Towards a remote animal tagging method with minimal detriment; how do animals react to new constructions in their environment?

Victoria Thomas.

Submitted to Swansea University in fulfilment of the requirements for the Degree of MRes Biosciences.

Swansea University, 2022.

Abstract

Tags that researchers attach to animals are equipped with a variety of sensory systems which allow us to quantify the ultra-fine scale movement of animals. Such technology aids conservation efforts by providing evidence-based data on the causal drivers behind animal space use. Yet, the attachment of tags sometimes requires individuals to be captured or restrained, which can cause acute stress and unusual movement patterns. To circumvent these issues, we are developing a remote tagging apparatus (TA), consisting of a gateway which deploys a tag when an animal walks underneath. A first step in investigating the feasibility of this tagging system in the wild is to investigate how animals react to a TA in their environment. Here, I deployed TAs at 22 field sites around Swansea, South Wales, and monitored the reactions of animals to them using camera traps. I focused on terrestrial carnivores (particularly foxes (*Vulpes vulpes*), otters (*Lutra lutra)* and badgers (*Meles meles*), but also recorded the reactions of other mammals and birds to the gateways. I aimed to examine (1) how animals react to the gateway, (2) whether animals showed neophobia to the gateway, and if this decreased over time, (3) how the siting of the gateway influenced whether animals were more likely to walk under it, and (4) whether baiting around the gateway increased the number of individuals that visited the site. I demonstrate that animals initially show neophobia of the gateway, but this decreases over time. Habitat specifics (i.e. vegetation cover and the density of the animal path/trail) made no difference in the number of times animals walked under the gateway, whereas baiting the gateway significantly increased number of animals visiting the site. These findings imply that remote tagging is possible but neophobia must be taken into account. Whilst still in the developmental stage, this remote tagging project shows potential for future studies wishing to tag animals without capturing or restraining them.

Lay summary

Researchers attach tags to animals via glue, collars, and tape to monitor their movement. Movement in animals alone can aid conservation efforts, as it allows us to see how the animal uses the space, where it may go in response to climate change and how it reacts to disturbances such as traffic. Yet, despite the incredible utility and importance of these tags in conservation, the way in which tags are attached can be stressful to the animal. As a result, data provided by the tags does not always represent normal animal movement, which then in turn affects conservation efforts. To combat this, a new tagging apparatus (TA) has been designed which remotely drops a tag onto the animal as it walks through. The TA models were left at fieldwork sites (22) to investigate how target animals, e.g. foxes (*Vulpes vulpes*), otters (*Lutra lutra*) and badgers (*Meles meles*), reacted to new object in their environment; if they feared the TA, and whether that lessened over time, and finally if baiting the area around the TA increased the number of animals that visited the site. After deploying the TA and camera system in sites across Swansea for five weeks it was found that throughout, animals did initially show fear when approaching the TA (by pausing and sniffing toward the TA). However, this decreased over time which also correlated with more instances of animals walking through the TA. Next, it was important to see whether habitat specifics such as vegetation cover affected the likelihood of animals walking through the apparatus (which it did not). Finally, bait was placed around the apparatus to see if this enhanced the number of animals that visited the site. The bait significantly increased the number of animals visiting the site per night. Investigating how animals react to new objects in their environment and how we can place the object or include bait to coax the correct animal to the apparatus is a vital first step in the remote tagging project. By understanding these elements, we can tailor the design of the apparatus to suit tagging rare or endangered animals. Whilst still in the developmental stage, the remote tagging approach shows huge potential for future studies wishing to tag animals without the associated stress from capture, which will enable collection of more precise data, and therefore successful conservation efforts.

Declarations

I declare that this work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

I declare that this thesis is the result of my own investigations, except where otherwise stated, and that other sources are acknowledged.

I give consent for my thesis, if accepted, to be made available online in Swansea University's Open Access Repository and for inter-library loan, and for the title and summary to be made available to outside organisations.

I declare the University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed: Victoria Thomas

Date: 06/01/2024

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed: Victoria Thomas
Date: 06/01/2024

I hereby give consent for my thesis, if accepted, to be available for electronic sharing
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The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.
Signed: Victoria Thomas
Date: 06/01/2024

Statement of Expenditure

Victoria Thomas

[redacted]

For the MRes project "Towards a remote animal tagging method with minimal detriment; how do animals react to new constructions in their environment?"

Category	Item	Description	Cost
Materials	Cameras	Wechamp 20MP 1080P HD trail cameras	£300
Materials		nium Aluminium to build version II of	£200

		the TA	
Total			£500

I hereby certify that the above information is true and correct to the best of my knowledge.

Signature: [redacted]

Signature: Victoria Thomas

Statement of contributions

Contributor Role	Persons involved
Conceptualization	RW, HN, VT
Data Curation	RW, HN, JR, VT
Formal Analysis	HN, VT
Funding Acquisition	N/A
Investigation	VT
Methodology	HN, VT
Project Administration	RW, HN, VT
Resources	RW, JR
Software	HN, JR

Supervision	RW, HN
Validation	RW, HN, JR
Visualization	VT, RW, HN
Writing - Original Draft Preparation	VT
Writing - Reviews & Editing	VT, RW, HN

Ethics approval and amendment confirmation

Approval number:

SU-Ethics-Student-[redacted]

Applicant Name: Victoria Thomas

Submitted by: Rebecca Stringwell

Full application details can be found in College Animal vertebrate review form .

Having examined the information included in the above application with Reference No. STU_BIOL_203625_170222114451_2, this Committee has decided to:

☑ **Approve this application**

with the following reputation risk to the University

☑ Low Risk ☐ Moderate Risk ☐ High Risk

Any amendments to approved proposals should be emailed to College Ethics Committee for review: cosethics@swan.ac.uk

☐ **Reject this application and allow for resubmission provided the ethical issues raised by the College Ethics Committee/AWERB Group below are addressed**

☐ **Return for minor amendment/clarification (please resubmit using the 'Resubmit minor amendment' option for a quick turnaround for approval)**

Comments:

The FSE ethics committee approve this application but request that modifications to the design or approach are submitted as amendments to [redacted] for consideration by the committee (16/03/2022):

*********** REVIEWER 1 - 09/03/2022 Recommendation: APPROVED (Low Risk)***********

Amendment regarding material change from wood to aluminium and the attachment of servo-motors for a later study

RS Rebecca Stringwell
To: THOMAS V. [redacted]

Tue 5/3/2022 11:21 AM

Hi Victoria,

I can approve that simple amendment and will add the approval to your original application.

With regards to not receiving the link – I can see that you have begun editing it so clearly found it 😊

Many thanks

Becky

...

Copy of fieldwork risk assessment conducted prior to beginning the project.

Risk Assessment Outcome:

Risk Rating: Negligible/Low risk **Submitted Date:** 16 Mar 2022
Approved Date: 16 Mar 2022 **Approved by:** Hazel Nichols

Student Details

Student Number:	[redacted]	**Project Supervisor:**	Dr Hazel Nichols
Course:	Biosciences	**Level:**	7

Assessor:	Victoria Thomas	**Assessment Date:**	16/03/2022
Contact Number:	[redacted]	**No. of Participants:**	4
Next of Kin:	[redacted]	**Next of Kin Contact Number:**	[redacted]

Name of field assistant(s) – Lone working is only permitted in exceptional circumstances, with the agreement of your Supervisor.

Izzy Stuart [redacted] Leah Walsh [redacted] Jack Williams [redacted]

Brief outline of the research / fieldwork activity*

Animals can be tagged to track animal movement and thus behaviour, which can provide key information for conservation and welfare studies. Such methods however, generally require the study animal to be caught and restrained and/or sedated (which, in extreme cases, can cause fatalities). Capturing an animal for tagging disturbs it and can result in atypical behaviours. The newly developed Japanese Gate (JG) is an automatic remote tagging apparatus, designed to mitigate negative behaviours associated with restraint/sedation. This project will assess whether and how wild animals approach these gates (although this project DOES NOT involve the deployment of any tags). The model (piccture attached - made from wood and aprox 42cm, heigh and 40cm wide) will be placed in Oxwich estate, Dr Nichols' garden, Dr Wilson's garden and Three Crosses farm. Camera traps will then be placed no more than 30cm away to record how, if any, animals interact with the apparatus. There will be three stages, with the appartus being placed in the habitat first, followed by the apparatus being baited, finally with the apparatus being both baited and having a small servo-motor attached to mimick the sound of deployment which will occur in real life.

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Acknowledgements

I give special thanks to James Williams, Dr Nichols, Prof Wilson, Mr Nick Adair alongside Ms Pippa Hardman and her team at Oxwich Nature Reserve (NRW). Permission to access your lands and sites has been invaluable to both my project and the wider studies that will be a part of the 'remote tagging of animals' series.

Thank you.

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Abbreviations

TA – Tag apparatus
TD – Tag dispenser
WTG – Walked through tag applicator

Definitions

Active interaction

Even if the animal did not go through the TA but exhibited behaviours such as sniffing, pausing, looking at the gate, was considered an active interaction and was detailed Y. If any animal moved rapidly through the TA without apparent interaction, it was detailed as N.

Attraction

A – Attracted, the animal looks/ pauses/ sniffs at the TA, and continues to approach the TA without backing away or flinching. 'Attracted' is also selected if the animal touches the TA with its snout when investigating it, climbs the TA, or perches upon the TA I – Indifferent, the animal passes by or under the TA without seeming to look/ pause or sniff without slowing its gait or changing direction.

R – Repulsed, selected when the animal looks/ pauses or sniffs toward the TA and freezes and backs away. Category also selected when the animal flinches when the TA first comes in to view and if it slinks back away from the TA by lowering its back when reversing, without taking its eyes off the TA.

Camera angle

F – In front of gate.

S – To the side of the gate.

Camera distance

1 = <1.5m.

2 = >1.5m.

Direction

The direction of the animal whilst walking to the TA:

D – Direct (directly walking to the TA).

O – Oblique (the animal approaches the TA from a sideways or diagonal direction)

Looks

The number of times the animal looks at the TA during the interaction (fig 4a).

Maximum plant height

Recording of the tallest plant/ shrub species taken from 25cm x 25cm quadrat placed over the TA.

Path density

1 – No path visible.

2 – Faint path wherein the ground beneath may have a slight imprint of an animal travelling

across, visible by grass species/lichen/reeds being comparatively more flattened than the flora surrounding.

3 – Well used track clearly visible by a trail of bare earth whilst the surrounding vegetation is growing uninterrupted nor trampled upon.

Pauses

The number of times the animal pauses at the TA during the interaction which is considered to be when the animal approaches the TA and stops moving (fig4b).

Site openness

1 – Very enclosed space, where it is obligatory for the animal to walk through the TA to get to the location on the other side. An example is a thicket with only one passageway, blocked on either side by impassable bramble species.

