Chapter 10
Health and Condition in Fish: The Influence of Lipids on Membrane Competency and Immune Response

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10.1 The Influence of Lipids on Health and Condition

Traditionally fisheries biologists have used various metrics to indicate the condition and, by implication, health of fish. These indices are usually based on relationships between length and weight (Anderson and Neumann 1996). Although such metrics can, under some circumstances, provide a quick estimate of a fish's condition, their ability to shed light on the underlying cause-and-effect relationship(s) governing a fish's health and nutritional status are limited. Biochemical measures (e.g. lipids including fatty acids (FA) and sterols, proteins and their constituent amino acids, and trace elements) offer complimentary measures to assess, in a more specific way, the condition and underlying health of fish. Fatty acids and other lipids affect the health of fish in many ways; including, but not limited to, their effects on growth, reproduction, behavior, vision, osmoregularity, membrane fluidity (thermal adaptation), and immune response. In this review, we focus on the latter two roles that lipids play in mediating the health and condition of fish.

10.2 The Influence of Lipids on Membrane Fluidity and Other Membrane Properties

10.2.1 Homeoviscous Adaptation

Aquatic organisms are exposed to varying and sometimes extreme environmental conditions (e.g., marked changes in temperature) that can induce strong and often debilitating effects on their physiology. Fish in temperate regions and at high altitudes must adapt to changing temperatures throughout the year. Behavioral and physiological

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adaptations provide an effective response to such stressors. Although in some circumstances, fish may be able to adapt behaviorally (e.g. by moving to warmer or colder water), biochemical and physiological adaptations, especially at the level of the cell membrane, provide the most specific, enduring, response to sustained changes in temperature. In effect, temperature can be regarded as a stressor to which cells must respond to establish a new equilibrium between their environment and the physiochemical properties of their membranous structures; a response termed "homeoviscous adaptation" by Sinensky (1974).

Fatty acids play important structural and functional roles in cell membranes. Although the composition of the cell membrane is specific to each cell type, all membranes must maintain an adequate level of "fluidity," particularly at colder temperatures (Fig. 10.1). The first targets of temperature changes are the biomembranes, which exist in a liquid-crystalline state at body temperatures (Singer and Nicholson 1972). In their free form, highly unsaturated fatty acids (HUFA; FA with ≥20 carbons and ≥3 double bonds; a subset of PUFA; FA with ≥2 double bonds) have a very low melting point (approaching −50°C) and thus have a much greater tendency to remain fluid in situ than do saturated FA (SAFA), which have much higher melting points (ranging from +58°C to +77°C for myristic (12:0) and arachidic (18:0) acid, respectively). Thus, the relative proportions of fatty acids matters a great deal because the lipid bilayer of cell membranes requires a certain degree of rigidity but must also be sufficiently fluid to permit lateral movement of the constituent lipids and embedded proteins. The large numbers of double bonds in HUFA enhance the ability of such FA to "bend" thereby increasing their flexibility, reducing order, and, at least in principle, leading to an increase in the fluidity of cell membranes (Fig. 10.2; Eldho et al. 2003).

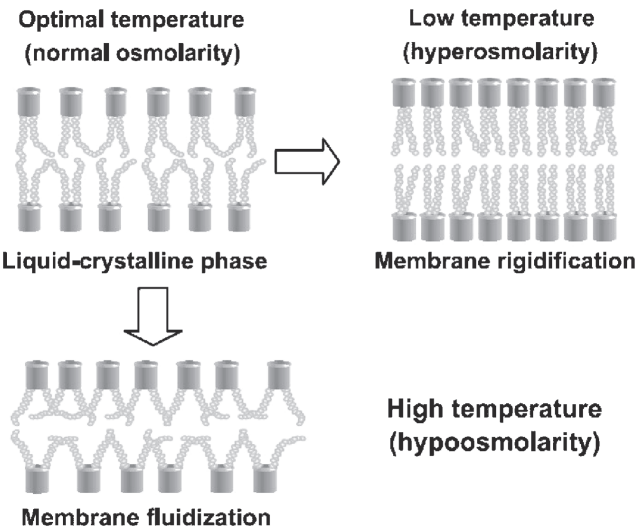

Fig. 10.1 Changes in membrane structure and the behavior of lipid bilayers under low and high-temperature stress. Low temperatures cause "rigidification" of membranes, while high temperatures cause "fluidization" of membranes. From Los and Murata (2004)

