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Assisted Recovery Following Prolonged Submergence in Fishing Nets Can Be Beneficial to Turtles: An Assessment with Blood Physiology and Reflex Impairment

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ABSTRACT. – **We conducted an experiment using freshwater painted turtles (*Chrysemys picta*) to determine if keeping turtles out of water for an hour enhances anoxia recovery following a simulated bycatch event in nets (i.e., 12 hrs of submergence at 25°C). Traditional blood physiology measures and the novel application of a reflex impairment index (e.g., responses to gravity, light, and tactile stimuli) indicated that keeping turtles in or out of water for an hour did not yield a significant improvement in anoxia recovery; however, when the majority of reflexes are impaired, in particular the tactile response (e.g., limb movements), it appears that assisted recovery (keeping turtles out of water) can reduce the chance of postrelease mortality. The use of the reflex impairment index is a simple and inexpensive way to determine turtle bycatch condition after submergence in nets and discern whether assisted recovery may be required.**

Turtle bycatch, the inadvertent capture of turtles during fishing activities, is regarded as a global conservation issue, particularly for marine turtles (Alverson et al. 1994; Hall et al. 2000; Lewison et al. 2004; Lewison and Crowder 2007). Although there are fewer bycatch studies in freshwater compared to marine environments (Raby et al. 2011), freshwater turtles are also prone to accidental capture and mortality from fisheries around the world (Beumer et al. 1981; Barko et al. 2004; Lowry et al. 2005; Carrière 2007; Larocque et al. 2012a). Freshwater turtles can also be captured incidentally during routine fisheries monitoring and research activities. Bycatch is a conservation issue because turtle populations are particularly sensitive to additional adult mortality due to their life-history characteristics (e.g., slow maturation, low reproductive success; Brooks et al. 1991; Congdon et al. 1993, 1994; Gibbons et al. 2002). Aside from potential immediate mortality, however, turtles caught in submerged nets (e.g., fyke nets) can be subjected to injury and stress that may lead to reduced fitness. For instance, even if nets are checked regularly enough to prevent immediate mortality, turtles may still be exhausted upon release from the prolonged lack of oxygen as well as efforts to escape. In this exhausted state, a released turtle may not be able to escape predators or remain at the surface to breathe, both of which could lead to delayed mortality.

Bycatch mitigation approaches usually aim to reduce bycatch or decrease mortality rates of discarded animals, typically through changes in fishing gear and practices (Hall and Mainprize 2005; Lowry et al. 2005; Soykan et al. 2008; Fratto et al. 2008; Larocque et al. 2012b, 2012c). To our knowledge, however, there has been no systematic research on strategies to facilitate turtle recovery (recuperation of physiological and behavioral conditions to the normal range) after prolonged captivity in submerged nets. This is in stark contrast to the abundant literature on recovery of fish captured in recreational and commercial fisheries (e.g., Farrell et al. 2001; Suski et al. 2006). For example, would releasing an exhausted turtle out of the water facilitate recovery? Bury (2011) found 12 lethargic freshwater turtles captured in hoop nets; after having their lungs drained and being placed on shore in protected containers overnight, all the turtles recovered. Similar recovery methods have been used on sea turtles (Balazs 1986; Gerosa and Aureggi 2001). This suggests that turtles could recuperate more rapidly if allowed to remain out of the water upon release; however, these reports are anecdotal and assisted recovery of freshwater turtles has yet to be assessed experimentally.

By adopting a conservation physiology approach, physiological tools can be applied to assess the recovery of turtles from anoxia, such as capture in submerged fyke nets (Wikelski and Cooke 2006). For instance, blood lactate increases and pH decreases with anaerobic metabolism, and both respond more rapidly as temperature increases (Herbert and Jackson 1985; Jackson and Ultsch 2010). Thus, blood lactate and pH are effective indicators of anoxia in turtles in warm temperatures (e.g., Larocque et al. 2012b). Another measure using reflexes has recently been incorporated into the evaluation of fish condition following fisheries interactions and is predictive of postrelease mortality (Davis 2010). Although not previously tested on turtles, reflexes such as a tactile response or righting response in water could serve as reflex impairment measures for evaluating turtle condition after submergence in nets. An added benefit of such reflex assessment is that, once validated, fishers as well as researchers can be trained to use reflexes to identify exhausted turtles that might benefit most from aided recovery (see Raby et al. 2012 for an example with Pacific salmon).

