

# Frequency of vateritic otoliths and potential consequences for marine survival in hatchery-reared Atlantic salmon

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## Abstract

Otoliths are inner-ear structures of all teleost fish with functional importance for hearing and balance. The otoliths usually consist of aragonite, a polymorph of calcium carbonate, but may also take the form partly or entirely of vaterite, a different polymorph of calcium carbonate. Vateritic otoliths occur sporadically in wild fish, but with a higher frequency in hatchery-reared fish. Abnormal otoliths have direct consequences for the inner-ear functions of fish and may be a symptom of environmental stress. In this study, the authors assess the differences in the frequency of abnormal otoliths and degree of abnormality (% vaterite) for different groups of hatchery-reared Atlantic salmon (*Salmo salar*) smolt and adults. The groups differed in parental broodstock origin (number of generations in hatchery) and treatment temperature. Smolt from the same groups were also released to complete their ocean migration. The otoliths of the returning and recaptured adults were subsequently extracted to assess the difference in frequency and degree of abnormality between the adults and the smolt from corresponding groups. Return rate varied among groups (0.2%–2.6%). The frequency of vateritic otoliths was high (11.4%–64.4%) and differed among smolt groups. The lowest return rates corresponded with the highest frequency of abnormal otoliths for the groups, suggesting that abnormal otoliths may have negative consequences for marine survival. Furthermore, indications of an effect of fast growth on the formation of abnormal otoliths were found for only one of the experimental groups, and for none of the groups after correcting for Type 1 error. This contradicts previous reports, suggesting rapid growth as the main cause of abnormal otoliths. Adult return rates were generally low, but abnormal otoliths were common, with high coverage (% vaterite).

## KEYWORDS

aragonite, crystalline otoliths, survival, vaterite

## 1 | INTRODUCTION

Anadromous Atlantic salmon (*Salmo salar* L.) individuals undergo vast migrations between their freshwater rearing habitat and the ocean feeding grounds in the North Atlantic. These migrations can be more than 2000 km long (Jonsson & Jonsson, 2011b; Thorstad *et al.*,

2011b, 2020). The marine part of the life cycle is dangerous, and mortality is generally high for Atlantic salmon (Chaput, 2012; Jonsson & Jonsson, 2004). Stocking of hatchery-reared fish to supplement natural populations is common, and it has been documented that such hatchery-reared fish usually have lower ocean survival than wild fish (Jensen *et al.*, 2016; Jonsson *et al.*, 2003). This is observed

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despite that growth often is considerably enhanced in hatcheries leading to larger size at age, and individual size is assumed to positively affect survival (Sogard, 1997). The lower survival rate of hatchery-reared fish relative to wild fish may be due to these fish not developing anti-predator behaviour and foraging skills that wild fish develop in natural conditions (Thorstad *et al.*, 2011). Recent studies indeed indicate that lower survival rates of domesticated salmon are due to their predation susceptibility, despite the potentially huge differences in growth. This may be because risk-taking traits are selected for in a domestic environment, and that predator-avoidance traits are costly to select for if no predator is present (Solberg *et al.*, 2020). In addition, hatchery-reared salmon may have been exposed to numerous stressors that may have lasting effects on performance. One substantial difference between hatchery-reared and wild salmon is the increased frequency of abnormal otoliths in hatchery-reared fish (Oxman *et al.*, 2007; Reimer *et al.*, 2017).

Otoliths are structures in the inner ear of all teleost fish that serve three purposes: detection of angular acceleration, detection of linear acceleration and hearing. The otolith organ generally consists of three semicircular canals and three otoliths (Schulz-Mirbach *et al.*, 2019). These structures are of great importance to the sensation of gravity, ability to hear, linear mobility and balance (Reimer *et al.*, 2016).

Detection of sound is possible for fish when the inner ear is stimulated by particle motion (Schulz-Mirbach *et al.*, 2019). Because fish tissue has a density similar to the surrounding water, this particle motion for fish is not detectable without otoliths (Popper & Hawkins, 2018). Otoliths are calcite structures and have a higher density than the water and surrounding tissue; therefore, sound pressure creates relative motion between the otolith and the sensory hair cells (Schulz-Mirbach *et al.*, 2019). This is how teleost fish with the help of their otoliths can detect sound directly. Some teleost fishes detect sound indirectly as well through sound pressure stimulating their gas-filled swimbladders, but they still depend on their otoliths for hearing. These fish hear a broader range of sound frequencies and are hearing specialists. Salmonid fishes are hearing generalists; they only detect sound directly and do not get any “help” from their swimbladder (Hawkins & Johnstone, 1978; Popper & Lu, 2000; Schulz-Mirbach *et al.*, 2019).

