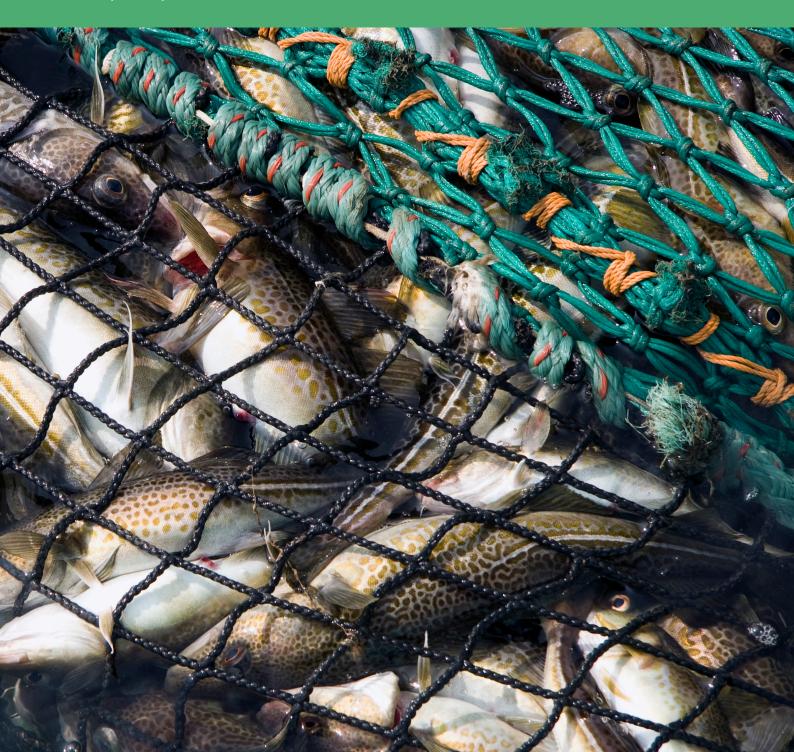# Eastern Baltic cod—New knowledge on growth and mortality

By Margit Eero (ed.)

DTU Aqua Report no. 341-2019

# Eastern Baltic cod—New knowledge on growth and mortality

By Margit Eero[1], Niels Gerner Andersen[1], Casper Willestofte Berg[1], Asbjørn Christensen[1], Jakob Hemmer Hansen[1], Kim Kær Hansen [3], Karin Hüssy[1], Kasper Kristensen[1], Anne-Mette Kroner [1,2], Lotte Kindt-Larsen[1], Henrik Lund[3], Karl Lundström[4], Lars O. Mortensen[1], Stefan Neuenfeldt[1], Morten Tange Olsen[2], Peter Ravn[5], and Jonna Tomkiewicz[1]

[1] Technical University of Denmark, DTU Aqua
[2] Copenhagen University
[3] Danmarks Fiskeriforening PO
[4] Swedish University of Agricultural Sciences, SLU Aqua
[5] Bioneer AS

DTU Aqua Report no. 341-2019

**HAV & FISK**

## Project resumé

The project "Eastern Baltic cod - new knowledge on growth and mortality is a way to improved management advice" focused on development and implementation of approaches and methods to describe growth and natural mortality of Eastern Baltic (EB) cod, to improve the knowledge on stock status and thereby the basis for fisheries management advice. The project was funded by the European Maritime and Fisheries Fund and the Danish Fisheries Agency.

The different activities and methods investigated in this project supplement one another and enable to improve the present stock assessment in short term, and also contribute to development of more longer term solutions to ensure solid scientific basis for fisheries management advice in future.

The ongoing tagging program is one of such activities directed to developing long term solutions to growth of EB cod. Good cooperation with fishing industry is essential for the success of the tagging program to provide the necessary information. Therefore, the present project has supported the tagging program by focusing on cooperation and communication with the fishing industry to establish best possible procedures for tagging and ensure return of the recaptured tagged cod.

Molecular-genetic analyses have been conducted that show low expression of growth hormones in EB cod. Such advanced technics have hitherto not been used in stock assessment context, and the pilot analyses conducted in this project therefore provide a basis for greater utilization of such innovative approaches and techniques in fisheries management context in future.

Analyses of the otolith daily rings, together with length distributions in the stock allowed quantifying the reduction in growth of smaller cod in later years. We also found a connection between growth and nutritional condition of fish that suggests that growth of EB cod likely has declined in line with reduced condition. Reduced growth of EB cod in later years is also explainable in a bioenergetics model that was developed to investigate the biological hypothesis related to condition, feeding and maturation. Reduced size at maturation, which was histologically confirmed is another factor that is expected to lower growth. Overall, all of the available investigations exploring cod growth suggest a reduced growth of EB cod in later years compared to the period in early 2000s or in the 1990s.

Several factors possibly contributing to an increased natural mortality were investigated as well. Seal predation was found to be important locally, though not explaining a high natural mortality of the entire EB cod stock. Analyses of stomach data show that cannibalism related mortality was high in years around 2010, but substantially declined in later years to a low level. Stomach data in combination with bioenergetic modelling suggest that mortality related to low feeding level and low nutritional condition occurs. Overall, natural mortality has likely increased in 2000s. Survey index describing stock development has also been improved.

Stock status in relation to proxy reference points has been estimated and applied in ICES advice using production model (SPICT), where specific version has been developed for EB cod to accommodate the change in production in later years. The progress with understanding growth and natural mortality of EB cod has allowed also for development of a quantitative assessment model (Stock Synthesis). Overall, the results of the project have contributed to increased understanding of the stock status, which has enabled establishment of the benchmark process for EB cod in ICES, with the benchmark assessment meeting scheduled for early 2019.

The work conducted in the project has links to Horizon 2020 PANDORA project that in combination with another EMFF project will address seal parasite infestation, which is another factor potentially causing reduced growth and mortality of EB cod.

# Contents

# Background and structure of the project

Biological advice based on stock assessment is a major input to fisheries management in the Baltic Sea. Therefore, it is important that stock assessments are based on solid scientific information and reflect the stock status as correctly as possible.

A number of changes in Eastern Baltic (EB) cod biology have been observed in later years, which include reduced nutritional condition of fish, maturation at a smaller size and increased parasite infestation. Also, relative abundance of larger individuals in the population has sharply declined since 2012 (Fig. 1.) This could be due to low growth and/or high mortality, which has not been possible to disentangle due to lack of reliable age information for cod to determine growth. Lack of reliable age readings and presumably non-constant natural mortality are the main reasons for lack of analytical stock assessment for EB cod, presently (Eero et al. 2015). Thus, biological understanding of processes potentially affecting changes in growth and natural mortality is required to elucidate the likely direction of change in these variables and possibly quantify the likely magnitude of change.



*Figure 1. Changes in i) mean Fulton's K condition factor for 40–60cm cod from BITS Q1 survey; the red bars showing the proportion of cod at condition below 0.8, ii) size at first maturation (L50) for females and males combined, from BITS Q1 survey; iii) parasite infestation (Eero et al. 2015); and iv) cpue of larger cod (>45cm in length) from BITS Q1 survey.*

The main focus of the present project is producing new information on these two parameters – growth and natural mortality, using alternative approaches and methods, independent of traditional age readings. The activities in the project include both exploring methods and approaches that could provide proxies for these two parameters to improve stock assessment in short term, and also contribute to methods that can provide validated information on these parameters in a longer term. Schematic illustration of the main activities in the project is provided in Fig. 2. The chapters below describe the work conducted and the main results from these different investigations.

*Figure 2. Schematic illustration of the activities and topics covered in the project focusing on providing new knowledge on growth and natural mortality and implementing this knowledge in stock assessment.*

# Results of the project

## 1. Towards a long term solution to growth & mortality –collaboration with fishing industry to support tagging

*By Karin Hüssy (DTU Aqua), Henrik Lund (DFPO) and Kim Kær Hansen (DFPO)*

Age determination of cod is traditionally conducted by counting annual rings on otoliths. For Eastern Baltic cod, reliability of this method has long been a subject of discussion (Hüssy et al. 2016). In recent years, likely due to reduced growth, the rings on otoliths have become increasingly difficult to interpret, and age determination of EB cod from otoliths is therefore considered unreliable. Therefore, alternative methods to determine cod growth are needed.

The project TABACOD (funded by Baltic2020) started in 2016 to develop new validated method for deriving information on cod growth that could inform growth in stock assessment models in future (http://www.tabacod.dtu.dk/).

To achieve this, two interlinked tasks are required:
  i)    Establishment of a spatially comprehensive sample of cod with "known growth" over a known time period to understand the present status of the stock;
  ii)   Development of an objective validated method that continuously allows deriving growth information in the future.

Two methods that will yield the necessary information are i) tagging of individual fish and ii) analysis of the chemical composition of their otoliths.

The aims of a tagging program is thus to provide information about size-specific growth of Baltic cod for stock assessment, but also to serve as validation for otolith chemistry-derived growth estimates. The chemical analysis of otoliths are expected to provide a cost-efficient tool for estimating growth in future after the termination of the tagging program.

The success of a tagging program to obtain the necessary information is entirely dependent on recaptures of the tagged cod in fisheries and return of the recaptured individuals to scientists. Thus, good cooperation with fishing industry is essential for the success of the tagging program to provide the necessary information to achieve long term scientifically solid solution to growth determination of EB cod. For that reason, the present project included a Workpackage (WP1) to support the tagging conducted in the TABACOD project by focusing on cooperation and communication with fishing industry. This is to establish best possible procedures for tagging and ensure return of the recaptured tagged cod. This work was done in collaboration between DFPO (tilsagn J .nr. 33113-B-16-049) and DTU Aqua.

Specifically, the activities included:
  • Informing fishing industry about the tagging program and calling for specific attention of tags when handling the catch, to be able to locate the tags.
  • Setting up information posters in habours about the tagging program.
  • Distributing brochures to fishermen who could possibly encounter tagged cod in their catches in the Baltic Sea.
  • Contacting larger fishing vessels directly that are fishing in relevant areas in the Baltic Sea to enhance the understanding of the importance of the tagging project, and the need for increased attention to discover the tagged cod in catches and return them.

- Continuous follow-up and contact to the fishermen to exchange information on tagging and recaptures.
- Ensuring that the recaptured tagged cod are returned to the scientists.
- Explaining the background and need for the tagging project to the fishermen and how the results will be used to improve the stock assessment and related basis for fisheries management advice.
- Participating in relevant meetings in the Baltic region, to inform about the project and discuss the results. This includes participation in DFPO general annual meetings around Denmark.
- Publishing frequent updates in Fiskeritidende and on relevant homepages concerning the tagging.
- Contributing to generating an overall positive attitude within fishing industry towards the tagging project, to increase willingness for an extra effort to look for and return the recaptured tagged cod to ensure success of the project.

TABACOD project continues until the end of 2019, thus some tagging activities and especially recaptures of tagged cod will continue also after the end of the present EMFF project. However, the efforts in terms of collaboration and communication with the fishing industry around tagging during the present project are expected to continue to have an effect also in the remaining part of the tagging program.

With the status of June 1, 2018, around 18 000 cod have been tagged in the Baltic Sea within TABACOD project (Table 1.1).

*Table 1.1. Number of cod tagged in the Baltic Sea in TABACOD project by country, with the status of June 1, 2018.*

| År | Land | Antal T-bar | Antal DST |
|----|------|-------------|-----------|
| 2016 | Danmark | 1915 | 50 |
| | Tyskland | 1446 | |
| | Polen | 1464 | |
| | Sverige | 1404 | 100 |
| 2017 | Danmark | 1466 | 174 |
| | Tyskland | 3110 | 127 |
| | Polen | 2172 | |
| | Sverige | 2193 | 175 |
| 2018 | Danmark | 940 | 132 |
| | Tyskland | 525 | 142 |
| | Polen | 989 | |
| | Sverige | May | May |
| | | | |
| | **Total** | **17533** | **909** |

# 2. Molecular-genetic analyses of cod growth

*By Jonna Tomkiewicz (DTU Aqua), Jakob Hemmer Hansen (DTU Aqua) and Peter Ravn (Bioneer AS)*

The purpose of this WP is to describe the growth of the Baltic cod at the molecular level. Growth and reproduction are closely linked physiological processes with *trade-offs* in energy allocation for growth and reproduction during life history. Here, the marked deterioration of the Baltic cod condition and sexual maturation led to the hypothesis that the growth rate cod of is reduced and therefore individuals mature and reproduce at a smaller size, which in turn lead to reduced growth and fitness. As this hypothesis presently cannot be verified through age determination, we applied molecular methods to study growth and reproduction in this stock. Thus, we compared cod caught in the Baltic Sea with the cod caught in Kattegat that show healthy growth and their size of sexual maturation is predictable. The objective was to analyse the expression of growth and reproduction genes and their receptors in tissue samples, as well as blood plasma levels of related hormones. By comparing the expression of such genes and levels of growth hormones in specimens at similar maturational stages in the two stocks, it will be possible to examine potential differences in growth processes in the two stocks. This may help an assessment of whether growth processes in Baltic cod contribute to the earlier maturation and deterioration of fitness in recent time.

## Methods and materials

### *Sampling and categorisation*

Cod in southern Kattegat (SD21) and the Baltic Sea (SD25) were collected during four research cruises in 2016-17. For each stock, sampling was conducted during two regular surveys, one before the onset of the spawning period (pre-spawning) and one after spawning (post-spawning) (Table 2.1). The cod were sampled at random from trawl catches to represent population demography and females identified. Maturity stage was judged visually (Tomkiewicz et al. 2003a) before sampling and later evaluated histologically in the laboratory. During pre-spawning cruises, sampling focused on female cod in maturing stages 3 (early ovarian development) and 4 (late ovarian development), while post-spawning cruises targeted stage 2 (late immature), 8 (spent) and 9 (resting).

*Table 2.1. Number of female cod sampled per area, month, period, cruise and maturity stage. Research cruises included Dana BITS 2017, Alkor 2017, Havfisken December 2016, Havfisken KASU March 2017. Maturity stage refers to histologically validated samples.*

| Area | Month | Period | Cruise | Maturity stage | | | | | Total |
|------|-------|--------|--------|---|---|---|---|---|-------|
| | | | | 2 | 3 | 4 | 8 | 9 | |
| Baltic | March | Pre-spawning | BITS 1 | | 38 | 13 | | | 51 |
| Baltic | August | Post-spawning | AL497/AL498 | 1 | | | 45 | 5 | 51 |
| Kattegat | December | Pre-spawning | Havfisken | | 35 | 15 | | 4 | 54 |
| Kattegat | March | Post-spawning | KASU | 1 | | 2 | 6 | 20 | 29 |
| Total number of fish sampled | | | | 2 | 73 | 30 | 51 | 29 | 185 |

Individual sampling included records of morphometric data (length, total body weight, $W_b$, somatic weight $W_s$, ovary weight, $W_o$, and liver weight, $W_l$), tissue samples for analysis of gene expression preserved in RNA-later (brain, pituitary, ovary and liver), ovarian sample for histology preserved in formalin (BiopSafe, Axlab) and blood plasma was obtained for hormone analyses. Morphometric data included calculation of Fulton's condition index, $K = W_b / L^3 * 100$, somatic condition index, $K_s = W_s / L^3 * 100$, the gonadosomatic index (GSI) GSI = $W_o / W_s * 100$ and the hepatosomatic (HSI) HSI = $W_l / W_s * 100$.

The maturity staging at sampling was revisited in the laboratory analysing the formalin preserved ovarian samples. It often is difficult to separate stages by gross examination alone, as the stages 2, 3, 8 and 9 may appear similar to the naked eye. Therefore, histological analysis that applies microscopy and staining of the tissue was used to identify structures at the cellular level and correct the maturation stage, when needed. For this purpose, the formalin-fixed ovarian samples were dehydrated in ethanol using standard histological procedures, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin. Histological maturity stages were assessed using the definitions of Tomkiewicz et al. 2003b. The total number of specimens in the targeted stages was 185 and the numbers per stage are given in Table 2.1. Due to the low number of specimens (2) obtained for Stage 2, these were not included in the analyses.

### *Identification of relevant genes, RNA extraction and gene expression analysis*

A literature review formed the basis for selecting relevant candidate genes related to growth and reproduction. Thirteen candidate genes and three reference genes were selected for gene expression analyses (Table 2.2). RNA was extracted from pituitary and gonad tissue stored at -80 degrees in RNA-later buffer. cDNA was synthesised and gene expression analyses were carried out using a BioMark HD, using triplicate assays for each individual sample. Positive control samples, no-template controls and no-RT controls were included in each run. Results are reported as levels of detection (Ct), standardized to one of the reference genes (EF1a). Differences between the Kattegat and the North Sea were assessed with linear mixed effects models using the R package lme4 (Bates et al. 2015), and treating Area and Maturity as fixed effects and Cruise and Individual as random effects.

### *Analysis of plasma hormone concentrations*

The presence of hormones in the blood of the fish was investigated through Western blotting. A Western blot is an immunological method for detecting small amounts of specific proteins in complex mixtures. Proteins are separated by gel electrophoresis (SDS-PAGE), transferred to a membrane and detected using specific antibodies. The complete GH amino acid sequence is known (GenBank ACD46080), and was used for identifying potential immunogenic regions in GH. A peptide stretching from isoleucine74 to serine87 was selected as immunogenic in collaboration with the antibody subcontractor, Biogenes (Berlin, Germany). This peptide was synthesised and used to raise antisera in two rabbits. Plasma samples from cod were analysed in Western blots. Antisera from the two rabbits were combined and used as primary antibody in the blots. Goat anti-rabbit antibody, coupled to horseradish Peroxidase, was used as secondary antibody. Plasma samples from two stage 8 cod, A56 from the Baltic Sea and K36 from Kattegat, were used in the analysis. The Western blotting experiments are aimed at supporting or challenging these qPCR data.

*Table 2.2. Candidate genes analysed for gene expression. See listed references for details on primer design for the individual assays.*

| Gene | Gene abbreviation | Tissue expression | Reference |
|---|---|---|---|
| *Maturation* | | | |
| Gonadotropin releasing hormone 3 | GnRH3 | Pituitary | Hildahl *et al.* 2011 |
| GnRH receptor R2a | gmGnRH-R2a | Pituitary | Hildahl *et al.* 2011b |
| Gonadotropin | GP | Pituitary | Mittelholzer *et al.* 2009 |
| Follicle stimulating hormone | FSH | Pituitary | Mittelholzer *et al.* 2009 |
| Luteinizing hormone beta | LH-beta | Pituitary | Mittelholzer *et al.* 2009 |
| 20b-hydroxysteroid dehydrogenase | 20b-HSD | Gonad | Breton *et al.* 2012 |
| FSH receptor | FSHr | Gonad | Breton *et al.* 2012 |
| LH receptor | LHr | Gonad | Breton *et al.* 2012 |
| P450 aromatase | CYP19A1 | Gonad | Breton *et al.* 2012 |
| P450 side chain cleavage | CYP11A1 | (testis) | Breton *et al.* 2012 |
| | | | |
| *Growth* | | | |
| Growth hormone | GH | Pituitary | Kortner *et al.* 2011 |
| Insulin-like growth factor 2 | IGF-2 | | Lanes *et al.* 2012 |
| Insulin-like growth factor 1 receptor | IGF1-r | Pituitary, gonad | Lanes *et al.* 2012 |
| | | | |
| *Reference genes* | | | |
| Elongation factor 1 | EF1a | | Hildahl *et al.* 2011 |
| Acidic ribosomal protein | arp | | Lanes *et al.* 2012 |
| Ubiquitin | ubi | | Lanes *et al.* 2012 |

## Results and discussion

### *Morphometric analyses of samples specimens*

The length and weight of the sampled females differed between the Baltic Sea and Kattegat with only limited overlap in size, although in the same stages of maturation were targeted and fishes were sampled at random from the trawl catches, with the purpose to obtain a representative sample of the population (Table 2.3). Stage 2 was not included in the sampled trawl catches, which excluded an analysis of immature cod prior to sexual maturation.

