Effect of temperature and CO2 concentration on the morphogenesis of sagittal otoliths in Atlantic herring (*Clupea harengus*) larvae

Mahé Kélig [1, *], Joly Léa [1, 2], Telliez Solène [1], Zambonino Infante José-Luis [2], Meunier Cédric Léo [3], Mackenzie Kirsteen [1], Giraldo Carolina [1]

[1] Ifremer, Fisheries laboratory, Channel and North Sea Fisheries Research Unit, Boulogne-sur-mer, France
[2] Ifremer, Univ Brest, CNRS, IRD, LEMAR, Plouzané, France
[3] Helmholtz-Zentrum für Polar- und Meeresforschung, Alfred-Wegener-Institut, Helgoland, Germany

* Corresponding author : Kélig Mahé, email address : kelig.mahe@ifremer.fr

Abstract :

Otoliths are very useful biomarkers especially for fish growth. Climate change with the associated global changes in warming and acidification could affect the calcification and the shape of otoliths during the crucial larval period in teleost fish. To evaluate this predicted combined effect of temperature and CO2, Atlantic herring (Clupea harengus) embryos and larvae were reared from hatching to respectively 47 and 60 days post-hatching (dph), under present day conditions and a scenario predicted for the year 2100 (IPCC RCP8.5). Otolith morphogenesis was tracked by analyzing area and normalized Elliptical Fourier coefficients. We found that otolith area for fish of similar size increased under the predicted 2100 climate change scenario compared to the present day. Climate change does not, however, seem to directly affect the otolith shape. Finally, the onset of otolith morphogenesis is hardwired, but the relationship between otolith and fish size is environment-dependent.

Highlights

► Climate change do not affect the otolith shape of the herring larvae ► No directional bilateral asymmetry found between left and right otoliths ► Relationship between otolith and fish size is environment-dependent

Keywords : Otolith shape, Otolith area, Temperature effect, Ocean acidification, Elliptic Fourier descriptors, Directional asymmetry

1. Introduction

26 Otoliths are calcified biomineralised structures overlying the sensory epithelia in the inner ear of fish.

27 They are formed by calcium carbonate crystals embedded in a non-collagenous organic matrix

28 composed of acidic proteins and polysaccharides (Degens et al., 1969; Popper et al., 2005). They are

29 metabolically inert and do not resorb in periods of stress, but grow throughout an individual's life, in

30 correlation with its ontogenic growth (Casselman, 1987). Because of the positive correlation

31 between otolith and fish growths, otoliths have been widely used in fisheries science to understand

32 the fish growth and the potential effects which controlled it (i.e. environment, fishing pressure;

33 Enberg et al., 2011 ; Marty et al., 2014; Carbonara et al., 2022). In Atlantic herring (*Clupea*

34 *harengus*), otolith microstructure has been used to study environmental effects (temperature, salinity

35 or feeding activity) on the growth of the larvae (Folkvord et al., 1997 ; 2004 ; Johannessen et al.,

36 2000; Berg et al., 2017; Denis et al., 2017; Tonheim et al., 2020), and otolith shape used as a tool for

37 stock identification (Turan, 2000; Burke et al., 2008; Libungan et al., 2015). Atlantic herring are of

38 high commercial importance, with around 1,640,000 tons caught in 2016 (FAO FishStat data), but

39 are vulnerable to the effects of climate change, particularly at the larval stage (Hufnagl and Peck,

40 2011).

41 Otolith growth, and ultimately global otolith shape are well-known to result from the combination of

42 genetic heterogeneity, ontogeny (physiological processes) and the influence of environmental (biotic

43 and abiotic) factors (Vignon and Morat, 2010; Mahé, 2019; Hüssy et al., 2020). Global climate

44 change is projected to cause warming of the ocean surface by 3 to 5°C by 2100, and acidification of

45 waters with a drop of 0.4 pH units in the worst-case scenario (IPCC 2019). The speed at which global

46 change is occurring will not allow some species to adapt; in addition, early life history stages which

47 do not yet have fully functional physiological homeostatic mechanisms could be particularly affected

48   (Melzner et al., 2009). Some ontogenic processes, such as otolith morphogenesis, could thus be

49   particularly impacted by the alteration of environmental conditions.

50   A previous study on herring larvae testing four temperature conditions showed that the size and the

51   width of the otolith daily increment were both temperature-dependent (Folkvord et al., 2004). Other

52   studies on the effects of ocean acidification on fish otoliths have been mostly conducted on larval

53   developmental stages. Several studies have shown that elevated levels of CO_2 caused an increase in

54   otolith size or modification of the otolith shape for multiple species (Munday et al., 2011; Bignami et

55   al., 2013; Maneja et al., 2013; Reveillac et al., 2015; Coll-Llado et al., 2021). Consequently,

56   acidification can lead to decoupling of otolith and body growth for some species (Reveillac et al.,

57   2015; Franke and Clemmesen 2011; Frommel et al., 2013; Perry et al., 2021).

58   Here, we aimed to investigate in Atlantic herring how otolith shape and size variability is affected by

59   the warming and acidification conditions projected by 2100 under the worst-case IPCC scenario

60   during the first early life stages. This otolith shape was investigated within individual (i.e. directional

61   bilateral asymmetry) and within the population (i.e. between individuals).

62

2. *Materials and methods*

63

2.1 Experimental design

64

65   A experiment with temperature and CO_2 concentration (through the measure of pCO_2 as partial

66   pressure of CO2) as combined factors was conducted, one close to present day conditions of

67   temperature and pH in the winter in the English Channel (Amb; 11°C and pH 8.0, pCO_2 ~560 µatm),

68   and the other a global change scenario of ocean warming and acidification (IPCC 2019, "Changing

69   ocean, marine ecosystems and dependent communities") (OWA; 14°C and pH 7.6, pCO_2 ~1660

70   µatm) (Supplementary Table 1). There were 3 replicates (tanks) for each experimental condition (i.e.

71   temperature-CO_2). Most of the eggs hatched on the 9th day of incubation (starting point of the

72   experiment: 1 dph) and larvae were kept until 69 days post hatch (dph). Three days after hatching (3

73   dph), all larvae were counted and distributed equally in six 38 L conical black tanks (1500 larvae by

74   tank), constituting the replicates of the experiment. A continuous flow through system of 20 L h^{-1}

75   was used (i.e. open circulation system). To allow the acclimation of the larvae, the temperature in the

76   OWA tanks was progressively increased from 11 to 14°C over 48 h. Each OWA tank was supplied

77   by a 200 L header tank, where the heated water was enriched with CO_2 to achieve the target value of

78   pH 7.6.

