Diversification of Fads2 in Finfish Species: Implications for Aquaculture

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Abstract

The capabilities for biosynthesis of long-chain ($\geq C_{20}$) polyunsaturated fatty acid (LC-PUFA) of farmed fish have been extensively studied in order to determine qualitative requirements for dietary essential fatty acids and to ensure high levels of omega-3 LC-PUFA in the farmed products for human consumption. Although LC-PUFA biosynthesis comprises multiple steps catalyzed by several enzymes, rate-limiting reactions in the pathways are controlled by fatty acid desaturases (Fads), enzymes introducing new double bonds into fatty acyl chains. The repertoire of Fads-encoding genes varies among vertebrates. Mammals have two FADS with known roles in the LC-PUFA biosynthetic pathways, namely FADS1 with Δ5 desaturase activity and FADS2 with Δ6 activity. Interestingly, teleosts, the fish group which most farmed species belong to, appear to have lost *fads1* during evolution and therefore Fads2 is the sole enzyme able to account for the desaturation reactions in the LC-PUFA pathway in teleosts. Unlike mammals though, functions of teleost Fads2 have diversified remarkably as a result of species-specific evolutionary history and environmental factors including habitat (marine *vs* freshwater), trophic level and ecology. This paper reviews the recent progress made on molecular aspects underlying the functional diversity of Fads2 characterized so far from finfish species. Specifically, we discuss the potential implications that Fads2 functions have for the ability of fish species to efficiently utilize dietary fatty acids when fed on vegetable oil-based feeds. In addition, current developing technologies including genetic approaches (e.g. transgenesis) to improve the LC-PUFA biosynthetic capability of fish are discussed.

Keywords: Fads2, Finfish, Aquaculture

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Introduction

Fish and seafood are universally recognized as important components of a healthy diet as they supply high quality, easily digested protein, essential micronutrients including the minerals selenium and iodine, and vitamins (Tacon & Metian, 2013). However, the nutrients most associated with the beneficial effects of eating fish are the n-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Lands, 2014). These key conditionally-essential fatty acids have been the most studied and have key roles in neural development, and beneficial effects in cardiovascular and inflammatory diseases and some cancers (Calder, 2014). The physiological, biochemical and molecular mechanisms underpinning the critical roles of these "omega-3" fatty acids in human health are increasingly being elucidated and understood (Calder, 2015). Our understanding of the beneficial effects of dietary EPA and DHA on human health have been largely based on two main lines of evidence, epidemiological studies and randomized controlled (intervention) trials, although laboratory studies investigating biochemical and molecular mechanisms have also provided mechanistic support to these *in vivo* approaches (Gil *et al.* 2012; Calder, 2015). Based on all the evidence, many recommendations for EPA and DHA intake for humans have been produced by a large number of global and national health agencies and associations, and government bodies with those of over 50 organizations compiled recently by the Global Organization for EPA and DHA Omega 3s (GOED, 2014). While recommended levels vary between 250 and 1000 mg, the International Society for the Study of Fatty Acids and Lipids recommend a daily intake of 500 mg of n-3 LC-PUFA for optimum cardiovascular health (ISSFAL, 2004). Projecting this to a world population of 7 billion, this amounts to a total annual requirement for over 1.25 million metric tonnes (mt) of n-3 LC-PUFA and, as global supply cannot meet this level of requirement, there is a large gap between supply and demand (Naylor *et al.* 2009; Salem

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and Eggersdorfer, 2015; Tocher, 2015).

