

ARTICLE

Short-Term Physiological Disruption and Reflex Impairment in Shortnose Sturgeon Exposed to Catch-and-Release Angling

Daniel P. Struthers, Shannon D. Bower, and Robert J. Lennox

Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

Christine E. Gilroy and Elizabeth C. MacDonald

Department of Biology, Mount Allison University, 63B York Street, Sackville, New Brunswick E4L 1G7, Canada

Steven J. Cooke

Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

Matthew K. Litvak*

Department of Biology, Mount Allison University, 63B York Street, Sackville, New Brunswick E4L 1G7, Canada

Abstract

Sturgeons (Acipenseridae) are the most imperilled group of fishes globally. Yet, many species remain important targets of recreational anglers. In the Saint John River, New Brunswick, Shortnose Sturgeon *Acipenser brevirostrum* is a popular species targeted by recreational anglers. The International Union for the Conservation of Nature has placed Shortnose Sturgeon on the Red List as “Vulnerable,” and therefore this species is afforded protection from harvest in Canada by the federal Species at Risk Act. Here, we evaluated physiological stress using two principal components axes, RC1 and RC2, generated from blood lactate, glucose, and pH, hematocrit, and reflex impairment of sturgeon exposed to exhaustive chase experiments at a hatchery and angled sturgeon that were captured during an annual fall competitive angling event. Physiological indicators of stress increased with holding time for Shortnose Sturgeon transferred into a tank and for sturgeon exposed to chase trials. Circulating metabolite concentrations were not associated with reflex impairment. The odds of reflex impairment increased as a function of air exposure, with a 1-min increase in air exposure increasing the odds of impairment by 1.78. All sturgeon survived hatchery experiments. Most of the wild Shortnose Sturgeon captured in the fishing derby (71%) exhibited reflex impairment, and 38% had superficial injuries such as cuts or wounds. None of these factors were associated with physiological disturbance, although ordinated stress physiology axes were elevated both 1 and 2 h after initial sampling, which was predictive by the holding time prior to weigh-in and measuring fish size. All fish were hooked in the mouth and each was released alive from the derby. Taken together the evidence suggests that Shortnose Sturgeon are resilient to recreational angling interactions, yet managers could use this information to improve best practices of catch-and-release angling.

*Corresponding author: mlitvak@mta.ca
Received July 8, 2017; accepted June 28, 2018

The Acipenseriformes are a primitive order of megafunal bony fishes comprising the paddlefishes (Polyodontidae) and sturgeons (Acipenseridae). This group of species is considered the most frequently imperilled with extinction (Haxton and Cano 2016), and 25 of the 27 sturgeon species are listed by the International Union for the Conservation of Nature (IUCN). Imperilment of sturgeons has predominantly been associated with habitat fragmentation, which restricts the ability of sturgeons to migrate to spawning grounds (Birstein et al. 1997; Haxton and Cano 2016), as well as overfishing, which includes unprecedented levels of illegal harvest (Birstein et al. 1997; Pikitch et al. 2005). These fisheries often focus on areas or times at which populations congregate for foraging, overwintering, and spawning. What was once characterized as a highly exploitative fishery sector for harvesting meat and roe, sturgeon fisheries are increasingly shifting towards recreational fisheries in order to maintain the economic and cultural benefits afforded by sturgeon fishing (McLean et al. 2016); yet, research on the response of sturgeons to recreational angling is relatively scarce (Hühn and Arlinghaus 2011).

Although the broad benefits of recreational fisheries are well studied (see review paper by Arlinghaus et al. 2007), there has recently been greater scrutiny afforded to recreational fisheries interactions focused on imperiled species (Cooke et al. 2016). While legislation on species at risk generally affords protection from exploitation for sturgeons, recreational angling is often permitted where commercial harvesting is restricted, and recovery plans have been developed (Cooke et al. 2016). Indeed, fish captured by recreational fishers may die after angling, negating the potential benefits of catch and release (Cooke and Schramm 2007), often after the fish has been released and the angler has concluded that the fish has survived. Moreover, fish must return to the common population without enduring sublethal effects that negatively affect lifetime fitness (e.g., elevated risk of postrelease predation or reduced reproductive potential; see Wilson et al. 2014 for discussion of sublethal effects in fisheries). Responses to recreational angling often depend on biological and environmental contexts in which the fishery takes place (Arlinghaus et al. 2007; McLean et al. 2016; Raby et al. 2016). Characteristics of catch-and-release angling episodes, such as air exposure, fight duration, and water temperature, can cause unrecoverable physiological disruptions (Cooke et al. 2013), but scientific evaluation of best practices can provide information to minimize the impact of recreational fisheries on fish populations (Brownscombe et al. 2017).

There are many approaches to investigate the consequences of catch-and-release angling on fishes (Cooke et al. 2013). Inferences about immediate and delayed mortality can be evaluated by assessing the vitality and physiology of fishes after being captured by rod and reel.

Reflexes are involuntary movements that are initiated by a stimulus and provide information about the vitality of an animal, because reflexes become impaired in animals subjected to stress or exhaustion. In some species, the presence or absence of reflexes has been validated as a predictor of survival (Davis 2007; Raby et al. 2012). Furthermore, physiological disturbances in fishes after an angling episode can be assessed by measuring metabolites within the circulatory system (Wedemeyer and Wydoski 2008; Cooke et al. 2013). Elevated primary (e.g., blood cortisol) and secondary physiology responses (e.g., blood glucose and lactate) can result in greater susceptibility of fish to disease or depredation (Wedemeyer and Wydoski 2008) or may alter behavior (Cooke et al. 2000; Brownscombe et al. 2013). Particularly during live-release fishing derbies, when fish are frequently captured and extensively handled for weigh-in, these techniques can be applied to rapidly assess a large number of fish to investigate the consequences of angling practices of the targeted fishery (Lennox et al. 2015).

Catch-and-release angling of Shortnose Sturgeon *Acipenser brevirostrum* is popular in the Saint John River, New Brunswick. This population is listed as vulnerable by the IUCN, as well as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2015). The physiological responses of sturgeons to stressors such as critical thermal tolerances (Ziegeweid et al. 2008; Spear and Kieffer 2016), hypoxia (Baker et al. 2005a), and exhaustive exercise (Baker et al. 2005b) have been characterized; however, there is a paucity of information detailing the response of sturgeons to acute angling stressors. Here we evaluated the potential impacts of recreational angling on Shortnose Sturgeon. We evaluated exhaustive chase and air-exposure experiments using hatchery fish, as well as in situ angling events (i.e., assessing fish status at a derby) to evaluate reflex and physiological responses. The results will be used to provide managers with information to improve upon current recovery plans with regards to best practices for catch-and-release angling of Shortnose Sturgeon.

