Ocean acidification reshapes the otolith-body allometry of growth in juvenile seabream

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ABSTRACT

The effects of elevated CO_2 partial pressure (pCO_2) on otolith calcification and on the coupling between the somatic and otolith growth were investigated in juvenile gilthead seabream *Sparus aurata*. Six-month old individuals were raised during seven weeks under four pCO_2 conditions set according to projected future ocean acidification scenarios. Body and otolith biometric parameters were measured throughout the experiment along with the otolith biomineralization monitored using a radiotracer technique based on ^{45}Ca incorporation. Seabream exhibited somatic growth resilience to all treatments. In contrast, increased growth rate and shape complexity of otoliths were observed with a pH_T drop from 8.1 to 7.5. Hypercalcification was observed under lowered pH, with a rate of calcium incorporation increasing by up to 18% between pH_T 8.1 and pH_T 7.7. This work highlighted an uncoupling of otolith and body growth of juvenile seabream within 40 d at pH_T 7.9 projected to be reached by the end of the century. As the otolith is an essential tool used in reconstructing fish life history, this work suggests that information resulting from otolith studies should be interpreted with caution with respect to the potential impacts that ocean acidification projected modifications could have on otolith biomineralization.

1. INTRODUCTION

On 9th May 2013, the concentration of carbon dioxide (CO_2) in the atmosphere reached the symbolic threshold of 400 ppm in Mauna Loa, Hawaii (IPCC, 2013), a level never reached at this reference site. The increase of atmospheric CO_2 from a preindustrial value of 280 µatm is the result of fossil fuel combustion, cement production and land use change (Ciais et al., 2013).

51　IPCC projections suggest further increase in the coming decades with concentrations reaching

52　490 ppm in 2050 and more than 1370 ppm by 2100 (IPCC, 2013). The ocean is a major carbon

53　sink, absorbing about 25% of anthropogenic CO_2 emissions thus limiting the greenhouse gas

54　effects on climate (Le Quéré et al., 2013). The increase in pCO_2 in the ocean has already led to a

55　pH decline of 0.1 unit since the industrial revolution and to major shifts in the ocean carbonate

56　chemistry, *i.e.* increased concentrations of dissolved inorganic carbon and bicarbonate ions,

57　decreased carbonate concentration, altogether leading to 'ocean acidification' (Caldeira and

58　Wickett, 2005). The pH of the surface ocean is expected to decline by 0.06 to 0.32 units by the

59　end of century (Ciais et al., 2013), resulting in an unprecedented change of seawater chemistry

60　equilibrium since the last 800,000 years (Zeebe and Ridgwell, 2012).

61　For a decade, a growing body of experimental studies has examined the response of marine

62　organisms to decreased pH across multiple taxa. Impacts on early life stages have been of

63　particular concern in fish (Baumann et al., 2012) because it has been hypothesized that animal

64　embryos and larvae may not be as resilient to physiological stress as juveniles and adults

65　(Pörtner, 2008), and because recruitment cohorts lay the foundation for population success and

66　connectivity (Planes et al., 2009). Nevertheless juveniles of coastal fish may be more exposed to

67　high CO_2 levels than earlier life stages and deserves particular attention. Indeed, unlike in the

68　open ocean, seawater pCO_2 is known to vary considerably in coastal waters depending on land-

69　driven eutrophication, which adds to the uptake of atmospheric CO_2, locally amplifying ocean

70　acidification (Cai et al., 2011; Guinotte and Fabry, 2008). Juvenile fish settling in coastal areas

71　during their high metabolic and fast growing phase could be severely challenged by hypercapnic

72　conditions.

73　Based on the sparse current knowledge, adult physiological performance allows fish to cope with

74　extracellular acidosis caused by increasing pCO_2 (*e.g.* Melzner et al., 2009b). But in early-life

75　stages of multiple taxa including fish, increasing pCO_2 was shown to affect calcification of shells

and skeletons due to a drop in the carbonate availability (*e.g.* Gattuso et al., 1998; Riebesell et al., 2000). Munday et al. (2011a) observed no effects on spiny damselfish otolith calcification at 850 µatm, while Munday et al. (2011b) and Checkley et al. (2009) highlighted an otolith hypercalcification in white sea bass larvae exposed at 993 and 2558 µatm pCO_2 and in clownfish larvae at 1721 µatm pCO_2, respectively. In cases of calcification modulation, otolith morphology can be affected, which may have negative repercussions on the behaviour and acoustic function in fish, decreasing their survival probabilities (Bignami et al., 2013a; Popper et al., 2005). It has also been recently reported that otolith increment growth can be uncoupled from somatic growth in fish larva raised under high pCO_2 conditions, then having potential implications for the study of fish populations (Bignami et al. 2013b). Understanding the way ocean acidification can affect the process of otolith formation is decidedly important in many perspectives among which is seems crucial to delineate how the environment can affect its growth and influence its coupling with the structural skeleton growth. If otoliths indeed ensure the fish's biological hearing and balance functions, they are also an essential tool used in fisheries biology to reconstruct individual life history in terms of age and somatic growth relationship (age-length keys) and attended habitats (Campana and Neilson, 1985; Campana, 2005).

This paper aims at evaluating the impacts of ocean acidification on the calcification rate of fish otoliths and on the understudied coupling between otolith and somatic growth in juveniles. These questions were investigated experimentally using a nuclear tracking approach, following the [45]Ca incorporation in otolith for 7 weeks of exposure to 4 realistic pCO_2 levels projected for the near-future. The gilthead seabream *Sparus aurata* was chosen due to its ecology and high economic value. Temperate and widely distributed over the North-Eastern Atlantic Ocean and the Mediterranean Sea, this coastal species is subjected to recreational and professional fishing and is increasingly aquacultured (FAO, 2014). In the Mediterranean Sea, it is the first marine fish species cultured with more than 140,000 tonnes produced in 2010 in 17 countries for a worth *ca.*

101   US$ 785 million (GFCM, 2013).

102

103   2. MATERIALS AND METHODS

104   *2.1 Organisms, radiotracer and experimental procedures*

105   Six-month old juveniles of seabream *Sparus aurata* have been purchased at the "Cannes

106   Aquaculture" fish farm of Monaco and were placed for three weeks in an open-circuit 500 l tank

107   in the IAEA-EL premises for acclimation.

