JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

Research article

Sedation of red porgy (*Pagrus pagrus*) and black sea bass (*Centropristis striata*) using ketamine, dexmedetomidine and midazolam delivered via intramuscular injection

Emily F. Christiansen, Jodi M. Mitchell, Craig A. Harms and Michael K. Stoskopf

North Carolina State University, College of Veterinary Medicine, Environmental Medicine Consortium and the Center for Marine Sciences and Technology, Morehead City, NC, USA
Correspondence: Emily F. Christiansen, 303 College Circle, Morehead City, NC, 28557, USA; emily.f.christiansen@gmail.com

Keywords:
anaesthetic, handling, restraint, sedation, teleost

Article history:
Received: 4 November 2013
Accepted: 7 June 2014
Published online: 31 July 2014

Abstract
Handling and restraint of large teleost fish in public aquaria presents significant logistical and safety challenges and research into effective injectable anaesthetic protocols has been limited. A combination protocol of ketamine (K), dexmedetomidine (D) and midazolam (M) injected intramuscularly was evaluated at several dose combinations in red porgy (*Pagrus pagrus*) and black sea bass (*Centropristis striata*). The response of red porgy was extremely variable, and even at the lowest dose tested (1 mg/kg K/0.025 mg/kg D/0.2 mg/kg M), multiple fish exhibited a severe, often fatal lactic acidosis. The protocol was effective in black sea bass, and the fish were consistently sedated and easily handled when anaesthetised with 2 mg/kg K/0.05 mg/kg D/0.2 mg/kg M or with 4 mg/kg K/0.1 mg/kg D/0.2 mg/kg M. All black sea bass recovered well following reversal of the dexmedetomidine with atipamezole at 10x the dose given and no long-term negative effects were seen. This protocol appears to be a safe and effective approach to sedating black sea bass to facilitate handling and movement.

Introduction

Public aquariums frequently maintain large saltwater "ocean" tanks to display a variety of pelagic and semi-pelagic fish species. Some commonly displayed teleost species, such as cobia (*Rachycentron canadum*) and goliath grouper (*Epinephelus itajara*), may reach adult lengths of over 2 m and greater than 60 kg body weight. In freshwater exhibits, arapaima (*Arapaima gigas*) and some species of catfish (order Siluriformes) attain comparable size. In a high-volume aquarium system, capture and restraint of large free-swimming fish manually or with nets may be unachievable, or present considerable physical risk to the animal and handlers.

Some facilities have addressed this challenge by conditioning larger fish to enter a confined area or restraint device, often using food rewards, but this option is not always available. Consequently, handling and close evaluation of these animals is frequently limited to an examination as they are placed into the exhibit, usually as juveniles, followed by no more than visual observations until they either die or become so moribund that they cannot evade capture. Captive display fish are susceptible to a variety of conditions, including trauma, infectious disease and neoplasia, that may be effectively treated and in many cases cured if they are recognised and addressed in a timely fashion (Stoskopf 1993). The development of a sedative technique that can be delivered from a short distance and allow for safe approach and handling would avail these larger specimens of similar diagnostic and treatment options that are commonly provided to smaller teleosts.

Several injectable anaesthetic protocols have been utilised with some success in elasmobranch species. Ketamine in combination with xylazine hydrochloride has been used to restrain adult sandbar sharks (*Carcharinus plumbeus*) for prolonged transport (Andrews and Jones 1990), and a combination of medetomidine and ketamine administered via intramuscular (IM) injection has been used successfully to immobilise sandtiger sharks (*Carcharius taurus*) for minimally invasive diagnostics and handling (Snyder et al. 1998).

Unfortunately, the efficacy of these medications is variable in bony fish and undesirable side effects are frequently seen. Successful anaesthesia using medetomidine and ketamine intravenously has been demonstrated in sturgeon (*Acipenser* sp) (Di Marco et al. 2011) and both xylazine and ketamine immersion have demonstrated efficacy in carp (*Cyprinus*

carpio) at high doses (72 mg/L ketamine and 23 mg/L xylazine) (Al-Hamdani et al. 2010). However, both of these delivery methods are prohibitive when attempting to anaesthetise fish in large volumes of water. A limited number of studies have demonstrated the use of intramuscular injection anaesthetics in teleosts. Ketamine alone has been reported as clinically effective in a variety of teleost species at 66–88 mg/kg, though unspecified adverse effects were seen at doses >215 mg/kg (Williams et al. 1993). In smaller fish, these routes and doses may be viable options, but in larger specimens, the volume of drug needed to deliver this dose rapidly becomes problematic, especially if a remote delivery device (pole syringe or dart projector) is necessary.

