UNIVERSITY OF TURKU
Faculty of Science

Prevalence and molecular identification of acanthocephalan parasites (*Corynosoma* spp.) in the Baltic herring (*Clupea harengus membras*) and great cormorant (*Phalacrocorax carbo*)

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Ecology

Master's thesis

Credits: 30

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26.10.2022

Turku

Master's thesis

Subject: Ecology
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Title: Prevalence and molecular identification of acanthocephalan parasites (Corynosoma spp.) in the Baltic herring (Clupea harengus membras) and great cormorant (Phalacrocorax carbo)
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Number of pages: 26 pages
Date: 26.10.2022

Baltic herring (*Clupea harengus membras*) is one of the most abundant and commercially important fish species in the Baltic Sea. In the Archipelago Research Institute, a long-term monitoring program was established in 1984 with an intention to monitor herring's reproductive health and changes in the population. In 2014, some of the herring were infected with acanthocephalans or thorny-headed worms of genus *Corynosoma*. Thorny-headed worms are intestinal parasites that occur in vertebrates. Their life cycles include an amphipod as the intermediate host and a fish as a paratenic host. Acanthocephalans of the genus *Corynosoma* mature in the intestines of mammals such as seals. Between the years 2014 and 2019 total of 7002 herring and 65 Great cormorants (*Phalacrocorax carbo)* were examined and acanthocephalans were collected. Their species were identified using DNA-analysis. The prevalence of the *Corynosoma* infections in herring increased from 2014 to 2018. The prevalence was higher in the Archipelago Sea than in the Bothnian Sea. Infected herring and cormorant individuals were generally larger than non-infected. DNA-analysis showed that three different Corynosoma species caused the infections in herring: *C. semerme, C. strumosum* and *C. magdaleni.* 26 % of the cormorants were infected and DNA-analysis showed that all the *Corynosoma* in cormorants represented species *C. semerme.*

Key words: fish, herring, *Clupea harengus membras*, cormorant, *Phalacrocorax carbo*, parasites, acanthocephala, *Corynosoma*, parasitology

Contents

1. Introduction

In the Baltic Sea, Baltic herring (*Clupea harengus membras* Wulf*)* is the most important commercial fish species (Natural Resources Institute Finland 2022). In Finland alone, approximately 90 million kilos of herring are caught annually. Herring is a migratory fish, which overwinters in the open sea and returns to the spawning grounds in the inner archipelago in the spring (Parmanne 1990, Kääriä et al. 2001). In the Archipelago Sea, the spawning time of the herring lasts from early May to mid-July (Rajasilta 1992). The characteristics of the herring population that spawns in the inner Archipelago Sea have been monitored annually by the Archipelago Research Institute, University of Turku since the 1980s (Rajasilta et al. 2006). In 2014, few individuals of helminth parasites that were later identified as *Acanthocephalans* of genus *Corynosoma* were found during the routine treatment of herring samples. In total, 31 different parasite species have been found in the herring in the Baltic Sea (Arthur & Arai 1984; MacKenzie 1987). Some prior studies have described the prevalence of the *Corynosoma* parasites in grey seals (*Halichoerus grypus* Fabricius) and Baltic ringed seals (*Phoca hispida* Gmelin) as well as in different fish species (Sinisalo & Valtonen 2003).

Acanthocepahalans or thorny-headed worms are intestinal parasites that occur in vertebrates (Valtonen et al. 2012). Their life cycles include an amphipod as the intermediate host and a fish as a definite host. *Acanthocephalans* of the genus *Corynosoma* mature in the intestines of mammals such as seals. They require a fish as the paratenic host to transfer the larvae from the amphipod to the definite host. In the Baltic Sea, *Corynosoma* use *Monoporeia affinis* Lindström*,* a small bottom dwelling crustacean, as the intermediate host. In the crustaceans, the acanthor larvae develop in to cystacanth larvae. Larvae do not further develop until ingested by definite host. The cystacanths are capsulated in the body cavity of the fish (Figure 1) and can remain there for years waiting for a suitable definite host to eat the paratenic host. Many fish species eat *Monoporeia affinis* and get infected with the *Corynosoma* cystacanths. In the Baltic Sea, the primary hosts are the gray seal (*H. grypus*) and the Baltic ringed seal (*P. hispida*) (Sinisalo & Valtonen 2003). Corynosoma individuals have also been found in the great cormorant *(Phalacrocorax carbo* Linnaeus) in Germany (Oßmann 2008). They mature and mate in the intestines of the host and new acanthor larvae are released with the feces. Internal parasites may affect the health of the host (Bergeron et al. 1997) and are linked with for example septicemia and perinatal death (Siebert et al. 2007). However, studies performed on seals suggest that even heavy *Corynosoma* infection does not harm the host (Valtonen 1983b). There

are only few incidents where humans have been infected with *Corynosoma* parasites and they have been linked to eating raw fish such as sushi and sashimi (Fujita et al. 2016, Takahashi et al. 2016).

Figure 1. Corynosoma cystacanth larvae in the body cavity of the Baltic herring.

Identifying *Corynosoma* species is difficult due to their small size and because species are very similar. However, it is crucial to reliably identify species if they are to be used for example in stock identification. Hebert et al. (2003) demonstrated that mitochondrial gene cytochrome c oxidase (COI) can be used in molecular identification of species. Prior studies have successfully used COI to identify and discriminate *Corynosoma* species (Garcia-Varela 2009; Waindok et al. 2018).

In this study I aim to

1) determine the prevalence of *Corynosoma* parasites in herring and in cormorants

2) determine factors affecting the probability of *Corynosoma* infection

3) use DNA sequencing to identify *Corynosoma* species

2. Materials and methods

2.1 The environmental conditions in the Baltic Sea

The Baltic Sea is an intra-continental brackish sea (Nehring & Matthäus 1991) with the total area of 415 023 km^2 and mean depth of 52 m (Wasmund et al. 2003). There is a clear salinity gradient in the Baltic Sea varying from 30 PSU in the Danish Straits to only 1 PSU in the Bothnian Bay affecting its fauna. Both salt water and freshwater species thrive in the Baltic Sea. The Archipelago Sea which is situated between the Bothnian Bay and the Baltic proper (59°45'-60°45'N and 21°00'-23°00'E) in the northern Baltic Sea. There are more than 60 000 islands in the Archipelago Sea making it the biggest archipelago in the world (Väänänen et al. 2020). The Archipelago Sea covers more than 9000 km^2 and the water volume is more than 200 km^3. The salinity varies between 4 and 6 PSU. The Archipelago Sea is rather shallow with average depth of only 23 meters and the deepest parts being less than 150 meters deep (Voipio 1981). Strong seasonality is characteristic to the Baltic Sea. During the summer months the seawater can reach 20 °C but during winter there is a great probability of an annual ice cover (Leppäkoski & Bonsdorff 1989). During the study period, the seawater salinity has decreased, and surface temperature increased (Figure 2.). Changes in the environment can negatively affect the physiology of the herring and make them more susceptible to infections (Lafferty & Kurtis 1999).

