

# QUALITATIVE AND QUANTITATIVE NUTRIENT REQUIREMENTS OF FISHES: A REVIEW<sup>1</sup>

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## ABSTRACT

Qualitative and quantitative protein, amino acid, lipid, fatty acid, carbohydrate, vitamin, and mineral requirements are summarized for salmonids and warmwater fish species. Special emphasis is placed upon amino acid, vitamin, and mineral requirements of fishes, since recent research with these nutrients has contributed to a better understanding of fish physiology and nutritional requirements. Protein requirements of fishes briefly stated are as follows: 30 to 55% dietary protein dependent upon age and feeding habit and dietary essential amino acids which include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Dietary lipid concentrations as high as 12 to 24% have demonstrated sparing action of protein for growth rather than energy utilization in fishes. Essential fatty acids for fishes usually include linolenic acid or an elongated form in the  $\omega$ 3 fatty acid family, while some fish species appear to derive nutritional benefit from linoleic acid. Vitamins required by fishes in the diet include thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, ascorbic acid, choline, folic acid, cyanocobalamin, biotin, inositol, vitamin A, cholecalciferol, vitamin E, and vitamin K. Minerals required in the diet of fishes include phosphorus, magnesium, and trace amounts of manganese, zinc, iron, copper, selenium, and iodine. Carbohydrate is an important dietary energy source for herbivorous and omnivorous species and can be incorporated into diets of carnivorous species for protein sparing action at slightly higher concentrations than those occurring in their natural diets.

A paucity of information exists concerning qualitative and quantitative nutrient requirements of various life stages of several species currently reared in large quantities in fish hatcheries. Interactions of various macronutrients and micronutrients as related to artificial diet formulation for fish culture are also discussed. Additionally, recommendations for future research are outlined.

Determination of nutrient requirements for fishes fed formulated diets has contributed to healthier, more rapidly growing fishes cultured in hatcheries and commercial facilities. For instance, formulated diets designed specifically for several salmonid species and channel catfish, *Ictalurus punctatus*, have been instrumental in the recent growth of salmon, trout, and catfish commercial culture. Recent production values of catfish, trout, and salmon by aquaculture in the United States are listed in Table 1. Information has also been gained on many physiological similarities and differences between fishes and other aquatic and terrestrial species.

Reviews dealing with nutrient requirements of fishes include those by Halver (1972a) and the National Research Council (1973, 1977, 1981). Halver (1972a) dealt with macro- and micronutrient requirements of fishes, known and pro-

TABLE 1.—United States aquaculture production of catfish, trout, and salmon, 1979 and 1980.<sup>1</sup>

Species	1979		1980	
	Metric tons	Thousands dollars	Metric tons	Thousands dollars
Catfish	18,415.9	28,800	34,790.6	53,600
Salmon	1,088.6	900	3,447.3	3,400
Trout	11,339.8	21,000	21,772.5	37,500

<sup>1</sup>Adapted from Thompson (1981). Data represent live weight harvest for consumption. Excluded are eggs, fingerlings, and other intermediate products.

posed biochemical pathways in fishes, feed formulation strategy, fish cultural practices, and feeding aspects of fish culture. However, since this thorough description of nutrient requirements of fishes, much additional information has been gained on fish nutrition. Synopses have been compiled of chemical composition of feed ingredients commonly used in formulated fish feeds, recommended test diet ingredient composition for determination of nutrient requirements of fishes, and feed formulation strategies on use of various combinations of protein or lipid sources (National Research Council 1973, 1977, 1981). Brief reviews of qualitative and quantita-

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tive nutrient requirements for specific groups of fishes are available. Included are those for trout, salmon, and catfish (National Research Council 1973), warmwater fishes such as common carp, *Cyprinus carpio*, channel catfish (National Research Council 1977), and salmonids (National Research Council 1981). Cowey and Sargent (1979) reviewed recent advances in protein, lipid, amino acid, fatty acid, vitamin, and mineral requirements of fishes, since their earlier work (Cowey and Sargent 1972). Special emphasis was placed on biochemical pathways in fish. However, only selected vitamin and mineral nutrient requirements of fishes were discussed, and no attempt was made to summarize, in total, the nutrient requirements of fishes. Also, a brief review of qualitative amino acid, fatty acid, vitamin, and mineral requirements of fishes is available in tabular form (Ketola 1977).

Currently, there is a paucity of information on nutrient requirements of fry and fingerling stages of coolwater fishes having commercial or recreational value (Ketola 1978; Orme 1978). Such species include the largemouth bass, *Micropterus salmoides*; smallmouth bass, *M. dolomieu*; yellow perch, *Perca flavescens*; northern pike, *Esox lucius*; muskellunge, *E. masquinongy*; tiger muskie hybrid (male northern pike  $\times$  female muskellunge); and walleye, *Stizostedion vitreum vitreum*. Also, nutrient requirements for striped bass, *Morone saxatilis*, (a coolwater or warmwater species depending upon the region of occurrence) are lacking except for protein requirements of the fingerling stage as a function of dietary lipid content (Millikin in press).

The present review provides an updated summary of qualitative and quantitative protein, amino acid, lipid, fatty acid, vitamin, and mineral requirements for all groups of fishes. Nutritive values of various feedstuffs, e.g., protein and lipid sources, and their relative contributions to cost-effective feed formulation, are not covered in this review.

Some topics important in fish nutrition that are not reviewed in the present article are metabolizable energy values of specific nutrients and feedstuffs for fishes and antinutritional factors often occurring in certain feedstuffs incorporated into commercial fish feeds. Metabolizable energy values of rainbow trout, *Salmo gairdneri*, have been examined for carbohydrates (Smith 1971), proteins (National Research Council 1981), and feedstuffs (Smith 1976; Smith et al. 1980; National Research Council 1981). Also, variabil-

ity in heat increment as a portion of metabolizable energy resulting from protein, carbohydrate, or lipid ingestion was examined in rainbow trout and Atlantic salmon, *S. salar*, fingerlings (R. R. Smith et al. 1978). Important antinutritional factors often occurring in commercial-type diets for fishes are antitrypsin activity in soybeans (Sandholm et al. 1976), mycotoxins in peanut meal and cottonseed meal (Sinnhuber et al. 1977), gossypol in cottonseed meal (Ashley 1972), and cyclopropanoid fatty acids as cocarcinogens fed simultaneously with aflatoxins to rainbow trout (Sinnhuber et al. 1968). An informative review of antinutritional factors in fish feeds is included in a review of nutrient requirements of coldwater fishes (National Research Council 1981).

## PROTEIN

Optimal dietary protein concentrations for fish are dictated by a delicate balance of dietary protein-to-energy ratio, plus protein quality (amino acid balance), and nonprotein energy sources (i.e., amount of fat in relation to carbohydrate). Excessive nonprotein energy intake resulting from high digestible energy-to-dietary-protein ratios often causes cessation of feeding before sufficient protein is consumed, since ingestion rate is primarily determined by total available dietary energy content (Page and Andrews 1973). Conversely, slow growth rates may result from low nonprotein energy intake or formula diets may simply be less cost-effective for fish farming purposes. Dietary protein in excess of that required for growth is often utilized for energy in fishes (Cowey 1979). For example, increased gluconeogenesis was demonstrated, as a result of higher activities of the gluconeogenic enzymes, fructose diphosphate, and phosphoenolpyruvate carboxykinase, in rainbow trout fed high dietary protein concentrations (Cowey et al. 1981b). Finally, dietary amino acid imbalances may result in higher dietary protein concentrations than that required for maximal growth as well as antagonisms between some amino acids, such as isoleucine and leucine in chinook salmon, *Oncorhynchus tshawytscha*, (Chance et al. 1964), isoleucine, leucine, and valine in channel catfish (Robinson et al. 1982), or lysine and arginine in salmonids (Rumsey<sup>3</sup>).

<sup>3</sup>Gary L. Rumsey, Tunison Laboratory of Fish Nutrition, U.S. Fish and Wildlife Service, Cortland, NY 13045, pers. commun. December 1981.

Since dietary protein quantity and quality are major determinants of growth in fish, numerous investigations have been conducted to determine protein requirements for specific fish species. Protein requirement studies that examine various concentrations of dietary protein with higher carbohydrate concentrations being substituted for protein in lower protein diets often produce reliable approximations of quantitative dietary protein requirements (Table 2). However, accord-

generally decrease with increasing age or fish size. For example, salmonids require about 50% protein<sup>4</sup> during the initial feeding stage of fry, decreasing to 40% protein after 6 to 8 wk, with a further reduction to 35% protein for yearlings (National Research Council 1973). Channel catfish fry require a minimum of 40% protein, decreasing to 30 to 36% for fingerlings and 25 to 30% protein for subadults weighing >114 g (National Research Council 1977; Andrews 1977).

TABLE 2.—Quantitative protein requirements of several fish species.

Species	Initial mean weight	Protein requirement, % dry diet	Study duration (wk)	Rearing temperature (°C)	Criteria <sup>1</sup>	Reference
<i>Salmo gairdneri</i>	6.9	40	10	16-27	G,FC,BP	Satia (1974)
	1.3	45	10	10	G,FC	Halver et al. (1964)
	61.0	42	22	8-12	G,BP	Austreng and Refstie (1979)
	0.7	40	32	15	G,FC	Cho et al. (1976)
	6.5-7.0	<sup>2</sup> 40-45	10	9-12.5	G,PER,FC	Zeitoun et al. (1973)
<i>Oncorhynchus kisutch</i>	14.5	40	10	6.5-10.5	G	Zeitoun et al. (1974)
<i>O. tshawytscha</i>	1.5	55	10	15	G	DeLong et al. (1958)
	1.5	40	10	8	G	DeLong et al. (1958)
<i>O. nerka</i>	1.15	45	10	10	G,FC	Halver et al. (1964)
<i>Micropterus salmoides</i>	?	40	2-8	23	G,FC	Anderson et al. (1981)
<i>M. dolomieu</i>	?	45	4-9	20.5	G,FC	Anderson et al. (1981)
<i>Morone saxatilis</i>	2.25	<sup>3</sup> 55	6	24.5±2	G,FC	Millikin (1982)
	1.41	47	10	20.5	G,FC	Millikin (in press)
<i>Pleuronectes platessa</i>	14.5	50	12	15	G	Cowey et al. (1972)
<i>Fugu rubripes</i>	2.0	50	3	25-26	G,FC	Kanazawa et al. (1980b)
<i>Anguilla japonica</i>	3.1	44.5	8	25	G,BP	Nose and Aral (1972)
<i>Tilapia zilli</i>	1.65	35	3	24-26	G	Mazid et al. (1979)
<i>T. aurea</i>	0.4	<sup>3</sup> 36	12	26-29	G	Davis and Stickney (1978)
<i>Ictalurus punctatus</i>	?	35	26	?	G,FC	Lovell (1972)
	10-25	35	8	28	G,PER	Murray et al. (1977)
<i>Chanos chanos</i>	0.04	40	4	25-28	G,FC	Lim et al. (1979)
<i>Ctenopharyngodon idella</i>	0.15-0.20	41-43	6	22-23	G,PER	Dabrowski (1977)
<i>Cyprinus carpio</i>	5.8	38	4	?	G	Ogino and Saito (1970)
<i>Chrysophrys aurata</i>	2.6	38	16	?	G,FC	Sabaut and Luquet (1973)

<sup>1</sup>G = growth, FC = feed conversion, BP = body protein, PER = protein efficiency ratio.

<sup>2</sup>Protein requirement increased from 40 to 45% as salinity increased from 10 to 20‰.

<sup>3</sup>Highest protein concentration examined.

ing to Rumsey (1978), protein sources generally have higher metabolizable energy values than carbohydrate sources for salmonids. Thus, in many dietary protein requirement studies, fishes were probably offered higher metabolizable energy values in the high protein diets compared with the low protein diets. Since fish fed low protein diets may have had less available energy, additional protein may have been shunted for metabolic requirements other than growth. Many of the quantitative protein requirements, listed in Table 2, may be overestimated values due to this shift in utilization of protein in low protein diets (Rumsey 1978). Therefore, protein-energy studies examining several energy concentrations within each of several dietary protein concentrations provide better estimates of quantitative dietary protein requirements of fishes.

Dietary protein concentration requirements

The higher protein concentrations in these two ranges produce better growth of channel catfish fingerlings and subadults, whereas the lower protein concentrations provide better protein conversion (weight protein fed ÷ weight gain) (Andrews 1977).

Increased water temperature has variable effects on the minimal protein or energy requirement for maximal growth rate of fishes. For example, chinook salmon fingerlings require 40% protein at 8°C and 55% protein at 15°C (DeLong et al. 1958). Striped bass fingerlings (initial mean weight = 1.4 g) require 47% protein at 20.5°C, while additional dietary protein is required (about 55%) at 24.5°C for maximal growth of slightly larger fingerlings (initial mean

<sup>4</sup>Nutrient content of diets is expressed as percentage of the diet on a dry weight basis, unless otherwise noted.

weight = 2.2 g) (Millikin in press and 1982, respectively). In separate studies with rainbow trout fingerlings, 35% dietary protein provided as good a growth rate as 40 or 45% protein within any of several temperature regimes (National Research Council 1981). Fingerlings (mean initial weight = 2.0 g) fed either 35, 40, or 45% protein grew equally well within any one temperature regime (9°, 12°, 15°, and 18°C) over a 16-wk period (Slinger et al. 1977, cited in National Research Council 1981). Slightly larger rainbow trout (mean initial weight = 3.45 g) also grew equally well when fed 35, 40, or 45% dietary protein within any one of three temperature regimes (9°, 12°, or 18°C) over a 24-wk period (Cho and Slinger 1978, cited in National Research Council 1981). Growth rates were progressively higher at each successive increase in rearing temperature, regardless of dietary protein concentration, except for 18°C in the second study. Increased feed consumption of lower protein diets occurred when rainbow trout were reared at higher temperatures and probably satisfied higher protein requirements at elevated water temperatures (National Research Council 1981). Chinook salmon fry (0.4 g) require 53% dietary protein (dry weight basis) combined with 16% dietary lipid (dry weight basis) when reared at 5° or 12°C based upon weight gain and survival rates (Fowler 1980, 1981). However, growth rates were two to three times more rapid for chinook salmon fry reared at 12°C.

