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Research article

The effect of preference assessment-informed enrichment device colour on biting and foraging behaviour in loggerhead sea turtles

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Abstract
Although environmental enrichment procedures have demonstrated their benefit to non-human animals in zoos and aquariums, the field has given little attention to phylogenetic variables that might affect their efficacy. Recently, research with loggerhead sea turtles *Caretta caretta* has demonstrated that they have true colour vision and that colours may differentially strengthen certain response classes (e.g. phototaxis). Colour preference was assessed for four turtles and for three of the turtles background colour was varied to control for contrast effects. The effect of enrichment device colour on level and type of interaction was evaluated. Differences in colour preferences were found for all turtles and there was minimal effect of colour on interaction with enrichment devices containing preferred food. These results suggest caregivers may wish to consider individual preferences when developing enrichment or training devices for these animals.

Introduction

Declining global sea turtle populations have sparked widespread rehabilitation and conservation efforts (Burghardt 2013; Wallace et al. 2011). Reintroduction success largely depends upon the presence of basic behavioural skills, defined by the International Wildlife Rehabilitation Council and others as the animal's ability to move within complex environments, construct a living space, avoid predators, forage for food, interact in social groups and avoid conflict with humans (Miller 2012; Reading et al. 2013). Several studies have demonstrated the benefits of high-quality environmental enrichment programmes for captive animals (Maple and Perdue 2013; Mellen and MacPhee 2001; Young 2013) and preliminary evidence suggests that enrichment programmes implemented by zoos and aquariums can increase the chance of released sea turtles' success in the wild (Monreal-Pawlowsky et al. 2017; Oros et al. 2016). Environmental enrichment can prepare rehabilitated animals for release by instating natural contingencies in the animals' contrived environment, such as occasioning the search for food (i.e. foraging behaviour) and programming intermittent reinforcement in the form of food for such responding. The importance of such goals applies to permanently housed animals as well; training any of these behaviours may constitute enrichment insofar as the animal learns, makes choices and engages in problem solving strategies or if the outcome itself reinforces behaviour (e.g. Fernandez and Martin 2023; Melfi 2013).

In one of the few demonstrations of enrichment with loggerhead sea turtles, Therrien et al. (2007) presented a variety of enrichment devices including polyvinyl chloride (PVC) pipes and water jugs filled with food to four turtles. During presentation of the enrichment devices, stereotypic swimming decreased from 77% to 8% while random swimming and focused behaviour increased from 20% and 2% respectively to about 44% each (Therrien et al. 2007). Reducing stereotypic behaviour or abnormal repetitive behaviour with no obvious function (e.g. pacing, swimming in a repeated pattern, gnawing at cage bars) is important because it causes alarm to public visitors and can co-occur with poor welfare (Mason 1991; Mason and Latham 2004; Mason and Rushen 2006).

The type of enrichment programme or device that benefits a given animal depends upon species characteristics and individual preferences. Bostwick et al. (2014) demonstrated that a shark model caused loggerhead sea turtles to turn their carapace toward the stimulus and pause longer prior to food retrieval compared to a sphere or a bare food item. The study demonstrated that the shape of devices placed in the animal's environment might play a role in their efficacy for enrichment or preparation for survival post-release. Other studies have demonstrated the role of individual preference, which is gauged through assessments that present stimuli in counterbalanced singles, pairs or groups and measure some choice response such as the percentage of trials in which the stimulus was selected (touched or manipulated) or the latency to approaching the stimulus (see Fernandez and Martin 2023 for a review of preference assessments conducted in zoos).

While not all animals have colour vision, some species use it as an important tool that aides in foraging, courtship, predator avoidance and other functions that prove crucial to survival (Gerl and Morris 2008). Turtles possess photoreceptors, which enable them to discriminate changes in stimulus dimensions such as wavelength (colour) and light intensity (Young 2013). In fact, one recent study (Noh et al. 2024) found that colour played an important role in sea turtle selection and complete ingestion of coloured jellyfish presented in a manner that replicates plastics found in the ocean that sea turtles might consume. The jellyfish were presented as coloured squares and texture was also manipulated and found to affect consumption. In this study, the sea turtles were more likely to approach and consume the lighter squares and the authors noted that the turtles were attracted specifically to the colour yellow, further supporting the potential usefulness of colour preference assessments. While studies have assessed preferences of other types of turtles and tortoises (e.g. Hall et al. 2018; Learmonth et al. 2021; Passos et al. 2014; Spiezio et al. 2017), to the authors' knowledge only one study exists evaluating colour preference with loggerhead sea turtles. Piovano et al. (2013) presented red, yellow and blue sacks to 38 turtles. They hung the sacks above the tank and filled the sacks with a weight, mackerel or squid. For each turtle, the authors recorded the colour of the sack the turtle attempted to bite on the first trial and whether it attempted to bite the same colour on the subsequent trial. The authors reported they observed colour preference for some turtles (i.e. higher probability of biting the same colour on subsequent trials) but no colour emerged as more highly preferred by the group.

Given the loggerhead sea turtle's ability to detect colour and that certain shapes may elicit species-specific behaviours, it is reasonable that phylogenetic relations (i.e. based on the evolutionary history of the animal) between specific colours and adaptive behaviours may exist, as has been shown with other species (e.g. Dong et al. 2010; Griebel 2002). The present study aimed to determine whether individual colour preference among blue, green, orange or yellow would affect the allocation of a biting response toward enrichment devices containing food in four loggerhead sea turtles at an aquarium in southeastern Georgia. A differential preference would suggest that colour should be considered when conducting preference assessments and planning environmental enrichment programmes; it would also suggest that incorporating other stimulus dimensions might be of value. First a paired-choice preference assessment modelled after Fisher et al. (1992) was used to detect idiosyncratic food and colour preference hierarchies. This assessment presents a fast and easy method of identifying preference hierarchies that other researchers have successfully used to detect preference hierarchies in animals (Mehrkam and Dorey 2014; Vicars et al. 2014). Then, with three turtles, the study evaluated whether enrichment devices built with high- versus low-preference colours would differentially influence species-typical foraging behaviours, such as swimming, biting or otherwise exploring the device. This latter assessment represents a reinforcer assessment, which offers qualitatively different information than a preference assessment (e.g. Piazza et al. 1996). While a preference assessment measures response allocation among choice options, a reinforcer assessment detects response rate, a measure of response strength, thereby providing information not just that an animal prefers a stimulus but that the stimulus will strengthen behaviour (see Patterson-Kane et al. (2008) for a discussion of the role of this and other operant procedures on gauging strength of animals' preferences). Previous research was improved upon by systematically training the biting response and controlling for reinforcement rates across colours and positions in the tank. Herein a methodology for assessing individual preferences and applying those preferences to individualise enrichment devices for each animal is presented.