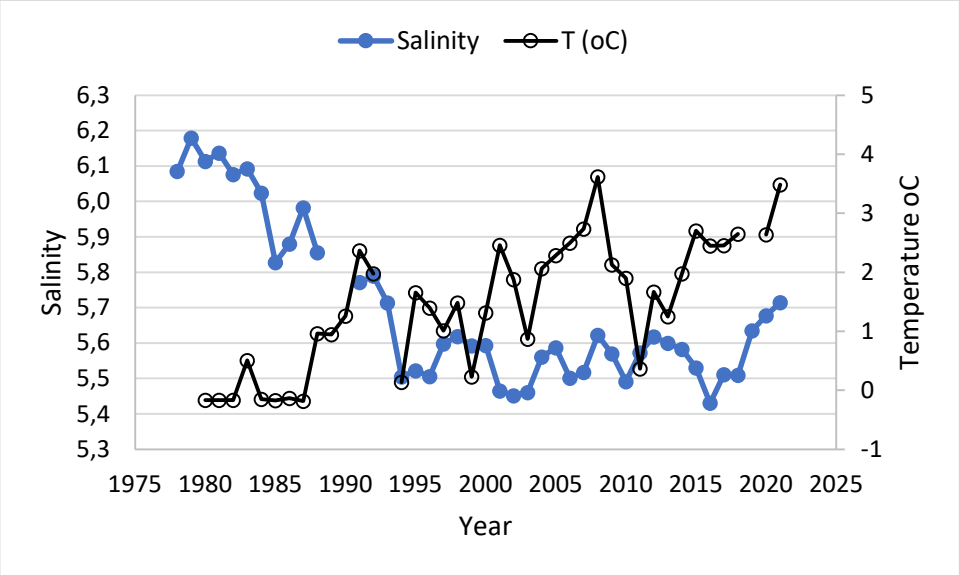

Figure 2. Mean annual salinity (psu) and temperature (T °C) of the winter months (January – April) in the Bothnian Sea during 1980-2021. (Data from depths of 0-50 m; source: ICES).

2.2 The seals of the Baltic Sea

Three seal species, the grey seal (*H.s grypus*), the ringed seal (*P. hispida*) and the harbour seal (*Phoca vitulina* Linnaeus) inhabit the Baltic Sea (Waindock et al. 2018). The population of grey seals declined from about 90 000 individuals a century ago to about 20 000 in 1940s due to heavy hunting pressure (Harding et al. 2007). Even after the hunting ceased the population kept declining reaching only about 3000 individuals in mid-1970s (Harding & Härkkönen 1999). Decline has been linked with low fertility due to high concentrations of organochlorines.

The seal populations started to recover at the end of the 20th century as DDT and PCB concentrations started to decline (Olsson 2000) and the reproductive health of the seals improve (Bergmann 1999). Since 2003 the population has grown approximately 5 % each year (Natural Resources Institute Finland 2017). In 2020, more than 40 000 grey seals were counted in the Baltic Sea. About 17 000 of those were calculated in Finnish waters. In addition to grey seals, approximately 13 000 ringed seals were counted in Bothnian Bay.

High prevalence of *Corynosoma* infection has been detected in ringed seals in Bothnian Bay (Helle & Valtonen 1981). 95.6 % of animals were infected with either *Corynosoma semerme* Forssell or *Corynosoma strumosum* Rudolphi. There is a seasonal variation in the number of worms and the authors argue that the intensity of infection may even increase during active feeding season.

2.3 The Great cormorant (*Phalacrocorax carbo*)

The populations of cormorants in the Europe declined in the 19th century due to human activities and harassment and it disappeared from many Baltic countries. The persecution continued until 1960s and especially the populations *Phalacrocorax carbo sinensis* declined drastically. The European population size was only about 4000 nesting pairs and half of those nested in Germany and Poland. In addition to harassment, pollutants such as DDT and PCB are believed to have affected the decline (Herrmann et al. 2019). Eggshell thickening, hatching difficulties and increased embryotic mortality have been linked with pollutant concentrations (Dirksen et al. 1995) as well as changes in adult behavior (Fry 1995). Due to banning of PCB and DDT the populations started to increase in the 1970s and 1980s (Herrmann et al. 2019). The number of nesting pairs soon reached 150 000. The first recorded nesting in Finland occurred in 1996 when ten nests were found in Tammisaari in the Gulf of Finland (Rusanen et al. 1998). Until the beginning of 21st century cormorants were only nesting in the southern coast of Finland but soon

they dispersed to all coastal areas (Lehikoinen 2003). In the first ten years the nesting population of cormorants increased annually by 124 % on average. The population of cormorants in Finland is currently approximately 25 000 pairs (Finnish Environmental Institute 2022).

Cormorants eat fish and crustaceans. An adult individual consumes approximately 300-500 grams of fish per day. They can dive up to 20 meters in search for food. The diet consists mainly of perch (*Perca fluviatilis* Linnaeus), Viviparous eelpout (*Zoarces viviparus* Linnaeus) and roach (*Rutilus rutilus* Linnaeus) as well as some herring (*Clupea harengus membras*) and pikeperch (*Zander lucioperca* Linnaeus) (Salmi et al. 2013).

2.4 Ethics statement and regulations concerning sampling of the study material

The great cormorant (*Phalacrocorax carbo* spp.) is a protected species by the EU Birds Directive (Article 9, 2009/147/EC) and hunting them of them is subject to license. We applied for a special license from the Southwest Finland Centre for Economic Development, Transport and the Environment for research purposes and were granted to shoot 50 individuals outside of breeding colonies (VARELY/362/2018). The shooting was conducted by the commercial fishermen following strictly prevailing hunting regulations for protected species. Only 24 individuals were shot under this special license. Later, a commercial fisherman applied for a comparable license to shoot harmful cormorants that were causing economical damage to his fish farming. He was also granted a license by the authorities (VARELY3509/2019) on a condition that shot cormorants were forwarded to benefit our study. This produced 41 additional cormorants for the research.

Besides our study, the cormorants that were shot benefited also other research. For example, cormorant's heads were collected for the use of Finnish Environmental Institute to determine which subspecies (*P.sinensis* or *P.carbo*) they represented, their stomach contents were used in a study by Åbo Akademi to determine the integration of invasive species to the local food web and their livers and muscles were collected for possible heavy metal analysis.

No living fish were used in this study as all samples were received from the professional fishermen as a part of their commercial catch, caught under the national and EU regulations.

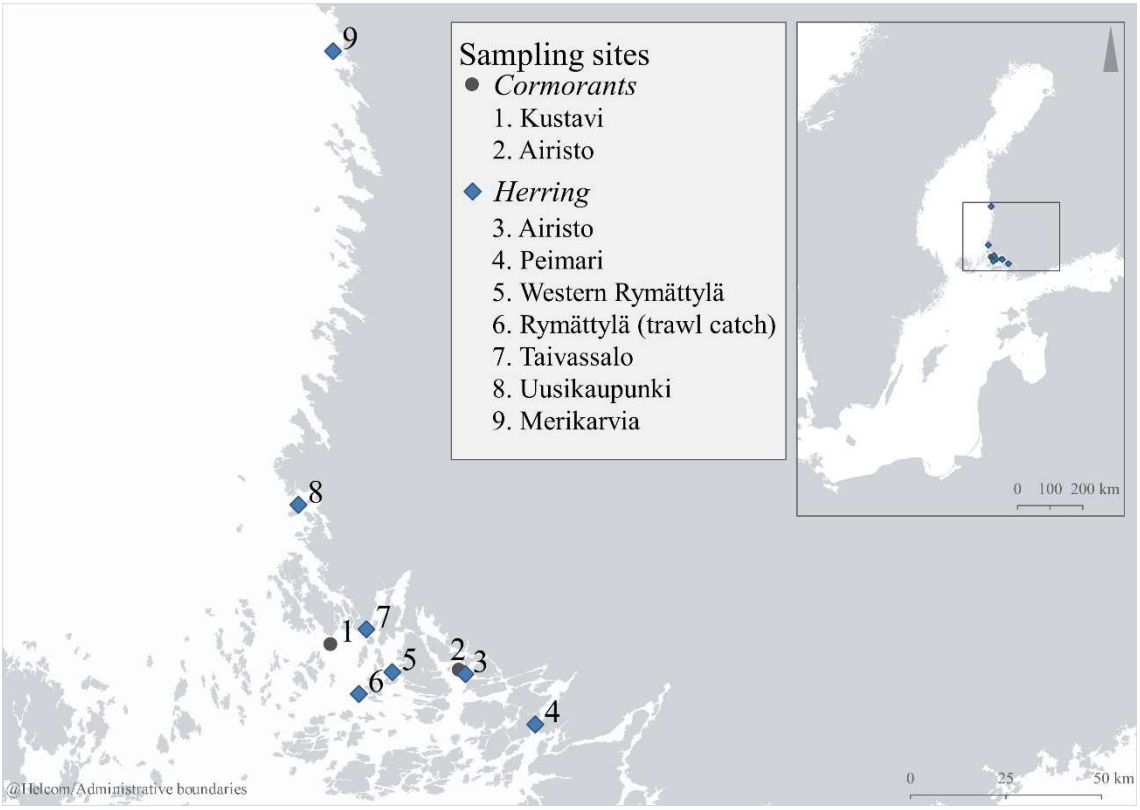

Figure 3. Sampling sites for cormorants and herring in the Archipelago Sea and in the Bothnian Sea.

