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Master of Science in Zoology

Master's Thesis

Impact of spawn concentration on the egg development of Baltic herring (*Clupea harengus* L.)

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Abstract

Western Baltic spring spawning (WBSS) herring annually migrates into shallow coastal estuaries of the southern Baltic Sea for spawning. One prominent spawning ground is the Greifswalder Bodden, the major investigation area in this study besides the Warnow river.

WBSS herring attach their demersal eggs to submerged vegetation. As a consequence of successive spawning waves or multiple events of egg mass deposition, the formation of multiple egg layers is regularly observed. It is a common suggestion that eggs inside egg masses are disadvantaged due to oxygen depression and accumulation of toxic metabolic excrements resulting in malformations of embryos or increased egg mortality rates. However, information on effects of defined numbers of egg layers on the reproduction success for WBSS herring is not yet available.

Hypothesizing that spawn concentration has a negative effect on fertilization success and egg survival, influences of multiple egg layers in a range of 1-10 layers were investigated. Thereby, experiments were repeated including herring from different spawning cohorts and spawning grounds. Additionally, two different densities of egg concentration within single layer spawn were investigated. All egg layers and densities were artificially spawned on glass substrates and incubated until shortly before hatching under controlled environmental conditions in the laboratory.

The results generally revealed that multiple egg layers influence the fertilization success and egg survival. Significantly higher fertilization and survival rates were recorded for single layer spawn than for multiple egg layer spawn. Furthermore, egg mortality rates stepwise increased with increasing number of egg layers, indicating a linear relationship. Additionally, differences in fertilization patterns and egg mortalities between spawning cohorts and spawning grounds were present. Effects of egg layering became rather distinct within the initial spawning cohort in the Greifswalder Bodden. Different densities within single layer spawn did not influence the fertilization success or mortality rate of herring spawn.

The study outcomes indicate that spawn concentration in terms of multiple egg layer deposition is an important factor, affecting WBSS herring reproduction success particularly in the initial spawning cohort. This becomes even more relevant as the submerged vegetation in inner coastal areas of the Greifswalder Bodden is reported to decline due to decades of eutrophication. Less spawning substrate and fragmentation of spawning grounds potentially result in increased egg concentrations per area on residual substrates. According to the present findings this cascade could negatively affect the overall reproduction of WBSS herring.

Zusammenfassung

Für das Laichgeschäft ziehen frühjahrslaichende Heringe der westlichen Ostsee jährlich in die flachen südlichen Küstengewässer ein. Ein großes und bedeutsames Laichgebiet ist der Greifswalder Bodden, welcher neben der Warnow das Hauptuntersuchungsgebiet der vorliegenden Arbeit darstellt.

Heringe laichen bevorzugt auf submerse Pflanzen. Dabei wurde mehrschichtiger Laich als Folge von wiederholten Laichaktivitäten oder massenhaftem Ablaichen beobachtet. Es ist eine gängige Annahme, dass die Eientwicklung in mehrschichtigem Laich durch mangelnde Sauerstoffversorgung und Ansammlung toxischer Abfallprodukte angrenzender Eier benachteiligt ist. Dennoch sind die Effekte von mehreren Eischichten auf den Reproduktionserfolg der frühjahrslaichenden Heringe der westlichen Ostsee weitgehend unbekannt.

Mit der Vermutung, dass die Laichkonzentration die Befruchtungs- und Überlebensrate der Eier negativ beeinflusst, wurden in der vorliegenden Arbeit die Effekte von 1-10 -schichtigem Laich untersucht. Dabei wurden die Experimente mit unterschiedlichen Laichergruppen und Laichern aus zwei unterschiedlichen Habitaten wiederholt. Zusätzlich wurden zwei Laichkonzentrationen innerhalb einschichtigen Laiches miteinander verglichen. Alle Eischichten und -konzentrationen wurden künstlich auf Glassubstrat gelaicht und bis kurz vor der Schlupfphase unter kontrollierten Umweltbedingungen im Labor inkubiert.

Die Resultate zeigten, dass mehrschichtiger Laich sowohl den Befruchtungserfolg als auch die Überlebensrate der Eier beeinflusst. Einschichtiger Laich erzielte signifikant höhere Befruchtungs- und Überlebensraten als mehrschichtiger Laich. Weiterhin stieg die Sterblichkeitsrate mit zunehmender Schichtung der Eier an und ließ einen linearen Zusammenhang vermuten. Darüber hinaus wurden Unterschiede zwischen Laichergruppen und –habitaten festgestellt. Der Effekt der Eischichtung wurde innerhalb der ersten Laichergruppe im Greifswalder Bodden besonders deutlich. Unterschiedliche Eikonzentrationen innerhalb einschichtigen Laiches hingegen hatten keinen Einfluss auf die Befruchtungs- oder Sterblichkeitsrate.

Die Ergebnisse deuten darauf hin, dass mehrschichtiger Laich ein zentraler, negativer Einflussfaktor auf den Reproduktionserfolg des frühjahrslaichenden Herings der westlichen Ostsee ist, besonders innerhalb der ersten Laichergruppe. Dieser Umstand gewinnt seit dem Rückgang der submersen Vegetation durch jahrzehntelange Eutrophierung im Greifswalder Bodden an Relevanz, da dies zu verstärkter Laichaktivität an verbleibendem Substrat führen könnte, was wiederum eine erhöhte Laichkonzentration zur Folge hätte. Vor dem Hintergrund der eigenen Ergebnisse könnte dies die Rekrutierung des frühjahrslaichenden Herings der westlichen Ostsee negativ beeinflussen.

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A	Appendix
ANOVA	Analysis of variance
Df	Degrees of freedom
DW	Dry weight
et al.	And other
Exp.	Experiment
FAO	Food and Agriculture Organization of the United Nations
Fert.	Fertilization
Fig.	Figure
FRV	Fisheries research vessel
H_0	Null-Hypothesis
HSD	Honestly significant differences
IC	Initial spawning cohort (Greifswalder Bodden)
ICES	International Council for the Exploration of the Sea
Lx	x represents the number/position of layers
Mort.	Mortality
n	Total number (of replicates)
p	Significance value
Pers. comm.	Personal communication
R^2	Coefficient of determination
SC	Second spawning cohort (Greifswalder Bodden)
SD	Standard deviation
Tab.	Table
Tx	x represents the number of egg layers within a treatment
WBSS	Western Baltic spring spawning
WH	Warnow herring
°d	Degree-Days (°C incubation temperature x d after fertilization)

1 Introduction

In general, Atlantic herring (*Clupea harengus*, Linnaeus 1758) is amongst the most economically important fishery species (FAO 2016) and the different stocks along the Baltic Sea support some important regional fisheries. In the Baltic Sea three major stocks in the Bothnian Sea, the Central Baltic Sea and Western Baltic Sea can be distinguished (von Dorrien *et al*. 2013). However, distribution areas of Western Baltic and Central Baltic herring were reported to overlap (Gröhsler *et al*. 2013).

According to time of reproduction, Western Baltic herring stocks can be classified as spring and autumn spawners. Western Baltic spring spawning herring (WBSS) supports a large over-regional fishery from spawning grounds in the western Baltic to feeding grounds in the Skagerrak and Kattegat. Since the International Council for the Exploration of the Sea (ICES) started monitoring and stock assessment of WBSS herring in the 1990s, a constant decrease in spawning-stock biomass and recruitment was reported (ICES Advice 2018). In 1991 the spawning-stock biomass incorporated 300.000 t but decreased about approximately 50 % within only five years (ICES Advice 2018). Recruitment decreased most probably due to unfavourable environmental conditions reaching lowest values of the time-series in 2014 and 2016. In order to decrease the fishing mortality, herring quotas were reduced resulting in a constant decline of commercial catches since the early 1990s. However, fishing mortality was relatively constant since 2010 and spawning-stock biomass showed no recovery. Due to the extraordinary low recruitment within the last years, it is fundamental to identify drivers and stressors of early life stage development modifying reproduction success of herring.

Western Baltic spring spawning herring perform seasonal migration from feeding grounds in the Skagerrak and Kattegat to spawning grounds in the coastal areas of the western Baltic Sea, showing a certain fidelity to specific spawning areas (Aro 1989, Moll 2018). One of the favoured spawning areas, the Greifswalder Bodden, is a shallow retention area incorporating several spawning sites and therefore plays a key role for recruitment of WBSS herring (Biester 1989).

For the Warnow river estuary migration of ripe herring is also recorded, although spawning sites are not yet located. Due to intensified anthropogenic impacts, spawning, egg development and early life stage survival are supposed to be challenging in this area.

Probably due to their spawning ground fidelity, the so-called component of the Rügen herring is genetically distinct from other components of western Baltic herring (Bekkevold *et al*. 2005, Jøergensen *et al*. 2005). Initial cohorts of spawning adults, usually immigrating into the Bodden from January to April, are considered older and larger in average body size compared to later cohorts (Kändler 1952, von Dorrien *et al*. 2013 and literature therein). WBSS herring mature at approximately 2 years of age showing a significantly lower fecundity

than larger herring (Anwand 1962). The herring spawning season includes multiple spawning peaks which can contribute differently to the annual recruitment (Polte *et al*. 2014). Spawning behaviour was observed by Aneer *et al*. (1983) and described as following: Single females leave large schools of herring descending to the substrate and position their genital opening close to the substrate for egg release. Males follow the females, releasing milt that sinks down onto the eggs for fertilization. Fertilization success and subsequently embryonic development in particular are depending on several intrinsic and extrinsic factors:

Egg and sperm quality is determined by the spawning season, parental effects and genotype combinations (Blaxter and Hempel 1963, Rajasilta *et al*. 1997, Laine and Rajasilta 1999, Geffen and Nash 2012). Sperm cell density and quality are documented to decrease over the spawning season (Rajasilta *et al*. 1997, Laine and Rajasilta 1999). Moreover, eggs of females with a higher condition factor and fat content yield higher survival rates and hatching success (Laine and Rajasilta 1999). Stationary attached eggs are exposed to local environmental effects particularly related to the nature and exposure of spawning substrate (von Nordheim *et al*. 2018).

Water temperatures have major effects on spawning behaviour and subsequent embryonic development. Certain water temperatures initiate and terminate the spawning season (Ojaveer and Simm 1975). For the WBSS herring a favoured water temperature of 5-8 °C for spawning activities is reported (Blaxter 1956 in von Dorrien *et al*. 2013). However, initial spawning is usually observed at about 4°C (Klinkhardt 1996, Moll 2018). Herring eggs subsequently develop in water temperatures in a range between 5-14°C and tolerate even more extreme values (Aneer *et al*. 1983, Haegele and Schweigert 1985). However, the optimum range for viable egg development of WBSS herring is between 7 and 13°C (Peck *et al*. 2012). The close connection between temperatures and egg development was already discovered by Apstein (1909) who introduced the unit degree days (°d = temperature (°C) x days (d)) for the estimation of the age of herring eggs and the prediction of their hatching date. Herring spawn generally tolerates rather low levels of dissolved oxygen. However, oxygen demand increases with ongoing embryo development due to the increasing metabolism and growth (Braum 1985, Aneer 1987, Kiørboe and Møhlenberg 1987). The water body of the Greifswalder Bodden is usually well oxygenated during spring time with rare occasions below 80 % saturation, providing favourable conditions for egg development (von Dorrien *et al.* 2013). Inadequate oxygen supply result in embryonic malformations or increased mortality rates. The definite saturation threshold for hatching was detected at 20 % (Braum 1985). Also, salinity is supposed to have major effects on early life stages which are poorly equipped with fully functional osmoregulatory organs and therefor are particularly sensitive to changes in salinity (Illing *et al*. 2016). Several studies reported a wide salinity tolerance for Atlantic herring (McMynn and Hoar 1953, Holliday and Blaxter 1960, Haegele

and Schweigert 1985). However, Illing *et al*. (2016) revealed a salinity threshold of 3-5 for fertilization of Atlantic herring eggs.

The spawning substrate has crucial impacts on egg development and hatching success. WBSS herring favours submerged vegetation which declined with increasing urban development of coastal areas since the early 20th century to a coverage of less than 10 % of the Greifswalder Bodden nowadays (Kanstinger *et al. 2016*). Moreover, the increasing eutrophication and turbidity forced a vegetation-shift from mainly macrophytes reacting sensitively to light limitation towards phytoplankton (Munkes 2005). Besides the severe decline in macrophytes-covering, the depth limit of macrophytes decreased from 14 m to 6 m in the Greifswalder Bodden (Munkes 2005). It is stated that a decline of vegetation coverage and submerged plant substrates might result in a decrease of spawning intensity and therefore threatens the reproduction of herring in this area (Scabell 1988, von Nordheim *et al*. 2018). Another assumption is that a decline and fragmentation of adequate spawning substrate leads into repeated spawning activities on remaining plants resulting in higher egg masses per area. With increasing human impact, a modification of coastal zones introducing artificial substrates such as harbour walls or wave breakers made of concrete occurs. Although, spawning on gravel and stones was observed (Scabell 1988, Rajasilta *et al*. 1989), reproductive success was low for gravel and concrete units in corresponding experiments (von Nordheim 2014). Moreover, von Nordheim *et al*. (2018) revealed that structural complexity of spawning substrates and submerged vegetation in particular provides a prerequisite for reproductive success.

Earlier studies reported multiple egg layers due to spawning intensity and repeated spawning activity on already egg-covered substrate (Parrish *et al*. 1959, Taylor 1971, Ojaveer 1981, Messieh and Rosenthal 1989). It is a common suggestion that eggs inside egg masses are disadvantaged due to oxygen depression and accumulation of toxic metabolic excrements resulting in malformed embryonic development and increased mortality rates (Rannak 1971, Taylor 1971, Klinkhardt and Biester 1984, Klinkhardt and Biester 1985, Messieh and Rosenthal 1989).

Evidence for the negative effect of high egg concentration is provided by other aquatic animals: In firm egg masses of the yellow spotted salamander (*Ambystoma maculatum*) a gradient of increasing oxygen saturation to innermost embryos was evidenced, indicating that diffusion alone is inadequate to provide oxygen (Pinder and Friet 1994). For the relatively loosely spawned egg masses of the wood frog (*Rana sylvatica*) however, water convections allowing gas exchange are considered sufficient (Pinder and Friet 1994). Back to herring eggs, it is known that egg samples taken from various layers revealed a similar fertilization success (Parrish *et al*. 1959, Messieh and Rosenthal 1989). But the rate of development is related to the position within the egg mass showing an advanced stage of

development in surface layers while eggs in lower layers appear in a retarded stage of development (Parrish *et al*. 1959, Messieh and Rosenthal 1989). Embryos in layers near the surface but not directly in the topmost layer were reported to hatch with serious body malformations whereas eggs near to bottom layers died at an early stage of development (Messieh and Rosenthal 1989). Hempel and Schubert (1968) recorded living eggs to appear in the blastodisc stage (about 1 day after fertilization) and no differences in state of development were observed in different egg layers of the investigated egg samples, most probably due to the minor importance of oxygen for egg development within the first days after fertilization. The quantification of dead eggs in this state of development revealed an average egg mortality of 50 % throughout the entire egg sample with highest mortality rates in dense packed parts of eggs, particularly in lower egg layers with up to 80 % dead eggs (Hempel and Schubert 1968). They considered the high egg mortality to be a consequence of low egg fertilization instead of oxygen depression, indicating that egg fertilization rates are influenced by multiple egg layer expansion.

However, Parrish et al. (1959), Hempel and Schubert (1968) and Messieh and Rosenthal (1989) surveyed *in situ* egg concentrations grabbing and investigating egg samples. To my best knowledge, Taylor (1971) was the only scientist so far empirically investigating the effect of egg concentration on reproduction success by artificially creating a defined number of egg layers (2, 4, 6, 8 and 10 egg layers). In general, Taylor (1971) found a decrease in hatching success with increasing number of egg layers indicating that intensity of egg deposition is the major factor modifying reproductive success. However, this study was focused on Pacific herring (*Clupea pallasii*, Valenciennes 1847) and does not include information on the effects of certain egg layers on fertilization success and egg development. For WBSS herring no records on the influence of intensity of egg deposition on reproductive success in terms of fertilization success, subsequent embryonic development and hatching success are available.

To address this knowledge gap, the present study investigated the effects of three dimensional egg masses, which are defined by the number of egg layers, on the fertilization success and egg mortality rate. Thereby, experiments were repeated including herring from the initial and second spawning cohort in the Greifswalder Bodden and from a different spawning ground, the Warnow river. Comparisons between this spawning groups should show if the effect of egg layering becomes rather distinct under certain conditions.

Additionally, the effects of egg density within single layer herring spawn on fertilization rate and egg development are investigated. Therefore, experiments on fertilization success and mortality rates of herring spawn were performed. A defined number of egg layers in a range from 1 to 10 layers and two different densities were performed by artificial strip spawning of ripe herring according to test the following hypotheses:

H_0: The amount of egg layers does not influence the herring reproductive success.

To test the null hypothesis, the following questions were investigated:

i. Is the egg fertilizations success influenced by the number of egg layers?

ii. Is the egg mortality influenced by the number of egg layers?

iii. Are the fertilization rate and egg mortality rate influenced by egg density in single layered spawn?

iv. Do the effects of egg layering become rather distinct in certain spawning cohorts and habitats?

2 Material and methods

2.1 Investigation areas

Fish were sampled in the Greifswalder Bodden and the Warnow river estuary. The Greifswalder Bodden is an inshore coastal lagoon in the southern Baltic Sea. The lagoon is formed by the German mainland in the south and the island of Rügen in the north, leaving two passages connecting the Bodden to the Baltic Sea (Fig. 1). The bigger opening of the lagoon is between Thiessow on Rügen and Peenemünde on Usedom in eastern direction. Westwards the Strelasund separates the island of Rügen from the German mainland.

