



**Diet and distribution of the Eurasian otter, *Lutra lutra* and
American mink, *Neovison vison* on the River Hull**

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requirements for the degree of

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**I declare that to the best of my knowledge this thesis is my own original work. To the
best of my belief it does not breach copyright or other intellectual property rights of
another party.**

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Abstract

Conflict between the invasive American mink *Neovison vison*, and native Eurasian otter *Lutra lutra* has been identified in several studies, however many questions remain surrounding factors contributing to possible co-existence. Furthermore, each species presence poses possible further economic and ecological threats. Links between mink predation and water vole, *Arvicola amphibius* decline have been listed as a key factor in the vole's high risk categorisation. Understanding of mink preference to endangered species therefore may be essential in future conservation efforts. In contrast, otter-human conflict has potential to negatively impact upon efforts to restore otter populations. Regular spraint collection and eDNA analysis have been combined to identify distribution, diet and species specific preferences. Whilst distribution analysis did not support the possibility of co-existence, mink diet has identifiably evolved to reduce conflict with otters in the region. Within this, mink were found to predate on endangered species, supporting removal efforts. Contrastingly, otter preference data was not concluded to display a preference for stocked species.

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Chapter 1 Introduction

1.1 Introduction

Invasive species can be particularly devastating in sensitive or rare ecosystems (Chornesky & Randall, 2003). The loss of species specific to such habitats also has a knock-on effect on wider global diversity (Chornesky & Randall, 2003; Polidoro, et al., 2010). Chalk streams have been recognised as a globally important rare habitat (Bowes, et al., 2005). However they are at risk, with an acknowledged link between the existence of invasive species and degradation (Bowes, et al., 2005).

The degradation of chalk stream habitat has a direct impact on the populations of rare and endangered species, including the water vole, *Arvicola amphibius* and brown trout, *Salmo trutta*. In particular, the preservation of chalk streams could be vital for maintaining reproductive success in trout. The sedimentary floor of chalk streams is an important spawning site for brown trout (Acornley & Sear, 1999) and the characteristics of chalk streams; low banks, submerged vegetation and unmodified channels, greatly compliment that required by the water vole (Barreto, et al., 1998). As a species facing decline across Britain (Rushton, et al., 2000), restoration of habitat, including chalk streams will be an important factor in preventing species extinction (Barreto, et al., 1998).

On a wider scale, understanding the broader ecology of chalk streams may be crucial for protecting at risk species. Information on the distribution of the invasive American mink, *Neovison vison* in this habitat and its interaction the native Eurasian otter, *Lutra lutra* could provide valuable insight to conservation efforts. One evolving ecological interaction in need of further exploration on chalk streams is the possible competition and aggression evolving between the two species has been following the release of the American mink peaking between the 1950s and 60s. The invasion of mink has also been linked to the decline of water voles and several bird species (Rushton, et al., 2000), which encouraged the widespread removal of American mink in conservation areas. Simultaneously, the decline of the American mink across Europe as a whole corresponded with an increase in otter populations (McDonald, et al., 2007). Therefore it has been suggested that the reintroduction and population expansion of the Eurasian otter is directly linked to the reduction of mink populations range makes

attention on restoring the species beneficial. However, whilst the wider literature often supports this theory, the suggestion of eventual co-existence between the two species is also widely expected. Evidence of diet adaptation in the Eurasian mink diet presents opportunity to reduce conflict between the species allowing co-existence to arise (Bonesi & Macdonald, 2004; Bonesi, et al., 2004).

Should aggression exist, counter-marking is to be expected (Johnston, et al., 2010). Counter-marking is described as when one individual leaves a deposit or spraint, on top of, or in close proximity to the spraint or deposit of a second individual (Johnston, et al., 2010). Such marking is commonly used in territory defence (Fisher, et al., 2003). Understanding the degree of countermarking however, comes with a number of difficulties. Prominently, due to both species occupying large areas the regularity of site checks to discover visible signs of counter-marking occurring is difficult to ensure in a small team (Copp & Roche, 2003; Roche, et al., 1995; Melero, et al., 2008). This is enhanced by sprainting type variety, as non-solid spraints degrade in a shorter time period (Gorman, et al., 1978).

1.2 Wider Context

When managing the restoration of species, and conservation of an area, it is important to understand how different species impact each other. Therefore the wider ecosystem must also be considered. Species within an ecosystem are adapted, often perfectly, to fill a specific ecological niche (Polechová & Storch, 2008). Consequently, the impact of losing or introducing a species can have a larger impact on the entire system (Charles & Dukes, 2008). Ecological networks are described as the interactions which occur between individuals within an ecosystem (Ings, et al., 2009). Nevertheless, not only must the presence or absence of these interactions be explored, but also the impact of differing interaction strengths of each interaction (Neutel & Thorne, 2014). Construction of meaningful networks is difficult, as traditional sampling is often inefficient which allows indirect interaction to be missed (McCann & Rooney, 2009). Predominantly, the interactions in a network are weak, with a few strong interactions such as that between specialist predators and prey (Bascompte, et al., 2005). Consequently, understanding the impact of population change in certain species, such as apex or invasive species is important for management and conservation in an area.

1.2.1 Trophic interactions

Ecological networks are maintained by negative feedback loops that promote stability and resist change (Robertson, 1991). In most cases, predator populations are largely prey dependent, balanced by bottom-up control (Wallach, et al., 2015). The resultant ecological pattern results in self-regulation; through systems including reproductive suppression, ensuring that top apex predators are unable to over-exploit valuable resources (Wallach, et al., 2015). Should a change occur within an ecosystem however, regulatory interactions can be lost often creating exponential population explosions or degradation, in lower tropic level species (Robertson, 1991; Barlas, 2007). This is a consequence of positive feedback loops; a feedback process with capability to vastly change aspects of a network by exacerbating the effects of a small disturbance (Robertson, 1991; Barlas, 2007).

The diet type of predators fall within one of two categories; specialists, a species with a homogeneous diet; consistent of mainly one species or group of species (Mody, et al., 2007), or generalists, species in which individuals have a highly diverse diet (Richmond, et al., 2005) Their impact on an ecosystem can therefore differ, with specialists species suppressing the populations of a fewer species, at a higher frequency (Simon & Toft, 1991) and generalists at a smaller frequency over many more species (Richmond, et al., 2005) . Equally, specialist species can be more vulnerable should prey species populations decline (Richmond, et al., 2005).

Apex predators can have substantial impact on an ecosystem, acting as top-down control on species in lower tropic levels (Wallach, et al., 2015). Their role in the control of mesopredators, (smaller predators (Johnson & VanDerWal, 2009)), and herbivores (Brook, et al., 2012) aids the regulation and balance of species diversity. Consequently, strict control or reduction of these species often causes trophic cascades such as changing the abundance and behaviour of mesopredators (Brook, et al., 2012).

As a result, predation is one of the strongest selection pressures acting on organisms in an ecological network, with the ability to impact the entire ecological community (Rizzari, et al., 2014). However, although apex predators are particularly important for ensuring balance is maintained in the ecosystem (Hollings, et al., 2016), understanding apex predator roles and interaction, must also consider a range of factors; e.g. climate,

population abundance, vegetation (Davis, et al., 2012). Predators are believed to be increasingly vulnerable to human persecution, environmental and anthropogenic changes (Pedersen, et al., 2007; Sozio & Mortelliti, 2016). For example, the human-induced introduction of feral cats, *Felis catus*, caused a decline population of the wild Tasmanian devil, *Sarcophilus harrisii*, due to the introduction and spread of DRTD (devil facial tumour disease). The impacts of these threats can be particularly devastating in apex predators. Characterised as a species with a conservative life history, such as, delayed sexual maturity and slow growth rates (Rizzari, et al., 2014), population sizes are often small. Therefore many populations only have a very limited buffer against extinction (Fagan, et al., 2001) so rapidly become vulnerable as threats increase.

Whilst many face threats, recent advances have highlighted that predators can play a key role in habitat restoration against processes such as climate change (Ritchie, et al., 2012). These impacts can be either direct linked between species or indirect. Whilst changes in a direct interaction between species can be essential for maintaining one group or aspect, indirect impacts have a broader impact, as they don't only impact solely on one individual group (Rizzari, et al., 2014). As a consequence, it is understood they will limit explosive population growth of both introduced species and also of invasive species (Wallach, et al., 2015).

Alternatively, competition may exist should there be overlap in predator prey preferences, such as foraging habitat or prey size range (Spitz, et al., 2006). Should overlap not be identified however, the impact of seasonal change can also impact aggression levels, with seasonal food limitation forcing overlap, and competition to exist (Jones & Barmuta, 2002). If overlap is found, this may also be a possible explanation for low competitive species density (Jones & Barmuta, 2002). This may in the case of otter and mink, explain a recorded decline in mink following otter reintroduction (Bonesi & Macdonald, 2004).

1.2.2 Competition

Competition exists in two forms. Inter-specific competition exists between individuals of different species (Cushman & Addicott, 1989), over aspects including food and shelter availability. Alternatively, intra-specific competition exists between members

of one species (Cushman & Addicott, 1989), such as competition for a mate (Paul, 2002).

In inter-specific competition, a decline in the population of the competitor species is likely to increase lifetime fitness, due to greater resource access. Within some species, these interactions have been found to influence not only the populations of species

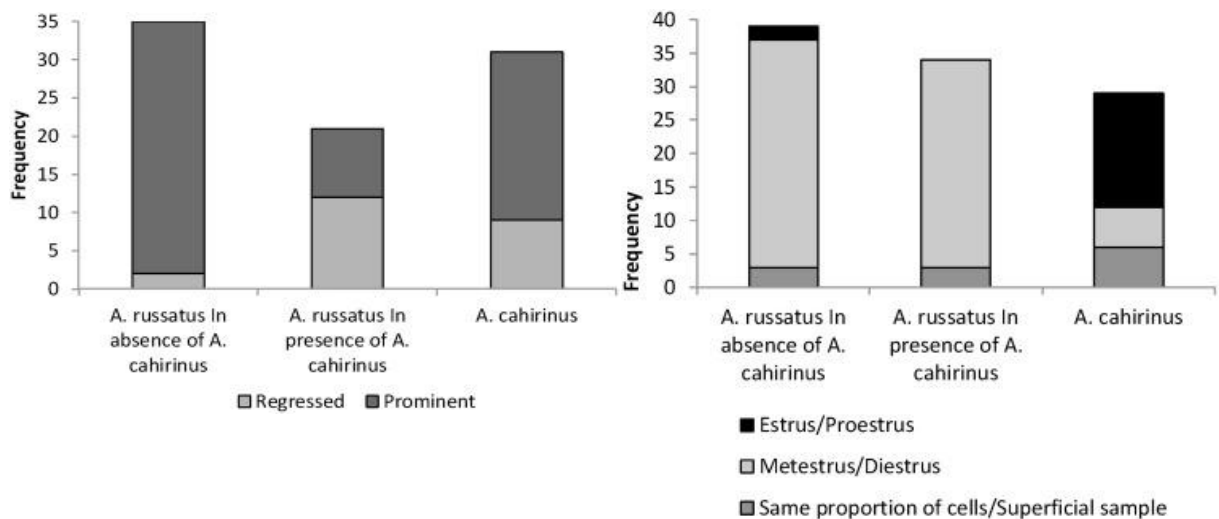


Figure 1: Two graphs displaying the impact on reproductive state in males (left) and females (Right) of the species *A. russatus*. Stage frequencies were identified to have caused significant differentiation between species in males, $p < 0.001$ (Fisher exact test). Alternatively no difference was seen between treatments in females, $p = 0.4953$ (Fisher exact test) (Katz, et al., 2018).

involved, but also wider carnivore species (Caro & Stoner, 2003). The subordinate species however is likely to suffer from declined fitness through reproductive suppression and resource loss (Katz, et al., 2018). Variation is also suggested to differ between sex and age. A study by Katz, et al. (2018) suggests that males and young are more clearly impacted by fitness decline in comparison to females in subordinate species (Figure 1). Therefore, inter-specific variation, including sex-ratio of the invasive and native competitors should be considered if possible. However in both cases, both sexes still face significant reproductive costs as a result of NIS introduction. Nevertheless, whilst invasions are seen as a direct cost, in comparison to factors like fragmentation and habitat loss, the impact of intra-specific on ecological communities has been found to be relatively minor (Sozio & Mortelliti, 2016).

Inter-specific competition has been further categorised in to two types; exploitation, a rivalry for resources, and interference, such as aggression (Liesenjohann, et al., 2011; Maurer, 1984). In either case, it is mutually beneficial for two competing species to evolve traits for competitor recognition (Grether, et al., 2009). As a result, this often

leads to the evolution of traits to allow individuals to avoid each other from visual or olfactory cues (Caro & Stoner, 2003). For this reason, encounter rates decline through behavioural change (e.g. alternate activity schedules) (Grether, et al., 2009). This often also impacts on morphology, physiology and selection for ecological divergence (Hoogland & Brown, 2016). Consequently, impacts on species will cause shift in habitat use, produce exploitive competition and promote aggression (Caro & Stoner, 2003). Measuring the impacts of inter-specific competition in the wild however is difficult(Hoogland & Brown, 2016). Therefore, the level of focus on investigation of the importance of variation in predator social structure and behaviour in influencing the interactions between tropic levels is insufficient (Ritchie, et al., 2012).

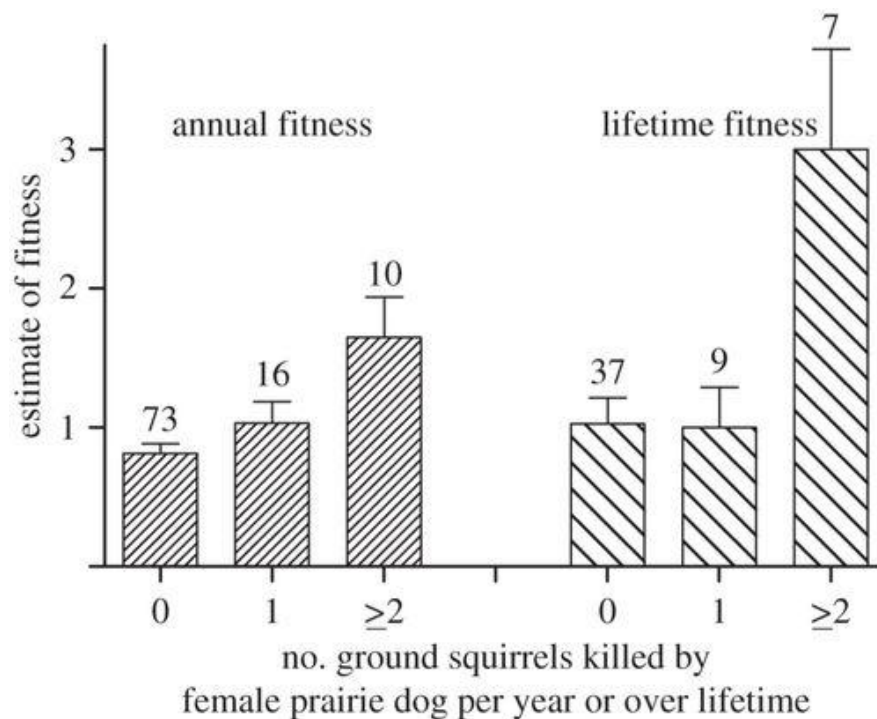


Figure 2: A graph displaying the relationship between active killings of ground squirrels by female prairie dogs on annual fitness (left) and lifetime fitness (right) (Hoogland & Brown, 2016).

The impact of interaction in prairie dogs (*Cynomys leucurus*) and ground squirrels (*Urocitellus elegans*) was analysed in a study by Hoogland & Brown (2016). Both species interact by inter-specific competition over vegetation for food. The results found that Prairie Dogs who actively killed squirrels had greater lifetime fitness (

Figure 2). This is likely due to decreased overall competition (Hoogland & Brown, 2016). These results are expected, with aggression echoed throughout ecosystems (Freeman, 2016; Anjos, et al., 2017) as the decline in competition increases reproductive success for the dominant species.

Comparatively, outcompeted species often evolve new traits or behaviours to avoid head to head confrontation such as; diet preference change, temporal overlap avoidance, or change in hunting ground (Křivan, 2003; Anjos, et al., 2017). Such adaptive foraging behaviours for example are often used by competing predators to allow for co-existence (Křivan, 2003).

1.2.3 Invasive Species

As one of the major man-made cause of ecosystem disruption, the introduction of an invasive species (Mooney & Cleland, 2001) can be harmful to an ecosystem. These species invade an ecosystem, their population expanding out of the natural range through human-mediated dispersion (Lee, 2002). Whilst only a small proportion of introduced species establish, invading an ecosystem (Williamson & Fitter, 1996), the impacts can be very harmful on the populations of native species (Lee, 2002). Consequently, NIS are widely associated with the degradation of native populations and are a leading cause of biodiversity loss (Hollings, et al., 2016; Didhama, et al., 2005). Impact occurs through new interactions (e.g. predation, disease), with no or limited regulation on invasive species population growth (Charles & Dukes, 2008).

In particular, NIS have been identified as one of the impacting factors in the extinctions of over 50% of species recorded by the IUCN (Clavero & García-Berthou, 2005; Hollings, et al., 2016). This data however, is increasingly being questioned, facing denialism; the rejection of undisputed scientific belief (Russell & Blackburn, 2017). This shift in belief is occurring as data proving a link is often supported by speculative data and limited observation (Gurevitch & Padilla, 2004). Nevertheless, despite debate, trends display that prey species are most heavily impacted, caused by a lack of the evolution of invasive predator avoidance behaviours (Hollings, et al., 2016).

As a result, this creates behavioural shifts in invasive species, with many studies highlighting their role in the evolutionary pathway of native species (Mooney & Cleland, 2001). Such adaptations can occur due to impacts including competitive exclusion,

introgression and predation (Mooney & Cleland, 2001). To predict and follow distribution use and spread by Invasive species, environmental conditions and niche theory is commonly used (Kulhanek, et al., 2011). This states that ecological conditions govern the area where species are able to establish or maintain the density of their population(Kulhanek, et al., 2011). Evolutionary change however, is not only seen within effected native species, but also in the invasive species themselves.

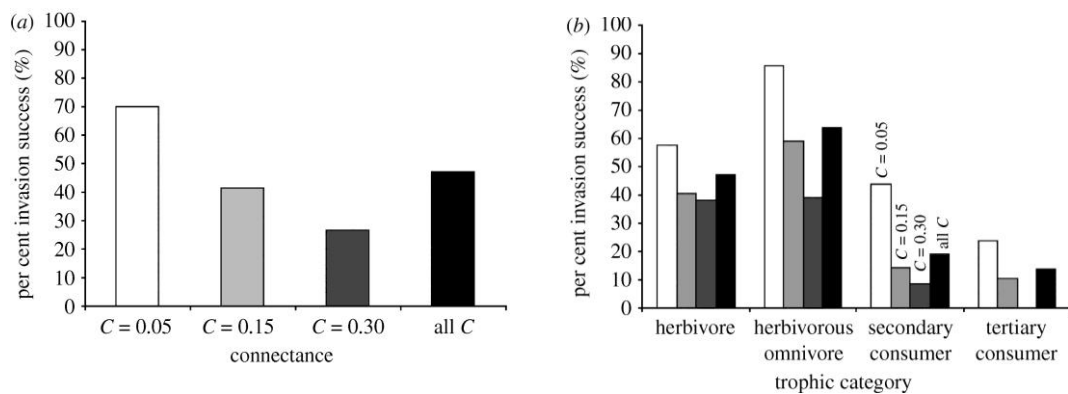


Figure 3: a) The impact of connectance on invasion success. b) Invasion success variation between different trophic categories.

