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Importance des acides gras dans la diète des Saumons d'Atlantique (*Salmo salar*) étudiés en milieu naturel.

par

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Importance des acides gras dans la diète des Saumons d'Atlantique (*Salmo salar*) étudiés en milieu naturel.

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Compte-tenu de l'impossibilité de donner aux jeunes saumons d'élevage une nourriture naturelle (insectes), les chercheurs se sont intéressés à trouver des aliments de substitut. Ils ont tenté de combler les besoins en protéines, en lipides, en vitamines et en minéraux obtenus dans la diète normale des poissons. Les moulés synthétiques, bien qu'ils favorisent la croissance des poissons d'élevage, n'assurent pas leur santé.

Des auteurs associent certains symptômes pathologiques chez le Saumon d'Atlantique (*Salmo salar*) et chez les autres salmonidés à des apports inappropriés de certains acides gras essentiels (AGEs) dont 18:3(n-3) et 18:2(n-6) et le rapport alimentaire de ces deux AGEs. Des études sur de nombreux poissons commerciaux marins ont apporté des informations pertinentes sur l'importance des AGEs dans l'alimentation.

Au cours des dernières années, il y eut de nombreuses tentatives pour expliquer les besoins en acides gras (AGs) des poissons, surtout chez le Saumon de l'Atlantique, et pour comprendre l'interaction entre les acides gras polyinsaturés (AGPI) et l'altération de la composition tissulaire en AGs par l'alimentation. Bien que le site actif de la synthèse lipidique soit majoritairement le foie chez les poissons et le tissu adipeux chez les mammifères, la biosynthèse (une voie enzymatique de désaturation/élongation) des acides gras est similaire.

Le rôle, la fonction et l'importance de ces lipides restent à étudier. Une connaissance plus approfondie de l'alimentation naturelle des saumons serait certainement plus désirable pour améliorer la biotechnologie d'élevage artificiel. Dans ce but, j'ai réuni la littérature sur le

métabolisme des AGPIs chez les saumons qui résume la source et la nature de leurs précurseurs et de leurs produits. Sans cette connaissance, le processus de biosynthèse des AGs et l'impact des AGEs apportés par l'alimentation naturelle des poissons sur la synthèse des AGPIs ne peut être comprise. J'inclus une analyse de la composition lipidique et des acides gras d'une variété d'insectes faisant partie de la diète des jeunes saumons afin de comprendre leurs influences sur la croissance de ces poissons. Peu d'études chez les insectes portent sur l'analyse d'AGs au niveau moléculaire. Ceci représente un désavantage si on cherche à associer leurs compositions en AGs avec la fonction métabolique chez les insectes. Néanmoins, l'interprétation et la compréhension de différents profils d'AG ont révélé certaines particularités dans la composition d'AGs chez les insectes. Les besoins métaboliques, caractéristiques pour chaque espèce, sont des facteurs majeurs dans la détermination de la nature et la diversité des AGs. De plus, le rapport entre les acides 18:3(n-3) et 18:2(n-6) présents chez les insectes tend à être sous la même influence. Finalement, les études sur la composition d'AGs chez les saumons seront utilisées afin de déterminer les AGs essentiels dans leurs tissus. Ceci permettra de mieux évaluer la valeur nutritive des AGs des insectes en fonction de leur impact sur les changements en AG des saumons.

Mots-clés: Salmonidés; Saumon d'Atlantique, Lipides, Acides Gras Essentiels, Insectes

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ABRÉVIATIONS

EFA	acides gras essentiels
FA	acide gras
HUFA	acides gras hautement polyinsaturés
Monoene	acides gras monoinsaturés
PUFA	acides gras polyinsaturés
LNA	acide linolénique
LA	acide linoléique
DHA	acide docosahexaénoique
EPA	acide eicosapentaénoique
PL	phospholipides
TG	triglycérides
TL	lipide total

Formule chimique C: x(n-i) exemple: 18:2(n-6)

C = le nombre d'atome de carbones composant l'acide gras;
x = le nombre de doubles liaisons carbone-carbone;
n = indique la présence d'au moins une double liaison;
i = correspond à la position d'un carbone avec une première double liaison compté à partir du méthyle terminal.

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INTRODUCTION ET PRÉSENTATION DU SUJET DE L'ARTICLE

Afin d'augmenter la production de Saumons d'Atlantique (*Salmo salar*) et d'autres salmonidés, différents types de programmes environnementaux tel la restauration d'habitats aquatiques naturels ont été entrepris (Roper *et al.*, 1997; Kauffman *et al.*, 1997). Les premiers programmes basés sur l'élevage artificiel avait laissé croire que la pisciculture pourrait augmenter la pêche de saumons (Netboy, 1980; Saunders, 1981). Des saumons de rivières et de ruisseaux furent donc mélangés avec des juvéniles nés et élevés artificiellement. On espérait qu'après avoir passé une partie de leur vie dans ces rivières, ces juvéniles migreraient vers la mer avec la population sauvage et reviendraient, à l'âge adulte, pour pondre. Cependant, alors que le programme cherchait à reduire la perte des poissons due à la destruction d'habitats et à une pêche excessive, il eut pour effet de diminuer les ressources naturelles de pêches (Hilborn, 1992; Utter, 1994). Il fut estimé que le taux de survie de ces animaux jusqu'à l'âge adulte n'était en général que de 10% et pourrait, sous des conditions optimales, atteindre un maximum de 45% (Wedemeyer *et al.*, 1980).

Un bon nombre de raisons peut expliquer ce faible taux de rendement des pisciculture dont le but étaient d'accroître les stocks naturels de poissons. Lors d'une croissance artificielle et rapide, il est fréquent de trouver des grandes proportions de jeunes mâles atteignant une maturité sexuelle précoce (Bailey *et al.*, 1980; Rowe and Thorpe, 1990; Saunders *et al.*, 1982, 1994). Bilton (1984) avait rapporté que les jeunes saumon coho (*Oncorhynchus kisutch*) provenant de pisciculture avaient une fréquence de maturité précoce assez élevée, même une fois relâchés dans la nature.

Cette maturité précoce est associée généralement à un faible taux de survie et une perte du nombre de mâles estimée à 60% dans la population des saumons (Myers, 1984). De plus, même si les jeunes saumons matures réussissaient à pondre, l'intégrité du pool génétique des poissons sauvages peut être compromise (Billington & Hebert, 1991; Waples, 1991; Evans & Willox, 1991; Kapuscinski & Hallerman, 1991; Hindar *et al.*, 1991).

L'introduction des saumons d'élevages en nature est parfois responsable de la réduction substantielle du nombre de saumons sauvages due à une prédation accrue, à la compétition ainsi qu'à la modification des habitats naturels (Krueger & May, 1991).

La haute densité de poissons dans les bassins d'élevages favorise l'apparition de maladies infectieuses qui peuvent être transmises à la population sauvage (Stewart, 1991; Hastein & Lindstad, 1991). Un manque de motivation chez les poissons élevés artificiellement et du comportement migratoire typique de ces poissons anadromes à retourner vers l'eau douce pour pondre pourraient expliquer la faible reproduction (Lund *et al.*, 1991).

Donc, la libération de juvéniles d'élevages de grand taille et apparemment en santé ne peut ni être bénéfique pour le milieu naturel ni contribuer à la pêche. Il peut être suggéré que l'échec de la pisciculture à produire de jeunes saumons en santé provient d'une absence de compréhension des influences environnementales et nutritionnelles affectant la physiologie et le comportement des saumons.

De nouvelles approches sont donc requises pour modifier la technologie dans la production de poissons. La création de conditions d'élevages en pisciculture similaires à celles que l'on retrouve dans l'écosystème naturel est d'une importance prioritaire. La réduction de la densité de saumons, le maintien de l'activité physique par la force des différents

courants d'eau (McDonald *et al.*, 1997), ainsi que la réduction de la compétition intraspécifique par l'élevage de deux espèces sont des facteurs amenant un taux de survie post-ensemencement plus élevé (Nortvedt & Holm, 1991). Le choix d'une alimentation naturelle au profit de l'alimentation commerciale pour ces poissons d'élevages avantagerait leur santé.

Le développement physiologique et somatique des jeunes saumons sauvages est déterminé génétiquement mais est sujet à des facteurs environnementaux: absence de nourriture, variété de nourriture, changements saisonniers, etc.. Les techniques d'élevages tendent généralement à maximiser le taux de croissance de saumons pré-sélectionnés en ne reproduisant pas des conditions environnementales comparables à celles retrouvées pour les individus sauvages en milieu naturel. Dans la préparation de nourriture artificielle, non seulement le manque d'énergie utilisable mais aussi son excès peuvent avoir d'importants effets sur la santé des poissons. Au lieu d'être utilisées pour la croissance, les protéines provenant de leur diète servent d'énergie quand celle-ci est déficiente. D'un autre côté, une diète trop riche en énergie peut restreindre la quantité de nourriture ingérée et prévenir la consommation adéquate en protéines et autres nutriments nécessaire pour une croissance optimale. De même si cette diète contient un rapport énergie/nutriment très élevé, elle peut causer une dépôt de grandes quantités de lipides dans les tissus (Shearer, 1994). Ceci peut toutefois être avantageux d'un point de vue marketing mais est indésirable pour les poissons destinés à la consommation humaine et pour ceux élevés pour être relâchés en nature (Ahlogren *et al.*, 1994). Donc, une connaissance inadéquate des besoins diététiques

pour le jeune saumon d'élevage peut être une cause majeure du faible développement des saumons.

Un aliment naturel composé de poissons, de crustacés, d'huile de poissons et autres représente une source excellente de nourriture pour les saumons. Il contient des lipides riches en acide gras (n-3) et faible en acide gras (n-6) qui sont appropriés pour leur diète. Toutefois, un approvisionnement incertain, la fraîcheur et le coût du matériel, le type et le niveau d'antioxydants ainsi que la manipulation et le stockage de ces produits aquatiques limitent l'utilisation de diètes artificielles. Ainsi, une grande variété, un faible coût et un accès facile sont la raison pour laquelle les plantes terrestres et les produits d'origine animale sont surtout utilisés. Bien qu'ils sont une source excellente en énergie et en acides aminés essentiels, ils ne sont pas adéquats en terme d'acide gras essentiels et ne complètent donc pas les besoins alimentaires (Tacon, 1994). Les lipides d'origine animale contiennent des acides gras saturés alors que les plantes terrestres ont des niveaux élevés en acide linoleïque (jusqu'à 40-60%) et acide oléique (jusqu'à 60%) (Price & Parsons, 1975; Yu & Sinnhuber, 1981; Grela & Günter, 1994). Ainsi une incorporation d'acides gras mono-insaturés et d'autres acides gras appartenant à la série (n-6), l'acide linoléique en particulier, ne peut complètement se substituée aux acides gras (n-3). Bell *et al.* (1994b) ont illustré les différences énormes dans la composition d'AG entre différents invertébrés qui constituent l'alimentation naturelle du saumon par rapport à leur substituts synthétiques d'élevages. Ils suggèrent que l'utilisation d'acides gras similaires à ceux retrouvés chez les invertébrés dont ils se nourrissent va promouvoir la croissance et prévenir des pathologies.

Ainsi, nous devons trouver des façons de préparer l'aliment afin qu'il ressemble à l'aliment naturel. Il est important que la composition des lipides dans l'alimentation artificielle (n-3), (n-6) et le ratio 18:3(n-3)/18:2(n-6) (acides gras essentiels (AGEs)) ressemble à la composition de la diète naturelle. Ceci aura une influence positive sur la croissance et sur la survie des saumons relâchés dans la nature.

L'objectif de cette étude est donc de ré-examiner le rôle des AGE dans la diète des saumons, particulièrement celui de l'Atlantique. Les points suivants seront abordés:

(1) Une détermination précise des besoins d'AGE sera tentée en se basant principalement sur les symptômes pathologiques des déficiences dues à ces AGE. Une emphase particulière sera mise sur les besoins alimentaires en acides 18:3(n-3) et 18:2(n-6). Non seulement les besoins absolus de chaque AGE seront examinés, mais l'équilibre optimal entre ces AGE également. Alors que cette recherche s'attardera davantage sur le saumon, des analyses comparatives seront faites avec d'autres poissons marins commerciaux (chapitre 3).

(2) Afin de comprendre les besoins lipidiques, l'implication d'AGE dans la synthèse de longs acides gras polyinsaturées (AGPI) sera étudiée. Aussi, des changements dans la composition en AGs du tissu qui sont influencés par le régime alimentaire seront expliqués (chapitre 4).

(3) Ceci nous amènera à un examen de la signification physiologique des AGPIs comme précurseurs pour la synthèse de molécules hautement actives biologiquement, tels les eicosanoïdes (chapitre 5).

(4) Finalement la composition de lipides totaux et des AGs des insectes sauvages constituant la diète des jeunes saumons sera examinée.

Cette observation sur la composition d'AGE chez ces insectes nous permet d'évaluer leur valeur diététique et leur influence sur la croissance des jeunes saumons (chapitre 6).

Dla Teresy, Joanny i Mateusza

Importance of fatty acids in the diet of Atlantic salmon (*Salmo salar*) studied in natural ecosystems

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Summary

In practice, it is difficult to supply natural food (insects) to young salmon reared artificially in aquaculture. Fish nutritionists have therefore been interested in finding suitable substitutes. They have given priority to meeting requirements for energy, protein, vitamins and major minerals. Such dietary strategies promote fast growth of the fish, but may not be optimal for their health. This may be explained by the fact that artificial diets have not met requirements in essential fatty acids (EFAs) such as 18:3(n-3), 18:2(n-6) which should be part of the diet formulation. Because they can exert beneficial effects on fish.

I have summarized the important points that relate specifically to pathological symptoms due to a lack of EFA and/or to inappropriate 18:3(n-3)/18:2(n-6) ratios used in juvenile Atlantic salmon and other salmonids species' dietary formulation. Although this summery will lead primarily to the role of EFA in salmon, other studies on other commercially important marine fish have also produced findings of major significance on the role of the EFAs in fish feeding. In recent years there have been increasing efforts to explain the requirements for specific fatty acid (FA) components. This in turn has lead to considerable efforts to understand the dynamic competitive turnover between individual physiologically important polyunsaturated fatty acid (PUFAs) (n-3) and (n-6) components, and changes in FA tissue composition which can be modified by dietary means. Although it has been determined that the active lipogenic site in fish is the liver rather than adipose tissue (unlike mammals), it is generally assumed that FA biosynthesis in freshwater fish proceeds via desaturation/elongation enzymatic pathways similar to those which operate in mammalian tissues.

The role, function and necessity of lipids is still not well understand, and an aquacultural breakthrough in the natural feeding habits of salmon is definitely desirable. I have therefore brought together earlier literature on PUFAs metabolism, and then summarize the source and nature of both its precursors and products. Without this knowledge the impacts of EFAs supplied in natural food on the PUFAs biosynthesis process in salmon cannot be understood. I also provide background information and discuss the significance of the total lipids in a variety of invertebrates which constitute part of the salmon natural diet and focus on the FA composition of the same invertebrates in relation to how it may influence the growth performance of young salmon. Relatively few of these studies have included analyses of fatty acids composition of all lipid categories in insects. This represents a disadvantage in any attempt to relate FAs function in insects and their FA composition. Nevertheless, my attempt to interpret and establish the meaning of a number of FA profiles in insects has revealed certain particular FA compositional distinctions. Metabolic requirement, which seems to be a species dependent characteristic, must be one of the major factors determining (a) occurrence of particular FA and (b) the relative proportion of 18:3 to 18:2. Finally, the studies covering FA composition of salmon have been interpreted to determine the desirable fatty acid profile, leading to an evaluation of the changes in the FA composition of salmon which can be attributed to the insect species in their diet.

Key words: Salmonids; Atlantic salmon; Lipids; Essential Fatty Acid (EFA); Insects.

1.0 Introduction

Different types of enhancement programs such as the restoration of natural aquatic habitats and their watersheds are currently being undertaken to increase the production of Atlantic salmon (*Salmo salar*) and other salmonids (Roper *et al.*, 1997; Kauffman *et al.*, 1997). The hatchery salmon programs established in the past led to the belief that aquaculture practices could substantially enhance the salmon fishery (Netboy, 1980; Saunders, 1981). Existing salmon-producing rivers and streams were thus stocked with hatchery-reared juvenile salmon. These juveniles, after spending a part of their life cycle in the streams, were expected to migrate to sea with the wild population and return as adults to spawn. Paradoxically, the hatchery perceived as a way to remedy the problem created by habitat destruction and overfishing, became itself a part of the problem and has been shown to have a negative effect on the natural fishery resources and was severely criticized (Hilborn, 1992; Utter, 1994). It has been estimated that survival rate of hatchery-reared salmon to adulthood under optimal conditions can reach a maximum of about only 45%, but it is usually less than 10% (Wedemeyer *el al.*, 1980).

A number of reasons could explain the low efficiency and poor performance of aquacultural programs which propagate "semidomesticated" juvenile salmon to enhance depleted native fish stocks. Under the accelerated development regimes of hatcheries, it is common to find high proportions of males maturing as parr (Bailey *et al.*, 1980; Rowe & Thorpe, 1990; Saunders *et al.*, 1982, 1994). Bilton (1984) has reported that the incidence of early maturation among large numbers of coho smolts (*Oncorhynchus kisutch*) increased enormously after hatchery release. This generally led to a low survival rate and a reduction in adult returns, causing an estimated 60% loss in the adult male population (Myers, 1984).

Furthermore, even if the young hatchery-reared fish succeed in spawning, the genetic integrity of natural stocks can be endangered (Billington & Hebert, 1991; Waples, 1991; Evans & Willox, 1991; Kapuscinski & Hallerman, 1991; Hindar *et al.*, 1991). Introduced salmonids are also sometimes responsible for a substantial reduction in native salmonid populations due to increased predation, competition and habitat modification (Krueger & May, 1991).

Also, the high occurrence of infectious diseases in farmed fish due to high fish densities in the hatcheries increases the probability of transmission of diseases to the wild stock (Stewart, 1991; Hastein & Lindstad, 1991). Finally, the low proportion of reared salmon inshore compared with that in coastal waters during the summer season is thought to be caused by a lack of motivation in farmed fish to enter freshwater to spawn or to the absence of migratory behavior typical of anadromous fish (Lund *et al.*, 1991). Thus, the release of large and apparently healthy hatchery-reared juveniles can be non-functional in a natural habitat, and their contribution to the fishery rather limited. It can therefore be suggested that failure to produce good quality smolts arises probably from the lack of understanding of the environmental influences and nutritional requirements affecting salmon physiology and behavior.

