Sveriges lantbruksuniversitet
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Department of Animal Environment and Health

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An observational study of McCord's snake-necked turtle (Chelodina mccordi) and Vietnamese pond turtle (Mauremys annamensis)

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Foderberiknings beteendepåverkan på sköldpaddor i fångenskap

En observationsstudie av McCord's ormhalssköldpadda (*Chelodina mccordi*) och Vietnamesisk dammsköldpadda (*Mauremys annamensis*)

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SUMMARY

McCord's snake-necked turtle (*Chelodina mccordi*) and Vietnamese pond turtle (*Mauremys annamensis*) are two critically endangered species of fresh water turtles originating from Asia. The aim of this study was to evaluate how the two species of aquatic turtles responded to food enrichment, if feed enrichment could reduce the stereotypic behaviour displayed by the McCord's Snake-necked turtles and if behavioural signs associated to good welfare increased in response to food enrichment. There are many different definitions of animal welfare. In this study, emphasis was put on the importance to look at species-specific behaviours and the response of the individual when assessing welfare. Few enrichment studies have been made on reptiles when compared to mammals. Two types of enrichments were used and evaluated through direct observation with focal sampling and video observation in this study. The enrichment, "The Egg", was a dog toy designed to contain treats, which could fall out of the toy when the animal manipulated it physically. The other enrichment was an automatic feeder, which expelled small amounts of feed multiple times during the one-hour long observations. Behavioural changes varied between individuals. On a group level, there was a significant difference ($p<0.05$) in the stereotypic behaviour "swim against glass" for the Vietnamese pond turtles. The behaviour was performed at longer durations when enrichments were used compared to normal feeding. A reduction of the stereotypic behaviour was seen in the McCord's snake-necked turtles when "The Egg" was used. The automatic feeder produced a decrease in foraging behaviour and increased stereotypic-, aggressive- and same-sex sexual behaviour for multiple individuals. More individuals displayed behavioural changes indicative of good welfare when "The Egg" was used compared to when the automatic feeder was used.

SAMMANFATTNING

McCord's ormhalssköldpadda (*Chelodina mccordi*) och Vietnamesisk dammsköldpadda (*Mauremys annamensis*) är två kritiskt hotade arter av medelstora sötvattens sköldpaddor med ursprung i Asien. Syftet med studien var att undersöka sköldpaddornas beteendeförändring vid foderberikning, minska ormhalssköldpaddornas stereotypa simmande mot inhägnadens glasvägg samt utvärdera om det skedde en beteende förändring som kunde indikera en ökad djurvälfärd. Det finns många olika definitioner av ordet djurvälfärd. Vid välfärdsbedömningen som gjordes i den här studien så lades vikt vid djurslagsspecifika beteenden och beteendeförändringar hos individen. Ett fåtal berikningsstudier är gjorda på reptiler i jämförelse med däggdjur. Två olika sorters berikning användes i den här studien, de utvärderades genom direkt observation av fokaldjur samt genom videoobservation. Berikningen som kallades ”Ägget” var en hundleksak utformad för att innehålla foder som kunde ramla ut när djuret fysiskt manipulerade den. Den andra berikningen var en foderautomat som delade ut små portioner av pellets flera gånger under de entimmes långa observationerna. Beteendeförändringarna skilde sig mellan individerna. På grupp-nivå ficks en signifikant skillnad ($p<0.05$) hos de Vietnamesiska dammsköldpaddorna av det stereotypa beteendet ”simma mot glaset”. En minskning av samma stereotypa beteende sågs hos de två McCord's ormhalssköldpaddorna när berikningen ”Ägget” användes. Foderautomaten minskade födosöksbeteende och ökade stereotypt, aggressivt och samkönat sexuellt beteende för flera individer. Fler individer visade beteendeförändringar som indikerade en god djurvälfärd när ”Ägget” användes jämfört med när foderautomaten användes.

CONTENT

INTRODUCTION

Maintaining a captive population for future conservation, education of the public and research are some of the goals that Nordens Ark pursue by keeping endangered species such as the McCord's Snake-necked turtle (*Chelodina mccordi*) and Vietnamese pond turtle, (*Mauremys annamensis*). The two species of turtles included in this study are critically endangered (Asian Turtle Trade Working Group. 2016. *Mauremys annamensis*.: Asian Turtle Trade Working Group. 2016. *Chelodina mccordi*). One of the species, the McCord's snake-necked turtle, demonstrated a stereotypic behaviour by interacting repeatedly with a transparent enclosure wall. The problem founded the idea of using enrichment as a way of reducing the behaviour. Enrichment is widely used in zoos as a way diminishing stereotypic behaviour and improving animal welfare.

The aim of this study was to evaluate how the two species of aquatic turtles responded to food enrichment. The following questions were addressed; Can feed enrichment reduce the stereotypic behaviour displayed by the McCord's Snake-necked turtles? Can behavioural signs associated with good welfare increase when the turtles are exposed to food enrichment?

LITERATURE REVIEW

McCord's snake-necked turtle (*Chelodina mccordi)*

This critically endangered (Asian Turtle Trade Working Group. 2016. *Chelodina mccordi*.) turtle is indigenous to the small Island of Roti in the south eastern Indonesian archipelago (Rhodin 1994b see Rhodin *et al*., 2008). The population has been diminished by intense collection of individuals from the wild for international pet trade. It lives in small, shallow eutrophic inland lakes, swamps and marshlands. These habitats are being reduced by agricultural development. The wet season is short and the dry season is long on the Roti Island, during which many of the small lakes dry out. During the dry season, according to local people, the turtle takes refuge under rocks or leaves in the surrounding forest, but does not bury itself in the mud to aestivate. Local people also reported that the activity of *C. mccordi* is nocturnal except during rains in the wet season, at which time the turtle can be seen wandering on land (Rhodin *et al.,* 2008). By looking at the osteology of the skull, it has been concluded that *C. mccordi* is adapted to be a generalised carnivore or piscivor which is further supported by local people reporting that *C. mccordi's* diet consists of small fish, insects, tadpoles, other small animals and water weeds (Maran & Coutard 2003: Symanski 2004 see Rhodin *et al*., 2008).

Vietnamese pond turtle, *(Mauremys annamensis)*

Little is known of the wild specimens of this species. It was first described in 1903 (Siebenrock 1903 see Van Dijk *et al.*, 2000). *M. annamnesis* is critically endangered (Asian Turtle Trade Working Group. 2016. *Mauremys annamensis*.) and endemic to the lowlands of central Vietnam. Collection for food, traditional medicine and pet trade and loss of natural habitat are the main reasons for the diminishing population (Van Dijk *et al.*, 2000). Its habitat consists of marshlands, streams and ponds.

The behaviour of wild Vietnamese pond turtles is mostly unknown according to McCormack *et al.,* (2014). Most observations of behaviour and diet are from captivity and their natural diet is not known. The Vietnamese pond turtle is most likely an omnivorous generalist feeder, but has shown a preference for a carnivore diet in captivity (McCormack *et al.,* 2014).

Reptile welfare assessment

Good feeding, good body weight and active reproduction are commonly perceived as indicators of good welfare in reptiles by their keepers and sellers (Warwick *et al.,* 2013). But the assessment of reptile welfare is far more complex than fulfilling of the criteria above. According to Warwick *et al.,* (2013), assessment of behaviour is an essential method for evaluating the welfare of a reptile.

Animal welfare is a widely used concept with many definitions. It can have different meanings for different people, depending on within which industry they work or what their personal ethical views are. Hewson (2003) has, in a review article, condensed the concept into three approaches. The first is the concept traditionally used by veterinarians and farmers; evaluation of the physical state of the animal's body and environment. This means that an animal that is healthy and producing well, fairs well. The second concept acknowledges that animals have feelings. The feeling-based concept concludes that if an animal feels well it fairs well. The third approach is the one of "natural living". This means that the animal fairs well if it can perform its natural behaviours. In this concept, fear of being preyed upon and feeling cold could be acceptable if it part of the animal's natural way of living. Hewson states that the most widely accepted definition of animal welfare is made by Duncan & Fraser (1997); "it comprises the state of the animal's body and mind, and the extent to which its nature (genetic traits manifest in breed and temperament) is satisfied" (Duncan & Fraser 1997 see Hewson 2003).

It is argued that there is no universal indicator of good animal welfare since there are no universal responses by animals to threats to their welfare (Dawkins 2001 se Hill & Broom 2009). Behavioural changes when exposed to a new condition in a captive environment, as well as comparison with the species normal pattern of behaviour is of great interest when conducting welfare studies (Hill & Broom 2009). Normal behaviour can be defined as "behaviour that should occur in healthy animals whose behavioural repertoires have developed under conditions that offer uninhibited opportunities, and are appropriate to the animals' needs for behaviour and resources." (Knierim *et al*., 2001 see Hill & Broom 2009). Such behavioural data lacks for most species in zoos today and the same goes for the two species of turtles included in this study (Knierim *et al.,* 2001 se Hill & Broom 2009; McCormack *et al*., 2014).

It is important to consider the species in question when assessing behaviour as an indicator of welfare. There are species-specific behaviours that have evolved to enable animals to cope with different conditions (Hill & Broom 2009). There has been a "taxa-bias" when it comes to studies on zoo animals, for example behaviour of mammals, primarily primates, has been far more evaluated then reptiles housed in zoos (Melfi 2005: Burghardt 1977). Historically, research on natural behaviour of reptiles has been a low priority, probably because of observational difficulty in the wild as well as the perception that reptiles are highly adaptable to captivity (Warwick 1990). Another reason for this neglect could be the widespread dated belief that reptiles are unintelligent and unable to learn due to their relatively small brain size. This has however been contradicted by numerous scientists (Burghardt 1997a and Jerison 1973 see Kuppert 2013). Stressed reptiles manifest an array of abnormal behaviours (Warwick *et al.*, 2013), but it is suggested that the behavioural responses of reptiles may be harder for us to recognize “intuitively” then the behaviour of mammals (Hill & Broom 2009).

Welfare can differ between individuals of the same species that are exposed to the same conditions (Hosey *et al.*, 2013a). Reaction to stimuli can be influenced for example by past experiences and the ability to cope with certain challenges. Reactions by an individual to changed conditions can be measured by using each individual as its own control (Hill & Broom 2009).

As a conclusion; it is important to document normal behaviour of the individual animal in question, to be able to document changes in behaviour when exposed to enrichment. It would also be ideal to have data on normal behaviour exhibited by a wild individuals living in its natural habitat.

Behavioural displays of captive reptiles

Reptiles are considered to be highly specialised physiologically to certain conditions and food sources. Warwick (1990) describes reptiles as environmentally inflexible since they are dependent on the ambient temperature of the enclosure due to their ectothermic nature. Apart from many mammals and birds, most reptiles do not foster their young (Burghardt 1977). Reptiles are born with innate knowledge of how to manage to survive in its natural environment, hence reptiles primarily receive their natural education innately, not socially (Warwick 1990). Although some educational elements are received pre-birth to mammals and birds a lot of the young’s education about the world it enters, is done by learning from the parents and/or the social group. Therefore, mammals and birds do not have the same pre-programmed expectations of the environment that it enters as a reptile does. It has been reported that reptiles bred in captivity for generations’ displays the same natural behaviour as their wild counterparts, when released into the wild. From this a conclusion can be drawn that the naturally innate characteristics and requirements a reptile needs to survive stay with reptiles for life and only small environmental adaptations can be expected to occur in captivity (Warwick 1990). From this knowledge, it can be argued that it is in some ways harder for a reptile to adapt to a suboptimal environment in captivity than it is for a mammal or bird.

Generally, reptiles react to highly inadequate or stressful environments in two ways, according to Warwick (1990). The first is by increased mobile activity. The second is hypoactivity and anorexia, which is a form of “waiting out” -strategy. Morgan & Tromborg (2007) summarises behaviours displayed by animals exposed to chronic captivity-related stress as increased abnormal behaviour, behavioural inhibition, hiding, aggression, and freezing behaviour. Behaviours that decrease under the same conditions are exploratory behaviour, reproductive behaviour, and behavioural complexity. Generally, for multiple species of zoo animals, the rate of abnormal repetitive behaviours has been considered a primary sign of poor welfare. These stereotypic behaviours may however, not diminish even if welfare is improved (Mason *et al*., 2007).

