# Seasonal influence of maternal effects and abiotic factors on early life stages of Atlantic herring *Clupea harengus*

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submitted by
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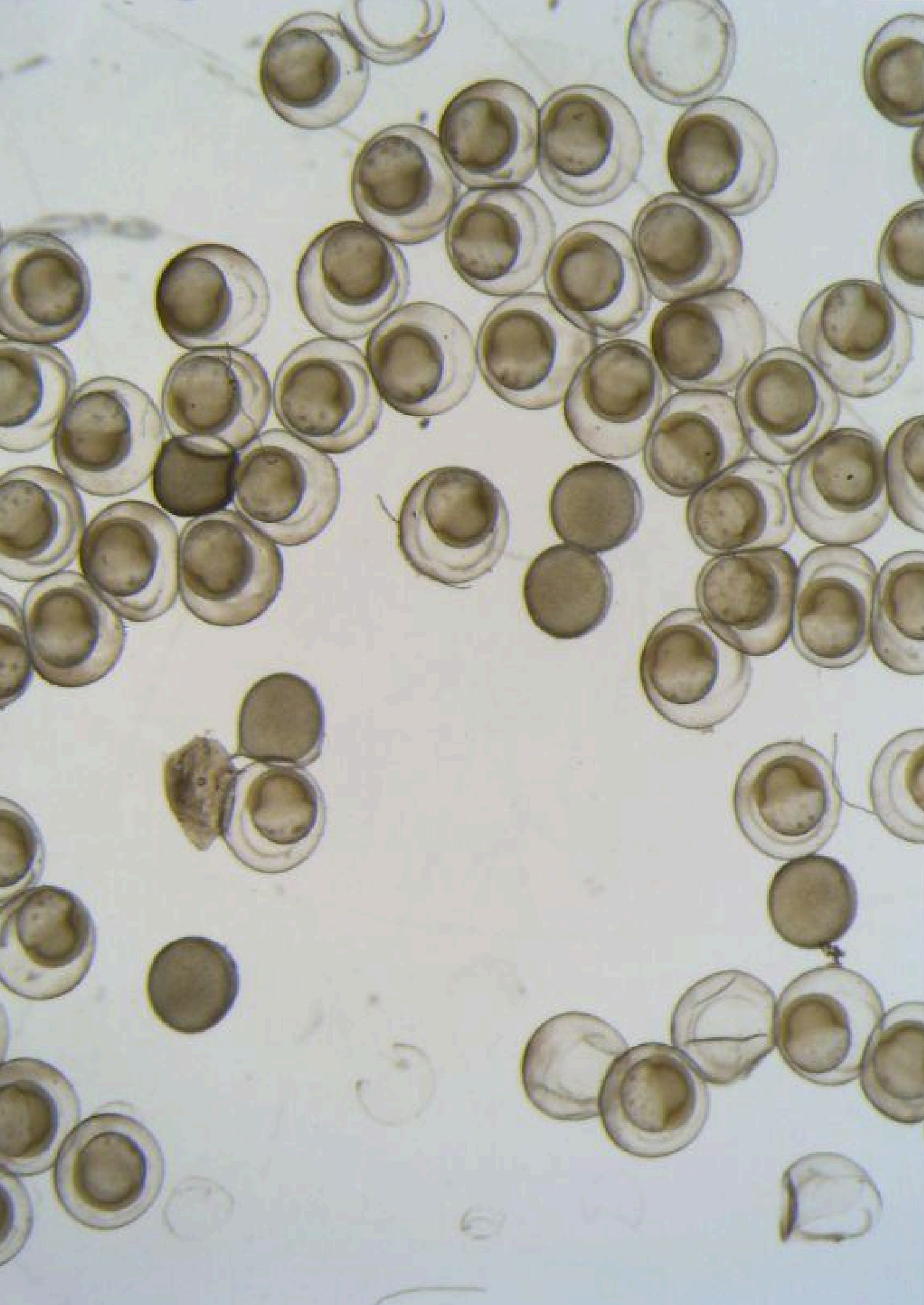

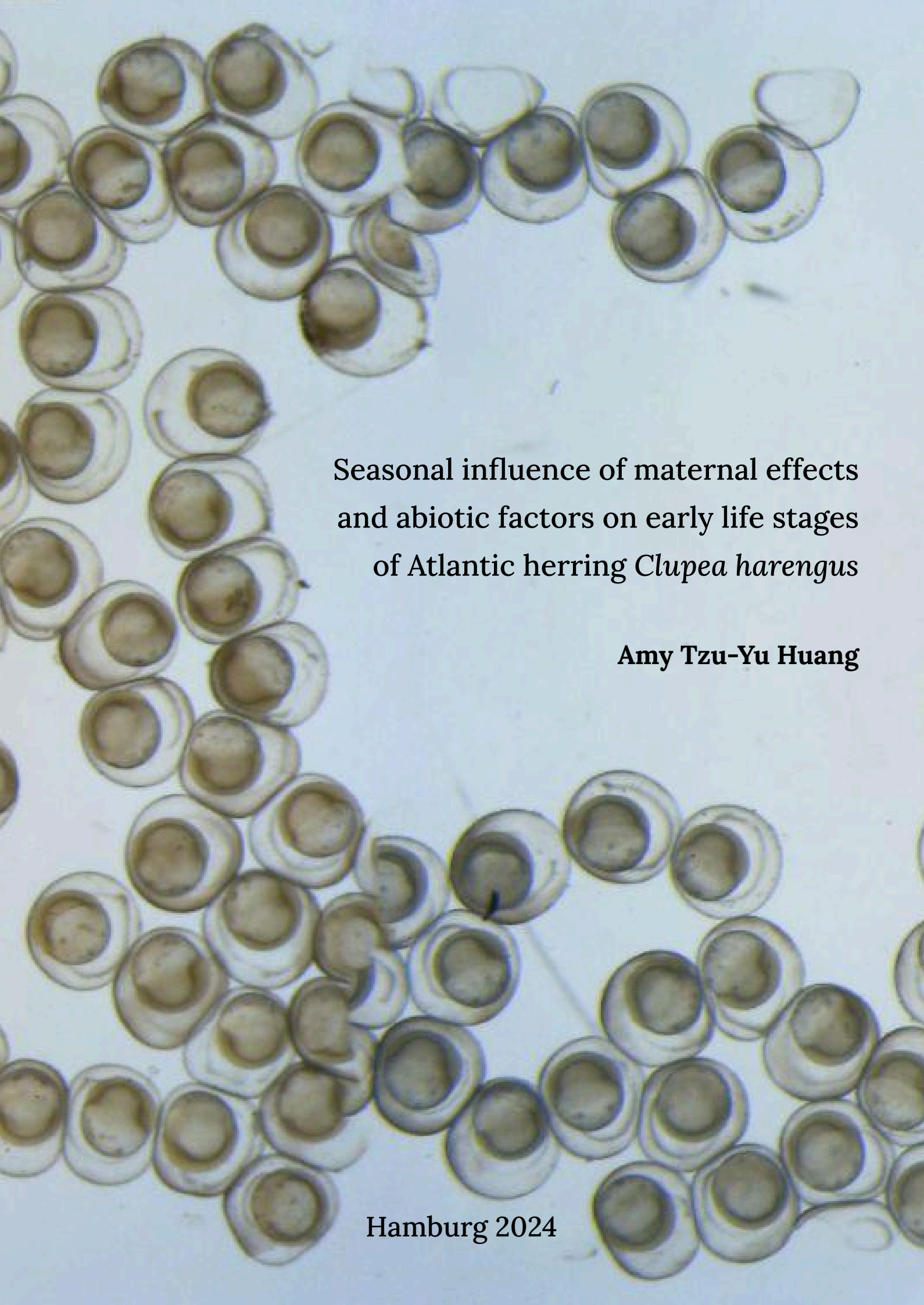

# Seasonal influence of maternal effects and abiotic factors on early life stages of Atlantic herring *Clupea harengus*

**Amy Tzu-Yu Huang**

Hamburg 2024

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# Summary

The North Sea and Baltic Sea rank among the fastest warming seas in the world, exhibiting the most pronounced warming rates during spring. This rapid increase in temperature may disrupt the life cycle scheduling of spring-spawning fish that depend on temperature cues for reproduction. Atlantic herring *Clupea harengus* is one of the most abundant and widely distributed small pelagic fish across the northern European region as well as a crucial prey species for piscivorous fish and mammals. In recent decades, elevated water temperatures prior to or during spring have been associated with reduced recruitment across various stocks of spring-spawning stocks. In particular, low recruitment rates in the western Baltic spring-spawning (WBSS) herring, compounded by overexploitation, have resulted in a steady decline in stock size since the 2000s. Long-term larval sampling (Rügen herring larvae survey) has been conducted annually in the main spawning and nursery ground of the WBSS herring, Greifswald Bay, where samples were taken weekly at various stations during spawning seasons and larvae were counted and measured. The survey revealed that the contribution of early-season cohorts to recruitment was low while the survival of larvae from late-season cohorts alone has been insufficient to rebuild the stock size.

Although field evidence implies larval cohorts from different times of the season have different survival rates, few studies have examined the explanatory factors. Seasonal differences in survival may be correlated with maternal and/or abiotic factors which also change with the progression of the season. In herring, larger, older females tend to spawn earlier than smaller, younger females, with first-time recruit females spawning last. Larger females also allocate more energy to reproduction and may produce larger and/or higher-quality eggs compared to smaller females. Moreover, larvae hatching at different times of the season encounter different environments such as different water temperatures. WBSS herring initiate spawning at a seawater temperature of ca. 4°C during spring while late-season females spawn ca. two months later. This translates to a temperature difference of more than 6°C between early- and late-season larval cohorts. As temperature governs the growth, development and metabolic rates of individuals according to their body size, larger and smaller larvae may use energy more (or less) efficiently at different temperatures.

The present thesis aims to disentangle maternal and environmental effects from seasonal effects on the survival of herring and size-at-age at early life stages. My co-authors

and I used field data on WBSS spawners to define the early, mid and late phases of the spawning season. We randomly selected 6–8 females from each seasonal timing and incubated their progeny in separate chambers throughout the egg (Chapter 2) and yolk-sac larval stages (Chapter 3) at early- (7°) and late-season (13°C) temperatures. The relative contribution of seasonal timing, females and incubation temperature to survival and mean size-at-ages was quantified. Finally, multi-year data on the size-at-ages and size-at-stages of larvae from WBSS as well as a winter- (Downs) and an autumn-spawning (Gulf of Riga) herring stocks were compiled. We quantified the variation in larval size through ages and ontogeny and examined the contribution of seasonal effects on the variation in larval growth and development (Chapter 4).

In WBSS herring (Chapters 2 and 3), survival of progeny from eggs until the end of the yolk sac larval stage was high (>85%) and no seasonal but small among-female differences were detected. Mean sizes of females, eggs and post-yolk larvae all decreased with the progression of the spawning season ($R^2$ = 0.31, 0.39 and 0.27, respectively). Compared with seasonal timing, individual females contributed equally ($R^2$ = 0.37) to the variances in mean egg size and 1.5 times more to that in mean post-yolk larval size. Although progeny size differed among different females, these maternal effects were, however, unrelated to female size, age or condition. Incubation temperature influenced the shape but not the yolk utilisation efficiency of yolk-sac larvae. In feeding larvae across herring stocks (Chapter 4), the (relative) variation in size-at-age increased after hatching to a maximum (10–15%) whereas the variation in size-at-stage decreased to a minimum (6%) prior to flexion, marking a threshold size required to enter the flexion stage. Larvae from colder months had a relatively shorter pre-flexion stage due to a larger initial size.

In conclusion, the present thesis identified the seasonal timing of spawning as a good predictor of egg and larval size, with size-at-ages of the early-season cohort being consistently larger than those of the late-season cohort in WBSS herring. Larvae with a larger initial size reached the threshold size for flexion more easily than smaller individuals. Although early-season cohorts had the advantage of a larger size, their low survival at the nursery grounds implies that other extrinsic processes such as seasonal match-mismatch with prey likely play more important roles in survival. While future studies should further examine the mechanisms of the seasonal variation in prey abundance and biotic dynamics,

management should implement plans to cease fishing until this stock has recovered and reduce nutrient input into coastal nursery grounds.

# Zusammenfassung

Die Nord- und die Ostsee gehören zu den sich am schnellsten erwärmenden Meeren der Welt, wobei die Erwärmung im Frühjahr am stärksten ist. Diese rasche Erwärmung kann den Lebenszyklus von Fischen stören, die im Frühjahr laichen und deren Fortpflanzung von der Temperatur abhängig ist. Der Atlantische Hering *Clupea harengus* ist einer der häufigsten und am weitesten verbreiteten kleinen pelagischen Fische in Nordeuropa und eine wichtige Beute von Raubfischen und Säugetieren. In den letzten Jahrzehnten wurden höhere Wassertemperaturen vor oder während des Frühjahrs mit einer geringeren Rekrutierung in verschiedenen frühjahrslaichenden Heringspopulationen in Verbindung gebracht. Die geringe Rekrutierung beim frühjahrslaichenden Hering der westlichen Ostsee (WBSS) und die Überfischung dieses Bestands haben seit den 2000er Jahren zu einem stetigen Rückgang der Bestandsgröße geführt. Im wichtigsten Laich- und Aufwuchsgebiet (Greifswalder Bodden) des WBSS-Herings wurden langfristige, jährlich Larvenbeprobungen (Rügen-Heringslarven-Survey) durchgeführt, bei denen wöchentlich während der Laichzeit an verschiedenen Stationen Proben entnommen und die Larven gezählt und gemessen wurden. Die Erhebung hat gezeigt, dass der Beitrag der frühsaisonalen Kohorten zur Rekrutierung gering war, während das Überleben der Larven aus den spätsaisonalen Kohorten allein nicht ausreichte, um die Bestandsgröße wiederherzustellen.

Obwohl Feldstudien darauf hindeuten, dass Larvenkohorten aus verschiedenen Saisonzeiten unterschiedliche Überlebensraten aufweisen, haben nur wenige Studien die erklärenden Faktoren untersucht. Saisonale Unterschiede in der Überlebensrate können mit mütterlichen und/oder abiotischen Faktoren zusammenhängen, die sich auch mit dem Verlauf der Saison ändern. Bei Heringen neigen größere, ältere Weibchen dazu, früher zu laichen als kleinere, jüngere Weibchen, wobei erstgebärende Weibchen zuletzt laichen. Größere Weibchen wenden auch mehr Energie für die Fortpflanzung auf und können im Vergleich zu kleineren Weibchen größere und/oder hochwertigere Eier produzieren. Darüber hinaus treffen die zu verschiedenen Zeiten der Saison schlüpfenden Larven auf unterschiedliche Umgebungsbedingungen wie z. B. unterschiedliche Wassertemperaturen. WBSS-Heringe beginnen im Frühjahr bei einer Meerwassertemperatur von ca. 4°C mit dem Laichen, während die Weibchen in der Spätsaison ca. zwei Monate später laichen. Dies bedeutet einen Temperaturunterschied von mehr als 6 °C zwischen den frühen und späten

Larvenjahrgängen. Da die Temperatur das Wachstum, die Entwicklung und den Stoffwechsel von Individuen in Abhängigkeit von ihrer Körpergröße steuert, können größere und kleinere Larven bei unterschiedlichen Temperaturen mehr oder weniger effizient Energie verbrauchen.

Die vorliegende Dissertation zielt darauf ab, mütterliche Einflüsse und Umwelteinflüsse auf das Überleben und die Größe im Alter der frühen Lebensstadien des Herings von saisonalen Effekten zu trennen. Meine Co-Autoren und ich haben Felddaten von WBSS-Laichern verwendet, um die frühen, mittleren und späten Phasen der Laichzeit zu definieren. Wir wählten nach dem Zufallsprinzip 6-8 Weibchen aus jeder Phase aus und bebrüteten ihre Nachkommen während des gesamten Ei- (Kapitel 2) und Dottersack-Larvenstadiums (Kapitel 3) in getrennten Kammern bei den Temperaturen der frühen (7°) und späten Saison (13°C). Der relative Beitrag des saisonalen Zeitpunkts, der Weibchen und der Inkubationstemperatur zum Überleben und zur durchschnittlichen Größe im Alter wurde quantifiziert. Schließlich wurden mehrjährige Daten über die Größe im Alter und die Größe im Stadium der Larven der WBSS-Heringe sowie einer im Winter laichenden (Downs) und einer im Herbst laichenden (Rigaischen Meerbusen) Heringspopulation zusammengestellt. Wir quantifizierten die Variation der Larvengröße über die Alters- und Ontogenesephasen und untersuchten den Beitrag saisonaler Effekte zur Variation des Larvenwachstums und der Larvenentwicklung (Kapitel 4).

Beim WBSS-Hering (Kapitel 2 und 3) war die Überlebensrate von den Eiern bis zum Ende des Dottersack-Larvenstadiums hoch (>85 %), und es wurden keine saisonalen, jedoch geringe Unterschiede zwischen den Weibchen festgestellt. Die durchschnittliche Größe von Weibchen, Eiern und Dottersacklarven nahm mit dem Fortschreiten der Laichzeit ab ($R^2$ = 0,31, 0,39 bzw. 0,27). Im Vergleich zum saisonalen Zeitpunkt trugen die einzelnen Weibchen in gleichem Maße ($R^2$ = 0.37) zur Varianz der mittleren Eigröße und 1,5 Mal mehr zur mittleren Größe der Larven nach dem Dottersackstadium bei. Die mütterlichen Auswirkungen auf die Größe der Nachkommenschaft waren jedoch unabhängig von der Größe, dem Alter oder dem Zustand der Weibchen. Die Inkubationstemperatur beeinflusste die Form, allerdings nicht die Dotterverwertung der Dottersacklarven. Bei der Fütterung von Larven aus verschiedenen Heringsbeständen (Kapitel 4) nahm die (relative) Variation der Größe im Alter nach dem Schlüpfen bis zu einem Maximum (10-15 %) zu, während die

Variation der Größe im Stadium vor der Flexion bis zu einem Minimum (6 %) abnahm, was eine Mindestgröße markiert, die für den Eintritt in das Flexionsstadium erforderlich ist. Larven aus kälteren Monaten hatten aufgrund ihrer größeren Ausgangsgröße ein relativ kürzeres Stadium vor der Flexion.

Zusammenfassend wurde in dieser Dissertation festgestellt, dass der saisonale Laichzeitpunkt ein guter Prädiktor für die Größe von Eiern und Larven ist, wobei die Größe im Alter der frühsaisonalen Kohorte beim WBSS-Hering durchweg größer ist, als die der spätsaisonalen Kohorte. Larven mit einer größeren Ausgangsgröße erreichten die Mindestgröße für die Flexion leichter als kleinere Individuen. Obwohl die frühsaisonalen Kohorten den Vorteil einer größeren Größe hatten, deutet ihr geringes Überleben in den Aufzuchtgebieten darauf hin, dass andere extrinsische Prozesse, wie z. B. die saisonale Nichtübereinstimmung mit der Beute, wahrscheinlich eine wichtigere Rolle für das Überleben spielen. Während zukünftige Studien die Mechanismen der jahreszeitlichen Schwankungen des Beuteaufkommens und der biotischen Dynamik weiter untersuchen sollten, müsste das Management Pläne zur Einstellung der Fischerei umsetzen, bis sich dieser Bestand erholt hat, und den Nährstoffeintrag in die küstennahen Aufwuchsgebiete reduzieren.

# Chapter 1

## General Introduction

Populations of marine fish have dramatically declined in size due to over-exploitation (Hutchings, 2000; Neubauer *et al.*, 2013) as well as shifted their productivity and distribution because of climate change (Hollowed *et al.*, 2013; Peck & Pinnegar, 2018; Philippart *et al.*, 2011). Winters are becoming milder and shorter, and extreme weather events such as heatwave occurs more often (Bindoff *et al.*, 2019; Frölicher *et al.*, 2018). While the EU has established goals to promote sustainable harvesting of marine fish (Froese *et al.*, 2018), managing marine resources remains challenging as habitats change at an unprecedented pace (Brander, 2010).

The North Sea and Baltic Sea are among the fastest warming seas of the world with the highest warming rate during the spring months (Dutheil *et al.*, 2021; Lima & Wethey, 2012). For example, in the Kiel Bight of the western Baltic Sea, water temperatures from January to February have risen by 3-5°C in recent years compared to two decades ago (Froese *et al.*, 2022). Historically, February had been the coldest month, with water temperatures dropping to below 3°C and cooler than January and March (Figure 1.1). In recent years, however, the temperature differences among the months from January to March have become smaller and smaller. An extreme case of this trend occurred in the year 2020, when there was almost no difference in water temperatures among these months.

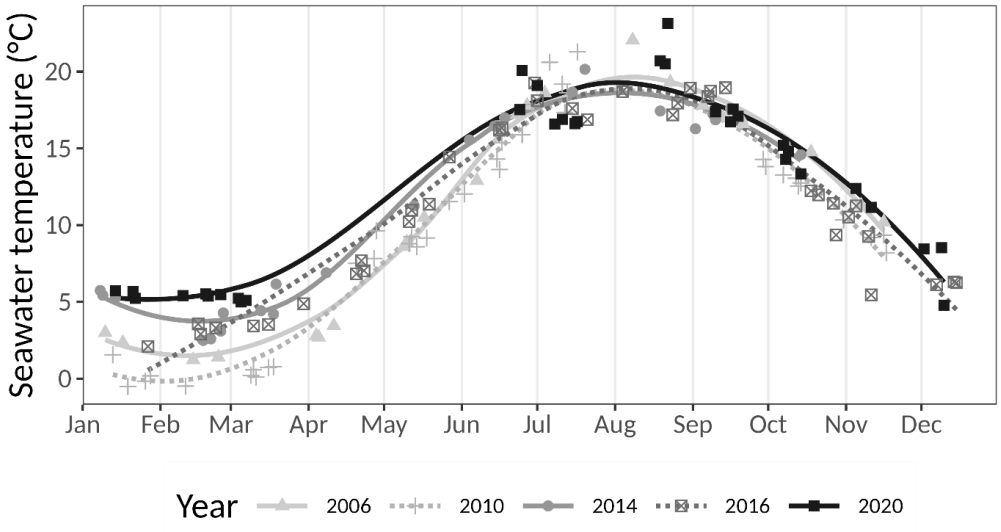


**Figure 1.1** Seawater temperatures in Kiel Bight of the western Baltic Sea. Data from ICES data portal.

Warming temperatures in spring pose a great challenge to marine fish stocks, which rely on temperature cues to synchronize spawning (Pankhurst & Munday, 2011). For instance, the spring-spawning herring stock in the western Baltic Sea begins spawning when water temperatures rise to 3.4–4°C during the spring months (von Dorrien *et al.*, 2013; Polte *et al.*, 2021). As this temperature threshold has been reached earlier in the year (Figure 1.2), the herring stock has started spawning activities several weeks earlier compared to five decades ago (Ory *et al.*, 2024). This trend has also been observed in another herring stock near the region (Arula *et al.*, 2019). The year 2020 was extremely warm, with monthly average water temperatures consistently meeting or exceeding 5°C (Figure 1.1). This resulted in the lowest recorded recruitment (the number of young fish surviving to join the adult population) of the western Baltic herring stock in history (ICES, 2022).

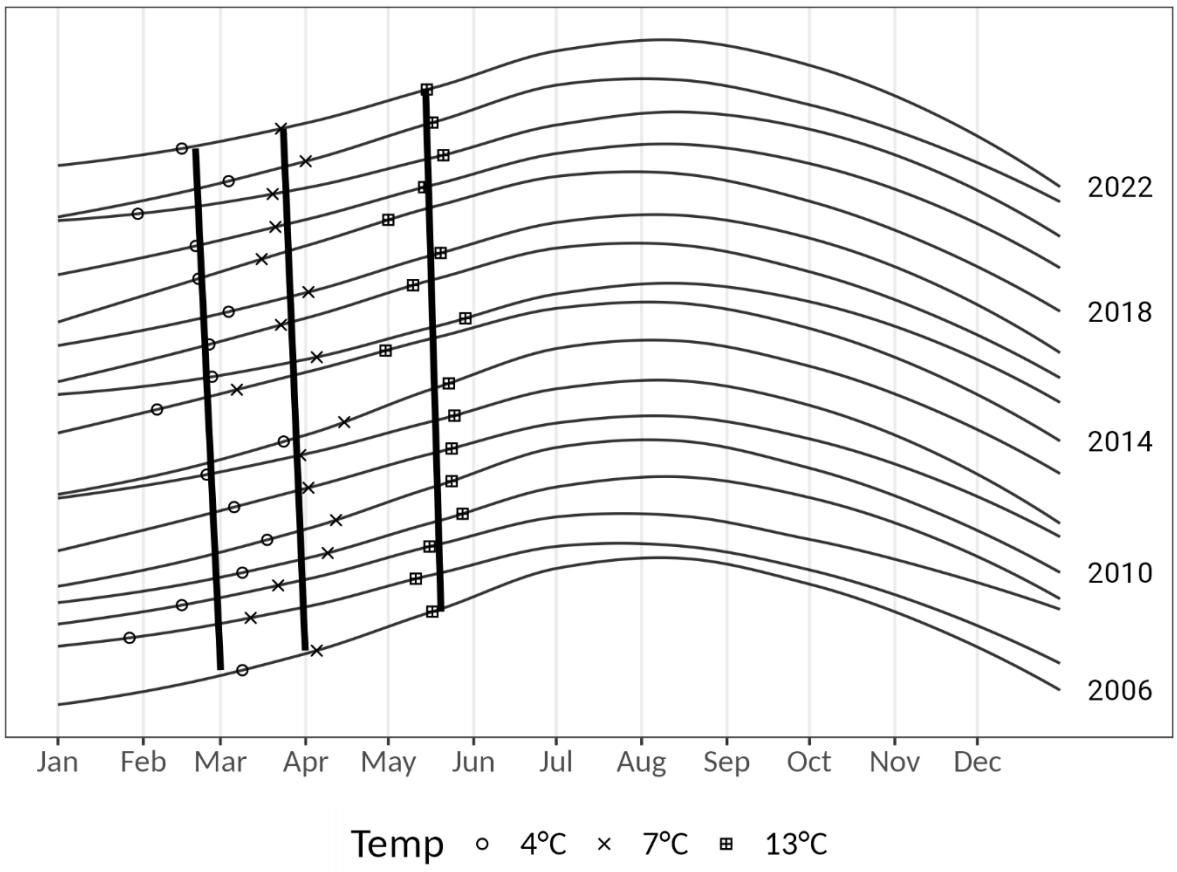


**Figure 1.2** Day of the year when water temperature thresholds (4, 7, and 13°C) are first reached in Kiel Bight of the western Baltic Sea. Data from ICES data portal.

The association between milder winter (or earlier warming in spring) and reduced recruitment levels has been documented in several spring-spawning herring stocks from the Baltic Sea and the Norwegian coast (Arula *et al.*, 2022; Polte *et al.*, 2021; Toresen *et al.*, 2019). These studies identified a prolonged spawning and hatching period after mild winter, along with a reduced subsequent abundance of larvae surviving to the advanced developmental stage, as potential causes for this phenomenon (Arula *et al.*, 2022; Polte *et al.*, 2021; Toresen *et al.*, 2019).

With an attempt to understand the implications of changes in marine fish phenology, the remainder of the chapter outlines the general seasonal environments of temperate and boreal seas, describes the reproductive strategy of marine fish in these regions with a focus on Atlantic herring *Clupea harengus*, and introduces the research questions and outline of the present thesis.

## 1.1 Seasonality of fish reproduction

### 1.1.1 Physical and biological seasonality

Marine fishes in the temperate and boreal zones are shaped by distinct seasonality (Fridolfsson *et al.*, 2023). Day length undergoes a fixed annual cycle, reaching its peak in summer and its nadir in winter. Correspondingly, water temperatures near the ocean surface warm and cool in tandem with changes in day length, whereas temperatures in deeper oceans remain cool and stable. As the temperature difference between the surface and deep waters increases, the ocean stratifies into warmer upper layers and cooler lower layers (Pohlmann, 1996). This stratification inhibits the mixing of nutrient-rich deep waters with the nutrient-depleted upper waters (Radach & Lenhart, 1995). Both sunlight and nutrients are required by phytoplankton, the ocean's primary producers, to perform photosynthesis. Since the nutrient concentration in the upper waters peak during the coolest months (Radach & Lenhart, 1995), phytoplankton populations are able to surge as soon as sufficient sunlight reaches the waters in spring (Sverdrup, 1953). This leads to a typical spring "bloom" of phytoplankton during which nutrients are rapidly consumed (Friedland *et al.*, 2016; Uitz *et al.*, 2010). The increased abundance of phytoplankton supports a corresponding rise in zooplankton biomass (Mackas *et al.*, 2012). As nutrients are depleted by early summer, both

phytoplankton and, subsequently, zooplankton gradually decrease in abundance until the next annual cycle begins. This interplay of physical and biological seasonality in temperate and boreal oceans defines a productive period from spring to autumn and an unproductive period during winter (Varpe, 2017).

### 1.1.2 Reproductive strategies of marine fishes

To confront the challenge of scarce food during the unproductive months, marine fish inhabiting higher latitudes have developed strategies to time their feeding and reproductive activities to specific parts of the year (Bye, 1990; Lowerre-Barbieri *et al.*, 2011). Most commercially important species (e.g. the clupeids, gadoids and flatfishes) are iteroparous; they can regenerate their gonads and reproduce multiple times throughout their lifespans. This contrasts with semelparous species such as capelin *Mallotus villosus* and Pacific salmons *Oncorhynchus* spp., which spawn once and then die (Murua & Saborido-Rey, 2003). Iteroparous marine fishes in temperate and boreal zones typically undergo annual cycles of gonad maturation. During these cycles, the number of oocytes destined to develop for the upcoming spawning season is determined and fixed at the initial stage of gonad development (Murua & Saborido-Rey, 2003). In other words, no additional primary oocytes will enter secondary maturation beyond those initially selected. This strategy, known as determinate fecundity (Brown-Peterson *et al.*, 2011; Lowerre-Barbieri *et al.*, 2011; Murua & Saborido-Rey, 2003), features a restricted and relatively shorter spawning season compared to that of tropical marine fishes (Brown-Peterson *et al.*, 2011; Lowerre-Barbieri *et al.*, 2011).

During the productive months, adult fish migrate to feeding grounds to gather energy, which can be used immediately or stored for winter (Dawson & Grimm, 1980; Schwalme, 1999; Slotte, 1999a). Females initiate oocyte development during these feeding months (Damme *et al.*, 2009), but the accumulation of yolk occurs later and relies on the surplus of stored energy reserves. The number of oocytes undergoing maturation can be reduced at any stage via resorption (atresia) by the females, depending on their nutritional status and the remaining energy reserves (Damme *et al.*, 2009; Kurita, 2003). In extreme cases, where spawning poses too great a risk or environmental conditions are unfavourable, females may resorb all of the oocytes and skip spawning for that year (Rideout *et al.*, 2000).

The season when spawning occurs varies among different stocks, even within the same species (Haegele & Schweigert, 1985). Day length serves as a reliable cue of the time and season of the year, while temperature regulates the final stage of gonad maturation (Pankhurst & Porter, 2003). For stocks that spawn in winter or spring, increasing day length and temperature signal the onset of spawning, whereas for those spawning in summer or autumn, it is the decrease in these cues that initiate spawning (Pankhurst & Porter, 2003). Regional populations begin and terminate spawning based on specific temperature thresholds, thereby defining the spawning season of each stock within a temperature window (Peck *et al.*, 2012a).

