Notes

Effects of Capture by Trammel Net on Colorado River Native Fishes

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Abstract

Trammel nets are commonly used to sample rare fishes; however, little research has assessed delayed mortality associated with this capture technique. We conducted laboratory experiments to evaluate the effects of capture by trammel net on bonytail *Gila elegans*, razorback sucker *Xyrauchen texanus*, and roundtail chub *Gila robusta*, at 15, 20, and 25°C. Fish (139–288 mm total length) were entangled in a trammel net for 2 h or captured by seine net and then monitored for mortality for at least 14 d. Blood samples were collected immediately after capture, and plasma cortisol levels were quantified as an index of capture-related stress. The cortisol response varied by species, but mean cortisol levels were higher for fish captured by trammel netting (295.9 ng/mL) relative to fish captured by seine netting (215.8 ng/mL). Only one fish (of 550) died during capture and handling, but 42% of the trammel-netted fish and 11% of the seine-netted fish died within 14 d after capture. In general, mortality after capture by trammel net increased with increased water temperature and at 25°C was 88% for bonytail, 94% for razorback sucker, and 25% for roundtail chub. Delayed mortality of wild-caught fish captured by trammel net has the potential to be high, at least under some circumstances. We suggest that sampling frequency, timing of sampling (relative to reproductive cycles), and water temperature all be considered carefully when using trammel nets to sample diminished populations of imperiled native fishes.

Keywords: bonytail chub; *Gila elegans*; razorback sucker; *Xyrauchen texanus*; roundtail chub; *Gila robusta*; humpback chub; *Gila cypya*; delayed mortality; trammel net

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Introduction

Capture and handling of fish can be critical for surveying fish populations, but sampling and monitoring practices should have minimal effects on the fish communities being studied. All aspects of sampling, including capture, handling, physical confinement, and time out of water, can cause injury (Kelsch and Shields 1996; Campbell et al. 2010), reduce growth (Paukert et al. 2005), or prevent spawning (Pickering 1993). In addition, these negative effects are additive (Wedemeyer et al. 1990) and can vary with water temperature (Davis 2006). The physiological response of fish to capture and handling has been evaluated for certain commercially and recreationally important fish species and for some common sampling gears, such as angling (reviewed in Muoneke and Childress 1994). However, much less is known about the response of rare fish species to

Table 1. Number of bonytail *Gila elegans*, razorback sucker *Xyrauchen texanus*, and roundtail chub *Gila robusta* that died within 14 d after capture in a trammel net or seine net (control) at 15, 20, and 25°C. Total number of fish used in each experiment and mean (SE) handling time, total length, and weight for each treatment group.

Species	Temp (°C)	Net type	Total no. of fish	No. of fish used for cortisol	No. of fish that died	Mean handling time (s)	Mean total length (mm)	Mean weight (g)
Bonytail	15	Trammel	24	11	2	77 (7.2)	235 (6.2)	84 (11.3)
		Seine	24	11	2	53 (7.3)	246 (5.8)	114 (11.6)
	20	Trammel	37	21	25	78 (6.5)	183 (5.1)	134 (10.3)
		Seine	29	17	3	59 (6.1)	266 (6.3)	144 (9.7)
	25	Trammel	26	11	23	78 (6.0)	254 (5.7)	81 (9.7)
		Seine	37	14	8	71 (5.9)	224 (5.4)	95 (9.3)
Razorback sucker	15	Trammel	30	19	0	84 (6.7)	233 (5.7)	233 (10.5)
		Seine	29	16	1	50 (6.4)	239 (5.8)	240 (10.1)
	20	Trammel	19	6	5	49 (6.7)	179 (5.0)	119 (10.5)
		Seine	30	15	1	75 (6.3)	240 (5.4)	112 (10.0)
	25	Trammel	30	21	28	77 (7.1)	234 (5.6)	142 (11.1)
		Seine	29	25	7	97 (6.8)	201 (5.0)	145 (10.7)
Roundtail chub	15	Trammel	35	18	4	80 (5.7)	229 (5.4)	61 (9.0)
		Seine	38	21	3	59 (6.1)	232 (6.1)	122 (9.7)
	20	Trammel	32	15	14	83 (5.6)	174 (5.5)	70 (8.8)
		Seine	44	14	3	50 (5.5)	238 (5.3)	103 (8.7)
	25	Trammel	24	15	6	76 (6.1)	235 (5.9)	65 (9.7)
		Seine	33	20	3	65 (7.8)	199 (6.5)	116 (12.4)

sampling and handling, and the physiological response of fish to less common capture gear, such as the trammel net. When rare fish populations are sampled using trammel nets, scientists and managers have no way to anticipate or project the mortality that may occur as a consequence of sampling.

