bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

1 **Full title:** Sex dimorphism in European sea bass (*Dicentrarchus labrax* L.): new insights into sex-related
2 growth patterns during very early life stages

3 **Short title:** European sea bass sex-related growth patterns during early life stages

4 Sara Faggion[1], Marc Vandeputte[1,2], Alain Vergnet[1], Frédéric Clota[1,2], Marie-Odile Blanc[1], Pierre Sanchez [1,2],
5 François Ruelle[1], François Allal[1]*

6 [1] MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots, France

7 [2] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France

8 *Corresponding author at: Ifremer, MARBEC, Chemin de Maguelone, 34250, Palavas-les-Flots, France.

9 Tel.: +33 4 67 13 04 04. E-mail: francois.allal@ifremer.fr (F. Allal).

10 ## Abstract

11 The European sea bass (*Dicentrarchus labrax*) exhibits female-biased sexual size dimorphism (SDD) early in
12 development. New tagging techniques provide the opportunity to monitor individual sex-related growth during
13 the post-larval and juvenile stages.

14 We produced an experimental population through artificial fertilization and followed a rearing-temperature
15 protocol (~16 °C from hatching to 112 days post-hatching, dph; ~20 °C from 117 to 358 dph) targeting a
16 roughly balanced sex ratio. The fish were tagged with microchips between 61 and 96 dph in five tagging trials
17 of 50 fish each; individual standard length (SL) was recorded through repeated biometric measurements
18 performed between 83 to 110 dph via image analyses. Body weight (BW) was modelled using the traits
19 measured on the digital pictures (i.e. SL, area, height, perimeter and volume). At 117 dph, the fish were tagged
20 with microtags and regularly measured for SL and BW until 335 dph. The experiment ended at 358 dph with
21 the sexing of the fish.

22 The sex-ratio at the end of the experiment was significantly in favor of the females (65.9% vs. 34.1%).

23 The females were significantly longer and heavier than the males from 103 dph (~30 mm SL, ~0.44 g BW) to
24 165 dph. A significant difference in the daily growth coefficient (DGC) was observed only between 96 and
25 103 dph, suggesting a physiological or biological change occurring during this period.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

26 The female-biased SSD pattern in European sea bass is thus strongly influenced by very early growth
27 differences between sexes, in any case long before gonadal sex differentiation has been started. This leads to
28 the hypothesis that early growth may be a cause rather than a consequence of sex determination in sea bass.

29 Introduction

30 The phenomenon of sexual size dimorphism (SDD) is common in animal species, and it is represented by the
31 differences in average body size of adult males and females [1]. Female-biased SDD is explained as a situation
32 where females are larger than males, while male-biased SDD is the reverse situation.
33 Male-biased SDD has been described in various teleost fish species: in different tilapiine strains, adult males
34 are much larger than adult females [2] and have a faster growth rate [3]; the same pattern has been observed
35 in cichlids [4], salmonids [5, 6] and catfishes [7]. Male-biased SDD is evolutionary linked to increased male-
36 male competition, territoriality or female-choice [8]. Conversely, female-biased SDD is linked to increased
37 fecundity of larger females and decreased male-male competition [8]; it has been observed, among the others,
38 in turbot *Psetta maxima* [9, 10] and in the European eel *Anguilla anguilla* [11].
39 Female-biased SDD is also a characteristic of the European sea bass (*Dicentrarchus labrax* L.), one of the
40 major aquaculture species in the Mediterranean area. The females of this species are known to be about 30%
41 heavier than the males from 300-400 g until over 1000 g [12, 13]. Furthermore, the common aquaculture
42 practice of size grading has shown that the largest fish selected at 86 days post-hatching (dph) later result to
43 be mostly females [14]. Previous studies exploiting individual tagging suggested that females are already
44 significantly heavier than males from 105 dph (1024 degree days above 10 °C), with a stable 40% difference
45 from 197 to 289 dph [15].
46 This supports the hypothesis that in European sea bass sex-specific growth may happen before gonadal sex
47 differentiation, as differentiation starts only around 128 dph [14]. In this species, there are no sex chromosomes
48 or “genetic sex”, as sex is determined by the combination of multiple genes and environmental temperature
49 (see the review by [16]).
50 New insights into the onset of sexual dimorphism in European sea bass during the post-larval stages can be
51 gained through the application of ultra-small tagging technologies to individually identify and monitor small-
52 bodied fish. 1 × 6 mm RFID glass microtags have been tested in European sea bass, allowing the tracking of

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

53 individuals from 96 dph (SL, standard length, ~ 36 mm; [15]). More recently, RFID microchip (0.5 × 0.5 mm)
54 tagging has been performed in fish aged 75 dph (SL ~ 20 mm; [17]).
55 This study exploited the techniques of microchip tagging and microtagging described in previous papers [15,
56 17, 18] to identify and follow European sea bass individuals from a post-larval stage (83 dph or 510 degree
57 days above 10 °C) until an age at which a reliable sex-identification age through gonads inspection was
58 possible (358 dph or 3229 degree days above 10 °C). Growth data were individually recorded during the
59 experiment, and related to the sex of the fish as observed at 358 dph. The aim of the study was to identify
60 differences in growth trajectories between males and females at the most precocious stage ever monitored and,
61 in any case, when the morphological sex differentiation of the gonads has not occurred yet.