Mature adults migrate from their overwintering grounds to specific spawning grounds, where they release and fertilize their eggs (Figure 1.3). Most temperate marine fish, including the gadoids, flatfishes and most clupeids, are batch spawners and release their eggs in multiple batches over a span of several weeks (Murua & Saborido-Rey, 2003). In contrast, Atlantic herring and capelin are total spawners and release a single batch of eggs within the spawning season. During the season, groups of adults arrive and release eggs at different times (Arula *et al.*, 2019; Lambert, 1987). The spawning period for both batch and total spawners extend beyond a few weeks and typically last for two to four months (Chambers, 1997). After releasing all the eggs, the adults leave the eggs to their fate and migrate to the feeding grounds, providing no parental care to progeny (Murua & Saborido-Rey, 2003).

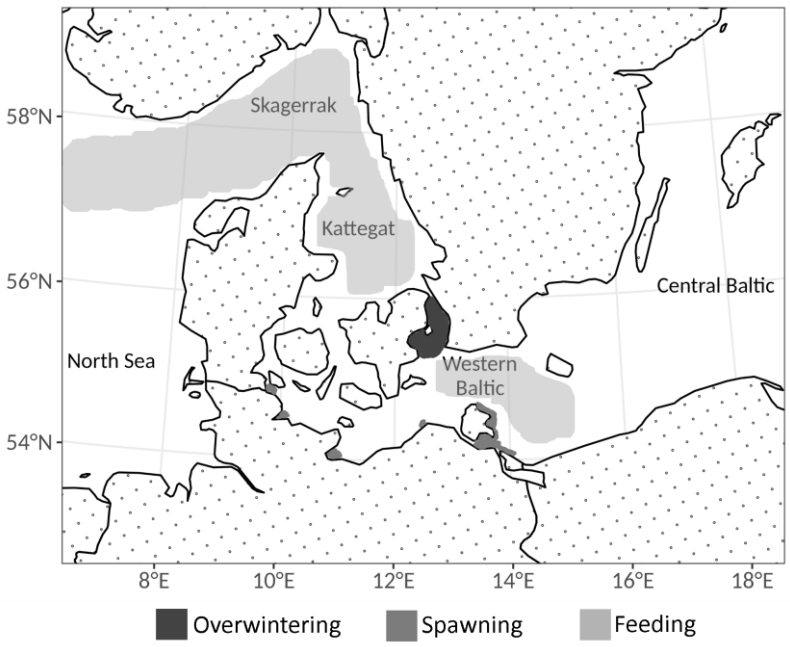


**Figure 1.3** Overwintering, spawning, and feeding grounds of the spring-spawning stock of Atlantic herring *Clupea harengus* in the western Baltic Sea.

Most species produce pelagic eggs that drift with the currents, whereas Atlantic herring produce demersal eggs which sink and adhere to subtracts such as seaweed or gravel (von Dorrien *et al.*, 2013). Depending on the temperature, both pelagic and demersal eggs hatch into planktonic, free-swimming yolk sac larvae after ca. two to five weeks (Olsen *et al.*, 2010). At this stage, the larvae rely entirely on their yolk reserves and are unable to ingest food until they develop simple digestive organs a few days later (Yúfera & Darias, 2007). Once the mouth, oesophagus and gut are functional, larvae begin to prey on zooplankton. Some larvae may still have residual yolk reserves, enabling them to feed both endogenously and exogenously (mix-feeding) for a few days (Busch *et al.*, 1996). The larval stage lasts ca. three months until metamorphosis into juveniles (Olsen *et al.*, 2010), which resemble adults but possess immature reproductive systems (Figure 1.4).

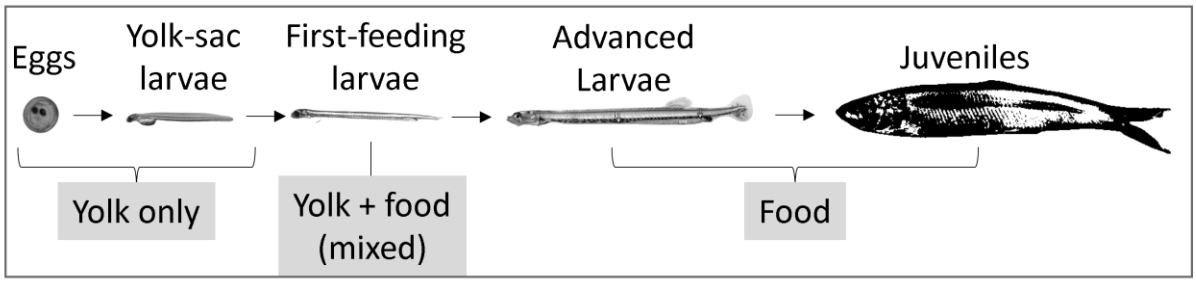


**Figure 1.4** Life cycle and energy sources for different life stages of Atlantic herring *Clupea harengus*. Photo of the advanced larvae from Norland *et al.* (2021).

## 1.2 Seasonal effects on progeny

### 1.2.1 Mortality in early life stages

The early life stages of marine fishes have extremely high mortality rates (Houde, 1987) and have drawn much attention in fisheries studies (Llopiz *et al.*, 2014). Eggs and larvae are relatively small and passive, making them vulnerable to a wide range of oceanic predators (Paradis *et al.*, 1996). After larvae develop simple digestive organs, their yolk reserves are nearly depleted (Yúfera & Darias, 2007), and their small bodies cannot withstand starvation for more than a few days (Yin & Blaxter, 1987). Larvae naturally experience high mortality rates due to predation and/or starvation, particularly during the transition from endogenous (yolk-based) to exogenous feeding (Houde, 2008). On average, >99% of all individuals produced will die by the end of the larval stage, before joining the adult population (Houde,

1997). As a result, marine fish populations exhibit substantial inter-annual variations in recruitment due, in principle, to environmentally driven changes in the growth and survival of young larvae (Houde, 2016). Even minor fluctuations in mortality rates can lead to significant variations in yearly recruitment level, a phenomenon that has remained at the heart of fisheries ecological research for more than 100 years (Hjort, 1914). The studies of Hjort (1914) showed that particular age group within a population could constitute a dominate portion the stock abundance over several years, highlighting the importance of recruitment variability in population dynamics.

The number of offspring that survive through the larval stage can provide a reliable estimate of recruitment. It has been demonstrated that larval mortality rate decreased as the larvae increased in size (Ware, 1975). Larger larvae typically exhibit improved swimming capabilities and foraging skills and are more resilient to starvation compared to smaller larvae (Clark *et al.*, 2005; Moyano *et al.*, 2016). Furthermore, increased size allows larvae to consume a broader range of prey, as their mouth gap can accommodate larger food items (Peck *et al.*, 2012a). Once larvae reach a certain size threshold where mortality rates have notably decreased and become stable, it is likely that a significant proportion of these survivors will eventually be recruited into the adult population. For example, in western Baltic spring-spawning herring, a size of 20 mm marks the size at which mortality stabilizes, and the abundance of larvae beyond this size serves as an estimate of recruitment for this stock (Oeberst *et al.*, 2009b).

### 1.2.2 Differences in cohort survival

As eggs are released at different times during the spawning season, they give rise to distinct larval and juvenile cohorts, each hatching at different points in the season. Variations in the survival of different cohorts have been documented for both batch-spawning and total-spawning fish species in the Northeast and Norwest Atlantic. Table 1.1 provides a review that highlights the differences in cohort survival and identifies intrinsic and extrinsic factors that may contribute to these variations. The similar patterns observed across both types of spawners suggest that the factors driving variation in cohort survival are likely comparable among them.

**Table 1.1** Differences in cohort survival observed in marine fish stocks in the Northern Atlantic Ocean. Abbreviations: d, day; F, field study; L, laboratory study, NE, Northeast Atlantic; NW, Northwest Atlantic.

| Species | Population | Higher survival | Correlation | Study | References |
|---|---|---|---|---|---|
| **Batch spawner** | | | | | |
| Haddock *Melanogrammus aeglefinus* | NE, North Sea spring | Early | Late: ↑first-time females | F, Feb–Jul 1994, 1996, 1999 | (Wright & Gibb, 2005) |
| Haddock | NW, Bay of Fundy spring | Early (at low prey) | Late: ↓feeding capabilities | L, 2001–2003 (adult Oct 2000), 0–20 d | (Rideout *et al.*, 2005) |
| Sole *Solea solea* | NE, Bay of Biscay spring | Early (first half) | | F, Feb–Mar 1992 | (Amara *et al.*, 1994) |
| Atlantic cod *Gadus morhua*, haddock | NW Atlantic, Georges Bank spring | Early | Early: ↓predators | F, Jan–Jun 1995–1999 | (Buckley *et al.*, 2010) |
| Winter flounder *Pseudopleuronectes amehcanus* | NW, Narragansett Bay spring | Early | | L, Feb–Apr 1968, 0–30 d | (Buckley *et al.*, 1991) |
| Mueller's pearlside *Maurolicus muelleri* | NE, Herdlefjorden autumn | Late | Early: ↓growth, ↓condition | F, Sep–Nov 1995 | (Folkvord *et al.*, 2016) |
| **Total spawner** | | | | | |
| Atlantic herring *Clupea harengus* | Norwegian spring | Early | Early: ↑northward drift, ↓growth, ↓temp | F, May–Dec 1991, 1992, 1996 | (Slotte *et al.*, 2019; Vikebø *et al.*, 2010) |
| Atlantic herring | Norwegian spring | Early (first half) | | F, Mar–May 1985, 1989, 60 d | (Moksness & Fossum, 1992) |
| Atlantic herring | Norwegian spring | Late | Late: ↑copepod abundance | F, Jan–May 1990 | (Fossum & Moksness, 1993) |
| Atlantic herring | Baltic spring | Early (first half) | Early: ↑prey, ↓predators | F, Jul–Nov 1993 | (Arrhenius & Hansson, 1996) |
| Atlantic herring | Baltic spring | Early (eggs) | Early: ↓temp, ↓depth, | F, May–Jun 1987–1989 | (Rajasilta *et al.*, 1993) |
| Atlantic herring | Blackwater Estuary spring | Early (not significant) | | F, 1979, 50 d | (Henderson *et al.*, 1984) |

The variation in cohort survival underscores the importance of examining how timing of spawning influences the characteristics of resulting cohorts. Previous studies suggest that, within the same spawning season, the quality of progeny can vary across different seasonal cohorts. First, older and/or larger females tend to arrive at the spawning ground earlier and release their eggs earlier than younger, especially first-time females, who tend to spawn last (Lambert, 1987; Slotte *et al.*, 2000). Second, older and/or larger females produce larger eggs of higher quality (Buckley *et al.*, 1991; Kjesbu, 1989; Trippel, 1998). Third, research on batch spawners indicates that eggs released earlier in the season are generally larger and contain more yolk reserves compared to those released later (Buckley *et al.*, 1991; Chambers & Leggett, 1996; Trippel & Neil, 2004).

### 1.2.3 Atlantic herring as total spawners

Atlantic herring *Clupea harengus* (Clupeiformes, Clupeidae) is a small pelagic fish of high ecological, economic, and cultural importance in northern Europe since medieval times.

Herring ranks top six of all marine species in global catch production (FAO, 2018) and serves as a critical link between lower (plankton) and higher (piscivores) trophic levels in the regional seas of northern Europe (Trenkel *et al.*, 2014). Its life cycle, characterised by nearshore spawning and offshore feeding migration, establishes unique teleconnections between separate food webs. Historically, herring has been a pivotal species in fisheries oceanographic research, arguably the most significant since the foundational studies by Hjort (1914).

Atlantic herring consists of many (meta- / sub-) populations which spawn at a unique time – with spawning in northern Europe occurring in every season (Hay *et al.*, 2001); for example, the spring-spawning stocks in the Baltic Sea, autumn-spawning stocks in the North Sea, summer-spawning stock near Iceland, and winter-spawning stock in the English Channel. However, these seasonal classifications are broad, as the specific spawning months vary according to local environmental conditions. In the Baltic Sea, spawning progresses from the southwest (beginning in January–February) to the northeast (in May), while in the North Sea, spawning starts with the northern stocks (in August) and then to the southern stocks (in December) (Haegele & Schweigert, 1985). Further, certain regions, such as the western Baltic Sea and Scotland's coastal waters, serve as spawning grounds for both spring- and autumn-spawning stocks (von Dorrien *et al.*, 2013; Frost & Diele, 2022).

Among common iteroparous marine temperate fishes, Atlantic herring is unique as the only total spawner (Murua & Saborido-Rey, 2003). Herring congregate in groups and spawn sequentially in waves (Lambert, 1987), which give rise to multiple larval and juvenile cohorts that originate from different females (Figure 1.5). This characteristics makes it relatively easier to disentangle extrinsic and intrinsic effects on the offspring of herring compared to other batch spawners, in which the same females may contribute to several cohorts. Consequently, Atlantic herring serves as an ideal species for investigating both inter- and intra-seasonal effects on progeny traits and survival.

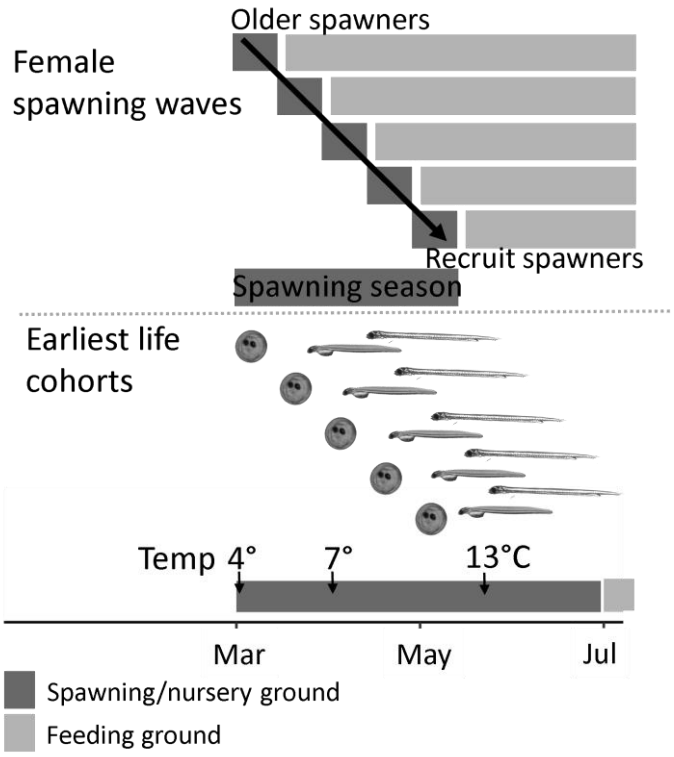


**Figure 1.5** Spawning waves of Atlantic herring *Clupea harengus* during the spring spawning season and the resulting successive cohorts at the spawning (also nursery) ground.

## 1.3 Objectives of thesis

### 1.3.1 Research gap

Numerous studies on herring have reported variations in survival rates among (early- and late-season) larval cohorts from the same spawning season (Table 1.1). Predominantly conducted as field studies before the 1990s, these investigations compared the hatching dates of survivors observed in a later life stage (e.g. late larvae or juvenile) with the hatching dates of newly hatched larvae sampled earlier. The findings from these studies suggest that early-season cohorts tend to exhibit higher survival rates compared to late-season cohorts. Despite the known differences in cohort survival, comparative studies on the environmental condition and characteristics of progeny from early, middle, and late phases of the season remain limited.

As numerous physical and biological factors coincide with seasonal timing, the true determinant of cohort survival are these factors rather than the timing itself. Thus, seasonal effects on cohort survival may arise from one or multiple factors, such as day length,

temperature, phyto- and zooplankton abundance, female status (e.g. size, age, condition), or egg size. In this context, examining the influence of these specific factors is crucial for unravelling the mechanisms behind differences in survival rates.

### 1.3.2 Hypotheses and outline of thesis

The present thesis examined how seasonal variations in maternal effects and abiotic factors, specifically temperature, interact to impact the early life characteristics of Atlantic herring and the temporal scale over which changes occurred. The seasonal scales under consideration included both within-season (early-, mid-, late-spring; Chapters 2 and 3) and among-season (spring, autumn, winter; Chapter 4) spawning periods. This research involved tightly coupled field surveys of spawning adults and controlled laboratory experiments. The laboratory results were designed to provide a mechanistic understanding of the patterns observed in long-term time-series data of herring larvae collected from Greifswald Bay in the western Baltic Sea.

The following five, inter-related research questions (Q) were investigated:

Q1. How does the seasonal timing of spawning impact progeny growth and survival?

Q2. How do female body size and condition impact progeny growth and survival?

Q3. Are size classes consistent throughout ontogeny, such that individuals with larger initial size remain larger at later developmental stages?

Q4. When and to what extent do differences in progeny traits (e.g. size and growth among conspecifics) emerge?

Q5. How do variations in spawning season used by metapopulations impact progeny growth?

Building on reported observations that larger females arrive earlier at the spawning grounds (Lambert 1987) and the theory that larger progeny tend to outperform smaller ones (Marshall *et al.* 2018), the following hypotheses (H) are formulated in alignment with each research question.

H1. Progeny of higher quality are produced earlier in the spring reproduction season.

H2. Progeny performance is influenced by both maternal traits and the relative timing of reproduction within the season; for instance, early-season progeny are expected to perform better at early-season temperatures than at late-season temperatures.

H3. Body size is an important performance trait and "bigger is better".

H4. Traits that enhance initial performance (e.g. a larger size) confer advantages throughout early development, from eggs to larvae transitioning to exogenously feeding.

H5. Patterns of progeny traits align with seasonal counter gradients within and among reproductive seasons.

In Chapters 2 and 3, females of the western-Baltic spring-spawning (WBSS) herring from early, middle and late phases of the spawning season were assessed. Their progeny were incubated in a controlled laboratory setting at two temperatures representative of early (7°C) and late season (13°C) conditions (H1, H2). The extent of variation in progeny characteristics was monitored both among individual females and across seasonal cohorts (H3, H4). In Chapter 4, multi-year laboratory data on larvae (from hatch up to 76 days post-hatch) of WBSS (spring-spawning), Downs (winter-spawning) and Gulf of Riga herring (autumn-spawning) were compiled to investigate patterns and variations in size-at-ages across different seasons (H4, H5).

# Chapter 2

# Disentangling seasonal from maternal effects on egg characteristics in western Baltic spring-spawning herring *Clupea harengus*

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**Keywords**

cohort mortality, life history, phenotypic plasticity, Rügen herring, spawning waves

## Abstract

In marine fishes, the timing of spawning determines the environment offspring will face and, therefore, the chances of early life stage survival. Different waves of Atlantic herring *Clupea harengus* spawn throughout spring in the western Baltic Sea and the survival of offspring from early in the season has been low in the most recent decade. We assessed changes in egg traits from early, middle and late phases of the spawning season to examine whether seasonal and/or maternal effects influenced embryo survival. At each phase, fertilised eggs of six to eight females were incubated at two temperatures (7 and 13°C) and egg size, fertilisation success, mortality and time to hatch were recorded. A compilation of data from 2017 to 2020 spawning seasons indicated that mean total length of females decreased with progression of the season and increasing *in situ* water temperature. For the sub-set of females used in the laboratory study, early spawners were 7.6% larger and produced 14.2% larger eggs than late-spring spawners. Fertilisation success was consistently high (>90%) and mortality to hatch was low (<3%). Neither the former nor latter were influenced by season but both were influenced by maternity. This significant female effect was, however, not related to any maternal trait measured here (total length, Fulton's condition factor or age).

There was no maternal effect on development rate at 7 or 13°C. Our results suggest that intrinsic differences among females or among spawning waves are unlikely to markedly contribute to the poor survival observed for progeny from early in the season in this population and point toward other, extrinsic factors or processes acting on eggs or early larval stages (e.g. seasonal match-mismatch dynamics with prey) as more likely causes of mortality.

## 2.1 Introduction

Marine fishes are characterised by high rates of mortality during the earliest life stages which decline at some point before or soon after larval–juvenile metamorphosis (Bailey & Houde, 1989). In marine fishes providing no parental care, the timing of spawning determines the environment the offspring will face and, therefore, the chances of early life stage survival. In the temperate zone, fish populations reproduce during specific seasons and spawning typically lasts for several weeks to three or four months (reviewed in Chambers, 1997). Atlantic herring *Clupea harengus* L. 1758 (Clupeiformes, Clupeidae) is a central species linking lower (planktonic) and upper (e.g. piscivorous) trophic levels in the northern Atlantic (Link & Garrison, 2002; Sveegaard *et al.*, 2012). Populations of *C. harengus* are distinguished by their spawning seasons, e.g. winter–spring or summer–autumn, and spawning areas (Haegele & Schweigert, 1985). *Clupea harengus* are iteroparous, total spawners with demersal eggs (Blaxter & Hunter, 1982). It has been demonstrated for some populations of spring-spawning *C. harengus* (e.g. Gulf of St. Lawrence, southern Iceland, southwestern Norwegian coast) that larger, repeated spawners tend to spawn earlier compared to smaller, first-time spawners which often spawn last, resulting in a series of egg depositions at the spawning ground across one season (Lambert & Messieh, 1989; Óskarsson & Taggart, 2009; Slotte *et al.*, 2000).

Larger *C. harengus* migrate longer distances than smaller individuals (Payne *et al.*, 2009; Slotte, 1999b) and encounter different environmental conditions during oogenesis as well as at the point of spawning. Depending on the location, eggs produced at the beginning or end of the spring spawning season can experience large temperature differences (reviewed in Haegele & Schweigert, 1985) such as 3 to 10°C in southern Norway and 5 to 13°C in northeastern Baltic Sea (Berg *et al.*, 2017; Ojaveer, 1981). In the transition waters between the

North Sea and Baltic Sea, salinities at spawning, feeding and overwintering grounds range from 7 to 35 (Leder *et al.*, 2021). Both abiotic factors, temperature and salinity, have inverse relationships with egg size within a population (Chambers, 1997). On the other hand, intraspecific differences in egg size have been commonly reported among females and, in some cases, these inter-individual differences have been related to maternal status, such that females that are larger, with more spawning experience, and/or in better condition produce larger eggs (reviewed in Chambers & Leggett, 1996; Green, 2008; Kamler, 2005). Egg size not only reflects seasonal and female characteristics in fish but can also be an indicator of offspring performance with impacts on the probability of survival at later stages (Chambers & Leggett, 1996; Chambers, 1997; Kamler, 2005). For example, larger eggs hatch into larger larvae which could survive longer without food and are less susceptible to predation (Bailey & Houde, 1989). Many laboratory studies have examined differences in the traits of *C. harengus* eggs between different seasons, i.e. winter–spring and summer–autumn, among different *C. harengus* populations (Blaxter & Hempel, 1963; Bradford & Stephenson, 1992; Hempel & Blaxter, 1967) and among females (size, age or condition) within populations (Bradford & Stephenson, 1992; Hempel & Blaxter, 1967; Óskarsson *et al.*, 2019; Zijlstra, 1973). Few studies, however, have considered both seasonal (early, middle and late phases) and maternal effects on eggs within a spawning season (e.g. Laine & Rajasilta, 1998; Temple *et al.*, 2000).

The western Baltic spring-spawning (WBSS) *C. harengus* is a well-studied stock with long time series data available (Oeberst *et al.*, 2009a). In contrast to other stocks in the central and eastern Baltic Sea that remained in waters of reduced salinity, WBSS *C. harengus* migrates to the North Sea and Skagerrak for feeding (Hay *et al.*, 2001). Their dominant spawning and nursery ground, Greifswald Bay, is a shallow, well-oxygenated, brackish lagoon (Figure 2.1; Munkes, 2005) where *in situ* water temperature during spring increases from approximately 4 to 9°C (Table 2.1) while salinity remains stable at 7 (Munkes, 2005). The recruitment strength and the size of the WBSS *C. harengus* stock have continuously declined since the early-2000s with the lowest recorded value occurring in 2019 (ICES, 2021). Polte *et al.* (2014) observed that during this period, the survival of offspring produced by early-season spawners has been low. Anecdotal reports exist that larger or older females tend to form the earliest spawning waves in this stock but the effect of female traits on spawning time or egg

characteristics has not been quantified. Moreover, although the low survival of the earliest life stages has been linked to warming water temperatures (Moyano *et al.*, 2020; Polte *et al.*, 2021), the processes causing losses in the productivity of this stock are unclear and both bottom-up and top-down processes are being examined (Moyano *et al.*, 2022).

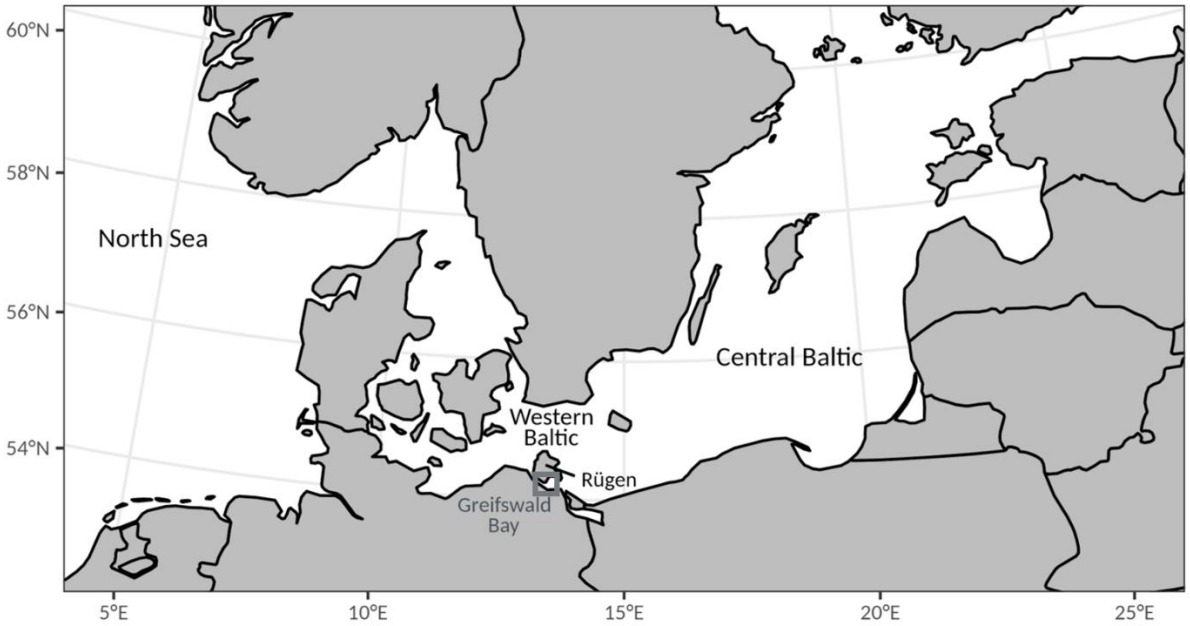


**Figure 2.1** Map of the Baltic and eastern North Sea showing the study area. The square represents our sampling site (54.25°N, 13.23°E) in Greifswald Bay of the western Baltic Sea where adult herring were captured.

**Table 2.1** *In situ* water parameters in Greifswald Bay in the western Baltic Sea and traits of spawning females at early, middle and late phases of the season. The difference between the earliest and latest sampling dates (in day of the year, DOY) across all years is defined as the potential duration of the spawning season and is then evenly separated into three periods. *In situ* water temperatures (T) and salinities (Sal) were calculated with pooled mean values of the years from 2015 to 2019 from ICES Ocean hydrochemistry dataset (2022).Female data from 2017, 2019 and 2020 were averaged per sampling date (N) before calculation and the number of total females sampled (n) per spawning time are provided. Values with brackets are grand mean (±SE). Abbreviations: $L_T$, total length; $M_W$, total wet mass; K, Fulton's condition factor; ND, not determined.

| Spawning time | Period (DOY) | *In situ* water | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | T (°C) | Sal | *N* | *n* | $L_T$ (cm) | $M_W$ (g) | *K* | Age (years) |
| **early** | 53–78 | 4.3 (0.1) | 8.0 (0.2) | 2 | 79 | 29.0 (0.5) | 187 (2) | 0.76 (0.03) | 6.8 (0.2)† |
| **middle** | 79–104 | 6.5 (0.8) | 8.2 (0.2) | 4 | 209 | 27.7 (0.4) | 168 (7) | 0.79 (0.01) | 5.5‡ |
| **late** | 105–130 | 8.7 (0.6) | 7.8 (0.3) | 3 | 100 | 26.6 (0.4) | 146 (4) | 0.78 (0.01) | ND |

† $n$ = 16

‡ $N$ = 1; $n$ = 8; SE not determined because of single sampling date

The present study examined the importance and relative contribution of seasonal and maternal effects on the size and performance (survival) of eggs of WBSS *C. harengus*. We sampled adult *C. harengus* in Greifswald Bay throughout the spawning season and reared the embryos of six to eight females from early, middle and late in the season at 7°C (a historical average temperature experienced during the mid-spawning season) and 13°C (above which egg survival declines during laboratory incubation (Peck *et al.*, 2012b)) to understand the causes of low survival in wild offspring from early in the season. We examined if female traits (total length, total wet mass, Fulton's condition factor and/or age) were related to spawning time or *in situ* water temperature and if seasonal, maternal effects, egg size and/or incubation temperature affected the success of embryonic development. Thus, this study tested whether "bigger is better" and whether warm temperatures experienced by eggs could be a reason for the decline in the productivity of the WBSS herring stock observed in the wild.

## 2.2 Materials and methods

### 2.2.1 Ethical statement

The handling and care of experimental animals in the present study complied with the German Animal Welfare Act and the Animal Welfare Regulation Governing Experimental Animals.

### 2.2.2 Field sampling and strip-spawning

We sampled adult *C. harengus* throughout the spawning season at Greifswald Bay (54.25°N, 13.23°E; Figure 2.1) in 2017, 2019 and 2020 during the Rügen Herring Larvae Survey (Oeberst *et al.*, 2009a). Sampling did not take place in 2018 due to a sudden drop in temperature in mid-spring and the formation of solid ice cover in Greifswald Bay. Adult *C. harengus* were caught using set gillnets with mesh sizes 25, 27 and 29 mm. Sampling started between late February and late March and lasted until the end of the spawning season which was 10 May in 2017 and 17 April in 2019. In 2020, sampling after the early season was not conducted due to COVID-19 pandemic restrictions and hence the end-point of the spawning season in that

year was not determined. The sampling dates were during days of the year (DOY) 81 and 130 ($n$ = 5) in 2017, 53 and 107 ($n$ = 3) in 2019 and on DOY 65 ($n$ = 1) in 2020. Across the 3 years, 388 females were sampled. At the sampling site, a conductivity-temperature-depth (CTD) instrument was deployed. *In situ* water temperature and salinity were calculated as the average of those measured just below the water surface and at the bottom (range of 6.1–7.1 m) of the sampling site. The difference between surface and bottom temperatures and salinities was <0.1°C and <0.05, respectively. The total length ($L_T \pm 0.1$ cm) and total wet mass ($M_W \pm 1$ g) of each specimen were measured. Fulton's condition factor ($K$) was calculated with the formula: $M_W \times L_T^{-3}$.

The early, middle and late phases of the spawning season were defined by taking the difference between the earliest and latest sampling dates (in DOY) across all years and then evenly separating the duration into three periods (Table 2.1). Within the total catch during the middle ($n$ of females = 39) and late ($n$ = 6) seasons in 2019 and early ($n$ = 70) season in 2020, we selected six to eight females representing the range in sizes captured and 5 to 26 males, all at spawning stage (maturity scale 6; Bowers, 1961), for the laboratory trials (Table 2.2). For the trial conducted early in the season, the strip-spawned fish were selected from the first half of the fish freed from the net. The sizes of randomly selected females were toward the upper part of the range of fish in the total catch which was unintended. The females we used were still representative of the size of early spawners captured at that time (Table 2.1). The age (years) of each of these randomly selected females was estimated from sagittal otoliths readings following the methods of McCurdy *et al.* (2005), by which the sagittae were dissected from fresh specimens, soaked in distilled water and then stored dried and the whole otoliths without polishing were examined under a binocular microscope for ring counting.

