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A breath of fresh air: avoiding anoxia and mortality of freshwater turtles in fyke nets by the use of floats

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ABSTRACT

1. Freshwater turtles are susceptible to drowning in commercial fishing nets and this is a major conservation concern. Methods to mitigate turtle bycatch mortality typically involve reducing the capture of bycatch using gear modifications. Another method to reduce mortality is to keep bycatch alive following capture.
2. Using physiological measures of anoxia, this study determined whether providing air spaces using floats within fyke nets could prevent turtles from drowning. In a controlled setting, blood lactate and pH of painted turtles (*Chrysemys picta*) experimentally introduced into submerged nets, nets with floats, and nets that breached the surface were compared. While emulating commercial fishing practices – where turtles and fish voluntarily entered nets – catch rates and compositions as well as blood lactate in turtles captured were compared in submerged nets with and without floats.
3. Painted turtles in submerged nets exhibited elevated blood lactate and pronounced acidosis compared with turtles from nets with floats and surfaced nets.
4. Catch rates and compositions from emulated fishing were statistically similar in nets with and without floats; however, total fish catches were roughly one-third less in nets with floats. The same pattern of physiological disturbance was observed with turtles captured in submerged nets with and without floats as in the controlled experiment.
5. Overall, blood physiology indicated that anoxia occurred in turtles in submerged nets while nets with floats reduced physiological disturbance. However, variation in blood lactate levels when fishing fyke nets with floats suggests that turtles were experiencing slight anoxia and so the size of air spaces may be important in allowing access to air. Creating air spaces in fyke nets using floats is a simple and cost-effective method to avoid the drowning of turtles.

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INTRODUCTION

Bycatch, the inadvertent capture of non-targeted fauna, is a growing conservation concern in commercial fisheries (Alverson *et al.*, 1994; Hall *et al.*, 2000; Lewison *et al.*, 2004; Lewison and Crowder, 2007). This concern is particularly acute when long-lived organisms with late maturation and naturally low recruitment, such as turtles, are incidentally captured as adults in a fishery and

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mortality ensues (Whitehead *et al*., 1997; Lewison *et al*., 2004). Slight additional adult mortality is often sufficient to put turtle populations in jeopardy (Brooks *et al*., 1991; Congdon *et al*., 1993, 1994). The pressure from additional annual mortality is especially serious when the majority of turtles, including marine and freshwater, are at risk and in decline (IUCN, 2011). Like marine turtle bycatch (Alverson *et al*., 1994; Lewison *et al*., 2004; Lewison and Crowder, 2007), 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*., 2011; Figure 1). Although the prevalence of freshwater bycatch is less publicized or documented than marine bycatch, freshwater bycatch can be a threat for aquatic turtle populations (Raby *et al*., 2011; Larocque *et al*., 2011).

Efforts to mitigate bycatch and associated mortality typically involve modifications to the fishing gear and/or methods (Broadhurst, 2000; Lewison *et al*., 2004; Gilman *et al*., 2010). In freshwater commercial fisheries, passive nets such as hoop nets (cylindrical nets distended by hoops and a funnel-shaped throat at the entrance), fyke nets (hoop nets with wings and a lead attached to ‘direct’ fish into the net; Figure 2), and trap nets (fyke nets except with rectangular frames) are commonly used (Hubert, 1996). As such, there have been attempts to modify hoop and fyke nets to reduce turtle bycatch (Lowry *et al*., 2005; Fratto *et al*., 2008a, b; Larocque, 2011). Exploiting physical and behavioural differences between the target fish species and bycatch turtle species, modifications can involve permanent alterations to the net that can prevent turtles from entering or that allow turtles to escape the net after entry (Broadhurst, 2000). Although some of these alterations appear to be effective (e.g. use of ring barriers, or escape chutes; Lowry *et al*., 2005; Fratto *et al*., 2008a, b; Larocque, 2011), net alterations also tend to be extensive, potentially expensive for commercial fishers to implement, and may not eliminate the problem of bycatch mortality completely.

Figure 1. Painted turtles (*Chrysemys picta*) captured in a submerged fyke net in Lake Opinicon, Canada (photo credit, S. M. Larocque).

Figure 2. Illustration of a typical submerged fyke net catching aquatic fauna (illustration credit, S. M. Larocque).