Trammel nets are often used when sampling populations of rare fishes because trammel netting is widely considered to be a nonlethal capture technique (Hubert 1996). A trammel net consists of three layers of multifilament netting with a small-mesh inner netting between two layers of larger mesh. Fish swim through the larger mesh and encounter the smaller mesh where they become entangled in a temporary "pocket" in the net. Unfortunately, fish captured in this manner often become so tightly wrapped in the net that movement of the gill cover is restricted, reducing water flow over the gills and hindering ventilation and oxygen exchange (Chopin et al. 1996). Prolonged struggling while entangled in the net also can result in extreme exhaustion and physical damage to tissues, both of which increase the physiological stress experienced by the entrapped fish. These unavoidable negative effects of trammel net capture suggest that delayed mortality may occur when fish are sampled in this manner.

In the Colorado River, bonytail *Gila elegans*, Razorback sucker *Xyrauchen texanus*, roundtail chub *Gila robusta*, and humpback chub *Gila cypha* populations are all commonly sampled using trammel nets (Kaeding et al. 1990; Valdez and Ryel 1995, *Supplemental Material*, Reference S1, http://dx.doi.org/10.3996/122011-JFWM-070.S1; Paukert 2004; Persons et al. 2012, *Supplemental Material*, Reference S2, http://dx.doi.org/10.3996/122011-JFWM-070.S2). Our observations of these imperiled fishes struggling to exhaustion when entangled and developing skin lesions as a result of capture led us to suspect that delayed mortality as a result of capture in a trammel net may be much higher than previously thought. It may be detrimental to sample rare fish populations if delayed mortality from capture in trammel nets is high. To address this issue, we conducted a series of controlled laboratory experiments using three native fishes from the Colorado River. Over a wide range of environmentally and physiologically relevant temperatures, we quantified physiological stress using blood plasma cortisol levels, an accepted index of physiological stress (Pottinger 2008), and we quantified the delayed mortality associated with capture by trammel net. With these data, we address the following questions. How does ambient water temperature impact stress and postcapture mortality of native fish caught by trammel nets? Can plasma cortisol levels provide a useful estimate of individual stress for these species? Are individuals with high plasma cortisol levels more likely to experience mortality after capture?

Methods

We evaluated physiological stress using blood cortisol levels and delayed mortality in three species of native fish, bonytail, razorback sucker, and roundtail chub, captured by a seine and a trammel net, at three temperatures, 15, 20, and 25°C (Table 1). A single experimental trial was conducted for each species at each temperature, for a total of nine trials. We obtained bonytail (n = 177) and razorback sucker (n = 167) from local hatchery stocks; roundtail chub (n = 206) were captured from the wild (Fossil Creek, Arizona) using hoop nets. Fish ranged from 139 to 288 mm in total length (Table 1). Experimental trials were conducted in a temperature-controlled greenhouse at the United States Forest Service, Rocky Mountain Research Station located in Flagstaff, Arizona, from June 1, 2007, to March 10, 2008.

Upon arrival at the research station, all fish were treated to remove external parasites by placing them in water with elevated salinity (3 ppt) at 22°C for 10 d, followed by a 2-h bath treatment of formalin at 26 ppm. All fish were then held for 2 wk and subsequently assigned to one of two treatment tanks, with an attempt to distribute equivalent body sizes and equal numbers into each tank. The two experimental tanks were 18,000-L rectangular, aboveground swimming pools (5.8 m long × 1.4 m tall × 2 m wide). Each tank was plumbed with a 1/2-hp high-efficiency centrifugal pump; bubble-bead filter (Aquadyne 2.2®); and an ultraviolet sterilizer. Water circulated though this filtration system at 227 L/min. To ensure oxygen remained at or near saturation, each tank was supplemented with an air pump attached to multiple air diffusers. Dissolved oxygen measurements at the highest temperature (25°C) confirmed that O_2 levels were at saturation. Each tank was stocked with 30 razorback sucker for 1 mo before the experiment to ensure that the biological filters had become established before initiation of the study. One of the two 18,000-L tanks was arbitrarily designated as the trammel net tank (treatment) and the other tank as the seine net tank (control). Trammel net and seine tanks were switched approximately halfway through the series of nine experimental trials to distribute any potential ''tank effect'' among treatments. Fish were held for 13 d before each netting experiment to allow fish to acclimate to the new environment and test temperature and to recover from any previous capture and handling events. We opened or closed windows in the greenhouse that contained the research tanks to achieve the desired water temperatures and lighting was not artificially controlled. Fish were fed a sinking, pellet feed ad libitum once daily during the acclimation period.

