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Comparative study of three alternative methods of aging Baltic flounder (*Platichthys flesus*)

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Abstract. Age reading is a major source of uncertainty in the assessments of fish stocks. The paper presents preliminary results of alternative approaches to estimating the age (year-class) structure of Baltic flounder, *Platichthys flesus*. The size structure of catches and the weight distribution of otoliths were analysed using Bhattacharya's method to distinguish between different cohorts (year classes). The traditional counting of annual growth zones on otoliths yielded the greatest number of age groups (12). Age determination by Bhattacharya's analysis of otolith weight frequencies enabled to distinguish between 7 cohorts. Bhattacharya's analysis of length frequencies of the same material allowed distinguishing 5 or 6 different groups. Theoretical weight distributions of otoliths, calculated by Bhattacharya's method for age groups 1 and 6, were not significantly different from the corresponding distributions of weights of otoliths obtained by the method of counting annual rings. The importance of random selection of individuals for the determination of the age structure of catch by annual rings of otoliths is emphasized.

Key words: age determination, Bhattacharya's method, flounder, weight of otolith.

INTRODUCTION

The quality of age determination plays a crucial role in age-structured fish stock assessment. However, as it has been stated in many cases (e.g. ICES, 2009a), consistency in age readings has been very difficult to achieve. Thus, for example the Baltic Fisheries Assessment Working Group of the International Council for the Exploration of the Sea (ICES) realized that there were long-standing problems with age determination inconsistencies in the assessments of Baltic cod and other demersal fish species. The group further concluded that substantial differences occur in age determination for fish from the same subdivision and year. The working group also realized that there were consistent differences between nations ('age reading schools') within and between years, indicating at different interpretation of growth zones on otoliths (ICES, 2009a). Regular exchange of otoliths and arrangement of workshops of age readers have been proposed (and proved to be useful) in order to improve the situation (ICES, 2008, 2009b). However, these costly measures can be effective only if taken regularly. Therefore, it comes as no surprise that looking for alternative methods to distinguish between cohorts (year classes) in analytically assessed fish stocks has become increasingly topical (e.g. Cardinale et al., 2000).

Virtual population analysis (VPA, e.g. Lassen & Medley, 2001) is a widely used method of age-structured stock assessment, deployed also by the ICES in its annual assessments. Stock assessment in the VPA is an analysis of the catches of commercial fisheries, obtained through fishery statistics, combined with detailed information of each age group (cohort) in the catch, which is obtained through sampling programmes and age readings. The idea behind the method is to back-calculate the population composition that must have been in the water to produce this catch (Sparre & Venema, 1992).

The counting of seasonal growth differences expressed on the otoliths as 'winter and summer rings' has been the most widely used method for age determination (ICES, 2008). In addition, analysis of otolith weight distribution (Pawson, 1990; Cardinale et al., 2000; Pilling et al., 2003; Pino et al., 2004; Cardinale & Arrhenius, 2004) and analysis of total length distribution of catches have been used to discriminate between the cohorts (Vitins, 1989; Sparre & Venema, 1992).

There are several techniques for age reading using otoliths, which depend on the species. Generally the method of stained slices is considered the most precise, and also burned and broken otoliths are considered to be better readable than whole otoliths (ICES, 2008). However, the more exact age reading methods (e.g. the method of stained slices) are also more time-consuming and expensive because special laboratory equipment is needed. As a result, the number of analysed specimens usually decreases.

The annual rings of otoliths of younger age groups are better readable than in older fish (ages 7 years and more). As the relatively young flounder (age groups 3–5) are dominating in the Estonian commercial catches, the counting of annual rings can be done effectively using the whole otolith (no slicing or breaking).

Several recent studies show that the weight of otoliths can be used for age determination (Pawson, 1990; Cardinale et al., 2000; Pilling et al., 2003; Pino et al., 2004; Cardinale & Arrhenius, 2004). Therefore, using different age determination methods simultaneously can be valuable in order to verify the age readings. In this respect, implementation of proper methods and criteria to discriminate different cohorts in the weight or length distribution is of crucial importance.