2.5 Sampling and treatment of fish

In this study, I used measurements of about 7000 individual herring (Table 1). During the years 2014-2018, commercial trap-nets were used to catch herring samples with the endorsement of the local fishermen for the monitoring program carried out by the Archipelago Research Institute. In 2017, the herring samples were collected from the Aristo inlet area and, in 2018, from the Archipelago Sea and Bothnian Sea (Figure 3). The samples were primarily collected during the spawning season between early May and early July with some additional winter samples. Each sample consisted of 100–200 randomly collected herring individuals. Fishes were stored in freezer in -20 °C until preparation.

Table 1. Study sites for herring catches and the number of individuals in fish samples

Year	Area	Number of individuals
2018	Peimari	249
	Taivassalo	570
	Western Rymättylä	348
	Rymättylä (trawl catch)	635
	Uusikaupunki (Bothnian Sea)	542
	Merikarvia (Bothnian Sea)	986
2017	Airisto	698
	Rymättylä (trawl catch)	175
2016	Airisto	1018
2015	Airisto	1031
2014	Airisto	750
total number of individuals		7002

Before preparation, the fish samples were thawed long enough in room temperature. I weighted each fish with the accuracy of 0.1 g and measured the body length with the accuracy of 1 mm. I dissected fish bellies and determined sex from gonads. I weighted the gonads with the accuracy of 0.01 g and stored them by freezing (-20°C) for later fat concentration analysis. I determined the reproductive phase of individuals from the gonads as well. I collected fish otoliths and preserved them as dried for later age analysis.

I inspected the body cavities of the herring for parasites. From the herring caught between 2017-2018 I calculated found parasites and from subset of 20-30 fish per sample, I collected and stored them in 70 % ethanol for later DNA analysis.

2.6 Sampling and treatment of cormorants

In 2018 and 2019, a total 65 cormorants were hunted for this study under special permits granted by authorities of the Southwest Finland Centre for Economic Development, Transport, and the Environment (VARELY/362/2018 and VARELY/3509/2019). Altogether, 15 cormorants were hunted from the northern Airisto Inlet area and 50 in the vicinity of a breeding colony of Tiiraletto, Kustavi.

For preservation, the birds were frozen in -20 °C soon after the shooting. Approximately 24 hours before dissection the birds were taken into room temperature to thaw. With the help of the taxidermist of the Zoological Museum in the University of Turku, Ari Karhilahti, I weighted the birds with the accuracy of 1 g and measured their lengths from the tip of the beak to the tip of

the tail with the accuracy of 1 cm, as well as the lengths of their wings. During the dissection, we determined the age of the birds according to Baker (1993). We cut off the bird heads and stored them in -20 °C for later identification of sub-species. We cut open the bird stomachs by using a sharp knife, and removed chest muscle, liver and stomach and stored the in -20 °C for later analysis. Finally, we removed the intestines, and determined sex from the gonads.

The intestines were stored in -20 °C until preparation. To preparate, the intestines were first thawed in room temperature a day before the parasite investigation. After that, we dissected the intestines starting from the cloaca by using sharp scissors. We cleaned the interior of the intestine carefully with water spraying. We inspected the cleaned intestine for Acanthocephala species. We calculated found Acanthocephalans and carefully removed them with tweezers and stored them in 70 % ethanol in 1.5 ml or 2 ml Eppendorf tubes.

2.7 DNA extraction

I performed the DNA analyses using QIAGEN Blood N Tissue -kit in the "Tick lab" in the University of Turku with guidance from the staff. From each fish sample, only one worm was used to extract DNA with PCR method. I cut the worm in half by using a sharp disposable knife. A piece of parafilm was used as a shield on a cutting board to avoid contamination from surfaces. I stored the worm head in 99 % ethanol in a separate Eppendorf tube for possible later morphological identification. I placed the worm tail in a 1.5 ml or 2 ml Eppendorf and pipetted 180 µl of Buffer ATL and 20 µl of proteinase K into the tube. I mixed the mixture was thoroughly with vortex machine and placed it into a heat box and incubated it in 50 °C for approximately 12 hours.

When the worm tissue was completely lysed, I mixed the mixture with a vortex machine for 15 seconds. Then, I added 200 µl of Buffer AL and mixed it with vortex machine. After that, the mixture was incubated in the heat block in 56 °C for 10 minutes. After incubation, I added 200 µl of 99 % ethanol into the mixture and mixed it thoroughly. Then I pipetted the mixture into a 2 ml DNeasy Spin Column collection tubes and centrifuged it at 8000 rpm for 1 min. I then placed the spin column into a clean 2ml collection tube in which I added 500 µl of Buffer AW1 and again centrifuged it at 8000 rpm for 1 minute. I then repeated this procedure and after that, I centrifuged the mixture at 14 0000 rpm for 3 minutes and transferred the spin column into a clean 1.5 ml or 3 ml Eppendorf tube. Next, I pipetted 50 µl of Buffer AE into the spin column membrane and incubated it for 1 min at room temperature. Finally, I centrifuged the membrane at 8000rpm for 1 minute after which I repeated the last step for maximum DNA yield.

2.8 PCR and purification

After the extraction of DNA, I prepared a 5mM primer mixture by mixing 270 µl of H_2O and 15 µl of forward primer (LCO1490GV: AGT TCT AAT CAT AAR GAT ATY GG (Nadler et al. 2006)) and 15 µl of reverse primer (HCO2198: TAA ACT TCA GGG TGA CCA AAA AAT (Folmer et al. 1994)). I used MyTaq RedMix for PCR. I prepared the master mixture by mixing 5.5 µl of MQ-H20, 7,8ul of MyTaq RedMix and 1.2 µl of primer mix per sample. Then, I pipetted 14 µl of master mix and 1 µl of extracted DNA into a tear-away tubes and ran a PCR program presented in Table 2 using Applied Biosystems 2720 Thermal Cycler.

Table 2. Temperatures, times, and number of cycles for PCR program.

	Temperature	Duration	Number of cycles
1.	95°C	5 min	
2.	95°C	1 min	
3.	48°C	1 min	
4.	72°C	1 min	35 cycles of steps 2-4
5.	72°C	5 min	

After PCR run, I pipetted 2 µl of PCR product into wells in gel electrophoresis to ensure successful DNA extraction and detect possible contaminations. To the remaining 13 µl of PCR product, I added 1ul of Shrimp Alkaline Phosphatase (rSAP) and 1 µl of Exonuclease I (Exo I) and ran a PCR program presented in Table 3 using Applied Biosystems 2720 Thermal Cycler to purify the PCR product.

Table 3. Temperatures, times, and number of cycles for PCR purification program.