2- Enclosed, but no obligatory passage as in '1'. Avoidance of the TA necessitated a substantial deviation to progress, generally by finding a climbable or passable route around the TA.

3 – Fairly open, ground flora species are those of differing heights but there are many trails in the site.

4 – Open in a big space such as a managed field.

Sniffs

The number of times the animal sniffs at the TA during the interaction which is sighted by the movement of the snout and body as the animal breathes in and out to scent the habitat (fig 3a&b).

Species

Animal species observed in the video recording. Due to the quality of the camera recordings, species were easy to identify, aside from some bird species. To identify bird species, a handbook by Holden & Gregory (2021) was used.

Target species

Only the animals with a similar height to the TA were studied in-depth to ensure the investigation into how wild animals react to novel objects was standardized. These target species were badgers, foxes (*Vulpes vulpes*) and otters (*Lutra lutra).*

Taxonomic grouping

Individual animals were grouped into; large Mustelidae, Rodentia, Aves, domesticated animals, Canidae, Sciuridae and Erinaceidae

Time/ Seconds

The length of the entire interaction, to include the start time at which the animal sees/ sniffs or approaches the TA, to when the animal leaves the TA or stops investigating.

Vegetation %

Percentage of ground flora in the quadrat.

WTG

Whether the individual walked/ travelled through the TA, yes or no.

Towards a remote animal tagging method with minimal detriment; how do animals react to new constructions in their environment?

Introduction

Tags to track animals have been attached since the 1960s, after their development to monitor terrestrial wildlife in Yellowstone National Park (Craighead, 1982). There have since been distinct improvements in animal-tagging methods, with respect to size, sensors and volume of information. This has in turn transformed our understanding of wild animal behaviour and ecology, also providing a powerful tool to evaluate animal physiology and inform conservation (Holton, et al., 2021). Tags that researchers attach to animals today can be equipped with a wide variety of sensory systems, including accelerometers (Lopez et al., 2016), magnetometers (Williams et al., 2017), pressure (Carreno-Munoz et al., 2022), temperature (Baida et al., 2021) and light sensors (Whitford & Klimley 2019) as well as GPS (global positioning system which records animal locations with a high temporal frequency) (Handcock et al., 2009) and VHF (very high frequency) (Thomas et al., 2011) (Coulombe et al., 2009) or acoustic telemetry (Hughey et al., 2018). Such sensors are, today, even being used to quantify the energetics, gait adjustment and ultra fine-scale movement of animals (Gunner et al., 2021). Movement is an essential component of animal survival as it enables the acquisition of food, influences community dynamism and composition and enables reproduction (Williams et al., 2020). Thus, the data acquired by using tags can provide valuable information on ecological and evolutionary processes, in addition to aiding animal conservation and conservation policy (Bograd et al., 2010). Within conservation, tag use generally has four applications; quantifying disturbance, evaluating the effect of environmental and climate change, estimating energy expenditure, and investigating habitat use (Wilson et al., 2015). Given that conservation resources are limited, the use of tags can particularly further conservation efforts by providing precise, cost-efficient data which aids researchers to identify the causal drivers behind alterations in animal movement and behaviour. This helps ensure that funding benefits wildlife through evidence-based management.

The value of tags on animals can, however, be tempered by the process of tag attachment. Indeed, the manner in which tags are attached to carrier animals is highly varied, with

attached systems ranging from inter-muscular harpoons in some fish (Jepson et al., 2015), glue on marine mammals and reptiles (Johnson 2005), collars on terrestrial mammals (Santos et al., 2021), to tape attached to feathers in birds (Fijn et al., 2012). A consequence of the way in which tags are put on animals is that many systems require that the individual be captured and restrained, which can have behavioural, physiological and psychological consequences (Hawkins 2004; Nussberger & Ingold, 2006). At its simplest, the capture procedure involves the single, instant seizure of the animal by a researcher, such as capturing a bird at its nest (Seward et al., 2020) but methods can extend to walk-in traps (Schutz et al., 2006) and many forms of tag attachment involve sedating the animal for the safety of both the animal and researcher (Horning et al., 2019). There are many problems that occur when an animal is captured for tagging. First, traps are novel objects, and as such, may capture either the least risk-aversive or least healthy animals, which may not be representative of the general population (Stryjek et al., 2019). Second, tagging may directly impact on space use (Jung et al., 2019) as animals may be unlikely to visit the site of capture as readily as they do before capture if they relate the site to stress Stryjek et al., 2019). Finally, restraint and capture are stressful events, which can even cause posttraumatic stress disorder in animals, as seen in wolves in Mallonee & Joslin's (2010) study. This may result in unusual behaviour post tagging, which could prove problematic if the animal-attached tags are deployed to provide information on undisturbed animals because the data received is non-random, so could lead to false conclusions (Stryjek et al., 2019). In extreme cases (particularly if translocation is required post-tagging) capture myopathy can occur (defined as a malignant consequence of stress during animal capture) which contributes to high mortality rates (Breed et al., 2019). Indeed, Stabach's et al., (2020) found that short term effects of GPS collars and tri-axial accelerometers placed upon scimitar-horned oryx (*Oryx dammah*) resulted in aberrant behaviour that lasted several hours to several days, with significant increases of headshaking combined with elevated faecal glucocorticoid metabolites, indicating acute stress. Many tagged animals may therefore behave abnormally for some time after capture.

Still, capturing methods have improved throughout time, with researchers taking steps to minimise discomfort by ensuring species-specific placing of tags for comfort, for example on the second vertebral structure on juvenile loggerhead turtles (Snape et al., 2019). Tagging studies have also reduced stress and mortality through ensuring every attempt is made to release the subject animal at the same location in which it was captured (Horning et al., 2019). Automatic tagging efforts may be able to further reduce the impacts of tagging through

avoiding the need for capture, restraint or the presence of humans in the immediate environment. There have so far been two recorded trials of automatically tagging animals. Stryjek's et al., (2019) saw the development of an electronically triggered live trap. The mechanism used throughout the trial was based on rodents brushing a hanging live-trap. Following the trigger, individuals were covered with a transparent "sheet" that dropped to prevent the animal from fleeing. The animal itself could then be transported to the laboratory for mark-and-release. Whilst this method is not entirely remote (the eventual mark-and release following capture was not remote), it does remove the need for initial human interaction and greatly decreases non-random sampling. Otherwise, a remote tag applicator was designed by Wiart & Spady (2011) to tag cattle in effort to comply with US farming legislation, that advises all animals must be tagged with a radio frequency identification (Charlebois et al., 2014). Despite the efficacy, the design itself centres around the jaws of the tag applicator piercing through the desired part of the animal. Both of these projects provide significant advances in remote-animal ‘tagging’, but neither is suitable for a project which seeks to limit wildlife stress and human interaction during tag deployment.

My project aims to work towards developing tagging methods that avoid capture and restraint of animals to reduce stress during a tagging event. The design of the current project (the ‘bur tagging’ approach) centres around a tag dispenser (placed in the areas used regularly by wild animals), being set to release specifically designed “Alice tags” which attach themselves to

the study individual as it passes under the dispenser. Tags are attached using the same mechanism as vegetation burs, which are seeds or dry fruits with hooks or teeth (Bansal & Sen, 1981), hence the name ‘bur-tagging’. Whilst the method in which tags are deployed onto the animal are new, the design of the tagging apparatus (hereafter TA) itself partially resembles a standard trap. For example, cage traps used for badgers (*Meles meles*) in the United Kingdom have set dimensions (1000 mm long, 350 < mm wide, and 350 mm high) (Natural England, 2022), which is similar to the tagging apparatus models used during the fieldwork stage of this project (see ‘Methods’ for exact dimensions). The most apparent difference between the standardized traps used by Natural England and the models used in this study is that my models do not have the gauge mesh attached to the arms of the object to truly trap any wildlife. Still, animals have to travel under the TA structure, which is similar to badgers walking through a trap. As these objects are alike, legislation and standard operating procedures written by the Department for Environment, Food & Rural Affairs (DEFRA, 2022) were used as guidance when placing the TA at sites the expectation that results

regarding animal reactions during this stage may be similar to those published by DEFRA (see methods for exact guidance). So whilst being in a developmental stage, this project uses current guidelines outlined by environmental agencies in the UK to ensure tagging will be effective when the real-life apparatus is used in later trials.

An important factor outside of the design of the TA and guidance on trap placement, which may affect the efficacy of the remote tagging project is animal personality. The concept of animal personality was first applied to animals in the late 1930s (Gosling, 2001) and is defined as consistent behavioural and physiological differences across time and different contexts between individuals of the same species (Azevedo & Young, 2021). When discussing animal personality, the traits typically referred to are: activity (the tendency of an animal to move through a landscape), shyness/ boldness (the response of the animal when reacting to potentially risky events), aggressiveness (the exhibiting of antagonistic behaviours towards individuals), exploration (the act of collecting information in a novel situation), and sociability (the propensity of interacting with conspecifics) (Majenlantle et al., 2022). All of these traits are thought to be affected by life history traits, which include fecundity, age, size of the animal, growth pattern and longevity (Brown & Choe, 2019). Understandably, animal personality is therefore likely to be a factor which will influence individual reactions to the tagging apparatus. Whilst animal personality was not measured in the remit of this project, species-level differences are addressed.

Indeed, one such behaviour considered at the species level is neophobia, understood as fear of novel stimuli (Crane et al., 2019). This term refers to a behavioural mechanism which reduces exposure to danger in individuals by limiting the costs associated with ecological uncertainty (Moretti et al., 2015; Elvidge et al., 2016). In the context of my study, neophobia includes aversion to novel objects (specifically the TA) which could potentially aid survival by encouraging vigilance when investigating unfamiliar objects which may pose danger. Whilst individuals within the same population will differ widely in how they cope with environmental changes, including novel objects (Gibelli & Dubois, 2017), it is evident that time plays a key role in reducing neophobic responses (Crane & Ferrari, 2017). Further, time may also be used as a proxy for how long it may take a species to habituate to a novel object. For example, mice (*Mus musculus*) can be habituated to a novel object within 1 day due to their innate preference for novelty (Lueptow 2017). In contrast, habituation to a human observer in pigtailed macaques (*Macaca leonine*) takes nearly 13 months (Gazagne et al., 2020). Whilst a human observer is, of course, very different to an inanimate object, it is

apparent that factors such personality, home range and fission-fusion dynamics affected macaque habituation in the study by Gazagne et al., (2020). In my study, I expect that species level factors such as whether the species has a generalist diet and is thus more likely to explore novel places to obtain food (Webster and Lefebvre, 2001), will affect the likelihood of species interacting with the TA.

