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A review of welfare indicators for sea turtles undergoing rehabilitation, with emphasis on environmental enrichment

Running title: Welfare and environmental enrichment for sea turtles

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Abstract

For animals undergoing rehabilitation it is vital to monitor welfare in a way that is feasible, practical, and limits stress to the animal. The industry gold standard is to assess welfare under the Five Domains model, including nutrition, environment, physical health, and behaviour as the first four physical domains and mental domain as the fifth. Feasibility and effectiveness of these domains for assessing welfare of sea turtles undergoing rehabilitation were reviewed and it was determined that the mental state can be best assessed through behavioural changes. A scoping review of the literature was conducted using Scopus and Web of Science to investigate use of environmental enrichment devices (EEDs) as a measure of welfare in sea turtles. Behavioural assessments using EEDs were found to be well-documented; however, most EED studies pertained largely to livestock or zoo animals. Furthermore, studies rarely concentrated on reptiles, and specifically sea turtles. Results also showed that some welfare assessment methods may be less appropriate for short-term captivity experienced during rehabilitation. Additionally, the hospital environment limits the ability to address some of the domains (ie biosecurity, feasibility, safety of turtle, etc might be compromised). This review shows that only three of the nine environmental enrichment strategies described in the literature suit the specific requirements of sea turtles in rehabilitation: feeding, tactile, and structural. It is documented that turtles display behaviours that would benefit from EEDs and, therefore, more specific studies are needed to ensure the best welfare outcomes for sea turtles undergoing rehabilitation.

Keywords: animal welfare, behaviour, captivity, enrichment devices, marine turtle, testudine.

Introduction

Welfare for animals under human care is an evolving concept and one that is implemented by individual organisations (Flint et al. 2017), resulting in varied welfare outcomes for the animals. Accredited institutions of the World Association of Zoos and Aquaria (WAZA) or the Zoo and Aquarium Association (ZAA) Australasia, for example, are bound by regulated welfare standards. For animals undergoing rehabilitation, however, welfare standards are set by specific national or state legislation, which is not always so clear or well-regulated (Englefield et al 2019) and often aimed at terrestrial animals and too general to be of direct relevance to sea turtles.

There are multiple ways to consider welfare. Dawkins (2008) proposed that animal welfare be determined and defined by two questions: 1) Are the animals healthy? and 2) Do the animals have what they want? Ideally, the desire is for animals to experience 'good' welfare. Identifiable in the Five Freedoms of animal welfare (Farm Animal Welfare Council 1993), and recognised by Barnett and Hemsworth (2009), are three primary facets of welfare: basic health and functioning, psychological or affective states, and natural living. The current industry standard for welfare assessment is the Five Domains model (Mellor 2017), which assesses animals holistically based on four physical domains (nutrition, environment, physical health, behaviour) and a fifth mental domain. Originally this model was developed as an assessment of welfare compromise for animals held in research, teaching and testing environments (Mellor and Reid 1994). Subsequently, it has been updated to include additional categories of animals under human care, such as domestic, livestock and zoo, and to incorporate and emphasise positive states of welfare (Mellor and Beausoleil 2015).

There is no single, fully inclusive method in the determination of welfare specifically for sea turtles; however, a species-specific welfare assessment based on the Five Domains model could be beneficial for sea turtles. A similar assessment was developed by Clegg (2015) for captive cetaceans. A species-specific assessment metric for sea turtles would have to consider individual requirements of species due to the variation between the seven species in diet and behaviours observed naturally in the wild. Whitham and Wielebnowski (2009) developed a three-step process for the maintenance of welfare for the individual animal. These involve: (1) the development of a welfare score sheet (based on extensive knowledge of normal parameters for the particular species); (2) the validation of the score sheet through a 6-month behavioural and physiological assessment; finally resulting in (3) a welfare score sheet personalised to each species. Such an assessment tool would be useful in a rehabilitation setting for sea turtles to ensure positive welfare and therefore promote speedy recovery.

The rehabilitation setting is a specific environment that would require the assessment to have different considerations than if it were for sea turtles housed in zoos or aquaria without intention of release to the wild. Common causes of hospitalisation for sea turtles include boat strike, ingestion or entanglement in fishing gear or marine debris, limb damage or loss, fibropapillomatosis or other disease, and floating syndrome (Flint et al. 2017). Each cause of hospitalisation requires consideration when housing and treating the turtles during rehabilitation. The average time of sea turtles in rehabilitation centres has decreased over the last couple of decades but can range from 1 day to more than a year, with the average time to release after rehabilitation being approximately 4 months (Flint et al. 2017). Furthermore, since the aim of a rehabilitated turtle is to release it back into the wild, it is important to limit turtle-human interactions, which might be more common in an aquarium setting. Therefore, for an assessment of turtles undergoing rehabilitation, it is most important to determine the desirable state a turtle must reach before it can be released and how quickly this can be measured (Deem & Harris 2017).