The objective of this study was to determine if leaving turtles out of the water following extended submergence in fishing nets facilitates recovery. We used painted turtles (*Chrysemys picta*) because they are frequently encountered as bycatch in freshwater fyke net fisheries in eastern Ontario (Larocque et al. 2012a). We used blood physiology and reflex impairment to assess recovery. Given that this is the first study to adapt reflex assessments for the study of turtle condition, we provide a commentary on this technique and its potential as a tool for turtle bycatch management.

METHODS

Animals. — Using fyke nets, we captured 34 male painted turtles (*Chrysemys picta*; mean mass ± SE: 335 ± 5 g, range: 276–394 g; mean carapace length ± SE: 142 ± 1 mm, range: 121–151 mm) in Lake Opinicon, Ontario, Canada (44°34′N, 76°19′W) from 6 to 21 June 2011. Fyke nets were set for 24 hrs in water approximately 1 m deep and were fished such that the lead line and wings acted as guides into the nets (see Larocque et al. 2012a for net dimensions and set details). Floats in the nets were used to create air spaces for turtles and other organisms (Larocque et al. 2012b). After capture, turtles were held outdoors at the Queen's University Biological Station in flow-through tanks (1-m^3 circular holding tanks containing ~700 l of lake water) between 24 and 72 hrs prior to being used in the experiment. All tanks contained floating wood platforms onto which turtles could climb to bask. Turtles were not fed while in captivity, nor disturbed.

Experimental Procedure. — We used 4 treatment groups to elucidate whether turtles, after being completely submerged for a long duration, differed in recovery in or out of water: 1) unsubmerged controls (baseline response), 2) immediately after submergence, 3) recovery in water, and 4) recovery out of water. Turtles did not vary in mass or size between treatments (mass, $F_{3,26} = 0.82$, $R^2 = 0.09$, $p = 0.50$; carapace length, $F_{3,26} = 1.55$, $R^2 = 0.15$, $P = 0.15$). For each treatment, turtles were sampled for blood lactate and pH (indicators of anoxia) as well as reflex responses. Ten control turtles were sampled after the holding period in the flow-through tanks to obtain baseline values. Otherwise, the rest of the turtles were individually submerged within indoor tanks (155 by 55 by 60 cm) containing lake water for 12 hrs. Turtles were submerged within the tank by enclosing them in a cage (75 by 40 by 30 cm) made of 5.1-cm^2 plastic mesh. Water temperatures were maintained between 24°C and 26°C, and dissolved oxygen was at ambient levels (typically 6–8 mg l^{-1}). A 12-hr submergence period at these temperatures is near the critical limit that painted turtles can withstand anoxic submergence (Musacchia 1959; Herbert and Jackson 1985). It is worth noting that, in Ontario, fishers have to check nets at most every 48 hrs, so much longer submergence is possible in the commercial fishery. Nevertheless, we had to balance realism with animal care and conservation concerns. Four turtles were not capable of withstanding 12 hrs of submergence (see ''Results'') based on a sublethal endpoint (i.e., lack of movement). Ability to withstand the submergence period was periodically assessed visually and with gentle prodding (if necessary to determine movement) to try to ensure the turtle's survival (if a turtle appeared in distress, it was checked at least every half hour). After the 12-hr submergence period, we released turtles from the submerged cage. Six turtles were immediately sampled after the submergence period. The remaining turtles were placed in a tank (1 m^3) of water and allowed 1 hr to recover prior to sampling. During the recovery period, seven turtles recovered out of water while placed on a 25 by 20-cm floating Styrofoam platform, while 7 turtles recovered in the water with no floating platform provided. A 1-hr recovery period was chosen for 2 reasons: a recovery period longer than 1 hr is not likely to be practical for fishers and blood pH is typically restored to normal values by this time period at the water temperatures used (Herbert and Jackson 1985).

Blood Physiology. — Blood samples were obtained within 3 min of handling the turtle for each experimental group. Using sodium-heparinized 25-gauge needles of 2.54-cm length (10,000 USP units·ml^{-1}, Sandoz, Québec, Canada) with 1-ml syringes, we extracted 0.2 ml of blood from the caudal vasculature on the dorsal side at the base of the tail. Whole blood lactate was measured with a Lactate Pro™ meter (Arkay Inc., Japan) that has been validated with teleost fish (Brown et al. 2008). We measured blood pH on a 3-point calibrated minilab IQ128 Elite pH meter (IQ Scientific Instruments Inc., USA).