Bhattacharya's method, distinguishing parameters with normal distribution from a combined distribution pattern (Sparre & Venema, 1992), can be used for splitting composite distributions of otolith weight and fish length distributions into separate normal distributions, applicable to cohorts. Despite that the 'real' age of fish remains unknown and we do not know whether the weight of otoliths or lengths of fish are normally distributed within a cohort. However, normal distribution of both was assumed in the present work in order to apply Bhattacharya's method.

The aim of the work was to study and compare the differences between the results of age determination by three methods: counting annual rings on the whole otolith, Bhattacharya's analysis of weight distributions of otoliths, and analysis of length distributions of fish.

The *Manual of the Baltic International Trawl Surveys* (BITS) (ICES, 2007) suggests performing a certain number of age readings by annual rings of otoliths in each 1 cm length group. Mainly flounders of ages from 2 to 6 years are caught by this trawling programme. The length distributions of different cohorts are significantly overlapping starting from age groups 2 and 3 years. With increasing age the connection between the length and age of fish becomes weaker. The current work attempts to suggest a less biased method for estimating the age composition of flounder for stock assessment.

MATERIAL AND METHODS

The data were acquired from different sources. The length distributions of flounder age groups 0 and 1 were obtained from the results of Beach seine surveys in Luidja Bay (north-western Hiiumaa Island) in 1989 (P. Komissarov, unpublished data). The rest of the otoliths used in the analyses were collected during the Baltic International Trawl Surveys in 2006 and 2007 in the ICES Subdivisions 28 and 29 using the standard TV3-520 trawl for bottom surveys. The trawl hauls were performed near the north-western coast of Saaremaa Island at depths from 19 to 57 m (2006) and 31 to 91 m (2007).

All the analysed flounders were measured, weighed, and both otoliths (sagittae) were collected for later examination. Weights of otoliths can change, and the change depends on the humidity of the environment. Therefore, the otoliths were weighed 2–3 days after drying in paper envelopes using the 'Mettler Toledo' AB104 scales. The precision of the scales was 0.1 mg. The material from trawling in 2006 and 2007 was analysed separately by Bhattacharya's method. The otoliths of 375 flounders were weighed in 2006 and of 409 in 2007. The average difference in the weights of two sagittae of the same fish was 5.3%, varying from 0% to 36.3%. Only one of the two sagittae (left or right) was used for Bhattacharya's analysis.

Bhattacharya's method for estimating parameters for a mixture of normal distributions is well described by Sparre & Venema (1992). The method consists in separating normal distributions, each representing a cohort of fish, from the overall distribution, starting on the left-hand side of the total distribution. The first determined normal distribution is removed from the total distribution and the same procedure is repeated as long as it is possible to separate normal distributions from the total distribution. The process can be divided into several stages, which are described below.

– Stage 1: Determine an uncontaminated (clean) slope of a normal distribution on the left side of the total distribution.
– Stage 2: Determine the normal distribution of the first cohort by means of a transformation into a straight line.
– Stage 3: Determine the number of fish per weight group of otoliths (total length group of fish) belonging to that first cohort and subtract them from the total distribution.

– Stage 4: Repeat the process for the next normal distribution from the left, until no more clean normal distributions can be found.
– Stage 5: Relate the mean weights of otoliths (mean lengths of fish) of the cohorts determined in stages 1 and 4 to the age difference between the cohorts.

The meaning of the separation index is explained as follows in (Sparre & Venema, 1992). The separation index is a measure of separation of two adjacent normal distributions in standard deviation units (deviation from mean divided by standard deviation). According to investigations by Hasselblad (1966), the estimation of parameters of studied normal distributions is extremely difficult when the means are separated by less than 2.0 standard deviation units.