*Table 2.3. Length and weight data for cod sampled during the pre-spawning and post-spawning cruises in the Baltic Sea and Kattegat incl. average length, standard deviation and minimum and maximum length and weight.*

| | Length (cm) | | | | Weight (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | avg | stdev | min | max | avg | stdev | min | max |
| Baltic Sea pre-spawning | 39 | 6.1 | 29 | 55 | 641 | 287 | 230 | 1544 |
| Baltic Sea post-spawning | 37 | 6.2 | 24 | 49 | 407 | 219 | 98 | 1038 |
| Kattegat pre-spawning | 64 | 7.8 | 47 | 80 | 2672 | 961 | 876 | 5120 |
| Kattegat post-spawning | 62 | 7.2 | 43 | 73 | 2375 | 727 | 682 | 3986 |

The length and weight relationships illustrated in Figure 2.1 show the consistently lower weight at length of female cod in the Baltic Sea compared to Kattegat with a trend towards lower weight-at-length in post-spawning Baltic cod.
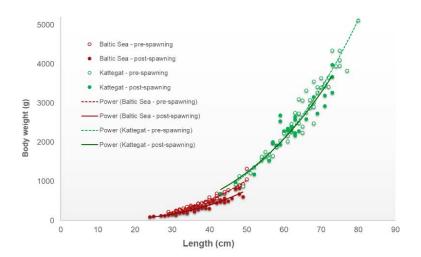


*Figure 2.1. Length-weight relationship from fish collected in the Baltic Sea and Kattegat during pre-spawning and post-spawning, respectively. Trend lines: Baltic Sea pre-spawning (y = 0.0088x$^{2.9826}$, R$^2$ = 0.9759) and post-spawning (y = 0.0051x$^{3.0524}$, R$^2$ = 0.9448); Kattegat pre-spawning (y = 0.0064x$^{3.1041}$, R$^2$ = 0.9512) and post-spawning (y = 0.0138x$^{2.9127}$, R$^2$ = 0.9072).*

While the sampled pre-spawning specimens distributed evenly on maturation stages 3 and 4, the post-spawning samples showed more stage 9 than 8 in Kattegat and more stage 9 than 8 in the Baltic Sea. This reflect the shorter and more concentrated spawning time of Kattegat cod. Condition indices, gonadosomatic and hepatosomatic indices of the females in relation to maturity stage were compared for the two areas. Despite the size difference between female from the two stocks, the condition indices based on body weight (Fulton's *K*) and somatic weight (*Ks*) were similar in stages 3 and 4. After spawning however, the average condition indices appeared lower in the Baltic than in the Kattegat. In contrast, the average GSI and HSI was consistently higher in Baltic cod than in Kattegat cod with the highest difference in pre-spawning stages. Considering the smaller size of cod, the observed pattern indicates a high investment in reproduction of Baltic cod compared to somatic body weight, but with substantial variability within stages in both stocks.
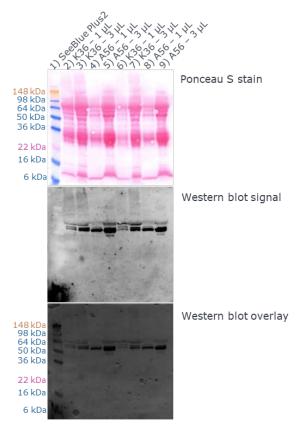
***Comparative analysis of the Baltic Sea and Kattegat growth and reproduction physiology***
We found highly significant differences in gene expression of the growth hormone gene in pituitary tissue from cod collected in the Baltic Sea and the Kattegat (P=0.0008). In contrast, we did not find a significant effect of maturity stage on the gene expression of the growth hormone gene (P=0.55), suggesting that the expression of this gene is not affected by the maturation trajectories of the fish. However, the functional consequences of these differences are not known at present.

The expression of the maturation related genes, follicle stimulating hormone (FSH) and luteinizing hormone (LH), showed less pronounced differences between areas. Thus, the relative gene expression levels of FSH in pituitaries from fish collected in the Baltic Sea and Kattegat were more closely related to maturity (P=0.006) than to area (P=0.01) although both were significant. Similarly, the relative gene expression levels of luteinizing hormone (LH) in pituitaries were more closely related to maturity (P=0.0006) than to area (P=0.05). The expression of these genes seemed to be more closely related to maturation schedules, as expected based on known gene function in maturation pathways (see Table 2.2 and associated references). Analyses of gonad tissue showed gene expression signals for relevant candidate genes. These results are promising for future work investigating these pathways in more detail.

### Plasma growth hormone concentrations

Preliminary blots were used to identify 1 µL and 3 µL as suitable volumes of samples. A Western blot from the experiments are shown in Figure 2.2. Ponceau S stain showed that the samples contained sufficient amounts of protein, that SDS-PAGE were running satisfactory, and that protein transfer to the membrane worked well. Plasma samples contained aggregates. Centrifugation of plasma samples to precipitate these aggregates did not affect the result. This is shown in lane 6-9, which can be compared to lane 2-5. This demonstrates that centrifugation is unnecessary. Furthermore, the identical results in lane 2-5 compared to lane 6-9 demonstrate the general reproducibility of the method. Antisera recognized a protein that migrated at 55 kDa. This should be compared to the predicted GH size, which is only 21 kDa. It is not possible to see any protein bands at 21 kDa. No protein glycosylations or other modifications, which can explain this size difference, are expected.



**Western blotting experiment, using anti-cod GH antisera.**
Proteins in cod plasma samples were separated in SDS-PAGE, and blotted onto a nitrocellulose membrane. The above pictures are showing different treatments of the same blot.
The upper picture shows Ponceau S staining. This is an unspecific protein stain, and it shows that loaded volumes of plasma samples contain appropriate amounts of protein, and that transfer from gel to membrane was satisfactory.
The centre picture shows the western blot. The Ponceau S stain was washed away, and the blot was hybridised with a mixture of rabbit anti-cod GH from two rabbits, and subsequently with goat anti-rabbit antibodies coupled to horseradish peroxidase. Finally, the blot was developed using Supersignal West Dura Extended Substrate.
The lower picture shows an overlay of the processed film from above, and the blot. This shows the position of the markers relative to the bands, which were recognized by the antisera.
• Lane 1 contains the molecular weight marker SeeBlue Plus2. Weight of marker bands are indicated to the left.
• Lane 2-5 contain raw plasma. Sample volumes is indicated.
• Lane 6-9 contains plasma, which is cleared by centrifugation. Sample volumes is indicated
K36 is blood plasma from fish K36 from the Kasu cruise (Kattegat)
A56 is blood plasma from fish A56 from the Alkor cruise (Baltic Sea)

*Figure 2.2. Example of Western blot used to detect the presence of hormones in blood plasma of the sampled cod. Here, the proteins are separated by gel electrophoresis (SDS-PAGE), transferred to a membrane and detected using specific antibodies.*

The protein band at 55 kDa can be explained by recognition of an unrelated, but cross-reacting protein, in cod plasma. This protein could be present in higher concentrations than GH, thereby masking GH presence. It is also possible that GH forms protein dimers, which are not resolved by SDS-PAGE. A database search (Entrez peptide blast) with the peptide used in rabbit immunization (GH Ile74 to Ser87) did not reveal any proteins with strong amino acid similarity, except GH from other fish species. The 55 kDa protein is present in higher concentration in A56 samples compared to K36 samples (compare lane 2 to lane 4, lane 3 to lane 5, lane 6 to lane 8 or lane 7 to lane 9). In contrast, a higher level of GH is expected in K36 (based on qPCR data). Further Western blot optimization is possible:

- Antisera from the two immunized rabbits can be used individually, instead of in combination. It is possible that only one of the sera recognize the 55 kDa protein, and that the other antiserum would preferentially recognize GH
- New antibodies, based on peptides from other regions in GH, can be developed. These would be unlikely to hybridize to the same 55 kDa protein.
- The Western blot membrane region corresponding to the expected GH size (21 kDa) can be excised and developed using methods that are more sensitive. This might reveal GH bands, which are obscured by the 55 kDa band in present blots.

## Conclusions and perspectives

The sampling was design was outline to cover the complete length range in catches. However, the size ranges of females obtained in the two areas differed to an extent that the specimens sampled hardly showed any overlap in length and weight within the maturity categories targeted in both sampling periods. This complies with the steady decrease in size-at-first maturity and disappearance of larger specimens in the Baltic cod stock in recent decades (Köster *et al.* 2017). The average condition indices appeared lower in the Baltic than in the Kattegat after spawning, while the average GSI and HSI was consistently higher in Baltic cod than in Kattegat cod, but with a high variability in both stocks. Further statistical and lab analyses will be performed on the sampled material addressing potential drivers. Considering the smaller size of cod, the observed pattern indicates a high investment in reproduction of Baltic cod compared to somatic weight; however, the relation between food intake, environmental factors and sexual maturation is still poorly understood, which argue for fundamental analyses of the mechanisms.

Gene expression analyses of candidate genes revealed different levels for the growth hormone gene when comparing the Baltic Sea and Kattegat populations. Here, the level was significantly lower for the Baltic Sea fish. These results indicate that growth processes may differ between the two populations, although it should be stressed that the functional consequences of the differences in gene expression are unknown at present. Consequently, the data can be used to further our understanding of the underlying physiological processes but cannot be directly translated to estimates of growth in any of the populations. Future work could combine gene expression analyses with experiments under controlled environmental conditions to obtain a better understanding of potential growth differences between the populations. Our results also establish gene expression analyses of a range of candidate genes for growth and maturation as a valuable and efficient tool for future work in cod.

Western blot was used in an attempt to support qPCR data. Anti-cod GH was raised in rabbits and used in Western blots. The antibodies recognized a protein with a size, which is however not consistent with cod GH. This protein was less abundant in a cod sample from Kattegat, compared to a sample from the Baltic Sea. Unfortunately, GH was not recognized. One explanation could be that GH levels in cod plasma are too low for detection by Western blotting.

# 3. Investigations of changes in natural mortality of cod

## 3.1 Seal predation on cod

### 3.1.1 Investigations of grey seal diet

*By Anne-Mette Kroner (DTU Aqua), Lotte Kindt-Larsen (DTU Aqua), Karl Lundström (SLU) and Morten Tange Olsen (KU)*

**Introduction**

During the past few decades, the Baltic grey seal (*Halichoerus grypus grypus)* population size has increased significantly (HELCOM Core Indicator, 2018). The grey seal is a top predator known to consume a wide range of fish species depending on prey availability, geographical area and season (Ampela and Ferland, 2006; Lundstrom *et al.*, 2010). An adult seal is estimated to consume approximately 4.5 kg of fish per day (Hammond and Grellier, 2006). However, the daily intake depends on both the individual energy requirement and the energetic content of the prey, with on average 4 kg of high energy sandeel required per day compared to an average of 7 kg of cod (Hammond, Hall and Prime, 1994).

As seal numbers increase, it has raised concerns over competition with fisheries and grey seals have been suggested to have a negative impact on cod stocks and hinder recovery of depleted stocks in other areas (Cook, Holmes and Fryer, 2015; Swain and Benoît, 2015). Therefore, understanding the diet composition of grey seals is critical to understanding how their predation may influence prey populations in the Baltic Sea including commercially important species such as Atlantic cod (*Gadus morhua*), Atlantic herring *(Clupea harengus)*, and sprat (*Sprattus sprattus*) (Sparholt, 1994). Furthermore, improved information on the grey seals' contribution to the natural mortality of the eastern Baltic cod stock is important to be able to improve the stock assessment of the cod stock.

The purpose of this work was to provide further data on the prey composition of Baltic grey seals. Scat content was evaluated from 820 seal scats collected over a four-year period (2014-2017) from four separate haul-out sites located in the Baltic Sea. Scat analysis is an extremely useful tool for examining mammalian diets because it is non-invasive, low-cost, and allows for the collection of large sample sizes (Bowen, 2000). Two methods were used to assess the prey composition – molecular analysis and otolith analysis of scats. Furthermore, the geographical, seasonal, and yearly differentiation between the prey species in the diet was examined.

**Molecular analysis**

Next Generation Sequencing methods have been used extensively in diet studies because they make it possible to identify numerous species from a large amount of samples using sequencing of DNA barcodes (Pompanon *et al.*, 2012). The 16S mitochondrial gene is the most commonly used, as multiple copies are present in each cell and makes it ideal for low-quality samples such as scats (Parsons *et al.*, 2005). It is especially useful if the otoliths are hard to identify, degraded and/or lacking due to seals only eating the soft parts of the fish. It has proven possible to find species even though very low concentrations of DNA was present in the scat (Matejusová *et al.*, 2008).

**Otolith analysis**

Scat content analysis based on visual identification of prey remains has been a popular method for analyzing and reconstructing the diets of seals and it has been used in numerous studies (Härkönen, 1987; Ampela and Ferland, 2006; Lundström *et al.*, 2007; Lundstrom *et al.*, 2010). While this method can be used to analyze any hard parts of prey remains (e.g. sagittal otoliths, vertebrae, cranial bones and other skeletal parts) most studies – including ours – focus on the identification of fish otoliths, which can

be used to reconstruct not just the prey species, but also number of prey consumed, their age, length and biomass.

The analysis contained following:

- Extraction of all otoliths and bony parts.
- Species identification of all otoliths.
- Photos, length and width measurements of all otoliths by use of Leica microscope and image analyzer.
- Evaluation of the level of erosion for each otolith and calculations of original otolith size.
- Calculation of weight and size of the fish belonging each otolith. For cod the size correction was based on cod from area 24 and 25 with in the same years of the scat collection. For other species, correction factors were collected from the available literature.

## Results

A total of 820 grey seal scats were collected across four study sites (Table 3.1.1). This include Måkläppen (Falsterbo), Sweden in 2014-2017 (N = 366), Tat (Christiansø), Denmark in 2015-2017 (N = 184), Rødsand, Denmark in 2017 (N = 159) and finally Utklippan, Sweden in 2016-2017 (N = 111). Of these samples, 300 contained otoliths, while DNA was extracted from 331 scats. Of these, 145 samples have been analyzed, and the rest is still in progress. The DNA analysis suggests that cod, garfish, herring, sprat, flatfishes and sandeel comprise a major part of the grey seal diet when estimated as frequency of occurrence (Table 3.1.2). Cod was also found to be the most frequently occurring species in the otolith analysis, followed by flatfishes, sandeel, unidentified codfishes (Gadidae), herring and whiting. However, in terms of biomass, cod comprised 83% of the total consumption by grey seals – more than ten times more than flatfishes (8%) as the second most abundant prey (Fig. 3.1.1; Table 3.1.3).The length of the cod consumed by grey seals varied from 129 cm and up to 909 cm. The most common length is between 550 and 650 cm as seen in figure 3.1.2.

*Table 3.1.1. The number of scats collected from each location and month. The numbers in parentheses are the number of samples successfully analyzed for DNA and the number in brackets are the number of samples containing hard parts. Samples from Utklippan 2017 have not yet been analyzed for hard parts. Not included in this table is 178 samples that has been DNA sequenced, and are in the process of being analyzed for fish species composition.*

| | Jan | Feb | Mar | Apr | Maj | Jun | Jul | Aug | Sep | Okt | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MÅK 2014 | | | | 16 (9) [7] | 32 (15) [6] | | 6 (5) [1] | 2 [1] | 4 (2) [2] | | 16 (7) [3] | | 76 (38) [20] |
| MÅK 2015 | | | | 66 (30) [12] | 30 (12) [5] | 4 (1) [2] | | | | 24 [4] | | | 124 (53) [23] |
| MÅK 2016 | 17 [8] | | | 30 [6] | 32 [6] | 16 [6] | | 16 [3] | | | | | 111 [29] |
| MÅK 2017 | 3 | | | | 47 [10] | 5 [2] | | | | | | | 55 [12] |
| TAT 2015 | | | | | 5 (4) [4] | 10 (7) [4] | | 10 (6) [1] | 5 (5) [4] | 19 (12) [4] | | | 49 (34) [17] |
| TAT 2016 | 18 (13) [11] | 18 (8) [8] | 18 (9) [8] | 28 [15] | | 6 [2] | 5 [2] | 3 [2] | | | 30 [18] | | 126 (30) [66] |
| TAT 2017 | | | 9 [2] | | | | | | | | | | 9 [2] |
| RØD 2017 | | | 32 [9] | | 21 [1] | | 6 [1] | 76 [74] | | 24 [8] | | | 159 [93] |
| UTK 2016 | | | | 4 [4] | 29 [27] | 1 [1] | | 5 [4] | 2 [2] | | | | 39 [38] |
| UTK 2017 | | | 17 | 17 | 24 | 5 | 2 | 5 | | | | | 70 |
| Total | 38 (23) [19] | 18 (9) [8] | 76 (32) [19] | 161 (73) [44] | 218 (75) [59] | 47 (26) [17] | 19 (16) [4] | 117 (28) [85] | 11 (9) [8] | 67 (24) [16] | 46 (20) [21] | 0 | **820 (145) [300]** |

*Table 3.1.2. Diet composition of the samples analyzed by DNA and otolith extraction. FO is the frequency of occurrence calculated by dividing the number of samples containing the species with the total number of samples containing prey. The total number of analyzed DNA samples is 145. The total number of analyzed otolith samples is 218.*

| Family | Species | Common name | FO$_{DNA}$ | FO$_{Otoliths}$ |
|--------|---------|-------------|--------|----------|
| Gadidae | *Gadus morhua* | Atlantic cod | 64.83 | 65.60 |
| Belonidae | *Belone belone* | Garfish | 38.62 | |
| Clupeidae | *Clupea harengus* | Atlantic herring | 37.24 | 10.55 |
| Clupeidae | *Sprattus sprattus* | European sprat | 33.79 | 4.13 |
| Pleuronectidae | *Pleuronectes/Platichthys* | Flatfishes | 18.62 | 21.10 |
| Ammodytidae | *Hyperoplus lanceolatus* | Great sand eel | 11.03 | |
| Zoarcidae | *Zoarces viviparus* | Viviparous eelpout | 7.59 | |
| Lotidae | *Enchelyopus cimbrius* | Fourbeard rockling | 5.52 | 3.21 |
| Gadidae | *Merlangius merlangus* | Whiting | 4.14 | 10.09 |
| Cyclopteridae | *Cyclopterus lumpus* | Lumpsucker | 3.45 | |
| Ammodytidae | *Ammodytes tobianus* | Lesser sand eel | 3.45 | |
| Scophthalmidae | *Scophthalmus rhombus* | Brill | 2.07 | |
| Anguillidae | *Anguilla anguilla* | European eel | 2.07 | |
| Salmonidae | *Salmo salar* | Atlantic salmon | 2.07 | |
| Salmonidae | *Salmo trutta* | Brown trout | 1.38 | |
| Gasterosteidae | *Gasterosteus aculeatus* | Three-spined stickleback | 1.38 | |
| Gobiidae | *Pomatoschistus minutus* | Sand goby | 1.38 | |
| Gadidae | *Pollachius virens* | Saithe | 0.69 | |
| Gobiidae | *Neogobius melanostomus* | Round goby | 0.69 | |
| Esocidae | *Esox lucius* | Northern Pike | 0.69 | |
| Percidae | *Perca fluviatilis* | European Perch | 0.69 | |
| Gobiidae | *Gobiusculus flavescens* | Two-spotted goby | 0.69 | |
| Gobiidae | *Gobius niger* | Black goby | 069 | |
| Ammodytidae | *Hyperoplus/Ammodytes* | Sandeel | | 13.76 |
| Gadidae | *Gadus/Merlangius* | Cod or whiting | | 12.84 |
| Clupeidae | *Clupea/Sprattus* | Herring or sprat | | 7.34 |
| Gobiidae | *Gobius/Neogobius* | Goby | | 5.05 |
| Pleuronectidae | *Limanda limanda* | Common dab | | 1.83 |

*Figure 3.1.1. Mean weight proportion of fish species in all samples analyzed for otoliths. Cod contributes to 83.4 % of the total biomass found in the seal scat samples. Numerical correction factors have been applied. Garfish consumption could not be measured because of the absence of otoliths.*
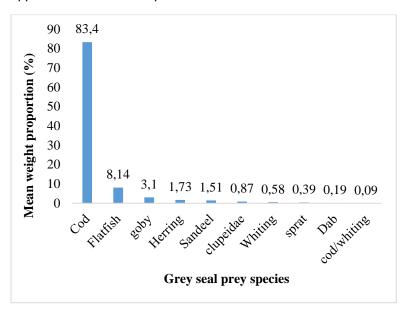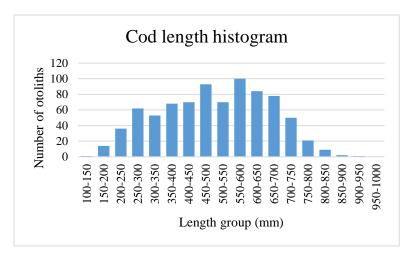


*Table 3.1.3. Total biomass of the primary prey species consumed by an individual grey seal in the southwestern Baltic Sea.*

| Prey species | Daily consumption (kg) | Annual consumption per seal (kg) |
|---|---|---|
| Cod | 3.753 | 1369.8 |
| Flatfish | 0.366 | 133.6 |
| Goby | 0.139 | 50.7 |
| Herring | 0.077 | 28.1 |
| Sandeel | 0.068 | 24.8 |
| Clupeidae | 0.039 | 14.2 |
| Whiting | 0.026 | 9.5 |
| Sprat | 0.017 | 6.2 |
| Dab | 0.009 | 3.3 |
| Cod/Whiting | 0.004 | 1.5 |

*Figure 3.1.2. Length proportion of the consumed cod in the southwestern Baltic Sea. The histogram is calculated from non-size corrected otoliths.*



## Discussion and conclusions on seal diet analyses

The recovery of the grey seal population throughout much of the Baltic signifies a conservation success story, but introduces new challenges because of the increase in interactions with fisheries (Königson *et al.*, 2007). The overall findings demonstrate a relatively varied diet of grey seals in the Southern Baltic Sea. A total of 23 species were identified from the molecular method, which for some has not been found in grey seal diet before. The most common prey species was cod, which confirms the competition that occurs between grey seals and fisheries.

