Effects of coastal habitat characteristics on the reproduction of Baltic herring (*Clupea harengus*)

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Lena von Nordheim

Cover images: Herring eggs in different developmental stages: shortly after fertilization in a petri dish (top), and advanced embryos attached to *Zostera marina* (right) and *Fucus vesiculosus* (bottom) on a natural spawning bed in the southern Baltic Sea.

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Dissertation

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SUMMARY

Environmental changes and habitat degradation due to anthropogenic stressors are the major threats to most wildlife all over the world. In marine coastal ecosystems, persistent eutrophication, ongoing coastal development and the consequences of climate change have led to severe alterations of habitats and correspondingly of living conditions for flora and fauna. The capability to counteract critical consequences, such as the decline of diversity and populations, through adapted management measures, strongly depends on a holistic understanding of the species' particular ecological needs and their interactions within the entire ecosystem.

In this context, the present studies focus on an economically and ecologically key marine species, the Atlantic herring (*Clupea harengus*), which represents a central component of marine food webs as forage fish, and at the same time one of the ten most important fisheries target species in the world. Herring perform annual migrations to specific spawning areas, where they attach their adhesive eggs to submerged structures. Independent of fluctuations of the spawning stock biomass, an extremely low recruitment of western Baltic spring spawning herring during recent years indicates the existence of critical bottleneck-phases during egg and larval development. Hence, eutrophication induced alterations of aquatic vegetation beds in their coastal spawning and nursery areas are evaluated within this thesis by investigating potential consequences for different early life stages of herring, with the help of intensive *in situ* and laboratory experiments and on-site observations.

A major consequence of eutrophication in general is a reduced depth and spatial distribution of perennial macrophytes and a shift of the local plant species composition. In order to evaluate potential impacts on herring reproduction, field experiments and investigations on the spawning substrate selectivity of herring were conducted. In herring spawning areas, 'artificial SAV beds' (i.e. artificially planted monocultures of selected plant species), and natural mixed vegetation beds, were sampled after natural spawning events occurred. The analysis of plant species specific egg coverage revealed a selectivity for branching and solid macrophytes, but also showed a certain plasticity to adapt their selection according to the available substrates and prevailing conditions. However, except from the essential function of perennial plant species that provide consistent, reliable spawning habitats over years (such as seagrass, pondweeds and perennial macroalgae), in general, the observed selection of certain plant species did not follow consistent patterns.

Since the natural selection as spawning substrate does not necessarily imply good quality in terms of egg survival and hatching success, further field experiments were carried out using the method of artificial spawning (i.e. eggs were manually attached to the different plant substrates). The analyses of egg survival rates on selected macrophyte species (*Zostera marina*, *Stuckenia pectinata*, *Fucus vesiculosus* and *Furcellaria lumbricalis*) showed no statistically significant differences. However, a trend for comparatively lower egg survival on the red algae *Furcellaria lumbricalis* was still visible.

In eutrophic systems, the above described perennial macrophyte species are often covered by fast growing epiphytic algal blooms. The results of this thesis revealed severe egg mortality rates on inner coastal spawning beds that were covered by such filamentous brown algal blooms. Corresponding field and laboratory experiments, presented here, proved that this is not a generic oxygen- or structure-related effect of epiphytic algae. Rather, the lethal effect could be specifically attributed to the filamentous brown algae *Pylaiella littoralis,* and became manifested in direct egg-algae contact as well as indirectly when those algae were present in the ambient water of the eggs. Since algal blooms are known to be promoted by high nutrient loads, this study revealed new aspects of eutrophication-consequences, and thus uncovered an additional stressor-cascade introduced by anthropogenic impacts on the coastal ecosystem and important fish resources.

The results of this dissertation confirm the importance of healthy natural aquatic vegetation beds with high structural complexity and an extensive three-dimensional structure, for different life stages of herring, and thus for overall herring reproductive success. As mentioned above, adult herring seem to prefer larger and solid vegetation for spawning. In addition, specific field experiments on the influence of the spawning substrate structure have shown that higher complexity is beneficial for egg survival. Finally, the investigation of larval distribution patterns revealed an active habitat selection of different larval stages for the shallow vegetated areas, presumably because they provide hiding places from predators, and at the same time larvae find suitable zooplankton prey.

Further field investigations combined with time series analyses revealed that climate induced changes in the seasonal timing of herring reproduction have several negative consequences on various herring life stages. A clear relationship was found between a late winter onset and a low recruitment, expressed as reduced larval and juvenile abundances. Additional field experiments in the beginning and the end of the spawning season allowed for a seasonal comparison of egg survival rates. The later experiment resulted in higher egg mortality, probably because fungi and algal blooms increase with rising water temperatures.

It can be summarized that eutrophication and changing temperature regimes could be identified as the major threats to herring reproductive success due to complex cascades that cause severe spawning and nursery habitat alterations. This underpins the urgent need to reduce causative anthropogenic stressors (i.e. nutrient inflows and greenhouse gas emissions), and to implement areas of particular importance in coastal zone management plans, in order to maintain or even restore the ecological function of coastal ecosystems as herring nursery areas in particular, and as habitat for numerous other fish species in general.

ZUSAMMENFASSUNG

Umweltveränderungen und Habitatzerstörung durch anthropogene Stressoren sind die größten Bedrohungen für Tier- und Pflanzenarten auf der ganzen Welt. In marinen Küstenökosystemen haben die anhaltende Eutrophierung, die fortschreitende Küstenentwicklung und die Folgen des Klimawandels zu gravierenden Veränderungen der Habitate und damit der Lebensbedingungen für Flora und Fauna geführt. Kritischen Konsequenzen, wie dem Rückgang von Diversität und Population, durch angepasste Managementmaßnahmen entgegenzuwirken, ist nur möglich, wenn umfassendes Wissen zu den ökologischen Anforderungen der Arten und den Wechselwirkungen innerhalb des gesamten Ökosystems besteht.

Mit diesem Hintergrund konzentrieren sich die vorliegenden Studien auf eine sowohl wirtschaftliche als auch ökologische Schlüsselart, den Atlantischen Hering (*Clupea harengus*). Er stellt als Futterfisch einen zentralen Bestandteil der marinen Nahrungsnetze dar und ist gleichzeitig eine der zehn wichtigsten Fischerei-Zielarten der Welt. Heringe wandern jedes Jahr zu bestimmten Laichgebieten, wo sie ihre Eier an Unterwasserstrukturen kleben. Eine in den letzten Jahren geringe Rekrutierung von Heringen aus der westlichen Ostsee, die unabhängig von Schwankungen der Bestandsgröße ist, deutet auf kritische Engpässe während der Entwicklung von Eiern und Larven hin. In dieser Arbeit werden daher eutrophierungs-bedingte Veränderungen der aquatischen Vegetation in Küstengewässern und damit in den Heringslaichgebieten und Habitaten von Larven und Jungfischen betrachtet. Dafür wurden mögliche Konsequenzen für verschiedene Herings-Lebensstadien mithilfe von *In situ*- und Laborexperimenten sowie Untersuchungen direkt in den Laichgebieten analysiert.

Eine Hauptfolge von Eutrophierung ist eine verringerte Tiefenausdehnung und räumliche Verteilung mehrjähriger Makrophyten und eine Veränderung der Artenzusammensetzung der Pflanzenwelt. Um mögliche Auswirkungen auf die Heringsreproduktion zu bewerten, wurden Feldexperimente und Untersuchungen zur Laichsubstratselektivität von Heringen durchgeführt. Dafür wurden nach natürlichen Laichereignissen die gemischte Vegetation im Laichgebiet und zuvor ausgebrachte „künstliche Laichbetten“ (gemeint sind selbst angelegte Monokulturen ausgewählter Pflanzenarten) beprobt. Die Analyse der pflanzenspezifischen Ei-Menge zeigte eine Präferenz für verzweigte und feste Makrophyten und gleichzeitig auch eine gewisse Flexibilität der Heringe ihre Auswahl, je nach verfügbaren Substraten und vorherrschenden Umweltbedingungen, anzupassen. Abgesehen von der Wahl mehrjähriger Pflanzenarten, die über Jahre hinweg dauerhafte Habitate bieten (wie Seegras, Laichkraut und mehrjährige Makroalgen), folgte die Selektion bestimmter Arten keinem konsistenten Muster.

Da die Wahl als Laichsubstrat nicht automatisch eine gute Qualität für das Ei-Überleben und den Schlupferfolg bedeutet, wurden gezielte Feldexperimente mit verschiedenen Pflanzenarten durchgeführt, auf die manuell Heringseier aufgebracht wurden („künstliches Belaichen“). Die Analyse der Ei-Überlebensraten auf den ausgewählten Makrophytenarten (*Zostera marina, Stuckenia pectinata, Fucus vesiculosus* und *Furcellaria lumbricalis*) ergab keine statistisch signifikanten Unterschiede. Auf der Rotalge *Furcellaria lumbricalis* wurde jedoch eine vergleichsweise geringere Überlebensrate der Eier beobachtet.

In eutrophen Systemen werden die oben beschriebenen mehrjährigen Makrophytenarten häufig von epiphytischen Algenblüten überwachsen. Die Ergebnisse dieser Dissertation ergaben massive Ei-Mortalitätsraten auf Laichbetten in inneren Küstenbereichen, in denen eine solche Algenblüte fädiger Braunalgen stattgefunden hat. In dieser Arbeit werden gezielte Feld- und Laborexperimente vorgestellt, die gezeigt haben, dass es sich dabei nicht um einen generellen sauerstoff- oder strukturbedingten Effekt epiphytischer Algen handelt. Der letale Effekt konnte spezifisch auf die fädige Braunalge *Pylaiella littoralis* zurückgeführt werden und manifestierte sich sowohl bei direktem Ei-Algen-Kontakt als auch indirekt bei Algen im Umgebungswasser der Eier. Da bekannt ist, dass Algenblüten durch hohe Nährstoffbelastungen gefördert werden, deckte diese Studie neue Aspekte der Eutrophierungsfolgen auf und zeigt damit eine zusätzliche Stressor-Kaskade, ausgelöst durch anthropogene Einflüsse auf Küstenökosysteme und wichtige Fischressourcen.

Die Ergebnisse dieser Dissertation bestätigen die Bedeutung gesunder aquatischer Vegetationsbestände mit hoher struktureller Komplexität und ausgedehnter dreidimensionaler Struktur für verschiedene Heringslebensstadien und damit generell für den Reproduktionserfolg der Heringe. Wie oben erwähnt scheinen adulte Heringe größere und feste Pflanzenarten zum Laichen zu bevorzugen. Darüber hinaus haben spezielle Feldexperimente zum Einfluss der Laichsubstratstruktur gezeigt, dass eine höhere Komplexität für das Überleben der Eier von Vorteil ist. Und auch eine Untersuchung der Larvenverteilungsmuster zeigte die aktive Habitatwahl verschiedener Larvenstadien für bewachsene Küstenzonen, vermutlich, weil dort Verstecke vor Prädatoren und gleichzeitig geeignete Zooplankton-Nahrung gefunden werden.

Die Kombination von weiteren Felduntersuchungen und Zeitreihenanalysen zeigte negative Auswirkungen klimabedingter Veränderungen des saisonalen Laich-'timings' auf verschiedene Lebensstadien des Herings. Dabei wurde ein deutlicher Zusammenhang zwischen späten Wintereinbrüchen und geringer Rekrutierung, gemessen an geringen Larven und Jungfisch

Abundanzen, festgestellt. Zusätzliche Feldexperimente zu Beginn und am Ende der Laichsaison ermöglichten einen saisonalen Vergleich der Ei-Überlebensraten. Während des späteren Experiments wurde eine höhere Ei-Mortalität festgestellt, wahrscheinlich weil Pilzbefall und Algenblüten mit steigenden Wassertemperaturen zunehmen.

Zusammenfassend lässt sich festhalten, dass Eutrophierung und sich verändernde Temperaturregimes als massive Bedrohungen für eine erfolgreiche Heringsreproduktion identifiziert wurden, da sie über komplexe Wirkkaskaden im Ökosystem zu gravierenden Veränderungen der Laich- und Nachwuchshabitate des Herings führen. Dies untermauert die dringende Notwendigkeit, ursächliche anthropogene Stressfaktoren (d.h. Nährstoffzuflüsse und Treibhausgasemissionen) zu reduzieren und Gebiete mit besonderer ökologischer Bedeutung in Küstenmanagementpläne zu implementieren, um ihre ökologische Funktion für die Heringsreproduktion im Speziellen und als Lebensraum für zahlreiche andere Fischarten im Allgemeinen zu erhalten beziehungsweise wiederherzustellen.

GENERAL INTRODUCTION

Reproductive strategies of fishes

Fishes pursue manifold reproductive strategies, combined with either regular migrations or rather territorial phases during their life cycles. A broad spectrum of different habitat utilization strategies can be found in freshwater and marine environments. However, the majority of marine pelagic fish species such as clupeids (e.g. sprat (*Sprattus sprattus*, Linnaeus 1758), anchovies (*Engraulis encrasicolus*, Linnaeus 1758) and sardines (*Sardina pilchardus*, Walbaum 1792)) spawn pelagic, i.e. free-floating eggs, committing the survival and development of their offspring to the prevailing current and hydrodynamic drift patterns (Shelton and Hutchings 1982, Nissling et al. 2003, Santos et al. 2004, Peck et al. 2009). In contrast, demersal spawning, i.e. releasing the eggs onto the bottom or attaching them to submerged structures is more common in freshwater ecosystems (Balon 1975, Potts and Wootton 1989). Here, the importance of the actual spawning ground conditions and suitable substrates for a successful reproduction of some important fish species is well known, which is a crucial prerequisite for a sustainable management of the ecosystem, for example through the establishment of protected areas (de Nie 1987, Cowx and Welcomme 1998, Schmieder 2004). Demersal spawning in shallow inshore waters such as bays, estuaries and lagoons has for decades been considered to represent the reproduction strategy of the minority of species in the marine fish community (Jackson et al. 2001). Hence, the importance of vegetated littoral zones as spawning grounds has so far been rarely in the focus of research. Although important ecosystem services of this zone are common knowledge in aquatic ecology (Petr 2000), they are often not studied in detail and receive little attention in most current coastal zone management plans. However, some crucial components of oceanic food webs and important fishery resources such as herring (*Clupea* spp.) rely on vegetated spawning grounds to a yet unknown extent.

The early life stages of herring are very vulnerable to environmental stressors as the eggs are fairly stationary and after hatch, larval distribution is considered to depend on local drift and current regimes in areas with high hydrodynamic forcing. Thus, the prevailing local environmental conditions on the spawning beds and juvenile habitats can highly influence the reproductive success of the population. For this reason, local ecosystem changes and habitat alterations on potential herring spawning grounds should be taken into account when evaluating the status of the population.

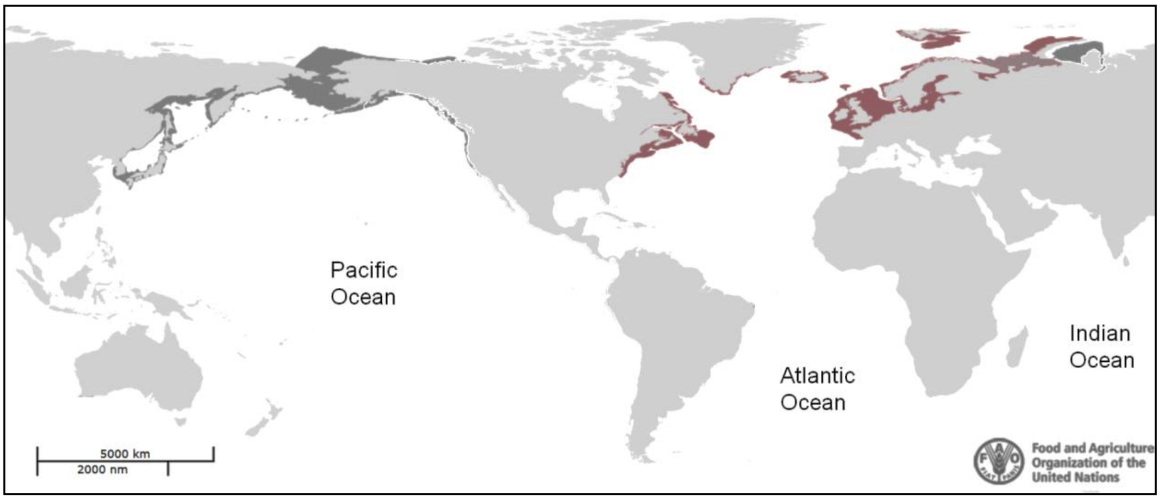


Figure 1. Geographic distribution of *Clupea harengus* (red) and *Clupea pallasii* (dark grey) after FAO (2019).

Herring in the world oceans

Generally, herring is not only economically but also ecologically extremely important. This planktivorous fish is a major food resource for higher trophic levels comprising a broad range of other marine animals and plays a key role in marine food webs (Blaxter and Hunter 1982, Möllmann et al. 2004, Bakun 2006). They can be found in big shoals all over the northern hemisphere (Fig. 1). With more than 100 species the family Clupeidae (herring) belongs to the Osteichthyes (bony fish) and are among the most common fishes in the world. The genus *Clupea* includes the Atlantic herring (*C. harengus,* Linnaeus 1758) and the Pacific herring (*C. pallasii*, Valenciennes 1847). Both species can be divided into several subpopulations mainly based on their distribution and spawning time but also referring to the morphology, growth and maturation (Haegele and Schweigert 1985).

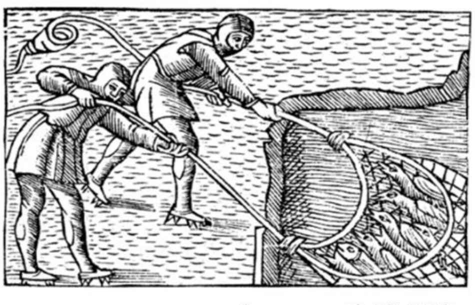
Balon (1975) defined 32 different guilds of fish according to their spawning and reproduction strategies. Herring subpopulations can be assigned to either one or the other of two of these reproductive guilds: ‘phytophilous’ herring attach their adhesive eggs to submerged aquatic vegetation, flooded terrestrial plants or appropriate replacement substrate (e.g. logs or branches and in altered habit), and ‘litho-phytophils’ are an intermediate guild and not restricted to vegetation but also use other structures such as logs, gravel and rocks. While the rather phytophilous Pacific species *Clupea pallasii* inhabits waters of the continental shelf and spawns along the shallow shoreline in springtime, Atlantic herring *Clupea harengus* is considered to be phyto-lithopilic (Haegele and Schweigert 1985, Klinkhardt 1996). Depending on the geographical distribution, the distinct groups and sub-populations, respectively, show different spawning modes from deposition of eggs on gravel or rocks on the deeper seafloor to migrations into very shallow coastal areas and spawning on vegetation and other submerged structures.

Herring in the western Baltic Sea and human history

Atlantic herring (*C. harengus*) is one of the most important commercial fish species in the Baltic Sea region since the medieval times. Herring could be found in unimaginable density close to the coast during their spawning migrations (Fig. 2) and were called “the silver of the sea”. As early as in the hanseatic period the fishes were the most crucial merchandise all along the Baltic coast and many occupational groups took part in the herring trade over wide regions (Klinkhardt 1996). Increasing fisheries and improvement of fishing gear and methods led to a constant rise of herring landings from year to year and resulted in heavy overfishing during past industrialization times.

In the western Baltic autumn spawning herring was the main target for commercial fishery during the first half of the 19th century until it decreased drastically in the 1970s due to unknown reasons. As a consequence, the spring spawning stock gained more importance (Parmanne et al. 1994). The spawning stock biomass of western Baltic spring spawning (WBSS) herring decreased from over 300 000 t in 1991 to less than half this amount in 1996. Since the early 1990s regular stock assessments are made for the WBSS herring and incorporated into annual fisheries advice. However, the recruitment has been low during the past decade and currently is at an historic low level since 2015 (Fig. 3, ICES Advice 2019). In 2019 the spawning stock biomass was approximately 70 000 t and thereby far below B_{lim} – the reference point for safe biological limits. As a consequence, the International Council for the Exploration of the Sea (ICES) advised a fisheries shut down in 2018, which was not implemented by the EU.

Olaus Magnus (1555)



unknown artist(1555)

Figure 2. Historic engravings, showing the rich abundance of herring that were fished at the Baltic Sea coast half a millennium ago.

Apart from a direct decline of the adult population due to high fishing pressure for many years, constant over-fishing might have also led to a decrease in the population's natural ecological buffering capacity towards environmental changes. The recruitment success of a fish population is decisively influenced by the survival of the early larval stages (Hjort 1914). Basically, two major bottlenecks during herring reproduction could be identified. First critical stage is the embryonic development until a successful hatch of larvae and second is the first feeding when larvae have consumed their yolk-sac (Polte et al. 2014). Although biological factors such as food availability and predation are primary influences on fish egg and larvae mortality (Houde 2008), the physical environment is also crucial for their growth and survival. However, research on this issue has traditionally more focused on larval stages resulting in a rather incomplete understanding, particularly concerning the embryonic development. Therefore, it is of fundamental importance to understand critical factors of herring reproduction by locating the drivers and stressors of the early lifecycle of herring.

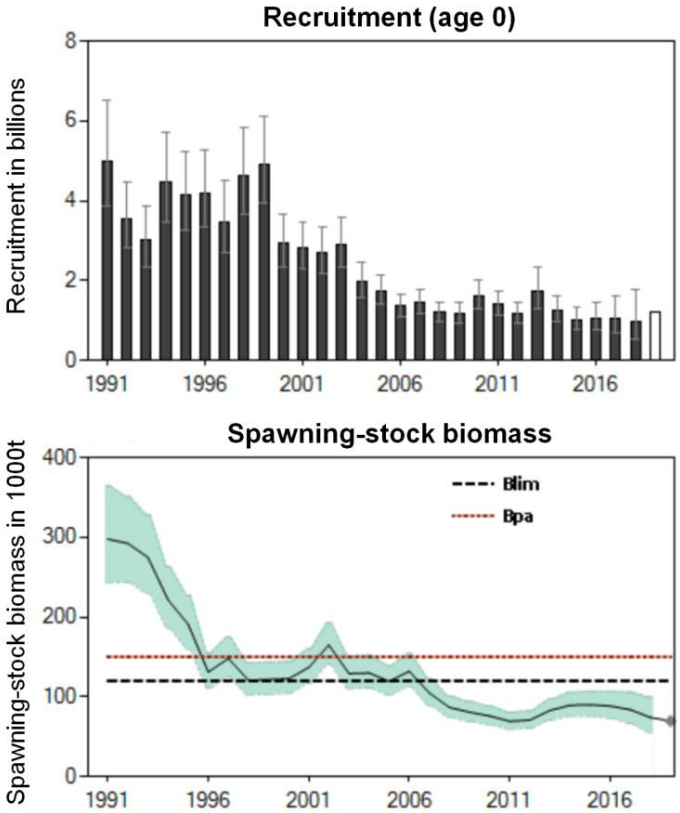


Figure 3. Development of herring in management subdivisions 20–24 of the Baltic Sea, spring spawners (WBSS herring), summary of the stock assessment. Modelled recruitment (top graph): unshaded 2019 value of the recruitment is the average value from 2013 to 2017; and Spawning-stock biomass (bottom graph): Blim is the reference point for safe biological limits of the stock, Bpa is the precautionary reference point, 2019 value is a predicted number; 95% confidence intervals are shown for SSB and recruitment. Modified after ICES (Advice 2019)

Herring ecology

LIFE CYCLE OF HERRING - Herring perform annual migrations between their feeding grounds and nursery areas (Blaxter and Holliday 1963, Aro 1989). The seasonal timing of the spawning period varies with the geographical distribution of herring populations (summarized in Sinclair and Tremblay 1984). After female herring have attached their eggs to a substrate and males released their milt into the water for fertilization, the adults continue their migration. Depending on surrounding water temperatures, the unguarded embryos develop in one to three weeks until they hatch as yolk-sack larvae (Fig.4). During this relatively long incubation period the eggs are susceptible to a variety of physical and biological stressors such as wave action and predation (Scabell 1988, Aneer 1989, Rajasilta et al. 1993, Kotterba et al. 2014, 2017, Moll et al. 2018). Herring eggs are highly vulnerable to fluctuations of the local environment due to the fact that the spawn is attached to stationary substrates. Generally, it is considered that herring eggs tolerate temperatures in the range of 5 - 17 °C and salinities in the range of 3 – 33 (Haegele and Schweigert 1985, Peck et al. 2012), but some studies in the Baltic Sea have proved that herring spawn is tolerant to even more extreme water temperatures (Aneer et al. 1983, Rajasilta et al. 1989). When the larvae hatch, they initially carry a yolk-sac which they consume within a few days. Then they start active predation on zooplankton, such as copepod nauplii and copepodite stages (Checkley Jr 1982, Möllmann et al. 2004, Paulsen et al. 2014). It is generally assumed that the distribution of fish larvae is passive due to wind and current driven drifts and water stratification (Hjort 1926, Lasker 1978, Iles and Sinclair 1982). Hydrographic modeling of passive particle dispersal in an important spawning ground in the Baltic Sea for example, suggested that wind and current forcing would retain most larvae in the particular spawning bay (Bauer et al. 2013). Thus, it can be assumed that the survival of early larval stages is subject to prevailing environmental conditions on the respective spawning grounds. Schooling behavior of larvae can be observed at the size of 25 to 30 mm (Rosenthal 1968) and when metamorphosis is completed at a total length of 35 to 40 mm, juvenile herring migrate to outer coastal areas (Blaxter and Hunter 1982, Klinkhardt 1996).

Figure 4. Images of herring eggs during different developmental stages and freshly hatched yolk-sack larvae (from left to right).

SPAWNING MIGRATIONS - As mentioned above, distinct herring stocks have different requirements regarding their spawning substrate. In the North East Atlantic, local populations are associated with particular spawning grounds even though spawning beds change over time (Cushing 1967). Due to the strong schooling behavior and regular homing, herring evolved a certain fidelity to specific sites (McQuinn 1997). It is reported that herring in the western Baltic Sea for example return to the same coastline and even to particular bays (Biester 1979, Aro 1989, Moll 2018, Moll et al. 2019). Returning to the same areas for spawning from year to year regardless of possible habitat changes involves severe risks for the survival of local populations. As for example, the decrease of seagrass (*Zostera marina*) beds at the Island of Sylt (Germany) in the North Sea led to the complete collapse of Sylt spring spawning herring population (Wohlenberg 1935) and a similar consequence is documented for the Zuiderzee (Netherlands), where the closure to what is today known as the Ijsselmeer lead to the extinction of the Zuiderzee herring population, as the fish were not able to enter their natal spawning ground anymore (Redeke 1939, Wolff 2000). Furthermore, in some Baltic Sea areas with highly degraded habitats such as the German Warnow estuary or Vistula lagoon on the Polish coast, herring spawning migration is still observed (Gruszka 2008 and P. Polte pers. comm.) despite of unfavorable ecosystem alterations. But neither it is clear which spawning substrates are used nor to what extent the recruitment success is affected.

SPAWNING BEHAVIOR - Herring attach their adhesive eggs directly to the spawning substrate. Some populations like the Norwegian spring and the North Sea autumn spawning herring are ‘lithophils’ and lay their eggs on gravel beds in offshore regions (Runnström 1941, Groot 1980, Maravelias et al. 2000). However, most herring migrate into shallow coastal areas, where they primarily spawn on submerged aquatic vegetation (SAV). In contrast to the offshore spawning, the spawning behavior of these subpopulations has been observed on several occasions in different areas. (Stacey and Hourston 1982, Aneer et al. 1983). It was described that large schools of herring circulate above the ground and then single females leave the school descending, close to the substrate. There they swim slowly in an arched position with the genital opening and tail slightly pressed against the substrate, start to vibrate and release the eggs (Fig. 5). Males show similar behavior, releasing milt that sinks down and fertilizes the eggs. The demersal eggs stick to the substrate and also to each other.

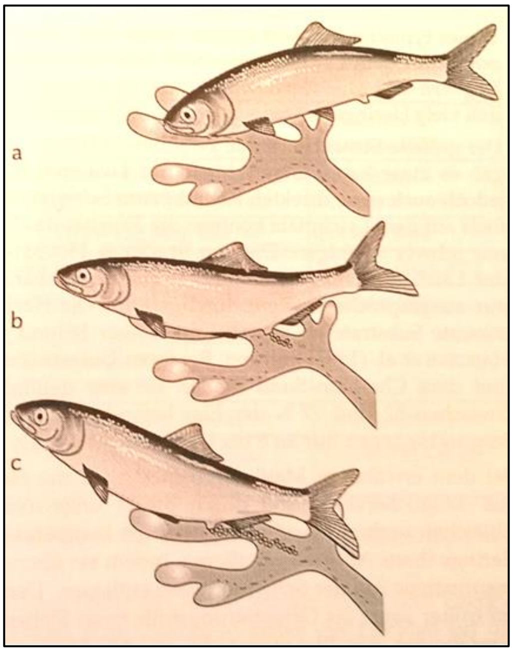


Figure 5. Important phases of the herring spawning process: **a)** females swim to the spawning substrate, **b)** during egg release, the abdomen keeps close contact to the substrate, **c)** after egg release, females swim back to their school
© Klinkhardt (1996)

Answers to the question if herring are selective in the choice of spawning substrate or if they would spawn on whatever they find in a selected area can hardly be found. An active substrate testing of female Pacific herring was described for captured herring in aquaria (Stacey and Hourston 1982) and *in situ* observations in the Baltic Sea suggested a preference for rather rigid and solid plants over softer ones (Aneer et al. 1983, Scabell and Jönsson 1984). Furthermore, an increased spawning intensity with higher vegetation coverage per area was documented (Scabell 1988, Rajasilta et al. 1989, Aneer 1989, Polte and Asmus 2006, Kanstinger et al. 2018) indicating a crucial importance of healthy SAV beds for herring reproduction. However, to the authors knowledge, no profound experimental studies exist on potential substrate selectivity of herring so far.

SPAWNING SUBSTRATES - Spring spawning herring in the Baltic Sea primarily use submerged vegetation as spawning substrates (e.g. Oulasvirta et al. 1985, Rajasilta et al. 1986, Aneer 1989). Different plant species preferences (e.g. *Ceramium* or *Pylaiella littoralis* and *Ectocarpus siliculosus* or *Zostera marina* and *Fucus vesiculosus*) were described for the Swedish coast, varying by year and investigation area (Aneer and Nellbring 1982, Aneer et al. 1983, Elmer 1983, Aneer 1989). From Finland preferences for filamentous algae (green: *Cladophora glomerata* or brown: *Ectocarpus siliculosus* and *Pylaiella littoralis*) were reported, while also perennial macrophytes were frequented but to a lesser extent (Oulasvirta et al. 1985, Kääriä et al. 1988, Rajasilta et al. 1989, 1993). In the Gulf of Riga and on the Lithuanian coast mainly red algae (*Furcellaria* spec., *Ceramium* spec. and *Polysiphonia* spec.) were used as spawning substrates and *Fucus vesiculosus* was reported to seemingly be avoided (Raid 1990, Šaškov et al. 2014). In contrast at the german Baltic coast, all present perennial macrophytes (*Furcellaria fastigiata*, *Fucus vesiculosus*, *Stuckenia pectinata* and *Zostera marina*) were reported to be mainly frequented as spawning substrates (Scabell and Jönsson 1984, Scabell 1988, Moll et al. 2018, Kanstinger et al. 2018). A few field studies reported that some eggs were additionally found on other substrates like gravel, stones, rock, sand, or on the shells of *Mytilus edulis* (Oulasvirta et al. 1985, Scabell 1988, Aneer 1989, Raid 1990, Šaškov et al. 2014), but no eggs were found on soft or muddy bottoms (Aneer et al. 1983, Oulasvirta et al. 1985, Kääriä et al. 1988). Aneer (1989) speculated that herring are relatively unspecific in their spawning substrate selection and that the preference for specific plant species can change over time, probably as a result of their natural succession. Furthermore, he recorded that spawning individuals generally prefer plants over non-biotic structures such as rocks. However, there are indications for herring spawning on every structure they find underwater, as fishermen often find their fishing nets covered by eggs. Scabell and Jönsson (1984) for example installed black and white vertical and horizontal nets on herring spawning grounds to investigate ‘alternative substrates’ and found selective spawning activity on the nets, as eggs were only attached to the black nets in vertical position and upon this up to a height of 40 cm above the ground.

Although those observations indicate that herring are not exclusively selecting vegetated spawning substrate, information on the substrate-impact on egg development is extremely rare. Aneer (1987) reported increased egg mortality caused by exudates, released by brown algae (*Pylaiella littoralis* and *Ectocarpus siliculosus*) in some specific stage of decomposition and Rajasilta et al. (2006) investigated the egg mortality on fresh and decomposed *Furcellaria* spp. and *Cladophora* spp. and showed that the red algae led to a significantly higher mortality. However, knowledge on potential substrate-impacts is currently of particular importance in order to enable a profound estimate to what extent ongoing coastal alterations and especially the change of plant species compositions might impact herring reproductive success.

***COASTAL WATERS – SPAWNING GROUNDS, NURSERY AREAS AND JUVENILE HABITATS* -** Coastal littoral zones provide important juvenile habitats for many invertebrate and fish species (Beck et al. 2001, Nagelkerken et al. 2015, Sheaves et al. 2015). The local benthic vegetation forms a complex habitat providing potential hiding places, and thus lowering predation risk as well as increasing food availability (Caddy 2007, Thiriet et al. 2014). Especially the structure of aquatic plants influences the associated fish community (Heck Jr and Orth 1980, Dionne and Folt 1991, Lillie and Budd 1992)

As mentioned above, submerged aquatic vegetation also provides spawning substrate for ‘phytophilous’ spawning fishes throughout a broad taxonomic range (Balon 1975). However, compared to the role as juvenile habitat this ecological function of marine SAV received little attention so far even though it is commonly considered in freshwater systems (Petr 2000).

Environmental changes – coastal habitat alterations

Coastal waters, lagoons and estuaries are amongst the most influenced marine ecosystems by human activities all over the world (Halpern et al. 2009, 2015). Being the link between land and ocean, those areas are affected by ubiquitous coastal modification, cultivation and eutrophication, often resulting in severe habitat degradations.

Eutrophication has been identified as the major concern for water quality for most of the freshwater and coastal marine ecosystems in the world (Nixon 1995, Selman et al. 2008, Howarth 2008, Smith and Schindler 2009, Rabalais et al. 2009, EEA 2015). A major consequence for the coastal littoral is the faster and more frequent growth of phytoplankton and epiphytic algae blooms, causing in reduced light levels for benthic macrophytes, and thus resulting in a plant species shift and the decrease of important habitat forming perennial macrophyte vegetation (Taylor et al. 1995, Duarte 1995, Valiela et al. 1997, Rabalais 2002, Gorgula and Connell 2004, Orth et al. 2006, Korpinen et al. 2007, Burkholder et al. 2007, Bricker et al. 2008, Teichberg et al. 2010).

As a consequence, the reduction of eutrophication has been in the focus of global political concern for decades and is addressed in regional conventions such as the Oslo-Paris-Convention (for the North-East Atlantic) and the Helsinki-Convention for the Baltic Sea. Although some effective regulations were enforced (particularly by EU directives - the Water Framework Directive and Marine Strategy Framework Directive), progress is only very slowly recognizable, because nutrients are stored in the sediments and the release will continue for decades (Gustafsson et al. 2012). In the Baltic Sea for example, the nutrient inputs were reduced and eutrophication actually decreased during the 1990s, but then remained on a constant level and today, the threshold values for achieving good ecological status ("GES") in the sense of the Marine Strategy Framework Directive of the EU by 2020 are still significantly exceeded (status report BMUB 2018, Reusch et al. 2018).

Recently, there is an even greater need for action, as consequences of eutrophication seem to be intensified by milder winters and steeper spring temperature curves. Synergy effects of climate change and eutrophication have only been in the focus of a few recent studies so far but is considered as a serious interaction (e.g. Oguz and Gilbert 2007, Lloret et al. 2008, Jeppesen et al. 2010, Suikkanen et al. 2013, White et al. 2018). Such synergistic effects of changing climate and eutrophication might have severe impacts on future ecosystem functioning and underlines the strong demand for reliable information on the full set of consequences.

BALTIC SEA - The Baltic Sea represents a transitional system that is extraordinarily predestined for studies on the effects of multiple anthropogenic stressors and the effectiveness of transnational management approaches. The semi-enclosed brackish water body is characterized by limited water exchange. The catchment area is formed by heavily utilized land (fourteen developed and industrialized countries) and the aquatic ecosystem is affected for decades by high nutrient inputs, reduced oxygen levels and warming (Reusch et al. 2018). These factors are particularly evident in shallower waters with little water exchange, but the same stressors also affect most other coastal areas in the world (EEA 2015, Halpern et al. 2015). For this reason, results of local studies on stressor consequences for the ecosystem and its ecological function for individual species can be transferred to numerous scenarios elsewhere on earth.
Studies in this dissertation focus on western Baltic spring spawning (WBSS) herring that migrate from feeding grounds in the Skagerrak and Kattegat to spawn in sheltered coastal areas in the western Baltic Sea (Aro 1989). The aim is the evaluation of possible consequences of coastal habitat alterations on the reproduction of the population.

Case example –a major spawning area of western Baltic herring

Research on factors influencing successful reproduction or the early herring life stages can best be conducted on natural spawning grounds. While the evaluation of single stressors and determination of critical ranges in laboratory studies can be very precise, only *in situ* experiments allow conclusions under the full set of stressors and cumulative impacts that naturally interact during embryonic development on the spawning site. Observations and field experiments for this work were conducted in the “Greifswalder Bodden”, hereafter referred to as “Greifswald Bay”, a major spawning area of the WBSS herring at the German coast.
During the 1980s those herring were subject of numerous regional studies including field observations and experiments on the spawning biology (e.g. Biester 1979, Klinkhardt 1984, 1986, Klinkhardt and Biester 1984, Scabell and Jönsson 1984, Scabell 1988). More recently, some detailed studies in the frame of doctoral theses and review articles on the biology of

WBSS herring have been published (e.g. von Dorrien et al. 2013, Kanstinger 2014, Polte et al. 2014, Kotterba 2015, Moll 2018).

However, even though so much research has been conducted on those herring there are several open questions regarding their ecology and they are currently gaining importance in the light of the above described environmental changes. In many of the earlier studies, observed spawning substrates were listed or at least parenthetically mentioned. However, a systematic evaluation of a possible active selectivity of adult fish or of the suitability of substrates in terms of egg survival was not delivered. Hence, the role of different plant species remained unknown, and thereby questions on consequences of changing coastal habitat conditions for herring are still not answered.

***CASE STUDY AREA: GREIFSWALD BAY* -** Because of its known importance for spawning of WBSS herring a variety of studies were conducted in this area, as mentioned above, investigating different features of herring biology and also a 30-year time series of monitoring the annual herring larvae production has been established. This continuous survey revealed a strong correlation between the local abundance of herring larvae and other recruitment indices of the entire stock (Oeberst et al. 2009, Polte et al. 2014). Furthermore, recent chemical analysis of the ear stones has confirmed that Greifswald Bay is a particularly important nursery for the WBSS herring population (Moll et al. 2019).

Each spring, successive groups of spawners gather in deeper waters off the entrance of Greifswald Bay and immigrate into the area in waves for spawning over a period of several months. The spawning period starts when the water reaches temperatures above 4 °C (Klinkhardt 1996, Moll 2018) and spawning usually occurs from March to June with the main spawning period in April and early May (Scabell 1988, Polte et al. 2014). The Bay is not only an important spawning area, but also a nursery area in which early life stages develop to juvenile fish (Polte et al. 2014). Hence, the entire life cycle of the fish can be studied in this system: from spawning and egg development, over all larval stages to the juveniles and finally to adults that return to the bay for spawning on their annual migrations.

As a result, local influences such as nutrient loads, global warming, extreme weather events and coastal modification potentially affect the dynamics of the entire western Baltic Sea population. The strong exposure of Greifswald Bay to the above influences and the shallow waters of the bay, which respond rapidly to physical variations, make the system an ideal model system to study human impacts and climate effects on the reproductive biology of coastal fish species.

The above described consequences of eutrophication, such as the reduction of vegetated areas and the decrease in depth limit of macrophytes are also noticeable symptoms of anthropogenic impacts in Greifswald Bay. In the early 20th century, the bottom was reported to be comprehensively covered by submerged plants, but with agricultural and urban development of the catchment areas, nutrient import into the lagoon increased from 1950 to 1990 and led to severe eutrophication (Schiewer 2001, Munkes 2005a). This caused a vegetation shift from a macrophyte-dominated to a phytoplankton-dominated system and perennial macrophytes are nowadays limited to the shallow coastal areas with a depth distribution down to only 3.5 m (Munkes 2005b, Kanstinger et al. 2018) causing the total vegetation ground coverage of the lagoon decreased to less than 10 % (Kanstinger 2014, Kanstinger et al. 2018).

Today the vegetation in Greifswald Bay is a mixture of marine, brackish and freshwater species. *Zostera marina* and *Stuckenia pectinata* are the most abundant flowering plants and according to bottom structure (sand or boulders) and water depth a variety of green, brown and red algae as well as extensive turf mats can be found (Munkes 2005a, Kanstinger 2014).

Aims of this thesis

In a time of changing aquatic habitats and decreasing submerged vegetation, often questions are raised, if such habitat alterations might impact the fauna at all and if mitigation and restoration measures would help to maintain a status quo and further on to re-establish an earlier and healthier status of the ecosystem. However, to answer questions on the consequences of environmental changes, it is necessary to understand the biological interaction cascades and their ecological background. A holistic understanding of the mechanisms behind the fundamental ecosystem functioning is a crucial prerequisite for identifying and reducing the main stressors.

Applying those questions to the Baltic herring spawning ecology, in this study, unique combinations of field experiments, observations and laboratory experiments were used to address important knowledge gaps in the functioning of western Baltic coastal ecosystems (see scheme Fig. 6). First, the actual spawning behavior of adult fish was examined with regards to plant species selectivity and vegetation depth limits (MANUSCRIPT I). Furthermore, potential factors influencing the herring egg survival were investigated: different plant species as spawning substrates (MANUSCRIPT II), the occurrence of eutrophication associated filamentous algal blooms (MANUSCRIPT III) and the effects of structural spawning substrate complexity and spawning time (MANUSCRIPT IV). Additionally, the habitat use of different larval stages and corresponding potential consequences were examined (MANUSCRIPT V) and effects of recent phenological shifts due to changes in regional climate were analyzed using a long term data series of larval production in the study area (MANUSCRIPT VI).

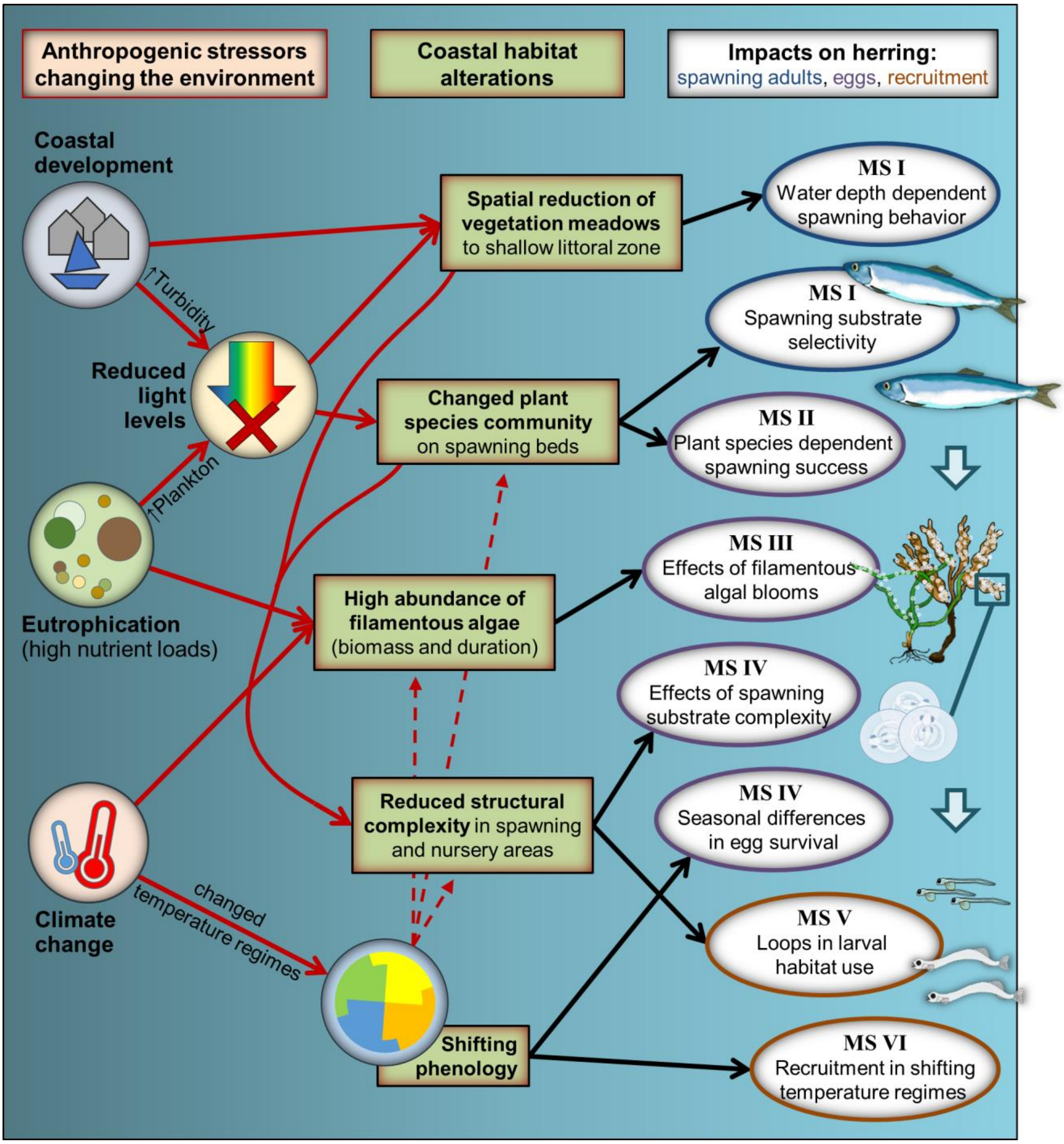


Figure 6 Conceptual diagram on anthropogenic stressor cascades. Changing environmental conditions led to habitat alterations in coastal waters, and thus might indirectly affect their function as herring spawning and nursery areas. Potential consequences for selected life stages of Baltic spring spawning herring were investigated within this dissertation and will be presented in the following manuscripts **MS I-VI**.

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Influence of the macrophyte composition on spawning substrate selection of spring spawning Atlantic herring (*Clupea harengus*) in the Baltic Sea

Lena von Nordheim, Paul Kotterba and Patrick Polte

Abstract

Pacific herring (*C. palasii*) and numerous Atlantic herring (*C. harengus*) populations rely on littoral spawning grounds in sheltered coastal and transitional waters, where they attach their adherent eggs to benthic structures. Those aquatic ecosystems are highly impacted by diverse anthropogenic stressors such as persistent eutrophication and ongoing coastal development. The consequence is a reduced depth limit for the growth of macrophytes, resulting in the areal decrease of vegetation beds and also a species shift in the plant community.

In order to evaluate potential consequences, the role of macrophyte species as spawning substrate was investigated by combining field experiments and *in situ* investigations in an important herring spawning area in the Southern Baltic Sea. On the natural spawning bed, 'artificial SAV beds' were created by diving, to provide monocultures of selected plant species (*Zostera marina*, *Fucus vesiculosus* and *Furcellaria lumbricalis*) to spawning herring. When spawning occurred, the 'artificial SAV beds' and the natural mixed vegetation beds were sampled. Analyses of egg numbers according to the plant species have shown that herring are selective in the choice of spawning substrate and seem to prefer branching and solid macrophytes. However, a certain plasticity to adapt their selection according to the available substrates and prevailing conditions was also revealed.

In general, the observed selection of certain plant species did not follow consistent patterns but emphasizes the essential function of plant species that provide consistently reliable spawning habitats over years (such as seagrass, pondweeds and perennial macroalgae). Considering the preference for extensive aquatic vegetation beds with high structural complexity, we conclude that ongoing changes of coastal ecosystems, including habitat fragmentation and losses of macrophyte meadows, have a high potential to negatively affect the successful reproduction of important ecosystem components, such as herring.