Behaviours associated with good animal welfare include exploration, species-specific behaviours (foraging and thermoregulatory behaviours) and behavioural diversity (Izzo *et al.*, 2011 see Bashaw *et al*., 2016). Warwick (2013) describes behaviour associated with a comfortable reptile to be; relaxed eating, relaxed drinking, relaxed breathing, unhurried body movements and locomotion and normal resting habits. A study by Case *et al.*, (2005) showed that environmentally enriched Box turtles who were less stressed then before, made fewer movements associated with the enclosure walls and spent more time resting and moving spontaneously about the enclosure.

Hyperactivity

Some captive reptiles may display hyperactivity, usually this is observed as constant escape attempts according to Warwick (1990). It is argued that some of these seemingly overactive animals are in fact engaging in activity, which are similar to the activity level as their wild counterparts. The activity is therefore not hyperactive by definition but can be argued to be excessive for the conditions the animal is subjected to in captivity. Two main reasons for hyperactivity is given by Warwick (1990); Innate drive as well as food and environmental searches. Carnivorous species of lizards as well as species who are browsers have evolved to be natural wanderers and have developed an innate need for activity. Successful tests have been done to decrease hyperactivity in carnivorous lizards by providing food enrichment in the form of hidden food in their captive environment (Warwick 1990).

The need for space can be quantified by looking at the reptiles normal" home range". It is a concept defined by Burt (1943) as an area around the home site, over which the individual normally travels in search for food. In a study of carnivorous mammals, a correlation has been found between stereotypic locomotion, pacing, and the size of the animal's natural home range size (Clubb & Mason 2003). Although no data could be found on the natural home range of Vietnamese pond turtle or McCord's snake-necked turtle, other medium sized fresh water turtles have been studied. Australian freshwater turtle, (*Chelodina longicollis*) has been shown to have a home range of 757 ±76 m in length (Roe & Georges 2008). Chinese stripe-necked turtle (*Ocadia sinensis*), a fresh water turtle found in Vietnam, has a home range average of 703 m and moved 5-245 m on a daily basis (Chen & Lue 2008). Furthermore, the Florida Red-Bellied Turtle (*Pseudemys nelson*) use an area of approximately 120 m in length (Kramer 1995). Mean home range length of yellow-blotched map turtle (*Graptemys flavimaculata*) has been estimated to 1,8 km (Jones 1996). And the home range of the wood turtle (*Clemmys insculpta*) was determined to be 28,3 ha (Arvisais *et al.,* 2001). Although these species of turtles are not alike, they are all small to medium sized turtles living in fresh water. It is not possible to compare the results of one species to another, but the home range studies above indicate that the turtles in this study probably move over a larger area in nature then is provided in captivity.

Hypoactivity

It can be challenging to determine if a naturally sedentary individual is abnormally hypoactive, but according to Warwick (1990), there are signs to look for. Anorexia for example, is a symptom that often accompanies hypoactivity. Reptiles who naturally aesivate in response to environmental treats are more prone to hypoactivity in response to an unsatisfactory artificial environment. To increase activity levels, successful tests have been done by providing larger, well-furnished enclosures as well as providing the animal's favourite food (Warwick 1990).

Aggression and Persecutions from other occupants

Aggressive behaviour towards cage-mates without apparent reason such as courtship or territorial conflicts is often a response to an unsatisfactory captive environment or situation. The behaviour can often be decreased by environmental or feeding enrichment (Warwick 1990).

Persecuted aquatic turtles are known to avoid conflict with fellow cage-mates by inhabiting areas in the enclosure unoccupied by other specimens, where an attack is less likely to occur. It can be both under water and on land. Individuals may also display hypoactivity accompanied by anorexia and less commonly, hyperactivity (Warwick 1990).

Sexual behaviour

"Nudging" is often the first courtship behaviour displayed by males of many freshwater turtles (Liu *et al*., 2013). It is a tactile signal performed by the male, by nosing or touching around the female's cloaca or on parts of the female's plastron or carapace with his head. The behaviour is thought to stimulate the female to turn and face the male (Murphy & Lamoreaux 1978: Liu *et al*., 2008). Nudging also is thought to have an olfactory function, by which the male can collect olfactory signals from the female's (Kuchling 1999). Some species perform "nudging" after mounting to leave a chemical signal from the male's chin glands on the female (Manton 1979 see Liu *et al.*, 2013).

Murphy & Lamoreaux (1978) describes courtship and mating behaviour of Common snake-necked turtle, *Chelodina longicollis*. This species performs a behaviour resembling the "nudging" described above. The male presses his chin against the female's vertebral grove, in the region of her postvertebral marginal scutes, and swims forward until his chin has passed along the carapace and the nuchal scute is reached. The male then proceeds to mount the female by pressing his plastron against her carapace and hooks himself with his hind limbs under her carapace.

Temperature preference

Since reptiles are ectothermic, they achieve thermoregulation by voluntary selection of different ambient temperatures. Warwick (1990) describes the well-known behavioural problems of some captive reptiles, who choose to occupy cold areas of the enclosure for long periods of time even though warm areas are provided. This behaviour can be accompanied by inactivity and anorexia. When the body temperature drops, so does the metabolic rate and the animal can enter a near hibernation-like state. The behaviour leads to slowed metabolism, a hibernation-like state that could be described as a physiological and behavioural shut down that the animal can use to cope with an unsatisfactory captive situation. The majority of the reptiles who display this abnormal behaviour are species that have a reputation for being highly unadaptable to captive environments. The problem with inactivity and anorexia usually continues even if there no longer are any cool areas in the enclosure to seek out, probably because the cause of the problem persists in an all warm environment. It is argued that the behaviour to seek out cool areas is an otherwise warm enclosure is due to an animal's innate drive for hibernation. Since this behaviour is displayed also by species without hibernation it can be dismissed as the only reason. The problematic behaviour usually disappears when the animal is provided with improvement of space, furnishing and foods.

Reptiles are known for seeking out warmer areas to elevate its body temperature when healing from physical injury or infection. This voluntary physical regulation of body heat is according to Warwick (1990) an indication that a voluntary psychological temperature regulation can occur as well. The thermoregulatory behaviour is hypothesised by Warwick (1990) to be influenced by the reptile's state of mind; "In short, the animal seeks an environmental temperature to match its mood.". This hypothesis dictates that an unsatisfied reptile will choose cold areas, whereas warmer climates are sought when the animal is content (Warwick 1990). In conclusion, increased basking behaviour could indicate a happier turtle.

Interaction with transparent boundaries

Transparent materials such as glass or acrylic plastic is often used as walls in enclosures for reptiles because of its practical qualities in maintaining temperature and moisture in the enclosure as well as it provides good observation of the animal. Many different species of reptiles can be seen repeatedly pushing their snouts against the glass or swim repetitively against it as can be read about in an article by Warwick (1990). Physical trauma, usually to the face, can occur due to this repetitive behaviour. The reason for this behaviour, in the majority of instances, is thought to be a due to the reptile's inability to interpret the barrier as impermeable. Inadequate environment in the enclosure may prompt environmental search behaviours in the reptile, and the reptile interacts with the glass as it attempts to seek out a new, more suitable environment. The transparent boundary can sometimes work as a mirror and the reptile may try to enter the appealing reflection of its own enclosure. To reduce the interaction with transparent walls, they can be masked by vegetation or replaced with non-transparent material. Unfortunately, the reptile often remembers that the wall used to be transparent and continues its attempts to penetrate it. The best way to decrease the behaviour is to remove the animal to a new enclosure without transparent boundaries (Warwick 1990).

Food enrichment

Food provided in captivity often differs from the food found in nature and generally takes much less time to eat than the animal's diet found in nature (Morgan & Tromborg 2007). It is widely perceived that food enrichment can increase zoo animal welfare by increasing the time spent foraging to mimic conditions in the wild (Shepherdson *et al.*, 1993: Izzo *et al.*, 2011 see

Bashaw *et al.,* 2016: Hill & Broom 2009), as well as increase the animal's physical condition and reduce obesity-related problems (Markowitz *et al.*, 1978 see Young 1997).

Several species of chelonians have been shown to be able to learn and problem solve in response to a small food reward (Davis & Burghardt 2007: Young *et al.*, 2012: Weiss & Wilson 2003). It has been shown that an aquatic turtle species had good visual ability and was able to distinguish between colours (Young *et al*., 2012). These characteristics make turtles good candidates to benefit from food enrichment.

Critical voices have been raised as to the positive impact of working for food. Young (1997) suggests that an increased foraging time could produce a higher level of psychological well-being but have harmful effects on the physical well-being. This assumption was made on the fact that energy is lost by the animal while foraging and therefore an enriched animal receives a lower net energy gain at each feeding then an animal fed by a bowl. A study was conducted with zebra finches that concluded that zebra fiches provided with easy accessible food lived longer and had a higher reproductive success then zebra finches that had to work for their food (Lemon & Barth 1992). From this point of view, introducing food enrichment could shorten the lifespan of the animal and therefore be seen as a negative impact on its welfare. This impact of food enrichment on longevity would be difficult to quantify properly according to Young (1997), because of the many factors affecting the husbandry of the individual zoo animal.

Another potentially problematic area of food enrichment is the fact that the enrichment itself can increase aggressive behaviour if provided to a group of animals. The enrichment can become an item to defend, which has been shown to be associated with aggressive behaviour in wild pigs by a study done by Schnebel & Griswald (1983) in pigs. The aggression decreased if the animals were provided with multiple feeding stations.

Enrichments effect on stereotypic behaviours performed by reptiles

Feeding enrichment has been proven to decrease the performing of abnormal behaviour in numerous zoo animals (for example see Shepherdson *et al.*, 1993). The results of the impact enrichment have had on stereotypic behaviours displayed by reptiles are varied.

Two studies of turtles have shown a decrease in stereotypic behaviour when the turtles were exposed to environmental enrichment in the form of novel objects and food enrichment (Burghardt *et al.,* 1996: Therrien *et al.,* 2007). It has also been shown that repetitive interaction with enclosure boundaries can be reduced when Eastern Box Turtles (*Terrapene carolina carolina*) were exposed to an enriched environment where natural behaviour could be performed (Case *et al.,* 2005). However, stereotypic interaction with cage barriers did not decrease when leopard geckos were exposed to feeding enrichment (Bashaw *et al.,* 2016), but it was concluded by the authors that welfare was increased for the lizards since exploratory behaviour, foraging and behavioural diversity increased.

MATERIAL AND METHODS

The study took place between the 26th of September and 12th of October in 2016 at Nordens Ark, Hunnebostand, Sweden.

The subjects, enclosure and normal feeding

Two groups of turtles were included in the study. Four Vietnamese pond turtles and two McCord's snake-necked turtles. All of the turtles in the study arrived at Nordens Ark in 2008. The four individuals of Vietnamese pond turtle were sisters hatched in 2007-01-01, and the McCord's snake-necked turtle subjects were two brothers hatched in 2007-06-22 (Jimmy Helgesson, personal communication 2016-10-10). This puts all of them at an age of around 9 years old at the time of the study.

Enclosure

The turtles were kept in enclosures with both land and water. Both enclosures had a transparent boundary facing the visitors. The layouts of the enclosures and placements of food enrichments can be seen in figure 1 and 2.

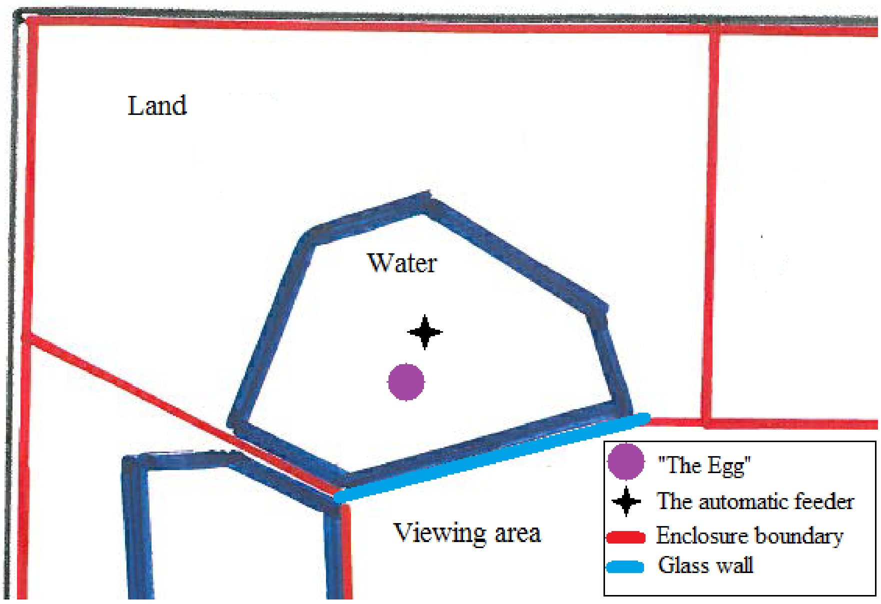

Figure 1. The McCord's snake-necked turtles' enclosure layout and placement of food enrichments. Origninal scetch "Våtmarken 2:a våning" by Christer Larsson, modified by author 2016.

