



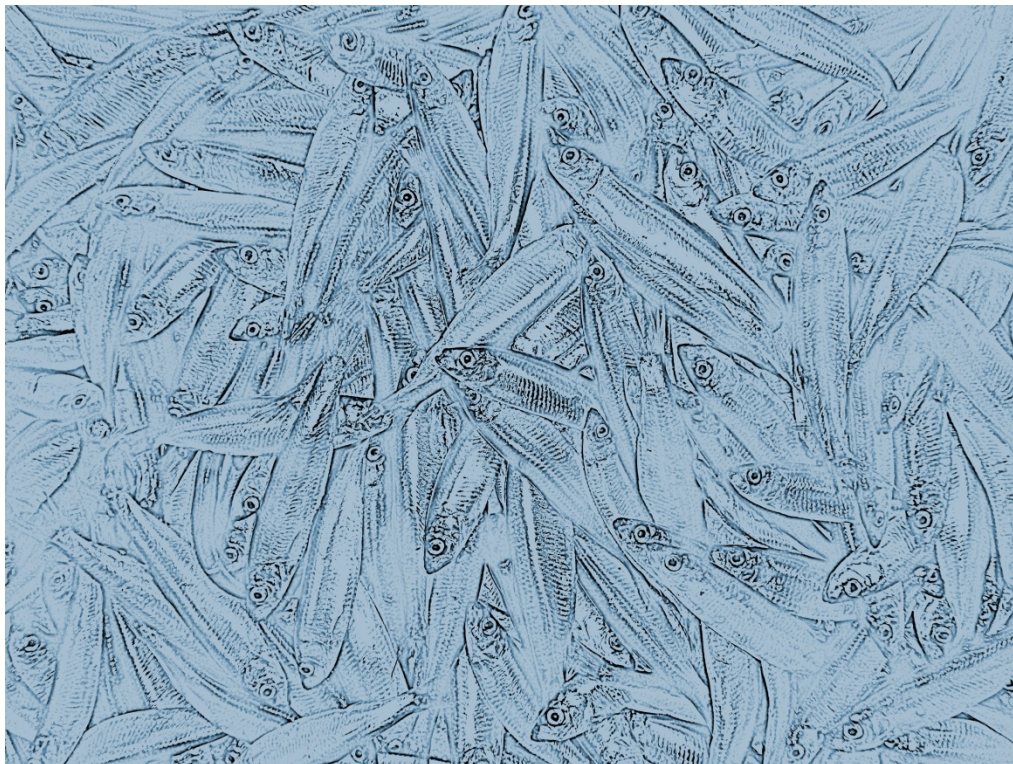
Sveriges lantbruksuniversitet  
Swedish University of Agricultural Sciences

Faculty of Natural Resources and  
Agricultural Sciences

# Morphological variation in herring (*Clupea harengus membras*)

– spring and autumn spawners in the Bothnian Sea

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Department of Aquatic Resources  
Master's thesis • 30 hec • Second cycle, A1E

Öregrund 2015

## **Morphological variation in herring (*Clupea harengus membras*)**

- spring and autumn spawners in the Bothnian Sea

Morfologisk variation hos strömming (*Clupea harengus membras*)

- vår- och höstlekare i Bottenhavet

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## Abstract

There are two spawning types of herring (*Clupea Harengus m.*) in the Bothnian sea. The aim of this thesis was to investigate if there are morphological differences between the spring and autumn spawners, and also study whether freezing affect the morphological measurements of herring. This was done by landmark based morphometrics.

150 herring were collected from the Forsmark area in the Bothnian Sea to investigate the effects of freezing. 316 specimens were caught in the area of Gävle and Hudiksvall, during spring and autumn in 2012. The results showed that freezing does affect morphological measurements mainly by shrinking the measurements connected to body length. Once herrings had been frozen, there were very little changes in morphology. The morphometric study of spawners confirms the claims by local fishermen, that there is a morphological difference between spring and autumn spawning herring. The spring spawners had a relatively larger eye, deeper head and thicker caudal peduncle, whereas autumn spawners overall had a relatively more elongated body shape.

This study confirms recent genetic studies (Barrio et al. submitted for publication) that the two spawning groups are different populations and might need different management plans. The method used in this study presents a cost-effective way of determining parts of the different populations in a mixed fishery.

*Keywords:* morphometrics, body shape, freezing, shrinkage



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# 1 Introduction

It is a challenging task to determine the boundaries of a fish population, especially in situations where populations mix at certain times of the year or during certain stages of their life history. In many situations the fish management unit is determined on the basis of some geographical area, rather than reflecting the appropriate structure of fish populations. Herring exhibit complex population structures with many individual spawning areas (Stephenson *et al.* 2001). Identification of intraspecific groups with different life histories is essential for understanding population dynamics and in the estimation of sustainable harvests (Cadrin and Silva 2014).