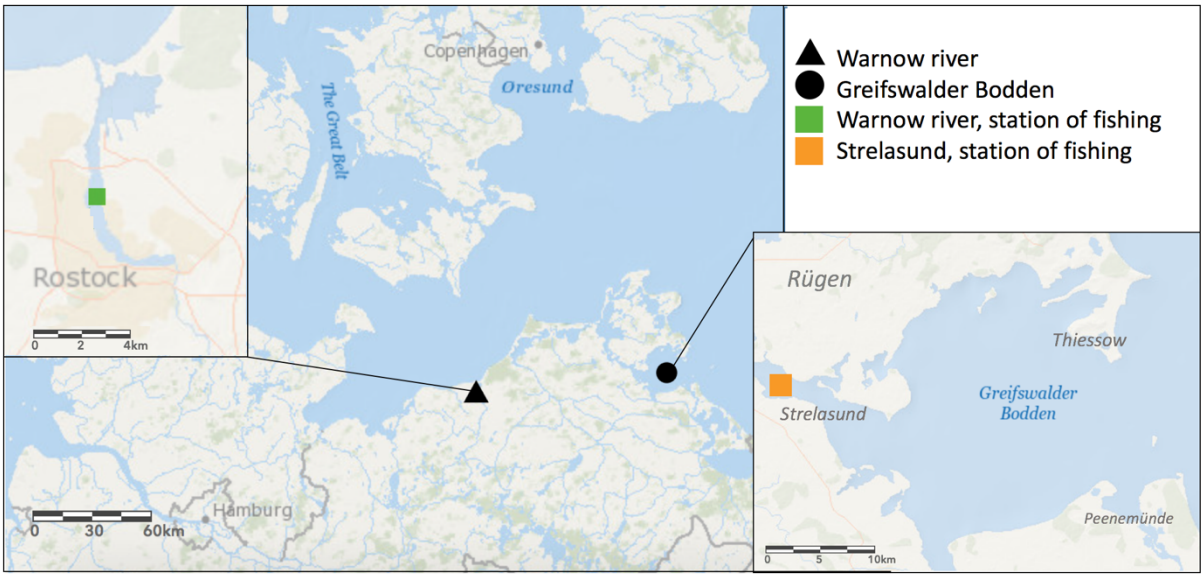

Fig. 1. Map of the investigation areas: Estuary of Warnow river (left) and Greifswalder Bodden (right), relevant for fish collection (orange and green squares) (Esri, AcrGIS 2018).

Due to the semi-enclosure, the Greifswalder Bodden is a shallow lagoon of brackish water with a mean depth of 5.6 m and a maximum depth of 13.5 m (Schiewer 2001). Because of wind induced water circulations the water column is mostly well mixed. Therefore, temperature and salinity stratification rarely occur and high saturation of dissolved oxygen prevail. The Greifswalder Bodden has an average salinity of 7.5 PSU (Schiewer 2001) and water temperatures varied between 3 °C and 12 °C during the fish collection in spring 2018.
The Greifswalder Bodden is known as one of the most important spawning and nursery areas for the Western Baltic spring spawning herring (Oeberst *et al*. 2009, Polte *et al*. 2017) and several herring cohorts migrate into inner coastal waters to spawning beds, located in the littoral zone of the lagoon.
The Warnow river originates near to the provincial capital Schwerin and ends in the southern Baltic Sea about 100 km westwards the Greifswalder Bodden (Fig. 1, middle). The estuary of

the river (Fig. 1, left) is characterized by urban development, industries and ports, tourism and a tunnel connecting the east bank with the west bank of the river. The water conditions quickly change due to the interaction of sea and fresh water showing a mean pH-value of 8.3 (min.: 7.8, max.: 8.7), an average salinity of 11.7 PSU (min.: 9.6 PSU, max.: 18.8 PSU) and water temperatures between 1.7 °C and 20.1 °C within the last three years (Landesamt für Umwelt, Natur und Geologie 2018). Every year, herring cohorts also enter the Warnow river estuary for spawning, however, explicit spawning grounds were not yet identified (although spawning was observed at the “Gehlsdorf ferry port” downtown of Rostock in 2010, Kotterba and Polte pers. observation).

2.2 Induced spawning and selected substrates

The aim of this study was to investigate the influence of multiple egg layers on its fertilization success and mortality rate. Therefore, laboratory experiments with treatments of different numbers of egg layers were performed. Because the number of egg layers is the dependent criteria in these experiments, the induced spawning was conducted with special care in order to gain smooth layers of a defined expansion.

2.2.1 Selected substrates

Glass is supposed to have minor influences on the fertilization and mortality rate of herring eggs and was chosen as spawning substrate (McMynn and Hoar 1953, Aneer 1987, Rajasilta *et al*. 1997, Laine and Rajasilta 1999).

The first experiments on fertilization success and mortality rate were conducted using microscope slides as substrate (Fig. 2, C). The slides were stacked together in a 90° angle for stabilization of egg layers and to get an insight into the layering through the vertical slide. But artificially spawning the eggs on slides was unpractical because eggs easily fell off the slide. Moreover, the exposure of eggs to surrounding water at the remaining three sides resulted in edge effects. Therefore, microscope slides were replaced by petri dishes for the following experiments (Fig. 5). A successful adherence of eggs on the petri dish was easy because eggs could not fall off the plate and adhered in remaining gaps by gently swinging and shaking the plate, producing a smooth layer.

2.2.2 Induced spawning

To gain a defined number of egg layers, herring eggs were attached to the substrates by induced strip-spawning, a common technique since the last decades (Taylor 1971, Ojaveer 1981, Aneer 1987, Laine & Rajasilta 1999, Rajasilta *et al*. 2006, von Nordheim *et al*. 2018). Therefore, herring were caught by gillnets (3 x 25 m x 5 m; mesh size 27, 28 and 29 mm),

set in the Greifswalder Bodden and the Warnow river for 20 min – 17 h (Tab. 1). Only mature fish in good conditions was used for the strip-spawning. Injured fish and fish with malformed ovaries were not included.

Tab. 1. Date of fish collection with location, number of hauls and time summarized of all hauls, mesh size and total catch.

Date	Location	Hauls (*n*, ∑ time)	Mesh size	Total catch
06.04.18	Strelasund	1, 17 h	28/29 mm	> 100 kg
27.04.18	Strelasund	3, 1h 40 min	28/29 mm	7.29 kg
13.04.18	Strelasund	1, 20 min	28/29 mm	nm.
02.05.18	Strelasund	2, 90 min	28/29 mm	7.43 kg
08.05.18	Warnow	3, 2h 15 min	27 mm	nm.

The substrate was placed in a bucket with habitat water and spawned with ripe and running female herring. Therefore, females were gently squeezed from head to anal opening while moving the fish constantly back and forth nearly above the substrate (Fig. 2, A). The stripping of the eggs was carried out carefully to avoid blood or broken eggs which can inhibit fertilization success. Unwanted egg clusters were diluted by moving the tail fin. For the petri dish eggs were stripped onto the plate and positioned by careful shaking.

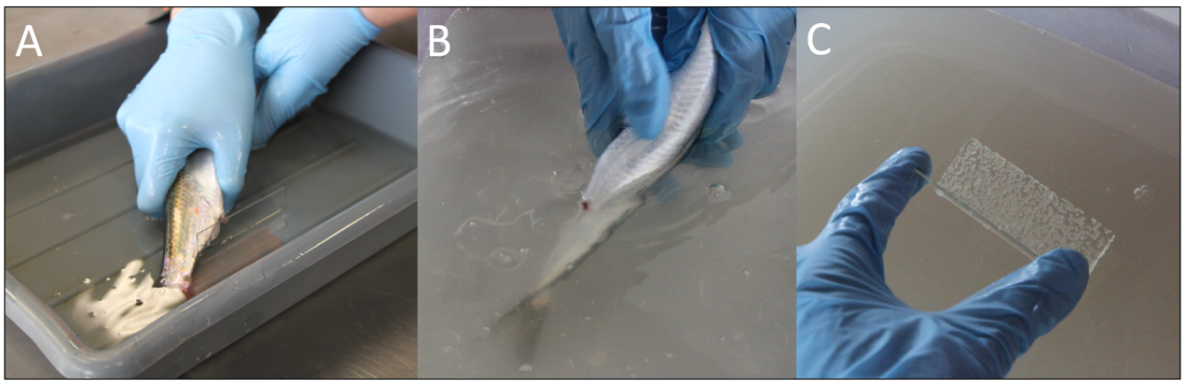

Fig. 2. Procedure of induced spawning: strip spawning eggs from female onto glass substrate (A), stripping milt from male into bucket of habitat water (B), incubating spawned microscope slide in milt-water-bucket for fertilization (C).

Depending on availability and conditions of male herring, the milt of six to ten males was mixed with habitat water in a bucket and used for the fertilization of spawned substrates (Fig. 2, B and C). If possible, three sperm buckets were provided so that only two replicates of each treatment were fertilized with the same sperm bucket to avoid paternal effects (Geffen

and Nash 2012). Also the female herring were used randomly, so that each treatment includes more than three individuals to avoid maternal effects (Geffen and Nash 2012). The incubation time of spawned substrates in the sperm buckets for fertilization was about 10 minutes. Afterwards, the substrates were rinsed with fresh sea water and stored in transport boxes filled with habitat water.

2.2.3 Number of egg layers in the experiments

In order to investigate the effects of egg layering, five treatments of different numbers of egg layers were prepared: Treatments with 1, 2, 4, 6 and 10 layers of eggs were compared in each experiment (Fig. 3, B to F). Each treatment included six replicates (n=6). In the following, the abbreviation Tx represents the treatment (T) of different numbers of egg layers (x: 1, 2, 4, 6, 10) and Lx represents a specific egg layer (L, x: 1, 2, …, 9, 10). Ten egg layers were set as maximum value because influences on fertilization and mortality rate were supposed to become visible within this range. Moreover, this layering was assumed to possibly occur *in situ* although it was not observed in the Greifswalder Bodden or the Warnow river estuary so far. However, multiple spawning activities leading to egg layering were reported although no precise number of layers was estimated (Klinkhardt 1996). Parrish *et al*. (1959) observed herring spawn up to 8 layers of eggs in the Firth of Clyde and Taylor (1971) created experiments on egg layers ranging from 2 to 10 layers.

In the course of the mortality experiments, additionally the influence of egg density on single layer spawn was investigated. Therefore, two different egg densities (loose and dense, Fig. 3, A and B) were compared and added to the experimental schedule. The single layer as mentioned before was set as dense and a second, more loose spawned single layer treatment was performed.

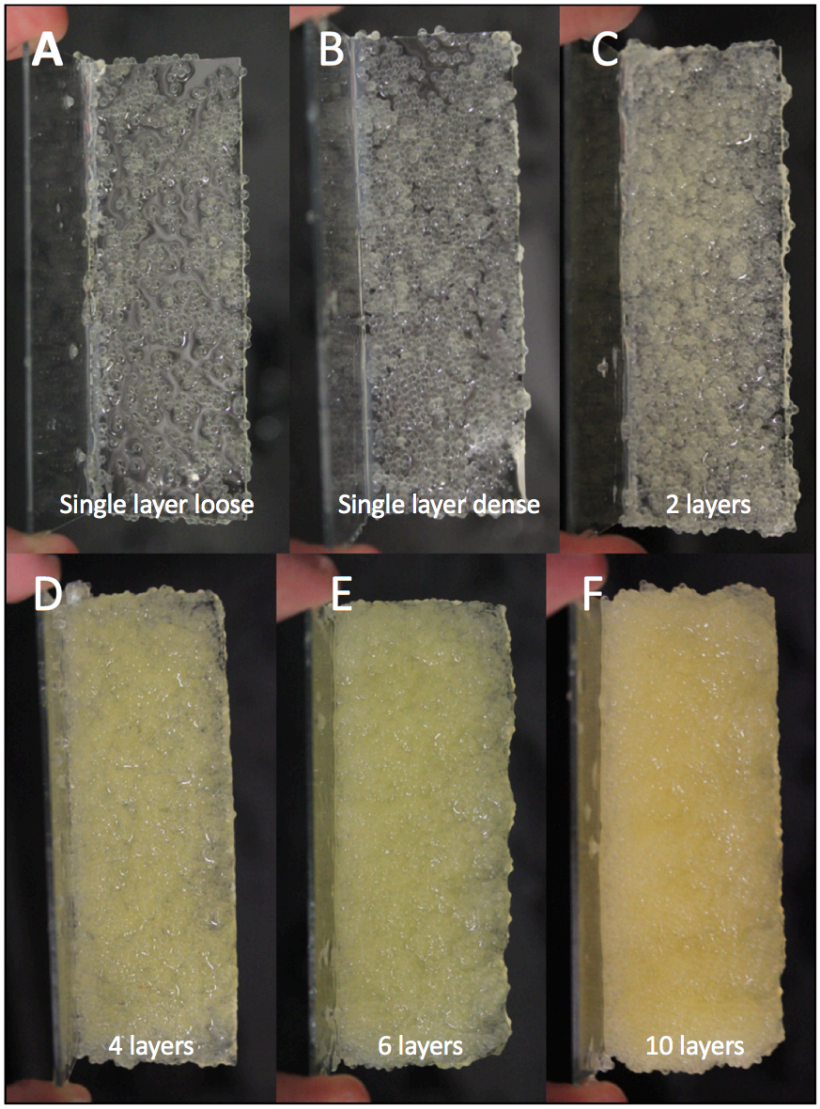

Fig. 3. Top view on different numbers of egg layers on microscope slide ranging from single layer to 10 layers (B to F) and different egg densities within single layers of eggs (A and B).

2.3 Experiments on fertilization success of herring spawn depending on the number of egg layers

Experiments were performed to investigate if the number of egg layers has an influence on the fertilization success of herring spawn. Moreover, data were used to examine the within-layer egg fertilization of selected treatments. Two fertilization experiments were conducted throughout the spawning season including fish from the initial spawning cohort (IC: Initial cohort) and the second spawning cohort (SC: Second cohort) in the Greifswalder Bodden. The fertilization experiment IC was carried out from 06.04. to 09.04.2018 and fertilization experiment SC from 27.04. to 30.04.2018.

For experiment IC the induced spawning and fertilization was carried out at the Thünen Institute of Baltic Sea fisheries in Rostock about 4 hours after catching the fish. Whereas the experiment SC was directly prepared on the research vessel FRV CLUPEA. The induced spawning was performed as described (see 2.2.2, p. 8). According to the number of egg layers in each treatment, single layers of eggs were spawned onto the substrate and the procedure was repeated until the desired number of egg layers was gained. It was essential to avoid contamination with herring sperm during the assemblage of egg layers. Due to the low total catch of herring the desired number of treatments and replicates could not be achieved during the experiment SC. The treatment with six layers of eggs (T6) as well as two replicates of each of the remaining treatments had to be rejected (resulting in n=4). Moreover, only one sperm bucket including the milt of six males could be achieved. In the experiment IC the amount of ripe males provided three sperm buckets consisting of the milt of ten males respectively.

2.4 Experiments on mortality rate of herring spawn depending on the number of egg layers

Mortality experiments were performed to investigate if the number of egg layers has an influence on the mortality rate of herring spawn. Moreover, data were used to examine the within-layer egg mortality of selected treatments. To answer the question if egg density has an impact on mortality rate in single layer herring spawn, experiments of two different egg densities (loose and dense) were carried out. Three mortality experiments were performed, including fish from different spawning cohorts and investigation areas.

The first mortality experiment was conducted from 13.04. to 23.04.2018, including fish from the initial spawning cohort in the Greifswalder Bodden (IC: Initial cohort). The induced spawning took place at the Thünen Institute in Rostock, about 5 hours after catching the herring. Due to quantity and quality of caught fish the desired number of 6 treatments and 6 replicates (see 2.2.3, p. 9) could be achieved. Moreover, the amount of ripe males provided three sperm buckets each including milt of ten males.

The second mortality experiment was conducted from 02.05. to 08.05.2018 including fish from the second spawning cohort in the Greifswalder Bodden (SC: Second cohort). The induced spawning for mortality experiment SC was conducted on the research vessel FRV CLUPEA, directly after catching the fish. According to the low total catch, the treatment with 6 layers of eggs (T6) as well as two replicates of the remaining treatments had to be rejected (resulting in n=4). In addition to that only one sperm bucket including the milt of 5 males was provided. This experiment was terminated at an early stage (after 6 days) in favor of the third mortality experiment.

The third mortality experiment was carried out from 08.05. to 18.05.2018 including fish from the Warnow river estuary (WH: Warnow herring). For experiment WH spawning was conducted at the Thünen Institute, only two hours after the fish was caught in the Warnow river. Due to the amount of ripe herring the desired number of 6 treatments and 6 replicates could be achieved. Moreover, three sperm buckets could be provided, each containing the milt of 6 male herring. Because the aim of these experiments is to investigate the mortality rate depending on the number of egg layers, a fertilization rate of 100 % is required. Therefore, every single egg layer of each treatment was fertilized before the next layer of eggs was adhered to ensure the maximum sperm supply for all eggs. However, the induced spawning was performed similarly for all experiments.
The within-layer egg mortality was shown for treatments of mortality experiment IC, in which a high overall fertilization success was recorded and the full number of treatments and replicates was performed until 100°d. Because egg samples were horizontally sliced it was possible to quantify the mortality rate for each layer within selected treatments.

