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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 FAs 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 aggregation 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, with increased levels of linoleic acid (18:2n-6) and a 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 the present 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.

KEY WORDS: Fish farms · Impact · FADs · Trophic marker · Biomarker · Vegetable oils · Marine resources · Management · Fish assemblages

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INTRODUCTION: USE OF FORMULATED FEED WITH INCREASING TERRESTRIAL VEGETABLE INGREDIENTS

Most farmed marine fish are carnivorous species such as Atlantic salmon *Salmo salar*, gilthead seabream *Sparus aurata*, European sea bass *Dicentrarchus labrax* and Japanese amberjack *Seriola quinqueradiata*, among others, that require marine ingredients in their feed in order to achieve an optimal growth rate and health status. However, there are many reasons why the aquaculture industry has been researching alternatives to fishmeal (FM) and fish oil (FO), since these products are both increasingly difficult to obtain and their costs have increased considerably. One of the main reasons is the status of traditional fisheries. Captures of wild fish have remained stable since the 1980s despite technical improvements, indicating that fish stocks are being exploited at their maximum levels (FAO 2009). Although improvements to feed-grade fisheries exploitation have been made (Welch et al. 2010), it appears that such fisheries still need to realise important progress in terms of correct labelling—regarding

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both captured species and their origins — which may compromise the sustainability of this marine resource (Deutsch et al. 2007). In addition, due to increasing demand, not only by the aquaculture industry, but also by terrestrial animal farming, the prices of feed-grade marine fishery resources have risen; FM increased in price from US \$694 to US \$1379 t^{-1} between 2005 and 2006, and FO prices rose from US \$894 to US \$1700 between 2007 and 2008 (Tacon & Metian 2008). Industry access to feed-grade fisheries may be decreased due to global warming and world agreements to reduce poverty and to increase food security and sustainability, along with ethical issues (De Silva et al. 2010). As a consequence, the aquaculture industry may prefer to rely on more stable and reliable land-based plant production rather than the highly fluctuating marine resources.

This scenario has driven research for alternatives to FM and FO in formulating aquaculture feeds. Much research has focussed on determining the optimal proportions for the substitution of FM and FO by plant products, without compromising fish growth and health status (Turchini et al. 2009, 2010). However, vegetable oils (VO), like soybean, rapeseed, linseed, or palm oils are rich in saturated acids like palmitic (16:0) or stearic acid (18:0), monounsaturated fatty acids like oleic acid (18:1n-9), and polyunsaturated fatty acids (PUFA), especially linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), but lack the long-chain PUFAs (LC-PUFA) eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) characteristic of FO (e.g. Turchini et al. 2010). Other alternative lipid sources are also being investigated, including terrestrial animal fats or alternative marine oils (e.g. zooplankton), but these resources also have limitations, having only very low levels of n-3 LC-PUFA or by having very limited and insufficient production, respectively, to satisfy current industry requirements (Bureau & Meeker 2010, Olsen et al. 2010). Despite the lack of n-3 LC-PUFA, VO have been the replacement of choice for FO due to considerations of availability and sustainability, and so considerable research efforts and investments have been applied in this field. Consequently, significant advances in the substitution of fish products by plant proteins and VO have been achieved (Turchini et al. 2009, 2010).

The replacement of FO with alternative oils such as VO in aquafeeds can cause alterations in fish physiology, including the immunological status of cultivated fish. These effects have been extensively studied and can be controlled under laboratory or cage conditions in order to achieve the maximum levels of substitution without compromising fish performance (Turchini et al. 2009, 2010, Montero & Izquierdo 2010). However, the use of alternative ingredients in aquaculture is prompting further questions about their effects on the environment. Several studies have appeared highlighting that the FA composition 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 for the influence and 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 in 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 effects of FAs of terrestrial origin on wild fish communities, focusing on future research efforts and monitoring guidelines for using FAs as biomarkers and also considering the potential effects on fish biology.

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 etc.) or artificial (docks, jetties, oil platforms, fishing gears etc.), 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 >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 the 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 to 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.