The theoretical distributions for every age group were calculated from the otolith weight and length distributions of fish by Bhattacharya's method. Bhattacharya's method presumes a normal distribution, which can also be a source of bias as the true type of distribution of every particular age group (cohort) is unknown. For comparison the age determination of all studied fish was performed by the routine method of counting the annual rings. The distribution of otolith weights of a certain age group determined by counting annual rings was compared with the distribution of otolith weights of the same age group estimated by Bhattacharya's method. The same procedure was applied for fish length distributions.

The differences in the results of three age determination methods were assessed using the Kolmogorov–Smirnov two sample test (Plokhinskij, 1961: 62–64; Urbah, 1963: 281–284), which is nonparametric and does not require normally distributed data. After several tests, 0.5 mg, which was 5 times higher than the precision of the scales, was chosen as the group interval for otolith weights. For length groups, 1 cm intervals were applied. Smaller intervals resulted in a number of empty groups, not allowing the application of Bhattacharya's analysis, and larger intervals resulted in some loss of information.

RESULTS

The length composition of Beach seine catches from Luidja Bay (1989) demonstrated that there was no problem in distinguishing the 0-group flounder on the basis of length composition as the length group 17–30 mm, representing the 0-group, was clearly distinguishable from the rest.

The idea of using the weight of otoliths for age determination is based on the assumptions that the weight of fish increases faster than their length and that the weight of the otoliths is more stable than the total weight of fish (Fig. 1).

The age reading by annual zones of otoliths revealed the presence of age groups 1–12 in the survey catches of 2006 (Table 1). Bhattacharya's analysis of otolith weight distribution allowed us to separate age groups from 1 to 7 (separation index > 2) of 9 age groups distinguished between the same individuals by the method of counting annual rings (Table 1). According to the Kolmogorov–Smirnov test, the distributions of otolith weights in age groups 1 and 6 were

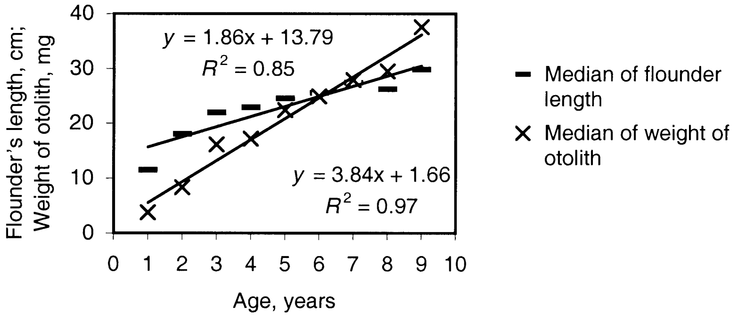

Fig. 1. Determination of the median length of flounder ($R^2 = 0.85$) and weight of otoliths ($R^2 = 0.97$) by age. Data from trawling in 2006.

Table 1. Mean weights of otoliths (W) and average total length (L) of flounder derived by different methods and separation index (I) from trawl hauls of 2006 and 2007

Age, years	W, mg			L, cm		
	Counting annual rings	Bhattacharya's weight analysis	I	Counting annual rings	Bhattacharya's length analysis	I
			2006			
1	3.6	4.1	2.5	11.4	11.1	17.4
2	8.8	7.2	3.9	17.4	19.7	9.6
3	16.1	11.8	2.1	21.8	23.2	11.6
4	18.4	14.4	3	22.9	27.3	8.7
5	23.1	19.2	6.2	24.4	30.7	
6	25.3	28.4	2.1	25.7		
7	26.7	30.3		26.6		
8	30.5			26.5		
9	37.6			29.8		
10	30.9			30.5		
11	45.4			32.7		
12	40.6			30.0		
			2007			
1	5.0	–	–	12.3	–	–
2	11.1	7.7	3.1	19.1	18.5	2.7
3	15.1	12.8	2.2	22.0	20.8	3.7
4	19.1	14.3	5.6	23.6	23.7	2.4
5	23.7	18.7	2.4	24.6	26.1	3.7
6	26.5	22.0	5.3	25.3	30.3	
7	29.1	29.8		26.2		
8	31.5			26.7		
9	30.8			28.4		
10	50.2			32.0		

not significantly different from the corresponding distributions estimated by Bhattacharya's analysis (Fig. 2a, b). The distributions of otolith weights in the rest of the age groups (2, 3, 4, 5, and 7) determined by counting annual rings (Fig. 3) and Bhattacharya's method were significantly different ($p < 0.01$). The variation of otolith weights inside the age groups from 1 to 7 years calculated by Bhattacharya's method was lower compared to that found by counting annual rings. The results of two methods showed a minor relative difference in the average otolith weight in the youngest and oldest age groups (Table 1).