Following cyclone Yasi in January 2011, in Australia's Far North Queensland, the region experienced a significant increase in sick, injured, and stranded sea turtles (Davis 2011; Meager & Limpus 2012). Several turtle rehabilitation centres opened in response to this increase, and the College of Public Health, Medical and Veterinary Sciences, James Cook University (JCU) was transiently part of this response. Close observation of these wild animals spurred research into environmental enrichment (EE) for sea turtles in rehabilitation (Lloyd et al. 2012), many of which have to spend months in plain plastic tanks whilst undergoing treatment. Newberry (1995) defined EE as an "improvement in the biological functioning of captive animals resulting from modifications to their environment." Hoy et al (2010) later organised enrichment strategies under eight classifications: feeding, tactile, structural, auditory, olfactory (ie exposing the animal to the smell of its prey), visual, social, and human-animal interaction. Maple and Perdue (2013) suggested that 'cognitive' also be included in this list. Ideally, one EED will be able to satisfy multiple different enrichment styles.

With an anticipated increase in hospitalised turtles following future cyclones and anthropogenically induced environmental damage, a thorough review to assess measures of welfare is critical, particularly in regard to how EE can increase speed of recovery and optimize chance of survival upon release back into the wild. This review covers suitable welfare assessment methods and how they can be adapted for turtles in rehabilitation, examples of past EE studies, and a discussion on the design of appropriate environmental enrichment devices (EEDs) for sea turtles in rehabilitation. Detailed explanations of auditory and olfactory EEDs are not included in this review, as there is little information on the uses of these in sea turtles.

Methods

A scoping review was conducted to explore the literature pertaining to use of environmental enrichment devices in turtles as a measure of welfare. Two databases were used for the search: Scopus and Web of Science. Ovid Medline was tested but yielded no relevant results so was excluded. Search terms were (environment*) AND (enrich* OR welfare OR entertain*) AND (turtle* OR cheloni* OR testudine* OR reptile* OR loggerhead* OR leatherback* OR hawks bill* OR Ridley OR terrapin*) AND (rehab* OR hospital* OR clinic* OR recover* OR captiv* OR recuperat*). Searches included the full date range of each database (Scopus: 1970 – present); Web of Science: 1965 to present) for articles related to environmental enrichment and welfare of non-pet testudines. The reference lists of the most relevant papers were used to look for additional papers that had been missed in the database search.

From the literature search, excluding duplicates, 87 articles were identified. Titles and abstracts were reviewed against the selection criteria, which narrowed the results to 15 articles. Any literature not directly pertaining to turtles interacting with environmental enrichment was excluded. All types of environmental enrichment were included and both marine and freshwater turtle studies were included; however, tortoises were excluded. Assessment of full texts reduced the total to 11 articles (Supplementary Figure 1), of which only 1 was specifically relating to environmental enrichment for rehabilitation of hospitalised sea turtles. Due to the lack of specific literature, this paper reviews wider literature in the context of the five domains as they relate to sea turtles.

Assessing sea turtle welfare in a rehabilitation setting

Physical health evaluation

Assessing physical health in sea turtles is met with many challenges, mostly due to the absence of reliable physical and biochemical reference values (March et al 2018). However, there are several general parameters that are relevant across all animal species and these can be considered in a modified version for sea turtles undergoing rehabilitation.

Presence of disease and injury in a captive setting are normally considered indicators of poor welfare (Barber and Mellen 2013); however, in the rehabilitation setting, this assessment of welfare may be less useful as turtles enter the establishment already diseased/injured. Therefore, it is more logical to assess recovery rate and absence of husbandry mutilations. These can be routinely evaluated by sea turtle carers and veterinarians in rehabilitation centres based on visual inspection, behaviour and activity levels. An unpublished example of a green turtle physical exam score card (Table 1) is provided from an Australian rehabilitation centre (courtesy of Dr Duane March). The level of epibionts and external parasites on admission can be visually assessed and easily treated with a freshwater bath on entry. Internal parasite infections are assumed and treated as a standard rule; however, these parasites may be resistant to treatment and therefore cause ongoing problems during rehabilitation.

Reproductive fitness may not be a reliable indicator of good welfare as captive animals have been known to reproduce well despite poor environments, and the opposite is also true (Wickins-Drazilova 2006). Specifically, for sea turtles undergoing rehabilitation, it is a poor indication of welfare as it would not be feasible to replicate the environmental conditions appropriate for successful reproduction in sea turtles. Furthermore, many of the individuals undergoing rehabilitation are sexually immature.

Stress has been linked to negative welfare (Broom & Johnson 1993) and therefore assessment of stress could be an indicator of welfare in sea turtles undergoing rehabilitation. Activation of the hypothalamic-pituitary-adrenal axis, and the subsequent release of glucocorticoids are commonly used to determine levels of stress (Hunt et al 2016; Stabenau & Vietti 2013). Glucocorticoid measurements may provide an indication of acute or chronic stress, depending on the chosen method of collection (blood, saliva and faecal/urine for acute stress, and samples of integumentary structures for chronic stress); however, there are numerous issues to this evaluation technique (Jessop et al 2004). Primarily, stress associated with reptile-capture and blood and saliva collection can interfere with results (Silvestre 2014). Additionally, glucocorticoids may be released in response to arousal, and not aversive stimuli (Latham 2010). Furthermore, there are incongruences as to the correlation of glucocorticoid levels to stress levels in sea turtle literature (Jessop et al 2002a, b; Gregory 1996). Finally, there seems to be a delay in green turtles' (*Chelonia mydas*) adrenocortical responses to stress (Jessop 2001). There may also be potential for adrenal fatigue in animals that are chronically debilitated (March et at 2018). Ironically, many of these parameters are obtained via invasive collection techniques, which may cause undue stress and actually decrease the welfare of the animal (Mason & Veasey 2010).

