THE EFFECT OF DIETARY LIPID AND VITAMIN E ON THE REPRODUCTION OF ARCTIC CHARR, *Salvelinus alpinus* (L.)

by

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ABSTRACT

This research was conducted in an attempt to improve embryonic survival of Labrador Arctic charr by altering the concentrations of lipid and vitamin E in the diets fed to broodstock. Four-year old Labrador Arctic charr broodstock were fed diets containing two levels of dietary lipid at two levels of vitamin E acetate (dl-α-tocopheryl acetate) supplementation. The diets were designated LLLE, LLHE, HLLE and HLHE to denote low lipid (12%)(LL), high lipid (19%)(HL), low vitamin E (30 mg/kg)(LE) and high vitamin E (600 mg/kg)(HE). For comparison, a fifth group was fed their normal diet - a commercial grower diet (COMM) containing 17% lipid and 100 mg vitamin E acetate/kg. Since Arctic charr broodstock used in this research were valuable, diets that might have a deleterious effect on the survival or fecundity of the broodstock could not be used. Dietary lipid concentrations were selected based on the requirements for juvenile Arctic charr. Dietary vitamin E concentrations were selected based on requirements stated in the literature for other salmonids.

Fish were fed for 71 days before withdrawal of feed prior to spawning in Year 1 and for 252 days in Year 2. Since fish fed the LL diets in Year 1 spawned 5 weeks later than those fed the HL diets, a crossover in diets was conducted to see if this would also occur in Year 2. Keeping the vitamin E level the

same, those fed LL diets in Year 1 were fed HL diets in Year 2 and vice versa. No effect of dietary lipid on spawning time was observed in Year 2.

The proportion of females failing to spawn was not related to the concentrations of dietary lipid or vitamin E. A significantly higher proportion of males fed the higher concentration of vitamin E produced milt in two successive spawning seasons.

Fecundity of 3894 and 4532 eggs in Year 1 and 4154 and 8305 eggs in Year 2 for the four experimental and COMM diets, respectively, was directly correlated with female weight and was not affected by the level of dietary lipid or vitamin E.

The fatty acid composition of the eggs reflected that of the diet and was not indicative of essential fatty acid deficiency. In Year 2, concentrations of total (22.3%), neutral (10.6%) and polar lipids (11.7%) in the eggs were not significantly affected by the concentrations of dietary lipid or vitamin E. The vitamin E concentrations of the eggs were 52, 202, 54 and 156 µg/g in Year 1 and 38, 208, 51 and 140 µg/g in Year 2 for the LLLE, LLHE, HLLE and HLHE diets respectively. The increase in dietary vitamin E resulted in a greater increase in vitamin E concentration in eggs from fish fed the LL compared to the HL diets.

Diets containing 30 or 600 mg vitamin E acetate/kg with 12 or 19% lipid met the vitamin E requirements of 4- and 5-year old broodstock to the extent that fertilization and embryonic survival was not affected significantly. Fertilization was 89% in Year 2 and 80% in Year 2. There was a high degree of within treatment variation in embryonic survival. Fertilization and embryonic survival were not correlated with vitamin E concentration of the eggs. Survival to swimup of fertilized eggs was 27% for the LE diets and 55% for the HE diets in Year 2. Embryonic survival to the eyed, hatch and swimup stages were negatively correlated with the percent neutral lipid and with 16:1n7 in the neutral lipid of the eggs.

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INTRODUCTION

As the aquaculture industry in Canada matures, there is the need to develop the technology to raise different species of fish and thus allow producers to raise different products for which they can develop markets. Beginning in 1978, stocks of Arctic charr [*Salvelinus alpinus* (L.)] were collected as eggs from wild fish in the Northwest Territories (NWT) and Labrador and transferred to the Rockwood Aquaculture Research Centre (RARC), Gunton, Manitoba. This facility became the major source of Arctic charr for producers across Canada. Travel costs to obtain eggs from wild stocks of Arctic charr, uncertain spawning times and lack of disease-free wild stocks make it impractical or impossible to continue obtaining eggs from wild stocks. This makes it necessary for producers to maintain their own broodstocks and they will require information on their dietary and environmental requirements.

Hatchability of Arctic charr from broodstock which have been raised in captivity has varied with the specific stock. Hatchability of the stocks originating from Nauyuk Lake in the NWT and from Norway has been high (Tabachek and de March 1990). Since these stocks are raised in a non-certified building at RARC, most producers cannot obtain permits to transport eggs from those stocks across provincial boundaries. One stock from the Fraser River, Labrador is raised in a certified building at

RARC and since these eggs can be transported across provincial boundaries, they have been distributed widely to producers throughout Canada. Unfortunately, embryonic survival of this stock has been variable and often low when fed the same diet and raised under the same environmental conditions as the Nauyuk Lake and Norway stocks at RARC.

Survival at first-feeding is also stock-dependent and some producers have experienced high mortality at first feeding with Labrador and Norway Arctic charr (Tabachek and de March 1991). Poor or uncertain hatchability and survival of fry forces producers to maintain a large inventory of broodstock and makes planning of production difficult. Therefore, there is a need to improve both the level and predictability of embryonic survival and survival at first-feeding of the Labrador stock of Arctic charr. Differences also existed in the duration of the spawning period with the Labrador stock of Arctic charr spawning over 4 to 5 months while the other stocks spawned within 6 weeks (Tabachek 1990).

There is a scarcity of information on the nutritional requirements of fish of reproductive age and on Arctic charr broodstock in particular. Therefore, it was not known if some nutrient(s) were related to the poor hatchability of Labrador Arctic charr broodstock.

Maternal nutrition affects embryonic and fry survival and can be influenced by levels of dietary protein and energy, carotenoids and specific minerals and vitamins (Luquet and Watanabe 1986). The levels of essential fatty acids and vitamin E affect hatchability of both fish (Watanabe 1985) and poultry (Jensen 1968). The level of unsaturated fatty acids influenced the vitamin E requirement of rainbow trout (*Oncorhynchus mykiss*) fry (Watanabe *et al.* 1981a). Juvenile Arctic charr require a high level of dietary protein (44-54%) and lipid (15-20%) (Tabachek 1986). Juvenile Arctic charr may have a requirement for a high level of essential fatty acids (Yang and Dick 1992) but the requirements for Arctic charr broodstock are unknown. It was hypothesized that if Arctic charr broodstock required a high level of dietary lipid and/or unsaturated fatty acids, they might also have a requirement for a high level of dietary vitamin E. The objective of this research was to examine the effects and interactive effects of both dietary lipid and vitamin E on the reproduction of Arctic charr. Reproductive parameters of interest included embryonic survival, growth and survival of fry, fecundity, proportion of the population that matured and duration of the spawning period.

LITERATURE REVIEW

2.1 Natural history of Arctic charr

Arctic charr have a circumpolar distribution throughout Arctic waters. In Canada, they are found in Labrador, Newfoundland, throughout the Northwest and Yukon Territories (Johnson 1980). Populations also occur in New Brunswick, northern Quebec and northern Manitoba. There are many differences in the natural life history between some of these populations including differences in growth rate, longevity, fecundity and age at maturity. Arctic charr used in the research described in this thesis originated from the Fraser River, Labrador. Charr may make several migrations to sea before they mature. Dempson and Green (1985) found that Labrador Arctic charr migrated to sea for the first time when they were 78-191 mm in length at 3 to 7 years of age. Charr migrated to sea in May and early June, spent 2-3 months at sea and returned to fresh water from the latter half of July through August. Larger fish returned first with a higher proportion of females returning early in the run. Annual length increments were 68 mm between 4-5 years, 103 mm between 5-6 years and 71 mm between 6-7 years of age. While 50% of males matured at 5 years of age when they were 245 mm in length, females matured at an older age with 50% mature at 7 years when they were 381 mm in length and 75% mature at 8 years. They estimated that 60% of the females returning to fresh water would spawn that year.

This is in contrast to charr in Nauyuk and Willow Lakes, NWT which spawn one year after returning from the sea and may spend up to two years in fresh water before returning to the sea (Johnson 1980).

Anadromous Arctic charr are iteroparous, spawning in alternate years. However, in nonanadromous populations they may spawn every year with only one anadromous stock in Norway spawning annually. While some tagged charr in an anadromous population were found to spawn every year, others in the same population were not recaptured at the same spawning site for two to five years. It is not known if the latter fish did not spawn during that interval or if they spawned at a different site but the former seems more probable. Arctic charr may spawn several times during their lifetime but frequency of spawning may decrease with increasing age.

In Labrador, water temperature during late July and August, when charr were returning to fresh water, varied between years but ranged from 8.7 to 12.9°C (Dempson and Greene 1985). Spawning occurred during a three week period in October at a water temperature of 1-3°C. It occurred in areas of fine and coarse sand and 40-50 mm gravel in 1.5-2.0 m of water. Fecundity of 7-13 year olds ranged from 2316-9245 eggs with a mean of 4665 ± 434 (std. dev.). Egg diameter was 4.5 mm.

The type of food consumed changes seasonally and annually according to availability. Small charr are benthic feeders and in fresh water they feed on invertebrates such as chironomids, amphipods, copepods and molluscs (Johnson 1980). When charr migrate to sea, they remain along the coastline and are pelagic feeders. When Labrador charr migrate to the sea, principal food items include amphipods (*Parathemisto libellula*), euphasid shrimp (*Meganyctiphanes norvegica*) and fish such as capelin (*Mallotus villosus*), sand lance (*Ammodytes americanus*) and young mailed sculpin (*Triglops pingeli*). During spawning, eggs are consumed by charr that may or may not have been involved in spawning. Charr can also be cannibalistic. Postspawning charr consume little, if any, food in fresh water and they can be very emaciated when they return to sea the following year.

Dempson and Green (1985) found few Arctic charr in Labrador that were older than 12 years with the oldest fish being 18 years. In other locations throughout the world, charr may live to be 30-33 years of age.

2.2 Arctic charr culture in Canada

Beginning in 1978, stocks of Arctic charr eggs were collected in the wild during several collections and transferred to the Rockwood Aquaculture Research Centre (RARC), Gunton, Manitoba. They were collected in the wild from Nauyuk Lake, N.W.T. in 1978 (Papst and Hopky 1984), Fraser River, Labrador in

1980 (Baker 1981, 1983), 1981 and 1984 (de March 1991) and Tree River, NWT in 1988. They were also collected from a hatchery stock from Sunndalsøra, Norway in 1980 (Baker 1983). Primarily, it has been the progeny from the Fraser River, Labrador stock collected in 1984 that have been distributed from a small certified facility at RARC. They have been transferred from RARC to producers, researchers and government agencies in every province and territory in Canada (Olson, personal communication).

When incubated at 6°C, survival of eggs collected from wild stocks was 88% to the eyed stage for the Nauyuk Lake, NWT stock (Papst and Hopky 1984), 93% to swimup for the Fraser River, Labrador stock collected in 1980 (Baker 1981) and 94% to eye and 87% to swimup for the Tree River, NWT stock (Olson, personal communication). While the hatchability of eggs collected in the wild from the Nauyuk Lake, NWT stock was high, the first generation domestic stock produced eggs which had only 17% survival to the eyed stage with 65% of eggs being unfertilized (Papst and Hopky 1984). Changes to the rearing and spawning techniques have increased the hatchability of this stock to 73-98% (Krieger *et al.* 1988) with consistent hatchability between fish and between spawning years. The latter authors showed that the eggs of Arctic charr over-ripened quickly and the chance of fertilization decreased significantly between the periods of 4-7 days and 11-14 days after ovulation. They also showed that

fertilization improved if the males had been raised in cold water (6.5°C) compared to warm water (8-17°C) prior to the first year of spawning. As with the Nauyuk Lake stock, survival of eggs from the Norway stock is high (> 80% eyed, Olson, personal communication). While hatchability of the Nauyuk Lake stock has improved, survival of stocks of Arctic charr originating from the Fraser River, Labrador have remained highly variable between fish and between spawning years at RARC. For example, survival to the eyed stage for one stock of Labrador charr was 50%, 72% and 35% in three successive years (Tabachek and de March 1990) and 68% the following year (Olson, personal communication). There has been varying success among producers who have raised Labrador charr to maturity (Tabachek and de March 1991). Hatchability of the Labrador stock of Arctic charr has been shown to be under maternal rather than paternal influence. When de March (1992) separated the eggs from individual Labrador females into groups and fertilized each group with the milt from a different male, the pattern of embryonic survival always indicated that survival was influenced only by the female. This observation suggests that the nutritional status and/or environmental conditions of the female should be investigated.

When Srivastava (1991) collected eggs from Labrador Arctic charr which had spawned for the first time, he found that those that spawned in the middle of the spawning season produced eggs that weighed significantly more, and had higher protein, lipid,

carbohydrate, ash and amino acids than those that spawned either 4 weeks earlier or 4 weeks later. In addition, eggs from this middle group also reached the eyed stage, hatched earlier and had fewer deformed fry than those spawned earlier or later.

Age of maturity and fecundity of stocks raised for one or two generations in captivity vary from one stock to another. The Nauyuk Lake stock produced 1200-2000 eggs while the Labrador stocks produced 3000-4000 eggs and the Norway stock produced 3000 eggs (Tabachek and de March 1990). The Labrador and Norway stocks start to spawn at 3 years of age, while the Nauyuk Lake stock does not start to spawn until at least 4 years of age with most fish spawning for the first time at 5-6 years of age. All stocks spawn for 4-5 years (Olson, personal communication). The mortality rate begins to increase at 6 years of age and fish are generally euthanized at 7-8 years of age.

Nauyuk Lake Arctic charr fingerlings raised at 12°C gained significantly more when fed a diet containing 54% protein and 20% lipid compared to those fed a diet containing 44% protein and 15% lipid (Tabachek 1986). Feeding diets containing 34% protein and/or 10% lipid resulted in significantly lower weight gain compared to feeding diets containing 44% protein and/or 15% lipid. The requirements for rainbow trout have been reported as 34% protein in diets with 15-20% lipid when raised at 15-18°C (Takeuchi *et al.* 1978) and as 40% protein in diets with 10%

lipid when raised at 16-27°C (Satia 1974). The essential fatty acid requirements of Arctic charr are discussed in Section 2.6.3. There has been little other research reported on the nutritional requirements of juvenile or broodstock Arctic charr.

Survival of swimup fry varies with the stock (Krieger 1987). Survival and growth of the Labrador and Norway stocks improved when rearing temperature at first feeding was increased from 7 to 10°C (Tabachek 1992) while there was only a slight improvement in survival and growth when fed a semimoist diet from one manufacturer compared to a dry diet from another manufacturer.

2.3 Physiology of reproduction

The physiology of reproduction in teleost fishes has been reviewed in detail (Nagahama 1983, Ng and Idler 1983). In summary, environmental factors such as photoperiod and temperature act upon the hypothalamus which is stimulated to secrete gonadotropin-releasing hormone (GRH). This hormone acts upon the pituitary causing it to secrete the gonadotropins a) lutenizing hormone (LH) and b) follicle-stimulating hormone (FSH). Thecal cells are stimulated to synthesize testosterone from cholesterol. FSH stimulates the development of the follicles and stimulates granulosa cells surrounding each oocyte to produce the enzyme that converts testosterone to estrogen. Estrogen is transported to the liver where it activates

hepatocytes to synthesize vitellogenin. Vitellogenin is transported to the ovary where it is selectively taken up by oocytes through micropinocytosis. Vitellogenin is taken up by the follicle cells, transported across the microvilli of the zona pellucida into the microvilli in the zona radiata of the oocyte. Nutrients such as vitamins and minerals are also taken up by the oocyte. Most vitellogenin, a glycolipophosphoprotein, is synthesized in the liver by exogenous vitellogenesis but there is evidence that a portion is synthesized in the ovary by endogenous vitellogenesis. It is hydrolyzed in the oocyte to form phosvitin (phosphoprotein) and lipovitellin (proteolipid which is 80% protein and 20% lipid). The lipid of lipovitellin consists of both polar and neutral lipids which Leger *et al.* (1981) found were 35% triacylglycerides. Yolk granules, containing lipovitellin and phosvitin, form in the periphery of the oocyte during the primary stage and fuse to form yolk spheres during the secondary stage. They coalesce to form a single yolk during late vitellogenesis. Oil droplets appear at the nucleus, migrate to the periphery and merge to form larger oil droplets. The oil droplets consist of triacylglycerides with traces of cholesterol and cholesteryl esters (Luquet and Watanabe 1986). When oocytes have matured and first meiosis ends, the microvilli of the follicle cells withdraw and the oocytes are released. Second meiosis then continues to metaphase and eggs are ready to be released from the body and fertilized.

2.4 Feed intake and reproduction

2.4.1 Feed intake

The effect of level of feed intake (ration) on maturation, fecundity, egg size and survival of eggs and fry has been reviewed (Roley 1983, Springate *et al.* 1985, Hardy 1985 and Luquet and Watanabe 1986).

Orr *et al.* (1982) found that adult rainbow trout consumed more feed and had higher weight gain when fed with demand feeders compared to those fed by hand. Fish feeding at high rates produced larger eggs and had higher fecundity but lower relative fecundity (eggs per body weight) than those who were hand-fed. There was no difference in survival of eggs to the eyed stage. Knox *et al.* (1988) found that feeding at full ration (0.7% body weight) resulted in rainbow trout producing progeny which were significantly larger at the eyed egg, yolk sac and swimup fry stages than fish fed at half-ration (0.35% body weight). Swimup fry from parents fed the full ration had significantly higher protein and lipid levels compared to the half-satiation group. However, ration had no effect on the fatty acid composition of polar or neutral fatty acids. Springate *et al.* (1985) reported on another part of the experiment of Knox *et al.* (1988) and stated that rainbow trout fed at full ration spawned 2-3 weeks earlier than those fed at half ration. The concentration of vitellogenin and testosterone in the serum were significantly higher at spawning in fish fed

at full ration. All fish fed at full ration spawned while 11% of those fed at half ration did not spawn. In addition, fish fed at full ration produced significantly more eggs (22% more) and these eggs were significantly larger than those from fish fed at half ration. Histological examination showed that there were significantly more atretic oocytes in fish fed at half ration. Ration did not affect fertilization rate or survival to the eyed stage.

Roley (1983) fed rainbow trout to satiation and half-satiation for one year prior to spawning. At cool water temperature, both egg size and survival increased for the half-satiation group compared to the satiation group. Feeding to satiation increased the variation in embryonic survival. In contrast to Orr *et al.* (1982), the high level of feeding resulted in smaller eggs with low survival although survival was independent of egg size. In contrast to Knox *et al.* (1988), spawning was delayed but occurred within a shorter interval in fish which had been fed to satiation but feeding level had no effect on the number of fish that spawned.

Jones and Bromage (1987) fed rainbow trout rations varying between 0.4 and 1.5% of body weight and found that while ration had a significant effect on fecundity beyond the effect of ration on body weight, it had no effect on egg diameter. This

is in contrast to the findings of both Orr *et al.* (1982) and Roley (1983).

2.4.2 Starvation prior to the spawning period

Ashton (1991) found no difference in fatty acid composition or survival of chinook salmon (*Oncorhynchus tshawytscha*) to the eyed stage when broodstock were starved for 7 and 14 days before spawning. Starvation of rainbow trout for 40 days before spawning did not affect fecundity, hatchability, egg weight, egg diameter or proximate composition (Ridelman *et al.* 1984).

2.5 Protein and energy

The influence of dietary protein and energy levels on reproduction has been reviewed by Hardy (1985) and Luquet and Watanabe (1986). It was believed that requirements for protein and energy might increase during the time of ovarian development. However, Hardy (1985) pointed out that somatic growth ceases during ovarian development and that there may not be an overall increase in the requirements for protein and energy.

Roley (1983) fed rainbow trout diets containing protein levels of 27 to 57% at 3.8 kcal metabolizable energy (ME)/g diet for 8 months prior to their first spawning at 2 years of age. There were no significant differences in hatchability, egg diameter, fecundity, duration of spawning or prespawning

mortality. He concluded that the requirement was 37 to 47% protein at 3.8 kcal ME/g. Washburn (1989) investigated the effects of dietary protein and energy on rainbow trout broodstock and found that feeding high dietary protein (58%) and energy (3.23 kcal/g) concentrations resulted in significantly lower hatchability compared to feeding a diet that was low in protein (30%) and energy (2.64 kcal/g). This latter combination was below the level of energy assessed by Roley (1983).

Smith *et al.* (1979) fed rainbow trout for 6 months before their first spawning season and then through 2 more consecutive spawning seasons. Test diets contained low, medium and high levels of protein (36, 42 and 48%) and energy (2.52, 2.85 and 3.44 kcal/g). Feeding rate was 0.8% body weight/day until feed intake decreased as spawning approached. The high protein-high energy diets had a positive effect on weight gain, fecundity and egg size but no significant effect on survival to the eyed stage.

Long-term feeding of rainbow trout from the fingerling stage through the second spawning season on diets containing 35% protein with 16-18% lipid or 45% protein with 15% lipid (Takeuchi *et al.* 1981b) resulted in no significant difference in fecundity, egg diameter or survival to the eyed (90%) and hatch (87%) stages. This conflicts with the findings of Watanabe *et al.* (1984b) who observed poor hatchability when broodstock were

fed a diet containing 46% protein and 15% lipid (57%) compared to 36% protein and 18% lipid (86%) or 28% protein with 21% lipid (70%).

2.6 Lipids, fatty acids and vitamin E

2.6.1 Chemistry of lipids and fatty acids

Neutral lipids include mono-, di- and triacylglycerides (TAG), free fatty acids, cholesterol and cholesterol esters with TAG providing the major source of energy. Polar lipids, which include phosphoglycerides, glycolipids, sphingomyelins and plasmolagens, are required for the formation of cellular and subcellular membranes.

Fatty acids are an integral part of these lipids. The position of the first double bond from the methyl end of a fatty acid is denoted by its "n" number with n3 fatty acids having their first double bond after the third carbon from the terminal end. Additional double bonds are interrupted by one saturated carbon atom. Double bonds increase the flexibility of the molecule and also result in a decrease in the melting point. Both these factors are important in maintaining the flexibility and function of membranes, especially in poikilotherms which live in cold environments. This is the reason there are high levels of unsaturated fatty acids in the polar lipids of biological membranes in fish. Fatty acids with 2 to 4 double bonds are called polyunsaturated fatty acids (PUFA) while those

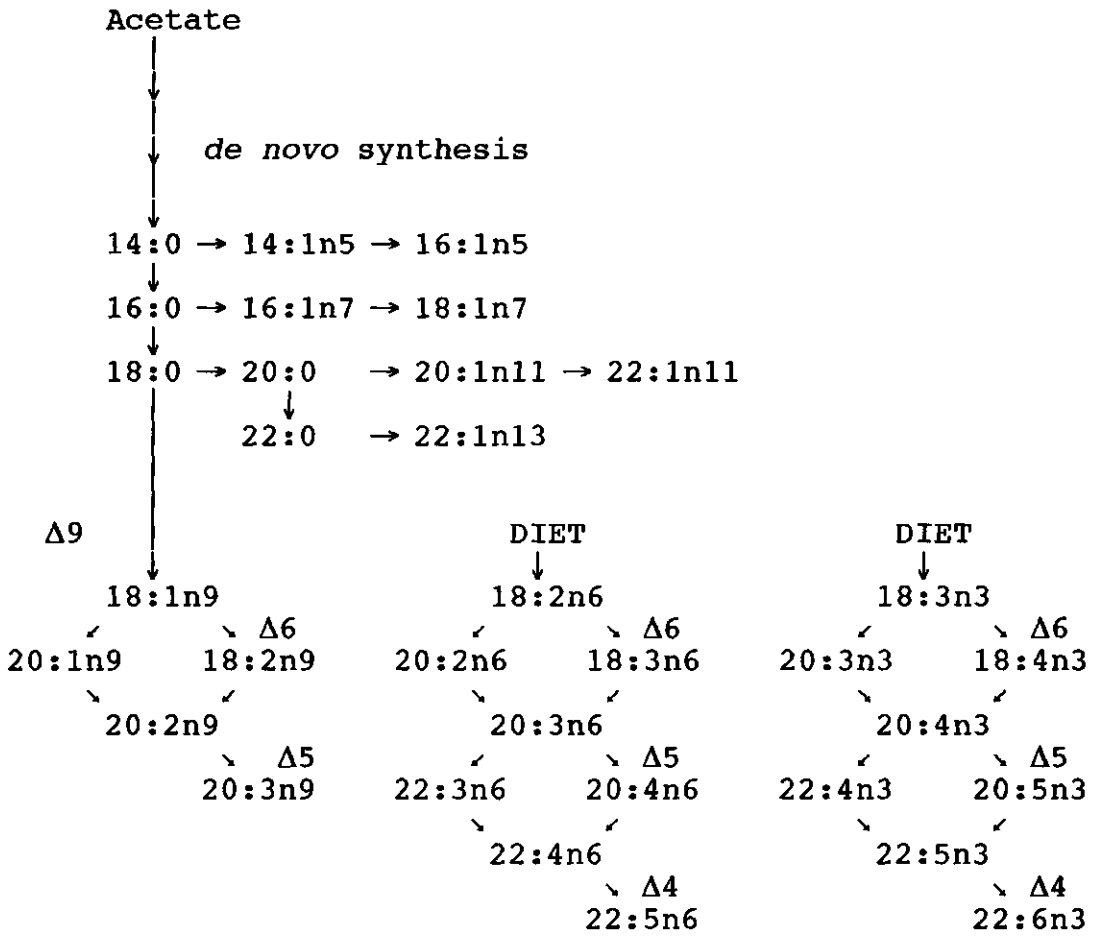
with greater than or equal to 5 double bonds are referred to as highly unsaturated fatty acids (HUFA). In fish, PUFA consist mainly of n6 fatty acids while HUFA consist mainly of n3 fatty acids. In TAG, saturated and monoenoic fatty acids occupy positions on the 1- (α) and 3- (γ) carbon of the glycerol molecule, while PUFA or HUFA occupy the 2- (β) carbon position. In polar lipids, monoenoic and saturated fatty acids occupy the α position and HUFA or PUFA occupy the β position.

Saturated fatty acids can be synthesized *de novo* from acetate (Castell 1979). Fatty acids that cannot be synthesized but which are necessary for the health and reproduction of the animal are considered to be essential fatty acids which must be supplied in the diet. Since fish cannot synthesize 18:2n6 or 18:3n3, the diet must supply these two fatty acids as precursors for desaturation and elongation into PUFA and HUFA or the diet must supply PUFA or HUFA (Figure 1). Desaturation and elongation of the n9, n6 and n3 series of fatty acids occurs through a common enzyme pathway of desaturases and elongases but with specific enzymes for each position (Figure 1). In mammals, the desaturase enzymes have a greater affinity for the most unsaturated fatty acid available (Leray and Pelletier 1985). That is, Δ5-desaturase has a greater affinity for 20:4n3 than 20:3n6 which has greater affinity than 20:2n9. Thus, there is the ability to desaturate 20:2n9 to 20:3n9 or 20:3n6 to 20:4n6 but in the presence of 20:4n3, Δ5-desaturase preferentially

converts 20:4n3 to 20:5n3. It is speculated that a similar mechanism occurs in fish and the presence of 20:3n9 is used as an indicator of essential fatty acid deficiency (Castell *et al.* 1972).

2.6.2 Digestion and absorption of lipids and vitamin E

In the gut lumen, an emulsion of lipid and lipid-soluble compounds occurs on contact with bile salts and bile phospholipids produced by the liver. In rainbow trout, triacylglycerides at the surface of the emulsion are hydrolyzed through the action of pancreatic lipase (Leger 1985) and possibly a colipase. Triacylglycerides are hydrolyzed in the 1- and 3-position into free fatty acids and 2-monoacylglycerides. The hydrolysis of the fatty acid in the 2-position also occurs if the acyl chain contains at least one double bond. This is different from mammals and birds where this latter hydrolysis does not occur. Micelles, consisting of bile salts produced by the liver, are amphipathic providing a polar exterior and neutral interior (Scott *et al.* 1982). This allows micelles to solubilize monoacylglycerides, long-chain unsaturated fatty acids and glycerol. The mixed micelle can then solubilize long-chain saturated fatty acids and fat soluble vitamins A, D, E and K and transport them to epithelial cells along the proximal intestine and/or pyloric cecae for absorption (Leger 1985). These cells project microvilli into the lumen giving them a high absorptive area.



Δ9, Δ6, Δ5, Δ4 = desaturases

Figure 1. Synthesis, desaturation and elongation of fatty acids in fish (Castell 1979, Henderson and Sargent 1985).

Tocopherols and their esters (Figure 2) have low absorption efficiencies of only 20-40% in mammals (Gallo-Torres 1980a) and this may be due to the long phytyl side chain. While absorption efficiencies have not been established for fish, α-tocopherol was absorbed more readily in rainbow trout than the β-, γ- or δ-forms of the vitamin (Watanabe *et al.* 1981b). Tocopheryl esters are hydrolyzed by pancreatic esterases to form tocopherol which can be solubilized in mixed micelles and absorbed (Leger 1985). Almost no tocopheryl esters are absorbed. Hung *et al.* (1982) found that absorption of d-α-tocopherol from dietary dl-α-tocopheryl acetate was slower than from dietary d-α-tocopherol and they speculated that this might be due to limitations in deesterification prior to absorption.

Re-esterification of glycerol, monoacylglyceride and lysophospholipids with fatty acids to form TAG and polar lipids (Leger 1985) occurs in the intestinal mucosa. End products pass through the intestinal wall, become incorporated into chylomicrons and very low density lipoproteins and are transported by the blood or lymphatic system (Sargent *et al.* 1989). Lipoproteins transported via the blood are transported to the liver by the hepatic portal vein and then to other tissues. TAG is taken up by adipose tissue (Henderson and Sargent 1985) where it is stored until required as an energy source while polar lipids are transported to other tissues for incorporation into membranes.

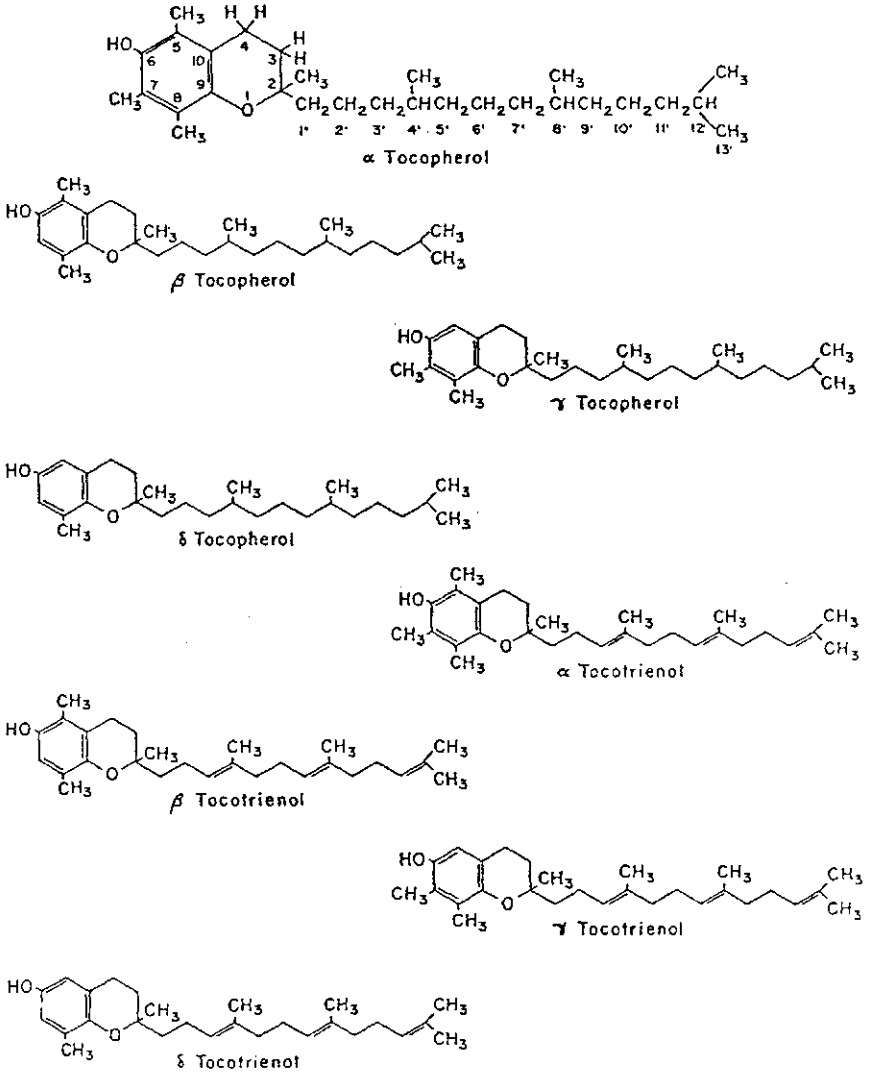


Figure 2. Forms of vitamin E (Scott *et al.* 1982).

Tocopherol is transported primarily in low density lipoproteins (Hung *et al.* 1982) and is taken up rapidly by plasma and liver but more slowly by white muscle, heart, spleen and erythrocytes (Cowey *et al.* 1983, Hung *et al.* 1982). When rainbow trout were fed a vitamin E-supplemented diet and then given an oral dose of d-α-tocopherol, initially it was found in high concentration in the cytosol of liver cells but this declined as it was taken up by mitochondria and microsomes (Cowey *et al.* 1981). In contrast, when fish had been fed a vitamin E-deficient diet, tocopherol was taken up most rapidly by mitochondria in the liver followed by microsomes, the nuclear fraction and cytosol implying that it is needed most by mitochondria.

2.6.3 Essential fatty acid requirements of fish for reproduction

The essential fatty acid requirements of immature fish have been reviewed by Kanazawa (1981). A recent report (Yang and Dick 1992) states that the essential fatty acid requirements of Arctic charr fingerlings were met with a diet supplemented with 1.7% n3 and 0.5% n6 fatty acids. Both Olsen *et al.* (1991) and Yang and Dick (1992) showed that Arctic charr were capable of desaturating and elongating 18:3n3 and 18:2n6. The former authors observed that n3 fatty acids were used in preference to n6 fatty acids for desaturation and elongation.

The requirements of broodstock for fatty acids have been reviewed by Watanabe (1985) and Ashton (1991) but no work with Arctic charr broodstock has been reported. Yu *et al.* (1979) fed rainbow trout experimental diets throughout their entire life history and assessed the hatchability of the eggs and the growth of the fry. They found that 18:2n6 was not required in the presence of 1.0% 18:3n3 fatty acids and both groups had equal hatchability (60%, n=4). Leray *et al.* (1985) fed a commercial diet and a semipurified diet to rainbow trout female broodstock for one year before spawning with the two diets differing in many respects including fatty acid composition. By recalculation of the data presented, the commercial diet contained 2.5% n6 and 1.3% n3 while the semipurified diet contained 5.4% n6 and no n3. The hatchability from the group fed the commercial diet averaged 79% (n=3) while that of the n3 deficient group was 20% (n=4). Those fed the n3-deficient diet produced eggs which had developmental disorders that could be seen at the 16-32 cell stage and hatched fry exhibited numerous types of spinal deformities and a shorter time to resorb the yolk sac (50 vs. 65 days). Watanabe *et al.* (1984b) observed that fecundity and embryonic survival decreased when rainbow trout were fed a diet with no n3 or n6 (54% eyed and 36% hatch) compared to a diet supplemented with 5% 18:2n6 (88% eyed and 82% hatch). Red sea bream (*Pagrus major*) fed n3-deficient diets also produced eggs with very low hatchability and egg and fry abnormalities were observed (Watanabe 1984a).

As discussed in Section 2.3, vitellogenin is a very high density lipoprotein containing 80% protein and 20% lipid which is high in essential fatty acids (Leger *et al.* 1981), especially 20:5n3 and 22:6n3. Feeding trout an n3-deficient diet for 6 months prior to spawning decreased the amount of vitellogenin (Fremont *et al.* 1984) as well as the levels of 20:5n3 and 22:6n3 in vitellogenin while it increased the level of 20:4n6. These changes were similar but less pronounced in other serum lipoproteins and were most pronounced during early vitellogenesis indicating that feeding a diet sufficient in essential fatty acids is important for vitellogenesis.

The fatty acid composition of the eggs reflected that of the diet. In both Leray *et al.* (1985) and Watanabe *et al.*'s (1984b) experiments, feeding trout diets with no n3 fatty acids resulted in eggs which had lower levels of n3 fatty acids (20:5, 22:5 and 22:6) and higher levels of n6 (18:2 and 20:4) in both the polar and neutral lipid fractions. Watanabe *et al.* (1984b) also found that the fatty acid composition of the milt reflected that of the diet in the same way as for the eggs and suggested that the effect of this change on fertilization should be investigated. While 20:3n9 was not found in the eggs of parents fed diets lacking in n3 fatty acids for 3 months (Watanabe *et al.* 1984a) or one year (Leray *et al.* 1985) prior to spawning, it was found in the milt. When trout were fed diets containing 18:3n3 alone or in combination with 18:2n6 for their entire life

history, 20:3n9 was observed in the eggs from both groups but was lower in the group fed the diet containing both fatty acids (Yu *et al.* 1979).

The transfer of 16:0 and 22:6n3 from the yolk sac to the body of the developing fry was shown by their increase in the body and concurrent decrease in the yolk sac of brook trout (*Salvelinus fontinalis*) (Atchison 1975) and rainbow trout (Hayes *et al.* 1985). Hayes *et al.* (1985) found that 18:0 as well as 16:0 and 22:6n3 were preferentially retained (74%) in rainbow trout at the yolk sac stage while fatty acids such as 16:1, 18:1, 20:4 and 20:5 showed only 41% retention. They postulated that those fatty acids which had high retention were required for specific purposes. Moreover, these purposes might be related to inclusion into polar lipids with the highly unsaturated 22:6n3 incorporated into the β position and the saturated 16:0 and 18:0 incorporated into the α position on the glycerol molecule.

2.6.4 Vitamin E

2.6.4.1 Chemistry of tocopherols

The chemistry of the tocopherols has been well documented by Kasparek (1980). Isomers of tocopherol exist which differ in the presence and location of methyl groups on the chromanol ring giving rise to α-, β-, γ- and δ-tocopherols (Figure 2). Stereo-isomers also exist with different centers of symmetry around the

C-2 carbon of the ring and the C-4′ and C-8′ of the isoprenoid chain. The d- stereoisomers are found in nature while those manufactured synthetically are racemic mixtures of d- and l-tocopherols. Watanabe *et al.* (1981b) found that α-tocopherols accumulated in tissues of rainbow trout, especially in the liver, while β-, γ- and δ- forms did not. No interconversion of these isomers occurred in the muscle or liver. Vitamin E is a generic term referring to all tocol or tocotrienol derivatives exhibiting the biological activity of α-tocopherol (Kasparek 1980). Unless otherwise specified, the term vitamin E in this thesis refers to α-tocopherol or its acetate derivative.

One of the major functions of vitamin E is its action as an antioxidant and the phenolic hydroxyl group provides the functional group. Based on effectiveness in preventing resorption of embryos in the rat, the isomers and their esters differ in biological activity (NRC 1987):

d-α-tocopherol	1.49 I.U./mg
d-α-tocopheryl acetate	1.36 I.U./mg
dl-α-tocopherol	1.1 I.U./mg
dl-α-tocopheryl acetate	1.0 I.U./mg
d-β-tocopherol	0.12 I.U./mg
d-γ-tocopherol	0.05 I.U./mg

Oxidation of tocopherols is enhanced by exposure to light, heat, alkali, iron and copper ions. Since α-tocopherol is

easily oxidized (Bauernfeind 1980), it is added to animal feed in its esterified form as α-tocopheryl acetate, a more stable form of the vitamin. The ester form has no antioxidant properties until the acetate group has been replaced by a hydroxyl group during hydrolysis initiated by pancreatic esterases. Since tocopherols are manufactured by plants, they are present in feed ingredients of plant origin. They are also present in ingredients of animal origin where animals have consumed plant material containing tocopherol.

2.6.4.2 Function of vitamin E

Vitamin E functions in preventing lipid oxidation and attack by free radicals, compounds with one or more unpaired electrons. In the peroxidation of lipid, a hydrogen atom is abstracted from the methylene carbon of a methylene-interrupted unsaturated fatty acid to form a lipid radical (R.)(Cowey 1986). This abstraction is initiated only by radicals with a high degree of reactivity, such as a hydroxyl radical (.OH), peroxy radical (ROO.) or possibly a hydroperoxy radical (HOO.). The double bond shifts resulting in a lipid radical which can react with oxygen to form a peroxy radical (ROO.) which can then abstract hydrogen atoms from other unsaturated fatty acids. The process is autocatalytic because generation of more radicals keeps the cycle going. The process destroys the function of PUFA by converting them into other compounds. It also results in the formation of (hydro)peroxides which move into all parts

of the cell reacting with membranes and sulfhydryl-containing enzymes (Tappel 1980). Vitamin E acts as a hydrogen or electron donor, donating the hydrogen from its 6-hydroxyl group to the peroxy radical converting it to the lipid hydroperoxide (ROOH). A vitamin E radical is formed but this is not active enough to abstract hydrogen from PUFA as ROO. can. ROOH is converted to ROH through the action of glutathione peroxidase in the presence of glutathione (GSH). The hydroxy radical (OH.), which is another radical that can initiate the oxidation process (Cowey 1986), is formed when the superoxide radical (O_{2}-.) and H_2O_2 react to form OH. and OH^- in the presence of Fe^{+3}. This reaction is prevented by the action of glutathione peroxidase which catalyzes the conversion of H_2O_2 to H_2O. Vitamin E and glutathione peroxidase function in different ways in preventing oxidation. Vitamin E functions within the membrane to protect polyunsaturated fatty acids with electron transport functions against free radical attack. Glutathione peroxidase acts within the cytosol to prevent the formation of hydroxy radicals and it destroys hydroperoxides which form.