FA profiles of adult fish

Initially, Skog et al. (2003) found that wild saithe *Pollachius virens* feeding around a salmon farm in a Norwegian fjord had FA profiles similar 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 (Fig. 1). Norwegian fishermen have traditionally argued that salmon farms affect the behaviour and taste of wild saithe (Carss 1990), and controversy still exists (e.g. Skog et al. 2003, Dempster et al. 2011).

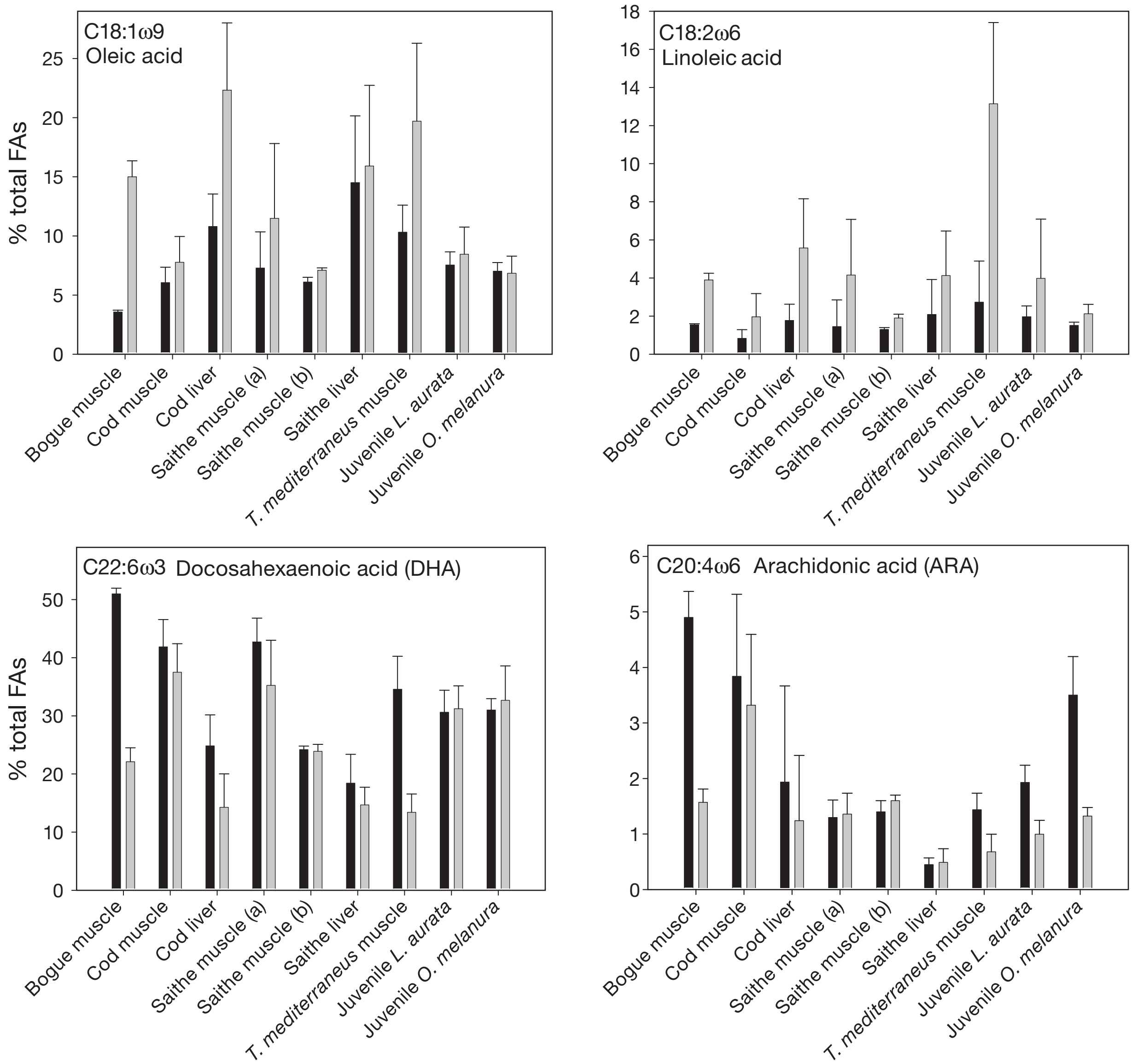

Fig. 1. Literature review of fatty acid (FA) profiles (as percentage of total FAs) of different fish species. Means (±SE) of non-associated control fish (black bars) and farm-associated wild fish (grey bars). Sources are: Arechavala-Lopez et al. (2010a) for bogue *Boops boops*; Fernandez-Jover et al. (2011) for cod *Gadus morhua* muscle, cod liver, saithe *Pollachius virens* muscle (designated Type a) and saithe liver; Skog et al. (2003) for saithe muscle (designated Type b); Fernandez-Jover et al. (2007) for *Trachurus mediterraneus* muscle; Fernandez-Jover et al. (2009) for juveniles of *Liza aurata* and *Oblada melanura*. Data from Fernandez-Jover et al. (2011) are pooled from 2 different localities. Data from Skog et al. (2003) consider as control fish the wild saithe from a fjord with no farming activity

