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Behavioral and Clinical Pathology Changes in Koi Carp (*Cyprinus carpio*) Subjected to Anesthesia and Surgery with and without Intra-Operative Analgesics

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Fish surgery is becoming increasingly common in laboratory and clinical settings. Behavioral and physiologic consequences of surgical procedures may affect experimental results, so these effects should be defined and, if possible, ameliorated. We document behavioral and clinical pathology changes in koi carp (*Cyprinus carpio*) undergoing surgery with tricaine methanesulphonate (MS-222) anesthesia, with and without intraoperative administration of the opiate butorphanol (0.4 mg/kg intramuscularly) or the nonsteroidal antiinflammatory analgesic ketoprofen (2 mg/kg intramuscularly). For all fish combined, surgery resulted in reduced activity, lower position in the water column, and decreased feeding intensity at multiple time points after surgery. The butorphanol-treated group was the only one not to experience significant ($P < 0.05$) alterations from presurgical behaviors. Clinical pathology changes at 48 h after anesthesia and surgery included decreased hematocrit, total solids, phosphorus, total protein, albumin, globulin, potassium, and chloride and increased plasma glucose, aspartate aminotransferase, creatine kinase, and bicarbonate. The only clinical pathology difference between treatment groups was a lower increase in creatine kinase in the ketoprofen-treated group. No adverse effects of butorphanol or ketoprofen at these doses were identified. These results suggest a mild behavioral sparing effect of butorphanol and reduced muscle damage from the antiinflammatory activity of ketoprofen.

Use of fish in biomedical research has increased considerably in recent years, and their care and use has been highlighted in recent laboratory animal forums (5, 34). Surgical procedures have been performed on fish in laboratory and field research settings for many years (30, 36) and are now being performed regularly on client-owned and display aquarium fish (11). Nonlethal surgery in fish research has been reviewed recently (10) and includes procedures for tissue or organ ablation, catheterization for fluid collection or drug delivery, and implanting instrumentation. The Animal Welfare Act (AWA) currently exempts cold-blooded animals from Institutional Animal Care and Use Committee (IACUC) coverage, but institutions receiving Public Health Service funding and other institutions extend IACUC coverage to all vertebrate animals used in research (1, 38). Because the AWA requires appropriate use of anesthetic, analgesic, or tranquilizing drugs to minimize pain and distress of animals used in experimental procedures (1), confusion can arise in the IACUC review process when "appropriate" analgesics are unknown for the species in question. Evaluations of fish anesthetics have focused on immobilization and safety, and the postsurgical analgesic effects of commonly used fish anesthetics such as tricaine methanesulfonate (MS-222) are hypothetical (33).

Fish studies involving surgical intervention either in the laboratory or in the field do not typically incorporate any protocols to provide postoperative analgesia. Although the neuroanatomy of fish has called into question anthropomorphic interpretations of pain perception (2, 28), fish do exhibit strong aversion to acute noxious stimuli and subtle behavioral changes and color changes in response to chronic stimuli (33). Polymodal nociceptors have been characterized on the face of rainbow trout (*Oncorhynchus mykiss*) that when stimulated by injection of acetic acid into the lips result in increased respiratory rate, reduced feeding, and behavioral effects of rocking back and forth on the pectoral fins and rubbing the affected area against the substrate, all of which actions were interpreted as manifestations of a pain response (32). Administering morphine at the time of the noxious stimulus reduced the anomalous behaviors and the increased respiratory rate, indicating an analgesic effect (31). A counter-argument is that because fish lack a neocortex, they are psychologically incapable of experiencing pain (28). In laboratory animal settings, the debate over whether or how fish perceive pain may be less relevant than the questions of what physiologic and behavioral effects are produced by noxious stimuli (such as surgery) that could impact research results and whether those effects can be ameliorated by therapeutic intervention. Effects of surgery on fish behavior and physiology that could alter research results are under-investigated. In particular, analgesic techniques that could alleviate surgical impacts have not been evaluated in fish. Butorphanol has been recommended at a dose of 0.1 to 0.4 mg/kg intramuscularly (i.m.) once perioperatively and causes no ap-

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parent harm but has not been evaluated rigorously (9, 11). We document behavioral and physiologic changes in fish undergoing surgery with and without perioperative administration of the opiate butorphanol or the nonsteroidal antiinflammatory analgesic (NSAIA) ketoprofen.

