BONUS BIO-C3

Biodiversity changes: causes, consequences and management implications

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BIO-C3 overview

The importance of biodiversity for ecosystems on land has long been acknowledged. In contrast, its role for marine ecosystems has gained less research attention. The overarching aim of BIO-C3 is to address biodiversity changes, their causes, consequences and possible management implications for the Baltic Sea. Scientists from 7 European countries and 13 partner institutes are involved. Project coordinator is the GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, assisted by DTU Aqua, National Institute of Aquatic Resources, Technical University of Denmark.

Why is Biodiversity important?

An estimated 130 animal and plant species go extinct every day. In 1992 the United Nations tried countering this process with the "Biodiversity Convention". It labeled biodiversity as worthy of preservation – at land as well as at sea. Biological variety should not only be preserved for ethical reasons: It also fulfils key ecosystem functions and provides ecosystem services. In the sea this includes healthy fish stocks, clear water without algal blooms but also the absorption of nutrients from agriculture.

Biodiversity and BIO-C3

To assess the role of biodiversity in marine ecosystems, BIO-C3 uses a natural laboratory: the Baltic Sea. The Baltic is perfectly suited since its species composition is very young, with current salt level persisting for only a few thousand years. It is also relatively species poor, and extinctions of residents or invasions of new species is therefore expected to have a more dramatic effect compared to species rich and presumably more stable ecosystems.

Moreover, human impacts on the Baltic ecosystem are larger than in most other sea regions, as this marginal sea is surrounded by densely populated areas. A further BIO-C3 focus is to predict and assess future anthropogenic impacts such as fishing and eutrophication, as well as changes related to global (climate) change using a suite of models.

If talking about biological variety, it is important to consider genetic diversity as well, a largely neglected issue. A central question is whether important organisms such as zooplankton and fish can cope or even adapt on contemporary time scales to changed environmental conditions anticipated under different global change scenarios.

BIO-C3 aims to increase understanding of both temporal changes in biodiversity - on all levels from genetic diversity to ecosystem composition - and of the environmental and anthropogenic pressures driving this change. For this purpose, we are able to exploit numerous long term data sets available from the project partners, including on fish stocks, plankton and benthos organisms as well as abiotic environmental conditions. Data series are extended and expanded through a network of Baltic cruises with the research vessels linked to the consortium, and complemented by extensive experimental, laboratory, and modeling work.

From science to management

The ultimate BIO-C3 goal is to use understanding of what happened in the past to predict what will happen in the future, under different climate projections and management scenarios: essential information for resource managers and politicians to decide on the course of actions to maintain and improve the biodiversity status of the Baltic Sea for future generations.

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I. Executive Summary

In 2017, the population genetic and experimental analysis of selected key native and invasive taxa was completed according to the work plan set out in task 1.3. Studies focused on selected native and non-indigenous species (NIS). For key zooplankton species (the native copepods *Eurytemora affinis* and *Temora longicornis*) a series of common-garden experiments was performed across different locations/populations to determine local tolerance and adaptation potential. It was found that both copepod species display pronounced local adaptation across the sampled locations with respect to temperature (*Eurytemora affinis*) and salinity (*Eurytemora affinis* and *Temora longicornis)*, implying evolutionary potential on the one hand, but also prompting the need to conserve populations with their particular response traits rather than species *per se*. In the invasive comb jelly *M. leidyi* an assessment of ocean currents as drivers of (re-)introductions and population structure (using microsatellites) was completed (also contributing to Task 2.3); it was found that genotypes in the Baltic changed throughout 2010-2013, and that prevailing currents determine secondary spread and re-seeding of genotypes in northern European seas, including the Baltic.

For the economic and ecological key fish species Atlantic cod (*G. morhua*) two studies described patterns in spatio-temporal population structure of cod in Arkona Basin and the Eastern Baltic Sea and assessed possible correlations with environmental drivers such as major inflow vents (collaboration with WP3 providing hydrodynamic modelling data). No mixing of eastern and western Baltic stock could be detected, and the major inflow of 2015 had no detectable influence on the proportional contributions of both stocks in the Arkona basin. This spatial genetic pattern seems to be remarkably stable over time (past 21 yrs). Time series data on the decrease of length at maturity in the eastern stock are thus due to endogenous factors and not influenced by immigration or emigration of particular genotypes. Additional experimental work assessed the vulnerability of larval cod to ocean acidification, and the role of acclimation and trans-generational effects to possibly mitigate impairment of larval survival. It was found that without evolutionary adaptation, the effects of ocean acidification levels expected for end of the 21st century on the survival of cod larvae of two separate stocks are severe and will translate to recruitment declines in the fished population of up to 90%. On the other hand, but only in a tested aquaculture stock, some buffering was observed towards ocean acidification effects in the offspring when the parental generation was already exposed to ocean acidification, but only under high food supply.

For the invasive round goby (*N. melanostomus)* a large spatial sampling programme was completed and a curated sample archive is now available for further analysis. Results from D1.3 are used in particular within WP3 (Task 3.2, 3.3 and 3.4).

II. Introduction

Populations of species may evolve quickly in response to environmental drivers (Schoener 2011; Reusch 2014). This relatively new insight in marine ecology is particularly relevant in the Baltic Sea with its steep environmental gradients and an ongoing change in salinity, temperature and other global change associated parameters that are exceeding rates in other world oceans. The overall objective of D1.3 was to fill critical gaps in our understanding of how such eco-evolutionary dynamics may play out in critically important Baltic species. To understand those dynamics, one must not only address evolutionary adaptation processes at any one location, but also consider the connectivity among sub-populations that may complicate local adaptation processes.

Due to the (long) generation time of most species, and in particular fishes, observing evolution in action via evolution experiments is seldom feasible (but see Lohbeck et al. 2012). However, there are a number of approaches that allow indirect inferences on the evolutionary potential, such as common garden experiments to assess the heritable component of tolerance traits, which in turn allows inferences on local adaptation (Sunday et al. 2104). Such an approach was undertaken in two thorough studies on the key native copepod species. In both species, it was found that across sampling stations in the Baltic, the performance at native vs. foreign temperatures and salinities was better, including growth and egg production.

The arrival of non-native species is not only an ecological but also a large-scale evolutionary experiment. It is thus not surprising that some of the most striking examples of rapid evolutionary adaptation come from NIS in their new habitat (Whitney and Gabler 2008). In addition, the repeated influx of novel genotypes may enhance genetic diversity and foster evolutionary adaptation (Dlugosch and Parker 2008).

Population connectivity and the resulting mixing of gene pools may also destroy locally adapted gene pools (Kawecki & Ebert 2004). Hence, high-resolution markers such as SNPs (single nucleotide polymorphism) may be very useful to determine whether gene pools can be treated as evolving units, or whether hybridization occurs (Therkildsen et al. 2013). It has long been established that many Baltic species including fish populations reveal steep genetic clines when entering the Baltic Sea (Johannesson & Andre 2006), but the fine-scale pattern and its stability over time in Baltic Eastern and Western cod (*Gadus morhua*) populations had not been assessed. A particular knowledge gap is the role of forcing by major inflow events, which may complicate the genetic structure between East and Western stocks. Such an analysis is also mandatory if we are to make inferences on fisheries induced selection within any one stock, since the alternative of genetic immigration from adjacent areas needs to be ruled out. In cod, reliable ageing in the central Baltic Sea is impossible (Eero et al. 2015), hence one has to rely on length-maturity relationships as "best of a bad" job. These relationships show a strikingly stark decrease over time that that could be

explained in the light of fisheries induced selection, but also in terms of food shortage and nutritional status (Köster et al. 2017).

One alternative route to adaptation lies in trans-generational effects, the non-genetic but nevertheless partially heritable transfer of (acquired) traits from the parental to the offspring generation. Evidently, these effects have created a lot of attention as a third way beside classical evolutionary adaptation and physiological plasticity for species to cope with climate change (Miller et al. 2012). While the mechanisms are poorly understood, a proper experimental design nevertheless allows us to make solid inferences on the presence and magnitude of such phenomena at the phenotypic level. This was done in D1.3 for the first time for a commercially important fish, Atlantic cod, in the parental and filial generation exposed to ocean acidification, fully crossed with ad libitum and restricted food supply.

The WP 1.3 produced many different studies and many extensive datasets. The main results are highlighted in the section III Core Activities, while more detailed descriptions as paper manuscripts are provided as appendices.

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III. Core Activities

1. Assessing phenotypic plasticity of salinity tolerance in the copepod *Temora longicornis*.

Authors: Anette Maria Christensen (P02 – DTU Aqua), Jörg Dutz (P02, also, IOW)

Abstract: Common garden experiments were conducted to test whether a broad physiological plasticity and acclimation account for the significant greater salinity tolerance of *Temora longicornis* of mesohaline origin compared to populations from polyhaline regions of the Baltic Sea. Using full sib clutches from the Kattegat and the Bornholm Sea split across a salinity gradient of 8-20 offspring survival revealed that the tolerance of low salinity results from genotype variation and local adaptation as a result of selection rather than a physiological euryhalinity of the species. This adaptation has relevant implication for the understanding of the environmental control of the species' dynamics.

Progress: All experimental work is completed. Data analysis and manuscript preparation is in progress. A detailed report of current status is attached as Appendix 1.

Deviations: No deviations from the work plan.

Introduction: Despite being largely mesohaline, the open basins of the Baltic Sea are dominated by copepods of marine origin which are of vital importance for the food web dynamics and fishery production. The understanding of the mechanisms underlying this euryhalinity, i.e. a broad physiological plasticity or genetic adaptation, is central to project the response and resilience of populations to future environmental change. At the margin of the species distribution, acclimation through a broad physiological plasticity might be associated with loss in fitness, increased vulnerability or hampered adaptation through genetic loss. While the few published studies on the salinity tolerance of polyhaline *Temora longicornis* suggest limits that contrast the presence of the species in the Baltic, experiments testing the physiological response of populations isolated along the salinity gradient in the Baltic showed significant differences in life history traits that could indicate local adaptation. Common garden experiments were, therefore, conducted to compare the phenotypic plasticity in two populations of mesohaline and polyhaline origin.

Methods and Results: The study used a polyhaline population isolated from the Kattegat (S=25) and a mesohaline population from the Bornolm Sea (S=7.9). Both populations were acclimated and grown for one generation at a salinity of 15 under identical conditions. Nauplii from eggs of 30-36 full-sib clutches produced by F2 females of each population and hatched at common garden conditions were distributed among four salinity treatments (about 3 to 15 N each) of S = 8, 10, 15 and 20. Each nauplii clutch was maintained at otherwise identical conditions to mother generations and fed in excess. Visual inspection was performed daily and moulting/survival was followed until metamorphosis to copepodite stage 1. Survival and development time of survivors were used to test for clutch and population effects in response to treatment salinity. The results show that the mean survival of nauplii until metamorphosis significantly differed among the populations. While the Bornholm population displayed only a slightly reduced survival at the lowest S=8 compared to the common garden conditions S=15, no survival was observed at S= 8 in offspring from the Kattegat population (Fig. 1.1). Mean development time to metamorphosis significantly increased with deceasing salinity, but population differences were not observed (Fig. 1.1).

Recommendations: The clear population differences in survival at low salinity suggest that the wide distribution of *Temora longicornis* is not based on a broad physiological plasticity and that *Temora longicornis* from the mesohaline Bornholm Basin represents a local adapted population. Although the Kattegat population displayed a rather broad tolerance range with survival and development down to a salinity of 10, developmental acclimation to conditions in the central Baltic Sea could not be achieved within one generation. Reaction norms for survival for the Bornholm population showed high phenotypic variance across the entire salinity gradient and suggest a resilience of the population to changing salinity by genotype x environment interactions on which selection could act.
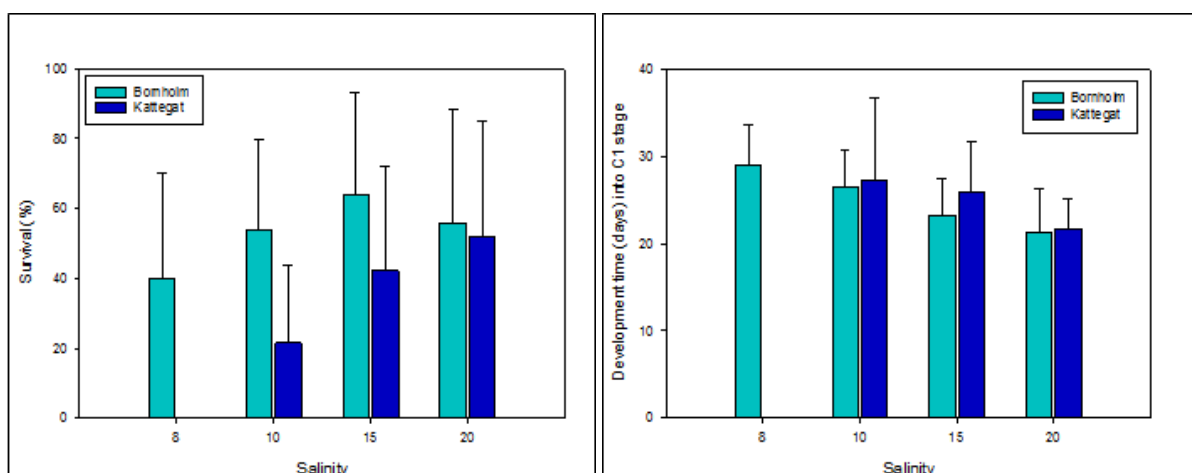

Figure 1.1: Mean survival (left) and mean development time into copepodite stage 1 (right) across the salinity gradient for the two populations from Bornholm and Kattegat; error bars denotes standard deviation. No individuals from the Kattegat population reached metamorphosis at the salinity of 8.

2. Genetic differentiation and adaptive capacity to climate change of the Baltic Sea zooplankton

Authors: Konrad Karlsson, Monika Winder, P04 - Stockholm University

Abstract: Genetic species differentiation between spatially dispersed populations can arise over a gradient of environmental factors due to different local selective pressures. As a result some populations might be better prepared to tolerate future environmental conditions than others. This is particularly relevant for the Baltic Sea, which has a spatial gradient of salinity and temperature. Here temperature is expected to increase more compared to world oceans, while salinity is expected to decrease because of increased river runoff. We sampled different populations of the copepod *Eurytemora affinis* across a temperature and salinity gradient and investigated their response to varying environmental factors in common garden experiments. *E. affinis* is warm adapted, thus populations from warmer areas had higher developmental rates in warm temperatures. The interaction of high temperature and low salinity had less adverse consequences in warm adapted populations. In addition, survival of warm adapted populations with short developmental time was more positively affected by high food quality, but more negatively affected by low food quality than in populations with longer time of development. Our findings suggest that development time is related to selection by temperature rather than seasonal time constraints, and that high food quality is important for populations with fast developmental rates. In conclusion, warm adapted populations could be better prepared for future elevated temperatures, but food quality and lower salinities could hamper their relative benefits.

Progress: Experiment, sample and data analysis completed, two manuscripts are currently prepared for publication. A detailed report of current status is attached as Appendix 2.

Deviations: No deviations from work plan.

Introduction: Genetic differentiation between spatially dispersed populations can arise over a gradient of environmental factors due to different local selective pressures. As a result some populations might be better prepared to tolerate future environment conditions than others. This is particularly relevant for the Baltic Sea, which has a spatial gradient of salinity and temperature. Here temperature is expected to increase more compared to world oceans, while salinity is expected to decrease because of increased river runoff. Our aim with this study was to investigate how populations of the copepod *Eurytemora affinis* that evolved under differing temperature regimes respond in survival and development time depending on temperature and salinity. We investigated tradeoffs in different traits by genetic correlations, quantify the genetic influence on the phenotype by heritability, and investigated for differences in phenotypic plasticity between genotypes. The three populations in this study originated from: the northern Baltic Sea (Bothnian Bay), Stockholm archipelago (Northern Baltic Proper, Askö), and the Gulf of Riga.

Methods and Results: Three different populations of the calanoid copepod *Eurytemora affinis* were sampled across the Baltic Sea salinity and temperature gradient, from the Northern Baltic Proper (Askö), Gulf of Riga, and Bothnian Bay. Species were exposed to different salinities and temperatures in common garden experiments and life history responses investigated over their development stages.

The development reaction norms as a response to temperature show genetic differentiation between the populations (Fig. 2.1). The Gulf of Riga population has faster development in high temperature. In a situation with seasonal time constraints (counter gradient variation), the opposite pattern would be more likely, with faster development in northern population from cold areas. We suggest that the present reaction norm is due to warm adaptation, that the populations are adapted to the warmest temperature they experience.

Correlations of genetic variance (indicating response to selection) in development time of the three populations between temperatures show how temperature adaptation arises (Fig. 2.2). Results show that selection for fast development in 22 °C does not select for fast development in 12 and 17 °C, indicated by the negative correlation. In contrast, fast development in 12 and 17 °C is positively correlated.

Results of survival reaction norms in response to temperature and salinity also indicated that low salinity has a negative effect on survival. The populations interact with temperature and salinity differently. In low salinity, the population from the warmer environment has higher survival in high temperature than the one from colder, which has higher survival in cold temperature.

Finally, response in development time depending on food quality and temperature indicated that in addition the type of food had different effect on development time. This suggests that food quality is another factor affecting copepod development time. Similar, survival particularly of the fast growing population (Gulf of Riga) is highly dependent on food quality (data not shown). In high temperature low food quality has negative effect on survival, whereas high food quality has a positive effect on survival.
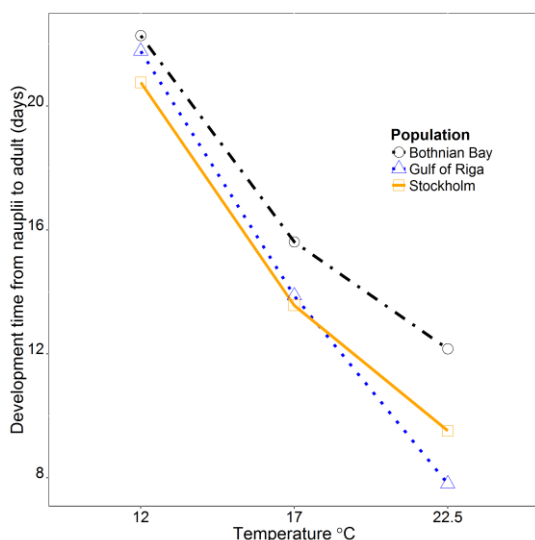

Figure 2.2 Development reaction norms as a response to temperature for three *E. affinis* populations of the Baltic Sea.
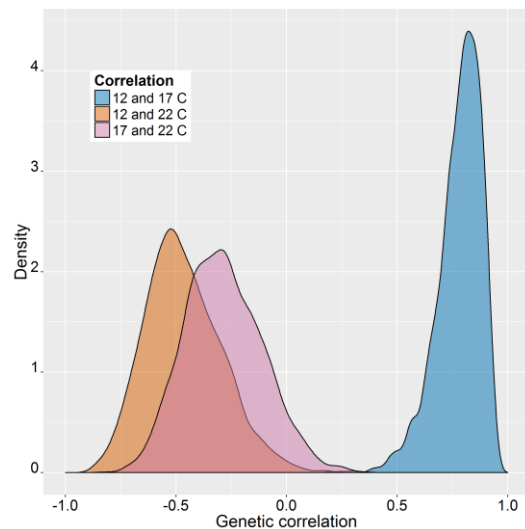

Figure 2.3 Correlations of genetic variance in development time between temperature treatments.

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Recommendations: Understanding the capacity of biological adaptation is critical for predicting climate change effects on ecosystems. The gradual variation in salinity and temperature in the Baltic Sea make it a stressful habitat for many organisms. A prospective scenario is that the Baltic Sea will become warmer and less saline. Our results suggest that some copepod populations likely have low tolerance levels to future desalination. Concomitant of warming of the Baltic Sea is the competitive advantage this will give to warm adapted populations, since they will have shorter development time in warmer water and therefore a higher population rate of increase compared to populations from colder areas. It is important to point out that the need of high food quality for warm adapted populations might counteract their competitive advantage in areas that are less productive. Investigating further populations will clarify the potential of adaption to salinity tolerance in spatially separated populations. Adaptation in different populations will give valuable knowledge about the future fitness of this key zooplankton species in a changing Baltic Sea.