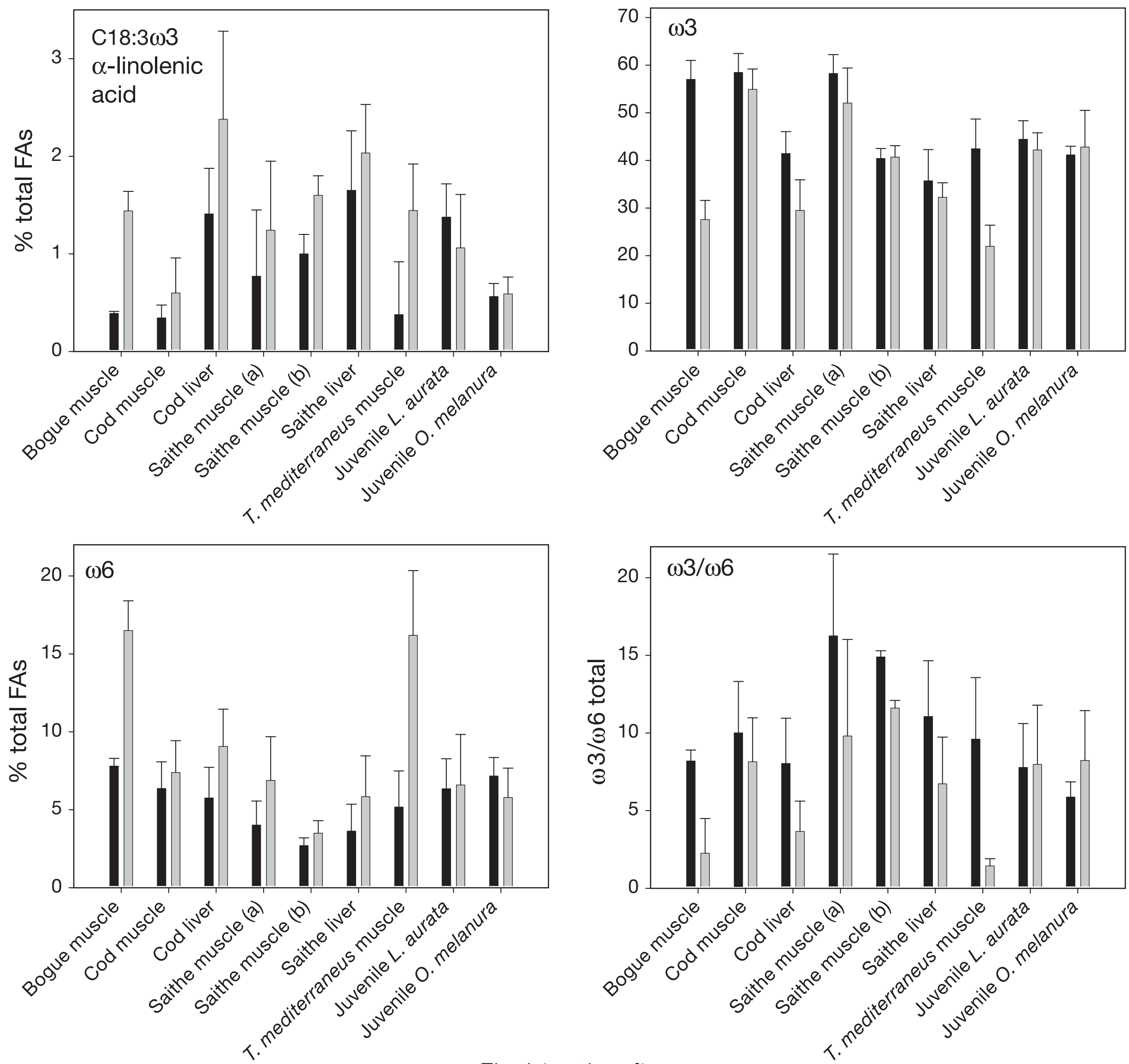

Fig. 1 (continued)

Along with saithe, the FA profiles of cod *Gadus morhua* around fish farms in Norway have also been studied (Fernandez-Jover et al. 2011). The present study supports the results by Skog et al. (2003) that higher levels of linoleic acid (Fig. 1) are found in farm-aggregated individuals of both species; therefore, this FA appears to be a strong indicator of food pellets in the diet. The present 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 20 or more carbons) and the n-3/n-6 PUFA ratio. In the case of saithe, in addition to increased levels of linoleic acid in the 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 2 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 (Fig. 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 kilometres from the nearest farm. In contrast, values of DHA, arachidonic acid (ARA; 20:4n-6) and the n-3/n-6 PUFA ratio were lower in fish sampled near fish farms (Arechavala-Lopez et al. 2010a). Similar changes have also been found in the liver, gill, gonad, adipose tissue and brain of *B. boops* (L. Martinez-Rubio unpubl. data). Due to the key role of brain in the regulation of 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 the brain highlight the importance of this dietary change, proving that the presence of aquafeed in the diet occurs regularly, and the magnitude of this change opens the question as to what may be the extent of the effect on fish health and performance.

FA profiles 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*, 2 common species in 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 the 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 PUFAs α-linolenic (18:3n-3) and linoleic (18:2n-6) acid 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 near coastal farms (Fernandez-Jover et al. 2009), and LC-PUFAs may be a key factor in obtaining high fecundity, egg quality, fertilization and hatching success (Pavlov et al. 2004). Spawners of cultured