The addition of an alpha-2 adrenergic agonist has been used as a means to decrease the necessary dose of ketamine and reduce the potential for side effects. Xylazine has been reported to be effective in elasmobranch species, but has consistently caused ventilatory collapse, cardiac disturbances and convulsions in trout (*Salmo* spp.), and is therefore not recommended for use in teleost fish (Oswald 1978; Ross and Ross 1999). A combination of ketamine (K) and medetomidine (M) injected intramuscularly at a dose of 6 mg/kg K and 0.06 mg/kg M has been reported effective for short-term anaesthesia of Gulf of Mexico sturgeon (*Acipenser oxyrinchus de soti*), and apnoea or prolonged recovery times were not reported (Fleming et al. 2003). In the bonito (*Sarda chiliensis*) and the Pacific mackerel (*Scomber japonica*), the same drug combination resulted in variable depth of sedation across a wide range of doses (Williams et al. 2004). The character of recovery in these fish was not described, but many of the bonitos required between three and 12 days for return to normal behaviour. The combination of ketamine and medetomidine has been anecdotally reported in additional bony fish species, including lungfish (subclass Dipnoi), snappers (*Lutjanidae* spp.), cobia (*Rachycentron canadum*) and tuna (tribe Thunnini), at doses ranging from 0.05 to 4.2 mg/kg medetomidine and 4 to 228 mg/kg ketamine (Neiffer and Stamper 2009).

We adapted a partially reversible sedation protocol including ketamine, dexmedetomidine and midazolam (KDM) for delivery via intramuscular injection to teleost fish. This protocol was used in a clinical setting for three individual cobia, ranging in weight from 4 to 43 kg, and in each case the drug combination provided adequate sedation to allow for easy capture and relocation of the fish. Target doses for each of these fish were 2 mg/kg ketamine, 0.05 mg/kg dexmedetomidine, and 0.2 mg/kg midazolam, but accurate body weights could not be obtained prior to anaesthesia, and actual back-calculated doses delivered ranged from 1.88–4.6 mg/kg ketamine, 0.048–0.07 mg/kg dexmedetomidine, and 0.19–0.23 mg/kg midazolam. If a deeper plane of anaesthesia was necessary for invasive or potentially painful procedures, the fish was transferred to a smaller holding tank containing tricaine methanesulfonate (MS-222). Encouraged by success with cobia, we sought to evaluate this combination in two additional teleost species with different natural histories to broaden our understanding of the efficacy and safety of the KDM combination across teleost species.

Methods

Fish and housing

Ten captive-bred and reared red porgy (*Pagrus pagrus*), representative of relatively pelagic teleosts, were obtained from a research laboratory following a growth rate study, and nine wild-caught black sea bass (*Centropristis striata*), representative of more demersal teleosts, were collected from Bogue Sound, North Carolina. Red porgy body weights ranged from 0.05 to 0.307 kg (median 0.145 kg). Black sea bass body weights ranged from 0.148 to 0.374 kg (median 0.253 kg). All fish were of undetermined sex.

Fish were maintained in a 6500 L recirculating system with filtered natural seawater maintained at ambient room temperature (20–22° C). Water quality testing was performed weekly, after the initial cycling of the system, using commercially available colorimetric assays (API Saltwater Master Kit, Mars Fishcare, Chalfont, PA, USA) to ensure that parameters remained within acceptable limits (pH 8.0–8.2; ammonia <0.25 mg/L, nitrites <0.5 mg/L, and nitrates <40 mg/L). Temperature was monitored using a submerged mercury thermometer located in the attached sump tank. Small water changes (<10% volume) were performed approximately twice a week to siphon debris from the tank, and larger 30 to 50% water changes were performed as needed (at least weekly during initial cycling, and approximately every two weeks for the remainder of the study). All fish were fed a commercial pelleted diet (Aquamax Grower 500, PMI Nutrition International, LLC, Brentwood, MO, USA) every other day with fasting periods of 48–72 hours prior to experimental trials. Fish were fed as much as they would consume within two minutes, and both groups were feeding well throughout the duration of the study.

Because the sea bass were collected from the wild with an elevated risk of ectoparasitism, each fish was treated with a freshwater and formalin dip (250 ppm for five minutes) prior to introduction to the system. They were then allowed to acclimate for two weeks prior to beginning experimental trials. Fish showed no evidence of ectoparasitism when routine screening of mucus and gill biopsies were done at the completion of the study.

