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[Article]

The Physiological Response of Port Jackson Sharks and Australian Swellsharks to Sedation, Gill-Net Capture, and Repeated Sampling in Captivity

LORENZ H. FRICK AND RICHARD D. REINA*
School of Biological Sciences, Monash University, Wellington Road, Clayton, Victoria 3800, Australia

TERENCE I. WALKER
Department of Primary Industries, Queenscliff Centre, Marine and Freshwater Fisheries Research Institute, 2a Bellarine Highway, Queenscliff, Victoria 3225, Australia

Abstract.—Studying postrelease effects of fisheries capture on chondrichthyans in the wild poses considerable logistical challenges. We report a laboratory-based technique to (1) simulate gill-net capture of sharks, which allows monitoring the condition of animals during recovery from a controlled capture event, and (2) assess effects of sedation, serial blood sampling, and repeated exposure to experimental treatment on stress-related blood variables. Exposing Port Jackson sharks *Heterodontus portusjacksoni* and Australian swellsharks *Cephaloscyllium laticeps* to 30 min of simulated gill-net capture elicited behavioral stress (struggling and elevated ventilation rate) and minor physiological stress (elevated plasma lactate) responses but did not cause any mortality. Sedation of Australian swellsharks affected some stress-related blood variables. Repeated handling of Port Jackson sharks and Australian swellsharks at short intervals may result in elevated stress levels, but repeated exposure to simulated capture does not affect the physiological response of these two species to the treatment. Overall, the results of this study demonstrate the feasibility of simulated capture events as a technique to investigate the physiological response of sharks to capture stress.

Postrelease mortality is of serious concern for fisheries management of bycatch, protected species, and harvested populations where a component of the catch must be returned to the water (Suuronen 2005; Moyes et al. 2006; Skomal 2007). It is commonly agreed that intense stress endured during a capture event can lead to delayed mortality, but the physiological cause responsible for such mortality has not been fully identified (Wood et al. 1983; Wedemeyer et al. 1990). Understanding the physiological response of sharks to capture stress and the circumstances under which such a response can carry an organism beyond its homeostatic limits is essential to predicting the fate of an animal after it has been caught, handled, and released.

The postrelease fate of sharks will ultimately have to be studied in the animals' natural environment, but understanding the physiological mechanisms underlying the phenomenon of delayed mortality from data collected in the wild is very difficult. Many factors, including duration of stress exposure (Wells et al. 1986; Chopin et al. 1996; Olla et al. 1997; Manire et al. 2001; de Lestang et al. 2004), water temperature and dissolved oxygen (Olla et al. 1998; Carlson and Parsons 2003), and possibly also the nutritive state of an animal, can have profound effects on exercise-related physiological processes but cannot be controlled in the wild. These sources of variation, along with potential cumulative effects of multiple stressors, prevent an unambiguous interpretation of data. Furthermore, obtaining repeated blood samples and monitoring the condition of an animal for several days after release are not feasible in the wild.

Several studies have successfully simulated capture of teleost fishes in a controlled laboratory setting with different types of fishing gear to investigate factors contributing to immediate and delayed mortality (e.g., Olla et al. 1997; Davis and Olla 2001; Ryer 2002). However, applying such an approach to sharks has not been attempted.

Various potential problems with this approach should be considered. Due to their size and nature, certain species of sharks may require immobilization prior to handling or blood sampling. The effects of sedation on blood constituents of sharks have not been examined, but it has been well documented that exposure to anesthetics can induce a stress response in teleosts (e.g., Iwama et al. 1989; Small 2003; King et al. 2005). Naturally, such an effect is undesirable when investigating the physiological response to a different stressor. Also, data obtained through repeated blood sampling during recovery from capture stress are likely to yield important information on any delayed effects

* Corresponding author: richard.reina@sci.monash.edu.au

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of exposure to capture stress, but handling alone can cause changes in stress-related blood variables (Barton et al. 1986; Gingerich and Drottar 1989).

Using a captive animal for more than one experiment may be an attractive option, given that the capture and transport of sharks to the research facilities incur considerable expense and logistical challenges. However, the reaction of an individual animal to an experimental treatment may be influenced by memories of previous treatments (Routley et al. 2002; Moreira and Volpato 2004; Hosoya et al. 2007), and multiple exposures to a certain stressor may result in a cumulative physiological stress response (Barton et al. 1986).

The overall objective of our study was to devise and evaluate a laboratory-based simulation of fisheries capture to measure and understand the short- and intermediate-term consequences of capture stress. This information will assist us in evaluating the likely effects of targeted or incidental capture of sharks in their natural environment.

We conducted four experiments with two benthic shark species, the Port Jackson shark *Heterodontus portusjacksoni* and the Australian swellshark *Cephaloscyllium laticeps*, both of which are common bycatch species in southeastern Australian fisheries (Walker et al. 2005; Walker and Gason 2007). We evaluated sampling technique, assessed adequate poststress monitoring duration and blood sampling frequency, and investigated potential habituation effects associated with repeated exposure of an individual animal to experimental treatment.

Methods

Animals and husbandry.—Port Jackson sharks and Australian swellsharks were caught by a commercial fisherman using gill nets in coastal waters near Mallacoota, Victoria, Australia, and were transported to research facilities in Queenscliff, Victoria, by use of trailer-mounted fish transport tanks (3 tanks; 1,000 L each) containing chilled, aerated seawater. Animals were transferred to circular, 19,000-L holding tanks connected to a flow-through seawater system running at ambient seawater temperature and were left to acclimatize for at least 7 d prior to experimentation. They were fed chopped pilchards *Sardinops neopilchardus* three times per week (approximately 1% of body mass per feeding event) but were unfed for 4 d before experimentation to reach a postabsorptive state. All sharks appeared to be in good condition and displayed normal behavior in the holding tank, including extended resting periods on the tank bottom, occasional periods of swimming, and vigorous struggling when caught out of the holding tank with a dip net.

Reference values from untreated sharks.—To obtain reference blood samples from untreated animals, Port Jackson sharks ($n = 6$; total length [TL], mean $\pm$ SE = 1,075 $\pm$ 44 mm) and Australian swellsharks ($n = 10$; 820 $\pm$ 18 mm TL) were caught in their holding tank with a dip net and were brought up to the side of the tank. Each fish was positioned on the water surface along the tank wall with its ventral side up and with its head and gills submerged to allow respiration, and 2.5 mL of blood were drawn from the caudal vessels with a heparinized needle (1.2 $\times$ 38 mm [18-gauge, 1.5 in]) and syringe. This procedure, including dipnetting, positioning the shark, and collecting blood, was completed in less than 1 min. Each shark was then released back into the holding tank. Since handling was very brief, stress to the animal was assumed to be minimal during this procedure, and the samples were used to establish reference values of resting sharks.

Experiment 1: effects of sedation.—Blood samples taken from Australian swellsharks treated by sedation with the clove-oil-derived anesthetic AQUI-S (isoeugenol; AQUI-S New Zealand Ltd.) were compared with samples taken from untreated sharks (reference values) and control sharks to identify changes in plasma constituents associated with immobilization.

