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Physiological disturbances and behavioural impairment associated with the incidental capture of freshwater turtles in a commercial fyke-net fishery

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ABSTRACT: Turtles are caught as bycatch in commercial fisheries in both inland and marine waters. Turtle mortality associated with bycatch is concerning, as life-history characteristics of turtles, including high juvenile mortality and delayed sexual maturity, make them particularly susceptible to population declines following small increases in adult mortality. In eastern Ontario, Canada, freshwater turtles are encountered as bycatch in an inland commercial fyke-net fishery. Although some temperate turtle species can tolerate prolonged submergence, their ability to withstand submergence decreases as water temperatures increase such that turtles may experience severe physiological disturbances and mortality following prolonged forced submergence. The purpose of our study was to evaluate the sublethal physiological consequences and related behavioural impairments associated with fyke-net capture for 3 species of freshwater turtles (eastern musk turtle *Sternotherus odoratus*, northern map turtle *Graptemys geographica* and painted turtle *Chrysemys picta*). Individuals that were entrapped for 3 h at elevated water temperatures (23 to 29°C) displayed considerably higher blood lactate and lower blood pH compared to free-living individuals. This trend was consistent across species and sexes. Despite having the largest increase in blood lactate, musk turtles did not exhibit behavioural impairment from entrapment, whereas both map and painted turtles displayed low responsiveness to behavioural assessments following entrapment. Our results suggest that sub-lethal responses can be used to identify potential harm or fitness impacts even in the absence of immediate mortality. Assessment of behavioural impairments, which could compromise activity and potentially result in post-release mortality, is important for protected and at-risk species that exhibit high juvenile mortality and delayed sexual maturity.

KEY WORDS: Bycatch · Conservation physiology · Entrapment · Submergence · At-risk · Sub-lethal · Lactate

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INTRODUCTION

Bycatch, the capture of non-targeted species, frequently occurs in commercial fisheries (Crowder & Murawski 1998, Hall et al. 2000, Hall & Mainprize 2005). Bycatch of marine species, especially turtles, has attracted considerable scientific and media attention (Lewison et al. 2004, Wallace et al. 2010). A variety of marine turtle bycatch reduction strategies have been developed and tested (UN FAO 2009), including the turtle excluder device, which has prevented the accidental capture of turtles in

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the shrimp trawl fishery (Seidel & McVea 1982). Despite being less well documented (Raby et al. 2011), bycatch of freshwater turtles also occurs in a variety of inland fisheries (Barko et al. 2004, McClellan et al. 2009, Larocque et al. 2012a). Turtles typically exhibit high juvenile mortality and delayed sexual maturity (Congdon et al. 1993). Thus, slight increases in adult mortality, from sources such as bycatch, have the potential to cause population declines (Congdon et al. 1993, Gibbons et al. 2000). This is particularly concerning given the at-risk status of many marine and freshwater turtles (Burke et al. 2000).

Interactions with fishing gear can cause stress and injuries that alter the health, condition, behaviour, and potentially the survival of animals, something well studied in fish (e.g. Chopin & Arimoto 1995, Davis 2002). These interactions have been comparatively poorly studied (aside from immediate mortality) in other taxa such as mammals, birds and reptiles, including marine turtles. Air-breathing organisms are at particular risk of bycatch-related stress and mortality as a result of entrapment and entanglement in fishing gear that impedes their ability to reach the water surface and respire. The capacity to withstand the effects of submergence will dictate the likelihood of mortality for a given species following capture in commercial fishing nets. Many freshwater turtle species can spend extended periods submerged. Some species, such as painted turtles *Chrysemys picta*, are able to remain submerged with little to no oxygen for extensive periods during hibernation (Belkin 1963, Gatten 1981, Milton & Prentice 2007). In addition to reduced metabolic rate during submergence, painted turtles rely on anaerobic metabolism to further reduce oxygen requirements (Jackson 2000a). The mechanisms and ability to withstand low oxygen for prolonged periods is species-specific (Reese et al. 2001). For instance, some species have a better ability to sequester CO_2: painted turtles appear to be among the most proficient species and are able to tolerate extensive periods in true anoxic conditions by using carbonate from their shells to buffer blood acidosis (Ultsch 1989, Ultsch & Jackson 1995, Jackson 2000a). Another mechanism frequently used by some turtles to acquire oxygen is the use of extrapulmonary gas exchange (e.g. eastern musk turtle *Sternotherus odoratus*: Ultsch et al. 1984, Ultsch & Cochran 1994). Individuals use their highly vascularized skin lining the cloaca and buccopharynx cavity to obtain oxygen from water (Feder & Burggren 1985, Ultsch 1988, Stone et al. 1992). Collectively, these mechanisms all contribute to a turtle's ability to withstand submergence associated with incidental capture.

For freshwater turtles, prolonged entrapment within nets is most likely stressful, particularly when water is elevated (Fratto et al. 2008, Larocque et al. 2012b). Elevated water temperatures and ensuing increased metabolic rates reduce the length of time a turtle can cope with submergence (Musacchia 1959, Herbert & Jackson 1985, Ultsch 1989). In addition, as water temperatures increase, dissolved oxygen levels decrease, thus limiting the available oxygen for uptake, resulting in rapid lactate accumulation even for species capable of extrapulmonary gas exchange (Frankel et al. 1966, Dejours 1994). Finally, entrapment exposes turtles to a variety of additional stressors, in particular the exertion associated with trying to escape from nets and interactions with other species, both target and bycatch, in a confined area.

The objective of our study was to assess the sublethal consequences of fyke-net entrapment on 3 species of freshwater turtles. In particular, we evaluated blood lactate and pH, as well as behavioural responsiveness following experimental entrapment. We also evaluated whether male and female painted turtles differed in their physiological and behavioural responses to entrapment. We focused on freshwater turtles that are incidentally captured in a small-scale commercial fyke-net fishery in eastern Ontario, Canada (Carrière et al. 2009, Larocque et al. 2012a,b). Upon capture, fishers are required to discard turtles (whether alive or dead) along with other bycatch (e.g. gamefish) as soon as possible after landing. Commercial fishing in that region occurs on inland lakes and rivers, with sunfish *Lepomis* spp. being the primary targeted species, in addition to bullhead *Ameiurus* spp., yellow perch *Perca flavescens*, black crappie *Pomoxis nigromaculatus* and rock bass *Ambloplites rupestris* (Burns 2007, Larocque et al. 2012a). The habitats of freshwater turtles and targeted commercial fish overlap, thus increasing the possibility of turtle entrapment (Carrière et al. 2009, McClellan & Read 2009, Larocque et al. 2012a). Presently, the Committee on the Status of Endangered Wildlife in Canada lists 7 of the 8 turtle species present in Ontario as species at risk. Of these 7 at-risk species, 2 are most likely to interact with commercial fishing gear and hence were the focus of our study: the eastern musk turtle and the northern map turtle *Graptemys geographica* are both recognized as special concern. In addition, the non-threatened painted turtle was used as a model, as it is the most commonly captured species in this fishery.