	Temperature	Duration
1.	37°C	5 min
2.	80°C	10 min
3.	10°C	30 min

I pipetted the final product into two separate wells on a 96-well microplate (7.5 µl each) and shipped them to the Macrogen lab in Amsterdam, The Netherlands, where the final sequencing was performed. The received sequences were cleaned and trimmed, and forward and reverse sequences were combined using Geneious R6 6.1.8 software. Final sequences were compared to known *Corynosoma* sequences using BLAST Basic Local Alignment Search Tool available at National Center for Biotechnology Information (Altschul et al. 1990).

2.9 Statistical analysis

I calculated quantitative descriptors of parasites according to Bush et al. (1997). Prevalence was calculated by dividing the number of infected host individuals by the total number of hosts. Intensity of infection was calculated by counting the number of Corynosoma individuals per infected host.

I conducted all statistical analyses using RStudio statistical software (RStudio Team 2022). To investigate the factors affecting the probability of a *Corynosoma* infection in cormorants, I used generalized linear model (GLM) (function "glm" in package lme4) (Bates 2015) with logit link function. The binomial variable infected=1 and non-infected=0 was used as a dependent variable and using stepwise correction protocol (function "stepAIC" from package MASS) (Venables & Ripley 2002) the best fitting model was constructed. Body length, age class (juvenile/adult), sex, maximum wing length and interactions between body length and sex and sex and wing length as well as age class and wing length were left in the final model:

glm(formula = corynosoma ~ length + ageclass + sex + wingmax + length:sex + sex:wingmax + ageclass:wingmax, family = binomial

I chose to use multiple variables describing physical size of the cormorants because it might affect cormorants´ prey type. Length of the intestines was measured from some of the birds, but due to data missing only from the Airisto area I ended up not including it. Including only the birds with measured intestines would have resulted in an even more skewed data as there were already more cormorants from Kustavi.

I used Fisher's exact test (function "fisher.bintest" from package RVAideMemoire) (Hervé 2022) to test the differences in *Corynosoma* prevalence in cormorants between Airisto and Kustavi areas, sexes, and age classes. I used Fisher's exact test due to small sample size.

I used either T-test or Wilcoxon test to test differences in body characteristics between sexes, sampling areas, age classes and between infected and non-infected individuals. T-test was used when sample size was large, variables normally distributed and when variances didn't differ between areas. Wilcoxon test was used when variables weren't normally distributed, or their variances differed.

I used Pearsons' correlation test to test correlation between years and *Corynosoma* prevalence in herring. I used Chi-squared test to test difference in prevalence between the Archipelago Sea and the Bothnian Sea.

3. Results

3.1 Prevalence of *Corynosoma* in the herring

Total of 13.1 % of herring caught from the Archipelago Sea and the Bothnian Sea were infected with Corynosoma. Corynosoma prevalence in herring varied between years and study sites ranging from 3.2 % to 24.1 % (Table 4.)

Table 4. Years, study sites, number of individuals, number of infected individuals and Corynosoma prevalence.

Year	Area	N of individuals	N of infected individuals	Prevalence %
2018	Peimari	249	55	22.1
	Taivassalo	570	111	19.5
	Western Rymättylä	348	84	24.1
	Rymättylä (trawl catch)	635	46	7.2
	Uusikaupunki (Bothnian Sea)	542	82	15.1
	Merikarvia (Bothnian Sea)	986	130	13.2
2017	Airisto	698	134	19.2
	Rymättylä (trawl catch)	175	6	3.4
2016	Airisto	1018	38	3.7
2015	Airisto	1031	151	14.6
2014	Airisto	750	83	11.1

Between the years 2014 and 2018 the prevalence of Corynosoma infection in herring in the Archipelago Sea increased from 11.1 % to 21.4 % (Figure 4). There wasn't statistically significant correlation between the years and prevalence (Pearsson's correlation $r=0.57$, $p=0.32$, $n = 5$) but when the year 2016 with much lower prevalence was left out, there was a significant correlation ($r = 1.0$, $p < 0.05$, $n = 4$).

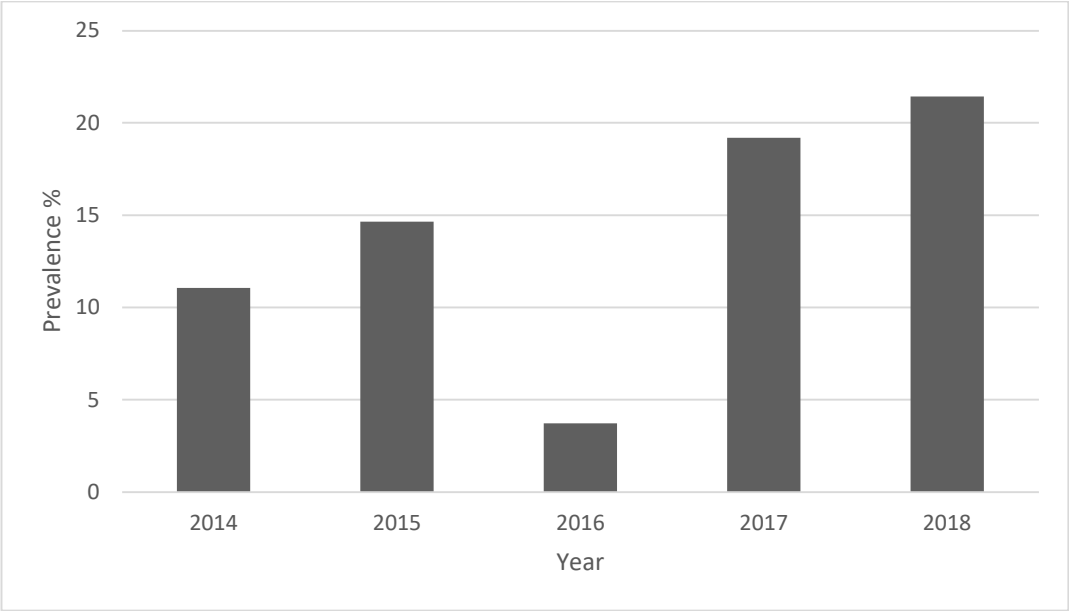

Figure 4. Prevalence (%) of Corynosoma infection in the Baltic herring in the Archipelago Sea in 2014-2018 (total n=7002).

In 2018 when herring samples were collected from both the Bothnian Sea and the Archipelago Sea, fish in the Archipelago Sea were significantly more likely to be infected with Corynosoma parasites (χ2, $\chi^2=26.16$, n=2695, df=1, $p<0.001$). In the Bothnian Sea less than 14 % of herring were infected whereas in the Archipelago Sea more than 20 % had Corynosoma parasites (Figure 5)

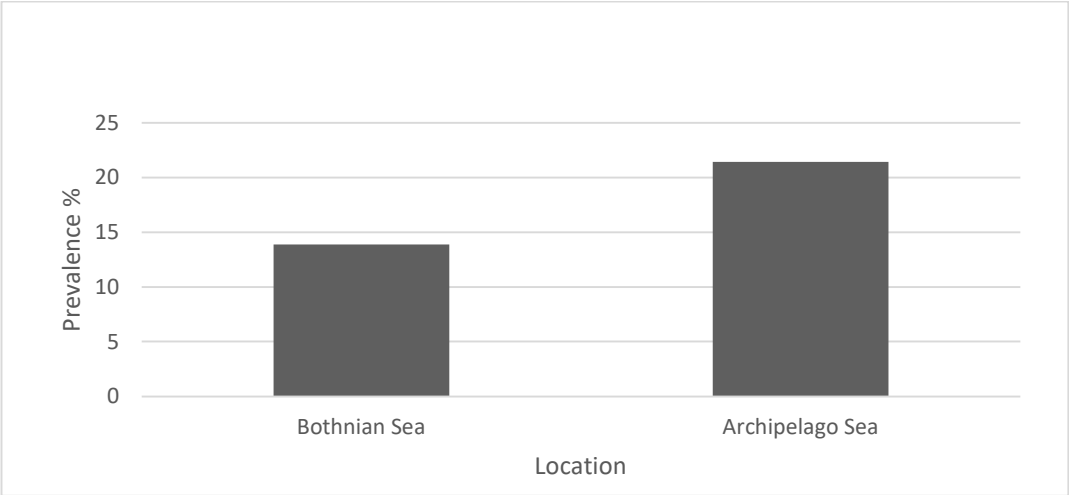

Figure 5. Prevalence (%) of the Corynosoma infection in the Baltic herring in the Bothnian Sea (n = 1528) and the Archipelago Sea in 2018 (n=1167).