Sharks ($n = 10$; 852 $\pm$ 15 mm TL) were removed from the holding tank with a dip net and were transferred into a black plastic tub (1,200 $\times$ 600 $\times$ 800 mm) filled with 200 L of sedative solution (24.3 mg of isoeugenol/L of seawater). Sharks were placed into the sedation tub in pairs. Sedation was achieved when sharks showed total loss of reactivity to external stimuli (after Tyler and Hawkins 1981). Once sedated, 2.5 mL of blood were drawn following the procedure described above. Sharks were then released into a separate holding tank and were left to recover.

A control group ($n = 10$; 812 $\pm$ 17 mm TL) was treated the same way as the sedation group, but control sharks were transferred into a sedation tub containing ambient seawater only and were left there for 27 min. This duration was the mean induction time we measured for sedation of Australian swellsharks.

Experiment 2: recovery from 30 min of gill-net exposure.—We tested various aspects of our capture simulation methods and established a general timeframe for recovery from the capture event. Gill-net capture was simulated in a circular, 5,000-L fiberglass aquaculture tank. A section of a gill net (length = 3 m; mesh diameter = 152 mm [6 in]) was attached to a wooden frame suspended from a metal tripod sitting on top of the tank. To record struggling activity of sharks in the gill net, we modified a method used by Chopin et al. (1996) to monitor struggling activity of hooked teleosts. A tension meter (S-Beam load cell; Sentran,

Ontario, California) was placed between the wooden frame and the tripod, so that movement of the suspended net was detected by the tension meter and data were sent to a computer via a serial connection to be recorded with a custom-made data acquisition software (programmed with LabVIEW, National Instruments, Austin, Texas). Load cell data were used to determine the duration of the initial struggling bout, the total amount of time spent struggling, and the total number of struggling bouts during the 30-min gill-net exposure.

Port Jackson sharks ($n = 10$; 1,120 ± 21 mm TL) and Australian swellsharks ($n = 10$; 841 ± 39 mm TL) were removed from their holding tanks with a dip net and were transferred to the experimental tank containing the gill net as described above. To ensure immediate entanglement, the leadline of the gill net was pulled over the side of the tank so that the gill net formed a horizontal pocket. Sharks were placed into this pocket and the leadline was dropped into the tank as soon as the shark started to become entangled. Each shark was left in the gill net for 30 min; during gill-net exposure, ventilation rate was recorded at 5 min (starting value), 15 min (intermediate value), and 25 min (ending value) by counting the number of gill beats for a period of 30 s. After 30 min, the shark was brought up to the side of the tank while still entangled, and a blood sample was obtained following the same procedure as described above. The animal was then placed into a separate recovery tank that was identical to the experimental tank but without a net. Additional blood samples were taken at 15 and 30 min posttreatment and at 1, 3, 6, 12, 24, 36, 48, and 72 h posttreatment. Ventilation rate was recorded immediately before (presampling) and immediately after (postsampling) obtaining each of these blood samples except those taken at 15 and 30 min and 1 h. Presampling ventilation rates were considered to reflect values at rest, since sharks had been undisturbed for at least 2 h prior to measurement. Sharks were not fed for the duration of this experiment. Based on preliminary results from Port Jackson sharks, collection of the 15- and 30-min blood samples was abandoned for Australian swellsharks.

Experiment 3: effects of serial blood sampling.—To assess the effect of serial blood sampling, we repeatedly collected samples from Port Jackson sharks ($n = 6$; 1,075 ± 44 mm TL) and Australian swellsharks ($n = 10$; 820 ± 13 mm TL) that had not been exposed to capture stress. A total of five blood samples were taken for Port Jackson sharks (0, 15, and 30 min and 1 and 3 h), and a total of three samples were taken for Australian swellsharks (0, 1, and 3 h).

Experiment 4: habituation to experimental treatment.—To investigate whether or not sharks could habituate to our experimental treatment if used for more than one experiment, we repeatedly subjected Port Jackson sharks ($n = 10$; 1,088 ± 25 mm TL) and Australian swellsharks ($n = 10$; 820 ± 18 mm TL) to 30 min of gill-net exposure. The capture simulation procedure was identical to the one described for experiment 2. After a shark was held for 30 min in the gill net, a blood sample was taken and the shark was transferred into the recovery tank. After 1 h, another blood sample was taken and the shark was returned to the holding tank, where it was left to recover for 48 h before being subjected to the same gill-net capture treatment again. This was repeated for a total of six gill-net exposures. Sharks were fed about 0.5% of their body mass after each treatment.

Blood analysis.—Immediately after obtaining a blood sample, approximately 10 μL of whole blood were drawn into a microcapillary tube and centrifuged for 10 min at 10,000 revolutions per minute (rpm) to determine hematocrit. Hematocrit was not determined for Australian swellsharks during experiment 1 or for Port Jackson sharks during experiment 2 due to equipment failure. The rest of the sample was centrifuged for 10 min at 10,000 rpm. The plasma portion was then removed and kept frozen at −20°C for later analysis. Plasma constituents were analyzed using a Beckman Synchron CX-5 analyzer (Beckman Coulter, Fullerton, California). Plasma samples of Port Jackson sharks were analyzed for lactate, glucose, potassium, urea, iron, chloride, magnesium, and phosphate. Plasma samples of Australian swellsharks were analyzed for lactate, glucose, potassium, and urea.

Statistical analysis.—Comparison of means between treatment groups in experiment 1 was conducted with single-factor analysis of variance (ANOVA). Pairwise comparisons of means were performed with Welch's two-sample *t*-test or a paired *t*-test. Relationships between struggling effort and ventilation rate or peak blood variable response were analyzed with linear regression. Time series data were analyzed with repeated-measures (rm) ANOVA. When the assumption of sphericity was violated for time series data, *P*-values were adjusted using either the Greenhouse–Geisser epsilon or the Huynh–Feldt epsilon. Two-sample *t*-tests (Bonferroni-adjusted *P*-values) were used to determine significant differences between individual time series data and reference values of untreated sharks if rm ANOVA results indicated significant changes over time. Some variables were log transformed prior to statistical analysis to meet assumptions of the respective tests. Statistical data analysis was performed using the R software package (R Core Development Team 2005). A significant difference was

TABLE 1.—Blood plasma constituent concentrations (mean ± SE; Hct = hematocrit) of Australian swellsharks subjected to four laboratory experiments (10 fish/treatment group) examining (1) sedation with AQUI-S (isoeugenol; 24.3 mg/L of seawater) versus holding in ambient seawater (control); (2) 30 min of simulated gill-net entanglement (samples collected at 0–72 h posttreatment); (3) serial blood sampling in the absence of capture stress; and (4) habituation to six 30-min gill-net exposures, where samples were collected immediately after each exposure (0 h) and at 1 h postexposure (fish were given a 48-h recovery interval prior to each successive exposure). If repeated-measures (rm) analysis of variance (ANOVA) results indicated significant changes over time, two-sample *t*-tests (Bonferroni-adjusted *P*-values) were used to determine significant differences (indicated by lowercase letters; values without a letter are not significantly different from each other) between treatment values for experiments 1–3 and reference values (blood constituent levels of untreated sharks); Tukey's post hoc analysis was used to determine differences between individual exposures for experiment 4.

