ESTONIAN UNIVERSITY OF LIFE SCIENCES

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ESTONIAN NESTING OSPREY (*PANDION HALIAETUS)* POPULATIONS' HEALTH AND RELATION TO POLLUTANT

EESTIS PESITSEVATE KALAKOTKASTE *(PANDION HALIAETUS)* POPULATSIOONIDE TERVIS JA SEOS SAASTEAINETEGA

Final Thesis

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Supervisor: Madis Leivits, DVM

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Osprey is a sentinel species around the world for assessing and monitoring environmental contamination of aquatic ecosystems. In different researches it has been found that they are sensitive to a wide range of bioaccumulative contaminants. Hence being strictly piscivorous, it is possible to link their diet to sources of localized contaminant exposure. However, there are no such studies conducted in Estonia. Also, not much is known about the overall health parameters of ospreys, including biochemical and hematological values. 11 birds from different Estonian areas were included in this study.

Blood samples from all birds were subjected to haematological and biochemical blood analysis and they were also tested for traces of mercury, lead and cadmium. The results were compared with previous studies and also regionalized between populations and gender. Overall, the results remained in within the range of previously studied references. There were some exceptions, which can be attributed to the variation in the ages of the birds involved in the studies. There were also some differences between two populations in biochemical analyses, which could be related to nutritional indicators. Further collection of samples and testingi s advised in order to have a better understanding of the differences between these Estonian populations.

Keywords: haematology, biochemistry, heavy metals, *Pandion haliaetus*

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Kalakotkas (*Pandion haliaetus)* on üle maailma üks indikaatorliikidest, kelle abil on võimalik jälgida veeökosüsteemide olukorda ja keskkonnasaastet. Erinevates uuringutes on leitud, et kalatkotkad on tundlikud paljude bioakumuleeruvate saasteainete suhtes. Kuna nad on ainult kalatoidulised, on nende toitumist võimalik siduda kohaliku saasteainete kokkupuute allikatega. Eestis aga selliseid uuringuid varasemalt läbi viidud ei ole. Samuti ei ole palju teada kalakotkaste üldistest vereparameetritest, sealhulgas biokeemilistest ja hematoloogilistest näitajatest. Antud uuringusse kaasati 11 lindu erinevatest Eesti piirkondadest. Kõikide lindude vereanalüüse hinnati hematoloogiliste ja biokeemiliste näitajate suhtes. Lisaks uuriti elavhõbeda, plii ja kaadmium sisaldust veres. Tulemusi võrreldi varasemate uuringutega ning lisaks ka regiooniti populatsioonide vahel ning emas- ja isaslindude vahel. Laiemas pildis jäid Eesti kalakotkaste vereproovide vahemikud varasemalt uuritud referentside vahemikku. Olid mõned erandid, mida võib seostada uuringutes kaasatud lindude vanuste varieeruvusega. Ka populatsioonide vahel oli erinevusi biokeemilistes analüüsides, mida on võimalik seostada toitumuslike parameetritega. Kuid täpsema ülevaate saamiseks oleksid vajalikud edasised uuringud.			
Märksõnad: hematoloogia, biokeemia, raskemetallid, *Pandion haliaetus*			

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LIST OF ABBREVIATIONS

Alb- albumin

ALP- alkaline phosphatase

ALT- alanine transaminase

Amyl- amylase

AST- aspartate aminotransferase

Bil- bilirubin

Ca- calcium

Cd- cadmium

Chol- cholesterol

CK- creatine kinase

Crea- creatinine

DDE- dichlorodiphenyldichloroethylene

Fe- iron

Glob- globuliin

Hg- mercury

K- potassium

LDH- lactate dehydrogenase

MeHg- methylmercury

Mg- magnesium

Na- sodium

OC- organochlorine pesticides

P- phosphate

Pb- lead

PBDE- polybrominated diphenyl ethers

PCB- polychlorinated biphenyls

PCDD-polychlorinated dibenzodioxins

PCDF- polychlorinated dibenzofurans

PCV- packed cell volume

PFOS- perfluorooctane sulfonate

TP- total protein

TPP- total plasma protein

Trigl- triglycerides

UA- uric acid

WBC- white blood cells

INTRODUCTION

About 90-100 pairs of ospreys nest in Estonia. More densely populated regions are in North-East and South-Estonia (Keskkonnaamet, 2019). Ospreys primarily feed on larger lakes rather than smaller ones. They have shown a preference in eutrophic and dyseutrophic lakes over other types (Lõhmus, 2001). The choice of prey depends on its availability (Jamieson, 1982).

Ospreys have been a sentinel species worldwide for assessing and monitoring environmental contamination of aquatic ecosystems. They are sensitive to a wide range of bioaccumulative contaminants (Grove *et al.,* 2009). Due to being strictly piscivorous and on the top of the food web, their diet is easy to link to sources of localized contaminant exposure (Lazarus, 2014). However, none of these studies conducted have been related to ospreys in Estonia.

There has been a correlation between usage of dichlorodiphenyldichloroethylene and a decrease of ospreys' population in Estonia in the 1960s and 1970s. The concentrations were measured in the sea eagles and the parallels were drawn from this for ospreys as well (Keskkonnaamet, 2019).

When aquatic organisms are exposed to contaminants with immunotoxicity, it results in poor health. This can make the organism more susceptible to parasites, viruses, bacteria, and fungi (Kataoka and Kashiwada, 2021). During fieldworks in 2015, there was a noticeable difference between 2 ospreys' populations. Population in North-East region had been infected with external parasites. Therefore, there can be expected possible pollutants around lake Peipsi altering the immunity of the birds.

This study focuses on 2 ospreys' populations in Estonia and compares the differences between those populations based on blood samples. Although they have received much attention in toxicological and biomagnification studies, there is still a lack of information about ospreys' plasma biochemistry or haematology (Muriel *et al.,* 2013). This study will analyze haematologic and biochemical reference intervals in ospreys' populations.

1. LITERATURE ANALYSIS

1.1. Osprey (*Pandion haliateus*)

The osprey (Figure 1.) (*Pandion haliateus)* or fish hawk, is a fish- eating raptor that is found on all continents except Antarctica. It is the sole member of the family *Pandionidae* (Meredith *et al.,* 2012). Osprey is one of the smallest of the Estonian eagles and is recognizable by its pale abdomen, black or brown back, and wingtips. The species is characterized by a spotted head and a flight pattern similar to seagulls. The wingspan ranges between 145- 160 cm, the weight of the female bird is up to 2 kg, the male bird weighs approximately 1.5 kg (Männik, 2006). It has rounded talons and spiculae on the digital pads. Ospreys are able to reverse digit four in order to grasp prey with two digits pointing forwards and two pointing backwards (Meredith *et al.,* 2012).

Osprey is a protected species, placed under I category protection which includes species that are rare and at risk of extinction (Keskkonnaamet, 2019).

Figure 1. The pair of ospreys (*Pandion haliaetus*). Female on the left and male on the right

1.1.1. Distribution and migration

Osprey is distributed mainly in Europe and Asia (apart from the tundras), but has spread in North Africa, Australia, North America and several islands of the Pacific Ocean (Männik, 2003).

Northern European ospreys winter in tropical West Africa (mainly south of the Sahara) and also in western, southern and eastern Mediterranean. Of the 9 ospreys equipped with GPS transmitters in Estonia, Latvia and Pskov regions, 4 wintered in the western part of Equatorial Africa, 3 in East Africa, 1 in West Africa and 1 in South Africa (Keskkonnaamet, 2019).