3. Salinity impact on distribution and adaptation potential in *Mnemiopsis leidyi*

Authors: Cornelia Jaspers (P02 – DTU Aqua), Thorsten Reusch (P01 – GEOMAR), contributions by P03 – UHH, P07 – SYKE, P05 - NMFRI

Abstract: With continuous monitoring activities from the BIO-C3 consortium, we can show that the invasive comb jelly *Mnemiopsis leidyi* has not established permanent self-sustaining populations in the low saline central and northern Baltic Sea. Further, work which contributes to deliverable 2.3 has shown that winter temperatures have a significant effect on persistence of *M. leidyi* in northern Europe. This indicates that salinity as well as temperature currently set limits to the range occupancy of *M. leidyi* in western Eurasia.

Progress: The work is compiled in a manuscript (Jaspers et al. submitted) but the major contribution of that manuscript delivers to Task 2.3 and will be reported with the Deliverable 2.3 report reported there. Additionally, a common garden experiment with *Mnemiopsis leidyi* from native and invasive origin has successfully been carried out in autumn 2016. The samples will be analyzed and inform about differences in size specific reproduction rate of native American versus northern and southern invasive populations.

Deviations: Common garden experiments are delayed due to difficulties arranging algae and copepod cultures for conducting the experiments. Results will be included in the final report.

Introduction: Up to now, *M. leidyi* has not established self-sustaining populations in the low saline central and northern Baltic Sea (Schaber *et al.*, 2011; Haraldsson *et al.*, 2013; Jaspers *et al.*, 2013). This is likely due to the documented effect of salinity on reproduction rates (Jaspers *et al.*, 2011). Similarly, the salinity levels in other invaded areas of western Eurasia,

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such as the northern Caspian Sea, prevents *M. leidyi* from establishing (Jaspers et al. submitted – to be reported as part of the Deliverable 2.3 report). And in the shallow, low saline Sea of Azov, *M. leidyi* populations die out after cold winter conditions and are dependent on re-seeding from the Black Sea (Shiganova *et al.*, 2001). Further, work which contributes to deliverable 2.3 has shown that winter temperatures have a significant effect on persistence of *M. leidyi* in northern Europe (Jaspers et al. submitted). This indicates that salinity as well as temperature currently set limits to the range occupancy of *M. leidyi* in western Eurasia.

Phenotypic plasticity or evolutionary change has been highlighted as important components which may enable species to circumvent barriers to its current habitat range (Sydeman *et al.*, 2015). Additionally, invasive species are often characterized by re-current invasions (Seebens *et al.*, 2016), which could lead to a constant delivery of new genotypes into certain areas. This can lead to a higher genotypic diversity within such populations as well as admixture of different sub-populations, potentially increasing the environmental envelope of the species in question and thereby its persistence (Rius and Darling, 2014). Therefore, not only range expansion but also adaptation potential of invasive sub-populations are important components to be considered in marine invasive species risk assessments.
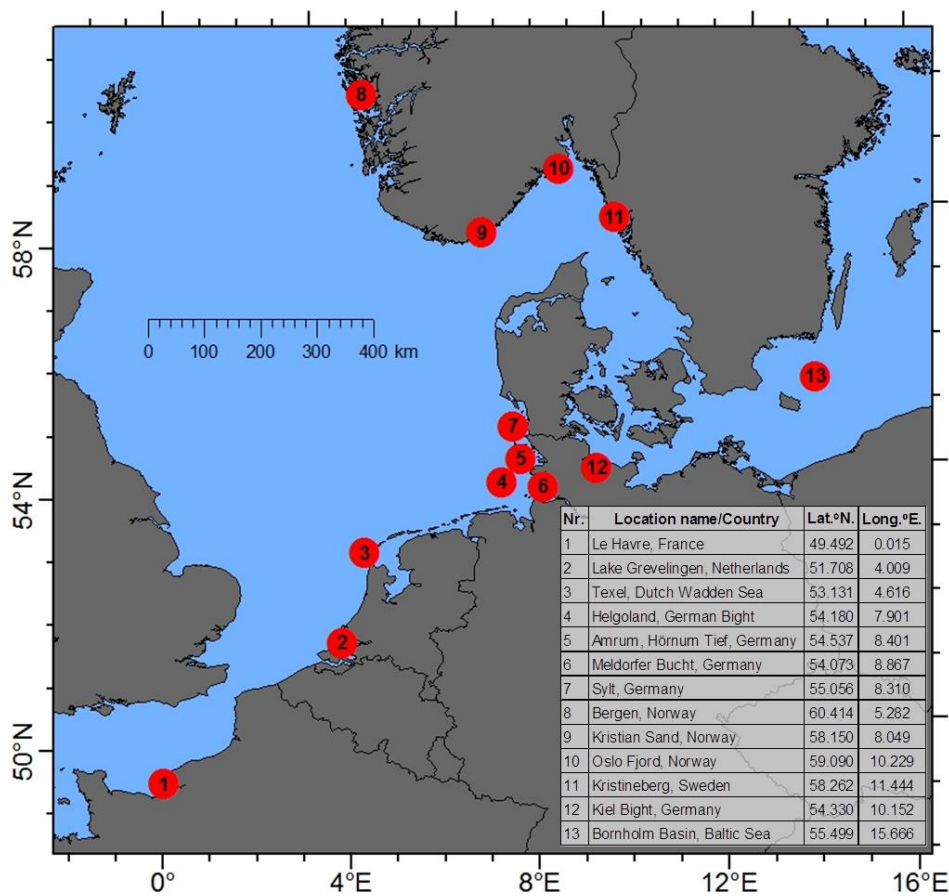

Nr.	Location name/Country	Lat.°N.	Long.°E.
1	Le Havre, France	49.492	0.015
2	Lake Grevelingen, Netherlands	51.708	4.009
3	Texel, Dutch Wadden Sea	53.131	4.616
4	Helgoland, German Bight	54.180	7.901
5	Amrum, Hörnum Tief, Germany	54.537	8.401
6	Meldorfer Bucht, Germany	54.073	8.867
7	Sylt, Germany	55.056	8.310
8	Bergen, Norway	60.414	5.282
9	Kristian Sand, Norway	58.150	8.049
10	Oslo Fjord, Norway	59.090	10.229
11	Kristineberg, Sweden	58.262	11.444
12	Kiel Bight, Germany	54.330	10.152
13	Bornholm Basin, Baltic Sea	55.499	15.666

Figure 3.1 Network of monitoring stations and regions in Northern Europe for the invasive comb jelly *Mnemiopsis leidyi.*

Methods and Results: Due to intensified and coordinated monitoring activities within BIO-C3 we were fortunate and could document that the invasive comb jelly *Mnemiopsis leidyi* disappeared from the Baltic Sea after consecutive cold winters (Jaspers et al. submitted). Monitoring stations in the Bornholm Basin, Kiel Bight as well as 11 other stations in Northern Europe (Fig. 3.1) allowed for testing of significant temperature effects on the occurrence of *M. leidyi* in Northern Europe.

First of all, we document a significant effect of winter temperature on the likelihood of presence/absence in Northern Europe (Jaspers et al. submitted). Secondly, we can document that the genotype which has been present in the Baltic Sea and Norway before 2011 got extinct and replaced by a new, modified genotype from the North Sea during 2014.

Recommendations: These results show that the SW North Sea is highly interconnected with large areas of Northern Europe, including the Baltic Sea, which makes dispersal of new genotypes with different adaptation potential from some of the largest ports of the world, present in the SW North Sea region, a likely scenario (Jaspers et al. submitted). This highlights that ballast water treatment as well as dispersal via ocean current connectivity on a continental scale is important for understanding invasion dynamics and persistence of species in non-native habitats – including the Baltic Sea as a recipient area.

References (Note: background, not BIO-C3 contributions)**:**

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Resulting BIO-C3 manuscript:

Jaspers C, Huwer B, Antajan E, Hosia A, Hinrichsen H-H, Biastoch A, Angel D, Asmus R, Augustin C, Bagheri S, Beggs SE, Balsby TJS, Boersma M, Bonnet D, Christensen JT, Daenhardt A, Delpy F, Falkenhaug T, Finenko G, Fleming N, Fuentes V, Galil B, Griffin DC, Haslob H, Javidpour J, Kamburska L, Kube S, Langenberg V, Lehtiniemi M, Lombard F, Malzahn A, Marambio M, Mihneva V, Møller LF, Niermann U, Okyar MI, Özdemir ZB, Pitois S, Reusch TBH, Robbens J, Stefanova K, Thibault D, van der Veer H, Vansteenbrugge L, van Walraven L, Woźniczka A (in press) Ocean current connectivity sets the scene for secondary spread of a marine invader across western Eurasia. Global Ecology and Biogeography.

 Note: The manuscript is included in the BIO-C3 Deliverable 2.3 report.

4. Cod spatio-temporal population mixing in ICES SD24

Authors/Contributors; partner institutes: Jakob Hemmer-Hansen, Dorte Bekkevold, Einar Eg Nielsen, Bastian Huwer, Karin Hüssy, Henrik Mosegaard, Margit Eero (P02 - DTU Aqua, Denmark); Jan Dierking, Hans Harald Hinrichsen, Burkhard von Dewitz, Thorsten Reusch (P01 - GEOMAR, Germany).

Abstract: Here, we have used a panel of high resolution single nucleotide polymorphism genetic markers to investigate spatio-temporal patterns of population mixing of eastern and western Baltic Sea cod populations within the Arkona Basin region. We confirm the presence of an east-west gradient in mixing proportions and find evidence for similar overall patterns in contemporary samples from 2014/2015 and archived samples from 2003/2004. We did not observe a strong effect of high salinity inflow events, a potential driver of population distributions. Future analyses should use higher geographical and environmental resolution to explore relationships between population distributions and potential drivers.

Progress: All work completed; resulting manuscript in preparation is attached as Appendix 3.

Deviations: No deviations from work plan.

Introduction: Genetic analyses of contemporary tissue samples have shown that western and eastern Baltic cod populations mix in SD24, and that there is an east-west gradient in mixing proportions within the area. The aim of this task was to examine effects of oceanographic conditions, salinity and Major Baltic Inflows (MBIs) in particular, on the proportions of the two population components in SD24.

Methods and Results: Based on time series of average salinity in SD 24, the years 2003 and 2004 were chosen to represent high salinity and stagnation years. In addition, the Major Baltic Inflow in December 2014 (Morholz *et al.* 2015) was used to track effects on the mixing proportions by comparing data from 2014 with data collected in 2015 after the inflow. For 2003 and 2004, we analysed DNA extracted from a total of 260 otoliths distributed in space and time in SD24. For the contemporary samples from the recent inflow event, we compared 666 samples from 2014 to 480 samples collected in 2015. We used 39 Single Nucleotide Polymorphism (SNP) markers which provide high statistical power for assigning fish to eastern and western Baltic Sea cod populations (Eero *et al.* 2014) to identify population of origin for the individual fish from SD24. As baseline, we used western (SD22) and eastern (SD25) fish collected in spawning season in 1996 and 2007. In general, the results confirmed the presence of an east-west gradient in mixing proportions, with high proportions of eastern fish in the eastern part of SD25 and low proportions in the west (Figure 4.1; see also Hüssy *et al.* 2015). These patterns appear to be temporally stable across the years included in this study, suggesting that also prior to 2004 there was a high proportion of eastern fish in the eastern part of SD24. Temporal stability also indicates that there is no direct relationship between the marked environmental shifts and mixing proportions in the years included here, as proportions of eastern fish were also high in the high salinity year 2003 and following the strong MBI in December 2014. More detailed investigation of the geographical distribution of the two population components for a subset of the samples also indicated similar distribution patterns before and after the inflow (Figure 4.1). A first analysis on a subset of samples suggested that there was no direct relationship between bottom salinity and proportion of eastern fish in catches (data not shown). It was, however, somewhat surprising that salinity did not seem to be markedly higher after the inflow in 2015. Thus, more detailed investigation of potential effects of environmental conditions will be needed in the future, including broader coverage in both space and time.

Recommendations: Results from these analyses suggest a general pattern of temporal stability of population mixing despite considerable environmental fluctuation in the years under study. However, as the area is characterized by high complexity with respect to hydrography and bathymetry, more elaborate analyses including additional samples/cruises as well as information on fish age and fine scale geographical and environmental resolution will be needed to explore potential factors affecting the distributions of the two populations in this area in more detail. For example, as seen from Figure 4.1, the deeper areas in the Arkona Basin as well as shallow areas in the eastern part of SD24 did not seem to have been covered very extensively with the samples analysed here. Further analyses of hydrographic data will also be needed to examine the geographical and temporal distribution of effects from the high saline water inflow.
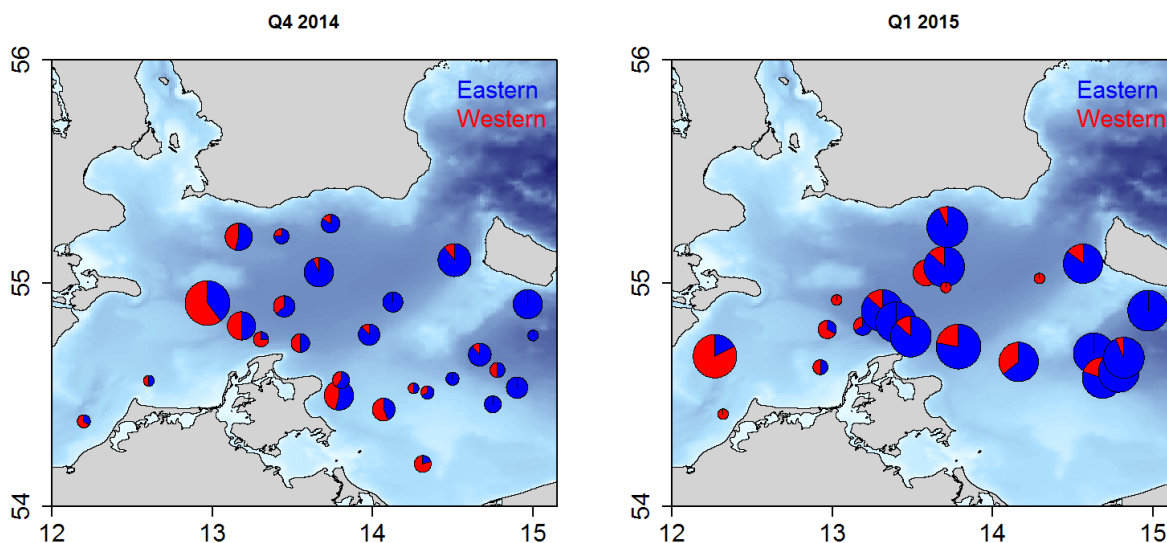
Figure 4.1 Proportions of eastern (blue) and western (red) cod in SD24 before (left) and after (right) the major Baltic inflow in December 2014. Size of pie is proportional to sample size.

References (Note: except for Eero et al. 2014, not BIO-C3 contributions)

Eero M., Hemmer-Hansen J., Hüssy K. (2014) Implications of stock recovery for a neighbouring management unit: experience from the Baltic cod, ICES Journal of Marine Science, 71, 1458-1466.

Hüssy K., Hinrichsen H.-H., Eero M., Mosegaard H., Hemmer-Hansen J., Lehmann A., Lundgaard L.S (2016) Spatio-temporal trends in stock mixing of eastern and western Baltic cod in the Arkona Basin and the implications for recruitment. ICES Journal of Marine Science, 73, 293–303. doi:10.1093/icesjms/fsv227.

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Resulting BIO-C3 manuscript attached as Appendix 3.

Jakob Hemmer-Hansen, Karin Hüssy, Henrik Baktoft, Bastian Huwer, Dorte Bekkevold, Holger Haslob, Jens-Peter Herrmann, Hans-Harald Hinrichsen, Friedrich W. Köster, Uwe Krumme, Henrik Mosegaard, Einar Eg Nielsen, Thorsten B. H. Reusch, Marie Storr-Paulsen, Andres Velasco, Burkhard von Dewitz, Jan Dierking, Margit Eero (in preparation for the journal "Evolutionary Applications"). Genetic analyses reveal complex dynamics within a population mixing zone.

5. Cod spatio-temporal population structure (1996-2016) in the Eastern Baltic Sea

Authors/Contributors: Jan Dierking, Hans Harald Hinrichsen, Burkhard von Dewitz, Thorsten Reusch (P01 - GEOMAR); Jakob Hemmer-Hansen, Einar Eg Nielsen (P02 - DTU Aqua)

Progress: All analyses are completed, a draft manuscript is in preparation and will be submitted in fall 2017 and submitted with the final report.

Deviations: Most analyses going according to plan. Current problems with the correct ageing of Eastern Baltic cod have prevented some data analyses on the cohort level (backcalculation from catch year to year of birth), that would have been of interest to assess the role of environmental drivers in more detail.

Introduction:
The population structure of marine fishes can provide crucial information on stock delineation and connectivity. Most studies to date address spatial patterns of population structure at single or few time points. This study takes advantage of a 20 year sample series from the central Baltic Sea on the commercially and ecologically important marine fish species cod.

Methods and Results: Here, we assessed whether temporal changes in stock structure from 1996-2016 correlated with fluctuating salinity and oxygen concentration. We employed single nucleotide polymorphism (SNP, 81 loci, of which 23 loci were identical to SNPs employed by Hemmer Hansen et al. – see Section 4. above) of DNA isolated from archived otoliths. We also used additional microsatellite analyses to identify and exclude contaminated historical samples from further analyses.

Spatio-temporal patterns are summarized in Figure 5.1. Our results confirmed the strong genetic differentiation of Eastern and Western Baltic cod and the role of the Arkona Basin as mixing zone, with presence of eastern and western cod genotypes (also see Hemmer Hansen et al. Section 4.). Temporally resolved data then revealed the absence of significant differentiation between the three eastern spawning locations Bornholm and Gotland Basin and Gdansk Deep over time. Interestingly, western Baltic cod genotypes likely representing migrants were detectable but very rare in the eastern Baltic (0 to 3% per year), and vice versa, with little evidence for the presence of hybrids. Overall, the high temporal stability and near complete integrity of Eastern vs. Western Baltic cod would be consistent with continuous negative selection against eastern cod in the west and western cod in the east and their hybrids in all locations.

Recommendations: For management, the rarity of "out of place" eastern and western genotypes in all locations except Arkona Basin, and the lack of further sub-structure between eastern locations suggests that the current separate management as two stock components is valid. Moreover, the consistent absence of Western Baltic cod in the Eastern Baltic, and Eastern Baltic cod in the Western Baltic suggest that a "rescue" of stocks by

immigration from neighbouring areas (e.g., Eastern Baltic cod providing spawners in the West after potential stock collapses) are unlikely.
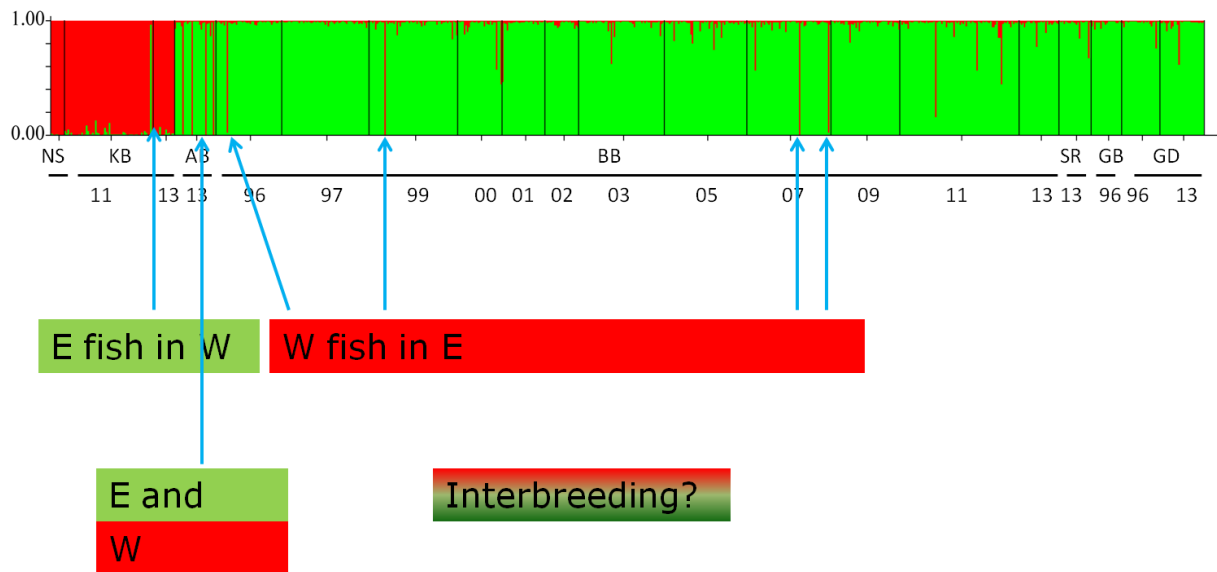

Figure 5.1 Bayesian clustering of all individuals based on the E-W SNP set (n = 23) obtained with the program STRUCTURE 2.3, assuming the presence of two clusters (K = 2). Each vertical bar represents one individual, and the two colors denote the inferred proportional genotypic contributions of each cluster. Sampling locations sorted from left to right by geographic area from west to east, and chronologically by sampling year within location. Examples of putative E fish in the W, W fish in the E, and the AB location with mixing of E and W individuals, pointed out by arrows.