A number of blood parameters normally used to assess health in mammals were found to be of limited prognostic value for green turtles undergoing rehabilitation in Australia (March et al 2018). Although some of the parameters would provide a general indication of health such as heterophil count and haematocrit level, none were correlated to recovery. This could be because of the particular suite of diseases encountered locally. The heterophil to lymphocyte ratio and blood glucose levels have been used to assess stress response (Davis et al. 2008; Krams et al. 20120), but it is clear that more research is needed to provide reliable prognostic biomarkers for each species of marine turtle in rehabilitation.

With all of these inconsistencies in mind, as well as the expense, specialised skillset, and human-turtle contact required, measurement of glucocorticoid levels and other blood parameters are not ideal adjunctive methods of health assessment for determining welfare status of sea turtles. Of course, they are necessary for determining the health and rehabilitation status of the turtles.

Nutritional evaluation

Sea turtles entering rehabilitation centres are frequently emaciated and therefore weight gain is a priority. Some literature has shown that adult green turtles appear to do very well on high protein diets in captivity (Wood & Wood 1981; Amorocho & Reina 2008). High weight gain is achievable on such diets, which can be either animal matter (Caldwell 1962) or commercially prepared high-protein, readily digestible pellets (Wood & Wood 1981). However, it is important to consider the optimal diet for sea turtles undergoing rehabilitation. There is a natural variation in the diets of wild sea turtles of different species and life stages (Limpus & Limpus 2000; Arthur et al 2009). Therefore, diet needs to be tailored to the specific nutritional requirements of the individual to reflect their natural preferences. Some rehabilitation centres have been known to feed turtles a high protein diet to encourage rapid weight gain, irrespective of species (Pers comm). For a predominantly herbivorous species such as the green turtle, this does not reflect a natural diet and may lead to uraemia and hypercholesterolaemia (March et al 2018).

Weight gain by itself is not necessarily an indicator of welfare; however, it can be used in conjunction with body condition scoring (Limpus et al 2012) to show progress for rehabilitation of emaciated sea turtles. Body condition reflects not only the availability of appropriate and nutritious food items in the captive setting, but also appetite and physiological ability to convert food to build muscle and support activity. This method can be applied for sea turtles, where body condition index (BCI) of turtles are recorded regularly, and release is dependent on having achieved a BCI consistent with wild populations (Bjorndal 1980). A more accurate method of scoring body condition would be bio-impedance analysis as that would differentiate between weight gain caused by fluid, fat or muscle (Kophammel submitted). However, this requires specialised equipment and training, as well as additional human-turtle interactions. Melvin et al. (2021) have also found evidence that malnutrition is a key factor in mortality of sea turtles undergoing rehabilitation and suggest monitoring metabolomic profiles for earlier diagnosis and treatment of metabolic failure. Whilst poor body condition/weight loss is often precipitated by stress, it is also influenced by diet, activity levels (Mason & Mendl 1993), and disease. Cachexia is a common finding in sea turtles presenting to rehabilitation clinics (March et al. 2021). Ideally, in a rehabilitation setting, each turtle’s diet would be formulated to cater for maintenance, whilst taking activity levels and disease status into consideration. Overall, measuring weight in conjunction with body scoring, is a useful method to assess welfare. It is minimally invasive and can be obtained on a weekly basis by rehabilitation staff and carers.

Environmental evaluation

The environmental domain for a captive turtle can be evaluated in two stages: 1. the initial set-up of the tank; and 2. the ongoing maintenance of tank conditions. Considerations when designing an enclosure for sea turtles should include substrate, structure/shape, size, depth, material and colour (Stamper et al 2017; New South Wales Government 2020). Substrate, structure and material for a sea turtle tank should consider that turtles are likely to ingest anything small enough (Hoopes et al 2017.). Particularly in a rehabilitation setting, it would be disadvantageous to put turtles in an environment where they may do more harm to themselves through ingestion or scraping against rough surfaces. Juvenile green turtles showed a preference toward the colour blue under experimental settings; therefore, implementation of blue tanks may improve their comfort (Hall et al 2018). Tanks should be deep enough to provide refuge, but weak turtles are at risk of drowning, and so fitness of the turtle needs to be considered (Stamper et al 2017). These features of the environment are likely to remain constant throughout the entire rehabilitation period and so anticipated length of time in captivity (as well as species) should be considered at set-up. This is particularly relevant to enclosure size as turtles must have sufficient space to manoeuvre and engage in positive natural behaviours (Stamper et al 2017).

