Up-scaling validation of a dummy regression approach for predictive modelling the fillet fatty acid composition of cultured European sea bass (*Dicentrarchus labrax*)

G. F. Ballester-Lozano[1], L. Benedito-Palos[1], M. Mingarro[2], J. C. Navarro[3], J. Pérez-Sánchez[1]

[1]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal, IATS-CSIC, Castellón, Spain

[2] Andromeda Ibérica Acuicultura S.L, Castellón, Spain

[3]Live Preys, Larviculture and Ecotoxicology Group, Institute of Aquaculture Torre de la Sal, IATS-CSIC, Castellón, Spain

Corresponding author: Jaume Pérez-Sánchez

E-mail: jperez@iats.csic.es

Phone: +34 964319500

Fax: +34 964319509

Running title: fillet fatty acid composition

Keywords: European sea bass, gilthead sea bream, turbot, sole, fillet, fatty acid composition.

Abstract

The aim of the study was to validate a dummy regression approach for predictive modelling the fillet fatty acid (FA) composition of cultured European sea bass with dietary FA composition and lipid fillet content as independent variables. The model used our own data on gilthead sea bream as reference subgroup dataset and data from turbot, sole and European sea bass as dummy variables. Most of the observed variance within and among species was explained by the regression model without statistical significant interactions on blocks between diet composition and fish species subgroups. For the validation of European sea bass FA descriptors, predictive values derived from data on fish reared at laboratory scale were plotted against those obtained in farmed fish harvested at commercial size. A close linear association near to equality was found for 12 representative FAs, including saturated FAs, monoenenes and polyunsaturated FAs. This finding reinforces the possibility to produce tailored and healthy seafood products according to the guidelines of essential FA requirements in humans. FA algorithms for all the species in the model are hosted at www.nutrigroup-iats.org/aquafat as a multispecies tool to interrogate the nutritionally regulated FA composition of four cultured marine fish species of a high added value.

Introduction

Nowadays, more than half of the world's fish and fish-based foods are supplied by the aquaculture industry, and the growing demand for seafood products will require in 2030 an additional production of 40 million metric tons just to maintain the current human consumption (McGuire 2013). This is because fish and fishery products represent a very valuable source of selenium, iodine, taurine, vitamins D and B12 and highly nutritive proteins, but also of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), specifically eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids. Marine fish are in fact the most important source of EPA and DHA in the human diet, and most production systems, even those of salmonids and freshwater fish, are facing increasing pressures to include relatively high levels of fish oil in commercial diets to meet the daily human requirements in EPA and DHA (500 mg day^{-1}). However, both fish meal and fish oil are finite natural resources, and the inclusion level of such marine feedstuffs in fish diets has been steadily declining over the course of the last 10 years (Hardy 2010; Tacon, Metian, Turchini & De Silva 2010; Shepherd & Jackson 2013). Terrestrial plant ingredients are the most obvious alternative, but vegetable oils are devoid of n-3 LC-PUFAs, and fish fed these oils have a fillet reduction in EPA and DHA content (Turchini, Torstensen & Ng 2009; Nasopoulou & Zabetakis 2012). Therefore, it is of relevance to guarantee a relatively high content of n-3 LC-PUFAs in fish meat to ensure the human healthful benefits of consuming farmed fish as a whole (Lund 2013). This is the rationale for the multispecies predictive modelling of fillet fatty acid (FA) composition, and interestingly the robustness of linear regressions with dietary FA composition and fillet lipid content as independent variables was proven highly informative in gilthead sea bream (*Sparus aurata*) (Ballester-Lozano, Benedito-Palos, Navarro, Kaushik & Pérez-Sánchez 2011; Benedito-Palos, Bermejo-Nogales,

Karampatos, Ballester-Lozano, Navarro, Diez, Bautista, Bell, Tocher, Obach, Kaushik & Pérez-Sánchez 2011). What is more, a dummy regression approach with the gilthead sea bream dataset as a reference subgroup has been used by Ballester-Lozano, Benedito-Palos, Riaza, Navarro, Rosel & Pérez-Sánchez (2014) for predictive modelling the fillet FA composition in turbot (*Scophthalmus maximus*) and sole (*Solea solea*) (www.nutrigroup-iats.org/aquafat). One of the assumptions of dummy regression is that the contribution of each fish species to the total variance will be best estimated by pooling all the data from the different species subgroups (Kutner, Nachtsheim & Neter 2004). Furthermore, the absence of a statistically significant interaction between fish species and diet composition spares the necessity of the use of a vast array of diets for all the species included in the model. However, most result outputs are species-specific, and each new species in the model should be introduced as a dummy regression variable and the resulting FA descriptors validated thereafter with additional data. This interactive procedure is carried out herein with European sea bass (*Dicentrarchus labrax*), using fish reared at the laboratory scale for the definition of the specific FA descriptors and farmed fish for the up-scaling validation of the predictive results of fillet FA composition.