For the on-site fertilisation and the later egg incubation in the laboratory, we used offshore North Sea seawater (salinity 33) that was sand-filtered, UV-sterilised, and diluted with tap water to salinity 7. A salinity of 7 represents the average salinity of the local waters (Munkes, 2005). The years from 2015 to 2019 showed a higher salinity of 8 probably due to less formation of ice at the spawning ground in the early months of the years. Eggs from each female were strip-spawned onto eight glass plates submerged in seawater at 10°C, with 63–379 and a mean (±standard deviation, SD) of 224 (60) eggs per plate. Eggs were spread

gently with the caudal fins of the females to ensure that only a single layer of eggs adhered to each plate. The milt from a group of males was mixed and then activated in water at the same temperature and salinity to fertilize the eggs. After 30 min of incubation, the egg plates were rinsed with equivalent seawater and placed into insulated and aerated transport containers to ensure stable temperature (9.6–10.4°C) and oxygen conditions, respectively, during the 3-h transport to the Elbe Aquarium research facility at the University of Hamburg. It was logistically impractical to fertilize and transport the eggs from the ship to the laboratory at the two incubation temperatures (7 and 13°C). The 10°C temperature was chosen because this was the mean temperature of the two incubation temperatures, yet a 3°C increase may affect herring eggs differently than a 3°C decrease. The small amount of time needed for the adult measurements and to strip-spawn and fertilize the eggs (<1 h) has been demonstrated to not influence fertilisation success (Alderdice & Velsen, 1978).

**Table 2.2** Details of the three trials examining seasonal and maternal effects on embryos of spring-spawning *Clupea harengus* from Greifswald Bay in the western Baltic Sea. Abbreviations, T, temperature; Sal, salinity; *n*, number of individuals; $L_T$, total length; $M_W$, total wet mass; *K*, Fulton's condition factor; ND, not determined.

| Spawning time | DOY | *in situ* water | | Female | | | | | Male | | | | Sampling date |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T (°C) | Sal | *n* | $L_T$ (cm) mean (±SE) | $L_T$ (cm) range | $M_W$ (g) range | *K* range | Age (years) range | *n* | $L_T$ (cm) mean(SE) | $M_W$ (g) range | |
| **early** | 65 | 4.6 | 7.9 | 8 | 29.3 (0.3) | 28.7–31.3 | 178–248 | 0.75–0.85 | 6–9 | 26 | 28.5 (0.2) | 26.0–30.0 | 05 Mar 2020 |
| **middle** | 81 | 6.4 | 9.3 | 8 | 28.7 (0.3) | 27.7–29.9 | 171–202 | 0.75–0.83 | 4–7 | 10 | 28.0 (0.3) | 26.0–29.5 | 22 Mar 2019 |
| **late** | 107 | 7.6 | 8.1 | 6 | 27.2 (0.4) | 26.0–28.5 | 132–181 | 0.67–0.80 | ND | 5 | 27.5 (0.4) | 26.5–29.0 | 17 Apr 2019 |

### 2.2.3 Egg incubation

Upon arrival at the laboratory, the eight egg plates from each female were randomly and evenly distributed to either cold (7°C, $n$ = 4) or warm (13°C, $n$ = 4) 250-ml, glass incubation chambers which were placed in water baths (Figure 2.2). Each chamber was gently aerated and constantly supplied with seawater (ca. 40 ml $min^{-1}$) from head tanks connected to a 500-l recirculating aquaculture system (RAS) in a controlled environment room. The 7°C treatment was ambient room temperature while the 13°C treatment was created using heaters (600W titanium heaters with TRD thermostatic controllers, Schego, Germany) in the head tanks and water baths. All water was within a RAS. Water was filtered through a filter floss pad, a biofilter and activated carbon, sterilised by UV lights and cooled to 7°C (Titan 1500, 790W, Aqua Medic, Germany). The water salinity was maintained at 6.6–7.4 (Cond

3110, WTW GmbH, Germany) by adding freshwater to the RAS. A light regime of 14:10 h light:dark, the average condition during spring, was used in all trials.

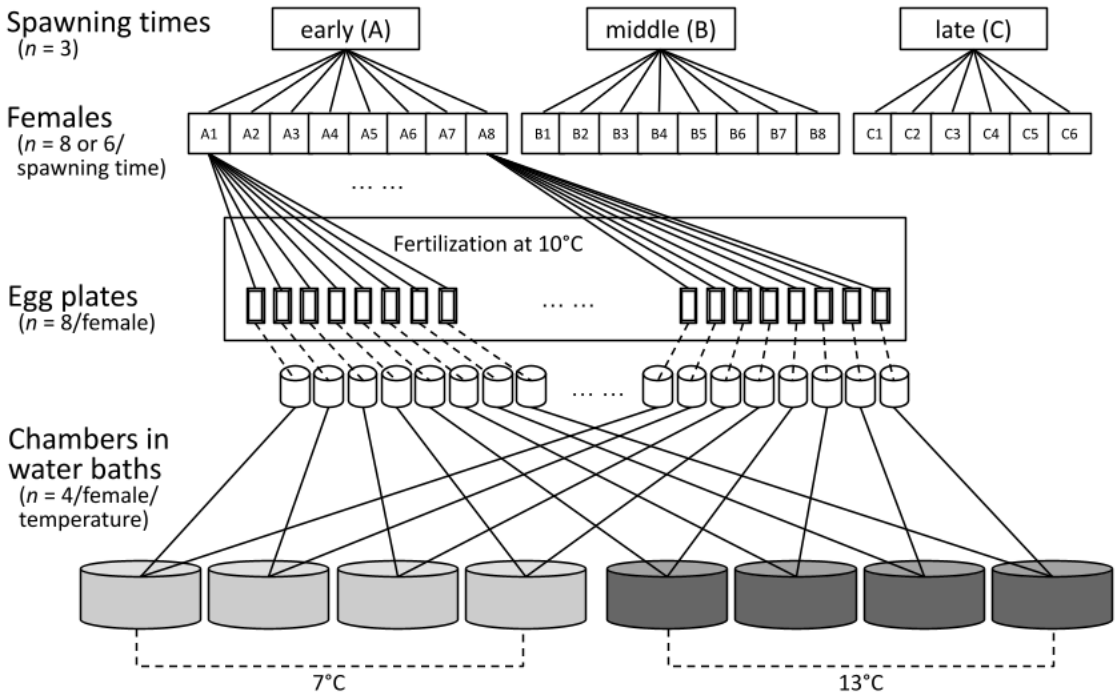


**Figure 2.2** Scheme of the experimental design. Replicates of two females from the early season were drawn as examples (e.g. Female A1 and A8). All females from all spawning times followed the same scheme.

One day after the eye-darkening stage, each egg plate was transferred from its 250- to a 1000-ml chamber and water inflow was switched off at night. This was done to prevent the outflow of hatched larvae since clupeid larvae primarily hatch at night (Alderdice & Velsen, 1971). Water temperatures were continuously recorded using temperature probes (TLog64-USB, ±0.5°C) in one random chamber per water bath. The temperature and salinity in each chamber were measured daily using a hand-held probe (Cond 3110, WTW, Germany) to verify similar conditions among chambers and water baths. Through all trials, the mean (±SD) temperatures for the warm and cold treatments were 12.6 (0.3)°C and 7.2 (0.2)°C, respectively. The dissolved oxygen was measured each day (Oxi 3210, WTW GmbH, Germany) and was >90% air saturation. Total ammonium/ammonia, nitrite and nitrate concentrations in the RAS were measured once per week and remained below 0.02, 0.001 and 0.05 mg $l^{-1}$, respectively (Tropic Marin $NH_4^+/NH_3$ test and $NO_2/NO_3$ Test Professional, Dr. Biener GmbH, Germany).

### 2.2.4 Egg characteristics

Egg plates were examined daily under a stereomicroscope (Leica M165 C, Germany; at 5.84× magnification). Eggs without cell division were considered unfertilised. Fertilised eggs that stopped developing and turned dark at a later embryonic stage were considered dead. Images of the whole egg plate were taken with a digital camera (Leica MC170 HD, Germany) connected to the stereomicroscope 1 day post-fertilisation (dpf) to measure egg size and to assess fertilisation success (%). Since almost all dead eggs appeared during the mid-blastula stage, digital images of all eggs were taken again at this stage (2–3 dpf) and at the eye darkening stage (16 and 6 dpf at 7 and 13°C, respectively) to confirm our mortality estimates. Areas of fertilised eggs at 1 dpf, as a proxy for egg size, were measured using ImageJ software (version 1.52, Wayne Rasband, NIMH, USA). The contrast of digital images was increased and eggs that touched one another were separated semi-automatically using the ImageJ "watershed" tool. All measurements were made using the ImageJ "particle analysis" tool with 49–164 eggs per plate measured (representing ca. 50% of the eggs per plate; frequency histograms of egg area measured per plate are shown in Appendix Figures A2.1–A2.3). Eggs on all digital images were counted and specified as fertilised, unfertilised or dead using the ImageJ "multi-point" tool. Fertilisation success was calculated as the percent of fertilised eggs in total eggs and mortality was the percent of dead eggs in fertilised eggs. After completion of the eye-darkening stage, eggs were checked for hatching at 2, 6, and 10 h (three intervals of 2-4-4 h) after darkness each night until peak hatch (50% viable hatch) occurred. Hatching during daylight hours (assessed in 2019) was minimal (<2.5% of eggs per chamber in total). Time to peak hatch was expressed in degree-days (°d; temperature in °C x time in days) post-fertilisation.

### 2.2.5 Data analyses

All analyses were performed in R version 4.0.3 (R Core Team, 2020). Linear mixed-effect models (LMMs) were used to account for the variance within replicates. For the female data from the field work in the years 2017, 2019 and 2020, we tested whether female $L_T$ and $K$ changed throughout the season with spawning time (in DOY) or *in situ* water temperature, which were included separately as the fixed effect because of collinearity (variation inflation factor > 3). Sampling year and date within year were included as random intercepts. For data from the three laboratory trials, we assessed whether egg area, fertilisation success and

mortality were influenced by spawning time, female traits, egg size (on fertilisation success and mortality) and/or incubation temperature (on mortality). Fertilisation success and mortality percentages were arcsine square root-transformed to meet the normality assumption assessed via QQ plots. In all analyses, we included spawning time, an ordinal variable with three levels (early < middle < late), as a fixed (seasonal) effect. Since the magnitudes of seasonal effects might differ early, middle and late in the season, we estimated both linear and quadratic trends of spawning time effect. For fixed (maternal) effects, we included female $L_T$, $K$ and age as continuous variables in all models, while female $M_W$ was not added because it was collinear with $L_T$ (variation inflation factor > 3). Age data for females from late in the season were missing and two sets of analyses (including and excluding age) were performed. The variance among individual females and among egg plates within females (in egg area model) was addressed as random intercepts. In addition, egg area was included as another fixed effect in models on fertilisation success data while egg area and incubation temperatures (categorical variable: cold and warm) were added as fixed effects in models on mortality data. The best model structures based on conditional Akaike's information criterion (AICc) were determined using the "MuMIn::dredge" function, which fitted models in all possible combinations of all fixed effects (Barton, 2020). Selected models were then fitted with the restricted maximum likelihood (REML) approach using the "lme4::lmer" function (Bates *et al.*, 2015). Data of egg development time were averaged at each spawning time and incubation temperature and analysed with incubation temperature as the only (fixed) effect using the "lm" function. All models were validated by plotting residuals *vs.* fitted values.

The significance level was set at $\alpha = 0.05$. *P*-values of each fixed variable were obtained from *t*-statistics with approximate degrees of freedom calculated by Satterthwaite's method with "LmerTest" (Kuznetsova *et al.*, 2017). The variance explained by random effects was represented by intraclass correlation coefficients (ICCs; Nakagawa & Schielzeth, 2010). Three types of $R^2$ were calculated using "MuMIn" and "r2glmm" to assess the amounts of variation explained by the model (Barton, 2020; Jaeger, 2017): partial $R^2$ for each significant fixed effect, marginal $R^2$ for all fixed effects, and conditional $R^2$ for the whole model including random effects (Nakagawa & Schielzeth, 2013).

## 2.3 Results

The mean (±standard error, SE) $L_T$ of females captured across sampling dates in 2017, 2019 and early in 2020 were 27.0 (0.4), 28.4 (0.6) and 28.6 (SE not determined because of single sampling date) while $K$ was 0.80 (0.00), 0.75 (0.02) and 0.79, respectively. Pooling all data from all years, $L_T$ of females decreased linearly (Table 2.1) with the progression of spawning time ($t$ = −8.899, $P$ < 0.001, partial $R^2$ = 0.311, Table 2.3, Figure 2.3a) and with increasing *in situ* water temperature ($t$ = −7.495, $P$ < 0.001, partial $R^2$ = 0.190, Table 2.3, Figure 2.3b). In the spawning time model, little variance was explained by the random effects (intraclass correlation coefficients, ICC, of year = 0.019, ICC of date:year = 0.009) while in the *in situ* water temperature model, additional variance was explained by the random effect of year (ICC of year = 0.132, ICC of date:year = 0.006). Comparing the years 2017 and 2019, females in the former were estimated to be 0.2 cm smaller in $L_T$ on the same DOY (Figure 2.3a) and 1.0 cm smaller at the same *in situ* water temperature (Figure 2.3b). No relationship was found between $K$ and spawning time *(*$P$ = 0.823, Table 2.3) or *in situ* water temperature ($P$ = 0.498, Table 2.3). While all females sampled from 2017 to 2020 were caught on DOY between 53 and 130, at water temperatures between 3.2 and 9.2°C and ranged from 23.0 to 31.5 cm in $L_T$, the females selected for our laboratory study were caught on DOY between 65 and 107, at water temperatures between 4.6 and 7.6°C and ranged from 26.0 to 31.3 cm in $L_T$ (Table 2.2, Figure 2.3).

### 2.3.1 Egg size

In the laboratory trials, the mean (±SE) egg area across spawning times ($n$ = 3) was 1.43 (0.06) mm$^2$, equivalent to an egg diameter of 1.35 (0.03) mm. Egg area decreased linearly throughout the season (linear trend of spawning time effect in the best model: $t$ = −4.570, $P$ < 0.001, partial $R^2$ = 0.392; Table 2.4) and eggs from late in the spawning season were estimated to be 12.5% smaller than those from early in the season (Figure 2.4). In addition, similar variance in egg area was explained by the random effects, by which variance among individual females (ICC = 0.592) was 18.5 times the variance among replicated plates within females (ICC = 0.032). However, neither $L_T$ nor $K$ was related to the female effect on egg area ($P$ > 0.05 or not selected in models; Table 2.4). Similarly, age had no effect on egg area (not selected in models; Appendix Table A2.2). Although the mean $L_T$ of females was progressively

larger earlier compared to later in the season, ranges of $L_T$ overlapped among different spawning times (Figure 2.3a) and females from earlier in the season produced larger eggs regardless of $L_T$ compared to those from subsequent spawning times (with a few exceptions; Figure 2.5).

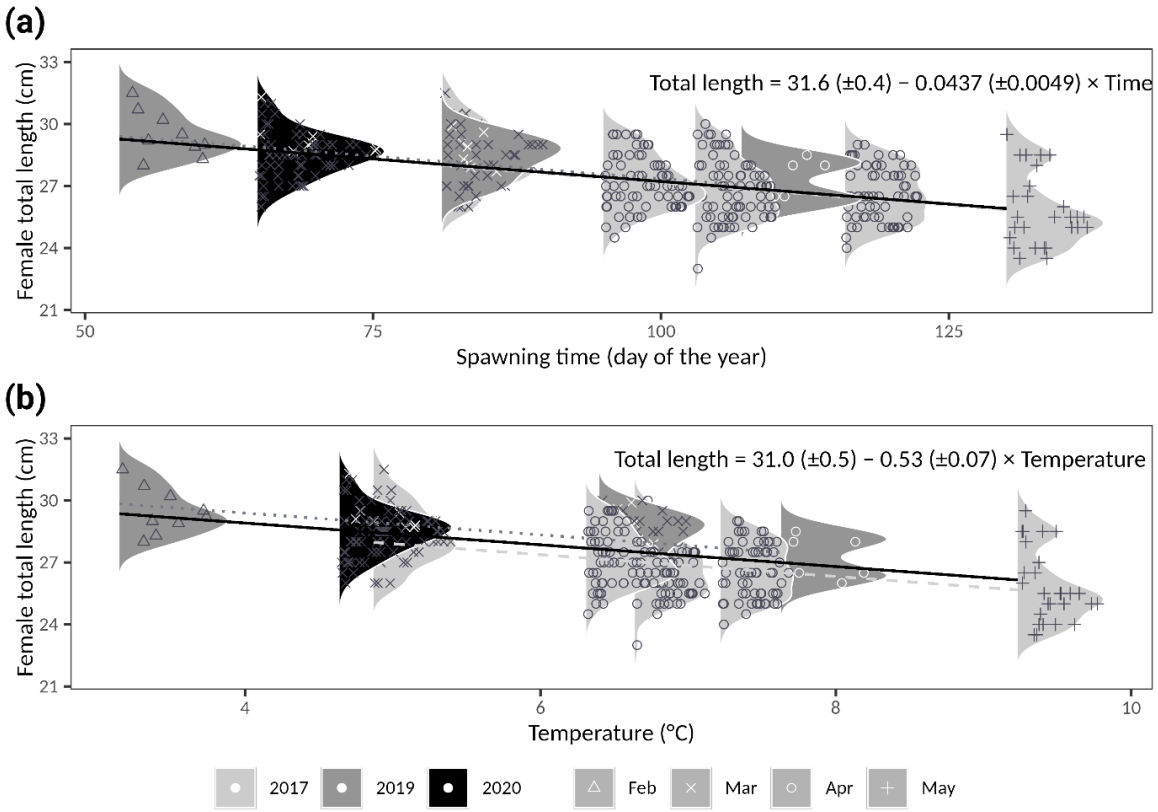


**Figure 2.3** Total length (cm) of *Clupea harengus* females *vs.* spawning time (day of the year, panel a) and *in situ* water temperature (°C, panel b) in Greifswald Bay, Germany in 2017, 2018 and 2020. Symbols (Δ, ×, ○, +) represent individual females that were sampled while white symbols are females that were strip-spawned for the laboratory experiments. Black lines show the regression across all data, while dashed and dotted lines show the different estimated intercepts for 2017 and 2019, respectively (not applicable for 2020 because of single sampling date). For both regressions, *n* of years = 3, *n* of sampling dates = 9 and *n* of females = 388.

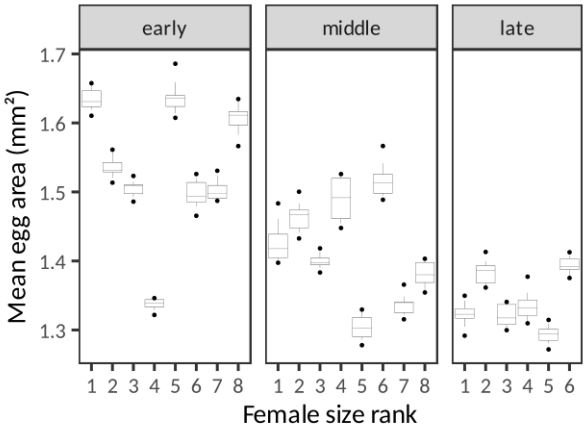


**Figure 2.4** Mean egg area (mm²) *vs.* size rank (1 = largest) of the six to eight female *Clupea harengus* for each of the three phases of the spawning season. Boxes display the 25th and 75th percentiles and whiskers display the 10th and 90th percentiles. The dots indicate values outside the whisker range. The line displays the mean value (*n* of egg plates = 8 per female, range of eggs measured = 49–164 per egg plate). Total lengths of females are listed in Appendix Table A2.1. Frequency histograms of egg area measured per plate are shown in Appendix Figures A2.1–2.3.

**Table 2.3** Summary of the linear mixed-effect models (LMM) to assess the influence of time (in day of the year, DOY) or *in situ* water temperature (°C) on total length ($L_T$) and Fulton's condition factor ($K$) of spawning females in Greifswald Bay in the western Baltic Sea. Time and *in situ* water temperature were included in separate models because of collinearity (variation inflation factor > 3). Comparisons of each model to the intercept-only (no fixed effect) model are provided with conditional Akaike information criterion (AICc). Models for comparisons were fitted by maximum likelihood while models for estimates were refitted by restricted maximum likelihood (REML). Year:date denotes nested random effect of dates within years. Values are estimated coefficients (±SE) for fixed effects and variance (in ±SD) for random intercepts. A dash indicates the effect was not included in the full model while an estimate of zero denotes the effect was assumed to be zero. Significant codes: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

| | Fixed effects | | | Random effects | | | Marginal/ conditional $R^2$ | Model selection | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Intercept | Time | Temperature | (1\|year) | (1\|year:date) | Residual | | *df* | AICc | weight |
| Female $L_T$ | 31.6 (0.4)*** | −0.0437 (0.0049)*** | – | (0.2) | (0.1) | (1.3) | 0.311/0.330 | 5 | 1318.69 | 1.00 |
| | 27.8 (0.6)*** | 0 | – | (0.8) | (0.9) | (1.3) | 0/0.441 | 4 | 1342.44 | 0.00 |
| | 31.0 (0.5)*** | – | −0.53 (0.07)*** | (0.5) | (0.1) | (1.3) | 0.190/0.302 | 5 | 1325.06 | 1.00 |
| | 27.8 (0.6)*** | – | 0 | (0.8) | (0.9) | (1.3) | 0/0.441 | 4 | 1342.44 | 0.00 |
| Female $K$ | 0.78 (0.02)*** | <0.01 (0.00) | – | (0.01) | (0.01) | (0.05) | <0.001/0.069 | 5 | −1134.11 | 0.27 |
| | 0.78 (0.01)*** | 0 | – | (0.01) | (0.00) | (0.05) | 0/0.055 | 4 | −1136.11 | 0.73 |
| | 0.77 (0.02)*** | – | <0.01 (0.00) | (0.01) | (0.01) | (0.05) | 0.002/0.068 | 5 | −1134.49 | 0.31 |
| | 0.78 (0.01)*** | – | 0 | (0.01) | (0.00) | (0.05) | 0/0.055 | 4 | −1136.11 | 0.69 |

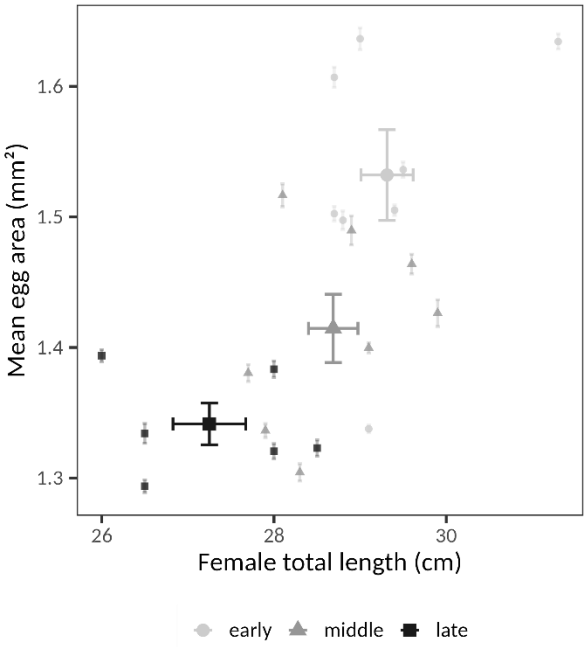


**Figure 2.5** Relationship between *Clupea harengus* egg area (mm²) and female total length (cm) at early, middle and late phases of the spawning season. The large symbols represent the grand mean (±SE) for both egg area and female total length ($N$ = 6 or 8) at each spawning time. The small symbols are the mean (±SE) egg area of each female ($n$ of egg plates = 8 per female).

### 2.3.2 Fertilisation success and mortality

The mean (±SE) percent (%) egg fertilisation of all females ($n$ = 22) across spawning times was 92.8(0.8)%. Out of all the females, the eggs of one female from early in the season had 13.9% lower fertilisation success than the mean (Figure 2.6). Egg mortality occurred predominantly during the mid-blastula stage and was negligible at later egg stages (Appendix Figure A2.4). The mean percent egg mortality during the blastula stage of all females ($n$ = 22) across spawning times and rearing temperatures (7 and 13°C) was 2.4(0.8)%. Eggs of one female from late in the season had a 15.0% higher total mortality than the mean (Figure 2.7). The overall percent egg viability, the amount of fertilised eggs that survived until hatch, across spawning times was >85% at both temperatures. The overall percent viability for eggs from the aforementioned early and late spawning females was 75.1 and 77.0%, respectively. The differences in egg fertilisation success and mortality were only explained by random effects of females (the best models: ICC = 0.691 and 0.718, respectively) and were not related to female $L_T$, $K$ or egg area ($P$ > 0.05 or not selected in models; Table 2.4) nor to age (not selected in models; Appendix Table A2.2).

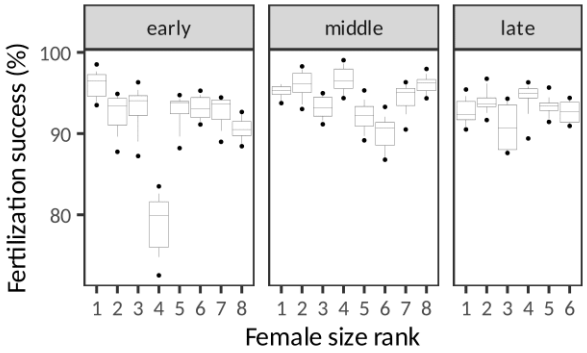


**Figure 2.6** Box and whisker plots of fertilisation success (%) among *Clupea harengus* females. Females are ordered by size rank (1 = largest) for each of the three phases of the spawning season. Whiskers show the 10th and 90th percentiles while boxes are the 25th to 75th percentiles. The dots indicate values outside the whisker range. The mean value is displayed as a line in each box ($n$ = 8 per female). Total lengths of females are listed in Appendix Table A2.1.

**Table 2.4** Summary of the three best linear mixed-effect models (LMM) to examine whether *Clupea harengus* egg area, fertilisation success and mortality at the blastula stage were influenced by spawning time and/or female traits (and/or egg area and/or incubation temperature if included). Models were fitted by maximum likelihood to compare conditional Akaike information criterion (AICc) and then the best models were refitted by restricted maximum likelihood (REML) for estimates. Abbreviations: TIME.L, linear trend of spawning time; TIME.Q, quadratic trend of spawning time; $L_T$, total length; $K$, Fulton's condition factor; AREA, mean egg area; $T_I$, incubation temperature; ID, individual female. ID:plate denotes nested random effect of plates within individual females. Values are estimated coefficients (±SE) for fixed effects and variance (in ±SD) for random intercepts. A dash indicates the effect was not included in the full model while an estimate of zero denotes the effect was not selected (assumed to be zero). Significant codes: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

| | **Fixed effects** | | | | | | | **Random effects** | | | **Marginal/ conditional $R^2$** | **Model comparison** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Intercept** | **TIME.L** | **TIME.Q** | **$L_T$** | **$K$** | **AREA** | **$T_I$** | **(1\|ID)** | **(1\|ID: plate)** | **Residual** | | ***df*** | **AICc** | **weight** |
| **Egg area** | 1.429 (0.017)*** | −0.135 (0.030)*** | 0.018 (0.028) | 0 | 0 | – | – | (0.077) | (0.018) | (0.061) | 0.399/0.773 | 6 | −43519.01 | 0.324 |
| | 0.835 (0.563) | −0.104 (0.041)* | 0.025 (0.029) | 0.021 (0.020) | 0 | – | – | (0.077) | (0.018) | (0.061) | 0.409/0.777 | 7 | −43518.33 | 0.230 |
| | 1.086 (0.383)** | −0.122 (0.033)*** | 0.025 (0.029) | 0 | 0.442 (0.492) | – | – | (0.077) | (0.018) | (0.061) | 0.408/0.778 | 7 | −43517.98 | 0.193 |
| **Fertilisation success** | 1.307 (0.013)*** | 0 | 0 | 0 | 0 | 0 | – | (0.059) | – | (0.039) | 0/0.691 | 3 | −569.24 | 0.162 |
| | 1.307 (0.013)*** | 0.018 (0.022) | −0.034 (0.021) | 0 | 0 | 0 | – | (0.057) | – | (0.039) | 0.099/0.709 | 5 | −568.62 | 0.119 |
| | 1.482 (0.148)*** | 0 | 0 | 0 | 0 | −0.122 (0.102) | – | (0.064) | – | (0.039) | 0.030/0.735 | 4 | −568.24 | 0.098 |
| **Blastula Mortality** | 0.128 (0.017)*** | 0 | 0 | 0 | 0 | 0 | 0 | (0.078) | – | (0.049) | 0/0.718 | 3 | −489.43 | 0.132 |
| | 0.701 (0.407) | 0 | 0 | −0.020 (0.014) | 0 | 0 | 0 | (0.076) | – | (0.049) | 0.063/0.726 | 4 | −489.41 | 0.131 |
| | 0.716 (0.415) | 0 | 0 | −0.028 (0.016) | 0 | 0.147 (0.144) | 0 | (0.078) | – | (0.049) | 0.078/0.739 | 5 | −488.31 | 0.076 |

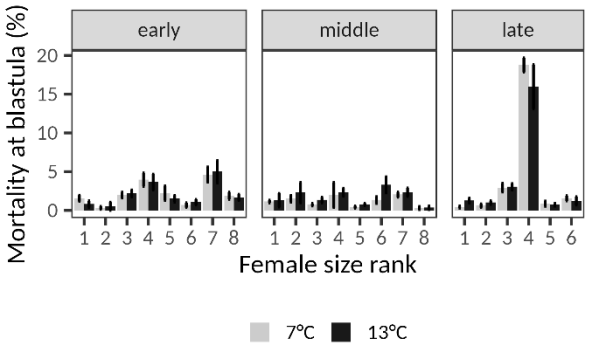


**Figure 2.7** Bar plots of mean (±SE) percent (%) egg mortality ($n$ = 4 per female per temperature treatment) at blastula stage among *Clupea harengus* females in the cold (7°C) and warm (13°C) treatments. Females are ordered by size rank (1 = largest) for each of the three phases of the spawning season. Total lengths of females are listed in Appendix Table A2.1.

### 2.3.3 Development time

The mean (±SE) time to peak hatch from early, middle and late in the season at 7°C was 132(1), 127(1), 117(0)°d, respectively, and at 13°C, values were 114(1), 104(0), 99(0)°d at 13°C, respectively. Eggs at 13°C were estimated to hatch 18.7°d earlier than those at 7°C ($df$ = 4, $t$ = −3.170, $P$ = 0.034, marginal $R^2$ = 0.668).

## 2.4 Discussion

In marine fish with protracted spawning seasons, it is important to identify and attempt to disentangle the processes that impact embryo quality, particularly because of the high rates of mortality experienced by early life stages (Houde, 1987). The size of the WBSS *C. harengus* population has continuously declined since the early 2000s and the causes have been linked to low survival of the earliest life stages (Polte *et al.*, 2014). The present study helps to disentangle the effects of spawning time and among-female differences on egg characteristics in *C. harengus* in the western Baltic Sea. Although it appeared that larger females produced larger eggs, our results indicate that similar-sized females had larger eggs earlier in the season at colder *in situ* water temperatures than females that spawned later. In the following, we discuss the roles of female traits, *in situ* water temperature and salinity in the seasonal dynamics of egg quality and whether these factors may be responsible for the relatively low survival of early cohorts observed in this population (Polte *et al.*, 2014).

