

**Influence of temperature and salinity on sprat (*Sprattus sprattus*) eggs and  
yolk sac larvae from contrasting environments**



Dissertation  
zur Erlangung des Doktorgrades  
der Mathematisch-Naturwissenschaftlichen Fakultät  
der Christian-Albrechts-Universität  
zu Kiel

vorgelegt von

Christoph Petereit

Kiel, 2009

Referent/in: Prof. Dr. D. Schnack

Korreferent/in: Prof. Dr. R. Hanel

Tag der mündlichen Prüfung: Kiel, den 27.01.2009

Zum Druck genehmigt: Kiel,

Der Dekan



## I Summary

The present thesis investigated the effects of manipulated temperature (range from 5-19°C) and salinity (range from 5-37.2) levels on survival, egg buoyancy, growth, developmental durations and important morphological traits of eggs and non feeding yolk sac stages of sprat (*Sprattus sprattus*) originating from two populations from contrasting environments. Experiments were performed with Baltic Sea sprat (brackish) and Adriatic Sea sprat (fully marine) populations and compared to data for the North Sea (English Channel, marine) sprat population extracted from literature data. The aims of the present study were to analyse and parameterize the reaction of the above mentioned traits to different abiotic levels on population basis and to study inter-population similarities and differences.

Results of Chapters 2 and 3 showed significant negative correlations of developmental durations and temperature increase in all populations. Egg survival in the Baltic was characterized by upper and lower thermal thresholds but no such limits were detected in Adriatic sprat egg survival suggesting high thermal plasticity for this population. Taking the slopes of egg developmental rates as a proxy for thermal sensitivity for each sprat population significant results revealed distinctive inter-population differences. Baltic Sea sprat showed cold adaptation which was potentially related to the low ambient temperature levels in the water layers where sprat eggs occur. Based on available literature this result of cold adaptation in Baltic sprat seems to be so far unique within Clupeiformes.

Contrary to a latitudinal gradient, Adriatic sprat showed intermediate thermal sensitivity compared to North Sea (English Channel) sprat. This was explained by sprats' boreal origin in the Adriatic Sea and the resulting spawning time in winter, the season with lowest water temperatures. Sprat spawning season in the North Sea is

temporally more extended towards summer and, therefore, more variable and warmer leading to an expected warm adaptation of this population.

Chapters 4 and 5 analyzed the effects of different salinity conditions on sprat egg and non feeding larval stages from the Baltic and the Adriatic Sea. As has become evident from experiments with the Baltic population, egg buoyancy was significantly negatively influenced by increased fertilization/incubation salinity. This implies that salinity conditions during the spawning season appear to be most relevant for modification of the buoyancy of eggs and yolk sac larvae determining their vertical habitat and consequently survival success of these early life stages. Buoyancy measurements of field caught Baltic sprat eggs in 2007 and 2008 showed annual as well as seasonal differences in egg specific gravity which were potentially associated with changes in adult sprat vertical distribution. Adriatic sprat eggs' neutral buoyancy was achieved at much higher salinity conditions compared to Baltic sprat eggs.

Different salinity levels did not influence egg developmental duration from fertilization to hatch in both populations. Egg survival showed an increasing tendency to a maximum hatching success between salinities of 14 - 18 in the Baltic Sea, whereas survival in Adriatic Sea sprat eggs increased with elevated salinity level with a maximum at 37.2. For both populations, salinity of 5 restricted complete egg development. Larval development was not restricted by salinity levels in the Baltic population.

But although embryos developed into viable larvae at salinities  $\geq 10 - 25$  in the Adriatic population, a significant contribution of these larvae to recruitment would not be probable, since a large proportion of the larvae either failed in emerging from the egg shell or showed deformations. However, under normal conditions salinity values exceed these in

the North Adriatic Sea. For salinities higher than 30, a high plasticity in development and survival has been observed, with a maximum larval survival at ambient (37.2) salinity conditions.

The time to eye pigmentation and isochronous mouth gap opening was taken as a proxy for first feeding. This duration was highly negatively correlated with increasing temperature but not influenced by salinity levels and was slightly earlier in the Adriatic sprat compared to Baltic sprat. Adriatic sprat larvae were found to generally have a shorter 'Window of Opportunity' (WOO), which describes the potential time to establish successful first feeding, compared to Baltic Sea sprat larvae. The WOO in Adriatic sprat larvae was shifted to higher temperatures compared to the Baltic larvae.

As water temperature is predicted to increase in the future, it will potentially reduce the WOO of the larvae from both populations. But Adriatic sprat larvae could possibly be effected more seriously than the Baltic larvae, since a e.g. 4°C temperature increase could possibly lead to a 50% reduction, which equals only a three to four day long WOO compared to over one week duration in Baltic larvae. To judge the relative importance for the final year class success, additional research on later larval and juvenile life stages is required since these life stages have been shown to be critical in other sprat populations.

This thesis provides new information on temperature and salinity influenced survival and developmental durations of non feeding early life stages of sprat from different populations. The parameterized results may now be implemented and applied in recruitment studies and individual based models (IBMs).

## II ZUSAMMENFASSUNG

Die vorliegende Arbeit befasst sich mit dem Einfluss von manipulierten Temperaturniveaus (Bereiche von 5-19°C) und Salzgehaltsniveaus (Bereiche von 5-37.2) auf die Überlebensrate, Ei-Schwebefähigkeit, Wachstum und Entwicklungsdauer sowie auf wichtige morphometrische Merkmale bei den Eiern und den noch nicht fressenden Dottersack-Larven-Stadien der Sprotte (*Sprattus sprattus*). Die Experimente wurden mit Populationen der Ostsee Sprotte aus dem Bornholm Becken (Brackwasser) und der Adriatischen Sprotte (salin bis hypersalin) aus dem Golf von Trieste durchgeführt, demnach aus Gebieten mit deutlich unterschiedlicher Hydrographie. Als Vergleichswerte konnten bereits publizierte Daten von Nordsee Sprotten (Englischer Kanal) herangezogen werden. Die Ziele der vorliegenden Arbeit waren zum einen die Analyse und Parametrisierung der beobachteten Reaktionen und Merkmale auf die verschiedenen veränderten abiotischen Einflüsse innerhalb einer Population und zum anderen die vergleichende Analyse zwischen den Populationen.

Die Ergebnisse aus den Kapiteln 2 und 3 zeigten signifikant negative Korrelationen der Entwicklungsdauer mit Temperaturerhöhung bei allen untersuchten Populationen. Das Sprottei-Überleben in der Ostsee Population wies ein unteres und ein oberes Temperaturlimit auf, wohingegen solche Limitationen nicht im untersuchten Temperaturbereich für die Adriatischen Sprotteier gefunden werden konnten, was auf eine erhöhte thermische Plastizität dieser Population schließen lässt. Auf Basis des Steigungskoeffizienten der Ei-Entwicklungsrates als ein Maß für die Temperatur-Sensitivität, konnten signifikante Unterschiede zwischen den Populationen bestimmt werden. Die Ostsee Sprott Population zeigte eine Kalt-Adaptation, was mit den niedrigen Wasser-

temperaturen innerhalb des Reproduktionsvolumens innerhalb der Wassersäule erklärt wurde. Die Adriatische Sprotte zeigte eine eher generelle, nicht spezialisierte Adaptierung, was sich mit der borealen Abstammung der Sprotte erläutern ließe, die sich unter anderem durch eine Laichzeit während der Periode mit kältester Wassertemperatur auszeichnet. Eine Warm-Adaptierung war hingegen für die Nordsee Sprotten Population gefunden worden, was durch eine längere Laichzeit mit durchschnittlich wärmerer und auch stärker schwankender Temperatur erklärt wurde.

Diese Studie beschreibt anhand des Beispiels der Sprotte Adaptierungsmuster die im Zusammenhang mit speziellen hydrographischen und phenologischen Besonderheiten zu stehen scheinen und nicht mit einer erhöhten Temperatur-Sensitivität von Populationen in einem geographischen Gradienten.

Die Inhalte in den Kapiteln 4 und 5 beschäftigten sich mit den Effekten von unterschiedlichen Salzgehaltsniveaus auf die Ei und die Dottersack-Phase bei der Ostsee Sprotte und der Adriatischen Sprotte. In Experimenten mit der Ostsee Sprotte konnte ein signifikant negativer Einfluss von erhöhter Befruchtungs- und Inkubationssalinität auf die Ei-Schwebefähigkeit festgestellt werden. Dieses weist darauf hin, dass die Salzgehaltsbedingungen zur Laichzeit relevant für die Modifikation der Ei-Schwebefähigkeit sind und damit eine wichtige Rolle für die Tiefenverteilung und somit den Überlebenserfolg von Eiern und Dottersack-Larven spielen könnten. Messungen der Ei-Schwebefähigkeit im Bornholm Becken in den Jahren 2007 und 2008 zeigten sowohl jährliche als auch jahreszeitliche Unterschiede, die potentiell mit unterschiedlichen Vertikalverteilungen der ablaichenden Elterntiere assoziiert sein könnten. Generell benötigten Adriatische Sprotteier einen sehr viel höheren

Salzgehalt zum Schweben als Eier der Ostsee Sprotte.

Versuche mit Eiern aus beiden Populationen bestätigten, dass unterschiedliche Salzgehalte die Ei-Entwicklungsdauer nicht beeinflussen. Der Ei-Überlebenserfolg war am höchsten bei Salinitäten von 14-18 bei der Ostsee Sprotte. Bei der Adriatischen Sprotte verbesserte eine erhöhte Salinität das Überleben mit Maximalwerten bei einer Salinität von 37.2. Salinitäten von 5 führten bei beiden Populationen nicht zu einer erfolgreichen Ei-Entwicklung. Bei der Ostsee Sprotte war die Larvenentwicklung nicht durch Salzgehalt beeinträchtigt. Obwohl sich bei der Adriatischen Sprotte bei Salinitäten von  $\geq 10 - 25$  lebensfähige Embryonen entwickelten, ist ein signifikanter Beitrag zur Rekrutierung eher unwahrscheinlich, da ein Großteil dieser Larven entweder nicht erfolgreich schlüpfen konnte oder Missbildungen aufwies. Jedoch bei Salzgehalten  $>30$ , welche in der nördliche Adria beobachtet werden können, ist eine hohe Plastizität in der Entwicklung und dem Überleben der Larven beobachtet worden. Die maximale Überlebensdauer wurde dennoch bei der Salinität von 37.2 gefunden, also unter den dort durchschnittlich vorherrschenden Salzgehaltsbedingungen.

Die Zeit bis zur Augenpigmentierung, welche mit der Öffnung des Mauls der Larven circa zeitgleich auftritt, wurde als Maß für die Zeit bis zur ersten potentiellen Nahrungsaufnahme definiert. Die Dauer bis zu dieser Pigmentierung korrelierte zwar stark negativ mit steigender Temperatur, jedoch nicht mit unterschiedlichen Salzgehalten, und erfolgte früher bei Larven der Adriatischen Population. Für diese Population wurde im Vergleich zur Ostsee Sprotte ein kleineres "Window of Opportunity" (WOO) ermittelt, was die vorhandene Zeit beschreibt, um erfolgreich die erste Nahrungsaufnahme zu erlernen, bevor die Energiereserven erschöpft sind und es zu

Hungertod käme. Das maximale WOO existiert für Adriatische Sprottlarven unter wärmeren Temperaturen als für Ostsee Sprottlarven. Bei einer prognostizierten klimabedingten Temperatur-Erhöhung würden sich diese Zeitfenster bei beiden Populationen verringern, jedoch könnte dieser Effekt sich stärker und ungünstig auf Adriatische Larven auswirken, da beispielsweise ein Anstieg von  $4^{\circ}\text{C}$  das Zeitfenster um 50 Prozent und damit auf nur 3-4 Tage reduzieren würde. Dies wäre nur die Hälfte der Zeit, die Larven aus der Ostsee zur Verfügung stände. Um abschließende Aussagen über die relative Bedeutung für den Rekrutierungserfolg machen zu können, sind weitere Studien an älteren, fressenden Larven und juvenilen Sprotten unumgänglich, da diese Lebensstadien sich bei anderen Untersuchungen als ebenfalls kritisch herausgestellt haben.

Die vorliegende Arbeit bietet neue Informationen über Temperatur- und Salzgehaltseinflüsse auf das Überleben und die Dauer von Entwicklungsprozessen von Ei-Stadien und nicht-fressenden Larven-Stadien verschiedener Sprotten Populationen. Die parametrisierten Ergebnisse finden zum Teil schon Anwendung, bieten jedoch auch die Möglichkeit, zukünftig in Sprotten-Rekrutierungs-Modellen und individuenbasierten Modellen (IBMs) implementiert zu werden.

# CONTENT

## III CONTENT

<b>I SUMMARY</b> .....	<b>II</b>
<b>II ZUSAMMENFASSUNG</b> .....	<b>IV</b>
<b>III CONTENT</b> .....	<b>VI</b>
<b>1 GENERAL INTRODUCTION</b> .....	<b>1</b>
1.1 PREVIEW.....	1
1.2 LIFE HISTORY OF MARINE FISH – THE EARLY LIFE STAGES.....	1
1.3 VARIABILITY IN MARINE FISH EARLY LIFE STAGES.....	3
1.4 THE ROLE OF ABIOTIC FACTORS TEMPERATURE AND SALINITY.....	5
1.4.1 <i>Temperature</i> .....	5
1.4.2 <i>Salinity</i> .....	6
1.5 SPRAT AS BIOLOGICAL MODEL.....	8
1.5.1 <i>Distribution, life-history-strategy and reproduction</i> .....	8
1.5.2 <i>Commercial importance</i> .....	9
1.5.3 <i>Ecological role</i> .....	9
1.6 INTER-POPULATION COMPARISON AND THERMAL SENSITIVITY.....	10
1.6.1 <i>Inter-population comparison</i> .....	10
1.6.2 <i>Thermal sensitivity</i> .....	10
1.7 THE TWO MAJOR STUDY AREAS.....	11
1.7.1 <i>Baltic Sea</i> .....	11
1.7.2 <i>Adriatic Sea</i> .....	11
1.8 STRUCTURE OF THE THESIS.....	12
1.8.1 <i>Temporal structure</i> .....	12
1.8.2 <i>General hypotheses</i> .....	12
1.8.3 <i>Aims and applications</i> .....	12
<b>2 THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF BALTIC SEA SPRAT (SPRATTUS SPRATTUS) EGGS AND YOLK SAC LARVAE</b> .....	<b>13</b>
2.1 ABSTRACT.....	13
2.2 INTRODUCTION.....	13
2.3 MATERIAL AND METHODS.....	16
2.3.1 <i>Larval growth</i> .....	18
2.4 RESULTS.....	18
2.4.1 <i>Egg phase</i> .....	18
2.4.2 <i>Larval phase</i> .....	18
2.5 DISCUSSION.....	20
2.5.1 <i>Egg phase</i> .....	20
2.5.2 <i>Larval phase</i> .....	23
2.5.3 <i>Population specific future implications</i> .....	24
<b>3 THE EFFECTS OF TEMPERATURE ON EGG DEVELOPMENT, GROWTH, MORPHOMETRIC TRAITS AND SURVIVAL OF ADRIATIC SPRAT (SPRATTUS SPRATTUS PHALERICUS) YOLK SAC LARVAE</b> .....	<b>27</b>
3.1 ABSTRACT.....	27
3.2 INTRODUCTION.....	27
3.3 MATERIAL AND METHODS.....	29
3.3.1 <i>Egg phase</i> .....	29
3.3.2 <i>Larval phase</i> .....	30
3.3.3 <i>Statistics</i> .....	32
3.4 RESULTS.....	32
3.4.1 <i>Egg phase</i> .....	32
3.4.2 <i>Larval phase</i> .....	34
3.5 DISCUSSION.....	38
3.5.1 <i>Egg phase</i> .....	38
3.5.2 <i>Larval phase</i> .....	40
3.5.3 <i>Conclusions related to recruitment problem, modeling purposes and outlook</i> .....	40



## CONTENT

<b>4 THE INFLUENCE OF DIFFERENT SALINITY CONDITIONS ON EGG BUOYANCY, - DEVELOPMENT, - SURVIVAL AND MORPHOMETRIC TRAITS OF BALTIC SEA SPRAT (<i>SPRATTUS SPRATTUS BALTICUS</i> SCHNEIDER) YOLK SAC LARVAE</b> .....	<b>42</b>
4.1 ABSTRACT.....	42
4.2 INTRODUCTION .....	42
4.3 MATERIAL AND METHODS .....	44
4.3.1 <i>Egg buoyancy</i> .....	45
4.3.2 <i>Field sampling</i> .....	45
4.3.3 <i>Larval morphometrics</i> .....	46
4.3.4 <i>Statistics</i> .....	46
4.4 RESULTS .....	47
4.4.1 <i>Egg phase</i> .....	47
4.4.2 <i>Larval phase</i> .....	50
4.5 DISCUSSION.....	52
4.5.1 <i>Egg development duration and survival</i> .....	52
4.5.2 <i>Consequences of different salinities for yolk sac larvae</i> .....	54
4.5.3 <i>Conclusion</i> .....	54
<b>5 EFFECTS OF REDUCED SALINITY CONDITIONS ON ADRIATIC SPRAT (<i>SPRATTUS SPRATTUS PHALERICUS</i>) EGG AND YOLK SAC LARVAL DEVELOPMENT</b> .....	<b>56</b>
5.1 ABSTRACT.....	56
5.2 INTRODUCTION .....	56
5.3 MATERIAL AND METHODS .....	58
5.3.1 <i>Biological material and experimental procedure</i> .....	58
5.3.2 <i>Egg development duration, relative buoyancy observations, survival and malformations</i> .....	58
5.3.3 <i>Larval morphometric traits and survival</i> .....	59
5.3.4 <i>Statistics</i> .....	59
5.4 RESULTS .....	60
5.4.1 <i>Egg buoyancy and egg development duration</i> .....	60
5.4.2 <i>Egg survival, yolk sac larval buoyancy and malformations</i> .....	60
5.4.3 <i>Larval morphometric traits</i> .....	61
5.4.4 <i>Yolk sac larval survival</i> .....	62
5.5 DISCUSSION.....	62
5.5.1 <i>Egg buoyancy</i> .....	62
5.5.2 <i>Egg development duration and egg survival</i> .....	63
5.5.3 <i>Morphometric traits</i> .....	64
<b>6 SYNTHESIS AND CONCLUSIONS</b> .....	<b>65</b>
6.1 SUMMARIZED RESULTS OF GENERAL HYPOTHESES .....	65
6.1.1 <i>1) Temperature has no effect on the survival, development and duration of non feeding stages (eggs and yolk sac larvae) of sprat</i> .....	65
6.1.2 <i>2) Duration and survival of non feeding stages of sprat (egg and yolk sac larvae) is not altered by changes in salinity regime during development</i> .....	65
6.1.3 <i>3) Sprat non feeding stages (egg and yolk sac larvae) from different populations are identically affected under exposure of same thermal history</i> .....	67
6.2 POPULATION DIFFERENCES .....	69
6.2.1 <i>Thermal sensitivity, plasticity and latitudinal gradient</i> .....	69
6.2.2 <i>Changing conditions and Window of Opportunity</i> .....	71
6.3 APPLICATION AND OUTLOOK .....	72
<b>IV REFERENCES</b> .....	<b>73</b>
<b>V ANNEX</b> .....	<b>90</b>
LIST OF FIGURES: .....	90
LIST OF TABLES: .....	95
APPENDIX CHAPTER 3 .....	97
<b>DESCRIPTION OF THE INDIVIDUAL SCIENTIFIC CONTRIBUTION TO THE MULTIPLE-AUTHOR PAPERS</b> .....	<b>99</b>
<b>DANKSAGUNG</b> .....	<b>101</b>

## CONTENT

---

<b>CURRICULUM VITAE.....</b>	<b>103</b>
<b>ERKLÄRUNG.....</b>	<b>109</b>

## CONTENT

---

## 1 General Introduction

### 1.1 PREVIEW

This thesis is structured into a general introduction part, specific result chapters compiled in manuscript form at different stages in the peer-review publication process (already published-submitted-in preparation) and a general conclusion section.

Each introductory part is closed with specific statements or relevant questions to be addressed within this study.

The introduction shortly covers life history of fish and in particular the early life stages. It condenses major hypotheses developed in fishery sciences to explain recruitment variability with focus on the early life stages. In the following, temperature and salinity as important abiotic factors are presented, affecting general and more specific patterns in aquatic animals and in particular fish early life stages. This leads to a more extended summary of the distribution, life-history, reproduction and economical as well as ecological importance of sprat as the key species and biological model in focus. The basic idea of thermal sensitivity is explained with relevance to the projected inter-population comparison of different sprat populations. Subsequently, morphology and hydrography of the two major study areas are presented. Finally, the general hypotheses and aims of the study are presented after a brief note on the temporal aspect concerning the development of this thesis.

### 1.2 LIFE HISTORY OF MARINE FISH – THE EARLY LIFE STAGES

Fishes are the largest vertebrate group on earth with >24.600 living species in 482 families and 85 orders (Nelson 1994). 41% of them living in freshwater, about 1% moves between both fresh- and saltwater and 58% are marine species (Cohen 1970). The life history of marine fish (Figure 1-1)

can be divided into five primary periods: embryo, larva, juvenile, adult and senescent (Fuiman 2002). Before releasing the gametes (eggs and sperm) into the free water column, some species have developed mating behaviours. For clupeids, spawning aggregations of male and female fish are generally assumed. The following summary of life history traits will concentrate on aspects relevant for clupeids. With some exceptions (e.g. herring), most marine fish species spawn pelagic eggs which become fertilized in the free water column.

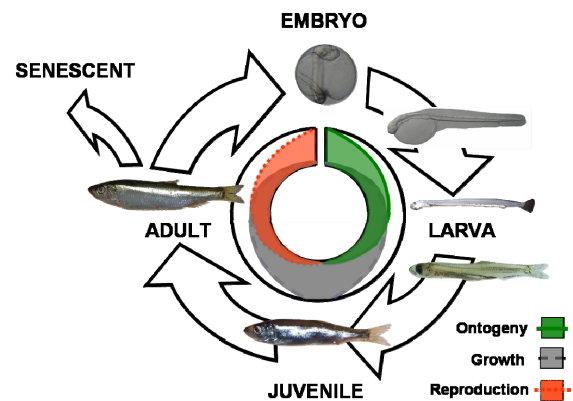


Figure 1-1: General life cycle of a marine fish, using sprat (*Sprattus sprattus*) as an example. Major periods and the corresponding dominant intrinsic processes are shown. The circular chart shows that ontogenetic changes (green color with solid lines) begin at fertilization of the egg and gradually diminish toward the juvenile period. Growth (grey color with long dashed line) also begins at fertilization and diminishes toward adulthood. Reproduction (red color and dotted line) begins when gonads differentiate and diminishes in senescence, modified after Fuiman (2002). Pictures of feeding larva are courtesy of Bastian Huwer and late larva/juvenile/adult sprats of Myron Peck.

Ontogeny begins with activation of the oocyte when sperm penetrates the chorion through the micropyle. Egg activation induces the delamination of the eggs' enclosing membrane, dehiscence of cortical alveoli around the periphery of the yolk, and rapid uptake of water from the surrounding environment (Fuiman 2002). This water uptake leads to the formation of the fluid-filled perivitelline space and a decrease in egg specific gravity. Simultaneously, micropyles close to

prevent polyspermy. The hardened chorion and the perivitelline space provide a barrier for the embryo against mechanical or chemical stressors. After merging of nuclei from egg and sperm, development follows the general scheme of embryology in vertebrates: cleavage, morula, blastula, gastrula, neural crest and so forth (Figure 1-2). Hatching begins when the egg membrane and the chorion are dissolved by hatching enzymes. In sprat larvae at hatch are undeveloped with unpigmented eyes and a large yolk sac. These larvae maintain predominantly motion-less in the water column or are subject to passive drift and transport through physical forcings and hydrographic features. For a short period after hatch (with duration depending on water temperature) larvae feed exclusively on their yolk reserve provided by the spawning female.

Kamler (2008) provides a recent review on resource allocation in yolk-feeding fish, where she focused on physiological as well as on ontogenetical development. Important events during the enduring development are the visual perception (distinct functionable eyes) in combination with mouth gap opening (developed jaw) and the evolving digestive system. In some species (e.g. sprat), first feeding occurs when yolk is still present. This period is called 'mixed-feeding period' where successful foraging performances should be achieved before yolk resources are depleted. The duration from theoretical first feeding (mouth gap opening) and starvation induced mortality can be called 'window-of opportunity' (following Peck *et al.*, unpublished a). However, this window can be reduced since there is general evidence for an additional 'point-of-no-return' which defines the point, where 50% of the larvae were too weak to feed (Yin and Blaxter 1987a, b). Egg and yolk sac larval stages are usually referred to as 'non feeding stages'. This last until larvae reach sufficient morphological, physiological and behavioural ontogenetic changes that enables them to feed on

external food resources. Survival of individuals of any stage of life requires adequate levels of performance against a variety of ecological challenges, such as obtaining food, evading predators, and locating and remaining in suitable habitats. The first feeding phase can be associated

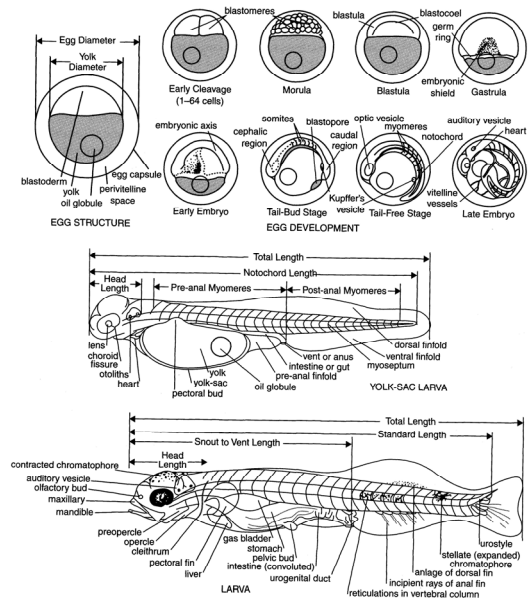


Figure 1-2: General anatomy of teleost fishes. Graphics taken from Fuimann (2002), original by Hardy *et al.* (1978). Egg developmental phases and fish egg and embryo structure (upper part). Yolk sac larva anatomy with important morphometric measurements. Feeding larva with pigmented eyes and open mouth gap (lower part). In this thesis, notochord length (= standard length) was measured (middle part). Note that sprat eggs have no oil globule.

with a habitat switch in some species, or diurnal and or feeding migrations. With the increase in body size and the acquisition of improved sensory systems feeding larvae can initiate schooling behaviour. In some taxa, the development of a functional swim bladder is a prerequisite for directed and immediate vertical movements or predator avoidance.

As individuals grow they pass through different allometric changes in growth patterns (e.g Peck *et al.* 2005, for sprat). As energy storage and metabolic rates change during the transition from larval to juvenile stage increased starvation resilience is assumed for juveniles (Fuiman

2002). In clupeid species high mobility of juvenile fish schools is frequently observed. After the first or second year of life most of clupeid fish initiate to mature. In herring, adult stocks perform extended feeding migrations but also exhibit a high potential to return to previously established stock specific spawning sites. Individual egg production is usually increasing with weight and age, if previous feeding conditions were appropriate for partitioning enough energy into gonadal maturation. The species specific stock structure can determine or modify the temporal extension of spawning season since experienced (individuals which have spawned already multiple of times) spawners usually spawn first. The age of the fish at first spawning is species and stock specific and fish have the potential to grow life-long (indetermined). However, the fishing pressure on almost all commercially important and exploited (through the 'bycatch' problematics also unexploited) species prevents the senescent-induced death of old individuals. Recognizing the multitude of life stages in fish showing more or less vulnerable phases on different temporal scales, this thesis concentrates on the early, non feeding phase of sprat. Experimental manipulating trials are used in general, although wherever and whenever possible field observations are included.

### 1.3 VARIABILITY IN MARINE FISH EARLY LIFE STAGES

Stock dynamics and vital rates of adult fish populations alone can not sufficiently explain the observed variability in recruitment success. Thus, additional to stock specific characteristic parameters of adult fish (e.g. growth, mortality, fecundity or egg production) there is evidence for other life stages to influence and shape population dynamics (Rothschild 1986, 1990). This is especially relevant because low proportions (0.01%) of originally spawned eggs finally succeed in surviving

and recruiting to the spawning population. Thus small changes in survival of the first vulnerable stages can result in high stock variability (Heath 1992; Cowan and Shaw 2002; Houde 2002).

Houde (2008) has reviewed relevant literature on recruitment related studies on early life history stages (Figure 1-3) and about the scientific progress made almost a century past Johan Hjort had presented his studies on "fluctuations in the year classes of important food fishes" (Hjort 1914, 1926).

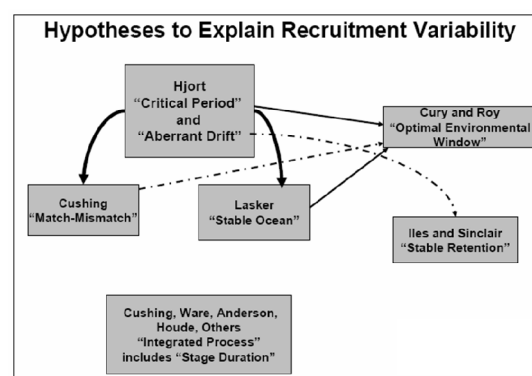


Figure 1-3: Hypotheses to explain recruitment variability in fish originating from Hjort's (1914) concepts of "critical period" and "aberrant drift". Solid arrows indicate direct and broken arrows indirect derivations from Hjort's hypotheses, whereas thickness of arrows indicates strength of the relationship (taken from Houde 2008, modified).

Hjort (1914) suggested that the earliest larval stages are the most critical period ('critical period concept') during which later year class strengths are largely determined. He hypothesized two major factors influencing early mortality rates i) scarcity of food during the early larval life and ii) transport by currents into unfavorable areas. These ideas influenced fishery research for long time and led to new concepts. These considered density dependent-mortality (Ricker 1954; Beverton and Holt 1957) to be more important compared to density dependent growth and reproduction. For fish larvae, the incidence of first feeding and the

connectivity to a larval cohort have been postulated to be important prerequisites for survival. Cushing (1974, 1990) focused with his 'match/mismatch hypothesis' on the exact seasonal and spatial occurrence of prey for the developing larvae. Match situations occur if fish larvae meet synchronously with appropriate food of suitable size in time and space whereas mismatch situations appear if this predator-prey interaction is uncoupled. To understand these interactions it is of overwhelming importance to investigate the temporal and spatial dynamics of the organisms in focus. This space-time variation in ecological events is largely driven by abiotic factors with temperature probably being the most important one (Sissenwine 1984).

Further hypotheses on explaining recruitment variability focused on physical forcings at either large, meso or small scales. Lasker (1975, 1981) observed the strongest year-class in Anchovy (*Engraulis mordax*) originating from stable (non-turbulent) conditions and formulated his 'stable ocean hypothesis', where he postulated that turbulent conditions destroy food patches (subsurface chlorophyll maximum) and dilute potential food organisms to below feeding threshold concentrations of first-feeding larval anchovies. Bakun and Parish (1982) found consistent patterns of avoidance in the reproductive strategies of anchovies, in areas with maximum upwelling since these are characterized by intense turbulent mixing and offshore transport. This concept has been specified by Cury and Roy (1989) who described an 'optimal environmental window' which postulates a dome-shaped relationship between recruitment and upwelling intensity. Highest recruitment would be achieved at moderate levels of upwelling creating micro-turbulences which enhance the encounter rate of feeding larvae. Alternative explanations for high mortality during the larval phase were given by Iles and Sinclair (1982) and Sinclair (1988)

with their 'larval retention- / membership-vagrancy- hypothesis' which states that physical retention of early life stages and not levels of available prey is critical in the recruitment process. Low mortality will be observed if adult fish are spawning in appropriate places with high retention potential for eggs and larvae.

Predation *per se* can control or regulate recruitment levels (Bailey and Houde 1989) and there are several other hypotheses, which focus on the factors of individual size, growth-potential, individual size-at-stage and the duration per stage. Slow growing and small fish larvae remain comparatively longer vulnerable to predation ('stage-duration-hypothesis') than their faster growing conspecifics which have the potential to grow out the most dangerous phases earlier (Houde 1987). Fast growth leading to larger size-at-stage would improve survival expressed in the 'bigger-is-better-hypothesis' by Houde (1987) and Anderson (1988). Emerging from this, the 'size selective predation-hypothesis' pointed out that larger larvae have higher escape potentials from predators compared to smaller larvae (Takasuka *et al.* 2003, 2004) and thus would have higher survival probability. However, other studies found fast growth prejudicially (Litvak and Legett 1992) under predation pressure.

In conclusion, there is rising evidence, that no single life stage controls or regulates recruitment, meaning that recruitment levels are not determined at a particular ontogenetic stage (Houde 2008). Depending on species and population this can either be non feeding stages (eggs and yolk sac larvae), first feeding larvae, late larvae or within the juvenile phase (see Houde 2008 for examples). These vulnerable stages can even be shifted if environmental forcings change due to increased environmental variability or ecosystem regime shifts as observed for walleye pollock (*Theragra chalcogramma*) (Bailey 2000).

Tools to describe and follow environmental variability in terms of the physical and biological processes acting and consequences emerging from this have been developed since the last decade. Coupled biological physical models (CBPM's) have recently been developed and applied, to explain mechanisms that generate recruitment variability (North *et al.*, in press). These approaches can contribute significantly to the understanding of factors influencing recruitment and are valuable tools in addition to extensive field monitoring and sampling programmes. Additionally they provide the opportunity to incorporate climate change related aspects in modeling e.g drift or potential prey fields. If CBPM's are coupled with individual-based models (IBM's) (Daewel *et al.* 2008a; Peck and Daewel 2007; Kühn *et al.* 2008) which mimic and investigate development, growth and survival on individual basis of early life stages of fish, the combination provides new powerful tools to simulate the influence of climate variability on potential growth and survival of larval fish (Daewel *et al.* 2008b). However, the application of IBM's requires detailed knowledge on the early life stages and the response of the species in focus to changing abiotic and biotic conditions, which can be simulated e.g. in laboratory experiments.

This thesis was designed to uncover important and relevant issues raised by some of the above mentioned authors for the species in focus. It analyzes the gap of knowledge on stage duration time, e.g time to first feeding or maximum survival time, which is both in a broader sense linked to Hjort's 'critical period' and Cushing's 'match-mismatch' hypotheses. As a step further, this study differentiates such traits on the population level, parameterizes the traits and makes them directly applicable for recruitment related studies on individual basis.

## 1.4 THE ROLE OF ABIOTIC FACTORS TEMPERATURE AND SALINITY

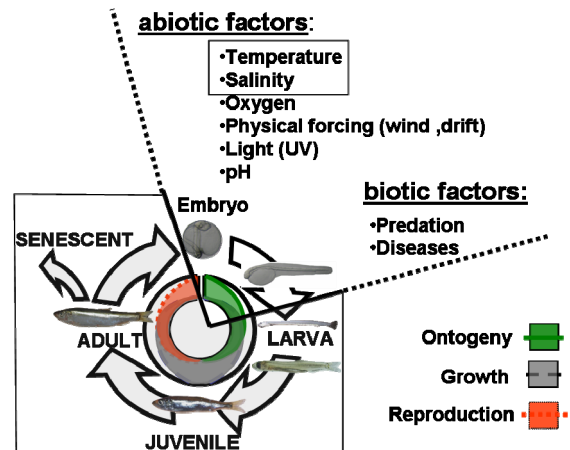


Figure 1-4: Abiotic and biotic factors effecting life stages of fish. Highlighted are only factors influencing non feeding phases (egg and yolk sac phase) of fish with relevance to ontogeny (green, solid line) and growth (grey, dashed line). Effects of temperature and salinity as abiotic effects were analyzed in particular within this study.

### 1.4.1 Temperature

It has long been observed that temperature shapes species and population distribution and that change in temperature can cause changes in spatial distribution or species composition. In aquatic ecosystems this has been confirmed for fish in freshwater (Sharma *et al.* 2007; Buisson *et al.* 2008), estuarine (Maree *et al.* 2000; Roessig *et al.* 2004; Harrison and Whitfield 2006) and marine systems (McFarlane *et al.* 2000; McGinn 2002; Roessig *et al.* 2004; Rose 2005; Poulard and Blanchard 2005; Ojaveer and Kalejs 2005; Sabatés *et al.* 2008). Effects of temperature are on the individual level. The rate of most physiological processes (metabolism, swimming, digestion, growth, etc.) generally declines with temperature in poikilotherms (Cossins and Bowler 1987). In addition in marine fishes temperature is one of several factors that have an effect on larval duration (Houde 1989, 1994).



Based on a literature review O'Connor *et al.* (2007) compared the effect of temperature on planktonic larval duration as a key component of marine life cycles among a geographically and taxonomically diverse group of marine fish and invertebrates. As a result, they developed a unified, parameterized model for the temperature dependence of larval development to assess the length of time that larvae are subject to movement by currents and exposed to sources of mortality. They concluded as “the duration of the larval period is known to influence larval dispersal distance and survival, changes in ocean temperature could have a direct and predictable influence on population connectivity, community structure and regional-to-global scale patterns of biodiversity” (O'Connor *et al.* 2007).

Performing experiments testing varying temperature levels to assess the durations of the planktonic phase and egg development times is a frequently used approach not only in fish related studies (reviews e.g. in Pauly and Pullin 1988; Pepin 1991; Kamler 2002; Fuimann 2002; Peck *et al.*, unpublished b) but also for zooplankton, reptiles, birds, mammals (reviews in Gillooly and Dodson 2000a) or insects (Gillooly and Dodson 2000b) or in combinations of all groups. Gillooly *et al.* (2002) linked embryonic development time and temperature to body size or neonate mass, respectively, and developed a general model, based on first principles of allometry and biochemical kinetics, that predicts the time of ontogenetic development as a function of body mass and temperature. But beside the overall picture, detailed research needs to be conducted on the species in focus in as many as possible of its different populations or stocks and on individual basis.

The extrinsic factor temperature controls the rate of ontogenic development within a viable range (Blaxter 1969; Blaxter 1992; Fuimann 2002; Kamler 2002), whereas

beyond that range temperature is a lethal factor (Brett 1979). Temperature can also effect morphometric traits like size-at-hatch (e.g. Blaxter 1992; Chambers 1997; Pepin *et al.* 1997; Peterson *et al.* 2004) or muscle development (Johnston *et al.* 2001; Johnston 2006).

But not only immediate responses to single factors can be found. It has become increasingly apparent, that within-species variation is inherent in natural systems and that at least some of this variation may reflect differences in embryonic or larval experiences. Such experiences may include delayed metamorphosis, short term starvation or short term salinity stress and cause latent effects. Although these effects have their origins in early development, they are first exhibited in juveniles or adults. This has been documented among gastropods, bivalves, echinoderms, polychaetes, crustaceans, bryozoans, urochordates, and vertebrates (Pechenik 2006). For fish it was shown that the temperature regime during the early life stages (until eye-pigmentation) can impact e.g. growth performance reflected in the adult stage. This was revealed for Atlantic salmon (*Salmo salar* L.) by Macqueen *et al.* (2008) who found significantly higher number of muscle fibres in three years (!) adult fish which had grown at 5°C compared to fish grown at lower or higher temperature regimes.

This thesis focuses on temperature-mediated direct effects on eggs and yolk sac larvae (Figure 1-4). It analyzes if or to what extent temperature influences morphological traits related to body size or area. In addition it provides new information about temperature induced shifts in non feeding sprat early life phenology.

#### 1.4.2 Salinity

Salinity is one of the factors largely contributing to shape species` distribution on an ecological scale. Increased salinity can modify species composition in fresh and brackish water zooplankton (Cervetto

*et al.* 1999; Hall and Burns 2001; Sarma *et al.* 2006; Jeppesen *et al.* 2007) or can cause detrimental effects in the worst case leading to extinction (Dana and Lenz 1986). Salinity also determines the distribution range in estuaries or brackish systems of other vertebrates such as decapods, amphipods, gastropods and bivalves (Kinne 1963; Westerbom *et al.* 2002).

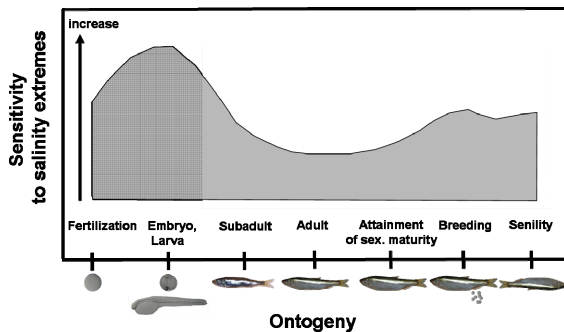


Figure 1-5: Schematic illustration of changes in sensitivity to upper and lower salinity extremes during ontogenetic development. Originally exemplified for trends found in numbers of estuarine animals (decapods, amphipods, mussels, gastropods), modified from Kinne (1963). Illustration was adapted to the life-cycle of sprat and dark squared area (fertilization to end of yolk-sac phase) refers to period considered in this thesis.

Also in fish, effects of salinity on distribution patterns or spawning areas have been observed. For example mackerel (*Scomber scombrus*) perform regular feeding migrations from the North Sea to the Western Baltic Sea, but the comparably low salinity averts successful reproduction (D. Schnack, personal communication).

The effect of water of a particular salinity on egg and larvae (Figure 1-5) may be the result of one or more factors like the osmotic concentration, the incidence and concentration of particular ions and the specific gravity of different salinities which affect organisms' relative buoyancy (Holliday 1969). For example, for marine fish in the Baltic Sea it is likely that they have experienced adaptational processes, which allow them to reproduce and to survive in a brackish water system (Voipio

1981; Parmanne *et al.* 1994). This includes the production of sperm, which can be activated and are mobile under low saline conditions (Griffin *et al.* 1996). And accordingly pelagic eggs need to be adapted to achieve neutral buoyancy to persist in the water column and to avoid potentially harmful hypoxia conditions in the bottom layers (Nissling and Westin 1991; Nissling *et al.* 2002, 2006).

Among other factors, many studies have reported an influence of salinity on fish development (review in Holliday 1969). In most species, egg fertilization and incubation, yolk sac resorption, early embryogenesis, swim bladder inflation and larval growth are dependent on salinity (review in Boeuf and Payan 2001).

The osmotic and ion regulation in larvae can be affected by changes in salinity (Alderdice 1988) severely, and numerous studies have shown that 20 to >50% of the total energy budget (at least for adult fish) are dedicated to osmoregulation (Boeuf and Payan 2001). Insufficient salinity conditions have shown to impact yolk utilization efficiency (review in Heming and Buddington 1988). High salinity conditions can also modify morphometric characteristics (review in Blaxter 1969) like vertebrae or myotomes. Hempel and Blaxter (1961) showed that the mean myotom counts of larval herring (*Clupea harengus*) hatched from eggs incubated in salinities ranging from 5 to 50psu were highest in higher salinity. But other authors doubt the effect of salinity on the numbers of vertebrae (Fahy and O'Hara 1977). Also malformed developments during embryogenesis are frequently observed, where salinity values are outside of the tolerable range of the species. Abnormal development of the caudal notochord development resulted in bending of the tail and occurred during high-salinity incubation (>34psu) of early Atlantic halibut (*Hippoglossus hippoglossus*) yolk sac larvae (Ottesen and Bolla 1998). Higher salinity (18psu compared to <5 psu) during early development was shown to increase

growth and survival potential significantly in Florida red Tilapia (*Oreochromis* - hybrids) grown in both brackish and saltwater for 37 days (Watanabe *et al.* 1989)

As evident from the above presented variety of factors, salinity can directly impact fish early life stages. Specific questions addressed by this thesis are: do altered salinity conditions induce changes in egg buoyancy and do they affect egg and larval survival? A focus is set on potential modifications in ontogenetic rates and on the occurrence of malformations.

## 1.5 SPRAT AS BIOLOGICAL MODEL



Figure 1-6: Adult sprat caught during a scientific research cruise onboard RV Alkor in May 2006

### 1.5.1 Distribution, life-history-strategy and reproduction

Sprat (Figure 1-6) is a small pelagic schooling zooplanktivorous fish species, inhabiting European coastal waters and marginal seas. This species is distributed from Norway, around the British Islands, the North Sea down to the Bay of Biscay and from the Skagerrak in the Baltic Sea up to the Kvarck region (Aro 1989). Spawning stocks are established in the Western Mediterranean Sea in the Gulf of Lyon (Palomera *et al.* 2007), the North Adriatic Sea (Zavodnik and Zavodnik 1969; Dulčić 1998; Tičina 2000) and the Black Sea (Daskalov 1999). They can tolerate a wide range of salinities (Parmanne *et al.* 1994) and have low individual biomass, early reproduction (between age 1 and age 2, Alheit 1988; Haslob, unpublished) and short lifespan (rarely >5 years, Bailey 1980) which characterize them as R-selected species (Peck *et al.*, unpublished a). Sprat are

indetermined batch spawners, producing up to 10 batches (George 1987) or more (Makarchouk 2002; Alekseev and Alekseva 2005; Haslob, personal communication) throughout the spawning season. Numbers of eggs per female and batch are increasing with age and size. Generally several thousand eggs are spawned per batch but specifications about egg numbers vary considerably between different studies, areas and populations (Zavodnik and Zavodnik 1969; Alheit 1987; Alekseev and Alekseva 2005). Spawning occurs both coastal and offshore. In the Baltic Sea sprat spawns from March to August (Parmanne *et al.* 1994) with peak spawning in May in the central parts (Figure 1-7), whereas in the North Sea (Wahl and Alheit 1988) sprat spawning season lasts from May to August.

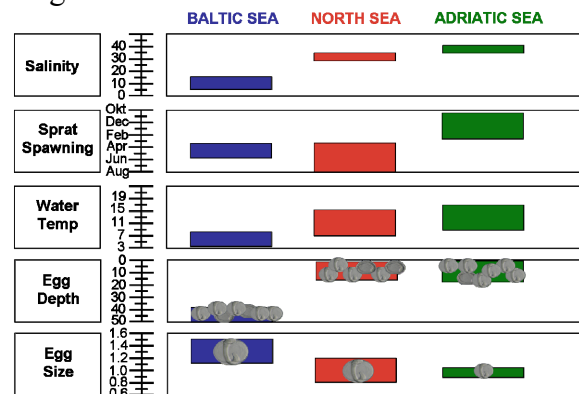


Figure 1-7: Hydrographic features of Baltic Sea, North Sea and Adriatic Sea characteristic for spawning time and occurrence of sprat eggs. Sprat spawning season is in spring and early summer in the central Baltic Sea (Bornholm Basin) and was found to continue until August in the North Sea. Spawning time in the North Adriatic is from November to March in winter. The range of water temperatures (°C) refers in all areas to the water layers in the depth, where sprat eggs were found. Vertical egg distribution (m) is expressed as mean depth value for the Bornholm Basin, Baltic Sea, for the North Sea as found in the literature (Conway *et al.* 1997). No studies were performed on vertical egg distribution in the Adriatic Sea. The range of the egg size (diameter, mm) for the Baltic Sea and the Adriatic Sea were obtained from this study, North Sea values were obtained from Alheit *et al.* 1987.