Materials and methods

Laboratory scale feeding trial

Juvenile European sea bass of Mediterranean origin (Andromeda Ibérica Acuicultura S.L, Castellón, Spain) were acclimatised for 20 days to laboratory conditions at the Institute of Aquaculture Torre de la Sal (IATS). A long-term trial was then undertaken

over the course of a 20-month productive cycle from November 2011 to July 2013. Four hundred and fifty fish were fed in triplicate groups to visual satiety with commercial pellets (Starter Plus, 1.5 mm; EFICO YM558, 3-4.5 mm) manufactured by Biomar (Dueñas, Palencia, Spain). Water flow (37‰ salinity) in 500-3,000 L fibreglass tanks was 10-30 L min^{-1}, the oxygen concentration remained higher than 85% saturation, and unionised ammonia was below toxic levels (<0.02 mg L^{-1}) with rearing densities less than15 kg m^{-3}. Total mortality was less than 2%, and day-length and water temperature followed the natural changes at IATS latitude (40º5′N; 0º10′E). All procedures were carried out according to national and institutional regulations (Consejo Superior de Investigaciones Científicas, IATS Review Board) and the current European Union legislation on handling experimental animals.

Lipid composition analysis

At regular intervals (each 6–8 months), 12 individuals were randomly selected for fillet sampling and killed by a blow to the head. Deboned left side fillets were vacuum packed in plastic bags and stored at −80 °C until analysis. The lipid content in freeze-dried fillet samples (0.5 g) was determined gravimetrically using the Soxhlet 4001046 Auto extraction apparatus (Selecta, Barcelona, Spain) with 50 mL of diethyl ether at 120 ºC as the extracting solvent. Total lipids for analyses of fillet FA composition were extracted in freeze-dried samples by the method of Folch (Folch, Lees & Stanley 1957). FA methyl esters were obtained by acid transmethylation (Christie, 1982) and analysed by gas-chromatography on a Fisons Instruments GC 8000 Series (Rodano, Italy) gas chromatograph, equipped with a fused silica 30 m × 0.25 mm open tubular column (Tracer, TR-WAX; film thickness: 0.25 µm, Teknokroma, Barcelona, Spain) using

123 nonadecanoic FA (19:0) as an internal standard. For more details of lipid and FA
124 analysis see Ballester-Lozano *et al*. (2014).
125
126 *Multilinear regression equations*
127
128 Our own data on fish FA composition together with those generated in the present study
129 were compiled for dummy regression analysis, using gilthead sea bream as the reference
130 subgroup, and turbot, sole and European sea bass as dummy regression variables fitted
131 to multiple regression equations:
132 $Y'_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X^{-1}_{i2} + [\beta_3 X_{i3} + \beta_4 X_{i4} + \beta_5 X_{i5}] + [\beta_6 X_{i1} X_{i3} + \beta_7 X_{i1} X_{i4} + \beta_8 X_{i1} X_{i5}] + [\beta_9 X^{-}$
133 $^{1}_{i2} X_{i3} + \beta_{10} X^{-1}_{i2} X_{i4} + \beta_{11} X^{-1}_{i2} X_{i5}]$
134 Where Y'_i = forecasted fillet FA in mg g lipid^{-1}; β_0 = Y axis intercept; $\beta_{1...8}$ = regression
135 coefficients; X_{i1} = dietary FA composition (mg g lipid^{-1}); X^{-1}_{i2} = inverse of fillet lipid
136 content (% wet matter); X_{i3} = 1 if turbot, 0 otherwise; X_{i4} = 1 if sole, 0 otherwise; X_{i5} = 1
137 European sea bass, 0 otherwise; $X_{i1}X_{i3}$ = interaction effect between X_{i1} and X_{i3}; $X_{i1}X_{i4}$ =
138 interaction effect between X_{i1} and X_{i4}; $X_{i1}X_{i5}$ = interaction effect between X_{i1} and X_{i5}; X^{-}
139 $^{1}_{i2}X_{i3}$ = interaction effect between X^{-1}_{i2} and X_{i3}; $X^{-1}_{i2}X_{i4}$ = interaction effect between X^{-1}_{i2}
140 and X_{i4}; $X^{-1}_{i2}X_{i5}$ = interaction effect between X^{-1}_{i2} and X_{i5}. The $\beta_3 X_{i3}$, $\beta_4 X_{i4}$ and $\beta_5 X_{i5}$
141 terms are written inside brackets for stressing that they correspond to dummy variables.
142 Brackets were also used for the interaction terms of dummy variables and independent
143 variables ($[\beta_6 X_{i1} X_{i3} + \beta_7 X_{i1} X_{i4} + \beta_8 X_{i1} X_{i5}]$, $[\beta_9 X^{-1}_{i2} X_{i3} + \beta_{10} X^{-1}_{i2} X_{i4} + \beta_{11} X^{-1}_{i2} X_{i5}]$) that were
144 analysed as blocks with a given statistical significance for each.
145
146 *Up-scaling validation*
147

For the up-scaling validation of European sea bass FA descriptors, 18 fish from the Mediterranean fish farm of Andromeda Ibérica Acuicultura (Castellón, Spain) were harvested at commercial size (330–400 g) in June of 2013. At the harvest fish were fed with EFICO YM557 (Biomar). Deviations from the model were analysed using a statistical t-test to determine if predicted FA values (results from the regression equations) were statistically distinguishable from observed values at the significance level of 5%. The *D* coefficient of distance (McIntire, Tinsley & Lowry 1969) was used to evaluate the paired fish species differences in FA descriptors (reference fish *vs* species subgroup):

$$D_{(h-j)} = [\Sigma^{n}_{i=1} (P_{ih} - P_{ij})^2]^{1/2}$$

Where $D_{(h-j)}$ is the distance between species *h* and *j*, and P_{ih} and P_{ij} are the output predictions of FA*i* in species *h* and *j*, for each *i* FA. For the calculus of the predicted fillet FA composition, the same diet FA composition (BIOMAR, EFICO YM557) was considered for all the fish species. The theoretical fillet lipid content was fixed at 9%, 7%, 8% and 11% for European sea bass, turbot, sole and gilthead seabream, respectively.