Environmental conditions that can be regularly and simply monitored to ensure comfort for sea turtles include temperature, light, UV, salinity and other water quality parameters (Stamper et al 2017). Sea turtles have a range of tolerability for each of these parameters; if they are not well-monitored and maintained, it is possible that sea turtles already in a weakened state might become further compromised by sub-optimal environmental conditions. Turtles undergoing rehabilitation are already in a weakened state and are therefore more sensitive to these environmental factors. For example, as ectotherms, reduced temperatures will reduce the efficiency of the digestive and immune system, which would be detrimental for underweight sick turtles (Hoopes et al 2017). These are all environmental conditions that are always essential to the physical wellbeing of sea turtles; however, variety in non-essential environmental stimuli have been shown to positively affect welfare of other animals (Burghardt 2013) and should, therefore, be considered for use with sea turtles. Environmental enrichment devices (EEDs) can be introduced to do this and the change in behaviour of the turtles can be used to assess the impact on welfare.

Behavioural evaluation

It has commonly been perceived that stereotypic behaviour is indicative of either past and/or present poor welfare (Mason 1991; Mason & Latham 2004; Garner 2005; Mason et al 2007). Indeed, the presence or absence of stereotypic behaviour remains as one of the best validated measures of animal welfare (Maple & Perdue 2013). Mason et al (2007) proposed that stereotypic behaviour, as a broad term, should refer to "repetitive behaviour induced by frustration, repeated attempts to cope and/or central nervous system (brain) dysfunction". In the rehabilitation setting, changes in behaviour could be due to brain damage caused by parasites such as spirorchiid flukes (Glazebrook et al 1989) or coccidia (Gordon et al 1993), or alternatively, it could be environmentally-induced, as a result of boredom or reduced welfare. This is particularly likely if the turtles are kept in sterile, empty hospital tanks, devoid of environmental enrichment.

Abnormal behaviours indicating stress in turtles include grafting of jaw (rasping of ramphotheca), pseudo-vocalization (squeaks or whines), pattern swimming, poor posture when resting at the bottom of the tank (flopped and lifeless rather than propped up on front flippers), and boundary exploration (related to exploratory and escape activity) (Arena et al. 2014; Tynes 2010). Leatherback turtles (*Dermochelys coriacea*) are particularly difficult to keep in captivity due to their inability to register boundaries. They are continuous swimmers and can cause additional daage to themselves if allowed to swim into the sides of a rehabilitation tank (Jones et al 2000; Levy et al 2005). Turtles recently hospitalised, or handled in and out of the water, may display behavioural floating for a period. This could be as a response to stress or a preference to be at the surface due to weakened physical condition (Manire et al 2017). Buoyancy disorder due to gas accumulation within the ceolemic cavity will be discussed later. Associated with the presence of or contact with humans, other stress-related behaviours include cloacal evacuations upon handling, projection of penis or hemipene, voluntary regurgitation of food, and human-directed aggression. Often these signs are related to fear and are common in overly restrictive and inappropriate environments (Warwick et al 2013).

Usually stereotypic behaviour is assumed to be associated with negative welfare in healthy animals (ie in zoos/aquaria), but in the case of sick turtles, it can actually show improvement of health via increased energy levels. However, if they are to be kept longer for full rehabilitation, stereotypic behaviours should be discouraged. EEDs are a useful tool, commonly used in captive settings to discourage stereotypic behaviours and encourage positive behaviours (Mason et al 2007). Consequentially, observing animals for the presence or absence of negative behaviours could be used as a proficient welfare evaluation measure, and potentially as a means of determining the effectiveness of EEDs, particularly in turtles that have spent several months in rehabilitation. Additionally, comparing captive animal behaviour with wild animal behaviour (Burghardt et al 1996; Smith & Litchfield 2010; Phillips et al 2011) is another measure of welfare. The more a captive-held animal engages in wild behaviour, the better its welfare is deemed. Similarly, the effectiveness of EE can be deduced by comparing the proportion of time an animal is engaged in a type of behaviour before and after introduction of an EED (Therrien et al 2007; Lloyd et al 2012).

Mental evaluation

The physical domains (health, nutrition, environment and behaviour) all contribute to the mental state of the turtles (Mellor 2017). The affective state of an animal can be assessed via study of its behaviour (Bracke & Hopster 2006). Stress fever and tachycardia, both physiological responses associated with emotion in other vertebrates, have been observed in iguanas (Cabanac 1999) and wood turtles (*Clemmys insculpta*) (Cabanac & Biernieri 2000). Cabanac (1999) also discovered that rather than venture into a cold environment to obtain food, iguanas preferred to remain in a warm environment, suggesting that their motivation was influenced by sensory pleasure. Therefore, it appears that basic affective states exist in reptiles, turtles included. In the assessment of affective states, there is a potential issue of over-anthropomorphosis and evaluator bias.

Using EEDs to monitor welfare

Modification of the environment to provide more opportunities and promote positive behaviours can be used to infer the affective state of the turtles and assess their welfare. EEDs should be designed to increase positive affective state of turtles but must be also be suitable for the rehabilitation setting. EEDs are all designed to enhance environmental opportunity and choice, but depending on the specific device, could also promote positive behavioural expression, increase fitness and aid nutrition. Thus, contributing to a positive affective state for the turtles and improved welfare. It is on this premise that EEDs may be able to contribute to a speedier recovery and shorter rehabilitation time of hospitalised turtles.