The advantage of the molecular method is that it provides results that are independent of any visually identifiable prey remains (Lundström, 2012). The major drawback of this method is the absence of information about the sizes of consumed prey items and the difficulty to accurately relate the number of prey DNA molecules identified in dietary studies to the relative biomass of different prey items consumed (Matejusová *et al.*, 2008).

The hard part identification method has been widely used, but a large source of bias is the erosion caused by digestion in the stomach, which especially affects small and fragile otoliths (Härkönen, 1987; Lundstrom *et al.*, 2010). In addition, prey species without otoliths or very small otoliths will be under-represented. Thus, due to the differing rates of digestion among the prey species, scat analysis may be biased towards species with large and robust hard parts (Bowen, 2000). Garfish does not leave behind any otoliths and therefore needs to be identified from other hard parts. The data from this study has not yet been analyzed for other hard parts and therefore the number and biomass of garfish in seal diet cannot be estimated. As seen in the molecular analysis, garfish are present in 38.62 % of the seal scats, so the biomass of garfish is potentially also substantial. The total consumption of cod is therefore overestimated, because of the absence of the garfish biomass.

In all, this work has proved promising conditions of dietary monitoring of grey seals by collection and analysis of scats from a number of haul outs. The results in this report are preliminary and have to be interpreted with caution. The hard part data from Utklippan 2017 as well as the DNA results from Rødsand and Utklippan is missing. Nevertheless, the data based on more than 300 samples suggest that Atlantic cod contributes to a substantial part of the diet of grey seals from the south-western Baltic Sea. This estimated high proportion of cod in the diet of grey seals in the south-western Baltic calls for further assessments of the impact of the seals on cod.

### 3.1.2 Approximate predation mortality on cod from grey seals

*By Margit Eero (DTU Aqua)*

Mortality rate of cod due to seal predation depends on i) seal abundance in the area overlapping with cod, ii) cod abundance, iii) daily consumption of an individual seal, and iv) the proportion of cod in seal diet.

The investigations of seal diet described in the section above have mainly focused on elucidating the proportion of cod in seal diet where information has been scares, especially for the southern Baltic Sea, where cod abundances are highest. Concerning the other information necessary for quantifying seal predation, literature information has been used.

## Abundance of grey seals

Data on counts of grey seals in the Baltic Sea are available from HELCOM. These data show that grey seals are mostly concentrated in central and northern parts of the Baltic Sea, with relatively small fraction in the southern Baltic Sea, i.e. in the area of overlap with cod. The estimates up to 2016 (presented at ICES WKBEBCA 2017, Lundstrøm, K., SLU) show below 5000 grey seals in the areas where most of the cod are currently distributed. The counts of seals generally underestimate population sizes (approximately 70% of the total population size is counted). Therefore, in the calculation of possible seal predation of cod, we used the value of 7000 individuals for representing the predator abundance.

## Amount of fish eaten

Grey seals are considered to consume 4.5 kg of fish per day, as an average (Hammond & Grellier 2006; Hammond & Harris 2006).
Thus, applying the seal abundances as described above results in ca 11500 tons of fish consumed by grey seals in the southern Baltic Sea per year (4.5 *365*7000).

## Predation mortality of cod

The tons of cod consumed depends on the proportion of cod in the seal diet. As the investigations described in this report showed, proportion of cod in grey seal diet can be high locally. Also, different sources of bias may affect this estimation, as described above. We applied 85% of cod in the grey seal diet in the calculation here, representing a maximum estimated value. This implies a consumption of cod by grey seals ca 9780 t per year.

Translating this into natural mortality estimate on cod depends on cod population size, as well as size structure of the consumed cod. Information on size structure of the prey is very difficult to obtain, and the available data shows relatively wide range of prey sizes (Fig. 3.1.2). Thus, for a rough approximation on possible predation mortality, we have not accounted for size composition of the prey.

Absolute stock size for EB cod for recent years is not available. However, survey data shows similar or higher catch rates in recent years until 2017 than the average in years 1997-2006 (Fig. 3.1.3). For these years, analytical stock assessment based on age readings is available and considered reliable, showing an average biomass around 135 000 tons. Thus, using the same biomass level for recent years until 2017 represents a minimum estimate. Consumption of 9780 t at such biomass level would imply a mortality rate of 0.07. It should be noted that this represents a maximum estimate, as several values used in this calculation where chosen from that end of the possible range that corresponds to highest possible predation rate. Thus, the average seal induced predation mortality of EB cod in its entire distribution area in recent years has likely been substantially below 0.1, thus not explaining the anticipated high natural mortality of EB cod in later years. However, the predation mortality could be

high locally. Also, if seal abundances continue to increase in future, predation mortality on cod would likely increase as well.
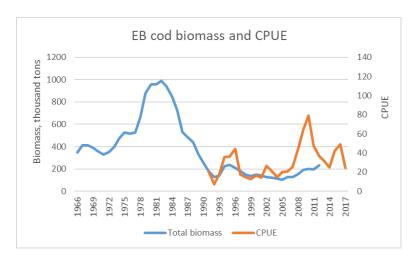


*Figure 3.1.3. Cod biomass from stock assessments, compared to CPUE (in weight) from BITS Q1 survey (data from ICES WGBFAS).*

## 3.2 Cod cannibalism

*By Stefan Neuenfeldt (DTU Aqua)*

Large database of cod stomach content data is available for the Baltic Sea, covering the period from 1960s-2010s. The stomach data have been collected and worked up within a number of national and international projects over the past decades, some of the most recent efforts with this database were carried out within EU BONUS INSPIRE project.

In the present project, this database was used to address a specific question related to cod cannibalism as a source of natural morality. Given the poor nutritional condition of EB cod in later years, which indicates food limitation, it is relevant to consider whether cannibalism related mortality has subsequently increased. Historical estimates of predation mortalities related to cod cannibalism back to 2011 are available from multispecies model (SMS). The estimates from this model have however not been possible to update for more recent years due to lack of age-based assessment for EB cod, which is needed for the former SMS model. Analyses of stomach data alone show that frequency of occurrence of cod in stomachs was high in years in the period 2007-2012 (Fig. 3.2.1). However, to translate this into mortality estimates, cod abundance needs to be taken into account as well. Therefore, an alternative approach was developed to get a rough indication of relative change in cannibalism related mortality, using survey data as a relative measure for cod abundance, combined with stomach content data:

$$N(eaten) = CPUE\ (pred)*freq*(365/tau)$$

$$M2 = \ln(N(end)/N(start))$$

$$= \ln(CPUE(prey)-N(eaten)) / (CPUE(prey)-N(eaten))$$

The results suggest that cannibalism related mortality was relatively high around 2010, however has substantially declined in later years (Fig. 3.2.2). This is likely related to low abundance of larger individuals in the cod stock that can use cod as prey. It should be noted that the exact values of natural mortality from these analyses are relatively more uncertain than the relative dynamics, which we mainly

focus on. These results suggest that cannibalism could have contributed significantly to natural mortality of smaller cod in early 2010s, however presently the cannibalism related mortality seems relatively low.
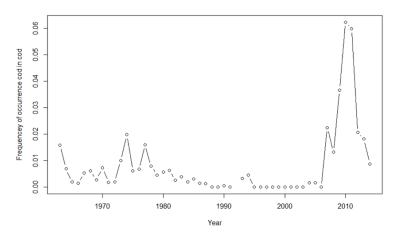


*Figure 3.2.1. Frequency of occurrence of cod in cod stomachs (Neuenfeldt et al. in prep).*
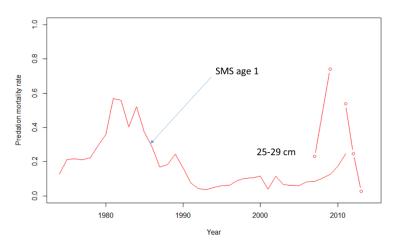


*Figure 3.2.2. Estimates of predation mortality for age 1 cod from SMS model until for 1974-2011, compared to the recent estimates for 25-29 cm cod, based on BITS survey and new stomach data (Neuenfeldt et al. in prep).*

## 3.3 Bioenergetic modelling of growth and survival of EB cod

*By Asbjørn Christensen, Stefan Neuenfeldt, Niels G, Andersen and Margit Eero (DTU Aqua)*

**Introduction**

A substantial amount of hypotheses have been proposed about the casual relations behind the commonly identified biological problems of the EBC stock, i.e. poor early growth and survival and earlier maturation. Up to now a substantial effort has been put into quantifying the physiological processes of cod, as well we the relation between feeding opportunities and fish size at one side, and growth, survival and maturation on the other side. Even though substantial uncertainties remain in the quantitative representation of cod life processes, the science has been consolidated sufficiently to form the basis

for a quantitative exploration of alternative hypotheses of the EBC issues. The quantitative uncertainty implies that we will not be able definitely to discriminate alternative hypotheses, but rather rank them as more or less significant. Thereby process-based models become circumstantial evidence in our understanding of the current stock problems, as well as future management perspectives.

## Materials and methods

Most process-based models for growth and survival take as starting point the physiological energy budget of the fish,

$$\Psi = \gamma I - M \qquad (1)$$

where I is the food intake, γ er efficiency at assimilating energy from the ingested food, M is the metabolic cost associated with life processes (excluding SDA, which is part of γ). The remainder

Ψ is a free energy that the fish may invest in growth, reproduction or storage. We take as starting point the implementation of (Jørgensen og Fiksen, 2006), which was originally developed for the North East Atlantic cod (NEAC) stock. We have maintained the the appealing basic strucutre of the model, but adapted the parameterization taking into account the specific knowledge for the EBC, including specific knowledge pertaining to the Baltic ecosystem. The model has been sketched in Fig 3.3.1.
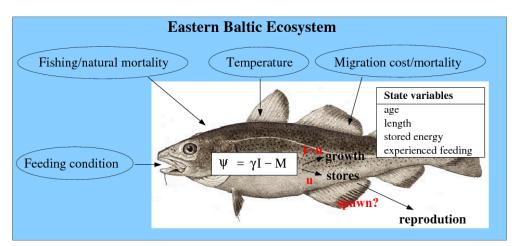


*Figure 3.3.1. Sketch of the (Jørgensen og Fiksen, 2006) model for growth, survival and maturation of cod.*

The highlights of the model are as follows:

- Medium complexity in the process representation (30 biological and physical parameters) and a minimum of state variables (4: age, length, energy storage and experienced past feeding level). This gives the model sufficient biological realism to contribute to the disentanglement of causal hypotheses, yet avoiding that the model drowns in its own complexity. Model state variables are observable, and the model parameters are directly measurable by independent experiments, not opaque aggregated parameters without clear biological meaning.
- The cod condition number is an emergent prediction from the model, not imposed directly or indirectly, e.g. through a length-weight key. The model is thus able to simulate how the condition number change under various conditions, as a synthesis of incorporated processes.
- There is insufficient knowledge about how growth and maturation is regulated in relation to cod life conditions; the model therefore determines growth and maturation dynamics form the principle of optimal behavior: given the state of the fish (age, length, energy storage and experienced past feeding level) and given the current ambient conditions, it will behave in such a way that it optimizes its life-time reproductive output. The principle of optimal behavior is quite

well-established in Ecology as a prior model, when insufficient knowledge about actual fish behavior under given conditions is available. Technically, the optimal behavior model is implemented by dynamical programming, where the decision surfaces leading to optimal life-time reproductive output is mapped for all state variable space with parametric ambient conditions. The model thus gives as output what is expected from a cod stock evolutionary adapted to its hosting ecosystem by natural selection.

- The model covers juvenile and adult age classes and covers the cod life cycle in time steps of one month, where seasonal effects are included.

In relation to the starting point (Jørgensen og Fiksen, 2006), the following amendments have been undertaken to optimize the description of EBC specifically:

- Maximum food intake, maintenance (food intake corresponding to no growth) and metabolism have been reparameterized as to reproduce a comprehensive data set (Hansson, 1996) and (Jobling, 1988;1982) covering relevant fish sizes and ambient temperatures.
- Application of natural and fishing mortalities, in accordance with recent WGBFAS assessment
- Temperature dependence if physiological processes are included dynamically.
- Shorter migration distance and other current regimes for EBC, compared to NEAC.
- The food intake is described by a size dependent stochastic model. The variance structure in the feeding level is chosen consistent with stomach fullness data sets (see section 3.2). It is important to represent the feeding level variance realistically, as the variance structure determines the risk taking behavior under various circumstances. The model is simulated with two feeding regimes: (i) a historic regime (to which the cod is adapted), and (ii) a recent feeding regime. The two feeding regimes are shown in Figure 3.3.2.

The model implementation is generic, i.e. it will repameterizable to other cod sub stocks, as well as other medium and long lived marine species, where sufficient knowledge is available. We denote this implementation and extension of (Jørgensen og Fiksen, 2006) *Gadiform.* The implementation is object-oriented and written in the popular language Python.
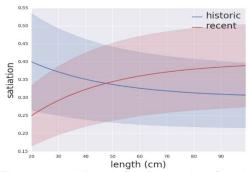


*Figure 3.3.2. Historic feeding regime (to which the cod is adapted) and the recent feeding regime (2005-2015). The red/blue lines correspond to average feeding level in each regime, the red/blue zones correspond to variance around average feeding level at different sizes.*

## Results

The scientific hypotheses we are exploring in this section is whether we are able to understand changed patterns of apparent natural mortality, condition and maturity as a plastic response to demonstrated changed feeding level patterns over the last 20 years, or whether liver worms and/or increased hypoxic zones are more likely explanations.

Figure 3.3.3 shows the so called probabilistic maturation reaction norm (PMRM, Heino et al., 2002) for EBC, as calculated by the Gadiform model. The color contours in the figure indicate the probability of

maturation at a given age and length, corresponding to optimal adaptation to the historic Baltic life conditions. The figure displays two average growth trajectories: one corresponding to historic and one to recent feeding levels at age and size. The figure indicate that maturation at smaller size, but higher age, is expected, so that slower growth for juvenile cod leads to later maturation. This PMRM is qualitatively similar to those found for other cod stocks (with negative slope), and it is therefore expected that this conclusion is robust toward potential variations in process representations within the Gadiform model.
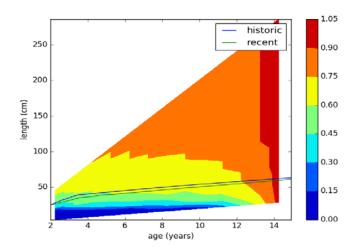


*Figure 3.3.3. Probabilistic maturation reaction norm (PMRM) for EBC, as calculated by the Gadiform model.*
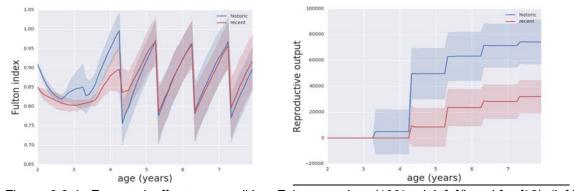


*Figure 3.3.4. Expected effect on condition Fulton number (100\*weight[g]/length[cm]^3) (left) and reproductive output (right) on population level, when the feeding level as only factor is changed from historic to recent. Lines indicate average, colored zones correspond to variations expected at population level.*

Figure 3.3.4 indicates the expected effect on condition number and reproductive output on population level, when the feeding level as only factor is changed from historic to recent. The overall features of the condition factor through the cod life cycle displays the phases of life: in the juvenile period, somatic growth is prioritized, with a controlled decline of condition number (as to leave a margin in case of adverse feeding opportunities); close to maturation, somatic growth slows, and reserves are build up to prepare for spawning. After first spawning, the cod (in most cases, unless spawning is skipped, which is emergent under certain combinations of life conditions and fish state), the cod enters an annual cycle, where the fish build-up reserves over the year after being spent, giving lower priority to somatic growth. When going from historic to recent size dependent feeding levels, a lower average feeding level for

juveniles is apparent; however, the effect of poorer early feeing conditions are leveled out toward older ages, where recent feeding levels are more generous (see Figure 3.3.2). The accumulated life time reproductive value drops, when switching from historic to recent feeding levels as isolated change, mainly due to delayed first maturation, which increases the risk of death before spawning.
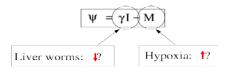


*Figure 3.3.5. Bioenergetic representation of liver worms and hypoxia respectively.*

Figure 3.3.5 indicates how liver worms and hypoxia respectively are expected to affect vital rates in Eq. 1. Liver worms are mainly expected to reduce nutritional intake (due to lower predation efficiency and nutritional uptake, Behrens, pers. comm.), whereas hypoxia is mainly expected to increase metabolic expenses on average (Plambech et al., 2013).
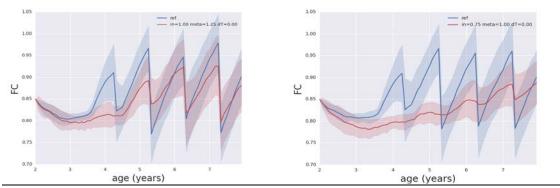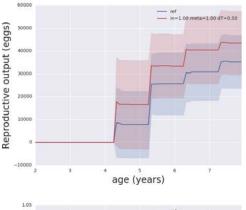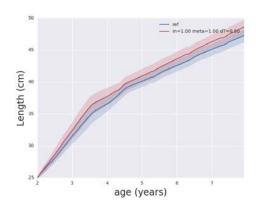


*Figure 3.3.6. Effect of hypoxia (left) and liver worms (right) on the Fulton index of cod under recent feeding levels. Lines indicate population average levels, colored zones indicate population variance levels.*

Figure 3.3.6 indicates the effect of hypoxia and liver worms on the Fulton index of cod under recent feeding levels, when the estimated increase in metabolic expenses (Plambech et al., 2013) and reduced nutritional uptake (Behrens, pers. comm.) is applied, i.e. the effect of hypoxia and liver worms are considered in isolation. Both mechanisms led (assuming the estimated impact on cod bioenergetics) to a drop in cod condition number at a level corresponding to the observed; the effect of liver worms has more distinct effect on older cods than hypoxia.