METHODS

Hatchery experiments.—Cultured Shortnose Sturgeon (F₁ generation) were sampled at the Mactaquac Fish Culture Station, New Brunswick, to evaluate physiology and reflex impairment prior to experiencing an exhaustive chase episode. This method for developing a control was considered appropriate here because the cultured fish were progeny from the Saint John River population. Furthermore, determining a baseline from wild fish is difficult to achieve without introducing handling biases in the data (Pankhurst 2011). Past work has suggested cultured Shortnose Sturgeon may be used as surrogates for wild

conspecifics based on similarities in habitat use, movement, and thermal preferences between wild and cultured fish (Trested et al. 2011). Additionally, water temperatures were similar between the fishing derby location (12.5°C) and Mactaquac Fish Culture Station (14°C). The sampled cohort was randomly selected from a population of approximately 550 individuals that were being held in a large (8 × 8 × 2.5 m) cement circulating tank.

First, 32 individuals (FL, 67 ± 13 cm [mean ± SD]) were netted from the captive population and placed in the circular holding tank. Four groups of eight sturgeon each were administered air exposure of either 0, 2, 5, or 10 min in duration. After air exposure, each fish was reflex tested, except for the eight individuals in the 0-min air exposure group, which were immediately reflex tested without air exposure treatment.

Sixteen Shortnose Sturgeon (FL, 69 ± 7.8 cm) were removed from the cultured population for serial blood sampling following a standardized exercise stressor. Measurement of blood metabolites and assessment of reflex actions proceeded as described later in the Methods. After baseline sampling, each of the 16 sturgeon were tagged using numbered flagging tape that was tied loosely around the caudal peduncle to identify the individuals. These fish were placed into a 3-m-diameter, circular, flow-through tank (flow, 36 L/s) and immediately forced into continual exercise by manually chasing them for a duration of 5 min to simulate the level of exhaustion that they would experience during angling. After the chase, each fish was removed from the tank to extract a second blood sample and undergo a second reflex assessment test. Individuals were then released back into the holding tank and resampled on an hourly basis for four consecutive hours.

A control group of Shortnose Sturgeon ($n = 8$; FL, 68 ± 13 cm) were placed into the circular holding tank but were not exposed to the experimental treatments designed to simulate angling and were sampled on the same schedule as those subjected to the exhaustive chase trials. This experiment was conducted to evaluate the effects of tank confinement and repeated blood draws in the hatchery and fishing derby experiments. The responses from these fish were then used to adjust the physiological outcomes in the exhaustive exercise and fishing derby experiments. In total, approximately ≤6 mL of blood tissue were collected during these experiments, and samples did not exceed 0.1% of the body weight (i.e., 1 mL/kg) given that the sampled fish weighed greater than 7 kg. This threshold is a guideline recommended by the Canadian Council of Animal Care for collecting blood samples from fish (Batt et al. 2005).

Fishing derby.—Recreational fishing data were collected during a fishing derby that commenced over a 7-h period (0800–1500 hours) on October 4, 2015, on the Kennebecasis River, which is a tributary of the Saint John River.

Most of the fishing effort was confined to a small section of the river system located near the Kennebecasis–Hammond River confluence. Previous studies have identified this site as an important aggregation site for Shortnose Sturgeon where they forage and overwinter (Li et al. 2007; Usvyatsov et al. 2012).

Sturgeon were captured by derby participants with medium- and heavy-action spinning rods using baited J-hooks. Fish were fought by anglers from boats and held in water for weigh-in using a rope tethered around the caudal peduncle and fastened to the side of the fishing vessel. The field crew intercepted the angled fish ($n = 33$) in an ad hoc manner by boating continuously through the area where derby participants were fishing. Upon retrieval, each fish underwent a blood draw and reflex impairment assessment using the methods described below. The derby participants were surveyed to determine the fight time (min) and holding duration alongside the boat prior to the arrival of the field crew (min) for each fish that was caught. Owing to difficulties establishing an accurate continuous variable for angling duration, it was treated as a factor with two levels, categorized as either *brief* (i.e., <2 min) or *long* (i.e., ≥2 min). Fish were measured (cm FL), and the hooking location was categorized as either hooked in the mouth or foul hooked. Fish were assessed for angling injury by surveying the body for fresh lacerations from the hooking and fishing line abrasions; injuries were categorized as none, some, or substantial.

The first 21 captured fish were transferred to two holding pens (dimensions, 2.5 × 1.2 × 1.2 m) made from high-density, polyethylene, flat-mesh netting (Vexar) to evaluate postangling recovery. The holding pens were placed on the boundary of the main channel to allow for continual flow of river water through the netting. Each fish was individually marked with flagging tape tied loosely around the caudal peduncle. An identification (ID) number and the time of initial sampling were written on the tape. Fish were placed in the net pens and sampled twice for reflex action mortality predictors (RAMPs) and blood using the same methods described below at approximately 1 and 2 h after the initial sample. Each fish was tagged with a T-bar anchor tag (Floy) and released 500 m downstream from the fishing derby area. In total, approximately ≤3 mL of blood were collected during these experiments, and samples did not exceed 0.1% of the body weight (i.e., 1 mL/kg) given that the sampled fish weighed >7 kg.

Physiological and reflex assessment protocol.—The physiology of Shortnose Sturgeon was evaluated from the exhaustive exercise trials and recreational fishing derby by measuring blood plasma chemistry and reflex actions following standardized stressors. Blood samples of 1 mL were drawn for each sampled time point by venipuncture on the caudal peduncle with an 18-gauge needle and 3-mL

heparinized barrel (Vacutainer) from sturgeon held supine in a padded trough. The needle was inserted into the caudal vasculature ventrally at the anterior insertion of the anal fin. Pressure was applied to the puncture site once the needle was removed, which eliminated any passive blood loss.