Vitamin E is mainly associated with the mitochondrial and microsomal membranes and to a lesser degree with the plasma and nuclear membranes (Cowey *et al.* 1981). Mitochondrial membranes contain 35-40% lipid and 90% of this is comprised of polar lipids (Pike and Brown 1967) which are high in PUFA and HUFA. The inner mitochondrial membrane contains proteins that allow

the passage of fatty acids, pyruvate, oxygen and some ions such as Ca^{+2} and PO_4^{-3} from the intermembrane space into the matrix space (Alberts *et al.* 1983) where enzymes involved in oxidation of fatty acid and pyruvate to acetyl CoA are located. In the matrix space, acetyl-CoA is metabolized via the citric acid cycle. Enzymes in the inner membrane include those involved in the electron transport system and oxidative phosphorylation (ATP synthetase complex). Vitamin E is associated with the inner membrane and influences the degree of unsaturation of the membrane. For example, PUFA have been found to be were lower in hepatic mitochondrial membranes from ducklings fed a vitamin E-deficient diet compared to a control diet (Molenaar *et al.* 1980). The fatty acid composition of the membrane affects the transport of compounds across the membrane by influencing the rigidity or fluidity of the membrane and the translocation of transport proteins. It is possible that free radicals form in this area because mitochondrial membranes are high in PUFA and oxygen is being supplied to mitochondria for oxidative reactions. Vitamin E deficiency has been shown to be associated with increased membrane fragility of both cell membranes such as erythrocytes in fish, birds and mammals and mitochondrial membranes in birds.

In mammals, vitamin E radicals are converted to tocopherone, hydrolyzed to tocopheryl quinone (Gallo-Torres 1980b) and reduced to tocopheryl-p-hydroquinone. The latter

compound is conjugated with glucuronic acid in the liver and excreted in the bile.

In some cases, the need for vitamin E in the membrane can be reduced by the presence of selenium which, through its action in glutathione peroxidase, can limit the formation of some of the products in the cytosol which keep the autocatalytic peroxidative process going. There is also evidence that carotenoids act as antioxidants (Tacon 1981) and there may be interactions between vitamin E and carotenoids. If this interaction did exist for Arctic charr, addition of carotenoids to the diet might reduce the requirement for vitamin E. Carotenoids were not added to the diets in this experiment in order to investigate the effects of vitamin E alone.

2.6.4.3 Mobilization and requirement of vitamin E for reproduction

King (1985) monitored the temporal changes in distribution of vitamin E in mature female rainbow trout. During the month of June, fish were fed a diet not supplemented with vitamin E. At the beginning of July, she found there was considerable storage of vitamin E in the liver and this constituted 45% of the total body storage of vitamin E (25 mg). Distribution of vitamin E was monitored in fish fed diets supplemented or not supplemented with the vitamin. In July and August, vitamin E was transported from the liver mainly to the muscle with some

transported to the ovary. Between August and October, the vitamin was transported from the muscle to the ovary and adipose tissue, and then from the adipose tissue to the ovary between October and December. During this latter period, vitamin E was deposited in the eggs from the ovarian tissue and there was a marked increase in the vitamin E content of the eggs. By December, there was a significant difference in the vitamin E content of the eggs from fish fed the vitamin E-supplemented diet compared the unsupplemented diet. Fish spawned between January 6 and 27. At spawning, fish fed the unsupplemented diet and supplemented diets, contained a total body burden of 25 and 35 mg vitamin E, respectively and the ovarian tissue contained 54.0% and 49.0% of this body burden, respectively. After spawning, the body burden was reduced to 11.5 and 18 mg vitamin E for those fed the unsupplemented and supplemented diets, respectively. At this rate, one or two additional spawning seasons would be required to reduce the body stores of vitamin E drastically.

Kinumaki *et al.* (1972) found that the vitamin E concentrations of ovarian tissue increased 4- to 12-fold when rainbow trout were fed diets supplemented with large amounts of vitamins A, D and E for 10-17 weeks prior to the start of spawning. At spawning, vitamin E was found to be distributed mainly in the muscle, skin and ovaries with a greater proportion of vitamin E present in the ovaries of supplemented fish (57-

59%) compared to the unsupplemented fish (39%). In the latter group, 17% of the vitamin was stored in the liver compared to 3-7% in the supplemented fish.

The requirement of fish for vitamin E depends on many factors. These include the species and stage of development, form of the vitamin, water temperature (Cowey *et al.* 1984), level of PUFA in the diet (Watanabe *et al.* 1981a), presence of selenium (Scott *et al.* 1982) and the presence of antioxidants such as ethoxyquin (6-ethoxy-1,2-di-hydro-2,2,4-trimethylquinoline). This makes it difficult to establish requirements and compare the results of experiments.

Vitamin E has been associated with reproduction since Evans and Bishop (1922) discovered that vitamin E prevented resorption of fetuses in pregnant rats. The word "tocopherol" was derived from the Greek "tokos" meaning "offspring", "pherein" meaning "to bear" and "ol" to denote the alcohol group (Scott *et al.* 1982). The vitamin protects the integrity of membranes in the female reproductive system of some animals and the vascular system of the embryo in the hen, turkey, rat, cow and ewe. It also prevents male sterility through its effect on the seminiferous tubules of the male rat, guinea pig, hamster, dog and chicken.

Several researchers have investigated the effects of dietary vitamin E on the reproductive performance of different species of fish. For example, King (1985) fed rainbow trout purified diets containing 0 or 90 mg vitamin E acetate/kg with 8% lipid for different periods of time of up to 6 months followed by starvation for 1 month prior to spawning. At spawning, there was a significant difference in vitamin E concentration of eggs only between those always fed the unsupplemented diet (5.39 μg/egg) compared to those always fed the supplemented diet (7.37 μg/egg). There was significantly greater survival of eyed eggs after shocking if the females had been fed the diet supplemented with 90 mg vitamin E acetate/kg for 6 months. Overall, hatchability was excellent with all treatments with only 11% mortality in eggs from females fed the deficient diet for 6 months and 2% mortality with the supplemented diet. Survival was poorest at levels of < 5 μg vitamin E/egg and best when eggs contained > 7 μg vitamin E/egg. Survival was poorest when vitamin E concentration was < 54 μg/g and best at > 82 μg/g. A critical period existed for the maximum effect of vitamin E deprivation on the rainbow trout egg. Fish fed the unsupplemented diet between August and October produced eggs with lower vitamin E levels than those from fish fed that diet later than October. Vitamin E was transferred efficiently from the yolk to the fry with swimup fry having similar vitamin E content to the eggs. King believed that the broodstock had sufficient body stores of vitamin E at

the beginning of the experiment to supply the ovaries without any supplementation of the diet. Further, she stated that the diets would need to be fed for a longer period of time before the body reserves of vitamin E would be depleted sufficiently for there to be a significant effect of dietary vitamin E concentration on hatchability.

Spawning time was delayed one week and fish did not all spawn successfully if they had been fed the unsupplemented diet for 6 months. Of the 8 fish in this treatment group, 3 spawned successfully, 3 did not ripen, 1 produced abnormal eggs and 1 died prior to spawning. The abnormal eggs had dispersed lipid globules and convoluted membranes. All the fish in the supplemented group spawned normally. There were no significant differences in egg weight or diameter.

Kinumaki *et al.* (1972) fed practical diets to rainbow trout broodstock. They fed diets containing 66, 4600 and 8200 mg vitamin E/kg for 19 weeks. The control diet (66 mg vitamin E/kg diet), which contained only 10% of the levels of vitamins A and D compared to the other two diets, resulted in survival to the eyed stage of 51% compared to 46 and 37% for the other diets respectively. Vitamin E in the eggs was 4.4 µg/egg (46 µg/g) for the control group compared to 23 and 32 µg (240 and 330 µg/g) for the groups fed diets supplemented with 4600 and 8200 mg vitamin E/kg. When the yolk sac had been absorbed, the fry

contained 3.5, 13 and 30 μg vitamin E/fry, respectively or 27, 114 and 166 μg/g. Thus, vitamin E had been transferred from the diet to the egg and absorbed from the yolk sac by the fry. The multiple differences between the levels of fat-soluble vitamins in the diets prevent making any firm conclusions about this research.

Takeuchi *et al.* (1981a) fed ayu (*Plecoglossus altivelis*) for 3 months prior to spawning with diets containing graded levels of vitamin E acetate plus endogenous vitamin E (total 10-2390 mg/kg). Feeding unsupplemented diets (10 mg endogenous vitamin E/kg) decreased survival to the eyed stage, hatchability and survival of fry immediately after hatching. Spawning was delayed or failed to occur in one-third of the female population fed the unsupplemented diet while all fish spawned when they were fed diets containing more than 34 mg total vitamin E/kg.

Watanabe and Takashima (1977) fed carp (*Cyprinus carpio*) broodstock semipurified diets containing either 0 or 700 mg vitamin E acetate/kg for 17 months. They found that the fish fed the unsupplemented diet had a very low gonadosomatic index (ovary weight/body weight). Oocyte development was delayed with oocytes lacking large yolk granules and yolk vesicles even though the oocytes had reached a size at which these granules and vesicles should have been deposited. It appeared that oocytes had begun to develop and then became atretic.

Histological examination of the pituitary gland indicated decreased secretory activity of the gonadotrophs, which produce FSH and LH, and thyrotrophs, which produce thyroid-stimulating hormone, and the epithelial cells in the thyroid tissue were also reduced in height. Spawning was probably delayed or prevented in a larger portion of the population as it was for the ayu (Takeuchi *et al.* 1981a) and rainbow trout (King 1985). In addition to endocrine gland dysfunction, the fatty acid composition of the carp ovarian tissue resembled that of fish with essential fatty acid deficiency since the tissue contained increased levels of 18:1n9 and 20:3n9 and decreased levels of 20:4n6, 20:5n3 and 22:6n3. If the eggs had been analyzed for fatty acid composition, they probably would have reflected this essential fatty acid deficiency as well. Watanabe and Takashima (1977) postulated that the α-tocopherol deficiency caused a reduction of 18:2n6, one of the essential fatty acids for carp, and that this created a secondary deficiency in n3 fatty acids.

Watanabe *et al.* (1991) evaluated the influence of vitamin E acetate concentration on reproduction in red seabream. Fish were fed a control diet containing 500 mg vitamin E acetate/kg for 90 days and subsequently they were fed either the control diet or a diet containing 2000 mg vitamin E acetate/kg for 10 days prior to and 30 days after spawning. The rate of buoyant eggs, hatching success and normal larvae were directly influenced by dietary treatment. Production of normal larvae

increased from 35% to 76% for seabream fed the diet containing 2000 mg vitamin E acetate/kg.

At the time that the current experiment began, most commercial broodstock diets available in Canada were supplemented with 300-500 mg (IU) dl-α-tocopheryl acetate/kg diet, while two were supplemented with 200 and 800 mg/kg. Although there are no reports on hypervitaminosis E in fish, March *et al.* (1973) showed that chicks had decreased growth rates and skeletal mitochondrial respiration rates when they were fed diets containing 2200 compared to 1000 mg vitamin E/kg. This led NRC (1987) to suggest a "tentative presumed safe use level of 75 IU/kg of BW/day".

MATERIALS AND METHODS

3.1 Location and time

The fish rearing and egg incubation portions of this research were conducted at Rockwood Aquaculture Research Centre (RARC) located 30 km north of Winnipeg (49°54′ N, 97°8′ W), Manitoba. Biochemical analyses and all other related activities (except as noted) were conducted at the Freshwater Institute Science Laboratory (FWISL), Winnipeg, Manitoba.

Throughout this thesis, Year 1 refers to the period from June 27, 1988 when the fish were weighed and distributed to the tanks until January 11, 1989 one week after the first spawning period was completed. Year 2 refers to the period from January 12, 1989 until January 3, 1990 when the second spawning period was completed.

3.2 Diets

3.2.1 Formulation

Four diets were formulated to contain two levels of lipid (12 and 19%) at each of two levels of dl-α-tocopheryl acetate (vitamin E acetate) (30 and 600 mg/kg). The formulations are presented in Table 1. Since broodstock used in this research were valuable, diets were formulated that were not likely to have a deleterious effect on the survival of the broodstock or their fecundity. The lower level of 30 mg vitamin E acetate/kg

Table 1. Formulation of experimental diets (g/kg dry diet, except as noted).

	Diet				
	LLLE	LLHE	HLLE	HLHE	COMM[1]
Herring meal, steam-dried[2,3] (CP=78%, TL=12%)[4]	395	395	395	395	
Soybean meal[2] (CP=47%, TL=2.5%)	195	195	195	195	
Corn gluten meal[2] (CP=68%, TL=8%)	86	86	86	86	
Whole wheat, ground[2] (CP=16%, TL=4%)	183	183	183	183	
Herring oil[5]	44	44	114	114	
Alpha-cellulose	70	70	-	-	
Vitamin premix[6]	10	10	10	10	
Mineral premix[7]	10	10	10	10	
Choline chloride (60%)	6	6	6	6	
Ascorbic acid (crystalline)	1	1	1	1	
dl-α-Tocopheryl acetate[8](mg/kg)	30	600	30	600	
Ethoxyquin (mg/kg)	35	35	-	-	

[1] Commercial rainbow trout grower diet contained all the ingredients in the high lipid diets plus blood meal, poultry meal, whey, brewers yeast, limestone, salt and methionine. COMM contained wheat shorts and middlings but no whole wheat or inositol.

[2] Four ingredients combined to form a basal mixture.

[3] Stabilized with ethoxyquin at 250 mg/kg.

[4] CP=Crude protein, TL= Total lipid

[5] Stabilized with ethoxyquin at 500 mg/kg.

[6] Vitamin premix supplied the following in mg or I.U./kg dry diet: vitamin A acetate (retinyl acetate), 10,000 I.U.; cholecalciferol, 2,400 I.U.; menadione sodium bisulfite, 25; thiamin mononitrate, 30; riboflavin, 60; calcium pantothenate, 120; niacinamide, 200; folic acid, 15; biotin, 3; pyridoxine hydrochloride, 30; cyanocobalamin, 0.06; inositol, 400.

[7] Mineral premix supplied the following in mg element/kg dry diet: Mn (as $MnSO_4.H_2O$), 86; Fe (as $FeSO_4.7H_2O$), 63; Cu (as $CuSO_4.5H_2O$), 25; Zn (as $ZnSO_4.7H_2O$), 144; I (as KIO_3), 8.

[8] Supplied courtesy of Hoffmann-La Roche, Etobicoke, Ontario.

1981). The 600 mg vitamin E acetate/kg concentration was chosen because it was above the range added to most salmonid broodstock diets (300-500 mg/kg). Tabachek (1984) showed that a dietary lipid concentration of 10% was below the growth requirements for Nauyuk Lake Arctic charr fingerlings while dietary lipid concentrations of 15-20% resulted in significant increases in weight gain. Lipid concentrations of 10-11% and 17-18% were chosen but upon analysis the diets contained 12% and 19% lipid. Throughout this thesis, the four diets have been denoted as LLLE (Low Lipid Low vitamin E), LLHE (Low Lipid High vitamin E), HLLE (High Lipid Low vitamin E), HLHE (High Lipid High vitamin E). The higher lipid level was achieved by replacing alpha-cellulose in the low lipid diets with additional herring oil stabilized with 0.05% ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4 trimethyl-quinoline). To equalize the concentration of ethoxyquin in the diets, additional ethoxyquin was added to the low lipid diets.

In addition to the above diets, a commercial rainbow trout grower diet (denoted as COMM) was fed to one tank of fish as a reference diet. The broodstock diet manufactured by the supplier (Martin Feed Mills, Elmira, Ontario) was a minimum of 6 mm in diameter. The wide variation in the size of charr in the population resulted in the 6 mm diet being too large for a portion of the population to consume. Therefore, the 5 mm grower diet had been used routinely at RARC until all the fish were large enough to consume the 6 mm broodstock diet.

3.2.2 Manufacturing

Soybean meal and whole wheat were ground in a Fitz mill and were ground finely enough to pass through an 18 U.S. mesh screen (1.00 mm) and for 60% of it to pass through a 30 U.S. mesh screen (0.60 mm). A basal mix of herring meal, corn gluten meal, ground soybean meal and whole wheat was prepared in a vertical mixer and was placed in double plastic bags with an outer brown paper bag. Minerals were ground to a fine powder in a coffee grinder prior to weighing. Both the mineral and vitamin premixes were prepared in pearlized cornstarch carriers, mixed in a Hobart mixer and stored in double plastic bags. Herring oil was placed in three 20 L plastic containers. All the above ingredients and mixes were prepared at UBC and shipped from Vancouver to Winnipeg under frozen conditions. Upon arrival in Winnipeg, the basal mix, ascorbic acid, choline chloride mixture, vitamin and mineral premixes were frozen at 35°C until required for manufacturing the diets during the two year period of the experiment. The dl-α-tocopheryl acetate was stored in a refrigerator while ethoxyquin was kept at room temperature. Herring oil was stabilized with the addition of ethoxyquin at 500 mg/kg (NRC 1981), flushed with nitrogen and stored at -35°C until required.

The diets were manufactured five times during the course of the experiment at 9-13 wk intervals (mean = 11 wk). Ingredients were weighed and mixed each time the diets were manufactured.

The commercial diet (COMM) was from one shipment in Year 1 and two shipments in Year 2. Since the herring oil did not reach a uniform liquid state when thawed at room temperature, a warm water bath (37°C) was used to liquefy the oil. After use, nitrogen was bubbled into the oil in the container before returning it to the freezer. Each time the four diets were made, all the oil used was only from one container. Both dl-α-tocopheryl acetate and ethoxyquin (when required) were mixed with the prescribed weight of herring oil before adding the oil mixture to the other dry ingredients. Diets were cold pelleted through a 5 mm die in a Superior-Templewood pellet mill located at the Department of Animal Science, University of Manitoba. Distilled water was added at 6-7% to aid in pelleting. Pellets were spread out in a thin layer on waxed paper and allowed to air dry for 4-5 hours at which time the moisture content was less than 9%. The diets were sieved using a Kason separator to pass over a No. 12 U.S. sieve (1.7 mm). Diets were placed in double plastic bags and an outer brown paper bag, transported to Rockwood Aquaculture Research Centre and stored at -10°C.

Herring meal and oil was purchased from B.C. Packers, Steveston, B.C. Approximately one month prior to this purchase, DFO's West Vancouver Laboratory purchased herring oil from the same supplier. A sample of oil from this first shipment was stabilized with 0.05% ethoxyquin and placed in two containers which were flushed with nitrogen. One container was placed in

the refrigerator and the other container was placed in a freezer at -15°C. The following day, the oil and a sample of basal mix were shipped on ice to Hoffmann-La Roche, Etobicoke, Ontario for analysis of α-tocopherol. Samples of herring oil used in this experiment were also shipped to Hoffmann-LaRoche but they were not analyzed. The herring oil used in this experiment was analyzed for peroxide value each time the diets were made (Woyewoda *et al.* 1986).

3.3 Parental fish

3.3.1 History of the stock

Arctic charr used in this experiment originated from the Fraser River in Labrador (56°39'N, 63°10'W). Charr were spawned at the site in October 1984 and fertilized eggs were transported to Winnipeg, Manitoba and incubated in a quarantine area at the FWISL. Once parental fish had been tested and found to be free of certifiable diseases, 1070 eyed eggs were transferred to the Rockwood Aquaculture Research Centre's (RARC) broodstock building which had a certified disease-free status. The number of fish was reduced over the years through culling, natural mortality and sampling of fish for certification testing once a year. Fish spawned for the first time as 3 year olds in the fall of 1987 with 143 females producing eggs out of the 476 male and female fish present at that time. An unknown number of fish that had not matured were maintained separately because they looked like "potential spawners" (Olson, personal communication)

with 48 females and 95 males in that group remaining the following year. By the time this experiment began in June 1988, 353 of the original population remained with an estimated 165 females, 134 males and 54 emaciated fish of unknown sex.

3.3.2 Selection of fish for the experiment

The majority of charr used in this experiment were selected from those that had spawned the previous year with 15 females and 10 males used for each of the five treatments. Emaciated fish or those with gross abnormalities were not used in this experiment. Fish were anaesthetized using 0.03% 2-phenoxyethanol and tagged with numbered Floy tags. Length was measured to the nearest millimeter and weight was taken to the nearest gram using a Mettler PE12 zero-tare balance. The wide range in the weight of the fish (345-2190 g) made it impossible to distribute the fish to the 5 tanks in a totally random manner. The total and mean weight of fish in each tank was affected dramatically if just 1 or 2 small fish were replaced by 1 or 2 large fish. Therefore, the tag number, sex, length and weight of all the fish considered for the experiment were recorded. Fish were distributed to each tank in a manner that provided each tank with the same weight distribution of male and of female fish.

3.4 Environmental conditions for broodstock

3.4.1 Tanks

Each of the five diets was assigned to one tank of fish. Five independent rearing units were used each consisting of a 1500 L light blue fiberglass tank (1.8 m x 1.8 m) (6 ft x 6 ft) mounted over a 1100 L biological filter [1.8 m (6 ft) diameter] containing 0.73 m^3 granite gravel (Papst and Hopky 1982). Flow rates to each tank averaged 105 L/min (range = 101-112). Recirculated water entered the tank through a spray bar to provide aeration. Fresh well water (6-7°C) was also added as makeup to replace a portion of the water being discarded. The flow rate of makeup water was approximately 6.5-7 L/min and was adjusted to achieve the desired temperature and to equalize the temperature between tanks. While filters were backwashed every 2 weeks during the feeding period, it was not necessary to backwash the filters during the spawning period when fish were not fed.

3.4.2 Lighting and photoperiod

Lighting for each tank was provided by 2 cool white fluorescent bulbs placed 1.2 m above the water. Light intensity was 165 lux when measured with a Minolta Autometer III and converted from electron volts into lux. It was 2.88 microeinsteins/m^2/sec when measured with a Lambda quantum photometer. The photoperiod was adjusted every 10 days to conform to the Winnipeg (49°54′ N, 97°8′ W) photoperiod. This

was similar to the photoperiod in Goose Bay, Labrador (Olson, personal communication) which is close to the spawning grounds of the parental charr (56°39'N, 63°10'W).

3.4.3 Temperature

On the ninth day of feeding in Year 1 (July 6, 1988), it was found that the water temperatures differed in the five tanks. At that time, the temperatures were 7.2°C (LLLE), 9.7°C (LLHE), 10.2°C (HLLE), 7.2°C (HLHE), 12.2°C (COMM). On July 7, temperature differences between tanks had been reduced to 1.5°C or less. Variation in temperature was due to differences in the amount of 7°C makeup water being added to each tank. The makeup water entered the tank below the water line and could not be adjusted because the flow valves were faulty. Changes to the makeup lines were made so that the water entered the tank above the surface of the water and a new control valve was installed for each tank. When this was corrected, lengths and weights of all fish were taken again on July 11, 1988. Thereafter, the temperature of water in each tank was measured each morning using a Fisher temperature meter. Adjustments to the amount of makeup water were made to equalize water temperatures in the five tanks. The amount of makeup water was adjusted to try and match the water temperature in Year 2 to that of Year 1.

3.4.4 Water chemistry

In Year 1, water samples were taken from each tank on July 13 and September 22. After the spawning season ended and feeding resumed, samples were taken on February 6, March 21, May 29, July 12, August 29, October 4 and December 6 in Year 2. Water samples were measured for pH and analyzed for ammonium-N, nitrite-N and nitrate-N according to the methods of Stainton *et al.* (1974). Temperature and dissolved oxygen were measured at the same time using a YSI-temperature oxygen meter. Un-ionized ammonia was calculated using the tables of Piper (1982) and the following equation:

Un-ionized ammonia (μg/L) =

$$\frac{\text{un-ionized ammonia (\%)} \times NH_4\text{-N (μg/L)}}{100}$$

3.5 Feeding regime

There appeared to be an effect of lipid level on the length of the spawning period in Year 1. To evaluate this further, a crossover in diets was carried out in Year 2 (Figure 3). The dietary vitamin E content of the diet fed to a given tank of fish was the same in both Year 1 and Year 2. However, fish fed a low lipid diet in Year 1 were fed a high lipid diet in Year 2 and those fed a high lipid diet in Year 1 were fed a low lipid diet in Year 2.

Originally, it was expected that the daily satiation ration could be established by feeding fish to satiation for 1 or 2

days. However, this did not prove to be satisfactory because fish did not consume the same amount each day. Fish consumed much more on the first day than they did on the second or third day of feeding to satiation. A regime was established of feeding to satiation twice a day for 3 consecutive days and using the mean as the daily ration to feed for the next 18 or 25 days. Feed was weighed for an 11 day and one or two 7 day periods with the latter portions stored in the freezer at -10°C until required. The weight of feed to be fed each day was converted to a volume per day for the convenience of hatchery staff. Hatchery staff offered this amount of feed over 3-4 feedings throughout the day. If it was not possible to feed the prescribed amount of feed, the feed remaining at the end of each period was weighed and net feed fed was calculated. In Year 2, there seemed to be 10% of the feed remaining at the end of each period. Therefore, the daily amount offered was set at 90% of the mean of the 3 day satiation amount.

In Year 1, fish were fed between June 28, 1988 and September 11, 1988. Feed consumption decreased as spawning time approached and feed was not offered from September 12, 1988 to January 11, 1989. When spawning was complete, feeding was resumed on January 12, 1989 and was offered until the end of the experiment. Fish were not fed on the day prior to, or on the day of weighing. Feed was offered for a total of 71 days in Year 1 of the experiment and 252 days prior to the first fish

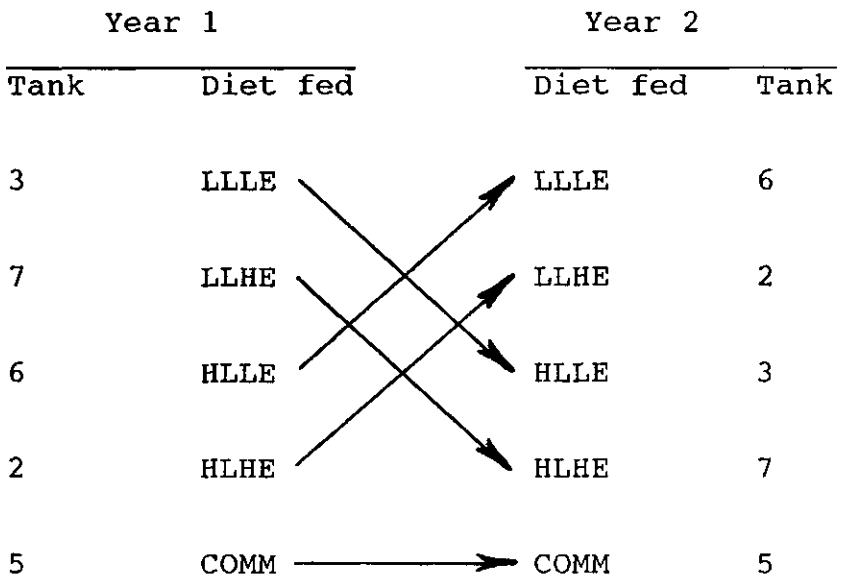


Figure 3. Experimental plan for feeding diets in Year 1 and 2.

spawning in Year 2. In Year 2, feed was offered throughout the spawning period on the 2 days following spawning.

3.6 Growth of broodstock

Fish were anaesthetized with 2-phenoxyethanol prior to taking length and weight measurements of individually tagged fish. Data were collected on the following dates: June 27, 1988, July 8, 1988, August 10, 1988, September 14, 1988, January 11, 1989, April 5, 1989, June 28, 1989, September 13, 1989 and January 3, 1990. Specific growth rate and condition factor were calculated.

Specific growth rate

$$= \frac{\log_e \text{final weight (g)} - \log_e \text{initial wt (g)}}{\text{time (days)}} \times 100$$

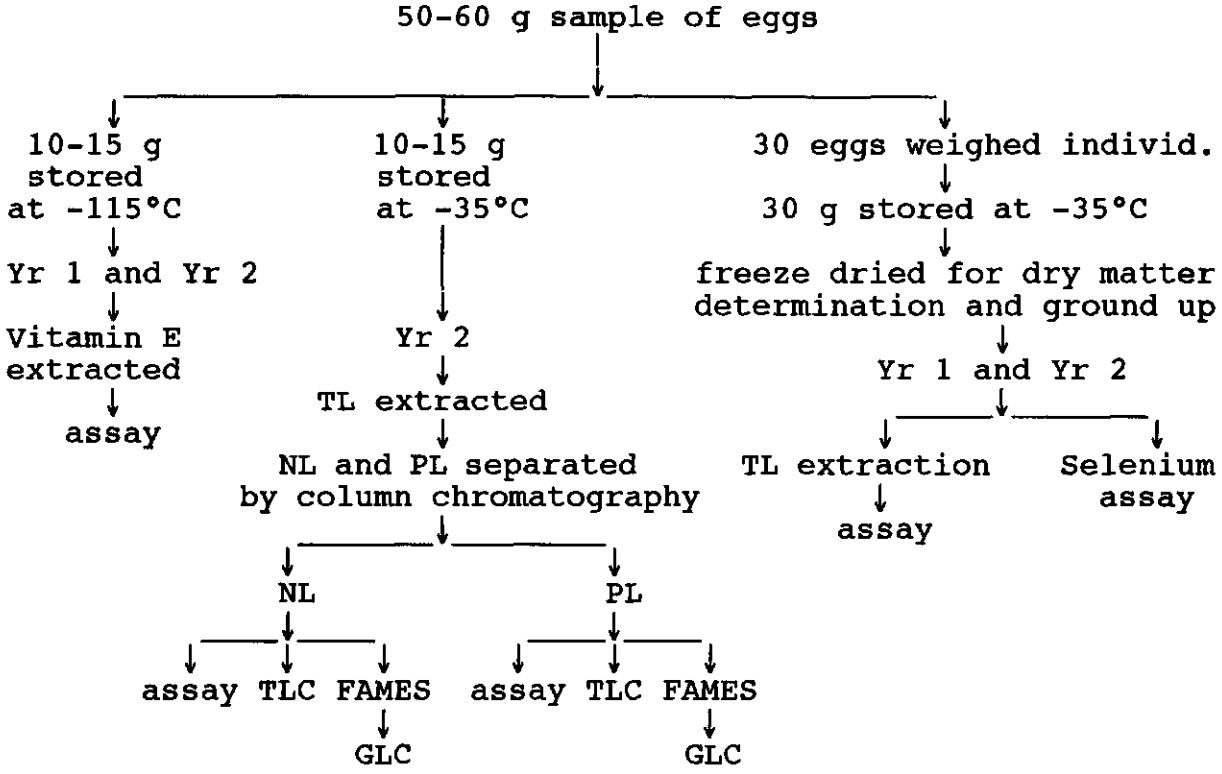
$$\text{Condition factor} = \frac{\text{weight (g)}}{\text{length}^3 \text{ (cm)}} \times 100$$

3.7 Spawning

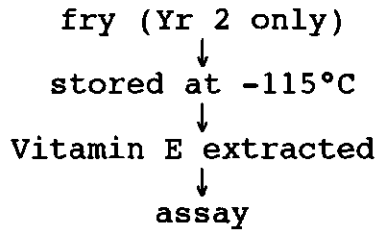
Krieger *et al.* (1988) found that Arctic charr eggs over-ripened quickly after ovulation and that the fertilization rate declined. Therefore once spawning commenced, all fish were checked at weekly intervals. Charr were anaesthetized using 0.03% 2-phenoxyethanol. The tag number and stage of development of each fish was noted. Males were judged as Not Ripe or Ripe while females were judged as No Development, Some Development, Well Developed, Ripe or Spawned. As fish were checked, female

fish that would release their eggs (Ripe) and males which would release milt (Ripe) were separated from the rest. After all the fish in a tank had been checked, the following spawning procedure was carried out. Each ripe female fish was anaesthetized and rinsed in fresh water to remove any anaesthetic. The tag number was noted and the fish was weighed to the nearest gram and fork length measured to the nearest mm. After drying the ventral side with a soft cloth, the eggs were released manually, collected and drained of ovarian fluid in 1 or 2 plastic pre-tared containers with aluminum screen bottoms. Ovarian fluid was collected in a plastic pan. The total weight of drained eggs was measured to the nearest gram. A 50-60 g sample of eggs was taken and processed as described in Section 3.11 (Figure 4). A rinse of 1.37% $NaHCO_3$ (250 ml) was added to the ovarian fluid and remaining eggs and then decanted. This rinse was evaluated as described in Section 3.13.1. Any abnormalities (discoloured eggs or blood in the ovarian fluid) were noted. Two male fish were anaesthetized and rinsed in fresh water before noting their tag numbers and measuring their lengths and weights. The ventral area of each fish was dried before collecting its milt in a dry plastic beaker. A 125 ml aliquot of rinse was divided between the two beakers, the contents of the beakers combined and added to the eggs. After mixing the sperm through the eggs gently for 2 minutes, the fluid was poured off and 6-7°C well water was used to rinse the eggs until the fluid was clear. A sample of 50 eggs was taken

ANALYSES OF EGGS



ANALYSIS OF FRY



TL = total lipid
NL = neutral lipid
PL = polar lipid
TLC = thin-layer chromatography
FAMES = fatty acid methyl esters
GLC = gas-liquid chromatography

All samples of eggs and fry were flushed with nitrogen prior to being frozen.

Figure 4. Flow diagram showing analyses conducted on eggs and fry in Year 1 and/or Year 2.

to assess fertilization rate as described in Section 3.8. Approximately 1000 eggs in Year 1 and 500 eggs in Year 2 were measured volumetrically and placed in the jar section of an incubator (described in Section 3.9). Jars were placed in a pan of 6-7°C well water and covered with black plastic. After 2 hours the jars containing the water-hardened eggs were transferred from the broodstock building to the main building and placed into the lower sections of the incubators. If fecundity was low, half the numbers of eggs were sampled and half the amount of rinse and extender was used. In Years 1 and 2, replicate groups of eggs were incubated from two and three fish, respectively. Excess eggs not required for the experiment were incubated in Heath trays within the broodstock building. In Year 2, Heath trays were compartmentalized and a comparison between survival to the eyed stage when incubated in Heath trays versus jar incubators was made. Eggs were not all released on the day the fish spawned since some eggs may not have ovulated and/or they remained in the ovarian cavity. To assess the total number of eggs produced, fish which had spawned were inspected during subsequent spawning checks and eggs were collected and counted. On three occasions in Year 1, large numbers of eggs were released during a subsequent check. These eggs were fertilized and incubated and data was used for the time when the highest number of eggs were released.

3.8 Fertilization rate and measurement of water-hardened eggs

A sample of 50 eggs (25 eggs if the fecundity was very low) were placed in a compartment of a perforated styrofoam egg carton (Ashton, personal communication). The egg carton was weighted down and placed in the top tray of a Heath tray (flow rate 8 L/min) and was covered with black plastic. After 26 hours (Year 1) or 48 hours (Year 2), the eggs were removed from the egg carton, drained and transferred to a vial containing 10 ml Stockard's solution (Velsen 1980). Eggs were measured after being in Stockard's solution for 68-70 days and 82-86 days in Year 1 and Year 2, respectively. To assess fertilization, the eggs were viewed under a Zeiss low power microscope. If no cell division had occurred, the egg was considered not fertilized.

Eggs were drained on a paper towel and the weight and diameter of 25 individual eggs were determined. The weight was taken to 0.0001 g on a Mettler AE160 and the diameter was determined by measuring under a Bausch and Lomb microscope fitted with a micrometer.

$$\% \text{ Fertilization} = \frac{\text{no. fertilized eggs}}{\text{no. fertilized} + \text{no. unfertilized eggs}} \times 100$$

3.9 Incubation of eggs

Eggs from each mating were incubated separately in upwelling 1 L jar incubators as described by Tabachek *et al.* (1993). Each lot of eggs was placed in an upper section made of ABS pipe with an aluminum screen bottom. The lower section of

each incubator contained aquarium gravel to disperse any air bubbles. Each trough of incubators received aerated well water at 6-7°C which passed through a household dirt and rust filter. The filters were cleaned every 5-10 days as required and replaced after 5-10 cleanings. In Year 1, eggs were incubated in four troughs each containing 24 incubators with a total flow rate of 14.6 L/min (608 ml/min per incubator). In Year 2, eggs were incubated in two troughs each containing 32 incubators with a total flow rate of 15.1 L/min (470 ml/min per incubator). Between 10 days after spawning and the eyed stage, eggs were treated with formalin (1/600 or 1667 ppm) for 15 min three times a week to control fungus growth (Hynes 1984, Fuze *et al.* 1985). Incubators were covered with black plastic and were exposed to yellow fluorescent and incandescent light only during removal of dead eggs and counting. Yellow fluorescent light was 350 lux or 8.2 and 10.1 microeinsteins/m^2/sec in Year 1 and Year 2, respectively. After reaching the eyed stage, the eggs were shocked and incubated one more day before removing the dead eggs. Dead and live eggs were counted at this time. Dead eggs and fry were removed and counted throughout the rest of the incubation period. Dead eggs were preserved in Stockard's solution in Year 1 but were inadvertently discarded in Year 2. In Year 2, records were also kept on the numbers of deformed fry and fry with blue sac, a condition in which a bluish liquid accumulates between the yolk and the membrane of the yolk sac (van Duijn 1973, Balon 1980). In Year 2, samples of fry with

blue sac and other abnormalities were preserved in Davidson's solution (formalin, 20 ml; ethanol, 30 ml; glycerine, 10 ml; glacial acetic acid, 10 ml; distilled water, 30 ml) for histological examination.

The equations used to calculate survival to the eyed, hatch and swimup stages are as follows:

$$\% \text{ Eyed} = \frac{\text{no. alive after shocking eyed eggs}}{\text{total no. eggs at beginning of incubation}} \times 100$$

$$\% \text{ Hatched} = \frac{\text{no. alive after hatching complete}}{\text{total no. eggs at beginning of incubation}} \times 100$$

$$\% \text{ Swimup} = \frac{\text{no. live fry at swimup}}{\text{total no. eggs at beginning of incubation}} \times 100$$

$$\% \text{ Eyed of fertilized} = \frac{\% \text{ eyed}}{\% \text{ fertilized}} \times 100$$

$$\% \text{ Hatched of eyed} = \frac{\% \text{ hatched}}{\% \text{ eyed}} \times 100$$

$$\% \text{ Swimup of hatched} = \frac{\% \text{ swimup}}{\% \text{ hatched}} \times 100$$

3.10 Growth and survival of fry

Fry were raised in the same type of system as described for the incubation of eggs in Section 3.9 except that no gravel was placed into the lower sections. Jars were placed into a trough with each jar connected to a header pipe running the length of the trough. Water was treated by a biological gravel filter

located beneath the trough. The filter (2.46 m x 93 cm x 88 cm), which contained 0.85 m^3 gravel, resulted in the water temperature increasing to 10°C with no additional heating. The system received approximately 15% fresh well water as makeup. The temperature was checked daily and was controlled by adjusting the amount of makeup water. The filter was backwashed once during the time that the culture system was used in Year 1 and in Year 2. The flow rate to each jar was 770-820 ml/min. For the first two days after placing fry in a jar, that jar was covered with black plastic. Illumination was from yellow fluorescent lights with an intensity of 127 lux and 1.94 microeinsteins/m^2/sec with a photoperiod of 10 hr of light and 14 hr of darkness.

The specific growth rate and mortality of fry from each mating were evaluated over a period of 41-42 days. Since fish spawned over a 3 month period, the evaluation of groups of swimup fry was initiated over a 3 month period. At swimup (123 and 125 d after spawning in Year 1 and Year 2 respectively), groups of fry were drained of water, batch weighed to 0.01 g and placed into the rearing unit described above. At this time, the yolk sac had not been fully absorbed. In Year 1, 50 fry were placed in each unit while 25 fry were used in Year 2 since the evaluation of the number of fry to raise in each rearing unit (see Section 3.13.2) showed that the growth rate of fry was significantly greater when fry number was 25 per unit compared

to 50. If adequate numbers of fry survived, two replicate groups from each female were raised; otherwise only one group was raised. Semimoist starter diet (Biodiet #1 manufactured by Bioproducts, Warrenton, Oregon) was used. Fresh feed was weighed weekly and it was placed in the refrigerator each night. The rest of the feed was kept frozen (-10°C) in a plastic bucket which was flushed with nitrogen before closing. Fry were not fed the day they were placed into the jars and they were fed lightly 3 times the next day and 6 times a day (between 0800 and 1600) to excess thereafter. Dead fry were removed and counted daily and the jars were cleaned weekly. At the end of the period, fry were counted and batch weighed to assess survival and specific growth rate.

$$\text{Survival (\%)} = \frac{\text{final no. fry}}{\text{initial no. fry}} \times 100$$

$$\text{Specific growth rate (\%/day)} = \frac{\log_e \text{final mean wt (mg)} - \log_e \text{initial mean wt (mg)}}{\text{no. of days}} \times 100$$

3.11 Collection of unfertilized eggs and fry for measurement and/or analysis

At spawning, a 50-60 g sample of eggs, which had been drained of ovarian fluid, was placed into a pre-tared brown glass jar and transported on ice back to Winnipeg. From this sample, two 10-15 g samples of eggs were transferred into clear

glass scintillation vials (Figure 4). Each vial was flushed with nitrogen and wrapped in a paper towel to protect the sample from exposure to light. One vial was stored at -35°C and the other was stored at -115°C. After obtaining the weights of 30 individual eggs to 0.0001 g on a Mettler AE160 balance, the total weight of eggs in the pre-tared jar was noted, the sample was flushed with nitrogen and stored at -35°C.