2.5 Laboratory setup and conditions

All experiments were conducted in the Zoological Institute, University of Rostock. The laboratory setup consisted of a freshwater circulation system which was connected to a climate-control unit and a water basin (Fig. 4, left). Each replicate of the different treatments was incubated in plastic tanks (Logiflex 1700 ml; Bikapack) (Fig. 4, right). The tanks were filled with habitat water either from the Greifswalder Bodden or the Warnow river. Up to 36 (6 treatments x 6 replicates) tanks were randomly placed in the temperature adjusted freshwater basin constantly cooling the tanks to 10 °C. Depending on the distance to the cold water influx, the temperatures between tanks varied about ±1 °C. This incubation temperature is considered to be adequate for vital development of herring eggs (Klinkhardt 1996, Peck *et al*. 2012). For oxygen supply and gentle water circulation an aeration system was installed that ended in pipette tips which were placed into every tank (Fig. 4, right). During the experiments a natural day and night rhythm and a daylight supply of 13-14 h per day were provided.

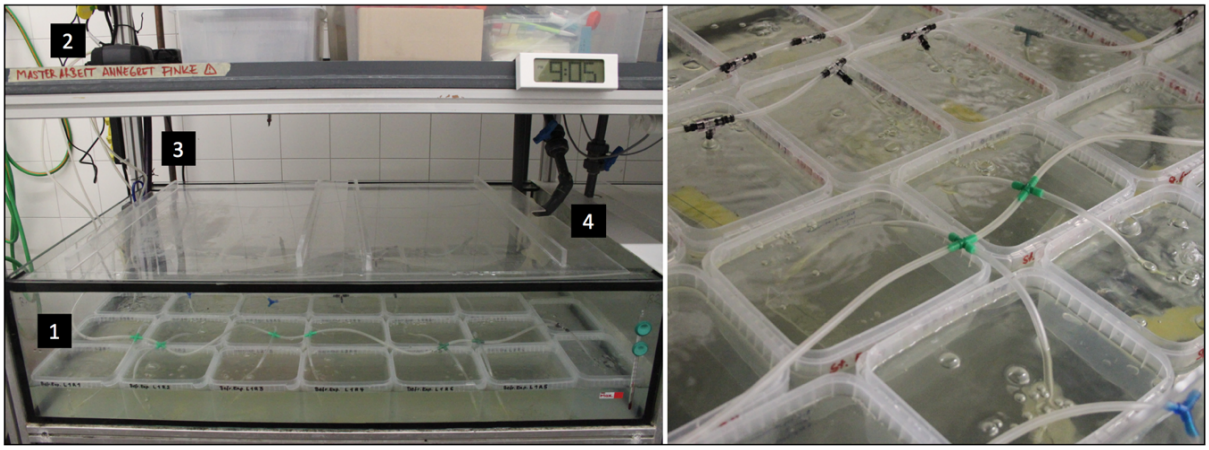

Fig. 4. Experimental setup: left: temperature adjusted fresh water basin (1), aquarium pump for oxygen supply (2), influx and efflux of cooled fresh water (3, 4), right: aeration system for subsequent oxygen supply in each plastic tank.

Water renewals were conducted using fresh habitat water every three days. Due to rather critical water conditions, water from Warnow river was filtered before exchange (100 µm). Water temperatures, salinities, pH-values and dissolved oxygen concentrations were measured using a handheld field probe (YSI Professional). Measurements were conducted every three days before every water renewal and egg sampling. Fresh habitat water had a higher temperature of 14.5 (fertilization experiment IC) to 17.9 °C (mortality experiment WH.) which was cooled down to 10 °C by climate control and kept stable in all tanks throughout the experiments (Tab. 36, p. A13). Oxygen level was near saturation level all the time with minor fluctuations. The pH-value was also constant throughout experiments and time, resting between 8.3 (mortality experiment IC) and 8.9 (mortality experiment WH). Salinity showed fluctuations between minimum values of 5.4 PSU (±0.7) (mortality experiment IC) and maximum values of 6.6 PSU (fertilization experiment IC) for the habitat water from Greifswalder Bodden (Tab. 36, p. A13). Salinity of water from Warnow river changed almost daily which resulted in an enormous increase or decrease of salinity in the tanks after water exchange (Tab. 36, p. A13).

2.6 Sampling and fixation of herring eggs

Throughout the experiments, egg samples were taken at the same time, based on the unit degree days (°d = temperature (°C) x days (d); with 10 °C) for the estimation of the age of herring eggs and the prediction of their hatching date, introduced by Apstein (1909).
During the fertilization experiments egg samples were taken at 25°d although the fertilization success of herring eggs became visible already after 30 minutes (Fig. 6, left). For fixation,

the whole spawned substrate (e.g. microscope slide or petri dish) was transferred into 4 % borax-buffered formalin.

In the course of the mortality experiments egg sub-samples of about one third of the entire spawn were taken at 20-25°d serving as fertilization control. To investigate subsequent embryonic development a second third of the entire spawn was taken at 60-70°d. The last egg sample was taken shortly before hatching at 100°d. All egg samples were preserved in 4 % borax-buffered formalin.

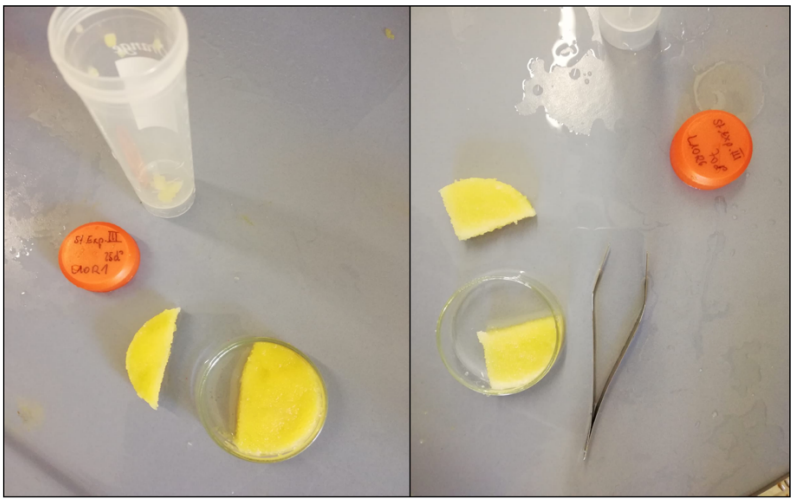

Fig. 5. Egg sampling of one third of the entire spawn at 25°d (left) and 70°d (right).

2.7 Data analysis and statistics

2.7.1 Analysis of egg samples

Egg samples were analyzed by observation through a dissection microscope and eggs were classified into four states: Fertilized or not fertilized at 20-25°d sampling and alive or dead at 60-70°d and 100°d. The states were classified according to Klinkhardt (1996) (Fig. 6, right) and quantified to investigate the fertilization or mortality rate. Figure 6 (left) displays own observations of different stages of development.

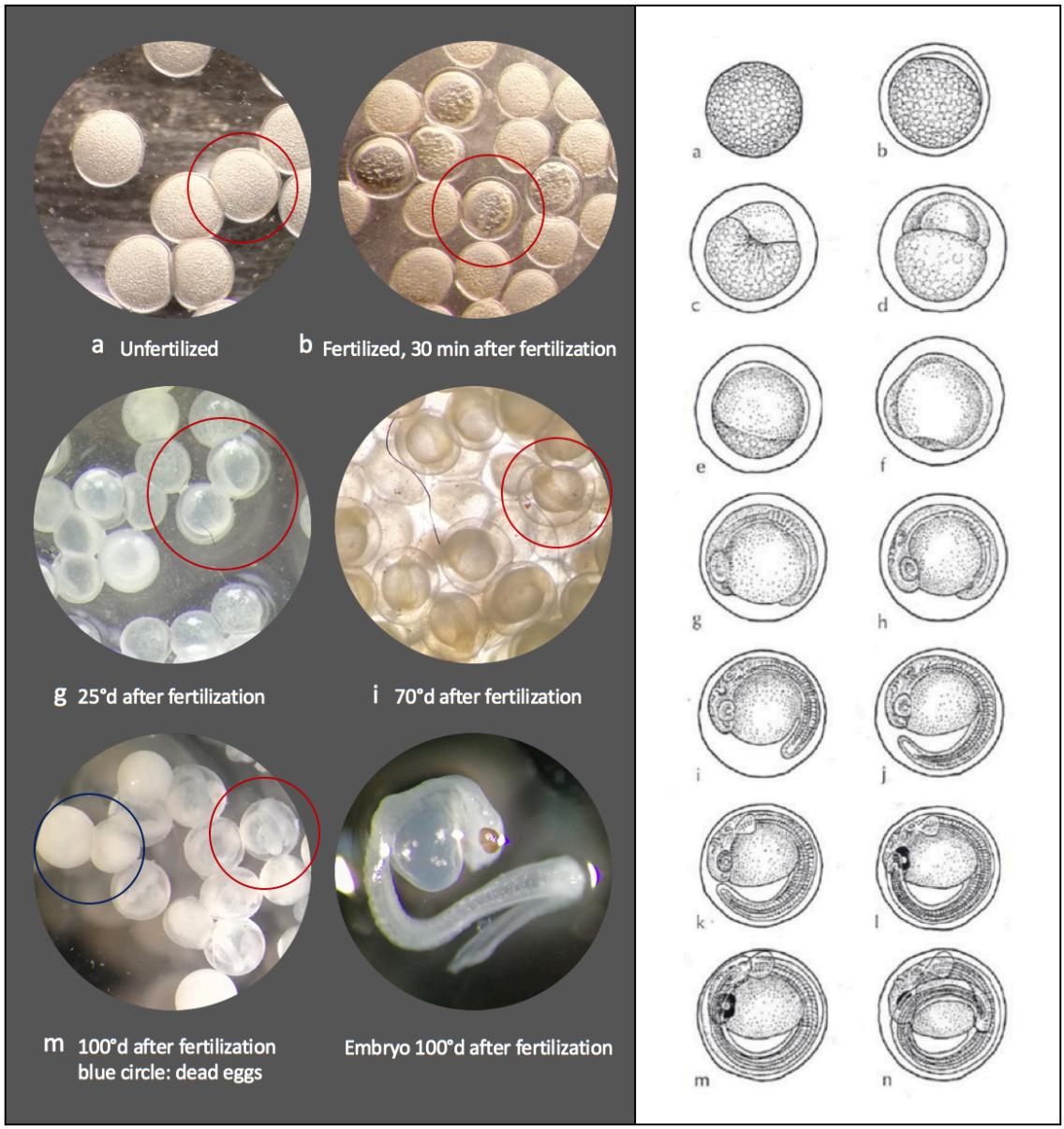

Fig. 6. Overview of developmental stages of eggs and embryos: Left: Own observations of unfertilized (a) and fertilized eggs in different stages of development (b, g, I, m), letters according to Klinkhardt (1996), right: Unfertilized egg (a), fertilized and swelled egg, yolk sac and chorion are separating (b), embryonic shield is visible, head gets distinguishable (g), embryo keeps growing and head region develops (i), embryo curls around yolk sac, eye pigmentation increases and movement of embryo starts (m).

For analysis of egg fertilization and mortality rates within layers, horizontally cuts were conducted within egg clusters consisting of more than two layers (T4, T6, T10). The number of cuts increased with increasing number of egg layers dividing the entire sample into slices of 2 egg layers (Fig. 7, A). For treatment T4 one cut was set at the half of the entire egg cluster. For treatment T6 two cuts were set, each at one third of the entire egg cluster. In treatment T10 more than 3 cuts were not possible to conduct in order to preserve the egg layers from damage. Moreover, it was difficult to count the specific layers and to visually

estimate where to cut. Therefore, the first cut was set at one half of the entire egg sample The second and third cut was set at the half of the newly formed two egg subsamples (Fig. 7, A).

For analysis of certain egg layers within selected treatments, the layers affected by cutting were summarized as L2-L3 and L3-L4 as well as L7-L8 and L8-L9 because mixing of these egg layers could not be excluded. All in all, the performance of cuts to analyze and quantify eggs throughout multiple egg layer treatments worked successfully as results showed. During the fertilization experiment SC (second spawning cohort) the procedure of cutting was not yet established. As a result, the within-layer egg fertilization in treatment T10 which was only cut at one half is shown by only 4 layers of eggs (L1, L4, L5, L10) (3.2.2., p. 27).

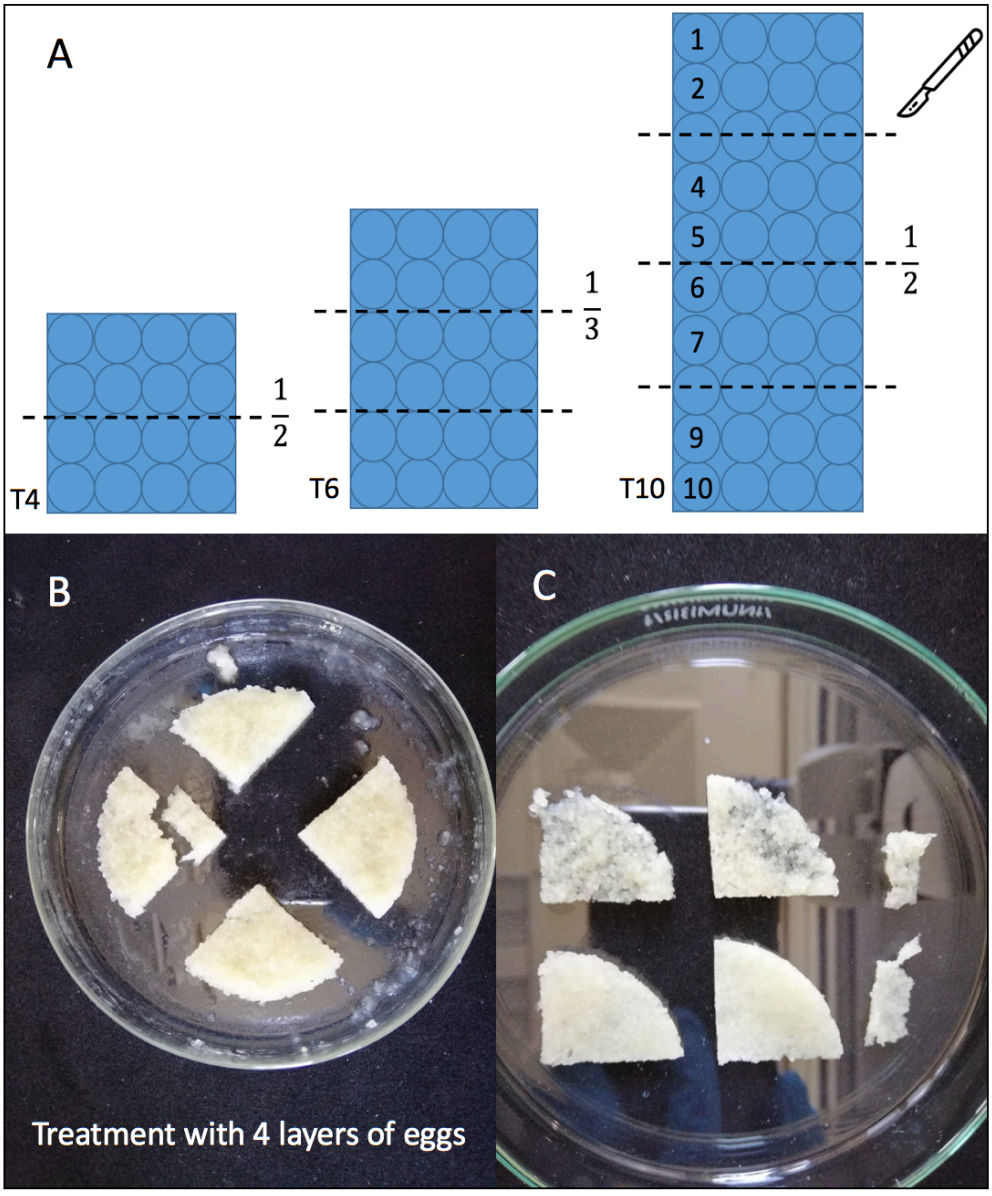

Fig. 7. A: Horizontal cuts within treatments consisting of more than two layers (T4, T6, T10), B: Egg sample (T4, 25°d) divided into four subsamples, C: Two subsamples were cut at the half respectively resulting in two surface (L1 + L2) and two bottom (L3 + L4) layers.

Fertilization success was calculated as the number of fertilized eggs in perspective to the total number of eggs so a relative percentage of fertilized eggs was determined.

$$(1)\ \%\ \text{fertilized eggs} = \frac{n\ \text{fertilized eggs} * 100}{n\ \text{of eggs}}$$

In the same way the percentage of unfertilized eggs was calculated and further used to correct the number of dead eggs in later samplings. Because unfertilized eggs were supposed to decease quickly it is important to exclude them from experimental effects. Therefore, the percentage of unfertilized eggs of the total number of dead eggs (counted) in later samplings was calculated resulting in the number of dead eggs due to the unfertilized state (unfertilized). This amount is then subtracted from the number of dead eggs to isolate the number of dead eggs according to the mortality effects of multiple egg layers (effected).

$$(2)\ n\ \text{dead eggs (unfertilized)} = \frac{\%\ \text{unfertilized eggs} * n\ \text{dead eggs (counted)}}{100}$$

$$(3)\ n\ \text{dead eggs (effected)} = n\ \text{dead eggs(counted)} - n\ \text{dead eggs (unfertilized)}$$

To estimate a relative percentage of the mortality of eggs, the modified number of dead eggs (effected) in perspective to the total number of eggs was calculated for samplings at 60-70°d and 100°d.

$$(4)\ \%\ \text{mortality of eggs} = \frac{n\ \text{dead eggs (effected)} * 100}{n\ \text{of eggs}}$$

2.7.2 Egg weight and number of eggs per treatment

In the course of the fertilization experiments the whole egg sample was extracted from the substrate after quantification. Dry weight was estimated after incubating eggs for 48 hours at 80°C. In the course of the mortality experiments subsamples taken at 20-25, 60-70 and 100°d were also extracted from the substrate after quantification. Dry weight was estimated

respectively and summarized for each replicate to gain the total dry weight of all eggs initially attached to the substrate.

