Long term changes in sex ratio and reproductive effort in the Baltic herring (*Clupea harengus membras*) in the south-western archipelago of Finland

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Abstract

Baltic herring (*Clupea harengus membras*) is a pelagic fish species that belongs to the pelagic food webs as a predator and as a prey. Herring is a key species in Baltic Sea ecosystem and a valuable fish for the fish industry around the Baltic Sea area. Because of this, monitoring of the herring is necessary for managing the fisheries and to see how the environmental changes affect the herring populations and the Baltic Sea.

In the present study, the aim was to examine the long-term developments in sex ratio and reproductive effort of males in the spawning shoals. The study material has been collected annually from the spawning sites of herring in the Airisto Inlet in 1984-2014. The data set includes individual measurements of about 38 000 herring and 1-38 samples taken randomly from the commercial trap net catches throughout the main spawning season (May –July). The sex ratio (Nr males/Nr females) in the spawning shoals was examined with *Generalized Linear Mixed Models* (GLIMMIX) for the annual trends and to find out the factors possibly affecting the sex ratio in the shoals (sampling procedure, environmental and stock variables). Gonadosomatic index (GSI), which was used as an indicator of the reproductive effort, was also examined with GLIMMIX, to provide additional information on the reproductive capacity of the population.

The results of the study indicate clear changes in the sex ratio of the spawning herring population. Firstly, the population is changing towards a female dominance. Secondly, the reproductive effort (gonadosomatic index) of the herring has decreased in males for which the change has been significant in all age classes. It seems that the present environmental conditions in the Baltic Sea and the ongoing climate change affect more on males than in females. This may change the population structure so that there are much more females than males in the population. If this trend continues for a long time, there is a risk for decrease in the population size in the future because the reproduction effort decreases.

KEY WORDS: sex ratio, gonadosomatic index, Baltic herring, *Clupea harengus membras*, Archipelago Sea, Baltic Sea

Contents

1. Introduction

The Baltic Sea is a shallow brackish-water basin, which is connected to the Atlantic Ocean in Danish straits. The fauna and flora of the Baltic Sea is a mixture of marine, freshwater and brackish water species, which are low in number, but which can have dense populations (HELCOM 2010). Herring (*Clupea harengus membras*) is one of the most abundant fish species in the Baltic Sea, which lives and reproduces in almost the entire sea area except for areas which have low salinity (<2-3 psu). This indicates a wide salinity tolerance of the species.

The Baltic Sea is considered as one of the most polluted sea areas in the world (HELCOM 2010). Another major concern in the Baltic Sea is eutrophication, which has increased the oxygen depletion in the Baltic Sea, especially in the deepest basins (HELCOM 2009) and caused massive blooms of blue-green algae, which have negative and unwanted effects in the ecosystem. After the major inflow of seawater from the Atlantic Ocean in 1976 (Matthäus 1993), vast hydrographic changes have occurred in the Baltic Sea. Reduced inflow of oceanic water together with increased precipitation and freshwater run-off from rivers have reduced the salinity of the water (Hänninen et al. 2000), and temperature of sea water has increased in all seasons (BACC 2013). Consequently, vast changes in the ecosystem took place at the end of the 1980s, known as the regime shift (Dippner et al. 2010). This was characterized by a decline of the abundances of marine fish and zooplankton species, and by a change in the predator-prey interactions in the pelagic ecosystem (HELCOM 2009b) In the Baltic herring, the regime shift resulted in a decrease of the growth rate and fish condition (Möllmann et al. 2004), and in large variations of the stock size (HELCOM 2013).

In the Archipelago Sea (Fig. 1), the characteristics of spawning herring have been monitored since 1984 with annual samplings from the spawning grounds (e.g. Rajasilta et al. 1999). As shown by the tagging experiments by Parmanne et al. (1990) and Kääriä et al. (2001), the herring spawning in this area belong mainly to the Bothnian Sea stock, which migrates to reproduce in the innermost parts of the south-western archipelago. Spawning fish have been analyzed every year by the same methods, but the intensity of sampling has varied between the years, depending on the resources available for the study.

There are some observed changes in the herring population from 1984 to 2015: the reduction of fish growth has led to a reduced body size (Rajasilta et al. 2015a), and the samples have also indicated an increasing number of gonadal abnormalities in herring during recent years (Rajasilta et al. 2015b). They are not evenly found from females and males, but slightly but significantly more from males. The preliminary findings also suggest that the sex ratio in the spawning population has not been stable, but varied strongly from year to year. As the state of the Baltic Sea has changed during the last 30 years, it is possible that females and males have responded to the changing environment in different ways (Rajasilta et al. 2015b). The reproductive effort of herring females, expressed as the gonadosomatic index (GSI), has remained stable in 1987-2002 (Rajasilta et al. 2015a). In males, it has not been studied, but the time-series on GSI (1987-2014) allows a more comprehensive examination of the potential change.

The aim of the present study was to examine:

a) Is the trend of the sex ratio during 1984-2015 statistically significant? If so, can the explanatory factors be found from the sampling procedure, from the environmental factors influencing the herring during the study years, or from the characteristics of the population?

b) Has the reproductive effort (GSI) of herring males changed? Is the change similar with both sexes?

According to the literature, the sex ratio in fish populations can vary for several reasons, starting from the possibility that females and males are born at different ratios (Wootton et al. 2015). Therefore, I first explore the mechanisms of sex determination in teleost fish in a literature review; then go through the life-cycle of the Baltic herring, and finally, analyze the sex ratio and GSI in the spawning population using Generalized Linear Mixed Models

(GLIMMIX), which are widely used for examination of time-series data (Hänninen et al. 2015).

2. Hypotheses of the study

2.1. Determination of sex in teleost fish

In teleost fish species, determination of sex can be divided into two main groups, (1) genotypic sex determination (GSD), in which the gender is determined at fertilization, and genetic differences between females and males can be observed, and (2) environmental sex determination (ESD), in which sex is determined after fertilization by the effect of environmental conditions such as temperature, pH, salinity, rate of growth and social environment.

Genotypic sex determination can be divided into three different systems. (1) In monogenic system, sex is determined by a gene located in a certain chromosome and genes in other chromosomes have little effect (Devlin and Nagahama 2002). Most of the fish species do not have morphologically differentiated sex chromosomes. (2) In multifactorial system, sex is determined by interactions between a gene or genes and the environment. (3) In polygenic systems, sex is determined with more than one gene, so that each gene provides a small, but additive effect to the result (Bull 1985). If there are signs of sex chromosomes in fish, the GSD is then considered to be the sex-determining factor rather than the ESD.

In fish, determination and differentiation of sex are different phenomena. According to Hayes' (1998), "sexual determination may be defined as the genetic or environmental processes that influence the definition of sex, while sexual differentiation refers to gonadal development once sex has been determined, i.e., if the undifferentiated or bipotential gonad follows the ovarian or testicular differentiation pathway". Worldwide there are about 24 000 different species of fish that live in different kind of habitats. Because fish are adaptable and plastic in their reproduction, they show a vast variety of mechanisms of sexual determination and patterns of sexual differentiation (Nelson 1994). To sum up, determination of the sex in teleost fish is complex and rather difficult to investigate, because the mechanisms behind it are species-specific and regulated by different factors; not just by one gene or one environmental factor (Table 1).

Table 1. Examples of environmental variables affecting sex determination in fish at different developmental stages. Ad.= adult; juv.= juvenile

Variable	Species	State	References
Growth			
	Anchoa Januaria	Ad. and juv.	Santos et al. (2006)
	Danio rerio	Juv.	Lawrence (2007)
	Rutilus rutilus	Egg and juv.	Paul et al. (2009)
Temperature			
	Apistogrammus spp.	Egg and Juv.	Römer et al. (1996)
	Danio rerio	Egg.	Abozaid et al. (2012)
	Oncorhynchus mykiss	Larvae.	Magerhans et al. (2009)
	Dicentrarchus labrax	Larvae.	Mylonas et al. (2005)
Water acidity			
	Pelvicachromis pulcher	Egg.	Rubin et al. (1985)
Density, social influence	*Paralychthys lethostegma*	Egg.	Luckenbach et al. (2003)
	Ichthyomon gagei	Juv.	Beamish (1993)
	Centropristis striata	Ad.	Benton et al. (2006)
Predation			
	Poecilia reticulata	Ad.	McKellar et al. (2011)
Time			
	Gnathopogon caerulescens	Egg.	Fujioka et al. (2015)

In a spawning fish population, sex ratio may be distorted for different reasons. For example:

a) Females and males are born at different ratios (and their mortality is constant and equal after sex is determined);

b) Females and males are born at equal ratios (but mortality is different among the sexes)

c) In mature fish, synchronization of spawning between the sexes is disturbed in the population.

d) Fish change their sex before or during the reproductive cycle.

In the Baltic herring, mechanisms of sex determination are unknown and, there are no results on the natural mortality of the species. Only the fishing mortality is known (Suuronen et al. 1996) and most likely, fishery doesn't select differently males and females, as their size and morphology is similar (Parmanne 1990). Sex reversal is known in many teleost fish species. Fish can change the sex due to a many reasons such as human activities, changing environment, population parameters and parental care of the offspring. This is done to make the individual survive and reproduce as well as possible, except if the reversal is due to a human activity (Wootton et al. 2015). Exact mechanisms of the sex reversal are not fully understood.

The assumption is that herring population follow's the Fisher's principle that is an evolutionary model explaining why the sex ratio of most species that produce offspring through sexual reproduction is approximately 1:1 (Fisher 1930).

Even if the sex ratio at birth is the 1:1 ratio, it is possible for either males or females to skip spawning because of the poor feeding conditions. Fish having low energy reserves and poor somatic condition cannot produce eggs or sperm every year for the spawning. Skipped spawning may cause cyclicality in the sex ratio.

2.2. Reproductive cycle of the Baltic herring

Herring spawns usually for the first time when it is 2-3 years old with an average of 13 to 15 centimeters in length and about 16 to 20 grams of weight. During the feeding period, fish collect energy resources for the next spawning. Feeding starts soon after the spawning is over and fish are migrating to the feeding grounds in the open sea. In summer, herring's main diet consists of copepods, such as *Limnocalanus macrurus*, *Eurytemora affinis*, and *Acartia bifilosa* (Flinkman et al. 1992), while during autumn and spring it eats macroscopic crustaceans such as mysids (Gorokheva et al 2004; Rajasilta et al. 2014; Rajasilta, pers.comm.). Summer and autumn are the main feeding time when fish gain their energy resources for upcoming spawning (Figure 1). The hardness of the winter and duration of the ice cover have a great influence on the herring reproductive cycle, especially on the timing of spawning (Rajasilta 1992).

Nutritional status and temperature of the water determine the starting point of the spawning (Rajasilta 1992). When there is sufficient amount of food available for the fish in the feeding areas they get ready for the spawning. If overwintering is successful it is possible for herring to spawn and reproduce every year. Females that are in good condition and have a high fat content in their muscle tissues are normally the individuals that produce more offspring than those in poor condition (Laine et al. 1999). High fat content in the muscle also tells about a successful dietary intake (Laine et al. 1999). When environmental conditions are unfavorable for reproduction, the female can reabsorb the eggs, or even skip the spawning. Although the amount of produced offspring is then reduced the female saves its own energy resources for the next spawning, when the feeding and reproduction conditions may be better (Kjesbu et al 1991; Kurita et al 2003).