The psychological and physical benefits of EEDs are well documented in captive mammals (Newberry 1995; Mellen & MacPhee 2001; Young 2013), but less so in the case of marine and terrestrial reptiles (de Azevedo et al 2007; Eagan 2019; Maple and Perdue 2013). Reptiles have previously been considered too sedentary to interact with, and thus benefit from, EE (Bennett 1982; Burghardt 2013). Turtles housed at JCU proved this to be a misconception by actively interacting with EEDs (Lloyd et al. 2012). Furthermore, a literature review by Lambert et al. (2019) found multiple studies that showed sentience in reptiles, including multiple turtle species. We therefore found it timely to conduct a thorough review of past reptile-specific EED studies as well as to draw from existing knowledge of wild sea turtle ecology to explore the potential for EEDs in assisting with rehabilitation of hospitalised turtles.

EEDs for turtles undergoing rehabilitation

At this point, it is necessary to make a distinction between EE for hospitalised turtles and those that are permanently captive (such as in public aquaria). For all captive turtles, it is desirable for their captive conditions to be as similar to their wild conditions as practically possible (Newberry 1995). Hospital settings, however, are often not conducive to this as they must remain sterile to reduce likelihood of infection, for example. As such, EEDs should aim to stimulate natural behaviours safely without jeopardising the necessary sanitation standards of a hospital setting or the safety of the turtle. Therefore, EEDs should encourage 'preferred' naturalistic living. The term 'preferred' is used to omit negative aspects of naturalistic living such as famine and predation (Hutchins 2006). Predatory avoidance behaviours correlated with stress could reduce longevity of animals in long-term captivity, which would be associated with negative welfare. However, anti-predator responses are necessary for temporarily captive turtles to ensure a good chance of survival on release. Turtles intended for release after rehabilitation, therefore, need to maintain a level of fearfulness, which could be promoted through subjection to occasional and temporary unpleasant stimuli (Guy et al 2013). With respect to this, it is difficult to prepare sea turtles for natural life in an artificial environment, especially in a rehabilitation setting where emphasis is on improving health and fitness. An ideal welfare evaluation plan for sea turtles in the rehabilitation setting would adhere to the following considerations:

1. Be **safe** for the turtle
2. Be **feasible** in the rehabilitation setting
3. Be **cost-effective**
4. Be **easily implemented** by carers without the requirement for specialised skills or training

5. Be **minimally invasive to induce little or no stress** on the turtles, which is especially important as these turtles are diseased and/or injured and added stress is likely to exacerbate immunosuppression, subsequently lengthening recovery time
6. Accurately measure **stress in conjunction with behavioural assessment**
7. Require **minimal human-turtle contact**
8. Require a **short-term** evaluation of welfare variables to provide a reliable indication of welfare

Feeding enrichment

Turtles in the wild appear to feed in bouts - early to mid-morning and mid-late afternoon (Ogden et al 1983) - and therefore reproducing this pattern in the captive setting to maintain the natural rhythm may be beneficial for release. Food-oriented devices appear to be a very effective form of EE (Maple & Perdue 2013). As a reflection of their natural foraging behaviour, hunting of live jellyfish, ctenophores, and squid would be a valuable EED for turtles in captivity or those undergoing rehabilitation. However, the ethical dilemma associated with live feeding, biosecurity, and the availability of such prey may exclude this EED. The lettuce feeders on the tank floor reported by Therrien et al (2007) may prove an interesting activity for turtles as this mimics grazing behaviour (Van de Merve et al 2009; Hart & Fujisaki 2010) and serves a dual purpose, as a hiding place.

Injuries and ailments of each individual turtle need to be considered when designing the EED. 'Floating syndrome', which affects the turtle's buoyancy, can be caused by air trapped in the lungs, coelomic cavity, or intestine of the turtle. The air upsets diving proficiency, which prevents the turtle from reaching the tank floor, resulting in major feeding constraints (Norton 2005). However, occasional bottom feeding for floating turtles would encourage them to try to dive down when they have enough energy. A possible alternative could consist of a frozen ice-block, containing squid and vegetable matter such as cos lettuce and nori, to encourage foraging and provide the turtles with a focused interactive activity for an extended period of time. Entanglement is another common cause of turtle hospitalisation. Entanglement may result in amputation of a flipper, causing restricted movement, which also needs consideration when designing EEDs. In general, natural foraging on the tank floor should be encouraged as well as a disassociation between humans and food.

Tactile enrichment

Hoy et al (2010) described tactile EE as "the provision of objects that are physically stimulating to the animal". To reflect their natural environment, turtles may benefit from the inclusion of muddy or sandy floor bottoms, perhaps contained within a tray to maintain ease of cleaning and water drainage; however, this is unlikely to be feasible in a sterile rehabilitation setting. Employment of stones too large to ingest, however, could provide excellent enrichment, for green turtles in particular, as they are attracted to rocky rubble to perform self-cleaning behaviours (Heithaus et al 2002; personal observation Ariel & Lloyd). Whilst captive turtles have been observed to swim under brooms in order to groom themselves (Brill et al 1995; Lloyd et al 2012), turtles have also been known to eat the broom bristles. Consequentially, this EED comes with risks and, if utilised, should only be provided under supervision. Provision of a 'waterfall', as well as toys such as hoops and balls, would provide valuable tactile enrichment (Burghardt 2005).