62 Materials and Methods

63 Production, rearing and microchip tagging of the experimental fish

64 The experiment was evaluated by the Ethical Committee (n° 036) and authorized by the French Ministry of
65 Higher Education, Research and Innovation (APAFIS#19713-2019010917222576v3). All experimental
66 procedures were conducted following the guidelines for animal experimentation established by the Directive
67 2010-63-EU of the European Union and the equivalent French legislation.
68 The production of the experimental fish is detailed in [17]. Briefly, artificial fertilization was performed in
69 March 2019 at the IFREMER experimental facilities (Palavas-les-Flots, France) using the eggs of two dams
70 and the cryopreserved sperm of five sires, from a synthetic F2 line originating from both Atlantic and
71 Mediterranean broodstock. After hatching, larvae were reared in a common garden under controlled conditions
72 (mean rearing temperature: 16.4 °C; salinity: 30.5‰). Fish were tagged with 0.5 × 0.5 mm microchips inserted
73 in the peritoneal cavity during five tagging trials between 61 dph (or 372 degree days above 10 °C) and 96 dph
74 (or 596 degree days above 10 °C; 50 fish in each trial; see [17]) and distributed each time to a new tank (N =
75 250 in total). The conditions of salinity and temperature of all the different tanks were the same as the common
76 garden tank and were maintained until 112 dph (or 720 degree days above 10 °C); the following 5 days the
77 temperature was gradually increased to 20 °C. Fish were then reared at a mean temperature of 20.3 °C (19.3–
78 21.2°C), salinity of 36.5‰ and photoperiod at 12L:12D (light:dark).

79 Growth monitoring, microtagging and sex recording

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

80 Biometric measurements were performed at 83, 89, 96, 103, 110, 117, 137, 150, 165, 180, 201, 223, 265, 302
81 and 335 dph. Each fish was anesthetized with MS-222 (Sigma-Aldrich, 0.07 g/l of seawater; [19]), recognized
82 through microchip ID reading, then placed over a light table (Ultra Slim Light Box, Microlight) and
83 photographed using a stand with a digital camera (12.2 megapixel). The measure of the standard length of each
84 fish were performed through image analysis (ImageJ software 1.51; [20]; see Supplementary material 1, Fig
85 S1).
86 When fish reached 117 dph (or 756 degree days above 10 °C), they were all weighed and tagged with a second
87 tag through intra-coelomic implantation of 1 × 6 mm glass microtags (Lutronics, Nonatec RFID, Lutronics
88 International, Rodange, Luxembourg) following the protocol by [18]. This was done to prevent loss of the
89 identity due to the increasing difficulties of optical microchip reading as fish grow, and thus to enable
90 individual growth data recording and correct sex assignment at the end of the experiment. The fish were
91 anesthesized using 32.5 µl from a 10% stock solution of ethyl-p-aminobenzoate (Benzocaine E1501, Sigma-
92 Aldrich) dissolved in 100% ethanol, per 100 ml of seawater solution. The tagging protocol consisted in piercing
93 a hole in the abdominal cavity of the fish with an 18-gauge needle, the microtag was picked up with a Dumont
94 n° 3 forceps, inserted and pushed inside into the abdominal cavity through the hole. The fish were then
95 transferred to a tank of isosmotic 0.2 µm filtered and sterilized seawater for recovery (to avoid osmotic stress
96 and prevent infections) and they were allowed to rest for 1 to 2 h before being returned to their rearing tank.
97 Fish were reared in a common garden tank from 117 to 358 dph with the same conditions described above
98 (mean temperature of 20.28 °C, 36.5‰ salinity and photoperiod at 12L:12D).
99 During the biometric measurement performed between 137 and 335 dph, fish were anesthetized as described
100 above, adjusting the anesthetic solution of ethyl-p-aminobenzoate and seawater according to the increasing
101 size of the fish, the microtag was read, the body weight and the standard length were individually registered.
102 At 358 dph (or 3229 degree days above 10 °C) fish were euthanized with an excess of benzocaine solution and
103 the sex was determined macroscopically through the direct observation of the gonads or using a gonadal squash
104 [21] when visual observation was ambiguous (see Supplementary material 2, Fig S2).

