Key environmental factors influencing the early life history of clupeoid fishes, adding nutrients and heat

Philipp Kanstinger

Hamburg 2014

Dissertation

with the aim of achieving a doctoral degree at the Faculty of Mathematics, Informatics and Natural Sciences Department of Biology. Institute for Hydrobiology and Fisheries science. University of Hamburg

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Cover photo illustration: Pristine seagrass meadow (*Zostera marina*) and shoal of forage fish (*Sardina pilchardus*) in an oligotrophic-mesotrophic (clear water) environment (Argolic gulf, Greece). In the foreground are herring eggs (*Clupea harengus*) attached to *Z. marina* (Greifswalder Bodden, Germany).

"It is not the most intellectual of the species that survives; it is not the strongest that survives; but the species that survives is the one that is able to adapt to and to adjust best to the changing environment in which it finds itself..... so says Charles Darwin in his "Origin of Species." (Megginson 1964)."

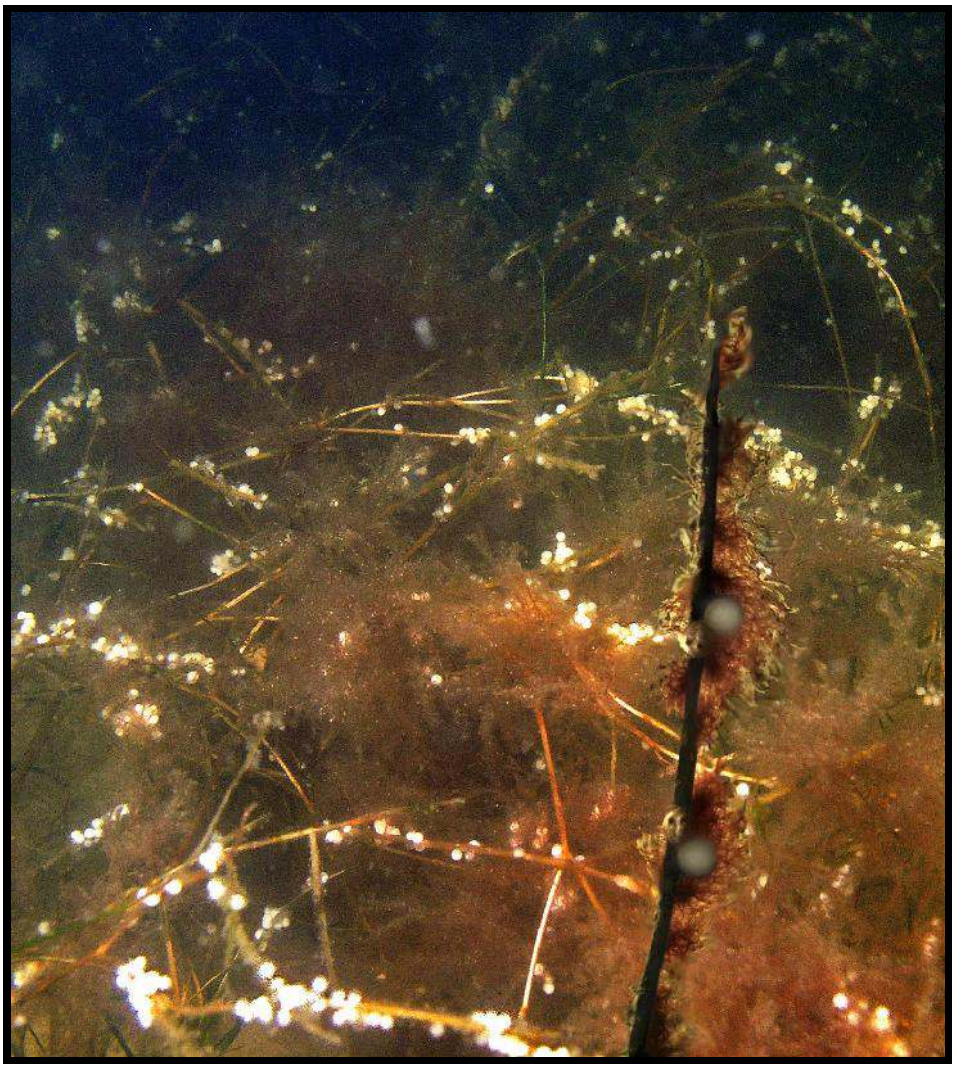


Photo illustration: Seagrass- and Pondweed-meadow (*Z. marina*; *Potamogeton pectinatus*) in an eutrophic environment (Greifswalder Bodden, Germany). Low water transparency caused by high phytoplankton biomass. A bloom of filamentous brown algae covers the spermatophytes. In the fore- and background are dead herring eggs that turned white during their degradation process.

Megginson, L. C. (1964). "Key to Competition is Management." *Petroleum Management*, 36(1): 91-95.)

Contents

Summary

The present PhD thesis focuses on the potential impacts of human-induced environmental changes on the early life history of clupeoid species in the North Sea and Baltic Sea. In addition to the more direct effects of fisheries, other anthropogenic impacts like eutrophication, habitat degradation or global warming directly and indirectly influence all six respectively four clupeoid species which inhabit the temperate waters of northern Europe. Allis shad (*Alosa alosa*), Twait shad (*Alosa fallax*), sprat (*Sprattus sprattus*), herring (*Clupea harengus*) are found in the North and Baltic Sea while European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) only occur in the North Sea and to some extent in the western Baltic Sea. It is projected that some of the anthropogenic impacts will increase in the future (e.g. warming) while others will remain at high level (e.g. habitat degradation, eutrophication) for a longer period of time. How these factors actually lead to stock changes, for example in abundance and distribution, is often not resolved in sufficient detail. The present dissertation aims to investigate the impacts of spawning habitat degradation in one of the key spawning areas of Atlantic herring in the Baltic Sea and to analyze the influence of warmer water temperatures on early life stages of this species. Additionally, the situation in the southern North Sea is investigated, where nowadays larvae of two "new" clupeoid species (anchovy and sardine) can be found within catches of sprat and herring.

The destruction of spawning habitats has already caused local extinctions of several clupeoid stocks (e.g. allies shad in the Rhine; Wadden Sea herring spring spawner) in Europe. Also the spawning grounds of Baltic Sea herring stocks are thought to be heavily impacted by eutrophication and other degradation effects. However, qualitative and quantitative studies analysing those effects are sparse and high-resolution spatial data are not available for most of the herring spawning areas in the Baltic Sea.

Chapters 2 and 3 focus on the spawning grounds within the Greifswalder Bodden, a key spawning area for the Western Baltic spring spawning stock (WBSS). By utilizing SCUBA-Diving based observations and samplings, aerial images, towed video transects, abiotic data collection, review of historic literature and laboratory experiments it is now possible to compare the historic and present situation of the spawning grounds in the bay. The study additionally identified indices suitable to predict spawning bed selection of WBSS herring and estimated egg mortality rates in different microregions throughout the 2009 spawning season. Compared to historic submerse vegetation data from the 1930´s, a 92% reduction in macrophyte coverage within the Greifswalder Bay was detected. Perennial macrophytes have become limited to shallow depths (0.2 to 3.5m) and are nowadays only found on a narrow fringe along the coastline and islands (Chapter 2). The decrease in macrophyte coverage has direct consequences for the availability of suitable spawning grounds for Atlantic herring in this region. Atlantic herring generally spawns on a variety of substrates across its wider range of distribution,. However the Baltic spring spawning stock in the Greifswalder Bodden appeared to utilize exclusively macrophytes as spawning substratum. The best spawning habitat selection model in this study was vegetation coverage within a wider radius (100m and 500m) indicating that large and dense underwater meadows are a key attraction for spawning herring shoals.

Egg mortality observed in 2009 was very high (55 to 91%) compared to older (>30 years) studies from the Baltic Sea that reported an average annual mortality between 2% and 30% (Chapter 3). Eggs transferred to the laboratory and incubated at ambient field temperatures exhibited relatively low mortality (27 to 38 %), indicating that endogenous factors were not the sole cause of the high in situ mortality. In situ mortality was significantly correlated to spawning intensity and may have been exacerbated by low oxygen conditions resulting from high egg densities and a concomitant bloom of filamentous brown algae. A positive-feedback scenario whereby habitat degradation and loss due to eutrophication (and warming) may lead to increased in situ mortality of herring eggs via density-dependent mechanisms.

In the North and Baltic Sea, sea surface temperatures are projected to increase by 2 to 3.5°C until the end of the century, if our emissions continue to rise at current rates. Shallow water areas like the Greifswalder Bodden are thought to be particularly affected by warming. To examine the potential direct effect of climate-driven warming on southwest Baltic herring, we quantified the survival, development, and biochemical condition of embryos (eggs and yolk-sac larvae) at ten tem-

peratures between 2.9 and 21.7°C (Chapter 4). Viable hatch was highest from 7 to 13°C and decreased sharply at temperatures below 3°C and above 21°C. Between 5 and 19°C, increasing temperature (T) decreased the time to 50% hatch (Ht (hrs)) according to: $Ht=4461.9*T^{-1.24}$. Using degree-days (°d=T (°C)*age (d)) could normalize some thermal effects. Most hatching occurred 90 to 120 °d post-fertilization, unfed larvae lost 0.33 µg dry mass (DM)·°d^{-1} and did not survive > 160 °d post-hatch. The nucleic acid (RNA:DNA) content of larvae were measured across all temperatures during the experiments in order to test and to calibrate these biochemical values as growth indices. RNA-DNA ratios rapidly decreased between 50 and 80°d post-hatch whereas $DNA*DM^{-1}$ increased throughout the yolk sac phase and likely provides a stronger indicator of irreversible starvation. The critical, "mixed feeding" stage appears to be 60 to 100°d post-hatch. The broad thermal tolerance of herring embryos makes "direct" negative effects of warming unlikely. However, a lack of common methods among the published studies makes it difficult to project how climate warming will affect embryos of different subpopulations. Moreover, secondary warming effects like a further degradation of spawning areas (due to temperature stress for seagrass meadows, or earlier summer blooms of filamentous algae), shifts in abundance and distribution of prey or predator organism, changes in drift patterns or low oxygen situations due to warmer water temperatures are likely to effect the various subpopulations with a different impact.

Besides the temperature dependent timing of critical periods in early larvae ontogeny, accurate parameterisations of the temperature effect on metabolism is essential to construct a reliable computer model of the life of a fish. In chapter 5 the routine respiration rates (R_R) of Baltic spring spawning herring at temperatures between 9 and 19°C for groups of larvae having mean body sizes from ~12.0 to 22.5 mm standard length (*SL*) are presented. This study is the first to measure respiration rates in herring larvae across a broad (ecologically relevant) range in water temperatures. The results demonstrate that temperature has a significant effect on the metabolic rate of Baltic herring, resulting in a Q_{10} of 1.5 for routine metabolism over the temperature range in the experiment. Larval R_R increased with increasing temperature (T) and dry body mass (DM, µg) according to:

$Log_{10}(R_R) = 0.0223+0.6557 \bullet log_{10}(DM)+0.0371 \bullet T$.

Relatively large larvae (>15 mm *SL*) exhibited diel differences in RR with daytime rates being ~twofold higher than those at night. Comparison of R_R reported here and in other studies on herring larvae highlights the need for researchers to apply standardized methods.

Changing temperatures do not only cause physiological changes on the organism-level, they also impact the population-level via changes in the balance between rates of mortality, growth and reproduction. A growing number of reports by European fishery scientists over the last 20 years demonstrate clearly that small pelagic fish populations in the shelf seas surrounding Europe are shifting their distributional borders to the North with dramatic changes in local abundance. Moreover, the fauna of the southern North Sea exhibited clear changes in the recent decades. European sardine and anchovy catches markedly increased since the 1990´s after a ~30 year absence and these clupeoids now co-occur together with sprat and herring. Consequently, little is known concerning potential interactions among these species in the region. Based upon parallel cruises conducted in the southern North Sea (Chapter 6), we compared the larval abundance, size distributions and RNA:DNA ratios of these three clupeoid species among 1) nearshore (Wadden Sea) areas and offshore (German Bight) areas that were either 2) vertically-mixed, 3) frontal zones, or 4) stratified water masses. In general terms, larval condition (RNA:DNA ratio) was relatively high at all stations. Although frontal zones clearly acted to concentrate larvae, larval conditions in those zones were not necessarily higher. For example, 15% of the sardines captured at the tidal mixing front were categorized as starving, while no starving sardine larvae were sampled in the stratified water masses. Habitats of sardine and sprat larvae were more similar to each other than to those of anchovies that were primarily restricted to nearshore areas. This is the first study examining the potential role of near- and offshore habitats as nursery areas and the extent to which resource (habitat) partitioning exists among the larvae of sprat, and the newly (re)established European anchovy and sardine stocks in the southern North Sea.

In conclusion, the results of this thesis answered

some of the open questions regarding how environmental factors influencing the early live of clupeoids. Moreover, the thesis raises also several new questions for future research. The results can be used for constructing habitat selection and individual based models. It is my hope that the results and concepts described here can be used to improve habitat selection models and individual based models in order to better predict future changes and to facilitate management responses.

Zusammenfassung

Untersuchung des Einflusses verschiedener Umweltfaktoren auf die frühen Lebensstadien heringsähnlicher Fische (Suborder Clupeoidei); die Rolle von Temperatur und Eutrophierung.

Heringsähnliche Fische sind ein integraler Bestandteil vieler mariner Ökosysteme. Sie nehmen Schlüsselpositionen im marinen Nahrungsnetz ein. Veränderungen in ihren Bestandsgrößen oder der geographischen Verbreitung haben häufig Auswirkungen auf das gesamte regionale Artengefüge. Clupeoide Fischbestände reagieren aufgrund ihrer hohen Reproduktionsfähigkeit und ihrem relativ schnellen Lebenszyklus stark auf Veränderungen in ihrer abiotischen und biotischen Umwelt, sofern diese Veränderungen während sensiblen Lebensabschnitten der Fische auftreten. Die hier vorliegende Arbeit untersucht die Auswirkung von Veränderung der Temperatur und des Eutrophierungsgrades auf die frühen Lebensstadien von clupeoiden Arten in der Nord- und Ostsee. In der Nord- und Ostsee existieren sechs bzw. vier heringsähnliche Arten; Maifisch (Alosa alosa), Finte (*Alosa fallax*), Sprotte (*Sprattus sprattus*), Hering (*Clupea harengus*), Europäische Sardine (*Sardina pilchardus*), Europäische Sardelle (*Engraulis encrasicolus*). Diese Arten durchliefen in den letzten Jahren und Jahrzehnten enorme Bestandsveränderungen, ausgelöst durch anthropogen verursachte Umweltmodifikationen. Neben der „direkten“ und teilweise existentiellen Fischereimortalität waren und sind die Bestände in der Nord- und Ostsee zusätzlich einer Reihe anderer, „indirekter“ anthropogener Einflüsse unterworfen. Hierzu gehören z.B. die Zerstörung von Laichgebieten, die Blockade von Wanderwegen, Wasserverschmutzung durch Chemikalien, Eutrophierung und die Auswirkungen der globalen Erwärmung. Die Mechanismen, wie sich diese Umweltmodifikationen auf die Größe und die Verbreitung der Clupeoidbestände auswirken, sind bis heute nicht ausreichend erforscht.

Beispielsweise sind vermutlich große Teile der Laichgebiete der Ostsee-Heringsbestände durch Umweltverschmutzungen zerstört oder stark beeinträchtigt. Es existieren bisher aber nur wenige qualitative und quantitative Studien zu diesem Thema. Für einen Großteil der Laichgebiete in der Ostsee sind keine hochauflösenden räumlichen Daten verfügbar. Das Kapitel 2 setzt sich mit den Laichplätzen im Greifswalder Bodden, einem der wichtigsten Rekrutierungsgebiete des Frühjahrslaichenden Heringbestandes in der westlichen Ostsee (WBSS) auseinander. Mithilfe von Luftbildaufnahmen, Unterwasservideos und tauchergestützten Beobachtungen konnte der gegenwärtige Zustand der Laichplätze erfasst, sowie relevante Abläufe im Heringslaichgeschehen identifiziert werden.

Obwohl Heringe bekanntermaßen auf einer Vielzahl von Substraten ablaichen, benutzt der in diesem Gebiet laichende Bestand ausschließlich Großalgen und Spermatophyten als Laichsubstrat. Insgesamt sind heutzutage etwa 7% des Greifswalder Boddens mit Unterwasserpflanzen bedeckt. Im Vergleich zum Jahr 1930 fand eine mehr als 90%ige Reduzierung der Makrophytenbestände im Bodden statt. Die Verbreitungszone mehrjähriger Pflanzen beschränkt sich aktuell auf eine sehr geringe Tiefe (0.2- 3.5m) und insgesamt bildet der submerse Pflanzenbestand nur ein relativ schmales Band entlang der Festland- und Inselküsten. Während der Studie konnten 12 Heringslaichplätze im Greifswalder Bodden lokalisiert werden. Mithilfe der erhobenen Daten wurden Modelle berechnet, um weitere geeignete Heringslaichpätze zu identifizieren. Das statistisch beste Ergebnis basiert auf der Vegetationsbedeckung in einem weiten Radius um den Laichplatz (Radius von 100m beziehungsweise 500m). Man kann folglich davon ausgehen, dass vor allem große und dichte Unterwasserwiesen Heringsschwärme zur Eiablage anziehen. Aus diesem Grund hat der extreme Rückgang an Makrophyten direkte Konsequenzen auf die Verfügbarkeit von geeigneten Laichplätzen für den Hering. Inwiefern der Verlust von Laichhabitaten und die starke Eutrophierung den Rekrutierungserfolg der Heringe beeinflussen können, wird in Kapitel 3 beschrieben.

Die beobachtete Eisterblichkeit im Greifswalder Bodden im Jahr 2009 war insgesamt sehr hoch (55 bis 91%), wenn man sie mit älteren (> 30 Jahre) Angaben aus dem Ostseeraum vergleicht (durchschnittliche jährliche Mortalität 2-30 %).

Herings-Eier, die aus dem Greifswalder Bodden in das Labor überführt und bei gleichen Temperaturen und Salinität inkubiert wurden, wiesen deutlich niedrigere Sterblichkeitsraten auf (27 bis 38 %). Daher ist ein ausschließlich endogener Sterblichkeitsfaktor (z.B. durch maternale Effekte) auszuschließen. Die Eisterblichkeitsrate auf den Laichplätzen erhöhte sich mit steigender

Intensität der Eiablage und es ist wahrscheinlich, dass viele Eier aufgrund schlechter lokaler Sauerstoffbedingungen eingegangen sind. Eine dichte Eiablage kann den Austausch von Sauerstoff im Inneren der Eiklumpen erschweren. Die zeitgleich aufgetretene Massenvermehrung an epiphytische Braun- und Grünalgen verschärft wahrscheinlich zusätzlich die schlechte lokale Sauerstoffsituation.

Es ist anzunehmen, dass der 90%ige Verlust an geeigneten Laichgebieten insgesamt die Reproduktionskapazität des lokalen Bestandes eingeschränkt hat. In Jahren mit einem hohen und zeitgleichen Aufkommen an laichreifen Elterntieren kann es zu erhöhten Laichintensitäten auf den noch vorhandenen Laichplätzen kommen. Diese Zonen mit sehr hoher Eidichte sind besonders anfällig gegenüber Sauerstoffunterversorgung und sind durch Pilz- und Bakterieninfektionen gefährdet, welche sich in den dichten Eiklumpen schnell ausbreiten können. Zusätzlich beschränken sich die vorhandenen Laichplätze auf die Flachwassergebiete des Boddens, die in besonderem Maße von Stürmen und Wellenschlag oder extremen Temperaturereignissen betroffen sein können. Durch hohe Eisterblichkeitsraten können diese Faktoren in ungünstigen Jahren zu einer starken Reduktion der Rekrutierung führen.

Flachwassergebiete wie der Greifswalder Bodden werden in Zukunft besonders stark von der globalen Erwärmung betroffen sein. Klimawissenschaftler sagen einen durchschnittlich Temperaturanstieg der Meeresoberfläche in der Nord- und Ostsee von 2-3,5°C innerhalb der nächsten 90 Jahre voraus, falls die Klimagas-Emissionen weiter mit dem derzeitigen Tempo ansteigen. Um abschätzen zu können, wie diese Erwärmung die westlichen Ostseeheringe beeinflusst, wurden bei Laborexperimenten mit Temperaturen zwischen 2,9 und 21,7°C die Überlebensraten, die Entwicklung und der biochemische Zustand von Herings-Embryonen (Eier und Dottersacklarven) untersucht (Kapitel 4). Für Temperaturen über 15°C sind bisher nur sehr wenige Beobachtungen über die Entwicklung der Eier und Larven veröffentlicht, obwohl dieser Temperaturbereich für einige Bestände (z.B. WBSS) sehr relevant ist. In den Experimenten traten die höchsten Schlupfraten zwischen 7 und 13°C auf. Aber auch in einem größeren Temperaturbereich (5°-19°C) waren die Sterblichkeitsraten relativ gering und erst bei Temperaturen von unter 3°C und über 21°C nahm die Überlebensrate der Eier sehr stark ab (20% bei 2,9°C bzw. 0% bei 21,7°C). Die Temperatur (T) hatte einen wesentlichen Einfluss auf die Entwicklungsgeschwindigkeit der Embryonen und der Zeit bis zum Schlupf (Ht, Std.). Dieser Zusammenhang kann wie folgt beschrieben werden: $Ht = 4461{,}9 * T^{-1.24}$.

Mithilfe von Gradtagen (°d = Temperatur (°C) * Alter (in Tagen d)) konnten einige beobachtete Temperatureffekte normalisiert und miteinander verglichen werden. Der Schlupf erfolgte 90 bis 120 °d nach der Befruchtung, nicht gefütterte Larven verloren daraufhin 0,33 µg Trockenmasse pro °d und verhungerten > 160 °d nach Schlupf. Die Experimente hatten auch zum Ziel, in den Fischen den Anteil an Nukleinsäuren (RNA:DNA) zu quantifizieren, um mit resultierenden Werten Entwicklungs- und Zustandsindikatoren zu kalibrieren. Die Verwendung von RNA:DNA Verhältnissen als Zustandsindikator ist bei der Feldforschung über marine Fischlarven weit verbreitet (siehe Kapitel 5). Laborexperimente, wie die hier vorgestellten, helfen die Ergebnisse der Felddaten besser interpretieren zu können. Das RNA:DNA Verhältnis in unseren Experimenten verringerte sich stark zwischen 50 und 80 °d nach Schlupf, wohingegen das DNA – Trockengewicht Verhältnis kontinuierlich anstieg. Das DNA–Trockengewicht Verhältnis scheint daher ein besserer Indikator für irreversible Hungerzustände als das RNA:DNA Verhältnis zu sein. Die kritische „mix-feeding“ Lebensphase, der Übergang zwischen endogener und exogener Ernährung, fand in unseren Versuchen zwischen 60 bis 100 °d nach dem Schlupf statt. Höhere Wassertemperaturen verringern maßgeblich das Zeitfenster zum Beutefang-Erlernen und machen die Larven anfälliger für Situationen, in denen nicht ausreichend Beuteorganismen zu Verfügung stehen.

Eine "direkte" Mortalität der Herings-Embryonen durch hohe Temperaturen in den Gewässern ist jedoch aufgrund der breiten Temperaturtoleranz unwahrscheinlich. Es ist aber anzunehmen, dass sekundäre Effekte einer Erwärmung das Potential haben, die jungen Lebensstadien von Heringen stark zu beeinflussen. Zu den sekundären Effekten gehören weitere Verluste von Laichplätzen (Absterben weiterer Seegraswiesen durch Temperaturstress, frühzeitigeres Auftreten von Braunalgenteppichen), Veränderungen der Meeresströmungen, zeitliche Veränderungen im Aufkommen der Beute- und Räuberorganismen und ein erhöhtes Risiko von Sauerstoffmangelzonen

durch schnellere Degradationsprozesse. Aufgrund der Vielzahl an lokal angepassten Hering-Subpopulationen sind aber allgemeingültige Aussagen, inwieweit Heringe durch die Klimaerwärmung beeinflusst werden, schwierig und selten sinnvoll.

Der Einsatz von Computer-Modellen ist ein viel versprechender Ansatz, um den Einfluss von Umweltveränderungen auf die Rekrutierung einzelner mariner Populationen vorherzusagen und zu quantifizieren. Für diese Modelle ist neben dem Wissen über den Einfluss von Temperatur auf die zeitlichen Abläufe der Embryogenese, auch eine genaue Parametrisierung der Temperatureffekte auf den Stoffwechsel vonnöten. In Kapitel 5 werden die Raten des Routinestoffwechsel (R_R) von ~ 12,0 bis 22,5 mm großen Heringslarven (Standardlänge SL) bei Temperaturen zwischen 9 und 19°C vorgestellt. Während dieser Studie wurden erstmals die Stoffwechselraten von Heringslarven im gesamten ökologisch relevanten Temperaturbereich gemessen.

Die Stoffwechselraten stiegen exponentiell mit der Temperatur (Q_{10} = 1.5) und der Zusammenhang zwischen Routinestoffwechsel, Temperatur, und Trockengewicht (DM, µg) konnte wie folgt beschrieben werden:

$\text{Log}_{10}(R_R) = 0{,}0223 + 0.6557 \bullet \log_{10}(\text{DM}) \; 0.0371 \bullet T$

Die Stoffwechselrate größerer Larven (> 15 mm SL) war am Tag in etwa zweifach höher als in der Nacht. Es wird davon ausgegangen, dass dies ungefähr dem Unterschied zwischen Standard- und Routinestoffwechsel entspricht. Ähnliche Werte wurden bei Experimenten mit narkotisierten Heringslarven festgestellt.

Eine Erwärmung der Wassertemperaturen kann sowohl physiologische Veränderungen auf dem einzelnen Organismuslevel, aber auch ganze Ökosystemlevel-Prozesse durch Veränderungen der Sterblichkeits-, Wachstums- und Fortpflanzungsraten beeinflussen.

Viele fischereiwissenschaftliche Veröffentlichungen aus den letzten 20 Jahren berichten von Veränderungen in der Verbreitung und den Bestandsgrößen von kleinen pelagischen Schwarmfischen in den Meeren rund um Europa.

Auch die Fischfauna in der südlichen Nordsee zeigt deutliche Veränderungen in den letzten 2-3 Jahrzehnten. Seit den 1990er Jahren werden neben Heringen und Sprotten immer häufiger Sardinen und Anchovis in den Fischerei- und Forschungsfängen gefunden. Bisher ist nur wenig über mögliche Wechselwirkungen zwischen diesen vier clupeoiden Arten bekannt. Wir untersuchten deshalb das Aufkommen der Larven während der Sommermonate (Juni/Juli) in der deutschen Bucht (Kapitel 6). Mithilfe von zwei parallelen Forschungsfahrten wurden gleichzeitig sowohl das Wattenmeer als auch Offshore-Gebiete beprobt, die entweder stratifiziert, vertikal gemischt oder Frontgebiete waren. Die Bongonetzfänge enthielten Sardinen-, Sprotten- und Anchovis-Larven, wohingegen Heringslarven in den Fängen fehlten. Das Auftreten von sehr jungen Sardinen- und Anchovie-Larven bewies, dass der komplette Lebenszyklus dieser Arten in der deutschen Bucht möglich ist, da in kommerziellen Fängen bereits juvenile und laichreife adulte Tiere gefangen wurden. In nahezu allen Gebieten konnte der generelle Ernährungszustand (basierend auf den RNA:DNA Verhältnissen) der Larven als gut eingestuft werden. In den Frontengebieten war die Anzahl an Sardinen- und Sprottenlarven deutlich erhöht, was wahrscheinlich auf dortige Advektionsprozesse zurückzuführen ist. Der Ernährungszustand der Larven war in diesen Gebieten aber eher schlechter, ein Anteil von 15% der dort gefundenen Sardinenlarven wurde als hungernd klassifiziert. Sardinen und Sprotten wurden größtenteils in den Offshore Bereichen gefunden wohingegen Anchovislarven nahezu ausschließlich in den Wattenmeerstationen vorkamen. Die hier präsentierte Studie ermöglicht neue Perspektiven im Hinblick auf die Ressourcen- und Habitat-Verteilung zwischen den Sprottenlarven und den „neu“ auftretenden Anchovie- und Sardinenlarven in der südlichen Nordsee.

Die hier vorliegende Arbeit beantwortet einige offenen Fragen im Hinblick darauf, wie heringsähnliche Fischbestände durch anthropogene Veränderungen beeinflusst werden. Zeitgleich werden aber auch neue Fragen aufgeworfen. Ich hoffe, dass die hier vorgestellten Beobachtungen dazu helfen, die Modellierung von Habitatselektions- und Rekrutierungsmechanismen clupeider Bestände voranzubringen. Diese Modelle könnten dazu beitragen, zukünftige Veränderungen besser vorherzusagen und Gebiets- und Bestandsmanagementmaßnahmen anzupassen.

General Introduction

Clupeoid species

Since the first herring-like fishes (Order Clupeiformes, Suborder Clupeoidei) evolved approx. 105 Million years ago (Klinkhardt 1996) the ancestors diversified into nowadays more than 300 species and thousands of locally adapted sub-populations (Munroe and Nizinski 2013).

Clupeoids can be found virtually worldwide in marine, brackish and freshwater environments and the biomass of single stocks can reach several millions of tons. In 2010 they accounted for approximately 27 % (> 17 Million tons) of the total reported global marine fish catch by weight (FAO 2012) and more clupeid fishes are caught than members of any other single group of fishes (Munroe and Nizinski 2013).

The different species typically have a relatively uniform appearance. They are small (less than 20 or 30 cm in length (Longhurst 1971)), have a silvery–greyish colour with a darker back and they tend to aggregate in large schools. The hotspot of clupeoid diversity lays in tropical waters where about 70 % of existing species live. With increasing latitude, species richness decreases while abundance (stock biomass) often increases (Longhurst 1971). The majority of clupeoids feed close to the base of the food chain and, thus, benefit more directly from nutrient-rich areas where there are strong seasonal or more continuous blooms of plankton.

They often represent a key link between planktic secondary production and higher trophic levels (Blaxter and Hunter 1982; Frederiksen et al. 2007; Kornilovs et al. 2001; Möllmann and Köster 2002) which include many top predators such as piscivore fishes, marine birds and mammals (Bagge et al. 1994; Engelhard et al. 2013; Greenstreet and Rogers 2006; Northridge et al. 1995; Pikitch et al. 2012; Thompson et al. 1991). In cases where their position at the mid-trophic level is dominated by a low number of species, e.g. such as in higher latitudes or in upwelling areas, clupeids can exert a wasp waist control (Cury et al. 2000). In these areas, the whole ecosystem can be particularly susceptible to fluctuations in the abundance of the clupeoids (Bakun 2006; Cury et al. 2000; Möllmann et al. 2005; Schwartzlose et al. 1999) and these fluctuations result from changes in environmental conditions (Beaugrand 2004; Cury et al. 2000; Frederiksen et al. 2006). Clupeoid populations naturally oscillate in abundance and sometimes fluctuate very drastically in number ("boom and bust"-dynamics) (Geffen 2009). They can function as sensitive "bio-indicators" of environmental changes on regional and basin scales due to their short life spans, high intrinsic growth rates, and tight coupling to meso-scale physical processes linked to climate processes (Borja et al. 1998; Cury and Roy 1989; Peck et al. 2012a; Peck et al. 2014). Since the beginning of industrialization approx. 100 years ago, stock oscillations of many clupeoid species have also reflected a wide range of human-induced environmental changes caused for example by direct overfishing (Lasker and MacCall 1983), depletion of top predators (Collie et al. 2013; Fauchald 2010; Köster and Möllmann 2000), destruction of coastal and riverine habitats (Doherty et al. 2004; Klauda et al. 1991), eutrophication of coastal waters (Aneer 1985a; Österblom et al. 2007; Turner 2001), toxic pollution (Carls et al. 2002; Karl et al. 2010), introduction of invasive species (Marshall 1991; Shiganova 1998) and global warming (Grant and Bowen 2006; Peck et al. 2012b; Rijnsdorp et al. 2009). Many examples of these human-induced changes in clupeoid stock abundances can be observed in the heavily industrialized areas of the North and Baltic Sea.

Overview of the clupeoid species in the North and Baltic Sea

Of the 300 species of clupeoids only six and four-species occur in the temperate waters of the North Sea and Baltic Sea ecosystems, respectively. In the Baltic Sea these species include allis shad (*Alosa alosa*), twait shad (*Alosa fallax*), sprat (*Sprattus sprattus*), herring (*Clupea harengus*). Additional clupeids in the North Sea include European sardine (*Sardina pilchardus*) and European (or Cape) anchovy (*Engraulis encrasicolus*). The clupeoid families of Chirocentridae (wolf herrings) and Pristigasteridae (Longhurst 1971; Nelson 2006) are absent in these temperate waters. The two species of shad were once widely distributed and abundant in Europe and commercial shad catches of more than 100 metric tonnes were recorded in the North and Baltic Seas as recently as the first half of the 20th century (Doherty et al. 2004). Allis and twait shad are anadromous fish that feed and grow in coastal and shelf waters and move to their natal river for spawning as far as 400 km upstream (Limburg and Waldman 2003). Due to this homing strat egy, habitat losses due to dams, pollution and

deterioration of the spawning grounds led to the drastic decreases in their abundances (Freeman et al. 2003) and distribution. Both species are now classified as vulnerable in Europe and have been placed in Appendix III of the Bern Convention (1979) that lists protected fauna species as well as in Appendix II and V of the European Community Habitats Directive (1992).

Another clupeoid species with distinct homing spawning behaviour in the North and Baltic Seas is Atlantic herring. Herring is a demersal spawner and different spawning populations can display large differences in life-history scheduling and physiological tolerance (Geffen 2009), allowing different stocks to spawn during autumn, winter, spring, or summer in salinities from 3 psu in the Baltic Sea to 33 psu in the North Sea. The discrete spawning season and the specific spawning locations represent the major characteristics traditionally used to name or identify the various herring (sub-) populations (Ojaveer 1990; Parmanne et al. 1994; Rechlin 1991).

Studies employing allozyme and mitochondrial DNA markers have found no or relatively low genetic differentiation between herring populations from open sea areas (Jørgensen et al. 2005; McPherson et al. 2004; Ryman et al. 1984; Turan et al. 1998). Nonetheless, herring populations inhabiting relatively closed marine areas such as fjords or living close to the edge of their environmental window (e.g. salinity in the NE Baltic Sea) are genetically distinct from other herring stock components (Jørstad et al. 1991; Turan et al. 1998).

Based solely on the spawning behaviour and not on genetic relationship, Atlantic herring stocks can be roughly divided into offshore autumn-winter spawners which utilize coarse sand, gravel or stones as spawning substrate and spring-summer spawning herring which mainly utilize submerged vegetation in coastal habitats (see Fig. 1, 1-17) (Aneer 1989; Schmidt et al. 2009). There are several exceptions to this rule, for example the e.g Norwegian - (17), Blackwater - (5) and some Swedish (11) spring spawning stocks spawn in deeper habitats below the vegetation zone, whereas some Baltic autumn spawners (Fig.1, 16) also utilize coastal vegetated nearshore areas (Fig.1, 9,10).

Like most clupeoids, autumn-winter and spring-summer spawners of Atlantic herring sup-populations exhibit natural long-term fluctuations in spatial diversity and stock productivity coupled to large-scale hydrographical and climate processes e.g. Bohuslän, Norwegian spring-spawning herring, the Russell cycle (Alheit and Hagen 1997). These variations can make local herring stocks very sensitive to additional anthropogenic impacts (Schmidt 2009) such as overfishing, spawning habitat degradation or changes in the hydrographical conditions due to global warming. Although clupeoid stocks can display very high population growth rates and rapid rebuilding when environmental factors are favorable (Geffen 2009), some stocks did not yet recover from the anthropogenic impacts in the last 150 years. The industrialized exploitation of herring as a marine resource goes back several centuries and the industry enabled empires to be build (e.g. Hanse 1100-1400 A.D.; Netherlands 1500-1700 A.D. (Alheit and Hagen 1997; Klinkhardt 1996). By the middle of the 20th century the herring fisheries had gone through periods of enhanced technological development, which resulted in high levels of fishing mortality for most European herring stocks (Saville and Bailey 1980). Their schooling behavior makes herring, similar to most clupeoids, particularly vulnerable to fishing techniques. Fish schools can easily be detected by acoustic devices such as sonar and echosounders, and can be effectively captured by large pelagic trawls or purse seines (Banks et al. 2001). This is especially problematic since the clustering and number of small pelagic fish schools was found to be density-independent and hence not to be related to their abundance (Petitgas et al. 2001). Even at a low stock size, herring were found to aggregate in large schools for spawning or feeding and could therefore still be detected and potentially fished towards stock collapse (Beare et al. 2002). Continued heavy fishing mortality in combination with low recruitment and poor year classes due to unfavorable environmental conditions will reduce populations below the critical minimum stock size within 2 to 4 years (Lasker 1985). After lack of management action the North Sea and Scandinavian stocks consequentially collapsed to approximately 1% of the post-war levels in the seventies (Bailey and Simmonds 1990; Simmonds 2007; Toresen and Østvedt 2000). After implementing a more precautionary management herring stocks in both areas recovered and are now approximately half of the spawning stock biomass (SSB) which were recorded as maximum in

1947 to 1950s (ICES 2012; ICES 2013). The herring stock of autumn spawners in the North Sea recovered after just five years, while the stock of spring spawner off Norway took almost 20 years to recover (Røttingen and Slotte 2001; Schmidt et al. 2009). But not all traditional spawning grounds were immediately recolonized by the re-expanding stocks. The southern Norwegian spring spawner and southern North Sea spawner appeared partially after 25 to 30 years of absence from their traditional spawning grounds (Røttingen and Slotte 2001; Schmidt et al. 2009) and some traditional spawning grounds in the North Sea (e.g. Doggerbank Fig.1, 3) are still not re-utilized (Schmidt et al. 2009). The processes that drive the re-colonisation of spawning grounds are thought to be linked to complex biological and environmental changes (Røttingen and Slotte 2001) and to be caused by large year classes or migrants "re-discovering" the grounds or "re-learning" the migration routes (Corten 2001; McQuinn 1997).

Similarly to overfishing also spawning habitat degradation can cause permanent stock collapse as the example of the eastern North Sea spring spawning stocks show. The near coastal spawning grounds along the Dutch, German and Danish coast more or less completely degraded after the Dutch Zuiderzee was closed by a damn what changed the hydrographical conditions within the Wadden Sea area in the beginning of the 20th Century (6). As a consequence, nearly all eelgrass meadows (*Zostera marina* and *Zostera noltii*), the main spawning substrates of local herring stocks, were eradicated by the wasting disease (Short and Wyllie-Echeverria 1996; Wohlenberg 1935) and the herring stocks consequently disappeared. Also the collapse of the Limfjord herring stock (7) is thought to be caused by a combination of overfishing, changed hydrographical condition and spawning habitat alteration (Poulsen et al. 2007; Ryves et al. 2004). On what scale habitat degradation, especially the eutrophication effects on the vegetation belt of the spawning grounds, influences the other spring spawning stocks is not yet resolved in detail. These spring spawning herring populations form a continuous chain extending from the North Sea to the northernmost parts of the Baltic Sea and for many areas local spawning grounds are not mapped and recent changes in the vegetation zones are not assessed. However, several reports from the main spawning grounds in the Baltic Sea report massive overall decline of macrophytes in the areas (Aneer 1985b; Domin et al. 2004; Munkes 2005; Ojaveer 1981b).

At the present time in the Baltic Sea, spawning primarily takes place in spring, while autumn-spawner are rare. At the beginning of the 20th century, autumn-spawner formed a high proportion of the commercial catches in the western and central Baltic but catches precipitously declined since the early 1970s (Arula et al. 2012). The cause of the disappearance of autumn spawner might be a combination of several factors: increased fishing mortality; changes in prey composition and density, resulting from the altered hydro-climatic environment; competition of autumn-spawner vs. spring-spawning herring and sprat; increased frequency of oxygen depletion situations in the spawning sites in deeper areas (Arula et al. 2012; Rechlin 1991; Von Dorrien et al. 2013). Spring spawning stocks in the Baltic Sea have not flourished in a similar manner as sprat, a species which has greatly benefited from changes in hydrographical conditions and the release from predation pressure by Atlantic cod (*Gadus morhua*). Only the herring stock in the Bothnian Sea has increased in the most recent decades (ICES 2012). The abundance of herring in the Eastern Baltic Sea (ICES Subdivisions 25-29, inc. Gulf or Riga, and 32) continuously decreased from 1974 to 2000 and then stabilized at low levels (ICES 2012) (Fig. 1, 10-15). The strong increase in sprat stock size since the early 1990s seemed exacerbated by inter-specific competition and lead to a decrease in herring weight at age especially in these northern areas (Casini et al. 2006; Möllmann et al. 2005).

For the western Baltic spring-spawning herring stock spawning stock biomass estimates are only available from 1990 onwards (Spawning grounds 8+9). SSB decreased ~ 30% from 1990-2012 caused by high fishing mortality and a low recruitment period in 2004–2009. There were no indications of systematic changes in growth or age-at-maturity, and reduced recruitment was probably due to increased mortality at the egg or the larval stage (ICES 2012).