In Year 2, samples of swimup fry were taken for analysis on the days the fry evaluations started. Fry were drained of excess moisture on paper towels, placed in plastic tubes with snap tops, flushed with nitrogen and immediately frozen on dry ice. They were stored at -115°C until analyzed for vitamin E (Figure 4).

3.12 Analyses of eggs, fry and/or diets

3.12.1 Vitamin E analysis of eggs and fry

Eggs and fry, stored at -115°C, were analyzed in duplicate for vitamin E (α-tocopherol) content (Figure 4) by using the extraction method of King (1983) and the adapted spectrophotometric method of the AOAC (1984) using bathophenathroline (Appendix 3a). Precautions were taken to minimize loss of vitamin E through exposure to heat, light and oxygen. Subdued fluorescent lighting was used (475 lux), vessels were flushed with nitrogen and the temperature was kept below 45°C during evaporation of solvents.

For comparison, samples of eggs which were collected from wild Arctic charr at Tree River, NWT were analyzed. These eggs were close to hatching when sampled.

3.12.2 Dry matter and selenium

The 30 g samples of eggs stored at -35°C were freeze-dried (Figure 4) with the difference in initial and final weight used to calculate percent dry matter. Three samples of finely ground eggs per treatment per sampling year were analyzed for selenium in the same way as the diets (Section 3.12.4).

3.12.3 Lipid and fatty acid analyses

The 10-15 g samples of eggs taken in Year 2 and stored at -35°C (Figure 4) were used for extraction of total lipids by a modified Bligh and Dyer (1959) method (Appendix 3a). Column chromatography with silica Sepak cartridges was used to separate total lipids into neutral lipid and polar lipid fractions by modification of the method of Ashton (1991). Total lipid was reduced to 60-70 mg and only one Sepak was used per sample. Neutral lipids were eluted with 30 ml 15% hexane in chloroform and polar lipids were eluted with 30 ml methanol followed by 30 ml chloroform: methanol (1:1). Duplicate aliquots of the fractions were assayed for lipid by the dichromate oxidation spectrophotometric assay (Fales 1971). Standard curves were conducted using neutral and polar lipids collected from Arctic

charr eggs in order to have a comparable level of oxidation. Results from this assay compared well with results obtained by weighing aliquots dried at 70°C. The relative percent of neutral and polar lipids in each sample of total lipid was established. To obtain percent total lipid on a dry matter basis, total lipid was also extracted from dry samples of eggs taken in Year 1 and 2 (Figure 4) and duplicate aliquots of lipid extracts were dried at 70°C and weighed. Neutral and polar lipids were converted to percent dry matter:

$$\text{Neutral lipid (\% dry matter)} = \frac{\text{neutral lipid (relative \%)}}{100} \times \text{total lipid (\% dry matter)}$$

$$\text{Polar lipid (\% dry matter)} = \frac{\text{polar lipid (relative \%)}}{100} \times \text{total lipid (\% dry matter)}$$

Since the precision of the neutral lipid and polar lipid fractionations was satisfactory, only one determination per sample was conducted.

The purity of the neutral and polar lipid fraction was checked by spotting aliquots of the fractions on Silica Gel G (0.25 mm) thin-layer plates and placing the plates in an ascending solvent system of hexane:diethyl ether:glacial acetic acid = 80:20:2 (Christie 1981). Lipid standards used for identification of the bands included phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, triolein, 1,2- and

1,3- diglycerides and monoglyceride. Location of the bands was visualized by spraying the air-dried plates with sulphuric acid and heating them at 110°C.

To determine the fatty acid composition of the neutral and polar lipids of the eggs collected in Year 2 and of the diets used in Year 1 and 2, 5 mg aliquots of total lipid were transesterified using the acid-catalyzed method with toluene and methanolic HCl and extracted in hexane (Yurkowski 1989). The extracts were purified by applying them on Silica Gel H plates and placing them in an ascending TLC tank with toluene. The methyl ester band was visualized by spraying with 0.02% Rhodamine 6G and viewing in ultraviolet light. The methyl ester band was removed from the plate, eluted with chloroform and concentrated in hexane. Fatty acid profiles were obtained by injecting the samples into a Varian Aerograph Model 3400 Chromatograph fitted with a Supelcowax silica capillary column (30 m, 0.32 mm I.D., 0.25 μm film thickness). Injector and detector temperatures were 235 and 250°C, respectively and the column temperature was held at 175°C for 25 min, increased 1°C per min for 35 min and held at 210°C for 30 min. Identity of each peak had been determined (Yurkowski, personal communication) by the methods described by Yurkowski (1989). Peak areas were measured by an electronic integrator (Varian Star Integrator, Revision A, Varian Associates, Walnut Creek, CA) and converted into area percent of each fatty acid. One

fatty acid determination per sample was conducted after determining that the precision of the test was satisfactory.

3.12.4 Analysis of diets

Major ingredients and diets were analyzed for crude protein by the macro-Kjeldahl method (AOCS 1989). Ash and moisture concentrations were also determined (AOAC 1984). The diets were analyzed for total Ca, Na, K, Mg, Mn, Zn, Cu, Fe by nitric acid-perchloric acid digestion followed by atomic absorption spectrophotometry. Selenium determination was by the method of Vijan and Wood (1976). Soluble reactive phosphorus was analyzed by the molybdenum blue method of Stainton *et al.* (1974).

3.13 Evaluation of methods

3.13.1 Evaluation of egg rinses and extenders

In Year 1, a total of 5 fish from tanks 2 (HLHE) and 5 (COMM) spawned between September 29, 1988 and October 12, 1988. The fertilization rate was low (mean = 59 ± 36) and ranged from 0-88%. Egg rinses and extenders were tested October 26, November 2, 1989 and December 27, 1989 on 2 fish which spawned each day to determine if the method being used was affecting the fertilization rate. The following were tested 1a) well water as rinse and extender on October 26, 1b) no rinse and well water as extender on November 2 (normal procedure at RARC), 2) lactose-$CaCl_2$-NaCl (Hamor 1987), 3) Tris-glycine.HCl (Billard 1977) and 4) $NaHCO_3$ (Wilcox *et al.* 1984). An equal weight (20 or 50 g) of

drained eggs was divided into 4 lots and 50 ml of rinse solution was poured into each pan, swirled and poured off. An equal volume (0.5 ml) of milt from each of the same two males was added to 25 ml of the solution and it was added to the eggs. Eggs were mixed gently for two minutes and then rinsed with well water until the water was clear. Samples of eggs were incubated as described to assess fertilization rate and survival to swimup. Based on the fertilization rate data, the decision was made to use the $NaHCO_3$ rinse. In Year 2, solutions 1b, 2 and 4 were re-evaluated on November 15, 1989 using 30-40 g eggs, 35 ml rinse, 15 ml solution to 0.4-0.7 ml milt from each of two males and fertilized and incubated as above.

3.13.2 Evaluation of number of fry to raise in each rearing unit

During Year 1, the number of fry that could be raised in each jar was evaluated. Swimup fry, all offspring of one female, were counted out into two groups of 10, 25, 50, 100 and 300 fry and batch weighed. The groups of 10 to 100 fry were placed into jars using a randomized design. Two groups of 300 fry were placed in 60 L fiberglass tanks as described by Tabachek (1983) with a flow rate of 1.8 L/min. Fry were raised in the manner described in Section 3.10.

3.14 Statistical analysis

In order to satisfy the conditions of the analysis of variance, the data must fit a normal distribution and have

variances which are equal and not related to the means (Li 1964). Arcsin transformation was carried out on all data from binomial distributions (fertilization and embryonic and fry survival). Log transformation was carried out on vitamin E concentration and content of eggs and fry as well as egg weights because variances were proportional to the means. Bartlett's test was used to test for homogeneity of variances prior to conducting analysis of variance.

Since most data sets were unbalanced, the general linear model (PROC GLM) was used to detect significant differences between means by analysis of variance (SAS Institute 1985). One-way analysis of variance was conducted on data for all five diets using the model:

$$Y = \mu + \alpha + \varepsilon$$

where Y = measurement, μ = mean of populations, α = effect of diet and ε = experimental error. Analysis of variance was used on data for the four diets comprising the 2 x 2 factorial design by using the model:

$$Y = \mu + \alpha + \beta + \alpha\beta + \varepsilon$$

where Y = measurement, μ = mean of populations, α = effect of lipid, β = effect of vitamin E, $\alpha\beta$ = interaction of lipid and vitamin E and ε = experimental error. Since no replicate tanks of broodstock were subjected to the respective treatments, variations among fish within each treatment served as the testing error. Duncan's multiple range test was used to detect

significant differences between means. The level of significance was set at 95% in all statistical analyses.

Regression analysis was conducted to detect correlation between selected parameters such as the effect of fish weight on fecundity or the effect of selected dietary fatty acids on the fatty acid composition of eggs (SAS Institute 1985). The level of significance was set at 95%. Regression analysis detected a correlation between embryonic survival and % neutral lipids in the eggs and it appeared that this relationship might also be affected by diet. Analysis of covariance was used to partition differences due to diet and neutral lipids on the intercepts and slopes of the regression.

Chi square analysis (Li 1964) was conducted to compare the effects of selected treatments (effects of vitamin E or lipid) on the proportion of females that spawned or males that produced milt. Data from the same dietary lipid or for the same dietary vitamin E treatments were pooled to increase the sample size for each group to obtain expected values of 5 (Li 1964). Data for the COMM diets were excluded from this analysis. When the expected number was too low for Chi square test to be valid (as in testing for the effect of diet on spawning time), Fisher's exact test was used (SAS Institute 1990).

Power analysis was conducted (Dixon and Massey 1969) as recommended by Peterman (1990) to determine the power of the analysis of variance given the variance that was observed in embryonic survival.

RESULTS

4.1 Feed composition

Experimental diets contained 46.9-47.8% crude protein and 7.5-7.8% ash, on a dry weight basis (Table 2). Total lipid contents of the low lipid diets were 11.8-12.6% while those of the high lipid diets were 18.8-19.0%. Metabolizable energy was estimated as 3.23 and 3.90 kcal/g for the LL and HL diets, respectively. COMM contained 42.4% protein, 16.8% lipid and 7.8% ash and was estimated to contain 120 mg total α-tocopherol /kg (Martin Feed Mills, personal communication), 223 mg ethoxyquin/kg and 3.47 kcal metabolizable energy/g. The four experimental diets contained total mineral concentrations of 16 mg Ca/g, 2.4 mg Na/g, 1.4 mg K/g, 1.5 mg Mg/g, 1.3 mg P/g, 91 μg Mn/g, 228 μg Fe/g, 26 μg Cu/g, 189 μg Zn/g and 1.02 μg Se/g on a dry weight basis. COMM had concentrations of Ca and K similar to the above, lower P of 1.1 mg/g and higher concentrations of the others: 2.8 mg Na/g, 1.9 mg Mg/g, 116 μg Mn/g, 42 μg Cu/g, 210 μg Zn/g and twice as much iron at 456 μg Fe/g. COMM used in Year 1 contained 0.75 μg Se/g while the two lots used in Year 2 contained 0.88 and 1.96 μg Se/g.

In addition to the supplemented amount of dl-α-tocopheryl acetate, endogenous α-tocopherol was present in the other ingredients. The basal mix contained 7.7 ± 0.2 mg α-tocopherol /kg. Herring oil, purchased one month prior to that purchased

Table 2. Composition of the diets (dry matter basis).

	Diet				
	LLLE	LLHE	HLLE	HLHE	COMM[2]
Moisture (%)	8.7[1] 0.80	8.8 0.60	8.6 0.56	8.7 0.72	7.1 1.16
Crude protein (%)	46.9 0.53	47.2 0.38	47.4 0.58	47.8 0.51	42.4 1.27
Total lipid (%)	11.8 0.34	12.6 0.59	19.0 1.11	18.8 1.20	16.8 1.25
Ash (%)	7.6 0.09	7.6 0.06	7.6 0.23	7.5 0.14	7.8 1.49
Fiber[3] (%)	9.5	9.5	2.5	2.5	2.5
Nitrogen-free extract[4] (%)	23.7	22.6	23.5	23.4	30.5
Ethoxyquin[5] (mg/kg)	160	160	160	160	223
[dl]-α-Tocopheryl acetate[6] (mg/kg)	30	600	30	600	-
[dl]-α-Tocopherol[7] (mg/kg)	14	14	24	24	-
Total [dl]-α-tocopherol + tocopheryl acetate (mg/kg)	44	614	54	624	120
Metabolizable energy[8] (kcal/g)	3.28	3.34	3.86	3.86	3.59

[1] Mean (upper line) ± standard deviation (lower line) for the 5 batches of experimental diets and 3 lots of COMM diet used throughout this study.
[2] Estimated values for the commercial diet on proprietary information supplied to the author by the manufacturer.
[3] Estimated using NRC (1981) tables on composition of ingredients.
[4] Estimated using 100-(total lipid + crude protein + ash + fiber).
[5] Total ethoxyquin in fish meal and oil.
[6] Taken from formulations in Table 1.
[7] Estimated from analysis of basal mix and herring oil as described in Section 3.3.3.
[8] Estimated using values of 4.2 kcal/g protein, 8.0 kcal/g lipid and 1.6 kcal/g raw starch (Brett and Groves 1979).

for this experiment, which was not frozen contained 184 ± 5.7 mg α-tocopherol/kg oil while the sample which was frozen contained 144 ± 0.7 mg α-tocopherol/kg oil. Samples of herring oil used in this experiment were also shipped to Hoffmann-LaRoche but they were not analyzed. Since the oil used in this experiment had been frozen before use, the level of endogenous α-tocopherol was estimated to be 144 mg/kg oil. Herring oil was estimated as contributing 14 mg α-tocopherol/kg to the low lipid diets and 24 mg/kg α-tocopherol to the high lipid diets. It is expected that much of the endogenous tocopherol would oxidize during the manufacturing process and on storage since α-tocopherol is easily oxidized (Bauernfeind 1980). Vitamin E acetate content of the diets was not analyzed. When vitamin E acetate, donated by Hoffmann-LaRoche for this research, was compared to vitamin E acetate obtained from Sigma Chemical Co., St. Louis, MO and ICN Biomedicals, Costa Mesa, CA, the spectra were identical and the absorbances were within 1.4% when ethanolic solutions of the same concentrations were compared in a spectrophotometer.

The peroxide value of the herring oil used in making the four experimental diets increased from 1.44 ± 0.38 meq/kg in July 1988 to 3.33 ± 0.11 meq/kg at the final analysis in September 1989. These values were within the guidelines for quality control of fish oils of < 5-10 meq/kg (NRC 1981, Canadian Working Group for Finfish Nutrition, personal communication).

4.2 Water temperature

As described in Section 3.5.3, variation in the temperatures between tanks was observed on day 9 of the experiment. At that time, water temperatures were 7.2°C (LLLE), 7.2°C (HLHE), 9.7°C (LLHE), 10.2°C (HLLE) and 12.2°C (COMM). That is, temperatures were low (7.2°C) for groups receiving one LL and one HL diet and for one LE and one HE diet. Similarly, temperatures were higher (9.7-10.2°C) for groups receiving one LL and one HL diet and for one LE and one HE diet. No data on water temperature was collected during the first 8 days of the experiment. Following changes to the water system to correct the problem, there was little difference in water temperature between tanks. Differences in temperatures between tanks after day 9 ranged from 0 to 1.5°C (0.27°C ± 0.11) (mean ± standard deviation) with differences of over 1.0°C occurring on 8 days during the 544 day period measured. Mean weekly water temperatures during Year 1 and 2 are shown in Figure 5 and Appendix 2a. During Year 1, mean weekly water temperature increased from 8.3 to 8.8°C during July and decreased steadily down to 7.1°C from August to mid-November as outdoor and room temperatures decreased. After room heaters were turned on in mid-November, water temperature increased to 7.7-7.8°C. In Year 1, spawning occurred between September 29 and December 27, 1989 when mean weekly temperatures were 7.1 to 7.9°C.

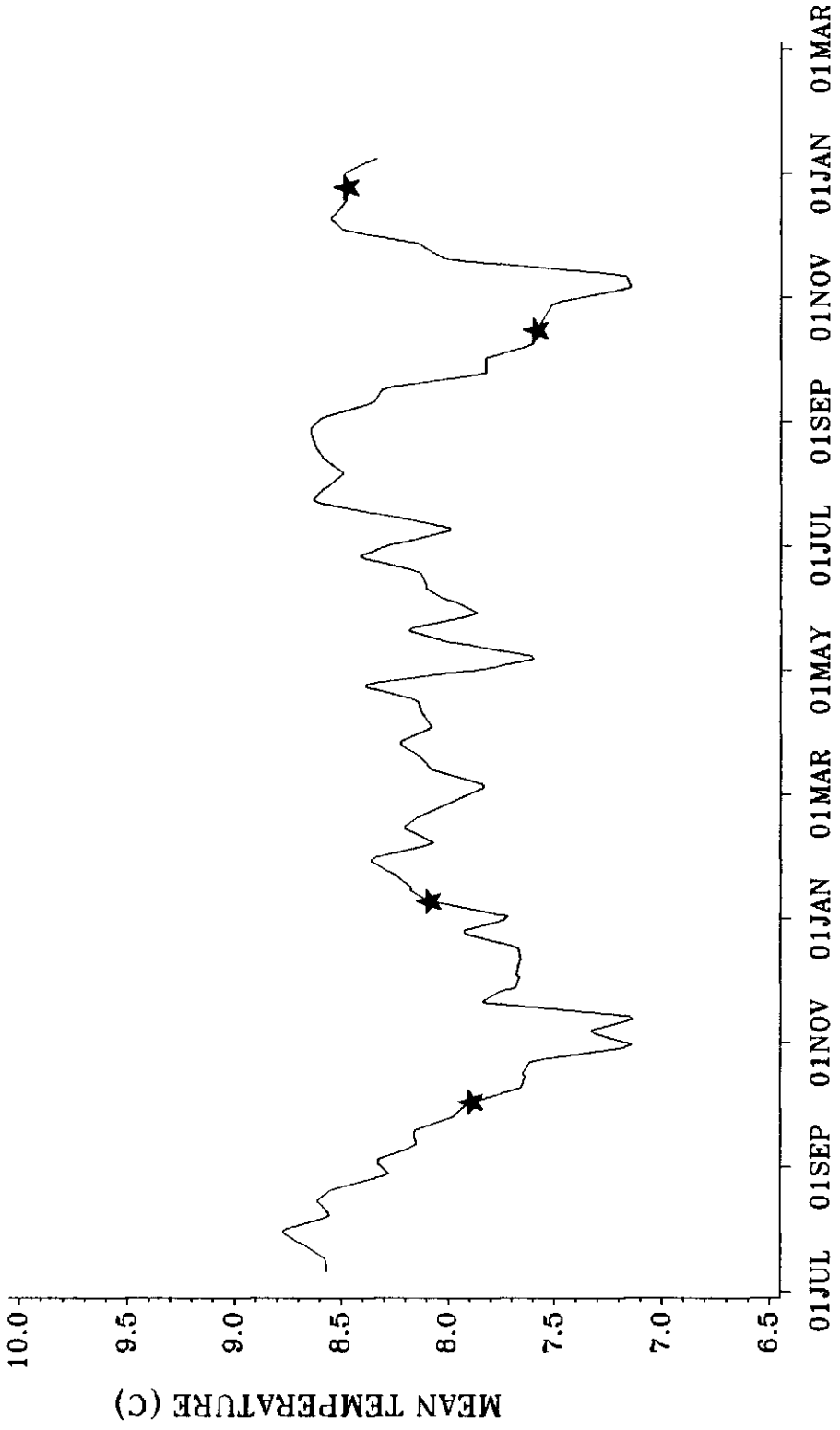


Figure 5. Mean weekly temperature during Year 1 and 2. Asterisks denote the beginning and end of the spawning period in Year 1 and Year 2.

In Year 2, mean weekly temperature varied between 7.7 and 8.4°C from January to May 1988, between 8.0 and 8.6°C from June to July (Figure 5) and declined from 8.6 to 7.2°C from August to November. As in Year 1, water temperature increased to 8.5°C from December 1989 to January 1990 when room heaters were used. Mean weekly water temperature was 7.1 to 8.5°C during spawning in Year 2. Simulation of water temperature in Year 2 to match that of Year 1 was good except between November 28 and December 25, 1989 when it averaged 0.6-0.9°C higher than corresponding dates in Year 1. Flow rate of the 6.5°C make-up water was increased as much as possible during this time but water temperature could not be decreased to match that of Year 1.

4.2 Water chemistry

Chemical composition of the incoming well water has been measured (Province of Manitoba 1987). Measurements indicated the following: pH, 7.75; conductivity, 918 µmho/cm; hardness, 424 mg/L; total dissolved solids, 560 mg/L (1982); alkalinity (HCO_3), 486 mg/L; total alkalinity ($CaCO_3$), 398 mg/L; Ca (extractable), 66.1 mg/L, Mg (ext.), 63.0 mg/L, Na (ext.), 40.8 mg/L; Fe (ext.), 0.114 mg/L (1982); Mn (ext.), <0.02 mg/L; SO_4 (soluble) 68 mg/L; Cl (sol.), 43 mg/L and F, 0.28 mg/L. These parameters are all within the suggested chemical values for hatchery water supplies used to raise trout (Piper *et al.* 1982) except for hardness. The hardness was 424 mg/L which exceeds the suggested range of 10-400 mg/L.

Mean values for water chemistry parameters for each tank over the 8 sampling times are shown in Table 3 and for the 5 tanks for each sampling time are shown in Table 4. Over the repeated samplings, there were no significant differences between tanks in any water quality parameters. Un-ionized ammonia was always less than that listed in the water quality criteria for optimum health of salmonids stated by Piper *et al.* (1982) as 0.0125 ppm (12.5 µg/L). The highest observed un-ionized ammonia level (8.96 µg/L) occurred on February 6, 1989. This occurred at a time when the efficiency of the biological filters may have been reduced when feeding resumed on January 12, 1989 after feed was withheld during spawning. The first backwashing of the filters occurred 4 days after taking the water samples. Nitrite-N was within the suggested criteria of 0.06 ppm (60 µg/L). While the suggested pH range for trout is 6.5-8.0, the pH of the recirculated water on most sampling days was 7.9-8.2 and 8.8-9.0 on two sampling days.

4.4 Feed fed and feed efficiency

Data were collected on the weight of feed offered to each tank of fish during each feeding period and these values were converted to dry weight of feed. Feed fed and weight gain between each weighing period in Years 1 and 2 are shown in Appendices 2b and 2c and are provided in Table 5. It is expected that differences in water temperature during the first 9 days of the experiment affected feed intake and ultimately

Table 3. Water chemistry data for each tank (n=8).

Tank	Temp.	D.O.[1]	pH	NH_4-N	NO_2-N	NO_3-N
	°C	mg/L		µg/L	µg/L	µg/L
2	8.3	11.4	8.2	28.8	2.25	455.3
3	8.5	11.4	8.4	26.3	1.00	366.0
6	8.6	11.4	8.3	35.8	0.88	418.5
7	8.4	11.4	8.3	28.4	0.88	404.5
5	8.2	11.2	8.3	38.5	1.13	364.8
Mean	8.4	11.4	8.3	33.8	1.23	401.8
SD[2]	0.14	0.15	0.05	4.84	0.58	38.1

[1] Dissolved oxygen
[2] Standard deviation

Table 4. Water chemistry data for each sampling day (n=5).

Date	Temp.	D.O.[1]	pH	NH_4-N	NO_2-N	NO_3-N
	°C	mg/L		µg/L	µg/L	µg/L
Aug. 22, 1988	8.5^{a}	11.7^{abc}	8.1^{b}	19.6^{bcd}	0.2	322.0^{cd}
Feb. 2, 1989	.	.	8.9^{a}	64.0^{a}	1.0	431.0^{bc}
Mar. 23, 1989	8.6^{a}	11.7^{abc}	8.2^{b}	54.0^{a}	1.0	468.6^{ab}
May 29, 1989	8.0^{bc}	11.4^{c}	8.1^{b}	30.8^{b}	3.0	442.0^{b}
Jul. 12, 1989	8.9^{a}	11.5^{c}	8.1^{b}	57.2^{a}	1.0	530.0^{ab}
Aug. 29, 1989	8.2^{b}	11.6^{bc}	8.1^{b}	25.8^{bc}	1.6	574.0^{a}
Oct. 4, 1989	7.9^{bc}	12.0^{a}	9.0^{a}	14.0^{cd}	1.0	220.8^{d}
Dec. 6, 1989	7.7^{c}	11.9^{ab}	8.2^{b}	14.6^{d}	1.0	226.0^{d}
Mean	8.3	11.7	8.3	33.8	1.2	401.8
SD[2]	0.43	0.21	0.38	22.0	0.81	132.6

[1] Dissolved oxygen
[2] Standard deviation
Means within a column which share a common superscript are not significantly different.

Table 5. Feed fed, weight gain and feed efficiency for each tank of fish fed for 71 days in Year 1 and 239 days in Year 2.

Diet	LLLE	LLHE	HLLE	HLHE	COMM	LL[1]	HL[2]
Total feed fed (kg, dry)[3]							
Year 1	15.2	14.9	16.4	17.1	20.1	15.0	16.7
Year 2	24.2	28.9	34.3	34.2	40.4	26.6	34.3
Total weight gain (kg)							
Year 1	9.75	8.12	12.24	12.28	15.45	8.94	13.87
Year 2	15.42	13.78	6.73	9.84	24.81	14.60	8.29
Feed efficiency (%)[4]							
Year 1	64.0	54.6	74.7	71.9	76.8	59.3	73.3
Year 2	63.7	47.8	19.6	29.4	61.4	55.8	24.2
Mean no. fish[5]							
Year 1	25	25	25	25	25	25	25
Year 2	21	23	22	24	24	22	24
Estimated dry weight feed fed per fish (g)							
Year 1	610	595	655	683	805	603	669
Year 2	1152	1254	1561	1425	1683	1203	1493

[1] LL = mean of the two LL diets
[2] HL = mean of the two HL diets
[3] Dry feed= feed, g as fed x (dry matter,% /100)
[4] Feed efficiency=(total weight gain, g x 100)/dry feed fed, g
[5] Average number of fish present during the feeding period

weight gain during this period. During these first 9 days, tanks of fish held at the higher temperatures had higher feed intakes and weight gains than their counterparts fed diets with the same lipid level. However, at the end of 71 days of feeding in Year 1, total weight gains were almost identical in both HL groups. In contrast, the LLLE group, which had been held at the lower temperature, had gained a total of 1600 g more than its LLHE counterpart held at the higher temperature. The effect that differences in this 9-day period had on weight gain during the remainder of the experimentcannot be estimated. In Year 1, the weight of dry feed fed in 71 days was slightly lower for the fish fed the LL diets (15.0 kg) compared to the HL diets (16.7 kg). Feed fed in Year 2 was also lower for fish fed the LL diets (26.6 kg) compared to the HL diets (34.3 kg). More COMM diet was fed in both Year 1 (20.1 kg) and Year 2 (40.4 kg) than all other diets. Having equal numbers of fish in each tank in Year 1 makes comparison of feed fed to each tank of fish simple. However, in Year 2 the numbers of fish varied between tanks and within a tank over the year. To compare feed fed on the basis of equal numbers of fish, a gross estimation of the feed intake per fish has been calculated and indicates that feed fed per fish was not markedly lower with the LL diets compared to the HL diets. In addition to the unequal numbers of fish in each tank in Year 2, a portion of the population did not feed and this was especially noticeable during Year 2.

Fish behaviour affected feed intake in two cases. On the twelfth day of the experiment, it was observed that fish in tank 6 stayed crowded together in the area under the water inlet. This behaviour appeared to inhibit feeding activity and water turbulence in this area made it impossible to determine if satiety had been reached. Dissolved oxygen was 10.4 mg/L and temperature was 8.5°C in both tanks. When the direction of water flow was altered, fish would disperse throughout the tank, but within a short time they resumed their positions under the water inlet. On day 43 of the experiment, fish from tank 6 were placed in tank 7 and those in tank 7 were placed in tank 6. Both groups acted normally and dispersed throughout the tank. Therefore, it appeared to be neither the effect of the tank nor the fish themselves but a tank-fish interaction that caused this behaviour to be exhibited. After this change was made, charr were left in these tanks and the diets each group had been fed were maintained. All records were changed to show that tank 7's fish and their respective diet were originally assigned to tank 6 and vice versa (Figure 3). It is expected that feed intake in tank 6 was reduced prior to their being moved to tank 7.

When feeding was resumed after spawning at the beginning of Year 2, one emaciated male fish in tank 3 (fed LLLE in Year 1 and HLLE in Year 2) became very aggressive. This fish, which was one of the smallest in the tank, became very territorial and forced all the other fish into the area under the water inlet

where water turbulence did not allow them to feed normally. If a fish ventured out of this area, the aggressive fish would challenge it. When the aggressive fish was removed from the tank on March 16, 1989, the remaining fish attained a more normal distribution in the tank and feed intake increased. The aggressive fish was not replaced.

Small numbers of fish were considered to be "loners" - fish that stayed outside of the main group of fish and always remained at the periphery of the tank. These fish generally did not feed. Even when a feed pellet was thrown directly in front of these fish, the pellet was either ignored completely or taken into the mouth and then spit out.

In Year 1, feed efficiency was higher for fish fed the HL diets (73%) and COMM diet (77%) than observed for those fed the LL diets (59%). The reverse occurred in Year 2 when feed efficiency was lower for the HL diets than the LL diets (56%) and COMM diet (61%). Feed efficiency was 29% for the HLHE diet and 20% for the HLLE diet. Part of the reason for the extremely poor feed efficiency of the latter group was the effect that the aggressive male fish had on the feeder's assessment of satiety. The feed offered was probably not all consumed.

Arctic charr took feed from the bottom of the tank as well as throughout the water column. One fish in each tank fed LLLE

and COMM in Year 1 and HLLE in Year 2 released almost all of its eggs into the tank. The small screen located in the middle of the tanks had perforations which did not allow either feed pellets or fish eggs to pass through it. When several eggs were placed in a tank to observe the response of the fish, the eggs were readily consumed.

4.5 Growth of broodstock

Fish had not been tagged during the previous spawning season and had no spawning colouration at the time this experiment was initiated. An experienced hatchery manager found that it was difficult to determine the sex of some fish. In Year 1, 11 out of 75 fish and an additional 3 fish in Year 2 (total of 2-5 fish in each tank), initially believed to be female, developed primary and secondary sexual characteristics of males (mean initial weight = 993 g, range = 690-1455 g). Eight of them gained weight throughout Year 2, while 6 lost weight. All those which gained weight produced milt while only 2 of the fish that lost weight produced milt. Fish which did not mature until Year 2 were large fish (2-3 kg at spawning time) and they had a very silvery appearance until they began to mature. The actual number of male and female fish in each tank and ultimately the length and weight distribution of males and females in each tank was different than expected. Initial weight distributions of male and female fish in each tank are presented in Table 6.

Table 6. Initial weight distributions of fish in each tank.

Tank	Females				Males				All			
	No.	Mean	SD	Range	No.	Mean	SD	Range	No.	Mean	SD	Range
		g	g	g		g	g	g		g	g	g
3	13	1010	222	680-1420	12	670	183	394- 931	25	847	265	394-1420
7	10	961	185	738-1264	15	770	287	353-1455	25	846	265	353-1455
6	13	974	222	699-1469	12	709	247	349-1269	25	847	266	349-1469
2	13	953	224	694-1490	12	731	281	345-1306	25	846	272	345-1475
5	12	1016	240	700-1509	13	690	192	395- 997	25	846	269	395-1509
Significance level from analysis of variance												
Source of variation												
All diets	ns				ns				ns			

ns = not significant, P>0.05

Initial and final weights of fish in Year 1 and 2 are presented in Tables 7 and 8. High dietary lipid content significantly increased the specific growth rate of females in Year 1 but not in Year 2 while dietary vitamin E had no influence (Table 9). There were no effects of lipid or vitamin E on the specific growth rates of males in either year. In Year 1, feeding the COMM diet resulted in significantly higher growth rates in females than for those fed other diets except HLLE and in significantly higher growth rates in males compared to those fed LLHE. There was a low incidence of fish losing weight in Year 1, but this incidence increased in Year 2 (Table 10). There was no significant difference between the proportion of males or females which lost weight and no significant effect of dietary lipid or vitamin E on the proportion of males or females losing weight. Fish which lost weight continually throughout Year 1 and/or Year 2 became emaciated but only 2 of these fish died prior to spawning in Year 2 (one fed LE and 1 fed COMM).

4.6 Spawning

4.6.1 Time of spawning

In Year 1, there appeared to be an effect of dietary lipid level on the initial spawning date and the length of the spawning period (Table 11). Fish fed the HL diets began to spawn on September 29, 1988 with six out of 26 fish spawning during the first 5 weeks. Those fed the LL diets did not begin to spawn until November 2, 1988. Fisher's exact test was used

Table 7. Initial[1] and final[2] mean weight and range in weight of males and females fed each diet during feeding period in Year 1.

Diet	Females				Males			
	Initial		Final		Initial		Final	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	g	g	g	g	g	g	g	g
LLLE	1010	680-1420	1480	912-2065	670	394- 931	974	463-1422
LLHE	961	738-1264	1380	1079-1866	770	353-1455	1031	494-2188
HLLE	974	699-1469	1573	1281-2256	709	349-1269	1079	725-1928
HLHE	953	694-1490	1523	1047-2248	731	345-1306	1137	509-2058
COMM	1016	700-1509	1768	1211-2511	690	395- 997	1184	615-1722

[1] Initial weight on June 28, 1988.
[2] Final weight on Sept. 13, 1988.

Table 8. Initial[1] and final[2] mean weight and range in weight of males and females fed each diet during the feeding period in Year 2.

Diet	Females				Males			
	Initial		Final		Initial		Final	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	g	g	g	g	g	g	g	g
LLLE	1120	841-1583	2072	853-2912	938	622-1784	1307	603-2271
LLHE	1141	880-1593	1644	738-3124	994	441-1868	1640	347-3423
HLLE	1129	827-1429	1773	540-2920	837	369-1188	1077	435-1966
HLHE	1064	746-1588	1499	607-2867	910	397-2062	1267	311-2346
COMM	1324	832-1849	2827	840-4490	1011	578-1446	2020	953-2947

[1] Initial weight on Jan. 12, 1989.
[2] Final weight on Sept. 13, 1989.

Table 9. Specific growth rate (mean ± standard deviation) of female and male fish fed the diets in Year 1 and 2.

	Specific growth rate			
	Year 1		Year 2	
	Female	Male	Female	Male
	%/day ± SD	%/day ± SD	%/day ± SD	%/day ± SD
LLLE	.485[c] ± .087	.466[ab] ± .291	.245 ± .167	.110 ± .207
LLHE	.464[c] ± .067	.360[b] ± .226	.105 ± .167	.142 ± .197
HLLE	.625[ab] ± .120	.560[ab] ± .201	.127 ± .246	.023 ± .225
HLHE	.604[b] ± .110	.511[ab] ± .332	.105 ± .195	.122 ± .210
COMM	.708[a] ± .139	.699[a] ± .308	.277 ± .153	.262 ± .126
	Significance level from analysis of variance			
Source of variation				
All diets	**	**	ns	ns
Excluding COMM				
Lipid	**	ns	ns	ns
Vitamin E	ns	ns	ns	ns
Lipid x Vit E	ns	ns	ns	ns

Means within a column which share a common superscript are not significantly different.
ns = not significant, P>0.05, ** = P<.01

Table 10. Number of male and female charr which lost or gained weight throughout Year 1 or 2.

Tank Dietary vitamin E	3 LE	6 LE	2 HE	7 HE	5 COMM	Total LE	 HE
Lost weight only in Year 1							
Male	1	0	0	2	0	1	2
Female	0	0	0	0	0	0	0
Lost weight only in Year 2							
Male	6	6	2	4	1	12	6
Female	5	3	5	4	1	8	9
Lost weight in both Year 1 and 2							
Male	0	0	2	0	1	0	2
Female	0	0	0	0	0	0	0
Lost weight in either Year 1 or 2							
Male	7	6	4	6	2	13	10
Female	5	3	5	4	1	8	9
Gained weight in Year 1 and 2							
Male	4	6	8	9	11	10	17
Female	6	10	7	6	9	16	13

Table 11. Number of females which spawned during the 14-week spawning period in Year 1 and the 11-week spawning period in Year 2.

Week of spawning	Date	Diet LLLE	LLHE	HLLE	HLHE	COMM	LL	HL
YEAR 1:								
1- 2	Sep. 29-Oct. 5/88	-	-	-	1	1	-	1
3- 4	Oct. 12-Oct. 19/88	-	-	1	2	1	-	3
5- 6	Oct. 26-Nov. 2/88	-	1	2	1	2	1	3
7- 8	Nov. 9-Nov 17/88	6	4	3	2	6	10	5
9-10	Nov. 23-Nov. 30/88	2	1	4	3	-	3	7
11-12	Dec. 7-Dec. 14/88	3	-	1	3	-	3	4
13-14	Dec. 21-Dec. 27/88	2	1	2	1	-	3	3
Total		13	7	13	13	10	20[1]	26
No. weeks of spawning		8	8	11	14	8	9	14
YEAR 2:								
1- 2	Oct. 11-Oct. 18/89	-	-	-	-	1	-	-
3- 4	Oct. 25-Nov. 2/89	3	-	-	1	2	3	1
5- 6	Nov. 8-Nov. 15/89	2	-	1	2	6	2	3
7- 8	Nov. 22-Nov. 29/89	3	4	3	3	-	7	6
9-10	Dec. 6-Dec. 13/89	1	2	2	-	-	3	2
11	Dec. 20/89	1	1	-	-	-	2	-
Total		10	7[2]	6[2]	6	9	17[2]	12[2]
No. weeks of spawning		8	5	6	6	6	8	8

[1] Including one fish which had ovulated eggs when found dead on floor.

[2] Plus 2 fish fed LLHE and 1 fish fed HLLE which contained maturing eggs when they died as a result of human error at 8 weeks.

and showed that there was a significant difference in the proportion of fish fed the HL diets spawning earlier than those fed the LL diets. In Year 1, fish fed both the LL and the HL diets finished spawning on December 27, 1988 with spawning ending 12 weeks after it had begun. The spawning period lasted 9 weeks for fish fed the LL diets and 14 weeks for fish fed the HL diets. Fish fed the COMM diet spawned over a 9-week period which began during the third week of spawning.

To observe if the effect of dietary lipid on spawning day was repeatable, fish fed the LL diets in Year 1 were fed the HL diets in Year 2 and vice versa while keeping the vitamin E levels the same (Figure 3). Spawning began a minimum of 12 days later in Year 2 compared to Year 1 beginning on October 11, 1989 for fish fed the COMM diet and not until on or after October 22 for those fed the other diets (Table 11). Dietary lipid had no effect on the spawning period in Year 2.

Spawning day was positively correlated with egg fertilization in Year 1 (r^2=0.143, Appendix 4) and negatively correlated with egg weight in Year 1 (r^2=0.143). There was no significant effect of spawning day on egg weight, egg fertilization or embryonic survival but fecundity was negatively correlated with spawning day (r^2=0.249).

Table 12. Number of females and males which produced eggs (spawned) and milt (ripe) in Year 1.

Diet fed in Year 1	LLLE	LLHE	HLLE	HLHE	COMM	LE	HE	LL	HL
Tank	3	7	6	2	5				
Females:									
Spawned	13	7	13	13	10	26	20	20	26
No development	0	3	0	0	2	0	3	3	0
Total	13	10	13	13	12	26	23	23	26
Fisher's exact test									
Vitamin E		P=0.10							
Lipid		P=0.10							
Males:									
Ripe	7	11	4	6	8	11	17	18	10
Not ripe	5	4	8	6	5	13	15	9	4
Total	12	15	12	12	13	24	32	27	14
χ^2 for vitamin E		0.292 P>0.05							
χ^2 for lipid		3.207 P>0.05							

Table 13. Number of females and males which produced eggs (spawned) and milt (ripe) in Year 2.

Diet fed in Year 2	LLLE	LLHE	HLLE	HLHE	COMM	LE	HE	LL	HL
Tank	6	2	3	7	5				
Females:									
Spawned	10	9	7	7	9	17	16	19	14
No development	1	2	4	3	1	5	5	3	7
Total	11	11	11	10	10	22	21	22	21
Fisher's exact test									
Vitamin E		P=1.00							
Lipid		P=0.16							
Males:									
Ripe	5	8	4	10	12	9	18	13	14
Not ripe	6	4	5	4	0	11	8	10	9
Total	11	12	9	14	12	20	26	23	23
χ^2 for vitamin E		2.738 P>0.05							
χ^2 for lipid		0.090 P>0.05							

4.6.2 Number of ripe males and females

Chi square analysis showed that dietary lipid or vitamin E concntration had no significant effect on the porportion of males which produced milt in Year 1 or Year 2 (Tables 12 and 13). Fisher's exact text showed there was no effect of dietary vitamin E concentration on the proportion of females which spawned in Year 1 or 2.

A total of 9 males did not mature in either spawning season with 5 fed the LE diets and 4 fed the HE diets (Table 14). A portion of the population which produced milt in Year 1 did not produce it in Year 2 and vice versa. Fish fed HE diets had a significantly higher proportion of males which produced milt in both years compared to the LE diets (χ^2=4.030). The low sample size and the closeness of the P value to significance suggests that this area should be investigated further. Diet had no significant effect on changing the proportion of males becoming ripe in Year 2 which had not been not ripe in Year 1 (χ^2=0.202).

4.6.3 Atypical spawning

In Year 1, one fish fed LLLE and one fed COMM released their eggs in the tank and both fish died within 2-3 weeks. Embryonic survival was poor being 14% eyed for the former and 0% eyed for the latter. One fish fed HLHE produced eggs which were spotted and appeared overripe (38% eyed) and there was blood in the ovarian fluid. This fish died within 2 weeks.

