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Search for parasites of exotic turtles in Hungary

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1. INTRODUCTION

In Hungary, only one native chelonian is found– the European pond turtle (*Emys orbicularis orbicularis*). It is widely distributed across the country, inhabiting most stagnant and slow flowing water habitats (Farkas, 2000). However, due to a high increase in the animal pet trade and lack of regulations, exotic turtles have started to invade the Hungarian waterbodies – mainly the pond sliders (*Trachemys)*. The disappearance of wetlands and fragmentation of habitats already poses a threat to the native pond turtles of Hungary, and having extra competition from alien invasive species could have a major impact on conservation projects (Farkas *et al*., 2013). These free-ranging individuals are found in many countries across Europe and have successfully established themselves in these ecosystems. They can cause direct competition for food, basking sites, and nesting sites, and tend to be quite aggressive, forcing the European pond turtles into less favourable habitats (Demkowska-Kutrzepa *et al*., 2018). They also may have a negative impact indirectly, through the introduction and transmission of new diseases to the area. This can occur through a co-introduced alien parasite species which may infect the native hosts, and in some cases, cause significant pathologies due to their naïve immunity. Another possibility is for the alien hosts to act as amplifiers or diluters of the native parasites, affecting the biodiversity and population dynamics (Lymbery *et al*., 2014). This could be an issue not only for the wild population of *E. orbicularis,* but also for those bred in captivity in reptile farms as parts of re-introduction programs, having a major impact on conservation projects and leading to an overall loss of biodiversity (Demkowska-Kutrzepa *et al*., 2018).

2. AIMS AND GOALS

The author's objective for this Diploma Thesis is to research the possible internal parasites of invasive turtles in Hungary, as at present there is no available data on this subect. This thesis will firstly review the available literature on internal parasites described in alien turtle species in other countries around Europe, as well as literature on the parasites of *Emys orbicularis,* with a special focus on the species that have been reported to switch hosts. This investigation will concentrate on internal parasites detectable through faecal or visual examination.

The practical part will be a first step in exploring the potential presence of parasites in a population of invasive turtles in a lake in Budapest. The content and results of this Diploma Thesis will hopefully aid in further research on the parasitic populations of freshwater turtles in Hungary and help conservation efforts of *E. orbicularis.*

3. LITERATURE REVIEW

3.1 Freshwater turtles of Europe

The pond slider (*Trachemys scripta*) is an ectothermic freshwater turtle of the *Emydidae* family originating from North America. There are three documented subspecies – the red-eared slider (*Trachemys scripta elegans*), the yellow-bellied slider (*Trachemys scripta scripta*) and the Cumberland slider (*Trachemys scripta troostii*). Their lifespan ranges from 20 to 50 years and their adult carapace size is on average 25-30cm; both factors that may make them complicated to keep as pets. They can be active all year round with a preferred temperature range of 10-21°C, but if the temperature drops too low, they will brumate. Females' sexual maturity is age-dependent and is usually reached at 5 to 7 years in the wild, whilst males' sexual maturity is size-dependent and reached between 9 and 11 cm in carapace length. Mating typically occurs between March and July in their natural environment, and eggs tend to be laid during May and June. It is notable that *Trachemys spp* (and other freshwater turtles) females can keep viable sperm for a year, a useful advantage for an invasive species. Pond sliders are opportunistic carnivores with a diet ranging from dandelions and hyacinths to earthworms and tadpoles. They tend to be more carnivorous when young and grow more omnivorous with age, they are aggressive hunters and efficient foragers, with a keen eyesight and olfactory sense to help them. In an ecosystem they act as both predator and prey, although, once they have reached adult size, the only true threat to them are humans. *Trachemys* is notorious nowadays as it is one of the world's top 100 worst invasive alien species (Burger, 2009; Héritier *et al.*, 2017; Rhodin *et al.*, 2017; van Dijk, 2010).

Trade in pond sliders started as early as the 1900s, where their sale in local flea markets lead to the beginning of farming by 1950 in the USA. In the 1980s and 1990s, over 52 million red-eared sliders were exported out of the USA towards Europe and Asia and sold as pets; they were very popular and sold as small and colourful hatchlings with relatively easy husbandry. Their demand also increased due to franchises like Teenage Mutant Ninja Turtles, a comic well loved by the children demographic. However, as the novelty of a new pet passes and they reach their adult size, living longer than expected and potentially develop health issues from improper keeping, owners seek to get rid of them. Many, potentially unaware of the ecological impact, turned to the solution of releasing them into the wild, as it is widely seen as more humane than euthanasia (Burger, 2009; Cadi and Joly, 2004; Meyer *et al*., 2015). Their trade was prohibited in the EU in 1997 (Wildlife Trade Regulation, Council Regulation 338/97 of the European Union), which unfortunately led to an increase in the release of many now illegal animals (Hulme, 2015).

Despite this ban, there was still a market demand for pet freshwater turtles by hobby keepers, which was met by the import of other species such as the yellow-bellied slider (*Trachemys scripta scripta*), the false map turtle (*Graptemys pseudogeographica*), the common musk turtle (*Sternotherus odoratus*) or the river cooter (*Pseudemys concinna*) – all of which originate from the USA (Kopecký *et al*., 2013). It was also reported that the other pond slider species can hybridize with the already present red-eared sliders and possibly increase its invasive potential (Urošević *et al*., 2019). A more thorough regulation was introduced in 2014 (Regulation 1143/2014) which placed a ban on importation trade, possession, breeding, transport, use, and release of a prioritised list of species which was made from evidenced-based risk assessments. Most of the North American terrapins were added to this list and were found to be the most common species voluntarily given up. Whilst this regulation reduced the sale and ownership of these species, it did not seem to deter the release of these species into urban lakes, despite this being a crime in most of Europe. This is likely due to a lack of education campaigns and of proactiveness of these legislations, only banning species once they are already found as invasive (Maceda-Veiga *et al*., 2019).

In Hungary, the only native chelonian present is the European pond turtle, *Emys orbicularis orbicularis*, recognisable by the yellow spots on its shell and body; it also belongs to the *Emydidae* family. It is the most common species in Europe but is considered 'Vulnerable' in the European Union and 'Nearly threatened' in Europe according to the IUCN. In Hungary, it is protected with a fine of HUF 50 000 for any harm caused to a specimen, but this does not apply to harm caused to its environment. The fragmentation of wetland habitats due to human expansion has had a big impact on their population, forcing them to concentrate on semi-natural and artificial lakes which are also inhabited by abandoned exotic turtles. Their adult carapace length is usually 12-20cm, which is a bit smaller than the pond sliders. It is believed that they can live for decades, with the average lifespan record at 15 years, but this is hard to evaluate as they are not usually kept in captivity (at least not legally). Mortality is at its highest during their first year of life due to them being an easier prey for predators at this age. They will brumate during the winter and will emerge in March to start mating in April, laying their eggs mostly in June. They possess the same sperm storing capacity as *Trachemys spp*, but this has led to some inbreeding issues in certain closed environments. They are omnivorous, with efficient hunting skills in the water, but this behaviour varies with their age like the pond sliders. Younger specimens have more carnivorous tendencies, with their diet becoming more plant based as they grow into adults (Bereznay, 2002; Farkas, 2000; Farkas *et al*., 2013; Magyar Madártani és Természetvédelmi Egyesület 2016; Rhodin *et al*., 2017).

A mapping program was launched nationwide by WWF-Hungary, which has started recording the population of *E. orbicularis,* in order to reach a more evidence-based consensus on their status. The research on this species so far is still limited to population surveys and monitoring; it is sadly lacking due to the Minister of Environment and Water's non-approval of the Action Plan towards the conservation of this species. Multiple projects have been set up around Hungary, tackling problems such as pollution, lack of basking and nesting sites, and notably the competition from red-eared sliders (Farkas *et al*., 2013). Multiple *T.s. elegans* specimens have been recorded in Hungary with old healed injuries, and some gravid females, which suggests that they successfully hibernate and reproduce (Kovács *et al*., 2004).

The other main native freshwater turtle in Europe is the *Mauremys spp*, pond turtles belonging to the *Geoemydidae* family with different subspecies according to location. It is found in the Balkans and in countries surrounding the Mediterranean Sea and is classed as 'Vulnerable' on the European Red List. No specimens of this species are found in Hungary; but they are present in Spain and France, - the countries where the most studies have been done on freshwater turtle parasites. In some environments, they coexist well with *E. orbicularis*, both having similar biological characteristics but different preferences in water deepth and basking sites. *Mauremys* are the most resistant of the two, however; reaching a larger adult size, they also have a high tolerance to pollution and a very adaptable diet, with adults becoming almost fully carnivorous in certain environments. Hence, they seem better equipped against the North American invaders. However, the biggest threat to this species is the same as *E.orbicularis* - habitat loss. They sometimes share the same parasites as *E. orbicularis* and may also be used to research the host switching abilities of alien parasites (Bertolero and Busack, 2017; Meyer *et al*., 2015; Rhodin *et al*., 2017).