The weight distributions of otoliths within the age groups from 2 to 7 (2006) determined by counting annual rings were complex due to overlapping. The extent of overlapping is shown in Table 2.

Bhattacharya's analysis of the length distribution from the survey in 2006 enabled distinguishing five age groups. Their length distributions were significantly different from those found for the corresponding age groups by the ring counting method except for the age group 1 (Table 1, Fig. 4).

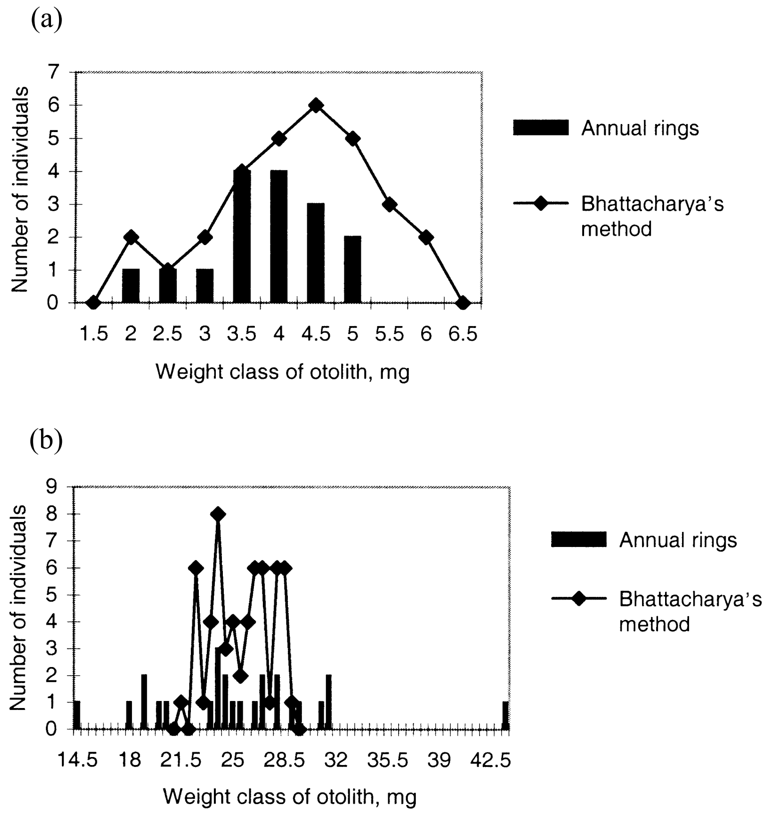

Fig. 2. Distribution of otolith weights in 2006. Age was determined by counting annual rings of otoliths and Bhattacharya's analysis of weight of otoliths. Differences are not significant. (a) Age group 1; (b) age group 6.

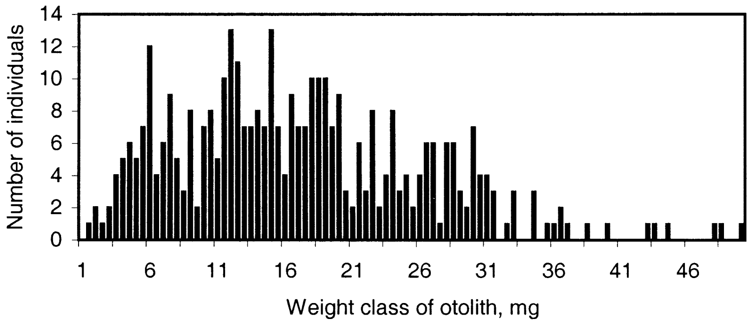

Fig. 3. Distribution of otolith weights in 2006. Total number of individuals 375.

