Animal Conservation

ZSL LET'S WORK FOR WILDLIFE

Animal Conservation. Print ISSN 1367-9430

Biologgers reveal post-release behavioural impairments of freshwater turtles following interactions with fishing nets

L. F. G. Gutowsky[1,†], L. J. Stoot[1,2,†], N. A. Cairns[1,2], J. D. Thiem[1,6], J. W. Brownscombe[1], A. J. Danylchuk[3], G. Blouin-Demers[2] & S. J. Cooke[1,4,5]

1 Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON, Canada
2 Herpetology Laboratory, Department of Biology, University of Ottawa, Ottawa, ON, Canada
3 Department of Primary Industries, Narrandera Fisheries Centre, PO Box 182, Narrandera, New South Wales 2700, Australia
4 Department of Environmental Conservation, University of Massachusetts, Amherst, MA, USA
5 Institute of Environmental Science, Carleton University, Ottawa, ON, Canada
6 Department of Primary Industries, Narrandera Fisheries Centre, PO Box 182, Narrandera, NSW, Australia

Keywords
sub-lethal; bycatch; behavioural effects; accelerometer; depth; temperature; overall dynamic body acceleration; fisheries.

Correspondence
Lee F. G. Gutowsky, Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON, Canada.
Email: lee_gutowsky@carleton.ca

†Shared first authorship.

Editor: Iain Gordon
Associate Editor: Lynne Shannon

Received 04 August 2016; accepted 01 November 2016

doi:10.1111/acv.12323

Abstract

Bycatch, the incidental capture of non-target organisms, occurs in most commercial fisheries. Although immediate bycatch mortality is frequently documented in fisheries, detrimental sub-lethal effects and potential post-release mortality remain largely unknown despite the potential population-level consequences. Turtles are captured as bycatch and their populations are vulnerable to slight increases in adult mortality. In eastern Ontario, turtles are frequently captured as bycatch in a small-scale freshwater commercial fyke-net fishery and, currently, the fate of discarded turtles is unknown. We wished to determine the effect of fyke-net capture on post-release survival and behaviour in eastern musk turtles *Sternotherus odoratus* and painted turtles *Chrysemys picta*. We used biologgers equipped with tri-axial acceleration, depth and temperature sensors to document locomotor activity, vertical distribution, and temperature use of entrapped (exposed to forced submergence for 4 h) and control turtles upon release. Overall dynamic body acceleration was used as a measure of post-release activity for the first hour, first 6 h, and first 48 h. Post-release mortality was not detected. Turtles subjected to entrapment exhibited lower activity during the first 6 h following release, and their vertical distribution and temperature use differed in the first 2 h following release, but these effects disappeared after 48 h, suggesting turtles have the ability to recover. Quantifying the post-release mortality and sub-lethal effects of entrapment is important for estimating the population effects associated with bycatch.

Introduction

Bycatch, the incidental capture and discard of non-target organisms, occurs in most commercial fisheries (Crowder & Murawski, 1998; Hall, Alverson & Metuzals, 2000; Hall & Mainprize, 2005). Fish bycatch accounted for *c.* 28% of total landings in the United States in 2002 (Harrington, Myers & Rosenberg, 2005). Although mortality frequently occurs, not all individuals caught as bycatch die immediately (Davis, 2002). Post-release mortality and detrimental sub-lethal effects have been reported in a variety of bycatch species including marine mammals, sea birds, sharks and marine turtles (Julian & Beeson, 1998; Lewison, Freeman & Crowder, 2004; Moore *et al.*, 2010; Finkbeiner *et al.*, 2011). Detrimental sub-lethal effects and post-release mortality remain largely undocumented, yet they can have considerable negative population-level consequences (Chopin & Arimoto, 1995; Davis, 2002; Lewison *et al.*, 2004). Unknown additional mortality rates are especially concerning for species that have slow maturation and long generation times (Crowder & Murawski, 1998; Hall *et al.*, 2000; Midwood *et al.*, 2015). Injuries and negative physiological effects sustained as a result of bycatch, such as net entanglement (e.g. Frick, Reina & Walker, 2010), hooking injuries and stress associated with prolonged submergence in air breathing organisms (e.g. Lewison *et al.*, 2005; Snoddy *et al.*, 2009) can be non-lethal, but can still impair behaviour. Behavioural impairments, such as reduced mobility and diminished foraging ability, can increase the risk of mortality (Davis, 2002). Various studies, mostly on fish, have focused on how sub-lethal

effects associated with capture manifest themselves behaviourally (reviewed in Wilson *et al.*, 2014). In addition, studies on fish have indicated that there are interspecific differences in the effect of sub-lethal stressors (Ryer, Ottmar & Strum, 2004). Identifying how behaviour is impaired following capture, especially locomotion, is crucial to estimate the overall negative population consequences of bycatch.

Biologgers are increasingly being used to study fine-scale, continuous animal movements in nature and to address conservation problems (Cooke, 2008; Rutz & Hays, 2009; Bograd *et al.*, 2010; Wilson *et al.*, 2015). While biologgers have been used to assess post-release behaviour and delayed mortality in marine turtles following interaction with fishing gear (e.g. Chaloupka, Parker & Balazs, 2004; Swimmer *et al.*, 2006; Snoddy & Williard, 2010), surprisingly little information exists on the post-release behaviour of freshwater turtle bycatch (Barko, Briggler & Osendorf, 2004; Larocque *et al.*, 2012*a*). Unlike marine turtles that are most frequently captured in long line and trawl fisheries, freshwater turtles are mostly encountered in trap net fisheries (Barko *et al.*, 2004; Larocque *et al.*, 2012*a*). Despite the ability of freshwater turtles to withstand extended periods of submergence, prolonged entrapment in fishing nets can result in drowning (Larocque *et al.*, 2012*a*). In addition, acute physiological and behavioural impairments can occur as a result of entrapment in fyke-nets (LeDain *et al.*, 2013; Stoot *et al.*, 2013), although this has not been assessed in free-ranging animals. Turtles are particularly susceptible to population declines following slight increases in adult mortality, such as bycatch mortality, because of their naturally high juvenile mortality and delayed sexual maturity (Congdon, Dunham & Van Loben Sels, 1993, 1994; Gibbons *et al.*, 2000).