Structural enrichment

In promoting naturalistic living, turtles should have access to shallow water for resting (Brill et al 1995). This can be achieved in the form of a platform suspended from the wall of the tank or positioned in the centre of the tank. Alternatively, water levels could be lowered for floating turtles, to enable them to reach the tank floor and right themselves with their flippers. Turtles should also have deeper parts in their tanks, ideally with 3D structures that could mimic caves (Brill et al 1995). A pipe on the tank floor, large enough for hiding their head, allows turtles to hide and/or exclude external stimuli during resting periods (Therrien et al 2007; Lloyd et al 2012). Hatchlings and young post-hatchlings are buoyant and so EEDs on the tank floor may not be appropriate. Therefore, mounting pipes to the side of the tank or in shallow water for young or floating turtles would provide a suitable refuge.

Social and visual enrichment

Sea turtles in restricted environments should be housed individually due to their typically solitary tendencies (George 1997; Heithaus et al 2002) and documented aggression in over-crowded facilities (Arena et al 2014) and during mating (Schofield et al 2007). However, cohabitation with other species, such as a green turtle and *Acanthurus nigrofuscus* or *Zebrasoma flavescens* (Balazs et al 1994) could potentially act as a form of social EE. Inter-species cohabitation would also provide visual enrichment (something to look at), whilst additionally satisfying the natural behaviour of the green turtle to be clean. However, Zamzow (1998) showed that whilst this cohabitation may be beneficial for control of ectoparasites, reef fish may serve as vectors in the spread of fibropapillomatosis or create an opportunity for infection if the turtle is wounded during cleaning. This would also require additional husbandry for the fish, which would be costly to the rehabilitation facility in terms of time and money.

Cognitive and human-animal enrichment

Maple and Perdue (2013 p 108) described cognitive enrichment as: “challenging and stimulating an organism’s memory, decision-making, judgment, perception, attention, problem-solving, executive functioning, learning and species-specific abilities.” A training routine using associative learning (Lopez et al 2001; Wilkinson et al 2007; Wilkinson et al 2009) would provide this type of enrichment and has been proven possible in marine turtles (Mellgren & Mann 1998; Bartol et al 2003). However, since rehabilitation turtles only remain in facilities temporarily, training may not be a worthwhile form of EE due to the potential time investment required for it to be successful. Additionally, although human-turtle interactions may be encouraged in aquaria to increase familiarity and reduce stress (Claxton 2011), they should be limited in temporary captive settings. Turtles may have extensive long-term memory (Bartol et al 2003; Davis 2009; Davis & Burghardt 2012); therefore, human-turtle interactions could cause potential overdependence and ‘trust’ towards humans. Lack of caution towards humans would be disadvantageous to the turtles after release as it could lead to injury (Addison & Nelson 2000).

Past examples of EE in captive turtles

A case study from a Spanish rehabilitation centre, based on the work of Therrien et al (2007), showed that EE aided in the successful rehabilitation and release of a sea turtle that was previously considered unfit for release due to a flipper amputation (Monreal-Pawlowsky et al 2017). Recognising the limitations of implementing EE in a rehabilitation environment, enrichment was based on feeding, tactile and structural stimuli. Enrichment primarily involved eating live food and aimed to prepare the turtle to avoid unnatural objects in the water, such as buoys. Despite being in captivity for 10 years, including a 2-year rehabilitation period, 2-months of EE was sufficient to prepare the turtle for release into the wild. This successful release was confirmed by 10-month transmission from a satellite tag that showed the loggerhead turtle crossed an expansive body of water. It is unknown how quickly a turtle might be released with a timelier introduction to EE as no specific studies for this were found in the literature. However, it is important to note that EE in this case study was administered over a short time period, easy to implement, cost-effective and required minimal human interaction as a webcam was used for monitoring.

Research was undertaken on the effects of EE on four captive display sea turtles (three loggerhead turtles (*Caretta caretta*) and one blind green turtle) in Florida (Therrien et al 2007). The behaviour of the turtles was assessed both with and without enrichment present. The EEDs were designed to stimulate their tactile sense, increase exploratory swimming, and satisfy their need to forage. The study showed that there was a significant increase in amount of time engaged in naturalistic behaviours with the use of EEDs. The devices for the blind turtle were modified to suit its special needs and successfully decreased the stereotypical behaviour and increased the exploratory behaviour of the animal. In an enrichment study of captive-raised, collectively housed green turtles intended for release, Kanghae et al. (2021) found that enrichment devices decreased negative behaviour. Specifically, the turtles exposed to enrichment had fewer bite wounds than turtles without enrichment and without other health parameters affected. EE appears to be just as effective for marine reptiles as it is for mammalian species, and should be encouraged for captive sea turtles, including disabled ones, and particularly when housed collectively.

