Otolith shape variations between artificially stocked and autochthonous pikeperch (*Sander lucioperca*)

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Abstract

Fish stocking is one of the most widespread and frequent management strategies in freshwater systems. However, the contribution of stocked fish to the population is seldom investigated, and hence the effectiveness of this strategy is virtually unknown for many populations. Understanding the contribution of stocked fish into the population is crucial because it allows the disentanglement of the confounding effects generated by allochthonous individuals into the estimation of survival and growth rates of the population. To discriminate between the allochthonous and autochthonous individuals in a population, the shape of sagittae otoliths from pikeperch (*Sander lucioperca*) where compared. Results indicated significant differences among stocked and non-stocked fish, with the former having smaller and wider sagittae otoliths than the later. Our results suggest that this technique can be used to discriminate the natal origin of fish in a much faster and cheaper way than commonly used techniques.

Key-words: fisheries, fish, freshwater, morphometry, stock discrimination

1. Introduction

Fish are extremely important components of aquatic environments. They provide essential ecosystem services to humans, including demand-derived ecosystem services such as the food resources and recreational activities, as well as fundamental ecosystem services such as resilience and nutrient cycling that ultimately affect water quality which is critical for humans (Holmlund and Hammer, 1999). With increasing threats to fish communities like habitat degradation, stock exploitation, pollution and climate change (Arthington et al., 2016; Gordon et al., 2018), many fish populations require operational management to mitigate the detrimental effects of human activities (Agostinho et al., 2008; Hansson et al., 1997; Welcomme and Bartley, 1998). Fish stocking (i.e. introduction of allochthonous individuals into the ecosystem) is one of the most widespread, frequent and cost-efficient management strategy adopted by stakeholders in freshwater systems (Hunt et al., 2017), which usually spend relatively large part of their budget in this activity (Welcomme and Bartley, 1998).

Given that the rearing conditions are fundamentally different from the wild conditions, and the homogeneity of behavioral and other biological traits of artificially bred fish, it is expected that the survival rates of stocked fish in the wild may differ considerably from the autochthonous individuals (Adriaenssens and Johnsson, 2013). Consequently, the managed populations receive an influx of individuals displaying unique set of characteristics that likely differ from the autochthonous fish, but their performance in the wild is not known, nor investigated, and hence the effectiveness of this strategy is virtually unknown in many cases. This knowledge gap is not easily disentangled because the discrimination between stocked and wild fish is not trivial. Different techniques can be used to mark stocked fish, including fin clipping, otolith staining and micro wire, VIE (Visual Implant Elastomer) and PIT (Passive Integrated Transponder) tags, but the results are not always satisfactory due to decreased capacity of mark detection, tag losses, differences detection and retention between sexes, influences on social behavioral and decreased survival rate of marked fish, biasing the stock

49 assessment (Buckley and Blankenship, 1990; Halačka et al., 2019; Jungwirth et al., 2019; Skalski et

50 al., 2009; Šmejkal et al., 2019; Svåsand et al., 1990; Taylor et al., 2005; Utter, 1998).

51 Fish otolith structures are considered natural tags (Bouchoucha et al., 2018; Campana, 1999;

52 Campana et al., 2000; Hamer et al., 2003; L'Abée-Lund and Jensen, 1993) and are excellent structures

53 to retrieve data on the chronologies of life history of fish. Otoliths grow continuously throughout

54 accretions of calcium carbonate ($CaCO_3$) aragonitic crystals on protein layers (Campana and Neilson,

55 1985), with the growth being acellular and metabolically inert, meaning that these natural tags are

56 neither reworked nor reabsorbed, even during times of starvation (Campana and Neilson, 1985).

57 Therefore the life history trajectory is preserved into the structure allowing researchers to trace back

58 important events on fish life. Otolith shapes are species-specific conservative and have been

59 successfully used in fisheries to discriminate fish stocks (Campana and Casselman, 1993; DeVries et

60 al., 2002; Paul et al., 2013; Schulz-Mirbach et al., 2008). The otolith morphometry is influenced by

61 both genetics and the environment where the fish lives (Vignon and Morat, 2010), with individuals

62 from the same population but living in different localities having different otolith shapes (Cerna et

63 al., 2019; Costa et al., 2018; Pérez and Fabré, 2013). Therefore, otolith shape is a powerful tool to

64 identify spatial distribution and natal origin of fish, because it integrates multiple variables in one

65 conservative structure.

66 Pikeperch (*Sander lucioperca*) is a very popular sportfishing target in European inland waters, but

67 the management of its population is often inadequate, leading to overexploitation which causes

68 significant ecological and economic impacts (Saulamo and Thoresson, 2005; Specziár and Erős,

69 2015). Pikeperch reproduction and overwinter survival of the newborns are highly sensitive and

70 dependent on various environmental conditions (Blabolil et al., 2016; Kokkonen et al., 2019;

71 Lappalainen et al., 2009). Therefore, stocking became a recurrent measure to mitigate the impacts of

72 poor management practices and natural recruitment failure (Specziár and Turcsányi, 2017).

73 Proper description of the contribution of stocked fish into wild populations is of paramount

74 importance because it would allow researchers to have a deeper understanding of the population

75 dynamics, stocking dependence, and provide adjustments on growth and survival rates of fish cohorts

76 taking into consideration their natal origin. Despite being widely used to discriminate wild stocks

77 (Campana and Casselman, 1993), otolith shapes are seldom used to differentiate autochthonous and

78 allochthonous individuals. In this context, the present study aimed at applying shape analysis to

79 discriminate between wild and stocked individuals of an ecologically and economically relevant

80 species (*S. lucioperca*) using an accurate and low-cost analytical tool.