Methods

Subjects, materials and setting

Assessments were conducted at the University of Georgia (UGA) Aquarium located on Skidaway Island, GA. The procedures were not invasive and were conducted in accordance with the standard operating procedures of the aquarium. All procedures used in the study received approval from both Institutional Animal Care and Use Committees (IACUC) housed in the first and third authors' institutions. Four loggerhead sea turtles referred to as Lefty, Rider, Neptune and Wiso served as subjects. Aquarium staff obtained the turtles from the Georgia Sea Turtle Cooperative (a division of the Georgia Department of Natural Resources), who found the turtles in nests where without intervention they would likely end up deceased. Veterinary staff routinely evaluated these 'stragglers' to ensure good health. Although received by the aquarium as hatchlings (i.e. less than one week old), at the beginning of data collection for each experiment the turtles' ages ranged from 6 to 25 months.

All turtles were experimentally naïve and researchers conducted the experiments in the same tanks that housed the turtles at the time in order to evoke normal responses and eliminate biases related to environmental changes. Turtles never shared tanks with one another, though some had fish inhabiting their tank during the time of the assessment. Researchers did not observe any interference from the fish with any of the experiments. Researchers presented stimuli and delivered reinforcers at the top of the tank where the public did not have access, even when the turtles resided in display tanks which had an acrylic viewing window.

Throughout the study, aquarium curators maintained daily food allotments with protocols in place for each turtle at the time. Investigators and aquarium personnel subtracted any food delivered during sessions from the daily food allotment to ensure that turtles consumed standardised proportions of their body weight each day. Additionally, investigators collected data at approximately the same time of day for individual turtles throughout each experiment phase. The food reinforcers used in the study included fish, shrimp and a nutritional supplement called gel food (a combination of gelatine, fishmeal, vegetables, whole eggs and vitamins) cut into pieces appropriately sized for each turtle.

Investigators delivered food reinforcers to the turtles using stainless steel tongs 29.9 cm long. Four different coloured wiffle balls (blue, green, orange and yellow) with 6.4 cm diameter functioned as the test stimuli in the colour preference assessment. Wiffle balls were made of plastic and had holes throughout to allow the turtle to easily bite the stimuli. These stimuli were chosen based on previously successful enrichment purposes (e.g. AdventureAquarium 2014). Caretakers present wiffle balls as enrichment devices because retrieving food from a wiffle ball topographically resembles working protein out of a shell. Observers and investigators consisted of two PhD-level behaviour analysts, two aquarium curators and several undergraduate and graduate student investigators who assisted in data collection. The faculty mentor trained students to collect data through verbal instruction and mock trials. The aquarium curators helped to develop the materials and protocols and trained the students and faculty mentor on feeding and care procedures for the turtles.

Experiment 1: Assessment of food preference

To identify food reinforcers used in subsequent phases, investigators assessed the turtles' food preference hierarchy among fish, shrimp, gel food and carrot using a pairwise preference assessment (e.g. Fisher et al. 1992). Prior to conducting sessions, the investigators removed any enrichment items from the tanks that might interfere with behavioural assessments. The prepared data sheets prescribed food and colour presentation order and position (see Supplementary Information A). The researcher commenced a trial when the turtle swam to a preexisting area in the back of the tank, to control for positional bias. Once at the back of the tank, the researcher presented two pieces of food equally distanced from each other and about 5 cm below the surface of the water. Choice was defined as a bite or any touch by the turtle's beak or tongue, regardless of consumption. Food position (left or right) was counter-balanced between trials, each item was presented an equal number of times and trials were presented at the middle of the tank about 15 cm in front of the tank wall. After a selection response occurred, the tweezers were removed and the next trial proceeded when the turtle swam to the centre of the tank near the wall opposite the investigators. Three sessions of 12 trials were conducted for each turtle for Rider, Lefty and Neptune and four sessions of 12 trials for Wiso. Wiso's data showed more variability in the selection response, therefore researchers included an additional session to increase the power of the results. A trial began when researchers presented the food in the tank and ended when the turtle selected the food item or after 60 s had passed.

Experiment 2: Assessment of colour preference

Based on the results of the food preference assessment, fish and shrimp were used as reinforcers to establish and maintain selection responses on the coloured balls.

This assessment attempted to identify if the sea turtles demonstrated a preference for certain colours. Stimuli included blue, green, orange and yellow wiffle balls. These colours were chosen based on previous research (Piovano et al. 2013). Additionally, these colours typically exist in the natural environment of loggerhead sea turtles and represent a continuous range of wavelengths on the colour spectrum. The selection response was trained by presenting one ball at a time and reinforcing bites (i.e. any touch by the turtle's beak or tongue to the stimulus) on the object with a piece of food identified from the food preference assessment. This continued until all ball colours received an equal number of reinforcers to control for accidentally establishing a preference from a history of reinforcement with the object.

This assessment was conducted in an identical fashion to Experiment 1 with the following exceptions. First, coloured balls were used as the stimuli to assess colour preference. Second, when the turtle made a selection, the investigator immediately removed the balls and delivered a piece of food as reinforcement to maintain selection response. If the turtle did not make a choice after 30 s, the investigator removed the balls from the tank, presented the next trial and repeated the skipped trial at the end of the block of trials. If no selection response occurred for three trials in a row the investigators would have terminated the session, but this never occurred. Lastly, a total of four 12-trial sessions instead of three were conducted over the course of two weeks.

