



Short Communication

Age determination of Atlantic halibut (*Hippoglossus hippoglossus* L.) along the coast of Norway: status and improvements

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This study re-evaluates the current ageing methodology for the Atlantic halibut, *Hippoglossus hippoglossus*. The traditional method is through surface readings of otoliths, but, based on new experiments with different preparation treatments and techniques, a more accurate and cost-efficient procedure for the age determination of Atlantic halibut is proposed.

The Atlantic halibut is distributed throughout the boreal waters in large parts of the North Atlantic Ocean (Godø and Haug, 1988a, b). It has long been an attractive target species for fishers because of its high market price. Halibut reach sexual maturity relatively late in life, making the stocks vulnerable to even moderate levels of fishing pressure as many individuals are harvested before they have the chance to reproduce (Sigourney *et al.*, 2006). This vulnerability is exacerbated when halibut aggregate to spawn and become easy targets for fishers (Høines *et al.*, 2009). During the last 10 years the total landings of halibut north of 62°N have increased considerably, while the catches in the south of Norway are still low (Høines *et al.*, 2009). Effective regulations are needed in order to ensure that the stock will again reach sustainable levels. This requires detailed knowledge of life history traits and age composition. Knowledge of age composition is one of the most important issues to consider in order to construct efficient management plans and to strengthen the basis for recovery strategies. Previous studies show similar growth rates in male and female halibut up to the onset of sexual maturity, after which females accelerate growth and attain greater maximum size (Haug and Tjemsland, 1986; Jakupsstovu and Haug, 1988; Armsworthy and Campana, 2010). The age and growth of Atlantic halibut have been rigorously validated, and they can reach at least 50 years of age (Armsworthy and Campana, 2010). The estimation of age is in most cases done by counting periodic

growth increments in otoliths. The age determination procedure most commonly used today at the Institute of Marine Research in Bergen (IMR) involves reading whole otoliths, immersing both the left and right otolith in water, photographing both using transmitted light, then counting the annual increments on both otoliths to estimate the age in years. Although others have used either sectioning (Armsworthy and Campana, 2010) or breaking and burning (Blood, 2003), reading whole otoliths is less time consuming and less costly, justifying the need for further validation of a new procedure. The main objective of this study was thus to compare different approaches of age determination, and to establish a new and improved, cost-efficient procedure for ageing Atlantic halibut. In order to improve the utilization of the information that the otoliths can provide in future management of the species, we also describe the relationship between age, length and weight, and spatial size variations.

Otoliths were taken from 345 halibut captured along the coast of Norway in the years 2004–2006 and 2008–2010, and otoliths from 264 of these were available for this study (Figure 1, Table 1). All otoliths collected between 2004 and 2006 had been stored dry in paper envelopes, whereas those collected between 2008 and 2010 were frozen in seawater. Otoliths collected between 2004 and 2006 had previously been aged by an experienced age reader either directly through a stereomicroscope or from digital images. The equipment and magnification used in