Marine microalgae and microbes are the primary producers of the vast majority of n-3 LC-PUFA (Harwood & Guschina, 2009), which consequently accumulate in the marine food web, underpinning why fish and seafood are the predominant sources of these nutrients in the diet (Tur *et al.* 2012; Sprague *et al.* 2017a). Indeed, consuming at least two portions of fish per week, of which one should be oily, is advised by global health authorities as a means of achieving the recommended daily intake of EPA+DHA in order to protect against cardiovascular and inflammatory diseases, among other health benefits (GOED, 2014). However, global fisheries are at, or beyond, exploitable limits and cannot satisfy the growing demand for fish and seafood (Worm *et al.* 2009). Consequently, aquaculture, which has been growing at over 6 % per year for around two decades, is filling the gap such that over 50 % of global fish and seafood is now farmed (FAO, 2016). Interestingly, high levels of n-3 LC-PUFA in farmed fish and shrimp were only assured by formulating feeds with high levels of fishmeal and, especially, fish oil, paradoxically themselves finite and limited marine resources derived from wild fisheries (Tacon & Metian, 2008; Shepherd & Jackson, 2013; NRC, 2011). Therefore, for aquaculture to continue to expand, alternatives to dietary fishmeal and oil have been sought, with plant meals and vegetable oils currently the only viable, sustainable alternatives (Turchini *et al.* 2011; Shepherd *et al.* 2017). However, these alternative ingredients do not contain n-3 LC-PUFA as the biosynthetic pathway for their production is not present in terrestrial plants (Harwood, 2005), and this has presented some major challenges for aquaculture.

Vegetable oils can contain high levels of the short-chain ($<C_{20}$) PUFA, α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), although these fatty acids have no major functional roles in most fish other than as precursors of the highly biologically active LC-PUFA, EPA, DHA and arachidonic acid (ARA, 20:4n-6) (Tocher, 2003). While PUFA in general are essential dietary components for all fish, the specific essential fatty acids (EFA)

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vary between species, with LNA and LOA able to satisfy EFA requirements in many freshwater and salmonid fish, whereas only the LC-PUFA themselves can satisfy EFA requirements in most marine teleosts (Tocher, 2010). However, in almost all species, dietary LC-PUFA support better growth performance than C_{18} PUFA (Tocher, 2010). Therefore, the critical importance of dietary LC-PUFA for optimal growth, development and health of all vertebrates including fish (Calder, 2014, 2015; Tocher, 2003, 2010) means that dietary fishmeal and fish oil replacement, with consequent reduction in dietary n-3 LC-PUFA, while entirely necessary, has potentially impacted aquaculture production and fish health (Tocher & Glencross, 2015). In addition, it has also resulted in levels of n-3 LC-PUFA in farmed fish declining in recent years with the associated impact on product/nutritional quality for human consumers (Sprague *et al.* 2016; De Roos *et al.* 2017). This has stimulated much research into mitigating and reversing this decline, and to increasing the global supply of EPA and DHA (Tocher, 2015; Sprague *et al.* 2017b). It is in this context that the considerable research effort into elucidating pathways of fatty acid metabolism in fish has been based with the overarching hypothesis that understanding the molecular basis of LC-PUFA biosynthesis and regulation will enable the pathway to be optimized to promote efficient and effective use of plant-based dietary ingredients in aquafeeds, and n-3 LC-PUFA contents of farmed fish to be restored (Leaver *et al.* 2008; Torstensen & Tocher, 2011).

Biosynthetic pathway of long-chain polyunsaturated fatty acids

The biosynthetic pathways of LC-PUFA in vertebrates including fish is restricted to conversion of C_{18} PUFA precursors including LA and ALA supplied in the diet. Two types of key enzymes, namely fatty acid desaturases (Fads) and elongation of very long-chain fatty acids (Elovl) proteins (commonly refer to as “elongases”), mediate the rate-limiting reactions in LC-PUFA biosynthetic pathways (Figure 1).

