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BRIEF COMMUNICATION

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Varying frequency of vateritic otoliths in the Baltic herring *Clupea harengus membras*

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Abstract
We report observations of vateritic crystallization in the sagittal otoliths of the Baltic herring *Clupea harengus membras* in the northern Baltic Sea. While the existence of vaterite in the calcium carbonate matrix of sagittal otoliths has been observed in various species globally, reports from the brackish Baltic Sea are few in number. Large variation in the frequency of vaterite in 1984, 1988, 1997, 2010 and 2017 was observed, suggesting that the phenomenon is not static and more long-term studies should be conducted in search of the ultimate causing factors.

KEYWORDS
aragonite, Baltic herring, mineral composition, otoliths, vaterite, XRD

The calcium carbonate ($CaCO_3$) matrix of sagittal otoliths is, in most cases, composed of aragonite, whereas the two other polymorphs, calcite and vaterite, are used relatively infrequently (Carlström, 1963; Gauldie, 1986; Reimer *et al.*, 2016; Strong *et al.*, 1986). Each of these polymorphs have their characteristic crystal structure, and a 'switch' from aragonite to either calcite or vaterite results in the formation or co-precipitation of a translucent or 'glass-like' crystalline matrix, which often also causes considerable distortion to the shape, density, brittleness, and size of the otolith (*e.g.*, Gauldie, 1986; Tomás & Geffen, 2003). This non-aragonite crystallization, also referred to in the literature as 'aberrant' crystallization, has been documented in the otoliths of several fish species in various environments (*e.g.*, Budnik *et al.*, 2020; Loeppky *et al.*, 2021; Melancon *et al.*, 2005; Reimer *et al.*, 2016; Tzeng *et al.*, 2007), including juvenile Atlantic herring *Clupea harengus* L. in the Celtic and Clyde Seas (Tómas and Geffen, 2003; Tómas *et al.*, 2004). Yet, the ultimate factors determining which $CaCO_3$ polyform is produced are still not completely understood (Thomas & Swearer, 2019).

Based on current knowledge, vaterite crystallization is associated with changes in the internal physiology of the fish and is possibly protein-mediated (as reviewed in Thomas & Swearer, 2019). For instance, Reimer *et al.* (2017) showed that fast-growing Atlantic salmon *Salmo salar* L. were three times more likely to have vaterite otoliths than slow-growing individuals were. The authors speculated that the difference could be caused, for example, by the greater energy content of fast-growing fish, which may lead to greater transport of bicarbonate ions (HCO_3^-) relative to Ca^{2+} into the endolymph and result in a lower (Ca^{2+})/(CO_3^{2-}) ratio conducive for vaterite formation. Studies have also shown that the environment (temperature, pH) can impact otolith biomineralization (Coll-Lladó, 2021; Loeppky *et al.*, 2021). In connection with this, physiological or environmental stress has been suggested to explain the occurrence of vaterite crystallization in some wild fish (Melancon *et al.*, 2005; Tzeng *et al.*, 2007; Yedier & Bostanci, 2019).

In the Baltic Sea, the existence of crystallized otoliths has long been recognized (*e.g.*, ICES, 2008), yet only few studies on the topic exist (*e.g.*, Lill *et al.*, 2020; Tzeng *et al.*, 2007). In these studies, the occurrence of vaterite was reported with respect to otolith microchemistry due to vaterite's capability to confound studies using otolith elemental signatures as biological tracers (Tzeng *et al*., 2007). Nevertheless, the semi-enclosed, brackish-water ecosystem of the Baltic Sea could also provide a unique model system to study causing environmental and physiological factors as its organisms are subjected to pronounced latitudal and vertical temperature and salinity gradients, which directly and indirectly dominate their physiological ecology. In addition, climate change is affecting the ecosystem of the Baltic Sea in ways that most coastal areas will experience only in the future (Reusch *et al*., 2018). To date, climate-induced hydrological changes have caused several changes in the ecosystem and food web that, for instance, have affected the growth rate and condition of the Baltic herring *Clupea harengus membras* L. (Casini *et al*., 2011; Möllmann *et al*., 2005; Rajasilta *et al*., 2019, 2021; Rönkkönen *et al*., 2004).

Here, we report observations of 'abnormal' or 'vateritic' crystallization in the sagittal otoliths of adult *C. harengus membras* from selected years, over the period 1984–2017. The mineral composition was studied from archival otoliths, collected in 1984, 1988, 1997, 2010, and 2017 as part of an annual monitoring program of the species spawning population in the northern Baltic Archipelago Sea (60°23′N, 22°05′E). The area encompasses one of the main spawning areas for the species in the northern Baltic Sea (Rajasilta *et al*., 1993). In the Baltic Sea, *C. harengus membras* is a dominant, shoaling fish species that lives and reproduces throughout the brackish-water basin and exhibits large variation in its characteristics. Sagittal otoliths were removed from the fish during the standard sample treatment for age determination (Eklund *et al*., 2001) and visually analysed as a whole under a stereomicroscope at 40× magnification. According to standard classification, described, for example, by Gauldie (1986) and Sweeting *et al.* (2004), the otolith was classified as 'vateritic' if any translucent and 'glass-like' crystals were clearly identifiable in either otolith, or as 'aragonitic' if the studied otoliths were opaque and no crystalline structure was evident (Fig. 1). The frequency of vaterite crystallization was defined as the percentage (%) of vateritic otoliths in fish in each study year (Table 1). In total 7792 fish were studied. Variation in the number of vateritic otoliths in each fish was not recorded during the monitoring.