In contrast to most of the herring stocks in the North and Baltic Seas, the spawning stocks of sprat (*Sprattus sprattus*) seem to be doing well and doubled respectively tripled their population size in the North Sea and the Baltic Sea since the 1970s (ICES 2012). However, the development of

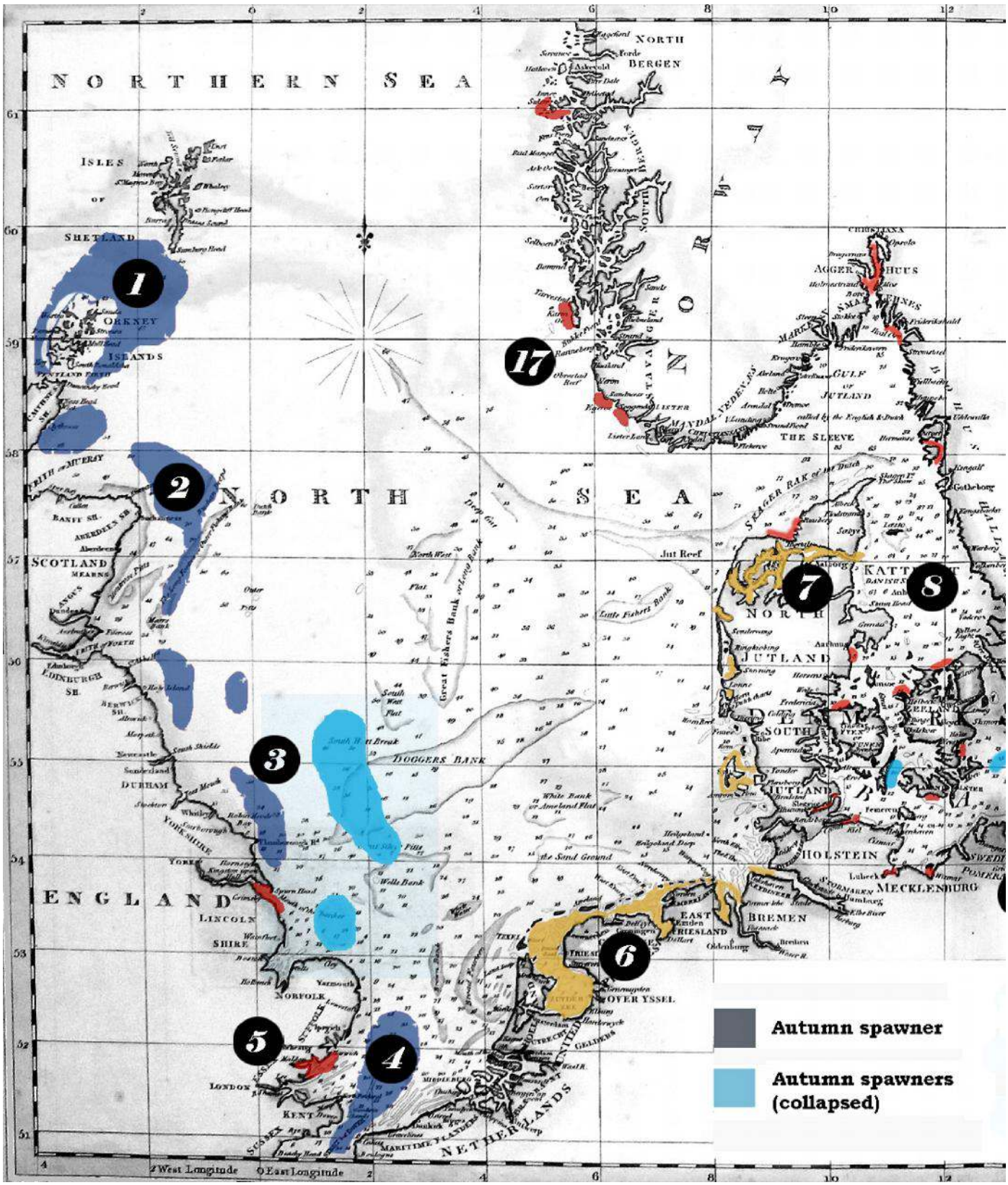
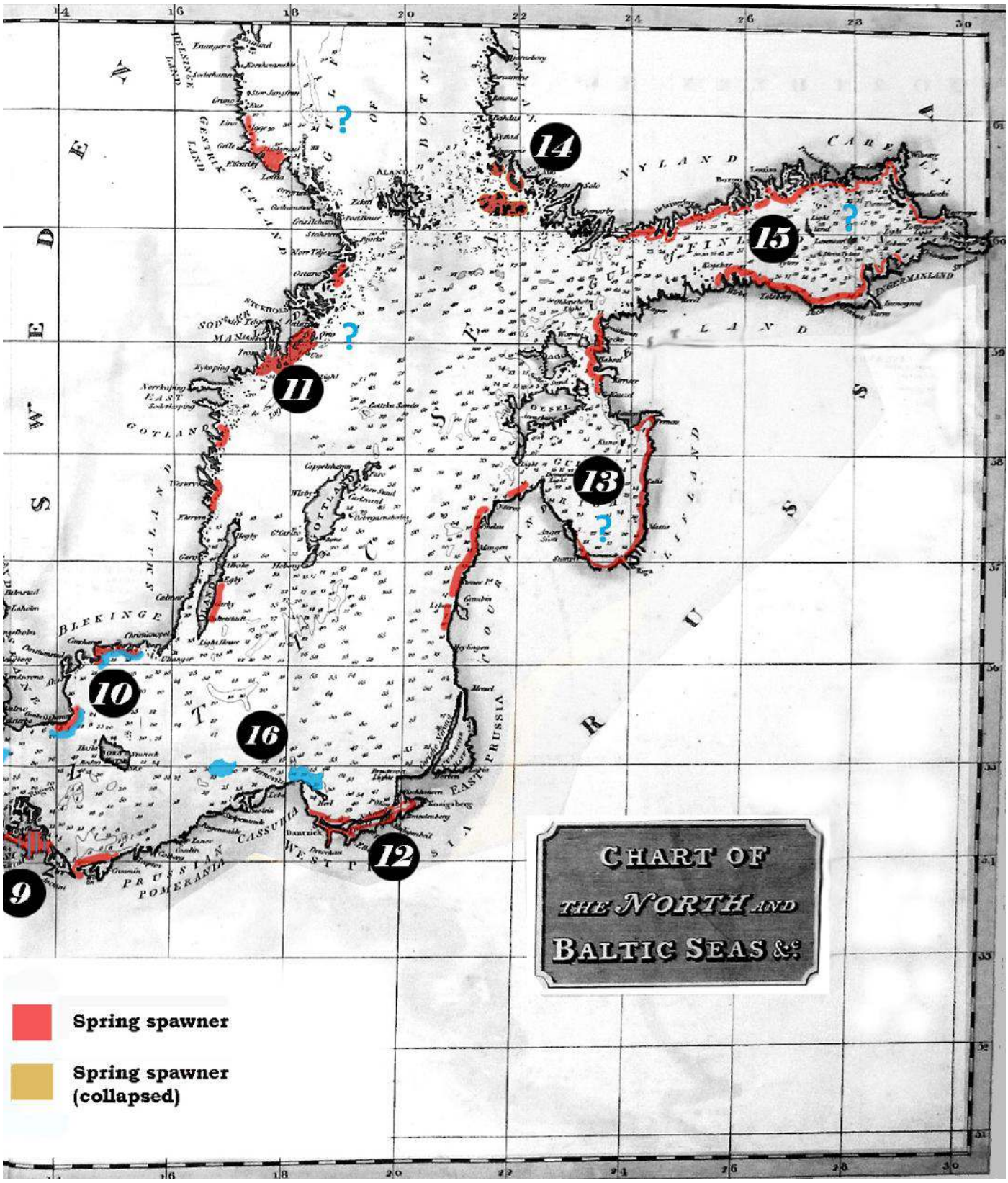


Fig. 1: Recent (dark-colour) and historic but collapsed (light-colour) spawning grounds of autumn- winter (blue) and spring-summer (red) spawning Atlantic herring (*Clupea harengus*). Spawning stocks and spawning areas: (1) Shetlands and Orkneys; (2) Buchanon; (3) Banks; (4) Central North Sea - Downs; (5) Blackwater; (6) Zuiderzee and North Sea; (7) Limfjord-herring; (8) Kattegat & Skagerrak ; (9) Ruegen and Western Baltic; (10) Blekinge Archipelago and coastal waters of Skåne; (11) Stockholm Archipelago; (12) Gulf of Gdansk; (13) Gulf of Riga; (14) Archipelago Sea; (15) Gulf of Finland; (16) Baltic Sea autumn spawner; (17) Norwegian Spring-Spawning Herring.



Spawning grounds redrawn and extracted in the North Sea from (Muenter 1863; Nielsen 1994; Poulsen 2008; Røttingen and Slotte 2001; Runnström 1941; Schmidt et al. 2009; Wood 1981) and in the Baltic Sea from (Alade et al. 2012; Aneer 1989; Aro 1989; Jørgensen et al. 2005; Kaaria et al. 1997; Korņilovs 1994; Muenter 1863; Nielsen 1994). Background map drawn by Thomson (1817).

the stock in the North Sea over time is uncertain due to the lack of a dedicated assessment in the North Sea for most of the period (ICES 2012).
Distinct sprat stocks exist in the North and Baltic Seas and the Skagerrak and Kattegat areas (ICES 2012). Main spawning areas are located in the inner German Bight, the British coast from Scotland to the English Channel and the northwest coast of Jutland in the North Sea (Aurich 1941) and from the Kiel and Mecklenburg Bays in the west to the major basins (Arkona, Bornholm, and Gotland Basins and the Gdansk Deep) in the central and eastern Baltic (Graumann and Krenkel 1986) in the Baltic Sea. In both seas peak spawning occurs between May and August, depending on water temperature (Peck and Hufnagl 2012).
As pelagic spawners which utilize mainly marine offshore areas sprat is not affected by habitat degradation to the same degree as shads or Atlantic herring. The North Sea and Baltic Sea stocks exist close to their high latitudinal limit (Peck et al. 2012a) and generally seem to profit from increasing water temperatures in the area (Haslob et al. 2012). The increase in sprat is thought to be a result of several factors including changes in hydrographical conditions (Alheit et al. 2005; Köster et al. 2003) and a release from predation pressure by overfishing of large predatory fishes e.g. cod (Fauchald 2010; Köster and Möllmann 2000). It is hypothesized that intensive harvesting of cod has released clupeids (e.g. sprat and herring) from predator control, and that a large population of clupeids suppresses cod recruitment through predation on cod eggs and larvae which reversed the predator–prey roles (Collie et al. 2013; Fauchald 2010; Köster and Möllmann 2000). In parallel to the increase in stock size, Baltic Sea sprat showed a decrease in individual growth and condition (Casini et al. 2006; Möllmann et al. 2005). Since the mid 1990s two "new" clupeoid species, European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis* encrasicolus), appeared increasingly in survey and fisheries trawls conducted in the North Sea (Beare et al. 2004). Both species are generally associated with warmer, southern European waters (Lusitanian ecoregion) e.g. Bay of Biscaya. However, literature reviews showed that both species also had northerly spawning sites and were targeted there by dedicated fisheries. Sardine was caught by the Cornish sardine fishery since the 16th century. Catch logbooks showed a strong positive association with warmer periods in the late 16th century (1590–1640) and 20th century (1930–1960) (Southward et al. 1988). The largest northerly Anchovy spawning area was in the Zuiderzee, a large shallow estuary in the Netherlands which was closed off by a dam in 1931 (Boddeke and Vingerhoed 1996) but they spawned also along the western Dutch Waddenzee (Boddeke and Vingerhoed 1996; Wallace and Pleasants 1972). Simultaneously to sardine landings in Cornish, the Dutch catches went up in the warmer period between 1930 and 1960. In the eastern North Sea episodes of increased abundance of anchovy and sardine were previously documented in 1948-1952 and 1958-1960 (Aurich, 1954, Postuma 1978). Thus, the current (re-) immigration of these warm-water clupeid species into the North Sea seems to be related to changes in water temperatures and circulation patterns (Corten and Van De Kamp 1996) that may be driven by relatively long-term climate cycles and/or climate change (Alheit et al. 2012; Petitgas et al. 2012). It has been hypothesized that the decline in the productivity of other species (such as herring) in the North Sea may have facilitated the increase in anchovy and sardine populations. In the Baltic proper sardines are absent probably due to unfavorable salinities (MacKenzie et al. 2007) while catches of anchovy are reported nowadays from the Kattegat, Skagerrak (Alheit et al. 2012) and central Baltic Sea (Draganik and Wyszyński 2004).

Why studying early life history ?

Changes in abundance of small pelagic fish species are mainly caused by recruitment variability that can be affected by changes in spawning stock biomass, reduced fecundity of adults and variation in early life stage (egg and larval) mortality (Dragesund et al. 1980; Lasker 1985). Above a certain size of a minimum spawning population (Blim) recruitment variability is often insufficiently explained by changes in spawning stock biomass or demographic (age-structure) differences (Dragesund et al. 1980). This emphasizes that recruitment is often determined by factors acting during the early life history of fish (Gulland 1965; Leggett and Deblois 1994). Egg and larval stage mortality rates are high and generally much less than 1% of fish larvae survive until they reach the juvenile stage

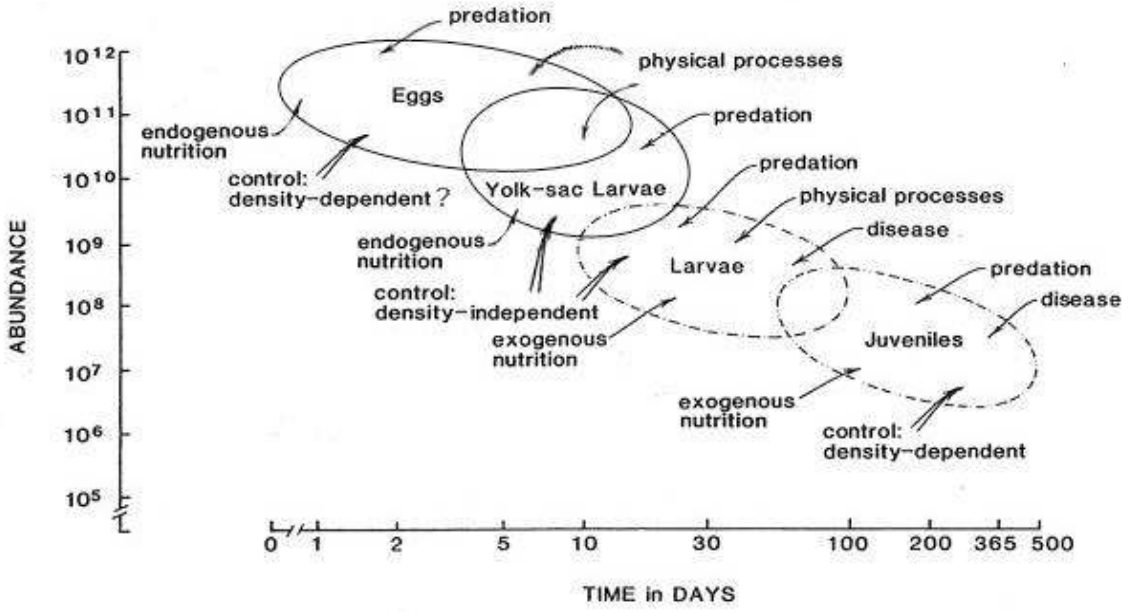


Fig. 2: A conceptualisation of the recruitment process in fishes including the sources of nutrition, probable sources of mortality, and hypothesized mechanism of control for early life history stages of Atlantic herring (*Clupea harengus*). Log_{10} scales are used on both axes (Based on (Houde and Hoyt 1987)).

(see Fig.2) (Fuiman and Werner 2002; Hjort 1914; Houde and Hoyt 1987). Small changes in mortality rates in early life stages can cause major, inter-annual differences in recruitment success (Sætre et al. 2002). Some processes and environmental factors act directly on early life stage mortality of clupeoids e.g. predation, diseases, extreme temperatures and low oxygen level events (Houde and Hoyt 1987; Neuenfeldt and Köster 2000). Other factors, like for example atmospheric climate oscillations such as in the North Atlantic (NAO) and the Atlantic Multidecadal Oscillation, influence larval survival indirectly due to their impact on drift patterns, water temperature or plankton production and concentration (Alheit et al. 2012; Gröger et al. 2010). Especially water temperature seems to be a key parameter by regulating development rates and energy consumption (Bernreuther et al. 2012; Haslob et al. 2012; Meskendahl et al. 2010; Peck et al. 2013).

Early life history stages of clupeoid species

The egg stage

Impacts of biotic and abiotic processes on egg stage mortality can vary widely between the clupeoid species and subpopulations covered in this study (Atlantic herring, sprat, European anchovy, European sardine) due to their different life histories.

Similar to most clupeoids, sprat, anchovy and sardine are broadcast (multi-batch) spawners of pelagic eggs which are transported passively by ocean currents (Lasker 1985). They spawn over long periods and over large areas, with spawning occurring when and where the sea temperature is within suitable limits and the adults have acquired sufficient energy reserves to allow maturation of ova (Armstrong and Shelton 1990).

The pelagic eggs are relatively small, e.g. anchovy eggs (prolate ellipsoids with mean dimensions of 0.7 mm x 1.5 mm), sardine (spheres of 1.6 mm diameter) or sprat eggs (spheres of 1 mm) and transparent. Eggs of these species are positively buoyant and are generally occurring in the upper 10–30 m of the water column (Coombs et al. 2004).

An exception are sprat eggs in low saline areas of the Baltic Sea, where they tend to sink down to intermediate water depths (30-60m) due to their specific gravity (Miranda et al. 1990; Nissling 2004). Time to hatch is strongly correlated to ambient water temperature and varies between 2-4 days for anchovy and sardine (Aldanondo et al. 2008; Miranda et al. 1990) and 6 days for sprat (Petereit et al. 2008) at 12-14°C water temperature.

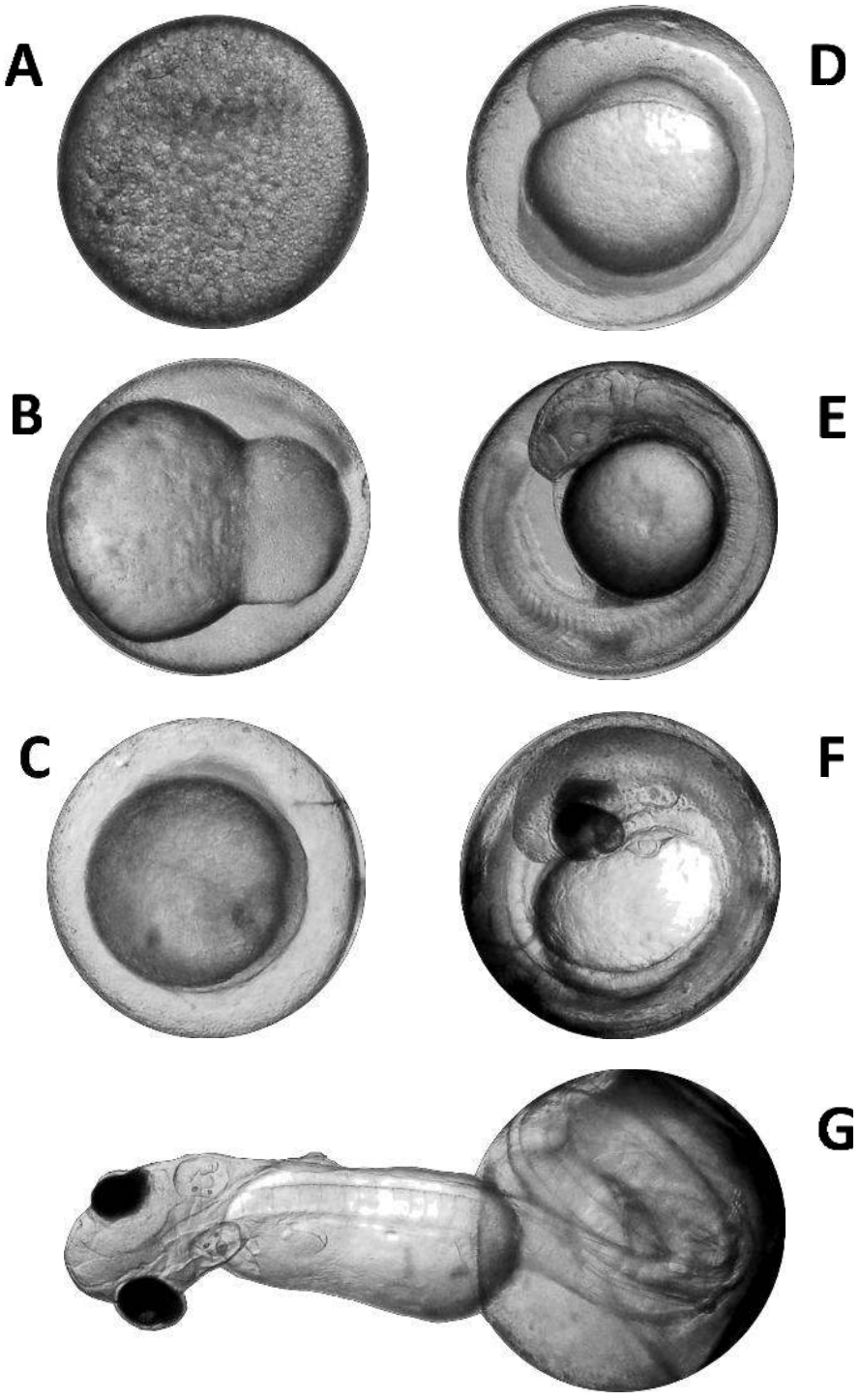


Fig. 3: Embryonic development of eggs and a newly hatched larva of Atlantic herring (*Clupea harengus*). Egg stages can be classified according to the scale of Klinkhard (1996) from 1 fertilized egg to 14 hatch. A. Unfertilized egg, B. Stage 2 Formation of the blastula, C. Stage 3 Gastrula stage; head mesoderm not yet differentiated from the trunk mesoderm. D. Stage 4 Head region shows some organization; head and trunk are still attached to yolk. E. Stage 8 Keel of anterior portion of head rises out clear of yolk; tail has grown out free; eye folds are visible and lenses start to develop, larva shows body movements and heartbeat. F. Stage 10 Behind the eyes, the othic vesicle and two otoliths are visible and the heart is clearly developed. The eye is approximately 70% pigmented with melanin; G. Stage 14 Hatching stage; embryo is completely formed; eyes heavily pigmented.

During this stage mortality rates are generally high (up to 67% d-l (Fossum 1988)) and variable between years and populations. Factors such as increased predator abundances, pollution, or environmental stressors that moderately increase mortality rates can have large impacts (Bunn et al. 2000). At hatch the larvae are blind, transparent and relatively small 3-4 mm (Petereit et al. 2008; Ré 1986).

Herring, unlike most other clupeoids, are total (single batch) spawner with a group-synchronous ovarian organization (Murua and Saborido-Rey 2003) and are unique among clupeoids in having a demersal egg phase. Herring eggs are 1.0 to 1.4 mm in diameter and have slightly negative buoyancy. They stick to the spawning substratum and to each other, gradually building a multi-layer mat (5 to 10 eggs thick) along the spawning ground (Napier 1993). The benefit from this benthic spawning strategy is that growth of the newly-hatched larvae occurs in a very specific region at a very specific time without large dispersal losses after a long embryonic incubation period (Blaxter and Hunter 1982). Eggs hatch 7 to 10 days after fertilization at 12 to 14°C (Klinkhardt 1996) but hatching can take up to 30 days at colder temperatures. Thus, the benthic egg phase of herring is often longer than the pelagic egg phase of sardine, sprat and anchovy. The mortality of benthic eggs is assumed to be low and typically ranges from 2 to 35 % in the Baltic Sea (Ojaveer 1981; Rajasilta et al. 1989; Rannak 1958; Scabell 1989). However, temporally and spatially distinct events such as oxygen depletion (Morrison et al. 1991) can lead to 100% mortality at spawning sites. The annual herring spawning time are pulses of prey at the specific locations (Willson and Womble 2006) which can attract large numbers of migrating predators including birds, fish and mammals (Rajasilta et al. 1993). Herring at hatch are relatively large (5.5 - 7.5mm) and far developed. Their eyes are heavily pigmented and the relation body size to yolk sac is smaller compared to sprat, anchovy and sardine.

The larval stage

The planctonic larval stage is probably the most dynamic life-history stage of marine fishes. A number of generic patterns are likely to be shared by the cohorts of marine fish early life stages including typical changes in spatial distribution, concentration, growth rate and abundance (Peck et al. 2012b). Radical morphometric changes, daily growth rates of the order of 5-30 % in weight and total mortality rates of high magnitude characterize the species-dependent period of time from hatching to metamorphosis (two weeks to two months) (Beyer 1980; Picquelle and Hewitt 1984). The morphometric changes lead to different behaviour, physiological performance and interactions between the larva and its surrounding environment (Fuiman and Werner 2002). Development of larvae is often saltatory and changes in morphometrics and behaviour happen rapidly between longer, more stable larval phases (Balon 2001). In the early stages of life, all clupeoid are subject to high mortality rates caused by a wide variety of predators e.g. larger fishes including adults of the same species, jellyfish, comb jellies (ctenophores) and arrowworms (chaetognathans) (Fuiman and Batty 1994; Holst 1992; Möller 1984). With each stage, larval length, swimming speed and maneuverability increase which improves the probability of survival due to size-selective predation ('bigger is better').

Figure 4 illustrates the change in lateral profile of herring and sprat starting from the eel-like form of the 11 mm yolk-sac larva (B) to the 102 mm juvenile (G) which has the final adult shape. In the first phase after hatch, larvae depend completely on endowed yolk reserves and are hindered in movement due to their large yolk sacs. However, in contrast to the egg stage they can actively change their position in the water column due to vertical movements and can also hold their position at a certain depth.

At the end of yolk sac stage, which lasts for all four species between 2-4 days at warm temperatures (12-14 C°), the larva has developed functional eyes, jaws and digestive system. Larval sizes at this stage range between 4.0-6.0 mm for sprat, anchovy and sardine and 8-10mm for herring (Klinkhardt 1996; Peck et al. 2012a). At this stage the larva has to switch from endogenous to exogenous food supply and has to successfully initiate first feeding. The transition to feeding in fish larvae is considered to be a "critical period" in which mortality is extremely high. As other marine fish larvae, clupeoids are visual feeders (Hunter 1977) and appear to select individual prey (Blaxter 1965). The main food item for first feeding clupeoid larvae is small zooplankton in particular the young stages of copepods (Blaxter 1965), but also smaller microzooplankton and even phytoplankton can be found in guts of first

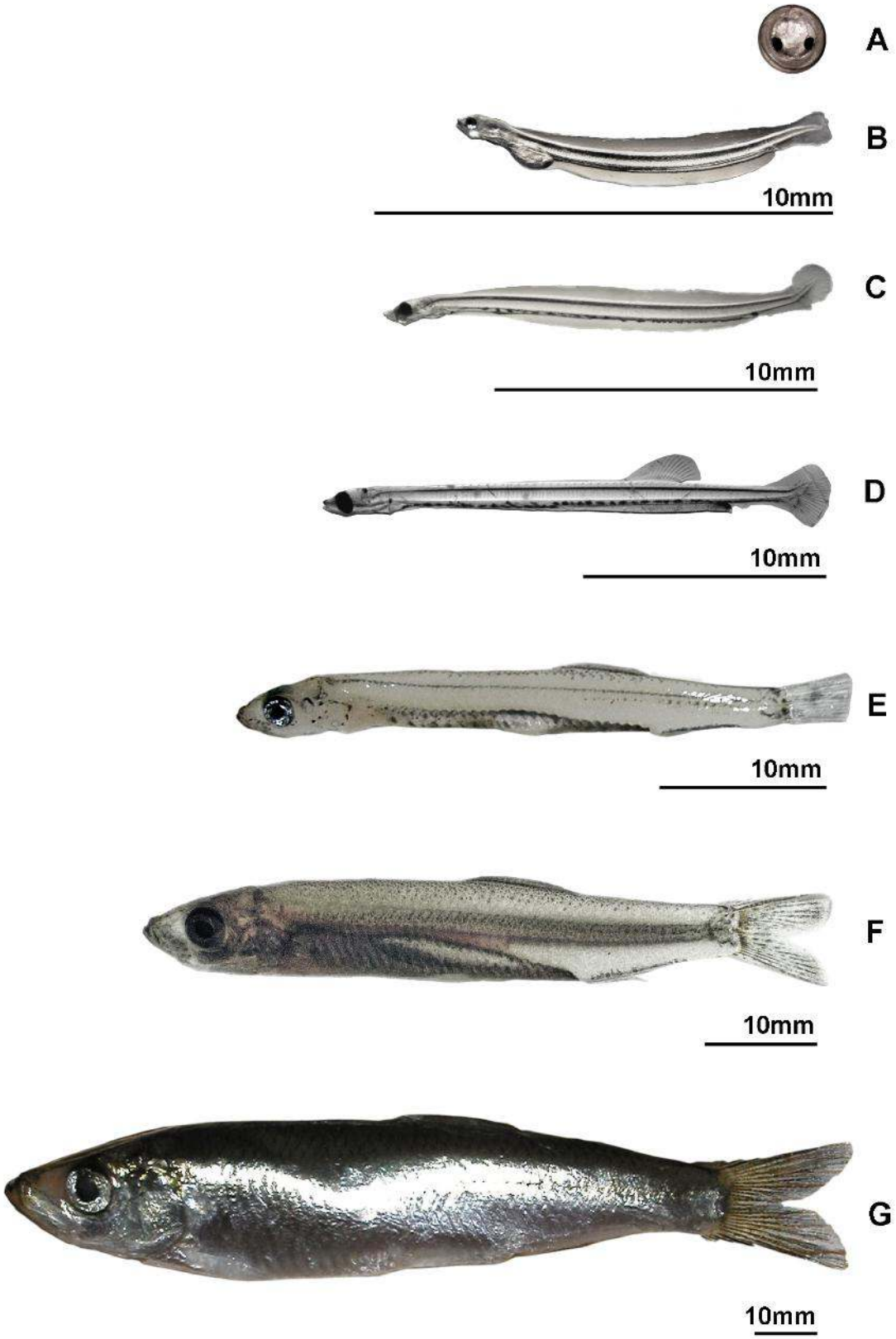


Fig. 4: Development overview of Atlantic herring (*Clupea harengus*) from egg to juvenile. Please note the changing scale. (A) Egg phase; (B) Yolk sac stage; (C) Flexion stage; (D) Swimming transition phase; (E)-(F) Post larval metamorphis; (G) Juvenile. Photos F and G by Claudia Günther.

feeders (Rosenthal and Hempel 1970; Splendens 1977; Voss et al. 2009). Feeding attacks consist of only one strike and initial feeding strike success is generally low, e.g. 10 % for northern anchovy (Hunter 1977) and 2-6 % for herring (Blaxter and Staines 1971; Rosenthal and Hempel 1970). Feeding success increases as a function of age and reaches 40%-60% after several days (Munk and Kiorboe 1985). In cases of no or too little food intake the "point of no return" is reached within a few days and the larvae will inevitably die (Blaxter 1963; Blaxter and Hempel 1961; Lasker 1985; Meyer et al. 2012). Thus there is a general importance of temporal match between the production and development of larvae and suitable prey ("Match-Mismatch Hypothesis") (Cushing 1990).

In the following a generalized description of subsequent development stages for all four species is presented although the length at certain stages varies between species. The given length estimates for the individual species were extracted from various reports (Klinkhardt 1996; Lebour 1921; Munk and Nielsen 2005; Peck et al. 2012a; Ré 1986). When first feeding was successfully initiated and the larva got not eaten by the various predators it enters the flexion stage at 10-12mm. At this stage the notochord associated with the tail fin on the ventral side of the spinal cord develops flexion (becomes flexible) and the pectoral fins become stronger. Until the larva exceeds a length of 15 mm it moves by undulatory locomotion and swimming speed is low with respect to the viscous forces of seawater. At 15mm the Reynolds number (*Re)* at searching speed exceeds 200, which is the transition region where the flow changes from viscous regime to inertial regime. During foraging, the size spectrum of ingested food particles increases in correlation to increase of body length and mouth gap diameter (García and Palomera 1996; Hauss and Peck 2009; Munk 1992). However, as a prey item approaches the maximum ingestible width, the probability of a successful attack approaches zero and therefore the optimal prey size is likely to be much smaller than the maximum ingestible size (Peck et al. 2012b). At larger size (15-25 mm) the caudal and dorsal fins develop what further increases the effectiveness of swimming by reactive forces through thrust generation, making movements less costly in terms of energy (Batty 1984). The subsequent change in swimming style (at approx. 20-25mm) coincidences with the onset of active schooling behaviour in sprat and herring (Peck et al. 2012a; Rosenthal 1968). At approximately 25 to 35 mm, the pelvic fins develop and tail and anal fins are well-forked. Subsequently, a period of post-larval / juvenile metamorphosis occurs when the body height increases in relation to length and the body profile becomes more like the adults. The body looses its transparency and turns whitish while pigmentation darkens the back. Synchronously the gill surface to body mass ratio increases rapidly while cutaneous oxygen uptake decreases. The fish finally enters the juvenile growth phase at around 40-60 mm.

Aims of the thesis

The present PhD thesis focuses on the potential impacts of human-induced environmental changes on the early life history of clupeid species in the North and Baltic Sea. It examines the impacts of spawning habitat degradation in one of the key spawning areas of Atlantic herring in the Baltic Sea and analyzes the influence of increased water temperatures for this species. Additionally, it explores the situation in the North Sea, where nowadays, due to warmer water temperatures, a mixture of sprat, herring, anchovy and sardine larvae can be found.

Degradation of spawning habitats has already caused local extinctions of some clupeoid subpopulations (e.g. allis shad in the Rhine, Wadden Sea spring spawning herring) in the North and Baltic Sea (De Groot 2002; Wolff 2000). For Baltic spring spawning herring, which depends on submersed vegetation as spawning substratum (Aneer 1989b; Scabell and Joensson 1989), eutrophication induced losses in macrophyte coverage and depth zonation are thought to have negative effects. However, the extension of habitat loss in many spawning areas of Baltic herring are unclear and therefore the present coverage and depth limits of macrophyte communities in one of the key spawning areas of Baltic spring spawning herring, the Greifswalder Bodden was assessed in chapter 2. By reviewing historic literature the present study intended to uncover the baseline of vegetation in the Greifswalder Bodden before the onset of eutrophication and to compare this baseline to the present situation. A further aim was to analyze the environmental data from spawning beds to develop a model predicting preferred spawning site areas. This may help fostering their protection against fur-

ther degradations e.g. by coastal development and construction, to ensure long-term recruitment success of this subpopulation. How spawning habitat loss and other environmental factors influence egg survival *in situ* was largely unknown.

By conducting and analysing laboratory experiments (e.g. temperature-related egg development rates; see chapter 4) and by observing egg mortalities in the Greifswalder Bodden, it is hoped to gain further knowledge on the egg mortality processes (chapter 3). In this region it was expected that temperature and other environmental factors and processes (e.g. eutrophication effects or utilized type of spawning substratum) would have a synergistic effect on egg survival. By repeated sampling of herring eggs and vegetation on six fixed transects in different spawning beds during a complete spawning season and by tracking abiotic environmental data, the study tried to gain insights into the mechanism behind the differences in egg mortality rates.

Temperature is the most important abiotic growth factor (Blaxter and Britain 1956) and thus ocean warming due to climate change is expected to directly influence fish stocks. It is thought that ocean warming is currently one of the main driving forces causing changes in species abundance, distribution and composition in marine ecosystems (Perry et al. 2005). Scientists estimate that regional sea surface temperatures in the North Sea increased by 0.9°C to 1.6°C within the last 50 years (Edwards and Richardson 2004; Wiltshire et al. 2010) and the Baltic Sea by 0.8°C from 1861 to 2000 (HELCOM 2007). The projections for future climate changes in the Baltic Sea and North Sea basin, with all of their uncertainties, indicate that atmospheric temperatures will continue to warm during the course of the 21st century (HELCOM 2007). Although climate change may influence the vital rates/productivity of a fish stock (growth, mortality, and recruitment), it is suggested that early life-history stages will be most sensitive to the effects of climate change (Rijnsdorp et al. 2009). Understanding the thermal niche of a species and the knowledge of the ecophysiology of different life stages is prerequisite to provide a strong basis to infer the response of a species to a change in temperature (and other climate-driven changes in abiotic factors) (Pörtner and Peck 2010). In chapter 4 the thermal windows supporting survival of the earliest life stages of Baltic herring (*Clupea harengus*) are presented. The objective of the study was to quantify the effect of temperature on the survival, time to hatch, and changes in biochemical condition (nucleic acids) of embryos (egg and yolk sac larvae) of southwest Baltic herring. Eggs of Baltic Sea spring spawner are expected to be heavily affected by ocean warming due to their coastal spawning areas and the fact that shallow areas will exhibit larger increases in sea temperatures than deeper waters (Rabalais et al. 2009). Measurements of the development of Baltic herring eggs and early larvae have been made at relatively low (3 to 14°C) water temperatures (Herra 1986; Laine and Rajasilta 1999; Ojaveer 1981a) and no previous study utilized warmer temperatures experienced by spring-spawned herring eggs and larvae in shallow areas of the Baltic Sea (up to 20.5°C (Oeberst et al. 2009)). Therefore, the thermal niche of the target species cannot be adequately described based on former experiments. By employing a wider range in temperatures (3–22 C°), we hoped to obtain a more complete picture of temperatures which constitutes the thermal window. In the light of efforts to project climate change impacts, the study focused on the temperature-dependent timing of critical periods during early ontogeny for herring in the southwest Baltic Sea and, more generally, on methods utilized to quantify thermal effects in early life stages.

After hatch and initiated first feeding, herring larvae rely upon sufficient prey abundances to maintain their metabolisms. Understanding how intrinsic and extrinsic factors affect rates of metabolic losses is a fundamental step towards understanding physiological constraints shaping life history strategies in poikilotherms. The rate of respiration (R), measured in terms of O_2 consumption, has been commonly used as an index for metabolic rate in aquatic and terrestrial animals (Brett and Groves 1979; Fry 1957). Factors have been published to convert respiration rates (O_2 consumption) into rates of energy (Brett and Groves 1979) or dry mass (Theilacker and Kimball 1984) losses. Intra- (stage-) specific differences in R have been documented such as changes in metabolic scaling (effect of DM) and thermal sensitivity (effect of temperature) (Klumb et al. 2003; Oozeki and Hirano 1994; Peck and Buckley 2008). These metabolic changes can cause differences in thermal windows supporting survival and growth (Pörtner and Peck 2010; Rijnsdorp et al. 2009). Since rates of growth and mortality are

inversely related during early life in marine fish, it is critical to measure and evaluate such inter- and intra-specific differences in growth (respiratory) physiology. In chapter 5 the growth physiology of larval herring in terms of routine respiration ($R_{R)}$ for groups of herring larvae ranging in mean standard length (*SL*) from 10.0 to 22.5 mm at temperatures from 9 to 19°C is presented. It was hoped that the measurements would yield better estimates of daily metabolic losses to understand the factors affecting the early survival of herring (Hauss and Peck 2009; Hufnagl and Peck 2011). Examining the physiological requirements of herring larvae at warmer temperatures may advance our understanding of the mechanisms acting during the early larval period to regulate recruitment in both autumn- and spring-spawning populations inhabiting the Baltic and North Sea (Nash and Dickey-Collas 2005).

Changing temperatures do not only cause physiological changes on the organism-level, they also impact the population-level via changes in the balance between rates of mortality, growth and reproduction (Rijnsdorp et al. 2009). An example hereof might be the increasing catches of adult sardine and anchovy in the North Sea during recent warmer years. The ecological impact that sardine and anchovy will have on populations of "resident" North Sea clupeid species such as sprat and Atlantic herring is currently unknown. In Chapter 6 the question is investigated if anchovy and sardine offspring can successfully forage in the North Sea and if therefore a life-cycle closure is possible in this area. Furthermore North Sea populations of sprat, herring, sardine and anchovy may exhibit resource competition and the degree of habitat partitioning among these species is unclear. From an early life stage perspective, all four species spawn in early summer to late spring (Alheit et al. 1987). The extent to which larval sardine, anchovy, herring and sprat use these different North Sea habitats could have important implications for competition and relative inter-specific productivity. Objective of the study was to examine the abundances, length distributions and biochemical conditions (*RNA:DNA*) of the occurring clupeoid species among offshore areas (German Bight) that were either well-mixed, stratified or frontal zones and shallow nearshore areas (Wadden Sea). Environmental data included both physical (temperature, salinity) and biological (zooplankton) characteristics in order to help interpreting any potential differences among these clupeoid species in the German Bight.

In summary, my thesis aims to identify and to illustrate mechanism how key environmental factors, like temperature and eutrophication, influence the early life history of European clupeoid species. European clupeoid species received scientifical attention since more than two centuries and a great knowledge about their life history and their ecological context has been accumulated. But their adaptability and diversification still surprise scientists and still cause new questions. I hope my thesis answers some of the open questions and simultaneously evoke new ones.

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Chapter 2

Macrophyte meadows and spawning bed selection of Atlantic Herring (*Clupea harengus*) in the Greifswalder Bodden, Baltic Sea

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Abstract

Coastal zones are productive areas of marine ecosystems and they are also hotspots of anthropogenic activity causing habitat degradation. The Greifswalder Bodden (Southwest Baltic Sea) is an estuarine lagoon which serves as an important spawning ground of the Western Baltic Spring-Spawning herring (*Clupea harengus*), which deposits eggs onto submerged vegetation. A gradual decline in the year-class success of this herring population has could therefore be due to losses in reproductive habitat resulting from reductions in macrophyte coverage due to eutrophication of Baltic coastal waters. Both aerial and SCUBA surveys conducted in spring 2009 revealed that only ~ 7% of the Greifswalder Bodden was vegetated. Herring eggs were observed on 12 of 32 SCUBA transects, at depths between 0.2 and 5 m and were attached to a variety of spermatophyte and algae species. A classification tree model fit to aerial and underwater data showed a statistically significant relationship between spawning sites and total vegetation within a 100 and 500 m radius. The model indicates that schools of herring preferentially spawn on dense and large underwater meadows. Only approximately ~ 5% of the Greifswalder Bodden falls into this category. Despite 20 years of efforts to reduce eutrophication, no increase in macroalgae and spermatophyte vegetation towards the historical level of 90% coverage is apparent. The availability of submerged aquatic vegetation could pose a density-dependent bottleneck limiting herring productivity (through increased egg mortality) in this and other Baltic Sea coastal regions.

Keywords: fish, coastal, eutrophication, reproduction, habitat, submerged aquatic vegetation, GIS

Introduction

Shallow coastal areas including lagoons and estuaries serve as breeding and nursery grounds for numerous aquatic animals and are therefore of high ecological value. Many such ecosystems are heavily affected by fisheries, eutrophication, chemical pollution, underwater noise and increased turbidity from shipping, tourism and harbours, and anthropogenic climate change (Halpern et al., 2012; Short and Wyllie-Echeverria, 1996). Degradation of coastal habitats has been observed world-wide (Lotze et al., 2006), but the coastline of the Baltic Sea is particularly disturbed by pervasive human activity (Elmgren, 2001; Korpinen et al., 2012). Over the course of the 20th century, intense benthic trawling and high river nutrient loads resulted in a dramatic change in the percentage cover and species composition of submerged aquatic vegetation in Baltic Sea coastal areas (Gibson et al., 2007; Olsen et al., 2007). Eutrophication has increased phytoplankton blooms, increasing turbidity, and reducing light at depth, which greatly limits the depth and geographical distributions of perennial macrophytes such as bladderwrack (*Fucus sp.*), Eelgrass (*Zostera marina*) and Pondweed (*Potomageton sp.*). On the other hand, such conditions have been associated with an increase in fast growing and short-lived filamentous epiphytic and drifting algae (Rönnberg and Bonsdorff, 2004).

