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Conservation biology

Physiological ramifications for loggerhead turtles captured in pelagic longlines

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Bycatch of endangered loggerhead turtles in longline fisheries results in high rates of post-release mortality that may negatively impact populations. The factors contributing to post-release mortality have not been well studied, but traumatic injuries and physiological disturbances experienced as a result of capture are thought to play a role. The goal of our study was to gauge the physiological status of loggerhead turtles immediately upon removal from longline gear in order to refine our understanding of the impacts of capture and the potential for post-release mortality. We analysed blood samples collected from longline- and hand-captured loggerhead turtles, and discovered that capture in longline gear results in blood loss, induction of the systemic stress response, and a moderate increase in lactate. The method by which turtles are landed and released, particularly if released with the hook or line still attached, may exacerbate stress and lead to chronic injuries, sub-lethal effects or delayed mortality. Our study is the first, to the best of our knowledge, to document the physiological impacts of capture in longline gear, and our findings underscore the importance of best practices gear removal to promote post-release survival in longline-captured turtles.

1. Introduction

The incidental capture, or bycatch, of loggerhead turtles (*Caretta caretta*) in commercial longline fishing gear has been identified as a significant source of mortality contributing to population declines [1]. The Mediterranean Sea is a region of high-intensity bycatch for loggerhead turtles, and mitigation of bycatch in this region is a conservation priority [2]. The southwestern Mediterranean serves as foraging habitat for immature loggerhead turtles originating from nesting beaches in North America and the eastern Mediterranean [3]. These waters are also heavily used by the Spanish pelagic longline fleet [4,5]. It is estimated that up to 10 656 loggerhead turtles were captured annually in this region from 2001 to 2006 [5]. The majority of sea turtles captured by shallow-set (less than 100 m) longlines are alive when retrieved from gear [6,7]; however, estimated post-release mortality probability is 0.29–0.37, with most turtles dying within one month of the interaction [4,5,8].

Post-release mortality estimates are based on observations of turtles transferred to holding facilities following capture [4,8] or analysis of satellite telemetry data for turtles released alive from longline gear [5]. Our goal was to investigate the physiological status of longline-captured loggerhead turtles, and to interpret results of blood analyses in the context of post-release mortality. Blood samples collected from loggerhead turtles captured by commercial longline or by hand at the sea surface were analysed for indicators of injury, stress and metabolic disturbance. Sea turtles may experience a variety of injuries as a

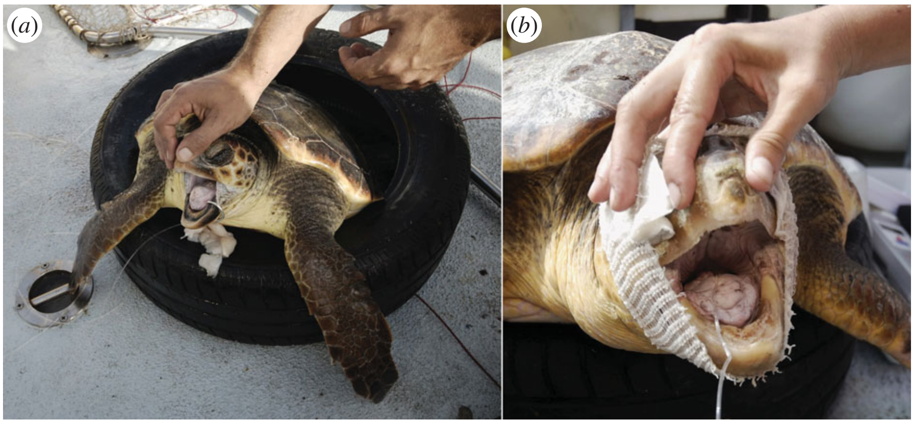

Figure 1. (*a*) A loggerhead turtle hooked in the mouth and (*b*) a loggerhead turtle that swallowed hook. Photo: J. Sanchez (SUBMON). (Online version in colour.)

result of gear interactions, and tissue damage owing to either hooking or landing may result in blood loss and a decrease in packed cell volume (PCV). Capture and restraint in fishing gear may trigger the systemic stress response, resulting in elevated levels of corticosterone and glucose [9,10]. Efforts to escape gear and reach the surface to breathe may result in metabolic disturbances, particularly an increase in blood lactate as turtles increase reliance on anaerobic metabolism [10–13]. We hypothesized that compared with hand-captured turtles, longline-captured turtles would exhibit higher concentrations of plasma corticosterone, glucose and lactate.