The total numbers of eggs in the (sub)samples of single layer treatments (loose and dense spawned) were counted. Relating the total number with the DW of eggs in the (sub)samples, a mean dry weight per egg was estimated for all single layer treatments. Depending on the number of single layer treatments and replicates in the experiments the mean DW per egg was based on 4-12 values. The mean egg DW was calculated for every fertilization and mortality experiment based on fish of different spawning cohorts and investigation areas.

Using the mean egg DW of a certain spawning cohort and habitat and the DW of each (sub)sample, the total number of eggs that were initially attached to the substrate of multiple layer treatments was calculated. To know the total number of eggs per treatment was an essential baseline for subsequent evaluation of the methodological success in composing multiple egg layers.

2.7.3 Statistics

Data have been processed with Excel 2016 (macOS) and were analyzed with R Studio (Version 1.0.143; RStudio Inc.). Different fertilization and mortality rates of single or multiple layer treatments were tested for significance, performing a one-way analysis of variance (ANOVA). Requirements for an ANOVA are in the order of their importance the independence of data within and among samples, balanced experiments with the same number of replicates (n), homogeneity of variances and normal distribution (Underwood 1997).

The Shapiro-Wilk-test was carried out to evaluate normal distribution and Levene's test was used to test homoscedasticity. If homoscedasticity was not accomplished by data transformation, ANOVA was performed anyway based on Underwood (1997) who describes the ANOVA to be robust against non-normal distribution and heteroscedasticity in particular when experiments were "large" (about 5 treatments and 6 replicates) and balanced. Multiple pairwise comparisons between mean values were performed using Tukey's Honest Significant Difference (HSD) test. For all statistical analysis the significance level was assigned as $p \leq 0.05$. The correlation between fertilization or mortality rate and egg layer thickness or specific egg layers was tested by linear regression analysis.

3 Results

3.1 Initial spawning cohort Greifswalder Bodden

During the initial spawning season, the first experiments on fertilization and mortality rate of herring spawn depending on the number of egg layers were conducted including herring from the Greifswalder Bodden. For the fertilization experiment IC, a mean egg DW of 216 µg ±28 µg was recorded. For the mortality experiment IC mean egg DWs of 254 µg ±37 µg, 233 µg ±56 µg and 184 µg ±36 µg were observed for the 25, 70 and 100°d sampling events. The comparison between mean egg DWs of different sampling events revealed a significant difference in mean egg DW between 25 and 100°d (p<0.001, df=21.96).

3.1.1 Fertilization success of herring spawn depending on the number of egg layers (IC)

The fertilization success was calculated as the number of fertilized eggs in relation to the total number of eggs in a sample (see 2.7.1, p. 17). Mean fertilization rates were compared between treatments including different egg layer expansion to discover any related effects.

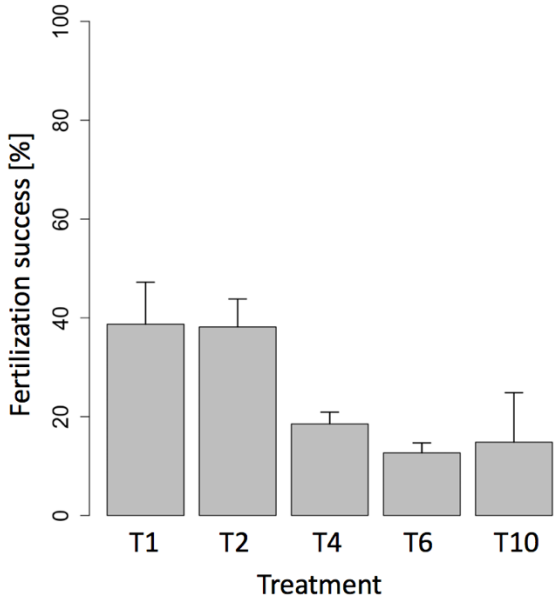

Fig. 8. Fertilization success [%] of herring spawn at 25 °d, analyzed by treatments of different numbers of egg layers (T1-T10, n=6) in experiment IC, bars show mean values and standard deviation. (Significances and data see Tab. 11, Tab 12, p. A1).

In the fertilization experiment IC fertilization success varies from 38.7 % (SD ±8.5) in T1 and 38.2 % (SD ±5.7) in T2 to 18.5 % (SD ±2.4) in T4, 12.7 % (SD ±2.0) in T6 and 14.8 % (SD ±10.0) in T10 (Tab. 11, p. A1). Significant differences between the mean number of fertilized eggs in treatments of different egg layer expansion could be detected (ANOVA, p<0.001;

F(4,25)=19.12). Tukey's HSD post hoc test showed a significantly higher fertilization rate for treatment T1 and T2 than in treatments T4, T6 and T10 (Tab. 12, p. A1; *df*=4). Between treatments T1 and T2 no significant difference was observed. The mean number of fertilized eggs in T10 is higher than in treatment T6 with a lower number of egg layers although it is not statistically proved.

3.1.2 Within-layer fertilization success depending on the total number of egg layers (IC)

Because egg samples were horizontally sliced it was possible to examine the fertilization success for each layer within a selected treatment. To show the fertilization success throughout layers, treatments of high numbers of egg layers (T6 and T10) were chosen from fertilization experiment IC, figures are emphasized by green coloring.

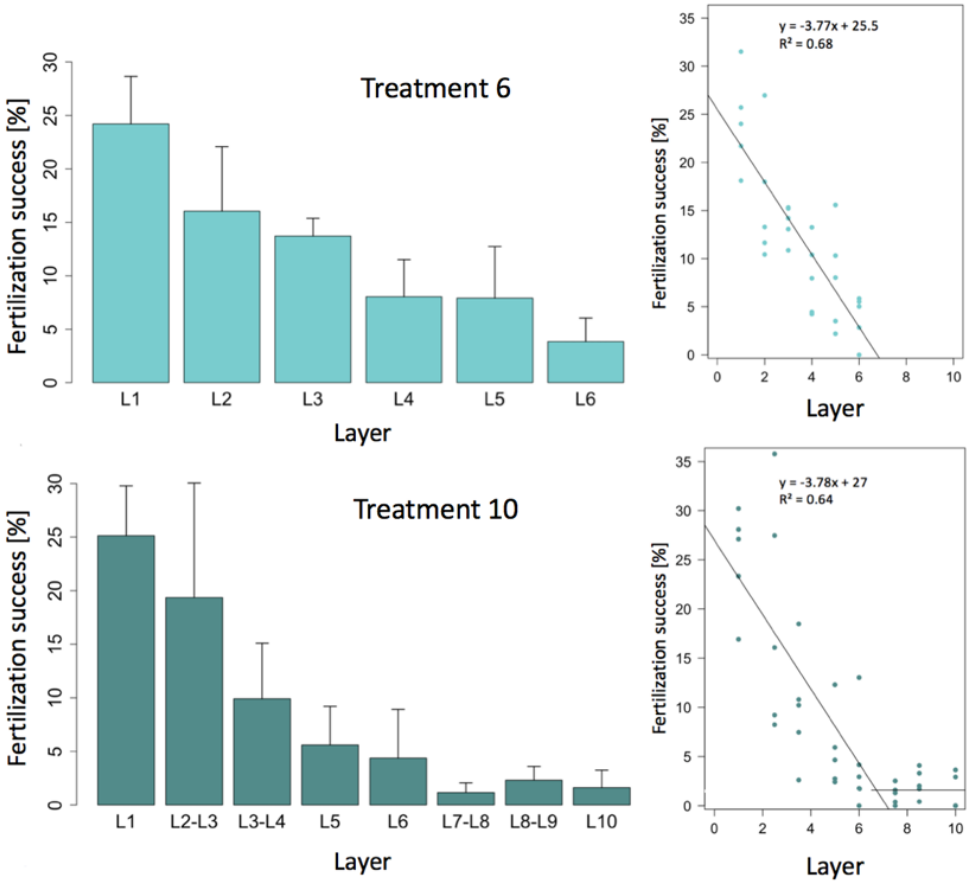

Fig. 9. Left: Fertilization success [%] throughout layers within treatment 6 (light green) and 10 (dark green), bars show mean values and standard deviation, right: Linear regression of fertilization success [%] in relation to layers within treatment T6 and T10 in experiment IC, colored dots represent replicates (Data see Tab. 13-15, p. A2-A3).

Tab. 2. Differences in mean egg fertilization [%] between layers within treatment 10 of experiment IC. Significance values (*p*-value) of multiple comparisons using Tukey's HSD post-hoc test.

Layer	L1	L2-L3	L3-L4	L5	L6	L7-L8	L8-L9	L10
L1	------	0.888	**0.003**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
L2-L3	0.888	------	0.431	**0.041**	**0.004**	**< 0.001**	**0.001**	**< 0.001**
L3-L4	**0.033**	0.431	------	0.922	0.431	**0.033**	0.186	**0.014**
L5	**0.001**	**0.041**	0.922	------	0.984	0.375	0.848	0.209
L6	**< 0.001**	**0.004**	0.431	0.984	------	0.888	1.000	0.701
L7-L8	**< 0.001**	**< 0.001**	**0.033**	0.375	0.888	------	0.992	1.000
L8-L9	**< 0.001**	**0.001**	0.186	0.848	1.000	0.992	------	0.940
L10	**< 0.001**	**< 0.001**	**0.014**	0.209	0.701	1.000	0.940	------

Dependent value: Fertilization success. Bold values show significance ($p \leq 0.05$).

Tab. 3. Comparison between mean egg fertilization [%] of layers L1-L6 within treatments T6 and T10 using the t.test.

	$L1_{L10}$-$L1_{L6}$	$L2\text{-}L3_{L10}$-$L2_{L6}$	$L2\text{-}L3_{L10}$-$L3_{L6}$	$L3\text{-}L4_{L10}$-$L3_{L6}$	$L3\text{-}L4_{L10}$-$L4_{L6}$	$L5_{L10}$-$L5_{L6}$	$L6_{L10}$-$L6_{L6}$
df	7.892	6.304	4.190	4.808	6.997	7.378	5.770
p-value	0.784	0.601	0.355	0.221	0.568	0.468	0.838

Dependent value: Fertilization success. Bold values show significance ($p \leq 0.05$).

The fertilization success of eggs in layers L1 to L6 within treatment 6 (light green) show an increase from the maximum value of 25.7 % (SD ±5.2) for L1 to a minimum value of 3.7 % (SD ±2.1) for L6 (Fig. 9. Left, light green). The fertilization rate in L2 is 17.6 % (SD ±6.4) and L3 is 14.6 % (SD ±2.5). The mean egg fertilization of 7.2 % (SD ±3.7) in L4 and 7.2 % (SD ±4.7) in L5 is nearly the same. Significant differences in mean egg fertilization were discovered (ANOVA, $p<0.001$; $F(5,24)=10.84$) and confirmed by Tukey's HSD post-hoc test between L1 and L4, L5 and L6 as well as between L6 and L2 and L3 (Tab. 13, p. A2). The R^2 value of 0.68 (Fig. 9, right) indicates a linear relationship (Fig. 9, right, light green). The fertilization success in treatment 10 shows an increase from L1 to L7/L8 and then a slight decrease to L8/L9 before the fertilization rate again increases in the lowest layer L10 (Fig. 9, left, dark green). A maximum value of 25.1 % (SD ±4.7) and a minimum value of 1.2 % (SD ±0.9) were recorded in L1 and L7/L8 (Tab. 15, p. A3). A fertilization success of 2.3 % (SD ±1.3) in L8L/9 and 1.3 % (SD ±1.6) in L10 was recorded. Significant differences between mean numbers of fertilized eggs were revealed (ANOVA, $p<0.001$; $F(7,32)=13.61$) and pairwise comparisons were carried out using Tukey's HSD post-hoc test (Tab. 2). The linear regression was applied on mean egg fertilization rates of T10 indicating a linear correlation between fertilization success and layers from L1 to L7/L8. As the bar plot demonstrates,

fertilization rate in L7/L8, L8/L9 and L10 are at a similar low level represented by a non-linear regression line (Fig. 9, left, dark green). Pairwise comparisons of mean egg fertilization in layers L1 to L6 between treatments T6 and T10 show no significant differences (Tab. 3).

3.1.3 Mortality rate of herring spawn depending on the number of egg layers (IC)

The mortality rate was calculated as the number of dead eggs in relation to the total number of eggs in a sample (see 2.7.1, p. 17). Mean numbers of dead eggs were compared between treatments including different egg layer expansion to discover any related effects. The overall fertilization rate in the first mortality experiment was more than 60 % in all treatments and did not affect the below results (Tab. 16, p. A3).

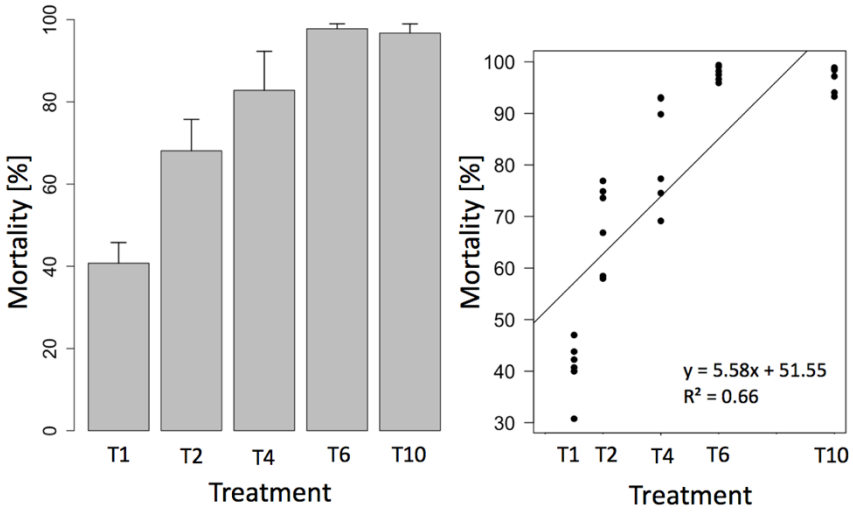

Fig. 10. Left: Mortality [%] of herring spawn at 70°d, analyzed by treatments of different numbers of egg layers (T1-T10, *n*=6) in experiment IC, bars show mean values and standard deviation, right: Linear regression model of mortality rate [%] in relation to treatments, colored dots represent replicates (Significances and data see Tab 17, p. A4).

Tab. 4. Differences in egg mortality of different treatments at 70°d in mortality experiment IC Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Treatments	T1	T2	T4	T6	T10
T1	------	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
T2	**< 0.001**	------	**0.011**	**< 0.001**	**< 0.001**
T4	**< 0.001**	**0.011**	------	**< 0.001**	**0.001**
T6	**< 0.001**	**< 0.001**	**< 0.001**	------	0.975
T10	**< 0.001**	**< 0.001**	**0.001**	0.975	------

Dependent value: Egg mortality. Bold values show significance ($p \leq 0.05$).

Tab. 5. Mortality rate of herring spawn at 100°d in mortality experiment IC analyzed by treatments of different layers of eggs (T1, T2, T4, T6, T10; *n*=6) with mean value and standard deviation (SD).

	Mortality [%]				
Treatments	T1	T2	T4	T6	T10
Mean ±SD	89.1 ±6.8	98.9 ±0.5	99.4 ±0.6	99.9 ±0.1	99.3 ±1.0

In the mortality experiment IC, egg mortality at 70°d shows a stepwise increase from T1 to T10 with mean values ranging from 40.8 % (SD ±5.0) in T1 and 68.1 % (SD ±7.7) in T2 to 82.8 % (SD ±9.5) in T4 (Tab. 17, p. A4). Highest mortality rate was recorded in treatments T6 and T10 with 97.8 % (SD ±1.2) and 96.7 % (SD ±2.3). Significant differences in mean egg mortality were detected (ANOVA, $p<0.001$; $F(4,25)=73.52$) and confirmed by Tukey's HSD test between every treatment accept for T6 toT10 (Tab. 4). The linear correlation between mortality and the number of egg layers of T1-T10 was tested applying a linear regression line (Fig. 5, top right). The R^2 value of 0.66 indicates a linear regression that would be even higher if T10 wasn't considered because data showed that there was no difference between mean mortality in T6 and T10**.** Egg mortality rate at 100°d was high in all treatments (Tab. 5). Significances in mean egg mortality were detected (ANOVA, $p<0.001$; $F(4,25)=18.87$) between T1 to all other treatments and confirmed by Tukey's HSD post hoc test ($p<0.001$) (Tab. 18, p. A4).

3.1.4 Within-layer egg mortality in particular egg layers depending on the total number of egg layers (IC)

The mortality rate in particular egg layers was shown for treatments T4 and T10 of mortality experiment IC. The overall fertilization success of all layers of T4 was above 70 % and above 65 % for T10 and did not affect the results below.