The trammel net (22 m long × 1.8 m deep), with two outer walls of 28-cm multifilament netting and one inner wall of 5-cm multifilament netting, was stretched across the length of the tank and gently moved from side to side to ensure all fish in the tank were entangled. The fish remained entangled in the net for 2 h, from 1800 to 2000 hours in every trial; this duration and time of day was chosen to replicate field conditions because 2 h is the standard maximum deployment time for humpback chub monitoring programs in both the Grand Canyon and Upper Colorado River Basin (Valdez and Ryel 1995, *Supplemental Material*, Reference S1, http://dx.doi.org/10.3996/122011-JFWM-070.S1; Paukert 2004). Fish in the seine tank were captured at the end of the entanglement time period using a seine (a lightweight, knotless nylon net with 1-cm mesh, a 230-g/m lead line on the bottom edge, and foam-filled floats along the top edge). Seines are not typically used to capture adult fish of these species in the Colorado River because adult fish typically reside in deep water with swift current where seining is largely ineffective, but seining served as an effective capture method for the control fish in our tanks because it allowed fish to be captured and released quickly. Fish were trapped within the seine net for less than 1 min. Two crews worked simultaneously to ensure that the capture of the last fish with the seine coincided with removal of the treatment fish from the trammel net.

After capture, all fish were removed from the nets and handled according to standardized fish handling procedures for the Colorado River in the Grand Canyon (Persons et al. 2012, *Supplemental Material*, Reference S2, http://dx.doi.org/10.3996/122011-JFWM-070.S2). After removal, fish were placed into a ''live well,'' a 76-L rectangular holding tank with supplemental aeration. Once all fish were placed in the live well, fish were individually weighed, measured (total length and fork length), and injected with a passive integrated transponder tag to allow for identification of individuals. Crews measuring and tagging the fish captured by the trammel net and crews measuring and tagging the fish captured by the seine net were alternated repeatedly after handling five fish to ensure there was no systematic bias in how fish were treated by individual handlers.

Blood samples were taken immediately after handling from approximately half the fish collected from the seine tank and half the fish collected from the trammel net tank (Table 1). Fish were randomly selected for blood extractions, with approximately equal numbers of blood samples drawn from each tank. The elapsed time for a fish to be removed from the live well, weighed, measured, implanted with a 12-mm passive integrated transponder tag, and have blood drawn (if performed) averaged 1 min and 15 s (Table 1). Blood samples were collected from the caudal vasculature into a heparin-treated 1-mL syringe (25-gauge, 15.9-mm needle), and the sample was placed into a 1.5-mL centrifuge tube. The centrifuge tube was spun at 10,000 rpm for 10 min to separate plasma from red blood cells, and plasma was removed and stored at −70°C.

Immediately after the handling procedures described above, fish were released into a 36,000-L recovery tank. The recovery tank was equipped in a similar manner to the control and treatment tanks, although it was attached to a larger filtration and pumping system to accommodate the greater volume of water. Fish were held in the recovery tank and monitored for at least 14 d to quantify postexperiment mortality. Dead fish were removed from the recovery tank daily, and their passive integrated transponder tag numbers were recorded. At the termination of the study (15 d after the last experimental trial and 276 d after the first experimental trial), all fish in the recovery tank were captured with a seine net, weighed, measured, and scanned to record the passive integrated transponder tag number.