79   Temperature and CO_2 were checked twice per day (pH meter 330i, WTW, Germany; Table S1).

80   Oxygen saturation (oximeter: WTW Oxi 340, Bioblock scientific) and salinity were measured once

81   per week, along with total alkalinity (TA) of each tank following the protocol of Anderson and

82   Robinson (1946) and Strickland and Parsons (1972). Oxygen concentration was always above 88%.

83   pCO_2 was calculated using the excel macro CO2sys (Lewis and Wallace 1998) with the constant

84   from Mehrbach et al., (1973) refit by Dickson and Millero (1987). To prevent any food limitation, the

85   daily food quantity was distributed four times during the day to maintain an ad libitum level (Strain

86   2002), ensuring that there were always prey in the tank during the day. To be sure to allow proper

87   feeding through time we used an increasing range of living prey sizes from phytoplankton to 24 h old

88   nauplii, before weaning with feed granules (as described in Joly et al., 2021). Throughout the study,

89   mortality was monitored daily, and was comparable between all ponds with no influence of

90   environmental conditions (for more details, Joly et al., 2021).

91   Experimental animal came from wild Atlantic herring from the Downs stock of the southern North

92   Sea and English Channel (Joly et al., 2021). Herring larvae were reared until they reached the last

93   larval development stage respectively from hatching to 47 days post-hatching in the OWA treatment

94   (14°C, 1660 µatm) and 60 days post-hatching in the Amb treatment (11°C, 560 µatm) to obtain the

95   same Growing Degree Days (GDD) for two temperature conditions. To follow the otolith growth and

96   shape variation through time, 351 Atlantic herring from 32 to ~662°C.day (GDD, Figure 1) were

97   sampled five times over the course of the experiment. At 32°C.day, 15 individuals were sampled, and

98   then 42 fish for each experimental condition were sampled at each of the next four samplings. To

99   assess the potential effect of temperature on the growth and shape of otoliths, we used the GDD

100   approach which quantifies the thermal opportunity for growth by aggregating temperatures relevant

101   to growth (McMaster and Wilhelm, 1997), and is thus more precise than the calendar time approach

102   when describing growth (Neuheimer and Taggart, 2007; Mahé et al., 2019). The first sampling

103   occurred at three days post-hatching (GDD=32°C.day). The four other samplings were then carried

104   out after different time periods depending on the rearing temperature (Figure 1).

105   **2.2 Otolith shape analysis**

106   After measuring the total length (TL± 0.1 cm) of fish, their sagittal otoliths (left and right) were

107   extracted from the cranial cavity and cleaned. The outline of each otolith was digitized using an

108   image analysis system. To compare the shapes of the left and right otoliths, mirror images of the right

109   otoliths were used. Otolith shape was assessed by analyzing first the otolith area (Oarea, µm²) and

110   Elliptic Fourier Descriptors (EFDs; e.g. Lestrel, 2008). Oarea seems to be a better univariate

111   descriptor than longest length. For each otolith, the first 99 elliptical Fourier harmonics were

112   extracted and normalized with respect to the first harmonic so as to be invariant to otolith size,

113   rotation and starting point of contour description (Kuhl and Giardina, 1982). To determine the

114   number of harmonics required to reconstruct the otolith outline, the cumulated Fourier power (F) was

115   used. Only the first 6 harmonics were necessary to ensure reconstruction of each otolith shape with a

116   precision of F=99.99% (Lestrel, 2008) and were thus used for further analyses.

117   The resulting matrix containing EFDs (as columns) for each otolith (as rows) was subjected to

118   Principal Components Analysis (PCA) (Rohlf and Archie, 1984). and the 3 first principal

119   components (PCs) were selected as otolith shape descriptors or shape matrix according to the broken

120   stick model (Legendre and Legendre, 1998), which, in this case, corresponded to a threshold of 3.4%

121   of the total variance explained (Borcard et al., 2011). In total, these 3 PCs explained 82.4% of the

122   total variance in the EFDs. This procedure allowed us to decrease the number of variables used to

123   describe otolith shape variability through EFDs while ensuring that the main sources of shape

124   variation were kept, and to avoid co-linearity between shape descriptors (Rohlf and Archie, 1984).

125   **2.3 Statistical analyses**

126   Each environmental condition was the combination of temperature and CO_2 concentration values.

127   Otolith area and larvae size differences were analysed using a post-hoc Tukey-HSD test among

128   several values of GDD. The relationship between larval total length (TL) and otolith area (Oarea) in

129   response to the environmental conditions (temperature and CO_2 concentration) was tested using

130   Analysis of Covariance after verifying the normality of residuals. Using the Fourier harmonics to

131   describe the otolith shape, pRDA were used to test the explanatory variables of interest (i.e., side,

132   combination of temperature and CO_2 concentration, GDD value) using total length to correct for fish

133   size. This pRDA was combined with permutation tests on the selected PC matrix and the explanatory

134   matrix consisted. To analyse the potential anatomical differences described by the directional

135   bilateral asymmetry between left and right otolith shape, partial redundancy analysis (pRDA) was

136   modelled on the selected principal components (PCs) matrix using otolith side (left/right) as the

137   potentially influential variable and the individual as the conditioned variable. To test the potential

138   effect of climate change, the pRDA was applied with the environmental explanatory matrix

139   (Temperature and CO_2 concentration). To visualise shape differences, average shapes were rebuilt

140   based on EFDs averaged for each group of individuals. Directional asymmetry and environmental

141   effect amplitude were then computed as the percentage of non-overlapping surface between the

142   reconstructed otolith average shapes relative to the total area they covered after superposition for 2

143 groups left side/right side or 2 environmental conditions as used for other otolith shape studies (Mahé

144 et al., 2018 ; Mahé et al., 2019 ; Mahé, 2019).

145 Statistical analyses were performed using the following packages in the statistical environment R:

146 'Vegan' (Oksanen et al., 2013), 'sp' (Bivand et al., 2013) and 'rgeos' (Bivand et al., 2013).

3. Results

3.1 Otolith growth

149 There is a positive relationship between otolith and fish larvae sizes. Otolith and larvae sizes

150 increased stepwise from 32 to 670°C.day (Figure 2). Over the course of the experiment, the ratio

151 between the dorso-ventral and antero-posterior axis increased, leading to the loss of the initial

152 circular shape of the otolith. Otolith morphogenesis was positively correlated with GDD. No

153 statistical approach was applied on this ratio between the dorso-ventral and antero-posterior axis or

154 otolith morphogenesis because it was only the difference automatically measured from the

155 reconstructed otolith average shapes which explained the shape evolution (because it was not

156 available (it was not possible to realize the automatic measures of individual length along the same

157 axis due to the shape close to the circle). Herring larvae were long and thin at hatching, then

158 progressively developed dorsal and caudal fins. Body height increased around 436°C.day, and the

159 pelvic fins were the last fins to differentiate at around 662°C.day. Otolith area and larvae size

160 differences were analysed among several values of GDD. Larvae size as otolith area grow during the

161 larvae life (i.e. with GDD value; Figure 3A). Larvae size increased significantly between 32°C.day

162 and 87°C.day, while the first changes in otolith area appeared only at 436°C.day. However, after this

163 GDD level, otolith area (i.e. otolith 2-dimensional measure) increased faster than larval size measure

164 (i.e. fish 1-dimensional measure). Neither larvae size nor otolith area were significantly different

165 between the two environmental conditions (temperature/CO2) at any value of GDD, with the

166 exception of larvae size at 436°C.day ($P<0.05$). The relationship between Oarea and TL was always

167   significant (P<0.05), but the slope was significantly higher in the OWA than in the Amb treatment

168   (slopes difference; P<0.05; Figure 3B). For the same fish length, the area of otolith was bigger for the

169   2100 scenario (14°C/CO2 1660 µatm) than under present day conditions (11°C/CO2 560 µatm)

170   (Figure 3B); this difference increased with fish size. Our experimental study showed that this

171   significant correlation between otolith and fish growth was still observed in present day

172   environmental conditions as well as for the 2100 scenario.