Herring in the Archipelago Sea were significantly larger than in the Bothnian Sea (t test, t=10.30, n=2696, p<0.001). In the Archipelago Sea mean length was 16.1cm and in the Bothnian Sea 15.3cm (Figure 6).

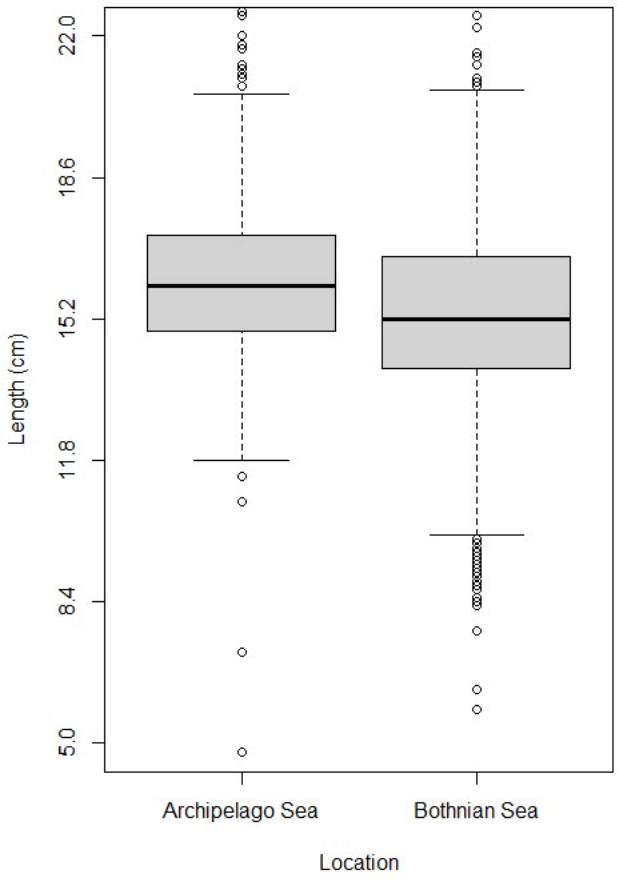

Figure 6. Body length of herring in the Archipelago Sea (n = 1167) and the Bothnian Sea (n = 1528) in 2018. Black line represents median length and grey box middle 50 % of the data.

Infected herring were significantly larger than non-infected (t test, t = -28.818, df = 1301.2, p-value < 0.05) with the mean length of infected individuals being 17.5 cm and non-infected individuals 15.6cm (Figure 7).

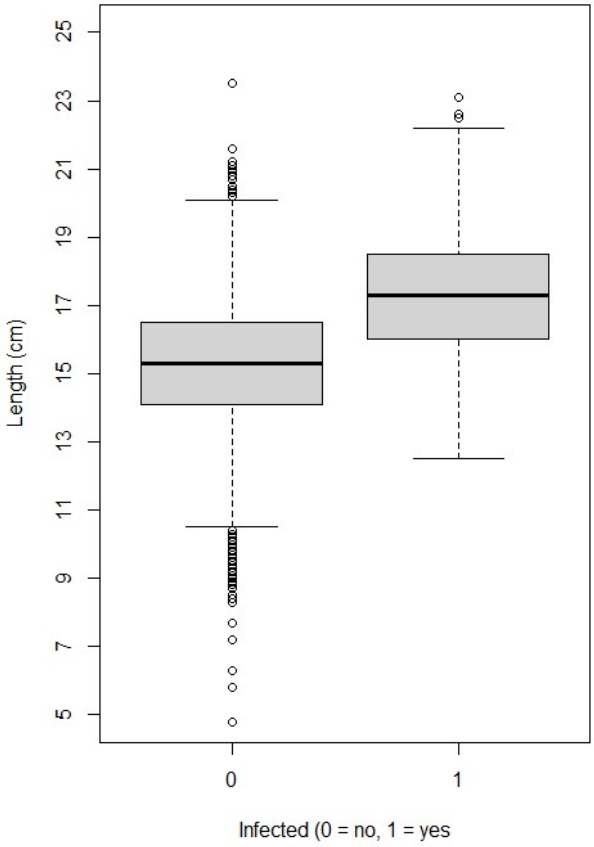

Figure 7. Body lengths of infected and non-infected herring. Black line represents median length. Grey box represents middle 50 % of the data (total n = 7002)

3.2 Prevalence of *Corynosoma* in the cormorants

In total, 26 % of cormorants were infected with Corynosoma parasites. The data indicated that the prevalence was higher in the Aristo area than in Kustavi, but the difference was not statistically significant (Fisher's exact test, n = 65, p = 0.07). 40 % of the cormorants in the Airisto area were infected (17 % in 2018 and 56 % in 2019), whereas in Kustavi, only 16 % of cormorants had *Corynosoma* parasites (Table 5.) The mean intensity of *Corynosoma* parasites varied from 1 in the Airisto are in 2018 to 29.4 in the same area in 2019.

The data indicated that male cormorants were more likely to get *Corynosoma* infection than females, but the difference wasn't statistically significant (Fisher's exact test, n = 65, p = 0.37). 27.3 % of males were infected whereas only 15.6 % of females had an infection.

Result indicate that adult cormorants were more likely to be infected than juveniles though the result isn't statistically significant (Fisher's exact test, n = 65, p = 0.11). Whereas 41.7 % of adult cormorants had *Corynosoma* parasites, only 17.0 % of juveniles had them.

Table 5. Study sites for cormorants and the total number of individuals, number of infected individuals, prevalence of Corynosoma infection and mean intensity.

Year	Area	N of individuals	N of infected individuals	Prevalence %	Mean intensity of parasites
2018	Airisto	6	1	16,7	1
2019	Airisto	9	5	55,6	29.4
2019	Kustavi	50	8	16,0	5.4

The cormorants in the Airisto area were significantly larger in terms of body length (t-test, t = 2.19, df = 63, p-value < 0.05) as well as in terms of body weight (Wilcoxon test, W = 589, df=63, p-value < 0.05) (Figure 8).