Results and discussion

Fish performance and fillet fatty acid composition

Fish reared at the IATS experimental facilities grew from an average body weight of 28.3 g in March of 2012 to 432 g in July of 2013 with a feed conversion efficiency of 0.8-1 for the active feeding period of March–November. Non-significant changes in fillet lipid content were found throughout all the productive cycle, though the trend of

lipid deposition rates followed the known seasonal changes in growth rates, feed intake and plasma levels of growth-promoting factors of this species in our latitude (Vega-Rubín de Celis, Rojas, Gómez-Requeni, Albalat, Gutiérrez, Médale, Kaushik, Navarro & Pérez Sánchez 2004), with the highest fillet lipid content at the end of the warm season in fish sampled in November of 2012 (Table 1). In contrast, as reported in a vast array of species, including salmonids (Bell, Tocher, Henderson, Dick & Crampton 2003), freshwater fish (Thanuthong, Francis, Senadheera, Jones & Turchini 2011; Wing-Keong, Cheong-Yew, Yang, & Romano 2013), and both cold and warm marine fish (Turchini *et al*. 2009), fillet FA composition highly reflects changes in diet composition. In the present study, dietary changes in FA composition occurred with the increase of pellet size from 1.5 to 3-4.5 mm, and largely affected myristic acid (14:0), palmitic acid (16:0), oleic acid (18:1n-9), linoleic acid (18:2n-6), linolenic acid (18:3n-3), EPA and DHA. As a general rule, the concentration of saturated FAs and n-3 LC-PUFAs was higher in the starting pellet (1.5 mm) than in finishing ones (3-4.5 mm), whereas the opposite was found for C18 monoenes and C18 PUFAs.

Fish specificity of fatty acid descriptors

Regarding European sea bass FA descriptors (Table 2), most of the observed variance in fillet FA composition was explained by dummy regression equations with highly significant ($P<0.0001$) coefficients of correlation, varying between 0.75 for 18:1n7 and 0.99 for 18:3n-3. It is noteworthy that the β_1 partial regression coefficients were statistically significant for all the FA descriptors ($P<0.001$), which indicates that the independent variable dietary FA composition highly contributes to the total variance of endogenous and non-endogenously synthesised FAs. However, this close association is theoretically better for non-endogenously synthesised FAs, especially in salmonids

200 (Sanden, Stubhaug, Berntssen, Lie, & Torstensen 2011; Turchini, Francis, Keast &
201 Sinclair 2011) and lean fish (Karalazos, Treasurer, Cutts, Alderson, Galloway,
202 Albrektsen, Arnason, MacDonald, Pike, & Bell 2007; Jobling, Leknes, Saether, &
203 Bendiksen 2008). In any case, the contribution of the second independent variable "fillet
204 lipid content" to the total variance is generally lower, which is supported in this work by
205 the observation that β_2 partial regression coefficients were not statistically significant
206 for 20:1n-9, 18:2n-6 and EPA.
207 The specific contribution of partial regression coefficients ($\beta_{3\text{-}5}$) to the total
208 variance is indicative of the distance between the reference species subgroup and each
209 particular dummy variable of the model. This is an indirect measure of the calculated
210 coefficient of distance *D* between gilthead sea bream (reference species subgroup) and
211 dummy variables, which was 51 for European sea bass and 96–106 for turbot and sole
212 (Fig. 1). Certainly, gilthead sea bream and European sea bass are carnivorous fish of the
213 order Perciformes that compete for a similar biological niche in temperate areas. In
214 contrast, turbot and sole are flat fish with different ecological environments and lipid
215 metabolic capabilities (Zheng et al. 2009; Morais, Castanheira, Martinez-Rubio,
216 Conceição & Tocher 2012), but even in the case of European sea bass the specific
217 coefficients (β_5) were statistically significant for almost all FAs with the exception of
218 20:1n-9 and EPA.
219 Focussing on the significance of blocks, the lack of statistically significant
220 interactions between diet and marine fish species subgroups was further corroborated in
221 the present study, and the corresponding block of interactions ($\beta_{6\text{-}8}$) was not included in
222 the regression equation of any FA descriptor. From a practical point of view, diet effects
223 can be, thereby, interpolated from a relatively low number of diets when a holistic
224 approach for fish meal and fish oil replacement in fish feeds has been considered for the
225 reference species subgroup. In contrast, both in this and in the previous study (Ballester-

