MEASURES OF EGG QUALITY AND HATCHERY PERFORMANCE OF ARCTIC CHARR (Salvelinus alpinus L.) AND ATLANTIC SALMON (Salmo salar L.)

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MEASURES OF EGG QUALITY AND HATCHERY PERFORMANCE OF ARCTIC CHARR (*Salvelinus alpinus* L.) AND ATLANTIC SALMON (*Salmo salar* L.)

by

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ABSTRACT

The present study examined how morphological, biological and biochemical characteristics of eggs correlated with overall performance (survival, growth) of eggs and alevins of Arctic charr (Salvelinus alpinus) and Atlantic salmon (Salmo salar). In addition, the effect of time of stripping on biochemical composition of laboratory-reared charr eggs was studied, and the biological, morphological and biochemical egg quality criteria of cultured and wild Atlantic salmon (anadromous) were compared and evaluated. Fertilization and hatching success, growth and survival of developing embryos, alevins and fry were recorded as biological measures of egg quality. These measures were compared to levels of protein, lipid, carbohydrate, moisture, ash, total and free amino acids as potential biochemical indicators, and to egg size, yolk-sac volume at hatching, alevin size at hatching and age at hatching as potential morphological indicators of egg quality.

For Arctic charr, eggs collected in the middle of the spawning period had the highest protein, lipid, carbohydrate and energy content, which were associated with higher fertilization and hatching success, and growth and survival of embryos, alevins and fry, than that of eggs collected early or late in the spawning period. Eggs collected from wild Atlantic salmon had higher protein, lipid, carbohydrate

and energy content and concomitantly higher fertilization and hatching success, faster development and greater growth and survival of embryos, alevins and fry than those collected from cultured stock. The total amino acid pool, and the protein, lipid, carbohydrate and energy content of eggs, alevins and fry decreased simultaneously during embryonic development of both Arctic charr and Atlantic salmon because they were utilized in metabolic processes.

Egg diameter and alevin length were positively correlated with egg weight and alevin weight, respectively. There was no correlation between egg weight and alevin weight or egg diameter and alevin length at hatching for either Arctic charr or Atlantic salmon.

The amino acids, serine, valine, tryptophan, lysine, isoleucine and threonine were important for growth and survival of embryos, alevins and fry of Arctic charr. However, alanine, aspartic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine were important for growth and survival of embryos, alevins and fry of Atlantic salmon.

It is suggested that energy level and/or amino acid content of eggs could be used as a condition index for the future development, growth and survival of embryos and alevins of salmonids.

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CHAPTER 1. GENERAL INTRODUCTION

In previous studies, many different definitions of "egg quality" have been used. To date, there does not appear to be a comprehensive definition of egg quality (Kjorsvik et al., 1989). The criteria used to assess egg quality have ranged from biochemical to biological to morphological.

Biochemical criteria have included amounts and types of proteins (Buckley, 1984; Craik and Harvey, 1984), lipids (Fraser et al., 1987, 1988; Fraser, 1989; Nomura et al., 1974; Soivio et al., 1989), amino acids (Fyhn and Serigstad, 1987; Fyhn et al., 1987) and carotenoid pigments (Craik, 1985; Craik and Harvey, 1986) of eggs. Recently, RNA-DNA (Buckley, 1984; Buckley and Lough, 1987; Clemmesen, 1987; Robinson and Ware, 1988) and triacylglycerol-sterol ratios (Fraser et al., 1987; Fraser, 1989) of eggs have been used as condition factors for growth of marine fish larvae.

Biological criteria have included fertilization rate (Kjorsvik and Lonning, 1983; McEvoy, 1984; Springate et al., 1984), hatching percentage (Thorpe et al., 1984), percentage starting to feed (Thorpe et al., 1984) and percentage of deformed larvae (Devauchelle et al., 1988).

Morphological measures have included egg diameter (Bagenal, 1967; Kjorsvik et al., 1984; Springate and Bromage, 1985), egg weight (Bagenal, 1969; Devauchelle et al., 1988), volume (McEvoy, 1984) and chorion appearance

(McEvoy, 1984, Springate and Bromage, 1985).

The most widely accepted definition of egg quality is "the egg's potential to produce viable fry" (Kjorsvik et al., 1989). Studies on egg quality have been investigated by a few workers (Mollah and Tan, 1983; Springate et al., 1984; Craik, 1985), but the above-mentioned biochemical, biological and morphological egg quality criteria have not been correlated together with growth and survival of embryos, alevins and fry.

In oviparous fishes, the yolk is the only source of nutrients and energy for developing embryos and alevins. As the egg water-hardens, the micropyle closes and there is no further chance for fertilization (Terner, 1979; Gilkey, 1981). After water hardening, the chorion permits gas exchange but is relatively impervious to most solutes, such as amino acids and nucleotides (Neyfakh and Abramova, 1974). Both the rate of yolk absorption and the efficiency of yolk utilization are important determinants for early development, growth and survival (Heming and Buddington, 1988). There are strong selective pressures synchronizing completion of yolk absorption, development of the capability of feeding, and the availability of suitable food (Bams, 1969; Rosenthal and Alderdice, 1976).

Successful embryonic development has been found to depend on several factors such as protein and RNA-DNA ratio (Bulow, 1970 and 1987; Ullrey et al., 1977; Buckley, 1984),

types and amounts of amino acids (Fyhn and Serigstad, 1987; Fyhn et al., 1987), digestive enzyme (Pedersen et al., 1987); oxygen (Gruber and Wieser, 1983), temperature (Peterson et al., 1977), types and amounts of lipids (Fraser, 1989; Soivio et al. 1989) and carotenoid pigments (Craik and Harvey, 1986). The egg's potential to produce a viable fry is determined by the above factors. If one of the essential factors is lacking or is inadequate, egg development will likely fail at some stage.

In fish, carbohydrates do not contribute much and the main source of energy is thought to be protein and lipid (Walton and Cowey, 1982). It would be speculated that eggs with higher caloric values would produce alevins with higher energy content at hatching.

Amino acids are precursors for many biological compounds, notably proteins, and are also substrates for energy production. In addition, the membrane transport system, specific acetylating enzymes, transfer RNA, cellular energy sources, and other substrates must be available for protein synthesis. Deficiencies or excess of one or more of these amino acids can limit the rate of protein synthesis. Imbalances of the amino acids are known to limit either protein synthesis (Tews et al., 1979) or growth of animal (Harper et al., 1970) or both (Tews et al., 1980). Following fertilization and during development of the embryo, the yolk proteins are resorbed and degraded, and the amino acids are

utilized for the synthesis of somatic proteins (Love, 1980).

The importance of amino acids to the embryonic development of fish has not been thoroughly investigated in spite of their significance for growth and survival. Essential amino acids can not be synthesized by animals and must be supplied in the diet (Walton, 1985). There are two main sources of amino acids, namely the diet and catabolism of body proteins which are in a state of constant turnover. Amino acids are primarily required for the synthesis of new body proteins and for other compounds with special properties such as hormones. The excess amino acids are rapidly deaminated, the amino group being ultimately excreted as ammonia and the carbon skeleton being oxidized via the tricarboxylic acid (TCA) cycle for energy or, in some cases, converted to glucose or lipid.

Lipids are major constituents for biomembranes and are important energy reserves in developing fish embryos (Atchison, 1975; Tocher and Sargent, 1984; Falk-Peterson et al., 1986). Triacylglycerol (TGA) is the major fraction of the storage lipid (Lehninger, 1975) and hence a form of energy storage in eggs (Kaitaranta, 1980; Kaitaranta and Ackman, 1981; Brind et al., 1982; Tocher and Sargent, 1984; Falk-Petersen et al., 1986), yolk-sac larvae (Eldridge et al. 1982; Vetter et al. 1983; Fraser et al., 1988) and adults (Love, 1980) of many fish species. Triacylglycerol is catabolized by pre-feeding, yolk-sac larvae of Atlantic

salmon, Salmo salar (Cowey et al., 1985), Atlantic herring, Clupea harengus L. (Fraser et al., 1987), Atlantic cod, Gadus morhua L. (Fraser et al., 1988), European sea bass, Dicentrarchus labrax (Quessada and Pionetti, 1987). Triacylglycerol continues to be depleted during the early stages of development, until the energetic demands of growth and metabolism are met from exogenous sources. When exogenously derived energy ultimately exceeds the immediate metabolic demands of a larva, excess energy can be stored as TGA (Clupea harengus L., Fraser et al., 1987; Pleuronectes platessa, Ehrlich, 1974a). In contrast, when exogenously derived energy is insufficient to maintain the basal metabolism of the larvae of these species, endogenous TGA is catabolized (Ehrlich, 1974a,b; Fraser et al., 1987).

Lipids are the most important chemical components among the constituents of broodstock diet which affect the composition of eggs (Watanabe, 1985). Diets deficient in essential fatty acids (EFA) resulted in low egg production and low egg quality in rainbow trout (Watanabe et al. 1984).

The fish oocyte synthesizes and accumulates many macromolecules (such as tRNA, mRNA) during oogenesis (Denis, 1977; Denis and Wegnez, 1977; Picard and Wegnez, 1979), which provide a reservoir of materials used during various biosynthetic activities essential for the early stages of embryogenesis. Several energy reserves, glycogen, lipid (triglycerides, neutral lipids, fatty acids) and protein

(mainly phosphoproteins, and lipoproteins) are accumulated within oocytes during oogenesis of teleost fish. After fertilization, the energy demand of the egg increases as the embryo develops. Embryonic respiration also continues to increase. The embryo undergoes intensive cellular multiplication (cleavage), and then cellular movements (gastrulation and epiboly) which require great quantities of energy rich molecules (ATP and ADP).

