College of Tropical Agriculture
and Human Resources
University of Hawai‘i at Mānoa

Foods and Nutrition
July 2012
FN-11

Nutrition Considerations in Aquaculture: The Importance of Omega-3 Fatty Acids in Fish Development and Human Health

Corilee Watters[1], Scott Iwamura[1], Harry Ako[2], Dong-Fang Deng[3]
[1]Department of Human Nutrition, Food and Animal Science, [2]Molecular Biosciences and Bioengineering, College of Tropical Agriculture and Human Resources, [3]Aquatic Feeds and Nutrition Department, Oceanic Institute

Aquaculture, the cultivation of fresh- and saltwater fish, shellfish, and algae, is being increasingly recognized as an important method of food production and accounts for close to half of the fish consumed worldwide (FAO 2010). For those who are raising fish, it is critical from a number of perspectives to consider what to feed them. For maximal growth, fish nutrition needs to be tailored to the species and the stage of development. Given that fish feed is one of the highest operating costs of an aquaculture system (FAO 2006), it is necessary to maximize the feed conversion ratio and use costly feed ingredients judiciously. Ultimately, the goal should be to optimize the nutritional composition of fish for consumption, since fish represent the main source of long-chain omega-3 fatty acids (LC ω-3 FA) in the human diet.

Consumption of the LC ω-3 FAs eicosapentaenoic acid (20:5 ω-3; EPA) and docosahexaenoic acid (22:6 ω-3; DHA) has been shown to have numerous health benefits. The American diet typically contains excessive amounts of ω-6 FAs, particularly linoleic acid (18:2 ω-6; LA), in relation to LC ω-3 FAs (Simopoulos 2006). This is of concern because high LA intakes inhibit integration of EPA and DHA into cellular membranes, which may result in increased production of pro-inflammatory arachidonic acid (20:4 ω-6; AA)-derived eicosanoids (Simopoulos 2006). Chronic inflammation promotes the development of many diseases, including heart disease, diabetes, and certain types of cancer (Libby 2007). LC ω-3 FAs, in contrast to LA, possess anti-inflammatory properties. Increased intakes of LC ω-3 FAs are associated with reduced incidence of heart disease and reductions in total mortality (Hu et al. 2003), improved clinical outcomes in inflammatory diseases such as rheumatoid arthritis (Calder 2006), and improved outcomes in depression (Freeman et al. 2006). Furthermore, DHA comprises approximately one third of the total fatty acid composition of the cerebrum, cerebellum, and retina (Neuringer 1988) and is needed for optimal cognitive and visual development.

The American Heart Association (AHA) and Academy of Nutrition and Dietetics (AND) recommend an intake of two 4-ounce servings of fish, preferably fatty fish, per week (Kris-Etherton et al. 2002; Kris-Etherton & Innis 2007). This amount of fish intake correlates roughly to 500 mg of EPA and DHA per day (Kris-Etherton & Innis 2007). In Hawai‘i, the median fish consumption per week is well below the AHA and AND recommendations at 5.3 ounces (Baker et al. 2012). Common animal protein sources in the Western diet, such as beef, chicken, and pork, do not provide adequate amounts of LC ω-3 FA in the human diet. Per 100 grams (~3.5 ounces), the approximate LC ω-3 FA content of beef, pork, and chicken is 70 mg, 26 mg, and 50 mg, respectively (Ponnamapal 2006; Blasbalg et al. 2011). Alpha-linolenic acid (18:3 ω-3; ALA), the ω-3 FA derived from plant sources such as flaxseed and walnuts, is not efficiently converted to LC ω-3 FA in humans (Brenna 2002).

Depending on feeding practices, other production methods, and choice of species in aquaculture, farmed fish may be significantly lower in LC ω-3 FA than wild-caught, open-ocean pelagic fish. Because aquaculture will be increasingly relied upon to meet future demands for seafood products, it is important to ensure that aquaculture products offer similar nutritional benefits as their wild-caught counterparts. The LC ω-3 FA content of farmed fish, however, can be increased by tailoring the nutritional composition of fish feeds to the physiological needs of the fish throughout their lifecycle. The purpose of this paper is to review the role of LC ω-3 FA in farmed fish and discuss how LC ω-3 FA can be incorporated into fish feed at key periods of development (Figure 1) to maximize fish production and to ultimately improve human health.