3.2 Invaders and their parasites

The biodiversity in freshwater environments is very rich; despite only amounting to 2% of the earth's surface area, they hold 10% of known free-living animal species. However, of these species, 24.6% of them are classified as threatened on the IUCN Red List, and one of the principal threatening processes are invasive species (Lymbery, et al., 2020).

Invasive species are non-native (alien) organisms that were introduced to a new environment, either accidentally or deliberately, and managed to survive and thrive. The impact of invasive species can be seen in two ways: directly with predation and competition, or indirectly through habitat alterations or a change in disease dynamics. They must overcome multiple hurdles in order to establish themselves, firstly needing to survive transport and release into the new ecosystem. There are then three main factors acting on the establishment of invasive turtles:

1. **Propagule pressure:** this takes into account the number of specimens released and the amount of release events. In this case, it is a continuous flow of low-sized propagule (individual pets being released by many unrelated people), which helps their spread, especially due to the long lifespan of turtles. There is also a wide genetic diversity in released specimens as they are often from different origins, and a high health quality of individuals due to their captive rearing.

2. **Ecological attributes:** as the pond sliders and other invasive turtles have a wide native range, this has led to them having a good tolerance for multiple environments. Their ability to brumate further helps them to survive in harsher winter environments. The fact that they are omnivores also helps them adapt to different resources, and the fact that they are amphibious allows them to travel across some land to find a different area with a potentially more generous environment.

3. **Characteristic of environment:** In this case, the Hungarian environment is comparable to their original one, with a temperate climate and similar ecosystems. Furthermore, the impact of humans on wetlands and ponds has led to the fragilisation of the native turtle species, creating a niche in the ecosystem for the invasive species to establish themselves.

At first, the impact of the invader should decrease as it spreads away from the original point of introduction, with a weaker invasion from the parasites as they depend on the original host's establishment and the population density of potential new hosts. For the most invasive species, they will continue to spread around to different local ecosystems in a 'hopping' pattern of invasion (Burger, 2009; Kolar and Lodge, 2001; Lymbery *et al*., 2014).

Even though it is widely acknowledged that invasive alien species pose a major threat to ecosystems globally, they are not well documented. An improvement in monitoring and reporting procedures are needed to better understand their impact (Kolar and Lodge, 2001, Burger, 2009; Shackleton *et al*., 2020; Stuart *et al*., 2020).

The United States of America is the top turtle-rich country, with 82 freshwater turtles and tortoise species. North American freshwater ecosystems often support multiple turtle species at the same time (up to six, seen in Alabama) and so their terrapins show high levels of competitive abilities. On the other hand, Europe's freshwater ecosystems are usually only inhabited by only one of two species, and if they do cohabit, their behaviours are varied enough that they do not impede each other. This difference in evolutionary pathway puts the more often mild-tempered native European turtles at a disadvantage. The main advantages *T. scripta* over the *E.orbicularis* are a lower maturity age, higher fecundity, and a larger adult size. They will compete for food, nesting sites and basking sites which are vital for the species' survival and procreation (Bertolero and Busack, 2017; Cadi and Joly, 2004; Meyer *et al*., 2015).

The impact of parasitic invasions on the world's biodiversity has been brought to the light over the past decade, with studies done on other vertebrates' host-parasite complexes translocations. The consequence of invasive turtles' parasites on the native European population is not well documented yet, but an increase in studies on the subject has been seen the past decade (Iglesias *et al*., 2015; Verneau *et al*., 2011). On a study on the competition between *E. orbicularis* and *T.s. elegans* by Cady and Joly (2004), it was proven that the concern on the exotic turtles' impact on the local population were valid. It was also suggested that part of the competitive dominance of red-eared sliders could be the effect of their imported pathogens on Eruopean pond turtles.

The stage of the parasite's bio-invasion seems to correlate with their impact on the local population; it is more likely to be negative as they start to establish and persist. However, due to the mostly cross-sectional nature of studies done so far, there is a lack of data on the impact of a parasitic invasions in one specific ecosystem over time. Hopefully this knowledge will be developed further over the coming years (Stuart *et al*., 2020).

If the introduced species is infected by the native parasites, it can impact the environment in two ways. If the invaders are more competent hosts, they can act as amplifiers, which can worsen the parasitic impact on the native species– this is referred to as "spillback". They can also lessen it; if they do not transmit the parasites as readily, or to the same extent it, can cause a dilution in the overall parasitic load of the habitat's population. Although this can be seen as a benefit for the native specimens, it may also lead to an overall loss of diversity as parasites struggle to survive. In the case of native species being infected with invasive parasites, this is called "spillover". Overall, an increase in the diversity of parasites has been linked to a decrease in body condition, fecundity and immunity of the host – reducing their overall fitness (Lymbery *et al.*, 2014; Stuart *et al.*, 2020).

One of the issues in documenting the incidence of invasive parasites is that international trade and movement of animals started long before the establishment of scientific monitoring programs and taxonomic divisions of parasites. Even once these controls did emerge, the methodology took some time to perfect and international communication was sometimes lacking. In order to fully know the origin of a parasite, one must look at their history, biology, geographic distribution, taxonomy and ecology – but this data is still unclear for many species (Lymbery *et al*., 2014).

Introduced specimens tend to have less parasites than local native species. This is often due to the anti-parasitic treatments used in captivity and captive-rearing outside of their natural habitats – they do not have the full range of parasites usually seen in the wild. This may give invaders an original competitive advantage at the beginning of introduction by improving their general fitness. If they do introduce alien parasites to the environment, these parasites must then overcome the same challenges to establish and spread themselves as the host invasive species. They must also be able to infect the native host species and complete their life cycles in them, and potentially intermediate hosts (Hidalgo-Vila *et al*., 2009; Lymbery *et al*., 2014).

There are some instances of parasites being introduced without their invasive host(s) or by translocation of the native host being infected during transport, but this is a rare occurrence.

Co-introductions with the alien host is the most common, with helminth being the most popular (as found in 49% of published literature), followed by arthropods (making up 17% of reported co-introduced parasites). It is important to note that a limitation to alien parasite data is that only established specimens are noticed and those that failed would not have been recorded. It occurs a lot more often for parasites with simple life cycles, with published data showing that 64% of established alien parasites had a direct life cycle. Even though the data is still lacking, it seems that the biggest hurdle for these co-invasive parasites is not the introduction, but more the inability to establish themselves in the new environment (Hidalgo-Vila *et al*., 2009; Lymbery *et al*., 2014). Invasive parasites may not necessarily need to infect a native host in order to spread, especially if the invasive host species is quite well established. Host-switching does not seem to halter their life cycles either. There has been no correlation observed between the complexity of a parasite's life cycle and their ability to host-switch (Lymbery *et al*., 2014). On the other hand, it has been reported that alien parasites may survive even if their original hosts do not. This would mean that even a short invasion may lead to unexpected long-term consequences on the native parasitic fauna (Solarz and Najberek, 2016).

The "naïve host" theory suggests that a parasite will be more pathogenic in the new host it has switched to compared to, their original one. This is likely because during its co-evolution with its native host, a balance was found between the parasite's virulence and the host's fitness and tolerance. However, this assumes that a co-introduced host and parasite complex necessarily has had a co-adaptation, and this may not always be the case. The new, native, host may also have sufficient immunity to not be impacted by this new parasite. Furthermore, this novelty goes both ways, and a parasite's virulence may be decreased when inside a new host and facing its own new challenges. Finally, if a newly introduced parasite causes too much damage to its new host, it will work against its establishment as this will limit the spreading potential (Lymbery *et al*., 2014).

Nevertheless, even if it is only by chance that a new host may show a higher level of pathogenicity in the native species, this may have disastrous effects on the local populations. It could also be worsened if the co-invading hosts remains fit when infected and act as reservoirs, while also competing in other aspects with the native hosts. Sadly this has been demonstrated in multiple studies with infectious disease being the main cause of negative impact in 25% of invasions. In the case of European native freshwater turtle, a severe outbreak of spillover parasites causing mass mortality in *E. orbicularis* has already occurred in Spain (Iglesias *et al*., 2015; Lymbery *et al*., 2014).

Overall, it has become clear that parasites being co-introduced with their alien hosts occurs rather commonly, and even for those with more complex life cycles, with freshwater ecosystems being potentially more prone to this due to their large biodiversity. Once they have established themselves, these invasive parasites may be amplified through their invasive hosts and even switch hosts. This may potentially be very pathogenic to the local population. Alien hosts may also impact the native parasite population by amplifying or diluting their numbers (Lymbery *et al*., 2014).