Changes in salinity may alter protein requirements of anadromous or euryhaline species. Rainbow trout fingerlings require 45% protein for optimal growth at 20‰ compared with a 40% protein requirement at 10‰ (Zeitoun et al. 1973). Since a salinity of 10‰ is almost isotonic with internal fluids (9‰) of rainbow trout fingerlings, the higher dietary protein requirement for rainbow trout reared in a salinity of 20‰ suggests that the higher dietary protein concentration may assist in osmoregulation in a hypertonic external environment for this species (Zeitoun et al. 1973). Conversely, coho salmon, *O. kisutch*, smolts require 40% protein in 10 and 20‰. Although maximum weight gain occurred at 40% protein in both salinities, maximum protein retention occurred at 40% protein in 10‰ and 50% protein at 20‰ (Zeitoun et al. 1974). The authors concluded that the hyperosmotic environment (20‰) did not stress coho salmon smolts in the same manner as previously shown with smaller rainbow trout fingerlings. Also, underyearling rain-

bow trout (mean weight = 70 g) require more dietary arginine (1.2% of the diet) when reared in freshwater than in those individuals reared in 20‰ (1.0% arginine of the diet) (Kaushik 1977, cited in Poston 1978). Further work is necessary to more firmly establish whether protein requirements change with salinity for specific life stages of various anadromous or catadromous fish species.

## AMINO ACIDS

### Qualitative and Quantitative Requirements

Examination of qualitative amino acid requirements of fishes has often been based upon growth and feed efficiency in long-term feeding studies. Typically, one of several amino acids is removed singly from a well-defined formula diet which is assumed to be nutritionally complete (i.e., positive control), to determine if significant reduction in weight gain occurs in fish fed the selected, amino acid-deficient diets compared with growth of fish fed the control diet. Thereafter, any group of fish fed a diet determined to be deficient in an amino acid, as indicated by reduced growth and feed efficiency, is separated into two subgroups: One subgroup is retained on the amino acid-deficient diet (control diet minus one amino acid), whereas the other subgroup is fed the control diet. Reduced growth rate or cessation of growth in fish fed the amino acid-deficient test diet versus the control diet is considered to be confirmation of a dietary requirement for the specific amino acid being tested. On the basis of such amino acid feeding studies, several fish species have been found to require the same 10 amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) as essential dietary constituents. Species requiring dietary inclusion of these amino acids include chinook salmon (Halver et al. 1957); rainbow trout (Shanks et al. 1962); sockeye salmon, *O. nerka* (Halver and Shanks 1960); channel catfish (Dupree and Halver 1970); Japanese eel, *Anguilla japonica*, and European eel, *Anguilla anguilla* (Arai et al. 1972b); common carp (Nose et al. 1974); red sea bream, *Chrysophrys major* (Yone 1975); and red-belly tilapia, *Tilapia zilli* (Mazid et al. 1978).

Qualitative amino acid requirements of plaice, *Pleuronectes platessa*, and sole, *Solea solea*, were investigated, using intraperitoneal injections of uniformly labelled - <sup>14</sup>C-glucose into individuals

of the two fish species (Cowey et al. 1970). Formation of radioactive labelled aspartic acid, glutamic acid, cysteine, serine, glycine, alanine, and proline over a 6-d period implied that sufficient amounts of these amino acids can be produced by underyearling plaice and sole through intermediary metabolism, thus suggesting dietary nonessentiality of those specific amino acids. On the other hand, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine were not incorporated from (U-<sup>14</sup>C) - glucose, thus implying dietary essentiality. Although the authors suggested that metabolic requirements for tyrosine were provided from hydroxylation of ingested phenylalanine, this still needs to be tested. Possible essentiality of tryptophan was not examined, thereby leaving the status of this amino acid unresolved for the plaice and sole.

Quantitative amino acid requirements determined for several fish species are generally based on weight gain, feed efficiency, and sometimes free amino acid plasma levels of individuals fed graded concentrations of a particular amino acid (Table 3). In addition to those values listed in Table 3, coho salmon have been shown to require 2.4% arginine of the dry diet (6.0% of the dietary protein), 0.7% histidine of the dry diet (1.7% of the dietary protein) (Klein and Halver 1970), and 0.2% tryptophan of the dry diet (0.5% of dietary protein) (Halver 1965). Many similarities exist between species in individual quantitative amino acid requirements when expressed as a percent of dietary protein (Table 3).

Amino acid composition of eggs and larval stages for a given species has been shown to be a

good guideline for estimating quantitative amino acid requirements of fry and fingerling stages. For example, diets formulated to contain the amino acid composition of Atlantic salmon eggs promoted better growth of Atlantic salmon fingerlings than use of an amino acid pattern based on the National Research Council's (1973) recommendations for salmonids (Ketola 1980). Ketola (1980) also observed the same pattern of accelerated growth in rainbow trout fry fed diets formulated on the basis of egg amino acid composition of that species compared with the National Research Council's (1973) recommendations. In a separate study, rainbow trout fingerlings were fed a diet containing soybean meal as the sole protein source or the same diet supplemented with amino acids (leucine, methionine, lysine, valine, and threonine) to provide a dietary essential amino acid profile similar to rainbow trout eggs. Improved weight gain occurred in rainbow trout fingerlings fed the amino acid supplemented diet, thereby suggesting the similarity between amino acid profiles of rainbow trout eggs and dietary amino acid requirements of rainbow trout fingerlings (Rumsey and Ketola 1975).

### Amino Acid Availability

A significant contribution to channel catfish diet formulation recently came from an extensive investigation of true amino acid availability (corrected for metabolic fecal amino acids) and apparent amino acid availability (digestibility) values of various feedstuffs commonly incorporated into commercial catfish diets (Wilson et al.

TABLE 3.—Quantitative dietary amino acid requirements for several fish species.<sup>1</sup>

Amino acid	Channel catfish <sup>2</sup>	Chinook salmon <sup>3</sup>	Japanese eel <sup>4</sup>	Common carp <sup>5</sup>
Arginine	4.3 (1.0)	6.0 (2.4)	4.5 (1.7)	4.2 (1.6)
Histidine	1.5 (0.4)	1.8 (0.7)	2.1 (0.8)	2.1 (0.8)
Isoleucine	2.6 (0.6)	2.2 (0.9) <sup>6</sup>	4.0 (1.5)	2.3 (0.9)
Leucine	3.5 (0.8)	3.9 (1.6) <sup>6</sup>	5.3 (2.0)	3.4 (1.3)
Lysine	5.1 (1.2)	5.0 (2.0)	5.3 (2.0)	5.7 (2.2)
Methionine	2.3 (0.6) <sup>7</sup>	4.0 (1.6) <sup>8</sup>	5.0 (1.9) <sup>9</sup>	3.1 (1.2) <sup>9</sup>
Phenylalanine	5.0 (1.2) <sup>10</sup>	5.1 (2.1) <sup>9,11</sup>	5.8 (2.2) <sup>11</sup>	6.5 (2.5) <sup>11</sup>
Threonine	2.3 (0.5)	2.2 (0.9)	4.0 (1.5)	3.9 (1.5)
Tryptophan	0.5 (0.1)	0.5 (0.2)	1.1 (0.4)	0.8 (0.3)
Valine	3.0 (0.7)	3.2 (1.3)	4.0 (1.5)	3.6 (1.4)

<sup>1</sup>Expressed as percentage of dietary protein with requirement as percentage of dry diet in parentheses.

<sup>2</sup>Based upon 24% dietary protein (Robinson et al. 1980a).

<sup>3</sup>Based upon 40% dietary protein unless otherwise noted (National Research Council 1973).

<sup>4</sup>Based upon 37.7% dietary protein (Nose and Arai Unpubl. data, cited in Cowey and Sargent 1979).

<sup>5</sup>Based upon 38.5% dietary protein (Nose 1979).

<sup>6</sup>Based upon 41% dietary protein (National Research Council 1973).

<sup>7</sup>In the absence of cystine which can replace 50 to 60% of methionine requirement (Harding et al. 1977).

<sup>8</sup>Methionine + cystine.

<sup>9</sup>In the absence of cystine.

<sup>10</sup>Phenylalanine + tyrosine requirement. Tyrosine can replace ca. 50% of phenylalanine (Robinson et al. 1980a).

<sup>11</sup>In the absence of tyrosine.

1981). Results generally suggested that reasonable agreement occurs between average apparent amino acid availability and protein digestibility values of any one specific protein source. However, individual amino acid availabilities were quite variable within and among various feed ingredients tested. Also, apparent amino acid availability values were considerably less than true amino acid availability values for feed ingredients containing relatively low protein content (e.g., 9 to 19%), such as rice bran, rice mill feed, wheat middlings, and corn.

### Lysine

Dietary lysine requirements for fishes range from 5.0 to 6.8% of the dietary protein. In addition to the quantitative lysine requirements listed in Table 3, rainbow trout fry require 6.8% lysine and lake trout, *Salvelinus namaycush*, fry require 6.0% lysine as a percentage of total dietary protein (Ketola 1980). Robinson et al. (1980b) reported a dietary lysine requirement of 5% of the dietary protein for channel catfish fed an adequate dietary protein concentration (30%); thus confirming a dietary lysine requirement (5.1% of the dietary protein) for channel catfish fed a marginal dietary protein concentration of 24% (Wilson et al. 1977). Excessive dietary lysine in the presence of marginal or adequate dietary arginine concentrations did not depress growth or feed efficiency of channel catfish, nor did excessive arginine depress growth or feed efficiency of channel catfish in the presence of marginal dietary lysine concentrations (Robinson et al. 1981). This is in contrast to lysine-arginine antagonisms reported for several terrestrial species (Maynard and Loosli 1969).

Lysine deficiency in fish may conceivably result in depressed rates of collagen formation. Hydroxylysine has been shown to be a constituent of collagen in several fish species (Mehrlé<sup>5</sup>). Fin rot occurred in rainbow trout fed a lysine-deficient diet containing corn gluten meal as the sole protein source (Ketola 1979a). Supplementation of a combination of lysine, arginine, histidine, isoleucine, threonine, tryptophan, and valine to the corn gluten meal diet resulted in improved survival, increased growth, and prevention of severe caudal fin erosion. At the same time,

removal of lysine from the amino acid mixture in the corn gluten meal supplemented diet increased mortality, reduced growth, and resulted in caudal fin erosion of rainbow trout.

### Methionine

Quantitative dietary methionine requirements for several fish species have been shown to depend upon dietary cystine concentration, since cystine can substitute for a portion of the methionine requirement. This is the result of the conversion of methionine to cystine being a common pathway of intermediary metabolism in many terrestrial animals (Maynard and Loosli 1969) as well as fish (National Research Council 1973). Rainbow trout fingerlings require 0.6% methionine (1.7% of the dietary protein) and 0.45% cystine (1.29% of the dietary protein), a total sulfur amino acid requirement of 1.05% of the diet (2.99% of dietary protein). This is based upon growth and feed efficiency as demonstrated by feeding studies, using a factorial design of 0.3 to 0.75% methionine and 0.04 to 0.6% cystine (Page 1978). Excessive cystine for rainbow trout (e.g., 0.6%) did not partially satisfy methionine requirements in methionine-deficient diets (0.3 and 0.45% methionine) based on weight gain and feed conversion. Chinook salmon require 0.5 to 0.6% dietary methionine (1.3 to 1.5% of the dietary protein) in the presence of 1.0% dietary cystine, whereas 1.6% methionine did not produce maximum growth in the presence of 0.05% dietary cystine (Halver et al. 1959). Channel catfish require 0.56% dietary methionine (2.34% of the dietary protein) in the absence of cystine, while 60% of the methionine requirement is replaceable with cystine (Harding et al. 1977).

Methionine deficiency has been shown to result in cataractogenesis in lake trout fingerlings and rainbow trout fingerlings. After 12 wk, rainbow trout fingerlings (initial mean weight = 1.5 g) fed 0.6% methionine plus 0.3 or 0.45% cystine did not develop any cataracts (Page 1978). Lower dietary methionine content (0.3 or 0.45%) combined with dietary cystine content ranging from 0.04 to 0.6% produced varying degrees of cataracts in rainbow trout. In another study, lake trout fingerlings were fed methionine-deficient diets (0.36% methionine = 0.96% of the dietary protein) containing soybean protein isolate as the sole protein source (40% of the diet). After 8 wk, lake trout fingerlings showed only initial signs of opacification of subcapsular areas (Pos-

<sup>5</sup>Paul M. Mehrlé, Columbia National Fisheries Research Laboratory, U.S. Fish and Wildlife Service, Columbia, MO 65201, pers. commun. December 1981.

ton et al. 1977). However, after 16 wk, 100% of lake trout fed 0.36% dietary methionine developed bilateral lenticular cataracts, while none of the individuals fed the control diet containing 1.2% dietary methionine (3.27% of the dietary protein) had cataracts.

Attempts to supplement suboptimal dietary concentrations of methionine plus cystine with dietary taurine or inorganic sulfate have proven unsuccessful with channel catfish fingerlings and rainbow trout fingerlings. Channel catfish fingerlings fed dietary taurine or inorganic sulfate as a partial replacement for methionine had reduced growth rates (Robinson et al. 1978), whereas rainbow trout fingerlings had reduced growth and developed cataractogenesis (Page et al. 1978). The absence of cataractogenesis in methionine-deficient channel catfish may be the result of the relatively shorter duration of this study (8 wk) compared with a 16-wk study on lake trout (Poston et al. 1977). Another explanation for the absence of cataract formation in channel catfish may be due to the slightly larger initial size of channel catfish (mean weight = 7.7 g) compared with lake trout (mean weight = 5 g). Either of these factors may have produced a slower turnover rate of amino acids in channel catfish.

According to a summary by Poston et al. (1977), insufficient methionine often results in reductions in sulfhydryl group concentrations, and lens glutathione synthesis also decreases rapidly during formation of most cataracts. The authors speculated that lens glutathione possibly protects the lens sulfhydryl groups from oxidation.