It is known that the size and weight of the eggs of fish can vary both within and between populations, and that the variations can be seasonal, geographical, genetic and age dependent (Bagenal, 1971; Hempel, 1979). The availability of food also affects egg size (Springate and Bromage, 1985; Springate et al., 1985). Intraspecific studies have concluded that large eggs produce large hatchlings possessing large yolk reserves relative to smaller eggs (Blaxter, 1969, 1988; Hempel, 1979). Dahl (1918 and 1919) found that, under the same environmental conditions, large alevins would grow more quickly than small ones. He further concluded that alevins from large eggs have a greater interval of time in which to establish exogenous feeding. The large eggs not only produced larger alevins but these larvae survived longer in the absence of external food (Blaxter and Hempel, 1963). This may be a selective advantage under the competitive conditions which prevail in wild stocks of fish.

In salmonids, not only do larger females produce more eggs, but they also produce larger eggs (Sargent et. al., 1987). Van den Berghe (1984) found in coho salmon, larger females compete for better oviposition sites, guard their nests more successfully and thus have higher rates of egg survival. Smith and Fretwell (1974) assumed that a female has a finite amount of energy to devote to egg yolk and that natural selection favours a female that allocates her investment in each egg so as to maximize the number of her offspring that survive to reproduce. In general, larger and older females produce greater number of larger eggs than smaller and/or younger individuals (Buss and McCreary, 1960; Gall, 1969).

Although there is considerable evidence among species that larger eggs have slower development rates (Balon, 1984; Paine, 1985), very little is known about the relationship between egg size and developmental rate within a species or population. Preliminary evidence, however, suggests that offspring from large eggs take longer to resorb their yolk sacs and enter the juvenile stage within species of salmonids (Salmo salar, Privol'Nev, 1960; S. gairdneri, Escaffre and Bergot, 1984; Oncorhynchus tshawytscha, Rombough, 1985). However, conflicting results have been reported as to whether egg size would confer any permanent or long-term advantages as far as growth and survival of the fry are concerned. Some authors reported no lasting effects

vitellogenin is subsequently processed into the yolk proteins, lipovitellin and phosvitin, and the receptors are either degraded or recycled to the surface of the cell to bind more of the ligand.

Proteins obtained from the maternal circulation later serve as amino acid and energy sources for the developing embryo (Tyler et al., 1988). The process of maternal-egg protein transport has been studied in only a few species of birds and amphibians, but little is known in fish (Tyler et al., 1988).

The present study examines how morphological, biological and biochemical characteristics of eggs correlate with overall performance (survival, growth) of eggs and alevins of Arctic charr (Salvelinus alpinus) and Atlantic salmon (Salmo salar). This study is divided into two topics:
(a) Egg quality, and effects of time of stripping on biochemical composition of eggs, and development, growth and survival of embryos, alevins and fry of Arctic charr (Chapter 3).
(b) A comparative study of egg quality of cultured and wild (anadromous) Atlantic salmon (Chapter 4).

of egg size on subsequent development (Zonova, 1973) whereas others found that these differential effects persist through the early life of the fish (Pitman, 1979).

Recent observations have found that hormones involved in sex maturation are also very important for egg development and survival (Brown et al. 1987, 1988, 1989; Leatherland et al., 1989a, 1989b; Greenblatt et al., 1989). Brown et al. (1989) reported that the presence of triiodo-L-thyronine (T_3) and L-thyroxine (T_4) in egg yolk of stripped bass (Morone saxatilis) were of maternal origin, and significantly increased hatching of the embryos and development of larval stages prior to the onset of endogenous thyroid hormone production. In salmonids, sexual maturation is mediated through the hypothalamic-pituitary-ovarian axis. During the reproductive period an increase in plasma proteins takes place when the female-specific protein vitellogenin (VTG, glycolipophosphoprotein) is synthesized in the liver and exported to the blood to be sequestered by the gonads for the formation of yolk proteins (Wallace, 1985). The stimulatory action of estrogen on vitellogenin production is universal throughout the nonmammalian vertebrate classes (Ng and Idler, 1983). The presence of vitellogenin in the blood depends on the transcription of the vitellogenin gene(s) and translation of the messenger RNA, and on the modifications of the protein and the export from the liver cells to the blood (Wallace, 1985). The

CHAPTER 2. GENERAL MATERIALS AND METHODS

2.1 Origin of Broodstock

The origins of broodstock of Arctic charr and Atlantic salmon are described in chapter 3.2 and 4.2 respectively.

2.2 Broodstock Identification and Stripping

Sexual identification was done on the basis of secondary sex characteristics such as presence of kype in males, and enlarged and red urogenital papilla in females. The fish were gently captured and wiped to prevent water and mucus from dripping into the container while stripping (Baker, 1980). The fish was stripped by hand using 3-4 long, smooth strokes from the pectoral fins to the vent.

Unlike females, the male can be spawned more than once during the spawning season. The male was captured and milt extruded into a clean, glass jar. Sperm viability was checked by placing a drop of milt on a microscope slide with a drop of water. The fluid activated sperm motility. If the male has viable sperm, the flagellum of sperm would be in motion.

2.3 Mating Design

For both species, eggs were stripped from four females and thoroughly mixed. Two thousand eggs were separated and fertilized with the mixture of milt collected from 3 males, and divided in half (two replicates). After 2-4 minutes, water was added. Eggs were stirred and water was poured off to remove excess milt and other debris. Eggs were disinfected with germicide (wescodyne @ 0.45%) after fertilization. The washing process was repeated 2-3 times. After that water was added and left for 1 hour to allow the egg to water-harden. The entire process of fertilization was done in dim conditions.

2.4 Incubation

After water hardening, the eggs were transferred to an incubator. Each tray of the incubator was divided into four equal parts. Each portion of the tray incubated 1000 eggs. Water temperature varied from $6\text{-}8^{0}$ C during incubation (Swift, 1965). The ammonia level was tested from time to time and dead eggs were removed and recorded regularly during incubation. After hatching, alevins were transferred to 12 litre buckets.

2.5 Sampling

Twenty eggs, alevins or fry were randomly selected for measurements (egg weight and diameter, fork length and wet weight of alevins and fry, yolk-sac dimensions of alevins at hatching) at the following stages; before fertilization, after fertilization, eyed-stage, hatching, first feeding, and one month after first feeding. The criterium for the various stages was when atleast 75% eggs were eyed, hatched or when at least 75% of the alevins began feeding. Yolk-sacs usually resemble prolate spheroids and their volume (mm^3) can be estimated using the formula $V = (\pi/6)L.H^2$ (Blaxter and Hempel, 1963) where L is the yolk-sac length and H its height in millimetres. Time to eyed-stage, hatching and exogenous feeding were recorded. Fertilization success, survival to eyed-stage, hatching success, number of deformed alevins at hatching, survival to first feeding and one month after first feeding were also recorded.

2.6 Rearing of Alevins and Fry

Rearing of alevins and fry of Arctic charr and Atlantic salmon are described in chapter 3 and 4 respectively.

2.7 Biochemical Analyses

For biochemical analyses, samples of 150 eggs, alevins or fry were randomly collected from each replicate (group) at the above mentioned stages. All eggs were thoroughly homogenized, and subsequent sub-samples (three from each replicate) were taken from the homogenized tissues for biochemical analyses.

2.7.1 Moisture Determination

Moisture content was determined by drying about 1 g of the sample into a pre-weighed foil plate. The sample was then dried in a forced-air oven at 105^0 C overnight or until a constant weight was obtained (AOAC, 1980).

2.7.2 Ash Determination

Ash content was determined by charring about 0.5 g of the sample in a weighed crucible over a Bunsen burner and then heating in a muffle furnace at 550^0 C until the ash had a white appearance.

2.7.3 Crude Protein Content

The content of crude protein in the sample was

determined by digestion of a known amount of sample in concentrated H_2SO_4 solution. The nitrogen was converted to ammonia in the form of ammonium sulphate. Upon the addition of base (NaOH) and distillation, the released ammonia was collected in a 4% boric acid solution and was subsequently titrated with a standard H_2SO_4 solution. The nitrogen content was then calculated (micro-Kjehdal method). The crude protein content was then calculated as %N x 6.25 (Bradstreet, 1965).

2.7.4 Lipid Determination

The lipid analysis was done by chloroform-methanol extraction (Bligh and Dyer, 1959; Folch et al., 1957). Ten g of tissues was homogenized with the mixture of 10 ml chloroform and 20 ml methanol for two minutes. To the mixture 10 ml chloroform was further added and after blending for 30 seconds, 10 ml distilled water was added and blending continued for another 30 seconds. The homogenate was filtered through Whatman No. 1 filter paper on a Coors No. 3 Buchner funnel with slight suction. The filtrate was transferred to a 500 ml graduated cylinder, and, after allowing about five minutes for complete separation and clarification, the volume of the chloroform layer was separated which contained the total lipid. The solvent (Chloroform) was evaporated under vacuum and the yield of

the extracted lipid was calculated.