All plasma samples were measured for cortisol with a commercially available enzyme-based immunoassay kit (Assay Designs Inc., Ann Arbor, Michigan). For each species, a serial dilution of plasma was found to be parallel with the standard curve. Samples were diluted 1:200 with the provided assay buffer; this dilution allowed the plasma cortisol samples to be within the

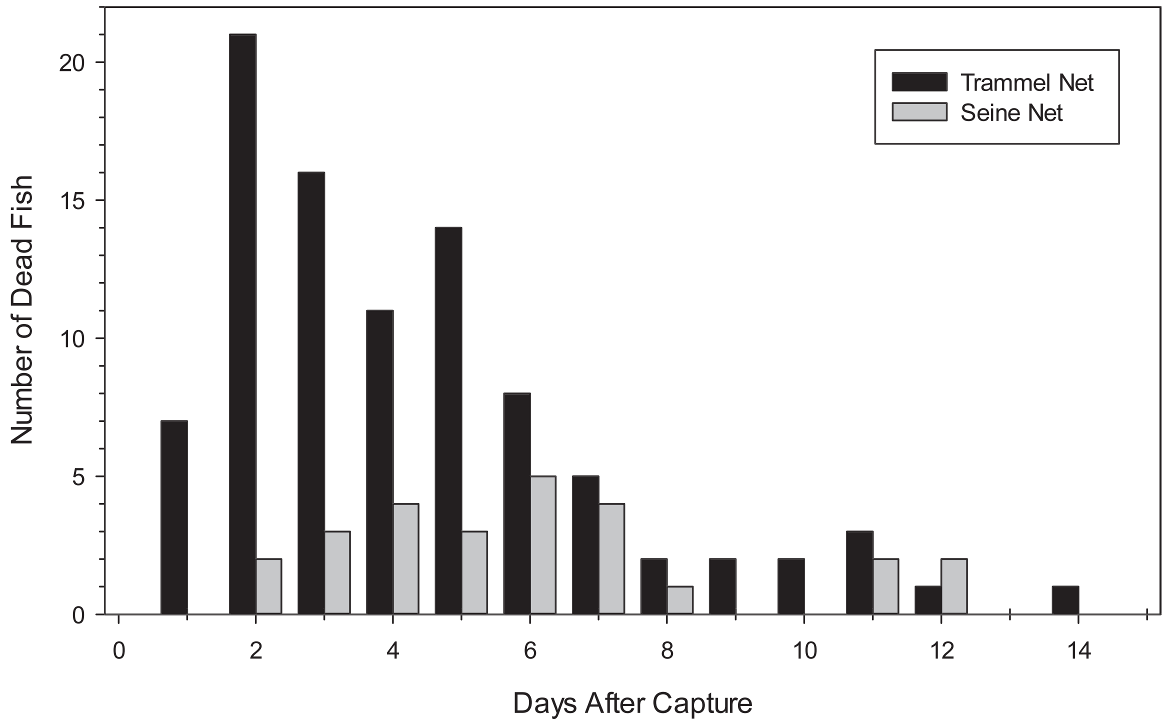

Figure 1. Total number of bonytail *Gila elegans*, razorback sucker *Xyrauchen texanus*, and roundtail chub *Gila robusta* that died within 14 d after capture in either a trammel net (treatment) or a seine net (control).

detection limits of the assay. All samples were measured in duplicate, and each plate contained eight pooled samples: four wells randomly placed on the plate were from one treatment sample and four wells randomly placed on the plate were from one control sample. Other than the dilution, the plasma was unmodified and no purification processes were performed. Plates containing samples were measured using a microplate reader with a wavelength filter of 405 nm.

We used *t*-tests to evaluate mortality, and plasma cortisol levels between trammel net and seine net at each temperature for all of the species grouped together ($n = 3$). A Kaplan–Meier survival curve was used to analyze probability of survival for fish caught in either the trammel net or the seine net during the 14 d after the treatment. Two-sample *t*-tests also were used to compare treatment and control groups related to weight loss, mortality from drawing blood, and time out of water. All statistical tests were evaluated at a significance level (α) of 0.05.

Results

Only 1 of 550 fish (a roundtail chub) died during the actual capture and handling process; all other mortality was delayed. Forty-two percent of the trammel-netted fish and 11% of the seine-netted fish died within 14 d after capture. Mortality of fish caught by a trammel net was highest from 2 to 7 d after capture (Figure 1). Trammel-netted fish died on average 4 d after capture, whereas seine-netted fish died on average 6 d after capture. For the fish that died within the first week after capture, fish caught by trammel net had a lower probability of survival than fish captured with a seine ($P < 0.05$, Kaplan–Meier survival curve). Fish in both groups experienced a decrease in mass over the course of the 276-d study. Fish caught by trammel net experienced a 3.8% weight loss, and fish caught by seine experienced a 1.8% weight loss; however, these two values were not statistically different (*t*-test, $P = 0.38$).

