



Otolith Weight in Age determination of Baltic Cod

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Summary

Through the co-operation between the Nordic countries Denmark, Sweden and Finland, major progress has been made towards solving the long lasting problem of creating an objective and reproducible method of ageing Eastern Baltic Cod.

Through the co-operation between the Nordic countries Denmark, Sweden and Finland, major progress has been made towards solving the long lasting problem of creating an objective and reproducible method of ageing Eastern Baltic Cod.

A database has been co-ordinate through Baltic Counties involved in sampling of the Eastern Baltic Cod stock (Denmark, Germany, Latvia, Lithuania, Estonia, Poland, Russia and Sweden). The database contains over 45 000 entries of with otolith weights including traditional ageing with the purpose to find a method how to infer age of fish from otolith weight.

The initiated co-ordination of the database has resulted in that registering of otolith weight is now more or less standard in all Baltic countries.

A review of the state of the art using otolith weight as a proxy for age has been made. This shows a requirement for a known age material for calibration of the method. Possible ways to obtain a calibration sample described are mark recapture experiments, natural time signal markers, traditional ageing, natural progression of otolith and fish size modes or otolith growth experiments in the field.

An investigation of the options for statistical treatment of the database has been conducted with the aim to develop and implement a method addressing the specific case of the Eastern Baltic Cod.

Based on the results of this project new funds has been granted from EU involving all relevant countries in the Baltic with the aim to further develop and refine the suggested method.

Sammanfattning

Ett samarbete mellan de Nordiska länderna Danmark, Sverige och Finland har resulterat i att framsteg har gjorts gällande ett långvarigt problem med att utveckla en objektiv och reproducerbar metod för åldersbestämning av torsk i Östra beståndet i Östersjön.

En databas har blivit upprättad genom samarbete med alla länder som tar prover på torsk i Östra Östersjön (Danmark, Tyskland, Lettland, Litauen, Estland, Polen, Ryssland och Sverige). Databasen innehåller mer än

45 000 uppgifter med otolit vikter kopplade till traditionell åldersbestämning. Syftet är att hitta en metod där man kan använda otolitvikt som ett mått på fiskens ålder.

Samarbetet har också resulterat i att registrering av otolitvikter i samband med provtagning är mer eller mindre standard i alla länder som utför provtagningen.

En utvärdering av de senaste metoderna som använder otolitvikt som ett mått på ålder har utvärderats. Dessa visar på behovet av kalibreringsprov med känd ålder på fisken. Möjliga metoder att få ett kalibreringsprov är märkning och återfångst försök, naturliga markörer för indikation av tid, naturliga progression av modalvärden för otolit och fisk storlek samt otolit tillväxt experiment.

Möjligheter att använda statistisk behandling av databasen har blivit undersökta med syfte att utveckla och genomföra metoder som är anpassade specifikt för torsk i Östra Östersjön.

Baserat på resultaten i detta projekt har nya medel beviljats från EU, med alla relevanta Östersjöländer som deltagare, med syftet att vidareutveckla och förfinas metoder som föreslagits.

1. Introduction

The Baltic cod stock is at present harvested outside safe biological limits. In a situation with a low cod stock, very narrow and young age distribution as well as a decreasing quality of the catch data, it is of crucial importance to use the best available methods to improve the precision of the stock assessment. Age estimation is a focal point in assessment where improvement is possible.

The initiative of investigating the possibilities of revising the age interpretation of the Baltic cod came about as a result of discussions during the ICES study group meeting in Riga May 2004 (SGABC). The agreed collaboration among Baltic Fisheries institutes on the age-revision provided a unique opportunity to solve a long time recognised problem. The present project was initiated to make progress in coordinating activities within the large network of ageing expertise from institutes around the Baltic Sea where all countries were involved in different constellations of responsibilities and means of funding. The present Nordic initiative provided the infrastructure and the organizational abilities in connection with scientific expertise to co-ordinate collection and analysing biometric data from cod otoliths.

The proposal included a co-operation between Denmark, Finland and Sweden to investigate the potential of otolith biometrics (mainly otolith weight) coupled with length frequency distributions to improve age determination of Baltic cod. The Institutes have been represented by Dr Henrik Mosegaard (Danish Institute of Fisheries Research), Dr Eero Aro (Finnish institute of Game and Fisheries) and Ms Yvonne Walther (Institute of Marine Research, Sweden). Other experts from the three institutes have been involved in different tasks e.g. data base management, analysis of otolith biometry and statistical analysis of age structure. The project was carried out in collaboration with the other national fisheries institutes around the Baltic Sea. The project directly addressed the following topics in the NAF work programme:

- Factors (i.e. human activities) in the sea that influences the living conditions of marine organisms.
- Methods and regulations that can lead to a more selective and sustainable fishery.
- Implementation and development of concepts in fisheries administrations such as, sustainable fisheries, precautionary approach and ecosystem-based fisheries management.

The present ageing method of Baltic cod is based on reader interpretation of annual rings in the otoliths of the fish. This method is subjective and without any proper validation. The problem in age reading of cod has been recognised since the beginning of the 1970ies. The first exchange program of Baltic cod otoliths was organised in 1980. The differences between age-readings have continuously been observed during the 1990ies and a succession of meetings and exchange programs with the countries around the Baltic Sea have tried to reach consensus between age-readers.

Since the use of otolith biometrics, particularly otolith weight has proven successful in other stocks with age-reading problems, preliminary investigations were carried out indicating that using otolith weight could be a potential method for facilitating improvement in assigning age classes to Baltic cod. The project was planned to aim at investigating and facilitating a series of steps towards a revision of the Baltic cod stock assessment by:

- Introduction of routine collection of the necessary otolith data
- Co-ordination of a database holding this information
- Reviewing the state of art in using otolith weight in combination with available analytical methods
- Investigation of the requirements for development and implementation of statistical methods specific for the Eastern Baltic Cod case.

As a basis for the project was the commitment by the fisheries laboratories around the Baltic to supply otolith weight data for the period 2001–2003. During the project period part of the activities would then further involve a continued updating of the time series of weighed otoliths and corresponding survey and fisheries data.

The project was granted by NAF in 2005.

2. Data Compilation

Data on some 44 183 otolith weight samples of cod from 1998 to 2005 have been provided by Denmark, Germany, Latvia, Poland, Lithuania, Russia and Sweden (Table 1). That is all the countries currently involved in commercial fishery of Baltic Cod. Finland, however, is not currently involved in otolith sampling in the Baltic but has made their contribution by expertise in age reading and coordination of the work. The data is compiled in a Microsoft SQL Server. The otolith weights are taken from Subdivisions 25, 26, 27 and 28 (Figure 1) and covers research surveys as well as commercial sampling.

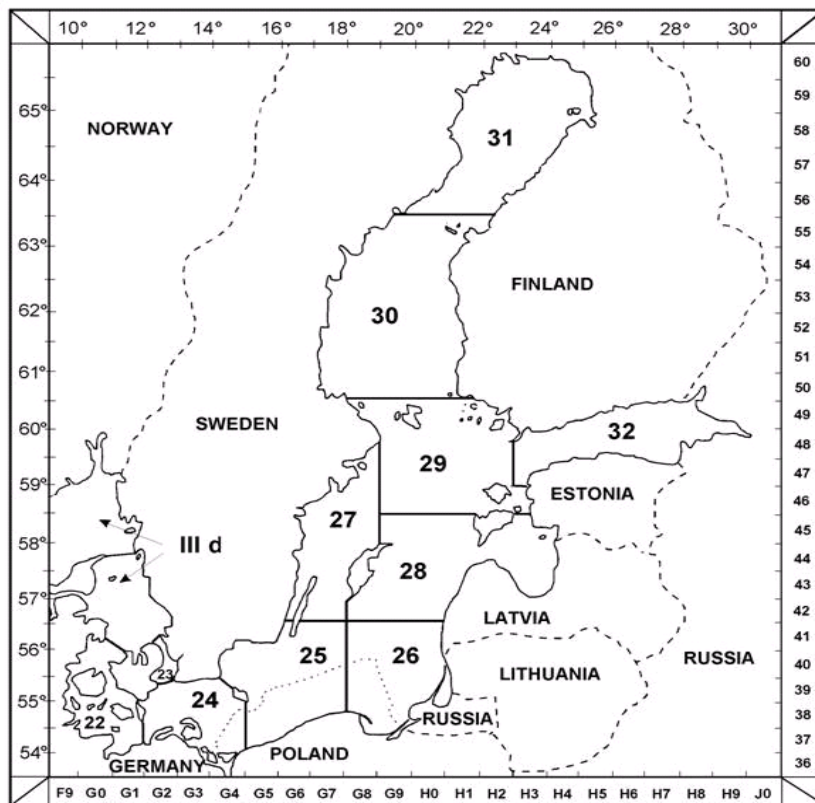


Figure 1. Baltic Sea with indications of ICES subdivision boards.

It was the general idea to primarily use survey sampling for obtaining otolith weights but it has a drawback as it covers a limited time period over the year and commercial sampling is continuous over the year. Including both types of sampling will give an opportunity to investigate if there are any differences in results with regards to the origin of the sam-

pling or if the sampling types are interchangeable with regards to the otolith weight parameter.

The database also contains individual ages (derived by traditional method of counting annuli) as well as per sample length frequencies. Most countries take a stratified sample of otoliths for ageing (e.g. 5 otoliths per length class of fish) and this makes the otolith sample in itself unrepresentative in comparison with the catch and needs to be raised to the length frequency sample which is catch representative.

Table 1 The number of otolith weight samples taken by various countries and in various ICES subdivisions per year.

Country	Subdivision/ year	1998	1999	2000	2001	2002	2003	2004	2005	Total
Denmark	25	1174	3454	1198	2731	2584	661	4192	1992	17986
Germany	25				2639	1622	2261			6522
Latvia	26				2227	1629	1812			5668
	28				229	829	481			1539
Poland	25				1421	758	1053	439	574	4245
	26				921	1053	1028			3002
Russia	26								815	815
Sweden	25				590		1168	528	690	2976
	26						268		3	271
	27				185		176	108	122	591
	28				60		295	50	163	568
Grand Total		1174	3454	1198	11003	8475	9203	5317	4359	44183

Overall, this point in the core task has exceeded our expectations. Thanks to the effort of the project finally the collection of otolith weight in connection with traditional age-reading has been implemented, more or less, as a standard procedure in several institutes.

There are still some issues about storage of data to be solved. The FishFrame database system (<http://www.fishframe.org>, see <http://dmz-web08.dfu.min.dk/BalticSea/FishFrame/Info/Documentation/documentation.aspx> for documentation and description of data exchange format) that contains the traditional commercial and survey length/age based catch at age data for Baltic Cod has been configured to contain otolith weight data. Several institutes surrounding the Baltic Sea registers already otolith weight data from 2006 in this database as a routine. The intention, then, is to let FishFrame hold the otolith weight data from all preceding years.

What considers the quality assessment of the otolith weight data uploaded as commercial data to FishFrame, it is expected that the individual countries are to be responsible. This also applies to the survey data for which the procedure is that they are submitted to the ICES database system by the individual countries, screened by the ICES data quality filter and, subsequently, transferred to the FishFrame database as an exact copy.



Figure 2. Front interface to the FishFrame database system

3. Otolith weight and age determination approaches

3.1 Review of published approaches using otolith biometrics

Templeman and Squires (1956) first documented the proportionality between size of the otolith and the size and age of the fish. But the interest in applying otolith biometrics as a proxy for age really caught on when Boehlert (1985) presented his work on how fish age could be derived from otolith measurements. Since then a number of methods has been published on different species and fish stocks with the attempt to make ageing methods more time and cost efficient as well as objective and reproducible. (e. g. Pawson ,1990, Pilling et. al. 2003, Araya, M et al 2001, Cardinale and Arrhenius 2004)

Each otolith weight- age relationship is population specific (Worthington, 1995) and should therefore be carefully implemented. Hence it is very important to have thorough knowledge about the underpinning biology and population structure of the species. In some cases the method has shown high potential. In a population of *Sardinella aurita* an index of age was obtained for individual fish by calculating the equivalent otolith weight at a particular fish length. The author concludes that this is possible on populations where the growth rate are known or assumed to be constant (Pawson, MG, 1990). Other species such as the *Sardinops neopilchardus* has shown direct relationship between otolith weight and age (Fletcher, 1991). In some cases otolith weight has been found very effective for age determination of young fish (for example Megalofonou 2006, Cardinale and Arrhenius, 2004).

In 2004 the state of art in using otolith biometrics for assigning age was summarized (Francis and Campana, 2004). A new method combining length mediation and otolith measurements in a length mediated mixture analysis was suggested and further illustrated in two simulation experiments (Francis et al 2005). The authors points out that age population structure can be obtained directly from a known age or validated sample (calibration sample) of individual fish and applied on a larger sample (production sample) representing the population. The methods discriminates otolith weight cohorts from on overall otolith weight distribution using statistical methods to refine the relationship between fish length and age and thus converting a length distribution to an age distribution.

As stated in Pauly (1987) the ultimate goal of ageing fish is usually to estimate growth or mortality parameters. Updating the method from traditional age-reading of Baltic cod on an individual basis to a more objective method directly targeting to estimate proportions at age in the population is considered quite sufficient for producing catch at age data for assessment purposes (SGABC 2006).

A high and significant correlation between otolith weight and fish age has been observed in Baltic cod (Cardinale et. al., 2000). The Bhattacharya method (1967) was used to discriminate normal distributions of otolith weight, each assuming to represent a cohort (i.e. age class) of fish from the overall size-frequency distribution. This method is however partially subjective as it uses traditional ageing to produce the calibration sample. This approach gives a more efficient method but does not completely fulfil the need of underlying objectivity. But it provides a criterion to evaluate the degree of separation between individuated normal distributions, known as separation index. Comparing age composition by the methods of weight splitting and traditional reading showed a much higher agreement with true age distribution in the weight based method, with a success rate of 98% (weight based) and 60–95% (traditional reading depending on individual reader experience) (ICES, 2004).