During our sampling early, middle and late in the spawning season, female $L_T$ and age decreased and $K$ remained similar with the progression of the spawning season. Comparison with earlier observations from the same spawning ground suggests decadal changes in the average $L_T$ of spawning females. The seasonal decrease in mean size ranged from 1.5 cm (29.4 to 27.9), 4.5 cm (27.4 to 22.9), 6.6 cm (28.8 to 22.2) and 2.9 cm (24.8 to 21.9 cm) in the 2000s, 1990s, 1970s and 1960s, respectively (Anwand, 1962; Biester *et al.*, 1978; Jørgensen *et al.*, 2005; Rajasilta *et al.*, 2006). These decadal differences in size are not simply due to the presence of larger females in recent years but could be due, in part, to differences in the sampling methods used across the decades. Trap nets and gillnets were used in the studies before 2000 and spawning and non-spawning fish were not separated (i.e. the total lengths of *C. harengus* at all maturity scales were included). Therefore, it is difficult to determine if the size (and/or age) of females has shifted in the most recent decade (when recruitment failure has been reported) compared to earlier decades when the stock was more productive.

When comparing females among sampling years, females in 2019 were caught earlier (DOY), larger in size ($L_T$), and appeared to be in lower conditions ($K$) than those in 2017. Indeed, recruitment was estimated to be lower in 2019 than in previous years, part of a negative trend with time that has been observed for about two decades (ICES, 2021). In the three sampling years, *in situ* water temperatures recorded early in the seasons were similar (differences < 0.4°C) to the documented range of the onset spawning temperature (3.5–4.5°C) in WBSS *C. harengus* from Greifswald Bay (Polte *et al.*, 2021). As *C. harengus* rely on temperature cues for migration to the spawning ground (Lambert, 1987), milder and shorter winters in the recent decade have led to a shortened overwintering period and earlier spawning in the Baltic Sea (Arula *et al.*, 2019; Polte *et al.*, 2021). Overwintering is when *C. harengus* rely solely on their energy reserves and when downregulation of oocytes (atresia) takes place (dos Santos Schmidt *et al.*, 2017). A shortened atresia process could explain the somewhat lower mean $K$ of the larger and earlier spawners in 2019 relative to those in 2017. While yearly fluctuation of female $K$ had also been observed in Norwegian spring-spawning herring, dos Santos Schmidt *et al.* (2017) demonstrated that a low mean $K$ of females does not necessarily relate to poor reproductive output of the same year since conditions of females can affect more than one subsequent spawning seasons. Thus, comparing winter duration

and differences in $K$ and fecundities of the early spawners among years could shed light on the reproductive status of this population.

Egg area provided a more precise metric of size than diameter and also allowed the comparison of its variance among replicated plates, females and spawning times as areas of single eggs were quantified in the present study. Within individuals, females produced eggs of consistent size but among different females and spawning times, differences in egg size were apparent. The mean egg size recorded in our study agrees with past observation for this population (from 1.4 to 1.3 mm from April to June; Scabell, 1988). The seasonal decrease in egg size has also been documented for many populations of *C. harengus* (Hempel & Blaxter, 1967; Rajasilta *et al.*, 1993) and has been associated with maternal differences such as size, condition and spawning experience throughout the spawning season (reviewed in Chambers & Leggett, 1996; Green, 2008). However, our study found no significant relationship between maternal status ($L_T$, $K$ or age) and egg size among the six or eight females sampled at the same spawning time. By separating year and age groups, (Zijlstra, 1973) reported only one out of the six comparisons between female size and egg mass exhibited a positive relationship within three North Sea *C. harengus* stocks. Similarly, this positive trend was uncommon in various *C. harengus* spawning sub-groups (populations) compared by Hempel & Blaxter (1967), 12 in 27 comparisons within 16 northeast Atlantic populations) and Bradford & Stephenson (1992), 2 in 12 northwest Atlantic populations). Although much of the variation in egg size in this and other studies could be assigned to a maternal effect (Green, 2008), our results are consistent with the conclusions of Chambers (1997), that egg size is unlikely to be correlated with female size.

Water temperature and salinity influence egg size both during oogenesis and at the point of spawning, after which the eggs harden within hours and the size stabilizes (Chambers, 1997). In the present study, temperature and salinity were maintained at the same condition across all trials throughout the season to eliminate their effects at the point of spawning. During oogenesis, larger and older individuals migrate further to the North Sea for feeding while the smaller individuals remain mostly in the Kattegat and Skagerrak (Payne *et al.*, 2009), suggesting that the larger *C. harengus* earlier in the season experienced higher salinity than those later in the season. Higher salinity resulted in decreasing egg diameters (Dushkina, 1973; Ojaveer, 1981). Yet, the earlier spawners still produced larger eggs in the

present study and the potential salinity effect on egg size during oogenesis appeared to be small. Water temperature, on the other hand, was likely related to the linear trend of spawning time effect on egg size because the recorded *in situ* water temperatures increased linearity with the progression of the season (Tables 2.1 and 2.2) and reflected the temperature females experienced at least during the pre-spawning period. Within marine fish species, eggs produced at colder temperatures tend to be larger than those produced at warmer temperatures (Atkinson *et al.*, 2001). When comparing six *C. harengus* populations with different spawning times in the eastern Atlantic and western Baltic, mean egg mass decreased from January to June and increased from August to December (Hempel & Blaxter, 1967). Our study demonstrated that egg size also changes within a population in a single spawning season. The dominant effect of temperature during oogenesis has, therefore, been overlooked in studies that showed inconsistent trends of maternal size and egg size (e.g. Bradford & Stephenson, 1992; Hempel & Blaxter, 1967). Chambers (1997) suggested that the inverse relationship between egg size and temperature may be due to different magnitudes of temperature effects on growth rate and developmental rate of embryos. As temperature increases, the increase in developmental rate of embryos is faster than the increase in growth rate, resulting in a smaller size of the same developmental stage at a higher temperature. Although Chambers (1997) pointed out that the change in egg size with temperature may only be a physiological response, Marshall *et al.* (2008) suggested that this relationship could also be adaptive because food is usually scarce in colder environments where a larger size helps individuals withstand harsh conditions, as opposed to warmer environments where food is more abundant and a higher number of smaller eggs can maximize survival. Hence, egg size is still an important trait that is linked to survival at subsequent larval stages.

During the egg stage, controlled laboratory experiments can identify key factors defining intrinsic performance such as fertilisation success and developmental mortality. The range of fertilisation success in the laboratory trials of our study agrees with that (70–90%) assessed by Rosenthal *et al.* (1988) for this population. A factorial experiment of *C. harengus* female-male pairs showed that eggs of the same female could be more easily fertilised by the sperm of some males (>75% compared to <55%) and vice versa, but no particular female or male resulted in an overall worse fertilisation success (Bang *et al.*, 2006), indicating the potential effect of genetics on fertilisation success. In our study, since all eggs from all females within

one spawning time were fertilised with milt from the same group of males, the lower fertilisation success of the eggs (which were also relatively small) from one early spawner could be caused by genetic differences of this and other females. On the other hand, egg mortality observed in the present study occurred predominantly during the mid-blastula stage, similar to several other fish populations in the Baltic Sea (Alter & Peck, 2021; Dahlke *et al.*, 2016; Laine & Rajasilta, 1999). The blastula life stage is the period during which maternal-to-zygotic transition takes place and when embryos start to produce their own mRNA (Hill & Johnston, 1997; Tadros & Lipshitz, 2009). Before this point, the embryos have been relying on mRNA provided by their mother, which explained the significant female effect on mortality. A multi-year study on *C. harengus* from the central Baltic Sea reported a low mean mortality of eggs (1.0 and 7.5%) in two of the years but a high mortality (32.8%) in one year when muscle fat of females was relatively low (Laine & Rajasilta, 1999). In that same batch of eggs, fertilisation success was high (>90%) indicating that mortality but not fertilisation success was influenced by female condition. While *K* showed no effect on mortality in the present study, lipid content may be a more accurate predictor. Furthermore, extrinsic factors in the wild could have higher impacts on egg survival than intrinsic factors. The highest egg mortality occurring in the laboratory trials conducted here (17.4%) is relatively low compared to the maximum mortality of 100% caused by layering of spawned eggs (Finke *et al.*, 2022). Moreover, 2.4-fold differences in egg mortality among batches were estimated to be due to predation (Kotterba *et al.*, 2017) and 1.6-fold differences in damage to eggs was documented after one storm event (Moll *et al.*, 2018). Those extrinsic factors, however, do not appear responsible for the relatively low survival of progeny early in the season since eggs at the surface layer (later-spawned) also experienced high mortality (Finke *et al.*, 2022), predation is highest late in the season (Kotterba *et al.*, 2014) and the intensity and frequency of storms at the spawning ground are similar throughout the season (Moll *et al.*, 2018).

The time to hatch in *C. harengus* embryos was not related to egg size but, similar to other marine fishes, development was more rapid as incubation temperature increased (Peck *et al.*, 2012a). In the field, eggs from later in the spring season will experience warmer temperatures and develop faster to hatch, which could be an advantage as sessile eggs are more susceptible to predators (Bailey & Houde, 1989). In our study, viable eggs of all females from the same

spawning time and incubated at the same temperature (7 or 13°C) hatched within the same night, irrespective of egg size. Likewise, Blaxter & Hempel (1963) compared the time required for *C. harengus* eggs to hatch among six *C. harengus* populations at temperatures between 5 and 15°C and found no effect of egg size. Compared to the results of other studies on the WBSS *C. harengus* population, the time to hatch observed here was similar to the values reported by Blaxter & Hempel (1963) and Peck *et al.* (2012a). The time to hatch at 7 and 13°C still differed after normalising the data by calculating physiological age (in degree-days) as embryos at the colder and warmer temperature hatched at greater and lesser physiological age, respectively. In the present study, the time required for embryos to hatch at both incubation temperatures slightly decreased in embryos from females spawning early, middle and late in the season. Given that egg sizes among spawning times overlapped, it is possible that water temperature experienced during oogenesis rather than egg size can exert a modest influence on the time required for eggs to hatch but this remains to be tested.

The present study quantified the change in maternal and egg traits occurring among females captured in early, middle and late spawning waves in WBSS *C. harengus*. The fertilisation success and viable hatch of eggs were relatively high and the modest maternal effect on egg performance was unrelated to spawning time, female size, condition or egg size. In WBSS *C. harengus*, extrinsic factors such as predation (Kotterba *et al.*, 2014, 2017) and/or storm events (Moll *et al.*, 2018) appear to have larger impacts on egg mortality and the "bigger is better" hypothesis does not apply during the egg stage. The results of the present study suggest that the lower survival of the early cohort observed in the wild is unlikely due to intrinsic differences in egg survival but could be due to differences in egg quality: fatty acids and other biochemical attributes were not measured in our study. After hatch, however, the larvae will not only face the same threats (from predation and storm events) as eggs but will also have the additional challenge of finding suitable food before their yolk reserves are exhausted (Houde, 1987). Differences in egg sizes among females in the present study suggest that newly-hatched larvae can be provided with different amounts and/or quality of yolk reserves which, in addition to extrinsic effects, may cause differences in survival at the first-feeding stage. Further study of first-feeding larvae is needed to better understand the intra-annual differences in survival in WBSS *C. harengus*.

# Chapter 3

# Maternal, temperature and seasonal effects in yolk sac larvae of Atlantic herring *Clupea harengus*

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Maternal influence, seasonal cohort, phenotypic variation, *Clupea harengus*, herring, Baltic Sea

## Abstract

In marine fishes that spawn at specific times of the year, maternal effects interact with seasonal abiotic factors to influence offspring phenotype that can affect growth, development and survival of the early life stages. The relative importance of maternal versus abiotic processes throughout ontogeny is unclear. We incubated the progeny of 22 Atlantic herring *Clupea harengus* females from either early-, mid- or late-spring at both early- (7°C) and late-season (13°C) *in situ* temperatures. After yolk sac larvae had hatched, changes in yolk sac area, notochord length, body depth, somatic body area, and cases of deformities were tracked until the point-of-no-return (beyond which starvation is irreversible), allowing somatic growth rate and yolk utilisation efficiency to be estimated. We then quantified the contributions of maternal effects, incubation temperature, and seasonal effects on offspring traits. Among newly-hatched larvae, the variance in body size was explained by seasonal (34%) and temperature (11%) but not maternal effects. As yolk sac larvae reached maximum size, egg size and individual females together accounted for half (49-59%) of the variance in body size while seasonal and temperature effects did not explain additional variance. The maternal effects were, however, unrelated to female size. As a result, size classes of females matched poorly (14-36%) with those of egg and yolk sac larval stages, while size classes of eggs matched well with that of maximum-sized larvae (59%) and less with size classes of

newly-hatched larvae (36%). Further, yolk sac larvae from later in the season or at the 13°C treatment had a relatively longer post-hatch, free-swimming yolk sac larval stage considering the whole yolk period. Yolk utilisation efficiency was similar and deformity percentage was low (<5.3%) across seasonal timing and temperature treatments. In summary, our study revealed that the seasonal effects on offspring size at the transition period from endo- to exogenous feeding were attributed to differences in egg size, with herring females from earlier in the season spawning larger eggs.

## 3.1 Introduction

In marine fish populations, high and unpredictable mortality of the early life stages can lead to large fluctuations in recruitment (Houde, 1987). Mass mortality of >50% occurs even under controlled, laboratory settings (e.g. van der Meeren & Næss, 1993; Pedersen *et al.*, 1990), especially during the transitional period from relying on yolk (endogenous) to feeding on external food (exogenous). It has been demonstrated that individuals with a larger body size have higher survival during this phase in both low and optimal prey conditions compared to those with a smaller body size (Garrido *et al.*, 2015; Rosenberg & Haugen, 1982). Reasons for this could be that a larger body size leads to better abilities to catch prey and more resistance against starvation (Ellertsen *et al.*, 1980; Knutsen & Tilseth, 1985). Understanding the factors that drive variation in body size at this stage, therefore, will help identify the underlying causes of differences in survival.

As most marine fishes do not perform parental care, body size at the transitional period from endo- to exogenous feeding is determined by the total energy available in the egg as well as the environment the egg experiences. The energy source of eggs is supplied by adult females, referring to a form of maternal effects using egg size as a proxy (Bernardo, 1996). The predetermined, fixed amount of energy reserve (yolk) supports embryos until first feeding, which happens when most of the yolk has been utilised to allow opening of the oesophagus (Kamler, 2008; Yúfera & Darias, 2007). After the eggs have been spawned, environmental factors such as temperature influence rates of development, growth and metabolism depending on body size and, thus, how much of the energy is allocated to growth (Kamler, 2008). Many studies have examined the effects of egg size or temperature on body size of yolk sac larvae. First, for some fish, egg diameter was found to be positively correlated

with length-at-hatch, but the explained variation ranged widely among studies of the same species (Atlantic cod *Gadus morhua*, 6 to 71%; Marteinsdottir & Steinarsson, 1998; Miller *et al.*, 1995; Nissling *et al.*, 1998; Pepin *et al.*, 1997), or was even absent in a different species (European sardine *Sardina pilchardus;* Garrido *et al.*, 2015). Second, increasing length-at-hatch has been associated with decreasing (Nissling, 2004; Peck *et al.*, 2012b) or increasing (Nissling, 2004; Pepin *et al.*, 1997) incubation temperatures. Patterns of changes in length-at-hatch and temperature are species-specific and differ among relatively cold (temperate) or warm (tropical) marine fishes (Peck *et al.*, 2012a). In summary, both maternal effects and temperature explained a large amount of variance in offspring body size but results are not uniform across studies. Quantifying the relative contributions of maternal effects and environmental effects on bod size helps to provide a clearer picture of the drivers of early-life developmental variation.

Maternal and temperature effects in temperate seas are linked with seasonal changes. Most temperate marine fish populations spawn in spring or autumn, with a protracted spawning season lasting from a few weeks up to three months (Wright & Trippel, 2009). Offspring from earlier in the spawning season tend to be produced by larger females (e.g. in cod (Kjesbu *et al.*, 2010) and Atlantic herring *Clupea harengus* (Lambert, 1987; Óskarsson *et al.*, 2002)). Also, water temperature rises (spring) or decreases (autumn) as the spawning season progresses. However, far fewer studies have considered how seasonal changes affect both females and their progeny. Miller *et al.* (1995) incubated Atlantic cod eggs throughout the spawning season from October to May at ambient temperatures and found that most (70%) of the variation in length-at-hatch was explained by temperature compared to egg size, with a consistent correlation among years. Another study on Atlantic silverside *Menidia menidia* showed that, when comparing eggs of similar diameter, newly-hatched larvae from early-season at colder temperature were longer than those from late-season warmer temperature (Bengtson *et al.*, 1987). Both cod and silverside, as well as most other temperate marine fishes, are batch spawners that release eggs multiple times within a season (Murua & Saborido-Rey, 2003). In batch-spawning fish, offspring released at different time periods of the season may be spawned by the same individuals. In contrast, Atlantic herring are total (single-batch) spawners, i.e. individuals release all of their eggs at once within a season (Murua & Saborido-Rey, 2003). In herring, offspring from different time periods of the season

are from different females. Hence, maternal effects in herring have a tighter link with seasonal abiotic factors compared to batch-spawning fish.

In the present study, maternal, temperature, and seasonal effects in herring offspring were tracked from hatch until the end of the yolk sac larval stage. We performed laboratory trials at early- (7°C) and late-season (13°C) temperatures with the offspring of early-, mid- and late-season females from the western Baltic spring-spawning (WBSS) herring that were spawned by early-, mid- and late-season females, which differed in size. Larval morphometric traits (yolk sac area, notochord length, body depth, and somatic body area), duration from hatch to growth thresholds (zero yolk reserves and maximum size) and morphological deformities were measured throughout ontogeny. The contribution of maternal, temperature, and seasonal effects on the measured larval traits was then quantified. The correlations between seasonal timing and size classes of different life stages (females, eggs, newly-hatched larvae, and yolk sac larvae reaching maximum size) were compared with one another. We expected that the variation in larval traits to be larger among seasonal cohorts than within seasonal cohorts. Our hypothesis was that the initial size class would be transferred to later life stages, such that size classes were consistent among eggs and yolk sac larvae.

## 3.2 Materials and methods

All experimental and sampling procedure complied with the German Animal Welfare Act and the Animal Welfare Regulation Governing Experimental Animals.

### 3.2.1 Adult spawning and pre-hatch incubation

The spawning season of western Baltic spring-spawning (WBSS) herring *Clupea harengus* L. was separated into three periods i.e. early- (day of the year; doy 53–78), mid- (doy 79–104) and late-season (doy 105–130; Huang *et al.*, 2022). Adult herring were caught using gill nets in Greifswald Bay (54.25°N, 13.23°E) within the western Baltic Sea at mid- and late-seasons in 2019 and at early-season in 2020, corresponding to doy 81, 107, and 65, respectively. Selected females of early- ($n$ = 8), mid- ($n$ = 8) and late-season ($n$ = 6) ranged from 28.7 to 31.3, 27.7 to 29.9, and 26.0 to 28.5 cm, respectively. The eggs of each female were stripped onto eight glass plates (63–379 eggs). Upon contact with water, herring eggs became

adhesive to the glass plates and then were fertilised with pooled milt from 5 to 26 males. The egg plates were placed into individual 250-ml cylindrical chambers. Per female, the eight egg chambers were randomly distributed to a 7 ($n$ = 4) or 13°C ($n$ = 4) 30-l water bath under a 14:10 h light:dark regime (Figure 3.1). The water baths were connected to a 500-l recirculating aquaculture system with a mechanical filter, a biofilter, UV lights, a protein skimmer, and a cooling unit (Titan 1500, 790 W, Aqua Medic, Bissendorf, Germany). After filtration, water flowed into six 45-l header tanks and then into each egg chamber at 40 ml $min^{-1}$. The system was maintained at 7°C and salinity 7, with only the header tanks and water baths heated for the 13 ° C treatments (600 W titanium heaters with TRD thermostatic controllers, Schego, Offenbach am Main, Germany). For a detailed description, see Huang *et al.* (2022) .

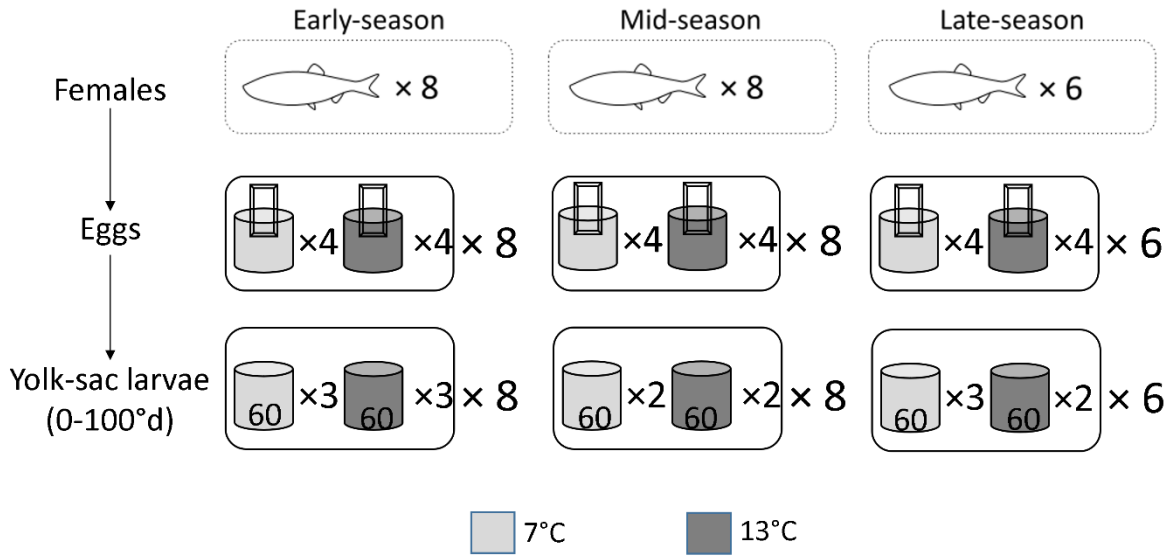


**Figure 3.1** Scheme of the experimental design. At each seasonal timing and temperature treatment (7 and 13°C), yolk sac larvae hatched from four egg chambers per female ($n$ = 6–8 per seasonal timing) were mixed to form two to three replicate chambers, each with ca. 60 larvae (Appendix Table A3.1).

### 3.2.2 Post-hatch incubation and sampling

Embryos from the same seasonal timing and incubation temperature group reached the embryonic stage of eye pigmentation on the same day. Starting from one day following eye pigmentation, egg plates were transferred into individual 1000-ml cylindrical chambers within the same water baths previously described. The chambers were supplied with gentle aeration but no water inflow to retain potentially hatched larvae. The chambers were checked for hatching at 2, 6 and 10 h after the onset of darkness. If more than five yolk sac larvae were present in any one chamber, all egg plates of that corresponding treatment were

transferred into new chambers. This was done to ensure the yolk sac larvae that were subsequently sampled were of similar ages (difference ≤ 4 hr). In case fewer than five larvae had hatched, all egg plates of the corresponding treatment were transferred to new chambers at the start of the next light period to maintain water quality. During the night of mass hatching, the most abundant larval batch across females (visual inspection) from the same hatching interval (0–2, 2–6, or 6–10 h) was chosen for both morphometric determinations and further incubation of yolk sac larvae. For morphometric measurements at hatch, ca. 40 larvae (10 larvae × 4 chambers per temperature; Appendix Table A3.1) per female were carefully siphoned via a 4-mm diameter tube into a 63 µm-mesh sieve cup on a petri dish. The petri dish contained seawater (salinity 7) with anaesthetic aquacalm (metomidate hydrochloride, Western Chemical Inc., Washington, USA, 20 mg $l^{-1}$). Yolk sac larvae were photographed on their left or right lateral side under a stereomicroscope (Leica M165 C, Leica Microsystems, Wetzlar, Germany) connected to a digital camera (Leica MC170 HD, Leica Microsystems, Wetzlar, Germany) for later measurements. After being photographed, yolk sac larvae were placed into a –80°C freezer.

For incubation, ca. 15 larvae from each of the four replicate chambers per female (total 42–65 larvae realised) were mixed to form new 1000-ml replicate cylindrical chambers ($n$ = 2 or 3; Figure 3.1, Appendix Table A3.1) with the same set up as the chambers for hatching. This was done to neutralize any chamber effects during egg incubation. The difference in the number of replicate chambers was due to an overlap of trial periods between mid- and late-season cohorts and limited laboratory space. The midpoint of the selected hatching interval for each seasonal cohort at each incubation temperature was defined as the hatch time for the respective cohorts. The duration from hatch time to the time at which individual photographs were taken was calculated as the age in degree-days (°d) post-hatch. For each larval chamber, ca.10 larvae were sampled, using the same method (careful siphoning) previously described. The sampling was repeated at a mean (±SD) interval of 18(5)°d through 100°d which is the point of no return (beyond which starvation is irreversible even if prey are added and feeding begins) in WBSS herring yolk sac larvae (Blaxter & Hempel, 1963) . After hatch, larvae were sampled five times, except for the mid-season cohort in the 13°C treatment which was sampled only four times due to the unintended loss of larvae during water exchange (Appendix Table A3.1).

Each replicate chamber had a 50 to 65% water exchange with seawater from the recirculating aquaculture system daily (13°C) or every other day (7°C). This ensured that total ammonium/ammonia, nitrite and nitrate concentrations remained below 0.02, 0.001 and 0.05 mg $l^{-1}$, respectively (Tropic Marin $NH_4^+/NH_3$ test and $NO_2/NO_3$ Test Professional, Dr. Biener GmbH, Wartenberg, Germany). During yolk sac larval incubation, the mean (±SD) water temperature in the chambers was 7.2(0.3)°C and 12.6(0.3)°C in the cold and warm treatment, respectively (TLog64-USB, ±0.5°C, Hygrosens Instruments GmbH, Löffingen, Germany). Salinity was maintained at a range from 6.6 to 7.4 (Cond 3110, WTW GmbH, Weilheim in Oberbayern, Germany). Dissolved oxygen was > 90% air saturation (Oxi 3210, WTW GmbH, Weilheim in Oberbayern, Germany).

### 3.2.3 Morphometric traits and deformities

Yolk sac area, notochord length, body depth, and body area were measured to the nearest 0.001 mm or 0.001 $mm^2$ on digital photographs of individual larvae (10× magnification) using ImageJ (version 1.52, Wayne Rasband, National Institute of Mental Health, Bethesda, Maryland, USA). Notochord length was measured from the tip of the notochord to the tip of the snout. Body depth was measured at the midpoint of the body. For body area, finfold area was excluded. Somatic body area was calculated as body area minus yolk sac area. Any morphological deformities such as curved bodies or yolk sac edema (Ojaveer, 1981, Appendix Figure A3.2) were noted and deformed larvae were excluded from further analyses.

### 3.2.4 Data analyses

To determine growth curves for each morphometric trait, Gompertz models were fitted to chamber averages at all sampling points (2–4 replicate chambers × 5–7 sampling points per female per temperature; Appendix Table A3.1). From the fitted values, we obtained the predicted size-at-hatch as well as the predicted age and size at which each trait reached its maximum value (length, depth, body area) or the yolk sac was fully depleted. Size-at-hatch was calculated to minimize the effects of the time differences (up to 7°d) between first- and last-sampled larvae within seasonal cohorts. A total of 44 Gompertz models (22 females × 2 temperatures) were fitted per trait (Appendix Table A3.2). Yolk utilisation efficiency was calculated by dividing the predicted growth in somatic body area by the initial predicted yolk sac area.

Variance in each morphometric trait was compared among replicate chambers, females, incubation temperatures and seasonal timing using random effects models. The proportion of variance at each level was estimated with intraclass correlation coefficients (ICC; Nakagawa & Schielzeth, 2010).

Linear-mixed effects models (LMM) were used to compare the predicted durations from hatch to yolk depletion, maximum body depth, and maximum somatic body area. In addition, LMMs were used to quantify the contribution of maternal, temperature, and seasonal effects on offspring traits. For this, we included female total length (cm), egg area ($mm^2$), incubation temperature (7 or 13°C), and seasonal timing (interval variable of 1–3 representing early-, mid- and late-season) as fixed effects. Seasonal effect was assumed to be linear because we observed linear relationships between seasonal timing and both *in situ* temperature and egg area (Huang *et al.*, 2022). Individual female was included as a random (intercept) effect to account for maternal effects that were not explained by female length or egg area. The contribution of each fixed effect was represented by partial $R^2$, while the contribution of the random effect was calculated with the difference between marginal and conditional $R^2$ (Nakagawa & Schielzeth, 2013).

To correlate size classes across life stages, we scaled the size ranges of (1) females (total length), (2) eggs (diameter, calculated from area), (3) newly-hatched larvae (notochord length), and (4) yolk sac larvae reaching maximum length (notochord length) between 0 and 1. For yolk sac larvae, average lengths across temperature treatments were used for scaling. The scaled sizes were then classified into quintiles from Q1 (largest 20 percentile) to Q5 (smallest 20 percentile). The correlation among seasonal timing and size classes of each stage was compared using Spearman's rank correlation.

All analyses were conducted in R version 4.0.3 (R Core Team, 2020). Gompertz models and LMMs (including random effects models) were fitted using the "minpack.lm::nlsLM" (Elzhov *et al.*, 2016) and "lme4::lmer" (Bates *et al.*, 2015) functions, respectively. Model assumption was checked with QQ plots and residuals vs. fits plots. *P* values and $R^2$ of models and effects were obtained using the packages "LmerTest" (Kuznetsova *et al.*, 2017), "MuMIn" (Barton, 2020) and "r2glmm" (Jaeger, 2017). The significance level was set at $P \leq 0.05$.

## 3.3 Results

Among replicate chambers of females, the variance in notochord length, body depth, and somatic body area of yolk sac larvae both at hatch and at maximum size was small (ICC = 0.027-0.059). The variance in yolk sac area at hatch was slightly higher (ICC = 0.098) but was half of the variance among offspring from different females (ICC = 0.198).

### 3.3.1 Growth during the yolk sac larval stage

After hatching, yolk sac area decreased gradually as notochord length, body depth and somatic body area of yolk sac larvae grew (Figure 3.2). Averaged across seasonal timing, the mean(±SE) duration from hatch to yolk depletion was 44.2(2.2) and 53.7(3.8)°d, which corresponded to 6.3 and 4.1 days at 7 and 13°C, respectively. Notochord length started to plateau on average 11.0°d before yolk depletion, when its growth rate fell below 0.01 mm per °d. Body depth reached its maximum 17.4°d before yolk depletion ($P < 0.0001$), whereas somatic body area of yolk sac larvae reached its maximum 6.3°d after yolk depletion ($P < 0.0001$; Table 3.1). After which, both body depth and somatic body area declined (Figure 3.2, Appendix Figure A3.1).