New approaches are required to modify the hatchery technology in fish management. An improvement and creation of aquaculture practices similar to the natural ecosystem is of primary importance in this case. Reduced fish densities and pre-release exercises with different flow regimes (McDonald *et al.*, 1997), as well as duoculture can lead to improved post-release survival (Nortvedt & Holm, 1991). Providing hatchery fish with some natural food may also be more effective than the exclusive use of defined diets.

In nature, the program of physiologic and somatic development of young salmon is genetically determined, but it is undoubtedly subject to environmental factors (e.g. lack of food, variety of food, seasonal changes, etc.). Hatchery practices generally maximize environmental advantages and minimize disadvantages, which in turn allow fish to reach greater growth rates than in the wild. In the diet formulation, not only dietary deficiency of useful energy but also its excess can have important effects on the fish health. Instead of being used for somatic development, dietary protein serves as energy when the diet is deficient. On the other hand, a diet containing an excess of energy can restrict food consumption and thus prevent the intake of the necessary amounts of protein and other nutrients for optimum growth. Excessively high energy/nutrient ratios can also lead to deposition of large amounts of body lipids (Shearer, 1994). This may be desirable from a marketing point of view, but it is undesirable in fish destined for human consumption and in fish raised for release (Ahlogren *et al.*, 1994). Thus, inadequate knowledge of the dietary-specific requirement of the young salmonids in aquaculture could be a major cause of the problems optimum development of salmon.

Aquafeeds (mainly fishmeal, fish oil, shrimp meal, etc.) represent an excellent source of nutrients for salmonids. They contain lipids rich in (n-3) FA and low in (n-6) FA, which are especially suitable for use in dietary formulations. However, cost, uncertain supply, freshness before and during processing, type and level of antioxidants, as well as handling and storage conditions of the aquafeeds limit the uses of artificial diets.

Therefore, wide variety, low cost and relatively easy access are the reasons why terrestrial plants (soybean, corn, wheat, etc.) and animal by-products are extensively used in feeds. Although they are excellent and rich sources of energy and essential amino acids, they are

not adequate to meet the essential fatty acids requirements (Tacon, 1994). Animal lipids contain highly saturated fat, while terrestrial plants have high levels of linoleic acid (18:2(n-6) (up to 40-60%) and oleic acid (18:1(n-9) (reaching up to 60%) (Price & Parsons, 1975; Yu & Sinnhuber, 1981; Grela & Günter, 1994). Thus, the incorporation of monoene and other fatty acids belonging to the (n-6) series, particularly linoleic acid, in salmonid artificial diets cannot completely substitute important (n-3) series components. Bell *et al.* (1994b) have illustrated the enormous differences in FA composition between various freshwater invertebrates which constitute the natural food of salmon parr and their farming food substitutes. They suggest that the use of dietary FAs similar to the FAs of freshwater invertebrates will promote growth and prevent pathological conditions. Therefore, efforts should be made to formulate feeds that mimics that found naturally. It is important that the lipid composition of artificial feeds, especially the (n-3) and (n-6) FAs and dietary specific 18:3(n-3)/18:2(n-6) ratio, closely resemble the composition of natural diets. This would have positive influences on the growth and survival of post-released salmonids.

The objective of this thesis is to review the role of EFAs in the diet of salmonids, particularly in Atlantic salmon. The research is organized as follows:

(1) On the basis mainly of the pathological symptoms of EFA deficiencies, an accurate determination of EFA requirements will be attempted with special emphasis on 18:3(n-3) and 18:2(n-6) acids. Not only will the absolute requirements of each EFA be established, but also the optimal balance between individual EFA components. Although the research is focused primarily on salmon, comparative data on other commercially important marine fish will complete the survey (chapter 3).

(2) In attempting to understand the requirements for specific lipid components, the involvement of individual EFAs in the synthesis of longer polyunsaturated fatty acids of physiological significance will be examined. Also, the changes in composition of fatty acids tissues which can be influenced by diet will be discussed (chapter 4).

(3) This will lead to an examination of the nature of the physiological significance of these longer polyunsaturated fatty acids as precursors for the synthesis of highly biologically active molecules such as eicosanoids (chapter 5).

(4) Finally in order to relate these observations to the field situation, the total lipid and FAs compositions of the natural (invertebrates) diet of young salmon will be examined. The observations are made emphasizing the EFAs composition of the same invertebrates in relation to how it may influence the growth performance of young salmon (chapter 6).

2.0 Methods

Data collected from the literature on lipid composition of insects is derived from whole body total lipid extracts. It should be noted that many of these studies did not explicitly state the extraction methods used nor the nutritive status of the insects. This limited the scope and depth of the analysis.

Variations in lipid content of insects can be attributed to the variety of extraction methods used, as well as to a wide variety of physiological and ecological factors, which influence each organism differently, sometimes making comparisons very difficult. Nevertheless, I believe that the information compiled allows the possibility of making a few general and important conclusions concerning the total lipid content of insects. In addition, this review may also be useful as a general reference, for estimations or for comparative purposes.

Characteristic fatty acid patterns emerge from analyses of whole insect body tissues. These are sometimes difficult to interpret because they mostly represent the lipid composition from an amalgam of all tissues. However, the exceptionally high occurrence of some individual fatty acids in body tissue can sometimes serve as a useful indicator of the biochemical particularity of insect lipids and can also provide an interesting indication of the nutritional value of this natural food for salmonids.

Linear regression analysis and the Wilcoxon-Mann-Whitney U-test were carried out on the data. Significant differences ($p < 0.0001$) were found between the orders for all the fatty acids. Comparisons were not carried out at the family level due to a lack of sample species in many of the families. For the statistical methods used to show the fatty acid compositional patterns, all available (from the same source) data were included.

3.0 Fatty acid requirements of fish

There has been a considerable increase of interest in cultured salmon and other commercial species for both human consumption and their release to enhance or restore natural fish stock. For this reason, a number of nutritional studies carried out under culture conditions have pointed out the important metabolic role of lipid and other (caloric, protein, vitamins, etc.) nutrient requirements for growth, survival and metamorphosis of salmonids (Halver, 1972; Cowey & Sargent, 1979; Cowey *et al.*, 1985; Lovell, 1989). Dietary requirements have also been elaborated by The National Research Council (1973, 1981). Aside from being a concentrated source of energy, the interest in lipids can be attributed to the fact that fatty acids are involved to a number of physiological functions, as structural elements in membrane bilayers and are precursors of active bio-molecules (eicosanoids)). These roles in fish have been studied and reviewed at length (Halver, 1972; Cowey *et al.*, 1977; Cowey & Sargent, 1979; Watanabe, 1982; Cowey *et al.*, 1985; Bell *et al.*, 1986; Henderson *et al.*, 1987; Lovell, 1989; March, 1992). Most previous studies described the role and dynamic nature of lipid composition affected by physiological and/or environmental conditions to emphasize the importance of the PUFA content of the diet. Here the determination of salmon nutrient needs will be to assess their EFA dietary optimal ratio. Although this work is mainly concentrated on salmon, the significance of EFAs and the characteristics of fatty acid deficiency will also be discussed below in regard to other related salmonid species. Work done on marine fish will also provide additional understanding of (n-3) and (n-6) FAs in fish feeding.

3.1 *Fatty acid composition in wild and hatchery-reared Atlantic salmon*

The failure to produce healthy juvenile salmon under aquaculture conditions is due in part to the use of inappropriate feed formulations that fail to provide the necessary overall nutrient profile, including all specific essential nutrients. The poor understanding of dietary fatty acids, for example, can be one of the causes of physiological deficiency in fish and of the appearance of pathological symptoms, which could in turn lead to poor growth and reduction of natural fish survival.

Ackman *et al.* (1986), while investigating the cause of a high incidence of dorsal, pectoral and caudal fin erosion in cultured salmon smolts, reported a distinctly different fatty acid composition in farmed salmon smolts compared to that in wild smolts (Table 1). The mean percentage of Σ (n-3) highly unsaturated fatty acids (HUFAs) (sometimes referred to as longer PUFAs, indicating C-20 and C-22 FAs), mainly 20:5(n-3) (EPA), 22:5(n-3) and 22:6(n-3) (DHA) in wild smolts constituted over 27% of total body fatty acids, while they represented no more than 15% in farmed salmon. Van Vliet *et al.* (1990) observed a similar high occurrence of Σ (n-3) FA relative to Σ (n-6) FA in wild *Salmo salar*, up to 2 to 3 times more than in cultured fish. Ackman and his co-worker have also reported a lower percentage of 18:2(n-6) (LA) in wild salmon than in the hatchery reared forms. The latter had 2 to 3 times more linoleate (18:2n-6) content in various lipid categories (phospholipid (PL), triacylglycerols (TG) and total lipid (TL)). The monoene acids accounted for 50% in farmed and 27% in wild salmon. The occurrence of monoene acids and LA in hatchery-reared salmon reflects their rich vegetable oil-based diet. The high amounts of LA and monoene FA found in hatchery reared smolts is due to an inefficiency of the desaturation/elongation enzyme system in synthesizing the longer unsaturated acids. The

wild form had a content 3 times higher of both 18:3(n-3) (LNA) and 20:4(n-6) (AA) FA in PL, TG and TL than the farmed one.

In nature, the wild salmon diet has an approximate 2:1 ratio of 18:2(n-6) to 20:4(n-6) (Hanson *et al.*, 1985). Therefore, it appears that wild fish accumulate 20:4(n-6) in body tissues selectively. Thus, a high content of arachidonic acids (20:4(n-6)) in fish tissues could be positively correlated with a good healthy growth and survival of young fish.

3.2 *(n-3) and (n-6) series of fatty acid requirement in salmonids*

The excessive consumption of artificial vegetable-based diets with high levels of oleic acid (OA 18:1(n-9)) (rather than diets containing fishmeal or other balanced supplement source of oil) resulted in high concentrations of oleic acid and lower concentrations of suitable PUFAs in tissue phospholipids. Skonberg *et al.* (1994) determined the fatty acid profiles of coho salmon and rainbow trout muscles: the fish were fed diets containing either herring oil or sunflower oil (high in monounsaturated and (n-6) FA). After 4 weeks, the muscles from fish fed the sunflower oil diet supplement contained twice the amount of 18:1(n-9) and approximately 65% of the HUFAs (20:4n-6, 20:5n-3, 22:6n-3) of the muscles of fishes fed herring oil. Bell *et al.* (1991) ascertained that *Salmo salar* heart and liver phosholopids had high levels of Σ (n-6) FA and low levels of 20:5(n-3) after 16 weeks of continued feeding on a diet containing the sunflower oil. This reflected high 20:4(n-6)/20:5(n-3) ratios which occurred most notably in phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE). Extended periods of feeding on this type of diet damaged the heart tissue (by thinning of the ventricular wall and by muscle necrosis), and caused a ~30% mortality due to shock syndrome. Thus, a diet containing low (n-3)/(n-6) ratio can be detrimental to salmon

health. On the other hand, a diet high in marine oils tended to produce lesions in Atlantic salmon. The coronary arteriosclerosis lesions seen in salmon do not contain lipid deposits. They are rather a collection of smooth muscle cells which have invaded the lumen and block the artery. Apparently, maturation and diet seems to have a significant effect on lesion incidence (Farrell *et al.*, 1986).

In practice, aquaculture nutritionists have to recognize the presence of an optimum level of lipids for fish feed, but it is important that lipid supplement must be always balanced in essential fatty acids. The importance of the (n-3) and (n-6) series of fatty acids was recognized by Burr *et al.* (1929, 1930) who first coined the name ''essential'' fatty acid and pointed to the importance of lipids for normal physiological function in animals as a result of feeding rats for several months on a fat-free diet.

For the two independent series of fatty acids, (n-3) and (n-6), the parent compounds are linolenic acid (18:3(n-3)) and linoleic acid (18:2(n-6)) respectively. Both are known to be essential for normal cell function. They cannot be synthesized *de novo*. As a result, these two acids must be supplied in the diet. Although 18:3(n-3) and 18:2(n-6), both polyunsaturated fatty acids, are the main fatty acids in the diet, their metabolites play more important roles and are metabolized by the same enzyme sequences (Fig. 1). Each fatty acid has a specific function, although the most important metabolites are long-chain highly unsaturated fatty acids, the dihomogamma-linolenic (DGLA, 20:3(n-6)) and arachidonic (AA, 20:4(n-6)) acids of the (n-6) series and eicosapentaenoic (EPA, 20:5(n-3)) and docosahexaenoic (DHA, 22:6(n-3)) acids of the (n-3) series (Singh *et al.*, 1976; Stacey, 1981; Holland *et al.*, 1985; Greene *et al.*, 1987; Tocher *et al.*, 1987). For the majority of terrestrial herbivores and omnivores, the requirement for (n-3) FA is much lower than that

for the 18:2(n-6) which is an essential element of the diet, acting much as 18:3(n-3) does in fish (Tinoco, 1982). However, production of longer polyunsaturated fatty acids from 18:2(n-6) appears to be limited in predators. Thus, felines mostly require longer (n-6) FA in their diet, such as 20:4(n-6) acid (Rivers *et al.*, 1975; Sinclair *et al.*, 1979; Pawlosky & Salem, 1996).

The dietary intake of both C-18 EFAs and the analysis of the extent of their bioconversion products show an efficient incorporation of C-20 and C-22 HUFAs into the phospholipid pool of salmonid tissues (Leger *et al.*, 1981; Fremont *et al.*, 1981; Hagve *et al.*, 1986; Henderson *et al.*, 1987; Turner *et al.*, 1989; Tocher *et al.*, 1990; Sowizral *et al.*, 1990). These findings indicate that salmonids do not accumulate dietary C-18 EFAs in their tissues without bioconverting them into longer chains of unsaturated fatty acids (C-20 and C-22 FAs).

The C-18 EFAs requirements of salmonids have been studied thoroughly. Castell *et al.* (1972a,b,c) have studied the nutritional aspects of EFAs in salmonids. They used trout to investigate the influence of four different diets: one fat-free diet and three others containing C-18 chain acids representing 18:3(n-3), 18:2(n-6) and 18:1(n-9) acids. Trout on fat-free and low (n-3) FA diets showed poor growth, and low feed conversion. They exhibited eroded tail fins and some of the tail fins were completely lost.

Symptoms such as swollen and pale livers, unusual heart conditions (such as enlarged heart with protuberances on the surface and acute local myocarditis) all appeared at the same time. Long term deprivation of EFAs in these salmonids additionally led to shock syndromes which were manifested in rapid swimming motion leading to unconsciousness, floating and sinking to the bottom. Adding oleic acids (18:1(n-9)) to the diet increased the

growth rate, however not substantially. The addition of 18:2(n-6) to the diet of the fish with swollen livers reduced the symptoms only by half. The return of the liver to a normal state resulted after an addition of 18:3(n-3) to the diet. Diets containing predominantly 18:3(n-3) provided the greatest growth rates, higher weight gains and low mortality rates. These experiments demonstrate that linolenic acid (1-2% of total FA) is the principal essential fatty acid to the diet to ensure the normal development and an optimum growth of the early fish stages.

Using the same experimental fish (trout), Yu & Sinnhuber (1972, 1975, 1976) confirmed the essential role of (n-3) FA in the fish diet. The high (n-3)/(n-6) ratios led to optimal fish growth and confirmed also that coho salmon (*Oncorhynchus kisutch*) performed better when fed with a diet containing 1% to 2.5% linolenate. However, food conversion and growth were depressed when the linoleate exceeded more than 1% in diet (Yu & Sinnhuber, 1979). Similarly, Watanabe (1982) pointed to a sensitivity of chum salmon (*Oncorhynchus keta*) to a diet deficient in EFAs. A diet supplemented with 1% of 18:3(n-3) and 1% of 18:2(n-6) produced the best weight gains and feed efficiency, without any of the EFA-deficiency symptoms typical for salmonids. Yang *et al.* (1994) confirmed that the EFAs requirements of arctic char (*Salvelinus alpinus*) are similar to those of trout. The *Salvelinus alpinus* 18:3(n-3) dietary requirements were higher and ranged from 2 to 2.5%. A diet containing more than 2.5% of 18:2(n-6), when fed to the arctic char, had negative effects on the digestibility of dietary amino and fatty acids. A smaller percentage of 18:2(n-6) in the diet (0.7-1%) had no adverse effects on fish growth.

3.3 *Fatty acid composition in freshwater and marine fish*

Fatty acid profiles of marine fish are usually characterized by a high content of long chain polyunsaturated fatty acids. The composition of longer PUFAs in marine organisms usually is dominated by the 20:5(n-3) and 22:6(n-3), which normally account for over 60% of the total HUFAs. The content of the Σ (n-3) series of fatty acids in freshwater fish ranges from 25 to 45% of the total body fatty acids with about 50% of 20:5(n-3) and 22:6(n-3) together (Henderson *et al.*, 1987). Freshwater organisms also contain relatively higher levels of both 18:3(n-3) and 18:2(n-6) compared to marine fish, suggesting a basic difference in dietary availability of these two acids (Ackman, 1967; Exler *et al.*, 1975). The Σ (n-6) series of fatty acids is more abundant in freshwater fish than in marine fish. The relative proportion of the (n-3) series of FAs to (n-6) is very low in freshwater fish, ranging from 0.5 to 3.9, in comparison to 4.7 to 14.4 found in marine fish (example is shown in Table 2) (Ackman, 1967; Kinsell *et al.*, 1977; Henderson *et al.*, 1984; Jahncke *et al.*, 1988). Gunstone *et al.* (1978), while comparing the fatty acid compositions of marine and freshwater fish, report that lipid content in saturated acids (16:0 and 18:0) was relatively lower and ranged from 17% to 19% for marine fish and up to 30% for freshwater fish. These compositional differences between marine and freshwater organisms have been attributed mainly to a varying diet intake (Gruger *et al.*, 1964; Ackman, 1967; Saddler *et al.*, 1972; Ackman *et al.*, 1994). These differences are apparent even within a given species. Salmon have two life stages: a freshwater and a marine stage. Many salmonids also have a landlocked form which remains in freshwater. Ozawa *et al.* (1993) have shown an evident difference in body fatty acid composition between the marine and freshwater forms of sockeye salmon

(*Oncorhynchus nerka*). Their findings confirm the general pattern, in which the landlocked form of sockeye has much lower (n-3)/(n-6) ratios compared to the marine forms.