Experiment 3: Colour contrast assessment

Only Lefty, Neptune and Wiso participated in this experiment. Rider was excluded because the other animals housed in his tank competed for access to the food reinforcers and devices

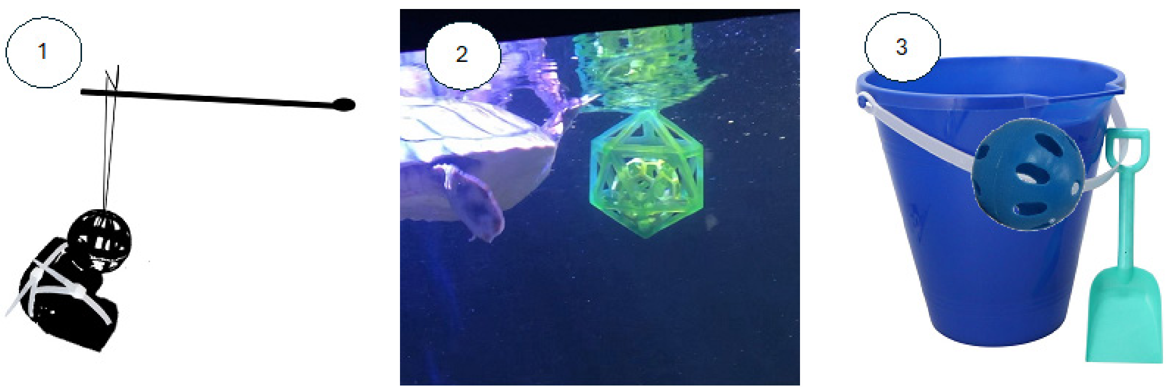

Figure 1. Example models of the enrichment devices used for: 1) Lefty, 2) Neptune and 3) Wiso

presented, threatening Rider's ability to access the prescribed allocation of food. Additionally, given Rider's size at the time of the assessment, the aquarium curators had safety concerns regarding the turtle's ability to bite through and potentially ingest coloured wiffle ball parts.

This assessment was conducted immediately after Experiment 2, in the same manner except the researchers varied the colour of the background. Between sessions, plastic background boards were rotated to control for the saliency of the wiffle ball colours against different backgrounds. The backgrounds consisted of yellow, black and white plastic sheets fixed against the back of the tank. The background colour remained constant throughout the 12-trial session and researchers rotated the background between sessions. Three sessions were conducted for each background colour. Observers recorded a selection response when the turtle's beak touched a ball, at which time the investigator removed the wiffle balls from the tank and delivered food in the same manner as Experiment 2. The coloured background remained in the tank. Observers recorded the selection on the data sheet and the investigator rotated the stimuli for the next trial. The investigator began the next trial after the turtle swam to the wall of the tank opposite the investigators.

Experiment 4: Free-operant comparison of enrichment devices

This final experiment evaluated whether stimulus colour would affect interaction with an enrichment device in a free-operant arrangement across 10-min sessions. This was evaluated by presenting a device created using the least and most preferred colours. These two conditions were evaluated along with a condition in which no enrichment device was present (i.e. control condition) using a multi-element design.

Dependent measures

Six categories of response were measured, adapted from Therrien et al. (2007). Resting was defined as "any period of inactivity" (e.g. turtle was motionless in the bottom of the tank). Pattern swimming was defined as "swimming in a repetitive pattern around the tank, scored within the interval when the pattern began the third time in a row" (e.g. if the turtle were to swim along the mirrored front of the tank along the same line back and forth). Random swimming was defined as "swimming in ways other than pattern swimming" (e.g. if the turtle is in front of the tank and then moves to the back wall of the tank). Interaction was defined as "investigation of the enrichment device excluding biting-pushing and rubbing" (e.g. if the turtle pushes the device with a closed beak). Biting was defined as "approaching the enrichment device with an open beak and touching the device with the beak" (e.g. if the turtle bites the ball, PVC pipe or food on the device). Any other behaviour was scored as not categorised (e.g. if the turtle pushed its flippers across its face). See Supplementary Information B for a copy of the data sheet.

Enrichment devices

The enrichment devices varied for each turtle, informed by their individual results from the preference assessments. Figure 1 displays a model example for each enrichment device. For Lefty, an enrichment device was created from yellow (i.e. most preferred) and blue (i.e. least preferred) PVC pipe. The device had ice cubes of fish and shrimp attached to it to encourage foraging behaviour. For Neptune, green was selected for the most preferred, based on consistent selection during the colour contrast assessment, and blue as the least preferred. The enrichment devices consisted of two rubber dog toys, one larger prism shaped ball that contained a smaller rubber ball that contained fish and shrimp. Interaction with the device resulted in pieces falling out of the smaller rubber ball.

Given the undifferentiated responses from Wiso, the researchers conducted brief preference assessments consisting of six trials prior to enrichment sessions. Based on the brief assessment, the researchers used the most and least selected stimuli for that day. For this reason, four enrichment devices were created of all colours (i.e. orange, yellow, blue and green). The devices consisted of plastic buckets that floated in the water with the wiffle balls attached to the middle with string. The balls contained food and interactions with the device caused the food the fall out of the ball for the turtle to access.

Procedures

Prior to each 10-min session, investigators rinsed the devices in fresh water and prepared the food. A session began when investigators positioned the devices in the centre of the tank so that it was partially submerged in the water. Observers recorded partial interval data in 10 s bins for random swimming, pattern swimming, resting and interaction with the device and recorded frequency data for biting the device.

The order of each condition was randomised across sessions. In the control condition (i.e. no device), the behaviour of the turtle was observed for 10 min without any external stimuli or interaction from the researchers. Most preferred sessions consisted of the yellow enrichment device for Lefty, the green enrichment device for Neptune and the colour enrichment device that corresponded with the most frequently selected colour during the brief preference assessment for Wiso. The least preferred session consisted of the blue enrichment devices for Lefty and Neptune and the colour enrichment device that corresponded with the least frequently selected colour during the brief preference assessment for Wiso. No interaction between investigators and the turtle occurred during any of the sessions.

Results

Experiment 1: Assessment of food preference

Figures 2–5 present the results of the food preference assessment for Rider, Lefty, Neptune and Wiso. The results are presented in two forms: in aggregate form using a bar graph to show the overall average of selections and on a standard celeration chart (SCC) that uses a ratio scale on the y-axis to examine proportional changes across selections. Celeration was calculated as a measure of learning (i.e. multiplicative growth across weeks), bounce as a measure of variability (i.e. the multiplicative value between the highest and lowest data point) and level as the measure of central tendency (i.e. the geometric mean). These metrics provided researchers with a more sensitive analysis across days to look at differences between these metrics across stimuli. While the use of SCCs for loggerhead sea turtles is novel, the authors based the study decisions on data displayed on the SCC. Therefore, the SCCs represent a component of the independent variable. The chart helps one determine the fluency of responses by looking at the frequency of responses across time (e.g. Evans et al. 2021). Researchers have used the chart to make decisions about animal training (e.g. Bulla et al. 2023).