Reflex Responses. — Each experimental group was sampled for reflex responses. Well-defined external stimuli such as gravity, light, sound, and touch evoke reflex responses in organisms. The types of reflexes tested on turtles were adapted from a Reflex Action Mortality Predictor (RAMP) developed for fish (Davis 2007) but also been applied to several invertebrates (Davis 2010). The measured reflexes involved orientation (whether turtles attempted to orient within water and on land when turned upside down), startle responses (whether turtles were startled by a hand-waving motion in front of the turtle and by hitting the side of the tank), an escape response (whether turtles attempted to escape when held), and physical responses (whether turtles responded to being touched on the limbs and head) for a total of 7 reflexes. To reduce subjectivity, we scored each reflex response as present or absent. Reflex responses for each turtle were converted into a Reflex Impairment Index (RII; adapted from RAMP used in Davis 2007) where RII = 1 − (sum of individual reflex scores/total possible score of 7). The RII ranges from a score of 1 indicating a turtle was fully impaired to a score of 0 indicating a turtle recovered all of its reflexes.

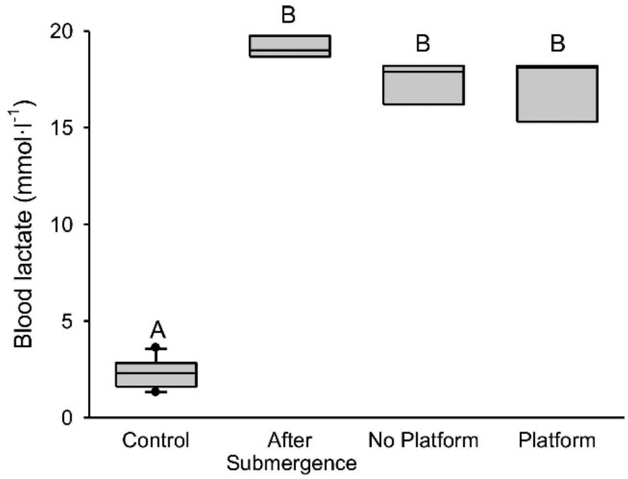

Figure 1. Blood lactate levels (mmol·l^{-1}) for painted turtles (*Chrysemys picta*) sampled when not submerged (controls), after being forcibly submerged, and after 1 hr of recovery from submergence with or without a platform. The box plots show the median within the box, with box boundaries being the 25th and 75th percentiles, whiskers representing the 10th and 90th percentiles, and dots being outliers. Box plots that were not significantly different according to Tukey's multiple comparisons are indicated by similar letters.

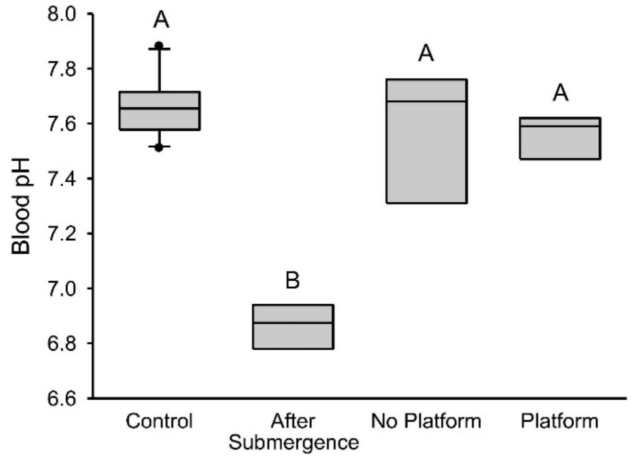

Figure 2. Blood pH for painted turtles (*Chrysemys picta*) sampled when not submerged (controls), after being forcibly submerged, and after 1 hr of recovery from submergence with or without a platform. The box plots show the median within the box, with box boundaries being the 25th and 75th percentiles, whiskers representing the 10th and 90th percentiles, and dots being outliers. Box plots that were not significantly different according to Mann-Whitney tests with a Bonferroni correction ($\alpha = 0.008$) are indicated by similar letters.

Statistical Analyses. — Blood lactate was square-root transformed and analyzed using an analysis of variance with post hoc Tukey's test. Both blood pH and RIIs did not meet either the assumption of homogeneity of variance or normality, thus we used the nonparametric Kruskal-Wallis test, with post hoc Mann-Whitney tests that used a simultaneous Bonferroni correction to determine whether experimental groups differed in blood pH and reflex responses. Significance was accepted at $\alpha = 0.05$, unless a Bonferroni correction was applied. All statistical analyses were performed using SPSS v.20.0.0 and values are reported as mean ± standard error.