### Tryptophan

Tryptophan deficiency symptoms have been described in rainbow trout; sockeye salmon; brook trout, *S. fontinalis*; and channel catfish. Tryptophan deficiency in rainbow trout and sockeye salmon has resulted in scoliosis (Shanks et al. 1962; Halver and Shanks 1960, respectively). In a separate study with rainbow trout, hyperemia, scoliosis, and abnormal deposition of calcium occurred in kidney and bony plates surrounding the notochord and sheath of fish fed tryptophan-deficient diets (Kloppel and Post 1975). The authors suggested that hyperemia might be attributed to a lack of serotonin resulting from a deficiency of its precursor, tryptophan. In discussing scoliosis, Kloppel and Post (1975) noted

that tryptophan is a major component of procollagen, a supposed precursor of collagen. However, the absence of scoliosis in channel catfish fingerlings fed tryptophan-deficient diets (Wilson et al. 1978), compared with studies on salmonids, probably resulted from a slower growth rate of channel catfish because of a larger initial mean weight (12.5 g).

Quantitative tryptophan requirements have been shown to be similar for three different salmonid species and channel catfish. Almquist-type plots of growth responses indicated dietary tryptophan requirements of 0.15 to 0.25% of the diet (0.4 to 0.6% of dietary protein) for chinook salmon and 0.20 to 0.25% of the diet (0.5 to 0.6% of dietary protein) for sockeye salmon and coho salmon (Halver 1965). Channel catfish fingerlings fed a tryptophan-deficient diet (0.05%) for 8 wk had significantly poorer weight gain and feed efficiency than individuals fed diets containing as low as 0.12% tryptophan (0.5% of dietary protein) (Wilson et al. 1978). Wilson et al. (1978) suggested that the lower dietary tryptophan requirement of fishes compared with that of terrestrial animals may be due to an inability of fish to convert tryptophan to niacin, thus reducing the metabolic need of tryptophan in fishes as compared with terrestrial species. Growth rate of brook trout and the low ratio of two enzyme activities (3-hydroxyanthranilic acid oxygenase to picolinic acid carboxylase) concerned with an intermediate of the conversion pathway of tryptophan to niacin indicated that dietary tryptophan is not an efficient niacin precursor for this species (Poston and DiLorenzo 1973). Additionally, a low ratio of the two liver enzyme activities exists in lake trout, rainbow trout, Atlantic salmon, and coho salmon compared with terrestrial vertebrates (Poston and Combs 1980).

### LIPIDS

#### Optimal Dietary Lipid Concentrations and Protein-to-Energy Ratios

Optimal dietary lipid concentrations for inclusion in formulated feeds for fishes involve consideration of several factors. The minimal dietary lipid concentration that maximizes dietary protein available for growth rather than energy (i.e., protein sparing) may be excessive for diet incorporation if fish are being cultured as a lean product for human consumption, or if freezer storage space is unavailable to retard develop-

ment of oxidative rancidity of the diets. However, if fish are being hatchery cultured for release into natural waters, high body lipid composition may be beneficial as an energy source during acclimation to natural food (Wedemeyer et al. 1980). Also, feeds that contain the minimal dietary lipid concentration required for maximal protein sparing action for a particular species are relatively cost-effective.

Protein sparing action of various dietary lipid concentrations has been examined for several fish species. Channel catfish fingerlings (mean initial weight = 1.25 g) cultured at 26.7° to 32.2°C and fed 35% protein diets grew faster on 8% dietary lipid, either as beef tallow or corn oil, than on 4% of either dietary lipid source (Dupree 1969a). Dietary lipid concentrations of 16% corn oil or beef tallow reduced growth rate and protein deposition of channel catfish compared with 8% dietary lipid. Therefore, maximal protein sparing action occurred at <16% of either dietary lipid source but ≥8% dietary lipid. In a separate study, larger channel catfish fingerlings (initial mean weight = 7.0 to 7.5 g), cultured at 30°C and fed 25% protein diets containing either 5, 8, 12, 15, or 20% bleached menhaden oil, had maximal weight gain and protein deposition when fed 25% protein with 15% lipid (Dupree et al. 1979). Channel catfish fingerlings (either 0.5 or 1.0 g initial mean weight in separate experiments) reared at 28°C grew faster when fed 35% protein plus 12% lipid, rather than 5% lipid combined with 25 or 35% protein or 12% lipid combined with 25% protein (Murray et al. 1977). Conversely, when channel catfish reared at 23°C were fed 25 or 35% protein combined with 5 or 12% lipid, 5% lipid plus 25 or 35% protein was sufficient for maximal weight gain and food conversion, probably due to lower metabolic requirements. Channel catfish with initial and final mean weights of 14 and 100 g, respectively, required 35% protein and 12% lipid, whereas subadults weighing 114 to 500 g required 25% protein and 12% lipid when reared at 27°C (Page and Andrews 1973). Protein-to-energy ratio requirements of channel catfish fingerlings (initial mean weight = 7.0 g) as a function of protein deposition were found to be 88 mg protein/kcal between dietary energy concentrations of 275 to 341 kcal/100 g when reared at 26.7°C (Garling and Wilson 1976). However, maximal weight gain occurred in fish fed 32 to 36% protein. Therefore, for the most cost-effective feed, the optimal dietary protein concentration for channel catfish

fingerlings reared at 26.7°C is between 24 and 32%, when considering weight gain, feed efficiency, and protein deposition. Rainbow trout fingerlings (initial mean weight = 4.8 g) reared at 12.2°C over an 18-wk period had equally good growth rates when fed 35% protein and 24% lipid as individuals fed 44 or 53% protein, each combined with either 8, 16, or 24% lipid. This indicated a minimal dietary lipid concentration of 24% for maximal protein sparing action and an optimal protein-to-energy ratio of 73 mg protein/kcal (Lee and Putnam 1973). Conversely, a study evaluating three dietary protein concentrations (33, 39, and 44%) combined with 22% lipid in each of three forms (22% herring oil, 14.6% herring oil plus 7.4% lard, or 11% herring oil plus 11% lard) in a 3 × 3 factorial showed that better growth was obtained in rainbow trout fingerlings (initial mean weight = 5.4 g) fed 44% protein and 22% lipid, regardless of the ratio of herring oil to lard when reared at 11.5°C over a 14-wk period (Yu et al. 1977). Protein efficiency ratios and protein retention values were similar, regardless of dietary protein concentration and lipid source combinations fed to rainbow trout fingerlings. The contradictory results of these two studies in the minimal dietary protein required (35% protein plus 24% lipid versus 44% protein plus 22% lipid) for maximal growth of rainbow trout fingerlings can be partially explained by differences in dietary fiber concentrations. A low dietary fiber concentration (6.5%) was incorporated in diets containing 36, 44, or 53% protein plus 24% lipid, whereas a high dietary fiber concentration (22.4%) was incorporated in diets containing 36, 44, or 53% protein plus 8% lipid (Lee and Putnam 1973). However, in the study by Yu et al. (1977), dietary fiber concentrations were held constant at 11.2% in all diets. Therefore, variable dietary fiber concentrations in the first study may have differentially affected amino acid absorption rates or feed utilization. Successive increases in dietary lipid concentration of 7, 11, or 16% in 30% protein diets or 9, 15, or 21% lipid in 40% protein diets resulted in increased weight gain and improved feed conversion of rainbow trout fingerlings (initial mean weight = 2 g) within each dietary protein concentration when reared at 11°C (Reinitz et al. 1978b). Minimal dietary protein and lipid concentration requirements were not determined, since the highest dietary protein (40%) plus lipid (21%) combination that was evaluated also produced maximal growth and optimal feed conversion ratios for rainbow trout fin-



gerlings. A separate study demonstrated that about 35% protein combined with either 23 or 27% dietary lipid provided better growth of rainbow trout fingerlings than 36% dietary protein combined with 14% dietary lipid (Reinitz and Hitzel 1980). However, the two high lipid concentration diets did not produce better growth rates of rainbow trout fingerlings than a 35% protein, 18% lipid diet. C. E. Smith et al. (1979) reported that rainbow trout brood stock fed high energy, high protein diets (16% lipid plus 48% protein or 17% lipid plus 49% protein) produced a greater volume of larger eggs than did fish fed diets low and intermediate in energy and protein (6% lipid plus 36% protein or 9% lipid plus 42% protein). However, considerable variation in protein (i.e., amino acid profiles) and lipid (i.e., fatty acid profiles) sources used between diets in this study makes interpretation of the data difficult. Striped bass fingerlings reared at 20.5°C and fed 37, 47, or 57% protein with 7, 12, or 17% lipid in a 3 × 3 factorial design showed maximal protein sparing action of lipid for growth when fed 12% lipid combined with 47% dietary protein or 17% lipid combined with 57% protein (Millikin in press). Juvenile turbot, *Scophthalmus maximus*, fed 35% protein combined with 3, 6, or 9% lipid plus 9 or 18% carbohydrate attained best growth and feed conversion when fed the diet containing 35% protein, 9% carbohydrate, and 9% lipid (Adron et al. 1976). Possibly, additional dietary lipid would have further spared protein for growth rather than energy. Also, a diet containing 35% protein, 9% carbohydrate, and 9% lipid produced similar growth and feed conversion of turbot when compared with a diet containing 55% protein, 9% carbohydrate, and 3% lipid. Juvenile blue tilapia, *Tilapia aurea*, with initial and final mean weights of 2.5 and 7.5 g, respectively, require about 56% protein and 460 kcal/100 g diet (123 mg protein/kcal) while fish >7.5 g required 34% protein and 320 kcal/100 g diet (109 mg protein/kcal) for maximum growth (Winfrey and Stickney 1981).

### Essential Fatty Acids

Inclusion of either linolenic acid (18:3 $\omega$ 3) or a more highly unsaturated fatty acid in the  $\omega$ 3 series in the diet of rainbow trout is essential. Rainbow trout fingerlings fed diets containing 1% 18:3 $\omega$ 3 as a supplement to 7.8% corn oil doubled their weight gain compared with individuals fed 10% corn oil as the sole dietary lipid

source (Lee et al. 1967). Castell et al. (1972a) determined that 1% 18:3 $\omega$ 3 or ethyl linolenate produced larger rainbow trout than 1% linoleic acid (18:2 $\omega$ 6) or ethyl linoleate. Additionally, 1% 18:3 $\omega$ 3 prevented essential fatty acid deficiency symptoms (e.g., fin erosion, heart myopathy, shock syndrome, and swollen, pale livers), while as much as 5% 18:2 $\omega$ 6 did not cure such symptoms (Castell et al. 1972b). Dietary linolenic acid's essentiality and growth enhancing ability for rainbow trout was confirmed in another study, wherein 1% methyl linolenate plus 4% methyl laurate (C 12:0) provided maximal growth and prevention of fatty acid deficiency symptoms compared with fish fed 5% methyl laurate (Watanabe et al. 1974). Rainbow trout fingerlings fed either 0.5% 20:5 $\omega$ 3 or 0.5% 22:6 $\omega$ 3 had better growth rates than individuals fed 0.5% 18:3 $\omega$ 3 in diets containing 5% total lipid, thereby showing a higher essential fatty acid efficiency of 20:5 $\omega$ 3 and 22:6 $\omega$ 3 at low dietary concentrations of these fatty acids (Takeuchi and Watanabe 1977). However, it is unknown whether evaluation of higher dietary concentrations (e.g., 1 to 3%) of each of these fatty acids would have produced similar differences in essential fatty acid efficiencies in terms of growth and feed efficiency of rainbow trout fingerlings. Yu and Sinnhuber (1972) found that 1% dietary 18:3 $\omega$ 3 or 1% docosahexaenoic acid (22:6 $\omega$ 3) provided similar growth rates in rainbow trout fingerlings. No higher dietary concentrations of either fatty acid were studied, nor were total dietary lipid concentrations >2% (dry diet basis) examined. The essentiality of 18:3 $\omega$ 3 for rainbow trout was further substantiated in a 34-mo feeding study in which fingerlings fed 1% ethyl linolenate plus 5% ethyl laurate grew to maturity and produced viable offspring which in turn had normal growth rates (Yu et al. 1979). Incorporation of 1.5% ethyl linoleate to a lipid mix of 1% ethyl linolenate plus 3.5% ethyl laurate did not confer any additional advantage in growth rate, percent fertile eggs, or percent viable fry for rainbow trout compared with individuals fed the 1% ethyl linolenate diet.

Closely related species such as coho salmon and rainbow trout have different quantitative dietary requirements for 18:3 $\omega$ 3 administered as the triacylglycerol, trilinolenin. Coho salmon fingerlings required 1 to 2.5% trilinolenin for maximum growth and feed efficiency when fed dietary trilinolenin concentrations ranging from 0 to 5%. High dietary trilinolenin concentrations (>1%) in the presence of the optimal trilinolenin

concentrations fed to coho salmon fingerlings resulted in reduced growth rate and feed efficiency values (Yu and Sinnhuber 1979). On the other hand, rainbow trout fingerlings had best growth rates and feed efficiency when fed higher trilinolenin concentrations combined with low trilinolein concentrations (5% trilinolenin plus 0% trilinolein or 3% trilinolenin plus 1% trilinolein) (Yu and Sinnhuber 1976). In a separate study, rainbow trout fingerlings fed a diet sufficient in 18:3 $\omega$ 3 (1% ethyl linolenate) actually had better growth rates and feed efficiency than fish fed 1% ethyl linolenate plus 1.5% ethyl linoleate (Yu and Sinnhuber 1975). Dietary supplements of 5% ethyl linoleate to feeds containing 0.1, 0.5, or 1% ethyl linolenate markedly reduced growth and feed efficiency of rainbow trout fingerlings.

Chum salmon, *O. keta*, fed 5% dietary lipid as either 5% methyl laurate, 4% methyl laurate plus 1% 18:2 $\omega$ 6, 4% methyl laurate plus 1% 18:3 $\omega$ 3, or 3% methyl laurate plus 1% 18:2 $\omega$ 6 and 1% 18:3 $\omega$ 3, showed best weight gain and feed efficiency when offered the 3% methyl laurate plus simultaneous supplements of 1% 18:2 $\omega$ 6 and 1% 18:3 $\omega$ 3 (Takeuchi et al. 1979). Also, simultaneous supplementation of 18:2 $\omega$ 6 (1%) and 18:3 $\omega$ 3 (1%) produced slightly better weight gain of chum salmon fingerlings than 4% methyl laurate plus 1% of a highly unsaturated fatty acid mix (containing 26.5% 20:5 $\omega$ 3 plus 42.1% 22:6 $\omega$ 3). However, minimal dietary requirements of 18:2 $\omega$ 6 or 18:3 $\omega$ 3 for optimal growth rate of chum salmon fingerlings were not determined.