A preliminary study on hospitalised sea turtles, conducted by Lloyd et al. (2012) arrived at similar conclusions. Lloyd et al. (2012) demonstrated that there was an overall decrease in pattern swimming and resting behaviours observed amongst the turtles in the presence of EE. Additionally, it was found that each turtle responded to different EEDs in their own specific ways, highlighting the apparent variances in natural behaviours and preferences between individuals. It is also important to consider the possibility that turtles will habituate to an EED if given unrestricted access to it. Consequentially, EEDs should be rotated and their use potentially supervised (Lloyd et al. 2012). Furthermore, the placement of structural elements of the captive environment should be altered two to three times a year to maintain their novelty factor (Hawkings & Willemsen 2004).

Relatively few studies on EE in sea turtles are published. For this reason, we have included studies on freshwater turtles. Case et al. (2005) assessed the preference as well as the physiological and behavioural effects of enriched versus barren environments on 38 box turtles (*Terrapene carolina*). Preference for the habitat-enriched environment was apparent. Following the preference tests, turtles were housed for a 1-month period in one of the two environments. Behaviourally, turtles with habitat enrichment spent less time engaged in negative behaviours, and physiologically they had significantly lower heterophil to lymphocyte (H/L) ratios than turtles in the barren environment. This illustrates that turtles prefer EE, that enrichment improves their welfare, and importantly, that this improvement can be observed in their behaviour. Similarly, Tetzlaff et al (2018; 2019a; 2019b) found that even captive-born *T. carolina* intrinsically preferred enriched habitats, and that enriched environments, along with time for growth in captivity, might aid survival post-release.

Food-centred enrichment for freshwater turtles has also been studied. Bryant and Kother (2015) used puzzle-based feeding enrichment devices to successfully increase time spent feeding and promote foraging behaviour of Fly River turtles (*Carettochelys insculpta*) on display at ZSL London Zoo. Bannister et al. (2021) introduced scented and unscented enrichment devices pre-feeding to reduce negative behaviour in a group of freshwater (*Pseudemys* sp. and *Trachemys scripta* ssp.) display turtles at Tynemouth Aquarium. Presence of enrichment devices pre-feeding successfully reduced escape behaviour and turtles showed greater interest in scented devices than unscented, indicating that olfactory enrichment is appropriate for captive turtles.

Burghardt (2005) observed ‘play’ behaviour in a captive Nile soft-shell turtle (*Trionyx triunguis*) that was introduced to five EEDs: two basketballs of different colours, a hoop, a rubber fill hose, and live fish for feeding. Burghardt (2005 p 82) defined play as "repeated, incompletely functional behaviour differing from more serious versions structurally, contextually, or ontogenetically, and initiated voluntarily when the animal is in a relaxed or low stress setting." These EEDs were introduced in an effort to reduce boredom-induced self-mutilation (Burghardt et al 1996). It was observed that this soft-shelled turtle played with the EEDs for 21% of observed time. This play is longer than juvenile captive mammals, including primates, which play between 1% and 10% of the time (Fagen 1981). Burghardt (2005) also mentioned object play behaviour in another two Nile soft-shelled turtles at Toronto zoo, as relayed by reptile curator Robert Johnson. Indeed, there are other examples of play in turtles, including object play in a loggerhead turtle (Satisky 1998; Satisky 2001 In Burghardt 2005), locomotor play in a wood turtle (*Clemmys insculpta*), and social play in Emydidae turtles (Burghardt 2005). Therefore, EEDs designed to encourage play should be considered for hospitalised turtles in order to increase welfare and reduce rehabilitation time.

Animal Welfare Implications

Maintaining positive welfare of animals under human care is of utmost importance. When considering appropriate methods to assess welfare status and promote positive welfare some distinctions need to be made specifically for sea turtles undergoing rehabilitation. Species-specific and life stage-specific considerations need to be made but also limitations due to the hospital environment should be considered. The five domains model of welfare can be applied to assess welfare of sea turtles, and reviewed for appropriateness, effectiveness and feasibility for application in the rehabilitation setting. Physical health evaluation methods are highly specialised, invasive and expensive and not easily implemented by rehabilitation staff. Nutritional evaluation should definitely be carefully considered with rehabilitation turtles and more research is needed to assess effects of poor diet on the physical health of sea turtles in captivity. The environmental implications on welfare of turtles undergoing rehabilitation can be difficult to manage due to the need for the environment to be sterile and easily cleaned, which makes this domain difficult to assess. The behavioural domain is easily assessed by rehabilitation staff and can be used to infer mental state of the sea turtles. For this reason, behaviour of turtles and mental affective states whilst undergoing rehabilitation should be widely implemented to promote positive welfare.

The limited literature shows that sea turtles respond to EEDs and can benefit from enrichment to improve their welfare whilst in captivity. They have been observed to have basic affective states, engage in play behaviours, and to respond positively to the introduction of EEDs. Through the use of EEDs (including devices to encourage foraging, complex multi-dimensional environments, and hides), designed according to the requirements of the rehabilitation centre and the needs of the individual turtle, it is possible to cover the three main facets of welfare, and thereby assist in the recovery and preparation of rehabilitated turtles for release back into the wild. The authors hope that this literature review will contribute to the recognition of the advantages and significance of EE in hospitalised sea turtles, and to encourage turtle rehabilitators to effectuate and employ EEDs. Future research projects may also assess the impact of various EEDs to determine the most beneficial of these on the welfare of hospitalised and other captive sea turtles, through welfare measures such as a reduction in stereotypic behaviour and faster recovery times, the ultimate goal being to improve the welfare of sea turtles held in confinement.