81

82 **2. Material and Methods**

83 Fish sampling were conducted separately for wild and stocked *S. lucioperca*. Wild individuals came

84 from the Lipno reservoir (Southwestern, Czechia), while stocked fish came from the rearing facilities

85 c.a. 150 km from the lake.

86 Young pikeperch were hatched at Pohořelice Fishery facilities and when they reach 4 cm (total length)

87 they were transported to the shallow rearing pond (Velké Rozběhlo – area = 13.2 ha) (Fig. 1). Fish

88 were kept under semi-natural conditions, being exposed to competition (intra and interspecific) and

89 predators (intracohort cannibalism and terrestrial species – birds and small mammals) and have access

90 to natural food represented mostly by plankton and later by young of the year stone moroco

91 (*Pseudorasbora parva*). In the shallow rearing pond the pikeperch had the company of adult common

92 carps (*Cyprinus carpio*) that were kept under relatively high densities (400 kg/ha).

93 Wild fish were sampled by European standard multimesh gillnets (CEN - Comité Européen de

94 Normalisation, 2005) in last week of August 2018 in Lipno reservoir, whereas stocked fish were

95 sampled during the fish stocking campaign in just before releasing to Lipno in mid October of the

96 same year. Stocked fish were randomly sampled using a scoop net and immediately euthanized by

97 thermal shock. The standard length (SL) of all fish were measured to the nearest 5mm using a fish

98   ruler. The sex of all individuals sampled in the current study was not determined because they were

99   all sexually immature. In the rearing facilities there was no separation between male and females at

100 the juvenile stage.

101 Only juvenile fish ranging from 190 to 245 mm of length (SL) were included into the analysis because

102 we could only ascertain that wild fish was not stocked until this size (currently, managers do not stock

103 fish smaller than this size in Lipno reservoir). The growth of wild individuals of the same cohort can

104 vary significantly (van Densen and Vijverberg, 1982) and for that reason, we selected for the analysis

105 only the wild fish with similar sizes of the stocked individuals (i.e. faster growers) to avoid bias due

106 to significant differential growth rates between stocked and wild fish. Additionally, using only

107 juvenile fish avoided bias due to sexual dimorphism on otolith shape (Vaux et al., 2019).

108 In laboratory the sagittae otoliths of *S. lucioperca* individuals were extracted from the cranial cavity

109 using a scalpel and tweezers. Otoliths were then mechanically cleaned and air-dried before being

110 photographed (15x magnification) under transmitted light using a camera (Optikam B3) mounted on

111 a stereomicroscope (STM 800). Otoliths were positioned over a flat surface with the sulcus facing

112 downwards (i.e. outer face up).

113 Contours of individual sagittae otoliths were digitally extracted from the photographs using the R

114 software (R Core Team, 2020) following the method described by Claude (2008). The standardization

115 of the otoliths positions was performed digitally by aligning them to a common center, orienting the

116 otoliths to remove discrepancies in positioning and scaling to centroid size using the functions

117 *coo_center*, *coo_rotate* and *coo_slidedirection* functions from the *Momocs* package (Bonhomme et

118 al., 2014). Due to otoliths shape irregularities, the outlines were smoothed to reduce the noise of

119 digitization using the function *coo_smooth* from the *Momocs* package (Bonhomme et al., 2014),

120 Otolith length (O_L), width (O_W), area (O_A) and perimeter (O_P) (Fig. 2) were computed using the

121 package *rgeos* (Bivand et al., 2019). Eight different shape indices (Burke et al., 2008; Leguá et al.,

122 2013; Rashidabadi et al., 2020; Škeljo and Ferri, 2012) were computed for each individual otolith

123 (Table 1). The aspect ratio (SI_{AR}) between the otolith length and width expresses the otolith

124 elongation. The circularity index (SI_{CI}), i.e., the ratio between the squared perimeter and the otolith

125 area indicates the circularity of the structure. The ellipticity index (SI_{EL}), i.e., the ratio between the

126 difference in otolith length and width and their sum expresses the similarity of the otolith shape to an

127 ellipse. The format factor (SI_{FF}) is the inverse ratio of the squared perimeter of the structure to the

128 squared perimeter of a circle of the same surface. The rectangularity index (SI_{RE}) is the ratio between

129 the area and the product of the length and width of the otolith. The roundness index (SI_{RO}) is the ratio

130 between the actual area and the area of a circle of the same circumference. The convexity (SI_{CO}) is

131 the ratio between the perimeter of the convex hull fitted into the otolith outline and the perimeter of

132 the otolith expressing the otolith edge roughness. The solidity (SI_{SO}) is the ratio between the otolith

133 area and the area of the convex hull fitted on the otolith outline expressing the concavity of the otolith.

134 SI_{CO} and SI_{SO} were computed using the functions *coo_convexity* and *coo_solidity* from the *Momocs*

135 package respectively (Bonhomme et al., 2014). The use of different shape indices in the same otolith

136 provides numerical evidence to describe spatial format of the structure (Tuset et al., 2003), and hence

137 is essential to a robust analysis on the 2D format of the calcified structures.

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139

140 *2.1 Statistical analyses*

141 The mean values, standard error and confidence intervals of the otolith descriptors and shape indices

142 were obtained after data bootstrapping with 1000 bootstrap replicates of indices using the functions

143 *boot* and *boot.ci* from the package *boot* (Canty, 2020). Bootstrapping assigns measures of accuracy

144 to the sample estimates by randomly sampling and replacing data, and hence providing a more precise

145 estimate of the values.