2.7.5 Carbohydrate Determination

Total carbohydrate was determined by the phenol - sulphuric acid procedure (Dubois et al., 1956) after extraction in hot 5% trichloroacetic acid as described by Barnes and Heath (1966). The colorimetric assay was carried out in triplicate.

2.7.6 Energy Content

In order to calculate total energy content per egg, alevin or fry, the amount of protein, lipid and carbohydrate were converted into calories as described by Crisp (1984). The conversion factors are as follows:

1 g protein = 5.65 Kcal,

1 g lipid = 9.45 Kcal,

and 1 g carbohydrate = 4.1 Kcal.

2.7.7 Amino Acids Determination (Total)

Amino acid levels were determined as described by Shahidi et al. (1990). Samples were freeze-dried and then hydrolysed for 24h at 110^0 C with 6N HCl (Blackburn, 1968). The hydrolysed amino acids were then separated and

identified (Shahidi et al., 1990). Tryptophan was determined separately according to the method of Penke et al. (1974). Cysteine and methionine were converted to cysteic acid and methionine sulphone respectively during the HCl hydrolysis (Blackburn, 1968). Therefore, they are reported as cysteine equivalent (cysteine eq. = 2 * cystine + cysteic acid), and methionine equivalent (methionine eq. = methionine + methionine sulphone).

2.7.8 Free Amino Acid Determination

For determination of free amino acid levels, samples were deproteinized with 10% sulfosalicylic acid (4 parts sample, 1 part sulfosalicylic acid) and diluted 1:2 with lithium citrate buffer pH 2.2, 0.3N Li (Mondino et al, 1972; Ohara and Ariyoshi, 1979). Deproteinized samples were analyzed with a Beckman 121 MB amino acid analyzer using Benson D - X8.25 resin and a single column, three buffer lithium method as per Beckman 121 MB - TB - 017 application notes.

2.8 Statistical Analyses

The Shapiro-Wilk statistic (N<50) was used to determine normality of the data. The data set were not normally distributed therefore nonparametric statistics were used.

Variables were compared using the Kruskal-Wallis test. When significant differences were found, the Mann-Whitney test was used to compare individual differences between groups. A regression analysis was used to determine the relationship between egg size and size of alevins at hatching. Procedures for these statistical analyses are described in Sokal and Rohlf (1981). A probability level of $P < 0.05$ was considered statistically significant. Computations were performed using the SAS (Statistical Analysis System, release 6.06) package.

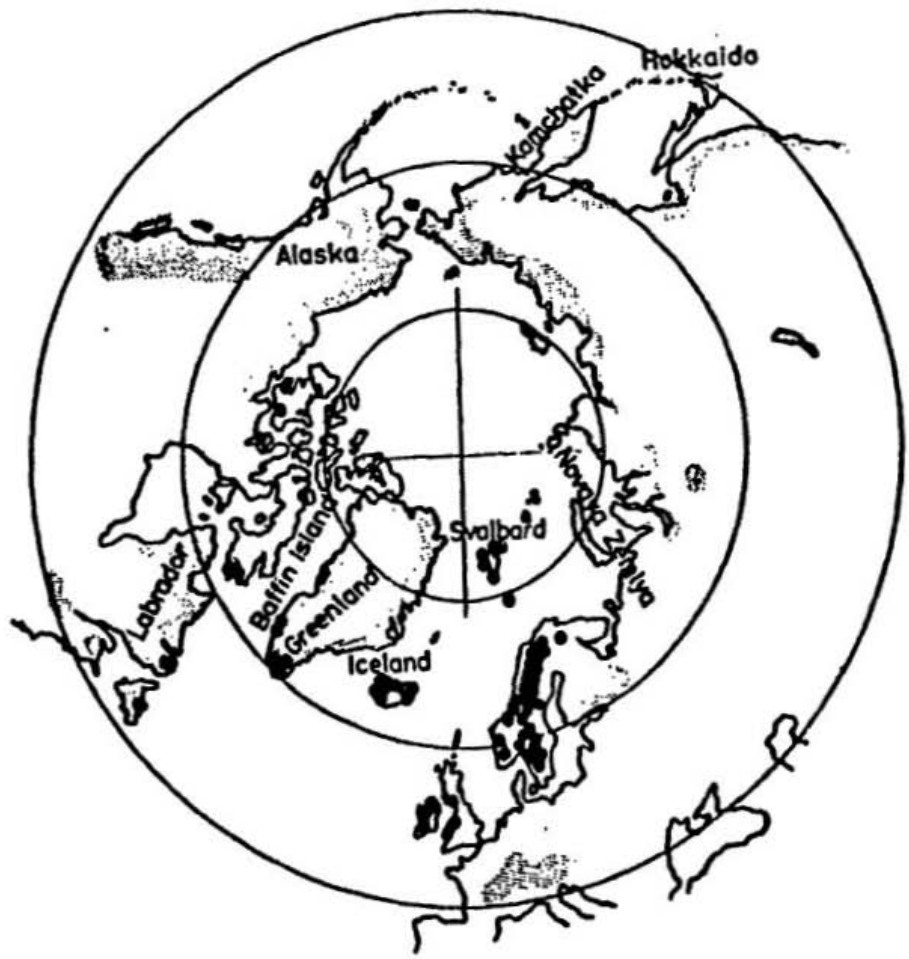
CHAPTER 3. EGG QUALITY, AND EFFECTS OF TIME OF STRIPPING ON BIOCHEMICAL COMPOSITION OF EGGS, DEVELOPMENT, GROWTH AND SURVIVAL OF EMBRYOS, ALEVINS AND FRY OF ARCTIC CHARR (Salvelinus alpinus L.).

3.1 Introduction

The Arctic charr (Salvelinus alpinus) has a circumpolar distribution in the northern hemisphere and occurs in nearshore marine waters, in lakes and rivers near the sea in North America, Asia, Europe, Novaya Zemlya, Iceland, and Greenland (Scott and Scott, 1988). Landlocked Arctic charr occur farther south than the anadromous form, including waters in the interior of the U.S.S.R., the Alps, the British Isles, and northern New England (Fig. 1)

In Canada, anadromous Arctic charr occur in Quebec (Vladykov, 1954; Saunders and Power, 1969; McAllister and Coad, 1974) and insular Newfoundland (Scott and Crossman, 1964; Rombough et al., 1978; Fig.1). Their distribution extends northward through coastal Labrador (Coady and Best, 1976; Dempson, 1982), the Arctic islands such as Baffin Island, in Hudson Bay, and westward to Alaska. Landlocked Arctic charr occur in New Brunswick lakes, widely in insular Newfoundland, Labrador, and lakes in extreme eastern Quebec and elsewhere through northern Canada, the Canadian

Figure 1: Circumpolar distribution of Arctic charr


Hokkaido
Kamchatka
Alaska
Novaya Zemlya
Svalbard
Baffin Island
Greenland
Labrador
Iceland

archipelago, and in Alaska in lakes and rivers near the coast (Scott and Scott, 1988). In Labrador, they are more abundant north of Hamilton Inlet being largely replaced by brook charr (Salvelinus fontinalis) and Atlantic salmon (Salmo salar) in southern Labrador (Dempson and Green, 1985).

Due to their circumpolar distribution, charr are well adapted to cold water (lethal freezing temperature 0.99^{0}C, Fletcher et al., 1988) and have a low optimum growth temperature relative to other salmonids (Swift, 1964 and 1965). Arctic charr are also capable of extremely high food conversion efficiencies in the wild, despite cold temperature and a short growing season (Johnson, 1980). These factors, combined with their gregarious nature (Jobling and Wandsvik, 1983 and 1986; Jobling, 1985; Wallace et al., 1988), high quality flesh and caviar (MacCrimmon and Gots, 1980) and high market demand (Iredale, 1984), make Arctic charr an ideal species for intensive culture utilization.

Arctic charr belong to the family salmonidae, subfamily salmoninae and genera Salvelinus (Johnson, 1984). It has the fusiform body shape and small head with terminal mouth (Bain, 1975). The body is somewhat rounded but shows great variability depending on size, sex, and state of maturity. Because of its wide geographical distribution, Arctic charr is quite variable and often occurs in different form with

differences in terms of fecundity and growth of anadromous and landlocked charr when raised under laboratory condition (Ringo, 1987 and 1988). On the other hand, Papst and Hopky (1984) have reported that wild Arctic charr had higher fecundity (mean fecundity = 4781) and larger egg diameter (mean egg diameter = 5.1 mm) than cultured stock (mean fecundity = 1769, mean egg diameter = 4.9 mm). Egg mortality prior to the eyed-stage was 83% in the cultured stock compared to 12% for wild stock, incubated under the same conditions.

Studies have reported that during the first cellular cleavages, the embryo depends upon the metabolic stores accumulated during the oocyte growth period to meet most of its energy demand (Terner, 1968a and 1968b; Terner et al., 1968; Boulekbache, 1981). Consequently, if fertilization of mature oocytes is delayed, part of the energy supply can be used up before fertilization, leading to an irreversible shortage of metabolic energy during the first cleavages of the egg (Boulekbache, 1981).