Usually, sagittal otoliths consist of aragonite, a polymorph of calcium carbonate, and are chemically inert with high purity (Falini *et al.*, 2004; Schulz-Mirbach *et al.*, 2019). This means that the otolithic structure already produced does not change; it only grows with daily accretion of new, permanently retained material (Campana, 1999; Schulz-Mirbach *et al.*, 2019). Sometimes, however, otoliths are formed partly or entirely of vaterite, a different, less dense polymorph of calcium carbonate. Such otoliths are categorized as abnormal (Falini *et al.*, 2004; Schulz-Mirbach *et al.*, 2019). These otoliths may consist of both aragonite and vaterite in different proportions. Studies on the two polymorphs in freshwater cultured pearls (produced by mussels in the genus *Hyriopsis*) (Soldati *et al.*, 2008) and in otoliths of reared juvenile herring (*Clupea harengus* L.) (Tomas & Geffen, 2003) have shown that the transition is sharp with a relatively short transitional area at the interface between vaterite and aragonite that consist of both polymorphs together. Characteristics of vaterite found in both studies

showed a higher concentration of Mn, and less Sr, N and K than in the aragonite part of the otolith. The herring study showed that although vaterite is less dense, the Ca concentrations did not differ, and the difference was found in the larger perimeter of the vateritic otoliths. Salmonids are especially susceptible to producing the vaterite form, and the differences in properties of the different polymorphs may have consequences for the movement of the otoliths in the inner ear (Sweeting *et al.*, 2004). The consequence of this vaterite replacement of aragonite in the sagittal otoliths is loss of hearing sensitivity across most of the hearing range (Oxman *et al.*, 2007; Reimer *et al.*, 2016). In addition, the more prominent the coverage of vaterite in the otoliths, the more severe the hearing impairment likely becomes. The density differences between vaterite and aragonite may also affect hearing directionality specifically (Reimer *et al.*, 2016).

Vateritic otoliths occur sporadically in fish in their natural habitats; nonetheless, studies indicate that the occurrence of such abnormal otoliths in farmed fish is much higher. Analyses on numerous species indicate that abnormal otoliths occur in c. 10% of wild fish, but in c. 50%–80% of hatchery-reared fish (Reimer *et al.*, 2016). Furthermore, Reimer *et al.* found vateritic otoliths in 48.7% of sampled farmed Norwegian Atlantic salmon and in only 8.6% of comparable wild salmon (Oxman *et al.*, 2007; Reimer *et al.*, 2016). Studies on lake trout (*Salvelinus namaycush* Walbaum) inhabiting a number of different lakes showed large variation in the frequency of abnormal otoliths among populations, and also a consistently much higher frequency of abnormal otoliths in hatchery-reared lake trout stocked in the same lakes (Bowen *et al.*, 1999). The prevalence of abnormal otoliths seemed to increase with increasing handling associated stress.

In order for the anadromous Atlantic salmon to survive the marine phase and successfully migrate back to their natal river to spawn, they depend on a fully functional set of sensory organs. A functional inner ear is one of these sensory organs. Given the importance of otoliths for the inner-ear functions of teleost fish and that abnormal otoliths have previously been shown to be common in hatchery-reared fish, a relevant question is to what degree otolith abnormalities can lead to differences in marine survival between hatchery-reared and wild fish. The primary objective of the present study was to quantify the prevalence of vateritic otoliths of hatchery-reared Atlantic salmon smolt and compare with adult salmon of the same cohort returning to the river after completing the ocean migration. This comparison can also give indications on selective survival at sea. In addition, the authors of this study investigate the potential link between growth rate during early life in different experimental smolt groups and their frequency of otolith abnormalities.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental design

The experiment was conducted more than 4 years at the NINA research station, Ims, located by the river Imsa in Rogaland in western Norway (58° 50'N, 6°E). The river drains into the Høgsfjord estuary is

c. 1 km long and contains a small population of anadromous Atlantic salmon (Jonsson & Jonsson, 2016). The salmon here migrate to the North Atlantic to feed in the ocean and return as adults to spawn, usually after 1 year. The river Imsa is equipped with a Wolf trap (Wolf, 1951) located 150 m above the river mouth, catching all descending fish over c. 10 cm long, and a box trap catching all ascending fish (Jonsson *et al.*, 2017).

During spring in 2016 and 2017, hatchery-reared Atlantic salmon smolt were released in the lower part of the river Imsa ( $N = 6958$  and  $N = 8933$ , respectively). In addition, a total of 1016 smolt of the 2016 cohort and 1047 of the 2017 cohort were euthanized using an overdose of benzocaine, and their otoliths were analysed to determine a baseline frequency of abnormal otoliths in the smolt. The hatchery-fish used in the 2016 and 2017 release consisted of fish from different experimental groups. Fish from these releases were later recaptured as adults when ascending to the river Imsa. The adults were euthanized (as above), and the otoliths of all the returning adult fish were collected (summary in Table 1).

The fish in this experiment originate from two different populations (rivers Imsa and Lone) and two different years of release (2016 and 2017). The groups differ in the number of generations in hatchery. The wild parental broodstock of the Lone population dates back around 30 years, whereas the smolt of the Imsa population are progeny of first-generation hatchery-reared parents (pers. comm., Knut Bergersen, Norwegian Institute for nature research). The two rivers are located quite close ( $59\text{--}60^\circ\text{N}$ ), in the western part of Norway.

Most salmon in both rivers mature after one winter at sea (1SW, called grilse), but a variable number also returns after two winters at sea (2SW). In general, fish of the Lone population are somewhat smaller at mature size (mean adult length  $\pm$  S.D.;  $575 \pm 46$  mm) than those of Imsa (mean adult length  $604 \pm 69$  mm) (Hansen & Jonsson, 1989; Jonsson *et al.*, 2007).

