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Do colours affect biting behaviour in loggerhead sea turtles?

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Sea turtles are considered to be primarily visual predators, but the influence of different colours on the probability of eliciting biting behaviour has not yet been fully investigated. We explored this colour influence in 38 wild loggerhead sea turtles *Caretta caretta* incidentally caught during fishing activities and rehabilitated in rescue centres. Our results showed for the first time that wild loggerheads tend to bite the same colour first chosen (i.e. each individual has its colour preference) but, considering the whole sample, no clear preference or avoidance for one of the three colours tested (yellow, red and blue) is shown. Such heterogeneity may be related to each subject's history and habits. These results suggest that changing the colour of baits as a mitigation measures may have little impact on sea turtles incidental capture rates. The length of captivity prior to the experiment affected the rate of the turtles' biting behaviour, suggesting that this parameter is a potential confounding factor that should be included in models for sensory behavioural studies.

KEY WORDS: visual stimuli, colours, feeding, captivity, *Caretta caretta*.

INTRODUCTION

Food detection is a complex task accomplished in animals by relying on a variety of sensory systems. Vision, hearing, echolocation, chemoreception, mechanoreception, thermoreception and electroreception are variously developed in different taxonomic groups and can be used with different levels of integration to achieve successful feeding.

Visual cues are considered to be of primary importance in the foraging behaviour of sea turtles (Southwood et al. 2008). Evidence obtained from studies of electrophysiology (Bartol et al. 2002) and retina morphology (Bartol & Musick

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2001) support the hypothesis that the loggerhead sea turtle *Caretta caretta* is capable of visually discerning underwater both large predators, such as sharks, and small prey, such as crabs (BARTOL et al. 2002). The ratio of cone-rod photoreceptors suggests that loggerhead vision is well suited to the photopic conditions (LEVENSON et al. 2004), typical of the well-lit waters where it usually forages (LUTCAVAGE & LUTZ 1997). The spectral sensitivity curve for the loggerhead sea turtle ranges from UV to red (340–700 nm, in HORCH et al. 2008). Colour vision and colour discrimination has been strongly suggested by the simultaneous presence of multiple pigments and oil droplets (LEVENSON et al. 2004).

Loggerheads tested for feeding behaviour in a dark environment showed limited ability to locate prey (SOUTHWOOD et al. 2007). On the other hand, loggerheads tested in a natural light environment showed to acknowledge the presence of artificial squid-like plastic baits (PIOVANO et al. 2004).

The aims of this study were to investigate the role of colours in eliciting biting behaviour in wild loggerhead sea turtles while in captivity and to ascertain the tendency of individual consistency in the colour choice if any.

MATERIALS AND METHODS

Subjects

We used 38 wild loggerhead sea turtles (30 immature-sized specimens and 8 adult-sized individuals) as our experimental subjects. The Curved Carapace Length (CCL) of the subjects, measured notch-to-tip (BOLTEN 1999), ranged from 22 to 88 cm (mean CCL ± SD = 49.2 ± 16.6 cm, Fig. 1). Turtles were incidentally captured in Italian seas (29% in 2001, 42% in 2002 and 29% in 2006) during fishing activities (longlines, trawls and trammel nets) and temporarily hosted in three Italian rescue centres of the TARTANET network (Cetaceans and Sea Turtle Rescue Centre Laguna di Nora, CTS Linosa Sea Turtle Rescue Centre and Fondazione Cetacea).