Ospreys leave their wintering grounds in early March, arriving in the northern breeding grounds by mid-April. They arrive back in Estonia in April, often before the water bodies are freed from the ice cover. The migration is liveliest between April and May. Ospreys leave the Northern breeding areas in August or September (Keskkonnaamet, 2019).

A significant number of female birds leave the breeding territory 2-3 weeks before the rest of the family. They'll fly to the first known stopover to gather strength for the migration. Based on Estonian data, the primary stop of female birds can also be relatively close to the nesting site (50-100 km), but then they still visit the nest site from time to time. Female birds leave the nesting sites a few weeks after the first flights of the chicks- at the end of the July or the beginning of August. Male birds that have failed to breed, start their migration to wintering grounds significantly earlier than successful breeding individuals (Keskkonnaamet, 2019).

1.1.2. Population in Estonia

Based on national monitoring and other works, 90-100 pairs of ospreys nest in Estonia. The species is common in North- Eastern, Eastern and Southern Estonia and over time the distribution area is moving across Northern Estonia towards the west. The growth of the osprey's population slowed down between 2006 and 2012, but based on the latest population estimate, the growth has accelerated again in recent years. The most densely populated regions in Estonia are: Peipsiveere Nature Reserve 3.9 nests per $100\ km^2$, Karula National Park 3.3 nests per $100\ km^2$ and Puhatu Nature Reserve 3.8 nests per $100\ km^2$ (Keskkonnaamet, 2019).

1.1.3. Nesting

Osprey starts nesting from the age of 3. Two-year-olds come to breeding grounds later than older birds and one-year-olds usually do not return from wintering grounds. The activities of pairs are clearly differentiated during the breeding season- the female bird is slightly larger and stays with the nest during the breeding period. The male bird makes sure that both the female bird and the chicks would be supplied with food (Keskkonnaamet, 2019).
Osprey often chooses swamp areas as its habitat in Estonia because there are suitable trees for building a nest in these areas (Lõhmus, 2001). More than 95% of European ospreys live in forest habitats (Saurola, 1997). Ospreys' hunting areas are large, being 5,1 km away from the nest on average and 21,5 km away at the maximum (Lõhmus, 2001).

1.1.4. Feeding habitats

1.1.4.1. Modes of hunting

Osprey feeds almost exclusively on a variety of fish. Fish are caught at a depth of up to a meter and sometimes the bird can completely disappear under the water (Männik 2006). While observing the prey, it flies over a body of water, occasionally using stationary or rappelling flight. Having spotted a suitable fish, the bird darts into the water with folded wings (Männik, 2003). Fish weighing 200-300 grams are preferred: it is difficult to fly far with larger ones and it is not efficient to catch smaller ones (Männik, 2006). Ospreys can carry prey to the nest from a distance of over 25 km. The male bird alone brings food to the family, while the female bird's task is to feed the offspring. Ospreys usually hunt individually, but in particularly good prey areas it is possible to observe multiple ospreys hunting together (Keskkonnaamet, 2019).

1.1.4.2. The choice of prey

The bird chooses the prey according to its availability- mostly fish with high abundance are caught. In this case, osprey has chosen a specific water from which it catches fish during the migration period (Jamieson 1982). According to this it can be concluded that ospreys prefer fish farms to natural water bodies for hunting because the fish population density in fish farming ponds is at least one order of magnitude higher. In natural eutrophic and dyseutrophic lakes, the possible amount of fish caught per hectare is 18- 20 kg of fish per year, in the fish farm ponds the same indicator is 80-120 kg. Based on observations, it has been found that the duration of the hunt is an average of 132 minutes from a natural body of water but only 22 minutes from a fishpond- the difference in time spent hunting is 6 times (Keskkonnaamet, 2019).

In Finland, the daily food requirement of an osprey is calculated to be 0,3- 0,4 kg of fish, while the daily norm for migratory birds is 0,5 kg of fish (Männik, 2006). In the absence of fish, when the water bodies are still frozen, osprey can sometimes also eat mice and frogs (Männik, 2003). In Estonia, Ospreys feed mainly on lakes and to a lesser extent on fishponds and rivers. The variability is greater during the migration when other water bodies are also used. Ospreys feed primarily on larger lakes rather than smaller ones and show a preference in eutrophic and dyseutrohphic lakes over other types. Unlike the rest of the world where ospreys feed a lot at sea, Estonian ospreys prefer lakes (Lõhmus, 2001).

Researching the species importance and weight composition of the bird's menu has been proven to be rather difficult. To determine the type of prey fish, certain so-called key bones are used, which are sorted out from the meal. Based on the key bones, it is relatively easy to determine the type of fish caught. With the help of the corresponding samples, the estimated size of the specimen can also be determined. Such parts are the protective bones covering the gills: *preopercula*, *opercula*, *subopercula* and *interopercula*, which together form the *operculum*. It is used to determine carp, salmon and perch. Predatory fish can also be characterized by their jaws. Fins and tail are also suitable for determining the type and size of the fish. According to the data of the nest cameras, it is quite rare that some part of prey remains uneaten but at the same time most of the species of the fish brought into the nest can be determined and the size of the prey can be estimated (Keskkonnaamet, 2019).

Inland, ospreys feed practically only on carp (91%) and only on a small extent on predatory fish (9%). In the coastal area the proportions are more equal: 64% feed on carp and 35% on predatory fish (Häkkinen, 1978). It can also be seen from the nest cameras that the species of prey differs over time. In spring, for example, there are more roaches and as the water warms, more tench and carp are added (Keskkonnaamet, 2019).

1.2. Environmental contaminants

Since the early 1900s, thousands of chemical compounds have been introduced into the environment polluting the air, soil, water and biota. Environmental contaminants' bioavailability, toxicity, concentration, duration of exposure and species sensitivity determine their physiological impacts on living organisms. Polyhalogenated aromatic hydrocarbons are the type of contaminants that are found to persist significantly in the environment today. These toxic substances such as perfluorooctane sulfonate compounds (PFOS), dioxins, furans, brominated flame retardants and organochlorine pesticides (OC) resist environmental or metabolic breakdown, are lipophilic and have the potential to bioaccumulate and/or biomagnify up the food web. When exposed to toxic agents, humans and vertebrate species living in the same local environment have similar biochemical, molecular and cellular responses. For centuries, animals have been used as an early warning system for environmental risks because of their recognized commonalities. A sentinel species is defined as an organism used to assess environmental contamination and its effects on environmental health. This is based on the organism's chemical sensitivity, position in the biotic community, exposure potential and geographic distribution or abundance (Grove *et al*. 2009).

Since the plant protection product used in Estonia must not cause pain to repellent vertebrate animals, have a harmful effect on non-repellent plants and animals as well as human health of the surrounding environment, the effect of their legal use cannot be high. However, the interaction of different plant protection products has not been studied (Keskkonnaamet, 2019). Environmental toxins can apparently reduce the food base in certain cases (such as leakage) and this in turn reduces the productivity of pairs feeding in the same area. Currently, pesticides are relatively little used in agriculture in Estonia due to their high price. Although the use and import of many pesticides dangerous to birds ((dichlorodiphenyldichloroethylene (DDE), aldrin, dieldrin, endrin, heptachlor, chlordane, parathion, carbaryl, inorganic and alkyl mercury compounds, paraquat)) are prohibited in Estonia, the use of others (several organochlorine compounds such as mirex or toxaphene, as well as many organophosphated and carbamates) are allowed (Keskkonnaamet, 2019).