3.3 Internal Parasites of Freshwater Turtles in Europe

A wide variety of endo- and ectoparasites have been found in free ranging reptiles, although most are not reported to have a major pathological impact. However, this could be because this topic has mostly been studied by taxonomists who did not report data on the hosts' response to infections. Many of the host-parasite relationships are still not fully known and some may need long term infections before a parasite has any clinical impact. Furthermore, without a necropsy it can be hard to see any potential lesions caused by parasites, but this is not always possible especially in native vulnerable specimens (Jacobson, 2007).

The trade of animals started long before any kind of scientific monitoring occurred, hence it is difficult sometimes to know the origin of some of the detected parasites. There has been multiple confusions over parasitic nomenclatures over the years, as this was previously based solely on morphology. This method is not always reliable as it depends on the quality of the equipment, as well as the parasite quality of the parasite and its preservation. Nowadays, with new molecular data and an increase in the number of studies on the parasites in freshwater turtles, we are starting to get a better picture of parasites' taxonomy and phylogenic relationships (Demkowska-Kutrzepa *et al*., 2018).

3.3.1 Trematodes

3.3.1.1 Polystomatidae

Members of the Polystomatidae family are monogenean flatworms often found infecting amphibians and freshwater turtles. Polystomes in chelonians are divided into three genera depending on the number of hamuli found between the posterior pair of suckers on the opisthaptor – *Polystomoides* (two pairs), *Polystomoidella* (one pair) and *Neopolystoma* (none). The suckers allow these parasites to anchor themselves inside the hosts. They follow a direct life cycle: operculated eggs are shed in the faeces and develop into free swimming larvae which infect new individuals. Different polystome species tend to be found in different microhabitats of the same chelonian – the urinary bladder and cloaca, the conjunctival sacs or the pharyngeal cavity. The shape of the egg is indicative of the location of the parasites - diamond shaped eggs are laid by parasites affecting the conjunctival sacs whilst pear-shaped eggs are laid by parasites found in either the urinary bladder or the pharyngeal cavity. Studies have shown that the American *Neopolystoma* and *Polystomoides* species have less host specificity than the European species (Héritier *et al*., 2017; Mehlhorn, 2016; Verneau *et al*., 2011)**.**

Polystomoides ocellatum is a parasite found infecting the native *E. orbicularis,* with no host-switching reported to date. If present in the buccopharyngeal cavity, the adult parasite worms can be collected by using long buccal swabs, which is an easy, fast, and harmless method. However, it is not possible to obtain all the parasites, and false negatives are quite frequent if there are too few parasites or if they are only present deep in the pharynx (Valdeón *et al*., 2013). *Polystomoides oris* is also found in the buccopharyngeal cavity, originally affecting *Chrysemis picta* specimens in North America. This parasite has also been reported in *T. scripta* and *E. orbicularis*, it has a clear capacity for host switching (Demkowska-Kutrzepa *et al*., 2018; Héritier *et al*., 2017).

Multiple *Neopolystoma* originally infecting North American turtles have been detected amongst the wild native turtles in European lakes: *Neopolystoma* sp3, which usually infects the urinary bladder of *T.s. elegans; Neopolystoma orbiculare* found in the urinary bladder of *C. picta*, or *Neopolystoma* sp4, previously found in the conjunctival sacs of *G. pseudogeographica* in North America. *Neopolystoma* sp6 was found in invasive and native specimens in France but it was not determined yet where it originated from. It is clear from different studies that these parasites are not very host specific and will switch readily with exposure. They may even have excluded local polystome species with their competition, as some native parasites are only now found in populations not sharing their habitat with invaders, but more research is needed (Demkowska-Kutrzepa *et al*., 2018; Héritier *et al*., 2017; Meyer *et al*., 2015; Verneau *et al*., 2011)

3.3.1.2 Spirorchiidiae

Spirorchiidae are the family of blood flukes (digenic trematodes) that affect turtles; they can be highly pathogenic and result in death. The knowledge of their specific life cycle is quite limited, especially as it is very variable amongst digenean. Their eggs are present in the water and are taken up by an unknown intermediate host (likely a snail), either directly or as a ciliated larva. Eventually, from daughter sporocysts, a cercariae will arise and they will be shed in the water by the snail where they will swim freely to infect the turtle (*per os* or *transcutan*). Adult worms in the final host will shed eggs in the faeces that can then be detected by sedimentation. Inside the host, the eggs migrate within the vascular compartment and become trapped at smaller, terminal vessels. This results in the formation of granulomas and extensive necrosis of the tissues, which leads to an acute inflammatory response. Often, secondary infections and gastrointestinal tract irritation also occurs, adding to the pathogenicity of the disease. In case of severe infection, eggs can be found microscopically in most of the organs (Iglesias *et al*., 2015; Jacobson, 2007; Mehlhorn, 2016).

There are 8 recognised species of the *Spirorchis* genus that affects North American freshwater turtles (although, there is controversy in the taxonomy) (Cribb *et al*., 2017; Iglesias *et al*., 2015; Taylor *et al*., 2015). Originally, there had only been evidence of this parasite being co-introduced with its host species but without any switch to a new host. This was seen in Japan, where the two parasites *Spirorchis artericola* and *Spirorchis elegans* were found in the invasive *T.s.elegans* (Demkowska-Kutrzepa et al., 2018; Oi et al., 2012).

In December 2012, in the Centeàns ponds of Galicia, Iglesias *et al*. (2015) found a mass mortality event in a population of *E. orbicularis,* and after some necropsies, they found specimens of *S. elegans*. This blood fluke had so far only been found in the vascular system of *Trachemys spp, C. picta* and *P. concinnna* in North America, or in imported specimens. The team extended their research to the general Galicean pond turtle population; collecting fresh faecal samples and using standard sedimentation, they found spirorchiid eggs in specimens from different areas than the original outbreak. They concluded that this was a result of a spillover from introduced alien species as *T. scripta* is a well-documented invader of the area.

Another parasite belonging to the *Spirorchis* genus is *Spirhapalum poelsianum* which has been documented in the vascular system of *E. orbicularis* in some countries sharing a border with Hungary - Poland, Ukraine and Romania (Iglesias *et al*., 2015).

3.3.1.3 Telorchiidae

This family of digenean trematodes, in the Plagiorchiidae order, are small lancet-like flukes found in the pancreatic and biliary ducts. Their life cycle is believed to be similar to the Spirorchiidae but is not yet well known. Eggs with a miracidium are shed in the faeces and are likely taken up by a freshwater snail (*Lymnaea spp*, *Planorbis spp* and *Physella spp* have been documented as intermediate hosts). A second host for the infective metacercaria to develop (according to typical digenean cycle) is likely a hemipteral larvae. It will be eaten by the turtles, causing an infection (Cardells *et al*., 2014; Taylor *et al*., 2015).

Telorchis trematodes are a rather common parasite of *T. scripta* and have been reported in multiple invasive specimens, for example *Telorchis attenuata* in Spain. In Japan, *Telorchis clemmydis* was also found in invasive red eared sliders, however, this parasite is native and hence cross contamination must have occurred from the native to the invasive specimens. In Europe, multiple species have been documented affecting *E. orbicularis*, for example *Telorchis temimi* (Spain) and *Telorchis parvus* (Austria). However, so far, no further cases of host switching have been documented (Cardells *et al*., 2014; Demkowska-Kutrzepa *et al*., 2018; Oi *et al*., 2012).

3.3.2 Nematodes

3.3.2.1 Ascarididae

Several *Ascarididae* species have been documented in freshwater chelonians, but only *Angusticaecum holopterum* has been fully described. The dioecious adult roundworms are found in the gastrointestinal tract of the turtles (oesophagus, stomach, large and small ntestine) while the larvae are found in the lungs. The adults may cause some injury to the gastrointestinal tract through obstruction, but most of the pathological damage is usually caused by the migration of the larvae. Transmission occurs through the intake of infective eggs containing a second stage larvae (L_2) which are shed in the faeces (Mehlhorn, 2016; Sprent, 1980; Taylor *et al*., 2015).

It is believed that *A. holopterum* occurs widely across Europe wherever land or pond turtles are present. It has been mostly described in the Mediterranean tortoises (*Testudo spp*) but has also been reported in *E. orbicularis* on numerous occasions. This parasite is not present in North America, but a few cases have been reported in *T.s. scripta* specimens, notably in a polish zoo where species were mixed. It is a possibility that there are other *Angusticaecum* species as identification was not done in multiple cases due to poor specimen preservation (Demkowska-Kutrzepa *et al*., 2018; Sprent, 1980).