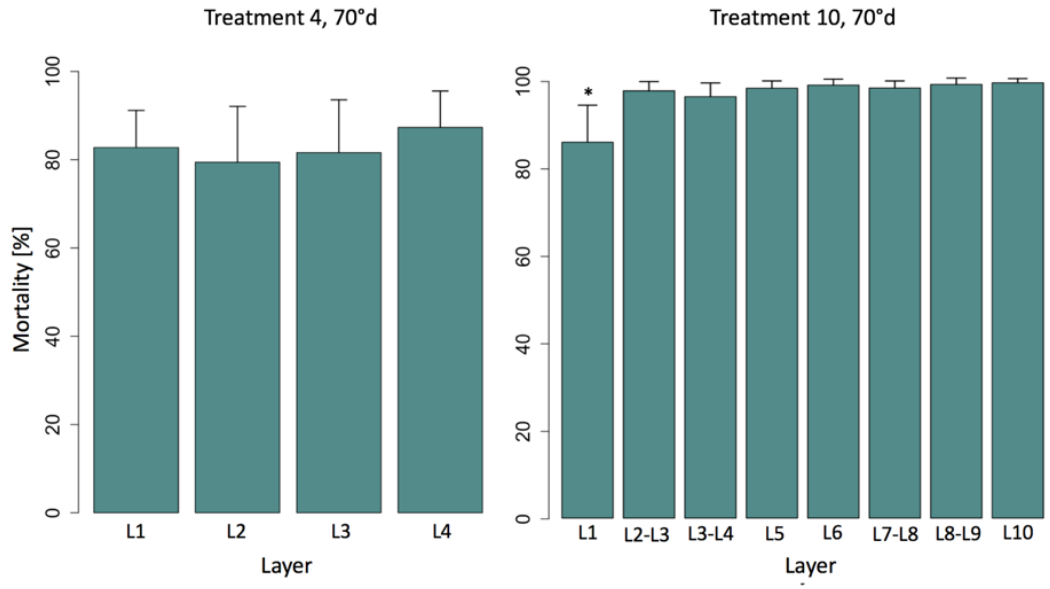

Fig. 11. Egg mortality [%] in different egg layers within treatment T4 (left) and T10 (right) at 70°d in experiment IC, bars show mean values and standard deviation (Significance and data see Tab. 19 - 21, p. A5 – A6).

In T4 egg mortality was high in all layers, ranging from 79.4 % (SD ±12.7) in L2, 81.6 % (SD ±11.9) in L3 and 82.8 % (SD ±8.4) in L1 to 87.3 % (SD ±8.3) in L4 (Tab. 19, p. A5). Significant differences in mean egg mortality were not detected (ANOVA, p=0.687; F(3,20)=0.5). In T10 egg mortality was more than 96 % in all layers accept for L1 (Fig. 11). In L1, lowest mortality rate of 85.8 % (SD ±8.4) was recorded (Tab. 31, p. A10). Significant differences in mean number of dead eggs were detected (ANOVA, p<0.001; F(7,40)=7.57). Tukey's HSD revealed a significant difference between L1 to all other layers (Tab. 21, p. A 6).

Tab. 6**.** Egg mortality [%] in different egg layers within T4 of mortality experiment IC at 100°d with mean values and standard deviation (SD).

	Mortality [%]			
Layers	L1	L2	L3	L4
Mean ±SD	98.6 ±1.1	99.4 ±1.4	99.8 ±0.4	100.0 ±0.0

Tab. 7. Egg mortality [%] in different egg layers within T10 of mortality experiment IC at 100°d with mean values and standard deviation (SD).

	Mortality [%]							
	L1	L2-L3	L3-L4	L5	L6	L7-L8	L8-L9	L10
Mean	97.5	98.6	99.8	99.6	99.3	100.0	100.0	99.7
±SD	±4.6	±3.1	±0.4	±0.8	±1.7	±0.0	±0.0	±0.7

Egg mortality at 100°d was high in all layers within T4 with a minimum value of 98.6 % (SD ± 1.1) obtained in L1 (Fig. 8). No significant differences between mean egg mortality were detected by ANOVA (p=0.130; F(3,20)=2.12). In T10 mortality varied between 97.5 % (SD ±4.6) in L1 and 100 % in L7-L8 and L8-L9 (Tab. 8). Beside L1 lowest mortality was achieved in L2-L3 with 98.6 % (SD ±3.1). No significant differences between mean numbers of dead eggs in different layers were detected (ANOVA, p=0.519; F(7,40)=0.90).

3.1.5 Mortality rate in surface egg layers (IC)

Surface layers of treatments of mortality experiment IC were used to show the mean mortality of eggs in surface layers depending on the total number of eggs in the treatments.

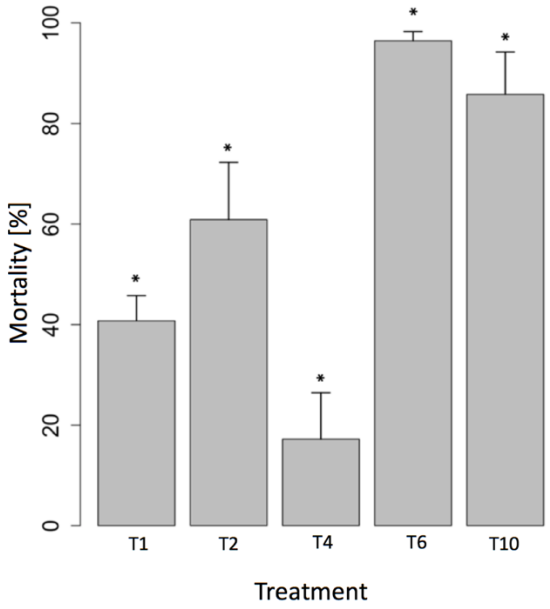

Fig. 12. Egg mortality [%] in surface egg layer of treatments of different numbers of total egg layers (T1-T10, n=6) at 70°d in experiment IC, bars show mean mortality and standard deviation, *: $p < 0.05$, ANOVA (Significances and data see Tab. 22, Tab. 23, p. A6 – A7)

The mean mortality of eggs in the surface layers of treatments T1 to T10 shows a high variability (Fig. 12). A minimum mortality of 17.2 % (SD ±8.4) was observed in T4, whereas maximum values of 85.8 % (SD ±8.4) and 96.4 % (SD ±1.8) were found in treatments T10 and T6 (Tab. 22, p. A6). In T1 a mortality rate of 40.8 % (SD ±5.0) and 60.9 % (SD ±11.4) in T2 was recorded. Significant differences were detected (ANOVA, p<0.001; F(4,25)=79.46)

and confirmed between all mean egg mortality rates of treatments T1 to T10 by Tukey's HSD post hoc test (Tab. 23, p. A7).

3.2 Second spawning cohort Greifswalder Bodden

The second fertilization and mortality experiments were conducted including fish from the second spawning cohort in the Greifswalder Bodden. For the fertilization experiment SC, a mean egg DW of 224 µg ±36 µg was recorded. In the fertilization experiment IC, a similar mean egg DW of 216 µg ±28 µg was found, showing no significant difference, tested by t.test (Tab. 35, p. A12). For the mortality experiment SC mean egg DWs of 200 µg ±20 µg and 368 µg ±41 µg were recorded for 20 and 60°d sampling. A significant difference between mean egg DW of 20 and 60°d was detected (p=0.002, df=4.30). Moreover, significant differences were detected between mean egg DWs in comparison to mortality experiment IC (Tab.35, p. A12).

3.2.1 Fertilization success of herring spawn depending on the number of egg layers (SC)

In the fertilization experiment SC, the overall egg fertilization was high in all treatments.

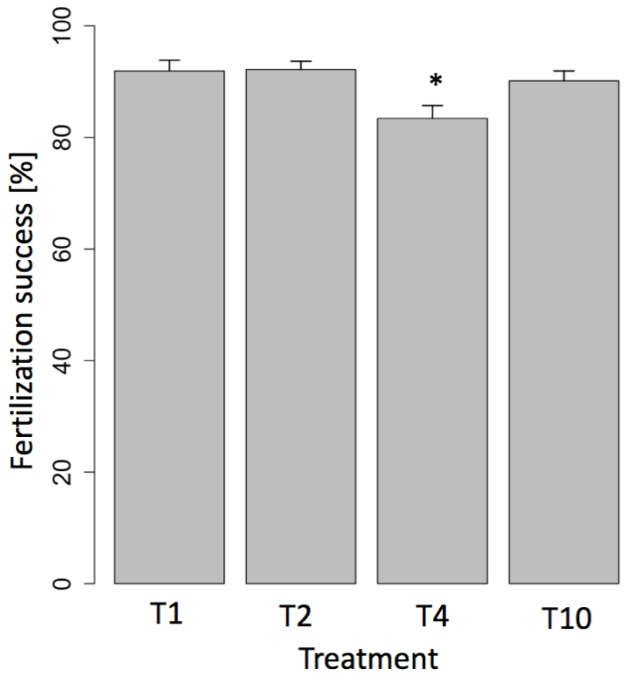

Fig. 13. Fertilization success [%] of herring spawn at 25°d, analyzed by treatments of different numbers of egg layers (T1-T10, n=4) in experiment SC bars show mean values and standard deviation, *: $p < 0.05$, ANOVA (Significance and data see Tab. 24, Tab. 35, p. A7).

The fertilization success (Fig. 13) ranges from 91.9 % (SD ±1.9) in T1, 92.2 % (SD ±1.5) in T2 and 83.4 % (SD ±2.3) in T4 to 90.1 % (SD ±1.8) in T10 (Tab. 14, p. A2). A significant difference is present between T4 compared to all of the other treatments (ANOVA, p=0.014; F(3,12)=14.09) (Tab. 25, p. A7).

3.2.2 Within-layer egg fertilization in a ten-layer treatment (SC)

In the fertilization experiment SC treatment T10 was chosen to display the egg fertilization success throughout the layers.

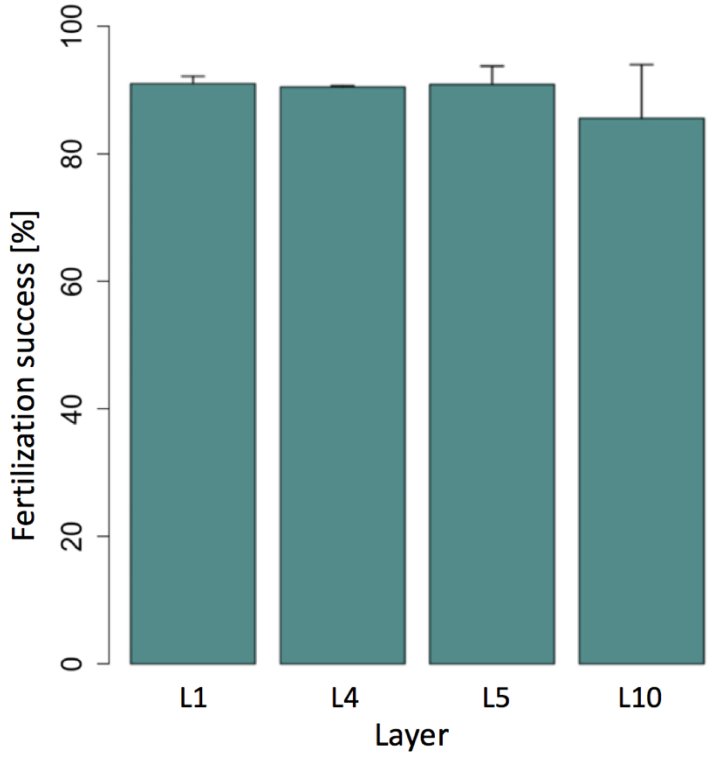

Fig. 14. Egg fertilization rate [%] at 25°d, analyzed by layers (L1, L4, L5, L10, n=6) within treatment 10 in experiment SC, bars show mean values and standard deviation (Data see Tab. 26, p. A8).

The specific layers (L1, L4, L5 and L10) of treatment 10 show a high fertilization success (Fig. 9). Mean egg fertilization rates of 91.6 % (SD ±1.2) in the surface layer L1, 91.1 % (SD ±0.2) in L4 and 91.5 % (SD ±2.9) in L5 were recorded (Tab. 26, p. A8). The lowest layer L10 shows a slightly reduced fertilization rate of 86.2 % (SD ±8.5), although this was not statistically significant (ANOVA, p=0.418; F(3,12)=1.02).

3.2.3 Mortality rate of herring spawn depending on the number of egg layers (SC)

Mean numbers of dead eggs were compared between treatments including different egg layer expansion to discover any related effects. The overall fertilization rate was more than

70 % in all treatments accept for T10 (50.4 % ± 10.9 SD) and did not affect the below results (Tab. 27, p. A8).

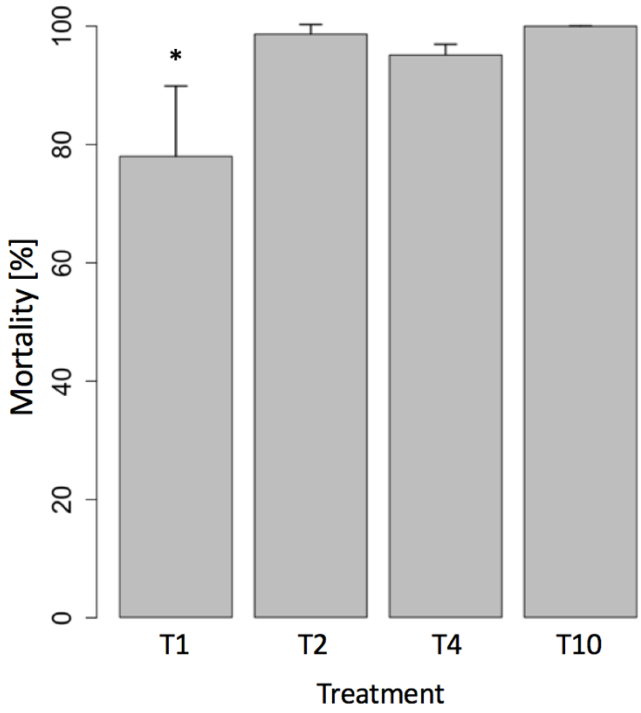

Fig. 15. Egg mortality [%] at 60°d, analyzed by treatments of different numbers of egg layers (T1-T10, n=4) in experiment SC, bars show mean values and standard deviation, *: p < 0.05, ANOVA (Significance and data see Tab. 28, Tab. 29, p. A8 – A9).

Egg mortality was high in all treatments ranging from 78.0 % (SD ±11.9) in T1, 95.1 % (SD±1.8) in T4 and 98.6 % (SD ±1.6) in T2 to 100.0 % (SD ±0.1) in T10 (Tab. 25, p. A7). Significant differences in mean egg mortality rates were detected (ANOVA, p<0.001; F(3,12)=15.57). Tukey's HSD test identified significant differences between T1 compared to all other treatments (Tab. 29, p. A9).

3.3 Warnow herring

The third mortality experiment was conducted including fish from the Warnow river. The mean egg DW found in this experiment was 170 µg ±14 µg, 144 µg ±27 µg and 158 µg ±29 µg for the 25, 70 and 100°d sampling events (Tab. 10, p. 32). A significant difference in between 25 and 70°d was revealed (p=0.011, df=16.40). Moreover, multiple significant differences in mean egg DWs in comparison the other experiments, based on herring from the Greifswalder Bodden are present (Tab. 35, p. A12).

3.3.1 Mortality rate of herring spawn depending on the number of egg layers (WH)

In the mortality experiment WH, fertilization controls show a stepwise decline in the mean number of fertilized eggs with increasing egg layer expansion. The overall fertilization did not exceed 25 % (Fig. 16, left).

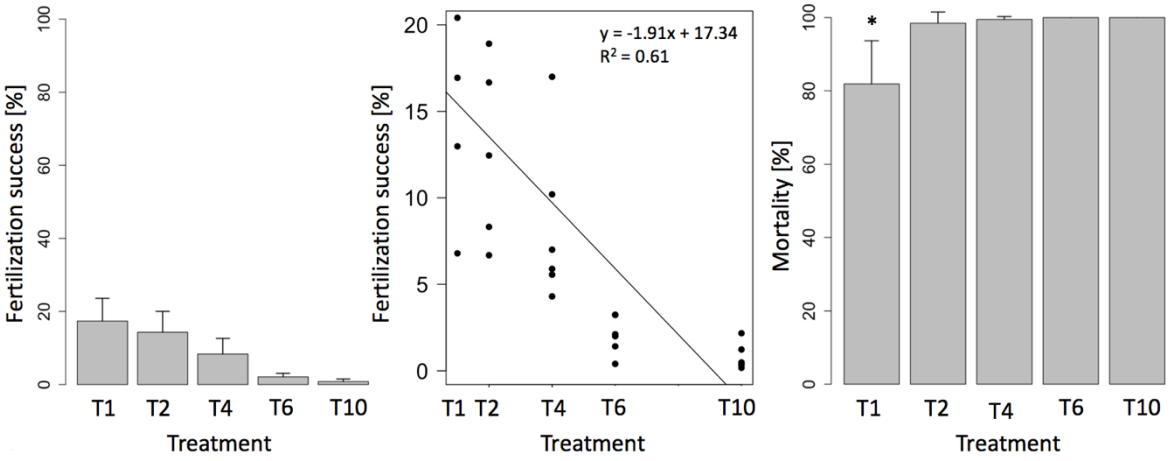

Fig. 16. Egg fertilization (Left) and mortality rate (Right) [%] at 25 and 70°d, analyzed by treatments (T1-T10, *n*=6) in experiment WH, bars show mean values and standard deviation, *: $p < 0.05$, ANOVA, Middle: Linear regression of egg fertilization in relation to treatments (Significance and data see Tab. 30 – 32, p. A9 – A10).

Tab. 8. Differences in egg fertilization [%] at 25°d of treatments of different egg layers in experiment WH. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Treatment	T1	T2	T4	T6	T10
T1	------	0.850	**0.031**	**< 0.001**	**< 0.001**
T2	0.850	------	0.231	**< 0.001**	**< 0.001**
T4	**0.031**	0.231	------	**0.015**	**0.001**
T6	**< 0.001**	**< 0.001**	**0.015**	------	0.679
T10	**< 0.001**	**< 0.001**	**0.001**	0.679	------

Dependent value: Fertilization success. Bold values show significance ($p \leq 0.05$)

Tab. 9. Mortality rate of herring spawn at 100°d, analyzed by treatments of different layers of eggs (T1-T10, *n*=6) in experiment WH with mean mortality and standard deviation (SD).