Eggs of the Imsa population were incubated in either natural water temperatures following seasonal fluctuations from c.  $2$  to  $8^\circ\text{C}$  in winter to around  $20^\circ\text{C}$  in summer (Imsa Cold), or in stable experimental water temperature conditions of c.  $7\text{--}8^\circ\text{C}$ , not following seasonal fluctuations (Imsa Warm) (Jonsson *et al.*, 2016; Jonsson & Jonsson, 2018). The eggs that were incubated in experimental water temperatures, not following the natural fluctuations, experienced on average c.  $3^\circ\text{C}$  warmer water temperatures (Jonsson & Jonsson, 2018). After initial feeding, the water temperature for fish in both treatments was kept at  $11.5^\circ\text{C}$ , and at natural water temperatures after the water reached a temperature of  $11.5^\circ\text{C}$ . All the different treatment groups were raised in 150 l tanks until they reached the smolt stage. The feeding regime was the same in all tanks: continuous feeding 24 h a day, with intervals of 8 min pause and 7 s distribution of food. The Lone 2016 tank contained 7410 individuals, the Lone 2017 tank contained 5760 individuals, the Imsa Cold 2016 contained 10,240 individuals, the Imsa Cold 2017 tank contained 9500 individuals, the Imsa Warm 2016 tank contained 10,880 individuals and the Imsa Warm 2017 tank contained 10,540 individuals. Because of the importance of temperature in developmental stages, one can expect size differences between the groups of smolt. In total,

**TABLE 1** Number of Atlantic salmon smolts and adults analysed, and number of smolts released in the river Imsa during 2016 and 2017

Year and group	No. of smolt sampled	No. of smolt released	No. of returning adults			Mean smolt length $\pm$ SD	No. of adults analysed		
			1SW	2SW	N total		N abnormal (%)	N total	N abnormal (%)
<b>2016</b>									
Imsa Warm	129	2981	12	5	120	47 (39.2)	186.2 $\pm$ 26.3	17	2 (11.8)
			<b>0.57%</b>						
Imsa Cold	5	1988	6	0	5	2 (40.0)	140.6 $\pm$ 20.3	6	3 (50.0)
			<b>0.3%</b>						
Lone	271	1989	2	2	261	170 (65.1)	155.5 $\pm$ 15.0	4	2 (50.0)
			<b>0.2%</b>						
<b>2017</b>									
Imsa Warm	350	4965	129	1	334	51 (15.3)	189.0 $\pm$ 19.1	121	25 (20.6)
			<b>2.62%</b>						
Imsa Cold	350	1992	40	1	324	37 (11.4)	182.6 $\pm$ 22.5	41	10 (24.4)
			<b>2.11%</b>						
Lone	347	1976	12	3	324	207 (63.9)	160.8 $\pm$ 13.4	13	7 (53.8)
			<b>0.76%</b>						
Total	2128	15,922	202	12	1368	514 (37.6)		202	49 (24.3)
			<b>1.34%</b>						

Note. Bold values indicate that there is no particular significance of the percentage of returning adults. Experimental group and year of release are given. "Cold" and "Warm" refer to the water temperature treatment in the hatchery for the Imsa population. The total number of smolt and adults included in the experiment (N total) and the number (and percentage) of these that had at least one abnormal otolith (N abnormal). Data are presented for each group and cohort separately, and summarized for the grand total.

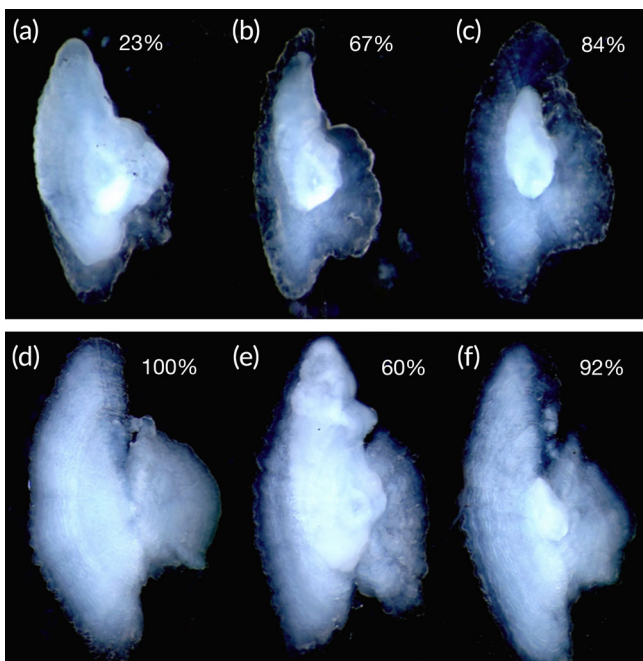
six different experimental groups were analysed as part of this experiment, differing in either temperature regime during early development in the hatchery, population of origin, or year of release.

Juveniles were raised to the smolt stage, and a number of randomly sampled smolt were euthanized for otolith extraction and measurement of total length (Table 1). The remaining smolt were released in Imsa below the trap and allowed to migrate to sea. The 2016 cohorts were tagged with Carlin tags (Carlin, 1955), and the 2017 cohorts were tagged with either Carlin tags or 12 mm passive integrated transponder (PIT) tags. The variation in the number of smolt released and tags used is due to the fact that the fish were part of different experiments not related to this one. The returning fish of the various groups were subsequently recaptured in the box trap located above the river Imsa mouth. Smolt released in 2016 were expected to return in 2017 and 2018, whereas smolt released in 2017 were expected to return in 2018 and 2019.