On the other hand Oeberst (2006) concludes that the Bhattacharya method, which assumes normal distributions, is inappropriate to use on Baltic cod as the distribution is skewed and skewness increases of the otolith weight distribution increases with the age of the fish.

The specific problem with age determination of the Baltic Cod puts certain requirements on the construction of the calibration procedure. The high variability and bias among institutes exclude the use of traditional counting of annual zones as means of calibration, hence this calibration method would induce an unwanted circularity (ICES, 2005).

It is also important how calibration samples are used, a need for continuous investigations and updating is necessary to ensure that the calibration sample is representative (Worthington et. al 1995b). Concerns about temporal and spatial variations in fish populations must be included in the sampling design for calibrations purposes. A substitute for known age material could be the construction of a growth model based on the measurements of otolith formation rate in relation to fish size and ambient environment. In the international EU funded research project CO-DYSSEY Baltic Cod were tagged and released carrying data storage tags (DST) registering information that could be used for this purpose.

3.2 Explore statistical approaches to age-determination with existing data

Due to the conservative nature of otolith accretion in relation to fish growth there is potentially an improved signal of age structure of a population contained in samples of otolith size and morphometrics compared to the corresponding fish size distribution. In a recent paper Francis and Campana (2004) has reviewed previous work on the use of otolith biometrics to estimate fish age and developed a new approach to estimate age composition using this information. The paper points to the fact that most frequently the purpose of inferring age is to estimate proportions at age at the population level, primarily to be used in a stock assessment model, rather than to assign ages to individual fish. The normal procedure for fish ageing is to estimate age from a 'calibration sample' with detailed information on age proportions, then use this to estimate the proportions at age for a larger 'production sample' for which only limited information (e.g. length distribution) is available.

The review by Francis and Campana (2004) identifies four different types of bias, statistical bias, discriminant bias, smoothing bias, heteroscedastic bias, and calibration bias, at least one of which is found in all traditional methods. The authors then demonstrate that their approach being based on mixture analysis produces unbiased estimates and is also technically superior as it uses information from the production sample as well as the calibration sample to simultaneously estimate age proportions in the population. The authors note that weighing otoliths can be five to ten times quicker than reading them, but that the final decision on the relative effort in the different components of the procedure should be evaluated using cost-benefit analysis, i.e. comparing the cost of obtaining the information with the quality of the results obtained.

Other approaches exist that circumvent the lack of known age material. A length based single species assessment model was derived assuming continuous reproduction, individual based and continuous time and length dependent mortality (Kristensen et al. 2006). Using Danish survey data on length frequencies for Baltic cod this spectra model was hierarchically tested and shown to fit well with progressing length modes from samples with a negative binomial distribution of catch per unit effort. The model was validated by its excellent prediction of the length frequencies of commercial catches. The results on Baltic cod indicate that fishing mortality on cod sizes above 45 cm may range between 1 and 3, considerably higher than the present perception in the ICES advice. With such high mortality only the slow growing individuals in the population will survive after age 3, therefore the first two year classes are the only distinct observable peaks of the length frequency distributions. With an assumed constant k in the von Bertalanffy growth equation (VBGE) the estimation of these year classes are more influenced by the assumptions

of the spawning time and recruitment distribution than older age classes. With the model output of an apparent high and unexplained among years variability in recruitment there is a need for more analyses of the population time series of surveys and catches before this model may be adapted for stock assessment.

3.3 Development of method for estimating age-length keys (ALKs) based on 2.1 and 2.2

Following the suggestion from Francis and Campana (2004) the optimal procedure to obtain age composition for stock assessment involves a simultaneous analysis of a calibration with known age material and a production sample with only fish size composition rather than the estimation of individual ages. ALKs will then emerge as models of age probability distributions by length class. The fundamental requirement of this approach is the achievement of known age material for the calibration. For Baltic cod this provides a specific problem since our investigations show that although some mark recapture experiments have been carried out in previous time no collections of known age otoliths exist. However, as suggested by Francis and Campana (2004) the maximum likelihood method may also be developed to allow for age information with some level of error, therefore indirect measures of year-class affiliation or proxies for age may be considered. The following section aims at investigating a number of alternative approaches for estimating age compositions of Baltic cod. Two options exist, one that under certain conditions indirectly provide known age material (allowing for some level of error at age) and another that build up the age structure model based on growth experiments or generic information on bioenergetics. The first group includes: mark recapture experiments, natural time markers, and natural progression of otolith- and fish size modes. The other option includes: otolith growth experiments in the field or in the laboratory and integrated otolith accretion - somatic growth models. These different approaches are evaluated in the present report.

3.3.1 Mark – recapture experiments as a source of known age material

Marking of known age cod (juveniles) and recapture at different ages has been shown to be a reliable method to produce known age otolith material (Faroe cod otoliths in the FAbOSA project). Unfortunately it has been a long time since such programmes were conducted in the Baltic, and to our knowledge no known age otolith collections exist. For theoretical considerations and the possibility to generalise findings of cod otolith growth for application to the Baltic we have reviewed the recaptured Faroe cod otolith material. This material has already been analysed

in several respects and significant genetic and environmental influences on otolith weight, size and shape have been found (Cardinale et al. 2004). The year-class separation in the otolith weight – cod length relationship in this material was analysed in Francis et al. (2005) and direct estimation from otolith weight – cod length relationship to age was found to be the most efficient use of resources to get a precise age distribution.

Together with colleagues from the FAbOSA project we have further analysed the Faroe cod otolith material (Doering-Arjesl, P., Cardinale, M., Kastowsky, M., Wickström, H., & Mosegaard, H. in prep.). Otoliths came from successive samples and recaptures from three different sources of Faroe cod. Samples taken once a year from Faroe Bank pen cage (FBP) and from Faroe Plateau pen cage (FPP) represented the two separate stocks raised at the same growing conditions (temperature and food intake) while recaptured individuals, continuously sampled from the fishery at the Faroe Plateau (FPR) represented the wild counterpart (recaptures from the Faroe Bank stock were very few and therefore not included in our analysis). Otolith weight and morphometric measurements along different axes of the otolith as well as Normalised Fourier Descriptors (NFDs) of otolith outline shape were recorded. Parameters were standardised according to cod length.

Classification of age was performed based on a set of significant parameters found by stepwise discriminant analysis. Linear discriminant analysis (DA) with cross-validation estimated the unbiased assignment of individual otoliths into age-classes using the combination of weight, morphometric variables and NFDs. The cross validated parameter estimates from each of the three DAs (calibration samples) were then used in a second step for testing the generality of the results on other sources/populations (test samples) for the common case where known age material would not be at hand.

Results from all six possible comparisons, using entire samples, indicated increased misclassification rates from about 10–20% for cross-validated classifications to about 20–50% when otoliths from one stock or environment was used to calibrate age analysis in another stock or environment. These results indicate that known age material may not be borrowed for age analysis of another stock with different genetics or environmental conditions. A further implication of these results is that rearing of Baltic cod for production of known age material will not necessarily yield otolith weights and morphometrics that directly translate into age classes in the field. Since Baltic cod and their environmental conditions deviate substantially from Faroe cod the available FAbOSA material is not suitable for age analysis of Baltic cod. The method by Francis and Campana (2004) is therefore not directly applicable to solve the Baltic cod age problem until a specific mark recapture programme in the Baltic has provided sufficient numbers of known age cod otoliths for calibration.

3.3.2 Natural time signal markers

Several candidates for time markers in fish otoliths exist following two different principles, either reoccurring events that produce marks with a known frequency (e.g. annual, lunar or daily) or specific marks coupled to unique periods in time that will identify individuals being in a specific life stage during this period (e.g. specific larval microstructure pattern, characteristics of 1st annual growth zone).

Besides the bipartite translucent – opaque otolith growth structures being the basis for counting of year rings, stable isotopes have been shown to exhibit annual fluctuations corresponding to temperature (δO^{18}) or metabolic variations (δC^{13}). In connection with the EU funded FP5 research projects IBACS and CODYSSEY Baltic cod otoliths from recaptured DST tagged cod were analysed with respect to stable oxygen isotope composition. Preliminary results indicate that variation in marginal values of δO^{18} does not directly correlate with logged ambient temperature. Further the slow otolith accretion rate in Baltic cod validated by the SEM detectable Sr band in the otolith induced by injection of SrCl_2 at release restricts the temporal resolution of any detectable stable isotope signal in this cod stock.

Natural marks may also exist as event specific otolith growth structures that may be extracted for later identification of one or several specific year-classes. Presently, the only available spawning area for Baltic cod is the Bornholm basin (Köster et al. 2005), this circumstance may yield low within year otolith signal variation due to the reduced environmental differences from a restricted large scale geographic distribution. Based on back-calculated hatch date distributions (Hinrichsen et al. 2003) the spawning period in the Baltic may cover almost half a year whereas hatch dates for survivors may show distinct peaks after settling as 0-group. With a strong temperature influence on larval and juvenile otolith microstructure (Otterlei et al. 2002, Hüsey et al. 2003a) it is likely that annual variation in seasonal temperature development along with density dependent effects on settling time, will create among year-class variation in otolith microstructure pattern during the early stages of Baltic cod (Hüsey et al. 2003b). With a multivariate approach this may provide a future robust basis for separation of year-classes to allow for a sufficiently precise calibration. It has to be investigated whether such signals exist in collections of 0-group or 1-group Baltic cod otolith material.

3.3.3 Traditional age reading as a source of “known age” material

Francis et al. (2005) suggest using traditional age reading (annulus counting) as a way to assign ages to an otolith weight calibration sample, in the present case where repeated age reading workshops have demonstrated errors and bias at all thinkable levels of stratification this appears to be a

procedure with an unpredictable outcome that will probably not solve the problem.

The overall result of several recent international age readings calibrations is that there is a general low agreement between readers. During the most recent SGABC reader interpretations of Baltic cod otoliths were scrutinized (ICES 2006). The use of an image analysis exercise clarified that the lack of agreement can be referred to several reasons, one being the position of O1. In 80% of the non-agreed otoliths the readers did among other things not agree upon which structure to point to as the first ring. Especially the younger ages had a high variability in the definition of O1 and these ages were associated with higher disagreement, thus in these cases a high disagreement could be related to a high variability in the definition of O1. Table 1 shows the average percent agreement, CV and relative bias by modal age and the variation within each modal age is clearly too high to use reader-determined ages as known-age material.

Table 1 Average percent agreement, CV and relative bias by modal age in recent age reading calibrations. Reading material was comprised of cod caught in SD 25 during Danish BITS cruises in 2003–2004.

MODAL age	Agree-ment	Mean bias	Mean CV
0	-	-	-
1	0.94	0.06	0.13
2	0.63	0.57	0.29
3	0.47	0.07	0.24
4	0.49	-0.20	0.23
5	0.55	-0.45	0.19
6	-	-	-
7	-	-	-
8	0.50	0.50	0.13

The image analysis, however, demonstrated variation in perception of age structures. In cases where a reasonably common interpretation of individual rings existed, disagreement arose where some readers choose to leave out specific rings identified by other readers as true annual rings. Identification of ring position is in general varying between readers, even in readings, which estimate age equal to the modal age, do not all have the same interpretation of ring position. Figure 3 shows a typical example of the variety in perception of age structures by otolith readers.

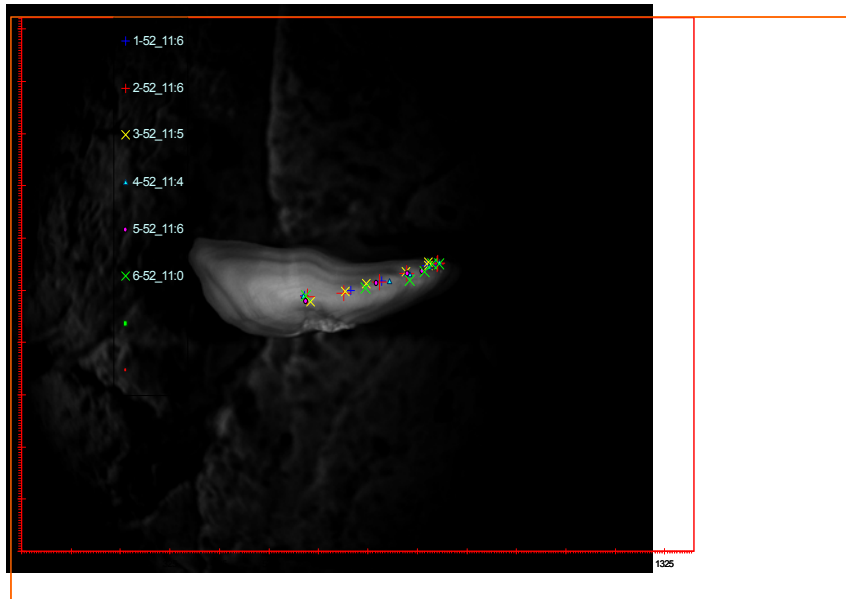


Figure 3. Example from the image analysis of an otolith where readers diverged to a high degree in interpretation of annual structures. Otolith from cod caught in sub.div. 25, March 2004.

Although the inconsistencies in reader interpretations have now been quantified, the results from the most recent age reader calibration exercise show no improvements compared to earlier exercises. This result points to some fundamentally unsolved problems in our understanding of the nature of Baltic cod otolith growth structures. Therefore we find that using traditional age reading for provision of known age calibration samples may introduce unknown levels error as well as bias.