For bonytail and razorback sucker caught by trammel net, delayed mortality increased directly with water temperature. At 15°C, delayed mortality for all trammel-netted bonytail and razorback sucker was similar to seine-netted fish (<9%), but at 25°C, mortality was 88% for bonytail, 94% for razorback sucker, and 25% for roundtail chub (Figure 2). At 25°C, significantly more fish captured with a trammel net died compared with fish captured using a seine (*t*-test, $P < 0.05$, pooled data for all species). We considered the possibility that drawing blood for the cortisol measurements resulted in additional mortality; however, there was no significant association between having blood taken and mortality (*t*-test, $P > 0.05$, data pooled for all species and temperatures). No significant differences were found in handling time between trammel and seine groups. Plasma cortisol levels varied widely (40.7–1,207.6 ng/mL) both within species and across species (Table 2). Roundtail chub caught in the seine generally had the highest mean cortisol levels at 283.5 ± 35.1 (SE) ng/mL, followed by razorback sucker at 227.9 ± 37.8, and bonytail at 215.7 ± 39.2 (Figure 3). In general, trammel-netted fish showed higher levels of plasma cortisol, relative to seine-netted fish (Figure 3), with the mean cortisol level for trammel-netted fish being 295.9 ± 16.7 ng/mL, and the mean cortisol level for seine-netted fish being 215.8 ± 10.1 ng/mL.