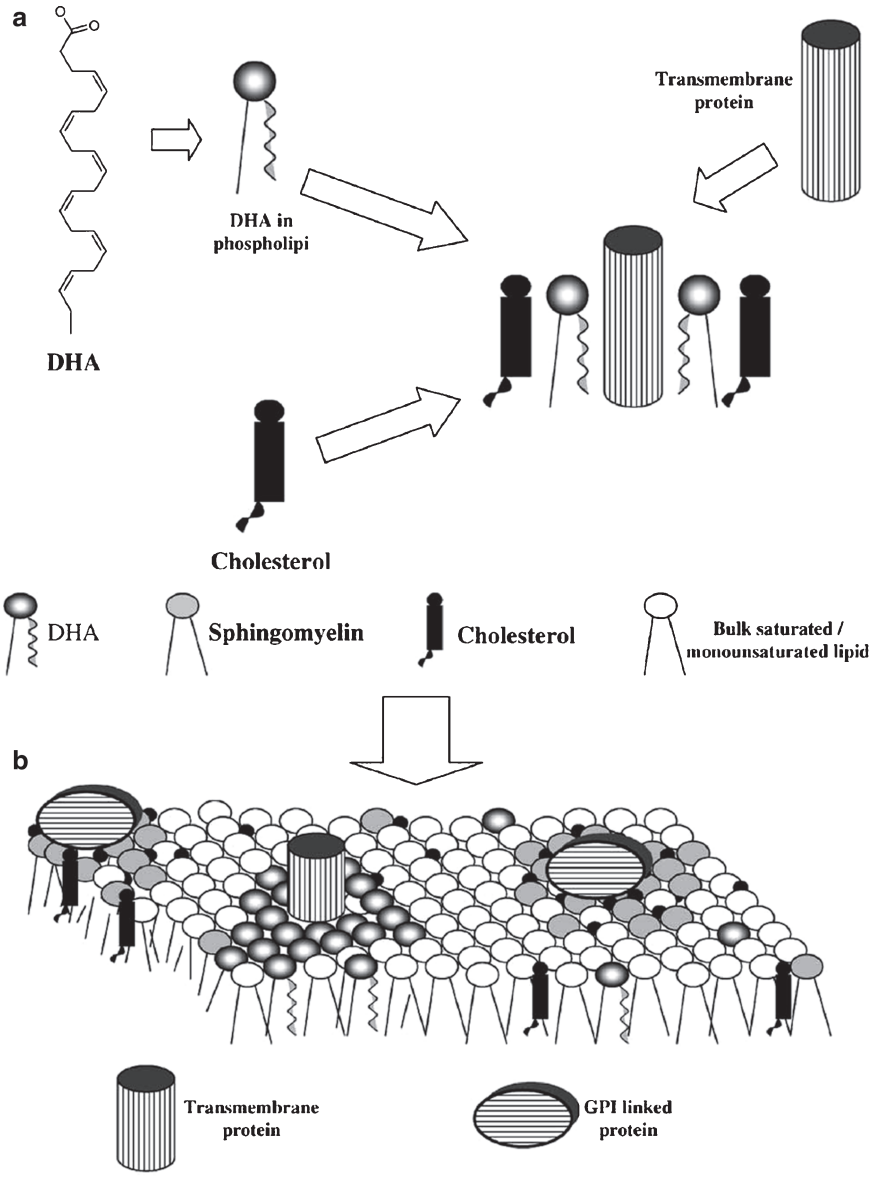

Fig. 10.2 (**a**) Schematic demonstrating the incorporation of docosahexaenoic acid (DHA) into the *sn-2* chain of a phospholipid and the association of this phospholipid with cholesterol. Also included is a transmembrane protein (e.g., rhodopsin) that has a high affinity for DHA. (**b**) Membrane phase separation into DHA-rich/cholesterol-poor (liquid disordered) and DHA-poor/cholesterol-rich (liquid ordered) domains. Different proteins partition into each domain. The liquid ordered domains are often referred to as "lipid rafts." *GPI* = Glycosylphosphatidyl-inositol. From Stillwell and Wassall (2003)

Docosahexaenoic acid (DHA; 22:6n-3) is especially important in this regard because it is the longest and most unsaturated FA commonly found in biological systems. The extremely high degree of acyl chain flexibility (Feller et al. 2002; Huber et al. 2002) conferred by the six double bonds in DHA are believed to be, at least in part, responsible for its effect on several important membrane properties including, but not limited to; membrane permeability, membrane fusion, and elasticity and vesicle formation (Stillwell and Wassall 2003; Wassall et al. 2004; and references therein). In addition, ion-transport processes of membranes as well as the functions of membrane-embedded, temperature-sensitive, enzymes (e.g. succinate hydrogenase, cytochrome oxidase and Na+/K+-ATPase) must be sustained (Hazel and Williams 1990; Hazel 1993; Adams 1999). Membrane competency is thus highly dependant on fluidity, ion transport and enzyme function, with temperature having a profound effect, both direct and indirect, on all of these properties.