146 The correlations between each of the eight shape indices were tested for correlation (Pearson's

147 correlation) using the *findCorrelation* function in the *caret* package (Kuhn et al., 2020). The non-

148   correlated indices were then selected for the training of a resilient backpropagation with back tracing

149   neural network using the *neuralnet* function from the *neuralnet* package (Fritsch et al., 2019). The

150   neural network is a type of supervised machine learning tool that is used to predict patterns among

151   multiple variables and that does not need to follow the parametric premises of traditional statistical

152   tests (Beck, 2018). This method characterizes the relationships among variables using an arbitrary

153   number of parameters (hidden layers) that are chosen through interactive training with the algorithm,

154   and hence, this tool can be classified as a hyper-parameterized non-linear model that can fit a smooth

155   function to any dataset with minimal residual error (Hornik, 1991). The number of hidden layers were

156   selected based on the number of input variables in the model (2/3 of the size of input layers plus the

157   size of output layers). Machine learning tools outperform traditional statistical classifiers in otolith

158   shape analysis and can improve the accuracy of fish stock discrimination studies (Smoliński et al.,

159   2019). The relative importance of each variable was assessed by the Olden's algorithm (Olden and

160   Jackson, 2002) using the *olden* function of the *NeuralNetTools* package (Beck, 2018). The relative

161   importance of input variables in neural networks is expressed by the sum of the product of the raw

162   input-hidden, hidden-output connection weights, with the values being only interpreted based on the

163   relative sign and magnitude between the explanatory variables (Olden and Jackson, 2002). The

164   dataset was randomly divided in two (training (60%) and testing (40%)) and the accuracy of the model

165   was assessed by comparing the observed values with the ones obtained by the model prediction using.

166   The recall (i.e. sensitivity) and precision of the model were evaluated using the *recall* and *precision*

167   functions from the *caret* package (Kuhn et al., 2020). The neural network analysis were performed in

168   R version 3.5.1 (R Core Team, 2020).

169   Additionally, the 2D shapes of the pikeperch sagittae otoliths were analyzed using the Elliptical

170   Fourier Analysis (*efourier* function) without the normalization of the coefficients. The number of

171   harmonics used was defined by applying the *calibrate_harmonicpower_efourier* function. The

172   Elliptical Fourier Analysis is commonly used to describe the fish otolith shapes with a high degree of

173 precision and success in capturing the shape of the otoliths, even those with rough contours (Assis et

174 al., 2020; Gagliano and McCormick, 2004; Karahan et al., 2014; Lestrel, 1997; Mahé et al., 2019;

175 Mérigot et al., 2007; Rashidabadi et al., 2020; Rodgveller et al., 2017; Smoliński et al., 2019).

176 The first coefficients derived from the first harmonic were excluded from the analysis because the

177 outlines reconstructed from these coefficients are simple ellipses used to standardize each outline for

178 size, orientation and starting point (Crampton, 1995). The normality of the coefficients were tested

179 using the *shapiro.test* function from the *stats* package (R Core Team, 2020). The coefficients that did

180 not follow a normal distribution were excluded from the analysis. The allometric relationships

181 between otolith length and each of the Fourier descriptors were tested using the ANCOVA test. The

182 descriptors that had a correlation between otolith length and the Fourier descriptors were excluded

183 from the analysis to remove the effects of allometry in otolith shape. Otolith length was used instead

184 of fish length because it is less prone to errors (Campana and Casselman, 1993; Rodgveller et al.,

185 2017).

186

187 The remainder Fourier descriptors were used to compare the shape of the otoliths between

188 autochthonous and allochthonous fish applying a multivariate analysis of variance (MANOVA) using

189 the *MANOVA* function. A linear discriminant analysis – LDA (*LDA* function) was applied to test the

190 correct classification of the otolith shapes based on the fish origin. The mean shape of the otoliths

191 from stocked and wild fish was obtained using the *mshapes* function and the differences between the

192 average shapes were obtained using the *coo_ruban* function. All analysis based on the Fourier

193 transformation method were performed on the *Momocs* package in R (Bonhomme et al., 2014).

194

195 **3. Results**

196 Overall, 158 otoliths were analysed (wild = 122, stocked = 36), with stocked fish having slightly

197 smaller body than wild individuals, with otolith area, length, width and perimeter being larger in the

198 former group (Table 2). All shape indices but SI_{CI} were larger in the wild pikeperch, with the strongest

199 differences between stocked and wild pikeperch being recorded for SI_{CI}, SI_{CO}, SI_{FF} and SI_{SO}, whereas

200 SI_{AR}, SI_{EL}, SI_{RE} and SI_{RO} having less prominent differences (Table 3).

201 The correlation analysis among the shape indices plus O_P (otolith perimeter) indicated that SI_{AR}, SI_{CI},

202 SI_{FF} and SI_{RO} were correlated and hence, O_P, SI_{CO}, SI_{EL}, SI_{RE} and SI_{SO} were selected for the training

203 of the neural network. The neural network used 4 hidden layers based on the selection of variables,

204 with only one hidden layer (H2) being positively linked to the output layer. The strongest positive

205 links were observed between SI_{EL} and the fourth hidden layer (H4) and SI_{RE} with the third hidden

206 layer (H3), whereas the strongest negative links were observed between OP and the fourth H4 and

207 SI_{RE} and H4 (Fig. 3). The relative importance of the input variables shows that the most important

208 variables were O_P (423.93), SI_{EL} (-368.60), SI_{RE} (-228.40), SI_{CO} (-192.14) and SI_{SO} (48.86)

209 respectively. The neural network showed a global accuracy of 79.59%, with the accuracy of the

210 classification of wild fish being 94.87%, whereas for the stocked fish of 10.00%. The model precision

211 was 0.78 and the recall 0.80.