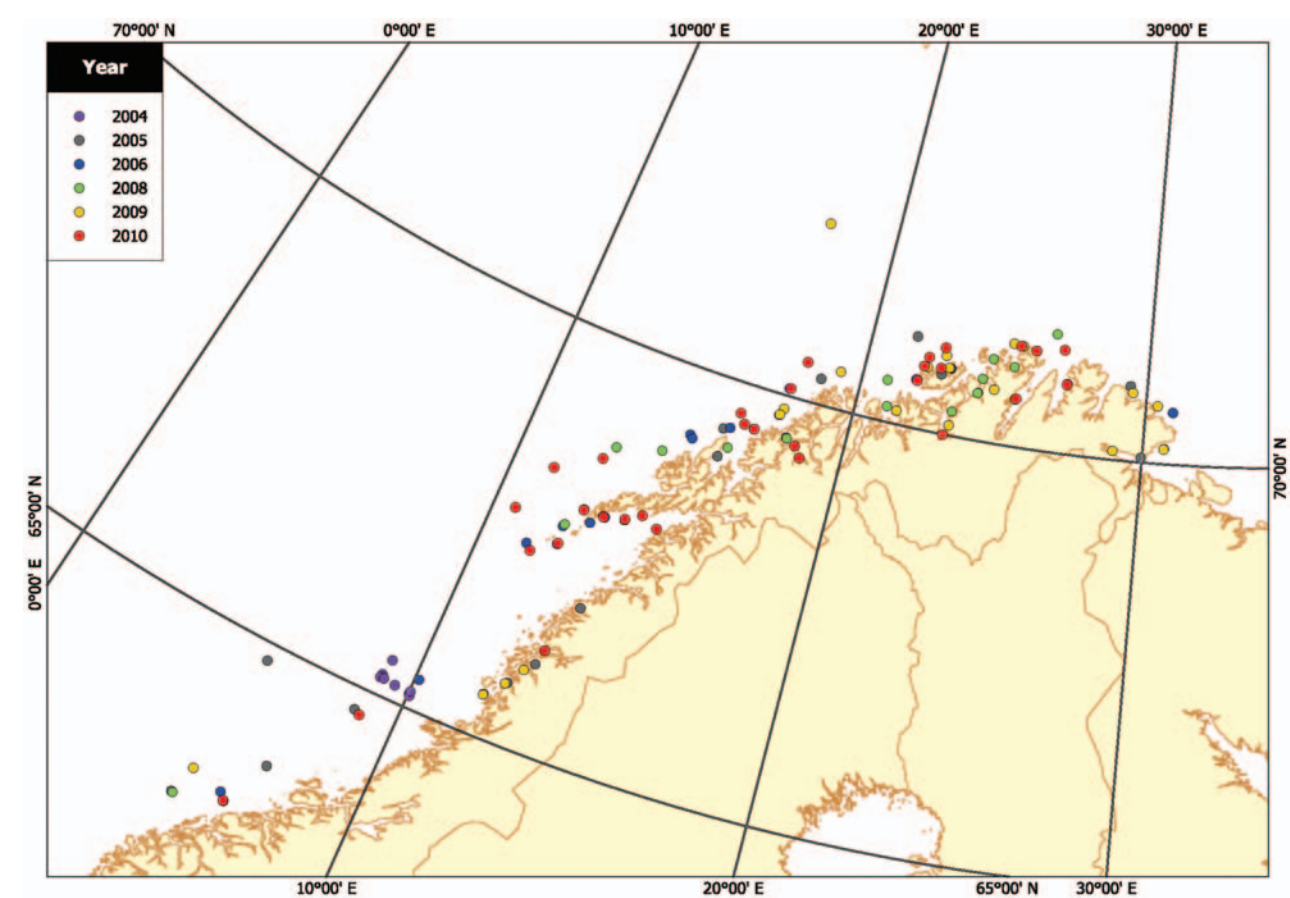


Figure 1. Sampling localities of Atlantic halibut along the Norwegian coast. The different symbols indicate the sampling year.

Table 1. Halibut otoliths collected in the years 2004–2006 and 2008–2010, indicating the number of otoliths pairs collected and number used in this study.

Year	Source/vessel	Date	No. of otolith pairs	No. used in this study ^a
2004	RV Johan Hjort	14.10–10.11	31	0
	RV Jan Mayen	22.10–07.11	16	0
	FV Førde Jr	19.09–03.10	48	44
	Fishermen		10	1
2005	Fishermen	24.02–25.02	8	0
	RV G.O. Sars	27.02–17.08	7	2
	Reference fleet	27.04–22.08	10	2
	RV Johan Hjort	23.10–04.11	17	17
	RV Jan Mayen	26.10–07.11	11	11
	FV Amigo	26.11–30.11	6	6
2006	RV Johan Hjort	12.02–16.11	23	23
	FV Amigo	01.08	22	22
2008	RV Johan Hjort	03.10–14.11	21	21
	NIFES ^b	20.02–11.12	23	23
2009	NIFES ^b	21.01	1	1
	RV Johan Hjort	06.10–25.10	17	17
	RV Jan Mayen	04.10–24.10	17	17
2010	RV G.O. Sars	24.08	1	1
	RV Johan Hjort	03.04–03.11	56	56
Total			345	264

^aSome otoliths were not available due to prior sectioning.

^bNational Institute of Nutrition and Seafood Research.

this study were the same as previously described in Karlson (2011). To determine what combination of clearing treatment and lighting gave the best view of increments, the otoliths collected in the years between 2004 and 2006 were photographed after receiving three different treatments. The whole otoliths were first taken directly from the paper envelopes, placed with their concave side facing the objective, in a Petri dish filled with water, and photographed. The same otoliths were then immersed in water for 24 h before transfer to 60% glycerol for 24 h. The otoliths were photographed using both transmitted and reflected light after each step.