In a study by Romanuk et al., (2009), niche models were used to estimate the success of species with varying connectivity to other species, and trophic categories. The resulting models predicted that invasive species with low connectance were most likely to be successful invaders (Figure 3. a). This was particularly true if the predation risk was low. Connectivity, between predator and prey, as well as competing predators, is often high causing predatory invaders, tertiary and secondary consumers to have considerably lower invasion successes (Figure 3. b). Success for these groups however is increased in ecosystems with fewer top predators. Although not clearly outlined by the study, is likely to be caused by greater completion for food, which is indicated to be an influence through connectivity level in all categories. These results support that adaptations to new abiotic factors and interaction with native species are key in the success level of NIS (Mooney & Cleland, 2001). Therefore, understanding interactions formed by invasive species may be vital in deciding appropriate conservation processes.

Both native- NIS and predator-prey interactions are impacted by and influence levels of competition(Morin, 1986). Competitive displacement is one of the key hypotheses for decline of native species (Bando, 2006). This occurs when niche overlaps occur

creating direct competition for food, shelter or territory between two species (Mills, et al., 2004). The success of invasive species can be influenced by their degree of resilience in a new habitat. Factors such as a larger adult body size, more diverse diet or greater tolerance to human presence can therefore be advantageous to either species involved (Mills, et al., 2004).

1.3 Molecular Analysis

Investigation of predation and competition between NIS and native species can be increasingly explored with the development of molecular analysis. As a result molecular analysis has propelled in use and development in recent years (Barnes & Turner, 2016). For example the sequencing and analysis of environmental DNA; genetic material extracted from environmental samples such as soil, water or air (Barnes and Turner, 2016). The development of environmental DNA (eDNA) analysis has enabled the detection of multiple predators in a single sample (Rodgers & Mock, 2015), allowing possible counter-marking to be identified. This is achieved with metabarcoding; the ability to relate DNA sequences to different taxonomic groups (Deiner, et al., 2017; Lamb, et al., 2019). The use of eDNA has seen a rapid increase in recent years (Rodgers & Mock, 2015), where many studies have focused on the ability to study species diet, without the identification specialisation required for traditional morphological analysis (Klymus, et al., 2017). Dietary overlap is a common cause of inter-specific competition, with aggression present when multiple predators share the majority, or part of their prey range (Spitz, et al., 2006).

Spraint analysis has also been increasingly used for dietary analysis (Taberlet, et al., 2012), containing DNA full or fragments of all the species in an environmental sample (Barnes & Turner, 2016). Analysis of these samples has the potential to display previously hidden preferences and overlap. In addition, there is a further need to evaluate the seasonal effect on diet (Bartoszewicz & Zalewski, 2003), this may also effect if and when conflict is strongest, as demand for certain food types may increase or decrease.

1.4 The Eurasian Otter, *Lutra lutra*

The Eurasian otter, *Lutra lutra* is a riverine apex predator found across the majority of Europe, as well as parts of Asia (Ruff, 2007). The species has been argued to be one of

the most important freshwater predators in Europe (Lyach & Čech, 2017). In many areas, the exact distribution and population levels of the Eurasian otters are simply unknown (Roche, et al., 1995; Bonesi & Palazon, 2007). Where levels are recorded there is a predominant focus on tracking reintroduced individuals, which have been shown to establish home ranges of up to 40km in length (Roche, et al., 1995).

The influence of competition however, could be a primary factor in the considerably reduced home ranges of otters in already established areas, with males maintaining an area of around 15km (Trowbridge, 1983). Comparatively, habitat ecology has already been found to impact on territory scale, with prey availability, and water quality impacting on population stability and structure (Ruiz-Olmo & Jiménez, 2009).

1.4.1 History of the British Otter Population and Current Status

Otter numbers within the United Kingdom (and across most of Europe and Asia (Li & Chan, 2018) saw rapid decline in the 1970s due to predominantly man-made threats, such as over-extraction of water, pollution and fragmentation (Hobbs, et al., 2006). This led to population decline, with many populations dying out completely (Lever, 1978). Despite previous concern that the Eurasian otter, along with several mustelids may become extinct in the UK, a study by McDonald et al. (2007), displayed that efforts to restore populations are having a positive impact. This trend has since continued with The Peoples Trust for Endangered Species (PTES) recording continued gradual increase of otter populations, estimated at 10,300 across Britain in (Species, 2020). This is in line with a growing number of reports each year by organisations and the public, with over 21,200 reports since 2010 in comparison to 11,200 between 1990 and 1999 (NBN Atlas, 2020). Recovery is largely as a result of legal action preventing purposeful killing of otters, as it was placed under protection of the Wildlife and Countryside Act 1981, as a European protected species (EPS) (Wildlife and Countryside Act, 1981). This has been proven to be successful with recent studies, finding British populations to be in steady and considerable recovery (Figure 4) (McDonald, et al., 2007; Mason & Macdonald, 2004).

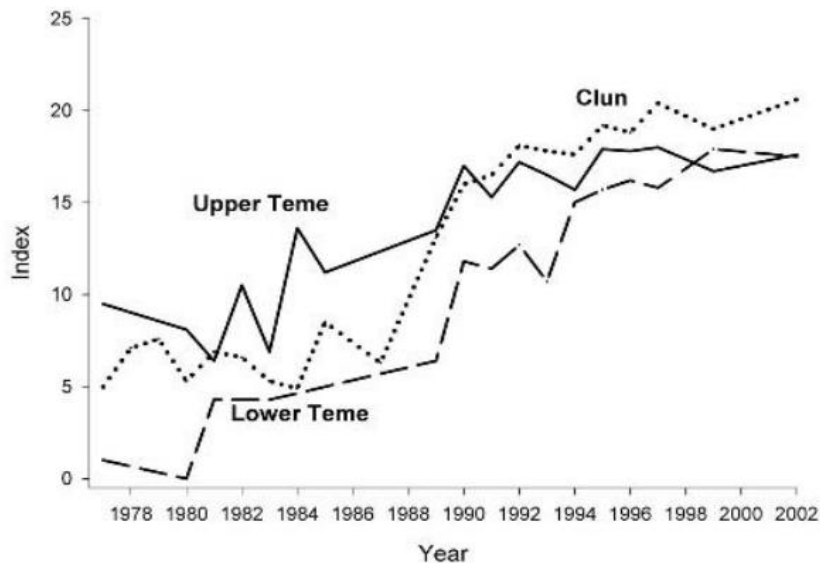


Figure 4: Otter population indexes for the River Teme Catchment (Mason and Macdonald, 2004).

Nevertheless, with much of the restoration in natural populations occurred as an indirect impact. The recovery of both fish and invertebrate populations has supported the recovery of Eurasian Otter populations *Lutra Lutra* (Wood, et al., 2014). However, this has resulted in relatively unknown otter population sizes and dynamics.

1.4.2 Feeding ecology

It is widely understood that the Eurasian otter are fish specialists but can have a varied diet, with high differentiation (Adrian & Delibes, 1987; Britton, et al., 2006; Ruiz-Olmo & Jiménez, 2009). A study completed between 1979 and 1984, by Adrian and Delibes (1987), in Lucio Bolin, found that by using spraint analysis, fish was the most abundant food choice by the Eurasian otter, followed by insects (32%) and amphibians (28%). Similar outcome was established by study in South West England by Britton et al., (2006) between 1999 and 2003. Nevertheless, debate exists concerning the cause for differentiation in otter diet, with earlier studies suggesting that it was habitat characteristics and structure that were the main cause for diet change (Jedrzejewska, et al., 2001).

A high level of fish in otter diet is considered to display a relatively stable and balanced habitat, with a less diverse diet seen (Ruiz-Olmo & Jiménez, 2009). Investigation in to effecting factors, predominantly size, found fish prey selection had a preference towards species in particular individuals in a 500g-1000g size range (Lanszki, et al.,

2001). The study established that species were disregarded above 1000g. Size was again found to be a key influence in a recent study by Britton et al. (2017), finding that pike *Esox Lucius* were the only species with specimens over 350mm found in otter spraint.

Size preference is commonly paired with belief that fluctuations in diet diversity are impacted by ecosystem stability and water availability (Clavero, et al., 2003). Aquatic species composition however, is also believed to have impact within this. A study in South West England by Britton et al., (2006) between 1999 and 2003, found the majority of fish consumption was of one species eel, *Anguilla anguilla*. The eel is believed to be favoured for both its size and high fat to weight ratio, and ease to capture (Carss, 1995; Beja, 1996). In contrast, in other UK regions, the eel is only a very minor diet component, replaced by cyprinid species (Copp & Roche, 2003). Consideration must also be taken for seasonal change, as diet preference was also found to be seasonally influenced, with fish populations being significantly more important in summer than winter months (Britton, et al., 2017).

Dietary data provides important information for informing conservation conflicts between otters and fishery interests (Britton, et al., 2017). The convenience of isolated fish populations in man-made constructions, such as ponds and fish farms have been found to be used by otters for convenient meals. Consequently, individuals appear to target fish ponds (Adámek, et al., 2003). A study by Adámek et al, (2003) in the Czech Republic, found that fish taken from these ponds, most commonly the common carp, *Cyprinus carpio*, made up around 80% of otter diet. Therefore, without the correct measures, due to long term memories and repetitive hunting behaviours, in many cases discovered areas are likely to become common hunting grounds (Lanszki, et al., 2001). As a result, conflict largely exists as anglers believe otters predate to an extensive level on preferred angler fish, emptying fish stocks (Lyach & Čech, 2017). This theory is supported by the findings of Almeida et al, (Almeida, et al., 2012) which found otter will prey on the most abundant species, even if less preferred. Therefore, well-stocked farms can become ideal in the dispersal of otter across Europe.

Debate however still exists around the extent of otter predating in fish farms. In 2017, Lyach and Čech (2017), found that otter and anglers caught significantly different fish

populations. In comparison, an alternative study of fish stocking in the Danish lowlands did find otter predation to increase on trout by 25%, suggesting a preference for stocked fish to wild fish (Jacobsen, 2005). Hatchery trout however, were shown to be ignored by otters (Jacobsen, 2005). Division in preference may be an outcome of different dominance levels, as in contrast younger and less dominant males are more likely to hunt non-native species (Jacobsen, 2005).

1.4.3 Behaviour

In the Eurasian otter distribution has been found to vary between sexes and age species (Koelewijn, et al., 2010). The distribution of offspring varies, with males being forced to migrate away from the area where they were born, whilst females commonly obtain the territory next to their mothers (Koelewijn, et al., 2010). Therefore, multiple females will be found within a dominant male's territory (Koelewijn, et al., 2010). As scent is used by otters to mark territory zones (Rostain, et al., 2004), and aid less dominant males, when avoiding more dominant males as meetings can be lethal, it can be hypothesised that dominant males will be the most common sample type.

One area concerning the European otter that appears relatively understudied is differentiation in spraint type. Spraint has been linked to preserving Resource Holding Potential (RHP), the ability for an individual to win an all-out fight, as it provides a sign of if an individual should be ignored, or is worth risking meeting (Kruuk, 1995). There are multiple acknowledged spraint types, mostly separated in to solid spraint, and more fluid anal jellies (Van den Brink & Jansman, 2006). Anal jellies are associated with fertile periods (2 weeks at a time) in both males and females (Laidler, 1982). With no set breeding period and embryonic diapause, a trait of all mustelids, with the exception of sea otters *Enhydra lutris*, is that it is common for the female to become pregnant immediately (Amstislavsky & Ternovskaya, 2000).

Females begin breeding at around two years old, and males four (Koelewijn, et al., 2010), likely due to a need to firstly establish territory. Both dominance and fertility have also been found to be linked to spraining location (Ruiz-Olmo & Jiménez, 2009). A study by Ruiz-Olmo (2009), found that most variation in spraint placement in a captive environment occurred between mating males and females; both leaving spraint in obvious landmarks. Secondly, dominant males would also use such

landmarks whilst remaining individuals prioritised holt entrances or ground placement. Observations have also detected that in areas where no landmark exits, they can be created with grass twists, or soil heaps (Woodroffe, 2007).

The lowest levels of spraint were found to be from females outside their fertile period (Ruiz-Olmo & Jiménez, 2009), suggesting that distribution of the two will differ. However, without individual DNA identification this will be difficult to track. Females are likely to wish to remain undetected as parental care in the Eurasian otter is carried out by the female (Woodroffe, 2007). As a result, they often maintain a considerably smaller home range than males, focused around a safe holt when young are present (Woodroffe, 2007). Care usually occurs for around one year, with pups remaining in the holt permanently for two months (Laidler, 1982). With this, sprainting trends are believed to differ with age, for example, females are known to eat the excrement of cubs up to fourteen weeks when weaning ends (Laidler, 1982). Again it appears relatively unknown how the female spraints at this time, although most studies suggest they will spraint in water to discourage detection (Ruiz-Olmo & Jiménez, 2009).

1.5 The American Mink, *Neovison vison*

The American mink is an invasive species introduced in to European countries, including Ireland and the United Kingdom, in the period between the 1920s and 1950s. The species was predominantly introduced for fur farming (Smal, 1988; Bonesi & Palazon, 2007; Kauhala, 1996). These farms predominantly existed within northern countries (Bonesi & Palazon, 2007), however following escape, mink were successful at establishing populations in the wild (Smal, 1988). As a result, the species is now found across the majority of Europe (Mitchell-Jones, et al., 1999).

1.5.1 Invasion History and Current Population Status in the UK

In the UK fur farming was banned in the UK in 2000, under the Fur Farming (Prohibition) Act, leading to the end of mass release of mink (Linzey, 2006). Population numbers are now declining in some European countries, the cause however is unknown (Bonesi & Palazon, 2007), although it is partly due to increased capture and kill efforts. It is believed that human development and otter repopulation may be partly responsible, as if competition is to exist otter is believed to be the stronger competitor (Kauhala, 1996; McDonald, et al., 2007). The impacts of invasive mink, both

ecological and economical are however still being seen, although to a lesser extent than previously believed (Smal, 1988; Bonesi & Palazon, 2007).

Population decline of mink is seen as beneficial, particularly on rare habitat types. The impact of the American mink has been linked to the degradation of chalk stream habitat (Søndergaard & Jeppesen, 2007). The impact is partially clear in the impact on water voles, *Arvicola amphibious*, are considered key players to the maintenance of rare ecotypes (McDonald, et al., 2007). Voles have direct benefits occurring through nutrient cycling and resource preservation (White, et al., 1997).

The impact of the American mink is highlighted in a recent study by (Brzeziński, et al., 2018), who identified that mink presence creates an observable behavioural change in water vole populations. The probability of vole occurrence overall, was found to be lower in areas with high mink populations (Figure 5). In conjunction, areas seemingly preferable to water voles still saw population decline significantly when mink were present (Figure 5). In spite of this, the impact of mink alone cannot be entirely blamed

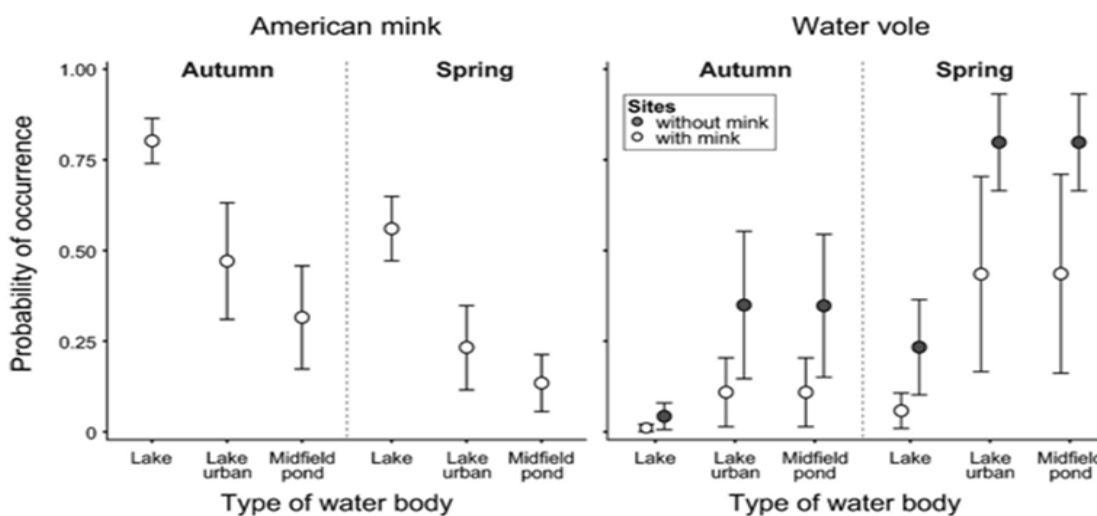


Figure 5: The probability of occurrence (accompanied by 95% CIs) of the American mink (two left-hand panels) and water vole (two right-hand panels) in relation to type of water body and season as predicted by the GLM models (Brzeziński et al, 2018).

for vole decline, with links found between fragmentation, degradation and population decline (Rushton, et al., 2000). Interestingly however, mink decline has been linked to otter population restoration, as it appeared in balance with the decline of the invasive American Mink, a subsequent increase of 18% to 80% in population numbers recorded. This was on par with mink population declines of between 20%-80% (McDonald, et al.,

2007). Therefore, it can be suggested that otter may play a role in the restoration of vole populations, through aggression towards invasive species.

1.5.2 Feeding Ecology

The diet of the American Minks is also affected by age, sex and seasonal variation. All have been found to influence diet but can also be affected by habitat type and prey behaviour (Birks & Dunstone, 1985; Bartoszewicz & Zalewski, 2003; Chanin & Linn, 1980). American mink are generalist, but their diet mainly consists of birds, fish and mammals, proportions of which are impacted by availability (Chanin & Linn, 1980). Yearly diet composition in females has been found to contain a higher percentage of fish and crustaceans than that of males (Birks & Dunstone, 1985). This was however, found to vary throughout the year due to seasonal change (Birks & Dunstone, 1985). A study by Bartoszewicz & Zalewski (2003) looked more closely at the influence of seasonal variation in the Słofsk Reserve, Poland. They identified that during the winter months, mammals and fish made up the mass majority of diet (up to 56% and 62% respectively). Comparatively, birds made up only 4-16%, which increased greatly to between 35-60% in the summer months. This was presumably due to higher bird population numbers, due to migration and breeding periods. Despite high consumption, American mink arrival was not shown to impact on the abundance of waterfowl, in all areas, in contrast to expectation (Bartoszewicz & Zalewski, 2003).

Dietary variation has also been displayed to occur at an individual level (Haage, et al., 2017). Haage, et al (2017), found that whilst the majority of individuals in mink populations were generalists, others specialised, for example, in fish and crayfish species. Individual specialisation type was seen to be effected by numerous factors (demographic traits, ecological opportunity, competition, learning, animal personality) and directly impacted the individuals fitness. To examine specialisation, a series of feeding trials were performed, where mink were given a choice of a house mouse *Mus musculus*, noble crayfish *Astacus astacus* or Baltic herring *Clupea harengus membras*. Of the two, only the fish was familiar to the mink, and therefore, as expected was the most heavily consumed in the first trials (Figure 6). As trials continued, most mink began to switch to prefer crayfish, yet the mouse was mainly ignored (Figure 6).