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216 The elliptical Fourier analysis was able to describe the outlines of *S. lucioperca* sagittae otoliths with

217 99.9% accuracy using 34 elliptical harmonics (136 Fourier descriptors). After the removal of the first

218 coefficients of the first harmonics (4 Fourier descriptors), the non-normally distributed Fourier

219 descriptors (30) and the descriptors with allometric influence (5), a total of 102 Fourier descriptors

220 were used in the analysis. The MANOVA test revealed that the otolith shapes were significantly

221 different between stocked and wild fish (F = 1.736, p < 0.05). Additionally, the LDA (linear

222   discriminant analysis) was able to correctly classify 77.9% of the otoliths based on fish origin, with

223   89.7% accuracy on the wild fish group and 32.0% on the stocked group.

224   The statistical tests support the visual inspection of the average otolith outlines from stocked and wild

225   fish, where it is possible to detect differences on shapes. The differences are especially pronounced

226   in the otolith rostrum, which is shorter in the otoliths from wild pikeperch in comparison with the

227   stocked individuals. The zone between the antirostrum and the postero-dorsal angle of in the dorsal

228   portion of otoliths is also different, with higher roughness being recorded in the otoliths from the

229   stocked fish and smoother contours in the wild pikeperch otoliths (Fig. 4).

230

231   **4. Discussion**

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234   Our results indicated that autochthonous and allochthonous fish have dissimilar otolith shapes,

235   suggesting that this feature can be applied in analytical tools to identify the natal origin of pikeperch

236   populations. In general, the otoliths from the stocked pikeperch were bigger, rougher and with a

237   longer rostrum compared to the wild individuals. Otolith shapes are influenced by many factors

238   (Mahé et al., 2019; Mille et al., 2016; Vignon, 2012; Vignon and Morat, 2010) and without

239   experimental studies it is difficult to disentangle the effects of multiple drivers. In this study we did

240   not attempted to investigate the drivers of otolith shape variation, but rather to test if there is any

241   signal of these drivers into the otolith shapes of fish of different natal origins. Using two different

242   methods and descriptors we were able to detect significant differences on the otolith shapes of

243   autochthonous and allochthonous individuals. The neural network applied to the shape indices spot

244   differences between the groups, with the 5 variables being important to classify the natal origin of

245   pikeperch individuals. Overall, the neural network was slightly more accurate than the LDA model,

246   with the accuracy at classifying the wild individuals being higher in the former, whereas the accuracy

247 of stocked individuals being higher in the later model. The higher accuracy of machine learning

248 methods over more traditional statistical tests observed in this study corroborates the finds of

249 Smoliński et al., (2019), but, in our case, the difference seems to be small and not true for all groups

250 (i.e. stocked fish). In both methods, the classification of wild individuals was much higher than the

251 stocked fish. It is a bit surprising that the accuracy of origin determination was higher with the wild

252 fish group. The higher accuracy of the models in this group of fish might suggest that the sample size

253 is an important factor to improve the accuracy of the methods, given that the number of wild

254 individuals used in this study is higher than stocked fish, and hence, in future studies the sample sizes

255 should be larger whenever it is feasible. Interestingly all individuals in stocked group were kept in

256 much more homogeneous condition than the wild fish. Alternatively, this result might indicate that

257 the rearing conditions lead to a higher variation on the otolith shape on *S. lucioperca*. Even though

258 the rearing conditions try to create homogeneous conditions for the fish, they do not allow the fish to

259 freely explore a broad range of micro-habitats that could theoretically allow the fish to actively seek

260 the optimal conditions for its growth. Given that the stocked fish were reared in semi-intensive

261 conditions (shallow ponds), they can be more vulnerable to other types of stresses than wild

262 individuals, which can cause shifts on the otolith shape (Fernandez-Jover and Sanchez-Jerez, 2015;

263 Koeberle et al., 2020; Vignon, 2018), because the later can perform vertical migrations to cope with

264 temperature shifts (Lehtonen, 1983; Lind, 1977; Saulamo and Lappalainen, 2007). Also, given that

265 captive fish inhabited different ponds before reaching Lipno reservoir, their growth and otolith shape

266 could be influenced by these different environments in a non-linear way (i.e. individual variability on

267 growth in different habitats), which might be reflected by the slightly higher diversity on the otolith

268 shapes in this group of individuals.

269 Differences in the food items consumption can have a remarkable effect on otolith shape (Mille et al.,

270 2016), and therefore can also be one of the factors influencing the observed differences between

271 autochthonous and allochthonous fish, given that the food intake and food items are likely to be

272 dissimilar between the groups. Mille et al., (2016) stated that the food item is more relevant than food

273 amount in affecting the otolith shape, therefore our results can indicate that fish reared in captivity,

274 even though in semi-intensive conditions, have different diet composition than wild fish. Wild fish

275 could have explored different habitats and areas of the reservoir throughout the year, adjusting their

276 food item selection according to their needs (Huuskonen et al., 2019), while stocked fish did not have

277 available a diverse set of food types in the ponds. In the same time the diet of pikeperch of the given

278 size in the Lipno reservoir is also quite uniform (100% percid fish fry, Vašek et al., (2018)), which is

279 believed to be the optimal diet for young pikeperch (Dörner et al., 2007).