There are two spawning types of herring (*Clupea harengus membras*) in the Bothnian Sea, spring and autumn spawners. The spring spawners aggregate in dense shoals in the coastal zone where spawning takes place. Autumn spawners on the other hand, have their spawning grounds in the steep coastal slopes or banks (Parmanne *et al.* 1994). During non-spawning time and feeding migration, the two groups mix in the off-coastal area which results in a problem with mixed fisheries.

Fish stocks can be identified on the basis of differences in characteristics caused by both genetic and environmental factors. Genetic differences mean that populations are reproductively isolated, while those that are due to a changing environment can be the results of groups being separated for large parts of their lives (Swain *et al.* 2005). Differences between groups due to the environment, can mean that these are variable tolerable to exploitation. Mark-recapture, life history characteristics, catch data, parasites, otolith microchemistry, genetics and morphometric variation have all been used as a method of stock identification for many fishery resources (Begg and Waldman 1999).

Herring has been an economically important species in the Bothnian Sea for centuries (Awebro 2003). At present most of the herring in the Baltic and Bothnian Sea spawn in the spring. A smaller group is spawning in the autumn. According to historical data from the Swedish agricultural society (Lundmark 2010) the autumn spawning herring population dominated from the middle of the 19th centu-

ry until the 1940s. During the 1940s there was a marked decline in fishing for autumn spawning herring. Sjöblom (1961) claimed in the 1960s that the spring spawning herring was the most economically important fish in the region, which thus may indicate on a marked shift from autumn spawners to spring spawners during 1945 to 1961. The reason for the decline in autumn spawning herring has not been resolved.

Herring can migrate long distances to feed. For example, herring that spawn near Rügen in Germany migrate to Kattegat and Skagerrak to feed (Aro 1989). Tagging experiments show that despite these migrations the spawning areas remain consistent (Iles and Sinclair 1982). It seems probable that the homing to spawning sites may represent some form of local adaptation to the environment for example different spawning time need different spawning grounds due to temperature and that there should be detectable population structure at spawning time (Ruzzante et al. 2006; Gaggiotti et al. 2009). A recent study (Barrio et al. submitted for publication) showed that there are genetic differences between spring and autumn spawning herring in the Bothnian Sea and this gives strong evidence that they belong to different populations. According to Aneer (1985) the spawning time, spring or autumn, is determined by feeding conditions during the adult phase and therefore not genetically fixed. Aneer claims that the absence of autumn spawners is thought to be the result of improved feeding conditions. Most of the reproductively mature parts of the herring stocks accumulate enough energy during late summer/autumn, to be able to spawn in the following spring and early summer (Aneer 1989). Jørgensen et al. (2008) made a genetic and morphologic comparison between four spawning locations in the Baltic Sea to examine whether morphological variation correlates with genetic and/or environmental factors. They suggested that shape may be more strictly genetically controlled, whereas size shows plastic response to environmental factors for herring in the Baltic Sea.

Meristics and otolith morphometrics have earlier been used to discriminate two groups of herring (*Clupea harengus membras*) in the Baltic Sea: spring and autumn spawners (Heincke, 1898; Ojaveer 2003). In the Gulf of Riga the morphological features of spring and autumn spawning herring have been compared. In the majority of the spring spawning herring the body is wedge-shaped with a comparatively large head and eyes, the body of the autumn spawners is spindle-shaped with clearly smaller head and eyes (Ojaveer and Gaumiga 1995). For a layman the two different spawning groups are hard to separate. Reports from fishers, argue that there are visible morphological differences and that spring spawning individuals have a bigger head (L. Berglund and S. Nordin 2013, pers.comm.).

The main aim of this study was to investigate if there are any morphological differences between spring and autumn spawning herring in the Bothnian Sea, and if this could be used as a cost-effective method in a mixed fishery.



In the study of morphological differences in the body of fish it is important to remove or minimize differences due to body size which can confound differences due to shape alone. It has previously been shown that physical variables such as total weight and total length in fish can change due to freezing of samples (Florin and Lingman 2008; Bucheister and Wilson 2005; Ajah and Nunoo 2003). An earlier study showed that freezing decreased the total lengths of herring (Giedz 1976). This study therefore also investigated if freezing affected the measurements that were used for morphological analyzes.

The following hypotheses were tested:

- H<sub>0</sub> Freezing does not affect the size of herring when doing morphological measurements.
- H<sub>0</sub> There is no morphological difference between spawning types of herring.
- H<sub>0</sub> There is no morphological difference in herring between Gävle and Hudiksvall.

## 2 Method

### 2.1 Sample collection

#### 2.1.1 Effect of freezing

A sample of 150 fresh herring was collected from a research survey during April 2012 in the area of Forsmark, in the Bothnian Sea (Figure 1). Within 10 hours after being caught, the fish were mounted on a Styrofoam and photographed together with an identification number from 1 to 150. Immediately after the picture was taken the fish was placed in a plastic bag and frozen. After 1 week, 1, 4 and 14 months fish with identity number 1-30, 31-60, 61-90 and 91-150 respectively, were thawed and photographed. There was substantial damage to the caudal fin in many of the fish frozen longer than one year. These individuals were removed from the study. Only 28 of the fish with identity number between 91-150 were therefore kept and photographed during the last session. A total of 118 fishes were photographed and analyzed for the entire study.