Measurement or result	Lactate (mmol/L)	Glucose (mmol/L)	K (mmol/L)	Urea (mmol/L)	Hct (%)
Reference value	0.33 ± 0.04	0.87 ± 0.22	3.64 ± 0.16		
Experiment 1: sedation					
Sedation	1.28 ± 0.19 z	0.97 ± 0.10	4.14 ± 0.08 z		
Control	0.41 ± 0.06	0.81 ± 0.08	3.73 ± 0.06		
Experiment 2: 30-min gill-net exposure					
0 h poststress	0.67 ± 0.09 z	0.59 ± 0.07	3.64 ± 0.05	357 ± 3	13.0 ± 0.3
1 h poststress	1.05 ± 0.16 z	0.68 ± 0.04	3.66 ± 0.11	353 ± 4	14.4 ± 0.3
3 h poststress	1.20 ± 0.19 z	0.78 ± 0.12	3.54 ± 0.11	353 ± 3	13.1 ± 0.4
6 h poststress	0.72 ± 0.13	0.78 ± 0.12	3.37 ± 0.11	352 ± 3	12.3 ± 0.5
12 h poststress	0.50 ± 0.07	0.77 ± 0.07	3.35 ± 0.08	348 ± 4	12.6 ± 0.3
24 h poststress	0.37 ± 0.09	0.66 ± 0.06	3.32 ± 0.09	351 ± 5	11.6 ± 0.3
36 h poststress	0.26 ± 0.05	0.72 ± 0.04	3.34 ± 0.08	348 ± 5	11.4 ± 0.4
48 h poststress	0.20 ± 0.07	0.51 ± 0.05	3.17 ± 0.06	346 ± 3	11.3 ± 0.6
72 h poststress	0.17 ± 0.06	0.47 ± 0.06	3.12 ± 0.10	351 ± 4	10.7 ± 0.6
rm ANOVA *P*	<0.0001	<0.05	<0.001	>0.05	<0.0001
Experiment 3: serial sampling					
0 h	0.54 ± 0.07	0.67 ± 0.10	3.89 ± 0.07		12.5 ± 0.3
1 h	0.55 ± 0.09	0.88 ± 0.12	3.99 ± 0.09		12.6 ± 0.3
3 h	0.67 ± 0.13	1.14 ± 0.16	3.68 ± 0.07		11.8 ± 0.4
rm ANOVA *P*	>0.05	<0.0001	<0.001		>0.05
Experiment 4: habituation					
Exposure 1 (0 h)	0.77 ± 0.11	0.67 ± 0.09	3.82 ± 0.10 z		12.7 ± 0.6
Exposure 2 (0 h)	0.66 ± 0.12	0.62 ± 0.09	3.94 ± 0.09 zy		12.0 ± 0.5
Exposure 3 (0 h)	0.69 ± 0.13	0.76 ± 0.07	3.92 ± 0.06 zy		11.9 ± 0.3
Exposure 4 (0 h)	0.73 ± 0.13	0.92 ± 0.15	3.92 ± 0.08 zy		11.4 ± 0.4
Exposure 5 (0 h)	0.61 ± 0.10	0.75 ± 0.09	3.93 ± 0.06 zy		11.3 ± 0.5
Exposure 6 (0 h)	0.85 ± 0.15	0.90 ± 0.15	4.29 ± 0.13 y		11.0 ± 0.5
rm ANOVA *P*	>0.05	<0.05	<0.01		>0.05
Exposure 1 (1 h)	1.06 ± 0.25	0.73 ± 0.08	3.78 ± 0.12 z		12.5 ± 0.5
Exposure 2 (1 h)	0.83 ± 0.21	0.81 ± 0.11	3.98 ± 0.14 zy		12.1 ± 0.5
Exposure 3 (1 h)	0.96 ± 0.21	0.96 ± 0.06	3.88 ± 0.09 zy		12.0 ± 0.4
Exposure 4 (1 h)	0.81 ± 0.15	1.09 ± 0.15	3.78 ± 0.10 z		11.0 ± 0.4
Exposure 5 (1 h)	0.84 ± 0.15	0.95 ± 0.11	3.89 ± 0.09 zy		11.0 ± 0.6
Exposure 6 (1 h)	0.96 ± 0.18	1.08 ± 0.15	4.30 ± 0.16 y		10.5 ± 0.6
rm ANOVA *P*	>0.05	<0.05	<0.001		<0.05

determined at *P*-values less than 0.05. Results are presented as means with SEs unless stated otherwise.

Results

All results of blood plasma constituent analysis of samples obtained from Australian swellsharks and Port Jackson sharks are presented in Tables 1 and 2, respectively.

Experiment 1: Effects of Sedation

For experiment 1, blood was collected from Australian swellsharks in September 2006. Water temperature was approximately 15°C.

Australian swellsharks generally thrashed briefly when placed into the sedation tub and then remained still until sedated. We did not record ventilation rate but observed an apparent initial acceleration and subsequent deceleration of gill beat frequency. Gill beat frequency was slow but steady during sedation, and no animal showed signs of equilibrium loss. Sedation was reached after 27 ± 2 min.

Mean plasma lactate levels of sedated Australian swellsharks were significantly higher than those of untreated and control sharks (single-factor ANOVA: df = 2, $F = 20.464$, $P < 0.0001$). Potassium levels of sedated Australian swellsharks were significantly

higher than those of untreated and control animals (single-factor ANOVA: df = 2, $F = 5.526$, $P < 0.01$). There were no significant differences in plasma glucose concentration between treatment groups. Resumption of voluntary movement indicated that all Australian swellsharks had recovered from sedation after 10 min in regular seawater.

Experiment 2: Recovery from 30 min of Gill-Net Exposure

For experiment 2, Port Jackson sharks were sampled in March 2006 and Australian swellsharks were sampled in September 2006. Water temperature ranged from 19.9°C to 21.1°C during the Port Jackson shark experiment and from 13.7°C to 16.3°C during the Australian swellshark experiment.

There were some species-specific differences regarding entanglement and behavior in the gill net. Most Australian swellsharks attempted to swim through the mesh of the gill net, which resulted in the net enmeshing around the gill region of the shark behind the jaw structures, thus tightly restraining the fish. Port Jackson sharks were not gilled in the net because their broad heads did not fit through the net mesh. They were usually entangled around the mouth region, pectoral fins, and dorsal spines. Most sharks struggled vigorously when placed in the gill net, but struggling usually ceased after 1–2 min and sharks then remained still. Port Jackson sharks generally thrashed much more violently than Australian swellsharks did. Struggling effort data for both species are presented in Table 3. There were often additional shorter struggling bouts at a later point during the 30-min gill-net exposure in Port Jackson sharks (e.g., Figure 1), but most Australian swellsharks remained still after the initial struggle. On average, Port Jackson sharks spent significantly more time struggling during the 30-min gill-net exposure than did Australian swellsharks (two-sample t-test: df = 9.419, $t = 2.386$, $P < 0.05$). One lightly entangled Port Jackson shark displayed slow swimming motions throughout the whole 30-min exposure, and the activity was registered by the load cell as 100% struggling. This shark was excluded from analyses involving struggling effort because its behavior was not typical of the other animals that were struggling intensely to free themselves from the net.