Finally, it is considered what magnitude of accumulated physiological response can be expected, when habitat temperature increases, due to global warming. Temperature increases in the Baltic region over the last 20 years are not heterogeneously distributed, but a representative upper limit average increase level seems to be 0.5 $^\circ$C (BACC II, 2015). Figure 3.3.7 shows the isolated effect of increasing the temperature level by 0.5 $^\circ$C from the historic reference level. The effect on growth, condition and reproductive output is beneficial, but this scenario does not consider the indirect effect of temperature increase on cod feeding levels, which is unknown, and therefore the net effect can not be estimated based on this exercise.
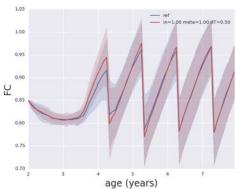
*Figure 3.3.7. Effect on growth, condition and reproductive output by isolated increase of the temperature by 0.5 °C from the historic reference level.*

## Conclusions

The *Gadiform* model is a generic model, which can simulate the juvenile and adult part of the life cycle for cod, focusing on bioenergetic and physiological processes, and thereby makes it possible to identify the most likely hypotheses and causal pathways behind observed changes in biological indicators of a stock. The model has been demonstrated on EBC, where all available knowledge and data about biological drivers have been included.

The conclusions that can be drawn based on the work above are as follows:

- Changes in the EBC stock condition index over the last 20 years can partially be understood as a plastic response to the changed feeding opportunities over the last 20 years, i.e. as a damage control (adapted behavior) to minimize the biological impact of changes.
- Changes in the EBC stock maturation pattern over the last 20 years can partially be understood as a plastic response to the changed feeding opportunities over the last 20 years; smaller size at first maturation is expected, whereas earlier maturation is *not* expected based on life history optimality; the latter conclusion is relatively robust toward reasonable model variations.
- Increased appearance of parasites (liver worms) and hypoxic areas over the last 20 years are both likely partial explanations of the decreased condition index of the EBC stock.

## 4. Cod growth and condition based on monitoring data

### 4.1 Quantifying growth of young cod, and its relation to condition

*By Karin Hüssy and Margit Eero (DTU Aqua)*

A number of studies have documented a decline in condition of eastern Baltic cod stock since the 1990s (Eero et al. 2012; 2015; Casini et al. 2016). Low nutritional condition, as well as maturation at smaller size and restricted size distribution of the stock suggest that growth rates of EB cod likely have decreased between the early 2000s and the 2010s. However, difficulties with age estimation prevent testing of this hypothesis in traditional ways. Therefore, an alternative approach was used where size at age was back-calculated from daily otolith growth patterns. This was supplemented by analyses of length frequency data in the population that can as well provide an indication of growth of smaller cod. These analyses are described in detail in Hüssy et al (2018).

**Quantification of growth from otolith daily rings**

Otoliths of fish grow on a daily basis, where daily growth layers are visible throughout the first years of a fish's life (Waldron and Kerstan 2001; Cermeño et al. 2003; Paul and Horn 2009). Counting the zones provides an estimate of the fish's age. Additionally, measurements of otolith growth between these zones allow back-calculation of fish growth from hatch to capture (Campana 1990; Li et al. 2008). In the present study, we utilized this technique to determine age and estimate individual growth patterns for eastern Baltic cod. For these analyses, individuals in the size range 15 - 35 cm were selected randomly from the Danish samples collected during the Baltic International Trawl Survey (BITS) from the first quarter of the years 2001, 2004 and 2013 in ICES Subdivision (SD) 25.

Growth rates in the known-age samples were estimated at 9.5, 7.8 and 5.7 cm per year for age classes 1, 2 and 3 respectively. Growth between age 2 and 3 decreased significantly from 8.8 cm in the 1997 year class to 7.6 cm in the 2010 year class (Fig. 4.1.1).



*Figure 4.1.1. Estimated growth by year-class, based on otolith daily rings (Hüssy et al. 2018).*

We also tested whether the known-age samples were representative of the population with respect to somatic condition within the same size range. This comparison showed that in 2001 and 2004, the average condition of the known-age fish and the population were similar. In contrast, the known-age sample from 2013 was biased towards individuals with a significantly higher condition than the

population average within the same size range. Thus, the growth in this sample may be overestimated. In the known-age samples, growth was positively related to somatic condition at capture.

**Growth indications from length frequency data**

Individual year-classes of cod can be detected in length frequency data only when stronger year-classes occur, following weaker ones. Thus, these analyses were restricted to 2003 and 2011 year classes. The length distribution of the population (from BITS survey), following the 2003 year class, showed a peak at 23 cm at age 2 (in 2005) and at 31 cm at age 3 in the following year (Fig. 4.1.2), corresponding to an average growth of 8 cm. For the 2011 year class, the peak in length distribution at age 2 was at 24 cm and at 29 cm at age 3, corresponding to an annual growth of 5 cm. Size at age 2 was thus similar between the two year classes. However, growth from age 2 to age 3 decreased from 8 to 5 cm in the 2011 year class. Growth of the 2003 year class estimated from the population length frequency corresponded with growth estimated from the known-age samples' 2003 year class (8.3 cm). However, for the 2011 year class, the length distribution derived growth of 5 cm is considerably lower than the estimates from the 2011 known-age year class (7.7 cm). This supports the hypothesis that the 2013 known-age sample was biased towards individuals with faster growth.

Similar magnitude of change in growth as in Q1 is estimated from length frequency data from Q4 (Fig. 4.1.2).
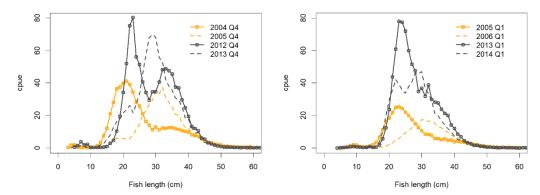


*Figure 4.1.2. Length frequency distribution of EB cod in BITS Q4 (left panel) and Q1 (right panel) survey in selected years, to track the peaks corresponding to stronger year-classes from 2003 and 2011.*

These analyses altogether suggest that growth of at least younger cod has declined in 2000s. The potential magnitude of change in growth of cod older than age 3 remains unclear, as back-calculating growth from daily increment patterns of the otoliths is not possible for older cod, and year classes of older cod cannot be identified from length frequency data either.

## 4.2 Links between growth and condition – information from other stocks

*By Lars O. Mortensen (DTU Aqua)*

In this investigation, we aimed to link nutritional condition of fish to growth in stocks for which this information is available, and could assist in the estimation of growth of the Eastern Baltic cod. To make generic assessment of the effect of condition on growth, multiple stocks and species from various areas in the Northern hemisphere were used (Table 4.2.1). It was hypothesised that above average condition would lead to above average growth as the improved condition would allow for an increased allocation of energy to somatic growth.

To do this, data on annual mean length-at-age (LAA) and weight-at-age (WAA) on 15 stocks were analysed, for which both weight and length at age were available (Table 4.2.1). The annual nutritional condition (C) of an age group within each stock was calculated by regressing the log LAA on the log WAA in each year and stock, and derive the residual deviance from the regression to each age group in each year. These residuals defined the annual condition of that age group and is an expression of the deviance of the length-weight relationship of an age-group each year. Negative residuals indicated a less than average condition of the fish, while positive residuals indicated a higher than average condition of the fish.

*Table 4.2.1. Overview of data used for analysis, including sample areas, years included and age range of each stock.*

| Stock | Specie | Area | Years | Ages |
|---|---|---|---|---|
| Arctic (AC) | Cod | 1 & 2$^{ICES}$ | 1985-2014 | 1-8 |
| East Baltic (EB) (*BITS*) | Cod | 22-28$^{ICES}$ | 1992-2005* | 1-6 |
| | Sprat | | | |
| | Herring | | 2001-2014 | 1-6 |
| | Plaice | | 1994-2015 | 1-4 |
| North Sea (NS) (*NS-IBTS*) | Cod | 3a. 4a.b.c & 7d$^{ICES}$ | 2001-2016 | 1-6 |
| | Haddock | | 2000-2015 | 1-4 |
| | Herring | | 2000-2015 | 1-4 |
| | Plaice | | 2003-2015 | 1-6 |
| | Sprat | | 2000-2015 | 1-3 |
| | Whiting | | 2000-2015 | 1-6 |
| Northern Canada (NC) | Cod | 2J3KL$^{NAFO}$ | 1982-2014** | 1-7 |
| Norwegian coast (NW) (*Costal survey*) | Cod | 1 & 2$^{ICES}$ | 1996-2015 | 1-9 |
| Southern Gulf of St. Lawrence (SG) (*DFO research survey*) | Cod | 3Pn, 4R, 4S$^{NAFO}$ | 1961-2013 | 1-11 |
| *data from after 2005 was removed from the analysis, as age readings after this period contained a disproportionate large variation. **data from 2008 was NA and was left out. | | | | |

Growth of the fish in each age-group was defined as the percentage change in average LAA from year y to year y+1. Thus, the annual growth in length of each age group was calculated by:

$$G_{a.y} = \frac{L_{a+1.y+1}}{L_{a.y}}$$

Where L is the length-at-age, a is the age group and y is the year.

The relationship between condition and growth was analysed, using linear regression, with condition as independent variable and growth as dependent variable. As growth decreases exponentially with length and length is an inherent part of the condition measure, the log(LAA) was also included in the model as independent variable to detrend the data.

Potential effects of condition on growth in different periods corresponding to large changes in LAA or WAA, were also investigated. This is because we hypothesized that more pronounced effects might occur at more extreme levels of growth or condition. To investigate periods of large changes in LAA and WAA, the time series of each stock was divided into two parts of different LAA or WAA regimes. The cutoff was done visually, to group the data sets into two more homogeneous periods of either temporal increase, decrease, stable or highly fluctuating LAA/WAA. Each stock was only split in two and each period for each stock was analysed. Effects of condition on growth in the two periods were subsequently compared.

Regressing the growth and condition, using the annual growth-at-age and condition-at-age for each stock, yielded significant models for all stocks, with length-at-age contributing significantly to each model. However, condition contributed significantly to eight of the stocks, with a partial R2 ranging from 0.01 to 0.27 and an average partial R2 of 0.145 (Fig. 4.2.1).

The analysis also divided all time-series into two sections, with different temporal development. This analysis demonstrated that 11 out of the 15 stocks had at least one period where condition had a significant influence on the growth.

In summary, these investigations demonstrated that there likely is a positive relationship between condition and growth across stocks, areas and time-periods. This is in line with the findings for younger individuals of EB cod (described in chapter 4.1). However, condition explained only a small part of the overall variation in growth patterns for most of the stocks, and the results were not entirely consistent across stocks. This makes is difficult to directly quantify growth of EB cod based on condition values.
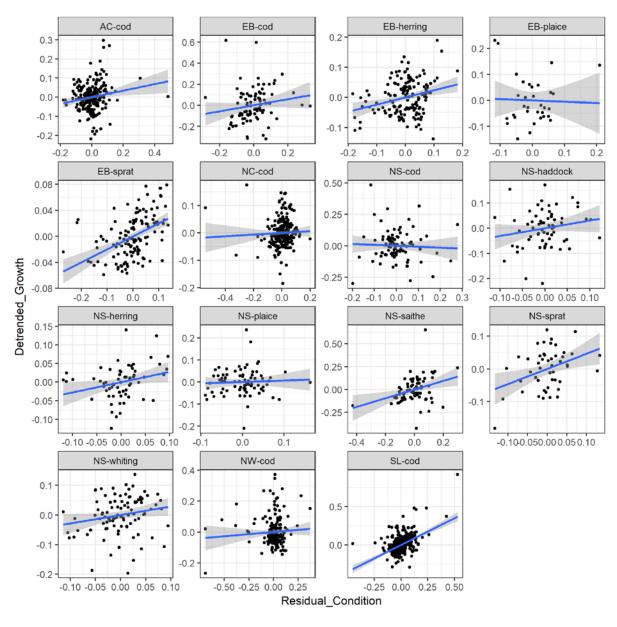


*Figure 4.2.1. Estimated effect of condition on growth of different stocks and species, where the data has been detrended by length and age.*

## 4.3 Proxies for growth change of Eastern Baltic cod

*By Margit Eero (DTU Aqua)*

In later years (since the mid-2000s), deviations in age-length-keys (ALK) between countries are severe (Hüssy et al. 2016), and major differences in stock trajectory and status could be obtained depending on which countries age information is used (ICES WKBALTCOD 2015). As precision of age estimation is known to decrease during unfavourable environmental conditions which affect fish growth (Yaragina et al., 2011), the increase in ageing problems may be the result of changing growth conditions in later years (Eero et al., 2015).

In years before 2007, differences in ALK between countries were less pronounced compared to later years and major inconsistencies in stock assessment were not detected at that time. Therefore, the age data from traditional age readings for before 2007 have been considered still applicable for stock assessment purposes (ICES WKIDEBCA 2018) and these data are used in the analyses to derive a proxy for plausible growth change. The data on age and length for individual cod from BITS surveys were used to obtain annual ALKs for years 1991-2006.

### Drivers & indicators for growth change

A number of changes in the Baltic ecosystem and in the cod stock have taken place in last decades, including a decline in nutritional condition of cod, reduced size at maturation and intensified hypoxia (Fig. 4.3.1). These changes are hypothesized to be associated with reduced growth (summarized in ICES WKBEBCA 2017), though direct relationships have not been demonstrated. Thus, there is a general consensus among the experts that the growth of EB cod must have declined as a result of the changes seen in cod biology and in the Baltic ecosystem.

The relative importance of the potential drivers/indicators for cod growth (condition, size at maturation, hypoxia) are not known, however major trends in respective time series are relatively similar, showing a decline especially in the period from late 1990s to 2010s, with some levelling off afterwards. Each of these time series was standardized (by subtracting the mean and dividing by standard deviation), and the standardized time series were subsequently averaged, to obtain an overall index (Fig. 4.3.1). The magnitude of change in this index from 1994-1997 to 1998-2001 was similar compared to the magnitude of change in the period from 2002-2006 to 2010-2014. No information on the type of relationship between these potential drivers/indicators and cod growth is available, and if present, such relationship would possibly not be linear. However, at lack of any other information, a simple assumption was made that the change in cod growth in the period from the average in 2002-2006 to the average in 2010-2014 is similar to the observed growth change from the average level in 1994-1997 to the average in 1998-2001.
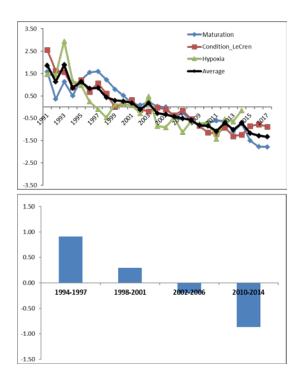
*Figure 4.3.1. Upper panel: Standardized time series of size at first maturation (L50) of cod, average condition of cod (Le Cren K) (estimated from BITS Q1 data in DATRAS) and extent of hypoxic areas (Casini et al. 2016). The time series of hypoxia is reversed to follow the same direction of trend as the other variables. The black line shows the average of the 3 standardized time series. Lower panel: The average index (shown as black line in the upper panel) averaged over the defined time periods.*

## Calculation of proxy ALK

The annual smoothed ALKs for Q1 BITS surveys were averaged over the years within the periods 1994-1997, 1998-2001, 2002-2006, corresponding to the time periods used for comparing changes in potential drivers/indicators for cod growth (Fig. 4.3.1). The resulting average length frequency distributions of age groups in the three time periods are shown in Fig. 4.3.2. As a next step, mean length at age was calculated for each age group, in the three time periods (Table 4.3.1).

The difference in mean length at age between time periods was calculated by dividing average mean length at age in 1998-2001 with the average mean length at age in 1994-1997, for each age group. Mean length at age of younger ages (1-2) was not consistently different between the two time periods. For age 3+ the mean length at age was lower for all ages in 1998-2001 compared to 1994-1997. The difference in mean length at age between the two periods was largest for middle age groups (3-5) (up to 10%), and less for older ages (6+) (2-6%). As an average for ages 3-9 (older fish not included due to few individuals), mean length at age was ca 6% lower in the later time period (Fig. 4.3.3).

A similar proportional change (6%) in mean length at age was assumed in the period from 2002-2006 to 2010-2014. The mean length at age for 2010-2014 was subsequently calculated by multiplying the mean length at age for 2002-2006 with 0.94, for ages 3+ (Q1) or 2+ (Q4). For younger ages, mean length at age in 2010-2014 was set to be the same as in 2002-2006. Subsequently, the corresponding difference in mean length at age in cm-s between 2002-2006 and 2010-2014 was calculated, shown in the table below:

| Age | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| diff_cm | 0 | 0 | 2.2 | 2.8 | 3.3 | 3.9 | 4.7 | 4.9 | 5.5 | 5.8 | 6.2 | 6.3 |

Next, to obtain ALK for 2010-2014, the percentage length distribution within each age group in ALK for 2002-2006 was moved towards smaller size-classes by the cm-s shown in the table above (rounded to the closest cm).

This resulted in average ALK for years 2010-2014 (Fig. 4.3.4). The average ALK for intermediate years (2007-2009) was calculated as an average of ALK in 2002-2006 and 2010-2014. This is considered to represent a minimum estimate for growth change, as for the more abundant age groups in the stock (3-5) the drivers/indicators suggest that the size at age could be lower than corresponding to the average 6% reduction applied here.

In subsequent years (2014 onwards), cod condition has remained stable, while size at maturation has further declined (Fig. 4.3.1). Consequently, the size at age may have further declined from 2014 onwards.



*Figure 4.3.2. Average length frequency distribution of cod age-groups (in percentage) in periods 1994-1997, 1998-2001 and 2002-2006, based on Q1 BITS data for SD 25-28. The length distributions are shown for ages 1-6.*

*Table 4.3.1. Mean length at age (cm) in different time periods, based on BITS Q1.*

| Age | 1994-1997 | 1998-2001 | 2002-2006 |
|-----|-----------|-----------|-----------|
| 1 | 11.7 | 12.2 | 11.9 |
| 2 | 23.9 | 24.1 | 24.2 |
| 3 | 39.4 | 35.8 | 36.3 |
| 4 | 51.8 | 46.4 | 46.3 |
| 5 | 59.6 | 54.6 | 54.3 |
| 6 | 67.7 | 65.0 | 65.7 |
| 7 | 79.1 | 77.7 | 77.5 |
| 8 | 89.3 | 84.1 | 82.3 |
| 9 | 92.3 | 90.3 | 91.2 |
| 10 | 99.6 | 98.2 | 96.7 |

*Figure 4.3.3. Ratio between average mean length at age in 1998-2001 (Period2) and average mean length at age in 1994-1997 (Period1).*



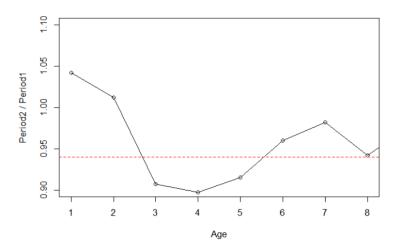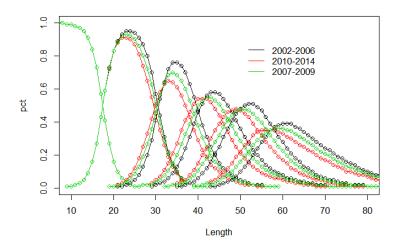*Figure 4.3.4 Average relative length frequency distribution (in pct) of cod age-groups in 2002-2006, based on age reading data from BITS Q1 in SD 25-28, compared to the constructed ALK for 2010-2015 and 2007-2009. The length distributions are shown for ages 1-6.*

## Validation of the proxy ALK with length frequency data

Given the unknown relationships between cod growth and the drivers/indicators for growth described above, also alternative indications of the magnitude of growth change in later years were explored. Length frequency distribution (LFD) data comparing the progression of two relatively stronger year-classes formed in 2003 and 2011 (based on Köster et al. 2017) were used as an indication of growth change in this period. The same data we also used to cross-check the growth estimates from daily otolith rings (Fig. 4.1.2).