To date, the biochemical composition of Arctic charr eggs has not been determined and correlated with their embryonic development, growth and survival. As far as egg quality parameters are concerned, same studies have been done in which biological (eg. Krieger, 1987) and morphological (eg. Wallace and Aasjord, 1984a) measures of egg quality have been discussed, but they have not been

various life-styles and appearances together with genetic differences (McCart and Craig, 1971 and 1973; Qadri, 1974; McCart, 1980). Morphological features, e.g. the number of gill rakers, pyloric caeca and vertebrae, have been used to distinguish between the various forms. Each charr form (dwarf, normal, and large (predatory)) differs from the others in one or several of the following characteristic : (1) age and size of sexual maturity, (2) time and place of spawning, (3) feeding habits, (4) distribution in the water column, (5) spawning colour, (6) morphological characteristics, (7) degree of being anadromous versus stationary (Yoshihara, 1973; Eriksson and Wiklund, 1989).

Anadromous female Arctic charr usually deposit 3000-5000 large eggs, 4.0-5.0 mm in diameter (Moore, 1975a; Scott and Scott, 1988). Landlocked charr are usually smaller than anadromous forms and deposit fewer and smaller eggs (Scott and Scott, 1988). Females spawn every second or third year, which varies by location.

Wallace and Aasjord (1984a) showed that larger charr eggs produced larger alevins which grew faster. Alevins from larger eggs also displayed less initial mortality than alevins from smaller eggs. They concluded that a good egg size was from 4.4-5.1 mm even though they had larger eggs. The largest eggs produced a high proportion of abnormal alevins.

Recent studies in Norway have shown that there are no

correlated with development, growth and survival of embryos and alevins. Therefore, these investigations are not enough to draw any concrete conclusion about the egg quality criteria.

The initial purpose of this study were: (1) to determine the relationship of biochemical, biological and morphological characteristics of the eggs to the overall performance (survival, growth) of eggs and alevins of Arctic charr (Salvelinus alpinus), and (2) to determine which egg quality traits or characteristics would be most appropriate as condition indices for the growth and survival of embryos and alevins. In addition, during initial egg collecting it was observed that fish spawned over an extended period of time. It has been noted that the time of stripping has significant effects on biochemical composition of eggs (Craik and Harvey, 1984) and survival (Springate et al., 1984). Maturation and egg development is environmentally and biologically controlled. After ovulation, eggs are held in the abdominal cavity until females respond to the appropriate environmental and biological stimuli. After completion of this phase, eggs are naturally spawned and fertilized. In contrast to the natural environment, when salmonids are kept in captivity, such stimuli are usually absent, and eggs remain in the abdominal cavity for a long period and undergo gradual deterioration in their appearance (Nomura et al., 1974; Statova et al., 1982), viability

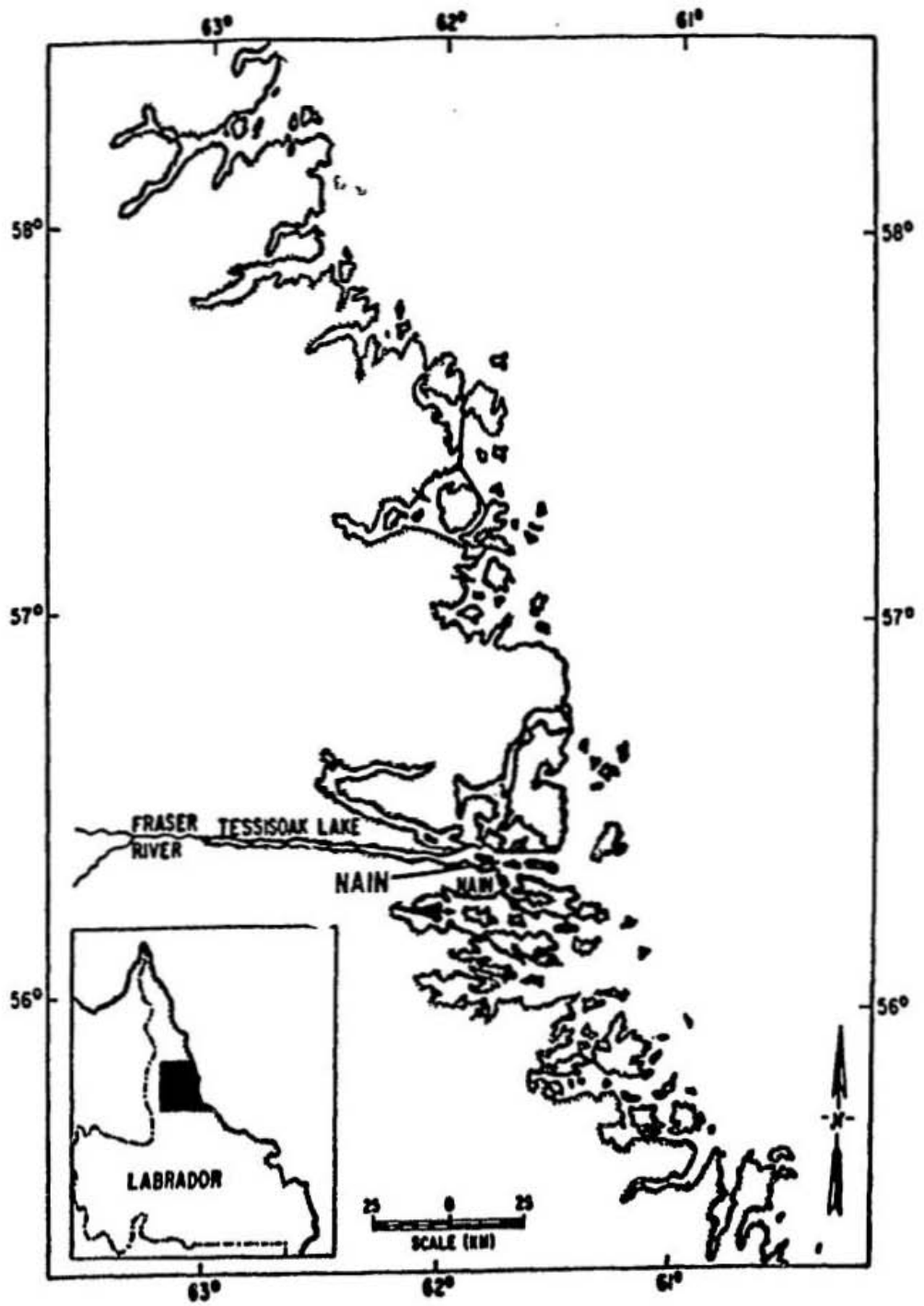
(Sakai et al., 1975; Hay, 1986) and biochemical composition (Craik and Harvey, 1984; Devauchelle et al., 1988). Over time, proteolytic breakdown of the yolk protein into small organic molecules, such as amino acids and peptides, may occur which would be lost through the chorion and resorbed into the maternal tissues (Takashima et al., 1975, Craik and Harvey, 1984). Therefore, over-ripening of eggs would lead to less nutrients and energy being available for embryonic development which may, in turn, result in poor survival of embryos and alevins. Therefore, in this study eggs were collected at three times (early, middle and late) over the spawning season to determine the effect that time of stripping may have on the biochemical composition and survival of eggs and alevins.

3.2 Materials and Methods

3.2.1 Origin of Broodstock

Broodstock originated from eggs collected from the Fraser River (56^0,39'N, 63^0,10'W, Fig. 2) and were raised at the Marine Sciences Research Laboratory, Logy Bay, Newfoundland. These broodstock were spawned for the first time. The broodstock were fed pelleted feed obtained from Corey Feed Mills Ltd., Fredericton, New Brunswick.

Figure 2: Origin of Arctic charr broodstock


63°
62°
61°
58°
57°
56°
FRASER RIVER
TESSISOAK LAKE
NAIN
NAIN
LABRADOR
25
0
25
SCALE (KM)

3.2.2 Mating Design

Fish were stripped three times (early, middle, and late) during the spawning season at 27 day intervals (November 17, December 14 and January 10). All the broodstock (n = 39, weight = 0.67 ± 0.17 kg, length = 39.3 ± 4.12 cm) were raised in a tank, and at each time four different fish were chosen. At each stripping, 2000 eggs from four females were fertilised with the milt of three males and divided in half (two replicates). After water hardening, the eggs were transferred to an incubator where water temperature varied from 6-8^0 C (Swift, 1965).

3.2.3 Incubation and Sampling

Incubation and sampling technique are described as in chapter two.

3.2.4 Rearing of Alevins and Fry

Yolk-sac fry were transferred to 12 litre buckets when most (>75%) of the yolk-sac was absorbed. Water temperature was maintained between 6-7^0 C with a light intensity of 50 lux and a 12h L: 12h D photoperiod (Wandsvik and Jobling, 1982; Jobling, 1987; Wallace et al., 1988). Fry were fed (feed obtained from Corey Feed Mills Ltd.) to satiation

throughout the experiment. Dead alevins / fry were removed regularly.

3.2.5 Biochemical Analyses

Biochemical analysis (protein, lipid, carbohydrate, free and total amino acids, dry matter and ash content) of eggs, alevins or fry was carried out as described in Chapter two.

3.3 Results

Data were not normally distributed (Shapiro-Wilk statistic), therefore nonparametric statistics (Kruskal-Wallis, and Mann-Whitney tests) were used. There were no significant differences between replicates of any of the variables studied ($P > 0.05$, Appendices 1, 2, 3, 4, 5 and 6), so replicates were pooled.