1.2.1. Ospreys as bioindicators

Adult ospreys are important worldwide as a sentinel species for assessing and monitoring environmental contamination of aquatic ecosystems (Grove *et al*., 2009). Their eggs and blood are unmatched matrices for documenting spatial and temporal trends and to clarify exposure, bioaccumulation and biomagnification of pollutants. As ospreys are strictly piscivorous and occupy the top of the food web, their diet is easy to monitor and link to sources of localized contaminant exposure. (Lazarus, 2014). They also have a global distribution, chemical sensitivity to numerous pollutants, capability to bioaccumulate lipophilic pollutants and tolerance for scientific activity. They are sensitive to a wide range of bioaccumulative contaminants, such as mercury (Hg), polychlorinated biphenyls (PCB), polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF) and DDE among others. Reproductive impact concentrations in osprey eggs are known to exist (for DDE). However, using osprey as a sentinel species has some disadvantages, including migratory behaviour (spending about half of its life away from the nesting area) and suitability for use in laboratory studies (there are no records of successful breeding of ospreys in captivity) (Grove *et al*., 2009).

1.2.2. Bioaccumulative contaminants

1.2.2.1. DDE

Pesticides used in agriculture were one of the reasons for the decrease in the number of ospreys in the 60s and 70s. The effects of toxic substances are amplified in aquatic ecosystems and the highest concentrations of the living substance DDE in the world have been measured in the sea eagles in the Baltic Sea and parallels can be drawn from this for ospreys as well. (Keskkonnaamet, 2019). It is a neurotoxic agent that either affects ion permeability or interacts with nerve receptors. Exposure to this pollutant increases adult mortality, reduces egg production, eggshell thinning, decreases fertility and hatchability. It also decreases survival of young (Grove *et al*., 2009). Older birds can exhibit abnormal behaviour such as abandoning the nest or acting aggressive towards the young. As a result, the population of ospreys in Estonia decreased to a few pairs (Keskkonnaamet, 2019). However, most osprey populations throughout their worldwide breeding range have now recovered (Grove *et al*., 2009).

1.2.2.2. PCB, PCDD and PCDF

The toxicity of PCB, PCDD and PCDF to biological organisms have been studied. They cause a variety of responses that include mortality, thymic atrophy, immunotoxic effects, reproductive impairments, porphyria and related liver damage. Compared to adults, bird embryos are much more sensitive to the adverse effects of PCB and this results in pericardial edema, cardiovascular abnormalities, liver lesions, external deformities, thymic hypoplasia, lack of lymphoid development and subcutaneous oedema. However, several studies found no evidence of PCB impact on osprey reproduction in eggs from which it can be concluded that they appear to tolerate higher concentrations of PCB with little effect on overall production rates. After DDE was banned, one study found osprey populations recovering rapidly despite continued high concentrations of PCB during the same period of time (Grove *et al*., 2009).

1.2.2.3. Mercury

Mercury (Hg) is a frequent contaminant of concern in aquatic ecosystems. Since ospreys' diet is comprised exclusively on fish, they are at greater risk for elevated methylmercury (MeHg) exposure, accumulation and toxicity. MeHg in the blood is bound to keratin during the growth of new feathers. The levels decrease as feather growth proceeds (an important detoxification process). The demethylated Hg is sequestered with selenium in the liver and kidneys and therefore providing additional protection from some of the more toxic MeHg (Grove *et al*., 2009). This process may impact the health of the bird as the entire plumage may contain as much as 85% of the total mercury body burden in chicks and as much as 93% of the total mercury body burden in adults. During the intermolt period levels continue to rise in internal tissues until the next molt, when the process is repeated (Hughes *et al*., 1997). In this sense, feathers are considered a good tool for biomonitoring. While feathers integrate exposure during their growth, blood provides a non- destructive measure of recent contaminant exposure (Espin *et al*., 2014).

Higher levels of mercury poisoning have been linked to neurological damage such as difficulties in walking ang flying and an inability to coordinate muscle movement. Birds are able to eliminate a substantial portion of their mercury body burden via their plumage. And, in case of females, also via the production and laying of eggs (Hughes *et al*., 1997).

Regarding the importance of bird species' egg production and laying in lowering the overall body burden of mercury, the available data is inconsistent. For some species, the amount of mercury transferred to eggs is a small proportion of the female birds' total burden and thus egg laying has been thought to be an insignificant pathway of mercury removal. However, some authors report that as much as 40% of the total mercury body burden may be removed. Deposition of mercury in the uropygial gland and salt gland and excretion are other pathways by which mercury is removed from the body (Hughes *et al.*, 1997).
Susceptibility to Hg is very variable within an among species and toxic levels vary for different endpoints, for example, deformities, hatching and cognitive effects (Burger *et al.*, 2013).

1.2.2.4. Lead

There aren't many studies on lead (Pb) exposure and accumulation in ospreys. It may be for a reason that ospreys often do not ingest fish bones (where Pb primarily accumulates) and if ingested, most are not digested (regurgitated as pellets). It also has limited transferability to eggs hence lowering the risk of embryonic toxicity (Grove *et al.*, 2009).

1.2.2.5. Cadmium

Cadmium (Cd) is a widespread environmental pollutant that accumulates in living systems and represents a significant global health hazard. Environmental pollution by Cd occurs widely. Cd exposure results in abnormal renal function indices. Some studies have shown that Cd is a known teratogen in avian species (Ge *et al.*, 2019).

1.3. Blood indicators in osprey

Haematology and plasma biochemistry studies are crucial for veterinary diagnoses of both wild and captive animals, as well as for a variety of other multidisciplinary tasks, including scientific study on animal ecology or the management of endangered species. Evaluating the biochemistry parameters in plasma levels offers essential information on the physiological condition of the animal of interest. This information is related to individual quality and therefore various other related factors, including survivorship, fecundity, spatial ecology or evolutionary ecology. Since the 1970s, reference values have been assessed for numerous species, body condition indices have been developed and blood parameters have been used as physiological indicators. Few of the studies, however, provided chemistry values for wild species (Muriel *et al.,* 2013). Less than 5% of the species of free- living birds have been studied (Ferrer *et al.*, 2023). Despite having much attention in toxicological and biomagnification studies, there is a lack of information about plasma biochemistry or haematology in osprey (Muriel *et al*., 2013). Numerous factors are known to have an impact on haematological variables, including chemical components. These factors are physiological state, age, gender, dietary status, seasonal variations, captivity, contaminants, and plasma storing methods. The majority of studies primarily focus on publishing reference intervals or questions of clinical interest (Ferrer *et al*., 2023).

1.3.1. Haematology reference values

The aim of establishing a reference interval is to determine an interval that captures a recognized proportion of the values within a healthy population (Geffre *et al.*, 2009). Meredith *et al.,* (2012) conducted a study to analyse haematologic and biochemical reference intervals for wild osprey nestlings. They concluded that mean value of white blood cells (WBC) in their study was similar to previously published reports (8.00 X 10^9/L), the mean haemoglobin (11.98 g/dL) was similar to wild nestling bald eagles and the median value of packed cell volume (PCV) was 40%. Overall, raptors with a PCV <35% are considered anemic, and raptors with a PCV >45% are considered dehydrated (Joseph, 1999).