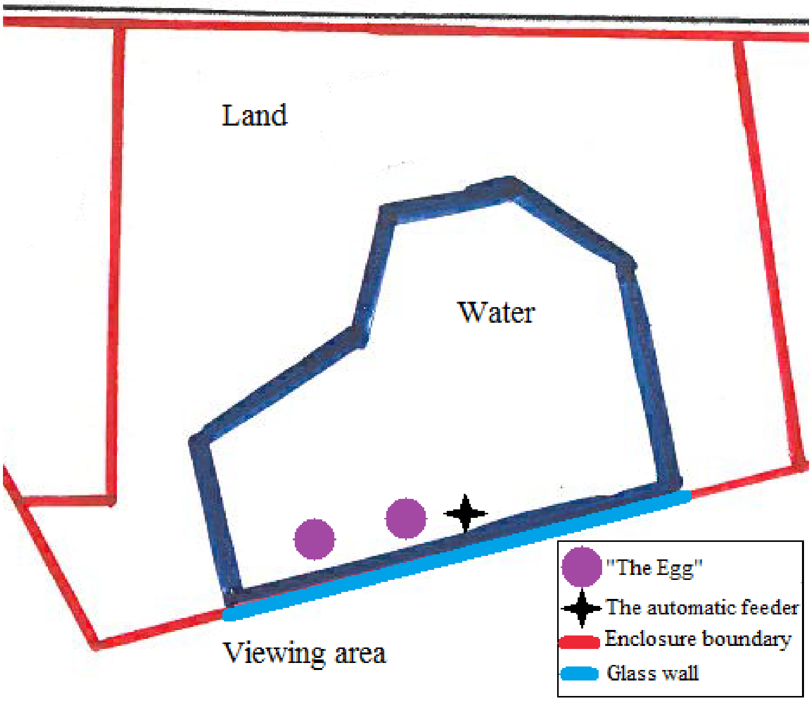

Figure 2. The Vietnamese pond turtles´ enclosure layout and placement of food enrichments. Origninal scetch "Våtmarken 2:a våning" by Christer Larsson, modified by author 2016.

Feeding

Normal feeding of the turtles took place in the mornings three times a week, on Mondays, Wednesdays and Fridays. Feeding consisted of zoo keepers throwing food directly into the water. A variety of feed was normally provided. In the study, only a pelleted feed was used. The pellets, "Natural Aquatic Turtle Food – Maintenance formula" by zoo med laboratories, inc., floats in water to facilitate for aquatic turtles who prefer feeding at the water surface (Zoo Med laboratories inc. 2016). It was estimated by a zoo keeper that 15 pellets per turtle was a normal ration. This meant 60 pellets for the Vietnamese pond turtles and 30 pellets for the McCord's snake-necked turtles per feeding.

Study layout

An observational study of the turtle's behaviour when exposed to different types of feeding was made. Observations were made for one hour at each feeding, both by direct continuous observation and by video filming. Feeding took place at 9:00 pm or 10:30 pm three times a week. Both species of turtles were observed when feeding was done the normal way, by the automatic feeder and with "The Egg".

The order in which the feeding was done for the Vietnamese pond turtles was; normal feeding, "Egg", automatic feeder, normal feeding. The McCord's snake-necked turtles feeding order was; normal feeding, automatic feeder, "Egg", normal feeding. See Table 1 for detailed layout.

Table 1. Feeding schedule

Week	Day	Vietnamese pond turtles	McCord's snake-necked turtles
1	Wednesday	Normal feeding	Normal feeding
	Friday	Normal feeding	Normal feeding
2	Monday	"The Egg"	Automatic feeder
	Wednesday	"The Egg"	Automatic feeder
	Friday	"The Egg"	Automatic feeder
3	Monday	Automatic feeder	"The Egg"
	Wednesday	Automatic feeder	"The Egg"
	Friday	Automatic feeder	"The Egg"
4	Monday	Normal feeding	Normal feeding
	Wednesday	Normal feeding	Normal feeding

If food was left in "The Egg" or automatic feeder at the end of an observation, it was released into the water for consumption. This was done to ensure that the turtles were given the same amount of food at every feeding.

Feed enrichment

In the study, two types of enrichment were presented to the turtles; "The Egg" and the automatic feeder. The placements of enrichments in the enclosures are visualised in figure 1 and 2.

"The Egg"

The enrichment called "The Egg" during this study, was a dog toy, "Kibble nibble" size XS/S, by ©Premier Pet Products LLC. A purple plastic toy in the shape of an egg, which could be screwed apart into two pieces and filled with treats. It had two orifices, one at each end of the egg, which were lined with plastic spikes to hinder treats from falling out by themselves (see figure 3). The animal had to manipulate the toy in order for the treats to escape the orifices.

The turtles would have to interact physically with "The Egg" in order for pellets to float out of an orifice. One "Egg" containing 30 pellets was presented to the McCord's snake-necked turtles. Two "Eggs" containing 30 pellets each was presented to the Vietnamese pond turtles.

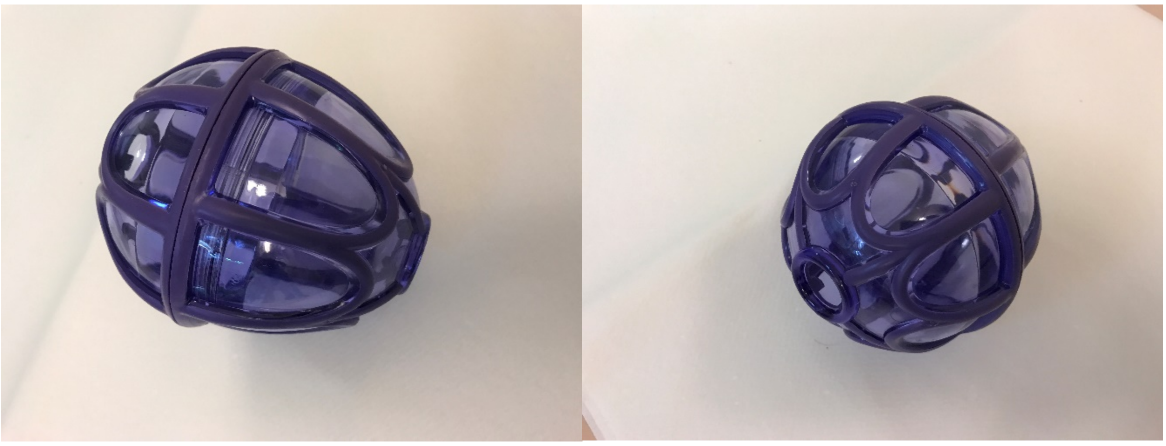

Figure 3. “The Egg”, seen from two angles. Kristofer Försäter, 2016.

The automatic feeder

EHEIM autofeeder, a battery driven machine designed to deliver feed at pre-set times during the day, see figure 4. It could be attached to the enclosure wall or enclosure furnishing. A built in timer made it possible to set feeding times. It had a rotatable vessel that could be filled with food. The vessel had an orifice at one side, which enable food to fall out when the vessel was rotated. A maximum of four feeding times could be programmed, and you could choose one or two rotations of the vessel per feeding time. (EHEIM turtlefeeder manual, 2016-12-07)

A small trial was made on the automatic feeder’s food-output. Based on the amount of pellets released in one rotation, settings were chosen that would be practical for both enclosures. The automatic feeder was pre-installed to deliver food 4 times during an observation with 15 min intervals. In the enclosure with 4 individuals of Vietnamese pond turtle, the automatic feeder was filled with 60 pellets and set to rotate twice at each feeding time. In the enclosure with 2 individuals of McCord’s snake-necked turtle it was filled with 30 pellets and set to rotate once per feeding time.

Figure 4. The automatic feeder, seen from two angles. Kristofer Försäter, 2016.

Preparations

The Egg and Automatic feeder were tested on both Vietnamese leaf turtles and McCord’s snake-necked turtles not included in the main study. After this test it was decided that getting pellets to float out of “The Egg” was a bit too difficult and the spikes around the orifices were cut off to facilitate pellet exit.

Observations were made for half an hour before the start of the study, on both species, to test the protocol. The protocol was then edited to include the most relevant behaviours according to the aim of the study, see table 2.

McCord’s snake-necked turtle

Due to an excessive interaction with the transparent boundary of their enclosure, scarring has developed on the heads of both individuals. The source of the stereotypic behaviour was not known, but it has been theorised by personal at Nordens Ark that the turtles associate people with food.

The turtle with the most scarring was called “1” and the other “2”. The turtle called “1” had scarring around both eyes, which formed the appearance of a white mask. “2”’s scarring was primarily located around and behind the left eye. There was also a small difference in size between the brothers. The individual called “1” had a slightly smaller skull then “2”, this difference could only be seen when the individuals were side-by-side.

Vietnamese pond turtle

Three of the four turtles were caught one day before the start of the study, and marked with either blue, green or pink nail polish on the front and back of the shell. The individuals were then named after their coloured marking; “Blue”, “Green” and “Pink”. The un-painted turtle was called “White”.

Direct continuous observation

The observational study was made using direct continuous observation on focal animals. The individuals were observed in a randomised order, for two minutes at a time and performed behaviours were noted. Behaviours were determined and defined before the start of the study and can be seen in table 2. The order by which the individuals were observed was determined by writing down their names on small paper notes, which were folded and mixed before drawing them one by one. Since the snake-necked turtles were only two individuals, the drawing was done on the first observation and then switched for the next one. The duration of each observation was one hour and commenced when food or enrichment was presented to the turtles. A mobile phone was used as a timer.

Table 2. *Ethogram over behaviours noted in direct observation*

Behaviour	Description
Eat	Intake of pellets in mouth, chewing. Same event when < 2 seconds has passed between displayed of described behaviour.
Forage/explore	Outstretched neck and side-to -side movement of head while swimming or remaining stationary. Can be done looking towards the water surface, ground or enrichment. When exploring a novel

	object, like "the egg" an outstretched neck with focus on the enrichment for > 2 seconds.
Touch with head/biting	Head is in contact with enrichment. Biting the enrichment. Contact within 2 seconds of each other counts as one event. Long contact without movement counts as one event.
Touch with extremity	Extremity in contact with enrichment. Multiple events within 2 seconds of each other counts as one event.
Swim	Movement under water, both swimming and walking. If behaviour is displayed again within 2 seconds it counts as the same event.
Swim against glass	Interaction with transparent boundary. Swims repeatedly against glass. Head and anterior extremity in contact with glass while swimming. Can be performed under water or at the water surface. Counts as the same event if the behaviour is repeated within 2 seconds.
Stationary under water	No movement under water. Movement of the head and small movements of extremities to maintain stationary are included. If behaviour is displayed again within 2 seconds it is counted as the same event.
Stationary at the water surface	Stationary with parts of the body over water level. Usually this behaviour is displayed when going up to the surface for air. Small movements of extremities to maintain position is included in the behaviour. If the behaviour is repeated within 2 seconds it is counted as one event.
Stationary on land	Stationary with majority of the body on land. If the behaviour is displayed again within 2 seconds it counts as one event.
Move on land	Walking or climbing when the majority of the body is above water level. If the behaviour is displayed again within 2 seconds it counts as one event.
Heat lamp /basking	Stationary under a heat source for more than 5 seconds. If the behaviour is displayed again within 2 seconds it counts as one event.
Head on other carapace	Pressing chin against the dorsal side of another turtle's carapace or neck. If the behaviour is displayed again within 2 seconds it counts as one event.
Other head on own carapace	An individual has another's chin pressed against its carapace or neck. If the behaviour is displayed again within 2 seconds it counts as one event.
Other	Behaviours not included above.
Not visible	Can not be seen or can not be identified. Duration of time not visible is noted.