226 Lozano *et al.* 2014), statistically significant interactions between fillet lipid content and
227 fish species subgroups were found for saturated FAs (14:0, 16:0, 18:0) and monoenes
228 (18:1n-9, 16:1n-7, 18:1n-7, 20:1n-9). Therefore, the contribution of the variable fillet
229 lipid content is highly dependent on fish species, probably reflecting the fish species
230 differences in tissue FA uptake and lipogenic enzyme activities, including among others
231 the stearoyl-CoA desaturase, evolved in teleosts as two different isoforms (Castro,
232 Wilson, Gonçalves, Galante-Oliveira, Rocha, & Cunha 2011), which are highly
233 nutritionally regulated at the transcriptional level in the skeletal muscle of gilthead sea
234 bream (Benedito-Palos, Calduch-Giner, Ballester-Lozano & Pérez-Sánchez 2013).
235 In addition to the above findings, statistically significant interactions on fillet
236 lipid content and dummy variables ($\beta_{9\text{-}11}$) where found for saturated FAs and monoenes,
237 but not for C18, C20 and C22 PUFAs, which is in accordance with the general
238 statement that marine fish have a limited capability for LC-PUFA biosynthesis due to
239 deficiencies in the PUFA desaturation/elongation pathway (Mourente, Dick, Bell &
240 Tocher 2005; Zheng, Ding, Xu, Monroig, Morais, & Tocher, 2009).
241
242 *Up-scaling validation: concluding remarks*
243
244 Given the fish species differences in lipid metabolism, the predictive modelling of fillet
245 FA composition becomes especially feasible for marine farmed fish. Concretely in the
246 present study, the up-scaling of predictive values to farming conditions allowed a close
247 linear association (R^2 = 0.98) near to equality for the regression plot of the observed (X-
248 axis) vs. predicted values (Y-axis) (Fig. 2). This regression feature was achieved for 12
249 representative FAs, including saturated FAs (14:0, 16:0, 18:0), monoenes (16:1n-7,
250 18:1n-7, 18:1n-9, 20:1n-9), C18 PUFAs (18:2n-6) and n-3 LC-PUFAs (22:5n-3, EPA,
251 DHA). Similarly, fillet FA composition of turbot and sole are also highly predictable

252 under farming conditions (Ballester-Lozano et al. 2014). Therefore, dummy regression
253 is confirmed as a powerful multispecies tool for predictive modelling the nutritionally
254 tailored fillet FA composition of marine fish. This skill is supported by the absence of
255 statistically significant interactions between dietary FA composition and fish species
256 subgroups, which reinforces the possibility to produce more tailored and healthy
257 seafood products according to the guidelines of essential FA requirements in humans.
258 For practical use of a wide range of stakeholders, including fish farmers, fish producers
259 and consumers, FA algorithms for all the species included in the model are hosted at
260 www.nutrigroup-iats.org/aquafat as an interactive tool to interrogate fillet FA
261 composition in four marine species (gilthead sea bream, turbot, sole and European sea
262 bass) of a high added value for the European and Spanish aquaculture in particular. This
263 type of information is useful for the implementation of selection programmes non-
264 merely based on growth biometrics modelling. Indeed, genotype and diet interactions
265 were found for body weight and lipid content in in European sea bass fed plant-based
266 diets (Le Boucher, Vandeputte, Dupont-Nivet, Quillet, Mazurais, Robin, Vergnet,
267 Medale, Kaushik & Chatain 2011), although we are still far of holistic approaches
268 considering how interact feed composition, and individual differences in muscle
269 adiposity and feeding behaviour on fish productive traits (Sih, Bell, Johnson & Ziemba
270 2004; Mas-Muñoz, Komen, Schneider, Visch, & Schrama 2011), also affecting the
271 human health benefits of consuming farmed fish fed alternative diets.
272
273
274 **Acknowledgements**
275
276 This study was founded by Spanish (AQUAFAT, AGL2009-07797, Predictive
277 modelling of flesh fatty acid composition in cultured fish species with different muscle

lipid content) and EU (ARRAINA, KBBE-2011-5-288925, Advanced research initiatives for nutrition and aquaculture) projects. Additional founding was obtained from the “Generalitat Valenciana” (research grant PROMETEO 2010/006). GFB-L was recipient of a Spanish PhD fellowship from the Diputación Provincial de Castellón.

References

Ballester-Lozano, G.F., Benedito-Palos, L., Riaza, A., Navarro, J.C., Rosel, J. & Pérez-Sánchez, J. (2014). Dummy regression analysis for predictive modelling the tailored fillet fatty acid composition of farmed flat fish using gilthead sea bream (*Sparus aurata* L.) as a reference subgroup category. *Aquaculture Nutrition*, DOI: 10.1111/anu.12095.

Ballester-Lozano, G. F., Benedito-Palos, L., Navarro, J. C., Kaushik, S. & Pérez-Sánchez, J. (2011). Prediction of fillet fatty acid composition of market-size gilthead sea bream (*Sparus aurata*) using a regression modelling approach. *Aquaculture* **319**, 81-88.

Bell, J. G., Tocher, D. R., Henderson, R. J., Dick, J. R. & Crampton, V. O. (2003). Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *Journal of Nutrition* **133**, 2793-2801.