3.4 *HUFA requirement in marine fish*

The ability to modify exogenous FAs in aquatic animals varies from species to species. Kanazawa *et al.* (1979) designed an experiment to compare the efficiency of enzymatic bioconversion of radioactive 18:3(n-3) acids among different fish species. Injections of 18:3(n-3) acids into the abdominal cavities of rainbow trout showed clearly that this fish has the ability to metabolize exogenously added 18:3(n-3) acid. This experiment demonstrated that *Plecoglossus altivelus* and *Anguilla japonica*, both freshwater species, do not convert efficiently 18:3(n-3) to longer PUFAs (20:5(n-3), 22:5(n-3) and 22:6(n-3)). The relative percentage of incorporation of these acids into body tissues (referenced to the rainbow trout as a 100%) were 36% and 20%, respectively.

For the marine fish, the bioconvertion of 18:3(n-3) acid to their corresponding longer acids was considerably lower: 7% for *Chysophrys major* and 15% for *Sebastiscus marmoratus*, (the trout being at 100%). Similar results obtained by Yamada *et al.* (1980) equally confirm the poor bioconversion of ^{14}C 18:3(n-3) to 22:6(n-3) in the bodies of marine fish, where the recovery of radioactive hexaene was from 1% to 5%, relative to rainbow trout which ranged from 4% to 15%. These studies show the inability of marine fish to desaturate and elongate C-18 EFAs into longer PUFAs at the same substantial and sufficient rates as the salmonids. This indicates that dietary fatty acid requirements are critical for marine organisms. Thus, their growth could not be enhanced by C-18 (n-3) nor by the (n-6) series

of fatty acids. Consequently, these C-18 fatty acids have low dietary value compared to longer chain unsaturated acids.

Cowey *et al.* (1976) have investigated the fatty acid composition of turbot (*Scophthalus maximus*) tissues and their pathological changes under different dietary oil supplements. They found that diets containing fatty acids from cod liver oil (rich in long chain PUFAs) satisfied the fatty acid requirements of the turbot. Turbot raised on the same oil supplements also showed better weight gains compared to fish fed on corn and hydrogenated coconut oil. Pathological anomalies, such as loss of adipose tissue integrity, ruptured cell membranes, and increased vascularization, occurred in turbot given hydrogenated coconut oil, while limited symptoms were observed in those fed the corn oil diet. In addition, the body tissues which had high concentrations of 18:2(n-6) in both triglycerides and phospholipids fractions demonstrated poor conversion of this acid into 20:4(n-6) and into other longer (n-6) series of FA homologues. However, in spite of different dietary treatments, the proportion of longer (n-6) PUFAs was similar with low levels of 20:4(n-6) in tissues of any of those fish.

Metabolic deficiency occurred in marine turbot when fed with large amounts of 18:3(n-3) and 18:2(n-6) acids. These acids were incorporated without modification into the body tissue. The enzymatic incapacity of this marine fish was caused by the low efficiency of the desaturation/elongation system in utilizing dietary 18:3(n-3) and 18:2(n-6) acids (Tocher *et al.*, 1989; Owen *et al.*, 1975). Thus, marine fish are unable to utilize C-18 PUFAs as essential fatty acids in order to convert them into highly unsaturated fatty acids even though this fish exhibited Δ 6 desaturase activity. This can be ascribed to a lack of active Δ

5 desaturase, an enzyme necessary for the biosynthesis of highly unsaturated acids (Tocher & Sargent, 1990; Mourente & Tocher, 1992; Tocher, 1993).

Salmonids require 18:3(n-3) and 18:2(n-6) FAs for good growth, but these fatty acids were found to be non-essential for turbot and other marine predatory fish. The (n-3) series HUFAs, especially 20:5(n-3) and 22:6(n-3) FAs, are of greater importance for this species (Watanabe, 1982; Ibeas *et al.*, 1994; Bell *et al.*, 1996a,b; Izquierdo, 1996). It seems though that the nutritional value of 22:6(n-3) may have a greater influence on growth and reproduction for marine organisms than 20:5(n-3). The ^{14}C-labelled study of extracellular fatty acid intake shows that the turbot have the ability to convert 20:5(n-3) into 22:6(n-3) acid (Linares *et al.*, 1991). However, endogenous enzymatic biosynthesis of 22:6(n-3) from 20:5(n-3) in turbot tissues is slow and is limited by insufficient levels of 20:5(n-3), which are necessary for adequate enzymatic bioconvertion (Mourente & Tocher, 1992; Tocher, 1993).

Essential fatty acid requirements of turbot and other marine fish are not satisfied only by the presence of these (20:5(n-3) and 22:6(n-3)) acids: they also require the appropriate dietary ratio. Bell *et al.* (1985a,b) recorded several signs of nutritional deficiency symptoms in turbot reared on diets with a negligible level of (n-3) HUFAs. Turbot fed diets containing high 20:5(n-3)/22:6(n-3) ratios (13.8) also had lower growth and high mortality rates. A marked reduction in mortality rates was observed when the 20:5(n-3)/22:6(n-3) ratio was 2.2 with 1.3% of Σ (n-3) PUFAs in diet. Feeding experiments using artificial diets by Furuita *et al.* (1996) on yellowtail larvae (*Seriola quinqueradiata*) have also shown that 22:6(n-3) given as a supplement with 20:5(n-3) decreases the mortality rates and improves the overall health of fish compared to those that were given 20:5(n-3) alone. Nutritional

studies carried out by Ibeas *et al.* (1997) and Rodriguez *et al.* (1997) pointed out the importance of different dietary 20:5(n-3)/22:6(n-3) ratios influencing the growth performance of the gilthead seabream (*Sparus aurata*). Favorable 20:5(n-3)/22:6(n-3) ratios considerably enhanced fish growth, but the ratios varied for different stages of gilthead seabream development. For example, growth performance was improved when the dietary 20:5(n-3)/22:6(n-3) ratios for larval and juvenile forms were respectively 1/1.3 and 2/1.

The inability of turbot and other marine fish to efficiently convert C-18 PUFAs into longer C-20 and C-22 FAs is most probably related to the biochemical composition of natural diets. Thus, an evolutionary break of the enzymatic bioconversion pathway to form HUFAs by loss of Δ 5 desaturation activity may not have been necessarily disadvantageous for marine fish in their natural habitat. Their prey contain and supply constant levels of highly polyunsaturated chains of fatty acids (20:5n-3 and 22:6n-3).

4.0 **The effects of dietary FAs upon lipid metabolism**

The dietary requirements for C-18 EFAs as well as their bioconversion into the longer C-20 and C-22 FAs cannot be determined and understood until the interaction between the (n-3), (n-6) and monoene fatty acids and the importance of enzyme such Δ 6 desaturation are defined.

The biotransformation of polyunsaturated fatty acids in animal tissues is produced by desaturations and elongations of FAs provided in the food or already formed in body previously. The bioconversion of FAs is regulated through competitive interactions

between almost all (n-3) and (n-6) FAs for the enzymes involved in the bioconversions process. In spite of the different positions of the double bonds in (n-3) and (n-6) series of FAs, the new molecule formed always retains the same number of carbon atoms from the methyl end to the nearest double bond in the respective series of FAs.

4.1 *Site of fatty acid synthesis*

Radiotracer studies involving the injection of radiolabeled acetate or fatty acids into fish have shown that the liver is an important site for fatty acid synthesis. Lin *et al.* (1977a,b,c) have investigated the activity of several lipogenic enzymes (fatty acid synthetase, citrate cleavage enzyme, malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP-isocitrate dehydrogenase) involved in the fatty acid synthesis in coho salmon (*Oncorhynchus kisutch*). They found that these lipogenic enzymes, in contrast to mammals, display little activity and are up to 20 times lower in mesenteric adipose tissue than in the liver.

Changes in the diet (e.g. increased lipid content) depressed the activity of enzymes in the liver, particularly the ATP citrate lyase. In contrast, the consumption of low fat diets increased the rate of fatty acid synthesis in fish liver up to 46%. However, unlike mammals, the lipogenic enzymes in the adipose tissue of fish did not respond to diet manipulations. Moreover, the wet weight of the liver generally remained unchanged whereas the weight of mesenteric adipose tissues increased proportionally to the intake of dietary lipid. Henderson & Sargent, (1981) confirm that the liver exhibits a substantial capacity for *de novo* synthesis of fatty acid and that adipose tissue can store triacylglycerols. When the rate of fatty acids synthesis incorporated into trout triacylgycerols was expressed in terms of DNA

(as an index of cell number), the FA synthesis in liver and adipose tissue was found to be similar. Although DNA content in adipose tissue was low per unit weight compared to the liver, the liver was a more productive lipogenic site than the adipose tissue.

4.2 *Effect of dietary (n-3) and (n-6)FAs on fish tissue lipids*

The literature is extensive concerning changes in tissue lipid content due to alteration of dietary lipids.

Diet is an important factor affecting greatly the fatty acid composition of membrane lipids and cell function (Innis & Clandinin, 1981a,b). Considering the fact that physical and structural properties of FA vary with chain length and degree of unsaturation, it can be expected that fatty acid compositional changes may affect the structure as well as a function of the membrane. The modulation of membrane fatty acids such as 20:3(n-6), 20:4(n-6), 20:5(n-3) and 22:6(n-3), which theoretically increase membrane fluidity, can alter the local microenvironment and thus may affect cellular function. For example, such changes can influence membrane-mediated cellular function including carrier-mediated transport, ions channels, activity and properties of membrane-bound enzymes, processes of phagocytosis, endocytosis, exocytosis, and consequently cause several adverse changes in the cell biology (Spector & Yorek, 1985). However, the specific effects of membrane FA modification on cell structure and function are very complex and vary from one cell type to another (Stubbs & Smith, 1984). The changes of the cell phospholipid bilayer dynamic are regulated by the availability of substrate and the capacity to biotransform dietary EFAs into longer unsaturated acids. The incorporation of dietary fatty acids into the phospholipid

membrane is competitively regulated at the cellular level by elongation and desaturation enzymes located in the endoplasmic reticulum membrane (Lands *et al.*, 1990).

Alternations in the composition of fatty acid are usually more pronounced at the body's PUFAs level. The presence or addition of saturated fatty acids (SFAs) in a diet exerts little effect upon fatty acid composition of fish tissue. For example, Stickney & Andrews (1971) observed that high SFA diets do not affect the concentration of total SFAs in fish body lipids. The level of saturated acids of two groups of catfish (*Ictalurus punctatus*) maintained at different water temperatures for 10 weeks and fed diets containing different lipid supplements (25% and 42% of SFAs) was relatively constant at ~20% of total body lipids. High levels of (n-3) and (n-6) series of FAs occurred in catfish carcasses and livers, only when the dietary sources of those fatty acids were provided. High (n-3) and (n-6) FAs diets not only increased the amount of these acids in the fish body but also reduced the levels of fatty acids belonging to the (n-9) and (n-7) series. An absence of (n-3) and (n-6) FAs in the diet results in an increase of the level of (n-9) FA and in a reduced interaction of other competitive substrates ((n-3) and (n-6)) FAs for enzymatic desaturation sites. The fish fed beef tallow (rich in saturated and monounsaturated fatty acids of 42% and 47% respectively) had high tissue concentrations of Σ (n-9) FA, which were particularly pronounced in the livers.

Yu *et al.* (1977) had similar results experimenting with trout. The diets containing 22% of herring oil (control) were replaced by varying proportions of lard (2 diets: 33% and 50%). The level of total SFA in the bodies of fish was constant at ~24% in all dietary groups (including control). The authors also provided interesting evidence for dietary FA modulation. The concentration of dietary SFAs was directly proportional to the level of

18:1 in fish body lipids, even though the concentration of dietary (n-3) and (n-6) series of FAs was inversely proportional to the concentration of 18:1 deposited in the fish body. These observations indicate that the fatty acid composition in fish reflects the FA composition of their diet, particularly the diets composed of unsaturated acids.

The maintenance of proper levels of SFAs in fish body tissue is regulated mainly through the β-oxidation and dehydrogenation of the Δ-9 position to form monoenoic acids. Since the unsaturated fatty acids are carried in large part at the *sn*-2 position, enzymes such as phospholipase A_2 showing specificity for the *sn*-2 position have an important role to play in the regulation of this substitution (Lehner & Kuksis, 1996).

4.3 *Desaturation of (n-3) and (n-6) FAs*

Some experiments *in vivo* have shown that fish are capable of modifying exogenous FAs and of synthesizing longer carbon chain acids in a way similar to that of mammals (Ninno *et al.*, 1974; De Torrengo & Brenner, 1976; Henderson & Tocher, 1987).

The unsaturated C-18 (n-3) and (n-6) EFAs can be metabolized by desaturation and elongation reactions to produce a variety of highly unsaturated fatty acid homologues. However, the elongation and desaturation pathways of dietary FAs in fish differ from those in mammals in that there is a greater importance in fish of dead-end elongation products such as 22:3(n-6), 20:2(n-6) and 20:3(n-3) acids. It has been suggested that such dead-end elongation products (which normally are not present in large amounts) are incorporated into triacylglycerols, then stored and later rapidly liberated for retroconversion by the desaturating and chain-elongating enzyme system. This indicates that these elongation

products are not preferred elements of phospholipid membrane components (Hagve *et al.*, 1986).

Sprecher & Lee (1975) established that dietary 18:2(n-6) and 18:3(n-3) metabolic biosynthesis pathways involved the same enzyme systems which follow via an alternating series of 6-, 5-, and 4- position-specific desaturase. Voss *et al.* (1991, 1992), who examined the same pathway of the polyunsaturated fatty acid biosynthesis in the rat liver, have shown new metabolic biosynthesis pathways to form 22:6(n-3), by replacing the hypothetical Δ 4 desaturase. It is also apparent that once 22:6(n-3) is synthesized, it may serve as a substrate for retroconvertion and give rise to 20:5(n-3) via β-oxidation, and then serve as a precursor for 22:6(n-3) biosynthesis. It was confirmed that the conversion pathway of 20:5(n-3) follows a first elongation into 24:5(n-3), then a Δ 6 desaturation into 24:6(n-3), and finally a chain shortening into 22:6(n-3) (Mourente, 1996) (Fig. 1).

4.4 *(n-3) and (n-6) FAs metabolic interactions*

Salmonids, like other vertebrates, do not possess the Δ 15 and Δ 12 desaturase enzymes necessary for the synthesis of 18:3(n-3) and 18:2(n-6) (both EFAs). As a result, these EFAs or their longer chain derivatives must be obtained from the diet. It is generally accepted that the substrate preference of the Δ 6 desaturase is the existence of a double bond between the C9-C10 positions of the hydrocarbon chain as well as C12-C13 and C15-C16. Thus, each type of C-18 FAs (18:3(n-3), 18:2(n-6), 18:1(n-9)) can interfere with the metabolism of the other. It seems that competition for the desaturating Δ 6 site increases with the number of double bonds. Studies on diets containing various ratios of different families of C-18 fatty acids have demonstrated that 18:3(n-3) is more effective in the

competition for desaturation and elongation enzymes than are the 18:2(n-6) and 18:1(n-9) acids. The activity of Δ 6 desaturase for the substrate (n-9) became specific unless the animal was maintained on a diet lacking in essential fatty acids. Like mammals, fish demonstrate a sequence of Δ 6 desaturase activity in the following order of reactivity: 18:3(n-3), 18:2(n-6) and finally 18:1(n-9) acid. Since these three acids are substrates for the liver microsomal Δ 6 desaturase, they react competitively when included in the diet (Brenner & Peluffo, 1966, 1969; Brenner, 1971; Henderson & Tocher, 1987). Thus, this competitive interaction plays an important role in the regulation of polyunsaturated fatty acids biosynthesis in organisms.

The effects of competitive interaction of unsaturated fatty acids are also observed at the Δ 5 desaturation step where a new double bond is introduced at position 5 in the direction of the carboxyl group. Ullman & Sprecher (1971) measured the *in vitro* effects of 18:2(n-6), 20:3(n-6) and 20:4(n-6) as potential inhibitors of the desaturation of the (n-9) (stearic, oleic, and eicosa-8,11-dienoic) series of acids in a comparative feeding study. All three (n-6) FAs were found to inhibit the desaturation of stearic acid (18:0). 18:2(n-6) as well as 20:3(n-6) effectively inhibited the conversion of 18:1(n-9) to 18:2(n-9) acid. However, the desaturation rate of 18:1(n-9) was not significantly altered by 20:4(n-6). Since both C-18 EFAs were desaturated by the same enzyme that introduced a double bond at position 6, it was surprising to find that only 18:3(n-3) inhibited the desaturation of 20:2(n-9) ,while 18:2(n-6) did not.

Sprecher (1974) demonstrated a similar specificity of Δ 5 desaturase for the C-20 FAs competitive substrate (20:3(n-9) and 20:4(n-6)) in feeding experiments with rats, whereas 20:4(n-6) was preferentially incorporated into membrane lipids. The biosynthesis of

unsaturated fatty acids in liver microsomes can by regulated by the competitive inter-relationships of fatty acids, which are members of the same FA series. In studying the conversion of (n-6) FA, Brenner (1969) has shown that the Δ 5 desaturation rate of 20:3(n-6) to 20:4(n-6) is higher than Δ 6 desaturation of 18:2(n-6) to 18:3(n-6) acid. However, the extent of conversion depended upon the initial concentrations of the fatty acids incubated. The 20:3(n-6) acid (a higher member of the (n-6) series of FAs) inhibits 18:2(n-6) desaturation to 18:3(n-6) acid. The conversion of 20:3(n-6) to 20:4(n-6) is also depressed by increasing the concentration of 18:2(n-6) substrate. Thus, regulatory mechanisms in the synthesis of both (n-3) and (n-6) series of FAs may be inhibited at the first step by the products of Δ 6 desaturation reactions, by the acids as intermediate members of the series, as well as by the highly unsaturated fatty acids (the last members of both EFAs metabolic pathways). Longer acids of the (n-3) and (n-6) series such as 22:6(n-3) and 22:5(n-6) can also exert strong feedback inhibition on Δ 6 desaturase and prevent the bioconversion of 18:3(n-3) and 18:2(n-6) EFAs to the higher members of (n-3) and (n-6) FAs.