280 A recent study showed that otolith shape is more sensitive to water temperature than to food quality

281 (Mahé et al., 2019). This fact can also be behind the observed dissimilarities on otolith shapes between

282 stocked and wild pikeperch in Lipno reservoir. The shallow depths of the rearing facilities compared

283 to the reservoir conditions might have constrained fish to a broader thermal range of conditions than

284 in the reservoir (due to the impossibility of spatio-temporal migrations to cope with thermal

285 fluctuations). Given that pikeperch do move to find better living conditions throughout the year

286 (Huuskonen et al., 2019), the individuals in the wild might experienced narrower thermal ranges than

287 stocked pikeperch, which might be reflected on the higher shape similarity among wild than stocked

288 group of fish. The water pH can be another variable behind the observed differences in otolith shape

289 between stocked and wild pikeperch individuals, given that lower pH values are associated with

290 bigger and rougher otoliths (Coll-Lladó et al., 2018; Holmberg et al., 2019), which are the most

291 striking differences between the two groups in our study. Alternatively, higher food availability during

292 early life (likely higher for stocked fish) can also cause higher otolith roughness (Hüssy, 2008).

293 Reared fish have a higher prevalence of otolith shape and mineral anomalies (vaterites) that can cause

294 important hearing impairments for the fish (Oxman et al., 2007; Reimer et al., 2016). The prevalence

295 of these anomalies is linked to stressful conditions present in the rearing facilities, such as high

296 population densities, temperature fluctuation, noise, vibration, diseases, poor water quality and

297   nutrition (Sweeting et al., 2004). In the present study, we did not detect the presence of vaterites in

298   the stocked nor in the wild fish, but otolith shape still differed between these fish. Other studies found

299   differences in otolith shapes in areas closely associated to fish farms but with low population density

300   (Fernandez-Jover and Sanchez-Jerez, 2015), suggesting that water quality might be linked to changes

301   in otolith shapes. High population density can significantly affect fish growth and bias fisheries'

302   assessments (Lorenzen and Enberg, 2002; Martino et al., 2019). Due to the tight connection between

303   somatic growth, food availability and otolith growth trajectory (Mahé et al., 2019; Vignon, 2012) it

304   is expected that population density can significantly influence the otolith shape as well, however the

305   role of population density dependence on otolith shape is yet to be scrutinized experimentally.

306   Many stock assessments studies neglect the share of stocked and wild fish in the population, which

307   can create biases in the interpretation of the population parameters extracted for the whole

308   metapopulation. For instance, a higher survival and cumulative dominance of stocked fish in the

309   population could wrongly indicate an accelerated somatic growth rate and early maturation of fish in

310   the population, given that fish in captivity often grow faster and mature earlier than wild individuals

311   (McDermott et al., 2011; Zupa et al., 2017), which is not possible to see in this study due to the

312   selection of wild fish with comparable sizes with the stocked individuals to avoid the bias of the

313   presence of slower and much smaller fish in the wild group (van Densen and Vijverberg, 1982). This

314   option allow us to compare individuals with more similar conditions, because the slow growing

315   pikeperch are in fact much smaller than the fast growing individuals in the studied area (Jůza et al.,

316   2013), but refrain us from assessing the full spectrum of otolith shapes in the wild population.

317   Nevertheless, differences in fish growth could potentially bias the assessment of long-term patterns

318   of a fish stock, masking the effects of other stressors like environmental carrying capacity, density

319   dependence, fishing pressure, shifts in management rules and climate change. In this regard, the

320   continuous monitoring of the contribution of stocked fish in the population could provide ways to

321   cope with the potential differences in conditions of the groups in the population.

322 In the present study, we did not perform any genetic analysis on the analyzed fish (from both

323 autochthonous and allochthonous individuals), therefore the genetic influence on the pikeperch

324 otolith shape cannot be determined. On the other hand, this method might not be the best option to

325 discriminate the fish origin in this case, because the ecosystem is continuously stocked and pikeperch

326 natural recruitment is successful, and hence the autochthonous fish genetics might contain the genetic

327 material of the allochthonous population due to the breeding between fish of different origins.

328 Additionally, genetics can be outperformed by other methods in stock discrimination studies

329 (Marengo et al., 2017).

330 Fish sex is believed to influence the otolith shape (Campana and Casselman, 1993), but recent studies

331 suggest that this can be a consequence of differences in the way of life between males and females

332 (Parmentier et al., 2018; Vaux et al., 2019). In the present study, individuals of both sexes were pooled

333 together because they were not adults yet and thus no behavioral sexual dimorphism would be

334 expected. So the effects of sex on otolith shape in this study are unlikely.

335 It is important to highlight that in our study, we focused only in fish of similar sizes with confirmed

336 natal origins and we did not attempted to identify the natal origin fish of larger sizes and with

337 unknown origin because we do not know if the observed differences in otolith shape are still

338 noticeable in stocked individuals after a certain period of residence in the lake. To classify older fish

339 of unknown origin and already living into the lake it is important to conduct further studies to describe

340 the consequences of habitat shift on the otolith shape through ontogeny, possibly combining shape

341 analysis with other analytical tools. Otolith shape is successful in identifying the natal origin of fish

342 in stock discrimination studies (Brophy et al., 2016; Burke et al., 2008; Campana and Casselman,

343 1993; Libungan et al., 2015; Paul et al., 2013; Schade et al., 2019). Given that the otoliths are

344 conservative structures (Campana and Neilson, 1985), the shape signal of early ages will invariably

345 be permanently record on the structure of older fish and detectable in stocked fish even after years

living in the wild (Negus, 1999; Vignon, 2018; Volk et al., 1999), and hence, otolith shape analysis is being successfully used to differentiate mixed-stocks.

