1 **Advances in understanding the mitogenic, metabolic and cell death signaling in teleost**
2 **development: the case of greater amberjack (*Seriola dumerili*, Risso 1810)**

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Abstract

Cell growth and differentiation signals of Insulin-like growth factor-1 (IGF-1), a key regulator in embryonic and postnatal development, are mediated through the IGF-1 receptor (IGF-1R), which activates several downstream pathways. The present study aims to address crucial in organogenesis and development pathways including Akt, MAPKs, heat shock response, apoptotic and autophagic machinery, and energy metabolism in relation to IGF-1R activation during five developmental stages of reared *Seriola dumerili*: 1 day prior to hatching fertilized eggs (D-1), hatching day (D0), three days post-hatching larvae (D3), thirty-three (D33) and forty-six (D46) days post-hatching juveniles. During both the fertilized eggs stage and larval-to-juvenile transition, IGF-1R/Akt pathway activation may mediate the hypertrophic signaling, while p44/42 MAPK phosphorylation was apparent at *S. dumerili* post-hatching process, and juvenile organs completion. On the contrary, apoptosis was induced during embryogenesis and autophagy at hatching day indicating a potential involvement in morphogenetic rearrangements and yolk-sac reserves depletion. Larvae morphogenesis was accompanied by a metabolic turnover with increased substantial energy consumption. The findings of the present study demonstrate the developmental stages-specific shift in critical signaling pathways during the ontogeny of reared *S. dumerili.*

Key words: fish ontogeny; IGF-1R; Akt; cellular signalling; metabolism

61 **1. Introduction**

62 Growth, a continuous biological process that commences from embryogenesis up until postnatal
63 adult stage, is the cumulative outcome of cell proliferation and cell differentiation (Duan et al. 2003). In
64 vertebrates, the genetically predetermined growth pattern and growth-related processes, such as energy
65 metabolism, are controlled by a hormonal regulatory network, while simultaneously affected by
66 environmental and nutritional factors (Duan 1998; Fuentes et al. 2013a). For instance, due to teleosts
67 ectothermic nature, developmental processes including hatching, sexual maturation and spawning are
68 triggered by conditions such as temperature, photoperiod and food availability (Duan 1998). In addition,
69 regulation of muscle growth is modified throughout fish development, depending on age and
70 reproductive stage, and annual cycles (Vélez et al. 2017). Growth in most fish species relies throughout
71 the life cycle on both the formation of new muscle cells and the increase in size of existing ones, known
72 as muscle hyperplasia and hypertrophy respectively (Johnston 1999). The aforesaid deviates fish from
73 higher vertebrates, in which postnatal growth is mediated exclusively via the hypertrophic mechanism
74 (Rowe and Goldspink 1969; Fuentes et al. 2011).

75 Somatic growth and development in vertebrates, including teleosts, is mainly governed by the
76 growth hormone/insulin-like growth factor (GH/IGF) axis (Picha et al. 2006; Zhong et al. 2012).
77 Secretion of IGF-1 is mainly GH-stimulated, although other hormones as well as nutritional, metabolic
78 and environmental signals greatly influence its expression (Triantaphyllopoulos et al. 2020). IGF
79 signaling system is involved in skeletal muscle growth and metabolism, and embryonic development in
80 teleosts (Eivers et al. 2004; Codina et al. 2008; Triantaphyllopoulos et al. 2020). IGF-1 ligand signals for
81 several functions including cellular growth and metabolism are mainly transduced through specific
82 binding to a tyrosine kinase receptor, the type 1 IGF receptor (IGF-1R) (Pozios et al. 2001; Eivers et al.
83 2004). The latter ligand-receptor interaction triggers the autophosphorylation-induced activation of IGF-
84 1R, which in turn phosphorylates a host of intracellular substrates, thus leading to the activation of two
85 major intracellular signaling pathways, the mitogen-activated protein kinases (MAPKs) and the
86 phosphatidylinositol-3 kinase/Akt-1 (PI3K/Akt-1) (Eivers et al. 2004; Fuentes et al. 2013a). Mitogenic
87 action of IGFs is mediated through both aforementioned pathways from early embryonic stages in
88 teleosts (Pozios et al. 2001). The MAPK pathway is immensely linked to skeletal muscle development,
89 whereas its activation stimulates muscle cell proliferation and terminal differentiation, hypertrophy and
90 IGF-1R-dependent mitogenesis (Haddad and Adams 2004; Li and Johnson 2006; Codina et al. 2008).

91 During early stages of differentiation, IGF-stimulated proliferation via MAPK pathway is decreased, 92 whereas IGF-1 signal transduction through activation of PI3K/AKT pathway modulates muscle 93 hypertrophy and myoblast differentiation (Coolican et al. 1997; Bodine et al. 2001). In addition to the 94 activation during differentiation and maturation, PI3K/AKT pathway mediates IGF-1 anabolic effects, 95 such as glucose and amino acid uptake in muscle, and stimulates protein synthesis (Negatu and Meier 96 1995; Castillo et al. 2004; Codina et al. 2008). Moreover, MAPKs seem to be involved in development 97 via another pathway: its activation is involved in the regulation of Hsps (Feidantsis et al. 2012), which 98 are required for two distinct developmental steps, oogenesis and early larval development (Jedlicka et al. 99 1997). A regulatory link between hormones such as IGF-1 and Hsp expression has been previously 100 shown in teleosts (Sathiyaa et al. 2001).

101 Programmed cell death is an equally important part in the growth pattern, involved in various 102 aspects of vertebrate development (Jacobson et al. 1997). Apoptosis and autophagy constitute the two 103 main processes of programmed cell death, acting synergistically or independently (Mariño et al. 2014). 104 Apoptosis counterbalances developmental errors, acting as a mechanism of quality control and repair, 105 while concomitantly contributing to the formation of new structures and degeneration of precedent ones 106 between developmental stages, and the structural flexibility towards functional adaptations (Jacobson et 107 al. 1997; Meier et al. 2000). In addition, equilibrium between anti-apoptotic and pro-apoptotic molecules, 108 which alongside caspases are the main apoptosis components, is a key-regulator in cell survival during 109 early embryonic development (Krajewska et al. 2002). Similar to apoptosis, autophagy is directly 110 involved in cell differentiation and tissue remodeling during embryogenesis, morphogenesis and 111 developmental events through degradation and recycling of cytoplasmic constituents (Lee et al. 2014; 112 Agnello et al. 2015). Autophagy's implication in development extends throughout ontogeny, constituting 113 the energy supplier during energetically-costly processes such as larval-to-juvenile transition (Mawed et 114 al. 2019).