Dietary supplements of either methyl linoleate or methyl linolenate at 0.5 or 1.0% concentrations provided better growth of Japanese eel fingerlings than a fat-free diet or 7% methyl laurate (T. Takeuchi et al. 1980). However, contradictory results in two separate studies by these investigators prevent any conclusions regarding minimal requirements of 18:2 $\omega$ 6 and 18:3 $\omega$ 3 for this species.

Common carp fry and fingerlings have demonstrated better growth when fed diets containing highly polyunsaturated fats (e.g., cod liver oil) than those containing 5% methyl laurate (Watanabe 1975a, b). Intermediate growth rates occurred in common carp fry fed either 1% methyl linoleate or 1% methyl linolenate plus 4% methyl laurate over a 22-wk period (Watanabe et al. 1975a), whereas 1% methyl linoleate or 1% methyl linolenate did not improve growth of common carp fingerlings over an 18-wk period (Wata-

nabe et al. 1975b).

High variability exists among various fish species in ability to elongate and desaturate dietary 18-carbon fatty acids to 20- or 22-carbon fatty acids. Several marine species appear to have lower enzymatic elongation-desaturation capabilities than freshwater fishes. Administration of (1 - <sup>14</sup>C) 18:3 $\omega$ 3 to individuals of red sea bream; black sea bream, *Mylio macrocephalus*; opaleye, *Girella nigricans*; striped mullet, *Mugil cephalus*; and rainbow trout indicated that only rainbow trout exhibited appreciable radioactivity in 22:6 $\omega$ 3 of body lipids (Yamada et al. 1980). Therefore, it was concluded that marine species have limited ability to elongate and desaturate 18:3 $\omega$ 3, resulting in dietary essentiality of eicosapentaenoic acid (20:5 $\omega$ 3) or 22:6 $\omega$ 3 and non-essentiality of 18:3 $\omega$ 3. In another study, injections of (1 - <sup>14</sup>C) 18:3 $\omega$ 3 into two individuals of each of several fish species resulted in intensive incorporation of 18:3 $\omega$ 3 into 20:5 $\omega$ 3 and 22:6 $\omega$ 3 in rainbow trout, while very low percent bioconversion of 18:3 $\omega$ 3 to 20:5 $\omega$ 3 and 22:6 $\omega$ 3 occurred in marine fish such as globefish, *Fugu rubripes rubripes*; Japanese eel; red sea bream; rockfish, *Sebasticus marmoratus*; and ayu, *Plecoglossus altivelis* (Kanazawa et al. 1979). The results of this study confirmed that pathways of elongation and desaturation of dietary 18-carbon fatty acids are poorly developed in marine fishes compared with rainbow trout. Earlier, Castell et al. (1972c) had reported elevated 20:4 $\omega$ 6 and 22:5 $\omega$ 6 concentrations in body lipids of rainbow trout fed 1% 18:2 $\omega$ 6 as well as elevated 22:6 $\omega$ 3 concentrations in individuals fed 1% 18:3 $\omega$ 3, suggesting an ability of rainbow trout to elongate and desaturate linoleic and linolenic acid. Also, rainbow trout, fed a fat-free diet or 5% oleic acid (18:1 $\omega$ 9) as the sole dietary lipid, accumulated high body lipid concentrations of eicosatrienoic acid (20:3 $\omega$ 9), an indicator of essential fatty acid deficiency in terrestrial animals. Furthermore, comparison of dietary fatty acid composition and initial and final body fatty acid composition of rainbow trout in another feeding study, suggests the ability of this species to elongate and desaturate 20:5 $\omega$ 3 into 22:6 $\omega$ 3 (Castledine and Buckley 1980). Cowey et al. (1976) concluded that turbot lack the necessary microsomal desaturases to effectively convert 18:1 $\omega$ 9, 18:2 $\omega$ 6, or 18:3 $\omega$ 3 into polyunsaturated fatty acids for deposition in neutral fats or phospholipids based upon growth and body lipid composition. Also, 1% dietary 18:3 $\omega$ 3 or 1% arachidonic acid (20:4 $\omega$ 6) in the pres-

ence of 4% 18:1 $\omega$ 9 promoted better growth of turbot than 1% 18:2 $\omega$ 6 plus 4% 18:1 $\omega$ 9. Additionally, 1% cod liver oil plus 4% 18:1 $\omega$ 9 produced better growth rates of turbot than all other dietary treatments (Cowey et al. 1976). Simultaneous supplementation of 0.45% 18:2 $\omega$ 6 and 0.45% 18:3 $\omega$ 3 as the only dietary constituents of the  $\omega$ 6 and  $\omega$ 3 series did not increase the level of 20:5 $\omega$ 3 or 22:6 $\omega$ 3 liver triglycerides in underyearling plaice (Owen et al. 1972). Growth studies of red sea bream indicated that a 2% dietary polyunsaturated fatty acid mix (38% 20:5 $\omega$ 3, 1.4% 22:5 $\omega$ 6, and 33.4% 22:6 $\omega$ 3) promoted significantly better weight gain than fish fed up to 4.2% dietary methyl linolenate (Fujii and Yone 1976). It was concluded that 18:3 $\omega$ 3 is not essential for red sea bream, since this species has little, if any, capability to elongate and desaturate 18-carbon fatty acids to 20- and 22-carbon fatty acids, based upon body fatty acid composition.

Specific qualitative and quantitative essential fatty acid requirements have not been determined for channel catfish fry (Yingst and Stickney 1979) and channel catfish fingerlings (National Research Council 1977; Stickney 1977). Nevertheless, growth rates of channel catfish in several studies comparing various lipid sources (e.g., beef tallow, menhaden oil, safflower oil, and corn oil) have consistently been high in individuals fed menhaden oil as the chief dietary lipid (Stickney and Andrews 1971, 1972; Murray et al. 1977; Dupree et al. 1979; Yingst and Stickney 1979, 1980). Typically, menhaden oil contains a large amount of fatty acids of the  $\omega$ 3 family (Stickney 1977), especially the polyunsaturated fatty acids (e.g., 3% 18:3 $\omega$ 3, 17% 20:5 $\omega$ 3, and 13% 22:6 $\omega$ 3). Therefore,  $\omega$ 3 fatty acids may be essential for channel catfish, especially in the fry and early fingerling stages.

Unlike most fish species studied, *Tilapia zilli*, fingerlings require 1% 18:2 $\omega$ 6 or 20:4 $\omega$ 6 for optimal weight gain as opposed to fatty acids of the  $\omega$ 3 series (Kanazawa et al. 1980a). However, this same species has a greater relative ability to elongate and desaturate 18:3 $\omega$ 3 to 20:5 $\omega$ 3 and 22:6 $\omega$ 3, than to elongate and desaturate 18:2 $\omega$ 6 to 20:4 $\omega$ 6 (Kanazawa, Teshima, and Imai 1980).

In a review of lipid requirements of fishes, Castell (1979) discussed differences in fatty acid composition of fishes due to salinity, temperature, diet composition, depth, seasonal variation, and reproductive stage; requirements, metabolism, and functions of dietary fatty acids for fishes are also discussed.

## CARBOHYDRATES

Carbohydrates are included in formulated feeds for fish primarily as a low cost source of energy to spare dietary protein for growth rather than energy. Protein sparing action of dietary carbohydrate was demonstrated in brook trout fed marginal concentrations of dietary protein (28 or 32%) with optimal protein-to-calorie ratios of 75 mg protein/kcal (Ringrose 1971).

Maximal dietary carbohydrate concentrations that can be fed to fish without reducing growth rate depend upon whether the fish species is carnivorous, omnivorous, or herbivorous. For example, maximal dietary dextrin concentrations that did not reduce growth rate were 10% for yellowtail, *Seriola quinqueradiata*, 20% for red sea bream and 30% for common carp (Furuichi and Yone 1980). Rainbow trout subadults can be fed 38% wheat meal or 41% cooked wheat (17 to 25% of dietary metabolizable energy) without deleterious effects on growth (Edwards et al. 1977). Similarly, rainbow trout fed 32% wheat meal or 21% wheat meal plus 13% glucose (15 and 26% metabolizable energy of the diet) did not have significant differences in growth rate (Refstie and Austreng 1981). However, dietary Cerelose<sup>6</sup> concentrations as low as 14%, substantially increased liver glycogen concentrations of rainbow trout compared with fish fed 0 or 7% dietary Cerelose (Hilton 1982). Additionally, low rearing temperatures (10° vs. 15°C) for rainbow trout resulted in increased liver glycogen concentrations. Therefore, Hilton (1982) suggested that stocking rainbow trout with high liver glycogen concentrations into natural waters could result in impaired liver function. Incipient lethal levels of waterborne copper were reported to be lower for rainbow trout fed higher concentrations of available carbohydrate, probably as the result of impaired liver function from high liver glycogen content (Dixon and Hilton 1981).

Digestibility of carbohydrates is generally inversely related to molecular complexity. Thus, monosaccharides are more available nutritionally to fishes than are disaccharides, which in turn are more available than are polysaccharides. Relative growth rates of chinook salmon fingerlings fed 20% carbohydrate were as follows: glucose > sucrose > fructose > maltose > dextrin > potato starch > galactose (Buhler and

<sup>6</sup>Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Halver 1961). Brook trout fingerlings (initial mean weight = 1.6 g) fed 14% glucose, galactose, or fructose over a 20-wk period had better growth rates when fed 14% glucose or fructose (McCartney 1971). Rainbow trout fingerlings had better growth rate, feed conversion, and protein efficiency when fed 30% glucose concentrations compared with 30% raw corn starch or 15% glucose plus 15% cellulose (Bergot 1979a). Dietary glucose concentrations as high as 30% doubled plasma glucose 6 h after the first meal, while normal plasma glucose concentrations were attained 24 h later (Bergot 1979b). Channel catfish fingerlings can utilize 2.25 g of dextrin in place of 1 g of lipid for growth equally well within dietary lipid concentrations of 5 to 12.5% and digestible carbohydrate concentrations of 5.6 to 22.5% (Garling and Wilson 1977).

Dietary fiber is not required by fishes and is considered to be a nonnutrient bulk component in fish diets. According to Leary and Lovell (1975), dietary cellulose in excessive amounts probably decreases absorption of essential nutrients by physical obstruction of enzymes and increased rate of passage through the digestive system. Obstruction of enzyme activity may result from ingested fiber chelating metal ions serving as cofactors of enzymes. Dietary cellulose concentrations as high as 8% did not inhibit growth of channel catfish, whereas 14% dietary cellulose depressed growth. No similar studies have been conducted with salmonids or other fish species to evaluate maximum tolerable, dietary fiber concentrations.

Cellulase activity in stomachs of several fish species and intestinal portions of stomachless Cyprinidae was positively correlated with amount of detritus consumed (Prejs and Blaszyk 1977). Microflora ingested along with detrital material is probably responsible for the cellulase activity in fish; however, further research should examine sources of cellulase activity, especially in herbivorous species.

## VITAMINS

Qualitative vitamin requirements of fishes (National Research Council 1973, 1977) and their biochemical and physiological functions are generally similar to requirements and functions demonstrated for terrestrial animals. Early qualitative vitamin requirement studies of fishes usually consisted of long-term feeding trials (e.g., 14 to 24 wk) in which laboratory cultured

salmonids were fed a positive control diet (i.e., assumed to be nutritionally complete) or that same diet omitting one of several vitamins (McLaren et al. 1947; Halver and Coates 1957; Coates and Halver 1958). Growth, survival, behavior, internal organ appearance, blood physiology, and histopathology were often examined to describe deficiency symptoms. Also, fish fed a vitamin-deficient diet were often divided into two subgroups during the course of the study. One subgroup remained on the vitamin-deficient diet while the recovery subgroup was fed the complete diet to detect positive responses such as accelerated growth rate and disappearance of deficiency symptoms.

More recently, biochemical criteria such as activities of specific enzymes requiring a given vitamin for coenzyme formation have been used to determine qualitative and quantitative vitamin requirements of fishes (C. E. Smith et al. 1974; Cowey 1976). According to C. E. Smith et al. (1974), biochemical defects in the form of reduced enzyme activity allow detection of pre-clinical vitamin deficiencies. A review of vitamin requirements of fishes was presented by Halver (1979).

## Thiamine

Essentiality of dietary thiamine has been verified for brook, brown (*Salmo trutta*), and rainbow trout (McLaren et al. 1947; Phillips and Brockway 1957), chinook salmon (Halver 1957), coho salmon (Coates and Halver 1958), channel catfish (Dupree 1966), rainbow trout (Kitamura et al. 1967a; Aoe et al. 1969), Japanese eel (Arai et al. 1972a), red sea bream (Yone 1975), and turbot (Cowey et al. 1975). Thiamine deficiency symptoms commonly observed in many of these fish species include anorexia, poor growth, depigmentation, and loss of equilibrium. Hemorrhage and congestion of fins has been noted in thiamine-deficient Japanese eel (Hashimoto et al. 1970; Arai et al. 1972a) and thiamine-deficient common carp (Aoe et al. 1969).

Quantitative thiamine requirements have been determined for turbot and channel catfish. Cowey et al. (1975) detected optimal erythrocyte transketolase (thiamine pyrophosphate serves as coenzyme) activity in turbot fed 2.6 mg thiamine/kg dry diet, whereas maximal growth occurred at dietary thiamine concentrations  $\geq 0.6$  mg/kg dry diet. Therefore, in addition to growth rates, functional evidence such as enzyme activity also

provides additional useful information for estimating quantitative vitamin requirements. Channel catfish fingerlings require a minimal dietary thiamine concentration of 1 mg/kg dry diet based upon maximal weight gain, feed efficiency, and prevention of thiamine deficiency symptoms such as anorexia, poor growth, dark coloration, and higher mortality (Murai and Andrews 1978a). Hematocrit values of channel catfish were unaffected by dietary thiamine concentrations ranging from 0.1 to 20.1 mg/kg dry diet.