In females, the maturation from oocytes to ripe egg cells takes approximately 8–10 months (Hagstrom Bucholtz et al. 2013). Then the fully developed egg cells are ovulated and released on to the substrate. Maturation of the egg cells starts for the first time when the juvenile herring reaches sexual maturation at about 1.5 years of age (Hagstrom Bucholtz et al. 2013). Research on males has been done less than on females, but most likely, the maturation and development of sperm cells follow the same pattern as that of the female oocytes. The development of fertilized egg cells to hatching lasts from several days up to weeks, mainly depending on the incubation temperature (Laine 1999).

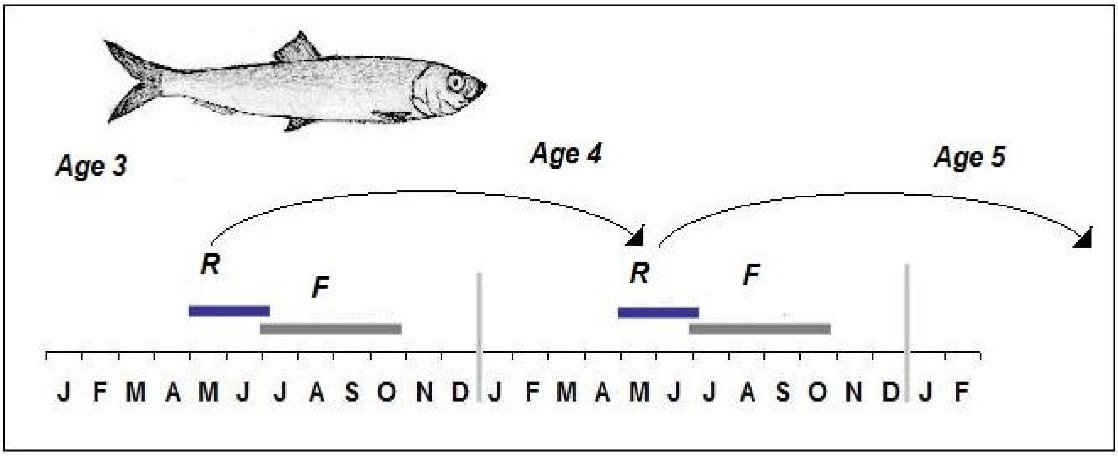


Fig. 1. The reproductive cycle of the herring in the Archipelago Sea. R= Reproduction period, F=Feeding period. (Figure: Herring project/Archipelago Research Institute).

2.3. Spawning

In the Archipelago Sea and the Gulf of Bothnia, most of the herring spawn during May and June. Start of the spawning is related to the latitudinal zones, so that it starts earlier in south and later in north. Large schools of herring arrive in the vicinity of the mainland a few days or weeks before the start of the spawning (Kääriä et al. 1996). In the Airisto area, spawning can last from April to the end of July, forming a continuum due to difference in the maturation rate of the individuals in the population (Rajasilta 1992).

Herring spawn on the coast, in vicinity of the mainland or in island regions, but not on the open sea areas. In Airisto, there are deep fracture–lines on the sea bed, which go through the area in the south–north direction. Majority of the herring's spawning grounds are located near these fracture lines (Kääriä et al. 1997). Studies also show that herrings migrate to the spawning grounds using these deep areas. This phenomenon is repeated every year and tagging experiments suggest that herring has a strong tendency for homing; i.e. they come every year to spawn in the same areas as in the previous years (Parmanne, 1990; Kääriä, 1997).

Baltic herring is a littoral spawner. Spawning depth is related to the spawning time. In Airisto, first shoals usually spawn in May at the depth of 0.5–3 m and later spawning shoals somewhat deeper at the depth of 1–7 m (Rajasilta et al. 1993). Herring prefers to spawn on aquatic plants growing on hard rock, stone or gravel bottoms (Rajasilta et al. 1993). On the herring spawning grounds, the vegetation consists of the green and brown algae (*Cladophora, Pilayella, Ectocarpus, Fucus*), red algae (*Furcellaria* and *Phyllophora*), as well as freshwater vascular plants (*Potamogeton*, *Myriophyllum, Ranunculus*, and *Zannichellia*) (Kääriä et al. 1996).

In Airisto, water temperature at the onset of spawning varies between 4-18 ºC, depending on the spawning time (Rajasilta et al. 1993). Herring is capable of producing offspring in rather low salinity, fertilization of the egg cells decreases if salinity is below 7-8 psu. Optimal salinity in spawning grounds for Baltic herring is somewhat 8 psu (Griffin et al. 1998).

2.4. Egg and larval phase

Shoals coming to spawn consist of different sizes and ages of fish. The early spawning herrings have bigger egg size than the later spawning herring (Rajasilta et al. 1993; Laine & Rajasilta 1998). The mortality rate of eggs on the spawning substrate increases during the summer. For example, the studies made in the 1980s showed that the average mortality rate of eggs in May was approximately 10 % and in June approximately 30 % (Rajasilta et al. 1993). Egg mortality depends partly on the plant species which the eggs are attached to. Field observations tell that the mortality rate is the lowest on pondweed (*Potamogeton pectinatus*) and the highest on red algae (e.g. *Furcellaria*). Red algae have also been shown to increase the mortality in eggs in experimental conditions; most likely the algae excrete some harmful compound (Rajasilta et al. 2006). Egg mortality is also caused by predators such as perch (*Perca fluviatilis*) which is one of the most efficient predators of the herring roe. In addition, eggs are also eaten by ruffe (*Gymnocephalus cernua*) and eelpout (*Zoarces viviparus*) (Rajasilta et al. 1993).

When herring larvae hatch approximately at the length of 6-7 mm they are translucent and very thin. Initially, first the larvae live with the energy that they get from the yolk sac. When the energy from the yolk sac is consumed, they begin to look for food and they eat everything that they can catch, mainly zooplankton (Rajasilta et al. 1992). The water temperature, the amount of available food resources, predation and many other environmental factors regulate the survival of the herring larvae after hatching (Hakala et al 2003; Peck ym.2012).

The larvae hatch in the littoral zone but soon after hatching they drift to pelagial waters where they grow a couple of weeks before returning to the shallow waters. When they reach the length of approximately 30 mm they go through metamorphosis and move back to pelagic waters (Urho & Hilden 1990). Very little is known about the herring juveniles at and after this phase. The consensus is that most of the juveniles spend the first winter near the spawning areas, although some of the juveniles are found in the outer archipelago in autumn and winter.

2.5. To grow or to reproduce

During the past 30 years, the reproductive features and growth rate of the Baltic herring have changed (Parmanne et al., 1994; Rönkkönen et al., 2004; Möllmann et al., 2005). Due to declining salinity and changes of the food webs of the Baltic Sea. Abundance of the large marine zooplankton species, like *Pseudocalanus sp.* has decreased and smaller-sized species like *Acartia spp.* and *Temora sp.* have increased (Möllmann et al 2004). At the same time, also the stock of the sprat (*Sprattus sprattus*) has increased, which together with the changes in zooplankton communities (Möllmann et al. 2004, 2005) affected the growth of the herring. Casini et al. (2006) concluded that the ultimate cause of the growth reduction is food competition, which is caused by the sprat in the central Baltic, and in the northern Baltic Sea by the herring stock itself (Lindegren et al. 2011).

Study of Rajasilta et al. (2015) shows that the reduction of the herring body size is strongly linked with reproduction, which is also a key life-history trait. Long-term data tells that the growth has decreased because fish channel their resources into reproduction, instead of increasing their body size. In years when feeding conditions were poor, the muscle tissue of the fish served as an energy source for reproduction, and due to this, fish were thin and had a low protein and fat content in the muscle tissue.

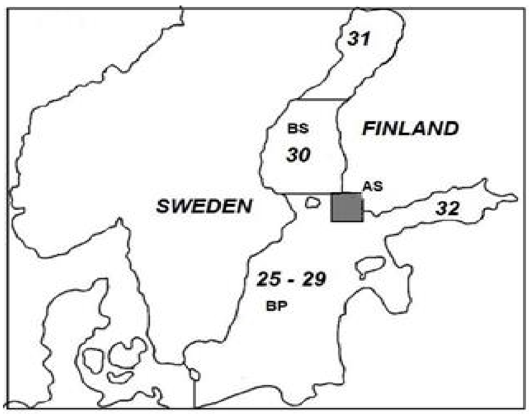
When salinity is low and fluctuates irregularly in the Baltic Sea, there is a possibility for the herring to allocate resources from somatic growth to reproduction and for this reason herring can distribute their reproductive effort for many years. Because of this, fish become smaller but still their gonadosomatic index (reproductive effort relative to body size) remains stable (Rajasilta et al. 2015). A long-term reduction in the size of the fish can be a sign of the herring population developing towards dwarf forms, normally this kind of development can be found in the freshwater species, like percids, salmonids and coregonids (Parker & Johnson 1991; Ridgway & Chapleau, 1994). Study of Ylikarjula et al. 1999 tells that dwarf populations of fish can be found from environments where there is very limited amount of food. In the Baltic Sea, this kind of environment is found when the salinity is low and the marine species cannot thrive. Due to the changing climate, this kind of phenomenon can occur anywhere and not just in the Baltic Sea ecosystems.

3. Materials and methods

3.1. Herring of the study area

In the Archipelago Sea, there are three medium-sized rivers, Aurajoki, Mynäjoki and Paimionjoki which form bays and estuaries where herring spawns and which are important as nursery grounds for larval herring (Fig. 2 a, b). Airisto and Mynälahti offer plenty of suitable spawning grounds, which produce the highest numbers of larvae on the Finnish coast (Parmanne 2001).

a)



(b)

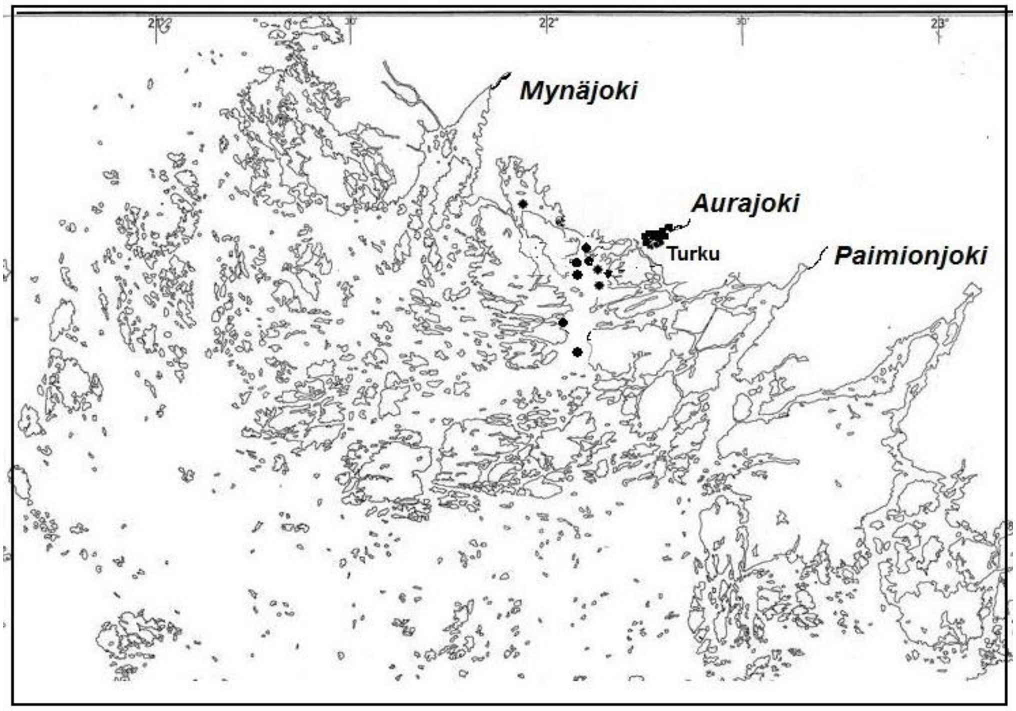


Fig. 2. (a) The Baltic Sea with its subareas (AS=Archipelago Sea, BS= Bothnian Sea and BP= Baltic Proper); the numbers indicate the subdivisions used in the stock assessment of the herring by the ICES (b) Fig. 3. The Archipelago Sea with its three main rivers (Mynäjoki, Aurajoki and Paimionjoki); black circles show the location of the trap nets used for herring sampling in 1984-2015.

