Intramuscular anesthesia of bonito and Pacific mackerel with ketamine and medetomidine and reversal of anesthesia with atipamezole

Thomas D. Williams, DVM; Megan Rollins; Barbara A. Block, PhD

Objective—To determine anesthetic effects of ketamine and medetomidine in bonitos and mackerels and whether anesthesia could be reversed with atipamezole.

Design—Clinical trial.

Animals—43 bonitos (*Sarda chiliensis*) and 47 Pacific mackerels (*Scomber japonica*).

Procedure—28 bonitos were given doses of ketamine ranging from 1 to 8 mg/kg (0.5 to 3.6 mg/lb), IM, and doses of medetomidine ranging from 0.2 to 1.6 mg/kg (0.1 to 0.7 mg/lb), IM (ratio of ketamine to medetomidine, 2.5:1 to 20:1). Doses of atipamezole equal to 1 or 5 times the dose of medetomidine were used. The remaining 15 bonitos were used to determine the anesthetic effects of ketamine at a dose of 4 mg/kg (1.8 mg/lb) and medetomidine at a dose of 0.4 mg/kg (0.2 mg/lb). The mackerels were given ketamine at doses ranging from 11 to 533 mg/kg (5 to 242 mg/lb) and medetomidine at doses ranging from 0.3 to 9.1 mg/kg (0.1 to 4.1 mg/lb; ratio of ketamine to medetomidine, 3:1 to 800:1). Doses of atipamezole equal to 5 times the dose of medetomidine were used.

Results—IM administration of ketamine at a dose of 4 mg/kg and medetomidine at a dose of 0.4 mg/kg in bonitos and ketamine at a dose of 53 to 228 mg/kg (24 to 104 mg/lb) and medetomidine at a dose of 0.6 to 4.2 mg/kg (0.3 to 1.9 mg/lb) in mackerels was safe and effective. For both species, administration of atipamezole at a dose 5 times the dose of medetomidine reversed the anesthetic effects.

Conclusions and Clinical Relevance—Results suggest that a combination of ketamine and medetomidine can safely be used for anesthesia of bonitos and mackerels and that anesthetic effects can be reversed with atipamezole. (*J Am Vet Med Assoc* 2004;225:417–421)

In fish, anesthetic agents are commonly administered by immersion, with IM, IV, and IP injection and oral administration used less commonly. Increased efforts to maintain captive collections of fish for research and commercial purposes, however, have led to the need for injectable anesthetic protocols that are reversible. Anesthetic protocols that involve IM administration would be particularly beneficial because of the ability to administer anesthetics by dart in the wild, in pens, and in large oceanarium-style multispecies tanks. Given the high value of scombrid fish, particularly yellowfin and bluefin tunas, as ranched fish with high market value and as captive specimens for exhibit at aquaria,[1] anesthetic protocols that involved administration of anesthetic agents by dart and reversal of anesthetic effects at the end of the anesthetic period would be most useful. Such protocols would not only increase the ease of transporting these fish, but also reduce the stress and pain they experience during handling.

Fish of the family Scombridae (bonitos, mackerels, and tunas) are well-known for their extraordinary physiology. In particular, tunas are endothermic and have a high metabolism and high cardiac output.[2,a] Most tunas are capable of migrating large distances, with many species known to migrate across ocean basins. Mackerels and bonitos are more primitive members of the clade and lack many of the physiologic characteristics that typify tunas. In particular, bonitos and mackerels are ectothermic, rather than endothermic. In addition, mackerels have low metabolic rates (172 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$). However, although perfusion of tissues is limited by capillarization in mackerels, aerobic capacity at the tissue level is high. Bonitos have a lower metabolic rate (107 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) than mackerels or yellowfin tunas (260 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$),[3] and they lack the vascular countercurrent heat exchange mechanisms found in tunas, but they have the aerobic capacity of the tuna genera *Thunnus* and *Euthynnus*. Thus, bonitos and mackerels are similar enough to tunas that they make good candidates for initial tests of anesthetic procedures that might eventually be adapted for use in tunas.

All teleost fish have an anatomic separation between aerobic, slow-oxidative muscles and anaerobic, fast-twitch, glycolytic muscles. Bonitos and mackerels have a distinct pattern of highly oxygenated, slow-oxidative muscle that runs along the midline of the body and is used for routine, sustained swimming.[4] Capillarization is increased in this region, suggesting that any anesthetic agents injected into these muscles would be rapidly absorbed, decreasing anesthetic induction time.