Benedito-Palos, L., Bermejo-Nogales, A., Karampatos, A. I., Ballester-Lozano, G. F., Navarro, J. C., Diez, A., Bautista, J. M., Bell, J. G., Tocher, D. R., Obach, A., Kaushik, S. & Pérez-Sánchez, J. (2011). Modelling the predictable effects of dietary lipid sources on the fillet fatty acid composition of one-year-old gilthead sea bream (*Sparus aurata* L.). *Food Chemistry* **124**, 538-544.

Benedito-Palos, L., Calduch-Giner, J., Ballester-Lozano, G. & Pérez Sánchez, J. (2013). Effect of ration size on fillet fatty acid composition, phospholipid allostasis and mRNA expression patterns of lipid regulatory genes in gilthead sea bream (*Sparus aurata*). *British Journal of Nutrition* **109**, 1175-1187.

Castro, L. F. C., Wilson, J. M., Gonçalves, O., Galante-Oliveira, S., Rocha, E. & Cunha, I. (2011). The evolutionary history of the stearoyl-CoA desaturase gene family in vertebrates. *Bmc Evolutionary Biology* **11**, 132.

309 Christie, W.W. (1982) *Lipid Analysis*: *Isolation, Separation, Identification and*
310 *Structural Analysis of Lipids*. 2nd edn., Pergamon Press, Oxford, UK.
311 Folch, J., Lees, M. & Stanley, G. H. S. (1957). A simple method for the isolation and
312 purification of total lipids from animal tissues. *Journal of Biological Chemistry*
313 **226**, 497-509.
314 Hardy, R. W. (2010). Utilization of plant proteins in fish diets: effects of global demand
315 and supplies of fishmeal. *Aquaculture Research* **41**, 770-776.
316 Jobling, M., Leknes, O., Saether, B. S. & Bendiksen, E. Å. (2008). Lipid and fatty acid
317 dynamics in Atlantic cod, *Gadus morhua*, tissues: Influence of dietary lipid
318 concentrations and feed oil sources. *Aquaculture* **281**, 87-94.
319 Kutner, M. H., Nachtsheim, C. J., & Neter J. (2004) Qualitative predictor variables. In
320 *Applied Linear Regression Models*, 4th edn, pp. 455-497. McGraw-Hill Press,
321 New York, USA.
322 Karalazos, V., Treasurer, J., Cutts, C. J., Alderson, R., Galloway, T. F., Albrektsen, S.,
323 Arnason, J., MacDonald, N., Pike, I. & Bell, G. (2007). Effects of fish meal
324 replacement with full-fat soy meal on growth and tissue fatty acid composition
325 in Atlantic cod (*Gadus morhua*). *Journal of Agricultural and Food Chemistry*
326 **55**, 5788-5795.
327 Le Boucher, R., Vandeputte, M., Dupont-Nivet, M., Quillet, E., Mazurais, D., Robin, J.,
328 Vergnet, A., Medale, F., Kaushik, S. & Chatain, B. (2011). A first insight into
329 genotype x diet interactions in European sea bass (*Dicentrarchus labrax* L.
330 1756) in the context of plant-based diet use. *Aquaculture Research* **42**, 583-592.
331 Lund, E. K. (2013). Health benefits of seafood; Is it just the fatty acids? *Food Chemistry*
332 **140**, 413-420.

333 Mas-Muñoz, J., Komen, H., Schneider, O., Visch, S. W. & Schrama, J. W. (2011).
334 Feeding Behaviour, Swimming Activity and Boldness Explain Variation in
335 Feed Intake and Growth of Sole (*Solea solea*) Reared in Captivity. *Plos One* **6**.
336 McGuire, S. (2013). WHO, World Food Programme, and International Fund for
337 Agricultural Development. 2012. The State of Food Insecurity in the World
338 2012. Economic growth is necessary but not sufficient to accelerate reduction of
339 hunger and malnutrition. Rome, FAO. *Advances in Nutrition: An International*
340 *Review Journal* **4**, 126-127.
341 McIntire, C. D., Tinsley, I. & Lowry, R. (1969). Fatty acids in lotic periphyton: Another
342 measure of community structure. *Journal of phycology* **5**, 26-32.
343 Morais, S., Castanheira, F., Martinez-Rubio, L., Conceição, L. E. C. & Tocher, D. R.
344 (2012). Long chain polyunsaturated fatty acid synthesis in a marine vertebrate:
345 Ontogenetic and nutritional regulation of a fatty acyl desaturase with Δ4 activity.
346 *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*
347 **1821**, 660-671.
348 Mourente, G., Dick, J. R., Bell, J. G. & Tocher, D. R. (2005). Effect of partial
349 substitution of dietary fish oil by vegetable oils on desaturation and β-oxidation
350 of [1-14C]18:3n−3 (LNA) and [1-14C]20:5n−3 (EPA) in hepatocytes and
351 enterocytes of European sea bass (*Dicentrarchus labrax* L.). *Aquaculture* **248**,
352 173-186.
353 Nasopoulou, C. & Zabetakis, I. (2012). Benefits of fish oil replacement by plant
354 originated oils in compounded fish feeds. A review. *Lwt-Food Science and*
355 *Technology* **47**, 217-224.
356 Sanden, M., Stubhaug, I., Berntssen, M. H. G., Lie, O. & Torstensen, B. E. (2011).
357 Atlantic Salmon (*Salmo salar* L.) as a Net Producer of Long-Chain Marine

omega-3 Fatty Acids. *Journal of Agricultural and Food Chemistry* **59**, 12697-12706.

