

1 **Effects of genetics and early life mild hypoxia on individual growth and size variation**
2 **in gilthead sea bream (*Sparus aurata*)**

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33

34 **Abstract**

35 The present study evaluated, in an 18-month gilthead sea bream trial, the time course effects of
36 genetics on individual size variation and growth compensation processes in the offspring of families
37 selected by growth in the PROGNSA breeding program. Families categorized as fast, intermediate
38 and slow growing had different growth trajectories with a more continuous growth in fast growth
39 families. This feature was coincident with a reduced size variation at the beginning of the trial that
40 clustered together the half-sib families sharing the same father. Correlation analysis evidenced that
41 the magnitude of compensatory growth was proportional to the initial size variation with no rescaling
42 of families at this stage. By contrast, the finishing growth depensation process can mask, at least
43 partially, the previous family convergence. This reflects the different contribution across the
44 production cycle of genetics and environmental factors in growth, production and welfare. How early
45 life experiences affect growth compensatory at juvenile stages was also evaluated in a separate cohort,
46 and intriguingly, a first mild-hypoxia pulse at 60-81 days posthatching (dph) increased survival rates
47 by 10% preventing growth impairment when fish were exposed to a second hypoxia episode (112-
48 127 dph). The early hypoxia experience did not have a negative impact on growth compensatory
49 processes at juvenile stages. By contrast, a diminished capacity for growth compensation was found
50 with repeated or late hypoxia experiences. All this reinforces the use of size variation as a main
51 criterion for improving intensive fish farming or selective breeding into practice.

52

53 **Keywords:** Size heterogeneity; Growth compensation; Early life hypoxia; Selective breeding;
54 Gilthead sea bream

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83 **Authors' contributions:** JPS coordinated and designed the study; EP, ERM, FNC, PSM, JCG farmed
84 fish from early life stages to the end of trial and participated in all samplings, JPS, ERM, EP analyzed
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86 **Introduction**

87 Growth trajectories of most animals are nearly identical when rescaled by body mass at maturity and
88 time to reach mature size (Sibly et al. 2015). However, how metabolic processes fuel and regulate
89 growth across life is under debate, though growth deceleration with increasing size appears mostly
90 related to a limited diffusion rate of nutrients and oxygen (O₂) (Glazier 2010). Besides, not all
91 individuals grow at the same rate and the large size variation of animals from the same cohort
92 illustrates the great propensity of individuals to develop and grow at different rates (Pfister and
93 Stevens 2002; Peacor et al. 2007; Teder et al. 2008). Growth variability is in fact a key ecological
94 attribute differentiating individuals, populations and species (Dmitriew 2011; Vincenzi et al. 2014),
95 contributing the habitat selection to maintain individuals on an optimal growth trajectory if habitat
96 variation through life stages allows different ecotypes (Rosenfeld et al. 2015). Indeed, the different
97 growth and life history traits of wild and farmed fish offer insights on the processes of growth
98 regulation under natural selection, and the extent to which such processes can be altered by artificial
99 selection or genetic modifications.

100 The overall high growth rates of domesticated fish stocks have been achieved by selection for
101 high rates of energy intake and allocation of metabolic energy to rapid biomass production at the
102 expense of a reduced energy expenditure on activity, predator avoidance or reproduction (Devlin et
103 al. 2004; Sibly et al. 2015). Other factors such as rearing density, O₂ availability and social
104 interactions also have major effects on growth phenotypic variability of farmed fish (Karplus et al.
105 2000; Barbosa et al. 2006; Azaza et al. 2013; Martos-Sitcha et al. 2019a). However, size
106 heterogeneity is often ignored as a selection criterion in Mediterranean fish farming, though growth
107 variability is considered one of the most important key performance indicators (KPI) to benchmark
108 the efficiency of productive systems within the framework of the PerformFISH H2020 EU project
109 (<http://performfish.eu>). Certainly, size heterogeneity is a good marker of environmental quality in
110 amphibians and terrestrial livestock animals (Peacor and Pfister 2006; Peacor et al. 2007; Teder et al.
111 2008), and both in plant and animal genetics, the reduction of environmental variance produces
112 animals that cope better with their environment (Mulder et al. 2008). Rabbits are a good example
113 since the environmental variance of litter size is genetically determined and closely related to the
114 viability of kits (Bodin et al. 2010; Blasco et al. 2017).

115 The growth rate of an individual organism has thereby ecological and evolutionary implications
116 (Arendt 1997; Dmitriew 2011; Shelton et al. 2013), and many species of amphibians and fish establish
117 dominance hierarchies, which usually reflect the larger size of socially dominant individuals (Symons
118 1971; Karplus et al. 2000; Grobler and Wood 2013). As a result of this, large size is often considered
119 advantageous under interference, but disadvantageous under exploitative competition because of the

120 higher metabolic costs of most competitive individuals (Van Buskirk et al. 2017). To better
121 understand the complex interplay and evolution of factors affecting fish size heterogeneity, we
122 assessed in a common garden system the effect of genetics on the dynamics of size variation in fish
123 families with different estimated breeding value for length at harvest (Perera et al. 2019).
124 Additionally, given the pleiotropic effects of O₂ availability on growth and energy metabolism
125 (Ratcliffe 2013), we also tested the impact of early life mild hypoxia on the achieved size variation.
126 Both approaches provide new insights on the processes driving compensatory growth and size
127 heterogeneity, serving as well to check the validity of size variation as a farming quality indicator in
128 an important fish species for the Mediterranean aquaculture with a production of 185,980 annual
129 tones in 2016 (FAO 2020).

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131 **Material and methods**

132 Ethics

133 All procedures were carried out according to IATS-CSIC Review Board, European (2010/63/EU)
134 animal directives and Spanish laws (Royal Decree RD53/2013) on the handling of experimental
135 animals.