To compare both the clarity and number of increments between sectioned otoliths and whole-mount otoliths, a subset of the 2006 collection comprising ten pairs that showed clear increments and ten pairs that showed relatively unclear increments were dried, embedded in a mixture of Epofix resin and hardener, and sectioned. Transverse slices were made using an Isomet 1000 low speed saw, producing sections of $\sim 500\ \mu\text{m}$ in thickness. Placing the section on the tip of a finger, one side was polished, using increasingly fine grades of abrasive paper and tap water on a mechanical rotating disk, ensuring that the section was ground to a uniform thickness. The section was attached with clear CrystalbondTM adhesive, pre-heated to $\sim 135^\circ\text{C}$, to a glass slide, polished side facing the glass. The second (unpolished) surface of the section was then polished, and the resulting section thickness was between 200 and 400 μm .

Digital images were taken of the prepared sections for both otoliths from all 20 halibut, and viewed in Photoshop. A new digital interpretation layer was created and a digital brush of defined colour and size was used to trace annual increments. Before marking the final annual band, the date of capture was considered

in order to decide whether or not the final increment was fully formed and could be counted as 1 year (with 1 January accepted as the birth date of all fish). Marked increments were counted on both left and right otoliths, and the results compared across treatments and light sources. The light source was evaluated by the number and clarity of increments and the otoliths were evaluated by which of the pair (right or left) consistently revealed the highest number of distinct increments. Comparing sections and whole-mount images of both left and right otoliths gave an indication of coherence of interpretation between the two methods. The resultant 'best practice' was performed on all otoliths collected between 2008 and 2010. The data analysis software system Statistica, version 10 (StatSoft Inc., 2010), was used for all figures and statistical analyses.

Images of otoliths exposed to different treatments (Figure 2) revealed that 24 h immersion in water gave the most defined increments (Figure 2b and e). Otoliths photographed directly after dry storage had a matte surface with less contrast between growth increments (Figure 2a and d), whereas otoliths photographed after 24 h in glycerol produced a refringent surface (Figure 2c and f). Separating true increments from false increments (non-annual additional opaque or translucent increments) was more difficult on images captured using transmitted light. Reflected light revealed a higher number of distinct increments, as well as more equivalence between left and right otolith interpretation. There was no significant difference between the numbers of increments counted for the two light sources (paired *t*-test, Table 2, $p > 0.05$).

The ages obtained from the left and right otolith were usually the same for a given fish. Where the increment count differed