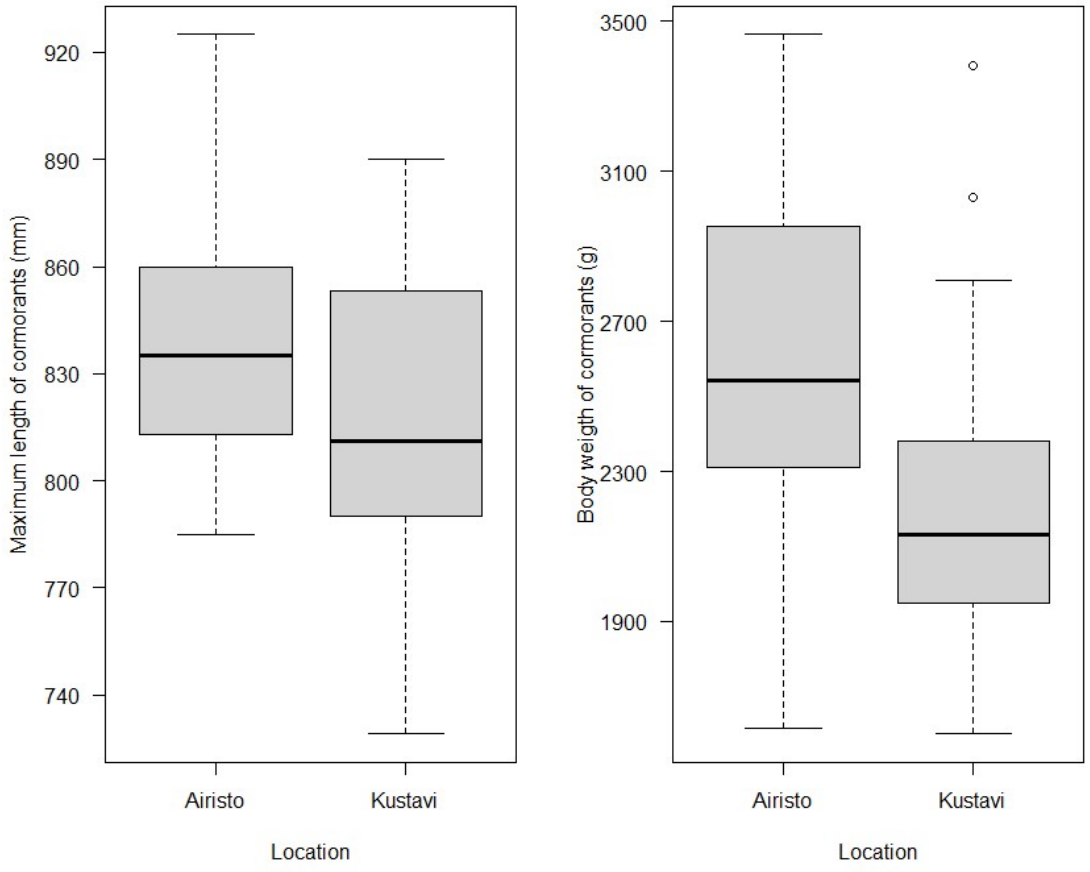

Figure 8. Size differences of cormorants between Kustavi and Airisto in terms of body length and body weight (n = 65). Black line represents median length and weight. Grey box represents middle 50 % of the data (total n = 65)

Male cormorants were larger than females and adults were larger than juveniles (Figure 9). Males were significantly larger in terms of wing length (W = 116, n = 65, p value < 0.05), body length (t = -7.66, df = 62.73, p-value < 0.05) and weight (W = 168, n = 65, p value < 0.05). Adults were significantly larger than juveniles in terms of wing length (Wilcoxon test, W = 504, n = 65, p < 0.05), body length (Wilcoxon test, W = 445, n = 65, p < 0.05) and weight (Wilcoxon test, W = 531, n = 65, p < 0.05)

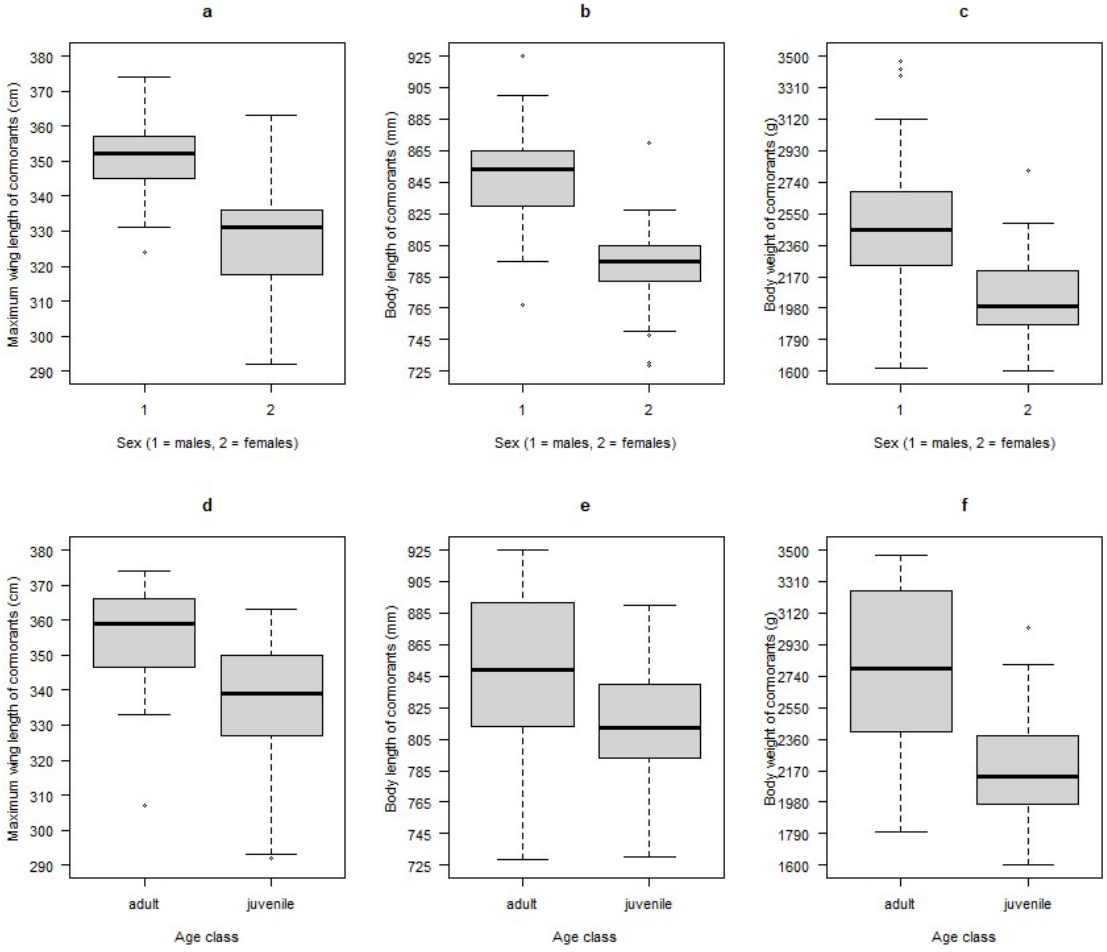

Figure 9. Size differences of cormorants between sexes (a, b, and c) and adults and juveniles (d, e and f). Black line represents median length (a, b, d and e) and median weight (c and f). Grey box represents middle 50 % of the data (total n = 65).

There weren't statistically significant differences between infected and non-infected cormorants in terms of wing length (t = 0.90, df = 18.37, p-value = 0.38) body length (t = 1.79, df

= 22.05, p-value = 0.09) or weight (W = 464, p-value = 0.09) but results indicate that infected individuals were generally larger (Figure 10).