Ketamine hydrochloride is a rapid-acting, nonnarcotic, nonbarbiturate anesthetic agent that induces a state of unconsciousness known as dissociative anesthesia. It is thought to induce anesthesia and amnesia by functionally disrupting the CNS through overstimulation or induction of a cataleptic state,[5] but is not capable of inducing a surgical plane of anesthesia on its

From the Tuna Research and Conservation Center, Monterey Bay Aquarium, 886 Cannery Row, Monterey, CA 93940 (Williams); and the Hopkins Marine Station, Stanford University, Oceanview Blvd, Pacific Grove, CA 93950 (Rollins, Block).

Supported by the Monterey Bay Aquarium.

The authors thank Steven Teo for assistance with statistical analyses.

Address correspondence to Dr. Williams.

own. Ketamine has been shown to have minimal hematologic effects in mammals.[6]

Medetomidine hydrochloride is an α_2-adrenoceptor agonist that has been extensively used in mammals to induce sedation, analgesia, and anesthesia[b]; it can be reversed with atipamezole hydrochloride, an α_2-adrenoceptor antagonist and competitive inhibitor of medetomidine. Although α_2-adrenoceptors have been widely studied in many species, research on α_2-adrenoceptors in fish is limited and further studies of α_2-adrenoceptor pharmacology in fish are needed to fully understand the mechanisms of medetomidine anesthesia in fish.

When administered to mammals in combination, ketamine and medetomidine induce a brief period of general anesthesia with good sedative and analgesic qualities that can be reversed with atipamezole.[b] The purposes of the study reported here were to determine the anesthetic effects of administration of a combination of ketamine and medetomidine in mackerels and bonitos and whether anesthesia could be reversed with atipamezole. In addition, we wanted to determine whether use of this combination would have any adverse hematologic effects. In this study, we were seeking to develop an injectable anesthetic protocol that required injection of only a small volume (approx 3 mL) of anesthetic agent, had an induction time of < 15 minutes, and had a total duration of effect of < 12 hours, whereby fish would become behaviorally responsive within that time span. Smooth induction and emergence with minimal adverse effects and minimal depression of cardiovascular and respiratory systems with no observable untoward effects were also important criteria for an acceptable anesthetic protocol.

Materials and Methods

A total of 43 bonitos (*Sarda chiliensis*) and 47 Pacific mackerels (*Scomber japonica*) were used in the study. During the study, the fish were housed in a 7,600-gallon, 30-m^3 tank at the Tuna Research and Conservation Center. Mackerels used in the study were collected in Monterey Bay during May, June, and July 2000; bonitos were collected in San Diego Bay during September 2000. Water was supplied to the tank at a rate that allowed turnover every 60 minutes to ensure 100% oxygen saturation. Water temperature ranged from 19.5° to 20.5°C, pH ranged from 7.8 to 7.9, and nonionized ammonia concentration was < 0.01 mg/L. Fish were fed 45.5 kg of squid and 3.7 kg of gelatin 3 times a week. Fish were not fed the day before any anesthetic trial, but were fed the next day. The Stanford University Institutional Animal Care and Use Committee approved the experimental protocol.

Drugs used in the study included ketamine[c] (100 mg/mL diluted with sterile saline [0.9% NaCl] solution to 10 mg/mL), medetomidine[d] (1 mg/mL), and atipamezole[e] (5 mg/mL). All injections were performed with 1-, 3-, and 5-mL syringes and 22-gauge, 3/4-inch or 25-gauge, 5/8-inch needles. All injections were made in the red lateral muscle.

Twenty-eight of the bonitos were used in a dose-response study. Doses of ketamine ranging from 1 to 8 mg/kg (0.5 to 3.6 mg/lb) and doses of medetomidine ranging from 0.2 to 1.6 mg/kg (0.1 to 0.7 mg/lb) were used. The ratio of ketamine to medetomidine ranged from 2.5:1 to 20:1. Doses of atipamezole equal to 1 or 5 times the dose of medetomidine were used. The induction time, duration of anesthesia, and recovery time were recorded for each fish.

Similarly, all 47 mackerels were used in a dose-response study. Doses of ketamine ranging from 11 to 533 mg/kg (5 to 242 mg/lb) and doses of medetomidine ranging from 0.3 to 9.1 mg/kg (0.1 to 4.1 mg/lb) were used. The ratio of ketamine to medetomidine ranged from 3:1 to 800:1. Doses of atipamezole equal to 5 times the dose of medetomidine were used. The induction time, duration of anesthesia, and recovery time were recorded for each fish.