Resulting BIO-C3 manuscript in preparation:
J. Dierking, B. v. Dewitz, J. Hemmer-Hansen, L. Elsbernd, S. Bracamonte, H. Schulz, C. Petereit, H.-H. Hinrichsen, E. Nielsen, T. Reusch (in preparation) Stability in spatial population structure and genetic diversity in Baltic cod over a period of strong fluctuations in environmental conditions and fishing pressure (1996-2016).

6. Declining length at maturity of Eastern Baltic cod over a 20 year period.

Note: Information in this section extracted from the peer-reviewed BONUS BIO-C3 paper Köster FW, Huwer B, Hinrichsen H-H, Neumann V, Makarchouk A, Eero M, Dewitz BV, Hüssy K, Tomkiewicz J, Margonski P, Temming A, Hermann J-P, Oesterwind D, Dierking J, Kotterba P, Plikshs M (2017) Eastern Baltic cod recruitment revisited - dynamics and impacting factors. ICES Journal of Marine Science 74:3-19. Attached to this report as Appendix 4.

This analysis of maturity data resulted from work also contributing to Task 2.1 and is reported here as a length based alternative to probabilistic maturation reaction norms that

would require reliable otolith-based age estimation. The analysis is exclusively based on data during 1995-2015 from Danish surveys that form part of the Baltic International Trawl Survey. Onboard the vessel, gonadal maturity of cod is visually judged on basis of a 10-stage maturity scale for cod developed and applied during all the surveys. This maturity scale is histologically validated and published as an illustrated manual (Tomkiewicz et al. 2003). Together, this makes the data series the most reliable information about the trend in cod maturation during the most recent 20 years.

Abstract

Mean length at which 50% of cod individuals in the Eastern Baltic Sea are sexually mature has drastically declined over the past 20 years for both females and males (Figure 6.1). However, at present the reason for this decline are not well understood, due to the range of simultaneous other changes that occurred over this time period. Understanding is further confounded by the current problems in Eastern Baltic cod age readings, which makes it difficult to assess different hypotheses (Hüssy 2010, Eero et al. 2015).
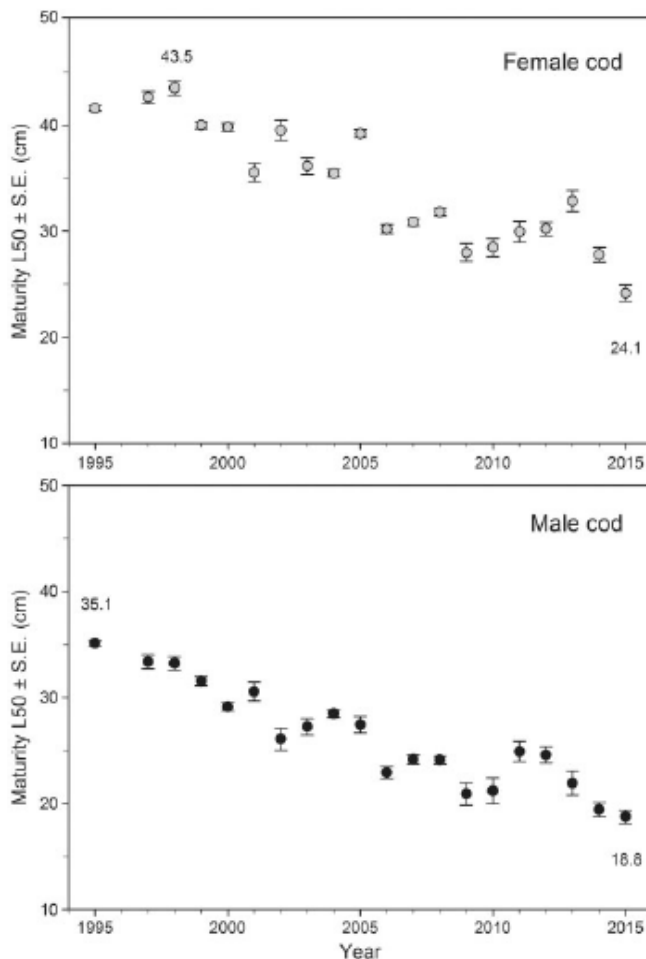
Figure 6.1 Mean length at 50% sexual maturity of female and male Eastern Baltic cod in ICES subdivision 25 during the years 1995-2015; error bars indicate s.e.. From Köster et al 2017.

Specifically, it is currently unclear whether the strong and highly significant decline in length at maturity is due to:

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a.) Slower growth of cod than in the past (i.e., age at maturity may have been stable, but fish have a lower mean size at a given age), which in turn could be related to the decreasing condition of cod over the past 15 years (Eero et al. 2015, Casini et al. 2016))

b.) Due to evolutionary adaptation or acclimation towards maturation at earlier age (i.e., age at maturity would have declined, and the younger age at which individuals reach maturity then corresponds to a smaller size) e.g., fisheries induced evolution due to strong fishing pressure (Vainikka et al. 2009).

Unfortunately, both possibilities can only be assessed if reliable age readings are available, which is currently not the case, and no solid conclusions can be drawn at present. This view was confirmed during in-depth expert discussions at the recent ICES Workshop on Biological Input to Eastern Baltic Cod Assessment (WKBEBCA) on March 1-2 2017. At the same time, given the generation time in cod of at least several years, the decline in length at maturity occurred so rapidly and was so strong that it seems unlikely that adaptive processes were the only drivers at play. Further analyses are ongoing, including the attempt to obtain improved age readings in the TABACOD project (http://www.tabacod.dtu.dk/), to ultimately be able to differentiate between the different factors at play.

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Eero M, Hjelm J, Behrens J, Buchmann K, Cardinale M, Casini M, Gasyukov P, Holmgren N, Horbowy J, Hüssy K, Kirkegaard E, Kornilovs G, Krumme U, Köster FW, Oeberst R, Plikshs M, Radtke K, Raid T, Schmidt J, Tomczak MT, Vinther M, Zimmermann C, Storr-Paulsen M (2015) Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES Journal of Marine Science: Journal du Conseil 72:2180-2186

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Köster FW, Huwer B, Hinrichsen H-H, Neumann V, Makarchouk A, Eero M, Dewitz BV, Hüssy K, Tomkiewicz J, Margonski P, Temming A, Hermann J-P, Oesterwind D, Dierking J, Kotterba P, Plikshs M (2017) Eastern Baltic cod recruitment revisited - dynamics and impacting factors. ICES Journal of Marine Science: Journal du Conseil 74:3-19

Tomkiewicz J, Tybjerg L, Holm N, Hansen A, Broberg C, Hansen E. 2002. Manual to determine gonadal maturity of Baltic cod. DTU Aqua Research Report 116-02, Charlottenlund: Technical University of Denmark. 49 p. (ISBN: 87-90968-38-7; http://www.aqua.dtu.dk/english/publications/research_reports/1996-2007)

Tomkiewicz J, Tybjerg L, Jespersen Å. 2003. Micro- and macroscopic characteristics to stage gonadal maturation of female Baltic cod. Journal of Fish Biology 62, 253-275.

Vainikka A, Gardmark A, Bland B, Hjelm J (2009) Two- and three-dimensional maturation reaction norms for the eastern Baltic cod, *Gadus morhua*. ICES Journal of Marine Science: Journal du Conseil 66:248-257

7. Transgenerational effects of parental acclimation to end-of-century ocean acidification on survival in cod larvae (*Gadus morhua*)

Authors/contributors: Martina H. Stiasny, Felix H. Mittermayer, Michael Sswat, Thorsten B. Reusch, Catriona Clemmesen (P01 – GEOMAR and Kiel University Dept. of Economics)

Abstract: Ocean acidification has been shown to have negative effects on early life stages of many fish species. However, recent research suggests that exposure of the parental generation to the same ocean acidification treatment, through the process of transgenerational acclimatization, can potentially reduce these negative effects, as in the case of *Amphiprion melanopus*. A transgenerational experiment was conducted to address the effects of parental acclimation to ocean acidification (OA) on the mortality of larval cod, as earlier experiments have shown that end-of-century levels of OA double daily mortality rates in several cod populations. Adult cod from an aquaculture stock were acclimatized to ambient (400 µatm) and predicted end-of-century pCO$_2$ conditions (1100 µatm according to the IPCC RCP 8.5) for six weeks prior to spawning. Eggs and larvae were exposed to the parental or opposed treatment and were kept under two different feeding regimes, high and low food. Preliminary results reveal the highest mortality occurs in the treatment with more than sufficient food when larvae from ambient parents were exposed to increased pCO$_2$ levels. But this effect was reduced when the parental generation was acclimated to the same high pCO$_2$, conditions and given a high food treatment. Larvae from the same pCO$_2$ treatment combination but under the low food regime showed no signs of reduced mortality compared to the direct effects; in contrary they showed the highest mortality of all treatment combinations. Transgenerational effects appear to potentially partially mediate the adverse effects of OA but only when the larvae are reared in energetically favourable condition.

Progress: Experiments performed, analysis ongoing. Manuscript in preparation attached as Appendix 5 to this report. Published BIO-C3 manuscript providing background information for this study, but also contributing to BIO-C3 Task 1.2, attached as Appendix 6.

Introduction: Ocean acidification (OA) is, as part of anthropogenic climate change, one of the major threats to marine biota and has already started to impact marine organisms and ecosystems. Fish have long been considered robust to the increased dissolution of atmospheric carbon dioxide into the oceans, since adult fish have been shown to manage pCO$_2$ levels far exceeding current predictions. But many recent studies have addressed the

effects of OA on early life stages of fish namely eggs and larvae, observing adverse effects in many of the investigated species. These adverse effects range from increased mortality (Stiasny et al. 2016), changed growth patterns and behaviour to differences in otolith formation and tissue damage . There appear not only to be differences between species but also between populations regarding vulnerability to OA. Our work compares the Western Baltic to the Barents Sea cod populations, but could not identify any differences in their reactions to end-of-century pCO_2 between the two in regard to larval mortality. However earlier studies on the Eastern Baltic population suggest that at least this population is robust to OA. Even though different parameters were quantified for the Eastern Baltic cod it is reasonable to assume that this population is robust to the effects of OA as they already have experienced high $pCO2$ naturally in their environment. This is due to the special environmental factors of saline stratification and resulting oxygen depletion, which creates water masses with high CO_2 concentrations to which the local cod might have adapted. Populations, which experience negative effects on the early life stages due to climate change, are thereby under another major stressor, next to exploitation, which could have detrimental effects on the population level and thereby also on the fisheries. A good scientific understanding of the effects is therefore vital. Yet so far only a few studies have taken transgenerational effects into account, as a short term way to possibly mediate the earlier mentioned adverse effects. As long term studies on the potential of adaptation are often not feasible due to long generation times, this study explores the potential of transgenerational acclimatization to reduce larval mortality in cod under predicted future pCO_2 conditions.

Methods and preliminary Results: Adult cod from an aquaculture stock, F4 generation, were acclimatized to an ambient pCO_2 treatment (~400 µatm) and a predicted end-of-century pCO_2 treatment (~1100 µatm) for six weeks. Fertilized eggs from each parental treatment were divided into halves; one half was moved into incubators with the same $pCO2$ as the parental treatment while the other half was moved to an incubator of the opposite treatment. Once the eggs had hatched 11 000 larvae were transferred into each of the 190 l rearing tanks, replicated six times for all parental and offspring CO_2 treatment combinations. Of these six replicates three were reared under an aquaculture feeding regime while the remaining three replicates were kept in a low food regime to assess whether an access of available food could help mediate the effects of ocean acidification. Both feeding regimes were similar until 13 dph after which the "High Food" treatment consisted of *Brachionus* feedings seven times a day, while the "Low Food" treatment was only fed three times a day with concentrations also roughly halved compared to the higher treatment. In order to address the mortality larval densities of each replicate were measured at 8, 12 and 16 days-post-hatch (dph) using a volumetric sampling method. Regression lines over the fraction of larvae in the tank at days 0, 8, 12 and 16 were fitted to every treatment with an intercept of 1 at 0 dph. A linear regression offered the best fit. The slope of the regression, i.e. the daily reduction in survival, was used for the statistical analysis. A three factorial ANOVA was

performed to examine the effects of parental and larval pCO_2 treatment as well as the effect of food and their interactions. The analysis confirms prior observations (Stiasny et al. 2016) of a highly increased larval mortality (Fig. 7.1) under direct exposure to end-of-century pCO_2 levels (larval pCO_2, F=13.040, DF=1, p<0.01). Additionally, for the first time in cod, a transgenerational effect was observed, which reduced the negative effects of OA on larval mortality (parental pCO_2 * larval pCO_2, F=8.732, DF=1, p<0.01). Nonetheless, the larvae in high pCO_2 conditions still had significantly lower survival than larvae in ambient pCO_2 conditions. Additionally, this effect was only observed in the high food treatment while no significant reduction in mortality was observed in the larvae from parents, which had experienced high carbon dioxide concentrations before under low food (see Fig 7.1 a and b respectively). In fact, here the trend was reversed with slightly lowered survival in the pre-treated high CO_2 treatment, compared to the direct CO_2 effect on the larvae (parental pCO_2 * larval pCO_2*larval food treatment, F=6.067, DF=1, p<0.05).

Conclusions and Recommendations: The preliminary results of this study suggest that a transgenerational effect can potentially reduce the adverse effects of ocean acidification on the survival of early life stages in cod after a short parental acclimation. Yet this appears only to be the case when the larvae are not energetically limited compared to their previous normal environment. In this case cod from an aquaculture stock that had been domesticated in the fourth generations and had most likely adapted to the plentiful feeding regimes applied in fry production (here implemented as the high food treatment). When given a low food treatment they can apparently not exploit the transgenerational acclimatization of their parents and experience even lower mortality as their peers under the direct effects of ocean acidification, e.g. without transgenerational acclimatization.

Data on ocean acidification effects on commercial species is still extremely limited and this is the first attempt to provide information on the potential of acclimation. There is still a lot more research to be done before this is understood. However, this study was able to show that there might be some mechanism for acclimatization. Nevertheless, the mediation through this is not strong enough to completely counteract the effect of ocean acidification and any positive effects rely strongly on the right circumstances in terms of energy supply, which is apparently needed by the larvae in order to fully utilize transgenerational effects. It is therefore still likely that ocean acidification may have effects on cod recruitment.
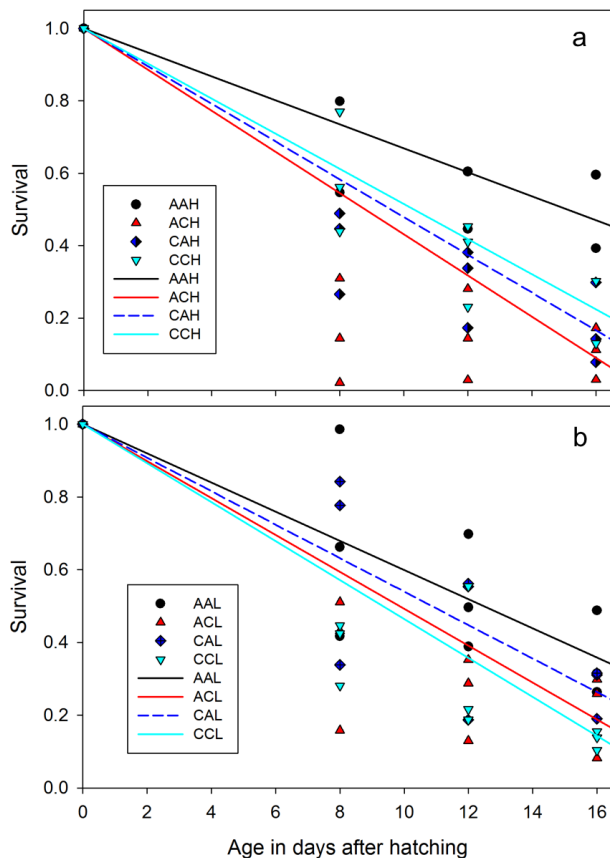
Figure 7.1: Mortality of cod larvae under ambient and predicted end-of-century ocean acidification levels from parents with and without parental acclimation under a) high food and b) low food treatment. Abbreviations in the legend: first letter describes parental pCO_2 treatment (A: ambient pCO_2 (~400 µatm) and C: end-of-century pCO_2 (~1100 µatm)), second letter describes larval pCO_2 treatment (A: ambient pCO_2 (~400 µatm) and C: end-of-century pCO_2 (~1100 µatm)) and last letter describes the food treatment (H: high food and L: low food)

Resulting BIO-C3 manuscripts:

Martina H Stiasny, Felix H Mittermayer, Michael Sswat, Thorsten B. Reusch, Catriona Clemmesen (in preparation) Transgenerational effects of parental acclimation to end-of-century ocean acidification on survival in cod larvae (*Gadus morhua*). (Note: this publication also contributes to Task 1.2, and results are in part reported there).

Stiasny MH, Mittermayer FH, Sswat M, Voss R, Jutfelt F, Chierici M, Puvanendran V, Mortensen A, Reusch TBH, Clemmesen C (2016) Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population. PLoS ONE 11:e0155448

8. Tissue sample database for invasive round goby *N. melanostomus*

Contributors: Dorte Bekkevold (curator), Jane Behrens, P02; Henn Ojaveer, P06; Felix Mittermayer, P01; Anastasija Zaiko, P08; Riika Puntila, P07; and partners from the NORDEN round goby network

Background. Analyses of samples collected across geographical areas with separate demographies and over time allow for a detailed description of invasion genetic history. It may e.g. be determined if invasions originate from single events or via multiple pulses in time and space. Likewise, it can be determined if invasions originate from single or multiple

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genetic source populations. Analyses of genetic variation also provide information about numbers of founders and local dynamics. Gaining such information is of importance to understanding risks and predicting dynamics of invasive species. In order to facilitate a detailed genetic study of round goby (*Neogobius melanostomus*) invasions in the Baltic Sea area, it was agreed to initiate collections of tissue samples among members of BIO-C3 in collaboration with members of the NORDEN round goby network. Depending on sample availability and funding opportunity, samples will be made available to analyses, once sampling has been completed.

Progress: Samples have been collected by a long list of participants, and in several cases samples represent temporal replicates (Table 8.1, Figure 8.1). Additional sampling is envisaged in the coming years, thus enabling very detailed insights into invasion genetics for the species. On-going activities relating to development of genetic resources, including SNP development, are closely followed in order to determine the best strategy for analysis. It is the expectation that funding will be obtained for performing a comprehensive population genetic analysis of samples. The collections are currently curated by DTU Aqua, and DNA extraction has been initiated but not completed. The collection constitutes a resource that can be made available to others interested in collaborating with BIO-C3 partners on developing analyses. The collection and data transpiring from analyses further constitute a valuable baseline that will allow for comparison with samples to be collected in the future, in order to gain understanding of the dynamics of round goby invasion across the Baltic Sea and adjacent waters. Amongst issues that can be studied is whether round goby will exhibit heritable adaptation to novel environments.