All tagging and handling of fish comply with Norwegian animal welfare laws and was approved by the Norwegian Food Safety Authority.

## 2.2 | Otolith categorization

Abnormal otoliths are in this context defined as otoliths partly or fully consisting of vaterite (Figure 1). Distinguishing between vaterite and aragonite is possible to do visually when looking at the otoliths in a



**FIGURE 1** Variation in per cent vaterite among abnormal otoliths. Top row (a–c) shows abnormal otoliths from three different smolt from the 2017 groups; a = Lone (161 mm), b = Lone (137 mm), c = Imsa Warm (198 mm). Bottom row (d–f) shows abnormal otoliths from three different adults; d = 2017 Imsa Cold, e = 2016 Lone, f = 2017 Imsa Warm

stereomicroscope. Each otolith was photographed, in water, with a Leica DC300 digital camera connected to a Leica MZ8 stereomicroscope connected to a computer. The software IrfanView (Škiljan, 1996) was used to view, photograph and save the photographs. A 2.5× zoom gave sufficient magnification for most otoliths, except for a few otoliths from the adults that were quite large and less magnification was needed.

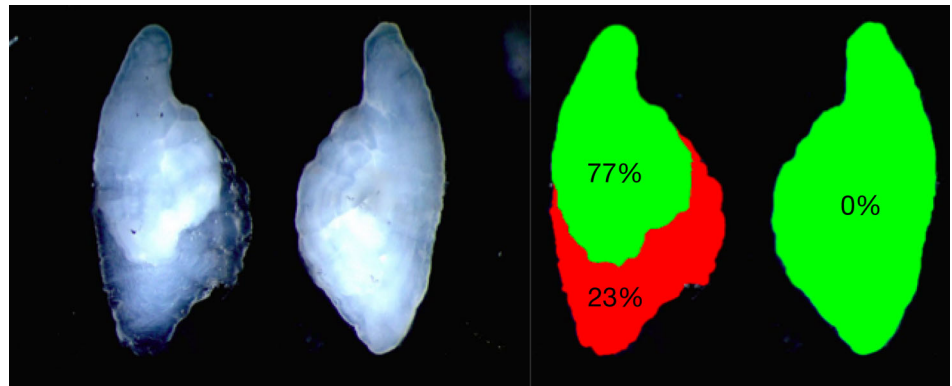
On each abnormal otolith, the part consisting of vaterite was quantified by outlining the part or parts consisting of vaterite and the part or parts consisting of aragonite using Adobe Photoshop™. The outlining was performed using the Quick Selection tool, which automatically outlines structures on the photograph; this automatic outlining was then overseen and adjusted in areas where the tool had done an insufficient job. The aragonite was then coloured in green and vaterite coloured in red in Photoshop, the colours having no significant purpose other than allowing a script to distinguish between the two (Figure 2). The amount of red and green pixels was then quantified, and the percentage of the total area of the otolith (no. of green pixels + no. red pixels) that consisted of vaterite was in that way measured. The pixels in the transition between the two more or less equally consisted of both colours and were decidedly divided by two. This quantification was performed using a custom script in Python (Python Software Foundation, <https://www.python.org/>) that reads the pixel data from the pictures as RGBA-channels; red, green, blue and alpha (transparent). Each of the abnormal otoliths was, using this method, assigned a number ranging between 0 and 1. The otoliths with the value of 0 had no visible vaterite and consisted only of aragonite, categorizing them as normal. The categorization of the individual otoliths was thus done in two different ways: normal/abnormal and degree of vaterite (% visible vaterite). An individual was classified as having abnormal otoliths if at least one of its otoliths had an identifiable proportion of vaterite.

To determine the reliability of the estimates, 60 randomly selected otoliths, stemming from 30 individuals with abnormal otoliths, were independently remeasured using the same pictures. The two measurements were highly correlated ( $r = 0.98$ ), indicating high repeatability.

## 2.3 | Statistical analyses

All statistical analyses were performed in R studio version 1.1.423 (RStudio, 2016). Linear models were checked for normality and homoscedasticity using diagnostic plots to check the model fit. The ggplot2-package (Wickham, 2016 in R was used to visualize the data. The effects of length and treatment group on probability of an individual having at least one abnormal otolith were tested using generalized linear models by implementing the glm() function with a binomial distribution and a logit-link. Having an abnormal otolith or not was used as the response variable, and length and group were used as the predictors. McFadden's  $R^2$  was calculated to determine model fit. McFadden's  $R^2$  is a pseudo- $R^2$  developed for logistic regressions,

**FIGURE 2** Example of the analysis process in Photoshop. Left: The picture displays two otoliths sampled from a 2017 179 mm long Lone smolt. The right otolith with no visible vaterite and the left otolith with a clearly visible vaterite part. Right: The same picture showing how the otolith was coloured with red and green to quantify to what degree the otolith was abnormal. The abnormal otolith in this example was estimated to be c. 23% vaterite