Table 14. Number of males which produced milt (ripe) in Year 1 which did or did not produce milt in Year 2.

Diet fed in Year 1		HLLE	HLHE	LLLE	LLHE	COMM		
Diet fed in Year 2		LLLE	LLHE	HLLE	HLHE	COMM	LE	HE
Tank		6	2	3	7	5		
Year 1	Year 2							
Ripe	Ripe	2	4	2	8	8	4	12
Ripe	Not ripe	2	2	5	2	0	7	4
Total		4	6	7	10	8	11	16
χ^2 for vitamin E		4.030	P<0.05					
Year 1	Year 2							
Not ripe	Ripe	3	4	2	2	4	5	6
Not ripe	Not ripe	4	2	1	2	0	5	4
Total		7	6	3	4	4	10	10
χ^2 for vitamin E		0.202	P>0.10					

Problems occurred with 3 fish fed HLLE in Year 2, with one fish releasing most of its eggs in the tank and blood occurring in the ovarian fluid of one fish which also released 50-60 browncoloured eggs. Brown debris was present in the ovarian fluid of a third fish. One fish fed LLLE had eggs which could not be released and they appeared to be in a solid mass. One fish fed LLHE produced eggs which included about 12 dark orange eggs and this fish died within 4 weeks of spawning. In Year 2, mortality, primarily of females, increased at spawning in all groups except those fed LLLE and COMM and this was due to fungal infection which spread rapidly over the skin.

4.7 Fecundity and egg size (diameter and weight)

Diet had no significant effect on unfertilized egg weight in either Year 1 or Year 2 (Tables 15 and 16, Figure 6a and 6b). There was no effect of diet on the weight and diameter of water-hardened preserved eggs except for a significant effect of vitamin E on egg diameter in Year 1 (Table 17). The coefficient of variation (CV) for eggs produced by each fish ranged from 4.5 to 24.1% (mean=8.5) for egg weight and from 1.2 to 6.3% (mean=3.7) for diameter in Year 1. There was little change in Year 2 with CV ranging from 3.0 to 16.6% (mean=7.5) for egg weight and 1.9 to 8.2% (mean=3.7) for diameter.

No significant effects of diet on the total weight of eggs or number of eggs produced per kg body weight (Table 15) were

Table 15. Egg production parameters (mean ± standard deviation) in Year 1.

Diet fed in Year 1	Wt. eggs produced	Wt. per egg	Estimated no. eggs released at spawning[1]	Estimated total no. eggs produced[2]	Fish wt.	Eggs prod. per kg fish wt.
	g ± SD	mg ± SD	no. ± SD	no. ± SD	g ± SD	no./kg ± SD
LLLE	227 ± 95	53 ± 7	3868 ± 1789	4346 ± 2060	1403 ± 355	3004 ± 1147
LLHE	248 ± 68	59 ± 10	3769 ± 1162	4337 ± 1645	1392 ± 270	3200 ± 1173
HLLE	240 ± 87	56 ± 6	4031 ± 1493	4253 ± 1435	1473 ± 292	2960 ± 1010
HLHE	227 ± 75	56 ± 7	3907 ± 1407	4504 ± 1391	1425 ± 344	2854 ± 615
COMM	249 ± 103	51 ± 6	4532 ± 1596	4752 ± 1682	1660 ± 418	2842 ± 694
Significance level from analysis of variance						
Source of variation						
All diets	ns	ns	ns	ns	ns	ns
Excluding COMM						
Lipid	ns	ns	ns	ns	ns	ns
Vitamin E	ns	ns	ns	ns	ns	ns
Lipid x Vit E	ns	ns	ns	ns	ns	ns

ns = not significant, P>0.05.
[1] Number of eggs released on spawning day.
[2] Number of eggs released on spawning day + eggs collected during subsequent weeks.

Table 16. Egg production parameters (mean ± standard deviation) in Year 2.

Diet fed in Year 2	Wt. eggs released		Wt. per egg[1]		Estimated no. eggs released at spawning[2]		Estimated total no. eggs produced[3]		Fish wt.		Eggs prod. per kg fish wt.	
	g	± SD	mg	± SD	no.	± SD	no.	± SD	g	± SD	no./kg	± SD
LLLE	384^{ab}	± 139	56	± 8	5838^{ab}	± 2119	6788^{ab}	± 2546	2111^{b}	± 516	3098	± 95
LLHE	209^{b}	± 131	55	± 4	2910^{b}	± 2453	3803^{b}	± 2462	1461^{b}	± 648	2396	± 92
HLLE	283^{b}	± 148	62	± 11	4197^{b}	± 2632	4865^{b}	± 2894	2159^{b}	± 650	2112	± 92
HLHE	234^{b}	± 118	61	± 11	3673^{b}	± 1665	4045^{b}	± 1953	1715^{b}	± 568	2172	± 82
COMM	505^{a}	± 187	55	± 6	8305^{a}	± 3313	9263^{a}	± 3728	2950^{a}	± 881	3067	± 56
Significance level from analysis of variance												
Source of variation												
All diets	**		ns		**		**		**		ns	
Excluding COMM												
Lipid	ns		ns		ns		ns		ns		ns	
Vitamin E	ns		ns		ns		ns		ns		ns	
Lipid x Vit E	ns		ns		ns		ns		ns		ns	

[1] Log transformation was conducted prior to analysis of variance on weight per egg.
[2] Number of eggs released on spawning day.
[3] Number of eggs released on spawning day + eggs collected during subsequent weeks.
ns = not significant, $P>0.05$, ** = $P < 0.01$
Means within a column which share a common superscript are not significantly different.

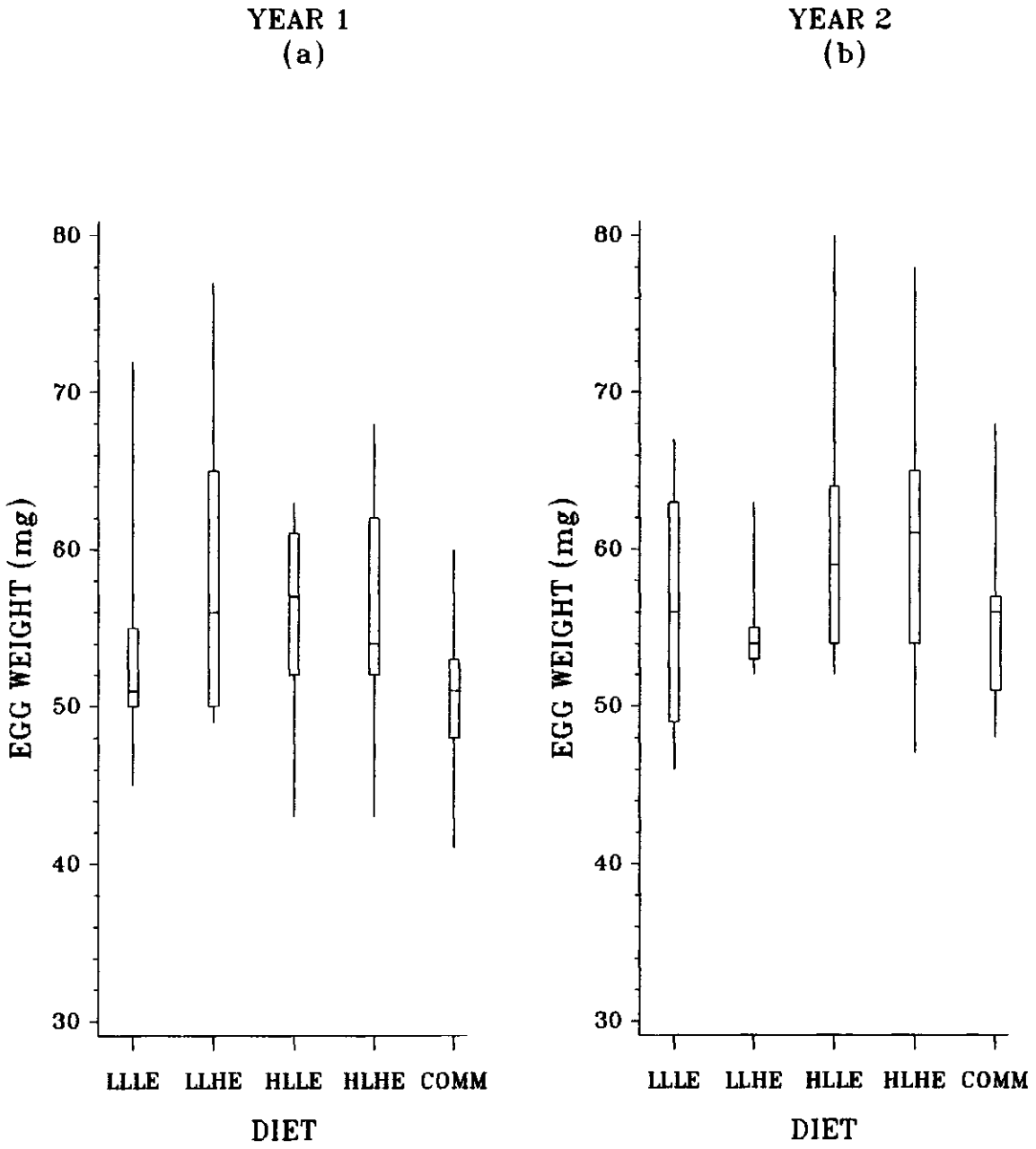


Figure 6. Egg weight in a) Year 1 and b) Year 2 showing minimum, 25, 50, 75 percentiles and maximum values.

Table 17. Egg weight and diameter of water-hardened preserved eggs (mean ± standard deviation) in Years 1 and 2.

Diet	Year 1				Year 2			
	Weight		Diameter		Weight[1]		Diameter[1]	
	mg	± SD	mm	± SD	mg	± SD	mm	± SD
LLLE	66.7	± 7.6	4.97	± 0.18	68.4	± 9.4	4.94	± 0.21
LLHE	73.3	± 10.2	5.15	± 0.23	64.4	± 4.3	4.90	± 0.10
HLLE	68.9	± 6.4	5.02	± 0.16	73.5	± 13.6	5.13	± 0.36
HLHE	68.3	± 7.1	5.00	± 0.16	73.7	± 9.9	5.04	± 0.22
COMM	64.5	± 7.9	4.94	± 0.19	66.1	± 5.7	4.91	± 0.15
Significance level from analysis of variance								
Source of variation								
All diets	ns		ns		ns		ns	
Excluding COMM								
Lipid	ns		ns		ns		ns	
Vitamin E	ns		*		ns		ns	
Lipid x Vit E	ns		ns		ns		ns	

[1] Log transformation was conducted prior to analysis of variance.
ns = not significant, P>0.05, * = P<0.05.

observed. Some eggs were released after spawning and there was no significant difference in the number of eggs released at spawning or the total number of eggs produced. InYear 2, fish fed COMM weighed significantly more than those fed other diets and they produced significantly more eggs and a greater total weight of eggs than those fed all diets except LLLE (Table 16). There was no significant difference in the number of eggs produced per kg body weight. The numbers of eggs produced were highly variable within each treatment in both years as shown in Figures 7a and 7b. The total number of eggs produced was directly correlated with fish weight in both Year 1 (r^2=0.334) and Year 2 (r^2=0.853) (Figures 8a and 8b, Appendix 4 but was not significantly correlated with egg weight.

Some fish lost weight throughout Year 2 and were not expected to produce any eggs. However, some of these fish produced small numbers of eggs. For example, 2 fish fed LLHE released less than 350 eggs with survival of 61 and 94% to the eyed stage and 1 fish fed HLHE released 525 eggs with 95% survival to the eyed stage. Fecundity was not correlated with fertilization, survival to the eyed, hatch or swimup stage or with survival or specific growth rate of fry.

Fertilization and survival to hatch and swimup stages was directly related to egg weight in Year 2 (r^2=0.11-0.12) (Appendix 4) but was not correlated in Year 1.

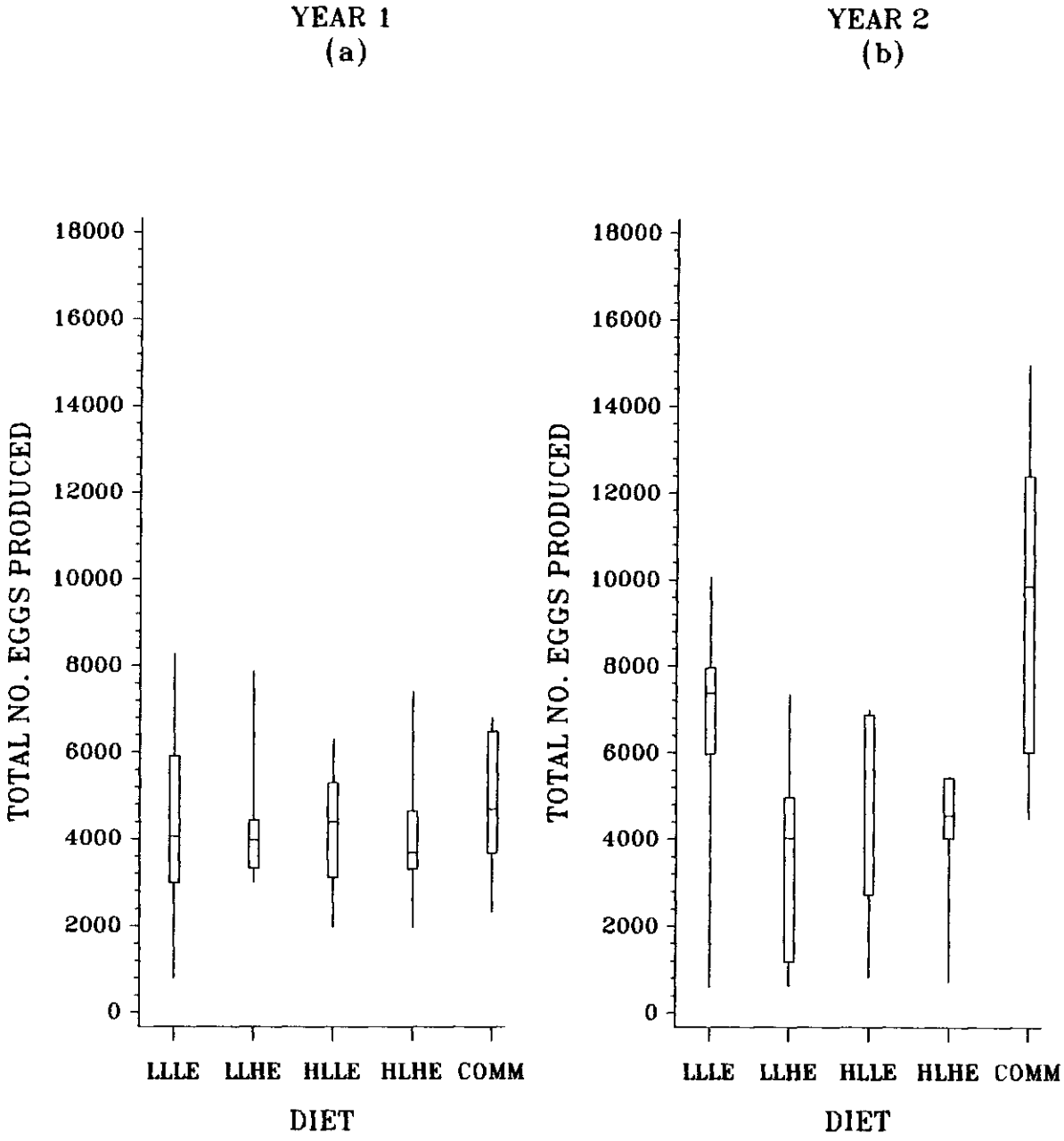


Figure 7. Total number of eggs produced in a) Year 1 and b) Year 2 showing minimum, 25, 50, 75 percentiles and maximum values.

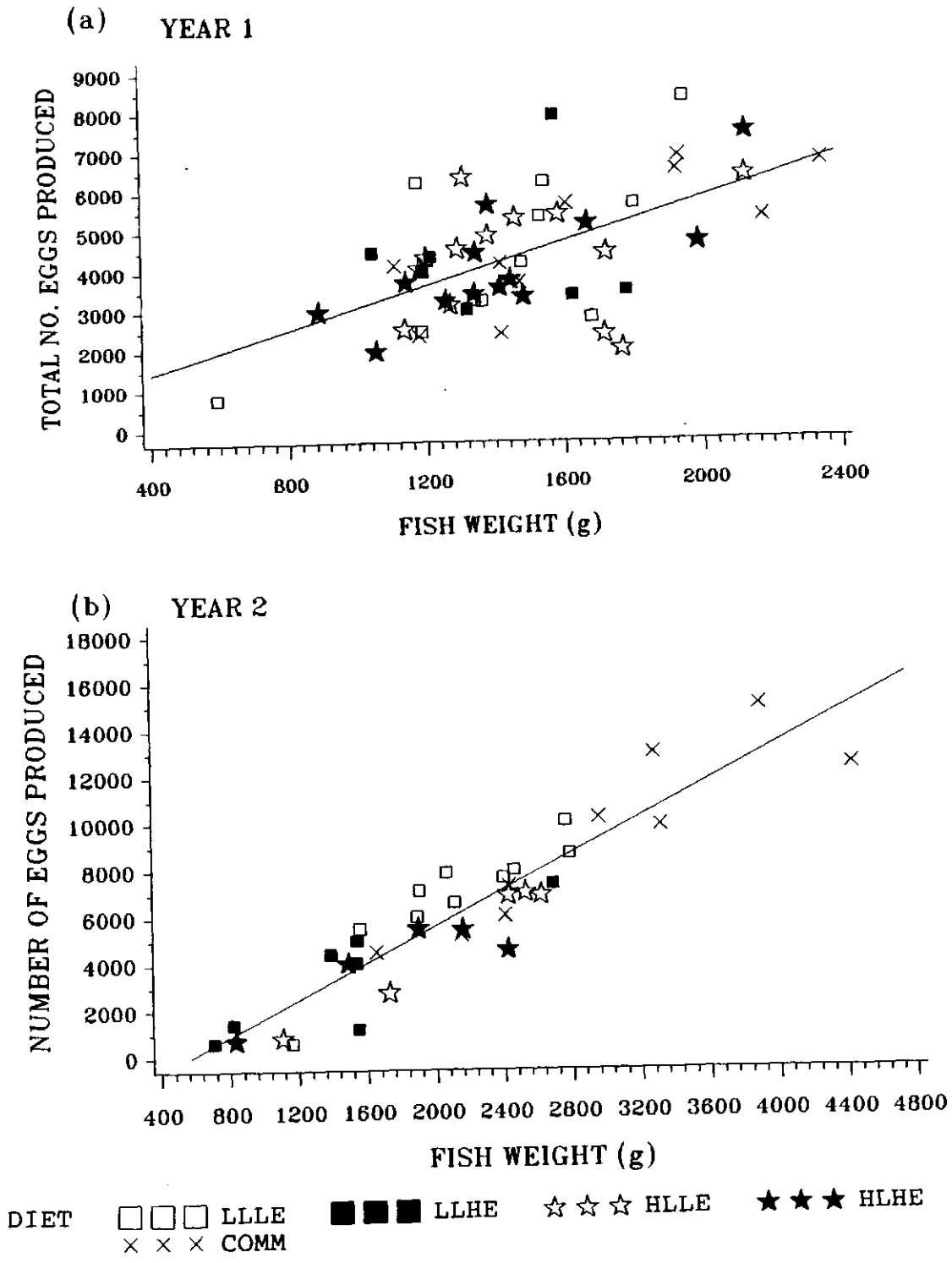


Figure 6. Relationship of total number of eggs produced and fish weight in :

a) Year 1 ($Y= 2.71X + 351.88$, $r^2=0.334$) and

b) Year 2 ($Y= 3.88X + 2202.25$, $r^2=0.853$).

4.8 Embryonic survival

More eggs were generally collected and fertilized than required for incubation for this experiment. Excess eggs were incubated in Heath trays until they reached the eyed stage and then they were distributed to producers. In Year 2, Heath trays were compartmentalized and eggs from each female were maintained separately. Survival to the eyed stage was comparable for eggs incubated in Heath trays compared to the jar system.

When fertilized eggs were viewed under a low power microscope, divided cells could be seen which varied from the 4-cell to 16-32 cell stage (Tables 18 and 19, Figures 9 and 10). In Year 2, 2 fish fed LLHE produced eggs in which abnormal cell division and they had vitamin E concentrations of 236 and 242 µg/g. Abnornal cell division was also observed in eggs from 3 fish fed HLLE and the vitamin E concentrations were 37, 54 and 59 µg/g. Cells appeared to have divided unevenly and/or were some distance apart. As the incidence of abnormal eggs increased, the percentage which reached the eyed stage decreased. Abnormal cells were similar in appearance to those illustrated in Leray *et al.* (1985) which were apparently due to an essential fatty acid deficiency.

There were no significant differences between the 5 dietary treatments in fertilization or survival to the eyed, hatch or swimup stage in Year 1 or 2 (Table 18 and 19, Figures 9 and 10).

Table 18. Fertilization[1] and embryonic survival[1] (mean ± standard deviation) in Year 1.

Diet fed in Year 1	n	Fertilized	Eyed	Hatch	Swimup
		% ± SD	% ± SD	% ± SD	% ± SD
LLLE	12	92 ± 5	67 ± 28	48 ± 32	42 ± 32
LLHE	7	90 ± 13	81 ± 14	76 ± 15	65 ± 31
HLLE	13	92 ± 8	77 ± 19	63 ± 25	58 ± 25
HLHE	12	86 ± 27	60 ± 34	46 ± 37	38 ± 36
COMM	10	87 ± 18	68 ± 37	63 ± 34	57 ± 31
		Significance level from analysis of variance			
Source of variation					
All diets		ns	ns	ns	ns
Excluding COMM					
Lipid		ns	ns	ns	ns
Vitamin E		ns	ns	*	*
Lipid x Vit E		ns	ns	*	*
			Eyed/Fert	Hatch/Eyed	Swimup/Hatch
			% ± SD	% ± SD	% ± SD
LLLE		-	71 ± 29	64[b] ± 27	84 ± 19
LLHE		-	90 ± 7	94[a] ± 6	82 ± 32
HLLE		-	83 ± 18	79[ab] ± 17	91 ± 7
HLHE		-	70 ± 31	66[b] ± 34	82 ± 24
COMM		-	74 ± 39	93[a] ± 6	91 ± 6
			Significance level from analysis of variance		
Source of variation					
All diets			ns	**	ns
Excluding COMM					
Lipid			ns	ns	ns
Vitamin E			ns	**	ns
Lipid x Vit E			*	**	ns

[1] Arcsin transformation was used on all data prior to analysis of variance.
ns = not significant, P>0.05, * = P < 0,05, ** = P < 0.01

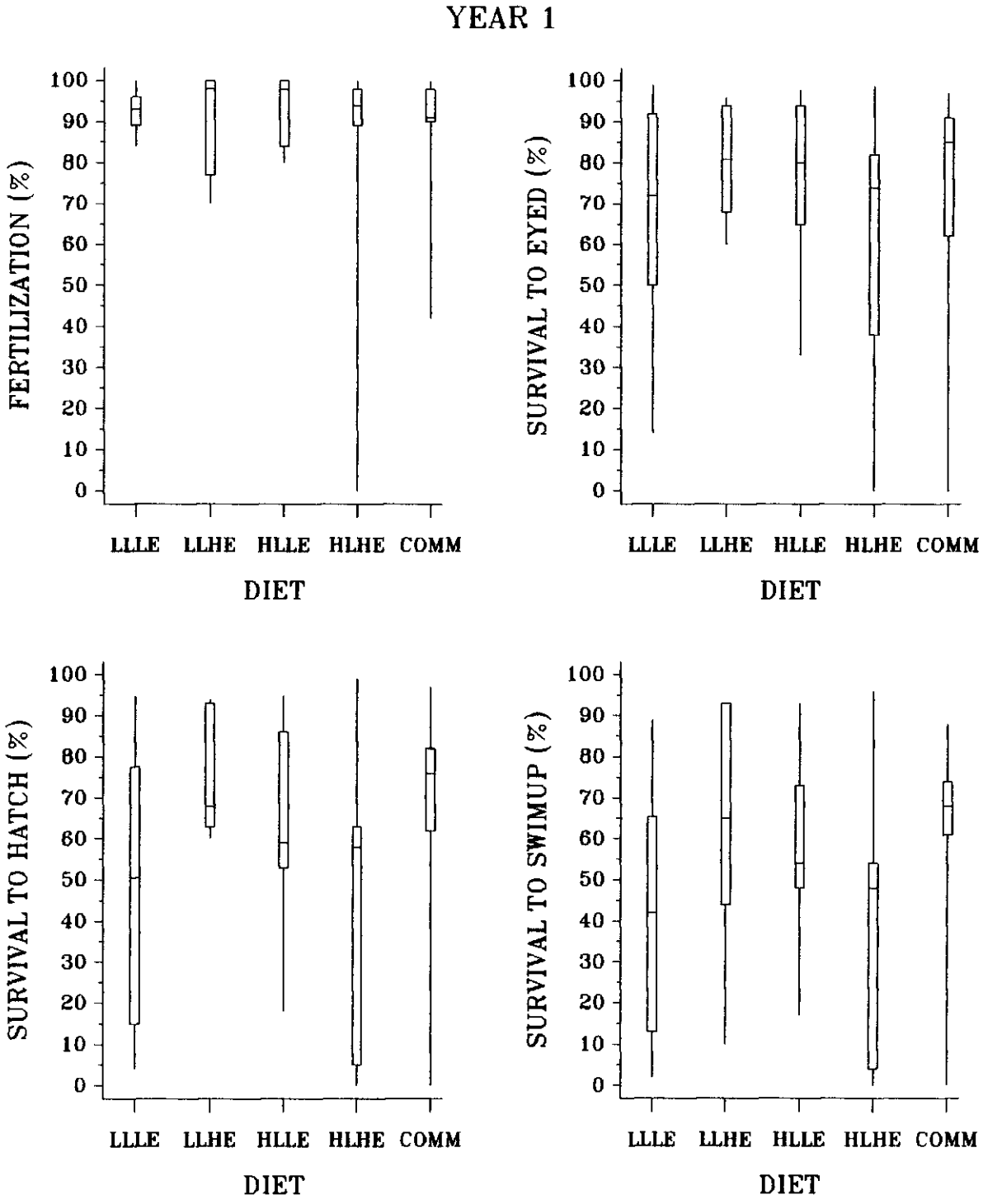


Figure 9. Fertilization and survival of eggs to the eyed, hatch and swimup stages in Year 1 showing minimum, 25, 50, 75 percentiles and maximum values.

Table 19. Fertilization and embryonic survival[1] (mean ± standard deviation) in Year 2.

Diet fed in Year 2	n	Fertilized	Eyed	Hatch	Swimup
		% ± SD	% ± SD	% ± SD	% ± SD
LLLE	10	87 ± 13	40 ± 35	31 ± 31	23 ± 30
LLHE	7	83 ± 11	53 ± 42	45 ± 39	42 ± 39
HLLE	6	69 ± 37	28 ± 40	24 ± 35	19 ± 32
HLHE	6	88 ± 11	65 ± 33	59 ± 34	51 ± 38
COMM	9	73 ± 28	37 ± 29	29 ± 25	24 ± 20
		Significance level from analysis of variance			
Source of variation					
All diets		ns	ns	ns	ns
Excluding COMM					
Lipid		ns	ns	ns	ns
Vitamin E		ns	ns	ns	ns
Lipid x Vit E		ns	ns	ns	ns
			Eyed/Fert.	Hatch/Eyed	Swimup/Hatch
			% ± SD	% ± SD	% ± SD
LLLE		-	44 ± 38	67 ± 21	74 ± 25
LLHE		-	56 ± 42	81 ± 24	82 ± 21
HLLE		-	37 ± 47	75 ± 34	45 ± 44
HLHE		-	75 ± 36	84 ± 15	77 ± 27
COMM		-	46 ± 32	76 ± 13	83 ± 9
		Significance level from analysis of variance			
Source of variation					
All diets		ns	ns	ns	ns
Excluding COMM					
Lipid		ns	ns	ns	ns
Vitamin E		ns	ns	ns	ns
Lipid x Vit E		ns	ns	ns	ns

[1] Arcsin transformation was used on all data prior to analysis of variance.
ns = not significant, P>0.05

YEAR 2

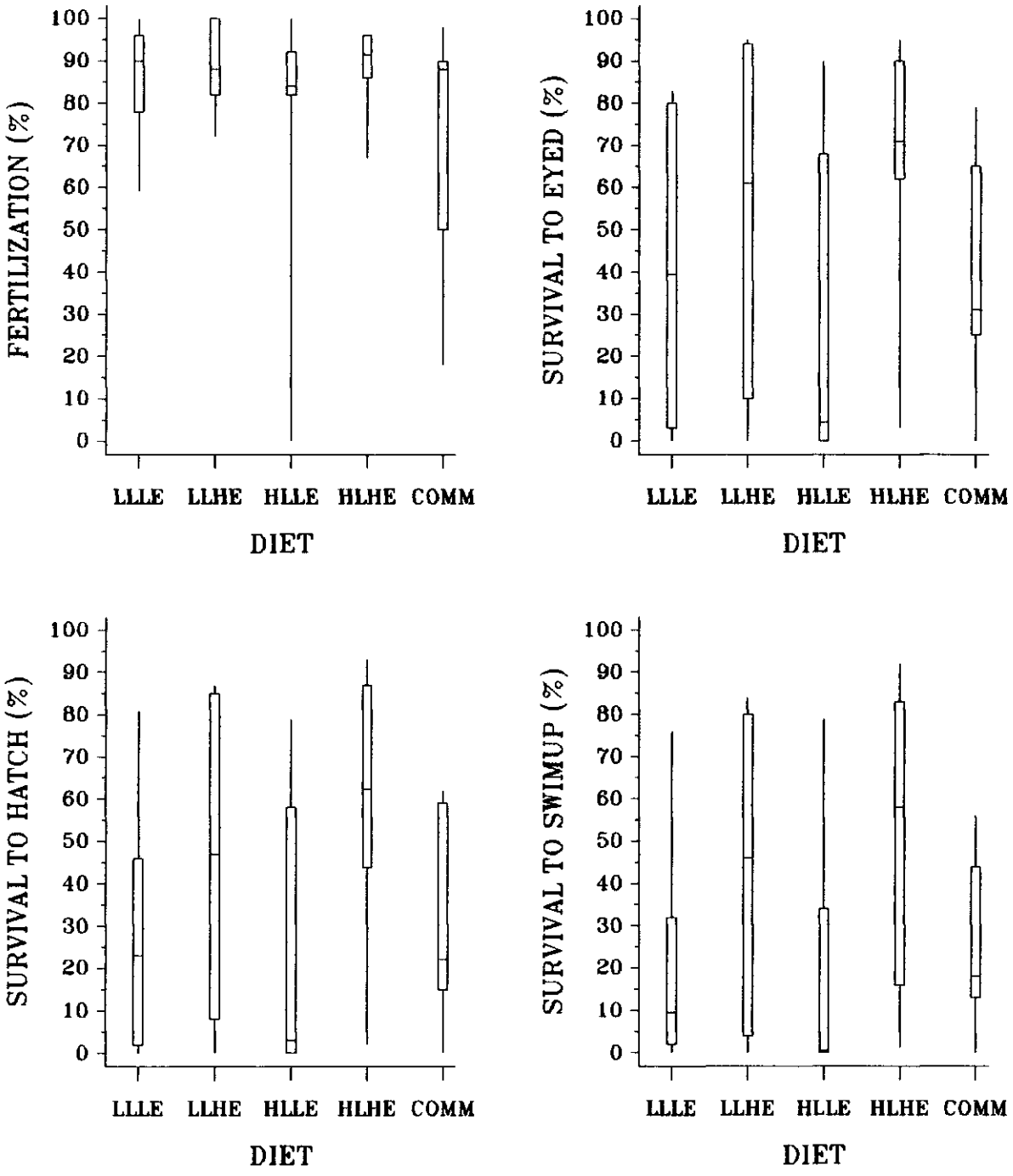


Figure 10. Fertilization and survival of eggs to the eyed, hatch and swimup stages in Year 2 showing minimum, 25, 50, 75 percentiles and maximum values.

In Year 1, there was a significant effect of dietary vitamin E. An interaction between lipid and vitamin E on survival to hatch and swimup resulted in an increase in survival as dietary vitamin E increased in the LL diets and a decrease in survival as vitamin E increased in the HL diets.

To ascertain if diet resulted in a difference in the stage at which embryonic development ceased, the following values were calculated and compared: Fertilized, Eyed/Fertilized, Hatch/Eyed and Swimup/Hatch (Table 18 and 19). A value was not included in the analysis if both the denominator and numerator were zero. In Year 2, most mortality occurred prior to reaching the eyed stage. Only 37-56% of eggs that were fertilized reached the eyed stage in all groups except the HLHE group, where 75% of those fertilized reached the eyed stage. Of the eggs that reached the eyed stage in Year 2, 67 to 84% hatched. Survival was low (45%) between hatch and swimup for fry from the HLLE group compared to other groups (74-83%).

When this experiment was initiated, approximately one-half of the stock of Arctic charr was used. The remaining fish were maintained as usual and fed the COMM diet (although it may have been from different shipments as the diet used in the present experiment). Although records were not kept on the survival of eggs from individual fish, the overall survival to the eyed stage was 72% in Year 1 and fish released 3300 eggs at spawning.

In Year 2, records were kept for individual fish and survival to the eyed stage was 35% ± 33 (std. dev.)(n=30) and fish released 4270 ± 2456 eggs at spawning. This was similar to survival to the eyed stage of 68% ± 37 in Year 1 and 37% ± 29 in Year 2 for fish fed the COMM diet in this experiment. Fecundity was higher for the fish fed COMM in the experimental group with them releasing 4532 ± 1596 eggs in Year 1 and 8305 ± 3313 eggs in Year 2.

Approximately 4-5% of fry had blue-sac and 4-5% had deformities in Year 2 but this did not appear to be correlated with diet. Most deformities were spinal deformities and conjoined twins. There was also evidence of "coagulated yolk" in at least 8 cases with proportions of over 20% of the fry affected. A hemorrhagic area was visible in the coagulated yolk in some cases. Fry from the HLHE group had the lowest incidence of coagulated yolk. Premature hatch was observed in 5 cases in Year 2 with 10-50% mortality occurring. A condition similar to blue-sac has been reported in sunapee (Salvelinus alpinus oquassa) and lake charr (Salvelinus namaycush) (Balon 1980) when they were incubated at a high temperature (9.5°C). Blue-sac has also been associated with low dissolved oxygen and toxicants. As reported by Balon (1980), histological examination showed the yolk sac separated from the yolk and became inflated as fluid accumulated.

4.9 Egg and fry composition

4.9.1 Vitamin E

Reproducibility of the assay for vitamin E concentration indicated a mean coefficient of variation of 3.7% for fry (n=29), and 6.1 (n=57) and 4.1% (n=38) for eggs in Year 1 and Year 2, respectively. When eggs were weighed out for vitamin E extraction and assay, they disintegrated as soon as the eggs began to thaw. This made vitamin E available for oxidation and resulted in a reduction of the reproducibility of the assay. Reproducibility improved when the antioxidant, butylated hydroxytoluene (BHT), was added to hexane, the extraction solvent, at 50 mg/L. Addition of BHT was not necessary during extraction of vitamin E from fry. The extraction blank indicated a need for a correction factor of 9.92 μg vitamin E per assay for the egg samples and 5.63 μg for the fry samples, with the difference being due to the addition of BHT for the analysis of the eggs.

Recovery was measured by spiking a sample of fry with a known amount of 95% pure dl-α-tocopherol (Sigma Chemical Company, St. Louis, MO). Recovery was 100.8 ± 0.54% (SD). A sample of the final ethanolic extract from fry from the HLHE treatment was subjected to a spectrophotometric scan in the ultraviolet range. The major peak occurred at a wavelength of 288-292 mμ which corresponded to α-tocopherol at 292 mμ (Budvari

et al. 1989). A small peak was evident at 280 mμ but no peaks occurred above 310 mμ indicating that retinol, with an absorption peak of 325 mμ was not present in the extract.

Increasing dietary vitamin E concentration resulted in significant increases in the vitamin E concentration and content of eggs and fry in both years (Table 20, Figure 11-13). In addition, in Year 2 there was also a significant effect of dietary lipid and an interaction between the effect of lipid and vitamin E on the eggs but not the fry. The rank of vitamin E concentration and content associated with the 5 dietary treatments remained the same in both years for eggs and fry, increasing in the order of LLLE, HLLE, COMM, HLHE, LLHE. In Year 2, there were significant differencesin vitamin E concentration and content of eggs from fish fed each diet while in Year 1 there were no significant differences in vitamin E of eggs from fish fed LLLE, HLLE or COMM.

At high dietary lipid, increasing dietary vitamin E had less impact on egg vitamin E concentration (2-3-fold increase) than at the low lipid concentration (3-5-fold increase). Except for the LLHE diet, swimup fry generally had a higher vitamin E concentration and content than the eggs.

Table 20. Vitamin E concentration and content[1] (mean ± standard deviation) of eggs in Years 1 and 2 and fry in Year 2.

Diet	Year 1 Egg		Year 2 Egg		Year 2 Swimup fry	
	µg/g± SD	µg/egg± SD	µg/g ± SD	µg/egg± SD	µg/g ± SD	µg/fry ± SD
LLLE	53^{c} ± 11	2.8^{c} ± 0.9	38^{e} ± 9	2.1^{d} ± 0.4	65^{d} ± 9	3.6^{c} ± 0.7
LLHE	202^{a} ± 48	12.1^{a} ± 4.1	208^{a} ± 61	11.7^{a} ± 3.9	209^{a} ± 73	11.2^{a} ± 4.8
HLLE	54^{c} ± 11	3.0^{c} ± 0.6	51^{d} ± 8	3.5^{c} ± 1.1	73^{cd} ± 23	4.5^{bc} ± 0.6
HLHE	156^{b} ± 20	8.6^{b} ± 1.6	140^{b} ± 28	8.2^{b} ± 1.8	153^{b} ± 20	9.6^{a} ± 1.2
COMM	69^{c} ± 16	3.5^{c} ± 0.8	68^{c} ± 7	3.8^{c} ± 0.7	86^{c} ± 13	5.4^{b} ± 0.7
	Significance level from analysis of variance					
Source of variation						
All diets	**	**	**	**	**	**
Excluding COMM						
Lipid	ns	ns	**	**	ns	ns
Vit E	*	**	**	**	**	**
Lipid x Vit E	ns	*	**	**	ns	ns

Means within a column which share a common superscript are not significantly different.
[1] Log transformation was used on all data prior to analysis of variance.
ns = not significant, P>0.05, *=P<0.01, **=P<0.01

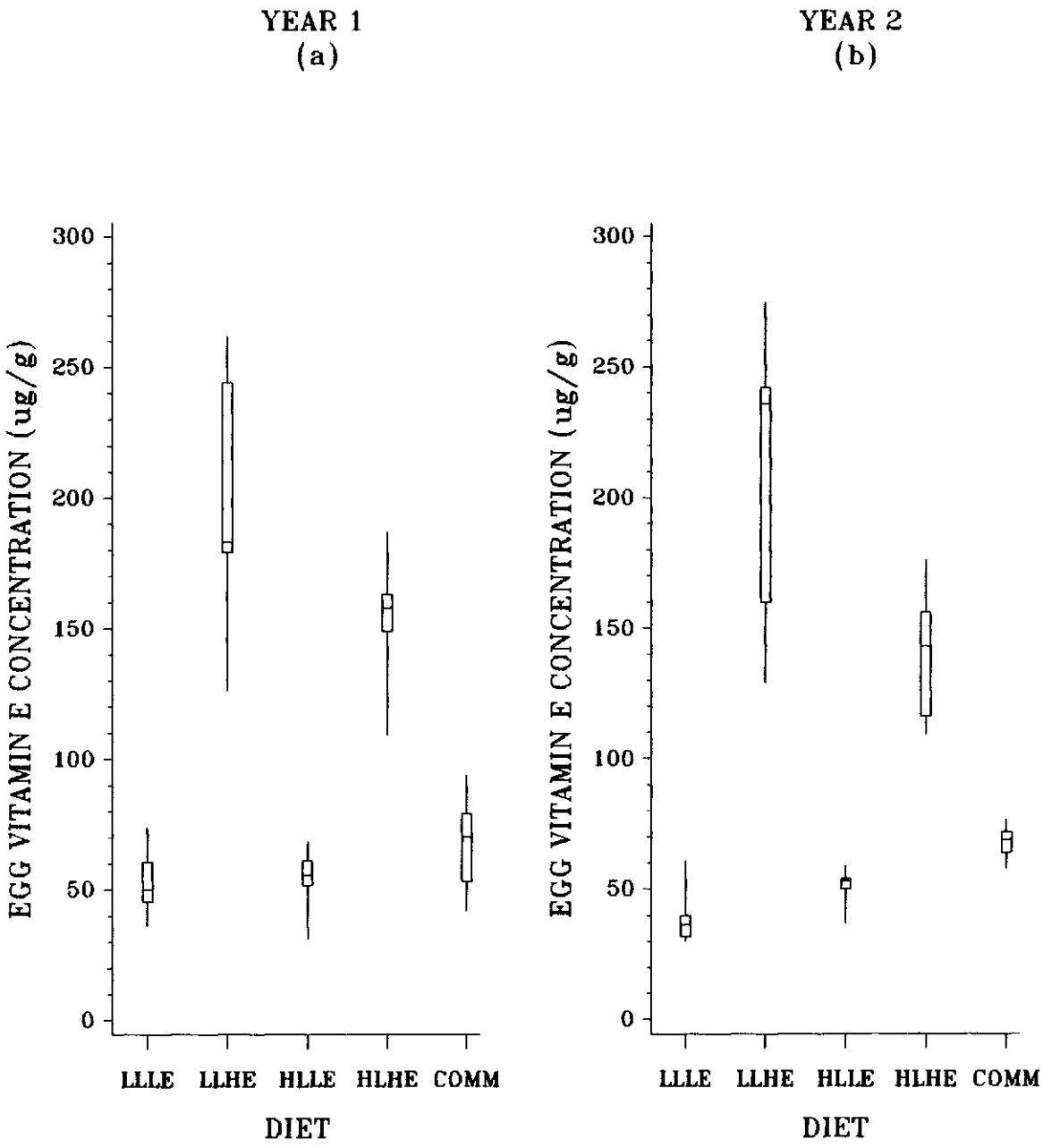


Figure 11. Vitamin E concentration of eggs in a) Year 1 and b) Year 2 showing minimum, 25, 50, 75 percentiles and maximum values.