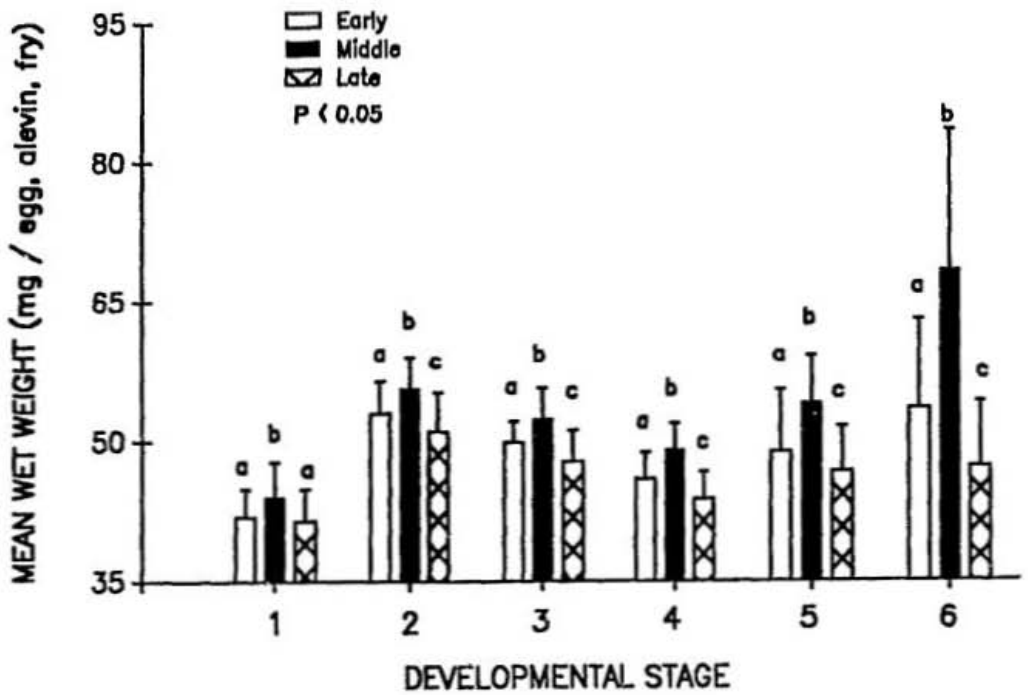
Eggs collected in the middle of the spawning season had significantly higher mean weight than eggs collected early or late season (Table 1; Fig. 3). Eggs collected in the middle of the spawning season also produced significantly heavier alevins at hatching compared to eggs collected early or late season (Table 1; Fig. 3). The percent increase in wet weight of fry from first feeding to one month after first feeding of the middle spawned group was the highest

TABLE 1.

Results of Kruskal-Wallis analysis (X^2) on egg variables of Arctic charr at three developmental stages (1 = before fertilization, 2 = after fertilization, 3 = eyed-stage) for eggs collected during the early, middle and late spawning season. S = Significance difference at $P < 0.05$, NS = No significance difference.

VARIABLES	STAGES 1		2		3	
	X^2	P	X^2	P	X^2	P
Overall (df = 2)						
Egg weight	10.88	S	23.69	S	27.86	S
Egg diameter	25.58	S	0.49	NS	0.93	NS
Protein	40.72	S	51.63	S	61.46	S
Lipid	44.29	S	50.96	S	72.92	S
Carbohydrate	66.91	S	24.51	S	15.02	S
Energy	42.95	S	50.89	S	66.53	S
Dry matter	40.56	S	48.97	S	55.47	S
Ash	42.95	S	23.69	S	15.29	S
Early vs. Middle (df = 1)						
Egg weight	7.09	S	9.61	S	9.12	S
Egg diameter	10.31	S	0.32	NS	0.06	NS
Protein	20.40	S	29.80	S	46.18	S
Lipid	20.40	S	21.52	S	46.18	S
Carbohydrate	33.64	S	12.94	S	13.96	S
Energy	20.40	S	29.17	S	46.18	S
Dry matter	20.40	S	29.80	S	36.05	S
Ash	11.54	S	9.61	S	1.39	NS
Early vs.Late (df = 1)						
Egg weight	0.26	NS	4.29	S	8.43	S
Egg diameter	3.67	NS	0.01	NS	0.56	NS
Protein	8.21	S	6.93	S	5.36	S
Lipid	14.97	S	14.66	S	12.06	S
Carbohydrate	15.43	S	2.23	NS	8.43	S
Energy	12.41	S	6.93	S	5.36	S
Dry matter	8.21	S	6.93	S	5.36	S
Ash	0.13	NS	4.29	S	8.43	S
Late vs. Middle (df = 1)						
Egg weight	8.89	S	21.62	S	24.43	S
Egg diameter	24.08	S	0.41	NS	0.78	NS
Protein	33.22	S	41.48	S	42.76	S
Lipid	33.22	S	41.48	S	53.87	S
Carbohydrate	53.09	S	21.62	S	0.20	NS
Energy	33.22	S	40.99	S	49.57	S
Dry matter	33.00	S	37.84	S	42.76	S
Ash	8.89	S	21.62	S	13.15	S

Figure 3: Mean wet weight (mg) of egg, alevin or fry at different stages of development for eggs collected during the early, middle and late spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.


Early
Middle
Late
P < 0.05
MEAN WET WEIGHT (mg / egg, alevin, fry)
95
80
65
50
35
a
b
a
a
b
c
a
b
c
a
b
c
a
b
c
a
b
c
1
2
3
4
5
6
DEVELOPMENTAL STAGE

(24.4%) compared to fry from the early (9.52%) or late (0.40%) spawned group. Before fertilization, eggs collected in the middle of the spawning season had significantly greater diameter than eggs collected early or late (Fig. 4). After fertilization, there were no significant differences in egg diameter among groups (Table 1; Fig. 4). Eggs collected in the middle of the spawning season produced significantly larger alevins and yolk-sac volume at hatching than eggs collected early or late ($P < 0.05$, Table 2; Fig. 5A), but there were no significant differences in alevin length and yolk-sac volume between early and late ($P > 0.05$, Figs. 5A and B). The same trend was noticed for fry at first feeding (Fig. 5A). One month after first feeding, fry from eggs collected in the middle spawning group were significantly larger than fry from eggs collected from late spawned group ($P < 0.05$, Table 2, Fig. 5A). The percent increase in body length of fry from the first feeding to one month after first feeding was highest (32.28%) for the early spawned group compared to fry from the middle (15.50%) or late spawned group (24.84%).

Egg weights and egg diameters were significantly positively correlated for all groups (Early: $r = 0.67$, $df = 1, 38$, $F = 31.37$; Middle: $r = 0.82$, $df = 1, 38$, $F = 80.78$; Late: $r = 0.71$, $df = 1, 38$, $F = 38.06$). There was a significant positive correlation between alevin weight and alevin length at hatching for the early and middle groups

Figure 4: Mean egg diameter (mm) at different stages of development for eggs collected during the early, middle and late spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