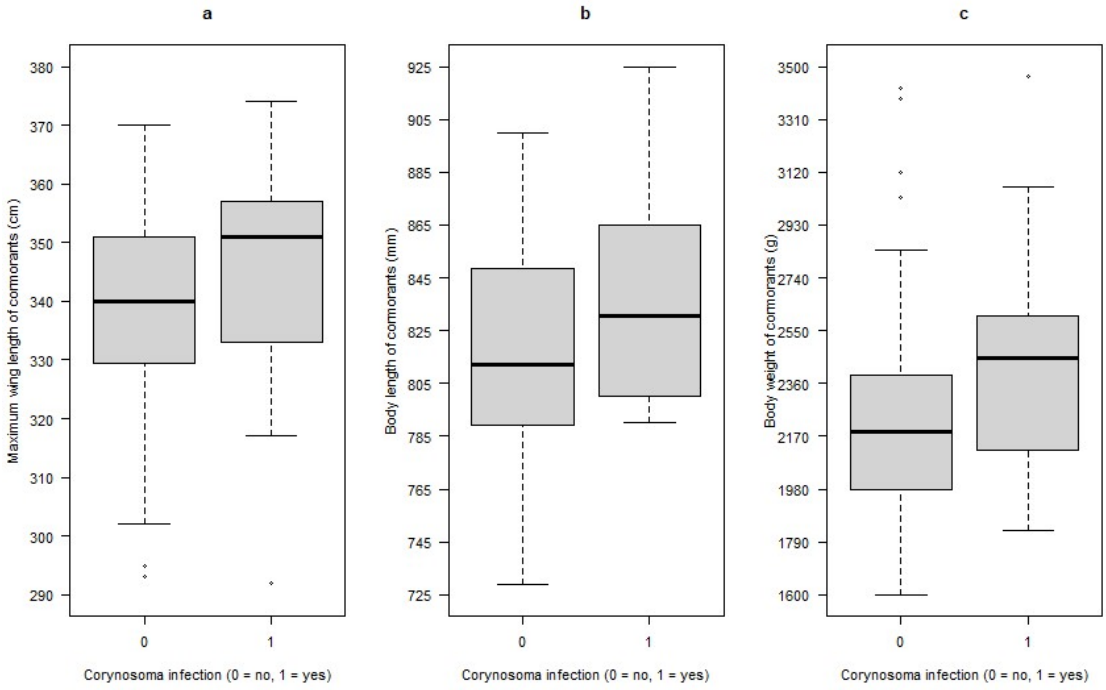

Figure 10. Size differences between infected and non-infected cormorants. Black line represents median length (a and b) and median weight (c). Grey box represents middle 50 % of the data (total n = 65).

According to generalized linear model, female cormorants were significantly less likely to get infected by *Corynosoma* parasites than males (Table 6).

Table 6. GLM output of the fixed effects on infection. Estimates and standard error values, with t- and p- values to fixed effects.

	Estimate ± SE	z-value	p-value
Intercept of full model	56.18 ± 27.8	2.02	<0.04
length: sex (female)	0.11 ± 0.05	2.49	<0.05
sex (female)	-72.74 ± 32.10	-2.27	<0.05
wing length	-0.16 ± 0.09	-1.90	0.06
age class (juvenile)	-56.08 ± 30.11	-1.86	0.06
length	0.00 ± 0.02	0.21	0.83
sex (female): wing length	-0.20 ±0.15	-1.36	0.17
age class (juvenile): wing length	0.15 ± 0.08	1.80	0.07

3.3 DNA sequencing

Total of 124 *Acanthocephalans* from 124 herring individuals collected from the Archipelago Sea (88 individuals) and Bothnian Sea (36 individuals) were sequenced. 121 of those represented species *C. semerme*, two species *C. strumosum* and one species *C. magdaleni* Montreuil (Figure 11). Individuals collected from the Bothnian Sea all represented species C. semerme.

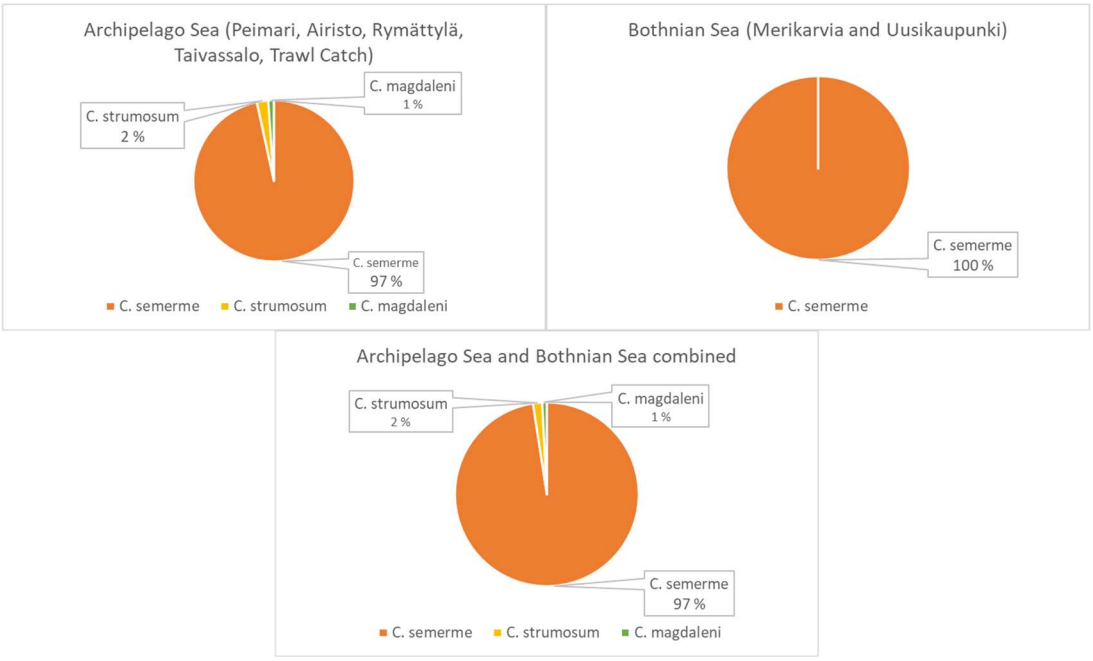

Figure 11. Proportion of different Corynosoma species collected from herring in the Archipelago Sea (n = 88), the Bothnian Sea (n = 36) and both areas combined (n = 124).

Total of 23 *Acanthocephalans* from 14 cormorant individuals were sequenced. All *Acanthocephalans* from cormorants represented species *Corynosoma semerme.*

4. Discussion

4.1 Prevalence of Corynosoma infections

Results show that infected herring individuals are generally larger than non-infected. This can be explained by their feeding habits. Herring feed on zooplankton and small crustaceans (Aneer 1975). Younger and smaller herring primarily feed on zooplankton (Arrhenius & Hansson 1993). When they grow larger, they start to feed on larger prey such as *Monoporeia affinis* which are intermediate hosts for *Corynosoma* and become susceptible to infection. *Monoporeia affinis* is more abundant in deeper waters of the open sea (Kangas 1976; Valtonen 1983a). Seals, the primary hosts of *Corynosoma* are also abundant in the open sea. As Valtonen (1983) suggests, it

is likely that *Monoporeia affinis* in the open sea are more frequently infected. Thus, it is likely that herring get the infection more likely at their winter feeding grounds at the open sea than in the shallow coastal waters. Older, larger herring have migrated to the feeding grounds more often so their higher parasite prevalence can be explained by their migration habits.

Corynosoma parasites don't mature in fish but rather stay in their body cavities as cystacanths. According to Valtonen et al. (2012) they can remain in fish for years. Thus larger, older fish are more likely to have been infected at some point of their life.

Herring in the Bothnian Sea were infected less frequently than herring in the Archipelago Sea. This is probably due to their slightly smaller size as the probability of the infection increases with size. There are mostly like other reasons as well and further studies are needed. There might be spatial variation in *Corynosoma* prevalence in *Monoporeia affinis* for example.

The prevalence of *Corynosoma* in herring increased from 2014 to 2018. Much lower prevalence in 2016 might be due to the research focusing primarily on morphological abnormalities in the gonads and the dissections being performed by different researchers. Thus, small worms might have gone unnoticed. The herring population in the Bothnian Bay increased rapidly from 1981 to 1994 (ICES 2017), then decreasing until 2002 after which it has steadily increased again. The year classes of 2014 and 2015 were extremely large. High population density causes stress and competition for food. As the large year classes grew and started to feed on *Monoporeia affinis*, the parasites became more common and were able to disperse.