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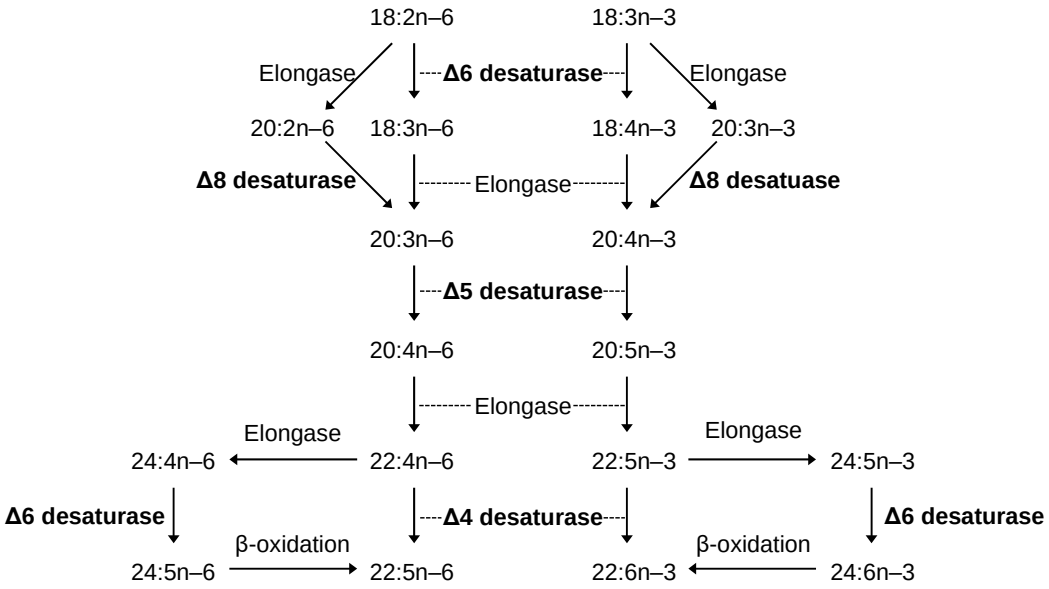

Figure 1. Complete biosynthetic pathways of long-chain polyunsaturated fatty acids (LC-PUFA) from the C_{18} precursors, namely 18:2n–6 and 18:3n–3 in vertebrates.

The Fads and Elovl enzymes involved in specific reactions of these pathways act on both the n-3 and n-6 series fatty acids, with n-3 fatty acids generally being preferred substrates (Castro *et al.* 2016). Using n-3 PUFA as an example, biosynthesis of EPA from ALA requires a Δ6 desaturation to produce 18:4n-3, which is then elongated to 20:4n-3. The latter goes through a Δ5 desaturation resulting in the production of EPA. An alternative pathway involves initial elongation of ALA to 20:3n-3, which is then converted to 20:4n-3 via Δ8 desaturation. As in the previous pathway, 20:4n-3 can be converted to EPA by the action of a Δ5 desaturase. In mammals, the production of DHA from EPA appears to proceed through the so-called "Sprecher pathway", a metabolic route that involves two consecutive elongations from EPA to produce 24:5n-3, the latter then being Δ6 desaturated to 24:6n-3 (Sprecher, 2000). While all the described reactions so far described take place in the endoplasmic reticulum (ER), a partial β-oxidation reaction of 24:6n-3 to DHA occurs in the peroxisomes and hence DHA biosynthesis through the Sprecher pathway is regarded as a more complicated route than the

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"Δ4 pathway", an alternative route for DHA biosynthesis proven to potentially operate in a number of fish species (Oboh *et al.* 2017). The presence of Fads enzymes enabling the Δ4 pathway in fish will be extensively covered in the following sections, although it should be noted that these pathways were recently proved to also potentially operate in human cells (Park *et al.* 2015).