MATERIALS AND METHODS

Study area

We conducted the study on Lake Opinicon (44° 34′ N, 76° 19′ W) approximately 100 km southwest of Ottawa, Ontario, Canada. Experiments were conducted between 30 June and 28 July 2011 when water temperatures ranged from 23 to 29°C. Free-living control turtles were captured between 14 June and 17 September 2011 when water temperatures ranged from 19 to 28°C and between 29 May and 3 June 2012 when water temperatures ranged from 20 to 25°C. For all experiments dissolved oxygen was at or near saturation (e.g. 6 to 8 mg l^{-1}).

Fyke nets

We used fyke nets similar to those used in the local commercial fishery to collect turtles following the methods outlined by Larocque et al. (2012a). Nets were fished in pairs and were attached by leads (see Fig. 1 in Larocque et al. 2012a). Each net contained 7 steel hoops, which were 0.5 m apart and 0.9 m in diameter, with 2 throats located in the second and fourth hoop. Two wings and a lead were attached vertically to the mouth of each net. Wings were 4.6 m long by 0.9 m high, while each lead was 10.7 m long by 0.9 m high. All gear was fabricated with 2.54 cm square, 5.08 cm stretch, nylon mesh.

Experimental procedure

We used 73 turtles of 3 different species for both treatment and control groups (Table 1). To assess sex-specific differences, we used only painted turtles despite an approximately equal sex capture ratio of 1:1 in the other species to minimize any potential sub-lethal effects on females. This was a condition of our Scientific Collection Permit and Species-at-Risk Permit from the Ontario Ministry of Natural Resources. We used 2 groups to determine the physiological effects and behavioural impairments associated with entrapment in fyke nets: a treatment group in which turtles were submerged in a fyke net for 3 h, and a control group of free-living turtles. This submergence time was chosen to provide sufficient time for blood physiology to respond to capture stress while avoiding immediate mortality (Larocque et al. 2012b). It should be noted that our treatment length of 3 h is not representative of typical entrapment length within the commercial fishery in Ontario, as fishers are required to check their nets every 2 to 7 d, depending on the season. Our estimates of physiological disturbance are therefore very conservative.

Control turtles were 'free-living' individuals which were caught via dip-net or snorkeling and sampled immediately (within 3 min of capture) to obtain baseline blood physiology values and behaviour tests and were transported back to the laboratory for morphometric measurements. Turtles were held outdoors in ~700 l fibreglass tanks at ambient temperatures until they could be returned to their capture location. Tanks were supplied with lake water using a flow-through system, and turtles were not fed, but were provided with basking platforms and exposed to ambient sunlight.

Treatment individuals were collected initially by fyke nets equipped with floats, which were checked daily, and captured turtles were transported back to the lab to obtain morphometric measurements. Turtles were held in outdoor ~700 l fibreglass tanks at ambient temperature for a minimum of 48 h before experimental trials to eliminate any potential effects of initial capture stress. After a minimum of 48 h, individuals were subjected to simulated entrapment for 3 h using a fyke net that was modified to prevent escape or entry. The net was completely submerged in the lake in approximately 1.5 m of water which ranged from 23 to 29°C over the course of this experiment.

Table 1. *Sternotherus odoratus*, *Graptemys geographica*, and *Chrysemys picta.* Sample sizes, carapace length (CL), and mass of 3 turtle species used to assess the sub-lethal consequences of entrapment in commercial fishing nets in Lake Opinicon, Ontario, Canada

Species	Sex	Total N (control, treatment)	Mean ± SE CL (mm)	Mean ± SE mass (g)
Eastern musk turtle	Male	18 (8,10)	107 ± 4	186 ± 16
Northern map turtle	Male	20 (10,10)	115 ± 6	178 ± 13
Painted turtle	Male	16 (6,10)	139 ± 2	330 ± 12
	Female	19 (9,10)	154 ± 3	481 ± 20

Blood physiology

All blood samples were taken within 3 min of capture or removal from the net. Approximately 0.5 ml of blood was taken from the caudal vasculature on the dorsal portion of the tail using a 1 ml Tuberculin slip tip with a sodium-heparinized (10 000 USP units ml^{-1}; Sandoz) coated syringe with a 25 gauge, 38 mm needle for painted and map turtles (Becton Dickinson). Smaller 28.5 gauge needles and syringes were used for musk turtles (Becton Dickinson). Blood lactate was measured immediately after collection using a Lactate Pro™ meter (Arkray) that was previously validated with teleost fish (Brown et al. 2008). Remaining blood was transported back to the field laboratory in the syringe on ice in a cooler. Blood pH was measured using a field physiology meter within 2 h of collection with a 3-point calibrated minilab IQ128 Elite pH meter (IQ Scientific Instruments) at ambient temperature.

Behavioural assessments

Both experimental and control groups were tested for behavioural responses. Involuntary response to a variety of stimuli such as touch, gravity and sound can be helpful in predicting mortality. These assessments to predict mortality have been used on a variety of vertebrates (Davis 2007, 2010), including freshwater turtles (LeDain et al. in press). We used 6 behavioural tests, similar to the ones employed by LeDain et al. (in press) that included escape ability, righting ability (both on land and in water), response to startles (audible/pressure and visual), and tactile stimuli to the head, limbs and tail (Table 2) The ability of turtles to complete these tests can serve as indicators of their condition. We combined tactile stimuli into a single response, as there was no variation between the reactions to head and limb/tail stimuli. If the individual responded to the stimulus, the behaviour was scored as present; lack of reaction indicated an absent response. Each behaviour was scored as present (1) or absent (0) and converted into a behaviour impairment index (BII) which is an overall score of impairment for each individual based on the number of tests performed where BII = 1 − (sum of individual test scores/total possible score of 6). The BII ranges from 0, which indicates that the individual was not impaired, to a maximum score of 1, which indicates that the turtle was completely impaired.