173   **3.2 Otolith shape**

174   No significant difference in shape was observed between left and right otoliths (Supplementary Table

175   2). The average percentage of non-overlapping surface between the two sides never exceeded 1%

176   (Figure 4). Similarly, the results showed no significant environmental effect on otolith shape in

177   herring larvae, with the average percentage of non-overlapping surface ranging from 0.73% to 1.48%

178   (Supplementary Table 2; Figure 4).

179   ## *4. Discussion*

180   The otolith shape is regulated by a complex combination of endogenous and exogenous factors,

181   including both abiotic environmental parameters (such as temperature and CO_2), and biotic

182   parameters (such as food availability). The influence of these factors is dependent on the ontogenetic

183   stage (i.e. the development stage of the individual). In addition, the otoliths can be different between

184   right and left inner ears as a consequence of potential developmental lateralization (e.g. side effect).

185   Environmental factors, especially temperature, have a greater influence than genetic differences for

186   Atlantic cod (Cardinale et al., 2004; Hüssy, 2008; Irgens, 2018). For seabass (*Dicentrarchus labrax*),

187   increased temperature speeds up otolith morphogenesis and modifies the developmental pattern of

188   the otolith shape (Mahé et al., 2019). Ocean acidification can also alter otolith shape (Holmberg et

189   al., 2019). For several species, individuals exposed to high CO_2 had a larger otolith area and

190   maximum length compared with controls; the increases were larger than could be explained by an

191   increase in $CaCO_3$ precipitation in the otoliths driven by the modification of the pH regulation in the

192   endolymph (Checkley et al., 2009; Munday et al., 2011; Réveillac et al., 2015; Coll-Llado et al.,

193   2018). Changes in extracellular concentrations of carbonate and bicarbonate caused by acid-base

194   regulation in a high CO_2 environment could increase the precipitation of $CaCO_3$ in the otolith (Payan

195   et al., 1998). In studies on other species including herring, high CO_2 had no effect on the larval

196   sagittal otolith (Franke and Clemmesen, 2011; Munday et al., 2011; Frommel et al., 2013; Perry et

197   al., 2015). Our experimental study showed that there is the relationship between otolith area and fish

198   length according to the environmental conditions and validated the results observed *in situ* (Folkvord

199   et al., 1997; Johannessen et al., 2000; Berg et al., 2017; Denis et al., 2017; Tonheim et al., 2020). A

200   previous study on herring larvae testing four temperature conditions (4°C, 12°C and two others

201   shifted twice with 4/8/4°C and 12/8/12°C) showed that the size and the width of the otolith daily

202   increment were both temperature-dependent (Folkvord et al., 2004). The otolith area per fish size in

203   our study increased with higher temperature and CO_2 (i.e. lower pH). It should be noted that the

204   feeding protocol chosen for this study provides *ad-libitum* feed, in order to avoid under-feeding

205   situations that could have interfered with the otolith study. Nevertheless, it cannot be excluded that

206   larvae under the 2100 scenario ingest more food than control larvae, which could also accelerate the

207   increase of the otolith area in the 2100 scenario group. While growth mechanisms of otoliths and

208   their morphogenesis during the early life stages of fish are poorly understood, shifts in otolith shape

209   are linked to physiological modifications due to environmental disturbance (Geffen, 1987; Vignon,

210   2018). Early life stages are more vulnerable to environmental challenges because they have higher

211   surface area to volume ratios and have not yet fully developed the homeostatic regulatory

212   mechanisms which are found in adult fish (Hurst et al., 2013). For Downs herring, a shift affecting

213   larval condition has previously been observed *in situ* for individuals at 13 mm, and was driven by

214   environmental conditions (temperature and prey concentration) (Dennis et al., 2017). Our

215   experimental study on Downs herring covered this length range but the otoliths did not show any

216   ontogenetic differences. Several previous studies showed that the increase in otolith area by fish size

217   could mainly result from water acidification (Checkley et al., 2009; Munday et al., 2011; Réveillac et

218   al., 2015; Coll-Llado et al., 2018). Moreover, the elevated seawater CO_2 can cause directional

219   asymmetry (Holmberg et al., 2019). For herring, the environmental factors we studied

220   (temperature/CO_2) did not affect the otolith shape between sides at the observed larval stage.

221   Consequently, it is likely that the level of response to CO_2 increase might be a species-specific

222   phenomenon. This capacity to maintain otolith shape in response to environmental changes is

223   probably due to efficient intracellular ionic-regulation mechanisms in this species (Ishimatsu et al.,

224   2008; Melzner et al., 2009). This result may suggest that herring larvae are well equipped to cope

225   with the environmental changes projected for 2100, as long as their energy and nutritional needs are

226   well covered, which would not necessarily be the case in the natural environment. The faster growth

227   of the larvae under the 2100 scenario may indeed lead to a greater prey requirement, and it cannot be

228   ruled out that the increase in the area of the otoliths may also have affected their hearing ability and

229   behaviour. Nevertheless, it should be noted that this species certainly has a good intracellular ionic

230   regulation because it is confronted with different environments (Ishimatsu et al., 2008; Melzner et al.,

231   2009). Indeed, herring larvae typically hatch in littoral regions where pH and temperature can

232   fluctuate because of freshwater inflow, so they may often experience such fluctuations and therefore

233   be quite resistant to them. For more extreme values, however, the homeostatic mechanisms might not

234   be sufficient to compensate for environmental stress and this could induce changes in otolith

235   morphological development (Coll-Llado et al., 2018).