Figure 8.1 Round goby sampling locations. Each sample is made up of fin tissue from 40-50 fish stored in 96% ethanol. 'Lithuanian transect' indicates the area where nine individual samples were collected along a North-South transect (see Table 8.1).

Table 8.1 Round goby sample database, curated by DTU Aqua. Each sample is constituted by fin-clips of 40-50 individual fish. See Figure 1 for the map of sampling locations.

Location	Sampling year
Smiltyne	2014 & 2015
Juodkrante	2014 & 2015
Karkle	2014 & 2015
Šventoji	2014 & 2015
Kiaulės nugara	2014
Vente	2014
Palanga	2014
Kleipeda	2014
Juodkrante	2014
Guldborgsund	2014 & 2015
Hel	2014 & 2015
Åland	2014
Muuga Harbour (southern Gulf of Finland)	2014
Northern Gulf of Riga	2014
Archipelago Sea	2014
Travemünde (Lübecker Bight)	2014
Gotland	2014
Göteborg	2014& 2015
Karlskrona	2014
Rostock	2014

IV. Appendices

Appendix 1. Christensen, Anette Maria, Dutz, Jörg. Population-specific salinity tolerance is key to the success of a marine copepod species in the Baltic Sea. (Manuscript in prep., currently under embargo).

Appendix 2. Karlsson, Konrad, Winder, Monika. Evolution of a high optimum temperature - a comparison of development time between populations of the Baltic Sea copepod *Eurytemora affinis* sampled over a temperature and food gradient. (Manuscript in review in the journal "Evolutionary Biology", currently under embargo).

Appendix 3. Jakob Hemmer-Hansen, Karin Hüssy, Henrik Baktoft, Bastian Huwer, Dorte Bekkevold, Holger Haslob, Jens-Peter Herrmann, Hans-Harald Hinrichsen, Friedrich W. Köster, Uwe Krumme, Henrik Mosegaard, Einar Eg Nielsen, Thorsten B. H. Reusch, Marie Storr-Paulsen, Andres Velasco, Burkhard von Dewitz, Jan Dierking, Margit Eero. Genetic analyses reveal complex dynamics within a population mixing zone (Manuscript in prep. for the journal "Evolutionary Applications", currently under embargo)

Appendix 4. Köster FW, Huwer B, Hinrichsen H-H, Neumann V, Makarchouk A, Eero M, Dewitz BV, Hüssy K, Tomkiewicz J, Margonski P, Temming A, Hermann J-P, Oesterwind D, Dierking J, Kotterba P, Plikshs M (2017) Eastern Baltic cod recruitment revisited - dynamics and impacting factors. ICES Journal of Marine Science: Journal du Conseil 74:3-19 (Published peer-reviewed manuscript)

Appendix 5. Martina H Stiasny, Felix H Mittermayer, Michael Sswat, Thorsten B. Reusch, Catriona Clemmesen. Transgenerational effects of parental acclimation to end-of-century ocean acidification on survival in cod larvae (*Gadus morhua*) (in review in the journal "Scientific reports", currently under embargo)

Appendix 6. Stiasny MH, Mittermayer FH, Sswat M, Voss R, Jutfelt F, Chierici M, Puvanendran V, Mortensen A, Reusch TBH, Clemmesen C (2016) Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population. PLoS ONE 11:e0155448. doi:0155410.0151371/journal.pone.0155448 (published peer reviewed manuscript)

MANUSCRIPT in preparation

Population-specific salinity tolerance is key to the success of a marine copepod species in the Baltic Sea.

Anette Maria Christensen , Jörg Dutz (Technical University of Denmark and IOW, P2)

Results are under embargo until publication. For more information, contact Jörg Dutz, e-mail: joerg.dutz@io-warnemuende.de

APPENDIX 2

MANUSCRIPT – In review with the journal "Evolutionary Biology"

Evolution of a high optimum temperature - a comparison of development time between populations of the Baltic Sea copepod *Eurytemora affinis* sampled over a temperature and food gradient.

Konrad Karlsson[1], Monika Winder[1]

[1]Department of Ecology, Environment, and Plant Sciences, Stockholm University, Stockholm, Sweden, 10691

APPENDIX 3

MANUSCRIPT DRAFT - In preparation for the journal Evolutionary Applications

Genetic analyses reveal complex dynamics within a population mixing zone

Jakob Hemmer-Hansen, Karin Hüssy, Henrik Baktoft, Bastian Huwer, Dorte Bekkevold, Holger Haslob, Jens-Peter Herrmann, Hans-Harald Hinrichsen, Friedrich W. Köster, Uwe Krumme, Henrik Mosegaard, Einar Eg Nielsen, Thorsten B. H. Reusch, Marie Storr-Paulsen, Andres Velasco, Burkhard von Dewitz, Jan Dierking, Margit Eero

Results are under embargo until publication. For more information, contact Jakob Hemmer Hansen, DTU Aqua Silkeborg, e-mail: jhh@aqua.dtu.dk

Appendix 4

Peer-reviewed manuscript:

Köster FW, Huwer B, Hinrichsen H-H, Neumann V, Makarchouk A, Eero M, Dewitz BV, Hüssy K, Tomkiewicz J, Margonski P, Temming A, Hermann J-P, Oesterwind D, Dierking J, Kotterba P, Plikshs M (2017) Eastern Baltic cod recruitment revisited - dynamics and impacting factors. ICES Journal of Marine Science: Journal du Conseil 74:3-19. (also contributing to BIO-C3 Task 2.1)

Review article

Eastern Baltic cod recruitment revisited—dynamics and impacting factors

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The Eastern Baltic cod abundance started rapidly to increase in the mid-2000s as evidenced by analytical stock assessments, due to increased recruitment and declining fishing mortality. Since 2014, the analytical stock assessment is not available, leaving the present stock status unclear and casting doubts about the magnitude of the recent increase in recruitment. Earlier studies identified main factors impacting on cod reproductive success to be related to the loss of two out of three spawning areas in the 1980s caused by lack of major Baltic inflows with a concurrent reduction in salinity and oxygen. Other important factors include prey availability for first-feeding larvae, egg predation by sprat and herring and cannibalism on juveniles, all in one way or the other related to the prevailing hydrographic conditions. These factors cannot explain increased reproductive success in the last decade, as the period was characterized by an absence of large-scale Baltic inflows since 2003 and persistent anoxic conditions in the bottom water of the deep Baltic basins. This questions the perception of the increased recruitment in later years and challenges our present understanding of cod recruitment dynamics in the Baltic Sea. In this contribution, we review evidence from the recent literature supplemented by information from latest research cruises to elucidate whether cod reproductive success indeed has increased during the last decade, and we suggest the key processes responsible for the recent dynamics in cod recruitment and outline directions for future research.

Keywords: Baltic inflows, early life stage survival, Eastern Baltic cod, oceanographic drivers, recruitment, reproductive success

Introduction

The Eastern Baltic cod, with its central distribution area in ICES Subdivisions 25–28 (Figure 1), started to recover from low stock sizes in the mid-2000s, as evidenced by analytical stock assessments, partly attributable to increasing recruitment (Eero et al., 2012a). An analytical stock assessment has not been available since 2014, due to increasing uncertainty in biological input parameters, leaving the present stock status unclear and casting
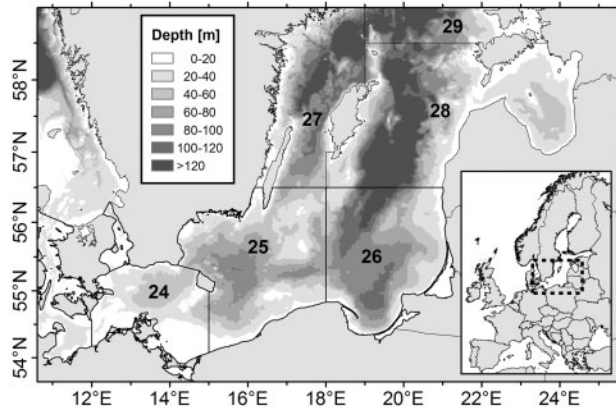
Figure 1. Study area in the southern Baltic Sea including ICES SD (numbers and thin black lines); SD 24 corresponds to the wider area of the Arkona Basin, SD 25 to the Bornholm Basin, southern SD 26 to the Gdansk Deep, northern SD 26 to the southern part of the Gotland Basin and SD 28 to the central Gotland Basin; inset: location of the study area in a European map.

doubts about the magnitude of increased recruitment including our understanding of processes affecting the reproduction of the stock (Eero *et al.*, 2015). The eastern Baltic ecosystem has commonly been considered "cod hostile" in the considered period, which should have resulted in low reproductive success of cod. In contrast, the biomass of small cod has increased up to fivefold in research surveys and is currently among the highest since the 2000s (ICES, 2014a; Eero *et al.*, 2015). The reasons for this apparent increase in recruitment are not clear. Improved knowledge on processes impacting on reproductive success is thus of fundamental importance for the management of the Eastern Baltic cod.

Earlier studies from the 1990s and early 2000s identified the key controlling factors for cod recruitment to be major Baltic inflows of saline and oxygen-rich water from the North Sea in combination with oxygen consumption (e.g., MacKenzie, *et al.*, 1996; Köster *et al.*, 2005a) and prey availability for first-feeding larvae, which is influenced by salinity and predation by sprat on zooplankton (Hinrichsen *et al.*, 2002; Möllmann *et al.*, 2005). Second-order regulating factors were identified as (i) prey availability for adults affecting egg production (Kraus *et al.*, 2002), (ii) egg predation by sprat and herring, depending on salinity/oxygen and timing of spawning defining spatial and temporal overlap between predator and prey, respectively (Köster *et al.*, 2005a), (iii) habitat availability for successful juvenile settlement (Hinrichsen *et al.*, 2003) and (iv) cannibalism on juveniles, depending on transport of juveniles, horizontal overlap with adults and abundance of alternative prey (e.g. Neuenfeldt and Köster, 2000, Uzars and Plikshs, 2000). More recent studies confirmed recruitment of Eastern Baltic Cod to be (i) significantly related to potential egg production, oxygen concentration in and below the halocline and predation related egg survival and larval prey availability (Köster *et al.*, 2009) and (ii) spawning stock biomass, the reproductive volume, i.e. the water volume sustaining egg development and the winter North Atlantic Oscillation index (Margonski *et al.*, 2010). The latter study included updated information until 2004.

At the first sight, above factors in combination with the prevailing environmental conditions cannot explain the increased reproductive success of cod from the mid-2000s (ICES 2014a, b). In this contribution, we review relevant available information to

elucidate whether the reproductive success of cod since the mid-2000s has indeed been higher than in preceding years, and whether it continues to be high in most recent years despite deteriorating nutritional condition of adult cod (Eero *et al.*, 2015; ICES 2015a, b). Our main focus is not to ascertain significant differences of recruitment in specific years, but to evaluate whether (i) the above-described factors and processes impacting on Eastern Baltic cod recruitment are still valid, (ii) there are indications that the relative importance of processes has changed and (iii) additional factors can be identified. To do this, we update available abundance, production and mortality estimates of early and juvenile life stages and relevant factors impacting on recruitment and review the state of the art knowledge on early life stages of Eastern Baltic cod through ontogeny from realized egg production to juvenile survival. We then evaluate the impact of physical/chemical conditions and biological interactions on survival of these life stages based on recent hydrographic, ichthyoplankton and trawl survey results, as well as published or on-going studies addressing specific life stages or physical/biological processes. We do not aim at an in-depth statistical analysis of the various interacting processes impacting cod recruitment, which is at present hampered by the absence of an analytical assessment and thus reliable recruitment estimates, but rather to guide future recruitment research on cod stock recruitment and collect available evidence of recruitment dynamics throughout the last decade.

Abundance and survival of early life stages
As a first step, available and updated abundance or production and survival estimates of eggs, larvae and juveniles are inspected for trends throughout the 2000s, utilizing information from the 1990s and if available earlier periods as reference (Table 1).

Egg stage
Daily egg production in the Bornholm Basin (Figure 1), the main spawning area of Eastern Baltic cod in the last decades (Köster *et al.*, 2009), increased from 2003 to 2008/2009 several fold and declined since then to similar low levels as encountered in the early 2000s (Figure 2). Egg survival was relatively high both in May/June and July/August since 2000 with a peak in 2003, and only occasionally low values. Comparing the 1990s and the 2000s, egg survival was generally higher in the latter period and predominantly higher late in the spawning season (Figure 2). Hydrographic conditions and predation by sprat and herring are investigated below as important processes impacting egg survival.

Larval stage
Larval abundance in the Bornholm Basin during the main spawning time was unexpectedly low in 2004–2010 and high in 2011–2012 (Figure 3) given the developments in egg production and survival (Figure 2). For most recent years, all ichthyoplankton surveys show a decline from 2012 to 2013, most drastically in August (Table 2) and a further decline from 2013 to 2014, with the exception of July/August. This may indicate declining recruitment success in most recent years. Larvae were regularly encountered in eastern spawning areas of the Gotland and Gdansk Basin, but in very low abundances (Figure 3). This implies a continued limited contribution of these spawning areas to recruitment of the stock as reported earlier (Köster *et al.*, 2009). Following specific cohorts through the egg to the larval stage in the Bornholm Basin in May/June and July/August 2010–2013 revealed differences in survival to and within the larval stage between years and

Table 1. Time series of variables used and sources of information, their temporal and spatial (ICES SD) coverage as compiled from literature and after update of survey data bases, as well as information on the method applied.

Time series	Period	Month	SD	Source	Method
Spawning stock biomass	1966–2013	Beginning of year	25–28	ICES (2013)	SAM assessment model (ICES, 2013)
Egg production (daily) and egg survival (stage Ia–III)	1990–2013[a]	May August	25	Köster et al. (2005a, b), 2000–2013 updated	Ichthyoplankton survey: Wieland (1988) Production/survival: Wieland et al. (2000a)
Larval abundance	1970–2014[b]	Main spawning season, from 2004: May–August	25, 26, 28	Köster et al. (2009), 2004–2014 updated	Huwer et al. (2011)
	2007–2014	Monthly March–August and November	25	BH pers. comm.	Huwer et al. (2011)
Catch rates of juveniles (<25 cm)	2001–2016[c] 2001–2015	February/March November	25, 26, 27, 28 25	ICES (2015c) updated with 2016 survey and 4. quarter 2015 survey Danish BITS	Extract from ICES Baltic International Trawl Survey (BITS) Datras database
Recruitment age 2	1966–2011	Beginning of year	25–28	ICES (2013)	SAM assessment model (ICES, 2013)
Mean length at 50% sexual maturity	1995–2015[d]	February/March	25	Plikshs et al. (2015)	Macroscopic inspection after Tomkiewicz et al. (2003) fitting a binomial logit function
Thickness of spawning layer, oxygen and temperature in the layer	1950–2013	Main spawning time, i.e. May until 1990 and afterwards August	25	BvD pers. comm.	Survival constraints: Plikshs et al. (1993) and temperature >2 °C acc. to Wieland et al. (1994) based on hydrographic station grid
Reproductive volume	1966–2013	Main spawning time, i.e. May until 1991 and afterwards August	26 and 28	Plikshs et al. (2015)	Plikshs et al. (1993)
Thickness of spawning layer	1980–2013	Western stock: February–May Eastern stock: June–August	24	FWK pers. comm.	Survival constraints for eastern stock as in SD 25, for western stock with salinity >15PSU acc. to Hüssy (2011)
Predation pressure on eggs	1990–1999 and 2004–2008	May and August	25	Neumann et al. (2014)	Köster and Möllmann (2000) with enhanced predator population estimation based on Baltic International hydroacoustic Surveys (ICES, 2015c)
Biomass anomalies of zooplankton	1975–2008	Spring (2. quarter) and summer (3. quarter)	25	ICES (2010) and unpubl. material	ICES (2010)

[a]May/June survey 2001 not yet analysed.
[b]Larval abundances in SD26 i 2007–2009 not available.
[c]Restricted to 2001 and onwards because of changes in survey design from 2000 to 2001 affecting the catchability of juveniles (ICES, 2015a).
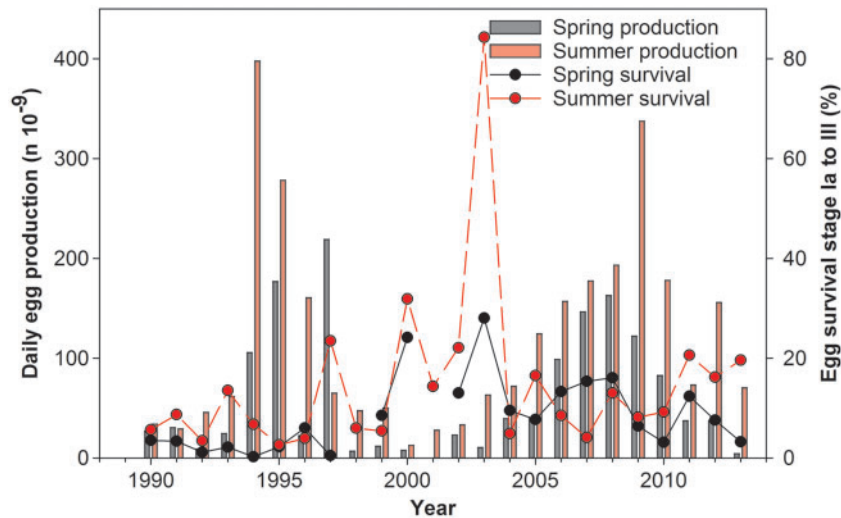[d]Restricted to 1995 and onwards as maturity sampling stage revised.

Figure 2. Daily egg production and egg survival in the Bornholm Basin estimated from ichthyoplankton surveys in spring (May/June) and summer (July/August) 1990–2013.
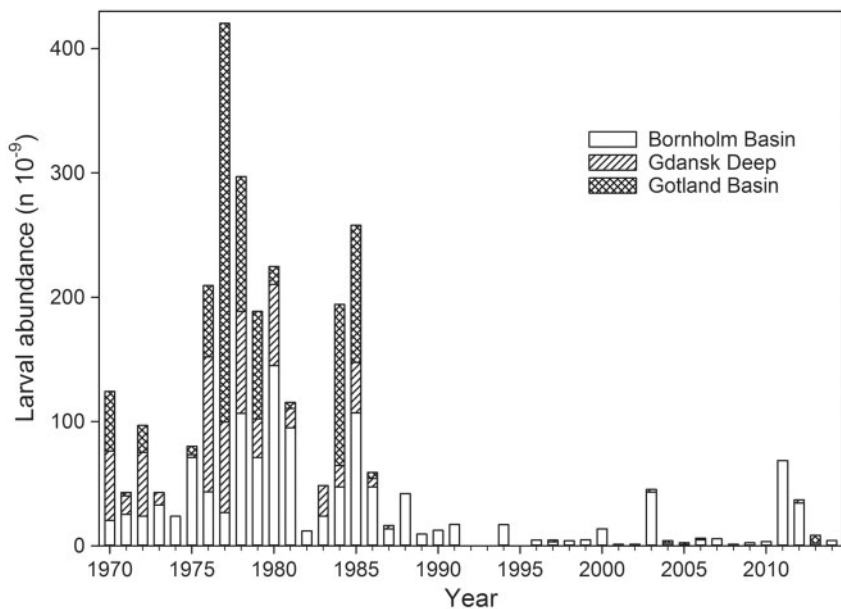

Figure 3. Larval abundances in the Bornholm Basin, Gdansk Deep and Central Gotland Basin from ichthyoplankton surveys during main spawning times 1970–2014.

seasons (Supplementary Figure A). Relatively low egg production in 2011 resulted in the highest abundance of larvae, while survival into the larval stage was lowest in summers 2010 and 2013. In all years covered, the ratio of young larvae to egg stage Ia abundance was highest in November indicating highest larval survival late in the spawning season. Among the processes affecting larval survival, prey availability and growth of larvae are considered below.