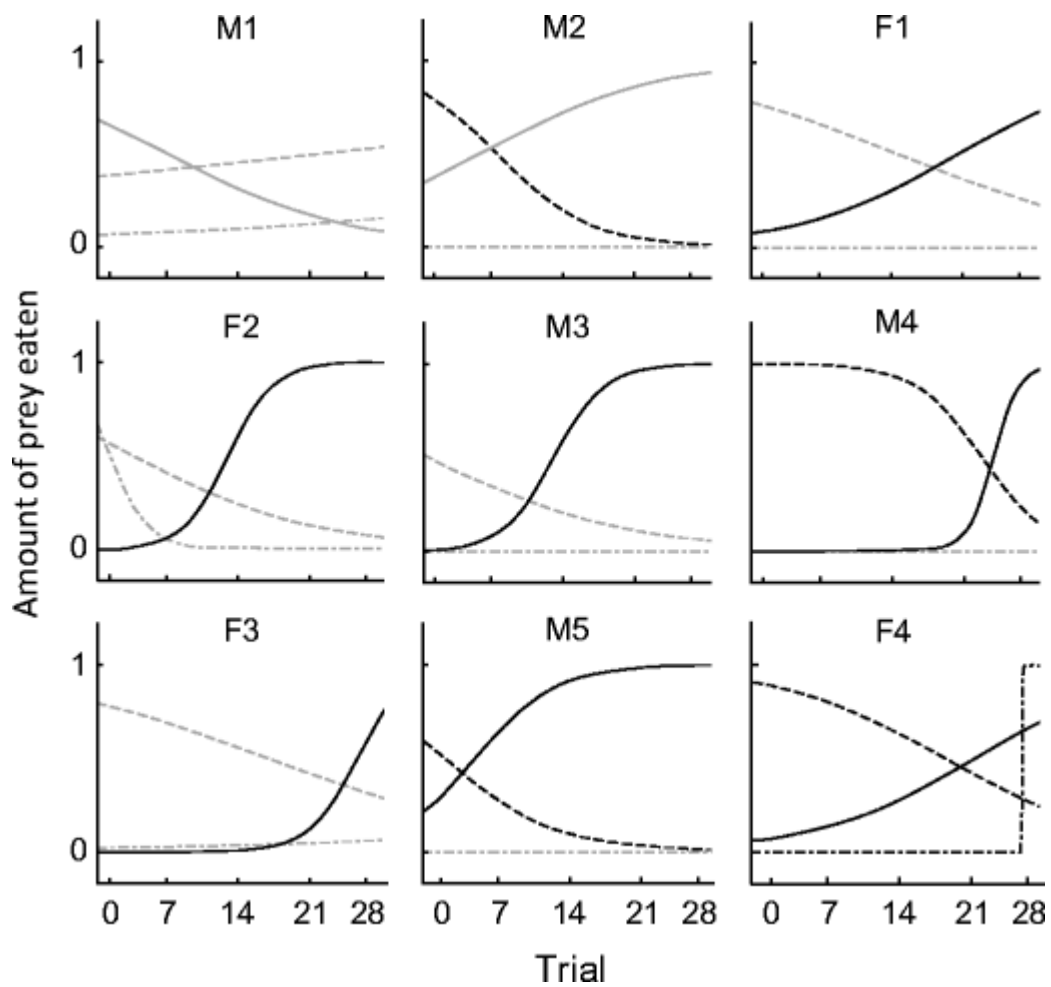


Figure 6: “The log-regression relationship between trial (N = 28) and prey items eaten by captive European mink (N = 9). Solid lines indicate crayfish, evenly dashed lines fish and unevenly dashed lines mouse. Black lines mark significance ($p \leq 0.05$) and grey lines mark non-significance according to logistic regressions. *F* represents females and *M* males” (Haage, et al., 2017).

This is in contrast to usual wild individuals, as the majority of studies found that in most cases diet is predominantly consistent of fish, and often a second prey group (i.e. birds, amphibians, water vole) (Bartoszewicz & Zalewski, 2003; Haage, et al., 2017; Chibowski, et al., 2019). This supports that, if available in high abundance, individuals will select preference to certain food types. Preference has also been identified between domestic and wild stocks. The percentage of domestic in comparison to wild stocks, as with otter, has been shown to be minimal in comparison to wild prey species (Chanin & Linn, 1980).

1.5.3 Behaviour

In the American mink, similarly to otter, activity levels are considerably greater at night, with levels showing the greatest increase at dusk (Zschille, et al., 2010). However, unlike in otter (Ruiz-Olmo & Jiménez, 2009), no sex-bias is described in scat levels

(Harrington, et al., 2009). These scat markings are relative to the home range of each individual (Helyar, 2005). Typically, within home ranges, members of both sexes maintain multiple dens which are used for shelter and rearing young (Gerell, 1970). As a result, preferred dens are close to locations with high prey abundance (Linn and Birks, 1981; Melquist et al., 1981)

Nevertheless, variation is seen in territory. Males maintain a much larger home range (Ireland, 1990) with scats used to mark territories (Helyar, 2005). This is paired with a higher activity level away from their den, in males (Ireland, 1990). Temporal variation however, also occurs between the two species. Unlike otter, mink have a set breeding period between February and April beginning at nine months of age (Hansson, 1947). Total activity following this period is greater in females, due to pup rearing. Female activity remains higher until August, when juvenile dispersal leads to an increase of territorial defence in males (Zschille, et al., 2010).

1.6 Otter-Mink Competition

For competition to exist between otter and mink there is likely to be overlap in predator prey preferences, such as foraging habitat or prey size range (Spitz, et al., 2006). This is a possible explanation for low competitive species density (Jones & Barmuta, 2002), and in the case of otter and mink, explain a recorded decline in mink following otter reintroduction (Bonesi & Macdonald, 2004). Should overlap not be identified however, the impact of seasonal change can also impact aggression levels, with seasonal food limitation forcing overlap, and competition to exist (Jones & Barmuta, 2002).

Evidence does support the theory that the re-establishment of otter populations can promote the decline of the American mink. Bonesi and Macdonald (2004) monitored the impact of the otter reintroduction, in Oxford, UK. The study identified that the re-introduction of Eurasian otter, lead to a direct decline in the percentage of sites occupied by mink (Figure 7). The interaction between mink and otter on areas

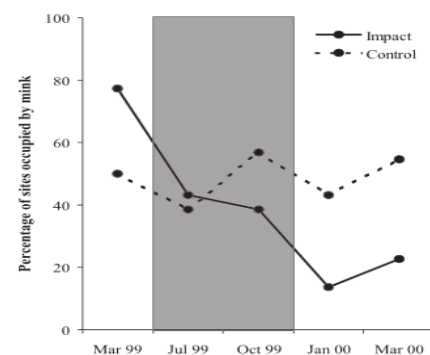


Figure 7: The percentage of sites occupied by mink before and after otter release (Grey). (Bonesi and Macdonald, 2004).

with existent otter populations however, is likely to display less of an impact as populations grow. This produced a hypothesis that in either case, the two species would eventually co-exist (Bonesi & Macdonald, 2004; Bonesi & W. Macdonald, 2004). A continuation of this study, identified that high habitat abundance, reducing reliance on aquatic prey can enable the two species to co-exist (Bonesi & Macdonald, 2004). However, due to the water constraints of both species the length of time in which species can co-exist is dependent of habitat characteristics (Bonesi & Macdonald, 2004). Understanding the dietary variance and habitat differentiation, particularly in areas with previously established areas, may still answer or highlight new questions.

1.6.1 Diet Overlap

Overlaps in the diet of Eurasian otter and American mink are likely to be a cause of competition, and therefore aggression between the two species. As already stated both can be predominantly generalist species, and whilst differences can be seen (e.g. otter consume a large number of invertebrates (Adrian & Delibes, 1987), both have high percentages of fish in their diet (Birks & Dunstone, 1985; Britton, et al., 2017). As both also displayed preference for native in comparison to stocked fish (Chanin & Linn, 1980; Lyach & Čech, 2017), there is potential for prey species overlap. However, as this preference had not been seen to be consistent at all sites, this cannot be guaranteed (Jacobsen, 2005). Advancements in understanding the impact of these preferences may also benefit from the use of molecular methods, with many previous dietary studies using morphological methods for identification.

Seasonal variation in habitat/hunting preference however, has been established with both species preferring water bodies tolerant to freezing (Brzeziński, et al., 2008). In the study by Brzeziński (2008), it was established both species had high occurrence in preferred areas. It was therefore hypothesised that mutual food tolerance can evolve between the two species, allowing for co-existence. In this case, co-existence has been predominantly explained by mink diet adaption (Bonesi, et al., 2004; Bueno, 1996; Clode & Macdonald, 1995) with overlap only occurring when mammalian prey presence was high. Several suggestions to the cause of this adaption have been given. Most studies reflect on the basis that mink are generalists, whilst otter are predominantly aquatic specialist (Bonesi, et al., 2004; Clode & Macdonald, 1995). To

further this, Bonesi et al., (2004), also found a direct link between otter density and shifts in mink diet. Competition was however, a recurrent theme in all three studies.

1.6.2 Aggressive Interactions

In the interaction of the Eurasian otter and American mink, initial aggression is evident (McDonald, et al., 2007). With most studies suggesting otter are the dominant species (Bonesi, et al., 2004). In comparison, a veterinary study carried out by Simpson (2006) found mink to be the second greatest cause of bites/injury on wild otter, suggesting aggression occurs in both directions. This is somewhat expected as the introduction of mink is disadvantageous to otter populations (Erlinge, 1972). An overlap in diet means the presence of mink restricts variety in otter diet over winter, when mink rely increasing on fish (Brzeziński, et al., 2008; Erlinge, 1972).

A second possible sign of aggression between otter and mink is the level of counter-marking; when one individual spraints on top of that of a second individual (Johnston, et al., 2010). Counter-marking has been observed in a wide variety of species, including mustelids (Rich & Hurst, 1999), and is heavily linked to territory defence (Hutchings & White, 2000). This is defended in a study by Palphramand and White (2007), who displayed how the presence of spraint allows, Eurasian badgers, *Meles meles*, to identify alien species. With sprainting predominantly carried out by males (Lisberg & Snowdon, 2011; Gosling & Roberts, 2001), counter-marking behaviour is believed to be evolutionarily stable, due to its difficulty to bluff (Fisher, et al., 2003). This therefore allows individuals to identify potential competitors (Rich & Hurst, 1998). With a correlation between the acknowledgement of an alien individual and aggression level (Palphramand & White, 2007). However, the degree to which detection extends past detection of an individual's species. Inter-specific detection is still being questioned, although is believed to occur (Harrington, 2007).

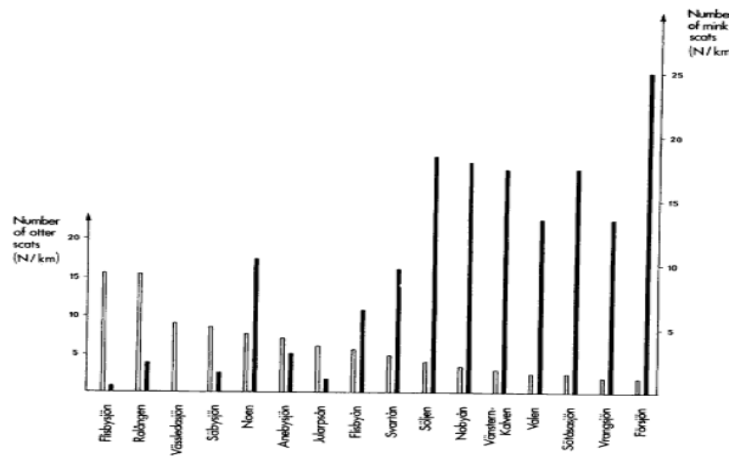


Figure 8: The relative density of otter and mink reflected by the number of scats by each species. To allow for better comparison the scales have been compensated, with a difference in the two ordinates. Open bars are otter and filled bars represent mink. ($r=0.76$, $p<0.001$). (Erlinge, 1972).

In otter and mink, inter-specific counter-marking is not heavily explored. Correlation in sprainting behaviour between the species however, was investigated in the 1970s by Erling (1972), whilst otter populations were in decline. The study established an inverse correlation between an increase in otter and the number of mink spraint (Figure 8). However, with otter populations re-establishing, and mink now in decline, uncertainty remains in if the same degree of aggression still remains.

1.7 The River Hull

The River Hull is predominantly consistent of chalk stream habitat (Wright, 1992), located in the East Riding of Yorkshire. Recognised nationally as an important site, the River Hull is the most northerly chalk stream in Britain, and also supports several other rare habitats, including riverside grassland and fen (Natural England, 1998). As previously described (1.1), the characteristics of chalk streams are idealistic for rare and endangered species, but are at a high risk from invasive species (Bowes, et al., 2005; Barreto, et al., 1998).

1.7.1 Ecosystem and its Endangered Species

The river Hull supports a diverse species composition, with particular interest in bird breeding communities (Natural England, 1998). Aside from more common waterfowl; mallard, *Anas platyrhynchos*, and mute swan, *Cygnus olor*, the catchment is inhabited by several waders; lapwing, *Vanellinae*, redshank, *Tringa tetanus*, and many other commonly occurring species (Natural England, 1998). Both the river and surrounding banks and area around the river itself, also offer habitat for bird species of concern

including the Grey partridge, *Perdix perdix* (The Wildlife Trust, 2020; Yorkshire Water, 2019).

Across the UK, river habitats are also described as critical for invertebrates such as the mayfly, *Ephemeroptera*, which depend on habitats with consistently high oxygen levels and cool water (Freshwater Habitats Trust, 2020). The presence of mayfly along the River Hull (NBN Atlas, 2020), is an important indicator of environmental health, implying high species diversity (Zedková, et al., 2015). This is emphasised by the presence of locally uncommon mayfly species including *Heptagenia fuscogrise* and *Caenis robusta* (Natural England, 1998).

In addition to high bird and invertebrate diversity, the River Hull is also critically important in the maintenance and re-establishment of declining mammal and fish species. Mammal diversity including endangered species, such as the Eurasian Otter and Water Vole, as already described, but also several other mustelid species; Stoat, *Mustela erminea*, weasel, *Mustela nivalis*, (The Wildlife Trust, 2020; The Wildlife Trust, 2020) and mouse species; Dormouse, *Muscardinus avellanarius*, wood mouse, *Apodemus sylvaticus* (The Woodland Trust, 2020). Similarly, the river ways are a vital habitat for once common fish species, now endangered and threatened species in the UK (Waterfowl and Wetland Trust, 2019). Both the European Eel, *Anguilla anguilla*, and River Lamprey, *Lampetra fluviatilis* in particular have displayed healthy populations in the River Hull (NBN Atlas, 2020; Canal and River Trust, 2016), despite growing threats of habitat degradation and over-fishing (Waterfowl and Wetland Trust, 2019; Canal and River Trust, 2016).

1.7.2 The Mink and Otter Population of the River Hull Catchment

Much of the East Yorkshire otter and mink population is believed to exist on the River Hull catchment (Wright, 1992). Similarly to the majority of the UK populations, otter populations have been seen to increase, whilst mink are believed to be in decline. Whilst otter populations never died out in the area, they did see a sharp decline in recordings until the late 1990s (NBN Atlas, 2020). However, despite the ecological benefits of otter, negative local perception has increased with otter population growth, with human-otter conflict appearing a concurrent theme across much of the Eurasian otters range (Kloskowski, 2011). With accusations of otter hunting artificial and

imported fish populations, understanding the changes in fish diet in comparison to location may reduce conflict.

In mink, comparatively, populations began to establish in the late 1970s (NBN Atlas, 2020), with the majority of farm releases occurring in the 1990s (Yorkshire Water, 2019). Release occurred from three farms in the catchment, one to the west of the city centre and a further two in the northern catchment (Yorkshire Water, 2019). Since then mink are believed to have established home ranges throughout the entirety of the River Hull, and then reduced with conservation organisations and capture. Much of this however is largely estimated.

1.8 Summary

Ecological networks are partially controlled, alongside bottom-up controls (Kominoski & Rosemond, 2012), through apex species such as the Eurasian otter. However, due to primarily man-made cause, the Eurasian otter has seen decline. In contrast, the introduction of invasive species can have detrimental impacts on the populations of native species. The American mink is an invasive species, which now inhabits much of the Eurasian otter's range. The impact of the species invasion is most heavily seen in the consequential decline of the water vole. Understanding how to control and limit the further impact of mink is therefore required.

Links have been found however, between the reintroduction, or natural range expansion, of otter and the recent decline in mink populations. In spite of this, the interaction between the species and any aggression present is poorly understood. Gaps also exist in understanding the cause of aggression; although it can be hypothesised that dietary overlap could be a key cause. Despite having traditionally variable diets, both species are believed to rely heavily on fish as prey. Nevertheless, variable recorded preference across studied sites and further analysis on identifying overlap in pre-established populations in to the evolution of co-existence is required as otter population dynamics continue to change.

1.9 Aims and Objectives

The distribution of otter and mink across Europe, and the degree of diet overlap requires further investigation (Roche, et al., 1995; Bonesi & Palazon, 2007). Yet the

possibility of otters acting as a deterrent for mink could be highly important in influencing conservation practices. Whilst studies have already evaluated distribution links between mink decline and otter recovery (McDonald, et al., 2007; Erlinge, 1972), and specifically the consequence of otter reintroduction (Bonesi & Macdonald, 2004), questions still lie around the aggression in areas where otter remain present, and mink populations are declining. The primary aim of this study is to understand the spatial distribution of the Eurasian otter and American mink along the River Hull, with the objective of mapping spraint distribution. Should competition be found, it can be hypothesised that there will be separation to some degree between the territory of otter and mink. With reports of aggression between the two species (McDonald, et al., 2007), it can secondly, be predicted, should it occur, counter-marking will predominantly be done by otters, which will be the dominant species.

The second aim is to investigate the impact of diet overlap between the two species, via diet analysis. With possible dietary overlap, varying aggression between individuals (due to difference in sex, age and fertility (Birks & Dunstone, 1985; Britton, et al., 2017; Rostain, et al., 2004; Koelewijn, et al., 2010)), it can be expected diet overlap will suggest strong competition for food. In either species, it can be hypothesised that the closer individuals are to the fish farms, the higher the percentage of stocked fish species will be in spraints with greater diet overlap, due to increase abundance. In addition, it can be predicted sprainting will occur at a greater frequency closer to the fish farm, as higher food availability presents greater opportunity for individuals to survive in the area.

In addition, such dietary analysis opens up the possibility to explore the presence of native species in spraint samples. Therefore, a final aim is to investigate impact of otter and mink on native species, hypothesising that mink are likely to have higher percentages of the endangered or at risk species in their diet than otter. Comparatively, it can also be hypothesised that the abundance of endangered species in both otter and mink diet, may be used as an indicator of a location's general biodiversity.