Statistical analyses

We compared blood lactate and pH levels of control and treatment groups among male turtles of the 3 species. Blood lactate and pH residuals did not violate the assumptions of normality and homogeneity of variance; therefore we used a 2-way ANOVA to test the effect of species and group on blood lactate and pH. To assess whether control and experimental groups differed within a species, we used follow-up 2-sample *t*-tests that assumed unequal variance. We also evaluated physiological differences between males and females in painted turtles from control and treatment groups. Both blood lactate and pH residuals did not violate the assumptions of normality and homogeneity of variance, so we used a 2-way ANOVA to test the effect of species and group on BII.

Table 2. *Sternotherus odoratus*, *Graptemys geographica*, and *Chrysemys picta.* Description of the 6 behavioural tests, adapted from LeDain et al. (in press), that were performed on 3 species of turtles and used to assess the sub-lethal consequences of entrapment in commercial fishing nets in Lake Opinicon, Ontario, Canada

Behavioural test	Description
Escape	Turtles were held posteriorly and completely submerged for 10 s; if any attempt to escape (moving limbs, moving neck/head) was observed, the behaviour was scored as present.
Righting (in water)	Turtles were placed on the their carapace, while being held underwater in a tub; if successfully able to right themselves or if any attempts were made (limb or head movement), the behaviour was scored as present.
Righting (on land)	Turtles were placed on their carapace, on land, and left for 10 s; if successfully able to right themselves or any attempts (limb or head movement) were made, the behaviour was scored as present.
Audible threat	Turtles were placed in a 14 gallon (~53 l) plastic tub, and were not held. The sides of the tub were gently tapped; if any signs of threat were evident (retraction of head, abrupt change in direction), the behaviour was scored as present.
Visual threat	Turtles were placed in a 14 gallon (~53 l) plastic tub, and were not held. A hand was waved within 10 cm of their face; if any attempt to retract the head was observed, the behaviour was scored as present.
Tactile stimuli	Turtles were held posteriorly and their tail, a limb and their head were gently pinched; if any attempt to retract the limb/head/tail was observed, the behaviour was scored as present.

Behavioural data were also analysed using a 2-way ANOVA since BII score residuals did not violate the assumptions of normality and homogeneity of variance. We again used follow-up 2-sample *t*-tests that assumed unequal variance to assess whether control and experimental groups differed within each species. All statistical tests were performed using JMP (Version 9.0.1, SAS Institute). Significance was accepted at $\alpha < 0.05$.

RESULTS

Of the 73 individuals sampled, all were able to complete the trial, and no turtles died. Our test of the physiological effects of entrapment in males of 3 species revealed a significant interaction between species and experimental group for blood lactate (partial $R^2 = 0.02$, $F_{2,48} = 14.19$, $p < 0.001$) and marginally significant for blood pH (partial $R^2 = 0.01$, $F_{2,48} = 3.18$, $p = 0.051$). These interactions indicate that the effect of treatment on blood lactate and pH differed by species. Post hoc 2-sample *t*-tests revealed that blood lactate was significantly higher in treatment than in control groups in all 3 species: map turtles ($t_{17} = 18.82$, $p < 0.001$), musk turtles ($t_9 = 20.81$, $p < 0.001$) and painted turtles ($t_{11} = 16.39$, $p < 0.001$; Fig. 1). Accordingly, blood pH was also lower in treatment groups than in control groups in all 3 species: map turtles ($t_{13} = 9.684$, $p < 0.001$), musk turtles ($t_{16} = 17.313$, $p < 0.001$) and painted turtles ($t_8 = 10.82$, $p < 0.001$; Fig. 1). Significant interactions resulted from musk and painted turtles having marked increases in blood lactate and decreases in pH between control and treatment groups, while map turtles exhibited less dramatic differences (Fig. 1).

We investigated behavioural impairment associated with entrapment in males of 3 species. There was a significant interaction between species and experimental group for the behavioural scores (partial $R^2 = 0.16$, $F_{2,48} = 14.07$, $p < 0.001$), indicating that the 3 species were not impaired to the same extent. Post hoc 2-sample *t*-tests revealed that the BII scores were significantly different between control and treatment groups for map turtles ($t_9 = 6.8206$, $p < 0.001$) and painted turtles ($t_{12} = 4.8179$, $p < 0.001$), but there was no significant difference in musk turtles ($t_9 = 1$, $p = 0.3434$; Fig. 2).

When we investigated sex differences in physiological responses to entrapment in painted turtles, there were no significant sex by group interactions for blood lactate (partial $R^2 < 0.001$, $F_{1,31} = 0.05$, $p = 0.83$) or blood pH (partial $R^2 = 0.01$, $F_{1,31} = 3.76$, $p = 0.062$). In addition, there was no significant difference between males and females in blood lactate (partial $R^2 < 0.001$, $F_{1,31} = 0.09$, $p = 0.77$) or blood pH (partial $R^2 < 0.001$ $F_{1,31} = 0.07$, $p = 0.79$, Fig. 1). There was a significant difference between painted turtle control and submerged groups when comparing both sexes in both blood lactate (partial $R^2 = 0.94$, $F_{1,31} = 809.95$, $p < 0.001$) and blood pH (partial $R^2 = 0.91$, $F_{1,31} = 336.37$, $p < 0.001$; Fig. 1).

BII scores between male and female painted turtles also lacked a sex by group interaction (partial $R^2 < 0.001$, $F_{1.31} = 0.014$, $p = 0.906$). In addition, there was no significant difference between males and females (partial $R^2 < 0.001$, $F_{1,31} = 0.027$, $p = 0.871$; Fig. 2). There was a significant difference between painted turtle control and submerged group behaviour scores (partial $R^2 = 0.57$, $F_{1,31} = 43.18$, $p < 0.0001$; Fig. 2).