It is unknown why channel catfish and common carp require lower dietary concentrations of thiamine compared with salmonids. Omnivorous fish would seemingly require higher amounts of thiamine for oxidative decarboxylation of pyruvate and the transketolase reaction of the pentose phosphate shunt, due to the ability of herbivores and omnivores to metabolize higher dietary carbohydrate concentrations than carnivorous fish such as salmonids (Murai and Andrews 1978a).

### Riboflavin

Dietary essentiality for riboflavin has been reported for rainbow trout (McLaren et al. 1947), brook, brown, and lake trout (Phillips and Brockway 1957), chinook salmon (Halver 1957), Atlantic salmon (Phillips 1959a), channel catfish (Dupree 1966), common carp (Aoe et al. 1967a; Ogino 1967), rainbow trout (Kitamura et al. 1967a; Poston et al. 1977; L. Takeuchi et al. 1980), Japanese eel (Arai et al. 1972a), and red sea bream (Yone 1975). Rainbow trout (mean initial weight = 5.9 g) fed riboflavin-deficient diets developed bilateral corneal and lenticular lesions after 11 and 15 wk of the experimental period (Poston et al. 1977). Further evaluations of cataract formations induced from riboflavin deficiency in rainbow trout fingerlings confirmed no retinal damage according to histopathological examinations (Hughes et al. 1981a). Fin necrosis, snout erosion, and spinal deformation also occurred in riboflavin-deficient rainbow trout (Poston et al. 1977). In a separate study, rainbow trout fingerlings (initial mean weight = 1.5 g) fed riboflavin-deficient diets displayed anorexia, poor growth, high mortality rate, lesion of fins, and cataract formation during an 8-wk study (L. Takeuchi et al. 1980). Common carp fingerlings fed riboflavin-deficient diets showed anorexia, poor growth, high mortality rate, and hemorrhage of skin and

fins (L. Takeuchi et al. 1980). Murai and Andrews (1978b) reported 100% occurrence of "short body dwarfism" due to shortening of individual vertebrae in riboflavin-deficient channel catfish after 20 wk. These authors speculated that abnormal vertebral growth may be related to hypothyroidism which in turn may be caused by riboflavin deficiency.

Quantitative riboflavin requirements have been determined for common carp, rainbow trout, and channel catfish. In several different studies, riboflavin requirements of carp fingerlings apparently declined with increasing fish size based on growth rate and liver riboflavin content. Common carp fingerlings with an initial mean weight equalling 1.5 g required 20 mg riboflavin/kg dry diet over a 6-wk period (Aoe et al. 1967a). Slightly larger carp fingerlings (initial mean weight = 2.8 g) required 10 mg riboflavin/kg dry diet over a 6-wk period (Ogino 1967), whereas individuals with an initial mean weight equalling 3.4 g required 5 to 7 mg riboflavin/kg dry diet (L. Takeuchi et al. 1980). Rainbow trout with an initial mean weight of 7 g required 12.2 mg riboflavin/kg for maximal growth, whereas 18.2 mg riboflavin/kg were required for saturation of head and posterior kidney tissue (Woodward 1982). However, larger rainbow trout fingerlings (initial mean weight = 11.2 g) required 3 mg riboflavin/kg dry diet according to growth, food conversion, and mean erythrocyte glutathione reductase activity coefficient or 12 mg riboflavin/kg dry diet for maximal liver riboflavin content (Hughes et al. 1981b). Rainbow trout fingerlings (initial mean weight = 1.5 g) required 4 mg riboflavin/kg dry diet based upon growth rate and feed efficiency and 6 mg riboflavin/kg dry diet based on liver riboflavin content (L. Takeuchi et al. 1980). Channel catfish fingerlings require 9 mg riboflavin/kg dry diet for maximal growth and 3 mg riboflavin/kg dry diet to prevent occurrence of short body dwarfism (Murai and Andrews 1978b).

### Pyridoxine

Dietary essentiality of pyridoxine has been reported for rainbow trout (McLaren et al. 1947), brook, brown, and lake trout (Phillips and Brockway 1957), chinook salmon (Halver 1957), Atlantic salmon (Phillips 1959a), coho salmon (Coates and Halver 1958), common carp (Ogino 1965), channel catfish (Dupree 1966), rainbow trout (Kitamura et al. 1967a), yellowtail (Sakaguchi et

al. 1969), Japanese eel (Arai et al. 1972a), red sea bream (Yone 1975), turbot (Adron et al. 1978), and gilthead bream, *Sparus aurata* (Kissil et al. 1981). Coates and Halver (1958) mentioned several pyridoxine deficiency symptoms for coho salmon including nervous disorders, hyperirritability, poor appetite, indifference to light, and rapid occurrence of postmortem rigor mortis. Halver (1957) reported the following additional pyridoxine deficiency symptoms for chinook salmon: ataxia, edema of peritoneal cavity, colorless serous fluid, blue-green coloration on dorsal surface, and excessive flexing of opercles. Clinical signs of pyridoxine deficiency in rainbow trout include hyperirritability, nervous disorders, erratic and rapid swimming, flexing of opercles, greenish-blue coloration, and tetany, just before death (C. E. Smith et al. 1974). Pyridoxine-deficient rainbow trout displayed normocytic, normochromic anemia, indicating that pyridoxine has a function in maintenance of normal erythropoiesis in this species (C. E. Smith et al. 1974). Also, rainbow trout fed pyridoxine-deficient diets for 7 d had lower aspartate aminotransferase activity in white muscle, whereas liver aspartate and alanine aminotransferase activity was reduced after 28 d (Jurss 1978, 1981). Pyridoxine deficiency symptoms in channel catfish fingerlings included nervous disorders, erratic swimming, opercle extension, and tetany (Dupree 1966). Andrews and Murai (1979) confirmed a pyridoxine requirement for channel catfish fingerlings, reporting that fish fed pyridoxine-deficient diets displayed anorexia, nervous disorders, tetany, and blue-green coloration on the dorsal surface. No anemia was detected in pyridoxine-deficient individuals, whereas a microcytic, normochromic anemia was observed in channel catfish fed 20 mg/kg or greater of pyridoxine. Chinook salmon fingerlings fed a high protein diet (65%) require about 15 mg pyridoxine/kg diet for optimal growth and disease resistance to *Vibrio anguillarum* (Hardy et al. 1979).

Quantitative pyridoxine requirements are known for channel catfish, red sea bream, gilthead bream, turbot, and common carp. Channel catfish fingerlings require a minimum of 4.2 mg pyridoxine/kg dry diet for maximal growth (Andrews and Murai 1979). Red sea bream required a minimum of 5 to 6 mg pyridoxine/kg dry diet for maximal glutamic oxaloacetic transaminase activity and maximal glutamic pyruvate transaminase activity (Yone 1975). A minimum of 2

to 5 mg pyridoxine/kg dry diet is required for maximal weight gain and pyridoxine liver content of red sea bream (Yone 1975). Kissil et al. (1981) reported optimal dietary pyridoxine concentrations for gilthead bream as a function of growth (1.97 mg pyridoxine/kg dry diet) and liver alanine aminotransferase activity (3.0 to 5.1 mg pyridoxine/kg dry diet). Pyridoxine deficiency symptoms in gilthead bream included hyperirritability, erratic swimming behavior, poor food conversion, retarded growth, and high mortality. Turbot fed pyridoxine concentrations of 1.0 mg/kg dry diet up to 30 mg/kg had similar growth rates, whereas individuals fed 0.26 or 0.50 mg pyridoxine/kg dry diet had reduced weight gain (Adron et al. 1978). Liver alanine aminotransferase activity and muscle and liver aspartate aminotransferase activity increased with higher dietary pyridoxine concentrations up to 2.5 mg/kg dry diet. Therefore, a dietary pyridoxine concentration of 2.5 mg/kg dry diet satisfied both maximal growth and liver aspartate aminotransferase activity for turbot. Common carp fingerlings required 5.4 mg pyridoxine/kg dry diet for maximal growth rate and prevention of deficiency symptoms (Ogino 1965).

### Niacin

Niacin has been shown to be an essential dietary constituent for rainbow trout (McLaren et al. 1947), brook and brown trout (Phillips and Brockway 1957), lake trout (Phillips 1959b), chinook salmon (Halver 1957), channel catfish (Dupree 1966), common carp (Aoe et al. 1967c), Japanese eel (Arai et al. 1972a), brook trout (Poston and DiLorenzo 1973), and red sea bream (Yone 1975). Hemorrhage and lesions of the skin have been reported in niacin-deficient channel catfish (Andrews and Murai 1978) and Japanese eel (Arai et al. 1972a). Dietary requirements for maximal growth include 28 mg niacin/kg dry diet for common carp (Aoe et al. 1967c) and 14.4 mg niacin/kg dry diet for channel catfish fingerlings (Andrews and Murai 1978).

### Pantothenic Acid

Essentiality of dietary pantothenic acid has been demonstrated for rainbow trout (McLaren et al. 1947), brown, brook, rainbow, and lake trout (Phillips and Brockway 1957), Atlantic salmon (Phillips 1959a), chinook salmon (Halver

1957), coho salmon (Coates and Halver 1958), channel catfish (Dupree 1966), rainbow trout (Kitamura et al. 1967a), Japanese eel (Arai et al. 1972a), and red sea bream (Yone 1975). Most of these species fed pantothenic acid-deficient diets displayed mucous covered gills, anorexia, reduced weight gain, and "clubbed gills."

Quantitative pantothenic acid requirements have been determined for channel catfish fry (250 mg/kg dry diet) and channel catfish fingerlings (10 mg/kg dry diet) (Murai and Andrews 1975 and 1979, respectively) and common carp fingerlings (40 mg/kg dry diet) (Ogino 1967). Murai and Andrews (1975) suggested that the relatively high dietary pantothenic acid requirements of channel catfish fry might be partially due to higher rates of micronutrient losses in small feed crumbles (high surface to volume ratio) fed to fry compared with larger feed particles fed to fingerlings.

### Ascorbic Acid

Ascorbic acid has several important physiological functions in fishes and is the vitamin most sensitive to processing and storage losses in fish formula feeds. Therefore, extensive research has been conducted on qualitative and quantitative ascorbic acid requirements for fishes. Ascorbic acid is a cofactor of an enzyme which is involved in hydroxylation of proline and lysine during collagen formation, thereby contributing to bone and skin formation. Ascorbic acid also has a role in iron metabolism and detoxification of organic pollutants such as toxaphene and polychlorinated biphenyls during accumulation in the liver.

Qualitative dietary ascorbic acid requirements have been reported for rainbow trout (McLaren et al. 1947; Kitamura et al. 1965; Hilton et al. 1978; Sato et al. 1978; John et al. 1979), brook trout (Poston 1967), coho salmon and rainbow trout (Halver et al. 1969), yellowtail (Sakaguchi et al. 1969), Japanese eel (Arai et al. 1972a), channel catfish (Lovell 1973; Wilson and Poe 1973), red sea bream (Yone 1975), mrigal, *Cirrhina mrigala* (Mahajan and Agrawal 1980a), and snake head, *Channa punctatus* (Mahajan and Agrawal 1979). Ascorbic acid deficiency symptoms in coho salmon and rainbow trout include reduced growth, distorted and twisted filament cartilage of the gill arches, acute lordosis and scoliosis, and eventual dislocation of vertebrae (Halver et al. 1969). Other physiological changes

in ascorbic acid-deficient rainbow trout include low hematocrit values (Hilton et al. 1978; John et al. 1979), and high plasma levels of triglycerides and cholesterol (John et al. 1979). Halver (1972b) reported that the rate of repair of experimentally induced wounds in salmonids is directly related to the amount of ascorbic acid intake. Rainbow trout fed ascorbic acid-deficient diets for 18 wk displayed impaired collagen formation in the skin according to an in vitro radioisotopic method with labeled proline (Yoshinaka et al. 1978). Brook trout fingerlings fed ascorbic acid-deficient diets over a 34-wk period developed scoliosis and/or lordosis, increased mortality rate, and internal hemorrhaging (Poston 1967). Scorbatic channel catfish experienced lordosis, scoliosis (and ultimately, broken back), hemorrhage within the vertebral column, and brittle vertebrae (Wilson and Poe 1973). Also, these investigators reported reduced serum alkaline phosphatase activity (65% lower), lower vertebral collagen content (42% less on a dry basis), and less hydroxyproline in the collagen of scorbatic channel catfish. Wilson and Poe (1973) speculated that reduced serum alkaline phosphatase activity may indicate reduced bone formation from lower osteoblastic activity. In addition to the aforementioned common ascorbic acid deficiency symptoms (e.g., lordosis, scoliosis, hemorrhage along spinal column, and poor growth), channel catfish had increased susceptibility to pathogenic bacterial infestation (*Aeromonas liquefaciens*) and occasional formation of hemivertebrae (Lovell 1973). Also, Halver et al. (1975) reported hyperplasia of nuclei of eye support cartilage in salmonids deficient in ascorbic acid. Vertebral collagen percentages of 24.5% or less and liver ascorbic acid concentrations of 50  $\mu\text{g/g}$  or less occurred in ascorbic acid deficient channel catfish fingerlings with an initial mean weight equalling 22 g (Lovell and Lim 1978). In contrast, channel catfish fingerlings fed sufficient ascorbic acid had 26% or greater vertebral collagen and 65  $\mu\text{g}$  or greater ascorbic acid/g of liver tissue. In a separate study, Lim and Lovell (1978) reported the following ascorbic acid deficiency symptoms in smaller channel catfish fingerlings (initial mean weight = 2.3 g): 1) anorexia after 9 wk, 2) scoliosis, lordosis, and darker pigmentation after 10 wk, and 3) lower hematocrit values after 18 wk. Also, liver ascorbic acid concentrations of 30  $\mu\text{g/g}$  and vertebral collagen percentages of 25% or less occurred in ascorbic acid-deficient channel catfish in this smaller size range. Snake heads with

ascorbic acid deficiency had an elevated liver cholesterol content after 150 d, in addition to the occurrence of scoliosis, lordosis, and decreased ascorbic acid concentrations in blood and kidney (Mahajan and Agrawal 1979). Ascorbic acid deficiency in fish from the same study resulted in normochromic, normocytic anemia after 120 d and normochromic, macrocytic anemia between 180 and 210 d (Agrawal and Mahajan 1980). Using  $^{45}\text{Ca}$  as a tracer, snake heads had reduced absorption of calcium from surrounding water by gills and skin and lower muscle and bone calcium content when fed an ascorbic acid-deficient diet for 210 d (Mahajan and Agrawal 1980b). Since distortion of gill filaments from cartilage malformation often occurs in ascorbic acid-deficient fish, decreased calcium absorption through the gills may have resulted from the ascorbic acid deficiency (Mahajan and Agrawal 1980b). Channel catfish (initial weight slightly  $>5$  g), fed either 670 or 5,000 mg of ascorbic acid/kg dry diet, did not receive any additional advantages in weight gain or backbone collagen concentration (Mayer et al. 1978). However, channel catfish exposed to increasingly higher concentrations of toxaphene were at least partially protected from growth retardation, vertebral development anomalies, and skin integrity problems, when higher dietary ascorbic acid concentrations were consumed. For instance, the no-effect toxaphene concentration on skin integrity (e.g., mucous cell numbers and epidermal thickness) was  $<37$  ng/l for channel catfish fed 63 or 670 mg ascorbic acid/kg dry diet, while the no-effect toxaphene concentration was between 68 and 108 ng/l for fish fed 5,000 mg ascorbic acid/kg dry diet (Mayer et al. 1978). Methods to measure rupture (the force level causing specimen failure) and elastic limit (the force level above which permanent structural damage occurs in a test specimen) according to Hamilton et al. (1981a) were used to evaluate effects of ascorbic acid deficiency on bone strength of channel catfish (Hamilton et al. 1981b). Channel catfish fingerlings (initial weight = 4 to 5 g) fed diets containing no ascorbic acid had significant reductions in length and weight after 150 d. Also, after 150 d, fish fed no supplemental ascorbic acid had reductions of 10% in backbone collagen and 16% in hydroxyproline concentration in collagen. Additionally, in channel catfish fed no supplemental ascorbic acid, 24% less force was required to cause permanent damage of vertebral centra (elastic limit) and failure of vertebral centra (rupture) occurred

at 16% less force than in individuals fed 500 mg ascorbic acid/kg, dry diet.