On newly-hatched larvae, maternal effects only influenced yolk sac area but not notochord length, body depth, or somatic body area (Table 3.2). Yolk sac area differed up to 56.3% among offspring of different females (female ID, $R^2 = 0.329$) within the same cohorts. The differences were not influenced by female total length ($P = 0.155$) but by egg area, with larger eggs giving rise to larger yolk sacs at hatch ($P = 0.005$, $R^2 = 0.278$). In contrast, seasonal effects influenced all metrics of somatic body size but had less impact on yolk sac area (Table 3.2). Mean notochord length, body depth and somatic body area decreased with subsequent seasonal timing (Table 3.1, Figure 3.3). The differences between early- and late-season larvae across both temperature treatments were 0.76 mm (11.5%, $P < 0.001$, $R^2 = 0.285$) for notochord length, 0.04 mm (12.6%, $P = 0.034$, $R^2 = 0.101$) for body depth, and 0.39 mm$^2$ (19.2%, $P = 0.019$, $R^2 = 0.122$) for somatic body area. Moreover, larvae at 13°C hatched at shorter lengths ($P < 0.0001$, $R^2 = 0.613$) and smaller somatic body areas ($P = 0.004$, $R^2 = 0.182$) but with larger yolk sacs ($P < 0.0001$, $R^2 = 0.259$) compared to larvae at 7°C.

**Table 3.1** Size at hatch, size at maximum during the yolk feeding period, and duration to yolk depletion and maximum size in *Clupea harengus* yolk sac larvae from early- ($n$ = 8), mid- ($n$ = 8), and late-season ($n$ = 6) females and incubated at 7 or 13°C. All values are mean (±SE). Tukey post-hoc tests were performed across seasonal timing and temperature treatments. Different lowercase letters indicate significant differences ($P < 0.05$). Abbreviations: Body, somatic body area; depth, body depth; length, notochord length; max, maximum; temp, temperature; yolk, yolk sac area.

| | **Grand mean** | **7°C** | | | **13°C** | | |
|---|---|---|---|---|---|---|---|
| | | **Early-season** | **Mid-season** | **Late-season** | **Early-season** | **Mid-season** | **Late-season** |
| *Size at hatch* | | | | | | | |
| **Yolk (mm²)** | 0.408 | 0.399 | 0.357 | 0.392 | 0.454 | 0.385 | 0.460 |
| | (0.018) | (0.012)$^{ab}$ | (0.018)$^{b}$ | (0.008)$^{ab}$ | (0.013)$^{a}$ | (0.021)$^{b}$ | (0.007)$^{a}$ |
| **Length (mm)** | 7.41 | 8.22 | 7.52 | 7.50 | 7.35 | 7.33 | 6.55 |
| | (0.22) | (0.08)$^{a}$ | (0.04)$^{b}$ | (0.05)$^{b}$ | (0.07)$^{b}$ | (0.09)$^{b}$ | (0.07)$^{c}$ |
| **Depth (mm)** | 0.326 | 0.362 | 0.289 | 0.318 | 0.342 | 0.338 | 0.307 |
| | (0.013) | (0.006)$^{a}$ | (0.018)$^{c}$ | (0.018)$^{bc}$ | (0.006)$^{ab}$ | (0.006)$^{ab}$ | (0.004)$^{bc}$ |
| **Body (mm²)** | 2.20 | 2.65 | 2.01 | 2.25 | 2.23 | 2.22 | 1.85 |
| | (0.12) | (0.05)$^{a}$ | (0.04)$^{c}$ | (0.02)$^{b}$ | (0.04)$^{b}$ | (0.05)$^{b}$ | (0.03)$^{c}$ |
| *Maximum size during yolk period* | | | | | | | |
| **Length (mm)** | 8.83 | 9.29 | 8.80 | 8.64 | 9.02 | 8.83 | 8.42 |
| | (0.18) | (0.12)$^{a}$ | (0.11)$^{bc}$ | (0.04)$^{bc}$ | (0.10)$^{ab}$ | (0.13)$^{bc}$ | (0.07)$^{c}$ |
| **Depth (mm)** | 0.392 | 0.405 | 0.380 | 0.382 | 0.418 | 0.376 | 0.392 |
| | (0.010) | (0.004)$^{ab}$ | (0.005)$^{c}$ | (0.003)$^{c}$ | (0.005)$^{a}$ | (0.005)$^{c}$ | (0.003)$^{bc}$ |
| **Body (mm²)** | 3.13 | 3.40 | 3.02 | 2.98 | 3.37 | 3.07 | 2.95 |
| | (0.13) | (0.08)$^{a}$ | (0.07)$^{b}$ | (0.05)$^{b}$ | (0.07)$^{a}$ | (0.08)$^{b}$ | (0.05)$^{b}$ |
| *Duration from hatch to growth thresholds* | | | | | | | |
| **Zero yolk (°d)** | 48.9 | 42.7 | 41.3 | 48.6 | 54.5 | 46.6 | 59.8 |
| | (3.0) | (3.3)$^{c}$ | (1.4)$^{c}$ | (0.7)$^{bc}$ | (1.5)$^{ab}$ | (0.6)$^{c}$ | (1.4)$^{a}$ |
| **Max depth (°d)** | 30.9 | 25.9 | 26.9 | 24.2 | 36.1 | 37.1 | 35.2 |
| | (0.7) | (2.1)$^{bc}$ | (0.9)$^{abc}$ | (3.5)$^{c}$ | (2.5)$^{ab}$ | (3.8)$^{a}$ | (2.2)$^{abc}$ |
| **Max body (°d)** | 55.3 | 48.5 | 43.9 | 64.5 | 57.0 | 60.3 | 57.9 |
| | (2.9) | (1.5)$^{ab}$ | (1.1)$^{b}$ | (7.0)$^{a}$ | (6.5)$^{ab}$ | (2.0)$^{a}$ | (1.9)$^{ab}$ |

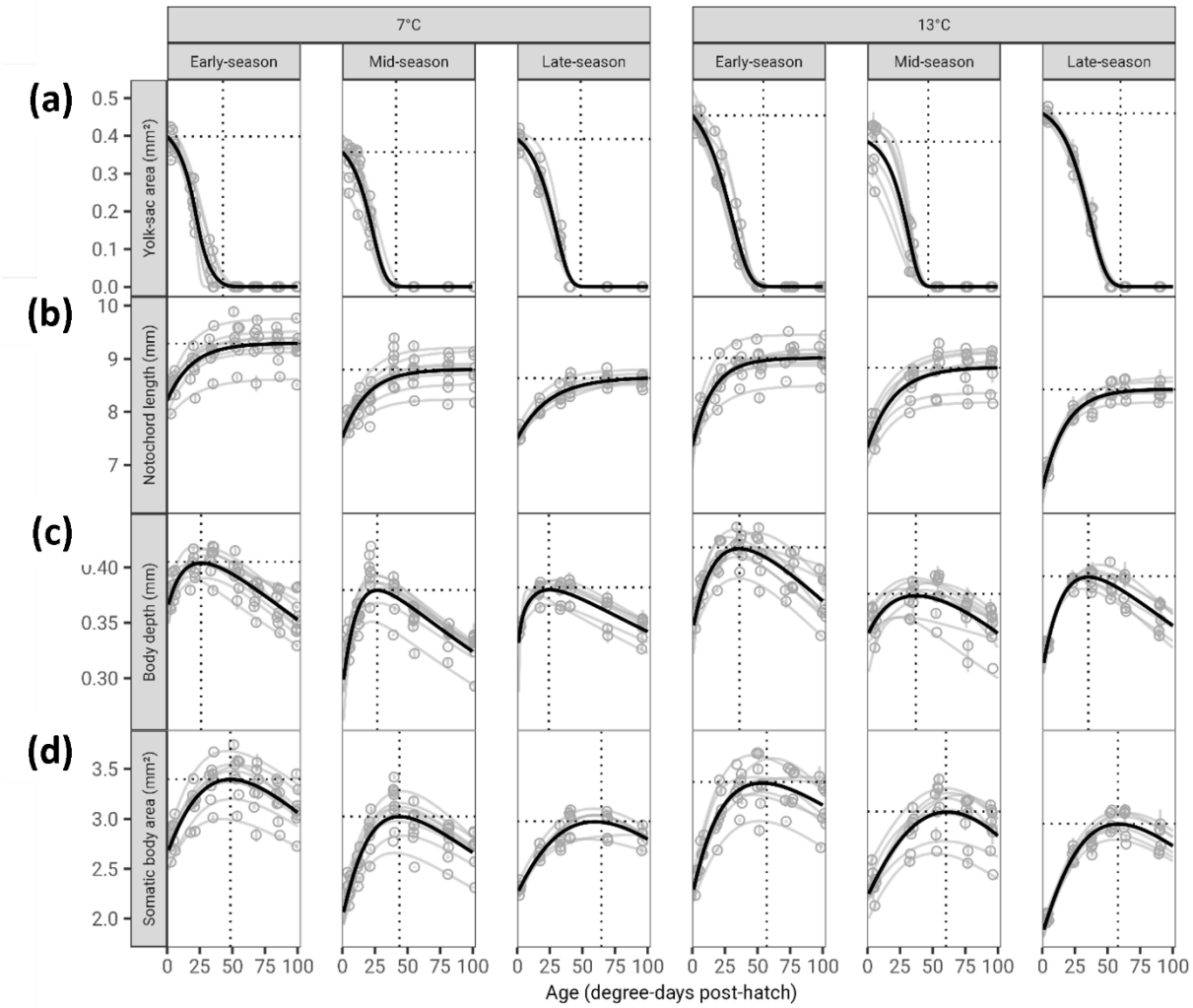


**Figure 3.2** Change in (a) yolk sac area, (b) body depth, (c) somatic body area, and (d) notochord length vs. age from 0 (hatch) to 100 (point of no return) degree-days post-hatch in *Clupea harengus* yolk sac larvae from eggs of early-, mid- and late-season females and incubated at 7 or 13 °C. Total *n* observations per female was 10–22 at each temperature treatment (Appendix Table A3.1). Circles are mean (±SE) observed values for offspring of individual females at each sampling point. Grey curves display Gompertz models fitted for yolk sac larvae of individual females while black thick curves represent the averaged curves within corresponding seasonal timing and incubation temperatures. Vertical dotted lines denote the average age at zero yolk reserves or maximum size. Horizontal dotted lines indicate the average maximum values for the given age range.

On yolk sac larvae reaching maximum size, maternal effects influenced all metrics of somatic body size (Table 3.2). Mean maximum notochord length, body depth and somatic body area differed among offspring of different females (female ID, $R^2$ = 0.169-0.315) and increased with egg size. Between larvae hatching from the largest and smallest eggs, differences were up to 0.96 mm (11.7%, $P < 0.0001$, $R^2$ = 0.505), 0.04 mm (12.0%, $P$ = 0.002, $R^2$

= 0.352), and 0.61 mm² (22.8%, $P < 0.0001$, $R^2 = 0.560$), respectively, within seasonal cohorts. Apart from the effect of egg area, seasonal timing explained no additional variance in maximum size (all $P > 0.05$; Figure 3.4). Averaged across seasonal timing, larvae incubated at 13°C had 0.006 mm deeper maximum body depths (1.6%, $P = 0.009$, $R^2 = 0.114$) and 0.14 mm shorter maximum notochord lengths (1.6%, $P = 0.001$, $R^2 = 0.053$) but similar maximum somatic body areas ($P = 0.960$) compared to 7°C larvae (Table 3.2, Figure 3.3).

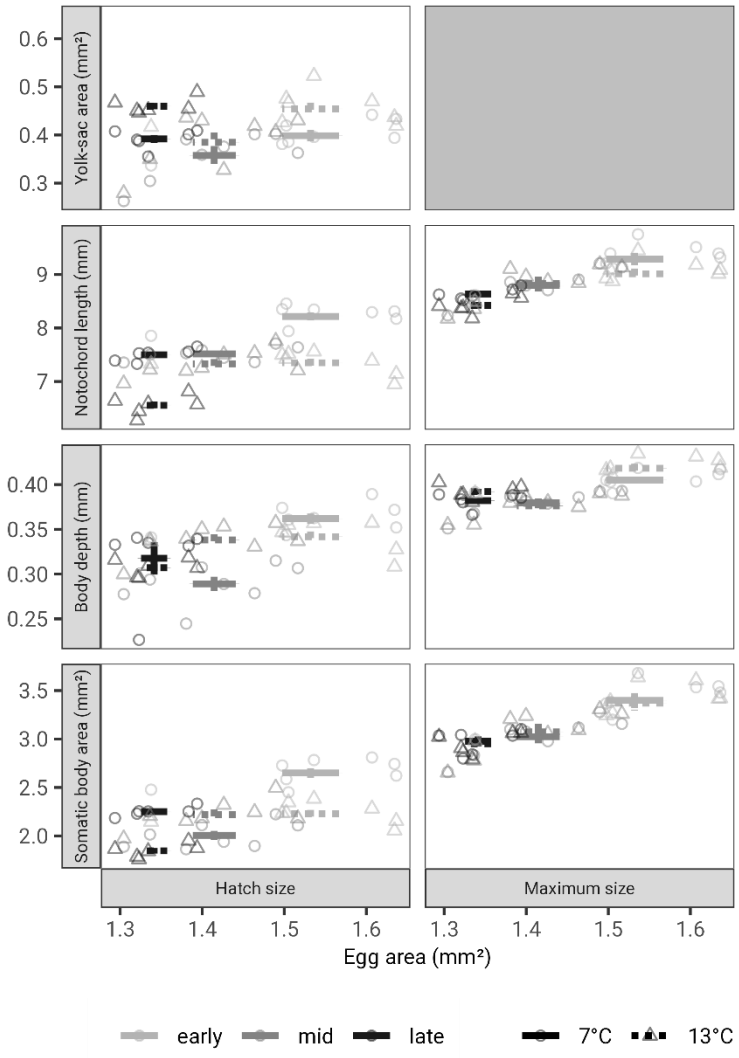


**Figure 3.3** Size (mm or mm²) at hatch and at maximum vs. egg area (mm²) for early-, mid- and late-season *Clupea harengus* yolk sac larvae incubated at 7 or 13°C. Circles (7°C) and triangles (13°C) are predicted values from growth curves (Figure 3.2). Solid (7°C) and dotted (13°C) lines represent the grand mean (±SE) for larval size and egg area ($n$ = 6–8) at each seasonal timing.

**Table 3.2** Summary of the linear mixed-effects models describing the effects of female total length, egg area, incubation temperature (7 and 13°C), and seasonal timing (early-, mid- and late-season) on size at hatch, maximum size during yolk period, and duration to yolk depletion and maximum size in *Clupea harengus* yolk sac larvae. Values of fixed effects are coefficient estimates (±SE) while random effects are in ±SD. Total *n* of observations per trait = 22 females × 2 temperatures = 44. Significant codes: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Abbreviations: Body, somatic body area; depth, body depth; length, notochord length; max, maximum; yolk, yolk sac area.

| | Range | Fixed effects | | | | | Random effects | | Marginal/ conditional $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | | Intercept | Female length | Egg area | 13°C | Seasonal timing | (1\|female) | Residual | |
| *Size at hatch* | | | | | | | | | |
| **Yolk (mm²)** | 0.263–0.523 | 0.233 | −0.014 | 0.360 | 0.049 | 0.017 | 0.032 | 0.027 | 0.434/0.763 |
| | | (0.309) | (0.009) | (0.111)** | (0.008)*** | (0.016) | | | |
| **Length (mm)** | 6.27–8.46 | 8.08 | −0.03 | 0.77 | −0.64 | −0.33 | 0.00 | 0.26 | 0.751/0.751 |
| | | (1.52)*** | (0.05) | (0.55) | (0.08)*** | (0.08)*** | | | |
| **Depth (mm)** | 0.227–0.389 | 0.453 | −0.009 | 0.107 | 0.008 | −0.019 | 0.000 | 0.028 | 0.329/0.329 |
| | | (0.165)** | (0.005) | (0.059) | (0.008) | (0.009)* | | | |
| **Body (mm²)** | 1.76–2.81 | 2.21 | −0.03 | 0.85 | −0.19 | −0.15 | 0.00 | 0.20 | 0.498/0.498 |
| | | (1.17) | (0.04) | (0.42) | (0.06)** | (0.06)* | | | |
| *Maximum size during yolk period* | | | | | | | | | |
| **Length (mm)** | 8.18–9.75 | 5.78 | −0.03 | 2.87 | −0.14 | −0.07 | 0.16 | 0.13 | 0.727/0.895 |
| | | (1.53)** | (0.05) | (0.55)*** | (0.04)** | (0.08) | | | |
| **Depth (mm)** | 0.351–0.434 | 0.281 | −0.003 | 0.137 | 0.006 | −0.003 | 0.011 | 0.007 | 0.554/0.869 |
| | | (0.103)* | (0.003) | (0.037)** | (0.002)** | (0.005) | | | |
| **Body (mm²)** | 2.65–3.68 | 1.19 | −0.03 | 2.09 | 0.00 | −0.05 | 0.12 | 0.06 | 0.748/0.955 |
| | | (1.06) | (0.03) | (0.38)*** | (0.02) | (0.06) | | | |
| *Duration from hatch to growth thresholds* | | | | | | | | | |
| **Max depth (°d)** | 8.7–51.9 | 10.7 | −0.8(1.3) | 25.9 | 10.4 | 1.1(2.3) | 2.4 | 6.5 | 0.401/0.471 |
| | | (42.8) | | (15.4) | (1.9)*** | | | | |
| **Max body (°d)** | 39.6–100 | 70.4 | −3.7(2.0) | 53.4 | 7.3(3.3)* | 5.4(3.4) | 0.0 | 10.9 | 0.271/0.271 |
| | | (64.2) | | (23.1)* | | | | | |
| **Zero yolk (°d)** | 28.3–63.7 | 36.0 | −2.2(1.1) | 43.9 | 9.3(1.4)*** | 4.4(1.9)* | 2.8 | 4.6 | 0.561/0.681 |
| | | (36.0) | | (13.0)** | | | | | |

Mean duration from hatch to yolk depletion was influenced, with decreasing importance, by incubation temperature ($P < 0.0001$, $R^2 = 0.429$), egg area ($P = 0.003$, $R^2 = 0.254$), and seasonal timing ($P = 0.033$, $R^2 = 0.137$) (Table 3.2, Figure 3.4). Considering all effects, early- and mid-season larvae hatching from larger eggs reached yolk depletion one to two days later than those hatching from smaller eggs. Late-season larvae, on the other hand, depleted their yolk on the same day at both temperature treatments. The mean (±SE) yolk utilisation efficiency was 2.28 (0.09) and was not significantly related to differences in egg area, incubation temperature, or seasonal timing (all $P > 0.05$). Within offspring of the same individuals, the somatic body area to yolk sac area ratio was similar between larvae reared at 7 and 13°C. In addition, the growth trajectories of larvae at both temperatures overlapped (Figure 3.5). The mean(±SE) larval yolk sac duration accounted for 26.0(1.7) and 33.6(2.0)% of the total yolk period (from egg fertilisation to yolk exhaustion) at 7 and 13°C, respectively.

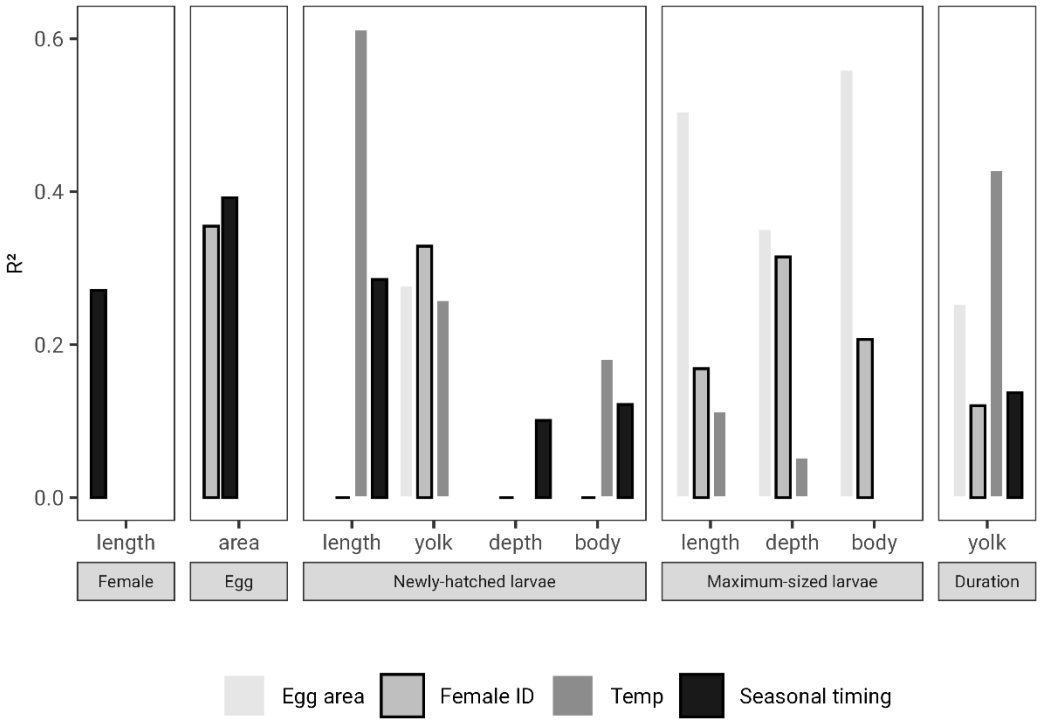


**Figure 3.4** Contribution of egg area, individual females, incubation temperature, and seasonal timing to total variance in morphometric traits of *Clupea harengus* (females, eggs, and yolk sac larvae) and duration from hatch to yolk depletion. Data on the female and egg stages are from Huang *et al.* (2022).

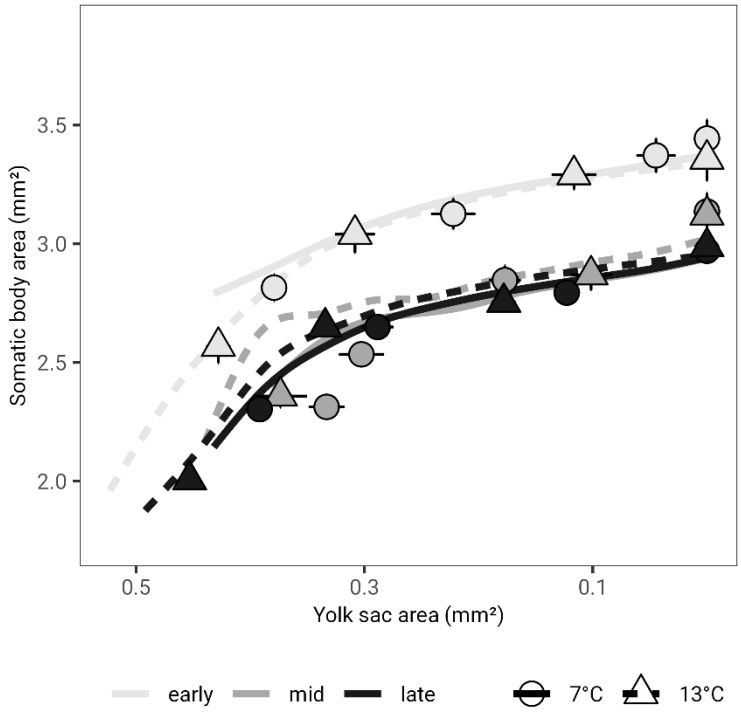


**Figure 3.5** Growth trajectories of somatic body area ($mm^2$) vs. yolk sac area ($mm^2$) in early-, mid- and late-season *Clupea harengus* yolk sac larvae. Circles (7°C) and triangles (13°C) are mean (±SE) observed values for offspring across females ($n$ = 6–8) within seasonal timing. Solid (7°C) and dotted (13°C) lines represent the average predicted values from Gompertz models (Appendix Table A3.2). The x-axis is reversed in direction (decreasing going to the right) to represent the assumption of yolk sac.

### 3.3.2 Matching of size classes across stages

Among the 22 females in the present study, early-, mid- and late-season individuals were in Q1 to 3, Q2 to 4 and Q3 to 5, respectively (Figure 3.6). Compared to the size classes of females, those of their eggs and yolk sac larvae were more ordered according to the timing of season (Table 3.3). At least 75% of the early-season eggs or larvae were in Q1 to 2, while 100% of the late-season larvae were in Q4 to 5. Mid-season eggs and yolk sac larvae reaching maximum size were distributed in Q2 to 5, but most (88%) of mid-season newly-hatched larvae were within Q3 to 4.

Comparing across life stages, 14 to 36% of females produced eggs and yolk-feeding larvae that fell within the same size classes. In contrast, 36 to 59% of eggs of individual females gave rise to yolk sac larvae that remained within the same size classes. Compared to females, size classes of eggs explained 1.7-fold and 3.3-fold more variance in size classes of newly-hatched larvae and yolk sac larvae reaching maximum size (all $P < 0.018$; Table 3.3), respectively. Shifts

in size classes across stages, when they occurred, were within one to two quintiles, except for one shift where a Q5 early-season egg produced Q2 newly-hatched larvae (Figure 3.6). The best correlation was between size classes of eggs and that of yolk sac larvae reaching maximum size, with 59% in the same quintile and the other 41% differing by only one quintile.

**Table 3.3** Pairwise comparisons of seasonal timing (early-, mid- and late-season) and size classes of *Clupea harengus* across four life stages: (1) females, (2) eggs, (3) newly-hatched larvae (Larv Hatch), and (4) yolk sac larvae reaching maximum size (Larv Max). Values are Spearman's rank correlation coefficients. Significant codes: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

| | **Season** | **Female** | **Egg** | **Larv Hatch** |
|---|---|---|---|---|
| **Female** | −0.68*** | | | |
| **Egg** | −0.72*** | 0.56** | | |
| **Larv Hatch** | −0.90*** | 0.59** | 0.76*** | |
| **Larv Max** | −0.72*** | 0.50* | 0.91*** | 0.81*** |

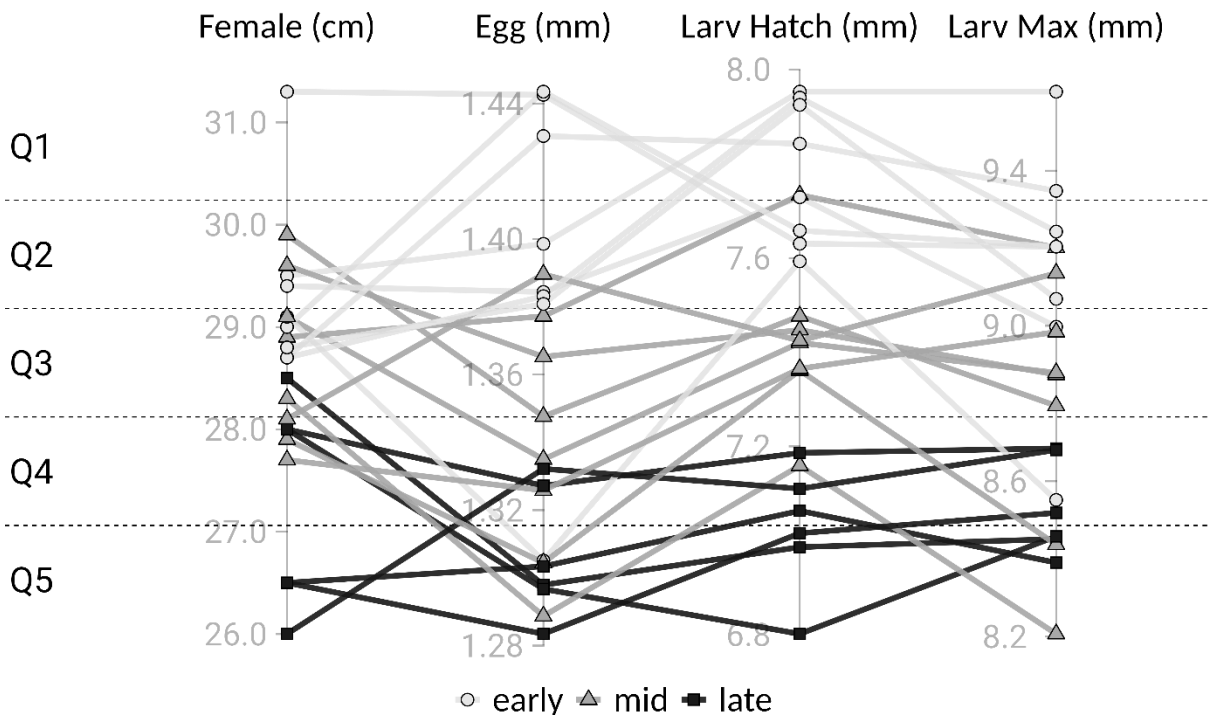


**Figure 3.6** Size classes (from large (Q1) to small (Q5)) of early- (pale grey circles), mid- (dark grey triangles) and late-season (black squares) *Clupea harengus* across four life stages, (1) females (total length), (2) eggs (diameter, calculated from area), (3) newly-hatched larvae (notochord length; Larv Hatch), and (4) yolk sac larvae reaching maximum length (notochord length; Larv Max). To standardize differences across stages, the size ranges of each life stage were rescaled between 0 and 1 and categorised into quintiles. Actual size scales are labelled in grey along vertical lines. Coloured lines connect females with their progeny.

### 3.3.3 Deformities

The mean(±SE) percent deformity in early-, mid- and late-season yolk sac larvae was 1.9(1.0), 0.4(0.1) and 1.0(0.2)% at 7°C, respectively, and 1.3(0.7), 1.0(0.3) and 5.3(1.5)% at 13°C, respectively. The highest percent deformity in progeny from any female was 8.4, 1.9 and 11.3% for early-, mid- and late-season larvae, respectively, whereas the majority (86%) had < 3% incidence of deformity. In mid- and late-season larvae, deformities occurred as black or curved bodies and were observed in larvae that had either hatched successfully or were unable to free themselves from the chorion. Deformed yolk sacs (edema) were only present in early-season larvae and were most prevalent in the 7°C treatment (92.6% of all deformed larvae).

## 3.4 Discussion

Understanding the interaction between environmental and maternal effects is essential for identifying influences on the early survival, growth, and development of marine fish (Chambers, 1997; Marshall *et al.*, 2008). The present study examined the contribution of maternal, temperature, and seasonal effects on body size variation in yolk sac larvae of a herring population. At hatch, somatic body size was largely controlled by incubation temperature and only yolk sac area was influenced by maternal effects. As larvae depleted their yolk sac and reached their maximum size, variation in somatic body size was attributed to egg size and individual females. We revealed that the seasonal decrease in body size of newly-hatched and maximum-sized larvae during the yolk period stemmed from seasonal differences in water temperatures and egg size, respectively.

The lengths at hatch we observed agree well with those reported in a three-year study using herring eggs collected from the same spawning ground as the present study (Busch *et al.*, 1996). The total hatching period lasted for around one to two months at the spawning ground between April and June (Busch *et al.*, 1996; Polte *et al.*, 2014). Mean length at hatch was the largest in early-season cohorts and decreased with subsequent seasonal timing, with a difference of 11-28% between early- and late-season cohorts (Busch *et al.*, 1996). In other studies on WBSS herring, the eggs used were from later spawners in the season, and their reported lengths at hatch matched with the late-season offspring in the present study (Franke & Clemmesen, 2011; Illing *et al.*, 2015; Moyano *et al.*, 2016; Peck *et al.*, 2012b).

Compared to which, lengths at hatch of the early- and mid-season offspring in the present study were 14% and 9% longer. The seasonal decrease in size at hatch during spring has also been observed in other temperate marine fish populations such as cod and Atlantic silversides, with reported seasonal differences of 9 to 12% in length (Bengtson *et al.*, 1987; Miller *et al.*, 1995).

Although both somatic body size (length, depth, area) at hatch and egg size decreased with the progression of the season (This study; Huang *et al.*, 2022), egg area did not influence the variation in size at hatch in the present study. A similar pattern was reported by Miller *et al.* (1995), who found that the correlation between egg diameter and length at hatch was not significant in half of the sampling cruises. By pooling data from the whole season across three years, they estimated that egg diameter was only weakly correlated with length at hatch. Further, in the present study, individual females also did not contribute to the differences in somatic body size at hatch. In studies of other marine fish populations that have followed the offspring of individual females until hatch, the reported contribution of individual females on variation in length at hatch was less than one-third of the female contribution on variation in egg diameter (Benoît & Pepin, 1999; Chambers *et al.*, 1989; Green & Chambers, 2007). These results were in line with our findings that female effects were stronger on egg size than on size at hatch.