Overall, the central aim of my project was to develop a tag-application protocol that maximized the likelihood that target animals could be tagged while minimizing stress to them (though no tags were to be deployed within the remit of this project). Specific objectives were; (1) to examine the extent to which wild animals reacted to the tag applicator, (2) to determine whether the animals showed neophobia and habituation, (3) to determine whether the site the tag applicator was placed (e.g. on a well-worn run *versus* faint trail) influenced the number of times animals walked under the TA, (4) to determine whether baiting the area round the tag TA enhanced the number of animals that visited that site per day. This project was therefore designed to provide fundamental data to inform best practice for the bur-tagging approach.

Methodology

To investigate how wild animals react to novel TAs, fieldwork was undertaken from March

June 2022 in Swansea County, Wales involving the use of the tag apparatus models, and camera systems to record interactions between wild animals and the object. TA models were initially placed in both rural and urban environments (Table 1), with cameras recording the interactions of animals with the TA, followed by a second stage wherein aluminium TAs were placed at sites with target animals both with and without bait to investigate if this changed their behaviour and likelihood to walk through the TA. See below for further details.

The tag apparatus (TA)

The basic design of the TA consisted of a flat arch shape resembling a miniature goal post that an animal is able to walk through/under. In future work, where a tag is deployed onto an animal, the TA will contain a tag dispenser with a trigger mechanism to detect the animal before dropping a tag, or remote logging device onto the animal. The tags "Alice tags" that will be used in combination with the TA system have been designed to be specifically small (ca. 1.2 g; 16 mm X 6 mm). However, in the current study, no trigger was present, and no tags were deployed. There were two iterations used within this study:

Version I – Wooden TA

This TA was made from three pine dowels (25 mm diameter) to form the sides and top of the arch with 2 cuboid pine feet (approx. 20 mm height x 40 mm width x 70 mm length) all held together using counter sunk wood screws. The wooden structure was held in place using galvanised steel tent pegs (approx. 220 mm length x 6 mm diameter). The complete dimensions of the gate were 420 mm height x 400 mm width and 40 mm depth (Figure 1).

Figure 1. Version I of the (wooden) tag applicator.

Version II – Aluminium TA

The second TA design (Figure 2) had the same basic structure as the first, but was made from Bosch Rexroth aluminium struts (20 mm x 20 mm with a 6 mm groove). The two sides and top were attached together using aluminium Bosch Rexroth Connecting Component, Angle Brackets, allowing the size of the gate to be adjustable. However, for this study the system was standardized to the limits of the TA, giving gate dimensions 400 mm height and 400 mm width. Two steel spikes were screwed on to the base of each side strut so the gate could be pegged/placed in the substrate (e.g. sand, soil).

Figure 2. Version II of the TA, made of aluminium.

Target species

The animals recorded were subdivided into target species and incidental species. This decision to subdivide these animals was because small species may not have recognised the TA as a threatening object at its current dimensions, but if the TA was altered to be at eye level, we may expect to see a difference in behaviour such as increased vigilance or avoidance. Therefore, only the animals with a similar height to the TA were studied in-depth to ensure the investigation into how wild animals react to novel objects was standardized. These target species were badgers, foxes (*Vulpes vulpes*) and otters (*Lutra lutra).*

The camera system

All interactions of animals with the TA were recorded using camera traps. The camera systems used were: Wechamp 20MP 1080P HD trail cameras with SanDisk ultra 32gb SD cards, and Amazon rechargeable AA batteries within. Cameras were set to record at 1080P for 30 seconds, with a 40 second lag and high motion sensitivity. Due to differences in visibility and topography at each site, it was not possible to standardise camera angle or distance to the TA, but the approximate camera angle and distance from the TA was recorded and was incorporated into the analyses.

Project stages

To cover all objectives, the project was split into two stages:

Stage 1 – Preliminary trials were conducted to locate well-used habitats/ trails which could provide as many recordings of animal interactions with the TA as possible. For this, version I of the TA was deployed across multiple sites for a five-week period (see below for details). Specific guidance on trap placement outlined by DEFRA (2022) which were used in this study are as follows:

- Traps must not be placed directly on spoil heaps.
- Traps must not be placed along fence lines where a lower strand of barbed wire would cause injury.

• The number of traps deployed must be recorded and location marked on a map (see fig. 3).

• Traps should be 'bedded in' on the ground to ensure that they are stable and securely positioned.

• To minimise disturbance and the risk of badgers deserting the site, all traps placed at a single location should be placed on the same day, avoiding dusk and dawn when badgers are likely to be active and above ground.

Stage 2 – The TA made from aluminium was deployed in the most utilized and accessible sites to determine to determine whether baiting the area around the tag applicator enhanced the number of times animals walked under the TA.

2.1 STAGE 1 – Study sites and materials

This stage of the project began on 17/03/2022 and ran for a five-week period. Initially, models of the TA made of wood were deployed across 19 sites (Table 1). These sites were selected based on their high suitability as habitat for my target species, and on the availability of landowner permissions to conduct the study at the site. When visiting the sites, trails of bare earth were searched for in hedges, fields, water runs, woodlands and parkland. Additional signs of animal presence included scat that contains white fragments due to partially digested bones and teeth which is typical of foxes and otters (Lloveras et al., 2011); and communal

latrines which are characteristic of badger territory boundaries (Delahay et al., 2001). Furthermore, at each site, I recorded habitat variables to be used to investigate how the siting of the TA influenced the number of individuals walking under it (Table 2).

Table 1. The site numbers, locations, names and habitat description for each site in which the models of the tag applicators were deployed.

Site numbe r	Location	Longitude/Latit ud e	Dates sites used	Urban or rural	Habitat description

Site 1	Three Crosses, Cefn Draw Farm	51.6293300, -4.0791420	17/03/2022 - 23/03/2022	Rural	Farmland, a field comprising of grass species
Site 2	Three Crosses, Cefn Draw Farm	51.6307993, -4.0792590	17/03/2022 - 23/03/2022	Rural	Farmland. There was a large tree within an unmanaged hedgerow with a trail surrounding it.
Site 3	Three Crosses, Cefn Draw Farm	51.6277341, -4.0741421	17/03/2022-20/04/2022	Rural	Woodland
Site 4	Three Crosses, Cefn Draw Farm	51.6297439,-4.0755254	25/03/2022-19/04/2022	Rural	Farmland, a well-used (typically by quadbikes and sheep) path
Site 5	Three Crosses, Cefn Draw Farm	51.631629, -4.076777	25/03/2022-15/04/2022	Rural	Farmland, a well-used (typically by

					quadbikes and sheep) path
Site 6	Three Crosses, Cefn Draw Farm	51.627875,-4.079841	25/03/2022-01/04/2022	Rural	A dried-up stream bed which still had some puddles
Site 7	Oxwich	51.565018, -4.161743	22/03/2022-04/04/2022	Rural	A trail in the midst of dead reeds beside a woodland
Site 8	Oxwich	51.565501,-4.165084	22/03/2022-26/04/2022	Rural	A grassy trail alongside the freshwater estuary
Site 9	Oxwich	51.563469, -4.164818	29/03/2022-14/04/2022	Rural	Marshland
Site 10	Hendrefoilan	51.623511, -3.998233	18/03/2022-24/03/2022	Urban	Managed garden
Site 11	Hendrefoilan	51.623259, -3.997961	24/03/2022-31/03/20	Urban	Patio in a managed garden

			22		
Site 12	Hendrefoilan	51.623302, - 3.997950	18/03/20 22- 24/03/20 22	Urban	Patio in a managed garden
Site 13	Three Crosses	51.628143, - 4.071750	01/04/20 22- 11/04/20 22	Rural	Managed garden
Site 14	Three Crosses	51.628091, - 4.071748	01/04/20 22- 11/04/20 22	Rural	Hedgerow of managed garden
Site 15	Singleton	51.608403, - 3.976783	18/03/20 22- 25/03/20 22	Urban	Open scrub bordering the university and adjacent road

Site 16	Singleton	51.608643, - 3.976636	18/03/20 22- 25/03/20 22	Urban	Sand dune

Site 17	Singleton	51.607173, -3.981315	26/03/2022-26/04/2022 Stage 2 16/05/2022-01/06/2022	Urban	A small pond located in Wallace Garden. The vegetation surrounding is managed by gardeners.
Site 18	Singleton	51.607011, -3.981202	26/03/2022-26/04/2022 Stage 2 16/05/2022-10/06/2022	Urban	A small stream with unmanaged vegetation surrounding either side
Site 19	Three Crosses, Cefn Draw Farm	51.629796, -4.075560	12/04/2022-19/04/2022	Rural	Farmland, a well-used (typically by quadbikes and sheep) path

Site 20	Oxwich	51.565501, -4.165249	10/04/2022-19/04/2022	Rural	A grassy trail alongside the freshwater estuary
Site 21	Singleton	51.606960, -3.981247	Stage 2 14/06/2022-29/06/2022	Urban	A small stream with unmanaged vegetation surrounding either side
Site 22	Hendrefoilan	51.595895, -4.013557	17/05/2022-28/06/2022	Urban	Fox run located in a managed garden

Table 2. Details all information concerning habitat variables that were recorded at each site, to be used during data analysis.

Category	**Description**
Date	Date of recording
Site number	Site number (described in Table 1).

Site openness	1 – Very enclosed space, where it is obligatory for the animal to walk through the TA to get to the location on the other side. An example is a thicket with only one passageway, blocked on either side by impassable bramble species. 2- Enclosed, but no obligatory passage as in '1'. Avoidance of the TA necessitated a substantial deviation to progress, generally by finding a climbable or passable route around the TA. 3 – Fairly open, ground flora species are those of differing heights but there are many trails in the site. 4 – Open in a big space such as a managed field.
Path density	1 – No path visible. 2 – Faint path wherein the ground beneath may have a slight imprint of an animal travelling across, visible by grass species/lichen/reeds being comparatively more flattened than the flora surrounding. 3 – Well used track clearly visible by a trail of bare earth whilst the surrounding vegetation is growing uninterrupted nor trampled upon.
Maximum plant height	Recording of the tallest plant/ shrub species taken from 25cm x 25cm quadrat placed over the TA.
Vegetation %	Percentage of ground flora in the quadrat.
Camera angle	F – In front of gate. S – To the side of the gate.
Camera distance	Camera distance categorised as: 1 = <1.5 m. 2 = >1.5 m.