Anaesthesia

Individual fish were weighed to the nearest gram in a 19 L plastic bucket (sea bass) or 2 L plastic bin (red porgy) containing a small amount of tank water using a tared scale (Ohaus GT4800, Ohaus Corporation, Parsippany, NJ, USA). Trials were conducted in a translucent rectangular plastic tank with 10 L of water, aerated with an air stone. The water was changed between each trial, and dissolved oxygen levels were measured periodically (range 6.20–6.67 mg/L). Due to the small size of some individuals, anaesthetics were diluted 1:10 using sterile water (Sterile Water for Injection, USP, Hospira, Inc., Lake Forest, IL, USA) as needed. Anaesthetic medications were injected into the right epaxial muscles lateral to the mid-dorsal fin using attached-needle syringes (1 ml / 27 ga x 1.3 cm needle, Terumo Medical Corporation, Elkton, MD, USA). The reversal agent, atipamezole (Antisedan® 5 mg/ml, Pfizer Animal Health, New York, NY, USA), was injected into the left epaxial muscles opposite the anaesthetic either 30 minutes (red porgy) or 60 minutes (sea bass) after the initial injection. Two of the red porgy did not receive anaesthetic injections; blood samples were collected from these fish as controls.

On the basis of the observed impact of the drugs on porgy, each red porgy was used for only one trial and received only one dose of the drug combination. Black sea bass were used in three separate trials in a cross-over design. Each black sea bass received one of three combinations of ketamine (Ketaset® 100 mg/ml, Fort Dodge Animal Health, Fort Dodge, IA, USA), dexmedetomidine (Dexdomitor® 0.5 mg/ml, Pfizer Animal Health, New York, NY, USA), and midazolam (Midazolam® 5 mg/ml, Baxter Healthcare Corporation, Deerfield, IL, USA), as listed in Table 1. There was a 1 week time period between trials to minimise the risk of drug residuals impacting later trials. The order in which each fish received each dose was randomised using coin-tosses.

Monitoring

Anaesthetic times were monitored using a stopwatch started immediately upon injection of the anaesthetic agents. Time to effect was reported as the time from injection to the first visible effect, usually a loss of normal posture or obvious disorientation. Recovery time was measured from the time of injection of the

Table 1. Low, medium and high KDM doses.

	Ketamine (mg/kg)	Dexmedetomidine (mg/kg)	Midazolam (mg/kg)	Atipamezole (mg/kg)
Low	1.0	0.025	0.2	0.25
Medium	2.0	0.05	0.2	0.5
High	4.0	0.1	0.2	1.0

reversal agent to the time the fish regained normal posture and obstacle avoidance. Opercular rate was measured through visual observation prior to injection and every five minutes thereafter. Sedation scores were assigned every 10 minutes using handling exercises adapted from Gladden et al. (2010) (Table 2). Each variable was rated from one to three, with one being no reaction and three being the response of a fish without sedation, for a possible score range between five (stage II–III anaesthesia) and 15 (no effect).

Blood sampling and processing

Immediately prior to anaesthetic reversal, approximately 0.1 ml of blood was collected from the caudal hemal arch using a 1 ml syringe with either a 22 or 25 gauge needle. Syringes were prepared by withdrawing approximately 0.1 ml of heparin into the syringe, then expelling all liquid, leaving a thin film of anticoagulant. Blood gases and lactate values were obtained using a portable point of care analyser (i-STAT® and CG4+ cartridges, Abbot Laboratories, Inc.). All samples were processed within three minutes of collection.

Recovery and follow-up

Regular monitoring of opercular rate and handling exercises continued for 30 minutes after reversal. The fish were then placed into a recovery basket floating in the housing tank. Fish that died during the recovery period and fish that appeared to recover well but were subsequently found dead after the experimental period were held on ice and a gross necropsy was performed within 12 hours.

Approval and disposition

The use of live fish in this study was approved by the Institutional Animal Care and Use Committee of North Carolina State University. Following completion of the study, the black sea bass were donated to public display aquaria and the red porgy were donated to the aquaculture programme of an educational facility, never to enter the food chain.

Statistical analysis

Summary statistics are presented as medians and ranges, because the data were not normally distributed. A Wilcoxon rank sum test was used to compare results between the red porgy and black sea bass receiving the same dose of anaesthetic (low dose). A Kruskal–Wallis test was used to compare results of anaesthesia and blood gases across three doses in black sea bass, followed by a Dunn all pairs post hoc test when a difference was detected. The Friedman test was used to evaluate changes in sedation depth and opercular rate over time in black sea bass. When a significant difference was detected, a 2-sided post hoc test was used to compare each time point with the 90 minute score for sedation depth and with the 0 time for opercular rate (Hollander and Wolfe 1972). Statistical analysis was performed manually or using JMP Version 10.0 (SAS Software, Cary, NC).