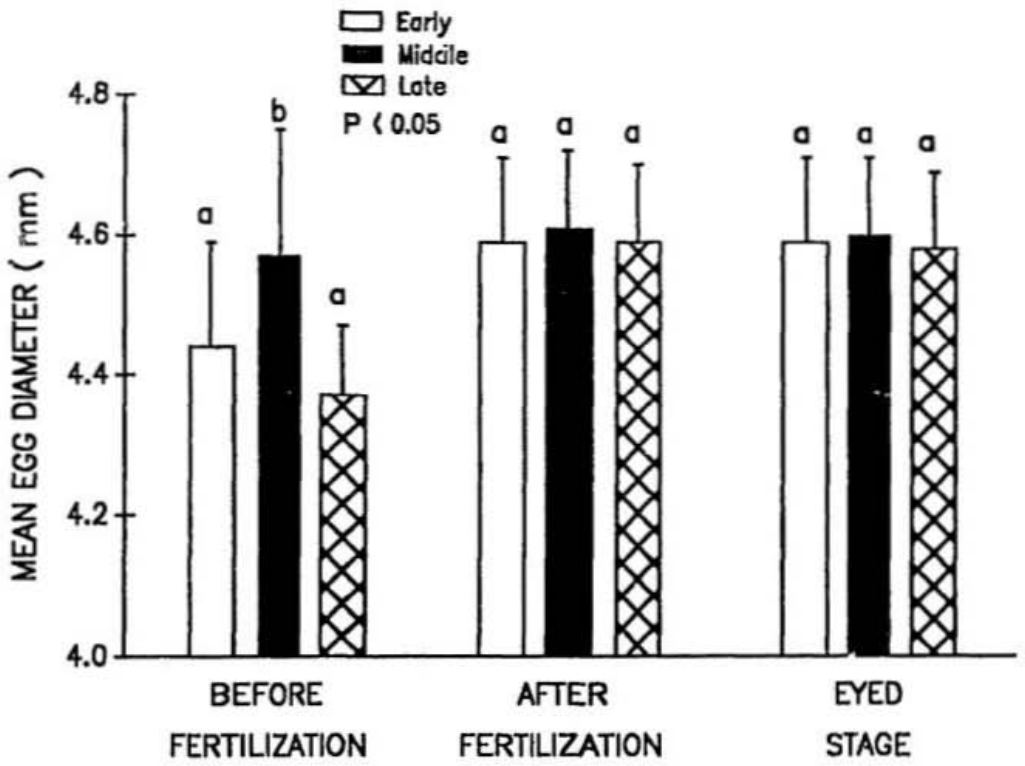
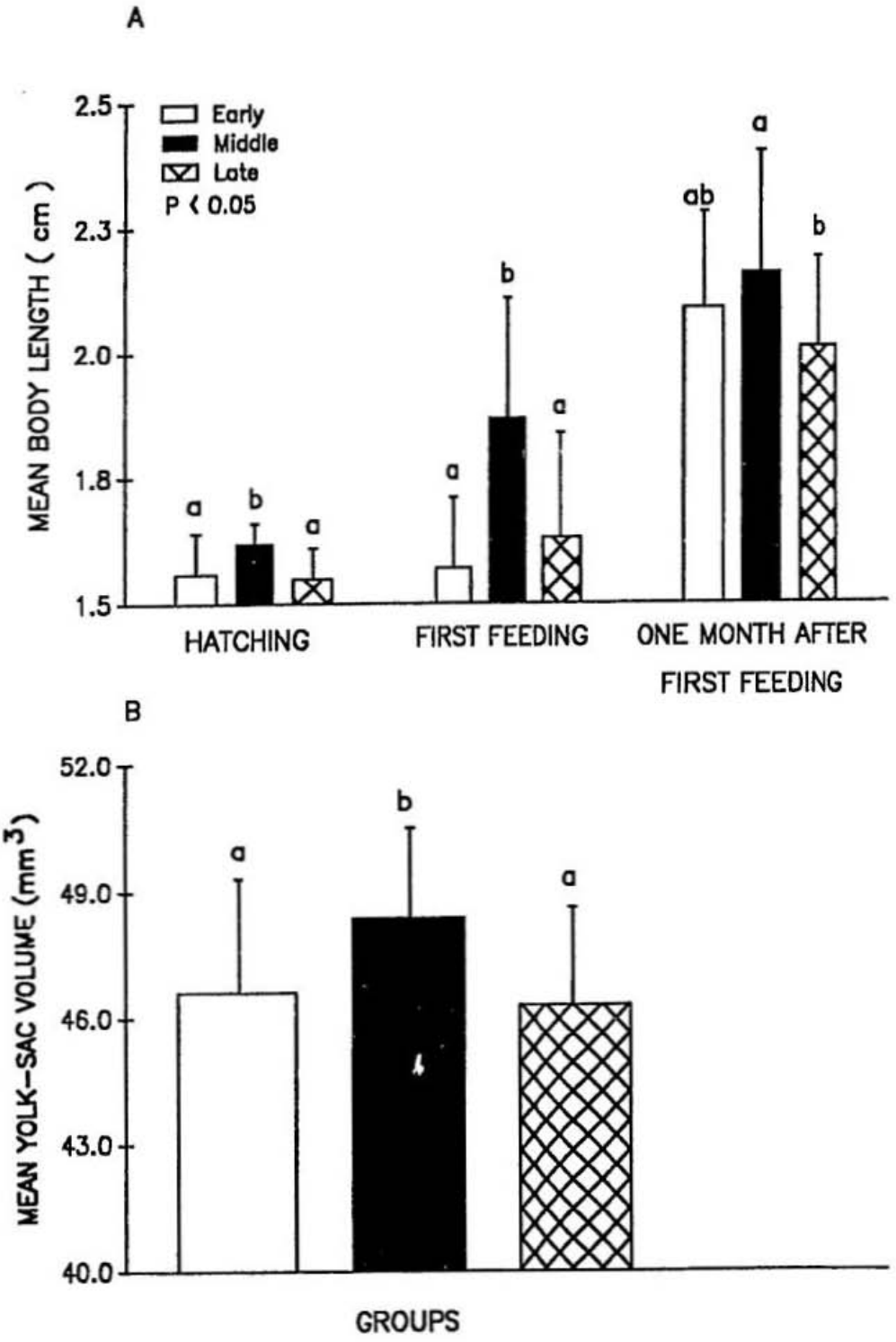

Early
Middle
Late
P < 0.05
MEAN EGG DIAMETER (mm)
4.8
4.6
4.4
4.2
4.0
a
b
a
a
a
a
a
a
a
BEFORE FERTILIZATION
AFTER FERTILIZATION
EYED STAGE

Figure 5: (A) Mean body length (cm) at different stages of development. (B) Mean yolk-sac volume (mm^3) at hatching for early, middle and late groups. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

TABLE 2.

Results of Kruskal-Wallis analysis (X^2) on fry variables of Arctic charr at three developmental stages (4 = hatching, 5 = first feeding, 6 = one month after first feeding) from eggs collected during the early, middle and late spawning season. S = Significance difference at $P < 0.05$, NS = No significance difference.

VARIABLES	STAGES 4		5		6	
	X^2	P	X^2	P	X^2	P
Overall (df = 2)						
Fry weight	45.58	S	35.91	S	40.31	S
Fry length	29.03	S	29.25	S	7.57	S
Protein	55.05	S	82.96	S	46.43	S
Lipid	81.31	S	98.85	S	43.64	S
Carbohydrate	19.70	S	9.16	S	62.79	S
Energy	66.08	S	89.53	S	45.63	S
Dry matter	47.81	S	81.12	S	46.64	S
Ash	3.26	NS	58.96	S	36.27	S
Early vs. Middle (df = 1)						
Fry weight	19.90	S	16.73	S	17.04	S
Fry length	16.76	S	24.92	S	1.88	NS
Protein	28.22	S	58.68	S	27.31	S
Lipid	51.82	S	59.27	S	17.05	S
Carbohydrate	0.87	NS	6.55	S	46.03	S
Energy	36.76	S	59.27	S	25.62	S
Dry matter	16.57	S	57.94	S	20.54	S
Ash	0.02	NS	30.83	S	20.20	S
Early vs. Late (df = 1)						
Fry weight	10.40	S	4.07	S	10.58	S
Fry length	0.84	NS	0.51	NS	3.12	NS
Protein	10.09	S	19.43	S	4.01	S
Lipid	16.97	S	43.71	S	14.01	S
Carbohydrate	10.40	S	7.05	S	0.04	NS
Energy	12.14	S	25.23	S	5.20	S
Dry matter	15.57	S	30.94	S	12.41	S
Ash	2.37	NS	9.84	S	3.49	NS
Late vs. Middle (df = 1)						
Fry weight	38.53	S	32.90	S	33.45	S
Fry length	28.47	S	18.19	S	6.47	S
Protein	44.99	S	50.85	S	38.41	S
Lipid	57.36	S	59.27	S	35.37	S
Carbohydrate	18.18	S	0.09	NS	47.74	S
Energy	51.68	S	57.21	S	37.81	S
Dry matter	40.22	S	37.93	S	37.93	S
Ash	2.49	NS	48.41	S	30.72	S

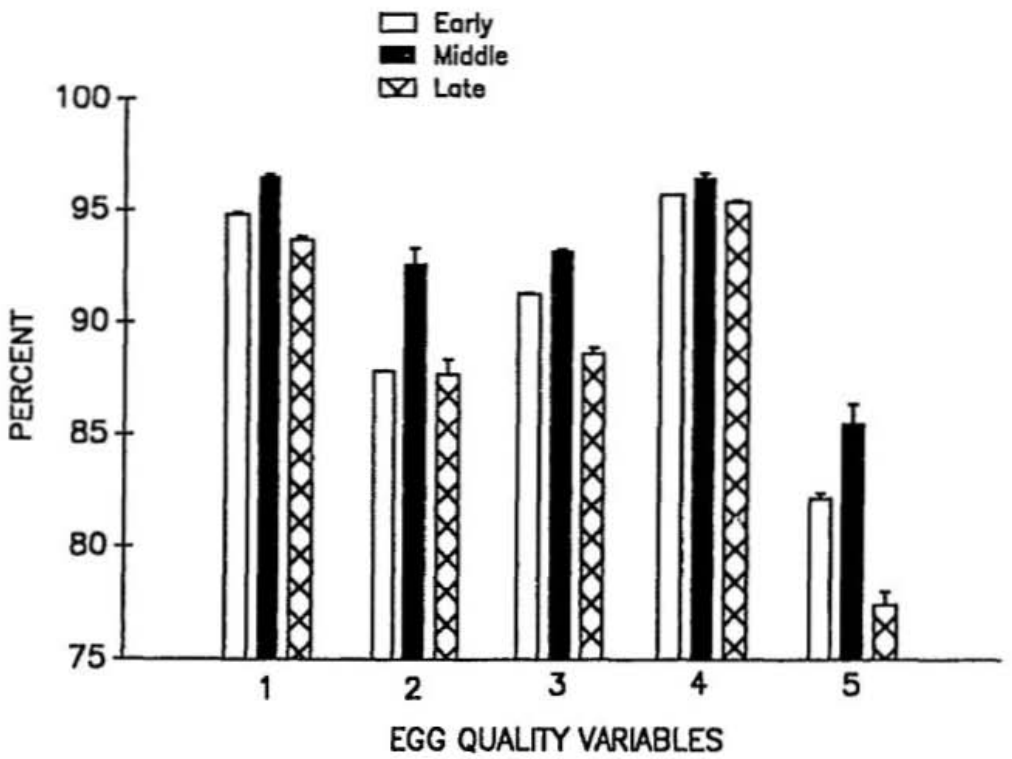

A
Early
Middle
Late
P < 0.05
MEAN BODY LENGTH (cm)
2.5
2.3
2.0
1.8
1.5
a
b
a
a
b
a
ab
a
b
HATCHING
FIRST FEEDING
ONE MONTH AFTER FIRST FEEDING
B
MEAN YOLK-SAC VOLUME (mm3)
52.0
49.0
46.0
43.0
40.0
a
b
a
GROUPS

(Early: r = 0.69, df = 1, 38, F = 33.94; Middle: r = 0.49, df = 1, 38, F = 12.29), but not in the late group (r = 0.19, df = 1, 38, F = 1.43). The correlation between egg weight and alevin weight at hatching was not significant (Early: r = 0.23, df = 1, 38, F = 2.23; Middle: r = 0.20, df = 1, 38, F = 1.60; Late: r = 0.00, df = 1, 38, F = 0.01). The correlation between egg diameter and alevin length at hatching was also not significant (Early: r = 0.0, df = 1, 38, F = 0.01; Middle: r = 0.14, df = 1, 38, F = 0.07; Late: r = 0.24, df = 1, 38, F = 0.24).