3.3.4 Natural progression of otolith- and fish size modes

Otolith or fish size distributions may be resolved by age group using the Bhattacharya method (1967). Another approach is the resolving of cohort wise multivariate distributions by methods like MULTIFAN etc. Theoretical considerations concerning the non linear relationship between otolith weight and cod length with an increasing standard deviation of otolith weight with cod length indicate that assuming cod size at age follows a normal distribution then otolith weight distributions will be increasingly skewed with increasing age. This will lead to large bias in estimated proportions at age using the Bhattacharya method (ICES 2005). However, when we analysed the otolith weight distribution at age in the FAbOSA known age cod material applying a symmetric distribution with increasing standard deviation no problems with skewness were found (Figure 4). Further the total deviation in estimated proportions at age from the true values was 2.2%. This result indicates that although a power function of otolith weight to cod length with an exponent of about 2 fits both the Baltic and the Faroe cod material, the underlying growth

relationship between somatic and otolith accretion allows otolith weight to follow almost symmetric distributions. However, our work on the Baltic otolith weight database show that for these types of methods to be robust there is a requirement of prior information on mean otolith and fish size at age and preferably also a measure of the distribution types and their variances. These requirements thus also points back to the problem of validation with known age material for the specific population in question. However in combination with validated growth information and an appropriate transformation of otolith weight an integrated approach based on maximum likelihood estimating age distributions directly appears most promising

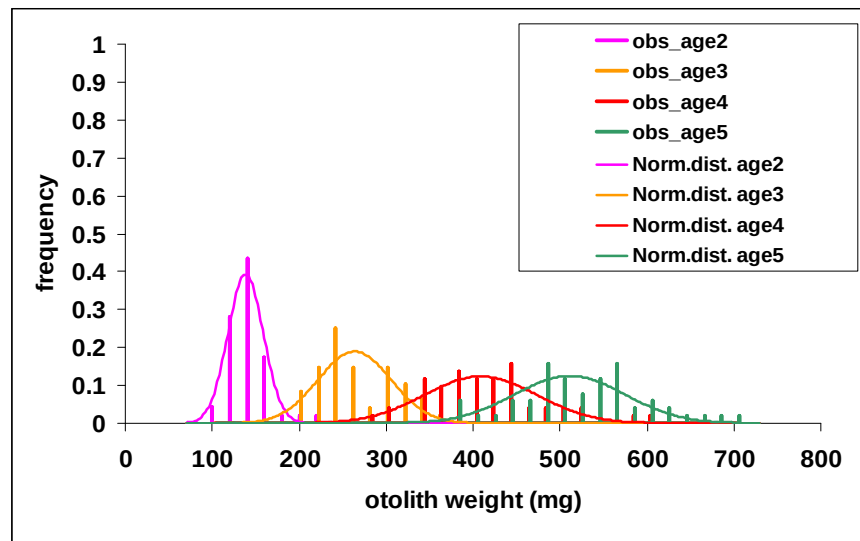


Figure 4. Otolith weight distributions by age from Faroe Plateau Pen cage reared cod (histogram) – fitted by Normal distributions (coloured lines).

3.3.5 Otolith growth experiments in the field

Otolith growth modelling using results from DST and Sr marked Baltic cod

In the EU FP5 project CODYSSEY, combined data storage tag and otolith mark – recapture experiments with mature Baltic cod were conducted, where Sr rich bands were incorporated into otolith growth structures and used for the analysis of otolith accretion rate under natural conditions in the Baltic.

A substitute for known age material could be the construction of a growth model based on measurements of otolith formation rate in relation to fish size and ambient environment. Baltic cod were tagged and released carrying data storage tags (DST) recording depth, salinity and temperature. In combination with the external tagging a solution of stable Sr was injected into the body cavity of the cod for the production of a Sr enriched band in the otolith indicating the temporal event of marking and

release. Both cod and DST were retrieved from the fishery and rewarded with 300 DKK for the tag and additionally 300 DKK for the fish at delivery.

Otoliths from a number of recaptured DST cod were processed to analyse accretion after formation of the Sr mark. Transverse sections (TS) through the centre of the Sagitta otolith were polished and carbon coated for backscatter imaging in SEM. A high electron dense band less than 10 microns wide indicated the Sr enrichment. Measurements were taken from the start of the band to the edge along the longest axis in the TS (Figure 5).

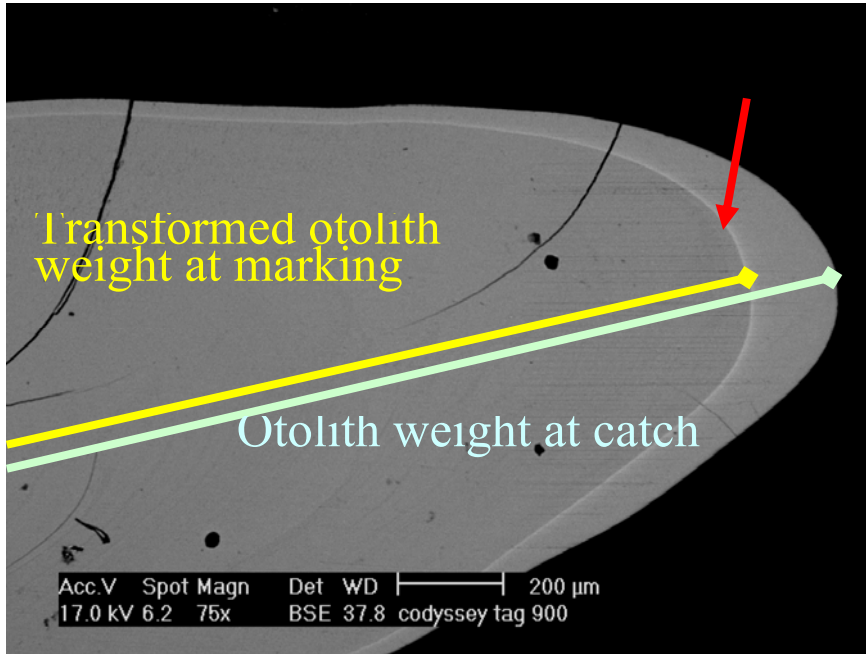


Figure 5 SEM backscatter image of Sr marked Baltic cod Sagitta otolith (red arrow electron dense Sr band)

To transform the increment after the Sr mark from a linear otolith radial scale (ΔOr) to a weight scale (ΔOw) otoliths were weighed and related to their measured height on a log-log scale, and in another series of measurements at the TS plane the height (Oh) was related to the radial distance from the centre to the edge (Or) using a mean geometric regression (Figure 6). Thus the weight increment could be calculated as:

$\Delta Ow = Ow_{edge} - Ow_{Sr} = \alpha \times (Oh^\beta - (Oh - g \times \Delta Or)^\beta)$ where α , β , and g were estimated from the following statistical relationships: $Ow = \alpha \times Oh^\beta$ and $Oh = g \times Or$.

The increment of otolith weight (ΔOw) in eight Sr marked cod that had spent more than sixty days in the Baltic before recapture were further analysed. When ΔOw was plotted against growth period in days a linear relationship was found with a good fit through the origin (figure XX2) and further cod length added significantly to the relationship as a second

covariate. It was therefore assumed that accretion rate was linked to fish size (e.g. length L) and a linear function was fitted to the relationship: $\frac{\Delta O_w}{\Delta t} = \lambda \times L + \varepsilon$ where λ is a coefficient giving annual otolith accretion in relation to fish length and ε is an error term.

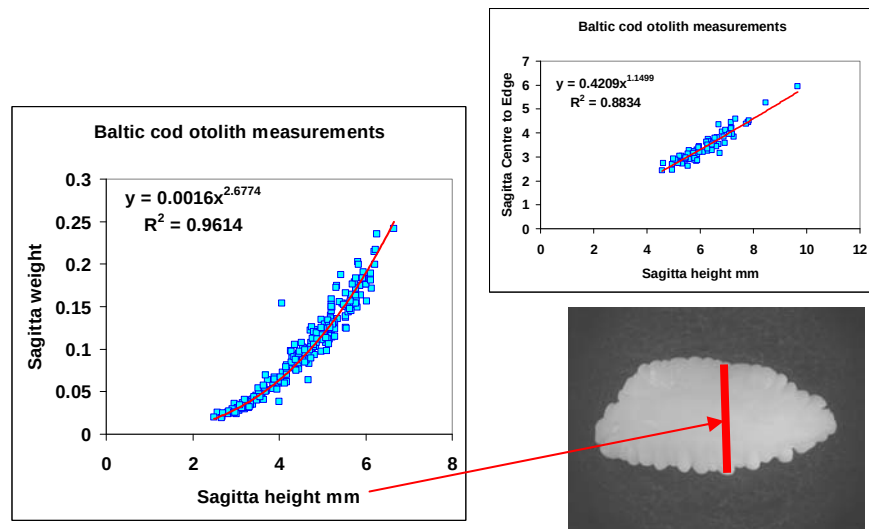


Figure 6 Measurements of the different dimensions of Baltic cod Sagitta otoliths.

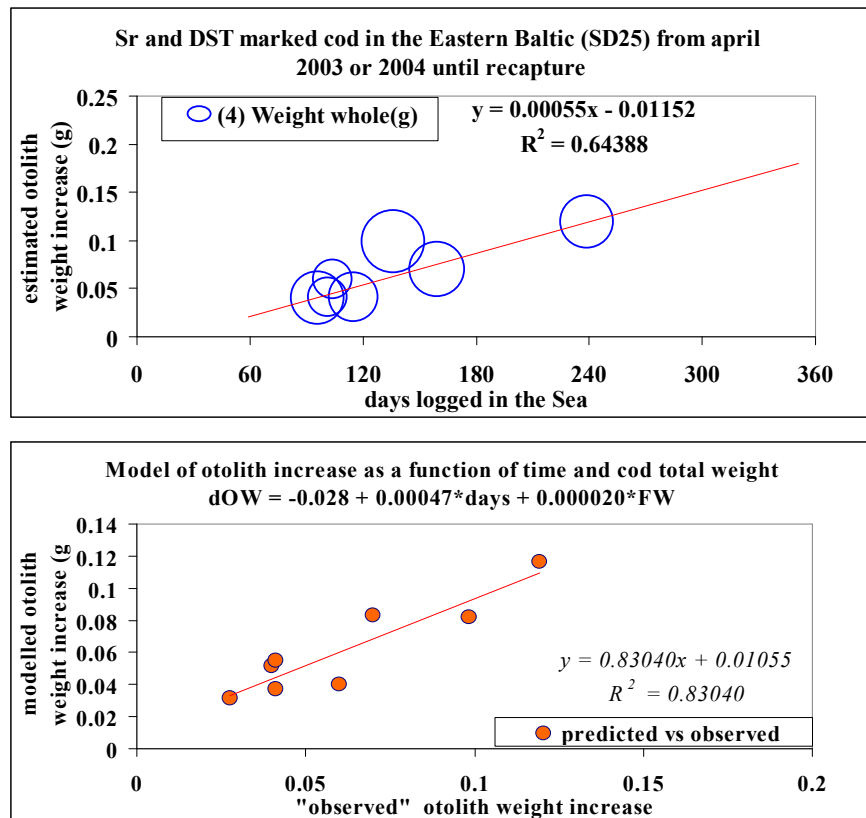


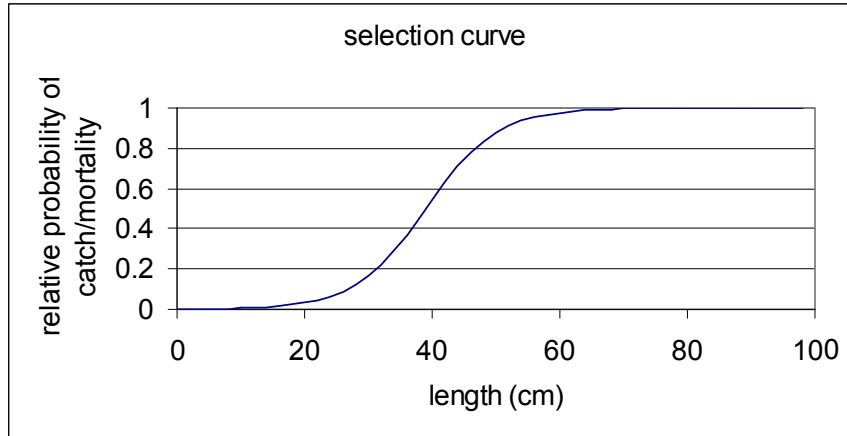
Figure 7 Upper panel: Relationship between days tagged, Baltic cod weight and otolith marginal accretion. Lower panel: estimated versus observed otolith weight increase.

Assuming a von Bertalanffy type model for cod length (L) growth the following differential equations are assumed:

$$\frac{\partial Ow}{\partial t} = \lambda \times L + \varepsilon \quad ; \quad \text{and} \quad \frac{\partial L}{\partial t} = \omega \times (1 - L / L_{\infty}) \Rightarrow$$

$$Ow_t - Ow_0 = \frac{L_{\infty} \times \lambda}{\omega} \times (L_{\infty} - L) + \frac{L_{\infty}^2 \times \lambda}{\omega} \times \ln \left[\frac{L_{\infty} - L_0}{L_{\infty} - L} \right] + \frac{L_{\infty} \times \varepsilon}{\omega} \times \ln \left[\frac{L_{\infty} - L_0}{L_{\infty} - L} \right]$$

where L is cod length at a given time t , Ow_t the corresponding otolith weight at t , L_{∞} the asymptotic length at infinity, ω is a constant related to initial growth rate (you often find the expression $K = \omega / L_{\infty}$), and L_0 and Ow_0 are cod length and otolith weight at an arbitrary initial time t_0 . The closed form can then be explored for different combinations of ω and L_{∞} given the coefficient λ of the linear relationship between L and otolith growth rate. To study the variation of otolith weight versus cod length in a population, combined variations in ω , L_{∞} and ε were applied. To achieve a realistic distribution corresponding to the one found in the samples of commercial catches from subdivision 25 a lower and upper thinning of the numbers at length was performed. The lower length tail was attained by a simulated logistic catch selection, and the upper length tail was achieved by a simulated mortality of individuals in the population integrating survival over time for a given growth trajectory assuming the same logistic selection curve.