Results

Induction and recovery times and blood lactate concentrations for all individual red porgy are presented in Table 3. One fish received the medications at the medium KDM dose, and due to poor recovery and eventual mortality, all subsequent red porgy received the low dose. Most of these fish exhibited a complete loss of equilibrium following injection, and remained ventral side up while attempting to right themselves throughout the study period. The abnormal posture and struggling swimming motions continued following administration of the reversal, and mortality rate was high (3/5). The remaining red porgy given the low dose KDM (n=3) showed minimal signs of sedation at the same dose, and did not lose righting ability at any time.

No gross abnormalities were noted on necropsy of the red porgy that died during the recovery period. Approximately 2–3 weeks following the completion of experimental trials, three other red porgy that appeared to recover well from the anaesthetics were found dead in the holding tank. One of these fish showed evidence of severe fungal nephritis, and the other two were emaciated at the time of death, though in good body condition at the time of the experiment.

Median and range of time to effect and recovery times for nine black sea bass given each of the three KDM doses are presented in Table 4. The peak depth of sedation for all doses was seen at

Table 2. Scoring criteria for sedation depth.

	1	2	3
Body position	Lying on bottom of tank in dorsally recumbent position	Lying laterally; difficulty maintaining equilibrium	Normal upright position
Lateral roll – place fish on lateral side	No response	Diminished response; some movement but easily restrained	Strong response; unable to restrain
Ventral roll – place fish in dorsal recumbency	No response	Diminished response; some movement but able to roll with little effort	Strong response; unable to restrain
Drag – gently pull fish backwards by tail	No response	Diminished response; some pectoral fin movement but still easily pulled back	Strong response; unable to drag backwards or restrain
Tail pinch	No response	Diminished response; some pectoral fin movement; unsuccessful attempts to escape	Strong response; fish escapes easily

Table 3. Anaesthesia times, individual lactate values, and outcome for red porgy given the low (1 mg/kg ketamine / 0.025 mg/kg dexmedetomidine / 0.2 mg/kg midazolam, fish #2–8) or medium KDM dose protocol (2 mg/kg ketamine/ 0.05 mg/kg dexmedetomidine, 0.2 mg/kg midazolam, fish #1).

Fish ID	Weight (kg)	Time to effect (minutes)	Recovery time (minutes)	Lactate (mmol/L)	Outcome
1*	0.862	5	N/A	18.8	Never regained righting ability; died
2	0.307	1.5	N/A	>20	Never regained righting ability; died
3	0.159	16	16	11.03	Only slight loss of equilibrium, remained upright throughout trial
4	0.132	9.5	~1440	5.19	Lost equilibrium quickly, and was unable to right self for approximately 24 hours post-reversal, but eventually recovered
5	0.050	13	19	0.56	Lost equilibrium, but recovered quickly
6	0.216	10	5	3.35	Only slight loss of equilibrium, remained upright throughout trial
7	0.078	5	4	1.71	Only slight loss of equilibrium, remained upright throughout trial
8	0.180	13.5	N/A	12.54	Never regained righting ability; died

*The first fish received the medium KDM dose and is not included in subsequent analysis.

30 minutes post-injection, and median and range at this time point is presented for each KDM dose. A dose-dependent effect on time to drug effect and peak depth of sedation is apparent, but no similar pattern is evident in recovery time. Table 5 and Table 6 present medians and ranges of sedation scores and opercular rates of black sea bass in each dose group, respectively. A similar trend in sedation depth is seen across doses, with the peak sedation between 20 and 40 minutes, and a rapid return to near normal responsiveness (maximal score of 15) following reversal with atipamezole at 60 minutes. The change over time was statistically significant for all three doses, and in all three groups depth scores from 20–60 minutes were significantly lower than scores at 90 minutes. At the medium dose, the sedation score at 10 minutes was also significantly lower than scores at 90 minutes. No mortalities or visible long-term side effects were seen in the black sea bass. Individual variation in opercular rates was wide, but there was a consistent decrease in rate following initial injection that remained relatively stable throughout the trial period. Opercular rate then increased towards baseline following reversal. A significant difference was seen among time points for each dose, with opercular rates at most time points during sedation being significantly lower than opercular rates before injection. Opercular rates at several time points following reversal were also significantly higher than opercular rates during sedation for each dose.

Table 4. Descriptive statistics (median and range) for black sea bass receiving three different dosages of ketamine (K), dexmedetomidine (D) and midazolam (M).