Most countries bordering the Baltic Sea recognised the negative effects of degraded coastal habitats on ecosystem services and responded by founding inter-governmental management and advisory committees (especially Helsinki Commission – Baltic Marine Environment Protection Commission) and by implementing European Union directives, e.g. Urban Waste Water Treatment, the Nitrates Directive and the Water Framework Directive in order to prevent further deterioration and to protect and enhance the environmental status of aquatic systems (Andersen et al., 2006). Twenty years after the initial restoration steps taken to decrease nutrient loads, there are some signs that nearshore areas of the Baltic Sea are recovering (Nilsson et al., 2005; Carstensen et al., 2006), although many reports describe a relatively unchanged state due to the stability of the newly established regimes (Weckström et al., 2004; Rönnberg and Bonsdorff, 2004). The Greifswalder Bodden (GWB) is the largest, shallow, semi-enclosed estuarine lagoon in the south-western coast of the Baltic Sea. Similar to the general situation in the Baltic Sea, the GWB has undergone substantial changes during the second half of the 20th century (Munkes, 2005). Urbanization of the catchment areas has dramatically increased nutrient levels in the estuary between 1950 and 1990 leading to eutrophication. This change has led to a phase shift from a macrophyte- to a phytoplankton-dominated ecosystem with increased turbidity and reduced light-at-depth (Munkes, 2005). The macrophyte percent coverage on benthic habitats sharply declined in the last 70 years from 90% (Seifert, 1938; Blümel et al., 2002) to between 4 to 15% in the 1980s and 1990s (Bartels and Klüber, 1998). The GWB is one of the key spawning areas of western Baltic Spring Spawning (WBSS) herring, Clupea *harengus* (Oeberst et al., 2009; Biester, 1989) and dense, large schools of herring have been documented for centuries in this region (> 700 years – (Biester, 1989)). Spawning usually occurs during a 12- to 14-week period from early March to early June and involves several spawning waves (Scabell, 1988). The WBSS herring use benthic spawning beds and depend on submerged vegetation or other surfaces to which the eggs can adhere (Scabell, 1988). Embryos hatch 6 to 30 days after fertilisation depending on ambient water temperatures (Peck et al., 2012) and hydrographical modelling suggests that most larvae are retained in the GWB area (Bauer et al., 2013) until they have developed strong foraging and swimming capabilities (~ 20 mm standard length). Herring is an essential component of the local and regional Baltic ecosystem, being an important predator of zooplankton (Casini et al., 2010; Cardinale et al., 2009) and an important prey item for various seabirds, marine mammals and predatory fish. Due to their natal homing, spawning areas such as GWB are used by WBSS herring year after year (Polte et al., 2013).

In the present study, we focus on the status of submerged macrophytes and on spawning sites of Western Baltic Spring Spawning herring (*Clupea harengus*) (WBSS) in the GWB lagoon. Our study aims to estimate the current percent coverage of macrophytes as well as the depth limits of macrophyte communities in the GWB during the herring spring spawning season to develop a model to predict preferred spawning site areas. We utilized data from aerial photography, video transects and SCUBA diver observations and

additional samplings during the spawning season 2009. The knowledge on the distribution of spawning grounds is a prerequisite to implement an ecosystem-based management. By identifying main spawning habitats, their importance for population size can be assessed and this may help fostering their protection to ensure long-term recruitment success.

Materials and Methods

Study area

The Greifswalder Bodden (54.2°N 13.6°E), with a surface area of 573 km^2, is the largest shallow lagoon on the southern coast of the Baltic Sea. Water depth is on average 5.8 m and reaches a maximum between 10 and 13.5 m in the eastern part (Fig. 1). The sediments in shallow areas are mainly sand and clay-gravel mixture while deeper areas tend to be muddy (Katzung, 2004). There are several extended boulder fields located in the central and western area of the Bay although hard substrate is generally sparse. The lagoon has two connections to the Baltic Sea, a large opening with a shallow sill in the east and a narrow channel on the western side (Strelasund). Salinity fluctuates between 5.3 and 12.2, with an average of 7.4. Water temperature changes seasonally from a minimum of -0.5°C in winter to a maximum of 24°C in summer. There are no measurable lunar tides and water circulation patterns are mainly wind-driven (Bauer et al., 2013). Wind-induced convection results in a well-oxygenated water column. Wind-driven advection of water masses between the GWB and Baltic Sea generates water level fluctuations of up to 1.5 m near the coast. During periods of high water, caused by north easterly winds, water masses flow from the Baltic Sea into the estuary, renewing its waters about 8 to 12 times per year (Munkes, 2005).

Aerial photography

Aerial survey was performed on 3rd April 2009 (Blom Germany GmbH) during stable atmospheric conditions and digital photographs were made at an altitude of 1,500 m using an UltraCamD delivering a ground resolution of 40 cm per pixel. The image survey covered a total area of 900 km^2 of the GWB and the surrounding landscape. The inner area of the GWB was omitted from the survey, since that area has water depths > 5 m and macrophytes were unlikely to be detected with aerial imagery (secci disk measurements made on 31st April 2009 indicated an average visual depth of ~4 m). A direct orientation approach was used for precise geo-referencing of the imagery, including a highly accurate GPS-receiver used in combination with an inertial system (GPS / INS). The expected accuracy

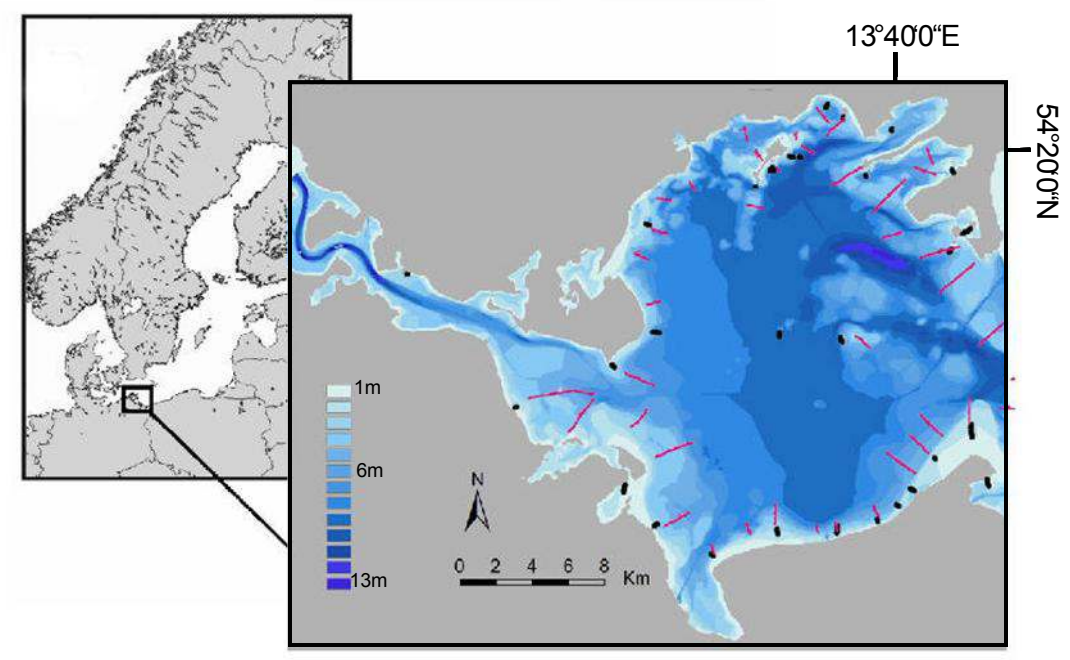


Fig. 1: Map of the Baltic Sea (left). Depth zonation in the Greifswalder Bodden (right). Red lines indicate video-transects; black points indicate diving transects.

of the direct orientation was approximately 1 m in the object space (real world coordinates) which was confirmed by comparative measurements. Both true colour (RGB) and infrared (CIR) images were obtained.

Macrophyte coverage model

Orthophoto mosaics were calculated using the best set of aerial photographs. The necessary digital terrain model for the orthophotos was derived from two different sources: 1) for the onshore portion the Digital Terrain Model 25 (DGM25) of the Office for Geoinformation, Surveying and Cadastre MV (LAIV) was used; 2) for the water body, a depth model based on the data from the German Maritime and Hydrographic Agency (BSH) was computed. The orthophoto mosaics were generated using Erdas Imagine 9.3 software.

The classification of macrophytes from the orthophoto mosaics was done semi-automatically in several steps. First, suitable training areas were defined based on the results of the SCUBA transects. The spectral properties of the training areas were identified through a "region growing approach" (Adams and Bischof, 1994). Subsequently, spectrally different macrophytes were assigned to a certain class (Algae_1 - Algae_n). Because of the different spectral characteristics of macrophytes, 5 to 8 different macrophyte classes were formed per image. Specific macrophyte species were not assigned to a specific class. The individual classes were then combined into an overall class "macrophytes". The image was classified with a maximum likelihood classifier. After the classification, isolated pixels were removed by a median filter and morphological filters (erosion / dilatation). Following these automated procedures, doubtful areas classified as non-vegetation areas were deleted in the manual quality control procedure to avoid misclassifications. As a result, the presented macrophyte coverage is estimated to be rather conservative.

Video transects

Towed camera surveys were conducted at 46 different sites within the GWB between the 21st April and 19th June 2009 to obtain a more precise estimate of underwater vegetation at depths ≥ 3m (Fig.1). Transects were directed perpendicular to the shore and were executed from 2m depth contour to 5 to 8m depth. To simplify the later estimation of degree coverage, one video frame per second was exported as a picture (jpeg) and overlaid by 4 x 4 grids. For calculation of the degree coverage, every 5th picture was analysed and the fractions of grids covered by vegetation were assessed (min. 0.1 grid; max. 16 grids).

SCUBA diver surveys

A total of 32 transects in the GWB were surveyed using SCUBA between the 25th April and 13th May to assess and quantify submerged vegetation and herring spawning activity (Fig.1). Transect locations were randomly chosen around the GWB. Visual observation and sampling procedures were conducted following standard methods (Bäck, 1999; Scabell, 1988). Transects were between 150 and 700 m in length and covered depths between 0.4 m and approximately 100 m behind the lower depth limit of the distribution of a phytobenthic zone. In large areas without a depth gradient, a transect was terminated when vegetation was absent for > 100 m or, in some cases, when SCUBA divers had relatively low volumes of remaining air.

Transects were perpendicular to the nearest shore and straight. A recreational GPS receiver towed directly above the dive team recorded the position every 5 s. GPS-time was synchronized with an underwater watch and a digital underwater camera (Olympus C-4040) to connect track-data with observations and images. Along each transect, a 1-m wide area on both sides of the transect line was visually inspected for a distance of ten fin kick-cycles (8-12m). Within each subsection, the diver noted time and depth (m). The composition of bottom sediments were classified as either: 1) Sand / Silt / Mud; 2) Clay, firm sand bottom; 3) single rocks and boulders < 20% ground coverage; 4) stone or boulder fields 20%-80 % ground coverage; 5) rock bottom >80 % ground coverage.

The percent cover of vegetation was estimated by projecting the outline of the shoots perpendicularly to the seabed. To categorize plant communities and species, a key of 9 types of algae groups / species based on abundance and thalli resemblance was used to facilitate data acquisition. The relative abundance of 9 plant types was noted including: 1) Eelgrass (*Zostera marina*); 2) Sago pondweed (*Potamogeton pectinatus*); 3) miscellaneous Angiospermophyta (e.g. *Ruppia maritima, Zannichellia palustris, Myriophyllum spicatum, Najas marina*); 4) *Furcellaria*

lumbricalis; 5) *Fucus* sp. (*Fucus vesiculosus, Fucus serratus*); 6) Filamentous *Rhodophyta* (e.g. *Bangia sp., Ceramium sp., Delesseria sp, Polysiphonia sp.*); 7) Filamentous Phaeophyta (e.g. *Chorda sp., Ectocarpus sp., Chorda filum*); 8) Miscellaneous Chlorophyta (*Cladophora sp., Ulva sp./ Enteromorpha sp. / Monostroma sp.)*; 9) Turf Mat - algae that form a dense mat or turf on the substrate (e.g. *Pilayella sp.*).

The intensity of herring spawning was assessed based upon 4 categories (O = no eggs; 1 = single eggs, 2 = single egg layer; 3 = multiple layers of eggs and complete plant covered). Unknown plant species with herring eggs attached were collected and identified later.

To obtain a precise species list along each transect, divers quantitatively sampled randomly placed 25 x 25 cm quadrates. A total of 10 quadrates was assessed on each transect. Benthic flora and fauna were collected to obtain species composition and biomass. Within each quadrate frame, the type of substrate, the dominant species composition and its percent cover, the presence or absence of herring eggs, and herring spawn intensity were determined. Shortly after collection, 3-4 samples collected from 0.0625 m^2 quadrates were chilled on ice and plants identified and the number of herring eggs was counted and their developmental stage was noted (Klinkhardt, 1996). All additional samples were preserved in 4 % formalin solution.

Statistical modelling

During exploratory data analysis, herring egg observations were screened for potential spatial-autocorrelation, effects of sample size or sampling date, and suitability for parametric statistical methods. Spatial variograms revealed a high degree of spatial auto-correlation among observations within sites, but similar variability between immediately adjacent and more distant sites. Data were thus pooled by site in all further analyses, and the presence or absence of herring eggs was used as the response variable. One site was excluded from analysis due to a combination of low sample size (n = 6) and no observed eggs, since further sampling may have changed this result. One site was included despite a very low sample size (n = 2), because eggs were present (which additional sampling could not have affected). Sample sizes at the remaining sites varied from n = 11 to 70 observations, with no significant association between sample size and the overall presence or absence of eggs (Kendall rank correlation tau = 0.16, $p > 0.05$). There was a significant effect of sampling date on the occurrence of eggs (Kendall rank correlation tau = -0.37, $p < 0.05$). This temporal effect could not be eliminated, but was explicitly accounted for in the interpretation of results. Finally, the data did not lend themselves to parametric analysis methods, so an entirely non-parametric approach was chosen.

Classification tree analysis (Venables et al., 1994) was used to model the statistical relationships between herring spawning activity and GIS-based descriptions of the environment as well as SCUBA diving observations of benthic vegetation and substrate. Classification trees recursively split the dataset into groups with the highest similarity in terms of presence or absence of herring eggs. GIS explanatory variables were: depth; distance from the 5 m isobaths; coverage of algae within a 50, 100, 200, and 500 m radius; and density of stones ("stone fields") within a 50, 100, 200, and 500 m radius. SCUBA explanatory variables were: mean occurrence of eleven vegetation types, mean occurrence of six substrate types, vegetation type of highest occurrence, substrate type of highest occurrence, and mean percentage of vegetation. Classification trees were constrained to a specific minimum group size and maximum number of splits (all feasible combinations were examined). Ultimately, a minimum of 8 sites per group was chosen, because it resulted in the most statistically conservative trees still resolving three groups of sites (low, medium, and high spawning probability).

Model performance was quantified in terms of the likelihood ratio positive (LR+), which is defined as the true positive rate divided by the false positive rate (of spawning classifications). To test the statistical significance of classification trees, a custom permutation test was designed. In this test, equivalent tree models were fit to 10000 datasets with randomly shuffled values of herring egg presence. If the actual model outperformed 95% of models fit to permuted data, it was considered significant at $p < 0.05$. All statistical analyses were conducted using the R programming language (Team, 2013).

Results

Historic macrophyte coverage

The vegetation of the Greifswalder Bodden received scientifically attention since the start of the 20th century. In total 9 studies of macrophyte coverage estimations were found for the time period between 1904 and 1997. Figure 2 displays historic macrophyte coverage and distribution of plant types based on observations from 1938 and 1955.

Macrophyte coverage and depth limits

A total of 40.4 km^2 (7.05 % of total GWB area) was classified as vegetation-covered bottom based on remote sensing and video transects. The classified macrophytes were not evenly spatially distributed and the vegetation zones existed mainly in a fringe along the coastlines and islands (Fig. 2).

The target scale of 1:5,000 and the image quality lead to a minimum size of a vegetation object of 10 m^2. The gray value differences between vegetation and non vegetation-covered surfaces in the model were generally very low. This was especially true for areas with low plant densities or plants in 3-4m water depths close to the maximum depth of visibility. A depth threshold of 3 m was set between the aerial image classification and the extrapolation from the video transects. This means that all areas mapped with aerial photographs < 3 m water depth were combined with extrapolated areas of the video transects ≥ 3m. The minimum detectable coverage in aerial images was about 10 to 30% canopy closure, depending on substrate, water depth and macrophyte species. A statistical comparison of the classification results to the SCUBA surveys provided an estimate of the classification accuracy. Detected macrophytes in the aerial images were geometrically overlaid with 1,783 vegetation observations during transects. Only 906 observations recorded a significant amount of vegetation cover (> 20%) while 528 of these points were also classified as vegetation in the macrophyte model based on aerial images. Nonetheless, similar patterns of macrophyte coverage were observed from the SCUBA diving transects and the macrophyte coverage model (Fig.3). The largest part of macrophytes were observed in shallow waters at depths of 1 to 2 m while macrophyte coverage rapidly decreased at depths > 3 m. Based on the SCUBA survey, the depth limit of the phytal zone was between 3.0 and 5.6 m depending on substrate and location. Different vegetation groups displayed only slight differences in the range of depths of occurrence (e.g., turf mats were not found at depths below 2 m, *Furcellaria lumbricalis* was found across the widest range of depths) (Figure 4). The degree of coverage was spatially variable and ranged from the sporadic occurrence of an individual specimen to insular and comprehensive algae or plant-stocks. Macrophyte meadows and beds were variable with respect to their plant communities and were composed of vascular plants and/or macroalgae.

On soft bottoms, the most abundant plant species were eelgrass (*Zostera marina)* and sago pondweed (*Potamogeton pectinatus)* and, to a much lesser extent, horned pondweed (*Zannichellia palustris*), *Ruppia maritima*, *Myriophyllum spicatum, Ranunculus baudotii* and *Zostera noltii*. The lower depth limit can be divided into a main depth limit and a maximum depth limit, where the main depth limit is defined as the depth where the number of shoots/coverage per area was significantly reduced (Bäck, 1999). Main depth limit of Spermatophytes living on soft bottom areas was between 2.5 and 3.0 m for *P. pectinatus* and *Z. palustris*, respectively, and from 3.0 to 3.5 m for *Z. marina.* Single specimens were found up to 3.6 m (*P. pectinatus*) and to 4.0 m (*Zostera marina*) depth. In areas where stones or boulders were present, a wide spectrum of red-, brown- and green- algae was present. The depth zonation of algae could be roughly classified as follows: Near shore rocks (0.3 to 1.5m) contained ephemeral filamentous and flat algae (e.g. *Enteromorpha* sp., *Polysiphonia nigrescens*, *Ulva* species, *Ceramium* sp. and *Cladophora glomerata, C. sericea*). Stones in deeper (1.5m < 3m) areas were mainly covered with erected perennial algae (e.g., *Fucus vesiculosus, Fucus serratus, Furcellaria fastigiata*). The proportion of filamentous and flat algae growing on suitable hard substrate increased again at depths > 3m (mainly *Polysiphonia nigrescens*). The percent coverage of algae sharply decreased at depths > 4 m and vegetation was replaced by bay barnacles (*Balanus improvius*) and blue mussels (*Mytilus edulis*). A maximum depth limit of 6 m was found for the vegetation on hard substrate. In deeper areas (sills and holes), rotting plant material was found, mainly consisting of *Z. marina* and turf mats and was not counted as vegetation cover.

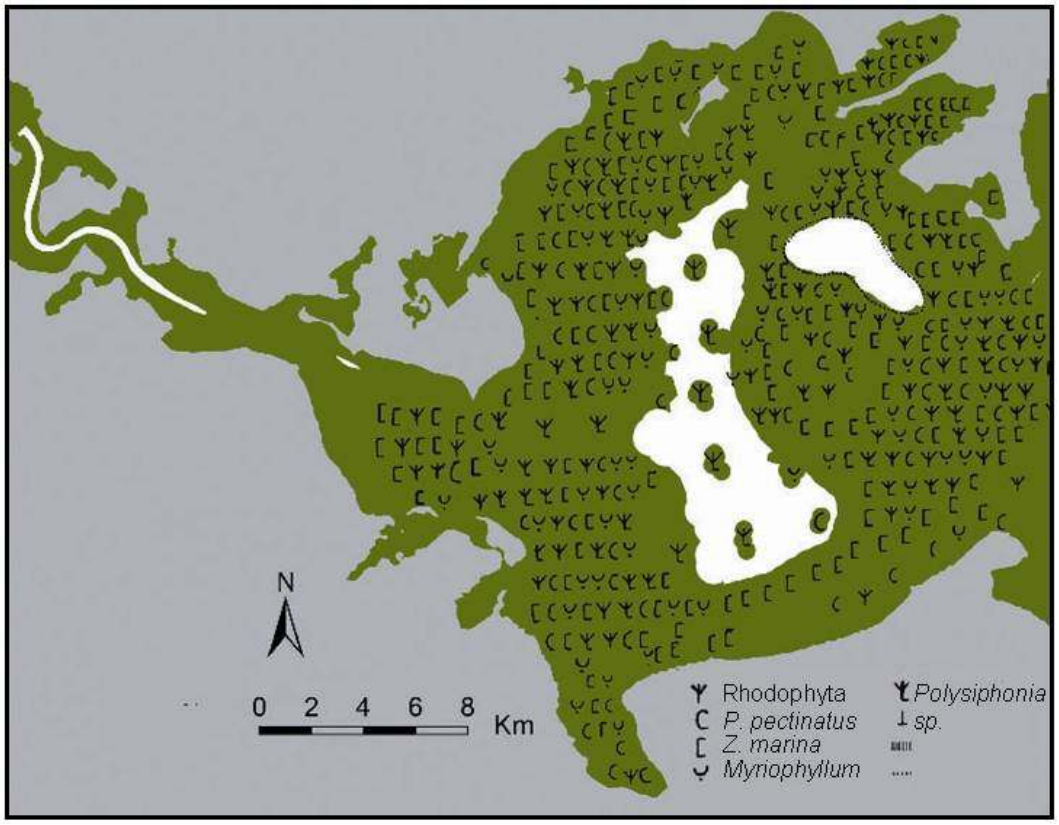


Fig. 2: Extrapolation of historic submers vegetation (green) around 1940-1950 based on Seifert 1938, Subklew 1955 (species distribution) and Munkes 2005.

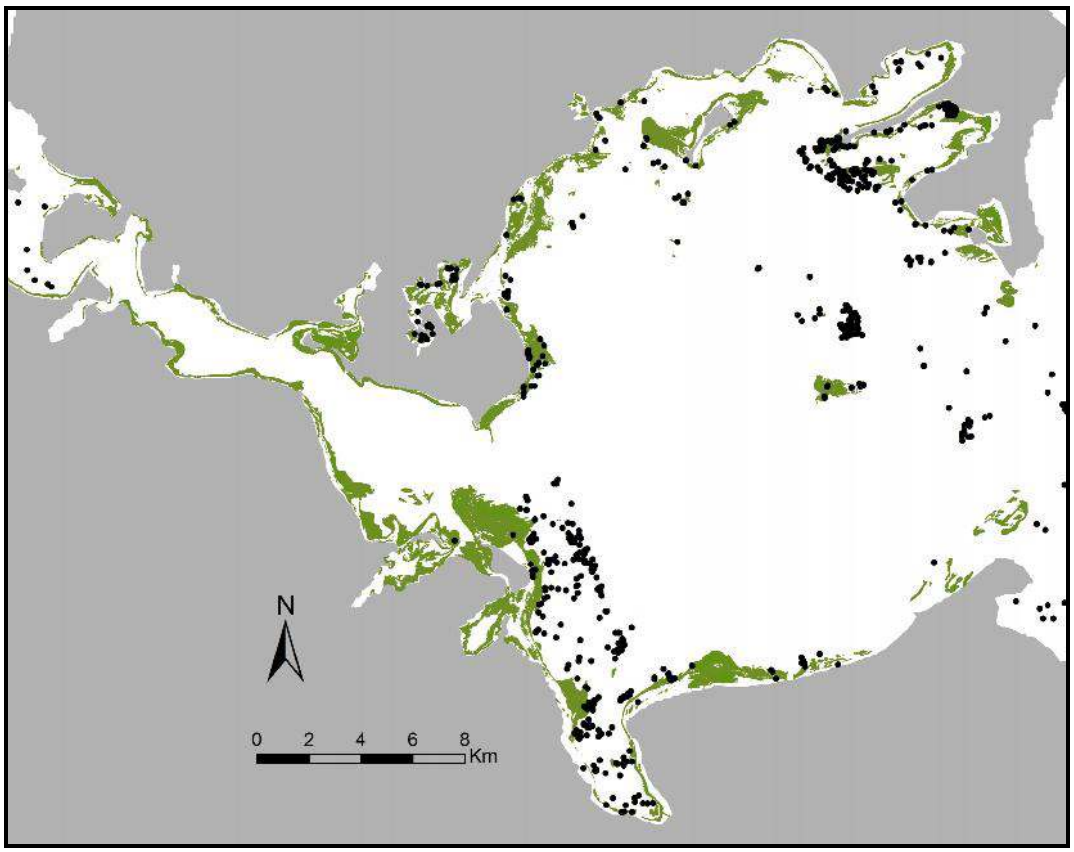


Fig. 3: Submers vegetation based on remote sensing (green) and stone fields >5m depth (points) located by sidescan.

Main and maximum depth limits of macrophytes varied strongly between locations.

Extensive turf mats accumulated as dense layers on the seabed occurred in shallow and protected bays and lagoons mainly but not exclusively in the southwest area of the GWB. These turf mats consisted of fast-growing annual algae such as *Cladophora* spp., *Enteromorpha* spp., *Ectocarpus* spp. or *Pilayella littoralis*. In most inspected areas, the percent coverage of epiphytes (e.g., Angiospermophyta and perennial habitat forming brown and red algae) was moderate to high.

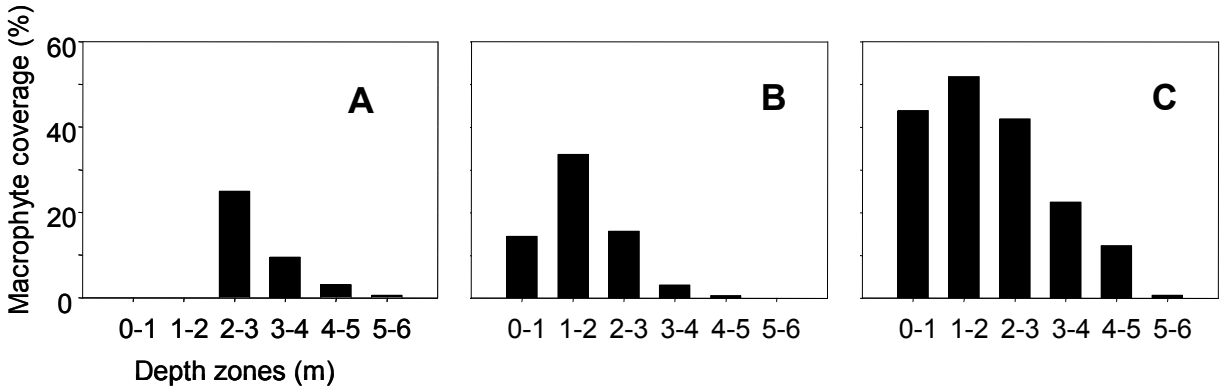


Fig 4: Macrophyte coverage (%) in different depth zones based on towed video (A), remote sensing (B) and SCUBA-diving (C).

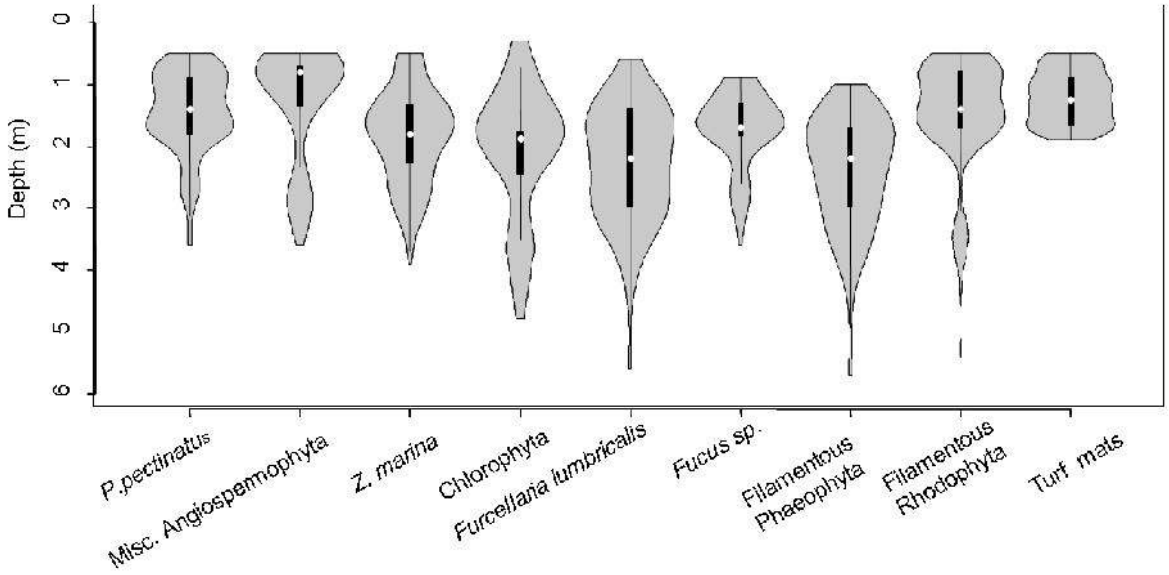
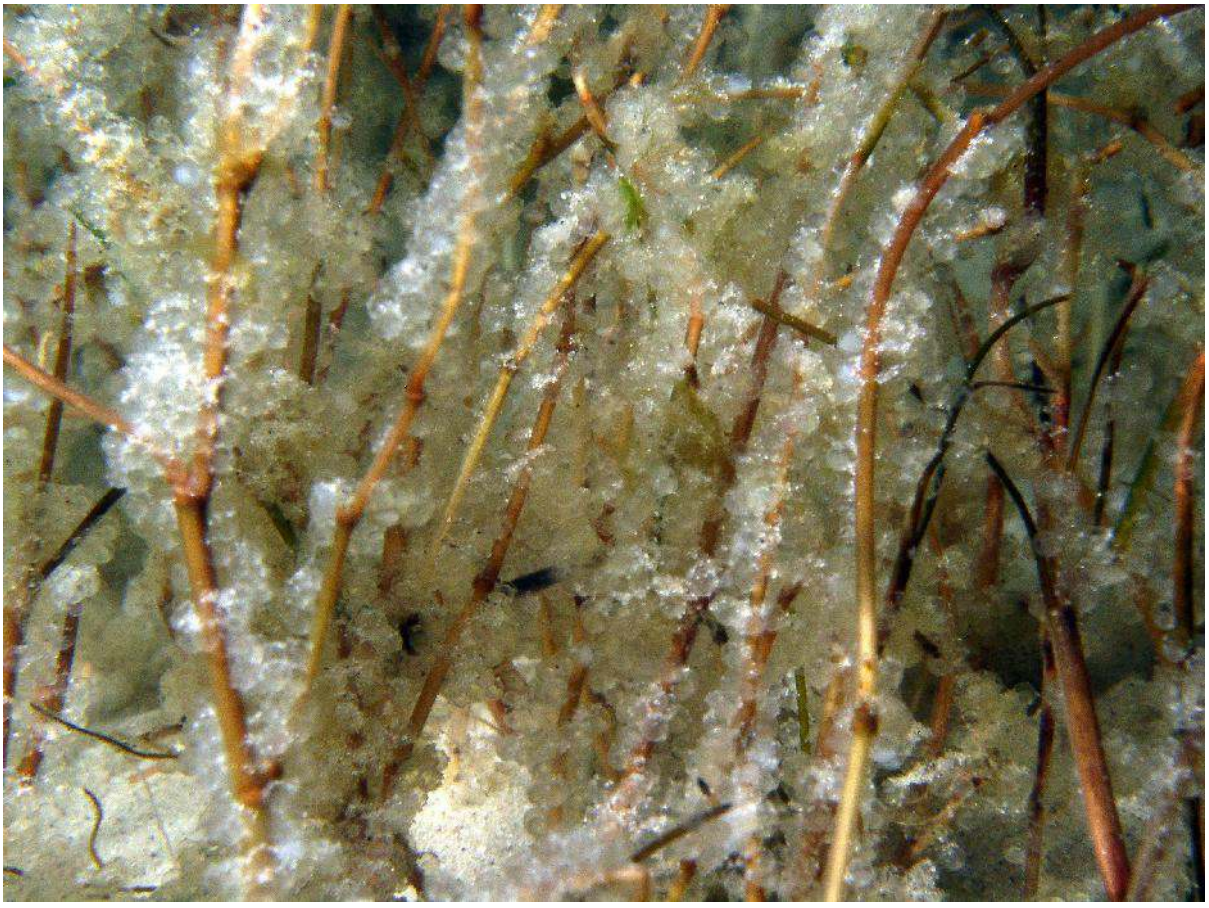


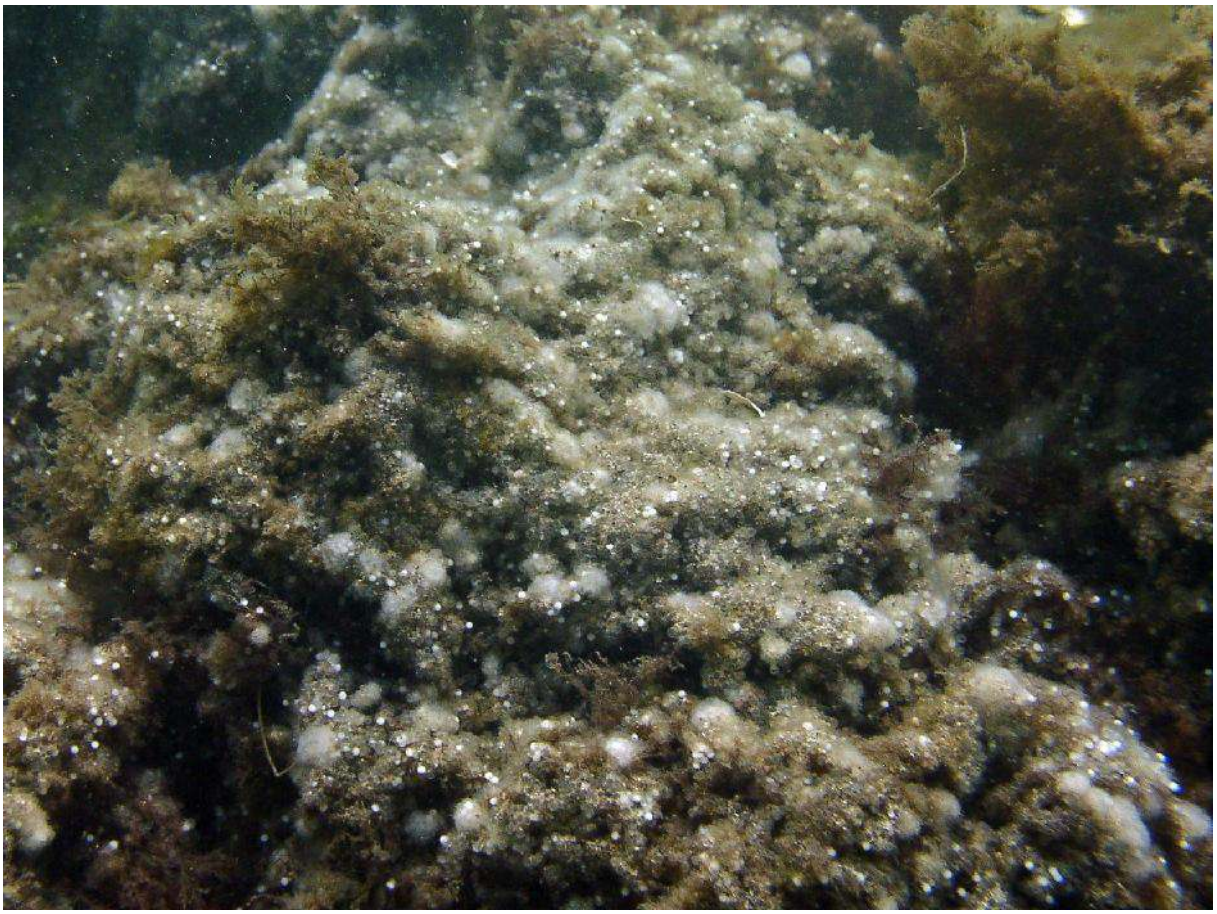
Fig. 5: Violin plot showing the depth distribution and abundances of different species/categories based on diving observations. Combination of box plot (median, interquartile range) and kernel density plot.

SCUBA observations of herring spawning habitat

Herring eggs were detected within 11 of the 32 SCUBA transects. Quantities varied from a single layer of eggs at concentrations between 500 and 20,000 eggs m^{-2} to centimetre thick clumps of eggs with maximum abundances of 1.3 million eggs m^{-2}. Most of the eggs were found at abun dances of 150,000 to 820,000 eggs m^{-2}. The extent of spawning sites ranged from 5 to 10 m^2 to very large stretches of vegetation belts (approximately 1500 to 2000 m^2). Macrophytes utilized as spawning substrate were mainly large perennial, habitat-forming plants such as eelgrass *Z. marina, Potamogeton sp., F. fastigiata* but also the filamentous thalli of *P. nigrescens.* Within larger meadows of the aforementioned species, other plants were also used as spawning substratum including *U. lactuca*, *F. serratus*, *Enteromorpha intestinalis*, *Cladophora sp.*, *Myriophyllum* sp, *Ruppia maritima , Zannichellia palustris,*
Myriophyllum spicatum, and *Najas marina.*



A = Herring eggs attached to black carrageen (*Furcellaria lumbricalis)*. Egg spawning intensity 1.
B = Herring eggs attached to sago pondweed (*Potamogeton pectinatus)*. Egg spawning intensity 2.



C = Herring eggs attached to filamentous red-alga (*Polysiphonia sp.*) Egg spawning intensity 3.
D = Herring eggs attached to eelgrass (*Zostera marina*). Egg spawning intensity 1.

Spawning sites were found between 0.3 and 4.5 m water depth - the main depth limit of macrophytes in the GWB. No living eggs were detected on mussels, bare stones or on soft sediment. Also, no eggs were found on drifting or epiphytic algae mats. However, at some locations, plants and algae with eggs were found below algae mats.
At three locations, herring eggs were found more-or-less exclusively on algae (*Polysiphonia nigrescens*, *Furcellaria fastigiata*) growing on hard bottom substrate while soft-bottom plant species (*Z. marina and P. pectinatus*) in the vicinity did not contain eggs.

Spawning habitat model

Classification trees performed well at explaining the distribution of herring spawning in the Greifswald Bodden, and both GIS- and SCUBA-derived explanatory variables resulted in statistically significant models (permutation test, $p < 0.05$). Each tree involved a primary split separating sites of low spawning probability from the rest and a secondary split between medium and high spawning probability sites (Fig.6). In the SCUBA-based tree, the low group was characterized by <25% mean vegetation, the medium group by >25% mean vegetation and 10-80% occurrence of "single rocks and boulders", and the high group by >25% mean vegetation and no "single rocks and boulders". This scheme correctly classified 6 sites as having eggs and 15 sites as having no eggs, with 3 false positives and 4 false negatives. The likelihood ratio positive (LR+) statistic was of 3.6, meaning that a predicted spawning site was 3.6 times as likely to have had eggs than not. In the GIS-based tree, the medium spawning probability group had a > 1500 m² vegetation coverage within a radius of 100m in combination with 1500m²- 88,500m² in a 500m radius and the high group had >1500 m² vegetation coverage within a radius of 100m as well as 88,500m² – 650,000m² vegetation coverage within 500 m radius. The GIS model correctly classified 7 sites as having eggs and 14 sites as having no eggs, with 4 false positives and 3 false negatives. Predicted spawning sites were LR+ = 3.15 times as likely to indicate observed eggs than not. Combining both SCUBA and GIS explanatory variables did not further improve the SCUBA model. While the SCUBA tree had a slightly better classification performance, the GIS model was clearly much more useful, since it could be combined with data from aerial photography to predict medium and high probability spawning areas not sampled in situ. Based on the GIS model, Fig. 7 provides a map of medium and high quality suitable spawning habitats of *Clupea harengus*. Resolution was restricted to 20 m x 20 m.

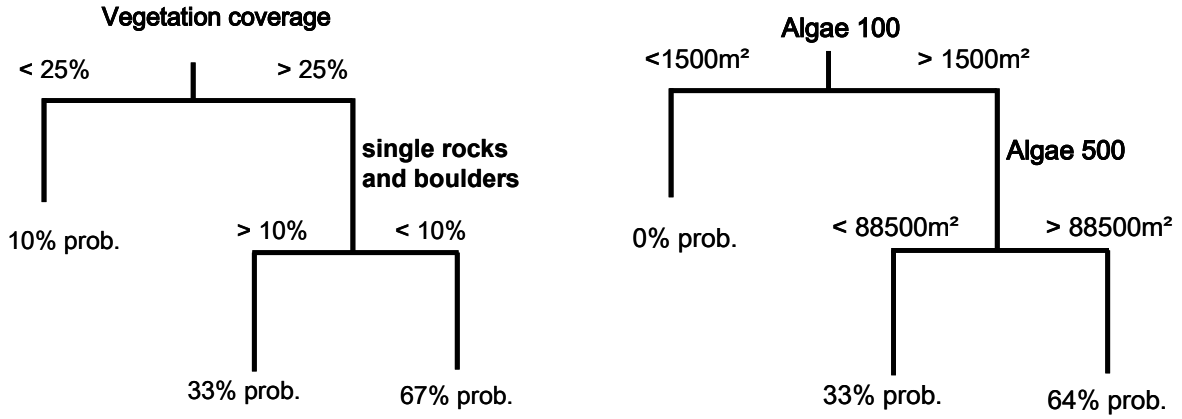


Fig. 6: SCUBA-based tree (left); low (10% spawning probability) was characterized by <25% mean vegetation, medium (33% probability) by >25% vegetation and 10-80% occurrence of "single rocks and boulders" and high (67% probability) by >25% mean vegetation and no "single rocks and boulders". GIS based (right) spawning habitat prediction model resulted in three groups of sites (no spawning 0% , low spawning 33%, and high spawning 64% probability) explained by vegetation density thresholds within a radius of 100m (100buffer > 1500m²) respectively 500m (500buffer> 88.500m²).