115 Disruptions in the aforementioned signaling pathways may result in reproductive dysfunctions 116 (Zupa et al. 2017), which have been reported in several species reared under aquaculture conditions, 117 including greater amberjack *Seriola dumerili* (Risso, 1810), a marine pelagic teleost species (Mylonas 118 and Zohar 2000). In regard to Mediterranean aquaculture diversification, greater amberjack is considered 119 as an excellent candidate due to high growth rate, high commercial demand and excellent flesh quality 120 (Papandroulakis et al. 2005; Nakada 2008). However, reproduction control in captivity has been one of

the major obstacles in this species commercialization due to the occurrence of several dysfunctions including failure of oocyte maturation and ovulation (Micale et al. 1999; Mylonas et al. 2004) as well as spermatogenesis impairment (Zupa et al. 2017). The present study aims to address the regulation of mitogenic, metabolic and cell death signaling during the development of greater amberjack under rearing conditions. In specific, crucial in organogenesis and development pathways, including MAPKs (p38, p44/42, JNK), AKT, heat shock response (Hsp60, Hsp70, Hsp90), apoptotic (Bax, Bcl-2, Caspases) and autophagic machinery (SQSTM1/p62, LC3 II/I, Ubiquitin), as well as, energy metabolism (AMPK, AMP/ATP ratio, metabolic enzymes' activity), were examined in relation to the activation of IGF-1R during five developmental stages of reared greater amberjack: 1 day prior to hatching fertilized eggs, hatching day, 3 days post-hatching larvae, thirty three days post-hatching young juveniles and forty six days post-hatching juveniles.

2. Material and Methods

2.1. Experimental procedure - Sampling

Samples were provided by a Greek commercial aquaculture facility, Galaxidi Marine Farm SA and scientists involved in animal handling and sampling were accredited by the Federation of Laboratory Animal Science Associations (FELASA) in categories A–D of competence. In specific, samples of 1 day prior to hatching fertilized eggs [day -1 (D-1)], hatching day [day 0 (D0)], three days post-hatching larvae [day 3 (D3)], and thirty-three and forty-six days post-hatching juveniles [day 33 (D33) and day 46 (D46), respectively] were collected. Fish developmental stages were as described in Tachihara et al. (1993). Sampling days were selected based on pivotal ontogenic and/or physiological events during greater amberjack development. Briefly, D-1 was selected to assess singaling pathways involvement in crucial embryonic processes including myogenesis, while D0 represents the onset exposure of newly-hatched larvae to the surrounding environment. D3 marks the mouth-opening stage, a fundamental switch period from endogenous to exogenous/mixed feeding, where yolk-sac absorption is almost completed (Masuma et al. 1990). At D33, greater amberjack undergoes the larval-to-juvenile transition (metamorphosis), while simultaneously marks the weaning from live feeds (rotifers) onto dry diet (fishfeed). Lastly, D46 was selected as a representative point during juveniles' rapid growth.

Two incubators, with a volume of 280 l, were each stocked with ~780g greater amberjack eggs. Borehole water was provided to the incubators, with a renewal rate of 50% per hour. The desired water

151 temperature (> 23°C) was achieved using heat pumps. During the egg and first larval stages, gentle
152 ventilation was provided for the smooth stirring of the water, to ensure maximum survival of eggs and
153 larvae. From each incubator, three biological replicates were collected (N = 6). At the same time, three
154 tanks, with a volume of 2,800 l, were each stocked with ~ 300 g fertilized eggs. Samples were collected
155 on both day 33 and 46 from tanks. From each tank, two biological replicates were collected (N = 6). Fish
156 were euthanized immediately after their removal from the tanks, using high concentrations of anesthetic.
157 Immediately, after collection all samples were placed in sterile 2 ml vials, frozen using liquid nitrogen,
158 and stored at -80°C until further analysis. Mean values of physicochemical data of the incubators and the
159 tanks are presented in Table 1.
160
161 *2.2. Preparation for immunoblotting*
162 The preparation of samples for SDS-PAGE, quantification of caspases and ubiquitinated proteins
163 and immunoblot analysis are based on well-established protocols. Prior to homogenization, pooling of
164 fertilized eggs and larvae was necessary to reach the required weight for immunoblotting. Specifically,
165 50 mg of frozen eggs, larvae and juvenile samples were immediately homogenized in 3 ml g^{-1} of cold
166 lysis buffer (20 mM β-glycerophosphate, 50 mM NaF, 2 mM EDTA, 20 mM Hepes, 0.2 mM Na_3VO_4,
167 10 mM benzamidine, pH 7, 200 μM leupeptin, 10 μM trans-epoxy succinyl-Lleucylamido-(4-guanidino)
168 butane, 5 mM dithiotheitol, 300 μM phenyl methyl-sulfonyl fluoride (PMSF), 50 μg ml^{-1} pepstatin, 1%
169 v/v Triton X-100), and extracted on ice for 30 min. Samples were centrifuged (10,000 × *g*, 10 min, 4°C)
170 and the supernatant was boiled with SDS/PAGE sample buffer (330 mM Tris-HCl, 13% v/v glycerol,
171 133 mM DTT, 10% w/v SDS, 0.2% w/v bromophenol blue) in a 3:1 ratio (40 μl buffer for 120 μl
172 supernatant). For the determination of LC3 II/I ratio and SQSTM1/p62 levels, samples were lysed in a
173 buffer containing 150 mM NaCl, 20 mM Hepes, 5 mM DTT, 0.3 mM PMSF, 0.2 mM leupeptin, 0.01
174 mM E64 and 1% Triton X-100. Lysates were incubated on ice for 30 min and then centrifuged at 4°C,
175 for 5 min at 3,000 × *g*. Protein concentration was determined by using the BioRad protein assay.
176 For the SDS-PAGE, equivalent amounts of proteins (50 μg), from samples of 5 individual batches
177 of each developmental stage, were separated either on 10% and 0.275% or 15% and 0,33% (w/v)
178 acrylamide and bisacrylamide, followed by electrophoretic transfer onto nitrocellulose membranes (0.45
179 μm, Schleicher & Schuell, Keene N. H. 03431, USA).