Government regulations often require all bycatch be discarded immediately upon landing. Given that post-release survival is not guaranteed when presumably alive bycatch is released (Raby *et al.*, 2011), post-release behaviour and survival must be properly assessed for released freshwater turtle bycatch. Here, we determined the fate and examined post-release behaviour in freshwater turtles caught as bycatch in commercial fyke-nets. Painted turtles *Chrysemys picta* and musk turtles *Sternotherus odoratus* were used because both are commonly encountered as bycatch (Carrière, Bulté & Blouin-Demers, 2009; Larocque, Cooke & Blouin-Demers, 2012*b*; Larocque *et al.*, 2012*a*). We compared individuals exposed to simulated entrapment to control individuals that were not entrapped. With tri-axial accelerometers, we measured fine-scale activity, vertical distribution and temperature use following release.

Materials and methods

Study area and turtle collection

All work was conducted on Lake Opinicon (44° 34′ N, 76° 19′W) *c.* 100 km southwest of Ottawa, Ontario, Canada between 14 May and 19 June 2012 when water temperatures ranged from 18 to 26°C (dissolved O_2: 6–8 mg L^{-1}). Turtles were captured with fyke nets (a type of passive trapping gear; details in Larocque *et al.*, 2012*a*) set in shallow bays (2–3 m depth) for *c.* 24 h with floats to provide air pockets. Upon capture, turtles were returned to the field laboratory where they were measured (mass and carapace length), and sexed (based on external characteristics). Individuals were held outdoors in ~700 L fiberglass tanks at ambient temperature for *c.* 48 h to let capture stress wane. Tanks were supplied with lake water via a flow though system. Turtles were not fed, but were provided with basking platforms exposed to ambient sunlight.

Experimental procedure

We used 32 males of each species evenly split between treatment and control groups. The treatment group consisted of turtles entrapped for 4 h in a closed fyke net set without air access in 1.5 m of water (23 to 29°C), and the control group consisted of turtles placed into a tank with access to oxygen and a basking platform exposed to ambient sunlight for 4 h. Four hours of entrapment was chosen to provide sufficient time for impairment to occur while avoiding immediate mortality, although actual entrapment duration typical of the fishery can be up to 7 days (Larocque *et al.*, 2012*b*). We used a matched pair design in which, for each individual undergoing the entrapment treatment, we selected a control individual that was matched for species, size, capture day and capture location. Both individuals from a given treatment/control pair were released simultaneously at the same location (their capture location).

After initial capture, attachment points were created for the accelerometers by drilling two small holes between the 10th and 12th marginal scutes on the left side (Fig. 1). Pairs of control and treatment individuals were then allowed to

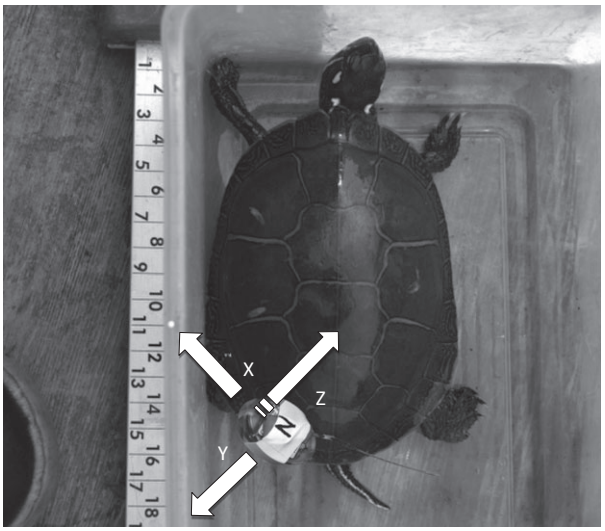

Figure 1 Placement of accelerometer logger on left side of a tagged painted turtle along with a radio transmitter to facilitate logger retrieval. Arrows show direction of *x*, *y* and *z*-axis.

recover for a minimum of 48 h, after which they underwent their respective treatments. After their 4 h experimental treatment, individuals were removed and tested for impairment using a series of basic behavioural responses (see below). Following behavioural assessment, tri-axial accelerometer loggers (model CEFAS G6a, 18 g in air, 10 Hz recording frequency, 1 Hz for temperature and depth; CEFAS Technology Limited, Suffolk, UK) were attached to the turtles, using a 13.6 kg stainless steel line. Tri-axial accelerometers measure both dynamic and static acceleration in units of gravity (g). Loggers also contained depth (herein referred to as vertical distribution, accuracy: ±1%; resolution: 4 cm) and temperature sensors (accuracy: 0.1°C; resolution: 0.03°C). Accelerometers were set to record for 48 h. To assist with tag retrieval, unique-frequency radio-telemetry transmitters (Model BD-2, 3.2 g in air, 20 cm trailing whip antenna, Holohill Systems Inc., Carp, ON, Canada) were tied to the accelerometers. The entire package weighed ~24 g in air and ~12 g in water. After logger and transmitter attachment (≤3 min procedure), turtles were immediately transported in a 95 L covered plastic container to their capture location for release. Forty-eight hours post-release, turtles were located using a hand-held radio-tracking receiver (Biotracker, Lotek Engineering, Inc.; Newmarket, ON, Canada) and 3-element yagi antenna (AF Antronics, Urbana, IL, USA). Recaptured turtles were released following retrieval of both tags.

Behavioural assessments

We used six behavioural tests (Table 2 in Stoot *et al.*, 2013) which assessed escape ability, righting ability (on both land and water), response to startles (audible/pressure and visual) and tactile stimuli to the head, limbs, and tail. The response was scored as present if the individual responded to the stimulus (1) or otherwise absent (0). Scores were converted into a behavioural impairment index (BII), which is an overall score of impairment for each individual based on the number of tests performed. The BII ranges from 0, which indicated that the individual was not impaired, to a maximum score of 1, which indicates that the individual was completely impaired and is calculated as BII = 1−(sum of individual test scores/total possible score of 6).

Data processing

Accelerometers were set to record total acceleration (g) at 10 Hz in three (x, y and z) axes. Total acceleration was calculated as the sum of static and dynamic acceleration. Tags were calibrated before deployment by rotating the device through known angles to real g (9.8 m s^{-2}; Gleiss *et al.*, 2010). Static and dynamic acceleration were separated by weighted smoothing at an interval of 3 s based on the methods of Shepard *et al.* (2008), using Igor Pro 6.0 software (WaveMetrics Inc., Lake Oswego, OR, USA). Median overall dynamic body acceleration (ODBA) values were calculated for both species for each time interval of interest. Vertical distribution and temperature data were logged every second for 48 h. For these metrics, medians were calculated for each 10 min interval beginning at the time of first release for a given matched pair of turtles. Data were processed in the R Statistical Environment (R Core Team, 2016).