3.2. Airisto

The Finnish Archipelago Sea is the most island-rich area in the northern Baltic Sea. The study area, Airisto Inlet is located in the southwest of Finland near the city of Turku (Fig. 2 b) and it is a part of the Archipelago Sea. Airisto is fairly shallow as is the entire Archipelago Sea. Mean depth is about 20 m while maximum depth in the deepest parts of the area is about 90 m. Salinity in the area varies currently between 4-6 psu throughout the water column (Fig.6). Airisto being free from the ice cover the temperature of the surface water varies between 0 and 20 °C. Permanent ice cover in the Baltic Sea varies yearly in between 49 000 and 405 000 km^2 (Fig.6). Highest temperatures in the area are usually found in August. Long term changes in surface water temperature in Airisto area can be seen in the figure 5.

All rivers discharging into the Archipelago Sea affect the water quality in the Airisto Inlet due to water circulation, but river Aurajoki contributes to it most, with the mean flow of 7.9 m3/s. Those rivers discharge a lot of water which is rich in nutrients, especially nitrogen (N) and phosphorus (P). Airisto area is to some extent eutrophicated, (Pitkänen 1986) due to industrial and communal waste waters and a heavy agriculture in its catchment area.

Airisto has a large littoral area with characteristic vegetation, which consists mainly of macroscopic algae (Fucus, Cladophora, Enteromorpha, Ectocarpus and Pilayella and common vascular plants (Potamogeton, Ranunculus and Zostera) (Mäkinen et al. 1984). A typical feature for the shores of Airisto is that they are rocky or stony, however sandy and clay shores can also be found. According to Heino (1973), Ancylus clay covers the bottoms of the Airisto basin.

According to tagging's, the herring populations that spawn in the Archipelago Sea belong to two main stocks, which have their feeding and overwintering areas either in the northern Baltic Proper or in the Bothnian Sea (Parmanne 1990). The populations reproducing in the estuaries of Mynäjoki and Aurajoki rivers mainly feed and overwinter in the Bothnian Sea (Parmanne 1990, Kääriä et al. 2001) During the past 30 years, the stocks have developed in opposite ways: in the Bothnian Sea (=ICES subdivision 30), the spawning stock has increased, while in the Baltic Proper (=ICES subdivisions 25-29 & 32) it has decreased (Fig. 4).

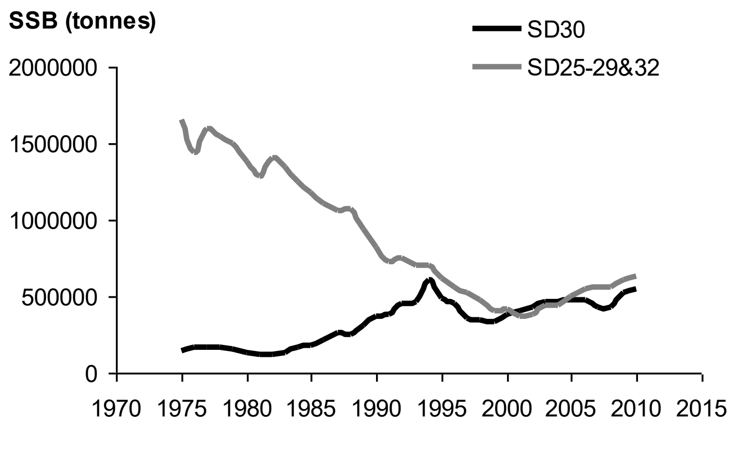


Fig. 4. The spawning stock biomass of the Baltic herring (SSB in tons) in the Bothnian Sea (SD30) and in the Baltic Proper (SD25-29 & 32) during 1975-2011 (source: ICES)

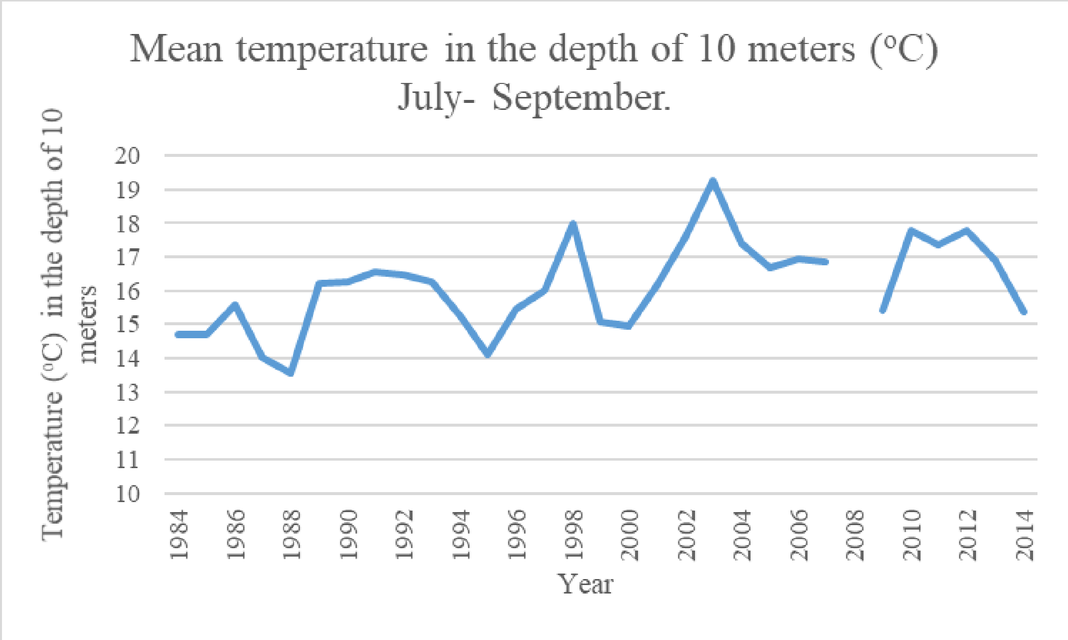


Fig. 5. Long term changes in surface water temperature in Airisto area. 2008 was not included in the dataset, because the project was not running in that year.

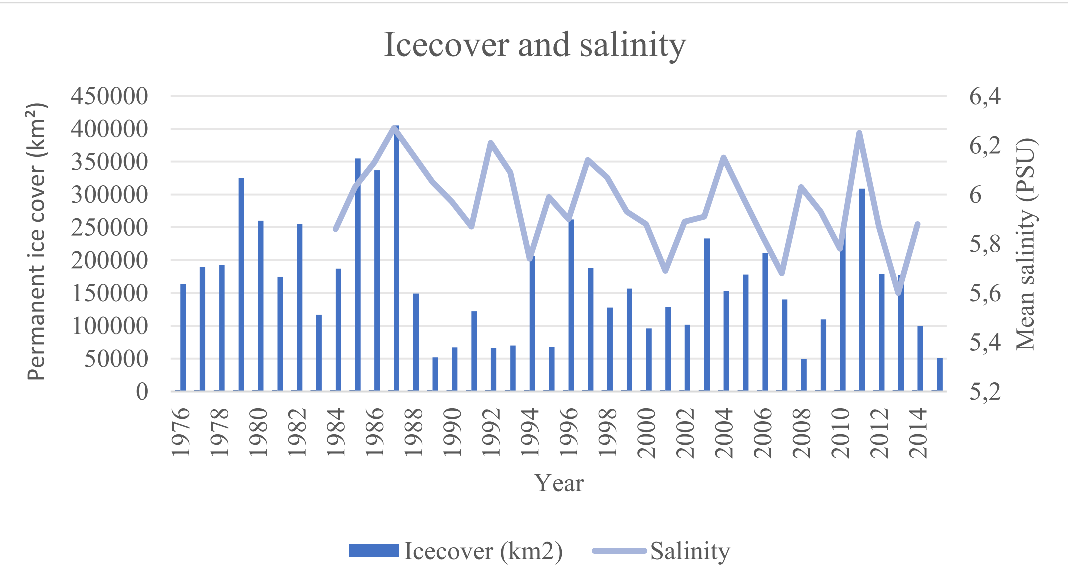


Fig. 6. Permanent ice cover in the Baltic Sea varies in between 49 000 and 405 000 km². Salinity in the area varies currently between 4-6 psu throughout the water column.

3.3. Sampling and treatment of fish

The data set analysed in the present study includes individual measurements of about 38 000 herring which were collected from the Airisto area in 1984–2015 (Fig. 2 b). The samples were taken exclusively by using trap–nets, which have a mesh size of 12-13 mm (knot-to-knot measure). These trap nets can be considered as non-selective fishing gear because they catch herring of all sizes, sex and reproductive stages present in the spawning shoals. Even small herrings (6-7 cm in length) are sometimes found in the trap nets.

From the beginning of the project in 1984 to 1999, the samples have been taken by the project personnel in all study years, when the trap-net catch was small, but if it was large then the fishermen helped in sampling or took the samples, according to orders given, before they emptied the gear. From 2000 onwards, the samples have been collected annually by the same fisherman, who has stored them at -20 ºC. After that they have been exported to the Archipelago Research Institute in Seili for treatment and analyses.

In sampling, the aim has been to cover the whole spawning season, but this has not always been successful. The sampling depends on when the fishermen set their trap-nets in to the sea, and on how long the fishery continues each season. Due to this, the length of the sampling period varies from 17 days to 91 days in the dataset. In some years, sampling has been more frequent than in others and therefore, the number of samples also varies from 1 to 38 per year, graphs about the day of the year and number of samples can be found from the appendices (Table 2 and appendix 1). Table of all measured variables can be found from the appendixes (appendix 2).

Table 2. Yearly information about the data and sampling from 1984 to 2014.

