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TECHNICAL NOTE

Efficacy and Physiological Responses of Grass Carp to Different Sedation Techniques: II. Effect of Pulsed DC Electricity Voltage and Exposure Time on Sedation and Blood Chemistry

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Abstract

Owing to the current absence of an approved “immediate-release” chemical sedative for use on fish, researchers have been exploring alternative methods that would allow treated fish to be released immediately after sedation, including the use of electro-sedation. To address the efficacy of this approach, we evaluated induction and recovery times, survival, and postsedation hematology of grass carp *Ctenopharyngodon idella* (291 ± 6.7 g, 30.6 ± 0.3 cm TL, mean \pm SE) sedated by exposure to 100, 150, or 200 V of pulsed DC (30 Hz and 25% duty cycle) for 5 or 10 s. Regardless of voltage strength or exposure time, all fish were sedated to Stage IV sedation within 0.75 min and recovered within 1.5 min. Although recovery times for fish exposed to electro-sedation for 10 s were longer than those for fish electro-sedated for 5 s using 100 and 150 V, the opposite trend was observed among fish sedated using 200 V. Overall, induction and recovery times were short: total time elapsed from induction to full recovery ranged from 1.0 to 2.1 min (mean, 1.6 min). No mortalities were observed 24 h post-sedation. Hematological changes observed were consistent with an acute stress response, but these effects were transient and few differences were observed among the electro-sedation protocols used. Our results indicate that pulsed DC electro-sedation is an effective strategy for quickly and easily sedating grass carp.

Grass carp *Ctenopharyngodon idella* are an herbivorous species introduced from China to the United States as a biological control for aquatic vegetation in aquaculture ponds and

other private and public waters (Masser 2002). As grass carp are a nonnative species, there is concern that these fish may reproduce and establish self-sustaining populations in U.S. waters. This has led officials from many states to ban stocking fertile (diploid) individuals (Kelly et al. 2011), whereas triploid grass carp may be allowed in some cases (Masser 2002). Triploidy induction results in “functional sterility” (Benfey 1999; Zajicek et al. 2011) and is recognized as an effective strategy to control undesired reproduction. A rapid detection method to verify triploidy was developed by Wattendorf (1986), and this simple blood test is used to verify the chromosome number of every grass carp prior to sale and transfer to states with diploid grass carp bans (Zajicek et al. 2011).

To facilitate testing, fish are typically sedated so that they can be easily handled during blood sampling. Currently, there are very few practical and effective chemical sedative options available to fish culturists to facilitate sample collection, and none of the sedative products available in the United States are legal (i.e., approved by the U.S. Food and Drug Administration [FDA]) for use on food fish (including fish that may be consumed after stocking in U.S. waters) without adhering to a lengthy withdrawal period (3–21 d) following exposure (see Gause et al. 2012, this issue). Although tricaine methanesulfonate (MS-222), benzocaine, eugenol, or carbon dioxide (CO₂) may be used as fish sedatives under certain circumstances, none of these are fully suitable for procedures such as blood sampling and triploidy verification of grass carp because they

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require lengthy withdrawal times, are difficult to use, or are not legally available for such use.

Given the constraints associated with chemical sedatives and the amount of time and resources required to gain FDA-approval of these compounds for use in fish, fish culturists have been exploring alternative sedation techniques, including electrosedation. Although electrofishing has been used as a field sampling technique for decades by fisheries professionals, the technique has recently been modified specifically for the purpose of sedating fish (Zydlewski et al. 2008; Trushenski et al. 2012). “Electroanesthesia”, or more accurately, electrosedation, can immobilize fish via electronarcosis (stunning) or electrotetany (tetanic muscle contraction) caused by electrically induced interference with neurotransmission. Although present electrosedation technology may be somewhat limited in fish with comparatively fragile vertebrae (e.g., salmonids; C. V. Burger, Smith-Root, personal communication), in many species it may offer several advantages over chemical sedatives in terms of withdrawal periods, chemical disposal, and potentially, ease of use. Perhaps more importantly, electrosedation of fish is currently not subject to FDA regulation and can be used legally without having to go through the arduous, multiyear, multimillion dollar process of getting a chemical sedative approved for use in fish. However, it is important to develop use protocols to ensure that fish can be effectively sedated with minimal risk of adverse postsedation outcomes, including mortality. Although preliminary experimentation suggested pulsed-DC electricity is suitable for sedating grass carp (described below), it is unclear whether different waveforms (e.g., different voltage strengths, frequencies) or exposure durations affect induction or recovery times, blood chemistry responses, or overall efficacy. Accordingly, we evaluated electrosedation effects (induction and recovery times, survival, and postsedation blood chemistry) on grass carp (a representative warmwater fish) using three different voltage strengths and two different exposure durations.