105 **Prediction of body weight from digital picture measurements and prediction of standard length**

106 The biometric measures performed on early stage fish (83 to 110 dph) relied on image analyses that allowed
107 the measurement of length, height, perimeter and area. To build a model to estimate the body weight from

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

108 digital picture measures, we followed the procedure detailed by [22]. During the biometric measurements
109 performed at 83, 89, 96, 103 and 110 dph, 50 additional fish from the stock rearing tank were randomly chosen
110 and sacrificed with an excess of anesthetic (MS-222) to directly measure the length and the weight of each fish
111 (total number of fish = 250). The standard length was obtained with a V-12B 12" vertical optical comparator
112 (Nikon) that allowed an accurate measure through magnification of the larva. The measure of the body weight
113 was achieved using a precision scale (to the nearest 0.01 g) after drying the fish with absorbent paper.
114 In addition, a digital picture of each fish was taken following the same procedure used for the experimental
115 fish. ImageJ software 1.51 (Rasband, 1997-2018) was used to perform image analysis obtaining the measures
116 of area, perimeter, length and height. The steps of image analysis are fully described in [22].
117 A volume index was calculated for each fish from height and length as:

118 $$Volume = \frac{\pi * Height^2 * \ Length}{12}$$

119 Pearson's coefficient of correlation (r^2) between measurements obtained from image analysis and
120 measurements obtained directly was estimated in R using cor.test function (package *stats*, R version 3.5.0,
121 [23]). Multiple regression models using length, height, perimeter, area and volume were tested using lm and
122 glm functions in R (package *stats*). The efficiency of the models and regression equations exploiting different
123 combinations of the traits to predict BW was evaluated through the coefficient of determination (R^2) and the
124 Akaike information criterion (AIC). The validation was performed as described in [22] dividing the dataset
125 into a "model set" (74% of the dataset) and a "validation set" (26% of the dataset; 13 randomly chosen fish for
126 each biometric measurement).
127 The r^2 between estimated and measured BW as estimated to assess the accuracy of the prediction model.
128 During the biometric measurement performed at 117 dph, only BW was directly measured on the fish; for this
129 reason, a model to estimate standard length using body weight was built. The data from the 50 additional fish
130 sacrificed during each biometric measurement was used; standard length and body weight were log-
131 transformed. The procedures followed were the same as the model built for body weight.
132 The predictive models were then applied to the experimental fish dataset to estimate the body weight of fish
133 aged 83, 89, 96, 103 and 110 dph and the standard length of fish aged 117 dph.

134 **Daily growth coefficient**

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

135 The daily growth coefficient was computed from the body weight data for each period between two biometric
136 measurements. The formula was the following:

137
$$DGC = \frac{BW_f^{\frac{1}{3}} - BW_i^{\frac{1}{3}}}{t} \times 100$$

138 where BW_f is the final body weight, BW_i is the initial body weight and t is the number of days.

139 **Statistical analyses**

140 The number of males and females in the population were compared through χ^2 tests.
141 Data for SL, BW and DGC were checked for normality and for homoscedasticity through Shapiro-Wilk and
142 Bartlett's tests. When the assumptions of normality and homoscedasticity were respected, data were compared
143 through ANOVA to check sex-related early growth patterns. Post-hoc analyses to adjust the p-values were
144 performed through Tukey's test. When data were assessed as non-normal and/or variances were not
145 homogeneous, non-parametric Wilcoxon-Mann-Whitney test was performed (one test at once; the p-value was
146 adjusted through Bonferroni correction). The significance threshold for the statistical tests was p-value < .05.
147 All the tests were performed in R version 3.5.0, package *stats* [23].

148 # Results

149 **Prediction of body weight from digital picture measurements and prediction of standard length**

150 Pearson's coefficient of correlation (r^2) between measurements obtained from image analysis and
151 measurements obtained directly were all high and significant, ranging from 0.9533 to 0.9963 (see table S1 in
152 Supplementary material 3 for details). The traits with the greatest correlation with BW were area (0.9898, p <
153 .0001) and volume (0.9963, p < .0001).
154 The model exploiting all the digital picture measurements (length, area, perimeter, height) and volume was the
155 one with the lowest AIC (-1070.9) and the greatest R^2 (0.9947; see table S2 in Supplementary material 3 for
156 further details). The model was the following:

157 *BW* = 0.1582 + 0.0037 *(Area)* - 0.0017 *(Perimeter)* - 0.0307
158 *(Height)* - 0.0073 *(Length)* + 0.0006 *(Volume)* (1)