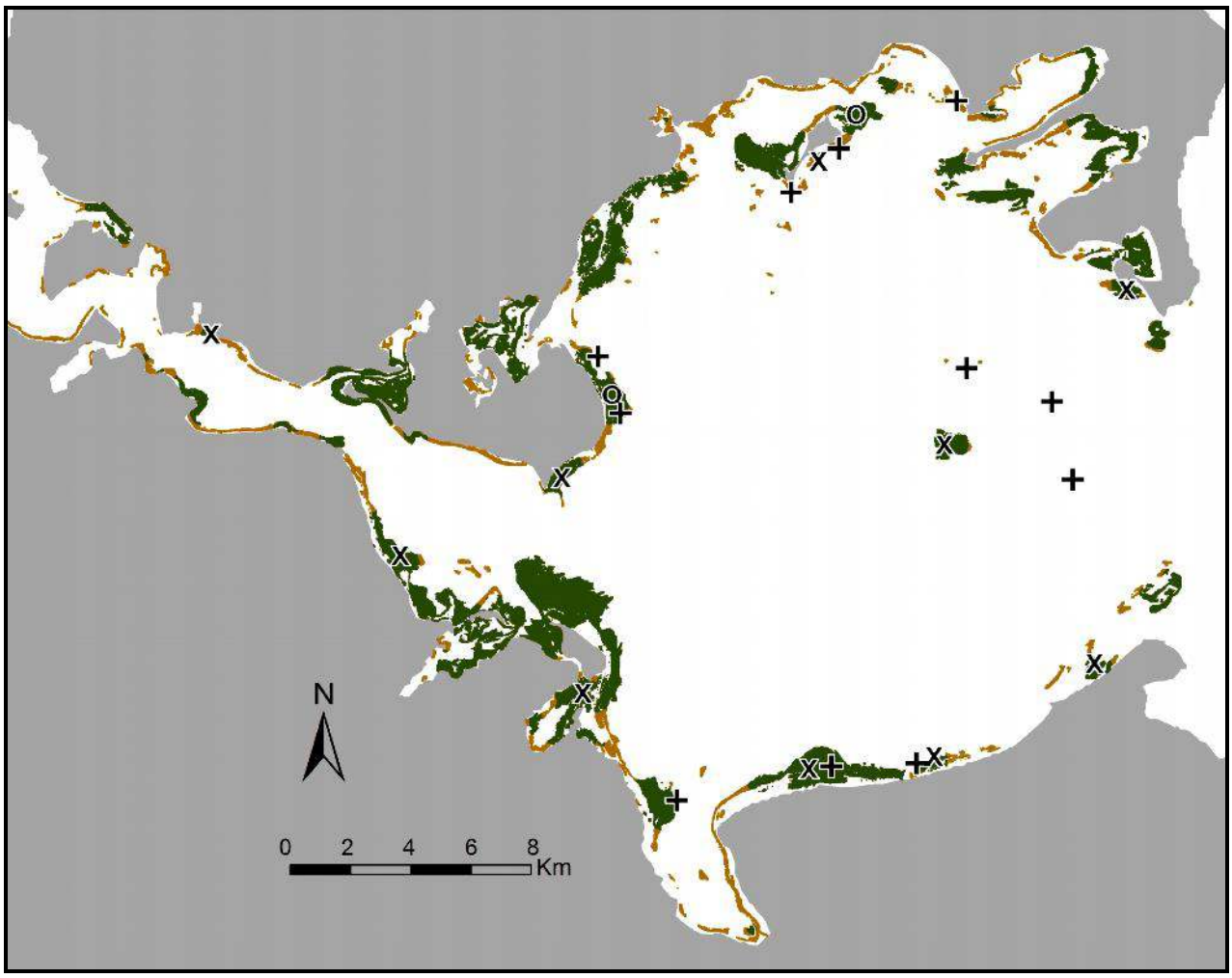


Fig. 7: The map visualizes results of the herring (*Clupea harengus*) spawning habitat prediction model in the Greifswalder Bodden. Colours indicate low (brown) and high (green) spawning probability. Known spawning grounds are marked (x = present study, o = studies in 2008 and 2011, + = Scabell (1988)).

Discussion

Vegetation Patterns

The first reports of the macrophytobenthos of the GWB originate from studies conducted using bottom trawls, dredges and rakes around the beginning of the 20th century (Schiemenz, 1898; Henking, 1904; Reibisch, 1904). Apart from their rather anecdotal character, these studies reported the predominance of eelgrass and red algae throughout the whole GWB lagoon with meadows so thick that demersal trawls became clogged or stuck (Reibisch, 1904). More extensive and detailed studies conducted in the 1930s confirm these observations. Seifert (1938) reported 90% vegetation cover with dense meadows occurring to 6 m depth of *Zostera marina*, *Ruppia maritima*, *Potamogeton pecitinatus*, *Ulva latissima* L. and other species. At depths up to 8 m, *Furcellaria fastigiata* and *Fucus vesiculosus* covered hard and soft bottoms (Fig 2). Studies conducted within the European Water Framework Directive to establish historical reference status (Domin et al., 2004) have confirmed these historical reports. At the beginning of the second half of the 20th century, macrophytes covered ca. 75% of the GWB (Subklew, 1955) and Engelman (1964) suggested that macrophytobenthos decreases were attributable to benthic trawling by the "Zeese"-fishery. In the 1980s, surveys using the same methods as the present study revealed a severe reduction in coverage of macrophyte vegetation (Geisel, 1986; Messner and Oertzen, 1990). The reduction included a shift to shallower depths of red algae (historic: 8-13 m, 1980´s: 4-6 m) and eelgrass (historic: 3-9 m, 1980´s: 3-4 m) and a more patchy distribution of plants. Total vegetation ground cover of vegetation was estimated to be only 3 to 15% (Geisel, 1986; Scabell, 1988; Messner and Oertzen, 1990) (Tab. 1). In the 1990's, extensive monitoring surveys of macrophytes in the GWB showed no clear increase in macrophytes cover and the total percent cover of vege-

tation was estimated to be 4 to 5% (Vietinghoff et al., 1995; Bartels and Klüber, 1998) and 10 % (Hübel et al., 1995). However, the maximum depth limits were reported to be slightly deeper than in the 1980´s (red algae 6.2 to 6.5 m and eelgrass: 3.7 - 5.0 m).

The present study, conducted 12 years after the last extensive survey, paints a mainly unchanged picture of the vegetation within the GWB with total vegetation coverage of 5.4 % and maximum vegetation depth of 4.0 m for *Zostera marina* and 6 m for red algal species. Our study was conducted in spring, prior to or at the beginning of the growing season. Shoot density and biomass of *Zostera marina* meadows increase twofold between March and August (Munkes, 2005), macrophyte biomass is 1.6-times greater in the summer compared to the winter (Schiewer, 2008) and red algae can extend their maximum depth distribution in the late summer when light intensities, temperatures and algal growth rates are highest (Domin et al., 2004). Not only temporal but also spatial variability can occur due to, for example, changes in sediment characteristics due to hydrography (Hübel et al., 1995). For example, vegetation depth limits differ between southwest and northeast GWB, areas dominated by sand and hard bottoms, respectively. In short, spatial variability can occur and our study may yield relatively low macrophyte coverage compared to previous studies conducted during more productive seasons, nonetheless, our estimates are valid during the spawning period of herring in the GWB and clearly indicates a lack of recovery of macrophytes within GWB.

The delay of recovery of macrophytes and macroalgae in the GWB despite 40 and 50% decreases in nitrogen and phosphate concentrations, respectively (Munkes, 2005) is thought to be caused by several factors. An important factor is bio-availability of sediment nutrient loads from the release of iron-bound phosphorus from sediments (Munkes, 2005). During summer, cyanobacteria are abundant which fuels phytoplankton productivity in the GWB (Munkes, 2005) and the increased sedimentation caused by excessive phytoplankton production is known to hinder the recruitment and settlement of fucoid plants (Eriksson and Johansson, 2005) and spermatophytes.

Tab. 1: Literature comparison of macrophyte coverage and vegetation depth limits in the GWB in the last 100 years.

Year	Max. depth eelgrass (m)	Max. vegetation depth (m	Macrophyt coverage GWB	Methods	Author
1904	8.0-9.0	8.0-9.0	100%	benthic trawl, dredges	Henking
1938	6.0	8.0	90%	dredges, rakes, grab samples	Seifert
1955	3.0	8.0	75%	dredges, rakes, grab samples	Subklew
1988	3.5	6.0	11%	scuba diving	Scabell
1990	4.0	4.0	10-15%	scuba diving	Messner & von Oertzen
1990	4.0	4.0	3%	scuba diving, aerial images	Messner & von Oertzen
1995	4.9	6.5	10%	scuba diving, aerial images	Vietinghoff
1997	3.7	6.2	4-5%	scuba diving, aerial images	Bartels and Klüber
2009	4.0	6.0	7%	scuba diving, video transects, aerial images	Present study

Moreover, shading by filamentous algae (Raberg et al., 2005) and high amounts of epiphyte biomasses may hinder the growth of perennial macrophytes (Folke et al., 2004). These various processes may be causing a hysteresis (Murray and Parslow, 1999; Steckbauer et al., 2011) in the trajectory and time course of recovery of the GWB macrophytobenthis community.

Herring spawning grounds

Although Atlantic herring utilizes a variety of spawning substrates across its wide range of distribution (Geffen, 2009), the WBSS population of this species spawns predominantly on macrophytes. The present study found no evidence of herring spawning on blue mussel (*Mytilus edulis*) shells in contrast to reports from the southwest Finish archipelago (Rajasilta et al., 1989). In the GWB, herring mainly deposited eggs onto large perennial algae *Furcellaria fastigiata* (Rhodophycea), *Zostera marina* (Spermatophyta) and *Potamogeton pectinatus*. Eggs were also attached to the filamentous brown algae *Polysiphonia nigrescens* which has been previously reported to be present at this spawning ground (Scabell, 1988). It appears that herring in more northern and eastern areas of the Baltic (coastal areas of Sweden and Finland) also attach their eggs to filamentous green and brown algae (*Cladophora* sp., *Ectocarpus* sp., *Pilayella* sp., *Chorda* spp.) and, only to a lesser degree, to larger species like *Potemageton sp., Zostera sp., Fucus sp. and Furcellaria sp.* (Kaaria et al., 1997; Aneer and Nellbring, 1982; Rajasilta et al., 1989). Aneer (1983) listed 28 different spawning substrates used by herring in the northern Baltic Sea areas and we found eggs on at least 13 species. Taken together, these reports suggest that herring can use a broad spectrum of potential substrates but this does not preclude that certain plant species are preferred. For example, Aneer (1983) observed herring actively searching for red algae (*Ceratium* sp.) within a vegetation belt. We also found a spawning site where a mixture of plant species occurred but eggs were only attached to *Furcellaria fastigiata*. The final choice of spawning substrate may depend on both the type and availability of different substrates (Rajasilta et al., 1989).

Our spawning model results agree with the results of other studies suggesting that plant type is a poor predictor for herring spawning activity (Rajasilta et al., 1993; Aneer et al., 1983). In our model the best spawning predictors were specific vegetation area thresholds within a radius of 100 m and 500 m, meaning that large underwater meadows attract spawning herring. Intense spawning activity is reported to occur within dense meadows (Scabell, 1988; Kaaria et al., 1997). Spawning habitat models constructed for Finnish and Swedish archipelagos indicated the importance of broad and rich vegetation zones (Kaaria et al., 1997; Aneer, 1989; Šaškov et al., 2011; Rajasilta et al., 1993). A second predictor for these archipelago areas was the close proximity of areas > 20 m in depth (Rajasilta et al., 1993; Kaaria et al., 1997; Aneer, 1989; Šaškov et al., 2011). Within the semi-enclosed GWB, however, the factor "distance to 5m isobaths" had low explanatory value.

Our spatial prediction of potential herring spawning grounds seems ecologically sound and agrees with most of the known herring spawning sites in the GWB, although the correlation between large and dense meadows and herring spawn is relatively basic as a model. As noted in the methods section, the fraction of sites with herring eggs decreased significantly with sampling date (Kendall rank correlation tau = -0.37, $p < 0.05$). Evidence for spawning was found at 9 of 15 sites from April 25 to May 11, but only at 3 of 16 sites on May 12 and 13. This apparent temporal effect probably resulted from a combination of residual spatial autocorrelation (some nearby sites were sampled on the same day), hatching of fertilized and degradation of unfertilized eggs over time. Complete degradation is unlikely to have been important. First, the highest category of spawning intensity was still observed on the last day of sampling. Secondly, the change in observed eggs was observed at three sites sampled in April and then again in May. At two sites, eggs were observed in 73-100% of samples initially and in 45-91% of samples 17 days later. At the third site, which had the lowest non-zero spawning intensity of the entire study, eggs were observed in 10% of samples initially and 0% eight days later. This suggests that the habitat of primary interest was easy to detect throughout the study, while only sites of very low spawning activity were not. In any case, the temporal pattern may have influenced SCUBA and GIS classification tree models. With respect to model performance, this likely resulted in damage as opposed to artificial inflation of the LR+ metric, because 100% of false positive classifications corresponded to sites sampled late (May 11-13). The

models were thus statistically significant despite potential confounding, not because of it. It is interesting to note that underwater measurements of vegetation only yielded a small increase in model performance relative to aerial photography and GIS (LR+ values of 3.6 and 3.15). This is consistent with the structure of the GIS model, in that higher resolution data (vegetation inside 50 m) did not dominate larger scale patterns (vegetation inside 100 and 500 m).

The role of stone and boulder fields at 3.0 to 4.5 m depth might be underrepresented in our study. These areas were often not classified as vegetation due to the limitation of remote sensing and some of these areas might be used as spawning grounds. In general, herring spawning at the GWB has been observed from March through June (Scabell, 1988) a slightly longer time period than the sampling period of our diving surveys. Despite the limitations of our study, our habitat prediction model can be a useful guide for further studies of herring spawning sites in GWB. It can also be used to better quantify the loss of preferred spawning habitat within the last 50 years and the consequences of local alterations to habitats within the GWB.

Herring return to the same spawning area year after year even if there are strong anthropogenic influences in the area (Rajasilta et al., 1999). It can be imagined that a loss of approximately 90% of potential spawning habitat has had large impacts on the potential productivity of herring in the GWB. Herring stocks collapsed in the southern North Sea shortly after the 'wasting disease' in the 1930s removed the entire subtidal seagrass stocks in the Wadden Sea (southern North Sea) (Wohlenberg, 1935). Also the decline in herring stocks in the White Sea after the 'wasting disease' events in the 1960s strongly suggest the importance of seagrass spawning habitats for herring productivity (Goscheva, 1970; Polte and Asmus, 2006). In the North-eastern Baltic Sea, herring has started to spawn on bare sediments, leading to an increased mortality of embryos (Raid, 1990). Herring may be able to compensate, to some extent, for changes in the composition of disappearance of bottom vegetation by depositing higher abundances of eggs on the remaining vegetation and/or by switching to different substrate. Nonetheless, changing spawning depth, plant species utilized, and/or egg concentrations deposited can have tradeoffs in terms of increased embryonic mortality. For example, increased thickness of egg masses restricts the supply of oxygen and increases concentrations of metabolic waste products both of which lead to higher embryo (egg) mortality (McMynn and Hoar, 1953). Oxygen supply might be further reduced by increases in algal turf mats and epiphytic algae (Norkko and Bonsdorff, 1996). In terms of shifting depth distribution, egg mortality on the shallow spawning beds in the littoral zone is thought to represent an important survival bottleneck for WBSS herring (Polte et al., 2013). Herring spawning beds are now concentrated in relatively shallow waters where eggs can suffer mortality from increased exposing to wave action (Haegele and Schweigert, 1985). A companion study (Kanstinger et al. In Prep) performed at these shallow spawning sites estimated 55 to 91% of the herring eggs in the GWB to be non-viable in comparison to 32% reported for the same area (Scabell 1988) and 4 -25 % in other regions in the Baltic (Ojaveer, 1981; Rajasilta et al., 1993; Aneer and Nellbring, 1982).

Future research is needed to identify the potential mechanisms delaying or hampering the recovery of macrophytes in the GWB and that underpin the relationship between macrophyte abundance and species composition and herring stock productivity. Changes in spawning beds are not only occurring in the GWB but also in other major spawning grounds in other areas of the Baltic Sea (e.g. Gulf of Riga, Gulf of Finland, western-Estonian archipelago (Rajasilta et al., 1999)). The results of this 2009 survey suggest a massive loss of potential spawning habitat compared to historical periods (pre 1950s) and it is likely that these losses have negatively affected herring recruitment. Given the importance of herring to the ecosystem dynamics of the Baltic Sea, until recruitment mechanisms are known, further losses of spawning habitat due to coastal construction e.g. power plants or gas pipelines should be avoided.

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Chapter 3

Severe mortality of herring (*Clupea harengus*) eggs at a key spawning ground in the southwest Baltic

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Abstract

We provide estimates of the magnitude and timing in embryonic mortality of Atlantic herring (*Clupea harengus*) using in situ observations and laboratory experiments. In the laboratory, the time course of embryo mortality was quantified across a wide range of temperatures (3-21°C). Mortality rates were highest between fertilisation and the gastrula stage as well as shortly before or during hatching. In the field, estimates of egg mortality were obtained from repeated underwater observations using SCUBA surveys conducted throughout the 2009 spawning season in the Greifswalder Bodden (southwest Baltic). The in situ mean total egg mortality was 55 to 91% compared to 27 to 38% for embryos transferred to the laboratory and incubated at ambient field temperatures. In situ mortality was up to 60% higher than previous estimates, does not appear to be solely due to endogenous sources, and was significantly correlated to spawning intensity (egg abundance). The ultimate source(s) of mortality are unknown but hypoxia resulting from a bloom of filamentous brown algae (*Pilayella littoralis* and *Ectocarpus sp.*) concomitant to herring spawning is a likely factor.

Keywords: fish, coastal, eutrophication, reproduction, habitat, submerged aquatic vegetation

Introduction

Small pelagic clupeid fishes are notorious for their large inter-annual fluctuations in spawning stock biomass and recruitment success (Schwartzlose et al., 1999; Kawasaki, 1992) and both Pacific herring (*Clupea pallasii*) and Atlantic herring (*Clupea harengus*) are not exceptions (Zebdi and Collie, 1995; Toresen and Østvedt, 2000). For effective stewardship of these and other fisheries resources, it is essential to gain estimates of the mortality rates of different life stages and to gain knowledge on the factors regulating these rates. The biomass of Pacific herring eggs and the extension of spawning beds in British Columbia (BC) and Alaska are regularly assessed by conducting surveys of the intertidal and upper subtidal zones. Since 1988, SCUBA diver transects have been the standard methodology to assess herring spawn depositions in BC (McCarter et al., 2005). However, much less is known regarding the biomass of spawned eggs and egg mortality in their subtidal spawning habitats in Atlantic herring stocks are demersal spawner and utilize spawning beds which are usually found in the littoral zone at depths of 0.5-20 m(Aneer, 1989a). They commonly spawn on vegetation growing on hard and soft bottoms, although bare stones, gravel, sand and blue mussels are reported to be used as substrate for eggs (Aneer and Nellbring, 1982; Rajasilta et al., 1989). The eggs adhere to the substratum until hatching and the time required for fertilized eggs (embryos) to hatch ranges from 4.5 to 25 days at 20 to 5°C, respectively (Peck et al., 2012). The mortality of Baltic herring embryos (fertilized eggs) has been reported to be relatively low, typically ranging from 2 to 35 % (Rannak, 1958; Ojaveer, 1981; Klinkhardt, 1996; Scabell, 1989; Rajasilta et al., 1989; Rajasilta et al., 1993b). Higher mortality rates can occur at the end of the spring spawning season when waters are warmer (Rajasilta et al., 1993b) or in association with distinct events such as oxygen depletion (Morrison et al., 1991) or runoff of land-based pollution (Aneer and Nellbring, 1982) occur. However, increased mortality can result from various factors, including intrinsic factors such as parental effects (Laine and Rajasilta, 1999; Geffen and Nash, 2012) and/or extrinsic factors such as wave action (Haegele and Schweigert, 1985b), predation by birds, fish and invertebrates (Rajasilta et al., 1993a), unsuitable spawning substrates (Aneer, 1987; Aneer, 1985; Rajasilta et al., 1989), pathogens such as bacteria, viruses and fungi (Sindermann, 1990), and/or oxygen depletion (Braum, 1985; Korņilovs, 1994)).

In the Baltic Sea, herring display a ´´traditional´´ use of specific spawning beds in littoral waters (Aneer, 1989a) with fish arriving at the same locations year after year and often successively utilizing the same spawning beds (Klinkhardt, 1996; Rajasilta et al., 1993b). In the Baltic Sea, herring commonly spawn on vegetation growing on hard and soft bottoms, although bare stones, gravel, sand and blue mussels have also been reported as spawning substrate (Aneer and Nellbring, 1982; Rajasilta et al., 1989). The western Baltic spring spawning (WBSS) herring aggregate on several coastal spawning grounds, of which the Greifswalder Bodden (GB) is an important one. The GB is the largest shallow bay (average depth = 5.8 m) on the southern coast of the Baltic Sea and has been well-known as a herring spawning ground for more than 700 years (Biester, 1989). Depending on the ice coverage, spawning by herring in the GB starts in March and extends to June with a peak in early May. During this 3.5-month period, temperatures increase from ~3°C to ~19°C (Klinkhardt, 1996). Changes in the abundance of larvae surviving to 20 mm length within the GB spawning area reflects the year-class dynamics of the entire WBSS stock (Oeberst et al., 2009). The authors concluded that year-class strength in this herring stock was mainly determined during the egg and early larval stage. Recruitment of WBSS herring has been severely reduced from 2004 to 2011, but the underlying mechanism causing lower survival of eggs or early larvae is not known (ICES, 2013).

The Greifswalder Bodden, with a size of 510 km² is the largest shallow bay (average depth = 5.8 m) on the southern coast of the Baltic Sea. It is a semi-enclosed system of estuarine character with relatively low salinity (6-11psu). Sediments consist of mud and, to a smaller extent of sand and clay gravel- mixture. Hydrodynamics are governed mainly by wind. Wind-mixing results in a well-oxygenated water column to the sea-bed (Stigge, 1989; Hubert et al., 1995). Nutrient levels in the estuary increased dramatically between 1950 and 1990 due to urban and agricultural development in the catchment of the estuary. Higher nutrient supplies have enhanced phytoplankton productivity, and decreased sub-surface light penetration in the Greifswalder Bodden

which has resulted in a approximately 90% loss of macrophyte coverage and induced a dramatic loss of potential spawning grounds for herring within the last 60 years (Kanstinger et al., in prep).

In the present study we evaluated in situ embryo (fertilized egg) mortality in the GB throughout the 2009 spawning season based upon repeated SCUBA sampling at spawning beds. Those field observations were combined with laboratory results on the time course of egg mortality over a broad range of constant incubation temperatures (3 to 21°C). We compare these and previous estimates of in situ mortality and speculate on the potential sources of mortality including the ongoing problems with eutrophication of littoral spawning beds and related changes in the GB ecosystem.

Material & Methods

Field study

Hydrography during spawning season

Surface and bottom temperature, salinity and dissolved oxygen concentrations were measured every week at 5 stations within the GB during larval herring surveys routinely conducted by the Thuenen Institute for Baltic Fisheries. Although sampling stations did not exactly match spawning sites monitored in this study (Fig. 1), the GB is a shallow, well mixed area and, thus, measurements made at these five stations were expected to represent the prevailing hydrographical conditions throughout the GB.

Spawning activity and sites

Six potential spawning sites were regularly monitored by SCUBA divers from March to July 2009 in the GB (Fig.2). All areas were selected based on a study by Scabell (1989), who reported on spawning activity from 1985 to 1987 in the three northern locations (F1-3) and categorized the three southern locations (F4-6) as potential spawning sites.

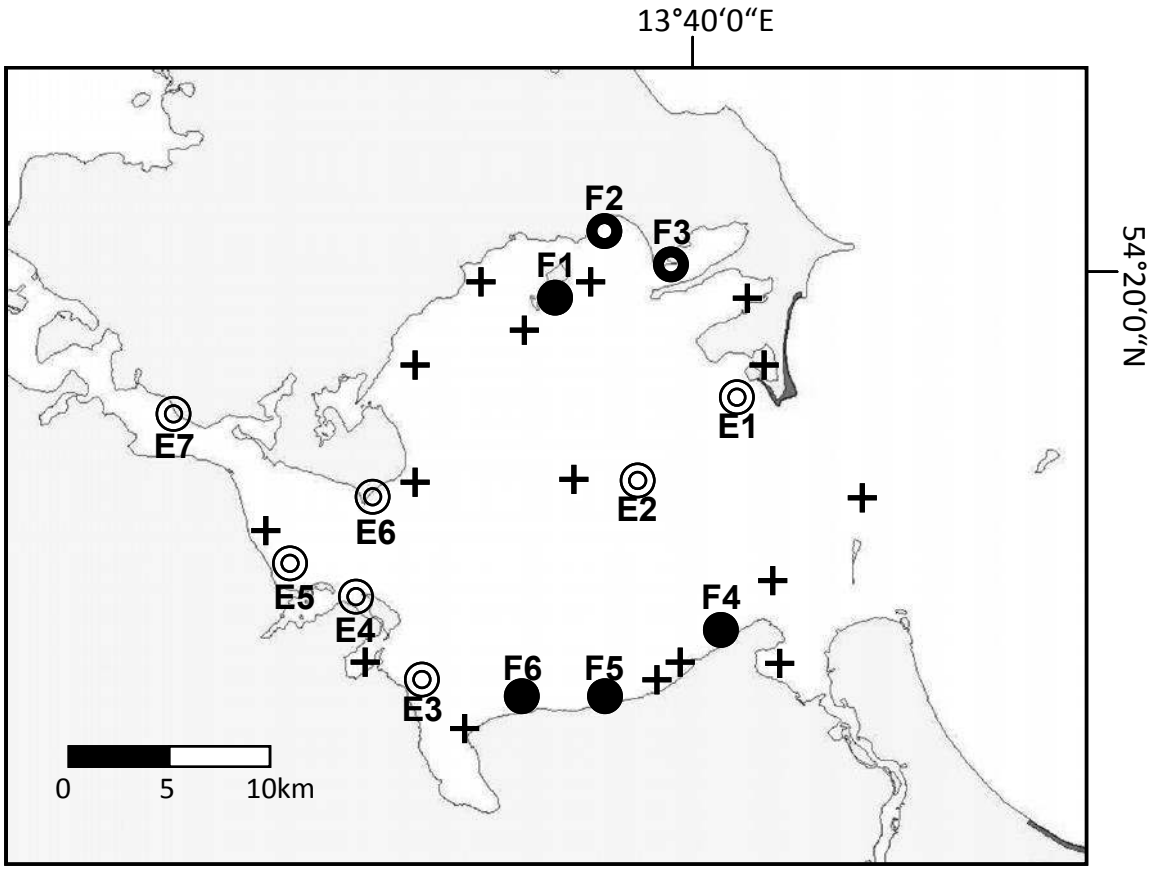


Fig. 1: Study area. Black circles= fixed transects; black points= fixed transects where eggs were found; transparent circles= free transects where eggs were found; crosses= free transects without eggs.

The survey method was based on a combination of three methods 1) Reef Check techniques (Hodgson, 1999), 2) guidelines for monitoring Baltic Sea phytobenthic plant and animal communities (Bäck, 1999) and, 3) methods used to assess herring spawning sites in the archipelago of SW Finland (Rajasilta et al., 1993b).

At each of the six locations, a starting point was randomly chosen in the 1- to 2-m depth zone to establish a 100-m fixed transect perpendicular to shore, start and end points marked by buoys. The line was colour coded every 5m point to mark the position of sampling points and to help the diver to locate his position.

On each transect, divers visually inspected a 1-m wide corridor on either side of the transect line for 5m and noted following information.

The depth, sediment type and spawning intensity was noted, with the latter based on four categories (O =no eggs; 1= single eggs, 2=single layer; 3=multiple layers of eggs) (Scabell and Jönsson 1984). Additionally the per cent cover of the vegetation was estimated by projecting the outline of the shoots perpendicularly to the seabed. To categorize plant communities and species a key of 9 types of algae groups / species based on abundance and thalli resemblance was used to facilitate data acquisition. Plant types were noted in order of their relative abundance. Plants types included: 1) Eelgrass (Zostera marina); 2) Sago pondweed (Potamogeton pectinatus); 3) Miscellaneous Angiospermophyta (e.g. Ruppia maritima, Zannichellia palustris, Myriophyllum spicatum, Najas marina); 4) Furcellaria lumbricalis; 5) Fucus sp. (Fucus vesiculosus, Fucus serratus); 6) Filamentous Rhodophyta (e.g. Bangia sp., Ceramium sp., Delesseria sp., Polysiphonia sp.); 7) Filamentous Phaeophyta (e.g. Chorda sp., Ectocarpus sp., Chorda filum); 8) Miscellaneous Chlorophyta (Cladophora sp., Ulva sp./ Enteromorpha sp./ Monostroma sp.); 9) Turf Mat - algae that form a dense mat or turf on the substrate (e.g. Pilayella sp.). Unknown plant species with herring eggs attached were collected into individually numbered bags and later identified.

To obtain a precise species list along transects divers sampled quantitatively using frames. A 1-m2 frame containing 16 squares (each 0.0625m2) was dropped every ten metres. Each time all squares were assessed visually and in three of those all benthic flora and fauna was sampled. The samples were stored in individually numbered bags to investigate the exact species composition as well as egg numbers and development stage. Within each frame additional visual observations were recorded:

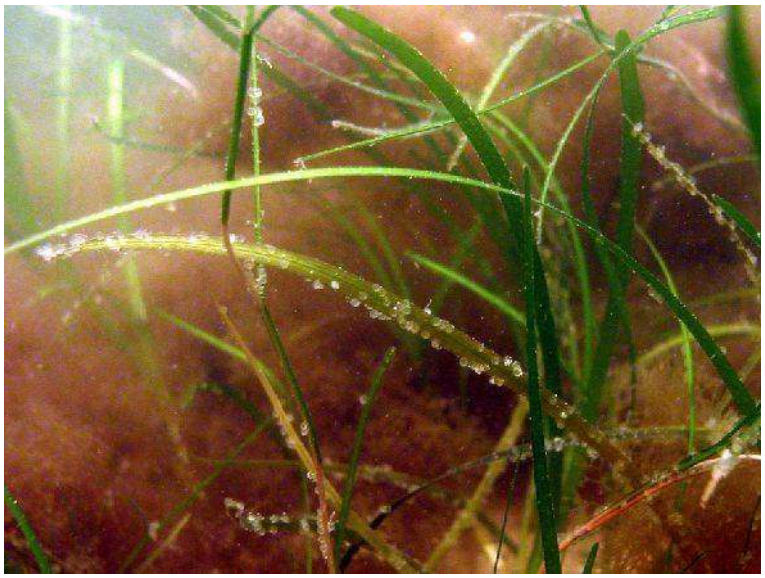
Type of substrate, the dominant species within the frame

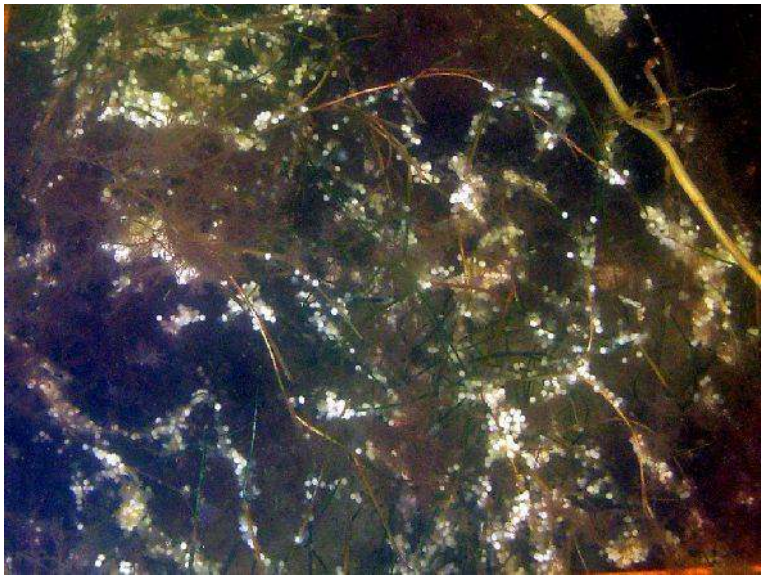
Presents / Absents composition, the per cent cover of the dominant species, the presents / absence of herring eggs, and the herring spawn intensity were visually estimated. A total of 3 to 4 samples of each transect containing vegetation and eggs was stored in ambient seawater which was analyzed within the next 12 hours (real time samples). All other samples were preserved in 4 % formalin solution.

A snapshot of the overall herring spawning activity in the area, a total ofwas gained by monitoring 24 “free’’, randomly located ’free’ transects between the 9th and 13th of May 2009 using the aforementioned methods. These transects were 150 to 700 m long and covered depths between 0.4 m and 100 m behind the lower depth limit of the phytobenthic zone. In large areas without a depth gradient, the transect was terminated when vegetation was absent for > 100 m or, in some cases, when SCUBA divers had relatively low volumes of remaining air. Towed buoys and attached GPS receivers record position coordinates every 5 s. GPS time was synchronized with the underwater watch and a digital camera to track observations. Transects were perpendicular to the shore and observations were made every ten kick cycles. If eggs were present, plant and eggs were sampled with 25 x 25 cm frames.

Processing field samples

Embryos of living “real time samples” were categorized into one of 13 stages based upon the developmental series presented by Klinkhardt (1996), empty chorions were classified as stage 14 (hatch). With known development stage and water temperature data it was possible to calculate spawning date and approximate hatching date. Peck et al. (2012) provide an equation that describes the effect of temperature (T) on the time (t (hrs)) required to reach each developmental stage (S, 1, 2, … 14, where 14 = hatch): $TSF = 3.51*ST - 0.046*T + 2.15$. This equation was used to plan the next sampling date to occur shortly before hatching, when the most precise estimate of egg mortality could be obtained. When no eggs were found, a sampling interval was used to assure intermediate spawned eggs were detected before hatching.





A = Spawning intensity 1. Living eggs attached to eelgrass (*Zostera marina*); B = Spawning intensity 2. Dead eggs attached to eelgrass (*Zostera marina*) and sago pondweed (*Potamogeton pectinatus)*; C = Spawning intensity 3. Dead eggs attached to eelgrass (*Zostera marina*) and sago pondweed (*Potamogeton pectinatus)*.

Four ´´real time samples´´ from locations F6 (Gahlkow) and F4 (Vierow) were transferred to aquarium facilities at the University of Hamburg for further incubation. Samples were placed in well aerated 60-l tanks at ambient temperature and salinity conditions during field collection (8°C and a salinity of 8). Embryos were incubated until hatching and mortality was estimated from per cent hatch.

All real time and formalin-preserved samples were examined for embryo development stage, the number of dead and living eggs (empty / broken egg shells were counted as living eggs that had hatched). At least 100 eggs were staged when samples contained large numbers of eggs (> 300). The visual spawning intensity was compared to the actual spawning intensity (biomass) calculated as the number of eggs gram spawning substratum (Eggs/SSW (gr)) and total egg abundance (number / m^2) according to the methods of Scabell (1989):. Plants were stripped of attached eggs, water was removed by spinning for 20 sec in a salad spinner, and weighed. The total number of eggs was counted or estimated from a weight-based subsample (when > 500 eggs were present).

Laboratory experiments to assess temperature effects on egg mortality

The laboratory experiment on southwest Baltic herring embryos was conducted prior to the fieldwork in the GB. Adult female and male herring were collected from a live trap in Kiel Bight (54.5°N, 10.20°E), transferred on ice to the University of Hamburg and strip spawned. Eggs from six females were fertilized using the milt from six males. Eggs were strip spawned in three (single egg) rows on one side of a square glass plate. All egg plates were randomly distributed into 1-l incubation tanks filled with 900 ml water and placed within a thermal gradient table, an aluminium block that was heated and cooled at separate ends by pumping temperature-controlled water through holes drilled in both ends. A total of 30 replicates were used (3 tanks x 10 temperatures). All replicates were initially stored at 8.0°C and the temperature was adjusted within < 8 h to the final test temperature. Fertilized eggs (embryos) were incubated at mean (±range) (measured) temperatures of 2.9, 4.9, 7.1, 9.5, 11.5, 13.6, 15.3, 17.4, 19.9 and 21.7 (±0.2)°C. The mean (± range) in water salinity was 19.0 (±1.0) psu. Frequent stirring and water renewal eliminated any thermal stratification and maintained dissolved O_2 levels at saturation. Eggs were incubated using a 14L:10D light regime.

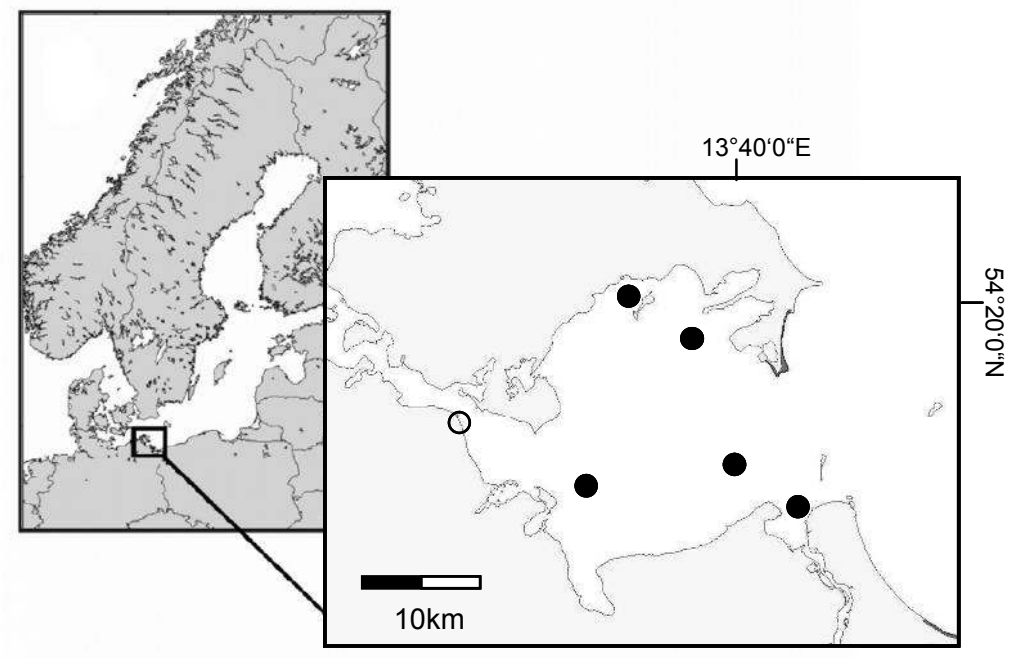


Fig.2: Area of investigation. The inserted map shows the location of the examined area. Points represent stations for hydrographical data, black dots: weekly measured stations; black circle: hourly measured permanent station.

During the experiment, eggs were counted and the developmental stage was assessed on a degree-day (°d = temperature (°C) * time (d)) schedule. Every 10 to 12°d (e.g., every 0.5 d at 20°C and 2.0 days at 5°C), the eggs in each tank were checked under a dissecting scope for developmental stage. Live and dead eggs were counted and new water at the test temperature was provided. At the end of the experiment, after the first hatch, one of the three replicates in each temperature was removed for use in another experiment, while the other two were further incubated until the end of the hatching phase.

Results

In situ estimates

Based on the weekly herring larvae sampling conducted by the Institute for Baltic Fisheries (TI-Rostock), newly hatched larvae (6-7mm) were found between mid-April (Julian day 105) until beginning of June (Julian day 155), with two peak hatches in mid- to late April and mid-May. Based on back calculations (Formula 1) spawning events started end of March and ended mid June. Therefore we concluded that our scuba based research covered the entire spawning period (Fig.3). Average temperature, salinity and oxygen-content during spawning season in the GB are displayed in Fig.4. Temperature at the start of spawning events was between 4-5°C and increased to15-17°C at the end of the spawning season. Salinity and oxygen content varied only slightly and average oxygen content stayed well above biotic critical levels. On average, the salinity was 7.3 psu. ± 0.9 StD. During the SCUBA based monitoring, herring spawn was found at 4 of the 6 regularly motored sites (F1, F5, F6, F7) and within 7 of the 24 free transects (E1-E7) sampled in mid-May. The quantity of eggs varied from a single egg to centimetre thick clumps with maximum egg maximum egg abundance between 20,000 and 1.5 Mio eggs /m² (see next page Table1). The eggs were not homogenously distributed within a spawning site. Instead, were deposited in centres of high spawning activity surrounded by areas with lower egg abundance. The overall size of most of the spawning areas were only roughly estimated, since spots of spawning beds might stretch over long distances along the coastline as indicated by short SCUBA sampling at locations around F4 and F6. The potential spawning area size was approximated from estimates of the size

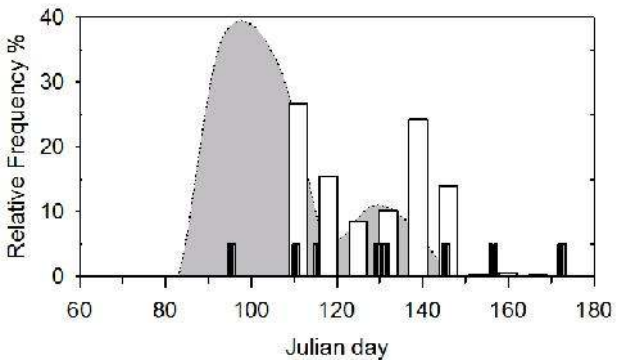


Fig.3: Relative frequency of newly hatched larvae (SL 5-7mm) based on weekly larvae sampling vTI (white filled area). Back calculated spawning date (grey filled area) including average mortality (cohort 1: 75%; cohort 2: 55%) and egg sampling dates (black bars)

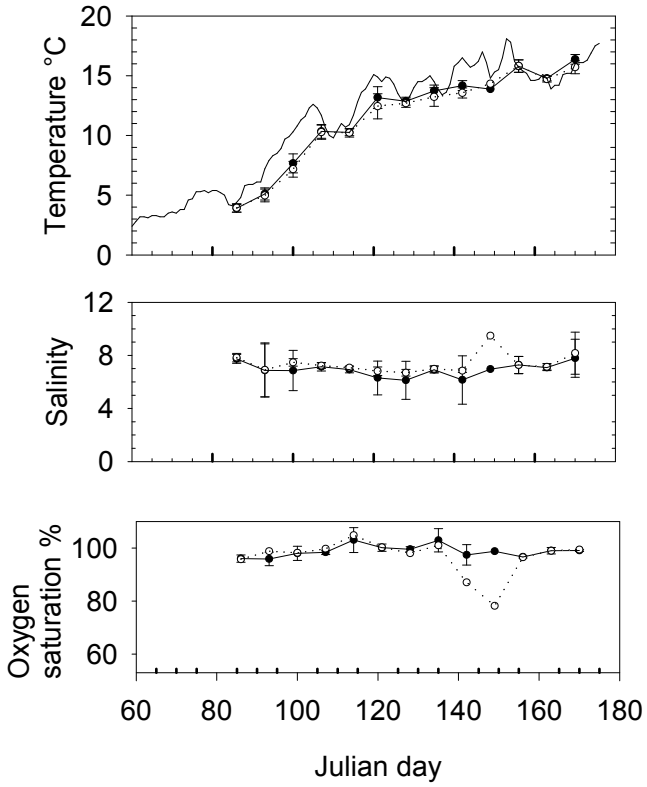


Fig.4: Hydrographic data (temperature, salinity, oxygen saturation) with standard deviation for bottom (dashed line) and upper (constant line) water layer. Temperature graph also includes hourly measurements from permanent station (Stahlbrode; continuous line without symbols).

of the phytobenthic community suitable for spawning based on aerial images.

Macrophytes utilized as spawning substratum

Plant species utilized as spawning substratum differed between spawning beds. The phytobenthic communities detected at the spawning sites

could roughly be classified in two different categories: On sandy bottoms (locations F4, F6) the main spawning and most abundant plant species were eelgrass (*Zostera marina)* and sago pondweed (*Potamogeton pectinatus)* and to a much lesser extent horned pondweed (*Zannichellia palustris*). On location F1 and F5 herring spawn was found more or less exclusively on algae attached to stones e.g. *Furcellaria fastigiata* at location F1 and *Polysiphonia nigrescens* at location F5. At these sites encircling seagrass plants had only few or no eggs attached. Other plant species only rarely utilized as spawing substratum included *Ulva lactuca*, *Fucus serratus*, *Enteromorpha intestinalis*, *Cladophora sp.* and *Myriophyllum sp.* . No eggs were recorded on the filamentous brown algae *Pilayella littoralis* and *Ectocarpus sp.* that formed dense carpets (0.5- 4 kg drained weight per m^2) at 7 out of 38 explored locations. Likewise, epiphytic brown- and greenalgae growing on Angiospermophyta or larger thalli of brown and red algae seemed to be avoided as spawning substrate although spawned eggs sometimes were overgrown by epiphytic filamentous algae.