Materials and Methods

Pilot study. A pilot study was conducted in conjunction with a fish medicine and surgery course for veterinary students under a protocol approved by the North Carolina State University IACUC. Six koi carp (*Cyprinus carpio*; weight [mean ± 1 standard deviation] = 67.8 ± 17.8 g; sex unrecorded) from a commercial producer (Blue Ridge Fish Hatchery, Inc., Kernersville, N.C.) underwent exploratory celiotomy according to standard protocols (9, 11, 17). Husbandry for the primary study was as described in the next paragraph, with the exception that individual fish tanks were not isolated from the common recirculating system after surgery during the pilot study. Surgeries were performed using the fish anesthetic MS-222 (Tricaine-S, Western Chemical Inc., Ferndale, Wash.) at 100 to 200 mg/liter on a recirculating fish anesthesia delivery system surgery table (17). Fish were divided randomly into two groups of three fish. During routine skin closure (11), one group received the opiate butorphanol at 0.4 mg/kg i.m., and the other group received an equivalent volume of sterile physiologic saline i.m. After surgery, fish were returned to individual 55-liter tanks, labeled A or B according to group. Student surgeons were blinded to treatment but could evaluate the behavior of the groups in aggregate. In light of subjective evaluation of the animals' greater activity levels, a higher position in the water column, and a more rapid return to feeding during the first 24 h postoperative period, observers were able to identify the butorphanol-treated group correctly.

Animals and husbandry. Thirty koi carp (126 ± 53 g; range, 58 to 265 g; 20 male, eight female, sex not determined for two) were obtained from a commercial producer (Blue Ridge Fish Hatchery, Kernersville, N.C.). They were placed in individual 55-liter experimental tanks a minimum of 14 days prior to initial blood collection and 28 days prior to surgery. Prior to surgery, tanks were linked in a recirculating system with a submerged biological filter (Fig. 1). Each tank was aerated individually. After surgery, valve settings isolated each tank from the recirculating system, and water quality was maintained by water changes as needed, so that any analgesics, pheromones, or metabolic products excreted by fish of one group could not affect fish of any other group. Sea salts (Crystal Sea, Marinemix, Marine Enterprises International, Inc., Baltimore, Md.) were added to tanks to maintain a salinity of 1 g/liter for 2 days after surgery to reduce osmotic stress that has been reported to follow anesthetic procedures (11). Water quality was monitored for temperature, ammonia, nitrite, and pH at least every 7 days prior to surgery and daily after surgery and remained within acceptable limits (temperature, 22.0 to 27.0°C; total ammonia nitrogen, 0.0 to 0.5 mg/liter; nitrite, 0.00 to 0.04 mg/liter; pH, 6.9 to 7.5). Fish were fed a commercial pelleted food (Koi's Choice, KayTee, Chilton, Wis.) on alternate days. All housing and protocols were approved by the North Carolina State University IACUC.

Study design. Koi underwent exploratory celiotomy by veterinary students in a surgery training laboratory. Surgery was performed using MS-222 at 100 to 200 mg/liter on a recirculating fish anesthesia delivery system surgery table (17). Procedure times were recorded as time removed from the individual holding tank until full recovery and return to the holding tank. Sex was determined by direct visualization of the gonads during surgery. Fish were divided randomly into three groups of 10 fish. During routine skin closure (11), one group received the opiate butorphanol at 0.4 mg/kg i.m., one received the nonsteroidal antiinflammatory agent ketoprofen at 2.0 mg/kg i.m., and the third received physiologic saline i.m.; all doses were adjusted to equivalent volumes per weight of fish. Observers were blinded to both treatment and group, and fish were evaluated for vertical position in the water column at 5-cm increments, caudal fin beat rate as an indicator of activity level, respiratory rate, and response to food (presentation of a single pellet) at 24 h before surgery and 0.5, 1, 2, 3, 6, 18, 24 and 48 h from the end of surgery. Response to food was scored according to whether the pellet was consumed within 3 min and on a scale of 1 to 4 as follows: 1) fish visually oriented to feed presentation and consumed pellet within 3 sec of the pellet hitting the water, 2) fish visually oriented to feed but was slower to respond, consuming pellet after 3 sec but before 20 sec, 3) fish did not orient visually to feed presentation but increased exploratory activity after apparently detecting feed by olfaction and consumed pellet between 20 sec and 3 min after pellet hit the water, 4) fish failed to consume pellet within 3 min, after which the pellet was removed. All observations were made by two investigators (CAH and JMK), and observations were calibrated for consistency between observers by practice runs prior to the experimental protocol.