In southern European waters sprat spawns during winter months. Spawning season starts in the Adriatic in October/November, peaks in December and is terminated in

March /April (Štirn 1969; Zavodnik 1969; Zavodnik and Zavodnik 1969; Teskerendžić 1983; Tičina 2000). In the Black Sea sprat spawn from October to March with peak spawning time in December and January (Daskalov 1999).

Three different sprat subspecies have been proposed over their distribution range and adults can be distinguished by numbers of postpelvic scutes as morphometric characteristic (Whitehead 1985). The subspecies considered in this thesis are i) *Sprattus sprattus balticus* Schneider, 1908, in the Baltic Sea ii) *Sprattus sprattus sprattus* Linnaeus, 1758, in the North Sea and northeast Atlantic and iii) *Sprattus sprattus phalericus* Risso, 1827, in the Mediterranean Sea and Black Sea.

Depending on the author (and area) sprat are classified as “cold-water species of boreal-Atlantic origin” (Rass 1949 cited from Daskalov 1999; Bombace 2001) or warm-water species (Nissling 2004; Morawa 1953). The time of spawning varies between populations and areas (Figure 1-7).

### 1.5.2 Commercial importance

The commercial importance (Figure 1-8) varies between the European sprat stocks and is high in the Baltic Sea with mean catches of  $>450 \cdot 10^3$ t per year since 1995. A regime shift occurred in the mid 1980<sup>th</sup> in the Baltic Sea (Alheit *et al.* 2005) with major consequences for the zooplankton and fish trophic level. Sprat stock increased since the mid 1980<sup>th</sup> as a combination of released predation pressure by its major predator cod (*Gadus morhua*), changed feeding conditions due to a shift in copepod species composition from a *Pseudocalanus* to an *Acartia* dominated community (Möllmann *et al.* 2000, 2003). This was also triggered by a change in hydrography which affected the reproductive volumes of both cod and sprat species, with conditions for cod reproduction degraded and for sprat improved (Köster *et al.* 2003). Contrary, in the Adriatic sprat, stock size has declined

drastically (Figure 1-8) since the mid 1980<sup>th</sup> with the consequence that catch statistics do not even cover this species in Croatia, a formerly important sprat fishing country (Vjecoslav Tičina, personal communication). In 2004 Adriatic sprat biomass was assessed by hydroacoustic methods to be less than 1000t (Tičina 2005). Climate change in combination with changed hydrographical conditions (temperature, salinity increase and a change in a major current system in the end of the 1980<sup>th</sup> (Eastern Mediterranean Transit, EMT)) and overfishing have been considered to be possible causes for the decline (Grbec *et al.* 2002, Azzali *et al.* 2002, Bombace 2001), but no real evidence for this is presented so far.

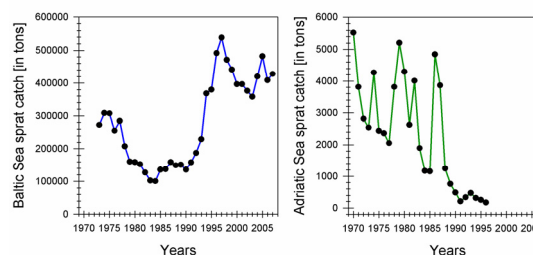


Figure 1-8: Sprat catch data for the Baltic Sea derived from the ICES website (<http://www.ices.dk/fish/statlant.asp>). Adriatic sprat catch data taken from Tičina (2000). Note that the magnitude between both data sets varies with a factor of 100! After 1997, sprat catch in the North Adriatic dropped further and no fishing data are reported since (V. Tičina, personal comment). In 2004 Adriatic sprat biomass was assessed by hydro-acoustic methods to be less than 1000t (Tičina 2005).

### 1.5.3 Ecological role

Sprat along with anchovies (*Engraulis encrasicolus*, *Engraulis ringes*, *Engraulis mordax*), sardines (*Sardina pilchardus*, *Sardinops sagax*, *Sardinella aurita*), herrings and others belong to the ‘small pelagics’, small schooling zoo- or phytoplanktivorous fish which do respond dramatically and quickly to changes in ocean climate (Hunter and Alheit 1995). Worldwide, they support economically very important fisheries especially in upwelling systems, are key species in their respective ecosystems, controlling the

lower foodweb dynamics and are also substantial prey for higher trophic levels (fish, birds, mammals).

## 1.6 INTER-POPULATION COMPARISON AND THERMAL SENSITIVITY

### 1.6.1 Inter-population comparison

Adult sprat populations experience different thermal histories (mean annual temperature range: Baltic Sea ~ 3-18°C; Adriatic Sea 9-25°C). These thermal conditions also influence metabolism, developmental rates and ontogenetic timing (Johnston and Wilson 2006) in ectotherms like fish for example.

Phenotypic plasticity is described as the capacity of a genotype to exhibit a range of phenotypes in response to variation in the environment (Fordyce 2006). Such adaptive responses are particularly evident in species which live in changing environments or experience large seasonal abiotic variations (Cossins *et al.* 2006).

The responses are (i) either maintaining normal levels of activity or of homeostatic potential ('capacity' adaptation) or (ii) increasing resistance against such potentially lethal effect of environment ('resistance' adaptation) (Cossins and Bowler 1987). This is important because temperature-induced developmental plasticity can influence the thermal tolerance of the species.

If large environmental changes occur during developmental or early life phases they may have long-life persistence (Cossins *et al.* 2006). If, on the other hand, adjustments for such changes occur during juvenile or adult phases, they might be reversible (Cossins *et al.* 2006).

The approach of this study does not only include the analyses of the effects of abiotic factors on non feeding stages of sprat within one population. It also includes the inter-population comparison on a latitudinal scale where populations with different ambient annual temperature and salinity regimes were selected. They

were compared under similar sets of incubation salinities and temperatures to detect if survival, embryonic development or timing to important ontogenetic events were different between the populations. Baltic Sea and Adriatic Sea populations were experimentally analyzed in this study, whereas data for the North Sea sprat population were extracted from published laboratory studies

### 1.6.2 Thermal sensitivity

In this thesis thermal sensitivity of egg development from three different populations was compared using the method presented by Pritchard *et al.* (1996) which was originally applied for aquatic insect eggs. In a review study on marine fish egg development this method was further used by Peck *et al.* (unpublished b). Instead of developmental time in days, the analysis of thermal adaptation considers the physiological time which is expressed as the thermal sum (degree-day) (Pritchard *et al.* 1996, Figure 1-9).

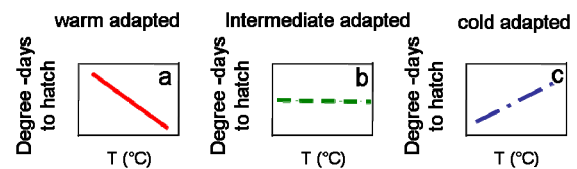


Figure 1-9: Thermal sensitivity concept. (a) Warm adaptation is considered if thermal sum in degree-days to hatch is reduced with increasing temperature. (b) Intermediate or general adaptation is given if neither increasing nor decreasing trend in developmental time is observed at increasing temperature. (c) cold adaptation is derived from decreasing developmental time with decreasing temperature. (Modified after Pritchard *et al.* 1996 and Peck *et al.*, unpublished b)

Warm-adaptation (Figure 1-9a) is considered when the thermal sum is plotted versus incubation temperature and the slope is negative. These individuals have to invest relatively more energy under colder temperatures. Slopes around zero indicate generalist (Figure 1-9b) individuals with a wide range of thermal adaptation (Pritchard *et al.* 1996). Positive slopes



indicate cold adaptation (Figure 1-9c) because development requires relatively less energy (less time) at colder temperatures. The slope of the average thermal reaction norm was used as an index of adaptation.

## 1.7 THE TWO MAJOR STUDY AREAS

### 1.7.1 Baltic Sea

The Baltic Sea is about 1600 km long, on average 193 km wide and has a mean depth of 55m (Figure 1-10b). It is characterised by a decreasing salinity regime from west to east and also by strong thermo-haline stratification with less saline water in the upper layer and higher salinity below the halocline. Major spawning grounds of important fish species like cod and sprat are the three deep basins (Bornholm Basin (Figure 10b, insert), Gdansk Deep and Gotland Basin). The renewals of oxygenated deep water in the basins are depending on strong inflow events from high saline water from the North Sea (Matthäus and Franck 1992, Matthäus and Schinke 1994). Salinity (Figure 10d) is an important factor for sprat reproduction potential since they spawn pelagic eggs which need to match sufficient density conditions in the water column to maintain neutral buoyancy. Water temperatures at these respective depths control the survival and duration of the eggs and yolk sac period (Figure 1-10d).

### 1.7.2 Adriatic Sea

The Adriatic Sea is 783 km long, on average 243 km wide and can be subdivided into three sections, North Adriatic, Middle Adriatic and South Adriatic (Figure 1-10c). The thermohaline properties of the Adriatic Sea are determined mainly by the air-sea interaction, water exchange through the Otranto Strait, river discharge, mixing,

currents and the topography of the basins (Zore-Armanda *et al.* 1999).

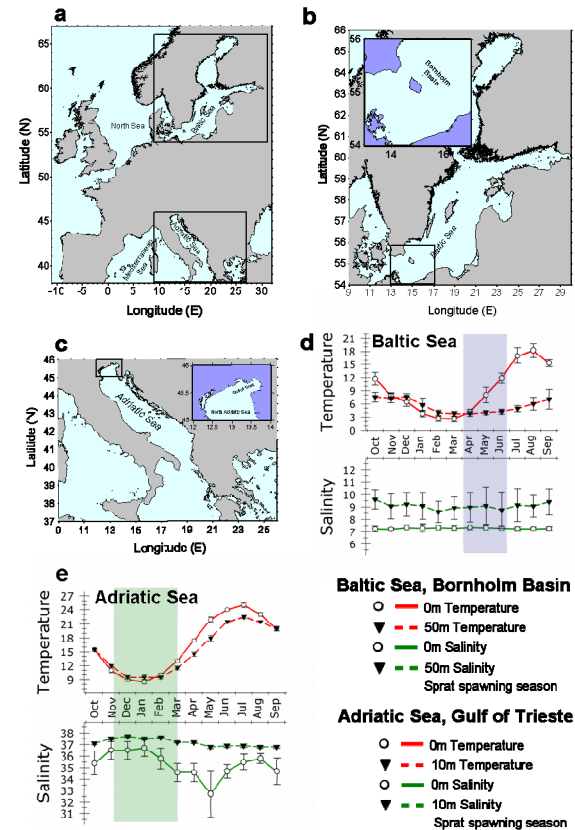


Figure 1-10: Map of Central European seas (a), map of the Baltic Sea (b) and the Adriatic Sea (c). The small inserts show detailed maps of respective sampling areas the Bornholm Basin in the Baltic Sea and the Gulf of Trieste in North Adriatic Sea. Mean monthly temperature and salinity values ((d) = Baltic Sea, (e) = Adriatic Sea) of either Sea surface (0m) or the depth were large numbers of sprat eggs are found during spawning season (Adria 10m, Baltic 50m). Timing of sprat spawning seasons are presented as semi transparent coloured background for each population. Temperature and salinity data from the Bornholm Basin are monthly mean values of 10 years (1996-2005, derived from ICES hydrographical database) and data for the Gulf of Trieste are redrawn from Malačić *et al.* (2006) for the period of 1991 -2003.

The high river discharge over the shallow shelf of the North Adriatic in combination with mixing of bottom sediments enables high productivity of this area and hence it is one of the richest fishing grounds in the Mediterranean (Zore-Armanda *et al.* 1999, Azzali *et al.* 2002). The largest input comes from Po river in seasons of ice melting in spring or frequent precipitation in autumn, but the high inflow variability

is the major factor determining temperature, salinity and transparency in the North Adriatic. Currents show central cyclonic circulation in combination with smaller cyclonic and anticyclonic eddies related to freshwater input and wind regimes (e.g. Bora = very strong and dry north-east wind). In the Northern Adria (Figure 1-10c, insert) monthly water temperatures vary from 9.2°C to 25°C at the surface and from 9.2°C to 22.6°C at 10m depth (Malačič *et al.* 2006, Figure 1-10e). Salinity of the Adriatic is relatively high and shows values from 38.4 to 38.9 in the open southern Adriatic Sea.

Salinity in the Northern part is more variable and also lower with annual mean (1991-2003) values between 35.3 at the surface and 37.1 at 10 m depth (Malačič *et al.* 2006, Figure 1-10e).

## 1.8 STRUCTURE OF THE THESIS

### 1.8.1 Temporal structure

The rapid communication of the research results was an important issue while preparing this thesis. In order to achieve a quick publication of the successively studied individual aspects, each chapter was developed as an independent manuscript. This includes repetitions in some parts of the thesis.

### 1.8.2 General hypotheses

This study analyses the effects of different temperature and salinity conditions on early non feeding stages of sprat. Following general hypotheses will be tested within the subsequent chapters:

1. Temperature has no effect on the survival, development and the duration of non feeding stages (eggs and yolk sac larvae) of sprat. (Chapter 2 and 3)
2. Duration and survival of non feeding stages of sprat (egg and yolk sac larvae) is not altered by changes in salinity regime during development. (Chapter 4 and 5)
3. Sprat non feeding stages (eggs and yolk sac larvae) from different populations are influenced in the same way when exposed to the same thermal history. (Chapter 2 and 6)

### 1.8.3 Aims and applications

From the analysis of important early life traits of sprat from different populations under the influence of varying abiotic factors temperature and salinity, the present thesis attempts to show, if and how regional sprat populations are specifically adapted to the hydrographical characteristics of the region. The results are meant to be directly applicable for recruitment related studies and implementation in population specific Individual Based Models (IBMs).

## 2 The influence of temperature on the development of Baltic Sea Sprat (*Sprattus sprattus*) eggs and yolk sac larvae

### 2.1 ABSTRACT

In spring 2004 and 2005 we performed two sets of experiments with Baltic sprat (*Sprattus sprattus Balticus* Schneider) eggs and larvae from the Bornholm Basin simulating 10 different temperature scenarios. The goal of the present study was to analyze and parameterize temperature effects on the duration of developmental stages, on the timing of important ontogenetic transitions, growth during the yolk sac phase as well as on the survival success of eggs and early larval stages.

Egg development and hatching showed exponential temperature dependence. No hatching was observed above 14.7°C and hatching success was significantly reduced below 3.4°C. Time to eye pigmentation, as a proxy for mouth gap opening, decreased with increasing temperatures from 17 days post hatch at 3.4°C to 7 days at 13°C whereas the larval yolk sac phase was shortened from 20 days to 10 days at 3.8°C and 10°C, respectively. Maximum survival duration of non-fed larvae was 25 days at 6.8°C. Comparing the experimental results of Baltic sprat with existing information on sprat from the English Channel and North Sea differences were detected in egg development rate, thermal adaptation and in yolk sac depletion rate. Sprat eggs from the English Channel showed significantly faster development and the potential to develop at temperatures higher than 14.7°C. North Sea sprat larvae on the other hand, were found to have a lower yolk sac depletion rate compared to larvae from the Baltic Sea. In light of the predictions for global warming, Baltic sprat stocks could experience improved conditions for egg development and survival.

### 2.2 INTRODUCTION

The stocks of many marine fish species are widely distributed and therefore experience different hydrographic conditions. For example, flatfishes such as turbot (*Psetta maxima*), plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) or other fishes like cod (*Gadus morhua*), sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) are distributed in the North Sea in an almost fully marine environment as well as in the brackish waters of the Baltic Sea. For this reason, stocks of the same species are specialized and adapted to prevailing hydrographical conditions in their habitat (Westernhagen 1970; Nissling and Westin 1991, 1997; Kåras and Klingsheim 1997) and these adaptations are considered specific for

the respective population as a result of long-term selection.

Early life stages are most susceptible to mortality and therefore affect recruitment success (e.g. Hjort 1914; Rothschild 1986, 2000; Trippel and Chambers 1997; Houde 2002). The early ontogenetic stages, i.e. eggs and larvae, are strongly influenced by abiotic factors such as temperature, salinity, oxygen or wind forcing (Grauman and Yula 1989; Blaxter 1992; Köster *et al.* 2003). Temperature plays a central role due to its importance in controlling physiological processes (Blaxter 1992; Fuiman 2002). The exact timing of critical transitions during early life history is extremely important for larval survival and thus the success of a cohort. For example, the duration of early development during stages not requiring food (eggs and endogenously feeding yolk-sac larvae) can vary by a factor of 2 – 5 depending on the species (e.g. Bisbal and



Bengtson 1995; Buckley *et al.* 2000). The speed of yolk depletion depends on the surrounding water temperature. As soon as endogenous reserves have been consumed and morphological changes, such as a functional visual system, functional jaw formations and mouth gap opening allow successful foraging

(Lasker 1964), prey availability is essential for the larvae. Therefore, knowledge about the duration and timing of early life stages is a prerequisite for understanding and interpreting match and mismatch situations between larval predators and their prey (Cushing 1972).

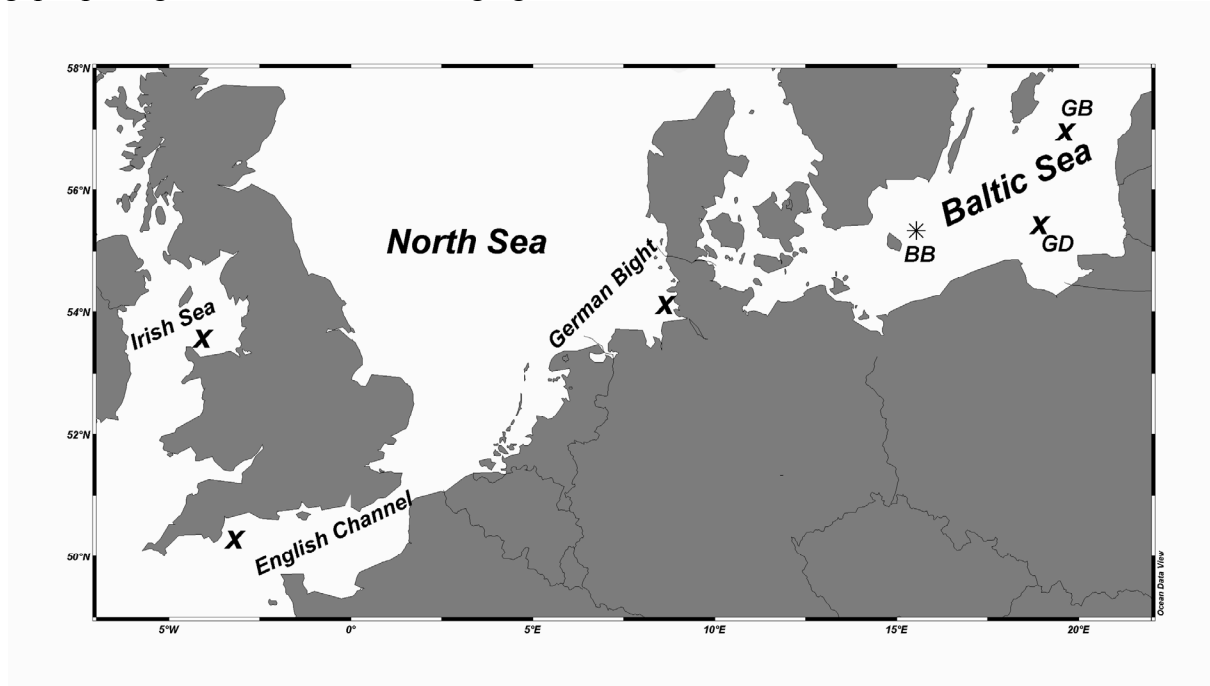


Figure 2-1: Map of hydrographic areas from which data on sprat early life history are available (crosses). From left: Irish Sea, English Channel, North Sea (German Bight), Baltic Sea (GD=Gdansk Deep (ICES Subdivision (SD) 26)), Baltic Sea (GB=Gotland Basin (SD 28)). The asterisk marks the origin of early life stages used for experiments in this study (Baltic Sea (BB=Bornholm Basin (SD 25))).

Temperature can mediate differences in timing and duration of critical life stages both between and within species. Due to the strong temperature influence on metabolism and ontogenetic development, phenotypic plasticity is frequently observed in ectotherms. Consequently, differences in thermal environments during life-history evolution could lead to a change in the optimal set of phenotypes expressed by a genotype (i.e., reaction norm: Pritchard *et al.* 1996; and references therein). In a study on the effect of temperature on the development of aquatic insect eggs, the slope of the average reaction norm was used as an index of adaptation, with positive slopes

indicating cold-adapted species, negative slopes indicating warm-adapted species, and slopes around zero indicating generalist species (Pritchard *et al.* 1996). The slope of this reaction norm can be taken as an indicator of thermal sensitivity. Within the present study, we calculated this index for thermal sensitivity for different sprat stocks to clarify whether a specific adaptation in accordance with temperature exists between North Sea and Baltic sprat. Possible consequences of these adaptations concerning a climate warming are discussed. While sprat are of high ecological and commercial value, little is known about temperature effects on their early life stages. Sprat are multiple batch spawners releasing several thousand pelagic eggs per spawning season, which lasts from

late March to July in the Baltic and from April to August in the North Sea. Due to the low salinity in the Baltic, egg buoyancy is restricted to the more saline, deeper and colder water layers

(e.g. 40-65m in the Bornholm Basin, one of the important spawning areas in the Baltic Sea). As the spawning season progresses the eggs were found higher in the water column.

Table 2-1: Stage specific egg developmental times in days post fertilization [dpf] for Baltic sprat eggs incubated under different temperature regimes. Shown are values of the last observed occurrence of a specific development stage and the first occurrence of hatched larvae.

Trial	1	2	3	3a	4
Month/year	March 2004	April 2004	April 2005	April 2005	June 2005
Method used to obtain fertilized eggs	Stripping	Stripping	Helgoländer Larval net	Helgoländer Larval net	Helgoländer Larval net
Position of sampling	φ : 55° 30.78' N λ: 016° 13.46' E	φ : 54° 59.82' N λ: 015° 35.91' E	φ : 55° 17.86' N λ: 015° 44.88' E	φ : 55° 17.86' N λ: 015° 44.88' E	φ : 55° 17.51' N λ: 015° 45.01' E
Date of sampling	29 <sup>th</sup> March	1 <sup>st</sup> April	27 <sup>th</sup> April	27 <sup>th</sup> April	8 <sup>th</sup> June
Eggs (no.)	2906	287	1125	64	165
Incubation temperatures [°C]	1.8; 3.4; 5.2; 6.8; 8.4; 10.0; 11.6; 13.1; 14.7; 16.0	8.4 and 10.0	3.8; 5.7; 7.6;	3.8; 7.6;	7.5; 9.4; 11.1; 12.9
Salinity	14.8	14.8	13.4	13.4	14.0
Light regime light/darkness [hours]	12/12	12/12	14/10	14/10	14/10
Replicates (beakers per temperature)	4	3	5	1	4
Image software	Image Tool 3.0	Image Tool 3.0	Image Pro-Plus 5.0	Image Pro-Plus 5.0	Image Pro-Plus 5.0
Imaged: alive/frozen	alive	alive	frozen	frozen	frozen
Egg phase: stage development	+	-	-	-	-
Egg phase: egg mortality	+	+	-	-	-
Egg phase: hatching success	+	+	+	+	+
Yolk sac larvae: yolk depletion	-	+	+	+	-
Yolk sac larvae: growth	-	+	+	+	-
Yolk sac larvae: eye pigmentation	-	+	+	+	+
Yolk sac larvae: mouth gape opening	-	+	+	+	+
Larvae: starvation 100% mortality	+	+	-	-	-

Alternately, in the North Sea sprat eggs float in the surface layers (5-20m, Conway *et al.* 1997). Historic observations of egg and larval development are limited to a few temperature regimes and the majority of studies use field caught sprat eggs and larvae which causes variation due to the difference in age (e.g. Ehrenbaum 1936; Morawa 1953). Experimental data on sprat egg and larval development from the English Channel and the North Sea are provided by Thompson *et al.* (1981) and Alshuth (1988). Shields (1989) investigated larval growth in feeding experiments with field caught sprat eggs from the Irish Sea. For the Baltic Sea information is even less abundant. Nissling (2004) provides information on egg developmental times, larval length at hatch, yolk sac depletion and mortality, originating from either strip spawned or field caught eggs from the Gotland Basin and the Gdansk Deep, respectively.

The aims of this study were the parameterization of the timing of

important ontogenetic milestones of sprat eggs and early larval stages from the Bornholm Basin, Baltic Sea, under different temperature scenarios. Such information can be used for example as input parameter for Individual Based Models (IBM's) (e.g. Kuehn *et al.* 2008). To our knowledge this is the first study in this specific spawning area, which (1) investigates in a comprehensive experimental approach Baltic sprat egg stage duration and mortality, as well as time to hatch, hatching rates and size at hatch, growth during yolk sac phase, eye pigmentation, and yolk sac depletion under a broad range of temperature conditions from 1.8°C to 16°C. (2) Results of the present study are compared to corresponding data on sprat early life history from the English Channel, North Sea and other regions of the Baltic Sea in order to assess whether possible population specific differences occur, and if so, (3) what implications this might have for the respective population under climate warming scenarios.

### 2.3 MATERIAL AND METHODS

Four experiments were conducted within the present study using material from the Bornholm Basin of the Baltic Sea. Figure 2-1 shows a map of hydrographic areas from which data on sprat early life history is available for comparison. Table 2-1 provides an overview on the methods and parameters measured in each trial. Sprat eggs were obtained from the Bornholm Basin either by

stripping of running ripe sprat (female sprat with hydrated, fully mature eggs ready to be fertilized) or by *in situ* sampling using a 'Helgoländer Larval net' (143cm in diameter; mesh size 300 $\mu$ m) towed vertically through the water column. For trial 1 and 2 hand stripped sprat eggs were obtained during one cruise with RV Alkor in March/April 2004 whereas experiments 3 and 4 were run with field caught eggs in April 2005 and June 2005, respectively.

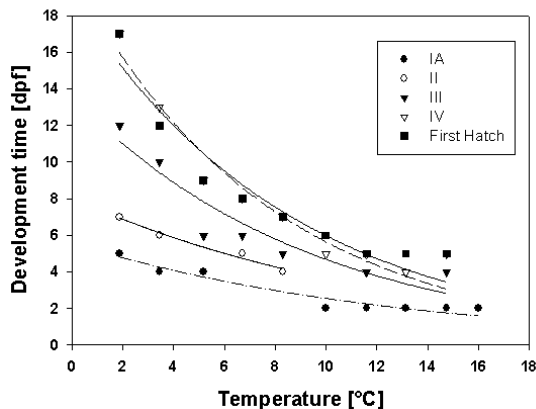


Figure 2-2: Stage specific egg developmental times in days post fertilization [dpf] for Baltic sprat eggs incubated under different temperature regimes. Shown are values of the last observed occurrence of a specific developmental stage and the first occurrence of hatched larvae.

Sprat used for stripping experiments were caught at night with a pelagic trawl (Engel Kombi-Trawl, codend mesh size: 10mm). Trial 1 consisted of eggs from one single female which were fertilized with a mixture of sperm from six males

for 30 minutes in unfiltered surface seawater at ambient salinity of 7.1. Trial 2 followed the same procedure with eggs from another single female and mixture of sperm from another six males. Subsequently eggs were transferred into a 500ml plastic box containing 1.0 $\mu$ m filtered Baltic seawater with a salinity of 18, which keeps fertilized eggs floating (Nissling et al 2003; Nissling 2004). Unfertilized eggs were negatively buoyant and consequently eggs which had sunk to the bottom were removed (Nissling 2004). Subsamples of the remaining floating eggs were checked under a stereo microscope after 12h to ensure fertilization success. The fertilized eggs were stored in darkness and cooled to 6°C in a climatic exposure test chamber (fluctuation max.  $\pm 0.5^\circ\text{C}$ ). All eggs were transported within 38 h to the Leibniz Institute of Marine Science in Kiel and upon arrival separated into 150ml beakers filled with 6°C and 5 $\mu$ m filtered water with a salinity of 14. The salinity of 14 kept the eggs floating at all temperatures. Each beaker containing 30 to 156 eggs was placed into a temperature gradient table. This incubation table was made of aluminium and was heated on one side and cooled on the other side, which created a stable temperature gradient. Up to ten different temperatures could be held at high accuracy (0.08 to 0.17 °C standard deviation) in six replicates. A functional diagram of such an incubation table can be found in Thomas *et al.* (1963) and a similar table was used for experiments with sprat eggs conducted by Thompson *et al.* (1981). After the beakers were placed into the table, they were gently acclimated to the chosen temperature by approximately 1 °C per hour. Trial 1 was performed with 10 different temperatures in 4 replicates (8.4°C and 10.0°C in 3 replicates) whereas trial 2 was restricted to 8.4°C and 10.0°C in 3 replicates due to the low egg numbers obtained for this trial (Table 2-1). Before incubation subsamples of eggs were checked again under a stereo microscope to determine the developmental stage at the start of the incubation. Egg stages were determined based on a modified scale developed by Thompson

*et al.* (1981) for English Channel sprat. All eggs in trial 1 were added to the temperature gradient table at the developmental stage IA. Since the eggs for experiment 2 were obtained one day prior to start they had developed further and had already reached the developmental stage II. The light regime was chosen to be 12L : 12D. Egg mortality was checked daily and dead eggs were removed from the beakers via pipetting (pipette with 5mm diameter). Every 24 hours, digital images of randomly chosen sub-samples (1-2 eggs/beaker) of eggs were recorded with a NIKON CoolPix 995 camera (3.4 Megapixel) under a stereo microscope (WILD M3 Z). Eggs were gently removed from the beakers with the pipette, carefully released in a drop of sampling water (with the same temperature and same salinity) on a petri dish and photographed. Due to the overall low numbers, photographed eggs were immediately and carefully replaced in the respective beaker. Egg developmental stages were determined from the images.

The duration of the egg stages I to IV was defined as days until the last egg of a temperature group reached the next stage. Egg development rate (EDR, % / d) until hatch was calculated, with hatching being defined as the time interval (T) from fertilization, until the first larvae within a temperature had hatched.

$$EDR = \frac{1}{T} * 100 \quad (1)$$

Additionally, daily sprat egg survival was determined.

Sub-samples of hatched larvae were photographed daily and morphometric measurements were taken using image analysis software Image Tool 3.0 UTHSCSA;

<http://ddsdx.uthscsa.edu/dig/itdesc.html>.

Yolk sac depletion rate expressed as (YSDR, % / d) was calculated from the time interval (T) from hatch to depletion of the yolk sac.

$$YSDR = \frac{1}{T} * 100 \quad (2)$$

Larval standard length (SL) of each larva was measured from the tip of the mouth to the end of the notochord. In addition, timing of completed eye pigmentation was recorded as a proxy for mouth gap opening and first feeding (Alshuth 1988; Fukuhara 1990). Occurrence of 100% larval mortality was noted. For trials 3 and 4 sprat eggs were obtained by tows with the 'Helgoländer Larvalnet' in the Central Bornholm Basin (ICES Subdivision 25) during two cruises with RV Alkor in April and June 2005, respectively (Table 2-1). Eggs of both trials were sorted into 500ml plastic containers and stored at 6°C. The fertilized eggs were transported within 48h to the Leibniz-Institute of Marine Science in Kiel. In the laboratory, eggs were transferred individually into 800ml beakers containing 5µm filtered Baltic Sea water using a syringe (5mm diameter). The different development stages of the *in situ* sampled eggs were separated into early (egg stage I and II) and late (egg stage III and IV) development stages and treated as two distinct trials. The beakers were transferred to the temperature gradient table and again temperatures were gently adjusted as was done for the first set of experiment. The photoperiod was adjusted to the time of the year at 14L : 10D (Table 2-1). Corresponding to trial 1 and 2 eye pigmentation was recorded and dead eggs and larvae were removed and counted daily. Larvae of the late developmental stage group of trial 3 were sampled on day 1, 2, 7 and 10 post hatch (dph). Larvae of the early stage group were sampled every 2 to 3 dph to analyse yolk sac area decrease and larval growth. Sampled larvae were immediately frozen at -70°C in seawater and digital images were recorded under a stereo microscope within 2 month of sampling. Morphometric measurements were taken as in trial 1 and 2.

### 2.3.1 Larval growth

In order to determine the effect of freezing and thawing on the length measurements of larvae, one shrinkage experiment was conducted. A total of 42 larvae was measured alive, frozen at -24°C for 540 days and were measured following thawing.

The growth curves for yolk sac larvae at different temperatures were estimated by fitting Laird-Gompertz growth equations:

$$L_t = L_0 * e^{\left( a * \left( 1 - e^{-b * t} \right) \right)} \quad (3)$$

where  $L_t$  is the length (mm) at age  $t$  (in days);  $L_0$  is length (mm) at hatch; and  $a$  and  $b$  are model parameters. This function has previously been used to model larval growth rates of clupeoid fish larvae (Munk 1993; Gaughan *et al.* 2001; Llanos-Rivera and Castro 2006)

## 2.4 RESULTS

### 2.4.1 Egg phase

Depending on the temperature, the time to first hatch varied between 5 days post fertilization (dpf) at 14.7°C and up to 17 days at 1.8°C (Figure 2-2). As expected, the duration of each developmental stage decreased with increasing temperature (Table 2-2). Above 14.7°C no successful egg development was observed. At the lowest temperature 1.8°C only two eggs survived, one larva having a malformed yolksac and the other hatching successfully. EDR until first hatch rose from 5.9% to 20% per day with increasing temperature (Figure 2-3). Egg survival varied between the experiments and showed no significant differences between the temperatures (Figure 2-4). In trial 1 survival until hatch was generally low, 1% to 6.5%, compared to 43% and 47% survival in trial 2. However, survival showed an increasing but not significant trend from the lowest temperature to 8.4°C in trial

1, while in trial 2 higher survival was observed at 10°C compared to 8.4°C.

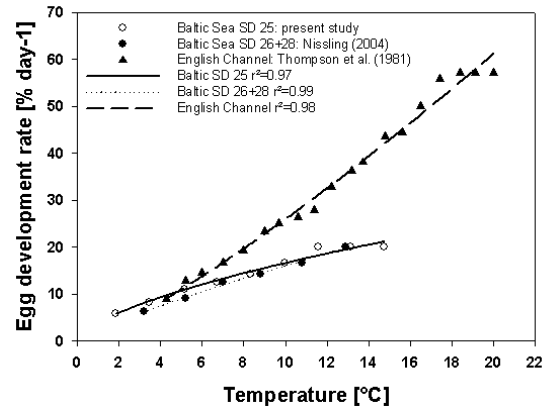


Figure 2-3: Egg development rates in percent per day versus temperature for egg incubation experiments from different areas. Literature values from Nissling (2004) represent 50%-hatch data whereas Thompson *et al.* (1981) and this study reflect first-hatch data for each temperature. Potential equations are fitted to the data.

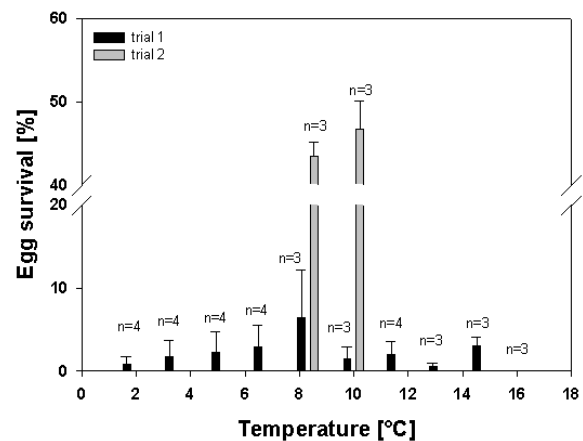


Figure 2-4: Temperature dependent egg survival for two different experimental trials at different incubation temperatures. No survival was observed at 16°C. Same temperatures (8.4 and 10.0°C) were used for trial 1 and 2. Shown are mean (+SD) values of 3 to 4 replicates (n).

### 2.4.2 Larval phase

In the preservation experiment, no significant differences in frozen standard length versus alive standard length were found. An analysis of covariance showed no significant difference in slope ( $p > 0.05$ ) or intercept ( $p > 0.05$ ) from a 1:1 ratio and residuals were normally distributed and showed no trend. Thus it was assumed that the morphometric measurements conducted on frozen material

were comparable to live measurements and no correction was applied.

Laird-Gompertz curves were fitted to larval growth data (Figure 2-5). The calculated length at hatch of 3.4mm and 3.5mm at 10.0°C and 8.4°C, respectively, agreed well with the

observed measured values of 3.5mm for both temperatures (Figure 2-5 a, b). For these temperatures eggs were obtained by fertilizing eggs of a single ripe female. The slopes of the growth curves at 10.0°C and 8.4°C were not statistically different (F-Test,  $p > 0.05$ ).

Table 2-2: Results of the parameterization during the egg stage of sprat egg temperature incubation experiments from the present study and from literature sources. Shown are coefficients of exponential ( $d = a * \exp(-b * T)$ ) functions fitted to the observed durations (d) in days post fertilization per temperature level T (°C). The potential ( $d = a * T^b$ ) equation described the daily proportion of total development time in percent per day (% day<sup>-1</sup>).

Stage duration	Stage /Area	Equation	Coefficients	Std error	r <sup>2</sup>	P	
This study	IA	exponential	a	5.5739	0.3759	0.93	<0.0001
			b	0.0763	0.0093		0.0002
This study	II	exponential	a	8.0628	0.3500	0.98	0.0019
			b	0.0763	0.0092		0.0131
This study	III	exponential	a	13.6062	1.2366	0.89	<0.0001
			b	0.1068	0.0149		0.0002
This study	IV	exponential	a	20.3280	1.4337	0.95	<0.0001
			b	0.1285	0.0126		<0.0001
This study	First Hatch	exponential	a	19.0949	1.4707	0.93	<0.0001
			b	0.1163	0.0131		<0.0001
<b>Egg development rate</b>							
This study	Baltic Sea	potential	a	3.9255	0.4101	0.97	<0.0001
			b	0.6296	0.0446		<0.0001
Nissling (2004)	Baltic Sea	potential	a	2.3657	0.1579	0.99	<0.0001
			b	0.8320	0.0284		<0.0001
Thompson et al. (1981)	English Channel	potential	a	1.5133	0.1713	0.98	<0.0001
			b	1.2353	0.0413		<0.0001

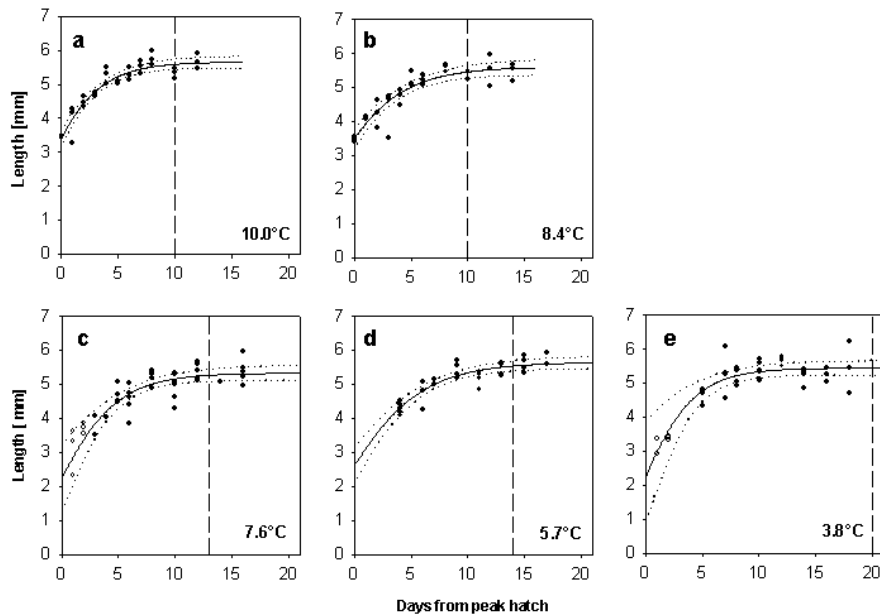


Figure 2-5: Individual length measurements of sprat yolk sac larvae incubated at 10.0°C (a), 8.4°C (b), 7.6°C (c), 5.7°C (d) and 3.8°C (e). Laird-Gompertz growth curves are fitted to the data. Open dots represent measurements from one single replicate only and were not included in the regression analyses. Dotted lines indicate 95% confidence interval of the nonlinear regression. Dashed vertical lines represent age in days at total yolk depletion.

Larvae from the third trial hatched from eggs obtained by *in situ* sampling. The fitted growth curves for 3.8°C, 5.7°C and 7.6°C are shown in Figure 2-5 (c, d, e). For these data length-at-hatch was not observed directly but were estimated based on fitted Laird- Gompertz functions. Neither growth curves nor lengths-at-hatch showed a temperature effect in this trial (Table 2-3).

The timing of total yolk sac depletion, marked by a dashed line within the growth curve plots (Figure 2-5), ranged from 10 days up to 20 days from 10.0°C to 3.8°C. In accordance with these results the observed yolk sac depletion rate increased with increasing temperature (Figure 2-6 and Table 2-3). Baltic sprat larvae showed a higher depletion rate at the same temperature compared to North Sea sprat larvae within the analyzed temperature range. The linear regression of the data obtained from the present study for the Bornholm Basin showed no significant difference to the data published by Nissling (2004) for the Gdansk Deep and the Gotland Basin. An analysis of covariance revealed significant differences in the slopes of linear regression lines between the Bornholm Basin (Baltic Sea) and the North Sea data on yolk sac depletion rate ( $p < 0.001$ ).

For every temperature examined, the time to complete eye pigmentation coincided well with the timing of mouth gap opening (Figure 2-7). Time to pigmentation decreased exponentially with increased temperature. Between 3.8°C and 10.0°C larval development was accelerated by a factor of 2.3 from 16 to 7dph for Baltic sprat larvae. Although the survival of starving sprat larvae showed high variability between different temperatures, a maximum survival was observed at 6.8°C with a mean of 24 dph (Figure 2-8). Temperatures above and below 6.8°C led to earlier mortality.

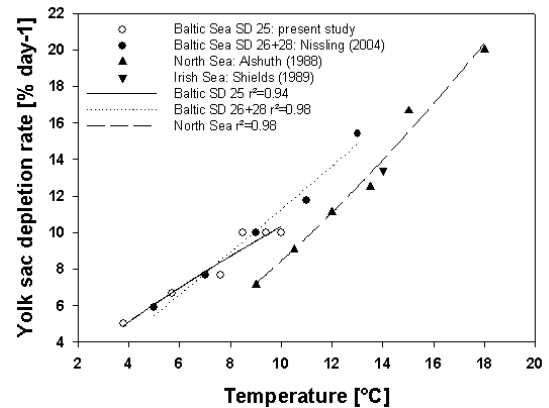


Figure 2-6: Yolk sac depletion rate for yolk sac larvae from different areas incubated at different temperatures. Potential equations are fitted to the data. Data from the North Sea, Irish Sea as well as from Subdivisions (SDs) 26 and 28 from the Baltic Sea are extracted from the literature.

## 2.5 DISCUSSION

### 2.5.1 Egg phase

Sprat egg development was temperature dependent. With increasing temperature, the incubation period from fertilization to hatch was reduced. For temperatures above 5°C, time to hatch was similar to data published by Nissling (2004) for Baltic sprat eggs. Differences between the studies were found at colder incubation temperatures (1-5°C), with Nissling obtaining a 2.5 day longer incubation period to hatch. This could be the result of the different definitions of the periods considered. While Nissling defined the period as the duration from fertilization to 50% hatch, we defined the hatch date where first hatching larvae were observed. By applying first hatch as indicator for the duration of the egg development period the results were shifted in the direction to the fastest developing embryos. Consequently, our model predicts the minimum time required for sprat larvae to hatch after fertilization. Additionally, we had to account for the first 38 hours post fertilization, where all eggs experienced 6°C temperature conditions (transport/acclimatization) which biased our 1.8°C and 3.4°C treatments and caused faster development of the eggs compared

to conditions with low constant temperatures from the time of fertilization. Colder temperatures increased the intra-specific variability of hatching, resulting in some eggs still being at stage IV while other larvae already had hatched. No successful egg development was observed at the lower and upper end of the tested temperature range. The lack of successful hatching at the temperature extremes agreed with results from experiments conducted by Nissling (2004). He found a significantly lower survival of sprat eggs at temperatures below 5°C. However, survival was generally low (0.6-6.5 %) in trial 1 compared to trial 2 (43-46%). Since, in both trials, incubated eggs were gently transferred to waters of higher salinity shortly after fertilization during the hardening phase of the egg membrane, differences in egg volume due to water loss through the membrane could not be excluded. Whether this had a potential effect on development or viability could not be assessed, but results from further experiments under different salinity regimes during incubation suggested the effect to be non-significant (Petereit *et al.*, unpublished data). Since eggs from only one female were used for each trial, the different survival rates might have reflected differences in egg quality due to maternal effects (Brooks *et al.* 1997; Chambers 1997; Trippel *et al.* 1997; Trippel *et al.* 2005).

Thompson *et al.* (1981) performed experiments on sprat egg development from the English Channel using 19 different temperatures from 4.5-20°C and found successful development over all temperatures, although from 17.4-20°C hatching occurred prematurely before many eggs reached stage IV. The authors stated that it was doubtful whether the larvae were sufficiently well developed to survive. Egg survival until hatch between 6° and 18.5°C ranged from 36% to 67%, with higher mortality at the extremes of the used temperatures. These findings of successful egg development above 14.7°C

contradict the findings of the present study and may be related to possible genetic differences, non-genetic adaptations or differences in incubation salinity between the two populations.

Baltic sprat and English Channel sprat showed obvious differences in egg developmental rate. Baltic sprat egg developmental rate increased from 5.9% to 20% per day depending on the temperature (Figure 2-3), whereas this rate for English Channel sprat eggs increased by a factor of five from 9% to 44% per day (temperature range 4.3°C to 14.8°C; Thompson *et al.* 1981). An analysis of covariance between the two linearized data sets of developmental rates revealed significant differences in the slopes ( $p < 0.001$ ) and intercepts ( $p < 0.001$ ).

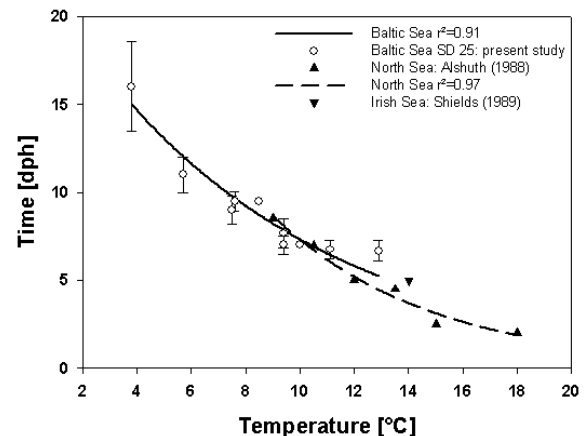


Figure 2-7: Time period from hatch to eye pigmentation of yolk sac larval sprat. Exponential functions were fitted to the data. North Sea and Irish Sea data were extracted from the literature. For Baltic sprat larvae, shown are mean ( $\pm$ SD) values of 3 to 6 replicates.