Besides producing feedback inhibition on the acid member of the same series, these longer chains of unsaturated acids can also exert crossed inhibition between substrates of different series of FAs. For example, Dato & Brenner (1970) have found that docosa-4,7,10,13,16-pentaenoic acid (22:5n-6) controls the biosynthesis of PUFAs, and inhibited 18:2(n-6) desaturation to 18:3(n-6) acid *in vitro*. Docosa-4,7,13,16,19-hexaenoic acid (22:6n-3) inhibited in a similar manner the desaturation of 18:3(n-3) to 18:4(n-3) acid. However, the authors also reported that the inhibitory effect of 22:5(n-6) was more pronounced on the desaturation of its own precursor (18:2(n-6)). 22:6(n-3) similarly produced an inhibitory effect on its own precursor (18:3(n-3)), but this inhibition was stronger on the desaturation

of its own precursor than 22:5(n-6) was on the same substrate. It was observed that in rats (Brenner *et al.*, 1969) and trout (Leger *et al.*, 1981), *in vivo* inhibitory effects of (n-3) FA were higher on (n-6) FA than vice versa. Under the condition of equal proportion of substrates the 18:2(n-6) desaturation was inhibited as well. However, the excess levels of 18:2(n-6) can block the unequal affinity to 18:3(n-3) for Δ 6 enzyme binding sites in order to synthesize the longer chain of the (n-3) series of FA.

The results of those studies clearly suggest that Δ 6 desaturase accommodates a wide range of chain length substrates. It seems to be the key enzyme for the biosynthesis of HUFAs and other biologically active compounds (eicosanoids) formed from dietary precursors.

5.0 **Eicosanoids formation and its significance in fish**

5.1 ***Occurrence and synthesis of eicosanoids***

Eicosanoids (Greek ''eicosa'' indicating 20) and their metabolites include a large number of physiologically active compounds which are formed in the body from dietary precursors. The initiation of eicosanoid biosynthesis occurs in response to physiological or pathophysiological stimuli. It is triggered by the release of fatty acid precursors esterified in the β-position of cellular phospholipids by the action of an intracellular phospholipase A_2 (Holtzman, 1992; Negishi *et al.*, 1993; Dennis, 1994). These physiologically active components are derived primarily as the oxygenated metabolites of C-20 fatty acids (20:5(n-3), 20:3(n-6) and 20:4(n-6)). Among them, the most well understood and probably the most important eicosanoid precursor is arachidonic acid, 20:4(n-6)). These acids are catalyzed enzymatically through the action of multi-enzyme complexes of membrane-bound

cyclooxygenases or specific lipoxygenases and give rise to three major groups of eicosanoids: prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT) (Needleman *et al.*, 1986; Willis, 1987; Diczfalusy, 1994).

Our understanding of the metabolism of C-20 FAs in fish is based upon investigations of mammals, such as the rat, which serves as a model system in particular. The interest in eicosanoid products came from physiological and clinical investigations in mammalian pharmacology in the early 1960s (Bergström *et al.*, 1968).

The biological significance of eicosanoids extends beyond their role in mammal physiology. These oxygenated metabolites of HUFAs were found in a large range of organisms including mammal, fish and invertebrate species (Christ & Van Dorp, 1972; Nomura & Ogata, 1976) and are known to play quite extensive and similar roles in the physiology and pathology of these organisms (Stanley-Samuelson & Pedibhotla, 1996).

In fish, these acid-derived substances have been identified in ovarian tissue and blood. They are directly involved in reproduction, including the stimulation of ovulation, and in behavioral changes in spawning females (Stacey, 1981; Stacey & Sorensen, 1991; Priddy & Killick, 1993). The PG metabolites (F-series) serve as postovulatory sex pheromones drawing the males toward ovulating females of the same species (Kitamura *et al.*, 1994a,b) as well as to promote homing behavior of migratory fish (Solomon, 1977). Moreover, eicosanoids also play an important role in the normal fertilization process by preventing polyspermic fertilization in many organisms (Schuel, 1984).

PGs are involved in osmoregulation. They regulate ion and water balance in fish (Spector & Yorek, 1985; Wales & Gaunt, 1996; Van Praag *et al.*, 1987; Brown *et al.*, 1991) and

control thrombocyte aggregation and blood clotting and are also powerful antinflammatory agents (Dyerberg & Bang, 1978; Kirtland, 1988; Horrobin, 1991).

Eicosanoid metabolites are implicated in the cellular defense response in fish and other vertebrates (Johnston, 1985; Kiron *et al.*, 1995). It has been proposed that some eicosanoids also play an important roles in the control of protein synthesis (Palmer, 1990; McLennan, 1991).

The 20:3(n-6) and 20:5(n-3) FAs are substrates which, when metabolized, yield the 1-series (PGE_1, $PGF_{1\alpha}$) and 3-series (PGE_3, PGF_3, PXA_3) prostaglandins. Endogenous 20:4(n-6) is metabolized to the 2-series homologues (PGD_2, PGE_2, PGF_2, PGG_2, PGI_2, TXA_2). It is thought that effects of 20:4(n-6)-derived eicosanoids demonstrate superior biological activity compared to the 1- and 3-series PG derived from 20:3(n-6) and 20:5(n-3) acids (Karmali, 1987).

5.2 *Modulator effect of dietary (n-3) and (n-6) FAs on eicosanoids production*

In most tissues of fish studied thus far, it seems that the preferred substrate for PG production *in vivo* is 20:4(n-6), followed by 20:3(n-6) and 20:5(n-3), although there is a predominance of the (n-3) series of FAs in freshwater fish body tissue. In salmonids, the 20:4(n-6) and 20:5(n-3) phosholipids are common constituents of all body tissues. However, the proportion of 20:4(n-6) exceeds that of 20:5(n-3) in total phosholipids. 20:3(n-6) content is generally lower, never reaching more than 2% of the total fatty acid content in freshwater species (Henderson & Tocher, 1987; Gunstone *et al.*, 1978). Additionally, as with the phospholipids, 20:4(n-6) is the predominant acid among all the (n-

6) series of FAs found in young wild salmon tissue (Ackman & Takeuchi, 1986). Thus, the relative abundance of all the FA precursors in body tissues follows the order of metabolic substrate preference for the cyclooxygenase pathway of the eicosanoids biosynthesis. This presence of 20:4(n-6) in membrane phospholipids results in a competitive relationship between peroxisomal β-oxidation and endoplasmic reticulum esterification. The 20:4(n-6) as a substrate is converted into the 22:4(n-6), which is subsequently retroconverted through β-oxidation and esterification into membrane lipids to finally give rise to the 20:4(n-6) again (Voss *et al.*, 1992; Sprecher *et al.*, 1995). Enzymatic studies by Verdino *et al.* (1964) and Sprecher (1967) have shown that rats raised on 22:4(n-6) and 22:5(n-6) incorporated small amounts of C-22 (n-6) HUFAs into the liver membrane. Instead of incorporating these components into body tissue, the rats preferentially retro-converted these acids into 20:4(n-6).

The production of eicosanoids from 18:2(n-6) and 18:3(n-3) cannot be assumed automatically. This is only possible if the organism in question is capable of converting 18:2(n-6) into 20:3(n-6) and/or 20:4(n-6) as well as 18:3(n-3) into 20:5(n-3); otherwise, eicosanoid precursors must be adequately present in the diet. Thus, the availability of those acid precursors may represent a key by which the biosynthesis of eicosanoids is made possible in organisms that are lacking EFAs. Indeed, Bell *et al.* (1985b) have demonstrated the effects of EFA dietary deprivation on the composition of turbot phosholipids and the content of 20:4(n-6) and 20:5(n-3), but the effects in different tissues are sometime quite diverse. The relative amount of 20:5(n-3) was the most affected; it decreased in all phospholipids of the tissues of fish raised on a diet totally deficient in PUFAs. In contrast, 20:4(n-6) is generally retained in the phospholipids (e.g. in phosphatidycholine from gut

and gill). However, the 20:4(n-6) content is almost double in muscle. Similar patterns were observed in phosphatidylinositol from the liver, gut and gills. This selective retention of 20:4(n-6) suggests the greater importance of this acid in fish physiology relative to 20:5(n-3) acid. Fish maintained only on a diet deficient in (n-6) PUFAs demonstrated a decrease in 20:4(n-6) levels in almost all phospholipids, with the exception of phosphatidylethanolamine, where it slightly increased in all the tissues. A marked decrease of 20:4(n-6) was noted especially in sphingomyelin in all tissues (up to 96% except in muscle tissue and up to 85% in phosphatidylinositol, a major phospholipid). Decreases in 20:4(n-6) content were compensated by increased levels of 20:5(n-3) acid. 20:5(n-3) levels were generally elevated in liver, gut and muscle phospholipids, but slightly lower (up to 30%) in the gills.

A competitive interaction between the (n-3) and (n-6) series of FAs for desaturation/elongation enzymes can have a great influence on the formation of eicosanoids precursors. This is due to the fact that Δ 6 desaturase is involved in the synthesis of the HUFAs, with a greater preference for the (n-3) FA as a substrate than for (n-6) and (n-9). The kinds of oils used in dietary supplements for salmon can significantly alter the long chain polyunsaturated fatty acid composition of fish phospholipids (Bell *et al.*, 1989). Thus, dietary factors directly or indirectly determine the availability of precursor acids which enhance or depress the biosynthesis of some eicosanoids products (Bell *et al.*, 1993a). These substances are actively implicated in the modulation of salmon immune response (Bell *et al.*, 1996a).

The modulator effect of dietary fatty acids on eicosanoids production and activity of phospholipase A in *Salmo salar* was reported by Bell *et al.* (1993b). Three diets consisting

of different lipid sources (sunflower oil, linseed oil, fish oil) representing various levels of 18:3(n-3), 18:2(n-6) and (n-3)/(n-6) ratios were supplied to post-smolts Atlantic salmon for 12 weeks. Fish fed sunflower oil supplements containing low dietary (n-3)/(n-6) ratios (= 0.2) as well as high levels of 18:2(n-6) resulted in lower body 18:3(n-3)/18:2(n-6) ratios (=0.01) and in the highest concentrations of 20:3(n-6) and 20:4(n-6) acids. These (n-6) FAs were found to be incorporated into all phospholipid classes of salmon heart tissues. In contrast, significantly lower levels of 20:3(n-6) and 20:4(n-6) were incorporated into the phospholipids of fish raised on linseed and fish oil supplements. This was due to a higher dietary occurrence of inhibitory 18:3(n-3) reflecting higher dietary 18:3(n-3)/18:2(n-6) and (n-3)/(n-6) ratios, 2.6 and 3.2 for linseed supplements respectively, and 0.9 and 7.8 for fish oil supplements. The sunflower diet induced a significant reduction of 20:5(n-3) in fish tissues in comparison with the other diets. This reduced synthesis caused an increased presence of dietary (n-6) FA (low 0.2 (n-3)/(n-6) ratio) as well as an extremely low proportion of 18:3(n-3) to 18:2(n-6) (= 1:70). The decreased capacity of 20:4(n-6) synthesis by fish fed linseed and fish oil supplements is therefore due to the competition of 18:3(n-3) with 18:2(n-6) for Δ 6 desaturase and of their metabolites for Δ 5 desaturase. As with 20:4(n-6), the production and concentration of thromboxane (TXB_2) and prostaglandin (PGE_2) in salmon plasma were also reduced in fish given a supplementary linseed diet relative to those fed a diet with sunflower oil. Similar findings were reported in mammals: the concentration of 20:4(n-6) and its derived PGs in rat blood decreased as the dietary 18:3(n-3) supplement increased (Hwang & Carroll, 1980). Additionally, Bell and his co-workers have also shown that an increased biosynthesis of 20:4(n-6)-derived eicosanoids by salmon leukocytes could be responsible for inducing a histopathological

cardiac lesion developed markedly in fish fed sunflower oil. Less severe effects were observed in fish fed fish oil, and no cardiac pathology was developed in fish fed linseed oil. The activity of cardiac phospholipase A was greater in fish given sunflower supplements (rich in 18:2(n-6) and (n-6) FA) relative to those fed either linseed or fish oil.

More recently, Bell *et al.* (1996b) have shown that low dietary (n-3)/(n-6) ratios with sunflower oil supplements resulted in a decrease in phospholipase A activity, but this time in salmon gills. The stimulation of eicosanoids production of one series in favor of another can therefore be achieved by supplying the appropriate FA precursors in the diet of fish.

In measuring the production of prostaglandins E and F of the 1-, 2-, and 3- series from exogenously added eicosanoid precursors, Bell *et al.* (1994a) demonstrated that 20:4(n-6) is the preferred prostaglandin substrate despite a supplemented excess of the others. Primary cultures of astroglial cells of turbot brain supplemented with 20:3(n-6) stimulated the production of PG E_1 and $F_{1\alpha}$, 4 and 2 times respectively, and also inhibited PGE_2 and $PGF_{2\alpha}$ production, 3 and 1.6 times respectively, by competing for the cyclooxygenase active site. Exogenous 20:5(n-3) did not significantly increase the 3-series PG, but competitively inhibited the production of PGE_2 and $PGF_{2\alpha}$, 4 and 14.6 times respectively, and significantly reduced $F_{3\alpha}$, itself a 3-series PG product.

It is interesting to note that although 22:6(n-3) is relatively abundant in fish tissues, it is not a substrate for eicosanoids production, and it has been found to interfere with an eicosanoid enzyme cascade. This C-22 acid is a strong inhibitor of the formation of 20:4(n-6)-derived prostaglandins but not of leukotriene (Corey *et al.*, 1983). Utilizing pure 22:6(n-3) and 20:4(n-6) in varying proportions in turbot diets, Bell *et al.* (1995) have also

observed impacts on PGs production. A trend of decreasing concentrations of PGE_2 and 6-ketoPGF$_{1\alpha}$ in turbot tissues was noted in fish fed the lowest dietary 22:6(n-3)/20:4(n-6) ratio, compared with those fed the highest levels of 20:4(n-6) acid.

Thus, the diet can influence the production of eicosanoids, which are bio-active molecules implicated in many physiological functions of cells, and as a result represent important mediators of health conditions in organisms. These substances *in vivo* exert no single over-riding effect upon cell growth. They can induce growth in one type of cell and may have opposite effects on other cells types. Therefore, improper dietary supplementation of lipids is often interpreted incorrectly, without enough attention given to the individual prostaglandins C-20 PUFAs precursors, which can contribute to diverse and sometimes physiologically negative effects. Thus, eicosanoids production demonstrates the importance of a balanced dietary supply of both (n-3) and (n-6) series of fatty acids in the maintenance of good growth and health of young salmonids.

6.0 **Total lipid and fatty acid compositions in insects**

The aim of this discussion will be to provide the significance of total lipids in insects, and to describe the present state of knowledge with respect to the metabolism and identification of insects' essential fatty acids. The EFAs composition of insects will be examined in relation to its effects on the formation of longer polyunsaturated fatty acids in salmon tissues and their influence on the growth of young fish. The emphasis of this work is to provide a critical assessment of the current state of knowledge in the literature and to point out areas

where I believe future studies would yield significant contributions to nutritional biochemistry.

6.1 *Total lipid composition in insects*

The body fats of insects as well as many other organisms are affected by their diet quantitatively. There is also a physiological/genetic source of limitations.

Organisms of the same genus and family in similar environments often show similar feeding habits. Consequently, species sharing a close ecological relationship may be expected to store lipids in a similar manner.

Tables 3 to 6 summarize the total lipid content of the different growth stages of various commonly studied aquatic and terrestrial categories of insects. These are based on an extensive research of the literature. Total body lipid content generally ranges from 1.5 to 12.7 % of the total wet weight of the insects (Tables 3 & 5) and 10.3 to 42.2 % of total dry weight (Tables 4 & 6). The mean fat content expressed per unit of wet weight of insects generally does not exceed 10 %.

The total lipid content (% of wet wt.) reported for terrestrial larvae is generally the highest, ranging from 3.8 to 12.7 % (Table 5). Aquatic larval forms of insects had a lower lipid content, ranging from 1.5 to 2.9% (Table 3).

The mean lipid content on a dry weight basis of terrestrial adult insects rarely exceeds 20%. The mean value for the terrestrial larvae stage is more variable and ranges between 17.56% and 42.40%, the one exception being terrestrial coleopteran larvae, which had lipid content of 42% (% dry weight, Table 6). The data indicates that the lipid content of insects reaches

a peak during the immature stages and decreases during the adult stages (see Hemiptera, Diptera, Coleoptera, Tables 4 & 6).

The previous research and cited data invite the following interpretations.

The total lipid content of insect bodies reported in the literature shows important changes and fluctuation during the insects' development. The total lipid content (as % of the dry wt.) generally remains constant during the early immature life stages. In II and III instar larvae, lipid content increases and reaches the first peak; in mid-IV and V instar larvae it steadily declines to a minimum value. At the end of the larval cycle and early pupal stage, a second peak is generally observed (Nakasone & Ito, 1967; Otto, 1974). These patterns of lipid content during the life cycle of the insect are observed in insect species with complete metamorphosis. Hemiptera, with an incomplete metamorphosis in various nymphal stages, do not show considerable variations in lipid content (% dry weight) (Lee e*t al.*, 1975).

The body fat of any organism is generally constituted of two fractions: a quite variable amount of storage lipids (neutral lipids), the majority being triacylglycerols (TG), and a more constant amount of structural phospholipids (PL) (polar lipids). TG function exclusively as an energy source while non-triacylglycerol lipids exert a variety of physiological and structural functions. For example, hydrocarbons and sterols are generally associated with the thin layer of cuticular lipids that serves in chemical communication and protects insects from dessication (Lockey, 1980; Blomquist *et al.*, 1987). PL are a component of cellular and subcellular biomembranes, which make up part of the structure of cells and tissues (Bridges, 1983).

The relative amount of neutral and polar lipids during various stages of development of insects is most likely due to autocorrelation (dividing by mass). During first larval and

nymph stages, the proportion of PL content is greater than TG (as % of total lipid weight). This pattern is probably due to the higher number of structural components (membrane bilayer) during the early immature stages of development. With subsequent growth and development, the PL content declines at the same time as TG content increases. The trend of increased TG in the later larval states is probably due to an accumulation of a large amount of lipids which are subsequently used during the non-feeding pupal stage. A higher TG storage also provides energy for metabolic processes occurring during adult metamorphosis (Beenakkers *et al.*, 1981). In general, TG are found to be the predominant lipid class that is deposited in the body of many insect species until somatic growth has ceased. The distribution of PL in the body is variable in and different among insects and is dependent upon the amount of TG that has been accumulated or used by an organism, and in turn is strongly influenced by a variety of environmental factors.