Mean ventilation rate did not change significantly during gill-net exposure in either Port Jackson sharks or Australian swellsharks (Figure 2) and was not correlated with the amount of time spent struggling. However, mean ventilation rate was significantly higher for Port Jackson sharks during gill-net exposure than at rest (presampling; paired t-test: df = 9, $t = 7.258$, $P < 0.0001$) and was temporarily elevated after blood sampling (postsampling) in both Port Jackson sharks (paired t-test on means of individual sharks: df = 9, $t = -8.026$, $P < 0.0001$) and Australian swellsharks (paired t-test on means of individual sharks: df = 9, $t = -11.630$, $P < 0.0001$). Ventilation rate returned to resting levels within 2 min after blood sampling in both species.

Mean plasma lactate levels were significantly elevated in both species immediately after 30 min of gill-net exposure (two-sample t-test; Port Jackson sharks: df = 12.81, $t = -5.98$, adjusted $P < 0.05$; Australian swellsharks: df = 17.72, $t = -3.49$, adjusted $P < 0.05$). Plasma lactate concentrations were decreasing in both species at 3 h and returned to resting levels at 12 h in Port Jackson sharks or at 6 h in Australian swellsharks (Figure 3). Changes in blood plasma lactate concentration during the 72-h monitoring phase were statistically significant (rm ANOVA; Port Jackson sharks: df = 10, $F = 16.03$, $P < 0.0001$; Australian swellsharks: df = 8, $F = 18.67$, $P < 0.0001$). There was no evidence for an effect of struggling effort on the magnitude of the peak lactate response (3 h poststress) in either species (Figure 4).

Changes in plasma potassium levels during the recovery period after capture were statistically significant in both species (rm ANOVA; Port Jackson sharks: df = 10, $F = 4.002$, $P < 0.01$; Australian swellsharks: df = 8, $F = 5.046$, $P < 0.001$), but individual time series results were not significantly different from reference values of untreated sharks. There was no significant change in mean plasma glucose concentration of Port Jackson sharks (rm ANOVA: df = 10, $F = 2.872$, $P = 0.059$). Changes in plasma glucose levels of Australian swellsharks were statistically significant (rm ANOVA: df = 8, $F = 4.980$, $P < 0.05$), but comparison of individual time series results with reference values of untreated sharks revealed no significant differences. Blood plasma concentrations of urea did not change significantly during the 72-h monitoring period after stress exposure in either species.

Plasma phosphate levels of Port Jackson sharks were stable until 12 h after stress exposure but then decreased, and levels at 72 h were significantly lower than reference values (two-sample t-test: df = 5.919, $t = 4.296$, adjusted $P < 0.05$). Changes over time were statistically significant (rm ANOVA: df = 10, $F = 8.4227$, $P < 0.0001$). Changes in plasma magnesium concentrations of Port Jackson sharks during the postcapture recovery period were statistically significant (rm ANOVA: df = 10, $F = 4.609$, $P < 0.001$), but comparison of individual time series results with reference values of untreated sharks revealed no significant differences. No significant changes were

TABLE 2.—Blood plasma constituent concentrations (mean ± SE; Phos = phosphate, Hct = hematocrit) of Port Jackson sharks subjected to laboratory experiments 2–4 (experiment 1 not performed with this species; see Methods). Experiment 2 ($n = 10$ fish) involved 30 min of simulated gill-net entanglement (samples collected at 0–72 h posttreatment). Experiment 3 ($n = 6$ fish) consisted of serial blood sampling in the absence of capture stress. Experiment 4 ($n = 10$ fish) assessed shark habituation to six 30-min gill-net exposures, where samples were collected immediately after each exposure (0 h) and at 1 h postexposure; fish were given a 48-h recovery interval prior to each successive exposure. If repeated-measures (rm) analysis of variance (ANOVA) results indicated significant changes over time, two-sample *t*-tests (Bonferroni-adjusted *P*-values) were used to determine significant differences (indicated by lowercase letters; values without a letter are not significantly different from each other) between treatment values and reference values (blood constituent levels of untreated sharks; $n = 6$ reference fish).