Fish used in the dose-response studies were captured as described.[1] Briefly, the water level in the tank was lowered to a depth of approximately 70 cm (2.5 feet). Several fish were separated from the school with a flexible vinyl partition, and individual fish were captured with a vinyl, water-filled sling. Once an individual fish was captured, the curved length of the fish was measured and an approximate weight was determined from a length-weight chart developed by the Tuna Research and Conservation Center. The appropriate doses of ketamine and medetomidine were then measured and injected into the red muscle of the fish, immediately ventral to the lateral ridge below the lateral line, posterior and dorsal to the end of the pectoral fin.

A colored plastic tag was inserted behind the second dorsal fin for visual identification of the fish, and the fish was then released from the sling and allowed to swim with the school. The behavior of the fish was observed at 1-minute intervals, and when the fish was no longer able to maintain equilibrium, orient, and swim independently, it was recaptured in a vinyl sling. Artificial ventilation of the fish was begun with a submersible pump. A fine stream of oxygen was bubbled into the intake of the pump.

Stage of anesthesia was determined (**Appendix**), and the highest stage of anesthesia was recorded. When individual fish reached stage 3 of anesthesia, a blood sample (2 to 3 mL) was collected from the bulbus arteriosis with a 5-mL syringe and 19-gauge, 1-inch needle. Atipamezole was then given to reverse the anesthesia. One milliliter of blood was placed into an EDTA-treated tube for subsequent determination of PCV and hemoglobin concentration. This sample was stored on ice until analyzed. The remainder of the blood sample was added to a serum separator vial with silica clot activator for subsequent chemical analysis. Serum was obtained by means of centrifugation, and samples were stored at –80°C until analyzed.

Immediately following collection of the blood sample, atipamezole was injected into the red muscle, ventral to the lateral ridge. The fish remained in the sling until it regained a strong tail beat and showed signs of righting and attempting to swim. The fish was then released, and another fish was captured. When the experiment was finished, the water level of the tank was restored.

All fish were observed 2, 5, and 10 minutes after release; every 15 minutes for the first 3 hours after release; and then daily until they had recovered completely or died. Induction time (time from administration of ketamine and medetomidine until the fish was observed to lose orientation and equilibrium), duration of anesthesia (time from administration of ketamine and medetomidine until administration of atipamezole), release time (time from administration of atipamezole until release of the fish from the sling), and recovery time (time from administration of atipamezole to recovery of equilibrium and orientation) were recorded.

Packed cell volume was determined by centrifuging an aliquot of the blood sample at 2,660 × *g* for 5 minutes. Hemoglobin concentration was determined by means of the cyanmethemoglobin technique, with absorbency measured at 540 nm. Serum calcium, chloride, phosphate, potassium, sodium, bilirubin, cholesterol, creatinine, and glucose concentrations and alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and creatine kinase activities were measured with automated analyzers.[f,g] Analyses were performed at 37°C.

In a follow-up study, 15 bonitos (mean ± SD body weight, 2.7 ± 0.6 kg) were anesthetized with ketamine at a dose of 4 mg/kg (1.8 mg/lb) and medetomidine at a dose of

Table 1—Anesthetic effects of various combinations of ketamine and medetomidine in bonitos.

Ketamine (mg/kg)	Medetomidine (mg/kg)	No. of fish	Induction time (min)	Anesthetic stage*	Duration of anesthesia (min)	Atipamezole (mg/kg)	No. of fish	Recovery time (min)	Comments
2	0.2	1	34.3	2a	34	1.0	1	3.7	Returned to normal behavior in 7 days
2	0.4	5	10.0 ± 4.9	3	14.9 ± 3.8	0.4	2	12.4	1 died; the other returned to normal behavior in 12 days
						2.0	3	3.1 ± 0.6	Returned to normal behavior in 3.7 days
2	0.8	4	11.8 ± 6.6	3	16 ± 7.4	0.8	1	30	Returned to normal behavior in 3 days
						4.0	3	5.5 ± 4.8	Returned to normal behavior in 9 days
4	0.2	2	22.2	2a, 2b	23	1.0	1	3.4	Returned to normal behavior in 7 days
4	0.4	4	6.6 ± 3.2	3	8.9 ± 2.2	0.4	1	2.7	Died
						2.0	3	2.0 ± 1.5	Returned to normal behavior in 3 days
4	0.8	3	10.4 ± 11.4	2b	17.9 ± 12.9	0.8	1	9.7	Returned to normal behavior in 5 days
						4.0	2	3.6	Returned to normal behavior in 5 days
4	1.6	1	12.5	3	35	1.6	1	46	Died
8	0.4	1	4.1	3	8	2.0	1	4	Returned to normal behavior in 7 days
8	0.8	1	10.0	2b	18	0.8	1	28	Died
8	1.6	1	7.8	2b	12	1.6	1	4.7	Died

Data are given as mean ± SD.
*Highest anesthetic stage attained; *See* Appendix for definitions of anesthetic stages.