Dose	Dosages (mg/kg)	Time to effect (min)[a]	Recovery time (min)	Peak sedation depth (at 30 min)[b]
Low (n = 9)	1.0 K 0.025 D 0.2 M	12 (8–28)	8 (6–23)	9 (7–13)
Medium (n = 9)	2.0 K 0.05 D 0.2 M	6 (4–18)	6 (2–23)	8 (7–10)
High (n = 9)	4.0 K 0.1 D 0.2 M	4 (3–13)	7 (2–14)	6 (5–8)

[a]Significant difference among doses (p=0.0413); high and low doses significantly different (p=0.0407).
[b]Significant difference among doses (p=0.0004); significant difference between low and high (p=0.0003) and medium and high (p=0.0404).

Blood gas, pH and lactate values (medians and ranges) are presented for red porgy at the low dose and black sea bass at three doses in Table 7. A blood sample was not collected from all individuals at all dose trials, and consequently group sizes differ. Temperature corrected values for blood pH and pCO_2 are presented for comparison. Median blood pH, pCO_2 and HCO_3 were lower for red porgy than black sea bass anaesthetised at the same dose (low KDM), but only HCO_3 differed significantly between species. The median lactate value measured for red porgy was higher than all measured values for black sea bass groups (low, medium and high KDM), but there was no statistically significant difference. There were no significant differences among blood gas values measured across three doses in black sea bass. When all red porgy were compared to all black sea bass, pH, pCO_2 and HCO_3 were significantly lower in red porgy, and lactate significantly higher.

Discussion

The behavioural response to the anaesthetic medications was dramatically different between these two species of teleost. The black sea bass consistently became sedate and markedly less responsive to stimuli and handling, similar to the reaction seen clinically in cobia. Many sea bass lost the righting reflex and rested calmly on the bottom of the experimental tank in a ventral side up position throughout the anaesthetic period, without obvious efforts to right themselves or reorient in the tank. In contrast, the red porgy that lost the righting reflex spent the entire trial period exhibiting active swimming motions and attempts to right

Table 5. Depth of sedation scores (median and range) over time for black sea bass at three different KDM doses (n = 9). Lower scores indicate greater depth of sedation. Maximum (non-sedated) score = 15, minimum (fully sedated) score = 5.

Dose	10 min*	20 min*	30 min*	40 min*	50 min*	60 min	70 min	80 min	90 min
Low (n = 9)	12[a] (9–15)	*9[a] (7–13)*	*9[a] (7–13)*	*10[a] (7–12)*	*10[a] (7–12)*	*10 (7–12)*	14 (7–15)	15 (7–15)	15 (9–15)
Medium (n = 9)	*9[b] (7–12)*	*8[a,b] (6–11)*	*8[a] (7–10)*	*8[a,b] (8–10)*	*8[a,b] (7–10)*	*8 (7–12)*	12 (9–15)	13 (12–15)	14 (13–15)
High (n = 9)	8[b] (6–11)	*7[b] (6–8)*	*6[b] (5–8)*	*6.5[b] (5–8)*	*7[b] (5–9)*	*7.5 (5–10)*	11 (7–14)	13 (11–15)	14 (12–15)

Time points in italics are significantly lower than scores at 90 minutes.
*Significant difference among doses.
[a,b]Values with the same superscript are not significantly different (p<0.05).

Table 6. Opercular rates per minute (median and range) over time for black sea bass at three different KDM doses.

Time (min)	0	5	15*	25*	35*	45*	55	60	65	75	85	90
Low (n = 7)	54 (42–66)	36 (30–54)	36 (30–48)	*36 (24–42)*	36 (30–42)	*30 (30–42)*	*30 (24–48)*	*30 (24–36)*	42 (30–48)	42 (36–60)	42 (24–66)	42 (24–60)
Medium (n = 9)	54 (42–60)	36 (30–48)	30 (24–36)	*30 (24–36)*	*30 (18–36)*	*30 (24–36)*	*24 (24–30)*	*30 (24–36)*	30 (24–48)	42 (24–60)	54 (24–60)	48 (30–60)
High (n = 8)	54 (48–72)	36 (30–42)	*30 (18–30)*	*24 (24–36)*	*24 (24–36)*	*24 (24–42)*	*24 (24–48)*	*24 (24–48)*	33 (24–36)	42 (30–60)	42 (30–72)	42 (30–66)

Time points in italics are significantly lower than respiration rates at 0 minutes.
*Significant difference between high and low doses (p<0.05).

Table 7. Median (and range) blood gas values for red porgy and black sea bass at three KDM doses collected immediately prior to anaesthetic reversal (30 min for red porgy, 60 min for black sea bass).