Declaration of interest statement

The authors wish to declare that they have no conflict of interest, or relationship, financial or otherwise, that might be perceived as influencing objectivity.

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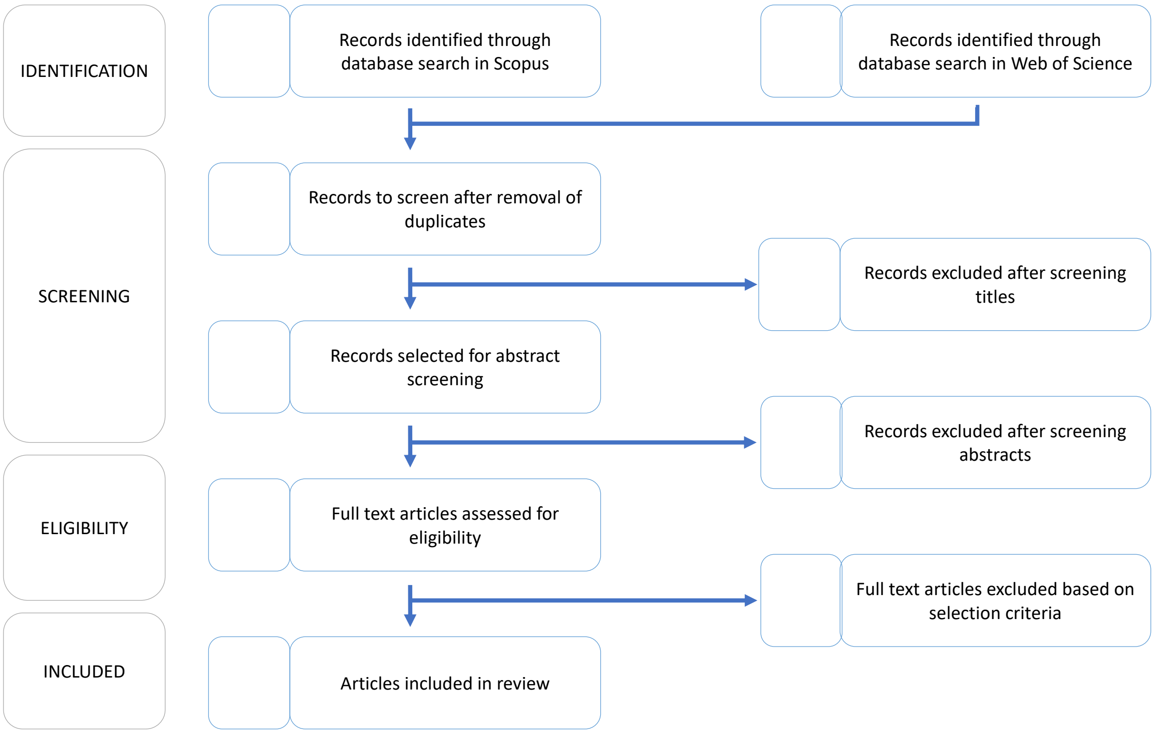
Table 1: Green turtle (*Chelonia mydas*) physical exam score card. Developed in consultation with participants in a workshop at the Turtle Health and Rehabilitation Symposium 2017, Townsville, Australia, facilitated by Duane March and implemented at Dolphin Marine Magic, Coffs Harbour, Australia.

Animal ID:					Location				
Comment:					Date		Date	Date	D
Demeanour	Bright, alert, responsive	0	Quiet, alert, responsive	1	Non-responsive	2			
Swim ability	Strong upright	0	Weak upright	1	Strong/Weak circling	2			
Skin Appearance	Healthy	0	Minor lesions	1	Generalised sloughing	2			
Skin Epibiotic load	X<10%	0	10<x<50%	1	50<x<100%	2			
Fibropapillomatosi s	Nil	0	Less than 5 lesions	1	Greater than 5 lesions	2			
Carapace Epiobiotic load	X<10%	0	10<x<50%	1	50<x<100%	2			
Carapace integrity	Firm	0	Soft at margins	1	Generalised weakness	2			
Plastron	Convex	0	0 <Concave<3 cm	1	3 cm <Concave	2			
Plastron integrity	Clean	0	Moderate damage	1	Marked damage	2			
Muscle tone	Strong	0	Poor	1	Absent	2			
Buoyancy	Neutral	0	Abnormal buoyancy with ability to dive	1	Abnormal buoyancy without the ability to dive	2			
Neurological exam	Jaw tone present	0	Jaw tone reduced	1	Jaw tone absent	2			
	Palpebral present	0	Palpebral reduced	1	Palpebral absent	2			
	Menace present	0	Menace reduced	1	Menace absent	2			
Total									

Welfare of sea turtles - Supplementary Material



Supplementary Figure 1: PRISMA flow diagram of scoping review search. Papers were excluded if they did not directly discuss enrichment of freshwater or sea turtles. Papers were included even if they were not in the context of rehabilitation. Only one paper directly discussed implications of environmental enrichment of turtles in a rehabilitation setting. Review papers were excluded.