Chapter 2 Distribution

2.1 Introduction

Variation in the degree of interaction between the Eurasian otter, *Lutra lutra*, and American mink, *Neovision vison*, has been found (McDonald, et al., 2007). The variability between studies necessitates further exploration of the possibilities of co-existence between the two species with little competitive interaction (Bonesi & Macdonald, 2004; Bonesi, et al., 2004). As an invasive species, linked to the decline of native species in the United Kingdom, decline in American mink populations are largely encouraged, with active culling schemes in place. Additionally, actions to promote Eurasian otter populations are often supported, as they are an important native apex predator (Lyach & Čech, 2017). Whilst both species already have some records along the River Hull, mink dating back to 1973, and otter 1993, these are fairly sparse (NBN Atlas, 2020) leading to an unsatisfactory understanding of distribution and/or interaction.

The primary aim of this study was to better understand the distribution of the Eurasian otter and American mink, along the River Hull in order to test a number of hypotheses surrounding the interaction between them. It can be hypothesised that there will be separation to some degree between the territory of otter and mink, due to expected aggression from niche overlap (Bonesi & W. Macdonald, 2004). Should aggression exist, countermarking can also be predicted between the two species, with otter expected to be the dominant species. Furthermore, it can be hypothesised that competition will be greater closer to fish farms, as higher food availability, presents greater opportunity for individuals to survive in the area (Freitas, et al., 2007). Therefore, it can be predicted that sprainting will occur at a greater frequency closer to the fish farm.

In addition, McDonald et al, (2007) proposed that a wider otter range expansion, primarily occurred in locations where mink had been actively removed. With culling occurring across the main river, variation in where co-existence has and has not occurred, may also be impacting on if sites do or do not develop co-existence. This was used to investigate two further hypotheses. Firstly, that mink capture will have a negative relationship with otter spraint count and secondly, that otter populations will be higher in areas with active mink trapping.

Investigation of otter and mink distribution will be identified with consistent spraint collection in the area. Co-occurrence analysis of collected data will be used to outline if, or if not, any degree of separation occurs between the two species, and if avoidance patterns occur. To also consider the impact of fish farms, with a possibly greater potential of coexistence, due to high availability of stocked fish, analysis of the correlation between spraint counts and distance to farms will also be considered. As a number of factors have been found to impact otter and mink density, further analysis was also run using mink trapping data provided but The Yorkshire Wildlife Trust and Natural England.

2.2 Methods

2.2.1 Data Collection

Physical site surveys and spraint collection were used to locate and estimate spatial distribution and activity of otter and mink populations within the River Hull catchment

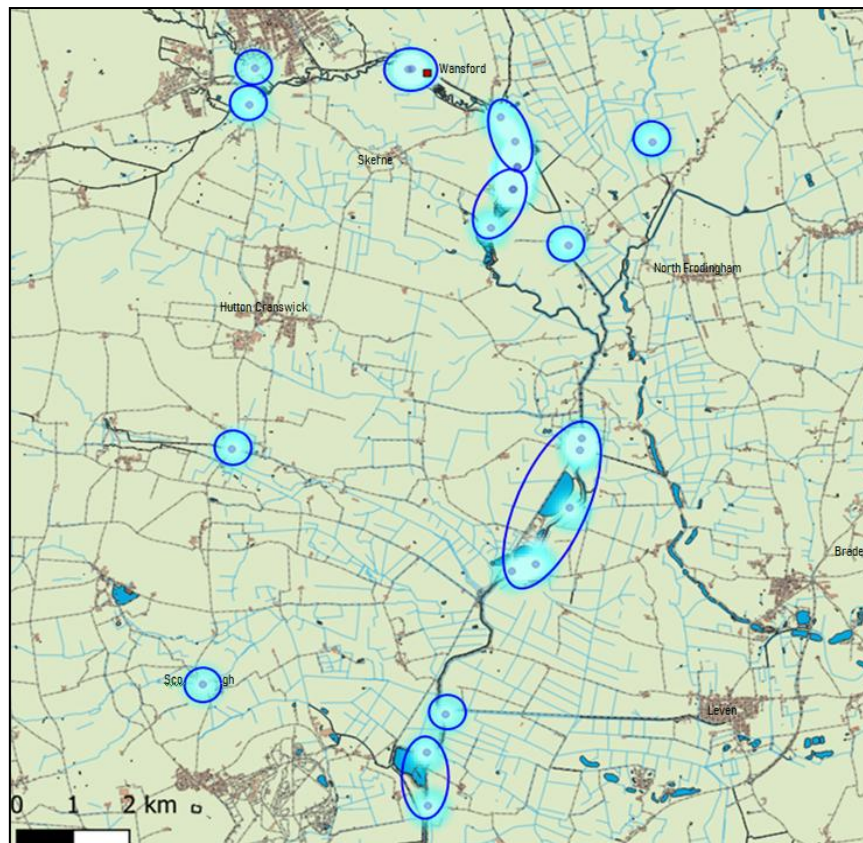


Figure 9: A map displaying the surveyed areas; Larger survey locations/groupings (Blue outline) and specific regular survey sites (blue dots) with the surveyed area indicated (Blue fill) can be seen. The trout farm is also visible (Red square).

(Kruuk, 1986, Mason and Macdonald 1987, Cho et al., 2009). Sites were chosen in conjunction with The Yorkshire Wildlife Trust. Survey sites were not systematically

chosen, but instead, were selected after being deemed to be optimal for detecting both signs of otter and mink activity. Predominantly, sites therefore contained established runs or sheltered locations, such as bridges. This increased the chances of spraints preservation between sampling cycles, whilst assuring efficient collection of a large sample size.

Twelve sample locations were identified along the River Hull catchment (Figure 9). Surveys were carried out at a total of 23 smaller sample sites within these locations. Surveys occurred between September 2019 and March 2020, at 3 week intervals, alternating over a three week period between three site groups (Figure 9). This time period was selected to increase the number of sites feasible to visit on a regular basis, without a high risk of causing disturbance to sites or behavioural patterns. Alternatively, regularity was maintained to reduce the impact of factors such as rain, or public use that may disrupt or remove spraints (Delibes-Mateos et al., 2014). To further reduce the impact of these factors, search areas focused on covered areas, such as bridges when possible. Equal sampling effort was maintained at each site.

Sites were surveyed for signs of mink and otter activity, such as runs, and spraints collected. A run can be identified as a defined path created by an animal, often regularly. Spraints were identified in the field on the basis of size, shape, and smell. Co-ordinates of the location for each sample were taken with the use of Solocator (Civi Corp Pty, 2019). Any counter marking, when an individual marks on top of another's markings (Thomas & Wolff, 2002), along with a number of other notable features (grass twists, spraint freshness), that may identify possible conflict between the two species were recorded. Spraint type (anal jelly, or solid spraint), surface type (solid ground such as concrete or grass) and run usage were also recorded as additional meta data. A maximum of two spraints were also collected at each site to reduce possible behavioural impact as removing a large quantity, or all spraint in an area, has the ability to impact aspects such as territorial marking. All samples were frozen for storage after collection. However, if more than one sample was found at a site all, all samples were documented.

2.2.2 Ethical Considerations

Ethical approval was granted by the University of Hull (U093). Precautions were put in place to avoid impacting on natural behaviour, by removing spraints too often, or discouraging otter/mink sprainting through heavy disturbance. Care was taken to avoid disturbing runs and surrounding wildlife.

2.2.3 Data analysis

Predator origin of samples found and collected between September and early November was confirmed with the use of eDNA metabarcoding as described in at the beginning of section 2.2. Any doubtful samples, where it was not apparently clear from visual inspection which species spraint should be assigned, found after this period were not included in the analysis. This resulted in a total of 256 samples were collected, documented and used to evaluate otter and mink distribution.

Statistical analysis was run in the statistical software R, version 3.6.2 ("Dark and Stormy Night") (R Core Team, 2019). The package GGLOT2 was also used for data visualisation. Distribution patterns were visualised with the aid of ArcGIS (ESRI, 2019), primarily identifying areas of high or low overlap between species, using a series of East Yorkshire shape-files.

A preliminary Mann Whitney U Test was used, to see if a significantly different number of spraints were identified between the two species. To analyse the studies primary aim; to understand the spatial distribution of the Eurasian otter and American Mink along the River Hull, a spearman's rank was used to identify if a relationship existed between the number of otter and number of mink spraints found. Preliminary spearman's rank correlations were used to investigate the prediction that sprainting will occur at a greater frequency closer to the fish farm. For this, two separate analyses were carried out for otter and mink respectively. Following this, a PERMANOVA was run using the package Vegan (Oksanen, et al., 2007) to identify if population number varied significantly between three distance groups; 0-3000m, 3000-6000m and 7000m+ from the fish farm. Distances were calculated as crow flies between plotted sites and the fish farms co-ordinates with the use of ArcGIS (ESRI, 2019).

To investigate the degree of overlap between the two species co-occur analysis was run in R using the Cooccur package (Griffith, et al., 2016). This analysed the likelihood

of co-existence between the two species at both higher and lower population frequencies than recorded. This was done using the total count of each prey species identified in otter and mink spraint per site.

To greater understand the movement of otter, temporal distribution patterns were analysed with a Generalised Linear Model (GLM), considering the change in spraint within each different distance group, over time. Samples were divided into four larger spatial groups at varying distances from the trout farm (Figure 10); 0-3000m, 3000-6000m, 7000-10000m and 10000-13000m. These groups were identified following a preliminary spearman's rank correlation between otter and mink spraint densities. The area between 6000-7000m from the farm was not included as there were no sample sites within this range. Mink was not analysed in this way due to insufficient sample size.

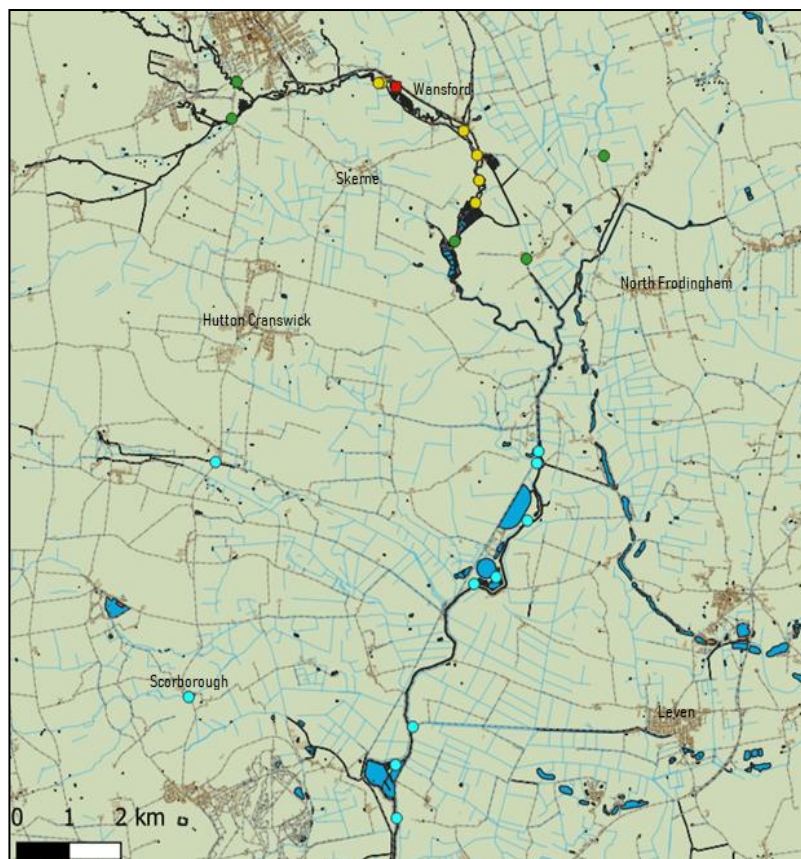


Figure 10: A map of the sample area, with distances from the fish farm displayed; 0-3000m (Yellow), 3000-6000m (Green) and 7000m+ (Blue). Fish farms are in Red.

In addition to direct interaction, the impact of trapping mink was also considered. For this, sites were grouped in the 12 larger sample areas (Figure 9). Larger location

grouping, rather than specified sites were used as trapping sites did not correlate with survey sites. This data was collected between January 2019 and February 2020. It was requested by Natural England that direct locations were not provided.

A spearman's rank correlation was used to identify the relationship between the number of mink trapped and number of mink spraints found. To identify if otter are moving in to locations with active mink removal, a Mann Whitney U Test was used. This analysed if trapping and removing mink had an impact the number of otter in an area. Specifically this was used to identify if there is a significantly higher otter spraint frequency in sites with or without trapping.

2.3 Results

Otter spraints were identified at all but two sites. The majority of spraints were found along the main river, but high densities were also discovered on the northern tributary, as on Figure 11. The total number of mink spraints found (14) were significantly lower than that of otter (257) ($W=499$, $P<0.05$).

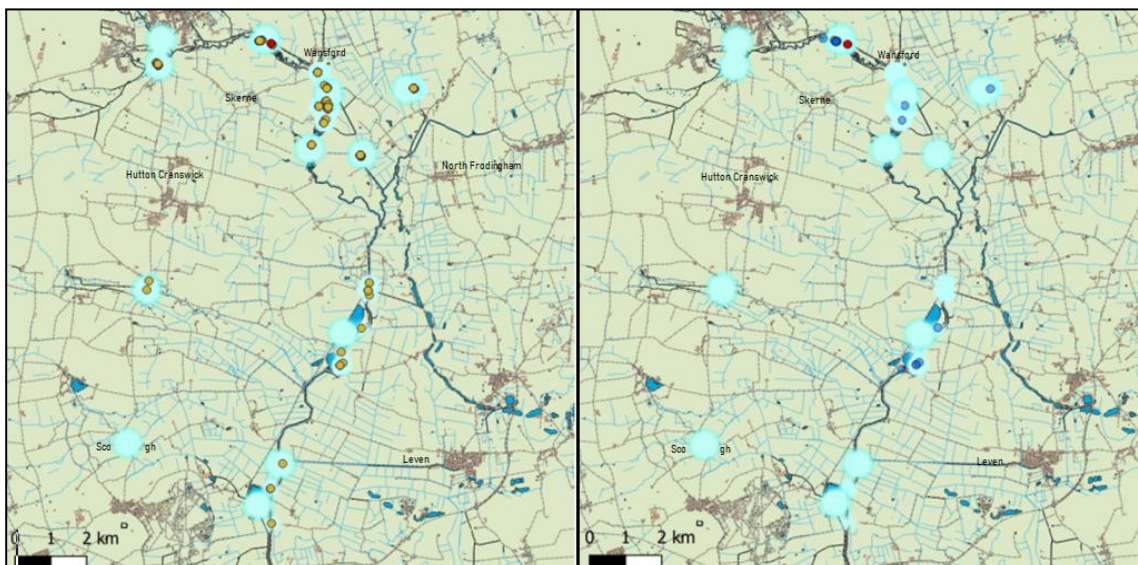


Figure 11: A map of sites where Otter spraint (Yellow) and Mink spraint (Dark Blue) were found and the Trout farm location (Red). Locations where sampling occurred are highlighted in light blue.

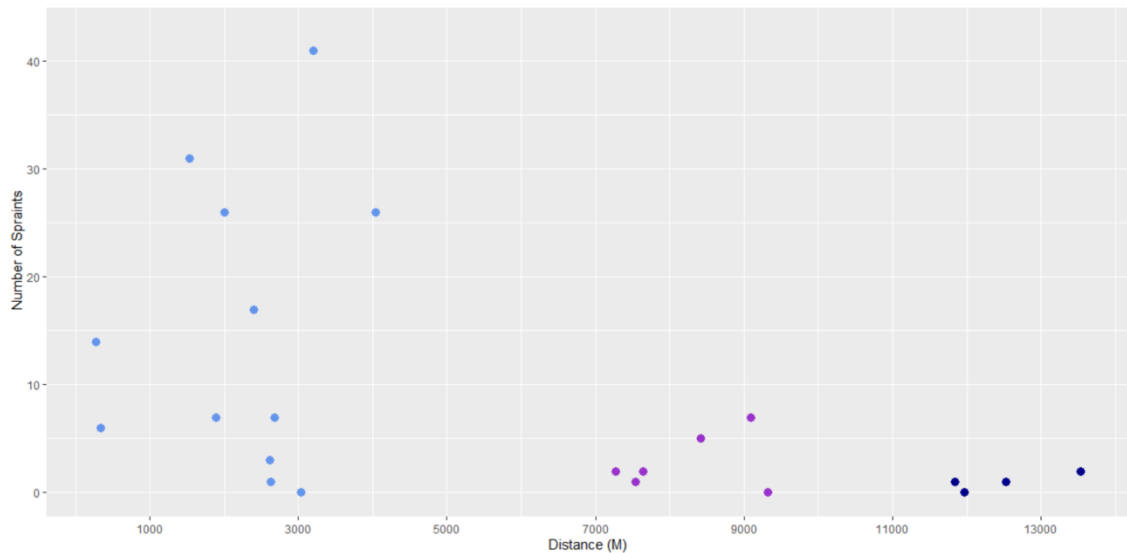


Figure 12: The correlation between distance from the Trout Farm and the number of Otter spraint found ($S=3104$, $p<0.05$). Three groups are identifiable, signified by different colours (Light Blue, Purple and Dark Blue)

2.3.1 The Impact of Trout Farms

Three distinct groups were identified with high spraint density from a preliminary spearman's rank plot within the closest group (0-4000 m) and then a decline in density to the next group (7000-10,000 m) and again to the final group (11,000-14,000 m) (Figure 12). Sites were found to have significantly different spraint count ($R^2=0.171$, $p<0.05$). The closest site (0.2km) to the trout farm did not record the highest density of spraint, however this is likely to be due to heavy flooding, often restricting collection at this site.

No correlation was identified between mink and the trout farm ($\rho = -0.245$, $p = 0.26$). This is possibly due to the impact of having a low sample size.

2.3.2 Species Overlap

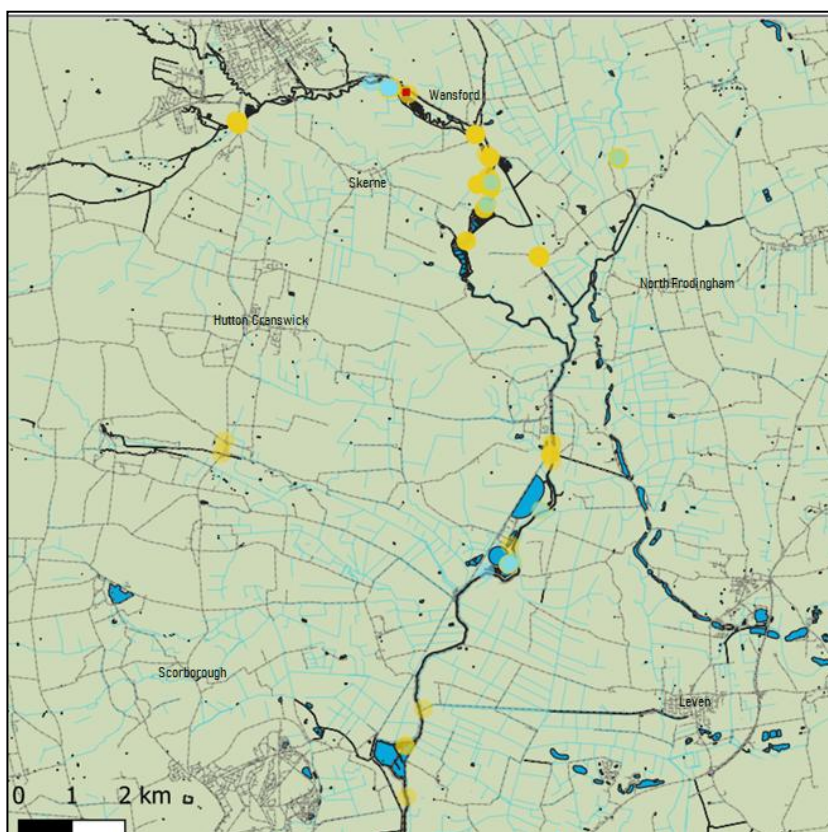


Figure 13: A map of the overlap between Mink (Blue (Heatmap and points)) and Otter (Yellow (Heatmap and Points)) spraint distribution. Sites are also indicated (see key).