Despite the absence of maternal effects on somatic body size at hatch in the present study, maternal effects did explain a large portion of variance in yolk sac area at hatch as well as the maximum size (length, depth and body) achieved after the yolk sac was depleted. A number of studies on marine fish populations had quantified the contribution of individual females on length and yolk sac size of larvae on day 0 and 5 after hatch. A pattern appeared that, with increasing larval age, the contribution of individual females on yolk sac size became smaller whereas their contribution on larval length became larger (Probst *et al.*, 2006; Rideout *et al.*, 2005), or *vice versa* (Trippel *et al.*, 2005). In the present study, we followed the yolk sac larvae beyond yolk depletion and detected significant contributions of egg size (50%) and individual females (30%) on maximum body size. This agrees with the results of previous studies which have reported that egg size explained 61 to 78% of the variance in length of older yolk sac larvae (Nissling *et al.*, 1998; Probst *et al.*, 2006; Rideout *et al.*, 2005). The absence and reappearance of maternal effects on yolk sac larval size from hatch to older ages were

also supported by the observation of Blaxter & Hempel (1963) on various herring populations, in which the positive linear relationship between egg dry weight and yolk sac larval length became stronger some time after hatching. In marine fishes that lack a free-swimming yolk sac stage (i.e. hatching without a yolk sac), the correlation between egg size and size at hatch was strong and consistent (e.g. mouthbrooders Segers & Taborsky, 2011) and wolffish *Anarhichas* spp. (Hansen & Falk-Petersen, 2001)). Thus, it appeared that maternal effects on somatic body size were confounded with maternal effects on yolk sac size, which explains the low contribution of maternal effects on size-at-hatch in the present and some previous studies.

Temperature, in contrast to maternal effects, showed the opposite trend of contribution to differences in somatic body size, with stronger influence on size at hatch than on maximum size during the yolk stage. Indeed, temperature was the dominant effect (18–61%) influencing body size of newly hatched larvae, but its contribution dropped to 0–11% as maximum body size was reached. Other studies that followed yolk sac larvae until yolk depletion showed that yolk sac larvae reached similar maximum lengths at different incubation temperatures (in herring (Overnell, 1997), haddock *Melanogrammus aeglefinus* (Martell *et al.*, 2005), and summer flounder *Paralichthys dentatus* (Johns *et al.*, 1981)). This is supported by the review in Kamler (2008) on freshwater and marine fishes, which reported that a temperature effect was detected in over 90% of studies on length at hatch but reduced to 65% of the studies on length at yolk depletion. In other words, the influence of temperature on variance in somatic body size decreased as yolk sac larvae grew.

We found that yolk sac larvae at 7 and 13°C followed the same growth trajectory of somatic body to yolk sac area ratio, only with those at 13°C hatching at an earlier stage with a smaller body. Larvae at 13°C grew to obtain the same body to yolk sac area ratio and eventually the same maximum body area compared to larvae at 7°C. Comparing embryos incubated at different temperatures, those that hatched at a smaller size possessed larger yolk sacs than those that hatched at a larger size (Galloway *et al.*, 1998; Johns *et al.*, 1981; Mäkinen *et al.*, 2023). In Atlantic silverside, embryos which had been incubated at temperatures colder than 30°C even hatched without a yolk sac (Austin *et al.*, 1975). The phenomenon of different body size and yolk sac size at hatch across incubation temperatures was similar to the asynchronous hatching of the same batch of eggs across several days

(Geffen, 2002; Laurel *et al.*, 2008; Politis *et al.*, 2014). Geffen (2002) compared the growth of herring yolk sac larvae that had hatched naturally and those that were released from the egg shells. The released larvae were smaller but grew faster and reached the same length as those that had hatched naturally. Taken together, these results suggest that temperature influences the specific time point along the growth trajectory (i.e. specific body to yolk sac area ratio) at which larvae hatch.

Offspring incubated at 13°C hatched with smaller bodies and spent a relatively longer portion (7.6%) of the yolk period as post-hatch, free-swimming yolk sac larvae compared to those at 7°C, which spend more portion of time as unhatched embryos. We used the data in Busch (1996) to calculate the relative duration of egg and yolk sac larval stages and found that later in the season, when water temperature was warmer, the portion of yolk period as eggs tended to be shorter (i.e. hatched earlier with a smaller body). This may benefit offspring at the spawning ground since predators are more active at higher water temperatures which occur later in the season (Kotterba *et al.*, 2014). A longer relative time as free-swimming yolk sac larvae rather than benthic static eggs may allow offspring to escape predators.

In WBSS herring, the sizes of females, eggs, newly-hatched larvae and yolk sac larvae reaching maximum size all decreased from early- to mid- to late-seasons. The size at each stage, however, did not show a consistent correlation with one another (e.g. sizes of eggs versus newly-hatched larvae). We showed that the inconsistency was caused by different factors influencing the size at hatch and size at maximum during the yolk stage. At hatch, temperature explained the most variance in body size along with additional seasonal effects. Early-season cohorts hatched with a larger size than subsequent cohorts. Since females from earlier in the season experienced colder temperatures during oogenesis than females that spawned later, the seasonal effect on size at hatch may also be a temperature effect (Miller *et al.*, 1995). At maximum size during the yolk stage, all of the seasonal effects could be attributed to the effect of egg size. Early-season eggs were larger than mid- or late-season eggs and gave rise to larger larvae at yolk depletion, a pattern also observed in haddock yolk sac larvae (Rideout *et al.*, 2005). To extend on the present study, future research may examine the extent of size-selective mortality in first feeding larvae from different time periods of the season and link the surviving larvae to the egg trait and eventually to the characteristics of

females that produced them. Such information helps us to understand the fluctuations in larval survival and population dynamics.

# Chapter 4

# Patterns of size variation reveal advantage of a larger size in Atlantic herring *Clupea harengus* larvae

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**Keywords**

Body size, coefficient of variation, spawning timing, cohort

## Abstract

In marine fish early life stages, survival to recruitment is often correlated with fast growth and/or developmental rates. Although individuals with different sizes have different chances of survival, many studies focused on the mean of body size but neglected the variation around it. The present study examined the changes in the relative variation (coefficient of variation, CV) of both size-at-ages and size-at-stages for larvae from three stocks of Atlantic herring spawning in winter, (early-, mid- and late-) spring as well as autumn. The seasonal gradient corresponded to a decreasing trend in the sizes of eggs and yolk sac larvae. Larvae from colder seasons possessed a larger initial size and reached the target size for flexion at younger ages, which may be favourable for survival. The pattern of CV in size reflects critical periods in early life stages and can be used to assess growth and developmental status within and among stocks.

## 4.1 Introduction

Early life stages of marine fish encountered a high mortality rate where a larger size and/or a shorter duration at a vulnerable stage are beneficial to survival (Houde, 1987). Many field and laboratory studies have examined larval growth and development by quantifying the mean size of larvae at a given age (size-at-age; e.g. size at 30 days post-hatch) or a developmental stage (size-at-stage; e.g. size at metamorphosis). However, the change in mean size does not reveal the differences in growth and development among individuals nor the nature of mortality such as magnitude and direction of size selectivity. The far less-addressed (relative) variation around those mean values, on the other hand, provides evidence to those processes. For example, the study of Chambers *et al.* (1988) showed that the size at a given age varied two times more than the size at metamorphosis, implying a threshold size window for larvae to enter metamorphosis.

Atlantic herring *Clupea harengus* spawn in waves of distinctive groups which give rise to successive larval cohorts within the reproductive season. Herring tend to produce larger (smaller) eggs during colder (warmer) months (Huang *et al.*, 2022) and larval cohorts from different seasonal timing have been shown to contribute unequally to recruitment (e.g. Polte *et al.*, 2014). To understand the growth and development among different seasonal larval cohorts, we extended the approach of Chambers *et al.* (1988) from among individuals to among seasonal cohorts and followed the variation in both size-at-ages and size-at-stages of herring larvae along ontogeny. It is essential to know when a change in variation occurs in order to assess the nature and degree of selective mortality.

## 4.2 Materials and Methods

### 4.2.1 Size-at-ages

We used laboratory data from growth trials on herring larvae between the years 2007 and 2014, as well as 2019 and 2020 (yolk sac stage) done at the Elbe aquarium, University of Hamburg. Spawning adults were collected at the spawning grounds of each stock at the respective spawning season. Females ($n$ = 6–24 per trial) were stripped spawned and their eggs artificial fertilised by milt of multiple males. The embryos and subsequent larvae were reared in replicated 90-L tanks with a light regime representative of the season, i.e. light:dark

of 10:14, 14:14, and 12:12 h in winter (Downs), spring (western-Baltic-spring-spawning, WBSS) and autumn (Gulf of Riga herring), respectively. Eggs and larvae were kept at water temperatures between 5 to 15°C, at salinity similar to the respective spawning grounds, and provided with algae *Rhodomonas baltica* at 10,000 cells $mL^{-1}$, dinoflagellates *Oxyrrhis marina* at 1,000 cells $mL^{-1}$, copepods *Acartia tonsa* and later brine shrimp *Artemia salina* ad libitum. Further details on the fertilisation, incubation and rearing conditions are described in Moyano *et al.* (2016) For WBSS herring, the trials were classified into early-, mid- and late-spring according to the fertilisation day of the year (Huang *et al.*, 2022) (Table 4.1).

### 4.2.2 Size-at-stages

Size-at-stages data for WBSS and Downs herring were from Fischbach *et al.* (2023) and Joly *et al.* (2023), respectively. Stages specified for Downs larvae are converted to the same system described for WBSS herring (Fischbach *et al.*, 2023)

### 4.2.3 Statistical analysis

We calculated the coefficient of variation (CV = standard deviation/mean) to represent relative variation. CV is a dimensionless and unitless value that is independent of sampling size. We used a loess function to fit the changes in CV of size-at-ages and size-at-stages with increasing mean larval size. Analysis was done in R (version 4.0.3).

## 4.3 Results

Herring larvae examined in the laboratory were between 6.6 to 22.3 mm in standard length, from 0 to 714-degree-day old (temperature × day, °d; corresponding to 60-day) (Figure 4.1a) and spanning three developmental phases: yolk sac, dorsal fin development (pre-flexion) and caudal fin development (flexion) (Figure 4.1b, Fischbach *et al.*, 2023; Joly *et al.*, 2023). Sizes of newly-hatched larvae followed a seasonal gradient, with the largest in winter (Downs) and the smallest in late-spring (WBSS) larvae (Table 4.1).

Relative variation (CV) of size-at-ages initially declined to a minimum (0.055) at age 11–84°d and then gradually increased 2-fold to a maximum at age 220–643°d. After which, the CV of size-at-age tended to decrease again (Figure 4.1c). The age at which the minimum CV occurred was earlier in autumn and early-spring larvae and the latest in winter larvae (Table 4.1), with the corresponding larval lengths at those ages exhibiting an increasing trend from autumn to winter (7.6–11.8 mm). On the contrary, the maximum CV occurred the earliest in early-spring and the latest in late-spring larvae, consistent with the smallest and largest larval size at the corresponding ages (Table 4.1).

The mean length of larvae at the end of the yolk sac stage (stage 4; Figure 4.1b) approximately corresponded to the larval length where the minimum CV in size-at-age and the maximum CV in size-at-stage occurred (Figure 4.1c, Table 4.1). On the other hand, the mean length of larvae prior to flexion (stage 7.5) marked the size with the opposite trend, accompanied by the maximum CV in size-at-age and the minimum CV in size-at-stage.

**Table 4.1** Laboratory studies on larval growth. Temperatures are rearing temperatures in the laboratory, with the temperature groups: cold = 5–7°C, med = 10–12°C, warm = 13–15°C. Abbreviations: CV, coefficient of variation; GoR, Gulf of Riga; NA, not determined; Temp, temperature; WBSS, western Baltic spring-spawning.

| Stock | Season | Year | Temp (°C) | CV | | | Size (mm) | | | Age (degree-day) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Hatch | Min | Max | at hatch | at min CV | at max CV | at min CV | at max CV | prior flexion |
| **Downs** | Winter | 2011 | cold | 0.089 | 0.059 | 0.114 | 8.51 (0.17) | 11.8 | 16.8 | 84 | 337 | 337 |
| **WBSS** | Early-spring | 2014, 2020 | cold, warm | 0.059 | 0.058 | 0.139 | 8.07 (0.13) | 8.4 | 12.5 | 25 | 220 | 365 |
| | Mid-spring | 2009, 2012, 2019 | cold, med, warm | 0.050 | 0.050 | 0.135 | 7.31 (0.26) | 8.2 | 15.2 | 56 | 458 | 470 |
| | Late-spring | 2007, 2013, 2019 | cold, med, warm | 0.059 | 0.052 | 0.125 | 7.02 (0.14) | 8.2 | 22.3 | 76 | 643 | 477 |
| **GoR** | Autumn | 2010, 2011 | med | 0.061 | 0.055 | 0.113 | 7.60 (0.24) | 7.6 | 14.3 | 11 | 319 | NA |

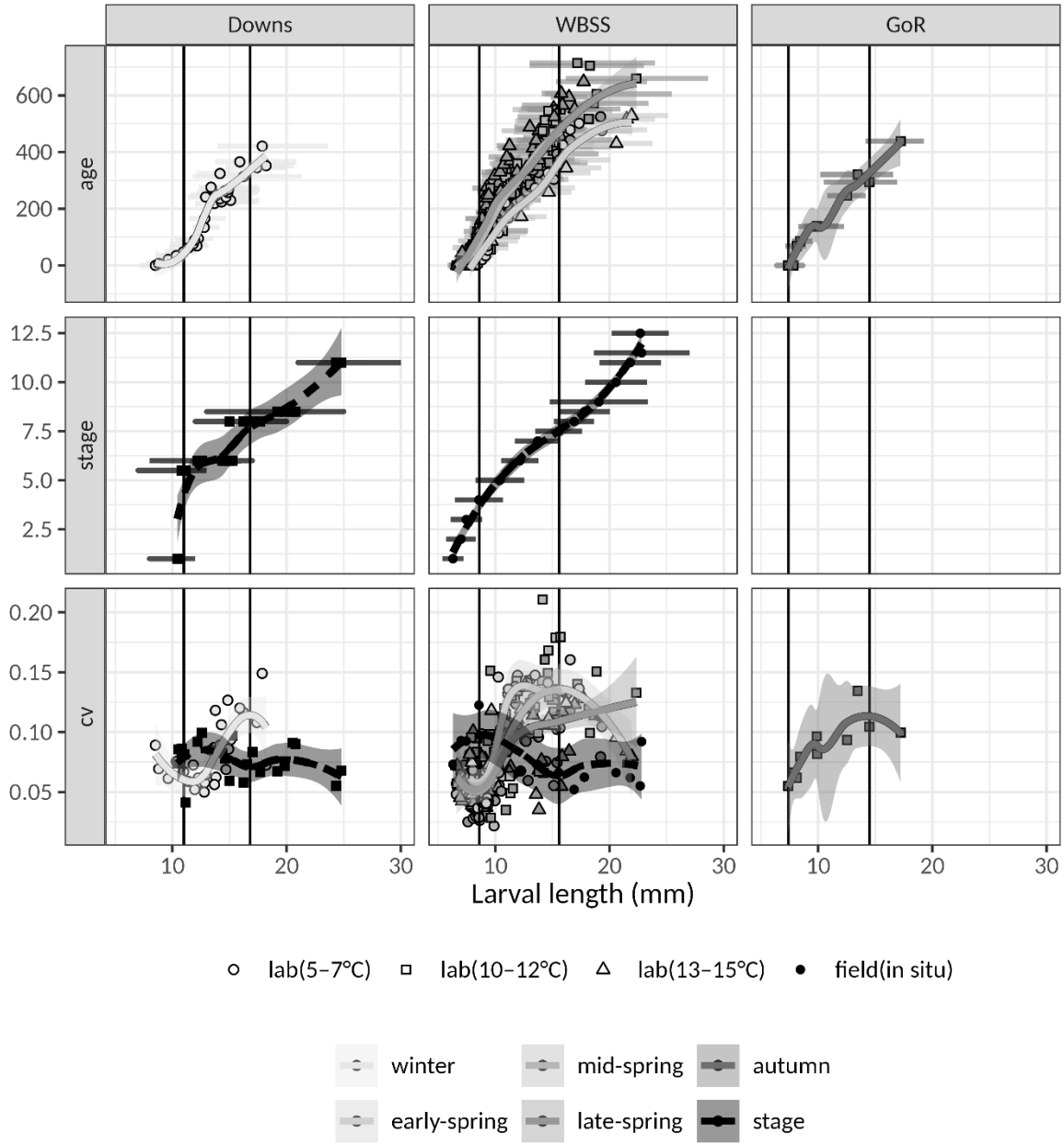


**Figure 4.1** Range in size-at-ages (a), range in size-at-stages (b) and CV of which (c) at respective mean larval length (mm). Vertical lines enclose the pre-flexion stage. Data are averaged among replicates for each trial.

## 4.4 Discussion

Analysis of the change in variation of body size helps to disclose the time when a change in growth rate or mortality among individuals occur, which may be critical periods for the early life stages of marine fish. As confirmed by the size-at-stage data, the patterns displayed by the (relative) variation in size-at-ages and size-at-stages were associated with the transitions

between developmental stages, where an increase or decrease in variation means a transition to the next developmental stage.

In size-at-stages, an initial increase and then decrease in CV to a minimum just prior to flexion indicated that, initially, larvae were more variable in size at each developmental stage but became more uniform in size by the start of the flexion stage. The decrease in CV was also reported by Chambers *et al.* (1988) for winter flounder *Pseudopleuronectes americanus*, in which the low CV at transitional stages indicated a target size at these developmental stages. A similar trend was found in the present study. The lowest CV in size of larvae occurred at the start of the flexion stage, agreeing with the interpretation of Chambers *et al.* (1988) that a threshold size was required for certain developmental stages. In herring, this is likely the flexion phase. The study of Gamble *et al.* (1985) revealed that the larvae of both autumn- and spring-spawning Clyde herring reached the same weight at flexion despite the former being only one-half the weight of the latter at hatch. Moreover, the duration of the final pre-flexion stage was greater than that of the start flexion stage in both autumn- (35% vs. 4%) and spring-spawning (15% vs. 5%) stocks. This means that although larvae were born with different initial sizes, they need to acquire the same size threshold in order to develop to the next stage (flexion).

An increase in the CV of size-at-age generally stems from the different growth rates among individuals, especially the relatively faster growth rates of larger larvae and/or faster growers. On the contrary, a decrease in the CV of size-at-age can be any combination of the following reasons: (1) slower growth rate of the larger individuals, (2) faster, compensatory growth rate of smaller individuals compared to larger ones and (3) mortality of smaller and/or larger individuals (size-selective mortality) (Chambers *et al.*, 1988). Several authors reported that mortality could occur even if larvae had fed, and those mortality events happened within certain periods, such as a few days after yolk sac depletion (Busch *et al.*, 1996; McGurk, 1984). For example, in Downs and Norwegian spring-spawning herring (two stocks with the largest initial size), 13 mm during the pre-flexion stage was defined as a critical size (Denis *et al.*, 2016; Fiksen & Folkvord, 1999). This marked the transition from an omnivorous towards a more carnivorous diet.

In Atlantic cod *Gadus morhua* and winter flounder, the maximum CV of size-at-age was ca 0.10 and remained relatively stable throughout the larval period (Chambers *et al.*, 1988)

compared to the CV in herring. Unlike herring, cod and flounder are already cannibalistic among larvae, which discourages large variation in size since small individuals are removed from the cohort by larger individuals. Therefore, the target size between transition stages may be a mechanism to prevent the variation in size among individuals from increasing beyond an asymptotic value. In conclusion, the present study shows that analysing the change in relative variation in size can help to identify critical periods where a change in growth rate or mortality within cohorts is likely to occur. With this information, one can assess the performance of certain cohorts or populations compared with those cohorts reared under controlled laboratory environments. When certain mechanisms can be identified to be responsible for the change in CV, we may use this to understand selective processes in the field.

# Chapter 5

# General Discussion

In northern European waters, marine fish populations have dramatically declined in size and shifted their productivity and distribution due to climate change and over-exploitation (Peck & Pinnegar, 2018). Rising spring temperatures have advanced the phenology of spring-spawning fish, leading to lower recruitment levels in numerous stocks over the past few decades. Understanding the patterns and factors that impact the reproductive success of fish helps to predict how fish populations will respond to ongoing ocean warming. This knowledge is critical for developing effective adaptation strategies that mitigate the social and ecological consequences of climate change.

The present thesis identified seasonal timing (early-, mid- and late-season) of spawning as a significant factor influencing progeny traits in Atlantic herring stocks (Figure 5.1). In western Baltic spring-spawning (WBSS) herring, progeny from early-season cohorts possessed larger body sizes compared to those from late-season cohorts, despite an earlier shift in spawning phenology driven by mild winters. However, the unexpectedly low survival rates of early-season larvae in the field suggests that extrinsic factors may exert a stronger impact than intrinsic differences, or the number of progeny, a factor not fully addressed in the present thesis, at different seasonal timings may also play a pivotal role in survival as body size does. While additional research is crucial to uncover the mechanism causing differences in cohort survival, the present thesis advocates for management strategies which aim at reducing fishing pressure and controlling extrinsic factors (e.g. nutrient loads to the nursery grounds) to aid in the recovery of the depleted WBSS herring stock.

The following content is organised into four sections. The first section examines the generality and the broader implications of the seasonal pattern revealed in the present thesis. The second section discusses the intrinsic and extrinsic mortality factors which contribute to variations in cohort survival. The third section highlights the methodological innovations and limitations of the present thesis that may help future studies. Finally, the fourth section discusses potential future research directions and management implications for the WBSS herring stock.

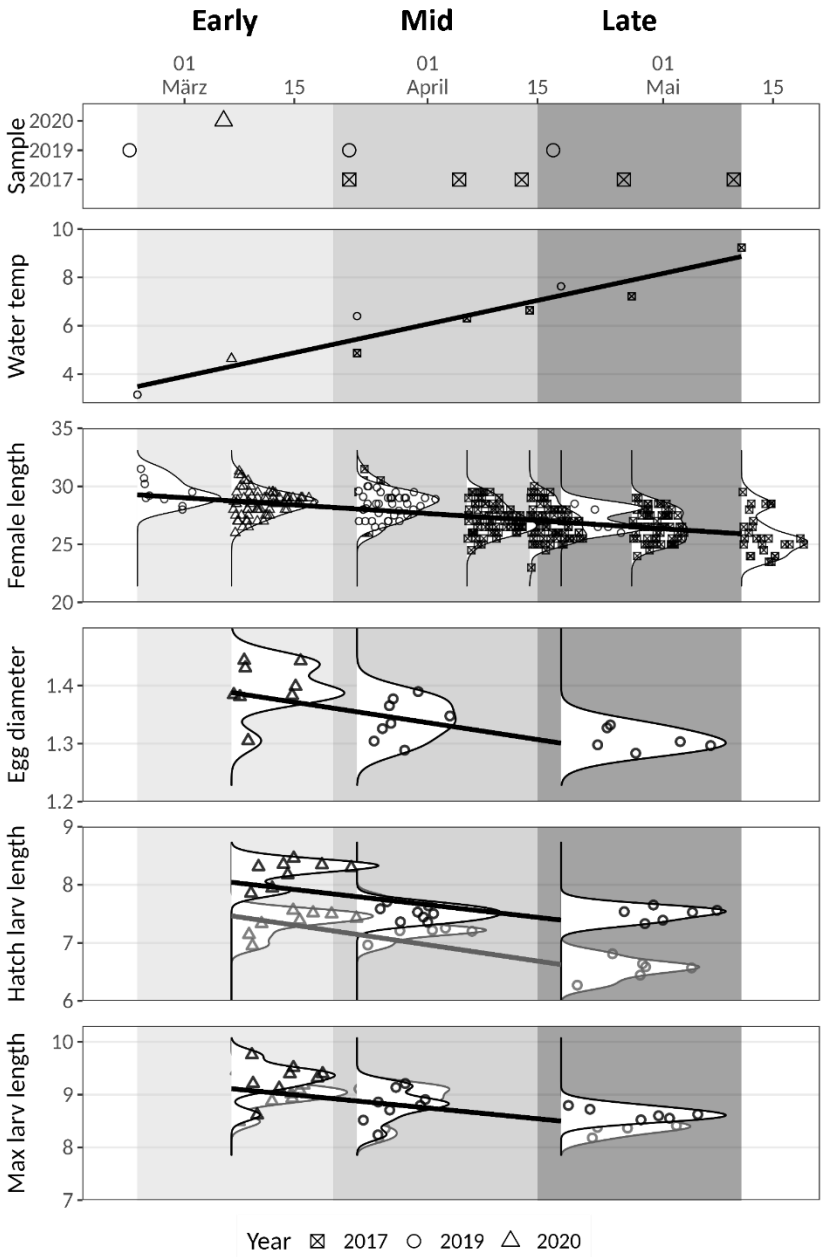


**Figure 5.1** Field sampling of Atlantic herring *Clupea harengus* spawners and *in situ* water temperatures (°C) in Greifswald Bay of the western Baltic Sea, along with the density graphs illustrating the seasonal decline in the size of females (cm) and their progeny (mm). For newly-hatched larvae (Hatch larv) and maximum-sized larvae during the yolk period (Max larv), results for temperature treatments at 7 (black) and 13°C (grey) are presented.

## 5.1 Generality of seasonal patterns in traits

### 5.1.1 Females

In Chapter 2, my co-authors and I reported that the mean length and age of spawning females decreased as the spawning season progressed. This pattern aligns with the observations in other spring-spawning herring stocks, including those in the Northwest

(Lambert, 1987) and Northeast Atlantic (Óskarsson *et al.*, 2002; Rajasilta, 2001), as well as in herring stocks that spawn in summer (Óskarsson & Taggart, 2009) and autumn (Lambert, 1987). The lower mean size of later-spawning females can be attributed to a higher percentage of younger females, who tend to spawn later than the lager or older conspecifics. A multi-year study of Norwegian spring-spawning herring revealed that earlier spawning was associated with a reduced percentage of recruit spawners within the spawning population (Husebø *et al.*, 2009). Specifically, when 20% of the spawners were recruits, progeny appeared ca. 10 days earlier than when 60% of the spawners were recruits. Research analysing the maturity stages of Central Baltic spring-spawning herring further supported this trend by showing that larger females were at more advanced maturity stages by early spring compared to smaller females (Bucholtz *et al.*, 2013). The body length of the females ranged from 17.5 to 25 cm, and this increase in length corresponded to a rise in the percentage of females reaching the final stage of oogenesis (late vitellogenesis) from 10 to 60%. This pattern could be explained by the observation that larger females allocated a relatively smaller proportion of their energy to growth and were more efficient in energy utilisation compared to smaller individuals (Folkvord *et al.*, 2014; Slotte, 1999a), allowing them to advance oogenesis faster.

### 5.1.2 Eggs

Similar to the mean sizes of females throughout the spawning season, the mean sizes of eggs produced by these cohorts also exhibited a decreasing trend (Chapter 2). The seasonal decline in both mean female sizes and mean egg sizes often leads to the conclusion that larger females produce larger eggs. However, this correlation is inconsistent within herring stocks, as indicated by various studies (e.g. Bradford & Stephenson, 1992; Hempel & Blaxter, 1967), with some supporting the correlation while others do not. Indeed, when comparing egg size and female size among individuals within specific cohorts (rather than the mean sizes across cohorts), no consistent trend was detected. Smaller females could even produce larger eggs than larger counterparts from the same cohort (Chapter 2). However, a clearer pattern emerged while comparing the eggs of similar-sized females across different cohorts. When female sizes were matched, those that spawned earlier consistently produced larger eggs than those that spawned later (Chapter 2, Figure 2.5). This suggests that factors other than female size significantly influence the egg size within stocks.

Chambers (1997) identified spawning temperature as a key factor influencing egg size and found that females spawning in colder months produced larger eggs than those spawning in warmer months, a pattern observed in various stocks of marine temperate fishes. The present thesis supports the conclusion of Chambers (1997), as the water temperature at the spawning ground increased linearly during the sampling period, while mean egg size decreased correspondingly. In contrast, autumn-spawning herring experience an opposite gradient of spawning temperatures (from warmer to colder) compared to spring-spawning herring (from colder to warmer). In autumn-spawning stocks, egg weight increased as the season progressed (Zijlstra, 1973). Consequently, larger females that spawned earlier in the autumn produced smaller eggs than their smaller counterparts that spawned later. This indicates an inverse trend: smaller females produce larger eggs.

The variation in egg size with seasonal temperatures is likely an adaptation to the environmental conditions that progeny will face later (Marshall *et al.*, 2008). In colder environments, prey availability is lower than in warmer ones, requiring that females provide more yolk per egg (Busch *et al.*, 1996). While both large and small females produce eggs of varying sizes in response to environmental factors, this suggests that maternal effects may be less important. However, another crucial trait in fish reproduction is fecundity. Several studies have compared the size and number of eggs produced by autumn- versus spring-spawning herring stocks (e.g. Damme *et al.*, 2009; Hempel & Blaxter, 1967). Both autumn- and spring-spawning herring initiated oogenesis around the same time in spring and undergo downregulation of egg number through resorption, a process called atresia (Damme *et al.*, 2009). The downregulation period was shorter in autumn-spawning herring compared to spring-spawning herring (dos Santos Schmidt *et al.*, 2017), resulting in a greater number of smaller eggs in the former. Nonetheless, within stocks, fecundity increased with female size regardless of spawning timing and season (Arula *et al.*, 2012; Óskarsson *et al.*, 2002; Zijlstra, 1973). For example, females of 24 cm in length achieved twice the potential fecundity compared to those of 20 cm, producing 40,000 eggs compared to 20,000 eggs in Central Baltic spring-spawning herring (Bucholtz *et al.*, 2013). In Northwest Atlantic herring, the fecundity of females of the size 30–36 cm increased linearly from 90,000 to 160,000 eggs in autumn-spawning females, and from 50,000 to 100,000 eggs in spring-spawning females (Bradford & Stephenson, 1992). Collectively, larger females of the spring-spawning stocks tended to

produce a higher number of larger eggs, whereas larger females of the autumn-spawning stocks produced a even higher number of smaller eggs earlier in the respective spawning seasons compared to their smaller counterparts within the same stocks. Given that larger females disproportionally contribute more to progeny production, the protection of these individuals within herring stocks remains an important conservation priority.