The overall PL content increases until the middle of the pupal period, and then gradually decreases until the end of the pupal stage (Bridges, 1983). The polar lipids represent mixtures of complex lipids, associated with the insect tissues, and as such they cannot be interpreted as homogenous body fat. It appears, however, that changes in the proportion of individual phospholipid content can take place during various phases of development (Fast, 1964, 1971; D'Costa & Birt, 1966).

Among the listed insects, the TG of Lepidoptera larvae varied from 45% to 82% of total lipid content and the PL between 3.3% and 18% (Fast, 1970). Wood *et al.* (1969) stated that neutral lipids consisting of TG appear to be relatively constant during all immature stages and reach approximately 93% of the total lipid content, whereas PL stood between 3.6-9.0%. Adult aphids can accumulate up to 80% TG of the total lipid content (Strong,

1963). The highest amount of total neutral lipids (most likely TG storage) was between 40-90% of the total lipid content as reported for both sexes of terrestrial adult and larval Coleoptera when artificially fed (Henson *et al.*, 1971, 1973)

Substantial proportions of the total lipid content of aquatic immature insects consisted of neutral lipids reaching up to 70%, the TG being a major component and making up 35% to 57% of the content (Lee *et al.*, 1975; Bell *et al.*, 1994).

The fatty acid composition of PL and TG differs considerably. Higher proportions of saturated and monounsaturated FAs are associated with TG. PL are characterized by a greater proportion of PUFA compared to TG. The polyunsaturated 18:3, 20:4 and 20:5 acids as well as saturated 18:0 are found predominantly in the PL (Fast, 1970; Grau & Terriere, 1971; Stanley-Samuelson & Dadd, 1983; Hanson *et al.*, 1983).

The distribution of highly unsaturated fatty acids in PL can vary considerably among certain groups of insects. It may suggest some particular physiological requirement for FAs. Due to the difference in FA composition within TG and PL lipids present in the organism, the degree of TG storage in the body could alter the ratio of some of the insect's fatty acids.

Investigations of the variations in the lipid content of insects focusing on the various extraction methods and a wide variety of physiological and ecological factors, which influence each organism differently, sometimes render comparisons very difficult.

Significant differences in body lipid content exist between males and females of many insect species. Females usually harbor a higher percentage of lipids than males of the same species (Lambremont *et al.*, 1964). This can be correlated with the storage of food reserves,

especially during reproduction (eggs production), since gravid females usually are more fatty than normal females.

The amount of lipids in insects can also be influenced by the size and social status of the individuals, where bigger organisms contain more fat per unit weight than smaller ones (Keller, 1989).

It is impossible to determine in the studies researched if an effort was made to clear gut contents of insects before analysis. Since all insects were analyzed whole, gut fullness, gut morphology and percentage of water in gut food compositions for wet weight all contribute to variability in body lipids. Hanson *et al.* (1985) suggested that detritivorous insects with possible large digestive tracts ingest considerable amounts of energetically deficient detritus food. Estimation of lipid content in these insects might therefore show smaller quantities than are actually present in the body.

6.2 *Fatty acid compositional distinctions in insects*

The fatty acids present in insect lipids are the same as those found in other animals, but exceptions do exist. The fatty acid composition of terrestrial insects is quite similar in a qualitative way. This is true for even numbered C-14, C-16 and C-18 chains of saturated acids, (n-7) and (n-9) monounsaturated acids and C-18 both (n-3) and (n-6) PUFAs (Thompson, 1973). The long-chain polyunsaturated fatty acids beyond 18:3(n-3) acid in homogenate body tissues were few or generally absent. However, the discovery of biologically active prostaglandins, similar to those in vertebrates, confirms that C-20 PUFAs must be a regular component in insect tissue (Loher *et al.*, 1981; Murtaugh & Denlinger, 1982; Stanley-Samuelson, 1987).

Effectively, a low occurrence of longer PUFAs among terrestrial insects has been reported by Stanley-Samuelson & Dadd (1983). These authors noted that C-20 PUFAs occur at low levels in whole body extracts. However, a removal of TG from lipid extracts and a fractionation of PL in certain tissues ensures the most efficient detection of larger levels of longer polyunsaturated fatty acids. The small distribution of even C-20 or other long polyunsaturated acids in terrestrial insects in particularly important organs, e.g. spermatophores or nerve tissue phospholipids, is physiologically more understandable than in other body parts.

Hanson *et al.* (1985) extensively examined the composition of fatty acids in numerous genera of aquatic insects. The composition and presence of shorter, even-numbered carbon chain acids were found to be similar to those of terrestrial insects. However, compared to terrestrial insects, aquatic insects have distinctly higher levels of longer PUFAs, especially the C-20 FAs. The widespread presence of longer PUFAs in aquatic insects suggests that they are important for the aquatic environment.

In spite of a fairly similar qualitative profile of fatty acids in all insects, the percentage of individual acids occurring in insects' tissue differs considerably. Taking into consideration that a number of both aquatic and terrestrial insect species present in fish ecosystems represent different fatty acid metabolisms, physiological requirements and ecological status, a variety of compositional forms of lipids should be expected. Thus, in a compilation of different compositional profiles of fatty acid in insects, it is easy to discover the characteristic pattern, already recognized many years before.

Among Homoptera, aphids are distinguished by a high occurrence of palmitoleic acid (16:0) (up to 60%) and 14:0 reaching 80% of the total fatty acid in some species. Another

closely related group, the coccids, is characterized by the presence of 10:0 and 12:0, both reaching 20% of the total fatty acid content. 18:1(n-9) comprise about 50% of all C-18 (18:3(n-3), 18:2(n-6) and 18:1(n-9)) FAs content in coccid species. This acid can reach up to 70 % in Cicadellidae. In larvae of Lepidoptera insects the levels of three C-18 FAs combined is extremely high, rendering this insect very atypical relative to other insects (Fast, 1970). In most of the Diptera insects, more than half of all fatty acid components are short-carbon chain acids. Palmitoleic acid (16:1n-7) has been proved to be exceptionally high in occurrence (up to 45% of the total fatty acid content) (Fast, 1966, 1970). Such high concentrations of palmitoleic acid are not restricted to the Dipteran species. Bracken & Harris (1969) studied fatty acid composition of different lepidopteran larvae. They observed that five of the eight species had high levels of 16:1(n-7), ranging from 18% to 53% of total fatty acid content. The unusually elevated concentrations of 16:1(n-7) in Lepidopterian bodies indicate their functional role, considering the fact that the ability to overwinter as larvae is correlated with high concentrations of palmitoleic acid.

The interpretation of the different compositional patterns of fatty acids in whole insects of various species gives rise to some difficulties in the determination of evident and unequivocal explications. It is known that the profile and changes of fatty acid composition is determined in part by the environmental factors. Adaptation in insects' evolution based on food acquisition was the concept adopted by Hanson *et al.* (1985). The different functional feeding groups (shredders, grazers, etc.) are an important element, which can help to explain fatty acid compositional patterns. Those authors found that almost all aquatic insects always have high proportions of longer PUFAs in body lipids at every stage of development. It is interesting to note that other noninsect freshwater invertebrate

organisms do not stand out from this general pattern (Desvilettes *et al.*, 1994; Dembitsky *et al.*, 1993). In addition, Hanson's work shows that there were high levels of 20:4(n-6) and 20:5(n-3) (both precursors for prostaglandins synthesis), always lower in grazer species and highest in predator and filter feeding insects. At first glance the exceptionally high levels of 20:4(n-6) and 20:5(n-3) acid in predator insects are held responsible for the existence of a metabolic pattern correlating positively with metabolic rates displaying high C-20 PUFAs contents in those species. Uscian *et al.* (1992) pointed to the unusually high level of 20:4(n-6) and 20:5(n-3) in the tiger beetle (*Cicindela circumpicta*) and robber fly (*Asilis* sp.), both terrestrial predatory species. The two C-20 PUFAs represent respectively over 5% and 12% of all phospholipid fatty acids in *Cicindela circumpicta* and *Asilis* sp. Continuing work is necessary and should focus on certain tissues of aquatic insects with lipid fractionation, otherwise the recognition of metabolic patterns of physiologically important fatty acids in whole-animal lipids extracts may prove to be difficult. Because the fatty acids synthesized by animals could be constantly diluted with similar components exogenously supplied in diet.

Hanson and his coworker have also recognized an interesting pattern of fatty acids within the same orders among insects' functional feeding groups. The mean level of polyunsaturated fatty acids of whole insect total fatty acid was negatively correlated with 16:1(n-7) among different feeding groups, especially within the Diptera, Ephemeroptera and Trichoptera. This finding suggests that a diet rich in high PUFAs tends to raise the total PUFAs proportion in body tissue and to concurrently decrease monounsaturated FAs, particularly 16:1(n-7) acid. Thus, in the following discussion I investigate if these patterns are constrained only by the diet.

The occurrence of palmitoleic acid in insect bodies and its relationship with the unsaturated components among a variety of aquatic insects shows an uncanny resemblance to other species of terrestrial origin. This relationship, particularly among aquatic Diptera, appears to be similar to Diptera insects representing terrestrial forms. The environmental influence on fatty acid compositional patterns found at the insect's whole body level may also have a biochemical meaning suggesting that occurrence, biosynthesis and incorporation of particular FAs are regulatory processes taking place at cellular, tissue and organ levels. Barlow (1964, 1965, 1966) has investigated fatty acid composition changes in different Dipterian insects reared on diets distinctive in fatty acid content. Regardless of stage of life and fatty acid composition even in fat-free diets, the content of palmitoleic acid still remains a relatively high component of body tissue. Additionally, data show that levels of 16:1(n-7) were negatively correlated (at 0.05) with the amount of 18:2(n-6) in the total fatty acid content in the Diptera species. The observed correlation therefore suggests the existence of an effective inhibition of the 18:2(n-6) present in body tissue on formation of 16:1(n-7) from exognenosly supplied 16:0 acid. It is possible that 16:1(n-7) is related in some manner to the polyunsaturated fatty acid. A reduction in the dietary intake of C-18 PUFAs resulted in the accumulation of monoene in insect bodies. Thus, the accumulation of monoene could be explained by the maintenance of desirable cellular properties which normally is achieved by synthesized or dietarily supplied PUFAs. Possibly the role of a high concentration of 16:1(n-7) in body lipids is associated with the insect's apparent lack of requirement for PUFAs. In fact, when a Dipterian insect colony was raised on pork liver (containing small amounts of 16:1(n-7), the body tissue showed considerable levels of 16:1(n-7) acid. Similarly, Barlow reported a particularly selective accumulation of fatty acids in

Homoptera, which showed approximately 55% of 14:0 in body tissue in spite of being fed bean leaves containing approximately 10% of this acid. While studying *D. melanogaster* Keith (1967a) has shown that the proportion of 16:1(n-7) and 16:0 in larval tissue were altered by the addition of 18:2(n-6) to the diet. Thus, the presence of 18:2(n-6) acts as a direct inhibitor, preventing the enzymatic conversion of 16:0 into 16:1(n-7) acid. Stanley-Samuelson *et al.* (1985) found that the supplementation of the *D. melanogaster* diet with various C-18 PUFAs affected the fatty composition of tissue phospholipids. An increasing proportion of C-18 PUFAs in the diet reduces the proportions of both 16:1(n-7) and 18:1(n-9) acids in phospholipids. However, the reduction of monoene synthesis in response to exogenously incorporated PUFAs resulted in an overall unchanged proportion of unsaturated fatty acids in the insect body. Physiological stability of tissues could be the most probable reason for a maintenance of a constant unsaturated/saturated ratio. The 18:2(n-6) and 18:3(n-3) are not synthesized *de novo* but their presence in insect bodies results in a direct incorporation into the body tissue. Thus, the maintenance of a specific proportion of C-18 PUFAs in the insect body can be considered neither important nor physiologically indispensable. The *Agria affinis* reared on a diet free of PUFAs grow as well as when fed a diet containing polyunsaturated supplements (House & Barlow, 1960). Keith (1967b) suggested that dietary C-18 PUFAs appeared not to be metabolized to longer-chained fatty acids in *Drosophila melanogaster*, but always caused a decrease of occurrence of 16:1(n-7) and 18:1(n-9) with corresponding appearence of 14:2 and 16:2 in the body. The addition of 18:1(n-9) to the diet increased the 14:1 considerably. Dietary stearate (18:0) greatly increased the 14:0 content in body tissues. Increased dietary level of 18:2(n-6) also resulted in a reduction of 18:3(n-3) in *Drosophila* body. In fact, the

inhibition of desaturation pre-existing 16:0 to 16:1(n-7) occurred in *Drosophila* with the presence of 18:2(n-6) in the diet. However, labelled acetate was readily incorporated through a dietary inhibitor into saturated and monounsaturated components, but not into unsaturated acids. The relatively constant distribution of labelled acid in the body of *Drosophila* was attributed to the existence of a monoenes formation pathway independent of dietary interaction. Those varied fatty acid interactions observed in *Drosophila* demonstrate that enzymatic chain-shortening and desaturation of C-18 occurs is regulated in some ways at the molecular level.

The cricket (*Acheta domesticus*) is an example of an insect species which retains a high amount of 18:2(n-6), regardless of the dietary access which renders this acid constantly available to the physiological processes. Studies by Cripps *et al.* (1986) and de Renobales *et al.* (1987) show that crickets are able to synthesize 18:2(n-6) from acetate, which contributes up to 20% of total fatty acid components of triacylglycerols. This insect does not respond to different dietary levels of polyunsaturated fatty acids by decreasing the content of monounsaturated acids, nor by an increased proportion of polyunsaturated components. The cricket tissue did not differ in fatty acid composition. The percentage of 16:0, 16:1(n-7), 18:1(n-9) as well as the proportion of 18:2(n-6) remained stable when insects were reared artificially on a lipid-free diet or a diet supplemented with 18:2(n-6) acid (Meikle & McFarlane, 1965). Thus, it may be suggested that this insect can synthesize 18:2(n-6), but does not require it in its diet.

We see that lipids and the fatty acids pattern of insect bodies which produces the particular features of an individual species can be determined not only by the variation in the composition (quality and quantity) of their natural food but also by the ability of an

organism to enzymatically modify exogenously supplied fatty acids. In addition, growth and development as well as the specificity of response of individual tissues in different physiological circumstances exert a strong influence upon the fatty acid compositional pattern. Environmental conditions are factors that may also alter fatty acid compositional profile of body tissues considerably. Thus, the occurrence of fatty acids which produce the some patterns in insect bodies is determined by external environmental conditions and physiologic-enzymatic factors as well.

6.3 *EFA components in insects vs Atlantic salmon*

Aquatic insects representing different stages of their life development are a major source of the salmons' essential fatty acid supply (Parker *et al.*, 1980). However, terrestrial species may also contribute as food in their freshwater phase.

The occurrence of PUFAs components and the body compositional ratio of (n-3)/(n-6) unsaturated fatty acids as well as the relative proportion of 18:3(n-3) to 18:2(n-6) occurring in all insects all seem to be species-dependent characteristics. In general, the lipids of aquatic insects contain higher levels of C-20 PUFAs compared to terrestrial species. The lipids of terrestrial insects containing substantial levels of 18:2(n-6) but less of 18:3(n-3) distinguish them particularly from the majority of aquatic insects (Fig. 2).

The low proportion of 18:3(n-3) for aquatic Heteroptera adults and nymphs may be related to their specific niche in the aquatic environment at the air-water interface. Heteroptera's low 18:3(n-3)/18:2(n-6) ratios similar to the rest of the terrestrial insects are determined by they prey of probable terrestrial origin, caught on the water surface. Among all terrestrial insects represented in this study, Lepidoptera larvae has substantially higher 18:3(n-3)

compared to 18:2(n-6) acid. Other terrestrial species represented by the Hymenoptera order appears also to have high 18:3(n-3)/18:2(n-6) ratio similar to the lepidopterian species (Young, 1967; Fast, 1970; Thompson & Barlow, 1974) (data not presented).

An interesting fact is that insects representing four aquatic orders (Ephemeroptera, Plecoptera, Diptera and Trichoptera) all have high proportions of 18:3(n-3) compared to 18:2(n-6) acid. Moreover, the ratios of those two fatty acids seem to be constant in all those four aquatic orders. Thus, the presence of these two essential fatty acids the above mentioned insects in the form of this particular ratio renders these insects nutritionally interesting with respect to the fatty acid requirement of young salmonids.

In wild salmon body, the proportion of C-18 EFAs to total PUFAs within TL (total lipids) as well as TG and PL is relatively low (less than 10%) (Table 1). Thus, the C-18 PUFAs are metabolized without being amply accumulated in fish tissue. However, the proportion of 18:2(n-6) in every lipid fraction of wild healthy smolt is greater than that of 18:3(n-3) acid. This situation is reflected in the low ratio of 18:2(n-6) to 18:3(n-3) in TL fatty acids, TG and PL (1.4, 1.1 and 1.7 respectively).

The 18:2(n-6)/18:3(n-3) ratio of corresponding lipid fractions in hatchery-reared salmon was extremely high (12.0, 13.3 and 12.8 respectively) (Table 1). This situation suggests that in the diet of cultured salmon, the level of 18:2(n-6) exceeds 18:3(n-3), whereas the reverse situation exists in the diet of wild salmon. Competitive interactions between 18:2(n-6) and 18:3(n-3) for an enzymatic desaturation site play an important role in the determination of the relative total proportion of both acids found in body lipids of salmon. Both essential fatty acids are incorporated into the tissue, but 18:2(n-6) seems to be retained and accumulated without further sufficient metabolism.

Most of the studies on the EFA nutritional aspect have indicated that salmonids require the 18:3(n-3) in their diet for optimal growth. The presence of 18:2(n-6) in the diet appears to be less essential, but its level should not exceed that of 18:3(n-3) acid. The small addition of a long chain C-20 PUFAs (20:5 and 22:6) in the trout diet was considered to be nutritionally efficient and appeared to have additionally positive effects on the growth of fish (Henderson & Tocher, 1987).

6.4 *Natural habitat and salmon EFAs metabolism*

To rank all environmental factors as to their respective, and perhaps interacting influence on fatty acids metabolism of the natural population of salmon is not possible at this time. However, it is obvious that diet is a main factor. Nutritionally, it is important to note that natural food of fish contains different proportions of the different series of fatty acids, and therefore the proportions of these series, particularly those of (n-3) and (n-6) FA in tissue lipids, change significantly depending on the choice and/or availability of foods.