Much of what is known concerning effects of specific FA and molecular species such as phospholipids on membrane fluidity come from studies on invertebrates, fish, and other vertebrates, especially mammals. Although ectothermic animals appear to increase the membrane content of unsaturated FA in response to colder temperatures, a clear and direct relationship between specific unsaturated FA and quantitative measurements of membrane fluidity has not commonly been demonstrated (but see Hall et al. 2002). However, such correlations do exist for some vertebrates. For example, experiments on rat platelet cells (Hashimoto et al. 2006), where the concentration of membrane-hardening cholesterol was manipulated, demonstrated that DHA has a more potent impact on membrane fluidity than eicosapentaenoic acid (EPA; 20:5n-3). Also, as vertebrates age, platelet membrane fluidity decreases as the DHA to arachidonic acid (ARA; 20:4n-6) ratio increases further supporting the critical role DHA plays in maintaining membrane fluidity (Hossain et al. 1999). Finally, there are cases where indirect evidence strongly points to a connection between n-3 HUFA in the diet and enhanced membrane fluidity and/or cold tolerance in fish (Kelly and Kohler 1999, Snyder and Hennessey 2003) and other vertebrates (Hagve et al. 1998) including humans (Lund et al. 1999).

10.2.2 Measuring Fluidity

In the ecological literature, the concept of membrane "fluidity" (or, more correctly, average membrane lipid order) is often invoked to explain mortality of fish due to cold exposure, however; actual physical measurements of membrane lipid order are often not done (Hall et al. 2002). Three main quantitative methods to assess membrane lipid order at the cellular level include steady-state fluorescence anisotropy measurements, electron spin resonance (ESR) spectroscopy, and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FT-IR). The first method relies on the degree of polarization of various synthetic fluorescent probes where the degree of polarization of the probe is inversely related to rotational fluidity (Kitajka et al. 1996, Hossain et al. 1999). The second method involves the incorporation of an electron spin label and subsequent measurements of the rate of motion of the

spin label using an electron spin resonance spectrometer (e.g. Buda et al. 1994). The third method is used in conjunction with fluorescence recovery after photo bleaching (FRAP) to determine the fluidity of the sample (Hull et al. 2005) and provides a more quantitative measure of the physical state of lipids embedded within the membrane. The interaction between lipids and proteins in the membrane is analyzed by monitoring the disorder of fatty acid acyl chains in terms of the stretching of bonds, in particular CH2 stretching in phospholipids. The fluid and rigidified states of lipids are represented by high and low frequencies of CH2 stretching, respectively; in other words, the higher the frequency, the more fluid the membrane lipids and the higher the degree of disorder.

10.2.3 Controlling Fluidity via Lipid Compositional Changes

It might appear that the most straightforward way to increase membrane fluidity would be to increase the overall concentration of HUFA, and especially DHA, in cell membranes. Although there is some evidence of modest increases in the proportion of DHA in fish in response to decreasing temperatures (Farkas et al. 2000) such a straightforward explanation does not take into account the structural details of the biologically relevant molecules (i.e., the phospholipids).

The situation is clearly more complex and must be viewed in the context of structural and chemical properties of the membrane phospholipids and their constituent parts. For example, it has been shown that the inclusion and position of the first unsaturated bond (especially in the *cis* configuration) in one of the two FA of the phospholipid molecule produces the greatest change in the physical properties of the molecule (e.g. melting point), with additional double bonds in the FA comprising the phospholipid molecule having progressively less effect (Coolbear et al. 1983; Stubbs and Smith 1984). Thus, the overall level of FA unsaturation in biomembranes is now known to play only a subordinate role in determining their overall fluidity. This is because it is now recognized that the physical properties of each molecular species of phospholipid, and their subsequent effects on membrane fluidity, largely depend on several interacting factors including; (a) the chemical structure of the headgroup, (b) FA chain length, (c) degree of unsaturation, and (d) positional distribution (*sn-1* vs *sn-2*) of the two FA within the molecules (Brooks et al. 2002). Perhaps most importantly (and, as yet, only partially understood) are the interactions between specific molecular phospholipids species and the configuration (packing) of membrane-bound proteins that contribute the greatest degree of membrane ordering state (i.e., a higher degree of disorder thereby resisting contraction) of cell membranes as temperatures fall (Buda et al. 1994).

Recent studies have revealed that organisms can adjust the fluidity of their membranes by modifying the proportions of specific combinations of monounsaturated (in the *sn-1*) and polyunsaturated (in the *sn-2* position) FA in phospholipid molecules such as phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE). For example, Farkas et al. (2000) concluded that the relative

amounts of these molecular species and their relative proportions are the major factors contributing to the maintenance of proper fluidity relationships in the brains of fish as well as helping maintain important brain functions such as signal transduction and membrane permeability. Similarly, Buda et al. (1994) found that, although the overall FA composition, including ARA and DHA, of carp (*Cyprinus carpio*) brain did not vary as an adaptive response to declining temperature, separation of PC and PE into their specific molecular species revealed a two to threefold accumulation of 18:1/22:6, 18:1/20:4, and 18:1/18:1 species in the latter, but no significant change in the molecular species composition of PC. Dey et al. (1993), in a study of the composition and physical state of fish liver phospholipids, found a two to threefold and a tenfold increase in the levels of 18:1/22:6 and 18:1/20:5 species of PE, respectively. Subsequent work with carp liver and with synthetic vesicles revealed that the placement of a *cis* Δ9 monounsaturated FA (MUFA) in the *sn-1* position, especially in PE, is amongst the most important factor determining the efficacy of the response of biomembranes to cold temperatures (Fodor et al. 1995). Similarly, exposure to cold induced precise changes to a limited number of phospholipid species in liver microsomes of carp including increases in the proportions of 16:1/22:6 and 18:1/22:6 for PC and PE, respectively (Brooks et al. 2002). The response for PI is a general increase in molecular species that had a MUFA in the *sn-1* position and, unlike the situation with PC and PE, a concomitant increase in either ARA or EPA in the *sn-2* position. Taken together, these studies point to a special and precise relationship between the molecular species composition of phospholipids and temperature, and especially to the critical role of the specific placement of particular MUFA in the *sn-1* position of PC and PE.