Another potential avenue to mitigate freshwater turtle bycatch mortality is to keep turtles alive in nets instead of focusing on avoiding bycatch in the nets. The drowning of turtles in submerged nets is the primary concern with commercial fishing bycatch (Figure 1). As turtles require air to breathe, the creation of air spaces within fyke nets could prevent anoxia (the lack of oxygen) and ensuing mortality (Grant *et al*., 2004). Bury (2011) showed that the use of air spaces in hoop nets and fyke nets largely prevent turtle mortality, yet Larocque *et al*. (2011) found that the use of floats in fyke nets (to keep the nets partially at the surface) was not always effective and substantial turtle mortality occurred. Thus, there is a need to verify whether air spaces created with floats in fyke nets minimize freshwater turtle bycatch mortality as well as whether such alterations affect fish catch rates, a factor influencing fisher acceptance of the use of floats in fyke nets.

Instead of using death as an end-point to determine the effectiveness of air spaces in fyke nets at negating turtle mortality, this study adopted a conservation physiology approach (Wikelski and Cooke, 2006) and used physiological variables to quantify anoxia. During anoxia, blood lactate accumulates as anaerobic metabolism occurs, and the increase in lactic acid results in a decrease in blood pH (Jackson, 2000; Hill *et al*., 2004). The ability of freshwater turtles to deal with anoxia decreases with increasing temperature (Herbert and Jackson, 1985). Therefore, blood lactate and pH levels should be effective, quick responding indicators of anoxia in turtles in warm water. The objective here was to determine, both in a controlled setting (when turtles were experimentally introduced into nets void of fish) and while emulating commercial fishing practices (when turtles and fish entered nets on their own), whether providing air spaces in fyke nets reduces signs of anoxia in freshwater turtles that are captured in these nets. A further objective was to determine whether the provision of air spaces reduces fish captures.

METHODS

Study site

The study was conducted on Lake Opinicon (44° 34' N, 76° 19' W) approximately 100 km south of Ottawa, Ontario, Canada. Lake Opinicon is a 788 ha shallow warm-water lake with a mean depth of 2.8 m. The controlled experiments were conducted in August 2009 during which time lake temperatures ranged from 18–21 °C. Commercial fishing practices were emulated in spring (late April–mid-June) 2010 during which time lake temperatures ranged from 12.7–25.9 °C.

Fyke nets

The fyke nets used had similar dimensions to those used in the commercial fishery (Figure 2). Each fyke net contained seven 0.9 m diameter steel hoops positioned 0.5 m apart. There were two throats per net, located at the second and fourth hoops. Each net had two wings and a lead attached to the front hoop that measured 4.6 m long by 0.9 m high, and 10.7 m long by 0.9 m high, respectively. All the nets, wings, and leads were constructed with 5.08 cm stretch nylon mesh.

Controlled experiment

Controlled conditions were used to determine whether turtles use air spaces provided in nets. Using fyke nets, 30 painted turtles (*Chrysemys picta*; 12 females, 18 males; mean carapace length ± SD: 140.67 ± 12.22 mm; mean mass ± SD: 353.53 ± 95.24 g) were captured. There were three net treatments and painted turtles were randomly assigned to each. The submerged treatment was a fyke net set completely under water at a depth of 1.5 m. The float treatment was a fyke net set with an air space created by putting at least two floats (e.g. water jugs; Styrofoam) in the cod-end of the net (ensuring that the floats and nets breached the surface). The surfaced treatment was a fyke net set at a depth of 0.5 m in which about half of the net was submerged. For all treatments, the opening of the net was closed after the insertion of a turtle. Trials involved putting an individual painted turtle into the net for 4 h. Preliminary trials using video surveillance indicated that after 4 h at ~20 °C, painted turtles reduced activity and showed signs of anoxia. After 4 h, turtles were retrieved and, within 2–5 min, a blood sample was taken to measure blood lactate and blood pH. Ten trials were completed for the submerged treatment, while nine trials were completed for the surfaced and float treatments as one turtle escaped in each.

Emulating commercial fishing

Nets with and without air spaces provided using floats were compared. Nets were set according to fishing practices commonly employed by commercial fishers in the area. Nets were set in pairs by adjoining two fyke nets (of the same treatment type) by their leads with the net openings facing each other and extending the wings 45° from the entrance of the net. Submerged nets (without floats) and nets with floats were simultaneously set in 30 locations within Lake Opinicon that were shallow (1–2 m) and vegetated. Submerged and float net pairs were set within 15 m of each other to reduce habitat variation. Net set durations varied (8–48 h) to minimize mortality of turtles; net set duration decreased as water temperatures increased based on reduced anoxia tolerance and survival durations found by Herbert and Jackson (1985). When lifting the nets, blood samples were taken from all turtles (all species) within 5 min to measure blood lactate. All organisms encountered were identified to species and tallied. Any mortality was documented.