The presence of a stronger year-class among weaker ones makes it possible to follow a specific year-class as peaks in LFD data, at least for younger ages. Due to low representation of <20 cm cod in BITS survey catches, these relatively stronger year-classes from 2003 and 2011 are first visible in Q4 survey

36

data as peaks in 2004 and 2012 (corresponding to age 1) and in Q1 survey data as peaks in LFD in 2005 and 2013, (corresponding to age 2)(Fig. 4.1.2). In subsequent years (2005 and 2013 for Q4 data and 2006 and 2014 for Q1 data), movement of these peaks towards larger sizes is detectable, corresponding to the particular year-classes at age 2 (Q4) or age 3 (Q1) (Fig. 4.1.2). The average length of cod corresponding to these peaks was calculated as the average of the 3 highest values in LFD:

| Year-class | Length at age 1 (Q4) | Length at age 2 (Q1) | Length at age 2 (Q4) | Length at age 3 (Q1) |
|---|---|---|---|---|
| 2003 YCL | 21 cm | 23 cm | 31 cm | 31 cm |
| 2011 YCL | 23 cm | 24 cm | 29 cm | 29 cm |

Length at smallest age (age 1 in Q4 and age 2 in Q1) does not appear to be lower for the 2011 year-class compared to 2003 year-class. In fact, mean length at age is somewhat higher for the later year-class for these youngest ages. However, this could be related to the long spawning time of the EB cod, as if the survivors originate from early in the spawning season, these are expected to have a larger length at age compared to those originating from the end of the spawning season. Similar length at age in the two periods is however in line with the proxy estimates based on observed changes in growth from 1994-1997 to 1998-2001, where no large changes in length at age were apparent for younger ages.

Mean length at age in subsequent year was however by 2 cm lower for the 2011 year-class (31 cm) compared to 2003 year-class (29 cm). This was consistent both for Q1 and Q4 data, implying a difference of 6 % (29/31=0.94) in mean length at age between in these periods. In terms of reduction in growth, this is a conservative estimate, as the 2011 year-class started out at a larger size than the 2003 year-class, as described above. The proportional change in mean length at age (6 %) indicated from length frequency data, is in line with the proxy change in mean length at age derived based on change in drivers/indicators for cod growth.

# 5. Standardized Length Based Survey Indices for Eastern and Western Baltic Cod

*By Casper W. Berg & Kasper Kristensen, DTU Aqua*

## 5.1 Data

The data is coming from the BITS survey in the DATRAS database. The data set used to fit the model used for the standardization consists of almost the entire BITS database, although the indices of interest are computed for smaller sub-regions. Hauls with "HaulVal" codes "V", "A","N" and "C" and with "StdSpecRecCode" equal to 1 or 3 are included in the analysis. All gear types are included, except those that have been used for less than 120 hauls (this criterion excludes also pelagic trawls, see figure 7). Data from ICES areas 20 and 21 are excluded, except those below 56.5° latitude (i.e. a northern edge of one extra ICES square going into Kattegat, to improve estimates near the edge of the domain boundary). A few north eastern hauls (east of 22° longitude and north of 58.15° latitude) have also been excluded, because including these would lead to inclusion of many unsampled grid cells with low abundance in the model, which would increase computation time without improving the indices.

At some stations with no oxygen (HaulVal code "N") no haul was performed (haul duration 0 or NA). In order to inform the model that the abundance are likely to be low at these stations, the haul duration was set to 10 min for these hauls, i.e. one third of the conventional duration of 30 minutes. This means that these zero observations are included in the model estimates of abundance, but they are not given as much weight as an actual performed haul.

Although most length data are registered using 1 cm resolution, the data are divided into coarser size bins at the tails of the size spectrum prior to the analysis in order to reduce the computational load. The following size groups (first one is 9 cm and below, second one is 10 and 11 cm, etc. ) are used:

```
[0,10), [10,12), [12,14), [14,16), [16,18), [18,20), [20,21), [21,22), [22,23), [23,24)
[24,25), [25,26), [26,27), [27,28), [28,29), [29,30), [30,31), [31,32), [32,33), [33,34)
[34,35), [35,36), [36,37), [37,38), [38,39), [39,40), [40,41), [41,42), [42,44), [44,46)
[46,48), [48,50), [50,52), [52,54), [54,56), [56,58), [58,60), [60,200)
```

### 5.1.1 Bathymetri

The Baltic Sea Bathymetric Database (BSBD, [1],500 m resolution) is used to define the domain of interest, and the survey indices are calculated by summing up standardized catch rates over all the grid cells. To speed up the calculations the resolution of the final grid was reduced to 2500 m. See figure 8 for a plot of the final grid.

## 5.2 Model

The statistical model estimates the expected catch of each size group $s$ at each point in space $x$ and time $t$ for all gear types. The model is known as a log-Gaussian Cox model, which describes large-scale abundance fields and local patchiness using correlated log-Gaussian variables, and if these were known, the catch numbers would be Poisson distributed [4]. Unlike the model in [4] we do not assume any population dynamics structure (this is the job of the assessment model that uses the results of this analysis). The objective of our model is to provide standardized estimates of the total catch at length, i.e. the result of a virtual survey where hauls were taken at the

exact same time in every grid cell using the same reference gear. Another way of formulating this is that we want the model to filter out the effects of changes in the survey design on the indices, most importantly gear effects and effects of unequal sampling intensity over the domain of interest. We also want to filter out random noise, which is also important especially for very patchy species where a single haul with a large catch can determine the value of an unfiltered survey index estimate. Rather than estimating a 4-dimensional field in (size, space x, space y, time) as in [4] we choose to estimate independent models for each size group in order to reduce the computational burden. The model is implemented in Template Model Builder [3].

### 5.2.1 Process equations

For a given size group the field $\eta(x, t)$ is a Gaussian zero-mean stochastic process which is correlated in space, and time. We assume a multiplicative correlation structure, such that the auto-covariance function of $\eta$ can be factorized into two terms a spatial correlation $\rho_{\mathrm{space}}$, and a time correlation $\rho_{\mathrm{time}}$:

$$\mathrm{Cor}\left(\eta(x, t) \cdot \eta(x + \Delta x, t + \Delta t)\right) = \rho_{\mathrm{space}}(x, x + \Delta x) \cdot \rho_{\mathrm{time}}(\Delta t)$$

Time is discretized in two steps per year (again to reduce computational demands). The correlation function $\rho_{\mathrm{time}}$ is as assumed to be that of an stationary AR(2) process, i.e. damped exponential and/or sine functions. This formulation is able to capture seasonal recurring patterns in the spatial distribution of a given length group. For example, the next year's distribution in quarter 1 can have a higher correlation with last year's quarter 1 than last year's quarter 4, even though the latter is closer in time. This is an extension compared to [4], where the correlation was exponentially decreasing function of time.

The geographical area discretized into cells in a uniform grid, assuming that the field $\eta(s, \cdot, t)$ is constant within each grid cell. Thus, the auto-covariance function $\rho_{\mathrm{space}}$ is replaced by a covariance matrix $\Sigma_{\mathrm{space}}$. The precision matrix $Q$, which is the inverse of the covariance matrix $\Sigma_{\mathrm{space}}$, is specified as follows:

$$Q_{ij} = \begin{cases} -q & \text{if cell } i \text{ neighbors cell } j \\ q \cdot (m_i + \delta) & \text{if } i = j \\ 0 & \text{else.} \end{cases}$$

Here, $m_i$ is the number of neighbors of grid cell $i$; typically 4 but less for boundary cells. $q$ and $\delta$ are parameters which are estimated. This precision structure implies that a cell is conditionally independent of a distant region, when conditioning on the cell's neighbors. The parameters $q$ and $\delta$ can be transformed into a variance parameter $\sigma^2$ and a decorrelation range parameter $H$ as follows:

$$\sigma^2 = \frac{1}{M}\mathrm{tr}\left(Q^{-1}\right) \quad , \qquad H = \frac{h}{\log(1 + \frac{\delta}{2} + \sqrt{\delta + \delta^2/4})} \quad . \tag{1}$$

Here $M$ is the number of grid cells and the trace $\mathrm{tr}\left(Q^{-1}\right)$ is the sum of diagonal elements of $Q^{-1}$, so that $\sigma^2$ is the spatially averaged variance of the field $\eta(s, \cdot, t)$. $h$ is the grid cell size. If the domain had been a one dimensional line, then $\sigma^2$ would be the variance while $H$ would be the decorrelation range, so $\Sigma_{ij} = \sigma^2 \exp(-|i - j| \cdot h/H)$. Similar for a 2D spatial field this implies that the correlation decreases with distance traveled through water.

### 5.2.2 Observation equations

If $N_i$ is the number of individuals in at a given size group in the $i$th haul, then

$$N_i \mid \eta(x_i, t_i) + \eta_0(i) \sim \quad \mathrm{Poisson}\Big( \exp\big(\eta(x_i, t_i) + \mathrm{t}(i) + \mathrm{Gear}(i) + f_1(\mathrm{depth}_i, \mathrm{Quarter}_i)$$

$$+ \mathrm{TimeOfDay}(i) + \log(\mathrm{HaulDur}_i) + \eta_0(i)\big)\Big)$$

where $\eta(x_i, t_i)$ is the random space-time field, and $\mathrm{t}(i)$ and $\mathrm{Gear}(i)$ are categorical fixed effect parameters of the time-step ( discretized into quarterly bins) and gear. The "TVL" gear is chosen as the reference gear, such that the "TVL" gear effect is set to zero and all other gears effects are relative to this gear type. The $f_1$ function is a second degree polynomial (re-scaled to have mean zero over when evaluated in the observed depths) with distinct parameters by quarter. The maximum of the second degree polynomial (assuming a negative coefficient for the quadratic term) can be interpreted as a "preferred depth" for a given length group. The $\mathrm{TimeOfDay}(i)$ term maps the time of day into one of three categorical levels { 1 = Mid-day (10-14), 2 = early/late day (7-10 and 14-17), 3 = night (the rest) }. The catch is assumed to be proportional to the haul duration, so this is included as an offset (linear term with assumed coefficient of 1). Finally, there is a haul specific independent random effect $\eta_0$, the so-called nugget effect. The resulting Lognormal-Poisson mixture is similar to a negative binomial distribution, due to the similarity of the Lognormal and the Gamma distribution and because the negative binomial distribution is the same as a Gamma-Poisson mixture.

For further details about the statistical methodology the reader is referred to [4].

### 5.2.3 Index uncertainties

This subsection describes how the uncertainty (coefficient of variation) for the total catch in numbers (summed over space and length groups for each of the time points) is computed. The resulting CVs can be used as data weightings in the assessment model.

The survey index is obtained by summing the expected values of the observation equation in every grid cell at the times of interest using fixed values for all nuisance parameters (Gear, time of day, haul duration). Since models are fit independently by length group, the error distribution around the survey index estimate can also only be estimated for one length group at a time, i.e. correlations between length groups cannot be estimated within the model. We compute the uncertainty for the log of the survey index for a given length group using the delta method (through the ADREPORT functionality in TMB). The log transformation is chosen, because the log index is typically better approximated by a normal distribution than the index without takin logs.

An approximate expression for the variance of the logarithm of the total abundance of all length groups (in numbers and for a given point in time), $\mathbb{V}(\log \sum I)$, in a given time-step can be found using the delta method, given that we have an estimate of the variance of the log of the individual length groups $\mathbb{V}(\log I_l)$:

$$\mathbb{V}\left(\log\left(\sum I\right)\right) = \frac{I}{\sum I}^T \Sigma_{\log I} \frac{I}{\sum I} \tag{2}$$

where $I$ is the vector of survey indices by length, and $\Sigma_{\log I}$ is the covariance matrix for the log of that vector $\log I$ (which is a diagonal matrix due to the independent model fitting). The square root of this expression ( standard deviation of the log indices ) is approximately equal to the CV of the total abundance, which may be used for data weighting in the assessment model.

## 5.3 Results

The survey indices obtained from the LGCP model are in overall agreement with the ICES indices in terms of the overall abundance trends (Figures 1 to 5). The largest discrepancies (and uncertainties) are found in the Q4 indices before year 2000 (see figure 3), because of reduced spatial coverage and mixture of gear types in this period. Two examples where the discrepancy is large are in 1994 Q4 and in 2008 Q4. At those time points ICES biomass estimates are 2.71 and 1.65 respectively (relative to a mean of 1), whereas the LGCP estimates are 0.87 and 2.45 respectively. The spatial plots (see Appendix) reveals that this discrepancy can be explained by the spatial coverage: in 1994 Q4 the majority of the assessment area was not covered, while in 2008 Q4 it seems that central areas predicted to contain high abundances by the LGCP model were sampled less intensively compared to other years.

ICES area 24 (see figure 9) is known to contain a substantial proportion of EB cod. Therefore figure 6 compares biomass indices (scaled to mean 1) computed from the LGCP model over two different regions, namely the standard EBcod assessment area (25-32) and one which also includes the eastern part of area 24 (area east of 13° longitude). It is seen that the indices are very similar, although the ratio between the two indices is above 1 around the time when the abundance of larger cod dropped drastically (year 2010), while it tends to be below 1 in the latest years. This implies that the decline in abundance is stronger in areas east of are 24. This could indicate that a small part of the drop in the standard indices can be explained by an increasing proportion of EB cod occupying area 24. The spatial plots confirm this pattern (e.g. Figure 23). The high concentration of abundances around the eastern and western stock border between area 24 and 25 is likely to cause problems due to mixing of the two stocks.
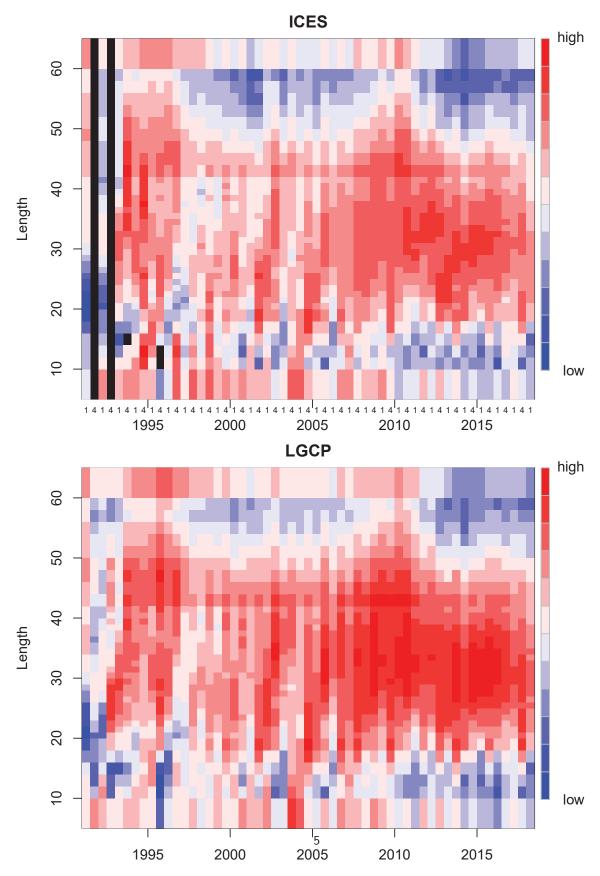
Figure 1: Top: ICES indices (aggregated to the same size bins as the LGCP index).
Bottom: Estimated indices from the LGCP model. The color scale is equidistant on a logarithmic axis, and the x-axis alternates between quarter 1 and quarter 4. Black means missing (or zero).

Figure 2: As figure 1, but rather than the log-abundances this figure shows the length proportions within years (i.e. each year has been divided by the sum on natural scale).

Figure 3: Top: Relative discrepancy between ICES and LGCP indices.
Bottom: Standard deviation of LGCP index on log scale, which is approximately equal to the coefficient of variation.

Figure 4: Time-series of total biomass (scaled to have mean 1 over time). Biomasses are calculated assuming a time-invariant length-weight relationship ($W = aL^3$).

Figure 5: As figure 4 but for selected subsets of size groups.

Figure 6: As figure 4, but comparing LGCP indices using the standard EBcod area (SD25+) with an alternative assessment area (east of 13 degrees longitude).

# References

[1] Baltic Sea Hydrographic Commission. Baltic Sea Bathymetry Database version 0.9.3, downloaded from http://data.bshc.pro/ on 02-01-2018, 2013.
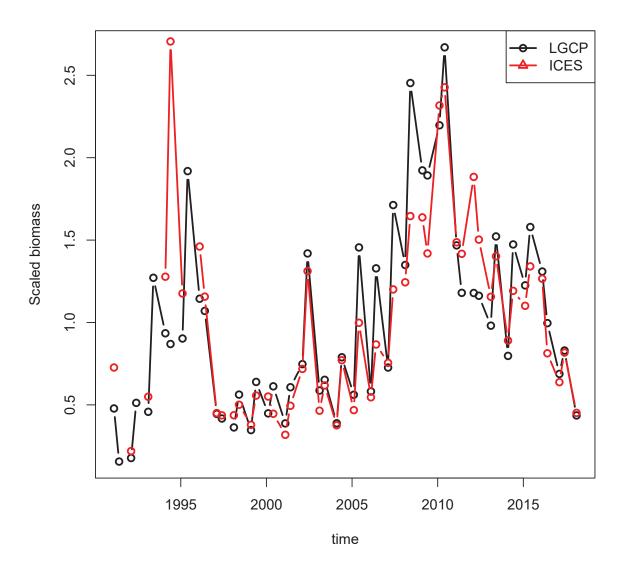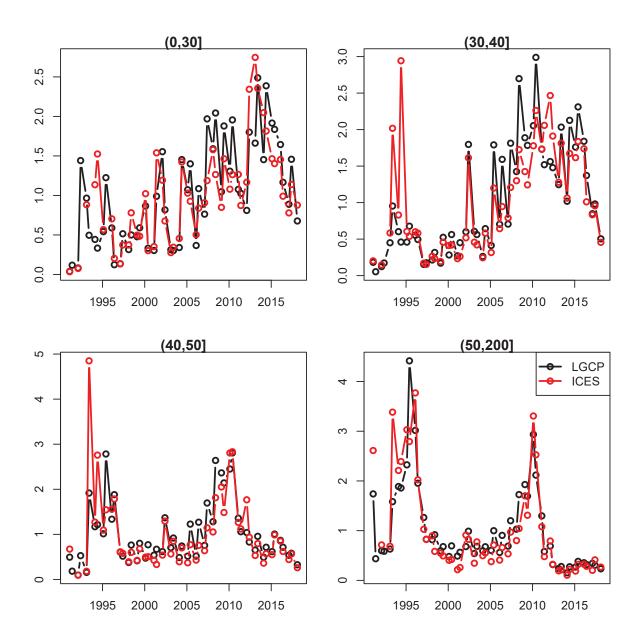
[2] Karin Hüssy, Krzysztof Radtke, Maris Plikshs, Rainer Oeberst, Tatjana Baranova, Uwe Krumme, Rajlie Sjöberg, Yvonne Walther, and Henrik Mosegaard. Challenging ICES age estimation protocols: lessons learned from the eastern Baltic cod stock. *ICES Journal of Marine Science*, 73(9):2138–2149, 2016.

[3] K Kristensen, A Nielsen, CW Berg, H Skaug, and B Bell. TMB: Automatic Differentiation and Laplace Approximation. *Journal of Statistical Software*, 70(5):1–21, 2016.

[4] Kasper Kristensen, Uffe Høgsbro Thygesen, Ken Haste Andersen, and Jan E Beyer. Estimating spatio-temporal dynamics of size-structured populations. *Canadian Journal of Fisheries and Aquatic Sciences*, 71(2):326–336, 2013.