Quantitative ascorbic acid requirements have been determined for several fish species. Rainbow trout (initial mean weight = 0.3 g) require 100 mg ascorbic acid/kg dry diet and coho salmon (initial mean weight = 0.4 g) require about 50 mg ascorbic acid/kg dry diet based upon blood and anterior kidney ascorbic acid concentrations and growth rate (Halver et al. 1969). The minimal level of ascorbic acid in the blood of coho salmon and rainbow trout accompanying normal growth and survival rate is  $35 \mu\text{g}$  ascorbic acid/g blood. Hilton et al. (1978) reported a lower ascorbic acid requirement (40 mg/kg dry diet) for larger rainbow trout (initial mean weight = 6.7 g), based upon growth, feed conversion, survival rate, and serum iron levels. Andrews and Murai (1975) estimated that channel catfish fingerlings (initial mean weight = 2.3 g) require 50 mg ascorbic acid/kg dry diet over a 28-wk period based on growth, feed conversion, and absence of ascorbic acid deficiency symptoms. Channel catfish fingerlings (initial mean weight = 2.3 g) required 30 mg ascorbic acid/kg dry diet over a 22-wk period for maximal growth, whereas 60 mg ascorbic acid/kg (the next highest experimental concentration) was sufficient to prevent distortion of gill filament cartilage and promote regeneration of skin and muscle in experimentally inflicted wounds after 10 d (Lim and Lovell 1978). Mahajan and Agrawal (1980a) concluded that mrigal fry and fingerlings require about 700 mg ascorbic acid/kg dry diet based upon growth, survival rates, and percentage occurrence of skeletal deformities.

Several sources of dietary ascorbic acid have been evaluated for relative nutritional value for channel catfish and rainbow trout. Channel catfish fingerlings (initial mean weight = 7.9 g) fed equimolar concentrations of 25 mg L-ascorbic acid (uncoated or ethylcellulose coated) or dipotassium L-ascorbate 2-sulfate dihydrate (AS) per kg dry diet over a 20-wk period did not show scoliosis, whereas 42% of the fingerlings fed a diet containing  $<5$  mg ascorbic acid/kg dry diet had scoliosis (Murai et al. 1978). Maximal weight gains and feed efficiency of channel catfish varied with dietary ascorbic acid sources. Only 50 mg of ethylcellulose coated or uncoated L-ascorbic acid were required for maximal growth and feed efficiency, while 200 mg of L-ascorbate-2-sulfate dihydrate were required for similar increments of weight gain. Generally, growth



reached a maximum when blood L-ascorbic acid concentrations reached 7  $\mu\text{g/ml}$  (Murai et al. 1978). Rainbow trout which were considerably younger (0.3 g) than channel catfish from the previous study, required about 80 mg of dipotassium ascorbic-2-sulfate (DAS)/kg dry diet over a 20-wk period to avoid ascorbic acid deficiency symptoms in the majority of fishes, and 160 mg DAS/kg dry diet to achieve normal growth (Halver et al. 1975).

### Choline

Dietary essentiality of choline has been demonstrated for rainbow trout (McLaren et al. 1947), brook and brown trout (Phillips and Brockway 1957), lake trout (Phillips 1959b), chinook salmon (Halver 1957), coho salmon (Coates and Halver 1958), channel catfish (Dupree 1966), rainbow trout (Kitamura et al. 1967a), common carp (Ogino et al. 1970a), Japanese eel (Arai et al. 1972a), and red sea bream (Yone 1975). Choline deficiency symptoms in fish include poor growth and feed efficiency, anorexia, fatty livers, and hemorrhagic areas in kidneys, liver, and intestine.

Quantitative choline requirements have been estimated for common carp fingerlings and lake trout fingerlings. Ketola (1976) examined relative growth rates of lake trout fed an unsupplemented diet (30 mg choline/kg dry diet) compared with equimolar supplements of aminoethanol, dimethylaminoethanol, methylaminoethanol, betaine-HCl, and choline (equivalent to 2,600 mg choline/kg dry diet). Lake trout fed the unsupplemented diet and aminoethanol and betaine supplements had reduced growth rates and high liver fat content. It was concluded that since betaine, a source of labile methyl groups, did not affect growth or liver fat content, any metabolic function of choline in regulation of liver fat in lake trout is unrelated to transmethylation. In a separate feeding study, a quantitative dietary choline requirement of 1,000 mg/kg dry diet was determined for lake trout fingerlings (Ketola 1976). Common carp require an estimated minimal dietary choline-CI concentration of 2,000 mg/kg dry diet based upon slightly reduced growth and fatty livers in individuals fed choline-deficient diets (Ogino et al. 1970a). The possible role of methionine as a methyl donor and its relative efficiency in preventing fatty liver and hemorrhagic degenerations of kidneys of choline-deficient fish has not yet been investigated.

### Folic Acid and Cyanocobalamin

Qualitative folic acid requirements have been demonstrated for brook, brown, and rainbow trout (Phillips and Brockway 1957), chinook salmon (Halver 1957), coho salmon (Coates and Halver 1958), channel catfish (Dupree 1966), rainbow trout (McLaren et al. 1947; Kitamura et al. 1967a), Japanese eel (Arai et al. 1972a), and rohu, *Labeo rohita* (John and Mahajan 1979). However, common carp fingerlings (mean initial weight = 2.5 g) fed several folic acid concentrations (0 to 15 mg/kg diet) over 16 wk did not show differential responses in growth, feed conversion, folic acid liver content, and erythrocyte counts (Aoe et al. 1967b).

Folic acid deficiency symptoms in chinook salmon include poor growth, anorexia, anemia, lethargy, dark coloration, and megaloblastic erythropoiesis (Halver 1957), while coho salmon displayed poor growth and anorexia (Coates and Halver 1958). Channel catfish fed folic acid-deficient diets displayed lethargy, anorexia, and increased mortality (Dupree 1966). Anemia in coho salmon fed folic acid-deficient diets was macrocytic with poikilocytosis of erythrocytes and a reduction in number of the erythrocytes (Smith and Halver 1969). Clinical folic acid deficiency symptoms in coho salmon in the same study included reduced growth, pale gills, exophthalmia, dark coloration, and distended abdomens with ascites fluid. These authors suggested that blood cell formation in fish is very sensitive to folic acid deficiency because of the importance of folic acid in incorporation of nucleotides into deoxyribonucleic acid. Phillips (1963) detected anemia (type was not reported) in brook trout fingerlings fed folic acid-deficient diets after 9 wk.

Qualitative cyanocobalamin requirements have been demonstrated for chinook salmon (Halver 1957) and channel catfish (Dupree 1966). Halver (1957) reported growth retardation and reduced hemoglobin concentrations and erythrocyte numbers in chinook salmon fed a cyanocobalamin-deficient diet for 16 wk. Channel catfish fed cyanocobalamin-deficient diets displayed growth retardation after 36 wk (Dupree 1966) or lower hematocrits after 24 wk (Limsuwan and Lovell 1981). Limsuwan and Lovell (1981) demonstrated that intestinal microorganisms synthesized about 1.4 ng of cyanocobalamin/g body weight per day. Lack of differences in growth, hemoglobin concentration, and erythrocyte numbers in fish fed either 21.8 ng cyanocobalamin/g

diet or 1.8 ng cyanocobalamin/g diet suggested that cyanocobalamin requirements are marginal for channel catfish (initial mean weight = 7.1 g) over a 24-wk period. However, channel catfish fry and early fingerling stages may have demonstrated a cyanocobalamin requirement under similar conditions.

The effects of feeding folic acid-deficient or folic acid plus vitamin B<sub>12</sub> (cyanocobalamin)-deficient diets have been examined with brook trout (Phillips 1963) and rohu (John and Mahajan 1979). During the time interval between 9 and 15 wk of a feeding study, anemia was more pronounced in brook trout fed a diet deficient in both vitamin B<sub>12</sub> and folic acid than in individuals fed a diet deficient in folic acid only. Lethargy, muscular loss, and poor growth rate were accentuated in rohu fed a diet concurrently deficient in folic acid and cyanocobalamin, compared with fish fed diets deficient in either folic acid or cyanocobalamin, singly. Megaloblastic anemia occurred in rohu fed a folic acid-deficient diet, a cyanocobalamin-deficient diet, and a diet deficient in both vitamins (John and Mahajan 1979).

### Biotin

Qualitative dietary requirements for biotin have been demonstrated for brook, brown, and lake trout (Phillips and Brockway 1957), chinook salmon (Halver 1957), coho salmon (Coates and Halver 1958), goldfish, *Carassius auratus* (Tomiyama and Ohba 1967), common carp (Ogino et al. 1970b), Japanese eel (Arai et al. 1972a), channel catfish (Robinson and Lovell 1978), and lake trout (Poston and Page 1982). Biotin deficiency symptoms generally occurring in salmonids include anorexia, poor growth, and depressed liver acetyl CoA carboxylase and pyruvate carboxylase (Poston and Page 1982). Biotin deficiency signs include spastic convulsions, fragmentation of erythrocytes, and muscle atrophy in chinook salmon (Halver 1957), depigmentation in channel catfish (Robinson and Lovell 1978), abnormal synthesis of liver fatty acids and high liver glycogen content in brook trout (Poston and McCartney 1974), pale-colored gills often with a mucous coating, protruding beyond the operculum in rainbow trout (Castledine et al. 1978), and high rates of deposition of glycogen in kidney tubules and short, thick gill lamellae in lake trout (Poston and Page 1982).

Biotin has been shown to be important in affecting growth and acetyl CoA carboxylase ac-

tivity in fish. Lake trout fingerlings required a minimum of 0.1 mg biotin/kg dry diet for optimal growth rate and a minimum of 0.5 mg biotin/kg dry diet for optimal swimming stamina (Poston 1976a). Acetyl coenzyme A carboxylase activity has been shown to be fully activated in livers of rainbow trout containing >3.3 μg biotin/g liver (Castledine et al. 1978). Dietary biotin concentrations of 8 mg/kg dry diet enhanced liver pyruvate decarboxylase activity in channel catfish fingerlings (Robinson and Lovell 1978), whereas 6 mg biotin/kg dry diet increased acetyl CoA carboxylase and pyruvate decarboxylase activities in brook trout fingerlings (Poston and McCartney 1974). Common carp fingerlings require 1 mg biotin/kg dry diet for maximal weight gain and biotin liver content (Ogino et al. 1970b).

Since biotin-containing lipogenic and gluconeogenic enzymes or both may have low activity in biotin-deficient trout, increased liver glycogen concentrations and altered liver fatty acid compositions may result (Poston and McCartney 1974; Poston 1976a). High concentrations of liver glycogen were reported in biotin-deficient lake trout (Poston 1976a; Poston and Page 1982) and rainbow trout (Castledine et al. 1978). Altered liver fatty acid composition occurred in biotin-deficient brook trout (Poston and McCartney 1974) and rainbow trout (Castledine et al. 1978). Liver lipid concentrations did not vary between channel catfish fed 0% or 8 mg biotin/kg dry diet over a 22-wk period (Robinson and Lovell 1978). Biotin-deficient diets resulted in larger liver size in brook trout compared with individuals fed sufficient dietary biotin (6 mg/kg dry diet) (Poston and McCartney 1974), whereas the presence (8 mg/kg dry diet) or absence of dietary biotin did not affect liver size in channel catfish (Robinson and Lovell 1978).

### Inositol

Qualitative requirements for inositol have been reported for rainbow trout (McLaren et al. 1947), brook, brown, and rainbow trout (Phillips and Brockway 1957), chinook salmon (Halver 1957), coho salmon (Coates and Halver 1958), common carp (Aoe and Masuda 1967), Japanese eel (Arai et al. 1972a), and red sea bream (Yone 1975). General inositol deficiency symptoms include poor feed digestibility and utilization, anorexia, reduced growth, and distended abdomens (Halver 1972a). Skin lesions occurred in common carp fed inositol-deficient diets and in-

cluded the following physiological and morphological changes: hemorrhage around the base of the dorsal fin, loss of skin mucosa, and "sloughing off" of scales and fins (Aoe and Masuda 1967).