**TABLE 2** Average length  $\pm$  S.D. (mm) of each group and cohort of smolt with and without abnormal otoliths

Group	Year	Average length (N)		Test statistics	
		With abnormal otoliths	Without abnormal otoliths	t-value	P
Imsa warm	2016	193.7 $\pm$ 24.4 (47)	181.5 $\pm$ 26.6 (73)	2.533	<b>0.013</b>
	2017	193.4 $\pm$ 16.5 (51)	188.2 $\pm$ 19.4 (283)	1.799	0.073
Imsa cold	2016	152.5 $\pm$ 24.8 (2)	132.7 $\pm$ 16.9 (3)	1.077	0.361
	2017	188.4 $\pm$ 25.3 (37)	181.9 $\pm$ 22.0 (287)	1.661	0.098
Lone	2016	155.6 $\pm$ 15.1 (170)	155.5 $\pm$ 14.9 (91)	0.051	0.959
	2017	161.2 $\pm$ 14.0 (207)	159.92 $\pm$ 12.4 (117)	0.824	0.410

Note. Bold values represent a P value that is  $<0.05$ , so there is a significant difference in length of the individuals with and without abnormal otoliths. T-tests were used to test for differences in average length.

where the higher McFadden's  $R^2$  indicates greater model likelihood (Veall & Zimmermann, 1994).

The effects of smolt length and treatment group on the degree of vaterite in the abnormal otoliths were tested using linear models using the `lm()` function. Degree of abnormality was used as the response variable, and length and group were used as the predictors. These models did not meet assumptions of normality and homoscedasticity based on diagnostic plots and the `ols_test_normality()` function from the `olsrr`-package (Hebbali & Hebbali, 2018). Preliminary analyses using this linear model showed that there was no effect of length, and therefore in this study the authors used Kruskal–Wallis-tests and Tukey's *post hoc* tests to test for the differences among treatment groups. Furthermore, they used a simple t-test to check whether individuals with abnormal otoliths were significantly longer on average than those without. To avoid Type 1 error, a Bonferroni correction was implemented by dividing the set alpha level (0.05) by the number of tests (6) to set a new and corrected alpha level (0.008).

### 3 | RESULTS

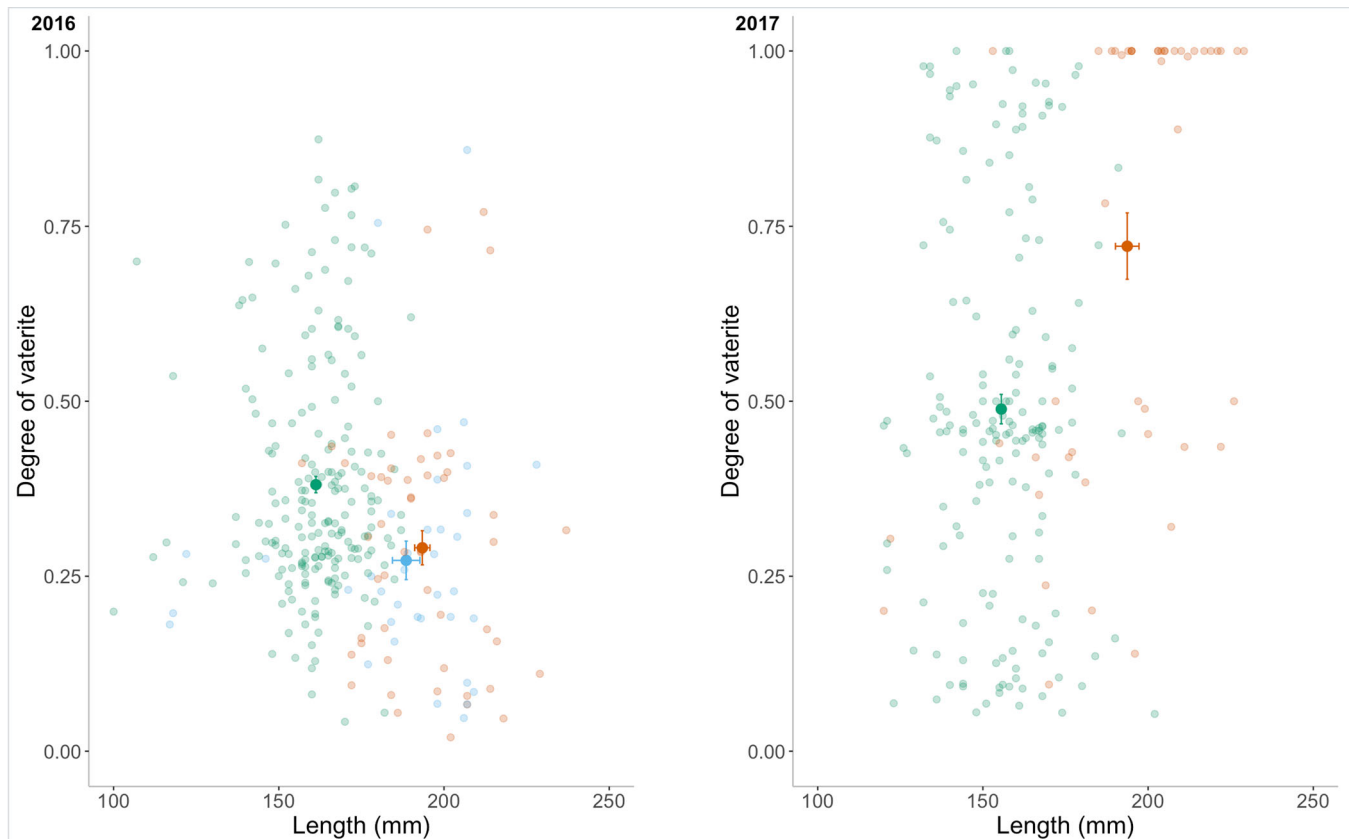
Although the adult return rate was low overall, the authors detected an overall lower occurrence of vaterite in otoliths of returning adult salmon than in out-migrating smolts, consistent with the expectations of reduced survival associated with vaterite. For most of the smolt groups, the frequency of individuals with at least one abnormal otolith