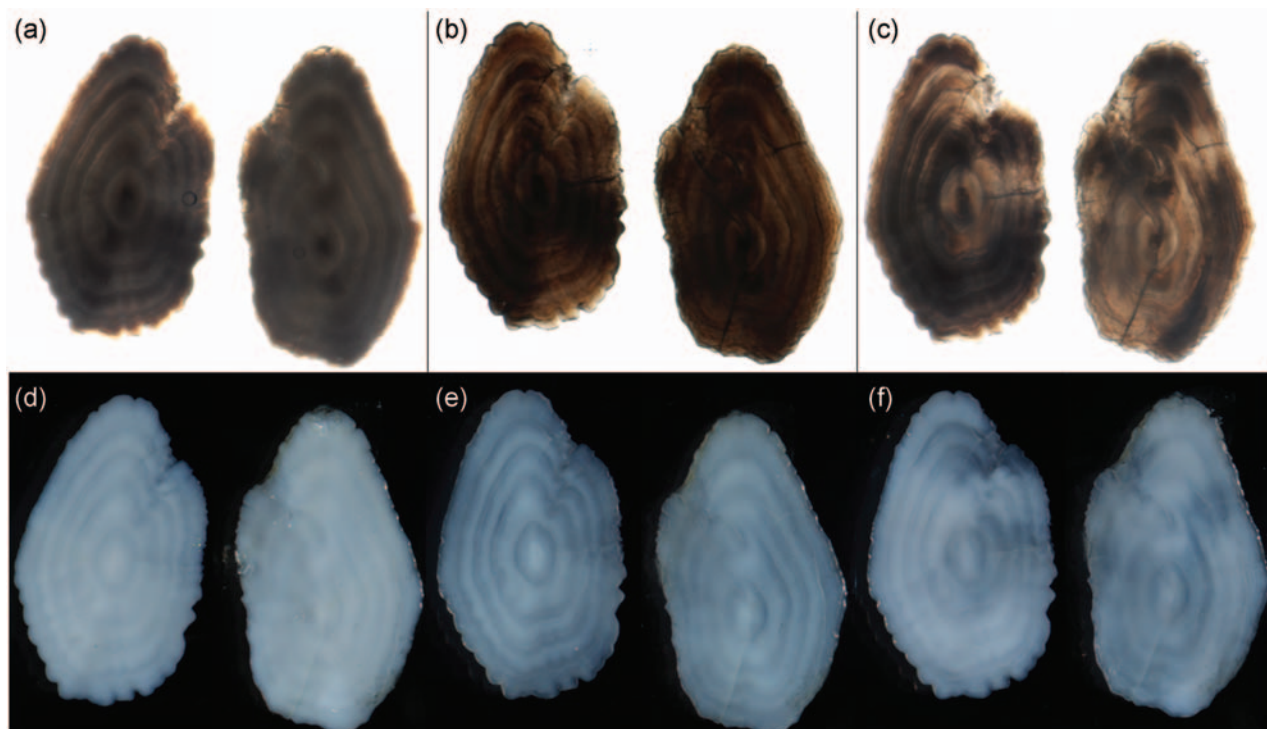


Figure 2. Examples of otolith pairs photographed after different treatments, and with different light sources. Images in the upper panel are photographed using transmitted light, while those in the lower panel are photographed with the use of reflected light. (a and d) Otoliths are photographed dry, displaying a rather matte surface. (b and e) Otoliths are photographed after a 24 h immersion in water, where increments are pronounced and clear. (c and f) Otoliths are photographed after 24 h in glycerol, producing a refringent surface.

Table 2. Overview of mean increment number counted when comparing across methods with corresponding difference and results of pairwise test.

	Gr 1 Mean	Gr 2 Mean	Difference	p-value
Left otolith, reflected light vs. transmitted light	8.16	7.99	0.17	0.054
Right otolith, reflected light vs. transmitted light	7.70	7.54	0.26	0.077
Left whole otolith vs. right whole otolith	7.45	7.19	0.26	<0.001
Left section vs. left whole	8.79	8.88	0.09	0.571
Right section vs. right whole	8.91	8.68	0.23	0.128
New age estimate vs previous age estimate	8.09	6.08	2.01	≪ 0.001

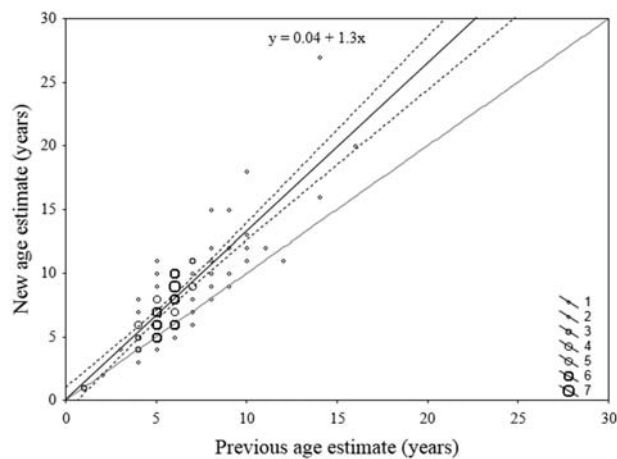


Figure 3. The difference in age interpreted for the same otoliths using the former and current method [regression line (solid line), confidence interval (dashed line), and the $y = x$ line (grey line)]. The size of the dots indicates the frequency of age observations.

