Monitoring the influence of marine aquaculture on wild fish communities: benefits and limitations of fatty acid profiles

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Abstract: Fatty acids (FA) have been applied as indicators of the influence of coastal sea-cage fish farming on wild fish communities in several recent scientific publications. Due to the relatively high conservation of FA composition throughout the food web, they are useful for characterizing trophic relationships. The increasing utilization of vegetable or alternative animal oils in the production of aquafeeds results in cultivated fish exhibiting higher levels of terrestrial FA in their tissues. As previously reported, wild fish ubiquitously aggregate around fish farms as a consequence of the introduction of new habitat and the easy availability of food - fish farms act as enhanced Fish Attraction Devices (FADs). The influence of food pellets on the composition of wild fish has been detected in recent studies on salmon, sea bass and sea bream aquaculture, showing increased levels of linoleic acid (18:2n-6) and low n-3/n-6 ratio as clear indicators of the consumption of food pellets from the farms. The potential ecological and physiological effects on wild fish are presently unknown. In this article, guidelines are proposed for the investigation and use of terrestrial FAs to track the effects of coastal aquaculture on wild fish communities and local fisheries as well as the benefits or limitations of this technique.

Keywords: Fish farms, Impact, FADs, Trophic Marker, Biomarker, Vegetable Oils, Marine Resources, Management, Fish assemblages.

1. Introduction: use of formulated feed with increasing terrestrial-vegetables ingredients.

Most farmed marine fish are carnivorous species such as, among others, Atlantic salmon (*Salmo salar),* gilthead sea-bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax)* or Japanese amberjack (*Seriola quinqueradiata*) that require marine ingredients in their feed in order to achieve optimal growth rate and health status. However, there are many reasons why the aquaculture

45 industry has been researching alternatives to fishmeal (FM) and fish oil (FO) since these products
46 are both increasingly difficult to obtain and their costs have increased considerably. One of the
47 main reasons is the status of traditional fisheries. Captures of wild fish have remained stable since
48 the 80's despite technical improvements, indicating that fish stocks are being exploited at their
49 maximum levels (FAO 2009). Although improvements to feed-grade fisheries exploitation have
50 been reached (Welch et al. 2010), it appears that these fisheries still need to make important
51 progress in terms of correct labelling – regarding both captured species and origin – which may
52 compromise the sustainability of this marine resource (Deutsch et al. 2007). In addition, due to
53 increasing demand, not only by the aquaculture industry, but also by terrestrial animal farming, the
54 prices of feed grade marine fishery resources have risen; FM increased in price from US $694 to US
55 $1379 per tonne between 2005 and 2006 and FO prices from US $894 to US $1700 between 2007
56 and 2008 (Tacon and Metian 2008). Industry access to feed-grade fisheries may be decreased due to
57 global warming, world agreements to reduce poverty and to increase food security and
58 sustainability, along with ethical issues (De Silva et al. 2010). As a consequence, aquaculture
59 industry may prefer to rely on more stable and reliable land-based plant production rather than the
60 highly fluctuating marine resources.
61
62 This scenario has driven research into alternatives to FM and FO for formulating aquaculture feeds.
63 Much research has focussed on determining the optimal proportions for the substitution of FM and
64 FO by plant products without compromising fish growth and health status (Turchini et al. 2009,
65 2010). However, vegetable oils (VO), like soybean, rapeseed, linseed or palm oils are rich in
66 saturated acids like palmitic (16:0) or stearic acid (18:0), monounsaturated fatty acids like oleic acid
67 (18:1n-9), and polyunsaturated fatty acids (PUFA), especially linoleic acid (18:2n-6) and α-
68 linolenic acid (18:3n-3), but lack the long-chain PUFA (LC-PUFA), eicosapentaenoic acid (20:5n-
69 3, EPA) and docosahexaenoic acid (22:6n-3, DHA), characteristic of FO (e.g. Turchini et al., 2010).
70 Other aternative lipid sources are also being investigated including terrestrial animal fats or
71 alternative marine oils (e.g zooplankton) but these resources also have limitations, having only very
72 low levels of n-3 LC-PUFA or by having very limited and insufficient production, respectively, to
73 satisfy current industry requirements (Bureau and Meeker 2010, Olsen et al. 2010). Despite the lack
74 of n-3 LC-PUFA, VO have been the replacement of choice for FO due to considerations of
75 availability and sustainablility and so considerable research efforts and investments have been
76 applied in this field. Consequently, significant advances in the substitution of fish products by plant
77 proteins and VO have been achieved (Turchini et al., 2009, 2010).
78
79 The replacement of FO with alternative oils such as VO in aquafeeds can cause alterations in the