species are fed a diet differing from that of fish reared for human consumption, namely, one optimizing 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. Clear indications of the importance of n-3 LC-PUFAs in larval development have been shown (Brown & 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 that aggregate around farms are mainly 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 unknown effects on spawning, egg quality, or larval survival.

FATTY ACIDS 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 FAs 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*.

Furthermore, wild fish with FA profiles modified by aquafeeds form an important component of the catch of artisanal fisheries in the SW Mediterranean, reaching local markets, as evidenced by Arechavala-Lopez et al. (2010a). Artisanal fishers increase their fishing effort around aquaculture cages where wild fish aggregate and therefore are vulnerable to capture (Akyol & Ertosluk 2010). Wild bogue aggregated at fish farms and those that were not aggregated but were captured within the same bay with trammel-nets presented modified FA profiles. The FA composition of individuals captured by artisanal fishing gears were always more similar to farm-aggregated than to control samples. To improve the capacity for differentiating fish origin, FA profiles can be used along with other techniques, like body morphology (Fleming et al. 1994, Grigorakis et al. 2002), condition indexes (Fernandez-Jover et al. 2007), trace elements (Yildiz 2008, Adey et al. 2009, Percin et al. 2011), stable isotopes (Serrano et al. 2007, Kharlamenko et al. 2008), or genetic methods (Danielsdottir et al. 1997). Fatty acid signature, however, presents advantages with respect to other techniques, since it can give a picture of the impact of farming on the environment as well as nutritional information (such as fat content or the n-3/n-6 ratio) that can aid in the correct labelling of fish products (Standal et al. 2007, Jacquet et al. 2010).