136 Long-term growth trial of selected families

137 Fish breeders belonging to the Spanish selection program of gilthead sea bream (PROGENSA) were
138 used to produce families with different heritable growth as reported in Perera et al. (2019). Briefly,
139 two mini-broodstocks, composed of nine (3 females, 6 males) and six (3 females, 3 males) fish with
140 estimated breeding value for length at harvest of -0.9 ± 0.2 cm and $+0.9 \pm 0.7$ cm, were moved to
141 spanning tanks to produce family crosses. Spawns occurred immediately after the formation of these
142 two mini-broodstocks, and two spawns corresponding to three consecutive days were taken and
143 incubated at 20 °C in cylinder conical tanks (400L). This procedure yielded 11 families that were
144 grown up from early life stages to 199-228 g (September 2017-September 2018) at the experimental
145 facilities of IATS-CSIC (Perera et al. 2019). In the present study, the trial was prolonged
146 overwintering to better assess the time course effects of genetics on the achieved size and growth
147 heterogeneity. Since no consistent differences in growth performance were observed with the two
148 dietary formulations tested in the trial, all biometric data were analyzed as a single dietary treatment
149 covering more than 2,500 individuals. All fish were individually tagged with passive integrated
150 transponders (PIT) (ID-100A 1.25 Nano Transponder, Trovan), and genotyped individuals were
151 reared together in replicate 3000-L tanks under natural photoperiod and temperature conditions (Fig.
152 1a) with well aerated seawater. The same family ratio was maintained in all replicated tanks, and the

153 concentration of water O₂ was always higher than 80 % saturation, varying fish density from 2.5
154 Kg/m³ at the beginning to 12 Kg/m³ at the end of trial. Fish were fed by automatic feeders 1-2 times
155 per day and 3-7 days per week according to fish size and season, being the ration adjusted weekly to
156 a level close to satiation. No significant mortalities occurred during all the trial and fish were weighed
157 and measured individually each 2-4 months, using a FR-200 FishReader W (Trovan, Madrid, Spain)
158 for data capture and pre-processing. For posterior analysis, three family groups with fast (c2c6, e5e2,
159 e6e1, e6e2), intermediate (c2c5, c2c7, c4c7, e4e1, e4e2) and slow (c4c3, c4c5) growth rate
160 phenotypes were established on the basis of their final weight in the 12-month trial of Perera et al.
161 (2019).

162 Mild hypoxia experiences during early life stages

163 Gilthead sea bream larvae were transferred at 45 days post-hatching (dph) (May 2018) from the
164 hatchery of Piscimar (Burriana, Castellón, Spain) to the facilities of IATS-CSIC. After an acclimation
165 period of 15 days, 60 dph larvae (137 mg average weight) were distributed in six 90-L tanks (n = 150
166 larvae per tank) connected to two re-circulatory systems with control of water temperature (22-23 °C)
167 and O₂ concentration (Martos-Sitcha et al. 2019a). Feeding was carried out to visual satiety several
168 times per day with commercial pellets (0.2, 0.3, 0.5 mm Skretting Gemma Wean; 0.8 mm Skretting
169 Perla Plus; 1.5 mm Biomar Intro Plus MT; 1.9, 3 mm Biomar EFICO YM 853). Water quality was
170 daily checked and O₂ concentration was maintained high (85% saturation; 5.8 ppm) in normoxic
171 tanks, whereas it was decreased gradually over the course of 24 h in fish exposed to mild hypoxia (50
172 %; 3.6-3.8 ppm). This reduced O₂ concentration was maintained for 21 days (60-81 dph) with the
173 restoration of normoxia up to 112 dph. Then, fish from each tank were distributed in two additional
174 tanks (40 fish per tank, 6.8-7.2 g average weight), and O₂ levels remained high or low for 15 days.
175 This resulted in four groups of fish according to their O₂ availability history (NNN, normoxic; NNH,
176 late mild hypoxic; HNN, early mild hypoxic; HNH: early and late mild hypoxic). At this end (July
177 2018), all fish were returned to normoxia, and 50 randomly selected fish (20-27 g) per experimental
178 condition were individually tagged and reared together in a 3000-L tank until September 2018.

179 Statistical analysis

180 Statistical analyses were performed using SigmaPlot version 14.0 (Systat Software, San Jose, CA)
181 with all P-values set to 0.05 unless otherwise stated. Normality and equal variance of data were tested
182 by Shapiro-Wilk and Levene tests, respectively. Specific growth rates were calculated as $SGR = 100$
183 $\times (\ln \text{ final body weight} - \ln \text{ initial body weight})/\text{days}$. Regression and Pearson correlation analyses
184 were used to describe the relationships between SGR and individual size. The significance of
185 regression slopes was tested by ANOVA and coefficient of determination were used as a measure of

186 relative goodness of fit of regression curves. Differences among slopes were assessed by ANCOVA,
187 making all pairwise contrasts with a Bonferroni adjustment of the significance level to correct for
188 multiple testing (Quinn and Keough 2002). Size heterogeneity was analyzed by measures of
189 frequency distribution, coefficient of variation (CV) and skewness.

190 **Results**

191 The time course of growth compensation and depensation processes

192 The offspring of fish families selected for fast, intermediate and slow growth showed different growth
193 trajectories. The highest difference in body weight among these groups increased from 15% in May
194 2018 to 26% one year later (April 2019), varying the average family weight at the end of trial from
195 258 g (slow growing families) to 347 g (fast growing families) (Fig. 1b). All families grew almost
196 equally over the course of summer, but the fast growing ones continued to grow at a slow rate during
197 overwintering whereas the slow growing families stopped growth completely or experienced a loss
198 of weight, which was exacerbated during the second cold season (Fig. 1c).