180 The resulting nitrocellulose membranes were subjected to overnight incubation with: polyclonal
181 rabbit anti-bcl2 (7973, Abcam), polyclonal rabbit anti-bax (B-9) (7480, Santa Cruz Biotechnology),
182 monoclonal mouse anti-HSP70 (H5147, Sigma), monoclonal mouse anti-HSP90 (H1775, Sigma), anti-
183 HSP60 (12165, Cell Signaling, Beverly, MA, USA), monoclonal mouse anti-phospho-SAPK-JNK
184 (9255, Cell Signaling, Beverly, MA, USA), monoclonal rabbit anti-phospho p44/42 MAPK (4376, Cell
185 Signaling, Beverly, MA, USA), polyclonal rabbit anti-phospho-p38 MAP kinase (9211, Cell Signaling,
186 Beverly, MA, USA), polyclonal rabbit anti-SAPK-JNK (9252, Cell Signaling, Beverly, MA, USA),
187 polyclonal rabbit anti-p44/42 MAPK (4695, Cell Signaling, Beverly, MA, USA), polyclonal rabbit anti-
188 p38 MAPK (9212, Cell Signaling, Beverly, MA, USA), monoclonal rabbit anti-LC3B (3868, Cell
189 Signaling, Beverly, MA, USA), polyclonal rabbit anti-p62/SQSTM1 (5114, Cell Signaling, Beverly,
190 MA, USA), monoclonal rabbit anti-phospho AMPK (2535, Cell Signaling, Beverly, MA, USA),
191 monoclonal rabbit anti-AMPK (5831, Cell Signaling, Beverly, MA, USA), anti-phospho-IGF-1R (3918,
192 Cell Signaling, Beverly, MA, USA), anti-IGF-1R (9750, Cell Signaling, Beverly, MA, USA) and anti-
193 phospho-Akt (9271, Cell Signaling, Beverly, MA, USA), anti-Akt (9272, Cell Signaling, Beverly, MA,
194 USA). Quality transfer and protein loading western blot, were assured by Ponceau stain and actin (anti-
195 β actin 3700, Cell Signaling, Beverly, MA, USA).
196 Concerning cleaved caspases and ubiquitination levels, protein samples were immunoblotted with
197 a dot blot apparatus employment (Hofmann and Somero 1996) and membranes were overnight incubated
198 with monoclonal rabbit anti-cleaved caspase antibody (8698, Cell Signalling) and monoclonal mouse
199 anti-ubiquitin conjugate (3936, Cell Signalling). Bands and blots were detected by enhanced
200 chemiluminescence, while quantification was applied through laser-scanning densitometry (GelPro
201 Analyzer Software, GraphPad).
202
203 *2.3. Determination of intermediate metabolism enzyme activities*
204 Activities of lactate dehydrogenase (L-LDH; E.C. 1.1.1.27.), citrate synthase (CS; E.C. 4.1.3.7.),
205 and 3-hydroxyacyl CoA dehydrogenase (HOAD;1.1.1.35) were estimated in samples according to well-
206 established techniques (Moon and Mommsen 1987; Sidell et al. 1987; Singer and Ballantyne 1989;
207 Driedzic and Fonseca de Almeida-Val 1996). For the analysis of L-LDH and HOAD activities, samples
208 were homogenized in a buffer containing 150 mM imidazole, 1 mM EDTA, 5 mM dithiothreitol (DTT)
209 and 1% Triton X-100, pH 7.4. For CS activity, tissue samples were homogenized in a buffer containing

210 20 mM HEPES, 1 mM EDTA, with 1% Triton X-100, pH 7.4. To avoid loss of enzyme activity during
211 sample preparation, procedures were performed on ice. Before analysis, homogenates were centrifuged
212 at 13,000 × *g* for 10 min at 4°C. Maximum activity levels were determined spectrophotometerically at
213 18°C. Activities of L-LDH and HOAD enzymes were measured following the oxidation of NADH at
214 340 nm (mM extinction coefficient = 6.22), while CS enzyme activity was determined based on the
215 reaction of free coenzyme A with DTNB (5.5 V dithio-bis (2- nitrobenzoic acid) at 412 nm (mM
216 extinction coefficient = 13.6). L-LDH was assayed in a medium containing 0.15 mM NADH, 1 mM
217 KCN and 50 mM imidazole, pH 7.4. The reaction was initiated by adding 1 mM pyruvate. CS was
218 assayed in a medium containing 0.4 mM acetyl CoA, 0.25 mM DTNB and 75 mM Tris buffer, pH 8.0.
219 The reaction was initiated by adding 0.5 mM oxaloacetate (OAA). 3-hydroxyacyl CoA dehydrogenase
220 was assayed in a medium containing 0.15 NADH, 1 mM KCN, 1 mM EDTA, 50 mM Imidazole, pH 7.4.
221 The reaction was initiated by the addition of 2.0 mM acetoacetate. Enzyme activities are expressed as
222 micromoles of substrate min/mg protein. Protein concentration in supernatants was determined by using
223 the BioRad protein assay.
224
225 *2.4. Statistics*
226 Changes in biochemical responses were tested for significance at the 5% level by using one-way
227 Analysis of variance (ANOVA) (GraphPad Instat 3.0). Post-hoc comparisons were performed using the
228 Bonferroni test. Values are presented as means ± S.D.
229
230 **3. Results**
231 *3.1. Insulin-like growth factor receptor (IGF-1R) and Akt*
232 The levels of IGF-1R and Akt phosphorylation in the five different developmental stages of the
233 greater amberjack are depicted in Fig. 1. Generally, compared to stage D-1, a significant decrease of
234 phosphorylation ratio of both IGF-1R and Akt levels was observed in stage D0 and D3 ($p < 0.05$).
235 However, the subsequent stage of D33 displayed a significant increase, while phosphorylation ratio of
236 both IGF-1R and Akt decreased in the following stage D46 (Fig. 1).
237
238

239 *3.2. Mitogen-activated protein kinases, MAPKs (p38 MAPK, p44 / 42 MAPK and JNK phosphorylation*
240 *ratios)*

241 Phosphorylation ratios of p38 MAPK, p44/42 MAPK and JNK followed a similar pattern,
242 showing statistically significant differences among the examined developmental stages ($p < 0.05$).
243 Specifically, the activation of p38 MAPK increased significantly at the stage D0 compared to stage D-1.
244 Nevertheless, a significant decline was observed at stage D3, followed by a new increase in the following
245 developmental stages D33 and D46, where the phosphorylation ratio maintained at similar levels to the
246 early developmental stages. Regarding the phosphorylation of p44/42 MAPK, increased levels were
247 observed in stage D0, compared to stage D-1, while a significant reduction was apparent in stage D3. In
248 the following developmental stages (D33 and D46), the phosphorylation ratio of p44/42 MAPK increased
249 significantly, exhibiting its highest levels in stage D46. Concerning JNK, its lowest phosphorylation
250 levels was recorded in stage D-1 while its highest in stage D0, followed by a significant decrease in the
251 later developmental stages (Fig. 2).
252

253 *3.3. Heat shock response (Hsp60, Hsp70 and Hsp90)*

254 The expression patterns of Hsp60, Hsp70 and Hsp90 in the five different developmental stages of
255 the greater amberjack are shown in Fig. 3. Hsp60 levels were significantly lower in the stages D-1 and
256 D0, compared to the later developmental stages ($p < 0.05$). Similarly, low expression levels of Hsp70
257 were also observed in stages D-1 and D0. However, a higher induction of Hsp70 was recorded in D3
258 stage compared to the early and later developmental stages ($p < 0.05$). Concerning Hsp90, a significant
259 increase was observed in stage D0, which was also maintained at high levels in stage D3 but decreased
260 significantly in the later stages ($p < 0.05$) (Fig. 3).
261