For a detailed overview of the depth zonations of the different macrophyte communities, please see Chapter 3 (Kanstinger in prep.).

The mean (± standard deviation, SD) egg abundance was highest on *F. fastigiata* and *P. nigrescens* with values of 1029(±289) eggs/SSWgr and 923(±489) eggs/SSWgr, respectively. The mean (±SD) abundance of eggs on *Z. marina* and *P. pectinatus* was lower with 463(±343) and 629 ±412 eggs/SSW gr, respectively. No living eggs were detected on bare rocks, stones, zoobenthos or on soft sediment. All spawning sites were found between 0.3-m and 4.5-m depth, which equals the average depth limits of the GB vegetation zone.

Egg mortality

Total mortality, including non-fertilized eggs, was only 3-5% in early egg development stages (locations F4 - F6) but increased to 55 to 91% for egg stages 11-14 (Table 1). Linear correlations between total egg mortality and egg densities per gram spawning substratum ($P=0.0077$, $R^2=0.243$, $N=92$) and the nominal factor spawning intensity ($R^2=0.640$; $P<0.0021$,$N=92$) were found.

Tab. 1: Characteristics of spawning beds in the GB. Errors indicate standard deviation

Position	F1	F4		F5		F6		E7	E1
Average Depth (m)	2.7	1.4		2.2		1.5		1.7	1.7
Bottom type	Stones. sand	Sand		Sand, single stones		Sand		Sand	Sand
Main spawning substratum	*F. fastigiata*	*P. pectinatus. Z. marina*		*P.nigrescens. Z. marina*		*Z. marina. P. pectinatus*		*Z. marina*	*Z. marina. P. pectinatus*
Potential spawning habitat area (km^2)	0.039	0.25		0.04		0.64		0.10	0.48
Julian Day	115	95	110	96	111	95	110	130	130
Egg Stage	11-13	2-3	13-14	1-3	12-14	2-3	13-14	12-13	5-6
Mortality (%)	75 ±33	3±2	74±36	6.±2	91±12	5±2	69±21	55±14	22±4
maximum eggs/m^2 in 1000	1217	582	759	1568	1297	812	770	84	697
Average eggs/m^2 in 1000	820 ±302	180 ±237	152 ±186	463 ±312	560 ±352	229 ±423	274 ±220	28 ±25	130 ±54
Average Eggs/SSW (gr)	941 ±302	376 ±366	421 ±445	793 ±522	914 ±354	534 ±492	732 ±402	92 ±50	223 ±252
Number of samples	11	20	24	20	24	10	9	9	6

Based upon the data from the 5 stations where mortality was could be assessed shortly before hatching, total mortality was best described using a model including the egg abundance (eggs/m^2) (increasing mortality) and Julian day (decreasing mortality) and the nominal factor plant species (r^2=0.377; Deviance explained=42.5%; GCV score = 210.75; Scale est.=192.42; n=92). A co-linearity was found for position and plant species and for spawning intensity (eggs/m^2) and Eggs/SSW.

The singular linear correlations between mortality and spawning intensity and egg densities per gram spawning substratum were confirmed by visual observations of the divers. When eggs were laid in multiple layers and formed clumps, often only the outer layer contained living eggs while the inner layers were dead. These clumps were frequently infected with various fungi.

For determination of the hatching success in location F6, nine samples had to be excluded from analysis due to a second spawning event at the same location. "New" eggs were spawned (approx. 50.000 eggs/m^2) directly on top of the older eggs and all samples containing eggs with different development stages were excluded from analysis.

Spawning beds containing only dead eggs were discovered in five locations during the snapshot campaign. These stations were excluded from the calculation of average mortality since it was unknown if larvae hatched successfully from these batches. The situation within these spawning beds can be described as follows: At Location E2 eggs were attached to *Polysiphonia nigrescens* growing on stones in 4.5-m depth holding high spawning intensity up to 10 layers with a maximum density of 1.5 Mio. eggs/m^2. At location E6 displaced eelgrass and large amounts of dead eggs were lying loosely on sand in 1.5m depth. We speculate that these eggs most likely drifted to this location and were not actively spawned here. At three stations (E3, E4, E5) a low abundance of dead eggs (5000-40000 eggs/m$^{2)}$ was found attached to *Z. marina* and *F. serratus* buried below thick carpets of *P. littoralis*. (see Figure 5 and .6 next page)

Loss of Eggs

Estimation of egg loss was difficult to assess due to the high variability of egg densities even within small areas. No significant differences in eggs/m^2 were found at locations F4-F6 between first and second sampling (Mann-Whitney t-test, P=0.832 ; P=0.227; P=0.959). Also the visual observations indicated no change in spawning intensity along the three transects, so a substantial loss by predation and/or wave action for these sites between first and second sampling time can be excluded.

During scuba-based surveys at the spawning sites no aggregations of predators (e.g. fish, invertebrates) were observed. Above the water an aggregation at spawning sites during SCUBA surveys. A flock of approximately 300 long-tailed ducks (*Clangula hyemalis*) was spotted at location F5 on Julian day 95 but no distinct signs of feeding such as large amounts of detached algae or bare rocks, were observed.

After hatching, the remaining dead egg aggregations in location F5 persisted for another three to four weeks period before being completely displaced by a storm event in mid-May (Julian day 134). Dead eggs disappeared within two weeks at location F4 and F6, which were characterized by lower egg densities and shallower depth (1.2 and 1.6 m).

Results Laboratory:

The total per cent (%) mortality of embryos was relatively high mortalities below 3.0°C (85% and 91%) and above 21°C (100%) (Fig.7). A 3-segmented regression over the temperature range can be used to describe the temperature related changes in total mortality.

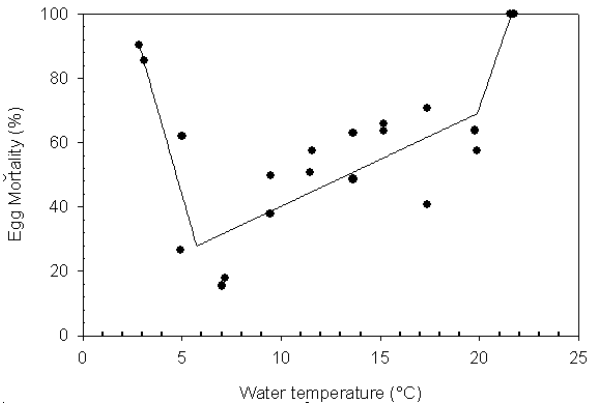


Fig.7 Egg mortality at the end of the experiment vs. temperature regime. Line =3-segmented regression

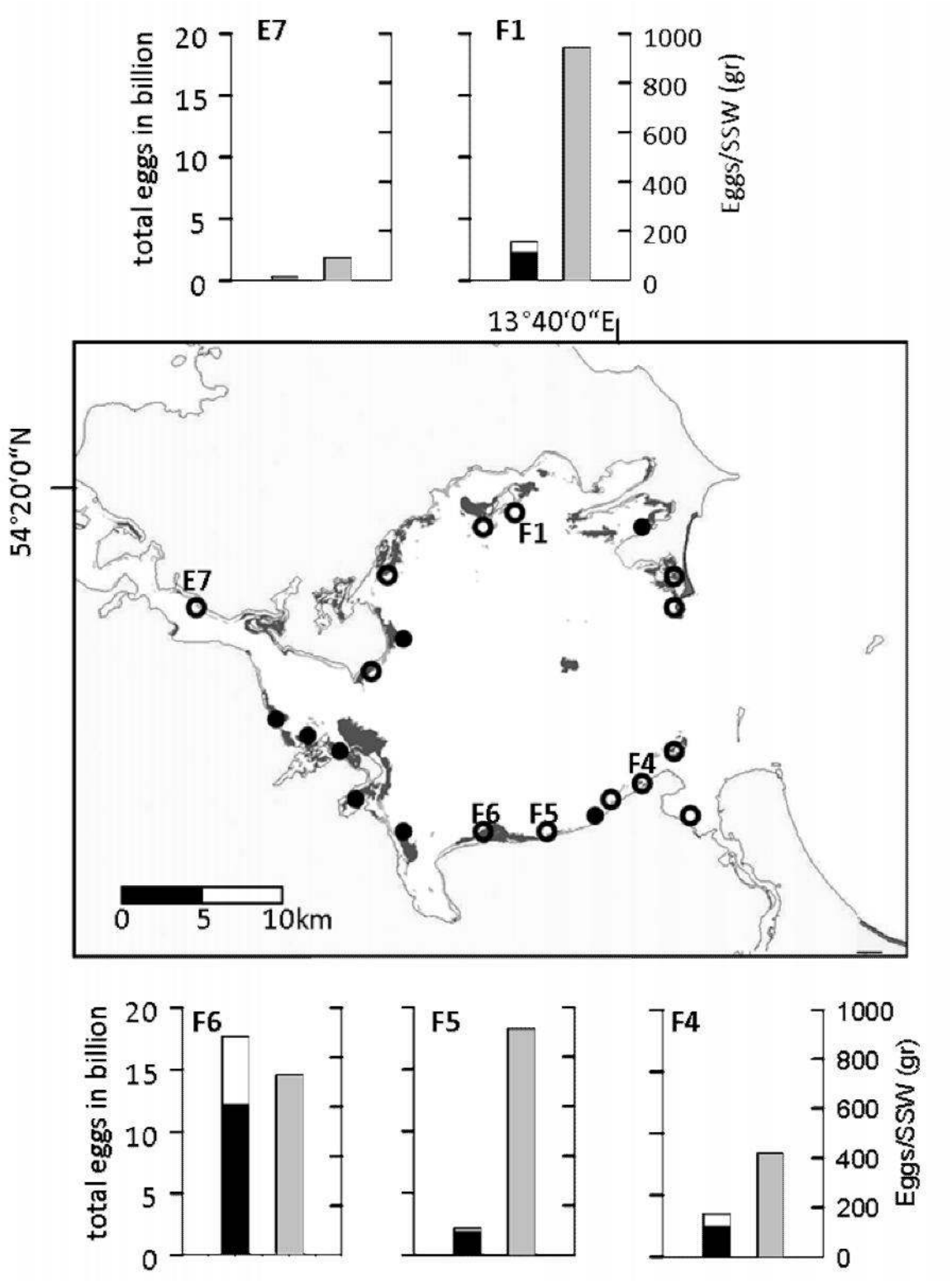


Fig. 5: The map indicates presence of filamentous brown and green algae in the investigated area. Empty circles represent the strong presence of epiphytic brown and green algae. Filled circles indicate presence of algae mats with high ground coverage (mainly *Pilayella sp.* and *Ectocarpus sp.*).
Bar charts display total egg amount of the whole potential spawning site (black bars: dead eggs, white bars: live eggs). Grey bars indicate Eggs/gram plant biomass (gr) within spawning site. Potential spawning areas are marked dark gray (based on Kanstinger et al. in prep.).

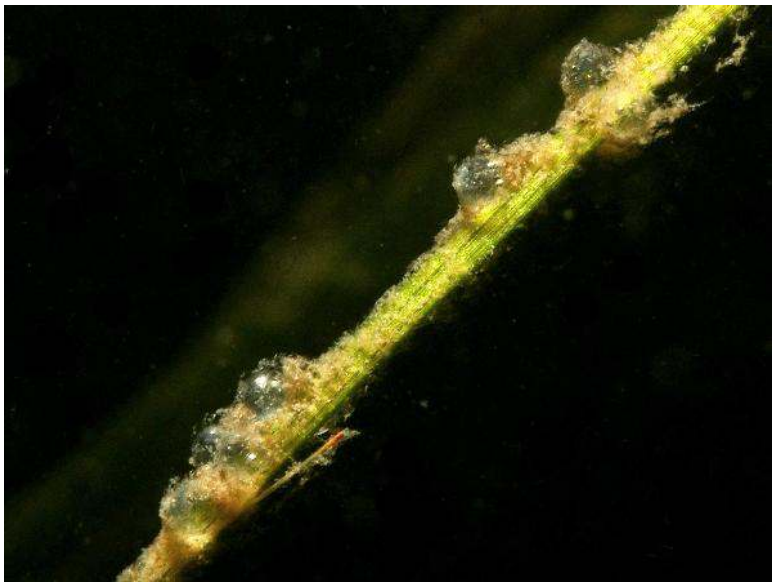


Fig. 6: Herring spawning beds affected by dense algae mats (*Pilayella littoralis*) (station E4), by epiphytic green algae (Chlorophyta) (station F6) and by marine snow (station F1).

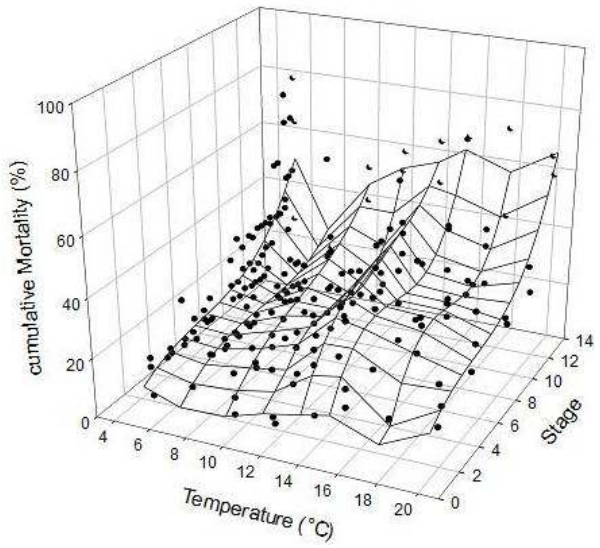


Fig.8 Cumulative herring egg mortality at different development stages and temperatures

Between 3 and 19°C, daily mortality was not constant over time. Instead, it changed during ontogenesis. The first high mortality phase occurred in the very early development stages between fertilisation and gastrula stage (stage 1-4). The second distinct mortality phase appeared shortly before or during hatching when embryos were fully developed (stage 12-14). Figure 8 displays temperature and developmental stage versus cumulative mortality. Time span of complete egg development ranged from 4.5 to 25 days between 19°C and 5°C. For a more complete overview of the results see (Peck et al., 2012)

Herring eggs from location F4 and F5 which were transferred after the first sampling to the University of Hamburg and incubated in a controlled environment under similar conditions (water temperature 10°C; 8 psu), displayed significantly lower mortalities than recorded in the field. An average mortality (± standard deviation) of 27% ± 7 and 38% ± 6% respectively were measured under experimental conditions compared to 74% ± 36 and 91% ± 12 in the field.

Discussion

Water temperatures in the Greifswalder Bodden ranged from 4-5°C to 19°C during the active spawning phase and egg development phase. This thermal window agrees with results from our laboratory temperature experiments and with studies of other authors (Herra, 1986; Blaxter and Hempel, 1961). It also coincidences with observations from various spawning sites of Baltic spring spawner (Scabell, 1989; Aneer, 1989b). It cannot be excluded that local temperature minima or maxima existed during the spawning season in the Greifswalder Bodden especially in the shallow areas which were not captured by the weekly measurements made at the rather offshore larval sampling stations. Moreover, temperatures displayed a higher variability when recorded on a daily basis (see Fig 3. Stahlbrode permanent station). However, Atlantic herring embryos can tolerate, at least over short time periods, a wider range in temperatures between 0.75 and 23°C (Blaxter, 1960). Therefore, a direct mortality due to unfavourable temperatures in the Greifswalder Bodden is unlikely in most years.

Still, increasing water temperature in the late spawning season can lead to increasing mortality rates (Klinkhardt, 1996; Ojaveer, 1981; Rajasilta et al., 1993a; Aneer, 1989a). The effect of temperature on mortality is indirect: relatively high temperatures accelerate the oxidative decomposition of substances in the milieu and also the oxygen requirements of the eggs. These two effects can act synergistically (Rajasilta et al., 1999). In our study we found the opposite trend with a better hatching success later in the spawning season when water temperatures were warmer. But this trend based only on observations of one spawning site which was sampled 3 weeks later than the others and which contained much less dense egg aggregations.

The accurate estimation of total mortality in the field is challenging. Unfortunately, not all eggs from the same spawning event simultaneously hatch due to differences in "micro-environment conditions" such as differences in temperature and oxygen concentration experienced by eggs spawned in differently sized patches. Even under the most controlled conditions, the hatching period can last from 2 to 8 days (Peck et al., 2012; Klinkhardt, 1996). Moreover, our experiment revealed non-constant mortality rates of herring eggs, with two distinct phases (after fertilization and shortly before hatch). High mortality rates at early and late stages of egg ontogenesis were also observed in other studies (Blaxter and Hempel 1963; Galinka 1973) and in other species e.g. Pacific cod (*Gadus macrocephalus*), Atlantic cod (*Gadus morhua*) or yellowtail flunder (*Pleuronectes ferrugineus*) (Geffen and Nash, 2012). There-

fore it is essential that sampling dates are chosen when embryos are about to hatch to obtain total mortality rates.

Based on the findings of broken egg shells in some of our samples we concluded that hatching had already started prior to sampling and these empty chorions were counted as hatched embryos. It cannot be ruled out, that some egg shells detached from the egg clumps or plants prior to, during, or shortly after hatching which would inflate our mortality estimates. On the other hand, empty chorions could be counted as mortalities resulting from predation by invertebrates. Finally, mortality might be underestimated if dead embryos detached from the substrate and were washed away due to waves and water currents (Rajasilta et al., 1999).

Estimations of mortality in early developmental stages are more robust than estimates of total mortality. The share of dead eggs in early development stages was low (2.9 - 6%) on spawning grounds F4-F6 and somewhat higher (22%) at location E1 later in the year. The low numbers of unfertilized eggs and dead early stage embryos observed agree with the results of other field studies from the Baltic Sea in which mortality during the first four stages was reported to be between 1 and 5 % (Rajasilta et al., 1993a; Scabell, 1989).

The average total mortality of 55-91% measured in the GB during the spawning season 2009 was high compared to older studies from the same region and to reports from other spawning areas in the Baltic Sea. In the GB average mortalities were approx. 32% in the years 1986 and 87 (Scabell, 1989), 4 -25 % in the gulf of Riga (Ojaveer, 1981), less than 15% in the inner Archipelago Sea of south-western Finland (Rajasilta et al., 1993a) and 7.8% to 18.4% in Gulf of Finland (Oulasvirta and Lehtonen, 1988). Two other authors only observed similar high annual mortalities. Aneer (1985) reported 33 to 75% mortality during peak spawning time in the Swedish Askö-Landsort area and Kornilovs (1994) found mortalities from 61 to 91 % in the south-eastern Gulf of Riga. Both authors suggested that mainly low oxygen levels caused the low level of egg survival. Interestingly, we were only able to locate one spawning site of the second larval peak, which can be seen in the weekly larvae abundance data from the TI (Fig. 6), although the spawning time coincided with the snapshot transect campaign. Reason for this might be high survival of eggs and therefore smaller spawning beds, spawning beds outside transects or a combination of both. Diving surveys in other years revealed significant lower mortalities.

Given the limited measurements made in this study, it is not possible to determine all factors causing the mortalities observed in the field. A single cause for the high egg mortality is highly unlikely given the large number of potential mortality sources that were not monitored but that may act simultaneously to reduce viable embryos. For example, fertilization success and mortality were reported to differ among eggs produced by different females in Baltic herring (Laine & Rajasilta 1999). However, the significantly higher survival rates of eggs that were transferred to the laboratory for incubation indicate that a solely endogenic factor for the high mortality in situ is unlikely.

The species composition of the plant communities on the spawning beds may affect the reproductive success of the herring as indicated by our GAM results. Field surveys and experimental studies showed that egg mortality can be higher on certain plant substrates, such as *Furcellaria lumbricalis*, Phyllophora species (Rajasilta et al., 1989; Rajasilta et al., 1993a; Rajasilta et al., 2006) or *Pilayella littoralis* (Aneer, 1987) due to toxic algae exudates. Concordantly, eggs attached to red algae species (*Furcellaria fastigiata* , *Polysiphonia nigrescens*) in our study experienced significantly higher mortality rates than spawn attached to vascular plants (*Z. marina, P. pectinatus*). However, both algae species displayed also significant higher egg densities in terms of eggs per gram spawning substratum when compared to vascular plants. When egg density and correspondingly egg mass thickness increases, the inner eggs may be lacking direct contact with surrounding water. This could either result in a restricted oxygen supply or a locally increased concentration of metabolic waste products as previously suggested by McMynn and Hoar (1953). Additionally, dead eggs influence the success of embryonic development of the neighbouring eggs by toxic decomposition substances. Furthermore, infestation of fungi on dead eggs and the spread within the egg clump (Rajasilta et al., 1999) might impede embryonic development. In our analysis, values of egg density based on visual spawning intensity or sampled eggs per gram spawning substratum had the best coefficient of determination for predicting egg mortality. The

correlation between high egg densities and low egg survival was confirmed by numerous field and laboratory studies (Blaxter and Dept, 1956; Hempel and Hempel, 1971; Rannak, 1971; Ojaveer, 1981; Haegele and Schweigert, 1985a; Scabell, 1989).

It has been suggested that density-dependence in herring operates at the egg stage since the substrates they require for spawning may be limited in area (Wood, 1981; Zheng, 1996; Iles and Beverton, 2000) and high spawning stock biomass leads to increased egg layer thickness. This has been demonstrated for Pacific herring but does not appear to be a common occurrence in Atlantic herring (Alderdice and Hourston, 1985; Bunn et al., 2000; Fox, 2001). Under natural conditions, eggs of Baltic herring are deposited sparsely on algae and not in several layers (Rannak, 1958). In contrast, we found on most spawning sites in the Greifswalder Bodden relatively high egg densities and eggs were often spawned in several layers. Therefore we hypothesize that a density-dependence might occur in the Greifswalder Bodden where underwater meadows suitable for spawning now only cover < 8% of their historical extent due to eutrophication-related processes (Kanstinger et. al. in prep.). Especially in years like 2009, when after a cold winter and a rapid warming of waters in March, adult herring receive a strong temperature signal which appears to synchronize their migration to the spawning sites in the GB and results in a relatively short, intense spawning season (Klinkhardt, 1996).

An additional eutrophication related source of mortality seemed to be blooms of filamentous epiphytic green- and brown algae and drifting brown algae mats (*Pilayella littoralis* and *Ectocarpus sp.*) that were found in many of the investigated spawning areas. Extensive mortality of benthic fish eggs can result from exposure to low dissolved oxygen concentrations during calm periods in areas with high macrophyte or macroalgal production (Breitburg, 2002). For the Baltic-Kattegat system, Aneer (1985) reported a strongly decreased survival of herring eggs due a combination of low oxygen and toxic exudates in dense stands of *Pylallelia littoralis*. Although Atlantic herring eggs can withstand unfavourable oxygen conditions for a short time period during early development (Braum, 1973), they might be susceptible at late development stages when embryonic activity and oxygen consumption reach their maximum (Braum, 1985; Rombough, 1988). The dissolved oxygen measurements during our study indicated no hypoxic or anoxic conditions, but these data were taken in offshore areas during daytime. It cannot be excluded that local minima in dissolved oxygen existed in the shallow spawning beds. Low oxygen concentrations may develop for short periods of time, especially during night. Diel-cycling hypoxia occurs within the shallow photic zone and is mainly driven by respiration by algae and can last for minutes to hours. In situations with high algal density, heavy cloud cover and calm wind these hypoxic events can be prolonged to several days (Tyler et al., 2009).

In addition to high egg densities due to low availability of suitable spawning substrate or local oxygen depletion due to blooms of filamentous algae, also another eutrophication related mechanism might cause elevated egg mortalities in certain years: Herring spawning beds are nowadays concentrated in relatively shallow waters and are more exposed to wave action during storm events. While egg losses due to storm events were not observed in 2009, the detachment of all dead eggs in location F5 proved that such events are possible and it is known for Pacific herring that wave action can lead to substantial egg losses (Haegele and Schweigert, 1985b).

However, low egg survival does not seem to be a constant problem in the GB. In 2008, a spawning bed located close to location F1 with low egg density and water temperature around 18°C had a total (visible) mortality of 34%. Mortalities of approx. 30 % were recorded in two spawning beds (locations F6 and E6) with intermediate to high egg densities at 12-14°C water temperature in 2011 (Patrick Polte, TI; personal comm.).

Since robust spawning stock biomass estimates for western Baltic spring spawner are available only from 1991 onwards (Cardinale et al., 2009), it is difficult to assess the role of increased egg mortality rates caused by eutrophication processes which started earlier and reached their current maximum in the 80´s. Nonetheless, our results support the hypothesis that eutrophication-related processes like the loss of spawning habitat and increased abundances of filamentous fast growing algae can lead to increased mortalities during egg development. The effect of particular mortality mechanisms during the egg phase might well vary between years and future research is needed to further evaluate the influ-

ence of the single stressors and potential synergistic effects. Results of the present study suggest that it will be important to monitor temperature and oxygen concentrations within the shallow spawning beds and to examine the impact of anoxia on late egg stages.

Acknowledgements

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Chapter 4

Thermal windows supporting survival of herring egg and larvae

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Abstract

Projecting climate-driven changes in marine systems will require knowledge on how thermal windows affect the vital rates of key species. To examine the potential, direct effect of climate-driven warming on southwest Baltic herring, we quantified the survival, development, and biochemical condition of embryos (eggs and yolk sac larvae) at ten temperatures between 2.9 and 21.7°C. Viable hatch was highest from 7 to 13°C, <20% at 2.9°C and 0% at 21.7°C. Between 5 and 19°C, increasing temperature (T) decreased the time to 50% hatch (Ht, hrs,): $H\text{t}=4461.9*T^{-1.24}$ ($r^2=0.98$, $p<0.0001$). Using degree-days (°d=T (°C)*age (d)) could normalize some thermal effects. Most hatching occurred 90 to 120 °d post-fertilization, unfed larvae lost 0.33 µg dry mass *(DM)* d^{-1}, larvae did not survive > 160 °d-post-hatch. RNA-DNA ratios rapidly decreased between 50 and 80°d-post-hatch whereas $DNA*DM^{-1}$ increased throughout the yolk sac phase and likely provides a stronger indicator of irreversible starvation. The critical, "mixed feeding" stage appears to be 60 to 100°d post-hatch. The broad thermal tolerance of herring embryos makes "direct", negative effects of warming unlikely; however, a lack of common methods among studies makes it difficult to project how climate warming will affect embryos of different fish populations and species.

Keywords: eggs, yolk sac larvae, temperature, survival, Baltic, herring

Introduction

The physiological responses of organisms to key environmental factors such as temperature are being examined with renewed vigour to gain a "cause and effect" understanding of the impacts of climate change on marine poikilotherms (Pörtner and Farrel, 2008). Understanding the thermal niche of a species and how additional environmental factors reduce or enlarge that niche is a fundamental prerequisite to understanding habitat requirements and how the productivity and distribution of species may be altered due to climate change (Pörtner and Peck, 2010). For species inhabiting brackish waters such as the Baltic Sea, salinity can be an important "masking factor" (Fry, 1971), one that acts to increase or decrease metabolic costs which changes the amount of energy available for growth and thermal windows of organisms in a variety of taxa from crustaceans (Kinne, 1971; Holste *et al.*, 2009) to various life stages of fish (for review see Boeuf and Payan, 2001).

Atlantic herring displays impressive differences in life history scheduling and physiological tolerance (Geffen, 2010) allowing different populations to spawn during the autumn, winter, spring or summer in shelf areas of the Northeast Atlantic and Baltic Sea. Herring produce demersal eggs that are attached to specific substrates at spatially-discrete spawning grounds used annually with varying intensity (Schmidt *et al.,* 2009; Nash *et al.,* 2009). In the southern Baltic Sea, the peak of spring- and autumn-spawning occurs in May and early September, respectively, and spawning grounds are typically shallow, coastal areas supporting the growth of macrophytes (Aneer, 1989)., Developing embryos of Baltic herring often experience a wide range of temperatures, particularly in the spring (Oeberst *et al.*, 2009a) and processes acting on young larvae (to 20 mm *SL*) appear to establish recruitment success (Oeberst *et al.*, 2009b). Furthermore, temperatures experienced during this early larval period have been significantly correlated with year-class success (Margonski *et al.*, 2010).

The objective of the present study was to quantify the effect of temperature on the survival, time to hatch, and changes in biochemical condition (nucleic acids) of embryos (egg and yolk sac larvae) of southwest Baltic herring. By employing a wide range in temperatures (3 to 22°C), we hoped to gain a more complete picture of optimal and sub-optimal temperatures (the thermal window) for early embryonic survival of herring in the southwest Baltic Sea. We discuss these and similar data collected on herring embryos from other Baltic sub-regions (Ojaveer, 1981; Herra, 1986; Laine and Rajasilta, 1999) and on other key Baltic fishes (e.g., Atlantic cod, *Gadus morhua*) and sprat (*Sprattus sprattus*). In light of efforts to project climate change impacts, we also comment on the temperature-dependent timing of critical periods during early ontogeny for herring in the southwest Baltic Sea and, more generally, on methods utilized to quantify thermal effects in fish early life stages.

Materials and Methods

Adult herring spawning and embryo incubation

Adult female and male herring were collected from a live trap in Kiel Bight (50.3°N, 10.13°E), transferred on ice to the University of Hamburg and strip spawned. In an attempt to minimize potential bias due to maternal effects (see Geffen and Nash, In Press), eggs from six females were fertilized using the milt from six males. The mean(±sd) fork length and wet mass for females were 22.5(0.9) cm and 165.8(14.6) g while males were 23.1(1.2) cm and 145.1(22.5) g. Eggs were stripped spawned in three (single egg) rows on one side of a square glass plate. Each plate contained the eggs of two or three different females. Egg plates were incubated with a mixture of milt from all males at 8.0°C and 19.0 psu for 15 min and then plates were put into water with no milt for an additional 15 min. This procedure yielded high (75 to 90%) fertilization success on egg plates.

Development of pre- and post-hatch embryos

For the incubation experiment, an attempt was made to utilize only plates with similar numbers of eggs and only those having eggs in single rows (no clumps). Egg plates were randomly distributed into 1-l incubation tanks (having 900 ml water) placed within a thermal gradient table (Thomas *et al.*, 1963), an aluminum block that was heated and cooled at separate ends by pumping temperature-controlled water through drilled channels. A total of 30 replicate (an egg plate within a tank) was used (3 tanks x 10 temperatures). All tanks were initially 8.0°C and the tem-

perature was adjusted within < 8 h to the final test temperature.

Fertilized eggs (embryos) were incubated at mean (±range) measured temperatures of 2.9, 4.9, 7.1, 9.5, 11.5, 13.6, 15.3, 17.4, 19.9 and 21.7 (±0.2) °C. The mean (± range) in water salinity was 19.0(±1.0) psu. Frequent stirring and water renewal eliminated any thermal stratification and maintained dissolved O_2 levels at saturation. Eggs were incubated using a 14L:10D light regime. During the experiment, eggs were counted and developmental stage was assessed on a degree-day (°d = temperature (°C) * time (d)) schedule. Every 10 to 12°d (e.g., every 0.5 d at 20°C and 2.0 days at 5°C), the eggs in each tank were checked under a dissecting scope for developmental stage, live and dead eggs were counted and new water at the test temperature was provided. Unhatched embryos were categorized into one of 13 stages (stage 14 = hatch) based upon the developmental series presented by Klinkhardt (1996) for Baltic herring, as opposed to the six stages utilized for North Sea embryos (e.g., Baxter, 1971).

Larvae were sampled at hatch (every 2 h) from each tank and their standard length (*SL*) was determined from digital photographs using image analysis (Leica MZ 16, Opitmas TM software). Directly after 50% hatch (the bulk of egg hatching on any day occurred about two h after the onset of darkness), the remaining eggs in one of the three replicate tanks at each temperature were discarded and newly-hatched larvae from the three replicate tanks were combined into the tank (transferred with a large mouth pipette) for continued incubation at the same temperature. Ten larvae from this tank were randomly removed every 10°d and rapidly transferred to individual cap vials and frozen at −80°C. Later, each larva was freeze-dried (Christ Alpha freeze drier, > 12 h) and its dry mass (*DM*) (Sartorius microbalance SE2-0CE, ± 0.1 µg) and bulk nucleic acid contents (see below) were measured.

Biochemical measurements

The bulk nucleic acid content of whole, individual yolk sac larvae was analyzed using a fluorescent-dye, microplate assay, modified after Caldarone *et al.* (2001). In short, a freeze-dried larva was homogenized in 1 % Sarcosil-Tris-EDTA-buffer using an ultrasonic disruptor, the samples were diluted with Tris-EDTA-buffer and loaded into 96-well plates with an aliquot of Ethidium-bromide (EB) stock solution, resulting in a final EB concentration of 1 µg*mL^{-1} and Tris-EDTA-buffer of 0.1%. Fluorescence of nucleic acid – EB complexes was measured at 520/605 nm (excitation/emission) in a microplate fluorometer (Xenius XC, SAFAS). Subsequent addition of specific restriction enzymes (R 6513 and D 4263, Sigma Aldrich) eliminated RNA and DNA from the samples. Concentrations were determined based on calibration curves using highly-purified 18S + 28S rRNA from calf liver and calf thymus DNA (R 0889 and D 4764, Sigma Aldrich). A standard homogenate (prepared from a large group of yolk sac larvae) was also measured on each microplate, providing an internal check on the accuracy of the assay.

Statistics

ANOVA's tested for significant differences in the mean number of embryos incubated among temperatures (to ensure random loading of replicates) and whether survival was affected by the number of embryos on plates (regardless of temperature). The time to peak (50%) hatch, total hatch success (HS, %), and the time course of hatching were calculated for n = 2, 2 and 3 tanks, respectively, at each temperature. The differences in tanks numbers was due to the sacrifice of one replicate tank at the time of peak hatch to allow incubation of a group of newly-hatched larvae (collected from all three tanks) and measurement of changes in larval DM and RD during the endogenous feeding period at each temperature. Although only one tank of yolk sac larvae was available at each temperature, pseudoreplication was avoided by pooling data from all temperatures into a combined analysis (via linear and non-linear regression analyses). In these regressions, time (age) was expressed in °d. All regression analyses were performed using SigmaPlot 9.0 (SPSS, 2001) or R (R Development Core Team, 2009). Parameter estimates were obtained using the sum of squared errors and regressions were considered significant at the alpha ≤ 0.05 level.

Results

Thermal Effects and Pre-hatch Development of Embryos

The mean (±SD) number of eggs on plates at each temperature was 337(±71) and there was no significant differences in the number of eggs incubated at each temperature (ANOVA, df = 9,

F = 0.65, P = 0.74) nor a relationship between percent hatch and the number of eggs incubated (across all temperatures). Egg development rates were significantly affected by temperature (*T*) and the time (*t*, h) required to reach each developmental stage (*ST*, 1, 2, 14, where 14 = hatch) was well explained by the function:

$$t_{STAGE} = 5.86(\pm 0.91) * ST^{-0.0495(\pm 0.002) * T + 1.964(\pm 0.066)}$$

where mean(±SE) parameter estimates are provided (n = 84, r^2 = 0.961, p<0.0001.) (Fig. 1). For example, embryos reached stage 7, the mid-point between fertilization and hatch, after ~20 and 120 h at 18.0 and 7°C, respectively.

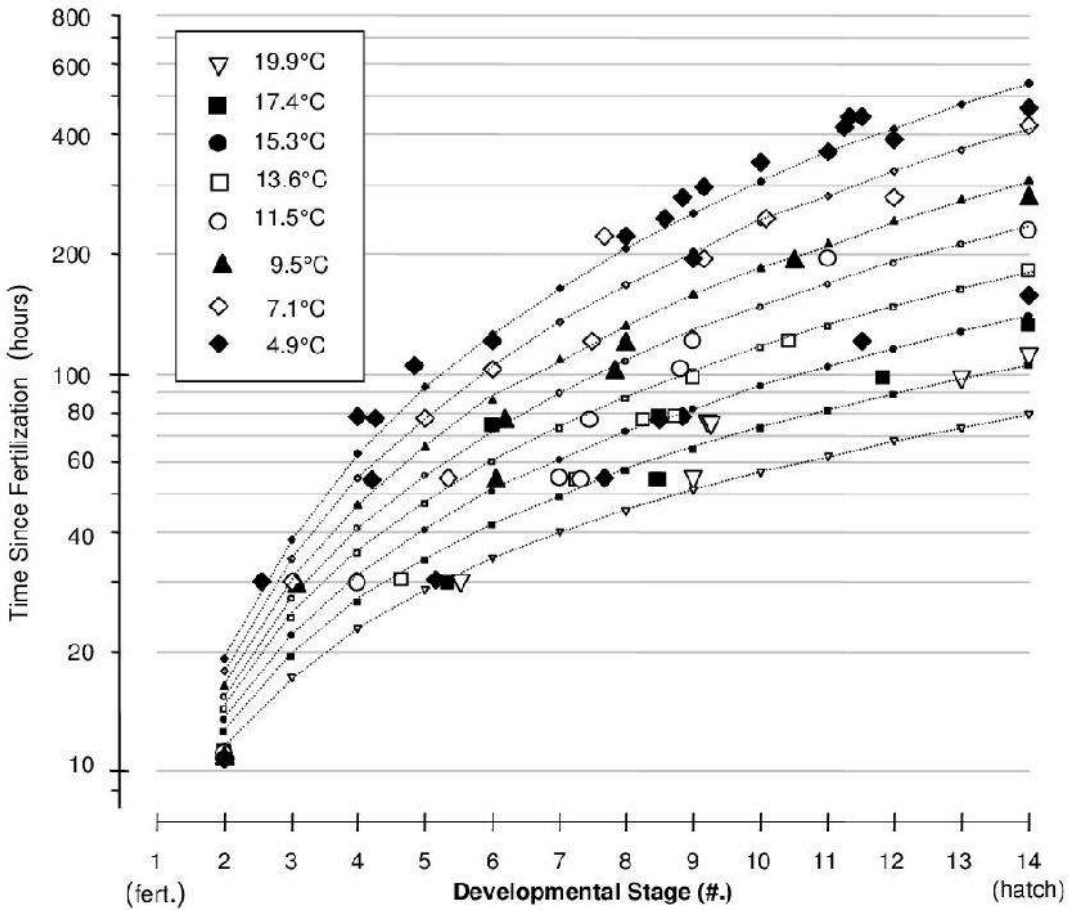

Fig. 1: Time required to reach different embryonic stages in southwest Baltic herring at each of eight different temperatures (different open and filled symbols) between 5.1 and 19.7°C. The regression equation was significant (p < 0.0001). Embryonic stages were based upon Klinkhardt (1996). The large symbols are observations, the small symbols refer to the temperature of each regression line. Note, data from the 2.9 and 21.7°C treatment groups are not shown. All embryos died in latter and only a few embryos survived in the former treatment group.

Thermal Effects and Hatching Characteristics

The percent hatch of embryos was variable but highest at intermediate temperatures (5 to 17°C) with relatively low hatching success (~15%) at 2.9°C and zero (no hatch) at 21.7°C (Fig. 2a). A 3-segment regression indicated low and high critical temperatures (join points of segments) at 2.8 and 18.2°C, respectively. Between 4.9 and 19.9°C, the effect of water temperature (°C) on the time to peak hatch *HP* hours post-fertilization) was well described by an allometric function:

$$HP = 4461.9(\pm 322.4) * T^{-1.232(\pm 0.031)}$$

where mean(±SE) estimates are provided (n =21, r^2 = 0.988, p<0.0001) (Fig. 2b). Between 4.9 and 19.9°C, the mean length of larvae at hatch decreased with increasing temperature from 7.5 to 5.5 mm *SL*. The decrease with increasing temperature could either be interpreted in a linear or nonlinear manner and regression equations are provided for both interpretations (Fig.2c).

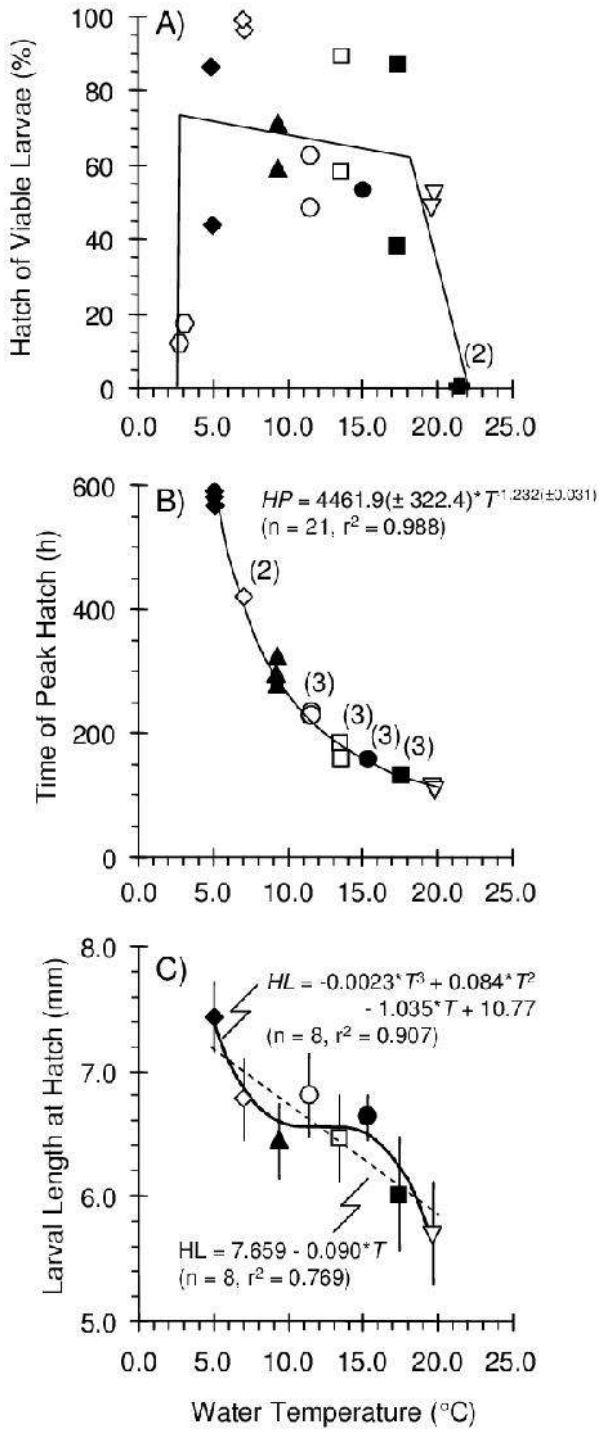

Fig. 2: The hatch success (panel A), duration of time required to reach peak (50%) hatch (panel B) and the larval length at hatch (panel C) for southwestern Baltic herring embryos. All regression equations were significant ($p < 0.001$). Panel A segmented regression: EQ1 H%= (0.22*(2.83-T) + 0.73*(T-t1))/(2.83-t1); EQ2H% = (0.73*(18.17-T) + 0.62*(T-2.83))/15.34; EQ3H%= (0.62*(t3-T) + 0.05*(T-18.17))/(t3-18.17), where t1 = min. T, and t3 = max. T, (n = 19, r2 = 561, p = 0.027). Panel A displays all 10 temperature treatments whereas panels B and C do not display data for embryos at the coldest and warmest temperatures due low (or no) survival.