Table 2. Overlapping of otolith weights between age groups

	Age groups					
	1 & 2	2 & 3	3 & 4	4 & 5	5 & 6	6 & 7
Extent of overlapping, mg	1.9	14	22	26	23	27

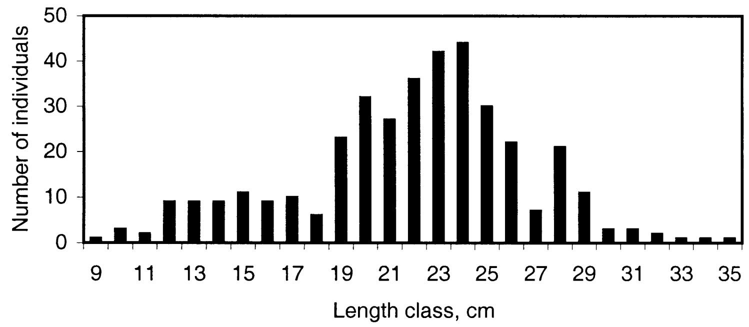

Fig. 4. Length distribution of flounder in 2006. Total number of individuals 375.

In 2007 the number of fish at age 1 was too small to perform Bhattacharya's analysis. Age groups 2 to 7 years were separated by Bhattacharya's analysis of otolith weight using the separation index > 2 as a criterion (Table 1). Among the same individuals age groups 2 to 9 were separated by counting annual rings of otoliths. The weight distributions of otoliths from the 2007 survey calculated by Bhattacharya's method were significantly different ($p < 0.01$) from the otolith weight distributions of the corresponding age groups found by counting annual rings (Table 1). Bhattacharya's analysis of length distribution allowed separating age groups 2 to 6 years from the survey conducted in 2007 (Table 1).

DISCUSSION

The distinguishing of the youngest age group and interpretation of the first annual ring on the otolith are the key elements in age determination. In this context the discrimination of the randomly appearing metamorphosis ring is essential (ICES, 2008). The available information on the size distribution and spatio-temporal pattern of flounder larvae and fry in the Northeastern Baltic (Kazanova, 1954; Grauman, 1981; Ojaveer & Drevs, 2003) suggests that the length distribution of smallest flounder caught in Luidja Bay in 1989, which was clearly separated from the rest of length groups, belonged to the age group 0. The average length of the age group 1 (59 mm) from the same catch corresponded approximately to values reported by Vitins (1989). So, by using the length distribution of flounder the age groups 0 and 1 can be separated easily when both these age groups are numerous in the catch.

The approximate size (diameter) of the first annual ring can be easily estimated using the relationship between flounder's total length (L) and the length of otoliths (L_O) $L_O = 0.0268L + 0.076$ (ICES, 2008). Additionally, this relationship allows of the conclusion that the length of the otoliths immediately after metamorphosis should be over 34 mm.

Analysis of otolith weights showed that the weight of otoliths increases relatively faster in successive age groups compared to the total length of fish because the weight of otoliths is a function of its volume (Fig. 1). Therefore the differences in the size of flounder of older age groups are more pronounced in the weight of otoliths rather than in its total length.

The oldest age groups (10 to 12) were represented only in very low numbers and therefore Bhattacharya's method could not be applied (Fig. 3). Neither could Bhattacharya's analysis be performed on the age group 10 in 2007 because the number of specimens of this age group according to the counting of annual rings was also too low.

In cases of an approximate agreement between the results of Bhattacharya's method and the annual rings counting method the resulting ages were considered as 'true ages'. In case of a disagreement, the method that included more biological information was considered to be preferable. Therefore, the method of counting annual rings, which enabled to distinguish more age groups, was considered more appropriate than Bhattacharya's analysis of otolith weights and that of length distribution of fish. It should be also mentioned that both Bhattacharya's method and annual rings counting involve subjective decisions, whose impact on the results depends on the peculiarity of the material.