262 *3.4. Apoptosis (Bax/Bcl-2 ratio and caspases)*

263 In order to investigate the apoptotic machinery in the examined developmental stages of greater
264 amberjack, the expression levels of the anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax, as
265 well as caspases were determined. Caspase levels exhibited higher levels in stage D33 comparing to the
266 other developmental stages ($p < 0.05$). Bax/Bcl-2 ratio was found to be higher in stage D3 compared to
267 the early and later developmental stages ($p < 0.05$) (Fig. 4).
268

269 *3.5. Autophagy (QSTM1/p62 levels, LC3 II/I ratio and ubiquitin)*

270 The results from the determination of autophagy machinery in the examined developmental stages 271 of the greater amberjack are presented in Fig. 5. Regarding protein ubiquitin conjugates, a sharp induction 272 was apparent in stage D0 comparing to stage D-1 ($p < 0.05$). Subsequently, levels of ubiquitination 273 decreased in stages D3 and D33 while an additional increase was observed in stage D46. During the 274 development of the greater amberjack, the SQSTM1/p62 levels and LC3 II/I ratio followed a reverse 275 pattern. Specifically, the decreased levels of SQSTM1/p62 and the increased LC3 II/I ratio among the 276 successively developmental stages D-1/ D0 and D3/ D33 indicated the triggering of autophagy (Fig. 5).

277

278 *3.6. Metabolic pathways (AMPK phosphorylation and AMP/ATP ratio)*

279 In Fig. 6, phosphorylation of AMPK, ATP and AMP levels, as well as AMP/ATP ratio are 280 illustrated in the five examined developmental stages of the greater amberjack. Although, AMPK 281 phosphorylation decreased in stage D0, compared to stage D-1, a significant activation was observed in 282 the following stages, exhibiting its highest levels in stage D33 ($p < 0.05$). ATP levels decreased 283 significantly in stage D0, compared to stage D-1, and exhibited its lowest levels. Thereafter, ATP levels 284 increased slightly and in stage D33 remained at levels similar to stage D-1. Subsequently, a significant 285 decrease was observed in stage D46. Compared to the ATP levels, AMP levels and AMP/ATP ratio 286 followed a similar pattern as depicted in Fig. 6.

287

288 *3.7. Metabolic enzymes' activity (L- LDH, HOAD and CS)*

289 The activity of L-LDH displayed its highest peak in stage D-1 but decreased significantly in stage 290 D0, remaining at constant levels throughout the following developmental stages ($p < 0.05$) (Fig. 7a). 291 Likewise, as displayed in Fig. 7b, the pattern of HOAD activity was similar to that observed for L- LDH, 292 except for stage D3 where HOAD activity levels returned to that of stage D-1 (Fig. 7b). In contrast to 293 LDH and HOAD, CS activity increased significantly in stages D0 and D3, compared to stage D-1 (Fig. 294 7c). Thereafter, a transient decrease in enzymatic activity was seen in stage D33, reaching similar levels 295 to stage D0, but CS activity increased again in stage D46 (Fig. 7c).

296

297

298

299 *3.8. Correlation analysis*

300 Table 2 illustrates the correlation analysis between the studied biological parameters of different 301 greater amberjack developmental stages. Most of the studied parameters were significantly correlated.

302

303 **4. Discussion**

304 Understanding signaling patterns during species development is fundamental in enlightening the 305 catalytic cell mechanisms and functions contributing in ontogeny. The present research demonstrates for 306 the first time the shifts in mitogenic, metabolic and cell death signaling occurring throughout the 307 development of a rearing teleost fish, *Seriola dumerili*, from fertilized eggs to juveniles. Additionally, 308 this research elucidates the developmental stage-dependent activation of IGF-1R and the regulatory 309 linkage among critical pathways such as IGF-1/Akt and IGF-1/MAPK, and major ontogenetic events and 310 nutritional shifts of *S. dumerili*.

311

312 *4.1. The IGF-1/Akt pathway during S. dumerili development*

313 IGF-1, as mediator of the pituitary GH effects or acting in an autocrine/paracrine manner, 314 stimulates muscle growth via a ligand-receptor interaction with IGF-1R (Le Roith et al. 2001; Bower et 315 al. 2008; Fuentes et al. 2011). In particular, IGFs are implicated, through the transduction of 316 miscellaneous signals, in hyperplasia (cell proliferation activation), hypertrophy (protein synthesis 317 increase), and differentiation during myogenesis and muscle regeneration (Vandenburgh et al. 1991; 318 Grounds 2002). According to the present results, during the fertilized eggs stage (D-1) of *S. dumerili*, 319 activation of both IGF-1R and Akt was prominent prior to a contemporaneous reduction on the hatching 320 day (D0). Expression of IGF-1R and receptor-ligand binding are linked to the development and the 321 growth rates in which an organism is subjected to, and therefore to the circulating IGF-1 levels (Mingarro 322 et al. 2002; Montserrat et al. 2007). Specifically, the activation of PI3K/Akt pathway in the fertilized 323 eggs stage, as indicated by the Akt phosphorylation (Rommel et al. 2001) herein, is in accordance with 324 previous studies that denoted the increase of phosphorylated Akt in the presence of IGFs (Montserrat et 325 al. 2007; Fuentes et al. 2011). In rainbow trout (*Oncorhynchus mykiss*), IGF-1-induced-activation of the 326 PI3K/Akt pathway was observed throughout skeletal muscle development, from myoblasts to myotubes 327 (Castillo et al. 2006), as well as during adipogenesis (Bouraoui et al. 2010). In regard to the fertilized 328 eggs stage, the appearance of melanophores on the embryo has occurred, while processes of embryonic

329 myogenesis facilitate muscle growth (Patruno et al. 1998) and the increase in myomeres number
330 (Tachihara et al. 1993). Thus, the Akt activation observed herein may mediate hypertrophy via induction
331 of downstream protein synthesis pathways, as has been previously described in mammals (Rommel et
332 al. 2001; Lai et al. 2004) and teleosts (Montserrat et al. 2007; Zhao et al. 2020).