Shepherd, C. J. & Jackson, A. J. (2013). Global fishmeal and fish-oil supply: inputs, outputs and markets. *Journal of Fish Biology* **83**, 1046-1066.

Sih, A., Bell, A. M., Johnson, J. C. & Ziemba, R. E. (2004). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology* **79**, 241-277.

Tacon, A. G. J., Metian, M., Turchini, G. M. & De Silva, S. S. (2010). Responsible Aquaculture and Trophic Level Implications to Global Fish Supply. *Reviews in Fisheries Science* **18**, 94-105.

Thanuthong, T., Francis, D. S., Senadheera, S. D., Jones, P. L. & Turchini, G. M. (2011). Fish oil replacement in rainbow trout diets and total dietary PUFA content: I) Effects on feed efficiency, fat deposition and the efficiency of a finishing strategy. *Aquaculture* **320**, 82-90.

Turchini, G. M., Torstensen, B. E. & Ng, W. K. (2009). Fish oil replacement in finfish nutrition. *Reviews in Aquaculture* **1**, 10-57.

Turchini, G. M., Francis, D. S., Keast, R. S. J. & Sinclair, A. J. (2011). Transforming salmonid aquaculture from a consumer to a producer of long chain omega-3 fatty acids. *Food Chemistry* **124**, 609-614.

Vega-Rubín de Celis, S., Rojas, P., Gómez-Requeni, P., Albalat, A., Gutiérrez, J., Médale, F., Kaushik, S., Navarro, I. & Pérez Sánchez, J. (2004). Nutritional assessment of somatolactin function in gilthead sea bream (*Sparus aurata*): concurrent changes in somatotropic axis and pancreatic hormones. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* **138**, 533-542.

Wing-Keong, N., Cheong-Yew, C., Yang, W. & Romano, N. (2013). Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative

stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil finishing diets. *Aquaculture* **372**, 97-110.

Zheng, X. Z., Ding, Z. K., Xu, Y. Q., Monroig, O., Morais, S. & Tocher, D. R. (2009). Physiological roles of fatty acyl desaturases and elongases in marine fish: Characterisation of cDNAs of fatty acyl Delta 6 desaturase and *elovl5* elongase of cobia (*Rachycentron canadum*). *Aquaculture* **290**, 122-131.

Table 1. Lipid content and fatty acid composition (% total fatty acid methyl esters) of diets and fillets of European sea bass sampled at different times over the production cycle (November 2011/July 2013). The range of variation is indicated for the two groups of pellet size (1.5 mm and 3-4.5 mm) with changes in lipid content and fatty acid composition. Data on fillet fatty acid composition and lipid content are presented as mean and standard deviations (n = 12). Data on fish with unlike superscript letters were significantly different (P<0.001; Holm-Shidack *post hoc* test).

	Diet		Fish					
	1.5mm	3-4.5mm	March 12[1]		November 12[2]		July 13[2]	
			Mean	SD	Mean	SD	Mean	SD
Body weight (g)			28.19[a]	6.95	288.2[b]	76.3	432.5[c]	58.0
Total lipids (g 100 g^{-1})	19.4-21.8	21.4-23.1	8.05	2.55	9.29	1.43	7.78	1.86
∑ FA (mg g $lipid^{-1}$)	661.4-730.7	575.2-766.9	614.7	72.8	567.5	68.94	564.5	12.03
FA (% FAME)								
14:0	5.8-6.6	1.8-2.1	4.32[a]	0.32	1.90[b]	0.10	1.72[c]	1.16
16:0	18.6-20.0	12.2-13.3	19.4[a]	0.92	16.3[b]	0.58	15.2[c]	0.61
18:0	3.9-4.0	3.2-4.5	4.21[a]	0.17	4.31[a]	0.29	3.87[b]	0.27
16:1n-7	6.8-8.1	2.3-2.5	7.44[a]	0.41	3.01[b]	0.22	3.17[b]	0.17
18:1n-7	2.9-3.0	2.2-2.5	3.44[a]	0.13	2.32[b]	0.04	2.24[c]	0.07
18:1n-9	11.3-14.2	25.5-26.4	21.2[b]	1.76	28.9[a]	0.79	28.7[a]	0.82
20:1n-9	1.4-1.6	1.7-2.0	1.79[c]	0.13	1.89[b]	0.09	2.05[a]	0.12
22:1n-11	0.9-1.6	1.2-1.5	0.67[a]	0.13	0.68[a]	0.05	0.58[b]	0.09
18:2n-6	7.7	29.2-30.5	6.02[c]	0.60	23.3[b]	0.98	24.6[a]	1.00
20:2n-6	0.12-0.13	0.28-0.30	0.50[b]	0.07	0.95[a]	0.07	0.95[a]	0.08
20:3n-6	0.06	0.10-0.11	0.16[a]	0.01	0.14[b]	0.01	0.11[c]	0.01
20:4n-6	0.69-0.96	0.25-0.27	0.75[a]	0.09	0.31[b]	0.03	0.35[b]	0.04
18:3n-3	1.0-1.1	3.7-4.0	1.06[c]	0.07	2.76[b]	0.10	2.87[a]	0.12
18:4n-3	1.7-2.2	0.51-0.53	0.96[a]	0.21	0.41[b]	0.04	0.38[b]	0.04
20:5n-3	11.1-12.8	2.9-3.0	7.87[a]	1.11	2.79[b]	0.26	2.80[b]	0.22
22:5n-3	1.2-1.7	0.63-0.70	1.33[a]	0.12	0.74[b]	0.06	0.69[b]	0.06
22:6n-3	8.9-12.6	3.4-3.9	11.2[a]	1.34	4.89[b]	0.54	4.89[b]	0.48

[1]Fish were fed with 1.5 mm pellet size from November 2011 to March 2012.
[2]Fish were fed from March 2012 to November 2012 with 3 mm pellet size and continued to be fed until July 2013 with 4.5 mm pellet size.