Fads enzymes catalyze the introduction of a double bond (unsaturation) between an existing double bond and the carboxylic group at the terminus of the fatty acid substrate and, therefore, are known as "front-end" desaturases (Castro *et al.* 2016). The repertoire of Fads varies among vertebrates (Castro *et al.* 2012). Mammals possess three Fads-like enzymes termed FADS1, FADS2 and FADS3. FADS1 is a Δ5 desaturase, whereas FADS2 is a Δ6 desaturase with the ability to utilize both C_{18} substrates at the beginning of the pathways (Figure 1) and C_{24} PUFA including 24:5n-3 involved DHA biosynthesis via the Sprecher pathway (Sprecher, 2000). Further activities of mammalian FADS2 include the above mentioned Δ8 desaturation (Park *et al.* 2009) and, at least in a cell culture system, Δ4 activity (Park *et al.* 2015). Although the precise function(s) of FADS3 in LC-PUFA biosynthesis remain largely unknown, a recent study using a *Fads3* knock-out murine model resulted in reduced levels of DHA in brain and a higher ratio of 22:5n-3 to DHA in liver compared to wild-type, which suggested that Fads3 may enhance liver-mediated DHA synthesis to support brain accretion (Zhang *et al.* 2017). Although being beyond the scope of the present review, it is important to note that, in addition to Fads, Elovl (elongases) also play major roles in LC-PUFA biosynthesis. Briefly, Elovl enzymes catalyze the first and rate-limiting condensation step in the reactions that result in the 2-carbon elongation of fatty acids (Jakobsson *et al.* 2006). Seven members of the Elovl protein family (Elovl1-7) have been described in vertebrates (Guillou *et al.* 2010). While Elovl1, Elovl3, Elovl6 and Elovl7 have saturated or monounsaturated fatty acids as preferred substrates, Elovl2, Elovl4 and Elovl5 elongate PUFA and therefore play important roles in LC-PUFA biosynthesis (Jakobsson *et al*. 2006;

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Guillou *et al*. 2010). Indeed, these enzymes have been the focus of investigation in a wide range of fish species and the repertoire and functions of Elovl enzymes in fish have been reviewed previously (Monroig *et al.* 2011a; Castro *et al.* 2016).

Diversification of Fads2 desaturase activity in fish

There is strong evidence supporting that virtually all teleosts have lost the *fads1* gene during evolution, although both *fads1* and *fads2* genes could still be identified in cartilaginous fish such as the smaller spotted catshark *Scyliorhinus canicula* (Castro *et al.* 2012). Loss of *fads1* in teleosts has been accompanied by the expansion of the *fads2* gene and the diversification of the enzymes' functions. These phenomena have been elucidated by cloning and functional characterization of *fads2* genes from a wide range of phylogenetically diverse groups of teleosts from different habitats (freshwater, diadromous, catadromous and marine) and trophic levels (from herbivores to top carnivores). We herein provide an updated list of all Fads2 cDNAs that have been cloned and functionally characterized from teleosts. As shown in Table 1, Fads2 from most of species showed Δ6 desaturase activity, consistent with the functions of the mammalian FADS2 (Guillou *et al.* 2010). Moreover, a comprehensive study investigating the Δ8 desaturase capability of teleost Fads2 confirmed that such desaturation ability occured in all teleost Fads2 enzymes tested (Monroig *et al.* 2011b), the list now expanded to include other Fads2 from meagre *Argyrosomus regius*, orange-spotted grouper *Epinephelus coioides*, and nibe croaker *Nibea mitsukurii* (Monroig *et al*. 2013; Li *et al.* 2014; Kabeya *et al*. 2015). Searches in genome assemblies from several commercially important marine species (e.g. Atlantic cod *Gadus morhua* and European seabass *Dicentrarchus labrax*) confirmed that only one copy of *fads2* exists in the genome indicating that desaturation abilities were restricted to Δ6 and Δ8. These findings were in agreement with the commonly accepted view that marine species have limited LC-PUFA biosynthesis capabilities (Tocher,

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2010), this being why most of commercially important marine species strictly require the supply of preformed LC-PUFA, particularly EPA and DHA, in their diet (Tocher, 2010). This situation was aggravated even further in species such as the two pufferfish species *Takifugu rubripes* and *Tetraodon nigroviridis*, which completely lack *fads*-like genes in their genome (Castro *et al.* 2012).

Table 1. Functionally characterized Fads2 in teleost. All activities were determined by the yeast heterologous expression.