We tested individuals that had been declared by the veterinary staff as having recovered and being in good health just before they were released at sea. None of them had suffered from mutilations or other visible handicaps. The period the turtles spent in captivity before the experiment varied from less than 1 month to more than 12 months (mean ± SD = 2.9 ± 4.6 months). During captivity, turtles were maintained individually in ad-hoc tanks inside the rescue centre. Windows were present in the area were the tanks were located, so turtles were able to see the light

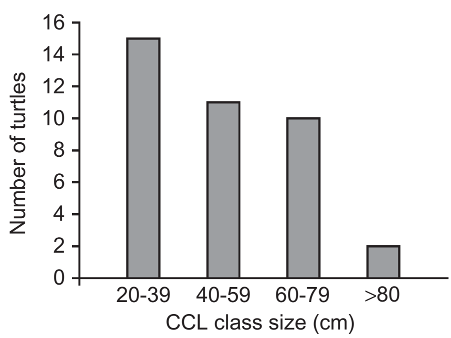

Fig. 1. — Number of turtles tested with respect to their CCL class size.

from outside. As a general rule, specimens were fed once a day at anytime during the daylight. As in PIOVANO et al. (2012), turtles were not fed with the species we later used as a source of chemical stimuli. In order to limit any possible associations between people and food no person was visible to turtles during feeding.

Test tanks

Tanks of different shapes were used during the test, according to availability in rescue centres. The surface area of tanks ranged from 15 m^2 to 80 m^2, according to the turtle CCL size. Tanks were filled with sea water to a depth of 1–1.5 m. The water temperature was between 24 °C and 28 °C. Tanks sides were opaque, so that turtles could not see through them. Following PIOVANO et al. (2012), each turtle was housed individually in the test tank for a minimum acclimatisation period of 12 hr before being tested and trials were run during daylight.

Experimental gear

A clear plastic tube was hung horizontally above the tank, from which three lines, each with an artificial prey item at its end, were attached at a distance of 10 cm from one another. The three artificial prey items were submersed 30 cm below the surface.

Fabric sacks sized 28 × 10.5 cm of three different colours (yellow, red and blue; Table 1) were used as artificial prey items. After appropriate calibration, colours were measured with a portable X-Rite spectrodensitometer (mod. 528) in a continuous scale using the CIE (Commission Internationale de l'Eclairage) L*a*b method colour system (WYSZECKI & STILES 2000), where L = lightness, a = red to green value, and b = yellow to blue value.

Colour choice trials

We ran two colour choice trials for each turtle: one with artificial prey items containing a weight, named 'colour-only trial', and one with artificial prey items containing a whole mackerel (*Scomber scombrus*) or a squid (*Ilex* sp.), named 'colour-chemical trial'. This way we accounted for possible lack of attractiveness of the artificial prey items, given that previous studies have highlighted the importance of chemicals in eliciting the biting behaviour of loggerhead sea turtles (PIOVANO et al. 2004, 2012), as well as the potential effects of the combining of colours and prey chemical cues on the response to different colours. In addition, we set up the experiment in such a way that allowed us to separate the role of colour from the role of the position of the artificial prey in the experimental gear.

Table 1.

Colours of artificial prey items (CIE L*a*b colour system, where L = lightness, a = red to green values, and b = yellow to blue values).

	Yellow	Red	Blue
L	84.64	37.97	41.85
a	−3.39	57.33	−13.20
b	56.04	34.29	−30.61

We recorded the biting behaviour of each specimen in both the colour-only trial and the colour-chemical trial. Each trial was composed of six repetitions, with the artificial prey items ordered according to one of the six possible colour combinations (i.e. B-R-Y, B-Y-R, Y-R-B, R-B-Y, Y-B-R, R-Y-B; where B = blue, R = red, Y = yellow). Each repetition lasted for a maximum of 10 minutes. A minimum of 30 minutes elapsed between two consecutive repetitions. Repetitions from the two trials were run in a random order. All three artificial prey items were taken out of the water soon after the turtle opened its mouth to bite, to avoid possible complications due to ingestion. We considered an attempt to bite as proof of biting behaviour. Turtles that attempted to bite the artificial prey items were not rewarded with food.

We recorded the turtles' biting behaviour with a remote controlled camcorder (Hi8 Sony CCD-TRV428E) on a tripod.