Statistical analyses

To test for differences in behavioural impairment scores, we used a generalized linear model where the outcome was assumed to be binomially distributed. Species, treatment group and their interaction were included as explanatory variables. Pairwise statistical differences were assessed using a Tukey post hoc test (Hothorn, Bretz & Westfall, 2008).

To assess the effects of entrapment on activity, we calculated median ODBA values over three time periods post release: 10 min intervals for the first hour, 1 h intervals for the first 6 h and 6 h intervals for the first 48 h. We used a repeated measures two-way ANOVA to test for the effect of time and experimental group on ODBA. Tests of sphericity were performed, using Mauchly's test, and if it was violated, degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity. Follow-up one-way ANOVAs were performed to assess the effect of treatment when there was indication of a statistical interaction or of a main effect. Since sample sizes were modest, we conducted follow-up one-way ANOVAs when $P < 0.150$.

Most records (96.4%) of vertical distribution were less than 3 m. Thus, data were truncated such that vertical distribution was ≤3 m. Two linear mixed effects models (LME, assuming a Gaussian distribution) were specified to evaluate: (1) overall vertical distribution during the first 2 h and; (2) overall vertical distribution during the first 48 h post-release. Fixed effects included species, treatment group, time interval and several interactions (Table 1). Because data were nested, turtle ID was included as random effects in each model. Furthermore, a correlation structure was included to account for the additional intra-class correlation observed in the residuals (Zuur *et al.*, 2009). Model validation followed Zuur *et al.* (2009). Statistical significance was evaluated by examining the effect size in relation to baselines automatically generated in the LME function of the R package 'MASS' (Venables & Ripley, 2002).

Data exploration showed nonlinear patterns in temperature use following turtle release. We therefore opted to use generalized additive mixed models (GAMM). Temperature was modelled assuming a gamma distribution with a log-link function. To capture the variation within individuals, a random intercept was specified for turtle ID. Fixed factors included treatment and species. Time was fitted with a spline smooth in the R package 'mgcv' (Wood, 2006; Zuur *et al.*, 2009). Two models were specified with temperature use during the first 2 h and during the first 48 h post-release. P-values are approximate for GAMM smoothers, thus statistical significance for smoothers was considered at $P < 0.001$ (Zuur *et al.*, 2009). A lack of confidence limit overlap was used to assess pairwise statistical differences.

Table 1 Parameter estimates and statistical significance of generalized additive mixed model and linear mixed effect terms used to explore post-release temperature use and vertical distribution in *Chrysemys picta* and *Sternotherus odoratus*

Model description	Model type	Species	Parameters	LME					GAMM		
				Estimate	SE	d.f.	*t* value	*P*-value	*F* value	edf	*P*-value
Vertical distribution over 2 h	LME	*C. picta*	Intercept	0.080	0.189	3125	0.411	0.681	–	–	–
		S. odoratus	Net treatment	0.092	0.230	26	0.402	0.691	–	–	–
			Musk turtle	0.679	0.237	26	2.86	0.008	–	–	–
			Net treatment: musk turtle	−0.482	0.237	26	−2.04	0.052	–	–	–
Vertical distribution over 48 h	LME	*C. picta*	Intercept	0.152	0.344	1326	0.442	0.658	–	–	–
		S. odoratus	Net treatment	0.536	0.486	26	1.10	0.280	–	–	–
			Musk turtle	0.684	0.471	26	1.45	0.158	–	–	–
			Net treatment: musk turtle	−0.536	0.666	26	−0.804	0.428	–	–	–
Temperature use at 10 min intervals for 2 h	GAMM	*C. picta*	Intercept	3.20	0.056	152	56.1	<0.001	–	–	–
			Control treatment	–	–	–	–	–	16.6	3.14	<0.001
			Net treatment	0.043	0.080	12	0.534	0.594	8.65	1.00	0.004
Temperature use at 10 min intervals for 2 h	GAMM	*S. odoratus*	Intercept	3.20	0.023	174	142	<0.001	–	–	–
			Control treatment	–	–	–	–	–	13.5	4.18	<0.001
			Net treatment	0.002	0.032	14	0.062	0.95	0.265	1.00	0.607
Temperature use at 1 h intervals for 48 h	GAMM	*C. picta*	Intercept	3.11	0.052	642	60.3	<0.001	–	–	–
			Control treatment	–	–	–	–	–	29.7	8.24	<0.001
			Net treatment	−0.002	0.073	12	−0.027	0.979	33.7	8.18	<0.001
Temperature use at 1 h intervals for 48 h	GAMM	*S. odoratus*	Intercept	3.17	0.018	734	172	<0.001	–	–	–
			control treatment	–	–	–	–	–	30.6	8.18	<0.001
			Net treatment	0.002	0.026	14	0.077	0.939	24.6	7.81	<0.001

Note that GAMMs have both an additive and linear component.

Results

Of the 16 pairs of turtles that underwent treatment, all but one painted turtle (from the control treatment) survived for 48 h. Therefore, we used 7 pairs of painted turtles and 8 pairs of musk turtles to document the effect of entrapment on activity. The mass of turtles was similar for control and treatment for both musk ($t_{14} = 0.121$, $P = 0.547$) and painted turtles ($t_{12} = 0.326$, $P = 0.625$), which indicates that tag burden was equal in both experimental groups for both species.

Behavioural impairment index

Both control and treatment group musk turtles frequently responded to each behavioural test. Entrapped painted turtles rarely reacted when tested (Fig. 2). We investigated the immediate impairment associated with entrapment using BII. Statistically, there was an interaction between species and treatment group ($Z = 4.59$, SE = 0.79, $P < 0.001$) where, relative to controls, painted turtles showed impairment following entrapment ($Z = 4.50$, SE = 0.54, $P < 0.001$), whereas musk turtles did not ($Z = 1.69$, SE = 0.8463, $P = 0.38$), Fig. 3.

Locomotory activity

We examined ODBA as a proxy for locomotory activity every 10 min for the first hour post-release and found no significant interaction between time and treatment in painted turtles ($F_{1.99,\ 23.93} = 0.668$, $P = 0.522$) or in musk turtles ($F_{5,\ 10} = 1.986$, $P = 0.167$). There was no significant difference in locomotion over time in painted turtles ($F_{1.99,\ 23.93} = 2.426$, $P = 0.110$) or in musk turtles ($F_{5,\ 10} = 1.283$, $P = 0.344$; Fig. 4). In addition, we did not find significant differences in locomotion between experimental groups in painted turtles ($F_{1,\ 12} = 3.454$, $P = 0.088$) or in musk turtles ($F_{1,14} = 2.837$, $P = 0.114$). Follow-up one-way ANOVAs revealed that experimental groups moved less than controls in musk turtles during 0–10, 10–20, and 30–40 min after release, as well as during 40–50 min after release in painted turtles (Fig. 4).