3.3.2.2 Kathlaniidae

The nematode genera *Falcaustra* is a roundworm of the *Kathlaniidae* family. So far, 68 species infecting reptiles have been documented around the world, 29 of which affect turtles. Of these, 16 have been reported on the North American continent in the *Trachemys spp*, *G. pseudogeographica* and *P. concinna*. The information on their taxonomy, life cycle and geographical distribution is inadequate, with frequent reporting of misinterpretations; this ought to become more precise moving forward with newly developed molecular based identification methods. Its presence has been linked to gastric nodules found during pathological dissections and eggs have been found in faecal samples from infected animals (Baker, 1986; Rakhshandehroo *et al*., 2020; Shayegh *et al*., 2016).

Falcaustra donanaensis is a parasite originally detected in the native pond turtle species. Recently, they have also been found in some invasive *T. scripta* specimens in Spain as a result of cross transmission from the native turtles. Other *Falcaustra* spp have also been documented in multiple parasitic studies across Europe in both native and invasive turtles, but they were not identified further. There have been multiple recent parasitic studies in Iran done on *E. orbicularis* and *Mauremys capsica* which suggest that the *Falcaustra spp* are a rather common parasites in the wild native turtles. Even if Iran is slightly separated from Hungary geographically, it is still part of the same temperate zone and this could be indicative of a similar parasitic population (Demkowska-Kutrzepa *et al*., 2018; Hidalgo-Vila *et al*., 2009; Rakhshandehroo *et al*., 2020; Shayegh *et al*., 2016).

Falcaustra armenica has also been collected from multiple European pond turtles in Romania, Poland, Turkey, and Bulgaria. It is likely that this parasite is also present in the native turtle population in Hungary due to the geographical proximity of these countries (Demkowska-Kutrzepa *et al*., 2018; Mihalca *et al*., 2007; Yildirimhan and Sahın, 2005).

3.3.2.3 Camallanidae

Around 40 species of nematodes belong to the Camallanoidea superfamily which affect amphibians and reptiles - especially chelonians. *Camallanus spp* and *Serpinema spp* are the two genera of the Camallanidae family reportedly found in turtles. They have very similar morphology, hence there have been recent changes in reports having reassigned many previously classified *Camallanus* parasites to the *Serpinema* genus. It appears that, currently, the most commonly found parasites in turtles are actually the *Serpinema,* with 12 species documented (Martinez Silvestre *et al*., 2015).

In their original habitat, the *Trachemys sp.* are infected by *Serpinema trispinosum,* which bares resemblance to *Serpinema microcephalum*, the European parasite infecting the native turtle species (well documented in *E. orbicularis* and *M. leprosa*). The now documented host transmission of *S. microcephalum* is therefore not surprising, as they fill similar ecological niches. While the exact life cycle of this parasite has not yet been fully described, it is likely similar to the others of its genus. First stage larvae (L_1) are excreted with the faeces into the water where they infect the first intermediate host, likely a copepod, and develop into third stage larvae (L_3). These copepods are often found around local vegetation and accidentally ingested by herbivorous adult turtles during grazing. It is also believed that fish, amphibians, and snails may act as paratenic hosts, as they also belong to the more omnivorous turtles' diets (younger specimens or certain invaders, such as *P. concinna*). The parasite will embed themselves in the small intestinal mucosa and reach maturity; this does not seem to cause major lesions in their original hosts. However, it was shown to cause enteritis in most invasive specimens (*Trachemys*, *Pseudemys* and *Graptemys*) caught in Spain, as well as pancreatitis (granuloma lesions) and hepatic lipidosis in some cases. No lesions have been documented in *Trachemys spp* infected with *S. trispinosum* in their natural environments in North America. It would seem that the introduced hosts are more susceptible to this new parasite than the native ones. Although, another factor may be that some introduced specimens may have a weaker immune system and general condition due to the sudden change in their environment at the release event. As of now, there are no reports of *S. trispinosum* in a native turtle, which could either be due to lack of exposure or a more host specific nature (Hidalgo-Vila *et al*., 2009; Martinez Silvestre *et al*., 2015; Oi *et al*., 2012; Yildirimhan and Sahın, 2005).

3.3.2.4 Spiruridea

Spiroxys contortus is a wide-spread parasite of multiple freshwater turtle species across North America, North Africa, and Palearctic Eurasia. While it has a more successful infection in some species over others, it is euryxenous and present in most freshwater turtles. The eggs are sensitive to a dry environment, but if shed in water they will hatch into an infective larva which attaches to some object in the aqueous environment. Their first intermediate host is a type of copepod which are then ingested by their second intermediate hosts, usually dragonfly nymph or tadpoles, although snails have also been recorded. In the turtles, their final host, they are found in the stomach where the larvae attach and embed themselves as they grow into adults. These later lay their eggs into the gastrointestinal tract to be passed in the faeces (L. Hedrick, 1935a; L. R. Hedrick, 1935b; Mascarenhas and Müller, 2015; Yildirimhan and Sahın, 2005). Experiments by Hedrick (1935a) on the parasites showed that infection may also occur from intake of the first intermediate host by the turtle. It has recently been reported in wild specimens of *E. orbicularis* in Romania, Austria, Turkey and Bulgaria (Demkowska-Kutrzepa *et al*., 2018; V. A. R. Hassl and Kleewein, 2017; Shayegh *et al*., 2016; Yildirimhan and Sahın, 2005). Considering this parasite is common to both *Trachemys spp* and *E. orbicularis,* it could be found in Hungary.

3.3.2.5 Oxyuridae

These pinworms are common parasites of herbivorous reptiles, often having a commensal relationship with their host. The adult worms can be found in the large intestines, and once fertilisation occurs, the females migrates down and lays eggs containing L_3 which are shed in the faeces; these can be detected by a flotation test (Jacobson, 2007; Rataj *et al*., 2011; Taylor *et al*., 2015).

Oxyurids nematodes were the most common parasites found in chelonians in the Slovenian study by Rataj *et al.,* (2011), with the *Tachygonetria* genus identified. Primarily found in *Testudo spp* specimens, it was also detected in *E. orbicularis* and *T. scripta.* The tortoises are herbivores which could explain their increased sensitivity to these parasites compared to freshwater turtles who are more omnivorous. This study, however, was conducted on populations coming from captive farms and as of now, this parasite has not been detected in the wild, but it has potential for co-introduction.

3.3.2.6 Physalopteridae

The Physalopteridae family has two genera: *Physaloptera* and *Abbreviata*, which may sometimes be confused as the taxonomy is often unclear. They are described as heteroxenous nematodes with an indirect life cycle. They have been documented to result in high and massive infections in wild reptiles, notably turtles, on the American continent. The eggs are ingested by arthropod intermediate hosts and develop into an infectious L_3 which will infect the host as it feeds on the insect. A smaller reptile may sometimes act as an intermediate host, this occurs especially in lizards. The larvae will attach to the stomach mucosa and grow into adults, feeding on the stomach content before finally shedding eggs that will pass with the faeces. They have not been found to cause pathologies but may hinder general host fitness(Goldberg *et al*., 2002; Hidalgo-Vila *et al*., 2009; King and Jones, 2016)..

They were first described amongst wild turtles in Europe by Hidalgo-Vila *et al.*, (2009), parasitising both the invasive *T.s. elegans* and the wild native *M. leprosa*. It is believed that they switched hosts from the local lizard population into the turtles, based on previous data on these reptiles. During the Slovenian study on parasites of reptiles, multiple exotic lizard species were found infected by *Physaloptera*. These were originally from pet shops, it could therefore be a possibility for a turtle to be infected in a captive environment by a lizard before release into the wild (Rataj *et al*., 2011).

3.3.3 Protozoa

3.3.3.1 Coccidia

Over 30 different species of coccidia have been reported in chelonians. However, there is still a significant lack in data on them, especially due to their intranuclear nature. *Eimeria* is the most common reported coccidia in turtles. Infection occurs *per os* with intake of infectious oocysts; these contain cell-penetrating sporozoites which are set free in the intestine and enter the host’s cells, where they develop into schizonts. This schizont formation damages the host’s cells which may lead to the appearance of clinical signs. Eventually, male and female gamonts are produced, which leads to a zygote and eventually a very resistant oocyst. It will be shed in the faeces and can be detected using a flotation test (Mehlhorn, 2016).

Many coccidia species have been detected in turtles in North America, with over 11 species of *Eimeria* in *T.s. elegans* alone. In Europe only 3 species have been recorded in *E. orbicularis: Eimeria gallaeciensis, Eimeria emydis,* and *Eimeria mitraria.* Recently, in a study done in a Spanish zoo, red-eared sliders were found to be infected by 4 alien parasites (*Eimeria graptemydos, Eimeria trachemydis, Eimeria marginate,* and *Eimeria chrysemis*) never recorded in Spain before (Buffoni Perazzo, 2017; Segade *et al*., 2006).