The majority of species distribution overlap occurred at three of the 23 sites sampled, two being the closest to the trout farm (L7,S1 and L7,S2), and the remaining at a Wildlife Trust reserve (L13,S5). Otters appear to be dominant across the river catchment, with the majority of sites displaying signs of only the one species (Figure 13).

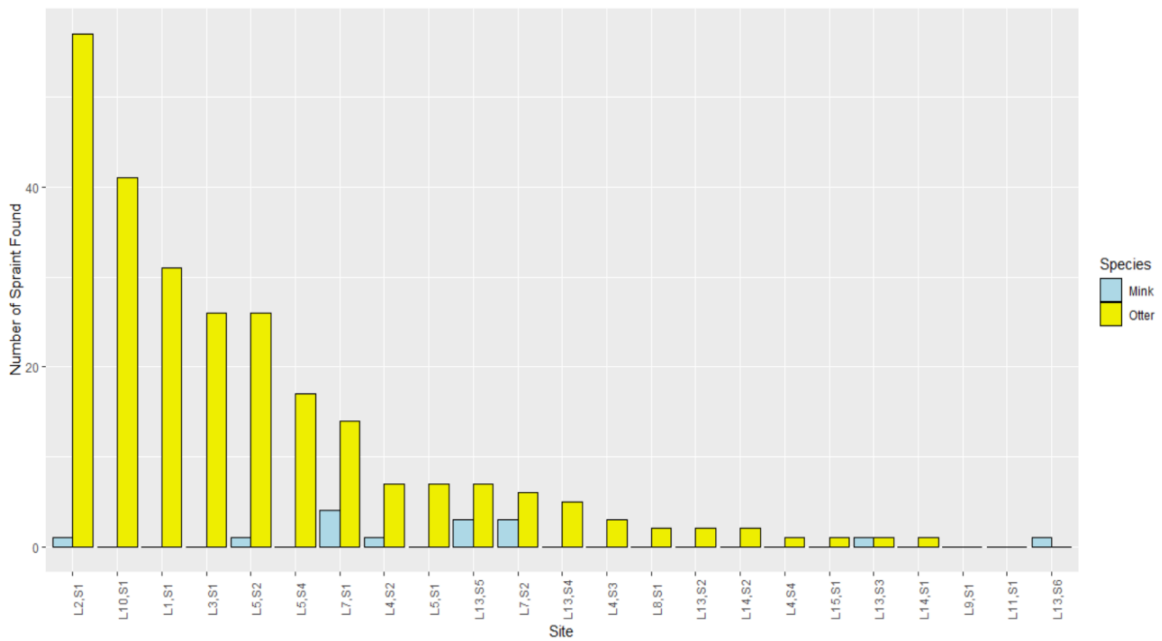


Figure 14: A bar chart of Otter and Mink Spraint counts. Found that there is no significant pairing or correlation between the two species ($p_{lt}=1$, $p_{gt}=0.32$), ($S = 1597.6$, $p\text{-value} = 0.3346$). At each site approximately 150m was surveyed, with the exception of bridges, where only the covered location was surveyed.

Co-occur analysis found that the overlap of otter and mink spraint across sites does not correlate with the probability of co-occurrence between the two species. The analysis described that lack of co-occurrence would also be maintained, should the frequency of spraints change, either with an increase ($p_{lt}=74$) or decline ($p_{gt}=0.73$) of spraints found in the area. This displays that the two species are not co-occurring.

2.3.3 Temporal Variation

A Generalised linear model displayed that temporal variation in distribution was not significantly different ($Df=34$, $Pr(>Chi)=0.9897$). However variation did vary significantly different over different distances ($Df=32$, $Pr(>Chi)=1.478e-15$).

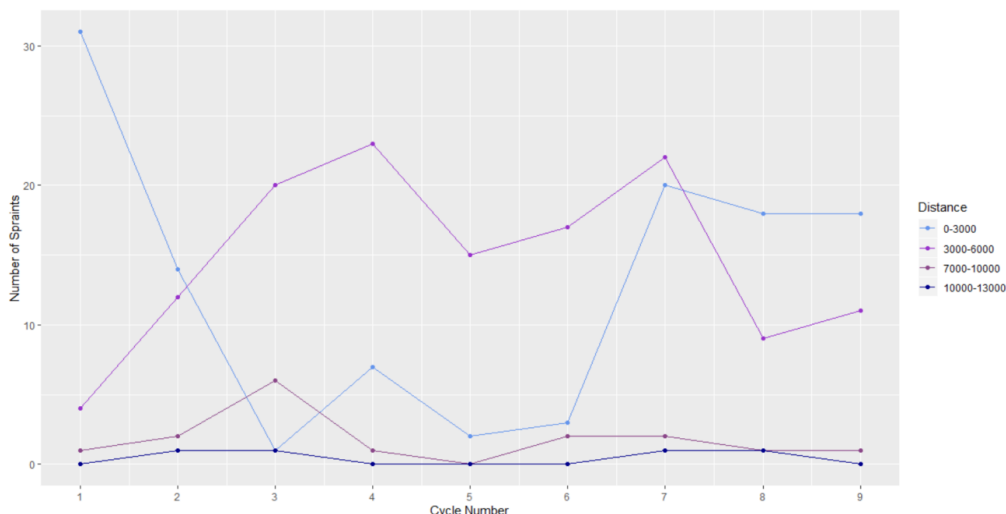


Figure 15: Temporal variation between four distinct distance groups ($Df=34$, $Pr(>Chi)=0.9897$)

2.3.4 The impact of active mink trapping

The number of mink captured was not correlated with either mink scats (rho=-0.195, p-value = 0.2591) or otter (rho=-0.094, p-value = 0.7328) (Figure 16:

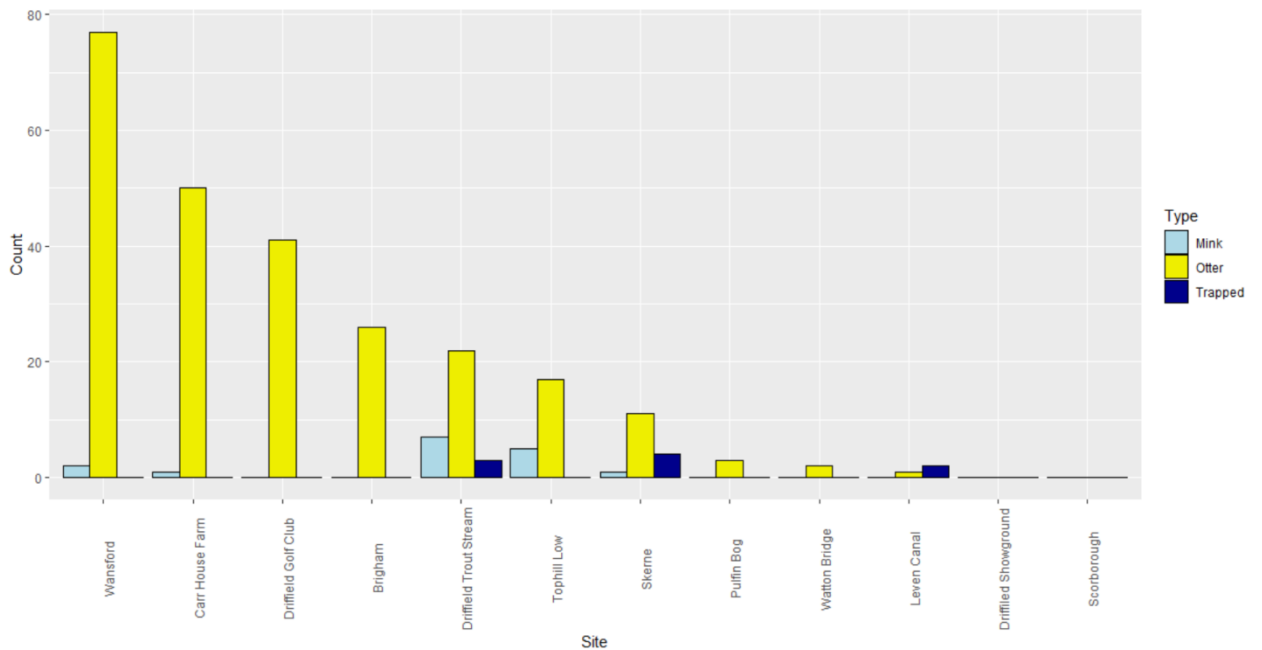


Figure 16: The number of otter spraint, mink spraint and mink captured between September and February 2020.

There was no significant difference found in the number of otter spraint at sites with and without mink trapping (W = 14, p-value = 0.8413).

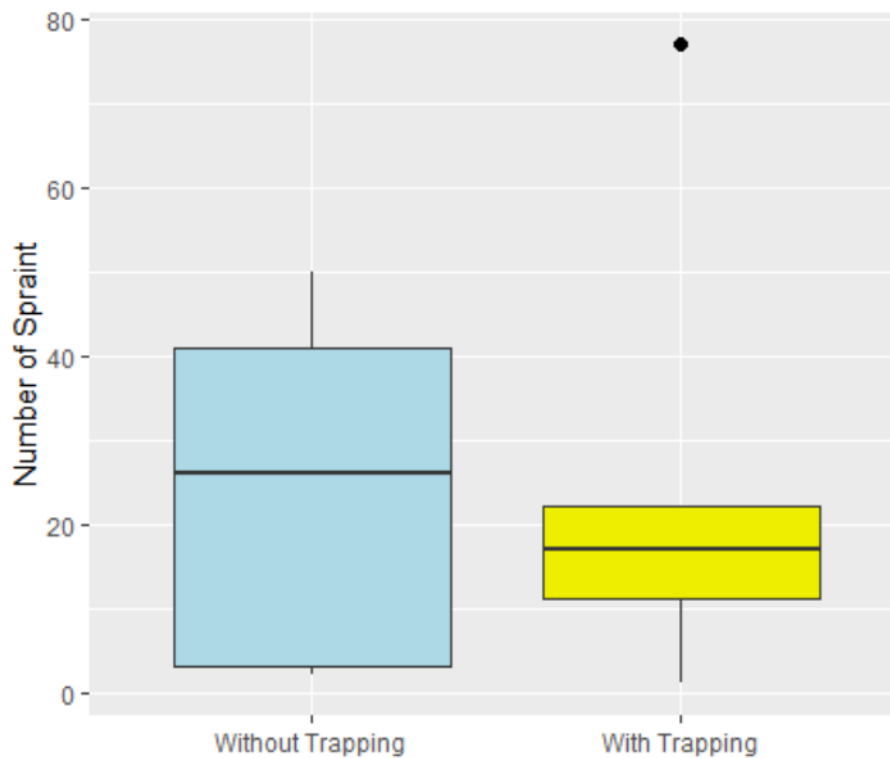


Figure 17: The impact of mink capture on the number of otter spraint found (W = 14, p-value = 0.8413).

2.4 Discussion

The results indicate that based on sprainting pattern otter are the more widely distributed in the River Hull catchment compared to mink. Under the assumption that the number of spraints is a good surrogate for density, it must be noted that the number of spraints in an area is impossible to correlate to a direct population size, so instead the number of spraints was used as a surrogate of population density. With spraint density in both species influenced by a variety of factors, including habitat features and prey availability, a number of unrecorded features could explain variation in the analysis as previously suggested by Jenkins and Burrows, (1980). The significantly greater number of otter spraints found could also suggested that, otters are the dominant species (Figure 11). This is hypothesised throughout a number of studies, with otter being the larger species and known reports of otter killing mink.

However, aggression between the two species cannot be stated as a conclusive explanation, in the River Hull populations. With no countermarking being identified at all throughout the sampled period, survey results suggest that in areas of overlap, aggression levels are low. Furthermore, no correlation was identifiable between otter and mink, in contrast to the majority of previous studies (Figure 11, Figure 14). As a result, the hypothesis that separation will exist to some degree between the territory of otter and mink, cannot be accepted.

Despite an initial hypothesis supporting the likelihood of co-existence, the study found no evidence this is occurring within the study area. There are several suggested reasons for why co-existence has not occurred. Primarily, this may be subject to the requirements of co-existence not being met. Habitat heterogeneity has been highlighted as a highly important reason for the co-existence of two species(Levin, 1974; Yodzis, 2013). In particular, Bonesi and Macdonald (2004) identified that in the case of the Eurasian otter and American mink, co-existence requires areas where segregation occurs between the dominant otter and sub-ordinate mink. To produce a more conclusive causation for population distribution on the river Hull catchment, the variety of habitat therefore warrants further investigation.

2.4.1 The Impact of Active Mink Removal

The findings are however displaying trends previously acknowledged in a study by Bonesi and Macdonald (2004) (Figure 11). The study identified that interaction between otter and mink differed between sites, with both areas of co-existence and species replacement. This again, focuses on the importance of habitat, highlighting the vast number of features that must be considered in conservation practice, to both eliminate the mink, and aid the repopulation of otter. This also brings into question the impact of current conservation work on the two species. Mink culling is active across the UK, primarily to reduce the impact of mink on endangered species, a theme replicated throughout countries where the impact of mink is well studied (Bonesi & Palazon, 2007).

As previously stated McDonald et al (2007), proposed active removal may also be beneficial in aiding the restoration of otter populations. Whilst it is unlikely mink would have a direct impact on a decline in otter populations, as the subordinate species (Bonesi, et al., 2004), the presence of inter-specific competition can be expected to negatively impact on otter populations to some degree (Amarasekare, 2002). Removal of mink is therefore also likely to be beneficial in otter repopulation, providing locations that fulfil niche requirements without the degree of completion in already populated locations. This may be particularly beneficial to the young, as they must obtain new territories.

Nevertheless, this theory was not supported in the results (Figure 17). With no significant variation in otter spraint was found between sites with and without culling, questions still remain about how culling is impacting on interaction between the two species. Mink have been found to be eliminated at high otter densities (Copp & Roche, 2003), reducing any competition, therefore has the possibility to allow greater repopulation rate to high density. It is possible however, that culling may also ease the number of sites able to promote co-existence. The ability for mink and otter to coexist has been found to be largely reliant on mink's ability to switch to a highly terrestrial based diet (Clode & Macdonald, 1995). By reducing, rather than entirely removing mink by culling, interspecific competition between mink is also reduced. Consequently, remaining mink have greater opportunity to rely on terrestrial prey alone, and

therefore greater opportunity to coexist. Whilst there is no sufficient proof of this, it may be worth considering in both future study and conservation planning.

In contrast, un-colonised locations, by either mink or otter were rarely expanded upon by either species, which may provide explanation for sites where neither species were located (McDonald, et al., 2007; Strachan & Jefferies, 1996) (Figure 11). This emphasises the importance of conserving locations where established otter populations already exist, in order to not negatively impact successful repopulation efforts.

2.4.2 Spatial Variation

Although mink samples proved to be minimal for further investigation, a number of interesting trends are identifiable in otter distribution. Spatial trends indicate three clear distance groups, to which sprainting variation exists (Figure 12). Preference is indicated to locations close to the fish farm, expected due to high density of preferred fish. The closest site (0.2km) to the trout farm did not record the highest density of spraint in otter. Due to heavy rain during the sampling period, this may in part be due to flooding restricting access to some sites during flooded periods. With a connection also found between areas with preferable habitat (White, et al., 2003), diet may not be the most important factor in otter distribution. Bank side cover and tree type, as well as high holt availability, low human disruption and stream gradient have all been identified as preferable features (Macdonald & Mason, 1983; White, et al., 2003).

The results however, still indicate preference to the area around the farm (Figure 12). Guter et al.,(2008), confirmed that sprainting events are correlated with the number of visits to an area in the Eurasian otter, supporting the hypothesis that otter populations will be greater in the area. However, the study also identified that it should not be assumed that the number of otter should be estimated by the number of spraint. A high population density however, is implied by a high sprainting number, with particularly high density at an area of overlapping home range (Guter, et al., 2008; Erlinge, 1968). High sprainting density in the sites within 3000m of the trout farm, therefore also supports the secondary hypotheses that aggression and competition levels will be higher close to stocked farms, due to high prey density.

A number of studies have also found that food availability is a dependent factor in the placement of sprainting (Macdonald & Mason, 1983; Prenda & Granado-Lorencio, 1996; Kruuk, 1992). High sprainting intensity along the river stretch, within 3000m of the farm indicates an area of high importance to individuals. In this case, lack of discrimination between areas with and without distinguishable features, such as, bridges may have caused a non-representative skew in the number of samples identified, in comparison to broader unsheltered spots.

2.4.3 Temporal Variation

From the data, temporal trends were not found to be significantly variable (Figure 15). Individuals are known to predominantly remain within a specific home range. With differentiation in the home range size between sexes, males occupying ranges around 15km and females 7km (Erlinge, 1967), minimal variation between groups could suggest individuals are remaining within distinct territories. To explore this further, future studies should also focus on identifying sexual dimorphism in collected spraint. Preference is however always identified towards the sites within 0-3000m of the farm, with holt potential and high food density.

However, a possible cycling effect is displayed between the most active spatial groups 0-3000, when divided down further to two equal spatial groups (Figure 15). A number of features have been identified as influencing factors on sprainting variation including population density, habitat characters, human disturbance and seasonal variation (Guter, et al., 2008). Interestingly, within this study, identification of these jellies is in line with the change in spraint frequency. Laidler (1982) found that the types of otter spraint could be a reliable indicator of fertility in both males and females. Specifically, fertile periods occur at two week frequencies, during which time anal jellies were deposited. Throughout the study, six anal jellies were identified, five within the 0-3000m group and one in the 3000-6000m group. Pregnant females have been identified in both captive and wild populations to have the lowest sprainting count (Kean, et al., 2011).

From this, it could therefore be suggested that, decline in sprainting in the 0-3000m group, after cycle two when the four out of five jellies were identified, was linked to fertile periods. A similar trend occurred with a single anal jelly identified in the 3000-

6000 groups in cycle six, after which spraint number declined. The gestation for the Eurasian otter is 60–64 days (Bonesi, et al., 2013). After this, females are known to try and disguise their presence by sprainting in water and eating the excrement of cubs for up to fourteen weeks, in line with when weaning ends (Laidler, 1982). This would estimate a five month period of low sprainting activity in a females territory, similar to the trend displayed in figure seven, in which sprainting is seen to be decreased between cycle two and seven (September-February) (Figure 15). Equally, the number of otter in a surrounded area may be expected to increase, as females with young are known to be incredibly protective of their young, with records of females even attacking dominant males (Simpson, 2006). This is a possible explanation for an increase in the 3000-6000m area.