fish physiology, including immunological status of cultivated fish. These effects are extensively studied and can be controlled under laboratory or cage condition in order to achieve the maximum levels of substitution without compromising fish performance (Turchini et al. 2009, 2010, Montero and Izquierdo 2010). However, use of alternative ingredients in aquaculture is prompting further questions about their effects on the environment. Some studies have appeared highlighting that FA compositions of sediments (Colombo et al. 1997), wild fish populations (Skog et al. 2003, Fernandez-Jover et al. 2007, 2009, 2011) and other associated fauna like shrimps (Olsen et al. 2009) can be altered as a consequence of food pellets that are not consumed by the cultured fish and are lost from the cages. Therefore, terrestrial FAs have been proposed as biomarkers of the influence and the impact of aquaculture on wild fish populations (Skog et al. 2003, Fernandez-Jover et al. 2007).

Wild fish aggregations around coastal sea-cage farms may reach high numbers and biomass (Dempster et al. 2002, 2009) and changes in the FA profile of this fauna have been detected for both adult and juvenile fish (Skog et al. 2003, Fernandez-Jover et al. 2007, 2009). This work presents the current status and knowledge of the effect of FA of terrestrial origin on wild fish communities focusing on future research efforts and monitoring guidelines for using FA as biomarkers and also considering the potential effects on fish biology.

2. Effects of lost food pellets on wild fish FA signature.

Fish are attracted towards floating objects, both moored and drifted. These objects, which may be natural (like logs, floating seaweed, jellyfish…) or artificial (docks, jetties, oil platforms, fishing gears…), are known as Fish Aggregation Devices (FADs) and have been traditionally used as methods for enhancing fisheries captures (Kojima 1956, Fonteneau et al. 2000, Dempster 2004). Fish farms also act as FADs (Carss 1990, Bjordal & Skar 1992, Dempster et al. 2002). Large numbers of species, estimated to be more than 160 worldwide (Sanchez-Jerez et al. 2011), have been recorded aggregating around floating cages of different farmed fish species including, among others, salmon, sea bass, sea bream, bluefin tuna and groupers. However, far from acting as traditional FADs, coastal cages function as enhanced aggregating devices principally due to availability of food in the form of lost food pellets that are not consumed by the farmed fish (Dempster et al. 2002, Tuya et al. 2006).

Most of these aggregated wild fish actively consume the lost particulate organic matter (POM), principally in the form of uneaten food pellets and faeces that fall from the cages. For most of the

studied aggregating species, it has been demonstrated that they change their diet while resident around farms (Fernandez-Jover et al. 2007, 2008, Dempster et al. 2009) and help to reduce the impact on the benthic system. Thus, wild fish feeding around fish farms reduce the total waste that reaches the environment by 40–80% (Vita et al. 2004, Felsing et al. 2005). Consequently, as wild fish substitute their natural diet by an elevated proportion of food pellets, it was hypothesised that they may present alterations in their FA profiles in a similar way as happens to cultured species.

2.1 FAs profile of adult fish

Initially, Skog et al. (2003) found that wild saithe (*Pollachius virens*) feeding around a salmon farm in a Norwegian fjord had similar FA profiles to the food pellets used at the farm, with increased levels of linoleic and α-linolenic acids as well as a comparatively low n-3/n-6 PUFA ratio, which reflected that in pellets (Figure 1). Norwegian fishermen have traditionally argued that salmon farms were affecting the behavior and taste of wild saithe (Carss 1990) and controversy still exists (e.g. Skog et al. 2003, Dempster et al. 2011).