DATA					SAMPLING				
Year	SexRatio	Nr of males	Nr of females	All	DOY1	DOY2	Period (days)	TotNr Fish	Nr of samples
1984	1,04	361	347	708	39	103	63	708	7
1985	1,10	518	468	986	44	94	50	986	9
1986	1,06	256	242	498	35	79	43	498	5
1987	1,19	1439	1208	2647	38	107	68	2647	18
1988	1,17	722	615	1337	40	97	56	1337	14
1989	1,04	393	378	771	26	118	91	771	8
1990	1,03	983	951	1934	27	124	95	1934	19
1991	1,03	966	937	1903	47	102	53	1903	18
1992	1,18	96	81	177	44	73	28	177	2
1993	0,86	68	79	147	78	78	0	147	1
1994	0,96	1110	1152	2262	56	114	57	2262	17
1995	1,50	90	60	150	59	59	0	150	1
1996	1,30	387	297	684	57	85	27	684	5
1997	0,80	2065	2588	4653	42	102	60	4653	20
1998	0,80	3322	4132	7454	41	126	84	7454	38
1999	0,84	531	634	1165	33	108	43	1165	6
2000	0,98	368	375	743	39	88	18	743	5
2001	1,01	944	939	1883	31	98	36	1883	10
2002	0,96	367	383	750	61	79	17	750	5
2003	1,09	469	431	900	53	93	39	900	6
2004	0,88	280	320	600	61	85	23	600	4
2005	0,95	219	231	450	71	93	22	450	3
2006	0,78	131	169	300	71	75	4	300	2
2007	1,08	78	72	150	58	58	0	150	1
2008									
2009	0,95	690	726	1416	50	94	43	1416	9
2010	0,89	428	481	909	56	92	35	909	6
2011	1,36	347	255	602	67	90	23	602	5
2012	0,81	231	285	516	60	77	16	516	3
2013	1,16	680	587	1267	53	95	41	1267	9
2014	0,83	324	390	714	61	94	32	714	4

In 2008, the project was not running and therefore there are no data from that year. The total number of examined fish per year varies from 147 to 7 454 (Table 2). Fish was measured from the tip of the nose to the end of the tail at 1.0 mm precision and fish were weighed at 0.1gram accuracy using a laboratory scale. Then the belly of the fish was cut open with a scalpel or preparation scissors. Gonads were taken out of the peritoneal cavity with caution, and their shape and structure was inspected visually. If malformations or morphological abnormalities were found they were described verbally and gonads were photographed. Sex and the developmental stage of gonads were determined according to Kesteven (1960; stages 1-8, (Table. 3)).

Table. 3. Kesteven's classification of gonadal maturity, stages 1-8.

Stage	Description
I juvenile	Gonads are small and close to the spine. Testis and ovaries are transparent, colorless or gray. Eggs are invisible to the naked eye.
II developing juvenile	Gonads are translucent, gray-red. In length, they are half or a little more than the length of the abdominal cavity. Individual eggs are shown with a magnifying glass.
III Developing	Gonads are opaque and reddish, they take up half the space of the abdominal cavity. There are also visible blood vessels. Eggs appear to the naked eye as light granules.
IV Developing	Gonads take about 2/3 of the abdominal cavity. Testis is white with reddish color and sperm does not flow when squeezed. Ovaries are orange-red. Eggs are distinct and dull.
V Ripe	Gonads fill the abdominal cavity. Testis is white and if squeezed, some sperm droplets are seen. Eggs are perfectly round, some already translucent and ripe.
VI Spawning	Eggs and sperm are running when squeezed. Most eggs are translucent, only a few faint eggs are visible in the ovary.
VII Spawning / Spawned	Gonads are not yet completely empty. Immature faint eggs are no longer visible in the ovary
VIII Spawned	Reproductive structures are flat and red, some reabsorbed eggs visible.

Gonadosomatic index (GSI; gonad Wt. / total Wt.) was calculated using fish total or somatic weight of the fish at maturity stages 4-6 (fully developed gonads).

Otoliths were removed from the head and they were prepared for reading the age. From 1984 to 1997 age was read from the whole otoliths that were preserved in Euparal. Age determinations were made under the microscope at 16 - 40x magnification in reflected light on a black background. Otoliths were viewed from the concave (outer) side. In reflected light, the growth zones of the otolith appear as alternating broad opaque zones (bright) and narrow translucent zones (dark). An opaque zone and the following translucent zone were together regarded as a year's growth, and the age was determined as the number of translucent zones (Eklund et al 2000).

In recent years, the growth of herring has been exceptionally slow and the annual rings therefore difficult to distinguish from each other. For this reason, reading the age from whole otoliths was substituted by a new method (Rekilä & Eklund 2012*). In this method, the otoliths are grinded and stained, which makes annual rings easily distinguishable (Fig 7). The new method has been applied in age readings since 1999. In our study, the ages have been read by two experienced otolith readers (J. Eklund and M. Elfving). Comparison of age estimates by the two readers showed that the difference was 0-1 years in 81% of the cases (n of fish = 150), the maximum difference (4 years) being in fish older than 10 years.

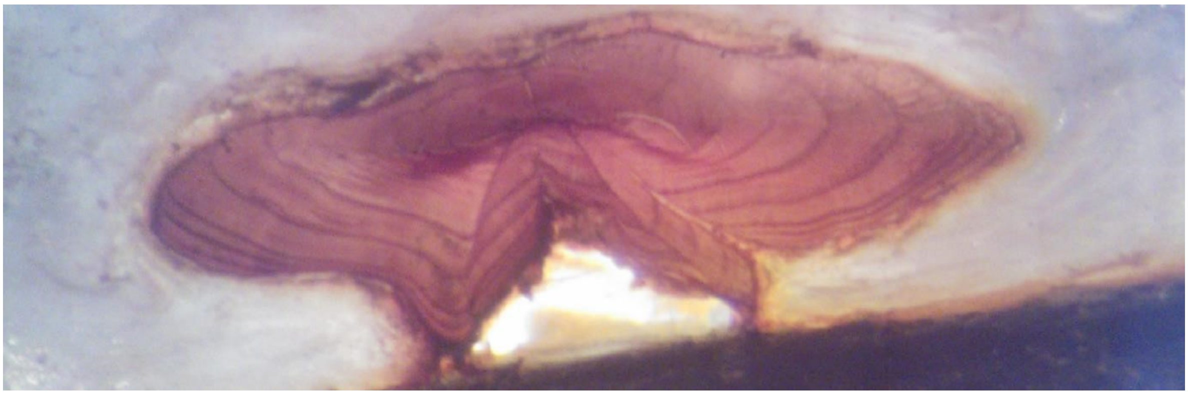


Fig. 7. Annual rings in the grinded and stained otolith of the Baltic herring. (Picture: Mikael Elfving).

4. Statistical analysis

In the statistical analyses, logistic regression models (General Linear Mixed Models; GLIMMIX procedure; SAS v. 9.3) were used. The method is widely implemented in the analyses of time series data in environmental and biological studies, due to the model properties in controlling autocorrelation and it doesn't expect the data to be normally distributed (Hänninen et al. 2015). GLIMMIX procedure can process auto-correlated time series data and random effects are considered (SAS Institute Inc. 2008).

4.1. Sex ratio

At the first phase, the effect of the sampling procedure on sex ratio was examined (Model I). Sex ratio was used as the response variable and different terms in the sampling procedure (Table 2) as explanatory variables.

In the second phase, the effect of the environmental factors such as salinity and temperature on sex ratio was examined (Model II). Sex ratio was used as the response variable and different environmental variables as explanatory variables. See table 1 from the appendices.

In the third phase, the effect of the stock parameters such as population size and gonadosomatic index on sex ratio was examined (Model III). Sex ratio was used as the response variable and stock parameters as explanatory variables. See table 1 in the appendices.

Distribution of the relative proportion of the males compared to all fish was defined as a binary distribution, and its linearizing link function was logit function. In every model of the three models the combined effect of the variables was also tested. In this model, Year was used in the class sentence and there was no random sentence in the model, because the difference between the years was in the main interest. The significance between the years were pairwise tested with glimmix.

I used Akaike's information criterion with a correction term (AICc) for small sample size (Johnson and Omland 2004). Based on those values, I excluded the non-significant explanatory interaction variables. At the end, I did a visual inspection of the residual distribution plots and generalized chi-square values to verify that the model reflected the data well.

4.2. Gonadosomatic index (GSI)

The annual trend of gonadosomatic index was also studied with GLIMMIX procedure. Year was used as a continuous variable and the gonadosomatic index as the response variable. Distribution of gonadosomatic index was defined as normal distribution and its linearizing link function was identity function. This was also done separately to males and females with the gonadal maturity stage of 4-6 and the age of 3-6. All statistical analyses were conducted with the statistical software SAS 9.3 and some sample means were calculated with Microsoft EXCEL 2010.

5. Results

5.1. Annual trend of the sex ratio

A total of 32 630 herrings were examined (15 574 males, 15802 females and 1254 unidentified or juvenile) during 1984-2014. The study reveals that the sex ratio (Nr males: Nr females) of the spawning population has significantly changed (GLIMMIX; $p = 0.0001$; Table 4) during the study (Fig. 8). In the first study years (1984-1992), the sex ratio was higher than that of Fisher's 1:1, indicating that there were more males than females in the spawning population each year (Fig. 8). Since then, the differences between the years have increased and the number of males in the population has decreased. The change appears to have occurred in the beginning of the 1990's, after which the spawning population has turned towards female -dominance (Fig. 8). When years were pairwise tested, differences in sex ratio between the years were found (Table 4).

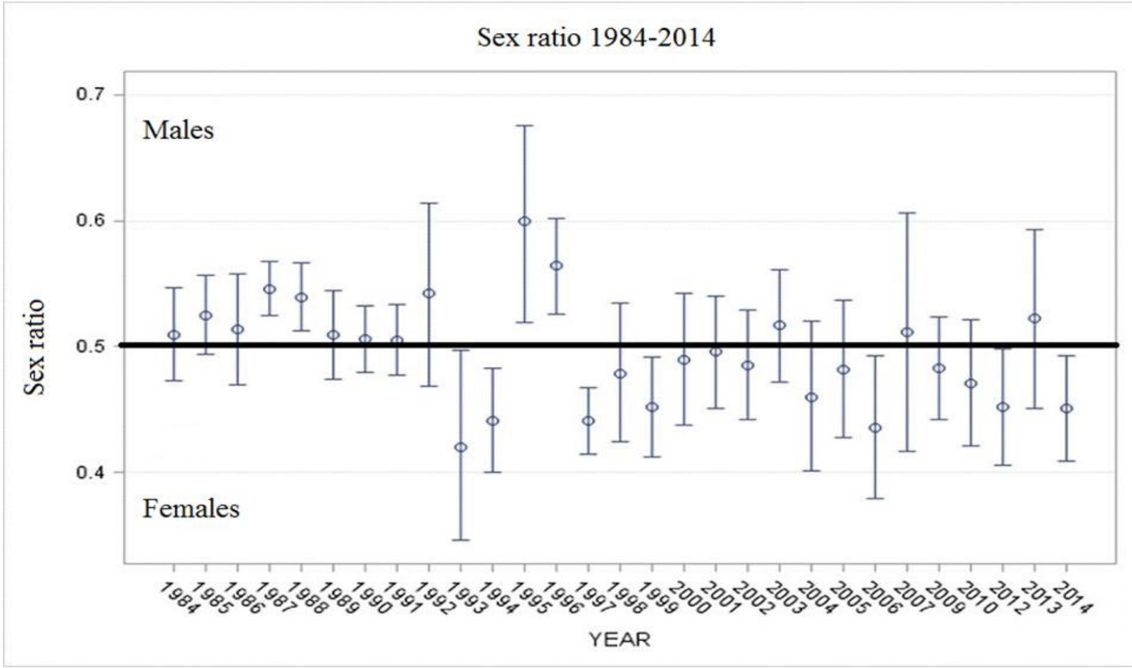


Fig.8. Sex ratio in the spawning population of the Baltic herring in the Airisto Inlet, SW Finland in 1984-2014. Mean of the samples are shown with standard deviation (vertical bars).