Survival at each stage was determined by comparison with the previous stage before collecting the sample for that stage. Fertilization success, survival to eyed stage, hatching success, survival to first feeding and one month after first feeding were significantly greater when eggs were collected in the middle of the spawning season than the early or late groups (Fig. 6). Eggs collected in the middle of the spawning season produced significantly fewer deformed alevins at hatching than the early and late groups (Early-Middle: F = 69.88, df = 1, 2; Early-Late: F = 111.97, df = 1, 2; Middle-Late: F = 151.64, df = 1, 2). The development of embryos and fry was faster when the eggs were collected in the middle of the spawning season compared to the early and late groups (Table 3).

The amount of protein and lipid per egg, alevin or fry from the middle group was always significantly greater than

Figure 6: Survival (percent) at various stages (from previous stage to present stage) of development for eggs collected during the early, middle and late spawning season. 1 = fertilization success, 2 = survival to eyed-stage, 3 = survival to hatching, 4 = survival to first feeding, 5 = survival to one month after first feeding. Values are means ± SD.


Early
Middle
Late
100
95
90
85
80
75
PERCENT
1
2
3
4
5
EGG QUALITY VARIABLES

PAGINATION ERROR.

TEXT COMPLETE.

ERREUR DE PAGINATION.

LE TEXTE EST COMPLET.

the early and late groups, and declined during development (Tables 1 and 2; Fig. 7). The amount of carbohydrate per egg, alevin or fry from the middle group was significantly higher than the early or late group before eyed-stage. At the eyed-stage and hatching, variation in carbohydrate content declined among groups, but it increased significantly after first feeding.

The dry matter content per egg, alevin or fry from the middle group was significantly higher than the early or late group, and declined through development (Tables 1 and 2; Fig. 8). The ash content per egg, alevin or fry from the middle group was also significantly higher than the early or late group, and declined until the eyed stage (Tables 1 and 4; Fig. 8).

The amount of protein, lipid and carbohydrate per egg, alevin or fry was converted into total caloric value in order to compare energy content. Eggs collected in the middle of the spawning period had significantly higher caloric value than those collected early or late, and these groups were significantly different in energy content at other stages of development (Tables 1 and 2; Fig. 9). Energy content of eggs declined during development with the minimum reached at one month after first feeding (Fig. 9).

Eggs collected in the middle of the spawning period had higher levels of serine, valine, aspartic acid, glutamic acid, threonine, proline, glycine, leucine, isoleucine,

Figure 7: Changes in the protein, lipid and carbohydrate content (mg / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different. $P < 0.05$.

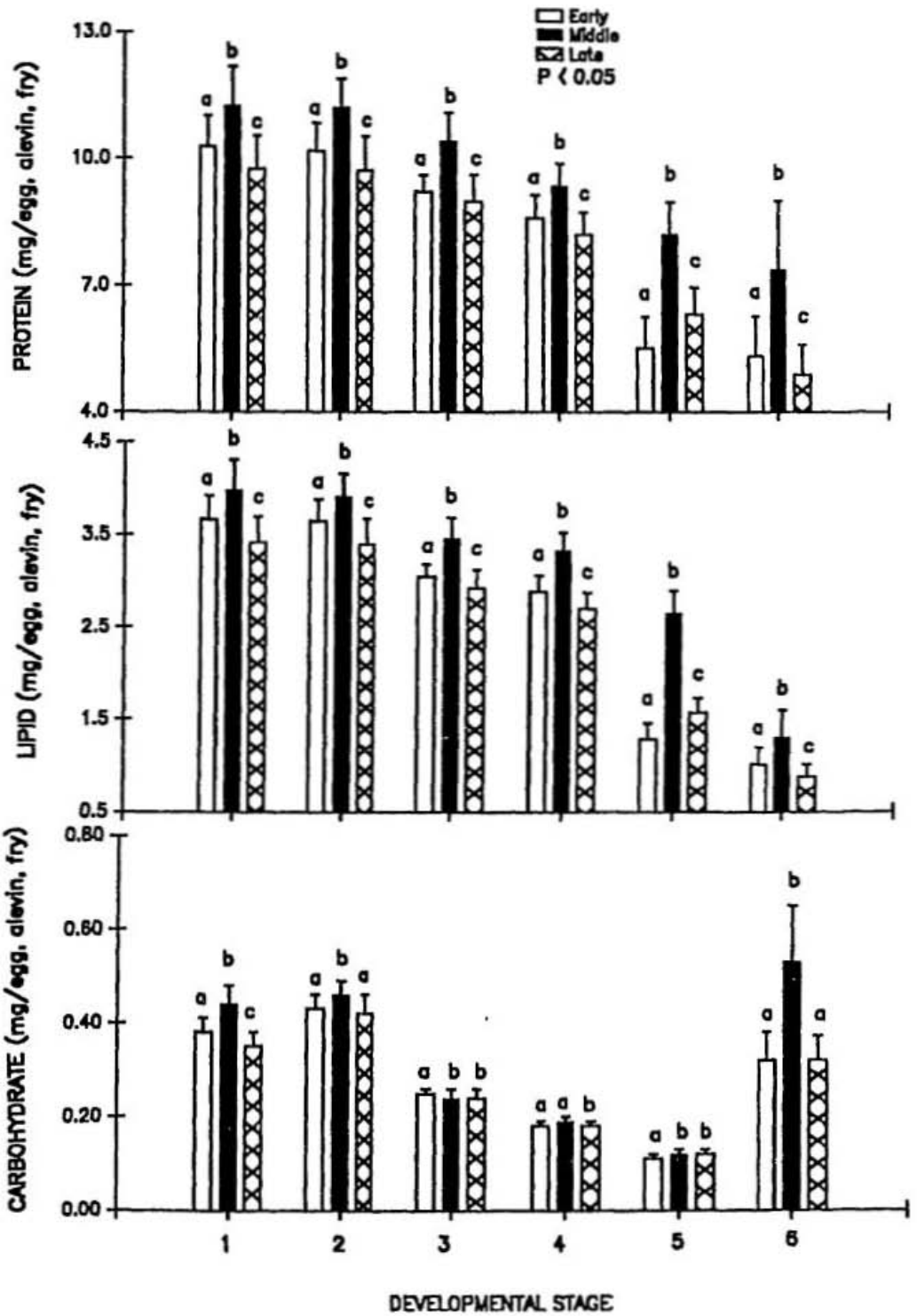

Early
Middle
Late
P < 0.05
PROTEIN (mg/egg, alevin, fry)
LIPID (mg/egg, alevin, fry)
CARBOHYDRATE (mg/egg, alevin, fry)
DEVELOPMENTAL STAGE

Figure 8: Changes in the dry matter and ash content (mg / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