Results of the food preference assessment show that Rider selected gel food the most consistently with increasing celeration and very little variability, selected fish the second most frequently with an increasing celeration across sessions and selected carrot the least with a deceleration across days. Lefty selected shrimp the most consistently with very little variability and the highest overall level compared to the other foods and selected carrot the least with only one selection response made. Neptune selected shrimp and fish the most consistently, with fish being selected two fewer times. Neptune selected carrot the least with only one selection response made. Wiso selected fish the most consistently

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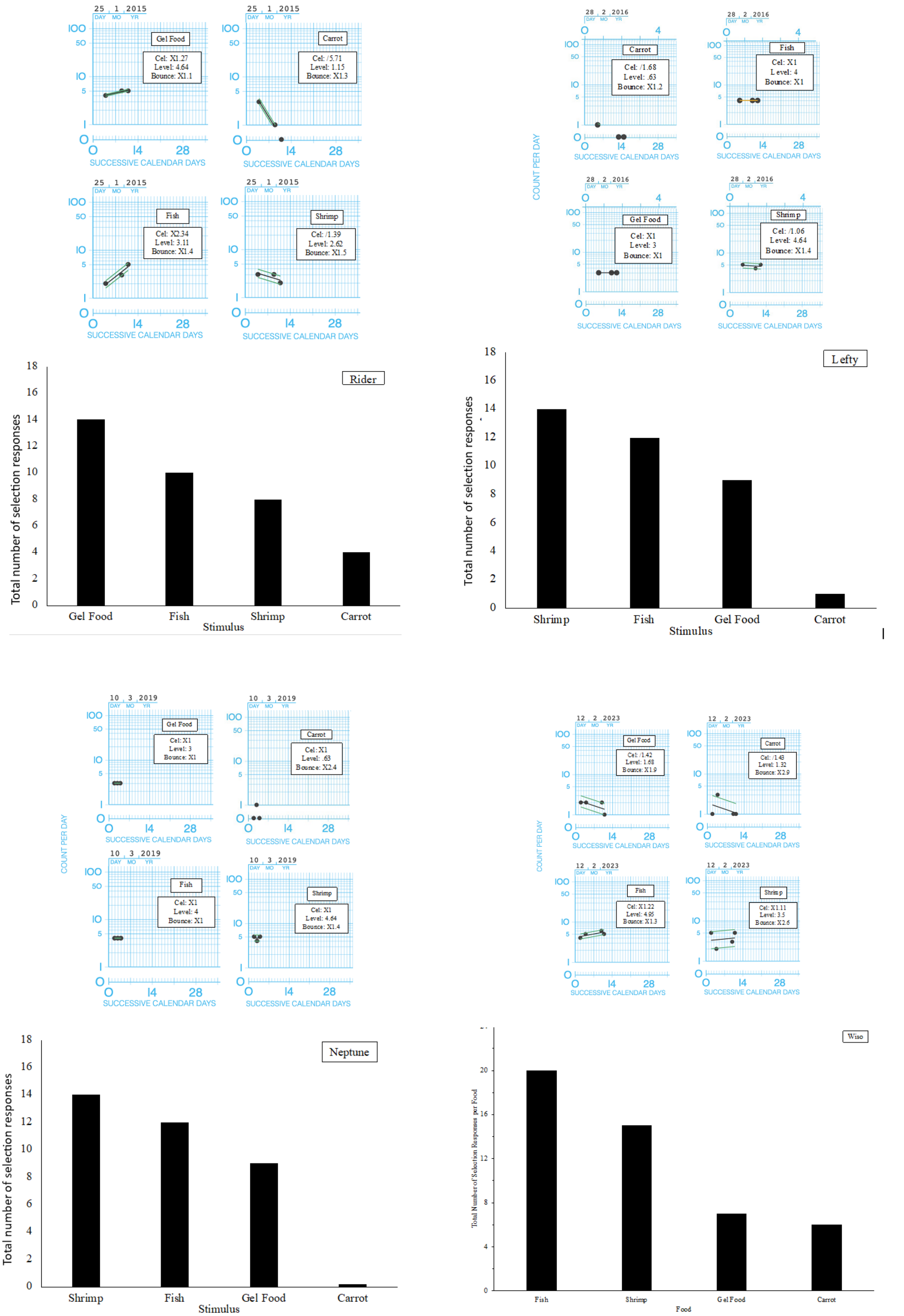


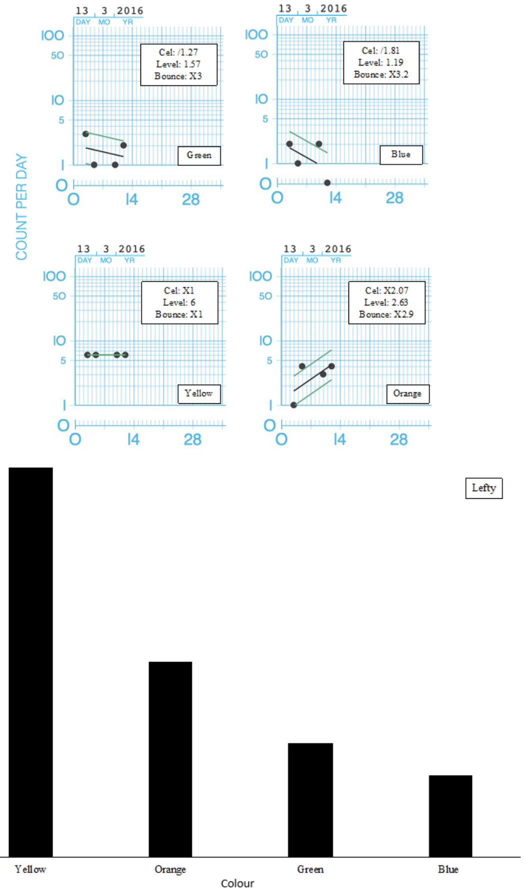
Figure 2 (Rider), 3 (Lefty), 4 (Nepture) and 5 (Wiso) Food preference assessment data: A) across-session data on segments of the standard celeration chart, dots represent selections; B) aggregate data across all sessions