At temperatures exceeding 5°C, Baltic sprat eggs developed more slowly compared to North Sea sprat eggs. This may have been the result of different salinities during incubation. Increased embryonic development rate at higher salinity has been demonstrated for other species e.g. turbot by Karås and Klingsheim (1997) who also reported a wider temperature range for optimal survival of North Sea turbot eggs (9-18°C) compared to Baltic turbot eggs (14-17°C).



Egg survival rates were highest between 20-35 salinity in the North Sea stock and very low at 15 and below, whereas eggs from the Baltic population had high survival rates at salinities between 10 and 15 (Kuhlmann and Quantz 1980; Karås and Klingsheim 1997). For herring, Holliday and Blaxter (1960) found a negative effect of low salinities on hatching. No significant effect of incubation salinity from 26-36 on 50% hatch was found for Atlantic cod eggs by Laurence and Rogers (1976). Additionally, results from a meta-analysis of the ontogeny of yolk-feeding fish by Kamler (2002) reported no decelerating effects of salinity on ontogenetic rates in the majority of the analyzed studies. However, no experiments examining the effects of salinity on the development rate of Baltic or North Sea sprat eggs have been published, which would have the potential to assess the magnitude of the factor salinity on time to hatch for this species.

The index of thermal sensitivity for Baltic sprat eggs was calculated to be 0.63, (Parameter b of power Function- slope of reaction norm, Table 2), which reflected cold-adaptation according to the gradient of the slope (Pritchard *et al.* 1996). Similarly, the temperature- dependent time-to-hatch data (50% hatch) reported by Nissling (2004) were used, fitted to the power function to obtain 0.83 for the parameter b (Table 2), which also reflected cold-adaptation. It can therefore be concluded, that the experimentally derived egg data from individuals from the three major spawning areas (Bornholm Basin, Gdansk Deep, Gotland Basin) in the Baltic have a cold-adapted egg development. In comparison, English Channel sprat eggs (first hatch data) showed a thermal sensitivity of 1.23 (Parameter b of power Function, Table 2) which reflected warm-

adaptation indicating thermal adaptation differences between the two populations.

According to Peck *et al.* (Prof. Myron Peck, University of Hamburg; unpublished b), no such thermal population differences were found for Gadiformes and Clupeiformes. They compared thermal sensitivity of six populations of Atlantic cod and stated that population specific differences may exist (specificly Baltic Sea) but in this case did not correlate with differences in latitudes. For Atlantic herring populations (Irish Sea / Baltic Sea; spring and autumn spawners) the thermal sensitivity did not differ much (Parameter b from 1.08-1.31), and always exceeded the b value of 1, which reflects more rapid egg development with increasing temperature. All considered Clupeiformes showed warm adaptation except for the sprat population in the Baltic. Fox *et al.* (2003) conducted temperature dependent development experiments with plaice (*Pleuronectes platessa* L.) eggs from the Irish Sea. They found more rapid egg development rates under similar temperature conditions in Irish Sea plaice populations compared to eggs from species from the North Sea. Larvae hatched up to two days earlier from Irish Sea plaice eggs. The authors suggested that known genetic differences between the two stocks could lead to the inter-stock differences in egg development rates. Also, maternal effects (egg size and spawning season) should be taken into consideration as egg development rates were shown to be affected by egg size. Since the application of incorrect egg development rates clearly has the potential to bias the assessment of spawning stock biomass (SSB) using egg production methods, Fox *et al.* (2003) recommended that egg development relationships should be evaluated separately for each stock.

Table 2-3: Results of the parameterization during the larval stage of sprat egg temperature incubation experiments from this study and from literature sources. Yolk sac larval growth was parameterized using Laird-Gompertz growth model, where  $L_t$  is the length (mm) at age  $t$  (in days);  $L_0$  is length (mm) at hatch; and  $a$  and  $b$  are model parameters. Shown are coefficients of exponential ( $d=a*\exp(-b*T)$ ) functions fitted to the observed durations ( $d$ ) in days post hatch until completed eye pigmentation per temperature level  $T$  ( $^{\circ}C$ ). The potential ( $d=a*T^b$ ) functions described the daily proportions of yolk consumption (% day<sup>-1</sup>).

Yolk sac larval growth	Temperature [ $^{\circ}C$ ]	Equation	Coefficients	Std error	$r^2$	P	
This study	10.0	Laird- Gompertz	a	0.5046	0.0366	0.89	<0.0001
Trial 2			b	0.3713	0.0592		<0.0001
			$L_0$	3.4158	0.1274		<0.0001
This study	8.4	Laird- Gompertz	a	0.4723	0.0432	0.82	<0.0001
Trial 2			b	0.2886	0.0609		<0.0001
			$L_0$	3.4964	0.1515		<0.0001
This study	7.6	Laird- Gompertz	a	0.7172	0.0910	0.78	<0.0001
Trial 3 + 3a			b	0.2886	0.0579		<0.0001
			$L_0$	2.6174	0.2609		<0.0001
This study	5.7	Laird- Gompertz	a	0.7762	0.2304	0.79	0.0023
Trial 3 + 3a			b	0.2621	0.0773		0.0022
			$L_0$	2.6019	0.6411		0.0004
This study	3.8	Laird- Gompertz	a	0.8952	0.1300	0.81	<0.0001
Trial 3 + 3a			b	0.3710	0.0677		<0.0001
			$L_0$	2.2970	0.3051		<0.0001
<b>Completed eye pigmentation</b>							
This study	Baltic Sea	exponential	a	23.2675	2.0871	0.91	<0.0001
			b	0.1157	0.0119		<0.0001
Alshuth (1988)	North Sea	exponential	a	40.3277	7.0769	0.97	0.0047
			b	0.1709	0.016		0.0004
<b>Yolk sac depletion rate</b>							
This study	Baltic Sea	potential	a	1.7621	0.4212	0.94	0.0139
			b	0.7688	0.1132		0.0025
Nissling (2004)	Baltic Sea	potential	a	1.0143	0.218	0.98	0.0187
			b	1.0462	0.0911		0.0014
Alshuth (1988)	North Sea	potential	a	0.2705	0.0773	0.98	0.0249
			b	1.4951	0.1061		0.0001
<b>Starvation induced mortality</b>							
This study	Baltic Sea	normal distribution	a	23.0473	1.1308	0.74	<0.0001
	100%		b	4.6151	0.5437		<0.0001
			$X_0$	6.1449	0.4378		<0.0001

### 2.5.2 Larval phase

Larval size at hatch is generally influenced by incubation temperature (Chambers 1997; Blaxter 1992) and larval length at hatch is an important parameter which influences initial locomotion performances such as swimming speed, escape response and predator avoidance (Blaxter 1992). However, in some cases larger larvae experienced higher vulnerability to predation pressure compared to conspecifics at the same age with smaller body size (Litvak and Legett 1992).

Eye pigmentation is essential for the developing larvae as it enables spatial orientation and allows controlled navigation. This event coincided well with mouth gap opening (this study; Alshuth 1988; Shields 1989). A functional visual system is also a prerequisite for successful feeding and improved predator avoidance (Blaxter 1992; Fuiman 2002). This ontogenetic event is positively related to

increasing temperature. As a consequence, eye pigmentation occurs sooner under warmer conditions and vulnerability to predation may be reduced. No prominent differences could be detected between sprat larvae from the Baltic Sea and the North Sea. The literature on North Sea sprat concurs with the results of this study and extends the temperature range at which eye pigmentation was completed within 3.5 dph up to 18.0 $^{\circ}C$  (Alshuth 1988). However, the ambient hydrographical conditions for the early life stages of sprat are not the same in both areas. Mean water temperatures during the time when early life stages of sprat occur are lower in the Baltic Sea (>3-7 $^{\circ}C$ , ICES Oceanographic Database; <http://www.ices.dk/ocean/>) compared to that of the North Sea (mean monthly temperatures 6.7-12.1 $^{\circ}C$  April to June; Loewe *et al.* 2005). Our results clearly indicate that the lower ambient temperatures in the Baltic may extend the

pre-visual phase, and hence protract the period of non targeted swimming and feeding behaviour of sprat larvae in this area.

For anchoveta (*Engraulis ringens*) from two different populations (13° latitude apart) from the coast off Chile, Llanos-Rivera and Castro (2006) found no differences in the duration of the yolk sac phase, yolk consumption rate and larval growth rate until yolk exhaustion when larvae were reared at the same temperature in the range between 12°C and 20°C. The opposite was found for sprat larvae in this study. Yolk sac depletion rates were differently affected under identical temperature conditions for Baltic and North Sea larvae. A higher depletion rate leads to an earlier demand for exogenous food resources and thus increases the risk of starvation.

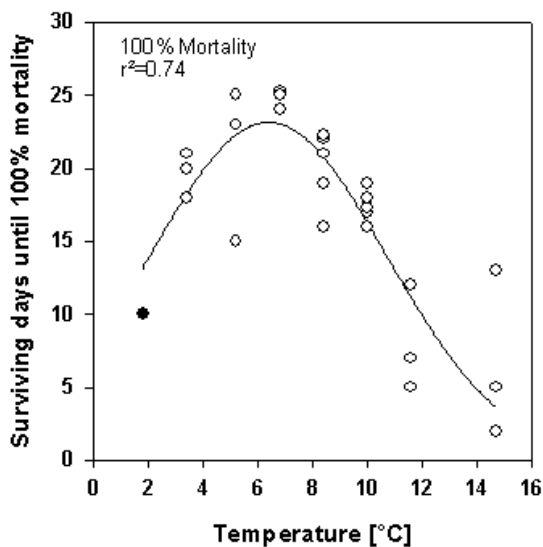


Figure 2-8: Starvation induced 100% mortality of sprat larvae in days after hatching. Shown is the fitted normal distribution curve for mortality (solid line). Each circle represents the time, until the last larvae within a single replicate (beaker) survived. At some temperatures (6.8°C, 8.4°C, 10°C) two replicates were terminated on the same day. Therefore, dots were offsetted graphically, which had no influence on the curve fitting. The single observation (black circle) was not included in the analysis.

For the yolk sac depletion rate, we have seen the same thermal adaptation pattern as

for the egg development. The b-parameters were 0.76 (present study), and 1.04 (Nissling 2004) for the Baltic and 1.49 for the North Sea.

The slopes of the regression lines were statistically different between Baltic Sea and North Sea. Therefore, it may be possible that different thermal adaptations on population level exist indicating that North Sea sprat larvae could cope better with warmer water than Baltic sprat larvae.

### 2.5.3 Population specific future implications

In this study we have shown the close coupling of temperature and the timing and duration of important ontogenic events during the egg and yolk sac larval stages of sprat from the Bornholm Basin (Baltic Sea). Our results suggest that increased temperatures predicted due to global climate change (IPCC 2002) would impact early life stages of sprat in the North Sea and Baltic Sea in different ways.

Assuming an increase of at least 2°C for the ambient water layers where sprat eggs and larvae occur, we would expect differences in the viability of Baltic and North Sea sprat eggs.

Alheit *et al.* (2005) compared temperature time-series for the Bornholm Basin from 1970-1987 and 1988-2003 and found an increase in the spring and autumn surface mixed layer temperature by about 1.5°C. On shorter time scales, temperatures in the upper halocline, i.e. the water layer where sprat eggs occur (Nissling *et al.* 2003) may be affected further due to the inflow of warm summer surface waters from the Kattegatt (Mohrholz *et al.* 2005). This may be advantageous (MacKenzie *et al.* 2007) as present average ambient conditions in the water layer where sprat eggs occur (45 to 65m; ~4°C) range well below the optimal survival temperature (8.4°C) found in this study and (5-13°C) as described by Nissling (2004). Additionally, low temperatures do not only directly influence survival but, as shown in the present study, also prolong the development time thus

increasing the susceptibility to predation or malformation of the embryos (Nissling 2004).

Contrary to the Baltic Sea, in the North Sea sprat eggs and larvae are distributed in the upper 5-20 m of the water column (Conway *et al.* 1997). Wahl and Alheit (1988) report the peak spawning time for North Sea sprat to be in May/June. Mean May temperatures from 1968 to 2005 were 9.1°C with a maximum of 10.9°C in 1990, whereas in June the mean temperature was 12.08°C with the maximum temperature of 14.2°C in 1992 (Loewe *et al.* 2005). Even for this temperature range lethal thermal values for successful egg development are unlikely, but a further warming would move temperature related survival rates further towards the descending leg of the curve.



### 3 The effects of temperature on egg development, growth, morphometric traits and survival of Adriatic sprat (*Sprattus sprattus phalericus*) yolk sac larvae

#### 3.1 ABSTRACT

The Adriatic sprat (*Sprattus sprattus phalericus*) population has severely declined from an important fisheries resource before the mid 1980's to a stock with a biomass <1000t assessed by a hydroacoustic survey in 2004. Causes for the decline are neither consistent nor well understood but are assumed to be related to climate change induced temperature increases. Early life stages (ELS) in fish are known to be susceptible to changes in environmental conditions, with temperature being the main abiotic factor influencing development duration, survival and growth since it controls the rate of physiological processes in ectotherms. Therefore, it is imperative to recognize critical temperatures and temperature-dependent durations of ontogenetic stages to interpret changes in abundance and dynamics of eggs and larvae of the species in focus.

In this study, sprat eggs were artificially fertilized and incubated in two trials at 11 different temperatures from 5 to 19 °C. Mean egg survival was high (>83%) and not significantly influenced by temperature. Duration of developmental stages, time to hatch, eye pigmentation (as proxy for first feeding) and yolk sac depletion were negatively correlated to increased incubation temperature and could be parameterized. Morphometric traits (standard length, yolk sac area, body area) at hatch showed no clear temperature related trends. A growth model for yolk sac larvae was developed with temperature as a controlling factor. Yolk utilization rate increased with rising temperature but no consistent trend with temperature was found for yolk utilization efficiency. The maximum survival potential in the larvae was highest between 8.5°C and 11°C with declining trends towards the upper and lower extreme temperatures. As the egg phase showed a high thermal plasticity, both egg development and survival are most likely not directly and severely impacted by an elevated temperature level. However, if temperatures were raised from 10.7 to 12.9°C yolk utilization rate nearly doubled and resulted in a 3-day reduction of yolk reserves. Maximum unfed larval survival time decreased simultaneously under elevated temperature which additionally reduced the potential time window of opportunity to successfully establish feeding. A temperature increase may have a crucial effect during the early first-feeding phase. To judge the relative importance for the final year class success, additional research on later larval and juvenile life stages is required since these life stages have been shown to be critical in other sprat populations.

#### 3.2 INTRODUCTION

Sprat (*Sprattus sprattus phalericus*) is a small pelagic, clupeid fish species, which is characteristic for the Mediterranean cold biota and was relatively abundant in the northern areas of the Mediterranean Sea (North Adriatic Sea and Gulf of Lyon), but recently became scarce (Bombace 2001). In the Adriatic Sea, sprat distribution is limited to the region ranging from the Istrian peninsula to Italy and the Kvarner Region (Gamulin and Hure 1983). In the Croatian fisheries sprat, together with sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*), represented the

third most important species for the small pelagic fisheries (Dulčić 1998, Tičina 2000), whereas it played only a marginal role for Slovenian fisheries (<35t year<sup>-1</sup>, Marčeta 2001). Despite the local commercial importance only little information on adult sprat biology is available to date, e.g. prey composition, growth characteristics and maturity status (Zavodnik 1969; Zavodnik and Zavodnik 1969; Tičina 2000). Autumn and winter spawning is typical for cold-water species of boreal Atlantic origin (Rass 1949; Daskalov 1999; Bombace 2001).

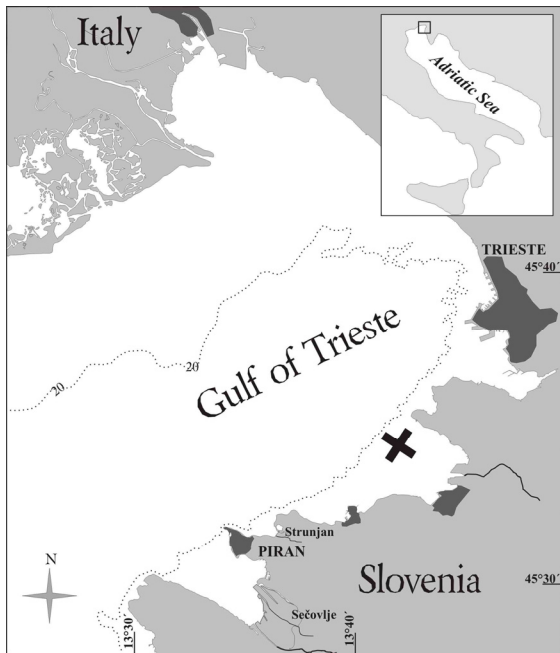


Figure 3-1: Area of origin of the experimental material, Northern Adriatic Sea, Gulf of Trieste, Bay of Koper. The black cross indicates the position at which the sprat used for the experimental trials were caught during the night of 11/12<sup>th</sup> January 2007.

Sprat spawning season in the Adriatic starts in October/November, peaks in December and is terminated in March /April (Štirn 1969; Zavodnik 1969; Zavodnik and Zavodnik 1969; Teskerendžić 1983; Tičina 2000). Water temperatures during this season range from 8.8 to 14°C (Zavodnik 1970; Teskerendžić 1983).

Developmental success of early life stages (eggs and larvae, ELS) are known to largely determine final recruitment success (Hjort 1914; Trippel and Chambers 1997; Houde 2002) due to their high susceptibility to suboptimal environmental conditions. To understand the underlying processes determining the overall ELS survival, it is important to study the influence of temperature, food availability and predation as most critical factors on the different developmental stages. Information on sprat egg and early larval

stages from Mediterranean populations is scarce (Palomera *et al.* 2007; review for NW Mediterranean small pelagics). Although a number of studies analyzed egg abundance data (Zavodnik and Zavodnik 1969; Teskerendžić 1983; Gamulin and Hure 1983; Tičina 2000) only one study has focused on otolith based growth during larval stages in Adriatic sprat (Dulčić 1998).

In recognizing the lack of information, the present study addresses the effect of temperature on eggs and larvae as the most important abiotic factor during early life stages (Blaxter 1992; Chambers and Legett 1987; Fuiman 2002). This is especially relevant as during recent years increasing water temperatures as an effect of climate change were observed in the North Adriatic Sea during the sprat spawning season (Malačič *et al.* 2006).

Petereit *et al.* (2008) recently found population specific differences in thermal adaptation patterns in early life stages of sprat originating from the Baltic Sea and North Sea. A note of caution should thus be added to comparison and transferability of results of temperature effects on timing and duration of important ontogenetic events (egg development duration, eye pigmentation as proxy for first feeding, yolk sac depletion, starvation potential) or larval morphometric traits (size-at-hatch, yolk sac larval growth, yolk utilization rate, yolk utilization efficiency) between sprat populations inhabiting regions characterized by different hydrographic regimes.

The existence of such differences requires population specific laboratory experiments, in which the temperature range should exceed the *in situ* observed ranges. In addition photographic documentation of sprat egg developmental stages and larval ontogeny is provided.

Table 3-1: Data defining the experimental set up: Biotic and abiotic factors, number of females from which eggs were obtained and experimental conditions in test trials for rearing early life stages of Adriatic sprat.

Trial	1	2
Number of sprat females ( <i>n</i> )	1	3
Number of replicates per temperature (beakers)	3	3
Number of eggs per replicate ( <i>n</i> )	24-80	36-98
Temperatures [°C]	5.0; 6.3; 7.3; 8.5; 9.7; 10.7; 11.8; 12.9; 14.1; 15.4	5.0; 6.3; 7.3; 8.5; 9.7; 10.7; 11.8; 12.9; 14.1; 15.4; 19.0
Salinity	37.0±1.5	37.0±1.5
Photoperiod light/dark [hours]	12:12	12:12
Time before start of experiment [dd°]	6	6

### 3.3 MATERIAL AND METHODS

Sprat was caught at night (11/12.01.2007) by purse seine with light-fishery onboard the commercial fishing boat “AMIGO” in the outer Bight of Koper/Slovenia (Figure 3-1). Hydrated eggs were gently stripped from each female (*n*=4) into separate Kautex containers with approximately 500ml of 0.2µm filtered Adriatic Sea water (~37 PSU), and fertilized by a mixture of sperm from 8-10 male sprat. Eggs were kept in a cool box (~10-12°C) and transferred to the Marine Biology Station in Piran within 4 hours after fertilization. Subsamples of eggs (>20 eggs per female) were checked for regular cleavage of the cells and egg sizes (50 eggs per female) were measured at 40x magnification (15/17 Heerbrugg okular with 0.025mm scale part resolution) under a stereomicroscope (Wild M3Z Typ S). From each Kautex container 25 to 100 eggs were carefully transferred with a pipette (5mm diameter) into 250ml glas beakers (= three replicates per temperature), containing 12°C 0.2µl filtered seawater (~37.5 PSU). Two trials were performed; trial 1 used eggs from a single female, while trial 2 used a mixture of eggs from three females (Table 3-1). Replicates of each trial were placed randomly in a temperature gradient table, which was designed for incubating small aquatic organisms, e.g., eggs and larvae of marine fish (Thomas *et al.* 1963; Thompson *et al.* 1981; Petereit 2004; Petereit *et al.* 2008) and gently acclimated (~ 1°C \* h-1) to the experimental

temperature gradient. Eggs from both trials were incubated at 10 different temperatures (5.0; 6.3; 7.3; 8.5; 9.7; 10.7; 11.8; 12.9; 14.1; 15.4°C) in three replicates. However, eggs from trial 2 were additionally incubated at 19°C in a separate water bath. Variation of temperatures was highest (±0.3°C) in the water bath and lower (±0.1°C) in the beakers embedded in the gradient table. Time from fertilization to start of the experiments (under temperature controlled conditions) was 12 hours (this equals 6 degree days) and was considered in all following calculations.

#### 3.3.1 Egg phase

Egg staging was performed following the scheme of Thompson *et al.* (1981) developed for English Channel sprat eggs. Subsampling (5 eggs per beaker) from all temperatures at the start of the experiment (6dd° post fertilization) revealed eggs to be in stage IB. We defined the transition into a new developmental stage as >50% of the eggs in a subsample having reached the new stage (Figure 3-2).

Duration of egg development until hatch was described as a function of temperature by the following equation:

$$f = a * e^{(-b*T)} \quad (1)$$

where T = temperature in °C, a and b are model parameters.

Hatch rates were defined as the percentage of eggs surviving from fertilization to hatch.



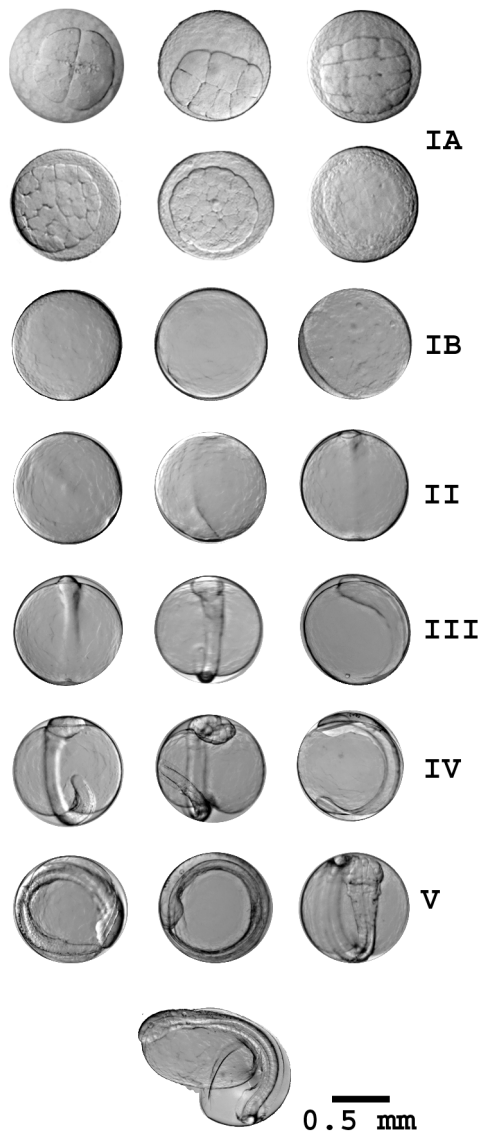


Figure 3-2: Sprat egg development until hatch. First egg cell cleavages in egg stage IA (4-, 8-, 16-, 32-, 64-, 128-cell stages) during the first twelve hours after fertilization [hpf] at a temperature of about 12°C (~6 dd°). From stage IB to V, three examples per stage are depicted in each row. Egg stages are exemplified for the development at 9.7°C. The time sequence in hpf is as follows: IB (12 - 32), II (32 - 56), III (56 - 84), IV (84 - 96), V (96 - 105) and hatch (105 - 108). Scale bar = 0.5mm.

Egg mortality was checked every 10 dd° post fertilization [ddpf] (at 8.5°C, 7.3°C, 6.3°C, 5.0°C additionally every 24 hours) for each beaker. Dead eggs were counted and removed (Table 3-2). 1-2 floating, alive eggs from each beaker were placed in sampling water on a petri dish and photographed after 0, 15, 20, 30, 35, 40

and 45 dd° under a stereomicroscope (Wild M3Z Typ S) at 16x magnification. Eggs were transferred into the respective beakers immediately after imaging (duration <30 sec). The degree day sampling approach was used due to the high number of different temperatures and beakers. Due to logistical problems (too many beakers in the beginning of the experiments at the same time), no staging was conducted at 19°C before 35 dd° after the start of the experiment. Additional staging was performed every 24h at cooler temperatures (Table 3-2).

### 3.3.2 Larval phase

After 30dd° post fertilization [ddpf] egg staging was intensified to a 5 dd° interval. (e.g. max. 24 hours at 5°C, min. 6.5 hours at 19°C, Table 2) This allowed a high resolution in checking for first hatched (FH) larvae. FH was defined as the occurrence of the first larvae in one of the three replicates for each temperature. Peak hatch (PH) was defined as >50% hatched larvae in a beaker.

Randomly, three larvae per beaker were sampled at PH ( $n=9$  per temperature for each trial) and subsequently every 10 degree days post hatch [ddph] and immediately placed onto a micrometer scale (0.1 mm scale bar resolution) in a drop of ambient water and photographed under a stereomicroscope (same equipment as for egg sampling, see above). After recording, larvae were fixed in a 25% ethanol-seawater solution (Gagliano *et al.* 2006) and immediately deep frozen at -70°C for later analysis. Maximum handling time per sample was about 1 minute. Larval mortality was checked every 10 ddph, backcalculated to age in days and dead larvae were counted and removed. Additional checks were performed every 24 hours at cooler temperatures (Table 3-2). Morphometric measurements and determination of eye pigmentation (EP) as a proxy for mouth gape opening and yolk sac depletion (YSD) were performed based on larval images.

Table 3-2: Schedule for egg and larval sampling, staging and mortality checks. Different temperature treatments started after 6 degree days [dd°] post fertilization. Egg staging and larval sampling followed a schedule of degree day (degree days post fertilization [ddpf] and degree days post hatch [ddph]).

Temperatures [°C]	Time equivalent to 10 dd° [hours]	Egg staging [ddpf] + 6	Additional Mortality check eggs [hours]	Larvae sampling [ddph]	Additional Mortality check larvae[hours]
5.0	48	0, 10, 20, 30, 35, 40	24	0, 10, 20, 30, 40, 60	24
6.3	38	0, 10, 20, 30, 35, 40	24	0, 10, 20, 30, 40, 60	24
7.3	33	0, 15, 20, 30, 35, 40	24	0, 10, 20, 30, 40, 60, 80	24
8.5	28	0, 15, 20, 30, 35, 40, 45	24	0, 10, 20, 30, 40, 60, 80	24
9.7	25	0, 15, 20, 30, 35, 40, 45	-	0, 10, 20, 30, 40, 60, 80	-
10.7	22	0, 15, 20, 30, 35, 40, 45	-	0, 10, 20, 30, 40, 60, 80	-
11.8	20	0, 15, 20, 30, 35, 40, 45	-	0, 10, 20, 30, 40, 60, 80	-
12.9	19	0, 15, 30, 35, 40, 45	-	0, 10, 20, 30, 40, 60, 80	-
14.1	17	0, 20, 30, 35, 40, 45	-	0, 10, 20, 30, 40, 60, 80	-
15.4	16	0, 20, 30, 35, 40, 45	-	0, 10, 20, 30, 40, 60, 80	-
19.0	13	0, 35, 40, 45	-	0, 10, 20, 30, 40, 50, 60	-

Larval ages expressed in degree days (dd°) were back calculated to age in days or hours depending on the measured trait. Standard length (SL, mm) was measured from the tip of the mouth until the end of the urostyl, yolk sac length (YSL, mm) and yolk sac heights (YSH, mm) as maximum extensions of the yolk sac. The yolk sac area (YSA, mm<sup>2</sup>) and body area (BA, mm<sup>2</sup>, excluding larval finfolds) were determined by circumscribing and integrating the area with image analysis software (Image Tool 3.0; <http://ddsdx.uthscsa.edu/dig/download.html>). Yolk sac volume (YSV) was calculated using a spheroid formula:

$$YSV = \left(\frac{\pi}{6}\right) \times YSL \times YSH^2 \quad (2)$$

where L is the length of the yolksac and H is the height (Blaxter and Hempel 1963, Hardy and Litvak 2004).

Yolk utilization rate (YUR) for each temperature and trial was determined by calculating slopes from linear regression analysis of the form of  $y = a x + b$  (Sigma

Plot) of YSV on age (YUR = slope of YSV regression).

Yolk utilization efficiency (YUE) was determined by comparing the BA increase (slope of the regression of BA on age: aBA) to the rate of yolk utilized (absolute value of decreasing slope of YSA on age: |aYSA|) until complete absorption of the yolk (YUE = aBA / |aYSA|). The calculation procedures followed the method described in Hardy and Litvak (2004) where they analyzed the YUR and YUE of two larval sturgeon species.

Growth curves for yolk sac larvae were estimated by fitting Laird-Gompertz growth equations, which have previously been used to model larval growth rates of clupeid fish larvae (Munk 1993; Gaughan *et al.* 2001; Llanos-Rivera and Castro 2006; Petereit *et al.* 2008).

$$L_t = L_0 * e^{\left(a * \left(1 - e^{-b * t}\right)\right)} \quad (3)$$

where  $L_t$  is the length (mm) at age  $t$  (in days);  $L_0$  is length (mm) at hatch;  $a$  is a

dimensionless parameter;  $b$  is instantaneous growth rate at age  $t_0$ ;  $t_0$  is the age at which the curve has an inflection point and at which absolute growth begins to decline.

### 3.3.3 Statistics

Data were analyzed using Statistica 5.0 software, Sigma Plot and Stat-Easy. All statistical comparisons were conducted on  $p=0.05$  significance-level. Egg sizes (diameters) were compared by t-test between the trials.

The egg stage duration was compared between the two trials using t-tests (multiple comparisons of means) for independent groups to test for egg size or maternal effects. Durations to FH and PH were compared by covariance analysis ANCOVA (Stat-Easy-software) between the trials (excluding 19°C) after applying ln- transformations on temperature and time (hours post fertilization).

Hatch rates were arcsine- transformed, tested for normal distribution and homogeneity of variances followed by variance components and mixed model ANOVA with “temperature” as fixed factor (excluding 19°C because it was analysed in trial 2 only) and “trial” as random factor.

The morphometric traits at hatch (SL, YSA, BA, BA:YSA ratio) were analysed by mixed model ANOVA with “temperature” as fixed factor and “trial” as random factor followed by post hoc tests. The temperature treatments 8.5 and 14.1°C had to be excluded from the analyses due to delayed sampling and the treatments of 15.4 and 19°C due to the lack of comparable data from the other respective trial.

Time to EP, YSD and YUR were compared between the two trials using covariance analysis (ANCOVA) after applying ln- transformations on temperature, days post hatch and the slope of yolk utilization (parameter “a”)

## 3.4 RESULTS

### 3.4.1 Egg phase

Eggs from trial 1 (single female) had an average diameter of  $0.99\pm 0.03$ mm (mean $\pm$ SD,  $n=55$ ) and eggs from trial 2 (three females) were on average  $0.98\pm 0.05$ mm (mean $\pm$ SD,  $n=71$ ) in diameter. The difference in diameter between trials was statistically not significant ( $p=0.25$ ).

Egg stage durations (Figure 3-3) were negatively related to temperature, where increasing temperature accelerated stage development. Stage IA was completed before 6 dd° after fertilization (Figures 3-2 and 3-3). As stage durations did not differ between trial 1 and trial 2 ( $p=0.92$ ) both experiments were analysed together. Stage durations were described as exponential functions of temperature (Table 3-3).

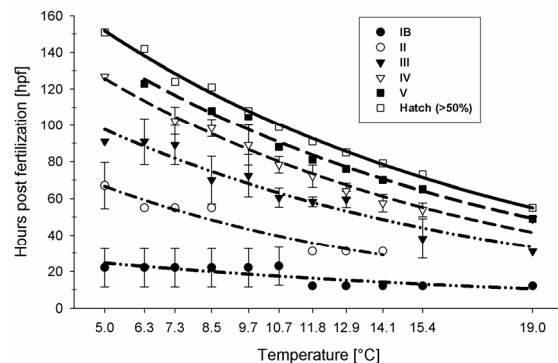


Figure 3-3: Developmental times (hpf) at which  $\geq 50\%$  of the eggs had reached the specified stages related to temperature. Shown are mean values for trials 1 and 2 combined ( $n=6$ ) with standard deviations and fitted exponential functions. Missing values (at 15.4°C and 19°C for stage II and at 5.0°C for stage V) are due to the fact that these stages could not be observed during two sampling intervals. Stage IA was observed between fertilization and start of the different temperature treatments (before 6dd° post fertilization).

FH and PH were temperature dependent (Table 3-4); time to peak hatch was delayed by a factor of  $\sim 3$ , from 55 to 151 hours, when temperature was reduced from 19°C to 5°C.

Table 3-3: Parameter values of equations describing the duration (d) of egg stages (i) in hours post fertilization as exponential functions of temperature (t):  $d_i = a \cdot \exp(-b \cdot t)$  as shown in Figure 3-3.

Egg Stage	Trial	Equation	Coefficients	Std error	p	r <sup>2</sup>	p	
IB	Combined	exponential	a	33.2571	4.9811	<0.0001	0.63	0.0019
			b	0.0609	0.0152	0.003		
II	Combined	exponential	a	105.5099	10.3953	0.0002	0.91	0.0004
			b	0.0918	0.0121	0.0006		
III	Combined	exponential	a	143.2889	10.04	<0.0001	0.93	<0.0001
			b	0.0767	0.0076	<0.0001		
IV	Combined	exponential	a	186.8616	7.5757	<0.0001	0.97	<0.0001
			b	0.0792	0.0041	<0.0001		
V	Combined	exponential	a	199.8196	8.573	<0.0001	0.97	<0.0001
			b	0.0738	0.004	<0.0001		

Table 3-4: Ontogenetic event table for the development of Adriatic sprat larvae incubated at 11 different temperatures. Shown are coefficients of exponential ( $d=a \cdot \exp(-b \cdot T)$ ) and power ( $d=a \cdot T^b$ ) functions fitted to the observed durations (d) per temperature level (T) in hours post fertilization for the events first and peak hatch, and in hours post peak hatch for the events eye pigmentation and yolk sac depletion. The results were calculated for each trial separately (n=3 per temperature level) and for both trial combined (n=6 per temperature level).

Developmental Events	Trial	Equation	Coefficients	Std error	p	r <sup>2</sup>	p	
FH First Hatch	1	exponential	a	227.31	16.21	<0.0001	0.92	<0.0001
			b	0.0798	0.0079	<0.0001		
	2	exponential	a	230.30	9.9461	<0.0001	0.97	<0.0001
			b	0.0775	0.0046	<0.0001		
	combined	exponential	a	228.29	9.07	<0.0001	0.95	<0.0001
			b	0.0783	0.0043	<0.0001		
PH Peak Hatch	1	exponential	a	227.96	8.717	<0.0001	0.97	<0.0001
			b	0.0754	0.0042	<0.0001		
	2	exponential	a	227.58	7.564	<0.0001	0.98	<0.0001
			b	0.00752	0.0035	<0.0001		
	combined	exponential	a	227.74	5.406	<0.0001	0.98	<0.0001
			b	0.0753	0.0025	<0.0001		
EP (MGO) Eye Pigmentation Mouth Gape Opening	1	power	a	1702.36	69.22	<0.0001	0.99	<0.0001
			b	-1.093	0.0202	<0.0001		
	2	power	a	1450.72	118.47	<0.0001	0.95	<0.0001
			b	-1.026	0.040	<0.0001		
	combined	power	a	1572.46	73.26	<0.0001	0.97	<0.0001
			b	-1.06	0.023	<0.0001		
YSD Yolk Sac Depletion	1	exponential	a	544.44	26.74	<0.0001	0.96	<0.0001
			b	0.108	0.005	<0.0001		
	2	exponential	a	620.39	31.17	<0.0001	0.94	<0.0001
			b	0.1126	0.006	<0.0001		
	combined	exponential	a	618.30	25.168	<0.0001	0.93	<0.0001
			b	0.1158	0.0046	<0.0001		

Overall egg survival rates were relatively high (min 83%, max 100% survival; Figure 3-4). The hatch rate from trial 1 was significantly higher compared to trial 2

( $p < 0.001$ ). There was no statistically significant temperature effect when using mean hatch rates (trial1 + trial2) for each temperature ( $p = 0.07$ ).

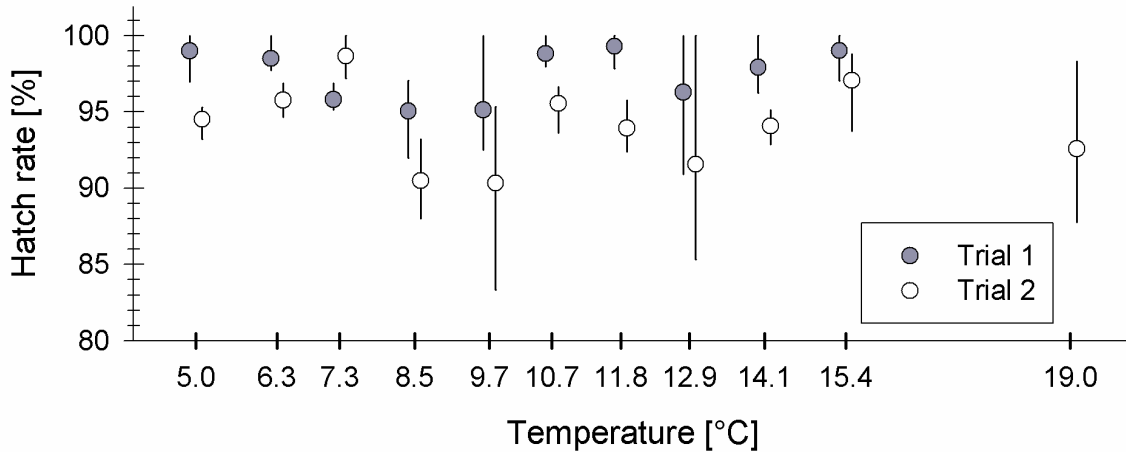


Figure 3-4: Hatch rates (percent survival from fertilization until hatch) of sprat eggs (trial 1 and trial 2 separately) incubated under different temperature levels. Shown are mean and range (minimum and maximum) values per temperature from three replicates. At 19°C only eggs from trial 2 were incubated. Both trials have identical temperature levels as indicates on the x-axis but points were slightly offset to prevent overlaying in the graph.

### 3.4.2 Larval phase

#### 3.4.2.1 Larval morphometric traits at hatch

SL-at-hatch (Figure 3-5a) was not significantly influenced by “temperature” ( $p=0.22$ ) nor by “trial” in general ( $p=0.11$ ). However, at temperatures from 5 to 7.3°C mean larval length was larger in individuals originating from trial 2 compared to trial 1.

YSA-at-hatch (Figure 3-5b) was not significantly influenced by “temperature” ( $p=0.26$ ). The random effect “trial” ( $p=0.058$ ), did not increase variability significantly, although larvae from trial 2 had larger mean YSA in 5 of 7 cases.

BA was larger in larvae from trial 2 in 6 out of 7 temperature treatments (Figure 3-5c). However, neither “temperature” ( $p=0.56$ ) nor “trial” ( $p=0.08$ ) had a significant influence on BA-at-hatch.

The ratio BA:YSA-at-hatch (Figure 3-5d) was significantly influenced by “temperature” ( $p=0.042$ ), and not by “trial” ( $p=0.13$ ). Colder temperatures (5-7°C) had a higher BA proportion compared to YSA.

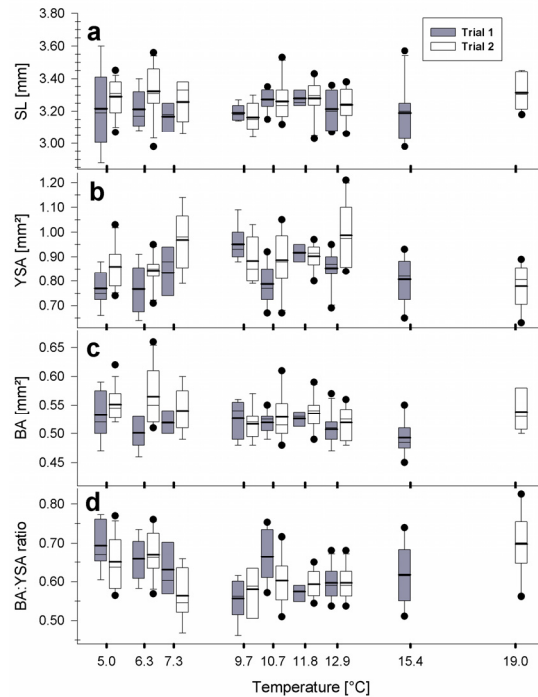


Figure 3-5: Morphometric traits-at-hatch of sprat yolk sac larvae incubated under different temperature levels and two trials. Box-Whisker plots show medians (= thin black lines), 25<sup>th</sup> and 75<sup>th</sup> percentiles indicated in the boxes, 10<sup>th</sup> and 90<sup>th</sup> percentiles indicated by the Whiskers, outliers (dots) and mean values (=thick black bars) for: (a) Larval standard length (SL), (b) yolk sac area (YSA), (c) body area (BA) and (d) the BA:YSA ratio at-hatch. 8.5°C and 14.1°C data were excluded from the analyses due to delayed sampling during the experiment. No larvae were sampled from trial 2 at 15.4°C because hatching from eggs of this trial was later than hatching from trial 1 at this temperature.

### 3.4.2.2 Yolk sac depletion, utilization and efficiency

Elevated temperature levels decreased the time to yolk sac depletion (YSD) in both trials significantly ( $p < 0.001$ ).

The time was increased from ~3 days to ~13 days at 19° and 5.0°C, respectively (Figure 3-6a). There was no statistically significant trial effect, though larvae from trial 2 showed higher potential to retain yolk reserves over a longer time period at temperatures lower than 10°C, with a maximum difference of 1.5 days at 5°C (Figure 3-6a).

YUR ( $\text{mm}^3 \cdot \text{day}^{-1}$ ) increased with raised temperatures (Figure 3-6b). The rate was not significantly different between the two trials.

YUE showed variability within and between temperatures and trials (Figure 3-6c). The highest YUE-range was detected in larvae from trial 2 incubated at 5 and 12.9°C. No significant differences were detected between trials ( $p = 0.91$ ) and temperatures ( $p = 0.16$ ).

### 3.4.2.3 Larval growth and eye pigmentation

Elevated temperature accelerated larval growth in both trials. For example at 15.4°C maximum length increment on yolk reserves was achieved after 5.2 days compared to 8 days at 5°C. Fastest growth occurred at 19°C leading to yolk sac depletion after about 3.2 days. Parameters  $a$  and  $L_0$  of the Laird Gompertz growth equation showed variability but no clear trend (Figure 3-7a and 3-7c) unlike the increasing trend (from  $>6.3^\circ\text{C}$ ) in parameter  $b$  with elevated temperature (Figure 3-7b). A general yolk sac larval growth model (3-7d) was developed based on the mean ( $\pm\text{SE}$ ) values of the Laird-Gompertz parameters  $a = 0.57 \pm 0.04$  and  $L_0 = 3.25 \pm 0.06$  derived from both trials and all temperatures. The parameter “ $b$ ”

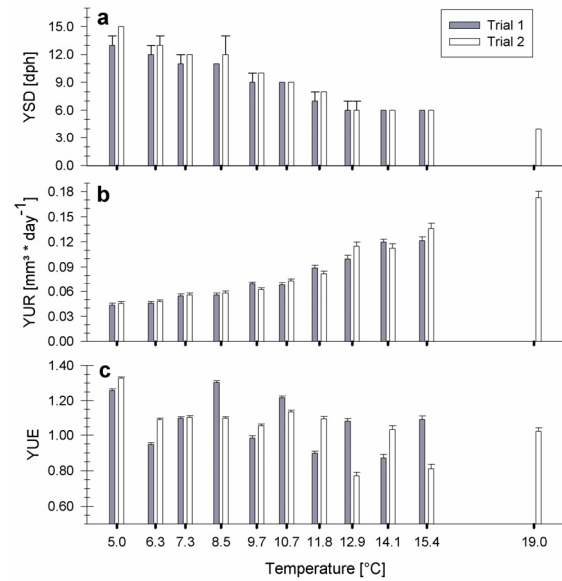


Figure 3-6: Sprat larval yolk resource-related proxies in relation to different temperature levels: (a) Time to yolk sac depletion (YSD) in days post fertilization in both trials (mean+SD). (b) Yolk utilization rates (+SE) (YUR,  $\text{mm}^3 \cdot \text{day}^{-1}$ ) from sprat larvae originating from two different trials vs incubation temperature. Shown are slope values ( $\pm\text{SE}$ ), calculated from the decrease of yolk sac volume (YSV) vs. time, for each temperature treatment and both trials. (c) Yolk utilization efficiency (+SE) (YUE) from sprat larvae originating from two different trials vs incubation temperature. Mean values ( $\pm\text{SE}$ ) were derived from body area (BA) to yolk sac area (YSA) ratios and reflect the conversion efficiency of yolk into somatic tissue.

was described as an exponential function of temperature on the basis of all observed  $b$  values:

$$b = 0.32 \pm 0.036 * e^{(0.059 \pm 0.008 * \text{Temp})}$$

$$(r^2_{\text{adj}} = 0.81, p < 0.0001).$$

Larval ontogenetic development (Figure 3-8a-e and 3-9a-e) and eye pigmentation (EP) was completed after about 3 days at 19°C and 14 days at 5°C (Figure 3-8d and 3-9d). This duration was best described by a power function of temperature (Table 3-4). This function was incorporated into the yolk sac larval growth model (Figure 3-7d, black dots) and length of larvae at this event can be calculated by incorporating this equation into the general growth model (see Appendix).