The lack of capacity of elongation and desaturation of C-18 PUFAs is a species specific characteristic. The lipid composition of the diet in the natural habitat directly influences the metabolism as well as lipid composition in fish, but this pattern is not common to all fish, even those of the same species. Moreover, the age of a fish appears to affect the activity of the enzymatic bioconversion system. Muje *et al.* (1989) have examined the composition of the fatty acids of the dorsal muscle of vendace (*Coregonus albula*) during the growth period in their natural habitat. They concluded that older fish selectively and more actively metabolize fatty acids. In contrast, the composition of the muscle lipids of young vendace resemble that of the fatty acid spectrum of their digested food. Environmental factors as

well as the presence of C-18 dietary EFAs appear to play a significant role in PUFAs composition and in the related proportion of C-20 FAs in fish.

The longer chain PUFAs includes 20:4(n-6) and 20:5(n-3) as metabolic precursors of 22:6(n-3) are common constituents of salmon as well as other fish tissues caught in freshwaters (Tables 1 & 2). Henderson & Tocher (1987) report that the relative proportion of these acids varies with the species. Similarly to the liver phospholipids of roach and pike, the proportion of 20:4(n-6) exceeds that of 20:5(n-3) in the whole body of wild Atlantic salmon smolts with respect to all the lipid fractions. The reverse situation exists in other freshwater fish. However, the comparison of fatty acids from the dorsal muscle of roaches caught in two different lakes shows that the 20:4(n-6)/20:5(n-3) ratios differ considerably between fish of the same species.

The 20:4(n-6)/20:5(n-3) ratios of two roaches (life weight 37.8g and 34.7g) caught in one lake were 0.52 and 0.55 respectively. In contrast, the corresponding value of fish weighing 191.1g and 175.1g from another lake were 1.34 and 1.65. Pikes from the lake had higher 20:4(n-6)/20:5(n-3) ratios (of 1.15 and 1.09) (383.4g and 243.6g respectively) than those from brackish waters, which were 0.24 and 0.31, corresponding to 2900g and 2000g respectively (Ahlgren *et al.*, 1994).

It is now well established that the major pathway of formation of longer polyunsaturated fatty acids begins with the Δ 6 desaturase between C-6 and C-7 of both 18:3(n-3) and 18:2(n-6), followed by a further desaturation between C-5 and C-6 by the Δ 5 desaturase, and between C-6 and C-7 again by a Δ 6 desaturase.

The pathway gives rise to C-20 and C-22 FAs of the (n-6) series, such as 20:4(n-6), 22:4(n-6) and 22:5(n-6), as well as 20:5(n-3), 22:5(n-3) and 22:6(n-3) of the (n-3) series

which are the most dominant PUFAs in the salmon body (Ackman & Takeuchi, 1986). The elongation/desaturation enzymes form the (n-3) and (n-6) series of PUFAs from the 18:3(n-3) and 18:2(n-6) dietary precursors. They are also responsible for the synthesis of (n-9) PUFAs from 18:1(n-9) of endogenous or dietary origin. The preferential substrate affinity of the Δ 6 desaturase generally follows a given order; 18:3(n-3) > 18:2(n-6) > 18:1(n-9). Thus, the desaturation of 18:1(n-9) and the formation of longer acids of the (n-9) series are competitively inhibited by both C-18 EFAs, allowing the synthesis of (n-9) occurring during a constant deficiency of EFAs in the diet. Theoretically, a large dietary excess of 18:1(n-9) can interfere with the desaturation of 18:3(n-3) and 18:2(n-6) acids. It has been estimated that the dietary amount of 18:1(n-9) at a 70% calorie content inhibits the synthesis of 22:5(n-6) from 18:2(n-6) by 50% (Holman, 1986). In practice, the amount of 18:1(n-9) in aquatic insects rarely exceeds 18:3(n-3) and 18:2(n-6) (Hanson *et al.*, (1985). It is interesting to remark that 18:1(n-9) in wild smolt represents less than 3% of total body FA while in hatchery-reared salmon reach almost 17% (Table 1).

The major HUFA (n-3) series of fatty acids in every lipid fraction of salmon tissue (even those from cultured ones) is 22:6(n-3) (the product of Δ 6 and Δ 5 desaturation of 18:3(n-3)). Levels of 22:6(n-3) are usually substantially greater than any (n-3) and (n-6) PUFAs in each lipid fraction, except for triacylglycerols in cultured salmon, where the level of 18:2(n-6) was higher than that of 22:6(n-3) acid. This evidence suggests that, when 18:3(n-3) enters the desaturation sequence to form 22:6(n-3), the process progresses through terminal Δ 6 and 5 desaturation without a significant retention of other (n-3) fatty acid desaturation products. The synthesis of 22:6(n-3) originally from 18:3(n-3) occurs normally, but a weak synthesis of 22:5(n-6) is formed from 18:2(n-6) in the salmon body.

This provides the evidence that Δ 6 desaturation has a higher affinity for (n-3) than (n-6) fatty acid substrate.

Essential fatty acids primarily come from insects, especially those containing high 18:3(n-3)/18:2(n-6) ratios. A strong presence of 18:3(n-3) fatty acids in the food is essential to assure the continuous synthesis of the (n-3) series of polyunsaturated fatty acids for salmonids. The competitive interaction among fatty acid substrates for desaturation is generally explained in terms of a strong affinity of the Δ 6 desaturase for 18:3(n-3). In theory, this may explain the decrease of (n-6) FA, including 20:4(n-6) in an organism reared artificially, which occurs normally during the high dietary intakes of (n-3) (including 18:3(n-3)). A similar inverse relationship exists between tissue levels of Σ (n-3) and dietary (n-6) FA. However, the level of 22:6(n-3) in salmon tissue was found to be substantial even when the proportion of 18:2(n-6) in the diet increased (Bell *et al.*, 1989). The amount of 22:6(n-3) in phospholipids was maintained at a high level in both wild and cultured salmon, and even higher in the cultured salmon if reared on diets containing greater nutrient supplies of 18:2(n-6) acid. The potential of greater influx of 18:3(n-3) from the natural diet permits tissues to synthesize more rapidly both 20:5(n-3) and 22:5(n-3) in amounts that could suppress by competitive interaction the conversion of 22:6(n-3) acid. However, its level always will remains elevated (Table 1). Thus, it is probable that 22:6(n-3) does not respond to dietary C-18 EFAs precursors in the same ways as do those of 20:5(n-3), 22:5(n-3) and 20:4(n-6) acids.

In a comparative approach to investigate the cause of pathology of hatchery salmon smolt, Ackman & Takeuchi (1986) found that the fatty acid compositions of cultured young salmon differed from those of wild salmon. The presence of 20:5(n-3) and 20:4(n-6) in the

total body lipids was comparatively at least 2 times and 16 times higher in wild salmon than in cultured fish. The phospholipid fraction of 20:5(n-3) was relatively constant among all fish analyzed, but 20:4(n-6) was 6 times higher in wild smolt compared to hatchery smolt. In salmon digesting natural food, where 20:4(n-6) never exceeded 20:5(n-3), it appears that this wild fish seems to selectively accumulate 20:4(n-6) or that elongation/desaturation operate to keep the 20:4(n-6) at a constant level. The hatchery diet is supplied with a high level of 18:2(n-6) as shown in levels within the triacylglycerols, which is 2 times higher than in wild fish. It appears that the young hatchery salmon are unable to convert 18:2(n-6) into 20:4(n-6) sufficiently. It is interesting to note that in wild salmon the level of 20:4(n-6) in every lipid fraction was higher than 20:5(n-3) acid. The relatively high proportion of 20:4(n-6) is correlated with the relatively low level of 18:2(n-6) acid. The phospholipids contained the larger 20:4(n-6) pool than the triacylglycerols in wild smolt. The inverse relationship occurs in cultured salmon (Table 1). Thus, a greater retention of 20:4(n-6) by wild young salmon makes it a good agent in the prevention of EFA deficiency lesions, a result of its higher efficiency compared to other acids. This was confirmed by the previous research of Mohrhauer & Holman (1963). Thus, the ability to elongate and desaturate the (n-6) precursor in the biosynthesis of 20:4(n-6) and its high presence in fish tissue may be correlated with the good health and better survival of juvenile salmon.

It has been noted that the body tissue of terrestrial insects has lower proportions of longer chain PUFAs in comparison with aquatic organisms. The widespread and probably constant occurrence of C-20 PUFAs, especially 20:5(n-3) and 20:4(n-6), in aquatic insects means that invertebrates are important nutritional elements in the aquatic food chain.

A negative correlation reported by Hanson *et al.* (1985) between the total PUFA and 16:1(n-7) of four orders (Ephemeroptera, Plecoptera, Diptera and Trichoptera) of insects reflects the substantial proportion of PUFAs in the majority of these aquatic species (Fig. 3). In the analysis of the PUFA proportion relative to palmitoleic acid in the whole body of insects, it is interesting to observe that the PUFAs are relatively more abundant in Trichoptera, but lower in the Ephemeroptera species. This high proportion of total body PUFAs in Trichoptera suggests a particular nutritional regime of those insects which may be the explanation thereof. The source of PUFAs of these insects can be of either exogenous origin or can come from the endogenous enzymatic bioconversion of shorter-chain PUFA precursors.

The Hanson *et al.* (1983) study based on the fatty acid composition of the Trichoptera *Clistoronia magnifica* (Limnephilidae) exposed to different food treatments showed an accumulation of both C-18 EFAs in larvae bodies, even in those insects reared on poor quality food. Whether *C. magnifica* is equipped with elongation/desaturation enzymes to synthesize the C-20 PUFAs is not known. The presence of microbial biomass in some dietary treatments suggests that microfloras contribute to the fatty acid pool of the *C. magnifica* diet. This species grew better when conditioned on a microbial innoculum treatment than when it was not so conditioned.

Another interesting point suggested by the authors is that this Trichoptera species has the ability to metabolize monounsaturated fatty acids from carbohydrate-derived acetate and also the ability of *de novo* biosynthesis of C-18 PUFAs. From the case of *C. magnifica* it cannot be determined if *de novo* biosynthesis of fatty acids is widespread among all Trichoptera species, as this has not been demonstrated. In addition, we cannot advance the

similar conclusion that all Ephemeroptera species lack the capacity to biosynthesize C-18 PUFAs. The evidence indicating that *Ephemerella walkeri* (Ephemerllidae) was not able to incorporate labeled acetate into linoleate was reported by Cripps *et al.* (1986). However, the fatty acid composition in the comparison of the two insect orders seems to reveal interesting information in light of the nutritional potential for fish.

The proportion of the total PUFAs in the Trichoptera order is significantly higher than in Ephemeroptera (U-test, $p= 0.0001$). This does not mean that higher levels of PUFAs in Trichoptera make them better food for salmon than the of Ephemeropterian species. The total body PUFAs content is difficult to interpret in terms of potential nutritional values for fish. A dilemma in the interpretation of the effect of a large number of chemically different components included in a macrocategory such as all PUFA is that any individually polyunsaturated component can interfere in the metabolism of another as well as be changed in the fish body. However, a detailed analysis of the proportions of individual components of PUFAs potentially represented in the salmon diet highlights some interesting points.

A significant linear regression relationship was noted between the total PUFAs content vs. the content of both C-18 EFAs components in all aquatic insects (with high 18:3(n-3)/18:2(n-6) ratios used in this study ($R^2 = 0.539$, $p< 0.0001$) (Fig. 4). An interesting fact is that the distribution of both 18:3(n-3) and 18:2(n-6) EFAs does not exceed 20% of all PUFAs in Ephemeroptera body tissue, although the whole body total PUFAs content can reach up to 40%. Similarly, a significant relationship of the total PUFAs vs. body C-18 EFAs content was found for both the Trichopterian and the Ephemeropterian species ($R^2 = 0.703$, $p< 0.0001$) (Fig. 5). However, no significant relationships were observed in the

Ephemeropterian species ($R^2 = 0.186$, $p < 0.073$) (Fig. 6a). A non-significant relationship was equally noted in the Diptera and Plecoptera insects (not shown). A different situation exists in the Trichopterian species ($R^2 = 0.787$, $p < 0.0001$), (Fig. 6b). Consequently, a regression analysis showing a strong relationship of C-18 EFAs vs. the total PUFAs for these Trichoptera organisms reveals that 18:3(n-3) and 18:2(n-6) are dominant components of all polyunsaturated fatty acids in the body tissue. In spite of similar body ratios of 18:3(n-3)/18:2(n-6) (Fig. 2) for the two groups of aquatic insects (Trichopterian, Ephemeropterian), it appears that an important distinction exists between the species. Trichopterian and Ephemeropterian insects may represent different categories of insects based on aspects of their polyunsaturated acid metabolism. The two groups of insects appear to differ in their ability or inability to utilize the PUFAs. The possible ability of *de novo* biosynthesis of fatty acids by a majority of the Trichoptera species (introducing double bonds into saturated C-16 and C-18 fatty acid at the Δ 9 position) implies a potential release from metabolic HUFAs dependence. On the other hand, the question is whether dietary C-20 PUFAs or other physiologically related fatty acids are required. Trichoptera species have a low level of C-20 FAs (20:5 and 20:4) relative to their total body PUFAs when compared with those of Ephemeropterian insects (U-test, $p = 0.0001$). The significant accumulation of C-18 EFAs in Trichoptera described as C-18 EFA/ΣPUFA ratios (U-test, $p = 0.0001$) suggests that they do not possess the ability to convert C-18 EFAs to longer unsaturated fatty acids. Thus, the fatty acid requirements for the majority of Trichopterian species for those dietary C-18 FAs may not be essential, i. e. they do not appear to have this need.

Stanley-Samuelson *et al.* (1988) report that larval mosquitoes (Diptera) and waxmoth (Lepidoptera) accumulate both dietary 18:3(n-3) and 18:2(n-6) without the capability of desaturation. However, 20:4(n-6) satisfied the dietary fatty acid requirement of this Dipterian species. Can it then be suggested that within the Trichopterian species, the majority represents a group of aquatic insects which do not biosynthesize PUFAs but do require C-20 PUFAs in the diet?.

In a comparison between Trichopterian and Ephemeropterian species, a significant (U-test, $p= 0.0001$) difference can be observed in the proportion of the total body PUFAs, the Ephemeropterian being at a distinctively higher level than the Trichopterian species. Also, in the Trichoptera species, the PUFAs are almost totally dominated by the C-18 PUFAs; in contrast, the polyunsaturated components in the Ephemeroptera species are mainly represented by C-20 FAs (20:5 and 20:4). And in spite of a lower proportion of total PUFAs in Ephemeroptera tissue, 20:5(n-3) is the predominating acid, reaching up to 50% of all PUFA body content, whereas in Trichopterian insects, it does not exceed more than 7% (Hanson *et al.*, 1985).

Both the (n-3) and (n-6) series are metabolized by the same desaturase, but the competitive affinity for the (n-3) is stronger than for the (n-6) series. Additionally, the rate of Δ 6 desaturation of both C-18 EFAs is regulated by the concentration of the reaction of the substrate and its product.

In mammalian tissue, the desaturation of 18:2(n-6) in rat liver microsomes is inhibited by a high concentration of 18:2(n-6) or by its C-18 and C-20 (n-6) homologues, but it is induced by a low concentration of 18:2(n-6) and (n-6) PUFAs. Similarly to Δ 6, the activity rate of Δ 5 desaturation is also inhibited by a high concentration of C-20 (n-3) and (n-6)

series of substrates, but activity is induced by a low concentration of C-18 and C-20 (n-6) FAs (Brenner *et al.*, 1969; Brenner, 1974, 1981; Naughton, 1981).

Christiansen *et al.* (1991) studied the effect of (n-3) and (n-6) FAs on the desaturation activity in rat liver. They showed that varying dietary 18:3(n-3)/18:2(n-6) ratios affect lipid composition considerably. The activity of Δ 6 desaturation expressed in the level of the bioconversion product of 18:3(n-3) used as a substrate was higher in animals reared on a diet with 18:3(n-3)/18:2(n-6) ratios of 5.3 and 0.9. On the other hand, with a 18:2(n-6) substrate, the activity of the same Δ 6 desaturation was also elevated in a diet even much lower with 18:3(n-3)/18:2(n-6) ratios corresponding to 0.3 and 0.9.

In the activity of Δ 5 desaturation using 20:3(n-6) as a substrate, the level of its bioconversion product as 20:4(n-6) was particularly higher in a diet with 18:3(n-3)/18:2(n-6) ratios of 5.3 and 0.9. A reduced activity of Δ 6 and Δ 5 desaturation and a low level of conversion products was observed in rats fed fish oil supplements representing low 18:3(n-3)/18:2(n-6) ratios (0.4) as well as high proportions of both 20:5(n-3) and 22:6(n-3), which might express feedback inhibition on desaturation enzymes.

Presumably therefore, *in vivo* substrate-product interactions exist to maintain an optimum level of important HUFA components in the salmon body.

A low level of ensemble of C-18 EFAs of total FAs, together with high proportion of 18:3(n-3) to 18:2(n-6), as well as high 20:5(n-3) occurring in Ephemeroptera make this insects specie nutritionally very attractive. These characteristics of a potential diet for salmon provide a beneficial metabolic selective accumulation of 20:4(n-6) in young fish and assure a good growth performance.

7.0 Conclusion

The dietary lipid requirement in fish, in particular fatty acids, varies from species to species. This may depend on the specific requirements for the (n-3) or (n-6) FAs as well as on the species' ability to metabolize 18:3(n-3) and 18:2(n-6) acids. The full role, function and necessity of these unsaturated fatty acids still remain to be learned, but based on this survey they are evidently associated with increasing the economy of the fishery. Obviously these acids are metabolized in common ways to the longer-chain unsaturated acids. In particular the C-20 and C-22 FAs possess distinct properties that are important to salmonids. They function in esterified forms and their essential role is determined by their relative concentrations in physiologically important phospholipids and other lipid fractions including triacylglycerols. Lack of or unbalanced C-18 PUFAs in diet lead to an insufficiency of the biosynthesis of longer polyunsaturated fatty acids related to certain biochemical changes that are connected to EFA deficiency. Consequently, an altered fatty acid composition of the membranes may account for gross histological and morphological lesions, symptoms which have been reported in nutritional studies. It cannot be excluded that these symptoms of EFA deficiency are also related to biologically active molecules such as eicosanoids.