### 5.1.3 Yolk sac larvae

While larger females did not consistently produce larger eggs than smaller females, larger eggs indeed gave rise to larger larvae that reached the end of the yolk sac (endogenously feeding) stage (Chapter 3). As a result, the average size of post-yolk larvae also exhibited a similar decreasing trend to that of egg size as the season progressed. It is important to emphasize that while egg size was correlated with larval size at the end of the yolk sac stage, this correlation did not hold during the earlier phases of the yolk sac stage. Previous studies have shown that correlations between egg size and size-at-hatch (the beginning of the yolk sac larval stage) have been inconsistent and sometimes weak, as observed in herring (Høie *et al.*, 1999) and cod (Miller *et al.*, 1995). This implies a primarily indirect relationship between these two factors. In Chapter 3, my co-authors and I demonstrated that size-at-hatch was influenced by incubation temperature, which determined the developmental stage at which embryos would hatch. Herring embryos may either continue developing within the eggs or hatch as free-swimming yolk sac larvae (Geffen, 2002). At higher temperatures, herring larvae tend to hatch at shorter lengths, typically with a larger yolk sac compared to those incubated at lower temperatures (Peck *et al.*, 2012b). On the contrary, the sizes of larvae at the end of the yolk sac stage were not influenced by incubation temperatures but, instead, were linked to seasonal variations in egg size (Chapter 3). Although less frequently reported compared to size-at-hatch, larval size at or near yolk exhaustion provides a more precise estimate of size at first feeding, as exogenous feeding begins once the yolk sac is nearly depleted (Busch, 1996; Yúfera & Darias, 2007). Nonetheless, determining the larval size at yolk exhaustion is more challenging, and size-at-hatch continues to be more frequently used as a proxy for initial size. This can be addressed by either measuring the size of the eggs or assessing newly-hatched larvae reared at colder temperatures, which results in less residual yolk sac at hatch, or by measuring both yolk sac and body size of newly-hatched larvae.

**Table 5.1** Body size of females (cm) and their progeny (mm) within and among various Atlantic herring *Clupea harengus* stocks at different incubation temperatures (°C), organised by season and month. The yolk sac stage is divided into newly-hatched larvae (Hatch) and maximum-sized larvae during the yolk period (Max). An asterisk (*) denotes measurements of dry weight (mg/100 eggs).

| Stock | Temp | Sal | Female length | Egg diameter | Hatch length | Max length | Reference |
|---|---|---|---|---|---|---|---|
| N Baltic spring | | | | | 6–8 | | (Urho, 1992) |
| NE Baltic spring | 7; 12 | 5–6 | | 1.3 | 7.4; 6.7 | 8.1; 8.3 | (Ojaveer, 1981) |
| Gulf of Riga spring | 7; 12 | 5–6 | | 1.2 | 6.5; 6.5 | 7.4 | (Ojaveer, 1981) |
| C Baltic spring | 13 | 6 | | | 7.2 | | (Arrhenius & Hansson, 1996) |
| W Baltic early spring | 6–9 | 7–10 | | | 7.1–8.2 | | (Busch *et al.*, 1996) |
| W Baltic early Mar | 7; 13 | 7 | 28.7–31.3 | 1.31–1.44 | 8.2; 7.4 | 9.3; 9.0 | This thesis |
| W Baltic late Mar | 7; 13 | 7 | 27.7–29.9 | 1.29–1.39 | 7.5; 7.3 | 8.8; 8.8 | This thesis |
| W Baltic late spring | 9–11 | 7–10 | | | 5.4–6.9 | | (Busch *et al.*, 1996) |
| W Baltic Apr | 7; 13 | 7 | 26.0–28.5 | 1.28–1.33 | 7.5; 6.6 | 8.6; 8.4 | This thesis |
| W Baltic Apr | 12 | 14 | 28 | | 5.9–7.0 | | (Franke & Clemmesen, 2011) |
| W Baltic Apr | 8.5–10 | 17–19 | 26.1 | | 7.2 | | (Moyano *et al.*, 2016) |
| W Baltic Apr | 9–10 | 15–16 | 23.0 | | 7 | 8.7 | (Illing *et al.*, 2015) |
| W Baltic spring | 5–20 | 19 | 22.5 | | 7.5–5.5 | | (Peck *et al.*, 2012b) |
| W Baltic Apr–May | 8 | 15 | 21–30 | 13* | 6.0 | 6.7 | (Blaxter & Hempel, 1963) |
| W Baltic spring | 8.5–10 | | | | 6.9 | | (Yin & Blaxter, 1987) |
| Clyde Feb–Mar | 8 | 35 | 23–32 | 30* | 7.5 | 10.5 | (Blaxter & Hempel, 1963) |
| Clyde spring | 7–8 | | | 1.76 | 8.8 | | (Yin & Blaxter, 1987) |
| Clyde Mar | 7 | | | | 8.8–9.3 | | (Geffen, 2002) |
| Clyde May | 5–12 | | | | | 10.6–11.9 | (Overnell, 1997) |
| Norwegian Mar | 8 | 35 | 26–39 | 29–35* | 8.2 | 10.5 | (Blaxter & Hempel, 1963) |
| Norwegian Mar | 4; 8; 12 | | 32–38 | 1.59–1.61 | 9.9; 9.5; 8.6 | 12.6; 12.1; 11.6 | (Høie *et al.*, 1999) |
| Norwegian spring | 8; 10 | | | | 9.6; 8.0 | | (Johannessen *et al.*, 2000) |
| NE Baltic Autumn | 7; 12 | 5–6 | | 1.2 | 7.0; 7.1 | 8.4; 8.9 | (Ojaveer, 1981) |
| Buchan Aug–Sep | 8 | 35 | 25–32 | 16* | 7.0 | 8.4 | (Blaxter & Hempel, 1963) |
| Norwegian Sep | 9.8 | | 30–35 | | 8.3–8.5 | | (Bang *et al.*, 2006) |

**Table 5.1 (continued)**

| Stock | Temp | Sal | Female length | Egg diameter | Hatch length | Max length | Reference |
|---|---|---|---|---|---|---|---|
| Dogger Oct–Nov | 8 | 35 | 23–32 | 27* | 7.2 | 9.5 | (Blaxter & Hempel, 1963) |
| Downs Nov | 13.5–15 | 33 | 27 | | 8–9 | 9–10 | (Joly *et al.*, 2021) |
| Downs Nov–Dec | 8 | 35 | 22–31 | 37* | 7.5 | 10.7 | (Blaxter & Hempel, 1963) |

Since eggs produced in colder months tend to be larger, and egg size is a reliable predictor of initial larval size, we can expect a seasonal gradient in which larger larvae are produced from winter to early spring, and smaller larvae are produced from late spring to autumn. Chapter 4 supports this hypothesis, showing that larvae from winter and early spring not only had a larger initial size but also experienced a shorter pre-flexion stage compared to those from late spring and autumn (supporting H5, Chapter 1). A comparative review (Table 5.1) on various herring stocks reveals that, both within and among stocks, progeny produced during the colder months (winter to early spring) are larger than those produced in the warmer months (late spring to autumn).

### 5.1.4 Summary

The body sizes of spawning herring and their progeny, at least up to the point of yolk exhaustion, were all correlated with spawning timing but influenced by different factors. The observed seasonal decline in the size of spawning females was linked to intrinsic differences in energy allocation and efficiency. Meanwhile, the seasonal variations in egg size and size-at-hatch were associated with spawning temperatures and incubation temperatures, respectively (Supporting H1, Chapter 1 hypotheses). Finally, the difference in post-yolk larval size corresponded to the seasonal variations in egg size (Supporting H4).

The seasonal patterns indicate that environmental factors such as temperature interact with maternal effects to significantly influence progeny traits in herring. To further illustrate these relationships, a comparative review of patterns observed in marine fish species is presented in Table 5.2. With the rapid warming observed in recent years, it is critical to examine how early-season females respond. Over the past decades, many spring-spawning stocks have begun spawning earlier, allowing their embryos to continue experiencing relatively consistent temperatures. However, in extremely warm years such as 2020, the lack

of a cooling followed by a warming temperature cue left day length as the sole determining factor for spawning. The seasonal patterns identified in the present thesis provide a basis for distinguishing seasonal cohorts within herring stocks in the field. This framework can be used to establish fishing closure periods aimed at protecting cohorts that produce progeny with a higher likelihood of survival.

**Table 5.2** Correlations between progeny traits and the seasonal timing of spawning, maternal effects or temperature. An upward arrow (↑) signifies a positive correlation, a downward arrow (↓) denotes a negative correlation, and a dash (-) indicates that no effects were detected on the trait. Autumn-spawning stocks are labelled with brackets, while unlabelled stocks are spring-spawning. An asterisk (*) means the adults were captive-bred. Abbreviation: cond., condition; yolk (effected stage), yolk sac larvae.

| Species | Effect stage | Earlier in season | Larger female | Well-fed female | Larger egg | Lower temp | References |
|---|---|---|---|---|---|---|---|
| **Batch spawner** | | | | | | | |
| Plaice *Pleuronectes platessa* | yolk | | -age at first feed<br>-survival | | ↑yolk vol. at hatch<br>-age at first feed<br>-survival | | (Kennedy *et al.*, 2007) |
| Winter flounder *Pseudopleuronectes americanus* | female | -size<br>-age<br>-ovarian weight<br>-GSI | ↑ovarian weight<br>↑GSI | | | | (Buckley *et al.*, 1991) |
| Winter flounder | egg | ↑size<br>↓number | ↑size<br>↑number | | | | (Buckley *et al.*, 1991) |
| Atlantic cod* *Gadus morhua* | egg | ↑size (individual)<br>-size (pooled) | -size | ↑size | | | (Chambers & Waiwood, 1996) |
| Atlantic cod | yolk | | | | ↑time to yolk depletion<br>↑time until death<br>↑length at hatch | | (Pepin *et al.*, 1997) |
| Atlantic cod | yolk | | | | | -size-at-hatch<br>-first feeding | (Jordaan *et al.*, 2006) |
| Arctic cod *Boreogadus saida*, Alaska pollock *Gadus chalcogrammus* | larv | | | | | ↑condition at low food | (Koenker *et al.*, 2018) |
| Haddock *Melanogrammus aeglefinus* | female | ↑age | | | | | (Wright & Gibb, 2005) |
| Haddock* | egg | ↑size<br>-fertilisation rate<br>-hatch rate | | | | | (Rideout *et al.*, 2005) |
| Haddock | yolk | | | | | ↑embryo size<br>-size at post-yolk | (Martell *et al.*, 2005) |
| Haddock | larvae | ↑size | | | ↑size<br>↑resist starvation | | (Rideout *et al.*, 2005) |
| Atlantic mackerel *Scomber scombrus* | yolk | | | | | ↑survival | (Mendiola *et al.*, 2007) |
| Atlantic wolffish *Anarhichas lupus* (autumn) | female | ↑size | | | | | (Gunnarsson *et al.*, 2016) |

**Table 5.2 (continued)**

| Species | Effect stage | Earlier in season | Larger female | Well-fed female | Larger egg | Lower temp | References |
|---|---|---|---|---|---|---|---|
| **Total spawner** | | | | | | | |
| Capelin *Mallotus villosus* | yolk | | | | -size<br>-yolk duration | | (Chambers *et al.*, 1989) |
| Capelin | egg | | | | | ↓malformation | (Shadrin *et al.*, 2020) |
| Atlantic herring *Clupea harengus* | female | ↑size<br>↑age | | | | | (Lambert, 1987) |
| Atlantic herring | female | -size | | | | | (Rajasilta *et al.*, 1993) |
| Atlantic herring | female | ↑size<br>↑age | | | | | This thesis |
| Atlantic herring | egg | ↑size | | | | | (Rajasilta *et al.*, 1993) |
| Atlantic herring | egg | ↑size | | | | | This thesis |
| Atlantic herring | egg | ↓size | | | | | (Zijlstra, 1973) |
| Atlantic herring | yolk | ↑size at post-yolk | | | ↑size at post-yolk | | This thesis |
| Atlantic herring | yolk | ↑yolk at size | | | | | (Busch *et al.*, 1996) |
| Atlantic herring | yolk | ↓diameter of white muscle fibres | | | | | (Temple *et al.*, 2000) |
| Atlantic herring | yolk | | | | ↑yolk sac size<br>-size | | (Blaxter & Hempel, 1963) |
| Atlantic herring | yolk | | | | | -yolk efficiency | (Overnell, 1997) |
| Atlantic herring | yolk | | | | | -yolk efficiency | This thesis |
| Atlantic herring | larvae | ↑mix-feed duration | | | | | (Busch *et al.*, 1996) |
| Atlantic herring | larvae | ↑growth rate | | | | | (Fey, 2001) |
| Atlantic herring | larvae | | | | | ↓number of inner muscle fibres<br>↑diameter of inner muscle fibres<br>-total cross-sectional area of muscles | (Vieira & Johnston, 1992) |
| Atlantic herring | juvenile | | | | | -total cross-sectional area of muscles<br>↓number of red and white fibres per myotome | (Johnston *et al.*, 1998) |

## 5.2 Mortality

### 5.2.1 Intrinsic differences

The present thesis investigated mortality resulting from intrinsic differences among herring eggs and yolk sac larvae. In controlled laboratory environments, intrinsic sources of mortality can be identified and distinguished from extrinsic sources of mortality (e.g. predation). Rather than exhibiting a constant mortality rate across all progeny ages, the present thesis identified "critical periods" during which elevated mortality rates occurred, a concept initially proposed by Hjort (1914). Outside these critical periods, mortality tended to be relatively low. The first critical period was observed at ca. two days post fertilisation during

the mid-blastula stage (Chapter 2). This phase marks an essential transition for embryos as they shift from relying on maternal mRNA to synthesising their own mRNA (Hill & Johnston, 1997). Mortality at this stage varied substantially among eggs from different females, ranging 2–17% (Chapter 2), and was found to be unrelated to incubation temperature (inconclusive H2, Chapter 1), female size or egg size (inconclusive H3). The second critical period occurred during hatching, where mortality primarily resulted from morphological deformities and unsuccessful hatching from the eggs (Chapter 3). Mortality rate could reach up to 11% and showed only a partial correlation with the mortality observed during the blastula stage.

During the transition from endogenous (yolk) to exogenous feeding in post-yolk larvae, a pattern of initially low mortality, followed by elevated mortality, and then a return to low mortality has been reported in young Pacific and Atlantic herring larvae (Busch *et al.*, 1996; McGurk, 1984). Both studies emphasised that starvation was not the sole cause of mortality since larvae might still die even when provided with adequate prey. In the experiments of McGurk (1984), by the time all larvae in the starving group had perished, mortality rates in the half-starved and fully fed groups reached up to 90% and 50%, respectively. Busch *et al.* (1996) further examined whether the larvae were actually feeding on the provided prey. They found that while 60–90% of larvae successfully consumed food, less than half of these larvae survived beyond the mass mortality period. This phenomenon, characterised by high mortality rates despite constant food supply and the absence of predators, has also been documented in other temperate marine fish species such as European sardine *Sardina pilchardus* (Garrido *et al.*, 2015). This phase constitutes the third critical period in the early life stages of herring.

Since the first and second critical periods were associated with developmental failures — specifically, the acquisition of functional structures rather than mere growth (biomass accumulation) — it is probable that the third critical period was similarly linked to a developmental bottleneck. Pacific herring *Clupea pallasii* larvae around the time of mass mortality possessed a length of 11–12 mm (McGurk, 1984), a size comparable to the critical size windows of 12–13 mm identified for Norwegian spring-spawning and Downs Atlantic herring larvae (Denis *et al.*, 2016; Fiksen & Folkvord, 1999). Fiksen & Folkvord (1999) used model simulations to show that larvae reaching this critical size might have perished not due

to a lack of food, but because of halted development. Furthermore, this critical size signified the transition from feeding on smaller prey items to larger ones, as well as a shift from an omnivorous diet to a more carnivorous one in Downs herring (Denis *et al.*, 2016). This change suggests that different prey types may be necessary for proper development. If larvae have failed to ingest enough food or not obtained the essential nutrients required for growth, those that cannot develop into the subsequent life stage are unlikely to survive.

The critical period following first-feeding occurred during the early pre-flexion phase (Fischbach *et al.*, 2023; Joly *et al.*, 2023). During this period, larvae at each developmental stage became increasingly uniform in size, especially compared to variability seen during hatching (Chapter 4). Fiksen & Folkvord (1999) proposed that larvae with greater yolk reserves were able to overcome developmental bottleneck more effectively than those relying heavily on external food sources. Indeed, larvae with a larger initial size were found to reach the threshold size for flexion more quickly than their smaller conspecifics (Chapter 4; supporting H4, Chapter 1). Thus, intrinsic differences continue to play a significant role in the developmental success of herring larvae.

### 5.2.2 Extrinsic factors

Unlike intrinsic sources of mortality that led to developmental "critical periods," most extrinsic factors affected eggs and larvae across all ages and stages. Given that herring produce demersal eggs, predation mortality can be substantial, particularly in shallow coastal areas. For instance, a previous study estimated that predation mortality for WBSS herring eggs could reach up to 11% within a week at depths of <14m (Kotterba *et al.*, 2014). In contrast, predation mortality for Norwegian spring-spawning herring eggs was estimated at only 4% over a period of 50 days at a depth >70 m (Toresen, 1991). Another key factor influencing egg mortality is the spawning substrate. Herring stocks that spawn in shallow regions typically deposit eggs on macroalgae, while those that spawn in deeper areas lay their eggs on hard structures such as rocks, gravels and sand (Frost & Diele, 2022). Certain algal substrates, especially red algae and filamentous algae, could lead to mass mortality rates of 30 to 100%, likely due to toxins produced by the algae (Nordheim *et al.*, 2020; Rajasilta *et al.*, 1993).

Numerous studies have highlighted the significance of seasonal match-mismatch dynamics in determining survival rates during the larval stage (Ferreira *et al.*, 2023). In contrast, larval density was found to have no significant impact on growth rates (Hakala *et al.*, 2003). This finding suggests that competition among larvae may be minimal, pointing instead to the importance of spatial and temporal alignment with available prey. As noted in the previous section, intrinsic differences may contribute to the mortality of feeding larvae that failed to progress to subsequent developmental stage (Fiksen & Folkvord, 1999). In this context, the mortality attributed to match-mismatch dynamics may be overestimated if some of which is due to these intrinsic differences. Evidence indicated that seasonal match-mismatch dynamics accounted for 23% of the variance in survival to recruitment (Fridolfsson *et al.*, 2023), showing the need for further investigation into the importance of intrinsic differences.

Biotic extrinsic factors can impact herring progeny across all developmental stages, but their effects tend to vary with the timing of the season. For instance, predator activity typically increases later in the spring as water temperature rises (Kotterba *et al.*, 2014). Likewise, mortality associated with algal blooms appears to peak later in the season (Nordheim *et al.*, 2020). In addition, prey concentrations are expected to be higher later in the season compared to early in the season, further influencing larval survival.

Physical processes during the egg stage can lead to direct mortality. For example, a single storm event resulted in the loss of nearly 30% of herring eggs (Moll *et al.*, 2018). Abiotic extrinsic factors also interact with biotic factors to shape herring survival. Herring eggs spawned in deeper waters had a greater chance of survival if their dispersal to nursery area coincided with times when predator populations were less active (Slotte *et al.*, 2019). In the western Baltic Sea, eutrophication caused by river runoff has stimulated the growth of filamentous algae and unstable microalgal blooms, which can create oxygen-depleted zones and affect zooplankton populations (Munkes, 2005). These changes in the environment may further complicate the survival prospects of herring eggs and larvae.

## 5.3 Innovations and limitations

The present thesis introduced three methodological innovations that facilitated the simultaneous examination of maternal, temperature and seasonal effects in herring. First, in

Chapters 2 and 3, a single recirculating system was employed for both temperature treatments (7 and 13°C). This design ensured that temperature remained the sole environmental variable, thereby eliminating the influence of other factors such as salinity. In addition, the recirculating system helped maintain the water quality of the egg chambers. The substantial water volume in the system also provided an effective buffer for water treatment. Second, the present thesis involved extensive individual-level measurements of both eggs (total $n$ = 18,602) and yolk sac larvae (total $n$ = 7,102). By employing image processing techniques, individual eggs were accurately identified, and a particle detection tool enabled precise measurements of all eggs on each plate (Appendix Method A2). For yolk sac larvae, macro-processing procedures were developed to automatically measure the total length, body area, and body depth from images after processing (Appendix Method A3). Third, in Chapter 3, a modified Gompertz model was developed to describe the decline in body depth and area of larvae following yolk depletion (Appendix Table A3.3). Unlike body length, both body depth and area showed significant decreases after yolk depletion and, to the best of our knowledge, no previous studies have focused on these traits in yolk sac herring larvae.

A major limitation of the present thesis was that the number of eggs was not quantified. Marine fish typically spawn a large number of eggs to ensure some of them will survive, and egg number at the cohort level is expected to be critical for recruitment success. Another limitation was that only one trial was conducted for each seasonal. Nevertheless, a pilot study conducted during the early season of 2019 supported the findings from the trial performed in early season of 2020.

## 5.4 Outlook

The abundance of the western Baltic spring-spawning herring has been in continuous decline for the past two decades. This stock once yielded the highest catches of any species in the region with nearly 200,000 tonnes in 1992, but catches dropped to ca. 10% of the aforementioned amount in 2020 (Froese *et al.*, 2022; ICES, 2022). A key factor contributing to this decline was the trend of decreasing recruitment of young fish, which reached a record low in 2020 (ICES, 2022). Field surveys conducted over these two decades have revealed that ca. 60% of the variation in recruitment numbers could be explained by the abundance of early

larvae. Specifically, a higher abundance of early larvae led to greater recruitment of juveniles (Polte *et al.*, 2014). While the majority of surviving fish originated from the latter half of the spawning season, this has been insufficient to rebuild the stock (Polte *et al.*, 2014).

Given the urgent need to understand the mechanisms underlying the unequal contributions among cohorts, the present thesis assessed the mortality rates and characteristics of eggs and larvae from the early, middle and late phases of the spawning season for WBSS herring stock. Survival rates were high across all cohorts until the end of the yolk sac stage, suggesting that the eggs produced by different cohorts shared similar quality attributes. However, it was evident that eggs from the cohort spawning earlier in the season were larger than those produced by subsequent cohorts.

From a stock management perspective, the goal is to implement fishery closures that protect the groups responsible for producing the largest number of surviving young in order to replenish the stock (van Overzee & Rijnsdorp, 2015). To achieve this, it is essential to quantify (1) the number of progeny produced by each cohort and (2) the percentage of these progeny that survive to later life stages. Some cohorts may produce offspring with higher survival rates, leading to a disproportionally greater contribution of survivors (Griffiths *et al.*, 2024; Trippel, 1999). Thus, it is important to assess both (1) and (2) in conjunction.

Regarding (1), it would be beneficial to assess the fecundity of early-, mid- and late-season females in relation to various body traits (e.g. size, condition factor *K*, gonadosomatic index), which can be evaluated through annual field surveys. This correlation could then be used to monitor the changes in fecundity over multiple years. For (2), the intrinsic mortality rate of progeny from different maternal origins could be investigated in the laboratory. For example, to further examine the impact of inter-female variation in egg size on progeny survival at later stages, a staining technique can be applied to the otolith of progeny during the egg stage. These otoliths can be extracted at a later life stage and analysed for the presence or absence of a stain under a fluorescent microscope. This method allows researchers to distinguish between progeny from two different groups while rearing them together. By staining the progeny from one group (e.g. progeny of a female producing larger eggs) and leaving the progeny from the other group (e.g. offspring of a female producing smaller eggs) unstained, researchers can identify the two groups at later life stages. It is crucial to disentangle intrinsic source of mortality from extrinsic ones in a controlled

laboratory setting to gain a better understanding of the mechanisms that influence survival and recruitment.

Although the early-season cohort produced larger eggs — providing advantages for larvae in terms of feeding and predator evasion — their consistently low contribution over several years implies that either their initial abundance is insufficient (Griffiths *et al.*, 2024), and/or they experience high mortality(Peck *et al.*, 2012a), which were related to the points discussed above for (1) and (2)

In addition to the WBSS herring stock, the population dynamics of the autumn-spawning (WBAS) herring stock from the same region merit consideration. Historically, the WBAS was the dominant stock over WBSS herring prior to the 1960s, after which the catch from the autumn-spawning stock became negligible (reviewed in von Dorrien *et al.*, 2013). A reverse trend has been reported for herring stocks from two other regions. In the west of Scotland, the spring-spawning stock dominated until the 1950s, but after decades of dominance by the autumn-spawning stock, the spring-spawning stock reemerged in large numbers at the spawning ground in the springs of 2018 and 2019 (Frost & Diele, 2022). In eastern Newfoundland, the spring-spawning stock accounted for 90% of the catch until the 2000s, when the autumn-spawning stock became the predominant population (Wilson *et al.*, 2018). This shift from spring- to autumn-spawning stocks has been linked to changes in the phenology of the major larval prey, which also transitioned from a spring bloom to an autumn bloom (Wilson *et al.*, 2018). To better understand these dynamics, it is important to examine prey availability in the nursery grounds across different seasons, as this may provide insight into the changes in local production.

The ICES has been recommending a zero catch limit for the WBSS stock since 2019 (ICES, 2019). Despite this advice, fishing continues under quotas set by management and primarily relies on cohorts that spawn early in the season. In light of the stock's low status and the threats posed by both exploitation and climate change, it is imperative to eliminate fishing pressure immediately and refrain from any catch until the stock has recovered (Froese *et al.*, 2022; ICES, 2019, 2022). In terms of the extrinsic factors affecting the spawning and nursery grounds, nutrient inflow is an important factor for egg survival and is a manageable influence. Nutrient inputs should be reduced to prevent large phytoplankton blooms, which compete with macroalgae (the spawning substrate for WBSS herring) and may lead to

unstable copepod production in the region. With climate change, a decline in stock production is anticipated. Thus, management must minimize anthropogenic pressures on the already vulnerable stock to ensure that fishing can resume sustainably once the stock has had the opportunity to recover.

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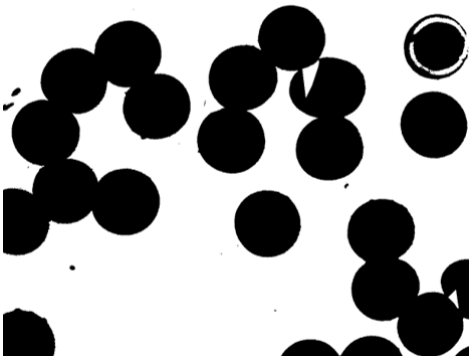
# Appendix: Methods

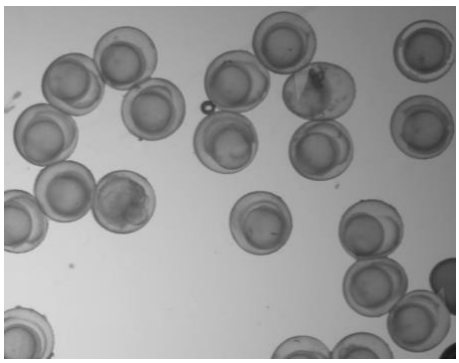
The present thesis used ImageJ software (version 1.52, Wayne Rasband, NIMH, USA) to perform automatic measurements of the morphometric traits of herring eggs and larvae. The image processing procedures outlined here are also applicable to other organisms from which morphometric traits can be estimated through image analysis.

## A2 Eggs: area

1. Create binary (black and white) images of the eggs, with the eggs representing in black and the background in white.

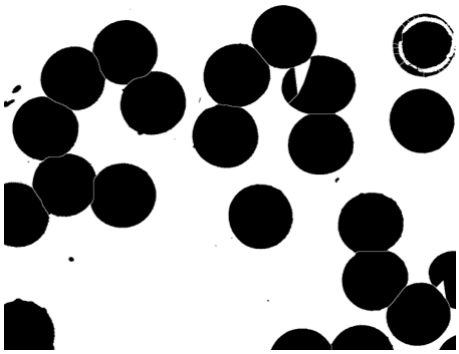


2. (Optional) Apply an overlay of the original colour photo of the eggs onto the binary image to identify and distinguish unwanted areas or objects (e.g. unfertilised eggs, broken eggs).

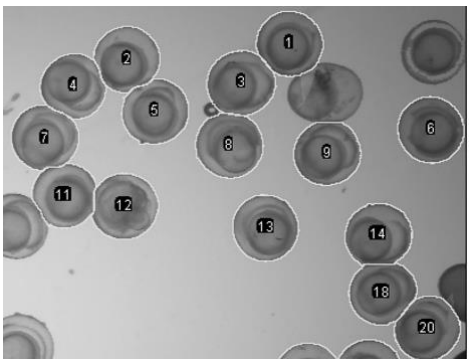


3. Remove any extraneous objects or background elements that should not be included in the measurements and fill any holes present in the eggs (for example, by using the "wand" selection tool followed by the "fill" function).

4. Use the “watershed” tool to separate the eggs from one another.

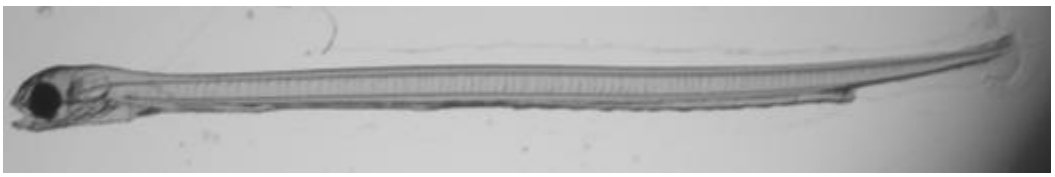


5. Set the scale and use “analyze particles” to estimate the areas of individual eggs.




## A3 Yolk sac larvae: body area and depth

1. Create binary (black and white) images of individual yolk sac larvae, with larval body in black and the background in white.
2. (Optional) Overlay the original colour photo of the larva onto the binary image to help identify unwanted areas or background (e.g. the finfold area).



3. Remove any background elements or portions of the body that should not be included in the measurements and fill any holes present in the larvae (for instance, by using the “wand” selection tool followed by the “fill” function).

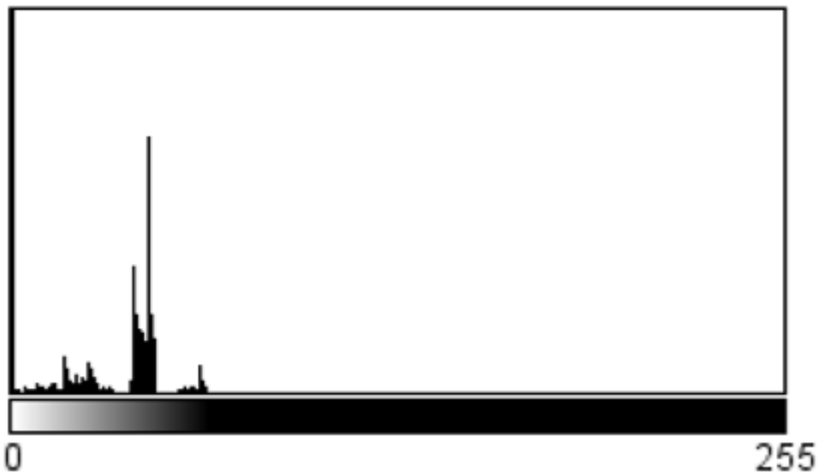
4. Set the scale and then select “measure” to obtain the body area.

5. Generate a distance map of the larval body, which visually represents the distance (in pixel) from any pixel on the body to its outer outline. The midline of the body, being the point farthest from the outer edge, will exhibit the highest value in the map.

6. Create a skeleton image of the body to illustrate the position of the midline.



7. Combine the distance map (step 5) with the skeleton image (step 6) to derive the distance from any pixel along the midline of the body to the outer outline, which represents half of the body depth. Use the x and y coordinates of the midline to determine the body depth at any specific location (e.g. at the midpoint).
8. Set the scale and use the ratio relative to pixels to calculate the body depth. For example, a value of 45 pixels, when divided by the scale of 209.375 pixels/mm and then multiplied by two, gives a body depth of 0.430 mm.
9. (Optional) Construct a histogram from the data obtained in step 7 to summarise the frequency of each estimated distance across the entire body. This illustrates the minimum, maximum and mode of larval body depth. Additionally, the total number of observations (pixels) will provide an estimate of the larval length.