#### 2.1.2 Spring and autumn spawners

A total of 316 herring samples were collected in 2012 during spring spawning and autumn spawning at two locations in the Bothnian Sea (Figure 1; Table 1). All samples were randomly collected from commercial catches. The fish was frozen by the fishers within a few hours after catch, transported frozen to the laboratory at the institute of coastal research and defrosted after a period of 6-18 weeks.

To minimize the possibility of ontogenetic effects to mask or exaggerate differences in morphometrics between areas or through time, the sizes of the sampled fish were limited to 17-20cm in body length.

Total length, total weight, sex, maturity and gonad weight were recorded for every individual after photographing. Only mature individuals were used in the study, according to established maturity scale (Bucholtz, R.H. *et al* (2008)).

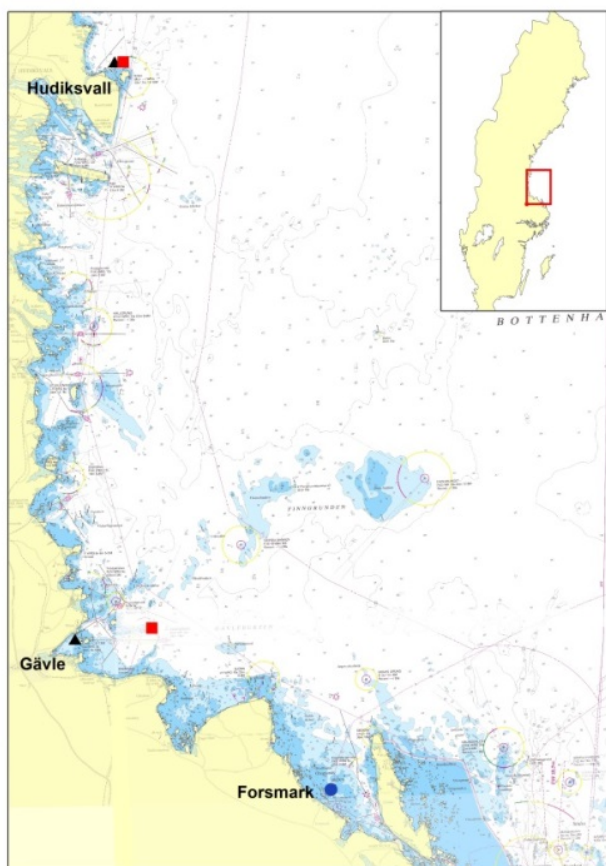


Figure 1. Location for sampling sites; Gävle, Hudiksvall (spring ▲ and autumn spawners ■) and Forsmark (study of freeze effects ●).

Table 1. Area, spawning type, number of fish, length and mean length in each sample for the spawning study.

Area	Spawning type	Number	Length (mm)	Mean length (mm)
Gävle	Spring	100	170-201	185,31
Gävle	Autumn	100	170-199	183,15
Hudiksvall	Spring	100	170-207	191,97
Hudiksvall	Autumn	16	171-204	184,75

## 2.2 Morphological analysis

Each individual fish was placed with the left side up on a piece of Styrofoam. As the fixation and/or preservation can distort the fish body, the Styrofoam was marked with a line, and the fish placed on this in its natural straight form. Individuals whose body shape could not in this way be manually straightened were excluded from the study. The dorsal and ventral fins of the fish were subsequently fixed with needles to the Styrofoam. The jawbone and the end of the skull were also marked with needles to facilitate the exact marking of landmarks on the photographs taken of each individual (Figure 2). A strip of millimeter paper was placed next to the fish and used for calibration. The fish were photographed with a Pentax K-7 digital camera, mounted on a tripod. The camera had the same setting for every taken picture.

All photos were imported into the software program tpsDig2 (F. James Rohlf 2005). 15 landmarks (lm) per fish individual were digitalized and the x and y coordinates of each landmark captured and transferred to Microsoft Excel. A total of 22 morphometric distances were used (Table 2).