Measurement or result	Lactate (mmol/L)	Glucose (mmol/L)	K (mmol/L)	Cl (mmol/L)	Mg (mmol/L)	Fe (μmol/L)
Reference value	0.25 ± 0.06	1.88 ± 0.08	4.65 ± 0.13	350 ± 11.84	1.40 ± 0.05	4.48 ± 0.87
Experiment 2: 30-min gill-net exposure						
0 h poststress	1.68 ± 0.33 z	1.80 ± 0.08	5.12 ± 0.19	272 ± 2	1.43 ± 0.09	2.06 ± 0.21
0.25 h poststress	1.67 ± 0.31 z	1.80 ± 0.08	5.18 ± 0.08	273 ± 3	1.49 ± 0.06	2.17 ± 0.18
0.5 h poststress	1.98 ± 0.37 z	1.83 ± 0.08	4.79 ± 0.18	274 ± 2	1.48 ± 0.05	2.07 ± 0.28
1 h poststress	2.04 ± 0.39 z	1.83 ± 0.07	4.86 ± 0.20	272 ± 2	1.43 ± 0.08	2.09 ± 0.28
3 h poststress	2.04 ± 0.39 z	1.71 ± 0.10	4.87 ± 0.21	273 ± 2	1.53 ± 0.08	2.02 ± 0.21
6 h poststress	1.47 ± 0.46 z	1.68 ± 0.09	4.93 ± 0.23	272 ± 1	1.48 ± 0.07	2.19 ± 0.23
12 h poststress	1.19 ± 0.39	1.93 ± 0.11	4.98 ± 0.15	270 ± 2	1.47 ± 0.06	2.53 ± 0.46
24 h poststress	0.57 ± 0.20	1.88 ± 0.11	4.92 ± 0.16	269 ± 2	1.42 ± 0.06	1.71 ± 0.18
36 h poststress	0.55 ± 0.12	2.09 ± 0.14	4.55 ± 0.17	268 ± 2	1.38 ± 0.06	1.84 ± 0.14
48 h poststress	0.46 ± 0.11	1.93 ± 0.12	4.73 ± 0.19	270 ± 2	1.36 ± 0.05	1.80 ± 0.15
72 h poststress	0.63 ± 0.19	1.73 ± 0.13	4.49 ± 0.14	270 ± 1	1.32 ± 0.05	2.05 ± 0.18
rm ANOVA *P*	<0.0001	>0.05	<0.01	>0.05	<0.001	>0.05
Experiment 3: serial sampling						
0 h	0.25 ± 0.06	1.88 ± 0.07	4.65 ± 0.12	350 ± 11	1.40 ± 0.04	4.48 ± 0.80
0.25 h	0.67 ± 0.19	1.88 ± 0.05	4.77 ± 0.16	344 ± 11	1.49 ± 0.05	4.22 ± 0.67
0.5 h	0.86 ± 0.20 z	1.97 ± 0.15	5.00 ± 0.14	331 ± 9	1.61 ± 0.11	4.33 ± 0.64
1 h	1.08 ± 0.35 z	1.87 ± 0.13	4.47 ± 0.11	336 ± 9	1.48 ± 0.08	4.58 ± 0.82
3 h	0.91 ± 0.25 z	1.88 ± 0.14	4.28 ± 0.11	341 ± 25	1.41 ± 0.04	4.33 ± 0.85
rm ANOVA *P*	<0.01	>0.05	<0.01	>0.05	>0.05	>0.05
Experiment 4: habituation						
Exposure 1 (0 h)	1.55 ± 0.50	1.38 ± 0.14	4.23 ± 0.10	358 ± 24	1.37 ± 0.07	4.16 ± 0.55
Exposure 2 (0 h)	1.28 ± 0.20	1.51 ± 0.22	4.40 ± 0.13	353 ± 24	1.39 ± 0.04	4.69 ± 0.72
Exposure 3 (0 h)	1.36 ± 0.20	1.44 ± 0.18	4.33 ± 0.14	346 ± 13	1.46 ± 0.04	5.34 ± 0.67
Exposure 4 (0 h)	1.13 ± 0.09	1.54 ± 0.15	4.41 ± 0.15	325 ± 5	1.47 ± 0.06	5.18 ± 0.52
Exposure 5 (0 h)	1.02 ± 0.14	1.63 ± 0.16	4.38 ± 0.14	349 ± 18	1.46 ± 0.05	4.94 ± 0.63
Exposure 6 (0 h)	1.32 ± 0.18	1.74 ± 0.18	4.31 ± 0.10	345 ± 17	1.43 ± 0.05	4.89 ± 0.67
rm ANOVA *P*	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Exposure 1 (1 h)	1.34 ± 0.40	1.25 ± 0.15	4.25 ± 0.28	365 ± 23	1.38 ± 0.04	4.07 ± 0.52
Exposure 2 (1 h)	1.22 ± 0.18	1.55 ± 0.20	4.38 ± 0.22	326 ± 12	1.40 ± 0.05	5.08 ± 0.88
Exposure 3 (1 h)	1.27 ± 0.16	1.46 ± 0.18	4.19 ± 0.14	309 ± 15	1.39 ± 0.05	5.20 ± 0.65
Exposure 4 (1 h)	1.25 ± 0.19	1.50 ± 0.14	4.43 ± 0.15	344 ± 13	1.45 ± 0.04	5.45 ± 0.79
Exposure 5 (1 h)	1.18 ± 0.21	1.73 ± 0.17	4.51 ± 0.17	339 ± 7	1.45 ± 0.04	5.10 ± 0.58
Exposure 6 (1 h)	1.36 ± 0.24	1.75 ± 0.17	4.51 ± 0.18	328 ± 8	1.42 ± 0.04	5.04 ± 0.72
rm ANOVA *P*	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

detected in circulating blood plasma concentrations of chloride or iron in Port Jackson sharks. There was a small but significant decrease in mean hematocrit of Australian swellsharks over the 72-h period (rm ANOVA: $df = 8$, $F = 10.998$, $P < 0.0001$).

Experiment 3: Effects of Serial Blood Sampling

Repeated blood samples were obtained from Port Jackson sharks and Australian swellsharks in April 2006 and October 2006, respectively, for experiment 3. Water temperature was 16.2–17.1°C during the Port Jackson shark experiment and 15.9–16.3°C during the Australian swellshark experiment.

Repeated blood sampling of Port Jackson sharks at the same intervals as described in the recovery experiment above but without any exposure to capture stress caused a small, significant increase in mean plasma lactate concentration (rm ANOVA: $df = 4$, $F = 13.058$, $P < 0.01$). Changes in plasma potassium concentration (rm ANOVA: $df = 4$, $F = 5.751$, $P < 0.01$) and hematocrit (rm ANOVA: $df = 4$, $F = 5.918$, $P < 0.01$) of Port Jackson sharks over time were statistically significant, but comparison of individual time series results with reference values of untreated sharks revealed no significant differences. There was no significant change in mean plasma concentrations of

TABLE 2.—Extended.

Measurement or result	Urea (mmol/L)	Phos (mmol/L)	Hct (%)
Reference value		1.39 ± 0.12	21.3 ± 1.6
Experiment 2: 30-min gill-net exposure			
0 h poststress	359 ± 9	1.33 ± 0.10	
0.25 h poststress		1.37 ± 0.08	
0.5 h poststress		1.39 ± 0.11	
1 h poststress	356 ± 8	1.34 ± 0.12	
3 h poststress	357 ± 8	1.44 ± 0.17	
6 h poststress	333 ± 15	1.28 ± 0.09	
12 h poststress	352 ± 12	1.13 ± 0.14	
24 h poststress	357 ± 11	1.03 ± 0.06	
36 h poststress	353 ± 11	0.98 ± 0.05	
48 h poststress	340 ± 13	0.97 ± 0.04	
72 h poststress	360 ± 6	0.89 ± 0.04	
rm ANOVA *P*	>0.05	<0.0001	
Experiment 3: serial sampling			
0 h		1.39 ± 0.11	21.3 ± 1.4
0.25 h		1.51 ± 0.14	21.9 ± 1.1
0.5 h		1.68 ± 0.22	23.2 ± 1.0
1 h		1.69 ± 0.13	22.0 ± 1.1
3 h		1.40 ± 0.08	19.4 ± 1.1
rm ANOVA *P*		>0.05	<0.01
Experiment 4: habituation			
Exposure 1 (0 h)		1.41 ± 0.08	19.3 ± 0.8
Exposure 2 (0 h)		1.24 ± 0.12	18.6 ± 1.0
Exposure 3 (0 h)		1.17 ± 0.20	18.7 ± 0.8
Exposure 4 (0 h)		1.37 ± 0.12	18.0 ± 1.1
Exposure 5 (0 h)		1.35 ± 0.07	19.1 ± 0.7
Exposure 6 (0 h)		1.20 ± 0.09	18.6 ± 0.8
rm ANOVA *P*		>0.05	>0.05
Exposure 1 (1 h)		1.38 ± 0.08	19.2 ± 1.1
Exposure 2 (1 h)		1.33 ± 0.11	19.3 ± 0.9
Exposure 3 (1 h)		1.26 ± 0.11	18.6 ± 0.8
Exposure 4 (1 h)		1.33 ± 0.11	19.6 ± 0.6
Exposure 5 (1 h)		1.29 ± 0.04	18.7 ± 1.0
Exposure 6 (1 h)		1.22 ± 0.05	19.7 ± 0.7
rm ANOVA *P*		>0.05	>0.05

glucose, chloride, magnesium, iron, and phosphate in response to repeated blood sampling of Port Jackson sharks.

