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Short Communication

Effects of chemicals from longline baits on the biting behaviour of loggerhead sea turtles

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The biting behaviour of the loggerhead sea turtle *Caretta caretta*, a high bycatch species by longline fishing gear, was studied on 30 wild specimens held temporarily at rescue centres. To account for repeated measures, the data were analysed using mixed-effects models. Chemicals from squid baits elicited significantly more biting behaviour than those from mackerel baits. Smaller turtles were more likely to bite than larger turtles. The findings add increasing evidence in support of the idea that the use of fish baits instead of squid baits could be a conservation measure to protect this endangered species from bycatch.

Keywords: bycatch mitigation,*Caretta caretta*, chemoreception, endangered species, fisheries

Introduction

The interaction between endangered species and fishing gear is an important issue in the conservation of marine life (Lewison et al. 2004a, Báez et al. 2007a). It involves a complex web of connections, which includes elements such as education, law enforcement, sustainable exploitation of resources, support to the local economy, and protection of biodiversity (FAO 2009).

Over the past decade, the interaction between the loggerhead sea turtle *Caretta caretta* and pelagic longline gear has been a particular matter of concern (Lewison et al. 2004b, Wallace et al. 2010). This Endangered sea turtle (IUCN 2010) is subject to a high level of bycatch by longline, estimated at more than 200 000 individuals worldwide in 2000 (Lewison et al. 2004b). Assuming a mortality rate ranging from 17% to 42% (NMFSSRSFD 2001), it is clear that immediate and concrete actions are needed to reduce the threat to this species.

Bycatch studies have shown that loggerhead sea turtles are generally hooked by longline gear when feeding on bait (Deflorio et al. 2005, Watson et al. 2005, Piovano et al. 2009). The two types of bait most often used by longliners are squid and mackerel. Size and type of bait are considered to be two of the main factors that influence both catch rate and size selectivity of the target species (Løkkeborg and Bjordal 1992, 1995, Ward and Hindmarsh 2007). In the laboratory, Kiyota et al. (2004) observed that captive loggerhead sea turtles behaved differently depending on the type of bait. They noted that squid were gulped down whole whereas mackerel were eaten piecemeal. This observation was suggested to be associated with the different textures of the two baits (Kiyota et al. 2004).

Tests with squid and mackerel simultaneously deployed at sea showed a lower sea turtle bycatch rate associated with mackerel bait (Watson et al. 2005, Yokota et al. 2009), which prompted a proposal for the use of mackerel baits instead of squid baits as a mitigation measure to reduce the capture rates of sea turtles in longline gear (Watson et al. 2005).

In view of the finding that squid is readily accepted by most species of turtles in captivity (Higgins 2003), supported by observations of wild loggerheads held temporarily in three different sea turtle rescue centres (M Affronte, Fondazione Cetacea, Italy, S Nannarelli, CTS Linosa Sea Turtle Rescue Centre, Italy, and G Ollano, Cetaceans and Sea Turtle Rescue Centre Laguna di Nora, Italy, pers. comm.), we decided to verify the loggerheads' preference for squid. To determine whether loggerheads were differentially attracted to the chemicals from mackerel and squid baits, we carried out experiments with those baits hidden in sacks, so that loggerheads could not visually recognise them as their usual preys. The focus of our study was to find new evidence concerning the feeding behaviour of wild sea turtles that could help explain reports from observations of longline fishing.

Material and methods

Choice experiment

We recorded the behaviour of 30 wild loggerhead individuals (22 juveniles and eight adults) held temporarily at the Laguna di Nora and CTS Linosa sea turtle rescue centres, Italy. Their curved carapace length (CCL) ranged between 29 and 82.5 cm (mean CCL = 48.7 cm, SD = 15.5) and all

of them were incidentally captured at sea by pelagic longline fishery. We selected individuals that had been kept for a short time in captivity (maximum four months) and declared fully recovered by the veterinary staff of each rescue centre. The experiment was performed in the days immediately prior to release of the turtles back into the sea.

We selected baits of similar size (26 cm in length) and focused on the two main longline prey items: mackerel *Scomber scomber* and squid *Ilex argentinus*. Each experiment consisted of an individual turtle and one type of bait: either mackerel or squid. Three individuals were double-tested using both types of bait. Neither of the bait types was given to the turtles prior to the experiments.

To prevent visual identification, baits presented to the turtles were hidden inside fabric sacks of size 28 cm × 10.5 cm (Figure 1). Based on the results from a preliminary test, each turtle was simultaneously presented with three whole baits of the same type (mackerel or squid) inside three sacks of different colours: blue, red and yellow. This was done to avoid biases due to individual heterogeneity in colour choice. For each experiment, the control was provided by testing the turtles' reaction in the presence of the same choice of three sacks with weights instead of baits.

To avoid problems due to bait and sack ingestion, each time a turtle opened its mouth to bite the sack, the bait was removed from the water. We considered an attempt to bite as proof of the biting behaviour.