METHODS

Electrosedation procedures.—A reference population of triploid grass carp (291 ± 6.7 g, 30.6 ± 0.3 cm total length, mean \pm SE) was held at Keo Fish Farm, Keo, Arkansas, in an outdoor raceway configured as a partial flow-through system (static raceway, periodically flushed with screened surface water) with supplemental aeration. Prior to experimentation, fish were fasted for a minimum of 24 h. To determine an appropriate “control” waveform from which to derive other waveforms for experimentation in the principal investigation, a preliminary experiment was conducted to determine whether a relatively mild waveform (based on our previous experience with electrosedation) would effectively sedate grass carp to Stage IV of sedation (see below). A group of 15 fish were randomly collected from the reference population and transferred into a 142-L cooler prefilled with 70 L of aerated culture water to achieve a depth of

approximately 8 cm and equipped with an electrosedation unit (PES Portable Electroanesthesia System, Smith-Root, Vancouver, Washington). These fish were exposed to 100 V of pulsed DC (60 Hz, 25% duty cycle, 5-s exposure). Using this waveform, mean sedation and recovery times were 0.5 and 1.8 min, respectively, and no overt signs of postsedation distress were observed. Accordingly, we determined that this waveform was effective and would serve as the lowest intensity waveform in the subsequent, principal investigation with one modification: given that higher voltages and exposure durations were to be investigated, we decided that the experimental protocols would use a lower frequency of 30 Hz.

For the principal investigation, groups of 15 fish were randomly collected from the reference population and transferred into the electrosedation unit configured as described above and filled with fresh culture water from the holding system. Fish groups were exposed to 100, 150, or 200 V of pulsed DC (30 Hz and 25% duty cycle) for 5 or 10 s in a 3×2 factorial design (100 V for 5 s, 100 V for 10 s, 150 V for 5 s, 150 V for 10 s, 200 V for 5 s, 200 V for 10 s). Culture water in the sedation chamber was aerated after sedating each group of fish but was not exchanged over the course of the experiment. A water sample was collected from the holding system at time (t) = 0, and the sample was analyzed in duplicate for the following: temperature and dissolved oxygen (YSI 550 meter, Yellow Springs Instruments, Yellow Springs, Ohio), conductivity, pH, salinity (Multi-Parameter PCSTestr 35, Eutech/Oakton Instruments, Vernon Hills, Illinois), hardness, alkalinity (digital titrator and reagents, Hach, Loveland, Colorado), total ammonia nitrogen, nitrite-nitrogen, and nitrate-nitrogen (DR 2800 spectrophotometer and reagents, Hach). All measured water quality characteristics were within ranges appropriate for grass carp (Masser 2002) (Table 1).

Fish were monitored during sedation to determine induction to Stage IV of “anesthesia” (Summerfelt and Smith 1990; although “anesthesia” is the term used by Summerfelt and Smith, we have used the term “sedation” throughout the manuscript to better reflect the behavioral responses we observed), which is

TABLE 1. Holding system water quality measured at the beginning of the experiment to examine electrosedation in grass carp.

Characteristic	Value
Temperature (°C)	18.6
Dissolved oxygen (mg/L)	7.98
Total ammonia nitrogen (mg/L)	0
Nitrite-nitrogen (mg/L)	0.003
Nitrate-nitrogen (mg/L)	0.9
Alkalinity (mg/L)	240
Hardness (mg/L)	374
Salinity (‰)	0.427
Conductivity (μ S/cm)	877
pH	7.5