1.3.2. Plasma biochemistry reference values

Alanine transaminase (ALT) is found in many different tissues and that's why it is not very useful as a diagnostic tool. Seasonal variations can be found, but the relevance of this is not quite clear (Joseph, 1999). In a study conducted by Muriel *et al.,* (2012), the minimum value of ALT was 2.1 U/L, and the maximum was 28.6 U/L.
The activity of aspartate aminotransferase (AST) is found in the liver, kidney, brain, heart and skeletal muscle (Joseph, 1999). It is difficult to interpret since its distribution in different tissues and organs can vary among species. It can be affected by factors as diverese as muscular and soft-tissue injury, physical activity, stress, nutritional status, hydration, hemolysis and liver

disease. The increase of AST could be related to higher physical activity and flight excersises prior to fledging, which may lead to muscular stress and result in increasing AST values (Muriel *et al.,* 2012). Elevations can also be observed with soft- tissue injury, bumblefoot, liver disease, septicaemia, and post surgically (Joseph, 1999). Lower levels of AST and also creatine kinase (CK) in nestling ospreys may be due to a combination of small body sice and lower muscular stress at nestling stage (Wink and Sauer- Gürth, 2000; Lener and Mindell, 2005). Younger raptors have lover values than the adults (Joseph, 1999). In a study conducted by Muriel *et al.*, (2012), the range of AST was 15.5- 180 U/L and in a study of Meredith *et al.,* (2012), the median value of AST was 38.5 U/L.

Activity of alkaline phosphatase (ALP) is found in the duodenum, kidney, liver and bone. The elevations of this enzyme are caused by osteoplastic activity (Joseph, 1999). It is associated with the elongation and ossification of skeletal structures (Vinuela *et al*. 1991; Tilgar *et al*. 2008). A similar absence of covariation in ALP with age has also been observed in other species (Muriel *et al*., 2012). Juvenile raptors have higher levels, as well as those raptors with a nutritional or secondary nutritional hyperparathyroidism disorder (Joseph, 1999). In the study of Muriel *et al*., (2012), the range of ALP was 613- 1300 U/L.

The range of amylase (Amyl) was 433-1180 U/L in the study conducted by Muriel *et al*., (2012).

Lactate dehydrogenase (LDH) is found in skeletal muscle, cardiac muscle, liver, kidney, bone, and red blood cells. It is not specific for organ disease, however, in raptors it may be used to evaluate muscle fitness (Knuth and Chaplin, 1994). A flight-trained raptor has low resting levels of lactate and LDH, and levels increase immediately after exercise in a similar fashion to CK levels (Meredith *et al.*, 2012). The range of LDH levels in Meredith *et al*., (2012) research was 1235- 3349 U/L and the median value was 1738 U/L.

Creatine kinase is mainly found in skeletal muscle, heart muscle and brain tissue. It is often used as a diagnostic indicator of muscle damage (Joseph, 1999). Muscle cell disruption and the release of enzymes can occur when muscle strength and elasticity is surpassed by exercise effort (Horobagyu and Denaham, 1989). Elevated CK levels in birds can be related to manual restraining and muscle damage (Meredith *et al.*, 2012). Trained individuals have lower CK levels because with training, muscle cells hypertrophy and neuromuscular coordination improves, consequently less cellular disruption occurs. Sampling location from the brachial vein may also elevate CK and LDH (Meredith et al., 2012). In the study of Muriel *et al.,* (2012), the range of CK was 20-712 U/L and in the study of Meredith *et al.*, 2012, the median value of CK was 2009.5 U/L with the range of 772- 4330 U/L.

The minimum value of creatinine (Crea) in a study conducted by Muriel *et al.,* (2012) was 0.56 U/L and the maximum was 5.12 U/L.

Blood urea nitrogen has limited value in the avian species. It can be used to evaluate pre renal (dehydration) causes of renal failure (Joseph, 1999). In bird species with poor fat reserves, such as birds of prey, urea concentration has frequently been used as an accurate indicator of nutritional state. Tissue proteins are actively mobilized as an energy source during starvation periods, which leads to an increase in the nitrogenous excretion components in blood derived from protein catabolism. Hence, when a bird is undernourished, plasma urea values tend to rise and recover after feeding (Garcia- Rodrigues *et al*., 1987). In the study of Muriel *et al.,* (2012) the range of urea was 0.87- 1.84 mmol/L and the males presented lower urea levels than females. In the study of Meredith *et al*., (2012), the median value of urea was 2.1 mmol/L and the range was 1.3-4.1 mmol/L.

Levels of uric acid (UA) are higher in carnivorous birds (Joseph, 1999). High values of urea and UA have proven to be good indicators of undernourishment (Ferrer *et al*., 1987).

Carnivorous birds have higher cholesterol (Chol) values than psittacines. Diagnostic interpretation may be difficult, however, higher values may be seen with starvation or high fat diets (Joseph, 1999). Both triglyceride (Trigl) concentration and cholesterol concentration are related to fat reserves, and are therefore relatively low in bird species with diets rich in protein, such as birds of prey (Griminger 1976). In the study of Muriel *et al.,* (2012) the range of Chol was 3.11- 11.49 mmol/L. In the study of Meredith *et al*., (2012), the median value of Chol was 4.84 mmol/L and the range was 3.2- 8.4 mmol/L.

In the study conducuted by Muriel *et al.,* (2012), the range of triglycerides (Trigl) was 5.82- 10.21 mmol/L. Osprey nestlings showed rising concentrations of Trigl with age, probably as a result of general increase in energy reserves and weight as well as a more active endogenous lipid metabolism associated with increasing physical activity during the growth stage prior to fledging (Muriel *et al*., 2012).

Muscular activity is known to increase intracellular potassium (K) and thus to reduce its plasma concentration (Wolf *et al.,* 1985). Therefore, the decreasing potassium values observed in the osprey nestlings with age might be related to increasing physical activity prior to fledging (Dujowich *et al.,* 2005). In the study of Muriel *et al.*, (2012), the range on K levels were 1.4- 8.64 mmol/L.

Calcium (Ca) concentrations in plasma have been related to chondrogenesis, osteoplastic activity, and bone mineralization, during the growth periood (Vinuela *et al.*, 1991; Tilgar *et al.*, 2004). Levels of Ca do not vary widely between species. Decreased values in raptors have been

Associated with deficiency of Ca in the diet (Joseph, 1999). In the study of Muriel *et al.*, (2012), the range of Ca was 0.24- 0.62 mmol/L.

Values of total plasma protein (TPP) are more accurate than the total serum solid levels measured with the refractometer. The larger the raptor species, the higher the TPP. A low TPP value may indicate malnutrition, or chronic intestinal parasitism. High values may reflect dehydration or infection (Joseph, 1999). In the study of Muriel *et al.*, (2012), the range of TPP was 11- 34 g/L. In the study conducted by Meredith *et al.*, (2012), the median value of TPP was 34.4 g/L, the median value of albumin (Alb) was 16.7 g/L and the median value of globulin (Glob) was 17.1 g/L.

The ospreys' specialized diet, based exclusively on fish, might influence its normal plasma chemistry pattern. In any case, care must be taken when interpreting interspecific variations in biochemical values, even within the same group. A lot of factors, such as, captivity, diet, physial activity, circadian cycles, and analytic methods may result in greater differences between species then expected (Ferrer 1993).

1.4. External parasites

External and internal parasites are one of the major determinants of population dynamics and adaptive processes, imposing fitness costs to their hosts and promoting genetic variation. The documented external parasites of raptors include lice, feather mites, ticks, hippoboscid flies and fly larvae. In addition, various ectoparasites are vectors of multiple rapine bird pathogens. While raptors infested with these ectoparasites may not exhibit any clinicala symptoms, a severe infestation, primarily caused by lice and feather mites, can result in ragged- looking feathers and self- inflicted trauma. Generally, the presence of mites does not determine negative effects in the health of raptors, with the exception of cases of mange caused by *Knemidocoptes* (Oliveira *et al.*, 2011).