Eimeria mitraria has been described quite extensively in North America in species such as *G. pseudogeographica* and *T.s. elegans,* and it is seen as an euryxenous parasites amongst turtles. Recently, it was reported amongst *E. orbicularis* in Spain in an area shared with *T.s. elegans,* however, it is not clear whether it is a parasite that has always been endemic to the area or if it was co-introduced. It has also been found in locally sourced alien red-eared sliders. More studies on whether it is present in areas with no invaders are needed in order to establish the origin of this parasite (Buffoni Perazzo, 2017; Segade *et al*., 2006).

Although there are few reports of *Cryptosporidium* in turtles, and no specific identified species, they have been documented in chelonians and are usually able to infect quite a wide range of hosts. Infection occurs by the ingestion of oocysts; these contain four sporozoites which are released in the host's intestinal tract and attach to the cells, entering them slightly, but stay extra-cytoplasmic, unlike *Eimeria*. There, they develop into schizonts and later undergo gametogony, leading to the formation of a zygote. It will encyst itself in the intestinal mucosa until it becomes an oocyst to be excreted into the faeces. Infection of *Cryptosporidium* has been shown to sometimes cause enteritis but is mostly asymptomatic. However, it has been observed that it tends to become severe and even fatal after big environmental stressors (Jacobson, 2007; Mehlhorn, 2016; Rzezutka *et al*., 2020; Taylor *et al*., 2015).

A recent study done in invasive *T. scripta* in Lublin (Poland) found *Cryptosporidium parvum* in faecal samples and intestinal scrapes, the latter of which proved more effective. Through sequence analysis, it was found to be identical to previously identified *C. parvum* in local cattle. The specimens did not show a lot of pathological changes and could be asymptomatic carriers. The lack of previous detection of *Cryptosporidium* in parasites could be caused by the intermittent shedding nature of the oocysts. Due to the zoonotic nature of *C. parvum*, this could be significant in the prevention of disease spread and maintenance of a good public health (Rzezutka *et al*., 2020).

3.3.3.2 Entamoeba

Multiple species of *Entamoeba* have been described infecting chelonians, the prominent ones for freshwater turtles being *Entamoeba terrapinae* and *Entamoeba invadens.* Infection occurs through *per os* intake of a 4-nucleated cyst, which will develop into uninuclear amoebae while encysting itself in the intestinal wall. They will become encysted in the colon and 4-nucleated cysts will be excreted in the faeces, but in low numbers, making diagnosis difficult. Turtles will often be quite asymptomatic; however, they may develop entamoebiasis especially if stressed. This will lead to necrotic enteritis and hepatic necrosis which may be fatal (Foreyt, 2002; Jacobson, 2007; Mehlhorn, 2016; Taylor *et al*., 2015).

4. MATERIALS AND METHODS

4.1 Location and study population

The trapping of turtles was established to safeguard the Hungarian European pond turtle population and is done by the Amphibian and Reptile Protection Division of the Magyar Madártani Egyesület (MME). It begun in Naplàs-tó (general GPS coordinates 47.510628,19.248028) in 2015, as this lake was determined as the model site by the original conservation efforts started by WWF-Hungary (Kovács *et al*., 2004). The overall number of individuals captured previously by the MME in Naplàs-tó is summarised in table 1 below. This capture was done before this study but gave us a good basis on what we may potentially trap. It must be noted that the number of European pond sliders was not checked for recapture, so the actual population should be smaller. Furthermore, as a high number of the invasive population has been captured over the years it is reported that the frequency of native turtles in the traps has increased significantly.

In 2016, there was a reported finding of polystomes in the oral cavity of a European pond slider. There were no findings of any in the invasive turtles, however, due to the aggressivity of these species, their oral cavities were not thoroughly searched. The polystomes were sent to Spain for genotyping and were found to be *Polystomoides ocellatum*.

The lake in Feneketlen-tó in Budapest (District XI) has had 79 sightings of *Trachemys Scripta Scripta* since 2011, according to the National Reptile and Amphibian Mapping page (Magyar Madártani és Természetvédelmi Egyesület, 2016). This artificial lake covers 1.1 hectares and reaches a depth of 4 to 5 metres. A water filtering system was installed in the 1980s to oxygenate the water and increase its quality (Cartographia, 2016). There is a railing around the banks, as well as reeds and trees providing shelter and coverage for wild animals. There are multiple basking spaces for the turtles, mostly in the form of big wooden logs in the water. Some of the vegetation on the banks is pulled out to provide appropriate nesting areas. A running tract surrounds the lake, and beyond the green area of this park there are roads and buildings. There is very little chance for wildlife to move in and out of the area by foot.

Trapping there was done in 2016-2017 and again in 2020 (this study could not be done on those specimens due to pandemic reasons), previously trapped specimens are summarised in

Table 2 below. Previously, *Polystomoides ocellatum* was found in European pond turtles caught in this location. Our specimens were therefore collected with this programme as they already had the trapping infrastructure in place and the authorisation to do so. Bàlint Halpern from the MME was very kind to provide his help with the material needed, and expertise in the best way to catch the turtles, and what could possibly be found. He also helped to gain the necessary authorisation. In order to sample the turtles, we had to get approval from Főkert Nonprofit Zrt., the organisation in charge of the maintenance of parks and green spaces in Budapest. Other locations for possible sampling would have been Margit-sziget Japánkert or Kopaszi-gát, especially the latter as it is a more open area connected to the Danube but time did not allow for this.

Signs (*Figure 1*) have been placed in the sampling locations, explaining to the public what the traps are and why turtles are being removed. This is done firstly to avoid possible confrontations, but also in order to educate them about the local wildlife and help prevent further release of invasive specimens.

Species		**Male**	**Female**	**Sum**
European pond slider	*Emys orbicularis*	-	-	139
River cooter	*Pseudemys concinna*	1	1	2
Chinese striped-necked turtle	*Mauremys sinensis*	1	2	3
Chinese Softshell turtle	*Pelodiscus sinensis*	0	1	1
Common musk turtle	*Sternotherus odoratus*	1	0	1
Mississippi map turtle	*Graptemys pseudogeographica*	2	4	6
Florida red-bellied cooter	*Pseudemys nelsoni*	1	0	1
Yellow-bellied slider	*Trachemys scripta scripta*	24	20	44
Red-eared slider	*Trachemys scripta elegans*	6	48	54
Non-native sum		**36**	**76**	**112**

Table 1 Summary table of exotics turtles trapped at Naplàs-tó since 2015 – Captures done and data provided by MME

Species		**Males**	**Female**	**Sum**
European pond turtle	*Emys orbicularis*	13	11	24
River Cooter	*Pseudemys concinna*	0	3	3
Mississippi Map Turtle	*Graptemys pseudogeographica*	1	3	4
Yellow-bellied slider	*Trachemys scripta scripta*	33	16	49
Red-eared slider	*Trachemys scripta elegans*	5	43	48
Non-native sum		**39**	**65**	**104**

Table 2 Summary table of previous captures at Feneketlen-tó 2016-2020 – captures done and data provided by MME

Figure 1 Sign at Feneketlen- tó explaining the trapping and its purpose to the public (Taken by author on day of sampling)

4.2 Trapping

Two types of traps were used for the catching of the specimens, which were kindly provided by Bàlint Halpern. He further helped with the catching of the turtles, as he is an expert in the matter. This proved an efficient way to catch the turtles, with the added benefit that they caused no harm to the health of the trapped specimens. The traps have flotation systems that provides the trapped turtles with access to oxygen at all times, so there is no risk of them drowning.

The **basking trap** (*Figure 2*) is a floating net with a plank of wood on top with sloping ramps. The turtles will climb onto it in order to a bask in the sun and when they jump off they are caught in the trap and can't exit due to the net underwater. This trap was placed strategically next to a branch that turtles are often seen basking on, so that if it is full, they would be likely to bask on our trap.

The **turtle hoop nest** (*Figure 3*) is set below the surface, with floaters to stop it from sinking too far down, and bait (pâté in this case) is placed inside. This trap was thrown a few meters further away from the basking trap and tied by a rope to a reed so we could reach it from close to the bank. The turtles come in, attracted by the food, but they cannot find the exit once inside.

The traps were checked daily, or every other day, over the span of two weeks from the 8th of August. The specimens were weighed, measured, and photographed for a population study. A full examination was done to check for the presence of any ectoparasites (even if they were not our focus, it would have still been of interest, leeches have been found previously) or other notable signs. They were kept in appropriate housing units in water. They were placed out in the sun a few hours per day for them to get UV rays, which is important to their health and growth. Their diet consisted of adequate turtle pellets along with fresh vegetables and fruits. After our study, they were sent to the Budapest Zoo where they now reside.