Tests were run in opaque tanks filled with sea water to a depth of 1–1.5 m. The water temperature was between 24 and 28 °C and the surface area of the test tanks ranged between 15 m^2 and 80 m^2. Each turtle was held individually in the test tank for an acclimatisation period (minimum of 12 hours) before being tested. The trials were run during daylight, when loggerheads mainly feed (Báez et al. 2007b). The behaviour of the turtles was recorded with a portable camcorder (Hi8 Sony CCD-TRV428E or Canon MV800i).

Statistical analyses

Because each turtle was tested twice within the same experiment, once with baits and once with controls, we accounted for individual repetitions during analyses by using mixed-effects models with occasion-specific random intercepts (McCulloch and Searle 2001).

First, we investigated what influenced a turtle's biting behaviour using a mixed-effects binary logistic regression (Pendergast et al. 1996). The binary response was defined as the event of biting (yes or no). This model is summarised as follows: for the ith turtle at the jth occasion, we modelled the probability of biting p_{ij} as

$$\log(p_{ij}/(1 - p_{ij})) = \alpha_i + \beta' x_{ij}$$

where the random intercept α_i is turtle-specific and assumed to be distributed like a Gaussian random variable, β is a vector of regression parameters and x_{ij} is the vector of covariates for the ith turtle at the jth occasion. Covariates were either the presence or absence of hidden baits or the type of bait (mackerel vs squid), the rescue centre (Laguna di Nora vs Linosa), the estimated size at sexual maturity (juvenile size vs adult size), the year (2001, 2002, 2006, 2010), the category of turtle length (smaller CCL < 42.5 cm vs larger CCL > 42.5 cm, where 42.5 cm was the median CCL), and the interaction between the type of bait and the category of turtle length.

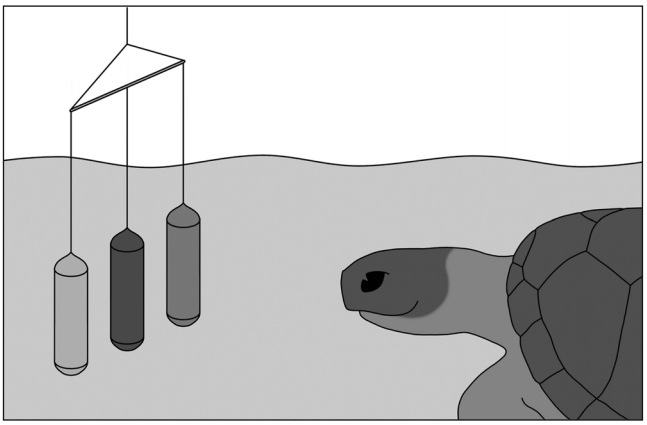

Figure 1: Choice experiment. The three sacks simultaneously presented to the sea turtle contained whole baits of the same type (mackerel or squid) or weights as a control. They were attached at a distance of 10 cm from one another

For each covariate we built a univariate model, with the random intercept and the fixed effect for that covariate. A multivariate model was then fitted using a forward stepwise selection procedure based on the Akaike information criterion (AIC). Analyses were performed in R (R Development Core Team 2009).

Results

Biting behaviour was significantly influenced by the presence or absence of bait (Table 1), with a greater probability of biting when baits were hidden inside the fabric sacks (log odds-ratio = 7.86, $p < 0.001$). In addition, there was a small but significant effect of CCL size (log odds-ratio = −0.16, $p = 0.025$) with a lower probability of biting for larger turtles. Other variables had no significant influence.

Overall, the highest frequency of biting behaviour (60%) was recorded for squid (Figure 2a, b). Between the two types of bait (Table 2), the probability of biting was significantly greater when using squid rather than mackerel (log odds-ratio = 1.32, $p = 0.025$). A more in-depth analysis of the biting behaviour provided further information on the significant variables of the final model selected (Table 3). We included the interaction between CCL and type of bait, which revealed that turtles smaller than 42.5 cm CCL (the median CCL of the sample) were more likely to bite. Conversely, larger turtles were less likely to bite and preferred squid to mackerel.

Discussion

The loggerhead sea turtle is an opportunistic carnivorous feeder (Bjorndal 1997, Polovina et al. 2003, Parker et al. 2005), able to exploit a variety of food resources, including non-indigenous species (Frick et al. 2009), discarded fish from fisheries (Plotkin et al. 1993, Tomas et al. 2001), crab-

Table 1: Results for mixed-effects models on the biting behaviour irrespective of the type of bait (selected model in bold)

Model	AIC
Empty model (only intercepts)	73.53
Predictors: presence of bait	63.73
Predictors: presence of bait, CCL	**58.06**
Predictors: presence of bait, CCL, maturity	58.28

Table 2: Results for mixed-effects models on the biting behaviour accounting for the type of bait (selected model in bold)

Model	AIC
Empty model (only intercepts)	69.41
Predictors: type of bait	61.22
Predictors: type of bait, CCL	**51.85**
Predictors: presence of bait, CCL, maturity	53.27