Video observation

A digital video camera on a stand was placed in front of the left side of the enclosure. Feed or enrichment were placed in the water straight after the record button was pushed. When the automatic feeder was used, the record button was pressed when the automatic feeder vessel began to rotate. The camera had a maximum video length of 30 min, therefore the record button had to be pressed again after 30 min and two videos were made in each hour of observation. Since the camera made a sound when ending a recording, the observer, who was sitting next to the camera, could restart the recording instantaneously.

The video films were manually analysed using a program called Solomon coder beta 16.06.26 to measure the duration of behaviours. The minimum duration of a behaviour was set to one second in the program. Only one behaviour could be recorded at a time. This propagated a priority order amongst the behaviours when multiple behaviours were performed at once. Since the aim of the study was to evaluate behaviour around feeding; eating, foraging and interaction with enrichment were prioritised over state behaviours such as swimming or being stationary. When an individual could not be identified it was recorded as “not visible”.

The ethogram used for the video observations was a simplified version of the ethogram used for the direct observations. The following behaviours were recorded in the videos: Eat, forage/explore, interaction with “The Egg”, swim against glass, swim, stationary in water, land, head on other carapace, other head on own carapace, other and not visible.

Two video observations were analysed per enrichemt. “The Egg” and the automatic feeder was used tree times, the first and last observation was included in the video analysis.

Statistical analysis

The results from the ethograms were put in Microsoft Excel. Statistical analysis was made using Minitab17. Friedman’s test was used to analyse behavioural differences between enrichments on a group level.

Since “The Egg” and the automatic feeder was used three times and the normal feeding and the normal feeding after exposure was conducted two times each, a mean frequency was calculated. Results from the direct observation are therefore presented as mean values of frequencies displayed during one feeding.

The total times for normal feeding, automatic feeder and normal feeding after exposure for the group of Vietnamese pond turtles were 28816 seconds each, for “The Egg” the total time was 28576 seconds. The difference in time is due to an technical error, it was considered to be neglectable when data was compared between enrichents. The total time of the video observations of the McCord’s snake-necked turtle for each type of feeding was 14408 seconds.

RESULTS

Vietnamese pond turtle

A summary of the video observations can be seen in figure 5. The behaviour “not visible” had its longest duration when normal feeding was conducted, but shortened during the other types of feeding. The behaviour was not divided equally amongst the individuals. One individual, “White”, produced a larger percentage of the “not visible” duration then the others throughout the study. “White” contributed to the total duration of “not visible” with the following percentages; 44 % during Normal feeding, 76,8 % with the automatic feeder, 73,5 % with “The Egg” and 63,4 % during normal feeding after exposure.

“Swim against glass” was performed for the shortest amount of time when normal feeding was condcted. When”The Egg” was used, the duration increased and the longest duration was recorded when the automatic feeder was used and persisted to be on a high level when normal feeding recommenced after exposure to enrichment.

The most time spent foraging or exploring occurred during normal feeding. When “The Egg” was used the duration of the behaviour “forage/explore” shortened, but with the behaviour “interact with “The Egg” added to “forage/explore”, the enrichment produced the longest duration. The shortest duration of foraging or exploring was measured when normal feeding recommenced after exposure to enrichment.

Swimming almost doubled in duration between normal feeding and normal feeding after exposure. Compared to normal feeding, swimming increased during both types of enrichment.

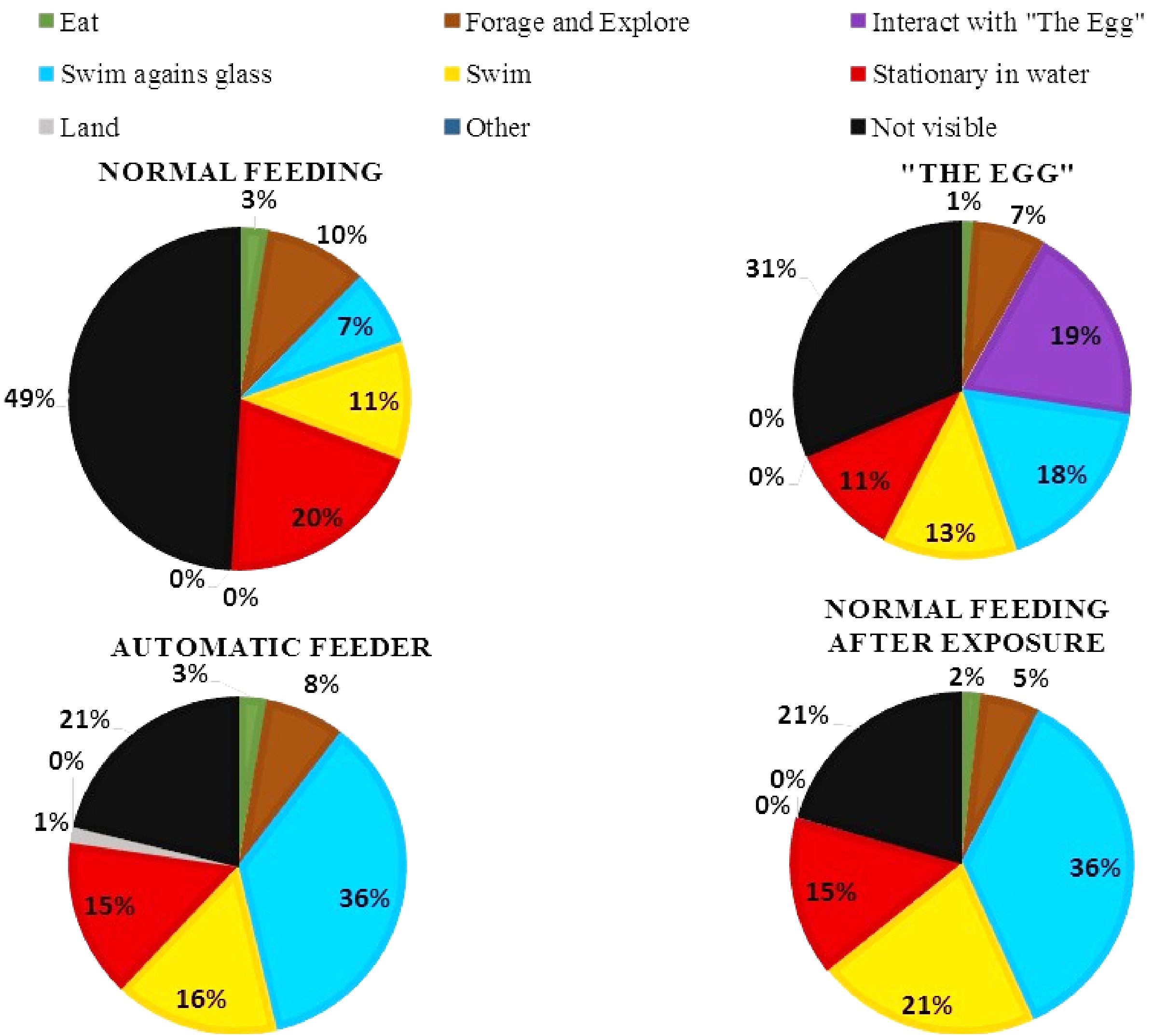

Figure 5. Summary of video analysis. Duration of behaviours displayed by the four individuals of Vietnamese pond turtle sorted by type of enrichment. In the pie charts "The Egg", "interact with "The Egg" is displayed as a separate behaviour. When statistical calculation was made and when forage duration was compared between enrichments, the values of the behaviour "interact with "The Egg" was included in the behaviour "forage and explore". Each pie chart represents approximately eight hours as 100 %.

Results from the direct observations can be seen in figure 6. The behaviour "movement on land" and "stationary on land", was performed during feeding with the automatic feeder and during normal feeding after exposure. The frequency for "movement on land" was 9 times with the automatic feeder and 4 times during normal feeding after exposure. "Stationary on land" was performed 4 times with the automatic feeder and 2 times during normal feeding after exposure. The behaviour "heat lamp/basking" was performed one time when fed with the automatic feeder.

"Head on other carapace" and "other head on own carapace" was not performed by the Vietnamese pond turtles, and therefore excluded from the result summary seen in figure 4.

How the Vietnamese pond turtles interacted with "The Egg" was noted during the direct observations, but since this behaviour could not be compared between observations, the categories “touch with head/biting” and “touch with extremities” were excluded from figure 6. The mean frequency of “touch with head/biting” was 10,3 times, “touch with extremities” was 52,3 times per feeding with “The Egg”.

Normal feeding and “The Egg” had the two lowest mean frequencies of “swim against glass”. “The Egg” had the highest mean frequency of “forage/explore” and the lowest was noted during normal feeding after exposure. The highest mean frequency of “swim” occurred when “The Egg” was used and the lowest frequency when normal feeding was conducted.

Behaviours observed and noted as “other” for the Vietnamese pond turtles were; yawning, biting another individual, biting on enclosure furnishing, displaying an open mouth towards another individual.

Aggressive behaviour in the form of biting the throat of another turtle and displaying an open mouth towards another individual was displayed by the individual “Pink” when fed by the automatic feeder and during normal feeding after exposure. When the automatic feeder was in use, she bit the individual “Blue” one time and displayed an open mouth towards the individual “Green”. During normal feeding after exposure she bit “Green” three times on the throat. All incidents were observed when the turtles were located near the glass wall of the enclosure.

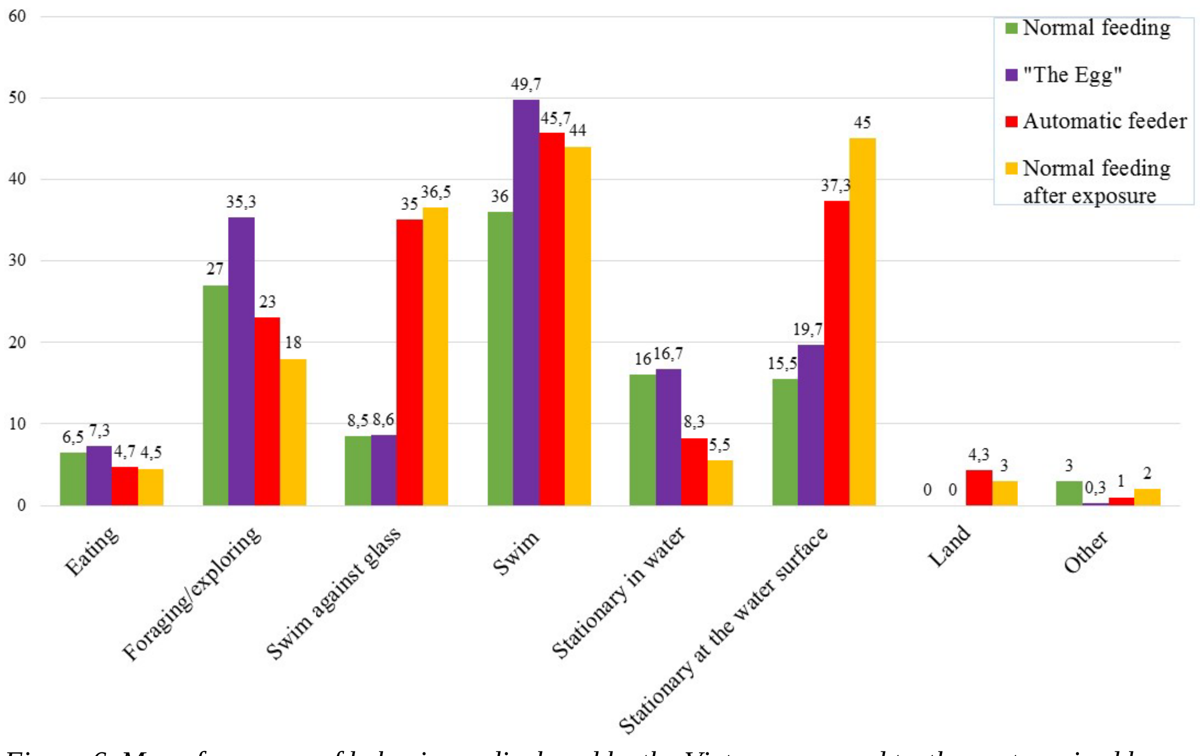

Figure 6. Mean frequency of behaviours displayed by the Vietnamese pond turtles, categorized by enrichment. The values from the behaviour “interact with “The Egg” was included in the behaviour “forage and explore”. The categories “movement on land” and “stationary on land” were combined into “land”. “Heat lamp/Basking” was included in the behaviour “other” in this figure.