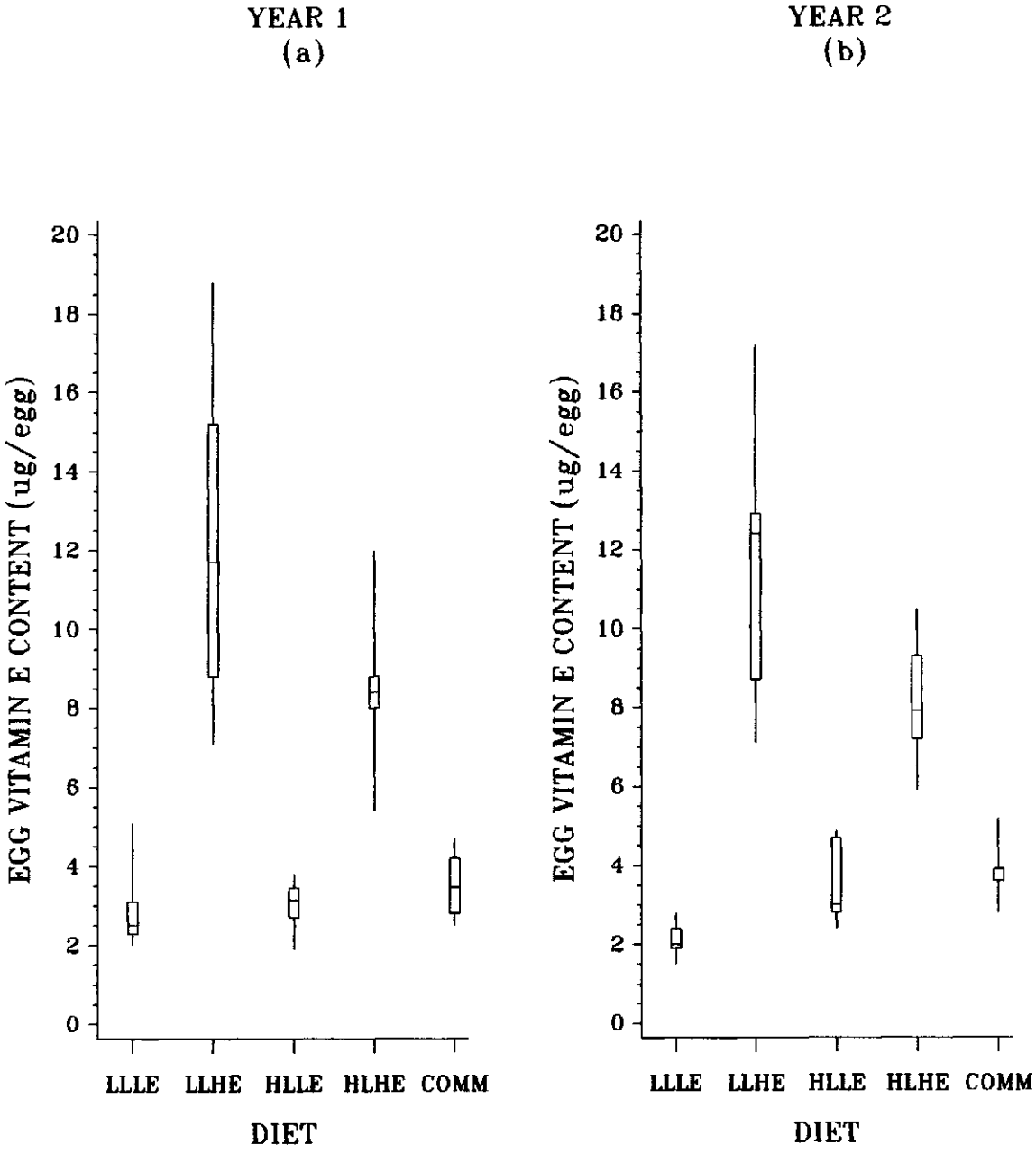


Figure 12. Vitamin E content of eggs in a) Year 1 and b) Year 2 showing minimum, 25, 50, 75 percentiles and maximum values.

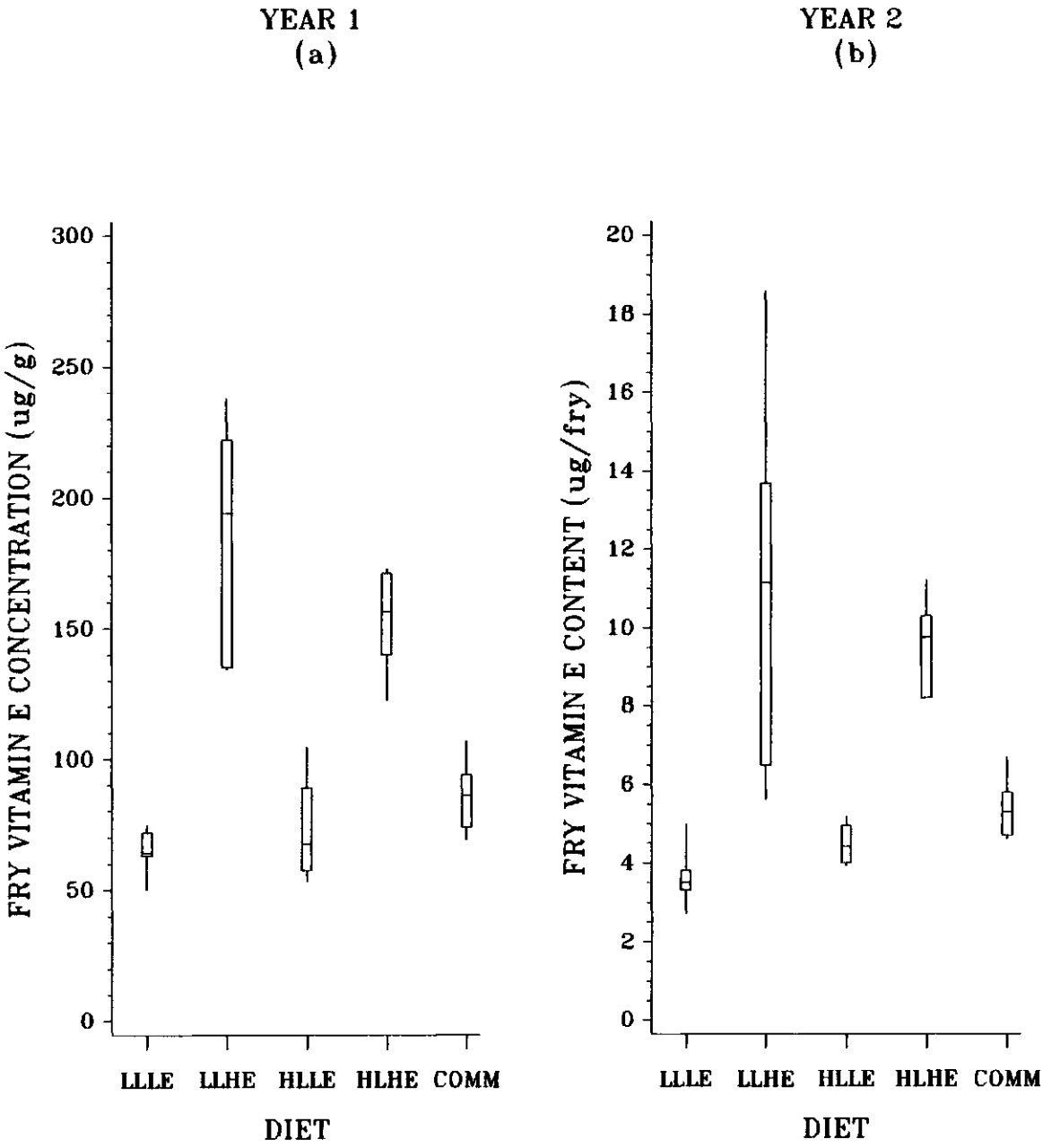


Figure 13. Vitamin E concentration and content of fry in Year 2 showing minimum, 25, 50, 75 percentiles and maximum values

There was no overlap in the range of vitamin E concentrations of the LE eggs (30-74 μg/g) compared to the HE eggs (109-275 μg/g). The COMM eggs were intermediate between the LE and HE eggs (42-94 μg/g). There was much greater variability in vitamin E concentration for the HE eggs compared to the LE eggs. Within the HE eggs, LLHE had greater variability than HLHE with eggs from fish fed LLHE ranging from 126-275 μg/g while HLHE ranged from 109-187 μg/g.

4.9.2 Lipid and dry matter

There were no significant differences in percentages of dry matter or total lipid in eggs from fish fed any of the diets in Year 1 (Table 21, Figure 14a). There was no correlation between total lipid or dry matter and survival of embryos or fry or specific growth rate of fry. In Year 2, eggs from fish fed COMM had greater percent dry matter than those of HLLE and significantly greater percent total and neutral lipid than noted for fish fed all other diets except LLLE (Table 22, Figure 14b and 14c). No significant differences existed in percent polar lipid (Table 22, Figure 14d). Neutral lipid was negatively correlated to survival to the eyed, hatch and swimup stages (r^2=0.26-0.34, P<0.003) (Figures 15a, 16a, 17a and Appendix 4). Analysis of covariance showed that there was no difference between diets in this relationship.

Table 21. Total lipid and dry matter contents (mean ± standard deviation) of eggs in Year 1.

Diet	Dry matter	Total lipid
	% ± SD	% ± SD
LLLE	37.0 ± 2.6	22.5 ± 1.1
LLHE	38.1 ± 1.5	22.2 ± 1.3
HLLE	38.4 ± 1.1	23.2 ± 1.0
HLHE	38.6 ± 0.9	22.3 ± 1.0
COMM	38.3 ± 0.8	22.2 ± 0.9
Significance level from analysis of variance		
Source of variation		
All diets	ns	ns
Excluding COMM		
Lipid	ns	ns
Vitamin E	ns	ns
Lipid x Vit E	ns	ns

ns = not significant, P>0.05

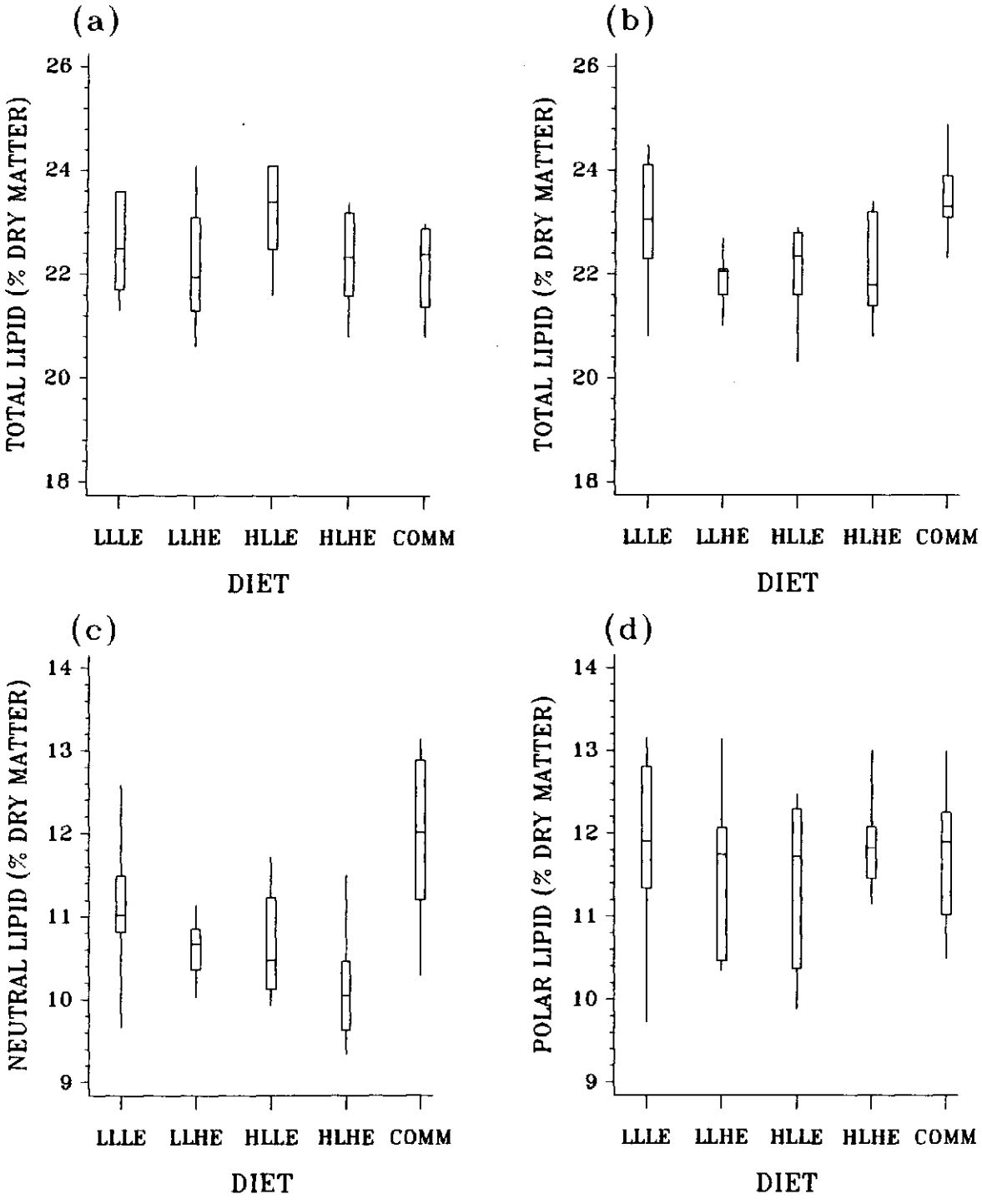


Figure 14. Total lipid of eggs in Year 1 (a), b) total, c) neutral and d) polar lipid in Year 2 showing minimum, 25, 50, 75 percentiles and maximum values.

Table 22. Lipid and dry matter contents (mean ± standard deviation) of eggs in Year 2.

Diet	Dry matter		Total lipid		Neutral lipid		Polar lipid	
	%	± SD	%	± SD	%	± SD	%	± SD
LLLE	38.0[a]	± 0.8	23.0[ab]	± 1.3	11.1[ab]	± 0.9	11.9	± 1.1
LLHE	36.1[ab]	± 3.6	21.9[b]	± 0.6	10.3[b]	± 0.8	11.6	± 1.1
HLLE	34.4[b]	± 4.6	22.0[b]	± 1.0	10.6[b]	± 0.7	11.4	± 1.1
HLHE	36.7[ab]	± 1.3	22.1[b]	± 1.0	10.2[b]	± 0.8	11.9	± 0.6
COMM	38.5[a]	± 0.9	23.5[a]	± 0.9	11.6[a]	± 1.0	11.8	± 0.8
	Significance level from analysis of variance							
Source of variation								
All diets	*		*		**		ns	
Excluding COMM								
Lipid	ns		ns		ns		ns	
Vitamin E	ns		ns		ns		ns	
Lipid x Vit E	ns		ns		ns		ns	

Means within a column which share a common superscript are not significantly different.
ns = not significant, P>0.05, *=P<0.01, **=P<0.01

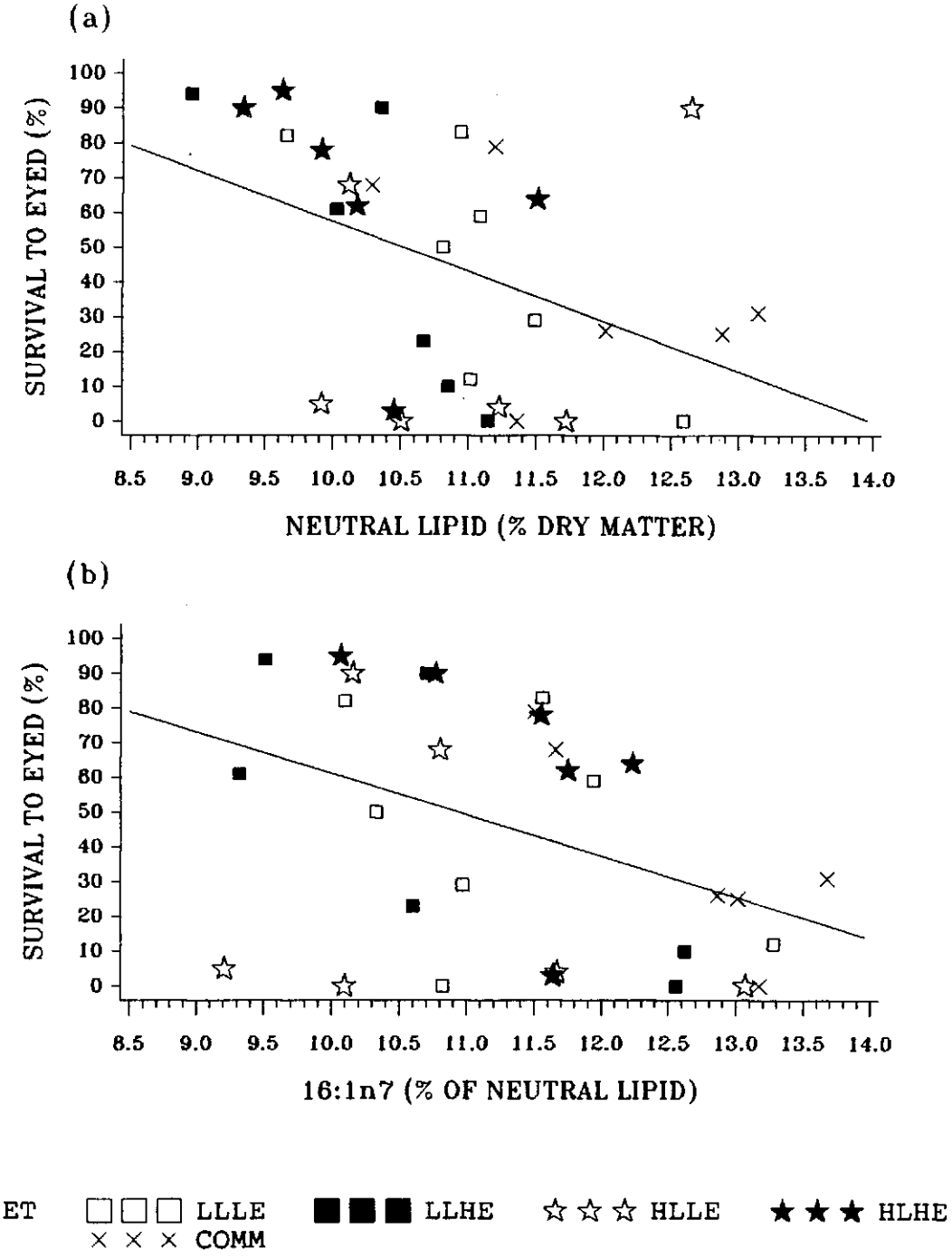


Figure 15. Relationship of :
a) neutral lipids (Y= -17.29X + 233.36, r^2=0.263) and
b) 16:1n7 in neutral lipids (Y= -13.68X + 202.52, r^2=0.238)
on survival to the eyed stage in Year 2.

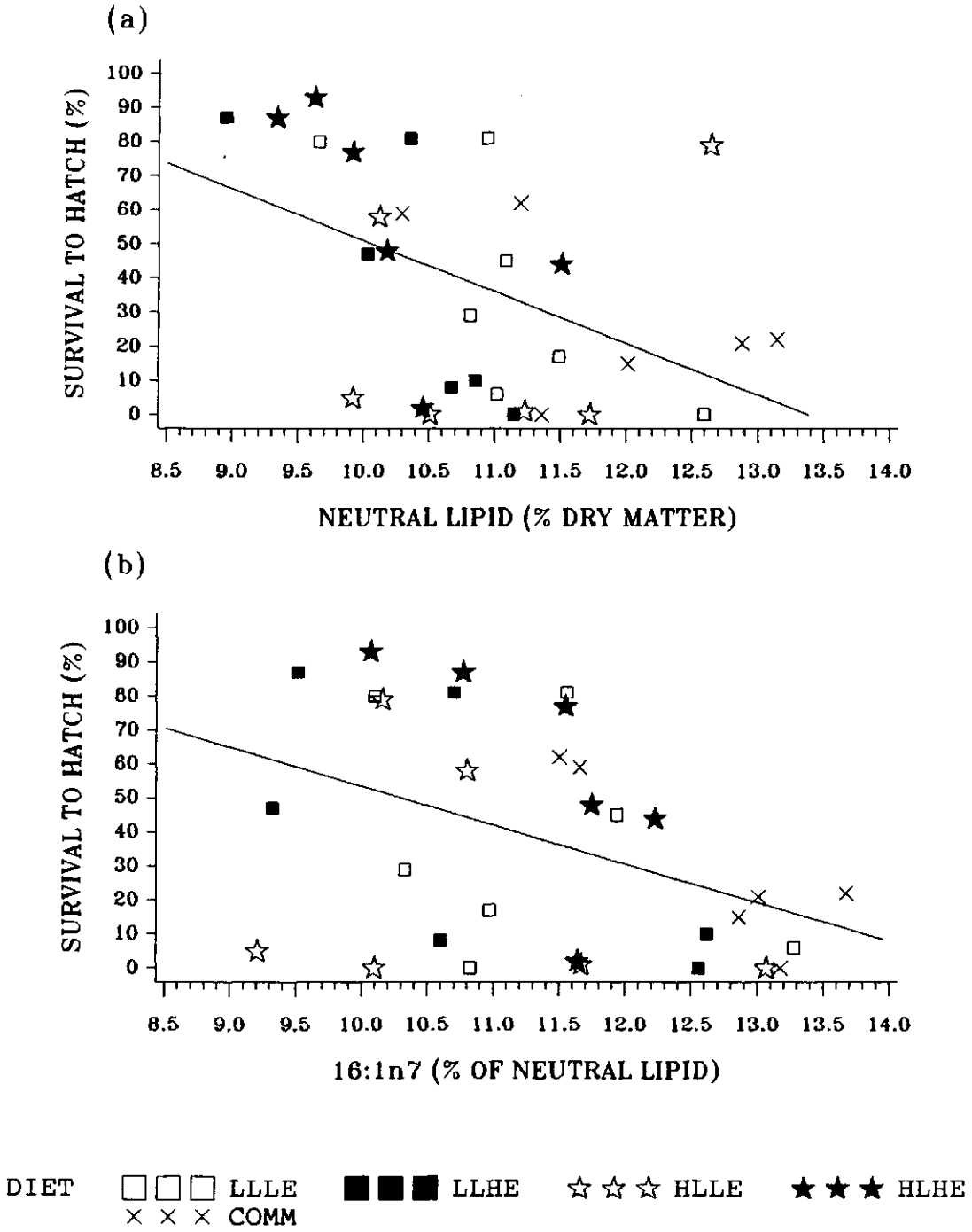


Figure 16. Relationship of :
a) neutral lipids (Y= -18.24X + 236.20, r^2=0.321) and
b) 16:1n7 in neutral lipids (Y= -12.92X + 186.58, r^2=0.234)
on survival to hatch in Year 2.

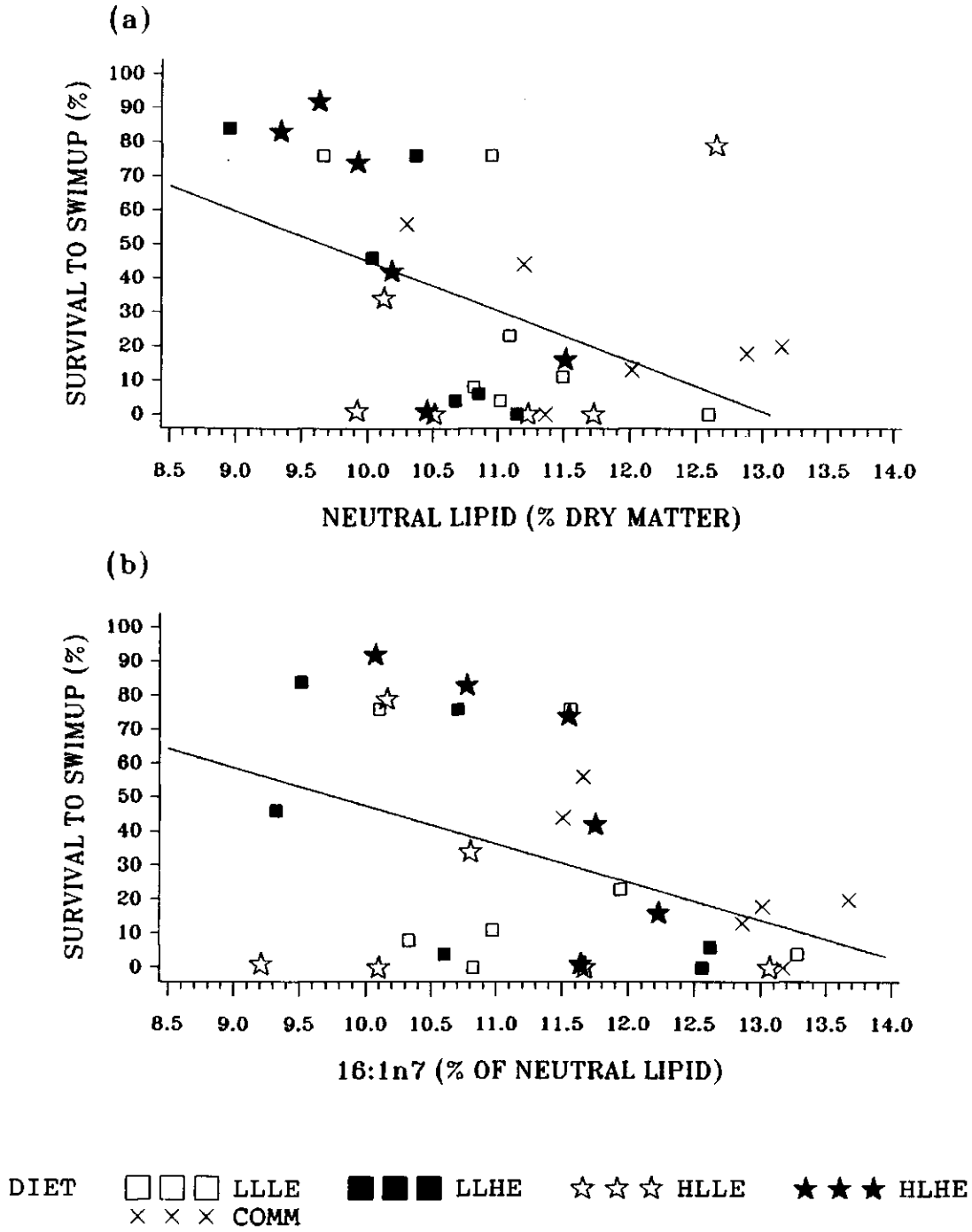


Figure 17. Relationship of :
a) neutral lipids (Y= -18.44X + 232.38, r^2=0.338) and
b) 16:1n7 in neutral lipids (Y= -12.57X + 176.66, r^2=0.228)
on survival to swimup in Year 2.

4.9.3 Fatty acid composition

The fatty acid compositions of the diets are shown in Tables 23 and 24 and Appendix 1a. Comparison of the fatty acid composition of the diets and the neutral and polar lipids of eggs are shown in Figures 18-20. The LL and HL diets were similar in most saturated, monoenoic, n6 and n3 fatty acids. Total lipids of the HL diets had significantly more total monoenoic and n11 fatty acids (specifically, 16:1n7, 20:1n11, 22:1n9, 22:1n11), 18:4n3, 20:5n3 and 22:5n3 compared to the LL diets. The HL diets had significantly less 18:2n6, 18:3n3, total n6 and total PUFA than the LL diets. The COMM diet fed in Year 1 differed dramatically in fatty acid composition from that fed in Year 2. Fatty acids in the COMM diet which differed the most between Year 1 vs Year 2 included 16:0 (15.8 vs 12.2%), 20:1n9 (4.5 vs 11.3%), 22:1n11 (5.4 vs 15.5%), 20:5n3 (10.5 vs 5.1%), total saturated (24 vs 18%) and monoenoic fatty acids (35 vs 52%).

Fatty acid composition of neutral and polar lipids of the eggs, at levels above 0.1% of lipid, appear in Appendices 1b and 1c. Selected fatty acids and total values for families of fatty acids present in the neutral and polar lipid fractions are shown in Tables 25-28 with indications of the significance of differences among them.

Table 23. Selected major fatty acids in the total lipid of the diets. Values are % of total lipid.

Fatty acid	Diet						LL[2]	HL[2]
	LLLE	LLHE	HLLE	HLHE	COMM Year 1	COMM Year 2		
	%	%	%	%	%	%	%	%
14:0	4.16	4.11	4.44	4.09	5.14	3.86	4.14	4.27
16:0	16.40	16.43	15.97	15.61	15.77	12.16	16.42	15.79
18:0	2.72	2.69	2.68	2.69	2.53	1.63	2.71	2.69
16:1n7	5.06	5.05	5.51	5.36	7.76	6.97	5.06[a]	5.44[b]
18:1n7	3.70	3.72	3.81	3.62	2.64	2.35	3.71	3.72
18:1n9	18.71	18.60	18.48	18.83	12.44	10.93	18.66	18.66
20:1n9	3.30	3.29	3.52	3.32	4.49	11.32	3.30	3.42
22:1n9	1.01	1.00	1.17	1.16	0.61	1.68	1.01[a]	1.17[b]
20:1n11	3.84	3.77	4.63	5.04	0.43	0.68	3.81[a]	4.84[b]
22:1n11	6.60	6.50	7.49	7.83	5.39	15.52	6.55[a]	7.66[b]
18:2n6	7.46	7.40	4.90	4.97	10.68	10.52	7.43[a]	4.94[b]
20:4n6	0.62	0.62	0.63	0.64	0.39	0.25	0.62	0.64
18:3n3	1.32	1.32	1.00	1.01	1.32	1.01	1.20[a]	1.07[b]
18:4n3	0.88	0.88	1.00	1.00	1.77	1.03	0.88[a]	1.00[b]
20:5n3	5.97	5.92	6.20	6.36	10.45	5.05	5.95[a]	6.28[b]
22:5n3	0.92	0.90	1.03	1.08	1.13	0.61	0.91[a]	1.06[b]
22:6n3	6.77	6.75	6.33	6.58	5.89	4.90	6.76	6.46

[1] Complete fatty acid profile appears in Appendix 1a.
[2] Values within a row which have different superscripts are significantly different ($P<0.05$).

Table 24. Totals for families of fatty acids in the total lipid of the diets. Values are % of total lipid.

Fatty acid type	Diet						LL[1]	HL[1]
	LLLE	LLHE	HLLE	HLHE	COMM Year 1	COMM Year 2		
	%	%	%	%	%	%	%	%
Saturated	24.87	24.88	24.80	24.06	24.26	18.49	24.88	24.43
Monoenoic	44.60	44.27	47.15	47.72	35.35	51.97	44.44[a]	47.43[b]
n5	0.50	0.52	0.55	0.53	0.48	0.63	0.51	0.54
n7	9.54	9.54	10.19	9.86	10.86	10.49	9.54	10.03
n9	24.12	23.94	24.29	24.46	18.19	24.66	24.03	24.38
n11	10.44	10.27	12.12	12.87	5.82	16.19	10.36[a]	12.50[b]
n6	9.04	9.02	6.62	6.69	12.46	11.57	9.03[a]	6.66[b]
n3	16.87	16.81	16.70	17.16	23.04	13.80	16.84	16.93
n3/n6	1.87	1.86	2.52	2.56	1.85	1.19	1.86[a]	2.54[b]
PUFA	11.70	11.70	9.15	9.21	16.33	14.06	11.70[a]	9.18[b]
HUFA	14.36	14.30	14.34	14.80	19.30	11.41	14.33	14.57

[1] Values within a row which have different superscripts are significantly different ($P<0.05$).

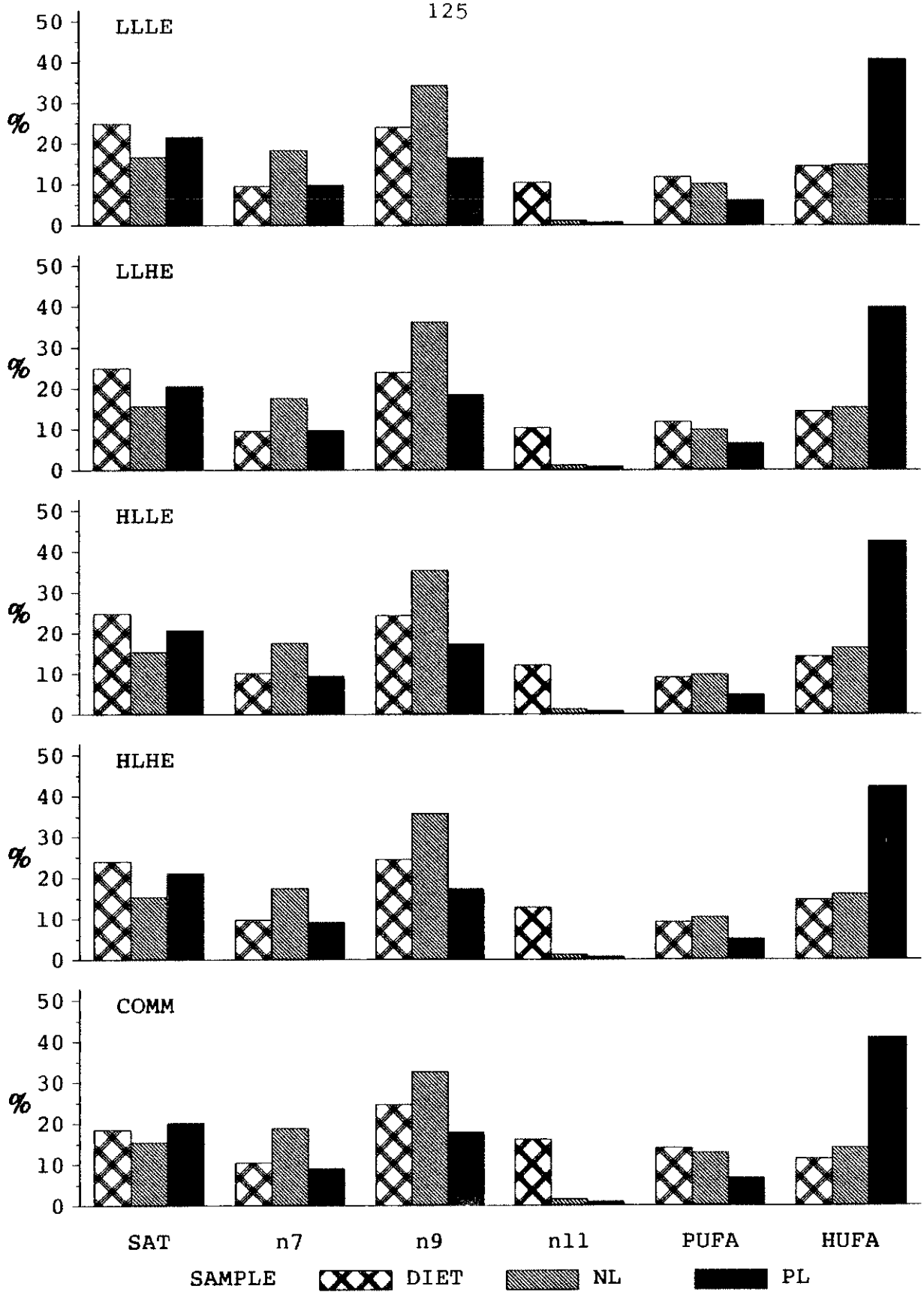


Figure 18. Comparison of saturated, n7, n9, n11 monoenoic acids, PUFA and HUFA of the total lipids of the diets with the neutral(NL) and polar lipids(PL) of the eggs in Year 2.

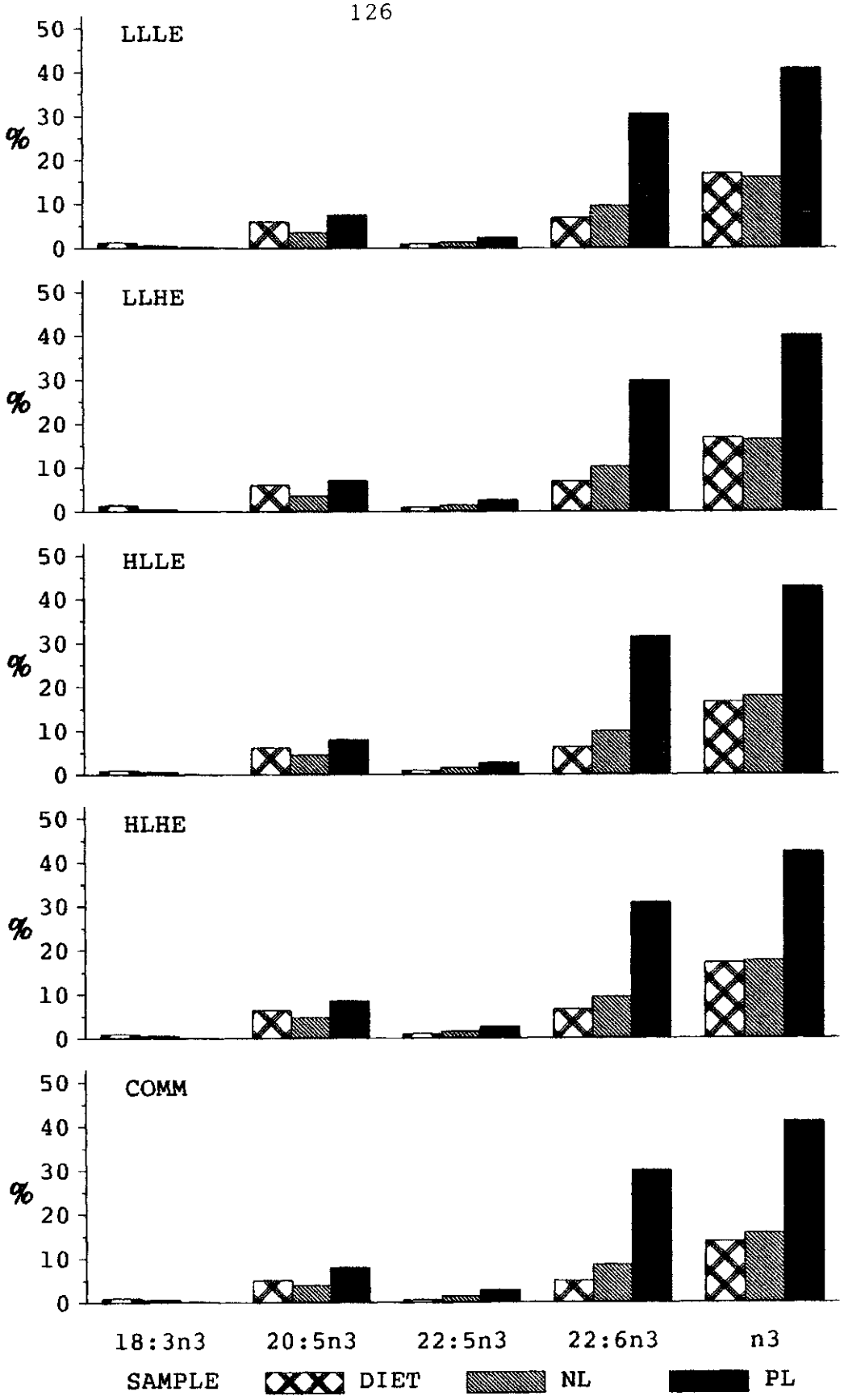


Figure 19. Comparison of n3 fatty acids of the total lipids of the diets with the neutral (NL) and polar lipids (PL) of the eggs in Year 2.