333 Furthermore, PI3K/Akt pathway activation appears to be immensely linked to IGF-1R activation
334 since Akt and IGF-1R showcased a similar phosphorylation pattern throughout *S. dumerili* development.
335 In contrast to fertilized eggs stage, IGF-1 involvement at the 3 days post-hatching larvae (D3) appears to
336 be lesser as observed by the decreased IGF-1R levels. The latter along with the simultaneous increase in
337 AMPK activation would suggest a reduction of protein synthesis in *S. dumerili* larvae due to AMPK's
338 negative regulatory effect on ATP-consuming anabolic processes via inhibition of TOR activation and a
339 positive regulatory effect on catabolic pathways including glucose and fatty acids uptake and oxidation
340 in order to provide ATP (Goodman et al. 2011; Fuentes et al. 2013b). Interestingly, in the present study,
341 IGF-1/Akt pathway activation displayed maximal increase during the larval-to-juvenile transition
342 (metamorphosis), which in reared *S. dumerili* occurred in thirty-three days post-hatching juveniles (D33)
343 and concurred with shifts in the feeding regime. During metamorphosis, restructure procedures at
344 morphological, physiological, molecular and behavioral level occur in order to remodel and reorganize
345 teleosts' larvae into juveniles (Islam and Tanaka 2006; Mawed et al. 2022). Previous studies have
346 reported modifications regarding head shape, fin spines length, intestinal area, and morphological and
347 functional development of stomach, pyloric caeca and gastric glands (Pedersen and Falk-Petersen 1992;
348 McCormick et al. 2002). Thus, in addition to a prominent role in embryonic myogenesis, IGF-1 may act
349 as a growth stimulator through the Akt phosphorylation in order to facilitate the rapid growth rate of *S.
350 dumerili* observed at this stage (Masuma et al. 1990; Papandroulakis et al. 2005). Muscle growth in
351 teleosts involves both fiber hyperplasia and hypertrophy, and among different stages one process may
352 prevail over the other one in terms of contribution, depending on body size, growth rate, fibre type and
353 environmental factors (Higgins and Thorpe 1990; Koumans and Akster 1995; Silva et al. 2009). In white
354 muscle of rainbow trout hyperplasia is the initial growth process, whose relative contribution gradually
355 decreases and this species growth by the end is entirely due to fiber hypertrophy (Stickland 1983).
356 Therefore, IGF-induced hypertrophic process via the PI3K/Akt pathway may occur in order to facilitate
357 the structural reorganization and development during *S. dumerili* metamorphosis.

358

359 *4.2. The IGF-1/MAPK pathway during S. dumerili development*

360 In addition to PI3K/Akt pathway, binding of IGF-1 to the IGF-1R activates the MAPK pathway, 361 which mediates the hyperplastic effect for cell proliferation through the activation of p44/42 MAPK 362 (Coolican et al. 1997). Herein, increase in the p44/42 MAPK phosphorylation was apparent on the 363 hatching day, in contrast to reduced levels in the before-after developmental stages. Ontologically, in *S.* 364 *dumerili*, as in many other teleosts, structures at hatching are undifferentiated including digestive tract, 365 which appears as a straight tube of simple cubic epithelium with no external anterior and posterior 366 opening (Masuma et al. 1990; Teles et al. 2017). Thus, both growth of existing cells and recruitment of 367 new ones through cell division act synergistically during organogenesis in postembryonic period 368 (Johnston and McLay 1997; Ostaszewska et al. 2008). Hyperplasia, for instance, has been demonstrated 369 as the crucial post-hatching process for the numerical increase of teleosts' slow muscle fibres (Veggetti 370 et al. 1990; Johnston 2006). Previous studies denoted a spatiotemporal activation of p44/42 MAPK 371 during embryogenesis in several species, including zebrafish (*Danio rerio*) (Krens et al. 2008; Wong et 372 al. 2019). In accordance with the aforementioned hypertrophy observations, hyperplasia seems to 373 predominate during the early *S. dumerili* development, while p44/42 MAPK activation may also act as a 374 regulator of genes involved in cell migration, differentiation and patterning, as has been previously 375 described in zebrafish (Krens et al. 2008). In addition, p44/42 MAPK signaling pathway mediates 376 odonto/osteogenic signals and thus may modulate skeletal and teeth development of *S. dumerili* larvae 377 (Ahi 2016).

378 Following p44/42 MAPK pathway activation at hatching day, a decrease was observed herein at 379 3 days post-hatching larvae stage. In accordance with our results, an apparent decline in hyperplasia has 380 been reported in other teleosts, such as blackspot seabream (*Pagellus bogaraveo*) (Silva et al. 2009), 381 during the first few days post-hatching, which coincides with the end of the endogenous feeding period. 382 Despite the aforementioned reduction, the activation of p44/42 MAPK pathway remained at high levels 383 throughout *S. dumerili* development, especially during metamorphosis and the subsequent forty-six days 384 post-hatching juveniles (D46). Similar to our results, larvae undergoing an excessive proliferation have 385 been previously reported in order to complete organs and tissues development and functionality (Yúfera 386 et al. 2014). For instance, during metamorphosis of redbanded seabream (*Pagrus auriga*), proliferative 387 events were involved in the development of several structures, such as gill and pseudobranch filaments 388 and gastric glands (Sánchez-Amaya et al. 2007). Organs' augmentation and establishment of functional

389 structures is fundamental in order for teleosts to cope with new regimes at the subsequent life stages
390 (Sánchez-Amaya et al. 2007; Ghasemi et al. 2020). It is well known that morphogenic transitions are
391 accompanied by changes in food consumption, which requires more complex digestive processes (Mai
392 et al. 2005; Islam and Tanaka 2006), such as *S. dumerili*'s shift from rotifers to formulated fishfeed (D33)
393 herein. Such dietary changes serve as supplier of the extra energy needed for the structural remodeling
394 during this stage (Islam and Tanaka 2006). Therefore, AMPK activation due to increase in the AMP/ATP
395 ratio (Goodman et al. 2011), as well as the activities of enzymes of the intermediate metabolism observed
396 during 3 days post-hatching larvae and/or thirty-three days post-hatching juveniles may indicate the
397 substantial energy amount consumed during morphogenic processes. However, glycolysis and lipid
398 metabolism (as indicated by L-LDH and HOAD respectively) seem to be the main source of ATP and
399 intermediate substrates required by biosynthesis during early development (Milman and Yurowitzky
400 1973; Tong et al. 2017). In addition, Segner and Verreth (1995) have observed a small delay in the
401 elevation of CS during the ontogenetic development in catfish, which coincides with the present results.
402 Therefore metabolic processes in *S. dumerili* development seem to involve carbohydrate and fatty acids
403 catabolism from fertilized eggs stage to cleavage stage, followed by fatty acids and amino acids
404 catabolism, reflecting a switch in fuel preferenda during organogenesis and tissues formation
405 (Lahnsteiner 2005). Thereafter, enzymatic activities in the present study decreased during both juvenile
406 stages (D33 – D46), highlighting their role in fish development, especially in the early developmental
407 stages.