236   Ontogeny and environment are the factors that control most of the otolith development during the

237   early stages of life. The otolith shape evolves from the basic round form to elongated shape due to

238   faster growth along the anterior-posterior axis than along the dorsal-ventral axis (Galley et al., 2006;

239   Mille et al., 2015; Bounket et al., 2019; Mahé et al., 2019). Our study showed that the otolith shape

240   of herring grew in this way during the larval period. At the adult stage, significant directional

241   asymmetry has been observed in herring (Bird et al., 1986), while no lateralization has been observed

242   at the larval stage. This bilateral effect increases over the life of the fish when considering a different

243   trajectory of otolith morphogenesis between left and right sides. However, the asymmetry between

244   left and right otoliths especially the fluctuating asymmetry may be underestimated in experiments

245   due to the small number of tested individuals which are experiencing stressful conditions and present

246   experimental conditions that do not require significant development of acoustic functions as in the natural

247   environment (Grønkjær and Sand, 2003; Diaz-Gil et al., 2015). Consequently, no lateralization has

248   been observed in the experimental condition for the herring larval stage does not mean that this result

249   will be the same in the natural environment. Although otolith shape is influenced by both abiotic and

250   biotic environmental parameters and depends on individuals' genotype, environmental effects can

251   also be perceived more with ontogeny, consequently, this developmental lateralization could be a

252   phenotypically plastic response to environmental drivers rather than the consequence of the

253   individual genotype, as previously suggested in *Boops boops* (Mahé et al., 2018). Sagittal otolith size

254   and shape for each species could therefore be adaptive traits to different habitats and ecological

255   niches (Lychakov and Rebane, 2000; Lombarte et al., 2010).

256   Conclusions

257   The growth and shape of the otoliths studied here did not appear to be affected by the environmental

258   conditions predicted for 2100. However, this experimental approach should be confirmed *in situ*.

259   Only the relationship between otolith and fish sizes seems to be environment-dependent. This

260   information is important to understand the otolith morphogenesis and consequently when the otolith

261   shape was used as a tool in fisheries science. In the future, to complete this approach, a crossed-effect

262   experiment (four different settings: control, temperature increase, acidification, temperature increase

263   and acidification) could be applied to quantify the effect of each environmental factor. In the same

264   way, it could be important to understand the link between the environment modifications and the

265   food consumption by fish to separate the direct and indirect effects of the climate change on the

266   otolith and fish growth.

267   ## *5. Acknowledgments*

280   ## *4. References*

281   Berg, F., Husebø, A., Aanestad Godiksen, J., Slotte, A., Folkvord. A., 2017. Spawning time of

282   Atlantic herring (Clupea harengus) populations within a restricted area reflects their otolith growth at

283   the larval stage, Fish. Res. 194: 68-75, doi:10.1016/j.fishres.2017.05.009.

284   Bignami, S., Sponaugle, S., Cowen, R., 2013. Response to ocean acidification in larvae of a large

285   tropical marine fish, Rachycentron canadum. Glob. Chang. Biol. 19: 996-1006,

286   doi:10.1111/gcb.12133.

287   Bivand, R.S., Pebesma, E., Gomez-Rubio., V., 2013. Applied spatial data analysis with R, Second

288   edition. New York: Springer.

289   Borcard, D., Gillet, F., Legendre., P., 2011. Numerical ecology with R. Springer, New York

290   Bounket, B., Gibert, P., Gennotte, V., Argillier, C., Carrel, G., Maire, A., Logez, M., 2019. Otolith

291   shape analysis and daily increment validation during ontogeny of larval and juvenile European

292   chub Squalius cephalus. J. Fish. Biol. 95: 444-452, doi:10.1111/jfb.13976.

293   Burke, N., Brophy, D., King, P.A., 2008. Otolith shape analysis: its application for discriminating

294   between stocks of Irish Sea and Celtic Sea herring (Clupea harengus) in the Irish Sea. ICES J. Mar.

295   Sci. 65: 1670-1675, doi:10.1093/icesjms/fsn177.

296   Carbonara, P., Ciccolella, A., De Franco, F., Palmisano, M., Bellodi, A., Lembo, G., Neglia, C.,

297   Spedicato, M.T., Zupa, W., Guidetti, P., 2022. Does fish growth respond to fishing restrictions within

298   Marine Protected Areas? A case study of the striped red mullet in the south-west Adriatic Sea

299   (central Mediterranean). Aquatic Conservation: Marine and Freshwater Ecosystems, 32(3): 417-429,

300   doi:10.1002/aqc.3776.

301   Cardinale, M., Doering-Arjes, P., Kastowsky, M., Mosegaard., H., 2004. Effects of sex, stock, and

302   environment on the shape of known-age Atlantic cod (Gadus morhua) otoliths. Can. J. Fish. Aquat.

303   Sci. 61: 158-167, doi:10.1139/f03-151.

304   Casselman, J.M., 1987. Determination of age and growth, p. 209-242. In A.H. Weatherley and H.S.

305   Gill [eds.], The Biology of Fish Growth. Academic Press.

306   Checkley, D.M., Dickson, A.G., Takahashi, M., Radich, J.A., Eisenkolb, N.R., 2009. Elevated CO_2

307   Enhances Otolith Growth in Young Fish. Science 324: 1683, doi:10.1126/science.1169806.

308   Chezik, K.A., Lester, N.P., Venturelli, P.A., 2014. Fish growth and degree-days I: Selecting a base

309   temperature for a within-population study. Can. J. Fish. Aquat. Sci. 71(1): 47-55, doi:10.1139/cjfas-

310   2013-0295.

311   Coll-Lladó, C., Mittermayer, F., Webb, P.B., Allison, N., Clemmesen, C., Stiasny M., Bridges, C.R.,

312   Göttler, G., Garcia de la serrana, D., 2021. Pilot study to investigate the effect of long-term exposure

313   to high pCO2 on adult cod (Gadus morhua) otolith morphology and calcium carbonate deposition.

314   Fish Physiol. Biochem. 47: 1879-1891, doi:10.1007/s10695-021-01016-6.

315   Coll-Lladó, C., Giebichenstein, J., Webb, P.B., Bridges, C.R., Garcia de la Serrana, D., 2018. Ocean

316   acidification promotes otolith growth and calcite deposition in gilthead sea bream (Sparus aurata)

317   larvae. Sci Rep 8: 8384, doi:10.1038/s41598-018-26026-y.

318   Díaz-Gil, C., Palmer, M., Catalán, I.A., Alós, J., Fuiman, L.A., García, E., del Mar Gil, M., Grau, A.,

319   Kang, A., Maneja, R.H., Mohan, J.A., Morro, B., Schaffler, J.J., Buttay, L., Riera-Batle, I., Tolosa,

320   B., Morales-Nin, B., 2015. Otolith fluctuating asymmetry: a misconception of its biological

321   relevance?, ICES Journal of Marine Science 72(7): 2079–2089, doi:10.1093/icesjms/fsv067.

322   Degens, E.T., Deuser, W.G., Haedrich, R.L., 1969. Molecular structure and composition of fish

323   otoliths. Mar. Biol. 2: 105-113, doi:10.1007/BF00347005.

324   Denis, J., Mahé, K., Tavernier, E., Monchy, S., Vincent, D., Vallet, C., Marchal, P., Antajan, E.,

325   Caboche, J., Lefebvre, V., Cordier, R., Loots, C., 2017. Ontogenetic changes in the larval condition

326   of Downs herring: use of a multi-index approach at an individual scale. Mar Biol 164: 154,

327   doi:10.1007/s00227-017-3180-3.