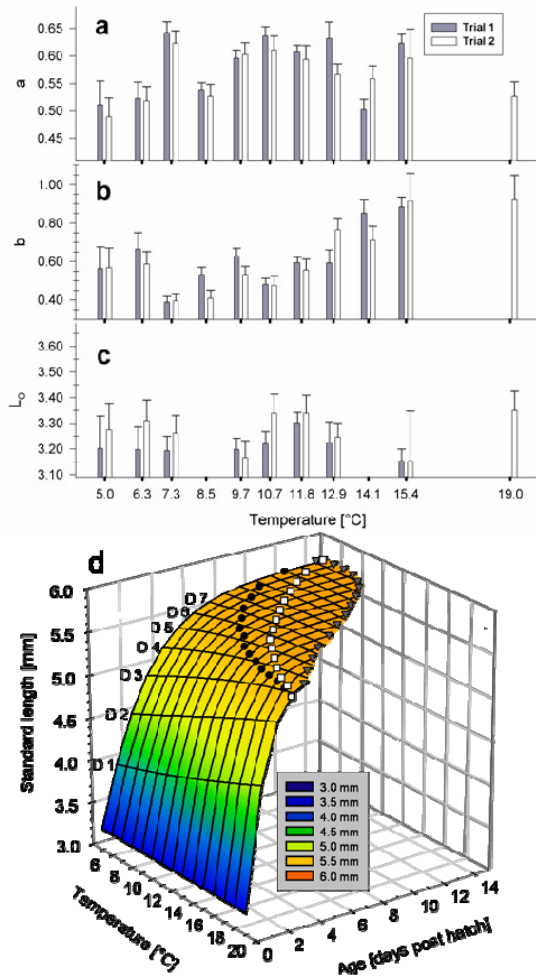


Figure 3-7: (a-c) Laird Gompertz growth parameters ( $a$ ,  $b$ ,  $L_0$  parameters  $\pm$ SE) derived from calculated growth curves based on larval length measurements taken every 10dd post hatch until yolk sac depletion from all temperatures and both trials. (d) Laird-Gompertz yolk sac larval growth model derived from mean values of parameters  $a$  and  $L_0$  from almost all temperatures (excluding 8.5°C and 14.1°C) and both trials. The parameter  $b$  was taken as an exponential function of temperature derived from least square curve fitting to all observed  $b$ , at temperature values (Figure 7b) of both trials. The interval (D) between each horizontal black line bar reflects the time of one day. The black dots represent larval sizes at eye pigmentation (EP) and the white squares larval sizes at yolk sac depletion (YSD) for each temperature. Grey triangles represent maximum survival time.

### 3.4.2.4 Larval survival

Survival of yolk sac larvae in both trials was influenced by temperature (Table 3-5) and the duration (days) of larval survival increased by a factor of 2.5 when temperature was lowered from 19°C to 9.7°C. Larvae incubated at 9.7°C showed maximum survival time (100% mortality) of 15.6 days. Longest mean ( $\pm$ SD) 50% survival was observed at 10.7°C with 11.1 ( $\pm$ 1.1) days for larvae originating from trial 2. Highest variability in 50% survival was observed at 9.7°C (9.6  $\pm$ 3.9 and 9.2  $\pm$ 2.6 days) in both trials and at 11.8°C (8.6  $\pm$ 2.5 days) in trial 1.

If the mean duration of 50% larval survival is expressed as experienced thermal sum (degree-days), survival is about twice as long in the temperature range 9.7-11.8°C compared to the lowermost (5°C) and uppermost (19°C) temperatures.

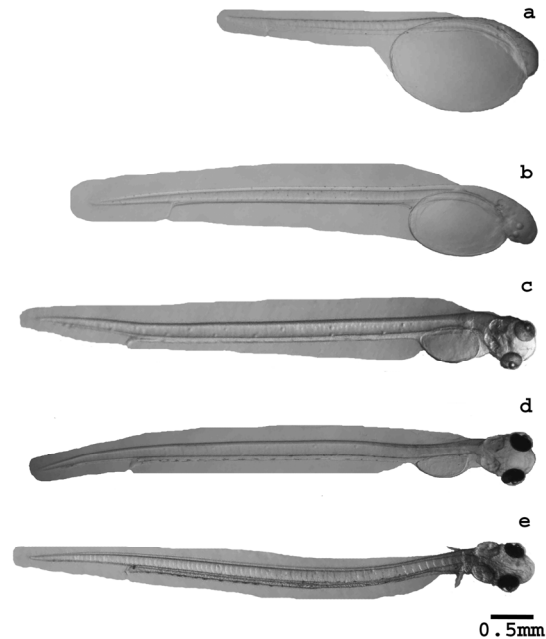


Figure 3-8: Development of sprat larvae, exemplified for development at 9.7°C: (a) newly hatched. (b) at 20 degree days post hatch (ddph) corresponding to 50 hour post hatch (hph). (c) at 40 ddp (100 hph). (d) at 60 ddp (150 hph), the time of eye pigmentation (EP) and mouth gap opening (MGO). (e) at 80 ddp (200 hph), the time of yolk sac depletion (YSD). Scale bar = 0.5mm

Table 3-5: Survival periods of unfed yolk sac larval sprat under different temperature conditions expressed in days post hatch (dph) and degree days post hatch (ddph). Shown are mean values  $\pm$  standard deviations (SD) for the periods up to 50% and 100% mortality as derived from three replicates from each of two trials. The 19°C level was tested in trial 2 only.

Temperature [°C]	Trial	Larval Mortality	Days [dph]	Days SD	Degree Days [ddph]	Degree Days SD
5	1		10.6	0.4	53.1	1.9
	2	50%	10.9	0.3	54.5	1.3
	1		14.0	0	70.0	0
	2	100%	15.0	0	70.0	0
6.3	1	50%	9.4	0.4	59.5	2.3
	2		9.3	0.2	58.8	1.2
	1		11.6	0.9	73.7	5.8
	2	100%	14.3	0	90.0	0
7.3	1	50%	8.9	0.7	64.9	5.0
	2		10.3	0.5	75.0	3.8
	1		14.2	1.6	103.3	11.5
	2	100%	15.1	0	110.0	0
8.5	1	50%	8.7	0.7	74.8	5.8
	2		7.8	0.6	66.5	5.2
	1		15.2	0	130.0	0
	2	100%	15.2	0	130.0	0
9.7	1	50%	9.6	3.9	91.8	37.5
	2		9.2	2.6	88.2	25.3
	1		15.6	0	150.0	0
	2	100%	15.6	0	150.0	0
10.7	1	50%	10.8	1.2	117.8	13.0
	2		11.1	1.1	121.1	12.1
	1		13.8	0	150.0	0
	2	100%	12.8	0.9	140.0	10.0
11.8	1	50%	8.6	2.5	103.5	30.1
	2		5.0	2.0	60.4	2.2
	1		11.1	1.7	133.3	20.8
	2	100%	11.4	0.5	136.7	5.8
12.9	1	50%	8.5	0.8	71.2	62.1
	2		5.8	1.7	73.4	21.0
	1		8.7	2.7	110.0	34.6
	2	100%	7.7	2.3	96.7	28.9
14.1	1	50%	6.1	0.4	86.6	6.2
	2		5.1	1.0	71.9	14.3
	1		8.0	1.6	113.3	23.1
	2	100%	7.6	2.2	106.7	30.6
15.4	1	50%	5.1	2.3	77.1	35
	2		3.3	0.3	49.6	4.5
	1		9.6	0.4	143.3	5.8
	2	100%	8.0	3.5	120.0	52.0
19.0	-		-	-	-	-
	2	50%	2.3	0.3	43.2	5.8
	-		-	-	-	-
	2	100%	6.0	2.4	110.0	43.6



### 3.5 DISCUSSION

#### 3.5.1 Egg phase

Egg development to hatch was accelerated by a factor of 3, from ~6.2 days to ~2.2 days, when temperature was increased from 5°C to 19°C. This is much faster compared to the egg development of Baltic Sea sprat, which takes about 11 days to hatch at 5°C and about 5.5 days at 13°C (Nissling 2004; Petereit *et al.* 2008). Thompson *et al.* (1981) performed incubation experiments with artificially fertilized sprat eggs from the English Channel within a temperature range similar to the present study (4.5-20°C). Compared to Adriatic sprat eggs, they found eggs to develop slower at temperatures below 8.8°C (e.g., ~ 9.3 days at 5°C), almost at the same rate between 9.7-13°C (e.g., ~4 days at 10.6°C) and faster at temperatures exceeding 14°C (e.g., ~1.8 days at 19.0°C). Since differences in sprat egg development duration exist it is recommended to use the population specific rate for application of egg production methods to assess spawning stock biomass (Fox *et al.* 2003). But beside the importance of embryonic duration, egg survival success finally determines the amount of newly hatching larvae.

Survival during the egg stages including subsequent hatching was high (range 83-100%) at all temperature treatments and for both trials with mean hatch rates of >90%. Zavodnik and Zavodnik (1969) found natural egg mortalities at peak spawning in North Adriatic field samplings varying from 10 to 15%. This rate increased during spawning season and could reach 80% mortality. They assumed temperatures lower than 10°C in combination with decreased physiological resistance of the eggs with progressing spawning season to be a potential explanation for the observed mortality increase.

Teskerendžić (1983) found sprat eggs from November until May in Kvarner Bay – Rijeka Region during field samplings and

reported proportions of dead eggs from 11 to 86%. This author excluded temperature variations in the environment as an influencing factor on sprat spawning intensity since she stated that meteorological and hydrographical environmental conditions were favourable at that period. However, there was no definition of “dead” eggs provided in the study and the author found no explanation why the percentages of dead eggs increased and decreased.

The present experimental results do not support the assumption of high mortalities at low temperature values within the observed range, but the survival rates observed by Zavodnik and Zavodnik (1969) at peak spawning matched the experimental observations (~10% mortality). At least eggs (naturally) spawned in December during peak spawning season (Štirn 1969; Zavodnik 1969; Zavodnik and Zavodnik 1969; Teskerendžić 1983; Tičina 2000) have the potential to successfully develop and hatch at temperatures higher than 5°C.

Survival was significantly higher in the trial with eggs from one female. The differences between the trials could be an effect of different egg quality per female (review in Brooks *et al.* 1997; Kamler 2005). An egg size effect could not be fully excluded although the egg size within both trials did not differ significantly, but the genetic contribution (and likely the yolk composition) was more diverse in trial 2 with eggs originating from three females. In comparison to experimental trials with other sprat populations, temperature induced mortalities during the egg phase was exceptionally low in the present study. Thompson *et al.* (1981) found 32-96% egg mortality until hatch under comparable conditions with highest mortality at the extreme temperatures (19-20°C and 4-5°C) and little variation (50-60% mortality) in between (6-18°C), in English Channel sprat eggs. For Baltic Sea sprat eggs Nissling (2004) reported significantly reduced survival at temperatures below

5°C and no differences in mortality from 5-13°C (33-39% mortality). Petereit *et al.* (2008) found high egg mortality (93-99%) from 1.8-16°C with no surviving eggs >14.7°C.

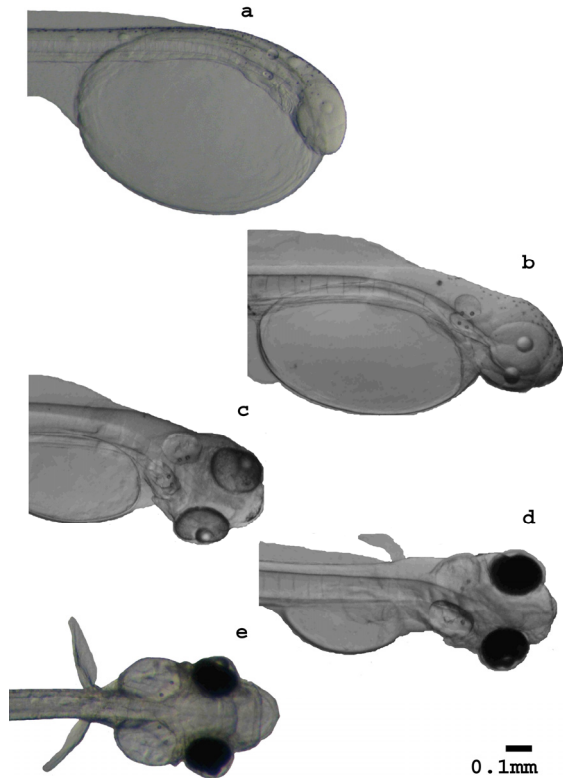


Figure 3-9: Development of the head of sprat larvae with special focus on eye and mouth development, exemplified for development at 9.7°C: (a) Newly hatched larvae. (b) 30 ddp (75 hph). (c) 50 ddp (125 hph) – eyes pigmented brown, mouth gape still closed. (d) 60 ddp (150 hph) – eyes pigmented black, mouth gape open. (e) 80 ddp (200 hph) – eyes fully developed, mouth fully functional, yolk sac depleted. Scale bar = 0.1mm.

The question arises what causes differences in experimental egg survival rates? One of the main differences between all experimental studies is the fishing gear used to obtain ripe sprat for artificial fertilization. While Thompson *et al.* (1981), Nissling (2004) and Petereit *et al.* (2008) used trawled individuals, this study used sprat caught by purse seine fishery, which were scooped out alive from the ring net. This method clearly has the advantage of treating the fish as gently as possible and avoids squeezing effects (increased

discarding of ripe eggs from the ovary due to physical stress) from the mesh and codend before eggs are taken for fertilization.

Incubation salinity during the egg phase could also influence egg survival. Whereas this study used salinities >37 psu, Baltic sprat eggs were incubated at 14 psu. However, Petereit (Chapter 5) found egg survival between salinities of 25, 30, 35 and 37.2 in another experimental approach (at 12°C) to range from ~90% to 100%, indicating that variation in salinity within this range is not likely to explain the observed variation in egg survival. Reasons for differences in mortality and egg development may also be related to genetical differences between the compared populations. Recent studies on population genetics have focused on European sprat population structure (Debes 2007; Limborg 2007; Debes *et al.* 2008). Analyses of nine microsatellite loci by Limborg (2007, Chapter 3) revealed a relative sharp genetic break between samples from North Sea and Baltic Sea corresponding to a steep salinity break and a strong differentiation of the Adriatic Sea population from all other samples. Using molecular markers on fragments of the mitochondrial control region as an alternative method, Debes *et al.* (2008) found that the populations of the two Mediterranean sites to be highly divergent in a comparison of seven sampling sites (Baltic Sea, North Sea, Bay of Biscay, Gulf of Lyon, Adriatic Sea, 2x Black Sea/Bosporus). Since both methods revealed large genetic differences independently between the Adriatic and other sprat populations, it seems possible that the observed differences in temperature related egg development and survival have a genetic basis.

As none of the experimentally analysed temperatures indicate lethal or even diminishing effects on egg survival, exposure of the eggs to expected scenarios of increased temperature (Christensen *et al.* 2007) is unlikely to impact the egg

survival directly. However, also indirect latent effects of temperature during the egg phase have recently been related (Macqueen *et al.* 2008) to significantly reduced numbers in muscle fibres in adult fish (salmon, *Salmo salar*), which demonstrates the importance of considering thermal history during early developmental stages.

As temperature mediates the duration of the egg developmental phase it determines the incidence of the vulnerable egg phase for predation and impacts egg survival indirectly. Whether predation is a cause for major egg mortality in the Adriatic Sea is so far unknown. Not only vertebrate predators occur (>420 fish species; Dulčić and Lipej 2002, Lipej and Dulčić 2004) but also gelatinous plankton blooms have increased recently and could impact egg survival (CIESM 2001, Hay 2006) in the field.

### 3.5.2 Larval phase

Different incubation temperatures did not significantly or consistently affect SL-, YSA- or BA- at-hatch. Contradicting patterns along a temperature gradient have frequently been described for other fish species in the literature. In our data neither a clear trend appeared towards larger (e.g. Martell *et al.* 2005, Chambers 1997), smaller (e.g. Alderdice and Forrester 1971) nor dome-shaped (e.g. Mediola *et al.* 2007) sizes-at-hatch with cooler temperatures. The absence of clear thermal optima of size in morphometric traits is subliminally supported by the high thermal plasticity already found during egg development and low egg mortality over the full range of temperatures.

Yolk resorption is mainly controlled by two factors; activity of hydrolytic enzymes and microvillar extensions (Skjaerven *et al.* 2003), which increase the surface of the syncytium layer (Kamler 2008). Enzyme activity is positively controlled by temperature which explains the increasing yolk demand (YUR) and decreasing time to YSD found in this study. The surface

area of the syncytium layer depends on egg size (Kamler 2008). Egg sizes were not statistically different between the trials. But individuals from trial 2 sustained longer on yolk reserves at temperatures below 9.7°C which might be related to the more diverse genetical contributions originating from three females.

Higher temperatures increased YUR and shortened time to YSD which is in agreement with the results found for sprat larvae previously (Kanstinger 2007; Petereit *et al.* 2008) and also for larvae from a multitude of other fish species (e.g. Houde 1974; Johns *et al.* 1981; Arul 1991; Polo *et al.* 1991; Kamler 1992; Collins and Nelson 1993; Klimogianni *et al.* 2004; Martell *et al.* 2005; Mendiola *et al.* 2007, Sähn 2008).

The increase in YUR at cold temperatures (from 5 to 8.5°C) was low compared to ambient (from 9.7 to 14.1°C) temperatures mirroring the *in situ* temperatures observed during sprat spawning season (Zavodnik 1970; Teskerendžić 1983; Malačič *et al.* 2006). YUR almost doubled from 10.7°C to 12.9°C resulting in a 3-day reduction until yolk reserves were depleted. This reveals that even small temperature increases (2.2°C) might have a major impact on the duration of endogenous feeding.

Non feeding larval survival was highly temperature dependent with longest starvation potential between 8.5°C and 11°C and decreasing trends to the upper and lower extreme temperatures. At 5°C and 19°C mean larval survival was barely restricted to complete yolk consumption which indicates severe thermal-activated derogations. It appears unlikely that such individuals would manage to initiate successful feeding.

### 3.5.3 Conclusions related to recruitment problem, modeling purposes and outlook

Our results did not indicate severe thermal limitations during the egg phase. Elevated temperature conditions accelerated egg development but were not found to be

critical for survival during the egg stage. However, in warming scenarios (Christensen *et al.* 2007) higher temperatures predicted for the spawning season would result in faster depletion of endogenous yolk reserves. This may result in faster larval development and earlier start of endogenous food intake under appropriate feeding conditions for the larvae.

At elevated temperature levels a decreased maximum survival time of larvae might lead to an overall reduction of the “time-window of opportunity” (Peck *et al.*, unpublished a) for first feeding larvae, which could be especially critical under suboptimal feeding conditions.

Voss *et al.* (2003) and Dickmann *et al.* (2007) have shown a generally low first feeding success and feeding frequency in sprat larvae in the Baltic Sea. A further time reduction during acquisition of successful foraging may severely impact the survival of a cohort also in Adriatic Sea sprat larvae.

This study provides parameterized ontogenetic early life history events which can be used as input variables for individual-based models (IBMs) of Adriatic sprat during non feeding stages. Such models allow backtracking and the fate of individuals during their development in time and require detailed data on important life stages and ontogenetic milestones of a species. IBMs have recently been developed and applied for other sprat population (for the North Sea: Kühn *et al.* 2008) or are under preparation for drift studies of non feeding stages (Peck, pers. comm.).

However, to comprehensively analyse recruitment relevant life stages feeding larval and early juvenile stages also need to be considered in future studies. They have been detected to represent important life stages in combination with transport patterns and appropriate temperature conditions for successful recruitment in the Baltic Sea sprat population (e.g. Köster *et al.* 2003; Voss *et al.* 2006; Baumann *et al.*

2006) and in Anchovy (*Engraulis encrasicolus*) population in the Adriatic Sea (review by Regner 1996). Since for Mediterranean sprat populations (North Adriatic Sea, Gulf of Lyon) no information on feeding larvae, early or late juvenile stages exist (Palomera *et al.* 2007; Ana Sabatés (for Northwest Mediterranean) and Vjecoslav Tičina (for Adriatic Sea), personal communications), further research is strongly recommended in that direction.

#### **4 The influence of different salinity conditions on egg buoyancy, - development, - survival and morphometric traits of Baltic Sea sprat (*Sprattus sprattus balticus* Schneider) yolk sac larvae**

##### 4.1 ABSTRACT

Sprat (*Sprattus sprattus*) is a key ecologic and economic player in the pelagic ecosystem of the Baltic Sea. However, longterm stock development is influenced by large variability in recruitment caused by fluctuations in abiotic and biotic conditions during the early life stage. This study concentrates on the influence of different ambient salinities on egg development time, egg buoyancy, egg survival and morphometric traits of early yolk sac larvae from sprat originating from the Bornholm Basin. Egg buoyancy was significantly negatively influenced by increased fertilization/incubation salinity. Buoyancy measurements of field caught eggs in 2007 and 2008 showed annual, as well as seasonal differences in egg specific gravity which are potentially associated with changes in adult sprat vertical distribution. Egg survival showed an increasing tendency to a maximum hatching success between 14 and 18 psu. Time to hatch was 9 days, irrespectively of salinity, but no larvae hatched at the lowest salinity treatment (5 psu). Yolk sac area (YSA) decreased almost linearly with increasing salinity and no difference in the duration of the yolk sac phase was found between all salinity treatments. At eye pigmentation (as proxy for time of first feeding) larvae showed high variance in standard length (SL) between individuals, but no statistically significant differences could be detected. Salinity conditions during the spawning season appeared to be most relevant for modification of the buoyancy of eggs and yolk sac larvae determining their vertical habitat and consequently survival success of these early life stages.

##### 4.2 INTRODUCTION

The population dynamics of sprat (*Sprattus sprattus*) are driven by large variability in recruitment caused by fluctuations in abiotic (Grauman and Yula 1989; Köster *et al.*, 2003) and biotic (e.g. Köster and Möllmann 2000) conditions during early life stages (ELS). Baltic sprat are located at the boundaries of their distribution area in terms of low salinity and low temperature conditions (Muus and Nielsen 1999) with the consequence that adaptations to the particularities of this environment were observed (Kändler, 1941; Parmanne *et al.* 1994). Abiotic conditions vary spatially and seasonally leading to changing conditions for survival and development of ELS. Baltic sprat have a prolonged spawning season which lasts from March-April to July-August (Aro 1989). In previous studies the influence of temperature on sprat eggs and larvae had been in focus (Nissling 2004; Petereit *et al.* 2008). However, for other fish species in

the Baltic Sea, salinity has been determined a critical factor for successful reproduction. Research on Baltic cod (*Gadus morhua*) has uncovered the effects of salinity on egg buoyancy. Recently, Govoni and Forward (2008) have provided a review on buoyancy patterns of eggs, yolk sac larvae and later larval stages of fish. They summarised the general assumptions and mechanisms on how egg density (buoyancy) is derived and controlled in marine fish eggs. It is achieved through passive physiological mechanisms due to the eggs' constituent compounds and through developmental events within the ovary of the female fish (Goarant *et al.* 2007; Govoni and Forward 2008 and references therein). It is known that brackish water eggs have a high water content of >96% (e.g. cod, Thorsen *et al.* 1996), due to the increased water uptake during final oocyte maturation (Craig and Harvey 1987) which is aquaporin-mediated (Fabra *et al.* 2005).

Table 4-1: Origin of biological material, experimental setup, applied methods and parameters measured of individuals used during salinity experiments with Baltic sprat from the Bornholm Basin. The symbol + indicates that data were used from this trial for the respective analysis and the symbol – indicates that no data were available.

	Fertilization Trials		Incubation Trial	Fertilization Trial
Trial	1	2	3	4
Year	2007	2007	2007	2007
Position of sampling	55° 03.53'N 015° 25.21'E	55° 17.10'N 015° 20.70'E	55° 18.32'N 015° 23.35'E	55° 17.78'N 015° 47.23'E
Date of sampling	19 <sup>th</sup> April	21 <sup>st</sup> April	21 <sup>st</sup> April	1st June
Time of sampling (hours)	10:00	18:30	23:00	22:30
No. females/males	4/4	4/12	4/12	4/1
Eggs per replicate (mean±stdev)	247±258	226±144	132±119	157±118
Incubation temperatures (mean±stdev)	6.0±1.0	6.3±1.2	6.3±1.2	8.9±1.0
Salinities:				
At fertilization	8, 14, 20	8, 14, 20	7.5	7, 8, 9, 10
During incubation	8, 14, 20	8, 14, 20	5, 8, 11, 14, 18, 30, 35	
Light regime light/darkness (hours)	24/0	24/0	24/0	24/0
Replicates (beakers per salinity)	3	3	3	3
Egg phase: time to hatch	+	+	+	-
Egg phase: egg survival	+	+	+	-
Egg buoyancy observation	+	+	+	+
Yolk sac larvae: size (SL)-at-hatch	+	-	+	-
Yolk sac larvae: body area (BA)-at-hatch	+	-	+	-
Yolk sac larvae: yolk sac area (YSA)-at-hatch	+	-	+	-
Eye pigmentation: SL, BA, YSA	-	-	+	-
Yolk sac depletion: SL, BA, YSA	+	-	-	-

This is caused by a high intracellular content of free amino acid (FAA), chloride anion (Cl<sup>-</sup>) and ammonia (NH<sub>4</sub><sup>+</sup>), where the high FAA pool is derived from hydrolysis of yolk proteins (Thorsen *et al.* 1996). In a study on Baltic cod eggs, Nissling *et al.* (1994a) found significant correlations of egg buoyancy with yolk osmolality and chorion thickness, but only weak correlations with egg size. One specific question addressed in our study is if fertilization or incubation salinity is influencing or modifying sprat eggs' neutral buoyancy.

Other studies on Baltic cod investigated egg mortality and hatching rates under different salinities (Nissling and Westin 1991a) and egg buoyancy was analysed from experiments and field samples (Nissling and Westin 1991b; Nissling *et al.* 1994a; Nissling and Vallin 1996; Nissling and Westin 1997). The effects of different incubation salinities on size at hatch and larval buoyancy (Nissling and Vallin 1996) or early larval survival, activity-level and feeding ability (Nissling *et al.* 1994b) was assessed from experimental work. For flatfishes like dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and flounder

(*Pleuronectes flesus*) (Nissling *et al.* 2002) or turbot (*Scophthalmus maximus*) (Nissling *et al.* 2006) spermatozoa mobility, fertilization rates or egg survival were analysed at different salinity levels and baselines for successful reproduction potentials could be set. For sprat a single study has concentrated on sprat egg specific gravity and vertical egg distribution (Nissling *et al.* 2003). But to our knowledge no study has been performed on the effects of salinity during fertilization and incubation from the egg phase until the end of yolk sac larval stage. The present study attempts to fill this gap. We have analysed egg and larval survival rates, egg buoyancy and the duration of the egg phase until hatching at different salinities and concentrated on differences in morphometric larval traits (length, yolk sac area, body area) at hatching, eye-pigmentation (as a proxy for first feeding) and yolk sac depletion. Our underlying hypotheses is that fertilization and incubation at different salinities will have an impact on i) the duration of the embryonic phase, ii) egg buoyancy and iii) on morphometric traits of yolk sac larvae. To compare experimental results with field

Table 4-2: Field sampling of sprat eggs from the Bornholm Basin, Baltic Sea: Dates, gears, numbers of stations and numbers of eggs used. The symbol + indicates cruises with successful artificial fertilization experiments, (experiments in 2008 were not successful). Vertical `Helgoländer Larven Netz` (HLN) hauls provided sprat eggs for egg buoyancy measurements in a salinity gradient column.

Cruise	Year	Month	Date	Gear	Experimental Trial	Stations, n	Eggs, n
Al 297	2007	April	14.-15.04	HLN + WP2	+	1	3
Al 297	2007	April	11.04	Multinet		1	5640
Al 299	2007	May/June	27.05	HLN + WP2	+	1	23
Al 299	2007	May/June	27.05	Multinet		1	17015
Al 318	2008	April	14.-15.04	HLN	not successful	3	31
Al 320	2008	May/June	1.-6.06	HLN	not successful	9	520

samples, sprat egg buoyancy and egg size measurements were performed in spring and early summer 2007 and 2008 on selected stations in the Bornholm Basin, Baltic Sea.

### 4.3 MATERIAL AND METHODS

In total, 4 different trials of experiments were conducted. Three of these trials (trials 1, 2 and 4) used different salinities during the egg fertilization and incubation process and therefore are labeled “fertilization trials”. In one trial (trial 3) eggs were fertilized at one salinity and immediately divided up into the different incubation salinities; this trial is labeled “incubation trial”. Details on locations, sampling times and abiotic conditions are listed in Table 4-1. Sprat were caught by pelagic trawl (Engel Combi Trawl, codend mesh size: 10mm) in the Bornholm Basin, Baltic Sea, with RV “Alkor” mid April and June 2007. For numbers of females/males used in each trial see Table 4-1. For trial 1 all eggs were first pooled in one test tube and then carefully allocated in equal amounts into three test tubes containing artificial seawater of salinities [psu] of 8, 14 and 20. One droplet of semen was transferred with a pipette into each of the tubes and gently stirred. Subsequently eggs of all experimental salinities were carefully decanted into beakers containing 150ml 6°C artificial seawater in three replicates. The same procedure was performed in trial 2 (Table 4-1). Eggs in trial 3 were activated in a beaker containing 1µm filtered Baltic seawater of a salinity of 7.5.

Within 5 min after activation, eggs were gently and randomly decanted in glass beakers containing 150ml 6°C artificial (Aquarium Brand) seawater of respective (Table 4-1) salinities in triplicates.

Eggs from trial 4 were only used for density observations and originated from the June 2007 cruise (Table 4-1). Eggs from 4 females were pooled in a dry test tube and gently mixed by turning it up side down once. Next, eggs were decanted randomly in similar numbers into 4 beakers containing salinities of 7, 8, 9 and 10. One drop of semen of one male sprat was activated with each of the salinities and poured carefully into each respective beaker immediately. Then eggs of each salinity treatment were divided up into three beakers filled with new pre-tempered water (8.9°C) of corresponding salinity. Fertilization success was not controlled before eggs were divided up. We defined the period from semen activation (at contact with the eggs) until hatching of the larvae from the chorion as egg phase and determined cumulative egg survival over the whole period. Dead eggs were removed with a pipette (5mm diameter) and 1/3 of the water from each beaker was exchanged daily and refilled with water of the same temperature and respective salinity. Eggs were staged following the scheme presented by Thompson *et al.* (1981) which was modified due to early hatching at the end of stage IV in the Baltic Sea. Every 24 h subsamples of eggs (1-2 \*replicate-1) were carefully extracted with a pipette (5 mm diameter) and checked for

Table 4-3: Mean egg survival rates and durations to important events during sprat egg and yolk sac stage. For each trial, bold labeled numbers of survival indicate significant differences ( $p < 0.01$ ) among salinities and different superscript letters indicate which ones differ. N is the number of independent replicates (beakers) per salinity treatment. "n.d." stands for not determined.

Trial	Salinity	n	Egg survival (%) mean $\pm$ stdev	Duration (days) Fertilization to hatching	Duration (days) Hatching to eye pigmentation (EP)	Duration (days) Hatching to yolk sac depletion (YSD)
1	8	3	6.08 $\pm$ 6.91	9	n.d.	11
1	14	3	21.45 $\pm$ 11.34	9	n.d.	11
1	20	3	12.15 $\pm$ 2.98	9	n.d.	11
2	8	3	<b>0.42 <math>\pm</math> 0.48<sup>a</sup></b>	9	n.d.	n.d.
2	14	3	3.41 $\pm$ 0.50 <sup>b</sup>	9	n.d.	n.d.
2	20	3	3.43 $\pm$ 0.63 <sup>b</sup>	9	n.d.	n.d.
3	5	3	0	no hatch	-	-
3	8	3	7.77 $\pm$ 5.34	9	9	n.d.
3	11	3	8.26 $\pm$ 3.89	9	9	n.d.
3	14	3	9.87 $\pm$ 4.52	9	9	n.d.
3	18	3	10.43 $\pm$ 4.42	9	9	n.d.
3	30	3	7.80 $\pm$ 4.71	9	9	n.d.
3	35	3	8.38 $\pm$ 1.18	9	9	n.d.

developmental stage under a stereomicroscope at 16x magnification. Due to the low overall number of eggs per trial, eggs were carefully placed back into the respective replicate beaker after staging (within <20 s). For ship-technical safety reasons the temperature controlled laboratory onboard of the RV "Alkor" had to be illuminated for 24 h. To proceed with the starting conditions, the same light regime was applied after arrival and transport to the institute's laboratory.

#### 4.3.1 Egg buoyancy

For trials 1, 2 and 3 the positions of the eggs within the glass beakers were determined and categorized (bottom, floating, surface) daily by visual observations. Additionally, (for trials 1 and 3) densities of three eggs (at developmental stage III) per salinity treatment (one egg per replicate) were determined in a salinity gradient column (Coombs 1981; Nissling *et al.* 2003). The positions of the eggs in the column were compared with the positions of 4 density floats of known specific gravity (correlation coefficient of the calibration floats >0.99) at 6°C in a temperature controlled laboratory.

Each egg at stage I from trial 4 was placed at 8.9°C in a new beaker which contained filtered seawater of respective fertilization salinity. For trials with salinity of 7 and 8, water of higher salinity was gently poured with a pipette as long as eggs layed on the bottom of the beaker. Addition of water was immediately stopped when eggs began to float up. Egg density values were calculated from salinity and temperature values if eggs floated for at least 60 min in the middle of the beaker. For the 9 and 10 salinity treatments, water of lower salinity was added since eggs already floated near the surface. As eggs consequently sank towards the bottom, the procedure of pouring higher saline water was performed as described above.

#### 4.3.2 Field sampling

Eggs were caught by either WP-2 (60 cm diameter, 200  $\mu$ m meshsize) or 'Helgoländer Larven Netz' (HLN, 143 cm diameter, 300  $\mu$ m meshsize) towed vertically from 5 m above the bottom to the surface. Eggs were sorted immediately from samples with pipettes (3 mm diameter), staged and size measured at 16x magnification under a stereomicroscope (Wild M3Z). Due to the ships movement, only size classes (small 1.27-1.38 mm;



medium 1.39-1.51 mm; large 1.52-1.58 mm) could be determined. The number of stations sampled and eggs measured differed between cruises (Table 4-2). Whereas in April 2008 just three stations in the Bornholm Basin could be analyzed (31 eggs) the May/June 2008 cruise (9 stations, 520 eggs) covered the northern, central and southern parts of the Bornholm Basin and yielded about 20-30 eggs per size class for density-measurements in the salinity gradient column (see above) in a temperature controlled laboratory (6°C). The depth at which each egg would have had potential density equilibrium was calculated from buoyancy values compared to CTD data (temperature, salinity, oxygen, sigma-t) derived at each sampled station.

In April and May/June 2007 repeated Multinet samplings (MN, 335  $\mu\text{m}$  mesh size, 0.5 m<sup>2</sup> net opening, 5 m depth interval) were performed at the HLN and WP-2 stations and analyzed for egg vertical distribution (Haslob *et al.*, 2007; Harjes 2008). The experimentally derived egg buoyancy data set from May/June 2007 was compared to the observed egg vertical distribution from multinet samples (Table 4-2, Harjes, 2008) and showed high overlap in the depth distribution between both independent methods. Since the sample size of buoyancy measured eggs for April 2007 was too low to cover the whole range of egg vertical distribution the vertical sprat egg distribution derived from Multinet samplings (between 50-70m depth where >90% of eggs were located; from Harjes, 2008) was used instead. Temperature and salinity conditions of respective CTD (ADM CTD) casts were used to calculate sigma-t density values in the depth range of the observed vertical egg distribution.

### 4.3.3 Larval morphometrics

Larval morphometrics were analyzed for experimental trials 1 and 3. From each salinity treatment 8-19 larvae were

randomly sampled at peak-hatch (>50% occurrence of hatched larvae). At eye pigmentation (EP, as proxy for first feeding) at day 9, (trial 3) and yolk sac depletion (YSD) at day 11 (trial 1) sample sizes had to be reduced due to low numbers of remaining larvae. At salinities of 30 and 35 larval mortality was high which resulted in a single surviving larva per treatment. These were excluded from further analysis.

Larvae were placed on a micrometer scale (1/10 mm scaleparts) and photographed under a stereomicroscope (WILD M3Z) equipped with a digital camera system (Canon Ixus Digital Camera). The duration of this procedure was <30 s. Morphometric measurements and determination of EP as a proxy for mouth gap opening and YSD were performed based on larval images. Time of YSD was defined as larvae having less than 5% of original yolk sac area left over. Standard length (SL, mm) was measured from the tip of the mouth until the end of the urostyl. The yolk sac area (YSA, mm<sup>2</sup>) and body area (BA, mm<sup>2</sup>, excluding larval finfolds) were determined by tracing and integrating the area with image analysis software (Image Tool 3.0; <http://ddsdx.uthscsa.edu/dig/download.html>).

### 4.3.4 Statistics

All statistical analyses were performed using the program STATISTICA, STAtEasy and SigmaPlot. No statistical tests were applied to compare egg development durations, durations to EP and time to yolk sac depletion since all larvae reached the respective development stage in all respective salinities at the same (sampling-) day. A 2-factorial (trial and salinity) ANOVA was applied to compare egg survival rates among trial 1 and 2. However, marginal deviations from the homogeneity of variances (Cochran test) were detected, irrespectively of transformations on the data, but normal

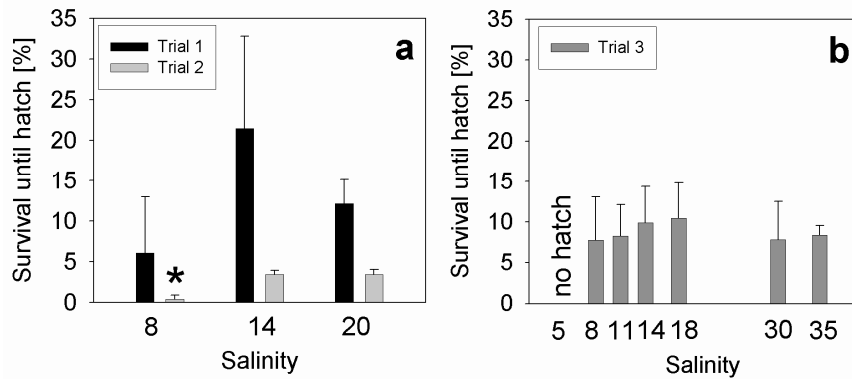


Figure 4-1: Cumulative egg survival from fertilization to hatch ( $n=3$  per salinity; mean  $\pm$  stdev). (a) Survival of eggs fertilized and incubated at different salinities, asterisk marks significant difference between salinities in trial 2 (b) survival of eggs fertilized at 7.5 and subsequently (<5 min) incubated at different salinities.

distribution assumptions were accepted. As a consequence, egg survival of each trial was additionally compared by 1-factorial ANOVAs separately to test for differences among salinities. Egg buoyancy derived from fertilization/incubation experiments, egg densities values among egg size classes and larval morphometrics (SL, YSA, BA) were compared among salinity treatments by one factorial ANOVAs, after meeting the assumptions of variance homogeneity (Levene's or Hartley F-max test) and normality (Shapiro Wilks W test). When significant differences were detected, Tukey HSD tests were applied. Seasonal differences of egg density values, where all egg size classes had been pooled, were compared using a Mann Whitney U-test among April and May/June 2008.

## 4.4 RESULTS

### 4.4.1 Egg phase

#### 4.4.1.1 Egg development and survival

Egg development time from fertilization until hatching was 9 days at 6.0-6.3°C in all experimental salinities (Table 4-3). There was no variation due to different fertilization salinities. Survival from fertilization to hatch was influenced by salinity in fertilization trial 2 ( $p=0.0007$ , Figure 4-1a, Table 4-3 and 4-4). Significantly lower survival (Tukey HSD,  $p=0.001$ ) was seen in the treatments

incubated at salinities of 8 compared to salinities of 14 and 20. The survival of eggs in trial 1 was significantly higher ( $p<0.05$ ) compared to trial 2. In the incubation experiment (trial 3) no hatch occurred at the lowest incubation salinity (5) and among the other salinities treatments in the range from 8-35 psu survival did not differ significantly ( $p=0.95$ ; Figure 4-1b, Table 4-3 and 4-4).

#### 4.4.1.2 Egg buoyancy derived from fertilization/incubation experiments

Irrespective of fertilization salinity (7.5 vs 8, 14, 20) eggs were buoyant at salinities of 14 and above. At 14 approximately 50% of the eggs were floating in the midwater and 50% in the surface layer. At salinities of 5, 8 and 11, eggs were lying at the bottom and at 18, 20, 30 and 35 eggs floated in the surface layer.

Experimental sprat egg buoyancy (egg stage III, Table 4-5) in April 2007 differed significantly between salinity levels 8 and 14 ( $p<0.05$ ), and between 8 and 20 ( $p<0.006$ ). The buoyancy decreased with increasing fertilization salinity (Figure 4-2a) and was best described by a linear function (Table 4-6). This increasing trend was also visible after fertilization at salinity 7.5 and immediate (<5 min after egg activation) transfer to higher saline incubation conditions in trial 3 (Figure 4-2a). In this case however, it was best

described by a polynomial relationship (Table 4-6). The best fit to all experimentally derived density values in relation to salinity was obtained from applying a non linear polynomial second order function to both fertilization/incubation data sets (Figure 4-2a, Table 4-6). A significant increase of

egg density (stage I) with increasing salinity was also found during the June fertilization experiments (Figure 4-2b, Table 4-5). The application of polynomial fitting yielded the highest value for explained variance, being however lower than those in April (Table 4-6).

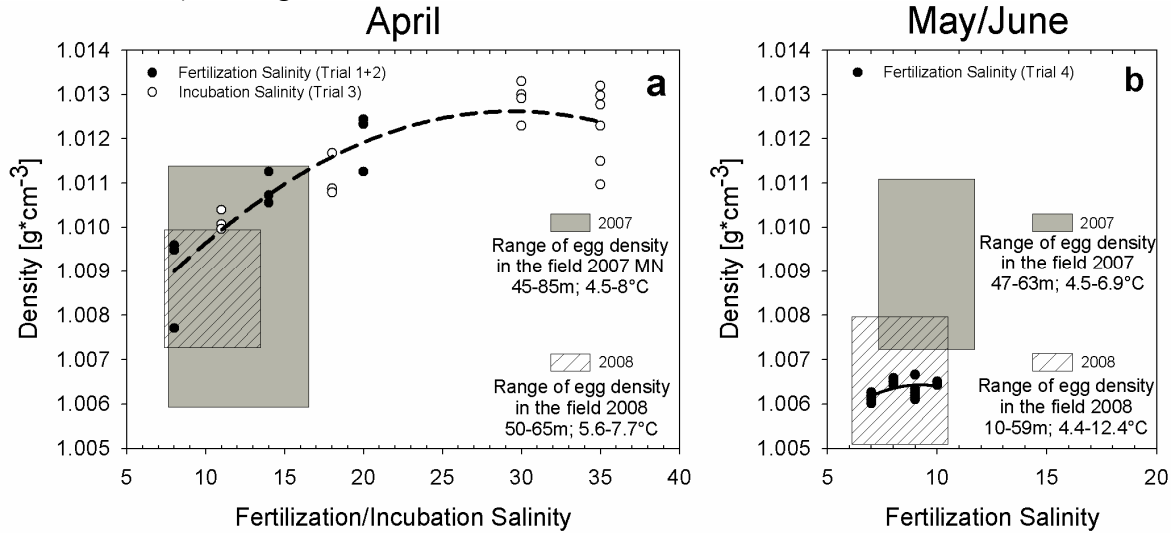


Figure 4-2: (a) Sprat egg density values derived from experimental trials in April 2007 (black dots: fertilization trial (trial 1 + 2); transparent dots incubation trial (trial 3)). Every single data point represents a density value of a single egg from an independent replicate, exceptions at salinity 30 and 35, where more than one egg was taken from each replicate. Second order polynomial regression is fitted to the combined data set (trial 1 + 2 + 3). Box areas (grey=2007; shaded=2008) show vertical range of egg density values and salinity derived from experimental density measurements of field sampled eggs. April 2007 egg densities were calculated from egg vertical distribution obtained by Multinet (MN) sampling (Harjes 2008). Depth and temperature range of the field caught eggs are presented. (b) Sprat egg density values derived from experimental trial in May/June 2007 (fertilization trial 4). Second order polynomial regression is fitted to the data. Box areas show vertical range of egg density values from field samples (as explained above).

#### 4.4.1.3 Egg buoyancy derived from field samplings:

Egg density ( $\text{g}\cdot\text{cm}^{-3}$ ) ranged from 1.006 to 1.011 in April 2007 and from 1.0072 to 1.0112 in May/June 2007 with a mean of 1.009 in both month (Figure 4-2a, b, grey boxes, Table 4-5). The egg density had a narrower distribution in April 2008 compared to April 2007 (Figure 4-2a, Table 4-5) and had about the same mean as the year before. Later in the season in May/June 2008 egg density ranged from 1.005 to 1.008 displaying the lowest density (=highest buoyancy) values of all samplings (Figure 4-2b, Table 4-5). Egg density showed significant differences between the sampling occasions in April

and May/June 2008 (Table 4-4). Mean egg density was higher in April compared to June. Comparison of field caught eggs vs experimentally derived egg densities showed an overlap with buoyancies from fertilization/incubation salinities of 8, 11 and 14 in April 2007 (Figure 4-2a, grey box), however this overlap was low for eggs which were incubated at salinities of 8 and 11 in April 2008 (Figure 4-2a, shaded box). Whereas a high overlap between experimentally and field caught egg derived densities could be observed for the May/June situation in 2008 (Figure 4-2b, shaded box), the situation in 2007 showed no overlap with much higher density values found in field sampled eggs (Figure 4-2b, grey box).

Table 4-4: Statistical results of the analysis of egg survival, egg buoyancy from experimental and field data and morphometric traits of sprat. Relative egg sizes refer to unpreserved, alive eggs and were between 1.27-1.38 mm for small, 1.39-1.51 mm for medium and 1.52-1.58 mm for large eggs. Larval morphometric traits are measured at three important ontogenetic events (hatch, eye pigmentation (EP) and yolk sac depletion (YSD)). SL is standard length, YSA refers to yolk sac area and BA describes larval body area excluding larval finfolds. Significant differences between salinities are contrasted in bold colour.