Along with saithe, the FA profiles of cod (*Gadus morhua*) around fish farms in Norway have also been studied (Fernandez-Jover et al. 2011). This study supported the results found by Skog et al. (2003) due to higher levels of linoleic acid (Figure 1) found in farm-aggregated individuals of both species, therefore, this FA appears as a strong indicator of food pellets in the diet. This study also analyzed the profiles of livers of associated cod and saithe, showing that the influence of VO was more marked in this tissue than in fish muscle. In this way, significant differences were found for oleic acid due to higher levels in farm-associated cod and significantly decreased levels of DHA (22:6n-3), total LC-PUFA (PUFA with chain lengths of twenty or more carbons) and n-3/n-6 PUFA ratio. In the case of saithe, in addition to increased levels of linoleic acid in muscle and liver of aggregated fish, a lower n-3/n-6 PUFA ratio was also detected. In addition, the total amount of n-6 PUFA was significantly higher in farm-associated fish. These results were consistent between two localities along the Norwegian coast.

Similarly, Fernandez-Jover et al. (2007) highlighted that farm-aggregated Mediterranean horse mackerel (*Trachurus mediterraneus*) drastically changed their feeding behaviour while resident around farms, since food pellets averaged 90% of total stomach contents while their non-aggregated counterparts mainly consumed juvenile fish, crustaceans and cephalopods. This was clearly reflected in the FA profile of the fish muscle; which showed significantly increased levels of linoleic and oleic acids and decreased DHA in farm-associated fish (Figure 1). Similar results were

obtained with Mediterranean bogue (*Boops boops*); muscle samples taken from individuals of this species captured closely associated or near farms presented higher percentages of linoleic, α-linolenic, oleic and palmitoleic (16:1n-7) acids than samples taken many kilometers from the nearest farm. In contrast, values of DHA, arachidonic acid (ARA; 20:4n-6) and n-3/n-6 PUFA ratio were lower in fish sampled near fish farms (Arechavala-Lopez et al. 2010a). Those changes have been also found in liver, gill, gonad, adipose tissue and brain of *B. boops* (Martínez-Rubio unpublished data). Due to the key role of brain in the regulation of the physiological functions, its chemical composition is relatively constant and more resistant to the influence of external factors than other organs (Odutuga 1977). Therefore, modifications found in brain highlight the importance of this dietary change, proving that the presence of aquafeeds in the diet is not occasional, and the magnitude of this change opens the question of what could be the extent of the effect on fish health and performance.

2.2 FA profile of juvenile fish

The role of coastal sea-cage fish farms as habitat for the settlement of fish in early developmental stages or juveniles and its influence on their FA composition in the Mediterranean has also been described. The FA profile of farm-associated juvenile fish is, as happens with adult fish, perceptibly altered (Fernandez-Jover et al. 2009). Again, high levels of linoleic acid and, in this particular case, decreased levels of ARA, are the main changes in the FA profiles of the juvenile mugilid *Liza aurata* and the juvenile sparid *Oblada melanura*, two common species of the Mediterranean that usually settle on shallow rocky shores or seagrass meadows. The staple diet of juvenile fish, zooplankton, also showed a modified FA profile. Therefore, it is still not completely clear if the altered FA signature of juvenile fish is a consequence of them feeding on zooplankton, or the direct consumption of fine particulate food pellets, or both.