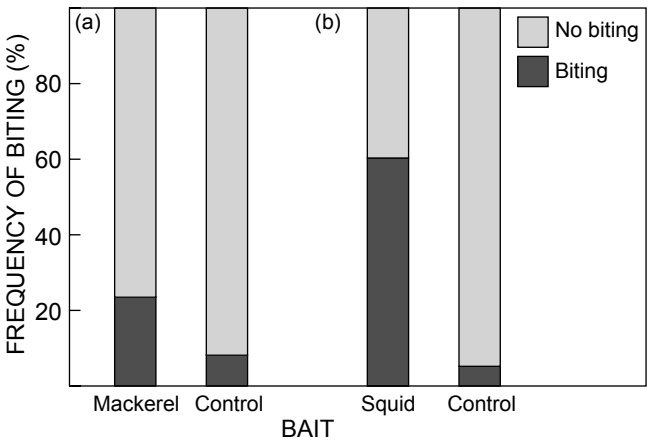

Figure 2: Frequency of biting recorded during experiments with (a) mackerel ($n_{turtles}$ = 13) and (b) squid ($n_{turtles}$ = 20), and their controls

Table 3: Final mixed-effects binary logistic regression model plus interaction

	Log odds-ratio	SE	*p*
(Intercept)	−38.836	3.973	<0.001
Squid baits	37.830	0.553	<0.001
Mackerel baits	−0.827	1.165	0.484
Smaller CCL	23.624	5.217	<0.001
Squid baits × smaller CCL	−16.733	0.553	<0.001
Mackerel baits × smaller CCL	19.336	1.165	<0.001

pot baits (Avissar et al. 2009) and longline bait (Revelles et al. 2007). However, while there is growing information on their diet (Boyle and Limpus 2008, Wallace et al. 2009, Carranza et al. 2011), little is known about the cues on which loggerheads base their feeding choice.

Sea turtles are considered to be highly visual predators (Bartol and Musick 2003), but their feeding behaviour relies on multiple cues, involving vision and chemoreception, in a complex relationship that is not yet understood. In the present study, chemicals from longline baits elicited significantly different biting behaviour in loggerhead sea turtles. Squid appeared to be preferred over mackerel even when concealed from sight. How this behaviour under laboratory conditions relates to behaviour in the wild during an incidental capture is not known. However, our findings support field observations that the type of bait can influence the loggerhead bycatch rate (Echwikhi et al. 2010) and higher loggerhead bycatch rates occur when using pelagic longline gear deploying squid baits compared to gear using mackerel baits (Báez et al. 2010).

It has been hypothesised that different bycatch rates could be related to different biting behaviours towards squid and finfish baits (Kiyota et al. 2004, Gilman et al. 2006). Stokes et al. (2011) reported that loggerheads reared in captivity attempted to swallow squid more often than sardine. Moreover, they found that the hook was more shielded when squid was used as bait and suggested that hook size affected where the turtles were hooked. Our findings provide an additional explanation for the difference in bycatch rate observed at sea between squid and finfish baits, as the chemicals from squid baits may elicit significantly more biting behaviour in loggerheads than mackerel baits. Squid appears to be the more attractive of the two types of bait and is also the type of bait that has a higher probability of hooking a turtle. The use of finfish such as mackerel could provide benefits by reducing bait attraction and the probability of hook ingestion, with the associated inability to remove the hook and branchline on board, which seriously affects sea turtle post-hooking survival (Valente et al. 2007, Casale et al. 2008).

The life cycle of loggerheads is characterised by shifts from the oceanic habitat, mainly for post-hatchlings and small juveniles, to the neritic habitat, mainly for large juveniles and adults (Bolten 2003, McClellan and Read 2007). Small juvenile loggerheads usually prey on epipelagic organisms, whereas larger individuals mainly prey on benthic organisms (Bjorndal 1997, Bolten 2003). In our study, the loggerheads were captured during pelagic longline fishing activities and the smaller turtles showed a higher frequency of biting on both types of bait (squid and mackerel). If we assume that the smaller loggerhead sea turtles captured in pelagic longline gear were in the pelagic phase, the difference we observed in the frequency of biting behaviour related to the size of the individual appears to be in line with the known habitat use and predator behaviour for this species. Smaller turtles appear to be more eager to feed on epipelagic prey than larger turtles. Notably, the size of the turtles more likely to bite was close to the average size of turtles reported to be more affected by pelagic longline fishing in the Mediterranean Sea (e.g. 46–47 cm on average in Laurent et al. 1998; 41 cm in Deflorio et al. 2005; 43 cm in Casale et al. 2008).

In the past decade there has been increasing interest in turtle sensory behaviour with regard to identifying characteristics that could be useful in reducing their rate of interaction with fishing gear (Piovano et al. 2004, Swimmer et al. 2005, Wang et al. 2007). Different types of baits have been tested at sea to verify their effectiveness in mitigating turtle bycatch without affecting the catch of target species (Echwikhi et al. 2010). In addition, artificial baits are currently being

developed and to that end further investigation of the relationships between chemical cues and prey appearance could be of value.

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