391

Table 2. Correlation coefficient (R^2) and partial regression (β_1- β_{11}) coefficients of European sea bass FA descriptors with dietary fatty acid composition (mg g^{-1} lipid) and the inverse of fillet lipid content (g 100 g^{-1} fillet) as independent variables.

FA	R^2	β_0	P^1	β_1	P^1	β_2	P^1	β_5	P^1	β_{11}	P^1
14:0	0.948	11.98	<0.001	0.62	<0.001	-47.20	<0.001	-9.13	<0.001	35.74	0.003
16:0	0.760	55.18	<0.001	0.64	<0.001	-141.04	<0.001	-12.94	0.014	78.58	0.046
18:0	0.773	10.90	<0.001	0.65	<0.001	-16.14	0.012	-2.21	0.049	-0.99	0.908
SFA	0.804	77.07	<0.001	0.65	<0.001	-202.57	<0.001	-23.06	0.001	112.84	0.038
16:1n-7	0.980	16.78	<0.001	0.90	<0.001	-40.02	<0.001	-12.95	<0.001	17.76	0.082
18:1n-7	0.749	18.66	<0.001	0.35	<0.001	-36.13	<0.001	-8.17	<0.001	29.81	0.005
18:1n-9	0.932	115.92	<0.001	0.59	<0.001	-329.02	<0.001	-42.39	<0.001	264.94	<0.001
20:1n-9	0.840	2.89	<0.001	0.67	<0.001	-5.36	0.115	0.30	0.680	5.59	0.211
MUFA	0.831	205.39	<0.001	0.42	<0.001	-518.29	<0.001	-82.29	<0.001	470.06	<0.001
18:2n-6	0.951	30.13	<0.001	0.67	<0.001	15.83	0.345	-24.83	0.001		
20:4n-6	0.973	0.24	0.036	0.83	<0.001	4.08	<0.001	-0.40	<0.001		
n-6 PUFA	0.960	32.06	<0.001	0.66	<0.001	19.40	0.0182	-0.25	<0.001		
18:3n-3	0.993	1.41	0.001	0.77	<0.001	-5.23	0.030	-2.95	<0.001		
20:5n-3	0.948	6.58	0.005	0.62	<0.001	-21.34	0.053	-1.07	0.497		
22:5n-3	0.983	8.09	<0.001	1.75	<0.001	-8.71	0.035	-11.72	<0.001		
22:6n-3	0.970	-0.44	0.751	1.05	<0.001	68.80	<0.001	-7.539	<0.001		
n-3 LC-PUFA	0.980	12.85	<0.001	0.81	<0.001	61.55	<0.001	-14.25	<0.001		

[1]*P*-values of regression coefficients.

Statistically significant contribution in analysis on blocks is not detected (P<0.05) for n-3 and n-6 PUFAs, and the corresponding interactions coefficients are not reported.

394 **Figure Legends**

395

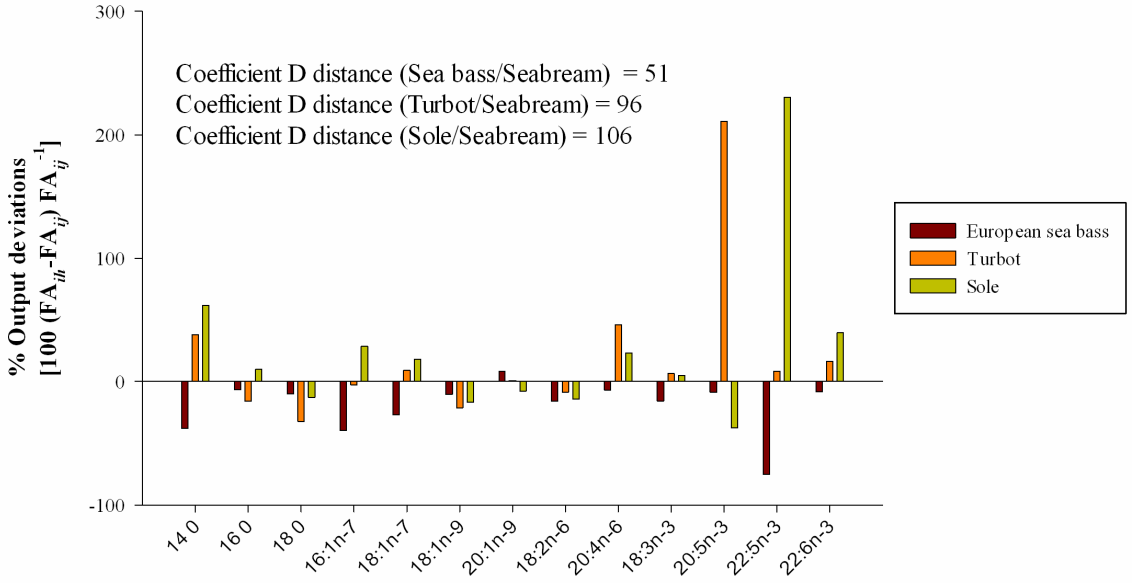
396 **Figure 1**. Predicted output deviations at marketable fish size on fillet FA composition
397 [100 (FA_{ih}-FA_{ij}) FA_{ij}^{-1}, FAi in fish species subgroup (*h)* and reference species (*j)* for
398 each *i* FA]. The calculations are made using dummy regression equations with the same
399 dietary FA composition and fillet lipid contents (9% European sea bass*;* 8% turbot; 7%
400 sole; 11% gilthead seabream) as independent variables.