Repeated blood sampling of Australian swellsharks at the same time intervals used during the recovery experiment but without exposure to capture stress did not have a significant effect on mean plasma lactate concentrations. Changes over time in plasma potassium (rm ANOVA: df = 2, $F = 21.393$, $P < 0.001$) and glucose (rm ANOVA: df = 2, $F = 26.286$, $P < 0.0001$) concentrations of Australian swellsharks were statistically significant, but comparison of individual time series results with reference values of untreated sharks revealed no significant differences. Mean hematocrit of Australian swellsharks was not affected by repeated blood sampling.

Experiment 4: Habituation to Experimental Treatment

During experiment 4, Port Jackson sharks and Australian swellsharks were subjected to six consecutive 30-min gill-net exposures separated by 48-h intervals in April 2006 and September 2006, respectively. Water temperature was 14.9–17.0°C for the Port Jackson shark experiment and 15.2–18.1°C for the Australian swellshark experiment.

Mean struggling effort of Port Jackson sharks, as indicated by the percentage of time spent struggling during each 30-min gill-net exposure, was considerably lower for exposures 4 and 5 than for the first three exposures, but the differences were not significant (Figure 5). There were no differences in struggling effort among capture exposures of Australian swellsharks (Figure 5).

No significant differences were detected in mean plasma concentrations of metabolites, electrolytes, or hematocrit between individual gill-net exposures in Port Jackson sharks.

Mean plasma lactate concentrations of Australian swellsharks did not differ significantly among gill-net exposures. Plasma glucose concentration of Australian swellsharks differed significantly among exposures (rm ANOVA; 0-h samples: df = 5, $F = 3.259$, $P < 0.05$; 1-h samples: df = 5, $F = 3.164$, $P < 0.05$), but Tukey

TABLE 3.—Variables (mean ± SE) describing the duration and number of struggling bouts exhibited by Port Jackson sharks and Australian swellsharks ($n = 10$ fish/group) during 30 min of simulated gill-net entanglement in the laboratory. Values in parentheses represent percentages of total exposure time. Lowercase letter indicates significant difference between species for the given variable (two sample *t*-test: $P < 0.05$).

Variable	Port Jackson shark	Australian swellshark
Mean length of initial struggling bout (s)	99 ± 43	49 ± 9
Mean number of struggling bouts	4.0 ± 0.7	2.3 ± 0.4
Mean cumulative struggling duration (s)	255 ± 63 (14 ± 3%) z	101 ± 19 (6 ± 1%)
Range of cumulative struggling duration (s)	59–592 (3–33%)	22–176 (1–10%)

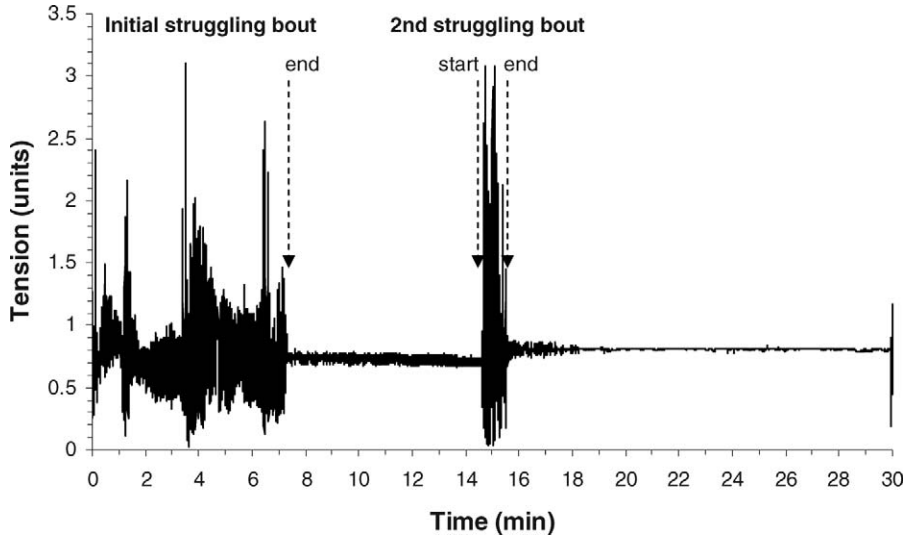

FIGURE 1.—Struggling profile (recorded with a tension meter) of a Port Jackson shark subjected to 30 min of gill-net entanglement in the laboratory. The initial struggling bout lasted 7 min 17 s and was followed by a resting period that coincidentally was also 7 min 17 s. The second struggling bout duration was 1 min, after which the shark remained still until the end of the exposure.

post hoc analyses with adjusted *P*-values revealed no significant differences between individual exposures.

Mean plasma potassium concentration of Australian swellsharks measured immediately (0 h) after gill-net exposure 6 was significantly higher than that measured 0 h after exposure 1 (Tukey's test: $t = 3.632$, $P < 0.01$) and was significantly higher than potassium levels measured 1 h after exposures 1 and 4 (Tukey's test: $t = 2.996$, $P < 0.05$). There were no statistically significant changes in mean hematocrit of Australian swellsharks measured 0 h after stress exposures, but hematocrit

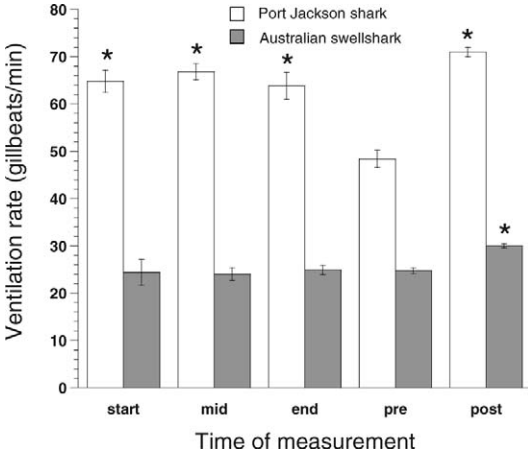

FIGURE 2.—Mean (±SE) ventilation rate (gill beats/min) of Port Jackson sharks and Australian swellsharks during a 30-min gill-net exposure in the laboratory (start = observed rate at 5 min; mid = rate at 15 min; end = rate at 25 min) and before (pre; resting values) and after (post) blood sampling during a 72-h recovery period from the simulated capture event. Asterisks denote significant differences (Tukey post hoc analysis: $P < 0.05$) from resting values of the respective species.