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

159 The global r^2 of the regression between measured and estimated BW using model (1) was 0.9969 ($p < .0001$).
160 The BW in the "model set" was estimated with an r^2 of 0.9974 ($p < .0001$), in the "validation set" with an r^2
161 of 0.9953 ($p < .0001$).
162 The coefficient of correlation between the logarithm of the measured SL and the logarithm of the measured
163 BW was significantly high (0.9873, $p < .0001$). The logarithm of SL was estimated for the fish aged 117 dph
164 (when only BW was directly measured) with the following model, having an AIC equal to -1111.5 and an R^2
165 equal to 0.9708:

166 $$log(SL) = 0.7350 + 0.2838\ (log(BW)) \quad (2)$$

167 The global r^2 of the regression between measured and estimated SL using model (2) was 0.9872 ($p < .0001$),
168 the "model set" r^2 was 0.9853 ($p < .0001$), the "validation set" r^2 was 0.9907 ($p < .0001$). For further details,
169 see Figs S3, S4 and S5 in Supplementary material 3.

170 **Proportions of males and females and sex-related growth patterns**

171 The reliable identification of the sex was possible, either through visual observation of the gonads or gonadal
172 squash, for the 98.4% of the fish; for the remaining 1.6%, the gonadal differentiation was not completed yet,
173 entailing some degree of uncertainty in the assignment of the sex. These fish were then removed from the
174 dataset. Globally, at the end of the experiment, 87 females and 45 males were detected, with a sex-ratio in
175 favor of the females of 65.9% vs. 34.1% for males (Table 1 and Table 2), which was significantly different (χ^2
176 $= 13.364$, p-value $= 3 \times 10^{-4}$).
177 Differences in terms of growth patterns were observed between females and males (Table 1). On average,
178 females were longer compared to males from 103 dph, when females were 6% longer than males, and a
179 significant difference was maintained until 165 dph, with females close to 4% longer than males. From 180
180 dph until the end of the experiment, the difference in length between females and males was small (around
181 2.5% in favor of females), and not significant.
182 Body weight followed approximately the same pattern (Table 2): females were on average heavier than males
183 at 103 dph (females were 20% heavier than males), between 117 and 165 dph (females were about 10% heavier
184 than males), and at 265 dph. From 180 dph until the end of the experiment, the difference in weight between

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

185 females and males was stable, and close to 8% in favor of females, although not significant most of the time
186 (except at 265 dph).
187 During the first three biometric measurements (at 83, 89 and 96 dph), even though the differences were not
188 significant, females were already around 2 to 6% longer and 13% heavier than males.
189 The daily growth coefficient (DGC) was higher in females in almost all the periods analyzed, with the only
190 exception of the interval between 103 and 110 dph (Table 3). Significant differences between males and
191 females were detected only during the interval between 96 and 103 dph, where the DGC of females was 32.5%
192 higher than that of males.

193 **Table 1**

194 Number and percentage of males and females, mean standard length (SL, mm) ± standard deviation and
195 mean body weight (BW, g) ± standard deviation for each age. Asterisks indicate significant differences
196 between males and females ($p < .01$ '**'; $p < .05$ '*').

Age (dph)	N (%)		SL (mm) ± SD		BW (g) ± SD	
	F	M	F	M	F	M
83	11 (61.1%)	7 (38.9%)	23.3 ± 1.3	22.8 ± 2.2	0.17 ± 0.04	0.15 ± 0.04
89	21 (70.0%)	9 (30.0%)	25.9 ± 1.9	25.2 ± 2.5	0.25 ± 0.06	0.22 ± 0.06
96	28 (62.2%)	17 (37.8%)	28.2 ± 1.7	26.7 ± 2.9	0.34 ± 0.08	0.30 ± 0.10
103	45 (62.5%)	27 (37.5%)	31.3 ± 2.2**	29.4 ± 3.0**	0.48 ± 0.12**	0.40 ± 0.13**
110	40 (60.6%)	26 (39.4%)	33.6 ± 2.3*	32.2 ± 2.9*	0.63 ± 0.14	0.56 ± 0.16
117	87 (65.9%)	45 (34.1%)	35.7 ± 2.2*	34.5 ± 2.8*	0.78 ± 0.17*	0.70 ± 0.19*
137	87 (65.9%)	45 (34.1%)	57.3 ± 3.7*	55.3 ± 5.2*	2.38 ± 0.44*	2.16 ± 0.52*
150	87 (65.9%)	45 (34.1%)	62.2 ± 4.3	60.6 ± 6.1	3.21 ± 0.62*	2.93 ± 0.78*
165	87 (65.9%)	45 (34.1%)	74.4 ± 5.0*	71.9 ± 6.9*	5.35 ± 1.06*	4.89 ± 1.36*
180	87 (65.9%)	45 (34.1%)	83.7 ± 5.4	81.2 ± 7.8	8.26 ± 1.73	7.63 ± 2.18
201	87 (65.9%)	45 (34.1%)	95.1 ± 8.5	93.7 ± 11.1	14.27 ± 3.14	13.19 ± 4.08
223	87 (65.9%)	45 (34.1%)	118.8 ± 8.2	115.9 ± 11.0	23.17 ± 5.18	21.55 ± 6.46
265	87 (65.9%)	45 (34.1%)	142.3 ± 10.3	138.3 ± 14.2	40.97 ± 9.44*	37.58 ± 12.2*
302	86 (65.6%)	45 (34.4%)	161.7 ± 11.8	158.1 ± 16.9	59.71 ± 14.0	55.11 ± 18.5
335	86 (65.6%)	45 (34.4%)	176.6 ± 12.9	172.0 ± 17.5	79.19 ± 18.9	73.44 ± 24.8