0.4 mg/kg (0.2 mg/lb). The induction time, duration of anesthesia, and recovery time were recorded for each fish.

Data analysis—Data are reported as mean ± SD. The effect of medetomidine and ketamine on the induction times of mackerels was analyzed by use of ANOVA, with first-order interactions. The induction times of the mackerels were not significantly different from a normal distribution ($P > 0.05$; Kolmogorv-Smirnov goodness-of-fit test).

Results

In the dose-response study with bonitos, combinations involving medetomidine at the lowest dose (0.2 mg/kg) resulted in unacceptably long induction times and only resulted in stage 2 anesthesia (**Table 1**). Similarly, administration of combinations involving medetomidine at the 2 highest doses (0.8 and 1.6 mg/kg [0.4 and 0.7 mg/lb]) resulted in variable depths of anesthesia, with 5 of the 10 fish receiving these combinations achieving stage 3 anesthesia (surgical anesthesia), but the other 5 achieving only stage 2 anesthesia.

Stage 3 anesthesia was achieved in all 13 bonitos that received 1 of 3 anesthetic combinations: ketamine at a dose of 2 mg/kg (0.9 mg/lb) and medetomidine at a dose of 0.4 mg/kg (0.2 mg/lb), ketamine at a dose of 2 mg/kg and medetomidine at a dose of 0.8 mg/kg (0.4 mg/lb), and ketamine at a dose of 4 mg/kg (1.8 mg/lb) and medetomidine at a dose of 0.4 mg/kg. Although fish that received ketamine at the higher dose (4 mg/kg) appeared to have shorter anesthetic induction times, no significant differences were found among groups. The results of biochemical tests from fish that received the 3 combinations appeared to be generally similar (**Table 2**).

All 14 bonitos given atipamezole at a dose equal to 5 times the medetomidine dose recovered completely (Table 1). In contrast, only 3 of the 8 bonitos given atipamezole at a dose equal to the medetomidine dose survived. No attempt was made to distinguish fish that died as a result of anesthesia from those that died as a result of handling.

In the follow-up study, the 15 bonitos were anesthetized with ketamine at a dose of 4 mg/kg and medetomidine at a dose of 0.4 mg/kg. Mean induction time was 8.9 ± 2.1 minutes, mean duration of anesthe-

Table 2—Results of hematologic and serum biochemical testing in bonitos anesthetized with ketamine and medetomidine.

Variable	Combination A	Combination B	Combination C
PCV (%)	32 ± 8.9 (5)	35 ± 5.1 (3)	32 ± 8.6 (3)
Hemoglobin (g/dL)	13.8 ± 3.8 (5)	17.3 ± 2.4 (3)	14.4 ± 4.5 (2)
ALP (U/L)	34 ± 0 (2)	40 (1)	28 ± 2.8 (2)
AST (U/L)	27 ± 20 (4)	19 ± 1.7 (3)	22 ± 6.9 (3)
ALT (U/L)	3 ± 2.2 (5)	0.4 ± 0.3 (3)	4 ± 1.7 (3)
Total bilirubin (mg/dL)	0.1 ± 0.1 (5)	0.2 ± 0.3 (3)	0.4 ± 0.3 (3)
Cholesterol (mg/dL)	219 ± 60 (5)	297 ± 31.1 (3)	225 ± 3.4 (3)
CK (U/L)	29 ± 21.2 (2)	4 (1)	9 ± 7.0 (2)
Creatinine (mg/dL)	0.2 ± 0.08 (5)	0.2 ± 0 (3)	0.9 ± 0.6 (3)
Glucose (mg/dL)	164 ± 48 (4)	154 ± 3.4 (3)	190 ± 59 (3)
Calcium (mg/dL)	13.1 ± 2.0 (5)	9.9 ± 3.2 (3)	12.1 ± 0.8 (3)
Chloride (mEq/L)	135 ± 30 (4)	170 ± 13.8 (3)	159 ± 3.4 (3)
Phosphate (mg/dL)	6.5 ± 2.4 (4)	6.3 ± 3.1 (3)	5.6 ± 0.8 (3)
Potassium (mEq/L)	3.8 ± 1.4 (4)	3.7 ± 0.6 (3)	3.7 ± 0.6 (3)
Sodium (mEq/L)	168 ± 6 (4)	208 ± 34.6 (3)	195 ± 5.1 (3)