Parameters	Trial	Traits	Duration / Salinity egg size / events	Time (days)	Statistical test	F	p
Egg survival	1	survival	fertilization – hatch	0	1 factorial ANOVA	F <sub>2,6</sub> = 2.91	0.130
	2			1 factorial ANOVA	F <sub>2,6</sub> = 30.78	<b>0.0007</b>	
	3			1 factorial ANOVA	F <sub>5,12</sub> = 0.21	0.950	
	1+2			2 factorial ANOVA, trial	F <sub>1,17</sub> =16.96	<b>&lt;0.05</b>	
	1+2			2 factorial ANOVA, salinity	F <sub>2,17</sub> =4.07	>0.05	
Egg buoyancy / density EXPERIMENT	1	salinity	8-14-20	0	1 factorial ANOVA	F <sub>2,6</sub> = 12.99	<b>0.007</b>
	3		11-18-30-35	1	1 factorial ANOVA	F <sub>3,12</sub> = 12.49	<b>0.0005</b>
	1+3		8-11-14-18-20-30-35	1	1 factorial ANOVA	F <sub>6,18</sub> = 13.88	<b>0.0001</b>
	4		7-8-9-10	1	1 factorial ANOVA	F <sub>3,20</sub> =11.87	<b>0.001</b>
Egg buoyancy / density FIELD	May/June 2007	egg size	small-medium-large	0	1 factorial ANOVA	F <sub>2,20</sub> = 0.2611	0.77
	April 2008		small-medium-large	1	1 factorial ANOVA	F <sub>2,28</sub> =0.273	0.97
	June 2008		small-medium-large	1	1 factorial ANOVA	F <sub>2,517</sub> =39.38	<b>0.0001</b>
	April 2008 vs May/June 2008		all eggs sizes combined		Mann Whitney U-test		<b>&lt;0.05</b>
Morphometric traits	1	SL	Hatch	0	1 factorial ANOVA	F <sub>2,37</sub> = 2.36	0.10
	1	SL	YSD	11	1 factorial ANOVA	F <sub>2,33</sub> = 0.84	0.43
	1	YSA	Hatch	0	1 factorial ANOVA	F <sub>2,35</sub> = 31.79	<b>0.0001</b>
	1	YSA	YSD	11	1 factorial ANOVA	F <sub>2,33</sub> = 2.79	0.07
	1	BA	Hatch	0	1 factorial ANOVA	F <sub>2,37</sub> = 2.06	0.140
	1	BA	YSD	11	1 factorial ANOVA	F <sub>2,33</sub> = 2.36	0.86
	3	SL	Hatch	0	1 factorial ANOVA	F <sub>5,83</sub> = 4.53	<b>0.001</b>
	3	SL	EP	9	1 factorial ANOVA	F <sub>3,24</sub> = 1.01	0.40
	3	YSA	Hatch	0	1 factorial ANOVA	F <sub>5,84</sub> = 30.40	<b>0.0001</b>
	3	YSA	EP	9	1 factorial ANOVA	F <sub>3,24</sub> = 2.20	0.11
	3	BA	Hatch	0	1 factorial ANOVA	F <sub>5,85</sub> = 1.71	0.14
	3	BA	EP	9	1 factorial ANOVA	F <sub>3,24</sub> = 2.44	0.08

Table 4-5: Mean (± standard deviations), maximum and minimum density values of sprat eggs derived either from experimental trials or from field samplings. Field data from April 2007 were derived from Multinet (MN) samplings with 5 m depth resolution (Harjes, 2008). N represents the number of analyzed eggs per sampling or treatment. Relative egg sizes refer to unpreserved, alive eggs and were between 1.27-1.38 mm for small, 1.39-1.51 mm for medium and 1.52-1.58 mm for large eggs.

Year month	trial	salinity	n eggs	Densities (g*cm <sup>-3</sup> )			Year month	Relative egg size	n eggs	Densities (g*cm <sup>-3</sup> )			
				maximum	minimum	mean ± stdev				maximum	minimum	mean ± stdev	
EXPERIMENT							FIELD						
2007	April						2007	April MN	total	5640	1.011	1.006	1.009
		1	8	3	1.0095	1.0077							
		3	11	3	1.0103	1.0099							
		1	14	3	1.0112	1.0105	2007	May/June	small	12	1.0112	1.0072	1.0090 (±0.0012)
		3	18	3	1.0116	1.0107			medium	7	1.0110	1.0080	1.0090 (±0.0011)
		1	20	3	1.0124	1.0112			large	4	1.0107	1.0076	1.0095 (±0.0013)
		3	30	4	1.0133	1.0122			total	23	1.0112	1.0072	1.0091 (±0.0012)
		3	35	6	1.0131	1.0109							
2007	May/June						2008	April	small	8	1.0097	1.0079	1.0089 (±0.0006)
		4	7	5	1.0062	1.0060			medium	16	1.0099	1.0073	1.0089 (±0.0007)
		4	8	5	1.0065	1.0064			large	7	1.0101	1.0075	1.0089 (±0.0008)
		4	9	5	1.0066	1.0061			total	31	1.0101	1.0073	1.0089 (±0.0007)
		4	10	5	1.0065	1.0064							
							2008	May/June	small	181	1.0080	1.0052	1.0065 (±0.0005)
									medium	151	1.0077	1.0052	1.0062 (±0.0005)
									large	188	1.0074	1.0051	1.0060 (±0.0004)
									total	520	1.0080	1.0051	1.0063 (±0.0005)

Table 4-6: Functions, parameters and statistics of sprat egg density values obtained from different salinity fertilization and incubation experiments in April and May/June 2007.

Salinity	Function	Trial	Month	r <sup>2</sup>	Y <sub>0</sub>	Y <sub>0</sub> SE	p	a	a SE	p	b	b SE	p	DF	p
8_14_20 Fertilization	f=y0+a*x linear	1+2	April	0.76	1.007	0.0007	<0.0001	0.0003	0.0000	0.0012	-	-	-	F <sub>1,8</sub> =27.40	0.0012
11_18_30_35 Incubation	f=y0+ax+bx <sup>2</sup> polynomial	3	April	0.66	1.0063	0.0016	<0.0001	0.0004	0.0002	0.0214	-0.000	0.000	0.0647	F <sub>2,13</sub> =15.65	0.0003
8_11_14_18 30_35 combined	f=y0+ax+bx <sup>2</sup> polynomial	1+2+3	April	0.77	1.0058	0.0008	<0.0001	0.0005	0.0001	<0.0001	0.0000	0.0000	0.0005	F <sub>2,22</sub> =43.15	<0.0001
7_8_9_10 Fertilization	f=y0+ax+bx <sup>2</sup> polynomial	4	May/June	0.25	1.0022	0.0022	<0.0001	0.0009	0.0005	0.0921	0.0001	0.0000	0.1187	F <sub>2,21</sub> =4.88	0.0181

#### 4.4.1.4 Egg size effect on buoyancy

The material from May/June 2007 and April 2008 did not indicate significant dependence of egg buoyancy on egg size (Table 4-4). However, in May/June 2008 egg density was inversely related to egg size. A post hoc test (Tukey HSD) revealed differences between all size groups. Differences in the density of field sampled eggs corresponded to differences in the abiotic conditions as revealed from CTD casts, i.e. mean temperatures of 5.3°C, 6.0°C and 6.3°C and salinities of 8.3, 8.05, and 7.85 for the small, medium and large size class respectively.

#### 4.4.2 Larval phase

##### 4.4.2.1 Morphometric traits of larvae fertilized and reared under different salinities

Larval SL was not significantly different between the salinity treatments, neither at hatch ( $p>0.1$ ) nor at YSD on day 11 ( $p>0.4$ ) (Figures 4-3a, b). Table 4-7 provides a summary of all measured values of larval traits from trial 1 and Table 4-4 provides results of the statistical analysis. However, YSA at hatch was significantly different between salinities treatments with largest mean area at salinity of 8 with  $2.37\pm 0.26\text{ mm}^2$  (Figure 4-3c). On day 11, YSA was reduced to less than 5% of the original area-at-hatch and did not differ

significantly ( $p=0.07$ ) between the salinities groups (Figure 3d). BA was not significantly affected by salinity, neither at hatch ( $p>0.1$ ) nor at yolk depletion ( $p>0.8$ ) on day 11 (Figures 3e, f).

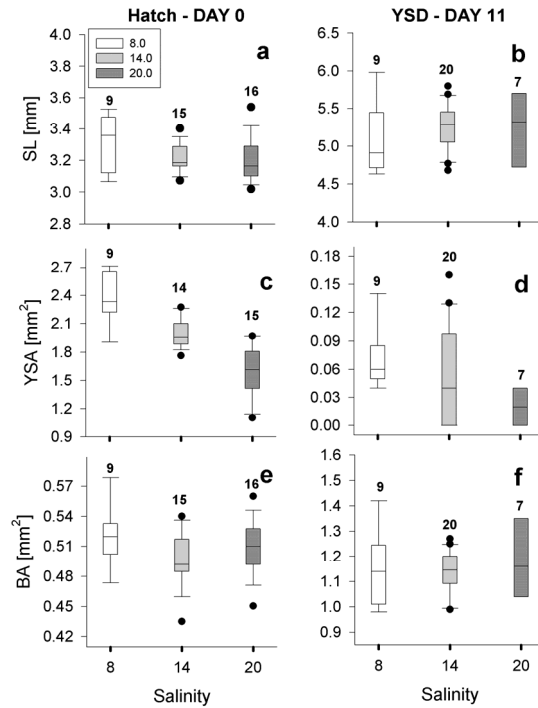


Figure 4-3: Yolk sac larval morphometric traits (standard length (SL, mm), yolk sac area (YSA, mm<sup>2</sup>) and body area (BA, mm<sup>2</sup>)) of Baltic Sea sprat larvae originating from the fertilization trial (trial 1), at hatch (Day 0, left panel) and at yolk sac depletion (YSD -Day 11, right panel). Box-Whisker plots show the median (black bar), the 25<sup>th</sup> and 75<sup>th</sup> percentiles as vertical boxes with 10<sup>th</sup> and the 90<sup>th</sup> percentiles as error bars. Numbers of measured larvae are presented above each Whisker.

Table 4-7: Sprat larval morphometric traits measured in fertilization trial 1. Shown are the mean  $\pm$  standard deviations of n individual larvae per salinity treatment at either hatch or YSD (yolk sac depletion). SL is standard length, YSA refers to yolk sac area and BA describes larval body area excluding larval finfolds. Bold labeled numbers of larval area measurements indicate significant differences ( $p<0.01$ ) among salinities and different superscript letters indicate which ones differ.

Trial	Traits	Event	Time (days)	Salinities					
				8	n	14	n	12	n
1	SL (mm)	Hatch	0	3.31 ( $\pm 0.17$ )	8	3.21 ( $\pm 0.08$ )	15	3.20 ( $\pm 0.13$ )	16
1	SL (mm)	YSD	11	5.07 ( $\pm 0.45$ )	9	5.25 ( $\pm 0.45$ )	20	5.29 ( $\pm 0.53$ )	7
1	YSA (mm <sup>2</sup> )	Hatch	0	<b>2.37 (<math>\pm 0.26</math>)<sup>a</sup></b>	9	<b>2.00 (<math>\pm 0.15</math>)<sup>b</sup></b>	14	<b>1.60 (<math>\pm 0.26</math>)<sup>c</sup></b>	15
1	YSA (mm <sup>2</sup> )	YSD	11	0.07 ( $\pm 0.03$ )	9	0.05 ( $\pm 0.05$ )	20	0.02 ( $\pm 0.02$ )	7
1	BA (mm <sup>2</sup> )	Hatch	0	0.52 ( $\pm 0.03$ )	9	0.50 ( $\pm 0.03$ )	15	0.50 ( $\pm 0.03$ )	16
1	BA (mm <sup>2</sup> )	YSD	11	1.13 ( $\pm 0.14$ )	9	1.14 ( $\pm 0.08$ )	20	1.16 ( $\pm 0.14$ )	7

#### 4.4.2.2 Morphometric traits of larvae fertilized at one and reared under different salinities

The statistical comparisons between salinity treatments in trial 3 are presented in Table 4-4 and a summary of the morphometric trait measurements is compiled in Table 4-8. Larval SL at hatch was largest at a salinity of 14 ( $3.24 \pm 0.09$  mm) compared to 18 and 35 (Figure 4-4a). This significant difference in larval length ( $p=0.001$ ) was abrogated at EP on day 9 (Figure 4-4b). YSA was significantly affected by salinity ( $p=0.0001$ ), where larvae incubated at salinity of 8 had the largest area ( $2.04 \pm 0.2$  mm<sup>2</sup>) and differed from larvae in all other salinities (Figure 4-4c). No significant differences in YSA were observed among larval groups at salinities of 11, 14 and 18, and among those at 30 and 35 (Table 4-8). However, at EP yolk reserves of surviving larvae did not differ significantly ( $p=0.11$ ) among treatments (Figure 4-4d). BA (Figure 4-4e) did not significantly differ at hatch ( $p=0.14$ ). At EP largest body area was measured at the salinity treatment of 8 ( $1.13 \pm 0.09$  mm<sup>2</sup>) and a decrease with increasing salinity is indicated but is statistically not significant ( $p=0.088$ ) (Figure 4-4f).

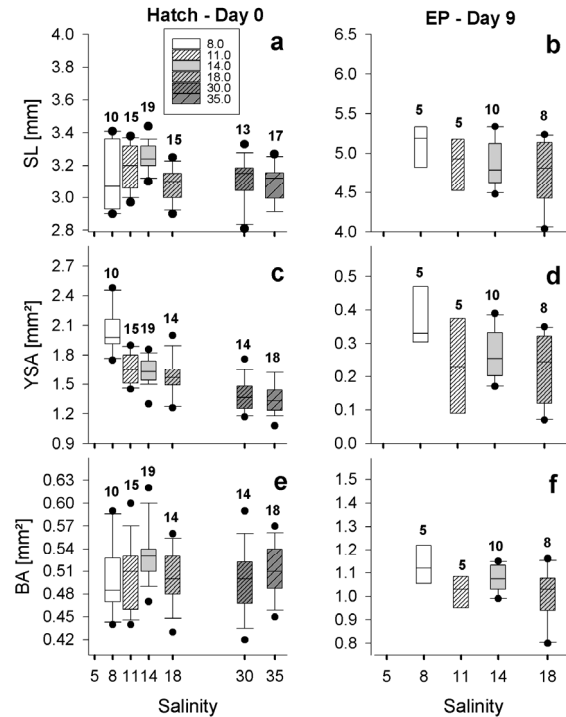


Figure 4-4: Yolk sac larval morphometric traits (standard length (SL, mm), yolk sac area (YSA, mm<sup>2</sup>) and body area (BA, mm<sup>2</sup>) of Baltic Sea sprat larvae originating from the incubation trial (trial 3), at hatch (Day 0, left panel) and at eye pigmentation (EP- Day 9, right panel). Box-Whisker plots show the median (black bar), the 25<sup>th</sup> and 75<sup>th</sup> percentiles as vertical boxes with 10<sup>th</sup> and the 90<sup>th</sup> percentiles as error bars.) Numbers of measured larvae are presented above each Whisker. Too few larvae were left in the 30 and 35 salinity treatments, values are not presented.

Table 4-8: Sprat larval morphometric traits measured in incubation trial 3. Shown are the mean  $\pm$  standard deviations of n individual larvae per salinity treatment at either hatch or EP (eye pigmentation). SL is standard length, YSA refers to yolk sac area and BA describes larval body area excluding larval finfolds. Bold labeled numbers of larval length or area measurements indicate significant differences ( $p < 0.01$ ) among salinities and different superscript letters indicate which ones differ.

Trial	Traits mean $\pm$ stdev	Event	Time (days)	Salinities											
				8	n	11	n	14	n	18	n	30	n	35	n
3	SL (mm)	Hatch	0	3.12 ( $\pm 0.19$ ) <sup>a</sup>	10	3.18 ( $\pm 0.14$ ) <sup>a</sup>	15	<b>3.24 (<math>\pm 0.09</math>)<sup>a</sup></b>	19	3.07 ( $\pm 0.10$ ) <sup>b</sup>	15	3.10 ( $\pm 0.13$ ) <sup>a</sup>	13	3.10 ( $\pm 0.07$ ) <sup>b</sup>	17
3	SL (mm)	EP	9	5.10 ( $\pm 0.32$ )	5	4.86 ( $\pm 0.33$ )	5	4.87 ( $\pm 0.30$ )	10	4.74 ( $\pm 0.43$ )	8	-	-	-	
3	YSA (mm <sup>2</sup> )	Hatch	0	<b>2.04 (<math>\pm 0.20</math>)<sup>a</sup></b>	10	1.68 ( $\pm 0.15$ ) <sup>b</sup>	15	1.63 ( $\pm 0.13$ ) <sup>b</sup>	19	1.57 ( $\pm 0.19$ ) <sup>b</sup>	14	1.37 ( $\pm 0.16$ ) <sup>c</sup>	14	1.34 ( $\pm 0.14$ ) <sup>c</sup>	18
3	YSA (mm <sup>2</sup> )	EP	9	0.37 ( $\pm 0.09$ )	5	0.23 ( $\pm 0.14$ )	5	0.26 ( $\pm 0.07$ )	10	0.25 ( $\pm 0.10$ )	8	-	-	-	
3	BA (mm <sup>2</sup> )	Hatch	0	0.49 ( $\pm 0.04$ )	10	0.50 ( $\pm 0.04$ )	15	0.53 ( $\pm 0.03$ )	19	0.50 ( $\pm 0.03$ )	14	0.50 ( $\pm 0.04$ )	14	0.50 ( $\pm 0.04$ )	18
3	BA (mm <sup>2</sup> )	EP	9	1.13 ( $\pm 0.09$ )	5	1.02 ( $\pm 0.07$ )	5	1.07 ( $\pm 0.05$ )	10	1.00 ( $\pm 0.12$ )	8	-	-	-	

## 4.5 DISCUSSION

### 4.5.1 Egg development duration and survival

Egg development duration of 9 days, independent of salinity, matched well with the calculated 9.5 and 9.2 days at 6°C and 6.3°C incubation temperature based on results of Petereit *et al.* (2008) in previous studies on Baltic sprat for first hatch, where eggs were fertilized at 7.1 and incubated at 14.8 salinity. The independence of hatching time on salinity corresponds to the majority of published reports (Kamler 2002). In contrast Holliday and Blaxter (1960) found two-day retardation to peak-hatch in herring (*Clupea harengus* L.) at salinities of 5.9 and 11.5 compared to 22-45. Laurence and Howell (1981) stated that “it is hard to attach any significance in an ecological sense to differences spanning only 0.6 days”, which they found as a statistically significant results of accelerated egg development at increased salinity (range 28-38) in yellowtail flounder (*Limanda ferruginea*). But for the situation of sprat in the Central Baltic Sea, reduced egg development time could be beneficial for survival due to a high predation pressure on eggs by clupeids (Köster and Möllmann 2000). Sprat eggs in this study were also incubated in salinity conditions exceeding the natural experienced range in the central Baltic Sea (30 and 35), but development time remained the same in all experiments. This implies that mainly other factors like temperature, oxygen or egg size control the duration of embryonic development and related mortality effects. However, there may also be a direct effect of salinity on egg survival, which could be relevant for recruitment

Egg survival differed significantly between both fertilization trials (1+2), showing that the conditions were in some unknown way different, but in both trials mean egg survival was lower at a salinity of 8 compared to salinities of 14 and 20. Though a corresponding effect was not

obvious from trial 3 at salinities ranging from 8 to 35, a trend to higher survival at salinities >8 might exist, probably with a peak around 14-18, but confirmation from further experiments is required. For the situation in the Central Baltic this would imply a higher egg survival in deeper water masses compared to less saline conditions in intermediate water layers or on the slopes of deep basins where spawning occurs. The successful development under high saline (30-35) conditions proves the euryhalin capacity of this species at least during the phase of egg development. Salinity of 5 however, did not lead to completion of egg development. Those eggs were fertilized at a higher salinity, and the question remains open, whether successful fertilization would at all be possible under this low salinity conditions. Sperm motility experiments, using 8 male sprat captured in the Bornholm Basin, revealed that only 2 of them produced viable sperm at salinities of 5 (Petereit, unpublished data). Even if eggs could have been fertilized under low-salinity conditions, no larvae would have survived from these eggs (Sjöblom and Parmanne 1980). Egg survival can also be influenced by low egg buoyancy. Sprat eggs are assumed to have a minimum buoyancy limit of 6 (Kändler 1941) or 6.5 (Hempel 1979). This implies that eggs would sink to the bottom and die if water density would fall below this threshold. At present salinities of 5 or less are very unlikely to occur on the main spawning grounds of sprat in the Central Baltic Sea, but a tendency towards lower salinity may be expected in the future (BALTEX, 2006; Meier 2006; Meier *et al.* 2006).

The importance of abiotic factors on egg buoyancy after releasing the eggs from the ovary into water has been questioned. Hempel (1979) stated that incubation salinity (via perivitelline water) does not contribute to the buoyancy of the eggs in general. This is supported by Nissling *et al.* (2003) who found no significant difference in egg specific gravity in fertilization

experiments with Baltic Sea sprat eggs at salinities of 10 and 15.

However, our study showed clear positive relationships ( $r^2 > 0.75$ ) of increased egg density resulting from increased fertilization (and incubation) salinity at least for the April situation, indicating that salinity as a factor cannot be neglected. Density adjustments of typical teleost eggs, after they had been transferred to markedly different salinities have been described earlier ((Jacobsen and Johansen 1908; Kändler and Tan 1965) both cited in Coombs *et al.* 1985)). A mechanistic explanation based on the permeability of the chorion for water and salts is given by Alderdice (1988). Egg swelling continues as water and ions enter the forming perivitelline fluid until a steady state ensues between the chorion and the increasing hydrostatic pressure (Alderdice 1988). The change in neutral buoyancy of the eggs has been estimated, corresponding to approximately 2 psu at a change in ambient salinity of 10 psu (Coombs *et al.* 1985) and agreeing well with the results from our data.

In principle, the observed differences in buoyancy could have also been derived from differences in egg size. An inverse relation between egg size and egg density has been shown by Nissling *et al.* (2003) for Baltic sprat eggs sampled from the sea. Our results from field samples in May/June 2007 and April 2008 did not support an inverse relation between egg size and egg density; but the May/June 2008 situation revealed a size effect on egg buoyancy. At that time, sampling was performed at 9 stations covering northern, central and southern stations in the Bornholm Basin. In 8 of 9 stations, there was a trend of increased buoyancy with increased egg size. But on two stations, egg buoyancy of smallest eggs was almost identical to medium and large sized eggs. Thus, a sampling effect can not be excluded and may have influenced the result in May/June 2007, with only one station sampled. Otherwise our experimental eggs

originated from several sprat females, but all eggs were pooled and carefully mixed before they were distributed to the respective fertilization/incubation salinities. Thus, we could exclude any potential effect of egg size on the observed density increase with increased incubation salinity at least for the experimental results.

Also, a clear seasonal difference in egg buoyancy could be observed in April and May/June 2008 concurrent to the progressing spawning season. The cause of the seasonal phenomenon is not really understood to date (Nissling *et al.* 2003). This effect might be partly explained by marked differences observed in the vertical distribution of sprat in the course of the spawning season as derived from hydroacoustic survey data (Stepputtis 2006). This also modifies the ambient salinity conditions for egg fertilization. The residence depth of sprat (weighted mean depth) decreased from 60-80 m (halocline and below) in March /April to 40-70 m (halocline, intermediate water layer) in May and to 20-60 m (upper halocline, intermediate and surface layers) in June to August (Daniel Stepputtis, unpublished data). This means that at least during daytime sprat experienced different salinities changing from higher saline to lower saline conditions over the spawning season (e.g in 2007 April 12-17; May 8-15; August 7.3-11). Spawning of Baltic sprat does not seem to be restricted to night times or darkness only, (Holger Haslob, unpublished data) which supports previous reports of daytime independent continuous spawning (Alekseev and Alekseeva 2005). Spawning then might occur in all water layers, especially within the deep layers and within the halocline under higher salinity conditions early in the spawning season. If eggs were spawned under these conditions, they would be activated with higher saline water compared to intermediate or surface layer water. And with progressing spawning season proportionally more eggs would be



activated at lower saline water. Spatial variation in egg density has been described for eggs of Bay of Biscay anchovy (*Engraulis encrasicolus*) where egg density increased with increased sea surface salinity (Goarant *et al.* 2007). For other Baltic species (cod, plaice, flounder and dab) Hohendorf (1968) performed micro measurements of the concentration of blood, urine and ovarian fluid in a decreasing salinity gradient (from west to east in the Baltic Sea). He found a significant decrease in ovarian fluid concentration and a general slight increase in blood concentration from west to east. He concluded that the final adaptation of the specific gravity of the pelagic eggs to the outer medium took place during spawning in seawater in the phase of activation. To our knowledge there is no information on blood or adult body fluid concentrations of sprat available which could help answer how slow or fast osmolality is regulated for this species under changing salinity conditions. Further research in this direction would be desirable. It would be especially relevant to know if such regulative mechanism would react on ambient salinity differences which are found between intermediate water layers or within the halocline. An impact on the osmolality of adult body fluid in response to changes in ambient salinity was hypothesised by Ponwith and Neill (1995) to be the cause for significantly different egg buoyancy values in red drum eggs (*Sciaenops ocellatus* L.). Eggs from two experimental trials originated from a broodstock which was adapted to a salinity of 28 to the first and for the second trial to salinity of 32. They suggested that “even if osmoregulation is marked in fish, euryhaline species may conform to their environment to a certain extent before the cost of active osmoregulation is warranted.”

#### 4.5.2 Consequences of different salinities for yolk sac larvae

Salinity during egg phase had a significant effect on YSA at hatching, with lower values at higher incubation salinities, but the duration of the yolk sac phase was not affected. This implies that suboptimal salinities did not lead to an earlier depletion of endogenous resources, at least in combination with moderate temperature conditions. But the size of the yolk sac also regulates larval buoyancy. Within the first days post hatch, yolk sac larvae stay in the same water layers as the eggs but with progressing development yolk sac volume reduces and neutral buoyancy is not longer achieved. Increased salinity reduces passive sinking speed in larval Baltic cod (Rohlf 1999). Whether higher salinity conditions are favourable for sprat larvae from energetically aspects (i.e. to counteract their sinking speed) was not analyzed within this study but would be an interesting topic to work on in the future. But as for Baltic cod larvae no influence of different salinity conditions was detected on larval swimming performance (Nissling *et al.* 1994b; Rohlf 1999), an increased distance to and extended duration of the vertical migration towards surface layers would be likely under low salinity conditions. Contrary to the YSA, mean BA in all trials was not significantly different between salinities, but varied considerably at EP and YSD in all experimental salinities. This might be explained by phenotypic plasticity differences in the offspring from the several females used.

#### 4.5.3 Conclusion

We have demonstrated that fertilization/incubation salinity can influence egg buoyancy patterns. An egg fertilization/incubation salinity difference of ~12 (8 to 20 or 18-30) resulted in an approximate 0.002 - 0.003 g\*cm<sup>-3</sup> change of egg density and could be described by a polynomial function. Although seasonal egg buoyancy differences could not be

satisfactorily explained by fertilization salinity alone, adult vertical distribution during oocyte maturation might be an important factor. It has long been recognised that vertical distribution of early life stages of fish can strongly influence transport outcomes where current velocity is not vertically uniform. Generally, minor differences in egg buoyancy could cause large differences in the vertical position of the eggs and may expose them to different flow schemes in terms of current magnitude and direction due to vertical current shear. This may result in spatio-temporal differences of transport from spawning grounds to areas with suitable or unsuitable habitat for eggs as well as for larval and juvenile fish. Especially in the Baltic Sea, showing a

strong west-eastward oriented density gradient, transport of eggs from west to east may lead to high fractions of dead eggs. This could be due to the fact that in the water column, the water density reached the minimum buoyancy limit of fish eggs and as a consequence the eggs could sink into less favourable habitat (e.g. less oxygen concentration) or could sink down to the bottom and die. The quantification of eastward-oriented drift of eggs and larval fish and the corresponding contribution of juveniles to eastern Baltic fish stocks may be helpful for management advices especially during periods for which the ratio between the spawning stock biomass of western and eastern stocks is relatively high compared to longterm means.

## 5 Effects of reduced salinity conditions on Adriatic sprat (*Sprattus sprattus phalericus*) egg and yolk sac larval development

### 5.1 ABSTRACT

Sprat (*Sprattus sprattus phalericus*) is considered a relict species of boreal cold biota in the Mediterranean with an established spawning population in the Northern Adriatic Sea. Its stock size has, however, dramatically decreased since the late 1980's for reasons still poorly understood. A laboratory study was designed to detect effects associated with reduced salinity levels on buoyancy, survival, growth and developmental rates of eggs and yolk sac larvae. The experimental results may help explore potentially negative impacts on sprat early life stages. Artificially fertilized eggs (~37 psu) were exposed to 8 different salinity levels ranging from 5 to 37.2 psu. Eggs were positively buoyant at salinities  $\geq 35$  psu. While egg survival was positively related to increased salinity, with no survivors at 5 psu, the time to hatch was not influenced by different salinity regimes. Although embryos developed into viable larvae at salinities  $\geq 10 - 25$  psu, a significant contribution of these larvae to recruitment would not be probable, since a large proportion of the larvae either failed in emerging from the egg shell or showed deformations. At 10 hours post hatch, larval standard length and body area were smallest in individuals incubated at the lowest salinity (25 psu) trial, considering only larvae without malformations. Conversely, yolk sac area was smallest at the highest salinity (37.2 psu). But the small initial size is not likely to have caused an earlier shortage of endogenous energy supply since maximum larval survival time (9.6 days) was observed at 37.2 psu compared to 35 psu (6.3 days).

In conclusion, a general high plasticity in development and survival has been observed. A small decrease in surface salinity during progressing sprat spawning season might impact egg and larval survival, but is considered to be of minor importance as long as positive egg buoyancy remains.

### 5.2 INTRODUCTION

European sprat (*Sprattus sprattus*) is a widely distributed small pelagic fish species with high economical and ecological importance, particularly in the Baltic Sea with mean annual catch of 420000 tonnes (mean annual catch in last decade, ICES 2008). An isolated population (Limborg 2007; Debes *et al.* 2008) of this "cold adapted species" of boreal origin (Rass 1949 cited from Daskalov 1999; Bombace 2001) inhabits the North Adriatic Sea, where it supports local commercial fisheries (Dulčić 1998; Tičina 2000) with a mean annual catch of about 3000t during 1979-1988 (Grbec *et al.* 2002). From the end of the 1980's there was a sharp declining trend in reported landings in this species (Grbec *et al.* 2002) with an actual estimated stock

size derived from hydroacoustic surveys of <1000t in the Eastern Adriatic in 2004 (Tičina *et al.* 2005). However, potential reasons and causes for this decline remain so far ambiguous and indecisive (Grbec *et al.* 2002).

It is a well known fact that early life stages (ELSs) of fish (eggs and larvae) are most susceptible to mortality and therefore affect recruitment success (e.g. Hjort 1914; Rothschild 1986; Trippel and Chambers 1997; Houde 2002). Since these ontogenetic stages are strongly influenced by abiotic factors like temperature (e.g. Blaxter 1992; Fuiman 2002) and salinity (Holliday 1969; Alderdice 1988) unfavorable conditions may account for a substantial variability in survival. ELS of sprat in Mediterranean populations have not been analyzed comprehensively, as pointed out by Palomera *et al.* (2007) in a

review on small pelagics of the Western Mediterranean. Published studies on Adriatic sprat eggs and yolk sac larvae are restricted to egg abundance and distribution data (Zavodnik and Zavodnik 1969; Teskerendžić 1983; Gamulin and Hure 1983; Tičina 2000) and only one study has focused on larval stages in Adriatic sprat (Dulčić 1998) analyzing otolith based growth rates. Recently, one laboratory study has focussed on temperature dependent egg developmental rates, survival and yolk sac larval growth compiled in a large range (5 -19°C) of 11 different temperatures (Chapter 3). A significant negative relation of egg developmental time and temperature increase was found, but egg survival was not significantly related to temperature. If unfavorable salinity conditions might negatively effect egg development and survival is so far unexplored.

The Gulf of Trieste in the North Adriatic Sea is characterized by annual mean ( $\pm$ standard deviation) surface salinities of 35.3 ( $\pm$ 1.1) and 37.2 ( $\pm$ 0.4) at about 10m depth, respectively (Malačić *et al.* 2006). However, the seasonal variability can be substantial due to large river freshwater input during the ice melting season leading to regional surface salinities of 29 to 37 (Malačić *et al.* 2006). The variability increases towards spring which is isochronical with the end of the sprat spawning season lasting from November/December to March/April (Zavodnik 1969; Zavodnik and Zavodnik 1969; Teskerendžić 1983; Tičina 2000).

During field samplings in Kvarner Region (Rijeka Bay), Teskerendžić (1983) observed highest sprat egg mortality coinciding with the lowest observed salinity values (35.7). However, she did not relate the high egg mortality to the low salinity levels.

In salinity exposure experiments with Baltic Sea sprat, Petereit *et al.* (Chapter 4, submitted) measured egg buoyancy, egg development duration, survival rates, larval morphometric traits at hatch, eye

pigmentation and yolk sac depletion and found egg buoyancy decreasing with increasing incubation salinity. Egg survival had a tendency to higher survival at intermediate (14-18 psu) salinity conditions in two of three trials. However, to date no study on the effect of different incubation salinities on eggs and yolk sac larvae is available for Adriatic sprat. This study tries to close this gap with an experimental approach where eggs were incubated under 8 different salinities ranging from 5 to 37.2 psu. Salinities of 30, 35 and 37.2 cover the natural range, whereas the lower salinities can be compared to conditions experienced by the sprat population in the Baltic Sea.

The specific hypotheses addressed in this study are, that different salinity conditions have no impact on (1) the egg buoyancy, (2) egg developmental duration, (3) egg survival, (4) yolk sac larval morphometric traits post hatching and (5) yolk sac larval survival.

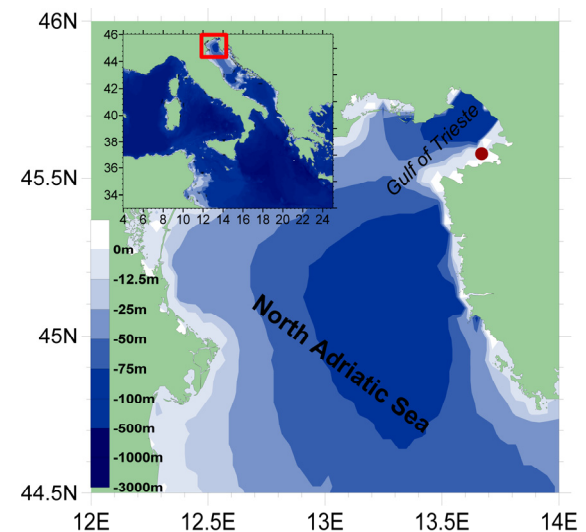


Figure 5-1: Map of the Western and Central Mediterranean with the Adriatic Sea (small insert). The red box indicates the enlarged part from the Northern Adriatic Sea (large map). The red dot shows the position where adult sprat was caught at night from 11-12<sup>th</sup> January 2007 in the Bay of Koper, Gulf of Trieste. These sprat provided the eggs for the salinity experiments.

Table 5-1: Summary of the biological sprat material used, experimental setup and the adjusted abiotic factors during the experimental incubation under different salinity treatments of sprat eggs and yolk sac larvae.

<i>Trial</i>	<i>Salinity incubation experiment</i>
Sprat females ( <i>n</i> ) contributing eggs	3
Egg size (mean±stdev; mm)	0.96±0.03
Beakers ( <i>n</i> ) per salinity treatment	3
Eggs (mean±stdev) per beaker ( <i>n</i> )	30±25
Salinities (psu)	5, 10, 15, 20, 25, 30, 35, 37.2
Temperature (°C)	11.3±0.3
Photoperiod light/dark (hours)	12:12
Egg stage at start of experiment	IB
Time expired before start of experiment (hours)	12

### 5.3 MATERIAL AND METHODS

#### 5.3.1 Biological material and experimental procedure

Sprat was caught at night by purse seine fishing in January 2007 in the Gulf of Trieste, Northern Adriatic Sea (Figure 5-1). Immediately after catch, eggs from several female sprat were strip-spawned into 500 ml plastic Kautex containers filled with 0.2 µm pre-filtered seawater of ~37 psu. Within 5 min after stripping, eggs were fertilized with one drop of a mixture of sperm from 8-10 males per container. Eggs were brought cooled (10-12°C) to the Marine Biological Station in Piran, where subsamples of eggs were checked for regular cleavages (>20 eggs) and size measured (> 10 eggs) at 40x magnification (Table 5-1). Consequently, eggs from three females were pooled and 30±25 (mean±stdev) eggs were randomly transferred carefully with a pipette (5 mm diameter) into 250 ml glass beakers containing 0.2 µm filtered artificial seawater (Aquarium Brand) of 5, 10, 15, 20, 25, 30, 35 and 37.2 salinity. All salinity treatments were processed in triplicates. The temperature controlled room was adjusted to 11.3(±0.3)°C. The eggs had an age of 12 hours post fertilization and were in the developmental stage of IB (Thompson *et al.* 1981) at time of partitioning in the treatments. One egg per replicate was carefully extracted daily, checked for developmental stage and

subsequently carefully replaced into the respective beaker.

All observations, handlings of eggs and larvae and water changes were made on a daily basis (every 24 hours). About 50% of the water was replaced with water of the same salinity, which led to increased salinity conditions of about 1 psu in each of the beakers at the end of egg phase.

#### 5.3.2 Egg development duration, relative buoyancy observations, survival and malformations

The egg development duration was followed until the time where >50% of larvae had hatched from the egg shell, but no additional samplings were approached which would have allowed for a higher resolution of the time to hatch. Dead eggs and larvae were removed and the vertical location of eggs and subsequently hatched yolk sac larvae was observed in the beakers and noted qualitatively (bottom, floating, surface). This was denominated as “relative egg buoyancy” for the location of the egg stages and as “larval buoyancy” for the vertical occurrence of the yolk sac larval stages.

Cumulative egg survival was defined as the number of eggs surviving from loading into the beakers until hatching from the egg integument. No exact numbers of dead eggs could be determined for the 10 and 15 psu trials at the latest egg stages. In a considerable number of cases it was not possible to distinguish if the egg had

already died before or during the hatching process. Based on close observations under a stereomicroscope the proportion of dead eggs was estimated for these trials.

Malformations of dead larvae were monitored after larvae had been removed from the beakers. However, the exact proportions of malformed larvae within each treatment or beaker were not calculated, unfortunately, since this was beyond the initial scope. But relative occurrence based on “yes/no” among the salinity treatments could be estimated. In some cases, unusual swimming performances of individual larva were detected during observations of the study.

### 5.3.3 Larval morphometric traits and survival

Randomly, 2-4 larvae per replicate were sampled 12 hours post hatch with a pipette, yielding in total 7-10 larvae per salinity treatment. Yolk sac larvae were immediately placed on a micrometer scale (0.1 mm scale bar resolution) in a drop of ambient water and photographed under a stereomicroscope (WILD M3Z equipment with a NICON camerasystem). Maximum handling time per sample was about 1 minute. Morphometric measurements were performed based on larval images. Standard length (SL, mm) was measured from the tip of the mouth until the end of the urostyl, yolk sac length (YSL, mm) and yolk sac heights (YSH, mm) as maximum extensions of the yolk sac. Yolk sac volume (YSV, mm<sup>3</sup>) was calculated using a spheroid formula adopted from Blaxter and Hempel (1963) and Hardy and Litvak (2004):

$$YSV = \left(\frac{\pi}{6}\right) \times L \times H^2 \quad (1)$$

where L is the length of the yolk sac and H is the height.

The yolk sac area (YSA, mm<sup>2</sup>) and body area (BA, mm<sup>2</sup>, excluding larval finfolds) were determined by circumscribing and integrating the area with image analysis software (Image Tool 3.0; <http://ddsdx.uthscsa.edu/dig/download.htm>). The BA / YSA ratio was calculated which reflected the proportion of how much yolk had been transferred into somatic tissue.

50% larval survival time was assessed as the time where 50% of the food deprived larvae remained in the beaker. 100% was the duration until the last larvae within a replicate had died.

### 5.3.4 Statistics

Data were presented as mean±stdev and all statistical analyses were performed using the program STATISTICA. Relative egg buoyancy, egg development time and larval buoyancy were not statistically compared among salinity treatments. Cumulative egg survival until hatch and larval survival potential (50% and 100%) were compared between the salinities from 25 to 37.2 using one-way-ANOVAs (significance level p<0.05) after meeting the assumptions of variance homogeneity (Cochran test) and normality. Fisher post hoc tests were applied to detect which respective salinities differed from each other. At a salinity of 20, distinguishing between live or dead larvae at the bottom of the beakers was in some cases difficult. To avoid overestimation of the larval starvation potential this salinity was excluded from the statistical analysis.

Morphometric traits were compared between salinities of 25 to 37.2 using non parametric statistic Kruskal Wallis ANOVAs (significance level p<0.05) and Median test, since assumptions for parametric tests were violated despite various data transformations.

5.4 RESULTS

5.4.1 Egg buoyancy and egg development duration

Eggs sank to the bottom in the salinity treatments of 5 to 30 psu after the transfer to the experimental salinities (Figure 5-2a) and remained there until larvae hatched. At 35 and 37.2 psu eggs remained in the surface layers which indicate that neutral buoyancy of the eggs is achieved somewhere between 30 and 35. The development time from fertilization until >50% larvae had hatched from the eggs was 4.5 days, if considered the time starting at partitioning in the different salinities 4 days from 10 to 37.2 psu.

5.4.2 Egg survival, yolk sac larval buoyancy and malformations

Cumulative egg survival was significantly different (ANOVA,  $p < 0.045$ ) between salinities from 25 to 37.2. Highest mean survival (100%!) was observed at 37.2 and was significantly higher compared to 25 and 30 with percentages survival of 90 and 92%, respectively (Figure 5-2b, Table 5-2). Successful egg development required salinities exceeding 5 psu, since all eggs at 5 psu died before reaching egg stage V (Figure 5-2b). At 10 psu approximately half of the eggs died before they reached the hatching stage and the majority of remaining individuals died shortly after the egg membrane was partly disrupted (Figure 5-4a). At 15 psu, also about 50% of the eggs died before hatching but successfully hatched larvae failed in completely emerging from the egg shell (Figure 5-4b, c). At 20 psu successful and complete hatching of larvae was observed, but a large proportion of these larvae exerted deformations in the tail region or notochord (Figure 5-4d). Furthermore, the 20 psu trial had to be excluded from the statistical analysis because of low (19%) survival within one replicate which lead to the violation of ANOVA assumptions of

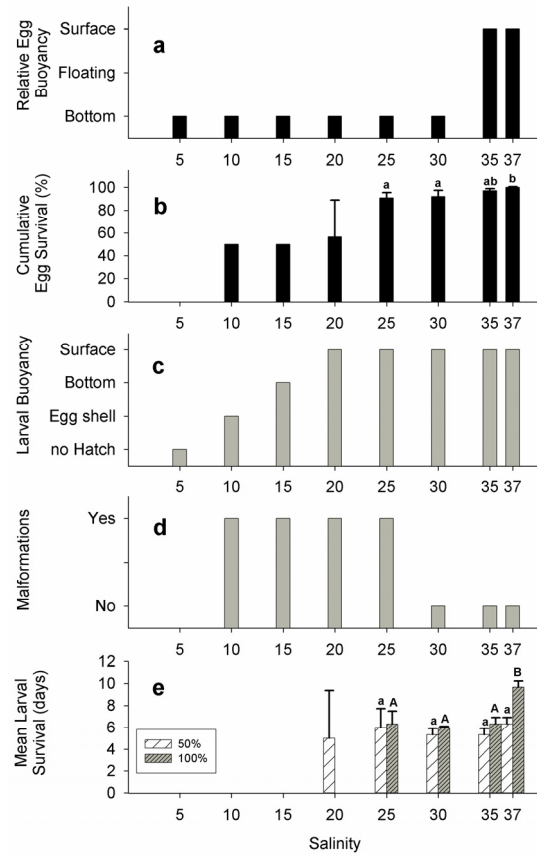


Figure 5-2: (a) Relative sprat egg buoyancy, categorized in floating, floating at surface layer or non floating (bottom). (b) Mean±stdev of cumulative egg survival from the 20-37 psu treatments of 3 independent replicates. No eggs survived at 5 psu and survival of 10 and 15 psu was estimated (see text). Significant differences ( $p < 0.05$ ) between the 25 to 37 psu treatments are indicated by different letters. (c) Larval neutral buoyancy during the first three days after hatch (at 20 and 25 psu larvae had to achieve neutral buoyancy actively). (d) Relative occurrence of malformations after hatch. (e) Mean±stdev survival days of non feeding yolk sac larvae after hatch derived from three independent replicates. Presented are 50% mortality and maximum survival time (100%) of individual larvae within a replicate. The 20 psu trial had to be excluded from the statistical analyses to avoid potential overestimations of survival time derived from uncertain determination. No comparisons were performed between levels of survival (50% and 100%) within a salinity treatment. Significant differences ( $p < 0.05$ ) between the salinity treatments 25 -37 psu are indicated by different letters.



Table 5-2: Mean±stdv of egg survival rates and larval survival potentials of n=3 replicates per salinity treatment and results of the ANOVA analyses. Significant values are typed in bold and differences between the treatments (p<0.05) are labeled through different letters. The 20 psu trial had to be excluded from the analyses.

Events	unit	Salinity (psu)												one-factorial-ANOVA	F	P
		20	n	25	n	30	n	35	n	37.2	n	37.2	n			
Egg survival	%	56.48 (±32.4)	3	90.9 (±4.5) <sup>a</sup>	3	92.1 (±5.1) <sup>a</sup>	3	97.1 (±1.8) <sup>ab</sup>	3	100 (±0.0) <sup>b</sup>	3	25 – 37.2 psu	F <sub>3,8</sub> = 4.23	<b>0.045</b>		
Larval survival 50%	days	5.0 (±4.3)	3	6.0 (±1.7) <sup>a</sup>	3	5.3 (±0.5) <sup>a</sup>	3	5.3 (±0.5) <sup>a</sup>	3	6.3 (±0.5) <sup>a</sup>	3	25 – 37.2 psu	F <sub>3,8</sub> = 0.75	0.55		
Larval survival 100%	days		3	6.3 (±1.1) <sup>a</sup>	3	6.0 (±0.0) <sup>a</sup>	3	6.3 (±0.5) <sup>a</sup>	3	9.6 (±0.5) <sup>b</sup>	3	25 – 37.2 psu	F <sub>3,8</sub> = 17.94	<b>&lt;0.001</b>		

variance homogeneity. Excluding this replicate would still result in significantly lower egg survival with 75% compared to all higher salinities (Table 5-2).