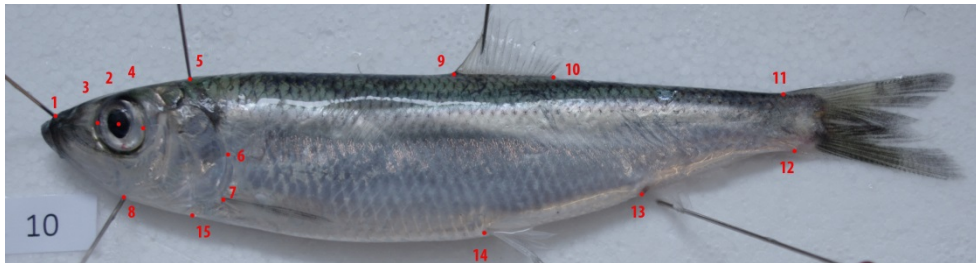


Figure 2. Digitalized morphometric landmarks: 1, mouth; 2, center of eye; 3, anterior edge of iris; 4, posterior edge of iris; 5, skull; 6, operculum; 7, insertion of pectoral fin; 8, jawbone; 9, anterior part of dorsal fin; 10, posterior part of dorsal fin; 11, first insertion of ventral caudal fin ray; 12, first insertion of dorsal caudal fin ray; 13, anterior part of anal fin; 14, anterior part of pelvic fin; 15, opposite landmark 5.

Table 2. Morphological measurements and associated landmarks.

	Morphological measurement	Landmark (lm)	Description
Skull region	MouthEye	1-3	mouth – eye
	MouthSkull	1-5	mouth – skull
	MouthOperc	1-6	mouth – operculum
	MouthPect	1-7	mouth - pectoral fin
	MouthJaw	1-8	mouth – jawbone
	EyeDiam	3-4	eye diameter
	SkullPect	5-7	skull - pectoral fin
	SkullJaw	5-8	skull - jaw bone
	SkullHeight	15-5	skull height
Body height	AntDorsPelv	9-14	anterior dorsal fin - pelvic fin
	PostDorsAnal	10-13	posterior dorsal fin - anal fin
	VentCaudDorsCaud	11-12	ventral caudal fin ray - dorsal caudal fin
Body length	MouthAntDors	1-9	mouth - anterior dorsal fin
	MouthPelv	1-14	mouth - pelvic fin
	DorsPect	9-7	dorsal fin - pectoral fin
	AntDorsDorsCaud	9-12	anterior dorsal fin - dorsal caudal peduncle
	AntDorsAnal	9-13	anterior dorsal fin - anal fin
	PostDorsVentCaud	10-11	posterior dorsal fin - ventral caudal fin
	PostDorsDorsCaud	10-12	posterior dorsal fin - dorsal caudal fin
	VentCaudPelv	11-14	ventral caudal fin - pelvic fin
	AnalPelv	13-14	anal fin - pelvic fin
	PelvPect	14-7	pelvic fin - pectoral fin

Using the Pythagoras' theorem  $a^2 + b^2 = c^2$ , the positions of each landmark were used to calculate the distances between the landmarks. For example:

Distance for eye diam =  $\sqrt{(\text{x coord. posterior edge of iris, landmark 4} - \text{x coord. anterior edge of iris, landmark 3})^2 + (\text{y coord. posterior edge of iris, landmark 4} - \text{y coord. anterior edge of iris, landmark 3})^2}$

## 2.3 Statistical analyses

### 2.3.1 Effect of freezing

Normality and equal variances for each morphological measurement were determined by histograms and Q-Q plots. T-test were subsequently performed to investigate if various times of freezing affected the distances between the landmarks. A paired two sampled t-test was used to investigate if different freezing treatments of 1 week, 1 month, 4 months & 14 months had effects on any of the morphological measurement in comparison to fresh fish. An unpaired two-sampled t-test, was performed to compare morphological measurement between different freezing treatments. All t-test were followed by Bonferroni correction to avoid Type I error. The statistical analyses on the effects of freezing were performed using SPSS statistics 21.

### 2.3.2 Spring and autumn spawners

In the understanding of morphological variation it is important to distinguish between differences due to different body shapes to those relative sizes of fish (Turán 1998). A PCA was performed to investigate groupings of morphological measurements including the potentially influential variables total length and age. To subsequently remove the effects of total body length and age on morphological measurement, each distance was normalized using standardized residuals from the log- log regression between the morphological measurement (dependent variable) and total length and age (explanatory variables). To investigate the potential differences between spawning types, a redundancy analysis (RDA) was performed with the normalized morphometric data as the dependent variables and spawning type as a class factor. Sampling area and sex were also added as covariates to control for any potential variation explained by these factors. Significance tests of explanatory of factors were computed with pseudo-F calculations from 999 permutations. The statistical analyses for the study of spring and autumn spawners were performed using the vegan package in R 3.0.3 statistical package (Oksanen et al. 2013).

## 3 Results

### 3.1 Effects of freezing

Landmark distances for 12-16 of the 22 morphological measurements were significantly different after freezing compared to fresh herring samples (table 3). The greatest effect was found on measurements related to body length, i.e. distances between landmarks in the direction from the anterior to the posterior part of the fish. The body height and particular the skull region were also affected by freezing, in particular after longer times of freezing, but not to the same extent as the body length. The percentage change in morphological measurement as a result of different treatments (table 4) were small; -6,6 – 5,4%. The character MouthEye (lm1-3) was an exception with a mean percentage change of 11,7%.