Blood sampling and analysis

Blood samples from turtles were taken from the caudal vein on the dorsal part of the tail using a 1 mL luer-lock sodium-heparinized (10 000 USP units mL^{-1}, Sandoz, Québec, Canada) syringe with a 25 gauge 38 mm needle (Becton Dickinson, Franklin Lakes, New Jersey). When both blood lactate and pH were measured (i.e. controlled experiment), a minimum of 0.2 mL of blood was obtained. If only blood lactate was measured (i.e. emulation of commercial fishing), 0.05 mL of blood was taken. All lactate and pH measurements were done on site. Whole blood lactate was measured using a Lactate Pro meter (Arkray Inc., Japan). Lactate Pro reads between 0.8 and 23.3 mmol L^{-1}, therefore when readings indicated 'low' it was assumed that lactate levels were 0.7 mmol L^{-1} (a conservative estimate that maintains lactate levels as a continuous variable). The use of Lactate Pro to measure lactate has been validated with teleost fish (Brown *et al.*, 2008), and it was assumed to be accurate for turtles. Blood pH was measured with a 3-point calibrated minilab IQ128 Elite pH meter (IQ Scientific Instruments Inc., California).

Data analysis

For the controlled experiment, blood lactate and pH for painted turtles among the three treatments were compared. Blood lactate level residuals did not meet the assumptions of normality and homogeneity of variance for an ANOVA, and so a non-parametric Kruskal–Wallis test was used. To determine which treatments differed, *post hoc* non-parametric Mann–Whitney U tests with a Bonferroni correction were used. For blood pH, however, the assumptions of normality and homogeneity of variance were not violated and an ANOVA was used to compare the three treatments. *Post hoc* Tukey's tests were used to determine which treatments varied in blood pH.

For the emulation of commercial fishing experiment, fish and turtle catch rates, catch composition, and blood lactate of turtles between nets with and without floats were compared. To compare catch rates for the two net types, catch per unit effort (CPUE – catch per hour) for each net pair was calculated to standardize for differences in net set duration. Catch per unit effort was calculated by taking the total catch from both nets in the pair and dividing it by the summed duration that each net was set. If one of the nets in a pair did not fish properly (e.g. holes in the net; wings twisted) that one net of the pair was removed from the calculation. The CPUE for fish and turtle catches were calculated. Fish catch rates were $\log_{10}$ transformed to meet the assumptions of normality and homogeneity of variance and nets with and without floats were compared using a paired samples *t*-test. Turtle catch rate residuals were non-normal for a paired samples t-test and a non-parametric Wilcoxon signed ranked test was used to compare net types. Whether nets with and without floats were catching the same composition of species was determined by comparing species catch rates from each net type ($N = 30$ per net type) using a multi-response blocked procedure (MRBP; blocking controlled for site variation) in PC-ORD 5.20 (McCune and Mefford, 2006).

Blood lactate in turtles was compared in submerged nets and in nets with floats. Both painted turtles and eastern musk turtles (*Sternotherus odoratus*) had sample sizes larger than six for each treatment and were thus used to compare net types with an ANOVA and a Tukey's *post hoc* comparison. All statistical tests were performed, unless otherwise stated, with SPSS 18.0.0 (www.spss.com). Significance was accepted at $\alpha = 0.05$, except when a Bonferroni correction was indicated. Values are reported as mean ± SE.

RESULTS

In the controlled experiment, painted turtle blood lactate levels were significantly different among treatments ($H_2 = 22.123$; $P < 0.001$). All treatments were significantly different from each other ($P < 0.005$; Figure 3(a)). Turtles from the surfaced net had the lowest lactate levels (1.0 ± 0.1 mmol L^{-1}), turtles from the float net had slightly higher lactate levels (2.2 ± 0.3 mmol L^{-1}), and turtles from the submerged net had the highest lactate levels (16.8 ± 0.6 mmol L^{-1}; Figure 3(a)). Blood pH levels in painted turtles also differed significantly among treatments ($F_{2,25} = 577.157$; $P < 0.001$). Turtles in both the surfaced and float nets had similar blood pH with 8.11 ± 0.02 and 8.05 ± 0.015 pH, respectively, while turtles in submerged nets had significantly lower pH (7.38 ± 0.02 pH; $P < 0.05$; Figure 3(b)).