2. AIMS OF THE STUDY

The aim of this study was to investigate:

- The different values of haematology and plasma biochemistry and samples compared to previous researches conducted
- The concentration and possible poisoning of different contaminants in ospreys in Estonia
- Possible differences between 2 Estonian osprey populations by comparing their blood samples

3. MATERIALS AND METHODS

3.1. Sample collection

Trapping of adult ospreys was done as an action of species research and protection plan for placing or removing GPS trackers or rings. The locations of the nests are shown in figure 2. Clinical examination and biological sample collection was permitted and done as additional activity during annual osprey monitoring program. Ospreys were trapped using suitable mist nest placed close to nest during end stage of breeding, when ringing of chicks was also performed. For baiting, a stuffed white-tailed eagle was placed close to the mist net.

The blood was collected by a licenced wildlife veterinarian from the basilic vein using a 3 ml syringe with a 22-gauge needle, which was then transferred directly into clean and heparinized tubes. The tubes were marked and labelled with the corresponding bird’s identification number. The clean tube was centrifuged, and serum was transported into clean tube. Samples were stored in the freezer at -20˚C.

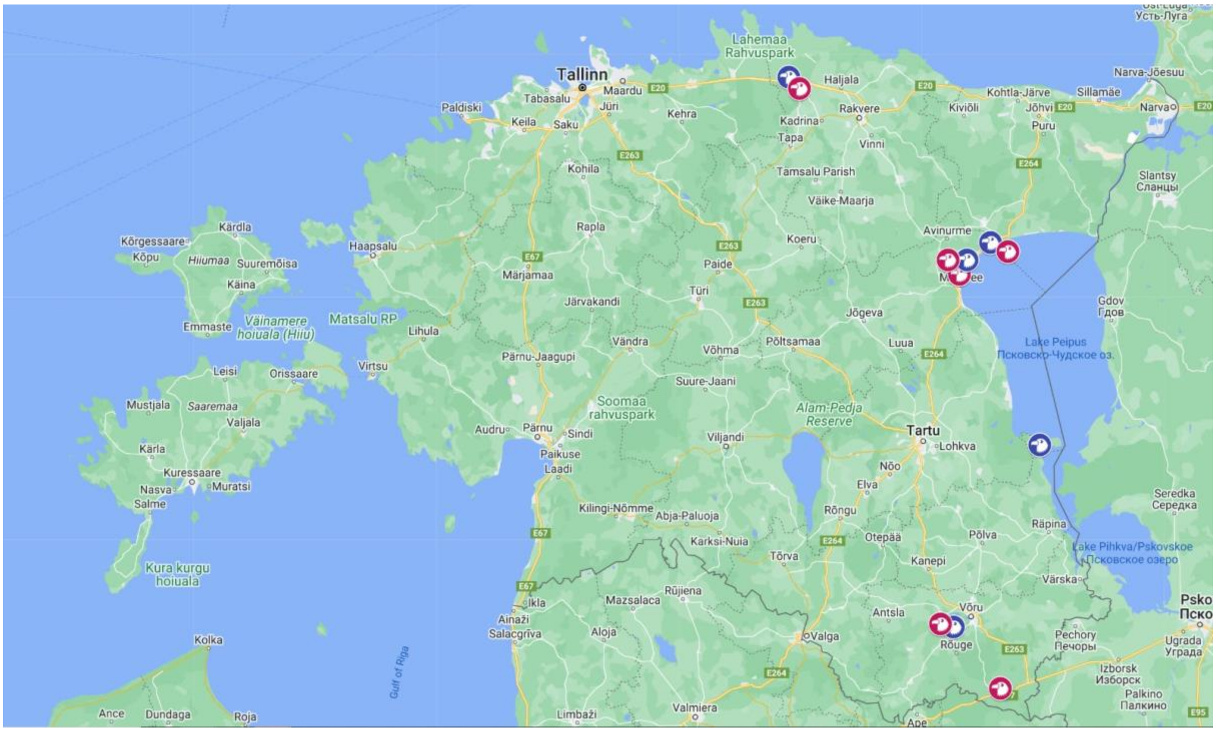

Figure 2. The locations of the ospreys in this study. Blue icons are representing male eagles and pink female eagles

3.2. Sample preparation and analysis for blood haematology

The blood sample was placed into a small hematocrit tube, the tube was sealed with clay and placed in the centrifuge. The total protein was measured from the centrifuged plasma with a refractometer.

3.3. Sample preparation and analysis of blood biochemistry

Frozen serum samples were sent to a commercial lab for blood biochemistry analysis in December 2015. The following 21 determinations were made in each plasma samples: alanine transaminase, aspartate aminotransferase, alkaline phosphatase, amylase, lactate dehydrogenase, creatine kinase, creatinine, uric acid, bilirubiin, cholesterol, triglycerides, sodium, potassium, calcium, magneesium, phosphate, iron, total plasma protein, albumin and globulin.

3.4. Sample preparation and analysis of heavy metals

The analysis for measuring the levels in the blood samples was performed at the Health Board Central Laboratory. The samples were prepared and analysed with inductively coupled plasma mass spectometer (ICP-MS), which is a method previously decribed by Kirk, E., 2020. LOD values for heavy metals were 0.009 ng/g for Pb, 0.003 ng/g for Cd and 0.004 ng/g for Hg. LOQ values were 0.0016 ng/g for Pb and 0.006 ng/g for Cd and Hg.

3.5. Declaration of ethical considerations

Ospreys were trapped and sampling was carried out during breeding season of 2015 as part of national monitoring program and under Estonian Environmental Board permit 26.06.2014 nr 1-4.1/14/298, “Nõusolek I kategooria kaitsealuste loomaliikide uurimis- ja kaitsetöödeks 2014–2015 aastal” (Agreement for I category protected species research and protection action for 2014-2015 year). Bloodsamples were collected by licensed veterinarian (license no. 0992).

4. RESULTS

All birds were ringed and an individual identification number was given. The identification numbers with the birds' nest location, gender, ringing year and origin have been pointed out in table 1. Three birds were from South Estonia and eight were from North-East region. Overall there were six female birds and five male birds. On clinical evaluation, all birds from North-East region had external parasites infestation.

A total of 11 blood samples from 11 different birds were taken. All results and data collected from the birds according to the sample type has been put together below in table 2. Biochemical analysis and analysis of heavy metals has been done on all eleven birds whereas the hematological analysis has been done on nine birds (A10334; A10479; A10483; A16183; ET 952; M47139; A16184; A8547, A16182).

Table 1. Identification numbers (ring number), nest locations, gender of the birds, ringing year and as some of the birds have been ringed as nestlings exact age and origin is known.

Nest ID	Gender	Identification number	Ringing year (age)	Origin
Laukasoo (North Estonia)	Female	M61123	2011 (5)	Finland, Kanta-Häme
Laukasoo (North Estonia)	Male	A16127	2015 (adult)	unknown
Nursipalu (South Estonia)	Female	A10334	2015 (adult)	unknown
Nursipalu (South Estonia)	Male	A10479	2015 (adult)	unknown
Selsi (South Estonia)	Female	A10483	2015 (adult)	unknown
Liin (Peipsi)	Female	A16183	2015 (adult)	unknown
Perajärve (Peipsi)	Male	ET952	2000 (16)	Latvia, Kemeri
Pilsi (Peipsi)	Female	A8547	2000 (16)	Estonia, Emajõe-Suursoo
Pilsi (Peipsi)	Male	A16182	2015 (adult)	unknown
Rannapungerja (Peipsi)	Female	M47139	2006 (10)	Finland, Virolahti
Rannapungerja (Peipsi)	Male	A16184	2015 (adult)	unknown

4.1. Haematology

A total of 11 haematology samples were taken and packed cell volume (PCV) and total protein (TP) was measured. Results are shown in table 2. Overall the minimum value of TP was 3 g/dL, maximum was 6.6 g/dL and the median value was 4.72 g/dL. As seen in appendix 1, in North-East region the median value of TP was 5.2 g/dL whereas in South Estonia it was 4.1 g/dL.
The overall median of PCV was 51%. The highest value was 55% and lowest 48%. In Northern regions the median value of PCV was 50% and in South Estonia 51%.