In this thesis I have attempted to summarize the evidence that many physiological functions of fish are intimately dependent on the type of PUFAs and optimal (n-3)/(n-6) ratio present in the diet. So far there are no publications that have investigated the lipid content of freshwater and terrestrial invertebrates that are the natural food of wild salmon, in order to

characterize a better manufactured diet which respects the precise nutritional requirement for both (n-3) and (n-6) FAs.

Therefore, we must conclude that both the nutritional and the economic success of salmon aquaculture definitely depend on incorporating such an exploitation of ecological knowledge into feeding biotechnology. The diet constitution of young salmon should resemble more closely the fatty acid content of freshwater insects (particularly the Ephemeroptera) and may be a desirable element in hatchery practices. Such diets with balanced (n-3)/(n-6) fatty acid components are of considerable importance in the rearing of healthy salmonids.

8.0 References

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9.0 Tables and Figures

TABLE 1.

Comparison of some fatty acids (wt. %) in the whole body tissue of wild and cultured juvenile Atlantic salmon.

Species	Atlantic salmon[a]			Atlantic salmon[b]		
Tissue	whole body			whole body		
Origin/caught	Wild Salmon/Nov. 13			Cultured Salmon/Nov. 21		
	TL	PL	TG	TL	PL	TG
14:0	1.5 ± 0.019	1.1 ± 0.137	3.1 ± 0.156	4.1 ± 0.294	1.6 ± 0.294	4.4 ± 0.235
16:0	14.2 ± 0.274	18.7 ± 2.038	12.8 ± 0.529	12.9 ± 0.313	18.6 ± 1.724	11.5 ± 0.392
16:1(n-7)	5.5 ± 0.196	2.7 ± 0.352	11.4 ± 0.901	8.2 ± 0.235	2.5 ± 0.313	8.6 ± 0.372
18:0	5.3 ± 0.078	5.2 ± 0.078	5.3 ± 0.098	2.5 ± 0.078	3.3 ± 0.196	2.4 ± 0.098
18:1(n-9)	12.6 ± 0.372	9.0 ± 1.117	19.2 ± 0.352	16.9 ± 0.392	8.4 ± 0.431	17.9 ± 0.254
18:2(n-6)	3.1 ± 0.117	1.7 ± 0.117	5.2 ± 0.313	9.6 ± 0.235	4.0 ± 0.098	10.3 ± 0.215
18:3(n-3)	2.2 ± 0.098	1.5 ± 0.098	3.0 ± 0.254	0.8 ± 0.039	0.3 ± 0.000	0.8 ± 0.039
20:4(n-6)	8.0 ± 0.117	10.4 ± 0.274	2.8 ± 0.274	0.5 ± 0.019	1.8 ± 0.117	0.3 ± 0.039
20:5(n-3)	4.6 ± 0.019	5.5 ± 0.196	2.1 ± 0.137	2.1 ± 0.019	6.6 ± 0.450	1.4 ± 0.058
22:5(n-3)	3.3 ± 0.294	3.4 ± 0.764	2.4 ± 0.196	1.1 ± 0.254	1.6 ± 0.176	1.0 ± 0.137
22:6(n-3)	15.4 ± 0.529	21.8 ± 1.313	4.1 ± 0.313	9.6 ± 0.176	36.2 ± 4.606	5.7 ± 0.137
Σ(n-3) PUFA	27.2 ± 0.784	33.2 ± 1.685	13.6 ± 1.234	14.8 ± 0.490	45.4 ± 5.115	10.2 ± 0.117
Σ(n-6) PUFA	15.8 ± 0.078	16.5 ± 1.195	10.8 ± 0.725	11.9 ± 0.450	7.7 ± 0.137	12.2 ± 0.215
Σ PUFA	45.3	52.0	28.2	28.4	54.7	24.4
Σ(n-3)/(n-6)	1.7	2.0	1.3	1.2	5.9	0.8
18:2/18:3	1.4	1.1	1.7	12.0	13.0	12.8

Mean ± 95% confidence intervals
TL, total lipid; PL, polar lipid; TG, triacylglycerols
a, b *Salmo salar* (n=3) adapted from Ackman & Takeuchi (1986).

TABLE 2.

Composition of total fatty acids (wt. %) in the muscle tissue of freshwater and marine fish.

Species Tissue Origin Capture	Red Drum[a] muscle marine Oct.	Capelin[b] muscle marine Aug.	Drum Freshwater[c] muscle freshwater Sep./Oct.	Sunfish Pumpkinseed[d] muscle freshwater Sep./Oct.
14:0	1.20	5.90	2.20 ± 0.784	2.30 ± 1.372
16:0	22.03	25.10	19.50 ± 4.116	18.80 ± 2.548
16:1(n-7)	6.68	8.30	16.60 ± 5.880	7.90 ± 4.900
18:0	6.33	1.20	3.30 ± 0.980	5.30 ± 1.372
18:1(n-9)	14.73	28.00	26.40 ± 11.564	13.20 ± 5.880
18:2(n-6)	1.41	1.80	3.10 ± 1.960	2.90 ± 1.176
18:3(n-3)	0.76	-	2.50 ± 3.920	1.90 ± 1.568
20:4(n-6)	2.95	-	4.90 ± 5.684	14.90 ± 5.684
20:5(n-3)	4.05	10.80	5.10 ± 2.744	7.10 ± 3.136
22:5(n-3)	2.45	-	2.20 ± 1.176	3.30 ± 0.980
22:6(n-3)	13.16	6.70	6.90 ± 8.232	13.70 ± 4.900
Σ PUFA	24.78	23.90	25.80	48.20
Σ (n-6)PUFA	4.36	1.80	9.10	22.20
Σ (n-3)PUFA	23.37	22.10	16.70	26.00
Σ (n-3)/(n-6)	5.36	12.30	1.83	1.17

Mean ± 95% confidence intervals

a *Sciaenops ocellatus* adapted from Jahncke *et al.* (1988).

b *Mallotus villosus* (n=8) adapted from Henderson *et al.* (1984).

c *Aplodinotus grunniens* (n=6), d *Lepomis gibbosus* (n=8) adapted from Kinsella *et al.* (1977).

TABLE 3.

Total lipids content of whole body (as % of wet weight) of various aquatic insects in the daily diet of salmon.

Organism	Stage	Lipids % (wet wt. basis)	95% Confidence intervals	No. of animals
[a] *Ephemeroptera* sp.	nymph	2.90	± 0.307	6
[b] *Trichoptera* sp.	larvae	1.51	± 0.254	6
[c] *Odonata* sp.	larvae	2.50	-	1

Adapted from:

[a, b] Herbes & Allen (1983).

[c] Fast (1964).

TABLE 4.

Total lipids content of whole body (as % of dry weight) of various aquatic insects in the daily diet of salmon.

Organism	Stage	* Lipids % (dry wt. basis)	95% Confidence intervals	No. of animals
a *Hemiptera* sp.	adult	17.31	± 3.654	16
b *Hemiptera* sp.	nymph	21.36	± 2.245	19
c *Ephemeroptera* sp.	nymph	20.53	± 2.821	16
d *Trichoptera* sp.	larvae	17.97	± 2.306	21
e *Plecoptera* sp.	nymph	16.22	± 4.221	6
f *Diptera* sp.	larvae	15.59	± 3.169	9
g *Coleoptera* sp.	larvae	20.32	± 5.945	8
h *Odonata* sp.	larvae	13.07	± 2.068	4
i *Megaloptera* sp.	larvae	16.45	-	2

* The 20 % of high and low values were "trimmed off" before the mean was calculated.
Adapted from:

a, b Lee *et al.* (1975).

c, d Herbes & Allen (1983); Hanson *et al.* (1985).

e, f, g, h, i Hanson *et al.* (1985).

TABLE 5.

Total lipids content of whole body (as % of wet weight) of various terrestrial insects and Arachnida in the daily diet of salmon.

Organism	Stage	* Lipids % (wet wt. basis)	95% Confidence intervals	No. of animals
a *Arachnida* sp.	adult	1.84	± 0.520	4
b *Homoptera* sp.	adult	6.56	± 2.023	3
c *Hymenoptera* sp.	adult	2.32	± 0.607	8
d *Coleoptera* sp.	adult	5.63	± 0.814	22
e *Diptera* sp.	adult	7.86	± 2.903	26
f *Lepidoptera* sp.	larvae	3.80	± 0.501	30
g *Diptera* sp.	larvae	6.00	± 0.841	11
h *Coleoptera* sp.	larvae	12.77	± 2.709	37

* The 20 % of high and low values were "trimmed off" before the mean was calculated.
Adapted from:

a Zandee (1967); Oudejans *et al.* (1971); Van Der Horst *et al.* (1972).

b, g Fast (1964).

c Scoggin & Tauber (1950); Fast (1964); Cavill *et al.* (1970); Summers & Schaefer (1988).

d Scoggin & Tauber (1950); Fast (1964); Henson *et al.* (1971); Henson *et al.* (1973).

e Fast (1964); Schaefer & Washino (1969).

f Scoggin & Tauber (1950); Fast (1964); Nakasone & It (1967); Wood *et al.* (1969).

h Scoggin & Tauber (1950); Fast (1964).

TABLE 6.

Total lipids content of whole body (as % of dry weight) of various terrestrial insects in the daily diet of salmon.

Organism	Stage	* Lipids % (dry wt. basis)	95% Confidence intervals	No. of animals
[a] *Hymenoptera* sp.	adult	11.25	± 1.130	9
[b] *Coleoptera* sp.	adult	20.32	± 5.945	8
[c] *Diptera* sp.	adult	17.85	± 4.013	16
[d] *Lepidoptera* sp.	larvae	17.56	± 2.453	14
[e] *Diptera* sp.	larvae	25.11	± 6.394	6
[f] *Coleoptera* sp.	larvae	42.40	± 9.201	4

* The 20 % of high and low values were "trimmed off" before the mean was calculated.
Adapted from:

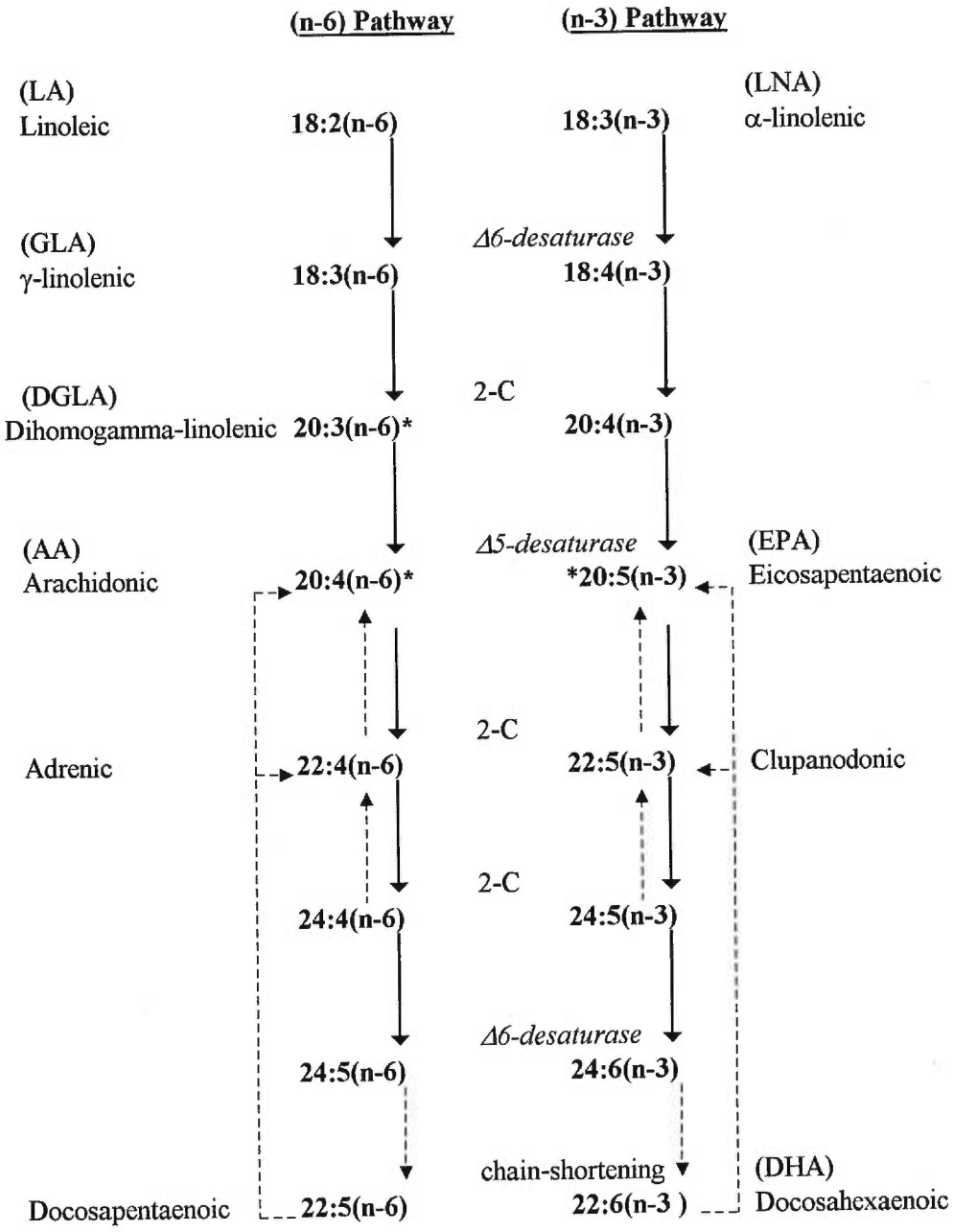
[a] Fast (1964); Keller & Passera (1989).

[b] Fast (1964); Summers & Schaefer (1988).

[c, f] Fast (1964).

[d] Fast (1964); Wood *et al.* (1969).

[e] Fast (1964); Wimer & Lumb (1967).



* Fatty acid which gives rise to eicosanoids

FIG. 1. Metabolism of the essential fatty acids α-linolenic (n-3) and linoleic (n-6) to the other fatty acids of the same family. Major steps denoted by bold arrows, known retro-conversion steps by dashed arrows. Elongation (2-C) of FA by addition of two new carbons to existing chain occurs alternately with desaturation.
Adapted from Sprecher & Lee (1975); Voss *et al.* (1991, 1992).

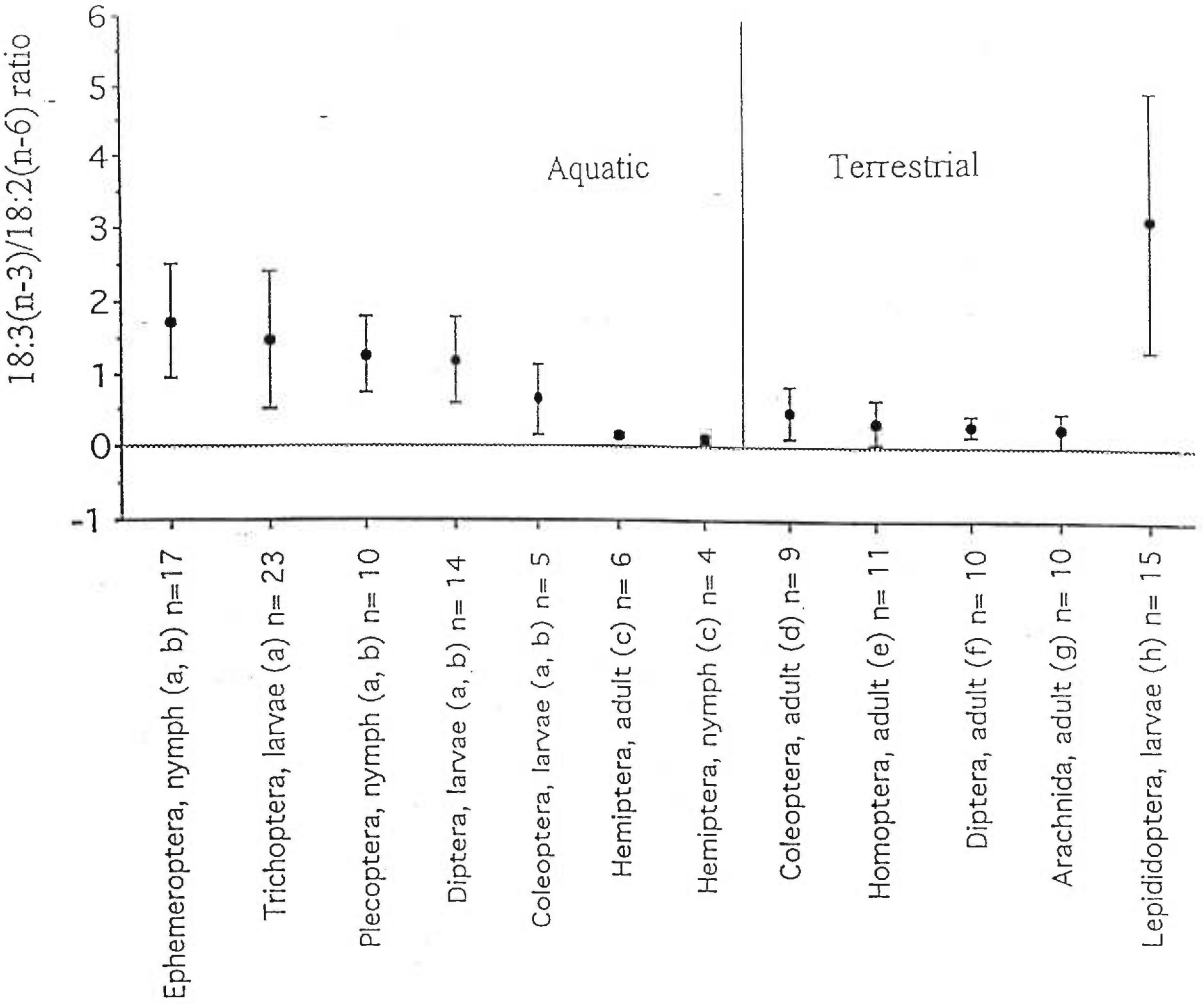


FIG. 2. Mean 18:3(n-3)/18:2(n-6) ratio in total body lipid of various aquatic and terrestrial insects species and Arachnida.

Adapted from: [a] Hanson *et al.* (1985).

[b] Bell *et al.* (1994b).

[c] Lee *et al.* (1975).