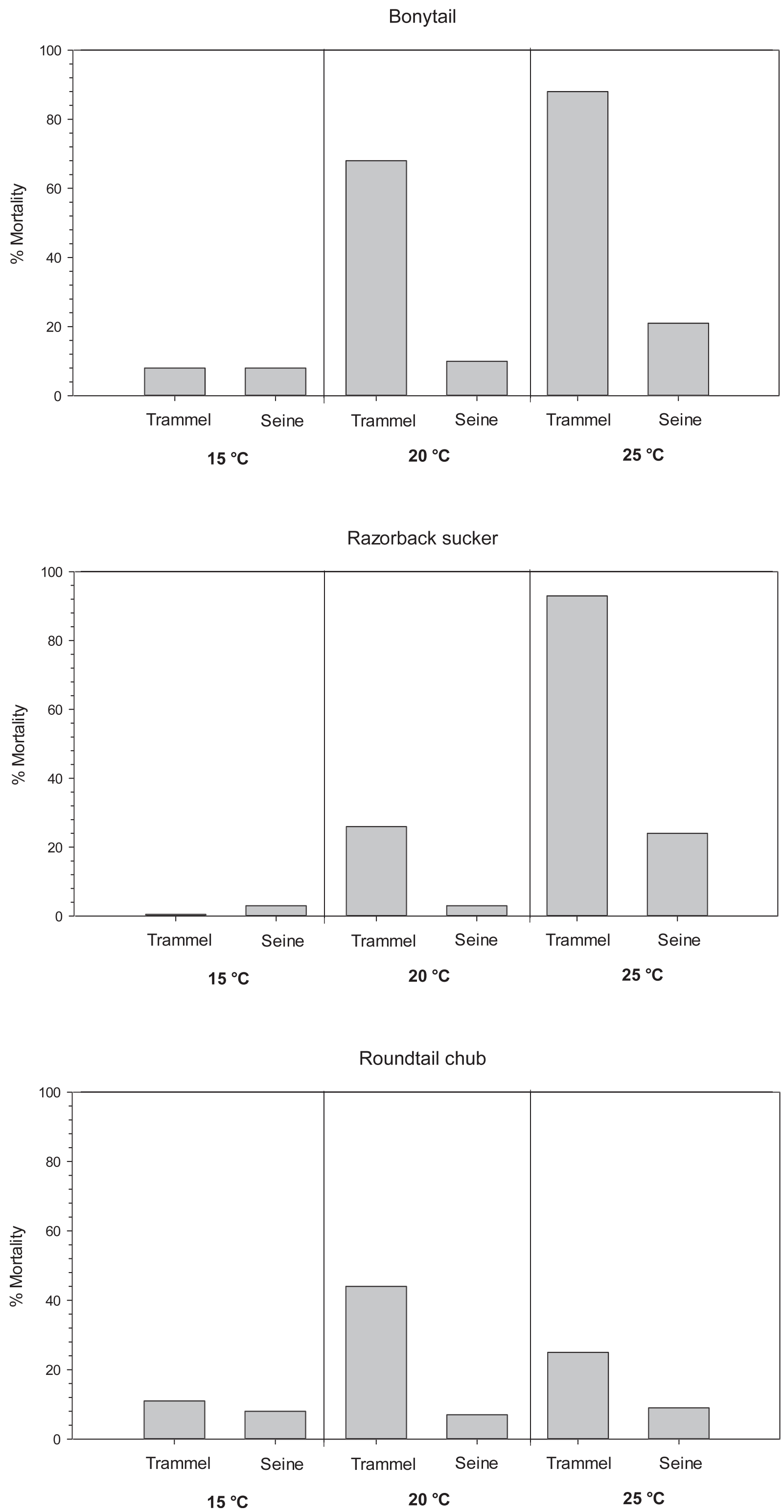

Figure 2. Percentage of mortality of bonytail *Gila elegans*, razorback sucker *Xyrauchen texanus*, and roundtail chub *Gila robusta* within 14 d of being entangled in a trammel net for 2 h (treatment) compared with fish captured with a seine net (control) at 15, 20, and 25°C.

Roundtail chub demonstrated a distinct mortality pattern relative to the other two species we tested. Mortality was low overall, did not increase with temperature, and was highest at the intermediate temperature (20°C). The roundtail chub used in this study also were the only individuals originally collected from the wild, which may have affected their response to capture and handling.

Table 2. Cortisol levels (mean ± SE) for bonytail *Gila elegans*, razorback sucker *Xyrauchen texanus*, and roundtail chub *Gila robusta* at three different temperatures for both treatment (trammel net) and control (seine) groups. Blood samples were collected immediately after fish were removed from nets, and then fish were weighed and measured.

	15°C		20°C		25°C	
	Control	Treatment	Control	Treatment	Control	Treatment
Bonytail	108.7 ± 43.4	108.2 ± 43.4	265.2 ± 34.9	333.5 ± 31.4	145.4 ± 38.5	334.4 ± 43.4
Razorback sucker	212.5 ± 36.0	182.8 ± 34.9	212.4 ± 37.2	224.6 ± 58.8	179.7 ± 28.8	355.6 ± 31.4
Roundtail chub	232.2 ± 31.4	379.9 ± 33.9	180.1 ± 38.5	145.4 ± 37.2	340.2 ± 32.2	422.9 ± 37.2

As temperature increased, so did plasma cortisol levels for both trammel-netted and seine-netted bonytail and razorback sucker but not for roundtail chub. In general, trammel-netted fish had both the highest cortisol levels and the highest delayed mortality. Seine-netted fish showed no direct association between cortisol and mortality. However, these results were affected by water temperature. Seine-netted and trammel-netted bonytail and razorback sucker exhibited differences in plasma cortisol at 25°C, but no differences were observed at 15 or 20°C (Figure 3).

Discussion

Bonytail, razorback sucker, and roundtail chub entangled in a trammel net for 2 h under controlled conditions experienced very little immediate mortality; however, delayed mortality was high, and it increased with water temperature. For razorback sucker, postsampling mortality exceeded 85% at the highest temperature (25°C). High mortality after capture in a trammel net also has been documented for sea bream *Pagrus major*, with 44% of fish dying within 18 d of capture under laboratory conditions at 19 ±1°C (Chopin et al. 1996). If similar mortality is experienced by fish captured by trammel net in natural settings, this may represent a previously unrecognized source of mortality that could adversely affect threatened fish populations. Although a large number of fish (550) were used in this study there was only one experimental trial conducted for each of the three species, for each temperature and capture method. Additional replication is therefore needed to confirm our results.

We note that fish caught with a seine still experienced up to 24% delayed mortality. This mortality is probably a result of the capture and handling stress that has been extensively documented for other fish species (reviewed in Kelsh and Shields 1996). However, the additional stress and injury due to being entrapped in a trammel net increased mortality by 3.6 times over that of the seine-netted fish. Postmortem examination of fish caught by trammel net revealed that, if fish died within the first 2 d after capture (10.9%), the cause of death seemed to be physical damage to the gills. In contrast, if a fish died 3–14 d after capture, secondary fungal infection seemed to be the cause of death. It is possible that fish held in our laboratory were particularly vulnerable to bacterial or fungal infections because of high fish densities and recirculating filtration.