Many mixed-stock discrimination studies are performed in the marine areas where there is no artificial stocking being conducted (Burke et al., 2008; DeVries et al., 2002; Paul et al., 2013; Vignon and Morat, 2010). They are targeted to detect natal areas of stocks with complex metapopulation structure, while our study targets a freshwater species that is artificially stocked by managers and do not perform long distance migrations. The scientific literature is much scarcer in this type of conditions (but see Pereira et al., (2019)) and our results shows that the otolith shape analysis is a cost efficient tool that can be successfully applied in actively managed freshwater fish stocks.

Our results highlights that the detection of the natal origin of fish based on their otolith shape is promising and the application of this method can provide a low cost and effective tool for managers to assess the efficiency of the stocking regime in their systems. Schade et al., (2019) compared the efficiency of four common stock discrimination tools and found out that the otolith shape analysis and stable oxygen analysis on the otolith nuclei were the most effective to distinguish the natal origin of mixed-stocks. However, sometimes the combination of shape analysis with other tools are needed to differentiate the origin of fish in the population (Marengo et al., 2017; Schade et al., 2019). For instance, the use of microelemental composition of fish otoliths can precisely indicate the origin of the fish in freshwater (Avigliano et al., 2017; Lazartigues et al., 2017; Spurgeon et al., 2018), but its application is financially costly, whereas the shape analysis is much more affordable, especially if used routinely. However, the combination of both tools could theoretically create the mechanisms to cut the costs in long-term, by developing a database of shape information that could be used to determine the natal origin of the fish using the different classification tools. It is also crucial to understand how the otolith growth trajectory is affected after the stocked fish is trans-located to the lake. The correct stock discrimination can uncover many hidden signals in fish population structure, which in turn will allow scientists to better guide stakeholders to improved management plans.

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Acknowledgments

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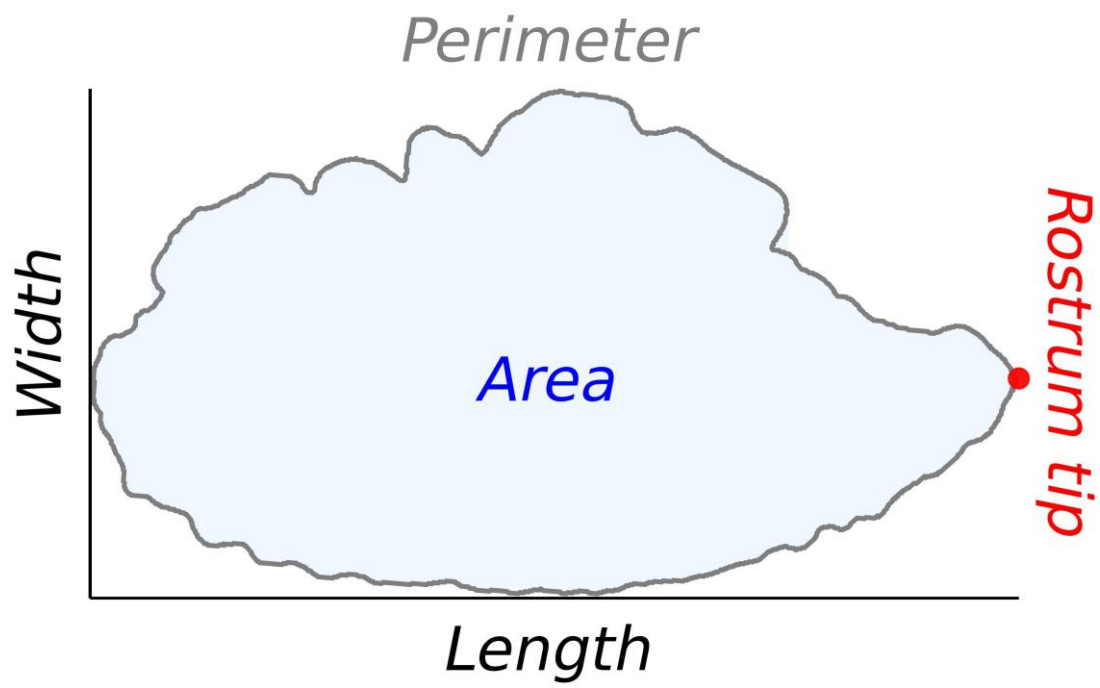
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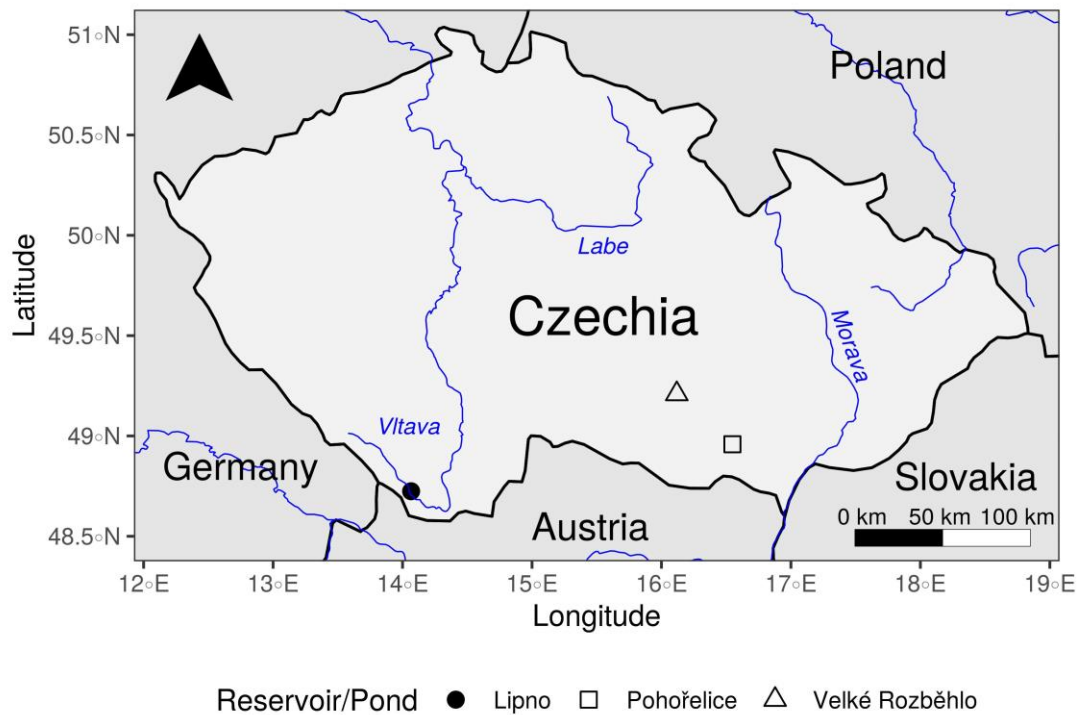
434   **Fig. 1.** Map showing the origin of the pikeperch (*Sander lucioperca*) individuals used in this study.
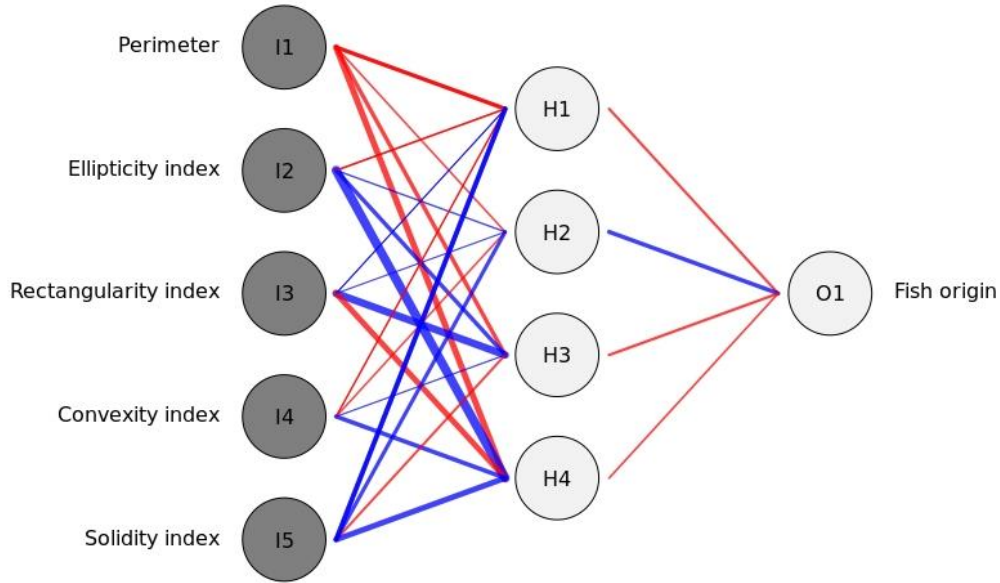
435   Reservoir = full symbol, rearing facilities = open symbols. Blue lines represent the largest riverine