108   Then, 200 fish juveniles of *ca.* 50 mm were randomly assigned in four 20 l circular tanks (one

109   tank per treatment) filled with filtered (0.45 µm) and UV-sterilized Mediterranean seawater (38

110   p.s.u.) pumped from 30 m depth in the Bay of Monaco. In each experimental tank (closed

111   system), seawater was constantly aerated. The light/dark cycle was 12h/12h. Fish were fed daily

112   *ad libitum* with pellets. After feeding, not ingested food has been removed and 80% of the

113   volume was renewed daily with sterilized and filtered seawater. Temperature was maintained at

114   21°C and controlled in each bath to within ± 0.5°C using temperature controllers connected to

115   300 W submersible heaters. Seawater pH (pH_T on the total scale, Dickson et al., 2007) was

116   adjusted to the desired level from ambient pH_T of 8.1 (corresponding to 475 µatm of pCO_2) to

117   low pH_T of 7.9, 7.7, and 7.5 (700, 1200 and 2000 µatm, respectively), as derived from various

118   models on trajectories of carbon emissions to the near-future (IPCC, 2013). The pH was

119   controlled in each bottle to within ± 0.05 pH unit using a pH-stat system (IKS, Karlsbad). The

120   experimental containers were continuously bubbled with CO_2-free air and discrete amounts of

121   pure CO_2 were added by the pH-stat system. pH and alkalinity were measured and set according

122   to Martin et al. (2011) using the R package seacarb (Proye and Gattuso, 2003).

123   Seawater in each bottle was spiked with ^{45}Ca (10 kBq l^{-1}). Radiotracers were purchased from

124   Radioisotope Centre Polatum, Poland, ^{45}Ca [as $^{45}CaCl_2$; $T_{1/2}$ = 163 d]. Stock solutions were

125   prepared in H_2O to obtain radioactivities that allowed the use of spikes of only a few microliters

126   (typically 5-10 µl). The radiotracer spikes were renewed at each water change and were checked

127   (i.e. counted in 1 ml of seawater) before and after each water renewal in order to maintain

128   radiotracer concentrations.

129   Fish were maintained for 40 d at the four pH treatments and continuously exposed to dissolved

130   ^{45}Ca in seawater. Five fishes were collected every 3 or 7 d in each tank, anesthetized with clove

131   oil prior to decerebration and then measured in length and weight to the nearest 0.1 mm and 1

132   mg, respectively. Left and right otoliths (sagittae) were extracted and photographed using a

133   Leica DFC420 camera mounted on a stereomicroscope (Leica LZ12). Otolith surface area (OSA,

134   mm^2), maximum Feret diameter (OF, mm) and roundness (OR) were calculated using ImageJ

135   software and weight measurement was made to the nearest 0.1 mg. ^{45}Ca content was then

136   determined. Paired radiolabelled sagittal otoliths from the same individual were pooled and

137   dissolved adding 300 µL of hydrochloric acid (HCl, 37%) at 80°C. After evaporation, the

138   residues were dissolved in 1 mL of distilled water. Biological and seawater samples were

139   counted after adding 10 mL of scintillation liquid, Ultima GoldTM XR (Perkin Elmer).

140   Emissions were measured with a liquid scintillation analyzer (Tri-Carb, Packarb 1600 TR or

141   Perkin Elmer 2900 TR) calibrated with an appropriate standard for each counting that was used.

142   Counting times were adapted to obtain relative propagated errors less than 5% (from 10 min to

143   24 h). Corrections for the physical half-life time and background noise were done in order to

144   determine the ^{45}Ca activities at the sampling time (Bq). The uptake of Ca in the otolith was then

145   expressed as the amount of Ca incorporated (Q_{Ca}, in µmol Ca g^{-1} of otolith dry wt) and following

146   the equation (Martin et al., 2011):

147   $Q_{Ca} = [(A_{cut} / A_{sw}) \times C_{sw}] \times 10^3$

148   where A_{cut} is the total ^{45}Ca activity in each otolith (in Bq), A_{sw} is the time-integrated activity (in

149   Bq g^{-1}) in seawater during the time of exposure and C_{sw} is the total Ca concentration in

150   Mediterranean seawater (0.0114 mmol g^{-1}).

151

2.2 Data Analyses

Due to compromises with experiment cost, radioprotection rules and waste management possibilities with respect to the use of radioisotope ^{45}Ca, only one 20-l tank has been dedicated to each pH conditions. Therefore, fish sampled along the experiment course at regular sampling time (n = 5 (and 10) per sampling time (at 40 d) per condition) have been considered as pseudoreplicates (Hurlbert, 1984). Accordingly, the effect of pH on measured physiological or morphological responses of fish has been tested through multiple linear regression considering the pH as a continuous covariate (Havenhand et al., 2011). Linear regressions were used to test the effects of pH (continuous covariate), on relationships between otolith biometrics (*i.e.* otolith surface area (OSA), weight (OW), maximum diameter (Feret's diameter, OF), roundness (OR)) and fish total length (FTL) or Time, including a FTL (or Time) x pH interactions. When FTL (or Time) x pH interaction was not significant, a simpler model without interaction (*i.e.* Time (or FTL) + pH) has been computed (Crawley, 2005).

To evaluate the effect of the pH treatment on the asymmetry of paired-otoliths (regarding left-right differences in OSA, OF and OR), log-transformed data were injected in a Brown-Forsythe robust Levene test of homogeneity of variances based on deviation from the median.

The amount of Ca incorporated in otoliths were standardized to the otolith weight and expressed according to the Time of experiment. Ca incorporation data (mmol g^{-1} otolith dry weight) have been fitted by multiple linear regression with Time x pH as continuous covariates.

Results are expressed as mean ± s.d. Significance was considered at $p<0.005$. All statistical treatments have been done using R (R Core Team, 2013).

3. RESULTS

3.1 Culture conditions

176   During the experiment, the fish biomass in each tank did not exceed 5 $g.l^{-1}$, *i.e.* far below the EU

177   recommendations for fish welfare (CEE n°2092/91) which limit the biomass to 25 $g.l^{-1}$ for fish

178   aquaculture in marine environment. Along the 40 d of ^{45}Ca exposure, pH_T was maintained at a

179   mean (± s.d.) of 7.50 ± 0.06, 7.69 ± 0.03, 7.89 ± 0.05 and 8.04 ± 0.05 (Table 1), corresponding to

180   pCO_2 of *ca.* 2000, 1200, 700, and 475 µatm, respectively. Mean temperature was 21.0°C ± 0.7.