Quantitative inositol requirements are available only for common carp and red sea bream. Common carp require 4 g inositol/kg dry diet for maximal weight gain, feed conversion, and prevention of skin lesions (Aoe and Masuda 1967). Red sea bream require between 550 and 900 mg inositol/kg dry diet in direct proportion to dietary glucose concentrations of 10 to 40% (Yone 1975). Currently, quantitative inositol requirements for salmonids are based upon dietary concentrations (250 to 400 mg/kg dry diet) that were included in the control diets used to determine qualitative vitamin requirements of chinook salmon (Halver 1957).

### Vitamin A

Vitamin A has been shown to be an essential dietary constituent for channel catfish (Dupree 1966), rainbow trout (Kitamura et al. 1967b), common carp (Aoe et al. 1968), goldfish (Jones et al. 1971), and brook trout (Poston et al. 1977). General physiological functions of vitamin A in fish include a role in maintaining normal growth rate, pigmentation, and vision. Long-term feeding studies have consistently yielded various eye malformations (e.g., popeye, cataracts, hemorrhage) in fish fed vitamin A-deficient diets. Rainbow trout fed no supplemental vitamin A in a semipurified diet developed corneal pitting and homogeneous clouding, thickening of the corneal epithelium, and degeneration of the retina (Poston et al. 1977). However, growth during the 22-wk period was similar regardless of dietary vitamin A content (0% vs. 10,000 International Units (IU) vitamin A/kg dry diet). Brook trout of a smaller initial weight (0.15 g) than the rainbow trout fingerlings (5.9 g) grew significantly faster over a 20-wk period when fed 10,000 IU vitamin A/kg dry diet compared with 0% vitamin A (Poston et al. 1977). Pronounced eyeball protrusions and dermal depigmentation occurred in brook trout fed vitamin A-deficient diets. Histopathological examinations of eyes of salmonids have shown that lens damage does not occur in vitamin A-deficient fish (Poston et al. 1977). Vitamin A deficiency symptoms in channel catfish include depigmentation, opaque and protruding eyes, atrophy, and death (Dupree 1970). Vitamin A-deficient goldfish developed exophthalmos, loss

of scales, anorexia, and eventual mortality (Jones et al. 1971).

Conversion efficiency of  $\beta$ -carotene to vitamin A has been examined indirectly for channel catfish and brook trout. Dupree (1966) indicated that 12 mg  $\beta$ -carotene/kg dry diet (equal to 20,000 IU of vitamin A/kg dry diet) were insufficient to prevent popeye in channel catfish fed vitamin A-deficient diets, whereas 450 IU of vitamin A/kg dry diet were sufficient to prevent occurrence of popeye in channel catfish fed diets devoid of  $\beta$ -carotene. These results suggested an inefficient conversion rate (if any) of  $\beta$ -carotene to vitamin A in channel catfish (Dupree 1966). In a separate study, channel catfish fingerlings (mean initial weight = 2.25 g) fed 1,000 IU vitamin A acetate had optimal weight gain and no occurrence of popeye or other vitamin A deficiency symptoms (Dupree 1970). Poston et al. (1977) demonstrated indirectly that brook trout can convert dietary  $\beta$ -carotene into vitamin A with conversion efficiency being greater at 12.4°C than at 9°C. Individuals fed 6 mg  $\beta$ -carotene/kg dry diet (10,000 IU vitamin A activity/kg for many terrestrial animals) without supplemental vitamin A did not develop depigmentation or pronounced eyeball protrusion. However, brook trout fed 10,000 IU vitamin A palmitate/kg dry diet without supplemental  $\beta$ -carotene grew significantly better than fish fed 6 mg  $\beta$ -carotene/kg dry diet. Additionally, brook trout fed 0.6 mg  $\beta$ -carotene/kg dry diet (1,000 IU vitamin A activity) during the same experimental period developed pronounced eyeball protrusion at either 9° or 12.4°C.

### Vitamin D

Qualitative requirements for cholecalciferol have been determined for channel catfish and rainbow trout. Lovell and Li (1978) demonstrated the essentiality of dietary cholecalciferol for channel catfish fingerlings via greater weight gain and bone mineralization (total body ash, phosphorus, and calcium) in individuals fed 500 IU cholecalciferol/kg dry diet compared with 0% dietary cholecalciferol. Hypervitaminosis was not detected since dietary cholecalciferol concentrations as high as 1,000,000 IU/kg dry diet did not suppress body weight gain nor body fixation of calcium and phosphorus. Barnett et al. (1979a) established the essentiality of cholecalciferol for rainbow trout fingerlings using two dietary concentrations (0% vitamin D<sub>3</sub> compared with 1,000 IU/kg dry diet). These investigators found that

symptoms of cholecalciferol deficiency included decreased weight gain and feed efficiency, marked increase in plasma triiodothyronine (T<sub>3</sub>) levels, lethargy, anorexia, increased lipid content of white muscle and liver, and clinical signs of tetany. Further study of cholecalciferol deficiency in rainbow trout indicated occurrence of tetany of the epaxial musculature (white muscle fibers) and changes in muscle ultrastructure, while red muscle fibers constituting the lateral line musculature appeared to be normal (George et al. 1979). These changes were interpreted as being indicative of disruption of calcium homeostasis. Bone ash, calcium and phosphorus, alkaline phosphatase, plasma calcium, and plasma magnesium were similar in rainbow trout fed either 1,000 IU cholecalciferol/kg dry diet or 0% cholecalciferol. Possibly, feeding dietary cholecalciferol concentrations >1,000 IU/kg to rainbow trout fingerlings would have influenced calcium, phosphorus, or magnesium content of bone or plasma. Plasma T<sub>3</sub> concentrations of rainbow trout were unaffected by calcium supplementation of vitamin D-deficient diets and plasma and skeletal calcium levels were unaffected by a limited range of dietary vitamin D content (0 to 1,000 IU cholecalciferol/kg dry diet) (Leatherland et al. 1980).

Relative efficacy of dietary ergocalciferol compared with dietary cholecalciferol was examined in channel catfish fingerlings (Andrews et al. 1980) and rainbow trout fingerlings (Barnett et al. 1979b; Leatherland et al. 1980). Dietary concentrations of 1,000 IU/kg or less promoted similar growth rates when identical amounts of ergocalciferol and cholecalciferol were fed to channel catfish. Comparison of identical dietary concentrations of cholecalciferol and ergocalciferol above 1,000 IU/kg (2,000 to 20,000 IU/kg) resulted in greater weight gain of fingerlings fed cholecalciferol. Based upon weight gain, channel catfish fingerlings (initial mean weight = 2.3 g) require dietary cholecalciferol at greater concentrations than 1,000 IU/kg dry diet, but ≤4,000 IU/kg dry diet (Andrews et al. 1980). Slightly larger channel catfish (initial mean weight = 6.0 g) require dietary cholecalciferol at greater concentrations than 1,000 IU/kg dry diet, but ≤2,000 IU/kg dry diet. Hypervitaminosis occurred in channel catfish fed 50,000 IU/kg of ergocalciferol or cholecalciferol as evidenced by reduced weight gain and feed efficiency. However, vertebral bone ash was not affected by various dietary ergocalciferol or cholecalciferol con-

centrations. Leatherland et al. (1980) reported that an inverse relationship between T<sub>3</sub>, a growth stimulating hormone, and dietary vitamin D concentration (cholecalciferol or ergocalciferol) existed in rainbow trout fingerlings. They speculated that hypersecretion of T<sub>3</sub> in fish fed vitamin D-deficient diets may be a compensatory response. Cholecalciferol concentrations of 200 or 800 IU/kg promoted slightly better growth of rainbow trout than identical concentrations of ergocalciferol (200 or 800 IU/kg). Also, 800 IU of cholecalciferol/kg was the only dietary vitamin D concentration which significantly reduced T<sub>3</sub> concentrations of fish compared with those fed a vitamin D-deficient diet. Barnett et al. (1979b) reported that rainbow trout fingerlings require between 1,600 and 2,400 IU of cholecalciferol/kg dry diet and that cholecalciferol is three times more effective than ergocalciferol in promoting weight gain.

### Vitamin E

Vitamin E has been established as an essential dietary component for chinook salmon (Woodall et al. 1964), brown trout (Poston 1965), channel catfish (Dupree 1969b; Murai and Andrews 1974), Atlantic salmon (Poston et al. 1976), common carp (Watanabe et al. 1970a; Watanabe and Takashima 1977), and rainbow trout (Cowey et al. 1981a). Vitamin E deficiency symptoms in channel catfish include poor growth, reduced food conversion, exudative diathesis, muscular dystrophy, depigmentation, fatty livers, anemia, and atrophy of pancreatic tissue (Murai and Andrews 1974). Dietary supplementation of DL- $\alpha$ -tocopherol (25 to 100 mg/kg) fed to channel catfish fingerlings removed all of these deficiency symptoms, whereas an antioxidant, ethoxyquin (125 mg/kg), did not significantly improve hematocrit levels or reduce incidence of muscular dystrophy. Vitamin E deficiency symptoms in chinook salmon included poor growth, exophthalmia, ascites, anemia, clubbed gills, epicarditis, and ceroid deposition in the spleen (Woodall et al. 1964). Brook trout fingerlings fed vitamin E-deficient diets had reduced growth rates, increased mortality, and lower microhematocrit values than did fish fed a diet containing 500 mg of DL- $\alpha$ -tocopherol acetate/kg dry diet (Poston 1965). Atlantic salmon fed vitamin E-deficient diets displayed anemia, pale gills, anisocytosis, poikilocytosis, exudative diathesis, dermal depigmentation, muscular dystrophy, and in-

creased carcass fat and water content (Poston et al. 1976).

Vitamin E has been shown to be important in reproductive physiology of fishes. Adult female common carp (initial mean weight = 100 g) fed a vitamin E-deficient diet for 17 mo displayed reduced weight gain, lower gonadosomatic index, apparent muscular dystrophy (degenerative epaxial muscles), higher muscle water content, lower muscle protein content, and lower concentrations of yolk granules and yolk vesicles in oocytes compared with individuals fed 700 mg  $\alpha$ -tocopherol/kg dry diet (Watanabe and Takashima 1977). Also, developing ovaries of common carp fed vitamin E-deficient diets had altered polar lipid fractions in the form of lower concentrations of 20:4 $\omega$ 6, 20:5 $\omega$ 3, and 22:6 $\omega$ 3 and higher concentrations of 18:1 $\omega$ 9 and 20:3 $\omega$ 9.

Quantitative vitamin E requirements of fishes depend upon interaction of several factors: 1) Dietary concentration of polyunsaturated fatty acids, 2) dietary selenium concentration, 3) dietary concentrations of prooxidants and antioxidants, 4) diet storage temperature, and 5) length of diet storage. Woodall et al. (1964) reported that a dietary vitamin E concentration of 5 to 30 mg  $\alpha$ -tocopherol combined with 5% herring oil provided satisfactory growth of chinook salmon and prevented the occurrence of clinical deficiency symptoms in this species. Common carp fingerlings (initial mean weight = 1.6 g) required about 100 mg  $\alpha$ -tocopherol/kg dry diet in order to maintain maximal growth rate and feed efficiency (Watanabe et al. 1970b). Slightly larger common carp fingerlings (initial mean weight = 6.4 g), fed 100 mg or less of DL- $\alpha$ -tocopheryl acetate/kg dry diet concurrently with 5% dietary methyl linoleate as the sole lipid component, displayed apparent muscular dystrophy and had less weight gain than did fish fed 300 mg DL-tocopheryl acetate/kg dry diet plus 5% methyl linoleate (Watanabe et al. 1977). Also, common carp fed 10 or 15% methyl linoleate plus 100 mg DL- $\alpha$ -tocopheryl acetate, had significantly less weight gain and higher occurrence of muscular dystrophy than fish fed 2 or 5% methyl linoleate plus 100 mg DL- $\alpha$ -tocopheryl acetate. Rainbow trout fingerlings (initial mean weight = 0.9 g) fed a diet containing 15% pollock liver oil (in the form of methyl esters) displayed general vitamin E deficiency signs (anorexia and reduced growth), after 6 wk (Watanabe et al. 1981). Diets supplemented with 50 mg  $\alpha$ -tocopherol/kg dry diet prevented anorexia and promoted growth

rates equal to those fish fed 100, 300, or 500 mg  $\alpha$ -tocopherol/kg dry diet. However, the minimal requirement may be slightly <50 mg  $\alpha$ -tocopherol/kg dry diet. Larger rainbow trout fingerlings (initial mean weight = 10 g) require 20 to 30 mg DL- $\alpha$ -tocopheryl acetate/kg dry diet when fed 1% 18:3 $\omega$ 3 plus 13% palmitic acid (Cowey et al. 1981a). Dietary vitamin E concentrations <20 mg DL- $\alpha$ -tocopheryl acetate/kg dry diet resulted in higher molar ratios of polyunsaturated fatty acid to tocopherol in rainbow trout livers. Also, in vitro ascorbic acid-stimulated peroxidation in mitochondria and microsomes was significantly higher in rainbow trout fed low dietary vitamin E concentrations (e.g., 0 and 5 mg DL- $\alpha$ -tocopheryl acetate/kg dry diet). Furthermore, these investigators suggested that the vitamin E requirement for rainbow trout is undoubtedly proportionally higher with increasing dietary concentrations of unsaturated fatty acids. Supplementary dietary concentrations of either 33, 66, or 99 IU of DL- $\alpha$ -tocopheryl acetate/kg dry diet added to 20 mg of dietary  $\alpha$ -tocopherol/kg dry diet, produced equal growth rates, feed efficiency, and whole body percentage protein, lipid, and moisture of rainbow trout fingerlings fed 12% dietary lipid (Hung et al. 1980). In another study, diets containing 24 mg of  $\alpha$ -tocopherol/kg dry diet combined with about 12% dietary lipid, which included 7.5% dietary, unoxidized herring oil, prevented vitamin E deficiency in rainbow trout fingerlings (Hung et al. 1981).

## Vitamin K

Dietary supplementation of vitamin K for salmonids has proven beneficial in increasing hematocrit values of brook trout (Poston 1964) and lake trout (Poston 1976b), whereas vitamin K supplementation of diets fed to channel catfish did not enhance blood clotting time or hemoglobin concentrations (Murai and Andrews 1977). Growth of each of the aforementioned species was unaffected by dietary vitamin K supplementation. A dietary concentration of 1 mg menadiolone dimethylpyrimidinol bisulfite/kg dry diet was sufficient to provide normal coagulation and packed cell volume of lake trout blood (Poston 1976b). Murai and Andrews (1977) concluded that channel catfish have an extremely low, if any, dietary vitamin K requirement, since individuals fed a diet devoid of vitamin K had similar weight gain, blood clotting times, prothrombin times, and hematocrit values to individuals fed

up to 1.2 mg menadione sodium bisulfite/kg dry diet.