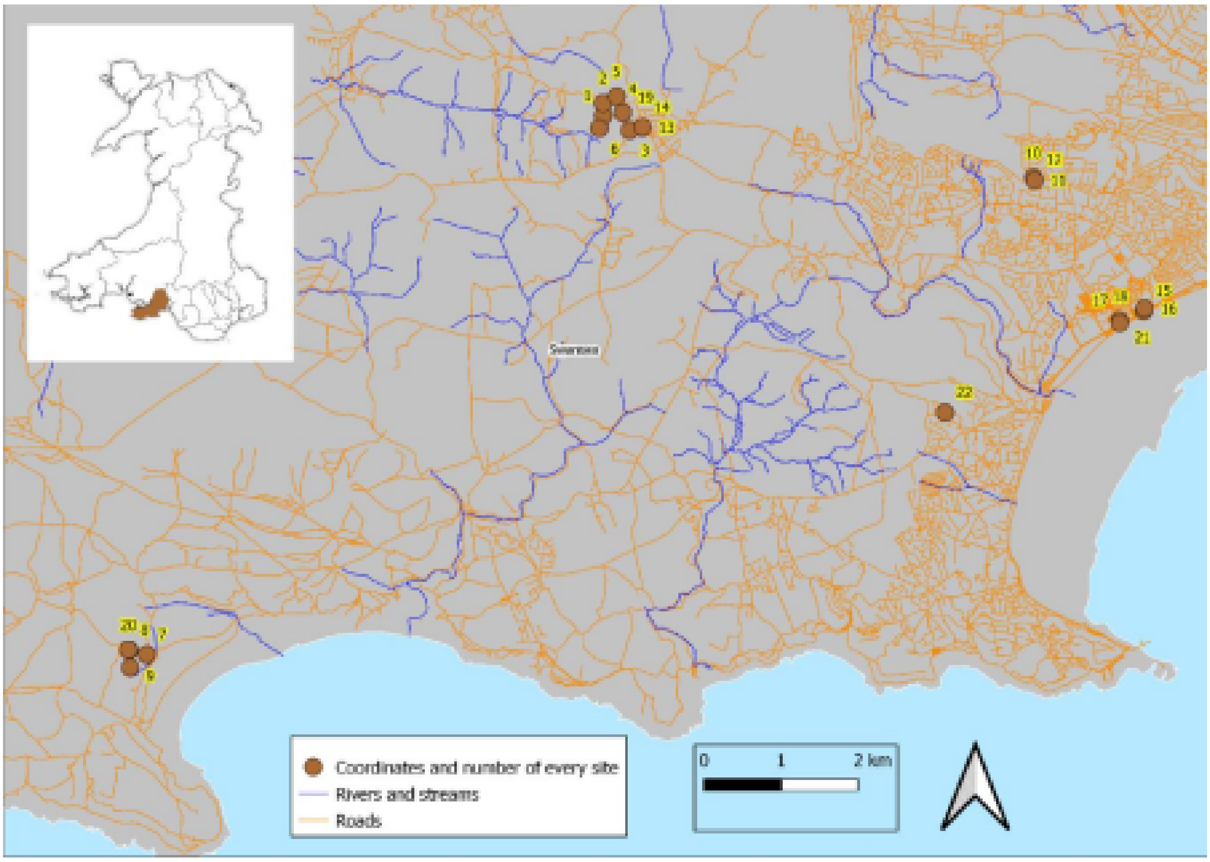

Figure 3. Each site (numbered 1-22) used throughout the project in Swansea County, with proximity to roads and water sources using a colour palette specified by the Web Content Accessibility Guidelines (2023) and Berisso (2018), as well as an inset map highlighting where Swansea County is within Wales.

The TAs were checked weekly to minimise any disturbance caused by repeat visits by researchers to the habitat and to account for animals which have a larger home range, such as the Eurasian otter which can range up to 40km (O'Rourke *et al.,* 2022) and therefore may appear only intermittently at the sites. Each week, the camera SD cards and batteries were replaced and the videos from each site were viewed. If sites had no animals or very few interactions (i.e. five or fewer per 7-day period), the TA model and camera were removed and placed elsewhere to increase the probability of animal interactions. An example of this is site 1 which only had three interactions during the first week, so the TA model was moved, and the site was not used again. By comparison, site 3 had 18 interactions during the first week, so the TA model was left for the entirety of stage 1 (5 weeks).

2.2 STAGE 2 – study sites and materials

Stage 2 focussed on deploying aluminium TAs in sites which were accessible at all times (i.e. not on private land) and had high numbers of interactions. Initially at these sites (17, 18, 21 and 22), the TAs were left for a week, with cameras being set to the same standards as those in stage 1. Videos of animal-TA recordings were then analysed.

Using the recordings, I investigated whether baiting the TA enhanced the frequency of animals visiting the site per day. As the majority of animals recorded in stage 1 were omnivorous, 'Brambles meaty hedgehog food' (https://www.littlepeckers.co.uk/p/brambles meaty-hedgehog-food-12-x-400g) was selected which is both RSPB endorsed and recommended for foxes and badgers (RSPB, n.d). Initially baiting was undertaken daily at 8am and 9pm respectively, to account for both diurnal and nocturnal activity. Due to practicalities in baiting regularly at these times, only sites at Singleton campus were utilized for this aspect of the project. After a week however, it was observed that only domesticated dogs (*Canis lupus familiaris*), insects (*insecta*), and magpies (*Pica pica*) tended to consume the bait placed at 8am. As this project focuses on remote tagging of terrestrial wild vertebrates, it was decided that baiting should be only undertaken at 9pm each day, to capture observations of target species such as foxes and badgers. The bait itself was weighed (7 g/portion) then placed directly below in the centre of the TA with an additional two portions placed 20cm either side of the TA. I anticipated that the time taken to explore the site, and potentially interact with the TA would increase due to individuals eating, so the recording time on the camera was increased to 50 seconds.

2.4 Data analyses

Films documenting interactions between animals and TAs were examined, and all variables listed in Table 3 were recorded. During fieldwork, 6 wild animal families were recorded, alongside domesticated animals (see table 4).

Table 3. Data recorded for each individual observation of an animal recorded by the camera traps.

Category	**Description**
Species	Animal species observed in the video recording. Due to the quality of the camera recordings, species were easy to identify, aside from some bird species. To identify bird species, a handbook by Holden & Gregory (2021) was used.
Time/seconds	The length of the entire interaction, to include the start time at which the animal sees/ sniffs or approaches the TA, to when the animal leaves the TA or stops investigating.
Active interaction	Even if the animal did not go through the TA but exhibited behaviours such as sniffing, pausing, looking at the gate, was considered an active interaction and was detailed yes. If any animal moved rapidly (less than 1 second) through the TA without apparent interaction (such as looking, pausing or sniffing), it was detailed as no.
WTG	Walked through TA, yes or no.
Direction	The direction of the animal whilst walking toward the TA: D – Direct (directly walking to the TA in a straight line so the TA is in front of its body). O – Oblique (the animal approaches the TA from a sideways or diagonal direction such as left or right, meaning it will have to angle its body or head to look straight at the TA)

Attraction	Attraction to the TA categorised as: A – Attracted, the animal looks/ pauses/ sniffs at the TA, and continues to approach the TA without backing away or flinching. 'Attracted' is also selected if the animal touches the TA with its snout or beak when investigating it, climbs the TA, or perches upon the TA I – Indifferent, the animal passes by or under the TA without seeming to look/ pause or sniff without slowing its gait or changing direction. R – Repulsed, selected when the animal looks/ pauses or sniffs toward the TA and freezes and backs away. This category also selected when the animal flinches when the TA first comes in to view and if it slinks back away from the TA by lowering its back when reversing, without taking its eyes off the TA.
Sniffs	The number of times the animal sniffs at the TA during the interaction which is sighted by the movement of the snout and body as the animal breathes in and out to scent the habitat (Figure 4). Sniffing typically identified when the head is inclined toward the TA and the nostrils of the animal flare in quick succession, whilst the body expands as the lungs fill with air. With birds the distinction is harder to decipher without nostrils which contract, however the beak would still be angle toward the TA and the body would contract slightly whilst breathing.
Looks	The number of times the animal looks at the TA during the interaction (Figure 5A). This behaviour is categorised when the animal looks directly at the TA, which often requires the animal to raise their head to see the very top of the TA, or look left/right if approaching the TA obliquely.

Pauses	The number of times the animal pauses at the TA during the interaction which is considered to be when the animal approaches the TA and stops moving (Figure 5B). Pausing is typically identified by an abrupt stopping in motion as the animal sees the TA, with the animal often looking frozen – i.e. no noticeable sniffing of the TA. An additional characteristic of pausing regularly observed is as the animal pauses, it may shift its weight to its hind legs, lower its back at the base (where the tail or feathers join) and raise its' head slightly to see the TA fully and there remain immobile whilst the animal considers the threat of the TA.

Figure 4a. A badger captured on camera at Oxwich site 8 sniffing the wooden TA model. Figure 4b. A mouse climbing upon the TA model and sniffing the top of the wood joiner, captured on camera at Oxwich site 9.

Figure 5a. An otter captured on camera at site 17 singleton, looking at a wooden TA. Figure 5b. An example of a fox pausing at an aluminium TA at site 18.

2.5 Statistical analysis

Data were analysed in R version 4.2.1 (2022). Packages used to explore aims and do statistical analysis were as follows;

ggpmisc (Aphalo, 2023)
ggplot2 (Wickham, 2016)
scales (Wickham & Seidel, 2022)
viridis (Garnier et al., 2023)
extrafont (Chang, 2023)
lubridate (Grolemund & Wickham, 2011)

plotly (Sievert, 2020)
lattice (Sarkar, 2008)
fossil (Vavrek, 2011)
lme4 (Bates et al., 2015)
Matrix (Bates et al., 2023)
lmerTest (Kuznetsova et al., 2017)

Model fitting and checking

GLMMs were fitted in R version 4.2.1 (2022) using lme4 (Bates et al., 2015). For models with a Poisson structure, I checked for overdispersion (by checking the residual deviance divided by degrees of freedom) and for any patterns in the residuals versus the fitted values, following Thomas' et al. (2013) study. For binomial models, overdispersion by definition cannot occur (Thomas et al. 2013). I also checked for the possibility of singular fit which can occur when there are too few data points relative to the number of model parameters which can result in model overconfidence (Oberpriller et al., 2022), thus invalid inferences. The details of each model fitted is given below.

Model 1

Using the principle that animals may exhibit neophobia through hesitation, avoidance or caution (Harris & Knowlton, 2001), recordings of looks, pauses, and sniffs during the wildlife-TA interactions were combined into 'total interactions' used as a proxy for detecting neophobia in individuals. A generalized linear mixed model (GLMM) with a Poisson distribution was run four times, one for each explanatory variable (days TA out, attraction, direction and species), with total interactions as the response variable. Separate models were run for each explanatory variable to avoid a singular fit. Data included 99 observations in total, with repeat sampling from 12 sites, so to avoid the effects of pseudoreplication, 'site' was included as a random factor in all models. The effect size and standard error associated with each response variable was obtained directly from the model, while the associated chi^2 and *p* values were obtained by comparing the full model to a model without the variable in question using a Chi^2 test. Where species, direction and attraction categories are included in the minimal model, effect sizes for the reference category (badgers, directionD, attractedA) were always zero.