Introduction

Herring are cosmopolitan fishes occurring in marine habitats all over the northern hemisphere. They annually migrate in big pelagic shoals from their feeding areas to specific spawning grounds, where they attach their adhesive eggs to benthic substrates (Blaxter and Holliday 1963, Haegele and Schweigert 1985, Aro 1989, Klinkhardt 1996). Thereby distinct herring stocks have quite different requirements regarding their spawning substrate. Pacific herring (*Clupea pallasii*) inhabit waters of the continental shelf where they spawn along the shoreline in springtime (Haegele and Schweigert 1985) .They obligatory require submerged aquatic vegetation (SAV) for spawning (Hay 1985). Balon (1975) defined different spawning guilds on the basis of substrate requirements and referred to *C. pallasii* as phytophilous spawners, while its Atlantic counterpart (*C. harengus*) was described as phyto-lithophilous, referring to spawning activity on vegetation and other benthic substrates. However, substantially different spawning modes can be found within subpopulations of *C. harengus* according to their spawning time and area (Fig. 1).

Outer coastal shelf spawners such as the Norwegian spring and North Sea autumn herring are reported to spawn lithophilous and attach their eggs to gravel beds (Runnström 1941, Groot 1980). In contrast, spring-spawning herring of the North and Baltic Sea migrate into shallow coastal and inshore areas where they attach their eggs to a broad variety of different benthic substrates (= phyto-lithopilous spawning, Balon 1975), preferably on aquatic vegetation (Scabell and Jönsson 1984, Oulasvirta et al. 1985, Rajasilta et al. 1986, Aneer 1989, Polte and Asmus 2006 and own observations over years).

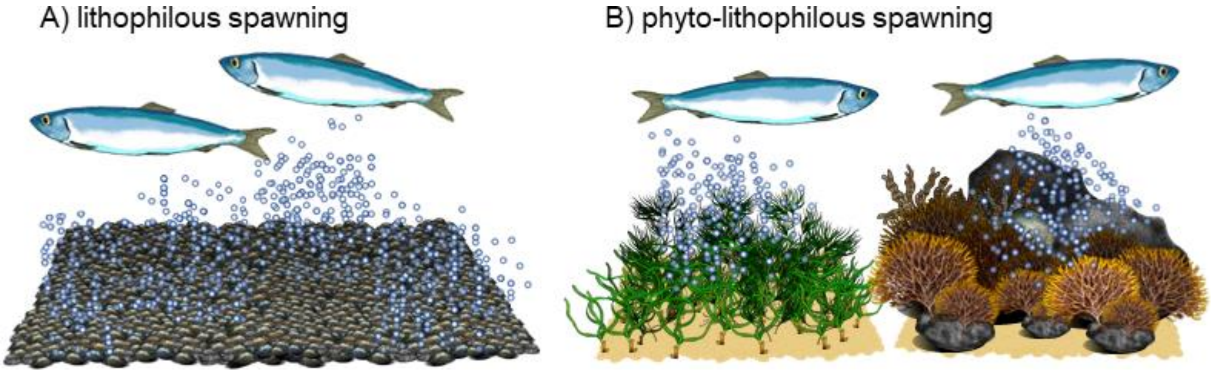

Figure 1. Spawning modes of Atlantic herring (*Clupea harengus*): **A)** lithophilous spawning on gravel beds in offshore waters (outer coastal shelf) e.g. Norwegian spring spawners or North Sea autumn spawners and **B)** phyto-lithophilous spawning on macrophytes and/or stones in coastal and inshore waters e.g. North Sea spring spawners or Baltic Sea spring spawners. © Kotterba

Due to homing behavior and site fidelity, local populations are associated with particular spawning grounds even though the particular spawning beds in those areas may change over time (Cushing 1967, McQuinn 1997). During recent decades, especially vegetated littoral zones are subject to alterations in species composition and habitat degradation as a consequence of eutrophication, climate change and coastal modifications (Selman et al. 2008, Howarth 2008, Courrat et al. 2009, Smith and Schindler 2009, EEA 2015, Halpern et al. 2015). Those factors resulted in a reduced depth distribution of perennial macrophytes and thereby the spatial extend of SAV meadows (i.e. potential spawning beds) decreased extremely in many coastal areas (Kautsky 1991, Vogt and Schramm 1991, Taylor et al. 1995, Valiela et al. 1997, Orth et al. 2006, Burkholder et al. 2007, Bricker et al. 2008). This might severely affect herring populations relying on SAV for spawning and has further implications for any other organism relying on this particular biogenic habitat.

The focus of this study is on western Baltic spring spawning (WBSS) herring, a herring population that relies on shallow coastal areas for spawning, egg development and as larval and juvenile habitat (manuscript V or Polte et al. 2017). WBSS herring annually migrate into shallow coastal areas and inshore lagoons where females attach their eggs almost exclusively to aquatic plants (Scabell and Jönsson 1984, Klinkhardt and Biester 1985, Scabell 1988, Aro 1989, Oeberst et al. 2009, Kanstinger 2014). The demersal spawn remains attached to the substrate during the complete embryonic development, suggesting a high influence and importance of the respective spawning substrate. This raises the question, if herring might selectively choose particular substrates. From observations of captured herring in aquaria, an active substrate testing of female herring was described in order to select a suitable substrate (Stacey and Hourston 1982) and *in situ* observations of Baltic spring herring indicate a preference for rather rigid and solid plants over softer ones (Aneer et al. 1983, Scabell and Jönsson 1984). However, to the authors' knowledge, no profound empirical studies exist on potential substrate selectivity of herring.

Applying those questions on Baltic herring spawning ecology, the following hypotheses were addressed by field observations and -experiments:

I) phyto-lithophilous spawning herring are non-selective in the choice specific aquatic plant species as spawning substrate, and

II) herring would spawn below the present depth limit of vegetation if suitable substrates were available (again).

Material and Methods

STUDY SITE - Investigations were carried out in Greifswald Bay, one of the key spawning areas for the WBSS herring with several spawning beds distributed in the bay (e.g. Scabell and Jönsson 1984, Klinkhardt and Biester 1985, Scabell 1988, Oeberst et al. 2009). The experimental sites were located in two different herring spawning beds close to the northern and southern shore respectively (Fig. 2). Greifswald Bay is a brackish inshore lagoon in the southern Baltic Sea, characterized by shallow water (mean depth: 5.8 m, maximum: 13.6 m; Reinicke 1989), low salinity (around 7 and 8; Schiewer 2001) and during spring, pH values are around 8.5. In this non-tidal system, water-level fluctuations (0 to 1.5 m), water exchange and mixing of the water column (spring oxygen-saturation: close to 100 %) are mainly wind-driven (Stigge 1989).

In the early 20th century, an almost complete ground coverage of submerged aquatic vegetation (SAV) was reported for Greifswald Bay (reviewed by Kanstinger et al. 2018). But already by the middle of the 20th century a sparser coverage and a decrease in vegetation was described (examined by dredge and bottom grab (Subklew 1955) and by SCUBA diving (Geisel 1986)). Increasing eutrophication caused a vegetation shift from mainly macrophytes to a high abundance of phytoplankton (Munkes 2005) and nowadays, only the shallow littoral zone can be found vegetated (Kanstinger et al. 2018).

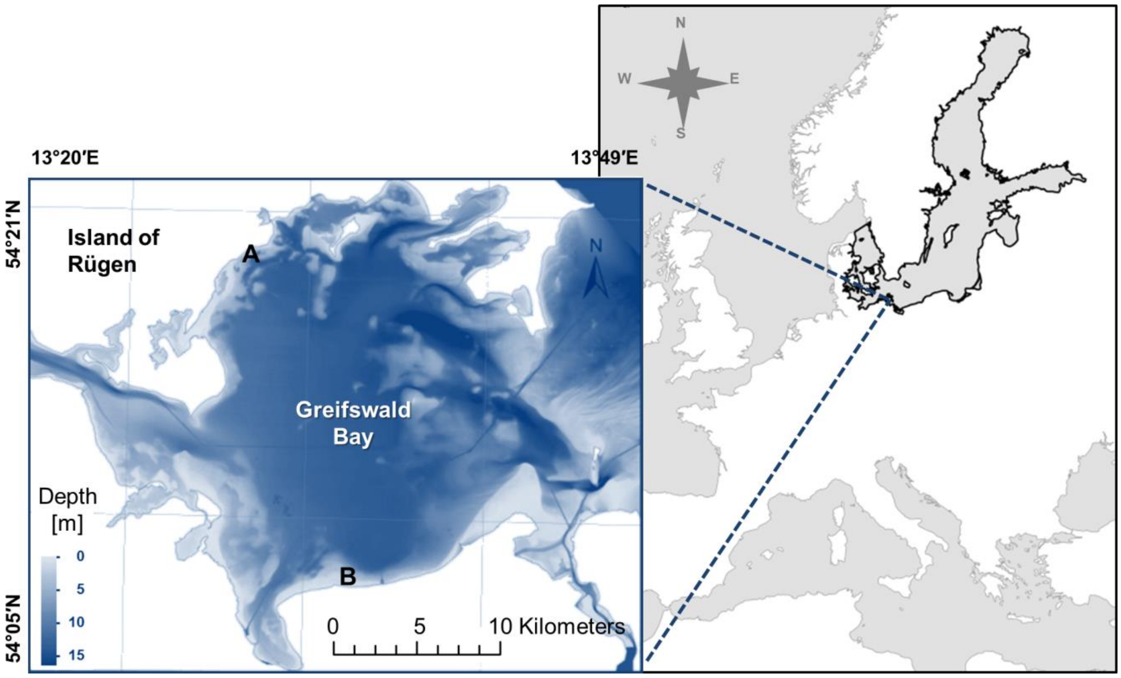

Figure 2. Investigation area Greifswald Bay and its location in the Baltic Sea (framed in black) with relevant locations for this study (A: ‘Altkamp’ and B: ‘Gahlkow’). Bathymetric data: received by courtesy of Federal Maritime and Hydrographic Agency (BSH) of Germany.

1 SAMPLING OF HERRING EGGS SPAWNED ON DIFFERENT PLANT SPECIES

Field studies were conducted at location B (‘Gahlkow’), where different depth zones are dominated by specific plant species: in shallow areas between 0.5 and 1.5 m, mainly pond-weeds (*Stuckenia pectinata*) are found, in deep areas between 2.5 and 3 m, seagrass (*Zostera marina*) is dominant, while in between, both species mix with a variety of other species with lower abundance. Around the vegetation bed, large areas of sand can be found (Fig. 3).

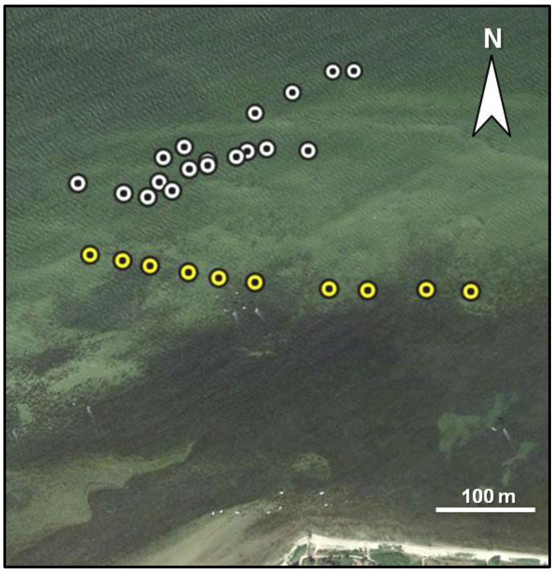

Figure 3. Aerial image of the Gahlkow area (location B): dots indicate sampling stations for vegetation and herring eggs in the mix zone A (white dots, n = 18) and shallow zone B (yellow dots, n = 10). Image © GoogleEarth

The spawn distribution on different plant species was investigated by **quantitative sampling** after the first spawning peak of herring spawning season 2016 (30/03/2016). Random samples were taken with a Van-Veen-Grab (Alu Bau GmbH, 400 cm² sampling area) in the mixed area (further referred to as “zone A”) and shallow area (“zone B”) (Fig. 3). The entire plant material and herring spawn of each sample was preserved in 4 % borax-buffered formalin. In the laboratory, plant species were separated, adhering eggs were counted for each substrate type ('filamentous algae' were determined as cross-species class, also including e.g. filamentous forms of *Ulva intestinalis*) and plant biomasses (dry weight) of each species were measured after drying at 80 °C for 48 hours.

The **egg density (ED)** was calculated from the number of eggs (EN) per plant biomass (PB) in gram dry weight. For evaluation of spawning substrate preferences, the **egg concentration (EC)** was assessed by relating the ED of each plant species (i) to the ED of the respective total sample (t).

$$\mathrm{ED} = \frac{\mathrm{EN}}{\mathrm{PB}} \quad ; \qquad \mathrm{EC_i} = \frac{\mathrm{ED_i}}{\mathrm{ED_t}} = \frac{\mathrm{EN_i} \times \mathrm{PB_t}}{\mathrm{PB_i} \times \mathrm{EN_t}}$$

ED: egg density
EN: egg number
PB: plant biomass
EC: egg concentration
t: total sample
i: plant species type

$\mathrm{ED_t}$ reflects a theoretical even egg distribution. Hence, $\mathrm{EC_i} = 1$ would represent an average egg density on i, $\mathrm{EC_i} = 2$ means twice as much, and $\mathrm{EC_i} = 0.5$ half as much eggs per PB of i.

2 ESTABLISHMENT AND SAMPLING OF SELECTIVITY EXPERIMENTS WITH ARTIFICIAL SAV BEDS

Field experiments were conducted in 2017 and 2018 at the location A (Altkamp) in order to investigate spawning preferences with regard to different plant species. The focus was on seagrass (*Zostera marina*), brown algae (*Fucus vesiculosus*) and red algae (*Furcellaria lumbricalis*) (Fig. 4A-C). Naturally, these species do only partially overlap due to a strong depth-dependent zonation in the Baltic littoral. To reduce the influence of water depth and spatial separation, SAV beds (= blocks) were artificially planted by arranging 1 x 1 m patches of each plant species (= replicates) with 3 to 5 m distance to each other (Fig. 4D). In contrast to seagrasses that are rooting in the seafloor, *Fucus vesiculosus* and *Furcellaria lumbricalis* are relatively easy transplantable due to their basal parts, which are attached to stones. Hence, appropriate natural patches of *Zostera marina* in approximately 2 m water depth (suitable depth for all chosen plant species) were selected by diving operations and marked with surface buoys. Subsequently, stones with attached algae were collected in the area and piled as *Fucus vesiculosus* and *Furcellaria lumbricalis* patches completing the experimental blocks. The whole experiment was arranged in a random block design (each block contains one replicate of each plant species = 3 different plant species per block) with 6 blocks in 2017 (start: 17/03) and 9 blocks in 2018 (start: 26/03).

Every 7 to 12 days (according to water temperatures and weather-dependent feasibility), the experiments were monitored for herring eggs.

Semi-quantitative documentation of the spawning intensity (ordinal scale) was conducted by section-wise visual evaluation (Fig. 5A) and classification of spawn density into “no eggs”, “single eggs”, “mono egg layers” and “multiple egg layers” (in accordance to an established monitoring methods: Hay and Kronlund 1987, Kotterba et al. 2017).

Additionally, **quantitative sampling** was conducted by randomly harvesting 3 samples of plant material and adherent eggs (75 cm² bottom area from 3 x 25 cm²) of each patch (Fig. 5B) and preserving it in 4 % borax-buffered formalin. In the laboratory, egg numbers were counted and fresh and dry weights (after drying at 80 °C for 48 hours) were determined for eggs and plant biomass of each sample.

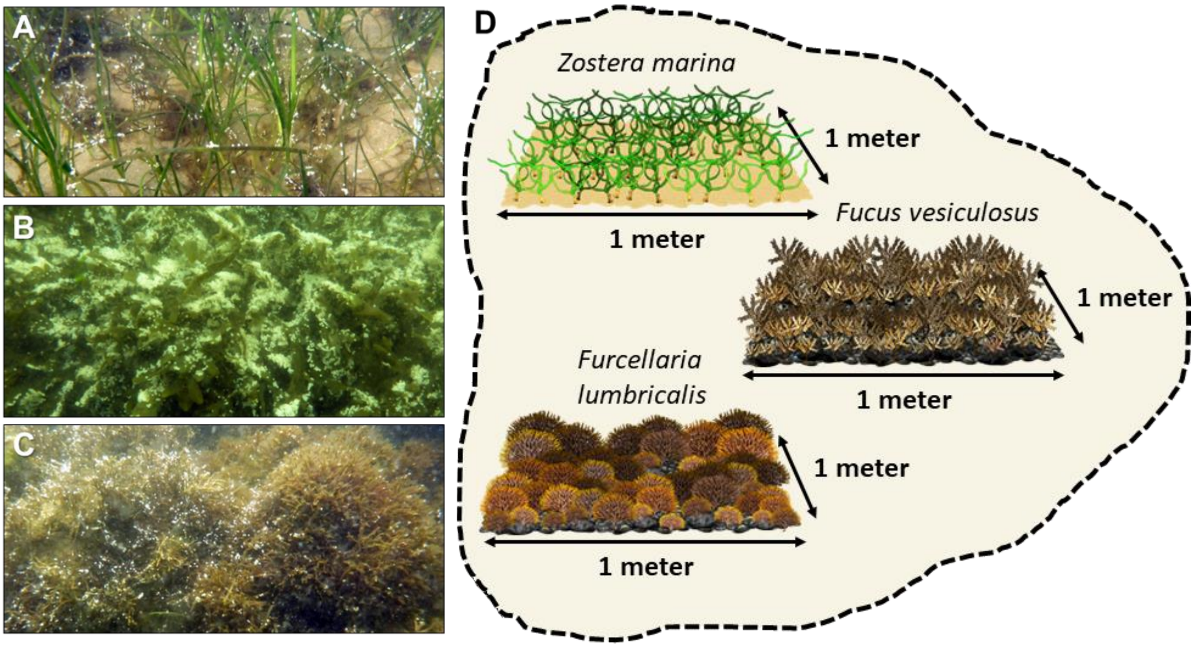

Figure 4. Underwater 'submerged aquatic vegetation' (SAV) beds for experimental investigation of spawning substrate preferences of herring: pictures of plant species patches with attached herring eggs (white dots) **A)** *Zostera marina*, **B)** *Fucus vesiculosus* and **C)** *Furcellaria lumbricalis* at locatin A in 2017, and **D)** illustration of a garden-block with one patch of each plant species Illustration © Kotterba.

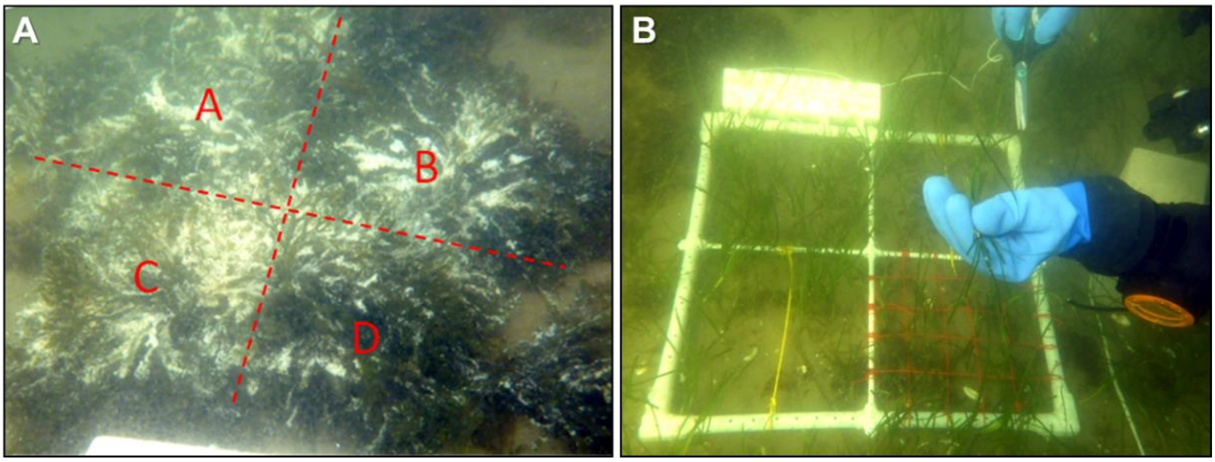

Figure 5. Sampling of experimental 'submerged aquatic vegetation' (SAV) beds with attached herring eggs (white dots): **A)** section wise (red A to D) semi-quantitative evaluation of spawn distribution, and **B)** quantitative sampling by harvesting macrophytes and adherent eggs for examination in the laboratory.

3 CONSTRUCTION OF ASBS - DEEPER THAN THE PRESENT VEGETATION DEPTH LIMIT

Artificial spawning beds (ASBs) were created by installing artificial spawning substrates (BALZER drop nets 1 x 0.5 to 1 m) at the seafloor in different depth zones in close proximity to known herring spawning beds at location A and B in Greifswald Bay (Fig. 2). The chosen artificial substrate has already been applied in earlier studies and was proved to be used by herring as spawning substrate (Kotterba 2015). Each ASB consisted of 3 x 6 nets that were positioned perpendicularly to the ground (with weights/hooks at the bottom and floating rope on top, Fig. 6). The complete experimental setup consisted of 3 ASBs: one in 4 m depth on sandy ground, one in 1 - 2 m depth on sandy ground and additionally a control in 1 - 2 m depth in a vegetated area (Fig. 7).

The setup was installed before the herring spawning season started (10/3/2016 at location B and 16/03/2017 at location A and B) and the experiment was run continuously until the end of the spawning season (in 2016 on 10/5 at location B and in 2017 on 15/5 at location A and 07/05 at location B). During that time, the ASBs were checked for herring eggs spawned thereon every 1 – 2 weeks. Sampling intervals were chosen according to the prevailing water temperatures to keep them shorter than the estimated egg developmental time (to avoid unnoticed hatching).

STATISTICS - For statistical analyses, SPSS 21 (IBM Statistics), R (version 3.2.3, R Foundation for Statistical Computing 2015) and Microsoft Office 2016 were used. For analyses of egg concentration (eggs per plant biomass and eggs per bottom area) on different plant substrate types, groups were compared by the non-parametric Kruskal-Wallis-Test, because homoscedasticity of variances was not initially fulfilled, or could be achieved by angular transformation. *Post hoc* comparison of egg concentration in relation to plant species was conducted with the non-parametric Mann-Whitney U-test (Mann and Whitney, 1947). The significance level for all statistical analyses within the study was set to $p \leq 0.05$.

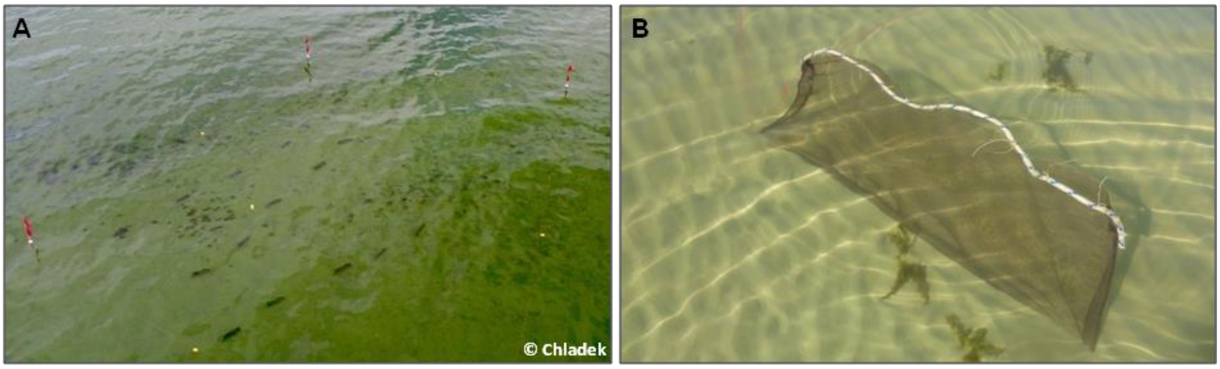

Figure 6. Images of artificial spawning substrates installed on sandy sea floor in 1 - 2 m water depth at location B: **A)** aerial view of an artificial spawning bed (ASB 2, Fig. 7) recorded with a drone and **B)** detail photo of one net fixed on the bottom with hooks and positioned upright with a floating rope.

Figure 7. Satellite image of the investigation area at location B with an overview of the artificial spawning bed (ASB) positions: ASB 1 in 4 m depth on sandy ground, ASB 2 in 1 - 2 m depth on sandy ground and ASB 3 in 1 - 2 m depth in a vegetated area as control.

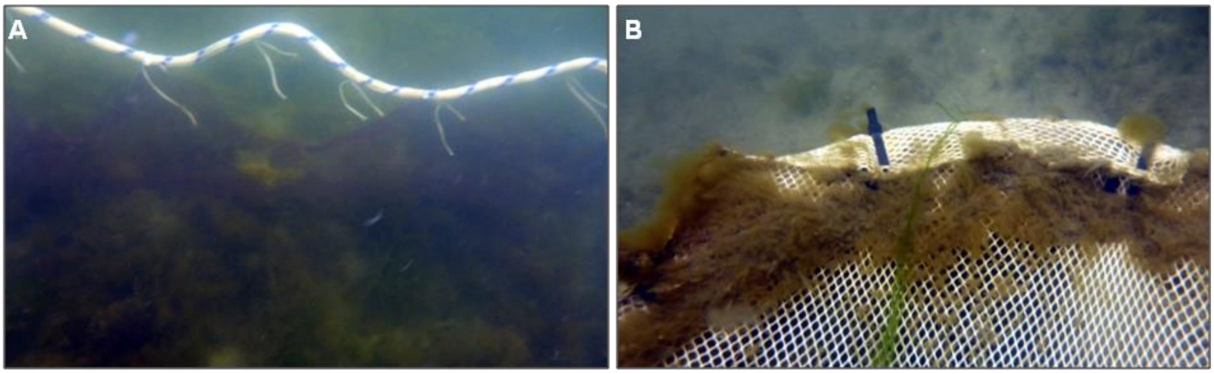

Figure 8. Under water images of the artificial spawning substrates installed at location B in 2016: **A)** the net was completely covered with filamentous brown algae and **B)** filamentous brown algae accumulated at the ropes but also covered the surrounding vegetation of this site.

Results

1 FIELD OBSERVATIONS OF SPAWNING SUBSTRATE SELECTIVITY

In zone A (Fig. 3), most samples were mainly composed of *Stuckenia pectinata* and *Zostera marina* to relatively equal parts (in terms of dry weight biomass) and in 14 of 18 samples filamentous green algae were found (Fig. 9A). Analyses of the relative "egg concentrations" reveal that in half of the samples (9 of 18) *Stuckenia pectinata* was clearly preferred and in one-sixth a majority of eggs was attached to filamentous green algae. In 75 % of the samples spawn concentrations on *Zostera marina* were much lower than average.

A comparison of the two dominating species (only samples in which both species occur; *n* = 16) showed on average 40 % higher egg concentrations (EC) on *Stuckenia pectinata* (mean EC = 1.39 ± 0.65), than on *Zostera marina* (mean EC = 0.58 ± 0.49). Considering the individual samples, in 12.5 % the egg distribution was equal (EC = 1 ± 0.2), in 12.5 % *Zostera marina* was preferred and in 75 % of the samples, eggs were preferably spawned on *Stuckenia pectinata*.

In terms of biomass, the plant community in zone B (Fig. 3) was clearly dominated by *Stuckenia pectinata* (Fig. 9B). However, in one-third of the samples *Ulva intestinalis* was dominantly frequented as spawning substrate, in another third filamentous brown algae were preferred and in the last third *Stuckenia pectinata* was frequented slightly more than the other species, even though *Stuckenia pectinata* constituted more than half of the biomass in 90 % of the samples.

Egg concentration values for the dominant plant species of each depth zone showed a clear preference for *Stuckenia pectinata* in zone A and rather low values in zone B (Fig. 9C). For *Zostera marina* extremely low egg concentrations in relation to a hypothetical equal distribution were found in zone A, and in both zones filamentous and epiphytic algae were comparatively excessively covered with eggs in some of the samples.

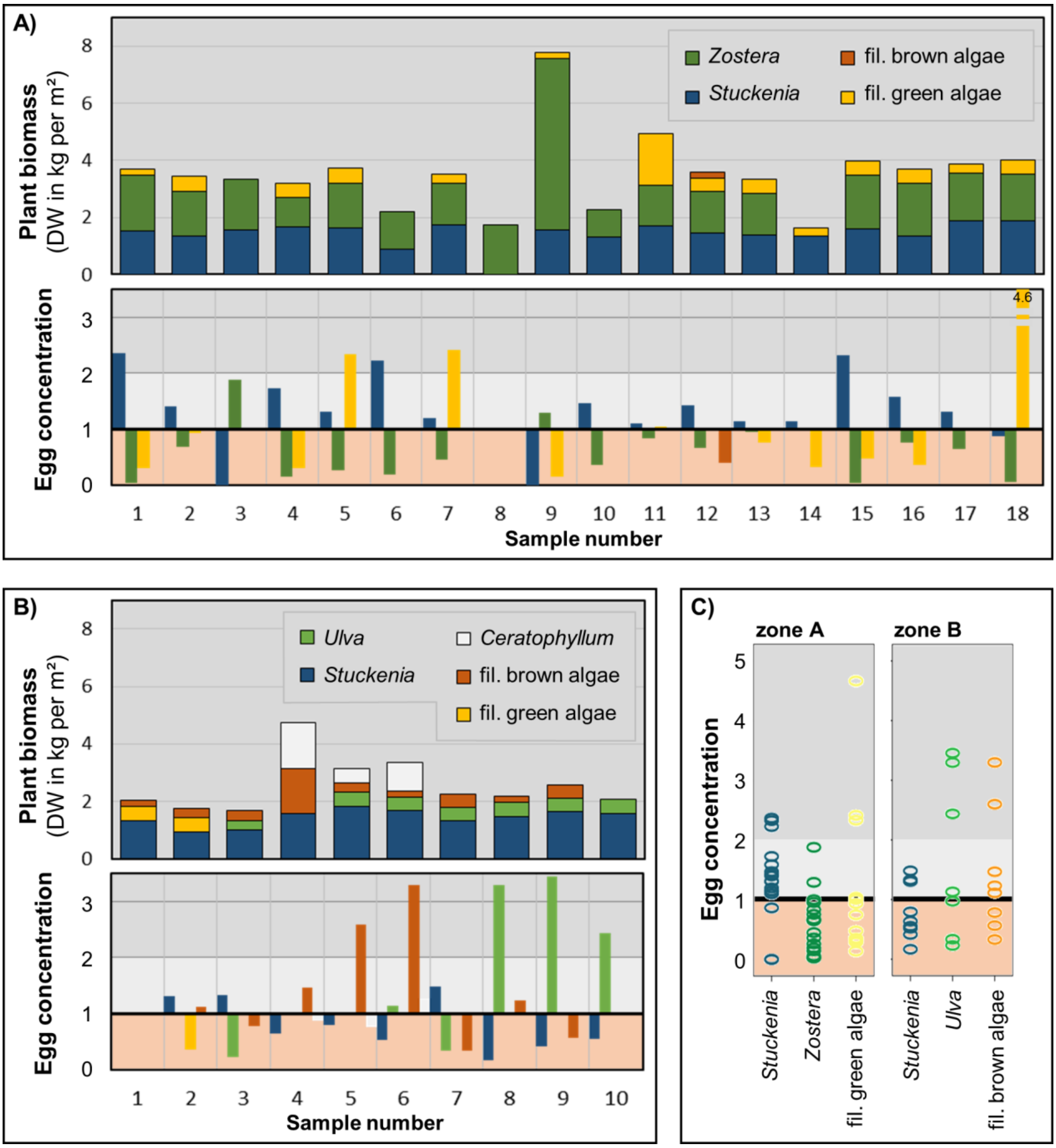

Figure 9. Herring spawn distribution analyzed by plant species substrate (i.e. *Zostera marina*, *Stuckenia pectinata*, *Ulva intestinalis*, and the cross-species classes filamentous green algae and filamentous brown algae) in two different water depth zones: plant biomass distribution (dry weight (DW) per area) and egg concentration on each plant species is shown for each sample of **A)** the mix zone A and **B)** the shallow zone B. Egg concentration was calculated from the number of eggs per plant species biomass in relation to the total number of eggs per plant biomass for each sample (1 would represent an average egg density, 2 means twice as much eggs and 0.5 half as much eggs per PB). Graphic **C)** shows an overview of the egg concentration values (circles) on the most abundant plant species in respective depth zone.

2 HERRING SPAWNING SUBSTRATE SELECTIVITY ON ARTIFICIAL SAV BEDS

Field experiments with distinct plant types offered as spawning substrate revealed different selectivity patterns in the consecutive years 2017 and 2018 (Fig. 10A&B). While all plant types were used as spawning substrate and did not differ with regard to the presence/absence of herring eggs, the spawning intensity ("single eggs" to "multiple layers") on different plant species clearly indicated preferences for certain species in both years. Semi-quantitative analyses indicate a preference for *Fucus vesiculosus* in 2017 and for *Furcellaria lumbricalis* in 2018.

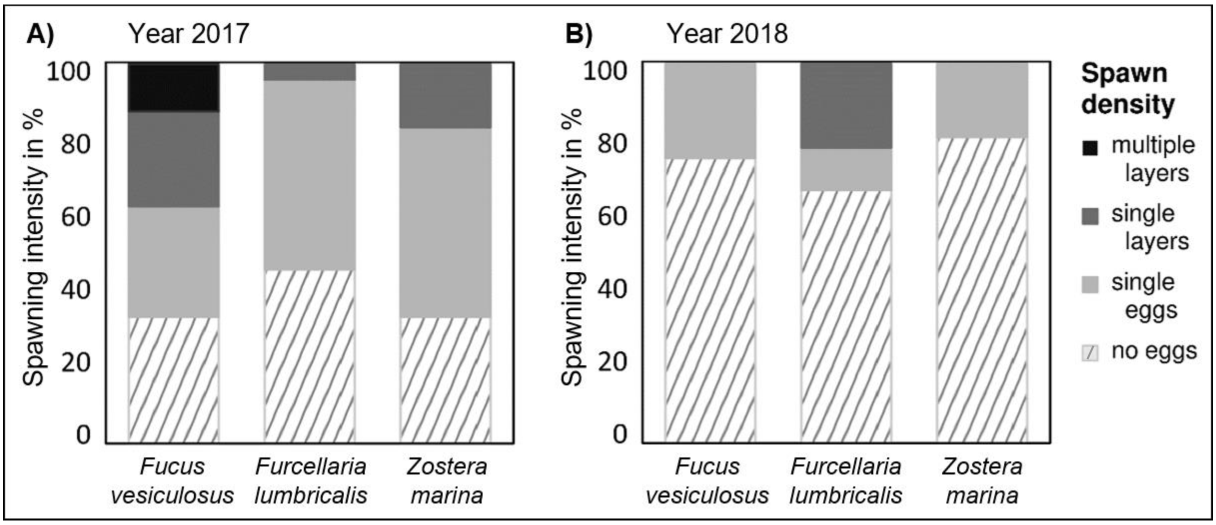

Figure 10. Herring spawning substrate selection analyzed by spawning intensity on different plant species: semi-quantitative sampling was conducted divers in **A)** year 2017 (n = 24 from 6 replicates x 4 sections) and **B)** year 2018 (n = 36 from 9 replicates x 4 sections) by section wise evaluation of the spawn density (see Fig. 5).

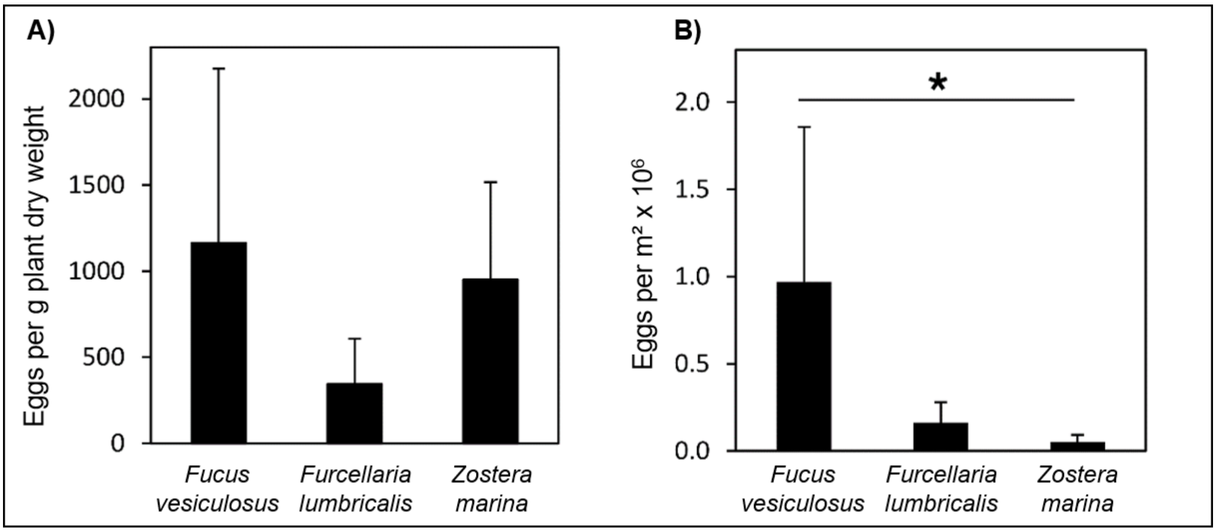

Figure 11. Herring spawning substrate selection analyzed by spawning intensity on different plant species: quantitative analyses of egg numbers per **A)** plant biomass (dry weight) and **B)** surface area in year 2017 (n = 4, 2 of 6 blocks with no spawning activity were excluded; * $p \leq 0.05$, U-test).

Quantitative analyses of herring egg concentrations in relation to the chosen spawning substrate resulted in non-significant differences of the number of eggs per plant species biomass (Kruskal-Wallis test, H(2) = 2.423, p = 0.298) (Fig. 11A). However, significant differences were found in plant species related egg numbers per bottom area (Kruskal-Wallis test, H(2) = 7.038, p = 0.030) (Fig. 11B). Thereby, significantly more eggs m^2 were found on *Fucus vesiculosus* patches than on *Zostera marina* beds (U-test, U = 0, p = 0.021) but egg density per bottom area was not statistically different between *Fucus vesiculosus* and *Furcellaria lumbricalis* patches (U-test, U = 3, p = 0.149) or patches of *Furcellaria lumbricalis* and *Zostera marina* (U-test, U = 2, p = 0.083).

3 SPAWNING ACTIVITY ON ARTIFICIAL SPAWNING BEDS (ASBS)

Experiments were successfully installed and subsequently, the ASBs were regularly checked for herring spawning activity. However, in both years and at both locations, no eggs were found on any of the nets, neither at the deeper position (ASB 1, deeper than the present vegetation depth limit) nor at the shallow positions (ASB 2 on a sand bank and control ASB 3 in a vegetated area) (positions see Fig. 7).

In 2016, eggs were only found in depth zones between 1.5 and 3 m, but the very shallows (where the ASB 3 was located) have apparently been avoided by the fish. The fish might have intentionally evaded that area because of a massive bloom of filamentous brown algae that occurred at that time in the shallows. However, in the same period, the same artificial spawning substrates (nets) were used for some different experiments a few kilometers down the coast, and there herring eggs were found directly attached to the nets (Kotterba pers. obs). Hence, the ASB setup was replicated in 2017 at the same location, and an additional one on the opposite shore of Greifswald Bay. The general spawning activity was again extremely sparse at both locations and again no eggs were spawned on any ASB.

Discussion

The often-used paradigm that “herring spawn on every structure they would find under water” has major relevance for assessing possible consequences of changing coastal habitats for herring reproduction. However, the paradigm is doubtful since indications for active substrate selection (Stacey and Hourston 1982, Aneer et al. 1983). For a profound evaluation of the utilization of herring spawning beds, knowledge on substrate selectivity of herring is an essential prerequisite. During this study, *in situ* experiments and observations of herring egg distribution, in dependence of the spawning substrate, revealed year- and site-specific selectivity patterns.

Different herring stocks in the Baltic Sea are reported to frequent different types of spawning substrates (e.g. Haegele and Schweigert 1985, Scabell 1988, Aneer 1989, Rajasilta et al. 1993, Klinkhardt 1996). The Central Baltic herring for example is assumed to spawn among other areas in coastal zones of Poland, like Vistula lagoon, where no SAV beds but mainly mud- and sand bottoms can be found. However, nowadays evidence for herring spawn was only found on blue mussel clusters that were covered with thick egg-layers (Gruszka 2008). For the Gulf of Riga and the Lithuanian coast, herring were reported to spawn on different red algae species and on sand on the bare sea floor (Raid 1990, Šaškov et al. 2014). In contrast to the rather phytohilous spawning spring herring, autumn spawning herring use offshore gravel beds for spawning (Ojaveer 1981, Oulasvirta 1988) In the past, the autumn spawners were dominant in Western Baltic until they decreased dramatically in the 1970s and the Western Baltic spring spawning (WBSS) herring, who primarily attach their eggs to SAV, gained more importance (Parmanne et al. 1994).

Regarding the spawning behavior, it is reported, that female Baltic herring seek direct contact with the substrate for spawning (Aneer et al. 1983, Scabell and Jönsson 1984). However, it is unclear if herring migration is directed to vegetated areas where they then chose a specific substrate to attach their eggs or if they release their eggs rather randomly. Investigations of WBSS herring egg distribution in the field (at location B in 2014), evidently show, that females are selective by attaching their eggs to SAV (with different spawning intensities) and strictly avoid the surrounding sand (no spawn was found) (Kotterba 2015, Kotterba et al. 2017). These results coincide with earlier observations in Greifswald Bay (Jönsson et al. 1984, Scabell and Jönsson 1984, Scabell 1988, Kanstinger 2014).

SPAWNING SUBSTRATE SELECTIVITY IN A MIXES VEGETATION BED - Our analyses of plant species related herring egg distribution after a natural spawning event at location B in 2017 showed a clear preference of female herring for *Stuckenia pectinata* in comparison to *Zostera marina*, indicated by much higher egg numbers per plant biomass. This might not only be a consequence of selectivity. As a matter of fact, in artificial spawning experiments (manuscript II) for example, it was noticed, that herring eggs seem to adhere not as good/quick to seagrass as they do to most other substrates. Furthermore, *Stuckenia pectinata* has a higher structural complexity and might thereby provide better egg developmental conditions (see manuscript IV or von Nordheim et al. 2018).

Filamentous and epiphytic algae were covered with comparatively high egg numbers in the field. This might be a consequence of those algae forming mats, which cover the actually targeted spawning substrates (e.g. *Stuckenia pectinata*). Furthermore, values of egg concentration (as numbers per plant biomass) might be disproportionally high on filamentous algae. Due to their special three-dimensional architecture, even low biomasses (dry weight) of algae may occupy a high area/volume where eggs can get trapped.

In several earlier studies, observations of Baltic herring eggs attached to different plant species were documented. For Sweden different preferences (e.g. *Ceramium* or *Pylaiella littoralis* and *Ectocarpus siliculosus* or *Zostera marina* and *Fucus vesiculosus*) and a variation from year to year was described (Aneer and Nellbring 1982, Aneer et al. 1983, Elmer 1983, Aneer 1989). In Finland filamentous algae (green: *Cladophora glomerata* or brown: *Ectocarpus siliculosus* and *Pylaiella littoralis*) seem to be preferred, while perennial macrophytes were frequented to lesser extent (Oulasvirta et al. 1985, Kääriä et al. 1988, Rajasilta et al. 1989, 1993), and from the Gulf of Riga an avoidance of *Fucus vesiculosus* was reported (Raid 1990). In contrast, at the german Baltic coast in Greifswald Bay, all present perennial macrophytes (*Furcellaria fastigiata, Fucus vesiculosus, Stuckenia pectinata* and *Zostera marina*) were reported to be mainly frequented as spawning substrates (Scabell and Jönsson 1984, Scabell 1988, Moll et al. 2018, Kanstinger et al. 2018). Some field studies reported that eggs were additionally found on other substrates like gravel, stones, rock, sand, or on the shells of *Mytilus edulis* (Oulasvirta et al. 1985, Scabell 1988, Aneer 1989, Raid 1990, Šaškov et al. 2014), but no eggs were found on soft or muddy bottoms (Aneer et al. 1983, Oulasvirta et al. 1985, Kääriä et al. 1988, Kotterba et al. 2017).

However, those earlier studies mainly provide qualitative information on plant species, utilized by herring as spawning substrates. Unfortunately, in those cases a numeric evaluation of potential substrate preferences is unfeasible, because references to abundance or biomass of respective species in the sampled areas are missing. Furthermore, the recorded egg numbers per area of this study were not compared to values of other studies, because spawning intensity was highly variable on small scales. Hence, egg numbers would not show a realistic picture. However, the relation of eggs spawned on different substrate types in each sample is still a reliable value.

The above described analysis of the relation between herring egg distribution and plant species was performed depth zone-wise and revealed zone dependent differences in spawning substrate selectivity. These were most likely induced by the occurrence of different plant communities in those zones. Haegele and Schweigert (1985) postulated, that herring do not favor one type of vegetation, and speculated the egg distribution to be a function of spawning depth and vegetation, present in respective area. Results of this study are contradictory, as in both depth zones *Stuckenia pecktinata* is present to the same biomass extend, but egg concentration (egg number on this substrate in relation to the egg number on all present substrates) was very different (high in mixed zone A and low in shallow zone B). This might be an indication for shifting preferences according to the available plant species.

SPAWNING SELECTIVITY ON MONO-SPECIES ‘ARTIFICIAL SAV BEDS’ - Thus, our results clearly emphasize an influence of depth and/or the present plant community. It might be speculated, that herring would spawn on the first suitable substrate they meet, when entering a potential spawning area. However, this theory was disproved by experiments with artificial SAV beds, planted in equal water depth and close spatial proximity to each other. Unfortunately, herring spawning activity was quite low since 2015 (ICES Advice 2019), resulting in limited generation of data (experiments were conducted in 2017 and 2018). Furthermore, for unknown reasons, all eggs found on the spawning beds in 2017 were not fertilized (see opaque color of eggs in Fig. 4 and 5). However, as the fertilization would occur after female herring attached their eggs to the substrates we assume that, whatever the reason, it did not affect the selection of spawning substrates.

Nevertheless, variable selectivity patterns were documented during a qualitative data collection in the first year and semi-quantitative samplings in both years. The latter revealed a visually noticeable preference for one of the three plant species each year, but contrary to expectations different species were preferred in the years (2017: *Fucus vesiculosus* and 2018: *Furcellaria* *lumbricalis*). This is an indication for some other mechanisms, besides the specific substrate type, being a trigger for spawning. Maybe, the selection is influenced by the incidence of light when herring arrive in the spawning area or by the current wave action, moving the plant species in a different manner. Nevertheless, both favored plant species have rather dark colors (brown and red) and are quite compact, solid and branched compared to the less frequented seagrass (*Zostera marina*), which is characterized by rather filigree, long and smooth leaves without branching.

Furthermore, the results prove the "first seen – first spawned"-hypothesis wrong, because in the study area, the fish first passed a natural seagrass bed and then arrived at the experimental gardens, where they preferably spawned on *Fucus vesiculosus* or *Furcellaria lumbricalis*, while on *Zostera marina* only few or no eggs were found. This is in accordance to recorded egg distributions in the Pacific, where spawn density increased closer to shore and was not influenced by the migration route (Haegele et al. 1981). Our depth-independent quantitative analysis of herring spawn distribution in 2017 revealed significantly more eggs per bottom area on *Fucus vesiculosus* than on *Zostera marina,* and egg numbers per plant biomass were also higher on *Fucus vesiculosus*. Those results clearly indicate active substrate selection of herring. This was also observed in other studies even though selected plant species were different. For the Wadden Sea at the Island of Sylt, 20 times higher egg densities per plant biomass were described and twice as many eggs per bottom area on *Zostera noltii* than on *Fucus vesiculosus* (Polte and Asmus 2006). However, the plant species beds evaluated in that study did not occur in direct spatial proximity. From all those results we conclude, that egg distribution patterns are indeed a function of spawning depth and the present vegetation, as Haegele and Schweigert (1985) assumed, but we would add that environmental factors seem to influence the attractiveness of particular plant species.