Whole blood was processed using point-of-care devices to measure plasma lactate (mmol/L; Lactate Pro LT-1710, Akray, Kyoto, Japan) and whole blood glucose (mmol/L; AccuChek Compact Plus, Roche Diagnostics, Basel, Switzerland). The use of these devices has been previously validated and reviewed for their use on fish (Cooke et al. 2008; Stoot et al. 2014) and have been used on sturgeon in previous field studies (Beardsall et al. 2013; McLean et al. 2016). Although the actual values might vary slightly from laboratory-based assays, the relative differences among individuals and treatments reflect meaningful differences in circulating blood metabolites. Hematocrit (ratio of the volume of packed red blood cells to the volume of whole blood) was determined at each time point for each sampled fish. Whole blood of each fish was drawn into three microhematocrit capillary tubes (75 mm length). One end of each tube was sealed with Critoseal, and the tube was then centrifuged for 10 min at 12,000 rpm (Sorvall Legend Micro 17 centrifuge, Hematocrit rotor, Thermo Scientific). Hematocrit is measured by comparing the results with the reading chart provided by Thermo Scientific; after centrifugation capillary tubes are aligned on the chart to determine the percentage of packed red blood cells. The pH of blood plasma was measured in triplicate for each fish (SevenCompact S220 pH/ion meter, Mettler-Toledo).

Reflexes were assessed using RAMP, (Davis 2010). The following reflex actions were assessed for each sampled fish: (1) *body flex*: fish was held in the middle of its body with both hands at the water surface and the investigator determined whether the fish flexed its body, (2) *equilibrium*: fish were held supine at the water surface to test for pronation within 3 s of being released, (3) *ventilation*: the investigator determined whether the fish had regular ventilation by observing whether the fish exhibited consistent frequency of opercular beats while held in water for 10 s, and (4) *tail grab*: the investigator determined the presence of burst swimming attempts by the fish within 3 s of grabbing the caudal peduncle prior to release. Reaction times for each reflex action was based on a cohort of untreated control fish ($n = 8$). For each of the four RAMP parameters, a binary response was recorded as either present or absent. For each sampled fish, impairment was calculated based on the overall outcome of the four RAMP parameters. A fish was considered impaired (i.e., *yes* = 1) if one or more of the RAMP parameters was absent. Alternatively, a fish was considered unimpaired (i.e., *no* = 0) if all reflexes were present.

Analyses.—For the air-exposed sturgeon, we modelled air exposure as a continuous variable and FL with a binary reflex impairment response using the glm function in R (R Core Team 2014), and we tested model fit with the Hosmer–Lemeshow goodness-of-fit test, implemented by the `hoslem_gof` function in the `sjstats` package (Lüdtke 2017).

Prior to analyzing the physiological response to the exhaustive chase and angling, the data were adjusted to account for the physiological effect of confining fish to holding tanks and serial blood draws on the individual sturgeon (raw data summarized in the Supplement available in the online version of this article). The values of the blood physiology variables for the physiological experiments were corrected using the control group from the hatchery experiments (i.e., not submitted to exhaustive chase). The mean values were calculated for each blood physiology response at each sample time point for the fish in the control cohort. Next, the responses of the physiological experiments were then adjusted by subtracting the mean values from the control cohort for each corresponding sampling period. This adjusted the experimental values by accounting for potential handling effects (i.e., repeated blood draws) and confinement stress of the fish that were being sampled. Ideally, a random block experimental design would be developed where individuals would be sampled once at each sampling time. However, due to logistical restrictions (holding tank availability and limited time available to complete experimental work) it was not possible to run this design.

Analyses for the circulating blood metabolites and reflex actions of the sturgeon was conducted for the exhaustive chase and fishing derby experiments. There were some missing values in the blood physiology data, and rather than delete these fish from the data set, we opted to impute them using the `imputePCA` function in the `missMDA` R package (Josse and Husson 2016). This generated a data set without any missing values. The four adjusted physiology variables—circulating lactate and glucose, pH, and hematocrit—were placed into a varimax rotated principal components analysis (PCA) using the principal function in the `psych` package (Revelle 2017) and the `GPArotation` package (Bernaards and Jennrich 2005) to deal with collinearity in these responses. The varimax rotated PCA was performed on the four blood physiology variables measured in sturgeon from the angling simulation and derby experiments. We used the first two rotated principal components axes (RC1 and RC2) as multivariate response variables that captured substantial variation in the blood physiology. These two axes explained 76% of the variation in blood physiology, with the RC1 axis positively loaded with lactate and negatively loaded with pH, and the RC2 axis positively loaded with glucose and negatively with hematocrit (see Table 1).

TABLE 1. Parameters for the varimax rotated principal component analysis (PCA) that was performed on the four blood physiology variables of the Shortnose Sturgeon angling simulation and derby experiments.

PCA parameters	RC1	RC2
SS loadings	1.81	1.21
Proportion variance	0.45	0.3
Cumulative variance	0.45	0.76
Proportion explained	0.6	0.4
Cumulative proportion	0.6	1

To investigate the relationship between physiological status and reflex impairment, we modelled binary reflex impairment with the two rotated principal components axes representing physiological status as fixed effects. Model performance was assessed with the Hosmer–Lemeshow test. To determine whether holding stress affected the physiological stress indicators of the sturgeon, RC1 and RC2 axes were both modeled by linear mixed effects modelling with individual ID as a random intercept and sampling time point and reflex impairment (binary) as fixed effects with the lme function from the nlme package (Pinheiro et al. 2015) in R. We used a similar approach to determine the effects of a standardized exercise stressor (i.e., angling simulation) on blood physiology; again, we constructed two linear mixed effects models, one each for RC1 and RC2 ordinated physiological variables, against the sampling time point and reflex impairment scores, with the individual ID of each fish incorporated as a random intercept. Using mixed-effects models allows the regression intercept change for each individual fish thereby accounting for the nested data structure, which was vital for our analyses given that the same individuals were sampled several times.

For fish captured in the fishing derby, the values of the RC1 and RC2 physiological response variables were compared at the three sampling time points using mixed-effects regression incorporating individual ID as a random intercept and the sampling time point as a fixed effect. For sturgeon sampled in the derby, we also modelled the influence of factors related to the angling event: the fish size, angling duration (either short or long), time the fish was held boat side prior to sampling (i.e., holding time), and presence of injuries on the two RC physiological response variables using generalized least-squares regression, gls, from the nlme package (Pinheiro et al. 2015) in R. Models were built for samples taken both after capture ($n = 33$) and before release of a subset cohort of fish held for 2 h ($n = 21$). Generalized least-squares regression was preferred to linear regression because it allows the incorporation of variance structures to account for heteroskedasticity of residuals (Pinheiro et al. 2015). We

also fit the same set of angling variables to reflex impairment scores taken after the sturgeon was retrieved from the anglers by using a generalized linear model, with the glm function in R, to test whether reflex impairment was predictable.