Larval buoyancy was different between the salinity treatments (Figure 5-2c). Larvae kept their neutral buoyancy only through active movements at 20 and 25 psu, whereas during the first three days larvae incubated at 30 to 37.2 psu remained in the surface water layer without active regulation of their vertical position (Figure 5-2c). Additionally, no abnormalities were observed in individuals from these high salinities compared to 25 psu and below (Figures 5-2d and 5-4a-d)

### 5.4.3 Larval morphometric traits

Larval morphometric traits at 10 hours post hatch showed significant differences between incubation salinities (Figure 5-3, Table 5-3). Mean SL with 3.81 mm was significantly (Kruskal-Wallis ANOVA, p<0.014) larger at 35 psu compared to 3.65 mm at 25 psu. YSA was significantly smaller at 37.2 psu compared to 30 psu (Figure 5-3b, Table 5-3). Consequently, YSV was smallest at the highest salinity but not significantly different any more. No significant influences of incubation salinity could be detected at BA (Figures 5-3c,d). The ratio of BA to YSA was largest at 37.2 psu, however this effect was at the margin of being significant (Figure 5-3e, Table 5-3).

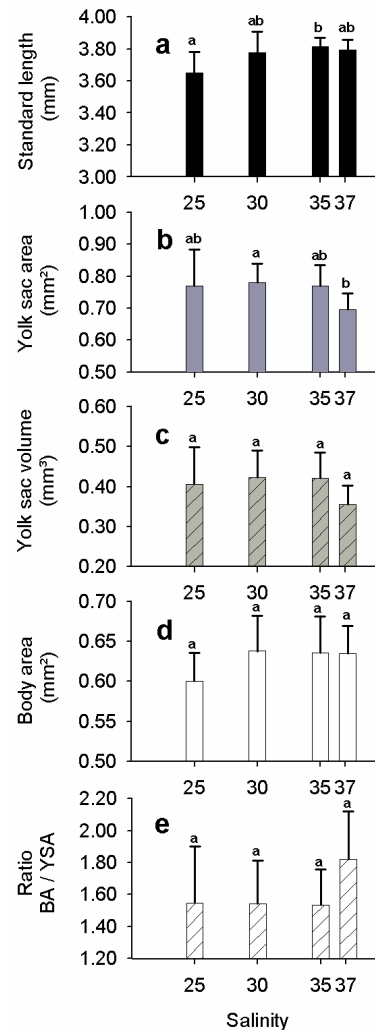


Figure 5-3: Morphometric traits of 10 hours post hatch sprat larvae (mean±stdv of 7-10 individuals per treatment) incubated under 4 different salinity treatments. Measurements were performed on larval digital images. (a) Standard length (SL) in mm. (b) Yolk sac area (YSA) in mm². (c) Yolk sac volume (YSV) mm³. (d) Body area (BA) mm². (e) Ratio of body area and yolk sac area. Significant differences (p<0.05) between the salinities are labeled with different letters.



Table 5-3: Mean±stdv of larval morphometric traits at 10 hours post hatch of n=7-10 individual larvae per salinity treatment and results of the Kruskal-Wallis ANOVA analyses and Median tests. Significant values are typed in bold and differences between the treatments ( $p < 0.05$ ) are labeled through different letters. (SL=standard length; YSA=yolk sac area; YSV=yolk sac volume; BA=body area; BA / YSA= ratio of body area and yolk sac area).

Traits	mean ± stdv	unit	Salinity (psu)						Kruskal-Wallis ANOVA	P	Chi <sup>2</sup>	df	P	
			25	n	30	n	35	n						37.2
SL	(mm)	3.65 (±0.13) <sup>a</sup>	7	3.77 (±0.13) <sup>ab</sup>	9	3.81 (±0.05) <sup>b</sup>	10	3.79 (±0.06) <sup>a</sup>	10	H <sub>3,36</sub> = 10.5	<b>0.014</b>	8.62	3	0.034
YSA	(mm <sup>2</sup> )	0.77 (±0.11) <sup>ab</sup>	7	0.78 (±0.05) <sup>a</sup>	9	0.77 (±0.06) <sup>ab</sup>	10	0.70 (±0.05) <sup>b</sup>	10	H <sub>3,36</sub> = 8.32	<b>0.039</b>	9.18	3	0.026
YSV	(mm <sup>3</sup> )	0.40 (±0.09) <sup>a</sup>	7	0.42 (±0.06) <sup>a</sup>	9	0.42 (±0.06) <sup>a</sup>	10	0.35 (±0.04) <sup>a</sup>	10	H <sub>3,36</sub> = 5.57	0.13	6.92	3	0.07
BA	(mm <sup>2</sup> )	0.60 (±0.03) <sup>a</sup>	7	0.63 (±0.04) <sup>a</sup>	9	0.63 (±0.04) <sup>a</sup>	10	0.63 (±0.03) <sup>a</sup>	10	H <sub>3,36</sub> = 4.20	0.23	3.98	3	0.26
BA / YSA	Ratio	0.79 (±0.12) <sup>a</sup>	7	0.82 (±0.08) <sup>a</sup>	9	0.82 (±0.06) <sup>a</sup>	10	0.91 (±0.07) <sup>a</sup>	10	H <sub>3,36</sub> = 7.58	0.055	5.14	3	0.16

#### 5.4.4 Yolk sac larval survival

The overall low number of hatched larvae (<7 individuals left per beaker after sampling) in combination with the frequent occurrence of malformed larvae prevented the statistical comparison of the 20 psu trial with the higher salinities (Table 5-2). Maximum larval survival duration of 9.6 days was detected at 37.2 psu and differed statistically (ANOVA  $p < 0.001$ ) from all other lower salinities (Figure 5-2e, Table 5-2). However, the duration where 50% of the larvae were still present, was not statistically (ANOVA,  $p < 0.55$ ) different between salinities of 25 to 37.2.

### 5.5 DISCUSSION

#### 5.5.1 Egg buoyancy

Positive egg buoyancy is important for marine fish eggs for survival and dispersion in the sea (Fabra *et al.* 2005) while negative buoyancy can be a potential cause for increased egg mortality e.g. due to sinking to layers with insufficient oxygen conditions (Nissling and Westin 1991; Bunn *et al.* 2000) which do not allow for successful egg development (Rombough 1988). In this study sprat eggs were neutrally buoyant at salinities  $\geq 35$  psu. Whether the egg accumulations on the bottom of the beakers during the experiment had caused serious oxygen deficiencies for the eggs in the lower salinity treatments was not directly measured. But the daily water exchange in

combination with moderate ambient water temperature conditions most likely provided sufficient abiotic conditions for survival. This is supported by high survival of eggs in the treatments of 25 and 30 psu, where the eggs were negatively buoyant.

The values obtained for neutral buoyancy (35 and 37.2psu) in the experiment are in accordance with the salinity range found in the field (Malačić *et al.* 2006) during sprat spawning season in the Adriatic. Occasionally, lower surface salinities were observed, but mean salinities at 10 m depth usually exceed these values and hence would prevent eggs from sinking to potential oxygen restricted layers or the bottom.

To our knowledge, no vertical distributed data are available for sprat eggs in the Adriatic Sea (V. Tičina, personal communication) which could provide information on egg buoyancies in the field as is available for sprat eggs e.g. in the English Channel (Coombs *et al.* 1985) or the Baltic Sea (Nissling *et al.* 2003; Petereit *et al.*, Chapter 4, submitted). At least for the stratified deep basins in the Baltic Sea this is especially relevant as different neutral buoyancies modify the vertical overlap between clupeid predators and eggs (Köster and Möllmann 2000). If this is also of importance for sprat eggs in the Adriatic Sea remains so far unclear. Further research in this direction would be desirable, especially as buoyancy measurements of other commercially

important pelagic fishes like Anchovy (*Engraulis encrasicolus*) and Sardine (*Sardina pilchardus*) have been conducted e.g. in the Bay of Biscay (Coombs *et al.* 2004) and revealed important insights on their vertical egg distribution.

### 5.5.2 Egg development duration and egg survival

The location and depth of the spawning event, in combination with modifications by drift and transport patterns, determine the ambient salinity conditions for the developing eggs in the field. The presented experiment reveals that time to hatch was not influenced by different salinity levels ranging from 10 to 37.2 psu. This supports previous findings for other fish species in the majority of studies (e.g. review from Kamler 2002; Shi *et al.* 2008) and has also been shown for Baltic sprat eggs, recently (Petereit *et al.*, Chapter 4, submitted).

However, egg survival was positively influenced by increased salinity conditions. At 37.2 psu not a single egg died during the experimental phase in all three replicates. This also asserts that the daily extraction and subsequent removal of individual eggs for checking of developmental stages had no detrimental effect on egg survival. Survival of >90% in salinities from 25-37.2 psu was at a magnitude comparable with egg survival (~85%) resulting from temperature (5-19°C) exposure experiments performed simultaneously and presented elsewhere (Petereit *et al.*, Chapter 3). However, survival was much higher compared to temperature or salinity exposure experiments with Baltic Sea sprat (Petereit *et al.* 2008; Petereit *et al.*, Chapter 4, submitted). The large salinity range for successful egg development characterizes Adriatic sprat as species with at high euryhaline capacity.

The minimum salinity conditions for successful egg development are salinities >5 psu and are in line with observations from another sprat population. Petereit *et al.*

(Chapter 4, submitted) found no development until hatching at 5 psu in egg fertilization/incubation experiments with Baltic sprat.

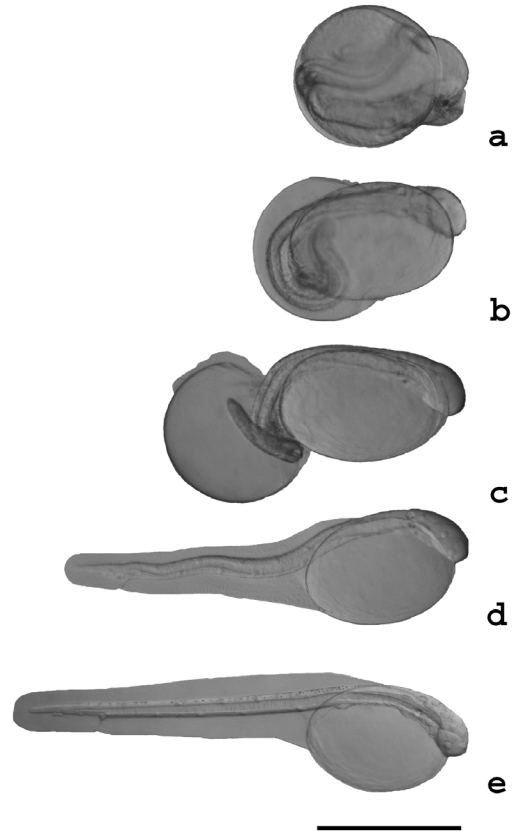


Figure 5-4: Typical habitus of sprat yolk sac larvae derived from eggs incubated at different salinity treatments. (a) Eggs incubated at 10 psu, larvae succeed to disrupt the egg integument but remained within the egg. (b) Eggs incubated at 15 psu, larvae failed to leave the egg integument and frequently got stuck with the yolk sac. (c) Eggs incubated at 20 psu, larvae regularly managed to hatch, but many deformations of the notochord and especially around the tail region were observed which inhibited hatching. (d) Eggs incubated at 25 psu, almost all larvae hatched successfully, but some larvae showed deformations on the notochord, which nevertheless did not prevent swimming. (e) Typical sprat yolk sac larvae hatched from eggs incubated at 30, 35 or 37.2 psu. No malformations were observed. Scale bar = 1mm.

However, as Baltic sprat stocks inhabit brackish water conditions (~6 to 20 psu), adaptations to lower salinity conditions are more likely compared to the Adriatic sprat population experiencing high salinity conditions. Whether Adriatic sprat eggs

could even be fertilized under such reduced salinity conditions was not assessed within this study, but experiments with other fish species like cod (*Gadus morhua*), herring (*Clupea harengus* and *Clupea pallasii*), dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), flounder (*Pleuronectes flesus*) and turbot (*Scophthalmus maximus*) have shown reduced fertilization success at decreased salinity conditions (Nissling and Westin 1997; Griffin *et al.* 1996; Nissling *et al.* 2002; Nissling *et al.* 2006).

Although embryos developed into viable larvae at salinities >10 – 25psu, a significant contribution of these larvae to recruitment would not be very likely, since a large proportion already failed in emerging from the egg shell or showed deformations which handicap swimming performances (Lee and Menu 1981; Shi *et al.* 2008). These handicaps are two-fold since the larvae have to accomplish compensation of negative buoyancy and hence have to invest more energy to prevent sinking (Moustakas *et al.* 2004). Additionally, larvae would most likely have to spent increased energy to balance osmotic differences (Kinne 1960). The sum of these additional physiological expenses are potentially expressed in the significantly longer 100% survival of larvae raised at the highest salinity, where they do not have such extra costs.

### 5.5.3 Morphometric traits

Salinity had significant effects on some larval morphometric traits early post hatch. SL and BA were smallest at the lowest salinity. If this trend of smaller body size would perpetuate until larvae switch to external feeding it could have an adverse effect since body size is generally assumed to be positively related to improved swimming performance and hence foraging abilities (Blaxter 1992). Additionally the escape potential in the sense of the 'growth-selective predation' hypothesis (Takasuka *et al.* 2004) states that small individual larvae are positively

selected by predators. However, it was shown for Baltic sprat larvae that a significant size advantage was caught up during ontogeny until the time of first feeding or at yolk sac depletion (Petereit *et al.*, Chapter 4, submitted). For other species, large variations of responses, including increase, decrease or constant size at increasing or decreasing salinity have been reported (Chambers 1997) leading Kamler (2008) to conclude in a review on yolk feeding fish that "growth patterns to salinity are dose- and species-specific and tolerance to salinity may be adaptive".

No consistent effect was found in YSA (and corresponding in YSV) which could be related to decrease or increase in size with salinity. This was also described for herring (*Clupea harengus*) yolk sac larvae (Holliday and Blaxter 1960) and recently for pomfret (*Pampus punctatissimus*) larvae by Shi *et al.* (2008).

YSA was smallest at the highest salinity but this was not likely related to advanced growth since larvae were neither significantly largest in SL nor in BA. Nevertheless, it led to the (non-significant) highest ratio of somatic tissue area compared to yolk area. Even if not measured in this study it indicates improved yolk utilization efficiency compared to other salinities. In combination with the longest maximum larval survival time it seems likely that ambient salinity (~37 psu) provide appropriate conditions for larval development and survival.

In conclusion, salinity influences egg buoyancy, egg survival, yolk sac larval morphometric traits but not the egg development duration. A decrease in surface salinity during progressing spawning season might impact egg and larval survival, but is considered to be of minor importance as long as positive egg buoyancy remains.

## 6 Synthesis and Conclusions

### 6.1 SUMMARIZED RESULTS OF GENERAL HYPOTHESES

#### 6.1.1 1) Temperature has no effect on the survival, development and duration of non feeding stages (eggs and yolk sac larvae) of sprat

This hypothesis has to be rejected. This study shows that temperature had an effect on survival (Figure 6-1), developmental rates, morphological traits and on timing of important ontogenetic stages (Table 6-1).

In all experiments, duration of developmental stages was negatively correlated with temperature. The results from Chapter 2 reveal that Baltic sprat eggs experience thermal threshold values ( $>3.4^{\circ}\text{C}$  and  $<14.7^{\circ}\text{C}$ ) after which no successful development occurs. Additionally, Baltic sprat egg survival showed a clear thermal optimum under the regional ambient conditions. No threshold temperatures were detected in the Adriatic sprat population within the range of temperatures tested ( $5\text{-}19^{\circ}\text{C}$ ) (Chapter 3).

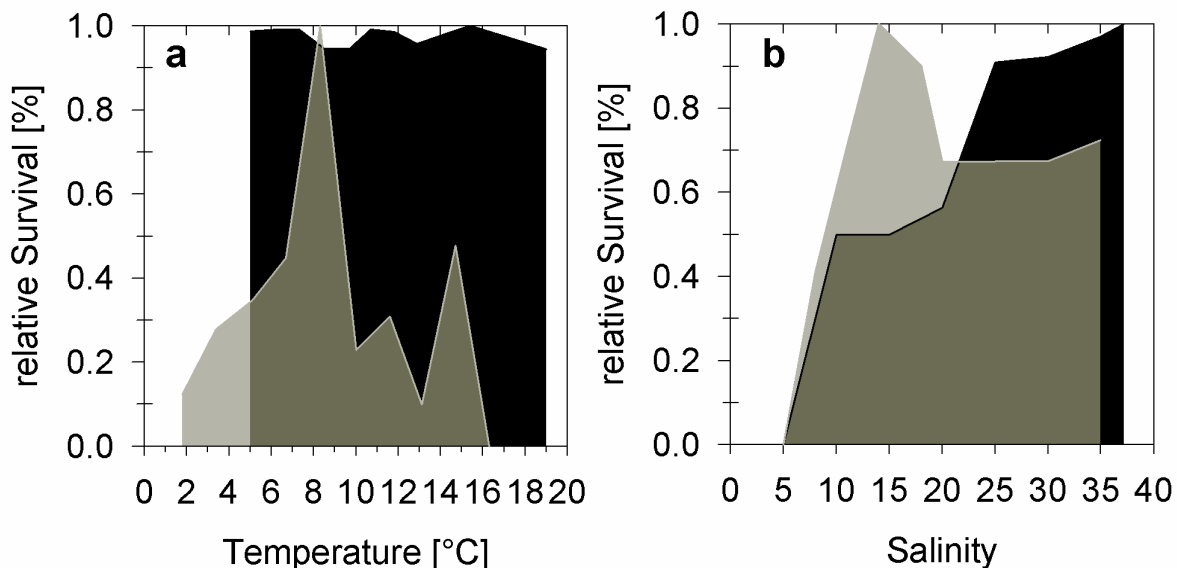


Figure 6-1: Relative mean egg survival (standardized to the maximum observed survival from fertilization until hatching) of sprat eggs incubation-experiment from different (a) temperature and (b) salinity treatments with two different sprat populations. Transparent grey colour represents mean egg survival of the Baltic Sea population and black colour represents egg survival of the Adriatic Sea population.

#### 6.1.2 2) Duration and survival of non feeding stages of sprat (egg and yolk sac larvae) is not altered by changes in salinity regime during development

This hypothesis has to be accepted for the duration of the developmental stages. In the present study, salinity was not found to alter the duration of sprat egg development

at optimum temperatures. Except for the lowermost salinity tested (5psu), eggs developed successfully in both populations (Chapter 4 and Chapter 5) over a high salinity range. Relative maximum survival was observed around ambient conditions in each population, respectively (Figure 6-1, Chapter 4 and Chapter 5).

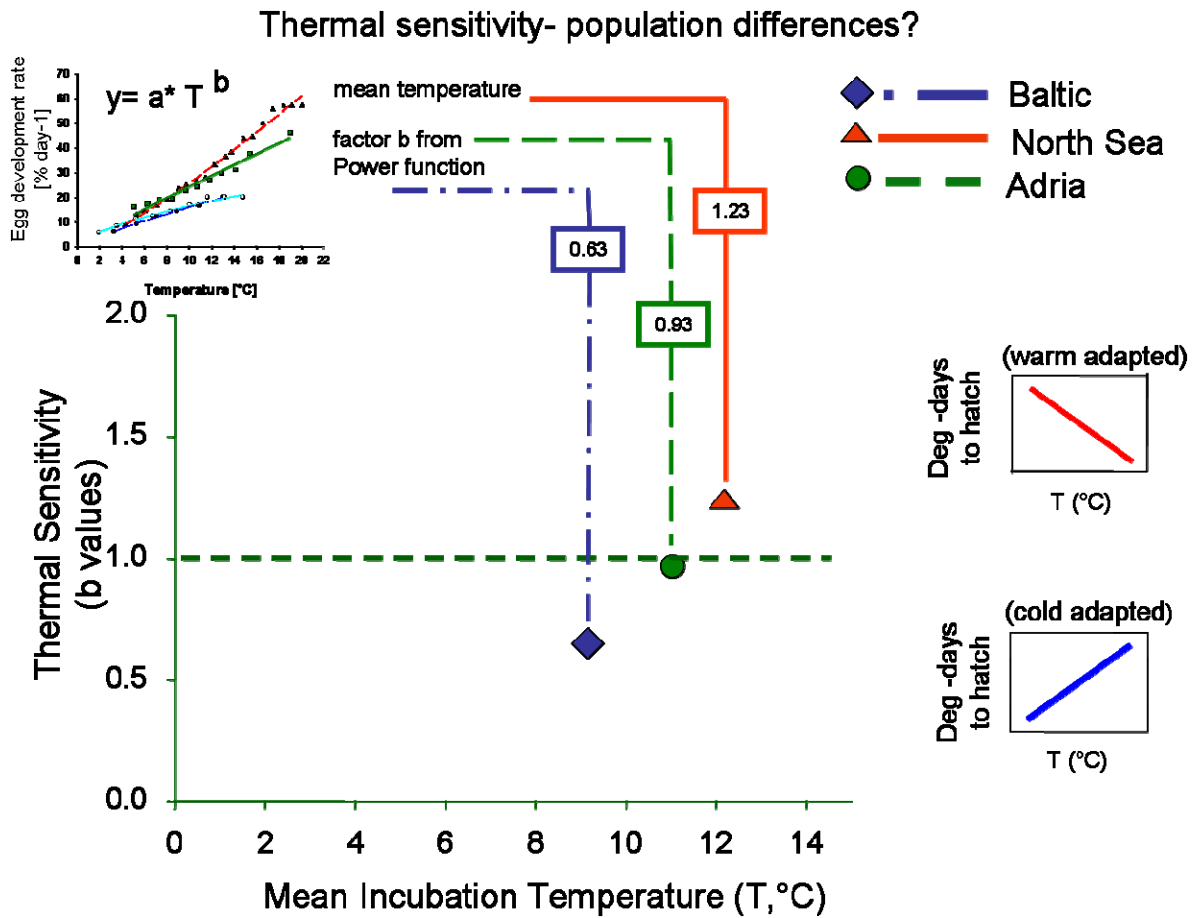


Figure 6-2: Thermal sensitivity values derived from sprat egg development rates (b values of power function) from the Baltic Sea, the North Sea and the Adriatic Sea sprat population. b-values above 1 indicate warm adaptation, which reflect a decrease in egg developmental time with increase in incubation temperature (Pritchard *et al.* 1996). b-values below 1 indicate cold adaptation with a decrease in developmental time with decreasing temperature. Accordingly, values of about 1 reflect general, intermediate temperature sensitivity.

Table 6-1: Summary of parameterized important ontogenetic events and developmental rates from sprat eggs and yolk sac larvae from the Baltic Sea, the North Sea and the Adriatic Sea. Egg development rate refers to time from fertilization to first hatch. Eye pigmentation reflects the time (in days) from hatching to fully pigmented eyes which is almost identical to the time of mouth gap opening (first feeding). Yolk sac depletion describes endogenous resource depletion, whereas the time from hatching until the last larva has deceased is defined as maximum larval survival duration. North Sea<sup>1</sup> - data were derived from Thompson *et al.* (1981) and North Sea<sup>2</sup> - data are derived from Alshuth (1988) and Shields (1989).

Trait	Function	Coefficients ± Std. Error	p	r <sup>2</sup>		
<u>Egg Development Rate</u>						
Baltic Sea	power	a	3.9255 ± 0.4101	<0.0001	0.97	
		b	0.6296 ± 0.0446	<0.0001		
Adriatic Sea	power	a	2.4037 ± 0.3416	<0.0001		
		b	0.9438 ± 0.0550	<0.0001		
North Sea <sup>1</sup>	power	a	1.5133 ± 0.1713	<0.0001		
		b	1.2353 ± 0.0413	<0.0001		
<u>Eye Pigmentation</u>						
Baltic Sea	exponential	a	23.2675 ± 2.0871	<0.0001	0.91	
		b	0.1157 ± 0.0119	<0.0001		
Adriatic Sea	exponential	a	21.229 ± 0.4686	<0.0001		
		b	0.1267 ± 0.0027	<0.0001		
North Sea <sup>2</sup>	exponential	a	40.3277 ± 7.0769	<0.0001		
		b	0.1709 ± 0.0160	<0.0001		
<u>Yolk Sac Depletion Rate</u>						
Baltic Sea	power	a	1.7621 ± 0.4212	0.0139	0.94	
		b	0.7688 ± 0.1132	0.0025		
Adriatic Sea	power	a	0.0087 ± 0.0008	<0.0001		
		b	1.1797 ± 0.0358	<0.0001		
North Sea <sup>2</sup>	power	a	0.2705 ± 0.0773	0.0249		
		b	1.4951 ± 0.1061	0.0001		
<u>Maxim. Larval Survival</u>						
Baltic Sea	peak	a	23.0472 ± 1.1308	<0.0001	0.74	
		b	4.6151 ± 0.5437	<0.0001		
		x <sub>0</sub>	6.1449 ± 0.4378	<0.0001		
Adriatic Sea	peak	a	14.5137 ± 0.3552	<0.0001		
		b	7.1698 ± 0.7365	<0.0001		
		x <sub>0</sub>	7.1536 ± 0.6535	<0.0001		

**6.1.3 3) Sprat non feeding stages (egg and yolk sac larvae) from different populations are identically affected under exposure of same thermal history**

This hypothesis has to be rejected. Results from the present thesis show for the first time that sprat populations have specific differences in egg developmental rates (Chapter 2 and Chapter 3) which are

caused by different thermal adaptations (Figure 6-2). Time to eye pigmentation (mouth gap opening, first feeding) appears earlier in the Adriatic population (Chapter 3) compared to the Baltic population (Chapter 2) (Table 6-1). Maximum larval survival differs significantly between both populations additionally (Chapter 2 and Chapter 3, Table 6-1). This leads to contrasting 'windows of opportunities' (WOO) (Figure 6-3).

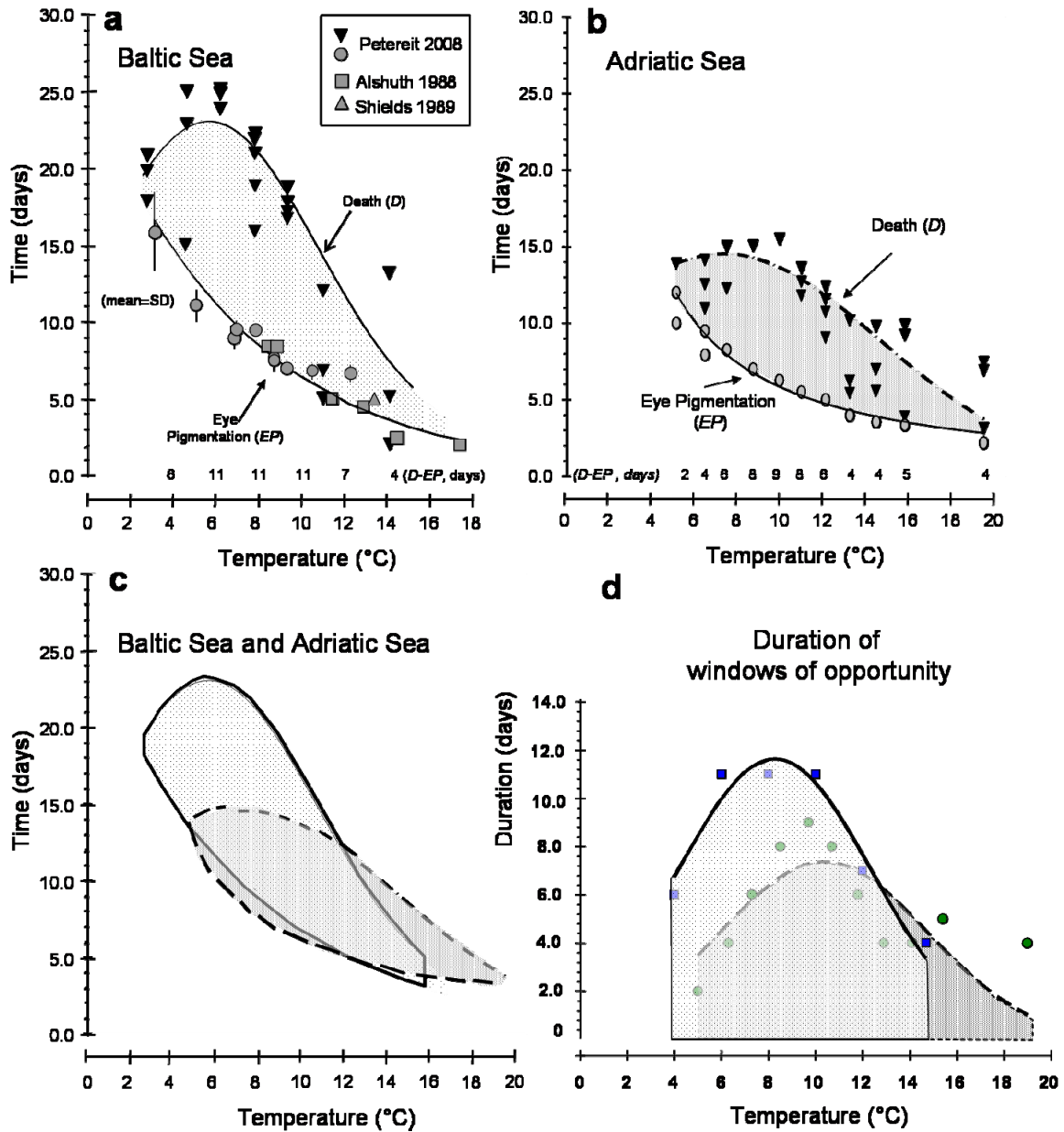


Figure 6-3: “Window of opportunity” (WOO) to successfully establish first feeding of sprat larvae from the Baltic Sea population and the Adriatic Sea population. (a) Duration from hatch to eye pigmentation (EP) (as proxy for first feeding) indicated by grey circles for sprat larvae from the Baltic Sea. Literature values for North Sea (grey squares/triangle) sprat larval time to eye pigmentation extend the temperature dependent function. Black triangles show maximum survival times from hatch until death (D) of individual larva from independent replicates. The enclosed area (dotted white) represents time WOO for Baltic sprat. (b) WOO (light grey area) of Adriatic Sea sprat larvae. (c) Overlapping WOOs from both populations (colours as above). (d) Exact temperature dependent durations of both populations (for parameterization see Table 6-1). Larval sprat from the Baltic sprat population experience longest WOO at about 8°C whereas larvae from the Adriatic population exhibit longest period between 10-11°C.

## 6.2 POPULATION DIFFERENCES

This study has shown differences in a number of traits between the populations. Egg neutral buoyancy is achieved under different salinity conditions. This is most likely due to the adaptation to much lower salinity conditions in the Baltic Sea sprat population compared to the Adriatic sprat population, especially during maturation and spawning season. Thermal sensitivity during the egg phase, egg survival as proxy for plasticity to thermal or salinity stressors as well as the time window for successfully established feeding differed between the populations:

### 6.2.1 Thermal sensitivity, plasticity and latitudinal gradient

The degree of thermal sensitivity (Figure 6-2) does not follow a latitudinal gradient directly. Intuitively, one may expect the Mediterranean sprat population to be the warm adapted population. However, this was not the case and could be partly explained by the different hydrographical situations in each of the areas. In the Baltic Sea specific gravity of sprat eggs (potentially modified by ambient salinity conditions) determines vertical distribution in the water column (Chapter 4) with eggs distributed around 40-80m depth. At this depth eggs experience ambient temperatures from ~4 to about 8°C during the spawning season. An increase towards surface layers can be observed with progressing spawning season. In comparison, sprat eggs are distributed in surface layers of the North Sea and the spawning season is temporally extended (Wahl and Alheit 1988), both resulting in more variable and higher incubation temperatures (Loewe *et al.* 2005). This fits to the warm adaptation as found in this study (Chapter 2). Contrary to both northern populations, sprat is a winter spawner in the Adriatic Sea (Chapter 3), matching the seasonal time window of lowest temperature (~9 to about 13°C) with comparably low temperature

variability. As is evident from Chapter 5, low surface salinity conditions towards the end of the spawning season might impact egg vertical distribution in extreme situations, but this is not likely to seriously alter the temperature conditions for egg development.

However, it is necessary to test the influence of other factors like salinity, egg size or genetical differences on EDR to affirm the existence of different thermal sensitivities.

For eggs from the Baltic Sea population (Chapter 4) as well as for the Adriatic Sea population (Chapter 5), no accelerating or decelerating effects of salinity on development could be observed within the applied daily sampling interval. Thus, potential effects of different salinities on EDR are unlikely, at least if such changes occur in combination with appropriate temperature conditions. Egg size differs significantly between the Baltic and the other two populations. Differences in egg sizes showed no effect on developmental time in herring (*Clupea harengus*) or cod (*Gadus morhua*) eggs (Blaxter and Hempel 1963; Pepin *et al.* 1997), but other studies delivered evidence that egg size influences egg development rates (Pauly and Pullin 1988; Peck *et al.*, unpublished b). Although egg sizes differed significantly between two Anchoveta populations off Peru, egg development duration was identical under the same incubation temperatures (Tarifeño *et al.* 2008). Sprat egg diameter of the North Sea data set ( $0.99 \pm 0.03$  mm, Thompson *et al.* 1981) and the Adriatic data set ( $0.98 \pm 0.05$ , Chapter 3) differed only slightly, however, the thermal sensitivity was significantly different. Thus it seems rather unlikely, that egg diameter causes the observed large differences in thermal sensitivity between the populations, even if Baltic sprat have generally larger eggs compared to both the Adriatic and North Sea populations.

Genetic differences between the populations have been detected recently by Limborg (2007) with microsatellite



markers and by Debes *et al.* (2008) with genetic structure analyses of the mitochondrial control region (compare discussion in Chapter 3).

Both studies confirm the findings that the Adriatic population is genetically different from the populations of the Baltic and the North Sea. Microsatellite loci revealed a break between the Baltic Sea and the North Sea population corresponding to a sharp salinity break (Limborg 2007). The Adriatic population showed low genetic diversity and a star-like haplotype network which indicates a recent demographic expansion scenario after a small population size (Debes *et al.* 2008). Usually, low genetic diversity indicates a reduced number of genotypes which does not necessarily reflect high phenotypic plasticity.

Nevertheless, the results of this thesis indicate a higher (thermal) plasticity (related to temperature induced egg survival) of the Mediterranean population compared to the Baltic population. Generally, higher thermal plasticity is assumed for species experiencing more changing environments or large seasonal abiotic variations (Cossins *et al.* 2006). The sea surface temperatures of the Baltic Sea and the Adriatic Sea have comparable annual amplitudes (covering about 15°C). Whether the water layers where the eggs occur vary greatly between the two systems needs to be resolved. However, other factors can potentially explain differences in egg survival, e.g. egg quality related to female age/nutritional condition, sampling time within the spawning season, or experimental sampling procedure (Chapter 3).

Comparing Baltic Sea and Adriatic Sea flounder populations (*Platichthys flesus*) Borsa *et al.* (1997) showed clear genetic differences. They assumed (relying on >45-90 year old literature sources) that no egg thermal tolerance differences between the flounder populations exist and concluded that the low temperature tolerance of the early planktonic stages

acted as limiting factor for southward dispersal and hence for the observed genetic differences (Borsa *et al.* 1997).

In comparison, this thesis reveals clear evidence for population specific thermal tolerances between sprat populations. Adriatic sprat eggs would likely tolerate higher thermal conditions appearing during potential southward dispersal, but the dispersal distance would be short due to the accelerated development time.

Such examples give evidence for the need of specific exposure experiments to uncover thermal plasticity differences between populations. Nevertheless, additional experiments with Adriatic sprat should approve the findings and should be designed to differ temporally (spawning season) or spatially (distribution range) from the performed study.

Other inter-latitudinal population comparisons with fish found no evidents for thermal adaptation differences. Tarifeño *et al.* (2008) analyzed egg development rates under different temperatures among anchoveta (*Engraulis ringens*) populations from the extremes of their distributional range along the coast of Chile. They found the time to hatch to decrease with increasing temperature, but found no inter-latitudinal differences in the temperature-development time relationship. Also Peck *et al.* (unpublished b), in a review on thermal sensitivity of fish eggs, found no differences within analysed Gadoid or Clupeid populations (see discussion in Chapter 2).

Van Doorslaer and Stoks (2005) compared thermal reaction norms in two congeneric damselflies that differ in latitudinal distribution at four different temperatures. They found the more northern oriented species to be more successful at cooler temperature during the embryonic development in accordance with their thermal adaptation. However, during the larval growth period, they did not find a consistent pattern of latitudinal compensation.

Additionally, growth rates are frequently compared as fitness related traits among populations incubated under different temperature treatments. Larsson *et al.* (2005) compared arctic charr (*Salvelinus alpinus* L.) from 11 European watercourses which ranged in latitudinal gradients from 54° to 70°N. They found no geographical or climatic trend in growth performances among populations and therefore no indication of thermal adaptation.

Since only non feeding sprat stages were considered in the present thesis, no direct comparison between thermal adaptiveness during later larval stages could be performed. Thus, precautions should be taken in generalizing this pattern as the adaptiveness of patterns in thermal reaction norms may not be sufficient for one developmental stage only (Van Doorslaer and Stoks 2005).

Further studies with respect to other fitness related traits as e.g. growth rate in feeding larvae would have been desirable and had been applied during this study. It turned out that first feeding was rarely established in any individual larva from both analyzed sprat populations although a variety of diverse food organisms were tested.

This is in agreement with other studies trying to grow sprat larvae, as performed during the GLOBEC Germany project (Prof. Dr. Myron Peck, University of Hamburg, personal comment; Kanstinger 2007).

### 6.2.2 Changing conditions and Window of Opportunity

Temperature increase (BALTEX 2006) will likely have a positive effect for recruitment success of the Baltic sprat early life stages (MacKenzie and Köster 2004; MacKenzie *et al.* 2007; Petereit *et al.* 2008) even if the magnitude of expected salinity decrease is challenging to predict (BALTEX 2006). However, such direct effect seems to be of minor importance as long as the threshold value of >5 psu is retained. But reduced salinity conditions during fertilization and incubation may modify egg buoyancy and

thus the eggs' vertical distribution (Chapter 4). Adriatic sprat non feeding stages are also likely to cope with temperature increase (Chapter 3) as predicted in future scenarios (Christensen *et al.* 2007). Salinity is predicted to increase in the North Adriatic Sea (Christensen *et al.* 2007). Due to the observed plasticity during egg and yolk sac larval stages for reduced salinity conditions (Chapter 5) it may be speculated that moderately increased salinity conditions will not dramatically impact the earliest life stages. Nevertheless, it is recommended that elevated salinity levels are tested in future research.

Furthermore, later (feeding) life stages might be very well influenced by temperature increases, due to reduced time during the critical first feeding period (Window of opportunity, WOO, Figure 6-3). The Adriatic population experiences its longest WOO at temperatures of 10-11°C yielding in about 7 days accomplishing successful feeding. An increase of 4°C would shorten the time to around half and therefore may have serious consequences for potential survival.

In comparison, the Baltic sprat population experiences the longest WOO (about 12 days) at 8°C and would have still 7 days if temperature would increase by 4°C.

Following Hjort's (1914) critical-period concept, this may make the Adriatic sprat larvae more vulnerable to food shortage due to the reduced time window and potentially makes match-situations (Cushing 1974, 1990) with suitable prey less likely under warming scenarios.

In other marine fish it has been shown that populations may react differently under changing abiotic conditions. For example, cod (*Gadus morhua*) populations are differently influenced by temperature and changing NAO conditions which force mainly temperature as well as wind stress and consequently influence prey availability and growth (Planque and Frédou 1999; Stige *et al.* 2006). Planque and Frédou (1999) showed that increased

temperature variability influenced recruitment of Atlantic cod stocks located in warm water negatively, cold water stocks positively, and that there was no relationship for stocks located in the middle of the temperature range.

This study shows for the first time that for sprat, as a member of the “small pelagic species”, different populations may be impacted differently under changing climatic conditions, when the thermal sensitivity during the egg phase is considered.

### 6.3 APPLICATION AND OUTLOOK

Temperature dependent egg development functions can be implemented in IBMs (e.g. Miller et al. 2006; Daewel *et al.* 2008a; Kühn *et al.* 2008) to model biophysical interactions. “Future challenges must bring oceanographers and ecologists together around specific dispersal problems if there is to be a significant improvement in the notable absence of hard data in this field of enquiry” (Largier 2003). Concerning ELS durations and temperature dependent mortality rates in sprat from different populations, this thesis has contributed new insights and data on the planktonic period over a wide temperature range. First results have already been published by Hinrichsen *et al.* (2007). They analysed the water masses in the Bornholm Basin, Baltic Sea, and showed a high correlation of surface water temperature in the beginning of the year to the water temperature in spring of the same year where zooplankton and ichthyoplankton develops. Temperature dependent development and mortality rates of secondary (copepods) and tertiary (ichthyoplankton- sprat, from this thesis) production were applied to predict the developing cohorts in the following spring season. The parameterizations of non feeding sprat stages from this study have been incorporated in a review paper on “The Ecophysiology of *Sprattus sprattus* in the Baltic and North Seas” by Peck *et al.*

(unpublished a) which will be submitted soon.

In a next step, early life history traits derived from this study will be integrated into a sprat population matrix model to be developed within the project RECONN (Haslob and Petereit, in prep.). This model will allow testing different scenarios for temperature dependent population development.

## IV REFERENCES

Alderdice, D.F. (1988): Osmotic and Ionic Regulation in Teleost Eggs and Larvae. In: WS Hoar and DJ Randall (eds.) Fish Physiology - The Physiology of Developing Fish - Part A – Eggs and Larvae. Volume XI, Academic Press, London, pp. 163-252.

Alderdice, D.F., Forrester, C.R. (1971): Effects of salinity and temperature on embryonic development of the petrale sole (*Eopsetta jordani*). J Fish Res Bd Can 28: 727-744.

Alekseev, F.E., Alekseeva, E.I. (2005): Batch fecundity and daily egg production of the Baltic sprat (*Sprattus sprattus balticus* (Clupeidae)) from the southeastern part of the Baltic Sea. J Ichthyol 45(1): 93-102.

Alheit, J. (1988): Reproductive biology of sprat (*Sprattus sprattus*) - Factors determining annual egg-production. J Cons Int Explor Mer 44: 162-168.

Alheit, J., Wahl, E., Cibangir, B. (1987): Distribution, abundance, development rates, production and mortality of sprat eggs. ICES CM/H:45.

Alheit, J., Möllmann, C., Dutz, J., Kornilovs, G., Loewe, P., Mohrholz, V., Wasmund, N. (2005): Synchronous regime shifts in the central Baltic and the North Sea in the late 1980s. ICES J Mar Sci 62: 1205-1215.

Alshuth, S. (1988): Bestimmung von Alter und Wachstumsraten bei Larven und Juvenilen der Sprotte (*Sprattus sprattus* L.). Dissertation, Alfred- Wegener- Institut für Polar und Meeresforschung, Bremerhaven, pp. 191 (in German).

Anderson, J.T. (1988): A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. J Northw Atl Fish Sci 8: 55-66

Aro, E. (1989): A review of fish migration patterns in the Baltic Sea. Rapp P-v Réun Cons Int Explor Mer 190: 72-96.

Arul, V. (1991): Effects of temperatue on yolk utilization of *Channa striatus*. J Therm Biol 16: 1-5.

Azzali, M., De Felice, A., Luna, M., Cosimi, G., Parmigianni, F. (2002): The state of the Adriatic Sea centered on the small pelagic fish populations. Mar Ecol 23 (Suppl.1): 78-91.

Bailey, R.S. (1980): Problems in the management of short-lived pelagic fish as exemplified by North Sea sprat. Rapp p-v Reun (Dan) 177: 477-488.

Bailey, K.M. (2000): Shifting control of recruitment of walleye pollock (*Theragra chalcogramma*) after a major climate and ecosystem change. Mar Ecol Prog Ser 198: 215–224.

Bailey, K.M., Houde, E.D. (1989): Predation on eggs and larvae of marine fishes and the recruitment problem. Adv Mar Biol 25: 1-83.

- Bakun, A., Parrish, R.H. (1982): Turbulence, transport and pelagic fish in the California and Perú current system. CALCOFI Rep 23: 99–112.
- BALTEX (2006): Assessment of Climate Change for the Baltic Sea basin. Summary: The BACC Lead Author Group (eds.), International BALTEX Secretariat, 35: 1-26, June 2006.
- Baumann, H., Hinrichsen, H.-H., Möllmann, C., Köster, F.W., Malzahn, A.M., Temming, A. (2006): Recruitment variability in Baltic sprat (*Sprattus sprattus*) is tightly coupled to temperature and transport patterns affecting the larval and early juvenile stages. Can J Fish Aquat Sci 63: 2193-2201.
- Beverton, G.J.H., Holt, S.J. (1957): On the dynamics of exploited fish populations. Ministry of Agriculture Fisheries and Food, Fishery Investigations, London, Series II-19, pp. 533.
- Bisbal, G.A., Bengtson, D.A. (1995): Effects of delayed feeding on survival and growth of summer flounder (*Paralichthys dentatus*) larvae. Mar Ecol Prog Ser 121: 301-306.
- Blaxter, J.H.S. (1969): Development: Eggs and Larvae. In: Hoar WS, Randall DJ (eds.) Fish Physiology: Reproduction, Growth, Bioluminescens, Pigments and Poisons. Volume III, Academic Press, New York, pp. 178-241.
- Blaxter, J.H.S. (1992): The effect of temperature on larval fishes. Neth J Zool 42: 336-357.
- Blaxter, J.H.S., Hempel, G. (1963): The influence of egg size on herring larvae (*Clupea harengus*). J Cons Int Explor Mer 28: 211–240.
- Boeuf, G., Payan, P. (2001): How should salinity influence fish growth? Comp Biochem Phys C 130: 411-423.
- Bombace, G. (2001): Influence of climatic changes on stocks, fish species and marine ecosystems in the Mediterranean Sea. Archo Oceanogr Limnol 22: 67-72.
- Borsa, P., Blanquer, A., Berrebi, P. (1997): Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. Mar Biol 129: 233-246.
- Brett, J.R. (1979): Environmental factors and growth. In: Hoar WS, Randall DJ, Brett JR (eds.) Fish Physiology: Bioenergetics and Growth. Volume VIII, Academic Press, New York, pp. 599-675.
- Brooks, S., Tyler, C., Sumpter, J.P. (1997): Egg quality in fish: what makes a good egg? Rev Fish Biol Fish 7: 387-416.
- Buckley, L.J., Bradley, T.M., Allen-Guilmette, J. (2000): Production, quality, and low temperature incubation of eggs of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in captivity. J World Aquac Soc 1: 22-29.
- Buisson, L., Thuiller, W., Lek, S., Lim, P., Grenouillet, G. (2008): Climate change hastens the turnover of stream fish assemblages. Global Change Biol 14(10): 2232-2248.

Bunn, N.A., Fox, C.J., Webb, T. (2000): A literature review of studies on fish egg mortality: Implications for the estimation of spawning stock biomass by the annual egg production method. Sci. Ser., Tech. Rep., CEFAS, Lowestoft, (111), 37pp.

Cervetto, G., Gaudy, R., Pagano, M. (1999): Influence of salinity on the distribution of *Acartia tonsa* (Copepoda, Calanoida). J Exp Mar Biol Ecol 239(1): 33-45.