associated with the total loss of equilibrium, muscle tone, and responsiveness to visual and tactile stimuli, but maintenance of a slow, steady, opercular ventilation rate. After the loss of equilibrium, slight manual pressure was applied along the trunk and caudal peduncle as a tactile stimulus. Fish were considered induced to Stage IV when they no longer responded to this stimulus, but the ventilation rate remained steady albeit reduced relative to unsedated fish. A tremor was observed immediately following electrosedation. Although fish were not responsive during this tremor (and were perhaps momentarily in Stage V or VI of sedation), induction was considered complete after the tremor had ceased and fish resumed ventilating. Immediately after induction, blood samples were collected from three fish using procedures described below. The remaining 12 fish were returned to holding tanks and monitored to determine recovery from sedation (return of normal equilibrium and tactile responsiveness). The group was considered recovered when the last fish recovered, (i.e., recovery time = time for last fish to recover). Assessment of induction and recovery can be somewhat subjective so bias was minimized by having the same observer apply all stimuli and assess when fish were sedated and recovered. Recovered fish were transferred to a second raceway (configured in the same manner as the one housing the reference population) and kept separated by raceway dividers positioned at ~1-m intervals along the length of the recovery raceway.

Sample collection and analysis.—In addition to collecting blood from fish sampled immediately after sedation ($t = 0$), blood samples were collected from three fish per group at 0.5, 1, 2, and 6 h postsedation (three fish per group per time point, individuals sampled once). Prior to sampling, all fish were immersed in a bath of metomidate hydrochloride (Aquacalm, Western Chemical, Ferndale, Washington; ~5–10 mg/L for ~30 s) to facilitate handling. Although additional sedation was not necessary for fish sampled at $t = 0$, these fish were also exposed to a metomidate hydrochloride bath to ensure consistent treatment of all fish. Once handleable, fish were weighed (to the nearest gram) and measured (total length to the nearest 0.5 cm), and a blood sample was collected from the caudal vasculature using heparinized, evacuated blood collection assemblies (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey). Although metomidate hydrochloride was selected, in part, because it limits or prevents corticosteroid increase during sampling (Olsen et al. 1995; Davis and Griffin 2004), all blood samples were collected within 5 min of capture to minimize the possibility of other confounding responses of handling and venipuncture. In addition to fish sampled at set time points after sedation, two fish from the reference population were also sampled every hour over the course of the experiment (experiment duration, 7 h; no fish were sampled more than once). After blood collection, all fish were returned to a separate area in the recovery system and monitored for adverse behavior and survival for 24 h. Blood samples were kept on wet ice (<36 h) until analysis for glucose, lactate, cortisol, and osmolality as described

by Gause et al. (2012). Although 36 h might be considered a lengthy period of time to hold blood samples prior to analysis, some assays could not be immediately conducted in the field and samples had to be transported back to the Fisheries and Illinois Aquaculture Center, Carbondale, Illinois. It is possible that levels of metabolically relevant molecules (e.g., glucose and lactate) could have changed slightly during this holding period; however, all samples were treated in the same manner to ensure validity of comparisons among treatments. Briefly, hematocrit (Statspin centrifuge, Fisher Scientific, Pittsburgh, Pennsylvania) and glucose (Freestyle Freedom Lite glucose meter, Abbott Laboratories, Abbott Park, Illinois) were determined using aliquots of whole blood, and then the remaining whole blood was centrifuged ($3,000 \times g$, 4°C , 45 min). Resultant plasma was collected and stored at -80°C until further analysis. Plasma samples were analyzed to determine lactate (Accutrend lactate meter, Roche, Mannheim, Germany), osmolality (Vapro 5520, Wescor, Logan, Utah), and cortisol (EIA 1887, DRG International, Mountainside, New Jersey). Although portable lactate and glucose meters such as those used in this study can slightly underestimate metabolite levels in fish blood relative to laboratory methods, they are considered precise and reliable for use in generating comparative data (Wells and Pankhurst 1999; Venn Beecham et al. 2006).

Although multiple fish were sampled from each treatment group at each time point, they were group-sedated and cohoused after sedation. Therefore, we determined that individual fish did not represent truly independent observations. Since the experiment lacked replicate experimental units, qualitative comparisons of within-group mean values were assessed rather than using statistical analysis to try to make quantitative comparisons.

RESULTS

All fish were successfully induced to Stage IV sedation in less than 1 min (mean = 0.6 min, range = 0.5–0.7 min), regardless of voltage strength or exposure duration (Figure 1). Recovery times were more variable, but recovery of equilibrium (mean = 0.7 min; range, 0.3–1.3 min) and tactile responsiveness (mean = 0.9 min; range, 0.5–1.4 min) were achieved in less than 2 min postsedation. Although a positive relationship was evident between longer exposure durations and increasing recovery times in fish sedated using 100 and 150 V, recovery times were shorter among fish sedated using 200 V. Overall, induction and recovery times were short, total time elapsed from induction to full recovery ranged from 1.0 to 2.1 min (mean = 1.6 min), there was minimal group-to-group induction or recovery variability, and no mortalities were observed 24 h postsedation.