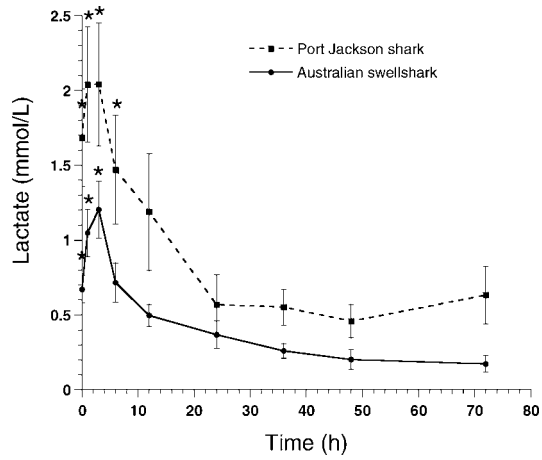

FIGURE 3.—Mean (±SE) plasma lactate concentration (mmol/L) of Port Jackson sharks and Australian swellsharks during a 72-h recovery period occurring after a 30-min gill-net exposure in the laboratory. Asterisks denote significant differences from reference values of untreated sharks of the respective species (two-sample *t*-test: Bonferroni-adjusted $P < 0.05$).

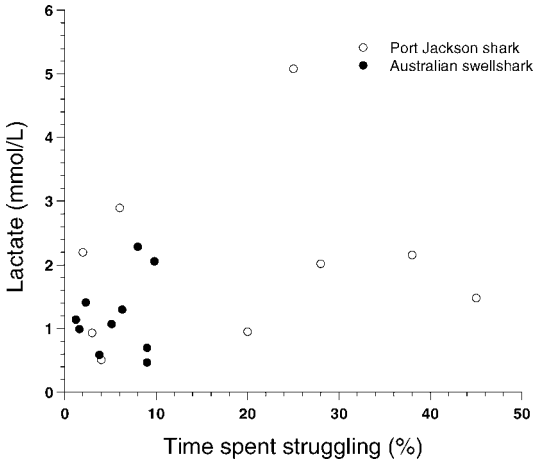

FIGURE 4.—Relation between plasma lactate concentration (mmol/L) measured at 3 h after a 30-min gill-net exposure and struggling effort (percentage of time spent struggling) during exposure for individual Port Jackson sharks and Australian swellsharks in the laboratory.

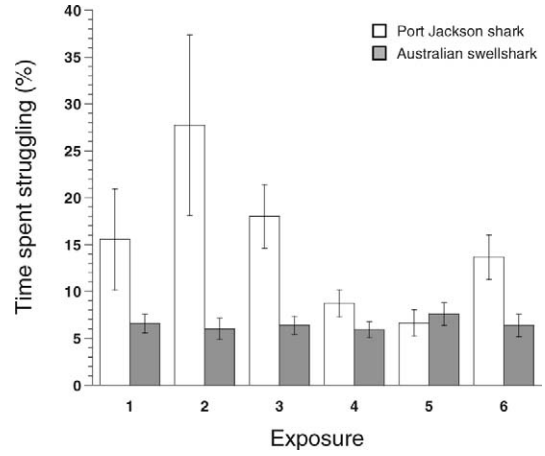

FIGURE 5.—Mean (±SE) struggling effort (percentage of time spent struggling) of Port Jackson sharks and Australian swellsharks during six consecutive 30-min gill-net exposures in the laboratory.

decreased significantly with increasing exposures in samples taken at 1 h postexposure (rm ANOVA: df = 5, $F = 3.958$, $P < 0.05$).

Discussion

Effects of Sedation

Average sedation induction time for Australian swellsharks was 27 min at an AQUI-S dose of 24.3 mg/L of seawater, and recovery time was less than 10 min. Cooper and Morris (1998) reported induction times of about 5 min for Port Jackson sharks exposed to an anesthetic dose (100 mg/L) of tricaine methanesulfonate (MS-222) during preparation for surgery. No other authors address the effects of anesthetics on blood constituent concentrations in sharks, but induction times for clove oil and other anesthetics in teleosts rarely exceed 5 min (Tort 2002; King et al. 2005; Velisek 2006). However, a reduction in induction time and an increase in recovery time with increasing concentration of anesthetic agent are well documented in teleosts for various types of anesthetics (e.g., Tort 2002; Woody et al. 2002; Cooke et al. 2004; King et al. 2005; Cunha and Rosa 2006). Therefore, the long induction times observed in the present study are probably due to the relatively low dosage we used.

Sedated Australian swellsharks showed elevated blood lactate in comparison with that of untreated or control sharks. Exposure to anesthetics has been shown to evoke a stress response in teleosts, as indicated by an elevation of plasma cortisol (Tort 2002; Small 2003; Davis and Griffin 2004; King et al. 2005). Accumulation of lactate in blood is well accepted as a secondary indicator of stress, reflecting an increased anaerobic metabolism due to fright or exertion (Wedemeyer et al. 1990). Small and Chatakondi (2005) also reported elevated plasma levels of lactate in channel catfish *Ictalurus punctatus* after anesthesia with AQUI-S. In the present study, the observed increase in blood lactate after sedation but not after confinement in a tub without anesthetic strongly suggests that the presence of an anesthetic agent in the sharks' environment evoked a stress response.

Plasma potassium concentration of Australian swellsharks was significantly higher in sedated animals than in untreated and control animals. Cliff and Thurman (1984) attributed a large increase in extracellular potassium in dusky sharks *Carcharhinus obscurus* after capture and transport to leakage from muscle cells induced by severe intracellular acidosis. The small changes in plasma potassium and lactate levels observed here in comparison with other studies (Cliff and Thurman 1984; Manire et al. 2001) suggest that exposure to the anesthetic did not result in severe intracellular acidosis.

A rapid increase of plasma glucose in response to a stressful event has been observed in teleosts (Barton and Iwama 1991), and exposure to anesthetics generally seems to have the same effect (Gingerich and Drottar 1989; Thomas 1991). However, we did not find elevated circulating glucose levels in Australian swellsharks after exposure to AQUI-S.

Overall, our data suggest that exposure to AQUI-S evokes a minor stress response in Australian swellsharks and that sedation of animals may confound

results of studies investigating aspects of stress physiology in elasmobranchs.

Recovery

Ours is the first attempt to quantify struggling effort of sharks exposed to capture gear and to include such data in quantitative analysis. Chopin et al. (1996) recorded struggling activity of hooked red sea bream *Pagrus major* but only used those records for descriptive purposes. Australian swellsharks seemed to fight much less once entangled, and mean cumulative struggling duration during the 30-min gill-net exposure was significantly lower for Australian swellsharks than for Port Jackson sharks. These differences may be related to species-specific behavior when exposed to a gill net or may be due to the higher ambient water temperature during the Port Jackson experiments, resulting in higher activity levels. Manire et al. (2001) also observed interspecific differences in struggling behavior in carcharhinid shark species exposed to gill-net capture.

The observation that both Port Jackson sharks and Australian swellsharks more or less stopped struggling after the initial thrashing is not surprising. Both species are capable of ventilating their gills by inhaling water through the spiracle and pushing it past their gills using respiratory muscles, a feature found in many bottom-dwelling sharks. This allows them to maintain gill ventilation even when entangled in a net, whereas shark species that rely completely on ram ventilation quickly face asphyxiation if restrained from movement. Since being restrained per se is not life threatening to Port Jackson sharks or Australian swellsharks, conserving energy by remaining still seems to be a reasonable strategy to cope with such a situation. Future work with ram-ventilating sharks should reveal whether this is the case.