199 During the first half of the trial (September 2017-July 2018), small fish of all families grew at a
200 higher rate than their bigger counterparts, and consequently, a consistent negative correlation between
201 SGR and initial body weight was found for two consecutive periods (Fig. 2a, b). The result was a
202 reduction in size heterogeneity of all families in July 2018, as revealed by close-to-zero skewness of
203 all frequency distributions of body mass (Fig. 3). This growth feature, referred as compensatory
204 growth by Ricker (1958), was marginally affected by the genotype because the slopes of the group
205 families categorized as slow, intermediate and fast growing phenotypes remained unaltered (Fig. 2a,
206 b, Supplementary Table 1). However, this growth compensation appears to be body size dependent,
207 because it stopped first in fast growing families with the achievement of 131-163 g of body weight
208 before the summer growth spurt (Fig. 2c). By contrast, the reduction of size variability continued
209 during the summer period in families with intermediate and slow growing features, until the final
210 cessation of such growth compensation process with the achievement of 181-227 g of body weight
211 during the second autumn season (Fig. 2d). Furthermore, the lack of growth compensation was
212 followed overwintering by a growth depensation process in fast growing families (Fig. 2e). This
213 resulted in a statistically significant positive correlation between SGR and initial body weight during
214 the finishing period (November 2018-Abril 2019), which favored the growth of big fish in
215 comparison to their small counterparts that shared an increased size heterogeneity. This feature was
216 evidenced by differences in frequency distributions of body mass, with changes in skewness at the
217 end of trial from positive values close to zero in slow growing families to negative values in
218 intermediate/fast growth families (Fig. 3). Further analysis of compensatory growth during the first

219 half of the trial (September 2017 to July 2018) revealed that initial size variation (i.e. CV) in half-sib
220 families sharing the same father was lowest in the fast growing families, but the close linear
221 association between initial CV and compensatory growth revealed that it was almost equal in all
222 families (Fig. 4).

223 Effects of early life mild hypoxia on growth, survival and size heterogeneity

224 In our experimental setup, episodes of mild hypoxia at 60-81 dph and 112-127 dph matched with key
225 periods of early life stages, corresponding to nursery and pre-fattening, respectively (Fig. 5). The first
226 mild hypoxia pulse did not affect growth rates, but enhanced by a 10% the survival rate of fish
227 growing at very high rates ($SGR \geq 9$). With the recovery of normoxia, growth and survival rates were
228 almost equal in NN and HN groups, but the response of these animals to a second episode of mild
229 hypoxia reflected the history trajectory. Thus, growth rates were lowered by a 15% in NNH fish in
230 comparison to NNN group, whereas both survival and growth in HNN and HNH did not differ
231 significantly and remained high.

232 The early life episodes of mild hypoxia (May-July 2018) also modulated later the achieved
233 compensatory growth during the second half of summer (August-September 2018) (Fig. 6). This
234 process was dependent on the number of exposures and the time window in which they occurred.
235 Thus, the trend for the slope of the regression line of SGR and initial body weight for the analyzed
236 period was as follows $NNN/N < HNN/N < NNH/N < HNH/N$, with a clear reduction of the slope from -
237 64×10^{-3} in NNN/N to -28×10^{-3} in HNH/N (Fig. 6, Supplementary Table 2). Accordingly, the final
238 CV of body weight was lowest in NNN/N (7.83) and highest in HNH/N (13.6) groups. Likewise, the
239 skewness changed from 0.006 in NNN/N fish to 0.626 in HNH/N fish, with intermediate values in
240 HNN/N fish, as a result of notable changes in the frequency distribution of body weight (Fig. 7).

241

242 **Discussion**

243 The present study confirms and extends the idea that growth selection in the PROGNSA breeding
244 program primes different growth trajectories and perhaps changes in standard metabolic rates as
245 reported in salmon, where strong selection for attributes in one life stage strongly influences or
246 constrains performance in another life stage (reviewed by Rosenfeld et al. 2015). Also, differences
247 in energy partitioning on growth, maintenance, activity, and perhaps social behavior might lead the
248 selection of animals that are not necessarily the fastest growing, but that have a positive effect on the
249 group as a whole (de Goede et al. 2013; Devlin et al. 2004). Breeding based on behavioral properties
250 are, however, difficult because the monitoring of social behavior is a costly and time consuming
251 process, though the use of small smart accelerometers attached to the operculum now make feasible

252 to obtain reliable measures of both respiratory frequency and physical activity of livestock fish
253 (Martos-Sitcha et al. 2019b; Rosell-Moll et al., unpublished results). Such approach contributes to
254 build up individual phenotypic variations, affecting growth, production and welfare that are primarily
255 conceptualized by changes in size variation. Certainly, fish welfare is compromised under intensive
256 culture, and structural environmental enrichment has the potential to improve gilthead sea bream
257 welfare (Arechavala-López et al. 2019). However, as pointed out by Balasch and Tort (2019), the
258 heterogeneity of stress responses varies within individuals and stressors, and become multimodal in
259 terms of typology, source and effects, being the intrinsic behavioral responses of each individual
260 dependent on genetics and stressful experiences in early life as discussed below more in depth.

261 Individual growth variation is a fundamental component of evolution, but factors that similarly
262 influence mean growth rate might affect differentially size variation (Peacor et al. 2007; Teder et al.
263 2008). This notion might be also extensive to livestock animals and early studies in gilthead sea bream
264 indicated that size variation during early life is affected by both behavioral interactions and nutritional
265 factors, though a general statement is that the improvement of culture conditions reduces the need for
266 stressful and labor intensive grading operations (Goldan et al. 1997; Karplus et al. 2000). This is
267 because different growth compensation mechanisms can become activated in a favorable
268 environment. Herein, this growth feature was exemplified during the first-half of the trial by
269 consistent negative correlations between growth rates and initial body weight. Besides, the achieved
270 compensatory growth was mostly independent of genetics since the slopes of regression equations
271 were almost equal in the families categorized as fast, medium or slow growth genotypes.
272 Accordingly, at this stage, a close linear positive linear association was found for of half-sib families
273 between initial size variation (expressed as CV) and the achieved growth compensation (% of CV
274 reduction). The same graphical representation displayed that the genetic component was a source of
275 initial size variation, since the half-sib families are closely grouped each other rather than to other
276 families. In any case, this growth compensation process is not extensive to all the production cycle
277 as it stopped when a critical size was reached, matching well with the size (130-220 g) at which
278 muscle hypertrophy is becoming the dominant mechanism of muscle growth in this species
279 (Rowlerson et al. 1995). Therefore, until this end, the capacity to reduce size heterogeneity has the
280 potential to be a suited KPI of the quality of farming conditions, helping us to discern improved or
281 impaired management protocols at the production level regardless of genetic source. This was further
282 supported by the observation that the capacity of growth compensation was compromised by early
283 life environmental disturbances. Despite this, our results also highlighted that early life exposure to
284 mild hypoxia serves to prepare the organism to better withstand stressful hypoxic episodes
285 experienced later on.