A summary of duration and frequency of foraging and exploratory behaviour can be seen in figure 7. The highest values of both duration and frequency occurred when “The Egg” was used as enrichment. The shortest duration and lowest frequency of “forage/explore” occurred during normal feeding after exposure.

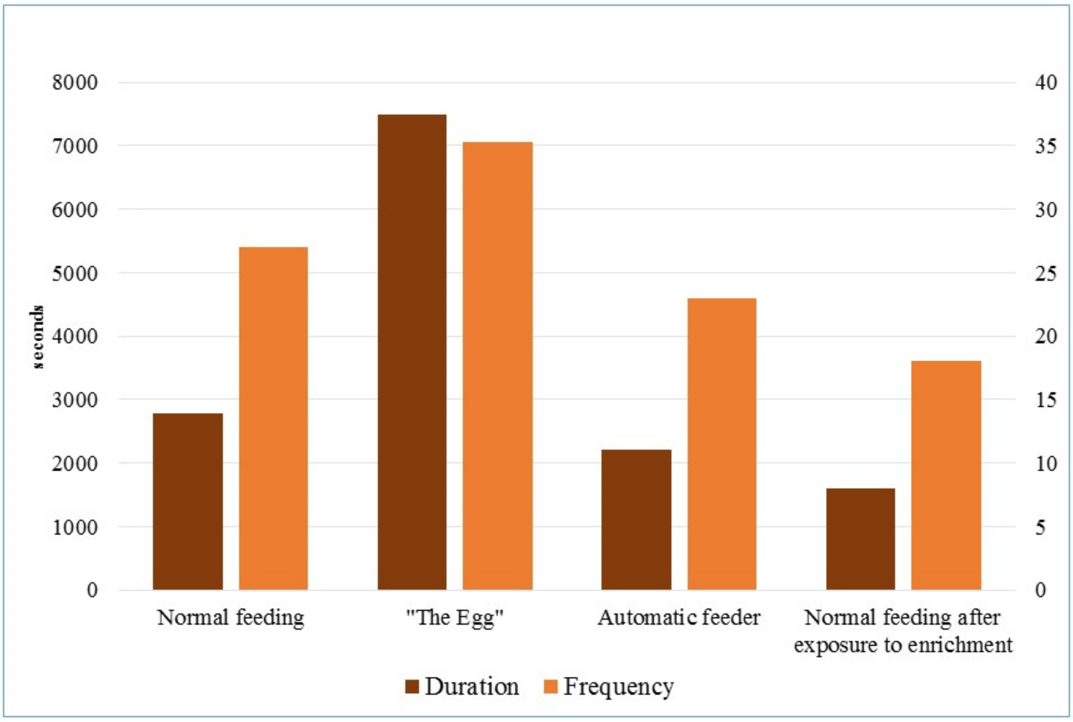

Figure 7. Summary of duration and mean frequency of foraging/exploration performed by all four individuals of Vietnamese pond turtle sorted by enrichment. The behaviour categorized as interaction with “The Egg” is included in foraging/exploration. The axis on the left describes duration of time in seconds. The axis on the right displays the mean number of times the behaviour was displayed.

When testing if there was a difference in the duration of the behaviour “swim against glass” between the different types of feeding for the group of Vietnamese pond turtles, a significant difference was found (S=8,1 and p=0.044), see figure 8. No significant differences were found when testing “eat” (S=5,7 and p=0.127) and “foraging” (S=3 p=0.392).

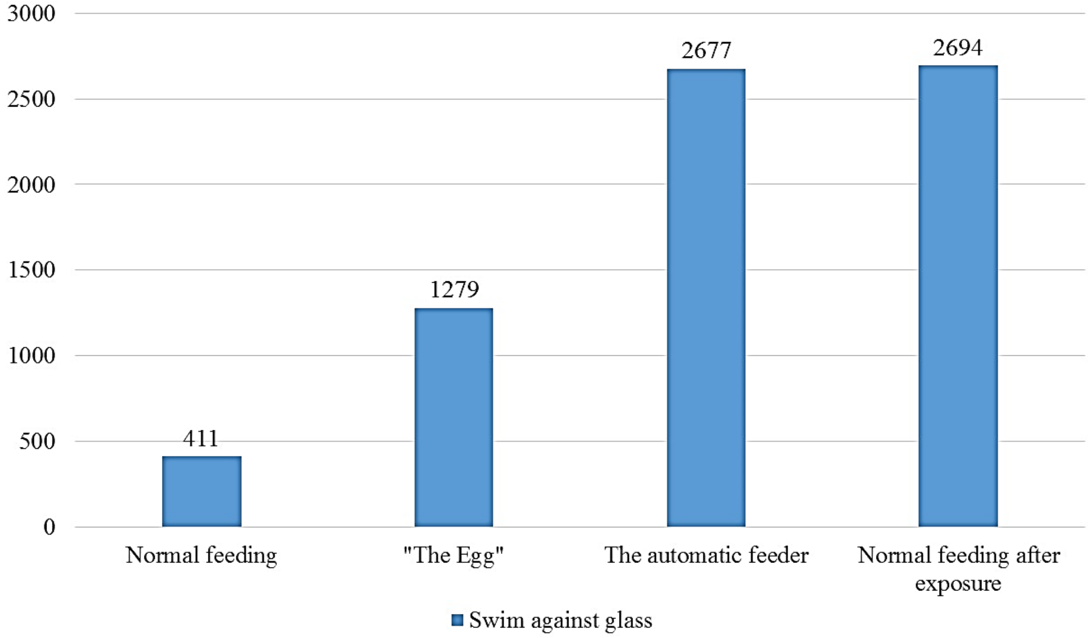

Figure 8. Medians of the duration of the behaviour "swim against glass" displayed by all four Vietnamese pond turtles sorted by enrichment. The axis represents duration of time in seconds.

The individuals behavioural change over time

An individual summary of a select number of behaviours, can be seen in table 3. The frequencies for the behaviours "touch with head/biting" and "touch with extremities" were summarised and input as the frequency of "interact with "The Egg" in table 3.

When "The Egg" was used after normal feeding, the individuals, "Blue", "Pink" and "White", decreased their eating time. "Blue", Green" and "Pink" increased their duration of "swim against glass". The change to the automatic feeder produced an increased duration of "swim against glass" for all four individuals. The durations of the total foraging and exploratory behaviours were shortened for all individuals but "White", who increased its duration. When normal feeding was conducted after exposure to enrichments, the duration and frequency of "forage/explore" decreased for the individuals "Blue", Green" and "Pink". The behaviour "swim against glass" increased in duration for "Green", "Pink" and "White.

The three individuals, "Blue", "Green" and "Pink", followed the same pattern of increased total foraging behaviour when "The Egg" was introduced and displayed a shortening of duration when the automatic feeder was used and an additional shortening of duration when normal feeding after exposure to enrichment was conducted. The longest duration of "forage/exploring" seen performed by "White" occurred when normal feeding was done after exposure to enrichment and the shortest duration occurred when "The Egg" was used.

Green" had twice as high frequency of "interact with "The Egg" as the individuals "Blue" and "Pink". The individual "White" had no note of interacting with "The Egg" physically.

The behaviour "swim against glass" was performed for the longest duration of time during normal feeding after exposure for three of the individuals. Only "Blue" had its highest duration during the automatic feeder.

Table 3. *Individual durations and mean frequencies of select behaviours displayed by the Vietnamese pond turtles.*

Individual	Behaviours	Normal feeding Week 1		"The Egg" Week 2		The automatic feeder Week 3		Normal feeding after exposure Week 4	
		Duration (s)	Frequency (n)	Duration (s)	Frequency (n)	Duration (s)	Frequency (n)	Duration (s)	Frequency (n)
"Blue"	Eat	249	1,5	105	1,7	243	1,3	108	0,5
	Forage/explore	879	8,5	416	10	679	7,7	612	6,5
	Interact with "The Egg"	-	-	1548	15,7	-	-	-	-
	Swim against glass	493	2,5	1487	1,3	3579	12	1338	5
"Green"	Eat	32	2	81	2,7	181	2,3	141	1
	Forage/explore	797	7,5	1094	11	548	7,7	398	3,5
	Interact with "The Egg"	-	-	1931	30,3	-	-	-	-
	Swim against glass	12	0,5	1388	3,3	2192	7	3694	13
"Pink"	Eat	318	2,5	138	3	337	0,7	225	1,5
	Forage/explore	800	7	466	12,7	503	2,7	116	1,5
	Interact with "The Egg"	-	-	1870	14,3	-	-	-	-
	Swim against glass	1580	5,5	2092	4	4444	15,7	5020	17,5
"White"	Eat	169	0,5	0	0	36	0,3	47	1
	Forage/explore	312	4	13	1,7	462	5	471	4,3
	Interact with "The Egg"	-	-	154	0	-	-	-	-
	Swim against glass	2	0	0	0	127	0,3	194	1

Other observations

The Vietnamese pond turtle's movements were generally perceived as faster and twitchier when enrichment was provided and during normal feeding after exposure to enrichment compared to the slower movements displayed during normal feeding in the beginning of the study.

McCord's snake-necked turtle

A summary of the video observations can be seen in figure 9. The pie charts display duration of behaviours displayed by the two individuals of McCord's snake-necked turtle when fed during this study.

The longest duration of "forage/explore" occurred when "The Egg" was used. The shortest duration of "forage/explore" was observed during feeding by the automatic feeder. The stereotypic behaviour "swim against glass" was performed for the longest duration when the automatic feeder was used. And the shortest duration of the behaviour was seen when "The Egg" was used. Swimming increased when both types of enrichment was presented, but the longest duration of was achieved when "The Egg" was used. The durations of the behaviours, "head on other carapace" and "other head on own carapace", had their longest durations when feeding was done by the automatic feeder and when normal feeding recommenced after exposure to enrichment. The shortest duration was seen when "The Egg" was in use.

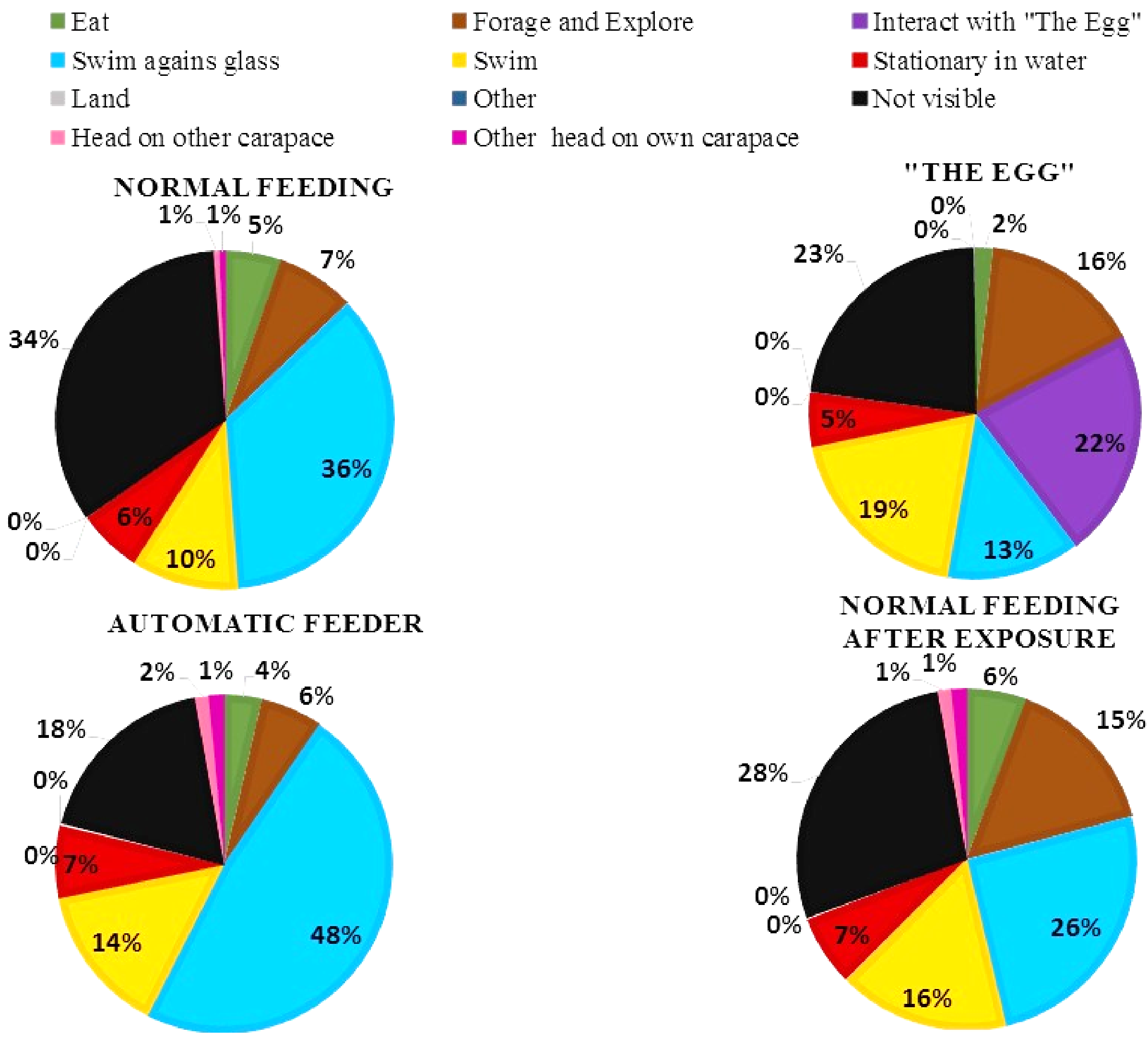

Figure 9. Summary of video analysis. Duration of behaviours displayed by the two individuals of McCord's snake-necked turtle sorted by type of enrichment. In the pie charts "The Egg", "interact with "The Egg" is displayed as a separate behaviour. When statistical calculation was made and when forage duration was compared between enrichments, the values of the behaviour "interact with "The Egg" was included in the behaviour "forage and explore". Each pie chart represents approximately four hours of video as 100 %.