Only one morphological measurement taken on samples frozen for different periods of time was significantly different (table 3.). The morphological measurement lm11-12 in the caudal peduncle had become smaller when comparing samples frozen for 4 and 14 months.

Table 3. T-test comparing distances between landmarks after different time periods of freezing. Values in bold indicate that the morphological measurement are significantly different at the 95% confidence level and after Bonferroni correction ( $p$ -value = 0,05/22).

	Morphological measurement	Land-mark (lm)	fresh vs. frozen 1w	fresh vs. frozen 1mo	fresh vs. frozen 4mo	fresh vs. frozen 14mo	frozen 1w vs. frozen 1mo	frozen 1mo vs. frozen 4mo	frozen 4mo vs. frozen 14mo
Skull region	MouthEye	1-3	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,9923	0,0038	0,5462
	MouthSkull	1-5	0,0027	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,3982	0,0123	0,1118
	MouthOperc	1-6	<b>0,0001</b>	0,0646	<b>0,0000</b>	<b>0,0000</b>	0,9315	0,0606	0,2336
	MouthPect	1-7	0,6567	0,5813	0,0099	<b>0,0030</b>	0,8068	0,1901	0,3276
	MouthJaw	1-8	<b>0,0000</b>	<b>0,0001</b>	0,4543	0,6611	0,2778	0,0676	0,0698
	EyeDiam	3-4	0,1376	<b>0,0001</b>	0,0446	<b>0,0000</b>	0,1220	0,1547	0,1481
	SkullPect	5-7	0,8936	0,6964	0,5505	<b>0,0000</b>	0,8669	0,2363	0,4487
	SkullJaw	5-8	0,0149	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,6425	0,0149	0,1106
	SkullHeight	15-5	0,0728	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,6174	0,0838	0,1208
Body height	AntDorsPelv	9-14	0,1309	<b>0,0009</b>	<b>0,0000</b>	0,8291	0,7515	0,0887	0,0273
	PostDorsAnal	10-13	0,8261	0,3236	0,9761	0,0512	0,8891	0,3948	0,0266
	VentCaudDorsCaud	11-12	0,4771	0,0314	0,0297	<b>0,0000</b>	0,6094	0,7162	<b>0,0018</b>
Body length	MouthAntDors	1-9	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,1466	0,6743	0,0796	0,1194
	MouthPelv	1-14	<b>0,0000</b>	0,0357	<b>0,0007</b>	0,1995	0,5018	0,1377	0,1795
	DorsPect	9-7	<b>0,0000</b>	<b>0,0000</b>	0,4881	<b>0,0000</b>	0,5543	0,1740	0,1340
	AntDorsDorsCaud	9-12	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,7542	0,2674	0,0179
	AntDorsAnal	9-13	<b>0,0000</b>	<b>0,0001</b>	0,0079	<b>0,0000</b>	0,7360	0,1342	0,0089
	PostDorsVentCaud	10-11	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,5456	0,4198	0,0225
	PostDorsDorsCaud	10-12	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,8491	0,7465	0,0562
	VentCaudPelv	11-14	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	<b>0,0000</b>	0,3496	0,3604	0,0200
	AnalPelv	13-14	0,0046	<b>0,0000</b>	<b>0,0004</b>	<b>0,0000</b>	0,8081	0,7609	0,0339
PelvPect	14-7	<b>0,0000</b>	0,0937	0,7632	0,3758	0,3296	0,1995	0,1897	



Table 4. The percentage change for each morphological measurement when comparing different treatments and the mean percentage change across all treatments.

	Morphological measurement	Landmark (lm)	fresh vs. frozen 1w	fresh vs. frozen 1mo	fresh vs. frozen 4mo	fresh vs. frozen 14mo	mean
Skull region	MouthEye	1-3	11,1	8,6	13,4	13,8	<b>11,7</b>
	MouthSkull	1-5	2,0	5,5	8,6	5,4	<b>5,4</b>
	MouthOperc	1-6	2,8	0,9	3,6	3,4	<b>2,7</b>
	MouthPect	1-7	0,2	-0,2	1,0	1,7	<b>0,7</b>
	MouthJaw	1-8	-4,5	-2,9	0,4	-0,3	<b>-1,9</b>
	EyeDiam	3-4	1,5	-4,0	-2,3	-4,5	<b>-2,3</b>
	SkullPect	5-7	0,0	-0,2	0,3	2,0	<b>0,5</b>
	SkullJaw	5-8	1,4	4,0	7,5	4,9	<b>4,4</b>
	SkullHeight	15-5	0,7	2,8	5,4	3,7	<b>3,2</b>
Body height	AntDorsPelv	9-14	0,7	1,4	3,6	0,1	<b>1,5</b>
	PostDorsAnal	10-13	0,2	-0,5	0,0	-1,3	<b>-0,4</b>
	VentCaudDorsCaud	11-12	0,7	1,4	1,6	-4,7	<b>-0,3</b>
Body length	MouthAntDors	1-9	-2,1	-1,4	1,0	-0,4	<b>-0,7</b>
	MouthPelv	1-14	-2,3	-0,6	0,9	0,4	<b>-0,4</b>
	DorsPect	9-7	-3,7	-1,9	-0,2	-1,2	<b>-1,8</b>
	AntDorsDorsCaud	9-12	-5,7	-4,8	-4,5	-6,8	<b>-5,5</b>
	AntDorsAnal	9-13	-1,5	-1,6	0,9	-2,7	<b>-1,2</b>
	PostDorsVentCaud	10-11	-7,0	-5,0	-6,1	-6,6	<b>-6,2</b>
	PostDorsDorsCaud	10-12	-6,0	-5,5	-7,5	-7,3	<b>-6,6</b>
	VentCaudPelv	11-14	-5,8	-3,8	-4,4	-6,9	<b>-5,2</b>
	AnalPelv	13-14	-1,7	-2,8	-3,3	-5,6	<b>-3,4</b>
	PelvPect	14-7	-4,6	-1,0	0,1	-0,4	<b>-1,5</b>