Data are given as mean ± SD. Numbers in parentheses represent numbers of fish.
Combination A = 2 mg of ketamine/kg and 0.4 mg of medetomidine/kg, IM. Combination B = 2 mg of ketamine/kg and 0.8 mg of medetomidine/kg, IM. Combination C = 4 mg of ketamine/kg and 0.4 mg of medetomidine/kg, IM. ALP = Alkaline phosphatase. AST = Aspartate aminotransferase. ALT = Alanine aminotransferase. CK = Creatine kinase.

Table 3—Anesthetic effects of various doses of medetomidine and ketamine in Pacific mackerel.

No. of fish	Weight (kg)	Medetomidine Amount (mg)	Medetomidine Dose (mg/kg)	Ketamine Amount (mg)	Ketamine Dose (mg/kg)	Induction time (min)	Duration of anesthesia (min)	Recovery time (min)	Comments
1	0.33	0.1	0.3	10	30	16	22	12	No effect
1	0.35	0.1	0.3	40	114	21	21	3	Stage 1
1	0.38	0.1	0.3	80	211	No effect	18	3	Stage 1
1	0.35	0.2	0.6	20	57	45	46	10	Long induction time
1	0.35	0.2	0.6	80	229	14	14	9	Stage 3
1	0.30	0.2	0.7	160	533	2	4	NA	Died
1	0.33	0.3	0.9	30	91	10	5	NA	Stage 2
2	0.36	0.4	1.1	4	11	50	52	2	Stage 2
1	0.35	0.4	1.1	8	23	12	42	2	Stage 2
1	0.35	0.4	1.1	20	57	13	13	5	Stage 3
5	0.36	0.4	1.1	40	111	8	9	11	Stage 3
5	0.37	0.4	1.1	80	216	4	7	6	Stage 3
7	0.39	0.7	1.8	30	77	9	1	2	Stage 3
2	0.36	0.8	2.2	4	11	55	45	2	Long induction time
2	0.35	0.8	2.3	8	23	56	48	3	Long induction time
2	0.35	0.8	2.3	20	57	8.5	7	5	Stage 3
7	0.40	0.8	2.0	40	100	6.5	5.0	2.8	Stage 3
4	0.36	0.8	2.2	80	222	9	7	7	Stage 3
1	0.38	1.6	4.2	20	53	14	15	6	Stage 3
1	0.35	3.2	9.1	10	29	50	52	0	Long induction time

Anesthetic effects were reversed with administration of atipamezole at a dose equal to 5 times the dose of medetomidine. NA = Not applicable.

sia was 19.0 ± 3.8 minutes, and mean recovery time was 7.7 ± 8.5 minutes.

Stage 3 anesthesia was achieved in mackerel given ketamine at doses ranging from 53 to 228 mg/kg (24 to 104 mg/lb) and medetomidine at doses ranging from 0.6 to 4.2 mg/kg (0.3 to 1.9 mg/lb; **Table 3**). The induction times for the mackerels were significantly affected by the medetomidine dose ($P = 0.008$), but not the ketamine dose ($P = 0.873$). However, the interaction between ketamine dose and medetomidine dose had a significant ($P = 0.014$) effect on induction time.

Discussion

Results of our study suggest that a combination of ketamine and medetomidine can be used to safely anesthetize bonitos and mackerels. Although promising doses were identified for both bonitos and mackerels, the lack of significant differences in induction, release, and recovery times precluded identification of the most effective doses of ketamine and medetomidine.

In the present study, there were substantial differences in the most effective doses of ketamine and medetomidine between bonitos and mackerels. In bonitos, the most effective dose of ketamine appeared to be 4 mg/kg, whereas in mackerels, the most effective dose of ketamine ranged from 50 to 200 mg/kg. Similarly, the most effective dose of medetomidine was 0.4 mg/kg for bonitos and ranged from 1 to 2 mg/kg for mackerels. The metabolic rate of bonitos is substantially lower than that of mackerels, and this difference may have accounted for the differences in effective doses of ketamine and medetomidine. However, both species have high tissue-level aerobic capacity[a]; thus, additional studies are needed to determine why the 2 species have such different dose requirements.