There is also a pontoon at Feneketlen-tó, which often has a few turtles swimming below it; this is due to the fact that many members of the public like to throw bread to feed them or the ducks, despite the educational signs explaining not to. We attempted to catch a couple specimens using a long net however this was not successful.

Figure 2 Basking trap used (picture by MME)

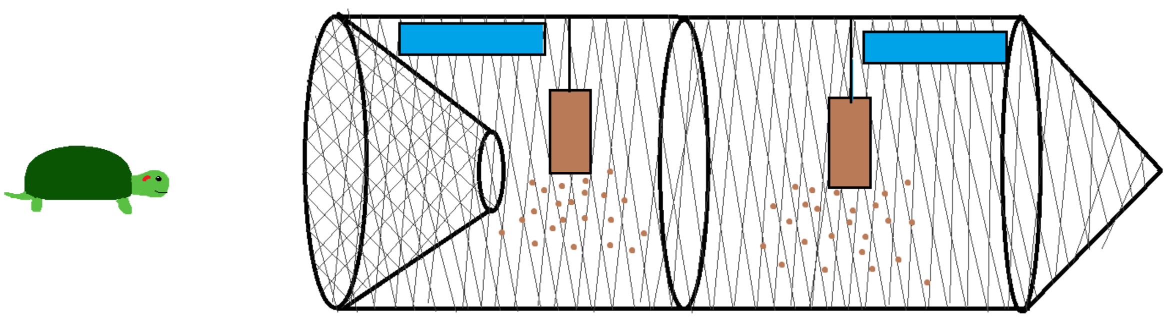

Figure 3 Hoop nest (drawn by author). Brown: bait feeders, Blue: floaters to keep trap at surface level

4.3 Faecal examination

Faecal samples were collected once the trapping was finished, it was done on two separate occasions five days apart. This is a non-invasive method that causes no discomfort to the specimens and is of minimal material and costs need. As turtles defecate in water, their faeces dissolve very quickly and it is very hard to collect direct samples. Our method for collection followed the one done by Verneau *et al*. (2011) in *E. orbicularis*. The turtles were kept in four separate groups in containers with 5cm of water. The groups were determined on their species, size, and temperament. If a group sample would have been positive, we would then have done individual sampling. Water was collected from each after 24 hours and carried to the laboratory in sealed containers for immediate examination. The laboratory used was at the Department of Parasitology and Zoology at the University of Veterinary Medicine Budapest.

The collected water was sieved (*Figure 4*)– first with a 180microns aperture sieve; this filtrate was discarded. That water was then filtered with a 63 microns aperture sieve. The deposits were rinsed off into a glass container to be further observed. We repeated this using a 38 microns aperture sieve, which was also rinsed and placed in a separate glass container. The apertures were chosen according to known parasite egg and oocyst sizes known from the literature. First, we performed a Sedimentation test. Samples taken were left to settle for 15-20 minutes. A few drops of the sediment were then collected with a glass pipette and placed on a microscope slide to be observed under a light microscope. Then we performed a classical flotation test. The sediment was then transferred to a test tube and saturated saline solution was added. After some time, a few drops were collected from the top and placed on a microscope slide to be observed using a light microscope.

Figure 4 Sieved used for the examinations of faecal samples (picture by author from day of experiment)

4.4 Throat and Cloacal swabs

Cloacal and Pharyngeal swabs were collected from the turtles using Q-tips swabs. For the pharyngeal swabs (*Figure 5*) and mouth observation, simply approaching their heads with the swabs would entice them to open their mouths and bite it; once inside the oral cavity, a gentle circular movement collected the tissue from inside. Some did not show as aggressive behaviour towards the swabs straight away, but with a few soft taps to the side of their mouths enticed them to open their mouths. During this we also looked inside of their oral cavities with a light in order to see if any parasites (notably polystomes) could be seen with the naked eye.

Cloacal swabs were collected by gently extending their tails and inserting the swabs into the cloaca. If they did not want their tail extended then the swabs were not taken, this only occurred for a couple of specimens. During this we also did a visual evaluation to see if any signs of parasites could be seen.

A smear was then made onto a microscope slide and left to dry. These were placed in a slide holder box to be carried to the lab and observed the next day. The samples were placed in Giemsa reagent for 30 minutes; whilst the eggs themselves do not stain, the outlines would be visible. They were observed under a light microscope.

Figure 5 Collection of a pharyngeal swab in a yellow-bellied slider (Picture taken day of sampling)

5. RESULTS

Originally, we targeted primarily *T.s. elegans,* as it is the most widely recorded invader across literature; however, the local exotic turtle population proved more diverse. Overall, 15 specimens were collected from four different species, summarised in *Table 3* below, some seen in *Figure 6*. We noted signs of hybridisation between *T.s. elegans* and *T.s. scripta* on some specimens. If in the future the specimens are dissected for a parasitic investigation, the Hungarian Natural History Museum would be interested in collecting DNA samples alongside it to explore this topic. The basking trap proved the most effective, capturing 10 of the specimens, whereas the hoop nest captured 5 turtles, which tended to be smaller (therefore younger). No native turtles were caught, even though the population of *E. orbicularis* in the Feneketlen-tó lake was a minimum of 10 specimens, as per previous observations in the spring. This could be explained by competition over the basking sites.

No signs of parasites were seen, during our general observation of the turtles. They were also all in good body condition and showed no clinical signs of any pathologies. Only one specimen was seen to have an old injury, with part of its tail missing – this could have been due to a predator or cannibalism maybe due to an overcrowded captive rearing. From the shell growth, it was established that most of the specimens had been in the lake for a minimum of one year, and much longer in some cases (notably the *P.concinna*). This could be seen due to the fact that the plastron shows different growth lines when a turtle brumates compared to when they don't. Usually, brumation is not seen in captivity as they are kept inside with no drop in temperature. But in the wild, their growth temporarily stops during the winter brumation, as their metabolism slows down, and a line is observed. Most of the specimens were fully grown adults older than a decade, only one younger specimen (*Trachemys spp*) was caught, estimated at four years old. Age is estimated by counting the growth lines on the dorsal side of the carapace, it is not completely accurate to the exact year but gives a general range.

During our observation of the faecal samples, we detected multiple objects such as prokaryotes, plant carbohydrates and even plastic particles. This showed us that our method was sensitive, however, no sign of parasites, oocysts or eggs were seen. Epithelial tissue, as well as faecal matter with bacteria, was observed from the slides made from the cloacal and oral swabs. This showed that they were effective in collecting tissue, however, no signs of parasites were detected.

It can be overall concluded that either they were not infected by parasites or that no shedding was occurring at the time of sampling. Our study findings are summarised in *Table 4*.

Species		**Male**	**Female**
River Cooter	*Pseudemys concinna*	-	1
Mississippi map turtle	*Graptemys pseudogeographica*	1	-
Red-eared slider	*Trachemys scripta elegans*	1	4
Yellow-bellied slider	*Trachemys scripta scripta*	4	4

Table 3 Summary of turtles caught at Feneketlen-tó for this study – August 2020

Species	**Sex**	**Eye observation**	**Sedimentation**	**Flotation**	**Cloacal swab**	**Throat swab**
P. concinna	Female	NPO	NPO	NPO	NPO	NPO
G. pseudographica	Male	NPO	NPO	NPO	N/A	N/A
T. s. elegans	Female	NPO	NPO	NPO	NPO	NPO
T. s. elegans	Female	NPO	NPO	NPO	NPO	NPO
T. s. scripta	Male	NPO	NPO	NPO	NPO	N/A
T. s. scripta	Male	NPO	NPO	NPO	NPO	NPO
T. s. elegans	Female	NPO	NPO	NPO	N/A	NPO
T. s. scripta	Male	NPO	NPO	NPO	NPO	NPO
T. s. scripta	Female	NPO	NPO	NPO	NPO	NPO
T. s. scripta	Female	NPO	NPO	NPO	NPO	NPO
T. s. scripta	Male	NPO – tail bitten off	NPO	NPO	NPO	NPO
T. s. scripta	Male	NPO	NPO	NPO	NPO	NPO
T. s. scripta	Female	NPO	NPO	NPO	NPO	N/A
T. s. elegans	Female	NPO	NPO	NPO	NPO	NPO
T. s. elegans	Male	NPO	NPO	NPO	NPO	NPO

Table 4 Summary of results – NPO: No Parasites Observed, N/A – No samples received

Figure 6 - 1 G. pseudogeographica (Biggest specimen on left), 2 T.s. elegans (middle specimens – upper one a likely hybrid), 2 T.s. scripta (on the right) (picture by author)

6. DISCUSSION

Our study showed that none of the caught invasive specimens seemed to be infected by parasites. The lack of co-invasive specimens in alien species is often due to their captive rearing, as they were likely exposed to anti-parasitic treatments in farms and pet shops. It is also possible that if some of these specimens were introduced originally with parasites, those could not establish themselves due to a lack of other final or intermediate hosts. Survivor bias may also play a role in our findings, as turtles released with parasites could potentially have a lower fitness and are less likely to survive release into the wild. Observation of the turtles' shells showed us that most had survived in the lake for a few years already; they were very fit individuals. It has also been shown in a previous study that turtles tend to have a higher parasitism in their first four years of life, decreasing thereafter. This is likely due to their diet, which is more carnivorous before reaching maturity where they become mostly carnivores, but may still accidentally ingest intermediate hosts (Cardells *et al*., 2014). Most of the specimens we caught were adults, based on their size and shell rings, with only one estimated at the age of 5 years old.