### 3.2 Study of spring and autumn spawners

There is a strong correlation of the distances between landmarks and the body length and age of the fish (figure 3). This correlation with the fish size and age will likely mask morphological differences between spawning types. A redundancy analyses (RDA) was thus performed on the standardized residuals in which the influence of length and age were removed, to determine the strongest correlations between the morphological measurements for spawning type, area and sex. There was a significant difference in morphology between spawning types (table 5). Redundancy component 1 (RDA 1), mainly driven by differences in spawning type explained 9 % of the variation morphology (figure 4). The autumn spawners were on average relatively longer in their anterior part of the body (lm 1-9, 1-14, 9-7 and 14-7) and the spring spawners had a relatively larger eye (lm 3-4) and higher body height (lm 5-8 and 11-12) (table 6, figure 5). Only 1% of the variance in morphology was explained by sampling area and sex.

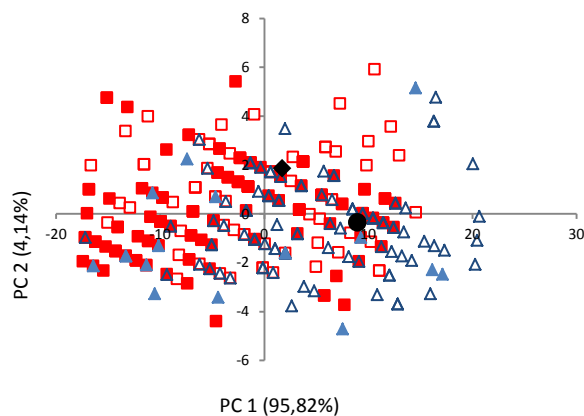


Figure 3. A) Individual scores (spring □ and autumn spawners ■ from Gävle, respectively, and spring Δ and autumn spawners ▲ from Hudiksvall, respectively) from the Principal component analysis with the loadings of total length: ● and age: ◆. ) All other morphometrics (lm-distances) loaded close to the origin and are therefore not included.

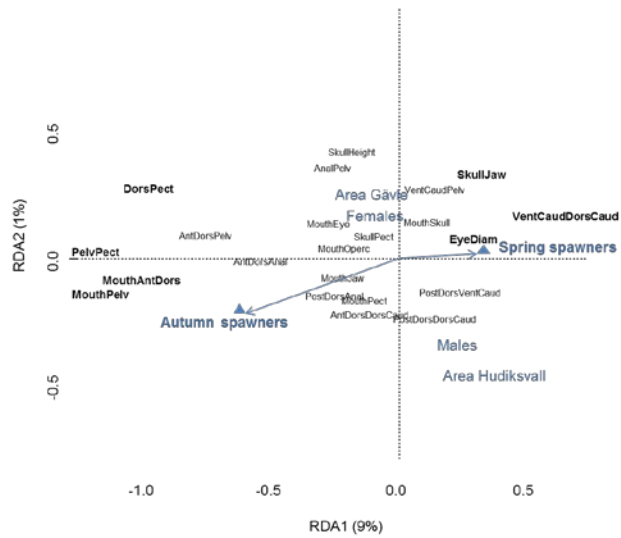


Figure 4. Results of the redundancy analyses (RDA). Morphological measurements marked in bold have the strongest correlation to spawning types (▲).