## MINERALS

### Calcium and Calcium-to-Phosphorus Ratios

Initial investigations indicated that calcium uptake in fish is primarily through imbibition and gill absorption rather than from dietary sources (Podoliak 1961; Simmons 1971). Nevertheless, since calcium and phosphorus are both major components of fish bone and scales, dietary calcium-to-phosphorus ratios were examined by several investigators to determine if any interactions occur between calcium and phosphorus, which might result in altered bone ash, calcium, and phosphorus content. Dietary calcium did not affect growth and feed efficiency of common carp (Ogino and Takeda 1976), channel catfish (Lovell 1977), and rainbow trout (Ogino and Takeda 1978). However, in a separate study with channel catfish, 1.5% dietary calcium induced maximal weight gain, whereas lower and higher dietary calcium concentrations produced less weight gain (Andrews et al. 1973). Optimal growth and feed conversion occurred in channel catfish fingerlings fed a 1.5:1 ratio of calcium to phosphorus (Andrews et al. 1973) and optimal feed efficiency and serum inorganic phosphorus were observed in red sea bream fed a 1:2 ratio of calcium to phosphorus (Sakamoto and Yone 1973). The difficulty in determining whether dietary calcium-to-phosphorus ratios are nutritionally significant in fish may be complicated by dietary factors such as magnesium and vitamin D or use of suboptimal calcium water concentrations in various studies. Based on the few species studied, it is unknown whether salinity determines if dietary calcium-to-phosphorus ratios are important dietary factors. Optimal dietary calcium-to-phosphorus ratios have been reported for one marine species (red sea bream) and one freshwater species (channel catfish), while no optimal ratios were reported for common carp or rainbow trout.

### Phosphorus

Dietary essentiality of phosphorus has been verified for channel catfish (Andrews et al. 1973; Lovell 1978), Atlantic salmon (Ketola 1975a), red

sea bream (Yone 1975), common carp (Ogino and Takeda 1976), and rainbow trout (Ogino and Takeda 1978). Deficiency symptoms in channel catfish include reduced growth, poor feed efficiency, low bone ash, and low hematocrit levels (Andrews et al. 1973), and reduced weight gain, bone ash, and bone phosphorus content (Lovell 1978). Red sea bream fed phosphorus-deficient diets contained lower vertebral ash, calcium, and phosphorus and more brittle bone structure (Yone 1975). Common carp and rainbow trout fed diets deficient in phosphorus had reduced calcium, phosphorus, and ash content of whole body and vertebrae (Ogino and Takeda 1976; Ogino and Takeda 1978, respectively). Also, Ogino and Takeda (1976) reported deformity of the frontal bone of the cranium of common carp and spondylolisthesis, brachyospondylie, and synostosis of vertebrae in phosphorus-deficient individuals.

Based on examination of limited sources of phosphorus, dietary phosphorus requirements have been reported as 0.4% or 0.42 to 0.47% for channel catfish (Gatlin et al. 1982; Lovell 1978, respectively), 0.6% inorganic phosphorus supplemented to a diet containing 0.7% phosphorus from plant sources for Atlantic salmon (Ketola 1975a), 0.68% for red sea bream (Yone 1975), 0.6 to 0.7% for common carp (Ogino and Takeda 1976), and 0.7 to 0.8% for rainbow trout (Ogino and Takeda 1978). Supplementation of 0.4 to 2% monosodium, monocalcium, or dicalcium phosphate to diets containing 0.55 to 0.65% available phosphorus did not improve growth or feed efficiency of rainbow trout fingerlings (initial mean weight = 21 g) over an 18-wk period (Reinitz et al. 1978a). Generally, inorganic phosphorus in formulated feeds is more digestible or available for fishes than organic forms of phosphorus occurring in soybean meal and fish meal (Ketola 1975a, b; Lovell 1978).

### Magnesium

Magnesium is an essential constituent of bone in fish and is interrelated with calcium metabolism. Whole body and vertebral calcium content were inversely related to dietary magnesium concentration in common carp (Ogino and Chiou 1976) and rainbow trout (Ogino et al. 1978), whereas whole body and vertebral phosphorus content were unaffected by dietary magnesium in the same species. Sakamoto and Yone (1979) concluded that marine fishes have very low (if

any) dietary magnesium requirements, since 12 versus 66 mg magnesium/100 g diet fed to red sea bream did not differentially affect growth, vertebral magnesium content, or vertebral calcium content.

Quantitative dietary magnesium requirements for rainbow trout (0.06 to 0.07%) and carp (0.04 to 0.05%) were established based upon one dietary calcium concentration and one calcium and magnesium concentration in ambient water in each study (Ogino et al. 1978; Ogino and Chiou 1976, respectively). Cowey (1976) reported that excessive dietary calcium (2.7%) in relation to dietary magnesium (0.04%) was accompanied by renal nephrocalcinosis in rainbow trout, while 0.1% magnesium fed to rainbow trout along with 2.7% calcium resulted in normal renal calcium concentrations. Further increases in dietary calcium concentrations to 4% required dietary magnesium concentrations of 0.1%, rather than 0.06% magnesium to prevent renal calcinosis (Cowey et al. 1977). Therefore a direct interrelationship was established between dietary calcium and magnesium fed to rainbow trout in freshwater (Cowey 1976).

### Manganese

Common carp and rainbow trout fingerlings have been found to have higher growth rates when fed 12 to 13 mg manganese/kg dry diet versus 4 mg manganese/kg dry diet (Ogino and Yang 1980). Manganese-deficient rainbow trout displayed abnormal curvature of the backbone and malformation of the tail.

### Zinc

Zinc has been shown to be an essential trace element for rainbow trout in separate studies (Ogino and Yang 1978; Ketola 1979b) and common carp (Ogino and Yang 1979). Dietary zinc concentrations of 15 and 30 mg/kg dry diet fed over an 8-wk period in the presence of 11  $\mu\text{g}$  zinc/l of rearing water promoted satisfactory growth of rainbow trout, while 5 mg zinc/kg dry diet produced slightly slower growth rates (Ogino and Yang 1978). In the same study, rainbow trout fingerlings fed 1 mg zinc/kg dry diet had poor growth, high mortality (46% vs. 0% in other treatments), high incidence of cataracts (49% vs. 0% in other treatments) and high incidence of fin erosion (86% vs. 0% in other treatments). Protein digestibility was appreciably reduced in rain-

bow trout fed 1 mg zinc/kg dry diet (Ogino and Yang 1978). Although carboxypeptidase activity was not tested, lower activity of this zinc-containing enzyme could explain lower protein digestibility (Ogino and Yang 1978). Ketola (1979b) determined that laboratory diets containing 40% white fish meal (60 mg zinc/kg dry diet) caused bilateral cataracts in rainbow trout, possibly as a result of excesses of other minerals in white fish meal (calcium, phosphorus, sodium, or potassium). Supplementation of 150 mg zinc/kg dry diet to the laboratory diet containing 40% white fish meal and 60 mg zinc/kg dry diet resulted in normal growth rates and prevented cataract formation in rainbow trout. Common carp, fed 1 ppm dietary zinc in the presence of 10  $\mu\text{g}$  zinc/l rearing water over 12- and 16-wk periods in separate studies, had high mortality rates, reduced growth rate, and had fin and skin erosion (Ogino and Yang 1979). No cataract formations were reported in zinc deficient common carp, however. Common carp fingerlings required between 15 and 30 ppm of dietary zinc for optimal growth.

### Iron

Dietary iron is essential for fishes to maintain normal hemoglobin content, hematocrit value, and mean corpuscular diameter. Hypochromic, microcytic anemia occurred in red sea bream (Yone 1975) and common carp (Sakamoto and Yone 1978b) as well as anisocytosis in red sea bream fed iron-deficient diets. Control diets fed to red sea bream and common carp, which prevented these iron deficiency symptoms, contained 1.2 g ferric citrate/kg dry diet and 199 mg iron/kg dry diet, respectively. A minimal dietary iron concentration of 150 mg/kg diet is required to prevent iron deficiency symptoms such as low mean corpuscular diameter and low blood iron content in red sea bream (Sakamoto and Yone 1978a).

### Copper

Dietary copper requirements have been investigated for channel catfish, common carp, and rainbow trout. Copper requirements, if any, for fingerling channel catfish are  $\leq 1.5$  mg/kg dry diet (Murai et al. 1981). Channel catfish fed 9.5 mg copper/kg dry diet while reared in water containing 0.33  $\mu\text{g}$  copper/l for 16 wk grew significantly slower than individuals fed diets containing only 3.5 mg copper/kg dry diet. Further

reductions in weight gain occurred in channel catfish fed diets containing 17.5 or 33.5 mg copper/kg dry diet compared with those individuals fed 9.5 mg copper/kg dry diet. Also, a slight reduction occurred in the number of erythrocytes and hematocrit levels in channel catfish fed 33.5 mg copper/kg dry diet resulting in slight anemia. Murai et al. (1981) suggested that since fish can absorb copper from the surrounding water, absorption of environmental copper may result in lower dietary copper requirements than that required by most terrestrial animals. Common carp fingerlings fed 0.7 mg copper/kg dry diet had lower weight gain than individuals fed 3.0 mg copper/kg dry diet (Ogino and Yang 1980). In contrast, no differential growth response occurred in rainbow trout fingerlings fed either 0.7 or 3.0 mg copper/kg dry diet (Ogino and Yang 1980).

### Selenium

Selenium is an essential dietary constituent for Atlantic salmon and rainbow trout. Poston et al. (1976) demonstrated dietary essentiality of selenium for Atlantic salmon fry and fingerlings. Deficiency of dietary selenium suppressed glutathione peroxidase activity, while supplements of both vitamin E (500 IU DL- $\alpha$ -tocopheryl acetate/kg) and selenium (0.1 mg/kg dry diet) prevented muscular dystrophy. However, no minimal dietary selenium requirement nor minimal selenium concentration causing toxicity was determined for Atlantic salmon. Dietary selenium concentrations as low as 0.07 mg/kg dry diet prevented selenium deficiency symptoms (i.e., degeneration of liver and muscle) in rainbow trout fingerlings concurrently fed 400 IU vitamin E/kg dry diet while reared in water containing 0.4  $\mu$ g selenium/l (Hilton et al. 1980). Since selenium is a component of glutathione peroxidase, it is of interest that maximal plasma glutathione peroxidase activity was obtained at a dietary selenium concentration of 0.15 to 0.38 mg/kg dry feed. On the other hand, selenium toxicity occurred in rainbow trout fed dietary selenium concentrations of 13 mg/kg of dry diet, causing reduced growth and feed efficiency and uncoordinated spiral swimming behavior 12 to 24 h before death. Hilton et al. (1980) emphasized the importance of reporting dietary vitamin E concentrations and water borne selenium concentrations when investigating dietary selenium requirements of fish.

### Iodine

Iodine has been shown to have a role in thyroid metabolism in fishes similar to that occurring in terrestrial animals. Woodall and LaRoche (1964) examined dietary iodide requirements of chinook salmon fed 0.1 to 10.1 mg iodide/kg diet during an initial 6-mo study and an additional 9-mo study. After 6 mo, no significant differences occurred in growth, feed efficiency, and body composition. However, iodine stored in the thyroid glands of chinook salmon fed 0.1 mg iodide/kg dry diet equaled only 40% of the iodide in individuals fed higher iodide concentrations (0.6, 1.1, 5.1, and 10.1 mg iodide/kg dry diet). The authors concluded that the minimal dietary iodide requirement of chinook salmon fingerlings was about 0.6 mg iodide/kg dry diet based upon the iodide content in thyroid glands. Additionally, they recommended a higher dietary iodide requirement for advanced parr (1.1 mg iodide/kg dry diet) and speculated that smoltification may be accompanied by increased thyroid activity. Increased thyroid activity has been demonstrated in several salmonids during the parr-smolt transformation (Wedemeyer et al. 1980).

### SUMMARY AND RECOMMENDATIONS

- 1) All fish species examined thus far in feeding studies require the same dietary essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine).
- 2) Optimal dietary lipid concentrations for maximal protein sparing action in most fish species range from 12 to 24%.
- 3) Qualitative essential fatty acid requirements and ability to elongate and desaturate fatty acids such as linoleic and linolenic acids are highly variable among fishes, indicating a need for more species-specific research.
- 4) Relative protein sparing action of carbohydrates and lipids is also highly variable among fish species, necessitating more species-specific research in place of approximating metabolic capabilities of an unstudied species based upon knowledge of other species.
- 5) Extensive research is needed to determine



- nutrient requirements for the striped bass and several coolwater fish species that support important commercial or recreational fisheries, or both.
- 6) A better understanding of the effects of high dietary fiber on fishes is necessary to evaluate the amount of interference of fiber with enzyme action, changes in transit time of ingested feed in the digestive tract with varying dietary fiber concentrations and effects of dietary fiber on nutrient absorption of fishes.
  - 7) Calcium and magnesium requirements in fishes should be further examined for individual species reared in different calcium and magnesium concentrations or salinities to further determine relative nutritional importance of dietary and water absorption routes of these minerals.
  - 8) Phosphorus requirements of fishes need to be evaluated in conjunction with calcium and magnesium requirements to determine optimal dietary ratios of each macromineral.
  - 9) Knowledge of requirements of fishes for trace elements such as selenium, copper, iron, and zinc is rare, thereby warranting additional research.
  - 10) Better definition of quantitative nutrient requirements of brood stock of various species is necessary to ensure better quality eggs and fry.
  - 11) Regarding better standardization of individual fish nutrition experiments, panelists from a recent fish nutrition workshop have recommended that measurements for metabolizable energy values for experimental diets and digestibility values for macronutrients should be monitored and reported. This is especially important in studies evaluating dietary protein concentration requirements and optimal protein to energy requirements of fishes. Metabolism chambers used by Smith (1971, 1976) are the preferred method.

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