The selection curve: $p = 1 / (1 + \exp(\alpha - \beta \times L))$ was applied simultaneously to a von Bertalanffy length growth expression with variation in both ω and L_{∞} as well with varying total mortality $Z = M + F$ to approximate the simultaneous length distribution and corresponding otolith weight distribution in the samples (commercial and scientific).

The approach did neither account for length stratification of samples nor the effect of different weightings of the different sample sources. The

presented procedure must therefore be seen as conceptual attempt to model the underlying processes of growth and mortality.

A semi automated minimisation routine was applied to maximise fit of model with observed data of cod length and otolith weight in subdivision 25. An individual based model approach (IBM) was used to give individuals with different age, length and otolith weight using different means and variation of ε , ω and L_{∞} . The only parameter that was taken as a constant was the coefficient λ of the linear relationship between L and mean daily otolith weight increase. The synthetic population was scaled to between 24000 and 32000 individuals which were then subjected to random individual mortality Z , and on top of that random catch probability both processes following the logistic selection curve. To decrease the stochastic noise introduced by random survival of very large individuals, both observed and model data on L were truncated at 90cm.

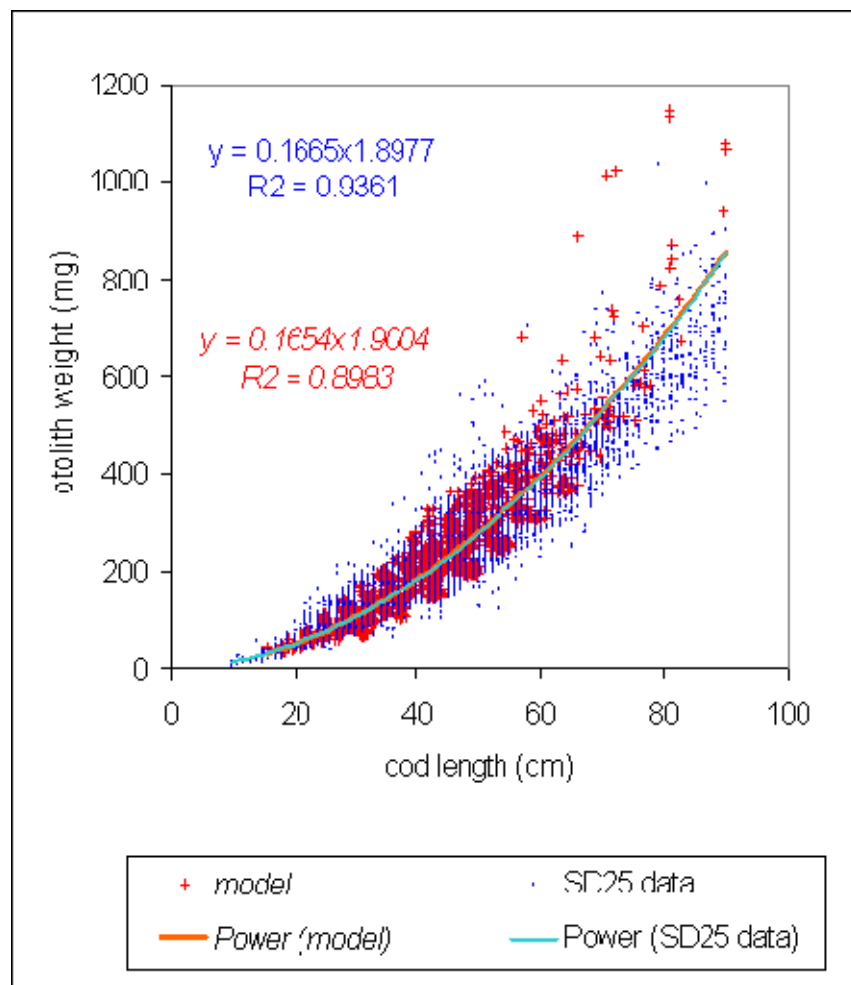


Figure 8 Plot of otolith weight versus cod length in material from 2001–2003 subdivision 25 (small blue circles) and similar data generated by the growth model (red crosses).

The parameters α and β of the selection curve were also varied in successive runs all to approach the length and otolith weight distributions and their individual relationship following a power law

$$\ln(Ow) = A + P \times \ln(L) + E \quad (\text{figures 8–10}).$$

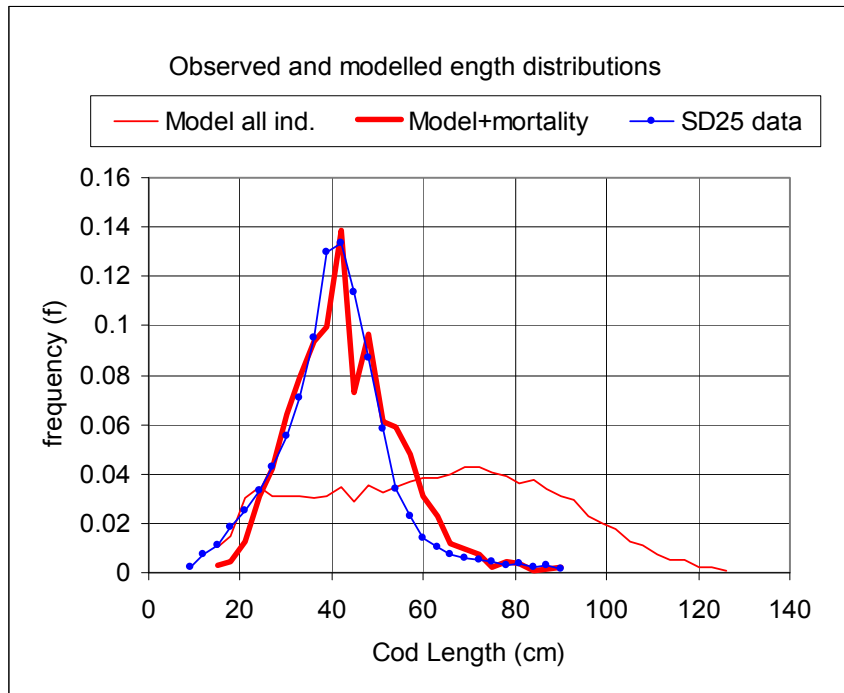


Figure 9 Model output of cod length distribution before and after applying mortality and catch selection compared to observed data from SD25.

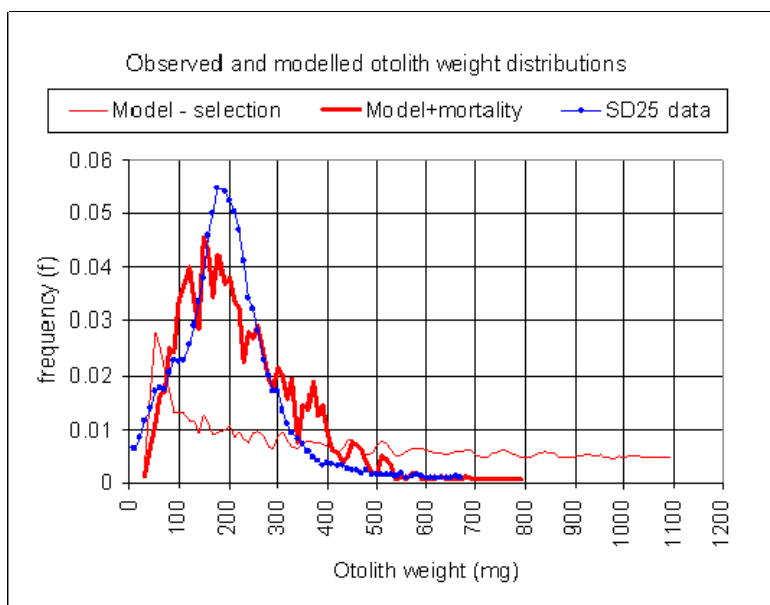


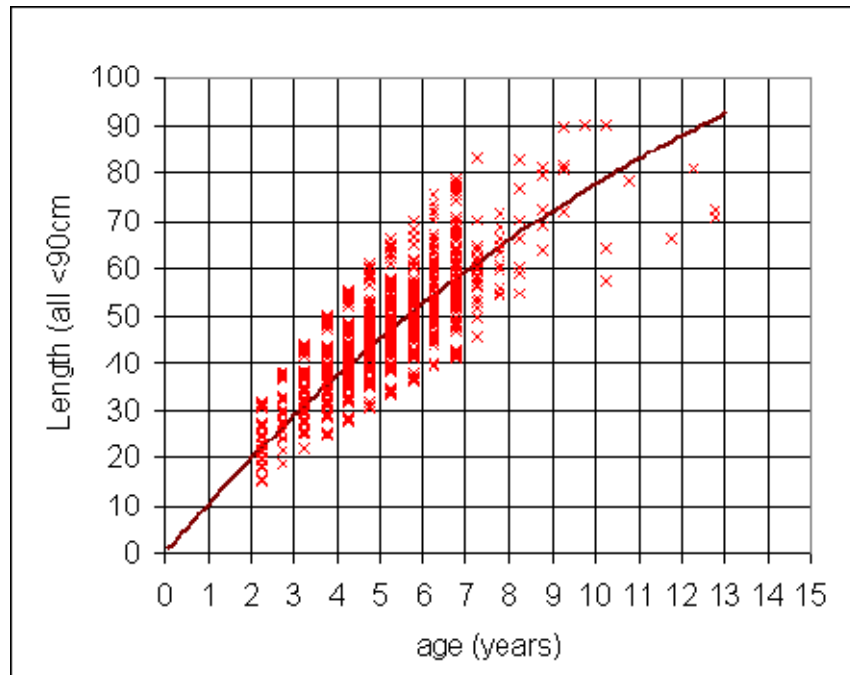
Figure 10 Model output of otolith weight distribution before and after applying mortality and catch selection compared to observed data from SD25.

Model stochastic explorations

An initial estimate of ε was achieved from the combined individual variation as well as model and measurement error in the experimentally found relationship between otolith weight increase and cod length. However applying this estimate gave a very high variation of the otolith weight – fish length relationship with R-square values around 0.2. A scaling factor ε -scale was therefore also applied and estimated in the procedure.

Low or no mortality lead to much steeper curvatures in the $Ow - L$ relationship and an apparently bad fit to the power model, however applying realistic total mortalities over 1 gave the same shape of the $Ow - L$ relationship as in the observed data. Simulation runs showed that parameters A and P describing the curvature of the otolith weight – fish length relationship could only be approximated allowing the model to give a higher variation E than in the observed data. This infers that the underlying relationship between otolith weight increase and cod length is more complicated than the suggested linear one. The field experiment of measuring otolith growth was performed on cod sizes larger than 40cm, and may therefore poorly represent small cod otolith formation both due to physiological differences (e.g. different maturation stages) as well as different habitat temperatures.

The estimated development of the variation in otolith weight at age may be overestimated, whereas the magnitude of the annual otolith weight increase for larger fish may still be captured by the model (Figure 11).



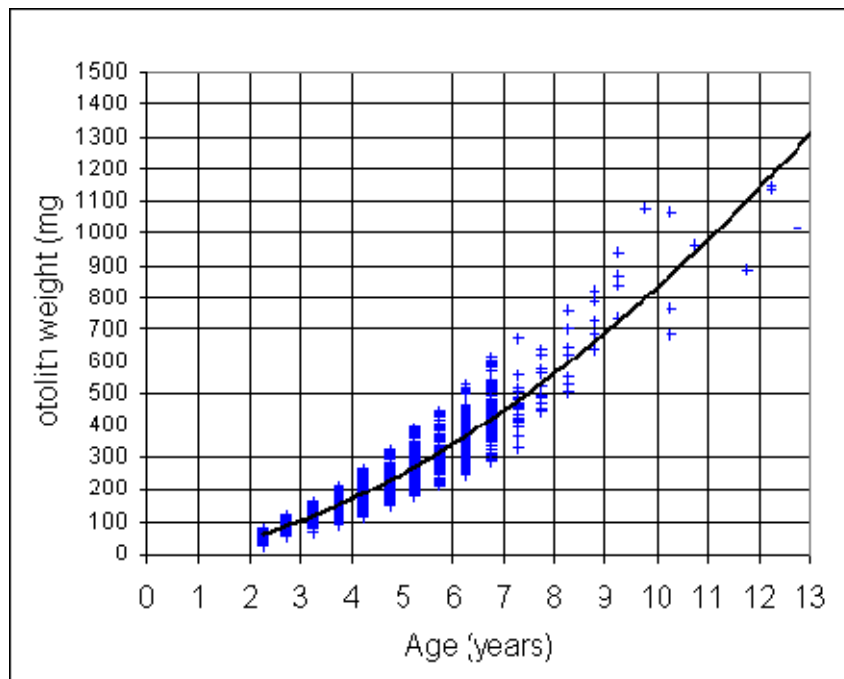


Figure 11 Model output of a) individual size at age, and b) individual otolith weights at age leading to the optimal fit.