DISCUSSION

Our main objective was to determine the sub-lethal consequences of fyke-net entrapment on 3 species of freshwater turtles. It was evident that simulated incidental capture causes significant changes to blood physiology compared to free-living individuals in all 3 species. Although the literature lacks baseline blood physiology values for musk and map turtles at temperatures similar to our study, all 3 species displayed control values similar to baseline blood lactate (~1.5 mmol l^{-1}) and pH (~7.8) found in painted turtles at similar temperatures to our study (~22°C; Keiver et al. 1992a,b, Warren & Jackson 2004). Blood lactate and pH trends associated with submergence were similar to trends found by Larocque et al. (2012b) where research focused on painted turtles submerged for 4 h. Clearly, treatment turtles in our experiment experienced stress related to prolonged forced submergence. Submergence of painted turtles for 12 h in small cages that prevented extensive movements also resulted in similar blood lactate and pH values to those we found (LeDain et al. in press). Entrapment in nets has the potential to cause exhaustion more rapidly in turtles as a result of the ability to move within the net and the active attempts to escape (Larocque et al. 2012b). All 3 species displayed a decline of 0.7 or greater in blood pH between control and treatment groups (Fig. 1). Decline in blood pH of approximately 1 unit can be lethal for the freshwater turtles assessed in this study (Ultsch et al. 1984). In addition, despite the relatively short entrapment period of only 3 h, lactate values of en-

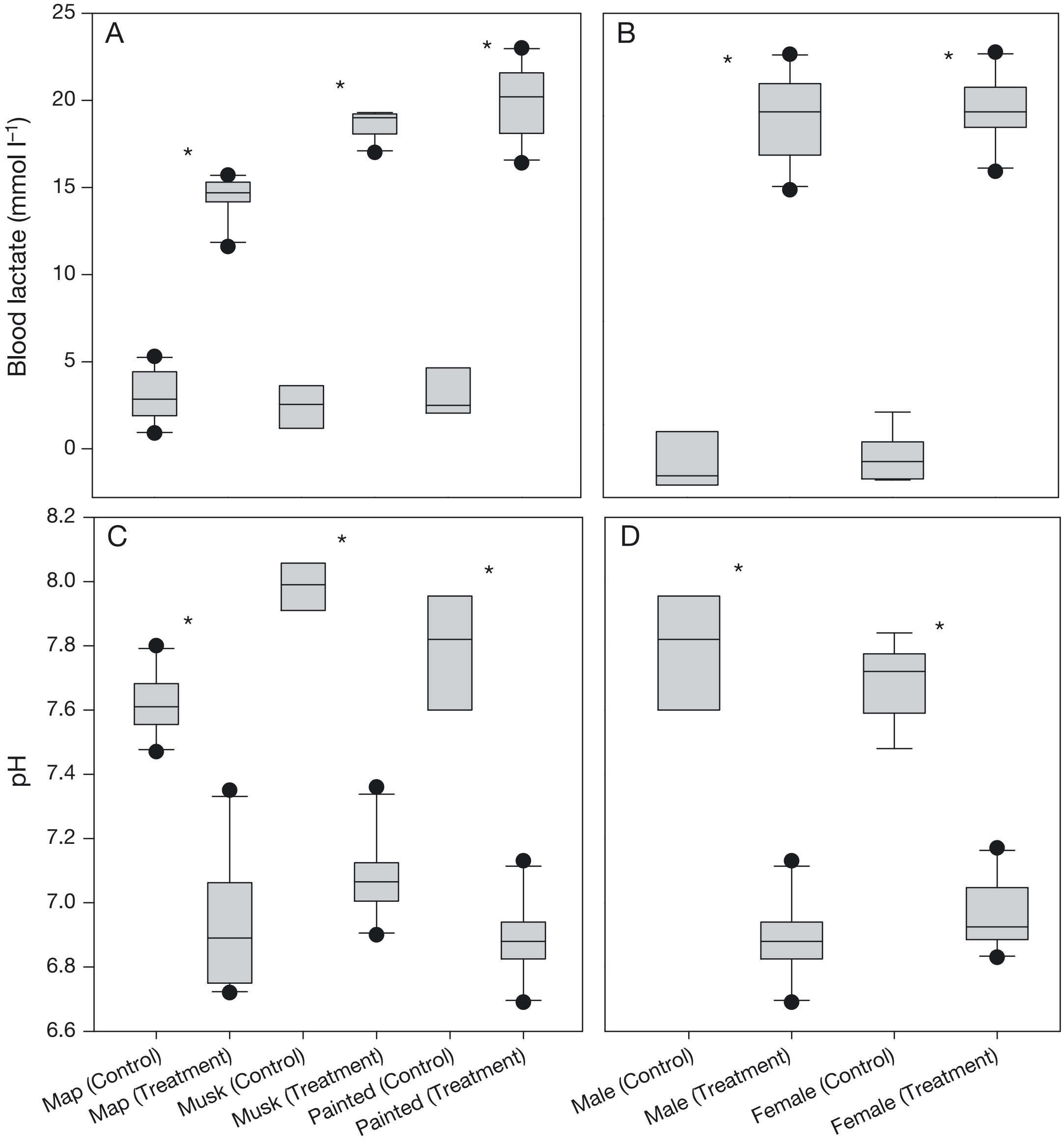

Fig. 1. *Graptemys geographica*, *Sternotherus odoratus*, and *Chrysemys picta*. Blood lactate (top panels) compared (A) in males among species and (B) between sexes (painted turtles only) and pH (bottom panels) compared (C) in males among species and (D) between sexes of control and submerged painted turtles. Median is reported along with the 25th and 75th percentiles. Bars represent the 10th and 90th percentiles, and any outliers (black dots) are shown. Significant differences ($p < 0.05$) between control and submerged individuals are represented by asterisks (*). Map: northern map turtle; Musk: eastern musk turtle; Painted: painted turtle

trapped turtles were similar to those found in hibernating painted turtles after 100 to 150 d (approximately 20 to 25 mmol l^{-1}, Reese et al. 2004) at 3°C. Additionally, increased rate and intensity of movement in the net as a result of increased temperature would result in an increased rate of oxygen consumption, thereby increasing acidosis (Robin et al. 1964, Jackson & Silverblatt 1974, Herbert & Jackson 1985). These findings agree with previous research suggesting that water temperature, regardless of the oxygen content, can limit duration of survival (Ultsch 1989). Similar studies have assessed the physiological effects of various types of fishing gear, such as trawl nets (Stabenau et al. 1991, Harms et al. 2003) and gillnets (Snoddy et al. 2009) on blood physiology of marine turtles and have shown similar effects of entrapment on blood physiology as our study.