181   Mean A_T of renewed seawater was 2.595 ± 0.003 $mmol.kg^{-1}$. It changed by a maximum of 0.2

182    $mmol\ kg^{-1}$ between two seawater renewals. C_T increased from 2410 to 2690 $\mu mol\ kg^{-1}$ whereas

183   the CO_3^{2-} concentration decreased from 240 to 80 $\mu mol\ kg^{-1}$ with decreasing pH. In all

184   conditions, the saturation state with respect to aragonite was higher than 1.

185

186   *3.2 Somatic versus otolith growth*

187   Fish somatic growth in total length and weight was significant over the 40 days period of the

188   experiment (p-value < 0.001, Table 2), slightly inflecting from day 30 (Figure 1, A,B). As a

189   mean, fish grew by 0.36 $mm.d^{-1}$ and 0.043 $g.d^{-1}$, a growth rate close to the 0.0534 $g.d^{-1}$ value

190   measured in juveniles seabream (1.24 ± 0.02 g) reared in closed system tank at a density of 1.5

191    $g.l^{-1}$ (Kalogeropoulos et al., 1992). Similarly Kim et al. (2012) reported a mean weight growth

192   rate of 0.0364 $g.d^{-1}$ for young juveniles of red seabream reared from 1.46 g for 42 days, arguing

193   for adequate rearing conditions in our experiment allowing a normal growth and development of

194   the juveniles (Figure 2). The pH treatments had no impact on those body growth parameters and

195   intercepts did not differ between treatments meaning the initial group of juveniles was

196   homogeneous (p-value > 0.05). In contrast, the allometric relationship between otolith surface

197   area and fish total length (Figure 3.A), though still linear, was altered by the decreasing pH (p-

198   value < 0.001, Table 2) with a lower slope value observed at pH_T = 8.1 compared to pH_T = 7.9

199   and 7.7, and even more compared to pH_T = 7.5 (Figure 3.A). The otolith's Feret diameter and

200   fish total length relationship tended also to be modified in the two lowest pH conditions though

201 not significantly (Figure 3.B, Table 2). The otolith roundness was not related to fish total length

202 except at pH_T 7.5 where the linear relationship was low but significant (Figure 3.C, p-value <

203 0.0046) indicating a faster evolution of the otolith toward a less round shape along with body

204 growth at the lowest pH than at more elevated ones. Finally, the otolith weight was linearly

205 correlated to the otolith surface area during all the experiment and whatever the pH treatment

206 (Table 2), indicating that the density of the otolith was not affected by pCO_2.

207

208 *3.3 Asymmetry*

209 Levels of both surface area and roundness asymmetry between left and right sagittal otoliths

210 (Figure 4) of juvenile seabream were not affected by pH treatments over the 40 days (Brown-

211 Forsythe robust Levene Test based on deviation from the median; $p > 0.05$). Disequilibrium of

212 the symmetry in the otolith surface area toward a greater OSA of the right otolith was observed

213 during the first half of the experiment whatever the pH (Figure 4.A.), but disappeared in animals

214 sampled during the last 10 days of experiment. No asymmetry of the otolith roundness was

215 observed (Figure 4.B.).

216

217 *3.4 Calcification*

218 The calcification of otolith was followed through ^{45}Ca accumulation throughout the 40 days of

219 experiment (Figure 5). The incorporation of Ca in otolith of juvenile seabreams followed a linear

220 equation whatever the pH (Table 3), which is consistent with the linear growth of fish over the

221 experiment. Based on the slope calculated for each pH, we could estimate that for the fish reared

222 at pH_T 8.1 rates of Ca incorporation were 13, 17 and 18% lower than rates performed at pH_T 7.9,

223 7.7 and 7.5, respectively.

224

225 4. DISCUSSION

226 Assessing whether elevated pCO_2 and lowered pH impact marine fauna and flora and to what

227 extent it can affect individual, population and ecosystem function is a major concern (Fabry et

228 al., 2008; Hilmi et al., 2013; Melzner et al., 2009a). Recent studies have reported that

229 hypercapnia can challenge fish physiology (e.g. Melzner et al., 2009b; Michaelidis et al., 2007),

230 affect the behaviour (Simpson et al., 2011) and growth (Baumann et al., 2012) and impact

231 calcified structures biomineralized from the early-life stages (Checkley et al 2009, Ries et al.

232 2009). Our study shows that responses can be contrasted at the sub-individual integration level.

233

234 *4.1 Effect of elevated pCO_2 on the coupling of fish somatic and otolith growth*

235 Juvenile seabream *Sparus aurata* reared under 4 pCO_2 treatments during 40 d exhibited no

236 variation in somatic growth. As documented, physiological performance of fish could be

237 expected to allow them to cope with elevated pCO_2 (Melzner et al., 2009b) without significantly

238 affecting the animal's growth (Hurst et al., 2012; Ishimatsu et al., 2008). Michaelidis et al.

239 (2007) demonstrated that long-term hypercapnia may cause a shift from aerobic to anaerobic

240 metabolism in seabream *Sparus aurata*, but only at dramatically elevated pCO_2 (~5000 µatm).

241 This suggests that this species is able to cope with realistic increasing pCO_2, according to IPCC

242 scenarios, without extra-energy demand and might thus maintain its juvenile somatic growth rate

243 as observed in this study.

244 In contrast, a modulation of the otolith biomineralization was observed, with an increase of the

245 calcification rate observed from 700 µatm after 40 days of exposure. This means that juvenile

246 seabream might be subjected to the hypercalcification of their otoliths by the end of the century

247 with respect to IPCC projected CO_2 levels. Only Bignami et al. (2013b) reported such a short-

248 term effect of increased pCO_2 under the threshold of 800 µatm, and this was at the larval stage of

249 cobia *Rachycentron canadum*. Here, the higher growth rate of the otolith surface area under

250 rising pCO$_2$ was directly linked with the increase of the net calcification rate calculated on the

251 basis of ^{45}Ca incorporation. The linearity of calcium incorporation with time was not disrupted

252 by pCO$_2$ treatments, but it did provide evidence of a critical difference in the mineralisation

253 slopes. The hypermineralization of calcareous structures in organisms maintained in decreasing

254 pH and in less favourable chemical condition for CaCO$_3$ precipitation (lower carbonate

255 saturation state, Ω_{arg}) has been already observed in species of various taxonomic groups

256 characterized by efficient capacities to maintain blood/hemolymph homeostasis, such as

257 crustaceans (Ries et al., 2009), cephalopods (Dorey et al., 2012; Gutowska et al., 2010) and

258 fishes (Bignami et al., 2013b; Checkley et al., 2009). Indeed, the active uptake of bicarbonate

259 ions in internal medium to compensate blood acidosis is expected to contribute at increasing Ω_{arg}

260 in the endolymph fluid surrounding the aragonitic otolith (Borelli et al., 2003). The precipitation

261 of HCO$_3^-$ with Ca^{2+} in endolymph leads in turn to active proton extrusion to avoid impeding the

262 calcification process (Allemand et al., 2007). Nonetheless, the cellular mechanisms underlying

263 the hypercalcification of otoliths in this context of ocean acidification remains poorly understood

264 (Munday et al., 2011b) and require further insights.