5.2. Factors affecting the sex ratio

At the next phase of the study, the effect of the sampling procedure and data describing the herring stock and environmental variables (Table 1) on the sex ratio were examined.

Table 4. Parameter estimates of the Generalized Linear Mixed Model showing the annual trend and effects of different variables (Model I sampling), (Model II environment) and (Model III stock) on the sex ratio of the Baltic herring collected from the Airisto Inlet in 1984-2014. Variables used in *Model I*: DOY 1, DOY 2, Period, Tot Nr of fish and Nr of samples. *Model II*: Ice cover, salinity, mean temperature (°C) during March-April and during July- September in the depth of 10 meters. *Model III*: Condition factor (CF), fish number in the stock and gonadosomatic index (GSI). Bold text shows the values judged to be significant ($p<0.05$); TotNr= total number of herring, Nr of samples, Ice cover, salinity, T (Jul.-Sept.), Condition factor (CF) and total number of the fish in the population.

Solutions for fixed effects						
Number of fish analysed = 32 630						
Effect	Estimate	SE Num	DF	DenDF	t	p
Year	0.436	0.47	1	28	8.66	**<0.0001**
Model I						
DOY 1	-0.00152	0.0014	1	24	-1.08	<0.2905
DOY2	-0.00642	0.0019	1	24	-3.23	<0.3602
Period	0.001545	0.0014	1	24	-1.09	<0.2875
Tot Nr of fish	-0.00014	0.00018	1	24	-8.01	**<0.0001**
Nr of samples	0.02679	0.0049	1	24	5.46	**<0.0001**
Model II						
Ice cover	-0.005	9.47	1	18	-4.97	**<0.0001**
Salinity	0.860	1.04	1	18	0.82	**<0.0008**
T (Mar.-Apr.)	0.604	0.37	1	18	-1.95	<0.0853
T (Jul.-Sept.)	-1.789	0.48	1	18	1.62	<**0.0001**
Sal* T (Jul.-Sept.)	0.8146	0.8660	1	17	0.94	**<0.038**
Sal*ice cover	0.611	3.858	1	17	1.59	**<0.0005**
T*T	0.140	0.1616	1	17	0.87	**<0.0029**
T (Mar.-Apr.)*Ice	4.04	0.0021	1	17	0.19	**<0.0001**
Model III						
CF	-0.614	5.563	1	22	-0.11	**<0.0001**
Tot Nr	0.042	0.020	1	22	2.09	**<0.0046**
GSI	-12.314	33.040	1	22	-0.37	<0.4913
CF*GSI	14.540	52.928	1	22	0.27	**<0.004**
CF*Tot Nr	-0.0821	0.032	1	22	-2.54	**<0.0114**
GSI*Tot NR	-0.268	0.201	1	22	-1.34	**<0.00134**

According to Model I (sampling), total number of fish examined (GLIMMIX, $p = 0.0001$) and number of samples taken in one season (GLIMMIX, $p = 0.0001$) had a significant effect on the sex ratio (Table 4). In addition, also the combined effect of total fish number and number of samples were statistically significant (GLIMMIX, $p = 0.0001$). The first (DOY 1) and last (DOY 2) days of sampling had no effect on sex ratio (DOY 1: $p = 0.2905$; DOY 2: $p = 0.3602$), and the number of sampling days (GLIMMIX, $p = 0.2875$) or the combined effect of the variables showed no significant effect (Table 4).

Model II (environmental factors) showed that salinity (GLIMMIX, $p = 0.0008$), average temperature of the sea water in July-September preceding the spawning year (GLIMMIX, $p = 0.0001$) and the area of permanent ice cover in the Baltic Sea (GLIMMIX, $p = 0.0001$) had a statistically significant effect on the sex ratio, while the effect of the average temperature in March-April was statistically insignificant (GLIMMIX, $p = 0.0853$). Combined effects of these factors were also tested and Salinity * average temperature in July-September (GLIMMIX, $p = 0.038$), salinity*Ice (GLIMMIX, $p = 0.0005$), average temperature in March-April * average temperature in July-September (GLIMMIX, $p = 0.0029$), average temperature in March-April * Ice (GLIMMIX, $p = 0.0001$) showed significant effect on the sex ratio.

Model III (stock related factors) indicated that the condition factor (CF) of spawning herring (GLIMMIX, $p = 0.0001$) and the total number of individuals in the herring population (individual's x 10^9) had a statistically significant effect on the sex ratio (GLIMMIX, $p = 0.0046$), whereas the effect of gonadosomatic index (GSI) was insignificant (GLIMMIX $p = 0.4913$). Combined effects of the stock related variables also showed significant effect on the sex ratio CF*GSI (GLIMMIX, $p = 0.0004$), CF*indiv. (GLIMMIX, $p = 0.0114$) and GSI* Indiv. (GLIMMIX, $p = 0.00134$).

5.3. Gonadosomatic index

The annual trend of gonadosomatic index was studied with GLIMMIX procedure, which shows that the reproductive effort of the herring population has decreased significantly (GLIMMIX, p = 0,0001) (Table 5) from 1984 to 2015 (Fig. 9).

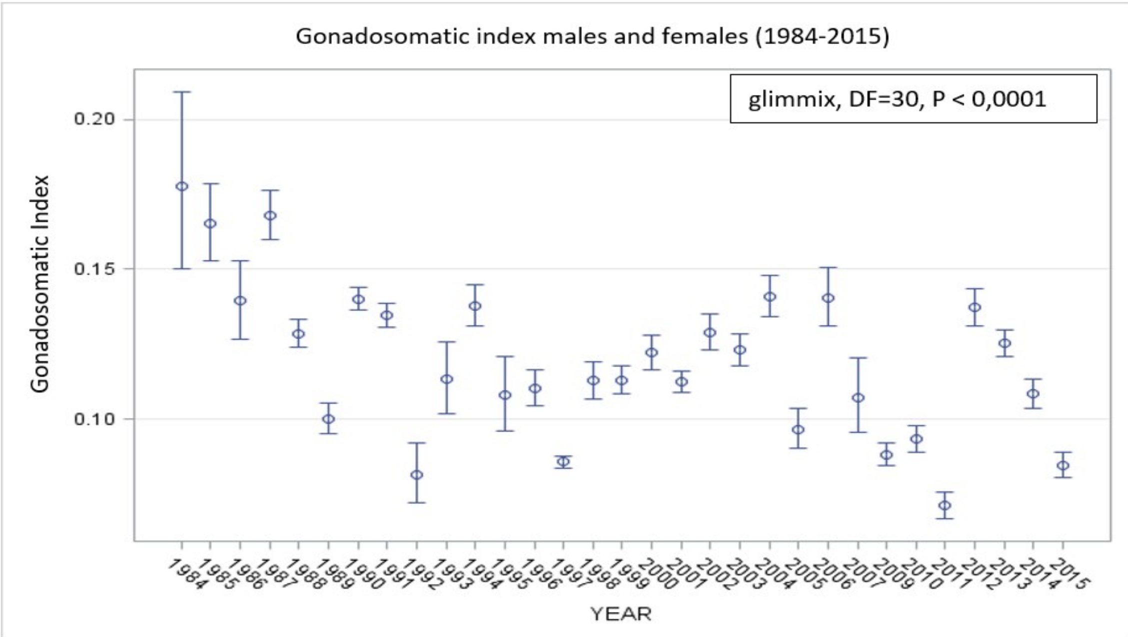


Fig. 9. The mean reproductive effort of the spawning herring population (GSI; females and males combined) in the Airisto Inlet 1984-2015. Vertical bars indicate standard deviation.

The analysis made with the sexes separately shows that the reproductive effort of males has significantly decreased during the study years (GLIMMIX, p = 0.0072), but in females, no significant trend was found (GLIMMIX, p = 0.6788). The analysis was also conducted for the age classes 3-6, which are the most common age groups in the spawning population. It shows that in females, the reproductive effort decreased significantly only in the age class 6 (Age 3, GLIMMIX, p = 0.1350) (Age 4, GLIMMIX, p = 0.2279) (Age 5, GLIMMIX, p = 0.8915) (Age 6, GLIMMIX, p = 0.0201) (Table 5). (Fig. 10), while in males, the reproductive effort decreased significantly in all year classes (Fig.11). (Age 3, GLIMMIX, p = 0.0280) (Age 4, GLIMMIX, p = 0.0052) (Age 5, GLIMMIX, p = 0.0039) (Age 6, GLIMMIX, p = 0.0001) (Table 5).

Table 5. Parameter estimates of the Generalized Linear Mixed Model showing the annual trend of gonadosomatic index and differences between the females and males. Bold text shows the values judged to be significant ($p<0.05$).

Solutions for fixed effects

Number of fish analysed = 27 005

Effect	Estimate	SE	NumDF	F	p
Intercept	0.1778	0.00972	30	76.33	**<0.0001**
Females					
Age 3	0.1246	0.01820	26	6.84	<0.1350
Age 4	0.1839	0.01258	26	14.62	<0.2279
Age 5	0.1869	0.01411	26	13.25	<0.8915
Age 6	0.2034	0.009827	26	20.69	<**0.0201**
Males					
Age 3	0.09350	0.01622	26	5.77	<**0.0280**
Age 4	0.1160	0.007198	26	16.12	<**0.0052**
Age 5	0.1293	0.006766	26	19.11	<**0.0039**
Age 6	0.1418	0.007465	26	18.99	<**0.0001**

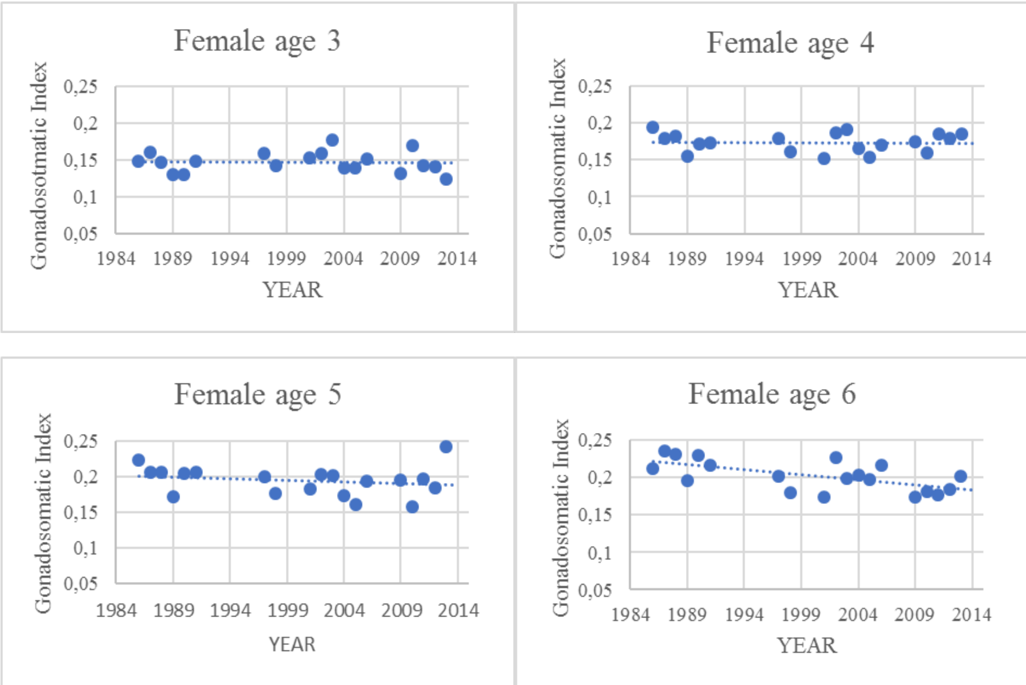


Fig. 10. Gonadosomatic index of females age 3-6.