# Appendix

|      | 1   | 4   |
|------|-----|-----|
| 1991 | 305 | 79  |
| 1992 | 203 | 50  |
| 1993 | 271 | 98  |
| 1994 | 277 | 91  |
| 1995 | 302 | 79  |
| 1996 | 349 | 92  |
| 1997 | 336 | 125 |
| 1998 | 370 | 99  |
| 1999 | 378 | 216 |
| 2000 | 369 | 228 |
| 2001 | 345 | 186 |
| 2002 | 262 | 210 |
| 2003 | 297 | 212 |
| 2004 | 302 | 207 |
| 2005 | 317 | 262 |
| 2006 | 278 | 210 |
| 2007 | 275 | 229 |
| 2008 | 282 | 234 |
| 2009 | 298 | 227 |
| 2010 | 286 | 224 |
| 2011 | 294 | 241 |
| 2012 | 272 | 227 |
| 2013 | 304 | 206 |
| 2014 | 252 | 226 |
| 2015 | 275 | 214 |
| 2016 | 279 | 267 |
| 2017 | 306 | 276 |
| 2018 | 307 | 0   |

Table 1: Number of hauls by year and quarter

|      | 21 | 22 | 23 | 24  | 25  | 26  | 27 | 28 |
|------|----|----|----|-----|-----|-----|----|----|
| 1991 | 0  | 76 | 0  | 68  | 64  | 119 | 15 | 41 |
| 1992 | 1  | 66 | 2  | 72  | 41  | 47  | 9  | 14 |
| 1993 | 1  | 55 | 5  | 81  | 111 | 71  | 12 | 33 |
| 1994 | 0  | 57 | 5  | 80  | 105 | 84  | 14 | 23 |
| 1995 | 0  | 34 | 5  | 77  | 111 | 99  | 11 | 44 |
| 1996 | 6  | 46 | 5  | 81  | 107 | 146 | 12 | 38 |
| 1997 | 2  | 59 | 1  | 77  | 124 | 148 | 15 | 35 |
| 1998 | 1  | 63 | 1  | 77  | 149 | 143 | 10 | 25 |
| 1999 | 11 | 61 | 4  | 129 | 205 | 120 | 11 | 53 |
| 2000 | 10 | 56 | 4  | 123 | 178 | 121 | 14 | 91 |
| 2001 | 11 | 64 | 4  | 111 | 146 | 95  | 19 | 81 |
| 2002 | 13 | 48 | 7  | 101 | 130 | 94  | 19 | 60 |
| 2003 | 12 | 44 | 6  | 92  | 154 | 121 | 24 | 56 |
| 2004 | 11 | 41 | 7  | 94  | 162 | 131 | 14 | 49 |
| 2005 | 11 | 56 | 6  | 88  | 179 | 148 | 23 | 68 |
| 2006 | 12 | 48 | 6  | 85  | 156 | 113 | 18 | 50 |
| 2007 | 13 | 49 | 4  | 87  | 148 | 120 | 18 | 65 |
| 2008 | 13 | 68 | 5  | 81  | 143 | 116 | 22 | 68 |
| 2009 | 9  | 50 | 6  | 99  | 172 | 101 | 22 | 65 |
| 2010 | 12 | 58 | 6  | 80  | 149 | 124 | 20 | 61 |
| 2011 | 13 | 59 | 6  | 95  | 175 | 109 | 21 | 57 |
| 2012 | 13 | 48 | 6  | 100 | 173 | 86  | 20 | 53 |
| 2013 | 13 | 49 | 5  | 92  | 171 | 105 | 15 | 60 |
| 2014 | 11 | 53 | 6  | 99  | 167 | 70  | 15 | 57 |
| 2015 | 12 | 61 | 8  | 88  | 167 | 85  | 17 | 51 |
| 2016 | 13 | 72 | 10 | 105 | 168 | 114 | 18 | 46 |
| 2017 | 13 | 72 | 11 | 98  | 205 | 109 | 19 | 55 |
| 2018 | 6  | 38 | 5  | 52  | 109 | 65  | 5  | 27 |

Table 2: Number of hauls by year and ICES area

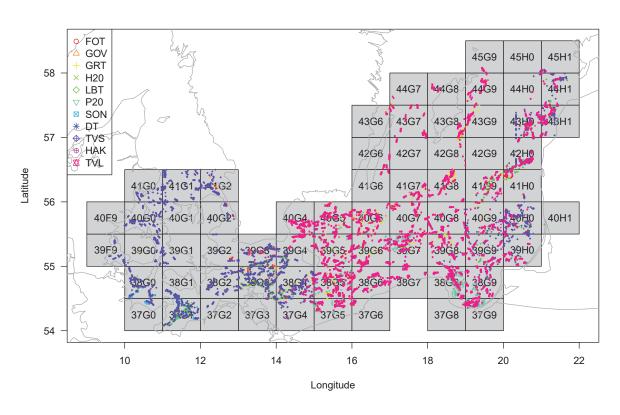| | FOT | GOV | GRT | H20 | LBT | P20 | SON | DT | TVS | HAK | TVL |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1991 | 59  | 6   | 64  | 48  | 59  | 72  | 76  | 0   | 0   | 0   | 0   |
| 1992 | 10  | 29  | 57  | 78  | 0   | 33  | 46  | 0   | 0   | 0   | 0   |
| 1993 | 44  | 32  | 71  | 119 | 25  | 50  | 28  | 0   | 0   | 0   | 0   |
| 1994 | 62  | 15  | 69  | 122 | 0   | 72  | 28  | 0   | 0   | 0   | 0   |
| 1995 | 31  | 46  | 68  | 104 | 0   | 47  | 18  | 67  | 0   | 0   | 0   |
| 1996 | 77  | 6   | 55  | 111 | 0   | 70  | 11  | 85  | 26  | 0   | 0   |
| 1997 | 89  | 0   | 49  | 79  | 20  | 141 | 20  | 0   | 22  | 41  | 0   |
| 1998 | 81  | 0   | 65  | 114 | 0   | 121 | 22  | 0   | 23  | 43  | 0   |
| 1999 | 52  | 39  | 59  | 114 | 39  | 61  | 10  | 0   | 72  | 40  | 108 |
| 2000 | 16  | 70  | 82  | 98  | 59  | 52  | 0   | 0   | 101 | 37  | 82  |
| 2001 | 0   | 0   | 0   | 0   | 0   | 11  | 0   | 0   | 224 | 0   | 296 |
| 2002 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 211 | 0   | 261 |
| 2003 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 205 | 0   | 304 |
| 2004 | 12  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 211 | 0   | 286 |
| 2005 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 233 | 0   | 346 |
| 2006 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 163 | 0   | 325 |
| 2007 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 171 | 0   | 333 |
| 2008 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 186 | 0   | 330 |
| 2009 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 212 | 0   | 313 |
| 2010 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 173 | 0   | 337 |
| 2011 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 188 | 0   | 347 |
| 2012 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 182 | 0   | 317 |
| 2013 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 176 | 0   | 334 |
| 2014 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 224 | 0   | 254 |
| 2015 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 184 | 0   | 305 |
| 2016 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 204 | 0   | 342 |
| 2017 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 198 | 0   | 384 |
| 2018 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 98  | 0   | 209 |

Table 3: Number hauls by year and gear

Figure 7: Map of all hauls (13402 in total) used by the model colored by gear type
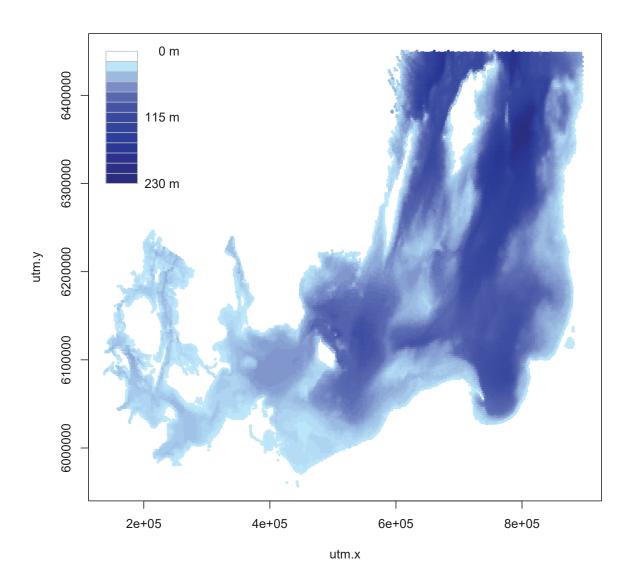
Figure 8: Bathymetric map (2500 m resolution) for the combined Eastern and Western Baltic cod areas.
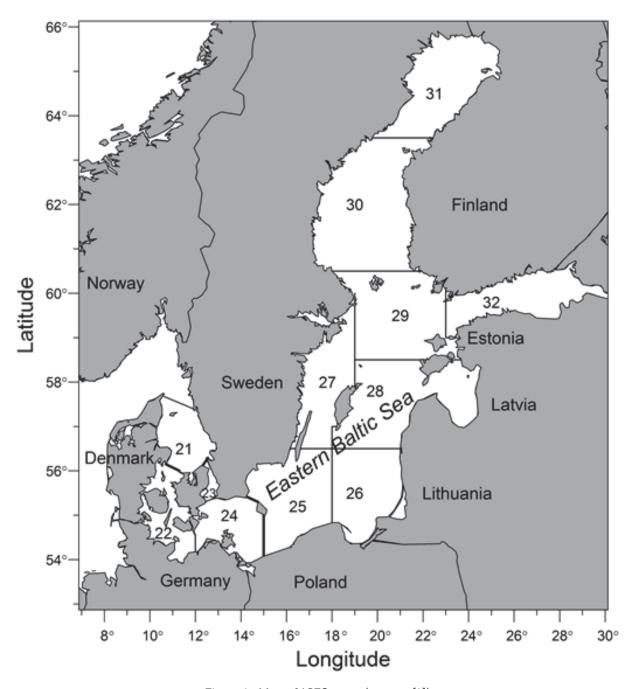
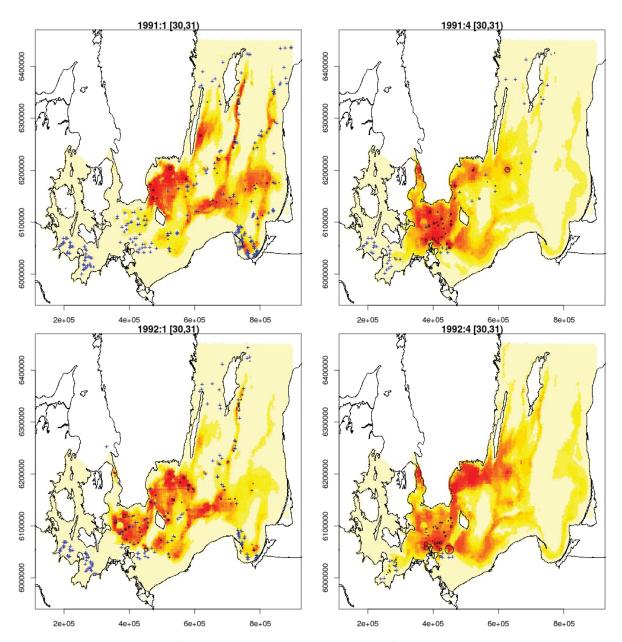Figure 9: Map of ICES areas (source: [2])

Figure 10: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
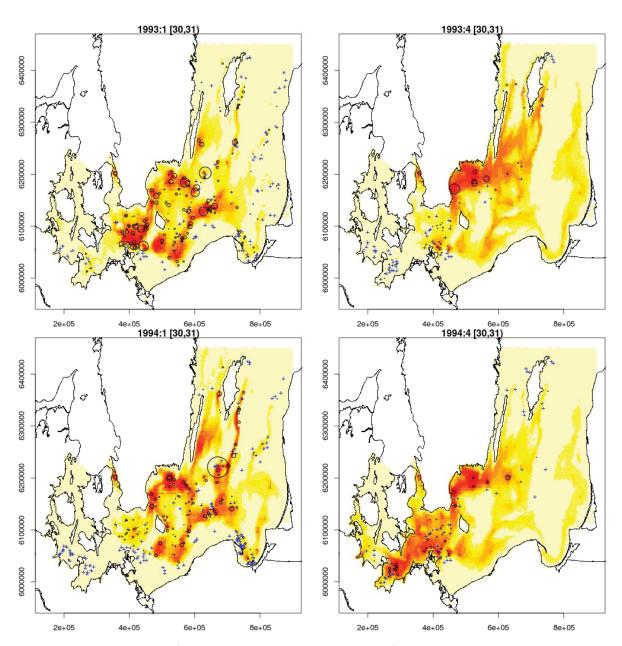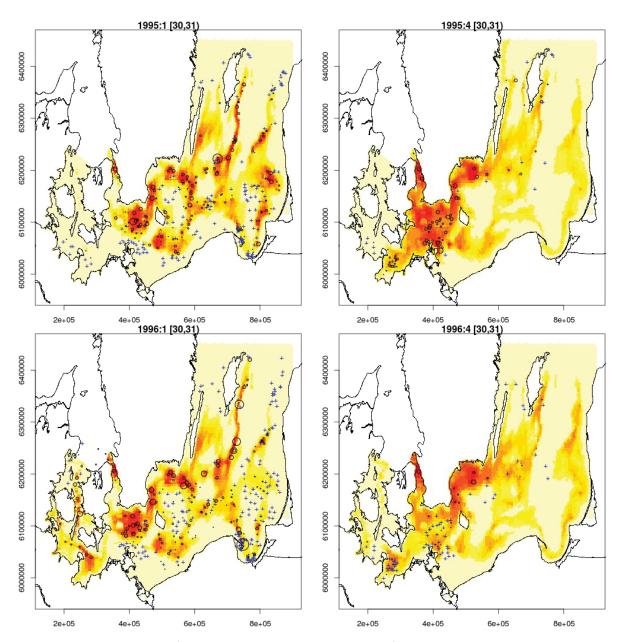
Figure 11: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.

Figure 12: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
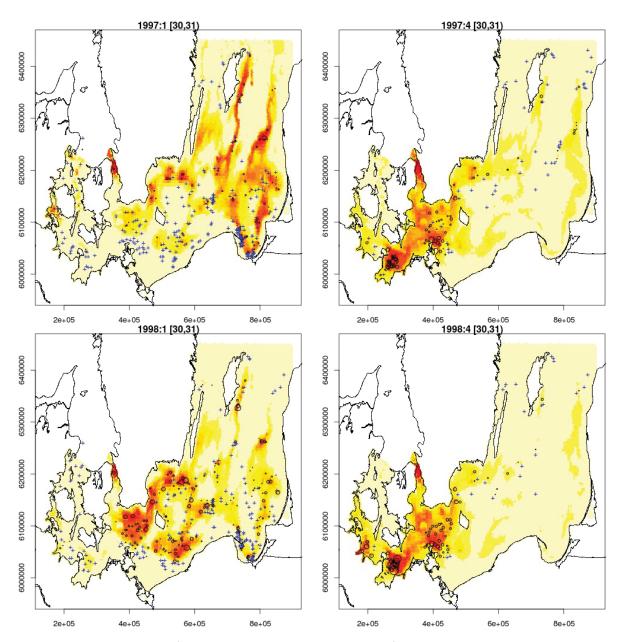
Figure 13: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
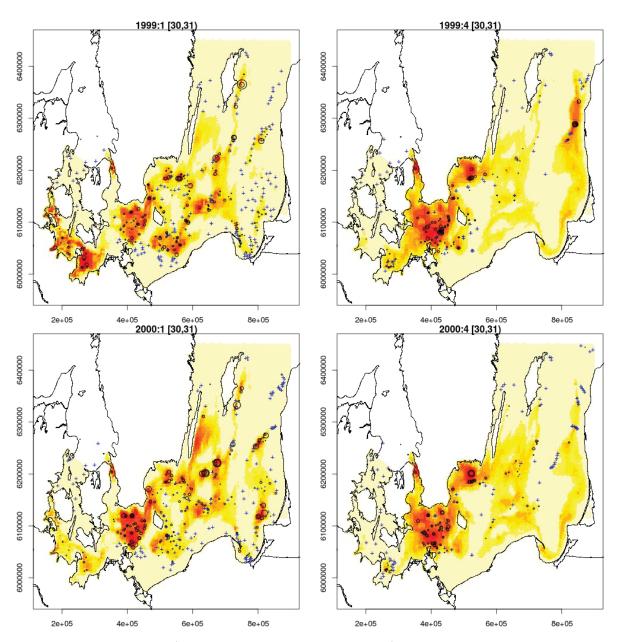
Figure 14: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
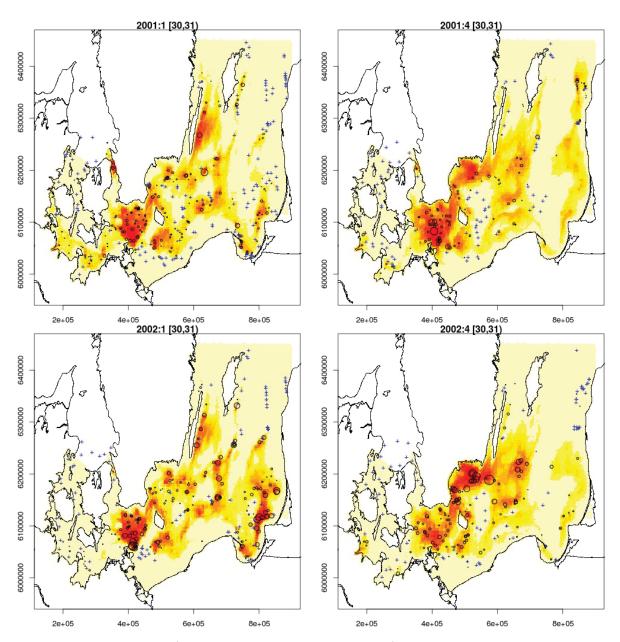
Figure 15: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.

Figure 16: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
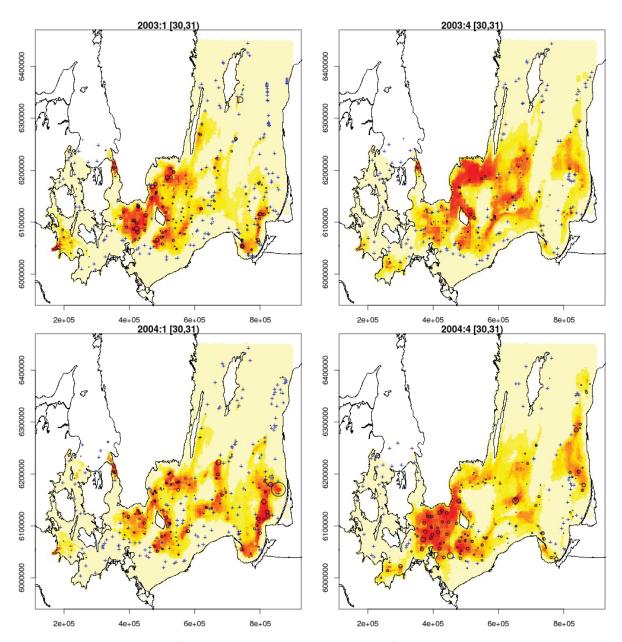
Figure 17: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.

Figure 18: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
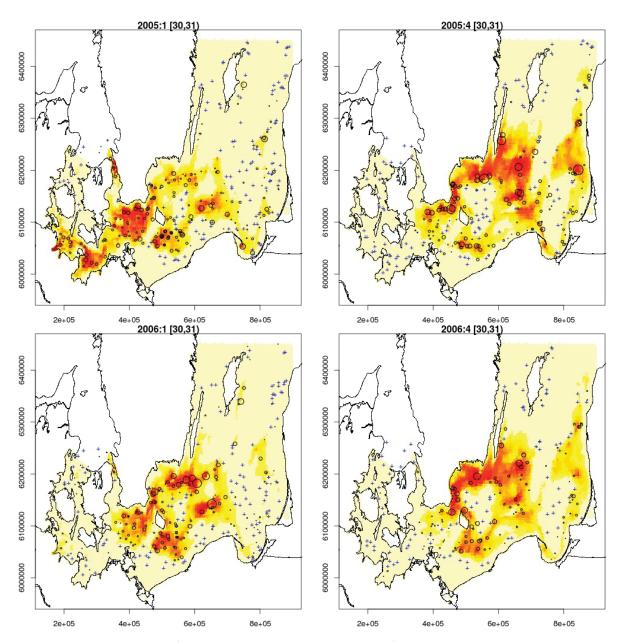
Figure 19: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
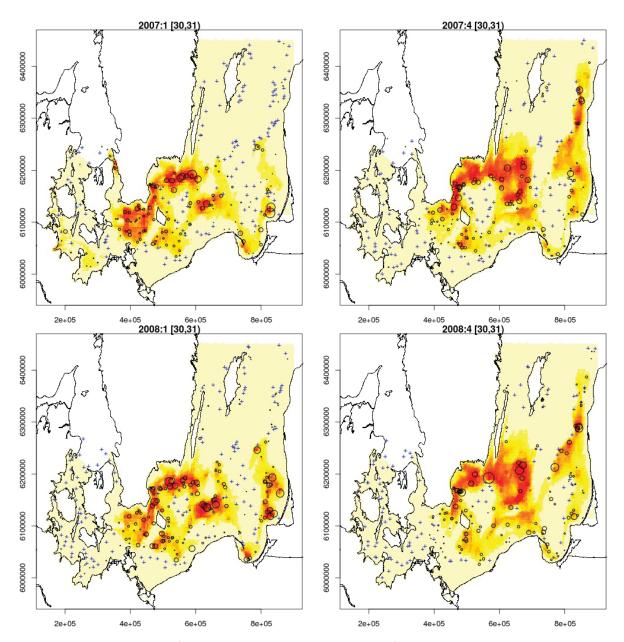
Figure 20: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.