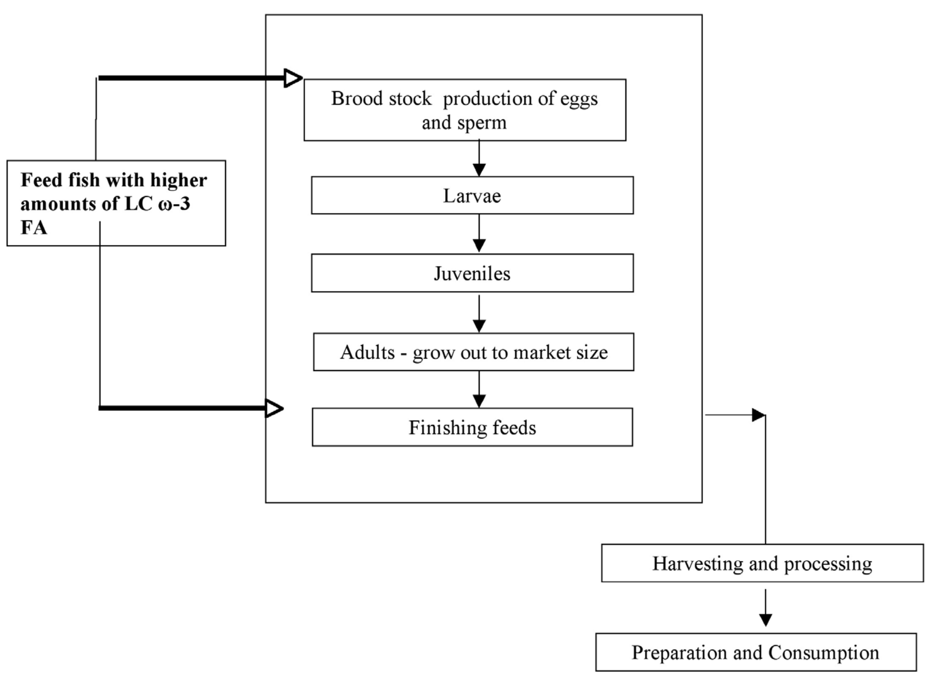
Nutrition Needs Throughout the Lifecycle of Fish

Nutrition requirements vary with the life stage of the fish. Many fish feed companies specifically market broodstock feed, which often contains higher amounts of LC ω-3 FA, vitamin C, vitamin E, and carotenoids, depending on the species of the fish. Feeds higher in LC ω-3 FA may offer developmental benefits for larval and juvenile fish and can improve the LC ω-3 FA content of the fish flesh. Finishing feeds, or feeds administered to mature fish prior to harvest, can also be used to improve LC ω-3 FA content (Bell et al. 2004; Glencross et al. 2003).

Broodstock

Fish, like other vertebrates, require LC ω-3 FA for growth and development. Broodstock feeds with increased LC ω-3 FA concentrations have been associated with improved egg morphology and viability (Izquierdo et al. 2001). Incorporation of ingredients providing high amounts of LC ω-3 FA such as squid meal and squid oil into feeds has been shown to improve egg quality in red sea bream, gilthead sea bream, and striped jack (Watanabe & Vassallo-Agius 2003). Other nutrient requirements may also be increased during reproductive periods; for example, gilthead seabream require increased amounts of dietary antioxidants (Fernandez-Palacios et al. 1998). Vitamin C, vitamin E, and carotenoids such as astaxanthin are purported to protect egg membranes from oxidative damage and improve egg quality in some aquaculture species (Izquierdo et al. 2001). Providing

Figure 1. Overview of production to consumption in aquaculture



milkfish larvae with vitamin C-enriched rotifers and artermia may reduce cartilage malformations (Cahu et al. 2003). However, similar to other vertebrates, fish are susceptible to nutritional toxicities. Excess dietary LC ω-3 FA has been noted to reduce the reproductive capacity of fish, perhaps by disrupting endocrine homeostasis (Izquierdo et al. 2001). High dietary retinoic acid levels can result in teratogenic effects and skeletal malformations in flounder larvae (Cahu et al. 2003).