197

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

198 **Table 2**

199 Number and percentage of males and females, mean daily growth coefficient (DGC) ± standard deviation for

200 each age interval. Asterisks indicate significant differences between males and females (p < .01 '**').

Age interval (dph)	N (%)		DGC ± SD	
	F	M	F	M
83-335	11 (61.1%)	7 (38.9%)	1.44 ± 0.14	1.38 ± 0.18
83-89	8 (53.3%)	7 (47.7%)	0.89 ± 0.34	0.87 ± 0.15
89-96	15 (71.4%)	6 (28.6%)	0.99 ± 0.24	0.87 ± 0.24
96-103	23 (60.5%)	15 (39.5%)	1.03 ± 0.27**	0.78 ± 0.25**
103-110	30 (57.7%)	22 (42.3%)	1.16 ± 0.25	1.21 ± 0.17
110-117	38 (59.4%)	26 (49.6%)	0.90 ± 0.38	0.81 ± 0.30
117-137	87 (65.9%)	45 (34.1%)	2.08 ± 0.17	2.03 ± 0.18
137-150	87 (65.9%)	45 (34.1%)	1.06 ± 0.25	1.05 ± 0.25
150-165	87 (65.9%)	45 (34.1%)	1.82 ± 0.23	1.75 ± 0.25
165-180	87 (65.9%)	45 (34.1%)	1.80 ± 0.36	1.79 ± 0.61
180-201	87 (65.9%)	45 (34.1%)	1.91 ± 0.31	1.84 ± 0.56
201-223	87 (65.9%)	45 (34.1%)	1.92 ± 0.27	1.90 ± 0.27
223-265	87 (65.9%)	45 (34.1%)	1.41 ± 0.23	1.32 ± 0.28
265-302	87 (65.9%)	45 (34.1%)	1.24 ± 0.23	1.21 ± 0.27
302-335	86 (65.6%)	45 (34.4%)	1.16 ± 0.29	1.15 ± 0.27

201

202 # Discussion

203 The miniaturization of fish tagging technologies has enabled the identification and tracking of individuals from
204 an early life stage, providing the opportunity of studying many biological and physiological changes occurring
205 during these sensitive phases.

206 Recent papers claimed the effectiveness of microtags [15] and microchips [17] as tagging tools for European
207 sea bass post-larval individuals. In this study, we used a combination of these two tagging methods to
208 efficiently identify the fish during the post-larval stage with microchips (from 83 dph and a mean SL of ~23
209 mm to 110 dph and a mean SL of ~33 mm) and during the juvenile stage with microtags (from 117 dph and a
210 mean SL of ~36 mm to 358 dph and a mean SL of ~171 mm). This allowed us to record individual growth
211 data through repeated biometric measurements. At the end of the experiment, the individual growth data were
212 related to the sex in order to gain knowledge about early sex-related growth patterns in the European sea bass.
213 Our study confirmed and strengthened the already known sex dimorphic growth pattern in the European sea
214 bass [12, 13, 14, 15], providing evidence of significant SSD in favor of the females in terms of body weight
215 and standard length. While the pattern of SSD after 10 months of age is well known, with a maximal difference

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

216 at ~1 year of age, followed by a slow decay [13, 24], its earlier dynamics remained poorly described, due to
217 the inability to tag fish before SSD builds up. The earliest tagging study to date was that of [15], which showed
218 that a 31% SSD for weight in favor of females was already established at 105 dph (0.59 g mean weight, 27–
219 53 mm total length). Other experiments with size graded groups have shown that SSD is already established
220 at 80 dph (36–45 mm total length) as sorting the largest individuals at that size resulted in a clear excess of
221 females, compared with the general population [14].

222 In the present study, we started measuring growth on the fish 22 days before [15] (83 vs 105 dph), but the
223 difference in terms of developmental stages was even greater, as [15] used a rearing protocol more similar to
224 hatcheries standard procedures, where temperature is raised from 16.5 to 22°C an earlier date (60 dph, Chatain,
225 pers. comm, vs. 112 dph in our study). We were thus able to individually follow the growth of future males
226 and females starting from 0.16 g instead of their 0.59 g.