Fyke-net entrapment and associated submergence led to species-specific physiological responses, pointing to the potential danger of using a single model

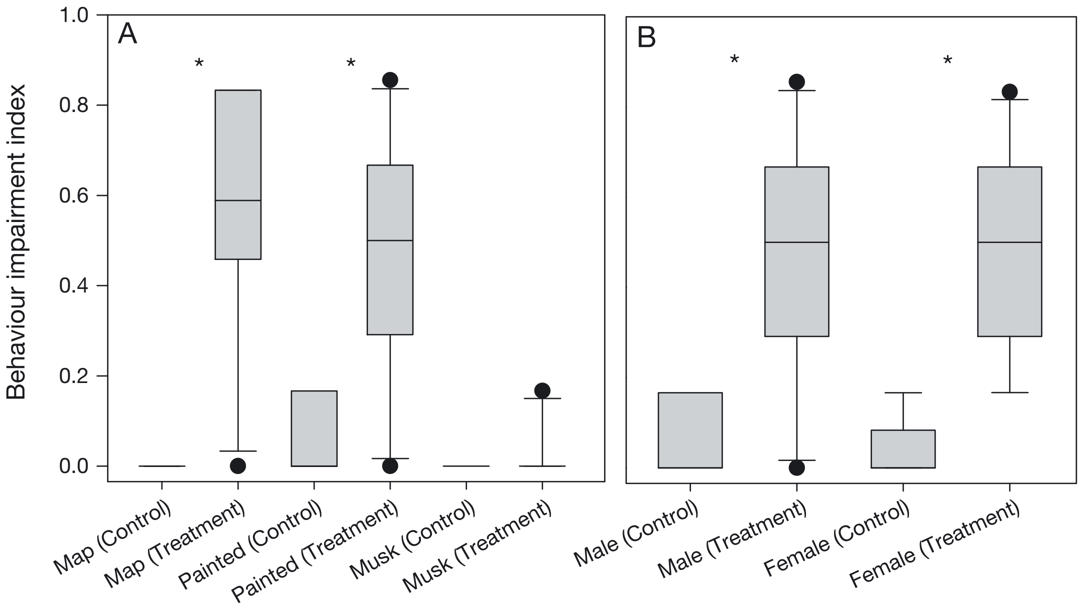

Fig. 2. *Graptemys geographica*, *Sternotherus odoratus*, and *Chrysemys picta.* Behaviour impairment index (BII) scores after 6 behavioural tests (see Table 2) of control and submerged individuals compared (A) in males among species and (B) between sexes of painted turtles. Scores range from 0 (individual was not impaired) to a maximum score of 1 (turtle was completely impaired). Median is reported along with the 25th and 75th percentiles. Bars represent the 10th and 90th percentiles, and any outliers (black dots) are shown. Significant differences ($p < 0.05$) between control and submerged individuals are represented by asterisks (*)

species as a surrogate for the various species potentially captured as bycatch (Fig. 1). Painted turtles exhibited a physiological response typical of a non-bimodally breathing turtle which relies on extreme anoxia tolerance. Denial of oxygen stimulates a physiological switch to anaerobic metabolism, which is characterized by the accumulation of blood lactate and decline in blood pH (Jackson 2000b). Despite having the ability to tolerate true anoxia in cold water, when submerged in elevated normoxic conditions (such as in this study), painted turtles were unable to acquire sufficient oxygen through secondary gas exchange mechanisms to remain aerobic (Ultsch & Jackson 1982, Jackson et al. 2000, Reese et al. 2001) as indicated in our study by elevated blood lactate and reduced blood pH. Therefore, painted turtles must metabolize energy anaerobically, resulting in blood acidosis (Ultsch & Jackson 1982, Ultsch et al. 1999, Jackson et al. 2000, Reese et al. 2001). Another mechanism of tolerating submergence is the use of gas exchange via extrapulmonary oxygen uptake or bimodal respiration, which is typified by musk and map turtles (Ultsch et al. 1984, Reese et al. 2001). Both species are known to be generally intolerant of prolonged submergence in anoxic conditions, but can survive extended periods of submergence in normoxic conditions during hibernation by remaining aerobic while accumulating relatively little blood lactate (Reese et al. 2001, 2003). Despite their tolerance of submergence in normoxic conditions, lactate accumulation in both species was relatively high in our study. We observed turtles swimming within the net and actively searching for exits. Increased activity, exacerbated by the elevated temperature, could be contributing to increased blood lactate and declines in pH (Dejours 1994). Gatten (1984) compared free-diving and forcibly submerged loggerhead musk turtles *Sternotherus minor* and found that involuntary submergence led to altered physiology and behaviour, including the accumulation of lactate. Stress associated with forced submergence may to some extent inhibit turtles from buffering acidosis, therefore altering associated blood physiology.

Furthermore, behavioural tests support our contention that the responses to simulated capture are species-specific. BII scores for submerged individuals were significantly lower than those for controls in painted and map turtles (Fig. 2). This indicates that physiological stress associated with submergence is manifested in behavioural impairments, such as righting ability (Fig. 3). Map turtles had the smallest percent increase in lactate and decrease in pH, despite being the most behaviourally impaired spe-