4.2. Plasma biochemistry

All overall results have been pointed out in table 2. The overall median of ALT was 16 U/L with the minimum value of 8 U/L and maximum of 23 U/L. In appendxix 2 it can be seen that in North-Eastern region the median value of ALT was 12.5 U/L whereas in South Estonia it was 17 U/L.
The overall median value of ALP was 396 U/L with the lowest value of 105 U/L and highest of 903 U/L. The overall median value of AST was 32 U/L with the minimum of 12 U/L and maximum of 51 U/L.
The overall median value of Amyl was 747 U/L with the lowest value of 214 U/L and highest of 1049 U/L. In North- East region, the median of Amyl was 875.6 U/L whereas in South Estonia it was 620. The median value of LDH was 494 U/L. The minimum value of LDH was 228 U/L and maximum 1124 U/L. The overall median of CK was 739 U/L with the minimum value of 183 U/L and maximum of 1353 U/L. In appendix 2 it is shown that in male birds the median value of CK was 1253 U/L whereas in female birds it was 683 U/L. The overall median value of crea was 20 µmol/L with the minimum value of 18 µmol/L and maximum of 26 µmol/L. The overall median value of urea was 2.5 mmol/L, the lowest value was 1.8 mmol/L and the highest was 3.8 mmol/L. In North-East region the median value of urea was 3.2 mmol/L whereas in South Estonia it was 2.3 mmol/L. The overall median value of uric acid was 746 µmol/L with the minimum value of 239 µmol/L and maximum of 1228 µmol/L. In North-East region the median value of uric acid was 852 µmol/L whereas in South Estonia it was 452 µmol/L. The overall median value of bilirubin was 6 µmol/L. In North-East region it was 4.5 µmol/L whereas in South Estonia it was 10 µmol/L. The overall median of cholesterol was 6.2

mmol/L with the minimum value of 4.7 mmol/L and maximum of 8.4 mmol/L. The overall median value of triglycerides was 1.97 mmol/L. The minimum value was 1.07 mmol/L and maximum 12.43. In North-East region the median value of triglycerides was 6.48 mmol/L whereas in South Estonia it was 1.3 mmol/L. The overall median value of potassium was 1.2 mmol/L with the minimum value of 1 mmol/L and maximum of 6.2 mmol/L. The median value of calcium was 2.08 mmol/L. The minimum value was 1.8 mmol/L and maximum 2.4 mmol/L. The median value of iron was 9.1 µmol/L, the minimum value was 5.9 µmol/L and maximum 19.7 µmol/L. In North-East region the median value of iron was 11.55 µmol/L whereas in South Estonia it was 6.7 µmol/L.

The overall median value of protein was 31.3 g/L with the minimum value of 20.6 g/L and maximum of 32.9 g/L. The overall median value of albumin was 13 g/L with the minimum value of 8 g/L and maximum of 18 g/L. The overall median value of globulin was 16.4 g/L. The minimum value was 12.6 g/L and maximum 19.8 g/L.

4.3. Heavy metals

All results of heavy metals have been pointed out in table 2. All analyzed ospreys had Pb and Cd residue concentrations in full blood below method LOD. For mercury, all birds had concentrations higher than used methods LOQ. Mercury concentration in ospreys blood average was 0,68 mg/kg, with median of 0,58 mg/kg, minimum 0,27 mg/kg and maximum concentration of 2,21 mg/kg. As shown in the figure 3, the female birds had lower concentrations of mercury compared to male birds. The birds are paired according to their nesting pairs.

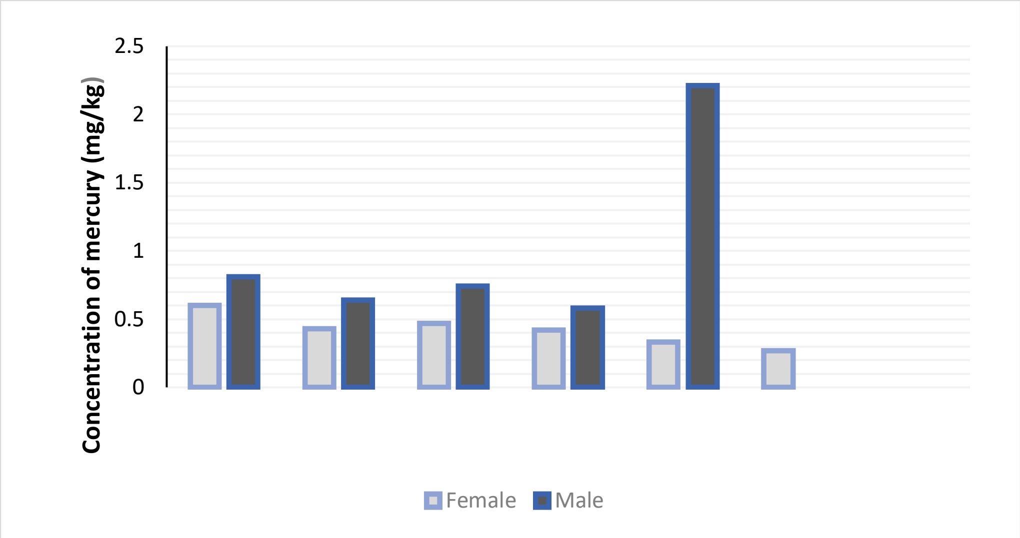

Figure 3. Mercury concentration levels in female and male birds' blood samples

Table 2. An overview of the 11 birds used in this study, their weight, biochemistry and haematology results and concetrations of heavy metals

Bird ID	**M 61123**	**A 16127**	**A 10334**	**A 10479**	**A 10483**	**A 16183**	**ET 952**	**A 8547**	**A 16182**	**M 47139**	**A 16184**	**MIN**	**MED**	**MAX**
Weight (kg)	1.74	1.48	1.7	1.36	1.82	1.74	1.6	1.78	1.6	1.82	1.48	1.36	1.7	1.82
ALT[1]	18	2	16	17	8	10	10	23	19	8	15	8	16	23
AST[2]	28	50	26	32	32	35	14	34	51	12	22	12	32	51
ALP[3]	463	336	349	192	105	386	903	527	204	456	436	105	386	903
Amyl[4]	620	747	526	672	214	884	812	1049	869	639	958	214	747	1049
LDH[5]	539	494	363	644	420	478	228	508	1124	472	643	228	494	1124
CK[6]	1182	1290	739	574	183	424	362	1094	1253	1353	627	183	739	1353
Crea[7]	26	23	20	19	21	21	20	20	26	18	19	18	20	26
Urea[8]	3.4	2.3	1.8	1.8	2.4	3.1	2.50	3.3	3.8	3.6	2.5	1.8	2.5	3.8
UA[9]	868	657	239	452	305	938	766	1087	746	1228	556	239	746	1228
Bil [10]	15	11	4	10	7	5	3	8	6	2	4	2	6	15
Chol [11]	7.1	8.4	6	5.5	4.7	5.8	7.2	7.8	6	7.3	6.1	4.7	6.2	8.4
Trigl [12]	1.97	3.02	1.07	1.3	1.17	7.08	6.77	6.16	1.79	12.43	1.14	1.07	1.97	12.43
Na [13]	149	149	147	146	145	147	152	145	148	147	153	145	147	153
K [14]	1.1	1.1	1.2	1.9	1	1.9	1	1.7	3.8	6.2	1	1	1.2	6.2
Ca [15]	1.9	2.04	1.94	2.09	1.85	2.08	2.4	2.18	2.27	1.8	2.19	1.8	2.08	2.4