Biological membranes are no longer considered to be homogeneous mixtures of lipid and protein, but rather are now known to be composed of lateral patches of PUFA-rich domains and cholesterol-rich membrane rafts (Wassall et al. 2004; Fig. 10.2). Sterols and, in particular, cholesterol in animal biomembranes interact closely with phospholipids in a way that moderates fluidity, i.e., they are thought to have an ordering influence (inducing a tighter lateral packing of phospholipids) on membrane fluidity when the membrane lipids are in their liquid-crystalline state (Cooper et al. 1978). This is known as the condensing effect i.e., the total area occupied by cholesterol plus phospholipids is less than the sum of the area occupied by each molecular type separately (Haines 2001). The kinks in unsaturated acyl chains limit the formation of bonds between those fatty acids and cholesterol resulting in a lipid driven mechanism to explain why there is lateral phase separation into PUFA-poor/sterol-rich and PUFA-rich (especially DHA-rich)/sterol-poor microdomains (Ohvo-Rekila et al. 2002; Wassall et al. 2004; Fig. 10.2). Thus, the extent to which cholesterol is present in membranes can have a pronounced effect on fluidity. For example, rainbow trout fed menhaden oil (containing cholesterol and HUFA) demonstrated reduced fluidity in their macrophages compared with macrophages of trout containing sunflower oil containing no cholesterol or HUFA (Bowden et al. 1994). A second important role of cholesterol in animal cells is to diminish energy loss by reducing cation (Na^+) leakage through lipid bilayers of biomembranes (Haines 2001). In summary, sterols are now known to be essential

nutrients (Martin-Creuzberg and von Elert – Chap. 3) since the deprivation of sterols is lethal to all animal cells. Although some of their functions are now known, currently the precise roles of cholesterol and phytosterols are only partially understood (Haines 2001).

10.2.4 Environmental Effects on HUFA Supply

In fish, depot triacylglycerols and flesh phospholipids typically contain ~95 and ~75% FA by weight, respectively (Arts et al. 2001). However, a review of fish oil triacylglycerols demonstrates that only a small proportion of these molecules contain EPA and DHA (Moffat 1995). This occurs because there is a fair degree of specificity in FA assembly in the triacylglycerol depot fats of fish (Brockerhoff and Hoyle 1963; Brockerhoff et al. 1964). Specifically, triacylglycerol synthesis starts with a phospholipid that usually has any one of five common FA (e.g., myristic, 14:0; palmitic, 16:0; palmitoleic, 16:1n-7; stearic, 18:0; or oleic, 18:1n-9) in the *sn–1* position. The placement of FA in the center (*sn–2*) position of the phospholipid molecule is directed by enzymes that preferentially put a HUFA (e.g., EPA or DHA) in this position but, depending on whether the fish is in either a biosynthesis or the catabolism energy status, the assembly process may have to again use a common FA. Fatty acids in the last (*sn–3*) position of the triacylglycerol molecule derive from whatever surplus FA are in the diet and are circulating in the blood when the triacylglycerol molecules are assembled (Arts et al. 2001). Thus, the FAs available in the diet when triacylglycerols and phospholipids are synthesized play an important role in their final molecular configuration.

Since diet affects the availability of specific FA and since this availability affects the final molecular configuration of membrane phospholipids, it follows that environmental perturbations that affect the availability of HUFA for fish may, in turn, influence a fish's ability to maintain adequate membrane fluidity and perhaps other aspects of membrane competency. Changes in the FA composition of phospholipids in response to a cold challenge take place quite quickly; on the order of days to weeks (Trueman et al. 2000).

Large-scale ecosystem perturbations in the Laurentian Great Lakes provide examples of how such changes have the potential to affect membrane fluidity in fish and their predators (Hebert et al. 2008). For instance, populations of the lipid-rich amphipod *Diporeia* sp., which can attain densities of >1,000 animals per m^2, have declined sharply in many areas of the Great Lakes, especially in Lakes Michigan and Huron (Nalepa et al. 2006, and references therein) potentially as a consequence of a not fully understood interactions with invasive mussels. *Diporeia* sp. are rich in EPA and DHA (Arts, unpublished data) and this fact, combined with their historically high densities, suggest that this species provides an important role in contributing essential n-3 HUFA, obtained from settling algae, to bottom foraging fish such as the commercially important lake whitefish (*Coregonus clupeaformis*). Lake whitefish spends a significant amount of time feeding in deep cold hypolimnetic waters where

the maintenance of membrane fluidity is likely important. Thus, the widespread loss of *Diporeia* sp. from their historic ranges is a key issue of concern in the Great Lakes in part because their loss is expected to have a strong effect on the availability of n-3 HUFA for bottom-feeding fish predators such as lake whitefish.