Annual rings on otoliths may be unclear (difficult to read) and also additional rings may exist. When using the method of annual rings counting it is important to consider also the available environmental information, as the growth conditions naturally affect the growth of otoliths. For example, a long-lasting upwelling, cooling the coastal water down to the conditions observed during the winter for several weeks, can result in the appearance of additional rings, marking the growth slowdown (Drevs, 2007).

Bhattacharya's analysis needs a sufficient number of individuals in every age group. Therefore, if only a small amount of material is available, the age should be determined by counting the growth zones of otoliths.

Bhattacharya's analysis of the weight of otoliths can add additional information for age determination in cases the structure of otoliths does not allow clear estimation of age. Analysis of length frequencies, which is used for age determination in cases the structure of otoliths is unclear, is less informative than the weight of otoliths.

Cardinale et al. (2000), pointing at the subjective factors involved in the process of age reading, claimed that age determination by counting annual rings is more like an art, while the weight of otoliths could provide more objective criteria. On the other hand, separating different age groups from a mixture of distributions, for example by Bhattacharya's analysis (Sparre & Venema, 1992), also includes subjectivity, and the extent of subjectivity depends on the peculiarities of the collected material. To illustrate the problem of subjectivity, the overall distribution of weights of otoliths of all studied age groups from the material of 2006 is presented in Fig. 3. The length distribution of flounder enabled separation of the smallest number of groups (Fig. 4) because it contains the smallest amount of information. This method is unsuitable for age determination of older flounder, which is essential for stock assessment purposes.

Random sampling is vital for age determination as it is the only fully satisfactory way to choose the samples (individuals) (Campbell, 1989). Older age groups are usually present only in small numbers in the catches. In order to get more information on older age groups, the total number of individuals, which are selected at random for age determination by annual rings of otoliths, must be increased to achieve a larger number of older individuals. Sorting fish by length before age determination (only part of the catch is usually analysed) results in biased age and length relationship as well as age composition.

CONCLUSIONS

In our comparison of three methods of age determination of Baltic flounder age determination on the basis of counting annual rings of otoliths proved to be the best tool for distinguishing between flounder's cohorts, enabling consideration of the specifics of each individual. Bhattacharya's analysis of otolith weight distribution enabled to separate the main age groups found in commercial catches of flounder. The method can be used as an additional source of information for age determination in case annual rings of otoliths are indistinct. However, the method requires a sufficient number of individuals in each age group under consideration. Bhattacharya's analysis of length frequencies did not give satisfactory results with respect to estimation of age composition of catches.

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Lesta (*Platichthys flesus*) vanuse määramise võrdlus kolmel erineval meetodil

Tenno Drevs ja Tiit Raid

Lesta vanust määrati otoliidi aastaringide järgi ja otoliidi massi ning kala pikkuse jaotuste Bhattacharya analüüsi abil. Suurim vanuserühmade arv kolmest meetodist määrati otoliidi aastaringide järgi. Bhattacharya meetod võimaldas otoliitide masside puhul eristada 7 vanuserühma, arvestades ka lahutusindeksi väärtust. Teoreetilised otoliidi massi jaotused, mis saadi Bhattacharya analüüsi puhul vanuserühmadel 1 ja 6 aastat 2006. aasta traalimistest, ei olnud oluliselt erinevad aastaringide meetodi tulemustest vastavalt Kolmogorovi-Smirnovi testile. Lestade pikkuste Bhattacharya analüüs võimaldas eristada 5–6 vanuserühma. Aastaringide halva loetavuse puhul sobib suguküpsetel lestadel vanuse määramiseks täiendava infoallikana otoliidi mass paremini kui kala pikkus. Saagi vanuselise struktuuri määramisel on edasiseks vanuse määramiseks otoliidi aastaringide järgi oluline järgida isendite juhusliku valiku printsiipi.