Figure 21: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
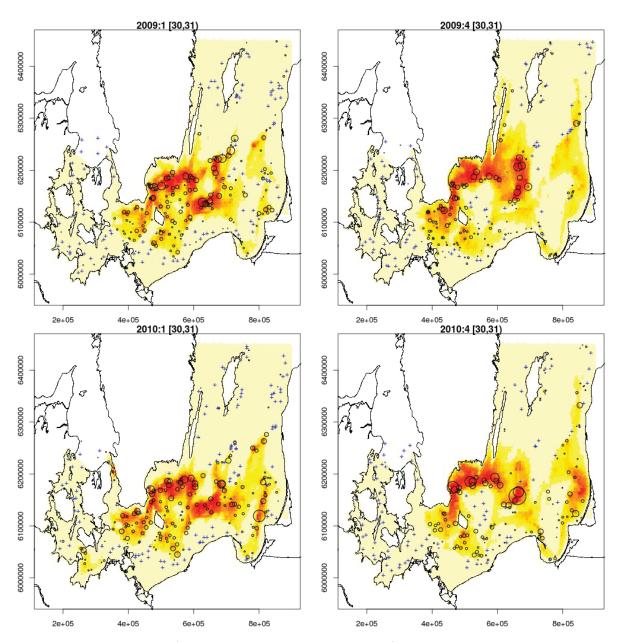
Figure 22: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
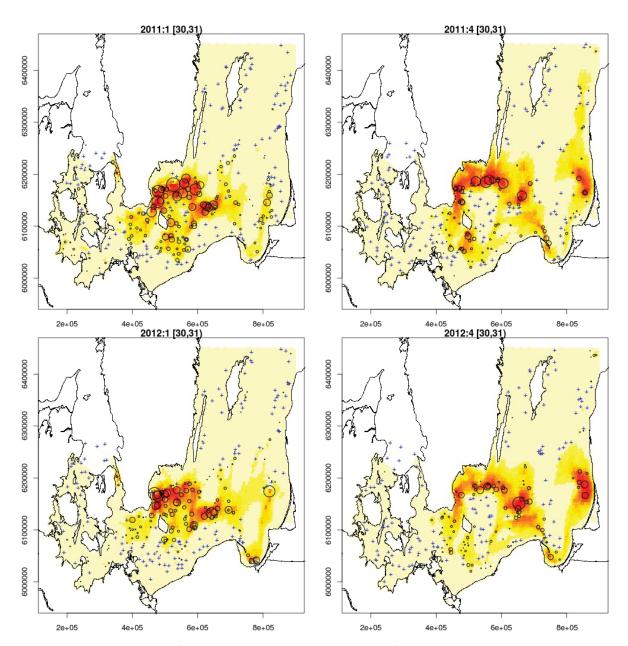
Figure 23: Concentration map (relative spatial abundance within a year) for the most frequently observed length group (30 cm). Hauls with positive catch rates are shown as circles with an area proportional to the numbers caught. Hauls with no catches are shown as blue plus signs.
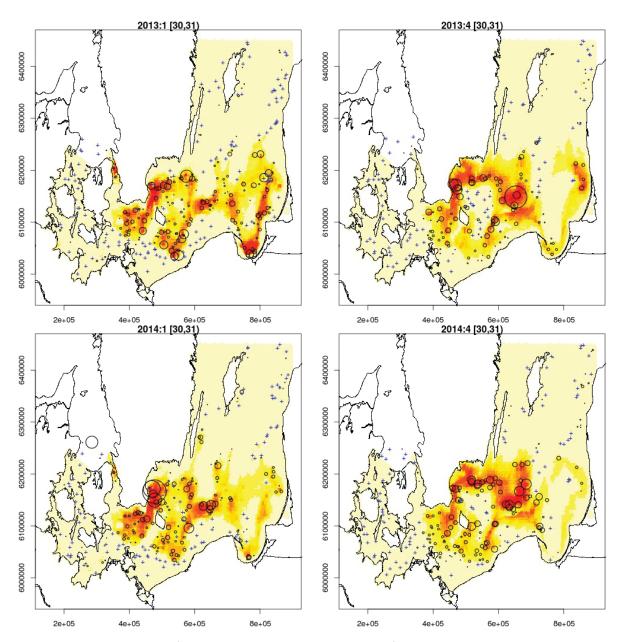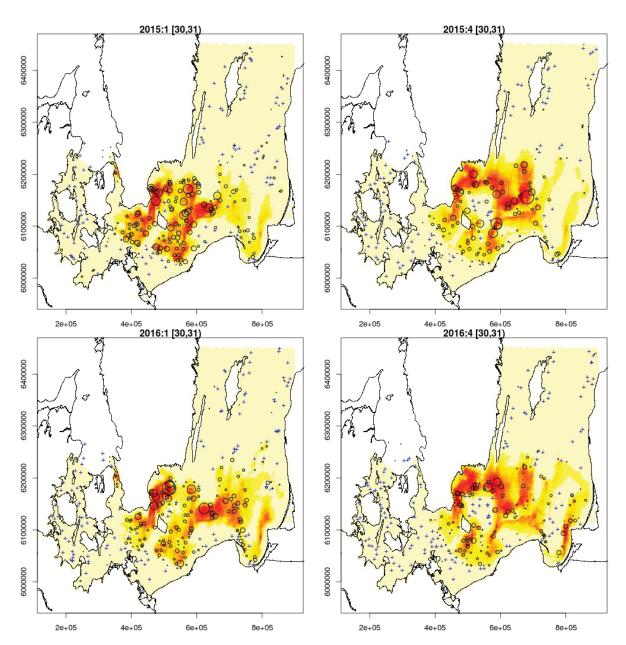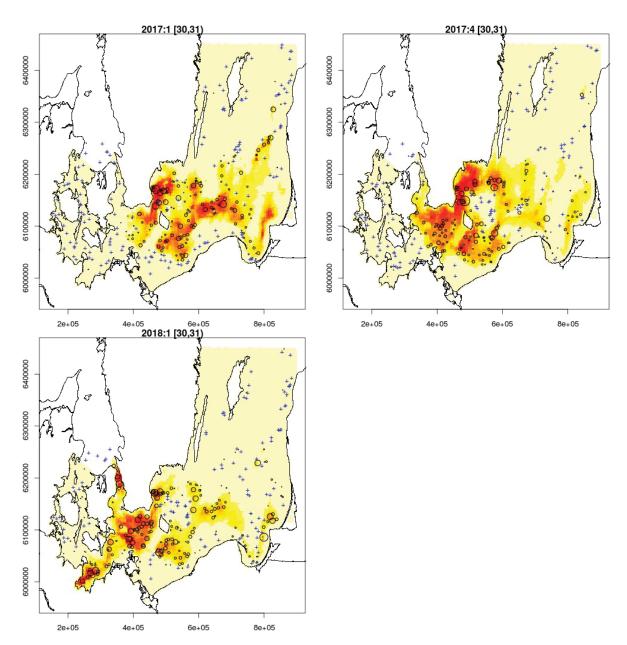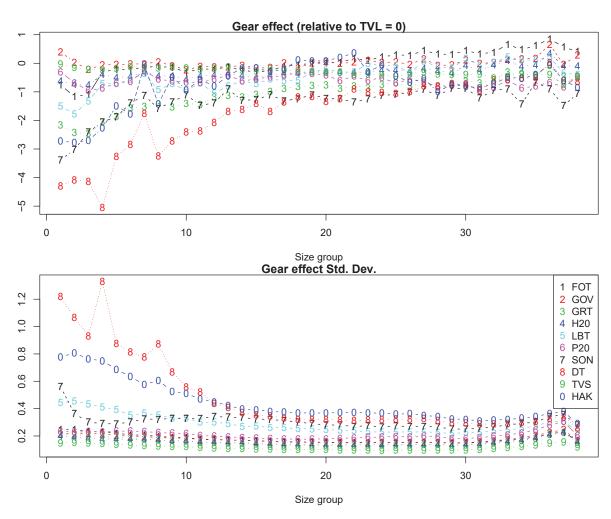
Figure 24: Estimated gear effect (log-scale) by length group.
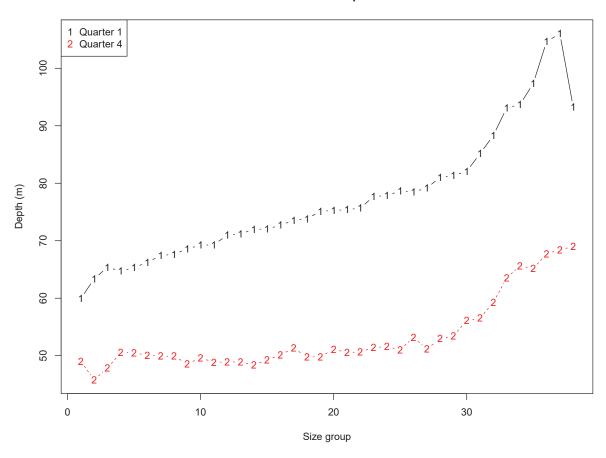
**Preferred depth**



Figure 25: Estimated preferred depth by length group.

# 6. Synthesis of information on cod growth and natural mortality

*By Margit Eero (DTU Aqua)*

Due to complexity of processes involved in growth and natural mortality of EB cod, understanding and quantifying these dynamics needs large scale dedicated research efforts. Therefore, aspects relevant for understanding and quantifying cod growth and/or natural mortality are addressed in a number of completed or presently ongoing national and international projects. The table below shows selected examples of recent or ongoing projects with most direct connections to the present EMFF project:

| Project | Focus in relation to EB cod |
|---|---|
| EU BONUS BIO-C3 | Understanding of ecological processes that affect changes in cod biology |
| EU BONUS INSPIRE | Understanding of ecological processes that affect changes in cod biology |
| TABACOD | Methods for estimating cod growth: tagging & microchemistry |
| EMFF Effects of seal parasites on cod | Understanding the effect of seal parasites on cod condition and growth |
| EU PANDORA | Quantifying the extent of seal parasite infestation, further analyses on growth |

The role of the present EMFF project focusing on cod growth and mortality is, in addition to generating new knowledge on the dynamics of these two parameters, also to synthesise and integrate the information produced in other relevant projects.

## 6.1 Synthesis of information on changes in growth

A number of investigations have addressed potential changes in growth. Most of the data and investigations presently available provide indirect indications for growth changes, however with some quantitative estimates available for younger cod (from otolith daily rings and length frequency data). For the older cod, no direct growth estimates for later years are currently available, as new methods that allow for direct growth estimates (based on taggings and microchemistry) are still under development (TABACOD project), and are expected to provide a long-term solution to growth estimation of EB cod (see chapter 1). The more indirect information can provide proxies for growth change in a shorter term, until the more long-term solutions become available.

For younger cod (up to age 3), some growth estimates are available from counts of daily rings on otoliths, as well as from inspecting length frequency distributions in the stock (chapter 4.1). These analyses suggest lower growth of year classes from late 2000s from age 2 to 3 compared to those from early 2000s or from the 1990s.

Concerning more indirect indicators for growth changes, the oxygen conditions have deteriorated in 2000s (Casini et al. 2016), which affects availability of benthic food for cod, and low oxygen can also reduce growth directly via altering metabolism (Plambech et al., 2013).

Several indicators measured on cod stock itself also point at reduced growth. Nutritional conditions is low, and this has been found to have at least some connection to lower growth as well, also in other stocks and species (section 4.2). Reduced growth of EB cod in later years is also explainable in a bioenergetics model (section 3.3). Molecular-genetic analyses indicated lower expression of growth hormones, and gonadosomatic and liver condition indices suggest high investment in reproduction (section 2). Also, reduced size at maturation, which was histologically confirmed is expected to lower growth due to energy being allocated to maturation.

The extensive database of stomach content data for EB cod, which have been intensively worked upon in later years in a number of projects (see chapter 3.2) allowed detailed insight into changes in diet composition and energy uptake of the cod. These data indicates that before 1990s, larger cod had generally lower feeding levels compared to smaller ones. However, in later years the feeding levels during life-history of cod successively reversed. While larger cod can compensate for the lack of benthic food in their diet by increasing predation on larger forage fish and cannibalism, smaller cod are not big enough for this compensatory behaviour. In consequence, many smaller cod have in later years had very low feeding levels that imply severe growth limitation or even starvation (ICES WKBEBCA 2017).

Cod in the EB Sea are also expected to be influenced by the increased abundance of grey seals. The seals cause mortality on cod through predation (see section 3.1). In terms of growth, the main impact is considered to be via infestation with the seal-associated liver worm *Contraceum osculatum,* which has increased in the Eastern Baltic (ICES WKBEBCA 2017). However, the effect of these parasites on cod growth and condition is not yet understood, and is being investigated in an ongoing EMFF project dedicated to this matter.

This summary of the available growth indications from recent studies is schematically illustrated in Figure 6.1.1. Overall, all of the available investigations exploring cod growth consistently point to the same direction, suggesting a reduced growth of EB cod in later years compared to the period in early 2000s or in the 1990s. This is a considerable progress compared to the situation some years ago, when there was no consensus whether growth has declined or remained stable (ICES WKBEBCA 2017). Presently, due to the number of investigations pointing in the same direction, there is an international consensus that growth of EB cod has declined since the 1990s, both in small and large fish (ICES WKIDEBCA 2018). However, the exact magnitude of growth change still remains to be clarified.



**ENVIRONMENTAL INDICATORS:**
• Poor oxygen conditions
Expected to reduce growth (benthic food & bioenergetics)

**COD INDICATORS:**
• Condition indices
Low condition: Associated with lower growth, also in other stocks. High investment in reproduction. Lower growth bioenergetically explainable
• Growth hormons
Low expression of growth hormons
• Small size at maturation
Histologically confirmed, expected to lower growth
• Stomach content data
Energy intake has reduced: suggests lower growth
• Seal parasites
Seal parasite infestation increased: possible effect on growth

**GROWTH MEASUREMENTS :**
• Otolith daily rings:
Lower growth of 2009-2010 year-classes compared to ycl. 1997, 2001 -2002
• Length frequency data:
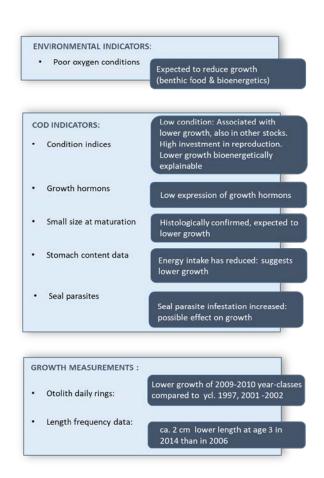ca. 2 cm lower length at age 3 in 2014 than in 2006

*Figure 6.1.1. Schematic overview of data sources and investigations indicating reduced growth of EB cod.*

## 6.2. Synthesis of information on changes in natural mortality

The ecosystem and biological factors which have been considered to possibly have increased the natural mortality of EB cod in later years include i) cannibalism; ii) predation by seals, iii) low nutritional condition and feeding level, iv) small size at maturation and v) infestation with seal parasites (ICES WKBEBCA 2017).For some of these factors, their possible contribution to a change in natural mortality of EB cod in later years has been addressed, described below.

### Cannibalism

Investigations of cod stomach data (section 3.2) indicate that natural mortality of smaller cod (<35 cm) due to cannibalism likely increased around 2010, but is indicated to have substantially declined in later years. This is likely related to few larger cod in the stock as predators. Thus, cannibalism related natural mortality is presently likely relatively low.

### Grey seal predation

At recent level of grey seals and the fraction of these found in southern Baltic Sea overlapping with cod, the mortality on cod due to grey seal predation can unlikely exceed 0.1, and most likely is lower than this (see section 3.1).

### Condition and feeding level

An association between poor condition/growth and natural mortality has been reported for a number of species (Adams et al., 1982; Henderson et al., 1988; Post and Evans, 1989; Thompson et al., 1991; Gislason et al., 2010). Experimental studies performed on Atlantic cod have also found a negative relationship between body condition and mortality (Dutil and Lambert, 2000). Thus, increased mortality of EB cod due to poor condition could be expected, also suggested by stomach data showing feeding levels corresponding to starvation (ICES WKBEBCA 2017). Using the results from the experiments by Dutil and Lambert (2000), possible condition related mortality of EB cod has been quantified (Casini et al. 2016b). These calculations showed that mortality due to poor condition could be approximately 0.1-0.2. This is in addition to the baseline natural mortality at "normal" conditions.

### Indications of increased mortality from sex ratio data

The proportion of female cod in the stock is generally higher than males for larger cod, as has been the case in the entire time series since the 1990s (Fig. 6.2.1). This is generally due to higher mortality of males. However, sex ratio estimated from BITS Q1 data shows that there is also a trend over time, with increases proportion of females in the stock also for those length groups where the sex ratio has earlier been more balanced. For example, sex ratio for 45-49 cm cod was close to 0.5: 0.5 in the 1990s, while the proportion of females is close to 80 pct in this length group in later years (Fig. 6.2.1). This change in sex ratio is likely related to both growth and mortality. Disappearance of males from the stock is explainable by their relatively higher mortality compared to females, while at which annual rate this happens depends on growth. Thus, estimating the level of mortality that could explain the sex ratios observed for different length groups, depends on the time it takes for the fish to grow from one length group to the next. For the exercise here to estimate possible mortality levels, we assumed 5 cm growth per year, which is in line with the growth analyses described in section 4.
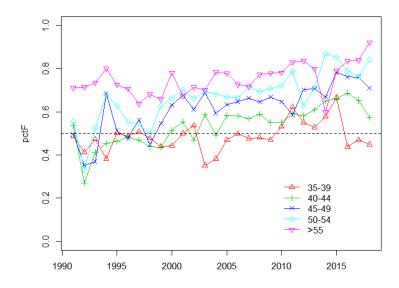
*Figure 6.2.1. Sex ratio (proportion of females in the stock), by length groups, estimated from BITS Q1 data.*

Accordingly, we looked at sex ratios by 5 cm groups (Fig. 6.2.1), which are assumed to represent subsequent age groups, and compared the recent values for proportion females in these length groups. Next, we calculated what percentage of the stock the males form that must have died to obtain the sex ratio that was observed for the next length group. Finally we converted these percentages to instant mortality rates. The values are shown in the table below:

| Length group | Average pctF in latest years | Proportion of stock numbers that have disappeared | Mortality |
|---|---|---|---|
| 35-39 | 0.5 | 0.23 | 0.26 |
| 40-44 | 0.65 | 0.12 | 0.13 |
| 45-49 | 0.74 | 0.11 | 0.12 |
| 50-55 | 0.82 | | |

Thus, under the assumptions of growth made in this exercise, mortality in the range 0.1-0.25 could be explained from the increasingly skewed sex ratio data in later years. It should be noted that this only represents the level by which the mortality of males could be higher than that of females, in addition to the base mortality of both sexes. Relatively higher mortality of males in later years could be due to small size at maturation and related energy investments. Small size at maturation could cause increased mortality of both females and males. This is not addressed in this investigation as the sex ratio only indicates by which factor the mortality of males could be higher than that of females. Fishing mortality is also considered to be generally higher in males than females, due to them spending longer time on spawning grounds. However, such effect is not expected to have increased in later years.

## Parasite infestation

Increased infestation with seal parasites is considered to likely cause increased natural mortality of EB cod, though the level of possible increase in mortality remains unclear (Horbowy et al. 2016).

**Summary**

Summary of the information from different investigations, which have tried to quantify potential changes in natural mortality is given in Figure 6.2.2. These indicate that an increase in natural mortality in recent period by ca 0.3 from the historical base value could be explained by the data explored. It should be noted that larger increase in M is possible, as mortality due to some factors (e.g. seal parasites, small size at maturation) have not been quantified.