[d] Barlow (1964); Young (1967); Fast (1970).

[e] Strong (1963); Fast (1970); Thompson & Barlow (1974).

[f] Young (1967); Fast (1970).

[g] Barlow (1964); Oudejans *et al.* (1971); Van Der Horst *et al.* (1972); Uscian & Stanley-Samuelson (1994).

[h] Fast (1964); Barlow (1964); Young (1967); Nakasone & Ito (1967); Fast (1970).

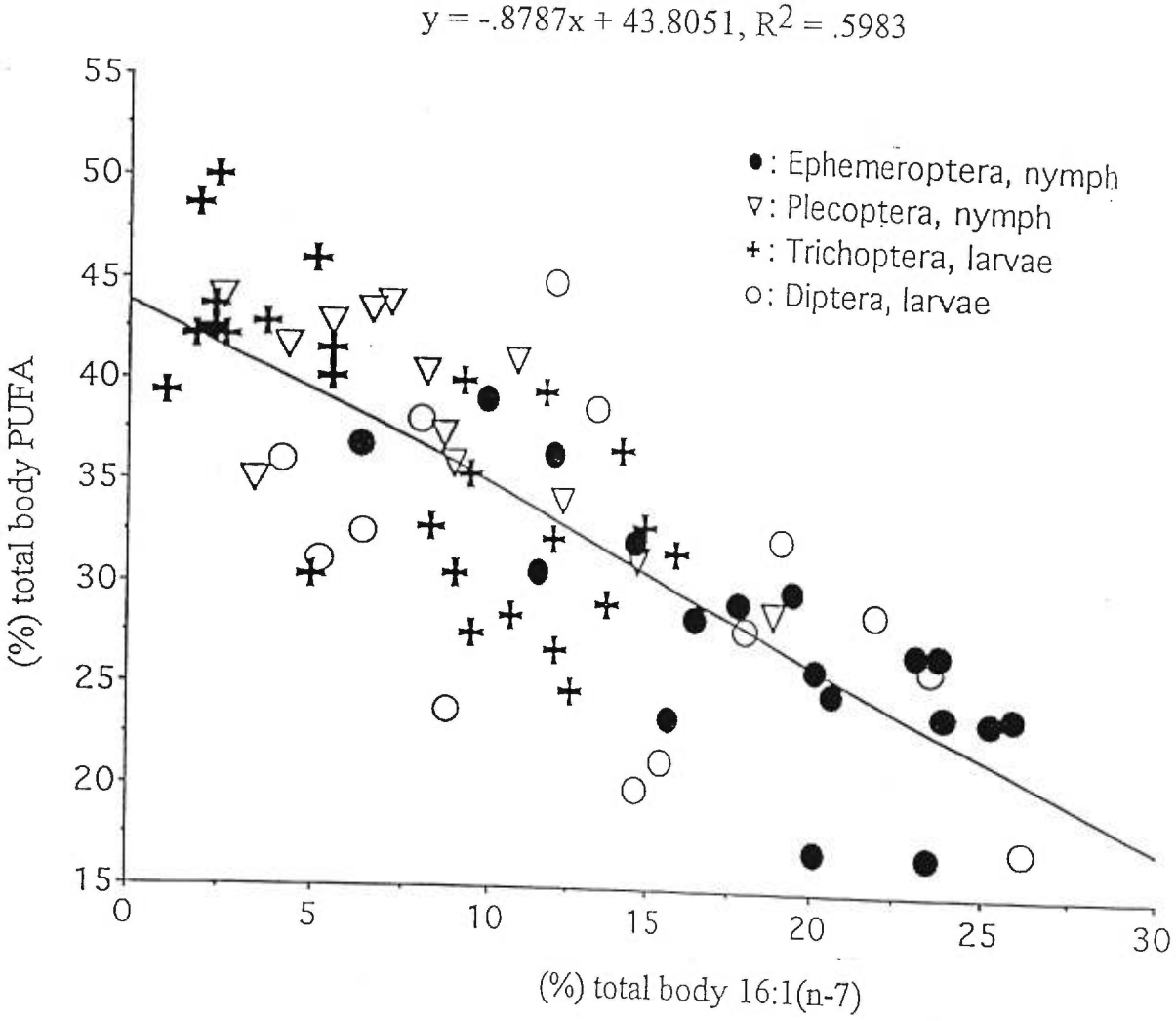


FIG. 3. Negative correlation between total PUFA and palmitoleic acid (16:1(n-7)) in aquatic insects species.
Adapted from Hanson *et al.* (1985), Bell *et al.* (1994b).

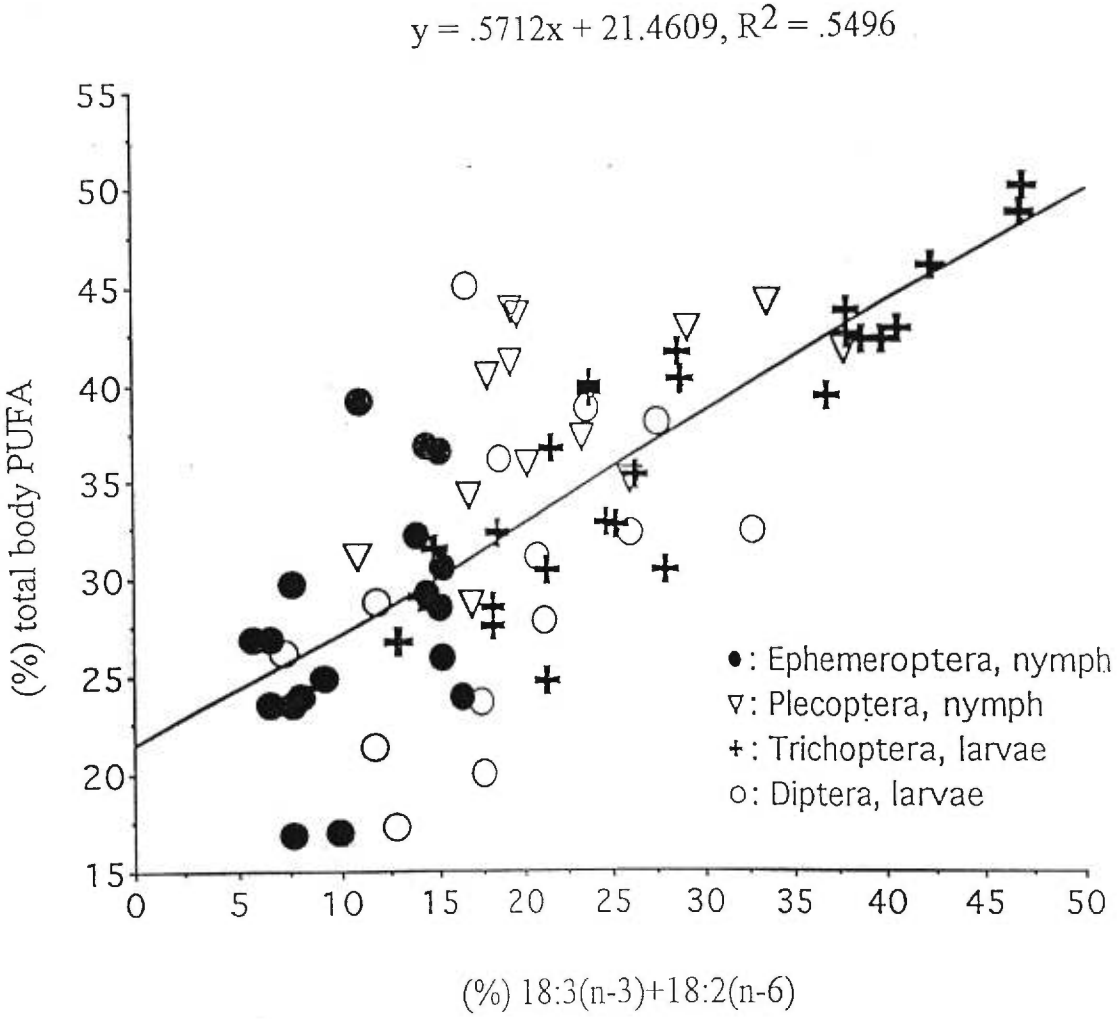


FIG. 4. Positive correlation between total PUFA and 18:3(n-3)+18:2(n-6) in aquatic insects species.
Adapted from Hanson *et al.* (1985), Bell *et al.* (1994b).

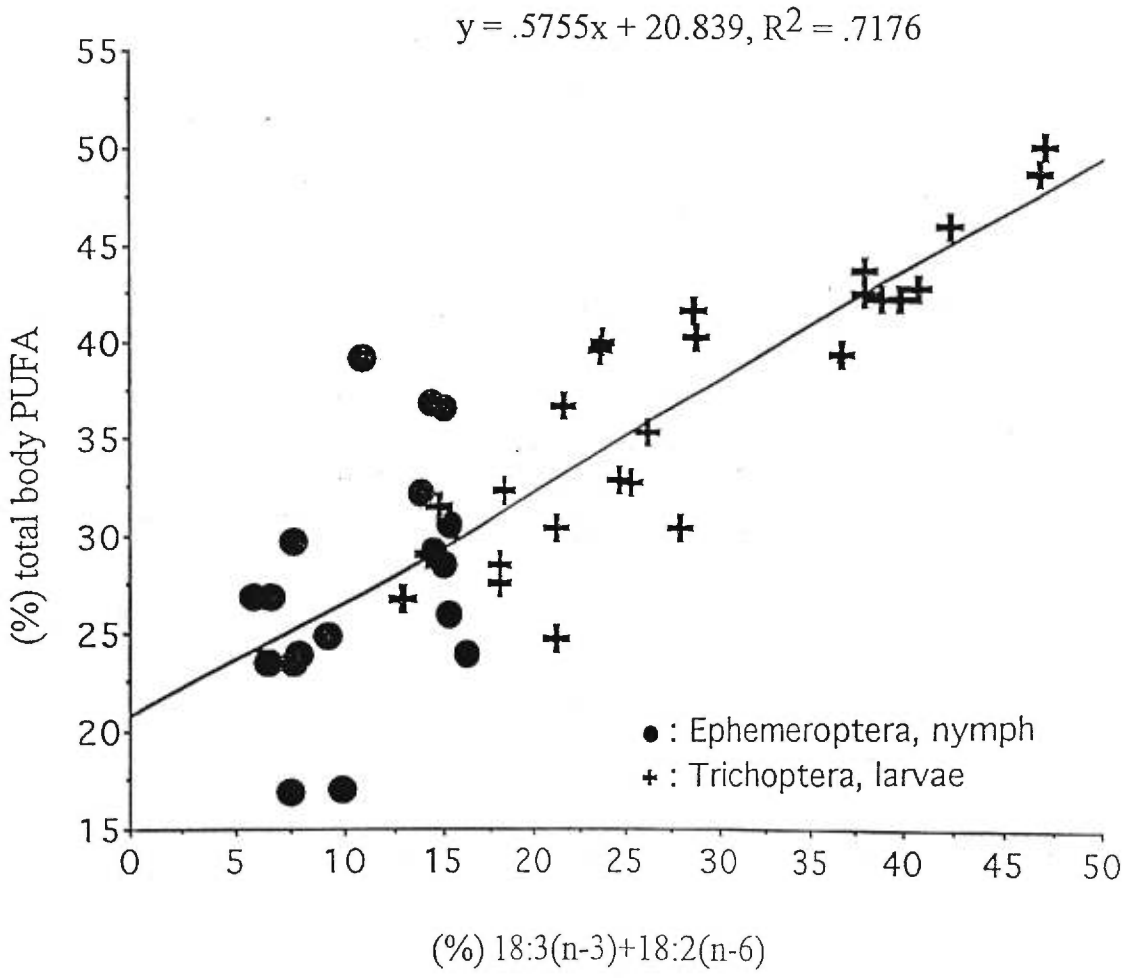


FIG. 5. Positive correlation between total PUFA and 18:3(n-3)+18:2(n-6) in Ephemeroptera and Trichoptera insects species.
Adapted from Hanson *et al.* (1985), Bell *et al.* (1994b).

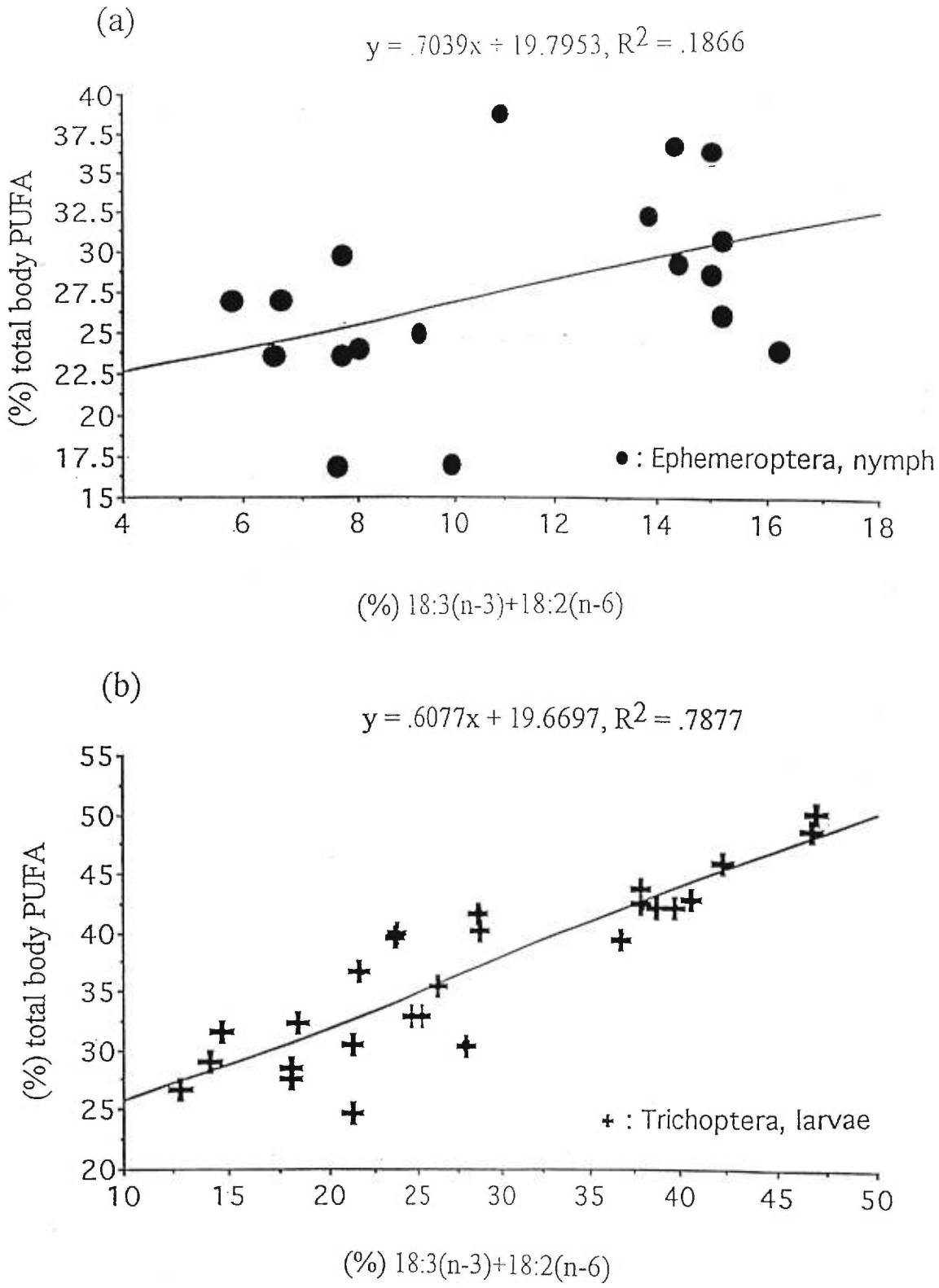


FIG. 6. Positive correlation between total PUFA and 18:3(n-3)+18:2(n-6) in Ephemeroptera (a) and Trichoptera (b) insects species.
Adapted from Hanson *et al.* (1985), Bell *et al.* (1994b).

CONCLUSION

Les besoins lipidiques, en particulier les acides gras essentiels, varient d'une espèce de poisson à l'autre selon leurs besoins spécifiques en acides gras (n-3) et (n-6) et leur habileté à métaboliser 18:3(n-3) et 18:2(n-6). Le rôle, la fonction et l'essentialité de ces acides gras insaturés restent encore à être étudiés, mais il est évident que les acides gras essentiels doivent être associés avec l'alimentation des poissons en pisciculture. Ces deux acides gras essentiels (18:3(n-3),18:2(n-6)) sont métabolisés de la même façon pour donner les longues chaînes d'acides gras polyinsaturées, en particulier les acides gras polyinsaturées C-20 et C-22, qui possèdent des propriétés physiologiques importantes pour les saumons. Les fonctions et les rôles essentiels des acides gras polyinsaturées dépendent de leurs concentrations dans les différentes classes de lipides telles que les phospolipides et les triglycérides.

Une diète non équilibrée en acides gras essentiels cause une insuffisance de la synthèse de acides gras polyinsaturées, responsable de certains changements physiologiques et biochimiques. Conséquemment, une altération dans la composition membranaire en acides gras polyinsaturées peut expliquer les lésions histologiques ou morphologiques; des symptômes qui ont été rapportés dans des études nutritionnelles. Il ne peut être exclu que certains symptômes reliés à des déficiences en acides gras essentiels soit associés à des molécules biologiquement actives telles que les eicosanoides.

Dans cette thèse, je montre que plusieurs fonctions physiologiques du poisson sont directement liées aux types de acides gras polyinsaturées et au rapport alimentaire entre (n-

3) et (n-6) acides gras présent dans la diète. Jusqu'à maintenant, il n'y a aucune publication qui a étudié la composition en lipides des invertébrés aquatiques et terrestres constituant l'alimentation du saumon sauvage. Ces connaissances permettraient une meilleure préparation de la diète qui respecterait le besoin nutritionnel pour (n-3) et (n-6) acides gras. Finalement, on doit conclure que le rendement économique de la pisciculture dépend d'une connaissance plus approfondie des acides gras essentiels dans l'alimentation des saumons. Ainsi, la diète des jeunes saumons doit se rapprocher davantage du contenu en acide gras des insectes d'eau douce (particulièrement les Ephéméroptères). Une telle diète équilibrée en acides gras (n-3) et (n-6) est d'une importance capitale dans l'élevage du saumons.

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