Chambers, R.C. (1997): Environmental influences on egg and propagule sizes in marine fishes. In: Chambers RC and Trippel EA (eds.) Early Life History and Recruitment in Fish Populations. Chapman & Hall, London, pp. 63-102.

Chambers, R.C., Legett, W.C. (1987): Size and age at metamorphosis in marine fishes: An analysis of lab-reared winter flounder *Pseudopleuronectes americanus* with a review of variation in other species. Can J Fish Aquat Sci 44: 1936-1947.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.-T., Laprise, R., Magaña Rueda, V., Mearns, L., Menéndez, C.G., Räisänen, J., Rinke, A., Sarr, A., Whetton, P. (2007): Regional Climate Projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis ZM, Averyt KB, Tignor M, Miller HL (eds.) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

CIESM (2001): Gelatinous Zooplankton Outbreaks: Theory and Practice. CIESM Workshop 14: 1-112.

Cohen, D.M. (1970): How many recent fish are there? Proc Calif Acad Sci, 4th Series, 38: 341-346.

Collins, L.A., Nelson, S.G. (1993): Effects of temperature on oxygen consumption, growth, and development of embryos and yolk sac larvae of *Siganus randalli* (Pisces: Siganidae) Mar Biol 117: 195-204.

Conway, D.V.P., Coombs, S.H., Smith, C. (1997): Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. ICES J Mar Sci 54: 136-147.

Coombs, S.H. (1981): A density-gradient column for determining the specific gravity of fish eggs, with particular reference to eggs from the mackerel *Scomber scombrus*. Mar Biol 63: 101-106.

Coombs, S.H., Fosh, C.A., Keen, M.A. (1985): The buoyancy and vertical distribution of eggs of sprat (*Sprattus sprattus*) and pilchard (*Sardina pilchardus*). J Mar Biol Assoc UK 65: 461-474

Coombs, S.H., Boyra, G., Rueda, L.D., Uriarte, A., Santos, M., Conway, D.V.P., Halliday, N.C. (2004): Buoyancy measurements and vertical distribution of eggs and sardine (*Sardina pilchardus*) and Anchovy (*Engraulis encrasicolus*). Mar Biol 145: 959-970.

Cossins, A. R., Bowler, K. (1987): The Temperature Biology of Animals. Chapman and Hall, London.

- Cossins, A., Fraser, J, Hughes, M., Gracey, A. (2006): Post-genomic approaches to understanding the mechanisms of environmentally induced phenotypic plasticity. *J Exp Biol* 209: 2328-2336.
- Cowan, J.H., Shaw, R.F. (2002): Recruitment. In: Fuiman LE, Werner, RG (eds.) *Fishery Science: The Unique Contribution of Early Life Stages*. Blackwell Science, Oxford, pp. 88–111.
- Craik, J.C.A., Harvey, S.M. (1987): The causes of buoyancy in eggs of marine teleosts. *J Mar Biol Assoc UK* 67: 169-182.
- Cury, P., Roy, C. (1989): Optimal environmental window and pelagic fish recruitment success in upwelling areas. *Can J Fish Aquat Sci* 46: 670-680.
- Cushing, D.H. (1972): The production cycle and the numbers of marine fish. *Symp Zool Soc Lond* 29: 213-232.
- Cushing, D.H. (1974): The natural regulation of fish populations. In: Harden-Jones FR (ed.) *Sea fisheries research*. John Wiley and Sons, New York, pp. 399–412.
- Cushing, D.H. (1990): Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Adv Mar Biol* 26: 250–293.
- Daewel, U., Peck, M.A., Kühn, W., St. John, M.A., Alekseeva, I.; Schrum, C. (2008a): Coupling ecosystem and individual-based models to simulate the influence of climate variability on potential growth and survival of larval sprat in the North Sea. *Fish Ocean* 17(5): 333-351.
- Daewel, U., Peck, M.A., Schrum, C., St. John, M.A. (2008b): How best to include the effects of climate-driven forcing on prey fields in larval fish individual-based models. *J Plankt Res* 30: 1-5.
- Dana, G.L., Lenz, P.H. (1986): Effects of increasing salinity on an *Artemia* population from Mono Lake, California. *Oecologia* 68: 428-436.
- Daskalov, G. (1999): Relating fish recruitment to stock biomass and physical environment in the Black Sea using generalized additive models. *Fish Res* 41: 1-23.
- Debes, P.V. (2007): Phylogeographie und Populationsstruktur der Europäischen Sprotte (*Sprattus sprattus*) mittels Sequenzdaten der mitochondrialen Kontrollregion. Diploma thesis, Univ Kiel, Germany (In German).
- Debes, P.V., Zachos, F.E., Hanel, R. (2008): Mitochondrial phylogeography of the European sprat (*Sprattus sprattus* L. Clupeidae) reveals isolated climatically vulnerable populations in the Mediterranean Sea and range expansion in the northeast Atlantic. *Mol Ecol* 17: 3873-3888.
- Dickmann, M., Möllmann, C., Voss, R. (2007): Feeding ecology of Central Baltic sprat (*Sprattus sprattus*) larvae in relation to zooplankton dynamics: implications for survival. *Mar Ecol Prog Ser* 342: 277-289.

- Dulčić, J. (1998): Larval growth of sprat, *Sprattus sprattus phalericus*, larvae in the North Adriatic. *Fish Res* 36: 117-126.
- Dulčić, J., Lipej, L. (2002): Rare and little known fishes in the eastern Adriatic Sea during the last two decades (1981-2001). *Periodicum Biologorum* 104(2): 185-194.
- Ehrenbaum, E. (1936): Naturgeschichte und wirtschaftliche Bedeutung der Seefischerei Nordeuropas. In: Lübbert H, Ehrenbaum E (eds.) *Handbuch der Seefischerei Nordeuropas*, Band II, Schweizerbart'sche Verlagsgesellschaft, Stuttgart. (in German)
- Fabra, M., Raldúa, D., Power, D.M., Deen, P.M., Cerdà, J. (2005): Marine fish egg hydration is aquaporine-mediated. *Science* 307: 545.
- Fahy, W.E., O'Hara, R.K. (1977): Does salinity influence the number of vertebrae in developing fishes? *J Cons int Expl Mer* 37(2): 156-161.
- Fordyce, J.A (2006): The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *J Exp Biol* 209: 2377-2383.
- Fox, C.J., Geffen, A.J., Blyth, R., Nash, R.D.M. (2003): Temperature dependent development rates of plaice (*Pleuronectes platessa* L.) eggs from the Irish Sea. *J Plank Res* 25 (11): 1319-1329.
- Fuiman L.A. (2002): Special Considerations of Fish Eggs and Larvae. In: Fuiman LE, Werner RG (eds.) *Fishery Science: The Unique Contributions of Early Life Stages*. Blackwell Science, Oxford, pp. 1-32.
- Fukuhara, O. (1990): Effects of temperature on yolk utilization, initial growth, and behavior of unfed marine fish-larvae. *Mar Biol* 106: 169-174.
- Gagliano, M., Kowalewsky, S., McCormick, M.I. (2006): An alternative method for the preservation of tropical fish larvae. *J Fish Biol* 68(2): 634-639.
- Gamulin, T, Hure, J. (1983): The spawning and spawning areas of pelagic fishes (*Sardina pilchardus*, *Engraulis encrasicolus*, *Scomber scombrus*, *Sardinella aurita* and *Sprattus sprattus*) in the Adriatic Sea. *Acta Adriat* 24: 97-131.
- Gaughan, D.J., Fletcher, W.J., White, K.V. (2001): Growth rate of larval *Sardinops sagax* from ecosystems with different levels of productivity. *Mar Biol* 139: 831-837.
- George, M.R. (1987): Ovarian maturation cycle of sprat, *Sprattus sprattus*. ICES CM/H:47.
- Gillooly, J.F., Dodson, S.I. (2000a): The relationship of neonate mass and incubation temperature to embryonic development time in a range of animal taxa. *J Zool* 251: 369-375.
- Gillooly, J.F., Dodson, S.I. (2000b): The relationship of egg size and incubation temperature to embryonic development time in univoltine and multivoltine aquatic insects. *Freshw Biol* 44: 595-604.



- Gillooly, J.F., Charnov, E.L., West, G.B., Savage, V.M., Brown, J.H. (2002): Effects of size and temperature on developmental time. *Nature* 417: 70-73.
- Goarant, A., Petitgas, P., Bourriau, P. (2007): Anchovy (*Engraulis encrasicolus*) egg density measurements in the Bay of Biscay: evidence for the spatial variation in egg density with sea surface salinity. *Mar Biol* 151(5): 1907-1915.
- Govoni, J.J. (2005): Fisheries oceanography and the ecology of early life history of fishes: a perspective over fifty years. *Sci Mar* 69(suppl.1): 125-137.
- Govoni, J.J., Forward Jr, R.B. (2008): Buoyancy. In: Finn RN, Kapoor BG (eds.) *Fish Larval Physiology*, Science Publishers, Enfield USA pp. 495-521.
- Grauman, G.B., Yula, E. (1989): The importance of abiotic and biotic factors in early ontogenesis of cod and sprat. *Rapp P-v Réun Cons Int Explor Mer* 190: 207-210.
- Grbec, B., Dulčić, J., Morović, M. (2002): Long-term changes in landings of small pelagic fish in the eastern Adriatic - possible influence of climate oscillations over the Northern Hemisphere. *Clim Res* 20: 241-152.
- Griffin, F.J., Pillai, M.C., Vines, C.A., Kaaria, J., Hibbard-Robbins, T., Yanagimachi, R., Cherr, G.N. (1996): Effects of salinity on sperm motility, fertilization, and development in the Pacific herring, *Clupea pallasii*. *Biol Bull* 194: 25-35.
- Hall, C.J., Burns, C.W. (2001): Effects of salinity and temperature on survival and reproduction of *Boeckella hamata* (Copepoda: Calanoida) from a periodically brackish Lake. *J Plankt Res* 23(1): 97-104.
- Hardy, J.D. Jr (1978): Development of fishes of the mid-Atlantic Bight: An atlas of egg, larval and juvenile stages. *Biol Serv Progr FWS/ OBS-78/12*, Fish Wildl Serv USA.
- Hardy, R.S., Litvak, M.K. (2004): Effects of temperature on the early development, growth, and survival of shortnose sturgeon, *Acipenser brevirostrum*, and Atlantic sturgeon, *Acipenser oxyrinchus*, yolk.sac larvae. *Env Biol Fish* 70: 145-154.
- Harjes, A. (2008): Saisonaler Einfluss abiotischer und biotischer Faktoren auf den Überlebenserfolg von Fischbrut im Bornholmbecken. Staatsexamen thesis, Univ. Kiel.
- Harrison, T.D., Whitfield, A.K. (2006): Temperature and salinity as primary determinants influencing the biogeography of fishes in South African estuaries. *Estuar Coast Shelf S* 66(1-2): 335-345.
- Haslob, H., Clemmesen, C., Schaber, M., Hinrichsen, H.-H., Schmidt, J.O., Voss, R., Kraus, G., Köster, F.W. (2007): Invading *Mnemiopsis leidyi* as a potential threat to Baltic fish. *Mar Ecol Prog Ser* 349: 303-306.
- Hay, S. (2006): Marine Ecology: Gelatinous Bells May Ring Change in Marine Ecosystems. *Cur Biol* 16(17): R679-R682.

- Heath, M.R. (1992): Field investigations of the early life stages of marine fish. *Adv Mar Biol* 28: 1-174.
- Heming, T.A., Buddington, R.K. (1988): Yolk absorption in embryonic and larval fish. In Hoar WS, Randall DJ (eds.) *The Physiology of developing Fish: Eggs and Larvae*. Volume XI - Part A, Academic Press, New York pp. 407-446.
- Hempel, G. (1979): Early life history of marine fish. The egg stage. University of Washington Press, Seattle Washington, pp. 1-70.
- Hempel, G., Blaxter, J.H.S. (1961): The experimental modification of meristic characters in herring (*Clupea harengus* L.). *J Cons int Explor Mer* 26: 336-346.
- Hinrichsen, H.-H., Lehmann, A., Petereit, C., Schmidt, J. (2007): Correlation analyses of Baltic Sea winter water mass formation and its impact on secondary and tertiary production. *Oceanologia* 49(39): 381-395.
- Hjort, J. (1914): Fluctuations in the great fisheries of Northern Europe viewed in the light of biological research. *Rapp P-v Réunion Cons Perm int Explor Mer* 160: 1-228.
- Hjort, J. (1926): Fluctuations in the year classes of important food fishes. *J Cons int Explor Mer* 1: 5-38.
- Hohendorf, K. (1968): Zur Schwebfähigkeit pelagischer Fischeier in der Ostsee – vorläufige Mitteilung. *Ber Dt Wiss Komm Meeresforsch* 3: 181-193.
- Holliday, F.G.T. (1969): The effects of salinity on the eggs and larvae of teleosts. In: Hoar WS, Randall DJ (eds.) *Fish Physiology: Excretion, Ionic Regulation and Metabolism*. Volume I, Academic Press, New York, pp. 293-309.
- Holliday, F.G.T., Blaxter, J.H.S. (1960): The effect of salinity on the developing eggs and larvae of the herring. *J Mar Biol Assoc UK* 39: 591-603.
- Houde, E.D. (1974): Effects of temperature and delayed feeding on growth and survival of larvae of three species of subtropical marine fish. *Mar Biol* 26: 271-285.
- Houde, E.D. (1987): Fish early life dynamics and recruitment variability. *Am Fish Soc Symp* 2: 17-29.
- Houde, E.D. (1989): Comparative growth, mortality and energetics of marine fish larvae. *Fish Bull US* 87: 471-495.
- Houde, E.D. (1994): Differences between marine and freshwater fish larvae: implications for survival. *ICES J Mar Sci* 51: 91-97.
- Houde, E.D. (2002): Mortality. In: Fuiman LE, Werner RG (eds.) *Fishery Science - The unique contributions of early life stages*. Blackwell Science, Oxford, pp. 64-87.
- Houde, E.D. (2008): Emerging from Hjort's shadow. *J Northwest Atl Fish Sci* 41: 53-70.

Hunter, J. R., Alheit, J. (1995): International GLOBEC small pelagic fishes and climate change program. GLOBEC Report 8: pp. 72.

ICES (2008): Data derived from ICES website: <http://www.ices.dk> and is based on FAO data: <http://www.ices.dk/fish/statlant.asp>

Iles, T.D., Sinclair, M. (1982): Atlantic herring: stock: discreteness and abundance. *Science* 215: 627–633.

IPCC (2002): Climate Change and Biodiversity- IPCC Technical Paper V:1-77. IPCC Secretariat, c/o World Meteorological Organization, 7 bis, Avenue de la Paix, Case Postale 2300, 1211 Geneva 2, Switzerland.

Jeppesen, E., Søndergaard, M., Pedersen, A. R., Jürgens, K., Strzelczak, A., Lauridsen, T.L., Johansson, L.S. (2007): Salinity induced regime shift in shallow brackish lagoons. *Ecosystems* 10: 48–58.

Johns, D.M., Howell, W.H., Klein-MacPhee, G. (1981): Yolk utilization and growth to yolk-sac absorption in summer flounder (*Paralichthys dentatus*) larvae at constant and cyclic temperatures. *Mar Biol* 63: 301-308.

Johnston, I.A. (2006): Environment and plasticity of myogenesis in teleost fish. *J Exp Biol* 209: 2249-2264.

Johnston, I.A., Temple, G.K., Vieira, V.L.A. (2001): Impact of temperature on the growth and differentiation of muscle in herring larvae. In: Atkinson D, Thorndyke M (eds.) *Environment and Animal Development: Genes, Life Histories and Plasticity*. BIOS Scientific Publishers Ltd, Oxford, pp. 99-120.

Johnston, I.A., Wilson, R.S. (2006): Temperature induced developmental plasticity in ectotherms. In: Warburton SJ, Burggren WW, Pelster B, Reiber CL, Spicer J (eds.) *Comparative Developmental Plasticity: Contributions, Tools and Trends*. Oxford University Press, pp. 124-138.

Kamler, E. (1992): *Early life history of fish: An Energetics Approach*. Chapman and Hall, London.

Kamler, E. (2002): Ontogeny of yolk feeding fish: An ecological perspective. *Rev Fish Biol Fisheries* 12: 97-103.

Kamler, E. (2005): Parent-egg-progeny relationships in teleost fishes: an energetics perspective. *Rev Fish Biol Fisheries* 15: 399-421.

Kamler, E. (2008): Resource allocation in yolk-feeding fish. *Rev Fish Biol Fisheries* 18: 143-200.

Kändler, R. (1941): Die Fortpflanzung der Meeresfische in der Ostsee und ihre Beziehungen zum Fischereiertrag. *Monatshefte f Fischerei* 11: 158-163.

- Kanstinger, P. (2007): Growth and condition of larval sprat (*Sprattus sprattus*). A combined laboratory and field investigation. Diploma thesis, Univ of Hamburg, Germany.
- Karås, P., Klingsheim, V. (1997): Effects of temperature and salinity on embryonic development of turbot (*Scophthalmus maximus* L.) from the North Sea, and comparisons with the Baltic populations. *Helgol Meeresunters* 51: 241-247.
- Kinne, O. (1960): Growth, food intake and food conversion in a euryplastic fish exposed to different temperatures and salinities. *Physiological Zoology* 33: 288-318.
- Kinne, O. (1963): Physiological aspects of animal life in estuaries with special reference to salinity. *Neth J Sea Res* 3(2): 222-244.
- Klimogianni, A., Koumoundouris, G., Kaspiris, P., Kentouri, M. (2004): Effect of temperature on the egg and yolk-sac larval development of common pandora, *Pagellus erythrinus*. *Mar Biol* 145: 1015-1022.
- Köster, F.W., Hinrichsen, H.-H., Schnack, D., St. John, M., MacKenzie, B., Tomkiewicz, J., Möllmann, C., Kraus, G., Plikshs, M., Makarchouk, A., Aro, E. (2003): Recruitment of Baltic cod and sprat stocks: identification of critical life stages and incorporation of environmental variability into stock-recruitment relationships. *Sci Mar* 67 (Suppl. 1): 129-154.
- Köster, F.W., Möllmann, C. (2000): Egg cannibalism in Baltic sprat (*Sprattus sprattus* L.). *Mar Ecol Prog Ser* 196: 269-277.
- Kuhlmann, D., Quantz, G. (1980): Some effects of temperature and salinity on the embryonic development and incubation time of the turbot, *Scophthalmus maximus* L., from the Baltic Sea. *Meeresforsch* 28: 172-178.
- Kühn, W., Peck, M.A., Hinrichsen, H.-H., Daewel, U., Moll, A., Pohlmann, T., Stegert, C., Tamm, S. (2008): Defining habitats suitable for larval fish in the German Bight (southern North Sea): An IBM approach using spatially- and temporally-resolved, size structured prey fields. *J Mar Syst* 74(1-2): 329-342.
- Largier, J.L. (2003): Considerations in estimating larval dispersal distances from oceanographic data. *Ecological Applications* 13(1): S71-S89.
- Larsson, S., Forseth, T., Berglund, I., Jensen, A.J., Näslund, I., Elliot, J.M., Jonsson, B. (2005): Thermal adaptation of Arctic charr: experimental studies of growth in eleven charr populations from Sweden, Norway and Britain. *Freshw Biol* 50: 353-368.
- Lasker, R. (1964): An experimental study of the effect of temperature on the incubation time, and growth of Pacific sardine embryos and larvae. *Copeia* 2: 399-405.
- Lasker, R. (1978): The relation between oceanographic conditions and larval anchovy food in the California current: identification of factors contributing to recruitment failure. *Rapp P-v Cons Perm Int Explor Mer* 173: 212-230.

- Lasker, R. (1981): The role of a stable ocean in larval fish survival and subsequent recruitment. In: Lasker R (ed.) *Marine Fish Larvae: Morphology, Ecology and Relation to Fisheries*. Washington Press, Seattle, pp. 80–87.
- Laurence, G.C., Rogers, C.A. (1976): Effects of temperature and salinity on comparative embryo development and mortality of Atlantic cod (*Gadus morhua* L.) and haddock (*Melanogrammus aeglefinus* L.). *J Cons int Explor Mer* 63: 220-228.
- Laurence, G.C., Howell, W.H. (1981): Embryology and influence of temperature and salinity on early development and survival of yellowtail flounder *Limanda ferruginea*. *Mar Ecol Prog Ser* 6: 11-18.
- Limborg, M.T. (2007): Genetic population structure of European sprat (*Sprattus sprattus* L.). Master's thesis, Univ Copenhagen, Denmark.
- Lipej, L., Dulčić J. (2004): The current status of Adriatic fish diversity. In: Griffiths HL, Kryštufek B, Reed JM (eds.) *Balkan Biodiversity. Pattern and Process in Europe's Biodiversity Hotspot*. Kluwer Academic Publishers, Dordrecht, pp. 291–306.
- Litvak, M.K., Legett, W.C. (1992): Age and size-selective predation on larval fishes: the bigger-is- better hypothesis revisited. *Mar Ecol Prog Ser* 81: 13-24.
- Llanos-Rivera, A., Castro, L.R. (2006): Inter-population differences in temperature effects on *Engraulis ringens* yolk sac larvae. *Mar Ecol Prog Ser* 312: 245-253.
- Loewe, P., Becker, G., Brockmann, U., Frohse, A., Herklotz, K., Klein, H., Schulz, A. (2005): Nordseezustand 2003 Ozeanographischer Zustandsbericht. *Berichte des Bundesamtes für Seeschifffahrt und Hydrography* 38: 217 pp. (In German)
- MacKenzie, B., Köster, F.W. (2004): Fish production and climate: Sprat in the Baltic Sea. *Ecology* 85(3): 784-794.
- MacKenzie, B., Gislason, H., Möllmann, C., Köster, F.W. (2007): Impact of 21st century climate change on the Baltic Sea fish community and fisheries. *Global Change Biol* 13: 1348-1367.
- Macqueen, D.J., Robb, D.H.F., Olsen, T., Melstveit, L., Paxton, C.G.M., Johnston, I.A. (2008): Temperature until the 'eyed-stage' of embryogenesis programmes the growth trajectory and muscle phenotype of adult Atlantic salmon. *Biol Let* 4(3): 294-298.
- Makarchouk, A. (2002): Changes in the survival of sprat eggs in the Eastern Baltic. *ICES CM/O*:13.
- Malačič, V., Celio, M., Čermelj, B., Bussani, A., Comici, C. (2006): Interannual evolution of seasonal thermohaline properties in the Gulf of Trieste (northern Adriatic) 1991-2003. *J Geophys Res* 111, C08009, doi: 10.1029/2005JC003267, 2006.
- Marceta, B. (2001): Status of Slovene research and fishery on small pelagics. In: Mannini P, Massa F, Milone N (eds.) *Priority topics related to small pelagic fishery resources of the*

- Adriatic Sea. FAO-MiPAF Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea. GCP/RER/010/ITA/TD-03. AdriaMed Tech Doc 3: 24-29.
- Maree, R.C., Whitfield, A.K., Booth, A.J. (2000): Effect of water temperature on the biogeography of South African estuarine fishes associated with the subtropical/warm temperate subtraction zone. *S African J Sci* 96(4): 184-188.
- Martell, D.J., Kieffer, J.D., Trippel, E.A. (2005): Effects of temperature during early life history on embryonic and larval development and growth in haddock. *J Fish Biol* 66: 1558-1575.
- Matthäus, W., Franck H. (1992): Characteristics of major Baltic inflows – a statistical analysis. *Cont Shelf Res* 12(12): 1375-1400.
- Matthäus, W., Schinke H (1994): Mean atmospheric circulation patterns associated with major Baltic inflows. *Dtsch Hydrogr Z* 46(4): 321-339.
- McFarlane, G.A., King, J.R., Beamish, R.J. (2000): Have there been recent changes in climate? Ask the fish. *Prog Ocean* 47: 147-169.
- McGinn, N.A. (2002): Fisheries in a changing climate. *Am Fish Soc Symp* 32, Bethesda, MD, 295 pp.
- Meier, H.E.M. (2006): Baltic Sea climate in the late twenty-first century: a dynamical downscaling approach using two global models and two emission scenarios. *Clim Dyn* 27: 39-68.
- Meier, H.E.M., Kjellström, E., Graham, L.P. (2006): Estimating uncertainties of projected Baltic Sea salinity in the late 21st century. *Geophys Res Lett* 33: L15705, doi: 10.1029/2006GL026488.
- Mendiola, D., Ibaibarriaga, L., Alvarez, P. (2007): Thermal effects on growth and time to starvation during the yolk-sac larval period of Atlantic mackerel *Scomber scombrus* L.. *J Fish Biol* 70: 895-910.
- Miller, C.M., Moloney, C.L., van der Lingen, C.D., Lett, C., Mullon, C., Field, J.G. (2006): Modelling the effects of physical-biological interactions and spatial variability in spawning and nursery areas on transport and retention of sardine *Sardinops sagax* eggs and larvae in the southern Benguela ecosystem. *J Mar Syst* 61(3-4): 212-229.
- Mohrholz, V., Dutz, J., Kraus, G. (2006): The impact of exceptional warm summer inflow events on the environmental conditions in the Bornholm Basin. *J Mar Sys* 60: 285-301.
- Möllmann, C., Kornilovs, G., Sidrevics, L. (2000): Long-term dynamics of main mesozooplankton species in the Central Baltic Sea. *J Plankt Res* 22: 2015–2038.
- Möllmann, C., Köster, F. W., Kornilovs, G., Sidrevics, L. (2003): Interannual variability in population dynamics of calanoid copepods in the Central Baltic Sea. *ICES Mar Sci Symp* 219: 220–230.

- Morawa FWF (1953): Beiträge zur Biologie des Sprottes (*Clupea sprattus*) in der Ostsee. Dissertation, Institute of Marine Sciences, Univ of Kiel, pp. 102 (in German).
- Moustakas, C.T., Watanabe, W.O., Copeland, K.A. (2004): Combined effects of photoperiod and salinity on growth, survival, and osmoregulatory ability of larval southern flounder *Paralichthys lethostigma*. *Aquaculture* 229: 159-179.
- Munk, P. (1993): Differential growth of larval sprat (*Sprattus sprattus*) across a tidal front in the eastern North Sea. *Mar Ecol Prog Ser* 99: 17-29.
- Muus, B.J., Nielsen, J.G. (1999): Die Meeresfische Europas in Nordsee, Ostsee und Atlantik. Franchk-Kosmos Verlags-GmbH & Co., Stuttgart.
- Nelson, J.S. (1994): Fishes of the world. 3<sup>rd</sup> Edition, John Wiley & Sons, New York.
- Nissling, A. (2004): Effects of temperature on egg and larval survival of cod (*Gadus morhus*) and sprat (*Sprattus sprattus*) in the Baltic Sea- Implications for stock management. *Hydrobiologia* 514: 115-123.
- Nissling, A., Westin, L. (1991a): Egg mortality and hatching rate of Baltic cod (*Gadus morhua*) in different salinities. *Mar Biol* 111: 29-32.
- Nissling A., Westin, L. (1991b): Egg buoyancy of Baltic cod (*Gadus morhua*) and its implications for cod stock fluctuations in the Baltic. *Mar Biol* 111: 33-35.
- Nissling, A., Kryvi, H., Vallin, L. (1994a): Variation in egg buoyancy of Baltic cod (*Gadus morhua*) and its implications for egg survival in prevailing conditions in the Baltic Sea. *Mar Ecol Prog Ser* 110: 67-74.
- Nissling, A., Solemdal, P., Svensson, M., Westin, L. (1994b): Survival, activity and feeding ability of Baltic cod (*Gadus morhua*) yolk sac larvae at different salinities. *J Fish Biol* 45: 435-445.
- Nissling, A., Vallin, L. (1996): The ability of Baltic cod eggs to maintain neutral buoyancy and the opportunity for survival in fluctuating conditions in the Baltic Sea. *J Fish Biol* 48: 217-227.
- Nissling, A., Westin, L. (1997): Salinity requirements for successful spawning of Baltic and Belt Sea cod and the potential for cod stock interactions in the Baltic Sea. *Mar Ecol Prog Ser* 152: 261-271.
- Nissling, A., Westin, L., Hjerne, O. (2002): Reproductive success in relation to salinity for three flatfish species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the brackish water Baltic Sea. *ICES J Mar Sci* 59: 93-108.
- Nissling, A., Müller, A., Hinrichsen, H.-H. (2003): Specific gravity and vertical distribution of sprat eggs in the Baltic Sea. *J Fish Biol* 63: 80-299.

- Nissling, A., Johannson, U., Jacobsson, M. (2006): Effects of salinity and temperature conditions on the reproductive success of turbot (*Scophthalmus maximus*) in the Baltic Sea. *Fish Res* 80: 230-238.
- North, E.W., Gallego, A., Petigas, P. (eds.) Manual of recommended practices for modeling physical-biological interactions during fish early-life. ICES Coop Res Rep, *in press*.
- Ojaveer, E., Kalejs, M. (2005): The impact of climate change on the adaptation of marine fish in the Baltic Sea. *ICES J Mar Sci* 62: 1492-1500.
- Ottesen, O.H., Bolla, S. (1998): Combined effects of temperature and salinity on development and survival of Atlantic halibut larvae. *Aquacult Int* 6: 103-120.
- Palomera, I., Olivar, M.P., Salat, J., Sabatés, M., Coll, M., Garcia, A., Morales-Nin, B. (2007): Small pelagic fish in the NW Mediterranean Sea: An ecological review. *Prog Ocean* 74(2-3): 377-396. [doi:10.1016/j.pocean.2007.04.012](https://doi.org/10.1016/j.pocean.2007.04.012).
- Parmanne, R., Rechlin, O., Sjöstrand, B. (1994): Status and future of herring and sprat stocks in the Baltic Sea. *Dana* 10: 29-59.
- Pauly, D., Pullin, R. (1988): Hatching time in spherical, pelagic marine fish eggs in response to temperature and egg size. *Environ Biol Fish* 22: 261-271.
- Pechenik, J. (2006): Larval experience and latent effects—metamorphosis is not a new beginning. *Integr Comp Biol* 46(3): 323–333.
- Peck, M.A., Clemmesen, C., Herrmann, J.-P. (2005): Ontogenic changes in the allometric scaling of the mass and length relationship in *Sprattus sprattus*. *J Fish Biol* 66(3): 882-887.
- Peck, M.A., Daewel, U. (2007): Physiologically-based limits to food consumption, and individual-based modeling of foraging and growth of larval fishes. *Mar Ecol Prog Ser* 347: 171–183.
- Peck, M.A., Baumann, H., Bernreuther, M., Clemmesen, C., Hermann, J.-P., Huwer, B., Kanstinger, P., Petereit, C., Temming, A., Voss, R.: The Ecophysiology of *Sprattus sprattus* in the Baltic and North Sea, *unpublished a*.
- Peck, M.A., Geffen, A.J., Chambers, R.C. Intra- and inter-specific effects of temperature on the development rate of marine and estuarine fish embryos, *unpublished b*.
- Pepin, P. (1991): Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Can J Fish Aquat Sci* 48: 503-518.
- Pepin, P., Orr, D.C., Anderson, J. (1997): Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (*Gadus morhua*). *Can J Fish Aquat Sci* 54(1): 2–10.
- Petereit, C. (2004): Experimente zum Temperatureinfluß auf frühe Entwicklungsstadien des Ostseedorsches (*Gadus morhua* L.). Diploma thesis, Univ Kiel, Germany (in German).



- Petereit, C., Haslob, H., Kraus, G., Clemmesen, C. (2008): The influence of temperature on the development of Baltic Sea sprat (*Sprattus sprattus*) eggs and yolk sac larvae. *Mar Biol* 154: 295-306 DOI 10.1007/s00227-008-0923-1.
- Petereit, C., Hinrichsen, H.-H., Voss, R., Kraus, G., Freese, M., Clemmesen, C.: The influence of different salinity conditions on egg buoyancy, -development, -survival and morphometric traits of Baltic Sea sprat (*Sprattus sprattus balticus* Schneider) yolk sac larvae, *submitted to Scientia Marina*.
- Peterson, R.H., Martin-Robichaud, D.J., Harmon, P. (2004): Influence of incubation temperature on body movements of Atlantic cod (*Gadus morhua* L.) embryos and on size at hatch. *Aquacult Res* 35(5): 453-457.
- Planque, B., Frédou, T. (1999): Temperature and the recruitment of Atlantic cod (*Gadus morhua*). *Can J Fish Aquat Sci* 56: 2069–2077.
- Polo, A., Yúfera, M., Pascual, E. (1991): Effects of temperature on egg and larval development of *Sparus aurata* L. *Aquaculture* 92: 367-375.
- Ponwith, B.J., Neill, W.H. (1995): The influence of incubation salinity on the buoyancy of red drum eggs and yolk sac larvae. *J Fish Biol* 46: 955-960.
- Poulard, J.-C., Blanchard, F. (2005): The impact of climate change on the fish community structure of the eastern continental shelf of the Bay of Biscay. *ICES J Mar Sci* 62: 1436-1443.
- Pritchard, G., Harder, L.D., Mutch, R.A. (1996): Development of aquatic insect eggs in relation to temperature and strategies for dealing with different thermal environments. *Biol J Linn Soc* 58: 221-244.
- Rass, T.S. (1949): Black Sea Ichthyofauna and its exploitation. *Proc Inst Oceanology, AS URSS* 4 (in Russian).
- Regner, S. (1996): Effects of environmental changes on early stages and reproduction of Anchovy in the Adriatic Sea. *Sci Mar* 60(Supl.2): 167-177.
- Ricker, W.E. (1954): Stock and recruitment. *J Fish Res Board Can* 11: 559-623.
- Roessig, J.M., Woodley, C.M., Cech Jr., J.J., Hansen, L.J. (2004): Effects of global climate change on marine and estuarine fishes and fisheries. *Rev Fish Biol Fish* 14: 251-275.
- Rohlf, N. (1999): Verhaltensänderungen der Larven des Ostseedorsches (*Gadus morhua callarias*) während der Dottersackphase. *Ber Inst Meereskd Christian-Albrechts-Univ Kiel*, 312: 1-52.
- Rombough, P.J. (1988): Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: Hoar WS, Randall, DJ (eds.) *Fish Physiology*. Volume 11, Part A, Academic Press, New York, pp. 59-161.
- Rose, G.A (2005): On distributional responses of North Atlantic fish to climate change. *ICES J Mar Sci* 62: 1360-1374.

- Rothschild, B.J. (1986): Dynamics of marine fish populations. Harvard University Press, Cambridge, pp. 277.
- Rothschild, B.J. (2000): Fish stocks and recruitment: the past thirty years. ICES J Mar Sci 57: 191-201.
- Sabatés, A., Martín, P., Lloret, J., Raya, V. (2006): Sea warming and fish distribution: the case of the small pelagic fish, *Sardinella aurita*, in the western Mediterranean. Global Change Biol 12(11): 2209-2219.
- Sähn, N. (2008): Temperature effect on Baltic larval cod (*Gadus morhua callarias* L.) derived from laboratory experiments. Diploma thesis, Univ Kiel, Germany.
- Sarma, S., Nandini, S., Morales-Ventura, J., Delgado-Martínez, I., González-Valverde, L. (2006): Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquat Ecol 40(3): 349-360.
- Sharma, S., Jackson, D.A., Minns, C.K., Shuter, B.J. (2007): Will northern fish populations be in hot water because of climate change? Global Change Biol 13(10): 2052-2064.
- Shi, Z., Huang, X., Fu, R., Wang, H., Luo, H., Chen, B., Liu, M., Zhang, D. (2008): Salinity stress on embryos and early larval stages of the pomfret *Pampus punctatissimus*. Aquaculture 275: 306–310.
- Shields, R.J. (1989): Studies of growth and nutritional status in 0-group sprat, *Sprattus sprattus* (Clupeidae), using otolith microstructure and lipid analytical techniques. PhD thesis, Univ Wales, Bangor, School of Ocean Science, Menai Bridge, Gwynedd, pp. 124.
- Sinclair, M. (1988): Marine populations. An essay on population regulation and speciation. Washington Sea Grant Program. University of Washington Press, Seattle, pp. 252.
- Sissenwine, M.P. (1984): Why Do Fish Populations Vary? In: May RM (ed.) Exploitation of Marine Communities, Springer-Verlag, Berlin, pp.59–94.
- Sjöblom, V., Parmanne, R. (1980): Sprat off the coast of Finland in 1977 and 1978. Annales Biologiques 35: 200-203.
- Skjaerven, K.H., Finn, R.N., Kryvi, H., Fyhn, H.J. (2003): Yolk resorption in developing plaice (*Pleuronectes platessa*). In: Browman HI, Skiftesvik AB (eds.) The big fish bang. Proceedings of the 26<sup>th</sup> Annual Larval Fish Conference, Bergen, 22-26 July 2002. Institute of Marine Research, Bergen, pp. 193-209.
- Stepputtis, D. (2006): Distribution patterns of Baltic sprat (*Sprattus sprattus* L.) - causes and consequences. Dissertation, Univ Kiel, pp. 152.
- Stige, L.C., Ottersen, G., Brander, K., Chan, K.-S., Stenseth, N.C. (2006): Cod and climate: effect of the North Atlantic Oscillation on recruitment in the North Atlantic. Mar Ecol Prog Ser 325: 227-241.

- Štirn, J. (1969): The North Adriatic Pelagial- It's oceanological characteristics, structure and distribution of the biomass during the year 1965, PhD thesis, Academia Scientiarum et Artum Slovenica, Classis IV: Historia Naturalis et Medicina, Ljubljana (English summary).
- Takasuka, A., Aoki, I., Mitani, I. (2003): Evidence of growth-selective predation on larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. *Mar Ecol Prog Ser* 252: 223-238.
- Takasuka, A., Aoki, I., Mitani, I. (2004): Three synergistic growth related mechanisms in the short-term survival of larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. *Mar Ecol Prog Ser* 270: 217–228.
- Tarifeño, E., Carmona, M., Llanos-Rivera, A., Castro, L.R. (2008): Temperature effects on the anchoveta *Engraulis ringens* egg development: do latitudinal differences occur? *Environ Biol Fish* 81: 387–395. DOI 10.1007/s10641-007-9208-7
- Teskerendžić, Z. (1983): The spawning of sprat, *Sprattus sprattus* (L.), in the Kvarner Region and Rijeka Bay. *Acta Adriat* 24(1/2): 13-24.
- Thomas, W.H., Scotten, H.L., Bradshaw, J.S. (1963): Thermal gradient incubators for small aquatic organisms. *Limnol Oceanogr* 8: 357-360.
- Thompson, B.M., Milligan, S.P., Nichols, J.H. (1981): The development rates of Sprat (*Sprattus sprattus* L.) eggs over a range of temperatures. *ICES CM* 1981/H:15.
- Thorsen, A., Kjesbu, O.S., Fyhn, H.J, Solemdal, P. (1996): Physiological mechanisms of buoyancy in eggs from brackish water cod. *J Fish Biol* 48: 457-477.
- Tičina V., Katavić, I., Dadić, V., Grubišić, L., Franičević M., Tičina, V.E. (2005): Acoustic estimates of small pelagic fish stocks in the eastern part of the Adriatic Sea: September 2004. *GFCM – SAC – SCSA*, Rome, 26-30. 09. 2005. [http://www.icm.csic.es/rec/projectes/scsa/Subcommittee\\_2005/Papers/No%2019-Anc,%20sar%2017.pdf](http://www.icm.csic.es/rec/projectes/scsa/Subcommittee_2005/Papers/No%2019-Anc,%20sar%2017.pdf)
- Tičina, V. (2000): Biology and commercial importance of sprat, *Sprattus sprattus phalericus* (L. 1758), in the Adriatic Sea. Doctoral Thesis, Univ Zagreb, pp. 1-133, (English Abstract).
- Trippel, E.A., Chambers, R.C. (1997): The early life history of fishes and its role in recruitment processes. In: Chambers RC, Trippel EA (eds.) *Early life history and Recruitment in fish populations*. Chapman and Hall, London, pp. xxi-xxxii (Introduction).
- Trippel, E.A., Kjesbu, O.S., Solemdal, P. (1997): Effects of adult age and size structure on reproductive output in marine fishes. In: Chambers RC, Trippel EA (eds.) *Early Life History and Recruitment in Fish Populations*. Chapman and Hall, London, pp. 31–62.
- Trippel, E.A., Kraus, G., Köster, F.W. (2005): Maternal and paternal influences on early life history traits and processes of Baltic cod (*Gadus morhua*). *Mar Ecol Prog Ser* 303: 259-267.
- van Doorslaer, W., Stoks, R. (2005): Thermal reaction norms in two *Coenagrion* damselfly species: contrasting embryonic and larval life-history traits. *Freshw Biol* 50: 1982–1990.

- Voipio, A. (1981): The Baltic Sea. Elsevier Scientific Publishing Company, Amsterdam.
- von Westernhagen, H. (1970): Erbrütung von Dorsch (*Gadus morhua*), Flunder (*Pleuronectes flesusus*) und Scholle (*Pleuronectes platessa*) unter kombinierten Temperatur- und Salzgehaltsbedingungen. Helgol wiss Meeresunters 21: 21-102.
- Voss, R., Clemmesen, C., Baumann, H., Hinrichsen, H.-H. (2006): Baltic sprat larvae: Coupling food availability, larval condition and survival. Mar Ecol Prog Ser 306: 243-254.
- Voss, R., Köster, F.W., Dickmann, M. (2003): Comparing the feeding habits of co-occurring sprat (*Sprattus sprattus*) and cod (*Gadus morhua*) larvae in the Bornholm Basin, Baltic Sea. Fish Res 63: 97-111.
- Wahl, E., Alheit, J. (1988): Changes in the distribution and abundance of sprat eggs during spawning season. ICES CM/H:45.
- Watanabe, W.O., French, K.E., Ernst, D.H., Olla, B.L., Wicklund, R.I (1989): Salinity during early development influences growth and survival of Florida Red Tilapia in brackish and seawater. J World Soc Aquacult 20(3): 134-142.
- Westerbom, M., Kilpi, M., Mustonen, O. (2002): Blue mussels, *Mytilus edulis*, at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea. Mar Biol 140: 991-999.
- Yin, M. C., Blaxter, J.H.S. (1987a): Feeding ability and survival during starvation of marine fish larvae reared in the laboratory. J Exp Mar Biol Ecol 105: 73-83.
- Yin, M. C., Blaxter, J.H.S (1987b): Temperature, salinity tolerance and buoyancy during early development and starvation of Clyde and North Sea herring, cod and flounder larvae. J Exp Mar Biol Ecol 107: 279-290.
- Zavodnik, N. (1969): An account on the sexual cycle and the spawning of the north Adriatic sprat (*Sprattus sprattus* L.). Medd Havsfiskelabor Lysekil 66: 1-3.
- Zavodnik, D. (1970): Comparative data on the spawning of sardine (*Sardina pilchardus*), sprat (*Sprattus sprattus*) and anchovy (*Engraulis encrasicolus*) in the North Adria. Ichthyologia 2(1): 171-178.
- Zavodnik, N., Zavodnik, D. (1969): Studies on the life history of Adriatic sprat. Stud Rev gen Fish Coun Medit 40: 26pp.
- Zore-Armanda, M., Grbec, B., Morović, M. (1999): Oceanographic properties of the Adriatic Sea- A point of view. Acta Adriat 40(suppl.): 39-54.

V ANNEX

List of figures:

- Figure 1-1: General life cycle of a marine fish, using sprat (*Sprattus sprattus*) as an example. Major periods and the corresponding dominant intrinsic processes are shown. The circular chart shows that ontogenetic changes (green color with solid lines) begin at fertilization of the egg and gradually diminish toward the juvenile period. Growth (grey color with long dashed line) also begins at fertilization and diminishes toward adulthood. Reproduction (red color and dotted line) begins when gonads differentiate and diminishes in senescence, modified after Fuiman (2002). Pictures of feeding larva are courtesy of Bastian Huwer and late larva/juvenile/adult sprats of Myron Peck. .... 1
- Figure 1-2: General anatomy of teleost fishes. Graphics taken from Fuimann (2002), original by Hardy *et al.* (1978). Egg developmental phases and fish egg and embryo structure (upper part). Yolk sac larva anatomy with important morphometric measurements. Feeding larva with pigmented eyes and open mouth gap (lower part). In this thesis, notochord length (= standard length) was measured (middel part). Note that sprat eggs have no oil globule. .... 2
- Figure 1-3: Hypotheses to explain recruitment variability in fish originating from Hjort's (1914) concepts of "critical period" and "aberrant drift". Solid arrows indicate direct and broken arrows indirect derivations from Hjort's hypotheses, whereas thickness of arrows indicates strength of the relationship (taken from Houde 2008, modified). .... 3
- Figure 1-4: Abiotic and biotic factors effecting life stages of fish. Highlighted are only factors influencing non feeding phases (egg and yolk sac phase) of fish with relevance to ontogeny (green, solid line) and growth (grey, dashed line). Effects of temperature and salinity as abiotic effects were analyzed in particular within this study. .... 5
- Figure 1-5: Schematic illustration of changes in sensitivity to upper and lower salinity extremes during ontogenetic development. Originally exemplified for trends found in numbers of estuarine animals (decapods, amphipods, mussels, gastropods), modified from Kinne (1963). Illustration was adapted to the life-cycle of sprat and dark squared area (fertilization to end of yolk-sac phase) refers to period considered in this thesis. .... 7
- Figure 1-6: Adult sprat caught during a scientific research cruise onboard RV Alkor in May 2006. .... 8
- Figure 1-7: Hydrographic features of Baltic Sea, North Sea and Adriatic Sea characteristic for spawning time and occurrence of sprat eggs. Sprat spawning season is in spring and early summer in the central Baltic Sea (Bornholm Basin) and was found to continue until August in the North Sea. Spawning time in the North Adriatic is from November to March in winter. The range of water temperatures (°C) refers in all areas to the water layers in the depth, where sprat eggs were found. Vertical egg distribution (m) is expressed as mean depth value for the Bornhom Basin, Baltic Sea, for the North Sea as found in the literature (Conway *et al.* 1997). No studies were performed on vertical egg distribution in the Adriatic Sea. The range of the egg size (diameter, mm) for the Baltic Sea and the Adriatic Sea were obtained from this study, North Sea values were obtained from Alheit *et al.* 1987. .... 8
- Figure 1-8: Sprat catch data for the Baltic Sea derived from the ICES website (<http://www.ices.dk/fish/statlant.asp>). Adriatic sprat catch data taken from Tičina (2000). Note that the magnitude between both data sets varies with a factor of 100! After 1997, sprat catch in the North Adriatic dropped further and no fishing data are reported since (V. Tičina, personal comment). In 2004 Adriatic sprat biomass was assessed by hydro-acoustic methods to be less than 1000t (Tičina 2005). .... 9

- Figure 1-9: Thermal sensitivity concept. (a) Warm adaption is considered if thermal sum in degree-days to hatch is reduced with increasing temperature. (b) Intermediate or general adaptation is given if neither increasing nor decreasing trend in developmental time is observed at increasing temperature. (c) cold adaptation is derived from decreasing developmental time with decreasing temperature. (Modified after Pritchard *et al.* 1996 and Peck *et al.*, unpublished) ..... 10
- Figure 1-10: Map of Central European seas (a), map of the Baltic Sea (b) and the Adriatic Sea (c). The small inserts show detailed maps of respective sampling areas the Bornholm Basin in the Baltic Sea and the Gulf of Trieste in North Adriatic Sea. Mean monthly temperature and salinity values ((d) = Baltic Sea, (e) = Adriatic Sea) of either Sea surface (0m) or the depth were large numbers of sprat eggs are found during spawning season (Adria 10m, Baltic 50m). Timing of sprat spawning seasons are presented as semi transparent coloured background for each population. Temperature and salinity data from the Bornholm Basin are monthly mean values of 10 years (1996-2005, derived from ICES hydrographical database) and data for the Gulf of Trieste are redrawn from Malačić *et al.* (2006) for the period of 1991 -2003..... 11
- Figure 2-1: Map of hydrographic areas from which data on sprat early life history are available (crosses). From left: Irish Sea, English Channel, North Sea (German Bight), Baltic Sea (GD=Gdansk Deep (ICES Subdivision (SD) 26)), Baltic Sea (GB=Gotland Basin (SD 28)). The asterisk marks the origin of early life stages used for experiments in this study (Baltic Sea (BB=Bornholm Basin (SD 25))). ..... 14
- Figure 2-2: Stage specific egg developmental times in days post fertilization [dpf] for Baltic sprat eggs incubated under different temperature regimes. Shown are values of the last observed occurrence of a specific developmental stage and the first occurrence of hatched larvae..... 16
- Figure 2-3: Egg development rates in percent per day versus temperature for egg incubation experiments from different areas. Literature values from Nissling (2004) represent 50%-hatch data whereas Thompson *et al.* (1981) and this study reflect first-hatch data for each temperature. Potential equations are fitted to the data. .... 18
- Figure 2-4: Temperature dependent egg survival for two different experimental trials at different incubation temperatures. No survival was observed at 16°C. Same temperatures (8.4 and 10.0°C) were used for trial 1 and 2. Shown are mean (+SD) values of 3 to 4 replicates (n)..... 18
- Figure 2-5: Individual length measurements of sprat yolk sac larvae incubated at 10.0°C (a), 8.4° (b), 7.6°C (c), 5.7°C (d) and 3.8° (e). Laird-Gompertz growth curves are fitted to the data. Open dots represent measurements from one single replicate only and were not included in the regression analyses. Dotted lines indicate 95% confidence interval of the nonlinear regression. Dashed vertical lines represent age in days at total yolk depletion. .... 19
- Figure 2-6: Yolk sac depletion rate for yolk sac larvae from different areas incubated at different temperatures. Potential equations are fitted to the data. Data from the North Sea, Irish Sea as well as from Subdivisions (SDs) 26 and 28 from the Baltic Sea are extracted from the literature. .... 20
- Figure 2-7: Time period from hatch to eye pigmentation of yolk sac larval sprat. Exponential functions were fitted to the data. North Sea and Irish Sea data were extracted from the literature. For Baltic sprat larvae, shown are mean ( $\pm$ SD) values of 3 to 6 replicates..... 21
- Figure 2-8: Starvation induced 100% mortality of sprat larvae in days after hatching. Shown is the fitted normal distribution curve for mortality (solid line). Each circle represents the time, until the last larvae within a single replicate (beaker) survived. At some temperatures (6.8°C, 8.4°C, 10°C) two replicates were terminated on the same day.