All of the fish included in these experiments lost weight during the study, despite supplemental feeding, suggesting that the laboratory environment was stressful. Tyus et al. (1999) reported minimal delayed mortality in razorback sucker held at 13°C after handling and invasive tissue collection procedures, which further indicates that our laboratory environment may have been unusually stressful and lead to higher than normal mortality. Increased stress is known to reduce growth and resistance to disease (Wedemeyer et al. 1990). Physical damage caused by entanglement plus the associated stress of capture and handling may together generate a reduced immune response and increased vulnerability to pathogens (Pankhurst and Sharples 1992, Lupes et al. 2006). Stress-induced immunosuppression and cumulative effects of additive stress may have been reasons that delayed mortality was high in our experiments.

Plasma cortisol is commonly used as an index to measure stress response in fish (Donaldson 1981, Pankhurst and Sharples 1992, Pickering 1993). Bonytail, razorback sucker, and roundtail chub captured with a seine all exhibited relatively high cortisol levels compared with published values from studies for other fishes (Delahunty et al. 1980, Redding et al. 1984, Barton 2002), but they were similar to baseline cortisol levels reported for bonytail from other laboratory studies (Sykes et al. 2011).

Fish captured with a trammel net in our study exhibited higher plasma cortisol levels relative to fish captured with a seine net at 25°C. This result suggests that trammel netting still triggers a large stress response at warm water temperatures, which could have detrimental impacts even if delayed mortality does not occur. Many rare native fishes are sampled when fish aggregate for spawning, and high stress levels can suppress gamete production by interrupting reproductive hormones (Pickering 1993, Haddy and Pankhurst 1999). For imperiled native fishes, interruption of spawning could be as detrimental to populations as individual fish mortality.

The negative effects of capture and handling on native fish species seem to be exacerbated at high water temperatures. The thermal optimum for the species we tested is 20°C (Bulkley et al. 1981, *Supplemental Material*, Reference S3, http://dx.doi.org/10.3996/122011-JFWM-070.S3). Above this temperature, the effects of trammel netting generally induced a higher cortisol response (Figure 3). Water temperature affects both oxygen availability and the metabolic rate of fish and is therefore one of the most important environmental factors