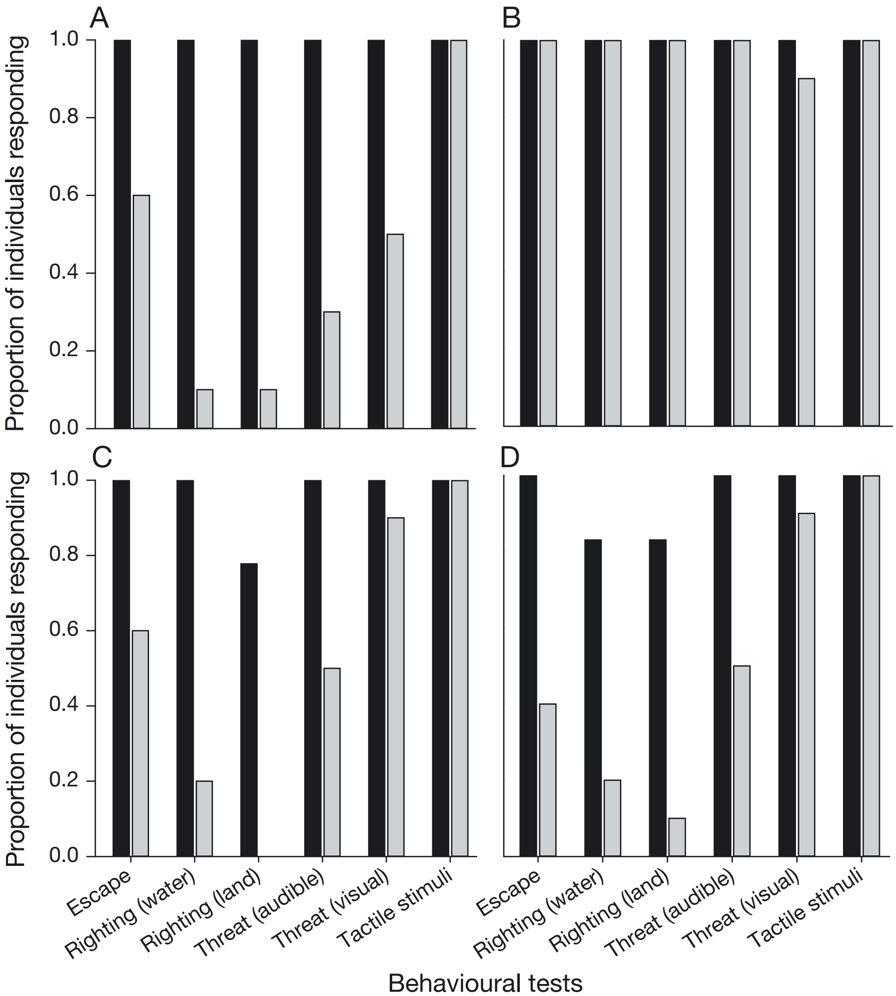

Fig. 3. *Graptemys geographica*, *Sternotherus odoratus*, and *Chrysemys picta*. Proportion of individuals that showed a positive response in each behavioural test (see Table 2) for each species: (A) male northern map turtle, (B) male common musk turtle, (C) female painted turtle and (D) male painted turtle. Black (grey) bars represent control (submerged) individuals

cies. This is not unexpected, as map turtles are known for their poor ability to tolerate blood acidosis (Reese et al. 2001). These results point to a species-specific sensitivity and some incongruence between blood physiology and behavioural indicators, again outlining the potential danger of relying on data from a single species to make management recommendations. Surprisingly, musk turtles did not have significantly different BII scores between control and treatment groups despite having significantly different blood physiology values. Out of the 6 reflex tests, the behaviour was present for all individuals from control and treatment groups for 5 of the tests (Fig. 3). Their ability to obtain oxygen from normoxic water (Ultsch et al. 1984) could account for their behavioural responsiveness, although we would expect to see corresponding physiological results. While accumulation of lactate occurs rather quickly, studies have shown that it takes several hours for lactate to be metabolized (Bennett 1978, Seymour 1982). Quick accumulation and potential lag time in metabolizing lactate could account for incongruence between blood physiology and behavioural responses. Increased activity as a result of elevated temperature could result in increased lactate levels and decreased pH, but our behavioural results do not support this idea. Compared to fish, for which several examples are available of incongruence between physiological and reflex (including some behaviours) metrics associated with fisheries interactions (Davis et al. 2001, Thompson et al. 2008), no published examples exist that have evaluated both types of endpoints in freshwater turtles to determine whether our observations are unique.

Sex appeared to have no influence on physiological or behavioural impairment following submergence in painted turtles, as similar responses were seen in males and females (Figs. 1 & 2). Significant differences were seen in blood lactate and pH as well as BII scores between control and submerged turtles in both sexes. Some studies investigating normal blood physiology profiles for various turtle species (e.g. *Chelonia mydas*, *Podocnemis expanssa*) have determined that males and females have similar physiological profiles at rest (Bolten & Bjorndal 1992, Oliveira-Júnior et al. 2009). Alternatively, some studies have suggested significant differences in haematology between sexes, including New Guinea snapping turtles *Elseya novaeguineae* (Anderson et al. 1997), bog turtles *Clemmys muhlenbergii* (Brenner et al. 2002) and Mediterranean pond turtles *Mauremys leprosa* (Hidalgo-Vila et al. 2007). Because turtles are ectotherms, 'normal' values can vary as a result of many factors such as activity, temperature and seasonality. Other species should be investigated to determine whether this pattern is consistent (including across sexually dimorphic or imperiled species), as the use of surrogates is clearly not ideal.

Although freshwater turtles are particularly adept at tolerating submergence, incidental capture causes significant species-specific changes in physiology and can lead to behavioural impairments. These changes in blood physiology were seen despite a submergence period much shorter than is typical in the commercial fishery in eastern Ontario. Despite lack of immediate mortality, some species exhibited behavioural impairments, which would compromise their activity and potentially result in post-release mortality, such as drowning. Additional non-lethal consequences associated with submergence are currently unknown, and should be the focus of future research. Our findings point to intrinsic differences in blood physiology and behaviour among species and reflect the need for management decisions to account for inter-specific variation. Painted turtles have previously been used as surrogates for at-risk species in the eastern Ontario fyke-net fishery (Larocque et al. 2012b). Our study highlights the limitations of using a surrogate species to gauge the physiological response to incidental capture. The use of a conservative approach with safe and pre-determined endpoints allowed us to determine the effects of incidental capture on at-risk species. Although mortality is an ecologically relevant endpoint for studying the effects of bycatch in an experimental context, it is not ethically appropriate when studying at-risk animals (Putman 1995, Minteer & Collins 2005). Analysis of sub-lethal consequences, such as physiological disturbance and behavioural impairments, is becoming recognized as an important tool in conservation science (Wikelski & Cooke 2006, Cooke et al. 2013a). In particular, physiological (e.g. Davis 2002, Cooke et al. 2013b) and reflex (e.g. Davis 2010) endpoints serve as objective indicators of animal welfare in fisheries (Diggles et al. 2011, Cooke et al. 2013b) and can be used to inform conservation actions (Wikelski & Cooke 2006, Seebacher & Franklin 2012) given their utility in defining cause and effect relationships and elucidating mechanisms of mortality (Cooke & O'Connor 2010, Cooke et al. 2013a). By determining how species respond to forced submergence associated with incidental capture, we can assist managers in the development of successful and sustainable bycatch reduction strategies. Given that most endangered species legislations extend beyond mortality to include sublethal disturbances (e.g. 'harm' and 'harassment' are forbidden by the Canadian Species at Risk Act), efforts to document physiological and behavioural impairments could provide an objective means of evaluating consequences of different types of fisheries activities while also providing information on animal welfare (Diggles et al. 2011).