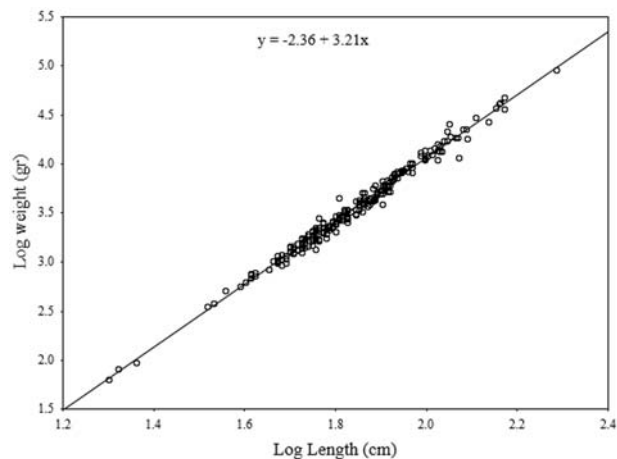


Figure 4. Regression of the relationship between log weight and log length. Data for both sexes are combined. $n = 247$.

between the right and left otolith, the left increment count tended to be higher (paired t -test, Table 2, $p < 0.001$) and typically showed better readability. Commonly there was a one increment (year) difference between the left and right otolith, but in some cases this was even more. There was no significant difference between the age estimates obtained from pairs of whole otoliths and that of their corresponding sections (paired t -test, Table 2,

$p > 0.05$). For otolith pairs where the age estimate differed between left and right whole-mount otoliths, their sections showed equivalence in 69.2% of the cases. The increment counts of sections were also more comparable with the increment count on left whole otoliths. This study's age estimates for halibut were almost always higher than previous estimates of the same otoliths, where both left and right otoliths had been aged directly after dry storage and photographed using transmitted light (paired t -test, Table 2, $p \ll 0.001$, Figure 3). This difference increased with age, giving a difference of up to several years for many of the cases.

The relationship between log total length and log wet weight for Atlantic halibut was close to allometric (Figure 4). The slope (3.21) was significantly different from 3 [general linear model (GLM), $p \ll 0.001$], indicating a non-isometric relationship. There were no differences in the length–weight relationship between male and female halibut in the size range studied (GLM, $p > 0.05$). Dividing our sampling sites into northern and southern regions gave us latitudinal locations ranging from 62.9°N to 71.2°N, with 66.5°N as the north–south boundary. In order to avoid any confounding errors due to latitudinal differences in sex-dependent size at age, we compared only males and females in northern latitudes which had wide overlapping size ranges and an approximately linear relationship between length- and weight-at-age, and found a difference in size-at-age where females were both longer and heavier at age (GLM, $p > 0.05$). The halibut caught in northern latitudes were larger than those sampled further south (GLM, $p < 0.05$), as were their lengths- and weights-at-age (GLM, $p < 0.05$, Figure 5). The weight and length appeared to increase continuously for both sexes as they grew older, and females were generally heavier and longer at a given age than males in the northern latitudes (GLM, $p < 0.05$).

In the present study, surface readings were performed on digital images of otoliths after different clearing treatments and under different lighting. Although glycerol was expected to enhance the contrast of the otolith growth increments (Forsberg, 2001), 24 h storage in 60% glycerol produced areas with increased transparency where the increments were in many cases almost erased. In contrast, 24 h immersion in water enhanced the appearance of the otolith increments in this study. Reflected light was also preferable to transmitted light. Our study also shows that the left otolith has clearer growth increments and a significantly higher number of distinct increments, in contrast to the recommendations of previous studies for the right otolith, which has the longest readable axis (Kvalsund and Albert, 2007). This is in agreement with Haug and Tjemsland (1986), who also found that the otoliths collected from the left side of the halibut show clearer growth increments.

No significant differences were found between the within-otolith surface and cross-section readings in this study.