The settings for the most successful series of runs (outputs shown in figures 8–11) are shown in the text table below:

epsi	annual error otolith growth rate	0.03290
sepsi	scaled annual error O_d/dt	0.00164
Z_scale	scaling of Z	1
Lo	L infinity cm	0
Oo	otolith weight mg	0
Yo	year start	2
eps_scale	scale of dO/dt error	0.05
Z	exponential annual mortality rate	1.3
rep_O	number of otoliths	18
rep_L	number of Loo	8
rep_k	number of omega	8
rep_Y	years	12
rep_M	periods per year	2
TOT_rep	N in the IBM	27648
TOT_L_surv	$N \cdot \exp(Z \cdot \text{sel} \cdot Y) \cdot \text{rand}()$	1598
a	selectivity alfa	40
b	beta	0.18

During the duration of the present project (OWABC) DST tagged Baltic cod have continuously been recaptured and their otoliths analysed whereby an increasingly more robust relationship between otolith growth rate and fish size is building up. This relationship may be used to improve

the precision of the growth based model. Analyses of total otolith increment from 42 DST marked cod are presently available for model parameterisation; data on the linear transverse otolith growth in relation to cumulated degree-days from release to recapture are plotted in figure 12.

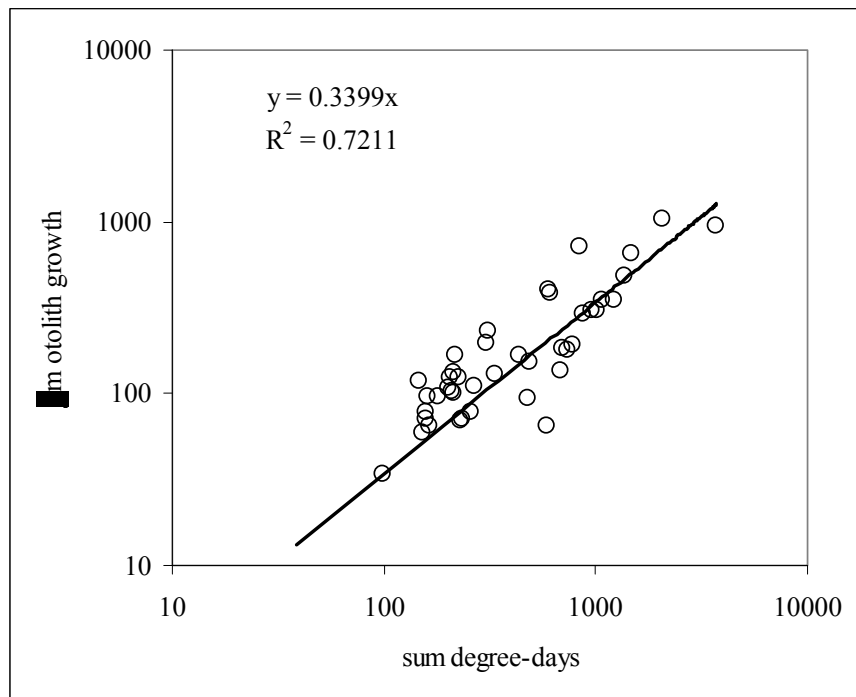


Figure 12 Baltic cod otolith growth along the dorso-ventral axis in relation to cumulated ambient temperature $\times$ time, on a log-log scale.

3.3.6 Integrated otolith accretion – somatic growth models

One approach that we have examined is the construction of integrated fish growth – otolith growth models from various sources of growth experiments in the laboratory and in the field. In the EU FP5 project IBACS, long and short term experiments were conducted where cod were monitored and otoliths were marked and measured for estimation of accretion rates at different environmental and physiological conditions.

One of the case studies focused on cod otoliths and material from Baltic cod mark – recapture experiments was used to analyse influence of natural environmental conditions on cod growth and otolith accretion (see 2.3.5).

The work had three main components: a) the formulation of an operational cod growth model based on bioenergetics b) the parameterisation of otolith weight accretion and optical density of growth structures, and c) the validation of the model by independent experimental data.

The main controlling factors for fish activity, growth and reproduction are food, temperature and light (Brett and Groves 1979). Other limiting factors like oxygen and salinity may create overall borders for scope of

existence. For the IBACS model development the effects of variation in the two most influential factors food and temperature was explored. Fish as exothermic animals exhibit progressively increasing oxygen consumption with increasing temperatures. Resting metabolism was experimentally found to be approximately 55 mg O₂/kg/hr for a 200g cod at 10°C with Q₁₀ of 2.6 from 5 to 10°C and a Q₁₀ of 1.9 from 10 to 15°C (Schurmann and Steffensen 1997).

There is basis for a general scaling of metabolism to mass of M^{3/4} in living organisms (Gillooly et al. 2001). However quite some variation among species may be found and the scaling of metabolism to body size may turn out differently for resting, and routine metabolism (Hermann and Enders 2000). Schurmann and Steffensen (1997), however, applied a relatively high value of 0.82 in recalculating their observed values for different sized cod to a standard size of 200g.

The capture, handling, intake, digestion and assimilation of food set the limits to the amount of energy available for metabolism and growth. Digestion rate in cod has been found to increase within the naturally varying range of temperatures, and Q₁₀ values are often higher than the above reported for resting metabolism, where e.g. Knutsen and Salvanes (1999) found a Q₁₀ of 3.4 in the range of 6–12°C. Since unlimited intake and thus successful feeding rate is expected to increase with digestion rate the energetic demands for the consumption processes may be investigated to find the reason for the generally observed dome shaped response of growth to temperature (Jobling 1994).

The defecation loss from consumed food that has not been absorbed during digestion and the excretion of primarily N-rich waste products from protein metabolism may constitute important amounts of the total energy consumed. These two sources together with mechanical and biochemical energy demand from processing a consumed meal (SDA) have in many models been set as constant proportions of total energy consumed (see e.g. Hanson et al. 1997); but their proportionality to consumption especially at high or low levels has been questioned (Bajer et al. 2004).

For the IBACS modelling approach the specific formulation of the model subcomponents was shown to be of some importance to the parameters driving otolith formation.

Even under low temperature conditions growth in cod is food limited under natural conditions (Bjørnsson 2002), this may partly be due to low energy content of invertebrates the most common natural food source and partly by the limited availability of food in general. Growth in cod is correlated with several indices of somatic condition e.g. hepatic index with a 44% correlation in well growing cod (Bjørnsson 2002).

With increasing size fish often spend an increasing fraction of energy input on gonad growth and reproduction (Yoneda and Wright 2005). The

IBACS modelling approach adopted a von Bertalanffy approach to the fraction of energy directed from somatic growth into gonad development:

Active metabolism

Following the ideas from Ware (1978) drag forces at fish size, swimming speed and temperature was described according to the following relationships:

$$(a) P = f \times \rho_w \times A \times C_T \times V^3 \times (2Q)^{-1}$$

Where:

f = a conversion factor ergs/sec $\rightarrow$ cal / sec: $2.39 \cdot 10^{-8}$ cal/erg

Q = dimensionless efficiency of chemical energy to locomotion energy: 0.20

ρ_w = density of water ($\text{g} \cdot \text{cm}^{-3}$)

A = wetted fish surface (cm^2) = $0.4 \cdot L^2$

C_T = Drag coefficient (dimensionless) = k/R_L^ω where k is a constant and

R_L is Reynolds number = $\rho_w \times L \times U \times \mu^{-1}$, where

μ is viscosity of water ($\text{g} \cdot \text{cm}^{-1} \cdot \text{s}^{-1}$) at T and salinity and

ω is a power dependent on flow = -0.5 at turbulent and -0.2 at laminar boundary layer flow.

U is body length per second ($\text{BL} \cdot \text{s}^{-1}$) and $V = L \cdot U$.

Substituting the dependence of C_T on L and swimming speed into (a) drag can be seen to be proportional to $P = \alpha \cdot L^\eta \cdot V^\beta$ where $\alpha = k \cdot f \cdot \mu^\omega$ for density of water being almost one ($\rho_w = 1$) with $\beta = 2.42$ and $\eta = 1.42$ in the example by Ware (1978) and $V = L \cdot U$.

From various workers resting metabolism has been estimated by the extrapolation of swimming metabolism at different swimming speeds back to the y-intercept; however scaling to body size demands knowledge about the body size scaling to swimming speed combined with the scaling at resting metabolism.

A relationship was set up of the following form:

$$(b) R = R_o + R_s$$

where R_o is resting metabolism and R_s is swimming costs estimated by scaling the drag force P to g wet weight respired per 24 hrs. Based on the argumentation by Ware (1978) that optimal cruising speed is close to observed swimming speed in pacific salmon, cod respiratory expenditure was calculated based on the swimming respiration measurements from Schurmann and Steffensen (1997).

The efficiency of swimming was expressed by the energy spent $E \cdot \text{s}^{-1}$ during a time interval with a swimming speed of $V \text{ m} \cdot \text{s}^{-1}$. The optimal

cruising speed without considering feeding was found by the minimum of R/V over V

$$(c) \delta [R/V] / \delta V = 0$$

$$\text{if } \delta^2 [R/V] / \delta V^2 > 0.$$

The components of total activity metabolism in (b) was expressed having the following relationship with size, swimming speed and temperature:

$$(d) R_o = \tau \cdot \text{EXP}(a+b \cdot T) \cdot c_F \cdot L^m$$

τ = is a constant transforming metabolic level into $\text{cal} \cdot \text{s}^{-1}$, and c_F is the condition factor scaling body wet weight to length in cm, $W = c_F \cdot L^m$, $m = \kappa \cdot \lambda$, where κ is the allometric exponent and λ is the weight exponent for resting metabolism.

$$(e) R_s = \alpha \cdot L^\eta \cdot V^\beta$$

where $\alpha = \eta = 2 - \omega$ and $\beta = 3 - \omega$ Respiration having units of $\text{cal} \cdot \text{s}^{-1}$ substitution into (c) yields:

$$(f) \delta [(\theta \cdot L^m + \alpha \cdot L^{2-\omega} \cdot V^{3-\omega}) / V] / \delta V = 0 \quad \text{where } \theta = \tau \cdot \text{EXP}(a+bT) \cdot c_F$$

Rearranging (f) and finding the derivatives gave the following expression for optimal swimming speed:

$$V_{\text{opt}} = (k \cdot L^{\omega+m-2})^{1/(3-\omega)}; \text{ where } k = (2-\omega)^{-1} \cdot \alpha^{-1} \cdot \theta \Rightarrow$$

$$(g) V_{\text{opt}} = k^{1/(3-\omega)} \cdot L^{(\omega+m-2)/(3-\omega)}$$

Data from Schurmann and Steffensen (1997) were used to fit the active metabolic equation

$R_{\text{tot}} = R_o + R_s$ combining (d) and (e). The weight exponent λ for cod was chosen equal to 0.82 and, κ , the allometric exponent was set to 3. The estimated parameter values are given in the text table and the fit to measured values in ob cit. are shown as correlation in Figure 13 and versus swimming speed in Figure 14.

0.064	b	estimated	Exponent to T in R_o
-5.77	a	estimated	Coefficient to T in R_o
0.594	ω	estimated	Exponent in drag
28.53	k	estimated	Coefficient in drag
0.82	λ	fixed	Exponent to W in resting metabolism
2.39E-08	f	fixed	Fixed cal/erg after Ware

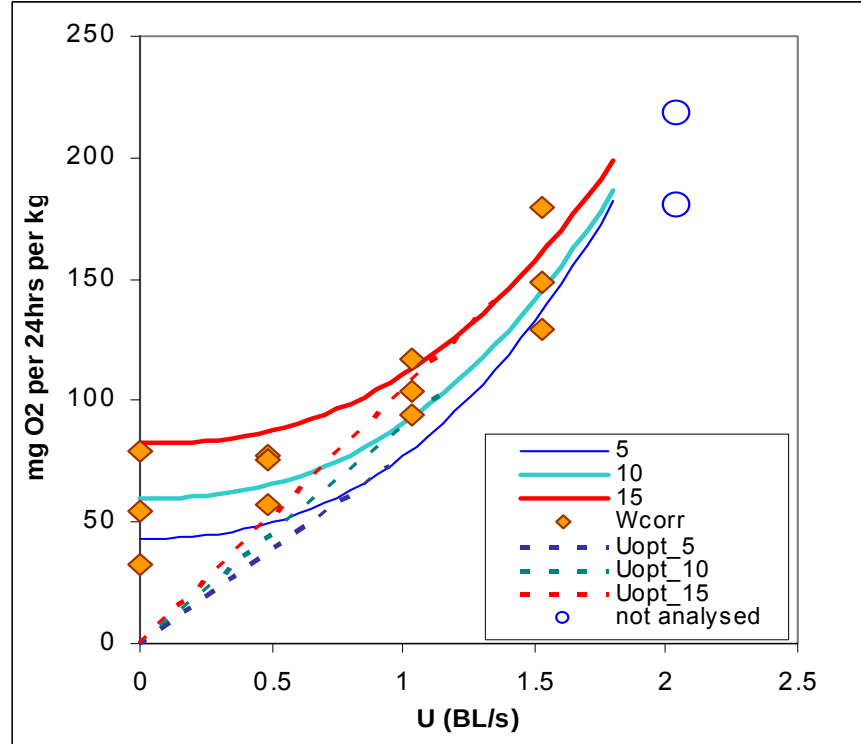


Figure 14 Swimming respiration for a 200 g cod (data taken from Schurmann & Steffensen 1997)

The aerobic scope is experimentally determined by the oxygen consumption at maximum sustainable swimming speed and may be expressed as (c_{OX}) a multiplier of resting metabolism. Data for cod data may be approximated by a parabolic relationship and the appropriate values used for the IBACS model were taken from Schurmann and Steffensen (1997).

Assuming equal respiratory regulations for all energy demanding processes aerobic scope also sets limits for the maximum consumption rate depending on the magnitude of energy expenditure in relation to amount of food consumed and the swimming speed determining R_S .