328 Enberg, K., Jorgensen, C., Dunlop, E.S., Varpe, O., Boukal, D.S., Baulier, L., Eliassen, S., Heino,

329 M., 2011. Fishing-induced evolution of growth: concepts, mechanismsand the empirical evidence.

330 Mar. Ecol., 33:1-25, doi: 10.1111/j.1439-0485.2011.00460.x.

331 Folkvord, A., Johannessen, A., Moksness, E., 2004. Temperature-dependent otolith growth in

332 Norwegian spring-spawning herring (Clupea harengus L.) larvae. Sarsia 89: 297-310,

333 doi:10.1080/00364820410002532.

334 Folkvord, A., Rukan, K., Johannessen, A., Moksness, E., 1997. Early life history of herring larvae in

335 contrasting feeding environments determined by otolith microstructure analysis. J. Fish. Biol. 51:

336 250-263, doi:0022-1 112/97/51A250+ 14 $25.0010.

337 Franke, A., Clemmesen, C., 2011. Effect of ocean acidification on early life stages of Atlantic herring

338 (Clupea harengus L.), Biogeosciences, 8(12): 3697-3707, doi:10.5194/bg-8-3697-2011.

339 Frommel, A.Y., Schubert, A., Piatkowski, U., Clemmesen, C., 2013. Egg and early larval stages of

340 Baltic cod, Gadus morhua, are robust to high levels of ocean acidification. Mar. Biol. 160: 1825-

341 1834, doi:10.1007/s00227-011-1876-3.

342 Galley, E.A., Wright, P.J., Gibb, F.M., 2006. Combined methods of otolith shape analysis improve

343 identification of spawning areas of Atlantic cod. ICES J. Mar. Sci. 63 : 1710-1717,

344 doi:10.1016/j.icesjms.2006.06.014.

345 Geffen, A.J., 1987. Methods of validating daily increment deposition in otoliths of larval fish, in Age

346 and growth of fish, Summerfelt RC, Hall GE editors. Iowa State University Press, 223–240.

347 Grønkjær P., Sand, M.K., 2003. Fluctuating asymmetry and nutritional condition of Baltic cod

348 (Gadus morhua) larvae. Mar. Biol. 143: 191-197. doi: 10.1007/s00227-003-1064-1.Holmberg, R.J.,

349 Wilcox-Freeburg, E., Rhyne, A.L., Tlusty, M.F., Stebbins, A., Nye, Jr.S.W., Honig, A., Johnston,

350 A.E., San Antonio, C.M., Bourque, B., Hannigan, R.E., 2019. Ocean acidification alters morphology

351 of all otolith types in Clark's anemonefish (Amphiprion larkii). PeerJ 7:e6152, doi:

352 10.7717/peerj.6152.

353 Hönisch, B., Ridgwell, A.J., Schmidt, D.N., Thomas, E., Gibbs, S.J., Sluijs A., Zeebe, R., Kump, L.,

354 Martindale, R.C., Greene, S.E., Kiessling, W., Ries, J., Zachos, J.C., Royer, D.L., Barker, S.,

355 Marchitto Jr., T.M., Moyer, R., Pelejero, C., Ziveri, P., Foster, G.L., Williams, B., 2012. The

356 geological record of ocean acidification. Science 335: 1058-1063, doi:10.1126/science.1208277.

357 Hufnagl, M., Peck, M.A., 2011. Physiological individual-based modelling of larval Atlantic herring

358 (Clupea harengus) foraging and growth: insights on climate-driven life-history scheduling, ICES J.

359 Mar. Sci. 68: 1170-1188, doi:10.1093/icesjms/fsr078.

360 Hurst, T., Fernandez, E., Mathis, J., 2013. Effects of ocean acidification on hatch size and larval

361 growth of walleye pollock (Theragra chalcogramma), ICES J. Mar. Sci. 70: 812-822,

362 doi:10.1093/icesjms/fst053.

363 Hüssy, K., 2008. Otolith shape in juvenile cod (Gadus morhua): Ontogenetic and environmental

364 effects. J. Exp. Mar. Bio. Ecol. 364: 35-41, doi:10.1016/j.jembe.2008.06.026.

365 Hüssy, K., Limburg, K.E., de Pontual, H., Thomas, O.L.R., Cook, P.K., Heimbrand Y., Blass, M.,

366 Sturrock, A.M., 2020. Trace Element Patterns in Otoliths: The Role of Biomineralization, Rev. Fish.

367 Sci. Aquac. 29(4): 445-477, doi:10.1080/23308249.2020.1760204.

368 IPCC, 2014. Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to

369 the fifth assessment report of the Intergovernmental Panel on Climate Change. IPCC 2014,

370 http://hdl.handle.net/10013/epic.45156

371   Irgens, C., 2018. Otolith structure as indicator of key life history events in Atlantic cod (Gadus

372   morhua). Ph.D. thesis. Univ. of Bergen.

373   Ishimatsu, A., Hayashi, M., Kikkawa, T., 2008. Fishes in high-CO_2, acidified oceans, Mar. Ecol.

374   Prog. Ser. 373: 295-302, doi:10.3354/meps07823.

375   Johannessen, A., Blom, G., Folkvord, A., 2000. Differences in growth pattern between spring and

376   autumn spawned herring (Clupea harengus L.) larvae. Sarsia 85: 461-466,

377   http://dx.doi.org/10.1080/00364827.2000.10414595.

378   Joly, L.J., Loots, C., Meunier, C.L., Boersma, M., Collet, S., Lefebvre, V., Zambonino-Infante, J.L.,

379   Giraldo, C., 2021. Maturation of the digestive system of Downs herring larvae (Clupea harengus,

380   Linnaeus, 1758): identification of critical periods through ontogeny. Mar. Biol. 168: 82,

381   doi:10.1007/s00227-021-03894-z.

382   Kuhl, F., Giardina, C., 1982. Elliptic Fourier features of a closed contour. Comput. Graph. Image

383   Proc. 18: 236-258, doi:10.1016/0146-664X(82)90.

384   Legendre, P., Legendre, L.F.J., 1998. Numerical Ecology. Elsevier Science.

385   Lestrel, P.E., 2008. Fourier Descriptors and their Applications in Biology. Cambridge University

386   Press.

387   Libungan, L.A., Óskarsson, G.J., Slotte, A., Jacobsen, J.A., Pálsson, S., 2015. Otolith shape: a

388   population marker for Atlantic herring Clupea harengus. J. Fish Biol. 86: 1377-

389   1395, doi:10.1111/jfb.12647.

390 Lombarte, A., Palmer, M., Matallanas, J., Gómez-Zurita, J., Morales-Nin, B., 2010.

391 Ecomorphological trends and phylogenetic inertia of otolith sagittae in Nototheniidae. Environ. Biol.

392 Fishes 89: 607-618, doi:10.1007/s10641-010-9673-2.

393 Lychakov, D.V., Rebane, Y.T., 2000. Otolith regularities. Hear. Res. 143: 83-102,

394 doi:10.1016/s0378-5955(00)00026-5.