227 At 83 dph, the fish were 23 mm SL and 0.16 g BW, and males and females were not yet statistically different
228 in size. Then, SSD built up and from 103 dph (645 degree days above 10 °C) the differences between males
229 and females became significant, until 165 dph (1241 degree days above 10 °C). The time when SSD builds up
230 is also shown by the difference in growth rate, measured as DGC, which was 32% higher in females than in
231 males from 96 to 103 dph. We cannot completely rule out that SSD existed before this time, as females were
232 (not significantly) larger than males as of 83 dph, and the lack of significance may be caused by the limited
233 samples size at those ages. Indeed, the sample size during the first period (83 to 110 dph) was rather low, due
234 to the fact that fish were not all tagged at the beginning of the experiment but at different ages, as we did not
235 initially know if they would survive and grow normally after such an early tagging (see [17] for details). Some
236 difficulties linked to the optical reading of the microchip, especially at 110 dph, also made that not all fish had
237 a complete set of growth measurements. Anyway, the main period for the onset of SSD in sea bass seems to
238 start around 96 dph (596 degree-days above 10 °C, with 0.33 g BW and 27.5 mm SL fish). This period is also
239 an important period for sex determination in sea bass, as rearing fish at cold temperature (< 17 °C) beyond that
240 time orients sex determination towards males, while earlier cold rearing promotes female sex determination
241 [Vandeputte et al., submitted]. In any case, it is clear that this phase of faster growth of females occurs well
242 before the start of histological sex differentiation, which occurs first in females, at a SL of 80-100 mm [14, 25,
243 26]. The first signs of molecular sex differentiation (higher expression of aromatase *cyp191a1* in future

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

244 females) are observed somewhat earlier, at a SL of 55 mm [27], but this still happens much later than the onset
245 of differential growth, which started around 27.5 mm SL in our experiment. This leads to two non-exclusive
246 hypotheses. Firstly, it may be that the differentiation pathway between males and females starts earlier than
247 what has been evidenced for the moment. Microchips could help the study of this in the future, giving access
248 to the future sex of fish as small as 23 mm, but this would have to be coupled with non-lethal sampling for
249 gene expression, which is far from simple at such a small size. Secondly, we should consider the possibility
250 that faster growth would be the cause and not the consequence of sex differentiation towards females. This
251 hypothesis has already been tested before, but with larger fish. It was shown that manipulating growth by food
252 restriction starting at 80 or 40 mm SL did not impact sex-ratios in the treated groups [28]. Taken together,
253 those results and ours suggest that very early (from 25 to 40 mm SL) growth may be the cause of female sex
254 differentiation in sea bass.

255 We observed a lower SSD between males and females compared to previous studies [13, 15, 24] which could
256 be linked to the fact that long cold rearing temperature also tends to decrease SSD in European sea bass
257 [Vandeputte et al., submitted]. This may also have been influenced by the population used, which is a mixed
258 population between Atlantic and Mediterranean sea bass. There are important differences in growth dynamics
259 between these two lineages [29], although population differences in SSD have not been investigated for the
260 moment.

261 Another aspect that may have affected SSD in the present study is the effect of microchip tagging on the fish.
262 It could have acted as a sorting event, selecting, *de facto*, the “stronger” fish characterized by a greater growth
263 potential, and thus eliminating the smaller fish, more likely to develop as males, which may at the same time
264 decrease SSD if the smallest males are removed from the population, and increase the proportion of females
265 in fish surviving until sexing.

266 Indeed, in our experiment, the sex ratio was significantly skewed towards females. This is in contrast with the
267 common observations of strongly unbalanced sex-ratio in favor of males in cultured sea bass; indeed, the
268 standard hatchery practices imply high rearing temperatures, that play a role in the masculinization of
269 developing fish [14, 30]. In our case, we followed a particular rearing-temperature protocol to obtain a roughly
270 balanced sex ratio. The experimental fish were exposed to low rearing temperatures (~16 °C) during the first
271 part of their life (from hatching to 112 dph), and to higher temperatures (~20 °C) during the second part of