SPAWNING BELOW THE PRESENT VEGETATION DEPTH LIMIT - It is a general assumption that the extent and distribution of herring spawning grounds has decreased in the study area Greifswald Bay. This is a consequence of eutrophication linked processes such as increased turbidity and accordingly limited light permeability resulting in a reduced depth distribution of vegetation (Munkes 2005, Kanstinger et al. 2018). However, to our knowledge it is not documented if herring actually spawned in deeper parts of the bay prior to the increased eutrophication and associated impacts in the 20th century. Furthermore, a potential spawning in deeper areas, if suitable substrate was available there, has not been sufficiently examined yet. To fill this knowledge gap, artificial spawning beds (ASBs) were offered in areas deeper than the present vegetation depth limit (on sandy sea floor) and as control in shallower vegetated and sandy areas. However, in two consecutive years (2016 and 2017), no herring eggs were found on any of the offered ASBs, allowing no conclusions about herring spawning depth and potential spawning below the actual vegetation depth limit. A possible reason could have been an avoidance of sandy areas by spawning herring, as Scabell (1988) recorded a close relation of spawning behavior to the distribution of spawning substrates and an increased spawning intensity with higher vegetation coverage per area. However, even on the control ASB 3 in the vegetated zone or on the surrounding vegetation no eggs were found. This was probably also a consequence of the very low spawning activity in combination with unfavorable environmental conditions at the experimental locations, even though spawning activity has been observed there in previous years 2012-2015 (Kotterba et al. 2017, Moll et al. 2018). However, we would still consider this method useful for analyses of a depth related spawning intensity especially in areas beyond the present vegetation cover. Nevertheless, we would propose an extended setup with more locations (which would include very high logistical effort) to cover more potential spawning areas and thereby compensate potentially low spawning activities.

In summary, observations and experiments of this study support the hypothesis, that herring are selective in the choice of spawning substrate. However, the selectivity seems to be influenced by a number of factors, while the on-site plant community, probably in combination with weather conditions, is the prominent one. During the present study, solid macro algae and the pondweed *Stuckenia pectinata* were frequently used as spawning substrate, while seagrasses were rather scarcely utilized. However, herring are attracted by extensive vegetation beds for spawning (Scabell 1988, Kääriä et al. 1997, Polte and Asmus 2006, Kanstinger et al. 2018), which are predominantly formed by perennial angiosperm species in the Baltic coastal zones. This suggests an important role of seagrasses and a major role of pondweeds in providing a consistent and reliable spawning area for herring form year to year, as they are the only constantly persistent plant species in coastal zones of the Baltic Sea. However, results of this study suggest that *Zostera marina* beds in Greifswald Bay might primarily function as attractant for spawning herring. Once arrived on the spawning ground, the fishes seem to prefer rather branching and solid species such as *Stuckenia pectinata* and the macro algae *Fucus vesiculosus* and *Furcellaria lumbricalis,* if available. Experiments on potential spawning below the present vegetation depth limit remained without results for the time being, probably due to very low spawning activity in the studied years, but the question should further be investigated.

From this study and a comprehensive literature review, we conclude that healthy perennial vegetation beds are the most important prerequisite for favorable herring spawning areas. Regardless of the exact plant species composition, herring will enter the area and find at that moment their preferred spawning substrate. However, the actual quality of the respective substrate in terms of egg development and hatching success has been evaluated in different studies (manuscripts II-IV).

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Developmental success of herring eggs spawned on different plant species

Lena von Nordheim, Paul Kotterba, Dorothee Moll and Patrick Polte

Abstract

Western Baltic spring spawning herring (*Clupea harengus*) annually migrate into shallow coastal areas for spawning. There, they attach their demersal eggs to submerged aquatic vegetation. Continuous eutrophication over the last decades led to a changed species composition and reduced depth distribution of the aquatic plant community. Nevertheless, herring regularly return to the same areas for spawning. It is assumed that herring spawning behavior is non-selective in respect of spawning substrates. However, little information is available on the effects of different spawning substrates on the egg development.

Hypothesizing that the substrate affects herring egg survival, field experiments were conducted in which eggs were artificially spawned on different perennial plant species that are frequently used by herring in the western Baltic Sea and on a control substrate respectively. All substrates were incubated directly on natural spawning grounds, regular sampling was conducted, and the egg survival rate was determined according to spawning substrates. The results indicate that all tested plant species led to egg survival rates well over 50 %, showing conspicuous but not significant differences. While comparatively high egg mortality was only found on the red alga *Furcellaria lumbricalis*, all other substrates resulted in high egg survival rates compared to the control (nylon-netting without physiological activity). The brown algae *Fucus vesiculosus* performed best in terms of continuously high egg survival, followed by the angiosperms *Zostera marina* and *Stuckenia pectinata*.

The results demonstrate healthy perennial macrophyte meadows being an important prerequisite for successful reproduction of Baltic spring herring. This emphasizes the need to preserve the remaining sedentary macrophyte beds and to improve regeneration capacity of the plant community by reducing anthropogenic stressors. This should be seen as a basis for effective management of coastal habitats and thus a foundation for resilient herring stocks.

Introduction

Most marine teleost fish are pelagic spawners with eggs that develop in the open water column (Balon 1975, 1981). However, some important components of oceanic food webs as well as important fishery resources such as herring (*Clupea* spp.) attach demersal eggs to benthic spawning substrates (Haegele and Schweigert 1985, Hay 1985, Klinkhardt 1996). Within those species and throughout a broad taxonomic range in general, many facultative and also obligatory phytophil spawners exist, relying on vegetated spawning grounds for reproduction (Balon 1975). Furthermore, they might depend on certain plant species for spawning and additionally, certain substrates might affect the egg development. Potential functions of phytophil spawning (e.g. physiological advantages or disadvantages due to photosynthetic oxygen enrichment or possible disadvantages due to oxygen depletion caused by nocturnal plant respiration) for the development of fish eggs are rarely studied.

During recent decades, aquatic habitats underlie severe alterations induced by omnipresent factors like coastal modification, cultivation and eutrophication, and global warming. In consequence of reduced light availability, aquatic vegetation can only be found in rather shallow waters and the specific depth distribution is mainly determined by water turbidity. Coastal ecosystems all over the world suffer from nutrient levels in the water that stimulate extreme phytoplankton growth (Bricker et al. 2008) and increase epiphyte coverage, resulting in reduced light levels due to overgrowth and shading of perennial macrophytes (Burkholder et al. 2007). It can be summarized that eutrophication leads to increased abundance (of fast growing algae), but decreased diversity of macrophyte species (Rabalais 2002, Worm et al. 2002). Under high nutrient supply seagrasses are replaced by macroalgae (Duarte 1995, Valiela et al. 1997, Pérez-Ruzafa et al. 2019), and additionally, sediment runoff, physical disturbance and global warming result in declining seagrass beads all over the world (Orth et al. 2006, Waycott et al. 2009). However, perennial macro brown algae, such as *Fucus vesiculosus*, are also reported to decrease in eutrophicated systems like coastal areas of the Baltic sea (Kautsky et al. 1986, Vogt and Schramm 1991). All those reports emphasize that structure and species composition of vegetated spawning grounds are subjects to an ongoing change, a development that most likely causes great risk for all species depending on specific habitat components for reproduction. However, knowledge on influences of certain spawning substrate on fish egg development in marine or limnic ecosystems in general is very limited.

To answer the question if a vegetation shift might influence the reproductive success of phytophilous spawners, we exemplarily tested the survival of herring eggs spawned onto different plant species. This study focuses on western Baltic spring spawning herring (*Clupea harengus*), which is considered to be rather unspecific in choice of spawning substrate but primarily uses the available aquatic vegetation (Aneer 1989). Earlier experiments revealed reduced herring egg survival rates induced by the filamentous brown algae *Pylaiella littoralis* (Aneer 1989, manuskript IV) and decomposing red algae *Furcellaria* sp. (Rajasilta et al. 2006), as well as a benefit of high spawning substrate complexity in general (manuskript IV or von Nordheim et al. 2018). However, detailed information on quality differences in terms of egg survival rates could not be found for western Baltic "common" herring spawning substrates. For the Baltic Sea, a variety of different aquatic plants was described as potential herring spawning substrates (e.g. Aneer and Nellbring 1982, Aneer et al. 1983, Elmer 1983, Oulasvirta et al. 1985, Rajasilta et al. 1989, 1993, Aneer 1989, Raid 1990, Kääriä et al. 1997, Šaškov et al. 2014). And four of those plant species were chosen for this study, known to be frequently used by herring in the Greifswald Bay (Scabell and Jönsson 1984, Scabell 1988, Kanstinger et al. 2018). However, previous studies were mainly conducted by random sampling after natural spawning events of herring. When determining egg coverage and mortality rates this method is prone to miscalculations because (dead) eggs from earlier spawning waves might lead to overestimations. Aneer (1989) for example observed the same dead eggs for weeks on some locations. For this reason we decided for experiments with the artificial spawning method where all eggs are directly attached to the substrates on one day and followed by a regular sampling.

Hypothesizing that herring egg survival is affected by vegetation taxon specific characteristics, we tested the flowering plants *Zostera marina* (seagrass) and *Stuckenia pectinata* (pondweed, a freshwater species) and the macroalgae *Fucus vesiculosus* (brown algae) and *Furcellaria lumbricalis* (red algae) as spawning substrates. They are dominating different water depth zones and differ in phylogeny and depth zonation (Fig 1). To include the full set of stressors (e.g. changes in water temperature and oxygen levels, wave action and biotic interactions) acting in natural environments, we conducted field experiments directly located on herring spawning grounds.

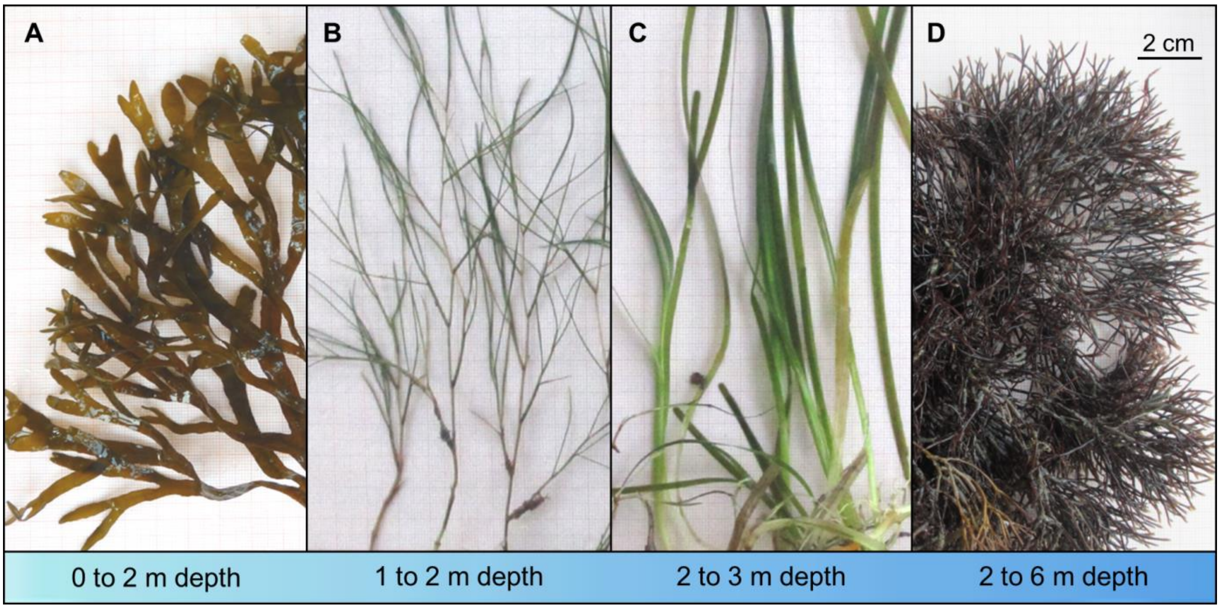

Figure 1. Macrophyte species naturally frequented by spawning herring in Greifswald Bay: A) *Fucus vesiculosus*, B) *Stuckenia pectinata*, C) *Zostera marina*, D) *Furcellaria lumbricalis* and their depth distribution (blue section below pictures) at the 2nd experimental location (according to own observations).

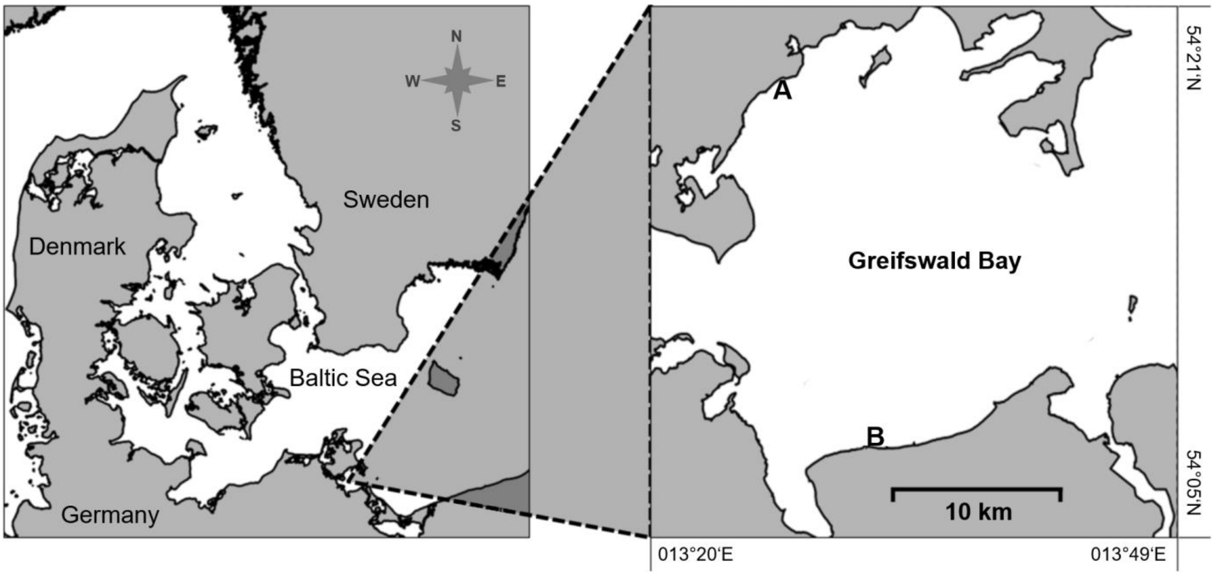

Figure 2. Investigation area Greifswald Bay and its location in the Baltic Sea with relevant locations for this study: A) 'Altkamp', 2nd experiment and B) 'Gahlkow', 1st experiment. Bathymetry data: received by courtesy of Federal Maritime and Hydrographic Agency of Germany.

Material und Methods

STUDY SITE - The study site Greifswald Bay (54°14'N, 013°33'E) is a brackish lagoon of the non-tidal Western Baltic Sea, enclosed by the German mainland and the Island of Rügen (Fig. 2). The water volume of 2.96 km³ is distributed over an area of 514 km² with a mean depth of 5.8 m (max. 13.6 m) (Reinicke 1989, Stigge 1989). The salinity ranges between 7 and 8 (Schiewer 2001), pH values are approximately 8.5 in spring. Water temperatures are closely related to seasonal changes in air temperature, fluctuating from above 20 °C in summer down to -2 °C with frequent closed ice covers in winter. Wind speed and direction are the main drivers of water level changes and additionally result in a mostly well mixed and oxygenated water body. Submerged aquatic vegetation is present from the very shallow littoral areas down to approximately 3.5 m water depth in the Greifswald Bay (Munkes 2005, Kanstinger et al. 2018). The macrophyte community is a mixture of marine, brackish- and freshwater species and in spring western Baltic spring herring annually migrate into the Bay for spawning (Scabell 1988, Oeberst et al. 2009, Polte et al. 2014).

PLANT COLLECTION AND ARTIFICIAL SPAWNING - For the experiments, selected plant species (*Zostera marina, Stuckenia pectinata, Fucus vesiculosus* and *Furcellaria lumbricalis*) were collected by snorkeling and diving in Greifswald Bay. Subsequently, ripe herring were caught in close proximity to their natural spawning grounds. To include a broad size range of mature fish (minimization of spawner size effects on experiments) gillnets of differing mesh sizes (3 nets of 25 m x 5 m with mesh sizes of 25, 27 and 29 mm) were used for fishing. Previous to installation in the experimental field set-up, the plant substrates and for the second experiment also a neutral control substrate (3 x 11 cm pieces of nylon netting, mesh size 4 mm) were covered with subsequent single layers of eggs by the well established method of artificial spawning (details described in manuscript IV or von Nordheim et al. 2018).

EXPERIMENTAL DESIGN - Two field experiments were conducted directly on natural herring spawning sites, located on the southern and northern shoreline in Greifswald Bay (Fig. 2), to include the full set of naturally interacting parameters that influence herring egg development. The aquatic plants, covered with fertilized herring eggs, were strapped onto metal frames and attached to the seafloor at a mean water depth of 1.0 m (Fig. 3). A randomized block design with 9 replicates ($n = 9$) was used to detect potential influences of natural gradients and small scale heterogeneity in the natural environment (Dutilleul et al. 1993). Hence, one replicate of

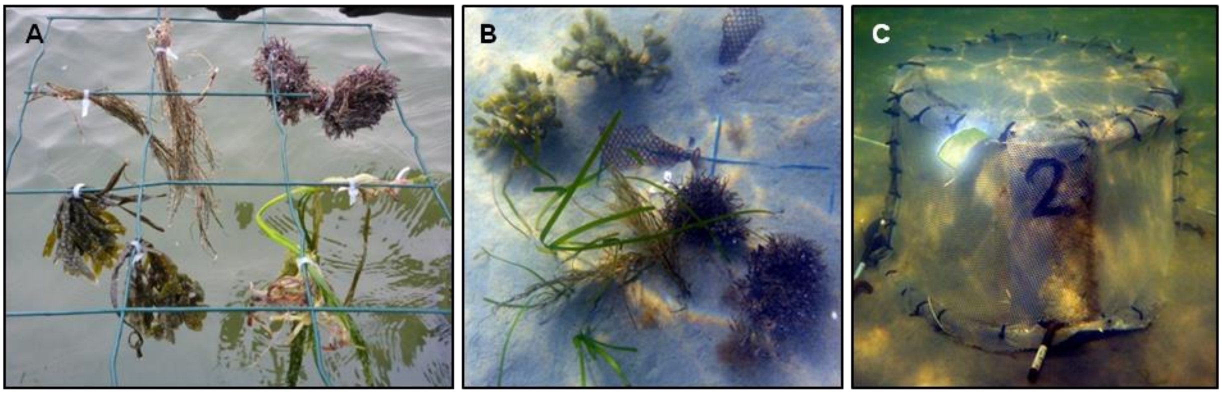

Figure 3. Field installation of experimental substrate types with attached herring eggs: A) grid with *Stuckenia pectinata*, *Furcellaria lumbricalis*, *Fucus vesiculosus* and *Zostera marina* (top left to bottom right), B) grid attached to sea floor with additional control substrate, C) substrates covered by predator-exclusion-cage.

each substrate type (consisting of two separately removable units, Fig. 3A and 3B) was randomly placed into a block (substrates were attached to a metal grid), and each block was covered by a cage of mesh (knot to knot mesh size of 5 mm, Fig. 3C) to prevent natural egg predation (Kotterba et al. 2014, 2017).

Samples from both experiments were taken at the start of the experiments (24 h post fertilization, *n* = 6) to investigate the fertilization success, after half of the egg development and shortly before the first larvae were hatching. The speed of development is highly depending on surrounding water temperatures. Hence, degree days (°d) were used for standardization, calculated as the product of temperature (°C) and egg development time in days (d) (Apstein 1909, Klinkhardt 1996). Since hatching experiments in the laboratory revealed no hatching before °d 90 at water temperatures below 13 °C (Blaxter and Hempel 1963, Peck et al. 2012), ‘final sampling’ was conducted between °d 55 and °d 90 in the field.

The first experiment (April 14th until April 22nd 2016) was conducted on the southern shore of Greifswald Bay (location B, ‘Gahlkow’) at water temperatures from 9 to 11 °C (Salinity 7.5, pH 8.3). A data logger (MiniDOT, PME), recording dissolved oxygen and water temperature, was installed in close proximity to the experiment for continuous monitoring. The second experiment (April 27th until May 5th 2016) was carried out at location A (‘Altkamp’), on the northern coast of Greifswald Bay, at water temperatures from 9 to 13 °C (Salinity 7.9, pH 8.3). A physiologically non-active standardized control (nylon netting) was added as a fifth substrate type, allowing comparison to other experiments containing that control (e.g. in manuscript III). In this second set-up, the data logger was mounted directly into one of the predator-exclusion-cages to check for any oxygen deficiencies right in the cage and for continuous monitoring of dissolved oxygen and water temperature. Unfortunately, two of the cages were washed away due to heavy wave action caused by a storm event. Hence, the replicate number was reduced to $n = 7$.

SAMPLING AND PROCESSING - Samples were taken by removing a unit of each substrate type from each block in the field, and immediately preserved in 4 % borax-buffered formalin. With this method the natural transparency of living eggs and the white color of dead eggs were conserved. Later on, in the laboratory, the distinction of originally vital and dead eggs was made and the percentage of living eggs was calculated. The fertilization success for each substrate type was determined by using a subsample of total $n \geq 400$ eggs. For determination of the egg survival on °d 55 and °d 90 it was aimed for a total $n > 200$ eggs. This could not be realized with all replicates (because of high egg loss rates on seagrass), but analyses over all replicates revealed no significant influence of reduced number of counted eggs in those cases (regression analysis). The counted percentage of living eggs in each replicate was normalized by the fertilization success according to the following formula: [percentage of living eggs * 100 / median percentage of fertilized eggs of the respective substrates type and experiment].

STATISTICS - Statistical and graphical analyses were performed with R (version 3.2.3, R Foundation for Statistical Computing 2015), SPSS 21 (IBM Statistics) and Microsoft Office 2007. The egg fertilization rate and later on the egg survival on different substrates was compared with one-way analysis of variance (ANOVA), and multiple comparisons of pairs (*post hoc* tests) between mean values were performed with Tukey’s Honestly Significant Difference (HSD) test. For analyses of egg survival on different substrate types, groups were compared by the non-parametric Kruskal-Wallis-Test and *post hoc* by the Dunn-Bonferroni-Test, because homoscedasticity of variances was not initially fulfilled, or could be achieved by angular transformation. The significance level for all statistical analyses within the study was set to $p \leq 0.05$.

Results

Oxygen saturation and water temperature followed strong diurnal rhythm at both experiment locations. Oxygen levels during daytime were higher during the 1st experiment at location B (up to 145 %, 'Gahlkow') than during the 2nd experiment at location A (maximum 130 %, 'Altkamp') but did not fall below 80 % at both locations (see Fig. 6 in 'General discussion', p. 181). Temperature differences mostly around 3 °C and up to 5 °C were recorded between day and night during both experimental periods.

***FERTILIZATION RATE* -** The fertilization rate (Tab. 1) was found to differ significantly between the tested plant spawning substrates (ANOVA: 1st experiment F(1,3) = 4.342, $p = 0.016$; 2nd experiment F(1,3)=6.911, $p = 0.002$). For both experiments, *post hoc* tests revealed a significantly lower fertilization on *Zostera marina* than on the other substrate types.

Table 1. Herring egg fertilization success on different spawning substrates in percent. Mean values with standard deviations and median values are shown for each substrate type and experiment ($n = 6$).

Substrate	**1st experiment**		**2nd experiment**	
	Mean	Median	Mean	Median
Fucus vesiculosus	97.82 (± 1.0)	97.79	97.81 (± 1.5)	97.63
Furcellaria lumbricalis	96.86 (± 0.8)	97.08	97.89 (± 2.1)	98.41
Stuckenia pectinata	97.72 (± 1.3)	97.64	98.24 (± 1.2)	98.55
Zostera marina	95.39 (± 1.9)	95.85	91.93 (± 5.5)	94.49
Control	-	-	100 (± 0)	100

***SUBSTRATE RELATED EGG SURVIVAL* -** data were normalized by the median fertilization success per substrate and experimental round. During the 1st experiment, egg survival rates were generally extremely low (Tab. 2). After half of the egg development, significant differences in the percentage of living eggs were found on the substrate types (Kruskal-Wallis test, H(3) = 10.563, $p = 0.014$), but only between *Furcellaria lumbricalis* and *Zostera marina* (Dunn-Bonferroni test, $z = 3.106$, $p = 0.011$). Shortly before the larvae would hatch, almost no surviving egg was found and no differences between the plant species were documented (Kruskal-Wallis test, H(3) = 6.171, $p = 0.104$).

Table 2. Substrate related herring egg survival in percent. Mean values with standard deviations are displayed for two experiments (Location B, 1st experiment: 8 days, 9 to 11 °C, salinity 7.5, pH 8.3, $n = 9$ and Location A, 2nd experiment: 8 days, 9 to 13 °C, salinity 7.9, pH 8.3, $n = 7$).

Substrate	**1st experiment**		**2nd experiment**	
	½ egg development	Shortly before hatch	½ egg development	Shortly before hatch
Fucus vesiculosus	8.20 (± 22.8)	0	89.36 (± 7.7)	82.41 (± 5.7)
Furcellaria lumbricalis	7.99 (± 6.0)	0.17 (± 3.4)	76.11 (± 9.6)	51.80 (± 38.0)
Stuckenia pectinata	2.87 (± 6.4)	0	76.22 (± 9.6)	71.88 (± 22.1)
Zostera marina	0.05 (± 0.2)	0	80.87 (± 13.9)	69.42 (± 32.7)
Control	-	-	81.69 (± 11.1)	62.78 (± 26.2)

During the 2nd experiment, mean egg survival rates remained above 50 % on all substrate types (Tab. 2). The percentages of living eggs varied a lot between the substrate groups but also within replicates of the same substrate type (**Fehler! Verweisquelle konnte nicht gefunden werden.**. No significant differences were detected after half of the egg development (Kruskal-Wallis test, H(4) = 7.420, $p = 0.115$) or shortly before hatch (Kruskal-Wallis test, H(4) = 2.914, $p = 0.572$). However, at both sampling dates, highest mean egg survival and lowest variation was found on *Fucus vesiculosus* and shortly before hatch, *Furcellaria lumbricalis* led to the lowest percentage of surviving eggs with highest variation.

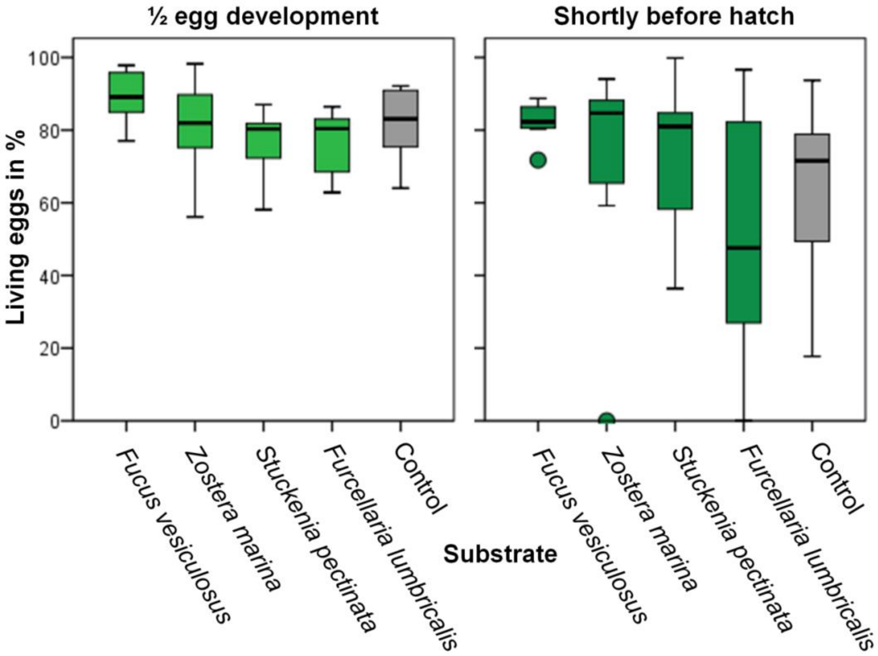

Figure 4. Boxplots showing the median survival rate of herring eggs on different spawning substrate types (Location A, 2nd experiment, 8 days, 9 to 13 °C, salinity 7.9, pH 8.3, $n = 7$). On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, whiskers show the 1.5 interpercentil range and dots represent outliers.

Discussion

Spring herring are assumed to be non-selective in the choice of spawning substrate and attach their eggs to all suitable substrates. However, in the Baltic Sea, submerged vegetation is reported to be the primary spawning substrate (e.g. Oulasvirta et al. 1985, Rajasilta et al. 1986, Scabell 1988, Aneer 1989). Aneer (1989) speculated that selected plants might change over time as result of changes in locally dominating species composition. During past decades aquatic vegetation in the Baltic Sea (in coastal areas all over the world) underlies severe changes due to eutrophication, light limitation and coastal modification. This study focuses on the question: What are the consequences for herring as a species that relies on submerged aquatic vegetation for spawning? Results of the experiments revealed no significant differences between Baltic herring egg survival rates on different naturally frequented perennial macrophytes as spawning substrates.

In the 1st experiment at ‘Gahlkow Haken’ extreme egg mortality with almost no surviving eggs was observed on all tested substrate types. We presume that a mass development of filamentous brown algae in the study was the reason for these extreme egg mortality values (see Fig. 5 in ‘General discussion’, p. 180). The species *Pylaiella littoralis* is already described to cause increased herring egg mortality (Oulasvirta et al. 1985, Aneer 1987) even though mortality rates to this entire extend have not been documented in experiments prior to this study. Furthermore, several earlier observations documented high amounts of herring spawn attached to filamentous brown algae but did not report increased mortality rates (Aneer and Nellbring 1982, Aneer et al. 1983, Elmer 1983, Klinkhardt and Biester 1985). However, directed own field and laboratory experiments on the effect of filamentous algae on herring eggs revealed a species specific lethal effect of the brown algae *Pylaiella littoralis* (manuscript IV or von Nordheim et al. 2018).

During the 2nd experiment mean survival of eggs did not fall below 50 % on any of the substrate types. However, only non-significant differences were observed and got more pronounced with ongoing development. The red algae *Furcellaria lumbricalis* is a common herring spawning substrate in the Baltic Sea e.g. in the Gulf of Riga and on the Lithuanian coast (Raid 1990, Šaškov et al. 2014) but resulted in the lowest egg survival rates during our experiments. This coincides with results of Rajasilta et al. (2006), who found a significantly higher egg mortality on *Furcellaria* spec. than on *Cladophora* spec. and reported the highest mortality (mean=14.4 %; n=20) on *Furcellaria* spec. that had decomposed over six days. Moreover, in an earlier study, the authors found significantly higher egg mortality on *Furcellaria* spec. than on *Cladophora* spec., *Potamogeton* spec. (pondweed) and blue mussels *Mytilus edulis* in the field

(Rajasilta et al. 1989). However, our findings were not statistically significant, and we did only test freshly harvested algae and no different decomposition stages. Furthermore, described low percentages of dead eggs on pondweeds in comparison to red algae (Rajasilta et al. 1989) coincide with our results for the pondweed *Stuckenia pectinata*. This species are freshwater angiosperms that are tolerant to moderate salinity levels in brackish water. Hence, they are no relevant spawning substrates for marine living herring but serves as common substrate for herring in the brackish Baltic Sea (Rajasilta et al. 1989, 1993, Kääriä et al. 1997, Moll et al. 2018, Kanstinger et al. 2018).

An unexpected result was the significantly lower fertilization success on *Zostera marina* compared to all the other substrate types, even though seemed to build even single layers on this species which should allow best possible fertilization rate. However, during artificial spawning this was one of the most challenging substrates, because eggs did not adhere to the blades of seagrass as easy as they did on all other substrates (including hands and equipment). These observations were even more surprising, since seagrasses are one of the most common spawning substrates of spring spawning Atlantic herring (Scabell and Jönsson 1984, Aneer 1989, Klinkhardt 1996, Polte and Asmus 2006). As seagrasses are sedentary over many years (Zipperle et al. 2009) and build extensive submerged meadows, they are particularly attractive for herring as the fish are known to prefer homogenous vegetation cover over wider areas for spawning (Scabell 1988, Kääriä et al. 1997, Kanstinger et al. 2018). However, it cannot be excluded that reduced egg attachment to *Zostera marina* was an artifact of artificial spawning. At the end of our experiments, the median egg survival rate on seagrass was still highest compared to the other tested spawning substrates.

After half of the developmental time, the control substrate (nylon netting) without physiological activity performed second best as spawning substrate, but shortly before hatch, the egg survival rate was second lowest and only one plant substrate (*Furcellaria lumbricalis*) led to a lower median egg survival, emphasizing early findings that suggest an increased oxygen need and sensitivity of herring eggs with ongoing development (Braum 1973, Klinkhardt 1996). Hence, during early development, the spawning substrate seems to be of minor importance, but later physiological interactions might severely influence the embryonic development (also indicated in manuscript IV). This would also explain higher differences in egg survival rates between the tested substrate types shortly before hatch than after half of the developmental time. However, statistical analyses of a potential increase in herring egg mortality with ongoing embryonic development resulted in no significant differences at all.

Only on the spawning substrate *Fucus vesiculosus*, the egg survival remained continuously high with low standard deviations during the whole experimental time, potentially indicating a physiological special feature of these algae. However, no statistical significance was found. *Fucus vesiculosus* is described to produce very effective anti-herbivore defense and antifouling protection (Rohde et al. 2004, Wahl et al. 2010), which could also impact the development of attached herring eggs. As no negative effects (or at least no higher mortality values) were observed, those mechanisms might have an indirect positive effect for the eggs by protecting them as well. In the Baltic Sea, *Fucus vesiculosus* serves as herring spawning substrate for decades (Aneer and Nellbring 1982, Elmer 1983, Scabell and Jönsson 1984, Klinkhardt and Biester 1985, Scabell 1988). However, *Fucus vesiculosus* has increasingly declined and occurs with reduced depth distribution in the Baltic Sea since the 20th century (Kautsky et al. 1986, Vogt and Schramm 1991, Torn et al. 2006, Vahteri and Vuorinen 2016). It can be seen an indicator species for water quality, responding quite sensitive to eutrophication (Bergström et al. 2003) and light limitation due to e.g. phytoplankton blooms and turbidity (Kautsky et al. 1986, Vogt and Schramm 1991, Berger et al. 2003).

It should be noted that none of the described differences between the tested spawning substrate types were statistically significant. Generally, very high variances were observed within the groups, impeding profound analyses of the data and making the interpretation of results rather speculative. However, results support earlier studies reporting comparatively high egg mortality on the red alga *Furcellaria lumbricalis*. Futhermore, speculations on potential negative impacts of *Fucus vesiculosus* exudates (i.e. herbivore defense) on herring eggs were proven incorrect, as the mean egg survival rate on this brown alga was highest compared to all other tested substrates. And finally, shortly before hatch of larvae, the angiosperms *Zostera marina* and *Stuckenia pectinata* also led to higher egg survival rates than the synthetic control substrate, suggesting a potential importance of some specific physiological activity of the spawning substrates during late embryonic development.

The results indicate that healthy macrophyte meadows, with plant communities consisting of seagrasses, pondweeds, bladderwrack or similar species are important prerequisites for successful reproduction of Baltic spring herring. Additionally to their specific quality as spawning substrates in terms of egg survival, these plant species are essential habitat structuring sedentary perennial macrophytes, which attract migrating herring from year to year for spawning (see manuscript I). Further research on substrate requirements and the role of particular plant communities (and on the physiological benefits of plant spawning on a microscale) for herring reproduction is required to evaluate consequences of environmental changes and anthropogenic alteration on coastal spawning grounds and their effects on population dynamics.

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Lethal effect of filamentous algal blooms on Atlantic herring (*Clupea harengus*) eggs in the Baltic Sea

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Abstract

In coastal marine ecosystems all over the world, eutrophication is a primary water quality issue, often resulting in mass developments of bloom-forming algae. Macro algal blooms have immense ecological effects as they alter the structure and the function of an ecosystem in the form of epiphytes and detached filaments accumulating in extensive mats of high biomass. Numerous fish species are affected as they depend on those coastal areas for spawning and as juvenile habitats. We investigated the effects of filamentous algae on the survival of demersally spawned fish eggs in one of the most important spawning grounds of herring in the western Baltic Sea which is subject to an intense and on-going eutrophication. Comparing the survival of herring eggs on two natural spawning beds, we found a mortality rate of nearly 100 % in the area with a filamentous brown algal bloom. Hypothesizing that the presence of filamentous algae facilitates herring egg mortality, we conducted field and laboratory experiments, revealing particular effects of distinct algal species. While filamentous forms of the green algae *Ulva intestinalis* induced no immediate effect, a significantly higher egg mortality was documented in experiments with the filamentous brown algae *Pylaiella littoralis*. Considering the ecological and economical importance of herring and other coastal spawners on the one hand and the persisting and increasing effects of eutrophication and climate change on the other hand, the results of our study clearly underpin the necessity to increase global efforts to reduce nutrient loads in coastal waters.

Introduction

Coastal waters, lagoons and estuaries are ecosystems that are highly influenced by human activities all over the world. At the same time, the littoral zone is a habitat inhabited by several life stages of diverse aquatic flora and fauna. Many fish species for example depend on those areas for spawning and as juvenile habitats. However, factors like coastal modification, cultivation and eutrophication are ubiquitous and can lead to severe habitat degradations.

(Smith and Schindler 2009) postulated, that eutrophication has become the primary water quality issue for most of the freshwater and coastal marine ecosystems in the world and their position was supported by a plethora of other studies (e.g. Nixon 1995, Selman et al. 2008, Howarth 2008, EEA 2015). A major consequence of eutrophication is an increasing abundance but decreasing diversity of aquatic vegetation (Rabalais 2002, Worm et al. 2002) and a plant community shift from slow growing macrophytes to fast growing filamentous algae and phytoplankton (Duarte 1995, Munkes 2005). Nitrogen enrichment stimulates an accelerated nutrient uptake, faster growth, and more frequent blooms (Gorgula and Connell 2004, Bricker et al. 2008, Teichberg et al. 2010, 2012), resulting in a limited depth distribution of benthic vegetation because of reduced light penetration, overgrowth and shading of perennial macrophytes by epiphytes (Kautsky 1991, Vogt and Schramm 1991, Taylor et al. 1995, Valiela et al. 1997, Orth et al. 2006, Burkholder et al. 2007).

Generally, the relation of perennial plants and filamentous algae indicates the nutrient status in an area (Kautsky 1991). Hence, an extensive occurrence of filamentous algae is globally seen as indicator for eutrophicated systems. In sheltered areas and systems with long water residence time, blooming filamentous algae tend to form massive floating mats, consisting of many different species (Duarte 1995, Valiela et al. 1997, Burkholder et al. 2007). Reports on ‘harmful algal blooms’ related to micro algae species and their effects on aquatic organisms, especially fish, are numerous (reviewed by Landsberg 2002, Anderson et al. 2012) but with regard to macro algae they are scarce. Valiela et al. (1997) summarized that harmful effects of macro algal blooms differ from micro algal blooms as they are usually not releasing toxic substances. However, those blooms last longer and have immense ecological effects as they alter the structure and the function of affected ecosystems. For example, early life stages of fish, inhabiting those coastal areas, are severely influenced by habitat changes (Caddy 2007). Green tide affected sites are reported to be less favorable nursery grounds depending on the macro algal density (Le Luherne et al. 2017). Effects on juvenile fish were investigated in the Baltic Sea and Skagerrak, revealing a negative effect of increasing vegetation cover or macro algal blooms, respectively, on different flatfish species developing in coastal areas (Pihl et al. 2005, Jokinen et al. 2016). However, the effect on fish species, spawning their benthic eggs directly on aquatic vegetation in shallow coastal areas is not comprehensively investigated yet, even though especially those ‘stationary’ eggs are highly vulnerable against changes in their direct environment. This study focuses on herring (*Clupea harengus*), being an important component of oceanic food webs on the one hand and important fishery resource on the other hand, and thereby an ideal model organism for evaluating wide-ranging eutrophication-linked effect chains. Exudates of the filamentous brown algae *Pylaiella littoralis* were assumed to have a toxic impact on herring eggs (Aneer 1987). However, during the above laboratory study the algal effect was not found particularly pronounced and the transferability of results to natural spawning grounds has not been tested. This reveals the lack of specific knowledge regarding the effect of filamentous algae on the survival of fish eggs. During recent years the local climate in the western Baltic Sea changed from several weeks of ice-cover to long periods of mild water temperatures in winter, which is promoting early and massive occurrence of *P. littoralis* (Kiirikki and Lehvo 1997) and thereby expanding the period of herring egg and algae overlap (L. von Nordheim, pers. observation). Such synergistic effects of changing climate and eutrophication might have severe impacts on future ecosystem functioning and underlines the strong demand for reliable information on the full set of consequences of eutrophication-linked macro algal blooms.

In this study, we focus on two algae species (*Pylaiella littoralis* and *Ulva intestinalis*) that are known to form massive spring blooms (Raffaelli et al. 1998, Salovius and Bonsdorff 2004) and are used as spawning substrate by herring (Aneer and Nellbring 1982, Kääriä et al. 1997). Hypothesizing that the presence of filamentous algae increases herring egg mortality, we (I) compared the survival of eggs on natural spawning grounds affected and un-affected by a filamentous brown algal bloom. Additionally, we conducted field and laboratory experiments, testing the effect of (II) the filamentous brown algae *P. littoralis* and (III) a filamentous form of the green algae *U. intestinalis* on herring egg survival.

Material & Methods

The study was conducted in Greifswald Bay, a major spawning and nursery area of western Baltic spring-spawning herring (Biester 1989, Polte et al. 2017). This shallow brackish lagoon in the western Baltic Sea (Fig. 1) has an average depth of 5.8 meters (max 13.5 meters), salinity ranges between 7 and 8 (Schiewer 2001) and pH values are approximately 8.5 during the spring herring spawning season. The lagoon is regularly covered by ice shields in winter and in summer the water temperatures regularly rise above 20 °C. In spring, the water column is mostly well mixed with oxygen levels remaining close to saturation (100 %) and the almost exclusively wind-driven fluctuations in sea level vary from 0 up to 1.5 meters (Stigge 1989). Greifswald Bay is affected by high nutrient levels above thresholds to achieve a “good ecological status” (Status Report BMUB 2018). After a vegetation-shift from a macrophyte dominated to a phytoplankton dominated system, the present vegetation is a mixture of marine, brackish- and freshwater species with perennial macrophytes limited to shallow areas of less than 3.5 meters water depth (Munkes 2005, Kanstinger et al. 2018).

***COLLECTION OF SPAWNING SUBSTRATES* -** Macrophytes and algae, used as spawning substrates in the experiments, were collected by snorkeling in 1 – 1.5 m deep water in Greifswald Bay. In all set ups, pieces of nylon netting (3 x 11 cm, mesh size 4 mm) were used as physiologically non-active control, hereinafter referred to as “neutral substrate”, allowing comparison between different experiments. Experiments on effects of dominant filamentous algae on herring egg mortality were conducted in two consecutive years. In spring 2016, we collected the filamentous brown alga *Pylaiella littoralis* at location B, while in 2017, the filamentous green alga *Ulva intestinalis* was collected at location A. In both years, the algae were separated from other plant material, rinsed in habitat water and stored in aerated barrels until they were deployed in the experiments on the next day.

***ARTIFICIAL SPAWNING* -** During spring spawning season, ripe herring were caught in Greifswald Bay with gill nets on board a research vessel. Mature fish were used to artificially spawn eggs onto different substrates. The different substrates were subsequently placed into a dish filled with habitat water and female herring were strip spawned onto their surface. When substrates were consistently covered with single egg layers, they were fertilized in buckets containing a sea water-sperm-mixture of the mixed milt of 10 males. Potential parental effects that might influence the fitness of embryos (Geffen and Nash 2012) were reduced by using eggs of one

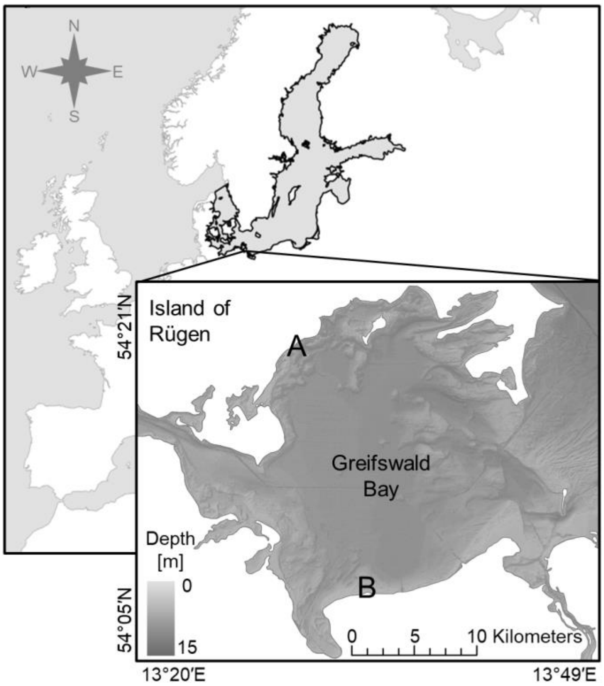


Figure 1. Investigation area Greifswald Bay and its location in the Baltic Sea (framed in black) with relevant locations for the experiments (A and B). Bathymetry data: received by courtesy of Federal Maritime and Hydrographic Agency of Germany.

female for only one replicate of each treatment, while the sperm mixtures (each derived from multiple males) were used for a maximum of three replicates. After 10 minutes fertilization time the substrates with attached eggs were rinsed with sea water and transported to the respective experimental sites (field and laboratory) on the same day.

EXPERIMENTAL PERIODS - For all experiments, degree days (°d) were used as a unit to standardize the egg development stages among experiments run under slightly different temperature conditions and were calculated by multiplying temperature (°C) with egg developmental time (d) (Apstein 1909, Klinkhardt 1996, Peck et al. 2012). The duration of the experiments was adjusted to weather conditions and water temperatures, as most herring larvae hatch between °d 90 and °d 120 (Peck et al. 2012), and samples of each replicate were regularly taken (see sample processing below) at the beginning (until °d 30), middle (°d 45 - °d 56) and end (°d 85 - °d 92, before larvae hatch) of the experiments.

FILAMENTOUS ALGAE EFFECTS ON SELECTED SPAWNING GROUNDS - In 2016, field experiments on the survival of herring eggs were conducted on two different locations in Greifswald Bay (Fig 1, locations A and B). On the southern coast of the Bay (location B: Gahlkow), filamentous algae were abundant (in particular the brown algae *Pylaiella littoralis*) and on the northern shore (location A: close to Altkamp), filamentous algae were scarce. Both locations were compared regarding the percentages of dead herring eggs (24 replicates: 7 units of 4 plant species respectively). To reproduce natural conditions as good as possible, different macrophyte species that are naturally frequented by spawning herring (*Fucus* spp., *Furcellaria lumbricalis, Zostera marina*, *Stuckenia pectinata*) were used as substrates. They were artificially spawned as described above, installed in the field at locations A (start: 27th April 2016, duration: 8 days, water: temperature 7 – 13 °C, salinity around 7.9 and pH around 8.3) and B (start: 14th April 2016, duration: 8 days, water: temperature 9 – 10 °C, salinity around 7.5 and pH around 8.3) and sampled one day after fertilization, at °d 45 and °d 80.During the experiment, dissolved oxygen was continuously measured in the water column at both locations. To analyze diurnal fluctuations of oxygen due to photosynthesis or respiration respectively, data were separated in day time values (4:00 am – 6:00 pm UTC) and night time values (6:00 pm – 4:00 am UTC).

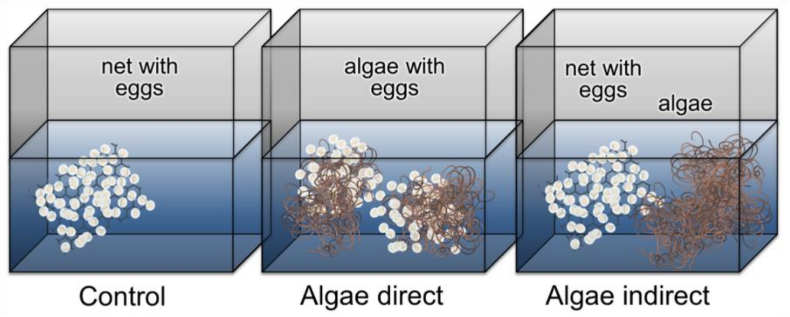
***FIELD EXPERIMENTS ON ALGAL EFFECTS ON EGG SURVIVAL* -** Herring eggs, directly attached to specific filamentous algae and eggs spawned on a neutral substrate as control were incubated in gauze cages (cubical metal frames, edge length 10 cm, covered with 500 µm gauze) were used for incubation. Each replicate was stored in a separate cage, protecting the eggs from predation and at the same time keeping the experiment positioned close to natural spawning beds (Greifswald Bay, location A) on the seafloor at 1 m water depth. A data logger (PME miniDO$_2$T Logger) was mounted besides the set-up for continuous monitoring of dissolved oxygen and water temperature.