RESULTS

Hatchery Experiments

Air exposure significantly increased reflex impairment of Shortnose Sturgeon ($z = 2.76$, $P = 0.01$; Hosmer–Lemeshow goodness-of-fit test: $\chi^2 = 8.94$, $df = 8$, $P = 0.35$). Odds of reflex impairment increased by 1.78 for 1-min increases in air-exposure intervals. The size of the fish (i.e., FL) was not a significant predictor of reflex impairment ($z = -1.13$, $P = 0.26$).

Only the second ordinated blood physiology variable was related to reflex impairment (RC1: $z = 0.72$, $P = 0.47$; RC2: $z = -2.63$, $P = 0.0090$) and provided a low to moderate model fit (Hosmer–Lemeshow goodness-of-fit test: $\chi^2 = 9.13$, $df = 8$, $P = 0.33$). Reflex impairment scores were incorporated into subsequent models to investigate the relationships for individual tests. For the cohort that experienced exhaustive chase, RC1 scores were elevated at all blood sampling time points following treatment compared with the baseline. (RC1: all $t > 2.00$, all $P < 0.05$; Figure 1). As for RC2 scores, they were elevated from the baseline up to 2 h after the treatment, then returned to baseline values (Figure 1). Reflex impairment score at the sampling time point was not predictive of the physiological stress response (RC1: $t = -0.97$, $P = 0.33$; RC2: $t = -0.11$, $P = 0.91$).

Derby Experiments

There were 33 Shortnose Sturgeon captured and sampled in the fishing derby (FL = 76 ± 14 cm [mean = SD]). During the fishing derby, the boat side holding duration ranged between 0 and 90 min with an average of 17 min (± 22 SD). All fish were hooked in the mouth, and none were foul hooked. Eight (24%) of the angled fish were held for >30 min by derby participants. When fish were grouped based on the angling duration (short, ≤ 2 min; long, > 2 min), there were 23 fish landed after a short duration and 11 fish that were retrieved after a long duration. Sturgeon of greater length fought for a longer duration than those of smaller size ($t_{1, 32} = 4.33$, $P < 0.01$). Of the sampled fish from the fishing derby, 13 (38%) exhibited superficial injuries upon collection from the derby participants.

The RC1 and RC2 values were significantly different at the 1-h (RC1: $t = 3.27$, $P < 0.01$; RC2: $t = -2.13$, $P < 0.05$) and 2-h (RC1: $t = 5.21$, $P < 0.01$; RC2: $t = -4.83$, $P < 0.01$) sampling time points compared with the initial sample. Upon capture, the first ordinated blood

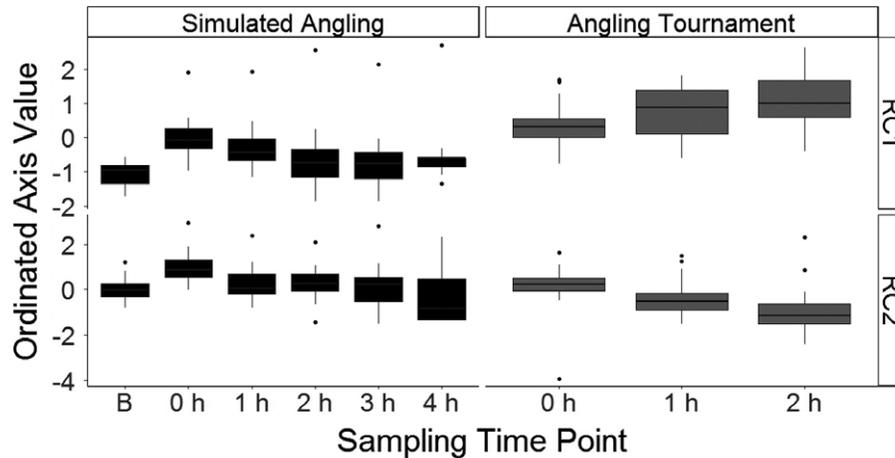


FIGURE 1. Boxplots of ordinated physiological statuses, RC1 and RC2 (see Methods), by experiment and treatment. Outliers are shown as dots. The two experiments in this study were (1) exhaustive chase, in which hatchery-origin Shortnose Sturgeon were chased for a 5-min period and blood sampled immediately after (0 h) and for four subsequent hours, and (2) an angling derby, in which wild Shortnose Sturgeon were captured by anglers and sampled by biologists immediately after capture and for two subsequent hours. The “B” sampling time point signifies sampling for baseline conditions prior to induction of stress.

physiology axis, RC1, was influenced only by the duration of boat side holding ($t = 3.80$, $P < 0.01$; see Table 2 for full model). For the second ordinated blood physiology dependent variable, RC2, fish length was a significant predictor ($t = 3.27$, $P < 0.01$; Table 2) and duration of boat side holding was a nearly significant predictor ($t = 1.84$, $P = 0.08$; Table 2) upon initial sampling after the angling event.

Furthermore, hooking injury was close to significant for predicting physiological responses of the RC1 axis (i.e., lactate and pH) after holding the sturgeon for ~2 h postcapture ($t = 2.00$, $P = 0.06$; Table 3). No fixed model terms were found to be predictive by way of the RC2 axis hematological responses (i.e., glucose and hematocrit) when sampled ~2 h postcapture (Table 3).

Neither length, injury, angling duration, nor holding time influenced observations of reflex impairment among the sturgeon that were angled during the fishing derby (see Table 4; Hosmer–Lemeshow goodness-of-fit test: $\chi^2 = 4.43$, $df = 8$, $P = 0.82$). There was no immediate mortality of sturgeon brought to the boat by anglers or delayed mortality of sturgeon held in net pens.