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

272 their life, targeting a balanced sex-ratio, following [31]. However, recent results show that continuing exposure
273 to cold temperature is likely to have an opposite effect on sex determination, progressively favoring males
274 with time spent below 17 °C beyond 55-75 dph [Vandeputte et al., submitted]. This may indirectly support our
275 previous hypothesis that tagging may have indirectly increased the proportion of females. However, it has to
276 be noted that the variation of sex-ratios in sea bass in different experiments using the same temperature
277 treatment remains very high, for reasons that are not identified for the moment [31, Vandeputte et al.,
278 submitted]
279 The fact that SSD in sea bass is established very early had already been evidenced indirectly by sorting
280 experiments [14, 26, 28], and using genetic links by repeatedly sampling the same families at different ages
281 [24]. This is more precisely documented by the present experiment, by monitoring the individual growth of
282 future males and females starting at 23 mm standard length, at 83 dph. For the first time, we could identify the
283 stage at which differential growth happens, which peaks between 96 and 103 dph (596 to 645 degree days
284 above 10 °C, 27.5 to 30.3 mm SL, 0.32 to 0.44 g BW). This provides key information to study the hypothesis
285 that faster growth may cause female differentiation in this species, which is plausible as SSD is established
286 long before the first known signs of sex differentiation.

287 **Funding**

288 The study was funded by the French Ministry of Agriculture (CRECHE2019 project).

289 **Acknowledgements**

290 We wish to thank Intellibio (Seichamps, France) for providing technical support and instrumentation.

291 **Declaration of competing interest**

292 The authors declare that they have no competing interests.

293 **Data Availability Statement**

294 The datasets underlying our findings are available in the institutional public data repository (SEANOE:
295 http://www.seanoe.org/).

296 Supporting information

297 **S1 Figure. Image analysis to recover length, height, perimeter and area of the fish** (DOCX)

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

298 **S2 Figure. Determination of the sex of the fish** (DOCX)

299 **S3 Tables and Figures. Prediction of body weight from digital picture measurements and prediction of**
300 **standard length** (DOCX)

301
References

302 1. Fairbairn D, Blanckenhorn W, Székely T. Sex, size, and gender roles. Evolutionary studies of sexual
303 size dimorphism. Oxford University Press; 2007.

304 2. Lind C, Safari A, Agyakwah S, Attipoe F, El-Naggar G, Hamzah A, et al. Differences in sexual size
305 dimorphism among farmed tilapia species and strains undergoing genetic improvement for body weight.
306 Aquacult Rep. 2015;1: 20-27.

307 3. Bhatta S, Iwai T, Miura T, Higuchi M, Maugars G, Miura C. Differences between male and female
308 growth and sexual maturation in tilapia (*Oreochromis mossambicus*). KUSET. 2013;8: 57-65.

309 4. Schütz D, Taborsky M. Giant males or dwarf females: what determines the extreme sexual size
310 dimorphism in *Lamprologus callipterus*? J Fish Biol. 2000;57: 1254-1265.

311 5. Quinn T, Foote C. The effects of body size and sexual dimorphism on the reproductive behaviour of
312 sockeye salmon, *Oncorhynchus nerka*. Anim Behav. 1994;48: 751-761.

313 6. Bonnet S, Haffray P, Blanc J, Vallée F, Vauchez C, Fauré A, et al. Genetic variation in growth
314 parameters until commercial size in diploid and triploid freshwater rainbow trout (*Oncorhynchus*
315 *mykiss*) and seawater brown trout (*Salmo trutta*). Aquaculture. 1999;173: 359-375.

316 7. Haffray P, Vauchez C, Vandeputte M, Linhart O. Different growth and processing traits in males and
317 females of European catfish, *Silurus glanis*. Aquat. Living Resour. 1998;11: 341-345.

318 8. Horne CR, Hirst AG, Atkinson D. Selection for increased male size predicts variation in sexual size
319 dimorphism among fish species. Proc Biol Sci. 2020;287: 20192640.

320 9. Imsland A, Folkvord A, Grung G, Stefansson S, Taranger G. Sexual dimorphism in growth and
321 maturation of turbot, *Scophthalmus maximus* (Rafinesque, 1810). Aquac Res. 1997;28: 101-114.

322 10. Aydin I, Sahin T, Kolotoglu L, Özongun M. The effect of sexual dimorphism on growth of the black
323 sea turbot, *Psetta maxima*. J. FisheriesSciences.com. 2011;5: 47-51.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