The *P. littoralis* approach was installed in the field (*n* = 9) on 27th April 2016 for a duration of 8 days with water temperatures from 9 to 13 °C, a salinity of 7.9 and pH 8.3. Samples were taken one day after spawning for, at °d 55 and shortly before hatch at °d 92.

The *U. intestinalis* approach was started on 13th April 2017 and ran 10 days at water temperatures between 8 and 9 °C, a salinity of 7.8 and pH 8.4. Samples were taken one day after spawning, at °d 43 and shortly before hatch at °d 85. Unfortunately, some of the cages were washed away by heavy wave action during a storm event, accordingly the number of replicates was reduced to *n* = 4.

***LABORATORY EXPERIMENTS ON ALGAL EFFECTS ON EGG SURVIVAL* -** Substrates with attached herring eggs were incubated in aerated aquaria with 2 liter of habitat water and a natural day-night rhythm of sunlight. Each replicate of a substrate type was incubated in a separate aquarium and abiotic parameters were monitored every 1 to 3 days and aquaria were kept under standardized conditions across replicates. Similar to the field experiments, the direct effect of algal substrates on egg survival was tested against a control (neutral substrate) and the indirect

Figure 2. Experimental treatments: substrates that were added into the aquaria/cages to analyze the direct and indirect effects of algae on herring eggs (“net” was used as neutral substrate).



effect of algae was analyzed in a third treatment with eggs spawned on the neutral substrate and algae added to the surrounding water (Fig. 2).

P. littoralis laboratory experiments were started on the 27th and 28th April 2016 investigating the direct and the indirect effect of those filamentous brown algae (n = 6). Experiments were run for 9 days at water temperatures from 6 to 13 °C, a salinity of 8 and pH 8. Samples were taken one day after spawning for determination of the fertilization success, at °d 56 and shortly before hatch at °d 89.

The laboratory experiment investigating the direct and indirect effect of *U. intestinalis* was started on the 26th April 2017 and ran 11 days under water temperatures between 6 and 10 °C, a salinity of 8.4 and pH 8.4 (n = 6). Samples were taken two days after spawning for determination of the fertilization success, at °d 45 and shortly before hatch at °d 80.

***SAMPLE PROCESSING AND DATA ANALYSES* -** In all experiments, sampling was conducted by removing a sub-unit of each replicate and preserving it in 4 % borax-buffered formalin. Fixed samples can be stored for a long time and further analyses can be done in the laboratory, since originally vital eggs remain transparent and dead eggs opaque white. The fertilization success was determined by counting fertilized eggs in each replicate and relating them to the total number of counted eggs (total $n \geq 100$).

The egg survival was determined for all samples taken during half time egg development and shortly before hatch. Random subsamples (of each sample) of at least 400 eggs (except for the first experiment (I), were subsamples contained at least 100 eggs) were processed and the percentage of living eggs was calculated. Samples of algal substrates were processed by carefully separating the eggs from each other and counting living and dead eggs under the microscope. Digital images were taken of the neutral substrates (pieces of netting: good visibility of eggs, since eggs were distributed homogeneously) and analyzed with imaging software (Image J 1.47v, Wayne Rasband, National Institutes of Health, USA) by determining the percentages for each replicate in 3 randomly placed rectangles (total ≥ 400).

STATISTICS - Statistical analyses were conducted using SPSS 21 (IBM Statistics), R (version 3.2.3, R Foundation for Statistical Computing 2015) and Microsoft Office 2007. Differences between egg survival rates in algae and control treatments were tested according to the given requirements of each dataset. For most data the *t*-test was applicable. However, if homoscedasticity of variances was not initially fulfilled, or could be achieved by angular transformation, Welch's t-test was used, as it is considered robust against deviation from such requirements. Cohen's *d* was used for interpretation of effect size and evaluated after Cohen (1988) where $d = |0.2|$ is considered as small, $d = |0.5|$ as medium and $d = |0.8|$ as large effect size. Results of the laboratory experiment with *U. intestinalis* (three different treatments) were compared with a one-way analysis of variance (ANOVA). The significance level for all statistical analyses within the study was set to $p \leq 0.05$.

Results

The fertilization rate was above 98 % in all treatments. Accordingly, impacts by limited fertilization success were considered negligible for further analyses. Oxygen saturation during field experiments and in all aquaria continuously stayed above 80 %.

(I) FILAMENTOUS ALGAE EFFECTS ON SELECTED SPAWNING GROUNDS

The comparison of herring egg survival rates in an area, affected by the mass occurrence of *P. littoralis* (location B) and an area that was almost unaffected by those filamentous brown algae (location A), shows a clear trend (Fig. 3). After half of the egg development, the mean egg mortality was significantly higher at location B with *P. littoralis* (95.67 % ± 13.04) than at location A (21.58 % ± 10.87, $t = 7.716$, df = 54, $p = 0.008$). Shortly before the larvae would hatch, almost no egg survived at the *P. littoralis*-location B (mean mortality 99.95 % ± 0.21) and a significantly lower mean mortality was observed at the location A (33.04 % ± 27.46, $t = 42.837$, df = 54, $p < 0.001$).

The oxygen saturation during the experimental period was constantly around 100 % in both areas. Some higher diurnal fluctuations were observed in the location B with filamentous algae but the values did not fall below 80 % at any time (minimum values: location A: night 91 %, day 93 %; location B: night 82 %, day 82 %).

Figure 3. Boxplots showing the median survival rate of herring eggs in two different areas (location A: scarce filamentous algae and location B: high abundance of filamentous algae). On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, whiskers show the 1.5 interpercentil range and dots represent spikes.

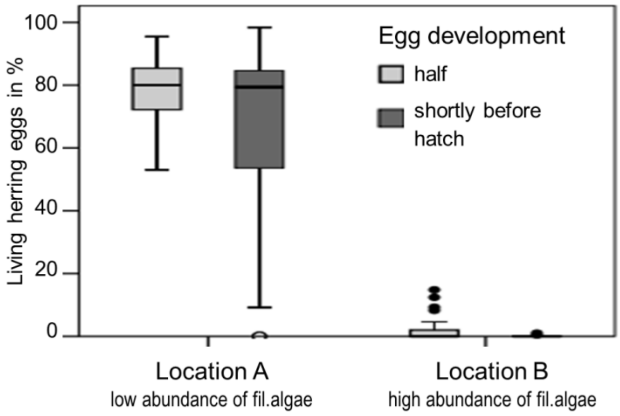


Table 1. Herring egg survival under the influence of filamentous brown and green algae in field and laboratory experiments (mean percentages of living eggs with ± standard deviations).

	Time & Treatments	Field values	Laboratory values
Pylaiella littoralis	**Half time egg development**		
	Control	94.23 (±6.67, *n*=9)	95.38 (±0.86, *n*=6)
	Algae direct	89.20 (±6.00, *n*=9)	45.04 (±6.85, *n*=6)
	Control		96.46 (±1.89, *n*=6)
	Algae indirect		46.09 (±29.34, *n*=6)
	Shortly before hatch		
	Control	96.61 (±2.51, *n*=9)	79.88 (±13.16, *n*=6)
	Algae direct	39.66 (±7.84, *n*=9)	5.93 (±7.82, *n*=6)
	Control		93.35 (±3.04, *n*=6)
	Algae indirect		7.40 (±14.53, *n*=6)
Ulva intestinalis	**Half time egg development**		
	Control	58.94 (±14.99, *n*=4)	88.40 (±7.26, *n*=6)
	Algae direct	75.43 (±13.42, *n*=4)	81.46 (±7.26, *n*=6)
	Algae indirect		81.46 (±27.68, *n*=6)
	Shortly before hatch		
	Control	37.72 (±12.55, *n*=4)	56.00 (±28.28, *n*=6)
	Algae direct	34.95 (±21.78, *n*=4)	70.76 (±19.52, *n*=6)
	Algae indirect		74.20 (±28.81, *n*=6)

(II) EFFECTS OF *PYLAIELLA LITTORALIS* ON HERRING EGG SURVIVAL

Generally, the survival in all herring egg treatments containing *P. littoralis* was significantly lower than without those filamentous brown algae (Fig. 4, Tab. 1). In the field experiment, after half of the egg development, survival in the control was significantly higher than on *P. littoralis* ($t = 2.191$, df = 16, $p = 0.044$) but with strong effect size ($d = 0.8$). In the laboratory experiments, a strong negative effect of *P. littoralis* already appeared after half of the egg development. The direct effect of *P. littoralis* led to a significantly lower egg survival than observed in the control ($t = 21.033$, df = 10, $p < 0.001$, very strong effect size $d = 10.3$). The laboratory experiment on the indirect effect also revealed a significantly better egg survival in the control than in *P. littoralis*–water (Welch-test (5.186) = 4.206, $p = 0.008$, strong effect size $d = 2.4$). Shortly before hatch, similar patterns became visible but revealed even higher significance levels. In the field experiment, survival in the control was significantly higher than on *P. littoralis* ($t = 21.028$, df = 16, $p < 0.001$, with a very strong effect size ($d = 9.8$). Similar tendencies were observed in the laboratory between the control and *P. littoralis* ($t = 9.975$, df = 10, $p < 0.001$), with a strong effect size of $d = 6.8$. The second laboratory experiment revealed a significantly higher survival of herring eggs in the control than on the neutral substrate indirectly influenced by *P. littoralis* ($t = 10.822$, df = 10, $p < 0.001$, very strong effect size $d = 8.2$).

(III) EFFECTS OF *ULVA INTESTINALIS* ON HERRING EGG SURVIVAL

Experiments on the effect of *U. intestinalis* as spawning substrate for herring eggs did not result in any significant differences between the control and the direct or indirect *U. intestinalis* treatments (field experiment: t-test, $p > 0.1$; laboratory experiment: ANOVA, $p > 0.1$) (Fig. 5, Tab. 1). In the field experiment, the egg survival rate in the control was lower than on *U. intestinalis* after half of the development, but slightly higher shortly before hatch. In the laboratory experiment, the percentage of living eggs after half of the development was almost similar in the control and on the *U. intestinalis* treatments. And shortly before hatch, a slightly lower egg survival was observed in the control compared to the *U. intestinalis* treatments.

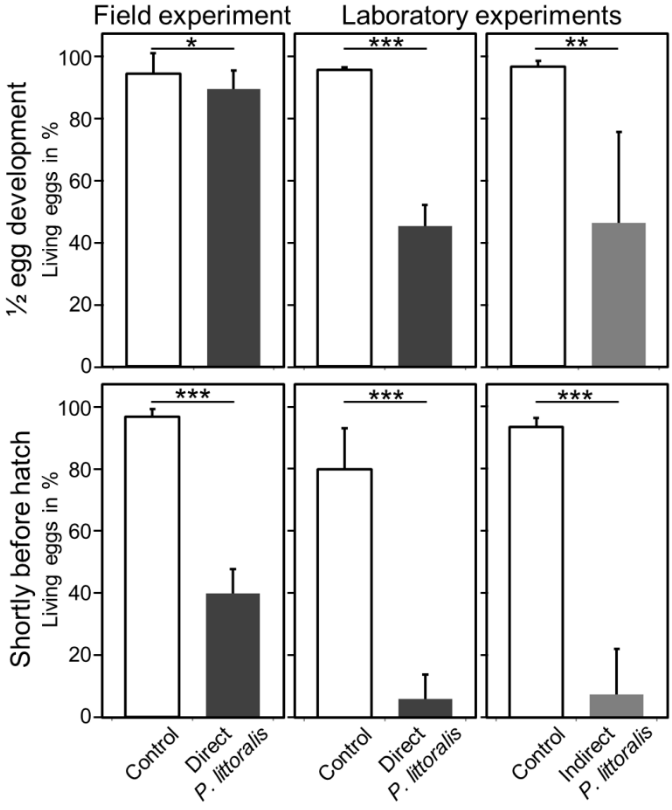


Figure 4. Percentage of living herring eggs (mean values with standard deviations indicated by error bars) after half of the egg development (top) and shortly before hatch (bottom) under different experimental treatments with *Pylaiella littoralis*:

field experiment with herring eggs spawned on neutral substrate (control) and on *P. littoralis* (n = 9); laboratory experiments on direct effects with similar substrates (n = 6) and on indirect effects with herring eggs spawned on neutral substrates and then incubated in water with *P. littoralis* (n = 6); *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.1$, t-test or Welch-test respectively.

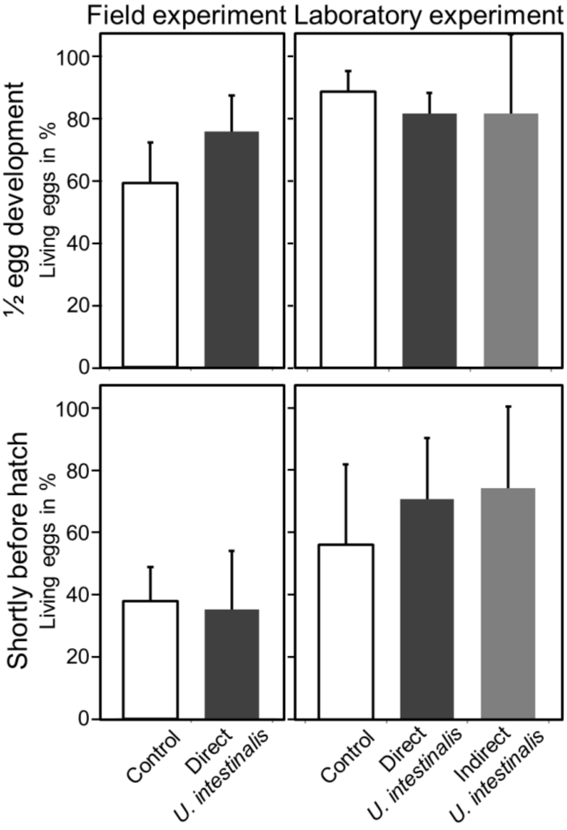


Figure 5. Percentage of living herring eggs (mean values with standard deviations indicated by error bars) after half of the developmental time (top) and shortly before hatch (bottom) under different experimental treatments with *Ulva intestinalis*:

field experiment with herring eggs spawned on neutral substrate (control) and on *U. intestinalis* (n = 4); laboratory experiment with similar substrates for direct effect analysis and with herring eggs spawned on neutral substrates, incubated in water with *U. intestinalis* for analyses of indirect effects (n = 6).

Discussion

This study demonstrates that particular filamentous algae can drastically affect herring egg survival even without direct physical contact. Results of the experiments clearly support the hypothesis, that the filamentous brown algae *Pylaiella littoralis* has a significantly negative effect on herring eggs in the Baltic Sea.

An exhaustive mortality of eggs was observed in combination with massive filamentous algae mats in the field. A mean herring egg mortality of almost 100 % shortly before the larvae would hatch, in contrast to 33 % (± 27) at a location with low filamentous algal abundance, shows the immense possible impact of those algae. The commonly assumed mechanism would be oxygen depletion in the water body because of excessive night time respiration of high plant biomasses or algal decay (Aneer and Nellbring 1982, Valiela et al. 1997, Burkholder et al. 2007, Anderson 2009). In shallow waters, hypoxia, induced by drifting algal mats, often causes major risks for the local fauna especially for non-mobile species (Berezina 2008, Arroyo et al. 2012, Lyons et al. 2014). Field observations in the Askö area (Baltic Sea, Sweden) revealed a significantly higher egg mortality on filamentous algae than on coarse algae in one of three study periods, and mortality was attributed to low oxygen levels at night time (Aneer 1985). However, Atlantic herring eggs are reported to be tolerant to oxygen fluctuations above 50 % saturation (Braum 1973). Since diurnal oxygen levels never fell below 80 % during our experiments, the observed egg mortalities are probably caused by other stressors. However, future studies should examine the oxygen regime on spatial micro scales (i.e. within egg clumps).

Experiments with filamentous algae, known for eutrophication-associated blooms in coastal waters, revealed specific effects on the survival of Atlantic herring eggs. While filamentous forms of the green algae *Ulva intestinalis* induced no immediate impact, significantly higher egg mortalities were documented in experiments with *P. littoralis*. Both, the direct and indirect influence (algae as immediate spawning substrate or as free floating fragments in the incubation tank) have been shown to significantly increase egg mortality rates compared to treatments without *P. littoralis*. Field experiments are a valuable tool to transfer results of laboratory experiments to *in-situ* conditions, which are not influenced by limited water exchange and a corresponding lower dilution potential. With high water exchange *in-situ*, the immense negative influence of those algae became visible only after a longer incubation period, emphasizing the importance of conducting experiments under both, laboratory and field conditions to gather comprehensive knowledge about biological processes.

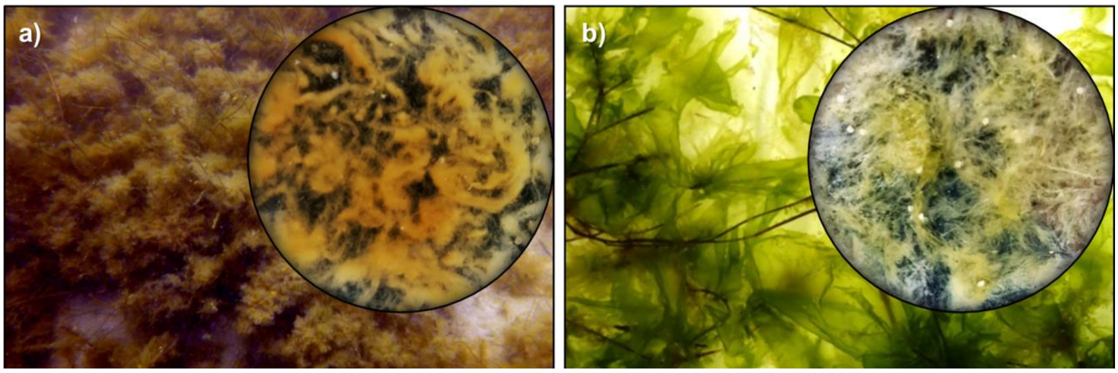


Figure 6. Pictures of epiphytic algae: a) filamentous brown algae *Pylaiella littoralis*, Order: Ectocarpales und b) filamentous form of the green algae *Ulva intestinalis*, Order: Ulvales.

Results of our study coincide partly with a study of Aneer (1987), where he detected negative effects of a "*Pylaiella littoralis*-*Ectocarpus siliculosus*-mixture" on herring egg survival in a laboratory experiment. The indirect effect was tested by leading water into egg chambers after it passed a cylinder with algae. A fresh algae mix resulted in 30 % of dead eggs (no algae treatment: 14 %), while the same algae mix in a decomposed stage had no significant effect (15 % of dead eggs compared to 13 % without algae). As explanation the author speculated on toxic exudates, released during a short time after detachment. However, this has never been experimentally verified. In contrast to Aneer's (1987) observation, this study systematically tested the influence of *P. littoralis* on herring eggs in field and laboratory experiments. Our results indicate that the lethal effect is not limited to fresh algae, because experiments were conducted with already detached algae that were washed into the littoral zone. Furthermore, the percentage of dead eggs was much higher in all brown algae treatments of this study with at least 60 % shortly before hatch and not more than 20 % in the respective control.

Generally, blooms of filamentous macro algae often have multiple negative consequences for affected ecosystems, e.g. light-dependent depth limitation of benthic macrophyte distribution due to shading or extreme oxygen consumption during decay of algae mats formed by detached filaments (Kautsky 1991, Valiela et al. 1997, Orth et al. 2006, Anderson 2009). Furthermore, particularly fish eggs attached to filamentous algae covering the benthic vegetation can be drastically affected, if those filaments are ripped off from their rooted part by hydrodynamic forces (storm events) and washed to shore or the open sea, where eggs hardly survive (Moll et al. 2018). While those effects are rather non-specific or act as part of ecological cascades, this study proves an explicit lethal effect of the filamentous brown algae *P. littoralis* on herring eggs. A filamentous form of the green algae *U. intestinalis* was investigated as second eutrophication promoted species. During our experiments, this species had a rather positive effect on the egg survival. However, regarding the negative effects of filamentous algae outlined above, they would most likely not be compensated by positive aspects under natural conditions on spawning grounds. Furthermore, egg survival was generally rather low at the end of the green algae field experiment, with mean values around 35 % on both substrates (algae and control). This might be due to unfavorable weather conditions during the experimental time as heavy storms resulted in increased wave action and silting. But as those factors were influencing both treatments to the same extend, the experimental result is still reliable.

It is common scientific sense that eutrophication linked mass blooms of filamentous, mat-forming algae have negative, often drastic effects on the resident community of the affected ecosystems. In contrast, some studies discuss the potential of algal mats formed by particular species to serve as temporal, nutrient-rich habitat for macrobenthos (Salovius et al. 2005) or as a refuge from hypoxia for some invertebrates (Coffin et al. 2017). This can probably be the case in heavily altered ecosystems with no or little remains of perennial benthic macrophytes. In those systems, the algae might, due to the lack of alternatives, serve as habitat or as spawning substrate for demersally spawning fishes such as Atlantic herring. However, considering all disadvantages of these algae including the direct negative effects on herring eggs demonstrated in this study, a spawning area merely offering filamentous algae cannot keep up with a pristine ecosystem that is dominated by perennial benthic macrophytes.

We can only speculate on the actual mechanisms causing high egg mortality related to the presence of *P. littoralis*. An explanation could be the immediate effects of exudates released by the algae (speculated by Aneer 1987). Phenols (Phlorotannins) for example are produced by brown algae as secondary metabolites, functioning as protection against herbivores and as antibacterial agents (reviewed by Targett and Arnold 1998, Creis et al. 2018). However, not all brown algae seem to have a negative effect, as for instance *Fucus vesiculosus* has a very effective antifouling mechanism (Wahl et al. 2010) but also turned out to be a favorable spawning substrate for *C. harengus* (Polte and Asmus 2006, L. von Nordheim pers. observation in the Baltic Sea). Furthermore, exudates are probably diluted quickly in the field and their physiological effect is presumably restricted to the spatial microscale. Possibly, high egg mortality rates are induced by reactive oxygen species (ROS) released by algae as component of stress responses during e.g. thermal stress, exposure to high radiation, pollutants or as pathogen defense (Mallick and Mohn 2000, Lesser 2006, Potin 2008, Bischof and Rautenberger 2012). To our best knowledge no information exist on potential vulnerability of fish eggs against oxygen radicals released by algae but this should be subject to future studies. High structural complexity of spawning substrates evidently benefits herring egg development (von Nordheim et al. 2018) but the mucilaginous and filamentous structure could cause the opposite effect. Potentially, eggs might be suffocated by dense algal accumulations, resulting in a lack of oxygen-rich surrounding seawater. However, this cannot explain the results in our experiment, because egg mortality was high in all *P. littoralis* treatments, even if those algae were not the direct spawning substrate, and the tested filamentous green algae had no negative effects. Potentially, the lethal impact might also be induced by protists (e.g. dinoflagellates or diatoms) associated with the algae. Both taxa are well known to include toxic species. A closer look at the diatom community on *P. littoralis* in the Baltic Sea reveals that filamentous algae host numerous diatom species (Snoeijs 1995). However, to our knowledge, a negative impact on vertebrates has not been documented yet. Furthermore, the dinoflagellate *Prorocentrum lima* is described to be attached to *P. littoralis* and induce diarrhetic shellfish poisoning in the northern USA (Maranda et al. 2007). But such incidents have not been described for the Baltic Sea so far.

Although the mechanism is yet unclear, this study proved the extreme negative impact of filamentous algae on herring egg survival and can therefore be regarded as a strong motivation to increase research efforts on the particular mechanisms. Preliminary findings indicate that *P. littoralis* benefits from mild and ice-free winters as Kiirikki and Lehvo (1997) documented maximum abundances after ice-free winters. Recently, we observed mass occurrences very early in years with warm winter-spring temperatures, e.g. 2016 in Mid-April and 2017 in Mid-March. The main reproduction period of *P. littoralis* is in winter. Accordingly, it grows rapidly in spring when the water temperatures rise and the light regime changes; during late spring and early summer the filaments degenerate and detach and during summer only the basal parts remain (Kiirikki and Lehvo 1997). Hence, this algae first benefit from high levels of nutrients which are constantly available in the eutrophicated study system Greifswald Bay (and in most other coastal areas all over the world as well), and second, is promoted by mild winters and rising water temperatures early in the year, as documented with increasing frequency for the temperate climatic zone (Schwartz et al. 2006, Haapala et al. 2015). Spring blooms of these algae frequently appear at the Baltic coast (Lotze et al. 1999) and recent mild winter conditions seem to increase the overlap of *P. littoralis* vegetation periods and the herring spawning season.

CONCLUSION - Macro algal blooms are promoted by eutrophication and their impacts on marine and estuarine ecosystem functioning and services are a global issue (Caddy 2007, Teichberg et al. 2012, Lyons et al. 2014). Immense herring egg mortality, for instance, linked to *P. littoralis* blooms on a natural spawning ground, and the validation of this lethal effect by field and laboratory experiments, emphasize the problematic current situation in coastal Baltic Sea habitats (indicated by Aneer (1985) and proved in this study). Anderson et al. (2012) defined harmful algal blooms (HABs) specifically as events causing injuries to humans, socioeconomics, or ecosystems. Since the latter two criteria are observed as consequence of the massive occurrence of *P. littoralis*, we would denote this species as harmful algae. The synergy of climate abnormalities with high levels of eutrophication has the potential to affect herring reproductive success e.g. via the cascade of these harmful algal blooms. This has important implications for coastal zone managements as it underlines the need for further nutrient reduction.

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Impact of spawning substrate complexity on egg survival of Atlantic herring (*Clupea harengus*, L.) in the Baltic Sea

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Abstract

Shallow shore zones are generally considered to provide juvenile habitats for many invertebrate and fish species and additionally serve as spawning grounds for important components of oceanic food webs and fishery resources such as herring (*Clupea* spp.). Herring attach their demersal eggs to benthic substrates, rendering reproductive success vulnerable to environmental changes and local habitat alterations. However, little information is available on the effects of different substrates on the survival of demersal eggs. Hypothesizing that the structural complexity of spawning substrates generally affects herring egg survival and that the effect-magnitude depends on the suitability of ambient environment, field experiments were conducted on a major spawning ground of *C. harengus* in the South-Western Baltic Sea. Herring eggs were artificially spawned on substrates of different structural complexity and incubated *in situ* under differing temperature regimes, at the beginning and the end of the natural herring spawning season, to include the full suite of stressors occurring on littoral spawning beds. Results of this study indicate a positive relation between high structural complexity of spawning substrates and herring egg survival. Mean egg mortality was three times higher on substrates of lowest complexity than on highly complex substrates. These differences became even more prominent under unfavorable conditions that appeared with rising water temperatures later in the spawning season. Although the mechanisms are still unclear, we conclude that structural complexity, particularly formed by submerged aquatic vegetation, provides a crucial prerequisite for the successful reproduction of substrate spawning marine fishes such as herring in the Baltic Sea.

Introduction

Shallow coastal zones are generally considered to provide important juvenile habitats for many invertebrate and fish species (Beck et al. 2001, Nagelkerken et al. 2015, Sheaves et al. 2015). While complex structures are known to be essential for the ecological function of coastal seascapes, their role as a spawning substrate has received little attention so far. Several studies highlight the importance of complex habitats for species diversity and abundance, as well as for the ecosystem function of littoral waters in general (Angel and Ojeda 2001, Gratwicke and Speight 2005, Kovalenko et al. 2012). Structural complexity, provided by benthic vegetation, promotes the abundance of associated fauna by increasing the number of potential hiding places (Caddy 2007). Furthermore, the complex architecture of macrophytes drives local patterns of juvenile fish abundances by increasing food availability and lowering predation risk (Thiriet et al. 2014). In this respect, investigations revealed a strong relation of aquatic plant structure and associated fish communities (Heck Jr and Orth 1980, Dionne and Folt 1991, Lillie and Budd 1992). Hence, complexity is not only determined by composition of seascape mosaics and plant canopy extension, but also by the morphology of habitat providing plant species. For example, different leaf and canopy architecture of e.g. seagrass species co-existing in a system can harbor a significantly differing fish diversity and size-composition (Hyndes et al. 2003). Furthermore, Heck Jr et al. (2003) reviewed previous studies on the nursery function of seagrass meadows and concluded structural composition to be the primary factor promoting the survival and growth of juvenile fauna.

Another important ecological function of submerged aquatic vegetation is providing spawning substrate for phytophilous spawners throughout a broad taxonomic range (Balon 1975). Compared to the role of vegetation beds as juvenile habitat, mentioned above, the function as spawning grounds is rarely in the focus of research, probably because most marine fish species spawn pelagic eggs and the amount of inshore spawning species with demersal eggs is rather considered a minority (Jackson et al. 2001). Although common knowledge in aquatic ecology (e.g. Petr 2000), this important ecosystem service is rarely studied in detail and receives little attention in current coastal zone management plans. However, some important components of oceanic food webs and important fishery resources such as herring (*Clupea* spp.) rely on vegetated spawning grounds to an unknown extent.

Within the genus of clupeid fish, spawning strategies differ slightly between Pacific herring (*C. pallasii*, Valenciennes 1847) and Atlantic herring (*C. harengus*, Linné 1756). Both species spawn demersal eggs that are actively attached to structures on the seafloor (Blaxter and

Holliday 1963). *C. pallasii* inhabits waters of the continental shelf where they spawn phytophilously along the shoreline during springtime, while *C. harengus* is phytolithophilous (spawns on vegetation and other benthic structures; Balon 1975) and different populations spawn in different spawning seasons throughout the year (summary in Sinclair and Tremblay 1984). Differences in spawning guilds as defined by Balon (1975) contain high ecological significance, since *C. pallasii* obligatory requires submerged aquatic vegetation in the shallow littoral zone for spawning (Hay 1985). It is possible that this also applies to spring spawning *C. harengus* in the Baltic Sea as this population shows a very similar spawning mode by directed spawning migrations into inner coastal waters and to vegetated spawning beds (e.g. seagrass, *Zostera marina* or pondweeds, Potamogetonaceae). Therefore, the assemblage of macrophytal spawning substrates and the structural complexity of plant architecture are potentially important drivers of embryonic development.

Many coastal herring spawning areas have changed drastically during the last decades. Anthropogenic influences have increasingly altered habitats by e.g. coastal modification, land cultivation and eutrophication. This led to increased water turbidity, a corresponding drastic decline of aquatic vegetation and a plant species shift from complex macrophytes, including perennial flowering plants, to filamentous algae and phytoplankton (Munkes 2005a).

Although spring spawning herring in the Baltic Sea primarily use submerged vegetation as spawning substrates (e.g. Aneer and Nellbring 1982, Klinkhardt and Biester 1984, Oulasvirta et al. 1985, Scabell 1988), some studies reported eggs attached to alternative substrates, such as gravel, stones, coarse sand or on bivalve shells (e.g. Scabell 1988, Rajasilta et al. 1989, Aneer 1989 and literature therein). Aneer (1989) stated that Baltic herring is rather unspecific in its choice of spawning substrates and that the dominating plant species selected for spawning might change over time, probably as a result of their natural succession. Some rather anecdotal records indicate that herring would spawn on every suitable substrate in the area since fishermen often find their fishing nets covered by eggs. Furthermore, autumn spawning herring in the North Sea for example, primarily spawn on gravel beds in deeper coastal shelf areas (Parrish and Saville 1965, Groot 1980, Maravelias et al. 2000).

Knowledge on direct effects of spawning substrate on marine and limnetic fish egg development in general is very limited. However, Aneer (1987) and Rajasilta et al. (2006a) respectively showed negative effects of exudates of filamentous brown algae (*Pilayella littoralis*, *Ectocarpus siliclosis*) as well as exudates of red algae (*Furcellaria* sp) on herring egg survival. Furthermore, the thickness of egg layers on the substrate can affect egg mortality by oxygen limitation, occurring in dense egg concentrations (e.g. Blaxter 1956, Parrish et al. 1959, Ojaveer 1981a, Klinkhardt 1984, Hay 1985). The egg concentration per area however seems to depend on plant species and might be related to substrate complexity. From the Wadden Sea (North Sea), it is known that herring egg abundance per area sea bed can be much higher in homogenous seagrass meadows (*Zostera noltii*) than on single aggregations of *Fucus vesiculosus*, even though the number of eggs per leaf area is higher on the latter (Polte and Asmus 2006). But remarkably, to our best knowledge, no records exist on the explicit effects of structural spawning substrate complexity on the embryonic development or mortality of herring eggs or any other demersal spawning fish species.

The superior effect of ambient water temperatures on the development of fish eggs is well described. Generally, herring eggs can tolerate temperatures in the range of 5 - 14°C and salinities in the range of 3 - 33 (Blaxter and Holliday 1963, Haegele and Schweigert 1985), but some studies in the Baltic Sea indicated that the eggs are tolerant to even more extreme water temperatures (e.g. 3.8 – 18.5°C, Aneer 1989; ≥ 3.5°C, Rajasilta et al. 1989). However, beside the direct effects on egg development, increasing water temperatures often trigger a suite of secondary stressors for fish egg survival (e.g. decreasing dissolved oxygen, algal blooms, increased loads of fungal spores) (Aneer 1987, Rajasilta et al. 1993).

To investigate the role of spawning substrate complexity within the full set of naturally interacting parameters, we conducted field experiments on a major spawning ground of *C. harengus* in the Southwestern Baltic Sea. The research was performed during different temperature regimes along the spawning season of two major spawning cohorts, hypothesizing that i) high structural complexity of spawning substrates positively affects herring egg survival and ii) that the magnitude of this effect increases with changing ambient environmental conditions in spring (i.e. along seasonal temperature gradients and temperature dependent factors like algae blooms and spread of fungi).

Material and Methods

Study site

The study site Greifswald Bay (54°14’N, 013°33’E) is considered a major spawning ground for spring-spawning herring in the Western Baltic Sea, since larval herring production in the bay regularly correlates with other recruitment indices of the entire stock (Oeberst et al. 2009, Polte et al. 2014). Greifswald Bay is a brackish and eutrophicated lagoon, formed by the German mainland and the Island of Rügen. Covering an area of 514 km² with a mean depth of 5.8 m

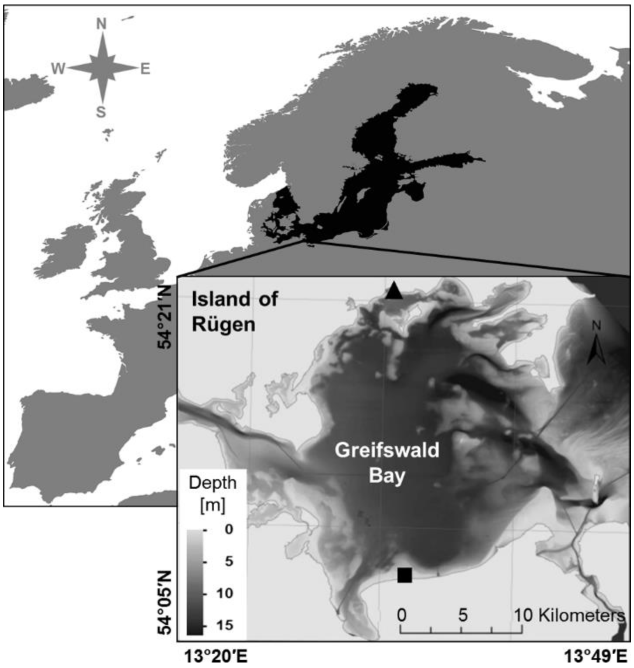


Figure 1. Investigation area Greifswald Bay and its location in the Baltic Sea (displayed in black) with relevant locations for experiments: ▲ field experiment 2014 and ■ field experiment 2015. Bathymetry data received by courtesy of Federal Maritime and Hydrographic Agency of Germany

(max. 13.6 m) and a water volume of 2.96 km³ it is the largest inshore water body of the German Baltic Sea (Reinicke 1989, Stigge 1989) (Fig. 1). The bay has an average salinity of 7.5 (Schiewer 2001), and water temperatures vary from -2 °C in winter to 24 °C in summer. Extensive and closed ice cover occurs frequently during winter and early spring. Water temperatures respond directly to seasonal changes in air temperatures because of the particularly shallow waters. In spring, vertical stratification is rather rare, since the water column is mostly well mixed by wind forcing. This usually results in a high saturation of dissolved oxygen during the herring spawning season.

In the early 20th century the seafloor of Greifswald Bay was extensively covered by submerged macrophytes, but during past decades the vegetation declined severely and presently perennial macrophytes are limited to the shallow littoral areas above 3.5 m water depth (Munkes 2005b, Kanstinger et al. 2016). The macrophyte communities are composed of marine, brackish- and freshwater species. *Zostera marina* and *Stuckenia pectinata* are the most abundant flowering plants, others are *Zannichellia palustris, Ruppia maritime* and *Myriophyllum spicatum* (Munkes 2005b). In shallow areas extensive mats of fast-growing annual algae like *Cladophora* spp., *Enteromorpha* spp., *Ectocarpus* spp. or *Pilayella littoralis* are common.

Experimental design: Herring egg survival on substrates of different complexity

This study investigates a sub-group of Atlantic herring in the Western Baltic Sea that undergoes seasonal migrations between feeding grounds in the Skagerrak and Kattegat and the spawning grounds in sheltered coastal areas (Aro 1989). In this area, herring spawning usually occurs in several waves starting in March (at water temperatures of about 4°C, Klinkhardt 1996), with the major spawning period in late March and April (Scabell 1988). During this short period water temperatures regularly rise by up to 12 °C. On natural spawning beds, numerous environmental stressors are linked to the seasonal temperature regime. Varying interactions of those temperature dependent factors (e.g. oxygen concentration, algae blooms and spread of fungi) and their combined impact on herring egg survival may complicate the interpretation of single-effect experiments in the laboratory.

Therefore, we conducted field experiments close to natural spawning beds of *C. harengus* in different temperature regimes that corresponded to the two major spawning waves of the dominant cohorts in the area. One experiment was carried out in the early spawning period (March 19th until April 04th 2015) at water temperatures from 4 to 6 °C and an additional one in the late spawning period (April 17th until April 26th 2014) at water temperatures from 11 to 13 °C. For logistical reasons, the experiments had to be conducted in consecutive years on two different stations in Greifswald Bay (Fig. 1), but we assumed no inter-annual differences, since in the bay the general seasonal patterns of environmental conditions are rather constant over the years.

SPAWNING SUBSTRATE COMPLEXITY

Artificial substrates (plastic aquarium plants) of differing morphologic complexity (Fig. 2) were used, to exclude potential physiological substrate differences (e.g. oxygen production, respiration or exudate release of distinct plant species). For complexity group C1, different substrates (plastic aquarium plants in 2015 and steel plates in 2014) were used in the two experiments, avoiding physiological substrate effects by using inert artificial materials with similar complexity values.

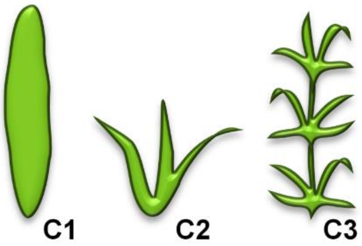


Figure 2. Scheme of substrates with increasing structural complexity (left to right).

For adequate classification and comparability between experiments, a measure of complexity was calculated for each substrate. We used the complexity index (I_{hv}) according to Dibble et al.

(1996) which is based on imaging analyses of the number and width of interstitial spaces between the object compartments (Fig. 3).

$$I_{hv} = f_h/l_h + f_v/l_v$$

Where: I_{hv} = complexity index; f = mean frequency of interstices per meter along an axis; l = mean length (mm) of interstices along an axis; h = horizontal axis; v = vertical axis. Each variable was calculated as the mean value of 6 transects along the respective axis (Fig. 3). Therefore, one unit of each substrate was placed on scale paper, a digital image was taken and six horizontal and six vertical transects were defined (Fig. 3a). Using an imaging program (Image J 1.47v, Wayne Rasband, National Institutes of Health, USA) the total length of the reading line (first substrate intersection to the last one) was measured for each transect, and the interstices (gaps in between substrate material) were counted and measured along the respective transect (Fig. 3b).

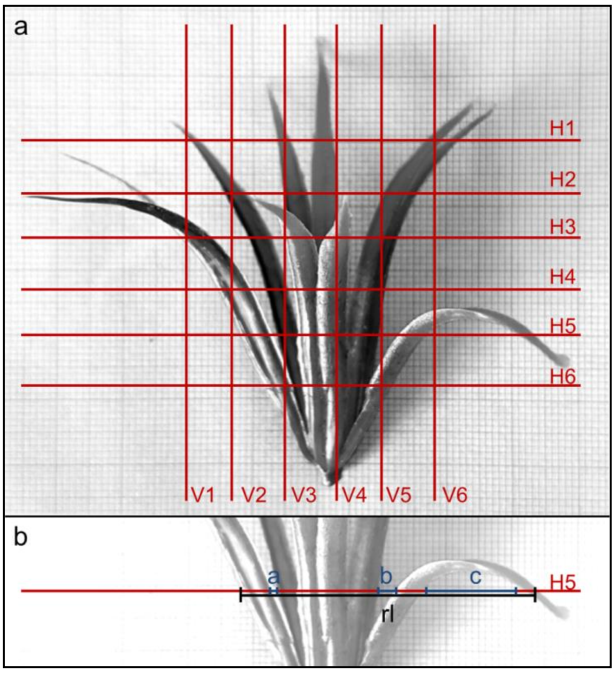


Figure 3. Digital images of an artificial spawning substrate, showing: **a)** horizontal (H) and vertical (V) transects (red) for determination of the complexity index (Tab S1, complexity group C2) and **b)** count example of transect H5, displayed with reading line (black) and interstices (blue)

High complexity index values reflect substrates with numerous small interstices in between the material and thus with a high complexity. According to the I_{hv}, substrates were classified into complexity groups C1: $I_{hv} < 1$, C2: $I_{hv} = 1 – 3$ and C3: $I_{hv} > 3$ (Fig. 2, Tab. 1). In the late spawning season no values for the medium-complex substrate group C2 were generated.

Table 1 Complexity indices of different artificial spawning substrates, respective interstice values and complexity group classification

Substrate (year)	f_h	l_h	f_v	l_v	I_{hv}	Complexity group
1 (2014)	0	0	0	0	0	C1
2 (2015)	0	0	6.84	0.32	0.22	C1
3 (2015)	82.19	0.43	48.11	0.66	2.63	C2
4 (2014, 2015)	99.55	0.47	108.71	0.54	4.14	C3

(f_h) mean frequency of interstices per meter along horizontal axis, (l_h) mean length [mm] of interstices along horizontal axis, (f_v) mean frequency of interstices per meter along vertical axis, (lv) mean length [mm] of interstices along vertical axis, (I_{hv}) complexity index.

***ARTIFICIAL SPAWNING* -** In Greifswald Bay ripe herring were caught by gillnets of differing mesh sizes (3 nets of 25 m x 5 m with mesh sizes of 25, 27 and 29 mm) to include a broad size range of spawners in the procedure and minimize intrinsic effects of spawner size on eggs. On board the research vessel, mature fish were separated according to gender.

Prepared artificial substrates of different complexity were subsequently placed into a bowl with habitat water and female herring were strip spawned onto their surface. Thereby, the best results were achieved by stripping from head to tail with light pressure while constantly moving the caudal fin through the water to dilute sticky egg clumps into consistent single egg layers. Thereafter, eggs were fertilized for about 10 minutes in buckets with a mixture of the milt from ≥ 10 males and sea water, rinsed and stored in transport boxes until installation in the field experiment. To reduce possible parental effects (Geffen and Nash 2012), we maximized the genetic mixture by using eggs of one female for only one experimental unit and particular milt-mixtures were used to fertilize not more than two units of each complexity group.

***EXPERIMENTAL SETUP* -** The field experiments were conducted in close proximity to natural herring spawning sites (< 1 km) in Greifswald Bay (Fig. 1). Each substrate complexity group was installed with 6 replicates (one replicate consisted of three sub-units of about 7 to 12 cm height; sub-units are displayed in Fig. 2). Predator-exclusion-devices (PEDs) with a mesh size of 5 mm (knot to knot) were used to eliminate the naturally high egg loss caused by predation (Kotterba et al. 2014, 2017). Therefore, in 2014 the experimental units were enclosed by tube PEDs, fixed in the water column (at 0.7 m, total water depth 1.5 m) and in 2015 the units were attached to the seafloor (mean water depth 1.0 m) and covered by cage PEDs (Fig 4). Units of different complexity groups were arranged in a randomized block design to compensate for potential influences of natural gradients and small scale heterogeneity in a natural environment

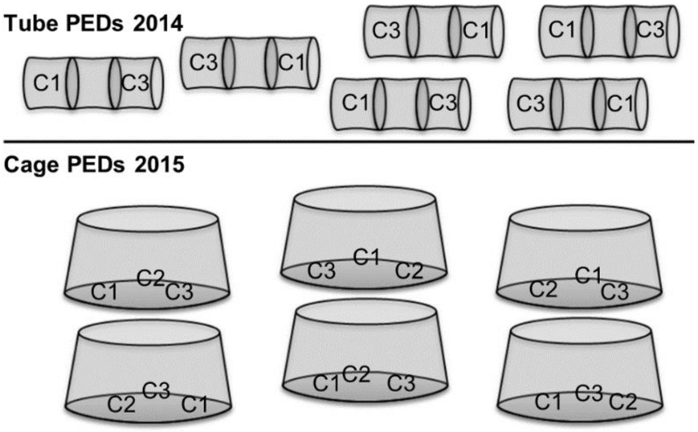


Figure 4. Experimental setup: arrangement of complexity group units (C1, C2, C3, $n = 6$) in predator exclusion devices (PEDs) and in the field experiments (2014, 2015)

(Dutilleul et al. 1993). Hence, one unit of each complexity group was randomly placed into each PED ($n = 6$).

To standardize the egg development stages for experiments run under differing temperature regimes, we used degree days (°d), calculated as the product of temperature (°C) and egg development time (d) (Apstein 1909, Klinkhardt 1996, Peck et al. 2012). Samples from both experimental set-ups were taken (see ‘data analyses’ for details) at the start, to investigate the fertilization success, and on °d 40 and °d 80 (shortly before hatch) post fertilization.

Data analyses

FERTILIZATION SUCCESS - The fertilization success was determined by counting the unfertilized eggs (Fig. 5) in initial samples (6 sub-units of each complexity group) that were taken three days post fertilization and expressed as percentage of the total number of eggs:

$$\text{Fertilization success [\%]} = \frac{n \text{ fertilized eggs} * 100}{\text{total } n \text{ of eggs}}$$

EGG SURVIVAL - In the experiment conducted in the early spawning season, samples (one sub-unit) of each replicate were preserved in 4 % borax-buffered formalin. This method allows firm distinction of viable and dead eggs since the natural transparency of originally vital eggs and cloudiness of dead eggs (white color) is conserved (Fig. 5). Digital images were taken in the laboratory and the percentage of viable eggs was determined by counting viable and dead eggs (total $n = 100$) with an imaging software (Image J 1.47v, Wayne Rasband, National Institutes of Health, USA).

$$\text{Viable eggs [\%]} = \frac{n \text{ viable eggs} * 100}{\text{total } n \text{ of eggs}}$$

In the experiment conducted in the late spawning season digital images of each replicate were taken in the field. The proportion of viable and dead eggs was macroscopically estimated by one individual observer (Meese and Tomich 1992, Dethier et al. 1993) to calculate an overall percentage of viable eggs attached to the particular substrate.