	Mortality [%]				
Treatments	T1	T2	T4	T6	T10
Mean ±SD	100.0 ±2.9	100.0	100.0	100.0	100.0

Egg fertilization was low in all treatments with maximum fertilization rates of 17.4 % (SD ±6.2) in T1, 14.3 % (SD ±5.7) in T2 and 8.3 % (SD ±4.3) in T4 (Fig. 16, left). Lowest egg fertilization was recorded in T6 with 2.1 % (SD ±1) and T10 with 0.8 % (SD ±0.7) (Tab. 30, p. A9). Significant differences between mean egg fertilization of treatments T1-T10 could be revealed (ANOVA, $p<0.001$; $F(4,25)=23.92$). Tukey's HSD post hoc test detected multiple significant differences in mean egg fertilization rates (Tab. 8). Because the bar plot indicates a linear correlation of fertilization success and treatments of different egg layer expansion (Fig. 16, left), linear regression was applied. The R^2 value of 0.61 underlines this assumption although this effect should have been prevented by fertilization of each single egg layer.
At 70°d egg mortality was higher than 80 % in all treatments (Fig. 16, right). 100 % mortality was recorded in treatments T6 and T10, while T2 and T4 reached mortality rates of 98.5 % (SD ±3.0) and 99.5 % (SD ±0.8) (Tab. 31, p. A10). Lowest mortality rate was recorded in T1 with 81.9 % (SD ±11.8). Therefore, significant differences in mean mortality rates were detected (ANOVA, $p<0.001$; $F(4,25)=8.44$). Tukey's HSD test identified significant differences between T1 compared to all other treatments (Tab. 32, p. A10). At 100°d egg mortality was 100 % in all treatments (Tab. 9). Therefore, statistical analysis was not performed.

3.4 Mortality rate of single layer spawn depending on egg density

To investigate the impact of egg density on single layer herring spawn experiments with a loose and dense layer of eggs were carried out in mortality experiments IC (Initial spawning cohort, Greifswalder Bodden) and WH (Warnow herring).

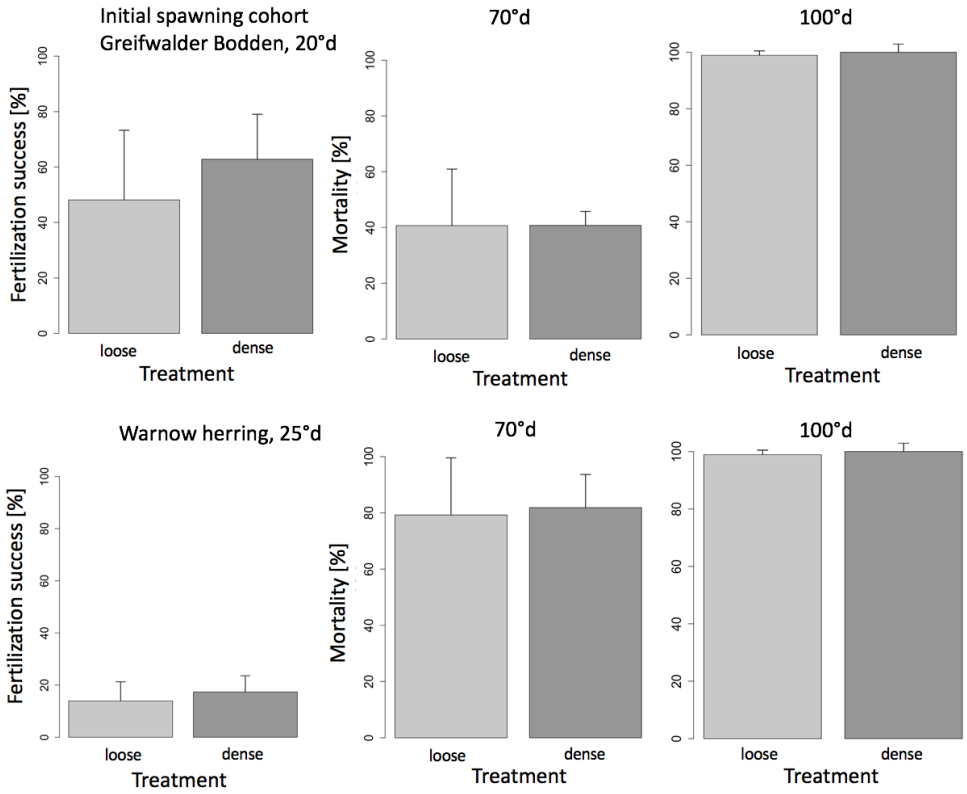

Fig. 17. Fertilization and mortality rate [%] of herring eggs in loose and dense spawned layers of herring from the Greifswalder Bodden (Top) and Warnow river (Bottom) (Data see Tab. 33, Tab. 34, p. A11).

In the mortality experiment IC (Fig. 15, top) egg fertilization was 48.1 % (SD ±25.1) in the treatment of loose spawned eggs and 62.8 % (SD ±16.3) in the treatment of dense spawned eggs (Tab. 33, p. A11). However, no significant differences in means were detected (ANOVA, p=0.299; F(1,10)=1.20). At 70°d egg mortality was 40.7 % (SD ±20.3) in the loose and 40.8 % (SD ±5.0) in the dense treatment and therefore no significant differences in mean mortality rate were revealed (ANOVA, p=0.994; F(1,10)=0). At 100°d egg mortality was high with 94.9 % (SD ±9.8) in the loose and 89.1 % (SD ±6.8) in the dense treatment (Tab. 33, p. A11), showing no significant difference in means (ANOVA, p=0.305; F(1,10)=1.17).

In the mortality experiment using Warnow herring egg fertilization was 13.9 % (SD ±7.3) in the loose and 17.4 % (SD ±6.2) in the dense treatment (Tab. 36, p. A12) resulting in no significant difference (ANOVA, p=0.439; F(1,10)=0.65). At 70°d and 100°d egg mortality was 79.2 % (SD ±20.4) and 98.9 % (SD ±1.6) in the loose treatments and 81.9 % (SD ±11.8) and 100 % (SD ±2.9) in the dense treatments (Tab. 34, p. A11). No significant differences between mean mortality were detected at 70°d (ANOVA, p=0.808; F(1,10)=0.06) and 100°d (ANOVA, p=0.279; F(1,10)=1.31).

3.5 Number of eggs per treatment

The total number of initially attached eggs onto the substrate (microscope slide: light grey, petri dishes: dark grey) was calculated for every treatment and replicate (Tab. 10).

Tab. 10. Total number of eggs on the microscope slide (light gray) and the petri dish (dark gray) in all fertilization and mortality experiments in treatments T1 (loose and dense), T2, T4, T6 and T10.

Treatments	T1 (loose)	T1 (dense)	T2	T4	T6	T10
Fert. Exp. IC		847 ±66	2242 ±384	4251 ±612	6052 ±497	9842 ±1821
Mort. Exp. IC	722 ±60	1093 ±139	2232 ±515	5832 ±446	8094 ±925	11447 ±1051
Fert. Exp. SC		1150 ±151	2992 ±439	5951 ±180		11495 ±742
Mort. Exp. SC		728 ±35	1838 ±275	2615 ±317		7209 ±305
Mort. Exp. WH	957 ±131	1486 ±125	4465 ±1047	9864 ±1547	14189 ±2053	19956 ±1794

In the fertilization experiment IC, about 1000 eggs per layer were attached to the microscope slides. In the mortality experiment IC two single layer treatments of different egg densities (e.g. loose and dense) were conducted clearly differing in total numbers of eggs. For T2 almost the same number of eggs (2232 ±515) was attached to the slide as in the fertilization experiment IC (2242 ±384). The total number of eggs in treatments 4, 6 and 10 was more than about 1000 eggs per egg layer. The overall low standard deviation of the mean values (n=6) reflects the successful consistent layering of eggs within treatments and experiments.
In the mortality experiment SC low numbers of eggs per treatment ranging from 728 ±35 in T1 to 7209 ±305 in T10 were recorded. In contrast to that, in mortality experiment WH a high number of eggs per treatment ranging from 957 ±131 in T1 (loose) to 19956 ±1794 in T10 were found.

4 Discussion

The results of the present study showed that three-dimensional egg masses have a negative effect on the fertilization success and survival rate and therefore on the reproduction of Atlantic herring (*Clupea harengus*) in the Baltic Sea.

Two laboratory experiments on egg layer dependent fertilization success and three on mortality rate including fish from different spawning cohorts and habitats were conducted. Results revealed that fertilization and survival rate in general were negatively correlated with the number of egg layers in a range of 1-10 layers of eggs. Moreover, differences in fertilization patterns and egg mortalities between spawning cohorts and investigation areas were present. Effects of egg layering on egg fertilization and mortality rate became rather distinct during experiments including fish from the initial spawning cohort in the Greifswalder Bodden.

It is a common suggestion that egg masses, attached on a small area, result in increased mortality rates due to a reduced oxygen supply or high concentration of metabolic waste products and exudates of adjoining eggs (McMynn and Hoar 1953, Rannak 1971, Braum 1985, Messieh and Rosenthal 1989). Although distinct experiments on effects of egg layering are rare, Taylor (1971) empirically investigated the effect of egg concentration on reproduction success recording a decrease in hatching success with increasing number of egg layers. However, for WBSS herring no records on the influence of intensity of egg deposition on reproductive success in terms of fertilization success, subsequent embryonic development and hatching success are available.

Egg fertilization depending on the number of egg layers

Results showed that the egg fertilization rate was affected by the number of egg layers. Fertilization success was significantly lower for treatments consisting of more than two layers of eggs compared to single- and two-layered treatments. Moreover, the within-layer egg fertilization showed a stepwise increase in fertilization success from surface layers to lower layers in the experiment of the initial spawning cohort, indicating a linear relationship. In the fertilization experiment of the second spawning cohort egg fertilization was high in all treatments except for a significant decline in the four-layered treatment. The within-layer analysis of egg fertilization in this experiment (second cohort) showed a not significant

decline in the ten-layered treatment, supporting the former results, that high numbers of egg layers result in lower egg fertilization. In contrast to that, in field studies Parrish *et al.* (1959) examined herring egg samples with an average thickness of four egg layers and found the proportion of unfertilized eggs not exceeding 1 %. Moreover, they did not observe a significant increase of unfertilized eggs up to eight layers of eggs. Although the influence of multiple egg layers on the fertilization rate could be proved in the present study, no definite explanation for this observation can be provided here. One probable explanation for the decreasing fertilization rate with increasing number of egg layers is the insufficient availability of herring sperm in lower egg layers, depending on the space between adjacent eggs. Hypothesizing that effects would be more distinct under unsaturated sperm conditions, the distinct increase in egg fertilization with increasing egg layers in the experiment of the initial cohort with an overall fertilization success below 40 % can be explained.

Egg mortality depending on the number of egg layers

Results showed that egg survival was negatively influenced by the number of egg layers. In the mortality experiment of the initial cohort, egg mortality showed a stepwise increase with increasing egg layers at 70°d (°d: incubation temperature x days after fertilization), indicating a linear relationship (R^2 = 0.66). The egg mortality rates in experiments of the second cohort in the Greifswalder Bodden and Warnow herring were high in all treatments at 70°d, showing significant differences of the single layer treatment in comparison to the multiple layer treatments, underlining that an increase in the number of egg layers results in higher egg mortality rates. Similar findings were recorded at 100°d and within specific egg layers of a ten-layered treatment in the mortality experiment of the initial spawning cohort in the Greifswalder Bodden.

Conducting field experiments to investigate the effects of egg layering on reproductive success of Pacific herring (*Clupea pallasii*), Taylor (1971) proved that hatching success depends on the intensity and deposition of eggs and found a negative correlation between hatching success and the number of egg layers. Hempel and Schubert (1968) investigated cross sections of multiple egg layer samples (3 cm thick, > 10 egg layers), taken from the North Sea and observed highest mortality rates in dense packed parts of eggs, particularly in lower egg layers with up to 80 % dead eggs. Because living eggs were recorded to appear in the blastodisc stage (about 1 day after fertilization), Hempel and Schubert (1968) considered the high egg mortality as a consequence of a low egg fertilization rate instead of oxygen depression, indicating that egg fertilization rates are influenced by multiple egg layer expansion. In contrast to that, Parrish *et al.* (1959) examined field samples of herring eggs of an average expansion of four layers of eggs and found the proportion of dead eggs not

exceeding 1 % or significantly increasing with increasing layers of eggs to a maximum expansion of eigth layers of eggs.

The observation of increasing mortality with increasing number of egg layers coincides with the common assumption that egg masses suffer from reduced oxygen supply and metabolic waste (McMynn and Hoar 1953, Rannak 1971, Braum 1985, Messieh and Rosenthal 1989). Packing of eggs as well as the extent of the egg surface are supposed to have major influences on the rate of gas exchange (Braum 1985). Within the first days after fertilization, eggs are swelling and space between eggs as well as the surface of eggs for respiratory contact is reduced. The process of swelling may lead into malformations or death of embryos (Klinkhardt 1996). In egg masses only 27 to 40 % of the entire egg surface was observed to be available for respiratory issues (Braum 1985). Eggs which are positioned in lower egg layers are therefore particularly challenged by oxygen depression. Moreover, the stage of development was observed to vary between egg layers with advanced stage in surface layers and retarded stage in lower layers (Messieh and Rosenthal 1989), which was related to oxygen depression. This becomes even more prominent with increasing oxygen demand with ongoing embryonic development (Braum 1985). Moreover, a retarded development may lead into increased mortality due to early yolk sac resorption. This may also explain Taylor's (1971) findings of a decreased hatching success with increasing number of egg layers.

Besides fish, amphibians are a prominent example for aquatic egg deposition in clumps or batches. All amphibians lay eggs with a jelly capsule, varying in form and thickness within species. As shown for herring egg masses, gelatinous egg masses of amphibians (up to 10-20 cm) also compete for adequate oxygen supply (Pinder and Friet 1994). Within the relatively loose structure of egg masses of the wood frog (*Rana sylvatica*) water convections are reported which allow gas exchange even with innermost embryos (Pinder and Friet 1994). In contrast to that, in the firm egg masses of the yellow spotted salamander (*Ambystoma maculatum*) no convection for oxygen delivery is possible (Pinder and Friet 1994). Measurements of oxygen levels throughout egg masses showed a gradient of increasing oxygen saturation to innermost embryos, indicating that diffusion alone was inadequate to provide oxygen (Pinder and Friet 1994). Similar as observed for herring eggs, inadequate oxygen levels lead into delayed development of amphibian eggs and become even more prominent shortly before hatching (Pinder and Friet 1994). However, the egg masses of many amphibians (also *Ambystoma maculatum*) are described to be cohabited by *Oophila ambystomatis*, a symbiotic green alga which provides oxygen by local production during light exposure (Gilbert 1944, Pinder and Friet 1994). The oxygen produced by local green alga is supposed to be the only source of oxygen for innermost embryos (Pinder and Friet 1994).

Abiotic requirements for subsequent egg development

As previously explained, one major factor modifying egg survival in egg masses is the oxygen supply. After fertilization, a constant increase of oxygen demand was recorded by Braum (1985), caused by the importance of oxygen for maintenance and growth. Malformations or increased mortality of embryos is most likely the consequences of oxygen depression. The experimental setup included an aeration system and therefore concentrations of dissolved oxygen were about saturation in each tank. The definite saturation threshold below which no hatching was observed, was detected at 20 % (8°C, 15 PSU) (Braum 1985) and did not occur in the measurements. However, concentrations of dissolved oxygen were not measured within multiple egg layers and therefore were possibly affecting egg development. Temperature is considered to be a second major factor influencing egg development from fertilization to hatching. Although herring eggs are adapted to fluctuations *in situ*, water temperature was constantly 10°C ±1°C in all tanks, creating favorable conditions for subsequent egg development. A wide salinity tolerance was observed for herring eggs (McMynn and Hoar 1953, Holliday and Blaxter 1960, Haegele and Schweigert 1985). However, Illing *et al.* (2016) revealed a salinity threshold of 3-5 for fertilization of Atlantic herring eggs. Salinity of habitat water from the Greifswalder Bodden varied only between 5.4 and 6.6 PSU during the experiments, whereas habitat water from Warnow river varied between 3.2 and 7.6 PSU. In this experiments the pH-value is considered to be related to the occurrence of microbial activities or metabolic waste of eggs. The pH-value was almost constant in all habitat water used for renewal and also during the experiment ranging between 8.0 and 8.9.

Egg mortality rate in surface layers

Using data from the mortality experiment of the initial spawning cohort in the Greifswalder Bodden, egg mortality rates in surface layers of treatments consisting of 1-10 layers of eggs were compared, showing significant differences between all treatments. This was surprising because surface layers in particular are supposed to have favorable exposure to the surrounding water and therefore experience a better oxygen supply and less concentrated egg metabolites. Therefore, generally low egg mortality rates were expected in all treatments. It is not clear, if the lower egg mortality in the surface layer of the four-layered treatment in comparison the higher egg mortality rates in the surface layers of the other treatments is a consequence of substrate effects (glass, egg layers) or an experimental artifact. However, the reasons for the differences in egg mortality rates in surface layers cannot be explained.

Mortality rate of single-layer herring spawn depending on different egg densities

In order to investigate if egg mortality is influenced by the egg concentration per area, effects of two different densities (loose and dense spawned) were compared. Results showed that neither fertilization success nor mortality rate at 70 and 100°d were influenced by different egg densities. Results do not coincide with findings indicating dependency of egg development on density of egg deposition in single and two layers of eggs (McMynn and Hoar 1953, von Nordheim *et al.* 2018). Munk and Rosenthal (1983) investigated different egg deposition patterns (single eggs, single egg row, double row) of Baltic spring spawning herring eggs (Langland, Denmark) and found them to influence the overall hatching success. They reported a significant higher number of viable, non-malformed hatchlings when eggs were deposited single or in a row in comparison to a double row of eggs. Munk and Rosenthal (1983) related these effects to a decrease of respiratory surface for sufficient oxygen supply. However, egg fertilization rate was not affected by egg deposition patterns ranging from 70 to 83 % in all their experiments.