436   systems in the country.

437

Fig. 2. Measurements extracted from the pikeperch (*Sander lucioperca*) sagittae otoliths and the landmark used to standardize the otolith positions.

Fig. 3. Neural network interpretation diagram showing the relationships between the otolith shape indices and the fish origin. The widths of the connections represent the strength of the relationship, with red color representing the negative weight connections and blue color representing the positive connection weights. Only the non-correlated shape indices were used in the analysis (i.e. roundness index (SI_{RO}), rectangularity index (SI_{RE}), format factor index (SI_{FF}) and solidity index (SI_{SO})). Letters inside the circles indicate the layer type in the neural network (I = input layer, H = hidden layer, O = output layer).

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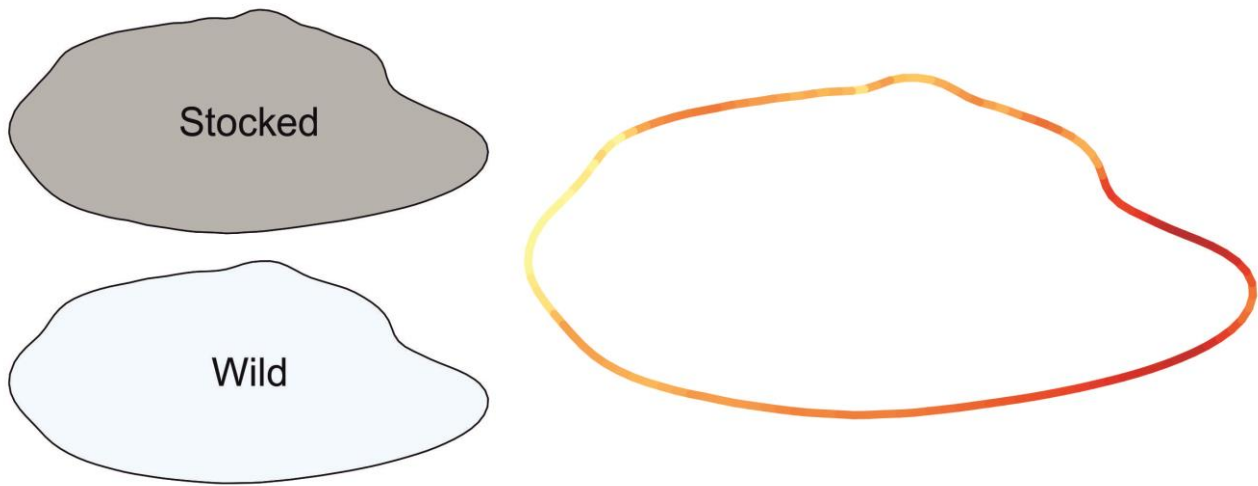
Fig. 4. Mean shape of the sagittae otolith from stocked (top left corner) and wild (bottom left corner) pikeperch (*Sander lucioperca*). Differences in the otolith average shape of stocked and wild pikeperch (right side) is expressed in color heatmap scale (small difference = light yellow, high difference = dark red). Otolith outlines were scaled to allow better visual comparison between groups.