Although most blood chemistry parameters did not vary substantially by electrosedation protocol at any single timepoint, hematocrit, blood glucose, and plasma cortisol, lactate, and osmolality varied over time following sedation (Figure 2A–E). Plasma cortisol and lactate concentrations initially increased

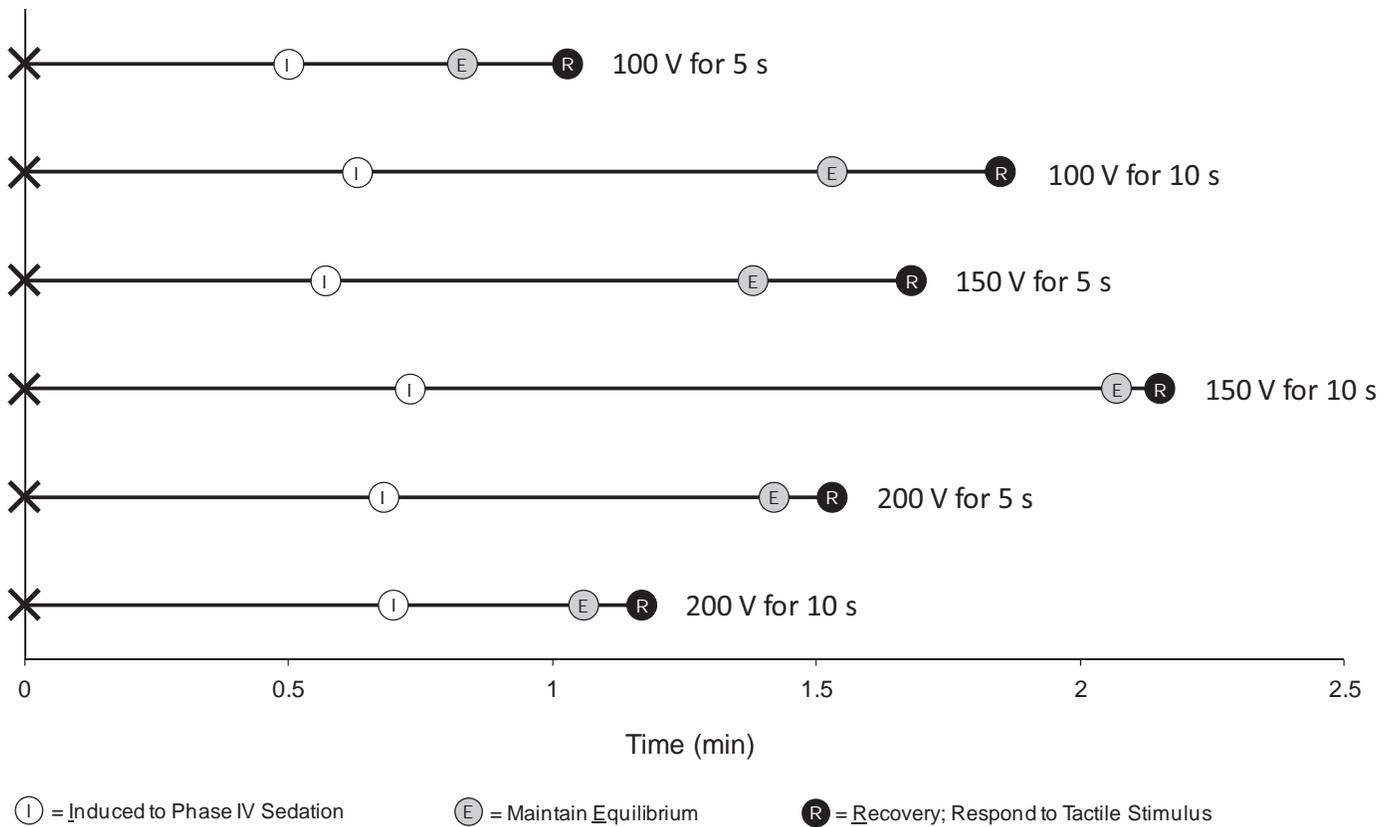


FIGURE 1. Schematic illustrating induction and various stages of recovery of grass carp electrosedated to Stage IV sedation using various pulsed DC voltage strengths and exposure durations.