This could also be reflective of the parasite population of the *E. orbicularis* sharing the lake; it is suggestive that the local population has a low parasitic load and has not yet been majorly affected by alien invasive species. The lack of evidence of spillover parasites in these invasive turtles could be a sign that they are not present in the lake and so there was no exposure. However, it could also be that there has not been enough exposure for those particular specimens as others were previously sampled in the year. It is also possible that the native population is infected with stenoxous parasites, for example *P. ocellatum* which has already been found. It would be interesting to do a study on both populations in the same ecosystem to assess exposures for the different host species.

Another reason these turtles might be without parasites is the fact that Feneketlen-tó is a rather isolated lake with no direct connection to the Danube or any other flowing water system. Even though turtles have been seen to wander across land and change habitat, this is almost impossible for them to do so here, as it is surrounded by roads (Magyar Madártani és Természetvédelmi Egyesület, 2016). As a closed, ecosystem this limits the changes in populations and diversity; if parasites are not present, it would be hard for them to enter the environment. On the other hand, if a parasite is present it will spread fast and wide due to a higher population density (Héritier *et al*., 2017).

However, the lack of parasites found in our study could be due to limitations in the methodology. First, a higher population size would be preferable, as we only observed 12.6% of the invasive turtles removed from Feneketlen-tó since the beginning of the program. Secondly, it would give better results to sample specimens from different locations in the country and compare the data of specimens in different locations and maybe more open ecosystems. There would be a possibility to sample turtles from Naplàs-tó where trapping already occurs, but also in other localities where conservation efforts are already in place. In Budapest we previously mentioned Margit-sziget Japánkert or Kopaszi-gát where the presence of exotic turtles has been noted. There are also some private landowners in other areas of Hungary that already remove the invasive specimens in their lakes with the help of the MME and could be willing to give these to a study.

Furthermore, most of the studies already carried out on invasive freshwater turtles and their parasites performed necropsies after humanely euthanising specimens, or using carcasses found. As the North American turtle species are invasive, approval for this is often granted. We could not succeed in obtaining the necessary authorisations in time for our study but it would be recommended to consider this in further studies in order to obtain more thorough results. Some of the owners or other organisations in Hungary removing exotic specimens from lakes already have authorisation and euthanise the specimens that they remove, unfortunately none lined up with the timing of our study, but it could be a useful resource for future ones. Necropsies were performed in the studies by Oi *et al,* in Japan; Héritier *et al.,* Meyer *et al,* and Verneau *et al.* in France; Hidalgo *et al*. and Domènech *et al*. in Spain; Hassl and Kleewein in Austria; and Mihalca *et al.* in Romania.

In a previous study in Spain on the parasites of *E. orbicularis*, helminths were found in far fewer amounts in faecal samples compared to the dissection of carcasses. However, this does not mean that faecal sampling and other methods are completely ineffective as they are commonly used for *E. orbicularis* as these vulnerable specimens are usually released after examination (Hidalgo-Vila *et al*., 2009). Blood sampling could also be quite informative, as blood smears done in Austria from caught invasive *Trachemys spp* specimens found some parasites (*Haemoproteus degiustii* and Aegytpianella) (Hassl and Kleewein, 2017). This could be set in place for turtles before they are placed in Budapest Zoo or before euthanasia, depending on given approval.

According to literature, freshwater snails act as an intermediate host for parasites of the *Telorchiidae*, *Spiruridea* and the *Spirorchiidae* family. They may also be paratenic hosts to *Camallanidae.* Therefore, collecting and studying snails present in an environment shared with wild turtles could also help give insight to the parasitic population (Cardells et al., 2014; L. Hedrick, 1935a; Iglesias et al., 2015; Martinez Silvestre et al., 2015).

There are two different situations for the presence of parasites in invasive turtles in Hungary. It is possible that they are also infected by alien parasites, which they may have encountered in captivity and brought with them; or infected with a species that has already established itself in the environment from a previous invasion. Another possibility is a parasite already present in the ecosystem, affecting the native population, may spillover into the invasive specimens. This host switching ability has already been established in multiple parasitic species, but it is important to keep in mind the parasites that have not yet been recorded in invaders; as it may only be due to lack of exposure. Due to the similarity in diet and behaviour of *E. orbicularis* and North American freshwater turtles, it is likely they would be exposed to the same infectious stages of parasites, whether free swimming in the water or in an intermediate host. The following are the parasites that should be considered in further studies of potential parasites in the exotic invasive turtles in Hungary.

In the *Polystomatidae* family, it has been shown that American parasites show less host specificity than their European equivalent (Héritier *et al*., 2017; Meyer *et al*., 2015; Verneau *et al*., 2011). So, even though *P. ocellatum* was previously found in the local *E. orbicularis*, it would not be expected in the invasive species, but should not be ruled out. However, in previous studies on *T.s. scripta*, around 50% of the caught specimens were infected by *Neopolystoma* or *Polystomoides* species of a mostly invasive nature (Héritier *et al*., 2017; Meyer *et al*., 2015; Verneau *et al*., 2011). When present, these parasites seem quite widespread, likely due to their lack of host specificity and direct life cycle. There was a wider incidence of “pear shaped” polystome eggs found in a study by Verneau *et al*. (2011) on invasive specimens; these are laid by parasites found in the urinary bladder and pharyngeal cavity which we could have potentially seen or swabbed if present. It is therefore possible that invasive *Polystomatidae* have not made it to Feneketlen-tó yet, especially as the only parasite found in *E. orbicularis* were *P.ocellatum*. Due to the higher spillover capacity of invasive polystomes, there is a real concern that they could replace *P. ocellatum* in European freshwater ecosystem and negatively impact the biodiversity (Héritier *et al*., 2017).

It is likely that the native turtles of Hungary already have a *Spirorchiidae* population, as *S. pelsianum* was already found in *E. orbicularis* in the neighbouring countries of Poland, Ukraine and Romania (Iglesias *et al*., 2015). This parasite has yet to have been seen in *T.s. scripta* or other invasive turtles, however, invasive specimens have been found infected by *Spirorchis* from North America (Demkowska-Kutrzepa *et al*., 2018). It is possible that this parasite has been less prevalent in invasive turtles due to its complex life cycle and need for an intermediate host; it is still not fully documented which gastropod exactly is required. However, there is a rising concern of host switching into the native turtle after a mass mortality event caused by *S. elegans* occurred in European pond turtles in Spain (Iglesias *et al*., 2015).

The small lancet-like flukes *Telorchiidae* could be present in the native *E. orbicularis* as multiple native *Telorchis* species have been recorded, such as *T. assula*, *T. parvus*, *T.temimi* and *T. stossichi* (Demkowska-Kutrzepa *et al*., 2018; V. A. R. Hassl and Kleewein, 2017). The snail species documented as intermediate hosts are also present in Hungary, so their indirect life cycle could be completed (Cardells *et al*., 2014). A case of spillover from native to invasive turtles was reported in Japan and Spain, therefore, this is a possibility and could lead to an increase in infection in the native turtles, which could negatively impact their fitness (Domènech *et al*., 2016; Oi *et al*., 2012). Furthermore, these trematodes are quite common in red-eared sliders in general, and some species (*T. attenuata* and *T. clemmydis)* have been reported as co-invaders (Demkowska-Kutrzepa *et al*., 2018; Domènech *et al*., 2016; Oi *et al*., 2012).

Currently, *Angusticaecum holopterum* is the only *Ascaris* described in chelonians. It is found infecting multiple species of land and pond turtles across Europe, including *E. orbicularis*. It therefore does not seem to show much host specificity amongst chelonians and has been recorded in *T.s. scripta* before. There is a lack of literature on this parasite, especially on specifics of its geographical location in *E. orbicularis,* but it was detected in nearby Poland so it should be kept in mind when studying the local freshwater turtle population (Demkowska-Kutrzepa *et al*., 2018; Sprent, 1980).