Dose	pH	pH (temp corr)	pCO2 mmHg	pCO_2 (temp corr)	HCO_3 mmol/L	Lactate mmol/L
Red porgy (n=7)[a]	7.19 (6.99–7.30)	7.35 (7.16–7.46)	10.1 (7.4–14.4)	5.3 (3.9–7.5)	3.5[b] (2.6–4.9)	4.27 (0.56–>20)
Black sea bass Low (n=4)	7.37 (7.14–7.62)	7.53 (7.30–7.78)	12.8 (8.8–17.2)	6.7 (4.6–9.0)	7.35[b] (5.8–9.0)	3.09 (<0.30–3.09)
Black sea bass Medium (n=6)	7.48 (7.13–7.60)	7.64 (7.29–7.77)	12.9 (10.5–16.1)	6.8 (5.5–8.4)	9.6 (5.3–10.4)	2.18 (<0.30–3.95)
Black sea bass High (n=2)	7.36 (7.24–7.47)	7.52 (7.40–7.64)	13.25 (12.2–14.3)	6.9 (6.4–7.5)	7.5 (6.1–8.9)	2.03 (0.88–3.18)

[a]All red porgy received the low dose of anaesthetics (1 mg/kg K / 0.025 mg/kg D / 0.2 mg/kg M).
[b]Significant difference between low dose red porgy and low dose black sea bass.

themselves. There was a general tendency to swim vigorously into corners of the rectangular tank, orienting nearly vertically with the tail towards the surface. We hypothesise that some of the difference in response to these medications is due to species-specific behavioural adaptations. While both species inhabit similar continental shelf environments in the wild, black sea bass are demersal, resting nearly motionless on the substrate or using tunnels and crevices for protection. Red porgy are more frequently seen hovering in the water column, having minimal contact with substrate or other permanent objects. It seems possible that the black sea bass are better able to adapt to a loss of equilibrium due to their regular contact with and use of substrate for orientation. A response similar to that of the black sea bass was seen in the cobia treated with these medications clinically. Despite their generally pelagic lifestyle, the cobia is another species that regularly shelters under and around natural and artificial structures (Shaffer and Nakamura 1989).

None of the fish that received these doses achieved a plane of anaesthesia that would allow for invasive or surgical procedures. Venipuncture required somewhat less manual restraint for sea bass that received the highest dose, but these fish still responded to the needle insertion. As has been seen in other studies using injectable anaesthetics in teleost fish, supplementary anaesthesia with another agent such as MS-222 would be necessary for invasive procedures (Tuttle and Dunn 2003; Neiffer and Stamper 2009). We did not investigate the effects of this protocol on subsequent immersion anaesthesia and whether it might allow for lower doses of MS-222, which may have utility in fish that poorly tolerate high doses of MS-222 or that exhibit a vigorous and potentially damaging excitement phase.

Anaesthetic studies in most species rely upon a painful stimulus such as a needle stick or extremity pinch to measure effectiveness and depth of anaesthesia. In many species, especially mammals, other cues such as eye position, blink reflexes and jaw tone are available to assess responsiveness, many of which have limited utility in fish. Anaesthesia in fish is conventionally divided into four separate stages, ranging from light sedation (stage I) through surgical anaesthesia (stage III) to medullary collapse (stage IV), with several stages further divided into light and deep planes (Stoskopf 1993; Ross and Ross 1999). None of the animals in our study attained stage III surgical anaesthesia, and nearly all remained reactive to gross stimulation (tail pinch) throughout the study. Depending on the criteria used, black sea bass given this sedation protocol achieved stage I (deep sedation) or stage II (narcosis) anaesthesia. Few assessments have been developed to quantify depth of sedation more specifically, necessitating the development of objective criteria to compare across doses in these fish. We adapted our handling measures from a recent study in koi carp (*Cyprinus carpio*) (Gladden et al. 2010), emphasising manoeuvres that would probably be encountered in capturing, transporting and examining larger specimens, such as rolling to visually examine all sides of the fish and moving horizontally through the water, as might be required if a fish came to rest in an awkward space in tank furnishings, or during manipulation into a stretcher.

An interesting, apparently benign, side effect noted in all fish was a patchy localised blanching of the skin at the site of intramuscular KDM injection. An opposite reaction occurred at the site of atipamezole injection, with the appearance of a small dark patch immediately over the site of injection. One aspect of skin coloration in fish is a physiological rearrangement of melanin pigment within melanophores, which are responsive to alpha-2 adrenergic stimulation. Medetomidine is a strong inducer of this reaction in fish, and in immersion studies with rainbow trout (*Oncorhynchus mykiss*) resulted in marked pigment aggregation and lightening of colour (Lennquist et al. 2010). As noted with immersion in rainbow trout, the colour reaction we saw following injection was temporary, and normal coloration returned within several hours.