For the analysis of ODBA every 1 h for the first 6 h post release, there was no significant interaction between time and treatment in painted turtles ($F_{2.25,\ 26.94} = 1.142$, $P = 0.339$) or in musk turtles ($F_{2.32,\ 32.48} = 0.515$, $P = 0.629$). Painted turtles moved less over time ($F_{2.25,\ 26.94} = 4.325$, $P = 0.020$), but not musk turtles

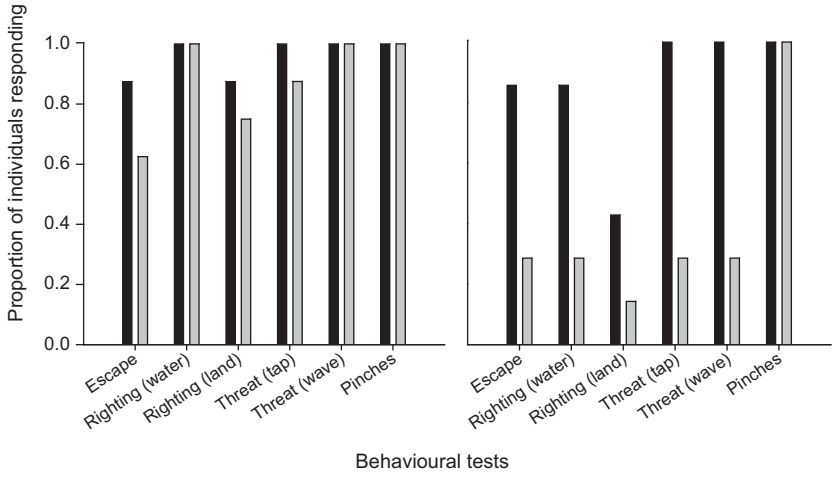

Figure 2 Proportion of individuals that responded with a positive response for each behavioural impairment index test for musk turtles (left) and painted turtle (right). Black bars denote control individuals and grey bars denote those subjected to simulated entrapment.

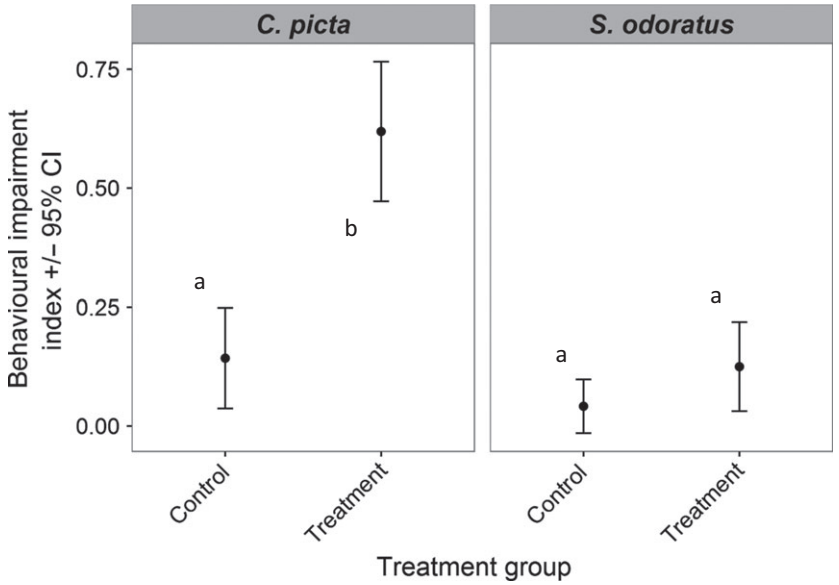

Figure 3 Fitted values (±95% confidence intervals) from the GLM of behavioural impairment index. Scores range from 0 (not impaired) to a maximum score of 1 (impaired). Statistical significant groups are denoted with a unique identifier.

($F_{2.32,\ 32.48} = 2.576$, $P = 0.084$; Fig. 4). Entrapped individuals moved less relative to controls in painted turtles ($F_{1,\ 12} = 5.912$, $P = 0.032$) and in musk turtles ($F_{1,14} = 4.588$, $P = 0.050$, Fig. 4).

For the analysis of ODBA every 6 h for 48 h post-release, there was no significant interaction between time and treatment in painted turtles ($F_{2.61,\ 31.33} = 0.102$, $P = 0.943$) or in musk turtles ($F_{7,\ 8} = 0.427$, $P = 0.861$). There was no difference in locomotion over time in painted turtles ($F_{2.61,\ 31.33} = 2.859$, $P = 0.059$) or in musk turtles ($F_{7,\ 8} = 2.273$, $P = 0.136$; Fig. 4). We found no significant difference in locomotion between experimental groups in painted turtles ($F_{1,\ 12} = 0.097$, $P = 0.761$) or in musk turtles ($F_{1,\ 14} = 2.823$, $P = 0.115$; Fig. 4).

Vertical distribution

Painted turtle vertical distribution was similar for the control [0.08 m ± 0.19 SE (−0.29, 0.45, 95% CI)] and entrapment treatments [0.17 m ± 0.19 SE (−0.19, 0.54, 95% CI)], whereas musk turtles in the control group were deeper [0.75 m ± 0.14 SE (0.48, 1.04, 95% CI)] than those subjected to entrapment [0.37 m ± 0.14 SE (0.09, 0.65, 95% CI)] for the first 2 h post-release (Fig. 5). Painted turtles in both the control and treatment groups spent time at the surface or out of water (Fig. 5). For both species, control and treatment groups covered a similar vertical distribution over 48 h post-release (Fig. 6; $P > 0.05$ in all cases).