A second example is the case of Chinook salmon (*Oncorhynchus kisutch*) and alewife (*Alosa pseudoharengus*) in Lake Michigan. Some fish species (e.g., salmonids) cannot elongate and further desaturate shorter chain (18 carbons) FA (ALA; 18:3n-3 and LIN; 18:2n-6) to their longer chain homologues (ARA, EPA, and DHA) in amounts sufficient to meet their needs (Sargent et al. 1995; and see Chap. 9). Historically, alewife comprised the main food source for Chinook salmon (Madenjian et al. 2005). This forage fish is known to be very rich in n-3 HUFA (Snyder and Hennessey 2003) and therefore likely supplied the abundant populations of Chinook salmon with much of their required dietary n-3 HUFA. Although the dramatic decline in the Chinook population in the late 1980s was associated with an epizootic disease outbreak, the underlying cause was thought to be nutritionally related (Benjamin and Bence 2003; Holey et al. 1998). It is therefore tempting to speculate that the underlying basis of this decline may have been, in part, due to a dietary n-3 HUFA deficiency, which contributed to the poor condition of the Chinook salmon rendering them more vulnerable to disease. Clearly, we require more information on the HUFA biosynthetic capabilities of key fish species and also on the distribution and relative abundance of these essential FA in their prey. Such information could be used to develop and refine specific biochemical indices of nutritional status and physiological competency, which, at least in theory, could provide a predictive tool to fisheries managers, forewarning them of impending fishery collapses.

Cultural eutrophication and climate change provide two other examples of large-scale perturbations with the potential to affect n-3 HUFA availability in aquatic ecosystem. Eutrophication generally results in a replacement of HUFA-rich taxa such as diatoms, cryptophytes, and dinoflagellates (Brett and Müller-Navarra 1997) with HUFA-poor taxa such as bloom forming chlorophytes and especially cyanobacteria (Müller-Navarra et al. 2004). Variations in the HUFA content of algae (i.e., availability of different taxa for consumption) can induce up to an eightfold difference in the PUFA concentration of the herbivore *Daphnia pulex* (Brett et al. 2006). Thus, cultural eutrophication can cause a marked impoverishment in the relative availability of n-3 HUFA rich food for uptake into the biomembrane phospholipids of herbivorous zooplankton, and, subsequently, for fish. This has important implications for fish condition and health since the availability of n-3 HUFA in fish diets is reflected in n-3 HUFA concentrations in fish tissues. For example, Estévez et al. (1999) found that the incorporation of ARA and EPA into fish eyes, brains, livers, and white muscle of turbot (*Scophthalmus maximus*) reflected the percentage of these FA in their diet. Masuda et al. (1999) demonstrated that ^{14}C-labeled DHA in the diet was incorporated directly to the central nervous system of yellowtail (*Seriola quinqueradiata*).

Climate warming also has the potential to affect the production of n-3 HUFA at the base of the food chain in cold water ecosystems. Algae react to lower temperatures by increasing the proportion of n-3 HUFA in their biomembranes (Chap. 1).

Compounding this, zooplankton, which are consumed by planktivorous fish and many larval fish, also demonstrate reduced concentrations of n-3 HUFA at higher temperatures in freshwater systems (and see Chap. 11 for a marine perspective). For example, members of the genus *Daphnia*, a keystone prey species for fish, demonstrate reduced EPA concentrations at higher temperatures (Schlechtriem et al. 2006). Thus, increased water temperatures are predicted, based on laboratory experiments, to exert a negative effect on the underlying availability of growth enhancing n-3 HUFA in the ecosystem. This also has implications for terrestrial systems because of their dependence on obtaining growth enhancing n-3 HUFA from aquatic systems (Chap. 8).

In conclusion, a fish's innate biochemical strategies and capabilities interact with the availability of key PUFA in its diet to maintain membrane competencies in response to varying environmental temperatures. As discussed earlier, the availability of some PUFA, in particular the n-3 HUFA, can be compromised under certain situations potentially leading to detrimental effects on membrane structure and, by consequence, fish health and condition. Clearly, this ability of fish to maintain membrane lipid order at physiologically advantageous levels affects their ability to withstand stress and disease. It is also clear that a fish's ability to withstand stress and disease is predicated on having a healthy, robust, immune system.