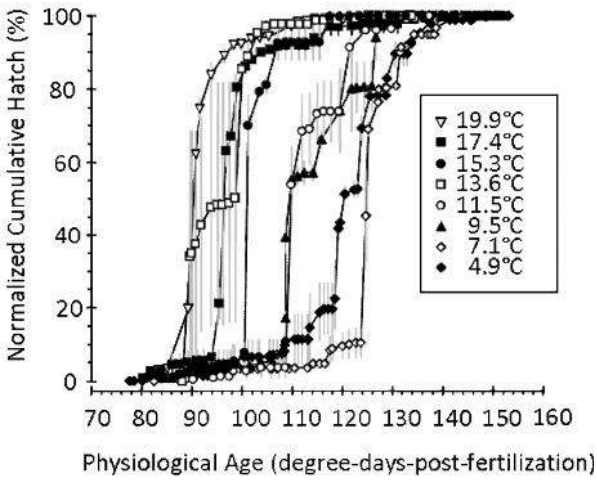

Fig. 3: Cumulative hatching (%) versus physiological age (degree-days post-fertilization) for southwest Baltic Sea herring embryos incubated at each of eight, constant temperatures (denoted by different symbols). Error bars represent the range of values based upon the 2 replicates at each temperature. Note, data from the 2.9 and 21.7°C treatment groups are not shown. All embryos died in latter and only a few embryos survived in the former treatment group.

At each temperature, embryos did not all simultaneously hatch. Hatching first occurred at 81°d and continued until ~140°d post-fertilization with the vast majority of hatching occurring between 92 and 124°d post-fertilization (Fig. 3). In this case, differences among temperature treatments in the age embryos at hatch were still apparent after expressing time in °d; embryos in the warmest (coldest) temperatures hatched first (last).

Thermal Effects and Post-hatch Embryos

The mean (±SD) dry mass (*DM*) at hatch was 85.4(±0.4) µg (averaged across temperatures). A regression of larval *DM* versus time (age, °d) predicted that unfed larvae lost 0.33 µg per °d and were no longer viable at ~110 to 140°d (Fig. 4a, see next page). Two biochemical measures (RNA-DNA ratio, and $DNA{\cdot}DM^{-1}$) changed in consistent ways among temperature treatments during the yolk sac larval period. RNA-DNA ratios decreased in a non-linear way with increasing physiological age (°d) and were half their initial value after 59°d post-hatch (Fig. 4b). RNA-DNA ratios remained unchanged and at were low after 120°d post-hatch. A linear increase with time was observed in $DNA{\cdot}DM^{-1}$ with initial values between 8 and 12 and final values as high as 20 to 25 (Fig. 4c).

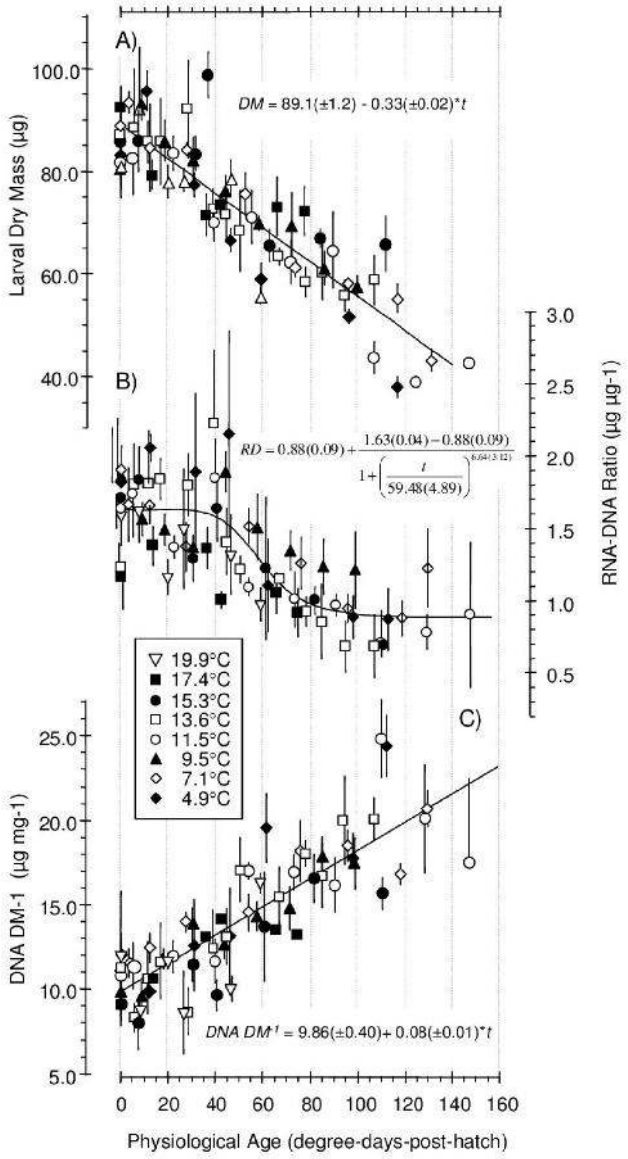

Fig. 4: Changes in herring dry mass (DM, panel A), RNA-DNA ratio (RD, panel B) and DNA per unit DM (DNA*DM-1, panel C) of yolksac larvae versus time (t, physiological age in degree-days (°C*days) post-hatch) at each of 8 temperatures between 4.9 and 19.9 (denoted by different symbols). All regressions were significant: Panel A) n = 67, r^2 = 0.812, p < 0.0001, panel B) n = 60, r^2 = 0.612, p< 0.01; panel C) n = 67, r^2 = 0.724 p < 0.0001.1982) of various studies have been thoroughly reviewed (e.g., Rombough, 1996; Kamler, 2002; Burt et al., 2010; Geffen and Nash, In Press). A common pitfall of experimental designs is that they do not include the full range of temperatures (including those that are lethal) experienced in the wild and, thus, they cannot adequately describe the thermal niche of the target species (Pörtner and Peck, 2010). A case in point is the research on herring embryos in which previous studies have not included the full range of temperatures experienced during spring and autumn spawning in the Baltic Sea. Furthermore, water salinity ranges from ~18 to 0 psu in different areas of the Baltic and is an additional factor that influences thermal windows (Klinkhardt, 1986).

Discussion

Climate change will have both direct and indirect effects on fish (Rijnsdorp et al., 2009; Pörtner and Peck, 2010) and a basic consideration is the effect of temperature on the timing and success of key developmental events during early life (e.g., the hatching and yolk absorption by embryos,). Numerous studies have evaluated the effect of temperature on the survival and rates of embryonic (egg and yolk sac larval) development of specific marine fish species (e.g., Lasker, 1964; Ryland *et al.*, 1975; Coombs and Mitchell, and the results of various studies have been thoroughly reviewed (e.g., Rombough, 1996; Kamler, 2002; Burt *et al.*, 2010; Geffen and Nash, In Press). A common pitfall of experimental designs is that they do not include the full range of temperatures (including those that are lethal) experienced in the wild and, thus, they cannot adequately describe the thermal niche of the target species (Pörtner and Peck, 2010). A case in point is the research on herring embryos in which previous studies have not included the full range of temperatures experienced during spring and autumn spawning in the Baltic Sea. Furthermore, water salinity ranges from ~18 to 0 psu in different areas of the Baltic and is an additional factor that influences thermal windows (Klinkhardt, 1986).

In the following, we discuss the results of the present and previous studies on Baltic herring, using this species in this region as a model for broader implications for studies attempting to reveal the direct and indirect effects of climate on the earliest life stages in fishes.

For Atlantic herring embryos, the developmental rates (times to hatch) reported at similar temperatures often differ among studies (Table 1). Herring hatched between 96 and 118°d (SW Baltic) in the present study, but shorter (66 to 75 °d, Gulf of Riga), similar (97 to 128°d, Gulf of Gdańsk), and longer (130 to 160°d, Gulf of Finland) times to hatch have been reported (Ojaveer, 1981; Herra, 1986; Laine and Rajasilta, 1999). Differences between the results of these studies cannot be explained by differences in salinity and appear to be due to methodological differences among the studies. Blaxter and Hempel (1963) reported that 130 to 150°d were needed at lower (5.5 to 8.5°C) and 100 to 120 °d at warmer (11.3 to 14.6°C) temperatures. Thus, studies utilizing different thermal ranges will likely provide different estimates of temperature effects. However, differences in reported development times

Tab. 1: Summaries of Studies performed on Baltic herring (Clupea harengus) investigating the effect of temperature on key developmental events.

			Timing of key developmental event (°d)			
Spring spawning stock	Salinity (psu)	Temperature (°C)	Hatching	Yolk absorbed	Starvation	Reference
Gulf of Riga	6.0	7	82[a]	146	–	Ojaveer (1981)
	6.0	12 and 17	67–69[a]	125 (17°C)	–	
	6.0	3, 7, and 12	75–81[a]	–	–	
	6.0	17	61[a]	118	–	
Gulf of Finland	6.0	10	139–204[b]	–	–	Laine and Rajasilta (1999)
Gulf of Gdańsk	6–8	3	97[c]	–	–	Herra (1986)
	6–8	6.4 and 8.0	127[c]	–	–	
	6–8	12	109[c]	–	–	
Western Baltic	3–10	4	160[c]	–	–	
	3–10	8 and 11	120[c]	–	–	
	8–10	4–14	100–136[c]	181 (8°C)	–	Klinkhardt (1986)
	15–35	5.5–8.5	130–150[c]	176 (8°C)	230–280	Blaxter and Hempel (1963)
	15–35	11.3–14.6	100–120[c]	–	240–264	

Time is expressed in degree-days [°C × time (d)].
[a]Time to first hatch.
[b]Time of first to last hatch.
[c]Time to 50% hatch.

among these Baltic studies may also be due to differences in the definition of "hatch" since a time span of at least 30°d was observed from the onset to end of hatching at most temperatures in the present study (see Fig. 4).

A secondary consideration of climate change is the effect of warming on key characteristics of larvae such as their size at hatch. In the present study, herring larvae were 5.5 to 7.5 mm *SL* at peak (50%) hatch and *SL*-at-hatch decreased with increasing temperature. Similar sizes-at-hatch were previously reported at intermediate temperatures (e.g., 5 to 12°C, 6.0 to 6.6 mm *SL*) by Blaxter and Hempel (1963) and Herra (1986). At 3°C, Herra (1986) reported that larvae were smaller but that the percentage hatch was extremely poor (~5%) which agrees with our results at that temperature (10 to 18% hatch). Body size at hatch has been demonstrated to increase (Alderdice and Forrester, 1971), decrease (Apostolopoulos, 1976) and remain constant (Lasker, 1964; Coombs and Mitchell, 1982) with increasing incubation water temperature. The differences in these trends may be related to a species' thermal window; with increasing temperature, larval size at hatch of species inhabiting relatively cold (1 to 10°C), intermediate (10 to 20°C) and warm (20 to 25°C) waters tend to increase, remain unchanged, and decrease, respectively (M.A. Peck, unpublished data compilation). Differences in the size of larvae at hatch are likely due to differences in the conversion efficiency of yolk proteins into somatic growth and that hatching of embryos can occur at more (or less) advanced developmental stages (Geffen, 2002).

A critique of the present study is that detailed morphometric measurements of yolk sac larvae were not made. Furthermore, although temperature can influence the size-at-hatch of marine fish larvae, maternal / parental effects can play a larger role as previously shown for herring (Blaxter and Hempel, 1963; Panagiotaki and Geffen, 1992; Evans and Geffen, 1998) and it is unknown whether parental effects interact with temperature to affect yolk utilization. Although this study utilized groups of females and males to provide fertilized embryos, adult herring spawning condition (e.g., lipid content) can display interannual variability (Laine and Rajasilta, 1999) with known consequences for embryo hatch success but unknown consequences for ranges in thermal tolerance of embryos.

In marine fish populations, large losses often occur during the mixed feeding period, a time when embryonic yolk reserves are nearly (or completely) exhausted and larvae are learning to forage for prey (Hjort, 1914; Cushing, 1990). Understanding how the timing of this mixed feeding period is influenced by temperature is critical for assessing climate-driven match-mismatch dynamics (Rijnsdorp et al., 2009). For our work on herring, in lieu of yolk sac measurements, we measured biochemical growth indices of protein-specific growth rate and nutritional condition. RNA-DNA ratios (*RD*) decreased to 50% of their initial values by 59°d post-hatch (see Fig 5c). The

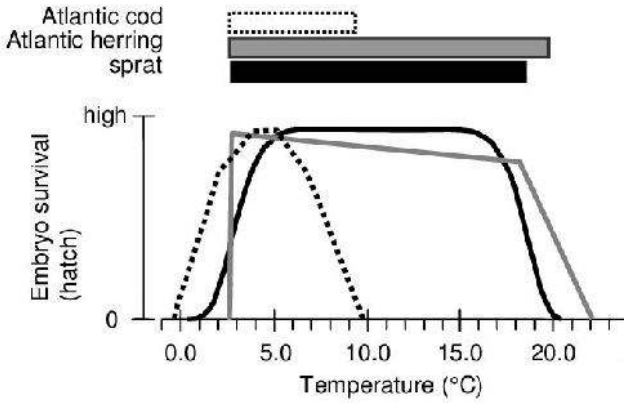

Fig. 5: Direct effects of temperature on embryo survival. Thermal windows supporting the survival and hatch of embryos of three, key Baltic Sea fishes: herring (this study), Atlantic cod (Geffen et al., 2006) and sprat (Peck et al., submitted). In each case, embryo survival reported in studies has been normalized (from 0 to 1.0).

timing of this biochemical change agrees well with the time of 95% yolk sac depletion (60 - 67°d) reported by Blaxter and Hempel (1963) at 8°C and Bang *et al.* (2007) at 12°C and to the temperature-dependent yolk sac duration (YS_{DUR}) calculated by Fey (2001). We speculate that timing of the rapid decrease in *RD* coincides with the onset of the "mixed feeding" stage, when larvae are relatively poor feeders and have utilized a large percentage of their yolk sac. In herring, the size of the yolk sac needs to be sufficiently depleted to allow the oesophagus to open so that prey organism can pass through it (Busch, 1996).

In the present study, newly-hatched herring larvae survived for 110 to 140 °d when incubated at temperatures between 4.9 and 19.9°C. Unfed yolk sac larvae from spring spawners in the Kiel bight have been reported to survive 127 to 153°d (15 to 18 days at 8 to 9°C) (Blaxter and Hempel, 1963). Herring are larger at hatch in other areas and contain larger yolk reserves allowing them to survive a bit longer. For example, Norwegian autumn-spawning and winter-spawning Downs herring survived 144 to 209 °d (8 and 12°C) and 187 to 247°d (8 to 9°C), respectively (Blaxter and Hempel, 1963; Bang *et al.*, 2007).

The "point of no return" (*PNR*), when larvae offered prey are too weak to feed and/or survive (Overton *et al.*, 2010), is a more critical threshold and was reported to occur at 100°d post-hatch for Baltic spring-spawning herring (Blaxter and Hempel, 1963). In Pacific herring, McGurk (1984) observed signs of starvation at 72 to 90°d post-hatch (6°C*12d, 8°C*11d, 10°C*9d). In this study, values of *DNA DM*$^{-1}$ > 17 occurred after 100 °d post-hatch, suggesting that *DNA DM*$^{-1}$ may provide a good biochemical proxy to use on field-caught larvae to identify individuals that are in poor condition and are beyond the *PNR*.

Climate change will influence the early survival of marine fishes by altering levels of abiotic factors (direct effect) and by disrupting the timing of key events (indirect / trophodynamic effects). Our results suggest that it is unlikely that climate-driven warming will cause direct mortality of herring embryos in the southwest Baltic due to developmental failure at warm temperatures. The broad range in temperatures tolerated by herring embryos is illustrated by comparing embryonic survival of herring and two other key, Baltic Sea fishes, Atlantic cod and sprat (Fig. 5). Embryos of cod have the most narrow range in temperatures suitable for development (Geffen et al., 2006) while sprat has a slightly narrower range in temperatures and does not successfully develop when waters are warmer than 17°C (Peck *et al.*, submitted).

It was beyond the scope of the present study to examine indirect effects of climate such as temperature-dependent changes in phenology and match-mismatch dynamics with prey, but our dataset does allows us to predict the timing and duration of critical periods in the early life of herring (Fig. 6). First, results of this study suggests that waters <5 and >20°C will cause direct mortality of herring embryos, which constrain profitable spawning periods. Results of various studies suggest an important period between 60 (yolk sac absorption) and 100°d post-hatch (point of no return) when a larvae must match with sufficient prey resources. For Kiel Fjord (the region where herring adults were collected for this study), average daily water temperature from 2004 to 2010 would not cause direct mortality of herring embryos during a 100 d period in the spring (day 90 and 190) while autumn spawning could occur between day ~220 to 330 (Fig. 6).

In the southwest Baltic, cool temperatures in early spring and late autumn provide a large window of opportunity for developing larvae to start feeding whereas herring have only a few days to start feeding at warm temperatures at the beginning and end of summer. Despite reduced time windows for successful first feeding in the late spring, larval herring appear to survive and growth well during that period (Oeberst *et al.*, 2009b). For autumn-spawned larvae in the Baltic Sea, interactions between water temperature, prey abundance, larval body size, and the

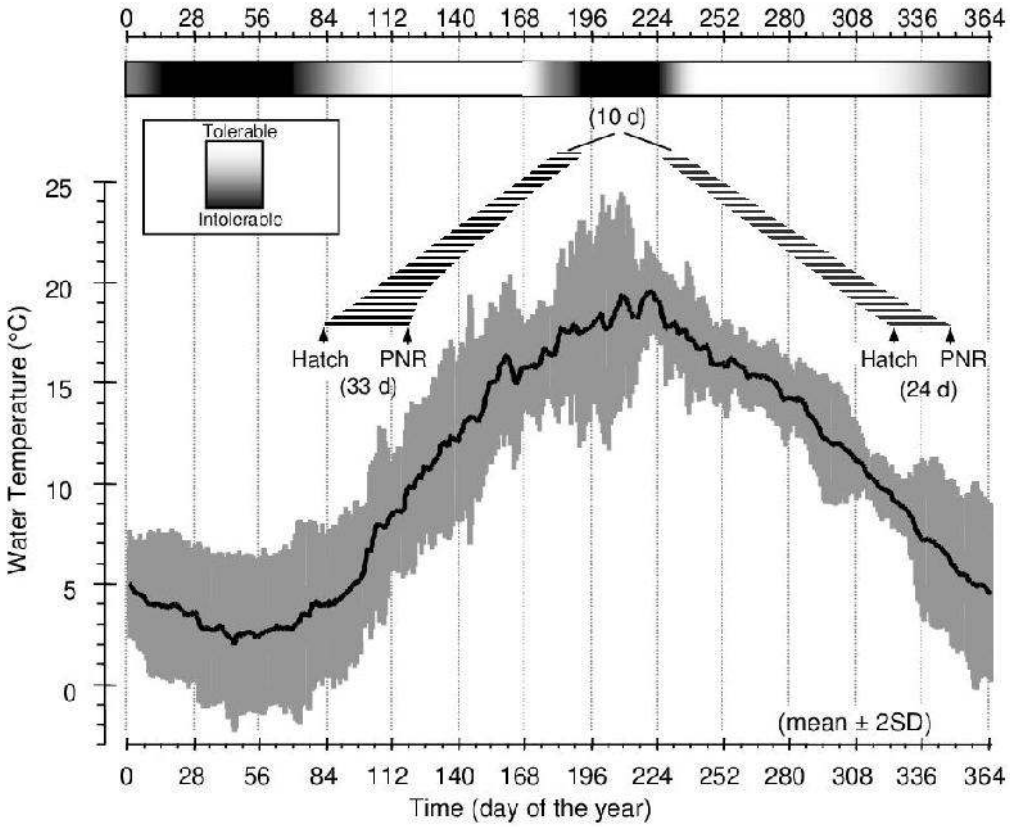

Fig. 6: Seasonal changes in the timing of spawning and potential feeding periods of southwest Baltic (Kiel Fjord) herring based upon the effects of temperature on herring embryos. The mean (±2SD) daily water temperatures are shown for the years 2006 to 2010 (n = 5). The shaded bar (top) indicates time periods when temperatures (on average) were tolerable or intolerable for survival of embryos. Theoretical durations of time between spawning (spawn) and the point of no return (PNR) are shown (horizontal lines) and were calculated from the mean, daily temperature climatology.

degree of overwinter mortality have not been studied. Overwinter mortality can be a strong factor constraining the range of latitudes (or altitudes) inhabited by fish species (Conover, 1992; Lankford and Targett, 2001).

In summary, we examined the effect of water temperature on southwest Baltic herring embryos and revealed clear, upper and lower thermal thresholds for survival, summarized the effect of temperature on developmental and biochemical changes, and compared these and other results of other studies conducted on Baltic herring embryos.

We recommend measuring *DNA DM*$^{-1}$ to detect young larvae beyond the point of no return. We demonstrated that a degree-day approach was an effective way to normalize certain aspects of growth physiology (e.g. see Neuheimer and Taggart, 2007) such as biochemical / metabolic changes of herring embryos at different temperatures. However, the use of degree-days has its shortcomings (e.g., see Bonhomme *et al.*, 2000) as we demonstrated for the timing of hatch windows; utilizing degree-days does not appear suitable at extreme temperatures - those that might be most interesting to examine in the context of climate change,

Potential pitfalls in methods commonly used to estimate thermal effects on fish embryos were recently highlighted by Geffen and Nash (In Press). That study provided advice on collecting data needed to apply egg production methods to estimate spawning stock biomass and highlighted stock-specific differences, the influences of source material, and inherent variability in the effects of temperature on development rate (Geffen and Nash, In Press). From the perspective of revealing direct effects of climate change, the present study also highlights the need for utilizing standard methods to address the central issue of how thermal sensitivity and adaptive capacity change across populations and species (e.g., Somero, 2010). In our example, one might expect thermal windows to differ between the SW and NE populations of herring that are genetically separate (Jørgensen et al., 2005) and experience

large differences in water salinities but a lack of common methods hampered inter-stock comparisons. Future studies on thermal impacts on fish embryos should use a wide range in temperatures and should consider the effects of additional factors on thermal windows including the magnitude of in situ variability in temperature (Helmuth et al., 2010) and the synergistic effect of multiple environmental factors (Pörtner and Peck, 2010). Another important research topic will be to examine whether and how intrinsic factors such as maternal condition (Laine and Rajasilta, 1999) and/or paternal (genetic) differences might modify thermal windows (Burt et al., 2010).

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Chapter 5

Respiration rates of Atlantic herring (*Clupea harengus* L.) larvae: Depicting energy losses in physiological-based foraging and growth models

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Abstract

The routine respiration rate (R_R, µl O_2 ind^{-1} h^{-1}) of larvae of spring-spawning Atlantic herring (*Clupea harengus* L., southwest Baltic population) was measured using a computer-controlled, closed-circuit respirometer at temperatures between 9 and 19°C for groups of larvae having mean body sizes from ~10.0 to 22.5 mm standard length (*SL*). The R_R of 47 small groups of larvae was measured every 0.5 to 0.8 hrs during 48-hr trials (12:12 L:D, light regime). Larval R_R increased with increasing temperature (T) and dry body mass (DM, µg) according to: $\text{Log}_{10}(R_R) = 0.0223+0.6557 \cdot \log_{10}(DM)+0.0371 \cdot T$, ($p < 0.0001$, r^2 adj = 0.771). Relatively large larvae (>15 mm *SL*) exhibited diel differences in R_R with daytime rates being ~twofold higher than those at night. This study is the first to measure respiration rates in herring larvae across a broad (ecologically relevant) range in water temperatures and to examine diel differences. Comparison of R_R reported here and in other studies on marine fish larvae highlights the need for standardized methods and advancements of techniques to measure the costs of swimming activity to parameterize physiological-based foraging and growth models.

***Key words*:** Larval fish, energetics, physiology, modelling, temperature, body size

Introduction

Understanding how intrinsic and extrinsic factors affect rates of metabolic losses is a fundamental step towards understanding physiological constraints shaping life history strategies in poikilotherms. The rate of respiration (R), measured in terms of O_2 consumption, has been commonly used as an index for metabolic rate in aquatic and terrestrial animals (Fry, 1957, Brett and Groves 1979). Respiration can be partitioned into various levels depending on the level of physiological activity or process occurring during the measurements, such as during anaesthetization (basal, R_B), quiescence (standard, R_S), routine movements (routine, R_R), digestion of food at known activity levels (specific dynamic action, R_{SDA}) and during active swimming (active, R_A) (Fry, 1957; Brett and Groves, 1979). Factors have been published to convert respiration rates (O_2 consumption) into rates of loss of energy (Brett and Groves, 1979) or dry mass (Theilacker, 1987). The latter are often employed in physiologically-based individual-based models (IBM's) depicting larval fish foraging and growth (e.g., Daewel et al., 2011; Peck and Hufnagl, 2012).

Atlantic herring (*Clupea harengus* L.) inhabits environments across the North Atlantic that have a broad range of water temperatures, prey characteristics and hydrographic conditions (Geffen, 2009). Atlantic herring exhibits a wide thermal tolerance with embryos displaying good hatch success from 5 to 19°C (Peck et al., 2012b) and later life stages surviving from -0.75 to 23°C (Blaxter, 1960). Spawning can occur in any season depending upon the population (Geffen, 2009; Hufnagl and Peck, 2011). Unlike other fish inhabiting the same environment such as Atlantic cod (*Gadus morhua* L.), herring larvae can survive while displaying little or no somatic growth (Johannessen et al., 2000; Folkvord et al., 2009) likely by down-regulating metabolism when feeding conditions are poor (see Kiørboe et al., 1987). Measurements of the growth physiology of larval herring (e.g., respiration rates) have been made at relatively cold (1 to 12°C) water temperatures (Holliday et al., 1964; Almatar, 1984; Kiørboe et al., 1987) and few data exists on warmer temperatures experienced in nature when larvae hatch in shallow areas of the Baltic Sea in spring (to 20.5°C, see Oeberst et al., 2009a). Examining the physiological requirements of herring larvae at warmer temperatures is needed to advance our understanding of the mechanisms that act during the early larval period (from hatch to 20 or 25 mm length) to regulate recruitment in both autumn- and spring-spawning populations inhabiting the Baltic and North Seas (Nash and Dickey-Collas, 2005; Oeberst et al., 2009b).

In the present study, we measured R_R for groups of herring larvae ranging in mean standard length (SL) from 10.0 to 22.5 mm at temperatures from 9 to 19°C. Herring were from the spring-spawners in the southwest Baltic Sea and test temperatures represented those experienced by this size range of larvae in nature. The respiratory data collected in this and previous studies were compared to estimate the effects of body size and temperature on R_R and to test for the presence of diel differences in R_R. It was hoped that these measurements would yield better estimates of daily metabolic losses needed within foraging and growth subroutines in Individual Based Models (IBMs) constructed to understand the factors affecting the early survival of herring (Hauss and Peck, 2009; Hufnagl and Peck, 2011).

Materials and Methods

Rearing Herring Larvae

Herring eggs were obtained from locations in the southwest Baltic Sea either by strip spawning adults caught in the Kieler Fjord (KF, 54.37°N, 10.17°E) or by collecting fertilized eggs in the Greifswalder Bodden (GB, 54.25°N, 13.68°E). Fertilized embryos were incubated until hatch and larvae were reared through the yolk sac phase at temperatures between 7 and 13°C in semi-static, 100-l tanks using a 14:10 L:D light regime with daytime light intensities of ~2.0 µE at a water salinity of 18. Exogenously feeding larvae were provided nauplii and copepodites of *Acartia tonsa* (Copepoda:Calanoida) and tanks were "greened" with algae (*Rhodomonas* sp.) throughout the rearing period. Specific details of methods used to rear copepods and herring larvae were previously reported (Peck and Holste, 2006; Hauss and Peck, 2009). Measurements of larval respiration rates reported in this study were made "ad hoc" (based upon the availability of larvae acclimated to different temperatures) during each of two rearing seasons (spring 2007 and spring 2008). Trials were conducted on larvae that were either reared at (or acclimated for at least 1 week to) one of the different test temperatures (Table 1). Only exogenously feeding larvae (those after yolk sac depletion) were used.

Tab. 1: Summary information for routine respiration (R_R) trials performed with Atlantic herring larvae. T = temperature, GB = Greifswalder Bodden, KF = Kiel Fjord, dph = days post hatch, SL = standard length, Rep = replicate, P = present, A= absent

Respiration Trial					Mean Dry Mass			Diel
(ID)	T (°C)	Reps. (No.)	Ind. Rep.$^{-1}$ (No.)	Spawning Site (GB or KB)	Age (dph)	Mean SL (mm) Min. to Max.	Mean Dry Mass (µg) Min. to Max.	Diel R (P, A)
1	15.2	2	5 to 6	GB	16	10.0 to 11.0	206.0 to 238.1	A
2	11.1	3	10 to 11	GB	19	11.6 to 13.2	197.4 to 314.1	A
3	9.0	2	9 to 10	GB	21	12.4 to 12.7	260.0 to 300.0	A
4	17.2	3	2 to 4	GB	24	12.5 to 17.5	227.2 to 1258.2	A
5	15.1	3	3 to 6	GB	26	14.7 to 18.6	752.3 to 1721.6	P
6	13.2	3	2 to 3	GB	30	11.5 to 15.3	161.9 to 423.9	P
7	13.2	3	2 to 3	GB	35	12.8 to 16.3	312.2 to 837.1	P
8	15.9	5	4 to 5	GB	38	14.0 to 17.4	343.2 to 930.2	A
9	19.1	1	5	GB	40	15,1	525.7	A
10	13.8	3	12	KF	41	14.9 to 16.3	569.3 to 876.5	A
11	16.1	3	5	KF	46	16.0 to 17.5	628.5 to 886.9	A
12	13.8	1	12	KF	50	15,7	737.8	A
13	15.2	5	5 to 6	KF	53	18.7 to 22.2	1671.2 to 3323.6	P
14	9.0	3	5	KF	54	17.7 to 19.9	1333.3 to 2188.5	A
15	9.0	3	5	KF	59	15.5 to 18.1	847.8 to 1267.9	A
16	19.3	2	5 to 6	GB	49	23.3 to 23.4	3036.1 & 3221.4	P
17	13.1	2	7	GB	47	20.7 to 22.5	2051.8 & 2094.4	P

Respiration Measurements

All respiration trials were started in the morning before prey items were offered to larvae in rearing tanks. Larvae were gently transferred by a large bore pipette to 50-mL chambers containing 0.3 µm filtered seawater and placed in a Micro-Oxymax respirometer (Columbus Instruments, Columbus Ohio, USA) for measurement of R_R (µl O_2 fish^{-1}h^{-1}). Czekajewski et al. (1994) outline the respirometer's operation and sensitivity to oxygen as detected by a paramagnetic sensor. This computer-controlled system and a similar measurement protocol have been previously employed to measure R_R in larval haddock (*Melanogrammus aeglefinus*), larval and juvenile Atlantic cod and a crangonid shrimp (*Crangon septemspinosa)* (see Peck et al., 2004; Peck and Buckley, 2007; Taylor and Peck, 2007; Lankin et al., 2008). Each respiration trial used 2 to 3 chambers containing 40 to 50 ml of filtered (1 µm) brackish (salinity 18) water and herring larvae and 2 blank chambers that contained only filtered water and a similar amount of water used to transfer larvae to chambers. The number of larvae placed in each chamber depended on larval body size and ranged from 2 to 12 fish (see Table 1). The system made measurements of the concentration of O_2 in the headspace of each chamber (in equilibrium with that in the water) every 0.5 to 0.8 hrs for approximately 48 h. The frequency of measurements depended upon the number of chambers utilized in an experiment (10 min per chamber). The light regime was set to 12L:12D with daytime light levels of ~2.0 µE.In every trial, larvae of approximately the same length were loaded in a chamber. The R_R data from a chamber were not used if 1) any larva died during the measurements, (this occurred in <5% of the chambers), or 2) mean O_2 consumption by fish was not > fourfold higher than that of the mean of the two blank (control) chambers. At the end of the trial, all larvae were digitally photographed and individually frozen at -80°C. Larval *SL* was determined using computer image analysis (Optimas, ±0.05 mm). Frozen larvae were subsequently freeze-dried (Christ-alpha 1-4, 12 to 24 h) and their dry mass (*DM*) was measured using a digital microbalance (Sartorius 1773, ±0.1 µg).

Calculation and Statistics

The mean respiration rate measured in each of the two blank chambers was subtracted from the respiration rate measured in each fish chamber at each measurement cycle. Next, the mean (48-h) respiration rate was calculated for each fish chamber and multiple linear regression analysis was used to examine the effects of T and mean DM on R_R. Statistics were performed using the software package "R". Diel differences in R_R were examined based upon significant differences (T-tests) in the R_R pooled for periods omitting measurement cycles made during the switch from day to night and vice versa.

Results

Hourly measurements of routine respiration rate (R_R) were made on a total of 47 groups of herring larvae having mean SL between 10.0 and 23.4 mm at water temperatures between 9.0 and 19.3°C (Table 1). The effect of dry body mass (DM) and temperature (T) on larval R_R was described best by a multiple linear regression:

$\text{Log}_{10}(R_R)=0.0223(\pm0.0903)+0.6557(\pm0.0541)\cdot\log_{10}(DM)+0.0371(\pm0.0068)\cdot T$

where mean ±SE parameter estimates are provided ($p < 0.0001$, r^2 adj = 0.771) (Fig. 1).

There was no significant interaction effect between temperature and body mass ($p < 0.05$). Observed and predicted values tended to agree well at cold and warm temperatures and for small and large body sizes. The model underpredicted R_R for fish in two chambers at an intermediate temperature (15.1°C). Those measurements stem from the same trial (trial 5, Table 1) and, for unknown reasons, the rates appear to be relatively high compared to larger fish measured at the same temperature.

Diel differences in R_R were apparent in six of the 17 trials (pooled RR data were significantly lower at night versus day periods, $p < 0.05$). Significant differences were only apparent in larvae that were > 12 mm SL in one trial conducted at 13.2°C and for larvae > 19 mm SL at colder and warmer temperatures (Table 1). Patterns of change in the moving average of R_R (three measurement periods averaged) followed the diel 12:12 L:D light regime with R_R decreasing shortly after the onset of darkness and increasing again shortly after the lights were turned on (Fig. 2). The magnitude of diel difference was on the order of 1.5 to 3.0-fold with the largest larvae tending to have the largest diel differences in R_R.

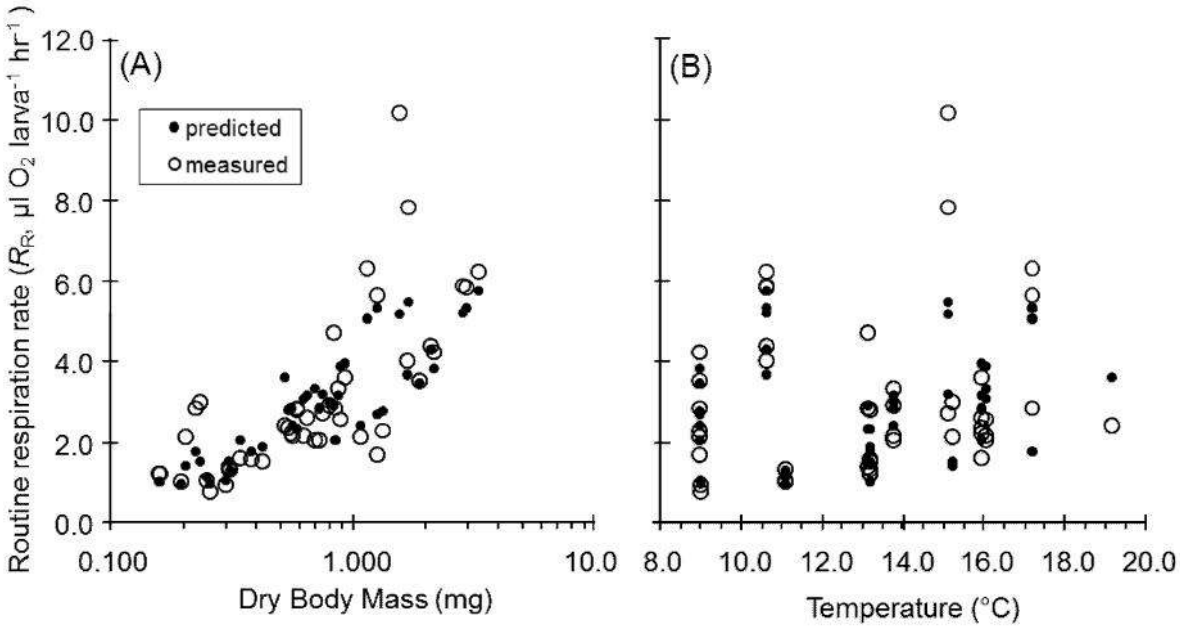

Fig. 1: Routine respiration rate (R_R, µl O_2 larva^{-1} h^{-1}) versus dry body mass (panel A) and water temperature (panel B) for larval herring (*Clupea harengus*) from the southwest Baltic Sea (16 psu). Both measured values (filled circles) and those predicted by a multiple linear regression (unfilled circles, see text) are shown.

Discussion

Gaining reliable estimates of respiration rates is critical to the development of physiological-based IBMs of larval fish foraging and growth (Peck and Hufnagl, 2012). Unfortunately, parameterizing energy losses within larval fish IBMs is challenging because respiration rates (and the effects of factors on those rates) can be considerably more variable in larvae (Giguère, et al. 1988; Houde, 1989) compared to juveniles and adults of the same species (Bochdansky and Leggett, 2001). Variability stems from both methodological issues (e.g., it is difficult to separate *R* into its various components (R_B, R_S, R_R, R_{SDA} and R_A)) and from "real" differences in growth physiology due to inter-specific differences in larval morphology, swimming activity, and/or rates of ontogenetic development (Fuiman et al., 1998; Peck et al., 2012a). Intra- (stage-) specific differences in *R* have also been documented such as changes in metabolic scaling (effect of *DM*) and thermal sensitivity (effect of temperature) (Oozeki and Hirano, 1994; Klumb et al., 2003; Peck and Buckley, 2007). Since rates of growth and mortality are inversely related during the early life of marine fish (Peck and Hufnagl 2012), it is critical to measure and evaluate such inter- and intra-specific differences in respiratory physiology. To the best knowledge, rates of respiration (O_2 consumption) by herring larvae have been previously measured in 5 studies (Holliday et al., 1964; Almatar, 1984; Kiørboe et al., 1987; Overnell, 1997; Bang, 2005). In the following, we attempt to compare our results and those reported in previous studies on herring. We also more broadly discuss the effects of key extrinsic (temperature) and intrinsic (body size and activity level) on respiration rates of marine fish larvae.

Temperature and body size are key extrinsic and intrinsic factors, respectively, affecting energy loss and the importance of correctly parameterizing their influence on respiration rates within physiological-based growth models for larval fish cannot be overstated. A variety of studies have examined the effects of temperature on *R* in early life stages of Atlantic herring. In unfed yolk sac larvae at 8°C, Holliday et al. (1964) reported rates between 0.10 and 0.20 µl O_2 $larva^{-1}$ h^{-1} larvae while Overnell (1997) measured rates that were a bit higher (between 0.20 and 0.30 µl O_2 $larva^{-1}$ h^{-1}). A Q_{10} of 2.0 described the increase in *R* with increasing *T* in newly-hatched herring larvae measured at 5, 8, 12, and 14°C (Holliday et al. 1964, see their figure 3b). Almatar (1984) examined the effect of acute temperature change (8, 13, and 18°C) on yolk sac and older (exogenously feeding) larvae and reported Q_{10} values of 2.7 and 2.4 for larvae maintained at relatively low and high salinity, respectively. The present study examined larger larvae at lower salinity and without acute temperature changes and found a lower effect of temperature (Q_{10} = 1.5) on R_R. This Q_{10} is at the lower end of values that have been reported for the larvae of other marine fish species in the Atlantic: Q_{10} values of 1.49, 2.56, and 3.00 have been reported for Atlantic mackerel (*Scomber scombrus*), alewife (*Alosa pseudoharengus*), and Atlantic cod (*Gadus morhua*), respectively (Giguère et al., 1988; Klumb et al., 2003; Peck and Buckley, 2007).