265 The observed uncoupling of fish body and otolith growth may have implications in fisheries

266 studies. Indeed, back-calculation of fish size from the otolith size is a common and quite highly

267 effective tool used in fisheries biology (Campana and Neilson, 1985; Mosegaard et al., 1988;

268 Payan et al., 1997). It can be used to determine fish-prey size spectra from otoliths collected in

269 the stomach contents of predators (Blanco et al., 2001; Markaida and Sosa-Nishizaki, 2003; Ross

270 et al., 2005). It is also commonly used to infer fish-size at age, based on otolith growth-

271 increments reading (Secor and Dean, 1989). However to be reliable, body and otolith growth

272 have to be uniquely and confidently linked throughout the developmental stage or time period of

273 interest. A body of studies has shown how complex this relationship can be (reviewed in Wilson

274 et al., 2009), being potentially impacted by environmental parameters fluctuations such as

275   temperature (Mosegaard et al., 1988; Takasuka et al., 2008). To date, "growth-effect" and "age-

276   effect" were the two major explanations of the uncoupling between somatic and otolith growth

277   (Wilson et al., 2009). This work presents the perspective that the distortion of the fish-otolith

278   allometric relationship can also originate from the modulation of the otolith growth induced by

279   variations of environmental parameters to which body growth is (more) resilient. Ocean

280   acidification, as a compound of the global change, appears here to alter this relationship.

281

282   *4.2 Effect of increased pCO_2 on otolith shape*

283   Ocean acidification has also been reported to affect other otolith metrics that were monitored

284   during this experiment. As otolith weight increased in the same way as the otolith surface area,

285   no effect of pCO_2 increase up to 2000 µatm was observed on seabream otolith density. These

286   results are consistent with Bignami et al. (2013b) who did not observe any density modification

287   of the otolith in cobia larvae until 800 µatm. Nevertheless, they reported an increase of 6%

288   density when they experimentally increased conditions to 2100 µatm, suggesting an alteration of

289   the otolith's mass-size relationship under extreme hypercapnia conditions. Seabream otolith

290   shape seems to be also sensitive to pH conditions. Sagitta of juveniles reared at 475 µatm where

291   indeed significantly shapeless compared to those mineralized at 2000 µatm. As no asymmetry

292   between left and right otoliths was recorded, no sign of "anomalous" calcification appeared,

293   being in agreement with Maneja et al. (2013) who did not observe a deviation from symmetry in

294   otoliths of Atlantic cod larvae raised at 1800 µatm and even at 4200 µatm. Then, the here-

295   observed lower otolith roundness with high pCO_2 seems to provide evidence that in response to

296   hypercapnia, the otolith not only grows, but also develops more rapidly, acquiring at a higher

297   rate its species-specific ornaments (Campana and Casselman, 1993; Popper et al., 2005).

298   All these otolith descriptors are important in the otolithic apparatus functioning, as the main

299   organ of the ear in the senses of balance, directed motion detection and hearing (Lychakov and

300   Rebane, 2000; Popper et al., 2005). The otolith features indeed determine the compromise made

301   between these three senses. In bottom-dwelling species such as *Sparus aurata*, otoliths tend to be

302   bigger relative to the body size than in pelagic species (Popper et al., 2005), improving balance

303   and hearing senses while impairing motion detection. Hearing in seabream has been shown to be

304   a developed sense that is critical for the welfare of juveniles (Filiciotto et al., 2013). Thus, as

305   reported in another Perciform and bottom-dwelling species (Bignami et al., 2013a), ocean

306   acidification, in increasing the size of the otolith relative to the size of the body, may improve

307   the hearing acuity of *S. aurata*. As highlighted by Bignami et al. (2013a) improving auditory

308   capacity could also be advantageous or deleterious depending on the sound spectra newly

309   accessible to the fish. As Filiciotto et al. (2013) reported, wild offshore sound at low pressure

310   levels are more beneficial to *S. aurata* with individuals being less stressed and faster growing

311   than individuals exposed to constant and high sound pressure levels in aquaculture tanks. In

312   increasing hearing acuity, ocean acidification could thus induce a threshold shift of sound

313   pressure tolerance (Smith et al., 2004) or displace the optimum window of frequency, making

314   the baseline sound of the environment more disturbing and stressful (Simpson et al. 2011, Caiger

315   et al., 2012; Filiciotto et al., 2013). As many other ecologically and economically important

316   species fished in the wild and cultured in offshore farms, the consequences of ocean acidification

317   on growth and survival performances related to balance and auditory capacities need to be

318   considered and evaluated.

319

320   ***4.3 Potential repercussions of elevated pCO_2 on the otolith as a recorder of the seawater***

321   ***chemistry***

322   Considerations have also to be raised in regard to the use by fisheries scientists of the chemical

323   composition of the otolith as a biological tracking tool (Campana, 2005; Miller et al., 2006).

324   Here, calcium incorporation into the otolith was modulated by seawater pH. This questions the

325   stability of the elemental:Ca ratio under environmental hypercapnia. During the

326   biomineralization of the otolith, chemical elements such as metals and metalloids are supposed

327   to substitute for calcium (Campana, 1999) or, at least for some transition metals, complex with

328   the organic matrix of the otolith via its constitutive metal-binding proteins (Miller et al., 2006).