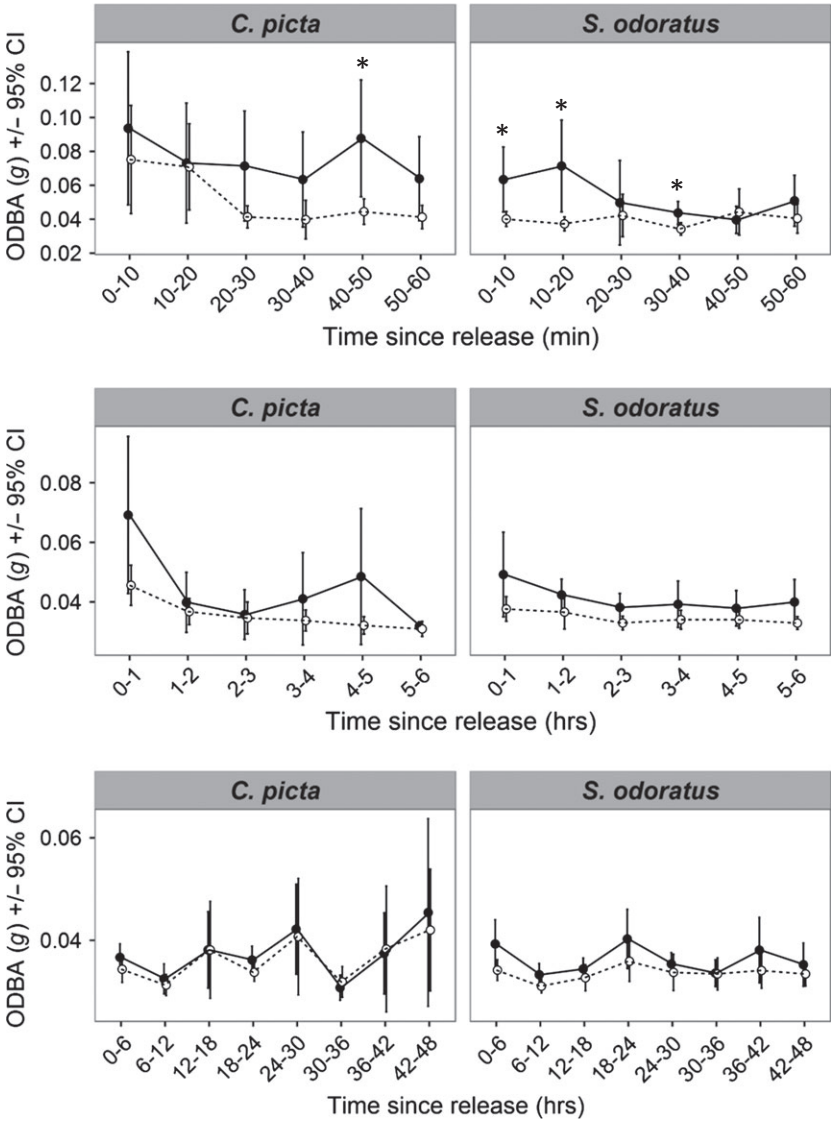

Figure 4 Least squares means of overall dynamic body acceleration (ODBA) (*y*-axis in *g*) ± 95% confidence intervals. Activity is assessed over three time periods: within the 1st h post-release (top row); 6 h post-release (middle row) and; 48 h post-release (bottom row). Control turtles (closed circles connected by a solid line) are compared to submerged individuals (open circles connected by a dashed line). Significant follow-up one-way ANOVAs are denoted with an asterisk (*).

Temperature use

For the first 2 h after release, painted turtles in the control group were at temperatures from 21.7 to 27.4°C (1.96 SD) while those in the entrapment group were at temperatures from 23.5 to 26.1°C (0.83 SD) with less variation (Fig. 7a, edf = 1.00, $P = 0.004$). The reduction in variation in the entrapment group was more pronounced in musk turtles (Fig. 7b). Immediately following release, control group musk turtle temperature followed a nonlinear pattern (edf = 4.18, $P < 0.001$) with relatively large variation across sampling periods (1.30 SD), whereas musk turtles in the entrapment group exhibited little temperature variation (0.09 SD, edf = 1.00, $P = 0.607$; Table 1). In the first 10 min interval, control musk turtles were at significantly higher mean temperatures (27.3°C, 25.7, 28.9, 95% CI) than turtles in the entrapment group (24.5°C, 23.1, 25.7, 95% CI).

Over a 48 h period following release, hourly temperature use was similar between the control (range: 19.8–25.7°C, 1.81 SD) and entrapment group (range: 20.1–26.0°C, 1.96 SD) in painted turtles and in musk turtles (control range: 22.6–25.4°C, 0.99 SD; entrapment range: 22.8–25.3°C, 0.86 SD). For both species, control and treatment groups exhibited statistically significant distinct cyclic patterns in temperature use (Fig. 7c and d, edf > 7.8, $P < 0.001$ in all cases; Table 1).

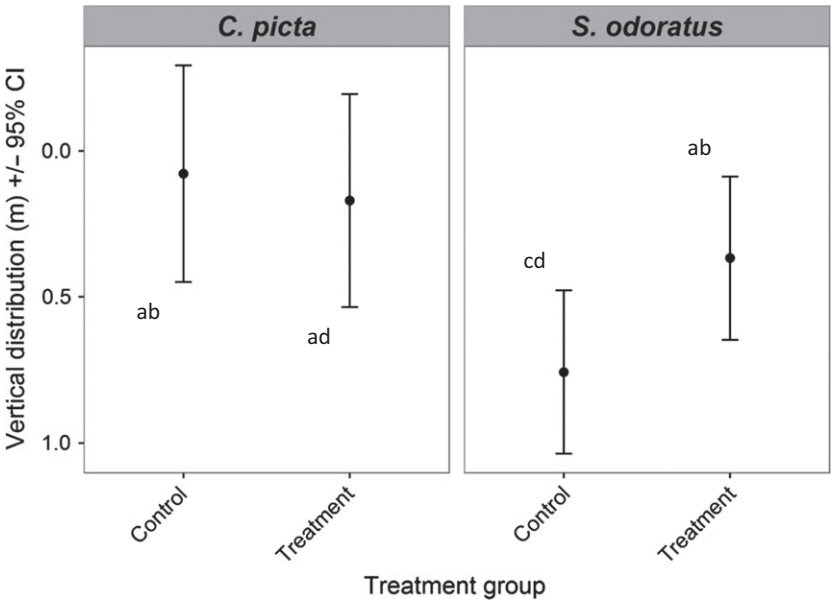

Figure 5 Linear mixed effects model estimates for swimming depth (m) ± 95% confidence intervals. Estimates were taken from turtles over the first 2 h post-release. Statistical significant groups are denoted with a unique identifier.