Currently, the potential consequences of altered FA composition on the development, health status and reproduction of aggregated adult and juvenile fish species remain unknown. On one hand, these species are consuming a high energy diet, providing higher lipid and energetic reserves that could be used, for instance, for the development of the gonads. As evidence of this, aggregated individuals usually present a higher corporal condition index than their not-aggregated counterparts (Skog et al. 2003, Fernandez-Jover et al. 2007, Dempster et al. 2009, 2011). However, the biologically active FA for fish are the LC-PUFA, DHA (22:6n-3), EPA (20:5n-3) and ARA (20:4n-6), and marine fish cannot endogenously synthesize these LC-PUFA from the short chain PUFA α-linolenic (18:3n-3) and linoleic (18:2n-6) acids and so they require LC-PUFA for optimal growth, health status, reproductive behaviour and successful larval development (Tocher 2010). It has been

estimated that, in the SW Mediterranean, at least 20 different fish species settle at coastal farms (Fernandez-Jover et al. 2009) and LC-PUFA may be key factors in order to obtain high fecundity, egg quality, fertilization and hatching success (Pavlov et al. 2004). Spawners of cultured species are feed with a diet which differs to that of fish reared for human consumption, which optimizes the requirements for reproduction in terms of gonad development, egg quality and larval survival. According to Van Der Kraak et al. (1998), ARA and other PUFAs are important regulators of steroid biosynthesis in fish. There are clear indications of the importance of n-3 LC-PUFA in larval development (Brown and Hart 2010) and eggs are generally considered to be of better quality if they present a higher content of total n-3 LC-PUFA, including enhanced levels of both DHA and EPA (Brooks et al. 1997). Wild fauna aggregated around farms mainly are adult fish of spawning size (Dempster et al. 2002) and their dietary requirements for optimal reproduction have never been studied. Changes in the FA profile of wild fish may have unkown effects on spawning, egg quality or larval survival.

3. FA as trophic markers of aquaculture influence on wild fish communities.

Fatty acids have often been used as dietary markers (Iverson et al. 2004). A trophic marker is a compound whose origin can be easily and unequivocally identified, that is inert and does not harm the organisms, is metabolically stable and not selectively processed, and transfers from one trophic level to the next in both a quantitative and qualitative manner (Dalsgaard et al. 2003). Although FA are not inert compounds, they accumulate over time and represent an integration of dietary intake over days, weeks, or months, depending on the organism and its energy intake and storage rates (Iverson, 2009). Many studies have inferred food web relationships from FA profiles with clear results (e.g. Graeve et al. 1994, Scott et al. 1999). Therefore, FAs have also been proposed as markers of aquaculture influence due to the change of the FA composition of associated fauna like sea-urchins (Cook et al. 2000, Barberá et al. 2011), mussels (Gao et al. 2006), shrimps (Olsen et al. 2009), fish (Skog et al. 2003; Fernandez-Jover et al. 2007) and also in sediment (Samuelsen et al. 1988; Henderson et al. 1997). Olsen et al. (2009) considered that only linoleic and α-linolenic acids can be used as clear aquafeed markers in shrimp (*Pandalus borealis*).

In addition, wild fish with FA profiles modified by aquafeeds are forming an important component of the catch of artisanal fisheries in SW Mediterranean, reaching local markets, as evidenced by Arechavala-Lopez et al. (2010a). Artisanal fishers approach the cages due to the increased vulnerability of aggregated species (Akyol and Ertosluk 2010). Wild bogue aggregated at fish farms and those non-aggregated but captured within the same bay from trammel-nets presented modified