was relatively high, varying from 11.4% to 65.1%. In total, 37.6% of the smolt had at least one abnormal otolith (Table 1). There were large differences among the three treatment groups (the 2 years pooled;  $\chi^2_2 = 336.1$ ,  $P < 0.0001$ ), with the Lone group having by far the highest proportion of abnormal otoliths (64.4% in total). The two Imsa groups had on average 11.8 (Imsa Cold) and 21.5% (Imsa Warm) of abnormal otoliths.

On average, there were no differences in size between smolt with or without abnormal otoliths. The exception was the 2016 Imsa Warm group, where individuals with abnormal otoliths were significantly larger than those without abnormal otoliths (Table 2). Nonetheless, when correcting for Type 1 error, no significant differences were found.

There were significant differences among smolt groups in the frequency of abnormal otoliths (Kruskal–Wallis rank sum tests indicated significant differences between groups for both years;  $P < 0.001$ ) (see Table 1). *Post hoc* tests showed that in 2016 the Lone group and the Imsa Warm group differed significantly from one another ( $P < 0.001$ ), whereas the Imsa Cold did not differ from Imsa Warm ( $P = 0.98$ ) and Lone ( $P = 0.51$ ). Similarly, the Lone group differed significantly from the two Imsa groups in 2017 ( $P < 0.001$ , both comparisons), whereas the Imsa Cold and Imsa Warm did not differ ( $P = 0.22$ ).

For individual smolt that had at least one abnormal otolith, the authors investigated the proportion of vaterite in the otolith. The average proportions of vaterite ( $\pm$ SD) in the abnormal otoliths for the 2016 smolt were  $0.49 \pm 0.27$  for the Lone group and  $0.72 \pm 0.32$



**FIGURE 3** The average coverage of vaterite and length (growth proxy) for each group for the two different cohorts (2016 and 2017). The slightly larger points indicate the average lengths and the average coverage of vaterite of the abnormal otoliths. The lines emerging from these points indicate the standard errors (—●—) Imsa Cold (—●—) Imsa Warm (—●—) Lone (—●—) Imsa Cold (NA) (—●—) Imsa Warm (NA) (—●—) Lone

for the Imsa Warm group ( $F = 24.44$ ,  $P < 0.0001$ ) (the sample size for Imsa Cold was very small for this analysis).

The average proportion of vaterite ( $\pm$ S.D.) for the 2017 smolt groups was generally somewhat lower than for the 2016 smolt;  $0.29 \pm 0.17$  for the Imsa Warm group,  $0.27 \pm 0.17$  for the Imsa Cold group and  $0.38 \pm 0.17$  for the Lone group ( $F = 10.48$ ,  $P < 0.0001$ ). Tukey's *post hoc* tests showed significant differences between the Lone group and Imsa Warm and Imsa Cold, whereas the Imsa Cold and Imsa Warm groups did not differ significantly. There was no relationship between smolt length and the per cent vaterite coverage of the affected otoliths for any of the groups (Figure 3; all  $P > 0.05$ ).