Larval Stage

LC ω-3 FA requirements may be twice as high in larvae as in juveniles, even within the same species, perhaps due to increased needs for neurological development (Izquierdo 1997). Larval fish, especially in marine fish production, are fed with live feed such as copepods, rotifers (*Brachionus sp.*), and brine shrimp (*Artemia sp.*) due to challenges associated with making efficient larvae feed. Brine shrimp are small aquatic crustaceans that produce dormant eggs called cysts that can be stored for prolonged periods of time and hatched out on demand. This has resulted in their extensive use as a larval feed in aquaculture production. The hatched form of brine shrimp are offered as a live food called nauplii. However, rotifers and nauplii are low in LC ω-3 FA and some vitamins and minerals. These live feeds can be enriched with microalgae that are high in DHA, such as *Isochrysis galbana* and *Schizochytrium* sp. (Table 1). Enrichment of LC ω-3 FA in larval feed may improve larval resistance to physical stress, as reported by Ako et al. (1994) in a study of striped mullet (*Mugil cephalus*).

Some fish larvae, such as those of pink snapper (*Pristipomoides filamentosus*), are too small to consume rotifers and require copepod nauplii as initial feeds for survival. Copepods have the advantages that they are able to synthesize LC ω-3 FA, do not require feed to be supplemented with EPA and DHA, and are higher in protein than rotifers. However, copepods are currently not available commercially, and methods for cultivation are labor intensive and exacting, including such considerations as the need to optimize microalgae density (VanderLugt et al. 2009).

Role of Feeds in Mature Fish

The nutritional composition of feeds can significantly alter the fatty acid profile of mature fish. Salmon receiving feeds containing high amounts of plant-based oils have significantly reduced tissue concentrations of LC ω-3 FA and higher ratios of ω-6 FA (Bell et al. 2003). Similar findings were noted in mature turbot (Regost et al. 2003), zebrafish (Tocher et al. 2001), and several species of tilapia (Tocher et al. 2001; Shapira et al. 2009; Karapanagiotidis et al. 2007). However, the incorporation of fish oil into finishing feeds has been shown to improve tissue concentrations of LC ω-3 FA in many aquaculture species, even if the fish have been previously raised on a plant oil-based diet. For example, Bell et al. (2004) reported that tissue LC ω-3 FA in Atlantic salmon was reduced after 40 weeks of feeding when 50% of the fish oil was replaced by linseed oil. The tissue LC ω-3 FA of these fish was restored to concentrations similar to that of salmon that had previously received fish oil-based feeds after the salmon were switched to a fishoil-based diet 24 weeks before harvest. Similar restoration of LC ω-3 FA has been observed in red sea bream raised on plant oil-based diets after 32 days of providing a finishing feed with fish oil (Glencross et al. 2003). Numerous feeds containing fish oil or fishmeal are commercially available (Table 1). Menhaden (*Brevoortia sp.*), an oily fish native to North Atlantic waters, is often a fishmeal source in dried feeds. Krill, a small, shrimp-like crustacean, is a source of fish oil used to enhance the nutritional properties of fish feeds.

Nutritional Considerations in Various Species

Some freshwater fish are capable of producing LC ω-3 FA from α-linolenic acid (18:3 ω-3; ALA), whereas in most marine fish, preformed LC ω-3 FA must be supplied in the diet since the conversion from ALA to EPA and DHA is negligible (Sargent et al. 1995). There are also species-specific differences. Herbivorous fish have the enzymes for endogenous production of LC ω-3 FA, but these enzymes are lacking in piscivorous or carnivorous fish, which must obtain LC ω-3 FA biomagnified in algae-consuming prey (Sargent et al. 1995). A summary of EFA requirements in various species of farmed fish is detailed by Tocher (2010).