221 FA profiles. The FA composition of individuals captured by artisanal fishing gears were always
222 more similar to farm aggregated than to control samples. To improve the capacity of differentiating
223 fish origin, FA profile can be use along with other techniques, like body morphology (Fleming et al.
224 1994, Grigorakis et al. 2002), condition indexes (Fernandez-Jover et al. 2007), trace elements
225 (Yildiz 2008, Adey et al. 2009, Percin et al. 2011), stable isotopes (Serrano et al. 2007) or genetic
226 methods (Danielsdottir et al. 1997). Fatty acid signature, however, presents advantages with respect
227 to other techniques, since it can give a picture on the scale of impact of farming on the environment
228 but also nutritional information (such as fat content or n-3/n-6 ratio) which can aid correct labeling
229 of fish products (Standal et al. 2008, Jacquet et al. 2010).
230
231 The amount of linoleic acid or the n-3/n-6 PUFA ratio may provide strong signals for measuring the
232 influence of fish farming on the local fish communities. However, there is not a single or a small
233 pool of FAs which can be exclusively labeled as 'food pellets originated'. For instance, linoleic acid
234 is also found in natural marine food but at low levels. Therefore, several studies have applied a
235 multivariate approach in order to improve the power of the analysis to discriminate the origin of
236 fish or the impact of VO on wild fish. Thus, Standal et al. (2008) applied linear discriminant
237 analysis (LDA) based on the scores of a previous principal component analysis (PCA) of liver oils
238 to differentiate reared and wild cod. Results revealed that LDA correctly grouped cod liver oils
239 depending on their wild or cultured origin (97 to 100% of individuals correctly grouped). Similarly,
240 Fernandez-Jover et al. (2011), applied LDA analysis to differentiate cod and saithe depending on
241 their farm-aggregated or non-aggregated origin. The analysis correctly classified 88.5% and 96.7%
242 respectively of cod muscle and liver. In the case of saithe, the analysis correctly differentiated
243 85.7% of saithe muscle and 96.7% of saithe liver. Non-correctly classified fish tissues may be due
244 to new arrivals, variation of the different tissues reflecting the diet, natural variability that decreases
245 statistical power, or even technique limitations.
246
247 Based on published studies, a pool of other different multivariate techniques can be applied for
248 obtaining discrimination of fish individuals according to their origin. These techniques may be
249 multidimensional scaling -MDS- (Fernandez-Jover et al. 2007), PCA (Skog et al. 2003, Fernandez-
250 Jover et al. 2011), multivariate analysis of variance –MANOVA- (Fernandez-Jover et al. 2007,
251 2009) or analysis of similarity ANOSIM (Hughes et al. 2005). Nonetheless, a univariate technique
252 may be initially used in order to detect which individual FAs may act as 'key-FA' for
253 discriminating the different fish and to avoid 'noise-FA' which will not aid to discrimination.
254 Moreover, FA signature analysis can be combined with other techniques, such as stable isotope
255 analysis, in order to improve the capacity of detecting fish farm influence and differentiating fish

origin as has been already applied in other fields (Cook et al. 2004; Kharlamenko et al. 2008). However, despite some clear field results, before giving specific guidelines for using FA as trophic markers in fish, several doubts require to be resolved.

4. Present knowledge gaps.

Further research using controlled experiments in the laboratory is necessary in order to better assess the incorporation rates of these FA in different species. The retention time of these FAs in fish tissues must also be analyzed if FAs are to be considered as potential biomarkers of the influence of fish farms on juvenile fish. A key issue is to quantify the minimum residence time of wild fish around the cages, and therefore, the minimum period and quantity of consumption of food pellets that enables detection of significant changes in the FA composition of fish tissues. Aggregated fish undertake seasonal migrations and, therefore, many species are not resident around the farms throughout the year (Valle et al. 2006, Fernandez-Jover et al. 2008). It has been estimated that 3 to 4 months is sufficient time to provoke a substantial change in the FA composition of Mediterranean horse mackerel, which was reflected in a strong increment of linoleic and diminished levels of DHA and the n-3/n-6 PUFA ratio (Fernandez-Jover et al. 2007). It is also known that wild cod and saithe may move among different fish farms within the same area, as has been studied in Norway (Uglem et al. 2008, 2009) and the Mediterranean with mugilids (Arechavala-Lopez et al. 2010b), thus increasing the duration of food pellet consumption. All of these variables have to be taken into account along with seasonal and spatial variation of FA (Fernandez-Jover et al. 2007, Tzikas et al. 2007) to clearly discriminate fish farm influence.