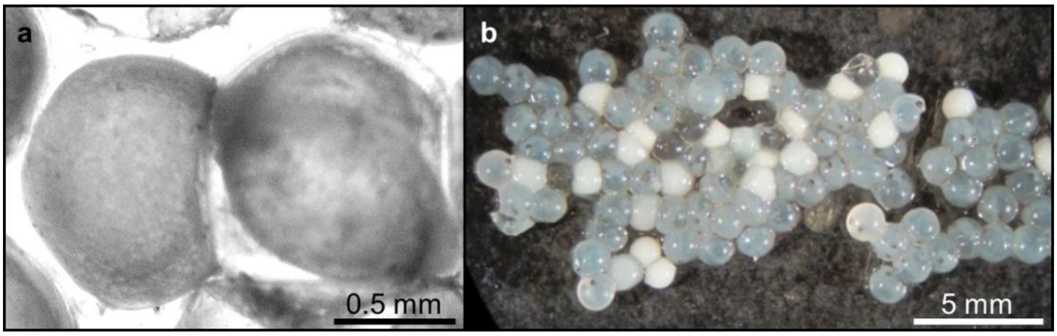


Figure 5. Stages of herring eggs observed under a microscope: **a)** unfertilized egg (left) and fertilized egg where chorion and yolk are separated (right) and **b)** several eggs in different stages (transparent) and dead eggs (opaque white)

STATISTICS - Statistical analyses were conducted using SPSS 21 (IBM Statistics), R (version 3.2.3, R Foundation for Statistical Computing 2015) and Microsoft Office 2007. Spawning substrates of different structural complexity levels were tested for differences in the percentages of viable eggs with a one-way analysis of variance (ANOVA). Multiple comparisons (*post hoc* tests) of pairs between mean values of all substrate groups were performed with Tukey’s Honestly Significant Difference (HSD) test. Potential block effects due to small scale environmental heterogeneity in the experimental setup were tested with a two-way ANOVA. For the comparison of viable egg percentages on °d 40 and °d 80 post fertilization, the Mann-Whitney U-test was conducted because not all requirements for a parametric test were fulfilled (Mann and Whitney 1947), and for the comparison of egg-mortality values on substrates of complexity groups C1 and C3, the t-test was used. The significance level for all statistical analyses within the study was set to $p \leq 0.05$.

Considering differences in sampling methods, years and seasons, statistical analyses were conducted independently for both experiments (early spawning season, 2015 and late spawning season, 2014).

Results

COMPLEXITY RELATED FERTILIZATION RATE - Generally, eggs on all complexity groups showed a high fertilization success, with mean values between 86 and 97 %, representing the initial amount of fertilized eggs at the beginning of the experiments (Tab. 2). The substrates had only minor effects, since in both experiments no significant difference in fertilization success was found between the complexity groups (ANOVA, experiment in the early spawning season: $p = 0.606$; F(2,15) = 0.52, experiment in the late spawning season: $p = 0.161$; F(1,10) = 2.30).

COMPLEXITY RELATED EGG SURVIVAL - In the experiment conducted during the late spawning season, the percentage of viable eggs was similar in all complexity groups at the beginning of the experiments and on °d 40 post fertilization (Fig. 6, bottom). A similar pattern was observed at the beginning of the experiment during the early spawning season (Fig. 6, top). However, although the difference was minor on °d 40 post fertilization in this period, on low substrate complexity C1 (93.3 % ± 1.5) egg survival was significantly higher than on medium complexity C2 (88.3 % ± 2.7) (ANOVA, $p = 0.022$; F(2,15) = 4.94).

On °d 80 post fertilization, much higher percentages of viable eggs were detected on more complex substrates in both experiments (Fig. 6). In the early spawning season, the differences were not significant (ANOVA, $p = 0.271$; F(2,15) = 1.43), but a trend of increasing egg survival was observed from complexity group C1 (65.2 % ± 27.3) to C2 (73.4 % ± 10.4) to C3 (82.7 % ± 10.3). During the late spawning season, differences in egg survival on C1 (14.3 % ± 17.4) and C3 (69.2 % ± 15.0) were highly significant (ANOVA, $p < 0.001$; F(1,10) = 34.22).

Table 2. Field data on herring egg survival on spawning substrates of different complexity (C1, C2, C3; n = 6). Presented are mean percentages of viable herring eggs in the course of development: start of the experiment (also represents the fertilization success), on degree day °d 40 and °d 80 post fertilization and the egg mortality from °d 40 to °d 80 in two spawning periods (temperature regimes) with standard deviations (±SD).

Spawning period	Substrate	Mean viable eggs [%] (±SD)			Mortality [%] (±SD)
		Start	°d40	°d80	°d40 to °d80
Early (4 to 6 °C)	C1	86.25 (± 6.53)	93.33 (± 1.51)	65.24 (± 27.28)	29.94 (± 29.56)
	C2	89.04 (± 3.19)	88.33 (± 2.66)	73.42 (± 10.37)	16.94 (± 10.96)
	C3	88.70 (± 5.28)	90.67 (± 3.67)	82.67 (± 10.34)	8.65 (± 12.44)
Late (11 to 13 °C)	C1	96.93 (± 5.02)	93.00 (± 9.44)	14.33 (± 17.41)	84.65 (± 17.74)
	C3	92.05 (± 6.07)	98.50 (± 1.38)	69.17 (± 14.97)	29.77 (± 15.20)

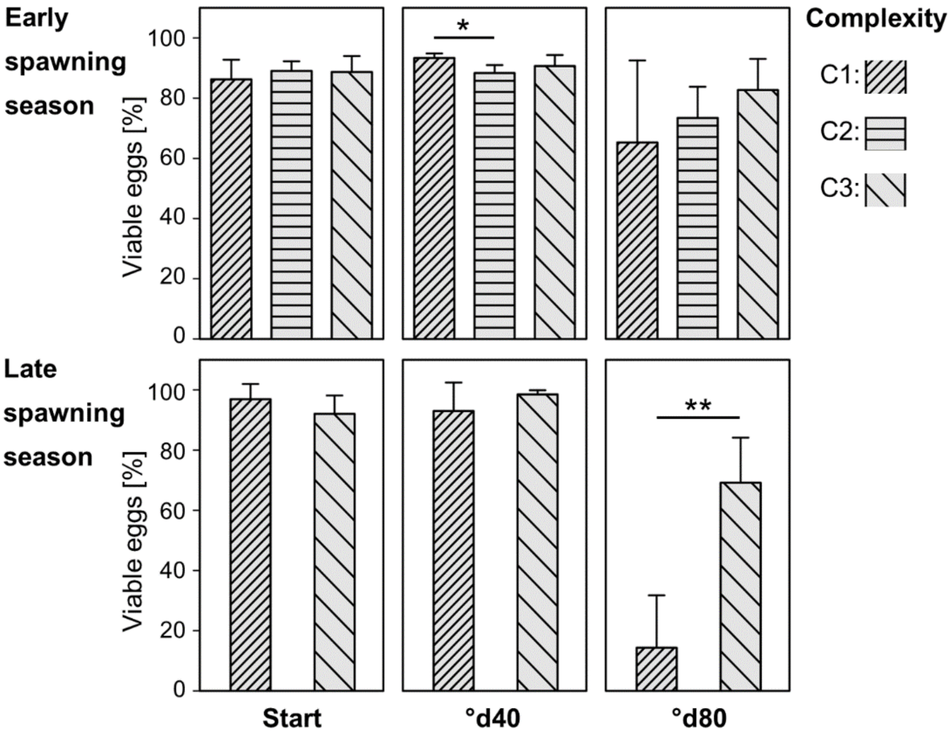
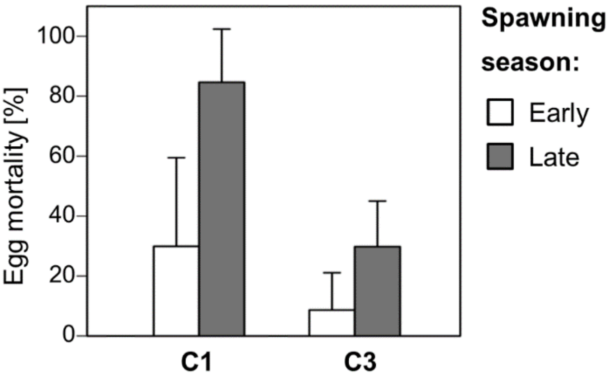


Figure 6. Percentage of viable herring eggs (mean values with standard deviations indicated by error bars) on spawning substrates with increasing structural complexity (C1, C2, C3; *n* = 6) displayed in the course of development: start of the experiment (equates the fertilization success), on degree day °d 40 and °d 80 post fertilization in two spawning periods (early and late spawning season) (*: $p \leq 0.05$, **: $p \leq 0.001$, Tukey's HSD-test) (data: Tab. 2)

EGG MORTALITY DURING ADVANCED DEVELOPMENT - During egg development, a decrease in the number of viable eggs was observed in all complexity groups (Fig. 6). A comparison of the percentages of viable eggs on °d 40 with °d 80 post fertilization revealed highly significant differences for eggs on all substrates except for complexity group C3 in the early spawning season (Tab. 1). During that period (°d 40 to °d 80), the mortality on substrates with low complexity C1 was almost three times higher compared to C3 in both experiments (Fig. 7). While this trend was not statistically significant for the early spawning season (t(10) = 1.63; p = 0.135), it was highly significant for the late spawning season (t(10) = -5.75; p < 0.001). Furthermore, the mortality on C1 (84.7 % ± 17.7) and C3 (29.8 % ± 15.2) substrates was three times higher in the late spawning season than it was earlier (C1: 29.9 % ± 29.6, C3: 8.7 % ± 12.4).

BLOCK EFFECTS - Experimental artifacts caused by individual PED characteristics or placement could be excluded in both experiments since during the whole period no significant block effects were detected by ANOVA.

Figure 7. Egg mortality (from °d 40 to °d 80) in percent (mean values with standard deviations indicated by error bars) on substrate complexity groups C1 and C3 in the early and late spawning season.



Discussion

EFFECT OF SUBSTRATE COMPLEXITY - The results of the present study support our hypothesis that high structural complexity of spawning grounds increases the survival of early Atlantic herring life stages. Generally, the effects of structural complexity on egg survival seem to depend on the stage of egg development and the ambient environmental conditions. In our experiments, the survival of eggs on all complexity groups was quite similar during the first half of the egg developmental period (until °d 40). However, during the second half (°d 40 to °d 80), the egg mortality was three times higher on the low complexity substrate C1 than on high complexity substrate C3. Our findings generally coincide with results of earlier *in situ* studies. Scabell (1988) stated that the sensitivity and mortality rate of eggs increases with proceeding ontogenetic stage. This might explain why complexity effects started to appear during advanced egg development. Furthermore, high variability between the mortality of eggs on small spatial scales is mentioned (Scabell 1988), and it is assumed that intrinsic factors (e.g. genotypic differences, hormones) can be even more important than moderate variations in the abiotic conditions (Ojaveer 1981b). Hence, variability in egg mortality on the differing complexity groups might be a result of parental effects rather than an experimental artifact. To minimize parental effects on the results of this study, a high number of adult herring was used during artificial spawning for the experiments. A high fertilization success of more than 86 % was achieved on all spawning substrates. These numbers match the ratios of natural spawning, observed in earlier field studies (e.g. Rannak 1971, Ojaveer 1981b, Aneer et al. 1983, Klinkhardt 1986, Scabell 1988). The number of fertilized eggs was not related to the substrate complexity, probably because of the intentionally generated even distribution of eggs in single layers. Under natural spawning conditions substrate complexity likely affects the pattern of egg aggregation. Thereby, multiple egg layers can reduce the fertilization success (Ojaveer 1981a).

To make sure that differences in egg survival were not caused by different fertilization rates, we intentionally avoided multiple egg layers and created a standardized single layer situation. In earlier studies, multiple layers and clustering of eggs were found to be related to limited embryonic development and high mortality rates (Blaxter 1956, Parrish et al. 1959, Ojaveer 1981a, Klinkhardt 1984, Kanstinger et al. 2016). Hence, the investigation of complexity dependent egg distribution represents an important future task to deepen our understanding of the role of spawning substrate complexity on herring hatching success.

Despite the intended single layer egg distribution, substrate complexity still affected the positioning of eggs (Fig. 8). On substrates with low structural complexity, all eggs adjoined side to side. However, eggs on substrates with higher complexity were scattered, thus plenty of the egg surface area was in contact with surrounding sea water, an essential prerequisite for adequate oxygen supply and disposal of metabolic products, which is crucial to avoid an autointoxication of the embryo (Parrish et al. 1959, Klinkhardt and Biester 1984). This might present an explanation for the documented higher egg survival on complex substrates, an effect that is becoming more apparent with ongoing embryonic development, which is connected with an increasing demand for oxygen (Braum 1973) and a rising amount of metabolic products (Klinkhardt 1996).

DECREASE OF AQUATIC VEGETATION AND POTENTIAL IMPACT ON HERRING ECOLOGY - In temperate coastal waters submerged aquatic vegetation contributes significantly to structural complexity of benthic habitats. During recent decades, increased nutrient inputs into coastal waters stimulated extreme phytoplankton growth, resulting in reduced light penetration and thereby limiting depth distribution of benthic vegetation (Bricker et al. 2007). In those nutrient rich systems a shift from slow growing macrophytes (such as seagrasses) to fast growing macroalgae and phytoplankton was observed (Duarte 1995). Multiple additional stressors, such as riverine sediment runoff, physical disturbance and global warming are causing seagrass declines on large scales (Orth et al. 2006, Waycott et al. 2009).

The results of our study indicate that complex structures decisively provided by aquatic vegetation have an essential function as spawning substrates for herring in the Baltic Sea. Consequently, an ongoing decline of submerged vegetation and shifts in species composition in inner coastal areas of the Baltic Sea potentially threaten the reproduction of substrate-spawning fish, and might have a major impact on the reproductive success of Western Baltic spring spawning herring. Potentially, a recovery of macrophyte beds would improve the depth

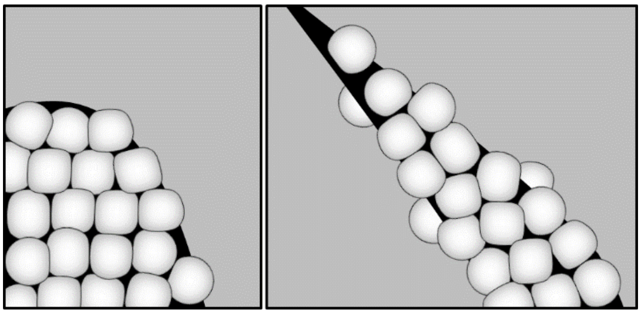


Figure 8. Schematic dispersal of the same number of eggs in single layers on substrates of low (left) and high (right) structural complexity

distribution of suitable spawning substrate, providing a less sensitive spawning habitat against steep temperature gradients and wave action. However, this is a rather slow process, lasting for years or even decades (Duarte 1995). If submerged aquatic vegetation in inner coastal waters of the Baltic Sea will ever recover, the potential to represent improved spawning grounds will depend on the remaining population size of Western Baltic herring and on the readiness of adult spawners to adopt deeper vegetation beds for egg deposition. The latter, however, is rather likely since e.g. observations from North Sea ecosystems indicated that herring adopted introduced seaweed (*Sargassum muticum*) in subtidal zones as spawning substrate, although they previously spawned exclusively in the intertidal zone (Polte und Buschbaum unpublished).

IMPACT OF AMBIENT ENVIRONMENTAL CONDITIONS - In this study, the environmental conditions in the late spawning season amplified complexity effects. While in the early spawning season (water temperatures between 4 and 6 °C) our data suggested a trend of decreasing egg survival in relation to decreasing substrate complexity, these differences became more pronounced in the experiment with water temperatures above 11 °C. Furthermore, the mortality on substrates of all complexities was three times higher in this experiment in the late spawning season. We refrained from conducting comparative statistical analyses between the early and late spawning season due to the different methods applied in both experiments, however, these trends are distinct and in good accordance with other studies.

Earlier experiments, for example, revealed a positive correlation between egg developmental rates and water temperatures (Apstein 1909, Blaxter and Hempel 1963). The course and seasonal timing of the Baltic herring spawning season is considered to be widely determined by the temperature regime. Spawning starts between 4 and 6 °C and takes place until water temperatures exceed values of approximately 15 °C (Ojaveer 1981a, Klinkhardt and Biester 1984, Oulasvirta et al. 1985). Highest amounts of viable hatchlings were found occurring at intermediate temperature levels between 7 and 13 °C and thereby most larvae hatched between °d 100 and 150 post fertilization (Peck et al. 2012). Our findings coincide with earlier studies, where mortality increased *in situ* with proceeding spawning season (Rannak 1971, Oulasvirta et al. 1985, Klinkhardt 1986, Rajasilta et al. 1993, 2006a) and in laboratory experiments with higher water temperatures (Blaxter and Hempel 1961, Ojaveer 1981b). From field studies in the Baltic Sea, mortality values from 5 % to 30 % were reported for the first half of the spawning season (Klinkhardt 1986, Rajasilta et al. 2006a). Later in the season mortality values of 77 % (Klinkhardt 1986) and even 100 % (Rajasilta 2006b) were found. These numbers correspond to our results on °d 80 post fertilization, where we found mean egg mortalities from 17 % (C3) to 35 % (C1) in the early spawning season and 30 % (C3) to 85 % (C1) in the late spawning season.

Oulasvirta et al. (1985) suggested that increasing temperatures alone did not explain the high *in situ* egg mortality in their study and mentioned an unfavorable environment as further explanation, because mortality was negligible in simultaneous laboratory experiments. This might also apply to our field experiment in the late spawning season with water temperatures from 11 to 13 °C. Right after the first days of the experiment, a high mortality of eggs was recorded. Initially single dead eggs were visible on the substrates. In a further stage, larger patches of dead eggs were covered by mucus until finally an almost complete coverage occurred. It seemed as if the eggs initially died and then were infested by fungi. As the spores of fungi are not visible macroscopically, another possibility is that fungal infection was not the consequence but the reason for egg mortality. Interestingly, egg mortality occurred with differing speed and to different degrees on the individual substrate complexity groups. Especially on the plainly structured substrate C1, the patches of dead eggs spread consistently and only some areas close to the substrate edges remained unaffected. In contrast, only small areas of dead eggs appeared on the complex substrate C3, and those patches did not spread much during the course of the experiment. Potential reasons for the positive effect of higher substrate complexity might be linked to differing egg distributions that appeared among the substrates of the complexity groups, even though the same strip-spawning technique was used (Fig. 8). This might be a structural effect, as eggs attached to a rather two dimensional structure cover wide areas homogeneously and thereby fungi can spread in a cascade infecting one egg after another. On more complex and multi-dimensional substrates the eggs occur in several patches without contact to each other, and this distance might have acted as an effective barrier against a fast spreading of the infestation.

Similar processes of fungal infestations are reported in several studies, mostly accompanied by rising temperatures in late spring and early summer (e.g. Ojaveer 1981b, Aneer and Nellbring 1982, Scabell 1988, Rajasilta et al. 1993, Kanstinger et al. 2016). In May 2012 and 2014, fungal infestation was also observed *in situ* in the investigation area Greifswald Bay, when water temperature exceeded 10 °C (pers. obs.). Accordingly, fungal infestations may generally be a frequent event that leads to an increase of egg mortality towards the end of the spawning season. Furthermore, high egg mortality in field observations was related to mass occurrence of filamentous algae (Aneer and Nellbring 1982, Aneer 1987). These events seem to affect herring spawn that is not deposited under optimal conditions in particular, meaning a reasonably robust and structured substrate and adequate circulation of fresh and well oxygenated water (own observations).

Larval herring production in Greifswald Bay is driven by multiple cohorts during the spring reproduction season. Although the mechanisms are not fully understood, the later cohorts are considered to contribute most to the year class strength of offspring, because numbers of hatched larvae correlate strongly with the overall recruitment of this population (Polte et al. 2014). Accordingly, egg survival during the late spawning season might be especially relevant for the annual reproductive success of this population. As effects of substrate complexity were most pronounced during this period, this underlines the potential impact on reproductive success.

CONCLUSIONS The results of this study indicate that structural complexity of spawning substrates positively affects herring egg survival. This became even more pronounced during the late spawning season experiment, when egg mortality generally increased. It can be concluded that complex structures in coastal waters have an essential function as spawning substrate for herring in the Baltic Sea. In temperate waters, submerged aquatic vegetation is an important biogenic provider of structural complexity. Accordingly, regional alterations and inconsiderate modifications within coastal ecosystems have the potential to negatively affect the reproductive success of important natural fish resources such as the Western Baltic spring spawning herring. It can be assumed that this also applies to numerous other aquatic species that rely on benthic structures as spawning substrates, but literature on the subject is rather scarce.

Although inshore lagoons and sheltered coastal habitats have been defined as key nursery areas for fish, the underlying complex ecological processes are not sufficiently incorporated into conservation approaches (Sheaves et al. 2015). Knowledge on the value of different potential spawning substrates is a crucial prerequisite for understanding the complicated processes of ecosystem functioning and hence should be implemented into coastal management plans.

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Ontogenetic loops in habitat use highlight the importance of littoral habitats for early life-stages of oceanic fishes in temperate waters

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Abstract

General concepts of larval fish ecology in temperate oceans predominantly associate dispersal and survival to exogenous mechanisms such as passive drift along ocean currents. However, for tropical reef fish larvae and species in inland freshwater systems behavioural aspects of habitat selection are evidently important components of dispersal. This study is focused on larval Atlantic herring (*Clupea harengus*) distribution in a Baltic Sea retention area, free of lunar tides and directed current regimes, considered as a natural mesocosm. A Lorenz curve originally applied in socio-economics to describe demographic income distribution was adapted to a 20 year time-series of weekly larval herring distribution, revealing size-dependent spatial homogeneity. Additional quantitative sampling of distinct larval development stages across pelagic and littoral areas uncovered a loop in habitat use during larval ontogeny, revealing a key role of shallow littoral waters. With increasing rates of coastal change, our findings emphasize the importance of the littoral zone when considering reproduction of pelagic, ocean-going fish species; highlighting a need for more sensitive management of regional coastal zones.

Introduction

Understanding dispersal mechanisms of larval fish is vitally important in determining whether fish during early life stages can grow and survive in marine habitats, a prerequisite to the successful recruitment of a population. For temperate waters, some general concepts or paradigms postulated decades ago set the direction of modern fishery science. Dispersal and survival of larval fish has been widely attributed to: passive drift along prevailing wind and current regimes (aberrant drift hypothesis, (Hjort 1926); stable retention hypothesis, (Iles and Sinclair 1982)), accumulation of larvae due to thermoclines and haloclines in stratified water bodies (stable ocean hypothesis, (Lasker 1978)), resulting in spatial and temporal overlap with suitable planktonic prey (match-mismatch hypothesis, (Cushing 1974, Cushing 1990)), particularly at the transition between yolk consumption and exogenous feeding (critical period hypothesis, (Hjort 1914)). Most of these principal concepts were developed based on the early life history ecology of small pelagic fishes, such as the clupeid species with a rather cryptic, translucent larval morphology. Studies have rarely discriminated between larval development stages although mobility and physico-chemical tolerance ranges can differ significantly in the early stages of life (Sogard 1997, Moyano et al. 2016). Although behavioural traits and active habitat selection are important drivers of larval settlement for many tropical reef fish (Paris and Cowen 2004, Leis 2010) and are increasingly implemented in coastal zone management (Hughes et al. 2005), these behavioural aspects are not often considered as mechanisms of larval dispersal in temperate waters (Houde 2008). However, in riverine systems including those with significant current velocities, larval dispersal appears to be not entirely passive but has an active, behavioural component (Schludermann et al. 2012). According to the distinct morphology of consecutive development stages it can be assumed that active habitat selection becomes increasingly pronounced along the early ontogeny and laboratory experiments have shown a significant increase of mobility in successive stages of larval Atlantic herring (*Clupea harengus*) (Moyano et al. 2016).

If active habitat selection is involved in larval dispersal of major fishery species such as herring, this would have significant impact on the interpretation and application of each of the paradigms outlined above. The overlap of larval distribution with suitable planktonic prey fields might not be exclusively determined by large scale oceanography but would also be influenced by the directed movement of larvae. A potential shift from passive to active dispersal at an advanced larval stage prior to metamorphosis to the juvenile fish would render dispersal models, integrated over the entire larval phase, imprecise to an unknown extent. However, the incorporation of behavioural aspects would potentially improve parameterization of such models.

In the Northeast Atlantic Ocean many commercially important fish species, such as plaice (*Pleuronectes platessa*) and Atlantic cod (*Gadus morhua*), recruit from pelagic eggs and larvae, moving towards coastal nursery grounds as post-larval juveniles (Gibson 1994, Linehan et al. 2001, Gibson et al. 2002). For herring that spawn benthic eggs in coastal areas, a reverse dispersal of hatched larvae from inshore and coastal shelf bank spawning grounds towards offshore habitats has been documented (e.g. Heath and Rankine 1988, Bartsch 1993). This unidirectional habitat shift might be contradicted by active habitat selection when i.e. larval fish frequent littoral zones for retention as is known from riverine fish larvae (Schiemer et al. 2001). Although current velocities and hydrodynamic forcing in rivers can be at magnitudes higher than in the ocean, limnetic fish larvae do not necessarily display linear unidirectional habitat use following the direction of the flow but primarily forage in sheltered retention zones in the littoral zone of river beds (Flore and Keckeis 1998, Schludermann et al. 2012). In freshwater systems, inshore retention areas and specifically the littoral zone with sheltered hydrodynamic conditions and macrophyte cover allows for better foraging (Flore and Keckeis 1998, Schludermann et al. 2012), increased prey density (e.g. Mavuti 1990, Meerhoff et al. 2007) and decreased exposure to predators (MacRae and Jackson 2001, Okun and Mehner 2005). The function of habitats located in the upper littoral zone for larvae of ocean-going species such as herring was reported on by Urho and Hildén (1990) 25 years ago. They attributed a decrease in abundance of larval herring in the outer coastal waters of the Baltic Sea to inshore migration rather than to mortality and suggested that knowledge of the spatial distribution of herring larvae must be broadened to understand the drivers of year-class strength.

The present study investigated habitat use of larval Atlantic herring in the Baltic Sea where the relative calm hydrology of retention areas provides a suitable model system to study stage-dependent habitat selection of differing larval development stages independent from large-scale physico-chemical stratification or the impact of major current regimes.

We adapted demographic distribution analysis from socio-economics to a 20 year time series of weekly larval distribution supplemented by field studies of stage-specific larval abundance in littoral and pelagic habitats. The objectives of the study were to test the hypotheses that i) spatial homogeneity of distribution is stage-specific, ii) vertical stratification of herring larvae occurring in shallow, well mixed water bodies point to an active positioning of larvae in the water column and iii) stage-specific shifts in habitat use are not unidirectional on the inshore-offshore gradient but the littoral zone is an important retention area for advanced larval stages.

Methods

STAGE-SPECIFIC SPATIAL DISTRIBUTION HOMOGENEITY - The distribution of herring larvae was studied in the Greifswald Bay (N54°14', E013°34'), a major spawning ground and retention area for Atlantic herring in the Western Baltic Sea (see supporting information (SI) and Fig. S1 for more details, p. 137 f.). The 'Rügen Herring Larvae Survey' (RHLS) is an ongoing complex stock assessment process where herring larvae are quantitatively sampled weekly at 36 stations in the bay and adjacent waters between March and July (Oeberst et al. 2009) (see SI and Fig. S1&S2A for details, p. 138 f.). Thirty stations from the RHLS were included in the present study. Stations located in the adjacent sound with a different hydrology and were excluded from the analysis. For the investigation of the spatial distribution of different herring size classes (Fig. S1, p. 138), data from the years 1992 to 2014 were used to initially visualize the relative distribution during the calendar weeks of the highest abundances of each length group and each year using the Esri® Geographic Information System ArcMap™ version 10.2 (see SI for further information on the selection process, p. 140).

The homogeneity of spatial dispersal of the larvae was then further characterized in detail by plotting the data into a Lorenz curve (Lorenz 1905). We then calculated the area below the curve as a measure of data homogeneity:

$$A = \sum_{i=1}^{30} \left(\frac{(y_i - y_{i-1}) \times (x_i - x_{i-1})}{2} + y_{i-1} \times (x_i - x_{i-1}) \right)$$

Where A is the area below the Lorenz curve in relative dimensions, y represents the cumulative proportion of larvae abundance ($0 \leq y \leq 1$) and x is the cumulative proportion of sampling stations (x = $^1/_{30}$; $^2/_{30}$; $^3/_{30}$;...;1). We used a Kruskal-Wallis test and corresponding post-hoc tests (Games-Howell test) to examine the differences between the larvae sizes.

ASSIGNING LARVAL SIZE CLASSES TO SPECIFIC DEVELOPMENT STAGES - Larval herring size classes (mm TL) were assigned to particular life stage categories according to literature on western Baltic herring (Moyano et al. 2016) and central Baltic herring (Arula et al. 2016). The transition between stages ranges between multiple millimeters in growth and differences in critical morphological traits (e.g. development of fin rays) could potentially affect larval distribution patterns. Therefore it was necessary to select size categories with definite stage classification and discard intermediate length classes to investigate stage specific habitat selection. Accordingly, larvae between 5 and 9 mm were categorized as yolk-sac-bearing hatchlings not yet feeding actively, while larvae between 14 and 18 mm were classified as having already passed the critical period of first feeding (shift from endogenous to exogenous nutrition). Larvae between 24 and 28 mm were classified as having already passed the main structuring bottlenecks of larval survival since their abundance relates linearly to the resulting numbers of juvenile fish caught in consecutive years (Oeberst et al. 2009). We used a Kruskal-Wallis test and corresponding post-hoc tests (Games-Howell test) to compare the means of the distinct groups.

LARVAL HERRING SAMPLING IN THE LITTORAL ZONE - Every second week between April and June in 2011, herring larvae abundances in the shallow littoral zone of the Greifswald Bay were investigated, using a plankton net mounted on an epibenthos sledge (see details in SI and Fig. S2B, p. 139 ff.). The data were normalized against the volume of filtered water and then compared to simultaneous RHLS catches of larvae from the pelagial of the bay.

VERTICAL DISTRIBUTION IN THE PELAGIC ZONE - In April 2012, the vertical distribution of herring larvae was investigated with standardized plankton samples at three distinct depths strata on three different stations within the Greifswald Bay (Fig. S1, p. 138). Sampling was repeated six times at each depth stratum at every station and larvae abundance was calculated by the volume of water filtered by the plankton net (see details in SI and Fig. S2C, p. 139 ff.). Larvae abundance from each depth strata from the stations were compared by ANOVA and corresponding post-hoc tests (Tukey's HSD test). As the level of cloudiness might affect depth stratification of the larval fish, weather data, derived from the federal meteorological service of Germany (DWD) were used to analyze the cloudiness during the depth-stratified sampling. For each sampling time, the mean hourly cloudiness ($n = 9$) was compared and an ANOVA was performed to test for significant differences in cloud cover. Furthermore, water probe profiles measuring conductivity, temperature and oxygen content of the water (CTD) were recorded to analyze the level of mixing in the water column. A total of 40 CTD profiles were recorded in the bay during the sampling period. We compared surface and bottom values of salinity, temperature and oxygen in order to identify possible stratification events of water masses.

SAMPLING OF ADVANCED LARVAL STAGES - Between March and June 2015 an additional sampling was performed weekly, specifically targeting herring larvae in advanced growth stages prior to metamorphosis. Sampling of larvae in the pelagial of the bay was conducted with a ring trawl equipped with a 1.5 mm mesh plankton net (see SI and Fig. S2D for detailed information, p. 138 f.). Sampling in the shallow littoral zone was conducted with a beach seine (mouth opening 7m, mesh size 5 mm (Fig. S2E p. 139), towed over a distance of 100 m. The exact area fished by beach seine hauls was determined by Global Positioning System. Principle environmental parameters such as sea temperature, salinity, oxygen saturation and weather data were recorded at the same time as the field sampling. All field samplings of larval fish were conducted under current licenses for wild fish sampling according to Mecklenburg-West Pomeranian (Germany) fishery law (§ 11 LFischG, Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern).

Results

SIZE-SPECIFIC LARVAE DISTRIBUTION - We found strong size-dependent differences in the spatial distribution of herring larvae in the study area (Fig. 1). Weekly distribution data of each larval length group (mm TL) were used to visualize the level of distribution homogeneity by a Lorenz curve (Fig. 2A). The size classes assigned to certain larval development stages based on information from the literature (Arula et al. 2016, Moyano et al. 2016) were found clearly reflected by the post-hoc test (Games-Howell test) of Lorenz curve areas of each mm size class (see Fig. S5 for details, p. 143). Therefore, shifts in distribution homogeneity could be assigned to particular size classes which translate to developmental stage with sufficient certainty. Fig. 2B reflects this pattern by showing aggregated measures of homogeneity for the years 1992 to 2014 represented by the mean areas below the corresponding Lorenz curves. Kruskal-Wallis test confirmed highly significant differences in spatial distribution homogeneity of distinct length classes ($\chi^2 = 477,447$, df = 29, $p < 0.001$). While there was a gradual increase of distribution homogeneity observed for the larvae prior to the first feeding stage (5-9 mm), larger larvae can be assigned to sharply delimited groups along their homogeneity of spatial distribution (Fig. 2B, Fig. S5, p. 134). Life-stage associated groups of length classes were also significantly different (Fig. 2C; $\chi^2 = 140,704$, df = 2, $p < 0.001$).

Larval size classes from 5-9 mm (yolk-sac) all occurred on a single patch resulting in a very heterogeneous spatial distribution over the entire sampling area. The intermediate stages represented by the size class of 14-18 mm (flexion stage) were distributed homogenously throughout the bay. The advanced stages of larvae in size class 24-28 mm (post-flexion) stage showed a high spatial heterogeneity.

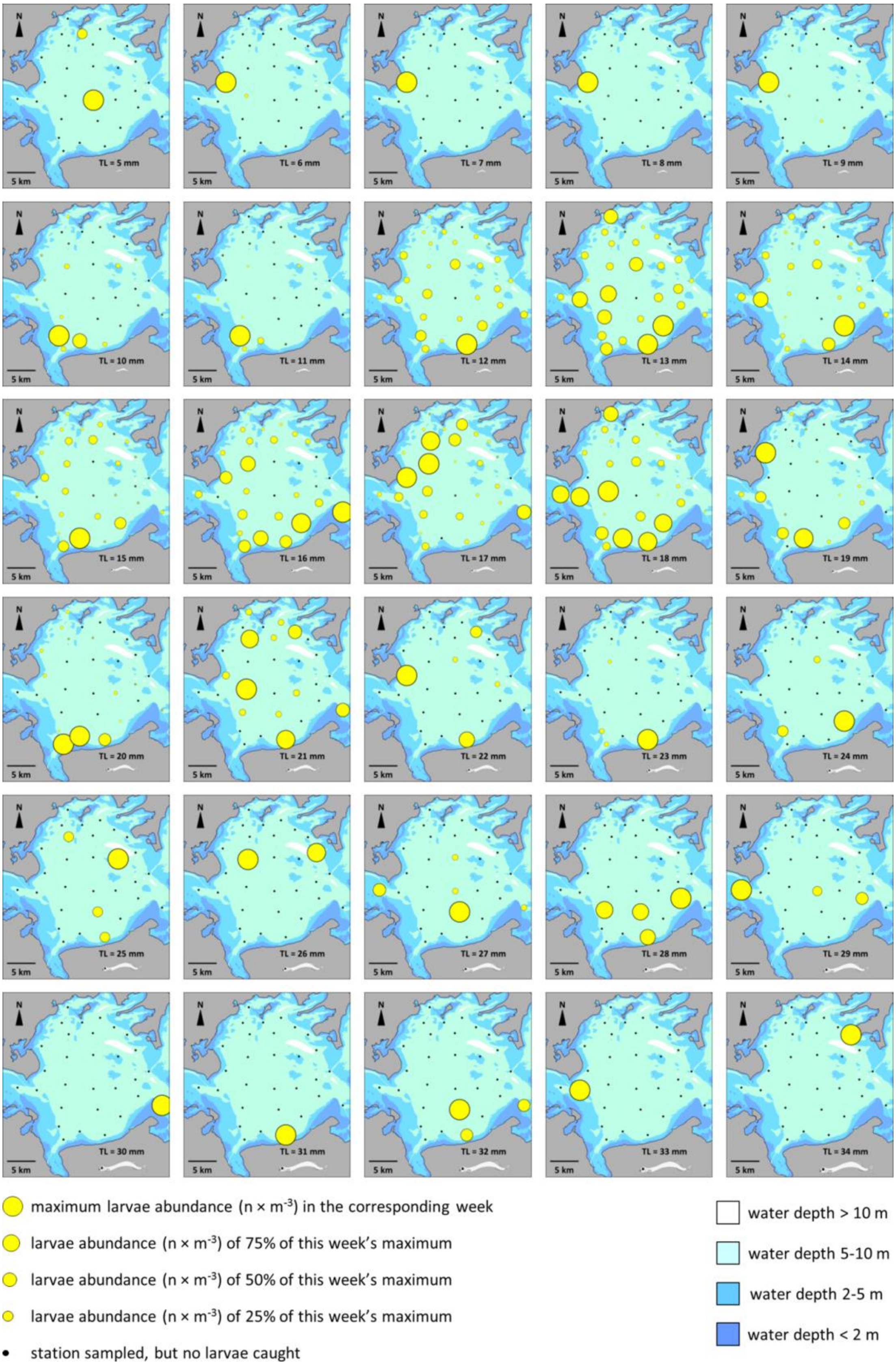


Figure 1. Relative distribution of larval herring size classes (mm total length) during weeks of their maximum absolute abundance in 2011. Data are based on the number of larvae per m3 filtered water. Size of yellow circles reflects the relative proportion of maximum abundance observed in the particular week. Source of elevation data: Federal Maritime and Hydrographic Agency (BSH), Germany.

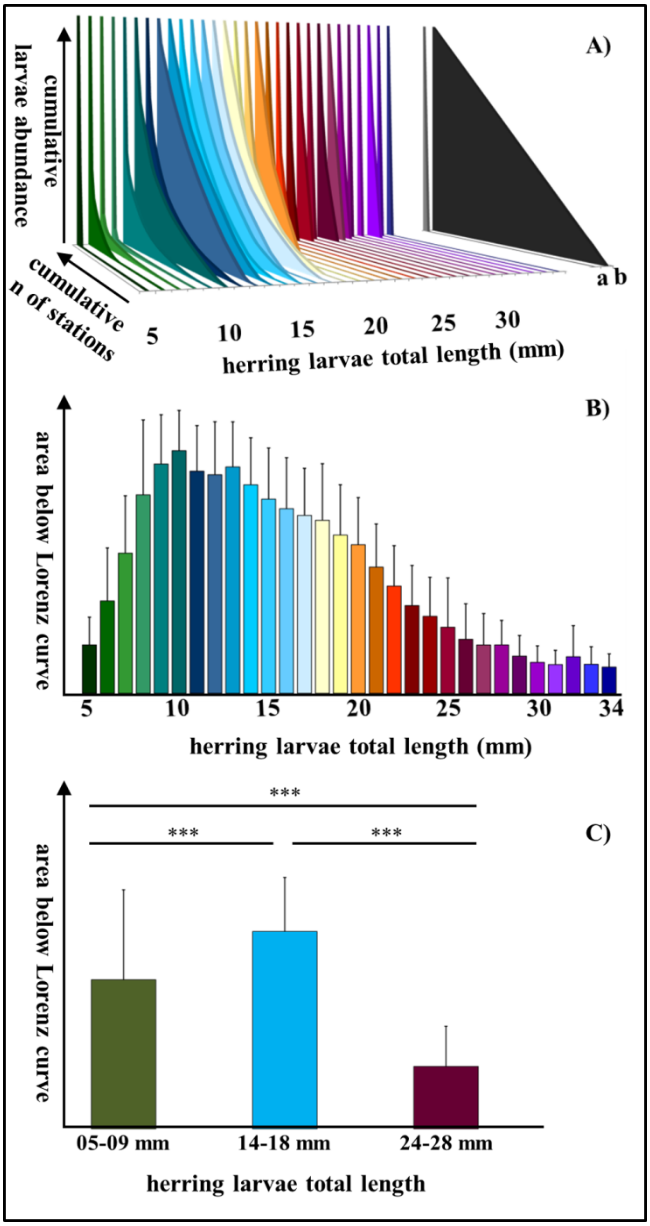
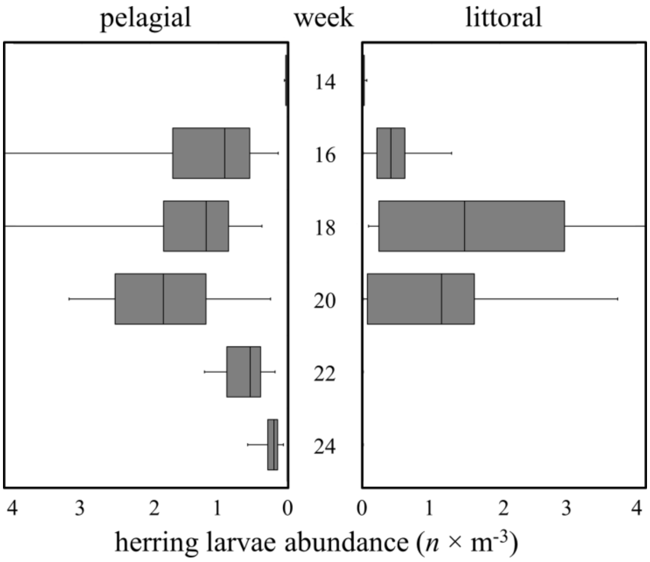


Figure 2. Size-specific spatial distribution homogeneity of larval herring. A) Spatial homogeneity of herring larvae of different size classes (5-34 mm) plotted in a Lorenz curve design (exemplarily shown for 2011): “a” and “b” represent theoretical curves for maximum heterogeneity and homogeneity, respectively. B) Mean Homogeneity of larval distribution for each 1 mm length class aggregated for the years 1992-2014. Bars represent arithmetic means, error bars represent standard deviations. C) Mean area (and standard deviations) below Lorenz curve for selected larvae length groups. Horizontal bars with asterisks indicate significant differences (*** represents a significance level of $p \leq 0.001$).

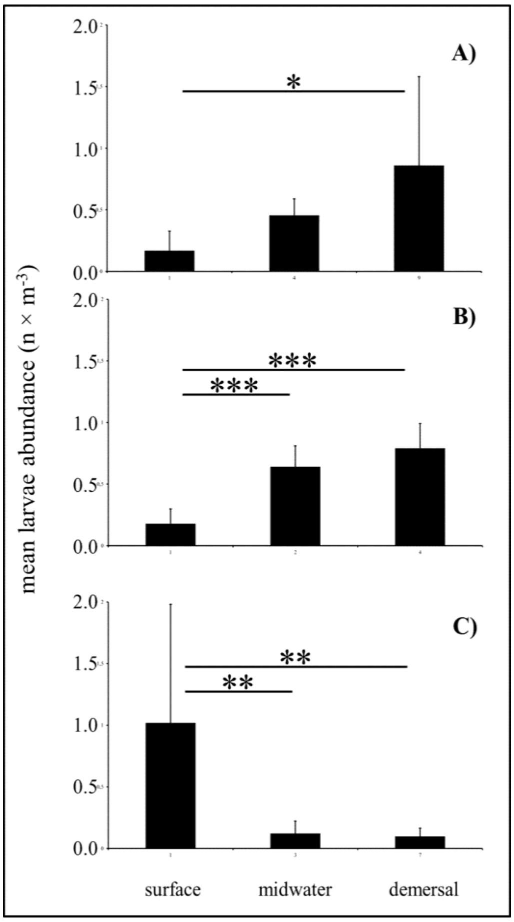
LARVAL HABITAT UTILIZATION - Quantitative sampling of larval herring in the pelagic zone and the respective littoral zones of the bay resulted in comparable numbers in both habitats (Fig. 3). However, the size distribution differed slightly (Fig. S6, p. 143). The majority of larvae found in the littoral zone during the sampling season (calendar week 14-24, 2011) was composed of yolk sac larvae (mean length 8.1 mm, SD = 2.3 mm). Throughout the season the abundance in the littoral zone (Fig. 3) peaked about two weeks earlier (calendar week 18) than in the pelagic zone (calendar week 20). With the passing of the season growing numbers of pre-flexion larvae (size 10-14 mm) could be observed in the littoral habitat but with increasing size their distribution shifted towards the pelagic zone. The mean size of larvae found in the pelagic zone during the sampling duration was 9.9 mm (SD = 3.4 mm).

Figure 3. Herring larvae abundances in the pelagic zone of Greifswald Bay (left panel) during selected weeks in 2011 compared with larvae abundances in the littoral zone (right panel) in corresponding weeks. Data are presented in boxplots with boxes reaching from the 0.25 quantile to 0.75 quantile (containing the median – given as horizontal line). Whiskers represent the absolute maximum and minimum values, respectively.



VERTICAL DISTRIBUTION - Pre-flexion and flexion stages (14-18 mm) of herring larvae were relatively homogeneously distributed within the bay but exhibited a significantly differentiated vertical distribution even in very shallow areas of the bay (Fig. 4). However, the orientation of vertical distribution patterns was not consistent for all stations: While larvae seemed to aggregate in bottom waters at two stations (Fig. 4A and 4B), the pattern was reversed in the third station (Fig. 4C).

Figure 4. Depth distribution of herring larvae (14-18 mm TL) on different sampling stations in the study area Bars represent mean values while the standard deviation is given by error bars (n = 6 hauls for each group). 'Surface' indicates larvae abundance close to the sea surface, 'demersal' refers to the sampling depth of 1 m above the sea bottom and midwater to the sampling in between. A) Northwestern station located in the narrow sound between the Island of Rügen and the mainland (maximum sampling depth = 9m). B) Station located at the western edge of Greifswald Bay (maximum sampling depth = 4m). C) Station at the southern coast of the bay (maximum sampling depth = 7m). Horizontal bars with asterisks indicate significant differences based on a one-way ANOVA and corresponding post-hoc tests (Tukey HSD-test; significance levels are given as: * $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$).



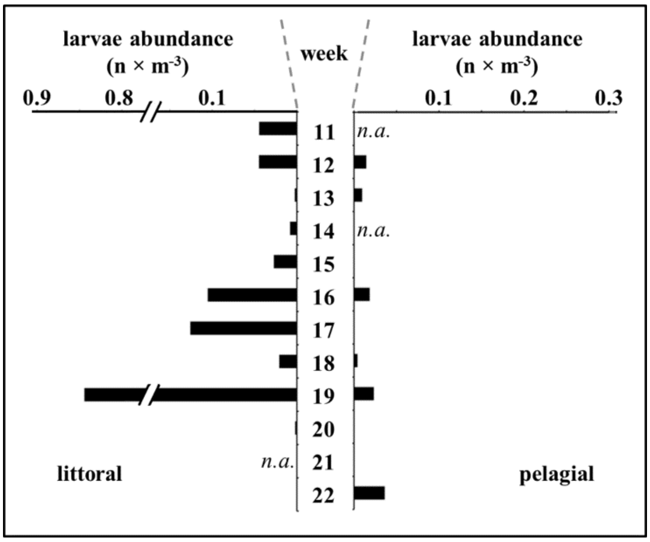


Figure 5. Depth dependent abundance of advanced herring larvae (> 20 mm) in Greifswald Bay. Left panel shows results of weekly beach seine samples in 2015 (littoral zone), while the right panel shows the abundances derived from ring trawl catches in corresponding weeks (pelagic zone). N.a. = not analyzed and sample not taken, respectively. Note the fracture of the y-axis for the littoral data.

DISTRIBUTION OF POST-FLEXION LARVAE - Advanced herring larvae of > 20 mm were found to form large shoals in the very shallow littoral (< 0.5 m depth) of Greifswald Bay during spring 2011. Beach seine sampling in spring 2015 revealed high abundances of larvae (up to a maximum of 0.84 larvae m^{-3}) in the littoral zone (Fig. 5). However, simultaneous ring trawl catches in the adjacent pelagic zone did not result in comparably high abundances (up to a maximum of 0.04 larvae × m^{-3}; Fig. 5).

Discussion

The Lorenz curve applied to the multi-decadal time series of weekly larval herring abundance revealed significant size-specific patterns in the spatial distribution of the herring larvae. Attributing larval size classes to development stages (e.g. Arula et al. 2016, Moyano et al. 2016), hatchlings (yolk sac stage) and advanced larvae (post-flexion) prior to metamorphosis showed high distribution heterogeneity, whereas the pre-flexion and flexion stage larvae were distributed more homogenously within the bay. In fish ecology, habitat requirements are generally assigned to ontogenetic levels (i.e. egg, larvae, juvenile, adult) rather than to development stages on a specific ontogenetic level. However, major fishery species in the Baltic Sea, such as cod, sprat (*Sprattus sprattus*) and herring all have complex life cycles including several larval stages that differ significantly in morphology and motility traits (Guan et al. 2008, Moyano et al. 2016). It is obvious that morphological and ecological differences can be more pronounced between successive larval development stages than between respective post-metamorphosis juvenile and adult stages.