However part of the ingested food energy is lost in different processes; if e_F defines the fraction of non digested food wasted as faeces we may define the assimilated food A as $C \cdot (1 - e_F)$, after assimilation a fraction of the consumed energy is used for the standard dynamic action, e_{SDA} and a fraction for excretion, e_U , lost by trans- and deamination of proteins for production of ammonia or other Nitrogenous compounds.

Since cod will not survive if long term oxygen demands exceed c_{OX} the following inequality may hold:

$$(h) R_o + R_S + A \cdot (e_{SDA} + e_U) \leq R_{crit}$$

Under the assumption that cod are moving at the above defined optimum swimming speed for a fraction of the day, f_D , determined by food avail-

ability then the energy budget for consumption (C) including temperature may be expressed in the following way:

$$A \cdot (e_{SDA} + e_U) \leq R_{crit} - (R_o + R_S)$$

Let:

$$C_{scope} = (R_{crit} - (R_o + R_S)) / ((e_{SDA} + e_U) \cdot (1 - e_F)) \Rightarrow$$

$$C \leq C_{scope}$$

For any settings of T°C and V the calculated $C_{max} > C_{scope} \geq C$ and consumption will therefore have a maximum limit equal to C_{scope} and cod will not be able to sustain consumption at maximum evacuation rates. Thus growth at unlimited food conditions may be calculated as:

$$G = C_{scope} \times (1 - e_F) - C_{scope} \times (e_{SDA} + e_U) - (R_o + R_{V,hrs})$$

where $R_{V,hrs}$ is the respiration at a specific swimming speed $V = f_V \cdot V_{opt}$ proportional to the optimal speed V_{opt} during a fraction, f_{hrs} , of the 24 hrs.

$$R_{V,hrs} = f_{hrs} \cdot \alpha \cdot L^{2-\omega} \cdot (f_V \cdot V_{opt})^{3-\omega}$$

where V_{opt} is defined in eq. 4 ; and thus growth in the same is limited by aerobic scope and scope for consumption:

$$(i) G = (R_{crit} - (R_o + R_{V,hrs})) / (e_{SDA} + e_U) - R_{crit}$$

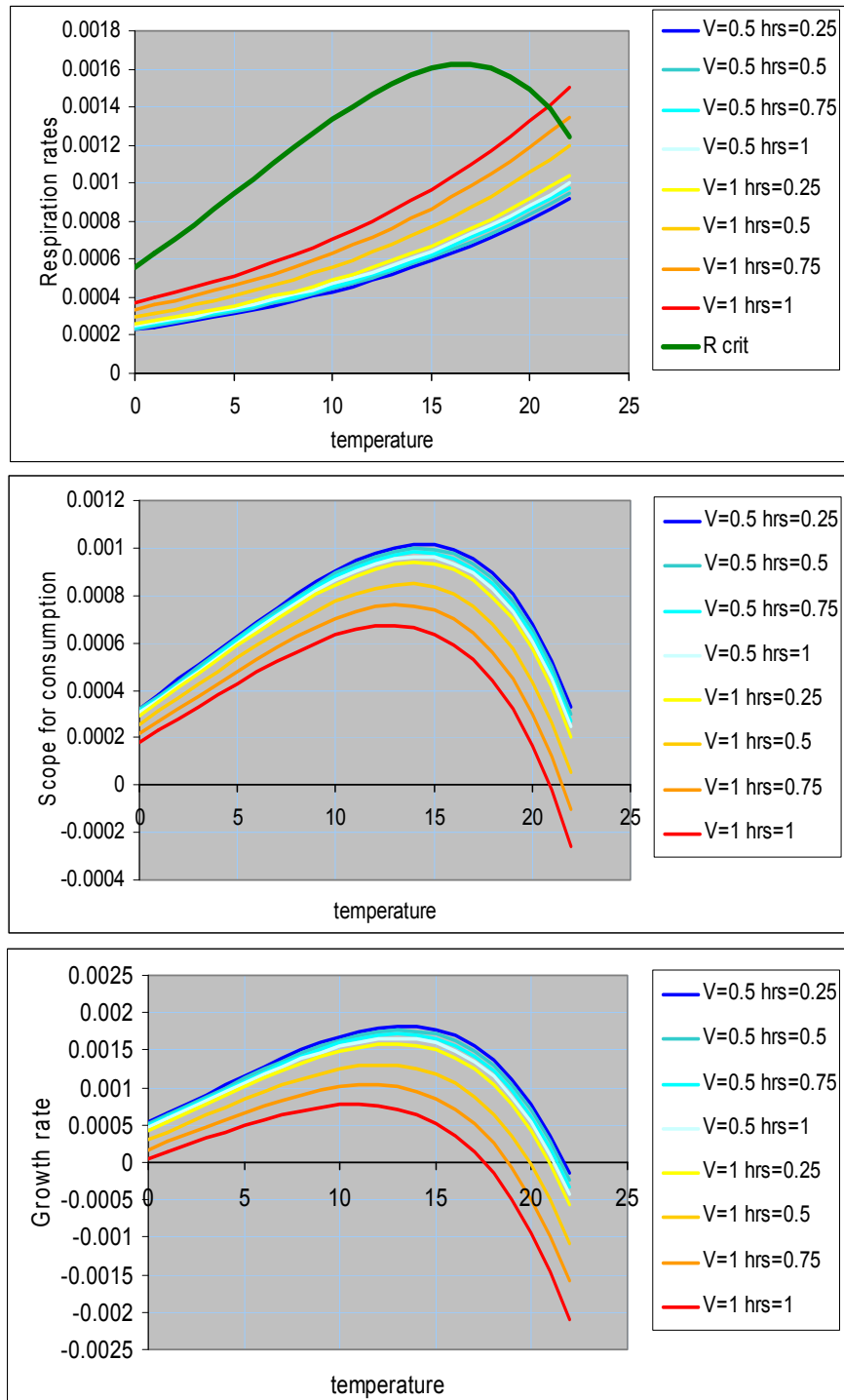


Figure 15: Metabolic relationships between temperature and a) Respiration rate, b) aerobic scope, and c) growth rate and temperature

Comparison of predicted and observed cod growth at different temperatures

Under the assumption that cod minimise risk of exceeding aerobic scope by separating searching for food from digestion, a dome shaped growth response to temperature at all feeding levels may be expected.

In an extensive series of long term experiments Bjørnsson and Steinarsson (2002) determined cod growth from early juvenile to large mature adult stages at different temperatures and under unlimited food conditions. They found that a three parameter equation of the following form adequately fitted their data:

$$(j) G = \delta_1 T \cdot W^{(\gamma + \delta_2 T)}$$

Although this model approach has a dome shaped temperature response growth rate only asymptotically approach zero at high temperatures and therefore may have a tendency to over estimate growth at super optimal temperatures. Further the study being performed at unlimited food supply gives no indication of how growth would scale to low feeding conditions.

The published results of Bjørnsson and Steinarsson (2002) were compared with the estimated relationships between growth and temperature using the IBACS bioenergetics approach. The estimated relationship between growth rate and scope for aerobic metabolism contain some parameters that may change under routine feeding behaviour. Since R_{crit} is measured under short term swimming experiments it may attain a lower value when limiting food intake at normal foraging behaviour. Further the suggested behavioural mechanism of shifting between searching and digestion will set $R_{V,hrs}$, the respiratory expenditure of active metabolism, at a value which is a fraction, $D24_{scale}$, of the value respired by constant 24 hours activity at swimming speed V . If cod is able to foresee its feeding conditions, optimal swimming speed may have a theoretical value different from what it would be if only distance swum was to be maximised.

The estimate of food consumption rate was therefore limited by a multiplier, f_{crit} , (less than one) times aerobic scope, and the best fit to the observed growth data was estimated by varying f_{crit} and $D24_{scale}$, the fraction of the day spent at foraging with the optimum swimming speed at the given combinations of cod size and temperature.

The different energy fractions of consumption not utilised for growth, e_{SDA} , e_U , and e_F were not available from the experiments by Bjørnsson and Steinarsson (2002). We therefore allowed all these parameters to be estimated within certain reasonable limits (details given in text table).

The adjusted equation (i) was then used to build an objective function F for minimisation, $\text{MIN}[F]$.

$$F = \sum \{G_{\text{obs}} - (f_{\text{crit}} \cdot R_{\text{crit}} - (R_0 + R_{V,D24\text{scale}})) / (e_{\text{SDA}} + e_U) - f_{\text{crit}} \cdot R_{\text{crit}}\}^2$$

where G_{obs} were the growth values of larger than 80 g cod in the data from Björnsson and Steinarsson (2002). The corresponding $F_{B\&S}$ estimating the sum of squared differences between equation (j) and G_{obs} was also found.

Text table

V_{scale}	1	multiplier of optimal swimming speed (fixed)
$D24_{\text{scale}}$	0.2	amount of time spent swimming at optimal speed (fixed)
e_{SDA}	0.12	Standard dynamic action. Limits=[0.1;0.25]
e_U	0.02	Excretion. Limits=[0;0.05]
f_{Repr}	0.25	fraction of G going into gonad production during reproduction. Limits=[0.25;0.75]
f_{Crepr}	0.87	Annual feeding during reproduction Limits=[0;1]
f_{crit}	0.8	Routine aerobic scope as a fraction maximum aerobic scope. Limits=[0.8;1]
e_F	0.02	Defecation. Limits=[0.1;0.25]
W_{mat50}	1000	WW where 50% of $f_{\text{Repr}} \cdot G$ go to reproduction (fixed)
b_{mat}	0.005	rate of change of % of $f_{\text{Repr}} \cdot G$ going to reproduction (fixed)

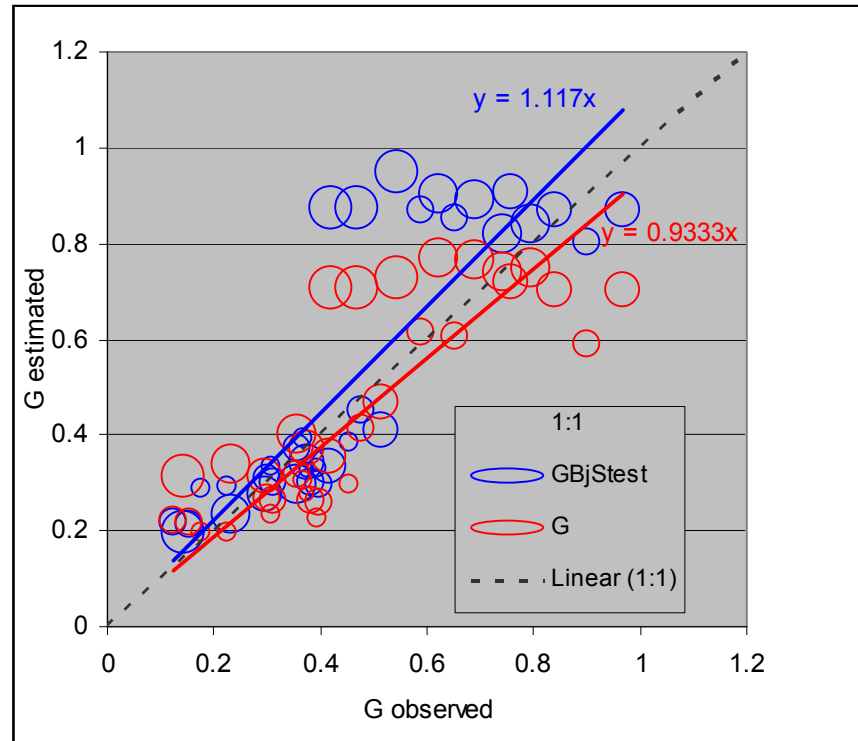


Figure 16 Estimated versus observed values from unlimited cod growth experiment. Blue = temperature – weight power function Björnsson and Steinarsson (2002). Red = Bionergetics relationship this study. Larger circles indicate higher temperatures

The estimated relationships are shown in figure 16, indicating a somewhat variable fit of both approaches. However $\text{MIN}[F] = 0.57$ with the estimated parameter values in the text table was considerably lower than $F_{B\&S} = 0.91$; indicating a somewhat better fit with the bioenergetics approach than with the original suggested temperature – weight power function. For each value the squared deviation $\{G_{B\&S} - G_{\text{obs}}\}^2$ was subtracted from the squared deviation $\{G_{\text{Resp}} - G_{\text{obs}}\}^2$ and plotted versus temperature. A negative value then indicates a better fit of the bioenergetics approach to growth G_{Resp} than the original $G_{B\&S}$ (Figure 17). It is clear that the bioenergetic approach has a better performance at higher temperatures, and we therefore chose this for continued model building.

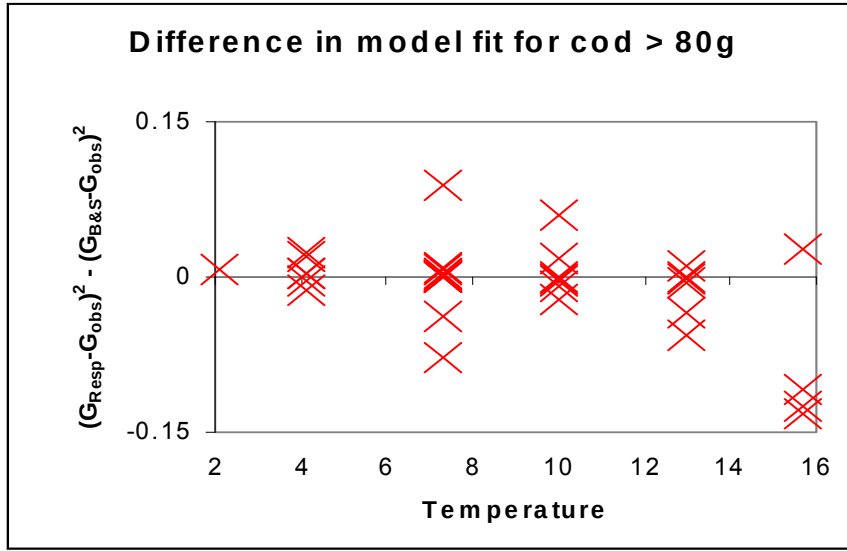


Figure 17 Comparison between bioenergetics approach (G_{resp}) to estimating growth rate G and three parameter T and W approach ($G_{B\&S}$) by Bjørnsson and Steinarsson (2002). Negative values indicate better fit of G_{resp} than of $G_{B\&S}$.