Juvenile stage

Significant relationships identified between larval abundance and 0-group recruitment in the Bornholm and Gdansk Basins suggest that the later larval and early juvenile stages are less critical for cod recruitment than the egg and the early larval stages (Köster

et al., 2003). The last accepted analytical assessment (ICES, 2013) indicated a nearly threefold increase in recruitment (age 2) from the lowest level of year-class 2002 to the highest value recorded since 1988 for year-class 2011. As age determination has been increasingly unreliable for the Eastern Baltic cod stock, being one of the reasons for failure in analytical stock assessment (Eero et al., 2015; ICES, 2015b), we use catch rates from International Bottom Trawl surveys for fish <25 cm in length as a rough approximation for illustrating variability in year-class strength (Figure 4). While juvenile cod are encountered throughout the central Baltic, by far highest catch rates are located in the area encompassing the Bornholm Basin (Subdivision 25). High catch rates in the fourth quarters 2012/2013 and in the first quarters 2013/2014, indicate

Table 2. Larval abundance (n 10^{-9}) in the Bornholm Basin from ichthyoplankton surveys in different months 2007–2014.

Month	March	April	May	June	August	November
2007	no data	0.0	2.4	No data	5.4	No data
2008	0.0	1.5	5.3	No data	8.7	No data
2009	0.1	0.5	1.3	No data	2.4	No data
2010	0.1	1.2	4.0	6.8	3.3	3.1
2011	0.5	0.3	12.4	18.7	68.6	3.2
2012	0.5	3.8	5.4	28.0	34.4	2.5
2013	0.1	0.9	4.6	no data	2.1	2.2
2014	no data	0.1	0.0	1.1	4.2	0.7

relatively strong year-classes 2011 and 2012. Low catch rates in the fourth quarters 2014/2015 and first quarter 2015/2016 indicate lower 2013 and 2014 year-classes; however, there is no general trend in the <25-cm fish abundance from 2000 to 2013 (Figure 4). During the juvenile stage of demersal fish stocks, year-class strength is suggested to be regulated primarily by density-dependent processes after settling mediated by competition for limited food availability and predation (e.g. Myers and Cadigan, 1993). Evidence for these processes is reviewed below.

Summary of trends

Earlier studies gave a coherent impression of high reproductive effort and success in early/mid 1980s, reduced reproductive effort and early life stage survival resulting in low recruitment in the second half of the 1980s and early and late 1990s (Köster *et al.*, 2005a). Within the period from 2000 to 2013, relatively high recruitment is reflected in high catch rates of juveniles from year-classes 2000, 2003, 2011 and 2012, all affiliated with intermediate to high egg survival and intermediate to high larval abundance (Figure 5). In turn, low catch rates of juveniles originated in 2007, 2009, 2010 and 2013 correspond to low egg survival (however not in May 2007) and low larval abundance. Intermediate recruitment from 2005, 2006 and 2008 is related to intermediate egg survival and no signal of increased larval abundance (Figure 5). The last analytical assessment suggests, in contrast, continuously increasing recruitment of year-classes 2003 to 2011, with spawning stock biomass increasing subsequently from the lowest levels on record in 2005 to levels recorded in the beginning and mid-1990s (Figure 5).

Factors influencing survival of early life stages

As a second step, we review the state-of-the-art knowledge on factors impacting on cod early life stages and evaluate the impact of physical/chemical conditions and biological interactions on survival of these life stages based on recent survey results and published or on-going studies (Table 1).

Parental characteristics
Maternal condition

Nutritional condition and likely also growth of adult Eastern Baltic cod have substantially declined in recent years (e.g. Eero *et al.*, 2015), which together with changes in the lipid content and composition of the adult cod diet (Røjbek *et al.*, 2012) may have impacted negatively on cod reproduction via reduced fecundity, atresia and viability of offspring. Relative fecundity of the Eastern Baltic cod has been reported to be independent of body length and spawning area or season, but to vary significantly between
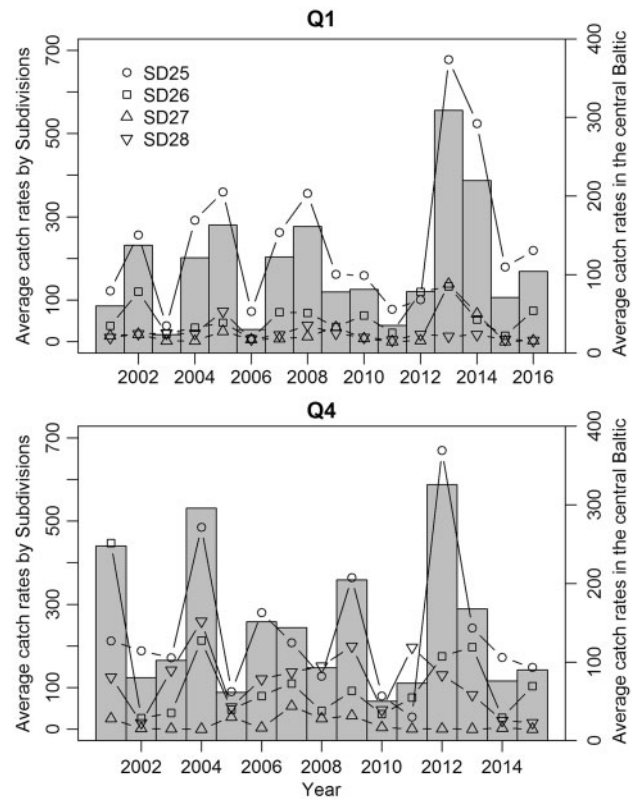

Figure 4. Average catch rates of juvenile cod (abundance <25 cm) in the first (upper panel) and fourth (lower panel) quarters from Baltic International Trawl Surveys 2001–2015/2016 in single Subdivisions 25, 26, 27 and 28 (lines), and the total central Baltic (bars) calculated as averages of the single Subdivisions weighted by the Subdivision areas; catch rates of age-group 0 in the fourth quarter and age-group 1 in subsequent first quarter bottom trawl catches cannot be considered representative (low catchability of too small and none-settled juveniles); recruitment signal is established for age-group 1 in the fourth quarter and age-group 2 in subsequent first quarter.

years (Kraus *et al.*, 2000). Existing fecundity models for the stock are based on relationships between the relative fecundity and either growth and temperature or food availability relative to stock size (Kraus *et al.*, 2002). However, it is unknown whether these relationships are still valid for the most recent years with reduced adult condition (e.g. Eero *et al.*, 2015; ICES, 2015b). Low maternal condition influences individual fecundity in other cod stocks (e.g. Lambert, 2008) and although atresia has earlier been reported to have a limited impact on realized egg production in Eastern Baltic cod, it is associated with maternal condition (Kraus *et al.*, 2008) and thus may have increased with decreasing female condition. Low condition is known to result in increased atresia (Thorsen *et al.*, 2006; Witthames *et al.*, 2013) and skipped spawning (Rideout *et al.*, 2006; Skjaeraasen *et al.*, 2009) in other cod stocks, but relationships are stock specific and cannot simply be transferred to the Eastern Baltic cod.

Essential fatty acids

Low maternal condition has been shown to impact not only the quantity but also the quality of spawning products and viability of offspring in other cod stocks (Marteinsdottir and Begg, 2002),
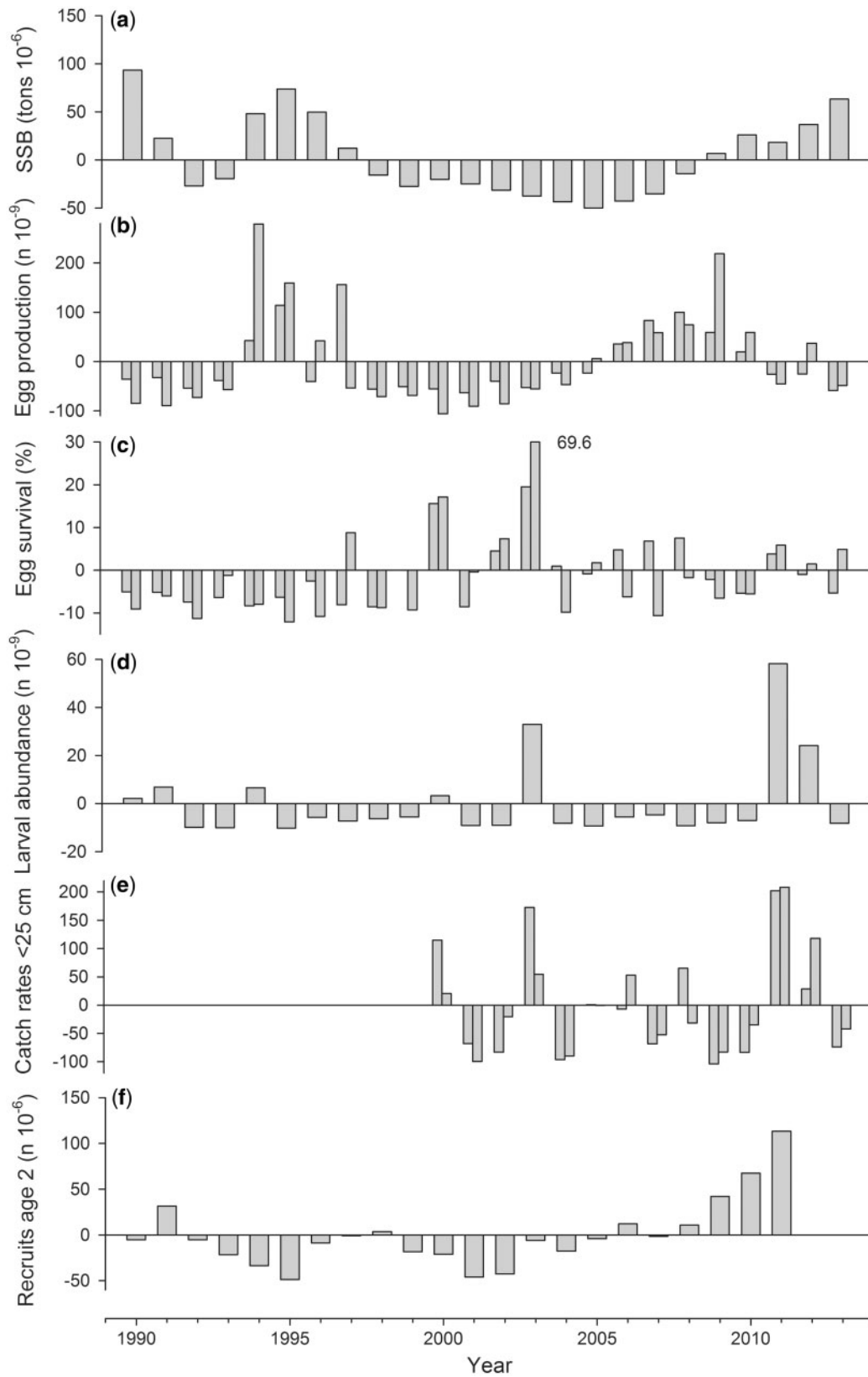
Figure 5. Anomalies of spawning stock biomass (a) and year-class strength (age group 2) (f) of Eastern Baltic cod (from ICES, 2013) during the 1990s and 2000s and from Baltic International Trawl Surveys (e) (data from figure 3 shifted to year of origin; right column is first and left column fourth quarter survey); daily egg production (b) and egg survival (c) in May/June (left columns) and July/August (right columns) and larval abundance (d) in the Bornholm Basin during main spawning time 1990–2013.

which is likely also the case for Eastern Baltic cod, whose diet is dominated by sprat and herring and few benthic species such as *Saduria entomon*. In a recent study, low arachidonic acid (ARA) levels were observed in cod livers and in whole fish of sprat and herring (Røjbek *et al.*, 2012), and low ARA levels have previously been observed in Eastern Baltic cod eggs (Pickova *et al.*, 1997). Feeding experiments with Atlantic cod in Canada have shown that eggs from fish on a diet with high ARA level had significantly higher ARA levels as well, and higher fertilization and hatching success than those fed low levels of ARA (Røjbek *et al.*, 2014a). Furthermore, larval survival was lowest in diets with low ARA level. This suggests that fatty acid composition of prey species may be critical for egg production, fertilization success and larval survival of Baltic cod. A correlation between dietary and ovarian fatty acid composition has been reported earlier for Northeast Arctic cod (Norberg *et al.*, 2009) and ARA as dietary supplement was found to improve egg production, percentage of buoyant eggs, hatching success and larval survival of Japanese flounder (*Paralichthys olivaceus*) when the proportion of ARA was increased (Furuita *et al.*, 2003). Lipid content and fatty acid composition differ significantly between sprat and herring (Røjbek *et al.*, 2014b) and *Saduria entomon* in the Baltic Sea, with the latter being richer in ARA. Recent changes in food availability for adult cod, specifically shortage of benthic prey (Eero *et al.*, 2015), may thus have impacted cod reproduction success via changes in lipid content and composition of the diet. Limitation of ARA in the food supply of cod may as well be associated with a delay in spawning resulting in the protracted spawning time of the stock (Røjbek *et al.*, 2012).

Protracted spawning time

The Eastern Baltic cod has a prolonged spawning period, which historically extends from March to September (Bagge *et al.*, 1994). This behaviour is likely an adaptation to the variable environmental conditions in the Baltic Sea (MacKenzie *et al.*, 1996). The time of peak spawning was between the end of April and mid-June in the 1970s and 1980s (MacKenzie *et al.*, 1996) and gradually changed to the second half of July during the 1990s (Wieland *et al.*, 2000b). In the 2000s, the relative egg production in May increased (Figure 2), while spawning continues as late as October/November (Baranova *et al.*, 2011). Timing and duration of spawning affect reproductive success through (i) seasonal variability in abiotic parameters (see above), (ii) temporal overlap with predators, (iii) temporal/spatial pattern of nauplii production determining the food availability for larvae and (iv) transport of larvae and early juveniles into suitable nursery areas (all addressed below). Timing and duration of spawning depends on sex and size with males generally ripening earlier than females, and large females ripening earlier and spawning longer than smaller females (Tomkiewicz *et al.*, 2009). Thus, changes in size at maturity and size/sex structure of the stock may have contributed to changes in reproductive success.

Size at sexual maturation

In Eastern Baltic cod, length at first maturity (L50) of sexes has decreased over the last 20 years (Figure 6), as egg size and buoyancy of cod eggs are associated with female size (Vallin and Nissling, 2000). This may imply an increased production of on average smaller eggs by smaller females in recent years as a consequence of attaining sexual maturation at smaller size, which in turn may have resulted in higher egg mortality in recent years (Hinrichsen *et al.*, 2016). However, this is not apparent from estimated egg mortality rates until 2013 (Figure 2). A comparative analysis of cod stocks across the Atlantic suggests that maturation patterns relate to growth potential and surplus production, however, that annual production of recruits per unit biomass was unrelated to average size at sexual maturation (Köster *et al.*, 2013). The combined consequences of declining growth, reduced condition and spawning at smaller sizes on individual egg production and viability of offspring in Eastern Baltic cod are to date not clear.

Parasitic infection

Another factor influencing the nutritional condition of sexually mature cod and thus potentially quantity and quality of offspring produced is parasitic infection. This is supported by Mehrdana *et al.* (2014) describing a significant, negative correlation between *Pseudoterranova* intensity in cod musculature and fish condition factor. Various species of seals are final hosts of *Pseudoterranova* anisakid nematodes. An increasing number of grey seals observed recently in the Baltic Proper (Härkönen *et al.*, 2013), including the main spawning area located in the Bornholm Basin is suggested to be responsible for an increasing intensity and prevalence of the parasitic infection of cod in the central Baltic Sea (Buchmann and Kania, 2012; Nadolna and Podolska, 2014). Based on samples collected in winter 2011, Nadolna and Podolska (2014) reported the presence of three species of anisakid nematodes (*Contracaecum, Anisakis* and *Pseudoterranova*) in the liver of cod, with *Contracaecum* being the most dominant species. The prevalence of infection has significantly increased compared with previous studies undertaken over the past few decades. However, reports on relationships between parasitic infection by anisakid nematodes and condition or mortality in cod are not conclusive. Lunneryd *et al.* (2015) did not detect a direct relationship between sealworm infection and nutritional condition in Baltic cod and a recent study on cod in the southern Gulf of St. Lawrence neither confirmed a negative effect on condition and growth (McClelland *et al.*, 2011), indicated in earlier studies on cod from the Cape Breton Shelf and Sable Island Bank (McClelland, 2002).

Hydrography in the spawning areas
Bornholm Basin

Hydrographic model results (Lehmann *et al.*, 2014) indicate that a number of smaller winter/spring inflows resulted in both increased salinity and oxygen in bottom water layers improving conditions for egg survival in 2000, 2001, 2007, 2011 and 2012. Less clear is the impact of baroclinic inflows during summer, transporting high-saline but warm and relatively low-oxygen water into the halocline, a process observed regularly since 1996 (Elken *et al.*, 2015). Hinrichsen *et al.* (2016) showed that the size of suitable spawning habitat is highest and stable throughout the spawning season for large eggs, being generally lower for midsized eggs and declining throughout the spawning season. Survival is lowest for small eggs, with best conditions late in the spawning season. This may be related to the effects of summer inflows (Hinrichsen *et al.*, 2016). During the last decades, no pronounced time trend in the thickness of the water layer sustaining cod egg development or in the oxygen content within this layer has been recorded (Figure 7). In contrast, the temperature within
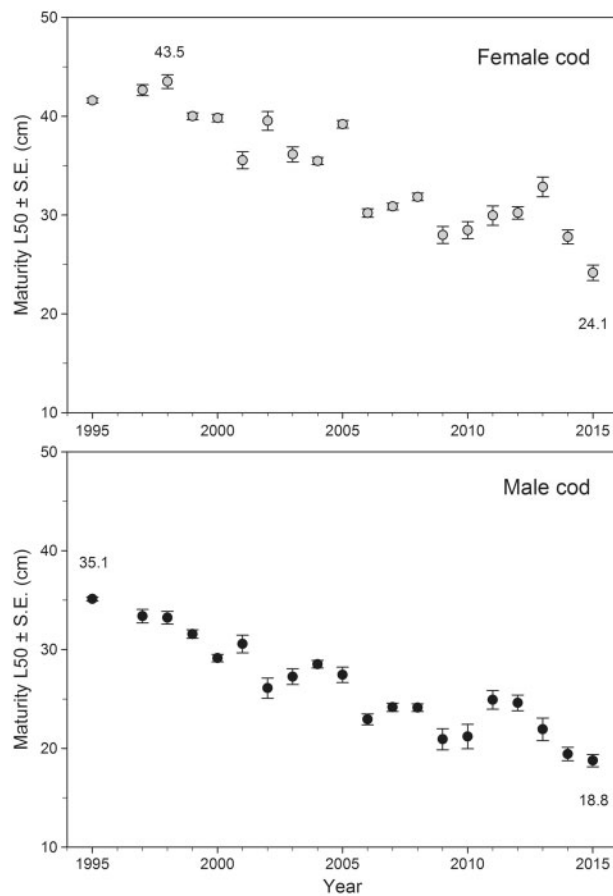
Figure 6. Mean length at 50% sexual maturity of female and male Eastern Baltic cod in Subdivision 25 during 1995–2015; error bars indicate s.e.

this layer shows an increasing trend from the mid-1980s to 2009, but relatively low values in most recent years (Figure 7). Concerning seasonal variability in egg survival, the traditional perception was that hydrographic conditions for egg survival are more favourable in May/June than in July/August (MacKenzie *et al.*, 1996). However, since 2004, the difference is marginal and in single years reversed (Supplementary Figure B), allowing high egg survival during the main spawning season in summer (Figure 2).