DISCUSSION

Shortnose Sturgeon is a social species (Buckley and Kynard 1981) and is often observed in large aggregations at foraging (Kynard 1997) and overwintering habitats (Li et al. 2007; Usvyatsov et al. 2012). Once an aggregation site is identified by fishers, the angler catch rates can increase dramatically as Shortnose Sturgeon are highly vulnerable to organic bait. Furthermore, Shortnose Sturgeon can be incidentally caught in shad, salmon, Striped

TABLE 2. Generalized least-squares regression output (gls) of wild Shortnose Sturgeon blood physiology, ordinated axes RC1 and RC2 (see Methods). Fish were sampled ($n = 33$) immediately after being captured by recreational anglers in a Shortnose Sturgeon fishing derby. Two factors, each with two levels, were included in the model: the angling duration (brief or long) and injury (absent or present). Baseline values for the factors are shown in parentheses.

Dependent variable	Fixed effect	Value \pm SE	t -value	P -value
RC1	(Intercept)	-0.30 ± 0.81	-0.37	0.71
	Length	$<0.01 \pm <0.01$	0.37	0.78
	Holding	$0.02 \pm <0.01$	3.80	<0.01
	Angling (brief)	0.10 ± 0.24	0.42	0.68
	Injury (present)	-0.03 ± 0.18	-0.01	0.99
RC2	(Intercept)	-3.42 ± 1.16	-2.94	<0.01
	Length	0.04 ± 0.01	3.27	<0.01
	Holding	$0.01 \pm <0.01$	1.84	0.08
	Angling (brief)	0.32 ± 0.34	0.92	0.36
	Injury (present)	$<0.01 \pm 0.26$	0.02	0.99

Bass *Morone saxatilis*, and Alewife *Alosa pseudoharengus* fisheries in the Saint John River and estuary (NOAA 1998; COSEWIC 2015). Although popular targets of fishing with dedicated derbys for catching the species, there is limited information that addresses stress physiology and reflex action for sturgeon related to catch-and-release fishing. Shortnose Sturgeon is a species of special concern in

TABLE 3. Generalized least-squares regression output (gls) of wild Shortnose Sturgeon blood physiology, ordinated axes RC1 and RC2 (see Methods). Fish were sampled after capture by recreational anglers in a Shortnose Sturgeon fishing derby ($n = 21$), and again after being held for ~2 h in a net pen. Two factors, each with two levels, were included in the model: the angling duration (brief or long) and injury (absent or present). Baseline values for the factors in the model are shown in parentheses.

Dependent variable	Fixed effect	Estimate \pm SE	<i>t</i> -value	<i>P</i> -value
RC1	(Intercept)	-1.50 \pm 1.45	-1.03	0.32
	Length	0.03 \pm 0.01	1.76	0.10
	Holding	<0.01 \pm 0.01	0.19	0.85
	Angling (brief)	0.24 \pm 0.47	0.51	0.62
	Injury (present)	0.72 \pm 0.36	2.00	0.06
RC2	(Intercept)	-2.15 \pm 2.34	-0.92	0.37
	Length	0.01 \pm 0.02	0.56	0.58
	Holding	0.03 \pm 0.01	0.23	0.82
	Angling (brief)	0.15 \pm 0.75	0.19	0.85
	Injury (present)	-0.21 \pm 0.58	-0.36	0.73

TABLE 4. Generalized linear model (glm) of reflex impairment in wild Shortnose Sturgeon sampled ($n = 33$) after being captured by recreational anglers in a Shortnose Sturgeon fishing derby. Two factors, each with two levels, were included in the model: the angling duration (brief or long) and injury (absent or present). Baseline values for the factors are shown in parentheses.

Fixed effect	Estimate \pm SE	<i>t</i> -value	<i>P</i> -value
(Intercept)	-0.65 \pm 2.08	-0.31	0.76
Length	0.01 \pm 0.02	0.72	0.47
Holding	-0.01 \pm 0.01	-0.62	0.54
Angling (brief)	-0.19 \pm 0.65	-0.28	0.78
Injury (present)	-0.01 \pm 0.49	-0.02	0.99

Canadian waters (COSEWIC 2015), yet the population within the Saint John River watershed remains open to recreational fishing. Recreational fishing may hinder normal behavior by causing physical injuries (Muoneke and Childress 1994) or by activating anaerobic metabolism leading to physiological stress that can reduce fitness (Kieffer 2000). Here we evaluated secondary stress responses (i.e., glucose, lactate) and reflex actions (i.e., RAMP) to rapidly assess Shortnose Sturgeon when captured by recreational anglers and monitored their short-term recovery (i.e., 2 h postcapture).

Angling results in increases of circulating metabolites characteristic of the secondary stress response in fish (Barton 2002). Indeed, the ordinated physiological axes in the

hatchery and wild sturgeon captured a substantial proportion of the variation in blood physiology (i.e., 76%). Lactic acid is produced by the white muscles during anaerobic exercise and dissociates to form lactate anions and a metabolic proton, which leak into the blood and reduce the pH; however, pH can also drop because of hypercapnia. Glucose is frequently used as a proxy for cortisol (Pankhurst 2011), the primary glucocorticoid stress hormone in fish, although there are multiple endogenous processes that explain variation in circulating glucose (Lawrence et al. 2018). Here, we found significant increases in stress indicators of sturgeon consistently after interventions, whether measuring tank effects, angling simulations, or derby fishing samples. In forced exercise experiments (Baker et al. 2005b), Shortnose Sturgeon were observed to elevate primary (i.e., cortisol) and secondary (i.e., lactate, glucose) stress metabolites in the blood tissue. However, sturgeons have been reported to have generally slow or small endocrine responses to stressors, having lower maximum thresholds relative to values found in teleost fishes and recover quickly (Kieffer 2000). The size of the fish was predictive of physiological effects for sturgeon that were caught in the fishing derby. Based on the RC2 axis, larger sturgeon were more likely to have elevated physiological responses (i.e., glucose and hematocrit) compared with those that were smaller in size. Smaller and/or younger individuals may be able to recover more readily from the angling event compared with larger and/or older sturgeon, although in the literature, the size of fish has not been found to be a significant predictor of hematological stress response variables (Beyea et al. 2005).

Boat-side holding time by the anglers led to an increase in the physiological disturbance, which may have been an artifact of the sampling timing given that the leakage of lactate from the white muscle into the blood is slow, and the peak of the physiological stress response is delayed relative to the onset of a stressor (Cooke et al. 2013; Lawrence et al. 2018). In support of this, the subset of sturgeon that were held for 2 h following capture exhibited a significant elevation of the physiological stress response after 1 and 2 h compared with the initial sturgeon sampled. However, since recovery was only monitored for 2 h postcapture this limits the temporal scope for assessing the time to full recovery after an angling episode. This is similar to the physiological status in the RC1 axis of the hatchery sturgeon that were sampled hourly for 5 h after simulated exercise but differs from other work with Shortnose Sturgeon (Baker et al. 2005b), which measured observed recovery from exercise within 2 h, albeit with younger and smaller fish (100 g). This is unlikely attributable to social stress given that the species aggregates (Buckley and Kynard 1981).