Results from the direct observations of the McCord's snake-necked turtle can be seen in figure 10. The highest mean frequency of "forage/explore" occurred when "The Egg" was used. The lowest mean frequency was noted when normal feeding was conducted. "Swim against glass" had its highest mean frequency when the automatic feeder was used and its lowest when "The Egg" was used. Lowest mean frequency of "swim" occurred during normal feeding and "The Egg" had the highest. The sexual behaviour of "head on other carapace" and "other head on own carapace" had the highest mean frequencies when the turtles were fed by the automatic feeder and when normal feeding was conducted after exposure to enrichment.

How the McCord's snake-necked turtles interacted with "The Egg" was noted during the direct observations, since this behaviour could not be compared between observations, the categories "touch with head/biting" and "touch with extremities" were excluded from figure

10. The mean frequency of “touch with head/biting” was 17,7 times, “touch with extremities” was 10 times per feeding with “The Egg”.

The behaviour “heat lamp/basking” was not performed by the McCord’s snake-necked turtle and was therefore not included in the summary.

Behaviours observed and noted as “other” for the McCord’s snake-necked turtles were; yawning, biting another individual, biting on enclosure furnishing, scratching or biting on own extremities or head. One individual ventured up on land at one time during normal feeding after exposure, this resulted in a mean frequency of 0,5 for “movement on land” and 0,5 for “stationary on land”.

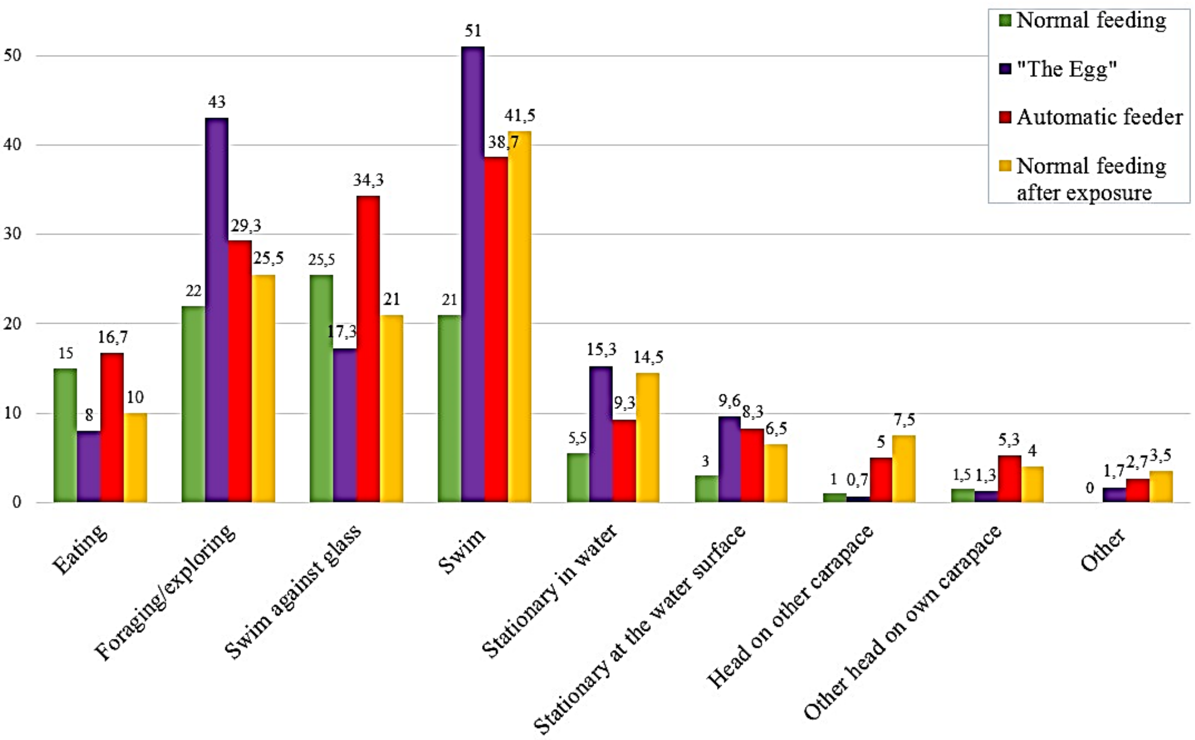

Figure 10. Mean frequency of behaviours displayed by the McCord’s snake-necked turtle categorized by type of enrichment. The values from the behaviour “interact with “The Egg” was included in the behaviour “forage and explore”. The categories “movement on land” and “stationary on land” were added to “other”.

A combination of the duration and mean frequency of the behaviour “forage/explore” is visualised in figure 11. The duration and frequency of “forage/explore” had its highest values when “The Egg” was used. The shortest duration was measured when the automatic feeder was used. The lowest frequency of “forage/explore” occurred during normal feeding.

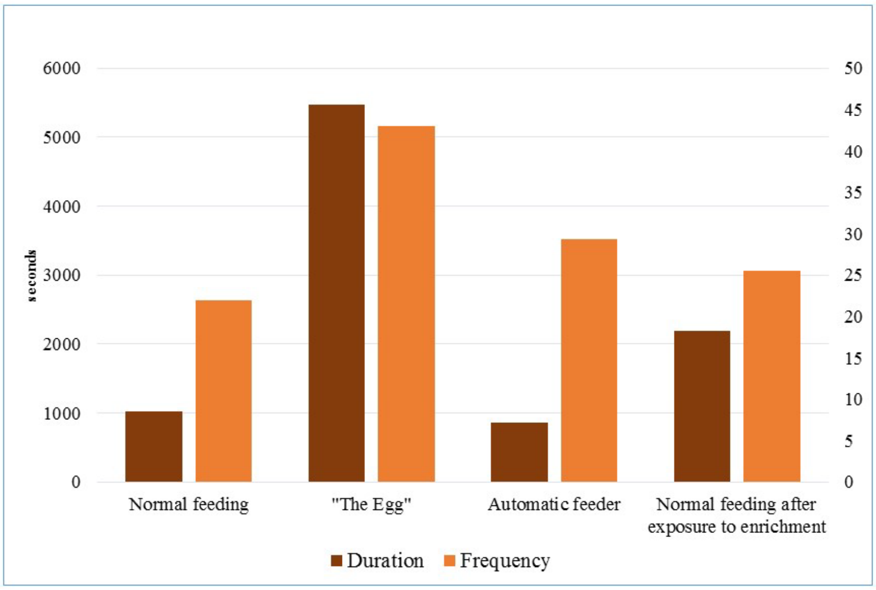

Figure 11. Summary of duration and mean frequency of forage/explore performed by the two individuals of McCord's snake-necked turtle sorted by enrichment. The behaviour categorized as interaction with "The Egg" is included as foraging/exploration. The axis on the left describes duration of time in seconds. The axis on the right displays the mean number of times the behaviour was displayed per observation and enrichment.

A summary of the duration and mean frequency of the stereotypic behaviour "swim against glass" can be seen in figure 12. The highest values of duration and mean frequency was noted when the automatic feeder was used. The shortest duration and lowest mean frequency occurred when "The Egg" was used as enrichment.

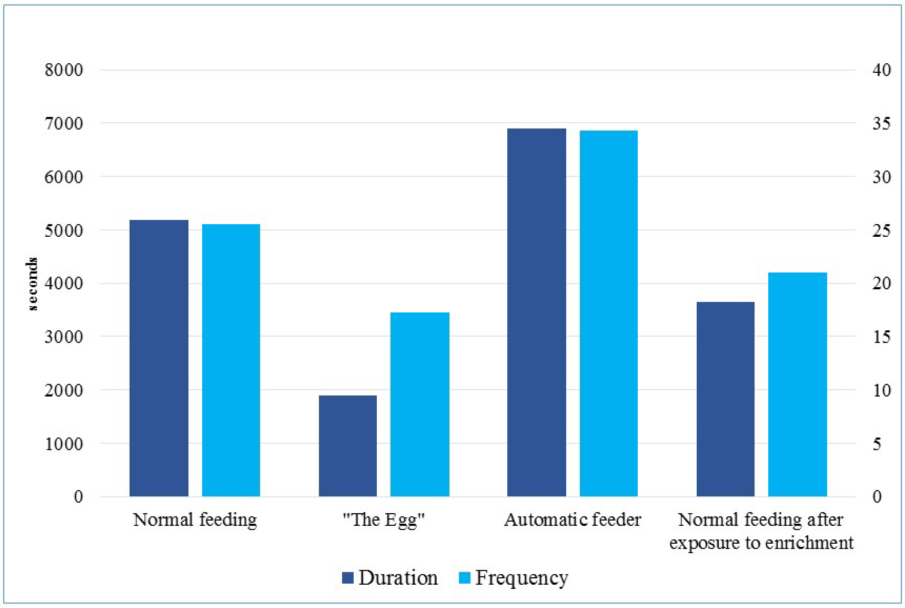

Figure 12. Summary of duration and mean frequency of "swim against glass" performed by the two individuals of McCord's snake-necked turtle sorted by enrichment. The axis on the left displays duration of time in seconds. The axis on the right displays the mean number of times the behaviour was recorded per observation and enrichment.

In figure 13, the duration and mean frequency of the sexual behaviour displayed by the two McCord's snake-necked turtles are summarised. The longest duration was measured when the automatic feeder was used. The highest mean frequency when normal feeding after exposure to enrichment was conducted. The lowest values on both duration and mean frequency occurred when “The Egg” was used.

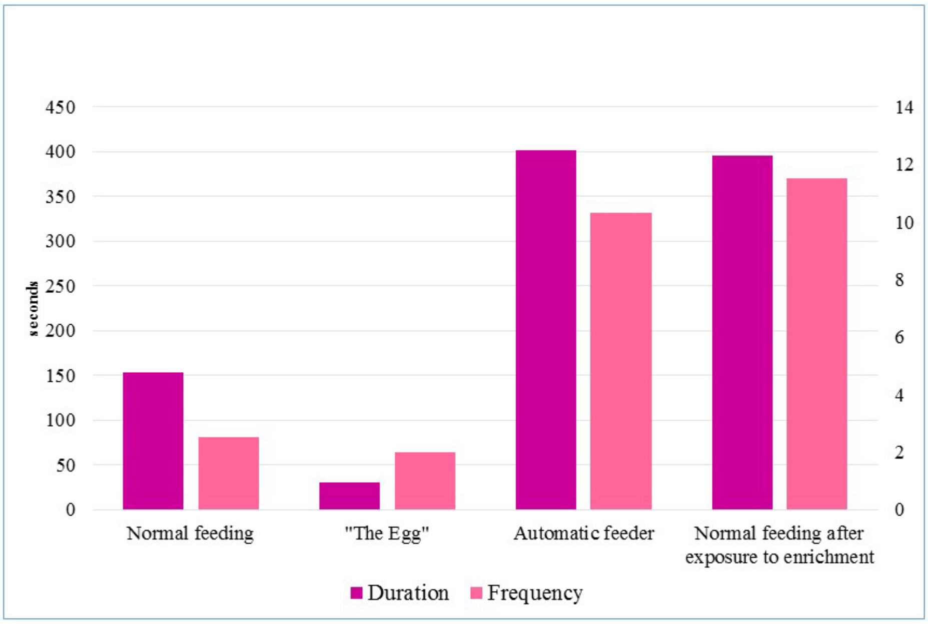

Figure 13. Summary of duration and mean frequency of “head on carapace” combined with “Other head on own carapace” performed by the two individuals of McCord’s snake-necked turtle sorted by enrichment. The axis on the left displays duration of time in seconds. The axis on the right displays the mean number of times the behaviour was recorded per observation and enrichment.