Portable point-of-care blood analysers are particularly useful for evaluating free-ranging and captive wildlife species, because they are easily transported into the field and require much smaller blood volumes than conventional laboratory analysers (McCain et al. 2010, Stoskopf et al. 2010). Blood gas reference intervals have been reported for a number of fish species, and comparisons with conventional analysers support their clinical usefulness in these animals, with some practical caveats (Harrenstien et al. 2005, Gallagher et al. 2010, Roth and Rotabakk 2012). The most common venipuncture site in teleost fish, the caudal hemal arch, is actually a complex of arteries and veins, and samples collected from this site contain varying amounts of arterial and venous blood. Some values measured by the iStat cannot be interpreted with mixed arterial and venous blood samples, namely pO_2, and these values were not presented here. Because this portable analyser is designed for mammalian species, it is calibrated to process samples at 37° C. Equations have been proposed to correct for temperature in ectothermic species, but a study using blood from sandbar sharks and smooth dogfish found results with these equations to be significantly different than those obtained from a tabletop blood gas analyser calibrated to the temperature of the samples (Gallagher et al. 2010). However, the authors noted a strong linear relationship between the raw values obtained from the iStat and the tabletop analyser, concluding that while the exact values provided by the iStat may be inaccurate, the interpretation of trends and differences between individuals is clinically reliable.

With the red porgy in this study, there was an obvious relationship between blood lactate at the end of anaesthesia and mortality: all fish with a value greater than 12 mmol/L died. No surviving fish exhibited such a severe elevation, and most were far lower, as were all of the black sea bass. Severe lactic acidosis has been reported following exhaustive exercise in multiple teleost species, often continuing to increase for some time after the cessation of exercise (Thomas et al. 1987, Tufts et al. 1991, Roth and Rotabakk 2012). The active swimming behaviour and attempts to turn upright seen in the red porgy throughout the trial periods appear to have driven these fish into anaerobic metabolism and caused the accumulation of lactic acid, resulting in severe metabolic acidosis. The observed blood gas values are consistent with a primary metabolic acidosis (decreased pH and decreased HCO_3), with partial respiratory compensation indicated by the lower pCO_2 values seen in red porgy as compared to black sea bass. The absence of a similar response and consequent acidosis in black sea bass may be a result of different behavioural reactions to equilibrium disruption and may help predict when this anaesthetic protocol would be appropriate.

This combination of anaesthetics appears to be safe and effective for black sea bass, and could be considered for other related species of teleost that exhibit similar behaviour. The adverse effects seen in red porgy at even the lowest dose tested warrant caution whenever using these medications on a new species, especially those that are wholly pelagic and/or do not make use of substrate or structures for shelter and resting. Sedation was more consistent with the medium and high KDM doses, and differences in time to effect and depth of sedation between the two are unlikely to be clinically relevant in most situations. If a shorter induction time is desired for any reason, using the higher end of the dose range would be appropriate. When working with large individuals in display aquaria, however, the volume of liquid drug that can be administered in a single injection is often a limiting factor and exact fish weight is likely unknown, so utilising the medium KDM dose as a starting point should provide acceptable effect with a margin of safety.

Acknowledgements

The authors thank Heather Broadhurst for extensive technical assistance throughout the project, and Dana Schmidt for assistance with animal care.

References

Al-Hamdani A.H., Ebrahim S.K., Mohammad F.K. (2010) Experimental xylazine-ketamine anesthesia in the common carp (*Cyprinus carpio*). *Journal of Wildlife Diseases* 46(2): 596–598.

Andrews J.C., Jones R.T. (1990) A method for the transport of sharks for captivity. *Journal of Aquariculture and Aquatic Sciences* 5(4): 70–72.

Clauss T., Berliner A., Brainard B. (2011) Chemical restraint in bonnethead sharks (*Sphyrna tiburo*): pilot studies with select sedatives and anesthetics. *Proceedings of the International Association of Aquatic Animal Medicine* 42: 121.

Di Marco P., Petochi T., Longobardi A., Priori A., Finoia M.G., Donadelli V., Corsalini I., Marino, G. (2011) Efficacy of tricaine methanesulphonate, clove oil and medetomidine-ketamine and their side effects on the physiology of sturgeon hybrid *Acipenser naccarii* x *Acipenser baerii*. *Journal of Applied Ichthyology* 27: 611–617.

Fleming G.J., Heard D.J., Francis Floyd R., Riggs A. (2003) Evaluation of propofol and medetomidine-ketamine for short-term immobilization of Gulf of Mexico sturgeon (*Acipenser oxyrinchus de soti*). *Journal of Zoo and Wildlife Medicine* 34(2): 153–158.