Figure 1. Racks of individual fish tanks on a shared recirculating system.

Hematocrit, total solids, plasma cortisol, and complete plasma biochemistry panels were compared between blood samples obtained 2 weeks prior to surgery and 48 h after surgery. Blood (10 ml/kg; range, 0.6 to 2.6 ml) was collected into heparinized syringes from the caudal hemal arch of anesthetized koi. Blood samples were held on wet ice until centrifuged within 1 h of collection to harvest plasma. Plasma was stored at –80°C until analyzed within 3 weeks of collection. Sixteen plasma chemistry analytes (glucose, calcium, phosphorus, total protein, albumin,

Table 1. Clinical pathology, cortisol, and weight values 2 weeks before and 48 h after surgery in 30 koi carp

	Presurgery median	Presurgery quartiles	48 h postsurgery median	48 h postsurgery quartiles	*P*
Packed cell volume (%)	36	32, 38	25	21, 28	< 0.0005
Total solids (g/dl)	2.2	1.9, 2.4	1.8	1.3, 2.1	< 0.0005
Glucose (mg/dl)	36	28, 43	82	62, 100	< 0.0005
Calcium (mg/dl)	8.4	8.2, 8.9	8.4	7.9, 8.8	ns
Phosphorus (mg/dl)	6.4	5.6, 6.6	5.3	4.7, 5.7	< 0.0005
Total protein (g/dl)	2	1.7, 2.2	1.9	1.5, 2.1	< 0.0005
Albumin (g/dl)	0.9	0.75, 0.95	0.8	0.7, 0.9	< 0.0005
Globulin (g/dl)	1.1	1.0, 1.3	1.1	0.8, 1.2	< 0.0005
Albumin:globulin ratio	0.74	0.65, 0.88	0.75	0.70, 0.84	ns
Aspartate transaminase (U/liter)	103	74, 138	600	423, 750	< 0.0005
Creatine kinase (U/liter)	3387	2628, 5676	5658	5532, 5750	0.003
Lactate dehydrogenase (U/liter)	132	93, 432	416	257, 649	0.046
Sodium (mmol/liter)	134	132, 137	134	131, 136	ns
Potassium (mmol/liter)	3.4	3.0, 3.7	3	2.7, 3.3	0.001
Chloride (mmol/liter)	115	113, 116	111	107, 113	< 0.0005
Bicarbonate (mmol/liter)	6	5, 7.5	8	8, 9	< 0.0005
Anion gap (mmol/liter)	17.4	14.9, 18.5	17.5	16.9, 19.6	ns
Cortisol (µg/dl)	23.7	16.3, 35.4	22.5	11.4, 29.9	ns
Weight (g)	123	77, 160	114	76, 152	< 0.0005

ns, not statistically significant.
Because of non-normal distribution of some data (Shapiro–Wilk test), all summary statistics are reported as median and lower and upper quartiles (25th and 75th percentiles). Pre- and postsurgical values are compared by Wilcoxon matched pairs signed ranks test. After application of the sequential Bonferroni correction, only the change in lactate dehydrogenase was no longer statistically significant. Creatinine was invariably below the limit of detection (0.1 mg/dl) and is not included in the table.

globulin, albumin:globulin ratio, aspartate aminotransferase [AST], creatine kinase [CK], lactate dehydrogenase [LD], sodium, potassium, chloride, bicarbonate, anion gap [(Na + K) – (Cl + HCO_3)], and creatinine) were determined using a Roche/Hitachi 912 Clinical Chemistry System (Roche Diagnostics, Indianapolis, Ind.). Plasma cortisol was measured by chemiluminescent immunoassay (Immulite 1000 analyzer, Modern Laboratory Services, Inc., Bakersfield, Calif.).