The biology and metabolism of lipids for each considered species may also be a source of variation since mobilization of the different FA may differ depending on fish species and tissue. For instance, the lipid content of the muscle of gadoids is very low, around 0.5%, with phospholipids as the major lipid class (Dos Santos et al. 1993, Jobling et al. 2008), thus indicating the predominantly structural role of fatty acid composition in this tissue, which generally presents a more conservative profile than other tissues like liver. Gadoids liver has a high lipid content consisting of triacylglycerols, with an energetic role, in which FA oxidation is a more dynamic process (Falch et al. 2006). Therefore, fish muscle may present a more conservative profile and may provide a clearer record of the fish diet during a longer period of time. The generally accepted idea is that the FA composition of fish tissues reflects, in a highly conservative form, the FA profile of the diet. However, some fish have the capacity, to a certain extent, to metabolize some important FAs. Nonetheless, marine fish are well supplied with EFAs in their natural diet and *de novo* biosynthesis

of LC-PUFA, as mentioned before, is likely to be suppressed in marine carnivorous (Tocher 2003) and some herbivorous like *Liza aurata* (Mourente and Tocher 1993, Sargent et al. 2002) which is an important species composing wild fish aggregations around Mediterranean farms (Fernandez-Jover et al. 2008)..

5. Conclusion and guidelines.

Strong evidence exists that FA signatures are modified in fish tissues when they aggregate around sea cages. The most suitable candidates for detecting this influence appear to be increased levels of linoleic acid along with decreased levels of DHA and the n-3/n-6 PUFA ratio. A multivariate approach should be also applied in order to obtain powerful and conclusive results when using FAs as biomarkers. It is also necessary to know in detail the 'natural' FA profiles of the analyzed species of wild fish as well as the spatial and temporal variability of their lipid composition. Parallel to the development of aquafeeds with alternative ingredients, research is also needed on the effects on wild communities in terms of health status or reproductive potential. This gap in knowledge on the effects on fish performance makes the precautionary principle of great value in this case and adds a further argument for the optimization of the use of food pellets in order to reduce organic wastes and to avoid economical losses and the effects on water quality, benthos and associated communities. The increased use of alternative oils in the formulated diets is posing new questions since it is possible that, while solving one problem, new issues are being opened. However, efforts to improve the efficiency of aquafeeds rich in VO are increasing and studies on new species are focusing mainly on carnivorous fish. Consequently, it is crucial to increase our knowledge on the degree of impact provoked by the FA composition of aquafeeds on the overall ecosystem.