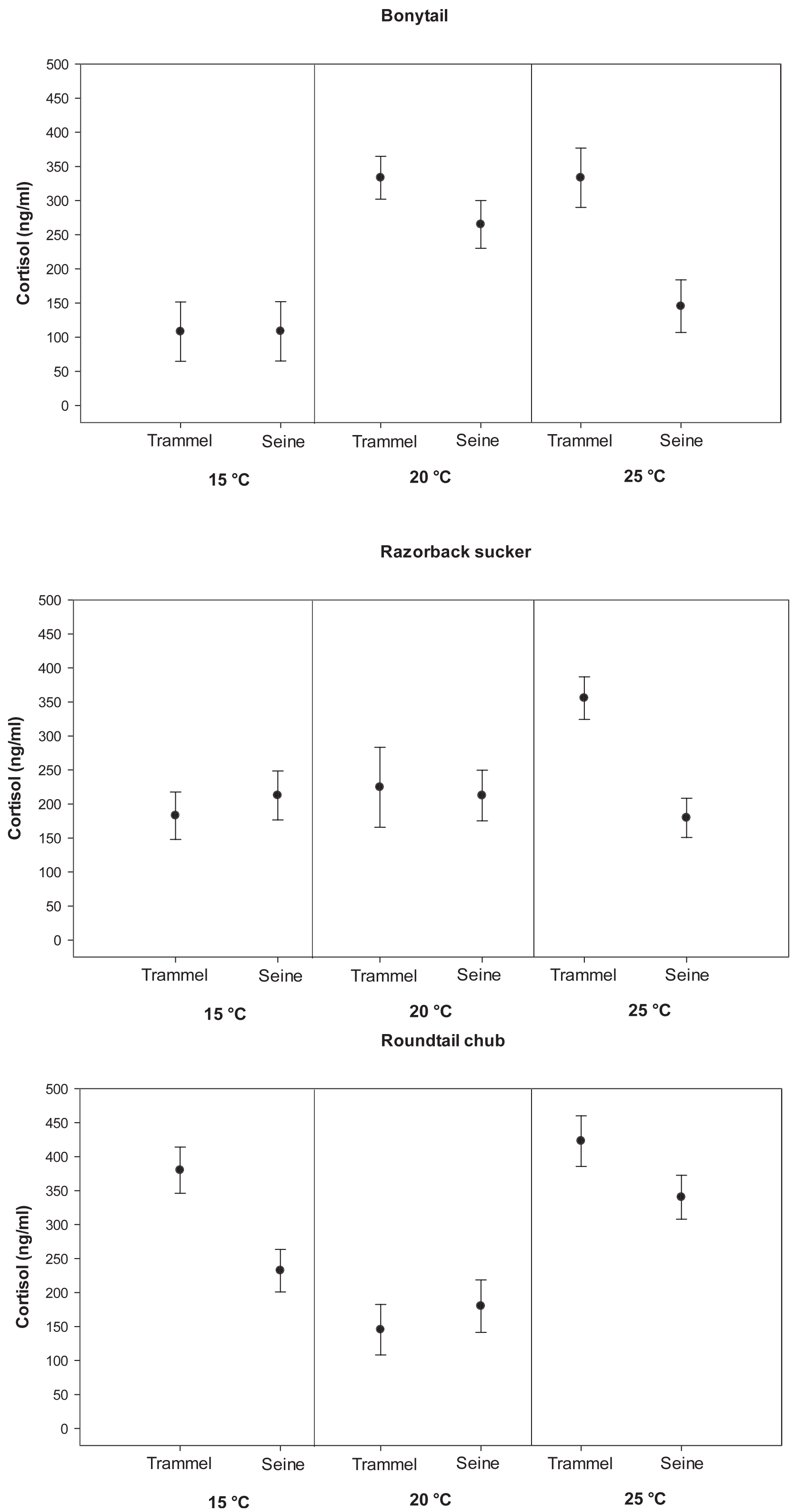

Figure 3. Mean cortisol levels for bonytail *Gila elegans*, razorback sucker *Xyrauchen texanus*, and roundtail chub *Gila robusta* entangled in a trammel net for 2 h compared with fish captured in a seine net at 15, 20, and 25°C. Blood samples were collected immediately after fish were removed from nets, weighed, and measured. Error bars represent 95% confidence intervals.

affecting fish physiology (Hoar and Randall 1978). Cold temperatures typically slow physiological function and can reduce stress in fish (Davis 2006), suggesting that temperature should be taken into account when considering the physiological stress caused by a particular capture method.

Based on the results presented here, we postulate that undocumented mortality probably occurs in wild fish populations as a result of capture by trammel nets and subsequent handling. This undocumented mortality may have harmful effects on small or frequently sampled fish populations. In addition, even if high mortality does not occur in the wild, increased stress is likely and may decrease the willingness of fish to feed and breed in the days and weeks after capture. For some native species such as bonytail and razorback sucker, high water temperatures (25°C) are likely to increase both stress and postsampling mortality in fish captured with trammel nets. We suggest that sampling frequency, timing of sampling (relative to reproductive cycles), and water temperature be considered carefully when using trammel nets to sample imperiled native fishes.

Supplemental Material

Please note: The Journal of Fish and Wildlife Management is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

Reference S1. Valdez RA, Ryel RJ. 1995. Life history and ecology of the humpback chub (*Gila cypha*) in the Colorado River, Grand Canyon, Arizona. Final Report to Bureau of Reclamation, Salt Lake City, Utah (contract 0-CS-40-09110).

Found at DOI: http://dx.doi.org/10.3996/122011-JFWM-070.S1; also available at http://www.gcmrc.gov/library/reports/biological/Fish_studies/Biowest/Valdez1995f.pdf (21.8 MB PDF).

Reference S2. Persons W, Ward DL, Avery LA, Burtner AC. 2012. Standardized methods for Grand Canyon fisheries research. U.S. Geological Survey, Grand Canyon Monitoring and Research Center, Flagstaff, Arizona.

Found at DOI: http://dx.doi.org/10.3996/122011-JFWM-070.S2 (1.5 MB PDF).

Reference S3. Bulkley RV, Berry CR, Pimentel R, Black T. 1981. Tolerance and preferences of Colorado River endangered fishes to selected habitat parameters. U.S. Fish and Wildlife Service, Utah Cooperative Fisheries Research Unit, Utah State University, Logan, Utah, Completion Report (contract 14-16-00018-1061 A-2).

Found at DOI: http://dx.doi.org/10.3996/122011-JFWM-070.S3 (9.6 MB PDF).

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