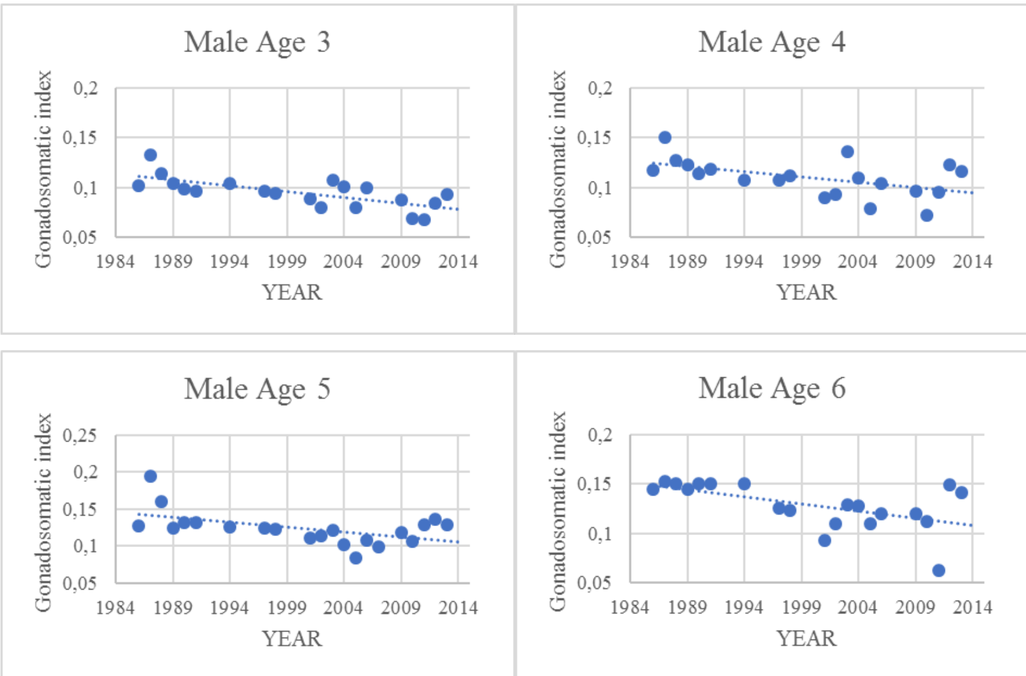


Fig. 11. Gonadosomatic index of males age 3-6.

6. Discussion

6.1. Sex ratio

This study reveals that the sex ratio in the spawning population of the Baltic herring has changed in the study area from 1984 to 2014. In the beginning of the study, the population sex ratio was near the normal Fisher's 1:1 ratio, but since then, the differences between the years have grown and the number of males in the population has decreased. This suggests that now there are more females than males in the population. The change appears to have occurred in the 1990's after which the number of males has steadily decreased. The change may have been caused by factors influencing the sex determination of the fish, or males and females come to the spawning grounds un-synchronized, which creates an uneven sex ratio in the spawning populations.

Worldwide there are about 24 000 different fish species which live in different kind of habitats. This is one of the reasons why fish show such a vast variety of mechanisms of sexual determination and patterns of sexual differentiation (Nelson 1994). Determination of the sex in teleost fish is highly complex, because fish are very adaptable and plastic in their reproduction. Often the sex is determined by the sum of several different factors affecting together and not just by one gene or environmental factor (Wootton and Smith 2015). In the Baltic herring, the basis of sex determination is not known, and results on the sex ratio in the herring populations are few. In the Bothnian Bay, the overall sex ratio was 1:1 in the spawning population, with some variation during the spawning time (Hahtonen et Joensuu 1984).

Sex ratios of worlds fish species have been studied little. Here are some examples of sex ratios of different fish species. Sex ratio of the swordfish, *Xiphias gladius*, in the northwest Atlantic is influenced by the temperature of the sea area, overall there are more females (69.49%), and is consistent for the temperate (76.37%) and the tropical areas (61.61%). However, for the subtropical area, the proportion of females (50.06%) is not different from the 1:1 ratio (Arocha et Lee 1993). In Southern Bluefin Tuna (*Thunnus maccoyii*) the sex ratio is tightly linked to the size of the fish. The sex ratio varied with fish length in all seasons, being significantly female-biased in length classes below 170 cm FL, and significantly male-biased in length classes above 170 cm FL (Farley at al. 2014).

Of the sampling variables, only the number of fish and number of samples affected significantly the sex ratio. This was most likely because of the large variation in the number of samples between the years. For instance, in 1998, 38 samples were taken including 7454 fish, while in 1993, 1995 and 2007, only one sample was taken each including about 150 fish.

Other sampling variables, DOY 1 and DOY 2 (Day of the year, beginning of the first of April) and number of sampling days did not have significant effect on sex ratio. To conclude, the sampling method may have affected the result to some extent, but it cannot be the only factor explaining the trend found in the sex ratio. For instance, in 1997 and 1998, sampling was frequent and number of samples high (see Appendix), but the spawning population was dominated by females throughout the spawning period in both years.

According to the GLM models, also salinity, temperature conditions and stock variables had a significant effect on sex ratio in the spawning population. This suggests that the sex ratio is environmentally induced and a result of natural long-term fluctuation in the conditions of the Baltic Sea.

In environmental sex determination (ESD) sex is determined after fertilization by the effect of environmental conditions such as temperature, pH, salinity, rate of growth and social environment (Wootton and Smith 2015). In many of the teleost fish species, temperature has a big impact on whether the fish is going to be a female or a male (Römer et al. 1996; Abozaid et al. 201; Magerhans et al. 2009; Mylonas et al. 2005). In some fish species, high temperature induces the sex ratio towards a strongly male –biased population (Fernando et al. 2008).

In the Airisto Inlet, the average temperature in July-September has steadily increased during the past 30 years (Fig. 5). The summer temperature had a statistically significant effect on the sex ratio, suggesting that warm summers on the spawning grounds increased the number of females in the spawning population. -In the study of Conover and Fleischer (2008), the sensitive period during which the temperature determined sex was the larval phase, and the timing of the effect seemed to depend on body size of the larvae rather than on the age. In the Baltic herring, the development of larvae to metamorphosis takes place during the summer season. If the sex is determined during this period, it is possible that in warm summers, a higher proportion of larvae are induced as females, and this pattern persists over the years when fish attain maturity and reproduce. This provides also, that mortality is equal in both sexes and stable through the reproductive age of the fish.

According to the GLM model (II), also the extent of the permanent ice cover in the Baltic Sea had a significant effect on the sex ratio. In the years of hard winter, there were more males in the population than females and vice versa. In young fish, the winter temperature can influence last summer's juveniles, on whose gonads have not yet been differentiated. In adult herring, the differentiation of the gonads begins in autumn and the sex of the fish is already determined. Hardness of the winter, which is reflected by the permanent ice cover of the Baltic, could therefore only effect on the maturing of the gonads and the formation of the shoals.

In this study, salinity had a statistically significant effect on the sex ratio of the herring. In declining salinity, the proportion of females increased, which may be only a result of parallel trends without any causative relationship.

There are very few studies about the effects of salinity on the sex ratio in fish. This is because the salinity of the oceans (Suckow et al. 1995) and the freshwater environments is much more stable than that of the Baltic Sea, which is a brackish waterbody and still geologically young and under a constant change (HELCOM 2013). Big ecological changes took place in the 1970's in the central Baltic of low salinity (HELCOM 2013 and BACC 2008). Not only the biological regime shift took place, but the period was followed by an increase in rainfall over the Baltic Sea area (Vuorinen et al. 2015).

Only a few studies have been made concerning the effects of salinity on the sex determination of teleost fish (e.g. Dunham 2011). For this reason, it is hard to reason if the changes in salinity determine the sex determination of the Baltic herring. However, the special features of the Baltic Sea must be taken into account when examining the sex ratio of the herring, as there are not many places in which the changes have happened as fast. Although it is unlikely that salinity alone causes the changes of sex ratio in the herring population, it is possible that the interaction between salinity and other environmental factors cause an effect on which male herring responds in a different way than females. This was suggested by the interaction of salinity and summer temperature and salinity and ice cover in the GLM models (II).

Stock related variables; condition factor (CF) and the number of individuals in the herring population (individual's x 10^9) showed a statistically significant effect on the sex ratio, while the effect of gonadosomatic index (GSI) was insignificant. Feeding conditions of the Baltic herring have changed drastically from the 1980's, as indicated by the growth rate (Möllmann et al. 2005; Casini et al. 2006; Rajasilta et al. 2015). Moreover, the herring population that spawns in the Airisto area has greatly grown in numbers from 1984 ($57x10^9$ individuals) to 2014 (177x $x10^9$ individuals) (ICES 2017). It is known that the social structure and the density of the population can influence the sex determination of the fish (Luckenbach et al. 2003; Beamish 1993; Benton et al. 2006).

Since the number of individuals in the population has increased threefold, also the population density may have had some influence on the sex ratio in the population studied. If the number of individuals increases in the population, there will be more competition on the spawning grounds (Luckenbach et al. 2003). Study of Taborsky (1998) tells that in many fish species, spawning males face fierce sperm competition which consumes their energy reserves and decreases their physical condition. One possible mechanism to reduce this problem in the population is sex reversal, which is hormonally induced. For instance, in the Black sea bass (*Centropristis striata*), females changed their sex when exposed to testosterone treatment (Benton and Berlinsky 2006). Together with changed feeding conditions, the increase of the population density could have induced sex reversal in males, resulting in a higher proportion of females in the population.