286 A number of adaptive processes, displaying hypometabolic states with reduced risk of oxidative
287 stress and improved exercise capacity, have been reported after acute or chronic hypoxia exposure in
288 juveniles of gilthead sea bream (Magnoni et al. 2017; Martos-Sitcha et al. 2017; 2019a). Additionally,
289 it is well known that early life hypoxia exposure increases the subsequent hypoxia tolerance at adult
290 stage in zebrafish (Robertson et al. 2014). Likewise, acute hypoxic stimulus in non-hatched embryos
291 displays a strong induction of glycolytic and glucose transport genes in trout at first-feeding (Liu et
292 al. 2017). O₂ levels also shape metabolic traits in European sea bass (Cadiz et al. 2017, 2018), though
293 in most cases the net balance is a growth depression (Zambonino-Infante et al. 2017) due to, among
294 other factors, higher incidence of opercula abnormalities (Cadiz et al., 2018), delay in the maturation
295 of digestive tract (Vanderplancke et al. 2015), with even transgenerational impairments of
296 reproduction in the case of acute hypoxia episodes in medaka (Wang et al. 2016). However, in our
297 experimental model, early exposure (60-81 dph) to mild hypoxia did not have a negative impact on
298 individuals growing at high rates (SGR>9). Indeed, the reduction of O₂ availability increased the
299 survival rates by about 10%. Since accelerated growth is by itself a source of oxidative stress
300 (Costantini 2014), a possible explanation of this beneficial effect is that our hypoxia protocol helps
301 to improve the oxidative balance. Certainly, the reduced O₂ availability would trigger a less active
302 behavior with also less aggressive interactions. In any case, a second episode of mild hypoxia (112
303 to 127 dph) reduced growth rate only when fish never faced this environmental condition before,
304 which is indicative of an effective environmental programming that produced different growth and
305 survival phenotypes depending on the time window of the hypoxia episode. This also applied to
306 compensatory growth feasibility, as the first early hypoxia episode did not compromise the growth
307 compensation process during early juvenile stages. By contrast, growth compensatory constraints
308 became evident with recurrent or late hypoxia episodes, which impaired, to a different extent, the
309 capacity to reduce size variation during the dominant phase of muscle growth hyperplasia. However,
310 it remains to be established the precise links between these phenotypic outputs and the early events,
311 which affect both physiological (Pittman et al. 2013) and social behavior (Garduño-Paz et al. 2010;
312 Bell et al. 2011; Langenhof et al. 2016) traits in fish.

313 Despite of the above limitations, it is plausible to postulate that the origin of the effects related
314 to O₂ availability are of epigenetic nature. Indeed, it is known from studies in higher vertebrates that
315 epigenetics plays a crucial role in the cellular response to hypoxia, involving the regulation of
316 hypoxia-induced transcription factor (HIF) family, or the long maintenance of a hypoxia-adapted
317 cellular phenotype after HIF has initiated the immediate response pathways (Watson et al. 2010). In
318 fish, an early hypoxic environment affected the DNA methylation of specific loci in rainbow trout
319 (Veron et al. 2018) and medaka (Wang et al. 2016). The link between coping style and reactivity of

320 the hypothalamic-pituitary-interrenal axis is also very important in terms of consequences for the
321 maintenance of oxidative balance and down-stream effects related to changes in oxidative stress
322 levels (Costantini et al. 2011). These issues warrant further examination, but intriguingly, measures
323 of growth compensation processes are highly informative of disturbances experienced during early
324 life, and would serve as a certificate of good management practices during the first half of the
325 production cycle. By contrast, the genetic source of size variation operates earlier during development
326 shaping the initial size variation of the population, and also during the finishing growth depensation
327 process that apparently favors adult individuals with larger size (de March 1995; Sogard and Olla
328 2000). Such information is relevant to discriminate the main source of variation in growth rates and
329 mean size along time or between different fish farms, helping to avoid the use of confounding criteria
330 for improving fish management and selective breeding (Power et al. 2011). In any case, size variation
331 is coming as a main criterion to evaluate and improve growth, production and welfare under the
332 intensive Mediterranean fish farming.

333

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496 **Figure captions**

497

498 **Fig. 1** Seasonal changes in temperature (solid line) and day length (dotted line) during the trial (a).
499 Body weight of fish categorized as slow (SG), intermediate (IG) and fast (FG) growing families.
500 Values are the mean of 3-5 replicate tanks (b). Changes in specific growth rates (SGR) of SG and IG
501 fish, expressed as the percentage of FG fish in each period(c). Values are the mean \pm sem of 3-5
502 replicate tanks

503

504 **Fig. 2** Regression plots between initial body weight (IBW) and specific growth rates (SGR) in fish
505 families of slow (SG), intermediate (IG) and fast (FG) growth rates at each period (a-e). Asterisks
506 mean statistically significant correlations ($p < 0.05$). Within each graph, letters to the right of asterisks
507 indicate statistically significant differences among slopes (ANCOVA)

508

509 **Fig. 3** Frequency distribution of body weight (BW) at three sampling points of fish families growing
510 at fast (a), intermediate (b) and slow (c) rates. Mean BW, skewness (Skw), and coefficient of variation
511 (CV) are shown for each group and sampling point. Dotted lines mark the mean BW of the group
512 with fast growth rates

513

514 **Fig. 4** Regression plot between initial body weight (IBW) and specific growth rates (SGR) of slow,
515 intermediate and fast growing families grouped into six half-sib families sharing the same father (a).
516 Close positive linear regression between initial size variation and compensatory growth as a function
517 of the reduction of coefficient of variation (b)