10.3 Modulatory Effects of Dietary Fatty Acids on Teleost Immune Response

10.3.1 Introduction

Lipids, FA, and their derivatives play a role in virtually every physiological process that occurs in vivo (Higgs and Dong 2000; Tocher 2003; Trushenski et al. 2006). For example, it has long been recognized in humans and other mammals that high dietary intake of saturated fats and cholesterol increase the likelihood of arteriosclerosis and heart attacks (Ulbricht and Southgate 1991). Saturated fats may have similar negatives effects on teleosts, as Atlantic salmon *Salmo salar* fed diets containing excessive quantities of saturated fats developed severe cardiomyopathy (Bell et al. 1991; 1993). However, it is with respect to immunity where the role of lipids and their constitutive FA in teleost health has been most clearly demonstrated (Rowley et al. 1995; Balfry and Higgs 2001; Sargent et al. 2002). Most of what we know about the immunomodulatory effects of dietary FA in teleosts has come from research using prepared diets with aquaculturally important fishes, most of which are carnivores. Because differences likely exist among species and trophic levels, major gaps exist in this body of research (see, generally, Blazer 1992; Rowley et al. 1995; Balfry and Higgs 2001). Nevertheless, the information gleaned in this manner provides useful insights on teleosts in the wild. Moreover, it would be nearly impossible to have obtained the current level of knowledge of teleost immunity and

FA if it were not for manipulative studies with aquaculture fish. Before reviewing the literature on the subject, a brief review of fish immunity is provided, with particular reference to those aspects dealing with FA derivatives.

10.3.2 Teleost Immune System

The immune system can simply be defined as a collection of mechanisms providing protection for an organism against pathogenic vectors (viruses, bacteria, fungi, parasites, etc.). To successfully meet the challenge of a vast array of potential pathogens, mechanisms must exist to distinguish pathogens from the organism's own proteins, and then to attack the pathogens before they manifest into debilitating disease. Immune strategies can generally be classified as innate (also called nonspecific) or adaptive (specific). Innate immune mechanisms are the more ancestral of the two, and function via self/nonself recognition of pathogens. Adaptive immune responses are based on production of pathogen-specific "populations" of antibodies, which can change in response to an individual's history of pathogen exposure. However, teleosts, though capable of adaptive immune responses, are more reliant on innate mechanisms due to the challenges of the aquatic environment (Tort et al. 2004).

10.3.3 Physical and Chemical Barriers

Mucous, scales (when present) and skin serve to seal the teleost and form the first line of defense against pathogens. These physical barriers may be breached by pathogens following injury or, in the case of stress, from impaired osmoregulatory ability. In the latter case, osmoregulatory failure leading to unregulated movement of water across the gill membranes provides pathogens, such as bacteria and viruses, with unimpeded access to the bloodstream. The skin may also secrete chemicals, antimicrobial peptides, and enzymes, to protect against infections. Antimicrobial peptides are short proteins, usually less than 50 amino acids long, which kill bacteria by disrupting membranes, interfering with metabolism, and/or targeting cytoplasmic components. Lysozyme is an enzyme that is sometimes classified as an antimicrobial peptide, though it is much longer in length. It acts as a self-induced antibiotic, destroying cell walls by hydrolyzing the polysaccharide component.

10.3.4 Innate and Adaptive Immune Systems

Those pathogens successfully invading a teleost will subsequently have to battle the innate immune system, including humoral (chemical secretions) and cellular agents. Upon penetration of the epithelium, inflammation occurs as a result of

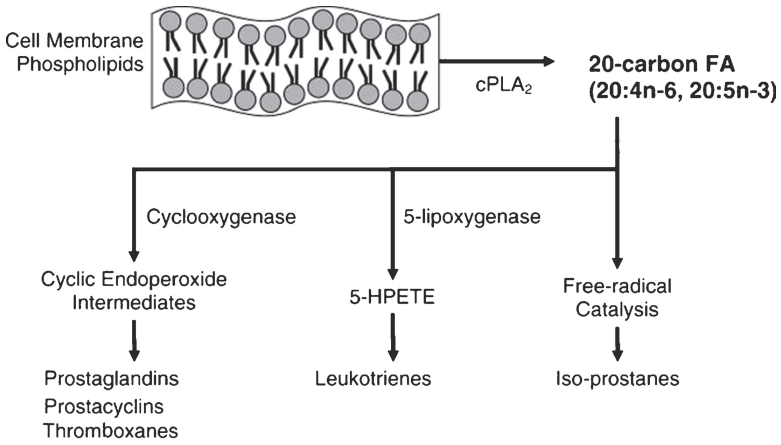

Fig. 10.3 Abbreviated diagram of eicosanoid synthesis pathways (*cPLA2* cytosolic phospholipase, *FA* fatty acid, *5-HPETE* hydroperoxyeicosatetraenoic acid). From Trushenski and Kohler (2008)

cytokines and eicosanoids being released by injured cells. Cytokines (e.g., interleukins, chemokines, and interferons) consist of proteins and peptides that bind to specific cell-surface receptors, each causing a cascade of intracellular signaling to alter cell functions. Eicosanoids, including prostaglandins and leukotrienes, are autocrines (hormone-like compounds), which serve, among other things, as modulators of inflammation and specific immunity responses. Eicosanoids are derived from 20-carbon n-3 and n-6 FA (Fig. 10.3), and are given specific attention in a subsequent section of this chapter. Teleosts possess leucocytes, (i.e. granulocytes, monocytes/macrophages, thrombocytes, nonspecific cytotoxic cells, and lymphocytes), all of which play vital roles in the innate immune system by patrolling the body in search of pathogens to be terminated. The adaptive immune response involves another class of leucocytes, called lymphocytes, which comprise a strong, complex defense mechanism, while also employing immunological memory. However, no immune response relationship of dietary FA to leucocytes, including lymphocytes, is apparent in teleosts.