Model 2

To investigate whether the probability of the animal walking through the TA changed over time, a GLMM with a binary distribution was fitted. The response variable was whether the animal walked through the TA, with 1 = walked through, and 0 = did not walk through. Days TA out, direction, species, attraction and total interactions were used as the explanatory variables which could influence an individual's likelihood of walking through the TA. This model included 99 data points, from 12 sites, so site number was included as a random effect. The effect size and standard error associated with each response variable was obtained directly from the model, while the associated $chi^2$and *p* values were obtained by comparing the full model to a model without the variable in question using a Chi^2 test. Where taxonomic grouping, direction and attraction categories are included in the minimal model, effect sizes for the reference category (badgers, directionD, attractionA) are always zero.

Model 3

To explore site-based factors (average plant height, quadrat %, camera distance, camera angle) which may influence animals walking through the TA, only data from the first week was used as I had data from a greater number of sites in the first week (sites with few observations were not observed for subsequent weeks). As the sample size was relatively low (22 observations from 5 sites), there was not sufficient data to run the binomial GLMMs with multiple explanatory factors without this resulting in a singular fit. The model was therefore run four times (one for each variable) and without site being included as a random effect (also to avoid singularity). A GLMM for path density was not run as 21/22 of videos were recorded at a site with a path density of 3, which could give biased results, thus inaccurate conclusions (Stuber et al., 2013).

Model 4

This model investigated whether baiting the area around the TA increased the number of animals visiting the site per night. At the sites available, the only target species recorded was the fox. The number of foxes that visited each site (4 altogether) was fitted as the response variable in a GLM with a Poisson distribution with trial type (baited and unbaited) as an explanatory variable. Initially, a GLMM was fitted, with site included as a random effect. However, due a 'singular fit' warning from R, which may have been due to a limited number

(35) of datapoints for the complexity of the model, I refitted the model as a generalized linear model (GLM) instead.

Results

Whilst data analysis was performed only on target species, there was a total of 194 observations over a five-week period in stage 1 which comprised of nine taxonomic groupings altogether (Table 3). Within these 9 groupings, the animals looked at the TA a total of 111 times (28.9%) on approach, paused at it 116 times (30.2%), and sniffed the TA 157 times (40.9%) (fig 3 & 4). When approaching, animals were primarily walking directly toward the TA with 156 counts (80.4%) and 38 (19.6%) counts of animals approaching the gate obliquely. In terms of path density, 73% of observations occurred on trails with a score of 3 (well-worn trails). During the interaction, individuals appeared mostly indifferent to the TA with 106 observations (54.6%) of indifference to the TA, 68 (30.1%) observations of animals showing attraction, and 20 (20.3%) observations of repulsion. There were 143 (73.7%) counts of individuals walking through the TA, and 51 (26.7%) counts of individuals choosing not to walk through the TA.

Table 4. Complete data on all taxonomic groupings of; the total time spent interacting with the TA, number of active interactions, number of times the animal walked under the TA, direction on approach, attraction type during interaction, and number of looks, pauses and sniffs per grouping.

Taxonomic grouping	Total observations that each taxonomic group comprised in the study %	Mean time/s of interaction	Percentage total of *active interactions* if 100% represents each group's total interactions	Percentage (%) of walking under TA	Direction of individual on approach D – direct O - oblique	Attraction A:attracted I:indifferent R:repulsed	Total number of looks	Total number of pauses	Total number of sniffs
Mustelidae	38	3.09	40.54	78.38	O – 8 D - 66	A- 23 R -9 I -42	38	61	51
Aves	25	7.95	41.66	72.91	O - 10 D - 38	A-18 R-1 I-29	30	10	5
Rodentia	4	9.37	75	50	O - 4 D - 4	A-7 I - 1	12	7	32
Domesticated	10	3.2	25	80	O - 1 D-19	A-2 R-1 I -17	3	5	4
Erinaceidae	3	6.4	60	100	O - 1 D - 4	A-3 I -2	4	5	12
Sciuridae	7	6.42	42.86	92.86	O - 2 D – 11	A-7 I - 7	8	6	21
Canidae	13	4.84	60	48	O - 11 D – 14	A-8 R-9 I-8	16	22	32

Aim 1: reactions to the TA

All target species interacted with the TA through looking at it, pausing and sniffing (Figure 6). Using the combination of looks pauses and sniffs (total interactions) as proxies for neophobia (hesitation, avoidance or caution) per target species, it was found the number of interactions between target species and the TA decreased over time since the TA was deployed (Table 4, Figure 7) and that individuals who exhibited indifference toward the TA displayed fewer total interactions. There was no impact on total interactions by species or approach style (direct/oblique).

Figure 6. Percentage of the number of times each target species looked, paused or sniffed towards the TA. Total number of interactions for badgers were 137; foxes 70, and otters 13

Table 5. Effect sizes and standard errors from the models run on total interactions, alongside the associated chi^{2}and *p* value for each GLMM per explanatory variable. The number of interactions (looks, pauses and sniffs) was the response variable.

Term	Effect	SE	df	X^2	*p*
Intercept	1.25	0.198			2.74E-10
Days TA out	-0.020	0.007	96	8.532	0.003
Intercept	0.939	0.236			7.07E-05
Species			95	8.236	0.016
SpeciesBadger	0	0			

SpeciesFox	0.219	0.201			
Intercept	1.029	0.205			5.49E-07
Direction			96	0.605	0.43
DirectionD	0	0			
DirectionO	0.139	0.177			

Intercept	1.486	0.085			<2e-16
Attraction		0	95	221.242	<2e-16
AttractionA	0	0			
AttractionI	-3.789	0.455			
AttractionR	-0.02	0.142			

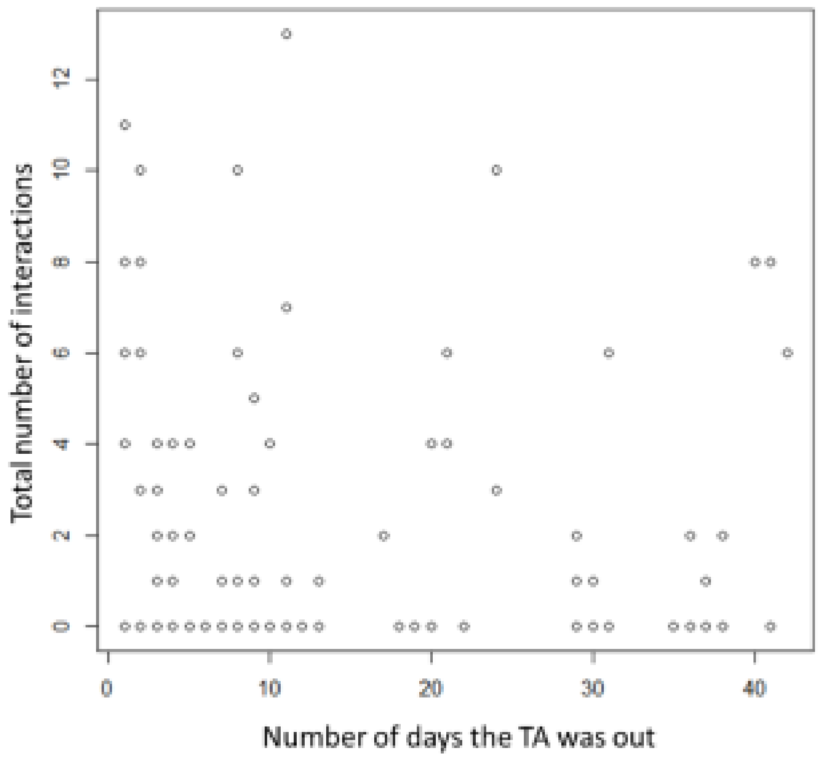

Figure 7.
Demonstrating that the total number of animal:TA interactions decreases as the number of days the TA is out increases.

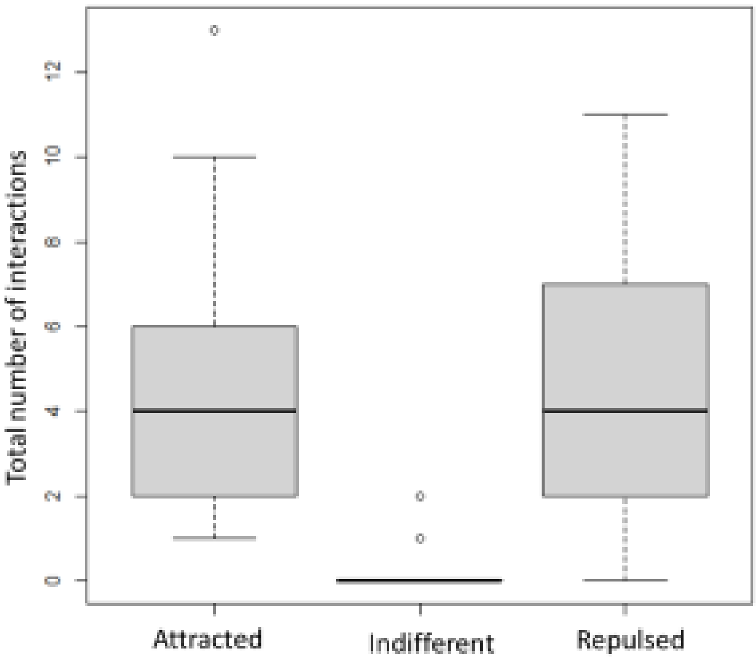

Figure 8. A visualisation showing that there are fewer interactions with the TA when animals appear indifferent.

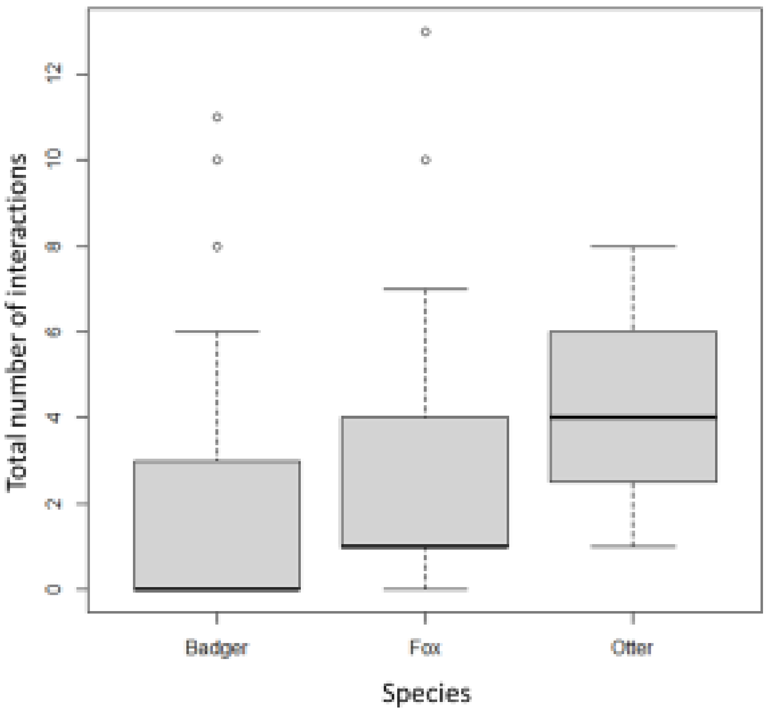

Figure 9. Displays that the number of interactions with the TA are highest for otters, followed by foxes. Badgers exhibit the least interactions.