Disturbance on the site however, is a secondary possible cause for changes in temporal change between the closest sites. Sprainting density was seen to increase between February and March (Figure 15). With the closest site being spring fed, it is also possible that otter sprainting increases with the density of fish, as otters increase visits to stocked areas. However, with the current data set and specifically a lack of ability to distinguish between sexes, neither theory can be concluded as an explanation. An attempt to investigate if young were present in the area was conducted, following identification of smaller otter-like footprints, with a camera set up alongside a known holt. However, due to isolation restrictions this was unsuccessful. Furthermore, the study would greatly benefit from the identification of individuals, providing an additional dimension to the study. Whilst the study currently displays area with higher and lower activity levels, quantification of population numbers is not attainable. Should further study continue, gene markers alongside eDNA analysis should be introduced. This would not only benefit understanding of temporal shifts, but enable individual home ranges to be identified.

2.4.4 Suggestions for Future Research

Despite the study highlighting a number of trends, further investigation is needed in all areas of the study. Predominantly a longer sampling period is required, particularly for temporal variation. Whilst fertility cycles may provide explanation for the decline in the closest group, other factors; such as disturbance or movement and population patterns of prey, could be an equally viable hypothesis for the change in spraint

density (Carss, 1995). This should be paired with reimplementing of video capture to incorporate individual identification and behavioural variation. Extending the study to examine if this cycle is annually repeated, could provide alternative conclusions for the change in spraint density. Furthering this, benefit may be seen by sampling the area 6000-7000m from the farm. This may provide further explanation surrounding the sudden changes in the two closest groups.

Secondly, an increased sampling period is likely to increase the number of mink samples. Mink samples remained consistently low throughout the entire sampling period (Figure 11). Due to the set breeding period, of mink, activity levels are lowest in the winter months (Hansson, 1947; Zschille, et al., 2010). One key shortcoming of the study therefore, is the sampling period is not optimal for surveying the distribution of mink. Future study should aim to optimise field surveys during this time if possible. To broaden this, the results and significantly lower number of mink spraints could be explained by a consequential change in mink behaviour, due to otter presence (Bonesi, et al., 2006). It is plausible that mink spraint, in this case, has become a negative signal of presence to otter, rather than an intended mark of territory.

This is complimented by the results. With no significant correlation identified between the number of mink spraint found and the number of mink trapped in a location (Figure 16). An inconsistency can be identified between the number of mink in the area, and the frequency of mink spraint. This supports that a behavioural change has occurred in mink along the River Hull. This result also identifies a further shortcoming of the study. A study by Bonesi et al, (2006) determined that signs of mink are in decline in locations adjacent to water bodies, but are not determined to correlate with a decline in mink populations. This therefore is also a highly possible explanation for the lack of evidence towards or against our main hypothesis. However it should be noted that trapping data was only used for a limited period.

Furthermore a relationship was found between the disappearance of mink spraint and an increase in terrestrial prey (Bonesi, et al., 2006; Clode & Macdonald, 1995; Bonesi, et al., 2004). To explore this further, spraint DNA analysis could be employed to determine if a significant diet variation exists between locations in mink. This implies that a higher sample size and broadened search locations are required. Taking these

finding into consideration, the results of the study must not be taken as conclusive, as to if mink are or are not present in an area, but rather, emphasis should be placed on signs of aggression. Incorporating physical and video trapping to identify if mink are present should be implemented in future study.

2.4.5 Conclusion

Concluding this chapter it can be argued that the Eurasian Otter, *Lutra lutra*, is seen to be, as hypothesised, the dominant species, in comparison to the American mink, *Neovision vision*, in the River Hull catchment. Analysis shows that from the mink and otter spraints collected, there is no evidence of aggression between the two species, rejecting the primary hypothesis. In contrast, there was also no co-occurrence found. These results open up a series of further questions for further study. Otter trends have also opened up areas for further investigation. Spatial variation has been identified; however, causation cannot be concluded. Further dietary and environmental consideration should be investigated in future study. Equally extended temporal data collection and video capture should be implemented.

Chapter 3 Diet

3.1 Introduction

Several factors including diet, human disturbance and holt availability have been found to significantly influence competition in mustelid species (Macdonald & Mason, 1983; White, et al., 2003). Additionally diet competition has been found to be a major factor between many native and invasive species (Bonesi, et al., 2004). This is also possible for the invasive American mink *Neovison vison* and Eurasian otter, *Lutra lutra*, a native apex predator. Both semi-aquatic mustelids, each has been found to display a preference for fish species in their native range suggesting diet overlap will occur (Haage, et al., 2017; Adrian & Delibes, 1987). However, some previous studies have found that mink predate heavily on terrestrial species in areas where the species co-exist, reducing the degree of competition and aggression (Bonesi & Macdonald, 2004).

As an invasive species, the American mink has been found to have a negative impact on British ecosystems (Bonesi & Palazon, 2007). Motivation to remove the mink is linked to decline in some native species, in particular, the water vole, *Arvicola amphibius* (Brzeziński, et al., 2018). It can be expected that if otter are present, mink will adapt their diet to consist of high percentages of mammals and amphibians (Chanin & Linn, 1980).

In contrast to mink, population recovery of the Eurasian otter is encouraged. This is due to their role in ecosystem regulation as an apex predator (Ruff, 2007). However, the feeding behaviour of the Eurasian otter has caused tensions to arise between fish farmers, anglers and conservationists (Freitas, et al., 2007). This is due to otter being opportunistic when hunting on prey (Bauer-Haáz, et al., 2014). Stocking fish in high densities, in areas accessible to otter, is therefore likely to result in otter predation of these stocks, which in turns can lead to loss of fish stock and therefore economic costs (Freitas, et al., 2007).

The primary aim of the study is to identify if a significant overlap in diet between the Eurasian Otter and American mink is present on the River Hull. Should no overlap be found it can be suggested that diet competition is low and increases the chance of co-existence occurring between the two species. A second aim is to identify the degree of

predation on endangered species in the diet of both otter and mink. It can be predicted that the percentage of endangered species will be higher in mink diet. Furthermore, to investigate if otter are having a direct impact on stocked population, a third aim is to identify preference to stocked fish populations. It can be predicted that if otter are predating at fish farms, a preference for stocked fish will be identified.

3.2 Method

3.2.1 Data Collection

Surveys for spraint occurred following the procedure described in Chapter 2. Sites were grouped as seen suffice for each hypotheses. For the majority of the study, data from all sites were merged, with exception for temporal and spatial analyses. To determine spatial variation in diet including the impact of a trout farm preference variation cause by fish farm presence, sample sites were divided in two groups. First an upstream catchment, at a distance of up to 7000m from the rainbow trout farm, distinguishing the salmonid region and a second downstream catchment, including

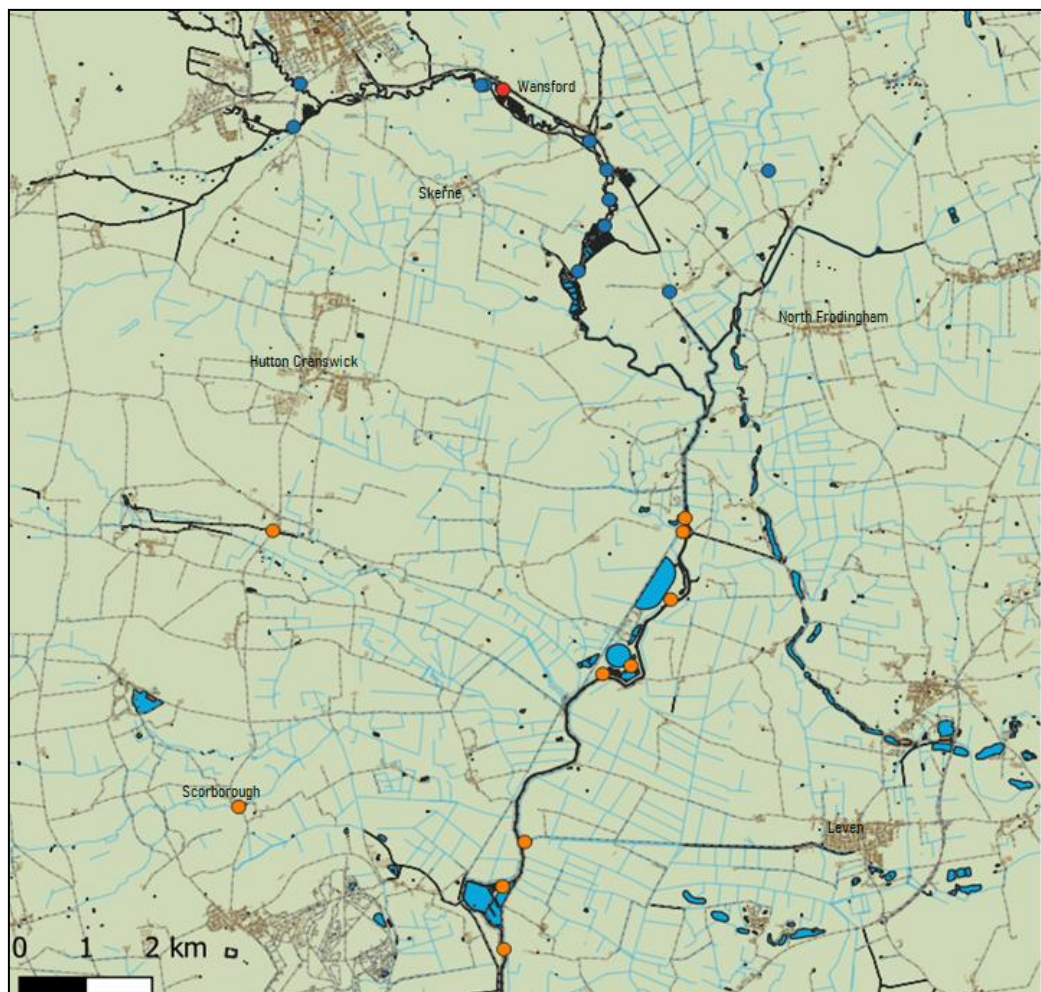


Figure 18: Collection sites with spatial groups distinguished; under 7000m (Blue) and over 7000m (Orange)

sites over 7000m.

When multiple spraints were identified at a single location, a minimum of one spraint was collected per collection cycle, unless the placement of a spraint was not safe to access. No more than two spraints were collected to minimise the impact of pseudoreplication. During spraint collection care was taken not to disrupt runs and part of each spraint was left at the site where possible to reduce possible impact on otter or mink behaviour. All spraints collected were stored at -20 degrees centigrade. Only samples collected between August and November 2019 were used due to laboratory use restrictions.

3.2.2 Ethical Considerations

Ethical approval was granted by the University of Hull (U093), as stated in chapter 2.

3.2.3 Spraint metabarcoding, eDNA metabarcoding and Bioinformatics

Diet analysis occurred on 60 collected spraint samples. From each spraint, 0.25g was used for DNA extraction, following the method for soil by Sellers et al (2018). A 106bp fragment of the mitochondrial 12S region was amplified using the vertebrate specific primers of Kelly et al (2014) as described in Harper et al. (2020). 2µl of extracted DNA was used for PCR. PCRs contained 12µl of Q5 2X (NEB #M0515), 0.5µl of BSA, 7.5µl of molecular-grade water and 1.5µl each of two primer. These primers were used as the majority of diet overlap is believed to be vertebrate species. PCR cycles were as follows: 98 degrees Celsius for 5 minutes, followed by 35 cycles of 98 degrees for 10 seconds, 58 degrees for 20 seconds, 72 degrees for 30 seconds, then a final elongation stage of 72 degrees for 7 minutes; 4 degrees for 10 minutes. Following PCR, 3µl of PCR product was run on a gel in an Ethidium bromide bath for 20 minutes to check if amplification was successful.

To determine diet preference, otter diet was compared to fish abundance in the river Hull as estimated from eDNA metabarcoding. The data were sourced from an ongoing PhD project (Griffith unpublished). 12 2l water samples were collected along the river Hull catchment (Figure 19). T Water samples were collected on ice and stored in sterile conditions. Samples were filtered within 16 hours of collection and extracted as outlined in Sellers et al. 2018 (<http://doi.org/10.3897/mbmg.2.24556>). Library preparation, sequencing and bioinformatics were carried out by Dr Graham Sellers and

Dr Robert K Donnelly following the protocols described in Harper et al 2020 (<https://doi.org/10.1111/1365-2664.13592>). Each library was quantified on a Qubit™ 3.0 Fluorometer using a Qubit™ dsDNA HS Assay Kit. Libraries were sequenced on an Illumina MiSeq®. Positive and negative controls were also included. Taxonomic labels were matched to successful forward sequences with the use of Kraken (Carss, 1995), to a minimum of order level. Reverse reads were not used due a clustering error in sequencing. As a result only forward read sequences could be matched to species

3.2.4 Statistical Analysis

All statistical analysis was run in the statistical software R, version 3.6.2 ("Dark and Stormy Night") (R Core Team, 2019) using the software package Vegan (Oksanen, et al., 2007). A false positive threshold was applied to total read counts from each sample, to remove taxonomic assignment that may have occurred as a result of contamination in

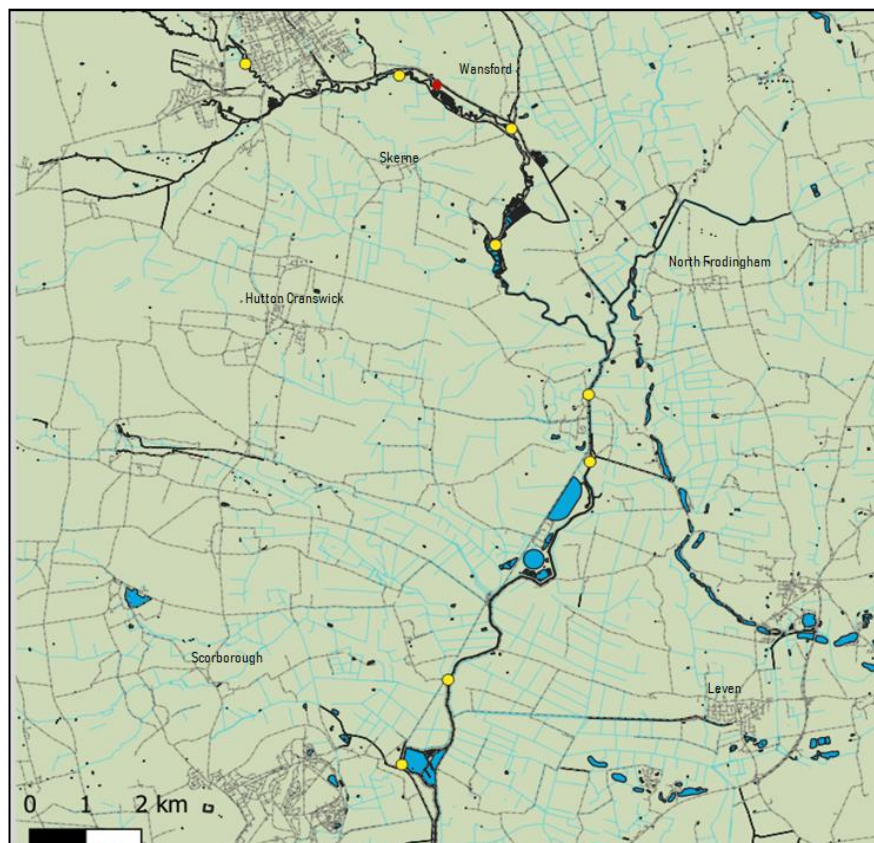


Figure 19: A map displaying water sample collection points (Yellow). Fish farm location is represented by the red square.

sequencing or preparation (De Barba, et al., 2014). For spatial analysis, where only the 2019-2020 dataset was used, the library threshold was set at 0.001 (0.1%) due to a smaller sample size and reduced reads from sequencing error. For species comparison

analysis the collated dataset was merged with a second dataset by Harper et al(2020), with data from 2015-2018 collections. When data was merged with the 2015-2018 dataset a threshold of 1.1% (0.011) was used due to high contamination in positive control. A 1.1% threshold was also applied on temporal analysis, where only samples from a single site in the 2015-2018 dataset were used. In both datasets, where samples contained DNA sequences from multiple predators, samples were only assigned to a predator if it occupied over 90% of reads from a single predator. Samples below the threshold were removed from the diet analysis.

The collected water samples filtered and processed as described in Li et al (2019) using the same vertebrate specific primers as used for otter spraint metabarcoding. As analysis was focused on preference within fish species, to determine if preference exists for stocked species, all non-fish species identified were removed. Read count data was set to a threshold of 0.001 (0.1%) and divided in to two groups; sites within 7000m and over 7000m from the fish farm mirroring the spraint groups. All statistics were set at a significance level of 0.05. All data was visualised using the package GGLOT2.

Firstly to evaluate diet overlap between the two species, data was converted to presence and absence. A PERMANOVA (Jaccard Index) was used to identify divergence between the diets of each species. A second PERMANOVA was also performed to effectively evaluate diversity between sites to compare the diversity of species composition. Alongside this stacked bar charts were produced to show variance in abundance occurred. To next identify if fish species composition also varied spatially this was repeated for the water sample data provided. A final PERMANOVA was used to identify variation in species composition between two distances, site within and over 7000m.

To investigate fish preference in otter in the Hull catchment Ivlev's index(Lanszki, et al., 2001) was used. This was run using the package selectapref (Richardson, 2017). Each species recorded in their diet had an index score between 1, preferred species and -1 avoided species (Lanszki, et al., 2001). This was calculated using total count data in water samples as an indication of available prey, and count data in spraint as consumed data. To analyses the spatial variation in preference this was repeated firstly,

samples collected in the 0- 7000m group, and then secondly for samples collected over 7000m from the fish farm.

To effectively identify the impact of each species in the wider ecological network identified prey species were categorised by extinction risk in Britain. Risk level was identified through the use of IUCN Red List, The Wildlife Trust and British AP resources (International Union for Conservation of Nature and Natural Resources, 2020; The Wildlife Trusts, 2020; Joint Nature Conservation Committee, 2020). These categories identified as Common (Least at risk), Concern, Endangered, Critically Endangered (Most at risk) and Non-native; species that had been introduced and had not since been considered to have become naturalised. Percentage comparisons of each group between otter and mink diet composition was used to identify the respective impact of each species.

3.3 Results

3.3.1 Data Filtering

Before threshold application, a total of 37 taxa were detected from 60 faecal samples. This included 16 fish, 6 bird, 4 amphibian and 14 mammal taxa. After threshold application the total taxa count was condensed to 31 including 16 fish, 6 bird, 4 amphibian and 6 mammal taxa.

3.3.2 The Overlap Between Otter and Mink Diet

Diet composition on a species level was found to vary significantly between predators ($F=5.426$, $R^2=0.033$, $P<0.001$) (Figure 20). Variation on the class level is also clearly visible, with otter showing a preference for fish prey whereas mink fed largely on mammals and birds (Figure 20).