408 Furthermore, IGF-mediated effects including cell migration and differentiation have been also
409 reported to involve activation of p38 MAPK signaling pathway (Zhang et al. 2005; Ren et al. 2010).
410 Herein, p38 MAPK phosphorylation was maintained at high levels during the developmental stages of
411 fertilized eggs and hatching day. Previous studies in both *Drosophila* (Suzanne et al. 1999) and zebrafish
412 (Fujii et al. 2000) have demonstrated implications of p38 MAPK in the establishment of the initial
413 asymmetry and axes patterning during embryogenesis. In addition, through the regulation of transcription
414 factors, p38 MAPK has been reported to be critical during skeletal myogenesis (Keren et al. 2006) and
415 morphogenesis (Adachi-Yamada et al. 1999) as well as in adipocytic, myogenic and neuronal
416 differentiation (reviewed in Nebreda and Porras 2000). In accordance with our findings, Krens et al.
417 (2006) observed constant p38a expression levels during zebrafish development. However, previous
418 studies have described a dual role of p38 signaling in the modulation of myogenesis, in which activation

419 induces myocyte differentiation while p38 suppression is essential for the subsequent alignment,
420 aggregation and fusion of the differentiated cells (Weston et al. 2003). From this standpoint, the reduction
421 in p38 phosphorylation observed herein at 3 days post-hatching larvae stage may be crucial for the
422 prevention of premature progression of muscle cells (Weston et al. 2003). Thus, p38 MAPK signaling
423 pathway may actively participate in several processes throughout *S. dumerili* development, from
424 regulation of early differentiation to juvenile muscle growth. On the contrary, JNK pathway activation
425 was prominent during the *S. dumerili* larvae and juvenile stages. Interestingly, a previous study in
426 zebrafish (Valesio et al. 2013) suggested a more potent role of JNK pathway at later stages of
427 organogenesis, where programmed cell death regulation is of greater importance and thus recruits both
428 pro-apoptotic and anti-apoptotic actions of JNK. In addition, during juvenile stages of *S. dumerili* p44/42
429 MAPK activation was also evident. The latter is consistent with previous studies in which hyperplastic
430 procedures occurs during larvae and juvenile stages, especially in species with higher growth rates, while
431 hypertrophy is more prominent at the adult stage (Veggetti et al. 1990; Chisada et al. 2011).
432
433 *4.3. Heat shock proteins in S. dumerili development*
434 Regarding Hsps, a significant induction occurred from the three days post-hatching larvae stage
435 onwards, except for Hsp90 which increased from hatching day. In accordance with the present results,
436 an induction in Hsp90 expression was observed during the first post-hatching stages of silver sea bream
437 (*Sparus sarba*) (Deane and Woo 2003). The involvement of Hsp90 during early development has also
438 been reported in zebrafish. In specific, Krone and Sass (1994) observed differences in the expression
439 patterns of the Hsp90α and Hsp90β isoforms during the early stages of embryogenesis. Specifically, no
440 changes in Hsp90α expression were observed at control temperatures, whereas induction of the Hsp90β
441 isoform was evident during somitogenesis.
442 Concerning Hsp70, similar results indicating an increase during early larvae stages have been
443 previously reported in zebrafish (Yeh and Hsu 2000, 2002). The induction of Hsp70 is triggered by a
444 developmental stage-dependent activation pathway and is considered to be mediated by signals of growth
445 or differentiation through the interaction of HSF-like factors with the promotor of the *hsp70* gene (Yeh
446 and Hsu 2000, 2002). In addition, a potential implication of Hsp70 in early zebrafish ontogenesis has
447 been reported by Blechinger et al. (2022), where *hsp70* was highly expressed during embryonic lens
448 formation. Thus, the Hsp70 levels observed herein in fertilized eggs may indicate a role in *S. dumerili*

449 early ontogenesis. Likewise, Japanese medaka (*Oryzias latipes*) exhibited non-inducible Hsp70 and
450 Hsp60 in early embryos, while levels increased later in post-hatching stages (Werner et al. 2001).
451 Among juveniles and adult rainbow trout, Rendell et al. (2006) have indicated differentiation, in
452 regard to the intracellular localization of Hsps, suggesting that the functions of these proteins may depend
453 on the stage of development. For instance, as *O. mykiss* develops, total Hsp90 in the nucleus decreases,
454 probably reducing the requirements for Hsp90 to function as molecular chaperones in the nuclear
455 compartment (Rendell and Currie 2005; Rendell et al. 2006). Considering that environmental conditions
456 in the aquaculture unit were at sustainable levels for *S. dumerili*, maintaining high Hsps levels during the
457 juvenile stages may be indicative of a greater need for the molecular chaperones function (Rendell et al.
458 2006). The importance of Hsps' function is also attributed to their involvement in signal transduction
459 processes, which interact with various components of signaling pathways that regulate growth and
460 development (Pratt and Toft 1997). Therefore, higher levels of Hsps may reflect higher growth rates,
461 higher nutritional requirements, and the constant renewal or replacement of proteins which occur at
462 juvenile stages. However, induction of Hsps during metamorphosis stage was also apparent at silver sea
463 bream (Deane and Woo 2003), thus indicating potential implication in cellular processes during the
464 transition from larvae to juveniles.
465
466 *4.4. Cell death in S. dumerili development*
467 Alongside growth, proliferation and differentiation, programmed cell death is crucial in early
468 animals' development, contributing to the sequential cell turnover mandatory for organs sculpting
469 (Penaloza et al. 2006). The apoptotic machinery seems to be active during the fertilized eggs stage of *S.*
470 *dumerili*, as observed herein by the Bax/Bcl-2 ratio, which displayed a subsequent reduction at hatching
471 day. Previous studies in zebrafish have reported rapid apoptotic clearance, which seems to be activated
472 at the gastrula stage and thereafter (Ikegami et al. 1999), to be coinciding with major developmental
473 events, such as initial outgrowth of retinal ganglion cell axons, lens separation from skin ectoderm and
474 Rohon-Beard neurons elimination (Svoboda et al. 2001; Cole and Ross 2001). In regard to *S. dumerili*,
475 several morphogenetic rearrangements occur during early embryogenesis that requisite cell death,
476 including Kupffer cells disappearance (Tachihara et al. 1993). Cell death continues to be differentially
477 recruited throughout development in order to facilitate the remodeling procedures such as changes in
478 zebrafish lateral line system (Cole and Ross 2001). In a previous study, cell apoptosis mediated critical

479 organs' transformative and functional changes, including eye movement and shift in swimming type,
480 during the metamorphosis of Japanese flounder (*Paralichthys olivaceus*) (Baolong et al. 2006). Thus, the
481 immense increase in caspases expression observed herein at the metamorphosis stage may be indicative
482 of the selective apoptotic process for *S. dumerili* organ-restructure. However, induction of the apoptotic
483 machinery as a cellular response to environmental stressors should not be excluded. The latter could be
484 supported by the fact that Hsps seem to modulate temperature-dependent cell death and defense
485 responses (Bao et al., 2014). According to Le Roith et al. (2001), anti-apoptotic signals in response to
486 environmental stimuli have been proposed to be mediated by IGF-1 through the Akt pathway.