Falcaustra is a commonly found roundworm in turtles across the world. There are 16 species recorded in North America in many of the turtle species there; it is therefore always a possibility for another of these to be co-introduced into European wetlands (Shayegh *et al*., 2016). In the European population, multiple native species have been documented, although not all have been fully identified. *F. armenica* was found in multiple countries around Hungary infecting the European pond turtles. *F. donanaensis* is another species described in *E. orbicularis,* with recorded spillover into invasive *T.s. scripta* specimens in Spain (Demkowska-Kutrzepa *et al*., 2018; Hidalgo-Vila *et al*., 2009). Despite the inadequate knowledge on this family of parasites, it is important to keep in mind its many species that are likely present in the country.

The *Camallanidae* are common parasites in reptiles, with turtles being no exception. They are mostly infected by the *Camallanus* and *Serpinema* species, which are quite similar and have had confused taxonomy. In Europe, *S. microcephalus* has been commonly reported in native turtles and is quite widespread; it is probably present in Hungary. This parasite has also been documented to infect the invaders and to cause pathological lesions in their gastrointestinal tract. In North America, freshwater turtles are infected by *S. trispinosus,* but so far, this parasite has not been reported in Europe.

The euryxenous parasite *Spiroxys contortus* has been documented in turtle species in North America and Europe and is quite widespread, despite its complex life cycle. It was documented in *E. orbicularis* specimens in multiple countries around Hungary such as Romania, Austria, Turkey, and Bulgaria. As it readily invades most exotic turtle species as readily as local ones, it is probable to have some presence in Hungary (Demkowska-Kutrzepa *et al*., 2018; A. R. Hassl and Kleewein, 2010).

The Oxyurid *Tachygonetria* has only been reported in turtles from Slovenian breeding farms and not in the wild. They were the most frequent parasite in that study population, present in 81.8% of chelonians belonging to 10 different species. Although they are more prevalent in tortoises, likely due to their more herbivorous diet, they have shown the ability to infect both *E. orbicularis* and *T.s. scripta* so they must be kept in mind as a potential parasite in Hungarian freshwater ecosystems (Rataj *et al*., 2011).

Nematodes of the *Physalopteridae* family are common parasites in wild turtles in North America (Goldberg *et al*., 2002). *Physaloptera abbreviata* has been reported in Europe, in both invasive and native species, in a spillover event believed to be from wild western green lizards (Hidalgo-Vila *et al*., 2009). This parasite could therefore make its way into the ecosystem through another reptilan route.

Coccidia is commonly reported in North American freshwater turtles, especially *Eimeria.* Some species of this genus have already been described in Europe amongst red-eared sliders (Buffoni Perazzo, 2017). *E. mitraria* was clearly identified in multiple *T.s. scripta* in Spanish freshwater ecosystems, where they were also present in the native *E. orbicularis,* it is still unclear whether it was already present before invasion by red-eared sliders (Segade *et al*., 2006). *Cryptosporidium* can also be quite prevalent in turtles, especially due to their wide host spectrum. Red-eared sliders have already been infected by *C. parvum* from local bovines in Poland, so it could be expected also in Hungary (Rzezutka *et al*., 2020).

Finally, there are multiple *Entamoeba* species that have been described infecting freshwater turtles, notably *E. terrapinae* and *E. invadens*. There are no reports of these in wild turtles in Europe at the moment, but they must be taken into consideration (Foreyt, 2002; Jacobson, 2007).

Co-introduction of a new and virulent parasite into the wild alongside exotic turtles may have a catastrophic impact on the local turtles and ecosystems. This may also be worsened by the direct competition from invaders, affecting the native turtles' fitness, which may worsen their immune system and gut flora. Native parasites may also be directly impacted if they switch into the exotics turtle. Their numbers may be amplified, which may further negatively impact the local population. However, they may also be diluted due to a higher variability of hosts, and maybe even replaced by co-introduced parasites. It is important to introduce more monitoring and control measures in order to limit the negative impact of alien invasive species and prevent a loss of biodiversity (Demkowska-Kutrzepa *et al*., 2018).

As of now, it is still too early to judge the efficacy of *ex-situ* reproduction and reintroduction of *E. orbicularis* specimens into the wild, but results seem promising. However, it is suggested for the focus to be placed on preserving the existing population by limiting the loss of habitat and competition from invaders (Mitrus, 2008). Capture and removal of exotic turtles has shown to be effective in decreasing efficiently the number of invasive specimens present in European wetlands but may not be enough to eradicate them. The hybridisation, something not encouraged in captive rearing, that we observed in our captured specimens alongside previous sightings of gravid females and exotic turtle nesting, suggests that these alien invasive species already readily breed in the Hungarian wetlands. Hungary, and Europe as a whole, needs to set up a proper alert and react system to tackle the issue and provide more long-term investments in researching the matter. Raising awareness and educating the public should also be a key focus. Not only teaching people to protect local habitats of freshwater turtles but also encouraging responsible pet ownership and preventing the further release of exotic animals into the wild. Like so many other conservation projects, having multiple approaches is crucial to succeed (Teixeira *et al*., 2014).

7. Conclusion

This thesis helped the author gain new knowledge on the parasites and conservation status of the wild freshwater turtle population in Hungary and Europe as a whole. Clearly, the trade of exotic pets has had major consequences on wildlife, notably in this case through the invasion of European ecosystems by North American alien species. Despite the high biodiversity of the freshwater ecosystems, there is still a lot of missing data especially when it comes to host and parasites interactions in reptiles. Previous studies have shown us that turtles can be parasitised by a variety of species, with variable abilities to switch hosts and cause disease. The impact of co-introduced parasites on the native population has started to be increasingly documented in recent years, especially in Europe. Through the already existing literature, it has become clear that this is a parameter that must be taken into consideration when setting up conservation projects.

Although the final scale of our study had to be reduced due to the pandemic, the samples taken were a good starting point to establish a technique and results for the future. Encouragingly, it was also suggestive that the local population has not yet been majorly impacted by the invasion of exotic turtles despite the fact they are clearly established. It can be recommended that further studies of this nature be carried out on freshwater turtle species in Hungary; these can be coordinated with the removal program already in place across the country. Collecting data at multiple locations, but also over certain periods of time, could prove extremely valuable in order to evaluate the parasitic profile of the Hungarian population of freshwater turtles. However, more thorough methods would be recommended, with blood sampling and dissection of removed specimens. Evaluating *E. orbicularis* parasites through faecal or blood sampling could also prove informative, although more care must be taken due to their more vulnerable nature.

8. Summary

An increase of the importation and trade of exotic animals in Europe over the past few decades has led to the invasion of North American freshwater turtles into the Hungarian wetlands. This poses a real threat to the local fauna and biodiversity, especially through direct or indirect competition with the native *Emys orbicularis*. One of the impacts of invasive species is through the co-introduction of alien parasites, or through a change in dynamics of already established, native parasites by amplification or dilution.

The aim of this study was to investigate the parasites of exotic turtles removed from "Feneketlen-tó", a lake of Budapest in Hungary, in order to evaluate the risk associated with the introduction of these animals. Faecal samples and oropharyngeal swabs were collected from 15 reptiles. Results showed that this turtle population was not parasitized at all, either by invasive or native parasites. However, it is suggested that this kind of data be collected from other areas in Hungary, with more thorough methods, and in the non native and native turtles, too. Getting an overall view of the parasites in freshwater turtles in Hungary could prove important in future conservation efforts.

Összefoglalás

Az egzotikus állatok Európába való behozatalának és kereskedelmének növekedése miatt az elmúlt évtizedekben az észak-amerikai édesvízi teknősök gyakran kerültek a magyarországi vizes élőhelyekre is. Ez a behurcolás valódi veszélyt jelent a helyi faunára és a biológiai sokféleségre nézve, különösen a mocsári teknőssel (Emys orbicularis) való közvetlen vagy közvetett versengés révén. Az invazív fajok egyik hatása az, hogy új parazitákat hurcolnak be, illetve a korábban már meghonosodott paraziták dinamikáját amplifikációval vagy hígítással megváltoztatják.

A tanulmány célja a budapesti Feneketlen-tóból kihalászott egzotikus teknősök parazitológiai vizsgálata volt, azért hogy megbecsülhessük a behurcolásukkal járó kockázatot. Az állatokból bélsármintákat és oropharyngealis tamponokat gyűjtöttünk. Ezzel a módszerrel a megvizsgált 15 állat közül egyetlen egyben sem találtunk semmilyen élősködőt. Mindamellett javasolható, hogy még alaposabb módszerekkel ilyen adatokat érdemes gyűjteni az ország más területeiről is, az őshonos és a behurcolt teknősökről is. A jövőbeni természetvédelmi erőfeszítések során fontos lehet az édesvízi teknősök parazitáinak általános vizsgálata Magyarországon.

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