# Appendix: Tables

**Appendix Table A2.1** Size rank of females within each spawning time and individual total length ($L_T$), total wet mass ($M_W$), Fulton's condition factor ($K$) and age. Size rank is based on $L_T$ from largest to smallest and, if the same $L_T$, on $M_W$. ND: not determined.

| Spawning time | Size rank | $L_T$ (cm) | $M_W$ (g) | $K$ | Age (years) |
|---|---|---|---|---|---|
| Early | 1 | 31.3 | 248 | 0.81 | 7 |
| | 2 | 29.5 | 219 | 0.85 | 9 |
| | 3 | 29.4 | 196 | 0.77 | 8 |
| | 4 | 29.1 | 194 | 0.79 | 7 |
| | 5 | 29.0 | 199 | 0.82 | 7 |
| | 6 | 28.8 | 178 | 0.75 | 6 |
| | 7 | 28.7 | 184 | 0.78 | 6 |
| | 8 | 28.7 | 182 | 0.77 | 6 |
| Middle | 1 | 29.9 | 201 | 0.75 | 5 |
| | 2 | 29.6 | 202 | 0.78 | 7 |
| | 3 | 29.1 | 189 | 0.77 | 4 |
| | 4 | 28.9 | 201 | 0.83 | 4 |
| | 5 | 28.3 | 173 | 0.76 | 6 |
| | 6 | 28.1 | 182 | 0.82 | 6 |
| | 7 | 27.9 | 175 | 0.81 | 6 |
| | 8 | 27.7 | 171 | 0.80 | 6 |
| Late | 1 | 28.5 | 181 | 0.78 | ND |
| | 2 | 28.0 | 158 | 0.72 | ND |
| | 3 | 28.0 | 146 | 0.67 | ND |
| | 4 | 26.5 | 149 | 0.80 | ND |
| | 5 | 26.5 | 145 | 0.78 | ND |
| | 6 | 26.0 | 132 | 0.75 | ND |

**Appendix Table A2.2** Summary of the three best linear mixed-effect models (LMM) on data including age (data from late in the season were excluded because of missing values). LMMs examine whether *Clupea harengus* egg area, fertilisation success and mortality at blastula were influenced by spawning time and/or female traits (and/or egg area and/or incubation temperature if included). Models were fitted by maximum likelihood to compare conditional Akaike information criterion (AICc) and then the best models were refitted by restricted maximum likelihood (REML) for estimates. Abbreviations: TIME.L, linear trend of spawning time; $L_T$, total length; $K$, Fulton's condition factor; AREA, mean egg area; $T_I$, incubation temperature; ID, individual female. ID:plate denotes nested random effect of plates within individual females. Values are estimated coefficients (±SE) for fixed effects and variance (in ±SD) for random intercepts. A dash indicates the effect was not included in the full model while an estimate of zero denotes the effect was not selected (assumed to be zero). Significant codes: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

| | Fixed effects | | | | | | | Random effects | | | Marginal/ conditional $R^2$ | Model comparison | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intercept | TIME.L | $L_T$ | $K$ | Age | AREA | $T_I$ | (1\|ID) | (1\|ID: plate) | Residual | | *df* | AICc | weight |
| **Egg area** | −0.382 (0.956) | −0.065 (0.031) | 0.037 (0.026) | 0.999 (0.708) | 0 | – | – | (0.082) | (0.019) | (0.061) | 0.324/0.765 | 7 | −29326.60 | 0.164 |
| | 0.705 (0.581) | −0.081 (0.030) * | 0 | 0.971 (0.733) | 0 | – | – | (0.084) | (0.019) | (0.061) | 0.274/0.759 | 6 | −29326.18 | 0.133 |
| | 1.473 (0.022) *** | −0.083 (0.031) * | 0 | 0 | 0 | – | – | (0.087) | (0.019) | (0.061) | 0.225/0.751 | 5 | −29326.15 | 0.132 |
| **Fertiliz. success** | 0.421 (0.596) | 0.052 (0.024) | 0.031 (0.021) | 0 | 0 | 0 | – | (0.063) | – | (0.040) | 0.191/0.765 | 5 | −404.80 | 0.134 |
| | 1.307 (0.017) *** | 0.038 (0.024) | 0 | 0 | 0 | 0 | – | (0.065) | – | (0.040) | 0.110/0.757 | 4 | −404.45 | 0.112 |
| | 1.307 (0.018) *** | 0 | 0 | 0 | 0 | 0 | – | (0.069) | – | (0.040) | 0/0.747 | 3 | −403.86 | 0.084 |
| **Mortality at blastula** | 0.116 (0.012) *** | 0 | 0 | 0 | 0 | 0 | 0 | (0.044) | – | (0.052) | 0/0.423 | 3 | −358.07 | 0.111 |
| | 0.116 (0.012) *** | −0.016 (0.017) | 0 | 0 | 0 | 0 | 0 | (0.045) | – | (0.052) | 0.028/0.440 | 4 | −356.97 | 0.064 |
| | 0.112 (0.013) *** | 0 | 0 | 0 | 0 | 0 | 0.008 (0.009) | (0.044) | – | (0.052) | 0.004/0.424 | 4 | −356.72 | 0.056 |

**Appendix Table A3.1** Sample size of *Clupea harengus* larvae for morphometric measurements. The number of larvae measured per female at each sampling point at 7 or 13°C is presented in mean number (±SE) among chambers ($n$ = 2–4). Deformed larvae were excluded. If the number of larvae measured was fewer than three within any chamber, these values at the respective sampling point were excluded. Abbreviation: ND, not determined.

| | **7°C** | | | | **13°C** | | | |
|---|---|---|---|---|---|---|---|---|
| | **At hatch** | | **Post-hatch** | | **At hatch** | | **Post-hatch** | |
| **Female no.(rank)** | ***n*** | **larvae per chamber** | ***n*** | **larvae per chamber** | ***n*** | **larvae per chamber** | ***n*** | **larvae per chamber** |
| *Early-season* | | | | | | | | |
| **1 (1)** | 4 | 9(0) | 3 | 12(1) + 12(2) + 12(1) + 8(1) + 10(1) + 6(3) | 4 | 12(0) | 3 | 10(0) + 11(1) + 9(1) + 11(1) + 6(3) |
| **2 (4)** | 4 | 11(1) | 3 | 10(1) + 11(0) + 11(1) + 7(1) + 10(1) + 5(1) | 4 | 13(1) | 3 | 10(4) + 11(0) + 9(1) + 13(2) + 10(0) |
| **3 (5)** | 4 | 11(1) | 3 | 11(1) + 10(1) + 12(1) + 8(1) + 12(1) + 7(0) | 4 | 12(1) | 3 | 10(0) + 11(1) + 9(1) + 11(1) + 9(2) |
| **4 (6)** | 4 | 12(1) | 3 | 11(1) + 10(1) + 11(2) + 7(2) + 11(1) + 7(1) | 4 | 10(1) | 3 | 11(1) + 10(1) + 11(4) + 11(1) + 7(2) |
| **5 (8)** | 4 | 11(1) | 3 | 12(2) + 8(1) + 9(1) + 9(1) + 11(1) + 4(1) | 4 | 11(1) | 3 | 11(1) + 10(1) + 9(1) + 10(1) + 8(2) |
| **6 (10)** | 4 | 13(1) | 3 | 11(1) + 10(0) + 10(0) + 7(1) + 11(0) + 9(1) | 4 | 12(1) | 3 | 10(1) + 10(0) + 7(2) + 9(0) + 9(1) |
| **7 (11)** | 4 | 11(0) | 3 | 9(0) + 10(0) + 11(0) + 8(2) + 11(0) + 6(2) | 4 | 16(3) | 3 | 10(1) + 8(2) + 8(1) + 11(1) + 10(0) |
| **8 (12)** | 4 | 11(1) | 3 | 10(1) + 11(1) + 11(1) + 8(1) + 11(1) + 7(2) | 4 | 10(2) | 3 | 11(0) + 11(1) + 9(1) + 13(3) + 7(2) |
| *Mid-season* | | | | | | | | |
| **9 (2)** | 4 | 9(1) | 2 | 10(0) + 14(2) + 10(0) + 10(0) + 12(2) + 10(0) | 4 | 5(0) | 2 | 9(1) + 8(2) + 9(1) + 10(0) |
| **10 (3)** | 4 | 11(3) | 2 | 10(1) + 10(1) + 10(0) + 11(0) + 11(0) + 10(0) | 4 | 4(1) | 2 | 9(0) + 8(2) + 5(2) + 3(0) |
| **11 (7)** | 4 | 12(2) | 2 | 10(1) + 13(1) + 10(1) + 11(1) + 12(0) + 10(1) | 3 | 4(0) | 2 | 11(1) + 9(1) + 8(3) + 8(4) |
| **12 (9)** | 4 | 12(1) | 2 | 9(0) + 13(1) + 10(0) + 10(1) + 11(1) + 10(1) | 4 | 3(1) | 2 | 9(1) + 11(2) + 10(0) + 7(1) |
| **13 (14)** | 4 | 10(1) | 2 | 13(3) + 12(1) + 10(0) + 11(1) + 10(1) + 10(0) | 4 | 5(2) | 2 | 7(3) + 12(ND)[a] + 8(4) + 6(ND) |
| **14 (15)** | 4 | 10(2) | 2 | 10(0) + 11(1) + 11(1) + 11(2) +12(1) + 7(1) | 3 | 6(1) | 2 | 9(1) + 9(1) + 10(0) + 11(1) |
| **15 (18)** | 4 | 10(2) | 2 | 10(0) + 12(1) + 10(0) + 13(2) + 8(3) + 6(3) | 3 | 5(0) | 2 | 11(0) + 8(1) + 8(3) + 4(1) |
| **16 (19)** | 4 | 11(1) | 2 | 7(2) + 9(1) + 10(0) + 11(1) + 12(3) + 5(1) | 4 | 5(0) | 2 | 10(1) + 9(2) + 9(2) + 10(ND) |

**Appendix Table A3.1** (continued)

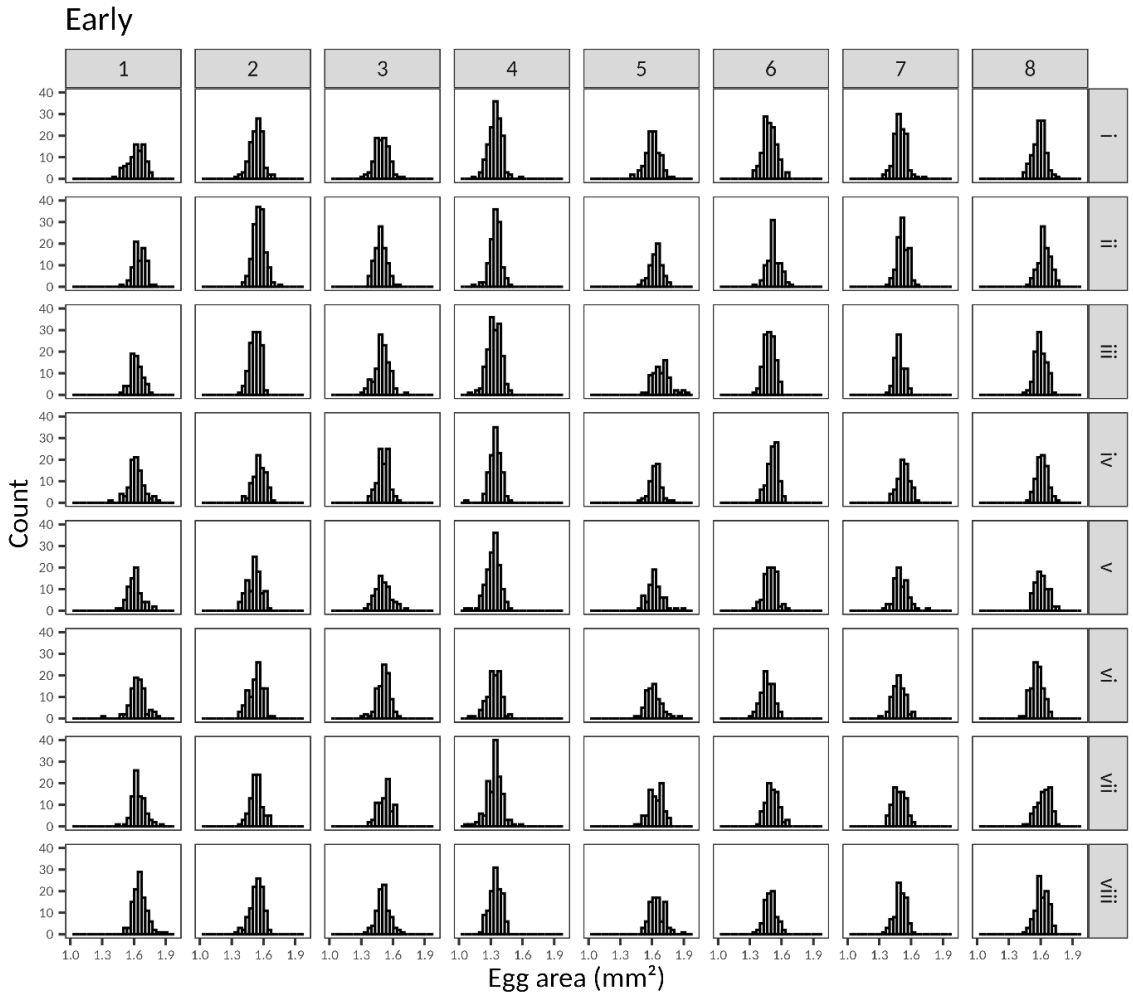
| | 7°C | | | | 13°C | | | |
|---|---|---|---|---|---|---|---|---|
| | At hatch | | Post-hatch | | At hatch | | Post-hatch | |
| Female no.(rank) | *n* | larvae per chamber | *n* | larvae per chamber | *n* | larvae per chamber | *n* | larvae per chamber |
| *Late-season* | | | | | | | | |
| **17 (13)** | 4 | 11(0) | 3 | 7(1) + 7(1) + 8(0) + 8(0) + 7(1) | 4 | 15(2) | 2 | 11(1) + 9(0) + 10(0) + 11(1) +12(1) |
| **18 (16)** | 4 | 12(1) | 3 | 8(1) + 7(1) + 7(1) + 7(1) + 5(1) | 4 | 14(1) | 2 | 12(1) + 10(1) + 11(1) + 10(0) + 11(1) |
| **19 (17)** | 4 | 11(1) | 3 | 6(0) + 8(0) + 7(0) + 7(1) + 5(1) | 4 | 12(1) | 2 | 11(1) + 11(1) + 12(2) + 10(1) + 7(2) |
| **20 (20)** | 4 | 10(1) | 3 | 7(1) + 8(0) + 6(0) + 6(1) + 4(0) | 4 | 10(0) | 2 | 8(1) + 10(1) + 10(1) + 10(1) + 10(0) |
| **21 (21)** | 3 | 11(1) | 3 | 7(0) + 6(0) + 6(1) + 8(1) + 7(1) | 4 | 12(1) | 2 | 10(1) + 11(1) + 10(0) + 10(0) + 10(1) |
| **22 (22)** | 4 | 12(1) | 3 | 8(1) + 6(1) + 6(0) + 7(1) + 8(2) | 4 | 12(1) | 2 | 11(1) + 12(0) + 11(1) + 10(0) + 10(0) |

[a] SE not determined because values were from one single chamber after exclusion of deformed larvae and data fewer than three.

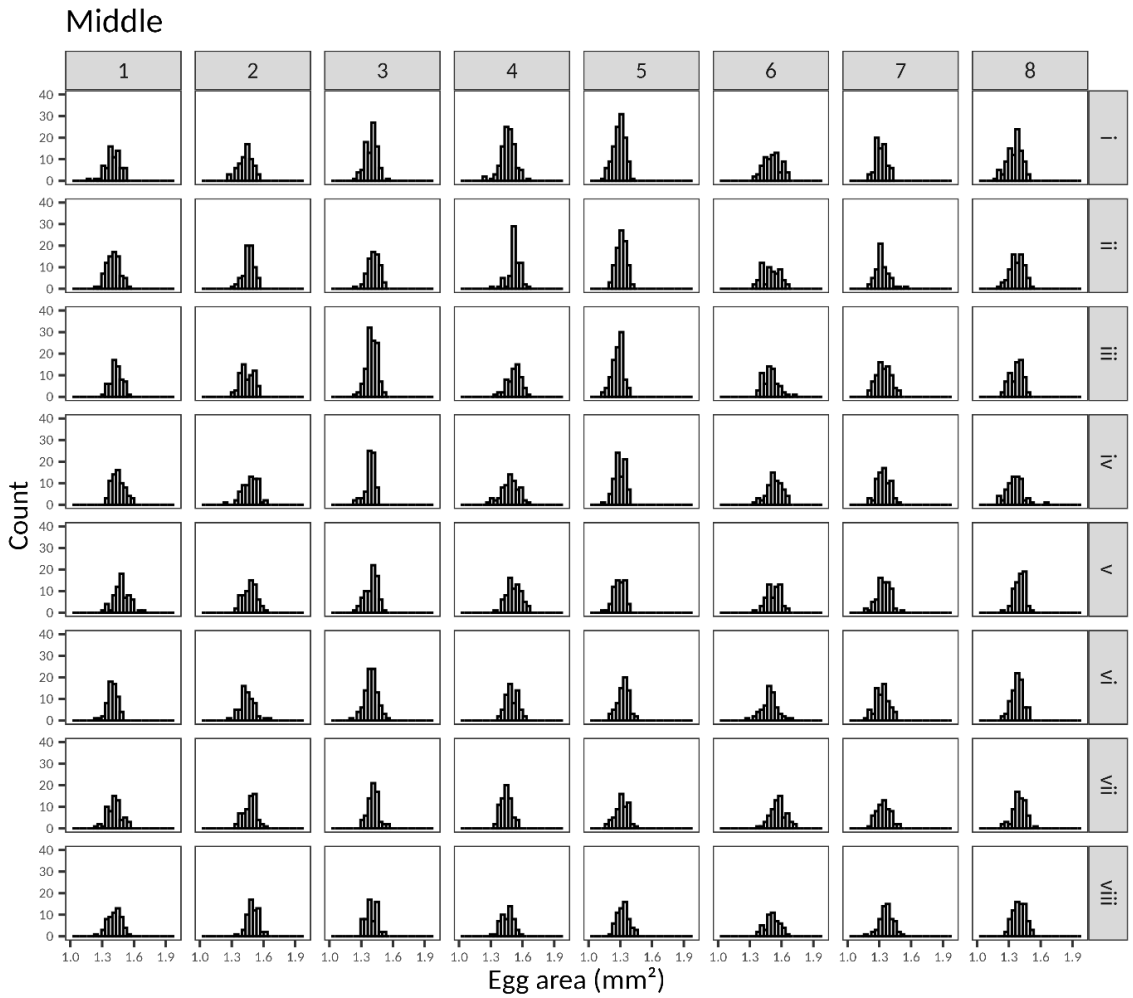
**Appendix Table A3.2** Parameter estimates of the models describing changes in notochord length, body depth, somatic body area, and yolk sac area with age.

| | Range | Mean (SD) |
|---|---|---|
| **Yolk sac area** | $\boldsymbol{Y_t = Ae^{-e^{-\beta(T_I - t)}}}$ | |
| $A$ | 0.307–0.649 | 0.447 (0.072) |
| $\beta$ | 0.054–0.280 | 0.100 (0.040) |
| $T_I$ | 20.9–38.6 | 28.2 (5.3) |
| **Notochord length** | $\boldsymbol{L_t = \alpha e^{\gamma(1-e^{(-\beta t)})}}$ | |
| $\alpha$ | 6.27–8.46 | 7.45 (0.51) |
| $\beta$ | 0.093–0.288 | 0.176 (0.050) |
| $\gamma$ | 0.026–0.113 | 0.057 (0.013) |
| **Body depth** | $\boldsymbol{M_t = \alpha e^{\gamma(1-e^{(-\beta t)} - \delta t)}}$ | |
| $\alpha$ | 0.227–0.389 | 0.327 (0.033) |
| $\beta$ | 0.066–3.438 | 0.460 (0.592) |
| $\gamma$ | 0.006–0.594 | 0.083 (0.086) |
| $\delta$ | 0.001–0.017 | 0.003 (0.003) |
| **Somatic body area** | $\boldsymbol{M_t = \alpha e^{\gamma(1-e^{(-\beta t)} - \delta t)}}$ | |
| $\alpha$ | 1.76–2.81 | 2.21 (0.27) |
| $\beta$ | 0.25–6.62 | 1.19 (1.41) |
| $\gamma$ | 0.006–0.108 | 0.035 (0.020) |
| $\delta$ | 0–0.025 | 0.006 (0.006) |

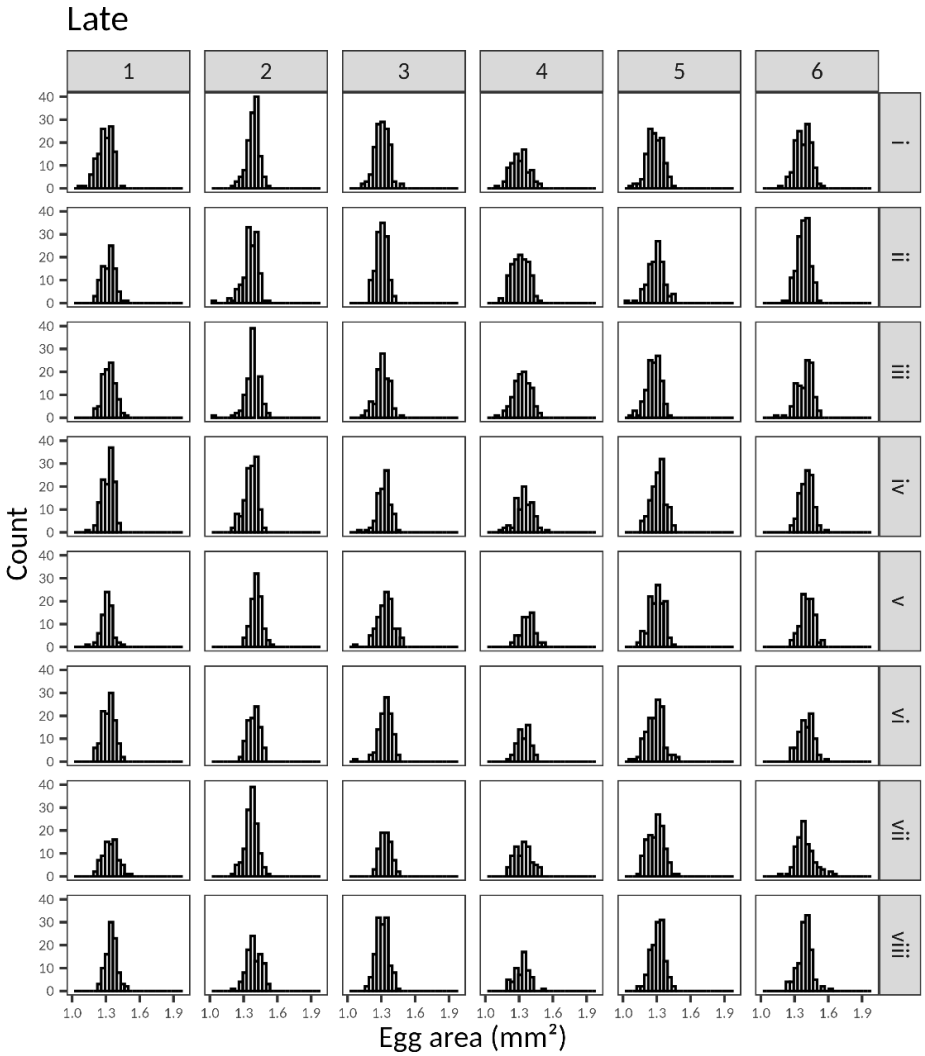
# Appendix: Figures



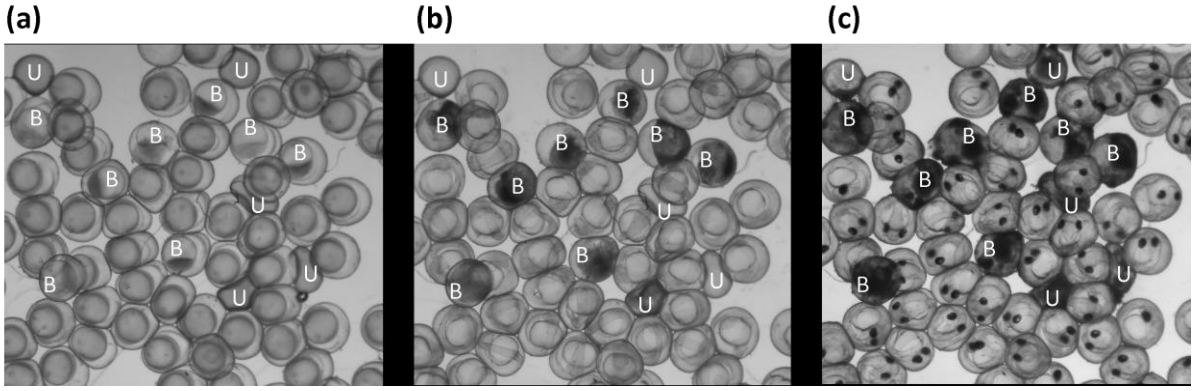

**Appendix Figure A2.1** Frequency histograms of egg area measured per plate for *Clupea harengus* females early in the season. Bars display the counts of the values within the respective bins. The columns from 1 to 8 represent female rank (Appendix Table A2.1) while the rows from i to viii indicate water baths in which eggs incubation chambers were placed.



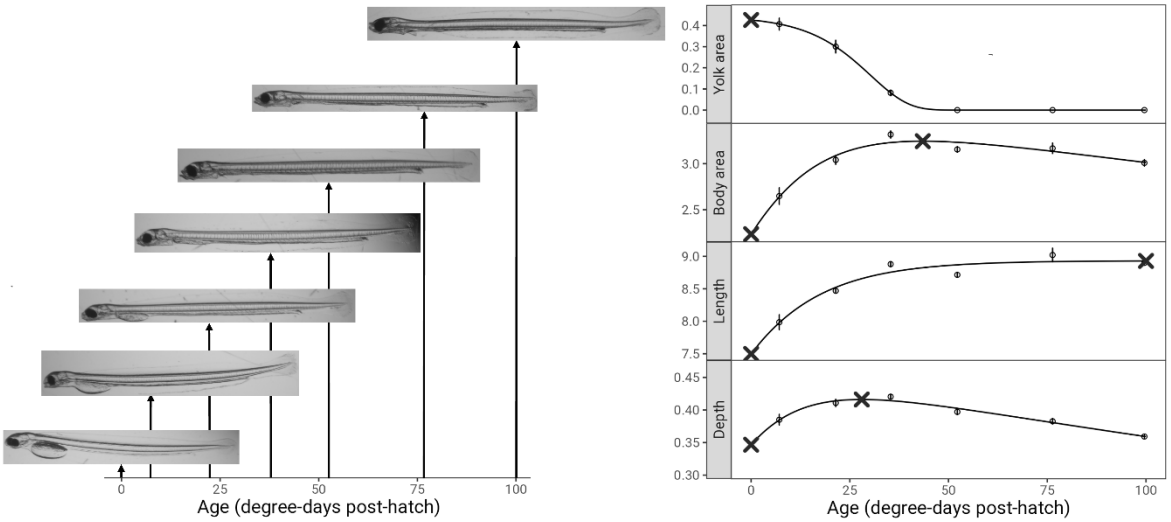

**Appendix Figure A2.2** Frequency histograms of egg area measured per plate for *Clupea harengus* females middle in the season. Bars display the counts of the values within the respective bins. The columns from 1 to 8 represent female rank (Appendix Table A2.1) while the rows from i to viii indicate water baths in which eggs incubation chambers were placed.



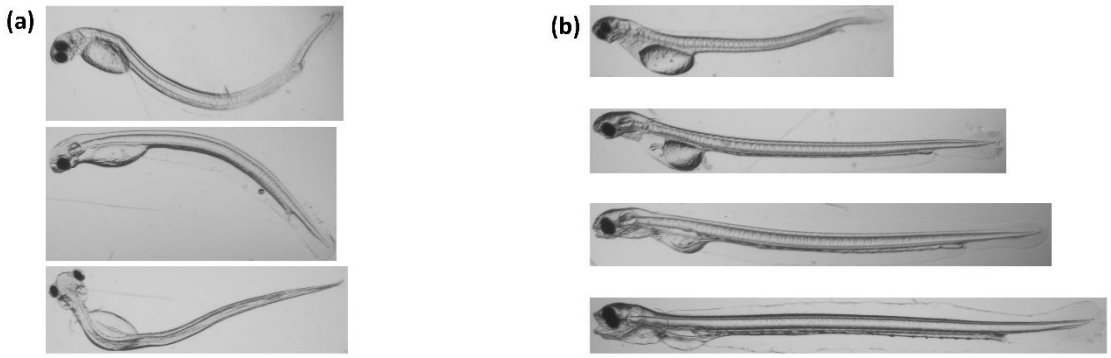

**Appendix Figure A2.3** Frequency histograms of egg area measured per plate for *Clupea harengus* females late in the season. Bars display the counts of the values within the respective bins. The columns from 1 to 6 represent female rank (Appendix Table A2.1) while the rows from i to viii indicate water baths in which eggs incubation chambers were placed.




**Appendix Figure A2.4** Atlantic herring *Clupea harengus* eggs incubated at 13°C at (a) one day post fertilisation, (b) three days post fertilisation, and (c) five days post fertilisation (the eye pigmentation stage). Unfertilised (U) and fertilised eggs that halted development during the mid-blastula stage (B) are labelled.




**Appendix Figure A3.1** Changes in yolk sac area, body area, total length and body depth of Atlantic herring *Clupea harengus* yolk sac larvae from 0 to 100 degree-days post-hatch.




**Appendix Figure A3.2** Deformities observed in Atlantic herring *Clupea harengus* yolk sac larvae, including (a) curved bodies or (b) yolk sac edema.

# Individual scientific contributions

## Abbreviations

A.T.H., Amy. T. Huang; K.A., Katharina Alter; M.A.P., Myron A. Peck; P.P., Patrick Polte; M.M., Marta Moyano; B.I., Björn Illing.

## Chapter 2

**Disentangling seasonal from maternal effects on egg characteristics in western Baltic spring-spawning herring *Clupea harengus***
Published in *Journal of Fish Biology*
Conceptualization and methodology: A.T.H., K.A., M.A.P.;
Resources: P.P., M.A.P.;
Field sampling: A.T.H., K.A., P.P.;
Investigation and experiments: A.T.H., K.A.;
Data analysis: A.T.H.;
Writing of the original draft: A.T.H.;
Review and revise of the manuscript: A.T.H., K.A., P.P., M.A.P.

## Chapter 3

**Maternal and seasonal effects on larval traits of Atlantic herring *Clupea harengus***
Submitted to *Journal of Experimental Marine Biology and Ecology*
Conceptualization and methodology: A.T.H., K.A., M.A.P.;
Investigation and experiments: A.T.H., K.A.;
Resources: P.P., M.A.P.;
Data analysis: A.T.H.;
Writing of the original draft: A.T.H.;
Review and revise of the manuscript: A.T.H., K.A., P.P., M.A.P.

## Chapter 4

**Patterns of size variation reveal advantage of a larger size in Atlantic herring *Clupea harengus* larvae**
Manuscript draft
Conceptualization and methodology: A.T.H., M.M., M.A.P.;
Investigation and experiments: M.M., B.I.;
Resources: M.A.P.;
Data analysis: A.T.H.;
Writing of the original draft: A.T.H.

**Supervisors' signatures** 19.03.2024

Prof. Dr. Myron A. Peck

Prof. Dr. Christian Möllmann

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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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Amy Tzu-Yu Huang

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Amy Tzu-Yu Huang