RESULTS

Of the 34 painted turtles tested, 4 turtles were not capable of completing the 12-hr submergence period. In these 4 cases, turtles were removed from the water prior to 12 hrs when showing no signs of movement (e.g., swimming) even after being handled by the researcher. The turtles were then left to recover out of the water. Two turtles died, while the other 2 slowly regained motor control and recovered completely (1 turtle took over 2 hrs to recover to the point that it was capable of active swimming).

Blood lactate in painted turtles differed among the four treatments ($F_{3,26} = 456.54$, $R^2 = 0.98$, $p < 0.001$). The post hoc Tukey's test revealed that blood lactate was lowest in the control group (2.33 ± 0.23 mmol·l^{-1}; Fig. 1), while platform, no platform, and immediately after submergence treatments had similar lactate levels at 17.20 ± 0.57, 17.37 ± 0.51, and 19.21 ± 0.35 mmol·l^{-1}, respectively (Fig. 1).

Blood pH was different among the four experimental groups ($\chi^2_3 = 15.3$, $\eta^2 = 0.53$, $p = 0.002$). Mann-Whitney tests with a Bonferroni correction of $\alpha = 0.008$ indicated that turtles immediately after submergence had the lowest blood pH (6.87 ± 0.03), while platform, no platform, and control groups had similar blood pH at 7.58 ± 0.05, 7.59 ± 0.08, and 7.67 ± 0.03, respectively (Fig. 2).

Of the reflexes tested, the tactile reflexes were least affected by the submergence period and were mostly present for all turtles in each treatment (Table 1). The remaining reflexes showed a relatively gradual return, as indicated by the proportion of reflexes present, in the order of: after submergence, no-platform, platform, and control treatments (Table 1). Overall, reflex responses were different between the treatments ($\chi^2_3 = 23.6$, $\eta^2 = 0.82$, $p < 0.001$). When using a Bonferroni correction of $\alpha = 0.008$, Mann-Whitney tests indicated that turtles in the control group had the most reflexes present (RII of 0.02 ± 0.02; Fig. 3). Both platform and no-platform treatments were similar at a RII of 0.29 ± 0.07 and 0.45 ± 0.07, respectively (Fig. 3). Turtles from immediately after submergence had the fewest reflexes present at an RII of 0.74 ± 0.04, but this was not statistically different from the no-platform treatment (Fig. 3).

DISCUSSION

Our objective was to determine if keeping turtles out of water can facilitate recovery after entrapment in fishing nets. Blood physiology and reflex responses suggested that recovery of painted turtles is not discernibly affected by whether a turtle recovers in or out of the water. Mean

Table 1. Proportion of painted turtles (*Chrysemys picta*) in each treatment that had a positive reflex response for each reflex tested.

Reflexes	Treatment			
	Control (n = 10)	After submergence (n = 6)	No platform (n = 7)	Platform (n = 7)
Orientation on land	0.80	0.00	0.29	0.43
Orientation in water	1.00	0.33	0.43	0.86
Audible startle	1.00	0.00	0.29	0.43
Visual startle	1.00	0.17	0.86	0.71
Escape response	1.00	0.17	0.14	0.71
Tactile startle (head)	1.00	0.33	0.86	0.86
Tactile startle (limb/tail)	1.00	0.83	1.00	1.00

RII values for turtles kept out of water were lower than those in the water, albeit not in a statistically significant manner. Turtles showing some reflex responses after submergence (RII $<$ 1) appear to be able to recover regardless of whether or not they were provided a basking platform. Unlike most other freshwater turtle species, painted turtles are known for their ability to withstand and recover from unusually long periods of submergence (Jackson et al. 1984, 2006; Ultsch et al. 1999; Jackson 2000). Thus, all the painted turtles may not have been ''exhausted'' after 12 hrs of submergence or recovered too quickly to show a treatment effect. However, 2 of the 4 turtles that could not withstand 12 hrs of submergence were able to recover when removed from the water. Due to individual variability in the ability to withstand submergence, these 4 turtles were submerged longer than capable and did not show any reflex responses upon removal from the water (RII = 1). The higher the RII values and severity of anoxia, the more likely a turtle will benefit from assisted recovery such as being left out of water (likely for longer than 1 hr as Bury 2011 found) and draining the lungs (Gerosa and Aureggi 2001). Thus,