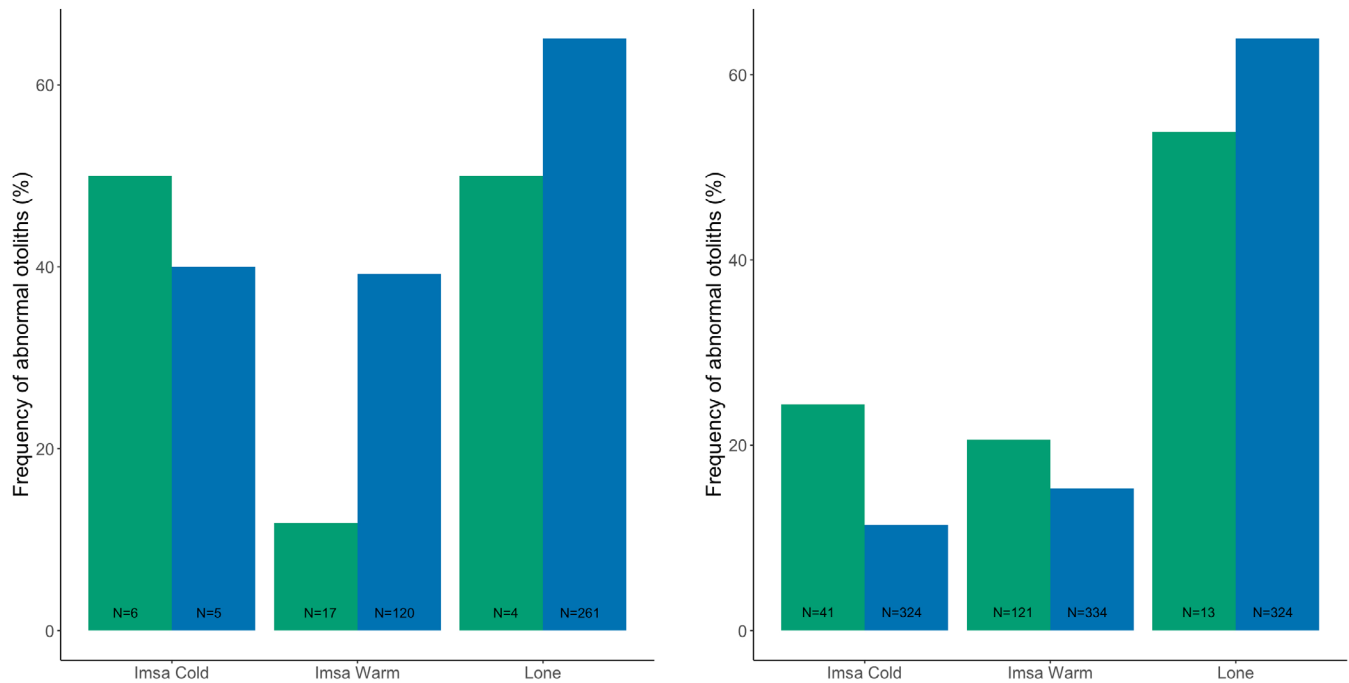
The return rate of adult salmon was generally very low (1.34%), but differed among groups (Table 1). In total, the returning adults had lower probability of having abnormal otoliths than the smolt ( $\chi^2 = 13.70$ ,  $P < 0.001$ ), based on the binomial visible vaterite/no visible vaterite classification. Nonetheless, again, there were large differences among groups (Figure 4). The 2016 Imsa Warm smolt had a significantly higher proportion of abnormal otoliths than the returning adults ( $\chi^2 = 4.87$ ,  $P = 0.020$ ), whereas the opposite relationship was significant for the 2017 Imsa Cold smolt and adults ( $\chi^2 = 5.43$ ,  $P = 0.020$ ). No significant differences in the proportion of abnormal otoliths were found for the 2017 Imsa Warm group ( $\chi^2 = 1.85$ ,  $P = 0.170$ ). The 2016 and 2017 Lone and the 2016 Imsa Cold group

had very low number of returning adults to compare smolt and adults (Table 1).

## 4 | DISCUSSION

In this study, the authors have quantified the prevalence of vateritic otoliths in groups of hatchery-reared Atlantic salmon smolt and compared with adult salmon of the same cohort returning to the river after completing their ocean migration. The smolt from different populations and treatments showed large variation in both the frequency of abnormal otoliths and the degree of vaterite in the abnormal otoliths, and overall the frequency of abnormal otoliths was high. In addition, the return rates were low; these observations in conjunction could possibly indicate a fitness consequence at sea. Furthermore, the smolt groups that were seemingly least successful in returning to the river were that same groups that had the highest frequency of abnormal otoliths as well.

The authors observed high levels of vateritic otoliths in all smolt groups investigated here. Fish are ectothermic and the formation of otoliths may be sensitive to environmental conditions. In particular, the production of vaterite may be influenced by individual differences in metabolic rate (Oxman, 2012; Sweeting *et al.*, 2004).



**FIGURE 4** The percentages of abnormal otoliths for each cohort (2016 and 2017) of each group for the smolt and the returning adults, and total N of each sample (■) Adult (■) Smolt (■) Adult (■) Smolt

Hatchery conditions are often designed to speed up growth through the use of commercial feed, and particular light conditions and temperature regimes. Recent studies have indicated that rapid growth may be the primary cause of abnormal otoliths, possibly because of changes in the composition of otolith membrane proteins, or lower  $(Ca^{2+})/(CO_3^{2-})$  ratio in the endolymph which favours the formation of vaterite (Reimer *et al.*, 2017). An earlier study of Norwegian salmon juveniles raised in hatcheries showed high frequency of abnormal otoliths as well as high degree of vaterite replacement with increasing growth (Reimer *et al.*, 2016). Therefore, environmental factors that result in enhanced metabolic activity may be important for the formation of vaterite otoliths (Gauldie, 1986; Reimer *et al.*, 2017). Nonetheless, in this experiment, the smolts with abnormal otoliths were not generally larger than those without abnormal otoliths; the exception was the 2017 Imsa Warm group. Thus, growth rate in itself is not the main explanation for occurrence of abnormal otoliths in hatchery fish. This suggests that there might be other and several stressors that induce the vaterite production. One of these stressors could potentially be density in the tanks, which differs for the experimental groups of this study. The authors could expect groups experiencing higher densities in the rearing-tanks to have higher frequency of abnormal otoliths. In general, the authors saw the contrary; the Lone groups experienced lower densities than the other experimental groups but a higher degree of abnormal otoliths. Nonetheless, when comparing the Lone group between the years, the authors observed a slightly higher frequency of abnormal otoliths in 2016 when they were kept at higher density than in 2017. This was also the case for Imsa Warm; a higher frequency of abnormal otoliths was observed in the year when they were

contained in tanks with more individuals. Although this suggests an effect of crowding, the data should be evaluated carefully.

