesthetic action of eugenol in the rat trigeminal system. Pain 144:84–94

Park MO, Hur WJ, Im SY, Seol DW, Lee J, Park IS (2008). Anaesthetic efficacy and physiological responses to clove oil-anaesthetized kelp grouper *Epinephelus bruneus*. Aquac Res 39:877–884

Pereira L, Fernandes MN, Martinez CBR (2013) Hematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone. Environ Toxicol Pharmacol 36:1–8

Popovic NT, Strunjak-Perovic I, Coz-Rakovac R, Barisic J, Jadan M, Berakovic AP, Klobucar RS (2012) Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. J Appl Ichthyol 28:553–564

Reinitz GL, Rix J (1977). Effect of tricaine methanesulfonate (MS-222) on hematocrit values in rainbow trout (*Salmo gairdneri*). Comp Biochem Physiol Part C 56:115–116

Shartau RB, Brauner CJ (2014) Acid–base and ion balance in fishes with bimodal respiration. J Fish Biol 84:682–704

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

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

Soivio A, Nynolm K, Westman K (1975) A technique for repeated sampling of the blood of individual resting fish. J Exp Biol 62:207–217

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

Swift DJ (1981) Changes in selected blood component concentrations of rainbow trout, *Salmo gairdneri* Richardson, exposed to hypoxia or sublethal concentrations of phenol or ammonia. J Exp Biol 19:45–61

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

Tomasso JR, Davis KB, Parker NC (1981) Plasma corticosteroid dynamics in channel catfish, *Ictalurus punctatus* (Rafinesque), during and after oxygen depletion. J Fish Biol 18:519–526

Tucker VA (1967) Method for oxygen content and dissociation curves on microliter blood samples. J Appl Physiol 23:410–414

Velisek J, Stara A, Li ZH, Silovska S, Turek J (2011) Comparison of the effects of four anaesthetics on blood biochemical profiles and oxidative stress biomarkers in rainbow trout. Aquaculture 310:369–375

Velíšek J, Stejskal V, Kouřil J, Svobodová Z (2009) Comparison of the effects of four anaesthetics on biochemical blood profiles of perch. Aquac Res 40:354–361

Wagner GN, Singer TD, Scott McKinley R (2003) The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac Res 34:1139–1146

Weber RA, Peleteiro JB, Martín 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

Wells RMG, Weber RE (1990) The spleen in hypoxic and exercised rainbow trout. J Exp Biol 150:461–466

Wood CM, McMahon BR, McDonald DG (1977) An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. J Exp Biol 69:173–185

Zilstra WG, Buursma A, Zwart A (1983) Molar absorptions of human haemoglobin in the visible spectral range. J Appl Physiol 54:1287–1291