The effect of body size on respiration rate is reflected in the metabolic scaling exponent (values of b in $R = aDM^b$). A variety of values of b have been reported in studies performed on marine fish larvae including both allometric ($b \neq 1$) and isometric ($b = 1$) values with a central tendency of b-values to range from 0.75 and 1.0 (Peck et al., 2012a). In some instances, the metabolic scaling factor changes during ontogeny from b values close to isometric during the early larval period to allometric ($b < 0.8$) at larger (post-metamorphic body sizes) (Bochdansky and Leggett, 2001; Klumb et al., 2003; Killen et al., 2007). Kiørboe et al. (1987) measured R_B (during anaesthesia) in 80 µg to 10 mg *DM* herring larvae at 8°C and reported b values between 0.79 and 0.81. Based upon fewer measurements that were made on smaller herring larvae, Almatar (1984) reported b-values between 0.737 and 1.331. The *DM* scaling factor was lower in the present study (0.665). Metabolic scaling factors have been reported to depend upon the activity occurring during measurements (Killen et al., 2010), thus direct comparison of the values in this and Kiørboe et al.'s (1987) study are not warranted. A meta-analysis of the *R* data collected on juveniles and adults of 89 teleost fishes revealed systematic variability in b values due to differences in life style (e.g., benthic versus pelagic), swimming mode (e.g., eel- or tuna-like) and ambient temperature (Killen et al., 2010) but other constraints, such as the costs associated with high rates of growth, are likely more important during the larval period (Weiser, 1991). The lack of standard protocols hamper comparisons here (for herring larvae) and among many other studies performed

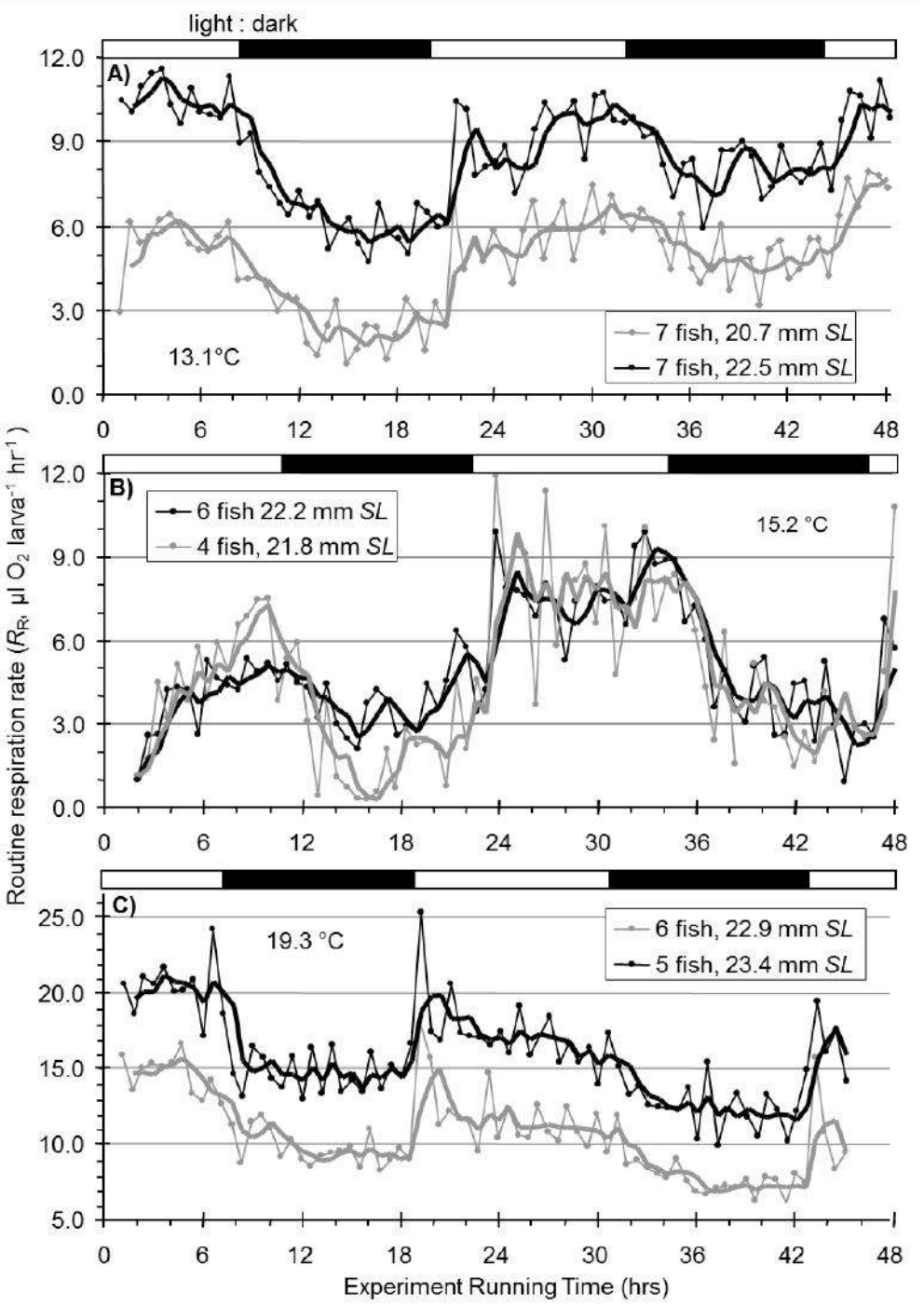

Fig. 2. Routine respiration rate (R_R, µl O_2 larva^{-1} h^{-1}) versus time (hr) for groups of larval herring (*Clupea harengus*). The mean respiration rate of two blank chambers at each measurement interval has already been subtracted from these rates. Each panel displays the R_R of two chambers containing 4 to 7 larvae. Larvae were between 20.7 and 23.4 mm *SL*. Data shown in panels A, B and C were collected in trials 17, 13 and 16, respectively (see Table 1). Lines display the running average (n = 3 measurements).

on marine fish larvae. The published measurements on herring larvae suggest that previous feeding or growth history can significantly influence *R* with larvae from high feeding conditions having anaesthetized respiration rates (R_B) nearly twofold greater than chronically poorly fed larvae (Kiørboe et al., 1987). For example, the R_B in a 500 µg *DM* larva was predicted to be 0.50 and 0.27 µl O_2 h^{-1} in "well fed" and starved larvae, respectively (see Kiørboe et al., 1987, their table 4, pg. 4) – note, larvae were unfed at the time of measurement so the increase in *R* was not due to R_{SDA}. At the same body size and temperature (8°C), our predicted R_R was roughly twofold greater (1.21 µl O2 larva^{-1} h^{-1}) than rates reported by Kiørboe et al. (1987) for well-fed, anaesthetized larvae. In

terms of routine losses, after one week of starvation at 7°C, 480 µg DM herring larvae lost 108 µg of DM (Hauss and Peck, 2009). Based upon our R_R measurements, the same size larvae at the same temperature would be expected to lose 125 µg DM (R_R-based, conversion from R to larval tissue provided by Theilacker (1987)). The good agreement between observed and predicted values suggests that our measurements provide reliable estimates for use in larval herring IBMs (Hufnagl and Peck, 2011). However, it is important to note that measurements of larval herring swimming activity in the presence and long-term absence of prey have not been compared (are unavailable) and that physiological models depicting larval growth are often most sensitive to parameters depicting activity costs (Peck and Hufnagl, 2012).

The importance of larval activity to respiration rates and energy losses in the larvae of herring and other marine fishes has been recognized for decades. For example, Holliday et al. (1964) stated that "the factor that was found to override all others in determining oxygen uptake was activity" (Holliday et al., 1964, page 713). Growth estimates obtained from larval fish IBMs are often most sensitive to changes in the parameter representing active (daytime foraging) respiration (e.g., Kristiansen et al., 2009; Hufnagl and Peck, 2011). Unfortunately, very few studies have quantified active respiration rates and metabolic scopes ($R_A - R_S$) in marine fish larvae. Some estimates are available for freshwater fish larvae (e.g. Dabrowski et al. (1986) performed measurements on whitefish (*Coregonus schinzi palea)* larvae) but newly hatched freshwater fish larvae are considerably larger and more active than marine fish larvae (Houde, 1997). Indirect measurements made by Ruzicka and Gallager (2006) on Atlantic cod larvae indicated that R in free-swimming individuals could be 3.8- to 5.0-times greater than that measured on individuals confined to small chambers. That study also suggested that respiration rates measured during routine foraging movements accounted for the vast majority (80%) of daily energy losses. Killen et al. (2007) reported that the factorial metabolic scope (R_A / R_S) of ocean pout (*Macrozoarces americanus*), lumpfish (*Cyclopterus lumpus*) and sculpin (*Myoxocephalus scorpius*) was only ~1.5 for larvae but increased to at least 2.5 in juveniles and adults, suggesting that larvae may be particularly limited in their aerobic capacity and, hence, quantifying the costs of activity is very important.

In the present study, larvae were measured in small groups allowing interactions to occur among individuals confined to swim within a relatively small (2 to 3 body length diameter) chamber. Working with 10 species of fish, Boisclair and Tang (1993) reported that turning movements were at least 6-fold more costly than linear swimming in flume tanks. Activity costs are expected to be high in small marine fish larvae swimming at relatively low Reynold's numbers. Therefore, although we did not quantify activity during measurements, it was expected that R measured here would be higher than that measured on isolated larvae in smaller chambers. In any case, only indirect information exists on the costs of activity (R_A) in marine fish larvae (although see Killin et al., 2010). In the present study, R_R during the day (in the light) tended to be ~2-fold greater than at night (in darkness) although diel differences were variable (Fig. 2) and mainly observed in trials testing relatively large larvae (Table 1). This magnitude of diel difference agrees well with the magnitude of 1) changes in activity of herring larvae at different light levels (Batty, 1987), 2) difference reported in R of marine fish larvae measured in light or darkness (Finn et al., 1995 & 2002), and 3) differences in R between anaesthetized and not anaesthetized larvae (Holliday et al., 1964).

Summary and Conclusions

Estimates of R_R obtained in the present study agree with those reported in other studies although differences in methods hamper direct comparison. Our estimates appear useful for modelling the foraging requirements and growth of herring larvae and are the only such estimates at relatively warm temperatures normally experienced by larvae in shallow coastal areas in northern Europe in the spring (end of April / beginning of May). Future studies should attempt to couple measurements of swimming activity and respiration rates in herring larvae having different recent and long-term growth histories. Such data would provide much-needed estimates for IBMs that hope to explore the costs and tradeoffs of foraging in different prey fields (Daewel et al., 2011; Hufnagl and Peck 2011). Basic, physiological data on herring larvae are needed to test the hypothesis that larvae of this

species can effectively regulate their metabolic costs depending upon growth needs and feeding conditions (Kiørboe et al., 1987). Models can then be used to explore how herring larvae in the Baltic Sea and elsewhere are capable of not only surviving long periods with little or no growth (Johannessen et al., 2000; Folkvord et al., 2009) but also displaying relatively rapid growth rates at warmer temperatures (Oeberst et al., 2009a) when prey are likely abundant.

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Chapter 6

Co-occurrence of European sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) larvae in southern North Sea habitats: Abundance, distribution and biochemical-based condition.

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Abstract

Spawning populations of European sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*) have become re-established in the southern North Sea after a ~30 year absence and now co-occur with sprat (*Sprattus sprattus*). Consequently, little is known about the potential interactions among these three species in this region. Based upon parallel cruises conducted in June/July 2005, we compared the larval abundance, size-distributions and *RNA:DNA* ratios of these three clupeid species among 1) nearshore (Wadden Sea) areas and offshore (German Bight) areas that were either 2) vertically-mixed, 3) frontal zones, or 4) stratified water masses. In general terms, larval condition (*RNA:DNA ratio*) was relatively high at all stations. Although frontal zones clearly acted to concentrate larvae, larval condition was not necessarily higher. For example 15% of the sardines captured at the tidal mixing front were categorized as starving, while no starving sardine larvae were sampled in the stratified water masses. Habitats of sardine and sprat larvae were more similar than those of anchovies that were primarily restricted to nearshore areas. This is the first study examining the potential role of near- and offshore habitats as nursery areas and the extent to which resource (habitat) partitioning exists among the larvae of sprat, and newly established European anchovy and sardine in the southern North Sea.

Keywords: *Sprattus sprattus*, *Engraulis encrasicolus*, *Sardina pilchardus*, larvae, RNA:DNA, North Sea

Introduction

Small pelagic fish species are one of the most sensitive "bio-indicators" of climate change on regional and basin scales due to their short life spans, high intrinsic growth rates (r), and tight coupling to meso-scale physical processes linked to climate processes (e.g., Cury and Roy 1989, Borja *et al.*, 1998, Roy *et al.*, 2007). Survey data indicate that European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) dramatically increased in the southern North Sea in the mid 1990's (Beare *et al.* 2004) and the first evidence of spawning activity of sardine and anchovy in the North Sea was detected during the cruises made in 2003-2004 as part of the German GLOBEC program (J. Alheit, IWO, Warnemünde Germany, pers. com.). However, episodes of increased abundance of anchovy and sardine in the North Sea have been previously documented during 1948–1952 and 1958–1960 (Aurich, 1954; Postuma, 1978). Thus, the current (re-) immigration of these warm-water clupeid species into the North Sea is likely related to changes in water temperatures and circulation patterns (Corten and van de Kamp, 1996) that may be driven by relatively long-term climate cycles and/or climate change.

The impact that sardine and anchovy will have on populations of "resident" North Sea clupeid species such as sprat (*Sprattus sprattus*) and Atlantic herring (*Clupea harengus*) is an area of active research due to both the potential ecological and economic consequences. North Sea populations of sprat, sardine and anchovy may exhibit resource competition and the degree of habitat partitioning among species is unclear. From an early life stage perspective, all three species spawn in the late spring and/or early summer (Alheit *et al.*, 1987). During this time period a variety of different habitats exist for eggs and larvae including: 1) nearshore environments that are high energy, tidally-dominated shallow water areas (Wadden Sea) as well as offshore areas that are either 2) permanently-mixed water masses, 3) frontal zones generated by tidal mixing and river plumes, or 4) stratified water bodies. The latter habitat is an example of a mesoscale hydrographical feature generally playing a major role in determining the patterns of larval fish abundance, distribution and sometimes growth rates in many areas (Nakata and Zenitani, 1996; Sabates and Olivar, 1996) including the North Sea (Munk, 1993; Valenzuela and Vargas, 2002). The extent to which larval sardine, anchovy and sprat utilize these different North Sea habitats could have important implications for competition and relative inter-specific productivity.

The objective of the present study was to examine the abundance, length-distribution and biochemical condition (*RNA:DNA*) of sprat, sardine and anchovy among 1) nearshore (Wadden Sea) areas and offshore areas (German Bight) that were either 2) well-mixed, 3) stratified or 4) frontal zones. Environmental data included both physical (temperature, salinity) and biological (zooplankton) characteristics to help us interpret any potential differences in distribution, abundance and condition among the three species in the four different areas. Despite decades of research on larval fish in the southern North Sea, the sampling scheme employed here (parallel cruises in near- and offshore areas) was the first to allow direct comparisons among these different habitats, and the first investigation of possible competition and habitat partitioning among the three co-occurring clupeiform species.

Materials and Methods

Field Investigation

The study area was located in the German Bight in the southern North Sea (Fig. 1). Samples were taken between June 28th and July 13th, 2005 as part of the GLOBEC-Germany and NUTEX Projects. In these surveys, the research vessels "Alkor" (cruise AL 260) conducted 11 hauls in the offshore area and "Ludwig Prandtl" con ducted 25 hauls in the nearshore area using two bongo nets with 500 µm mesh-size and a diameter of 40 cm in a double oblique tow. Bongo nets from "Ludwig Prandtl" were towed from 1 to 3 minutes at 0.5-3.5 knots and those on the "Alkor" were towed for 4 to 8 minutes at 2-3 knots. An estimate of the volume of water filtered was obtained using internal flowmeters mounted in the mouth of each net. The whole water column was sampled. The sample from one of the bongo nets was preserved in buffered 4% formalin. In the second net sample, clupeiform larvae were removed and immediately frozen at −80°C prior to preserving that net sample in 4% formalin.

In the Alkor 260 transects, the hydrographical situation in the sampling area was determined with a Video-Plankton-Recorder (Seascan Inc.) equipped with a CTD recording temperature, salinity, fluorescence and density.

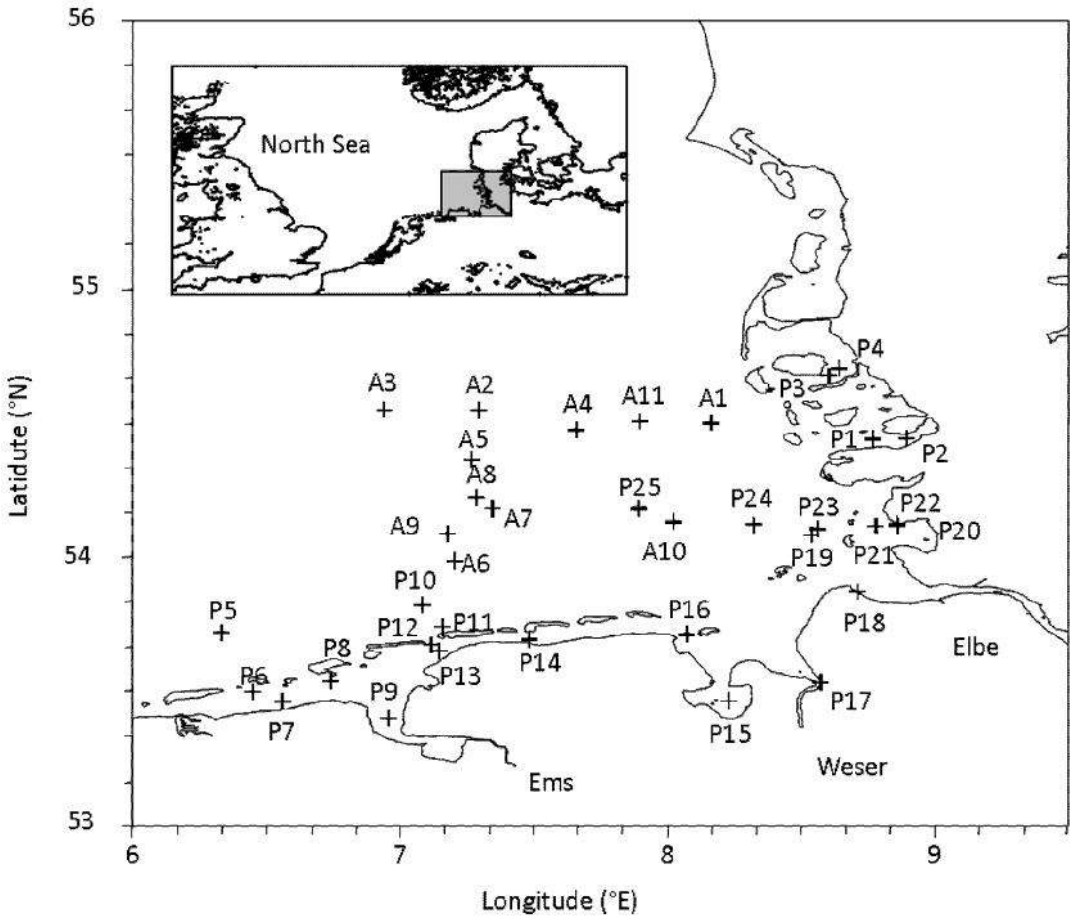

Fig. 1: Map showing positions of Alkor (A) and Prandtl (P) stations sampled in June/July2005 and the position of the sampling area relative to the North Sea (insert).

The "Ludwig Prandtl" used a "Ferry Box" to record hydrographical data and additionally deployed a CTD at 10 stations (Table1).

Sampling stations could be partitioned into four different categories: 1) offshore vertically-mixed, 2) tidal mixing zone (TMF), 3) stratified water and 4) Wadden Sea vertically mixed. The first three categories were based on the stratification variable (*f*) derived from vertical density profiles r(z) (Simpson *et al.*, 1979). The value of *f* represents a measure of the amount of energy required to mix the water column and thus increases with increasing water column stratification (mixed waters < 10 J m^{-3}, frontal waters 10–20 J m^{-3} and stratified waters >20 J m^{-3}) (Lee et al., 2007). The Wadden Sea is a flat high-energy system separated from the North Sea by a row of barrier islands and sandbanks. Deep tidal channels form the connection between the North Sea and Wadden Sea. Within the Wadden Sea these channels branch into numerous gullies and creeks. During ebb tides, vast areas of tidal flats emerge and, at low tide, about two third of the bottom of the Wadden Sea is exposed. Limits of the Wadden Sea were defined by the 10 m water depth profile along the German coast offshore of the islands.

Species Identification

In order to limit degradation processes of the frozen larvae, the vials were stored on ice and the following work was done as rapidly as possible. After defrosting, larvae and seawater were gently emptied into a small petri dish which was also placed on ice for cooling. Formalin preserved larvae were processed without cooling. Larvae were identified according to published keys (Halbeisen, 1988; Munk and Nielsen, 2005). Because of the close resemblance in terms of body shape and pigmentation of sprat and sardine, the identification was difficult for these two species, especially in cases where the larva suffered damage during capture. The most important characteristics used to distinguish sprat and sardine larvae were myomere counts (all numbers relate to preanal counts from first neck myomere to anus)

Tab. 1: – Stations sampled for clupeiform larvae within the southern North Sea during June / July 2005. The 36 stations were separated into four categories: Mixed (M), Wadden Sea (W), Stratified (S), and Tidal Mixing Front (F).

	Cluepiform Larval Sampling							Additional Data Coll.	
Station	Date	Time	Location		Depth	SW sampled			
(ID)	(Day-Month)	(Hour)	Lat. (°N)	Long. (°E)	(m)	Category	(m^{-3})	Copepods	CTD
A1	28-Jun	4:30	54.52	8.16	16	M	206.0	x	x
A2	28-Jun	12:31	54.55	7.29	32	S	130.1	x	x
A3	28-Jun	15:37	54.55	6.94	37	S	114.8	x	x
A4	28-Jun	13:04	54.47	7.66	25	S	250.0	x	x
P5	29-Jun	15:30	53.72	6.33	22	M	63.1	x	x
P6	29-Jun	10:41	53.50	6.45	2	W	23.7		
P7	29-Jun	11:50	53.46	6.56	3	W	22.9		
P10	30-Jun	15:36	53.82	7.08	20	M	26.7		x
P8	30-Jun	7:56	53.54	6.74	12	W	26.8	x	x
P9	30-Jun	9:56	53.40	6.96	11	W	28.5		
A5	1-Jul	16:13	54.36	7.27	43	S	211.4	x	x
A6	1-Jul	11:54	53.99	7.20	28	S	199.8	x	x
P11	1-Jul	9:52	53.74	7.16	5	W	45.4	x	x
P12	1-Jul	10:53	53.67	7.12	7	W	30.6		
P13	1-Jul	11:20	53.65	7.15	2	W	33.5		
A7	2-Jul	14:31	54.18	7.34	38	M	157.1		x
A8	2-Jul	16:29	54.22	7.29	38	F	57.2	x	x
A9	2-Jul	8:19	54.09	7.18	36	S	63.8	x	x
A10	4-Jul	14:17	54.13	8.02	28	F	82.9	x	x
P14	4-Jul	12:40	53.69	7.48	10	W	41.3	x	
P15	5-Jul	17:17	53.47	8.23	18	W	10.2		x
A11	6-Jul	8:44	54.51	7.90	19	S	157.9	x	x
P16	6-Jul	8:22	53.71	8.07	13	W	7.5		
P17	6-Jul	16:24	53.53	8.57	11	W	10.9		
P18	7-Jul	15:14	53.87	8.71	18	W	16.9	x	
P19	8-Jul	11:07	54.08	8.54	9	M	10.8		x
P20	8-Jul	14:41	54.12	8.86	8	W	58.7		
P21	8-Jul	15:22	54.12	8.78	8	W	25.6	x	
P22	8-Jul	15:46	54.11	8.86	8	W	44.0		x
P23	9-Jul	10:41	54.10	8.56	9	M	48.8		
P24	9-Jul	11:50	54.12	8.32	10	S	27.8		x
P25	9-Jul	15:15	54.18	7.89	40	S	41.1	x	x
P1	10-Jul	16:01	54.44	8.77	11	W	29.8	x	
P2	11-Jul	13:03	54.44	8.89	3	W	31.3		x
P3	12-Jul	10:52	54.68	8.60	11	W	31.3	x	
P4	12-Jul	11:38	54.70	8.64	9	W	39.4		

36-38 myomeres: sprat and 40-42 myomeres: sardine in the earliest stages. After flexion stage, starting at approximately 9 mm S_L the difference is 31-35 versus 36-41 myomere (Halbeisen, 1988). Identification by myomere count was impossible when the gut was detached from the anus region of the larval body. 56 % of all clupeid larvae could not be identified to species; net damage made it impossible to distinguish between sprat and sardine, especially in relatively small (4 to 8 mm S_L) larvae. Those potential mixtures of sprat and sardine larvae were placed within an "unidentified clupeids". Anchovy larvae were easier to distinguish from sprat and sardine due to differences in pigmentation patterns (a few groups of melanophores versus rows of melanophores) and in the proportion of pre-anal to post anal length (ca. 3:1 versus >4:1). In later stages, European anchovy develops a different position of the lower jaw and a more posterior position of the dorsal fin respective to the anal fin (Munk and Nielsen, 2005). After identification, the larvae were digitally photographed using a Leica DC

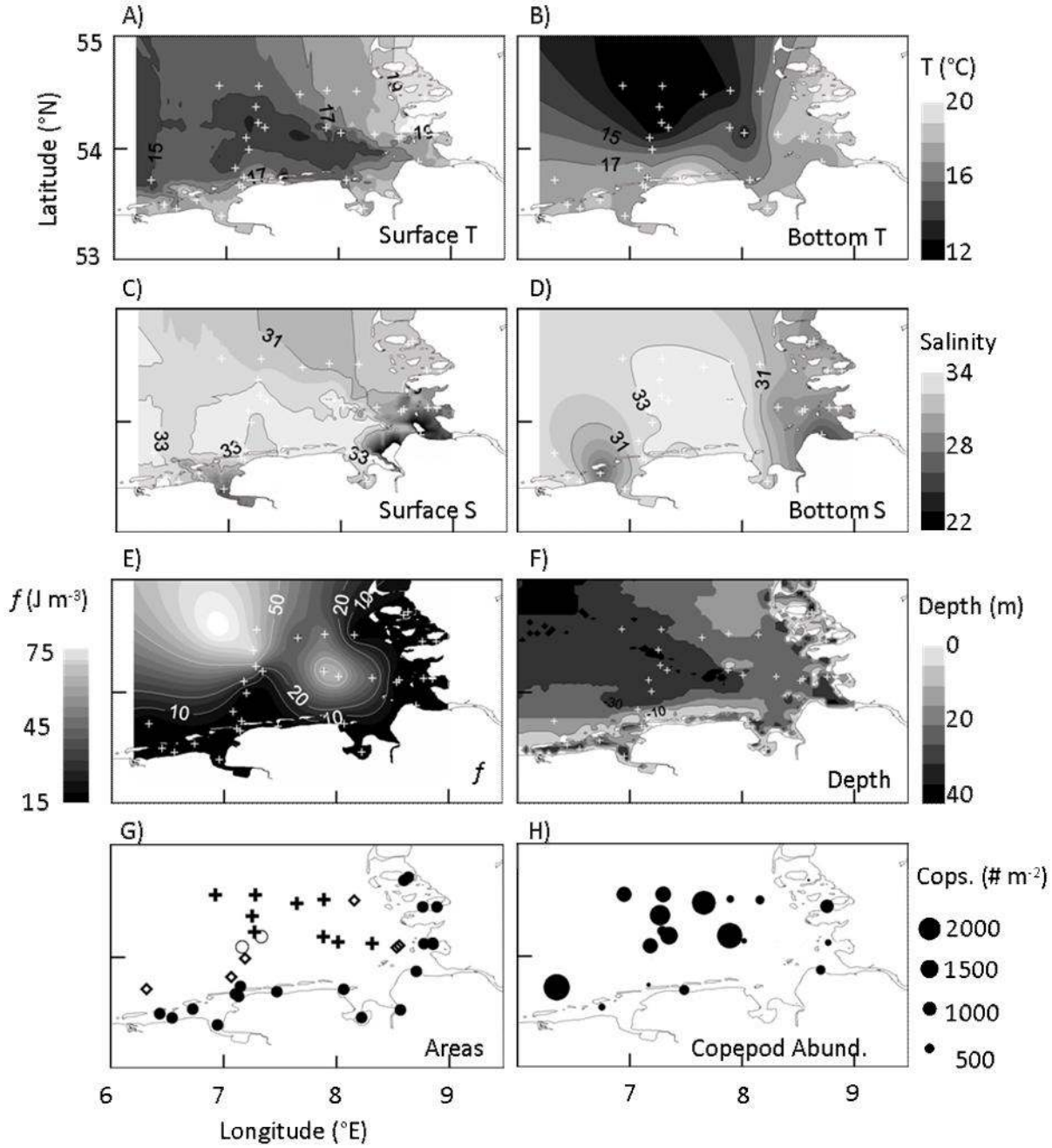

Fig. 2: Hydrographical situation within the sampling region during June/July 2005. Panels A - F display surface (ST) and bottom (BT) temperature (C°), surface (SS) and bottom (BS) salinity (psu), stratification index (f) and depth (m), respectively. Note, that within panel A-E, kriged values are shown. Panel G indicates the four station categories (nearshore/Wadden Sea (filled circles), well-mixed water masses (unfilled diamonds), tidal mixing front (unfilled circles) and stratified water masses (crosses)), and finally, the abundance of copepods (individuals m-2) captured using 500 µm Bongo gear is indicated in Panel H. Sampling locations are indicated by small, white crosses.

300 digital video camera connected to a Leica MZ 16 stereomicroscope.

Larvae for *RNA:DNA* measurements were re-frozen in individual vials at −80 °C. Standard length (S_L, ±0.05 mm) was measured with an image analysis system (Optimas 6.5). A preservation length correction was applied for specimen preserved in formalin based on a formula by Fey (2002) :SL_{live}= 0.910 $SL_{preserved}$ + 2.695. To establish an index of secondary production, copepods in the preserved bongo net samples of 19 stations were counted and abundances per square meter were calculated (Table 1, Fig.2).

Extraction and quantification of nucleic acids

Analysis of whole larval RNA- and DNA concentrations was performed by a modification of a protocol published by Caldarone (2001). Larval samples were freeze dried to constant weight (12 h, Christ Alpha 1-4 freeze drier, −51°C). The freeze-dried larvae were rehydrated in 150 ml 1% Sarcosil TRIS-EDTA buffer (STEP) and vortexed at high speed for 15 min. The larval tissues were homogenized by sonification on ice for 12 min, using an ultrasonic bath (Bransonic 221). Larvae >12 mm S_L were additionally homogenized by an ultrasonic pulse instrument (Sonoplus) before proceeding to quantifying nucleic acid concentrations.

Total nucleic acid concentrations (RNA and DNA) were determined fluorimetrically in a microtiter fluorescence reader (Spectrofluorometer Safas flx-Xenius) (excitation: 520 nm, emission 605 nm) using specific dye for nucleic acids (ethidium bromide). Estimates of the DNA and RNA content of each larva were taken from calibration curves established using standard DNA (calf thymus) and RNA (yeast). To evaluate reproducibility, a control homogenate (larval fish tissue) was added and processed in each microplate. To establish an inter-laboratory *RNA:DNA* calibration, the same homogenates were also processed and measured in the IFM-Geomar University of Kiel. Protein growth rates (*Gpi*, % day^{-1}) were then calculated from *RNA:DNA* and water temperature (*T*, °C) using the equation published by Buckley (1984):

$Gpi = 0.93\ T + 4.75\ (RNA:DNA) - 18.1.$

Data analysis

We employed both mutlivariate (all stations) and univariate (four station categories) statistics. In terms of multivariate statistics, non- parametric regression models (i.e., GAMs) were used to identify factors affecting larval abundances in the field. A GAM is a statistical model blending properties of multiple regressions (a special case of a general linear model) with additive models. In a GAM, the parameter terms bi and xi of multiple regression are replaced with functions f(xi): The functions f(xi) are arbitrary and often non-parametric, thus providing the potential for better fits to data than other methods. In a first step, we have used GAMs with single predictors to identify the relationships between individual hydrographic predictors and the probability of larvae presence. Each predictor can be analyzed with regards to the percent deviance explained, and its Generalized Cross Validation score (GCV). The GCV is a measure of predictive error of the model and thus takes into account not only the fit, but also the model complexity. In a second step, after the key environmental variables affecting larval abundance were identified, it was tested whether the inclusion of interaction between key variables increased the percentage of the explained deviance and reduced the GCV.

In terms of univariate statistics, larval abundance, standard length, and biochemical condition were compared among the four habitat categories using oneway Analysis of Variance (ANOVA). When significant differences were detected with an ANOVA, pair wise comparisons were made using a Tukey Tests. T-tests (assuming unequal variance) were also employed. Differences were considered significant at the alpha = 0.05 level. ANOVAs were followed by Tukey tests. A Bonferroni correction was used to adjust the level of significance to account for the effects of multiple comparisons.

Results

Hydrography

The hydrographic situation encountered during the cruises was in accordance with the typical summer conditions in the southern North Sea (Fig.2). A well-pronounced seasonal thermocline was found in the open areas of the German Bight at water depths of 10 to 15 m that separated 16°C surface water from a deeper, colder (12°C) water layer. In the southern Bight the tidal mixing front was well developed. A belt of cold surface water separated the well-mixed coastal regions from the thermally stratified offshore areas. In contrast the conditions in the eastern German Bight were most influenced by the plume of the Elbe River, characterized by sharp horizontal gradients in temperature and salinity. The surface temperature ranged between 21°C in the Wadden Sea and 15°C in the colder water belt. Salinity decreased from 33.5 psu offshore to 27 psu in near-shore areas. The Wadden Sea included 18 stations with a stratification *f*-value from 0.1 to 4.3 J m^{-3}, the mix water area contained 6 stations with a stratification *f*-value of 0.3 to 9.4 J m^{-3}. Two stations were categorized as frontal areas with stratification *f*-values of 11.4 and 17.9 J m^{-3} .

Tab. 2: - Results of Generalized Additive Model (GAM) analysis indicating the most relevent environmental factors that describe the abundance of all Clupeids, sardine and sprat at different stations within the southern North Sea. Both the percent explained deviance (ED, %) and the Generalized Cross Validation score (GCV) are provided. Single factor and multiple factor analyses were conducted.

Parameter	All Clupeids		Sardine		Sprat	
	ED (%)	GCV	ED (%)	GCV	ED (%)	GCV
Single Factors						
Depth	58.7	0.91	70.2	0.04	47.6	0.02
Bottom Temperature (BT)	32.1	1.50	41.9	0.09	32.7	0.02
Surface Temperature (ST)	29.7	1.55	40.8	0.04	31.4	0.02
Bottom Salinity (BS)	16.8	1.84	20.0	0.12	14.5	0.03
Surface Salinity (SS)	14.1	1.90	17.1	0.12	12.6	0.03
Copepod Index (CI)	23.3	2.61	15.3	0.18	4.2	0.05
Multiple Factors						
Depth +BT	59.4	0.95	70.4	0.047	47.7	0.018
Depth +BT+ST	63.3	0.92	76.5	0.040	51.6	0.017
Depth +BT+ST+SS	63.9	0.96	77.0	0.042	51.9	0.019
Depth +BT+ST+SS+BS	64.4	1.01	77.3	0.043	55.4	0.018
Depth +BT+ST+SS+BS+CI	68.4	2.16	83.5	0.070	55.8	0.047

whereas nine stations were considered stratified (f = 21.3 to 72.1 J m^{-3}).

Tidal currents were the most dominant current signal with up to 50 cm s^{-1}. Residual currents were approximately 4 to 5 cm s^{-1}, an order of magnitude less than the tidal signal. During the two-week sampling campaign, the net transport was eastward in surface waters, northward in the midwater depths and westward near the bottom. Hydrographic model runs (HAMSOM, T. Pohlmann, IfM, Univ. Hamburg, data not shown) indicated that the likelihood of sampling the same water masses on subsequent days at different stations was relatively low.

The concentrations of copepods (determined from samples taken with 500 µm nets) were highest in the stratified stations (2000 to 5500 copepods m^{-2}), intermediate in the TMF (560 to 780 copepods m^{-2}) and vertically- mixed waters (190 to 1650 copepods m^{-2}) and relatively low in the Wadden Sea (14 to 450 copepods m^{-2}). Only a relatively large size fraction of copepods was captured within Bongo nets, which did not cover the entire prey size spectrum of captured larvae. Thus, the concentrations of copepods reported here serve more as an index of secondary production rather than a quantity representing the total suitable prey abundance. Abundance and distribution of larvae A total of 1355 formalin preserved larvae of 24 sampling stations were analyzed.

At stations where larvae occurred, the minimum and maximum abundance of clupeid larvae, including sprat, sardine and unidentified clupeids, were 0.17 and 163.5 individuals m^{-2} a larval occurrence. Station features could be ranked according to the percentage of explained deviance and GCV scores for clupeid abundance. For all clupeids (containing unidentified clupeids, sprat and sardines), water depth was the best predictor of larval abundance (Table 2). Lower ranking predictors were bottom temperature, surface temperature, surface, salinity and bottom salinity. Zooplankton abundance was a poor indicator of sprat and sardine larval abundance. No GAM analyses could be made for anchovy due to the low number of stations where this species was captured. and bottom temperature (51.6% explained variance explained; 0.018 GCV).

In terms of univariate (station category) analyses, the abundance of clupeiform larvae was significantly different among the four habitats (ANOVA, df =3, 32, F=10.95, p<0.0001,) (Fig.3) with concentrations of clupeiform larvae significantly greater in frontal stations (f = 10-20 J m^{-3}) compared to mixed (P<00.5), stratified (P<0.01) and Wadden Sea stations (P<0.001) (Tukey HSD, $p < 0.05$). The same significant differences in abundance among the different areas were

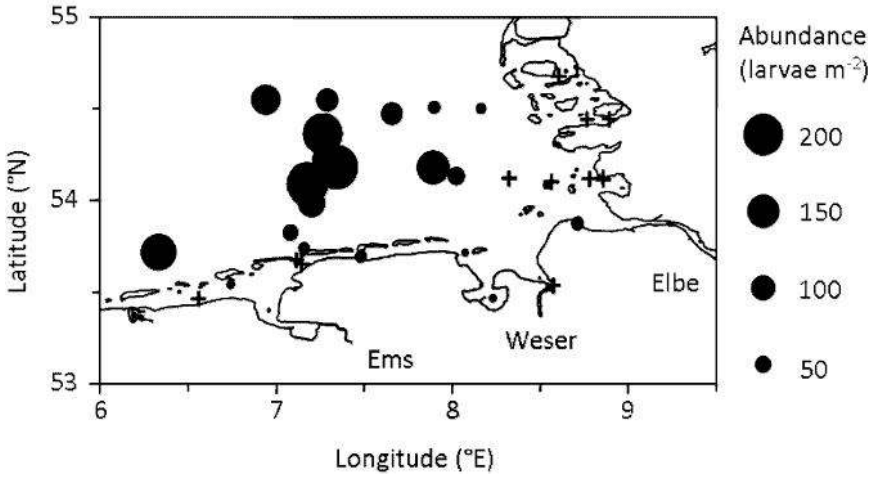

Fig. 3: Abundance (circles, individuals m^{-2}) of clupeiform larvae, including unidentified clupeids, sardine, sprat, anchovy, at different sampling stations in the German Bight. Crosses indicate stations where clupeiform larvae were not captured.

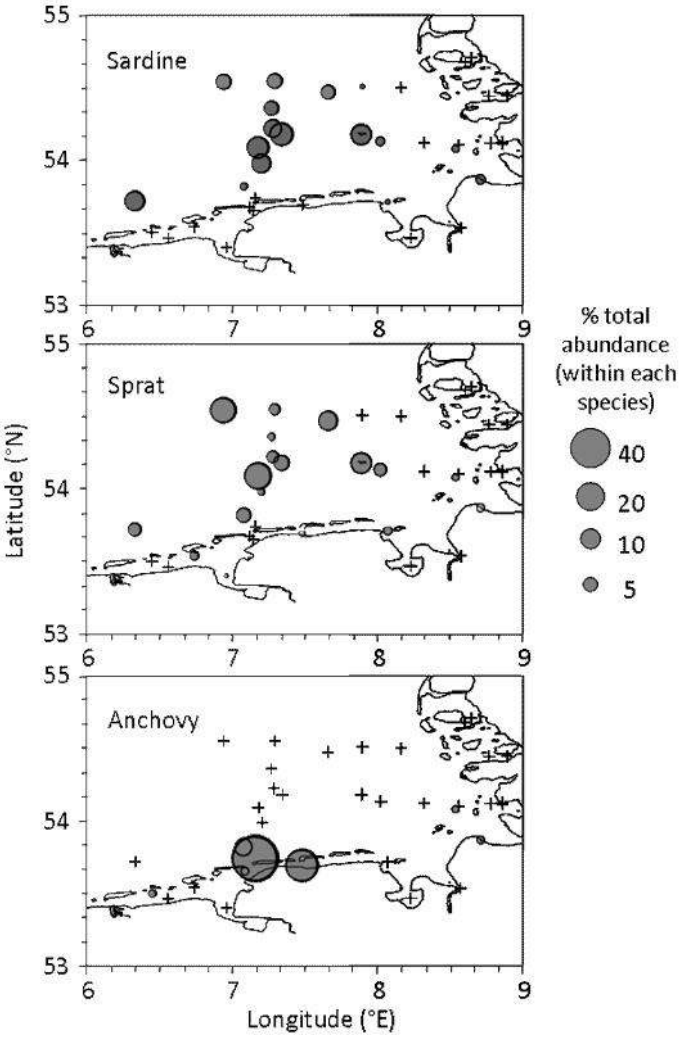

Fig. 4: Relative abundance of clupeiform larvae within each species in the German Bight, circles indicate percentage of total abundance, crosses indicate stations without larvae; (Panel A) sardine, abundance ranged from 0.64 to 45.34 larvae m^{-2}; (Panel B) sprat, abundance ranged from 0.17 to 24.74 larvae m^{-2}; (Panel C) anchovy, abundance ranged from 0.02 to 5.77 larvae m^{-2}.

detected for sardine (ANOVA, df =3, 32, F=18.99, p<0.0001) (Fig. 4).

Also sprat showed an inhomogeneous distribution pattern (ANOVA, df =3, 32, F=11.63, p<0.0001) with higher abundances in the TMF than in mixed water areas (P<0.01) or the Wadden Sea (P<0.05), but no significant difference between TMF and stratified water masses In the Wadden Sea, anchovy larvae were the most abundant clupeiform species. Sprat was found in low concentrations and sardines were completely absent except at station P18. No anchovy larvae were found offshore in the stratified and frontal areas (Fig.4). The abundance of anchovy in mixed and Wadden Sea areas was relatively low (between 0.05 and 5.61 larvae m^{-2}) compared to concentrations of clupeids in offshore areas.

In terms of length distributions, unidentified clupeids, sardine, sprat and anchovy were 4.5 to 20.3 mm, 4.6 to 20.7 mm, 6.9 to 17.6 mm, and 5.6 to 10.3 mm S_L (after correction for shrinkage) (Fig.5). Larvae > 16 mm S_L were excluded from further analysis due to possible net avoidance by larvae in these length classes. Compared to those captured in mixed waters, clupeid larvae found in stratified and frontal areas were significantly larger (unpaired t-test, t=4.524, df=573, p<0.0001; t= 5.804, df= 378, p<0.0001). Sprat larvae were larger in stratified and frontal areas than in the vertically mixed zone and the Wadden Sea (ANOVA, df = 161, F= 9.004, p<0.0001).

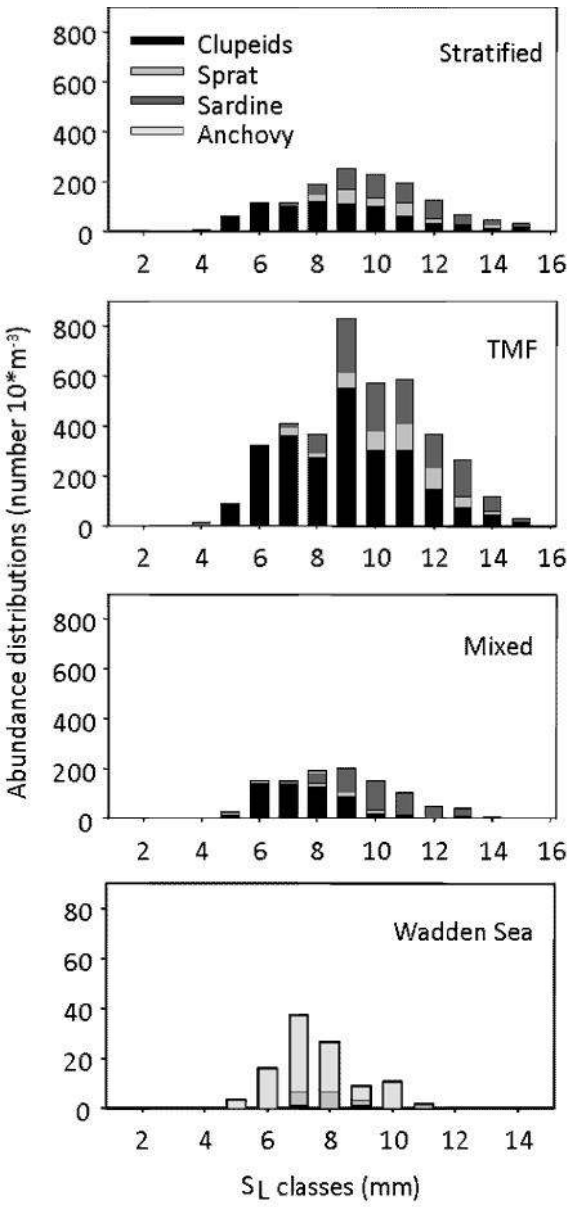

Fig. 5: Standard length (mm) abundance distribution of clupeiform larvae sampled in the tidal mixing front (TMF) (n=272), stratified areas (n=668), well-mixed water masses (n=354) and Wadden Sea (n=55). Note the smaller x-scale in Wadden Sea box

There was no significant difference in S_L of sardines (ANOVA, , df = 450, F=0.46, P>0.05) among the different areas. Also in anchovy no size differences were observed between mixed water masses and the Wadden Sea.