329   The changes of pH and seawater chemistry caused by increased CO_2 can modify the speciation

330   of metals and therefore their bioavailability to organisms (Millero et al., 2009). The

331   physiological response of fish to hypercapnia might in turn stimulate processes to compensate

332   for acidosis based on the key role of ion transporters such as the Na^+/H^+ exchangers (Hu et al.,

333   2013) that are hypothesized to be a major accumulation pathway for some cationic elements

334   (Webb and Wood, 2000). In this context, ocean acidification may interfere with trace element

335   uptake and body concentrations (Lacoue-Labarthe et al., 2009) and therefore could affect

336   microchemical signature recorded in fish otolith. Two previous studies observed that ocean

337   acidification has no effect on alkaline metals Ba-, Mg-, Sr-Ca ratio in the otolith of larval and

338   juvenile fish (Hurst et al., 2012; Munday et al., 2011a). In contrast, in statoliths of squid larvae,

339   the activity of the transition metal [65]Zn has been shown to increase with lowering pH (Lacoue-

340   Labarthe et al., 2011). Future research thus seems still needed to investigate the possibility that

341   ocean acidification could impact the incorporation of other trace elements used to track

342   movements of marine organisms (Arkhipkin et al., 2004; Campana et al., 2000), depending on

343   their chemical properties, molecular-binding affinities and incorporation pathway into the

344   otolith.

345

346   **5. Conclusions**

347   In conclusion, this study demonstrates that, even under projected near-future pCO$_2$ levels,

juvenile seabream exhibited an increase of their otolith calcification and development rates while their body growth rate was not affected. Highlighting an uncoupling of otolith and body growth rates which appeared within 40 d at a $p\mathrm{CO_2}$ of 700 µatm projected to be reached by the end of the century, this study shows that juvenile seabream could be more resilient to the ongoing ocean acidification in terms of somatic growth than in terms of structural calcification. As the otolith is an essential tool used in reconstructing fish life history in terms of age, somatic growth and attended habitats, this work suggests that information resulting from otolith studies should be interpreted with caution with respect to the potential impacts that ocean acidification projected modifications could have on otolith biomineralization.

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550 **TABLES**

551

552 **Table 1.** *Sparus aurata*. Carbonate chemistry and pH (mean ± sd) in the four pH levels applied

553 to reared juvenile of seabream for 40 d (1 tank per treatment). Partial pressure of CO_2 (pCO_2; μatm),

554 dissolved inorganic carbon (DIC; μmol.kg^{-1}), CO_3^{2-} concentration (μmol.kg^{-1}) and saturation state of seawater with

555 respect to aragonite (Ω_{arg}) are calculated from pH_T, temperature (21.0°C), salinity (38) and the total alkalinity

556 varying with time between two water renewals according the following equation: TA (μmol. kg^{-1}) = 2599 + 196 x

557 Time (day). Data are means ± s.d. of measurements taken every 15 min from Day 0 to Day 40; N = 24,724.

558

Treatment	pH_T	pCO_2	DIC	CO_3^{2-}	Ω_{arg}
pH 8.1	8.04 ± 0.05	477 ± 78	2412 ± 341	238 ± 22	3.65 ± 0.34
pH 7.9	7.89 ± 0.05	726 ± 101	2506 ± 247	178 ± 15	2.73 ± 0.23
pH 7.7	7.69 ± 0.03	1198 ± 105	2603 ± 153	121 ± 8	1.85 ± 0.13
pH 7.5	7.50 ± 0.06	1978 ± 202	2685 ± 244	80 ± 13	1.22 ± 0.19

559

560 **Table 2.** *Sparus aurata*. Parameters of the multiple linear regressions between fish total length

561 (FTL, mm) and Time (days), fish total weight (FTW, g) and Time (days) otolith surface area

562 (OSA, mm^2) and FTL, otolith weight (OW) and OSA, otolith Feret maximum diameter (OF,

563 mm) and FTL, and pH considered as a continuous co-variable, measured over the 40 days period

564 of experiment in 4 tanks maintained at pH 7.5, 7.7, 7.9 and 8.1. Significance of *p*-values [NS] >

565 0.05; [*] < 0.05; [**] < 0.01; [***] <0.001.

| Model	Parameter	Variable p-value	Model F-value	Model p-value
FTL *vs.* Time	Time	< 0.001***	117.1	< 0.001***
	pH	0.654 [NS]		
FTW *vs.* Time	Time	< 0.001***	85.8	< 0.001***
	pH	0.372		
OSA *vs.* FTL	FTL	0.002**	246.9	< 0.001***
	pH	0.046*		
	FTL x pH	0.021*		
OW *vs.* OSA	OSA	< 0.001***	737.3	< 0.001***
	pH	0.772 [NS]		
OF *vs.* FTL	FTL	0.002**	280.2	< 0.001***
	pH	0.636 [NS]		

566 Model degree of freedom: 3 and 186

567   **Table 3.** *Sparus aurata.* Daily rate of calcium incorporation (mmol.g$_{\text{otolith dry weight}}^{-1}$.d^{-1}; mean ±

568   sd) in otolith and parameters of the multiple linear regressions with pH as continuous factor.

| pH$_T$	N	Ca incorp. rate	R^2	Multiple linear regression		
				Parameters	Par. p-value	Model
pH 8.1	49	0.052 ± 0.001	0.962	Time	< 0.001	F test: 1753
pH 7.9	48	0.060 ± 0.002	0.969	pH	0.369	P < 0.001
pH 7.7	49	0.062 ± 0.002	0.974	Time x pH	< 0.001	
pH 7.5	50	0.062 ± 0.001	0.965			