487 On the other hand, mobilization of autophagy has been previously reported to occur during
488 developmental and differentiation pathways, acting simultaneously as an energy and nutrient supplier
489 (Mizushima and Levine 2010; Nuschke et al. 2014). The autophagic machinery is highly induced at
490 hatching day of *S. dumerili*, as observed herein by the elevated LC3 II/I levels, indicating a potential
491 involvement in yolk-sac reserves depletion. Prior to the nutritional switch from endogenous to exogenous
492 feeding, which occurs at 3 days post-hatching larvae in *S. dumerili* (Masuma et al. 1990), larvae constant
493 nutrients supply derives from the yolk-sac, which serves as the pivotal energy source for the highly
494 metabolically demanding processes of morphogenesis (Pelegri 2003; Lee et al. 2014; Mathai et al. 2017).
495 Implications of autophagy in early zebrafish morphogenesis and organogenesis have been proposed by
496 Lee et al. (2014), although it is not yet clear whether it is related to differentiation and/or metabolic
497 demands. However, induction of the autophagic process was also apparent in the subsequent thirty-three
498 days post-hatching juveniles stage as indicated by the increased LC3 II/I ratio and the decreased
499 SQSTM1/p62 levels. In addition to the potential involvement in the ontogeny and early development,
500 growing animals require high constitutive levels of autophagy in order to ensure proper cell proliferation
501 and functions (Mizushima and Levine 2010). Although ubiquitin represents a selective degradation
502 signal suitable for targeting various types of cargo (from protein aggregates to membrane-bound
503 organelles) (Kirkin et al. 2009), the present results showcase a differentiation in ubiquitination and
504 autophagy patterns in the thirty-three days post-hatching juveniles. Salmerón et al. (2015) have pointed
505 out that ubiquitin-related genes in gilthead sea bream are coordinately regulated during ontogeny, with
506 increased levels in fingerlings compared to juveniles. Similarly, ubiquitination herein is suppressed in
507 thirty-three days post-hatching juveniles, in which muscle growth is highly upregulated. Moreover, and
508 contrary to our results, it has been shown that in C2C12 muscle cells, increased ubiquitin levels suppress

509 proliferation, which can be associated with the poor healing potential in older individuals (Cai et al.
510 2004).

511 Furthermore, in contrast to previous studies demonstrating induction of autophagy due to
512 metabolic stress during the endogenous to exogenous feeding shift (Arevalo et al. 2018; Mawed et al.
513 2022), activation of AMPK in response to the increased cellular AMP/ATP ratio observed herein at the
514 3 days post-hatching larvae stage resulted in no elevation of the autophagic activity. Previous studies
515 have demonstrated that swimming activates AMPK in skeletal muscles, evidencing a critical role in the
516 metabolic and physiological adaptation to exercise (Magnoni et al. 2012, 2014). Subsequently, an
517 increase in the autophagic activity occurred during *S. dumerili* metamorphosis which is in accordance
518 with previous studies in other teleost species such as *Paralichthys olivaceus* (Gao et al. 2022) and
519 zebrafish (Mawed et al. 2022). During such a fundamental turnover stage, ontogenetic procedures may
520 recruit the autophagic machinery, as in the case of *P. olivaceus* where eye migration is driven by cell
521 death at the orbital tissue (Gao et al. 2022).

522 It is important to highlight that although both apoptosis and autophagy account for self-destructive
523 processes, a delay on the onset of apoptosis compared to autophagy was evident in the present study. The
524 latter could be attributed to the fact that in most cases autophagy acts as an inhibitor of apoptotic-induced
525 cell death, by recycling cell debris (Cooper 2018). However, due to the complexity of the corporation
526 between apoptosis and autophagy, which highly depends on the stimulus potency (Cooper 2018), the
527 aforementioned hypothesis needs thorough investigation.

528

529 **5. Conclusions**

530 Collectively, development in *S. dumerili* seems to recruit different and several signaling pathways
531 in each developmental stage, emphasizing their versatile roles in organogenesis and morphogenesis.
532 Specifically, hypertrophic signaling is stimulated via the IGF-1R/Akt pathway activation during the
533 fertilized eggs stage and larval-to-juvenile transition. Hyperplasia via p44/42 MAPK phosphorylation
534 mediates *S. dumerili* post-hatching process, and juvenile organs completion. The apparent induction of
535 Hsps at the juvenile stages suggest an immense chaperones recruitment due to higher growth rates, and
536 higher nutritional requirements. On the other hand, apoptosis was triggered during fertilized eggs and
537 autophagy at hatching day indicating an involvement in morphogenetic rearrangements and yolk-sac
538 reserves depletion. AMPK activation and AMP/ATP increase during metamorphosis indicate the

539 substantial energy amount consumed during this process (Fig. 8). Based on the present results and the
540 existing literature, Fig. 8 proposes signaling between the above-mentioned cellular processes.
541 Specifically, it seems that the activation of IGF-1R and Akt in the fertilized eggs' stage may be
542 responsible for processes observed in later developmental stages. Information in cellular physiology
543 regarding developmental procedures may help overcome reproductive dysfunctions which are a major
544 obstacle in this species commercialization. However, these signaling pathways need to be further
545 investigated in order to provide a more appropriate application in the aquaculture section ensuring the
546 proper and integrated teleost development.
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Ethics approval: The animal study protocol was performed in accordance with animal welfare guidelines of the Hellenic Aquaculture Producer Organization (HAPO), approved and validated by the Hellenic Ministry of Rural Development and Food (1558/198651), alongside the company's routine quality control samplings. All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the EU Directive 2010/63/EU for animal experiments.

Consent to participate: Not applicable

Consent for publication: Not applicable

Data Availability: The datasets generated and/or analysed during the current study are not publicly available because due to the nature of this research, accompanying data to the ones presented herein remain unpublished to date, but are available from the corresponding author on reasonable request.

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978 **Legends to Figures**

979

980 **Figure 1.** phospho IGF-1R / IGF-1R and phospho Akt / Akt levels (mean ± std) in different
981 developmental stages of greater amberjack (*S. dumerili*): 1 day prior to hatching fertilized eggs [day -1
982 (D-1)], hatching day [day 0 (D0)], 3 days post-hatching larvae [day 3 (D3)], and thirty-three and forty-
983 six days post-hatching juveniles [day 33 (D33) and day 46 (D46)]. Representative blots are shown.
984 Significant differences ($p < 0.05$) are presented between different developmental stages as lower case
985 letters.