518

519 **Fig. 5** Time windows of early life hypoxia exposure at different days posthatching (dph), leading to
520 four experimental groups (NNN/N, NNH/N, HNN/N, HNH/N) (a). Overall body weight of all fish
521 groups during the trial. Mean body weight at exposure times is indicated by dotted lines. Shaded area
522 represents the period analyzed for compensatory growth (b). Data on survival and specific growth
523 rates (SGR) after early life hypoxia exposure. Asterisks mean statistically significant differences
524 ($p < 0.05$) (c)

525

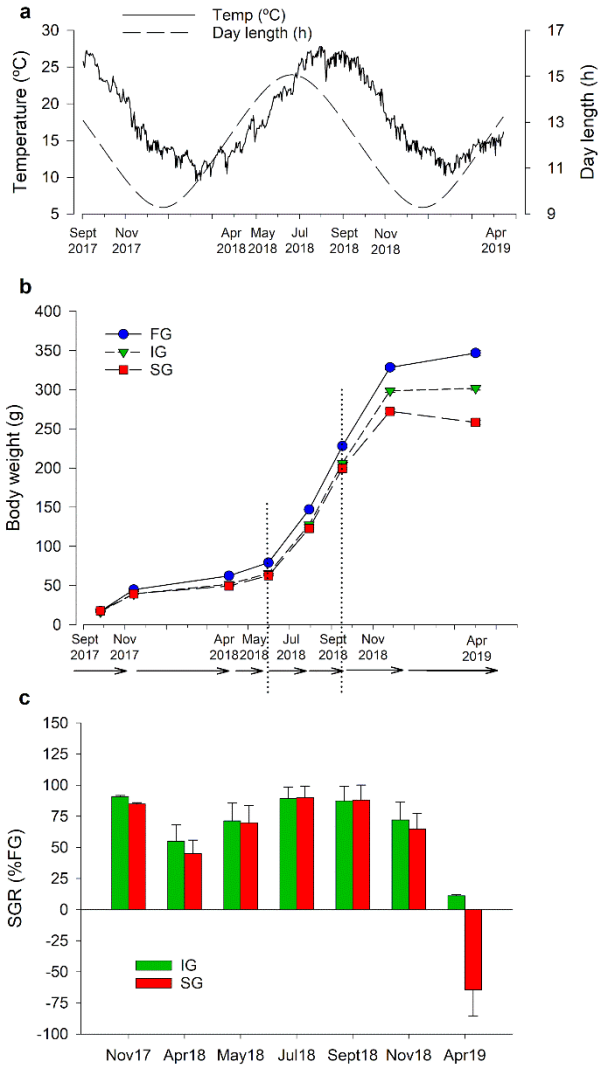
526 **Fig. 6** Regression plots between initial body weight (BW) and specific growth rates (SGR) at 147-
527 191 days posthatching (August-September 2018) in fish exposed to none (NNN/N) (a), one early
528 (HNN/N), one late (NNH/N) (b) or two (HNH/N) (c) mild hypoxia episodes during early life (60-81

529 dph and/or 112-127 dph). All correlations are statistically significant ($p < 0.05$). Letters to the right
530 indicate statistically significant differences among slopes (ANCOVA) of all four groups

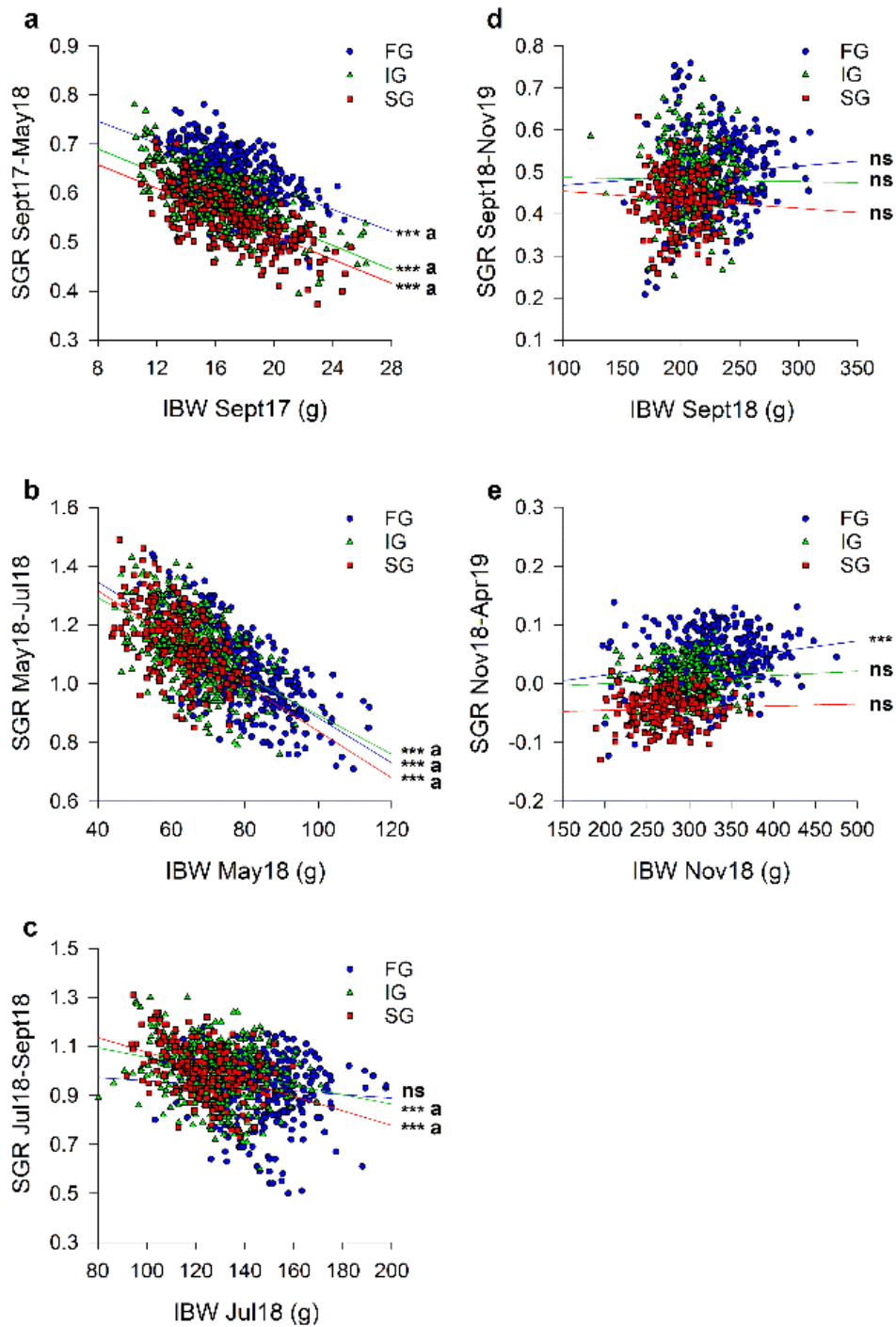
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532 **Fig. 7** Frequency distribution of body weight (BW) at 191 days post-hatching (dph) (September 2018)
533 in fish exposed to none (NNN/N) (a), one early (HNN/N) or late (NNH/N) (b) or two (HNH/N) (c)
534 mild hypoxia episodes during early life (60-81 dph and/or 112-127 dph). Mean BW, skewness (Skw),
535 and coefficient of variation (CV) are shown for each group and sampling point