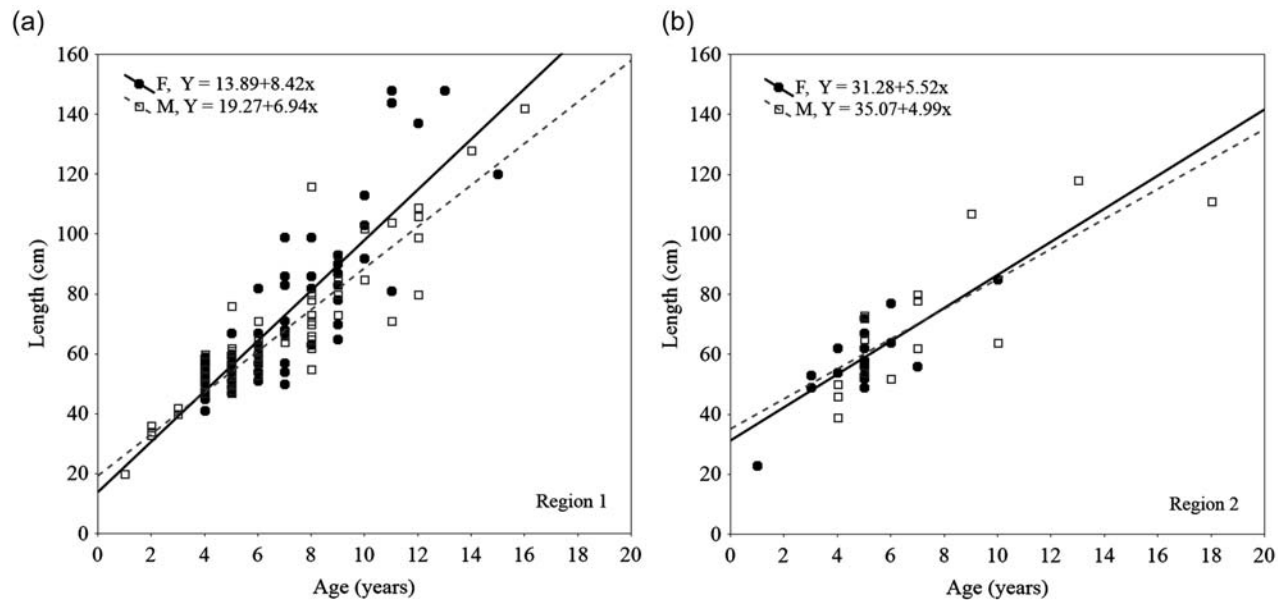


Figure 5. Regression of the relationship between length and age observed for male and female halibut caught at different latitudes. $n = 174$. Region 1 and 2 denote halibut collected at latitudes above and below 67.5°N , respectively.

Sectioning of otoliths was useful when the whole otoliths were damaged above or below the core, as an age can still be interpreted from the section in those cases. Previous ageing studies performed for a number of species have found that otolith surface readings underestimate age (Blood, 2003; Albert *et al.*, 2009; Lee *et al.*, 2009). Although we did not find any evidence of this in our study, most of our samples were from relatively young fish (<9 years). Because of the small number of older individuals, we can only conclude that surface readings are accurate for young fish. A cross-section reveals greater detail and may give a more reliable estimate of the age in older fish by improving the visibility of increments and reducing the difference between counts on the left and right otoliths in a pair.

Previous age determinations for Atlantic halibut may be underestimated, as our study shows that the number of increments recorded for both the left and right otolith was significantly higher than the number of increments recorded for the same otoliths using the older standard method from the IMR. Subjectivity is an element that is difficult to avoid in age interpretation, and therefore a potential source of error. It is not possible to conclude which of the two ageing techniques, the previous or the current, gives the correct estimate of age without validation of the ageing technique using methods such as bomb-radiocarbon assays (Armstrong and Campana, 2010) and chemical tagging of otoliths using oxytetracycline (OTC) followed by recapture (Treble *et al.*, 2008).

This study also found similar lengths and weights for male and female halibut up to 4–6 years of age. Male sizes-at-age appeared to level out at ~ 10 –12 years of age, at which point female growth accelerated. Females became significantly longer and heavier with age than males, in agreement with previous findings (Devold, 1938; Haug and Tjemsland, 1986; Jakupsstovu and Haug, 1988; Sigourney *et al.*, 2006; Armstrong and Campana, 2010).

This study further found latitudinal differences, such that Atlantic halibut collected in the more northern latitudes along

the Norwegian coast were significantly larger than their southern counterparts. Also, the lengths- and weights-at-age were significantly greater among individuals collected at the higher latitudes. Distinct variations in life-history strategies and biological characteristics have been documented in fish species inhabiting wide latitudinal ranges (Boehlert and Kappenman, 1980). Whether the observed growth patterns are due to genetic population differences among northern and southern halibut populations along the Norwegian coast remains to be documented.

We propose a new ageing procedure for Atlantic halibut. Otoliths that have been stored in paper envelopes should be immersed in water for at least 24 h and subsequently photographed using reflected light. If available and intact, the left otolith should be used for age interpretation, counting annual increments along the anterior–posterior axis. In cases where the increment count is >9 , the otolith should be sectioned and annual increments should be counted along the dorsal–ventral axis. We further emphasize the importance of validation studies, and suggest further work on this to achieve a cost-efficient and validated procedure.

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