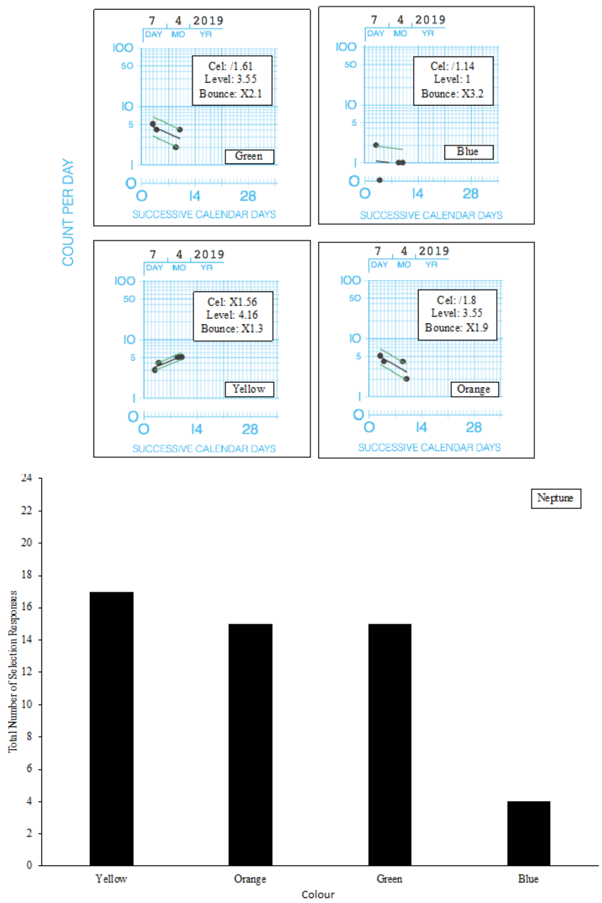
with very little variability and an accelerating trend. Wiso also selected carrot the least with a decelerating trend and a large degree of variability. The distribution of ranks of each food item were compared using the Friedman test and significant differences in ranking were found (Friedman $\chi^2=8.1_{(3)}$, P=0.044). However, a post-hoc analysis revealed no significant pairwise differences, likely due to the small sample size.

Results of Experiment 1 demonstrate that some commonalities amongst preferences exist between turtles, however data show idiosyncrasies which further support the need for individualised preference assessments. To further assess preferences, assessments were continued to identify if colour preferences exist within loggerhead sea turtles. The results of Experiment 1 were used to inform the selection of reinforcers to shape and maintain a selection response.

Experiment 2: Assessment of colour preference

Figures 6–9 present the results of the colour preference assessment for Rider, Lefty, Neptune and Wiso. Rider's within-session data did not transfer over when the university migrated to a new data system. Therefore, aggregate data previously displayed on a poster presentation was used to display the results of his assessment.





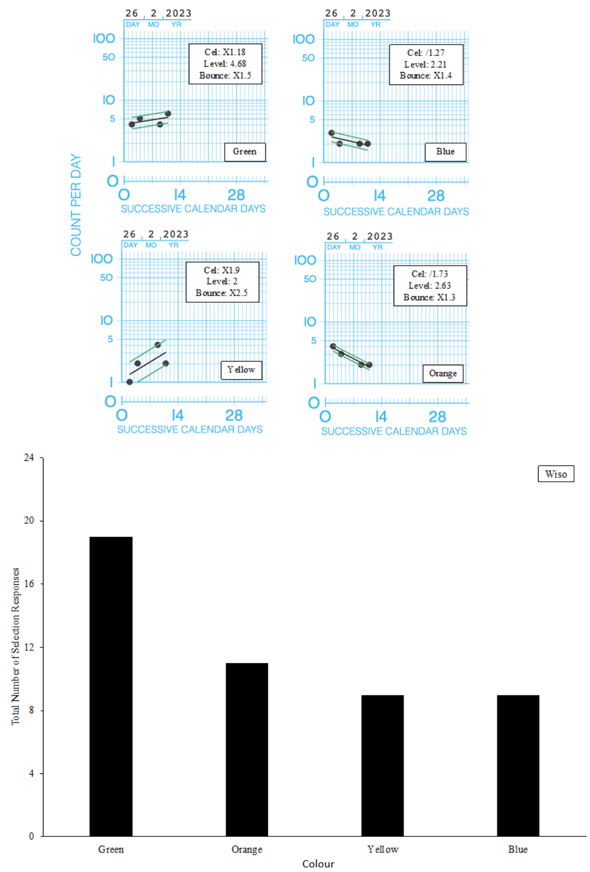


Figure 6 (Lefty), (Nepture) and 8 (Wiso). Colour preference assessment data: A) across-session data on segments of the standard celeration chart, dots represent selections; B) aggregate data across all sessions

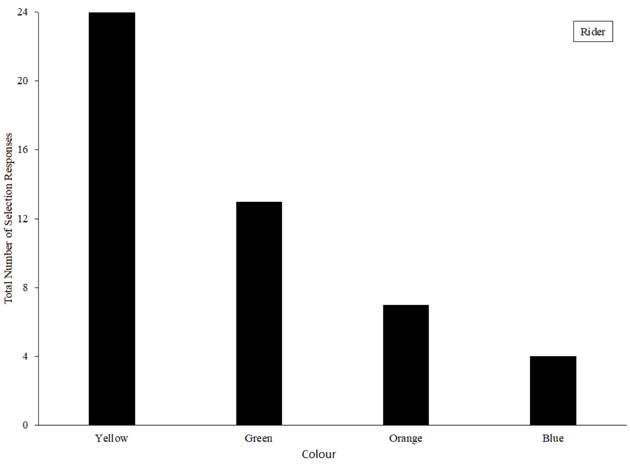


Figure 9 (Rider) Colour preference assessment data (aggregate data across all sessions)

Both Rider and Lefty selected yellow the most frequently across sessions and selected blue the least frequently. Every time the yellow ball entered the tank, Lefty selected it 100% of the time. Lefty's data also showed a deceleration in the selection of the blue ball across sessions. In aggregate form, Neptune does not demonstrate a clear preference between yellow, green and orange. However, inspecting the between-session data on the standard celeration chart, only yellow selections show an accelerating trend with very little variability. Taken together, this indicated to the researchers that Neptune showed a stronger preference for the yellow ball. Like Lefty and Rider, Neptune selected the blue ball the least frequently. Wiso selected green the most frequently with little variability and an accelerating trend across time. No major differences seem to exist among the other three colours, with blue and orange demonstrating a deceleration of selection responses across time.