The salinity of the Baltic Sea has decreased since 1970s (Hänninen & Vuorinen 2015). According to Boeuf and Pavan (2001) 10 to 50 percent of fishes' energy is used for osmoregulation. Decreasing salinity forces herring to adapt to new conditions and use more energy for osmoregulation. The fat content of the herring has decreased during the last thirty years (Rajasilta et al. 2018). Poor condition makes them more susceptible to infections (Lafferty & Kurtis 1999).

Cormorants nested on the Finnish coast for the first time in 1996 and by 2015 the nesting population was about 24 000 individuals (Ministry of the Environment 2016). At the same time populations of grey seals and ringed seals have also increased rapidly in the 21st century (Natural Resources Institute Finland 2017). Increase in the host populations has probably led to the increase of *Corynosoma* parasites as well.

Result show that male cormorants were larger than females and adults were larger than juveniles. Previous studies support this (Koffijberg & Van Eerden 1995). Results also suggests that males as well as adults were more likely to be infected with *Corynosoma* parasites which

may be due to their larger size. Previous studies have shown that male cormorants eat larger fish than females (Liordos & Goutner 2009). This might explain why males were more prone to *Corynosoma* infections because in most fish species larger fish tend to have *Corynosoma* parasites more often than smaller ones (Valtonen 1983a).

Cormorants in the Airisto area were infected more frequently than in Kustavi. Cormorants that were shot in Kustavi were smaller and there were more juveniles, because they were shot close to a breeding colony. This might explain the lower *Corynosoma* prevalence as juveniles have had less time to get infected and smaller cormorants eat smaller fish, which have less parasites. Kustavi is also situated closer to the Bothnian Sea where *Corynosoma* prevalence in herring was lower. The intensity of parasites was generally low (1-11 individuals per infected cormorant) but in one cormorant there were more than 121 *Corynosoma* parasites. That explains much higher mean intensity in 2019 in the Airisto area, where only 9 cormorants were shot that year.

Cormorants are also migratory birds overwintering in the southern, central, and western Europe (Lehikoinen 2003). Adult cormorants have already migrated during previous winter whereas juveniles born just months prior to being shot have not. Previous studies (Ossmann 2008) have shown that cormorants in Germany have *Corynosoma* parasites as well. It is possible that adults have been infected during their migration. However, since juveniles had *Corynosoma* infections as well the results suggest that it's not linked to migration.

Because cormorants are protected species and collecting samples was subject to special permit, the number of individuals available for this study was low. Only 65 individuals were used, and juveniles were overrepresented in the sample from Kustavi since most individuals were shot from a fish farm situated close to a breeding colony. On the other hand, in the Airisto area cormorants were shot farther from the breeding colony and adults were overrepresented. This makes it hard to reliably compare samples. Due to skewed data, outliers, small sample size and multiple variables GLM model wasn't very well fitted. Thus, tests like t-tests and Wilcoxon tests were performed to find some trends.

The most abundant *Corynosoma* species in both the Baltic herring and cormorants was *Corynosoma semerme* whereas only few individuals of *C. strumosum* and only one *C. magdaleni* were found. Previous studies have shown similar results (Sinisalo & Valtonen 2003). In their study the proportion of *C. semerme* was highest of all *Corynosoma* species in fourhorn sculpin (*Myoxocephalus quadricornis*) in the Bothnian Bay being 98.3 %. Cormorants' diet consists of variety of fish (Koffijberg & Van Eerden 1995; Finnish Environmental Institute 2018). Thus, it is logical that the dominant *Corynosoma* species in their prey is also dominant in the predators. The dominance of *C. semerme* has been observed in seals as well (Valtonen 1982).

Even though *Corynosoma* parasites are not harmful to humans, high number of parasites may affect the public opinion and decrease the value of fish in the eyes of the consumers. Because herring is a very important species for commercial fishing, it is important to monitor the abundance of parasites. Prior studies have shown that *Corynosoma* parasites can be found in other commercially important fish species such as cod *(Gadus morhua* Linnaeus*)* as well (Valtonen 1983a). Including more species to future studies is recommended.

4.2 Potential use of Corynosoma as a biological tag in herring studies

In the Baltic Sea, the herring stock size is assessed annually to set the catch quota. The herring stocks are managed by statistical sub-units (SD) defined by the ICES (International Council for the Exploration of the Sea). At present, the assessment protocol is based on the assumption that fish born within given SD-borderlines also recruit to the adult stock of the same SD unit and later form the stock to be fished. In the northern Baltic, herring studies and stock estimates made today mostly rely on the sampling performed on the open sea, where the stock consists of both juvenile and adult herring originating from different spawning grounds. The sampling on the spawning and nursery grounds, in turn, don't tell anything about the future environment of the larvae and young fish, after they have moved to the feeding grounds in the open sea (Rajasilta pers.com. March 2022) One possible way to identify stocks is to use parasites as biological tags or markers (Williams et al. 1992). In some cases, biological tags may be better than artificial tags, since they do not alter the natural behavior of the study species and artificial tagging is not suitable for some delicate species such as deep-water fish that have high mortality rate when being trawled. Catching fish for parasitological examination is also more cost efficient than artificial tagging. Prior studies have shown that parasites can be successfully used as biological tags for stock identification (Sherman & Wise 1961, Hislop & McKenzie 1976) and as indicators of migration (Williams et al. 1992). According to Sindermann (1983) parasites work best as natural tags when their prevalence or intensity of infections differ between areas and when they can be reliably identified.

The results of this study indicate thar *Corynosoma semereme* is the most abundant acanthocephalan species both in the Archipelago Sea and the Bothnian Sea. In the Archipelago Sea two *C. strumosum* and one *C. magdaleni* individuals were found as well, but due to rather small sample size from the Bothnian Sea it's likely that both species could be found there as well. Prior studies have also found all three species from samples collected from the Gulf of Bothnia (Nickol et al. 2002). More extensive sampling from different areas could show some differences

in species composition as there is evidence that geographical distributions of *Corynosoma* species differ (Leidenberger et al 2020). *C. magdaleni* is limited to Northern Atlantic coasts, *C. semerme* has a circumpolar distribution and *C. strumosum* has the broadest distributional range extending further south.

4.3 Conclusion

This study suggests that the probability of a *Corynosoma* infection increases with host size in both the herring and the cormorants. This is probably due to the feeding habits as larger herring feed on the *Monoporeia affinis*, the intermediate hosts of *Corynosoma* and larger cormorants feed on larger fish.

Future studies are required for more reliable results. I suggest a greater number of cormorants to be included in coming studies as that would result in more reliable models. There are also other fish-eating birds such as goosander *(Mergus merganser)* and seagulls which may have *Corynosoma* infections as well. I suggest those to be included in the future, too.

As the changing environment keeps affecting the future of herring, it is important to keep monitoring how the prevalence and the intensity of *Corynosoma* infections keep developing as it is one of the key species in the Baltic Sea.

5. Acknowledgments

This study was funded by the Finnish operational program of European Maritime and Fisheries Fund and The Fishery Leader group of the Archipelago Sea (project number 58254), the Southwest Finland Centre for Economic Development, Transport, and the Environment and by the Rotary Club Nauvo. The study has utilized research infrastructure facilities provided by FINMARI (Finnish Marine Research Infrastructure network).

I would like to express my deepest gratitude to the staff of Zoological Museum in the University of Turku for helping me whenever I needed help or guidance. Especially I'd like to thank the staff of the Archipelago Research Institute and my supervisors Jari Hänninen and Katja Mäkinen as well as Marjut Rajasilta for their endless support and patience with this thesis and for guiding me at the beginning of my career.

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