10.3.5 Eicosanoids

Eicosanoids are a class of biologically active, oxygenated molecules that have been incorporated as esters in phospholipids and diacylglycerols of the cell and nuclear membranes. Along with serving as messengers in the central nervous system, eicosanoids act like local hormones or signaling molecules to control inflammation and immunity. Eicosanoids largely consist of prostaglandins, prostacyclins, thromboxanes, and leukotrienes, with each having different biosynthetic pathways and physiological effects (Fig. 10.3).

Eicosapentaenoic acid, ARA, and dihomo-gamma-linolenic acid (DG-LIN; 20:3n-6) are the primary FA from which eicosanoids are derived via two enzymes, cyclooxygenase and lipoxygenase, both of which exist in several forms. Although a normal physiological product, excess eicosanoid biosynthesis is initiated by extreme stress/trauma, cytokines, or other stimuli, which trigger release of phospholipase at the cell wall. The phospholipase migrates to the nuclear membrane catalyzing ester hydrolysis of phospholipid or diacylglycerol to free EPA, ARA, and/or DG-LIN. These FA are subsequently oxygenated via a number of pathways into prostanoids and leukotrienes. Prostanoids, in general, mediate inflammation by acting upon platelet aggregation and vasoconstriction/relaxation, and can also directly influence gene transcription. Leukotrienes have a more neuroendocrine role in inflammation, particularly with respect to leucocyte activity.

Arachidonic acid-derived eicosanoids promote inflammation (vasodilators) while those from EPA and DG-LIN are described as less inflammatory, inactive, or anti-inflammatory (vasoconstrictors). Accordingly, dietary n-3 FA can counteract ARA-derived eicosanoids (see Bell et al. 1994) by (1) displacement (higher n-3/n-6 ratio in phospholipids), (2) competition (EPA and DG-LIN compete for cyclooxygenases and lipoxygenase), and (3) opposition (eicosanoids derived from EPA and DG-LIN counter/impede actions of ARA-derived eicosanoids).

10.3.6 Effects of Diet on Tissue Fatty Acid Composition

Marine oils derived from feed-grade, reduction fisheries often serve as the primary lipid constituents of aquaculture diets because of their high palatability to cultured fishes, attractant properties, and historically widespread availability and competitive pricing (Lane et al. 2006). On the one hand, tissue FA composition of fishes largely reflects the diet (Shearer 1994; Jobling 2003), and thus fishes fed marine-derived oils contain substantial amounts of bioactive HUFA, namely DHA, EPA, and ARA. On the other hand, tissues of fish fed diets containing plant or livestock-derived lipids will be higher in saturated fats. Several studies have shown reductions in HUFA when these latter diets have been fed to channel catfish (*Ictalurus punctatus)* (Manning et al. 2007; O'Neal and Kohler 2008), sunshine bass *(Morone saxatilis* x *M. chrysops)* (Kelly and Kohler 1999; Wonnacott et al. 2004; Lewis and Kohler 2008), rainbow trout (*Oncorhynchus mykiss)* (Caballero et al. 2002), Atlantic salmon (Bransden et al. 2003), gilthead seabream (*Sparus aurata)* (Izquierdo et al. 2003), and red seabream (*Pargrus auratus)* (Glencross et al. 2003). Marine oil-based "finishing diets" have been used to restore the tissue FA profiles of turbot (*Psetta maxima)* (Regost et al. 2003) and Atlantic salmon (Bell et al. 2003; 2004; Torstensen et al. 2004) previously fed nonmarine derived oils for the majority of the production cycle.

A simple dilution model describes FA turnover for medium-fat species following dietary fat modification (Robin et al. 2003; Jobling 2003, 2004a, b). Conversely, the dilution model did not adequately describe FA turnover in a "lean-flesh" fish (<2%

lipid), e.g., the sunshine bass (Lane et al. 2006). The deviations from the model in this lean fish suggest selective retention of n-3 HUFA along with preferential catabolism of saturates. The aforementioned studies have direct bearing on fishes in the wild in that they demonstrate tissue FA profiles which will essentially mirror seasonal profiles of the food chain, but that some selective retention of HUFA may occur on a species-specific basis (Dalsgaard et al. 2003).