The results of Experiment 2 indicated that the turtles exhibited a preference toward the yellow and green balls. To ensure the selection responses occurred because a preference existed and not because certain colours presented as more salient against the tank wall, colour stimuli were tested against contrasting background colours. Testing preferences against different controlled backgrounds would support preference or indicate selection responses based on sensitivity to stimulus intensity rather than colour.

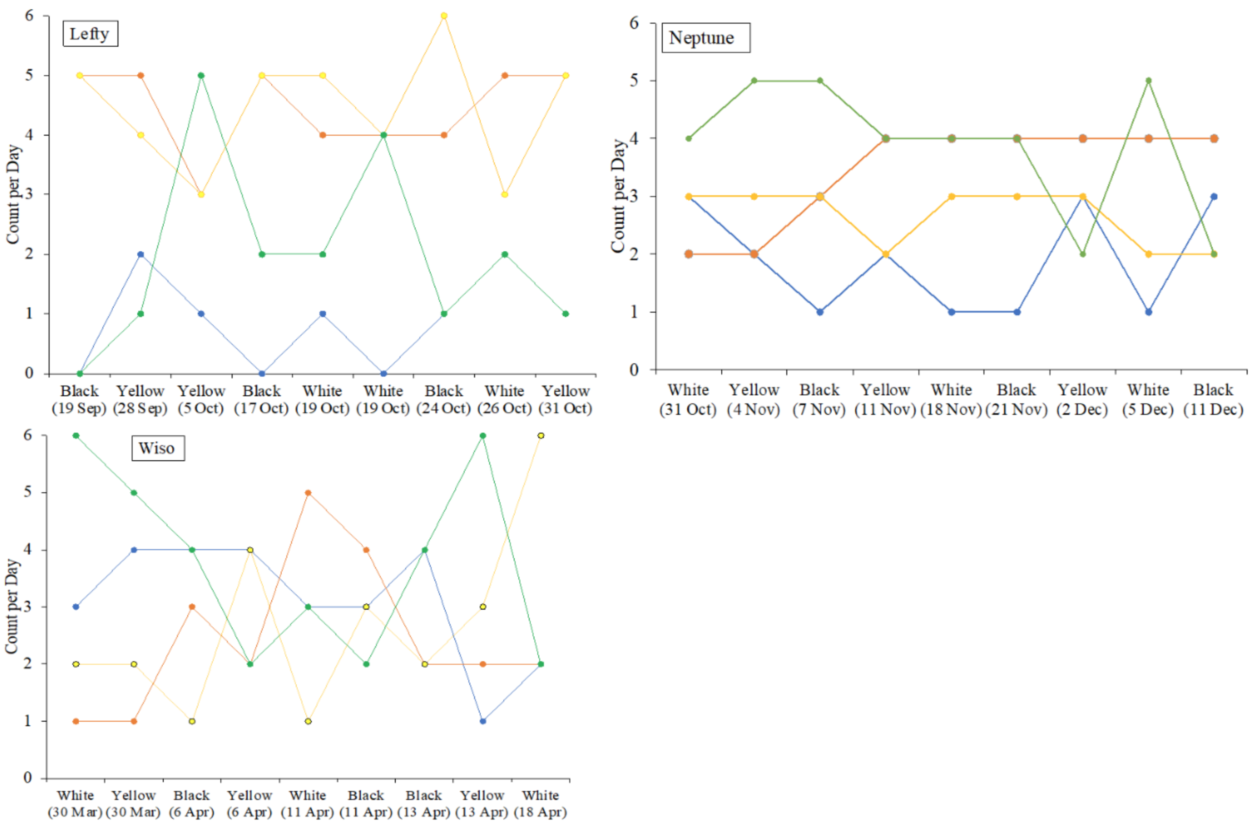
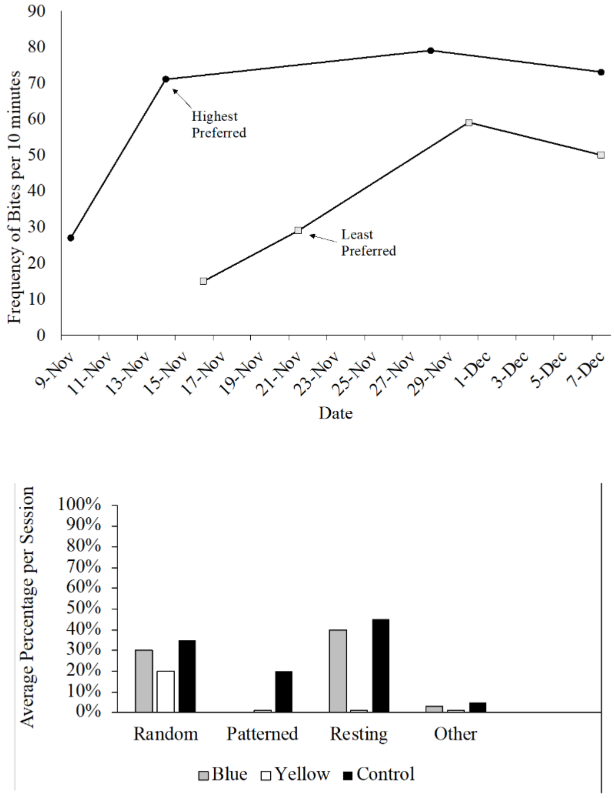