Aim 2: Neophobia over time

Model 2 (Table 6) found that the longer the TA was in place, the more likely animals were to walk through it. Animals were also more likely to pass under the gate if they reacted less to it (fewer total interactions) and if they approached it directly, rather than obliquely. Attraction was not fitted to the model due to this resulting in a singular fit, so these results are not presented.

Table 6. Effect sizes and standard errors from the model models run to test neophobia over time, alongside the associated chi^{2}and *p* values. Whether or not the animal walked under the TA was the response variable.

Term	Effect	SE	df	X^2	*p*
Intercept	1.222	0.724			0.091
Total interactions	-0.307	0.096	3	11.893	0.001
Intercept	-1.034	0.946			0.274

Days TA out	0.110	0.032	3	15.110	8.20E-05

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Intercept	0.745	0.644			0.247
Direction			1	6.260	0.001
DirectionD	0	0			
DirectionO	-1.638	0.654			
Intercept	0.961	0.674			0.021
Species			2	5.289	0.022
SpeciesBadger	0	0			
SpeciesFox	-1.557	0.680			

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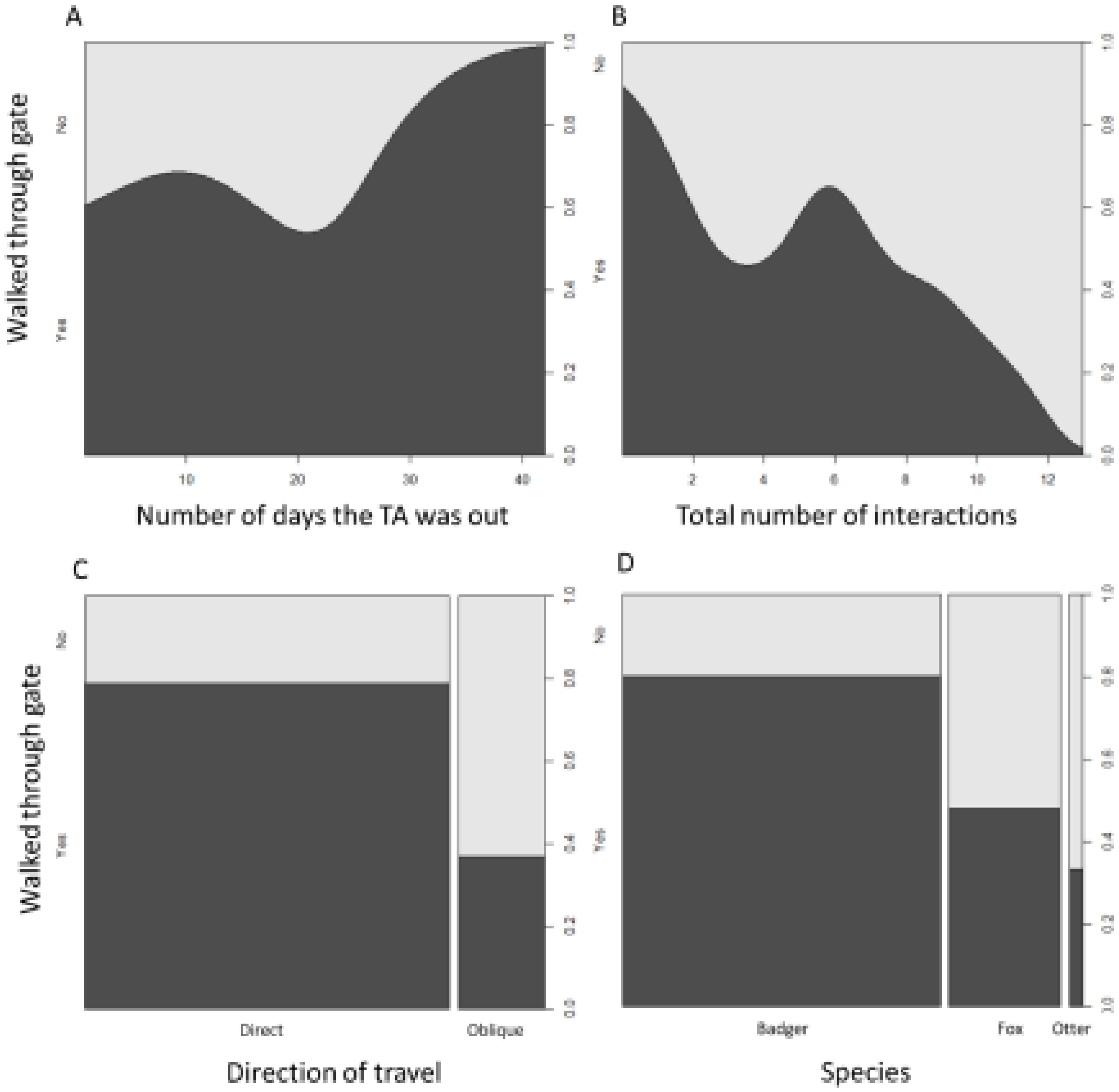

Figure 10a. Density plot showing number of instances of target species individuals walking through the TA after 0-40 days.

Fig 10b. Density plot showing total number of interactions correlated with if the individual walked through the TA.

Figure 10c. Raw population plot showing that if the individual approached the gate directly there are more instances of it walking through the TA.

Figure 10d. Raw population showing that badgers walked through the TA most, then foxes, then otters.

Aim 3: TA placement and environmental factors

When fitting the explanatory variables to the model, it was found that neither of the habitat variables (average plant height, quadrat %) nor camera distance and camera angle significantly influenced target animals walking through the TA. Whilst only the first week of data could be used to investigate this objective (to mitigate the potential effects of neophobia reduction over time), 86% of observations between target species animals and TAs occurred on trails with a score of 3 (well-worn trails).

Table 7. Effect sizes and standard errors from the models run to test the effects of TA placement and environmental factors on animals walking through the TA, alongside the associated chi^{2}and *p* values for each explanatory variable. Whether or not the animal walked under the TA was the response variable.

Term	Effect	SE	df	*p*
Intercept	-48.634	98879.221		
Average plant height	0.893	1486.117	20	0.001
Intercept	0.515	1.076		
Quadrat %	0.005	0.018	20	0.801
Intercept	76.70	138227.25		
Camera distance	-51.13	98871.41	20	-0.001
Intercept	2.708	1.033		
Camera angle	-22.274	4390.308	20	0.996

Aim 4: Baiting the TA

The trial which included baiting around the TA significantly increased the number of foxes that visited the site per night.

Table 8. Effect sizes and standard errors from model 4, alongside the associated chi^2 and *p* values for trialB (baited).

Term	Effect	SE	df	*p*
Intercept	0.406	0.204		
Trial			34	0.009
TrialA	0	0		
TrialB	0.620	0.246		

Discussion

Results summarisation

When examining the extent to which wild animals reacted to the TA, it was found that wild animals looked, paused and sniffed toward the TA during the recording. Badgers and otters paused most at the TA as opposed to look or sniffed, whereas foxes sniffed more than any other interaction type.

Evidence which suggests the target animals showed initial neophobia then habituation toward the TA was shown, as the total interactions decreased over time for foxes and badgers, which is consistent with initial neophobia followed by habituation (Honey et al., 1992). Unfortunately the sample size was too low to include otters in this statistical analysis. However, the target species most likely to walk through the TA compared to other species was the badger which is reflected in the data (fig. 10d); with badgers also exhibiting the lowest number of total interactions.

It was found that none of the habitat variables significantly influenced the number of times target species walked under the TA.

With regard to investigating whether baiting around the TA enhanced the number of target individuals that visited the site per night, it was found that baiting the area around the TA did increase the number of visits per night significantly.

Neophobia and habituation with respect to the TA

As a primary goal of this project was investigating how wild animals react novel objects in their environment, behavioural responses such as neophobia and habituation must be considered. As fig. 10a demonstrates, within the first 10 days, approximately 60% of animals walked through the TA. Considering neophobia refers to the avoidance of novel predators, objects and foods (Greggor et al., 2015), it seems plausible that individuals coming across the TA (which did not walk through the TA) may have been exhibiting neophobic behaviour. Displaying neophobia is thought to be a behavioural mechanism that serves to increase survival, as it reduces the probability of exploring potentially risky objects or resources (Crane & Ferrari et al., 2017). When investigating novel objects, animals might show behaviours such as wariness (Padovani et al., 2020) and alertness (Christebsen et al., 2021) if

they perceive a potential threat, which is something researchers should expect when placing TAs out in wild habitats. Indeed, as fig. 10b shows, animals that interacted the least with the TA (by looking, pausing or sniffing toward it) and were indifferent on approach were far more likely to walk through the TA than not. For example, with fewer total interactions, 70% of individuals walked through the TA, whereas with more interactions (e.g. 10), only 25% of individuals walked through the TA.

Habituation will be an essential factor when considering the success of the TA in tagging animals. Habituation is the process through which an individual becomes accustomed to novel stimuli through repeated exposure without experiencing sensory or motor fatigue (Rankin et al., 2008). Habituation is also an important form of behavioural plasticity which allows wild animals to conserve energy for other important tasks, such as obtaining food and courtship opportunities (Bell & Peeke, 2015). In terms of wildlife management, understanding the drivers behind animal habituation can further conservation as it allows researchers to understand how tolerant a species may be to disturbance and how they may react in terms of stress (Blumstein, 2016) which is something the development of the TA aims to minimize (Dennis & Shah, 2012).

Although some species habituate slowly, other species may show a preference for novelty, such as the rodents in Lupetow's (2017) study and mice in Christensen's (et al., 2021) study which saw individuals exploring both novel and non-novel objects equally. In this study, it was found that animals tended to habituate to the TA over time; so with respect to the TA as a novel object, if researchers wish to tag nearly 100% of animals per site regardless of preference for novelty (or not), then the TA should be left at the site for a minimum of 40 days, as demonstrated by fig 10a.

Species level behaviour with respect to the TA

I found that different species responded differently to the TA; otters interacted most with the TA and walked through the least, badgers interacted the least and walked through the TA most, and foxes were intermediate (see fig. 10d). Of course, number of total interactions with the TA were higher during the first few days in the target species, and this decreased over time. Badgers paused at the TA more than sniffed or looked at. When doing so, the badger appeared to stiffen in motion which is a typical form of vigilant behaviour (Macdonald et al., 2004) and is made in response to a threat such as predation (Tuyttens et al., 2001). It could be

possible that badgers paused regularly due to both increased vigilance with a novel object, and potentially due to their poor eyesight, despite being nocturnal (Buesching & Macdonald 2001). Subsequently, encountering a novel object that cannot easily be distinguished and identified in the dark (and may or may not smell of humans/surrounding mesocarnivores), would likely invoke neophobic behaviours such as pausing and assessing the potential threat before approaching.