Gdansk and Gotland Basins

Due to lack of major Baltic inflows and reduced salinity and oxygen, spawning conditions for the Eastern Baltic cod in these basins have deteriorated since the early 1980s (MacKenzie *et al.*, 2000; Köster *et al.*, 2009). Even when major Baltic inflows in 1993 and 2003 replaced part of the bottom water of the Gotland Basin, oxygen conditions in dwelling depth of cod eggs did not necessarily improve, with eggs floating in an intermediate oxygen minimum zone (Plikshs *et al.*, 2015). Although larger inflow events during winter/spring were missing from autumn 2003 to spring 2014 (Mohrholz et al., 2015), baroclinic summer inflows were recorded in 2002, 2006, 2009 and 2010 (Nausch *et al.*, 2014) and had a positive effect on the reproductive volume also in the Gotland and Gdansk Basin sustaining a certain egg development

(Figure 8). Besides restricted inflow events, oxygen consumption rates almost tripled in the deep water layers of the eastern Baltic basins since the 1940s, caused by increasing nutrient loads (Gustafsson, 2012; Carstensen, *et al.*, 2014) . Consequently, very low numbers of cod larvae were encountered in the Gotland and Gdansk Basin, implying a continued limited contribution of these spawning areas to recruitment of the stock (Plikshs *et al.*, 2015). Relatively good spawning conditions are usually observed in the Slupsk Furrow (connecting the Bornholm and the Gdansk basins), but considering the limited water volume of this area, it cannot contribute significantly to the recruitment success of the entire stock.

Arkona Basin

Utilization of the Arkona Basin, traditionally considered as a distribution area for the western Baltic cod stock, as spawning area of the eastern stock has been reported by Bleil *et al.* (2009) and Hüssy (2011). During the 2000s, the reproductive conditions for the Eastern Baltic cod during spawning time in June to August were generally better than in earlier decades, with several occasions of 10–15 m water layer sustaining egg survival (Figure 9). This is extremely seldom the case for the western Baltic cod stock (Figure 9). During the 1980s and late 1990s/early 2000s, reproductive success of the western stock was limited by low temperature as cold winter water often fills the Arkona Basin during the main spawning time of the stock in the first quarter, especially in inflow years. In contrast, salinity is the limiting factor for the eastern stock, even in inflow years as inflows have passed by the time of spawning further on into the central Baltic. Hydrodynamic modelling by Hüssy *et al.* (2015) suggests that, under the assumption of an evenly distributed egg production within the 25-m depths contour of the Arkona Basin, ca. two-third of the Eastern Baltic cod eggs are laid in unfavourable conditions not sustaining their development, i.e. are not fertilized, sink to the bottom due to low salinity or die due to low oxygen concentration in bottom waters. From the remaining one-third eggs, about 50% die due to bottom contact during their drift, while mortality due to low temperature is quite low. Survivors to the yolk-sac stage are mostly retained in Subdivision 24 (86%). Best spawning conditions for the Eastern Baltic cod prevail from mid-May to mid-August (Hüssy *et al.*, 2015), which implies that the observed shift to summer spawning in the early 1990s has enhanced spawning success of the Eastern Baltic cod in this area.

Predation by clupeids

Predation on early life stages is a major factor controlling recruitment success of marine fish stocks (e.g. Bailey and Houde, 1989; Heath, 1992). Field studies to quantify the predation on cod eggs by planktivorous fish have shown that the predatory impact by herring is quite limited in the North Sea (Daan *et al.*, 1985) and only slightly higher in the Northeast Arctic (Melle, 1985). In the central Baltic, predation on cod eggs by sprat and herring is intense, due to the vertical overlap between cod eggs and clupeids during their main daily feeding period (Köster and Möllmann, 2000). In contrast, predation on cod eggs by other predators and on cod larvae by clupeids is limited (Köster and Schnack, 1994; Köster and Möllmann, 1997). Comparison of diet compositions of sprat and herring in the Bornholm Basin during cod spawning seasons 2004–2008 with data from the 1990s suggest a reduced predation pressure on cod eggs in the recent period (Neumann *et al.*,
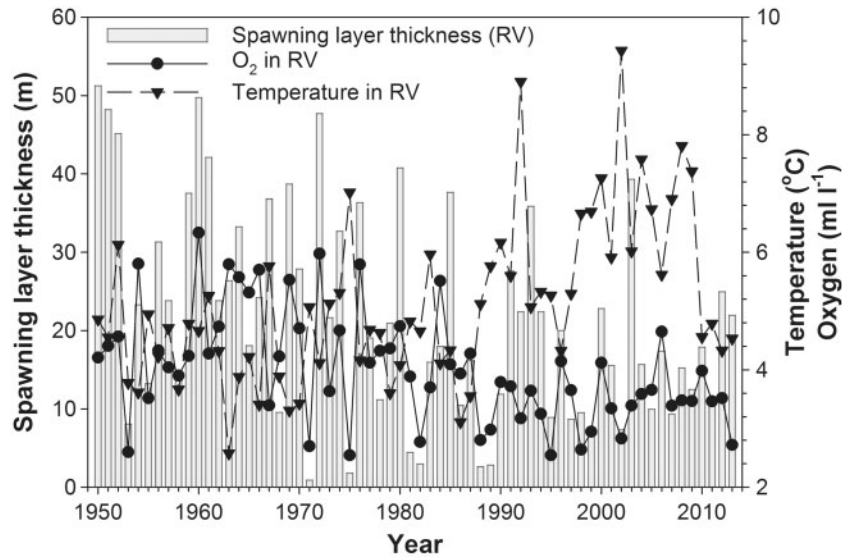
Figure 7. Thickness of water layer sustaining cod egg development in the Bornholm Basin 1950–2013 and average oxygen concentration and temperature in this layer during main spawning time.
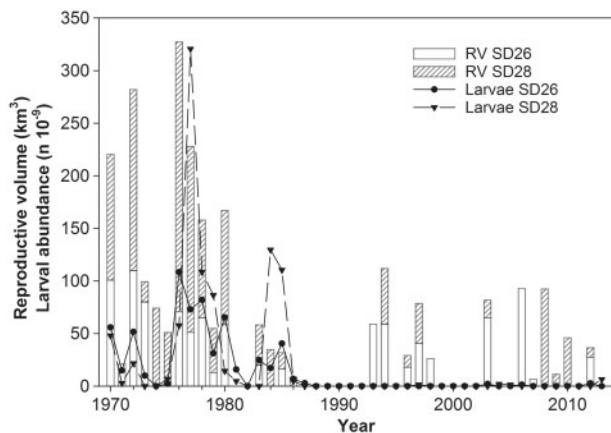

Figure 8. Reproductive volume (RV) sustaining cod egg development and larval abundance in SD 26 and 28 during main spawning times 1966–2013.

2014). This was further quantified in a more recent study comparing daily cod egg production and daily consumption rates by sprat and herring in the 1990s and 2000s indicating that the predation pressure in May/June was lower in 2005–2008 than in the early and late 1990s, and approximately on similar level as 1994/1995 (Figure 10). Similarly, predation pressure in July/August 2004–2006 and 2008 was in most cases lower than in the 1990s (Figure 10). The lower predation pressure is caused by a combination of reduced predator abundance (Eero *et al.*, 2012b) and lower daily rations of individual predators, mainly due to reduced spatial overlap between predator and prey as well as availability of alternative prey (Neumann *et al.*, 2014). While intra-, inter-annual and longer-term variability in predation on cod eggs by clupeids in the Baltic is obvious, consumption is at times high compared to egg production (Figure 10) and other sources of mortality (Voss *et al.*, 2011). Spatial overlap has been identified as a major factor impacting on predation of early life stages by pelagic fish also in other sea areas

(e.g. Garrison *et al.*, 2000; Hallfredsson and Pedersen, 2009). Especially in strongly stratified estuarine systems predation pressure may be high, as early life stages dwell in intermediate water levels utilized by pelagic fish for foraging, with examples being the Baltic, the Black Sea (Prodanov *et al.*, 1997) and the Gulf or St. Lawrence (Swain and Sinclair, 2000).

Larval growth and prey availability

Huwer *et al.* (2011) demonstrated that growth of Eastern Baltic cod larvae is poor compared to Norwegian cod larvae, and also below the level of poorly performing cod larvae on Georges Bank. This indicates severe food limitation for Baltic cod larvae confirming earlier modelling results (e.g. Hinrichsen *et al.*, 2002). The major prey of first-feeding cod larvae in the Baltic are calanoid copepod nauplii (Voss *et al.*, 2003), especially *Pseudocalanus acuspes* (Hinrichsen *et al.*, 2002). A higher survival into and through the larval stage in 2007 compared to 1994/1995 has been documented, which appears to be related to higher larval growth rates in the latter period (Huwer *et al.*, 2011). Back-calculated hatch positions of pelagic juvenile survivors in 2000 revealed highest larval survival at the slopes of the Bornholm Basin in summer (Huwer *et al.*, 2014), corresponding to modelled survival probability in a situation with low abundance of *P. acuspes* (Hinrichsen *et al.*, 2002). Thus, larval survival and growth in spring appears to be related to production of *P. acuspes* taking place mainly in the centre of the Bornholm Basin, while production of neritic copepods in slope regions of the basin (Hansen *et al.*, 2006) ensures reproductive success also at main spawning time. Prey availability for larvae in spring deteriorated since the 1980s due to reduced salinity, accelerated in the 1990s through increased grazing by sprat. Prey availability improved in the mid-2000s (ICES, 2010), with the biomass of *T. longicornis* and *Acartia* spp. showing positive anomalies and *P. acuspes* increasing to average values in the Bornholm Basin in spring 2004–2008 (Figure 11). Summer biomass values show a less positive development, except for *T. longicornis*, for which large positive anomalies where encountered in some years (Figure 11). In 2009 and 2010, average biomass values are apparent for all species in spring and slightly negative anomalies
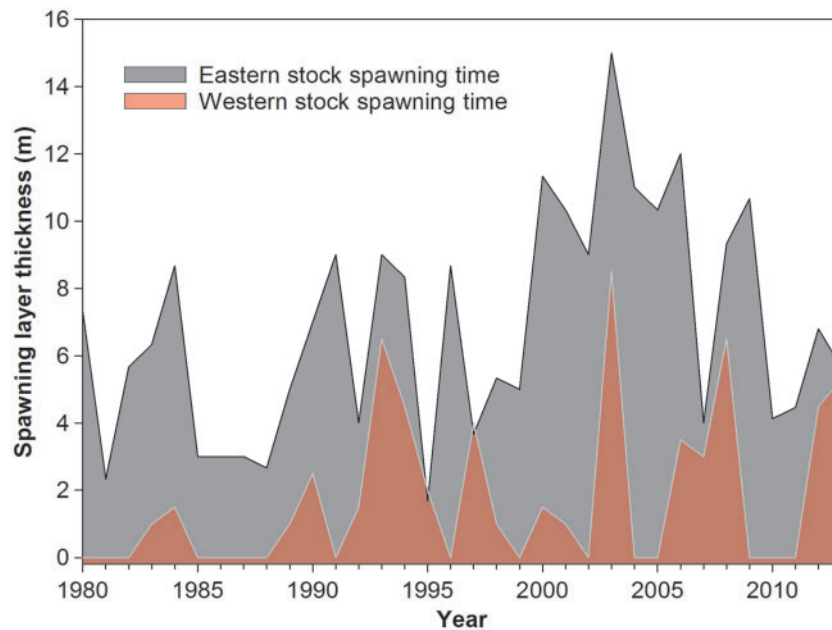
Figure 9. Thickness of water layer sustaining cod egg development in the Arkona Basin in February/March (Western Baltic stock spawning time) and June to August (main Eastern Baltic stock spawning time) 1980–2013.

in summer (http://www.st.nmfs.noaa.gov/copepod/time-series/pl-30102/html/zoom-wgzebox.html). In contrast, for most recent years (2011–2013), relatively low spring biomass was reported for all species in the central Baltic, partly associated to relatively cold winters (ICES, 2015b), while summer biomass was more on an average level, except for *Acartia* spp. exhibiting negative anomalies. Distinct temporal and spatial 'windows of survival' have been described as important for the recruitment of other species (e.g. Limburg, 2001; Lapolla and Buckley, 2005) and are as well obvious for the Eastern Baltic cod with the prolonged spawning season spreading risks (MacKenzie *et al.*, 1996).

Extension of juvenile nursery habitat

Growth and survival of juvenile Baltic cod in shallow waters have been suggested to be affected by density dependent processes such as competition for food (Hüssy *et al.*, 2003), cannibalism (Uzars and Plikshs, 2000) and the availability of suitable nursery areas (Hinrichsen *et al.* 2003). The latter study investigated the drift of larvae and pelagic juveniles from the spawning area of the Bornholm Basin to nursery areas by combining three-dimensional hydrodynamic model simulations and spatial distributions of juvenile cod from surveys covering the period from 1986 to 1999. Because of seasonal differences in circulation pattern, southern coastal nursery areas are most important for early and late spawned individuals, whereas larvae hatching in June to mid-July were transported to a greater extend towards the north or were retained in the Bornholm Basin (Hinrichsen *et al.*, 2003). This implies that shifts in spawning time change the importance of nursery areas. While in the 1980s and early 1990s northern nursery areas were of greater importance, transport to southern areas dominated in the remaining period. In most recent years above average egg production, egg survival and larval abundance were encountered in May/June and July/August indicating a spread of juveniles to both southern and northern nursery areas, which may have increased survival probability. The area

providing favourable settling conditions for juvenile cod, determined as size of the area with near-seabed oxygen saturation >40%, declined by more than 25% from the early 1950s to the 2000s, but with slightly improving conditions in 2006/2007 (Hinrichsen *et al.*, 2011). Transport to these suitable nursery areas and away from areas with high adult cod abundance is considered to be an important process governing juvenile survival. Growth of juvenile Baltic cod is known to differ between nursery areas with apparent size-selective control in certain areas (Hüssy et al., 2003). Since size-selective growth is known to affect survival (Tupper and Boutilier, 1995b; Gotceitas *et al.*, 1999), the impact of different drift patterns may influence recruitment through survival during the juvenile stage in several ways. Higher growth and survival are known to occur in structured habitats (e.g. eelgrass and cobble) (Gotceitas *et al.*, 1999; Cote et al., 2004; Lilley and Unsworth, 2014) with well-defined home ranges (Tupper and Boutilier, 1995a; Bradbury *et al.*, 2008). Exposure to predators affects habitat choice to avoid higher predation in less structured habitats at the cost of reduced growth (Gotceitas *et al.*, 1999; Linehan *et al.*, 2001), highlighting the importance of suitable habitat in the nursery areas.

Cannibalism

One of the major fish predators on cod is cod; cannibalism on juveniles is well documented in many Atlantic cod stocks (e.g. Bogstad *et al.* 1994; Palsson, 1994), including the Baltic (Sparholt, 1994; Neuenfeldt and Köster, 2000). However, the extent of cannibalism shows considerable differences between ecosystems and interannual and long-term variability (e.g. Palsson, 1994). The intensity of cannibalism is related not only to predator and prey abundance as well as the abundance of alternative prey, but also to the overlap in habitats (e.g. Anderson and Gregory, 2000; Steingrund and Gaard, 2005). Thus, in the Baltic, cannibalism is influenced by transport of larvae and early juveniles to nursery areas (see above) and hydrographic conditions impacting adult
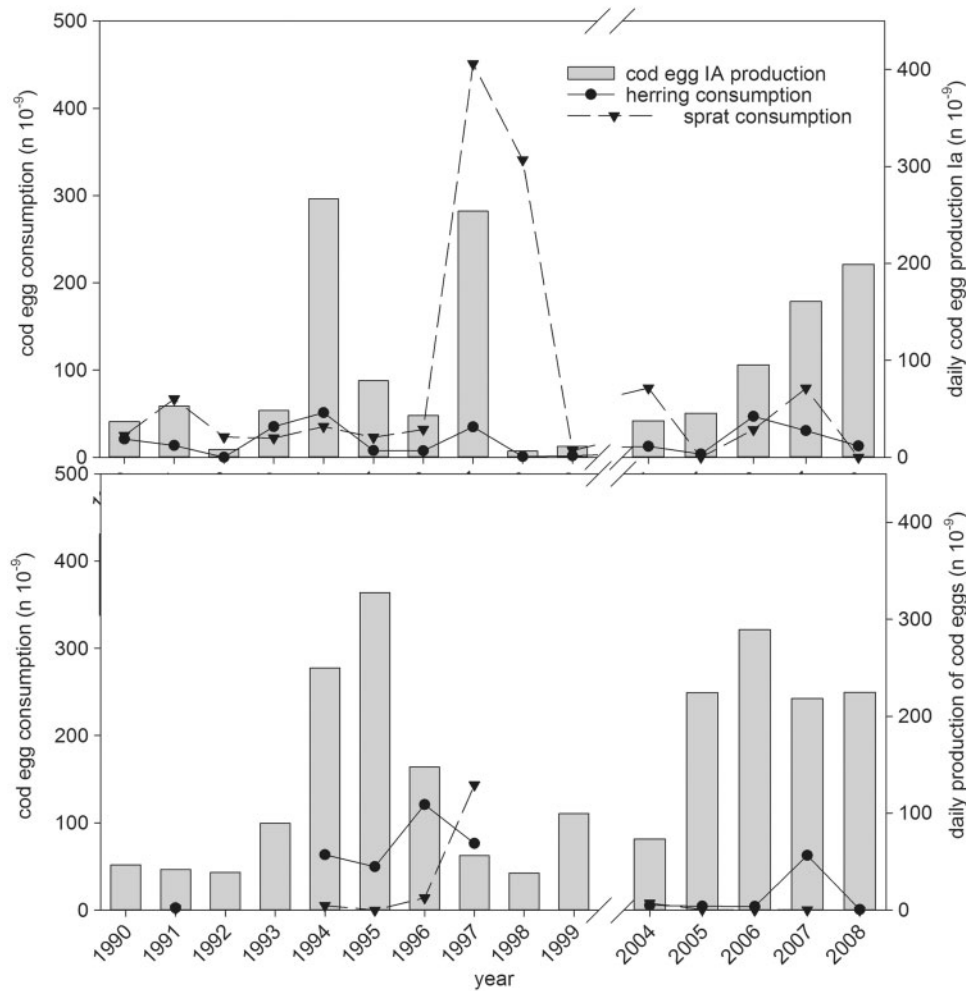
Figure 10. Daily cod egg consumption by herring and sprat populations in comparison to daily egg production (youngest egg stage: IA) in the Bornholm Basin in May/June and July/August 1990–1999 and 2004–2008.

and juvenile distribution (Uzars and Plikshs, 2000). For example, low oxygen concentrations in the deep Baltic basins may force the adults into shallower areas, and low temperature in shallow waters may force juveniles into deeper water thus increasing the spatial overlap between predator and prey (Köster *et al.*, 2005b). This hypothesis suggests that cannibalism is most pronounced in stagnation periods, especially after cold winters, and at high predator stock sizes. Part of these preconditions has been met in Subdivision 25 in the second half of the 2000s. Additionally, the abundance of alternative prey for adult cod has been limited (Eero *et al.*, 2015), resulting in high juvenile predation mortalities in Subdivision 25 compared to other areas of the eastern Baltic (Eero *et al.*, 2012b). Newly collected cod stomach content data confirm an increased frequency of occurrence of juveniles in adult cod diet in the late 2000s, specifically since 2008, while cannibalism was virtually absent in 1995–2003 (ICES, 2015b).

Recruitment dynamics and impacting factors

There is clear evidence that recruitment of Eastern Baltic cod has increased in the 2000s, despite major Baltic inflows being absent from 2004 to the end of 2014 (Mohrholz *et al.*, 2015). Egg production in the Bornholm Basin, the main spawning area of cod (Köster *et al.*, 2009) increased several fold until 2009, declining

since then, with increase and decline being more pronounced early in the spawning season (May/June) than at peak spawning time (July/August). Egg survival was mostly higher than in the 1990s, both early and at peak spawning time. Considering egg production and survival, larval abundances were lower than expected in 2004–2010, but higher than expected in 2011 and 2012, reaching levels last encountered in the 1980s. Available data for 2013–2014 indicate a dramatic decline in larval abundance in the Bornholm Basin throughout the spawning season. Catch rates of juveniles in the International Baltic trawl surveys confirm a number of relatively high (2000, 2003, 2011 and 2012) and intermediate year-classes (2005, 2006 and 2008), however without a clear temporal trend, followed by low recruitment originated in 2013 and 2014.