6.2. Gonadosomatic index (GSI)

Statistical analysis shows that the spawning effort of males had statistically significantly decreased, but females did not show a similar trend. Analysis was also conducted for the biggest spawning group which is males and females with the gonadal maturity of 4-6 and the age of 3-6. This analysis shows that in females from age 3 to 6, only in the age class 6 the reproduction effort decreased statistically significantly, whereas in males the reproduction effort has decreased statistically significantly in all year classes.

Reduced reproduction effort in males can be due to a fierce sperm competition because of the large population size. To a certain degree, the competition may increase the amount of sperm, but after a certain point it starts reducing (Stockley 1997). Environmental changes and the population parameters as they are at present, seem to reduce the reproduction and number of males.

Poor availability of food has resulted in slow growth and low body condition in the Baltic cod (*Gadus morhua*) (Yoneda & Wright 2005), suggesting that the changes in food availability would mainly affect the growth, condition and the level of gamete production, whereas temperature would affect the proportion of males that mature. In addition to slow growth, the herring of the study area suffers from gonadal malformations, which are more frequent in males than in females (Rajasilta et al. 2015). These results suggest that herring males are not coping well with the changing environment and this is seen also in their reproduction effort. One possible reason for the changed sex ratio is therefore, that males skip the spawning due to poor physiological condition (Rideout and Tomkiewicz 2011). Increasing number of non-spawning males in the population over the years could result in female-biased sex ratio in the spawning population.

6.3. Conclusions

The herring population that reproduces in the Airisto area have become female dominant in 1984-2014. Observed trend is statistically significant, but the reasons behind the trend could not be clearly demonstrated. The initial number of males and females in each new year class forms the basis of the sex ratio in the spawning population, but the final outcome is modified by fish mortality and environmental factors affecting the population after the year-class was born.

Reasons behind the changes in the sex ratio are likely the factors that effect on the state of the Baltic Sea (salinity and temperature), size of the herring population and the shape of the fish, but it is not possible to determine the mechanisms behind the changes due to the combined effects of the various factors. Sex determine mechanism in herring is unknown, but especially the effect of temperature and salinity to the sex should be investigated in herring and other fish species in the Baltic Sea. This requires experimental research, because the answer cannot be found from the samples gathered from the nature due to the combined effects of the various factors.

Temperature is known to influence the sex determination of fish, because of this the sex ratio of fish should be monitored in all fish species, as the climate change the rising temperatures can change the male-female ratio towards unfavorable population structures. Since the temporal trend was influenced by the number of samples taken in this study, the sampling rate should be extensive throughout the spawning season and the sample size large enough, so that the rate of males and females can be reliably determined.

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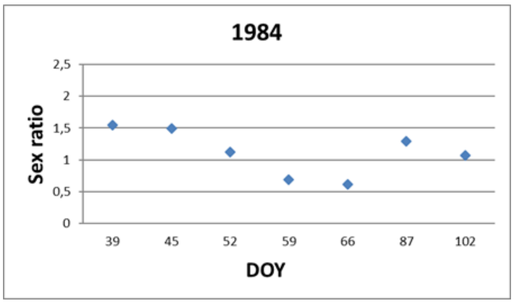
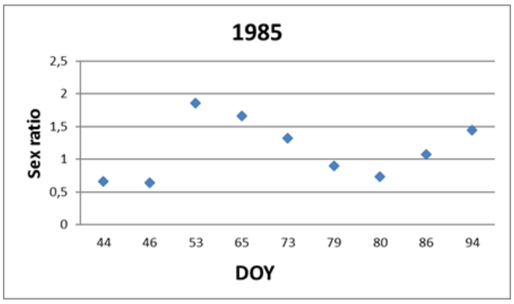
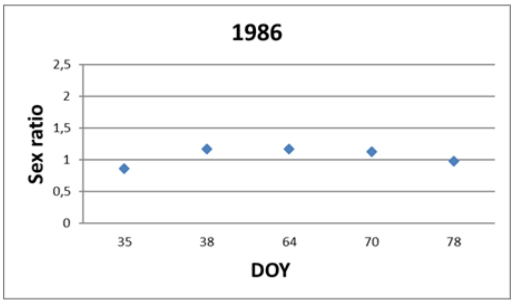
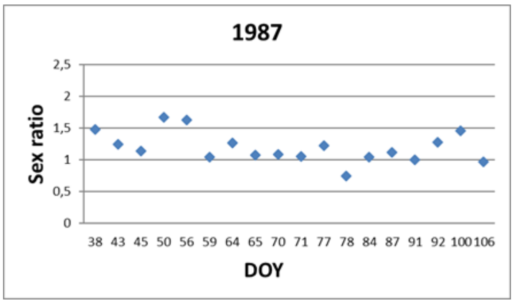
9. Appendices

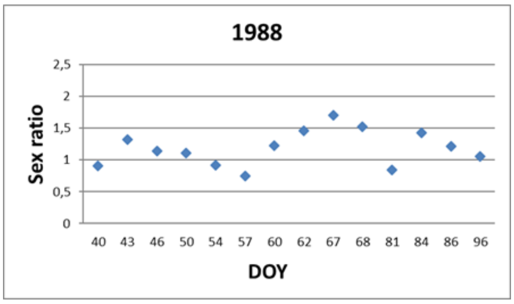
Appendix 1. Length of the sampling period varies from 17 days to 91 days in the dataset. In some years, sampling has been more frequent than in others and therefore, the number of samples also varies from 1 to 38 per year. In years 1992, 1993, 1995, 2006, 2007, 2008 there was only two or less samples taken, so they were excluded from the graphs.