Administration of atipamezole at a dose equal to 5 times the medetomidine dose appeared to be effective in achieving good anesthetic recovery and high survival rates. Lower doses of atipamezole were not as effective in bonitos.

Results of serum biochemical tests did not differ significantly among bonitos given 3 different doses of ketamine and medetomidine (ketamine at a dose of 2 mg/kg and medetomidine at a dose of 0.4 mg/kg, ketamine at a dose of 2 mg/kg and medetomidine at a dose of 0.8 mg/kg, and ketamine at a dose of 4 mg/kg and medetomidine at a dose of 0.4 mg/kg), suggesting that none of these combinations had significantly greater or lesser effects on physiologic stress or hematologic parameters.

In conclusion, results of this study suggest that IM administration of ketamine at a dose of 4 mg/kg in combination with medetomidine at a dose of 0.4 mg/kg is a safe and effective anesthetic protocol for bonitos and IM administration of ketamine at a dose of 53 to 228 mg/kg in combination with medetomidine at a dose of 0.6 to 4.2 mg/kg is a safe and effective anesthetic protocol for mackerels. For both species, administration of atipamezole at a dose equal to 5 times the dose of medetomidine reversed the anesthetic effects.

[a]Freund EV. *Comparisons of metabolic and cardiac performance in scombrid fishes: insights into the evolution of endothermy*. PhD dissertation, Department of Biology, Stanford University, Stanford, Calif, 1999.

[b]Jalanka HH. *Medetomidine, medetomidine-ketamine combinations, and atipamezole in nondomestic animals: a clinical, physiological, and comparative study*. PhD dissertation, College of Veterinary Medicine, Helsinki, Finland, 1991.

[c]Ketaset, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.

[d]Medetomidine, Wildlife Pharmaceuticals, Fort Collins, Colo.

[e]Antisedan, Wildlife Pharmaceuticals, Fort Collins, Colo.

[f]GemStar 2 analyzer, Schiapparelli Biosystems Inc, Columbia, Md.

[g]Express Plus clinical chemical analyzer, Chiron Diagnostics Inc, Emeryville, Calif.

Appendix

Criteria for determining stage of anesthesia in fish.[7]

Stage	Criteria
0	Normal behavior; reactive to external stimuli; equilibrium and muscle tone normal.
1a	Light sedation; slight loss of reactivity to external stimuli (visual and tactile); voluntary movement still possible; opercular rate normal.
1b	Deep sedation; complete loss of reactivity to external stimuli; slight decrease in opercular rate.
2a	Partial loss of equilibrium; partial loss of muscle tone; reactive only to strong tactile and vibrational stimuli; rheotaxi present, but swimming capability seriously disrupted; increase in opercular rate.
2b	Complete loss of equilibrium; total loss of muscle tone; reactive only to deep pressure stimuli; low opercular rate.
3	Loss of reflex activity; complete loss of reactivity to stimuli; slow opercular and heart rates (surgical anesthesia).
4	Medullary collapse; loss of respiratory movements, followed by cardiac arrest (overdosage).

References

1. Farwell CJ. Tunas in captivity. In: Block BA, Stevens ED, eds. *Tuna physiology, ecology, and evolution*. San Diego: Academic Press Inc, 2001;391–412.

2. Graham JB, Dickson KA. Anatomical and physiological specializations for endothermy. In: Block BA, Stevens ED, eds. *Tuna physiology, ecology, and evolution*. San Diego: Academic Press Inc, 2001;121–165.

3. Sepulveda CA, Kickson KA, Graham JB. Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative of the tunas (family Scombridae). *J Exp Biol* 2003;206: 2739–2748.

4. Ellerby DJ, Altringham JD, Williams TD, et al. Slow muscle function of Pacific bonito (*Sarda chiliensis*) during steady swimming. *J Exp Biol* 2000;203:2001–2013.

5. Plumb DC. *Veterinary drug handbook*. 3rd ed. Ames, Iowa: Iowa State University Press, 1999;362.

6. Hall LW, Clarke KW. *Veterinary anesthesia*. 8th ed. London: Balliere Tindall, 1983;91.

7. McFarland WN. A study of the effects of anesthetics on the behavior and physiology of fishes. *Pubs Inst Mar Sci (Univ Texas)* 1959;6:23–55.