The total amount of time spent struggling had no effect on mean ventilation rate during gill-net exposure in either species, and ventilation rate did not change during gill-net exposure. Aside from increasing oxygen uptake, an increased ventilation rate seems to be a behavioral mechanism to counteract respiratory and metabolic acidosis suffered during exhaustive exercise in some elasmobranchs (Heisler 1989; Wood et al. 1990). The elevated ventilation rate observed in Port Jackson sharks during gill-net exposure may therefore reflect some degree of blood acidosis, but the fact that mean ventilation rate was independent of struggling effort indicates that the acid–base balance was not severely affected by struggling activity.

The observed increase in ventilation rate during blood sampling indicates an increased oxygen uptake during an acute stress response to being handled. Ventilation rate seems to be a very sensitive indicator of the momentary state of alert in these sharks and is very easily measured when the sharks are resting on the bottom. Counting the gill beats per minute when a shark is moving is more difficult.

Elevated lactate levels resulting from capture stress have been well documented in sharks (Cliff and Thurman 1984; Wells et al. 1986; Hoffmayer and Parsons 2001; Manire et al. 2001; Moyes et al. 2006; Skomal 2006; Hight et al. 2007). Compared with the mean lactate concentrations reported in those studies, the peak mean values we present here are very low, especially given that repeated blood sampling applied at fairly short intervals (0, 15, 30, and 60 min) within the first hour poststress caused a rise in circulating lactate levels of Port Jackson sharks and thus most likely contributed to the magnitude of the response. This may indicate species-specific differences in the magnitude of the lactate response to capture stress or (more likely) that 30 min of gill-net exposure imposed minor physiological stress on Port Jackson sharks and Australian swellsharks. Maximum plasma lactate concentrations of Australian swellsharks recorded in the present study were slightly less than 2 mmol/L. Further investigation is required to determine whether Australian swellsharks generally do not build up as much lactate when stressed or do not release the lactate accumulating in white muscle cells into the blood stream to the same degree as other species.

Lactate concentrations of both species did not peak until 1–3 h poststress, which is consistent with the results of other studies. Hoffmayer and Parsons (2001) obtained repeated blood samples from Atlantic sharpnose sharks *Rhizoprionodon terraenovae* for 1 h poststress after hook-and-line capture and reported that the increase in circulating lactate concentrations never reached an asymptote during that period. Mean plasma lactate levels of dusky sharks reached a maximum at 3–6 h after capture and confinement (Cliff and Thurman 1984). This delayed increase in circulating lactate levels has been attributed to a slow leakage of lactate accumulating in the intracellular compartment of white muscle cells (Piiper et al. 1972; Milligan and Wood 1986).

Presumably due to logistical challenges associated with obtaining blood samples from animals released in the wild, information on recovery of sharks from capture stress is scarce. Cliff and Thurman (1984) published the only study that monitored blood variables over an extended period of time after capture stress exposure; they found that most variables, including lactate, returned to levels measured in minimally stressed sharks within 24 h. We found that plasma lactate levels of Port Jackson sharks and

Australian swellsharks returned to reference levels measured in untreated sharks at 6–12 h, but peak lactate concentrations in these species were much lower than those measured in dusky sharks (Cliff and Thurman 1984).

Increased levels of plasma potassium, glucose, magnesium, and hematocrit are expected responses of sharks to stress (Cliff and Thurman 1984; Wells et al. 1986; Hoffmayer and Parsons 2001; Manire et al. 2001; Moyes et al. 2006). Some of these variables did change during the 72-h recovery period after stress exposure in the present study, but none of the values measured during experiment 2 were significantly different from reference values measured in untreated sharks. This further supports the assumption that our experimental treatment imposed minor stress on Port Jackson sharks and Australian swellsharks. Similarly, Manire et al. (2001) reported elevated serum phosphate levels in severely stressed sharks after gill-net capture, but plasma phosphate levels of Port Jackson sharks were not elevated immediately after stress in our study. The subsequent decrease in circulating phosphate observed in the present study may be due to restoration of creatine phosphate and ATP stores during recovery (Wood 1991).

Plasma urea levels remained unchanged in both species, and urea concentrations measured in the present study corresponded well with values reported in other publications (Evans and Kormanik 1985; Cooper and Morris 1998; Moyes et al. 2006). No significant changes in plasma levels of chloride or iron were measured in Port Jackson sharks, which is consistent with the results of other studies investigating effects of capture on elasmobranchs (Cliff and Thurman 1984; Manire et al. 2001).

Repeated Blood Sampling

Repeated blood sampling of Port Jackson sharks at short intervals resulted in slightly elevated plasma lactate levels, indicating that the sampling procedure per se imposed some degree of stress on the animals. However, the effects of repeated blood sampling on blood variables of Port Jackson sharks were considerably less than those measured after 30 min of gill-net exposure. We found no effects of repeated blood sampling on circulating levels of lactate in Australian swellsharks exposed to a revised blood sampling regime; this finding suggests that blood sampling at a reduced frequency did not confound results of stress-related blood variables monitored during experiment 2.

Habituation

Our results did not provide any strong evidence for a habituation effect of repeated exposure to 30 min of gill-net capture stress. Barton et al. (1986) demonstrated that multiple exposures to stress at brief intervals can result in cumulative stress responses in Chinook salmon *Oncorhynchus tshawytscha*, but Tort et al. (2001) found that the stress responses of gilthead sea bream *Sparus aurata* subjected to handling and confinement were consistent if recovery periods between exposures were extensive.

None of the monitored blood variable responses of Port Jackson sharks differed between exposures, whereas the plasma glucose and potassium concentrations of Australian swellsharks were significantly higher after some exposures. The differences lacked any pattern to indicate that sharks habituated to our experimental treatment; thus, we ascribe the observed differences to natural variation.

However, we are not convinced that repeated exposure to a more stressful event than 30 min of gill-net exposure would not result in cumulative stress responses in these animals. Moreira and Volpato (2004) demonstrated that teleosts can recall the memory of a previously experienced stressful situation and can react to a conditioned nonstressful stimulus with increased plasma cortisol levels.

In conclusion, we found that light sedation of Australian swellsharks with AQUI-S can affect circulating levels of stress-related blood variables. We therefore recommend avoiding the use of sedation when studying physiological aspects of a stress response in elasmobranchs.

Elevated plasma lactate concentrations, as observed in studies using sharks caught in the wild, suggest that a simulated capture event elicits a physiological stress response in sharks. However, the responses measured in the present study were of much smaller magnitude than those reported in previous studies. Whether this is due to our experimental treatment or is a consequence of the metabolic scope of the two species used in this study requires further investigation. Given that lactate concentrations did not reach their peak until well after the end of the stress exposure, we emphasize the importance of monitoring circulating levels of plasma constituents for several hours or several days post-stress, but we caution that repeated blood sampling may affect stress-related blood variables.

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