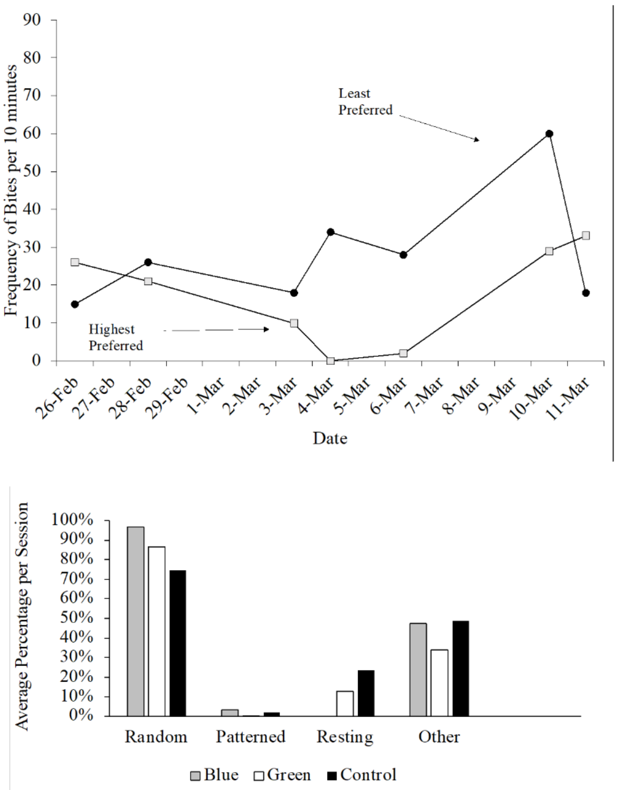
Figure 10. Results of the colour contrast assessment: the x-axis shows the background colour and the session data; the colour of the data points and series indicate the selection frequency of each colour



Experiment 3: Colour contrast assessment

Figure 10 presents the results of the colour contrast assessment for Lefty, Neptune and Wiso. In the colour contrast assessment, each colour was presented six times per session with three sessions of each background colour, yielding 18 trials per ball colour/ background colour combination. Lefty is the only turtle whose colour preference maintained against the different background. Lefty consistently selected the yellow ball across most sessions regardless of the colour background. Neptune selected green and orange more frequently and consistently across the coloured backgrounds. Wiso did not indicate a clear preference for any of the colours. Upon inspection, no covariation between colour background and stimulus selected appears to exist. However, the results do see different outcomes in the overall selection responses from the turtle, indicating a shift in preference for one turtle (Neptune) and undifferentiated responses from one turtle (Wiso). No major changes were observed in the preference selections for Lefty. The distribution of ranks of each colour were compared. The Friedman test was used and significant differences in ranking were found (Friedman χ^2=8.1 $_{(3)}$, P=0.044); however, a post-hoc analysis found no significant pairwise differences.

Results of the colour contrast assessment suggest that in general, preferences remained stable when controlling for stimulus saliency.



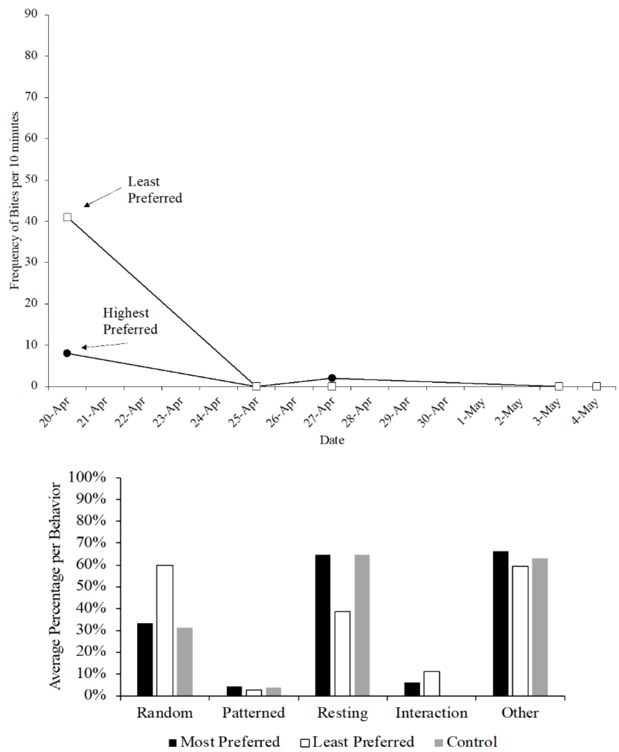


Figure 11 (Lefty), 12 (Neptune) and 13 (Wiso). Results of the free-operant enrichment device assessment: A) frequency of bites across both devices and B) aggregate percentage of 10 s intervals of random swimming, patterned swimming, resting and other behaviours

During the assessment, the turtles demonstrated behaviours that suggested the presence of the coloured background created a stressful environment. For example, the turtles would swim quickly to the back of the tank and would repeatedly headbutt the back of the tank. This prompted investigators to engage in desensitisation procedures prior to running this assessment. While this assessment provided the researchers with insight into the validity of the colour-preference assessment, zoological staff may not need to include this assessment in practical use. Taken together, data from the colour-preference and colour-contrast assessments were used to design enrichment devices.

Experiment 4: Free-operant comparison of enrichment devices

Figures 11–13 display the frequency of biting the device across sessions and the percentage of intervals in which the following behaviours occurred: resting, pattern swimming, random swimming and other (i.e. not categorised) for Lefty, Neptune and Wiso. Lefty was the only turtle that engaged in more biting behaviours on the enrichment device using the most preferred colour as identified through the previous assessments. Overall the yellow enrichment device was bitten more than the blue device. However, when looking at the relationship between enrichment devices compared to the control condition, both devices resulted in decreases in stereotypic behaviour with the largest effect observed between the yellow preference assessment and the reduction in resting.

Neptune and Wiso both demonstrated more interactions with the enrichment devices associated with the least preferred colour stimuli. Neptune engaged in consistently higher frequencies of bites to the blue device compared to the green device. Similarly, no robust differences seem to exist between the two enrichment devices on measures of stereotypic behaviour. Both devices produced minor reductions in stereotypic behaviours for Neptune. Wiso did not engage in many interactions with either of the enrichment devices. However, reductions in resting and increases in random swimming only occurred in the presence of the enrichment devices informed by the least preferred stimuli.

Discussion

Overall, the findings suggest that loggerhead sea turtles exhibit individualised tendencies for food and colour choice which are not always consistent given background colour change. Despite colour choice tendency this may not translate to a clear preference for enrichment, although these types of assessments may benefit the turtles by detecting their preferences and suppressing stereotyped swimming and resting for brief periods. Past research has determined that Caretta caretta have true colour vision (Young 2013) and that sea turtles may be particularly attracted to the colour yellow (Noh et al. 2024). This study extends those results, and together with Piovano et al. (2013) indicates that loggerheads also exhibit colour preference for certain colours. Piovano et al. found that some turtles did not exhibit colour preference; however, that study used a non-validated preference assessment methodology that confounded motivation for food with the assessment of colour and thus threw out a significant number of trials due to no response. In the present study, no assessment trials occurred where the turtle did not respond. These results also provide a protocol for aquarium personnel who may show interest in assessing loggerhead sea turtle preferences and implementing enrichment interventions. In addition, animal caretakers might use preference assessment procedure and devices as environmental enrichment for foraging behaviour (see also Woods et al. 2020).