Differences in fertilization patterns and egg mortalities between spawning cohorts and spawning grounds

Results revealed that differences in fertilization patterns between spawning cohorts were present: The overall fertilization success did not exceed 50 % but was more than 80 % in the experiments based on the initial and second spawning cohort in the Greifswalder Bodden. Moreover, the effects of egg layering became rather distinct in the initial spawning cohort. Although technically the procedure of artificial spawning and fertilization was carried out identically in both experiments there was a difference in the time period between fishing and strip spawning. For the experiment with the initial cohort strip spawning took place in the laboratory while for the second experiment spawning was directly conducted on the research vessel FRV CLUPEA. The delay of 4 hours between catching and induced spawning in the laboratory may contribute to the lower fertilization rate because egg fertilization was supposed to decline with increasing time of death of fish.

The different egg fertilization rates and responses to egg layer expansion observed in the fertilization experiments might be related to the influence of different spawning cohorts. Initial cohorts of spawning adults immigrating into the Greifswalder Bodden from January to April are considered older and larger in average body size compared to later cohorts (Kändler 1952). Moreover, young herring, matured at approximately 2 years of age were reported to show a significantly lower fecundity than larger herring (Anwand 1962). However, higher egg fertilization rates were observed within the second spawning cohort in the present study. In addition to that, a gradual increase in ovarian weight and total number of ripe eggs was observed during the spawning season (Anokhina 1971, Rajasilta *et al.* 2001). Anokhina

(1971) reported the individual condition of eggs and fecundity to be very variable, although Bode and Labbé (2010) could not reveal a significant difference between small and larger eggs. A decrease in sperm cell density and quality over spawning season (April/May to July/August) resulting in fertilization rates of 40-70 % in June and no fertilization success at all in July was observed by Rajasilta *et al.* (1997).

Results revealed differences in egg mortalities between spawning cohorts and habitats: Similar to the experiments on egg fertilization, effects of egg layering became rather distinct in the experiments based on the initial spawning cohort in the Greifswalder Bodden. This was underlined by the fact, that the overall egg mortality was comparatively low with a minimum egg mortality of about 40 % in the single layer treatment at 70°d. In comparison to that, egg mortality was higher, reaching more than 75 % in all treatments in the experiment including fish from the second spawning cohort in the Greifswalder Bodden.

In the experiments including fish from the Warnow river, generally lowest egg fertilization rates and highest egg mortality rates were recorded: Mortality of herring spawn depending on the number of egg layers was more than 80 % in all treatments at 70°d. At 100°d, egg mortality was about 100 % in all treatments, showing fungal covering of almost every replicate. Thereby it is not clear if the fungal infestation is caused by the number of dead eggs or *vice versa*. However, it is supposed that fungal infestation is caused by the high number of dead eggs due to their unfertilized state, particularly in the mortality experiment with Warnow herring (WH). Egg fertilization rate in control samples taken in the mortality experiment WH was below 20 % in all treatments. Although every single layer of eggs was fertilized before the next layer of eggs was attached in order to standardize fertilization before exploring effects on mortality, in experiment WH, fertilization rates linearly decreased with increasing egg layers (R^2 = 0.61). However, the mechanism behind this artifact cannot be explained. Microbial depletion of dead eggs as recorded in other studies (Klinkhardt 1996 and literature therein) was not observed. Additionally, a correlation between the number of egg layers and the extent of fungal covering was not observed, however it was not statistically tested.

Differences between herring groups from different spawning grounds were underlined by the multiple significant differences in the mean egg dry weight between Warnow herring eggs and Rügen herring eggs. Mean egg DWs of Warnow herring eggs at certain sampling events were significantly lower in comparison to the mean egg DWs of herring eggs from the Greifswalder Bodden. This is probably related to a different migration pattern of smaller sized herring into the Warnow river estuary. However, no information about condition or fecundity is available for Warnow herring. Multiple significant differences between mean egg DWs of

different spawning cohorts in the Greifswalder Bodden at certain sampling events are present. However, no distinct pattern can be revealed.

Abnormal ovary-phenomenon "Steinrogen"

It is supposed that annual differences in female and male condition and reproductive success are related to environmental variations. Winter conditions as length of ice period, extent of ice covering and water temperatures influence spawning patterns as timing or intensity of reproduction (Laine and Rajasilta 1999, Polte pers. comm.). Moreover, temperature regimes were found to modify ovarian weight and zooplankton production having an impact on reproductive properties (Rajasilta *et al.* 2001).

The maximum temperature was between 0 and 10 °C in January and early February 2018, providing acceptable conditions for spawning activities. In late February air temperature sharply dropped to -7 °C (Minimum: -13 °C) with ice covering on the Greifswalder Bodden (Tab. 18, p. A14), inhibiting spawning activity. Minimum temperatures exceeded the 0 °C – mark finally in early April and ripe herring were primarily caught for experiments a few days later, on the 06.04.2018. The sudden decrease in temperature causing a temporally break in spawning activity is supposed to negatively affect the overall herring condition leading to the abnormal ovary-phenomenon "Steinrogen".

The increasing prevalence of reproductive disorders and gonadal malformations due to anthropogenic influences is already reported for herring (Ojaveer *et al.* 2015, Rajasilta *et al.* 2015). Rajasilta *et al.* (2015) introduced several types of gonadal malformations as asymmetric, rudimentary, segmented, branched, hermaphroditic and miscellaneous abnormalities. During spring spawning season 2018, a large amount of female herring (over 80% in some catches) with the abnormal ovary-phenomenon "Steinrogen" was observed. Ovaries were hard and eggs could not be released anymore, not even by strip-spawning. Moreover, ovarian blood vessels and the ovarian membrane were injured. This type of gonadal malformation was also observed in earlier years, but usually did not exceed a proportion of about 1 % (Thünen Institute for Baltic Fisheries 2018). For some species the resorption of unreleased eggs usually at the end of spawning season was reported (Jakobson *et al.* 2016). However, herring was supposed to neither irregularly release or resorb the main part of eggs resulting in "Steinrogen". A prolonged period of unfavorable environmental conditions for spawning might promote the occurrence of this phenomenon, since female herring are considered to starve during spawning and starvation can lead to increased atresia (Ojaveer *et al.* 2015). Moreover, food restriction was proved to effect spawning success, gametogenesis, egg and larvae size (Rannak 1971, Rajasilta *et al.* 2001).

Method of egg layering

The artificial composition of egg layers by strip spawning under controlled conditions proved suitable to investigate effects of egg concentration on fertilization and egg survival. As multiple research questions remain, such as e.g. the impact of egg density on basic physiological processes, this method is a valuable tool for future studies. However, defined numbers of egg layers could only be achieved by artificial spawning. This was conducted earlier to address different research questions (Taylor 1971). During spawning, different levels of stickiness of eggs probably depending on female´s maturation were observed. Sticky eggs turned out to be favorable for attachment. In this study glass petri dishes as spawning substrates proved better than open-sided assemblages of microscope slides as eggs cannot detach as easily. Moreover, egg development on microscope slides showed edge effects caused by higher exposure to ambient water at the three remaining sides. Therefore, viable eggs for the lowest egg layers at 70 or 100°d mostly occurred along the sides of the egg masses, being not representative for the entire layer of eggs.

All in all, the egg layering was successful. The number of eggs was different in all treatments and values were in the same range in all experiments including fish from the Greifswalder Bodden. Only in the experiment with fish from the Warnow river mean egg number per treatment was comparatively high. For quantification of egg masses of more than 2 layers of eggs, cuts were conducted to get an insight into fertilization and mortality rate in all layers. The procedure of cutting was also successful which is also represented by results that show differences in means of two adjoining layers. All in all, specification of egg masses as a defined number of egg layers is a useful tool for comparability and reproducibility of experiments on egg layering.

Conclusion and relevance of findings

The present study showed that multiple egg layers influence the fertilization rate and survival rate of herring spawn. Results revealed that egg fertilization rates changed with increasing number of egg layers, showing a linear decrease in fertilization success from surface eggs to lowest layers of eggs. Egg mortality rates increased with increasing number of egg layers, indicating a linear relationship. Thereby, differences in fertilization patterns and egg mortalities between different spawning cohorts in the Greifswalder Bodden and in comparison to a second spawning ground, the Warnow river estuary: The effects of multiple egg layers on egg fertilization and mortality rate became rather distinct in the initial spawning cohort in the Greifswalder Bodden. In contrast to that, Warnow herring showed lowest egg fertilization and highest egg mortality rates in the experiment and significantly lower mean egg dry weights were recorded for Warnow herring eggs in comparison to eggs from the Greifswalder Bodden.

To minimize effects of fungal infestation due to deceasing of (unfertilized) eggs, water should be renewed more often or treated with fungi-inhibitory substances in future laboratory experiments. And experiments should be replicated *in situ* in order to confirm the results of the present study. Lower mortality rates than in the laboratory experiments are expected due to effects of natural substrates (plant respiration) and water circulation (wave actions and currents) resulting in better oxygen supply throughout egg layers.

The results of the present study showed that three-dimensional egg masses have a negative effect on the fertilization success and survival rate of herring eggs. This becomes relevant as coastal spawning grounds have changed and spawning substrates are reported to decline due to environmental and anthropogenic impacts (Kanstinger *et al.* 2016) in the Greifswalder Bodden. Less spawning substrate and fragmentation of spawning grounds potentially result in increased egg concentrations per area on residual substrates. According to the present findings this cascade could negatively affect the overall reproduction of Atlantic herring (*Clupea harengus*) in the Baltic Sea.

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Appendix

Fertilization and mortality experiments analyzed by treatments (T) and layers (L), initial spawning cohort Greifswalder Bodden

Tab. 11. Fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 25°d, analyzed by treatments (T1, T2, T4, T6, T10) in experiment IC with means and standard deviation (SD).

	Fertilization success [%]				
Treatments	T1	T2	T4	T6	T10
1	52.0	37.1	17.9	15.4	37.1
2	43.9	50.4	23.0	13.9	9.9
3	25.5	33.6	15.0	13.8	10.4
4	39.9	37.5	17.8	11.4	8.1
5	31.5	36.4	18.1	9.1	12.8
6	39.5	34.0	19.5	12.4	10.7
Mean ±SD	38.7 ±8.5	38.2 ±5.7	18.5 ±2.4	12.7 ±2.0	14.8 ±10.0

Tab. 12. Differences in fertilization success [%] between means of treatments (T1, T2, T4, T6, T10) in experiment IC at 25°d. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Treatments	T1	T2	T4	T6	T10
T1	------	0.100	**< 0.001**	**< 0.001**	**< 0.001**
T2	0.100	------	**0.001**	**< 0.001**	**< 0.001**
T4	**< 0.001**	**0.001**	------	0.619	0.921
T6	**< 0.001**	**< 0.001**	0.619	------	0.973
T10	**< 0.001**	**< 0.001**	0.921	0.973	------

Dependent value: Fertilization success. Bold values show significance ($p \leq 0.05$).

Tab. 13. Within-layer fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 25d°, analyzed by layers (L1-L6) within T6 in experiment IC with means and standard deviation (SD).

	Fertilization success [%]					
Layers	L1	L2	L3	L4	L5	L6
1	18.1	27.0	14.2	13.3	15.6	5.9
2	25.7	18.0	15.3	10.4	10.3	5.0
3	**33.1**	**25.0**	**19.1**	**2.9**	**3.5**	**2.7**
4	21.7	11.7	13.1	8.0	8.0	5.5
5	24.0	10.4	10.9	4.4	2.2	0.0
6	31.5	13.3	15.2	4.2	3.5	2.8
Mean ±SD	25.7 ±5.2	17.6 ±6.4	14.6 ±2.5	7.2 ±3.7	7.2 ±4.7	3.7 ±2.1

Bold letters show replicate which is not considered in the analysis and graph.

Tab. 14. Differences in fertilization success [%] between means of layers (L1-L6) within treatment T6 in experiment IC at 25°d. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Layers	L1	L2	L3	L4	L5	L6
L1	------	0.350	0.139	**0.002**	**0.001**	**< 0.001**
L2	0.350	------	0.993	0.172	0.129	**0.002**
L3	0.139	0.993	------	0.413	0.330	**0.006**
L4	**0.002**	0.172	0.413	------	1.000	0.330
L5	**0.001**	0.129	0.330	1.000	------	0.413
L6	**< 0.001**	**0.002**	**0.006**	0.330	0.413	------

Dependent value: Fertilization success. Bold values show significance ($p \leq 0.05$).

Tab. 15. Within-layer fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 25d°, analyzed by layers (L1-L10) within treatment T10 in experiment IC with means and standard deviation (SD).

	Fertilization success [%]							
Layers	L1	L2-L3	L3-L4	L5	L6	L7-L8	L8-L9	L10
1	**12.7**	**33.7**	**28.9**	**51.9**	**43.4**	**48.7**	**35.5**	**13.4**
2	27.1	16.1	10.2	4.7	4.2	0.4	1.7	0
3	30.2	8.2	10.8	2.8	0	2.5	3.3	3.7
4	23.3	9.2	7.5	2.4	3.0	1.3	4.1	0
5	16.9	35.8	18.5	12.3	13.0	0	2.0	0
6	28.1	27.5	2.6	5.9	1.8	1.6	0.4	2.9
Mean	25.1	19.4	9.9	5.6	4.4	1.2	2.3	1.3
±SD	±4.7	±10.7	±5.2	±3.6	±4.5	±0.9	±1.3	±1.6

Bold letters show replicate which is not considered in the analysis and graph.

Tab. 16. Fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 20d°, analyzed by treatments (T1, T2, T4, T6, T10) in mortality experiment IC with means and standard deviation (SD).

	Fertilization success [%]				
Treatments	T1	T2	T4	T6	T10
1	80.0	71.2	69.3	72.0	75.2
2	66.0	63.2	78.2	77.5	73.9
3	32.9	60.1	82.5	77.0	73.1
4	50.7	96.4	57.3	72.4	69.6
5	72.0	74.3	76.4	77.7	64.9
6	75.2	60.8	84.8	73.5	69.5
Mean ±SD	62.8 ±16.3	71.0 ±12.5	74.8 ±9.2	75.0 ±2.4	71.0 ±3.5

Tab. 17. Mortality [%] (number of dead eggs in relation to the total number of eggs) of herring spawn at 70°d, analyzed by treatments (T1, T2, T4, T6, T10) in experiment IC with means and standard deviation (SD).

	Mortality [%]				
Treatments	T1	T2	T4	T6	T10
1	40.0	73.6	89.8	96.6	98.4
2	43.8	74.9	69.1	97.5	93.3
3	30.8	58.0	77.3	98.2	97.2
4	47.0	66.8	74.5	95.9	98.6
5	42.2	58.5	93.1	99.4	94.0
6	40.7	76.9	92.9	99.0	98.9
Mean ±SD	40.8 ±5.0	68.1 ±7.7	82.8 ±9.5	97.8 ±1.2	96.7 ±2.3

Tab. 18. Differences in egg mortality [%] between means of treatments (T1, T2, T4, T6, T10) in experiment IC at 100°d. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Treatments	T1	T2	T4	T6	T10
T1	------	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
T2	**< 0.001**	------	0.840	0.329	0.676
T4	**< 0.001**	0.840	------	0.894	0.998
T6	**< 0.001**	0.329	0.894	------	0.974
T10	**< 0.001**	0.676	0.998	0.974	------

Dependent value: Mortality. Bold values show significance ($p \leq 0.05$).

Tab. 19. Mortality [%] (number of dead eggs in relation to the total number of eggs) of herring spawn at 70°d, analyzed by layers (L1-L4) within treatment T4 in experiment IC with means and standard deviation (SD).

	Mortality [%]			
Layers	L1	L2	L3	L4
1	90.1	88.8	92.6	87.8
2	75.5	64.8	66.2	71.1
3	69.5	68.7	79.7	89.4
4	79.7	67.0	65.9	84.7
5	90.2	93.6	93.9	96.2
6	91.5	93.4	91.4	94.7
Mean ±SD	82.8 ±8.4	79.4 ±12.7	81.6 ±11.9	87.3 ±8.3

Tab. 20. Mortality [%] (number of dead eggs in relation to the total number of eggs) of herring spawn at 70°d, analyzed by layers (L1-L10) within treatment T10 in experiment IC with means and standard deviation (SD).

	Mortality [%]							
Layers	L1	L2-L3	L3-L4	L5	L6	L7-L8	L8-L9	L10
1	91.8	97.9	98.1	99.1	99.5	98.5	100.0	100.0
2	72.7	93.1	90.8	97.1	99.1	95.7	96.6	98.5
3	85.4	98.8	95.8	99.4	98.3	100.0	100.0	100.0
4	91.5	99.7	100.0	99.6	100.0	98.9	100.0	100.0
5	77.0	97.5	93.9	94.9	96.1	96.4	97.2	97.4
6	96.2	98.2	98.5	98.5	100.0	99.5	100.0	100.0
Mean	85.8	97.5	96.2	98.1	98.8	98.2	99.0	99.3
±SD	±8.4	±2.1	±3.1	±1.7	±1.4	±1.6	±1.5	±1.0

Tab. 21. Differences in egg mortality [%] between means of layers (T1, T2, T4, T10) within treatment T10 in experiment IC at 70°d. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Layers	L1	L2-L3	L3-L4	L5	L6	L7-L8	L8-L9	L10
L1	------	**0.003**	**0.011**	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
L2-L3	**0.003**	------	1.000	1.000	0. 925	0. 999	0. 716	0. 574
L3-L4	**0.011**	1.000	------	0. 985	0. 696	0. 963	0. 414	0. 292
L5	**0.001**	1.000	0.985	------	0. 992	1.000	0. 915	0. 822
L6	**< 0.001**	0.925	0.696	0.992	------	0. 998	1.000	0.997
L7-L8	**< 0.001**	0.999	0.963	1.000	0.998	------	0. 957	0. 892
L8-L9	**< 0.001**	0.716	0.414	0.915	1.000	0.957	------	1.000
L10	**< 0.001**	0.574	0.292	0.822	0.997	0.892	1.000	------

Dependent value: Mortality. Bold values show significance ($p \leq 0.05$).