The increased reproductive success from 2000 to 2012 can be explained by a series of processes impacting egg survival: (i) the stock utilizes successfully the Arkona Basin as spawning area (Bleil *et al.*, 2009; Hüssy, 2011), where improved hydrographic conditions allowed increased egg survival, (ii) since 2000, minor inflows in winter/spring (Lehmann *et al.*, 2014) and baroclinic inflows in summer (Elken *et al.*, 2015) regularly enhanced egg survival in the Bornholm Basin both in spring and summer, (iii) increased recruitment into the spawning stock resulted in increased egg
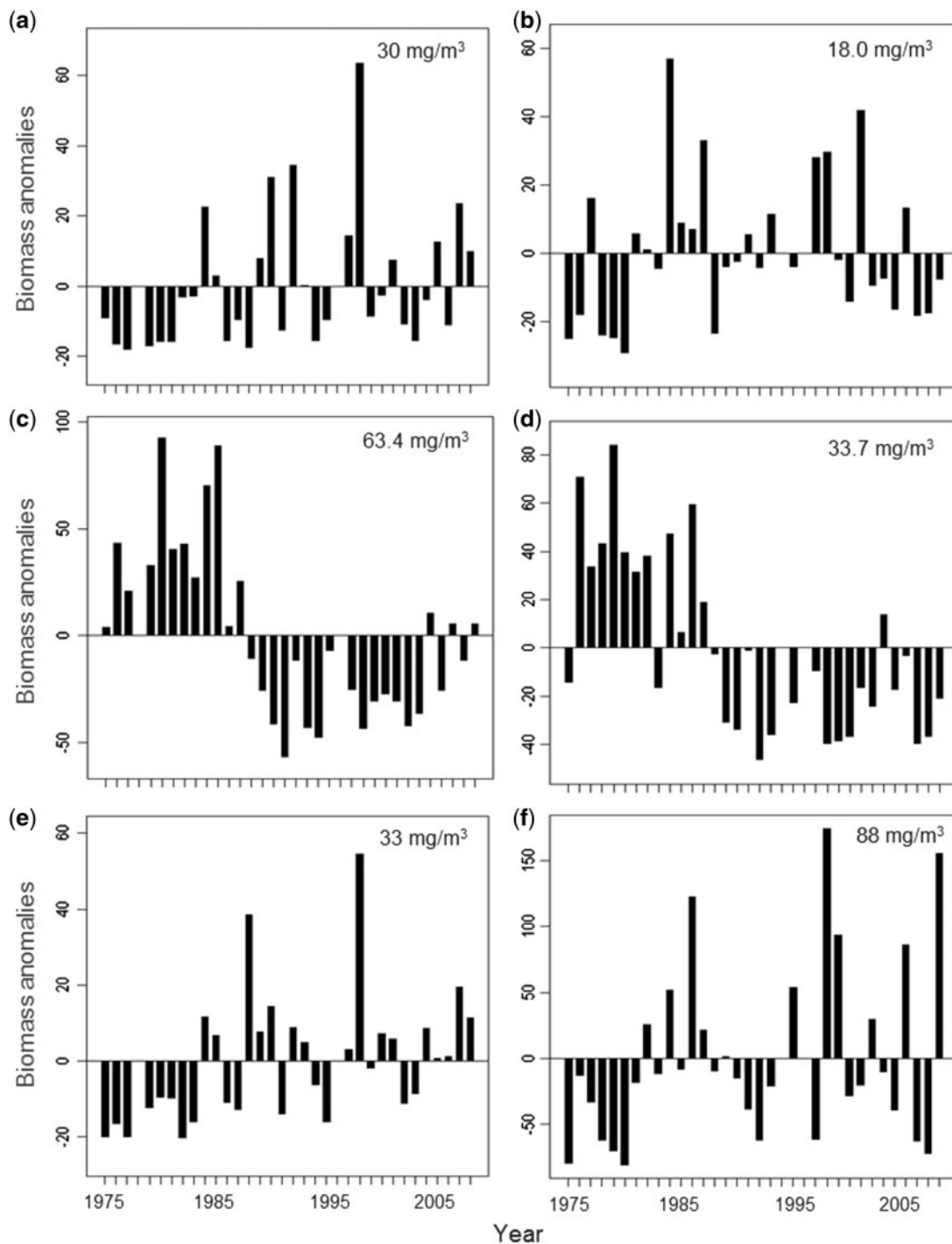
Figure 11. Biomass anomalies of *Acartia* spp. in spring (a) and summer (b), *Pseudocalanus acuspes* in spring (c) and summer (d) and *Temora longicornis* in spring (e) and summer (f) 1975–2008 in SD 25 including corresponding average biomass values.

production and (iv) egg predation by sprat and herring was consistently lower in 2000s than in the 1990s, caused by a combination of reduced predator abundance and lower daily rations by individual predators (Neumann *et al.*, 2014). Apart from the recognition that major Baltic inflows are not a prerequisite for high egg survival as suggested earlier (e.g. Bagge *et al.*, 1994; Köster *et al.*, 2005a), but that minor winter and summer inflows are sufficient to sustain successful egg development in the Bornholm

Basin, the present review also indicates that the traditional perception of hydrographic conditions for egg survival being more favourable in May/June than in July/August (MacKenzie *et al.*, 1996) does not apply for the last decade. Since 2004, the difference in hydrographic conditions in spring and summer is marginal and in some years even reversed. A lower egg predation by clupeids in summer compared to spring (Köster and Möllmann, 2000) has been confirmed, allowing in combination with the described

hydrographic conditions high egg survival rates during the main spawning period in summer. Another new issue is that the vertical overlap between cod eggs and their clupeid predators during their daily feeding period is not only directly related to major Baltic inflows (Köster *et al.*, 2005a) but may also be reduced in stagnation periods as a consequence of changed diurnal vertical migration behaviour of clupeids (Neumann *et al.*, 2014). In addition, a shift in the horizontal distribution of sprat to the east and north of the Baltic (Eero *et al.*, 2012b) has released the cod egg production from predation pressure.

The eastern spawning sites Gdansk Deep and Gotland Basin do only have a minor contribution to reproductive success (Plikshs *et al.*, 2015), but the stock extended its spawning area westwards into the Arkona Basin with spawning activity in contrast to earlier being partly successful, especially during peak spawning time in summer (Hüssy *et al.*, 2015). While the importance of these processes is reasonably understood, quantification of their impact on survival is largely confined to the egg stage and the main spawning areas of the Bornholm Basin. Regular ichthyoplankton surveys in the Arkona Basin are not available, as the importance of the basin as spawning area has been realized lately (Bleil *et al.*, 2009; Hüssy, 2011). A quantification of the contribution to successful egg production and larval survival requires an extension of the ichthyoplankton surveys conducted in the central Baltic (ICES, 2014b) in combination with experimental studies to determine the loss of egg production due to insufficient egg buoyancy (Petereit *et al.*, 2014).

Larval production occurs throughout an extended spawning season (April to November), creating windows of enhanced survival characterized by adequate prey availability (Huwer *et al.*, 2014) and transport to suitable nursery areas (Hinrichsen *et al.*, 2011). Prey availability for larvae in spring increased (at least until 2010) with larval hatch positions on the slopes of the Bornholm Basin sustaining sufficient survival in summer even in the absence of *P. acuspes*, resulting in improved larval growth performance compared to the mid-1990s (shown for 2007 and being under investigation for 2011). Timing of spawning in relation to the availability of suitable prey, especially for first-feeding larvae is important for larval growth and survival, with the phenology of relevant prey species showing relatively large inter-annual variability, as reported for *T. longicornis* in spring time (Dutz et al., 2010), and deserve closer investigation. Traditionally, prey availability for first-feeding larvae in spring has as well been linked to major Baltic inflows (Hinrichsen *et al.*, 2002), with the present review indicating that prey availability may also improve under stagnation conditions. However, information on larval prey availability is rather incomplete in the Bornholm Basin, as monitoring under HELCOM lacks the necessary spatial resolution (http://maps.helcom.fi/website/mapservice/index.html?config=configZooplankton.xml), data from large-scale international programs (e.g. STORE, 2003, BIO-C3, https://www.bio-c3.eu/), which may have the necessary spatial and temporal resolution, do cover only few years and national data are often not available in international databases. In our study, the lack of meso-zooplankton data from the Bornholm Basin in 2009 to 2014 renders interpretation of processes leading to high larval survival in 2011 and 2012 and low survival in 2013 and 2014 difficult. Zooplankton sampling is regularly conducted during ichthyoplankton surveys in the Bornholm Basin and effort should be allocated to enable analyses of the samples and inclusion in international zooplankton databases.

High reproductive success despite continued hypoxic/anoxic conditions may have impacted negatively on adult cod stock dynamics and subsequently recruitment through density-dependent effects (Eero *et al.*, 2015; ICES, 2015a, b). Anoxic conditions in the deep Baltic basins reduces benthic food availability and in combination with declining abundances of sprat in the southern central Baltic has likely negatively affected growth and condition of adult cod and increased also mortality (Eero *et al.*, 2015; ICES, 2015b). Reduced parental condition may also be caused by parasitic infection (Mehrdana *et al.*, 2014) and in combination with limited availability of specific essential fatty acids in the available diet (Røjbek *et al.*, 2012, 2014b) may have impacted negatively on individual egg production, fertilization and hatching success as well as post-hatch survival (Røjbek *et al.*, 2014a). Spawning at smaller sizes implies an increased production of on average smaller eggs by smaller females, which will have a negative impact on overall egg survival (Hinrichsen *et al.*, 2016) as well. These factors potentially explain the declining recruitment in the most recent years 2013 and 2014. However, the full consequences of declining growth, reduced condition and spawning at smaller sizes and their interactions on individual egg production as well as viability and fate of the offspring are not clear and need further investigation. Combined field and experimental studies are needed with deployment of modern molecular and histological techniques, inclusive subsequent raising of results from individual to population level, taking into account the size/sex and spatial structure in the stock.

Suitable nursery habitats for demersal juveniles have declined during the last decades due to persistent stagnation (Hinrichsen *et al.*, 2009, 2011). This trend likely increased juvenile mortality, counteracted only partly by a prolonged spawning season, resulting in wide-spread distribution of juveniles in the Central Baltic. In general, factors impacting juvenile survival and the role of suitable habitats for Eastern Baltic cod juvenile survival are not well understood and require studies contrasting different habitats in coastal and deeper water nursery areas. As an example, there are indications that cannibalism has increased (ICES, 2015b), however, whether or not also juvenile mortality has increased cannot be judged based on stomach data alone, as it may in fact only reflect increased abundance of small cod suitable as prey for larger cod (Neuenfeldt and Köster, 2000), caused by increased recruitment and/or reduced growth. A dedicated quantitative analysis is required to estimate cannibalism rates, considering predator and prey abundance and other sources of juvenile mortality, such as predation by the increased seal populations (Härkönen *et al.*, 2013) as well. Re-establishing an accepted analytical stock assessment (Eero *et al.*, 2015) is a pre-requisite to quantify recruitment and enable quantitative analyses of factors impacting on reproductive success, resolve the relative importance of various impacting factors and their interactions and testing earlier published recruitment models (Köster *et al.*, 2005a, 2009; Heikinheimo, 2008; Margonski *et al.*, 2010).

Acknowledgements

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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APPENDIX 5

Transgenerational effects of parental acclimation to end-of-century ocean acidification on survival in cod larvae (*Gadus morhua*)

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Abstract

Ocean acidification has been shown to have negative effects on early life stages of many fish species. However, recent research suggests that exposure of the parental generation to the same ocean acidification treatment, through the process of transgenerational acclimatization, can potentially reduce these negative effects. For the first time in a commercially exploited fish species, a transgenerational experiment was conducted to address the effects of parental acclimation to ocean acidification (OA) on the mortality of larval cod. In that species, earlier experiments have shown that end-of-century levels of OA double daily mortality rates in several cod populations and lead to severe tissue damage. Adult cod from an aquaculture stock were acclimatized to ambient (400 µatm) and predicted end-of-century pCO$_2$ conditions (1100 µatm according to the IPCC RCP 8.5) for six weeks prior to spawning. Eggs and larvae were exposed to the parental or opposed treatment and were kept under two different feeding regimes, high and low food. We found lower larval mortality in response to parental CO$_2$ pre-treatment only along with sufficient food. In contrast, under food limitation, pre-exposure of parents to high CO$_2$ exacerbated larval mortality under high pCO2, whereas larvae from ambiently exposed parents showed intermediate mortality levels. This is the first demonstration that transgenerational effects partially mediate the adverse effects of OA but only when the larvae are reared under ample resource supply. We hypothesize that excess metabolic resources are needed to build up transgenerational buffering effects in response to ocean acidification.

APPENDIX 6

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Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population

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Abstract

How fisheries will be impacted by climate change is far from understood. While some fish populations may be able to escape global warming via range shifts, they cannot escape ocean acidification (OA), an inevitable consequence of the dissolution of anthropogenic carbon dioxide (CO_2) emissions in marine waters. How ocean acidification affects population dynamics of commercially important fish species is critical for adapting management practices of exploited fish populations. Ocean acidification has been shown to impair fish larvae's sensory abilities, affect the morphology of otoliths, cause tissue damage and cause behavioural changes. Here, we obtain first experimental mortality estimates for Atlantic cod larvae under OA and incorporate these effects into recruitment models. End-of-century levels of ocean acidification (~1100 µatm according to the IPCC RCP 8.5) resulted in a doubling of daily mortality rates compared to present-day CO_2 concentrations during the first 25 days post hatching (dph), a critical phase for population recruitment. These results were consistent under different feeding regimes, stocking densities and in two cod populations (Western Baltic and Barents Sea stock). When mortality data were included into Ricker-type stock-recruitment models, recruitment was reduced to an average of 8 and 24% of current recruitment for the two populations, respectively. Our results highlight the importance of including vulnerable early life stages when addressing effects of climate change on fish stocks.

Introduction

The understanding of the effect of global change on fish populations is critical for sustainable exploitation and management of fisheries [1]. Ocean warming has already triggered poleward

range shifts of many marine fish populations caused by their thermal tolerance [2–4]. However, higher latitudes provide no refuge with respect to the concomitant pH decline, caused by the dissolution of the major greenhouse gas CO_2 in ocean waters. This "other CO_2 problem", also dubbed ocean acidification (OA) [5], is an inevitable consequence of anthropogenic release of CO_2. The potential consequences of ocean acidification on commercially important fish populations are intensely debated [6,7], but currently unresolved since data on population-level processes, e.g. recruitment to the stock, are almost entirely lacking [8–10].

Adult fishes have been shown to tolerate extreme CO_2 concentrations of up to 16,000 µatm [11], which led to the premature conclusion that fishes are less vulnerable to ocean acidification than for example calcifying organisms [12]. However, it is becoming increasingly evident that early life stages such as eggs and larvae are more susceptible to decreased ocean pH [7,13]. This is partly due to insufficient acid-base regulation prior to the formation of gills [14]. Recent studies have shown a diverse range of impacts of predicted future CO_2 concentrations on larval fish, particularly on sensory abilities like olfaction [15], behaviour [16,17], otoliths [18–20], development, tissue and organ structure [13,21]. Studies also found effects on survival of eggs, more specifically hatching success [22], and survival of very early larval stages [7,23]. Other studies were not able to find an effect on survival [24,25].

Survival, however, is the most important parameter to assess recruitment, thus of paramount importance for stock management. Recruitment to an exploited fish stock is defined as that point of time when a year-class enters the fished population, i.e. at an age of 1 year in the case of Western Baltic cod, and at an age of 3 years in Barents Sea cod. Here we assess larval mortality as a key variable to predict population growth and size [26,27] in Atlantic cod (*Gadus morhua*, L.) under end-of-century CO_2 concentrations. This is one of the most important species for commercial fisheries of the North Atlantic, It is of particular importance since landings of many cod stocks have decreased in the past decades with some stocks collapsing [28]. Any additional source of mortality, particularly one with a trend, should therefore be closely monitored and incorporated into management strategies.

We designed two experiments, in which the survival of cod larvae was quantified in direct response to increased $p$$CO_2$ levels as predicted for the end of the century. Atmospheric CO_2 concentrations have been continuously rising since the beginning of industrialisation and are currently exceeding 400 µatm. A third of the excess CO_2 is absorbed by the world's oceans, resulting in ocean acidification, leading to an estimated decrease in pH of 0.4 units ($p$$CO_2$ ~ 1,000 µatm) by the end of the century [5,29,30]. Eggs and larvae from the Western Baltic cod stock, caught in the Øresund, and from the Arcto-Norwegian Barents Sea cod stock were kept under control (~400–500 µatm) and high CO_2 (~1100 µatm) concentrations in two separate experiments until 25 and 22 days post-hatching (dph) respectively and survival was monitored closely.

Methods and Materials

For the Western Baltic experiment, adult cod were caught in the Øresund (55°58'N, 12°38'E) in March 2013 and strip-spawned. An equal volume of eggs was placed in 90 L rearing tanks at the Sven Lovén Centre, Kristineberg, Sweden. Three tanks were kept under ambient CO_2 concentrations of 426 ± 47 µatm and three tanks were kept under increased CO_2 conditions of 1033 ± 255 µatm. The temperature was kept constant at 7°C and the light regime was matched weekly to the ambient sun rise and sun set. After hatching the larvae were fed with natural plankton from the Gullmars Fjord under green water conditions with *Nannochloropsis*. (Food density estimates are given in Table A in S1 File). Survival was measured daily by collecting and counting all dead larvae from the bottom of the tanks. Initial number of larvae (on average

~800 larvae per tank) was then back-calculated to calculate survival in percentage. It was shown in separate experiments that dead larvae were easily found even after more than 24 hours post mortem in the tanks.

For the Barents Sea cod experiment adult fish were caught alive in the Barents Sea (70°15'N, 19°00'E) in March 2014 and transferred to the National Cod Breeding Centre, Tromsø. They were kept in large breeding tanks (25 m3) with flow-through from the fjord and at weekly matched ambient light regimes. All naturally produced eggs were collected using collectors behind the surface skimmer outflow. These were transferred to incubators with either ambient (503 ± 89 µatm CO_2) or increased CO_2 (1179 ± 87 µatm) concentrations. After peak hatch (more than 50% eggs hatched), 11,000 larvae were transferred into each of twelve 190 L rearing tanks with a constant flow-through of water from a common header tank. For the egg incubation and the start of the experiment the temperature was set to 6°C and was later raised to 10°C in all tanks at constant light conditions (24h). Larvae were fed with *Nannochloropsis* and *Brachionus* at different intervals for the high and the low food treatment (seven compared to three times daily), while the prey concentrations per feeding remained the same for both treatments. (For information on the feeding conditions, see Table B in S1 File). It should be noted, that even though the low food treatment only provided a fraction of the total amount of prey of the high food treatment, it is likely still higher than prey densities, which the larvae would experience in the field. However, this is difficult to compare, since we provided very high densities for short periods at the feeding times, which were then washed out of the tanks again. Therefore no steady density of prey was provided, but during feeding times prey densities were extremely high. This allowed for the exclusion of density and competition effects, which may have otherwise arisen due to different larval densities in the different treatments. Larvae in one tank in the ambient CO_2 treatment were abruptly lost over night, due to an unknown factor, resulting in six replicates for the high CO_2 treatment and five for the ambient treatment, each divided equally into the high and low food treatment. Starting on 8 dph survival was measured every four to six days by calculating the density of the larvae in the tanks. Five times 0.8 l of water was sampled from each tank over the whole water column using a pipe that could be closed at the bottom and the larvae contained in the pipe were subsequently counted in each sub sample. Prior to sampling an even distribution of larvae in the rearing tanks was achieved by increasing the aeration.

For both experiments the mean mortality coefficient was calculated after non-linear curve fitting of a negative exponential function for each replicate tank. Mean daily mortality rates (in percentage per day) were compared between treatments using a t-test (Western Baltic stock) and a two-way ANOVA (Barents Sea stock) after appropriate data transformation to achieve homogeneity of variances.

Ambient and increased CO_2 levels were achieved by controlling the pH values in a header tank with pH sensors connected to an IKS computer system. If the values deviated from the set target pH a magnetic valve opened automatically, which allowed a pulse of CO_2 from a CO_2 bottle to be injected into the header tank. The volume of the header tank ensured a thorough mixing and equilibration of CO_2 before the water entered the rearing tank thereby assuring constant conditions in the rearing tanks. The pH was furthermore manually checked every day in the rearing tanks with a separate pH sensor (WTW pH/Cond 340i/3320). Water chemistry, including DIC and alkalinity, was tested at the beginning and the end of the experiment for the Western Baltic cod experiment and weekly for the Barents Sea cod experiment based on the Best Practices Guide [31]. Further details regarding methods and carbon chemistry analysis are available in the Supporting Information.