The smolt from the Lone population had significantly higher frequency of abnormal otoliths than the smolt from the Imsa population. These groups were reared under the same temperature conditions, but they have a very different rearing history. The Lone population has had a much longer hatchery ancestry than the Imsa population; therefore, genetic drift in this population can have led to genetic changes as a result of the low selection pressure in the hatchery. For wild Atlantic salmon, survival from egg to smolt is only around 1.7% on average and may be 20 times higher in a hatchery (Araki *et al.*, 2008; Glover *et al.*, 2017; Jonsson & Jonsson, 2011a). The fish from the Lone groups had low return rates (0.2% and 0.5%) and higher frequencies of abnormal otoliths consistently in both years compared to the other groups. An important factor contributing to the low return rates for the Lone population is most likely the fact that these fish stem from numerous generations of hatchery-reared parents, and their wild ancestral broodstock dates back to the late 1980s. The Imsa population differs from the Lone population in that they are much closer to their wild ancestors. Therefore, the Lone population may have acquired lower fitness over generations and may generally be less conditioned for the unforgiving challenges of the ocean environment, possibly because of genetic adaptation to captivity (domestication) (Christie *et al.*, 2014).

Overall, there was in total a higher frequency of abnormal otoliths in the smolts than in the returning adults (37% and 24%, respectively). Separately, however, the results varied strongly among treatment groups. One reason for the variation in results among groups may be the very low return rate of mature salmon. The return rate of wild

Imsa salmon is generally low, and the return rate of hatchery-reared salmon released in the river is usually even lower than for the wild salmon (Jonsson & Jonsson, 2011a). Further, it is difficult to compare the 1SW and 2SW adults because of the low overall return rate, and in particular the very low numbers of 2SW salmon.

Abnormal otoliths may lead to reduced hearing sensitivity in fish. The primary issue with hearing loss may be predator avoidance. Underwater predators and prey produce sound in the infrasound range, below 20 Hz. Juvenile Atlantic salmon show awareness responses at 5–10 Hz, and avoidance responses at around 10 Hz, and hearing at these frequencies may indeed be impaired by vaterite (Knudsen *et al.*, 1992; Reimer *et al.*, 2016). In Norway, fish are protected by The Norwegian Animal Welfare Act (2009) (<https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/>), which states that animals must be kept in an environment which is consistent with good welfare. This includes the animal itself being able to properly both experience and respond to the environment in which it is confined. There is increasing evidence, including from this experiment, that practices linked to hatchery rearing result in a substantially increased frequency of otolith abnormalities compared to what would have been in the wild. Reimer *et al.* (2016) found hearing loss to be a consequence of this, in turn perhaps causing reduced ability to avoid predators; as they also addressed, this in itself could be seen as a conflict with good animal welfare. Nonetheless, this potential hearing loss is clearly not detrimental to Atlantic salmon homing because abnormal otoliths with high vaterite coverage were found in a number of the returning adults in this experiment. Abnormal otoliths have been observed in adults before, and it has been hypothesized that at least the loss of hearing sensitivity from abnormal otoliths may in some way be compensated for in fish (Oxman *et al.*, 2007).

In terms of homeward navigation, abnormal otoliths may also have an effect because of the importance otoliths have for gravity sensation and linear acceleration in the water column (Reimer *et al.*, 2016). Theoretically, otolith irregularities may impact on swimming performance and perhaps also navigational efficiency. Nonetheless, clearly, homeward migration is possible for individual with completely vateritic otoliths. McConnell *et al.* (2019) found that abnormal otoliths did not significantly affect homing ability in chum salmon [*Oncorhynchus keta* (Walbaum)], suggesting that the negative functional effects of otolith abnormalities may be compensated for by other factors (McConnell *et al.*, 2019). Atlantic salmon navigating homeward thus probably use multiple sensory organs that in combination allow for high precision.

An important factor that will affect results in experiments like this involving tagging is the mortality rates from tagging itself. In this experiment, the Imsa Warm group had a return rate of 0.6% and 2.6% in 2016 and 2017, respectively. In 2017, 2985 of the Imsa Warm group (total  $N = 4965$ ) were PIT-tagged, whereas the rest were Carlin-tagged. In 2016, all the Imsa Warm fish were Carlin-tagged. This might indicate that the higher return rate/survival may, at least in part, be attributed to lower tag-mortality of the 2017 Imsa Warm group.

A general take-away message from this experiment is that return rates were low for all smolt groups. Here also lies the main issue in determining the effect abnormal otoliths may have had; very few returned in general. This is not unique for the salmon of this experiment, nor unique only for hatchery-reared fish, although return rates of wild salmon are higher than for hatchery-reared salmon. The current decline in wild salmon populations is often attributed to low individual growth rates, reduced sea age at maturity (less multi sea winter individuals) and low marine survival (Jonsson & Jonsson, 2004). The smolt groups with the longest hatchery ancestry had by far the highest frequency of abnormal otoliths, as well as the lowest return rates. This may indicate that having abnormal otoliths is a symptom of reduced fitness.

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## AUTHOR CONTRIBUTIONS

B.A., L.A.V and A.F. conceived the study; B.A. collected and analysed the data; B.A. wrote the first draft, and all authors contributed to and accepted the final version.

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