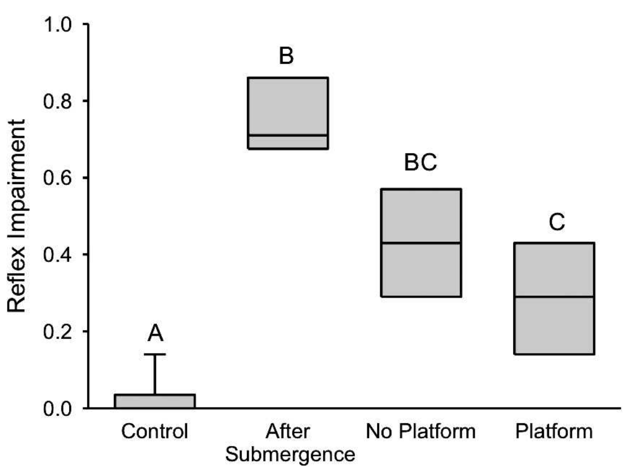

Figure 3. Impaired reflexes demonstrated by painted turtles (*Chrysemys picta*) sampled when not submerged (controls), after being forcibly submerged, and after 1 hr of recovery from submergence with or without a platform. The box plots show the median within the box, with box boundaries being the 25th and 75th percentiles, and whiskers representing the 10th and 90th percentiles. Box plots that were not significantly different according to Mann-Whitney tests with a Bonferroni correction (α = 0.008) are indicated by similar letters.

assisted recovery, such as being left out of the water, may be a useful approach to recuperate turtles severely impaired after prolonged submergence.

The 3 measures of impairment were all affected by the 12-hr submergence period, yet the recovery rate of each measure differed. Blood pH was recovered to baseline (control) levels within an hour, reflexes were in the process of recovering, and blood lactate had yet to show signs of recovery within an hour after submergence. Herbert and Jackson (1985) found similar results: after anoxia, painted turtles rapidly recovered blood pH while lactate removal took much longer. Reflex impairment represented blood pH and lactate responses by having an intermediate response rate. The presence or absence of reflexes can indicate signs of stress at the level of the whole animal (Davis 2010). Thus, behavioral and reflex responses were a good, simple, and noninvasive indicator of overall turtle stress when subjected to anoxia.

Adapting the use of RAMP from fish to gauge the severity of anoxia on painted turtles was effective. All the reflexes used in this study indicated some level of impairment following submergence, with some reflexes being more sensitive than others. The tactile responses to the limbs and head were the most insensitive to submergence: they were often the only remaining reflexes after submergence (Table 1). Thus, the absence of tactile responses could be indicative of turtles requiring assisted recovery the most. Other species of turtles may require different types of reflex responses that suit their morphology better (e.g., loggerhead turtles, *Caretta caretta*, are too large to use a hand holding ''escape response''). However, a tactile response is a universal reflex that can be used in all species. Employing a presence/absence scoring reduced subjectivity and will ease the training of commercial fishers, researchers, and government and nongovernment agencies on the use of the RII. In sum, we feel the RII is a very useful tool to assess turtle condition.

Turtles are particularly adept at withstanding long periods of submergence in water, yet they can drown in fishing nets, especially in warm waters. When removing turtles that exhibit minimal to no reflex responses (and may even appear dead) from submerged nets, in particular a tactile response, having turtles recover out of water will likely aid in preventing postrelease mortality. We attempted to minimize mortality by limiting the period of submergence to 12 hrs, but for longer submergence periods typical of commercial fisheries, assisted recovery may become even more important because turtles will be more exhausted than in our experiment. Although reduction of turtle mortality can be achieved through educating fishers on proper handling and recovery methods for turtles, the first course of action should be checking nets frequently and implementing seasonal or area restrictions. Bycatch reduction devices (turtle excluders and escape modifications) are also effective in reducing the number of turtles caught in nets while maintaining fish catch (Guillory and Prejean 1998; Lowry et al. 2005; Fratto et al. 2008; Hart and Crowder 2011; Larocque et al. 2012c). However, since turtle bycatch can still occur despite these conservation measures, albeit at reduced levels, examining reflex impairment is an effective and inexpensive way to discern whether turtles require assisted recovery after incidental capture in submerged nets.

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