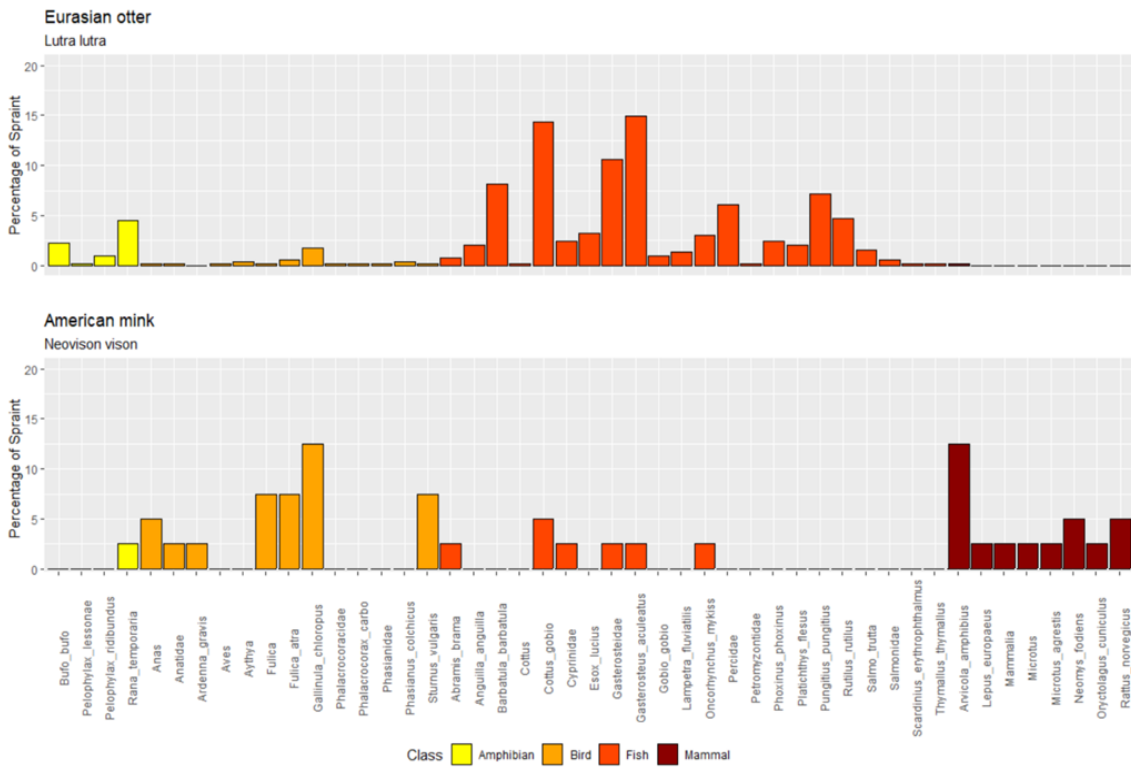


Figure 20: Diet composition of the Eurasian Otter and American Mink, with distinct class groups.

3.3.3 Impact on Endangered Species

The American mink appears to prey upon a higher percentage of endangered species (Table 1). This predominantly consisted of the water vole, *Arvicola amphibius* (Figure

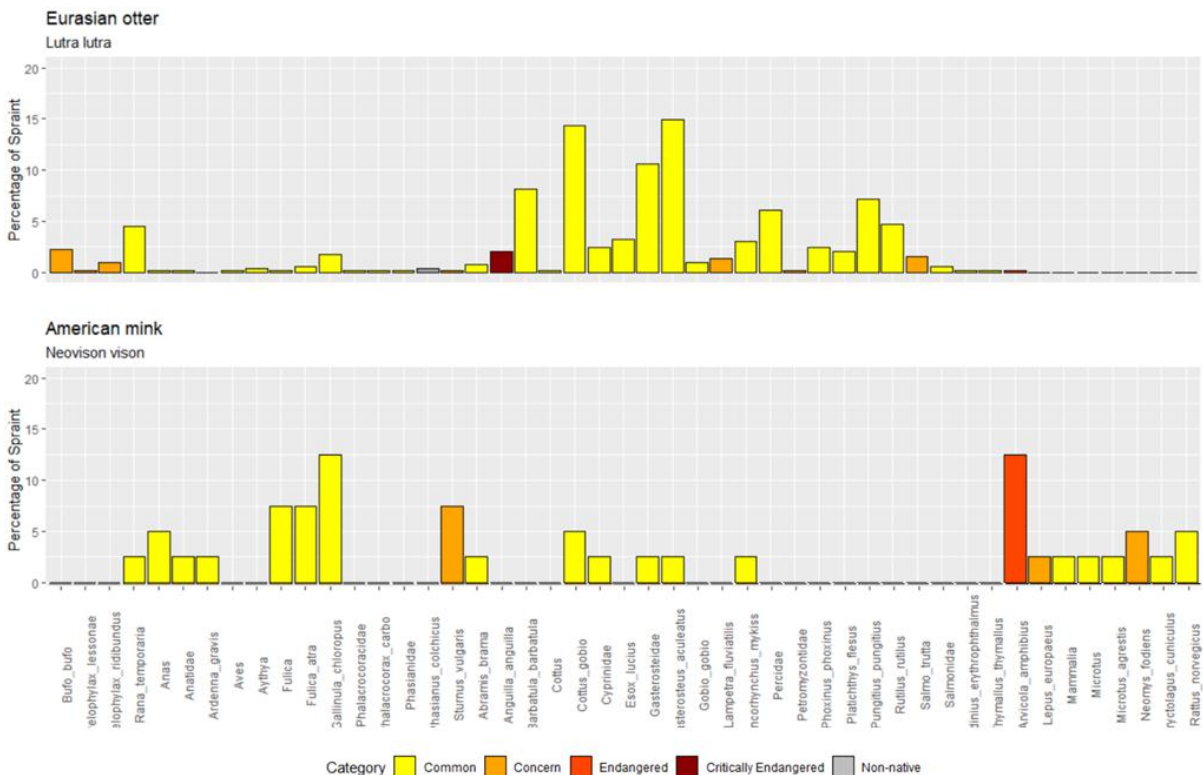


Figure 21: Diet composition of the Eurasian otter and American Mink, with distinct threat groups.

21). The Eurasian otter predominantly preyed on common species, however included a small percentage of critically endangered species, with the inclusion of the European eel, *Anguilla anguilla* (Figure 21). This is not seen in mink diet.

	Otter (%)	Mink (%)
Common	90.71	72.5
Concern	6.63	15
Endangered	0.19	12.5
Critically Endangered	2.08	0
Non-native	0.38	0

Table 1: Percentages of each threat level found in the diet of the Eurasian otter and American mink.

3.3.4 Variation in Otter Diet

3.3.4.1 Temporal Variation

Temporal variation was found to be significantly different between 2015, 2017 and 2018 ($F=3.0515$, $R^2=0.02961$, $p<0.001$) (Figure 22). The samples collected in both 2016 and 2019 were discounted due to very small sample size.

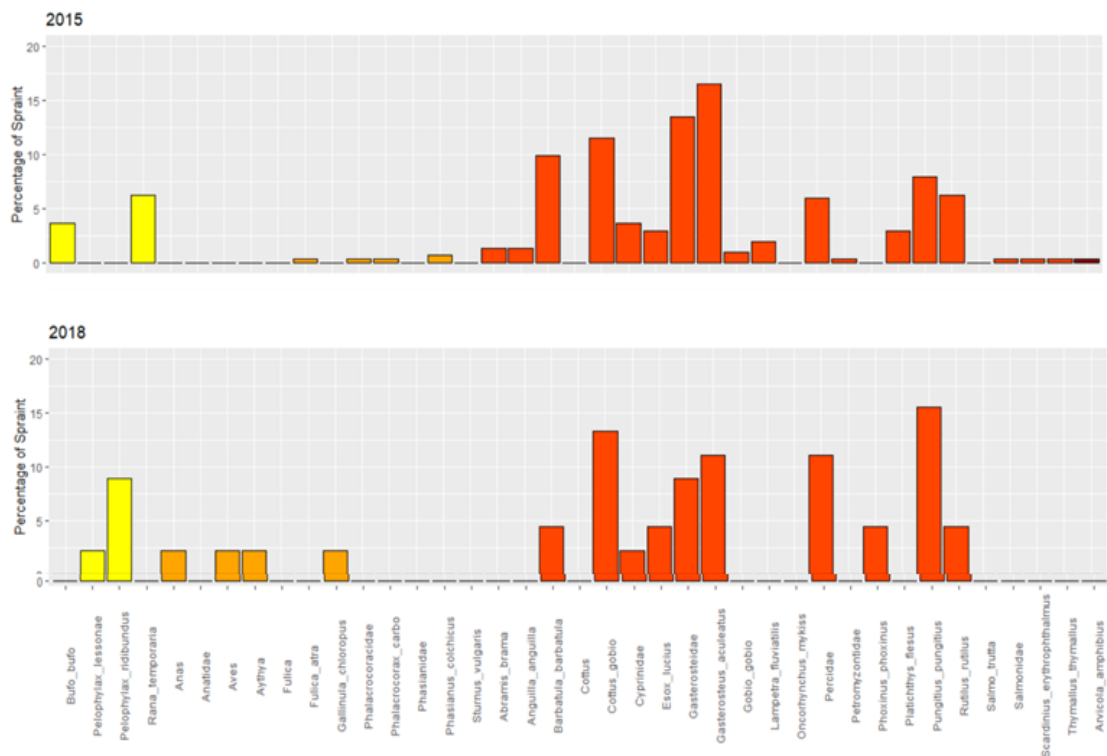
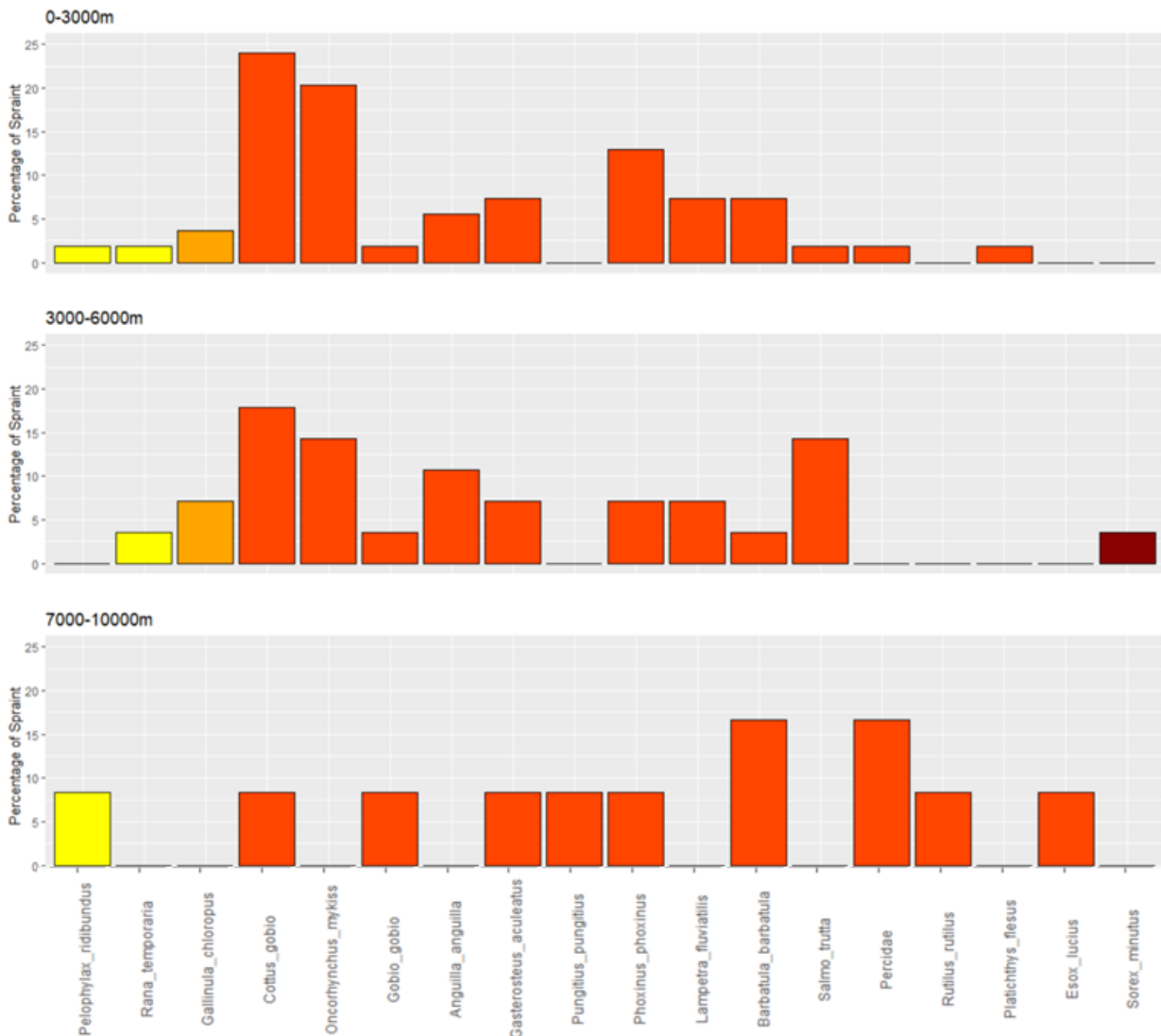


Figure 22: Temporal variation at Tophill Low Nature Reserve.

3.3.3.2 Spatial variation and preference

Spatial diet composition was found to vary significantly at different distances from the trout farm ($F=1.8534$, $R^2= 0.1388$, $p<0.05$) (Figure 23). Fish composition remained the most preyed upon class at all distances. Rainbow trout abundance in the spraint declined with increasing distance from the trout farm.



Fish composition was also significantly different between the two spatial groups (upper catchment vs. middle catchment, $F=4.577$, $R^2=0.337$, $P<0.05$) (Table 2). This is paired with variance in the preference for different species above and below the 7000m point. The species *Abramis brama* and *Anguilla anguilla* had decreased preference with distance. In contrast, preference for *Rutilus rutilus* and *Salmo trutta* increased. Preference for *Lampetra fluviatilis* and *Esox lucius* remained consistent.

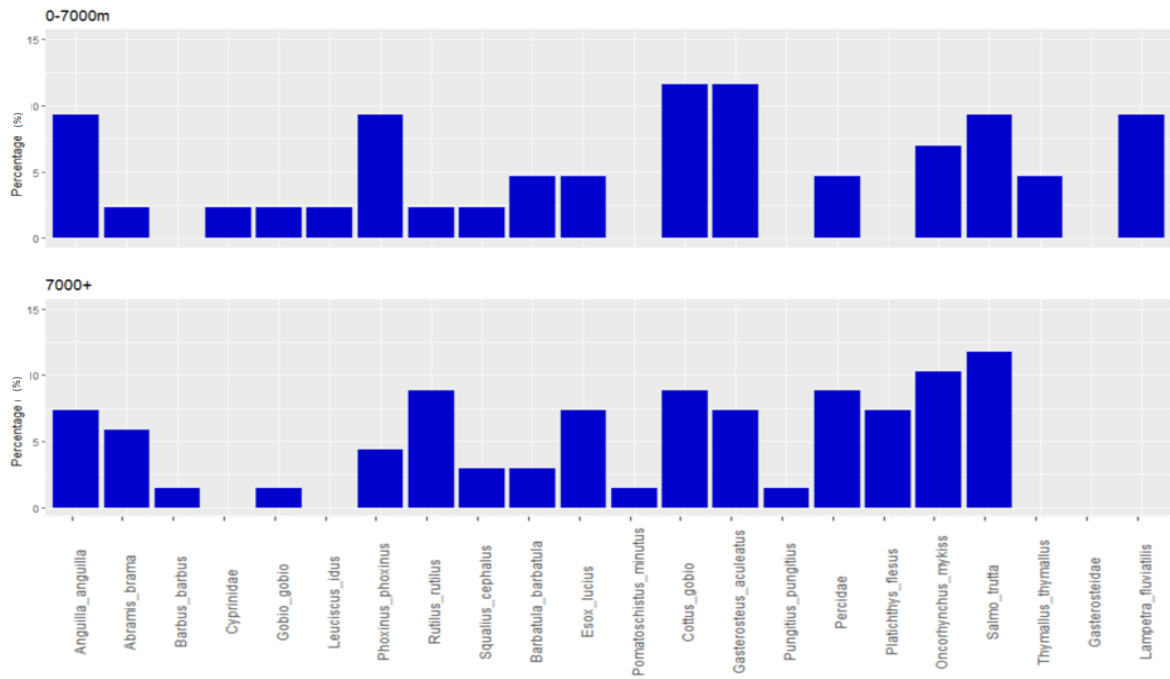


Figure 24: Fish species in water samples composition in the river Hull in two distinct groups, within 7000m of and over 7000m from the fish farm.

Species	0-7000m	7000+	Overall Preference
Abramis_brama	0.979	-1	0.972
Anguilla_anguilla	0.852	-0.434	0.747
Barbatula_barbatula	0.927	0.226	0.801
Cottus_gobio	0.275	0.951	0.52
Esox_lucius	0.957	1	0.827
Gasterosteus_aculeatus	-0.084	-1	-0.211
Gobio_gobio	-0.11	NaN	-0.135
Leuciscus_idus	-1	-1	-1
Lampetra_fluviatilis	1	1	1
Oncorhynchus_mykiss	-0.995	-0.678	-0.882
Percidae	0.916	-1	0.455
Phoxinus_phoxinus	-0.905	1	-0.779
Platichthys_flesus	1	0.969	0.84
Pomatoschistus_minutus	NaN	-0.901	NaN
Pungitius_pungitius	1	NaN	1
Rutilus_rutilus	-0.433	0.378	-0.704
Salmo_trutta	-0.975	0.796	-0.776
Scardinius_erythrophthalmus	1	NaN	1
Sturnus_vulgaris	1	-0.92	1
Squalius_cephalus	-1	0.948	-1
Thymallus_thymallus	-0.72	1	-0.652

Table 2: Ivlev index of species in three groups; 0-7000m and 7000m+ from the fish farm, and preference from the entire dataset.

3.4 Discussion

3.4.1 Diet overlap and competition between otter and mink

The analysis displays variation between the diet of otter and mink, with a preference for fish seen within the otter's diet. In comparison, mink diet consisted of mostly mammal and bird species (Figure 21). Temporal and spatial variation within otter diet, is also evident, however a preference for fish remains (Figure 23) (Figure 24). This however does not appear to directly link to the location of stocked fish species (Figure 24).

The data displays that otter predominantly predate on fish species on the catchment (Figure 20). Adapted as specialist fish hunters (Carss, 1995) this is as predicted (Figure 20). The remaining diet composition was consistent of amphibian and bird species, with mammals largely ignored. A similar diet composition trend was found in studies without mink present (Blanco-Garrido, et al., 2008; Smiroldo, et al., 2009). As a result, the analysis (Figure 21) indicates that there is no evidence that mink presence is creating an adverse impact on otter diet. As the larger of the two species, this also compliments the expectation that otter is the dominant species. With a similar diet study by Smiroldo et al (2009), identifying that otter increased consumption of alternative food types when fish populations were declined by an external factor. Therefore, high proportions of fish also may suggest a healthy fish population.

In contrast, the data suggest that mink rely more heavily on other aquatic and terrestrial species, than fish, compared to otter, in particular the moorhen *Gallinula chloropus* which makes up the largest percentage of diet composition (12.5%). This is contrasting to findings in numerous studies (Bartoszewicz & Zalewski, 2003; Haage, et al., 2017; Chibowski, et al., 2019), which found mink displayed preference to fish, with little interest in mammal prey. This is consistent with the hypothesis that mink diet might have adapted its feeding preferences to avoid competition with otter and allow species co-existence (Bonesi & W. Macdonald, 2004). Bird species however were not considered in a direct choice study by Haage et al, (2017), which may have caused significant variation to preference found.