324 11. Tzeng WN. Fisheries, stocks decline and conservation of anguillid eel. In: Arai T, editor. Biology and
325 ecology of anguillid eels. CRC Press Taylor and Francis group, Boca Raton, FL, USA; 2016. pp. 291-
326 324.
327 12. Chatain B, Peruzzi S, Saillant E. Sex determination in *Dicentrarchus labrax*, no evidence for male or
328 female heterogamety. In: Baroiller JF, Guerrier D, Guiguen Y, editors. Proceedings of the IVe Atelier
329 Déterminisme et Différenciation du Sexe. Station Commune de recherche en Ichtyophysiologie
330 Biodiversité Environnement, INRA, Rennes, France; 1997. p. 18.
331 13. Saillant E, Fostier A, Menu B, Haffray P, Chatain B. Sexual growth dimorphism in sea bass
332 *Dicentrarchus labrax*. Aquaculture. 2001;202 : 371-387.
333 14. Saillant E, Fostier A, Haffray P, Menu B, Laureau S, Thimonier J, et al. Effects of rearing density, size
334 grading and parental factors on sex ratios of the sea bass (*Dicentrarchus labrax* L.) in intensive
335 aquaculture. Aquaculture. 2003;221: 183-206.
336 15. Ferrari S, Chatain B, Cousin X, Leguay D, Vergnet A, Vidal M, et al. Early individual electronic
337 identification of sea bass using RFID microtags: a first example of early phenotyping of sex-related
338 growth. Aquaculture. 2014;426-427: 165-171.
339 16. Vandeputte M, Piferrer F. Genetic and environmental components of sex determination in the European
340 sea bass (*Dicentrarchus labrax*). In: Wang HP, Piferrer F, Chen SL, Shen ZG, editors. Sex control in
341 aquaculture: theory and practice. Wiley-Blackwell Hoboken, NJ, USA; 2019. pp. 307-320.
342 17. Faggion S, Sanchez P, Vandeputte M, Clota F, Vergnet A, Blanc M-O, et al. Evaluation of a European
343 sea bass (*Dicentrarchus labrax* L.) post-larval tagging method with ultra-small RFID tags. Aquaculture.
344 2020;520: 734945.
345 18. Cousin X, Daouk T, Péan S, Lyphout L, Schwartz M-E, Bégout M-L. Electronic individual identification
346 of zebrafish using radio frequency identification (RFID) microtags. J Exp Biol. 2012;215; 2729-2734.
347 19. Chatain B, Corraoa, D. A sorting method for eliminating larvae without functional swimbladders.
348 Aquaculture. 1992;107: 81-88.
349 20. Rasband WS. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. 1997-2018.
350 Available from: https://imagej.nih.gov/ij/.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.297598; this version posted September 15, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

351 21. Menu B, Peruzzi S, Vergnet A, Vidal M, Chatain B. A shortcut method for sexing juvenile European
352 sea bass, *Dicentrarchus labrax* L. Aquac Res. 2005; 36: 41-44.
353 22. de Verdal H, Vandeputte M, Pepey E, Vidal M-O, Chatain B. Individual growth monitoring of European
354 sea bass larvae by image analysis and microsatellite genotyping. Aquaculture. 2014;434: 470-475.
355 23. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical
356 Computing, Vienna. 2018. Available from: https://www.R-project.org.
357 24. Vandeputte M. Genetic variation of growth and sex ratio in the European sea bass *(Dicentrarchus labrax*
358 L.) as revealed by molecular pedigrees. PhD thesis, AgroParisTech. 2012. Available from:
359 https://pastel.archives-ouvertes.fr/pastel-00957623.
360 25. Roblin C, Bruslé J. Ontogenèse gonadique et differénciation sexuelle du loup (*Dicentrarchus labrax*),
361 en conditions d'élevage. Reprod Nutr Dev. 1983;23: 115–127.
362 26. Papadaki M, Piferrer F, Zanuy S, Maingot E, Divanach P, Mylonas CC. Growth, sex differentiation and
363 gonad and plasma levels of sex steroids in male- and female-dominant populations of *Dicentrarchus*
364 *labrax* obtained through repeated size grading. J Fish Biol. 2005;66: 938-956.
365 27. Blázquez M, Navarro-Martin L, Piferrer F. Expression profiles of sex differentiation-related genes
366 during ontogenesis in the European sea bass acclimated to two different temperatures. J Exp Zool.
367 2009;312B: 686-700.
368 28. Díaz N, Ribas L, Piferrer F. The relationship between growth and sex differentiation in the European
369 sea bass (*Dicentrarchus labrax*). Aquaculture. 2013;408-409: 191-202.
370 29. Vandeputte M, Garouste R, Dupont-Nivet M, Haffray P, Vergnet A, Chavanne H, et al. Multi-site
371 evaluation of the rearing performances of 5 wild populations of European sea bass (*Dicentrarchus*
372 *labrax*). Aquaculture. 2014;424-425: 239-248.
373 30. Piferrer F, Blázquez M, Navarro L, González A. Genetic, endocrine, and environmental components of
374 sex determination and differentiation in the European sea bass (*Dicentrarchus labrax* L.). Gen Comp
375 Endocr. 2005;142: 102-110.
376 31. Navarro-Martín L, Blázquez M, Viñas J, Joly S, Piferrer F. Balancing the effects of rearing at low
377 temperature during early development on sex ratios, growth and maturation in the European sea bass
378 (*Dicentrarchus labrax*). Aquaculture. 2009;296: 347-358.