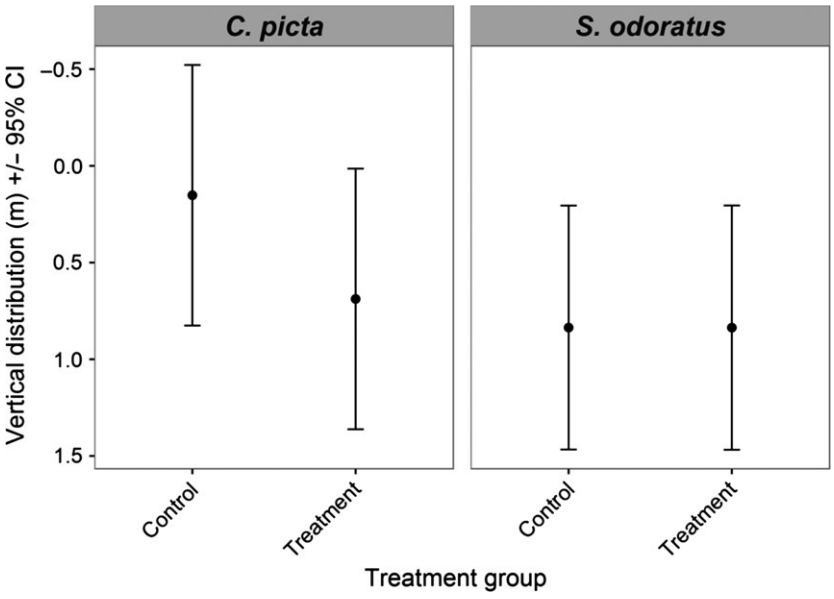

Figure 6 Linear mixed effects model estimates for swimming depth (m) ± 95% confidence intervals. Estimates were taken from turtles over the first 48 h post-release.

Discussion

Painted turtles entrapped for 4 h in fyke nets similar to those used in commercial fisheries showed short-term impairment, whereas musk turtles seemed unaffected. However, both species showed some evidence of impairment upon release. Therefore, behavioural tests failed to forecast the short-term behavioural impairment observed in musk turtles. This discrepancy further underscores the importance of species-based evaluations with multiple assessment techniques. Delayed mortality was not observed in entrapped turtles, but they experienced short-term behavioural impairment. Post-release mortality and behavioural impairment are considerable issues for marine turtles captured as bycatch (Chaloupka *et al.*, 2004; Swimmer *et al.*, 2006; Snoddy & Williard, 2010), and entrapped freshwater turtles should face similarly negative consequences.

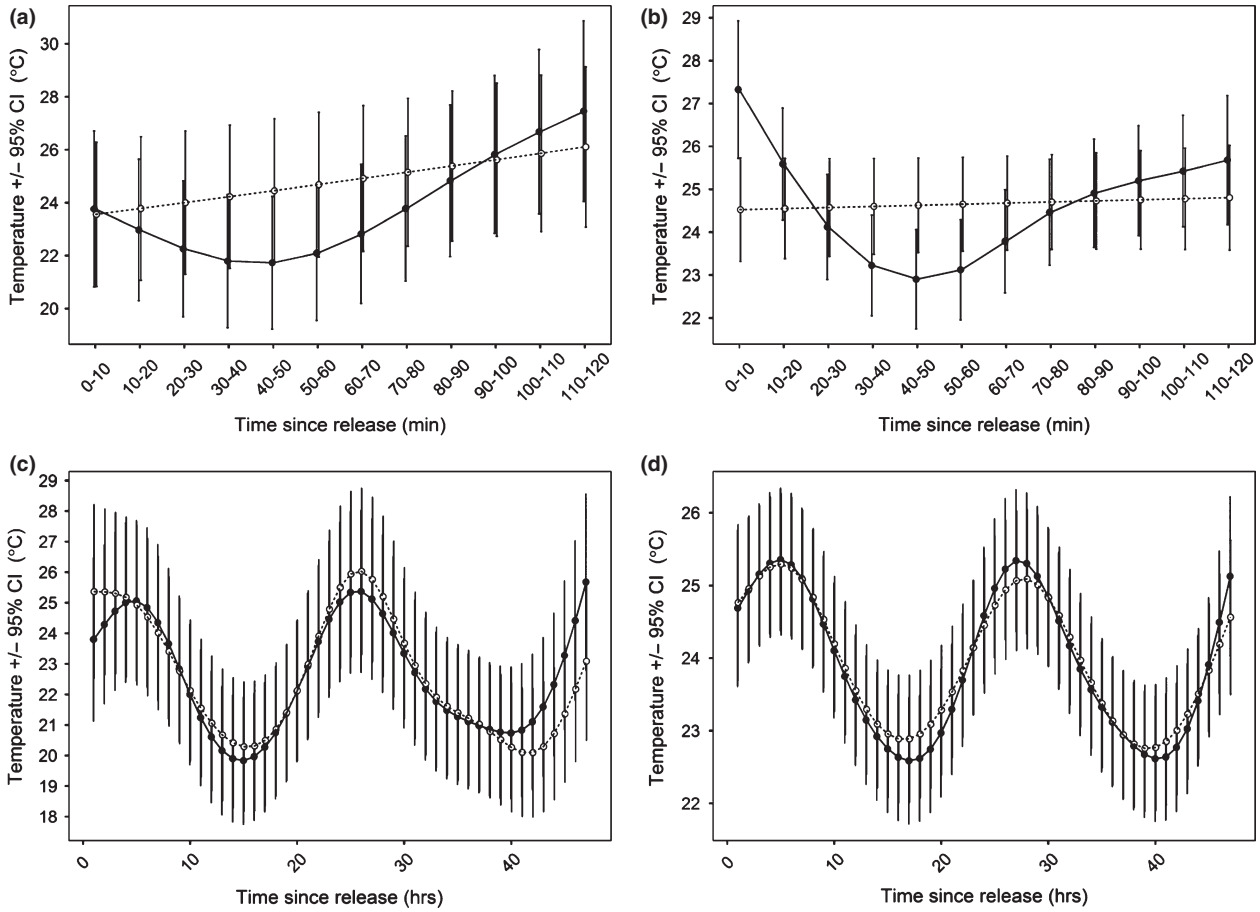

Figure 7 The contribution of the smoothers in generalized additive mixed models of post-release painted turtle and musk turtle temperature use: (a) Controls (closed circles, edf = 3.14, $P < 0.001$) and net treatment (open circles, edf = 1.00, $P = 0.004$) painted turtles across 10 min intervals for 2 h; (b) Controls (closed circles, edf = 4.18, $P < 0.001$) and net treatment (open circles, edf = 1.00, $P = 0.607$) musk turtles across 10 min intervals for 2 h; (c) Controls (closed circles, edf = 8.24, $P < 0.001$) and net treatment (open circles, edf = 8.18, $P < 0.001$) painted turtles across 1 h intervals for 48 h and; (d) Controls (closed circles, edf = 8.18, $P < 0.001$) and net treatment (open circles, edf = 7.81, $P < 0.001$) painted turtles across 1 h intervals for 48 h.