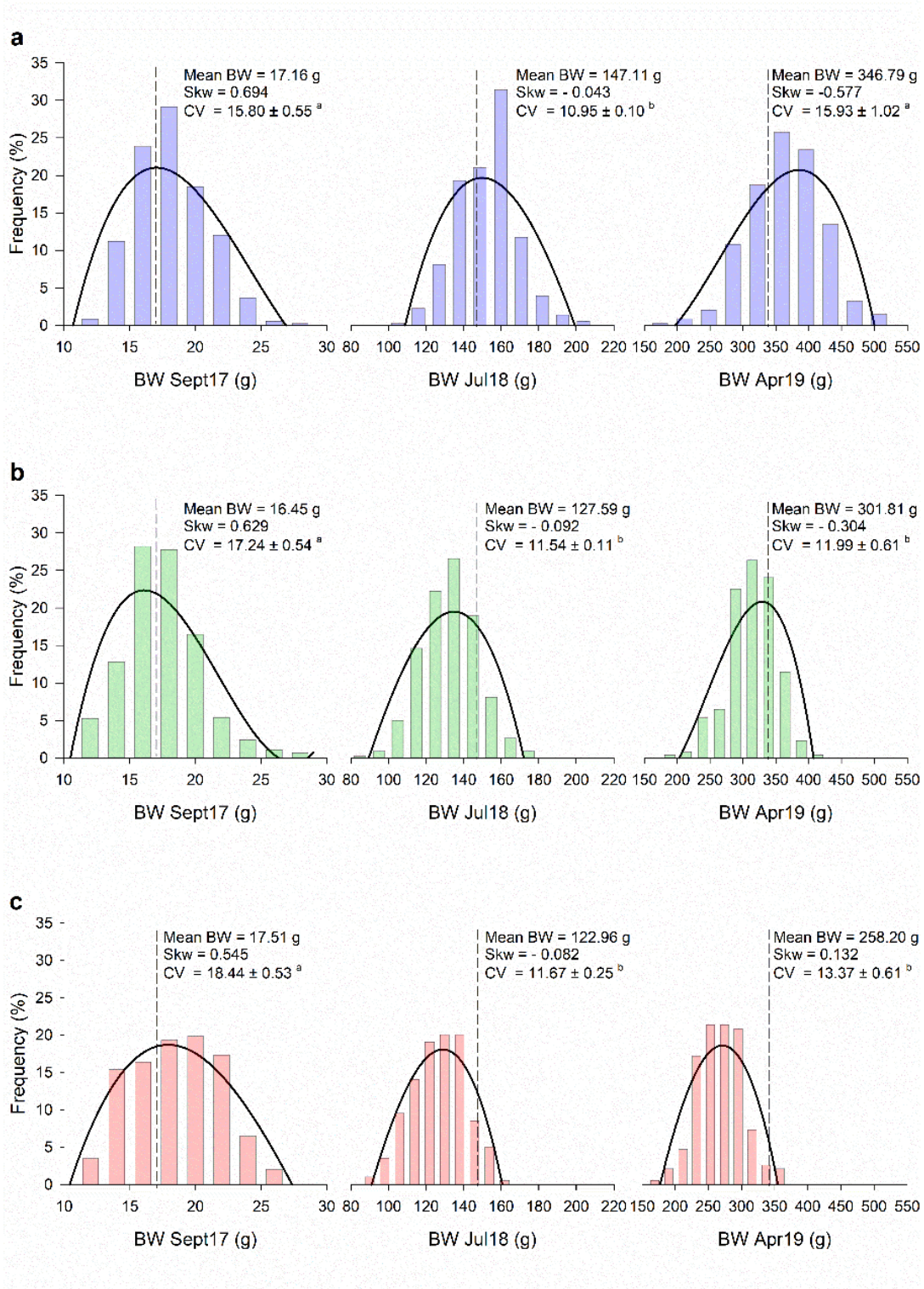
536 **Figure 1**



537

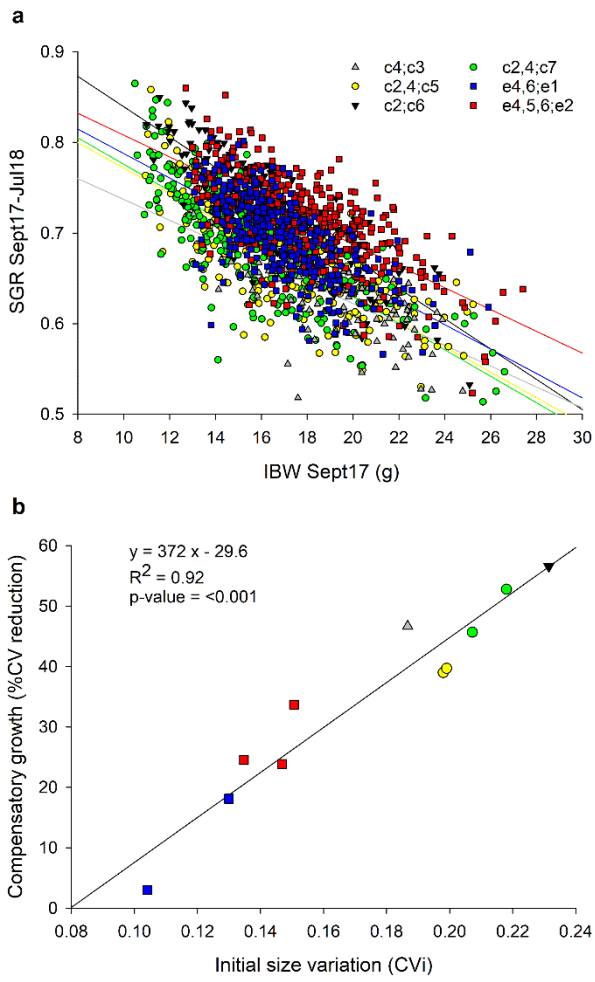


540 **Figure 3**