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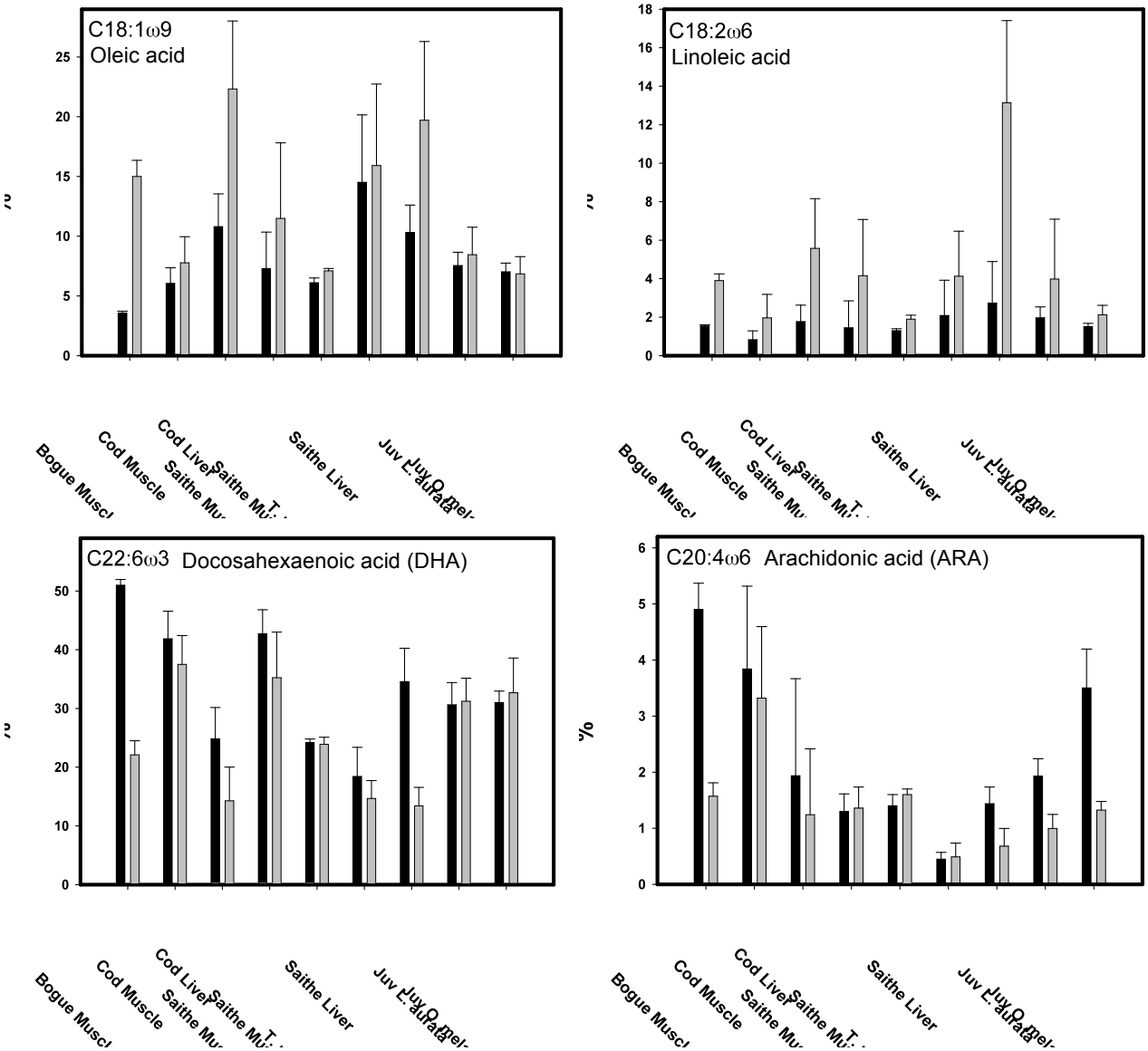
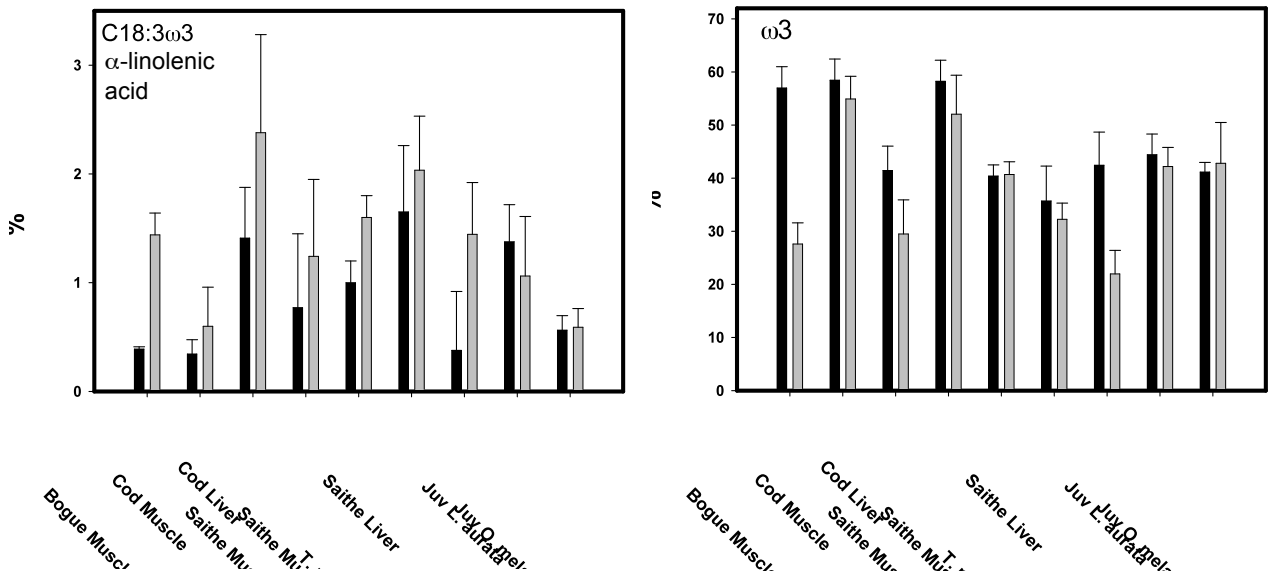
Figure 1. Literature review of fatty acid profiles of different fish species. Graphs show mean ± standard error of non-associated, control fish (black bars) and farm-associated wild fish (grey bars). Sources are Arechavala-Lopez et al. 2010a bogue (*Boops boops*):. Fernandez-Jover et al. 2011: cod (*Gadus morhua*) muscle, cod liver, saithe (*Pollachius virens*) muscle (a) and saithe liver. Skog et al. 2003: saithe muscle (b). Fernandez-Jover et al. 2007: *Trachurus mediterraneus* muscle. Fernandez-Jover et al. 2009; juveniles of *Liza aurata* and *Oblada melanura.* Data from Fernandez-Jover et al. 2011 are pooled from two different localities. Data from Skog et al. 2003 considers as control fish wild saithe from the fjord with no farming activity.