Despite exposure to the same conditions of simulated entrapment, musk turtles did not experience immediate behavioural impairment whereas painted turtles did (see also Stoot *et al.*, 2013). Although painted turtles are tolerant of anoxic conditions in cold water, submergence in warm normoxic conditions makes them incapable of sequestering sufficient oxygen through secondary gas exchange mechanisms to remain aerobic (Ultsch & Jackson, 1982; Jackson, Crocker & Ultsch, 2000; Reese *et al.*, 2001), thus requiring them to use anaerobic metabolism which leads to blood acidosis (Ultsch & Jackson, 1982; Ultsch *et al.*, 1999; Jackson *et al.*, 2000; Reese *et al.*, 2001). Unlike painted turtles, musk turtles are intolerant to submergence in anoxic conditions, but in normoxic conditions they use bimodal respiration to tolerate submergence, which is an alternative gas exchange strategy via extra pulmonary oxygen uptake (Ultsch, Herbert & Jackson, 1984; Reese *et al.*, 2001). Thus, the different strategies for coping with normoxic conditions are a plausible explanation for the species differences we documented in behavioural impairment following simulated entrapment.

Overall, we showed that freshwater turtle behaviour is impaired (i.e. decreased locomotory activity, distinct depth and temperature) during the 2–6 h window following accidental net entrapment. It is plausible that these results are linked: decreased locomotion in entrapped turtles leads to less variation in the depth of water used and, consequently, to the distinct water temperatures experienced. Our analyses of post-release behaviour indicated that entrapped turtles of both species appeared to return to normal behaviour after *c.* 2 h post-release. As with marine species, freshwater turtles released alive from commercial fishing nets may have reduced abilities to flee when faced with predators or rapidly approaching boats (Galois & Ouellet, 2007; Bulté, Carrière & Blouin-Demers, 2010). Within the eastern Ontario fyke-net fishery, turtles can typically remain entrapped in nets for up to 48 h at elevated water temperatures (similar to this

study) and up to 7 days at cooler temperatures (~10–12°C). Given that prolonged submergence times result in high mortality rates (Larocque *et al.*, 2012*a*), we would expect to see more pronounced impairments in both species when individuals are exposed to the submergence periods typical of this fishery.

In agreement with previous research, painted and musk turtles experienced significant impairment after simulated entrapment (Stoot *et al.*, 2013). Our findings further highlight the importance of evaluating post-release fate at several time points and for individual species. Behaviour score and activity differences between painted and musk turtles indicate that appropriate conclusions about post-release bycatch survival and behaviour can only be drawn following multiple lines of investigation. Electronic tagging techniques such as those used here hold much promise for the study of post-release behaviour and fate of individuals captured as bycatch (Cooke, 2008; Donaldson *et al.*, 2008).

Acknowledgements

We thank the Ontario Ministry of Natural Resources (OMNR) and the Canadian Wildlife Federation for funding. S.J.C. is supported by the Canada Research Chairs Program and S.J.C. and G.B.D. are supported by NSERC. S.J.C. is further supported by SSHRC through the Too Big To Ignore Program on small-scale fisheries. We also thank F. Phelan, M. Conboy and the staff of the Queen's University Biological Station for logistical support. We thank A. Weatherhead, S. Larocque and numerous volunteers for assistance in the field. All work was conducted with Scientific Collection Permits obtained from the OMNR for both fish (authorization no: 1067529) and turtles (authorization no: 1067660; species at-risk permit no: KV-B-006-12) and Animal Care Approvals from the Canadian Council of Animal Care as administered by Carleton University (protocol B10-01) and Queen's University (2010-042-R1).

References

Barko, V.A., Briggler, J.T. & Osendorf, D.E. (2004). Passive fishing techniques: a cause of turtle mortality in the Mississippi River. *J. Wildl. Manag.* **68**, 1145–1150.

Bograd, S.J., Block, B.A., Costa, D.P. & Godley, B.J. (2010). Biologging technologies: new tools for conservation. *Endanger. Species. Res.* **10**, 1–7.

Bulté, G., Carrière, M.-A. & Blouin-Demers, G. (2010). Impact of recreational boating on two populations of northern map turtles (*Graptemys geographica*). *Aquat. Conserv. Mar. Freshw. Ecosyst.* **20**, 31–38.

Carrière, M.-A., 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.

Chaloupka, M., Parker, D. & Balazs, G. (2004). Modelling post-release mortality of loggerhead sea turtles exposed to the Hawaii-based pelagic longline fishery. *Mar. Ecol. Prog. Ser.* **280**, 285–293.

Chopin, F.S. & Arimoto, T. (1995). The condition of fish escaping from fishing gears – a review. *Fish. Res.* **21**, 315–327.

Congdon, J.D., Dunham, A.E. & Van Loben Sels, R.C. (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.

Congdon, J.D., Dunham, A.E. & Van Loben Sels, R.C. (1994). Demographics of common snapping turtles (Chelydra serpentine): implications for conservation and management of long-lived organisms. *Am. Zool.* **34**, 397–408.

Cooke, S.J. (2008). Biotelemetry and biologging in endangered species research and animal conservation: relevance to regional, national and IUCN Red List threat assessments. *Endanger. Species. Res.* **4**, 165–185.

Crowder, L.B. & Murawski, S.A. (1998). Fisheries bycatch: implications for management. *Fisheries* **23**, 8–17.

Davis, M.W. (2002). Key principles for understanding fish bycatch discard mortality. *Can. J. Fish Aquat. Sci.* **59**, 1834–1843.

Donaldson, M.R., Arlinghaus, R., Hanson, K.C. & Cooke, S.J. (2008). Enhancing catch-and-release science with biotelemetry. *Fish Fish.* **9**, 79–105.

Finkbeiner, E.M., Wallace, B.P., Moore, J.E., Lewison, R.L., Crowder, L.B. & Read, A.J. (2011). Cumulative estimates of sea turtle bycatch and mortality in USA fisheries between 1990 and 2007. *Biol. Conserv.* **144**, 2719–2727.

Frick, L.H., Reina, R.D. & Walker, T.I. (2010). Physiological changes and post-release survival of Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity. *J. Exp. Mar. Biol. Ecol.* **385**, 29–37.