Our study is the first, to the best of our knowledge, to document the physiological status of loggerhead turtles immediately upon removal from longline fishing gear. These data allow us to gauge the condition of turtles prior to release, and further refine our understanding of sublethal effects and delayed mortality associated with this fishing method. Moreover, our data permit an assessment of the relative stress of longline hooking compared with other capture methods.

2. Material and methods

(a) Fieldwork

Fieldwork was conducted in the southwest Mediterranean Sea from mid-July to mid-August in five consecutive years. In 2009, commercial longlines targeting swordfish were set in the evening and retrieved at dawn, with a soak time of 9–11 h. Approximately 1600 branch lines terminating in baited hooks (either Mustad 7 J hooks or C16/0 circle hooks) were suspended from the main line within the top 50 m of the water column. Turtles captured on hooks were landed on the vessel with a dip net. The nature of hooking was documented (figure 1) and, when possible, gear was removed. All longline fishing operations were conducted by commercial fishermen. In 2008 and 2010–2012, turtles were captured by hand as they rested at the surface. Curved carapace length (CCL) was recorded for each turtle, and all turtles were released at the site of capture within 4–5 h. Sea surface temperature (SST) for the region in which turtles were captured was downloaded from the SOCIB database (http://socib.eu/).

(b) Blood analysis

We used a 4 ml heparinized vacuum collection tube and 21Gx1.5″ needle to obtain blood from the cervical sinus within 10 min of landing turtle. A subsample was transferred to a capillary tube for PCV determination. The remaining blood was centrifuged at 4000 r.p.m. for 5 min, and plasma was transferred to cryogenic tubes for storage at −80°C. Plasma corticosterone (CORT) levels were determined by radioimmunoassay [10]. Spectrophotometric techniques were used to analyse blood glucose and lactate [10].

(c) Statistics

We used generalized linear models to assess responses of blood variables to a combination of categorical and continuous explanatory variables (R v. 3.1.2). Data for CORT and glucose were fitted using a Gamma error distribution with log link function, and data for PCV and lactate were fitted using a Gaussian distribution and identity link function. The full model for all response variables included treatment (longline- or hand-captured), CCL, SST and year of capture (see the electronic supplementary material, table S1). We used Akaike information criterion adjusted for small sample size (AIC_c) and evidence ratios calculated from Akaike weights to assess best-fit models. ANOVA was used to compare the best-fit model for each response variable with a null model that included only the intercept term and the full model (see the electronic supplementary material, table S2).

3. Results

Blood samples were collected from 10 longline-captured and 13 hand-captured turtles. Four longline-captured turtles were hooked externally in either the mouth ($n = 3$, figure 1*a*) or flipper ($n = 1$); all gear was removed from these turtles. Six longline-captured turtles had swallowed the hook (figure 1*b*), and were released with hook intact and monofilament line cut as closely to hook as possible. We documented hook type for nine of the 10 turtles captured by longline; two turtles were captured on circle hooks and seven turtles were captured on J hooks. All turtles captured were immature, with CCL between 49.5 and 76.0 cm (62.6 ± 8.7 cm, $\bar{X} \pm$ s.d.). SST was within the range of 24.0–27.5°C (25.7 ± 1.4°C).

Turtles captured in longlines had lower PCV compared with hand-captured turtles ($\beta = -4.968$, $t = -3.643$, $p = 0.002$), and there was a positive relationship between PCV and CCL ($\beta = 0.182$, $t = 2.330$, $p = 0.032$; figure 2*a*). The best-fit model (PCV ∼ treatment + CCL) was significantly different from the null model ($p = 0.020$), but not significantly different from the full model ($p = 0.366$).

Blood corticosterone of longline-captured turtles was higher than that observed for hand-captured turtles ($\beta = 1.491$, $t = 8.401$, $p < 0.001$). Corticosterone increased with CCL ($\beta = 0.023$, $t = 2.231$, $p = 0.038$; figure 2*b*), particularly within the longline treatment group. The best-fit model (CORT ∼ treatment + CCL) was significantly different from