Species	Common name	Activities	References
Danio rerio	Zebrafish	Δ6, Δ5, Δ8	Hastings *et al.*, 2001; Monroig *et al.*, 2011
Oncorhynchus mykiss	Rainbow trout	Δ6, Δ8	Zheng *et al.*, 2004; Monroig *et al.*, 2011
O. mykiss		Δ5	Hamid *et al.*, 2016
Cyprinus carpio	Common carp	Δ6	Zheng *et al.*, 2004
Sparus aurata	Gilthead sea bream	Δ6, Δ8	Zheng *et al.*, 2004; Monroig *et al.*, 2011
Scophthalmus maximus	Turbot	Δ6, Δ8	Zheng *et al.*, 2004; Monroig *et al.*, 2011
**Salmo salar* (Δ5Fad)	Atlantic salmon	Δ5	Hastings *et al.*, 2005
S. salar (Δ6Fad_a)		Δ6	Zheng *et al.*, 2005
S. salar (Δ6Fad_b)		Δ6, Δ8	Monroig *et al.*, 2010, 2011
S. salar (Δ6Fad_c)		Δ6, Δ8	Monroig *et al.*, 2010, 2011
Gadus morhua	Atlantic cod	Δ6, Δ8	Tocher *et al.*, 2006; Monroig *et al.*, 2011
Rachycentron canadum	Cobia	Δ6, Δ8	Zheng *et al.*, 2009; Monroig *et al.*, 2011
Dicentrarchus labrax	European sea bass	Δ6	González-Rovira *et al.*, 2009; Santigosa *et al.*, 2011
Lates calcarifer	Barramundi	Δ6	Mohd-Yusof *et al.*, 2010
**Siganus canaliculatus* (Fad1)	Rabbitfish	Δ6, Δ5, Δ8	Li *et al.*, 2010; Monroig *et al.* 2011
S. canaliculatus (Fad2)		Δ4, Δ8	Li *et al.*, 2010; Monroig *et al.* 2011
Acanthopagrus schlegeli	Black seabream	Δ6	Kim *et al.*, 2011
Thunnus thynnus	Northern bluefin tuna	Δ6	Morais *et al.*, 2011
Solea senegalensis	Senegalese sole	Δ4	Morais *et al.*, 2012
Oreochromis niloticus	Nile tilapia	Δ6, Δ5	Tanomman *et al.*, 2013
O. niloticus		Δ4	Oboh *et al.*, 2017
Argyrosomus regius	Meagre	Δ6, Δ8	Monroig *et al.*, 2013
**Chirostoma estor* (Fads2a)	Pike silverside	Δ4	Fonseca-Madrigal *et al.*, 2014
C. estor (Fads2b)		Δ6, Δ5, Δ8	Fonseca-Madrigal *et al.*, 2014
Anguilla japonica	Japanese eel	Δ6, Δ8	Wang *et al.*, 2014

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Epinephelus coioides	Orange spotted grouper	Δ6, Δ8	Li *et al.*, 2014
Scatophagus argus	Spotted scat	Δ6	Xie *et al.*, 2014
Nibea mitsukurii	Nibe croaker	Δ6, Δ8	Kabeya *et al.*, 2015
Channa striata	Striped snakehead	Δ4, Δ5	Kuah *et al.*, 2015
C. striata		Δ6, Δ5	Kuah *et al.*, 2016
Clarias gariepinus	African catfish	Δ6, Δ5	Oboh *et al.*, 2016
Perca fluviatilis	European perch	Δ6	Geay *et al.*, 2016
Arapaima gigas	Arapaima	Δ6	Lopes-Marques *et al.*, 2017
Oryzias latipes	Japanese medaka	Δ4	Oboh *et al.*, 2017
Nibea coibor	Chu's croaker	Δ6	Huang *et al.*, 2017

*gene names referring to the corresponding publications in brackets.