Table 2 continues

Bird ID	M 61123	A 16127	A 10334	A 10479	A 10483	A 16183	ET 952	A 8547	A 16182	M 47139	A 16184	MIN	MED	MAX
Mg[16]	0.83	0.79	0.81	0.82	0.65	1.19	0.79	1.13	1.9	1.76	0.84	0.65	0.83	1.9
P[17]	0.5	0.5	0.5	0.5	0.72	0.5	0.5	<0.5	1.45	1.55	0.5	0.5	0.5	1.55
Fe[18]	10	9.1	6.4	6.7	5.9	13.9	10.4	12.7	7	19.7	7	5.9	9.1	19.7
TPP[19]	26.6	32.9	30	31.3	20.6	25.1	31.4	31.4	32.8	31.9	19.4	20.6	31.3	32.9
Alb [20]	11	14	13	14	8	12	15	14	13	18	13	8	13	18
Glob [21]	15.6	18.9	17	17	12.6	13.1	16.4	17.4	19.8	13.9	16.40	12.6	16.4	19.8
PVC [22]	-	-	55	51	51	48	49	48	51	52	53	48	51	55
TP [23]	-	-	4.1	4.4	3	4.6	5.6	4.8	5.6	4.7	6.6	3	4.7	6.6
Pb [24]	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024	0.0024
Cd[25]	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Hg[26]	0.27	2.21	0.6	0.81	0.43	0.33	0.58	0.42	0.74	0.47	0.64	0.27	0.58	2.21

ALT[1] – alanine transaminase (U/L)
AST[2-] - aspartate animotransferase (U/L)
ALP[3]- alkaline phosphatase (U/L)
Amyl[4]- amylase (U/L)
LDH[5]- lactate dehydrogenase (U/L)
CK[6]- creatine kinase (U/L)
Crea[7]- creatinine (µmol/L)
Urea[8]- urea (mmol/L)
UA[9]- uric acid (µmol/L)
Bil [10]- bilirubiin (µmol/L)
Chol [11]- cholesterol (mmol/L)
Trigl[12]- triglycerides (mmol/L)
Na[13]- sodium (mmol/L)
K[14]- potassium (mmol/L)
Ca[15]- calcium (mmol/L)
Mg[16]- magnesium (mmol/L)
P[17]- phosphate (mmol/L)
Fe[18]- iron (µmol/L)
TPP[19]- total plasma protein (g/L)
Alb[20]- albumin (g/L)
Glob[21]- globulin (g/L)
PCV[22]- packed cell volume (%)
TP[23]- total proteiin (g/dL)
Pb[24]- lead (mg/kg)
Cd[25]- cadmium (mg/kg)
Hg[26]- mercury (mg/kg)

5. DISCUSSION

Ospreys have been a sentinel species worldwide for assessing and monitoring environmental contamination of aquatic ecosystems. They are sensitive to a wide range of bioaccumulative contaminants (Grove *et al*., 2009). Although they have received much attention in toxicological and biomagnification studies, there is still a lack of information about ospreys' plasma biochemistry or haematology (Muriel *et al*., 2013). The main aims of this thesis were to investigate and compare the different values of haematology and plasma biochemistry to previous researches conducted and also to compare two different ospreys populations in Estonia.

In a study conducted by Meredith *et al.,* (2012), it was found that the median value of packed cell volume was lower compared to this current study.

In North- East region the median value of ALT was lower than in the South Estonia. Because ALT is found in many different tissues, it is not very useful as a diagnostic tool. Seasonal variations can be found, but the relevance of this is not quite clear (Joseph, 1999)

Muriel *et al.,* (2012) found that the minimum, maximum and median values of of AST were lower than in this study. AST is difficult to interpret since its' distribution in different tissues and organs can vary among species and it can be affected by many different factors. The increase of AST could be related to higher physical activity and flight excersises prior to fledging (Muriel *et al*., 2012). Lower levels of AST and also creatine kinase (CK) in nestling ospreys may be due to a combination of small body sice and lower muscular stress at nestling stage (Wink and Sauer- Gürth, 2000; Lener and Mindell, 2005). Joseph (1999) has also stated that younger raptors have lower values than the adults.

In a study from Muriel *et al.,* (2012), the minimum and maximum values of ALP were higher compared to this study. Elevation of this enzyme is caused by osteoplastic activity. Both juvenile raptors and raptors with a secondary nutritional hyperparathyroidism disorder have greater levels (Joseph, 1999).

In a study conducted by Meredith *et al.,* (2012), the median value and the minimum and maximum values of lactate dehydrogenase were higher compared to this study. LDH is not specific for organ disease, however, in raptors it may be used to evaluate muscle fitness (Knuth and Chaplin, 1994). A flight-trained raptor has low resting levels of lactate and LDH, and levels increase immediately after exercise in a similar fashion to CK levels (Meredith *et al.,* 2013).

In the study of Muriel *et al.* (2012), the range of creatine kinase was lower compared to this study whereas in a study conducted by Meredith *et al.,* (2012), the levels were higher. In this study, the male birds had higher levels of creatine kinase compared to female birds. Creatine kinase is often used as a diagnostic indicator of muscle damage (Joseph, 1999). Elevated CK levels in birds can be related to manual restraining and muscle damage. Also sampling location from the brachial vein may also elevate CK and LDH (Meredith *et al*., 2012).

In this study the minimum and maximum levels of urea were higher compared to the study of Muriel *et al.,* (2012) and the median level of urea compared to study of Meredith *et al.,* 2012 was also higher. In South Estonia the levels of urea were lower compared to the North-East region. High values of urea and uric acid have proven to be good indicators of undernourishment (Ferrer *et al*. 1987; Polo 1995), while total protein concentrations tend to decrease under conditions of malnutrition (Smith and Bush 1978).

In North-East region the levels of triglycerides were higher compared to South Estonia. Triglyceride values are related to lipid ingestion as well as endogenous synthesis (Ferrer *et al.,* 1978) and may decrease with prolonged food deprivation (Garcia-Rodriguez *et al.,* 1978).

The minimum and maximum range of calcium was higher in this study than in the study conducted by Muriel *et al.,*(2012) whereas the median value was similar to the median calcium level in the Meredith *et al.,* 2012 study. Calcium concentrations in plasma have been related to chondrogenesis, osteoblastic activity, and bone mineralization during the growth period in birds (Vinuela *et al*., 1991; Tilgar *et al.,* 2004).

For mercury, all birds had concentrations higher than used methods LOQ with the median value of 0.58 mg/kg. The female birds had lower levels of mercury in their blood samples compared to male birds. There is conflicting evidence about the importance of egg production and laying among bird species in reducing the total body burden of mercury. For some species, the amount of mercury transferred to eggs is a small proportion of the female birds' total burden and thus egg laying has been thought to be an insignificant pathway of mercury removal. But on the other hand, some authors report that as much as 40% of the total mercury body burden may be removed. Deposition of mercury in the uropygial gland and salt gland and excretion are other pathways by which mercury is removed from the body (Hughes *et al*., 1997).