Along the early herring ontogeny our results indicate a selective habitat use according to larval development stage including upper littoral and pelagic habitats of the system. After hatching, larvae in the yolk sac stage left the shore zone and moved towards pelagic habitats of the basin. However, they remained aggregated in the vicinity of the spawning beds. Larvae in the intermediate development stage (pre-flexion, flexion) were found to be increasingly dispersed throughout the pelagic zone of the bay. In contrast, fish in the advanced stages (post-flexion) were found abundant in the upper littoral zone while almost absent in the pelagic zone.

For technical reasons, differing sampling gear had to be used in the pelagic and littoral habitats. Because of potential bias introduced by gear type no direct statistical comparison of larval abundance in both habitats could be achieved but we demonstrated relative abundances of post-flexion larvae caught by the respective gear. As the ring trawl is standard gear to sample ichthyoplankton (e.g. McClatchie 2014), it can be suggested that the low larval numbers caught in the pelagic zone generally are a consequence of selective habitat use. Larvae at different developmental stages which have been observed repeatedly in the shore zones indicates a certain loop in habitat use (Fig. 6) that, to our best knowledge, has not yet been documented even for an otherwise intensively studied fishery species such as herring.

This habitat loop is even more surprising as most studies on larval dispersal commonly describe a rather unidirectional succession of habitats during the ontogeny of young fish. Generally, larval fish movement towards near shore habitats by either passive or active dispersal mechanisms has been widely described for ocean spawning fish (Leggett 1984, Drake and Arias 1991). However, “near shore” often refers to the open water body of estuaries, bays and lagoons but rarely includes the shore zone gradient encompassing the upper littoral zone just below the low tide mark.

In the Northeast Atlantic Ocean, many fishery species recruit from pelagic eggs and larvae. Species such as plaice and Atlantic cod are thought to disperse towards coastal nursery grounds as juveniles (Daan et al. 1990, Elliott et al. 1990, Pihl and Van der Veer 1992, Wennhage et al. 2007). For herring that spawn benthic eggs in the inner coastal areas, a reverse dispersal of hatched larvae towards outer coastal zones is generally assumed (e.g. Stevenson 1962, Bartsch 1993). Hence the majority of larval herring surveys conducted for stock assessment purposes are performed in outer coastal waters which substantially neglects the potential larvae habitats in the shore zones. This might represent a somewhat reversed scaling issue by ignoring the contribution of small scale habitats with significant ecological function but with limited geographic extension to overall system productivity. However, stressors to larval survival in inshore retention areas e.g. by altered habitat conditions would explain the discrepancies observed in classic analyses of stock recruitment relationships (e.g. Rothschild and Fogarty 1989, Iles 1994) and would help to clarify the stock-recruitment dilemma.

The example of the Western Baltic herring stock illustrates how local stressors on early life stage survival in spawning grounds and larval retention areas can affect entire population dynamics (Polte et al. 2014). In a system without significant tidal forcing or large scale current regimes, such as the Baltic Sea, behavioural traits might be important mechanisms of larval dispersal. As littoral habitats in temperate waters are underrepresented as important habitats for larval fish by coastal zone management, there remains a potential risk that important fish resources are being affected by habitat degradation before fishes grow into size classes relevant for current stock assessment models. Our case study on the Western Baltic herring could be considered a prime example of this problem because fishery mortality of the stock was drastically decreased during the past decade due to strict quota driven fishery restrictions. However, recruitment also continuously decreased during that period, driven by mortality during the early life stages (ICES 2015).

Even in well-mixed waters of the lagoon without pronounced thermoclines or haloclines, fish in the pre-flexion and flexion larval stages were not homogeneously distributed within the vertical water body. Despite site- and day-specific vertical zonation, the overall findings that larvae have shown a distinct distribution hints at the potential of these fish in the larval stages to be capable of active vertical positioning. According to Schnack (1974) the site- and date-specific differences in larval zonation patterns might be due to differing degrees of cloud cover where larvae are found closer to the surface on an overcast day. At station “C” at an intermediate depth (7 m), larvae were accumulated at the surface whereas at the other two stations they were found in mid-water or close to the sea bed. However, station “C” was sampled about one week later when cloud cover was significantly higher. This distribution difference would reflect the documented observation that vertical migration is driven by light levels (e.g. Blaxter 1974, Munk et al. 1989). Vertical migration is an important means of dispersal as ichthyoplankton might use certain vertical current regimes for dispersal (Laprise and Dodson 1989). Hence, behavioural traits should be considered in parameterizing spatial dispersal models for larval herring. The distinct larval distribution hints at an active habitat selection according to life stage. This suggests that transitional waters might offer important retention areas in the developmental stages of early herring until metamorphosis to the juvenile stage.

Since Johan Hjort (1914) postulated his critical period hypothesis it has been commonly accepted in fishery science that recruitment of fish stocks is often determined early in the larval stage of a species. The mechanisms causing most larval fish mortality are assumed to be

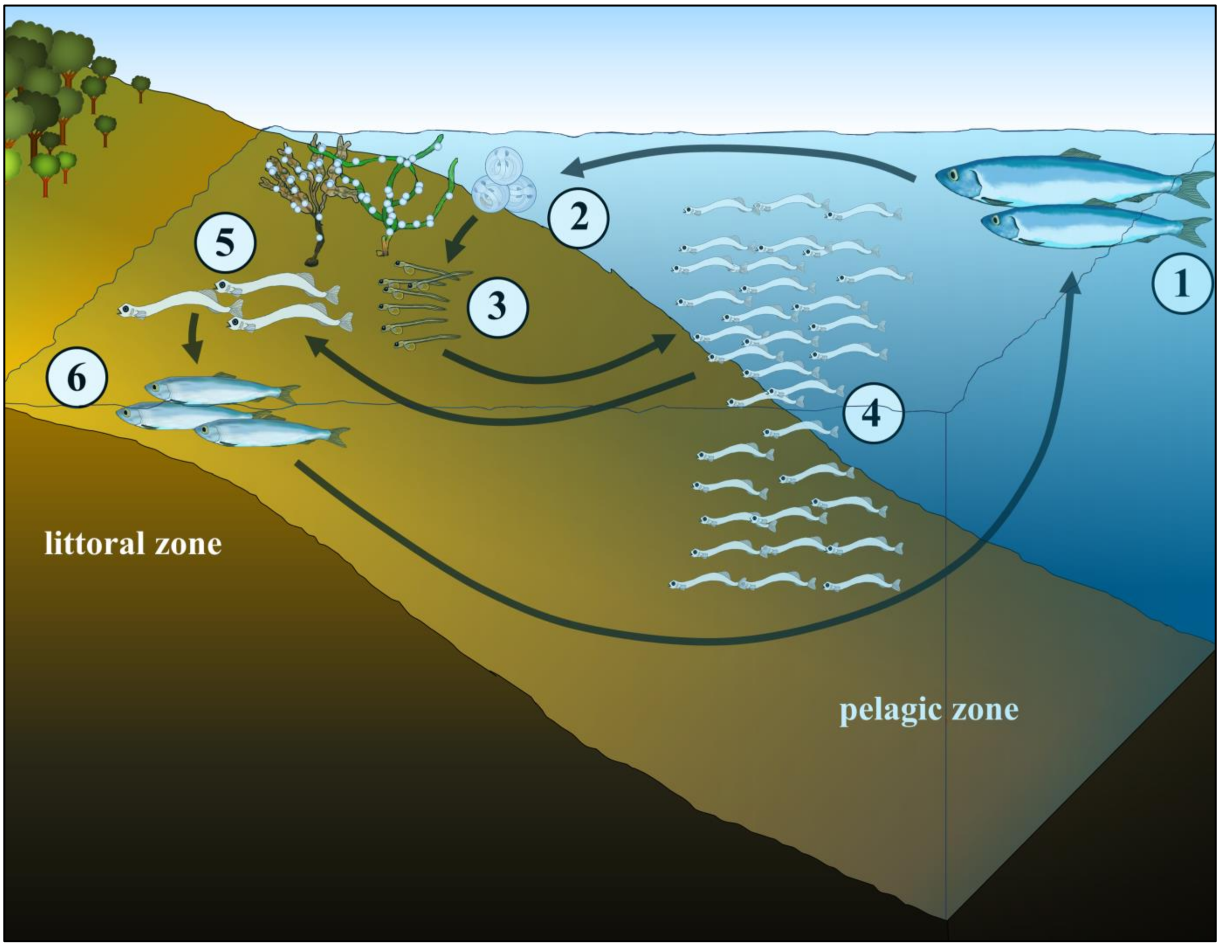


Figure 6. Ontogenetic habitat loop of herring in the Baltic Sea. Adult herring (**1**) migrate from the offshore pelagial into inshore waters to spawn their adhesive eggs on littoral substrates such as macrophytes (**2**). Yolk-bearing hatchlings (**3**) appear concentrated in the vicinity of the spawning beds, while medium sized larvae (**4**) are rather well horizontally distributed in the pelagic area of the bay; however, their vertical distribution in the water column is significantly heterogeneous. Advanced larvae (**5**) return to shallow littoral areas where they remain until after their metamorphosis to the juvenile fish (**6**). Growing juveniles migrate to offshore areas along their development and usually recruit to the spawning group after 2-3 years.

predominantly linked with predation (Bailey and Houde 1989) and food availability for early larvae (Hjort 1914, Cushing 1974). Habitat-specific mortality could potentially result in inhomogeneous distribution in the early life stages. Major mortality e.g. by starving after yolk consumption, occurs over a period from 6 to 8 days (McGurk 1986). Due to the high sampling frequency it is unlikely that we missed major mortality events related to habitat type. Concerning top-down control, earlier studies have shown that predation on herring larvae is of minor importance in the system (Kotterba 2015). Interpreting the different stage-specific patterns of distribution as being a consequence of active habitat selection, this study indicates that habitat requirements might vary according to the stage of larval development. This could introduce an undocumented suite of survival bottlenecks related to availability and connectivity of differing juvenile habitats. In addition to fishery impacts on the adult populations, coastal modification and habitat degradation might introduce significant stressors to important fish resources. An appropriate quantity of spawning stock biomass (SSB) is clearly necessary to sustain a population; however, this alone does not guarantee solid recruitment. It must also be considered that variability or a decrease in recruitment is widely structured by environmental conditions including biotic factors such as predation and competition as well as variation in the physico-chemical environment (e.g. Sparholt 1996, Begg and Marteinsdottir 2002). Many of the general theories and paradigms in larval fish ecology were developed by studying the clupeid species. The commonly accepted general concepts such as the stable ocean hypothesis (Lasker 1978) or the stable retention hypothesis (Iles and Sinclair 1982) are broadly based on the impact of the physical environment on larval dispersal. Leis (2010) questioned whether the role of behavioural patterns are important for larval dispersal and concluded that behavioural aspects are more pronounced in tropical species compared to species inhabiting colder waters, due to extrinsic physical and intrinsic physiological reasons. However, in the shallow retention areas of the Baltic Sea, our study indicates that even the fragile and cryptic clupeid larvae are able to actively select their habitats.

The ecological value of shallow temperate systems in general (e.g. Chícharo et al. 2012) and of littoral habitats in particular for larval fish have been only rarely incorporated in both scientific and marine policy plans, although 25 years ago Urho and Hildén (1990) described the importance of these considerations for Baltic Sea herring stocks. This gap is increasingly problematic as particularly shallow coastal habitats are exposed to the growing threat of drastically changing coastlines caused by human activity as well as numerous major environmental factors such as sea surface warming. Although the habitat function of shallow upper littoral zones as nurseries for fish communities has been broadly acknowledged in marine and freshwater systems (e.g. Werner et al. 1977, Beck et al. 2001), their role in the larval period of “wasp waist” (Bakun 2006) species in coastal food webs in temperate waters is not well understood. The findings of this study should not only provide a strong further impetus for investigation of the ecological functions and value of shore zones for early life stages of oceanic fish species, but will also encourage the implementation of these findings into current management of coastal zones.

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SUPPORTING INFORMATION

Ontogenetic loops in habitat use highlight the importance of littoral habitats for early life-stages of oceanic fishes in temperate waters

Patrick Polte, Paul Kotterba, Dorothee Moll and Lena von Nordheim

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STUDY AREA - Greifswald Bay located at the southern coast of the Baltic Sea is a shallow (mean depth of 5.6 m), mesohaline lagoon unaffected by any significant lunar tides (Fig. S1). Sea level amplitudes are irregular and mainly wind driven and the water exchange to the adjacent Baltic Sea is limited to a broad but shallow entrance in the east and a deeper but narrow sound in Northwestern part of the bay. The lagoon includes an area of approximately 514 km² and is considered to be a major spawning ground of spring-spawning herring in the Western Baltic Sea. Between March and May, herring immigrate into the bay in massive cohorts for spawning attaching adhesive eggs to benthic substrates such as submerged aquatic vegetation.

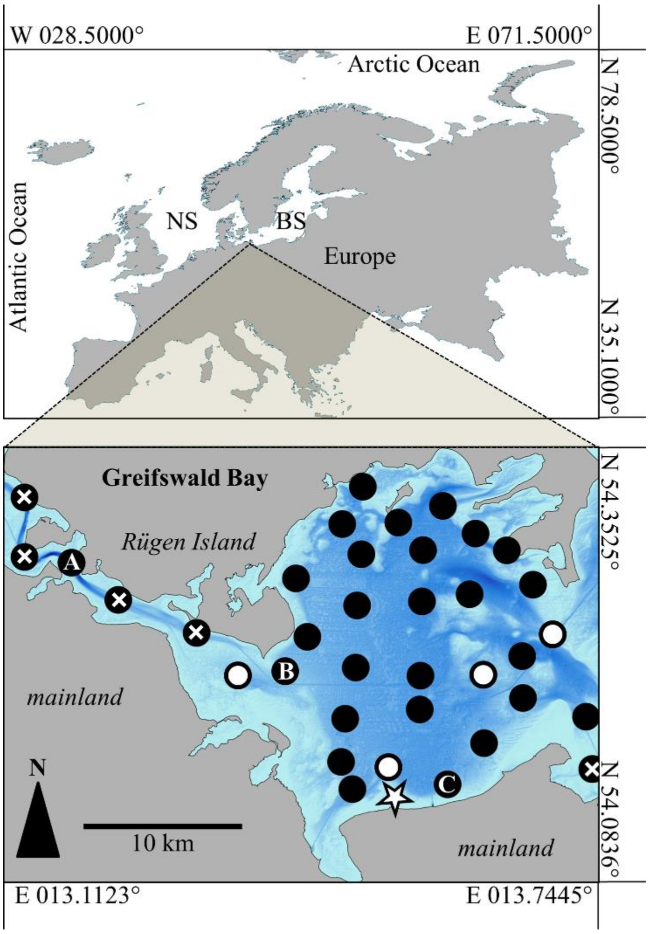


Figure S1. Location of Greifswald Bay in the Baltic Sea (BS). NS = North Sea. Standard stations of the Rügen Herring Larvae Survey (1992-2014) are shown in the lower figure of the bay and labelled corresponding to how the individual stations were included in this study: ● indicate survey stations sampled weekly during the herring larvae seasons. ⊗ are RHLS standard stations but not considered for the homogeneity analysis due to differing hydrology. Ⓐ,Ⓑ,Ⓒ represent RHLS standard stations selected for the 2012 case study on vertical larvae distribution (3 depth strata/station, n = 6/stratum).○ represent stations sampled weekly during spring 2015 with a ring trawl for advanced larval size classes. ☆ represent the shallow littoral site where larvae were sampled with an epibenthos sledge (every second week in 2011) and a beach seine (weekly in 2015). Source of bathymetry data: Federal Maritime and Hydrographic Agency of Germany (BSH). Maps were created using Esri® ArcGIS 10.2 software package (URL: www.esri.com).

RÜGEN HERRING LARVAE SURVEY (RHLS) - Since the bay is assumed to provide an important retention area for herring larvae, a regular ichthyoplankton survey was initiated in the 1970s to examine the inter-annual dynamics of herring larvae production. Since 1992, the survey consisted of a grid of 36 stations that were sampled weekly using a Bongo net with a mesh size of 335 µm and a round net opening of 60 cm in diameter. Catches with the Bongo net were conducted with oblique hauls at a speed of approximately 2 knots to assure a representative sampling of the entire water column from the surface down to 1 meter above the sea bottom (Fig. S2A). Only one of the two Plankton nets in the Bongo setup was used for the regular estimation of herring larvae abundance, while samples from the second one are usually used for case studies or additional analyses. The volume of filtered water was measured with mechanical and digital flowmeters (Hydrobios) installed in the center of each net. Each sampling has regularly been complemented by the recording of weather conditions, as well as turbidity, water temperatures, salinity and dissolved oxygen saturation.

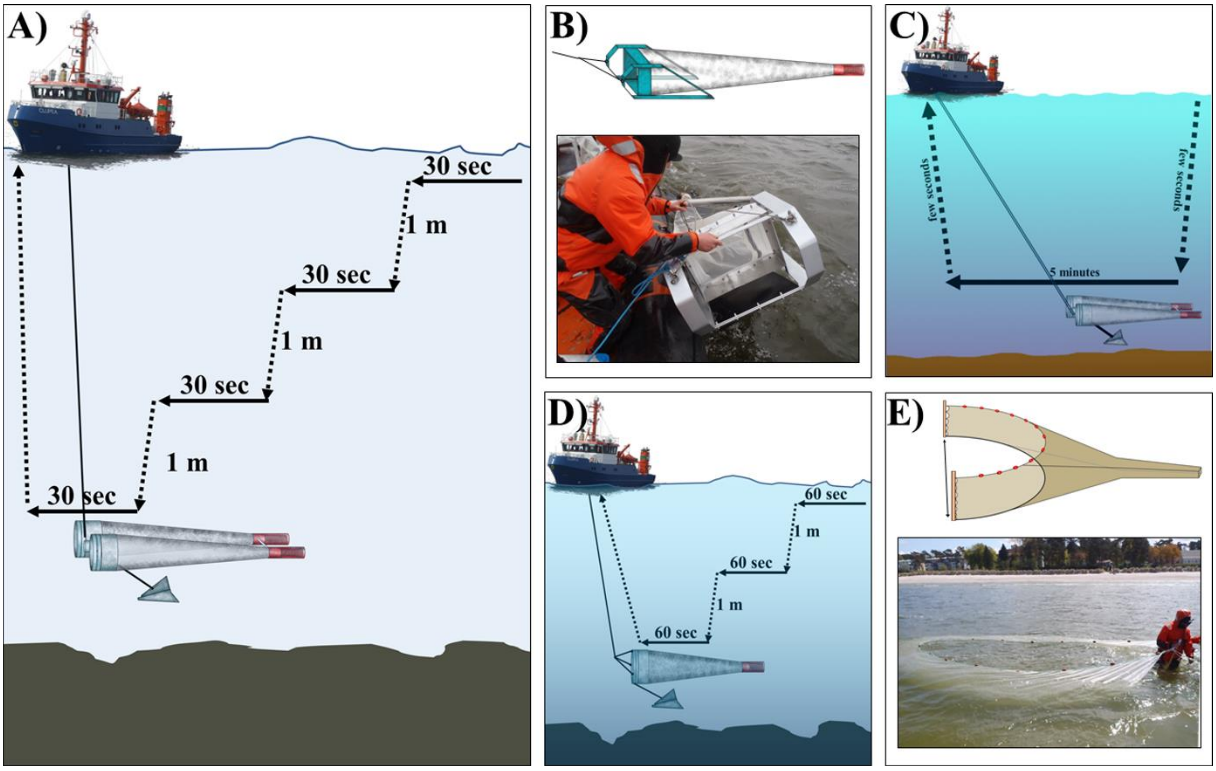


Figure S2. Overview of sampling procedures applied in the study. **A)** Schematic illustration of the standard sampling procedure of the RHLS sampling. At each station an oblique haul of the Bongo net is conducted down to 1 meter above the sea bottom. At each 1 meter step, the net is being towed horizontally for 30 seconds before it is lowered down for another meter. **B)** Modified epibenthos sledge equipped with a 500 µm Plankton net and a net opening of 56 × 40 cm (0.224m²). The cod end bucket is supplied with a 335µm mesh net. In the center of the net mouth, a mechanical flowmeter was installed to estimate the actual tow distance (not shown here). **C)** Schematic illustration of the sampling of herring larvae at a certain depth. This type of sampling was performed at three different depths strata with 6 replicates each resulting in 3 × 6 = 18 samples per station. **D)** Schematic illustration of the ring trawl sampling of large herring larvae in the pelagial zone of Greifswald Bay. **E)** *upper panel:* Schematic illustration of the beach seine applied to catch large larvae within the littoral zone of Greifswald Bay. *lower panel:* picture taken during the closing of the net at the end of the sampling process.

Pilot studies on herring larvae abundances revealed that stations sampled in the narrow sound connecting the bay with the Baltic Sea (“Strelasund”) and in the “Peene river” mouth in the south eastern part of the bay represent hydrologically different systems compared to Greifswald Bay with significant differences in physico-chemical conditions (Polte, unpublished). Therefore, only data from the 30 stations located within the bay were included in this study (Fig. S1).

***SIZE-SPECIFIC LARVAE DISTRIBUTION* -** The spatial distribution of each mm length group (total length = TL) of herring larvae between 5 and 34 mm was investigated using length distribution data derived from the RHLS data set. Detailed information on sample processing and larvae measurements is presented in Oeberst et al.[1]. For each mm-length group (TL) in every year, we selected the calendar week of highest mean abundance for a certain size class. Figure S3 exemplarily illustrates the selection of calendar weeks used for the year 2011. To verify the approach using maximum abundance as base line for distribution homogeneity, we conducted a similar analysis using the average abundance of the entire sampling period. Both analyses resulted in the same general pattern of spatial size-specific distribution homogeneity (Fig. S4). However, using the average-based approach resulted in an increase of zero values particularly for the more heterogeneously distributed size classes. Hence the maximum-approach was applied for further analyses.

Figure S3 also indicates, that the data set on larger larvae includes two different cohorts. To exclude cohort specific effects on our results, we compared the large larvae (25-34 mm) of both cohorts regarding their distribution homogeneity and found no significant differences (Mann-Whitney test, U=48.0; N=20; p=0.88). Hence we pooled the data of both cohorts to improve the sample size for these length groups.

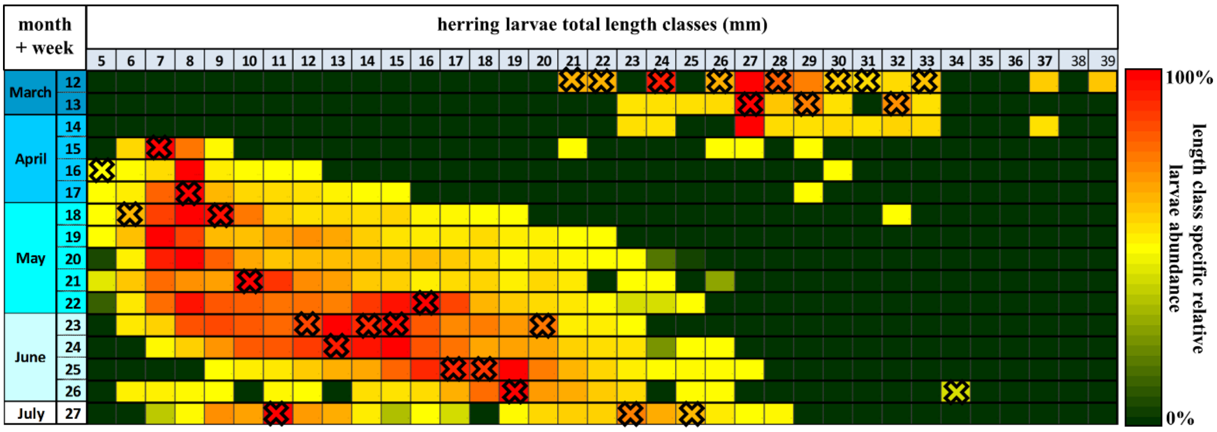


Figure S3. Illustration exemplarily showing the selection of calendar weeks in 2011 used for the analysis of spatial distribution homogeneity. For each length group, the week of highest abundance was selected (crossed fields), providing that all stations (*n*=30) have been sampled during that particular week. This selection has been done for all years available (1992-2014). The color ramp indicates the relative abundance of each herring larvae length class during all weeks.

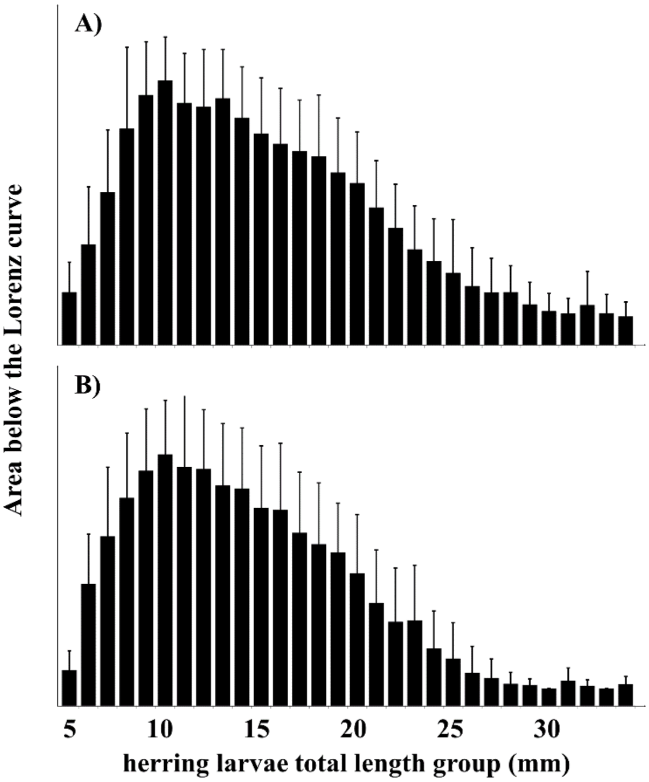


Figure S4. Size-specific spatial homogeneity of herring larvae in Greifswald Bay given as mean area (bars) below the Lorenz curve for the years 1992-2014 (error bars = standard deviation). A) Data selection (to be included in the analyses) based on the weeks of highest abundances of each length group in every year; B) Data selection based on the weeks of mean abundances.

LITTORAL SAMPLING OF HERRING LARVAE - In 2011, a modified epibenthos sledge equipped with a 500 µm Plankton net (Fig. S2B) was used to investigate the herring larvae abundance in a shallow littoral area in Greifswald Bay known to include herring spawning beds (Fig. S1). The sampling was performed by boat in every second week between April and June. Towing distance and filtered volume estimations were performed using a mechanical flowmeter (Hydrobios) installed in the center of the net and a handheld Global Positioning System unit (Garmin Vista HcX). The sample replicates varied in numbers between the weeks because of differing weather conditions and logistic limitations ($4 \leq n \leq 18$).

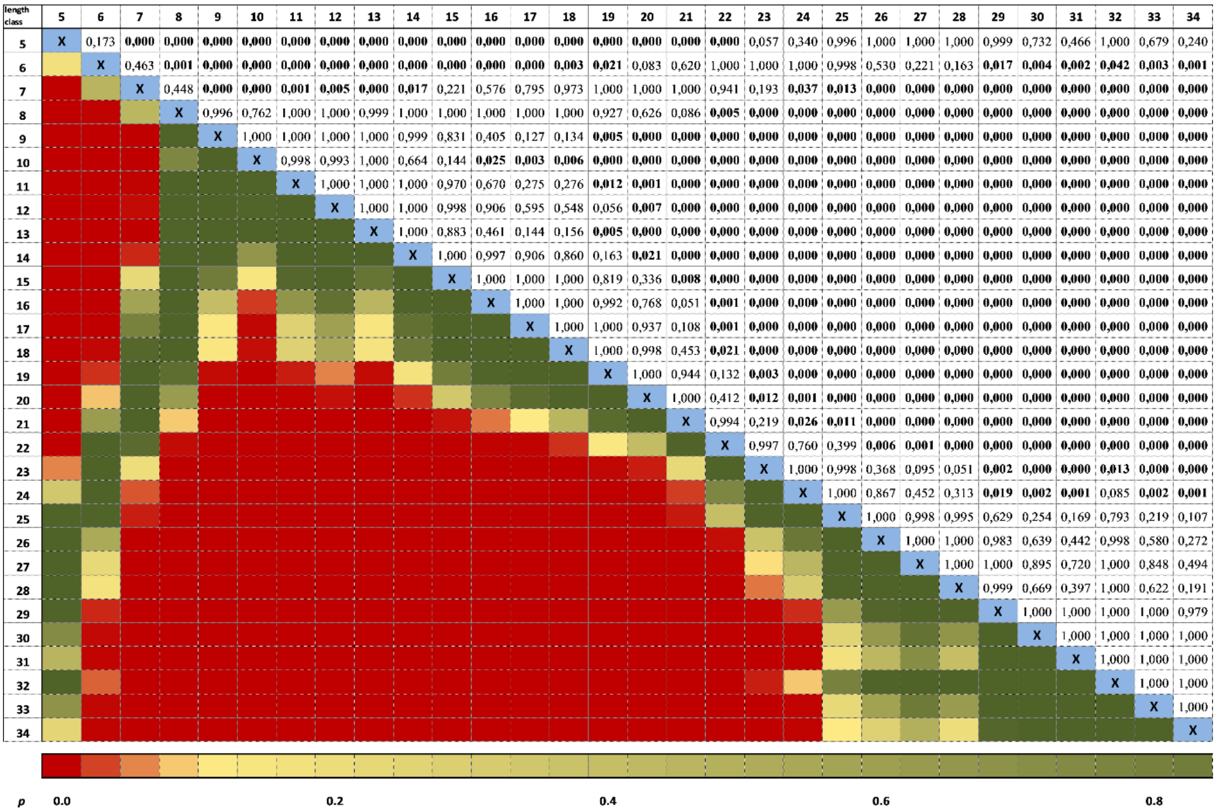
VERTICAL DISTRIBUTION OF HERRING LARVAE - In 2012, we sampled herring larvae at three different stations in the bay and the adjacent "Strelasund" (Fig. S1) in order to investigate the vertical distribution of larvae in the water column. The sampling was performed on three different depths zones on each station: 1m below the surface, 1 meter above the sea bottom and in midwater. The Plankton net was lowered down to the depth of interest and then towed horizontally for 5 minutes before it was recovered (Fig. S2C). For each depth zone, the sampling included 6 replicates, resulting in a total of 18 samples per station (6 replicates × 3 depth zones). Larval abundance was calculated per volume and then compared to each other.

SPATIAL DISTRIBUTION OF ADVANCED LARVAL STAGES - Between March and June 2015 (calendar weeks 11-22), an additional weekly sampling was conducted particularly targeting larger herring larvae. The pelagial of the bay was sampled with a ring trawl of 1 meter in diameter equipped with an ichthyoplankton net (mesh size 1.5 mm, Figure S2D). The net was towed at a speed of approximately 3 knots and for 1 minute at every meter step from the surface down to 1 meter above the sea bottom. With its larger opening and the increased towing speed compared to the Bongo net, the ring trawl is generally accepted to be more appropriate to catch larger fish larvae. Simultaneously, the littoral zone at the southern coast of the bay was sampled with a beach seine of 7 m opening and a mesh size of 5 mm (Fig. S2E). The beach seine was towed manually by two persons in a direction parallel to the shore line at a depth of approximately 1.0 meter. The towing distance was measured with a handheld GPS unit (Garmin Vista HcX). Two hauls in opposite directions were conducted at each sampling day in order to compensate for tow-direction related effects on the catch compositions (wind, waves and currents). Consequently, both catches were aggregated and treated as a single sample.

All statistical analyses were performed using SPSS 17.0 software. All post-hoc tests included a Bonferroni correction of significance levels to counteract cumulating type I error.

Table S7: CTD data of main physical variables comparing surface with bottom waters during the sampling period. Presented are mean values of water temperatures, salinity and dissolved oxygen. Mean differences between surface and bottom waters result from a pairwise analysis and the respective variability coefficients are presented.

	Mean value			Variability coefficients [%]	
	Surface	Bottom	Mean difference [%]	Surface	Bottom
Water temperatures [°C]	7.86	7.78	1.05 ± 1.49	8.75	9.08
Salinity	7.94	7.97	0.47 ± 0.78	3.59	3.73
Dissolved oxygen [%]	96.62	98.38	2.05 ± 1.51	2.05	2.79



length class	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
5	X	0,173	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	0,057	0,340	0,996	1,000	1,000	1,000	0,999	0,732	0,466	1,000	0,679	0,240
6		X	0,463	**0,001**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,003**	**0,021**	0,083	0,620	1,000	1,000	1,000	0,998	0,530	0,221	0,163	**0,017**	**0,004**	**0,002**	**0,042**	**0,003**	**0,001**
7			X	0,448	**0,000**	**0,000**	**0,001**	**0,005**	**0,000**	**0,017**	0,221	0,576	0,795	0,973	1,000	1,000	1,000	0,941	0,193	**0,037**	**0,013**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
8				X	0,996	0,762	1,000	1,000	0,999	1,000	1,000	1,000	1,000	1,000	0,927	0,626	0,086	**0,005**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
9					X	1,000	1,000	1,000	1,000	0,999	0,831	0,405	0,127	0,134	**0,005**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
10						X	0,998	0,993	1,000	0,664	0,144	**0,025**	**0,003**	**0,006**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
11							X	1,000	1,000	1,000	0,970	0,670	0,275	0,276	**0,012**	**0,001**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
12								X	1,000	1,000	0,998	0,906	0,595	0,548	0,056	**0,007**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
13									X	1,000	0,883	0,461	0,144	0,156	**0,005**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
14										X	1,000	0,997	0,906	0,860	0,163	**0,021**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
15											X	1,000	1,000	1,000	0,819	0,336	**0,008**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
16												X	1,000	1,000	0,992	0,768	0,051	**0,001**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
17													X	1,000	1,000	0,937	0,108	**0,001**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
18														X	1,000	0,998	0,453	**0,021**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
19															X	1,000	0,944	0,132	**0,003**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
20																X	1,000	0,412	**0,012**	**0,001**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
21																	X	0,994	0,219	**0,026**	**0,011**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
22																		X	0,997	0,760	0,399	**0,006**	**0,001**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**	**0,000**
23																			X	1,000	0,998	0,368	0,095	0,051	**0,002**	**0,000**	**0,000**	**0,013**	**0,000**	**0,000**
24																				X	1,000	0,867	0,452	0,313	**0,019**	**0,002**	**0,001**	0,085	**0,002**	**0,001**
25																					X	1,000	0,998	0,995	0,629	0,254	0,169	0,793	0,219	0,107
26																						X	1,000	1,000	0,983	0,639	0,442	0,998	0,580	0,272
27																							X	1,000	1,000	0,895	0,720	1,000	0,848	0,494
28																								X	0,999	0,669	0,397	1,000	0,622	0,191
29																									X	1,000	1,000	1,000	1,000	0,979
30																										X	1,000	1,000	1,000	1,000
31																											X	1,000	1,000	1,000
32																												X	1,000	1,000
33																													X	1,000
34																														X

Figure S5. Comparison of spatial distribution homogeneity of distinct herring larvae size classes (5-34 mm) for the years 1992-2014. Upper right part shows actual *p*-values of each post-hoc comparison (Games-Howell test). Lower left part represents a color ramp-based illustration of the pairwise comparisons between the different length groups.

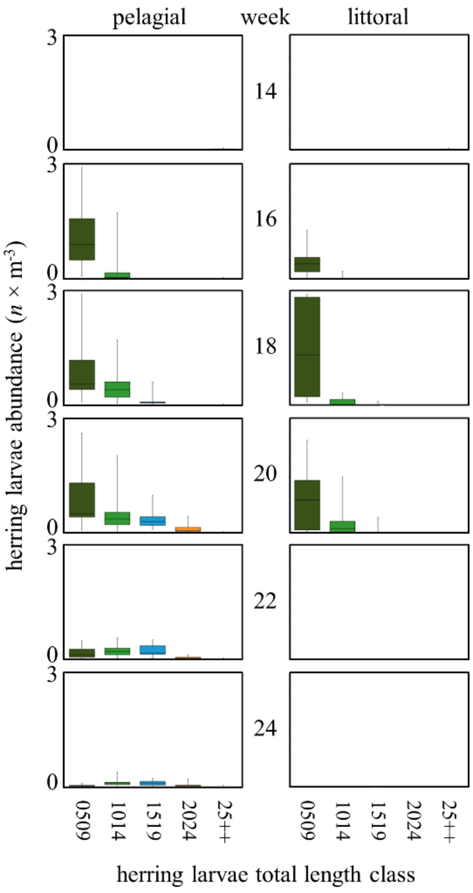


Figure S6: Abundances of distinct size classes of herring larvae in the pelagic zone of Greifswald Bay (left panel) in selected weeks of 2011 compared with larvae abundances in the littoral (right panel) during corresponding weeks. Data are shown in boxplots with boxes reaching from the 0.25 quantile to 0.75 quantile (containing the median – given as horizontal line). Whiskers represent the absolute maximum and minimum values, respectively. Total length classes: 0509 = 5-9 mm; 1014 = 10-14 mm; 1519 = 15-19 mm; 2024 = 20-24 mm; 25++ = > 25 mm total length.

Reduced recruitment of Western Baltic herring (*Clupea harengus*) as a response to warming winters

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Abstract

Shallow estuaries, bays, and lagoons are hot spots of ocean productivity. In the temperate zones, such systems often adjust rapidly to seasonal variations in atmospheric temperatures. During spring when biological reproductive processes begin, regional climate variability can be immense, and uncovering biological response, such as fish recruitment to changing temperature regimes, is a challenge.

Using herring as a paradigm for a response of coastal spring productivity to regional climate drivers, we demonstrate how the annual timing of winter onset can significantly affect reproductive success of spring-spawning herring (*Clupea harengus*) in the western Baltic Sea. We demonstrate that a temperature threshold range of 3.5–4.5 °C triggers initial spawning. Based on this, we applied model selection methods to a time-series of seasonal sea-surface temperatures and related the resulting best descriptor variables to annual reproductive success. The results reveal that a late seasonal winter onset contributed significantly to reduced larval production in a coastal nursery area and finally to a reduced abundance of juveniles in the entire distribution area. Using a combination of field research and a modeling approach, we present precedence for shifting regional winter regimes providing a present-day stressor to reproductive capacity of a central component of the coastal food web.

Introduction

There is increasing evidence that spring temperatures are rising in the temperate zones (Schwartz et al. 2006). As a result, shifts in the phenology of species in flora and fauna become increasingly obvious, especially in terrestrial ecosystems where many phenomena, such as flowering of plants or bird migration, are commonly observed (Kaspar et al. 2015, Socolar et al. 2017). On land, the immediate effect of changing climate regimes on the timing of seasonal succession and processes in nature is noticed as a present-day threat to species' reproductive success (Editorial Nature Climate Change 2018). In the marine environment, however, most attention is paid to the direct physiological effects of global ocean warming and acidification (Catalán et al. 2019). In aquatic systems, shifts in the seasonal order of natural phenomena are not as readily visible as in terrestrial systems, but have been reported from a variety of temperate oceans particularly for fish species subjected to a seasonal gradient in migration, feeding, and reproduction (Platt et al. 2003, Edwards and Richardson 2004, Rogers and Dougherty 2019). Recent suggestions that ecologists include regional climate regimes in their studies rather than relating changes in biota to average global warming (Bates et al. 2018) seem well justified considering that species phenology is often subjected to regional climate regimes.

Inshore, coastal systems are globally known as hot spots of ocean productivity and biodiversity (Cloern et al. 2014, Simcock 2017). In the temperate zones of higher latitudes, seasonal temperature regimes in shallow estuaries, bays, and lagoons fluctuate greatly owing to the effect of local air temperatures and wind mixing. The high temperature variability of transitional waters as a response to local weather regimes might obscure present effects of global ocean warming on coastal biota. However, shorter winters and steep spring temperature progressions might already affect these systems greatly, potentially influencing keystone species of coastal food webs with determined seasonal timing of reproduction processes.

Although the reproduction timing of marine fish has long been considered relatively constant (Cushing 1969), examples are increasing in temperate oceans, indicating that spawn timing is shifting with changing climate regimes (Wieland et al. 2000, Sims et al. 2004, Fincham et al. 2013). In many cases, it has not yet been clarified if those shifts in spawn timing can affect recruitment (Rogers and Dougherty 2019). The present study aims to deepen the insight into the relationship between spawn timing and recruitment success, which might be especially relevant in fish spawning all at once during a reproductive cycle (total or isochronal spawners (Hunter et al. 1985)), such as Atlantic herring (*Clupea harengus*, Linné 1758).

The search for the drivers of fish recruitment variability of ecological and economic keystone species such as Atlantic herring dates to the beginning of fishery science. Since Johan Hjort (1914) plausibly related the survival of early larval stages to the year-class strength of recruits, it has generally been accepted that larval fish mortality represents a major bottleneck in recruitment (Houde 2008, Oeberst et al. 2009a). In addition to biotic top–down and bottom–up control, the physico-chemical environment is of superior importance to larval growth and survival. The emerging awareness of climate-change-induced warming of the oceans fueled an abundance of studies of the physiological and metabolic responses of fish against rising ocean temperatures (Pörtner and Knust 2007, Pörtner and Peck 2010). According to former studies of larval herring physiology, *C. harengus* is considered relatively temperature tolerant and does not classify as exceptionally vulnerable to direct effects of climate change as they are predicted for the current century (Blaxter 1992, Peck et al. 2012). In addition to the direct temperature effect on the metabolism of the individual, changes in the temperature regime might greatly affect the reproduction phenology on the population level and, therefore, the match of susceptible early life stages with ambient environmental conditions.

Western Baltic spring-spawning herring is considered a meta-population of *Clupea harengus* (Bekkevold et al. 2005), migrating extensively from summer feeding grounds in the North Sea to constrained overwintering areas in the western Baltic Sea (i.e. Øresund, DK, SWE). In spring, they enter the inshore estuaries, bays, lagoons, and even artificial canals along the west and south coasts of the Baltic Sea for spawning. In late winter and early spring, herring schools aggregate in front of the inlets to inshore spawning grounds prior to spawning. Such aggregations are harvested by an industrial trawl fishery, while fish entering the shallow spawning ground are targeted by an artisanal gillnet fishery. For the early herring life stages those shallow systems are important nursery areas because they pass through the entire early life history in the area before they metamorphose and relocate to shore zone habitats of the outer coastal zone (Polte et al. 2017). As particular nurseries and inherent spawning grounds often make a greater-than-average contribution to the adult populations, local impacts can be readily transferred to the large-scale population level (Beck et al. 2001). Therefore, shallow coastal fish-nursery areas may provide suitable model systems to identify mechanisms and ecological pathways of climate effects from the very early life history at the inner coast until recruitment to the population in the ocean.

Using herring as a paradigm for the effect of regional climate on coastal productivity, we investigated the sensitivity of Atlantic herring reproduction in the Baltic Sea against regional winter characteristics. In contrast to relating global climate indices to fish recruitment dynamics (Cardinale et al. 2009, Gröger et al. 2014, Polte et al. 2014), the novel approach of this study was to identify an empirical temperature threshold for the onset of spawning that was then used to carefully dissect winter temperature patterns according to explanatory power for recruitment. By combining systematic ecological observation with an information-theory-based selection of regional winter descriptors, we addressed the following hypotheses: (i) initial spawning is subjected to regional temperature thresholds, and (ii) those thresholds, if applied to regional winter regimes, can explain a significant part of recruitment dynamics. Additionally, the stock recruitment relationship was investigated to evaluate potential impacts of spawning-stock biomass (SSB) variability on our results.

Material and Methods

Study system

Already in the 1930s Greifswald Bay, a shallow lagoon near the German island of Rügen was documented as a major spawning area for western Baltic spring-spawning herring (Biester 1989). The bay covers an area of 514 km^2, and the average water depth is 5.8 m with a maximum of 13.6 m (Reinicke 1989). Currents are predominantly wind-driven because tidal amplitudes are marginal (<10 cm, semi-diurnal). The annual mean salinity is 7.3 (Kell 1989). Seasonal temperature fluctuations are large, ranging from regular sub-zero °C sea-surface temperatures (SST), with a closed ice sheet in winter, to more than 20 °C SST during summer. Owing to the shallowness and frequent wind mixing, no thermoclines exist during spring, and there is no relevant difference between SST and bottom temperatures. Although the system is affected by eutrophication (Munkes 2005), extensive mixing by windforcing results in dissolved oxygen content close to 100% at the seabed.

Because the entire early herring life history, from spawning to advanced larval stages (post- flexion), can be investigated in Greifswald Bay, it represents an ideal model system for *in situ* studies of survival bottlenecks (Polte et al. 2014, Polte et al. 2017).

Arrival of adult herring on spawning grounds

The herring fishery in the area targets spawning aggregations exclusively. Although the ending of annual fishing effort is driven largely by annual catch quotas, the seasonal onset of the fishery and the seasonal distribution of landings act as informative proxies for when herring will arrive in the spawning area relative to other years. The fishery is composed of trawlers taking most of the biomass in the aggregation area in front of the entrance to the shallow spawning ground, and a small artisanal gillnet fishery is conducted on the immediate spawning ground in Greifswald Bay. The onset of both fisheries is heavily limited by weather conditions; the gillnet fishery in particular is subjected to ice cover in the bay. Taking all limitations into account, we consider the relative seasonal distributions of landings a suitable proxy to evaluate potential shifts in fishing behavior as a response to seasonal changes in fish migration.

INVESTIGATING THE TEMPERATURE RANGE FOR INITIAL SPAWNING - To determine if the onset of initial spawning is related to a particular temperature range (initial spawning range, ISR), we monitored defined transects located parallel with the south shoreline in Greifswald Bay and documented the first occurrence of eggs. The distribution of the spawning zone was surveyed by a towed video camera transmitting real-time images of the seabed and was assigned to three transects, representing different depth zones and plant communities. Each transect consisted of six fixed sampling stations, stretching on a latitudinal axis (from west to east) of 125 m total length (distance between sampling stations: 25 m). As soon as weather and ice cover allowed for systematic sampling of benthic substrata with a van Veen grab (250–400 cm2), each transect was sampled weekly from March to late May/early June in four consecutive years (2012–2015). If conditions did not allow for systematic sampling early in the season, a visual presence–absence control was conducted to make sure no spawning occurred before the transect sampling started. Herring eggs were counted from the grab samples and raised to mean egg concentration per square meter (Moll et al. 2018).

Biological response to regional winter characteristics

TIMING OF LARVAL HATCH - Weekly sampling of ichthyoplankton (see description of larvae index below) generally allowed a relatively exact determination of larval hatching periods. According to literature on the temperature-dependent duration of yolk consumption (Klinkhardt 1986), sampling intervals were set to prevent the yolk-sac stage from being passed between surveys; all larvae within a total length range of 5–9 mm were considered hatchlings (Polte et al. 2017, Dodson et al. 2019). To determine if the temperature regime affects the timing of larval hatch, two particular response variables focused on the earliest larvae stages (yolk-sac larvae). For this study, seasonal shifts of (i) peak hatching and (ii) analysis of hatching were investigated, based on the occurrence of maximum abundance of yolk-sac larvae and the number of days where yolk-sac larvae could be found in the system. In all, 16 years with full seasonal coverage of *in situ* data (periods 1992–1996 and 2007–2017) were included in the analysis. Previous studies indicate that early stage larvae are retained inside the Bay (Bauer et al. 2013, Polte et al. 2017). Therefore, significant export of larvae by current regimes is considered negligible.

Early life stage abundance indices

Two fishery-independent proxies for herring reproductive success in the western Baltic Sea (ICES 2018) were used in this analysis as response variables to determine the effects of the regional winter regime on western Baltic spring-spawning herring recruitment on different spatial scales.