395 Mahé, K., 2019. Sources de variation de la forme des otolithes : implications pour la discrimination

396 des stocks de poissons. Ph.D. thesis. Univ. of Littoral Côte d'Opale

397 Mahé, K., Gourtay, C., Bled Defruit, G., Chantre, C., de Pontual, H., Amara, R., Claireaux, G.,

398 Audet, C., Zambonino-Infante, J.L., Ernande, B., 2019. Do environmental conditions (temperature

399 and food composition) affect otolith shape during fish early-juvenile phase? An experimental

400 approach applied to European Seabass (Dicentrarchus labrax). J. Exp. Mar. Bio. Ecol. 521: 151239,

401 doi:10.1016/j.jembe.2019.151239.

402 Mahé, K., Ider, D., Massaro, A., Hamed, O., Jurado-Ruzafa, A., Gonçalves, P., Anastasopoulou, A.,

403 Jadaud, A., Mytilineou, C., Elleboode, R., Ramdane, Z., Bacha, M., Amara, R., de Pontual, H.,

404 Ernande, B., 2018. Directional bilateral asymmetry in otolith morphology may affect fish stock

405 discrimination based on otolith shape analysis. ICES J. Mar. Sci. 76(1): 232-243,

406 doi:10.1093/icesjms/fsy163.Maneja, R.H., Frommel, A.Y., Geffen, A.J., Folkvord, A., Piatkowski,

407 U., Chang M.Y., Clemmesen, C., 2013. Effects of ocean acidification on the calcification of otoliths

408 of larval Atlantic cod Gadus morhua. Mar. Ecol. Prog. Ser. 477: 251-258, doi: 10.3354/meps10146.

409 Marty, L., Rochet, M.-J., Ernande, B., 2014. Temporal trends in age and size at maturation of four

410 North Sea gadid species: cod, haddock, whiting and Norway pout. Mar. Ecol. Prog. Ser. 497: 179-

411 197. doi: 10.3354/meps10580

412 McMaster, G.S., Wilhelm, W., 1997. Growing degree-days: one equation, two interpretations. Agric.

413 For. Meteorol. 87; 291-300. doi:10.1016/S0168-1923(97)00027-0.

414 Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich,

415 M., Pörtner, H.O., 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals:

416 pre-adaptation through lifestyle and ontogeny?, Biogeosciences 6: 2313-2331.

417 http://www.biogeosciences.net/6/2313/2009/.

418 Mille, T., Mahé, K., Villanueva, C.M., de Pontual, H., Ernande, B., 2015. Sagittal otolith

419 morphogenesis asymmetry in marine fishes. J. Fish Biol. 87: 646-663, doi:10.1111/jfb.12746.

420 Munday, P.L., Gagliano, M., Donelson, J.M., Dixson, D.L., Thorrold, S.R., 2011. Ocean acidification

421 does not affect the early life history development of a tropical marine fish. Mar. Ecol. Prog. Ser. 423:

422 211-221, doi: 10.3354/meps08990.

423 Payan, P., Borelli, G., Boeuf, G., Mayer-Gostan, N., 1998. Relationship between otolith and somatic

424 growth: consequence of starvation on acid-base balance in plasma and endolymph in the rainbow

425 trout Oncorhynchus mykiss. Fish Phys. Bioch. 19: 35-41, doi:10.1023/A:1016064813517.

426 Perry, D.M., Redman, D.H., Widman Jr, J.C., Meseck, S., King, A., Pereira, J.J., 2021. Effect of

427 ocean acidification on growth and otolith condition of juvenile scup, Stenotomus chrysops. Ecol.

428 Evol. 5(18): 4187-4196, doi:10.1002/ece3.1678.

429 Popper, A.N., Ramcharitar, J., Campana, S.E., 2005. Why otoliths? Insights from inner ear

430 physiology and fisheries biology. Mar. Freshw. Res. 56 : 497-504,

431 https://dx.doi.org/10.1071/mf04267

432   Réveillac, E., Lacoue-Labarthe, T., Oberhänsli, F., Teyssié, J.L., Jeffree, R., Gattuso, J.P., Martin, S.,

433   2015. Ocean acidification reshapes the otolith-body allometry of growth in juvenile sea bream. J.

434   Exp. Mar. Bio. Ecol. 463: 87-94, doi:10.1016/j.jembe.2014.11.007.

435   Rohlf, F.J., Archie, J.W., 1984. A Comparison of Fourier Methods for the Description of Wing Shape

436   in Mosquitoes (Diptera: Culicidae). Syst. Biol. 33: 302-317, doi:10.2307/2413076.

437   Tonheim, S., Slotte, A., Andersson, L., Folkvord A., Berg, F., 2020. Comparison of Otolith

438   Microstructure of Herring Larvae and Sibling Adults Reared Under Identical Early Life

439   Conditions. Front. Mar. Sci. 7: 529, doi:10.3389/fmars.2020.00529.

440   Turan, C., 2000. Otolith shape and meristic analysis of herring (Clupea harengus) in the North-East

441   Atlantic. Arch. Fish. Mar. Res. 48: 283-295.

442   Vignon M., 2018. Short-term stress for long-lasting otolith morphology – Brief embryological stress

443   disturbance can reorient otolith ontogenetic trajectory. Can. J. Fish. Aquat. Sci. 75(10): 1713-1722,

444   doi:10.1139/cjfas-2017-0110.

445   Vignon, M., Morat, F., 2010. Environmental and genetic determinant of otolith shape revealed by a

446   non-indigenous tropical fish. Mar. Ecol. Prog. Ser. 411: 231-241, doi:10.3354/meps08651.

447

448

449

450

451

452   Figure 1: Main steps of the experimental design for herring with five samplings by the level of GDD

453   (Growing Degree Days) and by the number of dph (Days Post Hatching; grey value for 11°C; black

454   value for 14°C). Each condition of temperature and CO_2 concentration had three replicates. To test

455   the effect of global change, present day conditions (11°C and pH 8.0, pCO_2 560 μatm) versus the

456   ocean warming and acidification conditions predicted for 2100 (14°C and pH 7.6, pCO_2 1660 μatm)

457   were used.