Sturgeon can respond to stressors with a lower primary and secondary stress response than teleosts (Kieffer 2000;

Keiffer et al. 2001) and are known to be less responsive to stressful conditions such as hypoxia (McDonald and Milligan 1992) and forced activity (Baker et al. 2005b). Although sturgeon may not respond to stressful conditions in the same way as teleost fishes do, there was some evidence that holding fish with a rope tether for weigh-in was correlated to secondary stress levels in Shortnose Sturgeon by way of the RC1 axis of sturgeon immediately after capture. Holding studies have found that tethered Lake Sturgeon *Acipenser fulvescens* experienced greater stress and mortality rates than those that were held in net pens (Axelsen and Mauger 1993), and in general our findings support avoiding holding fish by rope tethers for prolonged periods to reduce physiological stress.

Reflex impairment correlated poorly with physiological stress indicators. On average, impairment occurred more frequently for angled fish than for the control group yet was found to be similar between angling duration levels (i.e., short versus long). However, the odds of reflex impairment did increase with longer intervals of air exposure, which is consistent with other species that lose vital function as oxygen circulation to organs and tissues declines (Cook et al. 2015). Reflex impairment was not predicted by hooking injury or the angling holding duration. Reflex impairment has been related to behavioral impairment and mortality of released fishes (Davis 2010; Raby et al. 2012; Brownscombe et al. 2013). However, this body of literature is typically directed towards teleost fishes rather than acipenserids, which have a less developed metabolic system due to its evolutionary history (Kieffer 2000; Kieffer et al. 2001; Baker et al. 2005a). Indeed, impairment frequency appeared to decline with recovery time, while physiological stress increased as fish were held to recover. This suggests that although reflex impairment may be an indicator of vitality for Shortnose Sturgeon, it should be avoided as a predictor of physiological stress, which is expected based on the trajectory of blood-based physiological metabolites following exposure to a stressor.

Our observations indicated that hooking injuries did not influence the reflex actions of the sturgeon, but weakly influenced their blood physiology (RC1: lactate and pH) after 2 h of holding in the net pens. Based on communications with recreational anglers, it was expected that foul hooking would occur somewhat frequently because of the active foraging behavior exhibited by Shortnose Sturgeon, which use their barbels and protractible lips to locate and identify organic food items on the substrate before taking the items into their mouths. Surprisingly, none of the angled fish were observed to be foul hooked in the rostrum, head, or body when captured during the fishing derby. However, 38% of the captured fish experienced injury from the angling episode. These injuries were commonly characterized as minor tearing of the mouth from the hook and line abrasions on the body and fins. Sturgeon may be more

susceptible to these types of injuries because they lack scales such as those found on teleosts and holosteans. Hooking injuries that cause bleeding and tearing of the fleshy mouth and lips could hinder feeding success, behavior, or cause infection. Hook style and bait selection have been found to influence hooking injury in salmonids (Bendock and Alexandersdottir 1993; Meka 2004) and black basses (Cooke et al. 2003) when recreationally fished, although these fish have terminal mouths and feeding modes that are not at all comparable with that of sturgeons. Hooking injury is considered a key factor in postrelease mortality of angled fishes (Muoneke and Childress 1994).

To limit disturbance to the fish and maximize sustainability of these recreational fisheries, anglers, especially those participating in live-release derbies, should strive to limit holding durations of sturgeon. As with most fish, it appears that returning a sturgeon to the water rapidly provides the best chance for them to recover and return to the common population. Anglers should also not attempt to hold fish in an effort to recover the captured fish since glucose and lactate values both increased with holding duration. Moreover, derby organizers should aim to implement rules and regulations that will reduce or eliminate the need to tether sturgeon next to boats. Alternatives may be onboard holding tanks or even mesh holding pens in which sturgeon can swim freely; however, given that we observed a holding stress response for fish simply transferred to net pens, even these alternatives should be minimized where possible. Ideally, fish measurements should be recorded immediately while the fish is submerged in water and released thereafter. Anglers and derby organizers that are educated about best practices can maintain these culturally notable events without significant harm to individual fish or the population.

Shortnose Sturgeon are targeted on the Saint John River basin but may also be inadvertently hooked by anglers who are targeting other recreationally valued species, such as Striped Bass, Atlantic Salmon *Salmo salar*, Alewife, and shad, which co-occur with Shortnose Sturgeon through their estuarine range. Although sturgeon have a lower physiological response to stressors than do teleosts, there is variability in physiology amongst sturgeon species (Baker et al. 2005a). Further work is recommended on sturgeon populations that are recreationally fished, with emphasis on those that are at greater risk. Future studies could help to improve upon the best practices for sturgeon fishing by quantifying injury frequency and severity of injury associated with terminal gear selection (i.e., hook type, rigging method).

ACKNOWLEDGMENTS

We acknowledge the Litvak Lab for their assistance with data collection efforts during the fishing derby and at the

Mactaquac Fish Culture Station. S.J.C. and M.K.L. are both funded through the Discovery Grant program by the Natural Sciences and Engineering Research Council of Canada and Habitat Stewardship Environment Canada Species at Risk Grant (HSP-7448). S.J.C. is also supported through the Canadian Research Chairs Program, and M.K.L. received funding from the New Brunswick Wildlife Trust Fund, the Canadian Foundation for Innovation, and the New Brunswick Innovation Fund. This work would not have been possible without the kind assistance of the staff at the Mactaquac Biodiversity Centre. All experimental manipulations performed during this study were conducted in accordance with Canadian Council of Animal Care regulations under permit number B13-02 (file 100105). There is no conflict of interest declared in this article.