The mineral composition and validity of the visual inspection was verified using powder X-ray diffraction (XRD), a common technique for characterizing crystalline materials (*e.g*., Dinnebier and Billinge, 2008; Fawcett *et al*., 2019). Six otolith samples (three 'aragonitic' and three 'vateritic'), taken from fish that were collected by commercial trawling in the Archipelago sea (AS) during May–July 1984 were ground as a whole and analysed individually to assess if any changes had occurred in the mineralogy of otoliths that would be invisible to the eye. The powder XRD measurements were performed with an Empyrean diffractometer (Malvern Panalytical Ltd, The Netherlands) in Bragg–Brentano configuration using Cu Kα radiation ($\lambda = 1.5418$ Å) and a PIXcel^{3D} detector. The mineralogical compositions of the otoliths were estimated from the diffractograms with Highscore Plus v4.9. software (Malvern Panalytical Ltd).

Vaterite crystallization was found in all study years with varying frequency. In 1984, the occurrence of vaterite was most frequent, with 48% of all studied fish having vaterite crystallization. The frequency varied also in the later study years, indicating a decline in frequency in 1997 and an increase in 2010 and 2017 (Table 1). The extent of vaterite replacement in each otolith as well as the number of vateritic otoliths in each fish was also observed to vary but was not quantitatively recorded during the monitoring. However, adding this information to future studies could be useful as it may have implications on how the otolith data should be analysed and interpreted (Vignon, 2020). The XRD analysis concurred that the studied otoliths from 1984 were composed of aragonite and/or vaterite as indicated by the visual inspection, while no calcite peaks were identified in the diffraction patterns. The otoliths that had been visually categorized as 'aragonitic' were determined to be composed only of aragonite as no vaterite peaks were identified in the patterns. In contrast, the otoliths categorized as 'vateritic' contained distinguishable vaterite peaks in addition to aragonite.

Recently, vaterite crystallization in sagittal otoliths has received attention as the polymorph-related changes in the otolith's mass symmetry have been suspected to negatively affect the auditory capacity and directionality, and thereby affect fish survival, growth and even stock replenishment (Reimer *et al*., 2016; Oxman *et al*., 2007; Vignon & Aymes, 2020). Nevertheless, the impact of the phenomenon

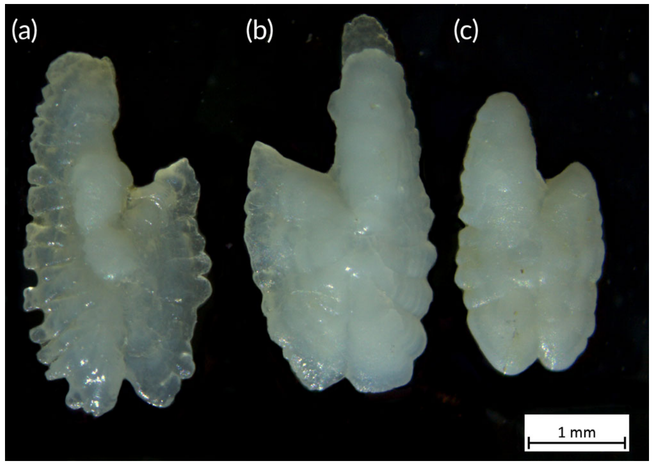

FIGURE 1 Examples of sagittal otoliths of *Clupea harengus membras* categorized visually as (a, b) 'vateritic' and (c) 'aragonitic' based on the occurrence of translucent calcium carbonate matrix in the vateritic otolith

TABLE 1 Percentage (%) of visually categorized 'aragonitic' and 'vateritic' otoliths in *Clupea harengus membras* during 1984, 1988, 1997, 2010 and 2017 ($n = 7792$, where n is the number of studied fish each year)

Year	Aragonitic	Vateritic	*n*
1984	51.94	48.06	697
1988	60.36	39.64	1289
1997	97.42	2.58	4736
2010	84.30	15.60	721
2017	88.30	11.70	349

on the survival of fish in the wild is inadequately understood, as most studies focusing on the ecological impact of the phenomenon have been conducted with farmed fish or in experimental conditions. In the wild, the occurrence of vaterite might not have any impact on individual fitness, or the fish might rely on other sensory organs or school behaviour to avoid danger and access food (Tómas & Geffen, 2003; Vignon & Aymes, 2020). The aim of this study was to provide important primary information on the inter-annual occurrence of vateritic otoliths in a *C. harengus membras* population due to its importance for fisheries and in the ecosystem. In the Baltic Sea, the occurrence of aberrant otoliths in *C. harengus membras* has been acknowledged in reports (*e.g.*, ICES, 2005, 2008) as well as recorded, to variable degree, in international and national databases, as it is generally agreed and recommended that the occurrence of vaterite otoliths is recorded but not used for age reading (*e.g.*, ICES, 2008). As vaterite replacement might have potential impact on the fish itself as well as to fish stock management practices, it is our belief that more studies using inter-annual otolith data are called for to aid in understanding the implications of the phenomenon.

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CONTRIBUTIONS

K.M. analysed the otolith data, wrote the first draft and finished the final version, and designed the study together with M.R., who also participated in writing the text. E.M. conducted the XRD analyses and wrote the respective text in the materials and methods section, and participated in revising the text. S.J. and J.H. participated in drafting and revising the text. All authors contributed to the article and approved the submitted version.

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APPENDIX A

The study area in the northern Baltic Sea. Baltic herring samples were collected from the inner region of the Archipelago Sea (AS) from trap-nets situated near known spawning sites.