In figure 14, a comparison between the duration of the stereotypic behaviour “interact with glass”, the sexual behaviour of “head on other carapace + other head on own carapace” and “forage/explore” is visualised. When the behaviour “forage/explore” increases, the other two decrease, and when “forage/explore” decreases, the other two increase.

Figure 14. The change seen over time in the duration of “forage/exploring”, “interact with glass” and “head on other carapace + other head on own carapace” in the two individuals of McCord’s snake-

necked turtle. The values from the behaviour "interact with "The Egg" was included in the behaviour "forage and explore". Both axes display the duration in seconds. The axis on the left is connected to "forage/explore" and "interact with glass". The axis on the right is connected to "Head on other carapace + other head on own carapace".

When testing if there was a significant difference in the duration of the behaviours "swim against glass" (S=3 and p=0.392), "eat" (S=6 and p=0.112) and "forage" (S=5,4 and p=0.145) between the different types of feeding in the group of McCord's snake-necked turtles, no significant differences were found.

The individuals behavioural change over time

An individual summary of the duration and mean frequency of select behaviours can be seen in table 4. The frequencies for the behaviours "touch with head/biting" and "touch with extremities" were summarised and input as the frequency of "interact with "The Egg" in table 4.

When the automatic feeder was used after normal feeding, the duration of "swim against glass" increased for both individuals. The behaviour "forage/explore" 's duration shortened for the individual "1" and increased for "2". The introduction of "The Egg" shortened the duration of "swim against glass" for both individuals, and increased their durations of "forage/explore". After exposure to enrichment, normal feeding was conducted. This resulted in longer duration of "swim against glass", and a shortening of the total duration of foraging and exploratory behaviours for both individuals.

When looking at the behaviour "forage/explore" as a separate behaviour, "2" had its longest duration when "The Egg" was used, and "1" had its longest during normal feeding after exposure. When "interact with "The Egg" was included, both individuals had their longest duration when "The Egg" was used. The shortest duration of the behaviour was measured during the automatic feeder for "1", and during normal feeding for "2".

"1" had almost twice as long duration of "interact with "The Egg" and approximately three times as high frequency as "2".

The stereotypic behaviour "swim against glass" was performed for the longest amount of time by "1", when the automatic feeder was used, and during normal feeding after exposure by "2". The shortest duration measured for "1" was when "The Egg" was used, the shortest for "2" was during normal feeding.

Table 4. *Individual durations and mean frequencies of select behaviours displayed by the McCord's snake-necked turtles.*

Individual	Behaviours	Normal feeding Week 1		"The Egg" Week 3		The automatic feeder Week 2		Normal feeding after exposure Week 4	
		Duration (s)	Frequency (n)	Duration (s)	Frequency (n)	Duration (s)	Frequency (n)	Duration (s)	Frequency (n)
"1"	Eat	52	4,5	5	0,3	51	2,3	87	1,5
	Forage/explore	676	11,5	777	20,3	314	11,7	1510	13
	Interact with "The Egg"	-	-	2028	20	-	-	-	-
	Swim against glass	4322	21	527	5,3	5072	24,7	1464	11,5
"2"	Eat	722	10,5	241	7	466	14,3	731	8,5
	Forage/explore	406	10,5	1479	22,7	544	17,7	683	12,5
	Interact with "The Egg"	-	-	1183	7,7	-	-	-	-
	Swim against glass	872	4,5	1360	12	1835	9,7	2194	8

Other observations

The McCord's snake-necked turtles seemed to react to sounds and visual stimuli of both observer, visitors and zoo keepers by moving towards the transparent enclosure boundary and perform the behaviour "swim against glass".

The McCord's snake-necked turtles had problems with ingesting dry pellets. They were often observed trying to chew and swallow the hard pellets, but ending up spitting them out multiple times. When time had passed and the pellets were softened by the water, ingestion was done swift and with ease.

DISCUSSION

Behavioural changes in response to enrichment

Eating and foraging

Three of the four individuals of Vietnamese pond turtles and both of the McCord's snake-necked turtles had their shortest eating durations when "The Egg" was used. The turtles were not able to empty "The Egg" of all pellets during the one hour long observations, therefore a smaller amount of pellets was available. The fact that less food was attainable when the enrichment "The Egg" was used, makes a comparison between eating times during the different enrichments impracticable.

There was a big difference in eating time between the individuals. "White" and "1" were the individuals who spent the least amount of time eating in each group. The reason for this is unknown. "1" ate during a short duration compared to "2" during all types of feeding in this study. The individual "White" had the second lowest duration of "eat" during normal feeding, but the lowest during all other types of feeding. Perhaps the enrichments produced a more competitive situation not suitable for this individual. The difference in eating time was not one of the main aims of this study, but a welcome knowledge to be forwarded to the zoo keepers.

The McCord's snake-necked turtles had problems with ingesting hard pellets and were observed having to chew, spit out and re-ingest the same pellets over and over again. Though this looked difficult and frustrating, it could be seen as a positive aspect of the pelleted feed since food given to zoo animals are often too soft and more easily ingested then feed in the wild (Morgan & Tromborg 2007).

None of the behavioural enrichment studies found on turtles evaluated the change in foraging behaviour (Burghardt *et al*., 1996: Case *et al.*, 2005: Therrien *et al*., 2007). This study showed similarities with the results from the study by Bashaw *et al*., (2016) on food enrichment for leopard geckos, where foraging behaviour increased when food enrichment was present. Foraging and exploratory behaviour increased in duration for five of the six turtles in this study when "The Egg" was used compared to normal feeding (see tables 3 and 4). Only two out of six individuals had an increase in foraging behaviour when the automatic feeder was used compared with the duration when normal feeding was conducted.

Interactions with “The Egg”

The two species of turtles interacted with the enrichment “The Egg” in different ways. When looking at the results from the directs observations, it was clear that the Vietnamese pond turtles used their extremities to manipulate it for the majority of times, while the McCord’s snake-necked turtles used their heads for the most part. The Vietnamese pond turtles managed to expel pellets from “The Egg” more readily then the McCord’s snake-necked turtles. Perhaps the McCord’s snake-necked turtles would benefit from using a different type of enrichment, more sensitive to manipulation.

How and for how long the turtles explored and interacted with the enrichment, “The Egg” differed greatly between individuals (see tables 3 and 4). The individual “White” in the Vietnamese pond turtles group, interacted with “The Egg” for less than a tenth of the duration compared to the mean duration of interaction with “The Egg” the other three individuals in the group performed. The individual “2” performed interaction with “The Egg” during little more than half of the time compared to the individual “1” in the McCord’s snake-necked turtle group. These individual differences were great but not noticeable when the results were summarized and presented on a group-level. This illustrates an important aspect of the cons of interpreting summarized behavioural data.

Interactions with the automatic feeder

The McCord’s snake-necked turtles gave no impression of understanding the concept of being fed multiple times by the automatic feeder. When pellets were poured into the water, they made no notice of it that the observer could detect. The Vietnamese pond turtles were, on the other hand, quick to respond when pellets were dropped into the enclosure and hurried up to the water surface to eat.

Some of the Vietnamese pond turtles were observed swimming with their heads above the water surface. It was interpreted as foraging behaviour directed towards the automatic feeder that was mounted approximately one meter above the water surface. Perhaps some individuals learnt that the pellets were expelled from the automatic feeder or at least that the pellets fell from somewhere above the water surface. It could also explain the increased frequency of the behaviour “stationary at the water surface”, that started when the automatic feeder was used (see figure 6).

Activity levels

In this study, the duration and frequency of swimming increased for both species on a group level when enrichment was present compared to normal feeding. The level of inactivity, in this study could be quantified by looking at behaviours named “stationary”. These inactive behaviours decreased in duration for the Vietnamese pond turtles when enrichment was present. The McCord’s snake-necked turtles had similar levels of inactivity throughout the study, but a small decrease when “The Egg” was used. This study was conducted during one hour sessions, a couple of days a week, if the enrichments had effects on activity levels throughout the day is unknown. Food enrichments given to two types of captive felid species showed an increase in their activity levels and decreased stereotypic pacing that persisted for several days (Shepherdson *et al.*, 1993). To assess whether or not an increased activity level would be a positive influence on the turtles' welfare, more information about the appropriate activity level of these species is wanted (Warwick 1990).

Sexual behaviour

The behaviours "Head on other carapace" and "other head on own carapace" is interpreted as a courtship behaviour described by Murphy & Lamoreaux (1978) in the species Common snake-necked turtle, (*Chelodina longicollis)* which is a member of the same genus as the McCord's snake-necked turtle. The two individuals of McCord's snake-necked turtle who displayed these behaviours were both adult males. For the majority of incidents, the individual "1" performed the behaviour "head on other carapace" and the individual, "2", was the one being mounted.

Bailey & Zuk (2009) describes possible evolutionary reasons for same-sex sexual behaviours in their review article on the subject. The article contains examples of same-sex interactions in mammals, birds, amphibians, reptiles and insects. The behavioural phenomenon is described as wide-spread with many possible underlying evolutionary reasons. The reasons presented in the study did not contain any examples or references to the occurrence in reptilian species. By extrapolation from the evolutionary reasons found in other types of animals, a number of plausible reasons were found that could apply to the situation of the McCord's snake-necked turtles; to establish or reinforce a hierarchy, mistaken identity, males deprived of interactions with the opposite sex and maladaptation (Bailey & Zuk 2009). The theory of maladaptation being a reason for same-sex interaction is interesting since the increase in sexual behaviour displayed in this study by "1" coincided with behavioural signs of poor welfare; decreased foraging and increased stereotypic behaviour (see figure 14). The reduction in foraging could produce a frustration in the animal which could lead to displacement or redirected behaviours and stereotypies (Duncan & Wood-Gush 1972). It would suggest that the individual "1" experienced an unsatisfactory situation when the automatic feeder was used and when normal feeding after exposure to enrichment was conducted.

Aggression

One of the Vietnamese pond turtles, "Pink", displayed aggressive behaviour when the automatic feeder was used and during the normal feeding that followed after exposure to enrichment. Aggressive behaviour can be a sign of an unsatisfactory situation according to Warwick (1990). The same article states that aggressive behaviour can be reduced by the use of enrichment, this was not the case with the use of the automatic feeder in this study. The decrease in foraging behaviour experienced when the automatic feeder was introduced after "The Egg" could be a factor contributing to the unsatisfactory situation. Aggression could be a displaced behaviour in response to the motivation to perform foraging behaviours which were no longer possible when "The Egg" was taken away (Duncan & Wood-Gush 1972).

It has been shown by Schnebel & Griswald (1983), in their study on wild pigs that enrichment can become an item to defend and increase aggressive behaviour. It is conceivable that the area under the automatic feeder became an area to defend and a reason for aggression. The aggressive behaviour observed did occur near the glass wall. The impact of a defendable object would, however, lead to the assumption that “The Egg” would produce the same aggressive behaviour, which it did not. Why the aggression continued the following week when normal feeding was conducted after exposure to enrichment could be explained by all three theories; absence of enrichment (Warwick 1990), a lingering belief that the area near the glass was where more food would be delivered (Schnebel & Griswald 1983) and frustration from decrease in foraging behaviour (Duncan & Wood-Gush 1972).