Differential formulation of otolith weight accretion

The IBACS model explore both otolith biomineralisation rate and organic influence on optical density, here the focus will be on the former.

Otolith weight accretion is considered to be composed of two elements (1) the inorganic mineral component growing as a function of resting respiration rate:

$$\delta O_M / \delta t = a_o \cdot g(R_o) + \delta W / \delta t \cdot P_{\text{eff}}$$

(2) The organic component incorporated in the otolith accretion assumed proportionally to fish protein synthesis rate raised to a power less than one:

$$\delta O_P / \delta t = (\text{MAX}(0 ; g_{P_o} \cdot \delta W / \delta t) + r_{P_o} \times R_o \times W^{s/v-1}).$$

Where r_{P_o} is the scaling to synthesis rate of re-circulated protein, and g_{P_o} is the scaling of otolith organic component to fish growth rate $\delta W / \delta t$.

Finally s/v is an exponent that scales as the active secreting epithelium surface in relation to whole fish body volume. However, the actual proportion active secreting surface area probably decreases with increasing fish size and age causing the flattening of the otolith 3D shape.

The fish bioenergetics model was fitted using Baltic environmental conditions (sub-division 25) with limited fish growth based on a proportion of day, P_{24} , feeding in relation to unlimited growth ($P_{24} = 0.2$) and ambient temperatures scaled from the recent 1990-ties time series by 1.1, the output was exemplified for a 1.7 kg 55cm cod caught January 2004 with an estimated age of 4 years, the model was started with an 0-group cod size of 6 g November 1st 2000 as typical size found in the BITS November survey in the Baltic.

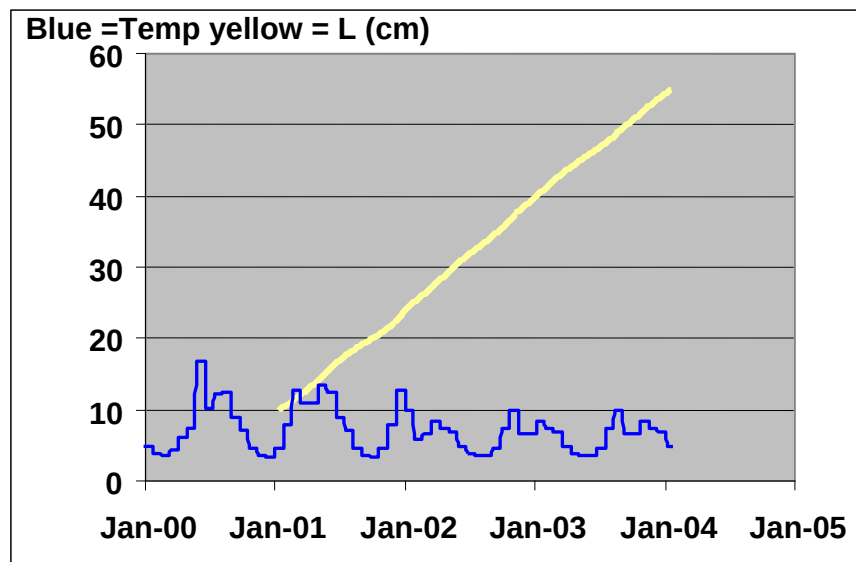


Figure 18 Ambient temperatures and cod growth in length

To make model output of the fish bioenergetics component fit the estimated size at age for the example cod (#3 in SD25 st19) model output for January 25 2004: Weight 1667 g, length 54 cm, P_{24} was estimated equal to 0.45.

Assuming the bioenergetics relationship to be realistic Baltic cod is then somewhat limited by food in addition to temperature. However periods of low food intake corresponding to migrations and spawning may be balanced by compensatory growth during other periods. The model was not adjusted for seasonal variation in feeding opportunity and growth in length is therefore a smooth almost linear function (figure 18).

The next step was to fit otolith growth rate r_{p0} to observed values of otolith growth in relation to cod size at age. The average otolith weight at each cod cm group in the Danish database from BITS surveys 2004 and 2005 were calculated and plotted, and the estimated power function used for calibration (Figure 19).

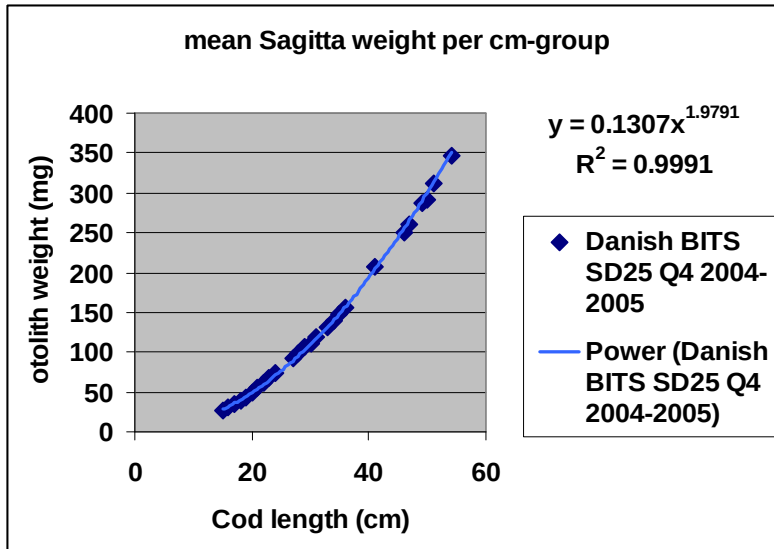


Figure 19 Observed relationship between length and mean otolith weight per cm group 15-54cm in Q4 DK BITS survey SD25 2004 and 2005

The optimized otolith model output with a volume to weight scaling of $2/3$ of R_o gave a higher exponent of 2.34 compared to the observed exponent of 1.98, indicating that either otolith accretion does not scale to resting metabolism with cod weight or that the estimate of cod size scaling of metabolism being 0.82 is too high (Figure 20a). If the cod weight exponent inherent in R_o is changed by multiplying with 0.66 according to a surface process the exponent changes some but insufficiently (Figure 20b); whereas a change in the multiplier from 0.66 to 0.54 fully adjusts the power relationship between otolith weight accretion and respiration (Figure 20c).

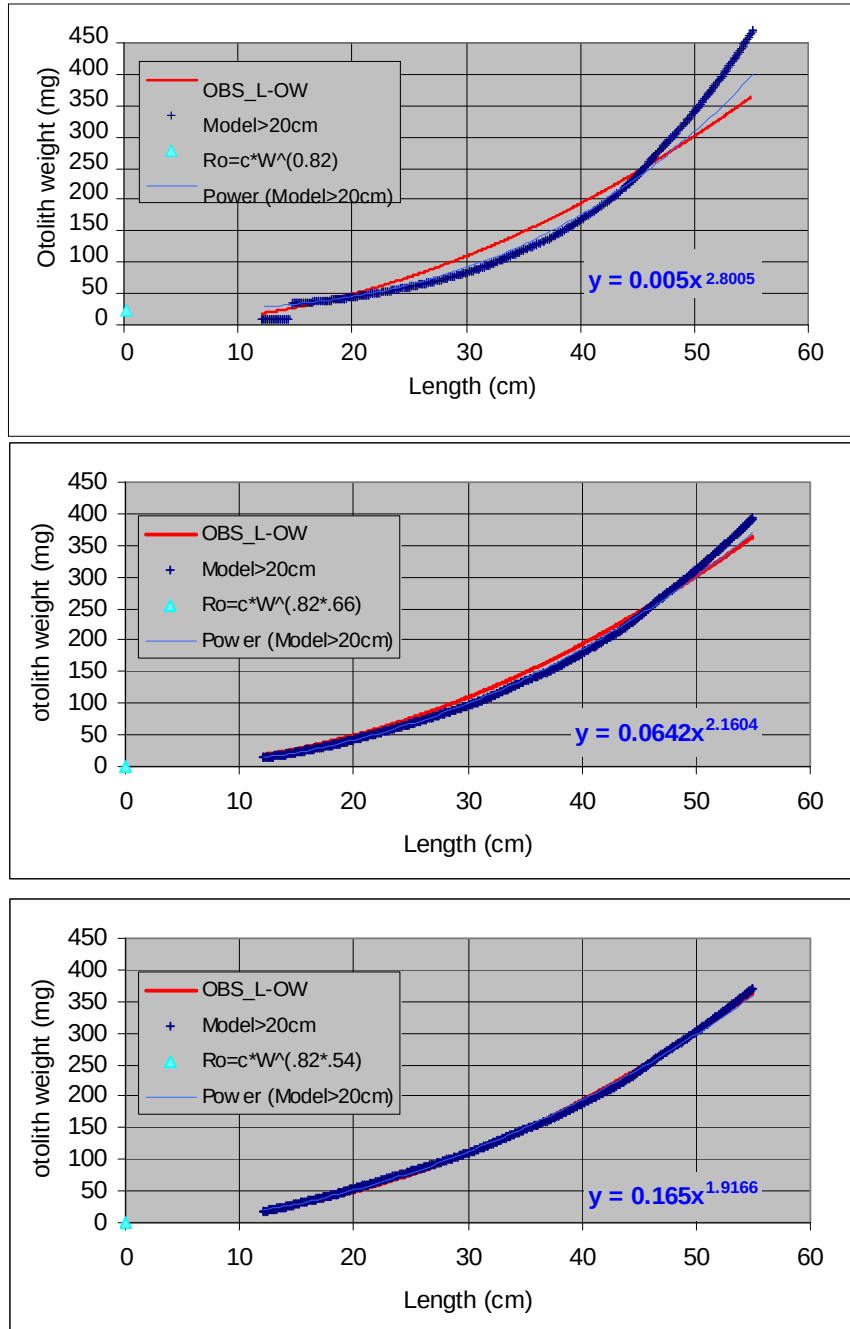


Figure 20 a) upper panel model output with original scaling of Ro (0.66) and W (0.82) b) middle panel rescaling of W to (0.75) c) lower panel rescaling of Ro to (0.44)

3.4 Validation of approach developed in 2.3

Different methods of validation exist depending on the final strategy for method application. 1) Model may be validated by predictions of otolith size, structure and morphology in relation estimated fish and age, and validated model inference of age structure tested by integration into population models with fishing mortality. 2) otolith and fish growth rates with estimated variation are simulated in population scenarios for comparison to observed progression of otolith size – fish size bivariate distributions.

The results from an IBACS short term experiment of cod growth and otolith formation at different temperatures and ad lib feeding were analysed in relation to model predictions of otolith growth. Data on otolith increment width formed during 2 weeks experimental periods were scaled to a standard axis in the otolith and were transformed to total otolith weight accretion per day for the different experimental periods with different temperatures. The log-log regression between the dorsal axis and otolith weight was used in the dOw back transformation, and therefore the values should be seen as relative to the true otolith weight increase, but reflecting the correct trend versus temperature and fish size (for the values to be accurate they have to be individually rescaled versus the correct reading axis). There was a good correlation ($R^2=0.70$) between observed and modelled values (Figure 21). Further the temperature relationship followed the same increasing trend all though observed Q_{10} based on mean dOw by temperature and averaged over successive temperature groups appeared to be somewhat higher for measured Q_{10} than for model Q_{10} , being 2.5 and 1.7 respectively. The model predictions are based on published values of resting metabolism, however the functional relationship to temperature is crucial for the shape of the response curve especially at low temperatures, forcing the intercept towards zero with a power function instead of the applied exponential may give more realistic values in this area. More research of these relationships is needed.

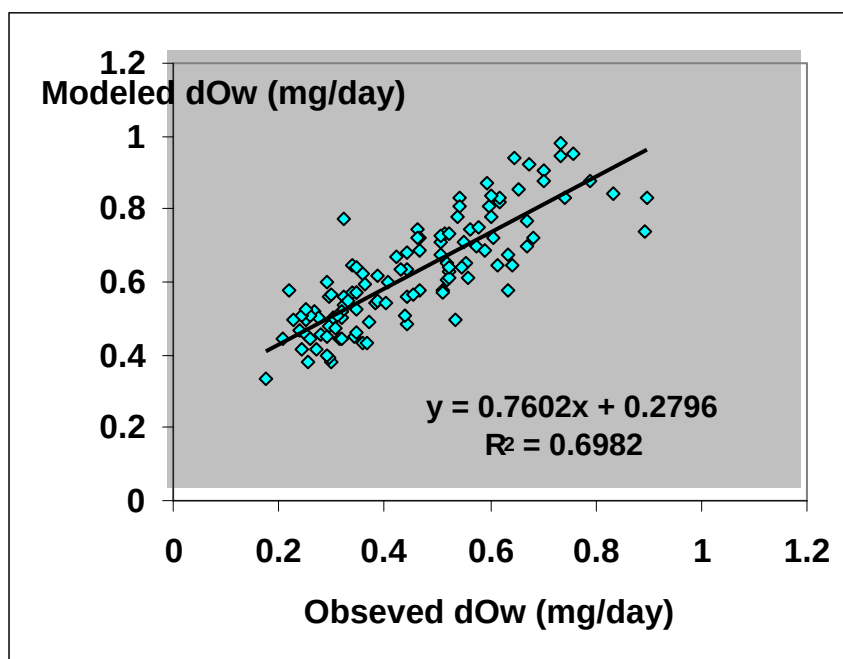


Figure 21 Modelled otolith weight increase per day versus observed otolith accretion based on back transformed otolith weight increase on standard scale (OBS X-axis should be re-scaled with corrected units)

3.5 Evaluate the possibilities to use the procedure described in 2.2–2.4 for implementation in assessment work

Given the establishment of validated otolith accretion models and information about ambient environmental conditions for cod distributions at size and season, otolith weight distributions from earlier periods may be transformed into age composition of the samples. Differences in earlier time environmental conditions like temperature and food influencing both cod growth and otolith accretion rates may change expected modal values of cod length and otolith weight at age. However, survey information on cod preferred habitat in combination with hydrographical reconstruction of ambient temperatures may yield enough information to estimate approximate otolith size at age. The raising of sample age composition to catches will require access to information about national sample stratification and total length distributions by year, quarter and area. Using theoretical parameter values as first approximations in multivariate mode splitting methods (e.g. MULTIFAN) or full population models with individual based growth and mortality will likely provide basis for an improved stock assessment of Baltic cod.