401

402 **Figure 2**. Plot regression of predicted and observed fillet FA values in the up-scaling
403 validation procedure. Values are the mean ± SE (*n* = 12) of farmed fish (Andromeda
404 Ibérica Acuicultura S.L, Castellón, Spain) harvested at commercial size (330-400 g
405 body weight).

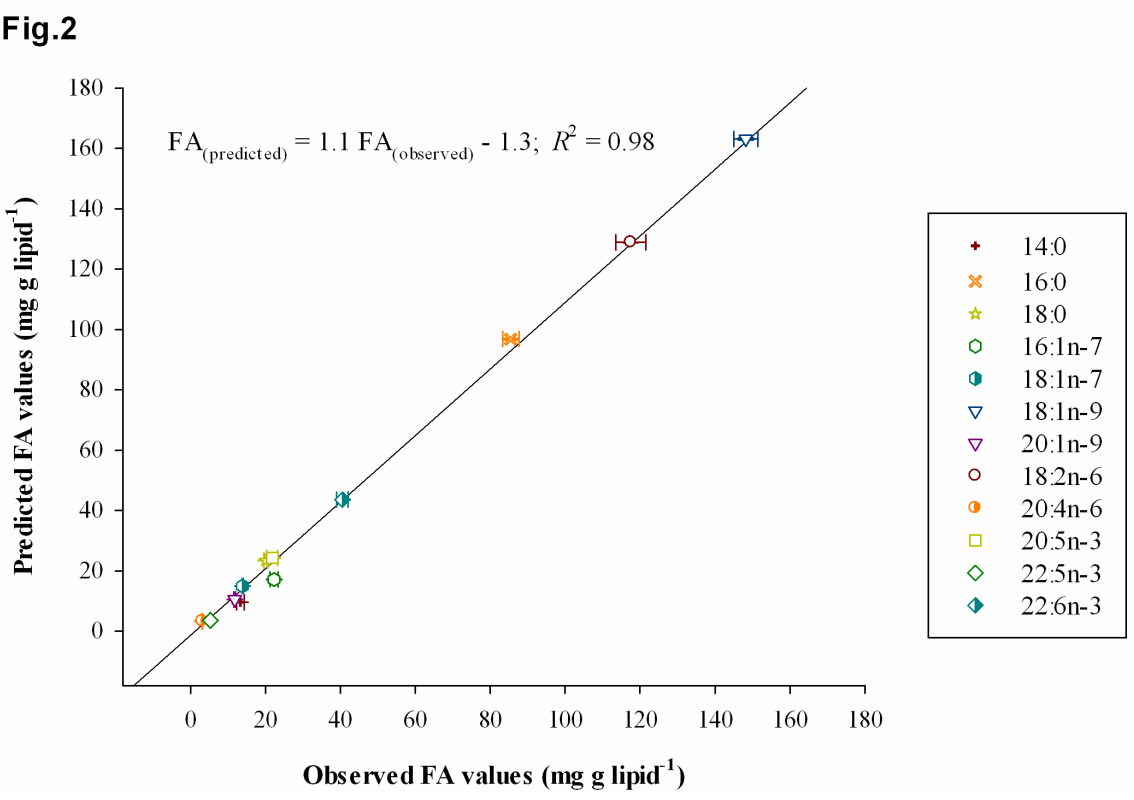
406

Fig.1


Coefficient D distance (Sea bass/Seabream) = 51
Coefficient D distance (Turbot/Seabream) = 96
Coefficient D distance (Sole/Seabream) = 106
% Output deviations
[100 (FAih-FAij) FAij-1]
300
200
100
0
-100
14:0
16:0
18:0
16:1n-7
18:1n-7
18:1n-9
20:1n-9
18:2n-6
20:4n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3
European sea bass
Turbot
Sole

407
408

Fig.2


FA(predicted) = 1.1 FA(observed) - 1.3; R2 = 0.98
Predicted FA values (mg g lipid-1)
Observed FA values (mg g lipid-1)
0
20
40
60
80
100
120
140
160
180
14:0
16:0
18:0
16:1n-7
18:1n-7
18:1n-9
20:1n-9
18:2n-6
20:4n-6
20:5n-3
22:5n-3
22:6n-3

409