Herbivorous and Omnivorous Fish Species

Herbivorous and omnivorous fish species have the ability to synthesize LC ω-3 FA endogenously and thus require lower amounts of EPA and DHA in their diet. This ability to elongate fatty acids such as ALA into LC ω-3 FAs suggests it may be possible to increase the LC ω-3 FA

Table 1. Sources of LC ω-3 FA for use in fish feed

Type	Commercial Availability	Total LC ω-3 FA (mg/g dry weight)	EPA (mg/g dry weight)	DHA (mg/g dry weight)
Microalgae *Schizochytrium* sp.				
Algamac 3050 Flake (Larval & post-larval feed) [a]	Aquafauna Bio-Marine Inc., Hawthorne, CA.	24.4	1.6	22.8
Algamac Enhance (Broodstock feed) [a]	Aquafauna Bio-Marine Inc., Hawthorne, CA.	10.2	0	10.2
Fish Oils/Fishmeals				
Ika Omega-3 (Squid Oil) [a]	Aquafauna Bio-Marine Inc., Hawthorne, CA.	14	13.4	0.6
Menhaden oil [b]	Animalfeeds International Corp., Clark, NJ. Omega Protein Inc., Houston TX.	14.7	8.5	6.2
Krill oil [c]	Krill Canada Sales Corp., Langley, BC.	6.9	5.7	1.2
DHA Enrichment Supplements				
Formulated Diet, ABM 4000 Series for larval/post-larval fish; Japonicus Formula [a]	Aquafauna Bio-Marine Inc., Hawthorne, CA.	14.5	9.1	5.4
Isochrysis galbana, microalgae DHA supplement[d]	Reed Mariculture Inc., Campbell CA.	3.5	0.3	3.2

[a] Data from Aquafauna Bio-Marine Inc. Web site, http://www.aquafauna.com/Products.htm.
[b] Data provided by Osman et al. (2001) for standardized menhaden oil.
[c] Data for Antarctic krill from Krill Canada Sales Corp Web site, www.krill.ca/analysis.html.
[d] Data provided by Tokusoglu & Unal (2003).

of fish by providing oils with high levels of ALA such as linseed oil.

Farmed mango tilapia fed a diet containing 8% fish meal supplemented with 7% extruded linseed oil attained increased levels of 18:3 ω-3 and slightly increased levels of DHA in comparison with tilapia fed the same diet not supplemented with linseed oil (Shapira 2009). In tilapia (*O. niloticus*), the extent to which ALA is desaturated and elongated into EPA is regulated by dietary fatty acid intake; fish fed a 5% fishmeal-based diet supplemented with 3% vegetable oil had twice the desaturation activity rate as fish fed the same diet supplemented with fish oil instead (Tocher et al. 2002). However, although tilapia are able to produce biologically adequate levels of LC ω-3 FA from precursors such as ALA, this process is inefficient, and supplementation of vegetable oils is not sufficient enough to raise LC ω-3 FA concentrations to those of tilapia fed fish oil (Tocher et al. 2002, Karapanagiotidis et al. 2007).

Research indicates that providing shrimp with feeds containing LC ω-3 FA can improve their LC ω-3 FA content and also helps to promote normal development. However, shrimp have a limited ability to convert LC ω-3 FA precursors to EPA and DHA. In a study investigating the effect of stearidonic acid (18:4 ω-3; SDA)-containing soybean oil on the growth of Pacific white shrimp (*Litopanaeus vannamei*), shrimp had decreased growth performance and significantly reduced tissue LC ω-3 FA levels when soybean meal replaced fishmeal in the feeds (Forster et al. 2011). This study suggests SDA-enriched oils cannot meet the LC ω -3 fatty acid needs of shrimp when fishmeal or fish oil is absent from the feed, because shrimp cannot efficiently convert SDA (or ALA) to EPA and DHA (Forster et al. 2011). A diet containing LC ω-3 FA is therefore necessary to improve EPA and DHA content of shrimp. A trial evaluating growth and fatty acid composition of juvenile shrimp (*Panaeus vannamei*) in response to different sources of dietary lipid showed that shrimp receiving a diet supplemented with menhaden oil had better weight gain, feed conversion, and survival than shrimp fed diets which were supplemented with different vegetable oils (Lim et al. 1997). Mature Pacific white shrimp receiving a diet supplemented with algal oil had higher DHA levels than shrimp receiving the control commercial feed after 4 weeks of feeding (Forster et al. 2010).