This demonstration also highlights recent examples of how animal caretakers, trainers and curators can use the science of behaviour analysis. The relationship between animal care/welfare and behaviour analysis has a long history (Fernandez and Martin 2023), including the use of preference assessments to inform enrichment devices (Mehrkam and Dorey 2014). The current study offers additional insight into how aquarium and zoological staff can use common assessment in behaviour analysis to further enhance the services they provide.

The colour of the enrichment device influenced biting rates in a free-operant arrangement for two of three turtles, although for Lefty the most preferred colour maintained the most responses while for Neptune the least preferred maintained the most responses. While this assessment included only three turtles, an important feature of this single subject methodology (Kazdin 2020) is that these assessments yielded many data points for each turtle to evaluate whether the findings remained stable or might be confounded by things like food motivation, stress levels (from, for instance, an empty versus busy aquarium) or undetected illness or fatigue. It is recommended that researchers and aquarium staff replicate these methods for clinical use, since controlling these types of confounding variables are just as important in clinical application as they are in research.

Resting rates also decreased across all three turtles in the presence of enrichment devices compared to the control condition. These results raise the question of whether different colours will elicit species-typical responses or yield faster conditioning. For example, a yellow device may evoke higher rates of foraging behaviour (e.g. biting) while a device of a different colour may evoke higher rates of grooming behaviour (e.g. rubbing against the device). Future research might evaluate device shape, size, odour and placement in the environment as well. While there do not seem to be any studies that evaluated these dimensions with respect to enrichment efficacy, researchers have found that captive loggerheads exhibited a defensive response to a shark model (Bostwick et al. 2014). When the researchers placed the squid below the shark model, the turtles took longer to eat the squid than when they placed squid with an arbitrary shape.

One would anticipate that a higher frequency of bites would occur in the presence of highly preferred stimuli, as higher rates of reinforcement occurred for biting those colours. This phenomenon was not observed across all turtles. Phylogenetic responses elicited by aversive stimuli represent one behavioural explanation for the increase in interactions with the least preferred stimuli for the turtles. Previous research has shown that unlearned aversive stimuli (e.g. pain, high temperatures) elicit, without training, behavioural responses that may have terminated that stimulus historically across the evolution of the species (e.g. Ulrich and Azrin 1962). It is feasible that a similar phylogenetically based behavioural relation exists between low preference colours and responses that remove or diminish aversive stimulus. While one cannot draw conclusions from the current study, this type of behavioural process might account for why two of the three turtles displayed higher amounts of biting towards the enrichment devices (e.g. if one interprets the bites as an instance of aggression rather than foraging). Future research on this is needed and could produce new paths for enrichment research that, complementary to opportunities for responding to produce appetitive stimuli, would evaluate choice opportunities for responding that remove aversive stimuli. Given Noh et al.'s (2024) finding that colour influenced consumption of plastic-like stimuli, future research might also explore interventions for rejection of characteristics like plastic either through stimulus discrimination training or Pavlovian conditioning. In this way preference assessments can help maintain the health, safety and dignity of captive animals.

Future research in this area should address several limitations. The enrichment device presentations occurred across relatively short spans of time, just 10 min. Recently researchers have evaluated enrichment devices over prolonged periods (e.g. Woods et al. 2020) but additional research seems warranted. In conjunction with longer evaluation periods, the addition of controls, such as the use of white or clear devices and prolonged evaluation of behaviour in the absence of the device, present additional research controls and variables to consider. Two turtles' biting increased across sessions, which may indicate the turtles began to learn different strategies for how to access food in the device. Future research might evaluate whether turtles learn such strategies, and, if so, whether these generalise to real prey. Future investigators may also explore whether the efficacy of the enrichment device might better sustain if researchers require the turtles to learn new strategies toward novel devices on a periodic basis.

Conclusion

Environmental enrichment represents one common practice at many zoos and aquariums, yet little evidence exists that demonstrates whether these modifications aid in the survival of animals that return to the wild, simply because at this stage it becomes very difficult to monitor the animal. More research is needed on the impact of behavioural preparation for rehabilitated animals being prepared for release. The aquarium released Rider, Lefty and Neptune soon after completion of the current study. The Georgia Sea Turtle Cooperative equipped each turtle with two metal flipper tags etched with a unique number and contact information and a passive internal transponder (PIT) tag. The PIT tag is an electronic tag measuring approximately 12 mm by 2.1 mm that was placed subcutaneously in the neck and contained a microchip that stored identifying information. Unfortunately, these methods of tagging provide no further data on location, behaviours or success after release, as the animals are not tracked and are only identified if they encounter humans again in close proximity, such as injury, stranding or nesting. However, as tagging and satellite tracking technologies become more readily available, it may become easier to gather data that sheds light on the survival rates and habits of rehabilitated animals (e.g. Mestre et al. 2014; Robinson et al. 2017). If researchers could track the animals' movements over time, there would be some level of post-intervention behavioural data available.

Lastly, several pragmatic benefits came about because of this project. First, it seems that preferences may function as a type of environmental enrichment. Anecdotally, none of the turtles engaged in stereotypic behaviours during the preference assessments used in the study. Similarly, the speed at which the turtles completed the assessments increased across sessions. It was anecdotally observed that the turtles began to 'restart' quicker between trials, one potential indicator of their assent to participate in the assessment. Also, these types of assessment may assist in the creation of individual enrichment devices for the animals, rather than a one-size-fits-all approach. Caregivers should consider individual preferences for various physical dimensions of devices (e.g. shape, colour, odour, location) used for general welfare, training and enrichment of captive animals. This ties into the last pragmatic benefit. By demonstrating individual preferences of animals in managed-care facilities, staff can use these data as part of their everyday educational efforts to help promote animal welfare and reduce the stigma around managed-care facilities. For example, because attendees of the aquarium could observe the assessments from the display windows of the tank, there was an increase in questions asked about the turtles and the type of work the staff does to ensure a high quality of life for the animals before release. These types of efforts demonstrate a two-fold benefit to both the long-term and immediate missions of the facility.

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