When emulating the commercial fishery using submerged fyke nets, 3025 fish of eight species and 50 non-fish fauna (three turtle species and one mammal species) were captured (Table 1). Fewer animals were captured with fyke nets with floats: 2040 fish of nine species, and 35 turtles of four species (Table 1). In submerged nets and float nets, 12.5% (4/32) and 23.5% (4/17) of northern pike (*Esox lucius*) died, respectively. Minimal mortality (<0.005%) of bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*) occurred for each net type. Turtle mortality ($N = 3$) only occurred in submerged nets, in which 12.5% (2/16) of painted turtles and 20% (1/5) of northern map turtles

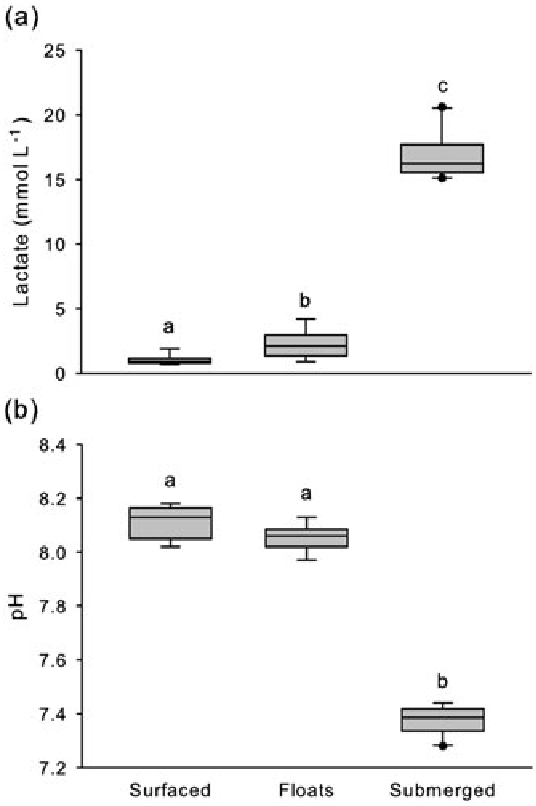

Figure 3. (a) Blood lactate (mmol L^{-1}) and (b) blood pH from painted turtles (*Chrysemys picta*) after being subjected to 4 h in one of three net types. Box plots accompanied by the same letters were not statistically different according to (a) Mann–Whitney U tests with a Bonferroni correction ($P < 0.01$) and (b) Tukey's multiple comparisons.

captured died. Mammals (two muskrats) were only captured in submerged nets and both died.

Catch rates were similar for fish ($T_{29} = 1.862$; $P = 0.073$), although submerged nets had slightly higher catch rates (3.05 ± 0.31 fish h^{-1}) than nets with floats (2.54 ± 0.34 fish h^{-1}). The inability to detect a significant difference may be due to a low sample size because a power analysis indicated that a sample size of 70 net sets would have been required to attain significance given variation in catches. Turtle catch rates were also similar between submerged nets (0.10 ± 0.04 turtles h^{-1}) and nets with floats (0.06 ± 0.02 turtles h^{-1}; $Z = -0.224$; $P = 0.823$). Species composition also did not vary between net types ($A = 0.012$; $P = 0.172$).

Blood lactate levels in turtles differed significantly between individuals captured in nets with floats and those without as well as between species ($F_{3,47} = 112.843$; $P < 0.001$; $R^2 = 0.870$). *Post hoc* Tukey's test revealed that painted turtles in submerged nets ($N = 16$) had significantly higher lactate levels than any other group (16.1 ± 0.6 mmol L^{-1}; Figure 4). Eastern musk turtles in submerged nets ($N = 19$) had significantly lower lactate levels (13.8 ± 0.5 mmol L^{-1}) than submerged painted turtles (Figure 4). Both painted turtles ($N = 8$) and eastern musk turtles ($N = 8$) in nets with floats had similar lactate levels (4.2 ± 0.9 and 1.9 ± 0.5 mmol L^{-1}, respectively) which were significantly lower than lactate levels in both turtle species from submerged nets (Figure 4).