569   R^2: determination coefficient; Model degree of freedom : 3 and 187 df.

570 **FIGURES**
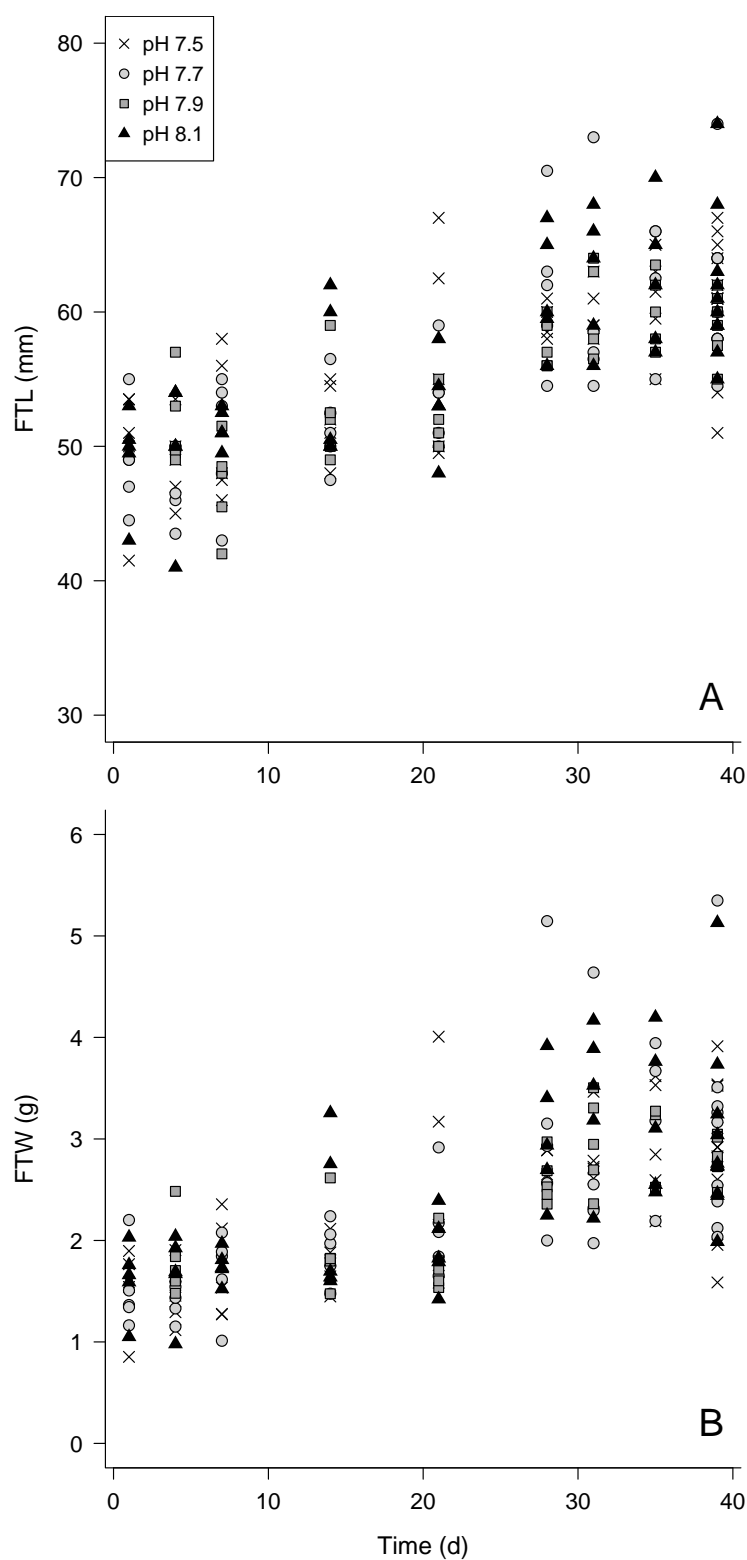
571
572 **Figure 1**. *Sparus aurata*. (**A**) Fish total length (mm) versus time (days of experiment) in the
573 different pH conditions. (**B**) Fish total weight (g) versus time (days of experiment) in the
574 different pH conditions.
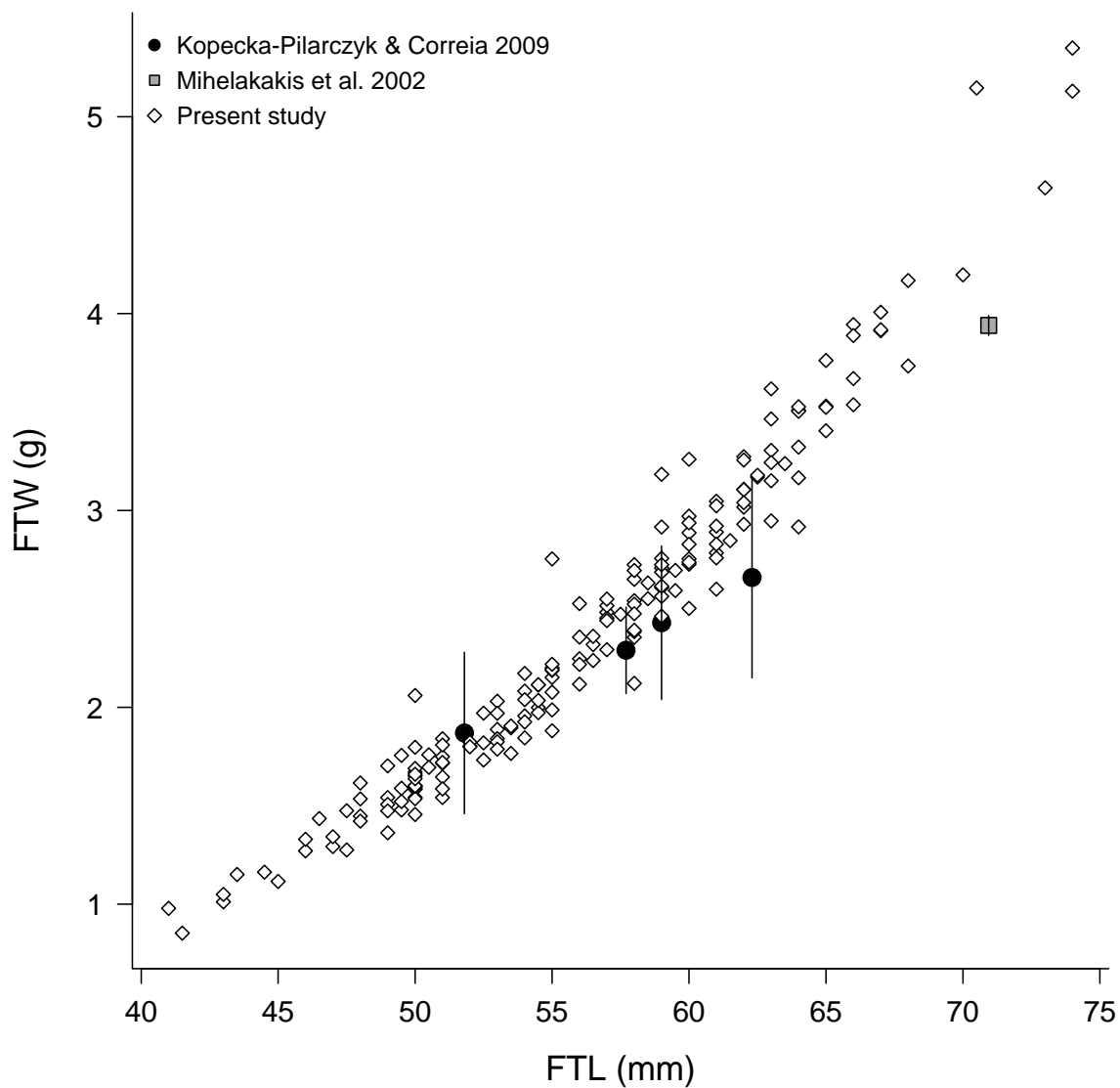
575
Figure 2. *Sparus aurata*. Fish total weight (g) versus fish total length (mm) recorded
individually in this study (all 4 pH conditions merged) and means (±sd for weight) reported by
Mihelakakis et al. (2002) and Kopecka-Pilarczyk and Correia (2009).
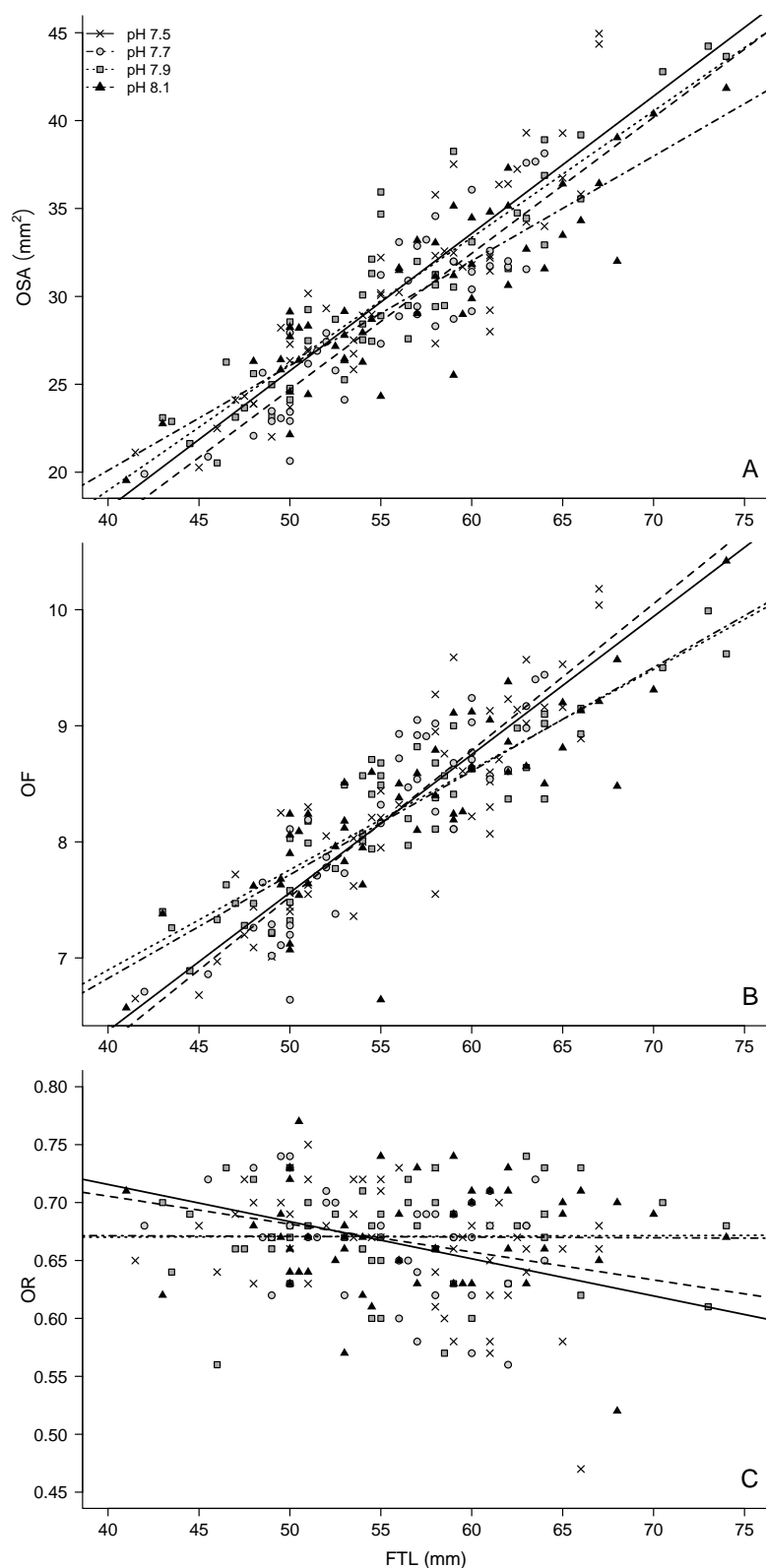