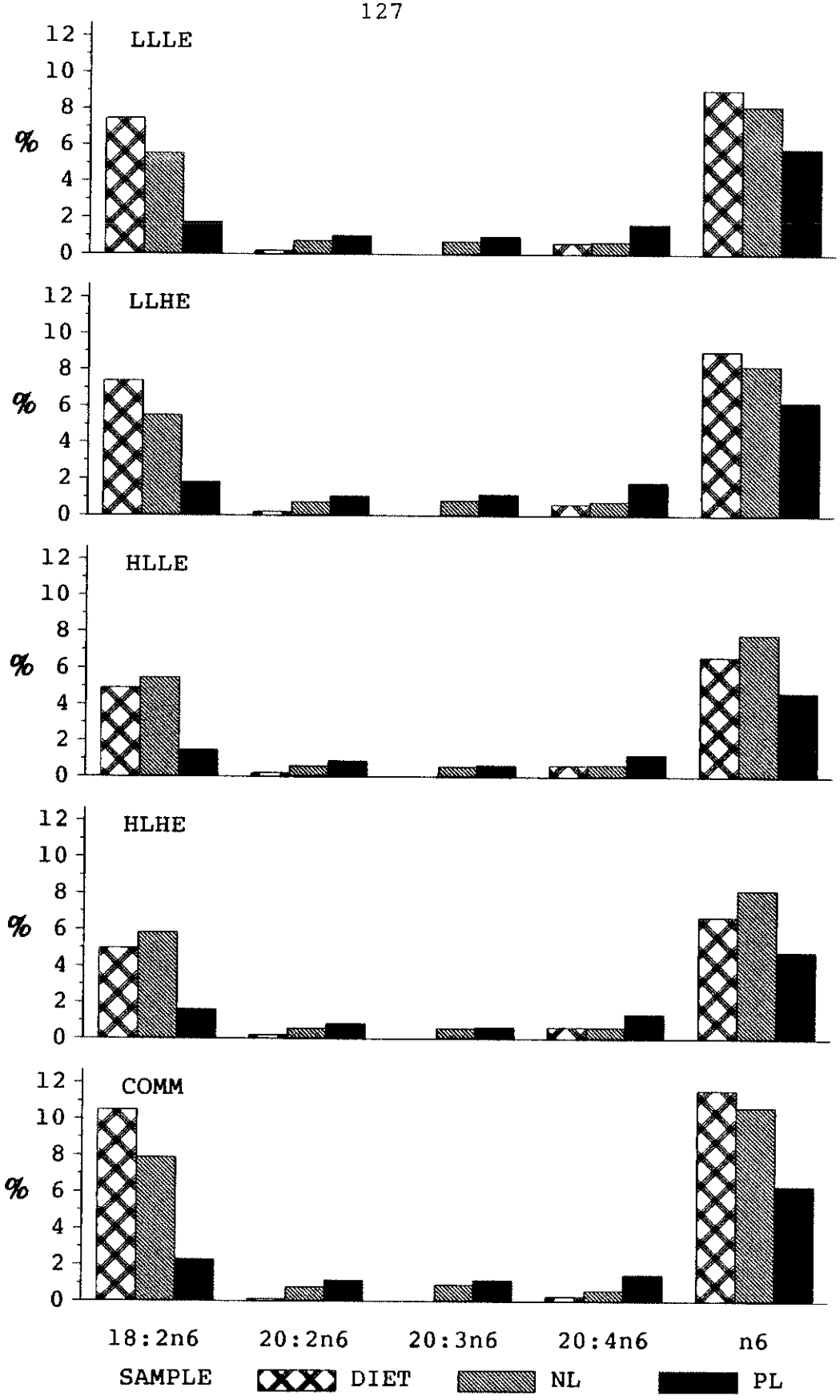


Figure 20. Comparison of n6 fatty acids of the total lipids of the diets with the neutral (NL) and polar lipids (PL) of the eggs in Year 2.

The fatty acid profiles of the neutral lipids show that eggs from fish fed HL diets had significantly more 20:5n3 than eggs from fish fed LL and COMM and more 22:5n3 than those of fish fed LLLE. Eggs from fish fed COMM had significantly higher 18:2n6, 20:1n9, total n6 and PUFA and lower 18:1n9 and n3/n6 than eggs from fish fed other diets. Negative correlations existed between 16:1n7 and total n7 in the neutral lipids and survival to eyed, hatch and swimup stages (r^2=0.23-0.24, P<0.0008) (Figures 15b, 16b, 17b, Appendix 4). The significant probability indicates that the slope was significantly different from zero. Analysis of covariance showed there were no differences between diets.

The fatty acid profiles show that the polar lipids of the HL eggs had significantly less 20:2n6, 20:3n6, total n6 and PUFA than in the LL and COMM eggs. Polar lipids of HLHE eggs had more 20:5n3 than found in LL eggs. There was an effect of dietary vitamin E concentration and an interaction between dietary lipid and vitamin E concentrations in 20:1n9 with LLHE eggs having significantly more 20:1n9 in the polar lipids than LLLE and HLHE eggs. COMM eggs were higher in 18:2n6 and 20:1n9 and lower in total saturated fatty acids in the polar lipids compared to all other treatments.

Table 25. Selected major fatty acids[1] in the neutral lipids of eggs. Values are % of neutral lipid.

Fatty acid	Diet LLLE	LLHE	HLLE	HLHE	COMM
	%	%	%	%	%
14:0	2.20	1.97	2.22	2.07	2.52
16:0	11.81	10.81	10.46	10.64	10.58
18:0	1.53	1.54	1.46	1.43	1.26
16:1n7	11.29	10.89	10.98	11.34	12.65
18:1n7	6.55	6.16	6.03	5.58	5.66
18:1n9	31.06^{a}	32.10^{a}	31.67^{a}	32.40^{a}	27.67^{b}
20:1n9	2.16^{bc}	2.65^{b}	2.27^{bc}	1.91^{c}	3.85^{a}
18:2n6	5.52^{a}	5.50^{a}	5.47^{a}	5.84^{a}	7.88^{b}
20:4n6	0.67	0.76	0.67	0.64	0.59
20:5n3	3.55^{b}	3.46^{b}	4.58^{a}	4.72^{a}	3.92^{b}
22:5n3	1.25^{c}	1.33^{bc}	1.63^{a}	1.51^{ab}	1.38^{bc}
22:6n3	9.50	10.19	9.88	9.49	8.58

Means within a row which share a common superscript are not significantly different.
[1] Complete fatty acid profile is given in Appendix 1b.

Table 26. Totals of families of fatty acids in the neutral lipids of eggs. Values are % of neutral lipid.

Fatty acid	Diet LLLE	LLHE	HLLE	HLHE	COMM
	%	%	%	%	%
Saturated	16.64	15.64	15.31	15.39	15.46
Monoenoic	54.79	55.47	54.89	54.96	53.71
n5	0.62	0.58	0.62	0.58	0.77
n7	18.38	17.60	17.54	17.48	18.81
n9	34.39	36.19	35.37	35.64	32.62
n11	1.07	1.10	1.35	1.26	1.52
n6	8.16[a]	8.26[a]	7.85[a]	8.17[a]	10.65[b]
n3	16.02	16.32	17.91	17.71	15.68
n3/n6	1.98[a]	2.03[a]	2.40[a]	2.21[a]	1.48[b]
PUFA	10.10[a]	9.81[a]	9.86[a]	10.34[a]	12.88[b]
HUFA	14.63	15.28	16.43	16.08	14.17

Means within a row which share a common superscript are not significantly different.

Table 27. Selected major fatty acids [1] in the polar lipids of eggs. Values are % of polar lipid.

Fatty acid	Diet LLLE	LLHE	HLLE	HLHE	COMM
	%	%	%	%	%
14:0	1.14	0.94	0.88	0.97	1.08
16:0	16.49	15.69	15.99	16.43	15.60
18:0	3.34	3.39	3.44	3.26	2.91
16:1n7	2.39	2.57	2.09	2.31	2.45
18:1n7	6.82	6.44	6.59	6.23	6.07
18:1n9	12.39	13.55	12.75	13.35	11.62
20:1n9	3.80^{c}	4.68^{b}	4.29^{bc}	3.64^{c}	6.04^{a}
18:2n6	1.74^{b}	1.80^{b}	1.49^{b}	1.62^{b}	2.29^{a}
20:2n6	1.01^{a}	1.06^{a}	0.89^{b}	0.82^{b}	1.15^{a}
20:3n6	0.99^{a}	1.15^{a}	0.62^{b}	0.62^{b}	1.15^{a}
20:4n6	1.63	1.79	1.24	1.37	1.48
20:5n3	7.46^{b}	7.05^{b}	8.10^{ab}	8.58^{a}	7.96^{ab}
22:5n3	2.32^{b}	2.58^{ab}	2.76^{a}	2.57^{ab}	2.82^{a}
22:6n3	30.51	29.86	31.38	30.87	29.99

Means within a row which share a common superscript are not significantly different.

[1] Complete fatty acid profile is given in Appendix 1c.

Table 28. Totals of families of fatty acids in the polar lipids of eggs. Values are % of polar lipid.

Fatty acid	Diet LLLE	LLHE	HLLE	HLHE	COMM
	%	%	%	%	%
Saturated	21.62	20.57	20.82	21.20	20.15
Monoenoic	27.60	29.48	28.20	27.86	28.61
n5	0.49	0.45	0.53	0.52	0.60
n7	9.89	9.72	9.43	9.17	9.12
n9	16.56	18.55	17.40	17.38	17.94
n11	0.65	0.77	0.86	0.79	0.95
n6	5.83[a]	6.25[a]	4.67[b]	4.81[b]	6.33[a]
n3	40.79	39.93	42.73	42.56	41.25
n3/n6	7.08[a]	6.42[a]	9.37[b]	8.93[b]	6.55[a]
PUFA	6.07[a]	6.42[a]	4.92[b]	5.13[b]	6.68[a]
HUFA	40.66	39.86	42.59	42.35	40.98

Means within a row which share a common superscript are not significantly different.

4.9.4 Selenium

Selenium concentrations ranged from 1.38-3.09 µg/g in the eggs in Year 1 and 1.70-3.58 µg/g in Year 2 on a dry matterbasis. Selenium concentrations were not influenced by dietary treatment and had no significant effect on embryonic survival.

4.9.5 Composition of wild Arctic charr eggs

Eggs which had been collected from wild stocks at Tree River, NWT were analyzed when they were close to hatching. Eggs weighed 122 mg and contained 73.8 µg vitamin E/g (9.0 µg/egg) and 3.97 µg selenium/g. Survival rates were excellent with 94% eyed and 87% swimup. Tree River eggs contained 67.3% dry matter, 22.7% total lipid, 11.9% neutral and 10.8% polar lipids on a dry matter basis. Compared to the Labrador eggs in this experiment, neutral lipid from the Tree River eggs contained 22:5n3 and less 18:1n9 and 18:2n6 (Tables 29 and 30). Overall, neutral lipids contained less HUFA and more PUFA, less n6 and more n3 resulting in a much higher n3/n6 ratio. The monoenoic fraction contained more n7 and less n9 fatty acids. Differences in the fatty acid profile of the polar lipid fraction between the Tree River andexperimental eggs were much less pronounced compared to the neutral fraction. Tree River eggs had more 18:0, 20:5n3, 22:5n3, total n3, n7 and an increased n3/n6 ratio, but less 18:1n9, 22:6n3, total n6 and n9.

Table 29. Selected major fatty acids[1] of the neutral and polar lipid of eggs of Arctic charr from Tree River, NWT.

Fatty acid	Neutral lipid	Polar lipid
	%	%
14:0	2.42	0.88
16:0	9.66	15.23
18:0	1.88	4.95
16:1n7	17.71	3.46
18:1n7	7.92	8.53
18:1n9	21.28	10.62
20:1n9	1.61	3.07
18:2n6	2.15	0.55
20:4n6	0.48	0.82
20:5n3	11.74	12.66
22:5n3	4.27	6.21
22:6n3	9.59	25.50

[1] Complete fatty acid profile is given in Appendix 1d.

Table 30. Totals of families of fatty acids of the neutral and polar lipid of eggs of Arctic charr from Tree River, NWT.

Fatty acid	Neutral lipid	Polar lipid
	%	%
Saturated	14.57	21.53
Branch	0.24	0.27
Monoenoic	50.56	27.94
n5	0.84	0.79
n7	26.02	12.63
n9	23.35	13.92
n11	0.35	0.60
n3	28.17	45.07
n6	3.71	2.41
n3/n6	7.59	18.70
PUFA	6.36	2.97
HUFA	25.94	44.61

4.10 Fry survival and growth

When different numbers of fry were raised in the 1 L jars described in Section 3.9.2, the specific growth rate decreased as the number of fry increased. It was significantly lower for 100 fry compared to 10 and 25 fry per jar (Table 31). In addition, the specific growth rate was significantly greater (2.68% body weight/day) when 300 fry were reared in 60 L tanks compared to any number reared in the jars. Although there were no significant differences in survival, cannibalism was observed in the jars containing 100 fry. Based on these results, the number of fry raised in the jar system was decreased from 50 per jar in Year 1 to 25 per jar in Year 2.

There were no effects of diet on specific growth rate or survival of fry in Year 1 (Table 32). Data for HLLE was excluded from all analyses of variance (Table 33) because only 2 lots of eggs in the HLLE group produced sufficient fry for use in the Year 2 growth trial. The specific growth rate was significantly lower for fry from the LLHE group than all other treatments. Overall survival of fry decreased from 82% in Year 1 to 76% in Year 2 which was similar to the decrease from 88% to 79% that was predicted from the decrease in the number of fry raised in the jars. With the exception of the LLHE group, the specific growth rate increased in Year 2 from 1.7% in Year 1 to 2.4% in Year 2 which was greater than the predicted 0.4% increase resulting from the decrease in number of fry in the

Table 31. Fry survival and specific growth rate when fry were stocked at different numbers (mean ± standard deviation of 2 replicates).

Rearing unit	No. of fry per unit	Initial weight	Final weight	Specific growth rate	Survival
		mg ± SD	mg ± SD	% body wt/day ± SD	% ± SD
1 L jar	10	49 ± 1.1	110[b] ± 14.6	1.93[b] ± 0.37	72.5 ± 3.5
	25	47 ± 2.3	103[b] ± 0.7	1.87[b] ± 0.10	79.0 ± 9.9
	50	49 ± 2.5	91[b] ± 5.8	1.47[bc] ± 0.03	87.5 ± 10.6
	100	50 ± 1.6	81[b] ± 14.0	1.11[c] ± 0.34	74.5 ± 17.0
60 L tank	300	51 ± 2.3	157[a] ± 13.3	2.68[a] ± 0.07	83.2 ± 1.1

Means within a column which share a common superscript are not significantly different.

Table 32. Egg and fry weight, survival and specific growth rate (mean ± standard deviation) of fry for 6 weeks at 10°C in Year 1.

Diet fed to parents in Year 1	n	Egg weight[1]	Initial fry weight	Specific growth rate	Survival[2]
		mg ± SD	mg ± SD	% body wt/day ± SD	% ± SD
LLLE	10	54 ± 8	56 ± 11	1.70 ± 0.37	86.6 ± 5.7
LLHE	7	59 ± 10	60 ± 7	1.69 ± 0.33	83.0 ± 9.2
HLLE	12	57 ± 5	59 ± 5	1.74 ± 0.39	80.1 ± 8.7
HLHE	8	53 ± 7	58 ± 7	1.78 ± 0.28	80.3 ± 8.6
COMM	8	53 ± 6	55 ± 5	1.69 ± 0.30	80.5 ± 10.1
		Significance level from analysis of variance			
Source of variation					
All diets		ns	ns	ns	ns
Excluding COMM					
Lipid		ns	ns	ns	ns
Vitamin E		ns	ns	ns	ns
Lipid x Vitamin E		ns	ns	ns	ns

[1] Weight of eggs resulting in fry used in this growth trial.
[2] Arcsin transformation used prior to analysis of variance.
ns = not significant, P>0.05

Table 33. Fry survival and specific growth rate (mean ± standard deviation) for 6 weeks at 10°C in Year 2.

Diet fed to parents in Year 2	n	Egg weight[1]	Initial fry weight	Specific growth rate	Survival[2]
		mg ± SD	mg ± SD	% body wt/day ± SD	% ± SD
LLLE	6	57 ± 6	64 ± 7	2.91[a] ± 0.49	78.0 ± 7.8
LLHE	4	57 ± 4	55 ± 7	1.84[b] ± 0.58	68.0 ± 10.5
HLLE[3]	2	70 ± 15	67 ± 16	2.94 ± 0.34	71.0 ± 21.2
HLHE	6	61 ± 11	66 ± 9	3.10[a] ± 0.21	77.0 ± 22.2
COMM	7	56 ± 7	65 ± 10	3.00[a] ± 0.20	86.6 ± 4.9
		Significance level from analysis of variance			
Source of variation					
All diets		ns	ns	**	ns

[1] Weight of eggs resulting in fry used in this growth trial.
[2] Arcsin transformation used prior to analysis of variance.
[3] Data for HLLE was excluded from the analysis of variance because the sample size was too low.
Means within a column which share a common superscript are not significantly different.
ns = not significant, P>0.05, **=P<0.01

jars. Environmental conditions were as similar as possible in both Year 1 and 2. Feed was purchased from the same company and analysis showed the proximate composition was 39.7% crude protein, 25.5% total lipid, 11.8% ash and 82.6% dry matter in Year 1 and 49.7% crude protein, 20.0% total lipid, 11.8% ash and 80.6% dry matter in Year 2. The differences in protein and lipid and/or changes in the ingredients used by the manufacturer in Year 2 may also have contributed to the differences in specific growth rate.

DISCUSSION

5.1 Growth of broodstock and feeding activity

In the wild, Arctic charr have the potential for a high rate of growth in a short period. They migrate to sea in May and early June, feed heavily in their short time at sea returning to fresh water in the latter half of July and August (Dempson and Greene 1985). They feed heavily while at sea and fish of all sizes have high specific growth rates during this short period (Johnson 1980). This indicates that they have the potential for a high rate of growth at least for this short period of time at sea.

There is little documented information against which to compare the growth rates achieved in this experiment. At this stage of life, growth rates are seldom monitored in hatcheries. Growth data collected on wild stocks are generally collected as length rather than weight data. Dempson (1982) showed that mark-recaptured 8-year old Labrador Arctic charr had increased in length by 52 mm (455 to 507 mm) in one year. In the current experiment, 4-year olds increased 23 to 38 mm (414 to 445 mm) in the 71 day feeding period in Year 1. In Year 2, charr fed the experimental diets increased from 42 mm (443 to 485 mm) which is slightly less than the increase found by Dempson (1982) while those fed COMM increased 76 mm (453 to 529 mm).

In Year 1, female fish fed the LL diets had significantly lower specific growth rates than those fed the HL and COMM diets. This effect of dietary lipid level was also observed when Nauyuk Lake Arctic charr fingerlings were fed diets containing 10% lipid compared to 15 and 20% lipid at dietary protein levels of either 34, 44 or 54% (Tabachek 1986). Dietary levels of 10% lipid and/or 34% protein not considered adequate to meet the growth requirements of Arctic charr fingerlings. There was a crossover in diets at the beginning of Year 2 and fish fed LL diets in Year 1 were fed HL diets in Year 2 and vice versa. Since mean weight of fish fed each diet differed (although not significantly) at the time of the crossover in diets, comparisons of the growth rates in Year 2 should be viewed with this in mind. Specific growth rates declined 20-51% from Year 1 for females and 24-39% for males fed 4 of the 5 diets and 4% for males fed HLLE.

Labrador Arctic charr have been shown to display high variability in growth rate during juvenile stages of life (Papst and Hopky 1983, 1989). This wide variation in growth rate and the small population size resulted in a wide variation in the size of fish at the beginning of the experiment. A portion of the male and female populations in each tank lost weight during some period or throughout the entire experiment. Although a particularly high proportion of males fed HLLE in Year 2 lost weight, other males in that tank had specific growth rates

equivalent to those of fish fed other diets. The high proportion of males which lost weight in that tank may have been affected by the presence of one aggressive male. Six out of 10 males and 5 out of 11 females continued to lose weight after removal of the aggressive fish. It is not known if this was caused by the after-effects of the aggressive fish or by the diet or some other unknown factor. Since aggressive fish were not observed in other tanks, some additional factor(s) must be considered.

The tanks used to raise the broodstock had been in use for many years. The position of the four tanks used for the four experimental diets were similar, all being along one wall and along the same catwalk. The building had limited access and staff were near these tanks only when they were feeding or spawning fish or maintaining the tanks. The position of the tank used for the COMM diet was in the opposite row and was not along the same catwalk. This tank may have had more disturbance since it was not along a wall and there were other tanks opposite from it where feeding and/or spawning occurred at different times from the other four tanks. Other than the problems with temperature differences between tanks during the first 9 days of the experiment, there were few differences in temperature, water chemistry and lighting between the tanks.

Fish gradually reduced their feed consumption as spawning

approached in Year 1. Feed was withheld from 14 days prior to spawning until all spawning was complete which resulted in feed being withheld for a total of 120 days. Whether this long period of feed withdrawal influenced their resumption of feeding in Year 2 is not known. It is a common practice to withdraw feed during spawning or for several days prior to spawning during each week of the spawning season. This is done to reduce the chance of fecal material contaminating the eggs and milt when males and females are stripped. At RARC, feed is withheld for 2 weeks prior to and during the entire spawning period. However, there are generally sufficient tanks available so that spawned fish can be placed into a tank and feed can be offered when fish are ready to resume feeding. Additional tanks were not available for this experiment. Records of the number of emaciated fish in the nonexperimental group (fish from the same stock as the experimental groups) were not kept but there was evidence that emaciated fish existed and that they were routinely culled during the annual sampling for certification purposes (Olson, personal communication).

In the wild, post-spawning charr consume little if any food in fresh water and hence they lose weight although they continue to increase in length (Johnson 1980). Condition factor, which is a measure of the plumpness of the fish, decreased from 0.97-1.00 in the prespawning run to 0.67-0.74 during the same fish's post-spawning migration the following summer. In the present

experiment, male and female charr that had lost weight had condition factors of 0.81-0.96 and 0.71-0.96, respectively, prior to spawning in Year 2, compared to condition factors of 1.08-1.48 and 1.35-1.73 for males and females that had gained weight. Fish that had lost weight generally produced few, if any, gametes and eventually these emaciated fish, referred to as "slinks", were expected to die of malnutrition. Regarding the weight gain of post-spawning "slinks" when they return to sea, Johnson (1980) states: "The recovery of emaciated charr is phenomenal when food is available." Therefore, one expects that they should be able to resume feeding and recover after their period of spawning and starvation. The failure of Labrador Arctic charr to resume feeding after spawning has been reported by other producers (Van Toever, personal communication). Broodstock survival and productivity are of importance to producers and techniques and/or diets which enhance the resumption of feeding after spawning need to be investigated.

Labrador charr broodstock consumed more COMM diet than the experimental diets and more HL compared to LL diets but the effects of dietary lipid on feed intake could not be analyzed statistically. In research with Nauyuk Lake Arctic charr fingerlings, dietary lipid did not influence feed intake (Tabachek 1986). One explanation regarding the higher intake of the COMM diet is that fish were more accustomed to this diet and therefore consumed it more readily. Since other Labrador Arctic

charr broodstock at RARC have adjusted well to a change in diet from the dry COMM diet to a semi-moist product manufactured by Bioproducts, it is unlikely that broodstock would not accept a "new" feed. COMM may have had a different flavour than the other diets and COMM did have a harder texture than the experimental diets, with the HL diets having the softest consistency.

Orr *et al.* (1982) and Ridelman *et al.* (1984) reported that starvation of rainbow trout for 40 days prior to spawning had no effect on fecundity, egg weight, diameter, proximate composition or hatchability. The effect that starvation prior to spawning had on resumption of feeding was not investigated. Ashton (1991) found that starvation for 7-14 days prior to spawning had no effect on the lipid content of eggs, condition factor or survival to the eyed stage in chinook salmon.

The general decline in feeding activity in Year 2 compared to Year 1 was observed in fish fed all diets including COMM. The experimental diets were manufactured at about 11 week intervals. Ingredients and feeds were kept frozen, as described in Section 3.2.2 and 3.5, and feeds were stored at room temperature only during the week they were fed. COMM used in Year 2 was from a later shipment than that used in Year 1. Hung *et al.* (1980) recommended using peroxide value to measure the oxidation of fish oils. Peroxide value showed that herring oil,

stored in a frozen state and flushed with nitrogen, had not deteriorated during storage over the length of the experiment. Off-flavours, that might have reduced feed consumption in all diets in Year 2, are not expected to have developed.

5.2 Relation of fecundity to ration and size of the female

The fecundity of stocks of Arctic charr from different locations differ widely both in the wild and in captivity. Johnson (1980) presents data on several stocks of wild charr of different ages which vary in mean fecundity from 2000-4954. At RARC, stocks of charr from Norway, Nauyuk Lake in NWT and Fraser River in Labrador produce 3000, 1200-2200 and over 3000-4000 eggs, respectively (Tabachek and de March 1990). Therefore, the data provided by Dempson (1982) and Dempson and Green (1985) in their sampling of Arctic charr from the Fraser River in Labrador provide very useful comparisons of wild and captive stocks which originated from the same location. Fecundity of fish spawned in Labrador, which were 7-13 years old (weight = 2.14 kg, length = 55.3 cm) ranged from 2316-9245 eggs (mean = 5242) (Dempson 1982). In the present experiment, the number of eggs released at spawning ranged from 763-7455 in Year 1 and from 327-13429 in Year 2 with the latter extending the range of Dempson (1982). Fish fed COMM released the highest number of eggs in both years, with ranges of 2183-6525 (4532 ± 1596) [mean ± standard deviation]) in Year 1 and 4047-13429 (8305 ± 3313) in Year 2. While the variance is high, 34 and 85% of this variance was

accounted for by the strong correlation between fecundity and fish weight in Year 1 and 2, respectively. Although females fed COMM had significantly higher fecundity than fish fed all other diets except LLLE in Year 2, they weighed more and did not have a significantly higher relative fecundity. Jones and Bromage (1987) showed that fecundity depended both on ration and fish weight. It is expected that the higher feed intake of fish fed COMM led to their higher weight which was related to their higher fecundity than those fed other diets. Initially, the population of charr at RARC was divided in half with one group allocated for the experiment and the remainder for the non-experimental group which were maintained as usual. Although the non-experimental group was fed COMM (although it may have been from different shipments for part of the time), fecundity did not reach the same high numbers as observed in the experimental group fed COMM. The non-experimental group had a mean fecundity of 3300 in Year 1 and 3900 in Year 2. The higher fecundity of the experimental group fed COMM was probably due to their being fed to satiation.

Jones and Bromage (1987) suggested an optimum ration of 0.75-1.0% body weight/day at 11.7°C for rainbow trout broodstock. In the current experiment, Arctic charr were fed to satiation but ration could be estimated from feed fed (dry feed fed, g x 100)/(no. days fed x (average body weight, g). When fed at water temperatures of 7.5-8.5°C, ration in Year 1 was

approximately 0.77-0.88% for the LL and HL diets and 0.98% body weight/day for COMM. In Year 2, this decreased to 0.31% for fish fed the LL diets and 0.50% and 0.42% for those fed the HL and COMM diets, respectively, which were much lower than the optimum ration suggested for rainbow trout of 0.75-1.0%.

Dempson and Green (1985) showed that there was a relationship between fecundity and fork length of wild Labrador charr. These authors also estimated fecundity to be 2450 eggs/kg body weight at 7-13 years of age. McGeachy and Delabbio (1989) showed that first-time spawning 4-year olds (mean weight=968 g) raised in captivity had a relative fecundity of 3850 eggs/kg. In the present experiment, fish released an average of 2842-3200 eggs/kg body weight in Year 1 (4-year olds) and 2112-3098 in Year 2 (5-year olds). Both ranges were lower than expected based on the findings of McGeachy and Delabbio (1989). The improvement in the correlation of fecundity and fish weight from 34% in Year 1 to 85% in Year 2 may be related to the fact that fish were fed to satiation for the entire season in Year 2 whereas they were fed to satiation for only 71 days prior to spawning in Year 1. Scott (1962) showed that restricted feed intake resulted in an increased proportion of atretic follicles. Springate *et al.* (1985) reported that feeding rainbow trout at full ration (0.7% body weight/day) resulted in significantly higher levels of vitellogenin in females and testosterone in males at spawning compared to those

fed at half-ration (0.35%). Significantly more atresia occurred in fish fed at half-ration. Females fed at full ration produced significantly more and larger eggs but had lower relative fecundity (eggs per body weight) and these findings were confirmed by Orr *et al.* (1982). During the period prior to the current experiment, it is possible that some fish may have received a smaller proportion of the ration than others. If ration was insufficient during the period prior to the experiment, eggs may have started to become atretic prior to the experiment. This may partially account for the fact that the data were more scattered about the regression line in Year 1.

5.3 Spawning time and proportion of fish that spawned

Arctic charr in Labrador (Dempson 1982) spawned in a 3-week period in October which peaked in the middle of October. In the current experiment, charr spawned over a much longer period of time. For example, they spawned over 14 weeks in Year 1 and 11 weeks in Year 2 peaking in the middle of November. This long spawning period is characteristic of Arctic charr raised at RARC from three collections in Labrador. In contrast, stocks of Arctic charr from Nauyuk Lake, NWT and Norway spawn earlier than the Labrador stocks and spawn within 4-6 weeks (Olson, personal communication). While dietary lipid had an effect on spawning time in Year 1, with fish fed LL diets spawning 5 weeks later than those fed HL diets, this pattern was not repeated in Year 2. It is assumed that this effect was not consistent or that

there was no direct effect of lipid on spawning time. Delayed spawning was not associated with diets that had low concentrations of vitamin E as it was with rainbow trout (Takeuchi *et al.* 1981b). Fish fed COMM spawned in 8 weeks in Year 1 and 6 weeks in Year 2. Roley (1983) found that spawning was delayed but occurred within a shorter period of time when rainbow trout were fed to satiation. In contrast, Arctic charr in the non-experimental group, fed at a lower ration, spawned 3-4 weeks later but over the same length of time compared to the population in the experiment in both years.

In Year 1, there was a small positive effect of spawning day on fertilization. Since this regression accounted for only 14% of the variance, it is expected that additional factors are responsible for the variation in fertilization. In Year 2, there was an negative effect of spawning time on fecundity, with those spawning late in the season producing the smallest numbers of eggs.

Arctic charr are iteropareous and may spawn several times during their lifetime in the wild although the frequency of spawning may decrease with increasing age (Johnson 1980). Charr in this experiment had spawned the year prior to the experiment. This iteropareous tendency makes it difficult to evaluate the effect of diet on the proportion of the population which spawned. The variance in this parameter is naturally high and

results in the need for large sample sizes to increase the power of the test (Dixon and Massey 1969).

While all fish spawned in Year 2 that had not spawned in Year 1, a portion of the population that spawned in Year 1 failed to spawn in Year 2. Dietary vitamin E had no significant effect on the proportion of females that spawned. This is in contrast to rainbow trout (King 1985) and ayu (Takeuchi *et al.* 1981a) where a portion of the population failed to spawn when fed diets with no vitamin E supplementation. The fact that similar proportions of the population fed the experimental diets failed to spawn in Year 2 supports the fact that charr do not all spawn every year. Continued studies with large populations of tagged Arctic charr and good record-keeping can ascertain if there are patterns in their spawning.

Roley (1983) showed that feeding level had no effect on the number of rainbow trout that spawned while Scott (1962) showed that increased feed intake increased the number of females that spawned. The proportion of Arctic charr that spawned in Year 2 was lower than in Year 1 even though they had been fed to satiation for all of Year 2.

Not all males matured and produced milt (ie. ripened) each year and 9 out of 59 males failed to mature in either year. Similar proportions of males became ripe when fed the LE versus

HE diets in Year 1 and Year 2. However, a significantly higher proportion (12/16) of males fed HE diets produced milt in two consecutive years compared to those fed LE diets (4/11). This area warrants further investigation with larger numbers of fish and with replicate tanks of fish. There are reports of vitamin E deficiency resulting in sterility in male rats, guinea pigs, hamsters, dogs and chickens (Scott *et al.* 1982) through its effect on the cell membranes of the seminiferous tubules. Male poultry were not affected by vitamin E deficiency unless they were fed the deficient diet for a long period of time or they were fed diets containing oxidized unsaturated oils (Jensen 1968). There are no reports of male infertility in fish resulting from a vitamin E deficiency. Watanabe *et al.* (1984b) found that the fatty acid composition of milt was affected by essential fatty acid deficiency but suggested further research was required to determine if this would have an effect on fertilization or hatchability.

5.4 Egg size

No significant relationship was found between fecundity and egg diameter in chinook salmon (Ashton 1991) or between fecundity and egg weight or diameter with Arctic charr in the present experiment. In contrast, Roy and Higgs (1987) reported an inverse relationship between egg weight and fecundity in chinook salmon (Roy and Higgs 1987).

There was a direct relationship of egg weight on survival in Year 2 but the low r^2 shows that the regression accounts for only 11-12% of the variance in survival. In addition, the slope of the line is so low that a 1 mg increase in egg weight would result in a 0.75% increase in fertilization and less than 1.5% increase in survival to all stages. Although no significant differences between diets were found, the greatest difference between means was 7 mg which translates into an increase of less than 10.5% in survival.

The diameter of eggs from charr sampled in Labrador was 4.5 mm (Dempson and Green 1985) which was smaller than those in Year 1 (4.94-5.15 mm) or Year 2 (4.90-5.13 mm) in this experiment.

5.5 Variation in embryonic survival

Labrador Arctic charr commonly produce eggs which exhibit wide variation in hatchability. de March (1992) showed that hatchability of Labrador Arctic charr was under maternal influence and was not affected by the source of the sperm. Therefore, its seems unlikely that survival was influenced by the male. The milt from two males was used to fertilize the eggs of each female to reduce the chance of a male effect. Records were maintained regarding the number of times each male was used and an attempt was made to equalize the number of times each male was used over the course of the long spawning seasons.

Variation in embryonic survival is not expected to be related to the method of incubation. A few replicates groups of eggs were incubated each year and no difference was found in survival between replicates. In addition, in Year 2, if more eggs were spawned than were required for the experiment, the excess eggs were incubated in compartmentalized Heath trays. Survival to the eyed stage was almost identical to that of the eggs incubated in individual jar incubators.

Springate *et al.* (1985) found no significant difference in hatchability or viability of fry from rainbow trout fed at half-ration compared to full-ration. However, Roley (1983) found that feeding to satiation increased the variation in hatchability and decreased survival at cool water temperature. Fish in the non-experimental groups, fed at a lower rate, produced eggs with the same mean survival to the eyed stage and the same variance as those fed COMM in this experiment. Since there was wide variation in the hatchability of Labrador Arctic charr, the effect of ration on hatchability is an area that warrants further investigation.

5.6 Lipid and fatty acid composition of eggs

The essential fatty acid requirements of Arctic charr broodstock are not known. The requirements of fingerlings are met when diets contain 1.7% 18:3n3 and 0.5% 18:2n6 (Yang and Dick 1992). The diets used here contained exceeded the

requirements of fingerling Arctic charr with LL diets containing 2.0% n3 and 1.1% n6 and HL diets containing 3.2% n3 and 1.2% n6. Takeuchi and Watanabe (1979) found that the growth of juvenile rainbow trout decreased when fed diets containing 2-4% n3 fatty acids. The effect of excess n3 fatty acids on Arctic charr is not known. COMM contained 3.6% n3 and 2.0% n6 in Year 1 and 2.5% n3 and 2.1% n6 in Year 2. COMM used in Year 2 was higher in 20:1n9 and 22:1n11 and lower in 20:5n3 than that used in Year 1. Atlantic herring oil contains more 22:1 (15-30%) than Pacific herring oil (10%) (Ackman 1982). Capelin oil is high in 20:1 and 22:1 while low in 20:5n3 and 22:6n3 (Sargent *et al.* 1989). Pacific herring oil may have been used in manufacturing COMM in Year 1 and Atlantic herring oil or a mixed fish oil containing a high proportion of capelin oil was used in Year 2.

Labrador and Tree River Arctic charr eggs contained 10-12% neutral lipid and 11-12% polar lipid. Eggs from wild as well as two strains of cultured chinook salmon had almost twice the concentration (18-19%) of neutral lipids and slightly more polar lipids (13-14%) (Ashton 1991) compared to Labrador and wild Tree River Arctic charr. Kaitaranta (1982) observed that neutral lipids made up over 50% of the total lipid of rainbow trout eggs. It is unfortunate that many authors who report fatty acid analyses on the neutral and polar lipid fractions of eggs fail to provide the percent of neutral and polar lipids that they observed. There is little information reported in the

literature for comparison of neutral and polar lipid contents of eggs in other salmonids. Craik and Harvey (1984) found that rainbow trout eggs with greater than 50% hatch had significantly more total lipid (25.7%) compared to those with 0% hatch (24.5%). Neutral lipids are utilized as energy sources throughout embryogenesis, especially in the later stages just prior to hatching (Sargent *et al.* 1989). While most polar lipids are incorporated into membranes, both triacylglycerol and phosphatidylcholine were utilized throughout embryogenesis by Atlantic salmon (Henderson and Tocher 1987).

Polar lipids contained higher levels of saturated fatty acids and HUFA and lower levels of monoenoic fatty acids than neutral lipids. Monoenoic acids, 16:1n7, 18:1n7, 18:1n9 and 20:1n9, were incorporated into both the neutral and polar lipids of the eggs, with high levels of 16:1n7 and 18:1n9 in the neutral lipids and 18:1n9 in the polar lipids. These two fatty acids can be synthesized *de novo* or obtained from the diet (Figure 1) (Castell 1979, Henderson and Sargent 1985). There was little incorporation of 22:1n9, 20:1n11 or 22:1n11 into either the neutral or polar lipids of eggs even though the two n11 fatty acids constituted 10.4-12.5% of the total lipid in the LL and HL diets. This was also the case for chinook salmon (Ashton 1991). According to Henderson and Sargent (1985), even when 22:1n11 is a major dietary fatty acid, it is not incorporated into the polar lipids of fish membranes and may

function mainly as an energy source through mitochondrial β-oxidation. Olsen *et al.* (1991) fed juvenile Arctic charr a diet containing 15.5% lipid with 12% of this lipid as 22:1n11 and found it made up only 1.2 and 1.8% of polar lipids of the liver and muscle, respectively and 6.4 and 12.3% of neutral lipids.

The major n6 fatty acid in the neutral lipids of eggs was 18:2n6 (28-32%) while there were low levels of 18:2n6 and 20:4n6 (1.2-2.3%) in the polar lipids and slightly less 20:2n6 and 20:3n6. Ashton (1991) found similar findings for neutral lipids of chinook salmon eggs although she observed higher levels of 20:4n6 than 18:2n6 in the polar lipids and minor amounts of 20:2n6 and 20:3n6. The findings with charr were in contrast to Atlantic salmon (*Salmo salar*) eggs, where 18:2n6 was the major n6 fatty acid in the neutral lipids and 20:4n6 was the major n6 fatty acid in the polar lipids (Cowey *et al.* 1985).

Egg polar lipids from the LL groups contained significantly more 20:2n6, 20:3n6, total n6 and PUFA compared to those from the HL groups. This increase in n6 fatty acids in egg lipids with increased dietary n6 is in agreement with Yu *et al.* (1979) and Watanabe *et al.* (1984b). Since the major dietary n6 fatty acid was 18:2n6 with only 0.6% 20:4n6, it appears that dietary 18:2n6 had undergone chain elongation and desaturation to 20:2n6 and 20:3n6 while 20:4n6 may have been from the diet or from desaturation of 20:3n6. Desaturation and elongation of the n9,

n6 and n3 series of fatty acids occurs through a common enzyme pathway but with specific enzymes for the desaturation of each position (Figure 1). In mammals, the desaturase enzymes have a greater affinity for the most unsaturated fatty acid available (Leray and Pelletier 1985). That is, Δ6-desaturase has a greater affinity for 18:3n3 than 18:2n6 which has a greater affinity than 18:1n9. Similar desaturases exist for longer chain molecules. Both Olsen *et al.* (1991) and Yang and Dick (1992) found that Arctic charr fingerlings could desaturate and elongate dietary 18:3n3 to n3HUFA and 18:2n6 and 20:4n6 to n6PUFA. The former authors observed that n3 fatty acids were elongated and desaturated in preference to n6 fatty acids. While HUFA, essential to maintaining membrane fluidity, made up 40-42% of the fatty acids in the polar lipids, they were present at much lower levels (14-17%) in the neutral lipids of the eggs. This is in agreement with data on rainbow trout (Watanabe *et al.* 1984b, Leger *et al.* 1985) and chinook salmon (Ashton 1991) eggs. Total lipids of the LL and HL diets contained approximately 6-7% 20:5n3 or 22:6n3 and there were minor amounts of 18:3n3, 18:4n3, 20:3n3, 20:4n3 and 22:5n3. While egg lipids contained less than 1% of the 18 carbon n3 fatty acids, 22:6n3 was incorporated into egg lipids at levels that were 2-5 times higher than present in the diets. Some of this highly unsaturated fatty acid probably came directly from dietary 22:6n3 while the remainder was synthesized by desaturation and elongation of the less highly unsaturated n3 fatty acids.

Dramatic differences between the fatty acid composition of COMM compared to the LL and HL diets were reflected in the composition of the eggs. The most striking differences consisted of COMM's being lower than the LL and HL diets in 16:0, 18:1n9 and 20:1n11 and higher in 18:2n6, 20:1n9 and 22:1n11. This was reflected in eggs from fish fed COMM having significantly less 18:1n9 in the neutral lipids and significantly more 20:1n9 and 18:2n6 in both the neutral and polar lipids. As was found with eggs from fish fed LL and HL diets, there was little incorporation of n11 fatty acids into the eggs of fish fed COMM even though total lipid contained 15.5% as 22:1n11.

Yang and Dick (1992) showed that Arctic charr produced 20:3n9 when either 18:3n3 and/or 18:2n6 were deficient. While 20:3n9 was not found in the eggs of rainbow trout fed diets lacking in n3 fatty acids for 3 months (Watanabe *et al.* 1984b) or one year (Leray *et al.* 1985) prior to spawning, it was found in the milt after 3 months (Watanabe *et al.* 1984b) of feeding. When trout were fed diets containing 18:3n3 alone or in combination with 18:2n6 for the entire lifetime of the fish (Yu *et al.* 1979), 20:3n9 was observed in the eggs from both groups but was lower from the group fed both fatty acids. It was also reduced in the milt of trout if the n3,n6-deficient diet was supplemented with 18:2n6 (Watanabe *et al.* 1984b). Since it takes a long feeding period before 20:3n9 is observed in

broodstock, Leray *et al.* (1985) suggested the use of another ratio as an indicator of n3 deficiency [20:3n9 + 20:4n6 + 22:5n6]/[20:5n3 + 22:5n3 + 22:6n3] with a high ratio (6-12) for deficient eggs and a low ratio (0.5) for non-deficient eggs. This ratio was low in the present experiment. Synthesis of 20:3n9 from 18:1n9 (18%) and/or 20:1n9 (3%) was probably inhibited by the presence of 18:2n6 (7%) and 18:3n3 (1%). Dietary lipids also contained 6% 20:5n3 and 6% 22:6n3 which could have reduced the requirement for desaturation of shorter chain molecules.