Data analysis. Statistical analyses were performed with a commercial software package (JMP 5.0, SAS Institute, Cary, N.C.). Data were tested for normalcy by the Shapiro–Wilk W test. Because many of these data were not normally distributed, nonparametric statistics were used, and summary statistics are reported as median and quartiles (25th and 75th percentiles).

Whether fish fed and the food response score were compared by time for all fish by using a chi-square test with correspondence analysis. The number of fish not feeding was too small at all time points to permit reliable feeding comparison between groups by chi-square analysis. Vertical position in the water column, caudal fin beat rate, and respiratory rate were compared by time for all fish by Friedman two-way layout followed by multiple comparisons with control presurgical values (14). Vertical position in the water column and caudal fin beat rate were compared further within treatment groups between presurgery values and multiple postsurgery values by using the Wilcoxon matched pairs signed ranks test. The Wilcoxon rank sum test followed by sequential Bonferroni correction for multiple comparisons (27) was used to compare weight, plasma cortisol, and clinical pathology analytes of all fish 14 days prior to and 2 days after surgery. The Kruskal–Wallis test was used to compare changes pre- and postsurgery in weight, plasma cortisol, and clinical pathology analytes between treatment groups. Statistical significance was accepted at $P < 0.05$.

Results

Procedures took place between the times of 12:04 and 15:57. Mean procedure time was 57 min (range, 31 to 95 min). All fish survived anesthesia and surgery, the postoperative monitoring period, and for several months after the study.

After surgery, according to evaluation of all fish by time, vertical position in the water column and caudal fin beat rate were reduced from presurgery values at multiple time points (0.5, 1, 18, and 48 h for vertical position and 0.5, 2, 3, 6, and 18 h for caudal fin beat rate; Friedman two-way layout, $P < 0.05$). Median (quartiles) vertical position in the water column for all fish presurgery was 15 (10, 20) cm above the bottom of the tank, compared with a median (quartiles) low of 10 (10, 15) at 0.5 and 18 h after surgery. Median (quartiles) caudal fin beat rate for all fish presurgery was 52 (35, 83) beats/min, compared with a median (quartiles) low of 38 (19, 54) beats/min at 18 h after surgery. Compared with presurgery values, there were no significant changes in vertical position or caudal fin beat rate at any time after surgery for the butorphanol treatment group, whereas significant decreases were recorded for vertical position at 0.5 h after surgery in the saline treatment group and at 1, 2, and 18 h postsurgery in the ketoprofen treatment group and for caudal fin beat rate at 2, 3, 6, and 18 h in the saline treatment group and at 0.5 and 18 h in the ketoprofen treatment group (Wilcoxon matched pairs signed ranks test, $P < 0.05$). Respiratory rates also were decreased overall from presurgery values at 6 and 18 h postsurgery (Friedman two-way layout, $P < 0.05$), but within treatment groups no changes were significant at any time. Median (quartiles) respiratory rate for all fish presurgery was 28 (16, 48) breaths/min compared with a median (quartiles) low of 14 (8, 33) breaths/min at 18 h after surgery.

Fewer fish consumed the feed pellet at 0.5 and 1 h postoperatively (21 and 25 fish, respectively, versus 28 to 30 fish at all other times), and more fish that did feed at those times did so less vigorously than at other time points (chi-square test with correspondence analysis, $P < 0.05$). The low rate of feed refusal at all times made comparison between groups unreliable.

Clinical pathology analytes that changed after surgery included significant decreases in hematocrit, total solids, phosphorus, total protein, albumin, globulin, potassium, and chloride and increases in plasma glucose, AST, CK, and bicarbonate (Table 1; Wilcoxon matched pairs signed ranks test followed by sequential Bonferroni test, $P < 0.05$). There were no significant changes in