Carnivorous Fish Species

Carnivorous fish have low delta5 desaturase activity and thus a poor ability to convert ALA to LC ω-3 FA. In addition to requiring more pre-formed LC ω-3 FA, carnivorous fish require a higher total amount of fat in their diet. Qin et al. (1998) reported that Chinese catfish growth was improved when the feed contained fishmeal and higher amounts of fat (36% protein, 24% lipid) in comparison to a lower-fat plant-based fish feed (49% protein, 18% lipid). In this study, the fishmeal-containing feed slightly improved the LC ω-3 FA content of diploid catfish but not that of triploids, even though the triploids had a higher overall fat content. For red sea bream juveniles, good growth can be achieved by using feeds containing 1% EPA and 0.5% DHA (Takeuchi et al 1990). The minimum requirement of red sea bream for EPA and DHA is 0.9% of the diet (Kalogeropoulos et al. 1992).

Fish Preparation Methods Influence Nutrient Composition

The LC ω-3 FA content reported for commonly eaten fish usually reflects EPA and DHA quantities present in the raw, not cooked fish. Cooking may decrease the LC ω-3 FA content of fish, and some cooking methods decrease its content to a greater extent than others. Bhouri et al. (2010) showed the LC ω-3 FA content of farmed sea bream was significantly reduced after cooking, especially when the fish was grilled or pan-fried in vegetable oils. Frying caused the greatest decrease in LC ω-3 FA, which can be attributed to (1) the absorption of the vegetable oils into the fish during frying, thus increasing the amount ω-6 FA and possibly displacing the LC ω-3 FA from the fish, and (2) the oxidation of EPA and DHA during the cooking process, since polyunsaturated fatty acids are highly susceptible to oxidation upon heating (Bhouri et al. 2010). Similar results were reported for fried gilthead sea bream (Amira et al. 2010) and for fried sardines and fried mackerel (Candela et al. 1998). These findings demonstrate that frying, especially deep-frying, decreases the nutritional benefits of fish consumption and should be used sparingly when preparing fish. However, moist-heat cooking methods, such as steaming, may result in less loss of LC ω-3 FA than grilling or frying (Bhouri et al. 2010). Roasting may also have a less pronounced effect on LC ω-3 FA content than frying (Echarte 2001). Thus, the health benefits of LC ω-3 FA can only be maximized if fish are prepared using cooking methods that preserve LC ω-3 FA content.

Conclusion

Fish, like other vertebrates, require LC ω-3 FA for optimal growth and development. Tailoring the nutritional composition of fish feeds to meet the physiological needs of fish can improve both fish development and the LC ω-3 FA content of the fish. Because fish and seafood products are an important source of LC ω-3 FA in the human diet and aquaculture will be increasingly relied upon to meet consumer demand for fish and seafood, it is vital that aquaculturists maintain LC ω-3 FA content in fish. Additional work needs to be done on optimizing the fatty acid composition of farmed fish by varying the composition of the fish feed throughout the lifecycle of the fish. Further research into judicious use of fish oil to optimize fatty acid composition, as well as alternate sources of LC ω-3 FA acids such as microalgae in fish feeds is also needed. Finally, consumers would benefit from education pertaining to the sources of LC ω-3 FA in our diet and cooking methods to optimize LC ω-3 FA intake.

Acknowledgement of Reviewers

Clyde S. Tamaru, Dept. of Molecular Biosciences and Bioengineering, College of Tropical Agriculture and Human Resources; Chengsheng Lee, Oceanic Institute.

References

Ako H, Tamaru CS, Bass P, Lee CS. 1994. Enhancing the resistance to physical stress in larvae of *Mugil cephalus* by the feeding of enriched *Artemia* nauplii. *Aquaculture* 122:81–90.