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LITERATURE CITED

Anderson NL, Wack RF, Hatcher R (1997) Hematology and clinical chemistry reference ranges for clinically normal, captive New Guinea snapping turtle (*Elseya novaeguineae*) and the effects of temperature, sex, and sample type. J Zoo Wildl Med 28:394–403

Barko VA, Briggler JT, Ostendorf DE (2004) Passive fishing techniques: a cause of turtle mortality in the Mississippi River. J Wildl Manag 68:1145–1150

Belkin DA (1963) Anoxia: tolerance in reptiles. Science 139: 492–493

Bennett AF (1978) Activity metabolism of the lower vertebrates. Annu Rev Physiol 40:447–469

Bolten AB, Bjorndal KA (1992) Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the southern Bahamas: size-specific and sex-specific relationships. J Wildl Dis 28:407–413

Brenner D, Lewbart G, Stebbins M, Herman DW (2002) Health survey of wild and captive bog turtles (*Clemmys muhlenbergii*) in North Carolina and Virginia. J Zoo Wildl Med 33:311–316

Brown JA, Watson J, Bourhill A, Wall T (2008) Evaluation and use of the Lactate Pro, a portable lactate meter, in monitoring the physiological well-being of farmed Atlantic cod (*Gadus morhua*). Aquaculture 285: 135–140

Burke VJ, Lovich JE, Gibbons JW (2000) Conservation of freshwater turtles. In: Klemens MW (ed) Turtle conservation. Smithsonian Institution Press, Washington, DC, p 156–179

Burns C (2007) Biological sustainability of commercial fishing in the inland waters of Kemptville District. OMNR Technical Paper. Ontario Ministry of Natural Resources, Kemptville

Carrière MA, Bulté G, Blouin-Demers G (2009) Spatial ecology of northern map turtles (*Graptemys geographica*) in a lotic and a lentic habitat. J Herpetol 43:597–604

Chopin FS, Arimoto T (1995) The condition of fish escaping from fishing gears — a review. Fish Res 21:315–327

Congdon JD, Dunham AE, Van Loben Sels RC (1993) Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. Conserv Biol 7:826–833
Cooke SJ, O'Connor CM (2010) Making conservation physiology relevant to policy makers and conservation practitioners. Conserv Lett 3:159–166
Cooke SJ, Sack L, Franklin CE, Farrell AP, Beardall J, Wikelski M, Chown SL (2013a) What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conserv Physiol, doi:10.1093/conphys/cot001
Cooke SJ, Donaldson MR, O'Connor CM, Raby GD and others (2013b) The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation, and relevance to stakeholders. Fisheries Manag Ecol 20:268–287
Crowder LB, Murawski SA (1998) Fisheries bycatch: implications for management. Fisheries 23:8–17
Davis MW (2002) Key principles for understanding fish bycatch discard mortality. Can J Fish Aquat Sci 59: 1834–1843
Davis MW (2007) Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. ICES J Mar Sci 64:1535–1542
Davis MW (2010) Fish stress and mortality can be predicted using reflex impairment. Fish Fish 11:1–11
Davis MW, Olla BL, Schreck CB (2001) Stress induced by hooking, net towing, elevated sea water temperature, and air in sablefish: lack of concordance between mortality and physiological measures of stress. J Fish Biol 58: 1–15
Dejours P (1994) Environmental factors as determinants in bimodal breathing: an introductory overview. Am Zool 34:178–183
Diggles BK, Cooke SJ, Rose JD, Sawynok W (2011) Ecology and welfare of aquatic animals in wild capture fisheries. Rev Fish Biol Fish 21:739–765
Feder ME, Burggren WW (1985) Cutaneous gas exchange in vertebrates: design, patterns, control and implications. Biol Rev Camb Philos Soc 60:1–45
Frankel HM, Steinberg G, Gordon J (1966) Effects of temperature on blood gases, lactate and pyruvate in turtles, *Pseudemyces scripta elegans, in vivo.* Comp Biochem Physiol 19:279–283
Fratto ZW, Barko VA, Pitts PR, Sheriff SL and others (2008) Evaluation of turtle exclusion and escapement devices for hoop-nets. J Wildl Manag 72:1628–1633
Gatten RE Jr (1981) Anaerobic metabolism in freely diving painted turtles (*Chrysemys picta*). J Exp Zool 216: 377–385
Gatten RE Jr (1984) Aerobic and anaerobic metabolism of freely-diving loggerhead musk turtles (*Sternotherus minor*). Herpetologica 40:1–7
Gibbon JW, Scott DE, Ryan TJ, Buhlmann KA and others (2000) The global decline of reptiles, déjà vu amphibians. Bioscience 50:653–666
Hall SJ, Mainprize BM (2005) Managing by-catch and discards: How much progress are we making and how can we do better? Fish Fish 6:134–155
Hall MA, Alverson DL, Metuzals KI (2000) By-catch: problems and solutions. Mar Pollut Bull 41:204–219
Harms CA, Mallo KM, Ross PM, Segars A (2003) Venous blood gases and lactates of wild loggerhead sea turtles (*Caretta caretta*) following two capture techniques. J Wildl Dis 39:366–374
Herbert CV, Jackson DC (1985) Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii.* I. Blood acid-base and ionic changes during and following anoxic submergence. Physiol Zool 58:655–669
Hidalgo-Vila J, Díaz-Paniagua C, Pérez-Santigosa N, Plaza A, Camacho I, Recio F (2007) Hematologic and biochemical reference intervals of free-living Mediterranean pond turtles (*Mauremys leprosa*). J Wildl Dis 43:798–801
Jackson DC (2000a) How a turtle's shell helps it survive prolonged anoxic acidosis. News Physiol Sci 15:181–185
Jackson DC (2000b) Living without oxygen: lessons from the freshwater turtle. Comp Biochem Physiol A Mol Integr Physiol 125:299–315
Jackson DC, Silverblatt H (1974) Respiration and acid-base status of turtles following experimental dives. Am J Physiol 226:903–909
Jackson DC, Crocker CE, Ultsch GR (2000) Bone and shell contribution to lactic acid buffering of submerged turtles *Chrysemys picta bellii* at 3°C. Am J Physiol Regul Integr Comp Physiol 278:R1564–R1571
Keiver KM, Weinberg J, Hochachka PW (1992a) The effect of anoxic submergence and recovery on circulating levels of catecholamines and corticosterone in the turtle, *Chrysemys picta.* Gen Comp Endocrinol 85:308–315
Keiver KM, Weinberg J, Hochachka PW (1992b) Roles of catecholamies and corticosterone during anoxia and recovery at 5°C in turtles. Am J Physiol 263:R770–R774
Larocque SL, Colotelo AH, Cooke SJ, Blouin-Demers G, Haxton T, Smokorowski KE (2012a) Seasonal patterns in bycatch composition and mortality associated with a freshwater hoop net fishery. Anim Conserv 15:53–60
Larocque SL, Cooke SJ, Blouin-Demers G (2012b) A breath of fresh air: avoiding anoxia and mortality of freshwater turtles in fyke nets by the use of floats. Aquat Conserv 22:198–205
LeDain MRK, Larocque SM, Stoot LJ, Cairns NA, Blouin-Demers G, Cooke SJ (in press) Assisted recovery following prolonged submergence in fishing nets can be beneficial to turtles: an assessment with blood physiology and reflex impairment. Chelonian Conserv Biol
Lewison RL, Freeman SA, Crowder LB (2004) Quantifying the effects of fisheries on threatened species: the impact of pelagic longlines on loggerhead and leatherback sea turtles. Ecol Lett 7:221–231
McClellan CM, Read AJ (2009) Confronting the gauntlet: understanding incidental capture of green turtles through fine-scale movement studies. Endang Species Res 10:165–179
McClellan CM, Read AJ, Price BA, Cluse WM, Godfrey MH (2009) Using telemetry to mitigate the bycatch of long-lived marine vertebrates. Ecol Appl 19:1660–1671
Milton SL, Prentice HM (2007) Beyond anoxia: the physiology of metabolic downregulation and recovery in the anoxia-tolerant turtle. Comp Biochem Physiol A Mol Integr Physiol 147:277–290
Minteer BA, Collins JP (2005) Why we need an 'ecological ethics'. Front Ecol Environ 3:332–337
Musacchia XJ (1959) The viability of *Chrysemys picta* submerged at various temperatures. Physiol Zool 32:47–50
Oliveira-Júnior AA, Tavares-Dias M, Marcon JL (2009) Biochemical and hematological reference ranges for Amazon freshwater turtle, *Podocnemis expansa* (Reptilia:

Pelomedusidae), with morphologic assessment of blood cells. Res Vet Sci 86:146–151
Putman RJ (1995) Ethical considerations and animal welfare in ecological field studies. Biodivers Conserv 4: 903–915
Raby GD, Colotelo AH, Blouin-Demers G, Cooke SJ (2011) Freshwater commercial bycatch: an understated conservation problem. Bioscience 61:271–280
Reese SA, Crocker CE, Carwile ME, Jackson DC, Ultsch GR (2001) The physiology of hibernation in common map turtles (*Graptemys geographica*). Comp Biochem Physiol A Mol Integr Physiol 130:331–340
Reese SA, Jackson DC, Ultsch GR (2003) Hibernation in freshwater turtles: softshell turtles (*Apalone spinifera*) are the most intolerant of anoxia among North American species. J Comp Physiol B 173:263–268
Reese SA, Stewart ER, Crocker CE, Jackson DC, Ultsch GR (2004) Geographic variation of the physiological response to overwintering in the painted turtle (*Chrysemys picta*). Physiol Biochem Zool 77:619–630
Robin ED, Vester JW, Murdaugh HV Jr, Millen JE (1964) Prolonged anaerobiosis in a vertebrate: anaerobic metabolism in the freshwater turtle. J Cell Comp Physiol 63:287–297
Seebacher F, Franklin CE (2012) Determining environmental causes of biological effects: the need for a mechanistic physiological dimension in conservation biology. Philos Trans R Soc Lond B Biol Sci 367:1607–1614
Seidel WR, McVea C Jr (1982) Development of a sea turtle excluder shrimp trawl for the southeast US penaeid shrimp fisheries. In: Bjorndal KA (ed) Biology and conservation of sea turtles. Smithsonian Institution Press, Washington, DC, p 467–502
Seymour RS (1982) Physiological adaptations to aquatic life. In: Gans C, Pough FH (eds) Biology of the Reptilia, Vol 13. Physiology D, physiological ecology. Academic Press, London, p 1–51
Snoddy J, Landon M, Blanvillain G, Southwood A (2009) Blood biochemistry of sea turtles captured in gillnets in the lower Cape Fear River, North Carolina, USA. J Wildl Manag 73:1394–1401
Stabenau EK, Heming TA, Mitchell JF (1991) Respiratory, acid-base and ionic status of Kemp's ridley sea turtles (*Lepidochelys kempi*) subjected to trawling. Comp Biochem Physiol A 99:107–111
Stone PA, Dobie JL, Henry RP (1992) Cutaneous surface area and bimodal respiration in soft-shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*), and mud turtles (*Kinosternon subrubrum*). Physiol Zool 65:311–330
Thompson LA, Donaldson MR, Hanson KC, Arlinghaus R, Cooke SJ (2008) Physiology, behavior and survival of angled and air exposed largemouth bass. N Am J Fish Manag 28:1059–1068
Ultsch GR (1988) Blood gases, hematocrit, plasma ion concentrations, and acid-base status of musk turtles (*Sternotherus odoratus*) during simulated hibernation. Physiol Zool 61:78–94
Ultsch GR (1989) Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. Biol Rev Camb Philos Soc 64:435–516
Ultsch GR, Cochran BM (1994) Physiology of northern and southern musk turtles (*Sternotherus odoratus*) during simulated hibernation. Physiol Zool 67:263–281
Ultsch GR, Jackson DC (1982) Long-term submergence at 3°C of the turtle, *Chrysemys picta bellii*, in normoxic and severely hypoxic water. I. Survival, gas exchange and acid-base status. J Exp Biol 96:11–28
Ultsch GR, Jackson DC (1995) Acid-base status and ion balance during simulated hibernation in freshwater turtles from the northern portions of their ranges. J Exp Zool 273:482–493
Ultsch GR, Herbert CV, Jackson DC (1984) The comparative physiology of diving in North American freshwater turtles. I. Submergence tolerance, gas exchange, and acid-base balance. Physiol Zool 57:620–631
Ultsch GR, Carwile ME, Crocker CE, Jackson DC (1999) The physiology of hibernation among painted turtles: the eastern painted turtle *Chrysemys picta picta*. Physiol Biochem Zool 72:493–501
UN FAO (United Nations Food and Agriculture Organization) (2009) Guidelines to reduce sea turtle mortality in fishing operations. UN FAO Fisheries Division, Rome
Wallace BP, Lewison RL, McDonald SL, McDonald RK and others (2010) Global patterns of marine turtle bycatch. Conserv Lett 3:131–142
Warren DE, Jackson DC (2004) Effects of swimming on metabolic recovery from anoxia in the painted turtle. J Exp Biol 207:2705–2713
Wikelski M, Cooke SJ (2006) Conservation physiology. Trends Ecol Evol 21:38–46

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