458

459

460
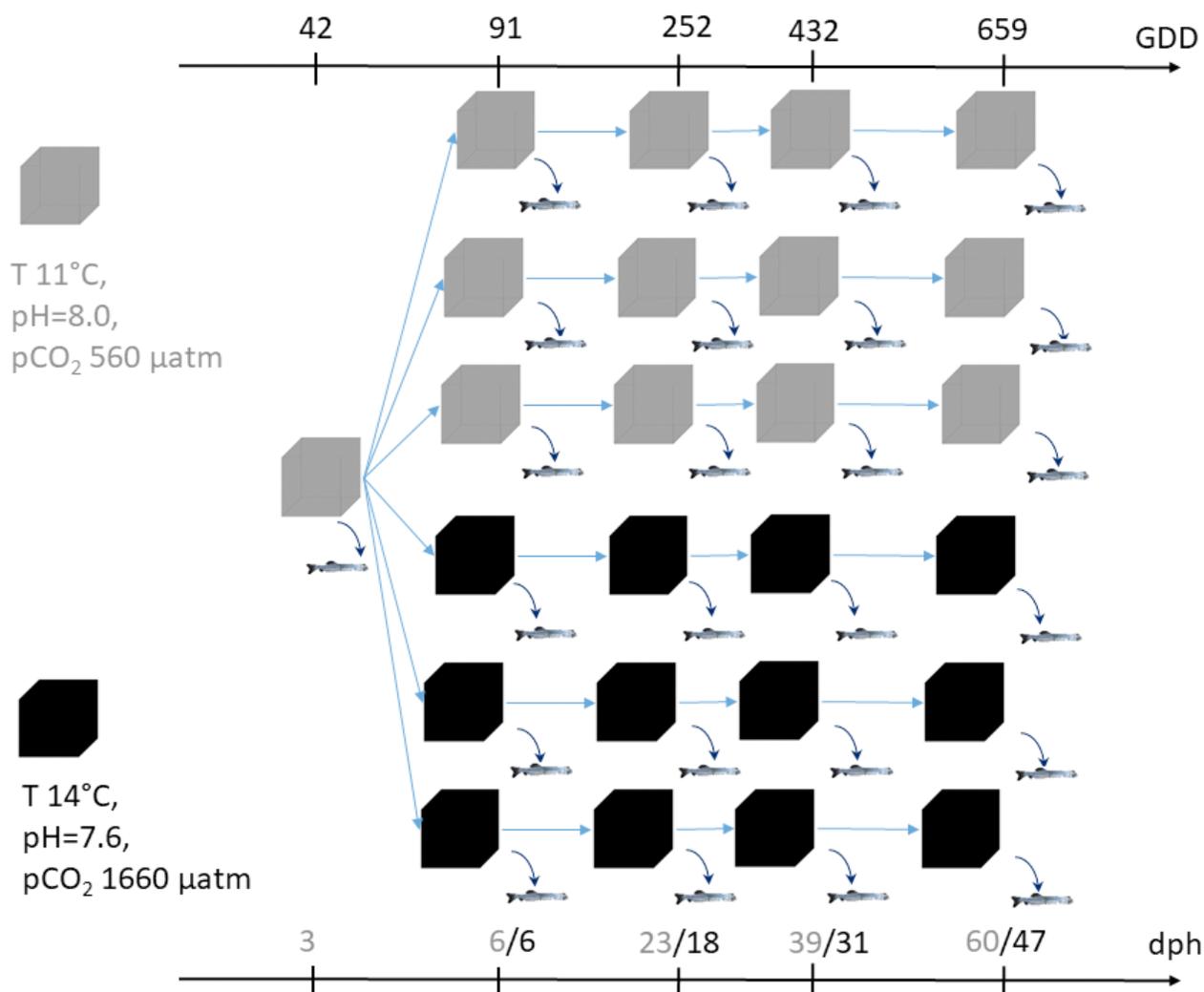
461   Figure 2: Growth of otolith shape and larval fish by GDD value (°C.day).
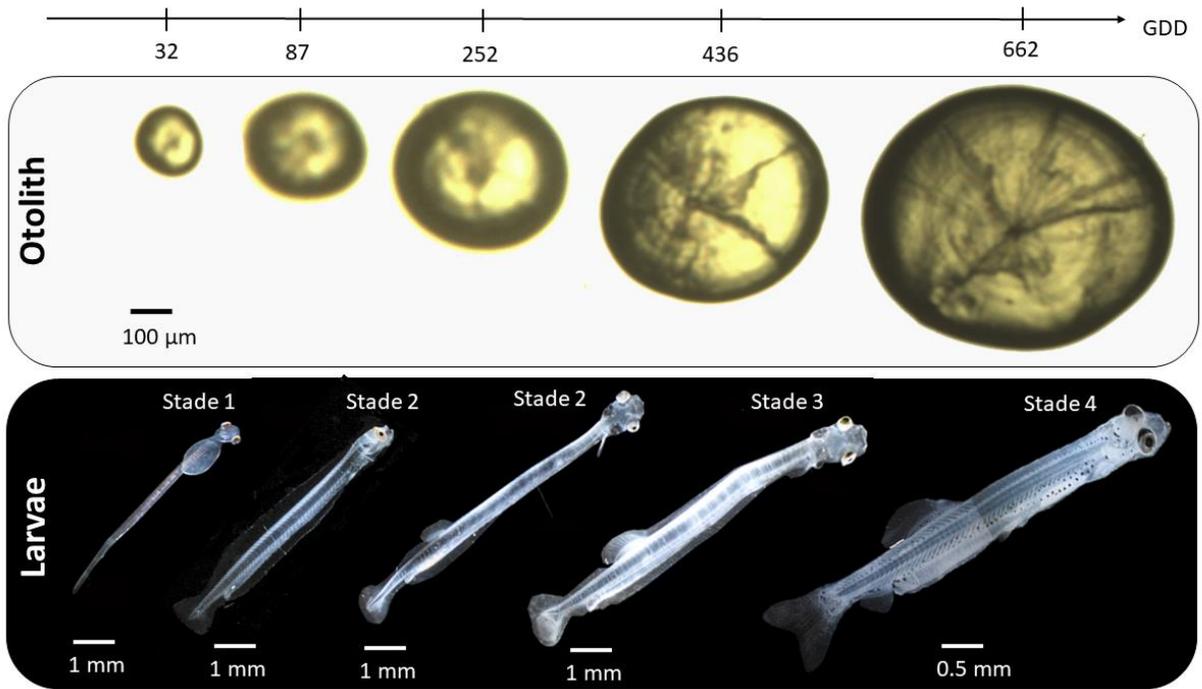
472   Figure 3: (A) Box-plot of otolith area (Oarea, µm²) and total length of fish (TL, cm) values by GDD

473   value (°C.day) and environmental conditions (red for 14°C and 1660 µatm, and blue for 11°C and

474   560 µatm). A different letter within each sampling time denotes a significant difference between the

475   two groups. (B) Relationship between otolith average area (Oarea) and larval body length (TL) in

476   response to the environmental conditions (red for 14°C and 1700 µatm, and blue for 11°C and 560