Gallagher A.J., Frick L.H., Bushnell P.G., Brill R.W., Mandelman J.W. (2010) Blood gas, oxygen saturation, pH, and lactate values in elasmobranch blood measured with a commercially available portable clinical analyzer and standard laboratory instruments. *Journal of Aquatic Animal Health* 22(4): 229–234.

Gladden J.N., Brainard B.M., Shelton J.L., Camus A.C., Divers S.J. (2010) Evaluation of isoeugenol for anesthesia in koi carp (*Cyprinus carpio*). *American Journal of Veterinary Research* 71: 859–866.

Harrenstien L.A., Tornquist S.J., Miller-Morgan T.J., Fodness B.G., Clifford K.E. (2005) Evaluation of a point-of-care blood analyzer and determination of reference ranges for blood parameters in rockfish. *Journal of the American Veterinary Medical Association* 226: 255–265.

Hollander M., Wolfe D.A. (1972) *Nonparametric Statistical Methods*. New York, NY: John Wiley & Sons, Inc.

Lennquist A., Mårtensson Lindblad L.G.E., Hedberg D., Kristiansson E., Förlin L. (2010) Colour and melanophore function in rainbow trout after long term exposure to the new antifoulant medetomidine. *Chemosphere* 80: 1050–1055.

Mandelman J.W., Skomal G.B. (2009) Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks. *Journal of Comparative Physiology B* 179: 267–277.

McCain S.L., Flatland B., Schumacher J.P., Clarke III E.O., Fry M.M. (2010) Comparison of chemistry analytes between 2 portable, commercially available analyzers and a conventional laboratory analyzer in reptiles. *Veterinary Clinical Pathology* 39(4): 474–479.

Neiffer D.L., Stamper M.A. (2009) Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR Journal* 50(4): 343–360.

Oswald R.L. (1978) Injection anesthesia for experimental studies in fish. *Comparative Biochemistry and Physiology* 60C: 19–26.

Ross L.G., Ross B. (1999) *Anaesthetic and Sedative Techniques for Aquatic Animals*. Oxford, UK: Blackwell Science Ltd.

Roth B., Rotabakk B.T. (2012) Stress associated with commercial longlining and recreational fishing of saithe (*Pollachius virens*) and the subsequent effect on blood gases and chemistry. *Fisheries Research* 115–116: 110–114.

Shaffer R.V., Nakamura E.L. (1989) Synopsis of biological data on the cobia *Rachycentron canadum* (Pisces: Rachycentridae). *NOAA Technical Report*, NMFS 82.

Snyder S.B., Richard M.J., Berzins I.K., Stamper M.A. (1998) Immobilization of Sandtiger sharks (*Odontaspsis taurus*) using medetomidine/ketamine. *Proceedings of the International Association of Aquatic Animal Medicine* 29: 120–121.

Stoskopf M.K. (1993) *Fish Medicine*. Philadelphia: W.B. Saunders Company.

Stoskopf M.K., Mulcahy D.M., Esler D. (2010) Evaluation of a portable automated serum chemistry analyzer for field assessment of Harlequin ducks (*Histrionicus histrionicus*). *Veterinary Medicine International*. doi: 10.4061/2010/418596.

Thomas S., Poupin J., Lykkeboe G., Johansen K. (1987) Effects of graded exercise on blood gas tension and acid-base characteristics of rainbow trout. *Respiration Physiology* 68: 85–97.

Tufts B.L., Tang Y., Tufts K., Boutilier R.G. (1991) Exhaustive exercise in "wild" Atlantic salmon (*Salmo salar*): acid-base regulation and blood gas transport. *Canadian Journal of Fisheries and Aquatic Science* 48: 868–874.

Tuttle A.D., Dunn J.L. (2003) Evaluation of intramuscular use of medetomidine (α-2 adrenergic agonist) as an alternative to tricaine methanesulfonate (MS-222, Finquel©) for sedation and anesthesia in teleosts and elasmobranchs. *Proceedings International Association of Aquatic Animal Medicine* 34: 43–44.

Williams T.D., Christiansen J., Nygren S. (1993) A comparison of intramuscular anesthetics in teleosts and elasmobranchs. *Proceedings International Association of Aquatic Animal Medicine* 24: 6.

Williams T.D., Rollins M., Block B.A. (2004) Intramuscular anesthesia of bonito and Pacific mackerel with ketamine and medetomidine and reversal of anesthesia with atipamezole. *Journal of the American Veterinary Medical Association* 225: 417–421.