(Leray *et al.* 1985) found that feeding rainbow trout broodstock an n3-deficient diet resulted in abnormalities in egg development visible at the 16-32 cell stage as well as a high incidence of deformities in the fry. Abnormalities in cell division were observed in eggs from 2-3 fish fed LLHE or HLLE but the fatty acid profiles of these eggs did not differ from other eggs in any consistent way. In addition, total n3 and n6 in these eggs ranged from 15.2-17.6% of the neutral lipid and 40.3-44.8% of the polar lipid which is not consistent with an n3 fatty acid deficiency. Leray *et al.* (1985) speculated that the incidence of egg abnormalities in their research might have been due to the high level of dietary 18:2n6 but levels of 18:2n6 in diets in the present experiment were much lower (0.6% in LL, 0.9% in HL and 1.6% in COMM) than those of Leray *et al.* (1985) (5.4%). Some other factor(s) must be responsible for the

abnormalities observed in this experiment.

While signs of fatty acid deficiency were observed in carp broodstock fed diets deficient in vitamin E (Watanabe and Takashima 1977), these were not observed in Arctic charr fed any of the diets. No changes in the fatty acid composition of eggs that would be indicative of an essential fatty acid deficiency were found in Arctic charr and it is expected that sufficient essential fatty acids were available from the diet and/or from body stores to meet the requirements.

There was a significant negative effect of neutral lipid and 16:1n7 and total n7 in the neutral lipids of eggs on survival to each stage of development with no significant difference between diets. The correlation with neutral lipid and 16:1n7 accounted for 26-34% and 19-21% of the variance in survival, respectively. The fact that the correlation was highly significant (P=0.0006-0.0032) for neutral lipid indicates that there is a low probability of this linear relationship being due to chance. However, the low regression correlation indicates that there are other covariate(s) that are not accounted for in this linear relationship. No reason for the effect of neutral lipid or 16:1n7 on hatchability is known but this is an area that warrants further investigation. When Leray *et al.* (1985) fed rainbow trout broodstock a semipurified diet containing no n3 and 5.4% n6 fatty acids, hatchability decreased

significantly compared to those fed a commercial diet containing 1.3% n3 and 2.5% n6 (by recalculation of the data in the paper). In the n3-deficient group, the neutral and polar lipids of the eggs and milt contained very low levels of n3 but increased levels of n6 giving them a significantly lower n3/n6 ratio (Leray and Pelletier 1985, Leray *et al.* 1985). There was also a decrease in n7 (15% vs 5%) fatty acids in the neutral lipids of eggs of the n3-deficient group. This leads one to speculate that if n7 fatty acids decreased in the n3-deficient group which had low survival, then n7 fatty acids were not negatively correlated with survival as they were with Arctic charr. When chinook salmon (Ashton 1991) were fed diets containing similar amounts of 16:1n7 to this experiment, the concentration of 16:1n7 in the neutral lipids of the eggs could be estimated from the data provided at 4-5% which is lower than observed in this experiment (9-14%).

5.7 Vitamin E in eggs

King (1985) found that the time prior to spawning during which dietary vitamin E was either present or absent affected the vitamin E concentration of the eggs. For example, when she sampled rainbow trout one month prior to spawning, she observed significant differences in vitamin E content and concentration of eggs from broodstock fed vitamin E-sufficient diets (90 mg/kg) from July to December compared to those fed deficient diets (0 mg/kg) from August or October to December. However, at

spawning time the only significant difference was in vitamin E content between those fed either the sufficient or deficient diet from July until spawning in January. While she stated that the greatest dietary effects on egg vitamin E at spawning occurred prior to October (ie. prior to the 3 months before spawning), her data seems to indicate that it was prior to the 6 months before spawning (ie. those fed deficient diets from July). However, she observed that the greatest rate of deposition of vitamin E into the egg occurred between October and December - ie. in the 3-4 months prior to spawning. In Year 1, Arctic charr were fed for 71 days before feed was withdrawn 18 and 32 days before the first HE and LE fish spawned, respectively. That is, feed was first offered 13 and 15 weeks prior to the commencement of spawning which was close to the time that the maximum rate of deposition of vitamin E occurred in King's work. One must be cognizant of the fact that there was a crossover of diets in Year 2. That is, keeping the dietary vitamin E levels the same, fish fed LL diets in Year 1 were fed HL diets in Year 2 and those fed HL diets in Year 1 were fed LL diets in Year 2 (Figure 3). It is interesting that the vitamin E levels observed in the HE eggs in Year 1 were very similar to those observed in Year 2 even though the diets were fed for a much longer period of time in Year 2. Since there were highly significant differences in the vitamin E content and concentrations of eggs from broodstock fed the LE versus HE diets and between those fed the two lipid levels at the high

level of vitamin E, the time that the diets were fed in Year 1 was sufficient for incorporation of vitamin E into the eggs. In Year 2, significant differences also occurred between those fed LLLE, HLLE and COMM which were not apparent in Year 1 with LLLE < HLLE < COMM. Initially, the reduction in vitamin E concentration in the LLLE eggs between Year 1 and 2 suggests that the additional year of being fed at the low level of lipid and vitamin E (LLLE) reduced the body stores of vitamin E and resulted in lower deposition in the eggs. While fish fed LLLE in Year 2 produced more eggs than those fed HLLE, there was no significant difference in the total vitamin E transferred to the eggs in the LLLE and HLLE groups in Year 1 (12.6 mg) or Year 2 (13.6 mg vs 15.2 mg). Fish fed LLLE also transferred a similar amount of vitamin E per kg fish weight to the eggs (6.4 mg/kg) as those fed HLLE (7.0 mg/kg). In contrast, fish fed LLHE in Year 1 deposited significantly more total vitamin E in the eggs compared to those fed HLHE (48.9 mg vs 35.0 mg vitamin E or 35.1 vs 24.6 mg vitamin E/kg fish weight) but the differences were not significant in Year 2 (54.6 mg vs 41.9 mg vitamin E or 37.4 vs 24.4 mg vitamin E/kg fish weight). While the diets were fed for a longer period of time in Year 2, there were no marked increases in the vitamin E concentration or content of the eggs. This confirms that the period of time for transfer of dietary vitamin E to the eggs was met during the shorter period of feeding in Year 1. While some fish had more time to mobilize vitamin E from storage sites to the eggs prior to spawning,

there was no significant relation between spawning day and vitamin E content.

King (1985) showed there was 25 mg of vitamin E in the body of rainbow trout at the beginning of her experiment in July with 45% of this stored in the liver. Vitamin E was transported from the liver mainly to the muscle in July and August. Between August and October, it was transported from the muscle to the ovary and adipose tissue and then from the adipose tissue to the ovary between October and December. Vitamin E was transferred into the eggs between October and December with fish spawning throughout January. At spawning, the body burden of fish fed the diet containing no vitamin E still contained 25 mg vitamin E while those fed the diet supplemented at 90 mg/kg contained 35 mg vitamin E. After spawning, the body burden was reduced to 11.5 and 18 mg vitamin E with fish having transferred half their body burden into the eggs. If the unsupplemented fish had been maintained on that diet for another year, one can only speculate that the vitamin E content of the eggs and/or the number of eggs might have decreased. King's experiment shows that it took at least one spawning season before the body stores were reduced by half. This was also observed in poultry where hatchability declined over time when they were fed a vitamin E-deficient diet and that 40 eggs had to be laid before a vitamin E deficiency developed (Jensen and McGinnis 1957). In the current experiment, diets contained 30 and 600 mg supplemental vitamin

E acetate/kg. If a diet with 0 mg vitamin E/kg had been fed, it might have resulted in a greater reduction in the concentration of vitamin E in the LE eggs in Year 2.

Watanabe *et al.* (1981a) found that the vitamin E requirement of rainbow trout fry increased as either the lipid level or degree of unsaturation of the dietary lipid increased. The vitamin E concentration of the liver decreased as dietary lipid increased with the greatest differences occurring between fry fed low lipid (3 and 5%) and high lipid (10 and 15%) diets. When fry were fed diets containing 50 mg vitamin E/kg, the vitamin E concentration in the liver was lowest when fry were fed the diet with the highest level of unsaturation (5.7% PUFA) and it was highest when they were fed the diet with the lowest level of unsaturation (1.6% PUFA). It was speculated that the decrease in liver vitamin E was the result of its increased requirement in preventing oxidation of PUFA and HUFA within the tissues. This is in agreement with research with ducklings and rats where vitamin E requirements increased with dietary PUFA only when dietary lipid was high (Fukaba 1980 cited by Watanabe *et al.* 1981a). The vitamin E concentration and content of the eggs were lower from Arctic charr fed the HLHE diet compared to the LLHE diet. This might be indicative of decreased body stores of vitamin E in broodstock fed the high lipid diets as occurred with fry in Watanabe *et al.* (1981a)'s experiment. While this was observed when dietary lipid increased in the HE

diets, it was not apparent when dietary lipid increased in the LE diets. Another possibility is that the vitamin E requirement was met at 600 mg/kg in the high lipid diet, but this level of supplementation was excessive at the lower lipid level, resulting in increased body stores of vitamin E and increased amounts of vitamin E to transfer to the eggs. A final possibility is that of decreased absorption of vitamin E in the high lipid diets which may have resulted in decreased transfer of vitamin E to the eggs. Decreased absorption of vitamin E was observed in rats fed fish oil compared to corn oil (Leka et al. *1989*).

King (1985) observed a decrease in the vitamin E content and concentration of rainbow trout eggs (5.39 µg/egg or 58.3 µg/g) when broodstock were fed diets containing no vitamin E compared to those fed diets containing 90 mg vitamin E/kg (7.37 µg/egg or 85.8 µg/g) for 6 months prior to spawning. Kinumaki *et al.* (1972) also observed increases in vitamin E from 4.4 µg/egg to 23 and 32 µg/egg (27 to 114 and 166 µg/g) when dietary vitamin E increased from 66 to 4600 and 8200 mg/kg. It is advisable to examine the trends that occur rather than to compare the actual vitamin E content since content is dependent on egg weight. Rainbow trout eggs in Kinumaki *et al.* (1972)'s experiment were almost twice the weight of Arctic charr eggs in the present experiment.

Vitamin E content and concentration in the fry was generally higher than that of the eggs in the present experiment. This is in contrast to the findings with Atlantic salmon (Cowey *et al.* 1985) and rainbow trout (King 1985, Kinumaki *et al.* 1972). King (1985) found that the vitamin E content of rainbow trout eggs was almost constant from the time of spawning until hatch while vitamin E concentration declined. Kinumaki *et al.* (1972) found slight reductions in vitamin E in the egg between spawning and the time the yolk sac had been absorbed. Cowey *et al.* (1985) showed that the vitamin E content of Atlantic salmon fertilized eggs decreased from 3.10 µg/egg (39.7 µg/g) to 2.29 µg per fry (32.7 µg/g) at the swimup stage. The higher vitamin E content and concentration in Arctic charr fry compared to eggs may have been due to the fact that less than only 2 months elapsed prior to analyzing the fry compared to 8-9 months for the eggs, although samples were flushed with nitrogen and frozen at -115°C. Tubes containing fry were immediately flushed with nitrogen and frozen on dry ice while egg samples were packed on ice but were not flushed with nitrogen and frozen until the samples were returned to the laboratory. Some groups of eggs did not result in sufficient fry for analysis and/or use in a growth trial and values for swimup fry are representative only of survivors from each lot of eggs. It is not known if the composition of survivors was different from the non-survivors from the same parent. King (1985) analyzed the dead eyed eggs and live eyed eggs from one

parent and found no difference in the content of the eggs although the dead eggs had a higher concentration because they were smaller than the live eggs.

King (1985) recommended a minimum of 7 μg/egg (82 μg/g) for best survival of rainbow trout and found survival was poorest at 5 μg/egg (54 μg/g). Eggs from the wild stock of Arctic charr from Tree River contained vitamin E at 73.8 μg/g or 9.0 μg/egg. This concentration is 1.3-1.9 times higher than that of Labrador charr eggs in either LE group, yet the content is similar to that of the HLHE group because the Tree River charr eggs weighed twice as much as the Labrador charr eggs. While Tree River charr eggs were close to hatch when sampled, both Cowey *et al.* (1985) and Kinumaki *et al.* (1981) showed that there was little decline in vitamin E between the unfertilized egg and hatch in Atlantic salmon and rainbow trout, respectively. In Year 2, fish in the present experiment produced eggs with 1.5-2.8 and 2.4-4.9 μg vitamin E/egg when fed the LE diets and 7.1-17.2 and 5.9-10.5 μg vitamin E/egg in the HE diets. Using King (1985)'s criteria, eggs in the LE group were all below the 5 μg/egg threshold while those in the HE group were almost all above the 7 μg/egg threshold. However, Arctic charr eggs were smaller than rainbow trout eggs and it is expected that a smaller embryo would require less vitamin E. Therefore, the threshold may be less for Arctic charr than recommended by King for rainbow trout.

The feeding of vitamin E-deficient diets to hens resulted in decreased hatchability in turkeys (Jensen *et al.* 1955, Atkinson *et al.* 1955) and chickens (Tengerdy and Nockels 1973). The involvement of vitamin E in survival of the egg was substantiated by showing that injection of vitamin E directly into vitamin E-deficient eggs increased hatchability (Jensen and McGinnis 1957). As has been found with fish, the vitamin E content of the chicken egg was directly related to maternal dietary vitamin E content (Bartov *et al.* 1965). King (1985) found eggs, from rainbow trout fed diets containing 8% lipid with 0 mg vitamin E/kg for 6 months prior to spawning, had significantly lower survival (89%) than those fed 90 mg vitamin E/kg (98%). Takeuchi *et al.* (1981a) also found hatchability decreased significantly when ayu were fed for 3 months prior to spawning on diets containing no vitamin E compared to more than 34 mg vitamin E/kg.

In the present experiment, significant differences were not observed in the embryonic survival of charr when fed the LE compared to HE diets in either year. In Year 2, the medians for survival to swimup were 45 and 57% for the HE diets indicating that half of these females produced eggs which had survival to swimup of greater than 45-57%. In contrast, 50% of the fish fed the LE diets had survival to swimup of greater than 9 and 1% and those fed COMM had a median of 17%.

The within treatment variance for survival of eggs to the eyed, hatch and swimup stages was very high and the sample size was low with 7-12 fish and 6-10 fish spawning per treatment in Year 1 and 2, respectively. The low sample size was due to mis-sexing of fish at the beginning of the experiment, failure of some females to spawn each year, premature release of eggs into the tank and a low number of fish mortalities. The high variances and low sample number resulted in the need for very large differences between means for a significant difference to be detected with an acceptable degree of certainty. Therefore, while the differences in the mean survivals in Year 2 lead one to suspect that there was a positive effect of vitamin E, these differences are not significant. For example, feeding the two LE diets resulted in 19 and 23% survival while the two HE diets resulted in 42 and 51% survival to swimup. Using power analysis described by Peterman (1990) and Dixon and Massey (1969) on data for survival to the eyed stage, there is a 40% chance of not rejecting (ie. accepting) the null hypothesis when the null hypothesis is false (μ=45, σ^2=1089, s^2=953, n=7.5, α=0.05). In other words, given the variance in embryonic survival in the population, if there was a significant difference to detect, the chance of detecting it would be 40%. Sample sizes of 15 and 25 would be required to increase the power to 0.75 and 0.95, respectively. The planned sample size was 15 but the actual sample size was as low as 6 in Year 2.

The case of low sample size often with a high degree of variation is common in broodstock nutrition with sample sizes of 3-6 fish per treatment reported (King 1985, Leray *et al.* 1985, Yu *et al.* 1979, Watanabe *et al.* 1984b). That is not to say that all broodstock nutrition is conducted on small groups of fish. Knox *et al.* (1988) used 40 females per tank with 2 replicates per treatment in his work on the effect of ration, but generally high numbers of broodstock are not available. In future, it will be necessary to reduce the number of treatments in order to have replicate tanks of fish and to increase the number of fish per tank. In addition, not all males produced milt each year and it is suggested that the number of males also be increased.

The fact that there was no direct correlation between vitamin E content or concentration of the eggs and survival to any stage in this experiment indicates either that there was adequate vitamin E even at the lower level of dietary vitamin E for adequate deposition in the egg or that there were other indirect effect(s) of vitamin E. Other indirect effects such as promoting a fatty acid deficiency were not found in any of the eggs. King (1985) reported one case of eggs with dispersed lipid globules and convoluted membranes in rainbow trout groups fed the unsupplemented diet for 6 months prior to spawning. Spawning abnormalities occurred in both years in this study but in Year 2, 4 of the 5 cases occurred in fish fed LE diets and 1 with the HE diets. These abnormalities included eggs which were

released into the tank, eggs which formed a solid mass and could not be released, the presence of blood in the ovarian fluid, brown particulate matter in the fluid, and small numbers of unusual brown and dark orange-coloured eggs with the orange eggs occurring in a fish fed a HE diet. While the presence of blood in the ovarian fluid might be indicative of a vitamin E deficiency in which there was deterioration of the walls of the capillaries, the incidence of this sign was very small. If dietary vitamin E had some other indirect effect, it was not measured in this experiment.

5.8 Fry growth and survival

Survival of fry decreased from 82% in Year 1 to 76% in Year 2 which was similar to the decrease from 88% to 79% that was predicted from the decrease in number of fry raised in the jars. With the exception of the LLHE group, the specific growth rate increased in Year 2 from 1.7% in Year 1 to 2.4% in Year 2 which was greater than the predicted 0.4% increase resulting from the decrease in number of fry in the jars. Environmental conditions were as similar as possible in both Year 1 and 2. Feed was purchased from the same company and analysis showed the proximate composition was 39.7% crude protein, 25.5% total lipid, 11.8% ash and 82.6% dry matter in Year 1 and 49.7% crude protein, 20.0% total lipid, 11.8% ash and 80.6% dry matter in Year 2. The differences in protein and lipid and/or changes in the ingredients used by the manufacturer in Year 2 may also have

contributed to the differences in specific growth rate.

There was no effect of vitamin E content or concentration of the fry on survival of fry in either year or on specific growth rate in Year 1. However, in Year 2 fry from the LLHE group had a significantly lower mean specific growth rate than those fed the other LL or HE diets. In addition, fry from the LLHE group had the lowest survival during the 6 week growth trial although this was not significantly different. Since the fry in the LLHE group had the highest levels of vitamin E, one must consider the possibility of hypervitaminosis but there was no correlation between specific growth rate or survival and vitamin E content or concentration. Although studies with chicks showed that growth rate and respiration rate of skeletal mitochondria were decreased when chicks received dietary levels of 2200 mg vitamin E acetate/kg compared to 1000 mg vitamin E acetate/kg (March *et al.* 1973), there have been no studies reported on hypervitaminosis E in fish. Kinumaki *et al.* (1972) fed diets containing 4600, 8200 mg and 10,000 mg vitamin E/kg and did not report increased mortality of eggs or fry.

CONCLUSIONS

Feeding 4-5 year old Labrador Arctic charr broodstock diets containing 12 or 19% lipid with 30 or 600 mg vitamin E acetate/kg diet did not result in significant differences in fertilization of eggs or survival to the eyed, hatch or swimup stages. In Year 2, fish fed the HE diets produced eggs with survivals 1.5-2.2 times higher than those fed the LE diets. The high degree of within treatment variation in survival to the eyed, hatch and swimup stages and the small number of females which spawned resulted in the need for very large differences in means in order to detect significant differences. Future research with Labrador Arctic charr broodstock requires higher numbers of fish per treatment and replicate tanks in order to increase the power of the test.

Feeding LL diets resulted in delayed spawning in Year 1 but this was not apparent in Year 2. The proportion of females failing to spawn was not related to concentrations of dietary lipid or vitamin E. A significantly lower proportion of males fed the higher concentration of vitamin E produced milt in two successive years. Fecundity was highly variable within treatments but variation was directly correlated with female weight and was not affected by the concentration of dietary lipid or vitamin E. Fish fed COMM consumed more feed, weighed more and produced significantly more eggs than those fed other

diets in Year 2 except for LLLE. Survival to swimup of the COMM group was half that of the HE groups.

Dietary vitamin E had a significant effect on the vitamin E concentration and content of the eggs even when fed the diets for only 71 days in Year 1. Increasing dietary vitamin E concentration had a greater impact on egg vitamin E concentration at the low compared to the high concentration of dietary lipid. This might be due to decreased absorption or increased oxidation of vitamin E within the tissues when the high lipid diet was fed. Fertilization and embryonic survival were not correlated with vitamin E content or concentration of the eggs. If the broodstock had been fed a diet containing 30 mg vitamin E/kg diet for more of their lifetime, they may have had lower stores of vitamin E at the beginning of the experiment and more pronounced effects of feeding a diet with a low concentration of vitamin E may have been observed.

Dietary lipid and vitamin E concentrations did not affect the total, neutral or polar lipid concentrations of the eggs. Feeding COMM resulted in significantly higher levels of total and neutral lipid in the eggs of fish fed all diets except LLLE.

The fatty acid composition of eggs did not indicate a deficiency of essential fatty acids. The fatty acid associated with essential fatty acid deficiency, 20:3n9, was not detected

in any eggs analyzed. The concentration of 22:6n3 was uniformly high in the polar lipids of eggs from fish fed all diets. The fatty acid composition of the eggs reflected that of the diet. The HL diets had significantly lower n6 and PUFA and a higher n3/n6 ratio than the LL diets and significant differences in these fatty acids and the n3/n6 ratio were apparent in the polar lipids of the eggs. COMM eggs were significantly higher in n6 and PUFA in the neutral lipids than eggs from all other treatments. Survival to the eyed, hatch and swimup stages was negatively correlated with the percent of neutral lipid and 16:1n7, one of the major n7 fatty acids in the neutral lipid fraction. The low coefficient of determination (r^2) for the effect of neutral lipid on survival indicates that additional covariate(s) must exist which also affect embryonic survival.

Eggs from wild Arctic charr from the Tree River, NWT had high survival to swimup. They had vitamin E concentration higher than those fed the LE diet and total, neutral and polar lipid similar to Labrador charr in this experiment.

Fry survival and growth were not affected by dietary vitamin E or lipid in Year 1 or Year 2 with the exception that fry from parents fed LLHE in Year 2 had significantly poorer specific growth rates than those fed other diets. Since these fry had significantly higher vitamin E concentration, their poor growth rate may have been related to hypervitaminosis.

LITERATURE CITED

Ackman, R.G. 1982. Fatty acid composition of fish oils. *In* Nutritional Evaluation of Long-chain Fatty Acids in Fish Oil. pp. 25-239. (S.M. Barlow and M.E. Stansby, eds.). Academic Press. London, England.

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson. 1983. Energy conversion: Mitochondria and chloroplasts. pp. 482-547. *In* Molecular Biology of the Cell. Garland Publishing, Inc., New York, NY.

American Oil Chemists' Society (AOCS). 1989. Nitrogen-ammonia-protein, official method Ba 4b-87. *In* Official Methods and Recommended Practices of the American Oil Chemists' Society. Vol. 1. Firestone, D. (ed.). AOCS, Champaign, IL.

Ashton, H. 1991. Lipid composition and fatty acid profiles of eggs from wild and cultured chinook salmon (*Oncorhynchus tshawytscha*). M.Sc. Thesis. University of British Columbia, Vancouver, B.C. 178 p.

Association of Official Analytical Chemists (AOAC). 1984. pp. 855-858. Official Methods of Analysis. Williams, S. (ed.) 14th ed. AOAC, Arlington, VA. 1141 p.

Atchison, G.J. 1975. Fatty acid levels in developing brook trout (*Salvelinus fontinalis*) eggs and fry. J. Fish. Res. Board Can. 32: 2513-2515.

Atkinson, R.I., T.M. Ferguson, J.H. Quisenberry and J.R. Couch. 1955. Vitamin E and reproduction in turkeys. J. Nutr. 55:387-397.

Baker, R. 1981. Report on Arctic charr egg take - 1980, Fraser River Labrador, pp. 45-53. *In* Waste Heat Aquaculture Interim Report. A report submitted by M.H. Papst, Aquaculture Project. Department of Fisheries and Oceans, Winnipeg, MB.

Baker, R. 1983. The effects of temperature, ration and size on the growth of Arctic charr (*Salvelinus alpinus* L.). M. Sc. Thesis. University of Manitoba, Winnipeg, MB. 227 p.

Balon, E.K. 1980. Early ontogeny of the lake charr *Salvelinus (Cristivomer) namaycush*. pp. 485-606. *In* Balon, E.K. (ed.) Charrs, Salmonid Fishes of the Genus *Salvelinus*. W. Junk bv Publishers, The Hague, Netherlands.

Bartov, I., P. Budowski and S. Bornstein. 1965. The relation between α-tocopherol content of the breeder diet and that of the newly hatched chick. Poult. Sci. 44: 1489-1494.

Bauernfeind, J. 1980. Tocopherols in Foods, pp. 99-167. *In* Machlin, L.J. (ed.) Vitamin E - A Comprehensive Treatise. Marcel Dekker, Inc., New York, NY.

Billard, R. 1977. Utilisation d'un systeme tris-glycocolle pour tamponner le dilueur d'insemination pour truite. Bull. Fr. Pisci. 264: 102-112.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Brett, J.R. and T.D.D. Groves. 1979. Physiological energetics, pp. 279-352. *In* Hoar, W.S., D.J. Randall, and J.R. Brett (eds.) Fish Physiology, Vol. VIII. Academic Press, New York.

Budvari, S., M.J. O'Neil and A. Smith. 1989. The Merck Index. 11th ed. Merck and Co., Rathway, NJ. 1606 p. plus Appendices.

Castell, J.D. 1979. Review of lipid requirements of finfish, pp. 59-84. *In* Halver, J.E. and K. Tiews (eds.) Finfish Nutrition and Fishfeed Technology. Schriften der Bundesforschungsanstalt fur Fischerei., Berlin, Germany.

Castell, J.D., R.O. Sinnhuber, J.H. Wales, and D.J. Lee. 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): Growth, feed conversion and some gross deficiency symptoms. J. Nutr. 102: 77-86.

Christie, W.W. 1982. Lipid Analysis. Isolation, Separation, Identification and Structural Analysis of Lipids. 2nd ed. Pergamon Press, New York, NY. 207 p.

Cowey, C.B. 1986. The role of nutritional factors in the prevention of peroxidative damage to tissues. Fish Physiol. Biochem. 2: 171-178.

Cowey, C.B., J.W. Adron, M.J. Walton, J. Murray, A. Youngson and D. Knox. 1981. Tissue distribution, uptake, and requirement for α-tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. J. Nutr. 111: 1556-1567.

Cowey, C.B., J.W. Adron and A. Youngson. 1983. The vitamin E requirements of rainbow trout (*Salmo gairdneri*) given diets containing polyunsaturated fatty acids derived from fish oil. Aquaculture 30: 85-93.

Cowey, C.B., J.G. Bell, A. Fraser and A. Youngson. 1985. Lipid and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). Lipids 20: 567-572.

Cowey, C.B., E. Degener, A.G.J. Tacon, A. Youngson and J.G. Bell. 1984. The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) grown at natural, varying water temperatures. Brit. J. Nutr. 51: 443-451.

Craik, J.C.A. and S.M. Harvey. 1984. Egg quality in rainbow trout: the relation between egg viability, selected aspects of egg composition, and time of stripping. Aquaculture 40: 115-134.

de March, B.G.E. 1991. Origins of cultivated Arctic charr in Canada, pp. 5-6. *In* Tabachek, J.L. (ed.) Arctic Charr Culture Bulletin, No. 1, Department of Fisheries and Oceans, Winnipeg, MB.

de March, B.G.E. 1992. Genetic aspects of Arctic charr culture. Proc. Ann. Meeting Aquaculture Assoc. Canada. Abstract.

Dempson, J.B. 1982. Ecology and comparative population dynamics of anadromous Arctic charr, *Salvelinus alpinus* (Linneaus 1758), in northern Labrador. M. Sc. Thesis. Memorial Univ. of Newfoundland, St. John's, Nfld. 189 p.

Dempson, J.B. and J.M. Green. 1985. Life history of anadromous Arctic charr, *Salvelinus alpinus*, in the Fraser River, northern Labrador. Can. J. Zool. 63: 315-324.

Dixon, W.J. and F.J. Massey. 1969. Introduction to Statistical Analysis 3rd ed. McGraw-Hill Book Co, New York, NY. 638 p.

Evans, H.M. and K.S. Bishop. 1922. On the relation between fertility and nutrition. Am. J. Physiol. Cited in Scott et al. (1982).

Fales, F.W. 1971. Evaluation of a spectrophotometric method for determination of total fecal lipid. Clin. Chem. 17:1103-1108.

Fostier, A., B. Jalabert, R. Billard, B. Breton and Y. Zohar. 1983. The gonadal steroids, pp. 277-372. *In* Hoar, W.S., D.J. Randall, and E.M. Donaldson (eds.) Fish Physiology, Vol. IX, Part A. Academic Press, New York.

Fremont, L., C. Leger, B. Petridou and M.T. Gozzelino. 1984. Effects of a (n-3) polyunsaturated fatty acid-deficient diet on profiles of serum vitellogenin and lipoprotein in vitellogenic trout (*Salmo gairdneri*). Lipids 19: 522-528.

Fuze, D.M., G. Kindschi and J.L. Banks. 1985. Notes on the formalin treatment of fall chinook salmon eggs for fungus, *Saprolegnia* sp. Technology Transfer Series 18: 5 p.

Gallo-Torres, H.E. 1980a. Absorption, pp. 170-192. *In* Machlin, L.J. (ed.) Vitamin E - A Comprehensive Treatise. Marcel Dekker, Inc., New York, NY.

Gallo-Torres, H.E. 1980b. Transport and metabolism, pp. 193-267. *In* Machlin, L.J. (ed.) Vitamin E - A Comprehensive Treatise. Marcel Dekker, Inc., New York, NY.

Hamor, T. 1987. Preliminary testing of sperm extenders and egg rinses for improved fertilization of rainbow trout eggs. Ann. Meet. Aquacult. Assoc. Canada. 1:54-55.

Hardy, R.W. 1985. Salmonid broodstock nutrition, pp. 98-113. *In* Iwamoto, R.N., and S. Sower (eds.) Salmonid Reproduction - An International Symposium. Washington Sea Grant Program, Seattle, WA.

Hayes, L.W., I.J. Tinsley and R.R. Lowry. 1973. Utilization of fatty acids by the developing steelhead sac-fry, Salmo gairdneri. Comp. Biochem. Physiol. 45: 695-707.

Henderson, R.J. and J.R. Sargent. 1985. Fatty acid metabolism in fish, pp. 349-364. *In* Cowey, C.B., A.M. Mackie and Bell, J.G. (eds.) Nutrition and Feeding in Fish. Academic Press, London, England.

Henderson, R.J. and D.R. Tocher. 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26:281-347.

Hung, S.S.O., C.Y. Cho and S.J. Slinger. 1980. Measurement of oxidation in fish oil and its effect on vitamin E nutrition and rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 1248-1253.

Hung, S.S.O., C.Y. Cho and S.J. Slinger. 1981. Effect of oxidized fish oil, DL-α-tocopheryl acetate and ethoxyquin supplementation on the vitamin E nutrition of rainbow trout (*Salmo gairdneri*) fed practical diets. J. Nutr. 111: 648-657.

Hung, S.S.O., T.W. Moon, J.W. Hilton and S.J. Slinger. 1982. Uptake, transport and distribution of DL-α-tocopherylacetate to D-α-tocopherol in rainbow trout (*Salmo gairdneri*). J. Nutr. 112: 1590-1599.

Hurley, D.A. and K.C. Fisher. 1966. The structure and development of the external membrane in young eggs of the brook trout, *Salvelinus fontinalis* (Mitchill). Can. J. Zool. 44: 173-190.

Hynes, J.D. 1984. *In* Castledine, A.J., C.Y. Cho, H.W. Ferguson, and S.J. Slinger (eds.) Handbook of Fish Culture. pp. 30. OMAF, OME, OMNR, Ontario.

Jensen, L.S. 1968. Vitamin E and essential fatty acids in avian reproduction. Fed. Proc. 27: 914-919.

Jensen, L.S. and J. McGinnis. 1957. Studies on the vitamin E requirement of turkeys for reproduction. Poult. Sci. 36: 1344-1350.

Jensen, L.S., M.L. Scott, G.F. Heuser, L.C. Norris and T.S. Nelson. 1955. Studies on the nutrition of breeding turkeys. I. Evidence indicating a need to supplement practical turkey rations with vitamin E. Poult. Sci. 35: 810-816.

Johnson, L. 1980. The arctic charr, pp. 15-98. *In* Balon, E.K. (ed.) Charrs, Salmonid Fishes of the Genus *Salvelinus*. W. Junk bv Publishers, The Hague, Netherlands.

Jones, J.G. and N. Bromage. 1987. The influence of ration size on the reproductive performance of female rainbow trout (*Salmo gairdneri*), p. 202. *In* Idler, D.R., L.W. Crim and J.M. Walsh (eds.) Proceedings of the Third International Symposium on Reproductive Physiology of Fish. August 2-7, 1987. Memorial University of Nfld., St. John's, Nfld.

Kaitaranta, J.K. 1982. Hydrolytic changes in the lipids of fish roe products. J. Food Technol. 17: 87-98.

Kanazawa, A. 1985. Essential fatty acid and lipid requirement of fish, pp. 281-298. *In* Cowey, C.B., A.M. Mackie and J.G.Bell (eds.) Nutrition and Feeding in Fish. Academic Press, London, Great Britain.

Kasparek, S. 1980. Chemistry of tocopherols and tocotrienols, pp. 7-65. *In* Machlin, L.G. (ed.) Vitamin E - A Comprehensive Treatise. Marcel Dekker, Inc., New York, NY.

King, I.B. 1985. Influence of vitamin E in reproduction in rainbow trout (*Salmo gairdneri*). Ph. D. Thesis. University of Washington, Seattle, WA. 152 p.

Kinumaki, T., K. Sugh, H. Iida and T. Takahashi. 1972. Addition of fat soluble vitamins to the feeding stuffs for parent rainbow trout with particular reference to the effect on the vitamin levels of eggs and fry. Bull. Tokai Reg. Fish. Res. Lab. 71: 133-160.

Knox, D., N.R. Bromage, C.B. Cowey and J.R.C. Springate. 1988. The effect of broodstock ration size on the composition of rainbow trout eggs (*Salmo gairdneri*). Aquaculture 69: 93-104.

Krieger, K.G. 1987. Early survival of three strains of hatchery-reared Arctic charr (*Salvelinus alpinus*). Proc. Ann. Meet. Aquacult. Assoc. Canada 1: 42-43.

Krieger, K.G., B.G.E. de March and R.N. Olson. 1988. Factors involved in spawning success of Arctic charr (*Salvelinus alpinus*) broodstock of the Nauyuk Lake strain. Bull. Aqua. Assoc. Can. 88-4: 34-36.

Leger, C. 1985. Digestion, absorption and transport of lipids, pp. 299-332. *In* Cowey, C.B., A.M. Mackie, and J.G. Bell (eds.) Nutrition and Feeding of Fish. Academic Press, London, England.

Leger, C., L. Fremont, D. Marion, I. Nassour and M.-F. Desfarges. 1981. Essential fatty acids in trout serum lipoproteins, vitellogenin and egg lipids. Lipids 16: 593-600.

Leka, L.S., K.M. Remaly, P.A. Bizinkauskas and M. Meydani. 1989. Effect of fish oil on intestinal absorption of vitamin E in the rat. Fed. Am. Soc. Exp. Biol. 3(4):A951. Abstract.

Leray, C., G. Nonotte, P. Roubaud and C. Leger. 1985. Incidence of (n-3) essential fatty acid deficiency on trout reproductive processes. Reprod. Nutr. Develop. 25: 567-581.

Leray, C. and X. Pelletier. 1985. Fatty acid composition of trout phospholipids: Effect of (n-3) essential fatty acid deficiency. Aquaculture 50: 51-59.

Li, J.C.R. 1964. Statistical Inference. Edward Brothers, Inc., Ann Arbor, MI. 658 p.

Luquet, P. and T. Watanabe. 1986. Interaction "nutrition-reproduction" in fish. Fish Physiol. Biochem. 2: 121-129.

March, B.E., E. Wong, L. Seier, J. Sim and J. Biely. 1973. Hypervitaminosis E in the chick. J. Nutr. 103: 371-377.

McGeachy, S.M. and J.L. Delabbio. 1989. The potential of Arctic charr (*Salvelinus alpinus*) as a Canadian aquaculture species. Bull. Aqua. Assoc. Can. 89-3: 40-42.

Molenaar, I., C.E. Hulstaert and M.J. Hardonk. 1980. Role in function and ultrastructure of cellular membranes, pp. 372-389. *In* Machlin, L.J. (ed.) Vitamin E - A Comprehensive Treatise. Marcel Dekker, Inc., New York, NY.

Nagahama, Y. 1983. The functional morphology of teleost gonads. pp. 223-275. *In* Hoar, W.S., D.J. Randall and E.M. Donaldson (eds.) Fish Physiology, Vol. IX, Part A. Academic Press, New York.

Ng, T.B. and D.R. Idler. 1983. Yolk formation and differentiation in teleost fishes. pp. 373-404. *In* Hoar, W.S., D.J. Randall and E.M. Donaldson (eds.) Fish Physiology, Vol. IX, Part A. Academic Press, New York.

NRC. 1981. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Coldwater Fishes. National Academy of Sciences, Washington, D.C. 63 p.

NRC. 1987. Vitamin Tolerance of Animals. National Academy Sciences, Washington, D.C. 96 p.

Olsen, R.E., R.J. Henderson and E. Ringo. 1991. Lipids of Arctic charr, *Salvelinus alpinus* (L.) I. Dietary induced changes in lipid class and fatty acid composition. Fish Physiol. Biochem. 9: 151-164.

Orr, W., J. Call, J. Brooks, E. Holt and J. Mainwaring. 1982. Effect of feeding rates on spawning performance of two year old rainbow trout brood stock. U.S. Dept. of the Interior Fish. and Wildlife Bozeman Info. Leaflet 24: 6 p.

Papst, M.H. and G.E. Hopky. 1982. Growth of rainbow trout (*Salmo gairdneri* Richardson) in a pilot commercial rearing system. Can. Tech. Rep. Fish. Aquat. Sci. 1112: iv + 18 p.

Papst, M.H. and G.E. Hopky. 1983. Growth of Arctic charr (*Salvelinus alpinus* L.) in a pilot commercial rearing system. Can. Tech. Rep. Fish. Aquat. Sci. 1182: iv + 16 p.

Papst, M.H. and G.E. Hopky. 1984. Development of an Arctic charr (*Salvelinus alpinus* L.) brood stock. Aquaculture 43: 323-331.

Papst, M.H. and G.E. Hopky. 1989. Aquaculture potential of Arctic charr. Bull. Aqua. Assoc. Can. 89-2: 15-19.

Peterman, R.M. 1990. Statistical power analysis can improve fisheries research management. Can. J. Fish. Aquat. Sci. 47: 2-15.

Pike, R.L. and M.L. Brown. Nutrition: an Integrated Approach. John Wiley and Sons. New York, NY. 542 p.

Piper, R.G., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler and J.R. Leonard. 1982. Fish Hatchery Management. U.S. Dept. of Interior, Fish and Wildlife, Washington, D.C. 517 p.

Ridelman, J.M., R.W. Hardy and E.L. Brannon. 1984. The effect of short-term starvation of ovarian development and egg viability in rainbow trout (*Salmo gairdneri*). Aquaculture 37: 133-140.

Roley, D.D. 1983. The effect of diet protein level, feeding level and rearing water temperature on the growth and reproductive performance of rainbow trout broodstock. M. Sc. Thesis. University of Washington, Seattle, WA. 227 p.

Roy, R.J.J. and D.A. Higgs. 1987. The effect of dietary lipid composition on growth and reproduction of broodstock chinook salmon (*O. tshawytscha*). Abstract. Proceedings of International Symposium on Feeding and Nutrition. Bergen, Norway, August 23-27, 1987 : 29.

SAS Institute. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc., Cary, NC. 956 p.

SAS Institute. 1990. SAS Procedures Guide, Version 6, Third edition. SAS Institute Inc., Cary, NC. 705 p.

Satia, B.P. 1974. Quantitative protein requirements of rainbow trout. Prog. Fish-Cult. 36:80-85.

Sargent, J., R.J. Henderson and D.R. Tocher. 1989. The lipids, pp. 153-218. *In* Halver, J.E. (ed.) Fish Nutrition. Academic Press, San Diego, CA.

Scott, D.P. 1962. Effect of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 19: 715-731.

Scott, M.L., M.C. Nesheim and R.J. Young. 1982. Nutrition of the Chicken. M.L. Scott and Assoc., Ithaca, NY. 562 p.

Smith, C.E., M.D. Osborne, R.G. Piper and W.P. Dwyer. 1979. The effect of diet composition on the performance of rainbow trout broodstock during a 3-year period. U.S. Fish and Wildlife Service. Bozeman Information Leaflet 8: 15 p.

Springate, J.R.C., N.R. Bromage and P.R.T. Cumaranatunga. 1985. The effects of different ration on fecundity and egg quality in the rainbow trout (*Salmo gairdneri*), pp. 371-394. *In* Cowey, C.B., A.M. Mackie, and J.G. Bell (eds.) Nutrition and Feeding in Fish. Academic Press, London, England.