Foxes were recorded sniffing (46% of the time) more than looking at or pausing at the TA. Such observations could be expected for foxes, as they have an acute sense of smell with both an olfactory system and vomeronasal system which is typical of mammals (Takigami et al., 2014), allowing them to detect specific odours (Ortiz-Leal et al., 2020). When encountering a novel object in its territory, a fox may display behaviour such as hesitancy, with Padovani's (et al., 2021) showing that dominant foxes tend to display more neophobic behaivours when approaching novel objects compared to subordinate foxes. To therefore limit neophobic behaviours that may be elicited when approaching the TA due to scent, TAs can be "weathered" before placing the apparatus in-situ. This process is listed by DEFRA (2022) when placing a trap out to trap wild animals, and can be applied to TA placement as well. Unfortunately, the precise time period in which human scent fades after visiting camera traps is largely understudied (Munoz et al., 2014), but it is clear that many species are highly sensitive to the scent of humans, for example, White-tailed deer (*Odocoileus virginianus*) can pick up the scent of a human from ½ mile away (NeSmith, 2020). Still, it might be expected that after a cooling period human scent may reduce and individuals may walk under the TA more frequently (in this study, after 25 days for 60% of individuals, and 40 days for nearly all individuals).

Otters walked through the TA the least, but interacted the most. Typically regarded as opportunists, the otters in this study may have interacted with the TA most as their exploratory nature allows them to exploit varied food resources (Duarte et al., 2022) so the TA may have been worth investigating in case it provided a resource. Clearly, there will be differences in how animals react to the TA at the species level when placed *in-situ*, and whilst the TA has not yet been deployed in real life, there are many studies discussing novel object reactions at the species level. If we take camera traps as an example for instance, Larrucea (et al., 2007) reports that coyotes (*Canus latrans*) behave aversively towards camera traps, whereas there is evidence to suggest that some large felids are actually attracted to camera trap flashes (Kelly et al., 2012). It was found in Glen's (2013) study that stoats (*Mustela*

erminea) frequently reacted to both models of camera traps (one type with a white flash, and one with infrared) by turning the head to look at the camera, or by approaching the camera. Rather interestingly in Glen's (2013) study, stoats did not show signs of wariness toward the cameras, nor retreat after they flashed. Researching into species level neophobic and exploratory behaviours toward novel objects will therefore prove valuable when predicting whether species may act negatively or positively toward the TA when it is deployed.

Future considerations

Gathering data during fieldwork was invaluable when investigating the extent to which wild animals reacted to the TA; whether the animals showed neophobia and habituation, if site factors influenced the number of times the animal walked under the TA, and determining whether baiting the area around the TA enhanced the number of times animals walked under the TA. However, for future studies there are several factors that could be improved when setting up the TA, alongside recommendations to enhance animal:TA tagging events and considerations which could benefit the future application of the TA.

Site selection

Despite gaining important insights into how wild animals react to novel objects, there were five study limitations that must be addressed. Firstly, site selection was based purely on what was available at the time (although this is a limitation that future researchers may also encounter, as having access to all private land is unrealistic). Ideally, there should be one TA per anticipated home range of the target species. Home range data in turn can help to establish territory boundaries which are defined as the area in which the animal regularly exploits resources and stores information cognitively (Spencer, 2012). By establishing the territory boundaries of the target species and placing only one TA per range, animals are less likely to be repeatedly exposed to the same equipment which would ultimately lead to a data bias (Stuber et al., 2013).

Experimental design and site selection

With respect to experimental design, during the first stage of this project, the TA and associated camera system equipment were placed on, or near, trails that appeared to be regularly used by wild animals, to capture as many wildlife-TA interactions as possible. This allowed sufficient data collection to investigate influences on the number of interactions with

the TA and on whether animals walked through the gate. However, the placement of cameras on trails inadvertently led to a sampling bias (Stuber et al., 2013), with data collected from 21 of 22 datapoints at sites with dense trails. However, future studies may wish to place the number TAs out equally during the first week on trails of varying densities (not primarily those which looked well worn) to thoroughly establish the effects of trail density on the number of animal observations.

Camera placement and range

An additional bias to consider is camera placement and range. Foxes for example, were first recorded interacting with the TA two weeks after deployment, which is consistent with Morton's (2022) study which saw that out of 87 foxes, 86 took two weeks to approach a novel object in their environment. However, it is possible that foxes, particularly with their keen sense of smell (Ortiz-Leal et al., 2020), had reacted to the TA from the first day in the form of avoidance and neophobia, but such responses were out of the range of the camera. It may be possible to place cameras in the surrounding area to record reactions of animals at a greater distance as opposed to only at the site facing the TA system, however this raises issues that are hard to correct for because, by placing cameras further away, animals may be influenced by these too.

Species phenology

The season in which stage 1 took place was late winter- early spring (March-April) and during this period badger activity levels tend to be low (Silk et al., 2017). Therefore, repeating this stage during summer would be beneficial when investigating species level responses to the TA, as there is elevated animal activity due to the long photoperiods and increased primary production (Humphries et al., 2017). In terms of species-specific activity levels, it is worth looking into current research to understand when the species is most active, as placing TAs during this period may increase the likelihood of the target animal walking through it. So, even though there is elevated animal activity during the summer, some species are more active during Autumn, such as foxes (Doncaster & Macdonald, 1996) meaning that it could be more appropriate to consider placing TAs during the Autumn as opposed to Summer.

Baiting practicalities

Lastly, due to practicalities in baiting daily at 9pm, only sites 17, 18, and 21 were utilized for this phase of the project as they were accessible (i.e. not private land, which is not always

easily accessible). However, the Oxwich reserve sites (7, 8, 9, 20) were the most ubiquitous in terms of animal observations, and had the most diverse range of species. Future studies may require permissions to access national nature reserves at off-peak times to enhance the number of species and individuals that may be attracted to the TA when baited. The sites used for baiting were in an urban environment (Swansea University, Singleton campus) which is in contrast to rural Oxwich. This potentially limited the number of observations, and species observed, thereby changing inferences derived from baiting, owing to anthropogenic disturbances which can restrict connectivity and animal movement (Doherty et al., 2021).

Enhancing the likelihood of animals walking through the TA

Baiting the area around the TA increased the number of times foxes visited the site. This then raises questions on how baiting can be applied to a specific taxon in future studies. What if for example, the species is wide-ranging and herbivorous, and the odor of the bait does not carry as pungently as meat-based foods? Yet, so long as the method in question enhances the number of animal-TA interactions per unit time, the specifics of the technique do not need to follow the same design used in this study. To illustrate, chemical communication could be used to successfully attract individuals to the area, as the release of pheromones often functions to advertise sexual receptivity and location (Coombes et al., 2018). The utility of this method is profound, as all species, regardless of how gregarious or solitary, need to coordinate their behaviour and activities with conspecifics to reproduce, which typically involves the use of pheromones (Brennan, 2010). One option may be to purchase pre-made or synthesized pheromones, but this could turn out to be costly, with research suggesting that the cost of the pheromone itself (if available) increases with relative effectiveness (Sramel et al., 2021). Fortunately, many species leave ultra-specific cues (termed infochemicals) *via* urine and anal secretions (Parsons and Blumstein 2010) which would only require the researcher collecting a sample *in-situ* and applying it to the area or TA system to attract an individual. However, if finding a sample *in-situ* is difficult due to the elusiveness of the species, it may be possible to get samples from captive individuals. For 5 years, rangers in Nagpur Forest department, India, have been utilizing urine from female tigers in local zoos, and using the samples to successfully coax problem tigers away from villages, or entice individuals to safer areas (Pinjarkar 2018). This same technique could be applied to enhance animal-TA interactions as a form of 'baiting' to attract conspecifics, however, it must be noted that some conspecific urine may repel individuals if it contains substances such as avoidance chemicals,

as seen in crayfish (*Procambarus clarkia*) so researching into chemical cues before using this method is important.

Acoustic cues and lures may also be an effective way to attract a target species. Typically, this method is associated with anurans, such as the Surinam golden-eyed tree frog (*Trachycephalus coriaceus*), with research demonstrating that the playback of choruses attracted frogs outside of natural breeding events (Fouquet et al., 2020). There is also a long history of feeding mimicry recorded in viperids. For example, juvenile rattlesnakes rattle their tails to lure in prey, with the acoustic signal given off mimicking that of insects (Schuett 1984). Such techniques have similarly been used in mammal studies. For instance, Charlton's et al., (2007) research found that female red deer (*Cervus elaphus*) will move preferentially toward simulated male roars with lower formant (resonance frequency) calls, which indicate larger body size. Thus, acoustic cues could present a fairly accessible, cost-effective way to attract terrestrial mammals to the TA sites as well. Yet, despite communication in animals being a dynamic, complex process (Hebets et al., 2016), it may be wise to use attractants, whether acoustic or chemical, individually. Indeed, Suarez-Tangil and Rodgriguez (2017) tested the effects of combining olfactory, visual and auditory attractants on luring mammals to sites for monitoring, and found that the use of all three together did not increase observations, and that the use of acoustic lures in particular seemed to have a negative effect on the presence of foxes and rabbits (*Oryctolagus cuniculus*). Of course, this result may link back to neophobia (as the acoustic signal played was a general sound, not taxon specific) but it is likely a consequence communication and sensory strategies which must always be considered when designing a method to enhance anima-TA interactions.

Feeding type differences and TA interactions

Target species may interact with novel objects differently depending on feeding strategies. For example, omnivores are considered generalists (Reuter et al., 2022) and in Morton's (2021) study it was found that raccoons (*Peocyon lotor*) were likely to explore novel objects, which could be explained by their opportunistic nature (Dohner, 2017). Contrastingly, herbivores may be more likely to exhibit caution when approaching a novel object, on account of being adapted to scrutinize for risk of predation whilst foraging (McArthur et al., 2014). One may therefore anticipate the time it takes a herbivore to approach the TA to be comparatively longer than that of a omnivore or carnivore. Carnivores in particular may exhibit more predatory behaviours towards novel objects, as they may perceive them as

potential food resources. Indeed, research examining captive snow leopard (*Panthera uncia*) personality traits in New York (Cassia & David, 2011) found that when introduced to a novel object, males were curious and investigative. In this knowledge it may therefore be reasonable to assume that carnivores may approach a TA in future studies comparatively quickly compared to the target species in this study, which were all omnivorous.