The amount of linoleic acid or the n-3/n-6 PUFA ratio may provide strong signals for measuring the influence of fish farming on local fish communities. However, no single or small pool of FAs exists which can be labelled exclusively as having been derived from food pellets. For instance, linoleic acid is also found in natural marine food, although at low levels. Therefore, several studies have applied a multivariate approach to improve the power of the analysis to discriminate the origin of fish or the impact of VO on wild fish. Thus, Standal et al. (2007) applied linear discriminant analysis (LDA) based on the scores of a previous principal component analysis (PCA) of liver oils to differentiate between reared and wild cod. Results revealed that LDA correctly grouped cod liver oils depending on their wild or cultured origin (97 to 100 % of individuals correctly grouped). Similarly, Fernandez-Jover et al. (2011) applied LDA analysis to differentiate between cod and saithe depending on their farm-aggregated or non-aggregated origin. The analysis correctly classified 88.5 and 96.7 %, respectively, of cod muscle and liver samples. In the case of saithe, the analysis correctly differentiated 85.7 % of saithe muscle and 96.7 % of saithe liver. Non-correctly classified fish tissues may be due to new arrivals, variation of the different tissues reflecting the diet, natural variability that decreases statistical power, or even technique limitations.

Based on published studies, a range of other different multivariate techniques can be applied to discriminate fish individuals according to their origin, including multidimensional scaling (MDS; Fernandez-Jover et al. 2007), PCA (Skog et al. 2003, Fernandez-Jover et al. 2011), multivariate analysis of variance (MANOVA; Fernandez-Jover et al. 2007, 2009), or analysis of similarity (ANOSIM; Hughes et al. 2005). Nonetheless, a univariate technique may initially be used in order to detect which individual FAs may act as 'key FAs' to discriminate between different fish and to avoid 'noise FAs' that do not aid in discrimination. Moreover, FA signature analysis can be combined with other techniques, such as stable isotope analysis, in order to improve the capacity for detecting fish farm influence and differentiating between fish origins, as has already been applied in other fields (Cook et al. 2004, Kharlamenko et al. 2008). However, despite clear field results in the FA profiles of wild fish previously consuming food pellets, several doubts need to be resolved before specific guidelines can be given for using FAs as trophic markers in fish.

PRESENT KNOWLEDGE GAPS

Further research using controlled experiments in the laboratory is necessary in order to better assess the incorporation rates of these FAs 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 for 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; thus, many species are not resident around the farms throughout the year (Valle et al. 2007, Fernandez-Jover et al. 2008). It has been estimated that 3 to 4 mo are 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 the seasonal and spatial variation in FAs (Fernandez-Jover et al. 2007, Tzikas et al. 2007) in order to clearly identify fish farm influence.

The biology and metabolism of lipids for each species considered may also be a source of variation, since mobilization of different FAs 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 representing the major lipid class (Dos Santos et al. 1993, Jobling et al. 2008). Thus, a predominantly structural role of the FA composition is indicated in this tissue, which generally presents a more conservative profile than other tissues like liver. Gadoid 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 provide a clearer record of 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, of metabolizing some important FAs. Nonetheless, marine fish are well supplied with biologically active FAs in their natural diet and de novo biosynthesis of LC-PUFA, as mentioned before, is likely to be suppressed in marine carnivores (Tocher 2003) and some herbivores, like *Liza aurata* (Mourente & Tocher 1993, Sargent et al. 2002), which is an important species in wild fish aggregations around Mediterranean farms (Fernandez-Jover et al. 2008).

CONCLUSIONS AND GUIDELINES

Strong evidence exists that FA signatures are modified in fish tissues when they aggregate around sea cages. The most suitable substances 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 also be applied in order to obtain powerful and conclusive results when using FAs as biomarkers. It is also necessary to obtain detailed information on 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 of food pellets on wild communities in terms of health status or reproductive potential. This gap in our knowledge regarding the effects on fish performance dictates great caution in this case and adds further urgency to the optimization of the usage of food pellets in order to reduce organic wastes and to the usage of avoid economical losses and negative effects on water quality, benthos and associated communities. The increased use of alternative oils in formulated diets poses new questions, since it is possible that, while solving one problem, new problems are being created. However, efforts to improve the efficiency of aquafeeds rich in VO are increasing, and studies on new species focus mainly on carnivorous fish. In conclusion, 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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