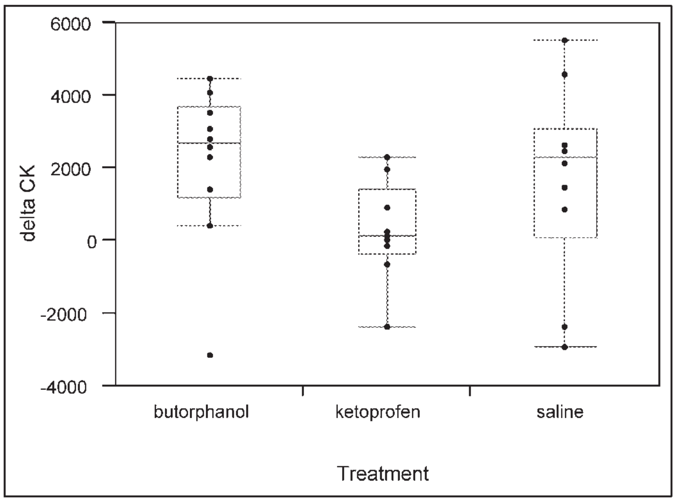

Figure 2. Change in creatine kinase (delta CK, in U/liter) by treatment group, showing a significantly lower elevation in the ketoprofen-treated group (n = 10 fish per group; Kruskal–Wallis, $P = 0.037$). Box plots depict median and quartiles (25th and 75th percentiles), and whiskers depict 10th and 90th percentiles.

cortisol, lactate dehydrogenase, calcium, sodium, or anion gap. Between treatment groups, the only analyte that differed was CK, which increased significantly less in the ketoprofen group (median increase of 90 U/liter) than in the butorphanol and saline groups (median increases of 2647 and 2267 U/liter, respectively; Kruskal–Wallis, $P < 0.05$; Fig. 2)

Discussion

Koi carp are not common laboratory animals, but they are sufficiently large to permit repeat venipuncture for monitoring potential adverse effects of surgery, anesthesia, and analgesic treatment on individual fish (unlike zebrafish, which require pooling of multiple individuals and lethal sampling to yield sufficient blood volume for analysis; 22) and are hardy surgical patients. Their size also facilitates surgical procedures and ease of postoperative monitoring, and the celiotomy approach used in the current study is a basic component of surgical procedures for other purposes, including gonadectomies for endocrinology investigations and transmitter implantation for field biology studies (10). In addition, they are commonly kept ornamental fish in garden ponds, and because of their size, value, and frequency of abdominal neoplasia (18), are seen relatively frequently as surgical patients in clinical practice. Analgesic protocols developed for koi could be extrapolated to other fish species.

Butorphanol is a synthetic opiate partial agonist active at the kappa and sigma receptors and is listed as a Class IV controlled substance by the United States Drug Enforcement Agency. It is used as an analgesic at doses ranging from 0.01 to 0.1 mg/kg (horses), 0.1 to 1.2 mg/kg (dogs), 1 to 5 mg/kg (small rodents), 1 mg/kg or 3 to 4 mg/kg (birds), and 0.4 to 1.0 mg/kg (reptiles) (4, 24-26). The duration of effect of butorphanol is around 2 h in many mammal species (20). It has demonstrated analgesic effects with rapid onset (by 20 min) and at least 1 h duration in African grey parrots (*Psittacus erithacus*) treated with 1 mg/kg i.m. (25). In green iguanas (*Iguana iguana*), butorphanol at 1 mg/kg did not have isoflurane-sparing effects (21). Variable effects of butorphanol between species may result from differences in butorphanol metabolism or opioid receptor expression (25, 39) and methods of assessment (21). In the current study, the butorphanol-treated group of koi was the only one not to exhibit any significant differences between pre- and any postsurgery observations of caudal fin beat frequency or vertical position in the water column, suggesting a mild behavioral sparing effect compared with the ketoprofen-treated and saline control groups. Fish have μ and κ opiate receptors throughout the brain (6), making it reasonable to expect some effect of opioid treatments in fish experiencing noxious stimuli. High doses of morphine (0.3 mg/g = 300 mg/kg i.m.) in juvenile rainbow trout have been shown to reduce the behavioral impacts of an injection of acetic acid into the lips, manifested in acid-injected saline-treated controls as increases in opercular rate, time to return to feeding, and frequency of rocking and rubbing affected areas against the substrate (31). Butorphanol, like other opioids, can cause respiratory depression in mammals, although there is a ceiling effect (26). Reduced respiratory rates were noted in the combined groups at 6 and 18 h postsurgery, but there were no differences between groups, so butorphanol at 0.4 mg/kg i.m. does not appear to cause respiratory depression in koi. The high doses of morphine employed in a study of rainbow trout (31), approximately 300-fold higher than an upper-end dose for dogs (26) would seem to imply that considerably higher doses of butorphanol also would be safe in fish. However, butorphanol administered at 1 mg/kg intravenously, moderately higher than the dose used i.m. here in koi, caused death in a tilapia (*Oreochromis mossambicus*) (37). Interspecies variation in opioid sensitivity, possibly influenced by water (and hence body) temperature may have marked effects on the therapeutic index of opioids in fish.