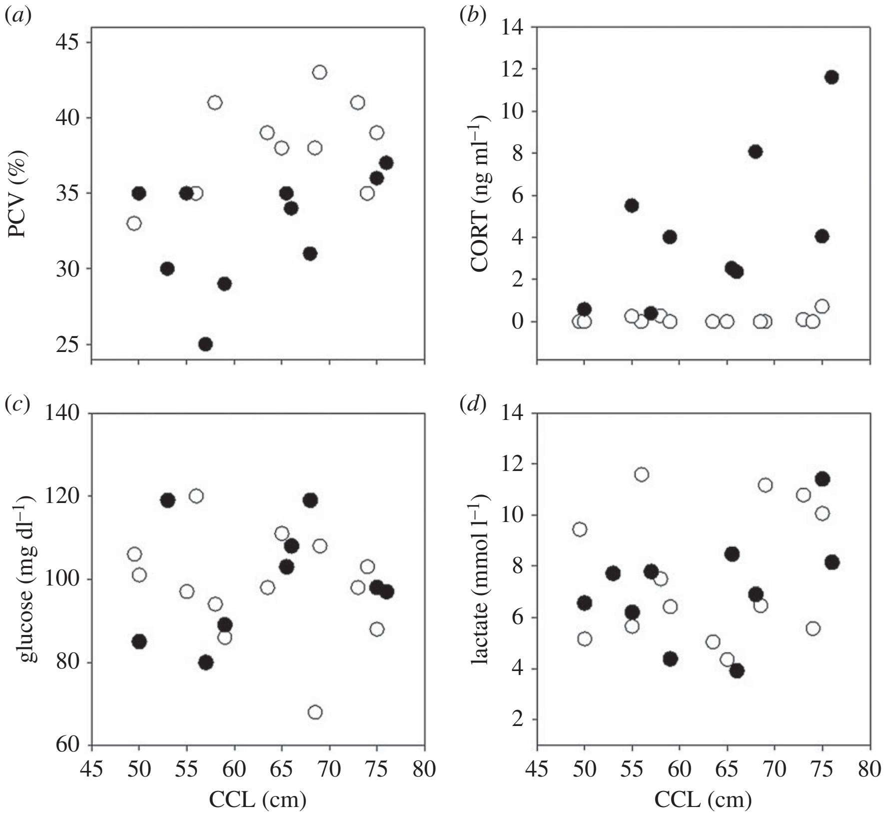

Figure 2. Longline-captured turtles (filled circles) had (*a*) lower PCV and (*b*) higher corticosterone compared with hand-captured turtles (open circles). Both PCV and corticosterone increased with size, measured as curved carapace length. No statistically significant trends were documented for (*c*) glucose or (*d*) lactate.

the null model ($p = 0.025$), but not significantly different from the full model ($p = 0.990$).

The null model yielded the best fit for both glucose and lactate (figure 2*c*,*d*); however, evidence ratios indicated uncertainty with regards to model selection. For these variables, the model with the second lowest AIC_c and evidence ratio < 2 (glucose ~ year; lactate ~ CCL) was compared with the null and full models. No significant differences were detected between the model for glucose and either the null ($p = 0.229$) or the full model ($p = 0.501$), or between the model for lactate and either the null ($p = 0.210$) or the full model ($p = 0.252$).

4. Discussion

Our study offers a snapshot of the physiological status of loggerhead turtles following a longline interaction. Analysis of PCV provides evidence that capture by longline results in blood loss, probably owing to internal and/or external injuries incurred during the process of hooking and landing. The PCV values for all turtles were within the reference range for Mediterranean loggerhead turtles [14], but PCV of longline-captured turtles was significantly lower than that of hand-captured turtles (figure 2*a*). We observed a positive relationship between PCV and CCL, consistent with other studies [14]. Injuries sustained by longline-captured turtles varied in severity and potential for long-term impacts. Turtles for which embedded gear was left in place are more prone to complications, such as reduced foraging capacity or internal lesions, which may lead to post-release mortality [15,16].

Capture in longlines resulted in induction of the systemic stress response in loggerhead turtles (figure 2*b*). Plasma CORT increases within 30 min of exposure to an acute stressor, and takes up to 180 min to peak in this species [9]. It was not possible to determine the exact duration of gear interactions in our study; consequently, observed CORT levels may not represent the maximal response to capture. Turtles removed from longlines had CORT values (4.3 ± 3.6 ng ml^{-1}) higher than that observed for hand-captured turtles (0.1 ± 0.2 ng ml^{-1}), but considerably lower than that of immature green turtles entangled in gillnets for less than or equal to 4 h (20.8 ± 16.5 ng ml^{-1}; table 1) [10]. Compared with sea turtles that are physically entangled in a submerged