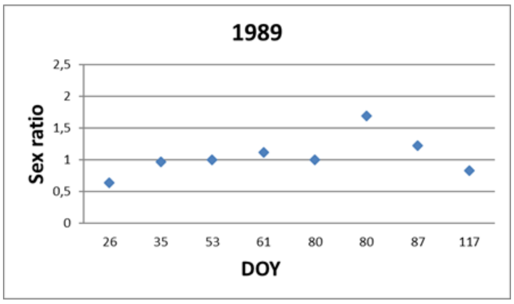


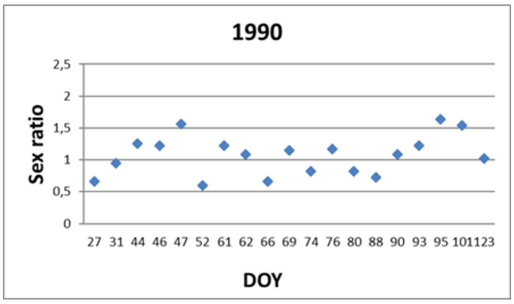


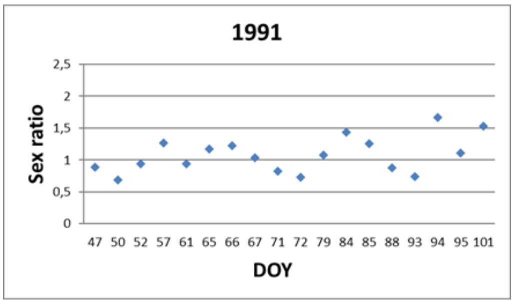


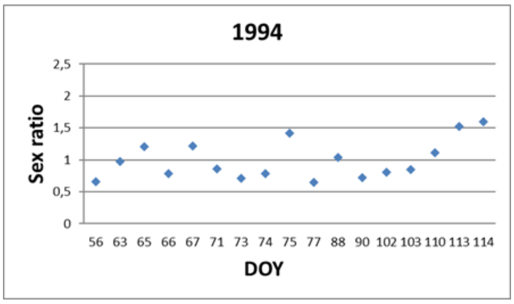


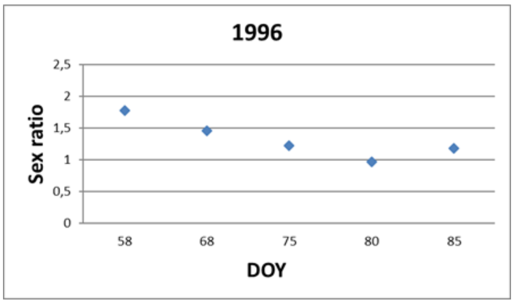


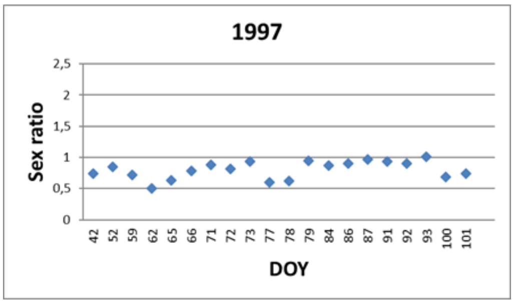


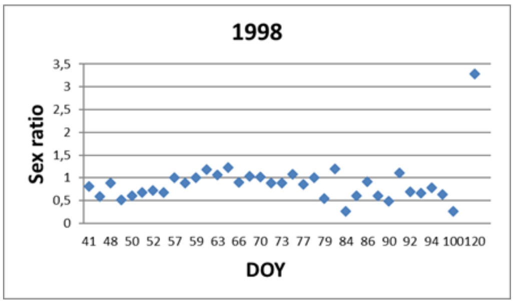

1990
Sex ratio
2,5
2
1,5
1
0,5
0
27 31 44 46 47 52 61 62 66 69 74 76 80 88 90 93 95 101 123
DOY

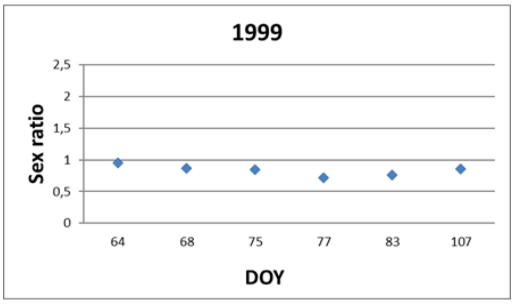

1991
Sex ratio
2,5
2
1,5
1
0,5
0
47 50 52 57 61 65 66 67 71 72 79 84 85 88 93 94 95 101
DOY

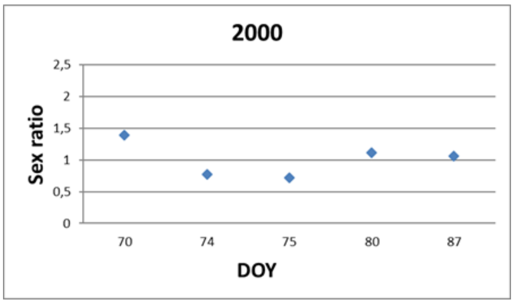

1994
Sex ratio
2,5
2
1,5
1
0,5
0
56 63 65 66 67 71 73 74 75 77 88 90 102 103 110 113 114
DOY


1996
Sex ratio
2,5
2
1,5
1
0,5
0
58 68 75 80 85
DOY


1997
Sex ratio
2,5
2
1,5
1
0,5
0
42 52 59 62 65 66 71 72 73 77 78 79 84 86 87 91 92 93 100 101
DOY


1998
Sex ratio
3,5
3
2,5
2
1,5
1
0,5
0
41 48 50 52 57 59 63 66 70 73 77 79 84 86 90 92 94 100 120
DOY


1999
Sex ratio
2,5
2
1,5
1
0,5
0
64 68 75 77 83 107
DOY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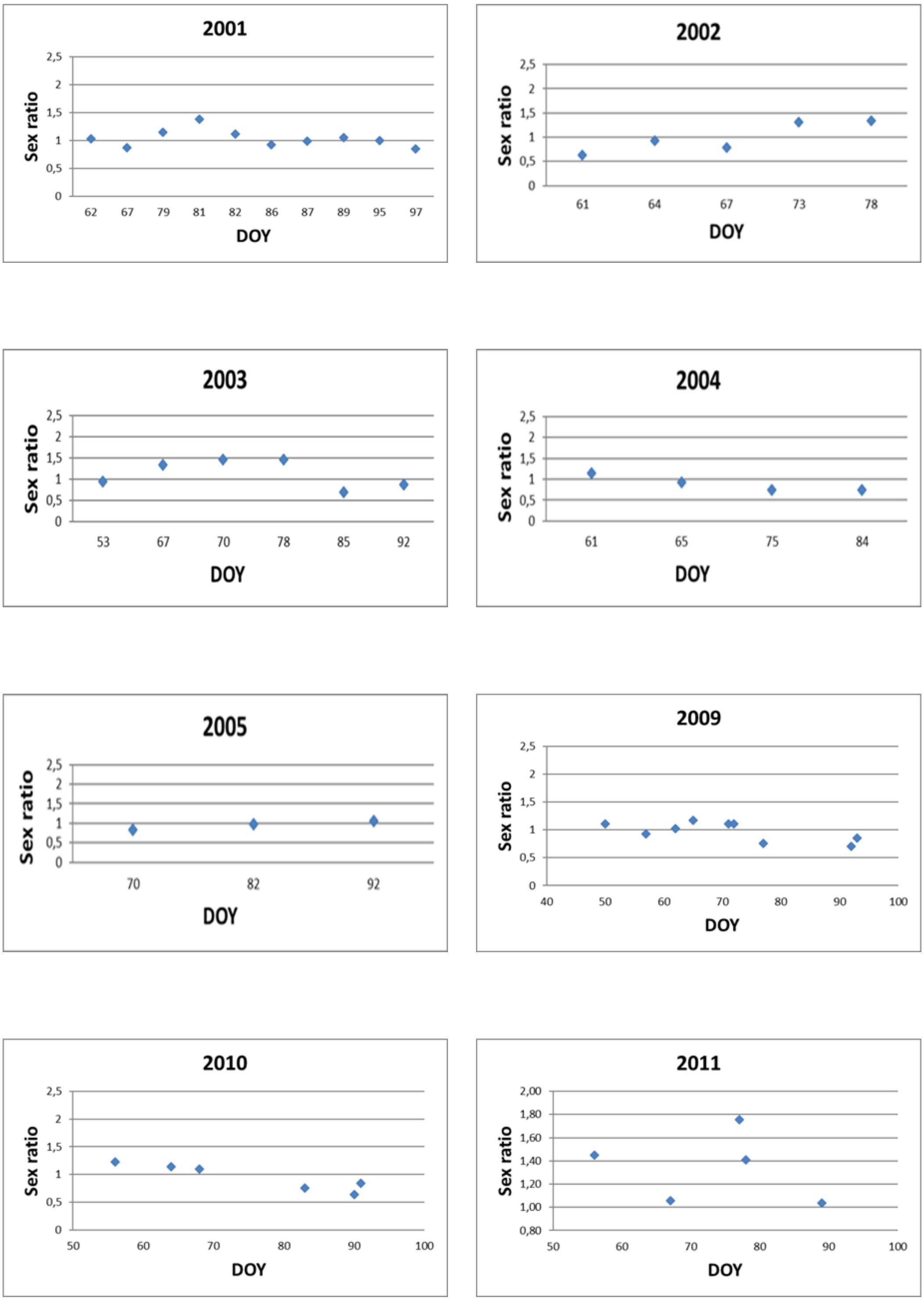
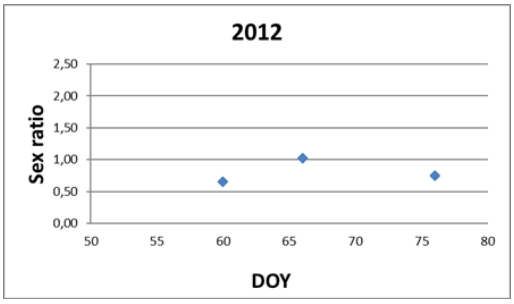
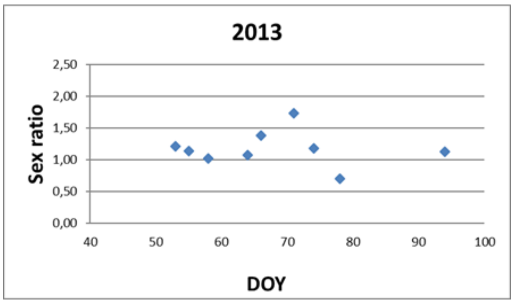
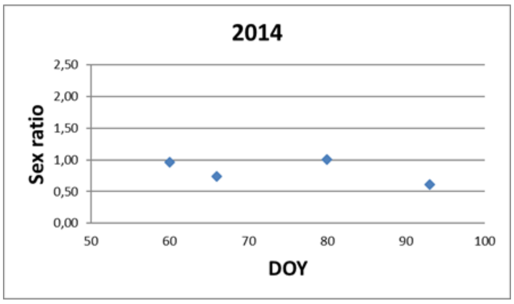
Sex ratio
2,5
2
1,5
1
0,5
0
70 74 75 80 87
DOY


2001
Sex ratio
2,5
2
1,5
1
0,5
0
62 67 79 81 82 86 87 89 95 97
DOY
2002
Sex ratio
2,5
2
1,5
1
0,5
0
61 64 67 73 78
DOY
2003
Sex ratio
2,5
2
1,5
1
0,5
0
53 67 70 78 85 92
DOY
2004
Sex ratio
2,5
2
1,5
1
0,5
0
61 65 75 84
DOY
2005
Sex ratio
2,5
2
1,5
1
0,5
0
70 82 92
DOY
2009
Sex ratio
2,5
2
1,5
1
0,5
0
40 50 60 70 80 90 100
DOY
2010
Sex ratio
2,5
2
1,5
1
0,5
0
50 60 70 80 90 100
DOY
2011
Sex ratio
2,00
1,80
1,60
1,40
1,20
1,00
0,80
50 60 70 80 90 100
DOY


2012
Sex ratio
2,50
2,00
1,50
1,00
0,50
0,00
50
55
60
65
70
75
80
DOY


2013
Sex ratio
2,50
2,00
1,50
1,00
0,50
0,00
40
50
60
70
80
90
100
DOY


2014
Sex ratio
2,50
2,00
1,50
1,00
0,50
0,00
50
60
70
80
90
100
DOY

Appendix 2. Variables measured in the dataset from 1984 to 2014. Doy 1 means number of days the first sample was taken after the first of April. Icecover means the permanent icecover in the Baltic Sea in km². Salinity and temperature values are average values measured in March-April in the depth of 0m and July-September in the depth of 10 meters. SSNr means the number of fish in the population. CF is the condition factor of the fish which is calculated by using fulton's formula. GSI stands for gonadosomatic index.

DATA					SAMPLING					ENVIRONMENT				STOCK		
		Nr of	Nr of				Period	TotNr	Nr of	Icecover		T °C (0m)	T °C (10m)	SSNr		
Year	SexRatio	males	females	All	DOY1	DOY2	(days)	Fish	samples	(km²)	Salinity	March-Apr	July-Sept	x10⁹	CF	GSI
1984	1,04	361	347	708	39	103	63	708	7	187000	5,86	0,47	14,7	57	0,642	0,010
1985	1,10	518	468	986	44	94	50	986	9	355000	6,03	0,32	14,7	66	0,606	0,232
1986	1,06	256	242	498	35	79	43	498	5	337000	6,13	0,18	15,6	82	0,624	0,047
1987	1,19	1439	1208	2647	38	107	68	2647	18	405000	6,27	0,23	14	85	0,624	0,039
1988	1,17	722	615	1337	40	97	56	1337	14	149000	6,16	0,48	13,55	88	0,632	0,124
1989	1,04	393	378	771	26	118	91	771	8	52000	6,05	1,28	16,23	96	0,664	0,096
1990	1,03	983	951	1934	27	124	95	1934	19	67000	5,97	2,00	16,25	110	0,651	0,123
1991	1,03	966	937	1903	47	102	53	1903	18	122000	5,87	0,76	16,56	125	0,635	0,131
1992	1,18	96	81	177	44	73	28	177	2	66000	6,21	1,27	16,48	149	0,597	0,082
1993	0,86	68	79	147	78	78	0	147	1	70000	6,09	0,57	16,28	150	0,625	0,113
1994	0,96	1110	1152	2262	56	114	57	2262	17	206000	5,74	0,11	15,26	145	0,645	0,137
1995	1,50	90	60	150	59	59	0	150	1	68000	5,99	0,58	14,11	128	0,578	0,108
1996	1,30	387	297	684	57	85	27	684	5	262000	5,90	0,08	15,46	118	0.603	0,11
1997	0,80	2065	2588	4653	42	102	60	4653	20	188000	6,14	0,63	16,01	111	0,616	0,085
1998	0,80	3322	4132	7454	41	126	84	7454	38	128000	6,07	0,73	18	113	0,658	0,112
1999	0,84	531	634	1165	33	108	43	1165	6	157000	5,93	0,68	15,08	114	0,595	0,112
2000	0,98	368	375	743	39	88	18	743	5	96000	5,88	0,93	14,95	127	0,634	0,123
2001	1,01	944	939	1883	31	98	36	1883	10	129000	5,69	1,22	16,18	130	0,625	0,11
2002	0,96	367	383	750	61	79	17	750	5	102000	5,89	2,10	17,63	128	0,509	0,129
2003	1,09	469	431	900	53	93	39	900	6	233000	5,91	0,12	19,25	129	0,583	0,123
2004	0,88	280	320	600	61	85	23	600	4	153000	6,15	1,26	17,41	136	0,598	0,141
2005	0,95	219	231	450	71	93	22	450	3	178000	5,99	1,23	16,7	156	0,559	0,097
2006	0,78	131	169	300	71	75	4	300	2	211000	5,83	0,14	16,95	156	0,53	0,141
2007	1,08	78	72	150	58	58	0	150	1	140000	5,68		16,85	157	0,503	0,107
2008																
2009	0,95	690	726	1416	50	94	43	1416	9	110000	5,93	1,28	15,4	180	0,557	0,087
2010	0,89	428	481	909	56	92	35	909	6	244000	5,78	0,61	17,77	177	0,532	0,098
2011	1,36	347	255	602	67	90	23	602	5	309000	6,25	0,58	17,38	185	0,534	0,066
2012	0,81	231	285	516	60	77	16	516	3	179000	5,87	0,60	17,8	169	0,616	0,128
2013	1,16	680	587	1267	53	95	41	1267	9	177000	5,60	0,30	16,89	190	0,571	0,126
2014	0,83	324	390	714	61	94	32	714	4	100000	5,88	2,30	15,37	177	0,608	0,177