As seen above, there was not enough data to make any scientific conclusions based on this thesis.

CONCLUSIONS

There are about 90-100 pairs of ospreys in Estonia. Most populated regions are around lake Peipsi and in South Estonia. They primarily feed on larger lakes rather than smaller ones. Their choice of prey depends on its availability.

Ospreys have been a sentinel species worldwide for assessing and monitoring environmental contamination of aquatic ecosystems. They are sensitive to a wide range of bioaccumulative contaminants. Due to being strictly piscivorous and on the top of the food web, their diet is easy to link to sources of localized contaminant exposure. When aquatic organisms are exposed to contaminants with immunotoxicity, it results in poor health. This can make the organism more susceptible to parasites, viruses, bacteria and fungi. Despite having much attention in toxicological and biomagnification studies, there is a lack of information about plasma biochemistry or haematology in osprey.

All haematological and biochemical sample results were compared with the previous two studies. There were some differences, including parameters that could be linked with birds' ages, but overall, no significant differences between all three studies were found. All tested 11 birds had some traces of heavy metals found in their tissues. The most noticeable difference appeared in mercury levels between female and male birds which can be possibly linked to the egg laying period. There were some biochemical analyse result differences between North-East and South regions connected to assumable nutritional parameters, but no final conclusions can be made. The weights of the birds did not differ so significantly.

The infestation of external parasites didn't seem to have any relation to overall blood parameters. Further research should be conducted to assess the differences between these populations.

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ÜLDKOKKUVÕTE

Kalakotkas (*Pandion haliaetus)* on üle maailma üks indikaatorliikidest, kelle abil on võimalik jälgida veeökosüsteemide olukorda ja keskkonnasaastet. Erinevates uuringutes on leitud, et kalatkotkad on tundlikud paljude bioakumuleeruvate saasteainete suhtes (Grove *et al.*, 2009). Kuna nad toituvad ainult kalast, on nende toitumist võimalik siduda saasteainete allikatega.

Eestis pesitseb umbes 90-100 paari kalakotkaid. Tihedamalt on asustatud Peipsi ja Lõuna-Eesti piirkond (Keskkonnaamet, 2019). Ükski varasemalt läbiviidud saasteainete uuring ei ole paraku olnud seotud Eesti kalakotkastega. Samuti ei ole palju teada kalakotkaste üldistest vereparameetritest, sealhulgas biokeemilistest ja hematoloogilistest näitajatest.

Antud töö käigus analüüsiti 11 linnu vereproove erinevatest Eesti populatsioonidest: Kirde-Eestist ja Lõuna-Eestist. Kõikide lindude vereanalüüse mõõdeti hematoloogiliste ja biokeemiliste näitajate suhtes. Lisaks uuriti elavhõbeda, plii ja kaadmium sisaldust veres. Tulemusi võrreldi varasemate uuringutega ning lisaks ka regiooniti populatsioonide vahel ning emas- ja isaslindude vahel. Laiemas pildis jäid Eesti kalakotkaste vereproovide vahemikud varasemalt uuritud referentside vahemikku. Olid mõned erandid, mida võib seostada uuringutes kaasatud lindude vanuste varieeruvusega. Ka populatsioonide vahel oli erinevusi biokeemilistes analüüsides, mida on võimalik seostada toitumuslike parameetritega. Täpsema ülevaate saamiseks oleksid vajalikud edasised uuringud.

Käesolev töö koondas endas hetkel kättesaadavad andmed kalakotkaste olukorra kohta Eestis.

APPENDIXES

Appendix 1. Results of haematology samples based on population and gender

		TP [1] (g/dL)	**PCV [2] (%)**
North- East	Median	4.8	51
	Range	4- 6.6	48.54
South	Median	4.1	51
	Range	3- 4.4	51-55
Males	Median	5.6	51
	Range	4.4- 6.6	49- 53
Females	Median	4.35	51.5
	Range	3- 4.8	48- 55

[1] TP- total protein

[2] PCV- packed cell volume

Appendix 2. Comparitive results of biochemical analysis according to nest location and gender

	North East		South		Males		Females	
	Median	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
ALT[1]	12.5	8-23	17	8-22	17	8-22	16	8-23
AST[2]	28	12-51	32	26-50	32	12-51	30	22-35
ALP[3]	446	204-903	336	105-463	336	192-903	411	105-527
Amyl[4]	876.5	639-1049	620	214-747	747	639-869	752	214-1049
LDH[5]	493	228-1124	494	363-644	494	228-1124	493	363-643
CK[6]	860.5	362-1353	739	183-1290	1253	362-1353	683	183-1182
Crea[7]	20	18-26	21	19-26	20	18-26	20.5	19-26
Urea[8]	3.2	2.5-3.8	2.3	1.8-3.4	2.5	1.8-3.8	2.8	1.8-3.4
UA[9]	852	556-1228	452	239-868	746	452-1228	712	239-1087
Bil[10]	4.5	2-8	10	4-15	6	2-11	6	4-15
Chol[11]	6.65	5.8-7.8	6.2	4.7-8.4	7.2	5.5-8.4	6.15	4.7-7.8
Trigl[12]	6.48	1.14-12.43	1.3	1.07-3.02	3.02	1.3-12.43	1.57	1.07-7.08
Na[13]	147.5	145-153	147	145-149	148	146-152	147	145-153
K[14]	1.8	1-6.2	1.1	1-1.9	1.9	1-6.2	1.2	1-1.9
Ca[15]	2.185	1.8-2.4	1.94	1.85-2.09	2.09	1.8-2.4	2.01	1.85-2.19
Mg[16]	1.16	0.79-1.9	0.81	0.65-0.83	0.82	0.79-1.9	0.83	0.65-1.19

Appendix 2 continues

	North East		South		Males		Females	
	Median	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
Phos[17]	0.5	0.5-1.55	0.5	0.5-0.72	0.5	0.5-1.55	0.5	0.5-0.72
Fe[18]	11.55	7-19.7	6.7	5.9-10	9.1	6.7-19.7	8.85	5.9-13.9
Protein[19]	31.4	25.1-32.8	30	20.6-32.9	31.9	31.3-32.9	28	20.6-31.4
Alb[20]	13.5	12-18	13	8-14	14	13-18	12.5	8-14
Glob[21]	16.4	13.1-19.8	17	12.6-18.9	17.8	14.9-18.3	15.5	12.6-15.5

ALT[1] – alanine transaminase (U/L)
AST[2-] - aspartate animotransferase (U/L)
ALP[3]- alkaline phosphatase (U/L)
Amyl[4]- amylase (U/L)
LDH[5]- lactate dehydrogenase (U/L)
CK[6]- creatine kinase (U/L)
Crea[7]- creatinine (µmol/L)
Urea[8]- urea (mmol/L)

UA[9]- uric acid (µmol/L)
Bil [10]- bilirubiin (µmol/L)
Chol [11]- cholesterol (mmol/L)
Trigl[12]- triglycerides (mmol/L)
Na[13]- sodium (mmol/L)
K[14]- potassium (mmol/L)
Ca[15]- calcium (mmol/L)
Mg[16]- magnesium (mmol/L)

P[17]- phosphate (mmol/L)
Fe[18]- iron (µmol/L)
TPP[19]- total plasma protein (g/L)
Alb[20]- albumin (g/L)
Glob[21]- globulin (g/L)

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