456 **Table 1.** Shape indexes (SI) computed for the pikeperch (*Sander lucioperca*) otoliths. O_A = otolith

457 area, O_{AC} = otolith convex hull area, O_L = otolith length, O_P = otolith perimeter, O_{PC} = otolith convex

458 hull perimeter, O_W = otolith width.

Shape index	Index acronym	Formula
Aspect ratio	SI_{AR}	$SI_{AR} = O_L/O_W$
Circularity	SI_{CI}	$SI_{CI} = O_P^2/O_A$
Convexity	SI_{CO}	$SI_{CO} = O_{PC}/O_P$
Ellipicity	SI_{EL}	$SI_{EL} = (O_L - O_W)/(O_L + O_W)$
Format factor	SI_{FF}	$SI_{FF} = 4\pi \cdot O_A/O_p^2$
Rectangularity	SI_{RE}	$SI_{RE} = O_A/(O_L \cdot O_W)$
Roundness	SI_{RO}	$SI_{RO} = 4 \cdot O_A/\pi \cdot O_P^2$
Solidity	SI_{SO}	$SI_{SO} = O_A/O_{AC}$

459

460 **Table 2.** Comparisons of fish standard length (SL) and the morphometrical measurements taken from

461 the otoliths of the stocked and wild pikeperch individuals (*Sander lucioperca*) from Lipno lake

462 (Czechia). O_A = otolith area, O_L = otolith length, O_P = otolith perimeter, O_W = otolith width, SE =

463 standard error, CI = confidence interval (5% – 95%), Range = (minimum – maximum).

	Stocked				Wild			
	Mean	*SE*	*CI*	*Range*	*Mean*	*SE*	*CI*	*Range*
O_A (mm^2)	8.28	0.74	6.83 – 9.73	1.39 – 17.34	7.91	0.39	7.19 – 8.68	1.13 – 15.23
O_L (mm)	4.97	0.25	4.46 – 5.45	1.93 – 7.3	4.83	0.14	4.56 – 5.09	1.79 – 7.43
O_P (mm)	14.62	0.83	12.98 – 16.25	5.27 – 24.48	13.88	0.4	13.04 -14.67	4.66 – 21.21
O_W (mm)	2.29	0.11	2.1 – 2.5	1.01 – 3.6	2.23	0.05	2.12 – 2.33	0.91 – 3.29
SL (mm)	208.46	2.29	204.4 – 213.3	195 – 245	217.32	1.36	214.6 – 219.9	190 – 245

464

465 **Table 3.** Average values for the shape indexes computed for the otoliths of stocked and wild pikeperch

466 (*Sander lucioperca*) individuals from the Lipno lake (Czechia). SI_{AR} = aspect ratio index, SI_{CI} =

467 circularity index, SI_{CO} = convexity index, SI_{EL} = ellipicity index, SI_{FF} = format factor index, SI_{RE} =

468 rectangularity index, SI_{RO} = roundness index, SI_{SO} = solidity index, SE = standard error, CI =

469 confidence interval (5% – 95%), Range = (minimum – maximum).

Otolith shape index	Stocked				Wild			
	Mean	*SE*	*CI*	*Range*	*Mean*	*SE*	*CI*	*Range*
SI_{AR}	2.139	0.037	2.070 – 2.214	1.858 – 2.727	2.142	0.017	2.109 – 2.175	1.889 – 2.823
SI_{CI}	20.014	0.36	19.30 – 20.76	16.643 – 24.824	19.349	0.15	19.06 – 19.63	16.011 – 23.627
SI_{CO}	0.903	0.008	0.887 – 0.918	0.777 – 0.962	0.924	0.003	0.918 – 0.930	0.841 – 0.989
SI_{EL}	0.361	0.007	0.348 – 0.375	0.300 – 0.463	0.362	0.003	0.356 – 0.367	0.308 – 0.477
SI_{FF}	0.633	0.011	0.611 – 0.656	0.506 – 0.755	0.653	0.005	0.644 – 0.664	0.532 – 0.785
SI_{RE}	0.683	0.004	0.676 – 0.691	0.653 – 0.728	0.687	0.002	0.683 – 0.692	0.630 – 0.748
SI_{RO}	0.409	0.006	0.398 – 0.420	0.334 – 0.477	0.411	0.003	0.405 – 0.416	0.328 – 0.464
SI_{SO}	0.950	0.002	0.944 – 0.955	0.918 – 0.974	0.954	0.001	0.952 – 0.957	0.924 – 0.981

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