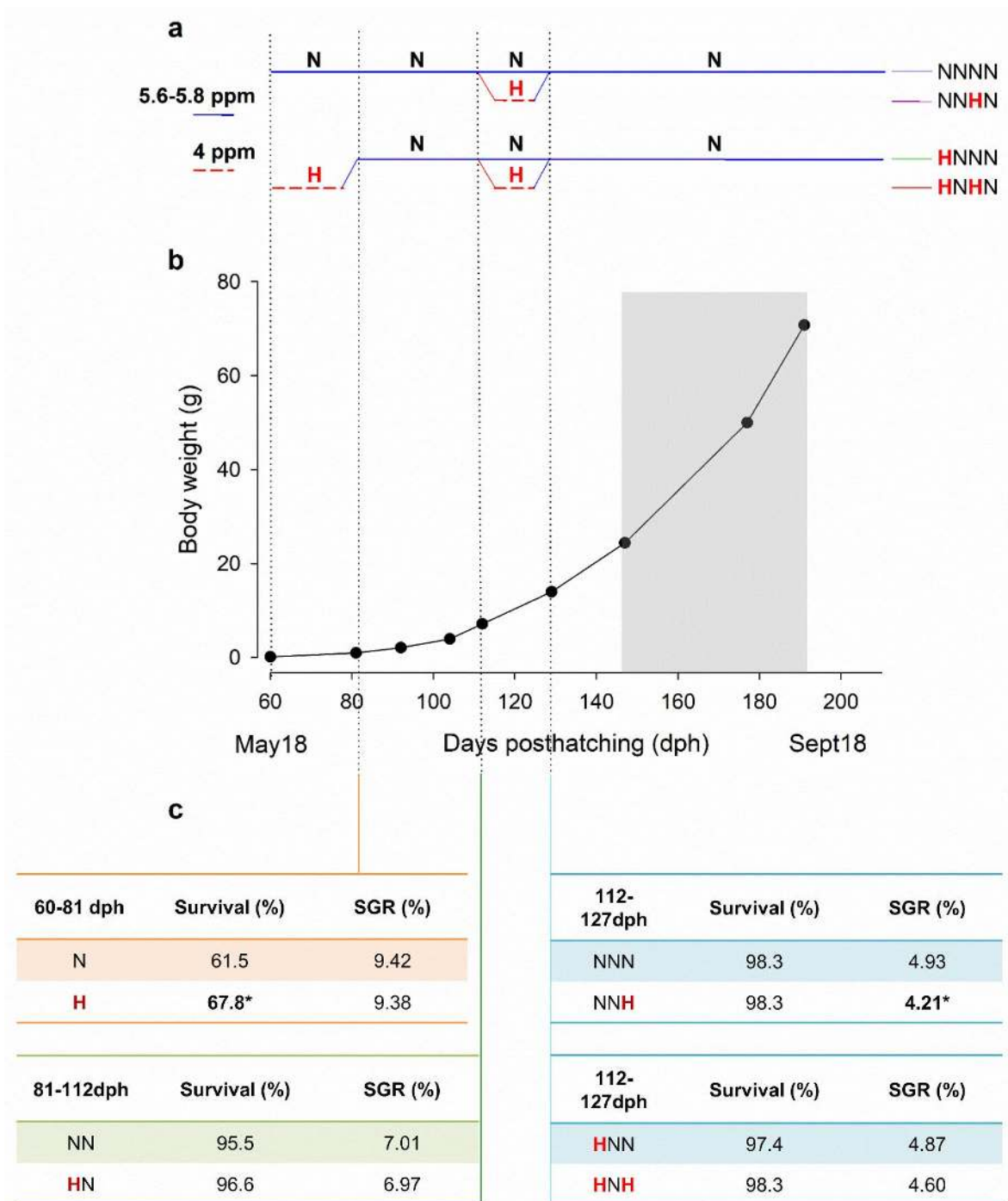
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542 **Figure 4**