- Cannibalism

  Increased in late 2000s and declined thereafter

- Low condition

  Can explain M increase by ~ 0.1

- Reduced proportion of males

  Can explain M increase by up to 0.25

- Seal predation
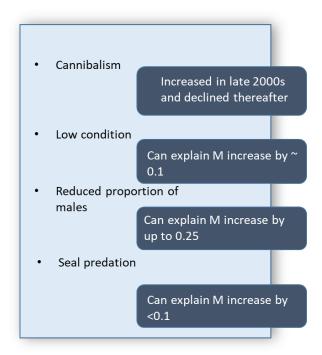
  Can explain M increase by <0.1

*Figure 6.2.2. Schematic overview of investigations indicating changes in natural mortality of EB cod in recent years.*

# 7. Stock assessment

*By Casper Berg and Margit Eero (DTU Aqua)*

## Surplus production model (SPICT)

Stock assessment on EB cod in ICES is currently based on DLS approach, i.e. looking at relative trends in BITS survey index. However, additional information has been incorporated in ICES advice since 2017, when the stock status in relation to proxy reference points has been evaluated as well. This is done using SPICT model, which name stands for a stochastic surplus production model in continuous time (Pedersen and Berg, 2017). SPICT does not need to separate between growth and natural mortality of the fish, which a strong advantage in situations where these are difficult to separate, like is presently the case for Eastern Baltic cod.

The model is using data on surveys and commercial catches, quarterly resolved.
Briefly, the model is based on a reparameterized version of the Pella-Tomlinson model (Fletcher, 1978) formulated as a stochastic differential equation such that it includes process noise:

$$dB_t = \left( \gamma m \frac{B_t}{K} - \gamma m \left[ \frac{B_t}{K} \right]^n - F_t B_t \right) dt + \sigma_B B_t dW_t,$$

K represents the carrying capacity, m represents the maximum sustainable yield (maximum surplus production), and n determines the shape of the production curve. $\sigma_B$ is the standard deviation of the process noise, and Wt is Brownian motion.

A specific version of SPICT has been developed for Eastern Baltic cod, to allow taking into account a potential change in surplus production over time (ICES WGBFAS 2017). The time period when a separate productivity "regime" was applied was estimated in the model, based on maximum likelihood value, thus not making explicit assumption on when the productivity change should take place and by which level. The new productivity regime was estimated in SPICT to start from 2010 (giving the best likelihood value). This is in line with the trends in major drivers considered to affect productivity changes (in terms of growth and natural mortality) (Fig. 4.3.1), as some of these were levelling off in the late 2000s.

SPICT operates internally with absolute values, but produces output, including the uncertainties also in relative terms (F/FMSY and B/BMSY), and the relative estimates are considerably more certain compared to the absolute ones. This is because the same parameters are included in both numerator and denominator of the relative values, which reduces the uncertainty in the relative estimates. The absolute catch corresponding to MSY is also reasonably well estimated, as the product of F*B is considerably better estimated than the F and B individually, because these estimates are strongly negatively correlated. Therefore, the absolute values for F, B, FMSY and BMSY are not recommended to be used, but the relative values for F/FMSY and B/BMSY are reasonably well estimated in the model for Eastern Baltic cod and can be used to define the stock status relative to the reference points (ICES WGBFAS 2017).

These relative values could also be used in forecast, and SPICT could thus potentially be used for providing MSY management advice. This option will be presented and further explored at ICES benchmark for EB cod scheduled for early 2019.

**Stock Synthesis (SS)**

During the last years, there have been efforts made to build an assessment model for the Eastern Baltic cod stock using SS. SS is a statistical age-structured population modelling framework that has been applied in a wide variety of fish assessments globally. It is widely tested, has a comprehensive manual and a dedicated website with about 250 scientists and a dedicated SS team to deal with bugs and continuously improve the model fitting and the representation of the model output through a dedicated R library (i.e. r4ss).

This software provides a statistical framework for calibration of a catch-at-age population dynamics model using a diversity of fishery and survey data. It is designed to accommodate both age and size structure in the population. Some key SS features include ageing error, estimation of growth, different spawner-recruitment relationships, movement between areas, the ability of incorporating tagging data and fishery discards, the use of environmental linkages, and allowing for time-varying parameters. SS can also be used to estimate natural mortality levels. The structure of SS consents for building of simple to complex models depending upon the data available. Thus, SS represents a flexible framework to accommodate specific cases such as the EB cod.

Developing an SS model for EB cod is a joint effort between different institutes, while preparing and quality checking the data inputs is mainly the responsibility of DTU Aqua, being responsible for the EB cod stock assessment in ICES. The latest status of the SS model for the Eastern Baltic cod stock was presented at ICES WKIDEBCA meeting (2018) and the model will be taken forward to the ICES benchmark scheduled for early 2019.

The results of this project to investigate and synthesize information on changes in growth of EB cod contribute to informing proxies for growth changes over time in SS. The information on possible levels of natural mortality synthesized in this project will be used to parametrize natural mortality estimation in SS and validate the derived values.

# 8. Communication and dissemination

*By Margit Eero (DTU Aqua)*

One of the important strengths of this project is the close national and international collaboration and communication within research community, as well as with relevant stakeholders. This has been among the tasks for this project to coordinate and integrate all relevant information also from other projects and research groups to ensure uptake of the latest international scientific developments in the process of improving stock assessment and management advice for the EB cod in ICES. This communication and collaboration work has largely utilised ICES as a platform, where dedicated meetings (WKBEBCA 2017 (Annex 3), WKIDEBCA 2018 (Annex 4)) have taken place to discuss the issues of EB cod. Regular meetings in the stock assessment group (WGBFAS) have also been utilized to move forward the process of improving the basis for management advice for EB cod. Stakeholders have participated in this process and discussions through these workshops, to ensure common understanding of the stock status and related possibilities for optimal utilization of the EB cod stock.

As a first step, an overview of relevant international ongoing and planned research activities was compiled (WGBFAS 2016). Effective information transfer from these research activites to the intersessional stock assessment work was considered essential to ensure progress with the assessment of eastern Baltic cod. For that reason, a Workshop on Biological Input to Eastern Baltic Cod Assessment (WKBEBCA 2017), was established, to facilitate discussion of the biological key issues among relevant experts, including the biology experts otherwise not participating in stock assessment work. Further, this WK was intended to serve as a platform for researchers working on EB cod to exchange and discuss new scientific information on the key biological processes relevant for stock assessment, to facilitate possible synergies and speed up the progress. The next workshop on Evaluation of Input Data to Eastern Baltic Cod Assessment (WKIDEBCA 2018) took a step further and discussed whether sufficient knowledge had been gained especially regarding growth and natural mortality, to proceed with benchmark assessment for the EB cod. Based on the available results, amongst others produced or combined in the present project, an international consensus was reached regarding reduced growth and increased natural mortality of EB cod. As a result of this meeting, benchmark process of EB cod was initiated, currently scheduled for early 2019. The reports of the dedicated workshops where the information on growth and natural mortality of EB cod have been discussed and synthesized are attached as Annexes 3 and 4.

In addition to the international meetings within ICES, national meetings have been held at DTU Aqua focusing on EB cod. These meetings have as well involved skakeholder participation to discuss and exchange information on stock status, including observations from DFPO. A specific focus regarding communication and collaboration with DFPO has been on tagging of EB cod, described in further detail in section 1. Other relevant stakeholders, incl. national management authorities have as well been informed about new information on EB cod stock status via written notes and discussion meetings.

Overall, the close connection to ICES and focus on collaboration and communication has ensured that the new findings on EB cod, including the outcomes of this project, are directly feeding into the assessment work in WGBFAS and have formed the basis for the upcoming benchmark assessment in early 2019.

# References

Adams, S. M., McLean, R. B., and Huffman, M. M. 1982. Structuring of a predator population through temperature-mediated effects of prey availability. Canadian Journal of Fisheries and Aquatic Sciences, 39: 1175–1184.

Ampela, K. and Ferland, A. (2006) 'Gray seal (Halichoerus grypus) diet composition in Nantucket Sound, U.S. as inferred from fecal remains', *Ices Cm*, pp. 1–14.

BACC II (2015), Second Assessment of Climate Change for the Baltic Sea Basin, Regional Climate Studies, Baltic Earth network

Baltic Sea Hydrographic Commission. Baltic Sea Bathymetry Database version 0.9.3, down-loaded from http://data.bshc.pro/ on 02-01-2018, 2013.

Bates D., Maechler M., Bolker B, Walker S. (2015) Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67, 1-48

Bowen, W. D. (2000) 'Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks', *Canadian Journal of Fisheries and Aquatic Sciences*, 57(5), pp. 898–905. doi: 10.1139/f00-032.

Breton T. S., Anderson J. L., Goetz F. W., Berlinsky D. L. (2012) Identification of ovarian gene expression patterns during vitellogenesis in Atlantic cod (Gadus morhua). General and Comparative Endocrinology, 179, 296-304

Campana, SE. 1990. How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Sciences 47: 2219–2227.

Cermeño, P., Uriarte, A., de Murguía, A.M., Morales-Nin, B. 2003. Validation of daily incremnte formaiton in otoliths of juvenile and adult European anchovy. Journal of Fish Biology 62: 679–691.

Casini, M., Käll, F. Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K. et al. 2016. Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. Royal Society Open Science doi: 10.1098/rsos.160416.

Casini, M., Eero, M., Carlshamre, S., and Lövgren, J. 2016b. Using alternative biological information in stock assessment: condition-corrected natural mortality of Eastern Baltic cod. ICES Journal of Marine Science,73: 2625–2631. doi:10.1093/icesjms/fsw117

Cook, R. M., Holmes, S. J. and Fryer, R. J. (2015) 'Grey seal predation impairs recovery of an over-exploited fish stock', *Journal of Applied Ecology*, 52(4), pp. 969–979. doi: 10.1111/1365-2664.12439.

Dutil, J. D., and Lambert, Y. 2000. Natural mortality from poor condition in Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences, 57: 826–836.

Eero, M., Hjelm, J., Behrens, J., Buchmann, K., Cardinale, M., Casini, M., Gasyukov, P., et al. 2015. Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES Journal of Marine Science 72: 2180–2186. doi: 10.1093/icesjms/fsv109.

Eero, M., Vinther, M., Haslob, H., Huwer, B., Casini, M., Storr-Paulsen, M., Köster, FW. 2012. Spatial management of marine resources can enhance the recovery of predators and avoid local depletion of forage fish. Conservation Letters 5: 486–492. doi:10.1111/j.1755-263X.2012.00266.x.

Fletcher, RI. 1978. On the restructuring of the pella-tomlinson system. Fish. Bull, 76(3):515-521

Gislason, H., Daan, N., Rice, J. C., and Pope, J. G. 2010. Size, growth, temperature and the natural mortality of marine fish. Fish and Fisheries, 11: 149–158.

Hammond, P. S., Hall, A. J. and Prime, J. H. (1994) 'The Diet of Grey Seals in the Inner and Outer Hebrides', *Journal of Applied Ecology*, 31(4), pp. 737–746.

Hammond P. S., Grellier K. 2006. Grey seal diet composition and prey consumption in the North Sea, 2006 Final report to Department for Environment Food and Rural Affairs on project MF0319.

Hammond, P.S. & Harris, R.N. 2006. Grey seal diet composition and prey consumption off western Scotland and Shetland. Report to SEERAD/SNH.

Hansson, S., Lars G. Rudstam, James F. Kitchell, Mikael Hildén, Barry L. Johnson, and Paul E. Peppard (1996). Predation rates by North Sea cod (Gadus morhua) – predictions from models on gastric evacuation and bioenergetics. ICES J. mar. Sci., 53: 107–114.

Härkönen, T. (1987) 'Seasonal and regional variations in the feeding habits of the harbour seal, Phoca vitulina, in the Skagerrak and the Kattegat', *Journal of Zoology*, 213, pp. 535–543. doi: 10.1111/j.1469-7998.1987.tb03724.x.

Heino M, Dieckmann U, Godø OR (2002). Measuring probabilistic reaction norms for age and size at maturation. Evolution 56(4):669-78.

HELCOM Core Indicator (2018) 'Population trends and abundance of seals', (July), pp. 1–24. Available at: http://www.helcom.fi/Core Indicators/Population trends and abundance of seals HELCOM core indicator 2018.pdf.

Henderson, P. A., Holmes, R. H. A., and Bamber, R. N. 1988. Sizeselective overwintering mortality in the sand smelt, Atherina boyeri, and its role in population regulation. Journal of Fish Biology, 33: 221–233.

Hildah J., Sandvik G. K., Edvardsen R. B., Norberg B., Haug T. M., Weltzie F. A. (2011b) Four gonadotropin releasing hormone receptor genes in Atlantic cod are differentially expressed in the brain and pituitary during puberty. General and Comparative Endocrinology, 173, 333-345.

Hildahl J., Sandvik G. K., Edvardsen R. B., Fagernes C., Norberg B., Haug T. M., Weltzien F. A, (2011) Identification and gene expression analysis of three GnRH genes in female Atlantic cod during puberty provides insight into GnRH variant gene loss in fish. General and Comparative Endocrinology, 172, 458-467

Horbowy, J., Podolska, M., Nadolna-Ałtyn, K. 2016. Increasing occurrence of anisakid nematodes in the liver of cod (Gadus morhua) from the Baltic Sea: Does infection affect the condition and mortality of fish? Fisheries Research. 179: 98–103.

Hüssy, K., Radtke, K., Plikshs, M., Oeberst, R., Baranova, T., Krumme, U., Sjöberg, R., Walther, Y., and Mosegaard, H. 2016. Challenging ICES age estimation protocols: lessons learned from the eastern Baltic cod stock. ICES Journal of Marine Science, 73: 2138–2149.

Hüssy. K., Eero, M., Radtke, K. 2018. Faster or slower: has growth of eastern Baltic cod changed? Marine Biology Research, DOI: 10.1080/17451000.2018.1502446

ICES 2015. Report of the Benchmark Workshop on Baltic Cod Stocks (WKBALTCOD). ICES CM 2015/ACOM:35

ICES 2017. Report of the Baltic Fisheries Assessment Working Group (WGBFAS). ICES CM 2017/ACOM:11

ICES 2017. Report of the Workshop on Biological Input to Eastern Baltic Cod Assessment (WKBEBCA). ICES CM 2017/SSGEPD:19

ICES 2018. Report of the Workshop on Evaluation of Input data to Eastern Baltic Cod Assessment (WKIDEBCA). ICES CM/ ACOM: 36

Jobling, M. (1982). Food and growth relationships of the cod, Gadus morhua L., with special reference to Balsfjorden, north Norway. J. Fish Biol. (1982) 21, 357-371

Jobling, M. (1988). A Review of the Physiological and Nutritional Energetics of Cod, Gadus morhua L., with Particular Reference to Growth Under Farmed Conditions. Aquaculture (70) 1-19.

Jørgensen, C. and Øyvind Fiksen (2006). State-dependent energy allocation in cod (Gadus morhua). Can. J. Fish. Aquat. Sci. 63: 186–199.

Kortner T. M., Overrein I., Oie G., Kjorsvik E., Barda T., Wold P. A., Arukwe A. (2011) Molecular ontogenesis of digestive capability and associated endocrine control in Atlantic cod (Gadus morhua) larvae. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology, 160, 190-199

Königson, S. et al. (2007) 'Seals and fyke nets: An investigation of the problem and its possible solution', Marine Biology Research, 3(1), pp. 29–36. doi: 10.1080/17451000601072596.

Köster, F. W., Huwer, B., Hinrichsen, Hans-H., Neumann, V., Makarchouk, A., Eero, M., Dewitz, B. V., Hüssy, K., Tomkiewicz, J., Margonski,P., Temming, A., Hermann, Jens-P., Oesterwind, D., Dierking, J., Kotterba, P. and Plikshs, M. 2017. Eastern Baltic cod recruitment revisited—dynamics and impacting factors. ICES Journal of Marine Science, 74 (1): 3-19.

Kristensen, K. Uffe Høgsbro Thygesen, Ken Haste Andersen, and Jan E Beyer. 2013. Estimating spatio-temporal dynamics of size-structured populations. Canadian Journal of Fisheries and Aquatic Sciences, 71(2):326–336.

Lanes C. F. C., Bolla S., Fernandes J. M. O., Nicolaisen O., Kiron V., Babiak I. (2012) Nucleotide Enrichment of Live Feed: A Promising Protocol for Rearing of Atlantic Cod Gadus morhua Larvae. Marine Biotechnology, 14, 544-558.

Li, L., Høie, H., Geffen, A.J., Heegaard, E., Skadal, J., Folkvord, A. 2008. Back-calculation of previous fish size using individually tagged and marked Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences 65: 2496–2508. doi:10.1139/F08-157.

Lundstrom, K. et al. (2010) 'Understanding the diet composition of marine mammals: grey seals (Halichoerus grypus) in the Baltic Sea', ICES Journal of Marine Science, 67(6), pp. 1230–1239. doi: 10.1093/icesjms/fsq022.

Lundström, K. et al. (2007) 'Estimation of grey seal (Halichoerus grypus) diet composition in the Baltic Sea', NAMMCO Scientific Publications, 6(0), p. 177. doi: 10.7557/3.2733.

Lundström, K. (2012) *Assessment of dietary patterns and prey consumption of marine mammals: Grey seals (*Halichoerus grypus*) in the Baltic Sea.*

Matejusová, I. *et al.* (2008) 'Using quantitative real-time PCR to detect salmonid prey in scats of grey Halichoerus grypus and harbour Phoca vitulina seals in Scotland - an experimental and field study', *Journal of Applied Ecology*, 45, pp. 632–640. doi: 10.1111/j.1365-2664.2007.0.

Mittelholzer C., Andersson E., Taranger, G.L., Karlsen O., Norberg B. (2009) Quantification of gonadotropin subunits GP alpha, FSH beta, and LH beta mRNA expression from Atlantic cod (Gadus morhua) throughout a reproductive cycle. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 153, 288-295

Parsons, K. M. *et al.* (2005) 'DNA-based identification of salmonid prey species in seal faeces', *Journal of Zoology London*, 266(3), pp. 275–281. doi: 10.1017/S0952836905006904.

Paul, L.J., Horn, PL. 2009. Age and growth of sea perch (*Helicolenus percoides*) from two adjacent areas off the east coast of South Island, New Zealand. Fisheries Research 95: 169–180.

Pedersen, M.W., and Casper W Berg, C.W. 2017. A stochastic surplus production model in continuous time. Fish and Fisheries, 18, 226–243

Plambech M,VanDeurs M,SteffensenJF,Tirsgaard B,BehrensJW.2013. Excess post-hypoxic oxygen consumption in Atlantic cod Gadusmorhua. J.Fish Biol. 83,396–403.(doi:10.1111/jfb.12171)

Pompanon, F. *et al.* (2012) 'Who is eating what: Diet assessment using next generation sequencing', *Molecular Ecology*, 21(8), pp. 1931–1950. doi: 10.1111/j.1365-294X.2011.05403.x.

Post, J. R., and Evans, D. O. 1989. Size-dependent overwinter mortality of young-of-the-year yellow perch (Perca flavescens): laboratory, in-situ enclosure, and field experiments. Canadian Journal of Fisheries and Aquatic Sciences, 46: 1958–1968.

Sparholt, H. (1994) 'Fish species interactions in the Baltic Sea', *Dana*, 10(June), pp. 131–162.

Swain, D. P. and Benoît, H. P. (2015) 'Extreme increases in natural mortality prevent recovery of collapsed fish populations in a Northwest Atlantic ecosystem', *Marine Ecology Progress Series*, 519, pp. 165–182. doi: 10.3354/meps11012.

Thompson, J. M., Bergensen, E. P., Carlson, C. A., and Kaeding, L. R. 1991. Role of size, condition, and lipid content in the overwinter survival of age-0 Colorado squawfish. Transactions of the American Fisheries Society, 120: 346–353.

Tomkiewicz,J., Tybjerg,L., Holm,N., Hansen,A., Broberg,C. and Hansen,E. 2003a. Manual to determine gonadal maturity of Baltic cod. DFU-rapport 116-02, Charlottenlund: Danmarks Fiskeriundersøgelser. 49 p.

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

Waldron ME, Kerstan M. 2001. Age validation in horse mackerel (*Trachurus trachurus*) otoliths. ICES Journal of Marine Science 58: 806–813.

Yaragina, N. A., Høie, H., Koloskova, V. P., Mjanger, H., Nedreaas, K. H., Senneset, H., Zuykova, N. V. et al. 2011. Precision of the Northeast Arctic cod age determination under variable environmental and information conditions. Marine Biology Research, 7: 599–607.