1) LARVAE ABUNDANCE - The "N20" larval herring index is an established metric of annual herring reproduction in the major nursery areas included in the international stock assessment procedure since 2008 (ICES 2018). The rationale for the index is based on the observation that herring larvae, and so their number, strongly correlate with later juvenile stages. Based on weekly ichthyoplankton samples taken over a three-month period, the N20 larval herring index is an annual estimation of larvae reaching a total length of 20 mm, having survived major early life stage mortality. The abundance of this particular size class provided a solid estimate for the year-class strength of recruits (see Oeberst et al. 2009a, Oeberst et al. 2009b for details). The N20 larvae index time-series dates back to 1992. Weekly ichthyoplankton surveys include a grid of 35 stations in Greifswald Bay sampled from March to June (2007–2017) or from mid-April to late June (1992–2006; Fig. 1). Herring larvae were sampled using a Bongo net (diameter 60 cm, mesh size 335 µm) equipped with flowmeters (HYDRO-BIOS). In the laboratory, all herring larvae were counted and measured to the nearest millimeter total length. If the number of larvae in the samples exceeded 1000 individuals, a random subsample of 600 larvae was measured to receive a weekly length distribution.

2) JUVENILE ABUNDANCE - The German autumn acoustic survey (GERAS) is part of an international program that provides annual information on stock size of small pelagic fish in the Baltic Sea coordinated by the International Council of Exploration of the Seas (ICES). The GERAS covered the western Baltic Sea from the inlets to the North Sea to the central Baltic basin of the Arkona Sea (Fig. 1). Survey details are provided at http://www.ices.dk/community/groups/Pages/WGBIFS.aspx. The abundance index GERAS first winter ring (1-wr) includes

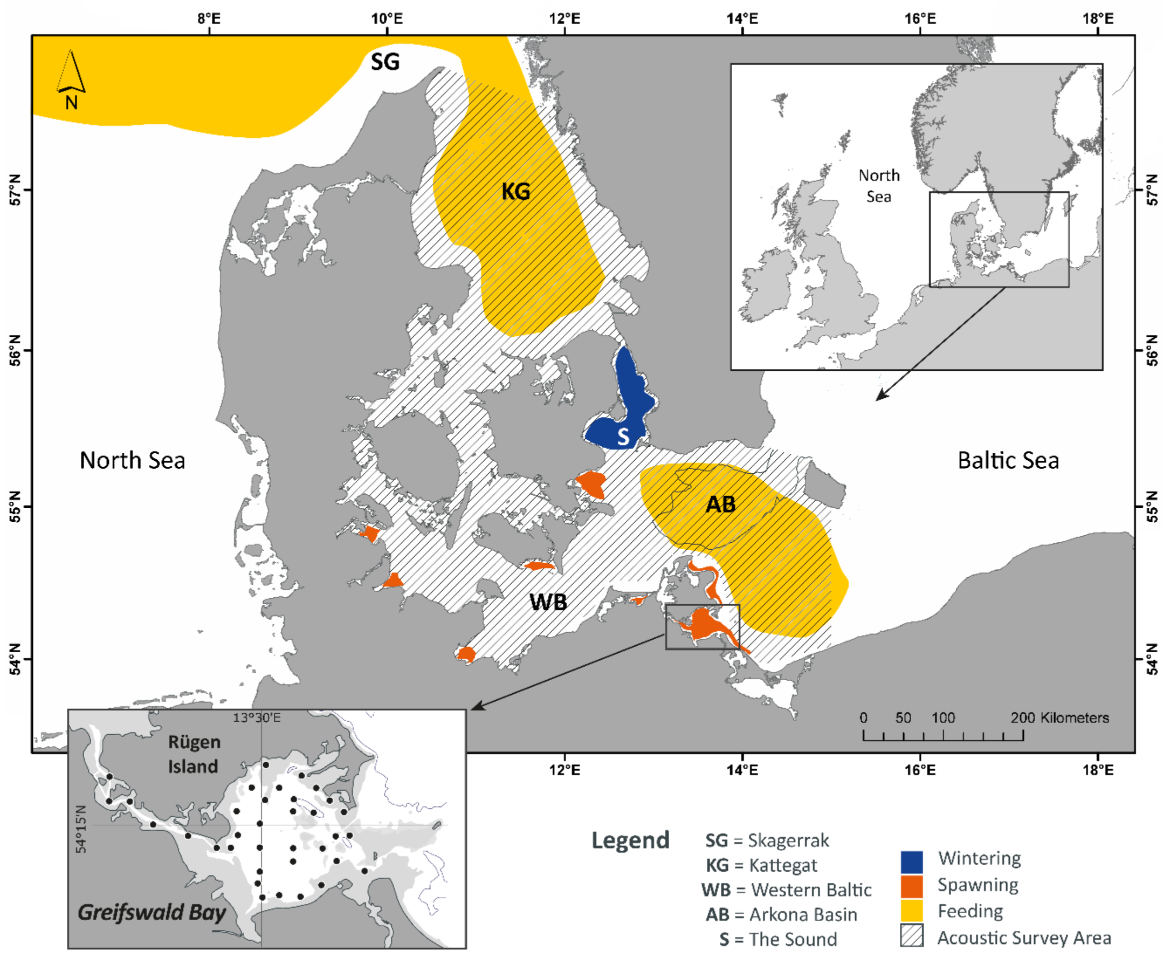

Figure 1. Study area, including the sampling area of the German acoustic survey (GERAS) in the western Baltic Sea (gray shading) and the sampling stations of the Rügen herring larvae survey (black dots) in Greifswald Bay.

1-year-old juveniles, as defined by the 1-wr microscopically detected in the otolith, as a proxy for herring recruitment in the entire distribution area. The GERAS 1-wr index follows on the larval production of the above N20 larvae index with a 1-year lag phase of fish metamorphosis and growth. Accordingly, the time-series 1993–2017 was used to determine if effects of local winter regimes, acting on the scale of a major nursery area (N20 larvae index), can be tracked to the large scale of recruitment in the western Baltic Sea.

Relationship of recruitment to spawning-stock biomass

A reduction in SSB (e.g., by overfishing) could affect the recruitment patterns studied. Therefore, the relationship of recruitment to SSB was tested by a linear regression model using the SSB data from the international stock assessment (ICES 2018), and the N20 larvae index and GERAS 1-wr juveniles as a baseline for interpretation of the model approach shown below.

Sea-surface temperature descriptors

Average daily SST data were extracted from NOAA daily Optimum Interpolation Sea Surface Temperature (OISST) with a spatial resolution of 0.25 × 0.25 degree and a temporal resolution from 1992 to 2017, available at https://www.esrl.noaa.gov/psd/data/gridded/data. noaa.oisst.v2. The original dataset was limited to those months potentially influencing herring migration, spawning, hatching, and larvae development (Polte et al. 2017). To determine the biological response to seasonal temperatures, we focused on the annual photoperiod instead of calendar periods, because the light conditions are evidently a major driver of Atlantic herring maturation processes (McPherson and Kjesbu 2012). Accordingly, the study period was set between the autumn equinox of the year *y-1* (September 22) and the first solstice of year *y* (June 21; Fig. 2). To characterize the annual variation of temperature regimes, we calculated several punctual descriptors within three different SST periods, defined as follows: The first period elapses from September 22, year *y-1*, until the day the SST falls into the *in situ* range for initial spawning ISR (see above: egg sampling). The second period starts the day the SST consistently falls below the lower limit of the ISR for seven consecutive days, and ends on the day the SST consistently ascends above the lower limit of the ISR for seven consecutive days. Finally, the third period elapses from the end of the second period until June 21 from year *y*. These three periods are referred to hereafter as prewinter, winter, and post-winter, respectively (Fig. 2). Annual SST descriptors used included: winter onset (number of days of the prewinter period), duration of the SST remaining within the ISR (3.5–4.5 °C), winter duration, and the post-winter duration. Other descriptors extracted from each period are the mean SST and standard SST deviation, the area under the SST curve (AUC) relative to the ISR or the SST slopes in prewinter and post-winter. The AUCs were calculated for each season by applying numerical integration based on the composite trapezoid rule, and the SST slopes were calculated using the Sens's method (Sen 1968). The complete list of descriptors extracted from the SST curves can be found in Table S1 in the Supporting Material section.

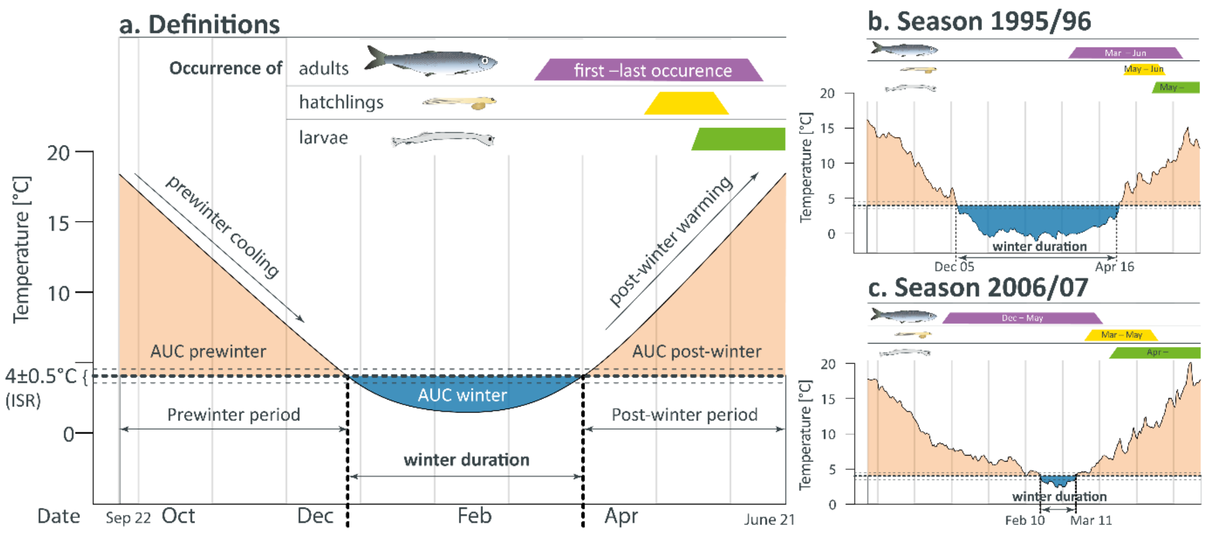

Figure 2. (a) Schematic of the best candidate winter descriptors identified by the model. Dashed horizon indicates the initial spawning range (ISR). AUC represents the area under the curve. Exact definitions of the descriptors are provided in Table 1. The particular seasonal periods are assigned to herring life stages in the system. Two opposing extreme years with differing regional SST curves are presented in **(b)** and **(c)** to illustrate the application of the concept.

***SELECTION OF SST PREDICTORS* -** The SST descriptors were analyzed for their potential as predictors in regression models to determine the influence of temperature regimes in Greifswald Bay on the local level of the nursery (N20 larvae index) and the larger spatial scale of the western Baltic Sea (GERAS 1-wr index). To determine climate effects on reproduction timing, the duration of the hatching season (hatching_DURATION) and the day of the year (counting from September 22 from year *y-1*), when the hatching peak was observed (hatching_PEAK), were included as response variables. The selection of SST descriptors to be used in the modeling section was conducted in two steps. First, an exploratory data analysis was conducted to identify significant pairwise linear correlation between the SST descriptors and the biological responses. Based on this exploration, only those descriptors demonstrating significant linear correlation were initially considered as predictors to model a given response variable *z*. In a second step, we assessed potential problems of multi-collinearity among the descriptors selected in the first step. This assessment was conducted based on the procedures described in Hair et al. (2013). Predictors indicating a variance inflation factor (VIF) greater than a value of 4.5 were directly discarded, while predictors indicating values within the range 3–4.5 were subjected to a deeper assessment to decide if they should be included in the model matrix (Hair et al. 2013). The remaining descriptors after the above two-step selection process were used to establish a full matrix to model the annual abundance indices and hatching characteristics.

MODELING THE BIOLOGICAL RESPONSE TO REGIONAL WINTER CHARACTERISTICS

The biological responses (N20 larvae index, GERAS 1-wr, hatching_DURATION, and hatching_PEAK) were modeled using generalized linear models (McCullagh and Nelder 1989),

$$g(\mu_i)=\boldsymbol{X}_i\beta \tag{1}$$

where μ_i is the expected value of the biological responses z at year i, g is a monotonic link function, $\mathbf{X_i}$ is a vector of predictors (added alone or as predictor interaction), selected after the procedure described in the previous section, and β is a vector of unknown coefficients associated with **X.** The N20 larvae and GERAS 1-wr indices were modeled using the log link function. The first modeling approach assumed a *Poisson* distribution of the residuals. In case of model overdispersion (*Deviance >>> d.o.f*), a negative binomial distribution was applied. The two hatching variables were modeled assuming a *Gaussian* distribution of the residuals.

The model matrix **X** in Equation 1 included the intercept term and a vector of descriptors of length *p*. Considering the model in Equation 1 as the full model, 2^p different submodels could be formulated leaving out one or more descriptors at a time, all of them considered potential candidates to model the variation of the biological response *z*. The candidate models were automatically ranked by a decreasing value of AICc (Hurvich and Tsai 1989), an Akaike Information Criterion (AIC) especially developed to avoid over-fitting when modeling data with small sampling sizes. The model with the lowest AICc was selected as the best candidate to describe variability of *z*.

Results

Relationship of recruitment to spawning-stock biomass

As an alternative hypothesis to the climatic effect on reproductive success, the potential impact of the SSB on the reproductive success was evaluated. However, along the 26-year time-series, no significant relationship between SSB and the N20 larvae index ($R^2 = 0.006$) or 1-year-old juveniles ($R^2 = 0.01$) was found.

Indication for herring arrival on spawning grounds

A relative indication for the seasonal timing of the initial spawning process of western Baltic spring-spawning herring can be derived by the annual onset of the trawlnet fishery on pre-spawning areas, because this fishery targets aggregations of the initial cohort of spawning fish in particular. Although the size of the fleet and number of trips per vessel remained unchanged, the fish biomass retrieved by the trawl fishery as early as January increased from

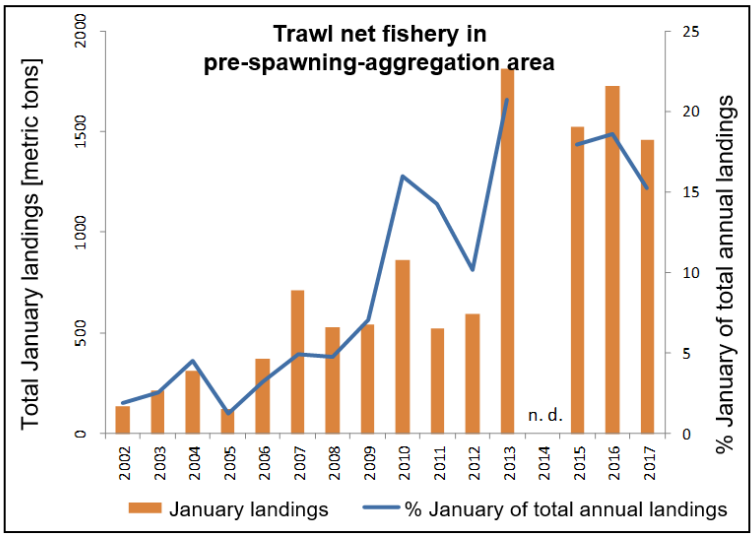

Figure 3. Metric tons of herring landings by the trawl net fishery in the pre-spawning-aggregation area (Pomeranian Bay in the vicinity of Rügen Island, ICES SD 24) during January. The increasing share (blue line) of the January landings from the total catch (at stable catch per unit effort, i.e. numbers of fishing vessels and numbers of trips per vessel) indicates earlier arrival of fish on the spawning ground.

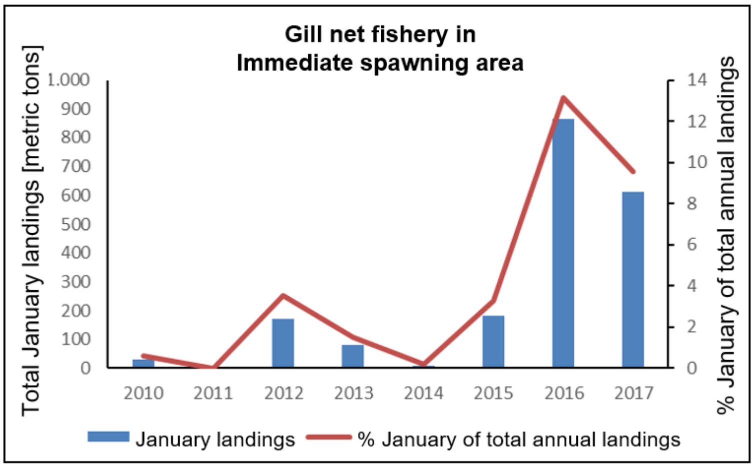

Figure 4. The artisanal gill net fishery on the immediate spawning ground of western Baltic spring spawning herring in Greifswald Bay landed an increasing share of their catch during January in recent years.

135 metric tons (2% of the total annual catch) in 2002 to 1500 metric tons (15% of the total annual catch) in 2017 (Fig. 3). Since 2012, January landings have increased sharply by >100% of the previous amount. As the gillnet fishery targets fish in full spawning condition, an earlier start of the gillnet fishery indirectly implies an earlier maturation of fish during the past decade. Similar to the shift in timing in the trawlnet fishery, the amount taken early in the season (i.e. January) has increased, particularly during the two most recent years, 2016 and 2017 (Fig. 4).

Definition of the temperature range for initial spawning

In four consecutive years (2012–2015), a particular temperature range for initial spawning (ISR) could be identified at 3.5–4.5 °C (Fig. 5). Although peak spawning in some years (e.g., 2012) occurred approximately a month later at an SST of >10 °C, during the above ISR the first eggs of the season were discovered by the systematic herring-egg survey on the spawning beds. Therefore, we used the ISR (3.5–4.5 °C) to define the three SST periods (prewinter, winter, and post-winter; Fig. 2). Owing to the weekly sampling interval, the exact day of spawning could

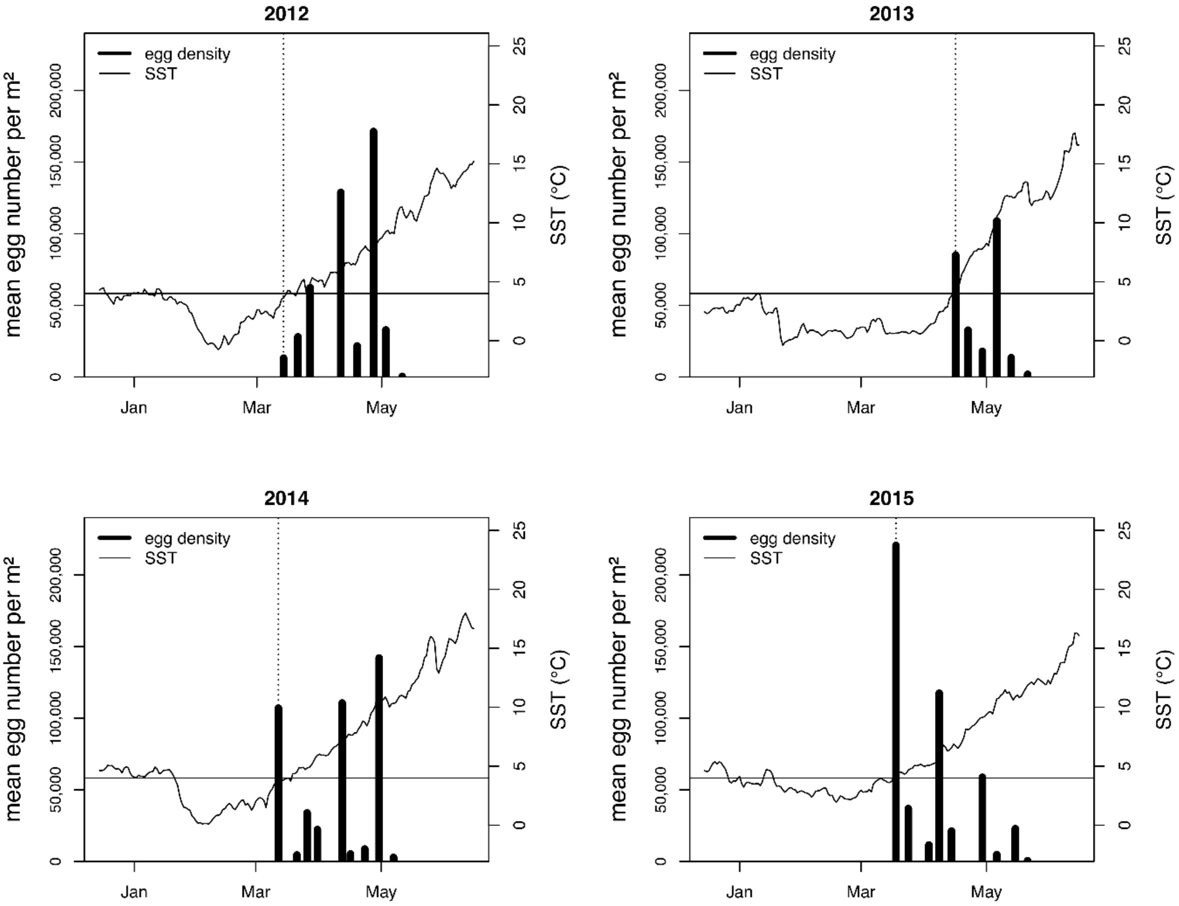

Figure 5. Mean number of herring eggs m−2 on a major spawning bed of ca. 1.5 m depth relative to SST (right y-axis). The black line indicates the 4°C literature value for initial spawning. Initial spawning was observed in 2012 at 3.6 °C, 2013 at 4.3 °C, 2014 at 3.7°C, and 2015 at 4.5°C.

not always be determined. The development stage of the eggs combined with regular controls on the spawning beds did not reveal any significant spawning underneath ice sheets or relevant spawning activity before the ISR was reached.

Response of herring productivity to particular winter descriptors

The exploratory data analysis revealed winter onset and winter duration to have the first and second strongest linear relationship with the N20 larvae index, respectively. However, both descriptors were found highly correlated ($R^2 = -0.87$). Therefore, to avoid problems with co-linearity of predictors, only winter onset was used in the subsequent modeling approach. After selection by the exploratory data analysis, the remaining descriptors were used to establish the following full model:

N20 ~ exp(winter onset * (AUC-prewinter + AUC-post-winter + prewinter slope)) (2)

The estimate of Equation 2, assuming a *Poisson* distribution, suffered from overdispersion. Therefore, a negative binomial modeling was finally applied. Based on the full model,

$2^7 = 128$ different candidate submodels were successfully estimated and ranked by decreasing AICc. The best candidate model resulted in $R^2 = 0.41$ (Table 1) and included only winter onset as SST predictor ($p < 0.001$; Table 2).

The curve predicted by the N20 model reveals that a late seasonal drop of SST below 3.5 °C (delay in winter onset) led to an average reduction in recruitment (Fig. 6a) as derived by larval herring productivity and abundance of 1-year-old juveniles 1 year later. The back transformation of the model coefficients (Table 2) revealed an expected decrease of ~3% (95% IC = 1.6–4.3%) in the N20 larvae index for each 1-day of delay in winter onset.

The same full model structure and modeling procedure applied to the N20 larvae index was used to model the GERAS 1-wr index. The best candidate model for GERAS 1-wr resulted in $R^2 = 0.44$ (Table 1) and included winter onset and AUC-post-winter as predictors. Although the effect of AUC-post-winter was found to be not significant ($p = 0.054$; Table 2), it was retained in the best candidate model. The GERAS 1-wr model predicted an expected decrease (~1.3% (0.6–2.0%)) in the abundance index for each 1-day delay in winter onset (Fig. 6b).

Table 1. Fit statistics of the best candidate models for the N20 larvae index, GERAS 1-wr, hatching_PEAK, and hatching_DURATION.

Response	Link function	Family	D0	D	*d.o.f*	R^2
N20 larvae index	log	Neg-binomial	45.48	28.18	24	**0.41**
GERAS 1-wr			46.06	26.94	22	**0.44**
hatching_PEAK	Identity	Gaussian	4023.75	2882.93	14	**0.28**
hatching_DURATION			1791.64	963.3685	14	**0.46**

Table 2. Summary of terms from the best candidate models for the N20 larvae index, GERAS 1-wr index, hatching duration, and hatching peak.

Response	Term	Coefficient	Error	z value	Pr(>|z|)
N20 larvae index	Intercept	11.5923	0.745	15.559	<0.0001***
	Winter onset	−0.03	0.0072	−4.1909	<0.0001***
GERAS 1-wr	Intercept	9.1238	0.5264	17.33	<0.0001***
	Winter onset	−0.0135	0.0037	−3.617	0.0003***
	AUC-post-winter	−0.0017	0.0009	−1.922	0.0546
DURATION_hatch	Intercept	5.5168	13.8352	0.399	0.696
	post-winter_duration	0.5155	0.1486	3.469	0.0037 **
Day_Peak_hatch	Intercept	171.5215	25.3838	6.757	<0.0001***
	AUC-post-winter	−0.1023	0.0434	−2.354	0.0337*

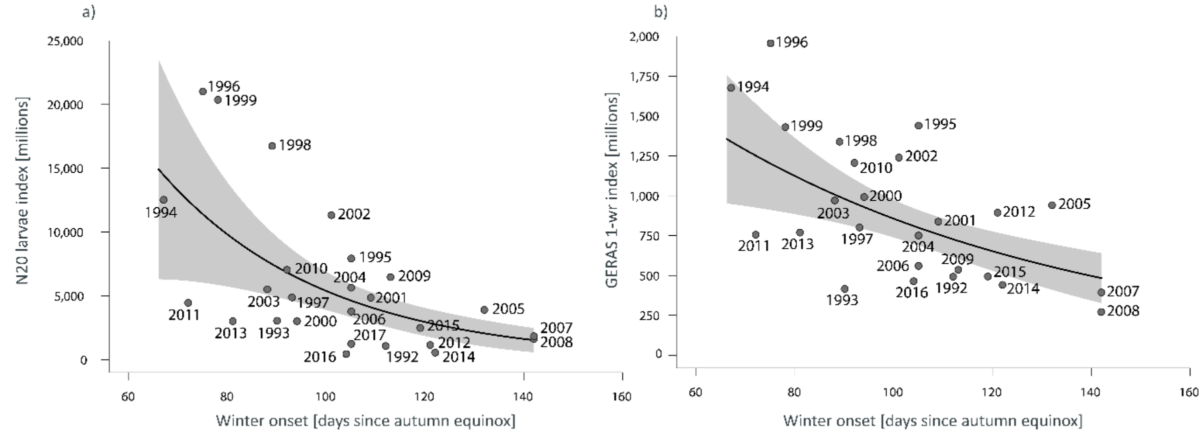

Figure 6. Predicted effect of winter onset (lines) and associated 95% CI (shadows) by the best candidate models on **(a)** the N20 larvae index in the nursery area, and **(b)** abundance of 1-year-old juveniles (GERAS 1-wr) in the western Baltic Sea. Points represent the annual estimates of the indices related to empirical values of the winter onset descriptor. In (b) the index is lagged 1 year in relation to the descriptor.

Impact of spawn and hatch timing on reproductive success

Our approach provided a full model for the timing of hatching peaks defined as:

hatching_PEAK~prewinter slope+AUC-winter+winter onset+post-winter slope +AUC-post-winter (3)

From Equation 3, 32 submodels were estimated and ranked by decreasing AICc value. The best candidate model yielded a $R^2 = 0.28$ (Table 1), and included only the AUC-post-winter ($p < 0.033$), which was found negatively related to the timing of the hatching peak (Table 2; Fig. 7a).

Although the post-winter period had no significant direct effects on the recruitment variables, it proved to affect the reproduction timing, because it was related to the seasonal setting of the hatching peaks and the duration of hatching. In detail, the AUC-post-winter was a significant descriptor of the seasonal setting of hatching peaks (Table 2). The model results indicate that an increase in the area below the seasonal temperature slope, as the integral of slope progression and the period in days until midsummer, indicate that steeper post-winter temperature curves in combination with shorter post-winter duration resulted in earlier hatching peaks (Fig. 7a).

The full model for hatching duration was established as follows:

hatch-duration~prewinter-meanT+AUC-winter +post-winter duration 8(4)

from which seven different submodels were derived and estimated. The best candidate model resulted in $R^2 = 0.46$ (Table 1), and included only post-winter duration ($p < 0.003$), positively influencing the duration of the hatching period (Table 2, Fig. 7b).

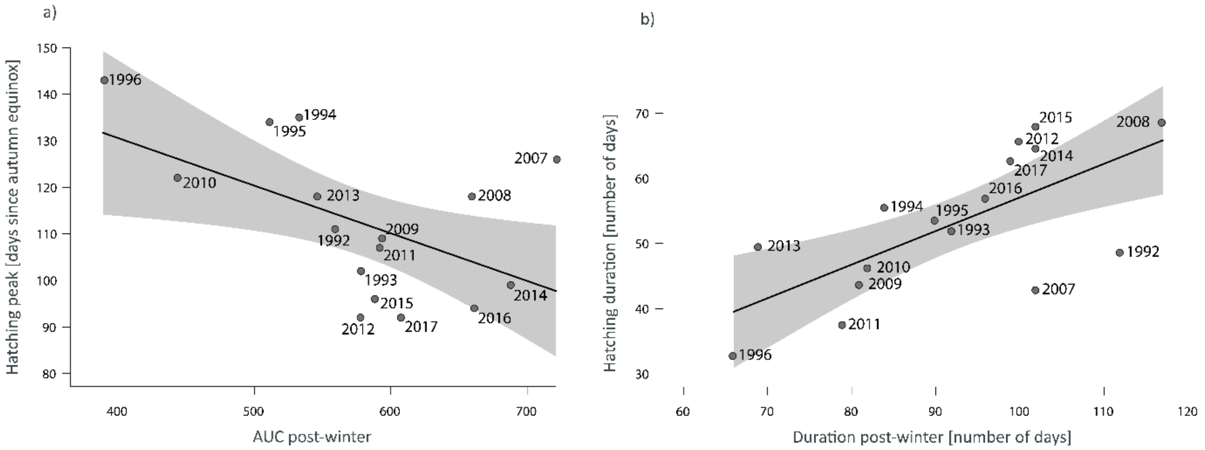

Figure 7. Predicted linear relationships (lines) and associated 95% CI (shadows) by the best candidate models on hatching responses. **(a)** Effect of AUC in the period after the increasing temperature curve crossed the initial spawning threshold range (post-winter) in the period from the autumn (9/22) equinox to the first solstice (6/21) when the major abundance of hatchlings (yolk-sac larvae) occurred. **(b)** Effect of the post-winter duration on the duration of the hatching period in days. Points represent the yearly estimates of the variables related to empirical value of the post-winter descriptors.

Using negative binomial models, we assessed potential relationships between hatching responses and the N20 larvae index. During the two periods (1992–1996 and 2007–2017) including data of the entire hatching period, the model revealed a negative correlation between the N20 larvae index (Fig. 8a) and the day in the photoperiod (September 22–June 21) where peak hatching occurred ($R^2 = 0.65$, $p < 0.0001$).

Furthermore, a significant trend indicated that hatching peaks occurred earlier along the time-series ($R^2 = 0.36$, $p = 0.01$). A direct comparison of the two investigation periods revealed a mean hatching peak at day 125 (SD ± 17.5) after the autumn equinox for the period 1992–1996, and day 106.6 (SD ± 12.8) for the past decade (2007–2017), indicating a mean of approximately 2.5 weeks of earlier occurrence. An analysis focusing on the past decade only resulted in a more pronounced significant trend toward earlier hatching ($R^2 = 0.6$, $p = 0.003$).

The post-winter duration was significantly related to the hatching duration (Table 2), demonstrating that the early onset of the post-winter period (i.e. a long post-winter period) resulted in an extension of the seasonal duration of hatching (Fig. 8b). An extended hatching duration resulted in a significantly reduced N20 larvae index ($R^2 = 0.38$, $p = 0.002$). During the two periods (1992–1996 and 2007–2017) no significant trend indicated that hatching duration became significantly extended over time ($R^2 = 0.08$, $p = 0.16$). A direct comparison of the periods revealed a mean hatching duration of 48.4 days (SD ± 9.1) for the period 1992–1996, and 55.0 (SD ± 11.4) for the past decade (2007–2017), indicating a slight extension of hatching duration of approximately 1 week.

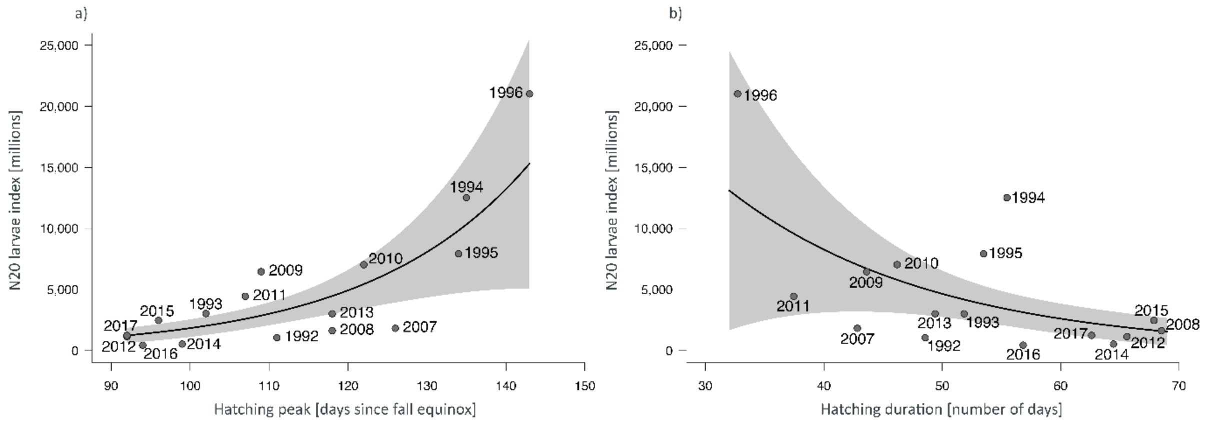

Figure 8. (a) Predicted effect of the date of the hatching peak in the period from the autumn equinox (9/22) to the first solstice (6/21) on larval herring production (N20 larvae index), and **(b)** predicted effect of the hatching duration in the same period on larval herring production (N20 larvae index). Points represent the annual estimates of the variables related to the empirical value of the post-winter descriptors.

Discussion

Earlier studies reveal a significant influence on variations in large-scale climate regimes on the reproductive success of fish, particularly on species that spawn all at once in a single sequence determined by a particular season, such as spring-spawning herring in the Baltic Sea (Cardinale et al. 2009, Gröger et al. 2014). However, the ecological mechanisms responsible for this relationship among global climate indices and local fish recruitment remained rather speculative. In general, our results now demonstrate that fish reproductive success in temperate coastal zones is subject to particular regional characteristics of seasonal temperature regimes relative to species- or population-specific temperature thresholds for reproduction phenology. These regional climate patterns are naturally not independent of broad-scale indices, such as North Atlantic Oscillation or Baltic Sea Index (Cardinale et al. 2009, Gröger et al. 2014). However, linking specific winter features to ecological (species-specific) thresholds might explain the underlying mechanisms of climate effects. A response to changing climate regimes in shallow coastal systems exposed to high atmospheric temperature variability must not necessarily manifest as a linear response to average annual temperatures, but can be uncovered by carefully splitting seasonal climate scenarios and in-depth knowledge of species reproduction ecology. In our approach, different periods during winter and spring temperature gradients were selected, covering important stages in the reproduction process and bottlenecks in the survival of early herring life stages (Fig. 2). Dissecting the SST curve along the light period from autumn equinox to first solstice into multiple variables inevitably resulted in a high level of co-linearity. In the process of selecting the descriptor variable for the model, co-linear variables were filtered out by keeping the ones with the highest correlation coefficient. This way, e.g., “winter onset,” was selected and “winter duration” abandoned, because those variables are strongly dependent. However, a late winter onset and a short winter duration have an equally negative effect on herring reproductive success. Our application of the SST range for initial spawning to define not only the winter ending but also the winter onset might appear somewhat arbitrary as a technical requirement of the winter descriptor model used. However, there is an indication that herring migration can indeed be initiated by temperature thresholds in the range 3–4 °C (Jakobsson 1969 and citations therein). Additionally, in the fishery data we observe an early (premature) arrival of herring in the area, where they aggregate before spawning. A late winter onset is therefore considered to affect the spawning phenology of the adult fish. The winter onset explained a significant amount of the variability of larval herring production in the major nursery area. This was reflected to the same degree in the abundance of 1-year-old juveniles on the larger spatial scale of the entire western Baltic Sea. The distribution of the years in the model result implicitly indicate that those relatively mild winter periods with a late winter onset occurred more frequently during the past decade. Recent estimates of the effect of climate change on the Baltic Sea identify an annual warming trend of 0.08 °C per decade, which is observed as a decrease in the number of very cold days during winter (HELCOM 2013). Although our limited and highly variable time-series does not (yet) reveal a significant linear trend of shorter winters, our results demonstrate that each day of winter delay results in a 3% reduction in the N20 larvae index and 1.3% of GERAS 1-wr.

In contrast to observations of different fish species and ecosystems (Rogers and Dougherty 2019), for herring in coastal spawning grounds, the occurrence of larval hatching peaks must not necessarily reflect the timing of spawning. For example, if early spawning resulted in increased egg mortality of initial cohorts, then a potential appearance of later hatching peaks produced by later cohorts would not reflect this phenological shift. This might explain why the timing of hatching peaks was affected by the predictor “AUC-post-winter” (summarizing: onset, duration, and progression of the warming SST curve after crossing ISR until the first solstice in summer). As spawning and the entire embryonic development are subjected to this season descriptor, hatch timing is probably a result of the combination of spawn timing and egg survival.

Earlier hatching was negatively correlated with larval production (N20 larvae index) indicating that a reduction in recruitment is mechanistically related to a phenological shift, potentially causing a temporal mismatch with adequate plankton prey. In larval fish ecology, the mismatch hypotheses, as postulated by Cushing (1975), originally assumed that the spawning time of fish remains rather constant, whereas the peaks of the annual plankton blooms might shift with the season (Cushing 1969, Durant et al. 2007). This can result in significant mismatch scenarios followed by starvation of larvae during their critical period (Hjort 1914), as e.g., documented for haddock (*Melanogrammus aeglefinus*) in Nova Scotia, Canada (Platt et al. 2003). However, the test of the match–mismatch hypothesis in the coastal herring nurseries must remain the subject of future research.

This study found the annual duration of larval hatching slightly extended with negative consequences for reproductive success. The negative effect of an elongated hatching duration is somewhat counterintuitive, because theoretically a spread of hatching over a longer period should result in increased potential to meet favorable conditions for growth and survival. This phenomenon potentially compensates for the disadvantages of premature hatching of the initial cohort by increasing the chances of hatchlings meeting favorable environmental conditions. Indeed, an increased contribution of larvae from later cohorts to overall production was observed in recent years but this occurred simultaneously with decreasing reproductive success (Polte et al. 2014). However, the extension of the hatching duration is the result of reproduction beginning earlier. In a scenario where initial hatchlings would starve as a consequence, the survivors would recruit from later cohorts, but overall productivity would potentially decrease. Rogers and Dougherty (2019) pointed out that, although the spawning of walleye pollock (*Gadus chalcogrammus*) in Alaskan waters shifted toward earlier spawning, the effects of timing on reproduction might be mitigated by fish demography. This refers to the situation where consecutive spawning is structured by multiple cohorts differing in size and age. Similarly, Baltic Sea spring-spawning herring enters the spawning grounds in successive cohorts where the initial cohort is represented by the largest, very fecund animals. However, it is not entirely clear if those size differences *de facto* represent different age groups or if they are caused by cohorts with differing growth patterns. A potential advantage enjoyed by later larvae cohorts is the chance to encounter better feeding conditions as they hatch closer to the peak of the spring plankton bloom and are experiencing elongated daylight periods promoting their success in plankton feeding (Hufnagl and Peck 2011). However, under current scenarios of steeper spring temperature gradients (Schwartz et al. 2006), later cohorts will be increasingly exposed to physiological thermal limits (Moyano et al. 2017). Additionally, we see high spring temperatures acting in synergy with high eutrophication levels on the littoral spawning beds. This synergy is already manifest in complex ecosystem cascades. A strong example is the retreat of aquatic plants, a major herring-spawning substratum, to a vertical depth limit of 3.5 m (Munkes 2005, Kanstinger et al. 2018). As a result, herring eggs are extremely exposed to storm-induced wave action, and increasing storm frequencies greatly affect egg mortality (Moll et al. 2018). Furthermore, some filamentous, epiphytic brown algae, such as *Pilayella littoralis*, can cause drastic egg mortality on herring-spawning beds (Aneer 1987) by an unknown toxic mechanism. Preliminary findings indicate that mild winter temperatures and eutrophication favor early and massive occurrence of this algae (Kiirikki and Lehvo 1997) and are able to expand the period of egg and algae overlap (L. von Nordheim, pers. observation). Cascade effects, as outlined above, might limit egg survival of later cohorts under warmer conditions. The sum of the direct and indirect stressors outlined above, together with additional top–down effects, especially by egg predation (Kotterba et al. 2014, Kotterba et al. 2017), demonstrates the diverse suite of environmental factors affecting herring reproductive success in coastal systems. Therefore, we consider the amount of 40% of the variability in larval production explained by the “winter onset” as a very strong effect. The impact is even intensified because it is transported to the population level as expressed by a similar effect on the 1-year-old juveniles on the large spatial scale. During four consecutive years with differing winter conditions, we found a consistent 4 °C temperature threshold (± 0.5 °C) for initial spawning. This is in line with anecdotal observations from the past (Klinkhardt 1996). Additionally, we found no evidence of herring spawning underneath a closed ice sheet in the bay as has often been anecdotally assumed. The identification of temperature-based spawning thresholds in our study is an important finding that might generally occur in other herring populations, although the precise temperature range might differ between populations according to latitude and season of spawning (Sinclair and Tremblay 1984). Such phenological thresholds, when identified, can provide a suitable baseline for future analysis of climate-induced response in recruitment success.

Considering the high plasticity of herring-spawning modes, the general relevance of our findings on the species level might be limited. However, the core of the subject – unearthing the present-day impact of local climate regimes on coastal productivity and economy – is underlined by the precedence we presented. Additionally, our approach might present an instrument to investigate the response of other species in temperate areas worldwide.

In this study, we found no relationship between recruitment strength and SSB over the time-series investigated. In fact, the SSB decreased drastically toward the mid-1990s before international management of the fishery was instituted (ICES 2018). After a certain transition period from 2000 to 2003, a particular period of low recruitment (<25% quantile of the time-series means) can be defined starting in 2004 and persisting until today. At this time, it can only be speculated that fishery pressure led to the stock's reduced reproductive capacity, but the unfavorable environmental conditions outlined in this study currently prevent recovery to strong year classes of recruits. Climate-induced changes in the species' phenology, including the seasonal timing of reproduction, might pose a present threat to biodiversity and sustainability of natural resources. Therefore, the mechanisms and consequences should become the focus of research. Although we cannot reverse current climate-change processes in the short term, directed coastal-zone management could mitigate the synergistic effects of shifting seasons with coastal eutrophication. To reduce the pressure on fish early life stages and maintain the functioning of nursery areas, eutrophication of coastal waters should be further restricted, and harvesting of affected fauna should be adjusted carefully under close observation of recruitment success.

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DATA AVAILABILITY - The datasets on abundance of herring larvae analyzed during the current study are available in the ICES Eggs & Larvae database available at http://www.ices.dk/marine-data/data-portals/Pages/Eggs-and-larvae.aspx. The data on the N20 larvae index, as well as the data on SSB, are available at http://www.ices.dk/community/groups/Pages/HAWG.aspx. The datasets on the GERAS 1-wr index analyzed during the current study are available at http://www.ices.dk/community/groups/Pages/WGBIFS.aspx. The datasets on fishery landings are compiled by the German Federal Office for Agriculture and Food and are available from the corresponding author upon reasonable request. The datasets on herring-egg densities on the spawning beds, as well as abundance data on the yolk-sac larvae generated in the current study, are available from the corresponding author upon reasonable request.

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GENERAL DISCUSSION

A changing world – Habitat loss and ecosystem degradation

The primary cause for the global loss of biodiversity is the massive growth of the human population and thus the increase in human demand for resources and the corresponding impacts on nature. This was stressed in textbooks as early as the 1980s (Ehrlich 1988) and became an even more prevailing topic when identifying causes of the ongoing decline and extinction of numerous species worldwide (Brooks et al. 2002, Hanski 2005, Sodhi et al. 2009, Keil et al. 2015). Nowadays, habitat degradation and habitat loss are the main threat to 85 % of all species listed in the IUCN's Red List (IUCN 2019). While approximately half of the terrestrial forests have disappeared throughout the ongoing process of deforestation, marine and especially coastal ecosystems are also heavily affected by human activities such as urbanization, industrialization and tourism (WWF 2019). One major agent for these habitat changes are the increasing unsustainable practices for food production worldwide, converting more and more pristine natural terrestrial habitats into intensively used agricultural land. Furthermore, agriculture and industry cause very high nutrient flows into aquatic ecosystems, leading to major changes of pivotal habitats. For example, for numerous fish species reports exist on the consequences of loss or degradation of coastal habitats that previously served as spawning areas and fish nurseries (e.g. Camhi 1998, Collins et al. 2000, Courrat et al. 2009, Rochette et al. 2010, Warren et al. 2010, Sundblad et al. 2014).

In order to evaluate negative consequences of habitat changes on certain species and for counteracting as early as possible with targeted mitigation measures, it is generally essential to gain knowledge about the different habitats that various life-stages of a species might inhabit and what properties they need to fulfill. Such important information can be collected for terrestrial species by monitoring the animals and their environment with adequate effort. In contrast to that, marine environments confront the observer with greater challenges. Particularly because easily only the 'tip of the iceberg' is seen or, in other words, the surface of the water body.

Especially coastal zones, lagoons and estuaries play an important role for many marine animals as they e.g. provide essential nursery habitats for numerous marine fish species (Blaber et al. 1995, Jackson et al. 2001, Beck et al. 2001, Nagelkerken et al. 2002, 2015). In order to uncover some of the often-speculated possible ecological interactions in such coastal spawning and nursery areas, an ecologically and economically important and cosmopolitan species was selected and investigated.

The focus of this work is on spring spawning Atlantic herring in the Baltic Sea, a pelagic fish species that migrates into shallow coastal areas, estuaries and lagoons for spawning (Haegele and Schweigert 1985, Aro 1989, Klinkhardt 1996). Herring are a major component of the marine food web as a link between the lower trophic levels and higher levelled predatory fish, birds, mammals (Blaxter and Hunter 1982, Kornilovs et al. 2001, Möllmann and Köster 2002, Möllmann et al. 2004). Moreover, herring are an important food resource for humans, being one of the most economically important fish species in the world (FAO 2018). At the same time, they provide an ideal case example for a species inhabiting a strongly impacted aquatic system with different environmental needs during distinct stages of their life cycle.

Habitat changes and impacts on herring reproduction

This cumulative dissertation is a summary of various studies on the habitat use of different life stages of western Baltic spring spawning (WBSS) herring and on indirect influences of changing environmental factors on the spawning substrate selection of adult fish and the survival rate of the eggs.

Herring life cycle and habitat use

Herring are pelagic swarm fishes that spend a large part of their life offshore, where they are feeding and overwintering (Haegele and Schweigert 1985, Klinkhardt 1996). However, spring spawning Atlantic herring in the Baltic Sea (as well as their Pacific counterpart *Clupea pallasii*) perform annual migrations into sheltered littoral zones for spawning (Blaxter and Holliday 1963, Haegele and Schweigert 1985, Scabell 1988, Aro 1989, Aneer 1989, Klinkhardt 1996). In the course of their life cycle, shallow coastal waters in particular play a crucial role for WBSS herring, as the eggs and several larval stages develop and grow up, respectively, in this area (manuscript V).

Herring eggs are attached to structures under water, making them most vulnerable to substrate properties and to the prevailing local environmental conditions. Once the eggs have been deposited, the adult herring continue their migration and the stationary eggs develop in the shallow littoral zone under the influence of various external stressors. Several studies were conducted to uncover direct impacts on this developmental stage, e.g. egg mortality due to unfavorable water temperatures, predation or storm events (Peck et al. 2012, Kotterba et al. 2014, 2017, Moll et al. 2018). Nevertheless, potential factors, which might indirectly influence the developmental success of herring eggs via more complex ecosystem cascades, have so far been rather neglected, and statements in this regard were mostly speculative rather than scientifically explored. This study was conducted to investigate selected aspects and cascades impacting herring survival during those life phases spent in shallow inshore waters. In the following, the major outcomes of the work will be discussed in more detail.