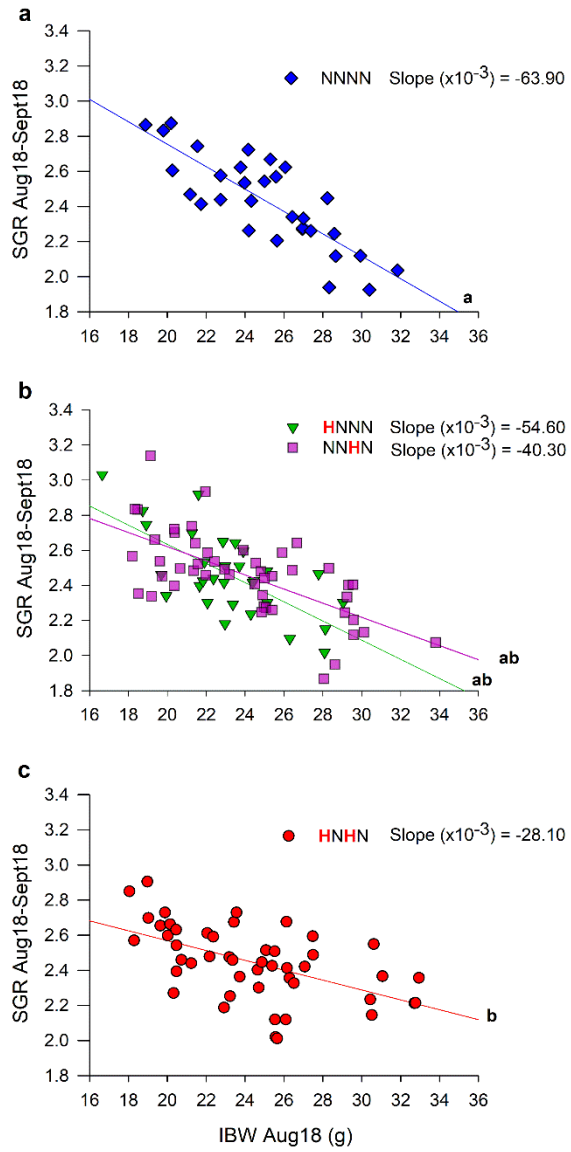
543

544 **Figure 5**



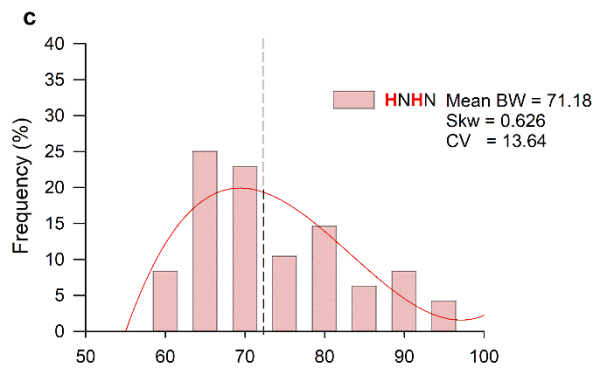
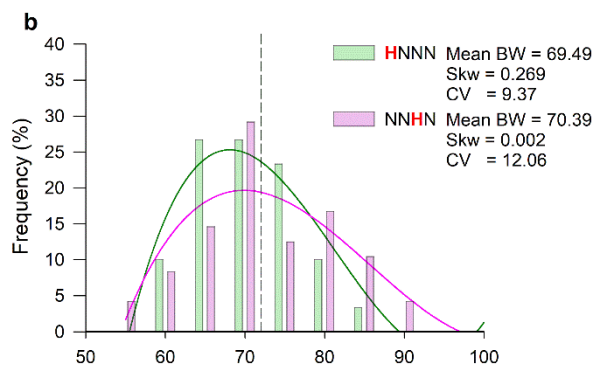
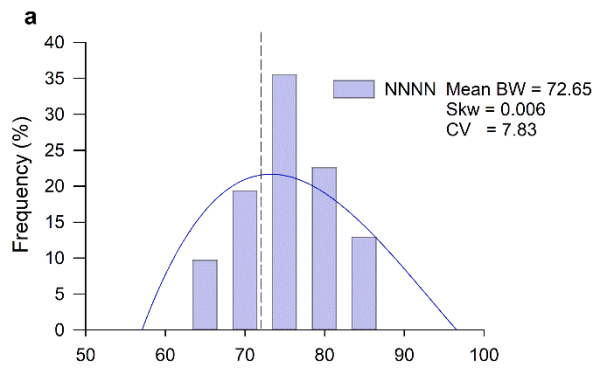
545

546 **Figure 6**



547

548 **Figure 7**



549

BW Sept18 (g)

550 **Supplementary Table 1.** Results of the regression analysis of specific growth rate (SGR)
 551 of gilthead seabream juveniles at the analyzed periods on the body weight at the beginning
 552 of each period. FG, IG, and SG mean fish families with fast-, intermediate-, and slow-growth
 553 rate, respectively. Letters next to the slopes mean statistically significant differences among
 554 slopes after ANCOVA. The Bonferroni adjustment of the significance level was used to
 555 correct for multiple testing. Only slopes from statistically significant correlations were
 556 compared.

Period	Group	N	F	P-value	Slope (x10 ⁻³)	R ²
Sept17- May18	FG	355	106.01	<0.001	-11.30 a	0.231
	IG	455	315.57	<0.001	-12.34 a	0.411
	SG	199	159.10	<0.001	-12.10 a	0.447
May18 -Jul18	FG	354	279.30	<0.001	-7.70 a	0.442
	IG	456	151.68	<0.001	-6.65 a	0.250
	SG	200	96.09	<0.001	-7.97 a	0.327
Jul18-Sept18	FG	347	2.66	0.104	-0.70	0.008
	IG	455	32.47	<0.001	-1.91 a	0.067
	SG	199	35.56	<0.001	-2.98 a	0.153
Sept18- Nov18	FG	331	0.77	0.381	0.17	0.002
	IG	259	0.05	0.830	-0.05	0.000
	SG	190	0.66	0.419	-0.20	0.003
Nov18-Abr19	FG	341	12.88	<0.001	0.19	0.037
	IG	261	1.27	0.261	0.07	0.005
	SG	190	0.36	0.551	0.04	0.002

557

558 **Supplementary Table 2.** Results of the regression analysis of specific growth rate (SGR)
 559 of gilthead seabream juveniles, from 147 days post-hatch (dph) (August 2018) to 191 dph
 560 (September 2018), on body weight at the beginning of that period. Fish experienced none
 561 (NNN/N), one early (HNN/N), one late (NNH/N), or two (HNN/N) mild hypoxia conditions
 562 during early farming (60-81 dph and/or 112-127 dph). Letters next to the slopes mean
 563 statistically significant differences among slopes after ANCOVA. The Bonferroni adjustment
 564 of the significance level was used to correct for multiple testing.

Period	Group	N	F	P-value	Slope (x10 ⁻³)	R ²
Aug18- Sept18	NNN	31	59.30	< 0.001	-63.90 a	0.672
	HNN	30	23.68	< 0.001	-54.60 ab	0.458
	NNH	48	35.51	< 0.001	-40.30 ab	0.436
	HNN	48	19.26	< 0.001	-28.10 b	0.295

565