DISCUSSION

In the controlled experiment, painted turtles in submerged nets had significantly higher blood lactate levels and significantly lower blood pH than both surfaced nets and nets with floats, thus indicating that turtles in submerged nets were experiencing anoxia. Previous studies of anoxic painted turtles at 20–22 °C (Keiver *et al.*, 1992a; Warren and Jackson, 2004) yielded lactate and

Table 1. Number and composition of organisms captured in fyke nets that were submerged or had floats ($N = 30$ paired net sets per net type) in Lake Opinicon, Canada

Group	Species	Submerged: Number caught	Submerged: %	Floats: Number caught	Floats: %
Fish	Bluegill (*Lepomis macrochirus*)	1519	49.40%	1044	50.31%
	Pumpkinseed (*Lepomis gibbosus*)	1091	35.48%	750	36.14%
	Largemouth bass (*Micropterus salmoides*)	135	4.39%	95	4.58%
	Rock bass (*Ambloplites rupestris*)	127	4.13%	59	2.84%
	Bullhead spp. (*Ameiurus* spp.)	95	3.09%	43	2.07%
	Northern pike (*Esox lucius*)	32	1.04%	17	0.82%
	Black crappie (*Pomoxis nigromaculatus*)	23	0.75%	29	1.40%
	Smallmouth bass (*Micropterus dolomieu*)	3	0.10%	2	0.10%
	Yellow perch (*Perca flavescens*)	0	0.00%	1	0.05%
Non-fish bycatch	Eastern musk turtle (*Sternotherus odoratus*)	27	0.88%	18	0.87%
	Painted turtle (*Chrysemys picta*)	16	0.52%	9	0.43%
	Northern map turtle (*Graptemys geographica*)	5	0.16%	7	0.34%
	Muskrat (*Ondatra zibethicus*)	2	0.07%	0	0.00%
	Common snapping turtle (*Chelydra serpentina*)	0	0.00%	1	0.05%
	Grand total	3075	100%	2075	100%

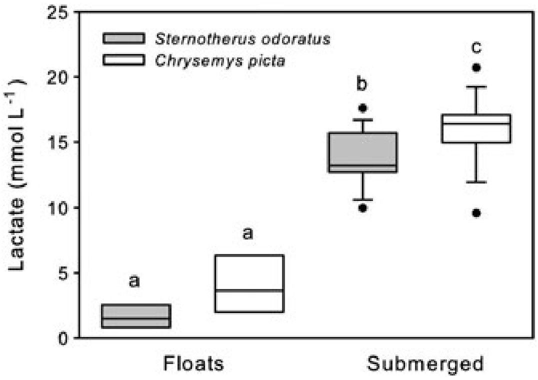

Figure 4. Blood lactate (mmol L^{-1}) in painted turtles (*Chrysemys picta*) and eastern musk turtles (*Sternotherus odoratus*) captured in fyke nets with floats, and in submerged fyke nets set in Lake Opinicon, Canada. Box plots accompanied by the same letters were not statistically different according to Tukey's multiple comparisons.

blood pH values that were consistent with the values observed here when turtles were sampled from submerged nets without air spaces, thus confirming that turtles in the submerged net treatment did indeed experience anoxia. Given that baseline lactate and pH levels for painted turtles are typically ~1.5 mmol L^{-1} and ~7.8 pH, respectively (Keiver *et al.*, 1992a, b; Warren and Jackson, 2004), the results here indicate that turtles were using the air space in both the surfaced net and the net with floats to breathe, and were not experiencing anoxia. Turtles in nets with floats had slightly (yet significantly) higher lactate levels and lower blood pH than turtles in surfaced nets, so it appears that turtles in nets with floats had more difficulty obtaining air. Lactate and blood pH levels from turtles in nets with floats still fell within the baseline measures from previous studies. Therefore, in comparison with submerged nets, nets with floats significantly reduced the risk of anoxia and ensuing drowning in painted turtles.