Considerations on future TA design

This study has been limited and rather specific in terms of focusing predominantly on terrestrial mammals. Nonetheless, a number of important findings point to how the TA approach might be expected to develop, and the relevance of it for tagging sensitive, elusive, or rare animals. In particular, I have only considered ground-dwelling species in my study, but it may be possible to tag arboreal species using this method. Indeed, since these animals may have to use specific branches for travel, their movements may be more constrained, making tagging easier. It may also be worth considering ventral attachment for birds and flying species like bats. Of course, it is important to recognize that an inappropriately placed tag may unbalance the individual, as seen in northern gannets (*Sula bassana*) fitted with GPS units, which caused knock-on effects relating to energy expenditure (Vandenabeele et al., 2014). Fortunately, the Alice tags used in combination with the TA system are small so the possibility of utilizing the TA system in arboreal species would depend entirely on the design of the TA apparatus itself. Further, in terms of design, it may be advantageous to combine both aluminum and titanium (or cheaper polycarbonate) during creation to waterproof certain components of the TA. Titanium and polycarbonate are commonly used in marine camera housings to combat the effects of extreme salinity and pressure (Purser, et al., 2020) – a concept that could be applied to TA construction if the target species is marine or freshwater.

Finally, outside of physical properties when designing the TA, it may be possible to link the TA system to AI considering the rapid development of image recognition, ensuring the correct species is tagged (and perhaps even be able to distinguish between male and female if that is relevant for the study). In the future, we might even see AI-enhanced, multi-loader TA type systems with moveable tag dispensers placed above waterholes in Africa so as to be able to tag specific species, for example zebras (*Equus quagga*) individually and to ignore wildebeest (*Connochaetes taurinus*). Such aspirations might seem lofty, but given that the security services can now track individual people as they move across the UK using only CCTV footage, we would be remiss not to consider it.

Summarized deployment considerations

Throughout this project, valuable insights into which factors do or do not impact animal-TA interactions have been identified. My results suggest that some species (such as otters) may interact with the TA more, so researching into species level neophobic/ exploratory behaviours will help when estimating how long it may take for an animal to habituate to the TA as ideally, researchers want minimal interactions with the TA as this increases the likelihood of the animal walking through it. Before placing the TA, researching into species home ranges and finding signs of animal presence (such as latrines) is important when finding an area which means the individual will actually come across the TA. Considering how the species uses space throughout time is also an important factor when placing TAs, as some seasons may have higher activity levels (such as Summer and Autumn). To enhance the number of individuals visiting the site in which the TA is placed, bait, pheromones and acoustic signals should be considered.

Conclusion

The findings of this study demonstrate that animals react to novel objects within their environment by looking, pausing and sniffing toward it, which is consistent with initial neophobia (Honey et al., 1992). Despite this, over time it was found that animals interacted less with the TA, and were more likely to walk under it following a period of habituation, and that significantly more individuals visited the site of the TA per night if baited. Thus, this part of the work - a first step in assessing the potential of the bur-tagging approach - indicates that it shows great promise, and that it can be modified according to target species to enhance its chances of success.

Future work needs to consider how to adapt the approach taken in this study according to the target species. As seen, there are species level differences when approaching the TA (for example, otters interacting the most with the TA, and badgers interacting the least) and previous research on the target species may elucidate whether the species is typically neophobic or exploratory which can help to predict habituation periods. Next, future work should also consider how the TA could be placed if the target species does not use trails, or leave obvious signs of inhabitance such as latrines. Whilst ways in which researchers may enhance the probability of tagging an animal (i.e., placing out the camera trap in an established home range before deploying the TA system) have been outlined, however, these considerations are on a general 'best-practice' basis. It may be that some of the suggestions differ substantially in their effectiveness depending on inter-individual space use, and individual neophobia.

Finally, the value of the bur-tagging depends on a number of other factors that were not looked at in my study including the accuracy with which the tag can be dropped in the right place on the animal, the adhesion of the tag to the animal, and the chances that the animal will not remove such a tag, with each of these being a study in their own. By understanding each of these aspects, we may hope to gain valuable and precise data from the tags on ecological and evolutionary processes in wildlife. In particular, quantifying the effects of environmental and climate change on species, and how best to further conservation efforts through evidence based management.

Appendices

Code

HABITAT ANALYSIS CODE

```
library(ggpmisc) #annotations for graphic
library(ggplot2) #graphics
```

```
library(scales)
library(viridis) #colour maps
library(extrafont)
library(lubridate) #good for dates as my R is in American
time library(plotly)
library(lattice)
library(fossil)
library(lme4) #fitting linear mixed effects models
library(Matrix)
library(lmerTest) #P values


setwd("C:/Users/COS-User/Documents/Research")
HA <- read.csv("first week habitat analysis.csv", sep = ",", header = T)


HA$R_Date <- as.Date(HA$Date, format = "%d/%m/%Y") # specify date format
HA$day <- as.numeric(strftime(as.POSIXlt(HA$R_Date), format = "%j")) HA <-
subset(HA, HA$R_Date > "2022-01-01") # specify 2022 HA$R_Date <-
as.Date(HA$Date, format = "%d/%m/%Y")
HA$day <- as.numeric(strftime(as.POSIXlt(HA$R_Date), format =
"%j")) HA <- subset(HA, HA$R_Date > "2022-01-01")
HA$Time.seconds[HA$Time.seconds == "<1"] <- 1


dim(HA)
names(HA)
str(HA)
```



```
head(HA)
tail(HA)
summary(HA)
```

```
HA$WTG_mod[HA$WTG == "No"] <- 0

HA$WTG_mod[HA$WTG == "Yes"] <- 1


> summary(m3a)
Call:
glm(formula = as.numeric(HA$WTG_mod) ~ Avg.plant.height, family = binomial,
    data = HA)


Call:
glm(formula = as.numeric(HA$WTG_mod) ~ Path.Density, family = binomial,
    data = HA)
> summary(m3c)
Call:
glm(formula = as.numeric(HA$WTG_mod) ~ Quadrat.., family =
    binomial, data = HA)
> summary(m4a)
Call:
glm(formula = WTG_mod ~ Camera.distance, family = binomial, data = HA)
> summary(m4b)
Call:
glm(formula = WTG_mod ~ Camera.angle, family = binomial, data = HA)
```


########Set WD for other excel spreadsheet

<u>WALKING THROUGH TA & NEOPHOBIA CODE</u>

```
S1 <- read.csv("STAGE1.csv", sep = ",", header = T)

S1$R_Date <- as.Date(S1$Date, format = "%d/%m/%Y")
S1$day <- as.numeric(strftime(as.POSIXlt(S1$R_Date), format =
"%j")) S1 <- subset(S1, S1$R_Date > "2022-01-01") # specify 2022
S1$R_Date <- as.Date(S1$Date, format = "%d/%m/%Y")
S1$day <- as.numeric(strftime(as.POSIXlt(S1$R_Date), format = "%j"))
S1 <- subset(S1, S1$R_Date > "2022-01-01")
S1$Time.seconds[S1$Time.seconds == "<1"] <- 1
summary(S1)


S1$WTG_mod[S1$WTG == "No"] <- 0
S1$WTG_mod[S1$WTG == "Yes"] <- 1
S1$WTG_mod <- as.numeric(S1$WTG_mod)
#### converting yes no into binary

###new model for total interactions. Total interactions is count data therefore the family
needs to be changed to poisson#####

Formula: Total_interactions ~ Days.TA.out + (1 | Site)
Family: poisson ( log )
Formula: Total_interactions ~ Species + (1 | Site)
 Family: poisson ( log )
Formula: Total_interactions ~ Direction + (1 | Site)
Formula: Total_interactions ~ Attraction + (1 | Site)

#getting p-values

> m12aa<-update(m12a,~.-Days.TA.out)
> anova(m12aa,m12a,type='Chi')
```

```
> m12bb<-update(m12b,~.-Species)
```

```
> anova(m12bb,m12b,type='Chi')

> m12cc<-update(m12c,~.-Direction)
> anova(m12cc,m12c,type='Chi')

> m12dd<-update(m12d,~.-Attraction)
> anova(m12dd,m12d,type='Chi')

#####new glm for WTG
S1 <- read.csv(“S1viva.csv”, sep = “,”, header = T)
View(S1)


S1$R_Date <- as.Date(S1$Date, format = “%d/%m/%Y”) S1$day <-
as.numeric(strftime(as.POSIXlt(S1$R_Date), format = “%j”)) S1 <-
subset(S1, S1$R_Date > “2022-01-01”) # specify 2022 S1$R_Date <-
as.Date(S1$Date, format = “%d/%m/%Y”) S1$day <-
as.numeric(strftime(as.POSIXlt(S1$R_Date), format = “%j”)) S1 <-
subset(S1, S1$R_Date > “2022-01-01”)
S1$Time.seconds[S1$Time.seconds == “<1”] <- 1
summary(S1)
S1$WTG_mod[S1$WTG == “No”] <- 0
S1$WTG_mod[S1$WTG == “Yes”] <- 1
S1$WTG_mod <- as.numeric(S1$WTG_mod)
#### converting yes no into binary


S1$Total_interactions<-as.numeric(S1$Looks.S) + as.numeric(S1$Pauses) +
as.numeric(S1$Sniffs)
```

```
m5 <- glmer(WTG_mod ~ Total_interactions + Days.TA.out + Species + Direction +
Attraction + (1|Site), data = S1, family = binomial)

summary(m5


m5 <- glmer(WTG_mod ~ Total_interactions + (1|Site), data = S1, family = binomial)

summary(m5)

Formula: WTG_mod ~ Total_interactions + (1 | Site)

  Data: S1

m6<-update(m5,~.-Total_interactions)

anova(m5,m6,type='Chi')


m5 <- glmer(WTG_mod ~ Days.TA.out + (1|Site), data = S1, family = binomial)

summary(m5)


m6<-update(m5,~.-Days.TA.out)

anova(m5,m6,type='Chi')

m5 <- glmer(WTG_mod ~ Species + (1|Site), data = S1, family =

binomial) summary(m5)


m6<-update(m5,~.-Species)

anova(m5,m6,type='Chi')


m5 <- glmer(WTG_mod ~ Direction + (1|Site), data = S1, family =

binomial) summary(m5)


m6<-update(m5,~.-Direction)
```

```
anova(m5,m6,type='Chi')


m5 <- glmer(WTG_mod ~ Attraction + (1|Site), data = S1, family = binomial)

summary(m5)

m6<-update(m5,~.-Attraction)

anova(m5,m6,type='Chi')

#######STAGE 2 ANALYSIS#######

setwd("C:/Users/COS-User/Documents/Research")

FBD <- read.csv("FOXBAITDAILY.CSV", sep = ",", header = T)


dim(FBD)
names(FBD)
str(FBD)
head(FBD)
tail(FBD)
summary(FBD)

FBD1<- glm(`No. fox` ~ Trial, data = FBD, family = poisson)
anova(m3)
anova_result <- anova(FBD1, m4, = "Chisq")
print(anova_result)
```

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