Statistical analyses

The variables considered were the length of captivity prior to the experiment, type of trial, turtle CCL and the interactions between these factors. In order to remove spurious effects due to a possible learning effect among the turtles, we adjusted all models for the effects of the ordered colour combinations presented in each of the two trials.

Since we had a repeated measures experiment, many basic and well-known inferential tools could not be used directly. We took into account groups of observations arising from the same turtle by building mixed effects models with turtle-specific random intercepts (McCulloch & Searle 2001). We assumed that random effects followed a Gaussian distribution, and effects relating to the same turtle were clustered in order to capture dependency arising from the repeated measurement. The effects corresponding to the other covariates were assumed to be fixed. For each covariate we built a univariate model, with the random intercept and fixed effect for that covariate; plus another fixed effect for the ordering of the colours presented, where applicable. We then fitted a multivariate model. Within the set of possible multivariate models, we selected the one that minimized the Akaike Information Criterion (AIC).

Colour choice. In order to investigate individual turtle behaviour with respect to colour choice, we restricted the sample to specimens that had bitten at least once ($N = 25$). In addition, for those turtles that had bitten at least twice ($N = 20$), on each occasion after the first bite we categorised their response into one of the following groups: not biting, biting the same colour as the first bite, biting a different colour. The responses were modelled using a multinomial mixed-effects logistic model (Hartzel et al. 2001), which can be summarized as follows: let $p_{\{ij0\}}$, $p_{\{ij1\}}$ and $p_{\{ij2\}}$ be respectively the probability for the i-th turtle on the j-th occasion of avoiding biting, biting the same colour, biting a different colour:

$$\log(p_{\{ij1\}}/p_{\{ij0\}}) = \alpha_{\{i1\}} + \beta_{1'x\{ij\}}$$

$$\log(p_{\{ij2\}}/p_{\{ij0\}}) = \alpha_{\{i2\}} + \beta_{2'x\{ij\}}$$

where $\alpha_{\{i1\}}$ and $\alpha_{\{i2\}}$ are independent normal random effects.

We used a multinomial mixed-effects logistic model also to model turtle responses with respect to the position of the colour within the gear.

What influenced individual turtle biting behaviour?. The binary response was defined as the event of biting, and the model we used was a mixed-effects binary logistic regression (Pendergast et al. 1996). This model can be summarized as follows: for the i-th turtle on the j-th occasion, we modelled the probability of biting p_{ij} as

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

where β is a vector of regression parameters, x_{ij} is the vector of covariates for the i-th turtle on the j-th occasion, and the random intercept α_i was assumed to be distributed like a Gaussian random variable.

The influence of the length of captivity prior to the experimental trials was also investigated with a classification and regression tree (BREIMAN et al. 1984).

What influenced the frequency of individual turtle biting?. We used a Poisson GLM to model the frequency of biting by the individual turtles. Since there were repeated experiments with the same turtle in different settings, as before, we used, a random intercept. We thus assumed the frequency of biting behaviour of the individual turtle was distributed like a Poisson random variable, whose parameter $\lambda_{\{ij\}}$ was modelled as

$$\log(\lambda_{\{ij\}}) = \alpha_i + \beta' x_{\{ij\}}$$

where, as before, β is a vector of regression parameters, $x_{\{ij\}}$ the vector of covariates for the i-th turtle under the j-th experimental setting, and the random intercept α_i is distributed like a Gaussian random variable.

Analyses were performed in R v. 2.9.1 (R DEVELOPMENT CORE TEAM 2009).

RESULTS

Colour choice

As a group, turtles chose the colours yellow, red and blue almost equally (Fig. 2). During repetitions, turtles bit the same colour previously chosen more frequently than a different colour (Fig. 3).

Results from a multinomial mixed-effects logistic model suggested that an individual was more likely to continue with the colour first chosen rather than change it.