All experiments were carried out in accordance to the national rules and regulations at the site of the experiments and all efforts where undertaken to minimize stress and suffering of the animals. Issues for work on vertebrate animals were obtained for each experiment and location.

For the experiment in Kristineberg with the Western Baltic cod the ethics permit number is 332–2012 issued by the Swedish Board of Agriculture (Jordbruksverket). For the experiment in Tromsø on the Barents Sea cod the ethics permit number is FOTS ID 6382, issued by the Norwegian Animal Research Authority (Forsøksdyrutvalget). In accordance with these permits animals were euthanized after the experiment or whenever some were taken out for density measurements using Tricaine methanesulfonate (MS222). No endangered or protected species were used in these experiments and no other special permits were necessary.

Population level effects

Considering the potential impact of ocean acidification on fisheries requires scaling from physiological responses to population-level processes. A simple way is to consider how ocean acidification could modify the parameters of growth, mortality and reproduction in a single-species. Here we concentrate on the modification of the parameters of the stock-recruitment relationship in an age-structured fishery model.

The effect of ocean acidification was assessed by modifying the density-independent parameter α of a Ricker type stock recruitment relationship. Ocean acidification causes a higher larval mortality rate. This leads to a density-independent mortality rate a caused by acidification. In the baseline scenario (no acidification) $a = 0$, while in the acidification scenarios, e^{-a} is the fraction of larvae surviving the effect of acidification. We used our experimental data to quantify this effect, and to compare scenarios (See Supporting Information). We used ICES data for Western Baltic cod for the years 1970 to 2014 and for Arcto-Norwegian cod for the years 1946–2014 to estimate the stock-recruitment relationship for the baseline scenario. We assume log-normal auto-correlated errors, and estimated the model. (Further details regarding the recruitment models are available as Supporting Information.) Because the severity of ocean acidification induced mortality on recruitment depends on the duration of the additional mortality, two developmental stages were chosen as termination for the enhanced mortality [20]. Based on the experimental temperatures at day 23 days post hatching the larval gut has reached its typical spiral form (and potentially altered function) while at 30 dph gills become visible on the gill arches. These two time points were used to evaluate the effect of increased mortality on recruitment success assuming the same mortality estimates until 30 dph as shown in the experiments until 22 dph and 25 dph. Mortality during the recruitment process consists of both density-independent and density-dependent effects. For simplicity we assume that the effect of ocean acidification on the survival will only influence the density-independent mortality during the recruitment phase potentially biasing the data to be on the conservative side.

Results

The effect of CO_2 was consistent among stocks and experimental conditions, i.e. different feeding conditions. At increased CO_2 concentrations the daily mortality rates had approximately doubled in both experiments, from 7 to 13% in the Barents Sea stock (Fig 1a) and from 9.2 to 20.4% in the Western Baltic Sea stock (Fig 1b) (Western Baltic experiment, T-test, t = -3.749, df = 2.41, $p = 0.024$; Barents Sea experiment Two-way ANOVA F = 8.434, df = 1, $p = 0.023$). In the Barents Sea experiment the food density had no detectable effect on mortality rate, neither as main effect nor in interaction with the CO_2-treatment (for additional statistics, see Tables C and D in S1 File). Cod larvae therefore appear to be negatively affected by ocean acidification even when *ad libitum* prey densities should ensure that energy is available for potential acid-base regulation mechanisms.

Next, the experimentally assessed larval mortality rates were incorporated into a Ricker-type stock-recruitment model that was parametrized for the two studied cod populations. We
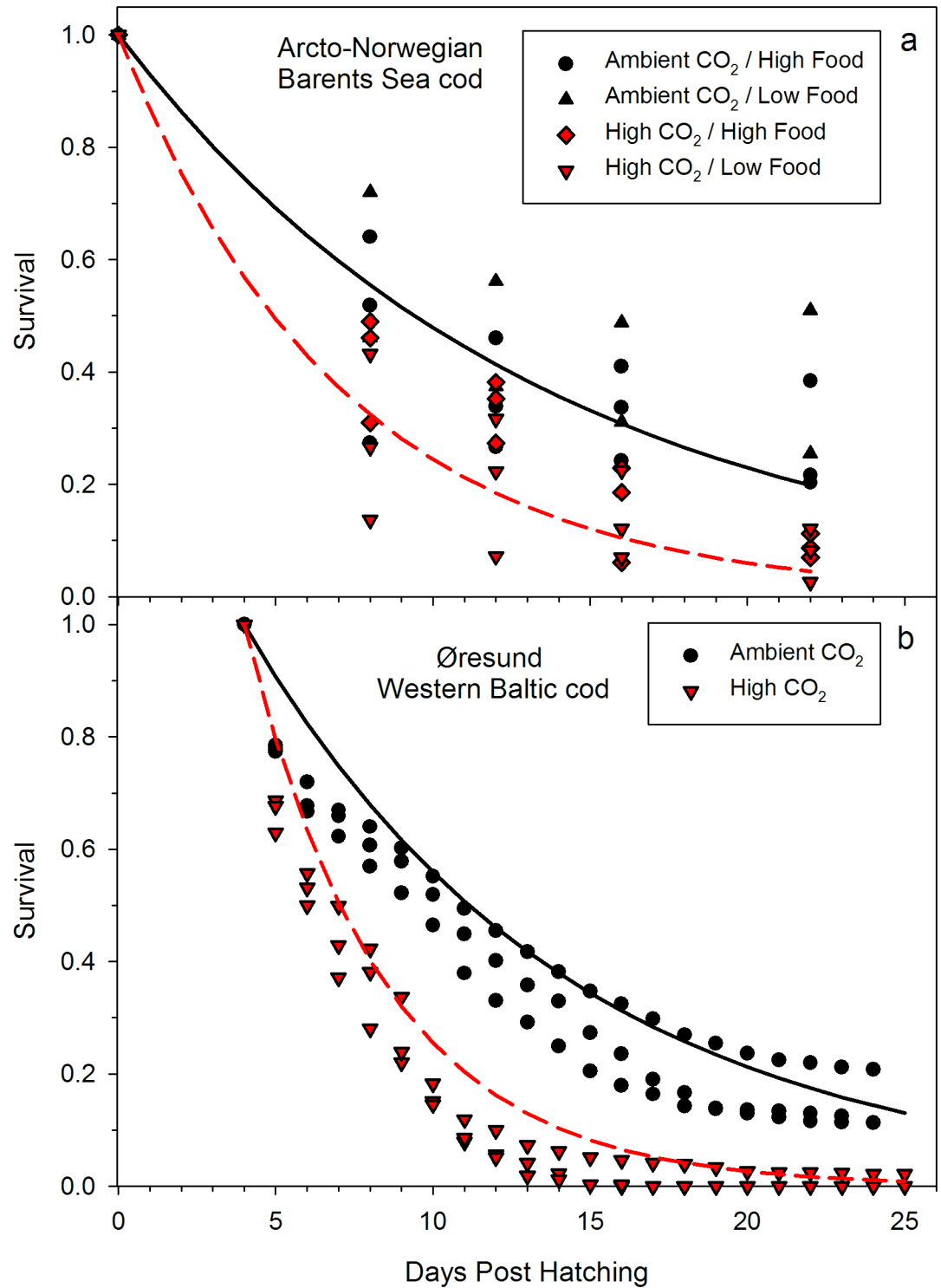
Fig 1. Effect of increased CO_2 on early life survival of *Gadus morhua* from (a) Barents Sea cod (b) Western Baltic cod. Each symbol represents the value of one replicate tank. Lines depict the number of survivors according to the fitted negative exponential function.

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concentrated on altering the larval mortality in order to evaluate the overall stock-recruitment relationship to assess their effects on population dynamics (for details see Supporting Information). The model results show that for both mortality scenarios increased larval mortality due to ocean acidification will reduce recruitment substantially. Recruitment levels will be reduced on average to only 8% of the baseline scenario in the case of Western Baltic cod for ocean acidification-induced mortality periods of 23 days (and 4% for a mortality period of 30 days), and to 24.5% (and 17% respectively) in Arcto-Norwegian cod (Figs 2 and 3).

Discussion

Under realistic scenarios of end-of-century ocean acidification, early larval survival of cod was significantly reduced in two separate experiments with two different Atlantic cod stocks. Results were consistent under different feeding regimes and strongly suggest that there is a severe effect of ocean acidification on Atlantic cod larvae and recruitment.

Mass spawning fishes such as cod have many offspring with low survival probability in nature. The salient question is whether our experimental conditions provide appropriate controls with reasonable natural mortality levels. Larval survival rates are naturally low even under ambient CO_2 concentrations and optimal feeding conditions. The mortality is mainly caused by the difficulty in a successful first feeding once the yolk sac is absorbed [27]. Other studies find similar mortality rates as our control values in the two experiments during early larval development [32,33]. Survival of larvae in our experiment from the Western Baltic stock was lower than for the Barents Sea stock, since they were fed with natural plankton in concentrations as provided by the fjord, while the larvae from the Barents Sea stock were kept under aquaculture conditions aiming for the production of the highest numbers of fingerlings for stocking of industrial scale production net pens.

Larval fish survival under ocean acidification has so far been shown in only one other study by Baumann *et al.* (2012) [7], albeit in a non-commercial fish species, the Atlantic silverside (*Menidia menidia*). In their study reduced larval survival was observed at 1100 *ppm*, a level of ocean acidification, which is predicted to occur globally at the start of the next century under the IPCC RCP 8.5, during the first week post hatch. Chambers *et al.* (2013) [22] found a decreased hatching success (reflecting embryonic development) of the summer flounder by 50% under 1860 *ppm*. This is a realistic ocean acidification level for the environment of this species within this century, even though values on a global average are predicted to be lower. Munday *et al.* (2015) [25] found no effect on the survival of yellowtail kingfish larvae. Other studies, like Munday *et al.* (2009b) [24]; Franke & Clemmesen (2011) [34]; Frommel *et al.* (2013) [35]; Hurst *et al.* (2013, 2015) [36,37], have addressed hatching success and have not seen any effects of ocean acidification. We are confident that this does not necessarily indicate that these species will not be affected or that our results present a contradiction. It is well known that early life stages of marine fish go through several bottlenecks with high mortalities during development and that different populations of the same species can react differently to CO_2 stress [35]. Our results show that the first days and weeks after hatching are a vulnerable phase to ocean acidification. So far studies on tropical fish have not seen an ocean acidification effect on survival [38]. This is not surprising, since early development in the studied species is very different from temperate fish and newly hatched larvae are further developed and physiologically more competent thus less vulnerable to physiological stressors. Furthermore the study by Munday *et al.* (2011), and other studies like Hurst *et al.* (2013), only quantified survival at a single day, which may not have been the final day of any additional mortality. Additionally, even if this was an end-point measurement, it does not allow for calculations of mortality rates.
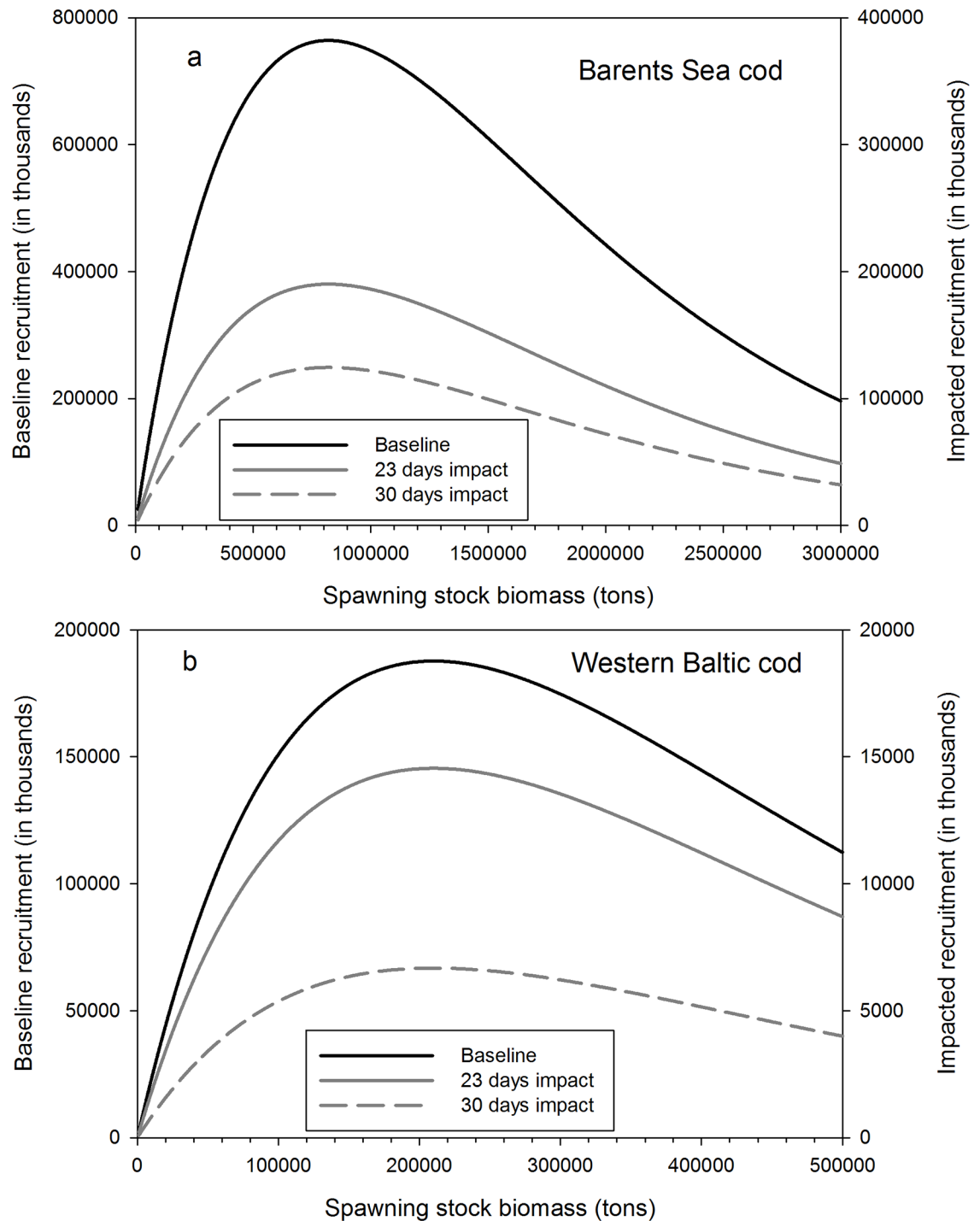
Fig 2. Recruitment functions under baseline and under ocean acidification scenarios for (a) the Barents Sea cod and (b) the Baltic Sea cod. The baseline scenario is based on no OA and spawning stock biomass at ICES precautionary biomass levels (B_{PA}) in dependence of the duration of OA-induced mortality. For better visualization a different scaling on the second y-axes was chosen for the impacted recruitment.
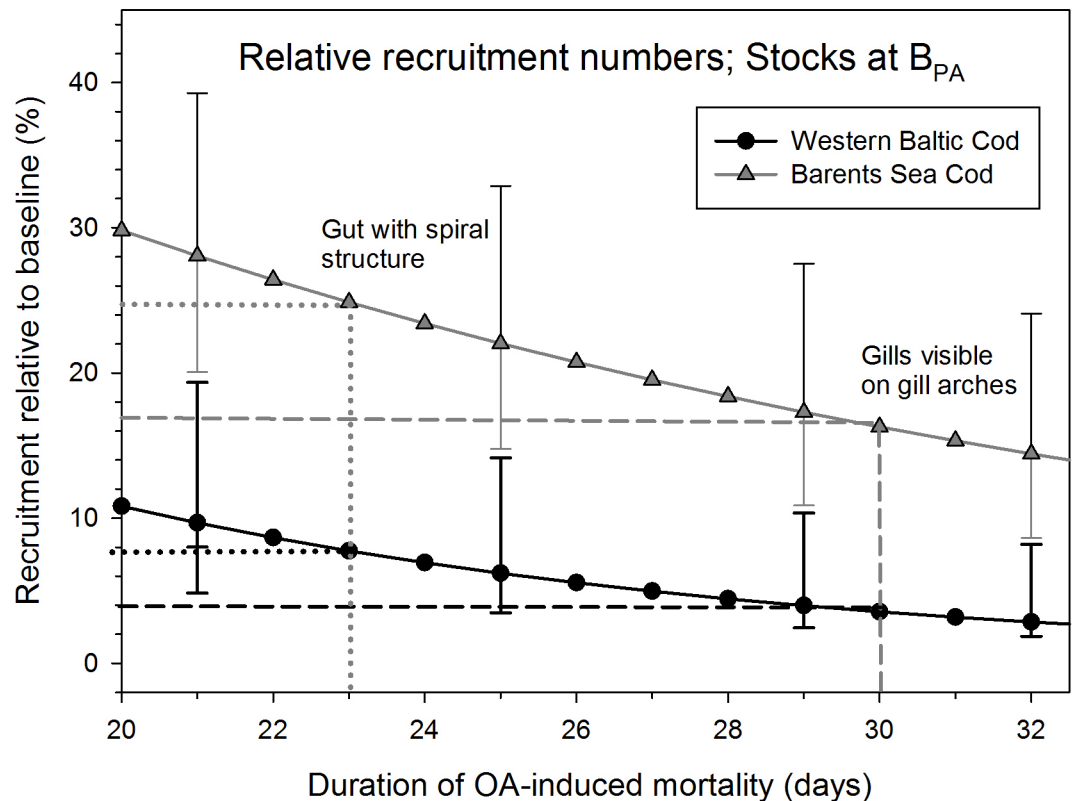
doi:10.1371/journal.pone.0155448.g002

Fig 3. Population recruitment under ocean acidification (OA) for Western Baltic cod (black line and symbols) and Barents Sea cod (grey line and symbols). Recruitment is given relative to a baseline scenario of no OA and spawning stock biomass at ICES precautionary biomass levels (B_{PA}) in dependence of the duration of OA-induced mortality. Two important points in larval development are highlighted. Standard deviations displayed only for selected days to improve readability.

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One factor that this study is not taking into account is possibility that parental exposure to the high CO_2 environment could limit the adverse effects of ocean acidification. This kind of transgenerational adaptation has been shown to mediate negative growth effects of OA in tropical reef fish [39]. However since most commercially important fish species are quite large and temperate fish species reach sexual maturity late, it will be difficult to perform experiments with long parental exposure time. Furthermore it cannot be ruled out, that ocean acidification might also have an additional negative effect on gonadal development in adult fishes, which might further reduce recruitment potential.

Range shifts are responses of many fish populations to track the poleward movement of their thermal range [2]. Unfortunately, this may exacerbate direct CO_2 effects identified here, since oceanic waters in higher latitudes will take up more CO_2 due to higher solubility and experience lower carbonate saturation [40]. Previously, ocean acidification has been shown to affect marine fish larvae's sensory abilities, morphology of the otoliths, cause tissue damage and behavioural differences [13,17,18,19,21].

Here we give the first demographic estimates for Atlantic cod under realistic end-of-century ocean acidification levels which are urgently needed to estimate whether these exploited fish populations could potentially expect population declines as a direct consequence of ocean acidification. The estimated recruitment declines shown are severe, of similar magnitude as population collapses due to overfishing [41] and have highly significant implications for the

governance of exploited fish populations. We show that indeed, increased mortality will affect recruitment at the population level, demonstrating that any future management of exploitation must directly consider effects induced by global change.

Supporting Information

S1 File. Supporting Information on experimental set-up, carbon chemistry, statistics and recruitment modelling.
(DOCX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: MHS CC MS. Performed the experiments: MHS FHM MS FJ CC VP AM. Analyzed the data: MHS TBHR. Contributed reagents/materials/analysis tools: MC RV. Wrote the paper: MHS TBHR FHM RV CC.

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