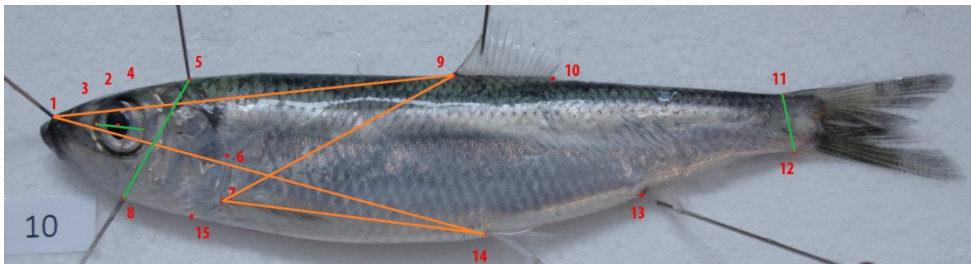


Figure 5. Morphometric measurements with the strongest correlation to the different spawning types. Measurements correlating to spring spawners are shown in green (lm 3-4, 5-8, 11-12 ) and those to autumn spawners in orange (1-9, 1-14, 7-9, 7-14).

Table 5. Results from redundancy analyses (RDA) for area, spawning type and sex.

	Df	Var	F	N.Perm	Pr(>F)
Area	1	0,407	7,7195	999	0,001***
Spawning type	1	1,261	23,9134	999	0,001***
Sex	1	0,222	4,1995	999	0,001***
Residual	312		16,4564		

Table 6. Morphometric scores from the redundancy analysis (RDA) for all morphological measurements along the two first RDA axes. Morphological measurements in bold indicate variables with the highest loadings for spring (positive loadings) and autumn (negative loadings) spawners, respectively, with RDA1. RDA1 explains 9% of total variation and RDA2 1%.

	Morphological measurement	Landmark (lm)	RDA1	RDA2
Skullregion	MouthEye	1-3	-0,24194	0,138011
	MouthSkull	1-5	0,15004	0,145576
	MouthOperc	1-6	-0,17763	0,037884
	MouthPect	1-7	-0,09844	-0,160461
	MouthJaw	1-8	-0,18287	-0,071141
	EyeDiam	3-4	<b>0,31081</b>	0,078026
	SkullPect	5-7	-0,06187	0,08832
	SkullJaw	5-8	<b>0,33757</b>	0,334002
	SkullHeight	15-5	-0,14304	0,419078
Body height	AntDorsPelv	9-14	-0,71848	0,093546
	PostDorsAnal	10-13	-0,21137	-0,143839
	VentCaudDorsCaud	11-12	<b>0,66073</b>	0,17432
Body length	MouthAntDors	1-9	<b>-1,00308</b>	-0,077657
	MouthPelv	1-14	<b>-1,15939</b>	-0,13178
	DorsPect	9-7	<b>-0,96658</b>	0,28041
	AntDorsDorsCaud	9-12	-0,06938	-0,216854
	AntDorsAnal	9-13	-0,50339	-0,008889
	PostDorsVentCaud	10-11	0,28915	-0,128103
	PostDorsDorsCaud	10-12	0,18808	-0,234686
	VentCaudPelv	11-14	0,18209	0,27278
	AnalPelv	13-14	-0,22254	0,361613
	PelvPect	14-7	<b>-1,18494</b>	0,023346
	% variance		8,6	1,1

## 4 Discussion

### 4.1 Effect of freezing

Freezing effected the morphological measurements of Bothnian Sea herring. Almost all of the morphological measurements for body length changed with freezing when compared to fresh, irrespective of the length of time specimens had been frozen. The change in the morphological measurements for body height and skull region increased with increased freezing time. The measurement mouth to the eye showed the largest change due to freezing when compared to fresh samples. This could be an effect of the mouth opening when the fish is thawed. There was no clear pattern in how the morphological measurement changed with freezing when compared to fresh fish.

When comparing freezing treatments, freezing had small effects on the measurements even after a long period of freezing. One single morphological measurement was affected by freezing, but only when comparing 4 month of freezing to 14 months of freezing. The same was shown when comparing fresh fish to those frozen for 14 months. The measurement represents the height of the caudal peduncle (lm 11-12) and as it is a narrow part of the body it might easily dry and in that way shrink.

The results in this study compare well with other studies (Geidz 1976) in how body length was affected by freezing. This study confirms that the body length shrank due to freezing in the comparison between fresh and frozen fish. This study however shows that all of the morphological measurements in the skull region expand due to freezing, except for MouthJaw and EyeDiam. This could indicate that freezing does not affect the bone structure in the skull and that the tissues in the skull lose its shape and expand due to freezing

In conclusion, it is important to be consistent choosing fresh or frozen fish, to avoid confounding results of morphometric differences. The study showed howev-

er, that once the fish individuals had been frozen, morphometric measurements showed very little or no variation with freezing time. From this it can be concluded that that the freezing will not bias the results in the study of morphology between spring and autumn spawners.

## 4.2 Study of spring and autumn spawners

There was a difference in the body morphology of herring in the Bothnian Sea between spawning types. Autumn spawners revealed a longer anterior body. The spring spawners on the other hand, showed a bigger eye diameter, deeper head and a deeper caudal peduncle. Overall, the differences in the morphological characters reveal that the spring spawners are more compact in their body shape and the autumn spawners have a more elongated body shape. The larger eye diameter of the spring spawners could be connected to feeding habits in the early spring, in preparation for spawning.