10.3.7 Tissue Fatty Acid Composition and Teleost Immunity

In addition to altering tissue FA profiles, a number of manipulative dietary lipid studies have noted attendant influences in immune system functioning, particularly with respect to eicosanoid production. For example, Bell et al. (1992) found diet related increases in ARA in post-smolt Atlantic salmon phospholipids induced production of ARA-derived eicosanoids. In a subsequent study, Bell et al. (1993) found, though the metabolic pathways involved are not fully clear, that a diet high in linolenic acid (ALA; 18:3n-3) (the biosynthetic precursor to EPA) resulted in higher levels of EPA in Atlantic salmon leucocyte phospholipids with a concomitant reduction in ARA-derived eicosanoids, ultimately resulting in increased antiinflammatory response and a reduction in cardiac lesions. Similar findings were obtained in related studies with juvenile turbot (S*cophthalmus maximus)* (Bell et al. 1994). Moreover, when diets high in ARA were used, ARA-derived prostaglandins were found to be significantly higher in tissue homogenates (Bell et al. 1995). These studies suggest dietary fats may have profound effects on nonspecific immune factors. In particular, diets high in n-3 FA will generally result in relatively higher levels of the antiinflammatory 3-series prostaglandins and 5-series leukotrienes and lipoxins derived from EPA, whereas high dietary n-6 promotes relatively higher levels of proinflammatory two-series prostaglandins and four-series leukotrienes and lipoxins derived from ARA (Balfry and Higgs 2001). Moreover, EPA competitively interferes with eicosanoid production from ARA and, ultimately, eicosanoid actions are largely determined by the ratio of ARA to EPA in cellular membranes wherein an imbalanced ratio can be damaging to fish (Sargent et al. 2002). Lin and Shiau (2007) found three nonspecific immune response indicators (respiratory burst, lysozyme activity, alternative complement activity) were significantly higher in grouper (*Epinephelus malabaricus)* fed a diet with a blend of fish oil and corn oil as the lipid source than fish fed diets with either fish oil or corn oil as the sole lipid source. The blend of corn and fish oils likely proved positive in this regard because of the complexity of the immune response, i.e., contradictions exist in immunostimulation and suppression resulting from dietary source of lipid. Thus, as previously suggested by Fracalossi and Lovell (1994), a species-specific dietary n-6 and n-3 HUFA ratio should optimize immune function. A dietary n-6 to n-3 PUFA ratio no greater than 4:1 and, preferably, closer to 1:1, is recommended for human health (Lands 1992), but no specific ratios for teleosts have been determined.

10.3.8 Tissue Fatty Acid Composition and Disease Susceptibility

Information on the relationship of dietary lipids to specific disease resistance is limited and seemingly contradictory. For example, Li et al. (1994) reported negative effects of high dietary n-3 HUFA levels in channel catfish, while Sheldon and Blazer (1991) found in the same species that bactericidal activity was positively correlated with dietary HUFA. Lodemel et al. (2001) observed higher survival in Artic charr (*Salvelinus alpinus)* fed dietary soybean oil (high in n-6) than fish oil, whereas Montero et al. (2003) found in gilthead seabream the inclusion of soybean oil reduced both serum alternative complement pathway activity and head kidney phagocytic activity. Montero et al. (2003) also determined in gilthead bream that rapeseed oil affected head kidney macrophages activity, while linseed oil altered stress response. In actuality, such conflicting results may be due to the different modes in which different dietary lipids and FA ratios may affect disease susceptibility in teleosts. For example, Sheldon and Blazer (1991) examined the effects of lipid types primarily on the specific immune response while Lodemel et al. (2001) evaluated immunological effects indirectly via influence of dietary lipid on gut microflora. A greater incidence of goblet cells in the midgut region of infected fish fed soybean oil was observed, prompting Lodemel et al. (2001) to theorize mucous sloughing is a protective response against bacterial infection, similar to what has been shown in the gills (Ferguson et al. 1992). These results further emphasize the importance of a proper dietary n-3 to n-6 ratio for health maintenance in teleosts.

10.4 Future Directions

A solid body of evidence exists for teleosts demonstrating that: (1) tissue lipids largely reflect their dietary intake; (2) several FA play pivotal roles in the health of fish and other aquatic organisms including, as reviewed here, effects of lipids on membrane competency and immunity; and (3) some species-specific balance of n-3 and n-6 FA is necessary to optimize immune functioning and general health (and see Chap. 7 for specific examples pertaining to fish and other aquatic organisms). For fishes in captivity, future research should be directed at determining the interaction between cholesterol (and other sterols) and fatty acid profiles on membrane fluidity and thermal tolerance through careful dietary manipulations. Specific attention should be paid to determining dietary thresholds of specific FA or combinations of FA and sterols on thermal tolerance and other aspects of membrane competency. In an aquaculture context, strides can be made in further boosting immunity via dietary lipid manipulation, particularly with respect to optimizing species-specific balances for n-6:n-3 FA. For fishes in the wild, trophic studies should focus on identifying how seasonal changes in the food chain create a cascade of FA alterations at each trophic level, ultimately altering tissue compositions of top carnivores. Research on how anthropogenic alterations of ecosystems, including eutrophication as well as global warming and exotic invaders, impact the bottom-up transfer of FA and associated health factors should also prove

quite illuminating. Clearly, great strides could be made if fish nutritionists, physiologists, and ecologists collaborated on such endeavors.

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