477   µatm) (smoothing method using level of confidence interval of 0.95).
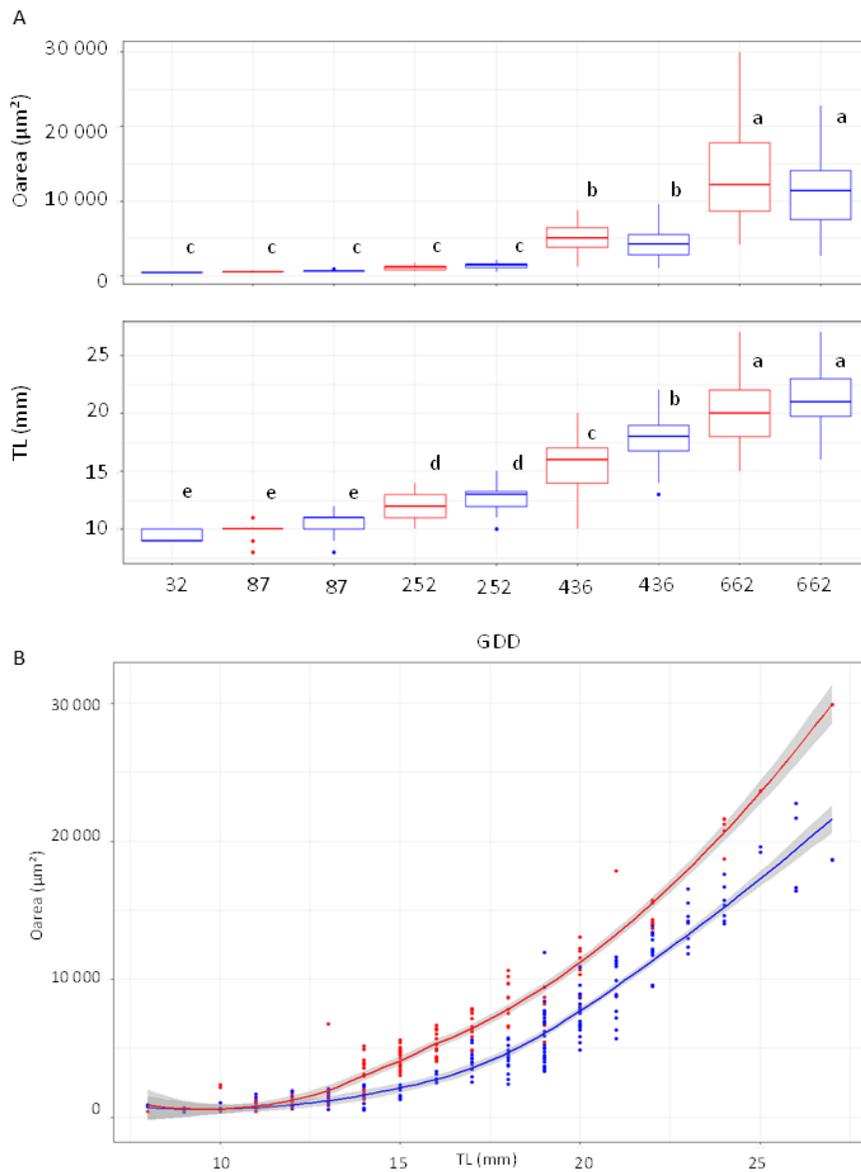
478

479   Figure 4: Percentage of non-overlapping surface between left and right otolith shape and for two

480   environmental conditions by GDD value (°C.day) (arrows identify the main areas of difference

481   between both otoliths).
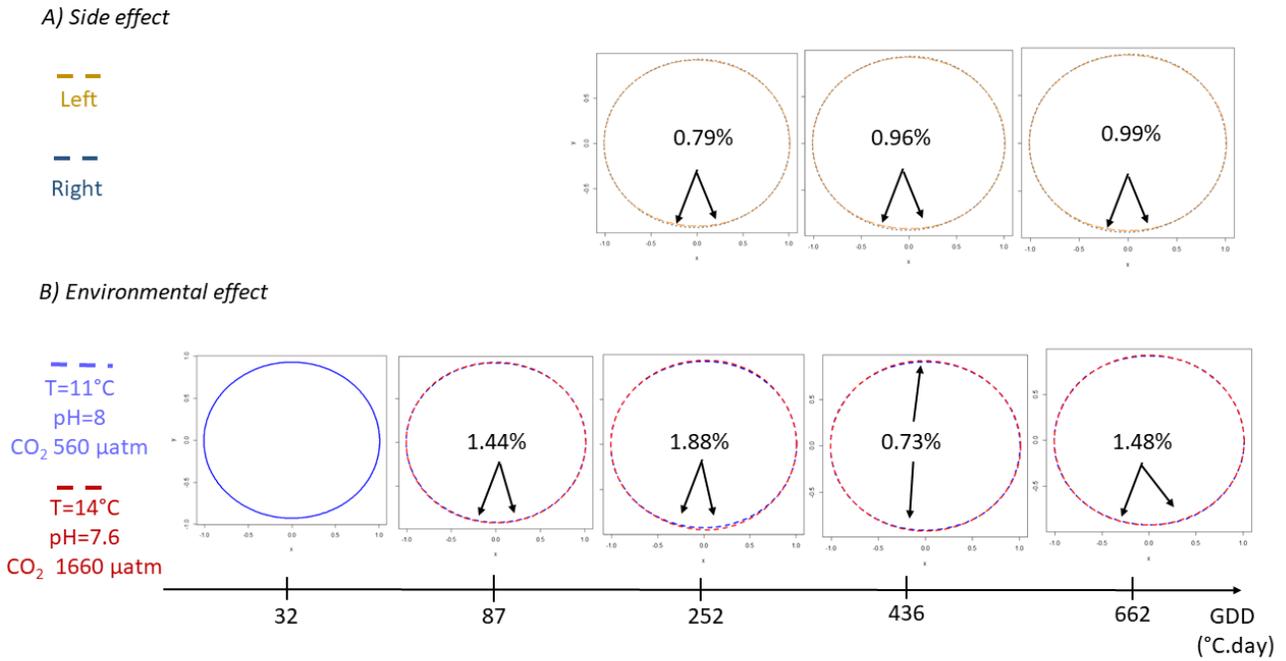
482

483

484

485

486

487

488

489

Effect of climate change on the morphogenesis of sagittal otoliths in Atlantic herring (*Clupea harengus*) larvae

Supplementary Material

Kélig Mahé[1*], Léa Justine Joly[1,2], Solène Telliez[1], José Luis Zambonino-Infante[2], Cédric Léo Meunier[3], Kirsteen M. MacKenzie[1], Carolina Giraldo[1]

[1]Ifremer, Fisheries laboratory, Channel and North Sea Fisheries Research Unit, Boulogne-sur-mer, France

[2] Ifremer, Univ Brest, CNRS, IRD, LEMAR, F-29280 Plouzané, France

[3]Helmholtz-Zentrum für Polar- und Meeresforschung, Alfred-Wegener-Institut, Helgoland, Germany

Supplementary Table S1. Water parameters (Mean ± sd) during the larval rearing of herring larvae from 32 to ~670°C.day

Condition	T (°C)	pH	S (psu)	TA (µmol/kgSW)	pCO$_2$ (µatm)
Amb (11°C, 650 µatm) n= 6	11.3 ± 0.4	8.03 ± 0.04	32.7 ± 0.4	2275.2 ± 139.3	559.1 ± 55.9
OWA (14°C, 1660 µatm) n = 6	14.2 ± 0.5	7.61 ± 0.12	32.7 ± 0.4	2280.3 ± 150.4	1661.6 ± 291.3

Supplementary Table S2: Summary of redundancy analyses of herring otolith shapes.

Factor	df	P-value
Side	1	0.756
T°C/pCO$_2$	1	0.097
GDD	8	0.118