REFERENCES

- Arlinghaus, R., S. J. Cooke, J. Lyman, D. Policansky, A. Schwab, C. Suski, S. G. Sutton, and E. B. Thorstad. 2007. Understanding the complexity of catch-and-release in recreational fishing: an integrative synthesis of global knowledge from historical, ethical, social, and biological perspectives. *Reviews in Fisheries Science* 15:75–167.
- Axelsen, F., and A. Mauger. 1993. Sturgeon holding cage trials on Lake Opawica and Lake Lichen. Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Direction de la Recherche Scientifique et Technique Document Recherche 93/06, Quebec.
- Baker, D. W., A. M. Wood, and J. D. Kieffer. 2005a. Juvenile Atlantic and Shortnose sturgeons (family: Acipenseridae) have different hematological responses to acute environmental hypoxia. *Physiological and Biochemical Zoology* 78:916–925.
- Baker, D. W., A. M. Wood, M. K. Litvak, and J. D. Kieffer. 2005b. Haematology of juvenile *Acipenser oxyrinchus* and *Acipenser brevirostrum* at rest and following forced activity. *Journal of Fish Biology* 66:208–221.
- Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* 42:517–525.
- Batt, J., K. Bennett-Steward, C. Couturier, L. Hammell, C. Harvey-Clark, H. Kreiberg, and G. Griffin. 2005. CCAC guidelines on: the care and use of fish in research, teaching, and testing. Canadian Council on Animal Care, Ottawa.
- Beardsall, J. W., M. F. McLean, S. J. Cooke, B. C. Wilson, M. J. Dadsell, A. M. Redden, and M. J. W. Stokesbury. 2013. Consequences of incidental otter trawl capture on the survival and physiological condition of threatened Atlantic Sturgeon. *Transactions of the American Fisheries Society* 142:1202–1214.
- Bendock, T., and M. Alexandersdottir. 1993. Hooking mortality of Chinook Salmon released in the Kenai River, Alaska. *North American Journal of Fisheries Management* 13:540–549.
- Bernaards, C. A., and R. I. Jennrich. 2005. Gradient projection algorithms and software for arbitrary rotation criteria in factor analysis. *Educational and Psychological Measurement* 65:676–696.
- Beyea, M. M., T. J. Benfey, and J. D. Kieffer. 2005. Hematology and stress physiology of juvenile diploid and triploid Shortnose Sturgeon (*Acipenser brevirostrum*). *Fish Physiology and Biochemistry* 31:303–313.
- Birstein, V. J., W. E. Bemis, and J. R. Waldman. 1997. The threatened status of acipenseriform species: a summary. Pages 427–435 in V. J. Birstein, J. R. Waldman, and W. E. Bemis, editors. *Sturgeon biodiversity and conservation*. Kluwer Academic Publishers, New York.
- Brownscombe, J. W., A. J. Danylchuk, J. M. Chapman, L. F. Gutowsky, and S. J. Cooke. 2017. Best practices for catch-and-release recreational fisheries—angling tools and tactics. *Fisheries Research* 186:693–705.
- Brownscombe, J. W., J. D. Thiem, C. Hatry, F. Cull, C. R. Haak, A. J. Danylchuk, and S. J. Cooke. 2013. Recovery bags reduce post-release impairments in locomotory activity and behavior of bonefish (*Albula* spp.) following exposure to angling-related stressors. *Journal of Experimental Marine Biology and Ecology* 440:207–215.
- Buckley, J., and B. Kynard. 1981. Spawning and rearing of Shortnose Sturgeon from the Connecticut River. *Progressive Fish-Culturist* 43:74–76.
- Cook, K. V., R. J. Lennox, S. G. Hinch, and S. J. Cooke. 2015. Fish out of water: how much air is too much? *Fisheries* 40:452–461.
- Cooke, S. J., M. R. Donaldson, C. M. O'Connor, G. D. Raby, R. Arlinghaus, A. J. Danylchuk, K. C. Hanson, S. G. Hinch, T. D. Clark, D. A. Patterson, and C. D. Suski. 2013. The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation and relevance to stakeholders. *Fisheries Management and Ecology* 20:268–287.
- Cooke, S. J., Z. S. Hogan, P. A. Butcher, M. J. W. Stokesbury, R. Raghavan, A. J. Gallagher, N. Hammerschlag, and A. J. Danylchuk. 2016. Angling for endangered fish: conservation problem or conservation action. *Fish and Fisheries* 17:249–265.
- Cooke, S. J., D. P. Philipp, J. F. Schreer, and R. S. McKinley. 2000. Locomotory impairment of nesting male Largemouth Bass following catch-and-release angling. *North American Journal of Fisheries Management* 20:968–977.
- Cooke, S. J., and H. L. Schramm. 2007. Catch-and-release science and its application to conservation and management of recreational fisheries. *Fisheries Management and Ecology* 14:73–79.
- Cooke, S. J., C. D. Suski, S. E. Danylchuk, A. J. Danylchuk, M. R. Donaldson, C. Pullen, G. Bulté, A. O'Toole, K. J. Murchie, and J. B. Koppelman. 2008. Effects of different capture techniques on the physiological condition of Bonefish *Albula vulpes* evaluated using field diagnostic tools. *Journal of Fish Biology* 73:1351–1375.
- Cooke, S. J., C. D. Suski, M. J. Siepker, and K. G. Ostrand. 2003. Injury rates, hooking efficiency and mortality potential of Largemouth Bass (*Micropterus salmoides*) captured on circle hooks and octopus hooks. *Fisheries Research* 61:135–144.
- COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2015. COSEWIC assessment and status report on the Shortnose Sturgeon *Acipenser brevirostrum* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa. Available: www.registrelep-sararegistry.gc.ca/default_e.cfm. (August 2018).
- Davis, M. W. 2007. Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. *ICES (International Council for the Exploration of the Sea) Journal of Marine Science* 64:1535–1542.
- Davis, M. W. 2010. Fish stress and mortality can be predicted using reflex impairment. *Fish and Fisheries* 11:1–11.
- Haxton, T. J., and T. M. Cano. 2016. A global perspective of fragmentation on a declining taxon—the sturgeon (Acipenseriformes). *Endangered Species Research* 31:203–210.
- Hühn, D., and R. Arlinghaus. 2011. Determinants of hooking mortality in freshwater recreational fisheries: a quantitative meta-analysis. Pages 141–170 in T. D. Beard, R. Arlinghaus, and S. G. Sutton, editors. *The angler in the environment: social, economic, biological, and ethical dimensions*. American Fisheries Society, Symposium 75, Bethesda, Maryland.
- Josse, J., and F. Husson. 2016. missMDA: a package for handling missing values in multivariate data analysis. *Journal of Statistical Software* 70:1–31.
- Kieffer, J. D. 2000. Limits to exhaustive exercise in fish. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 126:161–179.