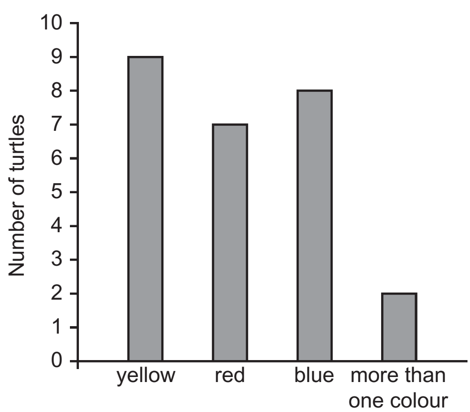

Fig. 2. — Preferred colour by individual turtles (sample restricted to individuals for which at least one instance of biting behaviour was recorded, N = 26).

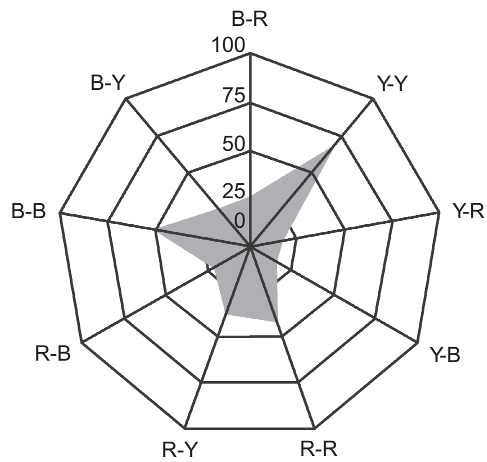

Fig. 3. — Consecutive colour choices, in percentage. (Code: left letter = first colour choice, right letter = consecutive colour choice; B = blue, R = red, Y = yellow).

The probabilities of recording (1) a bite to the colour first chosen and (2) a bite to a colour different from the one first chosen were significantly different ($P < 0.001$), and the first was significantly higher than the second (bite the colour previously chosen: intercept = −0.252; bite a colour different from the one previously chosen: intercept = −1.248). The position of the colour within the gear did not influence the response of individual turtles (bite the same position: intercept = −0.35; bite a different position: intercept = −0.41; $P > 0.05$).

What influenced individual turtle biting behaviour?

In general, we observed biting behaviour in 17 out of 38 specimens during colour-only trials and in 25 out of 38 specimens during colour-chemical trials. The probability of recording biting behaviour for a loggerhead during colour-chemical trials was significantly greater than during colour-only trials (log-odds = 1.754, $P < 0.001$).

In our sample of turtles the length of captivity prior to the experimental trials significantly affected the probability of an individual exhibiting biting behaviour, with a clear difference at a threshold period of 6 months (log-odds = −2.043, $P = 0.020$). The subsample of loggerhead held in captivity for more than 6 months (N = 9) showed a higher percentage of individuals exhibiting biting during both colour-only and colour-chemical trials than the subsample of loggerheads held in captivity for less than 6 months (N = 29) (Fig. 4).

What influenced the frequency of a turtle's biting?

We observed biting behaviour in 124 of the 456 repetitions. In general, the presence of the chemical stimulus elicited individual biting behaviour, with an increase of 0.779 bites on average per individual ($P < 0.001$).

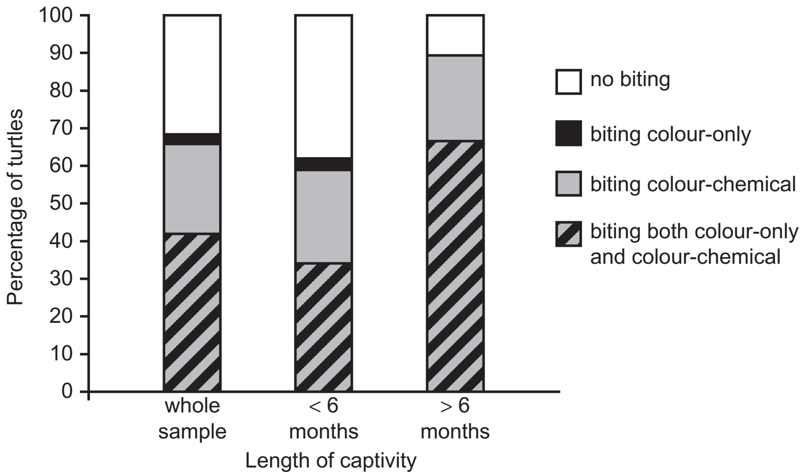

Fig. 4. — Frequency of biting and not biting behaviour recorded on the entire sample of loggerheads (N = 38) and on the subsamples of loggerheads held in captivity for less than 6 months (N = 29) and more than 6 months (N = 9).