Acquisition of alternative regioselectivities has occurred within teleost *fads2* (Fonseca-Madrigal *et al.* 2014; Castro *et al.* 2016). Indeed, zebrafish *Danio rerio* Fads2, the first functionally characterized Fads2 in a teleost, showed dual Δ6 and Δ5 desaturase activities (Hastings *et al.* 2001). Similarly, dual Δ6Δ5 desaturase activity has been reported in other teleost Fads2 including those from the rabbitfish *Siganus canaliculatus* (Li *et al.* 2010), Nile tilapia *Oreochromis niloticus* (Tanomman *et al.* 2013), pike silverside *Chirostoma estor* (Fonseca-Madrigal *et al.* 2014), African catfish *Clarias gariepinus* (Oboh *et al.* 2016) and striped snakehead *Channa striata* (Kuah *et al.* 2016) (Table 1). Interestingly, a recent study demonstrated that an Atlantic salmon *Salmo salar* desaturase, initially characterized as a monofunctional Δ5 desaturase (Hastings *et al*. 2005), also possessed Δ6 desaturase activity (Oboh *et al.* 2017). This result leaves the rainbow trout Δ5 desaturase as the only essentially monofunctional Δ5 Fads2 desaturase (Hamid *et al*. 2015). The distribution of dual Δ6Δ5 desaturases from species throughout the entire tree of life of teleosts (Betancur *et al.* 2013) suggested that this is a rather common trait among teleost Fads2. Importantly, the acquisition of Δ5 desaturase within a Fads2 can partly compensate the loss of Fads1 in some teleosts, allowing them to synthesize EPA and ARA from the corresponding C_{18} PUFA precursors, namely ALA and LA, respectively (Figure 1).

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Further cases of diversification of teleost Fads2 functions include the ability of some teleost Fads2 to act as Δ4 desaturases. Such activity was first discovered in the rabbitfish *Siganus canaliculatus*, at that time representing the first case of a Δ4 desaturase among vertebrates (Li *et al*. 2010). Further Δ4 Fads2 have been subsequently discovered in other teleosts (Table 1). It is important to note that, in some species such as rabbitfish, the Δ4 Fads2 co-exists with another Fads2 with Δ6/Δ5 desaturase activity, enabling all the desaturation reactions required for production of DHA from the precursor ALA. While its herbivorous feeding behavior was hypothesized to account for such a desaturase activity complement in rabbitfish (Li et al., 2010), the same pattern was later discovered in non-herbivoreous teleost species such as the pike silverside (Fonseca-Madrigal *et al.* 2014), striped snakehead (Kuah *et al.* 2015; Kuah *et al.* 2016) and Nile tilapia (Tanomman *et al.* 2013; Oboh *et al.* 2017), suggesting that confounding factors other than trophic level can also drive the functional diversification among teleost Fads2.

Fish can biosynthesize DHA through two possible pathways

As introduced above, Sprecher and co-workers demonstrated that the biosynthesis of DHA in mammals did not appear to proceed through a Δ4 desaturation from 22:5n-3 but rather 22:5n-3 was elongated to 24:5n-3 before the latter was further desaturated at the Δ6 position to produce 24:6n-3. Chain shortening of 24:6n-3 to DHA was achieved in the peroxisomes and therefore the Sprecher pathway involved translocation of fatty acids from endoplasmic reticulum (responsible for desaturation and elongation reactions) to peroxisomes where partial β-oxidation to DHA takes place. Although these studies were conducted in rats, the same pathways were accepted to mediate DHA biosynthesis in all vertebrates, and further studies confirmed that the Sprecher pathway was also active in rainbow trout (Buzzi *et al.*1997; Henderson *et al.* 1998). Later, the zebrafish Fads2, which had been proven to act as