When the final strategy for revision of Baltic cod age data has been decided guidance may be provided for updating of historic catch at age data.

Based on the results emerging from the present project under the auspices of the Nordic Council of Ministers and collaborative efforts among Baltic fisheries institutes within ICES have led to international initiatives of EU research and study projects. These initiatives aim at developing cost efficient methods of fish age estimation and provide a practical solution to long lasting problems of age determination of Baltic cod.

4. Conclusions

It was stated by the 2006 ICES study group on Age of Baltic Cod (SGABC) (ICES 2006) that the development of an otolith growth model is hampered by the lack of validation material (mark-recapture or other data on known age). This prevents not only validation but also the development of statistical methods to update historical data. Further SGABC (ICES 2006) again noted that the annual and intersessional age reading exercises have not improved the precision between age readers. There is still a large variation in the interpretation of ages both between laboratories but also within single readers. And finally SGABC observed that annual study groups do not provide a sufficient effort to overcome above difficulties. Instead a dedicated and focussed research programme should address the age reading problems.

The present small scale project under the auspices of the Nordic Council of Ministers has been able to accomplish progress along several lines.

Thanks to the effort within this project the collation of standardised data on otolith weights and corresponding fishing observations in an internationally accessible database has been initiated. Further, published literature on available methods and results from several international collaborations within EU research projects and ICES working/study groups have been put together and analysed in the context of Baltic cod age estimation. These results have been used during our exploration of the otolith weight frequencies extracted from the database to develop strategies for optimal estimation of cod population age structure. Several independent results point at otolith weight from known age cod as a robust basis for calibration of an objective estimation of Baltic cod age – length relationship.

However, we observe that there presently is a lack of known age Baltic cod calibration material and that no strategy for production of such material yet exists. We therefore propose an initiation of a long term continuous mark-recapture programme for Baltic cod where juvenile cod are raised to or caught at a known age, marked, released and recaptured in the fishery for the provision of otolith weights at different ages for the calibration samples.

Given the present situation with no known age otoliths we assess a number of alternative approaches, either providing indirect estimates of age/year-class with some error or setting up models of population age composition based on growth or bioenergetics. With the accumulating numbers of recaptured cod with Sr marked otoliths the usefulness of the growth based models increases. We find that otolith growth based meth-

ods may provide a preliminary means of adjusting the present perception of the age structure of Baltic cod.

Due to the collaborate effort between the project partners and their colleagues around the Baltic the focus has moved from adjusting the long time persisting age determination errors and bias by inter-calibration exercises of otolith interpretation towards more objective measures using cod size and otolith metrics.

Finally the project has provided a platform for conducting inter-sessional work on the otolith weight database as well as initiating international EU initiatives for research projects addressing this fundamental scientific problem for the regional fisheries management.

References

- Asano, M., and Mugiya, Y. 1993. Biochemical and calcium-binding properties of water-soluble proteins isolated from otoliths of the tilapia, *Oreochromis niloticus*. *Comp. Biochem. Physiol. B Comp. Biochem.* 104: 201–205.
- Beck, F., and Gropp, J. 1995. Estimation of the starvation losses of nitrogen and energy in the rainbow trout (*Oncorhynchus mykiss*) with special regard to protein and energy maintenance requirements. *J. Appl. Ichthyol.* 11: 263–275.
- Bhattacharya, C.G., 1967. A simple method of resolution of and distribution into gaussian components. *Biometrics* 23:115–135.
- Björnsson B 2002 Effects of anthropogenic feeding on the growth rate, nutritional status and migratory behaviour of free-ranging cod in an Icelandic fjord. *ICES J Mar Sci* 59(6) 1248–1255
- Björnsson B. and A. Steinarsson. 2002. The food-unlimited growth rate of Atlantic cod (*Gadus morhua*) *Can. J. Fish. Aquat. Sci.* 59: 494–502.
- Boehlert, GW, Using objective criteria and multiple regression models for age determination in fishes. *Fishery Bulletin.* Vol. 83, no. 2, pp. 103–117. 1985.
- Brett, J. R. & Groves, T. D. D. 1979. Physiological energetics. In *Fish Physiology*, Vol. 8 (Hoar, W. S., Randall, D. J. & Brett, J. R., eds), pp. 279–352. New York: Academic Press.
- Cardinale, M., Arrhenius, F., and Johnsson B. 2000. Potential use of otolith weight for the determination of age-structure of Baltic cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*). *Fisheries Research*, Volume 45, Number 3, pp. 239–252(14).
- Cardinale, M. and Arrhenius, F 2004. Using otolith weight to estimate the age of haddock (*Melanogrammus aeglefinus*): a tree model application *J Appl. Ichth.* Vol. 20 no 6 pp 470-
- Cardinale, M., Doering-Arjes, P., Kastsowsky, M., and Mosegaard, H. 2004. Effects of sex, stock and environment on the shape of Atlantic cod (*Gadus morhua*) otoliths. *Can. J. Fish. Aquat. Sci.* Vol 61(2) p158–167.
- Doering-Arjes, P., Cardinale, M., Kastsowsky, M., Mosegaard, H. Optimising fish ageing using otolith weight and shape confirmed with known age cod. ORAL PRESENTATION at the 3rd International Symposium of Fish Otolith Research and Application. Townsville, Australia 11–16 July 2004.
- Fletcher, WJ., 1991. A test of the relationship between otolith weight and age for the pilchard *Sardinops neopilchardus*. *CJFAS* Vol. 48, no. 1, pp. 35–38.
- Francis, R.I.C.C. and Campana, S.E. (2004) Inferring age from otolith measurements: a review and a new approach. *Can.J. Fish. Aquat. Sci.*, 61: 1269–1284.
- Francis R. I. C, Harley J. Campana, S and Doering-Arjes, P., 2005. Use of otolith weight in length-mediated estimation of proportions at age. *Marine and Freshwater Research* 56(5) 735–743
- Houlihan, D.F., Hall, S.J., Gray, C., and Noble, B.S. 1988. Growth rates and protein turnover in Atlantic cod, *Gadus*

- morhua. Can. J. Fish. Aquat. Sci. 45: 951–964.
- Hüssy, K., Mosegaard, H., Hinrichsen, H.H., and Böttcher, U. 2003. Factors determining variations in otolith microincrement width of demersal juvenile Baltic cod *Gadus morhua*. Marine Ecology Progress Series 258 (2003): 243–251.
- Hüssy, K., Mosegaard, H., Hinrichsen, H.H., and Böttcher, U. 2003. Using otolith microstructure to analyse growth of juvenile Baltic cod *Gadus morhua*. Marine Ecology Progress Series 258 (2003): 233–241.
- Hüssy, K., and Mosegaard, H. 2004. Cod (*Gadus morhua* L.) growth and otolith accretion characteristics, modelled in a bioenergetics context. Can. J. Fish. Aquat. Sci. 61 (6) p1021–1031.
- Hüssy, K., Mosegaard, H., Jessen, F. 2004. Effect of age and temperature on amino acid composition and the content of different protein types of juvenile cod (*Gadus morhua* L.) otoliths. Can. J. Fish. Aquat. Sci. 61 (6) p1012–1020.
- Megalofonou, P., 2006. Comparison of otolith growth and morphology with somatic growth and age in young-of-the-year bluefin tuna. J. Fish Biol. Vol. 68 no. 6 p 1867–.
- Huuskonen, H., and Karjalainen, J. 1998. A preliminary study on the relationships between otolith increment width, metabolic rate and growth in juvenile whitefish (*Coregonus lavaretus* L.). Arch. Hydrobiol. 142: 371–383.
- ICES, 2004. Report of the Study Group on Ageing Issues in Baltic Cod, 11–14 May 2004. ICES CM 2004/ACFM:21.
- ICES, 2005 Report of the Study Group on Ageing Issues of Baltig Cod (SGABC). ICES CM 2006/ACFM:02, Ref G,H.
- ICES, 2006 Report of the Study Group on Ageing Issues of Baltig Cod (SGABC). ICES CM 2006/BCC:08.
- 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.
- Jobling, M. 1994. Fish Bioenergetics. In *Fish and Fisheries (13) ed. T.J. Pitcher*, Chapman and Hall, London.
- Knutsen I, Salvanes AGV 1999. Temperature-dependent digestion handling time in juvenile cod and possible consequences for prey choice Mar Ecol Progr Ser 181: 61–79.
- Mosegaard, H., Clausen, L.A.W., Christensen, A., Thygesen, U.H., Hüssy, K., Paulsen H., and Beyer, J.E. Model analysis and numeric simulation of fish otolith accretion. ORAL PRESENTATION at the 3rd International Symposium of Fish Otolith Research and Application. Townsville, Australia 11–16 July 2004.
- Mosegaard, H., K. Hüssy and C. Sparrevohn, 1997. Back-calculating Baltic cod size at age from otolith measurements. ICES. C.M. 1997/S:09.
- Mosegaard, H; Reeves, SA, 2001. Revision of Baltic cod age determination based on otolith accretion characteristics and weight distributions International Council for the Exploration of the Sea, Palaegade 2–4 DK-1261 Copenhagen Denmark. [vp].
- Oeberst, R. 2006. Can the frequency distribution of otolith weight be used for estimating unbiased proportions of age groups of Baltic cod. Working doc. Presented to the SGABC, Gdynia, Poland in May 15–19, 2006.
- Pannella, G. (1971). Fish otoliths; daily growth layers and periodic patterns. Science 173, 1124–1127.
- Pauly, D, 1987. Application of information on age and growth of fish to fishery management. In age and growth of fish. Edited by R.C Summerfelt and G.E Hall. Iowa State University Press. Ames. Iowa. Pp 495–506.
- Pawson, MG, 1990. Using otolith weight to age fish. Journal of Fish Biology. Vol. 36, no. 4, pp. 521–531.
- Pilling, G.M, Grandcourt, E.M and Kirkwood, G.P. 2003. The utility of otolith weight as a predictor of age in the emperor Lehtinus mahsena and other tropical fish species. Fish. Res. 60: 493–506.
- Rollefsen, G. (1933). The otoliths of the cod. Fiskeridirektoratets Skrifter, Serie Havundersøkelser 4(3), 1–14.
- Salvanes AGV, Hart PJB. 2000. Is individual variation in competitive performance of reared juvenile cod influenced by haemoglobin genotype? Sarsia 85:265–274.
- Secor, D.H., and Dean, J.M. 1989. Somatic growth effects on the otolith – fish size relationship in young pond-reared striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 46: 113–121.

- Strand E, Jorgensen C, Huse G 2005. Modelling buoyancy regulation in fishes with swimbladders: bioenergetics and behaviour. *Ecol Model* 185 (2–4): 309–327
- Temming A, Herrmann JP 2003 Gastric evacuation in cod - Prey-specific evacuation rates for use in North Sea, Baltic Sea and Barents Sea multi-species models. *Fish. Res.* 63 (1): 21–41
- Templeman, W., and Squires, H.J. 1956. Relationship of otolith lengths and weights in the haddock *Melanogrammus aeglefinus* (L.) to the rate of growth of the fish. *J. Fish. Res. Board Can.* 13:467–487.
- von Herbing IH and White L 2002 The effects of body mass and feeding on metabolic rate in small juvenile Atlantic cod. *J. Fish Biol.* 61 (4) 45–958
- Worthington, D.G., Fowler, A.J., and Doherty, P.J. 1995. Determining the most efficient method of age determination for estimating the age structure of a fish population. *Can. J. Fish. Aquat. Sci.* 52: 2320–2326.
- Wright, P.J. 1991a. The influence of metabolic rate on otolith increment width in Atlantic salmon parr, *Salmo salar* L. *J. Fish Biol.* 38: 929–933.
- Wright, P.J. 1991b. Calcium binding by soluble matrix of the otoliths of Atlantic salmon (*Salmo salar* L.). *J. Fish Biol.* 38: 625–627.
- Wright, P.J. Fallon-Cousins, P., and Armstrong, J.D. 2001. The relationship between otolith accretion and resting metabolic rate in juvenile Atlantic salmon during a change in temperature. *J. Fish Biol.* 59: 657–666.
- Yamamoto, T., Ueda, H., and Higashi, S. 1998. Correlation among dominance status, metabolic rate and otolith size in masu salmon. *J. Fish Biol.* 52: 281–290.
- Yoneda M, Wright PJ 2005. Effects of varying temperature and food availability on growth and reproduction in first-time spawning female Atlantic cod. *J. Fish Biol.* 67 (5): 1225–1241