Spatial variability in condition

A total of 263 undamaged larvae were available for measurements of biochemical-based condition. No size correction for shrinkage due to preservation at −80°C was applied. *RNA:DNA* ratios for clupeid larvae captured within the study area were highly variable (Fig. 6) and ranged from 0.70 to 7.02, 0.53 to 6.62, and 0.79 to 6.27 in sardine, sprat and anchovy, respectively. A correlation between *RNA:DNA* (ln-transformed) and S_L was observed for sprat (Pearson correlation, n=163, Pearson r= 0.34, P<0.001) but not for sardine (n=71, Pearson r= 0.11) or anchovy (n=29, Pearson r= -0.12). Due to the low numbers of undamaged larvae, *RNA:DNA* values were pooled in a reasonable way to make inter-specific / within habitat comparisons as well as intra-specific / among habitat comparisons. Larvae of the same species that were sampled at similar station categories (e.g. Wadden Sea, TMF, etc.) were combined in two length categories (5 to 10 mm and 10 to15 mm S_L.) based on the development of the caudal fin (flexion stage) and behavioural changes (e.g., schooling) occurring at ~ 10 mm S_L in sprat, sardine and anchovy.

No significant differences in mean *RNA:DNA* ratio were found in different water mass for sardine or sprat (Fig.6), nor were there differences between pre-and post-flexion sprat and sardine larvae in the same water masses. A significant difference between anchovy and sprat *RNA:DNA* (P=0.006, t=3.722, df=40) and G_{PI} (P=0.0001, t=4.234, df=48) was detected inthe Wadden Sea samples, where anchovy displayed higher values than sprat. Protein growth rates in the frontal and stratified stations were calculated in two different steps. Since fish larvae collected during double oblique tows of Bongo gear, their vertical distribution was unknown. To account for potential differences in temperature, maximum and minimum G_{PI} values (max) were calculated for each larva using sea surface temperature and bottom temperature, respectively. G_{PI} was generally positive with mean values of 10.8 (max) and 8.9 (min) % d^{-1} for sprat, 12.2 (max) and 9.4 (min) % d^{-1} for sardine and 19.6 % d^{-1} for anchovy. Nevertheless, some individuals had lower *RNA:DNA* values that indicative of starvation during laboratory calibration trials performed using clupeid larvae (Clemmesen, 1994) and had negative G_{PI} values based upon Buckley's (1984) interspecific equation. Based on Clemmesen's (1994) formula, 10% of sardine and 3% of sprat and anchovy were in poor nutritional condition. The results of Buckley's (1984) equation (Fig.6) suggest an even lower percentage of starving larvae. 10% (max) and 3% (min) for sardine and < 1 % for sprat larvae. There were some differences in the percent-

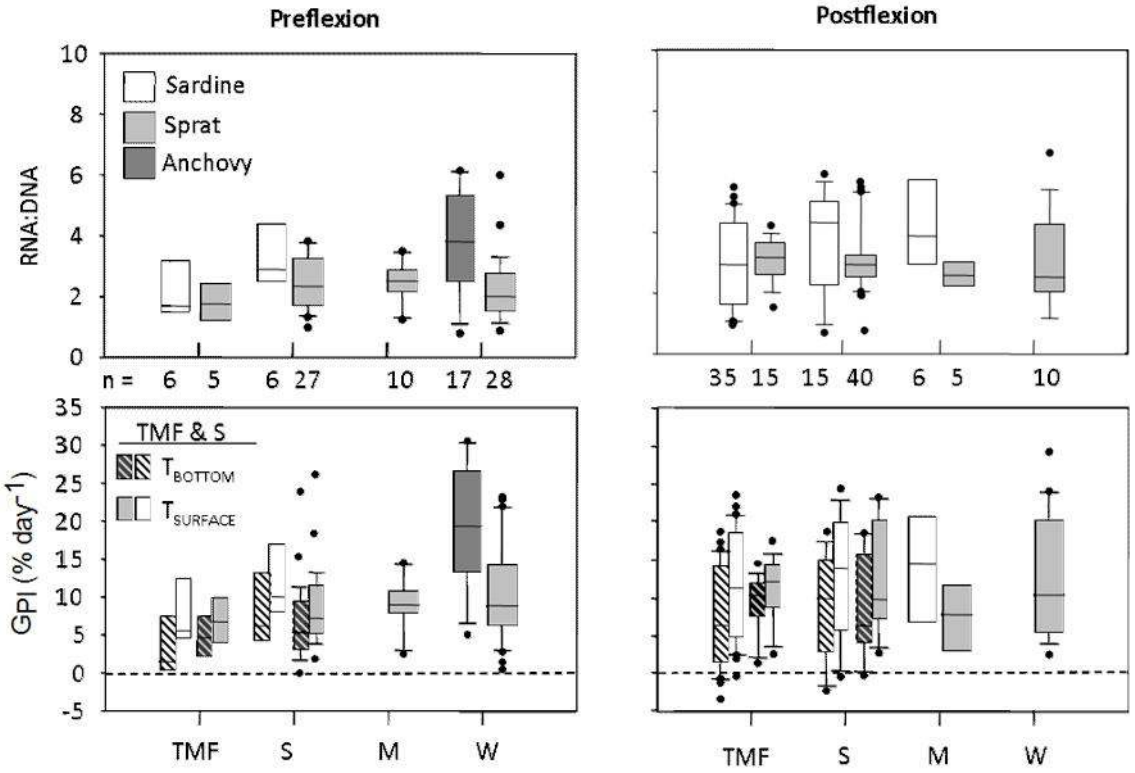

Fig. 6: Box and whisker plots of values of *RNA:DNA* ratio and protein-specific growth rate (*GPI*) for pre-and post-flexion clupeiform larvae collected in each of four different habitats (tidal mixing front (TMF), stratified (Strat.), mixed (Mixed), and Wadden Sea. For sprat and sardine sampled in frontal and stratified areas, minimum (light grey) and maximum G*PI* (calculated based upon minimum and maximum water temperatures that could have been experienced at each station) are shown (sprat, white background; sardine, grey background). The median, 10th, 25th 75th and 90th percentiles and outliers are indicated. The number of larvae analysed is shown between top and bottom panels.

age of starving larvae detected among the areas. The highest proportions of star ving larvae, defined by Clemmesen´s (1994) formula, were sardines at the frontal stations (15.6% starving larvae; stations A6, A9) and on the stratified side close to the river plume of the Elbe River (12.2% starving larvae; station P25). For larval sprat, average proportion of starvation was 5.3% in the stratified area and 7.8% in the Wadden Sea. Larval nutritional condition was not correlated to any environmental factors including depth, surface & bottom temperature, surface & bottom salinity and copepod abundance.

Discussion

Our results confirm the re-establishment of spawning populations of anchovy and sardine within the southern North Sea. While water circulation in the North Sea can transport eggs and foraging larvae from large catchments areas (including the English Channel) into the German Bight (Bartsch and Knust, 1994), the abundances of extremely young (pre-flexion) anchovy and sardine larvae obtained in the present study indicate adult spawning in the region.

Spawning events of sardines and anchovies in the German Bight were described by Aurich (1954) during the period 1948-1952 but not in the following 50 years. The first finding of early stages of sardines in the German Bight in recent times was noted by Huwer (2004). Aurich (1954) discussed climate change and above average summer temperatures for this phenomenon. A situation similar to the situation in 2003 and 2005 when summer temperatures in the North Sea were well above the long-term average (2003 was + 2 to 3°C; 2005 was + 1.0 to 2.5°C). It seems reasonable that warm water temperatures are at least a precondition for anchovy and sardine spawning. Also the recent changes in phytoplankton and zooplankton species composition of the North Sea (Reid *et al.*, 1998, Alheit *et al.,* 2005) might be favourable for sardine and anchovy. The increase in abundances of warm-water species, e.g. *Calanus helgolandicus* a predominantly Mediterranean species, might offer more suitable food for anchovy and sardine. The present study

was the first to synchronously sample the larval fish community in both nearshore (Wadden Sea) and offshore areas in the southern German Bight. In offshore areas, the highest abundance of larval sprat and sardine were found at frontal stations, whereas lower abundances were found in the well- mixed and strongly stratified areas. These findings agree well with studies examining the distribution of sprat in the same area (Munk, 1993; Valenzuela *et al.,* 2002). Advection and retention processes in different frontal areas have been described in a number of studies (e.g., Fortier and Gagne, 1990; Munk, 1993) and may have important consequences for the distribution and abundance of larvae in this study. Stratified and frontal areas contained significantly larger larvae than well-mixed areas, suggesting either an offshore movement of sprat and sardine with development or that the latter areas supported lower rates of growth and survival. In contrast to offshore areas, near-shore and/or coastal well-mixed habitats (such as the Wadden Sea) do not appear to be an important foraging area for the larvae of sprat (low abundance, 7.6 % starving) and sardine (one station, low abundance). These coastal areas, however, appear very important for anchovy larvae, which were absent at offshore stations. Aurich (1954) described a similar distribution pattern for anchovy larvae in the same area.

Although our GAM analyses indicated that stations supporting high abundances of sprat and sardine could not be easily separated (e.g., depth, surface & bottom temperature, surface & bottom salinity were associated with the abundance of both species), temperature likely plays an important role in habitat partitioning among the larvae of these three clupeid species. For example, maximum spawning activity for sprat, sardine and anchovy in European waters occurs at 6 to 12, 14 to 16, and 14 to 19 °C, respectively (de Silva 1973; Ré, 1990; Sola *et al.*, 1990; Motos *et al.*, 1996). These temperature preferences lead to seasonal habitat partitioning in the Mediterranean Sea, where anchovy spawns in summer and sprat and sardine spawn during the winter (Olivar et al., 2001). Planque et al. (2007) suggested that bottom water temperature was the strongest predictor for potential spawning habitat in anchovy (threshold > 12°C), whereas sardine appears to have a greater tolerance than anchovy for low bottom temperature (Planque et al., 2007). The role of salinity for sprat, sardine and anchovy larvae is not fully understood. Larval sprat can tolerate low salinity in the Baltic Sea (8 psu, Voss *et al.*, 2003) but sprat eggs in the North Sea are distributed mainly in waters with a salinity of 30-33 psu (Moksness and Torstensen., 1985) while larvae are likely to occur over a similar or slightly higher range of salinities (Moksness and Torstensen 1985; this study). Reid (1966) argued that anchovy (and other engraulids) have an affinity towards estuaries and other coastal areas, but not necessarily towards waters having a specific range in salinities. Whilst river plumes (i.e. low salinity) appear to be recurrent, preferential areas for spawning, anchovy also spawns in other areas such as slope water eddies or the shelf break which are characterized by high salinity throughout the water column (Motos *et al.*, 1996). Little is know about the impact of salinity on spawning and the larvae of sardine (Planque et al., 2007).

No data are yet available concerning adult spawning stock sizes of sardine and anchovy in the North Sea. We suggest that the relatively low abundance of anchovy (six inshore stations) compared to sprat and sardine (offshore areas) in our survey was likely due to a better match with the spawning period of sprat and sardine compared to that for anchovy in the southern North Sea. Latitudinal gradients in seasonal water temperatures will shift spawning seasons in different areas (Stratoudakis *et al.*, 2004). Peak spawning by anchovy occurs between May and June in the Bay of Biscay (Planque *et al.*, 2007) but likely occurs later (July/August) in the North Sea at the northernmost limit of the latitudinal range of this species. However it should be noted that abundances of clupeiform larvae in the northeastern inshore area of the German Bight found in the preset study were generally lower than those previously observed for sprat (Alshut 1988), sardine (Huwer 2004) and anchovy (Aurich 1954) in this region. These differences might be explained due to different currents and drift patterns among the sampling months and years.

In most marine fish species, the probability of survival during early life is thought to be positively correlated with growth rate as faster-growing individuals spend less time in stages particularly vulnerable to starvation and predation; larger larvae have enhanced ability to feed and avoid predators (Cushing, 1974; Rice *et al.*, 1993). The hypothesis, that growth of larvae might be promoted in frontal zones (Munk, 1991;

Nakata and Zenitani, 1996; Lee *et al.*, 2007) was not unconditionally supported in the present study. *RNA:DNA* ratios of sprat and sardine were generally high (albeit quite variable) and appeared unrelated to hydrographic conditions in the southern North Sea in June/July 2005. However, the finding that 15% of sardine at frontal stations appeared to be starving suggests, that competition for zooplankton could have been high in these waters at this time period. Moreover, potential predators also accumulate in the vicinity of fronts (Grimes and Kingsford, 1996; Munk, 1993), another tradeoff regarding residence (by either passive or active mechanisms) at frontal areas.

The finding in the present study, that anchovy had significant higher condition (*RNA:DNA* ratio) and growth rate (G_{PI}) than sprat in the Wadden Sea was unexpected. Sprat and anchovy consume similar prey items during the pre-flexion stage, mainly copepod nauplii (Dickmann *et al.*, 2007; Rossi *et al.*, 2005), although there is some evidence that anchovy larvae are opportunistic feeders that can consume protozoan (Rossi *et al.*, 2005). We speculate that anchovy larvae may be able to better exploit prey resources in the Wadden Sea. On the other hand, otolith studies suggest that growth potential of anchovy and sprat are slightly different, making it difficult to compare the "suitability" of the same habitat based solely on growth rates. Larval sprat growth rates between 0.30 and 0.36 mm d^{-1} were reported at 15°C in the North Sea (Alshuth, 1988) and 0.40 to 0.42 mm d^{-1} in the Adriatic Sea (Dulcic, 1998). Anchovy can reach higher growth rates in the same size class (6-20 mm), ranging from 0.5 mm d^{-1} at 16 to 18 °C (Garcia *et al.*, 1998; Somarakis and Nikolioudakis, 2007) to 0.9 mm d^{-1} at 20°C (Palomera *et al.*, 1988). These examples for sprat and anchovy also suggest that the larvae of the latter species might cope better with the relatively high temperatures (17.0 to 20.5°C) associated with near-shore areas such as the Wadden Sea.

In conclusion, we provide a snapshot of the distribution, abundance and biochemically-based condition (and growth) of sprat, anchovy and sardine larvae in both near- and offshore habitats of the southern North Sea. Naturally, temporal patterns may differ due to changes in extrinsic (environmental) and intrinsic (timing of life history events such as adult spawning and migrations) factors. Nevertheless, the present study suggests that larvae of these three species generally have high growth rates in the areas where they were found, but can potentially compete for prey resources in specific habitats (fronts and stratified waters, sardine and sprat). The three species exhibited a degree of habitat partitioning both spatially (anchovy occur in near-shore areas, sardine do not) and perhaps temporally (peak anchovy spawning will likely occur later than sprat). Ontogenetic changes are known to occur in habitat requirements and utilization, for example post-larval and juvenile stages of sprat are known to actively migrate to inshore areas for foraging (Beyst *et al.*, 1999). Accordingly, it will be important to continue to examine the inter-relationships between sardine, sprat and anchovy throughout their first year of life, since factors affecting recruitment are not only acting during the larval period but also during the early juvenile period in many fish species (Sogard, 1997).

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Chapter 7

General conclusions and outlook

The detailed knowledge on how environmental factors influence early life history is a prerequisite to understand and forecast stock size and distribution changes in clupeoid species. Due to the fact that clupeoids have a centuries long history of being one of the most important marine protein resource for human needs, members of this family of fishes have received a great deal of scientific attention since the earliest days of fisheries research (Mitchell, 1864; Heincke, 1874; Dodd, 1752; Cleghorn, 1854)

The information collected through numerous studies reveals that a high level of adaptability appears to be a basic trait for many clupeoid species (Geffen, 2009; Morote et al., 2008; Cury and Fontana, 1988; Roy et al., 1989). With more data available the picture of adaptability, plasticity and population structure becomes more and more complex and new questions arise. The present thesis answers some open questions with regard to habitat utilization and the role of temperature during early ontogenetic development. These questions have become increasingly important as research attempts to understand and forecast how anthropogenic impacts beyond fishing such as eutrophication or global warming (Peck et al., 2013; Rijnsdorp et al., 2009; Checkley et al., 2009) influence recruitment of clupeoid fish species.

Effects of eutrophication and habitat degradation

Degradation of shallow water coastal areas by fisheries, eutrophication, chemical pollution, traffic, harbours, tourism, and anthropogenic climate change have been observed worldwide (Short and Wyllie-Echeverria, 1996) and the populations of many aquatic species which utilize these areas as spawning and nursery grounds are heavily impacted. For example, the loss of suitable vegetation for spawning has led to a massive decrease in abundance in some Atlantic and Pacific herring spawning components (e.g. North Sea spring spawners, White Sea herring) (Klinkhardt, 1996; Wohlenberg, 1935; Cohen, 1995). Moreover, the spawning grounds of Baltic Sea herring stocks are thought to be impacted by eutrophication and other degradation effects (Aneer, 1985). However, qualitative and quantitative studies analysing those effects are sparse and, for most of the herring spawning areas in the Baltic Sea, high-resolution spatial data are not available.

The research presented in chapter 2 and 3 focused on the Greifswalder Bodden, a key spawning area for the Western Baltic spring spawning stock (WBSS) of herring. By utilizing SCUBA surveys, aerial images, towed video transects, abiotic data collection, review of historic literature and laboratory experiments, the historic and present situation of the spawning grounds in the bay can now be compared. These studies also revealed which indices are most useful to predict spawning bed selection of the WBSS herring, allowing insights to be gained on how eutrophication might influence stock recruitment.

Compared to historic submerged vegetation survey results from the 1930s we found a 92% reduction in macrophyte coverage within the Greifswalder Bay. The primary reason for this heavy loss is thought to be eutrophication and associated high phytoplankton blooms which lead to high turbidity (Munkes, 2005). Perennial macrophytes such as bladderwrack (*Fucus sp.*), eelgrass (*Zostera marina*) and pondweed (*Potomageton sp.*) have become limited to shallow depths (0.2-3.5m) and are nowadays only found on a narrow fringe along the coastline and islands, covering only 7 % of the total Greifswalder Bay. We could not find any community of loose-lying Black Carrageen (*Furcellaria lumbricalis*) which were reported to inhabit vast areas of sandy bottom in all deeper parts (4-10m) of the Greifswalder Bay in earlier times.

Despite 20 years of restoration efforts by governmental agencies and strongly reduced nutrient input, no increase towards the historical level of vegetation depth limits or total coverage of macroalgae and spermatophyte vegetation was apparent when comparing the present situation to studies conducted in the 80´s and early 90´s.

The decrease in macrophyte coverage has direct consequences to the availability of suitable spawning grounds for Atlantic herring in the region. Although Atlantic herring spawns on a variety of substrates across its wide range of distribution (Geffen, 2009), spring spawner appear to utilize more or less exclusively macrophytes in the Baltic Sea (Aneer and Nellbring, 1982; Scabell and Joensson, 1989; Kaaria, 1999). Also during the presented study divers did not

find any living herring eggs on mussels or on soft or hard sediments.

We observed eggs attached to 13 different macrophyte and spermatophyte species, indicating a relatively broad spectrum of plants that can be used as spawning substratum. However, this broad spectrum does not exclude that certain plant species are preferred or avoided as substrate. Macrophytes utilized as spawning substratum in the Greifswalder Bay were mainly large perennial, habitat forming plants, such as eelgrass, pondweed and Black Carrageen *(Furcellaria lumbricalis).* Drifting or epiphytic brown algae mats (*Pilayella littoralis and Ectocarpus sp.*) seemed to be actively avoided. The final choice of spawning substrate may depend upon both the types and availability of different substrates (Rajasilta et al., 1989). When all of these stations within the Greifswalder Bay were combined, statistical analysis did not show a general spawning preference for a certain plant species. This finding agrees with the results of other studies suggesting that plant type is a poor predictor for herring spawning activity (Rajasilta et al., 1993a; Aneer et al., 1983).

The best spawning predictors in the study were vegetation coverage thresholds within a radius of 100m respectively 500m, indicating that large underwater meadows attract spawning herring shoals. The spatial prediction of potential herring spawning grounds based on surrounding vegetation coverage seems ecologically sound and model results agree with almost all known herring spawning sites in the GWB.

A correlation between intense spawning activity and dense meadows has been also reported for herring spawning grounds in the Finnish and Swedish archipelagos and there is a general agreement that broad and rich vegetation zones are of primary importance for spring spawning herring (Rajasilta et al., 1993b; Kaaria et al., 1997; Aneer, 1989; Šaškov et al., 2011). Although the model is relatively basic, I believe it can be a valuable tool to locate spawning grounds of Baltic spring spawning herring in known spawning areas.

Due to the fact that robust spawning stock biomass estimates for WBSS are only available from 1991 onwards (Cardinale et al., 2009) and eutrophication reached its current maximum in the 1980´s, we were unable to quantify the consequences of the dramatic loss of potential spawning grounds with regard to stock variability. Additionally, the effect of reduction in spawning ground availability on stock size might be masked by increased food availability for larval, juvenile and adult herring through nutrient-enhanced primary production and associated increased secondary production. However, we assume that eutrophication has strong direct and indirect negative effects on the egg stages of Baltic herring. During the spawning season 2009 we found an average total egg mortality of 55 to 91% in the Greifswalder Bay (Manuscript 2). These rates are much higher compared to older studies from the Baltic Sea. Embryo (fertilized egg) mortality has been reported to be relatively low, typically ranging from 2 to 35 % (Lisivnenko, 1958; Rannak, 1958; Ojaveer, 1981; Klinkhardt, 1996; Scabell, 1989; Rajasilta et al., 1989; Rajasilta et al., 1993b). There are also reports of high or total mortalities from spawning grounds (Morrison et al., 1991; Aneer, 1985), but these reports normally describe spatially or temporally restricted events such as abnormally cold or warm temperatures, storm events or local oxygen depletion. In the case of this study, high mortalities were found over a wide area and were estimated for eggs deposited by different (temporally distinct) herring spawning waves. An intrinsic cause (e.g. maternal or paternal effects) for the observed high mortalities can be excluded since eggs transferred and incubated in the laboratory displayed significantly higher survival.

The analysis of the field data showed that values of egg concentrations were the best predictors for in situ egg mortality. This suggests that when eggs were laid in multiple layers and formed thick clumps, only the outer layer contained living eggs while eggs within the inner layers were dead. In general, when egg mass thickness increases, the inner eggs lacki direct contact with surrounding water which restricts their oxygen supply or causes a local increase in the concentration of metabolic wastes (McMynn and Hoar, 1953). A correlation between high egg concentrations and low egg survival has been confirmed by numerous field and laboratory studies (Blaxter and Dept, 1956; Hempel and Hempel, 1971; Rannak, 1971; Ojaveer, 1981; Haegele and Schweigert, 1985; Scabell, 1989). However, within pristine spawning grounds with large and dense areas of vegetation, one would envision that Baltic herring eggs would be more sparsely deposited in less layers as observed in earlier times

(Rannak, 1958). For Pacific herring (*Clupea pallasii*) stocks, which also utilize macrophytes as spawning substrate, it has been suggested that a density-dependence mechanism operates at the egg stage since the substrates they require for spawning may be limited in spatial extent and high spawning stock biomass leads to increased numbers of egg layers (Wood, 1981; Zheng, 1996; Iles and Beverton, 2000). In the case of the Greifswalder Bay, where underwater meadows nowadays only cover 8 % of their historical abundance, the egg carrying capacity of the spawning ground might be reached. When comparing predicted and observed spawning sites, 90% of the areas predicted to be utilized were actually utilized as spawning grounds. Moreover, multiple layers of eggs were encountered in most of these spawning sites, even relatively early in the spawning season. A density-dependent mechanism might exist particularly in years such as 2009 that were initially cold but then rapidly warmed. This temperature regime would be expected to synchronize the migration to spawning sites and result in a relatively short, intense spawning season (Klinkhardt, 1996).

An additional effect of eutrophication, which further reduces potential spawning site areas and might impact egg mortality, is blooms of filamentous epiphytic and drifting brown algae (e.g. *Pilayella littoralis* and *Ectocarpus sp.*). These algae were not utilized as spawning substrate by herring but they have overgrown vast areas of potential spawning habitats in the shallow bays in the Greifswalder Bodden. Additionally, the high metabolic rates of these species occurring within the spawning sites can result in low dissolved oxygen concentrations during calm periods especially at night (Breitburg, 2002). In combination with the observed high egg densities it is likely that local low oxygen phases during the final stages of herring egg incubation (Braum, 1985; Rombough, 1988) can be considered as the main reason for the high egg mortalities in 2009.

The reduced depth zone of macrophyte and spermatophyte vegetation has also increased the risk for recruitment failures on the egg levels in two other ways. Storm events in spring are likely to cause stronger impacts than in historic times due to the fact that almost all spawning areas are nowadays in depths above 3.5m and therefore increasingly affected by wave action. Secondly, shallow water systems are more affected by rapid temperature increases or warmer temperatures caused by global change (Rabalais et al., 2009). Warmer temperatures can have direct or indirect effects on egg mortality: relatively high temperatures, especially in spring, accelerate the oxidative decomposition of substances in the milieu, increase the metabolic rate of filamentous algae and also increase the oxygen requirements of the eggs. A recent publication (Polte et al., 2013) concluded that the egg phase is one of the key survival bottlenecks for herring in this region and it is therefore prerequisite to further monitor the environmental factors and herring spawning activity on the spawning grounds. Especially continuous oxygen measurements in shallow water areas during the spawning season need to be collected and analysed. Coastal management plans should minimize additional direct anthropogenic impacts like harbour dredging, coal power plant sewage and pipeline constructions in order to avoid a further reduction of the last potential spawning sites in the Greifswalder Bodden.

Effects of temperature

In the North and Baltic Sea, sea surface temperatures are projected to increase by 2.0 to 3.5°C by the end of the century, if our emissions continue to rise at current rates (Parry, 2007; Störmer, 2011). In order to better project climate change impacts on early life stages, in chapter 4, the effect of temperature on the survival, time to hatch, and changes in biochemical condition (nucleic acids) of Baltic herring embryos (egg and yolk-sac larvae) was quantified. Gaining reliable estimates of how long larvae can survive without feeding in a prey mismatch situation and how starvation rate and time until death are influenced by temperature is critical if one hopes to understand how bottom-up processes contribute to mortality in the sea (Meyer et al., 2012).

By employing a wide range in temperatures (from 3 to 22°C) in the presented experiments, it was hoped to gain a more complete picture of the optimal and suboptimal temperatures, which constitute the thermal window of this species. The results suggest that it is unlikely that climate-driven warming will cause direct mortality of herring embryos in the Greifswalder Bay or other spawning areas of Western spring spawning herring. The percent hatch of embryos in the experiments was variable. However, they were highest at intermediate temperatures (5–13°C) but did not decrease strongly until temperatures

were warmer than 20°C. Water temperatures in the Greifswalder Bay and Kiel Fjord (another spawning area of WBSS) during herring spawning season (April till early June) were reported to be well below 20°C in recent years and it is unlikely that future warming will generate these critical temperatures in spring and early summer. However, warmer temperatures greatly affect the temperature-dependent timing of critical periods during early ontogeny for herring. For example the duration of the egg phase decreased from 25 days at 5°C to 5 days at 20°C in the experiment. In terms of degree days, WBSS herring larvae reached the critical, "mixed feeding" stage at 60 to 100°d post-hatch and did not survive > 160 °d-post-hatch. Therefore, cooler temperatures provide a larger window of opportunity for developing larvae to start feeding, whereas herring have only a few days to start feeding at warm temperatures. Despite reduced time windows for successful first feeding at warmer temperatures e.g. in early-mid summer, larval herring appear to survive and grow well during that period in the Greifswalder Bay (Oeberst et al., 2009). Our results of the timing of important development stages agree mostly with earlier studies on herring larvae of different Baltic stocks (Ojaveer, 1981; Laine and Rajasilta, 1999; Klinkhardt, 1996; Herra, 1986; Hempel and Blaxter, 1963). However, it also became evident that there is a need for utilizing standard methods to address the central issue of how thermal sensitivity and adaptive capacity change across populations and species (e.g. (Somero, 2010)). In our Baltic herring example, one might expect thermal windows to differ between the Southwest and Northeast populations of herring that are genetically separate (Jorgensen et al., 2005) and experience large differences in water salinities. However, a lack of common methods hampered interstock comparisons.

The results of the study can also help to better interpret biochemical measures (RNA:DNA ratio (RD) and DNA × DM) when obtaining and analysing larvae from the field. The nutritional condition of marine fish early life stages has been evaluated using the ratio of nucleic acids (RD) for more than three decades (Buckley, 1984; Buckley et al., 1999; Buckley, 1979). Nonetheless RD is not usually considered an indicator of starvation because some life stages and species can compensate for extended periods of food deprivation, either by catabolising energy reserves within muscle and liver tissue or by utilizing embryonic yolk reserves, sometime leading to ambiguous patterns in changes in RD (Meyer et al., 2012). During the experiment, RD and DNA × DM-1 changed in consistent ways among temperature treatments during the yolk-sac larval period. RD values decreased in a non-linear way with increasing physiological age and were half their initial values after 59°d post-hatch. RD remained low and did not change after 120°d post-hatch. In contrast, DNA × DM displayed a linear increase and may provide a better biochemical proxy than RD to use on field-caught larvae to identify individuals that are in poor condition and are beyond the Point of no Return.

The use of Individual-based models (IBM) of larval fish foraging and growth has become a more and more valuable tool in order to analyse the reason for stock recruitment variability and to evaluate the potential impacts of climate change aspects (Peck and Hufnagl, 2012). Besides the temperature-dependent timing of critical periods in early larvae ontogeny, also accurate parameterisations of the temperature effects on metabolism is essential to construct a reliable model for fish species. In chapter 5 the routine respiration rate (R_R, µl O_2 ind^{-1} h^{-1}) of Baltic spring-spawning herring at temperatures between 9 and 19°C for groups of larvae having mean body sizes from ~12.0 to 22.5 mm standard length (*SL*) were presented. This study is the first to measure respiration rates in herring larvae across a very broad (ecologically relevant) range in water temperatures. The results demonstrate that temperature has a significant effect on the metabolic rate of Baltic herring, resulting in a Q10 of 1.5 for routine metabolism over the temperature range from 9-19°C. Estimates of R_R obtained in the present study agree well with those reported in other studies conducted on herring after taking into account differences in methods (Holliday et al., 1964; Almatar, 1984; Kiørboe et al., 1987; Overnell, 1997; Bang, 2005). Observed and predicted values in the experiment tended to agree well at cold and warm temperatures and for small and large body sizes. There was no significant interaction effect between temperature and body mass ($p < 0.05$). The overall analysis of both, temperature and body mass effects on standard metabolism resulted in a weight scaling exponent of b=0.665, which is lower than the weight scaling exponents for herring larvae reported from another study (Kiørboe et al., 1987).However,

Kiørboe measured R_S during anaesthesia and metabolic scaling factors have been reported to depend upon the activity occurring during measurements (Killen et al., 2010), thus direct comparison of the values in this and Kiørboe et al.'s study are not warranted.

The estimates of R_R appear to be reasonable estimates of routine energy losses appropriate for modelling the foraging requirements and growth of herring larvae at relatively warm temperatures normally experienced in the Baltic coastal areas in the early - late spring. Furthermore, it is important to note that measurements of larval herring swimming behaviour during active foraging and during starvation in the absence of prey are unavailable yet. Activity costs are expected to be high for small marine fish larvae with relatively low Reynold's numbers and swimming activity may override all other factors in determining oxygen uptake (Holliday et al., 1964). Although activity was not quantified during the measurements, indirect information on the costs of activity (R_A) was obtained. In the present study, R_R during the day (in the light) tended to be ~2-fold greater than at night (in darkness) although diel differences were not consistent and were only observed in trials testing relatively large larvae. These diel differences agree well with the magnitude of changes in activity of herring larvae at different light levels (Batty, 1987; Finn et al., 2002; Finn et al., 1995) and differences in R between anaesthetized and not anaesthetized larvae (Holliday et al., 1964). Future studies should attempt to couple measurements of swimming activity and respiration rates in herring larvae having different recent and long-term growth histories. Such data would provide much-needed estimates for IBMs that hope to describe the costs and tradeoffs of foraging in different prey fields (Rose et al., 2011; Hufnagl and Peck, 2011).

Interspecies interaction and habitat partitioning

A growing number of reports by European fishery scientists over the last 20 years demonstrate clearly that small pelagic fish populations in the shelf seas surrounding Europe are shifting their distributional borders to the North with dramatic changes in local abundance (Quero, 1998; Stebbing et al., 2002; Brander et al., 2003; Ter Hofstede et al., 2010; Simpson et al., 2011; Alheit et al., 2012; Alheit et al., 2013). Climate change can affect the distribution of fish populations by different mechanisms, for example by direct displacement of populations into new areas or increased productivity of fringe components of populations (Beare et al., 2004; Rijnsdorp et al., 2009). Moreover, the fauna of the southern North Sea exhibited clear changes in the recent decades. European sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) catches markedly increased since the 1990´s after a ~60-year absence and these clupeoids now co-occur together with sprat and herring. It is thought that the early life history stages of the occurring clupeoid species might be one of the keys for explaining the observed changes in distribution and abundance (Daewel et al., 2008; Raab et al., 2013; Petitgas et al., 2013). Chapter 6 examines the abundance, length distribution and biochemical condition (*RNA:DNA*) of sprat, sardine and anchovy among offshore and shallow nearshore areas in the southern North Sea. The extent to which larval sardine, anchovy, sprat and herring use the different North Sea habitats could have important implications for competition and relative inter-specific productivity. Herring larvae were absent during the sampling in June/ July, indicating a temporal partitioning of nursery grounds between larvae of herring and the other three clupeoid species. However, the situation might be different in late summer (August/September) when herring larvae from the western stocks are drifted into the German Bight where they might mix with late spawned sardine and anchovy larvae. The results of the presented study confirm the re-establishment of spawning populations of anchovy and sardine within the southern North Sea. While water circulation in the North Sea can transport eggs and foraging larvae from large catchment areas (including the English Channel) into the German Bight (Bartsch and Knust, 1994), the abundances of extremely young (pre-flexion) anchovy and sardine larvae obtained in the present study indicate local adult spawning activity. A clear degree of habitat partitioning was detected between the larvae of the three occurring clupeoid species. Sardine and sprat occurred in offshore areas, mainly in frontal- and stratified zones whereas anchovy larvae were found exclusively in shallow coastal areas. Aurich (Aurich, 1953) described a similar distribution pattern for anchovy larvae in the same area, which might be explained by the preferences of spawning anchovies towards warmer water temperatures (Petitgas et al., 2012). The

Biochemical indices suggested that larvae of all three species generally had high growth rates in the areas where they were found. However, the result that 9% of sardine at frontal stations had low RD values indicated food deprivation and potentially suggests a competition for prey resources in these waters.

It is thought that the expansion of anchovy in the North Sea was driven by pulses of successful recruitment of local subpopulations (Petitgas et al., 2012) connected with increases in water temperature as revealed through patterns in the Atlantic Multidecadal Oscillation (Alheit et al., 2013). Strong year classes are controlled by relatively warm summer temperatures of sufficient duration followed by favourable winter conditions (Raab et al., 2013). There is probably a balance between habitat suitability in terms of the pre-winter growth and winter conditions allowing sufficient survival at length (Raab et al., 2013; Petitgas et al., 2012). Since their peak in 2004, the abundance of European anchovy and European sardine in the North Sea has declined in likely in response to the several strong, cold winters. Populations of these species in the North Sea are now at relatively low levels (based on ICES-International Bottom Trawl Survey (IBTS) Data). Further research focussing on the overwintering situation of anchovies and sardines is needed to indentify critical thresholds in prey concentrations and water temperature. Also habitat preferences and potential habitat partitioning of the older stages (postlarvae, juvenile, adult) should be observed in greater detail. Also the example of the local North Sea anchovy population in combination with the Blackwater herring (Roel et al., 2004; Poulsen et al., 2007), Limfjord herring (Poulsen et al., 2007), Bay of Biscay herring (Alheit and Hagen, 1997) and Wadden Sea herring (Polte and Asmus, 2006) (all *Clupea harengus*) clearly show that clupeid species are able to persist in adverse conditions over many generations as self-recruiting remnant populations. Deeper knowledge of the factors influencing these remnant populations might help us to better understand marine evolutionary pathways and would improve the assessment of extinction risk of marine clupeoids and other marine fish species.

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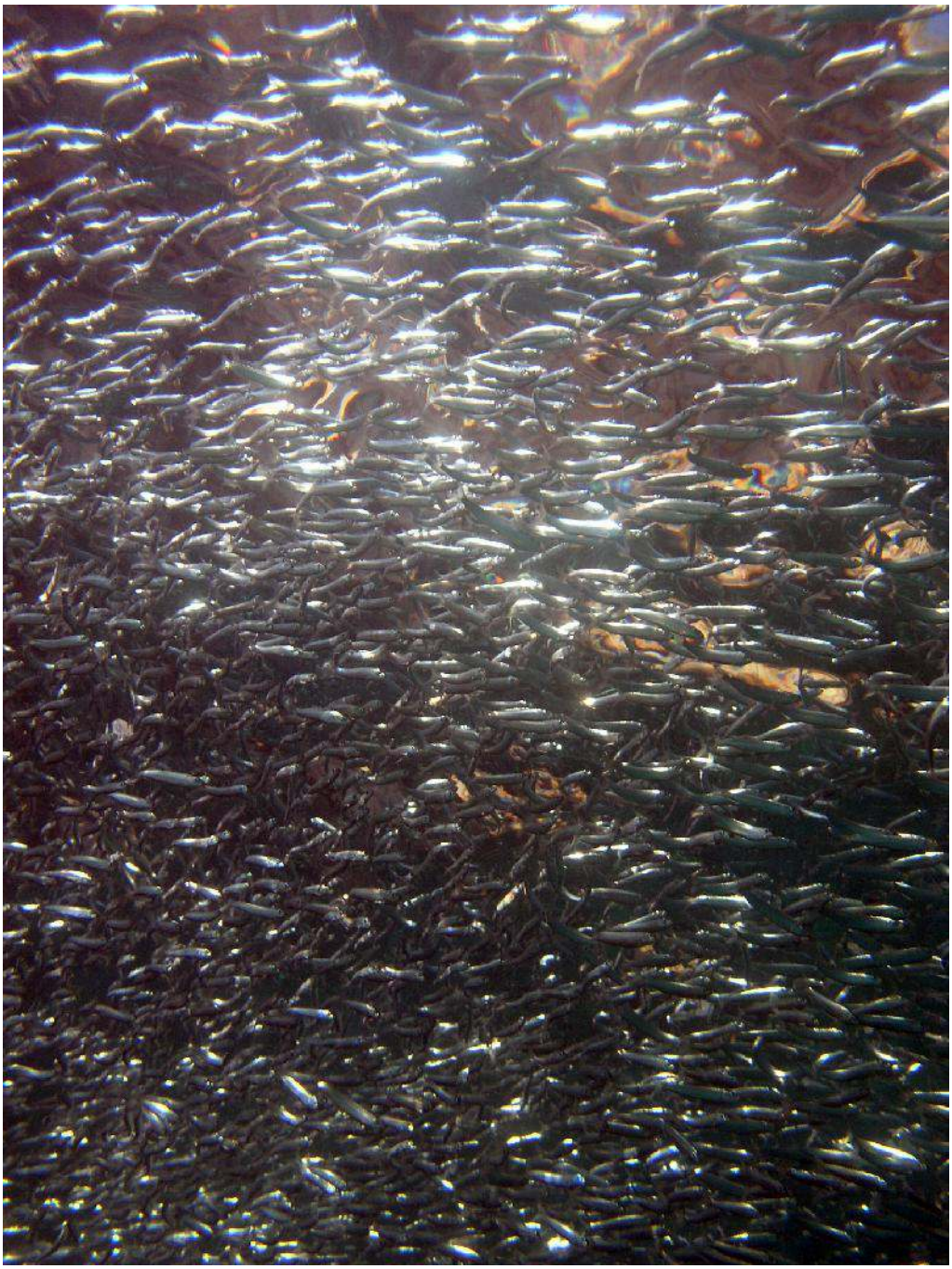

Juvenile sardines (*Sardina pilchardus*) Argolic gulf, Greece

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Individual scientific contributions to the multiple-author manuscripts and outline of publications

The chapters of this thesis are partly already published or written as manuscripts with multiple authorship. This list serves as a clarification of my personal contributions to each publication.

Macrophyte meadows and spawning bed selection of Atlantic Herring (*Clupea harengus*) in the Greifswalder Bodden, Baltic Sea

Submitted - Estuarine, Coastal and Shelf Science

Parts of the analyses, all text writing and graphical presentation were done by Philipp Kanstinger under supervision of Prof. Myron Peck and Cornelius Hammer. GIS analyses were conducted by Jutta Beher and Statistical Tree analyses by Klaus Huebert. Daniel Stepputis supervised and provided the towed video transect data and Görres Grenzdörffer processed and analysed the aerial images.

High Mortality Rates of Eggs at a Key Spawning Ground for Herring (*Clupea harengus*) in the southwest Baltic

All analyses, text writing and graphical presentation were done by Philipp Kanstinger under supervision of Prof. Myron Peck. Christof Schneider helped to organize the field sampling and to develop the sampling design.

Thermal windows supporting survival of the earliest life stages of Baltic herring (*Clupea harengus)*

Published – ICES Journal of Marine Science, 69: 529–536.

All analyses, text writing and graphical presentation were done by Prof. Myron Peck in close cooperation with Philipp Kanstinger and Linda Holste. Experiments were performed by Philipp Kanstinger, Linda Holste and Meike Martin under the supervision of Prof. Myron Peck.

Respiration rates of Atlantic herring (*Clupea harengus* L.) larvae and energy losses in IBMs depicting foraging and growth

Submitted - Journal of Experimental Marine Biology and Ecology

All analyses, text writing and graphical presentation were done by Prof. Myron Peck in close cooperation with Philipp Kanstinger. Experiments were performed by Philipp Kanstinger, Muriel-Marie Kroll and Maja Walter under the supervision of Prof. Myron Peck.

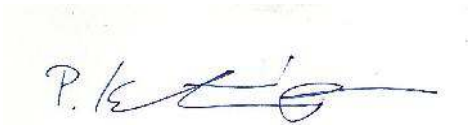
Co-occurrence of European sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) larvae in southern North Sea habitats: Abundance, distribution and biochemical-based condition

Published- Scientia Marina, Volume 73, No S1 (2009)

All statistical analyses, text writing and graphical presentation were done by Philipp Kanstinger under supervision of Prof. Myron Peck who developed the sampling design.

Declaration on oath

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.



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