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Δ6 desaturase towards 18:3n-3 (Hastings *et al.* 2001), was demonstrated to effictively desaturate 24:5n-3 to 24:6n-3 (Tocher *et al.* 2003). This study therefore confirmed that the same Fads2 enzyme could operate at both of the distinct Δ6 desaturation steps of the pathway (Figure 1), one on 18:3n-3 and the other on 24:5n-3. In contrast, Kabeya *et al.* (2015) showed that the Δ6 Fads2 from Nibe croaker *Nibea mitsukurii* was able to desaturate 18:3n-3 but not 24:5n-3, suggesting that the ability of fish to biosynthesize DHA through the Sprecher pathway varies among species. In order to test this hypothesis, a recent study investigated the prevalence of the Sprecher pathway among teleost fish by determining the Δ6 activity towards C_{24} substrates (24:5n-3 and 24:4n-6) by teleost Fads2 desaturases (Oboh *et al.*, 2017). The study concluded that, with the exception of the Nibe croaker Δ6 Fads2, all non-Δ4 desaturases had the ability to desaturate C_{24} PUFA substrates at Δ6 position and therefore both 24:5n-3 and 24:4n-6 were converted to 24:6n-3 and 24:5n-6, respectively. Importantly, such desaturase capability was demonstrated in Fads2 from species with different evolutionary backgrounds and with different desaturase activities including monofunctional Δ6 and bifunctional Δ6Δ5 desaturases (Oboh *et al.* 2017). Although none of the Δ4 Fads2 studied by Oboh *et al.* (2017) were able to desaturate C_{24} PUFA substrates, some of these Δ4 desaturases were present in fish species (e.g. rabbitfish and pike silverside) in which a further Fads2 had the ability to desaturate 24:5n-3 to 24:6n-3 as per the Sprecher pathway. Consequently, two pathways for DHA biosynthesis, namely the Sprecher pathway and the Δ4 pathway, could potentially coexist in some teleost fish species and, if both were functional, this would represent a clear advantage for satisfying DHA requirements through endogenous production from dietary precursors.

It is interesting to note that Oboh *et al.* (2017) further confirmed that the occurrence of the Δ4 pathway was more widespread than initially believed when the first vertebrate Δ4 desaturase was discovered in the rabbitfish (Li *et al.* 2010). Using a conserved region containing the YXXN motif responsible for Δ4 desaturase activity (Lim *et al.* 2014) as a

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search query, Oboh *et al.* (2017) identified the presence of putative Δ4 desaturases in 11 species and confirmed their functions in Nile tilapia and Japanese medaka as described above. The distribution of Δ4 desaturases within the tree of life of bony fishes suggested that Δ4 Fads2 appear to be restricted to teleost species within recently emerged lineages, suggesting that the acquisition of the Δ4 pathway occurred later during the evolution of teleosts, with more basal fish lineages (e.g. eels, carps, catfish, salmonids) having (if any) the Sprecher pathway as the sole route for DHA biosynthesis (Oboh *et al.* 2017).

Utilization of molecular information of LC-PUFA biosynthesis for aquaculture

The rapidly accruing molecular information of the LC-PUFA biosynthetic pathway in fish may enable implementation of new genetics-based approaches to optimize and maximize EPA and DHA levels in farmed species. For instance, genetic selection of fish strains with enhanced ability for LC-PUFA biosynthesis appears as a very promising strategy (Gjedrem, 2000) since the n-3 LC-PUFA content in flesh was confirmed to be a highly heritable trait in Atlantic salmon (Leaver *et al.* 2011). Our expanding knowledge of the genes encoding the fatty acyl desaturases and elongases directly associated with the n-3 LC-PUFA trait may therefore make them appropriate targets for selection when desirable alleles are identified. This could enable the development of fish strains with enhanced ability to thrive on more sustainable plant-based feed formulations. In this respect, wild stocks may represent a valuable genetic resource for improving the n-3 LC-PUFA trait as it was shown recently that land-locked strains of Atlantic salmon, which do not migrate to the sea, have increased LC-PUFA biosynthetic capacity (Betancor *et al.* 2016).