Galois, P. & Ouellet, M. (2007). Traumatic injuries in eastern spiny softshell turtles (*Apalone spinifera*) due to recreational activities in the northern Lake Champlain basin. *Chelonian Conserv. Biol.* **6**, 288–293.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S. & Winne, C.T. (2000). The global decline of reptiles, déjà vu amphibians. *Bioscience* **50**, 653–666.

Gleiss, A.C., Dale, J.J., Holland, K.N. & Wilson, R.P. (2010). Accelerating estimates of activity-specific metabolic rate in fishes: testing the applicability of acceleration data-loggers. *J. Exp. Mar. Biol. Ecol.* **385**, 85–91.

Hall, S.J. & Mainprize, B.M. (2005). Managing by-catch and discards: how much progress are we making and how can we do better? *Fish Fish.* **6**, 134–155.

Hall, M.A., Alverson, D.L. & Metuzals, K.I. (2000). By-catch: problems and solutions. *Mar. Pollut. Bull.* **41**, 204–219.

Harrington, J.M., Myers, R.A. & Rosenberg, A.A. (2005). Wasted fishery resources: discarded by-catch in the USA. *Fish Fish.* **6**, 350–361.

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.* **50**, 346–363.

Jackson, D.C., Crocker, C.E. & Ultsch, G.R. (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**, 1564–1571.

Julian, F. & Beeson, M. (1998). Estimates of marine mammal, turtle and seabird mortality for 2 California gillnet fisheries: 1990-1995. *Fish. Bull.* **96**, 271–284.

Larocque, S.L., Colotelo, A.H., Cooke, S.J., Blouin-Demers, G., Haxton, T. & Smokorowski, K.E. (2012a). Seasonal patterns in bycatch composition and mortality associated with a freshwater hoop net fishery. *Anim. Conserv.* **15**, 53–60.

Larocque, S.L., Cooke, S.J. & 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. Mar. Freshw. Ecosyst.* **22**, 198–205.

LeDain, M.R.K., Larocque, S.M., Stoot, L.J., Cairns, N.A., Blouin-Demers, G. & Cooke, S.J. (2013). Assisted recovery following prolonged submergence in fishing nets can be beneficial to turtles: an assessment with blood physiology and reflex impairment. *Chelonian Conserv. Biol.* **12**, 172–177.

Lewison, R.L., Freeman, S.A. & Crowder, L.B. (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.

Lewison, R.L., Nel, D.C., Taylor, F., Croxall, J.P. & Rivera, K.S. (2005). Thinking big-taking a large-scale approach to seabird bycatch. *Mar. Ornithol.* **33**, 1–5.

Midwood, J.D., Cairns, N.A., Stoot, L.J., Cooke, S.J. & Blouin-Demers, G. (2015). Bycatch mortality can cause extirpation of four freshwater turtle species. *Aquat. Conserv.* **25**, 71–80.

Moore, J.E., Cox, T.M., Lewison, R.L., Read, A.J., Bjorkland, R., McDonald, S.L., Crowder, L.B., Aruna, E., Ayissi, I., Espeut, P., Joynson-Hicks, C., Pilcher, N., Poonian, C.N.S., Solarin, B. & Kiszka, J. (2010). An interview-based approach to assess marine mammal and sea turtle captures in artisanal fisheries. *Biol. Conserv.* **143**, 795–805.

R Core Team. (2016). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org/. Accessed 15 August 2016.

Raby, G.D., Colotelo, A.H., Blouin-Demers, G. & Cooke, S.J. (2011). Freshwater commercial bycatch: an understated conservation problem. *Bioscience* **61**, 271–280.

Reese, S.A., Crocker, C.E., Carwile, M.E., Jackson, D.C. & Ultsch, G.R. (2001). The physiology of hibernation in common map turtles (*Graptemys geographica*). *Comp. Biochem. Physiol. A* **130**, 331–340.

Rutz, C. & Hays, G.C. (2009). New frontiers in biologging science. *Biol. Lett.* **5**, 289–292.

Ryer, C.H., Ottmar, M.L. & Strum, E.A. (2004). Behavioral impairment after escape from trawl codends may not be limited to fragile fish species. *Fish. Res.* **66**, 261–269.

Shepard, E.L.C., Wilson, R.P., Quintana, F., Laich, A.G., Liebsch, N., Albareda, D.A., Halsey, L.G., Gleiss, A., Morgan, D.T., Myers, A.E., Newman, C. & Macdonald, D.W. (2008). Identification of animal movement patterns using tri-axial accelerometery. *Endanger. Species. Res.* **10**, 47–60.

Snoddy, J.E. & Williard, A. (2010). Movement and post-release mortality of juvenile sea turtles released from gillnets in the lower Cape Fear River, North Carolina, USA. *Endanger. Species. Res.* **12**, 235–247.

Snoddy, J.E., 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.

Stoot, L.J., Cairns, N.A., Blouin-Demers, G. & Cooke, S.J. (2013). Physiological disturbances and behavioural impairment associated with the incidental capture of freshwater turtles in a commercial fyke-net fishery. *Endanger. Species. Res.* **21**, 13–23.

Swimmer, Y., Arauz, R., McCracken, M., McNaughton, L., Ballestero, J., Musyl, M., Bigelow, K. & Brill, R. (2006). Diving behaviour and delayed mortality of olive ridley sea turtles *Lepidochelys olivacea* after their release from longline fishing gear. *Mar. Ecol. Prog. Ser.* **323**, 253–261.

Ultsch, G.R. & Jackson, D.C. (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, G.R., Herbert, C.V. & Jackson, D.C. (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, G.R., Carwile, M.E., Crocker, C.E. & Jackson, D.C. (1999). The physiology of hibernation among painted turtles: the eastern painted turtle *Chrysemys picta picta*. *Physiol. Biochem. Zool.* **72**, 493–501.

Venables, W.N. & Ripley, B.D. (2002). *Modern applied statistics with S*. 4th edn. New York: Springer.

Wilson, S.M., Raby, G.D., Burnett, N.J., Hinch, S.G. & Cooke, S.J. (2014). Looking beyond the mortality of bycatch: sublethal effects of incidental capture on marine animals. *Biol. Conserv.* **171**, 61–72.

Wilson, A.D.M., Wikelski, M., Wilson, R.P. & Cooke, S.J. (2015). Utility of biological sensor tags in animal conservation. *Conserv. Biol.* **29**, 1065–1075.

Wood, S. (2006). *Generalized additive mixed models: an introduction with R*. Boca Raton, FL: Chapman and Hall/CRC.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009). *Mixed effects models and extensions in ecology with R*. New York: Springer Science & Business Media.