Aquafauna Bio-Marine Inc. 2012. Available at: http://www.aquafauna.com/Products.htm Accessed June 5, 2012.

Amira MB, Hanene JH, Madiha D, Imen B, Mohamed H, Abdelhamid C. 2010. Effects of frying on the fatty acid composition in farmed and wild gilthead sea bream (*Sparus aurata*). *International Journal of Food Science and Technology* 45:113–123.

Baker KK, Watters C, Onaka AT, Horiuchi B, Dannemiller J, Brooks B. 2012. Health Hawaii Survey: fish consumption for adults in Hawaii HHS, 2007 and 2008. Hawaii Department of Health. Honolulu, HI.

Bell JG, Tocher DR, Henderson RJ, Dick JR, Crampton VO. 2003. Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *J Nutr* 133:2793–2801.

Bell JG, Henderson RJ, Tocher DR, Sargent JR. 2004. Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar)* using a fish oil finishing diet. *Lipids* 39:223–232.

Bhouri AM, Harzallah HJ, Dhibi M, Bouhlel I, Hammami M, Chauouch A. 2010. Nutritional fatty acid quality of raw and cooked farmed and wild sea bream (*Sparus aurata*). *J Agric Food Chem* 58:507–512.

Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. 2011. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am J Clin Nutr* 93:950–962.

Brenna JT. Efficiency of conversion of α-linolenic acid to long-chain n-3 fatty acids in man. *Curr Opin Clin Nutr Metab Care* 5:127–132.

Borlongan IG. The essential fatty acid requirement of milkfish (*Chanos chanos* Forsskal). 1992. *Fish Physiology and Biochemistry* 9:401–407.

Cahu CH, Infante JZ, Takeuchi T. 2003. Nutritional components affecting skeletal development in fish larvae. *Aquaculture* 227:254–258.

Calder PC. 2006. n-3 Polyunsaturated fatty acids, inflammation and inflammatory diseases. *Am J Clin Nutr* 83:1505S–1519S.

Candela M, Astiasaran I, Bello J. 1998. Deep-fat frying modifies high-fat fish lipid fraction. *J Agric Food Chem* 46:2793–2796.

Echarte M, Zulet MA, Astiasaran I. 2001. Oxidation process affecting fatty acids and cholesterol in fried and roasted salmon. *J Agric Food Chem* 49:5662–5667.

FAO. State of World Aquaculture 2006. Available at: ftp://ftp.fao.org/docrep/fao/009/a0874e/a0874e05.pdf. Accessed June 15, 2012.

FAO. World Review of Fisheries and Aquaculture 2010. Available at: http://www.fao.org/docrep/013/i1820e/i1820e01.pdf. Accessed June 15, 2012.

Fernandez-Palacios, H., Izquierdo, M.S., Gonzalez, M., Robaina, L., Valencia, A. 1998. Combined effect of dietary α-tocopherol and *n*-3 HUFA on egg quality of gilthead seabream broodstock (*Sparus aurata*). *Aquaculture* 161:475–476.

Forster IP, Dominy WG, Obaldo LG. 2010. Effect of diet manipulation on cholesterol and docosahexaenoic acid levels in shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society* 41(2):240–249.

Forster IP, Dominy WG, Obaldo LG, Hartnell GF, Sanders EF, Hickamn TC, Ruebelt MC. 2011. The effect of soybean oil containing stearidonic acid on growth performance, n-3 fatty acid deposition and sensory characteristics of pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Nutrition* 17:200–213.

Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, Keck PE, Marangell LB, Richardson AJ, Lake J, Stoll AL. 2006. Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. *J Clin Psychiatry* 67:1954–1967.

Glencross BD, Hawkins WE, Curnow JG. 2003. Restoration of the fatty acid composition of red seabream (*Pagrus auratus*) using a fish oil finishing diet after grow-out on plant oil based diets. *Aquaculture Nutrition* 9:409–418.

Hu FB, Eunyoung C, Rexrode KM, Albert CM, Manson JE. 2003. Fish and long-chain ω-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. *Circulation* 107:1852.