Srivastava, R.K. 1990. Measures of egg quality and hatchery performance of Arctic charr (*Salvelinus alpinus* L.) and Atlantic salmon (*Salmo salar* L.). M.Sc. Thesis. Memorial University of Newfoundland., St. John's, NF. 166 p.

Stainton, M.M., M.J. Capel and F.A.J. Armstrong. 1974b. The chemical analysis of fresh water. Fish. Res. Board Can. Misc. Spec. Publ. 25: 25 p.

Tabachek, J.L. 1983. Evaluation of grower diets for intensive culture of rainbow trout (*Salmo gairdneri* Richardson). Can. Tech. Rep. Fish. Aquat. Sci. 1178:iv + 15 p.

Tabachek, J.L. 1986. Influence of dietary protein and lipid levels on growth, body composition and utilization efficiencies of Arctic charr, *Salvelinus alpinus* L. J. Fish Biol. 29: 139-151.

Tabachek, J.L. 1992. A review of the nutrition and feeding of Arctic charr, *Salvelinus alpinus* (L.) and priorities for future research. Abstract. Vancouver, B.C. June 1-3, 1992. Proc. Annual Meeting Aquaculture Assoc. Canada. Abstract.

Tabachek, J.L. and B.G.E. de March. 1990. Stock differences, pp. 8. *In* Tabachek, J.L. (ed.) Arctic Charr Culture Bulletin, No. 1, Department of Fisheries and Oceans, Winnipeg, MB.

Tabachek, J.L. and B.G.E. de March. 1991. Research priorities for the culture of Arctic charr, *Salvelinus alpinus* (L.), in Atlantic Canada. Can. Ind. Rep. Fish. Aquat. Sci. 211: iv + 15 p.

Tabachek, J.L., M.J. Foster, C.E. Engel and R.N. Olson. 1993. A system for the incubation of separate groups of salmonid eggs. Prog. Fish-Cult. Accepted.

Tacon, A.G.J. 1981. Speculative review of possible carotenoid function in fish. Prog. Fish-Cult. 43: 205-208.

Takeuchi, M., S. Ishi and T. Ogino. 1981a. Effect of dietary vitamin E on growth, vitamin E distribution, and mortalities of the fertilized eggs and fry in ayu *Plecoglossus altivelis*. Bull. Tokai Reg. Fish. Res. Lab. 104: 111-122.

Takeuchi, T., T. Watanabe and T. Nose. 1979. Requirement for essential fatty acids of chum salmon (*Oncorhynchus keta*) in freshwater environment. Bull. Jap. Soc. Sci. Fish. 45: 1319-1323.

Takeuchi, J., T. Watanabe and C. Ogino. 1978. Optimum ratio of protein to lipids in diets of rainbow trout. Bull. Jap. Soc. Sci. Fish. 44: 683-688.

Takeuchi, T., T. Watanabe, C. Ogino, M. Saito, K. Nishimura and T. Nose. 1981b. Effects of low protein-high calory diets and deletion of trace elements from a fish meal diet on reproduction of rainbow trout. Bull. Jap. Soc. Sci. Fish. 47: 645-654.

Tappel, A.L. 1980. Vitamin E and selenium protection from *in vivo* lipid peroxidation. Ann. N.Y. Acad. Sci. 18-31.

Tengerdy, R.P. and C.F. Nockels. 1973. The effect of vitamin E on egg production, hatchability and humoral immune response of chickens. Poult. Sci. 52: 778-783.

van Duijn, C., Jr. 1973. Diseases of Fishes. C.C. Thomas Publisher. Springfield, IL. 372 p.

Velsen, F.P.J. 1980. Embryonic development in eggs of sockeye salmon, *Oncorhynchus nerka*. Can. Spec. Publ. Fish. Aquat. Sci. 49: 19 p.

Vijan, P.N. and G.R. Wood. 1976. An automated submicrogram determination of selenium in vegetation by quartz tube furnace atomic absorption spectrophotometry. Talanta 23: 89-94.

Washburn, B.S. 1989. The effects of dietary protein and energy on the body composition and reproductive performance of rainbow trout broodstock (*Salmo gairdneri*). M. Sc. Thesis. University of California, Davis, CA. 55 p.

Watanabe, T. 1985. Importance of the study of broodstock nutrition for further development of aquaculture, pp. 395-414. *In* Cowey, C.B., A.M. Mackie, and J.G. Bell (eds.) Nutrition and Feeding in Fish. Academic Press, London, England.

Watanabe, T., T. Arakawa, C. Kitajima and S. Fujita. 1984a. Effect of nutritional quality of broodstock diets on reproduction of red sea bream. Bull. Jap. Soc. Sci. Fish. 50: 495-501.

Watanabe, T., T. Fujimura, M.-J. Lee, K. Fukusho, S. Satoh and T. Takeuchi. 1991. Effect of polar and nonpolar lipids from krill on quality of eggs of red seabream *Pagrus major*. Nippon Suisan Gakkaishi 57: 695-698.

Watanabe, T., A. Itoh, S. Satoh, C. Kitajima and S. Fujita. 1985a. Effect of dietary protein levels and feeding period before spawning on chemical components of eggs produced by red sea bream broodstock. Bull. Jap. Soc. Sci. Fish. 51: 1501-1509.

Watanabe, T., T. Koizumi, H. Suzuki, S. Satoh, T. Takeuchi, N. Yoshida, T. Kitada and Y. Tsukashima. 1985b. Improvement of quality of red sea bream eggs by feeding broodstock on a diet containing cuttlefish meal or on raw krill shortly before spawning. Bull. Jap. Soc. Sci. Fish. 51: 1511-1521.

Watanabe, T., C. Ogino, Y. Koshishi and T. Matsunaga. 1974a. Requirement of rainbow trout for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 40: 493-499.

Watanabe, T. and F. Takashima. 1977. Effect of α-tocopherol deficiency on carp - VI. Deficiency symptoms and changes of fatty acid and triglyceride distributions in adult carp. Bull. Jap. Soc. Sci. Fish. 43: 819-830.

Watanabe, T., F. Takashima and C. Ogino. 1974b. Effect of dietary methyl linolenate on growth of rainbow trout. Bull. Jap. Soc. Sci. Fish. 40: 181-188.

Watanabe, T., T. Takeuchi, M. Saito and K. Nishimura. 1984b. Effect of low protein-high calory or essential fatty acid deficiency diet on reproduction of rainbow trout. Bull. Jap. Soc. Sci. Fish. 50: 1207-1215.

Watanabe, T., T. Takeuchi, M. Wada and R. Uehara. 1981a. The relationship between dietary lipid and α-tocopherol requirement of rainbow trout. Bull. Jap. Soc. Sci. Fish. 47: 1463-1471.

Watanabe, T., M. Wada and T. Takeuchi. 1981b. Absence of interconversion of tocopherols in rainbow trout. Bull. Jap. Soc. Sci. Fish. 47: 1455-1462.

Wilcox, K.W., J. Stoss and E.M. Donaldson. 1984. Broken eggs as a cause of infertility of coho salmon gametes. Aquaculture 40: 77-87.

Woyewoda, A.D., S.J. Shaw, P.J. Ke and B.G. Burns. 1986. Recommended laboratory methods for assessment of fish quality. Can. Tech. Rep. Fish. Aquat. Sci. 1448: v + 156 p.

Yang, X. and T. Dick. 1992. Essential fatty acid requirements of Arctic charr, (*Salvelinus alpinus* L.). Proc. Annual Meeting Aquaculture Assoc. Canada. Vancouver, BC. June 1-3, 1992. Abstract.

Yu, T.C., R.O. Sinnhuber and J.D. Hendricks. 1979. Reproduction and survival of rainbow trout (*Salmo gairdneri*) fed linolenic acid as the only source of essential fatty acids. Lipids 14: 572-575.

Yurkowski, M. 1989. Lipid content and fatty acid composition of muscle from some freshwater and marine fish from central and Arctic Canada. pp. 547-557. *In* Chandra, R.K. (ed.) Health Effects of Fish and Fish Oils. ARTS Publishers. St. John's, Nfld.

Appendix 1a. Fatty acids in the total lipid in the diets.

Fatty acid	LLLE	LLHE	HLLE	HLHE	COMM Year 1	COMM Year 2
	%	%	%	%	%	%
14:0	4.16	4.11	4.44	4.09	5.14	3.86
15:0I	0.15	0.08	0.17	0.15	0.16	0.17
15:0	0.34	0.36	0.36	0.33	0.31	0.31
16:0I	0.11	0.14	0.13	0.10	0.09	0.09
Unknown	0.25	0.27	0.27	0.26	0.11	0.22
16:0	16.40	16.43	15.97	15.61	15.77	12.16
16:1n7	5.06	5.05	5.51	5.36	7.76	6.97
16:1n5	0.18	0.19	0.20	0.18	0.22	0.24
Unknown	0.29	0.33	0.34	0.31	0.30	0.15
16:2n9	0.15	0.17	0.17	0.16	0.13	0.10
Unknown	0.42	0.44	0.47	0.45	0.79	0.48
17:0	0.83	0.86	0.94	0.92	0.14	0.22
Unknown	0.24	0.26	0.26	0.24	0.22	0.15
17:1n9	0.32	0.31	0.32	0.31	0.23	0.15
18:0I	0.22	0.20	0.19	0.18	0.11	0.13
16:5n3	0.37	0.40	0.42	0.39	1.38	0.60
18:0	2.72	2.69	2.68	2.69	2.53	1.63
18:1n9	18.71	18.60	18.48	18.83	12.44	10.93
18:1n7	3.70	3.72	3.81	3.62	2.64	2.35
18:1n5	0.32	0.33	0.35	0.35	0.26	0.40
18:2n6	7.46	7.40	4.90	4.97	10.68	10.52
18:3n6	0.15	0.16	0.17	0.17	0.23	0.12
19:0	0.09	0.10	0.10	0.09	0.14	0.08
18:4n6	0.11	0.12	0.13	0.12	0.17	0.07
19:1n7	0.10	0.10	0.10	0.10	0.04	0.04
18:3n3	1.32	1.32	1.00	1.01	1.32	1.01
18:4n3	0.88	0.88	1.00	1.00	1.77	1.03
20:0	0.20	0.20	0.20	0.21	0.14	0.17
20:1n11	3.84	3.77	4.63	5.04	0.43	0.68
20:1n9	3.30	3.29	3.52	3.32	4.49	11.32
20:1n7	0.48	0.48	0.56	0.57	0.29	0.81
20:2n6	0.18	0.18	0.20	0.20	0.14	0.13
20:4n6	0.62	0.62	0.63	0.64	0.39	0.25
20:3n3	0.08	0.08	0.09	0.09	0.06	0.05
20:4n3	0.29	0.29	0.33	0.34	0.66	0.24
20:5n3	5.97	5.92	6.20	6.36	10.45	5.05
22:0	0.13	0.13	0.11	0.12	0.09	0.09
22:1n11	6.60	6.50	7.49	7.83	5.39	15.52
22:1n9	1.01	1.00	1.17	1.16	0.61	1.68
22:1n7	0.20	0.19	0.21	0.21	0.13	0.33
21:5n3	0.21	0.21	0.23	0.25	0.38	0.19
22:5n6	0.12	0.12	0.13	0.14	0.07	0.07
22:5n3	0.92	0.90	1.03	1.08	1.13	0.61
22:6n3	6.77	6.75	6.33	6.58	5.89	4.90
24:1n9	0.78	0.74	0.80	0.84	0.42	0.59
Unknown	0.17	0.16	0.12	0.11	0.07	0.06

Appendix 1b. Fatty acid composition (mean % and standard deviation) of the neutral lipids of the eggs in Year 2.

Fatty acid	Diet LLLE		LLHE		HLLE		HLHE		COMM	
	%	SD	%	SD	%	SD	%	SD	%	SD
14:0	2.20	0.14	1.97	0.24	2.22	0.35	2.07	0.36	2.52	0.23
14:1n5	0.11	0.01	0.09	0.02	0.10	0.02	0.07	0.04	0.11	0.02
15:0I	0.10	0.02	0.09	0.02	0.11	0.01	0.10	0.01	0.11	0.01
15:0	0.25	0.03	0.21	0.02	0.26	0.02	0.25	0.05	0.26	0.03
16:0I	0.11	0.03	0.09	0.01	0.11	0.00	0.09	0.02	0.08	0.01
16:0	11.81	0.87	10.81	2.97	10.46	1.11	10.64	1.29	10.58	0.41
Unkn.	0.15	0.03	0.15	0.06	0.17	0.02	0.14	0.02	0.28	0.04
16:1n9	0.69	0.14	0.78	0.19	0.65	0.10	0.59	0.11	0.55	0.30
16:1n7	11.29	1.09	10.89	1.43	10.98	0.93	11.34	0.78	12.65	0.87
16:1n5	0.19	0.02	0.17	0.01	0.20	0.01	0.19	0.03	0.26	0.02
Unkn.	0.23	0.04	0.19	0.01	0.21	0.03	0.20	0.04	0.08	0.05
16:2n9	0.19	0.04	0.16	0.01	0.22	0.04	0.17	0.01	0.22	0.03
Unkn.	0.16	0.03	0.16	0.03	0.19	0.02	0.20	0.03	0.30	0.03
17:0	0.21	0.06	0.16	0.08	0.23	0.03	0.21	0.04	0.01	0.01
Unkn.	0.14	0.04	0.11	0.01	0.13	0.00	0.13	0.02	0.10	0.01
17:1n9	0.52	0.05	0.43	0.03	0.56	0.05	0.55	0.09	0.30	0.11
18:0	1.53	0.14	1.54	0.45	1.46	0.10	1.43	0.12	1.26	0.12
18:1n9	31.06	1.04	32.10	2.65	31.67	1.49	32.40	2.94	27.67	1.98
18:1n7	6.55	0.35	6.16	1.25	6.03	0.46	5.58	0.76	5.66	0.58
18:1n5	0.32	0.01	0.31	0.04	0.34	0.00	0.31	0.04	0.39	0.01
Unkn.	0.25	0.07	0.33	0.17	0.20	0.06	0.24	0.10	0.16	0.05
18:2n9	0.17	0.03	0.18	0.02	0.10	0.04	0.13	0.01	0.15	0.01
18:2n6	5.52	0.48	5.50	1.37	5.47	1.03	5.84	1.04	7.88	0.75
18:3n6	0.22	0.02	0.20	0.02	0.23	0.02	0.23	0.03	0.18	0.09
19:0	0.64	0.21	0.85	0.82	0.57	0.33	0.69	0.38	0.71	0.32
18:4n6	0.20	0.01	0.20	0.04	0.22	0.01	0.24	0.02	0.24	0.01
19:1n7	0.12	0.01	0.11	0.02	0.11	0.02	0.19	0.20	0.07	0.00
18:3n3	0.61	0.07	0.46	0.11	0.62	0.09	0.65	0.12	0.56	0.07
18:4n3	0.55	0.11	0.42	0.11	0.55	0.04	0.67	0.10	0.64	0.13
18:4n11	0.19	0.03	0.17	0.04	0.21	0.01	0.24	0.02	0.35	0.03
20:1n11	0.77	0.13	0.70	0.20	0.90	0.18	0.84	0.27	0.66	0.09
20:1n9	2.16	0.21	2.65	0.61	2.27	0.38	1.91	0.17	3.85	0.40
20:1n7	0.42	0.04	0.45	0.07	0.41	0.03	0.39	0.07	0.43	0.05
Unkn.	0.32	0.09	0.42	0.13	0.18	0.07	0.19	0.08	0.17	0.09
20:2n6	0.72	0.10	0.71	0.07	0.60	0.06	0.56	0.03	0.79	0.08
20:3n6	0.71	0.16	0.80	0.16	0.57	0.19	0.58	0.12	0.90	0.02
20:4n6	0.67	0.09	0.76	0.14	0.66	0.05	0.64	0.09	0.59	0.08
20:4n3	0.33	0.10	0.26	0.06	0.41	0.06	0.41	0.11	0.38	0.06
20:5n3	3.55	0.65	3.46	0.50	4.58	0.22	4.72	0.36	3.92	0.47
22:1n11	0.32	0.06	0.40	0.23	0.45	0.06	0.42	0.09	0.86	0.20
22:1n9	0.17	0.01	0.23	0.08	0.21	0.04	0.19	0.01	0.26	0.05
21:5n3	0.22	0.02	0.20	0.02	0.25	0.01	0.27	0.02	0.24	0.01
22:5n6	0.11	0.02	0.10	0.01	0.10	0.01	0.09	0.02	0.06	0.02
22:5n3	1.25	0.24	1.33	0.14	1.63	0.12	1.51	0.07	1.38	0.22
22:6n3	9.50	1.59	10.19	1.24	9.88	0.75	9.49	1.41	8.58	1.46

Appendix 1c. Fatty acid composition (mean % ± standard deviation) of the polar lipids of the eggs in Year 2,

Fatty acid	Diet LLLE		LLHE		HLLE		HLHE		COMM	
	%	SD	%	SD	%	SD	%	SD	%	SD
14:0	1.14	0.09	0.94	0.11	0.88	0.13	0.97	0.15	1.08	0.07
15:0	0.27	0.03	0.22	0.02	0.24	0.02	0.28	0.02	0.25	0.03
16:0	16.49	0.59	15.69	1.13	15.99	0.28	16.43	0.51	15.60	0.95
Unkn.	0.18	0.06	0.18	0.06	0.10	0.03	0.12	0.10	0.27	0.05
16:1n9	0.58	0.12	0.67	0.18	0.45	0.15	0.48	0.14	0.54	0.09
16:1n7	2.39	0.14	2.57	0.24	2.09	0.21	2.31	0.31	2.45	0.18
16:1n5	0.10	0.01	0.09	0.02	0.09	0.04	0.10	0.01	0.13	0.01
Unkn.	0.23	0.03	0.18	0.01	0.21	0.01	0.21	0.03	0.17	0.02
16:2n9	0.11	0.01	0.09	0.01	0.10	0.01	0.10	0.01	0.08	0.01
17:0	0.17	0.04	0.14	0.01	0.13	0.02	0.12	0.03	0.08	0.02
Unkn.	0.16	0.01	0.12	0.01	0.17	0.03	0.15	0.02	0.12	0.02
17:1n9	0.24	0.02	0.20	0.03	0.22	0.02	0.23	0.01	0.17	0.02
18:0I	0.22	0.10	0.20	0.03	0.20	0.13	0.21	0.10	0.18	0.02
18:0	3.34	0.23	3.39	0.60	3.44	0.43	3.26	0.25	2.91	0.30
18:1n9	12.39	0.67	13.55	2.76	12.75	1.97	13.35	1.55	11.62	0.72
18:1n7	6.82	0.35	6.44	1.32	6.59	1.18	6.23	0.75	6.07	0.45
18:1n5	0.39	0.03	0.36	0.06	0.43	0.03	0.41	0.05	0.47	0.03
19:0A	0.18	0.09	0.21	0.09	0.12	0.04	0.16	0.06	0.10	0.06
Unkn.	0.11	0.04	0.09	0.02	0.08	0.00	0.08	0.00	0.09	0.00
18:2n6	1.74	0.23	1.80	0.17	1.49	0.34	1.62	0.16	2.29	0.15
18:3n6	0.11	0.01	0.08	0.01	0.11	0.02	0.11	0.02	0.10	0.01
19:0	0.12	0.04	0.13	0.08	0.06	0.03	0.08	0.03	0.11	0.03
19:1n7	0.26	0.02	0.21	0.05	0.25	0.08	0.22	0.05	0.15	0.02
18:3n3	0.15	0.02	0.11	0.02	0.14	0.03	0.16	0.04	0.12	0.02
18:4n3	0.14	0.03	0.10	0.02	0.09	0.01	0.13	0.01	0.13	0.03
20:1n11	0.30	0.06	0.27	0.14	0.34	0.12	0.35	0.13	0.31	0.07
20:1n9	3.80	0.33	4.68	0.96	4.29	0.88	3.64	0.47	6.04	0.49
20:1n7	0.42	0.02	0.49	0.09	0.49	0.10	0.40	0.06	0.46	0.05
Unkn.	0.12	0.06	0.20	0.08	0.04	0.06	0.08	0.03	0.09	0.02
20:2n6	1.01	0.12	1.06	0.13	0.89	0.14	0.82	0.08	1.15	0.14
20:3n6	0.99	0.30	1.15	0.07	0.62	0.28	0.62	0.11	1.15	0.14
20:4n6	1.63	0.27	1.79	0.47	1.24	0.18	1.37	0.37	1.48	0.29
20:4n3	0.13	0.05	0.11	0.03	0.14	0.03	0.14	0.05	0.14	0.03
20:5n3	7.46	0.76	7.05	0.69	8.10	1.11	8.58	0.60	7.96	0.61
22:1n11	0.10	0.03	0.14	0.05	0.16	0.04	0.14	0.03	0.26	0.04
21:5n3	0.09	0.06	0.12	0.01	0.12	0.01	0.12	0.01	0.09	0.06
22:4n6	0.10	0.05	0.13	0.04	0.08	0.01	0.06	0.03	0.04	0.03
22:5n6	0.28	0.03	0.24	0.06	0.23	0.05	0.21	0.04	0.12	0.08
22:5n3	2.32	0.30	2.58	0.17	2.76	0.20	2.57	0.18	2.82	0.29
22:6n3	30.51	0.84	29.86	1.42	31.38	1.45	30.87	0.99	29.99	0.88
24:1n11	0.24	0.17	0.36	0.07	0.36	0.07	0.30	0.16	0.38	0.06
24:1n9	0.13	0.02	0.12	0.01	0.13	0.01	0.16	0.08	0.12	0.01
26:0	0.10	0.02	0.07	0.04	0.08	0.04	0.07	0.03	0.11	0.03

Appendix 1d. Fatty acid composition of the neutral and polar lipid of eggs of wild Arctic charr from Tree River, NWT.

Fatty acid	Neutral lipid	Polar lipid
	%	%
14:0	2.42	0.88
15:0I	0.11	tr
15:0	0.22	0.22
16:0I	0.13	tr
16:0	9.66	15.23
16:1n9	tr	0.29
16:1n7	17.71	3.46
16:1n5	0.30	0.15
Unknown	tr	0.16
16:2n9	0.13	0.10
Unknown	0.24	tr
17:0	0.15	0.15
Unknown	tr	0.13
17:1n9	0.34	0.16
18:0I	tr	0.23
18:0	1.88	4.95
18:1n9	21.28	10.62
18:1n7	7.92	8.53
18:1n5	0.45	0.64
18:2n9	0.10	tr
18:2n6	2.15	0.55
18:3n6	0.23	0.12
19:0	0.16	tr
18:4n6	0.20	tr
19:1n7	0.06	0.12
18:3n3	0.77	0.15
18:4n3	0.64	tr
18:4n11	0.19	tr
20:1n11	0.18	0.11
20:1n9	1.61	3.07
20:1n7	0.33	0.52
20:2n6	0.29	0.41
20:3n6	0.30	0.27
20:4n6	0.48	0.82
20:4n3	0.88	0.34
20:5n3	11.74	12.66
22:1n11	0.17	0.06
22:1n9	0.12	tr
21:5n3	0.28	0.12
22:4n6	tr	0.12
22:5n6	tr	0.12
22:5n3	4.27	6.21
22:6n3	9.59	25.50
24:1n11	tr	0.43

Appendix 2a. Weekly water temperature (mean and standard deviation) in Year 1 and Year 2 (n=5).

Year 1				Year 2			
Week	Date	Temperature °C	SD	Week	Date	Temperature °C	SD
				28	020189-080189	8.09	0.05
				29	090189-150189	8.18	0.07
				30	160189-220189	8.28	0.06
				31	230189-290189	8.34	0.00
				32	300189-050289	8.07	0.07
				33	060189-120289	8.21	0.06
				34	130289-190289	8.10	0.20
				35	200289-260289	7.95	0.07
				36	270289-050389	7.84	0.11
				37	060389-120389	8.08	0.17
				38	130389-190389	8.14	0.17
				39	200389-260389	8.23	0.14
				40	270389-020489	8.08	0.04
				41	030489-090489	8.13	0.03
				42	100489-160489	8.18	0.15
				43	170489-230489	8.39	0.19
				44	240489-300489	7.87	0.53
				45	010589-070589	7.61	0.26
				46	080589-140589	8.01	0.09
				47	150589-210589	8.17	0.14
				48	220589-280589	7.87	0.15
				49	290589-040689	8.02	0.16
				50	050689-110689	8.11	0.10
				51	120689-180689	8.17	0.07
				52	190689-250689	8.42	0.13
1	280688-040788	-	-	53	260689-020789	8.20	0.20
2	050788-110788	8.57	0.58	54	030789-090789	8.00	0.26
3	120888-180788	8.58	0.12	55	100789-160789	8.35	0.14
4	190788-250788	8.69	0.08	56	170789-230789	8.64	0.24
5	260788-010888	8.76	0.18	57	240789-300789	8.56	0.12
6	020888-080888	8.55	0.16	58	010889-070889	8.50	0.22
7	090888-150888	8.61	0.16	59	080889-140889	8.59	0.28
8	160888-220888	8.50	0.12	60	150889-210889	8.63	0.19
9	230888-290888	8.28	0.10	61	220889-280889	8.65	0.18
10	300888-050988	8.33	0.15	62	290889-040989	8.56	0.26
11	060988-120988	8.15	0.14	63	050989-110989	8.35	0.31
12	130988-190988	8.16	0.13	64	120989-180989	8.27	0.25
13	200988-260988	7.97	0.05	65	190989-250989	7.83	0.15
14	270988-031088	7.89	0.14	66	260989-021089	7.83	0.29
15	041088-101088	7.66	0.21	67	031089-091089	7.61	0.32
16	111088-171088	7.65	0.22	68	101089-161089	7.59	0.14
17	181088-241088	7.55	0.14	69	171089-231089	7.55	0.36
18	251088-311088	7.14	0.22	70	241089-301089	7.48	0.40
19	011188-071188	7.33	0.09	71	311089-061189	7.15	0.49
20	081188-141188	7.15	0.14	72	071189-131189	7.28	0.55
21	151188-211188	7.84	0.16	73	141189-201189	8.02	0.50
22	221188-281188	7.69	0.13	74	211189-271189	8.14	0.10
23	291188-041288	7.68	0.06	75	281189-041289	8.50	0.20
24	051288-111288	7.66	0.09	76	051289-111289	8.54	0.07
25	121288-181288	7.71	0.05	77	121289-181289	8.49	0.10
26	191288-251288	7.93	0.16	78	191289-251289	8.43	0.12
27	261288-010189	7.72	0.03	79	261289-010190	8.49	0.07

Appendix 2b. Total gain, feed fed and feed efficiency of each tank of fish for each period in Year 1.

Initial date	280688	090888	110888	280688
Final date	080788	100888	130988	130988
Period	A TO B	B TO C	C TO D	A TO D
Days fed	9	30	32	71
Days not fed	2	2	2	6
Diet LLLE Tank 3				
Initial no. fish	25	25	25	25
Final no. fish	25	25	25	25
Total initial fish wt (g)	21172	21806	26709	
Total final fish wt (g)	21806	26709	30924	
Total gain (g)	634	4903	4215	9752
Feed fed (g, dry)	1274	6784	7190	15248
Feed efficiency (%)	49.8	72.3	58.6	64.0
Diet LLHE Tank 7				
Initial no. fish	25	25	25	25
Final no. fish	25	25	25	25
Total initial fish wt (g)	21160	21941	27766	
Total final fish wt (g)	21941	27766	33440	
Total gain (g)	813	3253	4050	8116
Feed fed (g, dry)	1452	6370	7043	14864
Feed efficiency (%)	56.0	51.1	57.5	54.6
Diet HLLE Tank 6				
Initial no. fish	25	25	25	25
Final no. fish	25	25	25	25
Total initial fish wt (g)	21162	22525	29789	
Total final fish wt (g)	21525	29789	36616	
Total gain (g)	985	5358	5893	12236
Feed fed (g, dry)	1228	7124	8022	16374
Feed efficiency (%)	80.2	75.2	73.5	74.7
Diet HLHE Tank 2				
Initial no. fish	25	25	25	25
Final no. fish	25	25	25	25
Total initial fish wt (g)	21160	21941	27766	
Total final fish wt (g)	21941	27766	33440	
Total gain (g)	781	5825	5674	12280
Feed fed (g, dry)	1127	7449	8503	18381
Feed efficiency (%)	69.3	78.2	66.7	66.8
Diet COMM Tank 5				
Initial no. fish	25	25	25	25
Final no. fish	25	25	25	25
Total initial fish wt (g)	21162	22525	29789	
Total final fish wt (g)	22525	29789	36616	
Total gain (g)	1363	7264	6827	15454
Feed fed (g, dry)	2054	8758	9322	20135
Feed efficiency (%)	66.4	82.9	73.2	76.8

Appendix 2c. Total gain, feed fed and feed efficiency of each tank of fish for each period in Year 2.

Initial date	120189	060489	290689	120189
Final date	230189	280689	130989	130989
Period	E to F	F to G	G to H	E to H
Days fed	82	82	75	239
Days not fed	2	2	2	6
Diet LLLE Tank 6				
Initial no. fish	24	23	23	
Final no. fish	23	23	22	
Total initial fish wt (g)	27709	36616	49114	
Total final fish wt (g)	36616	49114	52520	
Total gain (g)	5081	7239	3096	15416
Feed fed (g, dry)	7401	8627	8156	24184
Feed efficiency (%)	68.7	83.9	38.0	63.7
Diet LLHE Tank 2				
Initial no. fish	24	24	24	
Final no. fish	24	24	24	
Total initial fish wt (g)	25622	29160	34623	
Total final fish wt (g)	29160	34623	39405	
Total gain (g)	3538	5463	4782	13783
Feed fed (g, dry)	9497	10891	8470	28859
Feed efficiency (%)	37.3	50.2	56.5	47.8
Diet HLLE Tank 3				
Initial no. fish	23	21	21	
Final no. fish	21	21	20	
Total initial fish wt (g)	22467	24259	27288	
Total final fish wt (g)	24259	27288	29201	
Total gain (g)	1792	3029	1913	6734
Feed fed (g, dry)	10536	13804	10006	34347
Feed efficiency (%)	17.0	21.9	19.1	19.6
Diet HLHE Tank 7				
Initial no. fish	24	24	24	
Final no. fish	24	24	24	
Total initial fish wt (g)	25622	29160	34623	
Total final fish wt (g)	29160	34623	39405	
Total gain (g)	4587	3992	1258	9837
Feed fed (g, dry)	10009	12675	11523	34207
Feed efficiency (%)	45.9	31.5	10.9	28.8
Diet COMM Tank 5				
Initial no. fish	23	23	23	
Final no. fish	23	23	22	
Total initial fish wt (g)	27709	36616	49114	
Total final fish wt (g)	36616	49114	52520	
Total gain (g)	8907	12498	3406	24811
Feed fed (g, dry)	15474	15671	9248	40393
Feed efficiency (%)	57.6	79.8	36.8	61.4

Appendix 3a. Method of extraction (adapted from King 1985) and assay (adapted from AOAC 1984) of tocopherol in eggs and fry.

REAGENTS FOR EXTRACTION:
Ethanol, 99%
Hexane, distilled in glass (B.P.=68-69°C) - Caledon
Isooctane (2,2,4-Trimethylpentane) (B.P.=99-100°C) - Caledon
Acetonitrile, HPLC grade - Sigma

EXTRACTION:

Weigh sample directly into a 16 x 125 mm culture tube containing 1 ml 99% ethanol and 2 ml hexane containing 50 mg/l butylated hydroxy-toluene (BHT). Use 0.4 g eggs and 0.2-0.4 g fry. Eggs must be kept frozen during the weighing process or they will rupture and immediately expose the vitamin E to oxygen. Therefore, keep the sample vial in a styrofoam block and keep the weighing utensils cold throughout the weighing process. Flush the sample vial with nitrogen and return it to the freezer immediately. Weigh out and homogenize one sample at a time. Keep the samples on ice and flushed with nitrogen throughout all the steps in the extraction.

Homogenize sample 30 sec using a Polytron homogenizer. Sample must be kept in ice throughout the homogenizations.

Add another 1 ml 99% ethanol and 2 ml hexane.
Homogenize 15 sec with Polytron.

Transfer homogenate to a 16 x 125 mm screw-cap tube with a Pasteur pipette.
Add 1 ml ethanol and 2 ml hexane to the culture tube and rinse tube and Polytron generator by homogenizing for 15 sec.
Transfer the rinse to the above screw-cap tube and flush tube with nitrogen.
Keep samples on ice or in refrigerator until all the samples are extracted.

Centrifuge tubes 2 min in clinical centrifuge.

Transfer the upper hexane layer with a Pasteur pipette to a clean 16 x 125 mm screw-cap tube if it is to be evaporated in a heating block or a 50 ml flask if it is to be evaporated in a rotary evaporator.

Add 4 ml hexane and rehomogenize pellet by vortexing 1 min.

Centrifuge and combine hexane layer with the first hexane layer.

Evaporate hexane under nitrogen in a heating block or with a rotary evaporator at less than 50°C.

Extract twice with 2 ml acetonitrile and transfer extracts to a clean 16 x 125 mm screw-cap tube.

Extract acetonitrile extract with 3 ml isooctane by vortexing 1 minute followed by centrifuging at 6 for 1-2 min to separate the phases. Transfer the upper isooctane phase to a clean 16 x 125 mm screw-cap tube.

Extract the acetonitrile extract a second time with 3 ml isooctane by vortexing for 0.5-1 min and centrifuging as before. Combine the upper phase with previous extract.

Evaporate isooctane under nitrogen in a heating block or with a rotary evaporator at less than 50°C.

Dissolve extract in 3.0-4.0 ml 99% ethanol (eggs) or 2.0 ml ethanol. Adjust volume as required for the assay.

Assay the extract by the following AOAC (1984) spectrophotometric method.

Note: Throughout the extraction, all hexane and isooctane contained 50 mg/l BHT as an antioxidant. A blank should also be extracted to correct for interfering substances. Use of BHT will result in an increase in this correction value.

ASSAY:
REAGENTS FOR ASSAY:
1) Bathophenanthroline (Sigma) 0.003M
Weigh 100 mg bathophenanthroline. Bring up to 100 ml with 99% ethanol. Store in amber bottle at 5°C. Prepare fresh every 3 weeks.

2) Ferric chloride (Fisher) 0.002M
Weigh 55 mg $FeCl_3.6H_2O$. Bring up to 100 ml with 99% ethanol. Store in amber bottle at 5°C.

3) o-Phosphoric acid (Baker) 0.172M
Bring 1.1 ml concentrated phosphoric acid (86%) up to 100 ml with 99% ethanol.

4) Standard: Weigh 0.1 g α-tocopherol (95% purity) (Sigma) to 4-5 decimals and transfer to a volumetric flask with 99% ethanol. Make up to 100 ml with ethanol.

Dilute the above stock to 1 ml in 50 ml with ethanol.
Concentration (g/ml) = 0.95 x weight g/ 5000 ml
(approx 20 µg/ml).
Add volumes of up to 2.00 ml of the diluted stock tocopherol solution (0-40 µg) and make up to 2.0 ml with 99% ethanol.

Make up a blank with 2.0 ml ethanol.

METHOD:

Add tocopherol extract (in 99% ethanol) to a 13 x 100 mm culture tube.
Add 99% ethanol to make up to 2.0 ml.
Add 0.5 ml bathophenanthroline reagent and vortex.
Add 0.25 ml ferric chloride reagent. Start timing once last of reagent added. Vortex.
Add 0.25 ml phosphoric acid reagent exactly 15 seconds after the addition of the ferric chloride reagent. Vortex.
After 3 minutes the colour which has developed is stable for 90 minutes.
Read samples at 534 mμ against the blank.

NOTE: This method has been scaled down by half the volumes used by AOAC (1984).

CALCULATION:

Calculate slope of standard curve = absorbance / μg tocopherol.
Slope should be approx 0.0310.

$$\text{Vitamin E } (\mu g/g) = \frac{\text{absorbance}}{\text{slope}} \times \frac{2.0 \text{ ml}}{\text{vol assayed (ml)}} \times \frac{1}{\text{wt sample (g)}}$$

Vitamin E content of egg (μg/egg) =
vitamin E concentration in eggs (μg/g) x egg weight (g)

Vitamin E content of fry (μg/fry) =
vitamin E (μg/g) x fry weight (g)

Appendix 3b. Method of extraction and analysis of total lipid in eggs and diets.

REAGENTS:

Chloroform - distilled in glass
Methanol - distilled in glass
Concentrated sulphuric acid

Potassium dichromate (0.167M):
49.0 g reagent grade potassium dichromate
Bring to 1 liter with distilled water.

Lipid standard: 0.1 g/10 ml in chloroform.
In order to have a comparable level of oxidation in the standard and the samples, standard curve should be conducted with the samples being analyzed. In the current research, neutral and polar lipids extracted from Arctic charr eggs were used as standards.

EXTRACTION:

Weigh sample into large screw-cap tube (25 mm x 150 mm). Use dry or wet samples - 0.4 g dry feed or eggs or 1.0-1.5 g wet eggs.

Add 5.0 ml chloroform
10.0 ml methanol
4.0 ml distilled water for a dry sample*
or 3.4 ml for a wet sample.
Homogenize 20 sec.
Add 5.0 ml chloroform
5.0 ml distilled water.
Homogenize 10 sec.
Rinse homogenizer between samples with chloroform:methanol (1:1) and discard rinse.

Filter homogenate through a Stefi sintered glass funnel using Whatman No. 1 filter paper (42.5 mm) into a medium screw-cap tube (20 x 150 mm). Rinse funnel between samples with chloroform:methanol (1:1) and discard rinse.

Allow layers to separate overnight in refrigerator. Many samples can be processed to this step until ready to proceed with the assay. The upper layer will protect the lower layer from any chance of evaporation. When ready to conduct the colourimetric assay, aspirate off and discard upper methanol-water layer. Transfer chloroform layer to a small screw-cap tube (16 x 125 mm).

Appendix 3b. Continued.

ASSAY:

Transfer aliquot of the lower chloroform layer (ie. 100-250 µl or sufficient for approx 1 mg lipid) to a 20 mm x 150 mm tube (not screw-cap). Rinse pipette between samples.
Evaporate solvent in a stream on nitrogen or other gas or allow to dry in fume hood. While evaporating, place tubes in a warm water bath or a heating block at 70°C. Tubes must be heated for 10 min at 70°C after solvent has evaporated in order to ensure no traces of solvent remain. This is critical in order to obtain good replicates.

Add 2.0 ml of 0.167M potassium dichromate to each tube.
Add 4.0 ml concentrated sulphuric acid. Mix by vortex.
Heat in oven at 115°C for 30 min (set our oven at 225 F). Leave an empty row between tubes in the rack and do not overload oven with more than 2 racks.
Cool for 3 minutes at room temperature.
Add 2.0 ml distilled water and mix with vortex.
Cool in water bath or sink of cold water. Mix with vortex.
Read absorbance at 600 ηm in 1 cm cells using blank as a reference.

STANDARD CURVE:

Standard: 1.0 g/100 ml = 10.0 mg/ml
Add 25-200 µl standard ie. 0.25-2.00 mg lipid.

The standard curve is not linear beyond 2 mg lipid.

CALCULATION:

$$\% \text{ lipid} = \frac{\text{absorbance} \times \text{total volume chloroform used} \times 100}{\text{slope} \times \text{volume aliquot} \times \text{sample weight} \times 1000}$$

Where: volume of chloroform and volume of aliquot in ml
sample weight in gm
1000 converts mg in standard to gm.
slope = slope of standard curve = absorbance/mg lipid

Appendix 4. Statistically significant regressions of of various parameters on fecundity, fertilization and survival of eggs in Year 1 and Year 2.

Dependent variable Y	Parameter X	Regression equation	r^2	Signif.
Year 1:				
No. eggs released	Fish wt. (g)	Y = 2.40 X + 501.07	0.315	0.0001
No. eggs produced	Fish wt. (g)	Y = 2.71 X + 351.88	0.334	0.0001
% Fert.	Spawning day	Y = 0.299 X + 73.58	0.143	0.004
% Fert.	No. Eggs	Y = 0.0038 X + 72.88	0.147	0.004
% Eyed	No. Eggs	Y = 0.0067 X + 40.73	0.142	0.005
% Hatch	No. Eggs	Y = 0.0059 X + 32.18	0.083	0.033
% Swimup	No. Eggs	Y = 0.0059 X + 24.80	0.091	0.025
Year 2:				
% Fert.	Fish wt. (g)	Y = -0.009 X + 101.25	0.112	0.040
No. eggs released	Fish wt. (g)	Y = 3.47 X + 2061.01	0.844	0.0001
No. eggs produced	Fish wt. (g)	Y = 3.88 X + 2202.25	0.853	0.0001
No. eggs produced	Spawning day	Y = -109.74X + 11056.72	0.249	0.002
% Fert.	Egg wt (mg)	Y = 0.75 X + 41.10	0.118	0.037
% Hatch	Egg wt (mg)	Y = 1.43 X - 44.54	0.118	0.037
% Swimup	Egg wt (mg)	Y = 1.32 X - 44.29	0.108	0.047
% Eyed	NL (%)	Y = -17.29 X + 233.36	0.263	0.0032
% Hatch	NL (%)	Y = -18.24 X + 236.20	0.321	0.0009
% Swimup	NL (%)	Y = -18.44 X + 232.38	0.338	0.0006
% Eyed	NL 16:1n7 (%)	Y = -13.68 X + 202.52	0.238	0.006
% Hatch	NL 16:1n7 (%)	Y = -12.92 X + 186.58	0.234	0.007
% Swimup	NL 16:1n7 (%)	Y = -12.57 X + 176.66	0.228	0.008