Figure 3. *Sparus aurata*. (**A**) Allometric relationship between the otolith surface area (OSA, mm^2) and the fish total length (FTL, mm), (**B**) Otolith Feret maximum diameter (OF) and (**C**) otolith roundness (OR) measured in juvenile seabreams raised during 40 d at four different pH$_T$ treatments 7.5, 7.7, 7.9, and 8.1 (1 tank per treatment, see Table 2 for statistical significance). An otolith roundness value of 1 means a circular shape. For OR, only data recorded at pH 7.5 were significantly linearly distributed (full black line, p = 0.0046).
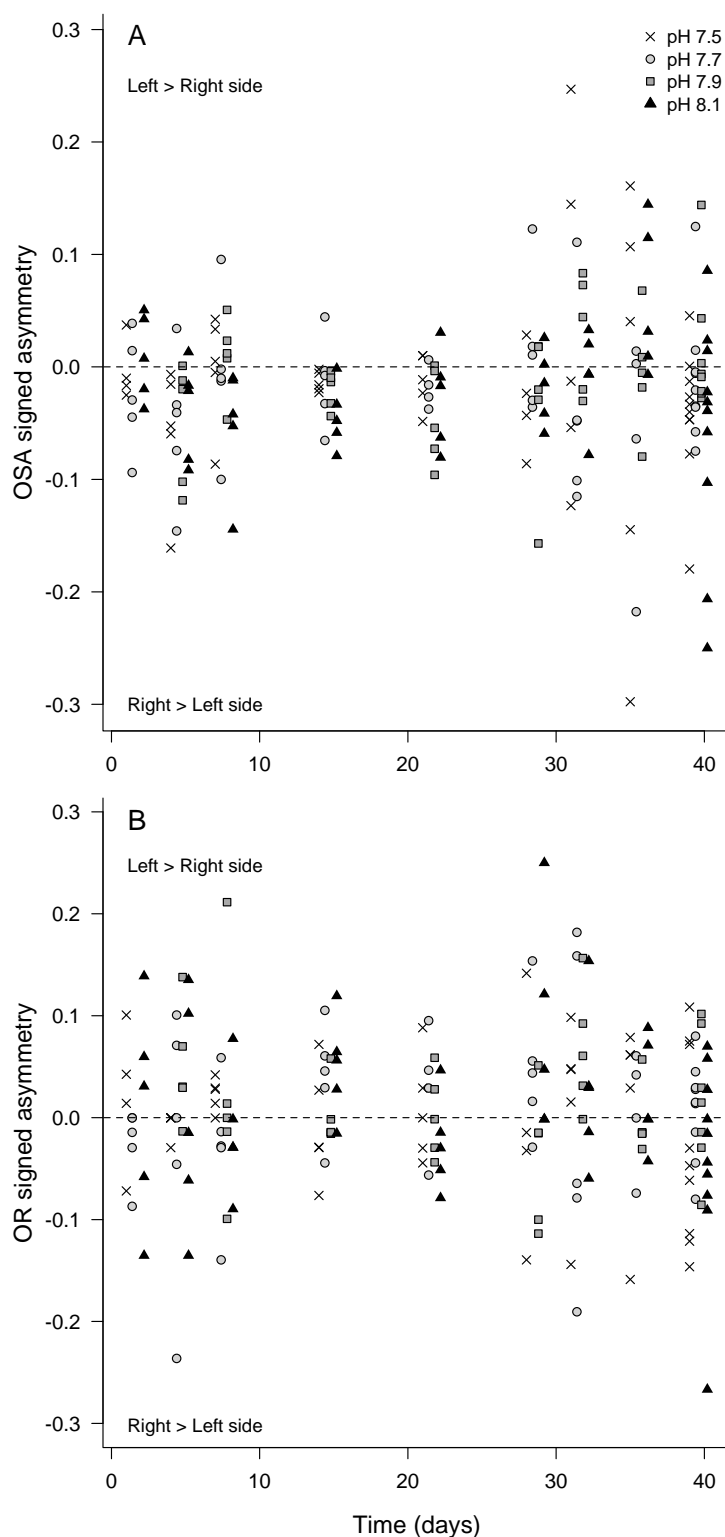
Figure 4. *Sparus aurata*. Asymmetry ([left - right]/mean$_{left,right}$) in (**A**) otolith surface area (OSA) and (**B**) otolith roundness (OR) between left and right