After hatching the larvae remain in coastal areas. This study could show that the very early larvae stay in the sheltered shallow vegetated area until they move to slightly deeper waters, when they reach a more mobile pre-flexion stage and later on return to the shallow parts again as post-flexion larvae (manuscript VI, Fig. 1). It can therefore be generally concluded that the shallow coastal waters and, in particular, the vegetated areas play a crucial role throughout the life stages of spring spawning herring in the Baltic Sea. This again underlines the importance of not only paying attention to factors that directly influence the eggs or larvae, but also to the potential consequences of ecosystem changes due to human impacts and changing environmental conditions.

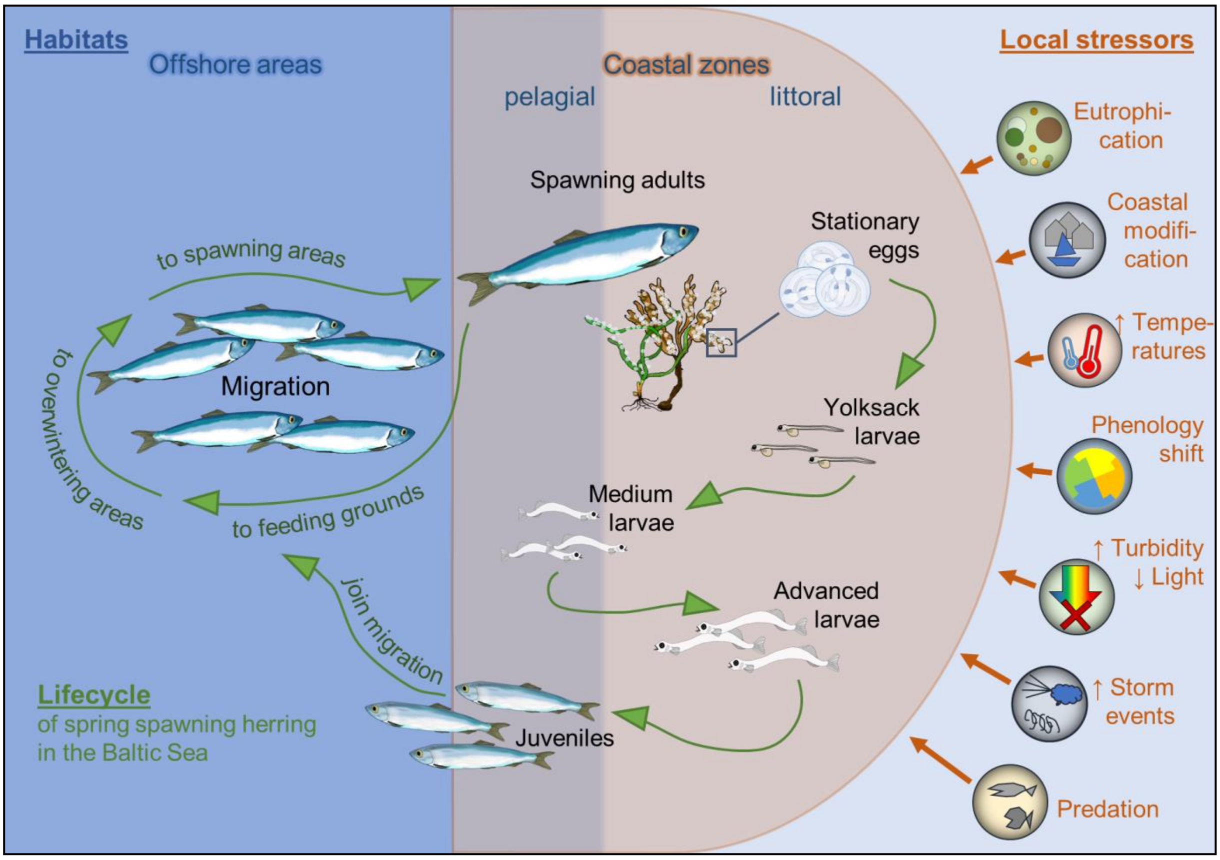
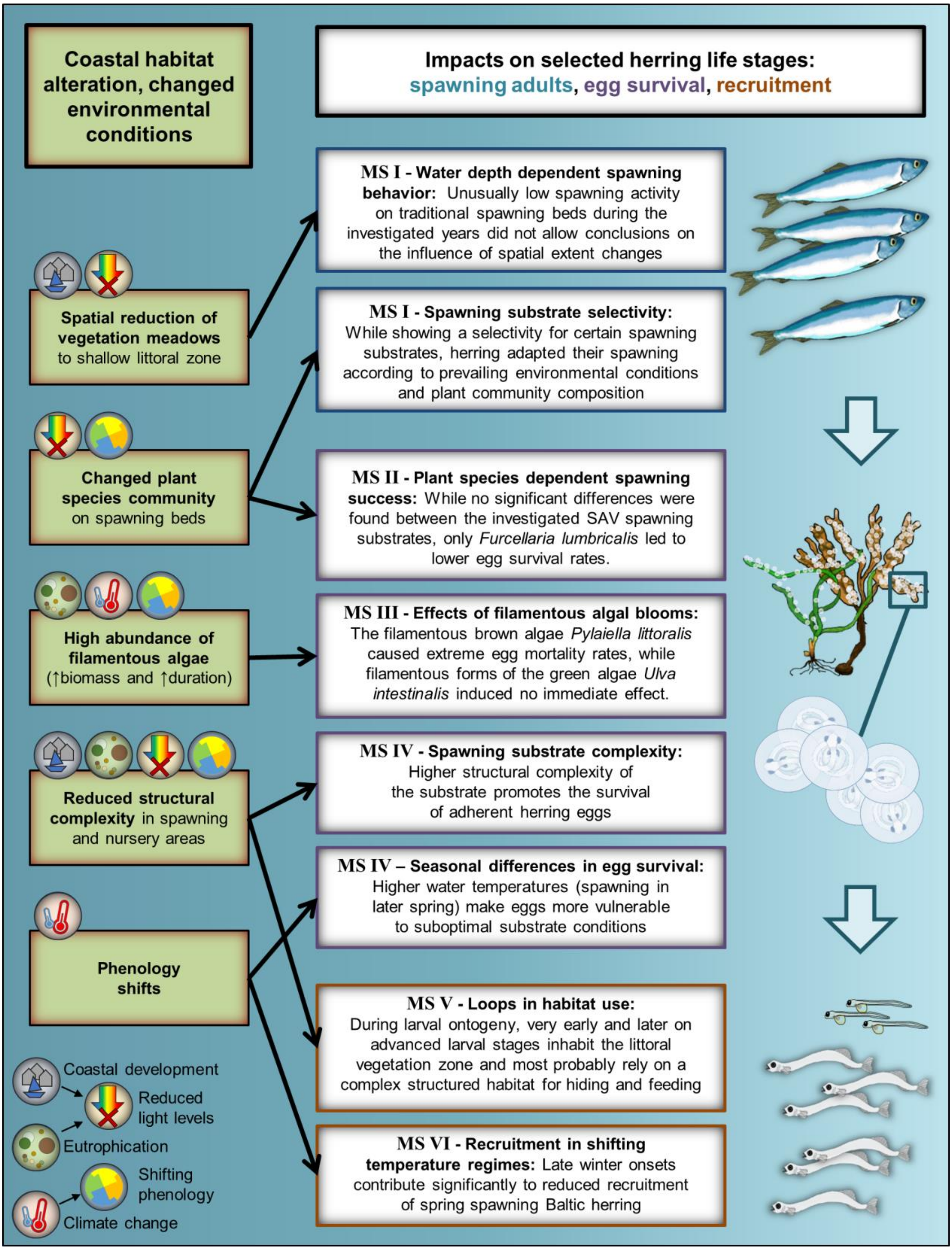


Figure 1. Herring habitat use (blue) during the lifecycle of spring spawners (green), showing the dependence of several early life stages on the shallow coastal littoral zone (manuscript V) and selected stressors (orange) that are present in this habitat, influencing herring reproduction through direct and/or indirect mechanisms.

Habitat alteration – A potential risk for herring recruitment?

For decades, coastal ecosystems suffer from anthropogenic influences, such as high inputs of nutrients from wastewater, agriculture and industry, but also from the progressing urban development at the coastlines and the corresponding coastal defense and construction measures within the waters. Those factors are known to change the marine plant species communities and their spatial coverage and hence are speculated to indirectly impact spring spawning herring recruitment by altering their nursery habitats. Studies included in this dissertation investigated selected aspects and potential mechanisms and the following relations were found (Fig. 2).



MS I - Water depth dependent spawning behavior: Unusually low spawning activity on traditional spawning beds during the investigated years did not allow conclusions on the influence of spatial extent changes

MS I - Spawning substrate selectivity: While showing a selectivity for certain spawning substrates, herring adapted their spawning according to prevailing environmental conditions and plant community composition

MS II - Plant species dependent spawning success: While no significant differences were found between the investigated SAV spawning substrates, only *Furcellaria lumbricalis* led to lower egg survival rates.

MS III - Effects of filamentous algal blooms: The filamentous brown algae *Pylaiella littoralis* caused extreme egg mortality rates, while filamentous forms of the green algae *Ulva intestinalis* induced no immediate effect.

MS IV - Spawning substrate complexity: Higher structural complexity of the substrate promotes the survival of adherent herring eggs

MS IV – Seasonal differences in egg survival: Higher water temperatures (spawning in later spring) make eggs more vulnerable to suboptimal substrate conditions

MS V - Loops in habitat use: During larval ontogeny, very early and later on advanced larval stages inhabit the littoral vegetation zone and most probably rely on a complex structured habitat for hiding and feeding

MS VI - Recruitment in shifting temperature regimes: Late winter onsets contribute significantly to reduced recruitment of spring spawning Baltic herring

Figure 2. Overview of the main findings and the environmental background of this dissertation: changes of coastal aquatic ecosystems (green) with the triggering anthropogenic stressors (circles) and their consequences for the resident fauna, using the example of spring spawning herring in the Baltic Sea. In the color framed white boxes, findings are presented for different life stages of herring (blue: spawning adults, purple: egg development, orange: larvae). For detailed illustration of the stressor cascades, see Fig. 6 in general introduction, p. 21.

REDUCTION OF SPAWNING AREAS

Decreasing macrophyte beds in number and extension entails the shrinkage of potential spawning areas for spring spawning herring. In the investigation area, a major spawning area of WBSS herring, the vegetation coverage has drastically decreased compared to the historical status. Nowadays macrophytes can hardly be found deeper than 3 meters (Kanstinger et al. 2018), resulting in a narrow vegetation belt at the coastline around the bay (Fig. 3). Such a decline of perennial macrophytes is a typical consequence of eutrophication in marine coastal waters as well as freshwater ecosystems all over the world (Kautsky et al. 1986, de Nie 1987, Duarte 1995, Cowx and Welcomme 1998, Rabalais 2002, Orth et al. 2006, Burkholder et al. 2007, Bricker et al. 2008, Waycott et al. 2009).

Herring are known to show a homing behavior and return to the same spawning areas year after year (McQuinn 1997, Wheeler and Winters 2011), most likely, regardless of local habitat changes. Hence, the reduction of suitable spawning substrate in a common spawning area would result in large numbers of herring spawning in a smaller area. The reduced availability of spawning substrate surface area could result in several layers of eggs spawned on top of each other, potentially resulting in a lack of oxygen and increasing mortality rates (Blaxter 1956, Parrish et al. 1959, Ojaveer 1981, Klinkhardt 1984, Hay 1985). However, taking into account the currently very low spawning stock biomass of WBSS herring and the low egg numbers found during the field studies (eggs spawned in patches, almost exclusively as monolayers and much vegetation without attached eggs), the phenomenon of thick egg layers probably does not currently influence the recruitment of herring from Greifswald Bay (manuscript I). However, it might well be a severe problem in areas, where the vegetation has declined more drastically or even disappeared completely.

However, in Greifswald Bay, the current distribution limit of the remaining flowering plants in the upper 3 meters of the littoral zone leads to a strong exposure to the waves of the spring storms causing high mortality rates to attached eggs because of high mechanical stress and also due to destruction and expulsion of plants. Field investigations revealed that up to 94% of the herring eggs attached to aquatic plants were transported out of the system by storms in 2012, as they were washed onto shore or disappeared into deeper waters (Moll et al. 2018).

While there is anecdotal information on herring spawning on alternative substrates such as harbor walls and wave breakers if no vegetation was available, this does not reveal anything about the developmental success of eggs thereon. Our experiments have shown that egg survival (especially in suboptimal developmental conditions) is significantly higher on structurally complex substrates (such as aquatic plants) compared to plain structures (manuscript IV).

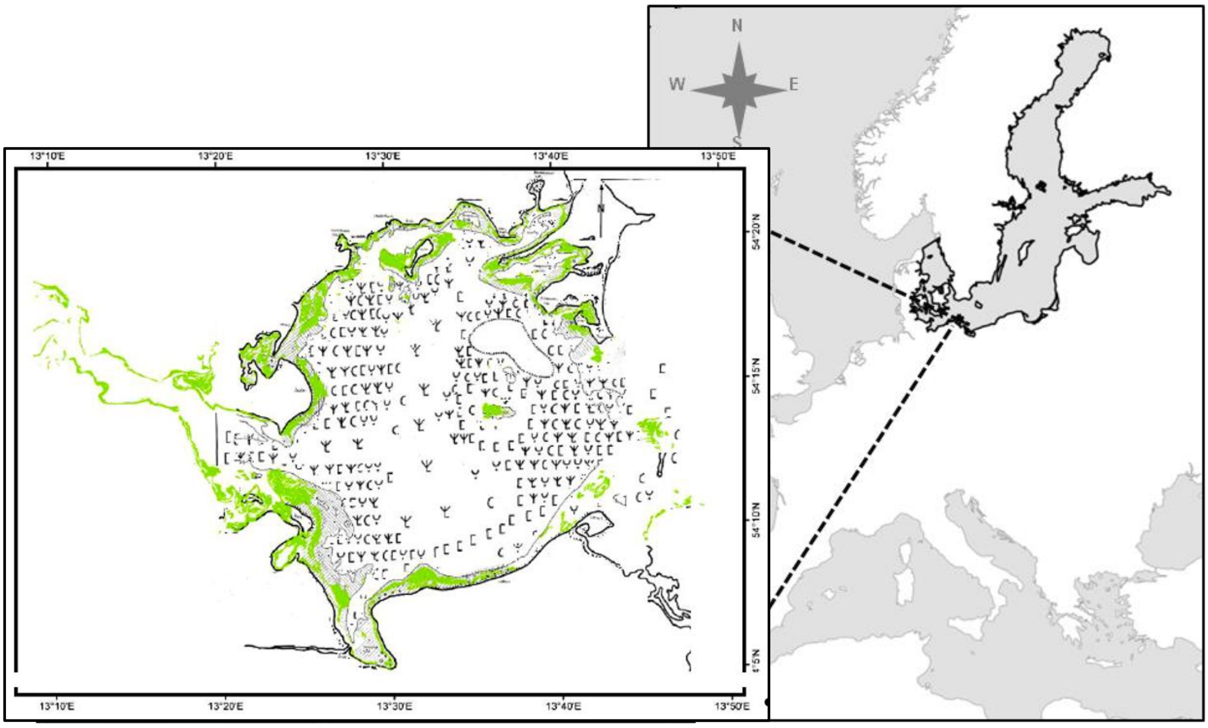


Figure 3. Decrease of submerged aquatic vegetation coverage of Greifswald Bay. Comparison of historical and actual vegetation coverage data from Subklew (1955) by dredge and bottom grab (plant symbols), from Geisel (1986) by SCUBA diving (shaded grey) and from Kanstinger et al. (2018) by analysis of aerial photographs (green). Illustration modified after Hammer et al. (2009).

Furthermore, fish and invertebrates seem to be attracted by aquatic vegetation and their abundance is often higher in vegetated habitats compared to unvegetated areas (Orth et al. 1984, Beck et al. 2001, Hyndes et al. 2003, Thiriet et al. 2014). The same seems to apply for herring, as they are reported to be especially attracted by large and dense macrophyte vegetation beds for spawning (Scabell 1988, Rajasilta et al. 1989, Aneer 1989, Kanstinger et al. 2018).

Combining the previous findings leads to the conclusion that complex vegetation beds with sufficient spatial coverage and depth distribution is an important prerequisite for maintaining key coastal spawning habitats for herring. Furthermore, an alteration of the local plant species community and available spawning substrates, respectively, might change the conditions for the embryonic development.

PLANT SPECIES SHIFT

Eutrophication and the corresponding limited light penetration cause not only a decrease of the spatial extent of the littoral vegetation beds but also a change of the species composition and habitat structure (Pérez-Ruzafa et al. 2019), Fig. 4). This was also reported for the study area Greifswald Bay (Munkes 2005, Kanstinger et al. 2018). It is very likely that species composition and distribution of vegetation in the spawning areas affect both, spawning behavior and reproductive success of herring. Until now, it was unclear, whether adult herring choose their spawning substrate selectively according to distinct plant species. However, Baltic spring spawning herring are known to spawn mainly on submerged aquatic vegetation (SAV), and there are a few earlier studies that have documented spawn to a lesser extent on stones and shells (Oulasvirta et al. 1985, Scabell 1988, Aneer 1989, Raid 1990, Šaškov et al. 2014). Hence, it was speculated that herring would use any available submerged substrate and especially each occurring plant species to attach their eggs.

However, for this study it was hypothesized that within a specific area the choice of spawning beds and spawning substrate, respectively, might be influenced by the local plant species composition. The present investigation of egg distributions after natural spawning events in the field has now shown that herring adapt their selected spawning substrates according to the existing substrates (manuscript I). Nevertheless, within the available vegetation, a preference for certain species could be observed, supporting the hypothesis that herring actively choose suitable substrates. This reveals the existence of certain requirements regarding the spawning substrate that might vary according to the prevailing weather and water conditions during the spawning process. The exact mechanisms could not be sufficiently detected during this study but should be subject of future investigations. However, especially branching and solid macrophytes (such as *Stuckenia pectinata*, *Fucus vesiculosus* and *Furcellaria lumbricalis*) were frequently used as spawning substrate, while more flexible species (such as seagrasses) were rather scarcely used throughout the period of this study (manuscript I). During visual inspections of the spawning beds, a high amount of eggs was also observed on more fragile and filamentous algae like for example *Pylaiella littoralis*, *Ulva intestinalis* or *Polysiphonia* spec., but since they grow as epiphytes on the previously mentioned macrophytes it might be considered as accidental attachment, trapping the eggs before they can stick to the solid thallus of the originally "targeted" macrophyte.

From own observations in the field and a comprehensive literature review (see manuscript I), it can be concluded that healthy perennial vegetation beds are the most important prerequisite for herring spawning areas. Although seagrass beds do not seem to represent preferred spawning substrates, they definitively play a key role in providing extensive and persistent vegetated areas and thereby in attracting herring to specific spawning beds (Zipperle et al. 2009). Regardless of the exact plant species composition, herring will enter the area and find the spawning substrate they prefer most under the current local conditions.

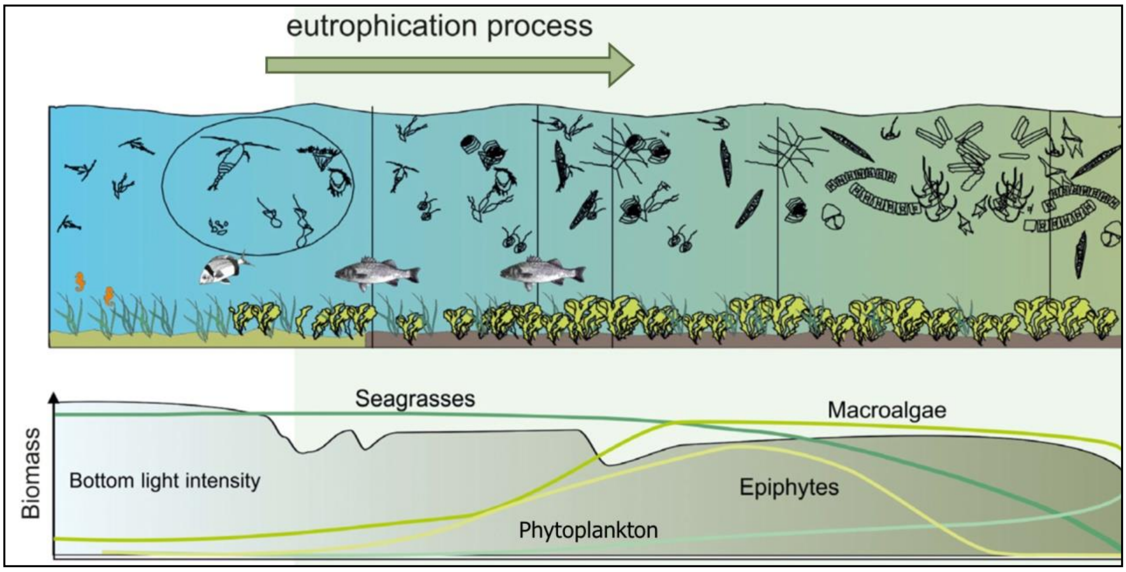


Figure 4. Evolution of the eutrophication process in aquatic habitats. Modified after Pérez-Ruzafa et al. (2019).

Further, the actual quality of different spawning substrates in terms of egg development and hatching success has been evaluated (manuscripts II-IV). Considering the survival of herring eggs attached to different plant species, field experiments revealed clear differences. While flowering plants like *Zostera marina* and *Stuckenia pectinata* and the brown algae *Fucus vesiculosus* as spawning substrates led to high egg survival rates, on the red algae *Furcellaria lumbricalis* a comparatively low egg survival was observed (manuscript II). Additionally, drastic egg mortality rates were found in association with the filamentous brown algae *Pylaiella littoralis* (manuscript III). The fact that those last two plant species have a negative effect on egg survival has already been supposed in earlier studies (Aneer 1987, Rajasilta et al. 1989, 2006) and could now be proved by the presented experiments. This is an alarming fact, as both species are rather resilient to or even benefit from poor water quality in terms of eutrophication. High nutrient levels in the water promote epiphytic fast-growing filamentous algae such as *Pylaiella littoralis,* as well as phytoplankton blooms leading to an increased turbidity and corresponding reduced light levels (Burkholder et al. 2007, Bricker et al. 2008). Furthermore, *Furcellaria lumbricalis* is one of the few macrophyte species growing down to deeper vegetation zones, probably due to their tolerance to lower light levels.

However, most macrophyte species respond to those altered environmental conditions by reduced depth distribution, lower resilience and decreasing abundance (Kautsky et al. 1986, Valiela et al. 1997, Orth et al. 2006, Waycott et al. 2009, Rabalais et al. 2009). From a long-term perspective, it seems very likely that decreasing abundance and epiphytic overgrowth of the optimal plants for spawning may have severe consequences for the herring reproduction.

MACRO ALGAL BLOOMS

Epiphytic macro algal blooms are well known phenomena in coastal areas. Those kinds of algae are promoted by eutrophication, and the impact of their massive blooms on marine and estuarine ecosystem functioning and ecosystem services is a global issue (Caddy 2007, Teichberg et al. 2012, Lyons et al. 2014). High nutrient levels crucially influence the biotic interactions in an aquatic system. In the Baltic Sea for example, the abundance of algal opportunists and epiphytes increased while the natural regulation by grazers is negatively affected (Reviewed by Korpinen et al. 2007). As mentioned above, these changes drastically affect the extension and composition of SAV meadows with cascading effects on the local fauna.

During field experiments for this dissertation, immense herring egg mortality of nearly 100% was observed on natural spawning grounds and was speculated to be linked to massive filamentous brown algal blooms in the area (Fig. 5, manuscripts II, III). This assumed lethal influence was than tested in specific field and laboratory experiments with different epiphytic algal species and could be confirmed for the filamentous brown algae *Pylaiella littoralis* (speculated by Aneer 1987, proved in manuscript III). While filamentous forms of the green algae *Ulva intestinalis* induced no immediate effect compared to the control, significantly higher egg mortalities were documented in experiments with *Pylaiella littoralis*. Actually, the egg mortality observed on natural spawning beds and in the experiments was so drastic that we would even conclude to classify a bloom of *Pylaiella littoralis* as a harmful algal bloom (HAB). HAB-criteria are met according to Anderson et al. (2012) who defined them as events causing injuries to humans, socioeconomics, or ecosystems. However, although substrate structure or a lack of oxygen could be excluded as cause of mortality, the underlying reason or mechanism is still unclear and deserves further attention in the framework of future research.

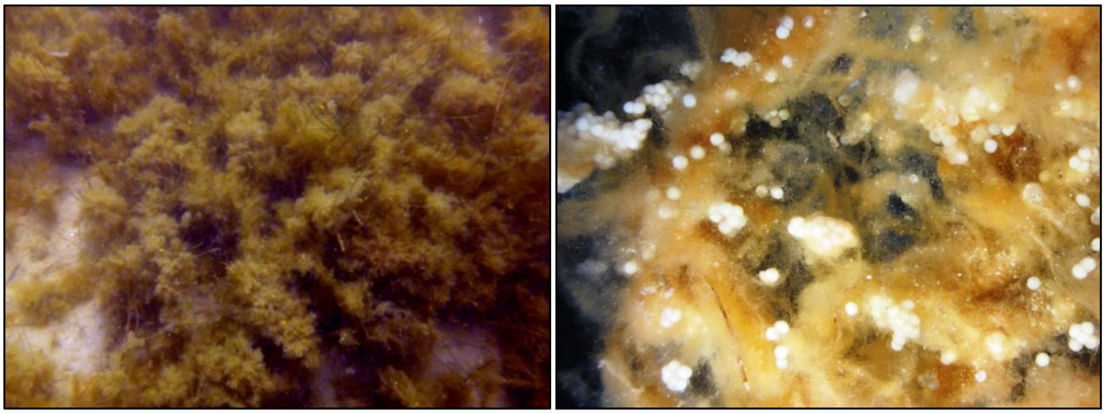
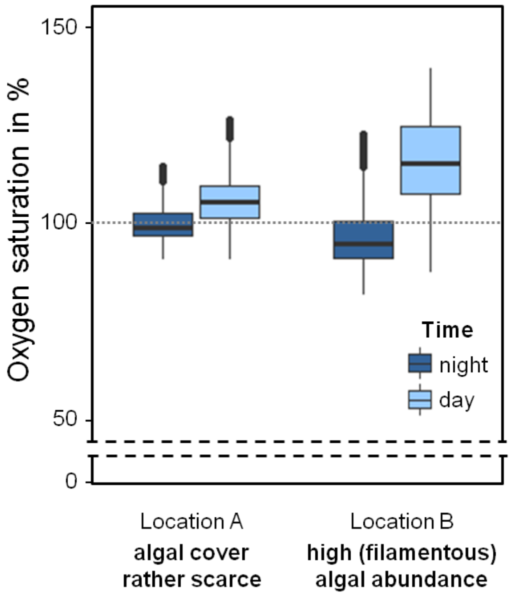


Figure 5. Images of *Pylaiella littoralis* on a herring spawning ground in Greifswald Bay: A) mass occurrence of *P. littoralis* on the macrophyte *Stuckenia pectinata* at location B (Gahlkow, April 2016) and B) dead (white) herring eggs attached to *P. littoralis*.

Figure 6. Median oxygen saturation in the water during day and nighttime in two areas (different algal abundance) during 10 days of experimental times in spring 2016 in Greifswald Bay (location A: Altkamp, location B: Gahlkow). Boxplot: in each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, whiskers show the 1.5 interpercentil range and dots represent outliers.



OXYGEN LEVELS IN SAV BEDS

From the outset of this study it was not clear whether the above described serious egg mortality was caused by a specific plant species-dependent effect, or whether it could generally be the consequence of massive algal blooms. Those blooms are known to produce high plant biomasses that often cause oxygen depletion in the water body because of algal decay or excessive nocturnal respiration (Aneer and Nellbring 1982, Valiela et al. 1997, Burkholder et al. 2007, Anderson 2009). In shallow waters, hypoxia, induced by drifting algal mats, often causes major risks for the local fauna especially for non-mobile species (Berezina 2008, Arroyo et al. 2012, Lyons et al. 2014).

In the northern Baltic Sea for example, observed egg mortality on filamentous algae was attributed to possible low oxygen levels at night time (Aneer 1985). Aneer (1987) then tested different saturation levels in the laboratory, found the eggs being tolerant to chronically reduced levels down to 25 % and concluded oxygen limitation not being a major cause for high mortality in the field. To exclude biases caused by local hypoxia, the field investigations for this dissertation were conducted with parallel on-site measurements of diurnal oxygen values on the spawning beds with and without filamentous algal blooms to assess potential oxygen limitations (Fig. 6). The recorded oxygen saturation did not fall below 80 % at any time during the experiments, suggesting that oxygen depletion was not an issue because Atlantic herring eggs are further reported to be tolerant to oxygen fluctuations of above 50 % saturation (Braum 1973). However, the saturation was only measured close to the seabed in the water column, allowing no conclusions regarding the oxygen regime on spatial micro scales (i.e. within egg clumps). This would be an interesting future investigation subject, especially with reference to different types of spawning substrates.

However, most laboratory studies on oxygen tolerance, as well as studies on tolerated temperature windows are carried out with constant test-values over the entire trial period (e.g. Blaxter 1956, Braum 1973, Aneer 1987, Peck et al. 2012). However, during this thesis recorded *in situ* data show that natural conditions are completely different with oxygen and temperature following strong diurnal fluctuations in aquatic habitats and especially in shallow waters with slow or limited water exchange (Fig. 7). The presented data allow the comparison of oxygen saturation in a shallow vegetated area (1 to 1.5 meters water depth) and a deeper unvegetated area (3 to 4 meters water depth) in about one kilometer distance clearly and reveal stronger fluctuations in the shallower area. Since spring spawning herring in the Baltic Sea attach their eggs to SAV with depth distribution limited to shallow parts of the littoral, the shallow logger data reflect the environmental conditions that those eggs naturally experience. In the respective area, day-night temperature differences up to 4 °C were detected and would very likely result in non-linear embryonic development. Therefore, the inclusion of diurnal changes of oxygen and especially of temperature values should be considered in future experimental setups in the laboratory or laboratory results should regularly be validated in the field under *in situ* conditions.

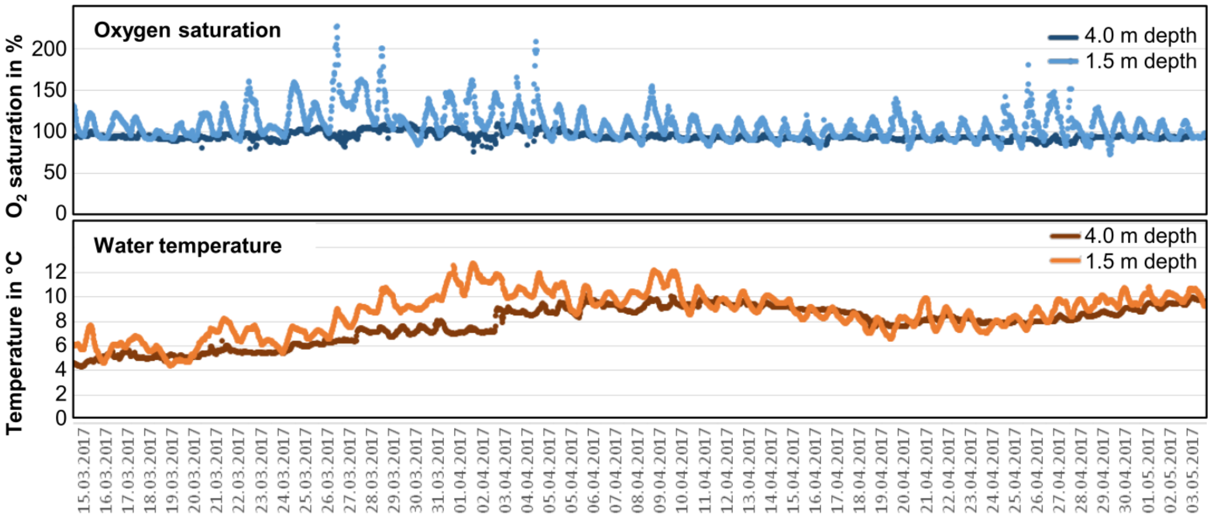


Figure 7. Diurnal oxygen saturation (top graphs) and water temperature (bottom graphs) in the unvegetated deep (4 meters) and vegetated shallow (1.5 meters) coastal zone at ‘Gahlkow’ (location B) from mid March to early May in 2017.

SEASONAL TEMPERATURE REGIME AND RECENT CHANGES

Spring temperatures in the temperate zones of the earth are rising (Schwartz et al. 2006) and shifts in the seasonal order of nature events (phenology) are observed in many phenomena, such as the flowering of plants or the migration of birds in terrestrial ecosystems (Kaspar et al. 2015, Socolar et al. 2017). The effects of climate change on the timing of seasonal succession and reproductive processes is already perceived as a threat to animal and plant populations and is therefore moving into the focus of ecological research (e.g. „Timing is everything“, Nature Climate Change 2018). Nevertheless, scientific attention is still very much in line with global climate average predictions (IPCC special report 2018), while the actual, already ongoing changes on the local scale receives too little attention (Bates et al. 2018). Such regional influences on important aquatic processes and resources are no future scenarios but highly up to date.

Especially in shallow and enclosed bay systems with limited water exchange, factors such as eutrophication and increasing water temperatures have strong and immediate impacts on diverse processes. Thus, in the following paragraph investigations on different life stages of herring will exemplarily be discussed in the light of indirect temperature effects. Starting with the earliest life stage, the eggs of WBSS herring are quite tolerant to different temperatures between 5 - 17 °C (Peck et al. 2012), indicating that seasonal differences in survival rates are rather caused by direct then indirect effects of temperature. Particularly in the later spawning season, when water temperatures rise, unfavorable conditions for eggs seem to develop. The field experiments revealed high egg survival rates at the beginning of the spawning season (manuscript IV) but increasing egg mortality with higher temperatures, probably due to fungus coverage of eggs (Fig. 8, also observed by Ojaveer 1981, Aneer and Nellbring 1982, Scabell 1988) and extensive algal blooms (Fig. 5). Further experiments proved the lethal influence of the bloom forming filamentous brown algae *Pylaiella littoralis* (manuscript III) that benefits from high nutrient loads and is promoted by mild winter temperatures (Kiirikki and Lehvo 1997). The combination of both factors leads to early and massive blooms, expanding the period of herring egg and algae overlap with drastic consequences (pers. observation, manuscript II). During spring season, herring enter the spawning area in several consecutive spawning waves. Although the mechanisms are not fully understood, the later groups are considered to determine the year class strength, because the observed numbers of hatched larvae correlate strongly with the overall recruitment of this population (Polte et al. 2014). Hence, it can be concluded that eggs spawned later in the season suffer from various factors that reduce their survival rates and that those eggs are at the same time very important in terms of larval survival and reproduction

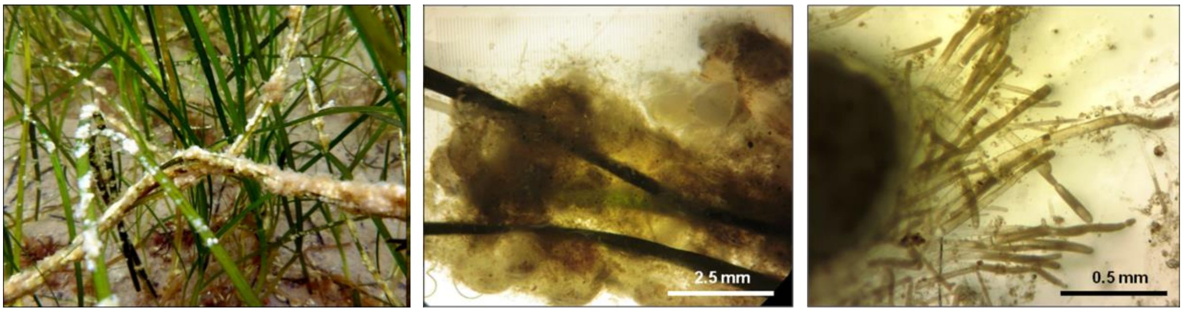


Figure 8. Pictures of fungus on herring eggs in the field (left) and eggs that got infested during experiments under the microscope (middle and right).

success, respectively. Such findings emphasize the importance of considering various interacting factors as a whole when evaluating ecosystem changes.

In addition to the effects of water temperature and related environmental conditions on the physiology and metabolism of fish egg and larval stages, a change in regional temperature regimes may have significant effects on the reproductive timing of fish populations. Preliminary findings indicated that the decreasing recruitment of the WBSS herring for about 15 years might be a result of changes in climate (Gröger et al. 2014, Dodson et al. 2019). Now, the present modeling analysis combining field research results and the time series of herring larvae monitoring data revealed that shifting winter regimes on a regional scale impact the reproduction of the whole herring population (manuscript VI). This study has shown a clear relationship between a late winter onset, a low abundance of larvae in the studied coastal nursery area (Greifswald Bay) and finally a reduced juvenile population in the entire distribution area.

Even minor seasonal shifts of developmental periods or the change of environmental conditions during certain stages of development can have extreme impacts on later life stages. For example, larvae hatching from later spawned eggs may experience steep temperature increases and water temperatures that exceed the physiological optimum or even their thermal limits (Arula et al. 2016, Moyano et al. 2017). In addition, shifts in phenology can lead to a mismatch of predators (such as herring larvae) and their prey (in this case zooplankton) and thus may result in an overall reduced recruitment (Edwards and Richardson 2004, Alvarez-Fernandez et al. 2015, Illing et al. 2018).

In conclusion, there are numerous stages in the course of the herring life cycle - from spawning to egg developmental success to larval survival and their development to juveniles - which may strongly be impacted by regional climate changes and where major impacts are already observed. In combination with diverse other stressors for early herring life stages, such as predation pressure or spawning habitat degradation due to eutrophication and coastal development, the observed extremely reduced reproductive success of the herring population seems to be an inevitable consequence and emphasizes the strong need for counteracting measures.

As mentioned at the beginning of this discussion, habitat loss is the major reason for the global decline of biodiversity and populations. This dramatic development is now amplified by the changes in climatic conditions (Jetz et al. 2007, Brook et al. 2008, Sodhi et al. 2009). Using herring as an example for an ecologically and economically relevant species that depends on ecologically intact coastal habitats for spawning, consequences of various stressor impacts and their amplification by climatic effects were shown. However, a further increase in knowledge about those connections and the synergistic effects is still of great importance to be able to incorporate appropriate mitigation (or at least adaption) measures into fisheries and coastal zone management (Mantyka-pringle et al. 2012).

Implications for management approaches

Understanding specific interactions within and between different ecosystems and the (synergistic) effects of omnipresent stressors is essential in order to protect the existing biodiversity as effective as possible. Furthermore, it is a prerequisite for improving environmental conditions, reaching a “good environmental status” (according to EU MSFD) and thus, a basis for enhancing the potential of buffering severe consequences of environmental changes that are definitely affecting ecosystems already and even more in future. Merging accurate knowledge about individual species and uncovering cross-species cascade effects is needed to understand the complex interrelationships in an ecosystem.

From the studies included in this theses and earlier literature it can be concluded that it is impossible to define only one major bottleneck with one associated stressor that determines the herring reproductive success from year to year. Because of high annual variability of possible stressor combinations it is a huge challenge to consider all of them as variables when trying to assess population dynamics. Nevertheless, it is essential to invest this effort. As shown in this study, some stressors do not directly affect the reproduction of herring but act indirectly via cascades. Thus, uncovering these cascades may allow a judgement of the weight of the initial stressors in holistic ecosystem analyzes. Awareness of the complexity of ecological processes should make clear that these must urgently be considered when developing and implementing sustainable management and conservation approaches.

However, it can be certainly stated that efforts in reducing each single stressor would help to maintain or even improve ecosystem functioning. Even though it may be impossible to stop current climate change processes in the short term, mitigation measures at the source level should still be enforced as soon and strict as possible. Especially in the light of cumulative effects with rising temperatures, the habitat conditions in eutrophic ecosystems must be improved by further reduction of nutrient inputs from the atmosphere, industrial and urban wastewater and agricultural land use. This again emphasizes the importance of a consistent implementation of existing regulations such as the EU Water Framework Directive and the EU Marine Strategy Framework Directive. Additionally, areas of high ecological importance, determined according to their function as local marine key habitats that are essential for ecological key stone species, should be part of future coastal zone management planning (e.g. for the establishment of specially designed protected areas and the implementation of measures supporting natural regeneration).

Outlook

While some knowledge gaps regarding factors that influence herring reproductive success were filled during this study, new research questions arose. These aspects are important in order to enable the setup of effective assessment and management measures and need to be answered in the coming years.

So, for example, it is still unclear when exactly the signal is given for gonad maturation and the start of adult WBSS herring spawning migrations. Examining the gonad maturity stages of fish along the annual migration and maturation cycle would help to answer that question. Furthermore, knowledge on herring migration timing and exact data on fish migrating into their spawning areas (e.g. by using telemetric observation methods) is needed to understand the annual influence of climatic variability and for implementing them in stock assessment. Present field studies on the spawning beds in Greifswald Bay have shown that the first spawning activity regularly starts at a water temperature of 3.5 - 4.5 °C (manuscript VI). Such a narrow temperature threshold makes the spawning timing extremely sensitive to seasonal temperature shifts and potential food mismatch situations as one consequence of changes in climate regimes and phenology. Such a mismatch affects the reproduction of predator species that depend on the availability of suitable prey during early life stages according to their requirements (Durant et al. 2007). In order to find out whether a mismatch situation appears in Greifswald Bay, the seasonal zooplankton community needs to be compared with the different food requirements in the course of larval herring development.

Furthermore, more comprehensive knowledge on the extension and location of herring spawning beds along the Baltic Sea coast is required. Although there are reports on WBSS herring spawning at the outer coastline in the Baltic Sea, so far it is completely unknown to what extent those areas might be used as spawning grounds and if this spawning is quantitatively contributing to recruitment. Eggs were found on the beach, attached to macrophytes that were washed ashore, but efforts aiming on finding herring larvae in the area remained without results. However, as the water exchange at the outer coastal areas is higher, in spring the temperatures increase moderately compared to in inner coastal waters and consequences of eutrophication seem to be less noticeable. Hence, the potential of outer coastal macrophyte beds as alternative spawning beds for herring should be evaluated by answering the following questions: Are those areas currently frequented by spawning herring on a larger scale? To what extent do inner, sheltered coastal waters differ as herring spawning areas (environmental conditions and plant community) from the Baltic Sea's outer coast? How are the spawn quality and egg survival rates on the outer coasts compared to the inner coastal waters? Is there sufficient food (quantity and quality) available for the larvae on the outer coasts? Do outer coast seagrass meadows (or other macrophyte beds) form important nurseries for the post-larval juvenile fish? And finally, as not much information is available on the spawning location and the ecological requirements of autumn spawning herring that were reported to spawn in deeper waters on the outer Baltic coast until a dramatic decrease in the 1970s (Aneer 1985, Parmanne et al. 1994), all the above listed questions should also be answered for all seasons in which ripe and running herring can be observed in the area.

Conclusion

This thesis has added new puzzle pieces to the overall understanding of herring ecology and crucial impacts of environmental stressors thereon. Eutrophication and changing temperature regimes were identified as the major threats to herring reproductive success. Direct influences on eggs or larvae seemed rather minor under *in situ* conditions but extreme indirect impacts were detected on site, caused by complex effect cascades that lead to severe habitat alterations.

Numerous anecdotal narratives and assumptions about herring reproduction exist, postulating a non-selective choice of spawning substrates and no relevance of specific plant species for spawning. However, this thesis presents empirical evidence for the importance of healthy natural aquatic vegetation beds with high structural complexity and an extensive three-dimensional structure. Perennial plant species in general (such as seagrasses, pondweeds and perennial macro algae) are essential for providing reliable spawning habitats over years, even if the observed substrate-selective behavior of spawners followed no strictly consistent pattern. The extensive field observations and experiments for this thesis represent a good complementation to previously conducted pure laboratory experiments by not just supporting earlier hypotheses with more empirical data but also considering cumulative effects of the diverse *in situ* conditions. Severe herring egg mortality rates were experimentally attributed to extensive filamentous epiphytic brown algal blooms which, in turn, are promoted by eutrophication. In addition to the global issues with pelagic harmful algae blooms (HABs), the results reveal the previously less known harmful effect of filamentous, benthic algal blooms. Furthermore, it was demonstrated that climate induced changes in the seasonal herring reproduction timing already impact several life stages of herring, proving the consequences of shifting temperature regimes not being a future scenario anymore but a serious present stressor.

Inshore lagoons and sheltered coastal habitats provide key nursery areas and important juvenile habitats for many invertebrate and fish species but local interactions and ecological processes are still not sufficiently incorporated into conservation and management approaches (Beck et al. 2001, Nagelkerken et al. 2015, Sheaves et al. 2015). In order to maintain or restore important coastal ecosystem functions and the potential buffer capacity against changing climatic conditions, the above displayed complexity of interactions once again emphasizes the need to take rapid action against further habitat degradation as well as the importance of an adapted management for a sustainable use of resources.

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DECLARATION OF AUTHORS' CONTRIBUTIONS

TO MANUSCRIPTS WITHIN THIS THESIS:

MANUSCRIPT I - Influence of the macrophyte composition on spawning substrate selection behavior of spring spawning Atlantic herring (*Clupea harengus*) in the Baltic Sea

Lena von Nordheim, Paul Kotterba and Patrick Polte

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L.v.N. and P.K. designed and performed research. Lv.N. organized and conducted the main part of the experiments and sampling, was responsible the laboratory processing, performed the data analysis and wrote the manuscript. P.K. assisted with field work and revised the manuscript. P.P. supervised the work, contributed to the conceptual design and revised the manuscript.

MANUSCRIPT II - Developmental success of herring eggs spawned on different plant species

Lena von Nordheim, Paul Kotterba, Dorothee Moll and Patrick Polte

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L.v.N. designed the study in agreement with the Co-authors. L.v.N. performed the experiments and data analysis and wrote the manuscript. P.K. and D.M. contributed to the concept and study design, assisted with field work and revised the manuscript. P.P. supervised the work and contributed to the conceptual design and manuscript writing.

MANUSCRIPT III - Lethal effect of filamentous algal blooms on Atlantic herring (*Clupea harengus*) eggs in the Baltic Sea

Lena von Nordheim, Paul Kotterba, Dorothee Moll and Patrick Polte

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Lena von Nordheim, Paul Kotterba, Dorothee Moll and Patrick Polte

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L.v.N. desgined the study in agreement with the Co-authors, performed the experiments and data analysis and wrote the manuscript. P.K. and D.M. contributed to the concept and study design, assisted with field work and revised the manuscript. P.P. supervised the work and contributed to the conceptual design and manuscript writing.

MANUSCRIPT V - Ontogenetic loops in habitat use highlight the importance of littoral habitats for early life-stages of oceanic fishes in temperate waters.

Patrick Polte, Paul Kotterba, Dorothee Moll and Lena von Nordheim

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P.P. and P.K. designed and performed research; D.M. contributed methods and data; D.M. and L.v.N. analyzed data, participated in conceptualizing the study and revised the manuscript. P.P. and P.K. wrote the manuscript.

MANUSCRIPT VI - Reduced recruitment of Western Baltic herring (*Clupea harengus*) as a response to warming winters

Patrick Polte, Tomas Gröhsler, Paul Kotterba, Lena von Nordheim, Dorothee Moll, Juan Santos, Paco Rodriguez-Tress, Yury Zablotski and Christopher Zimmermann

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P.P. and P.K. designed and performed research; D.M. contributed data on herring-egg sampling; D.M. and L.v.N. analyzed data, participated in conceptualizing the study, and revised the manuscript. J.S. developed and performed the modeling approach; T.G. contributed data and analyses of juvenile herring sampling. P.R.-T. and Y.Z. analyzed data and contributed to the data modeling. C.Z. contributed with conceptual development and design of graphics. P.P. and J.S. wrote the manuscript.

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Hereby, I confirm the accuracy of the statements above

Lena von Nordheim	Dr. Patrick Polte
(Doctoral candidate)	(On behalf of the supervisors)

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DECLARATION ON OATH

I hereby declare upon oath that I have written the present dissertation „Effects of coastal habitat characteristics on the reproduction of Baltic herring (*Clupea harengus*)“ independently and have not used further resources and aids than those stated.

EIDESSTATTLICHE VERSICHERUNG

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift mit dem Titel: „Effects of coastal habitat characteristics on the reproduction of Baltic herring (*Clupea harengus*)“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Rostock, 05.10.2019 Lena von Nordheim

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