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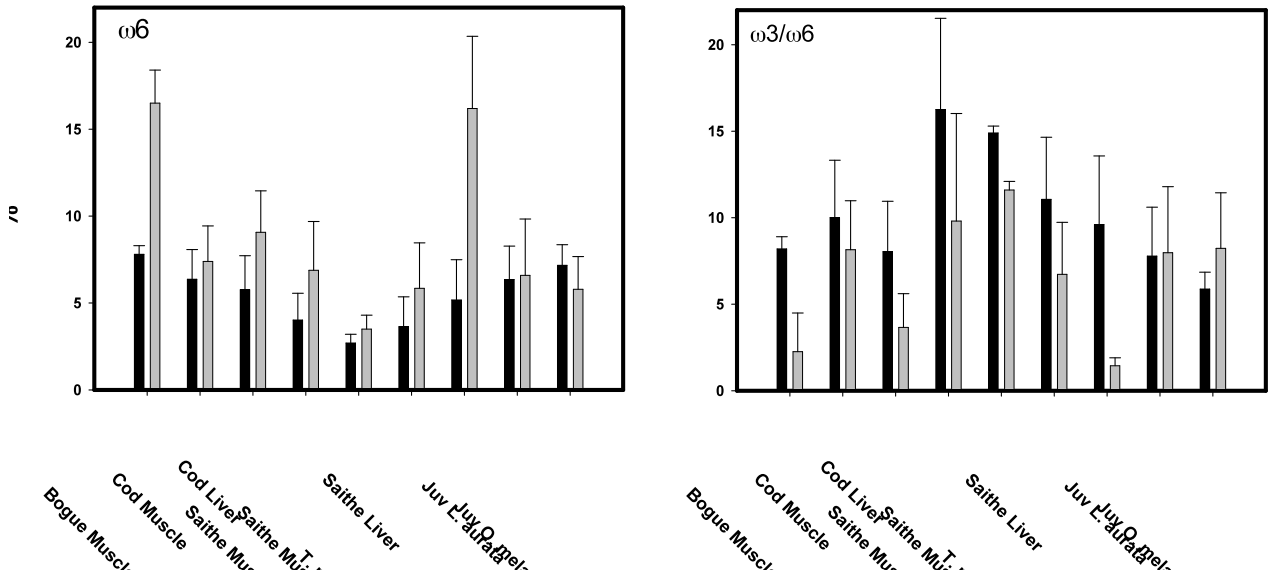
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