Ketoprofen is an NSAIA recommended for treatment and prevention of postoperative pain in dogs and cats at an initial dose of 2 mg/kg (20). It has also been demonstrated to have analgesic effects in mallard ducks (*Anas platyrhynchos*) at a dose of 5 mg/kg (19) and has been used empirically in reptiles at 2 mg/kg (4). It is a potent nonspecific inhibitor of cyclooxygenase. Duration of analgesic effect can be up to 24 h in dogs. In comparison with butorphanol, ketoprofen's longer duration of action (in mammals) and not being a controlled drug would be important advantages for routine use in fish, where opportunities for repeat dosing are often limited, and fish researchers may not be working as closely with veterinary support as might investigators using other model animal systems. In the current study, behavioral sparing effects of ketoprofen versus saline-treated controls unfortunately were not clearly evident. However, elevations in CK at 48 h after surgery were significantly less in the ketoprofen group than either the butorphanol or saline groups, suggesting that the anti-inflammatory effects of ketoprofen reduced muscle damage secondary to surgically induced inflammation. Because of NSAIA effects on hemostasis, preoperative use is controversial (15). Ketoprofen administered at 2 mg/kg prior to elective ovariohysterectomy in dogs inhibited platelet aggregation but had no effect on buccal mucosal bleeding time (16). Besides the potential to impair hemostasis, gastric ulceration and hepato- and nephrotoxicity are additional adverse effects that have been associated with NSAIA use (26). Clinical pathology measures that may be used to indicate hemorrhage and liver and kidney toxicity (hematocrit, proteins, AST, LD, Ca, P, electrolytes) in fish did not differ between the ketoprofen-treated group and the butorphanol-treated or saline control groups. Adverse effects of a single dose of ketoprofen at 2 mg/kg i.m. in koi therefore were not evident in this study. Necropsy and histopathology, which could have provided more definitive assessment of adverse effects, were not performed in this study.

Grouping all fish together, several behavioral and clinical pathology measures were altered significantly from presurgical values within the first 48 h of surgery, although time to complete resolution was not determined, because data collection was terminated after anesthesia for venipuncture to determine postoperative clinical pathology changes. Postsurgery reductions in activity (caudal fin beat rate), vertical position in the water column, and vigor during feeding at multiple time points were consistent with subjective observations during the pilot study as well as clinical impressions in the course of our surgical practice. Reductions in caudal fin beat rate, vertical position in the water column, and respiratory rate that were noted at 18 h likely had a diel cycle component, as this observation time was shortly after the timer turned on the lights in the morning. Diel cycles in behavior were not examined in this study, but the effects likely would have been minor at the other observation times.