after sedation and then decreased over time. The cortisol response was rapid and transient, peaking (162–288 ng/mL) at 0.5 h postsedation and returning to resting levels (0–100 ng/mL) between 2 and 6 h postsedation. The peak lactate response developed more slowly than cortisol, reaching maximum levels (6–9 mmol/L) between 0.5 and 2 h postsedation, but dropped below resting levels by 6 h postsedation. Peak levels of blood glucose (98–124 mg/dL) observed within the first 0.5 h postsedation, decreased slightly over the next 0.5 h and then increased slightly from 1 to 6 h postsedation. Hematocrit and plasma osmolality fluctuated near resting levels throughout the sampling period. There was no indication that any one combination of voltage strength and exposure duration consistently produced the highest or lowest blood chemistry responses.

During electrosedation, fish exhibited opercular flaring, fin extension, and body rigidity but regained normal posture after resolution of the postsedation tremor. Slight petechial hemorrhaging was observed along the lower flank and opercular area in a few fish during electrosedation.

DISCUSSION

Pulsed DC, applied at voltage strengths of 100–200 V and exposure durations of 5 or 10 s, was effective in sedating grass carp

to Stage IV sedation. Voltage strength and exposure duration had little effect in terms of time to induction and recovery from sedation. In addition, there was little variability among electrosedation protocols on the effects on blood chemistry responses following sedation. Although slight numeric differences were noted for induction and recovery times, the maximum difference observed (0.23 and 1.12 min, respectively) would probably not be considered practically relevant to most fisheries professionals. All fish recovered within 2 min of induction, which would probably be considered adequate for sedating grass carp to facilitate procedures such as triploidy verification. The observed changes in blood chemistry were consistent with an acute stress response (Barton 2002), but these physiological responses were resolved or nearly resolved within 6 h of sedation, and no postsedation mortality was observed. Slight epidermal hemorrhaging was observed in some electrosedated fish. However, we concluded that these relatively uncommon lesions were probably unrelated to electrosedation since petechiae were also observed in some fish prior to electrosedation. Based on these data, it would appear that juvenile grass carp are resilient to electrosedation at the range of voltage strengths and exposure durations tested in this experiment, and that the electrosedation protocols tested are reasonably safe with respect to postsedation survival and physiological status of these fish. In the present work, we