Enrichments effects on stereotypic behaviour

The behaviour “swim against glass” was considered a stereotypy in this study due to the seemingly functionless, repetitive and self-damaging nature of the behaviour (for review see Mason 1991).

The two McCord’s snake-necked turtles, who had a long duration of the behaviour “swim against glass” during normal feeding, showed a shortening of the behaviour when “The Egg” was used (see table 4). Both individuals shortened their performance of the behaviour from the week before, when the automatic feeder was used, and “1” shortened it compared to normal feeding as well. The reduction in stereotypic behaviour seen when “The Egg” was used, corresponded with the reduction of stereotypic behaviour seen in other food enrichment studies (Shepherdson *et al.:* 1993 Therrien *et al.*, 2007). The automatic feeder had a negative impact on the stereotypic behaviour for both individuals who increased their time spent swimming in front of the glass.

A significant difference in the behaviour “swim against glass” was found for the Vietnamese pond turtles. From the statistical analysis it can not be determined if there was a significant difference between all types of feeding or not. But by looking at the medians, the median for normal feeding has a much smaller value than the others, and one can draw a conclusion that this type of feeding differs from the others. That means that the introduction of feeding enrichments during this study have significantly increased the duration of the behaviour “swim against glass”. A stereotypic behaviour that was not considered to be performed to a high extent or to be a problem for this group. A theory as to why the Vietnamese pond turtles increased their activity in front of the glass is the positioning of enrichments (see figure 2). The two toys, “The Eggs”, were placed in close proximity to the glass in order for them to be visible. The automatic feeder could not be placed above the centre of the enclosure as it was first planned due to practical issues, and had to be mounted on the glass wall. The positioning of the two types of enrichments caused pellets to be delivered in close proximity to the glass wall. It can be theorised that the Vietnamese pond turtles learned to swim against the glass because that was where food could be found. Speculatively, a positioning of enrichments further away from the glass wall would reduce the turtle’s interaction with the transparent boundary. Another plausible reason for the increase of the stereotypic behaviour could be due to stress (Mason 1991). The change in feeding routine could produce a stressful situation for the individuals. Observations consisting with this theory are the faster body movements displayed by the Vietnamese pond turtles when the feeding enrichments were used, which could indicate stress according to Warwick (2013). The reduction in foraging behaviour could produce a frustration in the individual that could lead to an increase in stereotypic behaviour when the automatic feeder was used (Duncan & Wood-Gush 1972). It is also conceivable that the observer, who was sitting in front of the glass wall during the observations, affected the turtle's performance of the behaviour "swim against glass", however, studies on the visitor effect on reptiles were not found.

Did the use of enrichment increase signs of good welfare for the individuals in this study?

Behavioural signs of good animal welfare can be; increased foraging behaviour (Morgan & Tromborg 2007: Izzo *et al*., 2011 see Bashaw *et al*., 2016), reduced stereotypic behaviour (Mason *et al.,* 2007: Morgan & Tromborg 2007), appropriate activity levels (Morgan & Tromborg 2007: Warwick 1990), increased behavioural diversity (Morgan & Tromborg 2007), reduced aggression and reduced persecution of cage mates (Morgan & Tromborg 2007 : Warwick 1990) as well as unhurried relaxed body movements (Warwick 2013).

Since the observations in this study were conducted during a limited period during the day, an evaluation of the behavioural diversity and activity levels was considered unfit for the data collected.

Table 5 and 6 contains overviews of the effects enrichments had on behaviours that could be used as welfare indicators. The individuals "2", "Blue" and "Green" got marks in the category "increased sexual behaviour, aggression, or persecution" since they were the individuals being attacked and persecuted. The duration of the behaviour "interact with "The Egg" was included in the foraging behaviour.

It can be seen in the tables 5 and 6 that the McCord's snake-necked turtles displayed solely behaviour indicative of good welfare when "The Egg" was used as enrichment. The results from the individuals of Vietnamese pond turtles were not quite as uniform. Three of the individuals performed increased foraging behaviours but the stereotypic swimming in front of the glass increased as well. The automatic feeder provided solely behaviours indicative of poor welfare for one of the McCord's snake-necked turtles and three of the Vietnamese pond turtles.

Table 5. *Individual behavioural changes in duration seen when "The Egg" was used compared to the week before. "NO" stands for "no occurrence" it means that the behaviour was not performed neither on the week before or during the week the enrichment was used.*

"The Egg"	Indicators of positive welfare			Indicators of poor welfare		
Individual	Increased foraging	Decreased stereotypic behaviour	Decreased sexual behaviour, aggression or persecution	Decreased foraging	Increased stereotypic behaviour	Increased sexual behaviour, aggression or persecution
"1"	x	x	x			
"2"	x	x	x			
"Blue"	x		NO		x	NO
"Green"	x		NO		x	NO
"Pink"	x		NO		x	NO
"White"		x	NO	x		NO

Table 6. *Individual behavioural changes in duration seen when the automatic feeder was used compared to the week before. "NO" stands for "no occurrence" it means that the behaviour was not performed neither on the week before or during the week the enrichment was used.*

Automatic feeder	Indicators of positive welfare			Indicators of poor welfare		
Individual	Increased foraging	Decreased stereotypic behaviour	Decreased sexual behaviour, aggression or persecution	Decreased foraging	Increased stereotypic behaviour	Increased sexual behaviour, aggression or persecution
"1"				x	x	x
"2"	x				x	x
"Blue"				x	x	x
"Green"				x	x	x
"Pink"				x	x	x
"White"	x		NO		x	NO

Signs of stress were evident in the group of Vietnamese pond turtles when food enrichments were used. All four individuals displayed an increase in their interaction with the transparent enclosure wall and displayed faster body movements. One individuals displayed aggression. These behaviours could be interpreted as signs of stress according to Warwick (2013).

Reduced opportunities to perform foraging behaviours may be stressful to the animal and produce redirected behaviours and stereotypies (Duncan & Wood-Gush 1972: Morgan and Tromborg 2007). This argument is supported by the behaviours indicative of stress or discomfort that was displayed by several individuals in this study when foraging time was reduced. As described in earlier passages in this discussion, figure 14 shows the connection between foraging, stereotypic behaviour and sexual behaviours performed by the McCord's snake-necked turtles. The behaviours; “swim against glass” and the same-sex sexual behaviours “head on other carapace” and “other head on own carapace”, can be seen as behaviours associated to stress and poor welfare while foraging an indicator of good welfare (Bailey & Zuk 2009: Mason *et al.*, 2007: Morgan & Tromborg 2007). From this, an assessment could be made that “The Egg” had a positive impact on animal welfare for the McCord’s snake-necked turtles while the automatic feeder had a negative impact. Aggressive behaviour and “swim in front of glass” displayed by the Vietnamese pond turtle “Pink”, increased when the individual experienced a decrease in foraging behaviour when the automatic feeder was used. These behavioural changes would suggest that the welfare for this individual, and the individuals subjected to the aggressive behaviours, were reduced when the automatic feeder was used as enrichment.

This study provides a good example of the variation in assessed welfare described by Hosey *et al.,* (2013a), between individuals exposed to the same stimuli. Hosey *et al.*, (2013b) describes the needs of an individual to depend on factors like; age, sex, social rank, size and temperament. The groups in this study were quite homogenous. They consisted of siblings of the same sex who had been subjected to the same upbringing, and who were roughly of the same size. Factors like social rank and temperament, or personality, persisted as sources of differences in individual needs. Mehrkam & Dorey (2014) conducted a preference test between enrichments provided to Galapagos tortoises. It was found that individuals of the same species who had had identical development histories had different enrichment preferences. Furthermore, the individual differences continued when looking at the enrichment’s efficacy in promoting behavioural changes. The authors recommended that these individual differences were taken into account when implementing new enrichment strategies in a group of animals. And that the enrichment was evaluated not only on a species level, but at an individual level. The results of the difference in behavioural changes between the individuals of the same group in this study supports these conclusions.

Method discussion

The method of using direct continuous observation on focal animals, is described in a text by Altmann (1974), as being a common method of choice in behavioural observations and that the method can provide relatively unbiased data when used properly. It is a technique that has been used in field situations on a vast variety of animal species (see Altmann 1974 for review). This study was conducted by one person who did all observations, interpretations of behaviours and processing of data. As always, there is a risk of errors due to the human factor. Altmann (1974) addresses the issue of observer fatigue. It is suggested by the author that the upper time limit of each session should be limited by the observer’s capability of focusing on the task. The duration of time that an observer can stay focused before fatigue arises, is influenced for example by the observer’s familiarity of the species and the number of behaviours to be noted. The method used during the direct observations, taking notes manually with pen and paper, required a focused and swift observed. During note-taking, observation of the turtles could not be made, and some time loss was inevitable. The location where observations were made was not secluded. The observer sat in front of the currently observed enclosure and shared the exhibit with working zoo keepers and visitors of the park.

Enthusiastic visitors occluded the observer's view a couple of times. Focus was lost during short periods of time due to external distractions, the most frequent reason being questions about the exhibit by visitors.

The 30-minute time restriction of video recordings made a restart of the video camera mid-way through each observation necessary. This produced a short interruption of focus and a small shift of the second half of the observation. This resulted in the direct observation and video observation not ending simultaneously. In addition, the video camera was sometimes blocked by visitors which contributed to the duration of the behaviour "not visible".

Few animals were included in the study, and no control group existed. All female and all male groups were studied, and each species was therefore represented by one sex. Due to lack of data on the natural behaviour of these two species, the behavioural changes the animals displayed when exposed to enrichment was compared to the behaviour displayed during normal feeding or the type feeding conducted during the week before. These conditions limit the applications of the results on a larger population. The method of using the animals as their own control, does not make it possible to account for external factors affecting the turtle's behaviour over time. Examples of such external factors could be the observer effect and the shift to an unchanged diet over a long period of time.

A factor that affected the duration of "not visible" for the McCord's snake-necked turtles were the variation of the content of plants and branches as furnishing in enclosure. When more vegetation was introduced in the enclosure, less visibility was obtained.

Visitors and zoo keepers were observed to influence the behaviour of the McCord's snake-necked turtle. During the early observation time, 9.00 am, zoo keepers still worked and walked around in the amphibian exhibit. During the later staring time, 10:30 am, visitors had started to view the animals. The conditions of this human influence was not constant and the effects on the behaviours was not included in this study. The impact of the observer on the behaviour displays of the turtles is an important aspect to consider. The observer was sitting in front of the glass wall, and the McCord's snake-necked turtles had been reported, before the start of the study, to swim against the glass when people moved near the enclosure. How the observer affected the turtles, and if the turtles learned to ignore the observer was not evaluated in this study.

The placement of enrichments in the Vietnamese pond turtle's enclosure have been discussed earlier in this section. It is conceivable that the close proximity to the glass wall generated a higher frequency of the behaviour "swim against glass". It would have been more ideal if the enrichment were placed more to the centre of the enclosure as it was for the McCord's snake-necked turtles.

It is suggested, in a study of the visual wavelength discrimination of a marine species of turtle (Young *et al.*, 2012), that the purple "Egg" might not have been ideally coloured. In their study of the Loggerhead turtle, they found a lower association between food and the colour blue, compared to other colours. It would be interesting to see if the same difference in colour preference is seen in these fresh water turtles and if the colour of the enrichment affects the turtles' utilization of it.

CONCLUSIONS

In this study, the stereotypic behaviour "swim against glass", performed by the group of Vietnamese pond turtles, was significantly impacted by the use of food enrichment. No more statistically significant results were found, but numeric increases and decreases were presented.

" The Egg" reduced the duration of stereotypic behaviour, as well as sexual persecution, displayed by the group of McCord's snake-necked turtles. Both groups of turtles displayed more foraging and exploratory behaviour with "The Egg" compared to the other types of feeding. In summary, "The Egg" produced behavioural changes indicative of increased welfare for the majority of the individuals.

The automatic feeder numerically increased the stereotypic interaction with the glass wall and shortened the duration of foraging behaviour for both species in general. During feeding with this enrichment, sexual behaviour displayed by the group of McCord's snake-necked turtles increased and aggressive behaviour were displayed by a Vietnamese pond turtle. On an individual level, the majority of turtles displayed behavioural changes indicative of a decreased welfare when the automatic feeder was used.

Though this study was small and lacked statistically significant results for most of the behavioural changes, the result can be seen as an indication that some individuals of these two species of turtle could benefit from being provided with feeding enrichment such as "The Egg".

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