Table 1. Comparison of corticosterone (CORT), glucose and lactate for immature loggerhead turtles captured in pelagic longlines (≤50 m) and immature green turtles captured in shallow-set gillnets (≤2 m). (Blood variables for both longline and gillnet studies were analysed using the same techniques in the same laboratory. Data for PCV were not available for comparison. Data are presented as range ($\bar{X} \pm$ s.d.). Stress associated with enforced submergence may contribute to higher levels of CORT, glucose and lactate in turtles captured in gillnets compared with longline-captured turtles.)

	longline (maximum soak time of 11 h)	gillnet[a] (maximum soak time of 4 h)
CORT (ng ml^{-1})	0.6–11.6 (4.3 ± 3.6)	0.3–51.8 (20.8 ± 16.5)
glucose (mg dl^{-1})	80–166 (106 ± 25)	89–192 (136 ± 35)
lactate (mmol l^{-1})	3.9–11.4 (7.2 ± 2.1)	13.1–50.2 (30.6 ± 10.2)

[a]Data from Snoddy *et al.* [10].

net, sea turtles hooked on shallow set longlines may have a greater ability to swim and reach the surface to breath. In contrast to previous studies [9,17], we found a positive relationship between CORT and CCL. Larger turtles exhibited a more pronounced response to longline capture than did smaller turtles, but interpretation of this trend is complicated by uncertainty regarding the duration and depth of hooking interactions. Size-dependent variation in the magnitude of the stress response in immature turtles warrants further investigation. Elevated levels of CORT may benefit turtles in the short-term by coordinating physiological and behavioural adjustments to promote survival, and in the absence of a sustained stressor should abate over time [18]. However, turtles released from longlines with serious injuries or gear attached may experience continual stress owing to pain or restriction in movements, leading to chronically high levels of CORT and an increased risk of sublethal effects and delayed mortality [18,19].

Induction of the systemic stress response can trigger an increase in gluconeogenesis [18]; however, we did not observe elevated glucose in longline-captured turtles. Variation in nutritional status of individual turtles may account for the lack of a significant trend. Glucose of longline- and hand-captured turtles was within the range of reference values for immature loggerhead turtles in the Mediterranean [20].

Longline-captured and hand-captured loggerhead turtles exhibited similar blood lactate levels (range 3.9–11.6 mmol l^{-1}), which were higher than baseline values reported for Kemp's ridley turtles (0.7 $\pm$ 0.1 mmol l^{-1}) [11], intermediate to values for loggerhead turtles captured in poundnets (0.8–5.2 mmol l^{-1}) and trawls (8.5–20.0 mmol l^{-1}) [13], and lower than lactate for green turtles captured in gillnets (13.1–50.2 mmol l^{-1}; table 1) [10]. Capture by trawl and gillnet often results in forced submergence, which necessitates increased reliance on anaerobic metabolism and, consequently, more pronounced increases in lactate [10,13]. Lactate accumulation may require a recovery period, during which turtles could be vulnerable to boat strike or predators. Although turtles captured by shallow-set longline may struggle to escape, the moderate increase in blood lactate and the fact that turtles typically are landed alive suggests they are capable of surfacing to breathe while hooked. Moderate lactate levels in turtles hand-captured at the surface may indicate that these animals were recovering from diving bouts during which they relied on anaerobiosis.

The fate of turtles released alive from longlines depends on the nature of the interaction and condition at the time of release. Physiological disturbances experienced by longline-captured turtles leave them vulnerable to environmental threats in the short-term, but full recovery should be possible in the absence of persistent stressors. Guidelines for best practices in handling and removing gear from sea turtles captured by pelagic longlines have been developed and promoted by the Food and Agricultural Organization of the United Nations (http://www.fao.org/docrep/012/i0725e/i0725e.pdf). Adherence to these guidelines should greatly enhance survival probabilities of sea turtles released alive from longlines.

Ethics. Ethical approval was obtained through the UNCW Institutional Animal Care and Use Committee (no. A0809-023).

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. A.W., M.P., R.S. and Y.S. participated in study design and fieldwork, gave final approval for publication, and agree to be held responsible for the content included in the manuscript; A.W. conducted data analysis and wrote the manuscript; M.P., R.S. and Y.S. critically revised the manuscript; M.P. coordinated blood sampling; R.S. coordinated field logistics; Y.S. conceived of the study.

Competing interests. We have no competing interests.

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