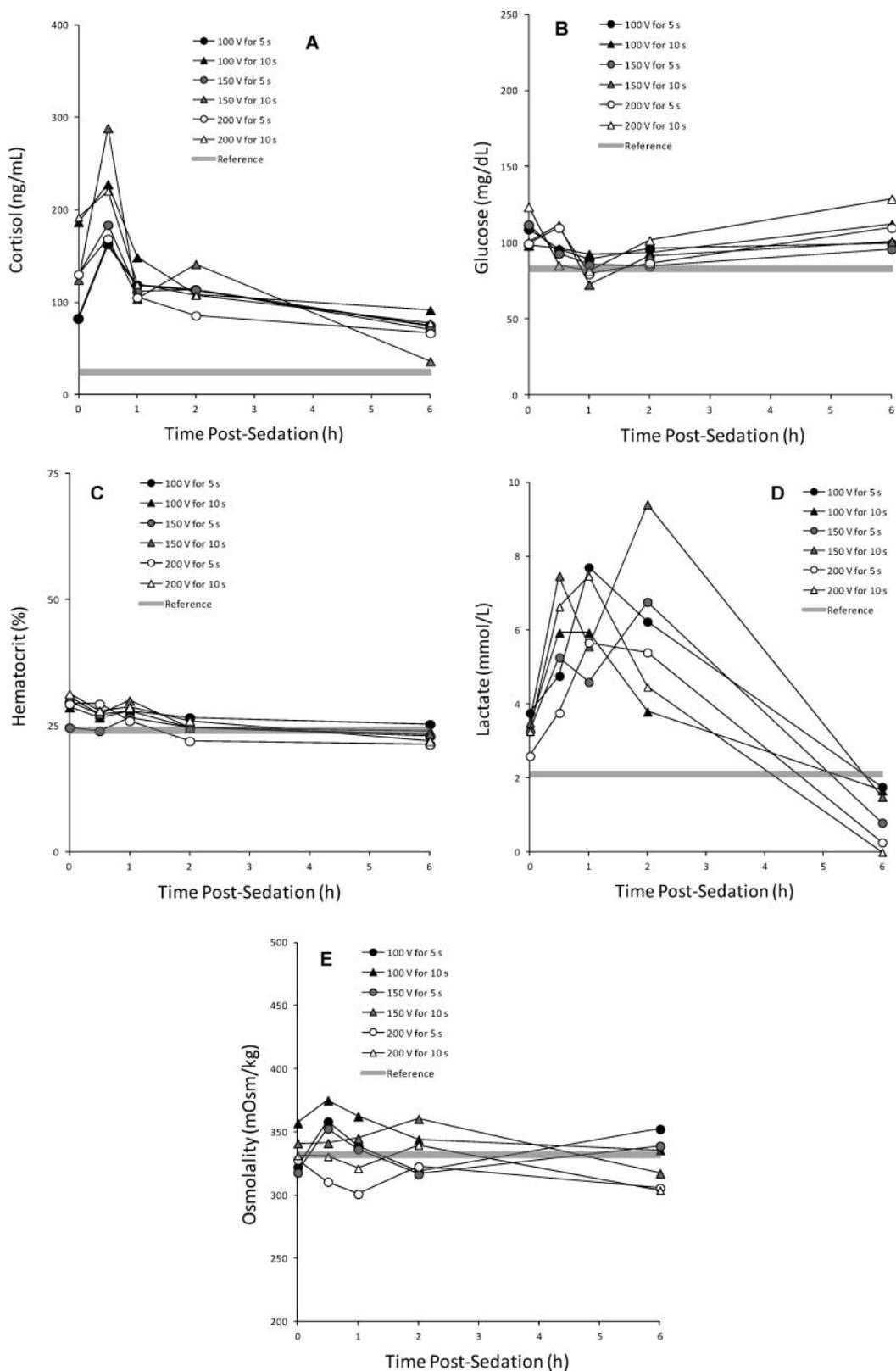


FIGURE 2. Time course of blood chemistry responses (A = plasma cortisol, B = blood glucose, C = hematocrit, D = plasma lactate, E = plasma osmolality) of grass carp following electrosedation using various pulsed DC voltage strengths and exposure durations. Points represent mean values; grey reference bars represent means of values observed for fish sampled from the reference population throughout the course of the experiment.

did not examine fish for vertebral or other internal injuries following electrosedation. These types of injuries have been observed following exposure to pulsed DC electrosedation in some (Gaikowski et al. 2001; Zydlewski et al. 2008) but not all fishes (Vandergoot et al. 2011). Electrically induced injury and mortality rates are a function of the type and strength of the waveform used, as well as the fish involved (Snyder 2003). In general, short duration exposure to low-intensity, pulsed DC waveforms is considered less risky than longer duration exposure to high intensity, AC waveforms, though the reported effects of these and other factors are “sometimes sparse, difficult to compare, and often questionable” (Snyder 2003). Regardless, the absence of direct or delayed mortality and overt signs of injury suggest that each of the protocols assessed in the present work are reasonably safe when used to sedate grass carp.