Tab. 22. Mortality [%] (number of dead eggs in relation to the total number of eggs) of herring spawn at 70°d, analyzed by topmost layers of treatments (T1, T2, T4, T6, T10) in experiment IC with means and standard deviation (SD).

	Mortality [%]				
Treatments	T1	T2	T4	T6	T10
1	40.0	73.7	9.9	94.8	91.8
2	43.8	64.8	24.5	94.6	72.7
3	30.8	58.0	30.5	99.0	85.4
4	47.0	47.7	20.3	94.6	91.5
5	42.2	46.1	9.8	98.5	77.0
6	40.7	74.9	8.5	97.2	96.2
Mean ±SD	40.8 ±5.0	60.9 ±11.4	17.2 ±8.4	96.4 ±1.8	85.8 ±8.4

Tab. 23. Differences in mortality [%] between means of topmost layers of treatments (T1, T2, T4, T6, T10) in experiment IC at 70°d. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Treatments	T1	T2	T4	T6	T10
T1	------	**0.018**	**0.001**	**< 0.001**	**< 0.001**
T2	**0.018**	------	**< 0.001**	**< 0.001**	**< 0.001**
T4	**0.001**	**< 0.001**	------	**< 0.001**	**< 0.001**
T6	**< 0.001**	**< 0.001**	**< 0.001**	------	**0.039**
T10	**< 0.001**	**< 0.001**	**< 0.001**	**0.039**	------

Dependent value: Mortality. Bold values show significance ($p \leq 0.05$).

Fertilization and mortality experiments analyzed by treatments (T) and layers (L), second spawning cohort Greifswalder Bodden

Tab. 24. Fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 25°d, analyzed by treatments (T1, T2, T4, T10) in experiment SC with means and standard deviation (SD).

	Fertilization success [%]			
Treatments	T1	T2	T4	T10
1	92.4	92.1	81.0	87.3
2	92.3	91.9	87.1	91.4
3	88.8	94.4	83.3	91.9
4	94.1	90.3	82.3	89.9
Mean ±SD	91.9 ±1.9	92.2 ±1.5	83.4 ±2.3	90.1 ±1.8

Tab. 25. Differences in fertilization success [%] between means of treatments (T1, T2, T4, T10) in experiment SC at 25°d. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Treatments	T1	T2	T4	T10
T1	------	0.998	**0.001**	0.668
T2	0.998	------	**0.001**	0.570
T4	**0.001**	**0.001**	------	**0.004**
T10	0.668	0.570	**0.004**	------

Dependent value: Fertilization success. Bold values show significance ($p \leq 0.05$).

Tab. 26. Fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 25d°, analyzed by layers (L1, L5, L6, L10) within treatment T10 in experiment SC with means and standard deviation (SD).

	Fertilization success [%]			
Layers	L1	L5	L6	L10
1	91.7	90.9	94.0	91.2
2	90.0	91.3	91.4	92.6
3	91.6	91.1	86.8	89.2
4	93.3	91.2	93.8	71.7
Mean ±SD	91.6 ±1.2	91.1 ±0.2	91.5 ±2.9	86.2 ±8.5

Tab. 27. Fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 25d°, analyzed by treatments (T1, T2, T4, T10) in experiment SC with means and standard deviation (SD).

	Fertilization success [%]			
Treatments	T1	T2	T4	T10
1	70.3	67.4	75.5	53.4
2	93.2	77.5	87.5	32.5
3	68.5	83.3	83.3	61.8
4	83.0	56.4	93.6	53.8
Mean ±SD	78.7 ±10.0	71.2 ±10.3	85.0 ±6.6	50.4 ±10.9

Tab. 28. Mortality [%] (number of dead eggs in relation to the total number of eggs) of herring spawn at 60°d, analyzed by treatments (T1, T2, T4, T10) in experiment SC with means and standard deviation (SD).

	Mortality [%]			
Treatments	T1	T2	T4	T10
1	71.6	98.5	96.4	99.9
2	62.4	100.0	96.6	100.0
3	93.4	100.0	95.4	100.0
4	84.6	96.0	92.1	100.0
Mean ±SD	78.0 ±11.9	98.6 ±1.6	95.1 ±1.8	100.0 ±0.1

Tab. 29. Differences in mortality [%] between means of treatments (T1, T2, T4, T10) in experiment SC at 60°d. Significance values (p-values) of multiple comparisons using Tukey's HSD test.

Treatments	T1	T2	T4	T10
T1	------	**0.001**	**0.023**	**< 0.001**
T2	**0.001**	------	0.272	0.756
T4	**0.023**	0.272	------	0.055
T10	**< 0.001**	0.756	0.055	------

Dependent value: Fertilization success. Bold values show significance ($p \leq 0.05$).

Fertilization rates and mortality experiments analyzed by treatments (T) and layers (L), Warnow herring

Tab. 30. Fertilization success [%] (number of fertilized eggs in relation to the total number of eggs) of herring spawn at 25°d, analyzed by treatments (T1, T2, T4, T6, T10) in experiment WH with means and standard deviation (SD).

	Fertilization success [%]				
Treatments	T1	T2	T4	T6	T10
1	21.3	16.7	4.3	0.4	0.2
2	20.4	22.8	17.0	1.4	1.2
3	6.8	8.3	10.2	3.2	2.2
4	13.0	12.5	7.0	2.1	0.5
5	26.0	6.7	5.9	3.3	0.3
6	16.9	18.9	5.6	2.0	0.4
Mean ±SD	17.4 ±6.2	14.3 ±5.7	8.3 ±4.3	2.1 ±1.0	0.8 ±0.7

Tab. 31. Mortality [%] (number of dead eggs in relation to the total number of eggs) of herring spawn at 70°d, analyzed by treatments (T1, T2, T4, T6, T10) in experiment WH with means and standard deviation (SD).

	Mortality [%]				
Treatments	T1	T2	T4	T6	T10
1	85.9	91.7	100.0	100.0	100.0
2	63.2	100.0	99.1	100.0	100.0
3	75.1	100.0	100.0	100.0	100.0
4	93.7	100.0	97.9	100.0	100.0
5	75.7	100.0	100.0	100.0	100.0
6	97.6	99.2	100.0	100.0	100.0
Mean ±SD	81.9 ±11.8	98.5 ±3.0	99.5 ±0.8	100.0	100.0

Tab. 32. Differences in mortality [%] between means of treatments (T1, T2, T4, T6, T10) in experiment WH at 70°d. Significance values (*p*-value) of multiple comparisons using Tukey's HSD test.

Treatments	T1	T2	T4	T6	T10
T1	------	**0.002**	**0.002**	**0.001**	**0.001**
T2	**0.002**	------	1.000	0.985	0.985
T4	**0.001**	1.000	------	0.997	0.997
T6	**0.001**	0.985	0.997	------	1.000
T10	**0.001**	0.985	0.997	1.000	------

Dependent value: Mortality. Bold values show significance ($p \leq 0.05$).

Tab. 33. Fertilization success [%] and mortality [%] of single layer herring spawn at 25°d, 70°d and 100°d, analyzed by treatments of different density (loose and dense) in experiment IC with means and standard deviation (SD).

	Fertilization success [%] 20°d		Mortality [%] 70°d		Mortality [%] 100°d	
Treatments	loose	dense	loose	dense	loose	dense
1	39.5	80.0	50.7	34.0	99.3	84.9
2	66.1	66.0	57.3	43.8	97.9	92.9
3	46.1	32.9	55.4	30.8	99.0	88.5
4	39.7	50.7	53.2	47.0	100.0	95.2
5	89.4	72.0	25.3	42.2	73.1	76.7
6	8.0	75.2	2.2	40.7	100.0	96.5
Mean	48.1	62.8	40.7	40.8	94.8	89.1
±SD	±25.1	±16.3	±20.3	±5.0	±9.8	±6.8

Tab. 34. Fertilization success [%] and mortality [%] of single layer herring spawn at 25°d, 70°d and 100°d, analyzed by treatments of different density (loose and dense) in experimentWH with means and standard deviation (SD).

	Fertilization success [%] 25°d		Mortality [%] 70°d		Mortality [%] 100°d	
Treatments	loose	dense	loose	dense	loose	dense
1	6.0	21.3	100.0	85.9	100.0	100.0
2	21.1	20.4	91.2	63.2	96.4	95.0
3	14.3	6.8	95.5	75.1	100.0	91.9
4	24.1	13.0	68.8	93.7	97.0	100.0
5	14.3	26.0	79.9	75.7	100.0	97.3
6	3.8	16.9	40.0	97.6	100.0	99.1
Mean ±SD	13.9 ±7.3	17.4 ±6.2	79.2 ±20.4	81.9 ±11.8	98.9 ±1.6	100.0 ± 2.9

Tab. 35. Differences in mean egg dry weight (DW) between experiments and different samplings therein (20-25, 60-70, 100°d). Significance values (*p*-value) of multiple comparisons using t.test.

Experiments		Fert. Exp. I.	Fert. Exp. II.		Mort. Exp. I.		Mort. Exp. II.			Mort. Exp. III.	
	Sampling	25	25	20	70	100	25	60	25	70	100
Fert. Exp. I.	25	---	0.747	**0.040**	0.444	0.073	0.393	**0.003**	**0.012**	**0.001**	**0.003**
	25	0.747	---	0.270	0.762	0.153	0.376	**0.004**	0.081	**0.024**	**0.044**
	20	**0.040**	0.270	---	0.331	**< 0.001**	**0.008**	**0.010**	**< 0.001**	**< 0.001**	**< 0.001**
Mort. Exp. I.	70	0.444	0.761	0.331	---	**0.031**	0.152	**0.003**	**0.006**	**< 0.001**	**0.002**
	100	0.073	0.153	**< 0.001**	0.031	---	0.309	**0.002**	0.253	**0.007**	0.085
Mort. Exp. II.	25	0.393	0.376	**0.008**	0.152	0.309	---	**0.002**	0.067	**0.006**	**0.022**
	60	**0.003**	**0.004**	**0.010**	**0.003**	**0.002**	**0.002**	---	**0.003**	**0.001**	**< 0.001**
Mort. Exp. III.	25	**0.012**	0.081	**< 0.001**	**0.006**	0.253	0.067	**0.003**	---	**0.011**	0.261
	70	**0.001**	**0.024**	**< 0.001**	**< 0.001**	**0.007**	**0.006**	**0.001**	**0.011**	---	0.229
	100	**0.003**	**0.044**	**< 0.001**	**0.002**	0.085	**0.022**	**0.001**	0.261	0.229	---

Dependent value: Mean egg dry weight. Bold values show significance ($p \leq 0.05$).

Tab. 36. Mean temperature, oxygen saturation, salinity and pH-values throughout the fertilization and mortality experiments with water renewals (bold). Bold letters show water renewal and fresh water parameters.

Experiment	**Day**	**pH-value**	**Salinity [PSU]**	**Oxygen saturation [%]e**	**Temperature [°C]**
Fert. Exp. I.	Day 0	8.6	6.6	99.9	14.5
	Day 3	8.6	6.2 (±0.6)	102.4 (±2.2)	10.0 (±0.2)
Fert. Exp. II.	Day 0	8.7	6.2	96.2	16.4
	Day 3	8.6	5.8 (±0.5)	102.2 (±0.2)	10.0 (±0.2)
Mort. Exp. I.	Day 0	8.3	6.5 (±0.2)	108.1(±14.7)	14.8 (±0.2)
	Day 3	8.5 (±0.1)	5.9 (±0.9)	101.6 (±3.3)	10.2 (±0.2)
	Day 6	8.4 (±0.1)	5.8 (±1.0)	95.6 (±12.8)	9.9 (±1.6)
	Day 6	**8,7**	**6.0**	**107.3**	**15.0**
	Day 7	8.6 (±0.1)	5.6 (±0.8)	95.6 (±11.0)	10.2 (±0.2)
	Day 10	8.5 (±0.1)	5.4 (±0.7)	99.4 (±16.5)	10.2 (±0.2)
Mort. Exp. II.	Day 0	8.7	6.2	98.6	14.6
	Day 3	8.5	5.4 (±1.2)	100.3 (±3.7)	10.3 (±0.2)
	Day 3	**8.6**	**6.1**	**96.5**	**14.7**
	Day 6	8.7	6.1 (±0.1)	110.4 (±22.8)	10.1 (±0.1)
Mort. Exp. III.	Day 0	8.9	3.5	118.7	17.9
	Day 3	8.8	3.4 (±0.4)	102.0(±17.9)	10.1 (±0.3)
	Day 3	**8.5**	**7.6**	**120.1**	**13.0**
	day 6	8.5 (±0.1)	5.9 (±1.5)	90.1 (±18.7)	10.4 (±0.2)
	Day 6	**8.7**	**4.9**	**82.4**	**14.6**
	Day 7	8.0 (±0.3)	4.5 (±0.2)	92.8 (±11.8)	10.0 (±1.7)
	Day 8	8.5 (±0.1)	4.5 (±0.3)	91.6 (±14.9)	10.3 (±0.2)
	Day 8	**8.6**	**3.3**	**90.0**	**15.0**
	Day 10	8.5 ±0.2	3.2 (±0.1)	90.2 (±23.2)	10.5 (±0.2)

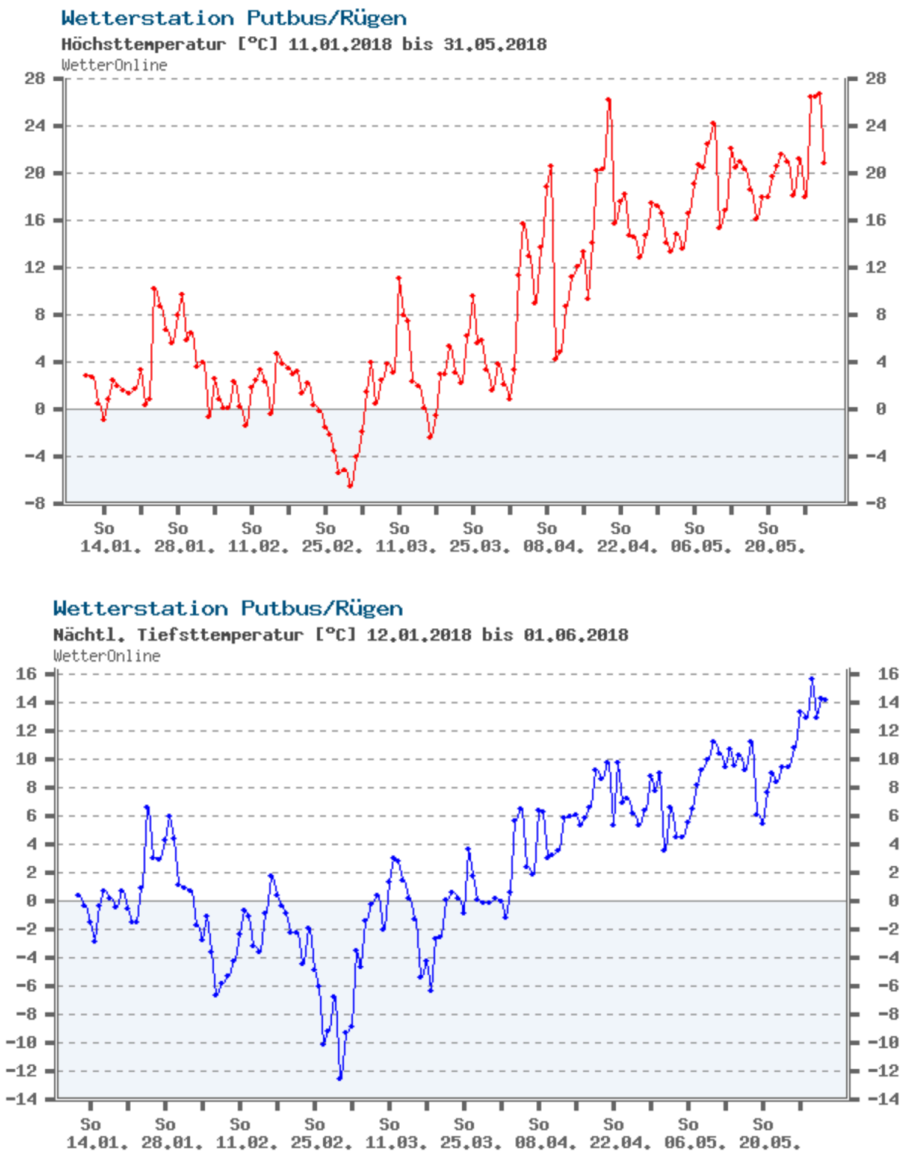

Fig. 18. Temperature in Putbus, Rügen island from January to May 2018 with maximum temperature curve (red) and minimum temperature curve (blue). (Wetter online, August 2018).

Declaration of Authorship

I hereby declare that the master thesis I am submitting was written only with the assistance and literature cited in the text. Only the sources cited have been used in this work. Parts that are direct quotes or paraphrases are indicated as such. The figures and photographs in this work have been prepared by me, if not labelled otherwise. I further declare that the thesis has not been previously submitted whether to the University of Rostock or to any other university.

03.07.2018

Place, date

Annegret Finke