Changes in clinical pathology analytes after anesthesia and surgery are consistent with surgically induced hemorrhage (reduced hematocrit, total solids, total protein, albumin, globulin, phosphorus) and tissue (primarily muscle) damage (combined increase in AST, LD, and CK, albeit lesser increase in CK in the ketoprofen-treated group). The reduction in hematocrit was greater than what would be expected from the presurgery phlebotomy of 10 ml/kg alone, even without compensatory mechanisms in the 2-week recovery period. Increased plasma glucose is consistent with a generalized stress response (12, 23) and with MS-222 anesthesia (2). Plasma glucose prior to surgery appears low compared with values for many other species but is consistent with previously reported values for a smaller sample size of common carp (8) and for southern stingrays (3). Sample handling was appropriate for avoiding artifactual decreases in plasma glucose. Despite increased postsurgery plasma glucose concentrations, plasma cortisol was not increased at 48 h, although it may well have been elevated in the hours immediately after surgery. Both baseline and postsurgery cortisol values were comparable to baseline values obtained for striped bass (*Morone saxatilis*) (12). Cortisol production is considered an equivocal indicator of pain and analgesic effect (29). The reductions in potassium and chloride took place despite recovering the koi in water supplemented with 1 g/liter sea salts in an attempt to avoid electrolyte imbalances associated with MS-222 anesthesia and surgical disruption of the epithelial barrier in freshwater fish (11). Sodium concentrations were not altered at 48 h after surgery, however. Postanesthesia and -surgery electrolyte imbalances in koi may be alleviated but not eliminated by recovery in water supplemented with 1 g/liter sea salts. It should be acknowledged that the clinical importance of some of the statistically significant clinical pathology changes would be considered minor when considering treatment of individual fish, but depending on the research protocol, the aggregate of alterations could have undesirable effects. Presurgical clinical pathology values generated during this investigation can be used as reference intervals for koi and represent an expansion in sample size and number of analytes previously available for comparison (8).

Both butorphanol and ketoprofen are available in parenteral forms, important for facilitating delivery to fish. They are also available in oral forms, which could be incorporated into a medicated gel food (7) for potential follow-up analgesic delivery. Neither drug is approved for use in food fish, so field studies involving fish surgery with analgesic use ideally should be targeted for fish outside of the legal size or season for sport and commercial fishing. We investigated effects of single intraoperative (nearly postoperative) doses of analgesics. Possible objections to this strategy include that the doses are 1) too late and 2) too few. Under the principles of preemptive pain management, analgesics administered preoperatively may reduce inflammation and reduce the need for postoperative analgesics (13). However, preoperative use of NSAIAs such as ketoprofen is sometimes discouraged due to its effects on hemostasis, and for comparative purposes, both analgesics were administered at equivalent times and while general anesthesia still prevailed. A single dose of analgesic may not provide optimal pain relief. Yet, recapture for additional doses may cause more pain from escape attempts and more physiologic damage from general capture stress than would be alleviated by more analgesics. Attempting oral delivery using medicated gel food would introduce uncertainties based on whether the fish were feeding or would accept the medicated gel food, degree of drug leeching into the water prior to consumption, and unknown enteral absorption. Furthermore, in a research and clinical field that has not previously addressed analgesia, simple protocols with measurable benefits are more likely to be readily adopted than are complex protocols.

The doses of butorphanol and ketoprofen used in this study were extrapolated from doses published for use in other species and from anecdotal information on clinical use in fish. The study did not include dose–response testing or pharmacokinetics, both of which would be valuable components of further investigations on provision of analgesia in fish. Safety and efficacy of the doses used in this study may vary between fish species and housing conditions.

Although the effects of analgesic treatments observed in this study were not dramatic, they should not be interpreted to mean that analgesics have little value in fish, particularly in light of the marked behavioral changes observed in aggregate after surgery. Assessing pain even in comparatively familiar domestic mammals is challenging (35). Evaluating analgesic efficacy is a greater challenge in less-well-known nondomestic species (33), particularly when they lack features such as muscles of facial expression, an ability to vocalize, and limbs or a flexible head and neck that can be directed towards areas of irritation. Different behavioral or physiologic measures, analgesic protocols, or experimental systems may prove more effective in demonstrating postsurgery analgesic effects in fish. Behaviors monitored in rainbow trout in response to a noxious stimulus (acetic acid injection into lips), with and without morphine analgesia, have included rubbing the affected area on the substrate, rocking on the pectoral fins, opercular rate, time to return to feeding, swimming frequency, and cover usage (32), with increased rubbing, rocking, and opercular rate proving to be the most reliable indicators of pain and attenuation of these effects indicators of analgesia. In the current study, rubbing and rocking were not seen in koi with ventral midline incisions, and respiratory rate did not increase in any group at any time point.

Results of this study suggest a mild attenuation of behavioral changes after surgery by using butorphanol at 0.4 mg/kg i.m. once intraoperatively and reduced muscle damage by using ketoprofen at 2 mg/kg i.m. once intraoperatively. No adverse effects were noted with either treatment protocol.

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