Blood chemistry responses observed in this experiment were comparable with those reported in two experiments conducted on hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*) using the same Portable Electroanesthesia System and in the experiment conducted by Gause et al. (2012) in which grass carp were sedated using a variety of chemical sedatives. Trushenski et al. (2012) reported a slightly greater plasma cortisol pulse and greater plasma glucose and lactate pulses, but similar hematocrit and osmolality levels, in hybrid striped bass (510 ± 12 g, 33.7 ± 0.2 cm, mean \pm SE) electrosedated at 100 V, 30 Hz, and 25% duty cycle for 3 s than we observed in grass carp in this experiment. In another experiment, Trushenski and Bowker (in press) used similar electrosedation protocols to sedate smaller hybrid striped bass (211 ± 4 g, 26.1 ± 0.1 cm total length, mean \pm SE), and found that grass carp plasma cortisol levels were lower than or comparable with those observed in the smaller hybrid striped bass at $t = 0, 1, 2,$ and 6 h. However, the plasma cortisol levels observed in grass carp were much lower ($150\text{--}300$ ng/mL) at $t = 0.5$ h than observed in the smaller hybrid striped bass ($400\text{--}650$ ng/mL) (Trushenski and Bowker, in press). Responses of lactate, hematocrit, and osmolality noted for grass carp were of a comparable or smaller magnitude than those reported for smaller hybrid striped bass by Trushenski and Bowker (in press), but followed the same basic patterns of acute response and resolution within 6 h of sedation. The somewhat attenuated lactate response observed in grass carp, in comparison with hybrid striped bass, is probably the result of the comparatively lower metabolic rate of grass carp (Tuncer et al. 1990; Brougher et al. 2005; Fu et al. 2009). Increased lactate formation results from anaerobic metabolic activity occurring during periods of limited or no oxygen availability, such as when environmental oxygen availability is limiting or during exhaustive physical activity when respiration is insufficient to meet tissue oxygen demand for aerobic metabolism (Bennett 1978; Burton and Heath 1980). Sedated fish exhibiting reduced ventilation rates may accumulate lactate, particularly if their metabolic rate and oxygen demand is high. Juvenile hybrid striped bass have a considerably higher oxygen demand at rest (132 mg $\text{O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) (Tuncer et al. 1990;

Brougher et al. 2005) than some other fish, including grass carp (56 mg $\text{O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; Fu et al. 2009); for purposes of comparison, Clarke and Johnston (1999) modeled the metabolic rate of 55 species of fish, and estimated the resting oxygen consumption of a 50-g fish at 15°C to range from 27 to 133 mg $\text{O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ depending on species. This may explain why electrosedated hybrid striped bass experience greater postsedation lactate pulses than do electrosedated grass carp. The blood glucose response of grass carp observed in this experiment fluctuated near 100 mg/dL compared with the smaller hybrid striped bass in the previous experiment by Trushenski and Bowker (in press); in that study blood glucose displayed a pulse from resting levels of approximately 55 mg/dL to a peak at $t = 1$ h of 180–220 mg/dL. The reduced metabolic rate and lower plasma cortisol levels of grass carp may explain the relatively minor glucose response observed in the current experiment. Cortisol can activate glycogenolysis and gluconeogenesis processes in fish, which cause increases in substrate levels (glucose) in the blood to produce enough energy to meet the demand of the organism (Barton and Iwama 1991; Martínez-Porchas et al. 2009), and since grass carp have lower metabolic demands and experienced a lower cortisol response to electrosedation than hybrid striped bass (and possibly a lower catecholamine response), a lower glucose response may be expected. This and other research with chemical sedatives and various methods of electrosedation (AC, continuous DC, pulsed DC) or electroshock (Schreck et al. 1976; Mesa and Schreck 1989; Barton and Grosh 1996; Barton and Dwyer 1997) have demonstrated that fish undergo the generalized stress response (Barton 2002) following sedation (Bourne 1984; Bernier and Randall 1998; Davidson et al. 2000; Davis and Griffin 2004; Woods et al. 2008; Feng et al. 2009; Neifer and Stamper 2009; Sattari et al. 2009; Carter et al. 2011; Trushenski et al. 2012; Gause et al. 2012). Because these effects also occur after exposure to sedatives in the absence of handling further emphasizes that the sedatives themselves act as stressors (Zahl et al. 2010). Differences in the magnitude of physiological responses aside, our present results are broadly consistent with our previous work sedating adult hybrid striped bass (Trushenski et al. 2012) and the majority of published works on the subject.

In conclusion, pulsed DC electrosedation is an effective strategy for sedating grass carp quickly and easily for routine handling procedures. Electrodesation offers one distinct advantage over other currently available options: fish can be released immediately after treatment. Like other sedatives, electrosedation induces an acute stress response in fish. Although electrosedated grass carp exhibited responses consistent with the generalized stress response in fish, none of the protocols used elicited responses that were particularly severe in comparison with the others or the reported effects of chemical sedatives (Gause et al. 2012), and fish were observed to recover from these effects within 6 h. Although slight differences in induction and recovery times were associated with different voltage strengths and exposure durations, all of the protocols used yielded sedation

patterns that would be considered acceptable for handling grass carp and testing them for triploidy. However, to minimize the incidence of unforeseen injuries or physiological alterations, we recommend that users employ the lowest voltage strength and exposure duration that yields effective electrosedation.

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