986

987 **Figure 2.** phospho p38 MAPK / p38 MAPK, phospho p44/42 MAPK / p44/42 MAPK and phospho JNKs
988 / JNKs levels (mean ± std) in different developmental stages of greater amberjack (*S. dumerili*): 1 day
989 prior to hatching fertilized eggs [day -1 (D-1)], hatching day [day 0 (D0)], 3 days post-hatching larvae
990 [day 3 (D3)], and thirty-three and forty-six days post-hatching juveniles [day 33 (D33) and day 46 (D46)].
991 Representative blots are shown. Significant differences ($p < 0.05$) are presented between different
992 developmental stages as lower case letters

993

994 **Figure 3.** Hsp60, Hsp70 and Hsp90 levels (mean ± std) in different developmental stages of greater
995 amberjack (*S. dumerili*): 1 day prior to hatching fertilized eggs [day -1 (D-1)], hatching day [day 0 (D0)],
996 3 days post-hatching larvae [day 3 (D3)], and thirty-three and forty-six days post-hatching juveniles [day
997 33 (D33) and day 46 (D46)]. Representative blots are shown. Significant differences ($p < 0.05$) are
998 presented between different developmental stages as lower case letters

999

1000 **Figure 4.** Cleaved caspases, Bax and Bcl-2 levels and Bax/Bcl-2 ratio (mean ± std) in different
1001 developmental stages of greater amberjack (*S. dumerili*): 1 day prior to hatching fertilized eggs [day -1
1002 (D-1)], hatching day [day 0 (D0)], 3 days post-hatching larvae [day 3 (D3)], and thirty-three and forty-
1003 six days post-hatching juveniles [day 33 (D33) and day 46 (D46)]. Representative blots and dots are
1004 shown. Significant differences ($p < 0.05$) are presented between different developmental stages as lower
1005 case letters

1006

1007 **Figure 5.** Ubiquitin conjugates, SQSTM1/p62 and LC3 II/I ratio levels (mean ± std) in different
1008 developmental stages of greater amberjack (*S. dumerili*): 1 day prior to hatching fertilized eggs [day -1
1009 (D-1)], hatching day [day 0 (D0)], 3 days post-hatching larvae [day 3 (D3)], and thirty-three and forty-
1010 six days post-hatching juveniles [day 33 (D33) and day 46 (D46)]. Representative blots and dots are
1011 shown. Significant differences ($p < 0.05$) are presented between different developmental stages as lower
1012 case letters

1013

1014 **Figure 6.** phospho AMPK / AMPK, AMP/ATP ratio, AMP and ATP levels (mean ± std) in different
1015 developmental stages of greater amberjack (*S. dumerili*): 1 day prior to hatching fertilized eggs [day -1
1016 (D-1)], hatching day [day 0 (D0)], 3 days post-hatching larvae [day 3 (D3)], and thirty-three and forty-
1017 six days post-hatching juveniles [day 33 (D33) and day 46 (D46)]. Representative blots are shown.
1018 Significant differences ($p < 0.05$) are presented between different developmental stages as lower case
1019 letters

1020

1021 **Figure 7.** L-LDH (A), HOAD (B) and CS (C) V*max* activity levels (mean ± std) in different
1022 developmental stages of greater amberjack (*S. dumerili*): 1 day prior to hatching fertilized eggs [day -1
1023 (D-1)], hatching day [day 0 (D0)], 3 days post-hatching larvae [day 3 (D3)], and thirty-three and forty-
1024 six days post-hatching juveniles [day 33 (D33) and day 46 (D46)]. Significant differences ($p < 0.05$) are
1025 presented between different developmental stages as lower case letters

1026

1027 **Figure 8.** Summarized model of metabolic and signaling pathways in different developmental stages of
1028 greater amberjack (*S. dumerili*): 1 day prior to hatching fertilized eggs [day -1 (D-1)], hatching day [day
1029 0 (D0)], 3 days post-hatching larvae [day 3 (D3)], and thirty-three and forty-six days post-hatching
1030 juveniles [day 33 (D33) and day 46 (D46)].

1031

1032 **Table 1.** Water physicochemical characteristics (mean ± std) during the five developmental stages (D-1,

1033 D0, D3, D33 D46) of greater amberjack (*S. dumerili*)

Stages	**T (°C)**	**pH**	**O_2 (mg l^{-1})**	**O_2 (%)**
D-1	21.7 ± 1.2	7.8 ± 0.2	10.4 ± 0.4	145 ± 22
D0	23.4 ± 1.5	7.8 ± 0.3	6.6 ± 0.23	97 ± 12
D3	23.3 ± 1.3	7.9 ± 0.1	7.0 ± 0.4	98 ± 11
D33	25.9 ± 1.7	8.0 ± 0.2	7.7 ± 0.3	97 ± 10
D46	24.1 ± 1.4	8.1 ± 0.2	7.9 ± 0.4	101 ± 15

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Table 2 Coefficients of linear correlation (Pearson test) between the biological parameters investigated in in different developmental stages (D-1, D0, D3, D33, D46) of greater amberjack (*S. dumerili*). Significant correlation at the 0.05 level is represented with asterisk (*).

	Hsp90	Hsp70	Hsp60	p38	p44/42	JNKs	Bax/ Bcl-2	Caspases	Ubiquitin	AMPK	AMP/ ATP	LC3 II/I	p62	Akt	IGF	LDH	HOAD	CS
Hsp90	1	0,49*	0,45*	-0,19	0,43*	0,9*	0,16	0,16	0,83*	0,38*	-0,19	0,59*	-0,26	-0,09	-0,14	-0,87*	-0,42*	0,68*
Hsp70		1	0,84*	-0,84*	-0,21	0,24	0,42*	0,42*	0,35*	0,39*	-0,13	0,49*	-0,38*	0,48*	0,4*	-0,09	0,37*	0,56*
Hsp60			1	-0,48	0,21	0,37*	0,16	0,48*	0,3	0,6*	0,26	0,39*	-0,37*	0,38*	0,41*	-0,28	0,02	0,22
p38				1	0,69*	0,18	-0,67*	-0,23	0,003	-0,22	0,19	-0,27	0,23	-0,44*	-0,28	-0,29	-0,74*	-0,53*
p44/42					1	0,75*	-0,58*	0,09	0,53*	0,17	0,19	0,18	-0,07	-0,26	-0,11	-0,78*	-0,92*	-0,14
JNKs						1	-0,25	0,23	0,87*	0,23	-0,16	0,6*	-0,31*	-0,09	-0,06	-0,96*	-0,77*	0,43*
Bax/Bcl-2							1	-0,45*	-0,13	0,39*	0,08	-0,34*	0,44*	-0,33*	-0,47*	0,19	0,68*	0,51*
Caspases								1	0,02	0,22	0,11	0,83*	-0,98*	0,91*	0,92*	-0,14	-0,21	-0,24
Ubiquitin									1	-0,12	-0,56*	0,48*	-0,1	-0,12	-0,12	-0,73*	-0,43*	0,79*
AMPK										1	0,78*	0,11	-0,17	-0,04	-0,04	-0,38*	-0,13	-0,11
AMP/ATP											1	-0,25	0,001	-0,1	-0,02	-0,04	-0,17	-0,66*
LC3 II/I												1	-0,91*	0,69*	0,65*	-0,49*	-0,34*	0,24
p62													1	-0,87*	-0,85*	0,23	0,25	0,14
Akt														1	0,98*	0,23	0,17	-0,17
IGF															1	0,2	0,05	-0,29
LDH																1	0,8*	-0,31*
HOAD																	1	0,23
CS																		1