Studies have indicated that endogenous LC-PUFA biosynthesis in fish may be enhanced by “nutritional programming” and that this may involve activation and/or optimization of gene expression (Clarkson *et al.* 2017; Vera *et al.* submitted). The nutritional

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programming concept involves exposing an animal to a dietary stimulus early in life that alters that individual metabolically and physiologically such that it becomes adapted and better able to respond to a similar nutritional challenge later in life (Lucas, 1998; Patel & Srinivasan, 2002). Very recently, an early nutritional intervention in Atlantic salmon at first feeding using a diet formulated almost entirely with plant meals and vegetable oils, with only very low levels of EPA and DHA, adapted fish to better utilize these feeds. Thus, fish given this challenging diet at first feeding showed greatly increased retentions of EPA and DHA compared to fish fed a diet with high levels of n-3 LC-PUFA at first feeding (Clarkson *et al.* 2017). Liver gene expression showed an up-regulation of all pathways of intermediary metabolism including LC-PUFA biosynthesis (specifically *fads2* and *elovl5* genes) in fish given the early nutritional stimulus that was consistent with a biochemical and physiological response/adaptation (Vera *et al.*, submitted). While these data also provide some insight, the precise nature of the molecular response is still under investigation and likely involves epigenetic mechanisms (Balasubramanian *et al.* 2016).

Transgenic technology has also been applied to enhance the capacity of LC-PUFA biosynthesis. Initial trials using the model species zebrafish were conducted by introducing genes encoding putative Δ6 (Alimuddin *et al.* 2005) and Δ5 *fads2* (Alimuddin *et al.* 2007), and *elovl5* (Alimuddin *et al.* 2008) from masu salmon *Oncorhynchus masou*. The resulting transgenic zebrafish carrying masu salmon putative Δ6 *fads2* showed a level DHA twice as high in comparison with non-transgenic counterparts (Alimuddin *et al.* 2005). The transgenic zebrafish carrying masu salmon putative Δ6 *fads2* and *elovl5* also showed higher LC-PUFA levels in comparison with non-transgenic counterparts (Alimuddin *et al.* 2007, 2008). Thereafter, humanized *Caenorhabditis elegans* ω3 desaturase (*fat1*) and Δ12 desaturase (*fat2*) were also introduced into zebrafish enabling the transgenic fish to produce PUFA *de novo* (Pang *et al.* 2014). As developing transgenic techniques have become applicable to non-model species, transgenic strains have also been established in commercially important

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species, namely the common carp *Cyprinus carpio* (Cheng *et al.* 2014) and the marine Nibe croaker (Kabeya *et al.* 2014, 2016). These studies have the potential, not only to understand molecular and physiological mechanisms underlying LC-PUFA metabolism *in vivo*, but also to generate specific strains of farmed fish that would possibly not require a dietary supply of preformed LC-PUFA for larval and on-growing stages. However, the applicability of these technologies to fish farming is still extremely challenging due, in part, to socio-political issues and food safety regulations.

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Conclusions

LC-PUFA, particularly those of the omega-3 series, are essential components of the human diet being primarily sourced from fish. Therefore, as aquaculture has continued to expand, the industry has had to confront the high demand for nutritious (high omega-3 LC-PUFA) fish and seafood products whilst increasing the utilization of non-marine ingredients in aquafeeds. Simultaneously, a number of research lines have been conducted to broaden the knowledge with regards to LC-PUFA biosynthesis in fish in order to understand their ability to utilise dietary fatty acids contained in alternative plant-based ingredients used in aquafeeds. In the present review, we have summarized the extent to which our understanding of teleost Fads2, one of the key enzymes of LC-PUFA biosynthesis, has helped to achieve this aim. In contrast to mammals, teleosts have lost the *fads1* gene (Δ5 desaturase) but possess different copy numbers of fads2. Importantly, rather than remaining as Δ6/Δ8 desaturases as in mammals, teleost fish Fads2 have functionalized as a result of species-specific evolutionary histories and ecological factors including habitat and trophic level. In addition, it is noteworthy that two distinct pathways of DHA biosynthesis, the so-called "Sprecher pathway" and "Δ4 pathway" have been confirmed to be spread widely among teleosts. However, since Fads2 regioselectivity could be completely different even within closely related species, the functional characterization of Fads2 will continue to provide valuable insights into LC-PUFA biosynthesis in fish and be vital for practical applications within the aquaculture industry.

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