sagitta of juveniles reared at 4 pH treatments 7.5, 7.7, 7.9, and 8.1 (1 tank per treatment) during 40 d. Dashed-line represents symmetry; offset in the *x*-axis was used to display the data per pH treatment.
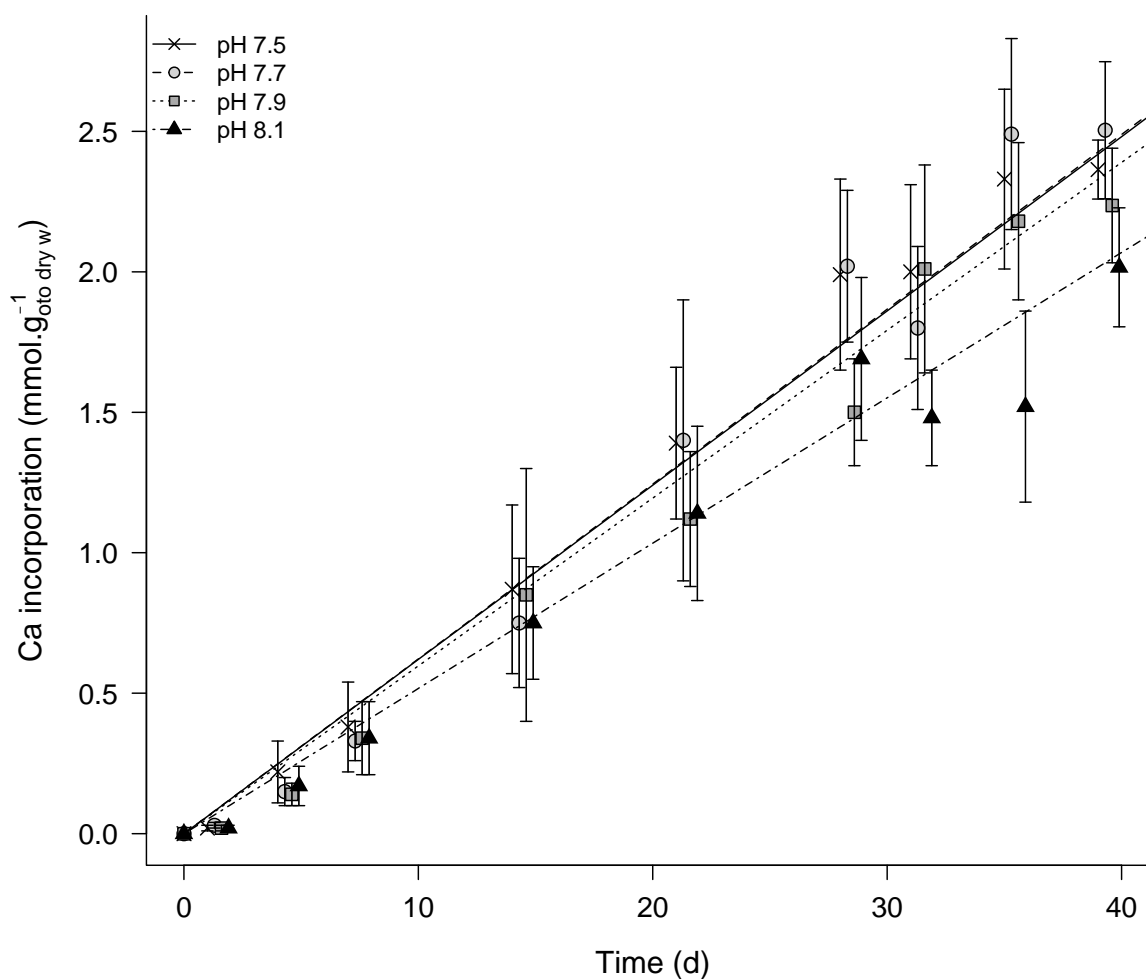
Figure 5. *Sparus aurata*. Incorporation of calcium (mmol.$g_{\text{otolith dry weight}}^{-1}$ ± sd) in sagittal otolith of juvenile seabreams reared at 4 pH treatments 7.5, 7.7, 7.9, and 8.1 (1 tank per treatment) during 40 d. Offset in the *x*-axis was used to display the data per pH treatment. See Table 3 for data and statistical comparisons. Rates of Ca incorporation calculated on pseudoreplicates at pH_T 8.1 are 18, 27 and 25% lower than rates at pH_T 7.9, 7.7 and 7.5, respectively.