Little morphological variability was observed in the body shape of herring between sex and area in the Bothnian Sea. The small difference in morphology for sex and area strengthens the theory that the morphological differences are related to spawning type.

Morphological variability may be a result of genetic differences (Griffiths *et al.*, 2010) and/or phenotypic plasticity (Olsson and Eklöv, 2005). Food availability has been observed in other studies as a factor affecting fish morphology and behavior. In experiments with perch, an increasing abundance of food resulted in a deeper body and a relatively smaller head, while low food levels gave a more slender body (Borchherding and Magnhagen, 2008). Consumption of more energy rich prey may also contribute to change in body shape development (Mahe *et al.*, 2014). If herring have the same response to food availability as perch, the morphology for the autumn spawning herring show that they have poorer food levels than the spring spawners. Whether there are differences in the food preference for spring and autumn spawning herring is not known.

The morphological difference in spring and autumn spawning herring is supported by studies showing that genetic factors have an important role for controlling spawning time for herring in the Bothnian Sea. (Barrio *et al.*, submitted for publication). This gives strong evidence that the spawning groups are two different populations. Morphological and life history differences used to define stocks may be especially important to consider even when only little genetic structure can be detected (Larsson 2008). At the moment the two groups are managed as one stock unit in the Bothnian Sea. Spawning takes place in different environment and due to their natal homing behavior they return to the same spawning ground (Iles and

Sinclair 1982). How the ecological response of eliminating herring from a spawning ground would affect the population structure is not known. It is therefore important to provide the best conditions for keeping them. Maintenance of the full diversity of spawning groups should be the default approach in management (Stephenson *et al.* 2001). In between spawning the two spawning types in the Bothnian Sea probably mix, making separate management difficult.

The result of this study confirms suggestions by local fishermen, that there is a morphological difference between spring and autumn spawning herring. The study could, however, be improved. The present study investigated the morphological differences in herring between two areas and one year only. In addition, the sample size of autumn spawners from the Hudiksvall area was small and therefore gives a weak insight in how the morphology differ in-between areas for autumn spawners. A question arising is whether there are in fact several populations of spawners along the coast of the Bothnian Sea. Future analyses should focus on studying samples from different areas, geographically and spatially apart from each other e.g. herring from the coast and the open sea. Shape analysis may give a better insight in the distribution of autumn spawners and a better explanation of the reasons behind the differences in the body shape of herring. To investigate if herring have a rapid morphological adaptation as in perch (Olsson and Eklöv, 2005), it would be of great value to repeat the same study but including also materials from different years. Diet studies would also be of interest to confirm if this could explain some of the morphological differences between spawning types. Age analyses could reveal if one of the two groups is growing more slowly and therefore could be variable tolerable to fishing.

In conclusion, freezing effects fish morphological measurements and needs to be considered in future studies. Once the specimen had been frozen, the length of time of freezing however, did not affect morphological measurements. A difference in morphology between the two spawning groups agreed with genetic studies that there are two different populations, which has evolved different prominent characters. Based on this study a few morphological identifiers could be selected for identification of spring and autumn spawners. The method used in this study presents a cost-effective way of determining parts of the different populations in a mixed fishery. This information together with genetic data could be of importance in a first step for separate management of different spawning types of herring in the Bothnian Sea.

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## Popular science summary

Herring is a common fish around the world. There are many things that could affect the way they look i.e. how old they are, what they eat and where they live. The time when the herring spawn is something that varies in different locations. In the Bothnian Sea we have herring spawning in the spring and herring that spawn in the autumn. The two groups have different spawning areas, but during non-spawning period they probably mix in the open sea. There have been many discussions in the scientific world whether these two spawning types are from the same population. Recent studies show that spring spawning herring and autumn spawning herring in the Gävle area in the Bothnian Sea belong to different populations. They are as different from each other as Baltic Sea herring and Atlantic herring. At the moment the two groups are managed as one unit which could be unfavorable for them. In order to preserve the two spawning types it's important to easily detect them. This can be done by analyzing genetics but this is usually a costly method and it is therefore interesting to find a more cost-effective method. The main aim of this study was to investigate if it is possible to distinguish the two spawning types by looking for differences in the body shape.

In my study I collected spring and autumn spawning herring from the Gävle region and spring and autumn spawning herring from the Hudiksvall region, in the coast of the Bothnian Sea. I did 22 different measuring's on each fish and compared the measurements between all fishes. Herring with different spawning time did vary in how their body is shaped. The results showed that the autumn spawners had a longer anterior body and the spring spawners showed a bigger eye diameter, a deeper head and a deeper tail. The spring spawners had larger eyes, which could be connected to feeding habits in the early spring, in preparation for spawning.

The method used in this study presents a cost-effective way to separate spring and autumn spawners. This information could be of importance in a first step for separate management of different spawning types of herring in the Bothnian Sea.