- Kieffer, J. D., M. Brilliant, A. Wakefield, and M. K. Litvak. 2001. Juvenile sturgeon exhibit low physiological responses to exercise. *Journal of Experimental Biology* 204:4281–4289.
- Kynard, B. 1997. Life history, latitudinal patterns, and status of the Shortnose Sturgeon, *Acipenser brevirostrum*. *Environmental Biology of Fishes* 48:319–334.
- Lawrence, M. J., S. Jain-Schlaepfer, A. J. Zolderdo, D. A. Algera, K. M. Gilmour, A. J. Gallagher, and S. J. Cooke. 2018. Are 3-minutes good enough for obtaining baseline physiological samples from teleost fish? *Canadian Journal of Zoology* 96:774–786.
- Lennox, R. J., J. W. Brownscombe, S. J. Cooke, A. J. Danylchuk, P. S. Moro, E. A. Sanches, and D. Garrone-Neto. 2015. Evaluation of catch-and-release angling practices for the Fat Snook *Centropomus parallelus* in a Brazilian estuary. *Ocean and Coastal Management* 113:1–7.
- Li, X., M. K. Litvak, and J. E. H. Clarke. 2007. Overwintering habitat use of Shortnose Sturgeon (*Acipenser brevirostrum*): defining critical habitat using a novel underwater video survey and modeling approach. *Canadian Journal of Fisheries and Aquatic Sciences* 64:1248–1257.
- Lüdecke, D. 2017. sjstats: statistical functions for regression models. R package version 0.9.0. Available: <https://CRAN.R-project.org/package=sjstats>. (August 2018).
- McDonald, D. G., and C. L. Milligan. 1992. Chemical properties of the blood. Pages 55–133 in W. S. Hoar, D. J. Randall, and A. P. Farrell, editors. *Fish physiology*, volume 12, part B. Academic Press, New York.
- McLean, M. F., K. C. Hanson, S. J. Cooke, S. G. Hinch, D. A. Patterson, T. L. Nettles, M. K. Litvak, and G. T. Crossin. 2016. Physiological stress response, reflex impairment and delayed mortality of White Sturgeon *Acipenser transmontanus* exposed to simulated fisheries stressors. *Conservation Physiology* 4(1):cow031.
- Meka, J. M. 2004. The influence of hook type, angler experience, and fish size on injury rates and the duration of capture in an Alaskan catch-and-release Rainbow Trout fishery. *North American Journal of Fisheries Management* 24:1309–1321.
- Muoneke, M. I., and W. M. Childress. 1994. Hooking mortality: a review for recreational fisheries. *Reviews in Fisheries Science* 2:123–156.
- NOAA (National Oceanic and Atmospheric Administration). 1998. Final recovery plan for the Shortnose Sturgeon (*Acipenser brevirostrum*). National Marine Fisheries Service–Fisheries, Silver Spring, Maryland.
- Pankhurst, N. W. 2011. The endocrinology of stress in fish: an environmental perspective. *General and Comparative Endocrinology* 170:265–275.
- Pikitch, E. K., P. Doukakis, L. Lauck, P. Chakrabarty, and D. L. Erickson. 2005. Status, trends and management of sturgeon and Paddlefish fisheries. *Fish and Fisheries* 6:233–265.
- Pinheiro, J., D. Bates, S. DebRoy, and D. Sarkar. 2015. nlme: linear and nonlinear mixed effects models. R package version 3.1–120. Available: <http://CRAN.R-project.org/package=nlme>. (September 2015).
- R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available: <http://www.R-project.org/>. (August 2018).
- Raby, G. D., M. T. Casselman, S. J. Cooke, S. G. Hinch, A. P. Farrell, and T. D. Clark. 2016. Aerobic scope increases throughout an ecologically relevant temperature range in Coho Salmon. *Journal of Experimental Biology* 219:1922–1931.
- Raby, G. D., M. R. Donaldson, S. G. Hinch, D. A. Patterson, A. G. Lotto, D. Robichaud, K. K. English, W. G. Wilmore, A. P. Farrell, M. W. Davis, and S. J. Cooke. 2012. Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild Coho Salmon bycatch released from fishing gears. *Journal of Applied Ecology* 49:90–98.
- Revelle, W. 2017. psych: procedures for personality and psychological research. Northwestern University, Evanston, Illinois. Available: <https://CRAN.R-project.org/package=psych>. (August 2018).
- Spear, M. C., and J. D. Kieffer. 2016. Critical thermal maxima and hematology for juvenile Atlantic (*Acipenser oxyrinchus* Mitchill 1815) and Shortnose (*Acipenser brevirostrum* Lesueur, 1818) sturgeons. *Journal of Applied Ichthyology* 32:251–257.
- Stoot, L. J., N. A. Cairns, F. Cull, J. J. Taylor, J. D. Jeffrey, F. Morin, J. W. Mandelman, T. D. Clark, and S. J. Cooke. 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conservation Physiology* 2(1):cou011.
- Trested, D. G., K. Ware, R. Bakal, and J. J. Isely. 2011. Microhabitat use and seasonal movements of hatchery-reared and wild Shortnose Sturgeon in the Savannah River, South Carolina – Georgia. *Journal of Applied Ichthyology* 27:454–461.
- Usvyatsov, S., J. Watmough, and M. K. Litvak. 2012. Age and population size estimates of overwintering Shortnose Sturgeon in the Saint John River, New Brunswick, Canada. *Transactions of the American Fisheries Society* 141:1126–1136.
- Wedemeyer, G. A., and R. S. Wydoski. 2008. Physiological response of some economically important freshwater salmonids to catch-and-release fishing. *North American Journal of Fisheries Management* 28:1587–1596.
- Wilson, S. M., G. D. Raby, N. J. Burnett, S. G. Hinch, and S. J. Cooke. 2014. Looking beyond the mortality of bycatch: sublethal effects of incidental capture on marine animals. *Biological Conservation* 171:61–72.
- Ziegeweid, J. R., C. A. Jennings, D. L. Peterson, and M. C. Black. 2008. Effects of salinity, temperature, and weight on the survival of young-of-year Shortnose Sturgeon. *Transactions of the American Fisheries Society* 137:1490–1499.

SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.