- Therefore, dots were offsetted graphically, which had no influence on the curve fitting. The single observation (black circle) was not included in the analysis. .... 24
- Figure 3-1: Area of origin of the experimental material, Northern Adriatic Sea, Gulf of Trieste, Bay of Koper. The black cross indicates the position at which the sprat used for the experimental trials were caught during the night of 11/12<sup>th</sup> January 2007. .... 28
- Figure 3-2: Sprat egg development until hatch. First egg cell cleavages in egg stage IA (4-, 8-, 16-, 32-, 64-, 128-cell stages) during the first twelve hours after fertilization [hpf] at a temperature of about 12°C (~6 dd°). From stage IB to V, three examples per stage are depicted in each row. Egg stages are exemplified for the development at 9.7°C. The time sequence in hpf is as follows: IB (12 - 32), II (32 -56), III (56 – 84), IV (84 – 96), V (96 – 105) and hatch (105 – 108). Scale bar = 0.5mm. .... 30
- Figure 3-3: Developmental times (hpf) at which ≥50% of the eggs had reached the specified stages related to temperature. Shown are mean values for trials 1 and 2 combined (n=6) with standard deviations and fitted exponential functions. Missing values (at 15.4°C and 19°C for stage II and at 5.0°C for stage V) are due to the fact that these stages could not be observed during two sampling intervals. Stage IA was observed between fertilization and start of the different temperature treatments (before 6dd° post fertilization). .... 32
- Figure 3-4: Hatch rates (percent survival from fertilization until hatch) of sprat eggs (trial 1 and trial 2 separately) incubated under different temperature levels. Shown are mean and range (minimum and maximum) values per temperature from three replicates. At 19°C only eggs from trial 2 were incubated. Both trials have identical temperature levels as indicates on the x-axis but points were slightly offset to prevent overlaying in the graph. .... 34
- Figure 3-5: Morphometric traits-at-hatch of sprat yolk sac larvae incubated under different temperature levels and two trials. Box-Whisker plots show medians (= thin black lines), 25<sup>th</sup> and 75<sup>th</sup> percentiles indicated in the boxes, 10<sup>th</sup> and 90<sup>th</sup> percentiles indicated by the Whiskers, outliers (dots) and mean values (=thick black bars) for: (a) Larval standard length (SL), (b) yolk sac area (YSA), (c) body area (BA) and (d) the BA:YSA ratio at-hatch. 8.5°C and 14.1°C data were excluded from the analyses due to delayed sampling during the experiment. No larvae were sampled from trial 2 at 15.4°C because hatching from eggs of this trial was later than hatching from trial 1 at this temperature. .... 34
- Figure 3-6: Sprat larval yolk resource-related proxies in relation to different temperature levels: (a) Time to yolk sac depletion (YSD) in days post fertilization in both trials (mean+SD). (b) Yolk utilization rates (+SE) (YUR, mm<sup>3</sup>\* day<sup>-1</sup>) from sprat larvae originating from two different trials vs incubation temperature. Shown are slope values (±SE), calculated from the decrease of yolksac volume (YSV) vs. time, for each temperature treatment and both trials. (c) Yolk utilization efficiency (+SE) (YUE) from sprat larvae originating from two different trials vs incubation temperature. Mean values (±SE) were derived from body area (BA) to yolk sac area (YSA) ratios and reflect the conversion efficiency of yolk into somatic tissue. .... 35
- Figure 3-7: (a-c) Laird Gompertz growth parameters (a, b, L<sub>0</sub> parameters ±SE) derived from calculated growth curves based on larval length measurements taken every 10dd post hatch until yolk sac depletion from all temperatures and both trials. (d) Laird-Gompertz yolk sac larval growth model derived from mean values of parameters a and L<sub>0</sub> from almost all temperatures (excluding 8.5°C and 14.1°C) and both trials. The parameter b was taken as an exponential function of temperature derived from least square curve fitting to all observed b, at temperature values (Figure 7b) of both trials. The interval (D) between each horizontal black line bar reflects the time of one day. The black dots represent larval sizes at eye pigmentation (EP) and the white squares larval sizes at yolk sac depletion (YSD) for each temperature. Grey triangles represent maximum survival time. .... 36

- Figure 3-8: Development of sprat larvae, exemplified for development at 9.7°C: (a) newly hatched. (b) at 20 degree days post hatch (ddph) corresponding to 50 hour post hatch (hph). (c) at 40 ddph (100 hph). (d) at 60 ddph (150 hph), the time of eye pigmentation (EP) and mouth gap opening (MGO). (e) at 80 ddph (200 hph), the time of yolk sac depletion (YSD). Scale bar = 0.5mm ..... 36
- Figure 3-9: Development of the head of sprat larvae with special focus on eye and mouth development, exemplified for development at 9.7°C: (a) Newly hatched larvae. (b) 30 ddph (75 hph). (c) 50 ddph (125 hph) –eyes pigmented brown, mouth gape still closed. (d) 60 ddph (150 hph) – eyes pigmented black, mouth gape open. (e) 80 ddph (200 hph) – eyes fully developed, mouth fully functionable, yolk sac depleted. Scale bar = 0.1mm. .... 39
- Figure 4-1: Cumulative egg survival from fertilization to hatch (n=3 per salinity; mean ± stdev). (a) Survival of eggs fertilized and incubated at different salinities, asterisk marks significant difference between salinities in trial 2 (b) survival of eggs fertilized at 7.5 and subsequently (<5 min) incubated at different salinities. .... 47
- Figure 4-2: (a) Sprat egg density values derived from experimental trials in April 2007 (black dots: fertilization trial (trial 1 + 2); transparent dots incubation trial (trial 3). Every single data point represents a density value of a single egg from an independent replicate, exceptions at salinity 30 and 35, where more than one egg was taken from each replicate. Second order polynomial regression is fitted to the combined data set (trial 1 + 2 + 3). Box areas (grey=2007; shaded=2008) show vertical range of egg density values and salinity derived from experimental density measurements of field sampled eggs. April 2007 egg densities were calculated from egg vertical distribution obtained by Multinet (MN) sampling (Harjes 2008). Depth and temperature range of the field caught eggs are presented. (b) Sprat egg density values derived from experimental trial in May/June 2007 (fertilization trial 4). Second order polynomial regression is fitted to the data. Box areas show vertical range of egg density values from field samples (as explained above)..... 48
- Figure 4-3: Yolk sac larval morphometric traits (standard length (SL, mm), yolk sac area (YSA, mm<sup>2</sup>) and body area (BA, mm<sup>2</sup>)) of Baltic Sea sprat larvae originating from the fertilization trial (trial 1), at hatch (Day 0, left panel) and at yolk sac depletion (YSD - Day 11, right panel). Box-Whisker plots show the median (black bar), the 25<sup>th</sup> and 75<sup>th</sup> percentiles as vertical boxes with 10<sup>th</sup> and the 90<sup>th</sup> percentiles as error bars. Numbers of measured larvae are presented above each Whisker. .... 50
- Figure 4-4: Yolk sac larval morphometric traits (standard length (SL, mm), yolk sac area (YSA, mm<sup>2</sup>) and body area (BA, mm<sup>2</sup>)) of Baltic Sea sprat larvae originating from the incubation trial (trial 3), at hatch (Day 0, left panel) and at eye pigmentation (EP- Day 9, right panel). Box-Whisker plots show the median (black bar), the 25<sup>th</sup> and 75<sup>th</sup> percentiles as vertical boxes with 10<sup>th</sup> and the 90<sup>th</sup> percentiles as error bars.) Numbers of measured larvae are presented above each Whisker. Too few larvae were left in the 30 and 35 salinity treatments, values are not presented. .... 51
- Figure 5-1: Map of the Western and Central Mediterranean with the Adriatic Sea (small insert). The red box indicates the enlarged part from the Northern Adriatic Sea (large map). The red dot shows the position where adult sprat was caught at night from 11-12<sup>th</sup> January 2007 in the Bay of Koper, Gulf of Trieste. These sprat provided the eggs for the salinity experiments..... 57
- Figure 5-2: (a) Relative sprat egg buoyancy, categorized in floating, floating at surface layer or non floating (bottom). (b) Mean±stdev of cumulative egg survival from the 20-37 psu treatments of 3 independent replicates. No eggs survived at 5 psu and survival of 10 and 15 psu was estimated (see text). Significant differences (p<0.05) between the 25 to 37 psu treatments are indicated by different letters. (c) Larval neutral buoyancy during the



- first three days after hatch (at 20 and 25 psu larvae had to achieve neutral buoyancy actively). (d) Relative occurrence of malformations after hatch. (e) Mean±stdev survival days of non feeding yolk sac larvae after hatch derived from three independent replicates. Presented are 50% mortality and maximum survival time (100%) of individual larvae within a replicate. The 20 psu trial had to be excluded from the statistical analyses to avoid potential overestimations of survival time derived from uncertain determination. No comparisons were performed between levels of survival (50% and 100%) within a salinity treatment. Significant differences ( $p<0.05$ ) between the salinity treatments 25 -37 psu are indicated by different letters. .... 60
- Figure 5-3: Morphometric traits of 10 hours post hatch sprat larvae (mean±stdev of 7-10 individuals per treatment) incubated under 4 different salinity treatments. Measurements were performed on larval digital images. (a) Standard length (SL) in mm. (b) Yolk sac area (YSA) in mm<sup>2</sup>. (c) Yolk sac volume (YSV) mm<sup>3</sup>. (d) Body area (BA) mm<sup>2</sup>. (e) Ratio of body area and yolk sac area. Significant differences ( $p<0.05$ ) between the salinities are labeled with different letters..... 61
- Figure 5-4: Typical habitus of sprat yolk sac larvae derived from eggs incubated at different salinity treatments. (a) Eggs incubated at 10 psu, larvae succeed to disrupt the egg integument but remained within the egg. (b) Eggs incubated at 15 psu, larvae failed to leave the egg integument and frequently got stuck with the yolk sac. (c) Eggs incubated at 20 psu, larvae regularly managed to hatch, but many deformations of the notochord and especially around the tail region were observed which inhibited hatching. (d) Eggs incubated at 25 psu, almost all larvae hatched successfully, but some larvae showed deformations on the notochord, which nevertheless did not prevent swimming. (e) Typical sprat yolk sac larvae hatched from eggs incubated at 30, 35 or 37.2 psu. No malformations were observed. Scale bar = 1mm. .... 63
- Figure 6-1: Relative mean egg survival (standardized to the maximum observed survival from fertilization until hatching) of sprat eggs incubation-experiment from different (a) temperature and (b) salinity treatments with two different sprat populations. Transparent grey colour represents mean egg survival of the Baltic Sea population and black colour represents egg survival of the Adriatic Sea population..... 65
- Figure 6-2: Thermal sensitivity values derived from sprat egg development rates (b values of power function) from the Baltic Sea, the North Sea and the Adriatic Sea sprat population. b-values above 1 indicate warm adaptation, which reflect a decrease in egg developmental time with increase in incubation temperature (Pritchard *et al.* 1996). b-values below 1 indicate cold adaptation with a decrease in developmental time with decreasing temperature. Accordingly, values of about 1 reflect general, intermediate temperature sensitivity. .... 66
- Figure 6-3: “Window of opportunity” (WOO) to successfully establish first feeding of sprat larvae from the Baltic Sea population and the Adriatic Sea population. (a) Duration from hatch to eye pigmentation (EP) (as proxy for first feeding) indicated by grey circles for sprat larvae from the Baltic Sea. Literature values for North Sea (grey squares/triangle) sprat larval time to eye pigmentation extend the temperature dependent function. Black triangles show maximum survival times from hatch until death (D) of individual larva from independent replicates. The enclosed area (dotted white) represents time WOO for Baltic sprat. (b) WOO (light grey area) of Adriatic Sea sprat larvae. (c) Overlapping WOOs from both populations (colours as above). (d) Exact temperature dependent durations of both populations (for parameterization see Table 6-1). Larval sprat from the Baltic sprat population experience longest WOO at about 8°C whereas larvae from the Adriatic population exhibit longest period between 10-11°C..... 68

## List of Tables:

Table 2-1: Stage specific egg developmental times in days post fertilization [dpf] for Baltic sprat eggs incubated under different temperature regimes. Shown are values of the last observed occurrence of a specific development stage and the first occurrence of hatched larvae.....	15
Table 2-2: Results of the parameterization during the egg stage of sprat egg incubation experiments from the present study and from literature sources. ....	19
Table 2-3: Results of the parameterization during the larval stage of sprat egg incubation experiments from this study and from literature sources.....	23
Table 3-1: Data defining the experimental set up: Biotic and abiotic factors, number of females from which eggs were obtained and experimental conditions in test trials for rearing early life stages of Adriatic sprat. ....	29
Table 3-2: Schedule for egg and larval sampling, staging and mortality checks. Different temperature treatments started after 6 degree days [dd°] post fertilization. Egg staging and larval sampling followed a schedule of degree day (degree days post fertilization [ddpf] and degree days post hatch [ddph]).....	31
Table 3-3: Parameter values of equations describing the duration (d) of egg stages (i) in hours post fertilization as exponential functions of temperature (t): $d_i = a \cdot \exp(-b \cdot t)$ as shown in Figure 3-3. ....	33
Table 3-4: Ontogenetic event table for the development of Adriatic sprat larvae incubated at 11 different temperatures. Shown are coefficients of exponential ( $d = a \cdot \exp(-b \cdot T)$ ) and power ( $d = a \cdot T^b$ ) functions fitted to the observed durations (d) per temperature level (T) in hours post fertilization for the events first and peak hatch, and in hours post peak hatch for the events eye pigmentation and yolk sac depletion. The results were calculated for each trial separately (n=3 per temperature level) and for both trial combined (n=6 per temperature level).....	33
Table 3-5: Survival periods of unfed yolksac larval sprat under different temperature conditions expressed in days post hatch (dph) and degree days post hatch (ddph). Shown are mean values $\pm$ standard deviations (SD) for the periods up to 50% and 100% mortality as derived from three replicates from each of two trials. The 19°C level was tested in trial 2 only. ....	37
Table 4-1: Origin of biological material, experimental setup, applied methods and parameters measured of individuals used during salinity experiments with Baltic sprat from the Bornholm Basin. The symbol + indicates that data were used from this trial for the respective analysis and the symbol – indicates that no data were available. ....	43
Table 4-2: Field sampling of sprat eggs from the Bornholm Basin, Baltic Sea: Dates, gears, numbers of stations and numbers of eggs used. The symbol + indicates cruises with successful artificial fertilization experiments, (experiments in 2008 were not successful). Vertical ‘Helgoländer Larven Netz’ (HLN) hauls provided sprat eggs for egg buoyancy measurements in a salinity gradient column. ....	44
Table 4-3: Mean egg survival rates and durations to important events during sprat egg and yolk sac stage. For each trial, bold labeled numbers of survival indicate significant differences ( $p < 0.01$ ) among salinities and different superscript letters indicate which ones differ. N is the number of independent replicates (beakers) per salinity treatment. “n.d.” stands for not determined.....	45
Table 4-4: Statistical results of the analysis of egg survival, egg buoyancy from experimental and field data and morphometric traits of sprat. Relative egg sizes refer to unpreserved, alive eggs and were between 1.27-1.38 mm for small, 1.39-1.51 mm for medium and 1.52-1.58 mm for large eggs. Larval morphometric traits are measured at three important	

ontogenetic events (hatch, eye pigmentation (EP) and yolk sac depletion (YSD)). SL is standard length, YSA refers to yolk sac area and BA describes larval body area excluding larval finfolds. Significant differences between salinities are contrasted in bold colour..... 49

Table 4-5: Mean ( $\pm$  standard deviations), maximum and minimum density values of sprat eggs derived either from experimental trials or from field samplings. Field data from April 2007 were derived from Multinet (MN) samplings with 5 m depth resolution (Harjes, 2008). N represents the number of analyzed eggs per sampling or treatment. Relative egg sizes refer to unpreserved, alive eggs and were between 1.27-1.38 mm for small, 1.39-1.51 mm for medium and 1.52-1.58 mm for large eggs..... 49

Table 4-6: Functions, parameters and statistics of sprat egg density values obtained from different salinity fertilization and incubation experiments in April and May/June 2007. 49

Table 4-7: Sprat larval morphometric traits measured in fertilization trial 1. Shown are the mean  $\pm$  standard deviations of n individual larvae per salinity treatment at either hatch or YSD (yolk sac depletion). SL is standard length, YSA refers to yolk sac area and BA describes larval body area excluding larval finfolds. Bold labeled numbers of larval area measurements indicate significant differences ( $p < 0.01$ ) among salinities and different superscript letters indicate which ones differ..... 50

Table 4-8: Sprat larval morphometric traits measured in incubation trial 3. Shown are the mean  $\pm$  standard deviations of n individual larvae per salinity treatment at either hatch or EP (eye pigmentation). SL is standard length, YSA refers to yolk sac area and BA describes larval body area excluding larval finfolds. Bold labeled numbers of larval length or area measurements indicate significant differences ( $p < 0.01$ ) among salinities and different superscript letters indicate which ones differ. .... 51

Table 5-1: Summary of the biological sprat material used, experimental setup and the adjusted abiotic factors during the experimental incubation under different salinity treatments of sprat eggs and yolk sac larvae..... 58

Table 5-2: Mean $\pm$ stdv of egg survival rates and larval survival potentials of n=3 replicates per salinity treatment and results of the ANOVA analyses. Significant values are typed in bold and differences between the treatments ( $p < 0.05$ ) are labeled through different letters. The 20 psu trial had to be excluded from the analyses..... 61

Table 5-3: Mean $\pm$ stdv of larval morphometric traits at 10 hours post hatch of n=7-10 individual larvae per salinity treatment and results of the Kruskal-Wallis ANOVA analyses and Median tests. Significant values are typed in bold and differences between the treatments ( $p < 0.05$ ) are labeled through different letters. (SL=standard length; YSA=yolk sac area; YSV=yolk sac volume; BA=body area; BA / YSA= ratio of body area and yolk sac area). .... 62

Table 6-1: Summary of parameterized important ontogenetic events and developmental rates from sprat eggs and yolk sac larvae from the Baltic Sea, the North Sea and the Adriatic Sea. Egg development rate refers to time from fertilization to first hatch. Eye pigmentation reflects the time (in days) from hatching to fully pigmented eyes which is almost identical to the time of mouth gap opening (first feeding). Yolk sac depletion describes endogenous resource depletion, whereas the time from hatching until the last larva has deceased is defined as maximum larval survival duration. North Sea<sup>1</sup> - data were derived from Thompson *et al.* (1981) and North Sea<sup>2</sup> - data are derived from Alshuth (1988) and Shields (1989). .... 67

Appendix Chapter 3

**Temperature dependent yolk sac larval growth model derived from two experimental trials and from 11 different temperature controlled treatments (5-19°C).**

$$L_t = L_0 * e^{\left( a * \left( 1 - e^{(-b * time)} \right) \right)}$$

This equation was applied to larval growth from each of the temperature treatments and trials separately. Growth was calculated from hatch until yolk sac depletion.

Larval size-at-hatch showed no temperature trend. Because larvae were sampled later at 8.5 and 14.1°C their values were not included into the mean value ( $\pm$ StDev).

$$L_0_{Growth} = 3.25 \pm 0.06$$

Since the “a” values showed no temperature dependent trend, all values from all trials were pooled and a mean value ( $\pm$ SD) was computed.

$$a_{Growth} = 0.57 \pm 0.04$$

The instantaneous growth rate at age t0 showed a clear positive temperature dependent trend and was parameterized by an exponential growth curve.

$$b_{Growth} = a * e^{(b * Temp)}$$

$$b_{Growth} = 0.32 \pm 0.036 * e^{(0.059 \pm 0.008 * Temp)}$$

Other important larval events such as eye pigmentation (EP), yolk sac depletion (YSD) and maximum survival time could also be parameterised for different temperatures. This makes it possible to calculate durations to and larval length for each of these events.

**For larval length at EP as proxy for first feeding:**

$$L_{t_{EyePigmentation}} = 3.25 \pm 0.06 * e^{\left( 0.57 \pm 0.04 * \left( 1 - e^{\left( - \left\{ 0.32 \pm 0.036 * e^{(0.059 \pm 0.008 * Temp)} \right\} * time_{EP} \right)} \right) \right)}$$

$$time_{EP} = \left( 1572 \pm 73 * Temp^{(-1.06 \pm 0.023)} \right) / 24$$

**For larval length at yolk sac depletion:**

$$L_{tYolkSacDepletion} = 3.25 \pm 0.06 * e^{\left( 0.57 \pm 0.04 * \left( 1 - e^{\left( - \left\{ 0.32 \pm 0.036 * e^{(0.059 \pm 0.008 * Temp)} \right\} * time_{YSD} \right) \right) \right)}$$

$$time_{YSD} = \left( 618 \pm 25 * e^{(-0.1158 \pm 0.0046 * Temp)} \right) / 24$$

**For larval length at maximum survival time:**

$$L_{tMaximumSurvivalTime} = 3.25 \pm 0.06 * e^{\left( 0.57 \pm 0.04 * \left( 1 - e^{\left( - \left\{ 0.32 \pm 0.036 * e^{(0.059 \pm 0.008 * Temp)} \right\} * time_{MST} \right) \right) \right)}$$

$$time_{MST} = 14.51 \pm 0.35 * e^{\left( -0.5 * \left( (Temp - 7.15 \pm 0.65) / 7.16 \pm 0.73 \right)^2 \right)}$$

Description of the individual scientific contribution to the multiple-author papers

One of the chapters of this thesis is already published (Chapter 2) and all are written in manuscript form with multiple authorships. This list serves as a clarification of my personal contribution on each manuscript.

Chapter 2:

**The influence of temperature on the development of Baltic Sea sprat (*Sprattus sprattus*) eggs and yolk sac larvae**

*Published in Marine Biology 2008, 154: 295-306*

Authors: C. Petereit, H. Haslob, G. Kraus and C. Clemmesen

All laboratory experiments and consequent data analysis were performed by Christoph Petereit. The text writing, graphical presentations and almost all statistical analyses were performed by Christoph Petereit. Holger Haslob assisted during the preparation of the first draft version. Dr. Gerd Kraus and Dr. Catriona Clemmesen- Bockelmann provided helpful comments to improve earlier versions of the manuscript.

Chapter 3:

**The effects of temperature on egg development, growth, morphometric traits and survival of Adriatic sprat (*Sprattus sprattus phalericus*) yolk sac larvae**

Authors: C. Petereit, G. Kraus, H. Haslob, R. Hanel, A. Ramšak and C. Clemmesen

All laboratory experiments and consequent data analysis were performed by Christoph Petereit. The text writing, statistical analysis and graphical presentations were performed by Christoph Petereit. Prof. Dr. Reinhold Hanel arranged the possibility to take advantage of the excellent research facilities at the Marine Biological Station in Piran, Slovenia in close cooperation with Dr. Andreja Ramšak. Colleagues provided helpful comments to improve earlier versions of the manuscript.

Chapter 4:

**The influence of different salinity conditions on egg development, egg survival and morphometric traits of Baltic Sea sprat (*Sprattus sprattus*) yolk sac larvae**

*Submitted to Scientia Marina*

Authors: C. Petereit, H.-H. Hinrichsen, R. Voss, G. Kraus, M. Freese and C. Clemmesen

The text writing, data and statistical analysis, graphical presentations and most of the laboratory experiments were performed by Christoph Petereit. Some laboratory data were provided by Marko Freese derived from his Semesterarbeit which was supervised by Christoph Petereit. Hans-Harald Hinrichsen and Dr. Rudi Voss added valuable comments and contributions while preparing the first draft version. Dr. Gerd Kraus, Prof. Dr. Dietrich Schnack and Dr. Catriona Clemmesen-Bockelmann provided helpful improvements on later draft versions.

Chapter 5:

**Effects of reduced salinity conditions on Adriatic sprat (*Sprattus sprattus phalericus*) egg and yolk sac larval development**

Author: C. Petereit

All laboratory experiments, consequent data analysis text writing, statistical analysis and graphical presentations were performed by Christoph Petereit.



### Danksagung

Ich möchte Herrn Prof. Dr. Dietrich Schnack für die Möglichkeit zur Promotion bei ihm und die Betreuung der Arbeit danken. Dieser Dank gilt insbesondere für die Endphase der Arbeit, in der er sicherlich einen Großteil seiner wohlverdienten „freien Zeit“ in Korrekturen und Verbesserungsvorschläge investiert hat.

Des Weiteren geht mein großer Dank an Catriona Clemmesen dafür, dass Sie mich als Mitarbeiter in Ihrem RECONN Projekt ausgesucht hat. Dafür, dass ich an ihrer großen Expertise teilhaben darf und sie mit mir zusammen Ideen und Experimente entwickelt hat, die letztendlich erst diese Arbeit ermöglicht haben, bin ich ihr sehr dankbar. Außerdem ist nicht zu vergessen, dass Konferenz- und Projektreisen ohne ihr Engagement und ihren Zuspruch nicht möglich gewesen wären, wie auch, dass ich die nötige freie Zeit für andere Projekte bekommen habe. Und wo gelegentlich ein Motivationsschub zur Förderung des Durchhaltevermögens not tat, hat ein Gespräch mit ihr gute Dienste geleistet.

Ähnliches gilt für Gerd Kraus, der immer ein offenes Ohr und ein freundliches Wort für mich hatte, auch wenn er die eine oder andere Frage zur Auswertung bekam oder er das „zweifelhafte Vergnügen“ der Korrekturen meiner First-Drafts hatte. Ich wollte schon immer einmal mit meinem Chef surfen gehen – das hat ja auch einmal geklappt – auch da warst Du Vorbild.

Ich möchte Herrn Prof. Dr. Myron Peck von der Uni Hamburg danken. Er hat mich während dieser Arbeit an seinem enormen Wissen über experimentelles Arbeiten, Fische, Copepoden und Modellierung partizipieren lassen und wertvolle Tipps und Inspirationen gegeben. Einhergehend möchte ich auch meinen Hamburger RECONN Projektkolleginnen und -kollegen Ute, Linda, Nike, Sonja und Philip für die gute Kooperation und Hilfe danken.

Experimentelles Arbeiten ist nur im Team möglich. Deshalb möchte ich allen Beteiligten innerhalb der Fischereibiologischen Abteilung und auch aus anderen Abteilungen danken: den Technikern Rudi Lühje, Dirk Jarosch und Svend Mees für die Logistik, Helgi Mempel und Thomas Hansen für alles im und ums Labor und Antje Burmeister für die Wissensvermittlung über die Copepoden-Bestimmung. Außerdem allen beteiligten HiWis im Laufe der Jahre (Jesco, Andrea, Andrea, Michael, Nicole, Marko, Wiebcke, Enno, Anja, Yuri, Matthias, Leni u.a.). Dies gilt auch für die kompetente und unkomplizierte Unterstützung durch die Mitarbeiter des Kieler Aquariums, der IFM-GEOMAR Bibliothek und unserer Sekretärin Brigitte Rohloff. Dies alles hätte aber nur begrenzt geholfen, ohne die Hilfe aller Kapitäne und Crews der jeweiligen Forschungsschiffe und kommerziellen Fischkutter, an deren Bord ich an meine Sprotten gekommen bin. Auch an diese ein großes Dankeschön.

I gratefully acknowledge all scientific and technical staff members of Marine Biological Station in Piran, Slovenia, for offering excellent support and experimental facilities during my scientific work in Jan-Feb 2007. In particular, I would like to thank Dr. Andreja Ramšak for enabling this not only scientifically successful and inspiring time. I also would like to thank Katja Stopar and Tjasa Kogovsek for their help and support during this stay.

Im gleichen Zusammenhang möchte ich Prof. Dr. Reinhold Hanel danken, ohne dessen Initiative dieser Forschungsaufenthalt in Piran sicherlich nicht stattgefunden hätte.

Ziemlich am Ende, dieses jedoch nicht wörtlich zu nehmen, möchte ich meinen Kollegen der Arbeitsgruppe danken: Hans-Harald Hinrichsen, Rudi Voss, Jörn Schmidt, Holger Haslob, Matthias Schaber, Jan Schröder, Malte Damerau, Eva Jakob, Enno Prigge, Lasse Marohn, Karsten Zumholz und Eske Evers. Es gab immer einen sehr guten Austausch (nicht nur



## DANKSAGUNG

---

wissenschaftlich), viel Unterstützung eurerseits und nötige Kaffeepausen. Ein besonderer Dank geht jetzt in der Endphase an Andrea Frommel für Ihre unermüdlichen Englisch-Korrekturen. Herzlichen Dank an alle für die schöne gemeinsame Zeit!

Zuletzt möchte ich meinen Eltern für die notwendige Unterstützung bis zum Abschluss dieser Arbeit danken und dafür, dass Ihr stets an mich geglaubt habt.

Zu guter Letzt aber möchte ich meiner Frau Tanja und unserer Tochter Emma für ihre Liebe und Unterstützung sowie für ihr Verständnis danken, dass Sie sehr viel Zeit ohne mich verbringen mussten, während Sprott-Eier und Sprott-Larven im Mittelpunkt standen.

Curriculum Vitae

**Christoph Petereit**

Muhliusstraße 70  
24103 Kiel  
Deutschland

E-Mail: [cpetereit@ifm-geomar.de](mailto:cpetereit@ifm-geomar.de)

Internet: <http://www.ifm-geomar.de/index.php?id=cpetereit>

PERSÖNLICHE DATEN

Geburtstag: 04. Januar 1977  
Geburtsort: Kappeln, Deutschland  
Familienstand: verheiratet, ein Kind  
Staatsangehörigkeit: Deutsch

AUSBILDUNG

Winter 2008: Abgabe der Doktorarbeit, Christian-Albrechts-Universität (CAU), Kiel  
2004: Diplom (Note: sehr gut) im Hauptfach Fischereibiologie, Christian-Albrechts-Universität, Kiel  
1996: Abitur (Note: gut), Klaus-Harms Schule, 24376 Kappeln

WISSENSCHAFTLICHER WERDEGANG

Seit November 2007: Wissenschaftlicher Angestellter und Promotionsstudent am Leibniz-Institut für Meereswissenschaften (IFM-GEOMAR) an der Christian-Albrechts Universität (CAU), Kiel im EU-Projekt "UNCOVER"

Nov. 2007 – Apr. 2008: Elternzeit

Jan. 2007 – Dez. 2008: Mitarbeit in der interdisziplinären Arbeitsgruppe (IFM-GEOMAR) "Rippenqualle, *Mnemiopsis leidyi*"

Nov. 2004 - Nov. 2007: Wissenschaftlicher Mitarbeiter und Promotionsstudent im DFG Projekt "RECONN2" im Rahmen des DFG-Schwerpunktprojektes "AQUASHIFT", am IFM-GEOMAR, Kiel

Mai 2004: Diplom an der CAU, Kiel (Fischereibiologie, Zoologie, Geologie)

April – Mai 2004: Wissenschaftlicher Mitarbeiter im EU Projekt "ECOCARP", IFM-GEOMAR, Kiel

Apr. 2003 – Feb. 2004: Diplomarbeit im Rahmen von "GLOBEC - Germany" (BMBF) am IFM-GEOMAR an der CAU Kiel

WISSENSCHAFTLICHES INTERESSE

Populationsdynamik früher Lebensstadien in marinen und limnischen Systemen unter Einfluss von sich ändernden abiotischen und biotischen Faktoren

Fischereirelevante ökologische und ökonomische Bedeutung einwandernder Fischarten

Nahrungsnetz-Interaktionen evertebrater Predatoren und Ichthyoplankton

PUBLIKATIONEN (begutachtet)

**Petereit, C.**, Haslob, H., Kraus, G., Clemmesen, C. (2008): The influence of temperature on the development of Baltic Sea sprat (*Sprattus sprattus*) eggs and yolk sac larvae. *Mar Biol* **154**:295-306. DOI 10.1007/s00227-008-0923-1

Hinrichsen, H.H., Lehmann, A., **Petereit, C.**, Schmidt, J. (2007): Correlation analyses of Baltic Sea winter water mass formation and its impact on secondary and tertiary production. *Oceanologia* **49**(39):381-395

Probst, W.N., Tan, D., Gao, Y., Drossou, A., **Petereit, C.**, Wecker, B., Xiong, M., Ueberschär, B., Chang, J., Rosenthal, H. (2006): Rearing of *Procypris rabaudi* during early life history stages. *J Appl Ichthyol* **22**:530-535.

Hinrichsen, H.-H., Schmidt, J.O., **Petereit, C.**, Möllmann, C. (2005): Survival probability of Baltic larval Cod in relation to spatial overlap patterns with its prey obtained from drift model studies. *ICES J Mar Science* **62**(5):878-885. doi:10.1016/j.icesjms.2005.04.003

PUBLIKATIONEN (eingereicht und in Vorbereitung)

**Petereit, C.**, Hinrichsen, H.H., Voss, R., Clemmesen, C., Haslob, H., Freese, M., Kraus, G.: The influence of different salinity conditions on egg buoyancy, -development, -survival and morphometric traits of Baltic Sea sprat (*Sprattus sprattus balticus* Schneider) yolk sac larvae. *submitted to Scientia Marina*

**Petereit, C.**, Clemmesen, C., Haslob, H., Kraus, G., Ramsak, A., Hanel, R.: The effects of temperature on egg development, survival and morphometric traits of yolk sac larvae of Adriatic sprat (*Sprattus sprattus phalericus*). *In prep*

Helland, I.P., Clemmesen, C., **Petereit, C.**, Mehner, T.: Starvation does not explain abundance decline during early life stage of vendace (*Coregonus albula*). *In prep*

PUBLIKATIONEN (nicht begutachtet)

Lehmann, A., Javid Pour, J., Clemmesen, C., **Petereit, C.**, Schmidt, J. (2008): *Mnemiopsis leidyi*, a new invader to the Baltic Sea: Possible Pathways of Distribution. *Baltex Newsletter* No. **11**

**Petereit, C.**, Haslob, H., Kraus, G., Clemmesen, C., Voss, R. (2006): Temperature dependent developmental success of sprat early life stage: comparing Baltic and North Sea sprat. *GLOBEC International Newsletter*, Vol.**12**, No.1

**Petereit, C.**, Clemmesen, C., Kraus, G., Schnack, D. (2004) High resolution temperature influences on egg and early larval development of Baltic cod (*Gadus morhua*) ICES CM 2004/K:03

**Petereit, C.** (2004): Experimente zum Temperatureinfluß auf frühe Entwicklungsstadien des Ostseedorsches (*Gadus morhua*). Mathematisch-Naturwissenschaftlich Fakultät aus dem Leibniz-Institut für Meereswissenschaften an der Christian-Albrechts-Universität zu Kiel, Diploma Thesis, 46 pp.

#### POSTER PRÄSENTATIONEN

Peschutter, J., Franke, A. and **Petereit, C.**: Growth, RNA:DNA ratio and prey of juvenile garfish (*Belone belone*) from Kiel Fjord, Baltic Sea- coupling experiments with field observations.

32<sup>nd</sup> Annual Larval Fish Conference, Kiel, Germany, 04-07.08.2008

Kanstinger, P., Peck, M.A., Huwer, B., Baumann, H., Clemmesen, C., **Petereit, C.** How slow can you grow? Variability in the timing of daily ring formation in larval sprat.

32<sup>nd</sup> Annual Larval Fish Conference, Kiel, Germany, 04-07.08.2008

**Petereit, C.**, Kraus, G., Clemmesen, C.: How do changes in temperature and feeding regime affect sprat early life history?

GLOBEC-Germany Final Symposia, Hamburg, Germany 14-15.11.2007

Clemmesen, C., **Petereit, C.**, Malzahn, A., Calderone, E., Harrer, D.: Temperature dependent decline in RNA/DNA ratios in food deprived fish larvae.

30<sup>th</sup> Annual Larval Fish Conference, Lake Placid, USA, 10-14.09.2006

#### VORTRÄGE

Haslob, H., **Petereit, C.**, Clemmesen, C., Kraus, G.: The effect of natural variability of life history parameters on Baltic Sea sprat population dynamics.

38<sup>th</sup> Annual Conference Gesellschaft für Ökologie – 5<sup>th</sup> Annual AQUASHIFT Meeting Konstanz, Germany 22-26.09.2008 (26.09.08)

**Petereit, C.**, Hinrichsen, H.-H., Voss, R., Kraus, G., Freese, M., Clemmesen, C.: The influence of different salinity conditions on egg development, egg survival and morphometric traits of yolk sac larvae of Baltic Sea sprat (*Sprattus sprattus*).

32<sup>nd</sup> Annual Larval Fish Conference, Kiel, Germany, 04-07.08.2008 (06.08.2008)

Helland, I.P., Clemmesen, C., **Petereit, C.**, Mehner, T.: Growth and recruitment of larval vendace (*Coregonus albula*) in a deep lake.

32<sup>nd</sup> Annual Larval Fish Conference, Kiel, Germany, 04-07.08.2008 (05.08.2008)

**Petereit, C.**, Kraus, G., Stransky, J., Clemmesen, C.: Match-mismatch between secondary and tertiary production in the Baltic Sea.

37<sup>th</sup> Annual Conference Gesellschaft für Ökologie – 4<sup>th</sup> Annual AQUASHIFT Meeting, Marburg, Germany 10-14.09.2007 (12.09.07)

**Petereit, C., Clemmesen, C., Haslob, H., Kraus, G., Ramsak, A., Hanel, R.:** Temperature Experiments with early life stages of sprat (*Sprattus sprattus*) from the Baltic Sea, North Sea and the Adriatic Sea.

31<sup>st</sup> Annual Larval Fish Conference, St.John`s, Canada 09-12.07.2007 (11.07.07)

**Petereit, C.:** Highly resolved temperature experiments with Baltic cod (*Gadus morhua*) eggs and larvae.

RESTOCK Project Meeting, Bornholm, Denmark, 27-28.03.2007 (27.03.07)

**Petereit, C., Clemmesen, C., Kraus, G., Haslob, H., Hanel, R.** Temperature Experiments with early life stages of sprat (*Sprattus sprattus*) from the Baltic Sea, North Sea and the Adriatic Sea.

Marine Biological Station Piran, Slovenia 07.01-11.02.2007 (02.02.07)

**Petereit, C., Kraus, G., Clemmesen, C., Donner, M. Harrer, D.:** Match – Mismatch between Secondary and Tertiary Production in the Baltic.

3<sup>rd</sup> AQUASHIFT-Workshop, Kiel, 14.11.2006.

**Petereit, C., Haslob, H., Kraus, G., Clemmesen, C.:** Simulating the effect of warming on the developmental success of sprat early life history stages: Are Baltic and North Sea sprat differently effected?

ASLO Summer Meeting 2006, Victoria, British Columbia, Canada, (07.06.2006)

**Petereit, C., Kraus, G., Clemmesen, C.:** Parameterizing individual-based models of early life stages of sprat (*Sprattus sprattus*).

AQUASHIFT Modelling Group Workshop, Hamburg, Germany, 21-22.02.2006 (21.02.06)

**Petereit, C. & Kraus, G.:** RECONN2: Resolving trophodynamic Consequences of Climate Change- Experimental Overview.

AQUASHIFT Fish Group Meeting, Hamburg, Germany 15.12.2005

**Petereit, C, Kraus, G., Clemmesen, C.:** Resolving trophodynamic Consequences of Climate Change – Match-mismatch between secondary and tertiary Production in the Baltic Sea.

2<sup>nd</sup> AQUASHIFT Workshop, Kiel, Germany, 28-30.09.2005 (29.09.05)

**Petereit, C., Clemmesen, C., Kraus, G., Schnack, D.** High resolution temperature influences on egg and early larval development of Baltic cod (*Gadus morhua*) ICES Annual Science Conference, Vigo, Spain 22-25.09.2004

#### AUSLANDSERFAHRUNG UND PRAKTIKA

Januar 2007 – Februar 2007: Marine Biologische Station Piran (MBS), Slowenien  
Laborexperimente mit frühen Lebensstadien der Adriatischen  
Sprotte (*Sprattus sprattus phalericus*)  
<http://www.mbss.org/>

Juni 2004 – Juli 2004           Praktikum in der kommerziellen Fischerei auf dem  
Krabbenkutter “Hartje“ mit Fangfahrten auf der Nordsee

April 2004 – Mai 2004:       Institut für Hydrobiologie (IHB), Wuhan, China  
Experimentelle Studien mit endemischen karpfenartigen  
Fischen als potentielle Kandidaten für die Aquakultur  
<http://159.226.163.238/english/default.aspx>

#### FORSCHUNGSFAHRTEN

Sechs wissenschaftliche Ausfahrten mit FS “ALKOR“ und FFS  
“Walther Herwig“ (ehemals BFA jetzt vTI) in die zentrale  
Ostsee (2003-2008):  
Beprobung von Fischlarven- und Copepodenverteilungen,  
Hydrographische Aufnahmen (CTD) und Fischereibiologische  
Grundlagenforschung an Bord.  
Experimentelle Arbeiten über Temperatur- und  
Salzgehaltseinflüsse auf Fischeier und Fischlarven von Sprotte  
und Dorsch.

Zwei wissenschaftliche Ausfahrten mit FS “POSEIDON“ in den  
zentralen (Azoren) und subtropischen (Kapverdische Inseln)  
Atlantik mit ozeanographischen und planktologischen  
Fragestellungen.

Fahrtleiter auf eintägigen Ausfahrten auf kleineren  
Forschungsschiffen

#### LEHRERFAHRUNG

Beteiligung an der Lehre im Rahmen des fischereibiologischen Großpraktikums  
(mit Dietrich Schnack, Friedrich Köster, Reinhold Hanel, Gerd Kraus, Rudi Voss und  
Catriona Clemmesen)

WS 2006/2007:           Großpraktikums Ausfahrt, allgemeine Fischbearbeitung  
und dem Kursteil Wachstum  
WS 2005/2006:           Großpraktikums Ausfahrt, allgemeine Fischbearbeitung  
und den Kursteilen Wachstum sowie Otolithen  
SS 2005:                 Seminar “Futter bei die Fische“: Vom Plankton zu den  
Fischlarven – Aufbau und Erhalt von  
Lebendfutterkulturen.  
WS 2004/2005:           Großpraktikums Ausfahrt, allgemeine Fischbearbeitung  
und dem Kursteil Wachstum  
WS 2003/2004:           Großpraktikums Ausfahrt, allgemeine Fischbearbeitung  
und Mitarbeit im Kursteil Otolithen

Beteiligung an der Planung und Durchführung von Diplom- und Masterarbeiten  
(mit Catriona Clemmesen)

Andrea Frommel (2008):

Influence of temperature on growth and biochemical-based indicators of growth in juvenile Gobiids of the Baltic Sea

Betreuung von Semesterarbeiten:

Jesco Peschutter und Andrea Franke (2006):

Biologische Beobachtungen und Untersuchungen des Effekts verschiedener Hälterungstemperaturen auf das Wachstum und das RNA/DNA- Verhältnis juveniler Hornhechte (*Belone belone*) aus der Kieler Förde.

Marko Freese (2008):

Fertilization and buoyancy experiments with Baltic Sea sprat (*Sprattus sprattus*) eggs.

Matthias Paulsen (2008):

Nahrungszusammensetzung der europäischen Sardelle (*Engraulis encrasicolus*) aus der Kieler Bucht (zusammen mit Matthias Schaber).

## ERKLÄRUNG

---

### Erklärung

Hiermit erkläre ich, daß die vorliegende Dissertation selbständig von mir angefertigt wurde. Die Dissertation ist nach Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel verwendet. Diese Arbeit wurde weder ganz noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Prüfungsverfahren. Die Promotion soll im Fach Fischereibiologie erfolgen.

Kiel, den 16.12.2008

Christoph Petereit