Izquierdo MS, Fernandez-Palacios H. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* 197:25–42.

Izquierdo MS, Fernandez-Palacios H. 1997. Nutritional requirements of marine fish larvae and broodstock. *Cah Options Mediterr* 22:243–264.

Kalogeropoulos N, Alexis MN, Henderson RJ. 1992. Effects of dietary soyabean and cod-liver oil levels on growth and body composition of gilthead bream (*Sparus aurata*). *Aquaculture* 104:293–308.

Karapanagiotidis IT, Bell MV, Little DC, Yakupitiyage A. 2007. Replacement of dietary fish oils by alpha-linolenic acid-rich oils lowers omega 3 content in tilapia flesh. *Lipids* 42:547–559.

Krill Canada Sales Corp. 2012. Available at: www.krill.ca/analysis.html. Accessed June 5, 2012

Kris-Etherton PM, Harris WS, Appel LJ. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106:2747–2757.

Kris-Etherton PM, Innis S. 2007. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. *J Am Diet Assoc* 107:1599–1611.

Libby P. 2007. Inflammatory mechanisms: The molecular basis of inflammation and disease. *Nutrition Reviews* 2:140–146.

Lim C, Ako H, Brown C, Hahn K. 1997. Growth response and fatty acid compositino of juvenile *Penaeus vannamei* fed different sources of dietary lipid. *Aquaculture* 151:143–153.

Neuringer M, Anderson GJ, Connor WE. 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Ann Rev Nutr* 8:517–541.

Osman H, Suriah AR, Law EC. 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. *Food Chemistry* 73:55–60.

Patil V, Kallqvist T, Olsen E, Vogt G, Gislerod HR. 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquacult Int* 15:1–9.

Ponnampalam EN, Mann NJ, Sinclair AJ. 2006. Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts, potential impact on human health. *Asia Pacific Journal of Clinical Nutrition* 15(1):21–29.

Qin JG, Fast AW, Ako H. 1998. Growout performance of diploid and triploid Chinese catfish *Clarias fuscus*. *Aquaculture* 166:247–258.

Regost C, Arzel J, Robin J, Rosenlund G, Kaushik SJ. 2003. Total replacemnt of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*). Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture* 217:465–482.

Rodriguez C, Perez JA, Badia P, Izquierdo MS, Fernandez-Palacios H, Lorenzo Hernandez A. 1998. The n-3 highly unsaturated fatty acid requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet. *Aquaculture* 169:9–23.

Sargent JR, Bell JG, Henderson RJ, Tocher DR. 1995. Requirement criteria for essential fatty acids. *Journal of Applied Ichthyology* 11:183–198.

Shapira N, Weill P, Sharon O, Loewenbach R, Berzak O. 2009. n-3 PUFA fortification of high n-6 PUFA farmed tilapia with linseed could significantly increase dietary contribution and support nutritional expectations of fish. *J Agric Food Chem* 57:229–2254.

Simopoulos AP. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomedicine & Pharmacotherapy* 60:502–507.

Tocher DR, Agaba M, Hastings N, Bell JG, Dick JR, Teale AJ. 2002. Nutritional regulation of hepatocyte fatty acid desturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). *Fish Physiology and Biochemistry* 24:309–320.

Tocher, D.R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research* 41:717–732.

Tokusoglu O, Unal MK. 2003. Biomass nutrient profiles of three microalgae: *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrisis galbana*. *J Food Science* 68:1144–1148.

Vanderlugt K, Cooney MJ, Lechner A, Lenz PH. 2009. Cultivation of the paracalanid Copepod, *Bestiolina similisi*. *Journal of the World Aquaculture Society* 40(5):616–628.

Watanabe T., Vassallo-Agius R. 2003. Broodstock nutrition research on marine finfish in Japan. *Aquaculture* 227:35–61.

Yone, Y. 1978. Essential fatty acids and lipid requirements of marin fish. Pp. 43–59 in Dietary Lipids in Aquaculture, Japanese Society of Scientific Fisheries. Tokyo, Japan: Koseisha-Koseik-Abu.