When using commercial fishing practices, there was no statistically significant difference in catch rates or catch composition between submerged nets and nets with floats. Of the turtle species captured, eastern musk turtles are threatened, and northern map turtles and common snapping turtles are of special concern both provincially and federally (COSEWIC, 2010). Thus 66.6% and 74.3% of individual turtles captured in submerged nets and nets with floats, respectively, were at risk. Both submerged and float nets experienced similar levels of fish mortality; minimal mortality of *Lepomis* spp. and substantial mortality (>12%) of pike. Even though the two net types fished similarly with no statistically significant difference, mean catch rates for fish and turtles were lower in nets with floats. These lower catch rates in nets with floats translated into a reduction of 1000 organisms (or 32.5%) in the total catches compared with control nets (Table 1). As commercial fishers typically invest much greater fishing effort, using floats in nets could result in a reduction in their overall catches and, thus, resistance to the adoption of this gear modification (Broadhurst, 2000). A possible explanation for this slightly lower catch rate is that floats in the cod-end of nets cause the nets to be set diagonally instead of horizontally. As set depth increases, the severity of the diagonal net set increases and this may make it less likely for organisms to swim through the funnels of the fyke net to be captured.

Both painted turtles and eastern musk turtles had significantly lower blood lactate levels in nets with floats compared with submerged nets when using commercial fishing practices. Turtle mortality experienced in submerged nets indicated that turtles were in nets long enough to experience anoxia, and variation in lactate levels (9.6–20.7 mmol L^{-1}) was probably due to the unknown duration that turtles spent in the submerged net prior to lifting (Figure 4). On the other hand, the absence of mortality and low blood lactate levels of turtles in nets with floats indicate that floats prevented anoxia in turtles. Mean lactate levels of painted turtles in nets with floats, however, were two-fold higher than when tested in controlled conditions. Variation in blood lactate levels (0.7–8.3 mmol L^{-1}) of turtles in nets with floats suggest that these turtles may still experience anoxia and/or some level of physical exhaustion while attempting to escape, albeit much less severe than in nets without floats. The size of the air space created by floats may play a role in effectively mitigating anoxia in turtles, as Larocque *et al.* (2011) found substantial turtle mortality even with the use of floats in fyke nets.

Painted turtles experienced higher lactate levels than musk turtles both in submerged nets and nets with floats. The difference in lactate levels is probably the result of morphological and physiological differences between the two species. Most aquatic turtles are bimodal breathers (exchanging O_2 and CO_2 in both air and water; Gage and Gage, 1886; Ernst *et al.*, 1994). Eastern musk turtles, however, excel at obtaining oxygen in water through various morphological adaptations compared with painted turtles (Ultsch and Wasser, 1990; Ernst *et al.* 1994). Thus, musk turtles can obtain more oxygen under water and lactate levels would therefore be less likely to increase as much as in painted turtles

(Ultsch and Wasser, 1990; Prassack *et al.*, 2001). Physiologically, painted turtles are anoxia tolerant and are capable of sustaining high lactate levels, whereas musk turtles are relatively anoxia intolerant (Jackson *et al.*, 2007). If trapped in nets in normoxic water, however, musk turtles may be able to withstand longer submergence as they are better able to extract oxygen from the water than painted turtles.

Using physiological parameters, it was determined that the use of floats in fyke nets was effective at mitigating the mortality of freshwater turtles and potentially other air breathing organisms, as has been seen in previous studies that used floats while capturing turtles (Vogt, 1980; Gibbons, 1990; Bury, 2011). Blood lactate levels, an indicator of anaerobic metabolism (including exercise) and anoxia, were reduced when using floats in fyke nets instead of submerged nets. Although a statistical difference was not detected in catch rates, the overall catch was roughly one-third less when floats were used while emulating commercial fisheries, so there may be some challenges with acceptance of float use by fishers. Using floats will also make the nets more visible than if they were totally submerged, although less so than if using surfaced nets in shallower waters, which are less likely to be fished. Net visibility is of concern to commercial fyke net fishers as there is the potential for an increased risk of vandalism and theft as well as a perceived (but not studied) risk of avian predation on fish when nets are visible. However, it has been suggested that floats could potentially be camouflaged using spray paint to avert the above concerns. Aside from commercial fisheries, other organizations (e.g. academic and government research, consultants) use fyke nets for research and monitoring of aquatic organisms (such as fish). These organizations and agencies, if not already doing so, should use floats or other net modifications (Lowry *et al.*, 2005; Fratto *et al.*, 2008a, b; Larocque, 2011) to help mitigate turtle mortality when using hoop nets, trap nets, and fyke nets. Although the use of floats to create air spaces of sufficient size will not reduce bycatch in nets, it is a simple, immediate, and cost-effective method to avoid the drowning of turtles, including species at risk that are encountered.

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