3.4.2 Human-Otter Conflict and Diet Preference

Fish are always the most abundant classification in otter diet (Figure 20). When analysing the 2019 data set alone (Figure 23), rainbow trout is one of the most abundant components of the otter's diet. Decrease in frequency in spraint with distance from the site, suggests that individuals close to the farm do maintain a preference for stocked fish, where fish populations are at high frequency. This is supposedly supported as the greatest temporal variation in otter exists between the closest two groups, (0-3000 and 3000-6000) and the final group (7000-10000), where both brown and rainbow trout are not present. Consumption shift may suggest that sites further downstream to farms are suggested to be less optimal to otter populations (Figure 23), with increase in stone loach, *Barbatula barbatula* found.

Evidence of predation on stocked species can fuel human-otter conflict due to the prediction that otter diet will show preference for stocked species. Consequently, to some degree this decline in consumption of both brown and rainbow trout should be expected. Due to the populations of both species being stocked, the composition of each is expected to decrease with distance from the farm. However, contrastingly, populations remain high across the entire catchment (

Figure 24), therefore suggesting otter predate on stocked species due to high abundance not preference. To some degree this change in preference may be influence by the size of rainbow trout in each location, with average individual in the species varying to between 500g, optimal for otter and over 1000g, which are generally avoided. Further support of this is evident in the combined data set, in which no preference for stocked rainbow trout is shown in otter (Table 2) and a decline in preference for brown trout closer to the fish farm (Table 2). Contrast in the degree of preference identified in otter in this dataset, may be explained with a shift in the sites where the majority of samples were collected.

Despite both otter and mink being present in the catchment, mink do not often face the same stigma of hunting stocked fish. This is most likely due to their reputation for preying upon mammals (Bartoszewicz & Zalewski, 2003). However, the percentage of both stocked fish; Brown Trout, *Salmo trutta* and Rainbow Trout, *Oncorhynchus mykiss*

are relatively low in not only otter (3%), but mink also (2.5%). Mink therefore may be equally benefiting from stocked fisheries, with rainbow trout responsible for almost the same percentage of diet as for otter, and one of only five fish species present in their diet. Fish that are present in mink diet are only constitute 12.5% of prey consumed (Figure 20), with *Abramis brama*, *Oncorhynchus mykiss* and *Gasterosteus aculeatus* each contributing around 2.5%. Of the fish consumed by mink, all were recorded to be highly populated species across the River Hull catchment (

Figure 24). Two of these species are also expected to be unfavourable to otter due to large size, possibly reducing the competition on these species between otter and mink (Lanszki, et al., 2001). This is supported by the preference data (Table 2), with the exception of *Abramis brama*, which is considered to be preferred by otter when analysing prey choice with exception to the 7000m+ group (Table 1). Further spatial segregation of mink diet preference may still identify that due to a contrast in otter preference between sites being reversed in mink, may again support mink diet adaptation to avoid conflict with otter.

Pungitius pungitius is indicated to be of high preference, with increase in consumption also identified between 2015 and 2018. Despite a preference for fish around 500g-1000g being previously found, high consumption of small fish around 50g was identified in an earlier study by Lanszki & Körmendi (1996), with fish over 1000g responsible for only 1% of diet. This is reflected in avoidance of *Pomatoschistus minutes*, *Leuciscus idus* and, in the overall catchment, *Squalius cephalus*. In contrast, the percentage of diet constructed of larger fish species in the whole of otter diet is approximately 11%. It is possible the hatching or breeding periods of fish influence the degree of predation on each species, as the age of individuals predated on cannot be determined in the eDNA approach. The Northern Pike, *Esox lucius*, is a preferred species which individually constitutes to 3% of otter diet, the largest percentage of any fish over 1000g. When considering the species wider ecology, sample collection and hatching periods overlap, in which period high numbers of individuals of more optimal hatchlings are present in waterways (McNamara, 1937). Such suggestion however can only be hypothesised with no supporting evidence.

The behavioural, ecological features of each species may however be able to explain preference where size of fish cannot. Of the species recorded (Table 2), most can be segregated into two groups; benthic or open water species. Initial grouping indicates that otter display preference for benthic species including *Barbatula barbatula* and *Lampetra fluviatilis* (Vowles, et al., 2018; Zweimüller, 1995) and avoidance for open water species such as *Leuciscus idus* and in all but 7000m+ range, *Squalius cephalus* (Gousskov, et al., 2016). This trend is anticipated as otter are opportunistic hunters (Bauer-Haáz, et al., 2014). Although there are limited studies on the impact of benthic behaviour on predation in the preference of the Eurasian otter, there is a known relationship between the distantly related sea otter *Enhydra lutris* (Koepfli & Wayne, 1998) and a diet of benthic prey (Strobel, et al., 2018). In this case the ability to dive directly on to bottom dwelling prey can be directly linked to the behavioural strategies used by individuals to both identify and capture prey (Strobel, et al., 2018). Overlap in this behaviour may exist between the two species, supporting the hypothesis that the Eurasian otter will display preference to benthic prey. However with rapid evolutionary change between otter species (Koepfli & Wayne, 1998), further data and analysis is required before this can be proven or rejected.

Furthermore, preference for benthic prey is not always supported as benthic species such as *Gobio gobio* (Zweimüller, 1995), are shown to be unfavourable prey. Secondary grouping of these species however indicates avoidance for gregarious prey, as avoided species are described as social if displaying a benthic ecotype (Frost, 1943). Developed as a defence mechanism, evolution of a social group decreases the chance of an individual being predated (Hamilton, 1971) upon but also increases the abundance of a species in a small area, making a sighting by a predator more likely (Morelli, et al., 2019). Nevertheless several aspects may contribute to a decreased ability to capture prey once detected such as increased vigilance of prey and possible predator confusion (Allan & Pitcher, 1986). Regardless, the development of shoaling behaviour appears to be advantageous in avoiding otter predation.

The degree to which cyclic events, such as breeding or spawning periods, impact on otter preference is difficult to determine with limited data on the composition of fish populations in the area. Gaps in the variation of temporal fish preference may be explained should further collection of spraint and water samples continue. With

current analysis only focused on one sampling event, the temporal variation in freshwater species composition cannot yet be considered. In continuation, using only water samples to defer preference is much less useful in mink populations which are suggested to have predominantly terrestrial based diet. Nonetheless, spatial variation in the preference of fish indicates that otter preference varies greatly in relation to species composition. Where preference for larger fish, such as *Abramis brama* may appear unusual in relation to known otter preference (Lanszki, et al., 2001), it is clearly indicated that in the sites furthest from the trout farm, the species is actively avoided. Increased preference for species in relatively low abundance in the sites where larger species are predated upon, suggest that larger fish may only be predated upon when more preferable species are in low abundance.

Whilst the impact of otter on fish farms is largely considered, the effect of fish farming on otters is regularly ignored. The cessation of fish farming has been identified to decrease otter reliance on fish stocks, with a shift to alternative prey (Lanszki, et al., 2001). In this case, fish are believed to become a secondary source of prey (Lanszki, et al., 2001), caused by a shift in vegetation type. With previous farms once in the lower section of the river a consequence it may hypothesize that the reliance or preference of fish may decrease with distance, however this is not reflected in the results (Table 2). High preference for fish may remain present throughout the entire catchment, which may be explained by season (Lanszki, et al., 2001) or time since farm closure. Substitution of prey type may also be being missed as invertebrate species were not identifiable in the eDNA results. Invertebrates have also been found to previously contribute to a large proportion of otter diet on the River Hull. Consideration of this factor, in comparison to the reliance on invertebrates of mink, should it be further explored may also identify further areas reducing otter-mink conflict.

Consideration should be applied to the possibility that fish choice in the 2019 sample may to some degree be impacted by the impact of flooding on both fish populations and otter prey choice of both the otter and mink. Otter have been found to avoid fish living in open water, in comparison to areas with aquatic plant coverage (Lanszki, et al., 2001). With a further preference for fish in shallow regions, water overflow in the habitat and open degree of waterways varied greatly at some time periods and sites. Advancing on this, there has already been several studies on the direct impact of

flooding events on fish populations, including increased mortality of less adaptable species (Power, et al., 2008; Booth, et al., 1988). In particular, the heavy flooding events of years previous to the sampling period have been highlighted as having possible irrevocable damage on freshwater communities in the United Kingdom (Office, 2015). As an implication, the composition of species will be adjusted, having a possible continued effect on the preference of otter and possibly mink. This may also provide a secondary explanation for why otter are preying on unpreferably sized species, although this is not conclusive from the data collated. Nonetheless, with continued intense flooding events, including within the study period the impact of such events must be considered in future studies and preference analysis.

3.4.3 Differential impact of both predators on species in the wider ecosystem.

Several studies have also linked mink populations to a decline in native species. In particular, the American mink is criticised for much of the decline in the water vole. It is evident however that both otter and mink are having impact on at risk species. Despite competition initially displaying positive cause for decline in mink population the shift in diet, caused by the presence of otter may be counterproductive. For example, it is possible that pushing mink towards a more terrestrial base diet be encouraging the mink to predate on water voles. Furthermore, whilst the percentage of at risk species predation is considerably greater in mink than otter (Table 1), it should be noted that critically endangered species are only existent in the otter's diet. Preference is shown for the European eel, *Anguilla anguilla* over the entire catchment, which makes up 2% of otter diet. Nevertheless, despite predation stable populations of the eel have been recorded in the River Hull catchment indicating otter populations are not having a detrimental impact on eel populations (East Yorkshire Rivers Trust, 2020).

Focusing on the impact of predation on birds, a second study by Ferreras and Macdonald (1999), directly investigated the impact on moorhen, *Gallinula* and coot, *Fulica*. Mink were found to have a direct negative impact on breeding periods of both species. In particular, the study found that of the two species, the nesting behaviour of coot is particularly vulnerable to mink predation (Ferreras & Macdonald, 1999). Preference for coot however is not identified in the River Hull catchment, with an equal preference identified for both species (Figure 20). To some degree, this is likely

to be due to little overlap in the sample collection and breeding period of both species, between March and September.

It is also notable that, although only responsible for a very small percentage of otter diet (0.38%), non-native species were identified in otter diet but were not present in mink diet (Table 1). Although not often of major concern, studies surrounding the negative impact of increasing pheasant *Phasianus colchicus* numbers indicate that high densities of the birds can be long term (Neumann, et al., 2015). This is most evident in their effect on hedgerow structure and invertebrate communities (Neumann, et al., 2015). Therefore evidence of predation by otter may be ecologically beneficial. Although low suggested preference to pheasant implies the impact is likely to also be limited. The low frequency of pheasant present in otter diet may be due to contamination in contrast to being prey items. The hunting ecology of otter is adapted to catch aquatic prey (Kruuk & Kruuk, 2006), of which pheasants are rarely considered. Alternatively, this percentage may be low due to the rarity of swimming events by pheasant.

3.4.4 Conclusion

The data supports several expected outcomes. Most apparent is further evidence that otter remain the dominant species in the catchment area. Conflicting evidence however is also existent, with all of the comparative diet data between otter and mink supporting co-existence (Figure 20, Table 1). Yet variation both spatial and temporal apparent in otter diet underlines the significance of wider ecological factors; such as prey composition in an ecosystem and changes in abiotic features. These factors and monitoring changes should therefore be identified as a key area of focus for continued study. Building upon this the impact of such factors on the degree of reliance in diet on native species could be critical in future conservation plans. Particularly within this, the possibility that otter may be negatively indirectly impacting on water vole must be scrutinised. In either case, the negative impact of mink on populations is evident and a requirement to maintain monitoring of mink populations remains.

Chapter 4 Discussion

Variation in the degree of co-existence between the Eurasian otter, *Lutra lutra*, and American mink, *Neovision vison*, cannot be easily determined by one single factor. This is increasingly apparent on the River Hull. From the analysis in chapter 2, it was found that countermarking was not found to be occurring and there is no reasonable evidence to suggest co-existence. In contrast, variation in the diet of the two species was found, supporting the hypothesis that the two species are co-existing. Both chapters did however support the hypothesis that the Otter, *Lutra lutra* is the dominate species over the American Mink, *Neovision vison*,

As no degree of co-existence evident from the distribution of the two species, divergence in diet may be unexpected. Lack of countermarking however indicates that lack of co-existence may be caused by the inclusion of sites where mink have never been present, or variation in the wider ecological factors as to if co-existence can exist, between sites. Alternatively, it is possible that difference between the found outcomes of each factor is a result of ongoing adaption from aggression to co-existence. With longstanding knowledge of both populations being present in the area, for this to be a viable hypothesis a prolonged change in mink diet must have occurred. The time necessary for co-existence to occur nevertheless is not fully understood, and the degree to which it can be sustained has been found to vary (Bonesi & W. Macdonald, 2004). New consideration of wider ecological factors known to influence if co-existence will occur, such as habitat variation, therefore could provide explanation for the contrast in distribution and diet results. In particular, from initial data, co-existence is not occurring away from the main river. Consideration of habitat and species composition change from main and tributary sites is an area should therefore be included in future study.

This suggestion is further supported by the results. Whilst a preference is found for the locations within a closer range to stocked farms, it is not identified for stocked fish. Instead habitat features and holt availability appear from the results to be the main aspect influencing the sprinting behaviour of otter. This emphasises the possible hypothesis that interspecific aggression is greater over habitat and territory protection, than of areas with large fish stocks. As stocked fish also display features both

previously found and suggested in the results to be only slightly preferable to otter, there is no indication otter are a large threat to farm populations. Resultantly neither otter nor mink can be pinpointed specifically as large financial threats to farms but will predate upon due to ease of access. In contrast, the greatest evidence of intra specific competition does exist in the areas with stocked fish and therefore guaranteed high fish abundance. With a slight preference for fish present in both species this should be expected. With DNA of these species in the diets of both otter and mink protection around farms should be used to avoid any economic loss from either species.

Variation in when, or if co-existence occurs is emphasised in the divergence in diet identified. With a more substantial data set it may be possible to look more specifically at the composition of fish is significantly different between sites where the two species are present and those where only one species exists. From the results it can already be suggested that mink may adapt their diet in order to co-exist with otter. This was already known in areas where otter were reintroduced and can now be concluded to also occur in areas with growing but pre-established populations. Lack of specific historic data nevertheless also brings question in to if areas without mink have been reclaimed by otter, are unpreferable to mink, or otter have acted as preventatives in the spread of mink populations. In either case, divergence in diet displays that otter are restricting the prey in which the generalist mink are choosing to predate upon. However, this may be counterproductive in conservation efforts with the possibility co-existence has resulted in emphasised negative implications. As a result, this can accentuate the ecological damage mink presence can cause.

From the results of the study it can be recommended that to reduce the level of predation on stocked fish, diversity of fish should be maintained in surrounding areas. As these fish are less preferable, having diversity, particularly of species with preferable traits will reduce predation on stocked fish. This is supported by both the found diet and distribution of otter. Whilst a positive correlation does indicate that otter have higher competition over sites closer to farms, for the majority of the sample period the highest sprainting activity is not at sites directly adjacent to the farm. The lack of desire for otter to particularly focus predation efforts on stocked fish is again emphasised here, with a dip in spraint numbers implying that an area with high stocked populations have a declined preference. Consequently, areas with high

populations of stocked fish appear to be possibly less beneficial in otter repopulation. High spraint count throughout the year in the adjacent sites and high preference at certain temporal periods displays that decline in diversity is not impacting otter populations greatly if at all. Nonetheless, with variation between the species composition of fish in the two distance groups identified where significant variation in distribution is found, it is indicated that diet is an influence on otter distribution. An increase in the populations of less preferable fish, and decline in preferred fish is seen. In contrast, the preference factors of mink however cannot be seen due to minimal mink spraint collection. With greater data, the factors influencing mink prey choice will be beneficial for understanding exactly how otter are affecting mink preference.

In future study, the possible impact of interspecific competition and therefore pressure applied on mink to predate on terrestrial prey by otter must be explored. Using the distribution data there are identifiable areas where mink or otter are less abundant. Two hypotheses can be applied here to gain a greater understanding of the direct impact mink have. Firstly, it can be predicted that mink are less likely to be present in areas with low abundance of terrestrial prey species; hypothesising that the population of species such as the water vole, *Arvicola amphibius* will be lower in sites where mink are not present. Secondly, the degree of preference for mink, given the results of this study should remain high across all sites. However, as it is suggested that mink have adapted to terrestrial prey to avoid prey competition, it can be hypothesised that preference for terrestrial species will increase with otter abundance in the area. Despite the possible role of otter on the percentage of at risk species in mink diet, mink are still a threat to native species. The percentage of mink diet composed of concern or endangered species is over a quarter of the total mink diet. Consequently, the results support continuation of work to remove mink, especially in areas with otter present.

Despite previous studies emphasising possible potential of otter to remove mink form an area, from collated spraint data, the degree of interspecific completion is not great enough. Resultantly, otter are not a long term solution to aid the removal or reduction of mink. Positively, mink however do not appear to be hindering or impacting upon the restoration of otter populations. Regardless, negative implications must also be considered and precautions must be applied in efforts to increase otter populations.

Should both populations remain in the area, and otter continues to incline without a reduction in mink there may be a risk of over population. Evidence of high levels of overpopulation does not appear to be a significant on the catchment, predation on at risk and endangered species may become a future concern. This therefore should be monitored, and may with future study become greater evidence for the required removal of mink. Alternatively, otter may have the potential to contain mink populations. No influence from the location of mink trapping on otter population supports belief that otter are the dominant territorial species. Therefore it remains possible that if otter populations continue to rise, the area in which mink occupy may continue to decline slowly. Consequently, conservation efforts to restore populations on the catchment should be continued.

Equally, future work must also be undertaken to examine the seasonal variation of diet and movements over the summer period of both species. The breeding period of otter displays annual variation, therefore impacting differing sprainting density throughout the year. Whilst results do not indicate that otter movements vary greatly the study period, indication of cyclic movements in upper stream locations may be more evident over an annual period. In relation to this, significantly difference in annual diet composition may be reflected by changes in movement and behaviour. The beneficial value of both distribution and diet data could be greatly increased should further meta data, such as sex, age or dominance be considered. As suggested from the distribution data, specific sections of the catchment have greater possibility to rear young. Should this be the case, the diet of young learning to catch prey is likely to differ from that of more experienced individuals. Similarly, dominant males are more likely to predate at sits with more preferable fish than males obtaining poorer quality habitats. Importantly, the degree of more or less preferable in each individual's diet has the potential to reflect on the ecosystem health. For instance, if a less dominant male has a low degree of impreferable fish in their diet this may be a reflection of good fish diversity in the entire catchment. Full understanding of this however cannot be explained by the results, highlighting where new data may be beneficial to ecological monitoring. Nevertheless, strong diversity is currently displayed both in water and spraint samples.

Conclusively, evidence supports the coexistence of the Eurasian otter and American mink at the majority of sites on the catchment. With a number of sites maintained by otter populations alone, sustainable or growing otter populations display signs of and future potential in containing the sites where mink populations occur. Precautions must be taken in conservation efforts, with threatened species in both otter and mink diets, with further possibility of otter forcing diet change in mink. Nevertheless, the study has highlighted a number of future questions on surrounding both diet and distribution in both species. For distribution in particular, the wider environmental factors and individual species ecology require further investigation. In comparison in diet, future focus on meta data alongside the preferences of both species should be the next step in to the study of otter and mink interactions.

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