Length of captivity played a significant role: the average frequency of biting elicited by colour-only and colour-chemical artificial prey items was 1.7 (SD = 1.5) and 3.4 (SD = 2.4) for turtles with more than 6 months in captivity and 0.8 (SD = 1.4) and 1.9 (SD = 2.2) for turtles with a shorter period in captivity. Results from the GLM model showed that turtles that spent less than 6 months in captivity had a significantly lower frequency of biting (coefficient estimate = −1.221, $P = 0.022$). Other variables had no significant influence on the frequency of biting, either alone or in the multivariate model ($P > 0.05$).

DISCUSSION

The analysis showed that results from the group as a whole can hide differences in individual colour choice. Turtles tended to bite the same colour they had first chosen; however, as a group, no clear preference or avoidance for the yellow, red or blue artificial prey items was shown. Our sample was composed of turtles ranging in size from small juveniles to adults incidentally captured in the same fishing areas. No sign of tagging was present, so information about their history was not available. We do not know how long they had been in those areas before being captured or what they were feeding on. Their previous experiences may have determined their preference for the colour they chose during our experiment. Moreover, individual heterogeneity in the choice of a colour fits well with the model proposed for the loggerhead sea turtle by Vander Zanden et al. (2011) of individual feeding specialists within a generalist population (Bjorndal 1997; Parker et al. 2005).

In recent years, attempts have been made to use a sensory-based approach to reduce incidental capture of sea turtles by longline fishing gear (reviewed in Southwood et al. 2008). The concomitance of photopic foraging environment and supposed colour vision suggested that the colour of baits could be changed to reduce their attractiveness or visibility. Unfortunately, after some encouraging results in captivity, no success was obtained in field trials (Swimmer et al. 2005; Yokota et al. 2009). Our findings suggest that it is difficult to identify a colour that is avoided by the majority of a turtle population because of strong individual colour preferences. For these reasons, changing the colour of baits as a mitigation measures may have very little impact on sea turtle incidental capture rates.

Sea turtles tested in vision and olfaction studies (reviewed in Bartol & Musick 2003; Southwood et al. 2008) had experienced different lengths of captivity, as either short-term guests of rescue centres or long-term guests of captive facilities. In this study, wild loggerheads from fishing bycatch showed a different rate of biting behaviour in relation to the length of captivity, and this phenomenon may be the result of conditioning. For example, operant conditioning methods have been successfully used in training juvenile loggerhead sea turtles to associate a stimulus with food reward (Bartol 1999). Our turtles were not specifically trained, but on average specimens that experienced a period of captivity longer than the 6 months were more likely to associate an object in the tank with something to eat. Of course we cannot say which of the two behaviours, the wariness of the first period or the greater confidence after 6 months is more similar to the turtles' natural behaviour at sea. Nevertheless, our results suggest that this difference is important, and should be taken into account. As the length of captivity is a potential confounder, this variable should be included in models or considered when selecting individuals to be tested.

Overall, we have very limited knowledge about the role played in sea turtles decision making processes by information from different sensory channels and the effects of their combination. Further research examining the relationships of visual cues and prey appearance is needed. These kinds of behavioural studies could help fill the gap in our knowledge of the sensory behaviour of marine vertebrate species. In addition, these studies could have a strong conservation impact because they may reveal measures that help to improve the recovery of endangered sea turtle populations worldwide.

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