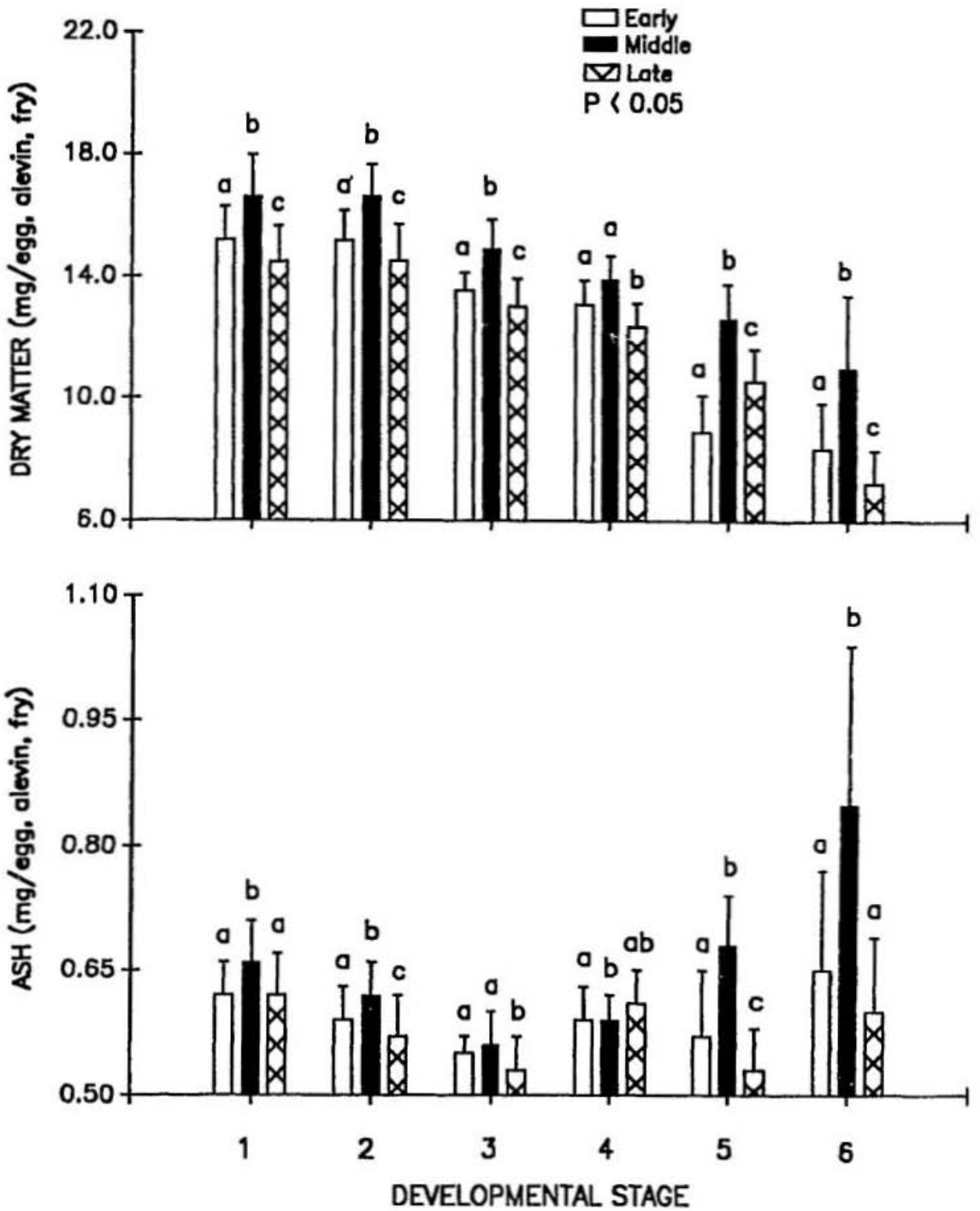
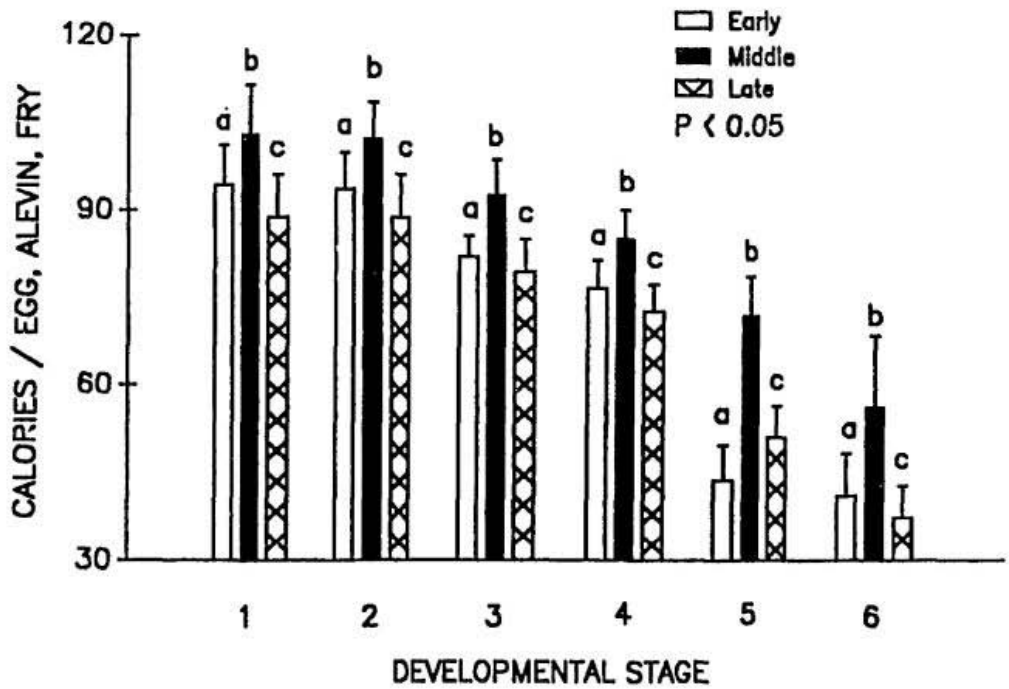

Early
Middle
Late
P < 0.05
DRY MATTER (mg/egg, alevin, fry)
22.0
18.0
14.0
10.0
6.0
ASH (mg/egg, alevin, fry)
1.10
0.95
0.80
0.65
0.50
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 9: Changes in the energy content (calories / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.


Early
Middle
Late
P < 0.05
CALORIES / EGG, ALEVIN, FRY
120
90
60
30
a
b
c
1
2
3
4
5
6
DEVELOPMENTAL STAGE

tyrosine, phenylalanine, tryptophan, histidine, arginine and lysine than the eggs collected early and late (Tables 4, 5 and 6). However, serine, valine, tryptophan, lysine, isoleucine and threonine were found to decrease during development in each group (Figs. 10, 11 and 12). Total essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, and valine; Walton, 1985) were higher in eggs collected in the middle of the spawning season (49487 nmoles / egg) than the eggs collected early (41907 nmoles / egg) and late (42808 nmoles / egg).

In all groups, the free amino acid pool was found to increase from before fertilization to one month after first feeding (Early: 404 nmoles/ egg to 8219 nmoles/fry ; Middle: 655 nmoles/egg to 4988 nmoles/fry; Late: 678 nmoles/egg to 1249 nmoles/fry). Total (free) essential amino acids were lower than the non-essential (free) amino acids in all the groups during development (Tables 7, 8 and 9).

During amino acid catabolism, the amino group is excreted as ammonia. The amount of ammonia formed by the developing embryo within eggs increased during this period with the highest value reached at hatching, and followed a rapid decline at first feeding (Fig. 13).

TABLE 4.

Levels of amino acid (nmoles / egg, alevin, fry) at different developmental stages of Arctic charr stripped early in the spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding, * = essential amino acids.

	Developmental Stages					
Amino Acid	1	2	3	4	5	6
Alanine	8959	9628	8537	8315	4461	4523
Arginine*	3310	3931	3672	3529	2093	2079
Aspartic acid	7350	7844	7126	6697	3315	3941
Cysteine eq.	1436	1369	1016	990	808	508
Glutamic acid	7338	8101	8161	7439	4542	5453
Glycine	3506	3952	4423	4410	4087	5417
Histidine*	1875	1979	1799	2033	1180	1054
Hydroxylysine	121	122	107	147	81	108
Isoleucine*	4759	5260	4841	4756	1682	2175
Leucine*	8003	10371	8102	7816	2760	3883
Lysine*	6085	6632	5555	5752	3151	3538
Methionine eq.*	1345	1565	1222	1210	918	879
Phenylalanine*	3225	3810	3506	3328	1684	1727
Proline	4233	5206	4927	3942	2016	1975
Serine	5385	5998	5338	5016	1954	2095
Threonine*	4382	4590	4420	4111	1978	2326
Tryptophan	371	504	431	364	186	181
Tyrosine*	2392	2661	2465	2316	988	944
Valine*	6530	7005	6206	6067	3388	2795
Essential	41907	47805	41789	40919	19822	21400
Non-Essential	39427	43978	40571	36851	19378	20759
Total	81334	91783	82360	77770	39200	42159

TABLE 5.

Levels of amino acid (nmoles / egg, alevin, fry) at different developmental stages of Arctic charr stripped in the middle of spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding, * = essential amino acids.

	Developmental Stages					
Amino Acid	1	2	3	4	5	6
Alanine	8274	7987	9479	8417	7652	5949
Arginine*	3990	4063	5300	3063	3254	2758
Aspartic acid	8024	8317	8217	7771	6327	5635
Cysteine eq.	1395	1669	1733	1272	1967	928
Glutamic acid	8151	8384	8471	7589	7139	6728
Glycine	4176	4209	3631	4256	5048	5861
Histidine*	1986	2007	1754	1803	2144	1560
Hydroxylysine	204	166	137	133	167	116
Isoleucine*	5816	5753	5192	3556	4300	2952
Leucine*	9373	9284	9555	7647	7379	5324
Lysine*	7256	7153	6606	5856	5592	4580
Methionine eq.*	1514	1835	1688	1411	1643	1149
Phenylalanine*	3807	3803	3105	2923	3114	2363
Proline	4876	5271	4130	3267	3513	2570
Serine	5852	5858	5011	3882	4421	3288
Threonine*	4908	4989	3780	3427	3691	3096
Tryptophan	526	512	265	243	239	282
Tyrosine*	2863	2834	2042	1861	2162	1382
Valine*	7973	8145	6581	6381	5787	3989
Essential	49487	49866	45602	37927	39067	29152
Non-Essential	41478	42372	41075	36830	36473	31357
Total	90965	92238	86677	74757	75540	60509

TABLE 6.

Levels of amino acid (nmoles / egg, alevin, fry) at different developmental stages of Arctic charr stripped late in the spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding, * = essential amino acids.

Amino Acid	Developmental Stages					
	1	2	3	4	5	6
Alanine	9085	8588	8973	8451	6271	3667
Arginine*	3475	3450	3726	3417	2733	1977
Aspartic acid	6949	6724	7557	6539	5244	3979
Cysteine eq.	1420	1492	1096	903	737	601
Glutamic acid	7245	7195	8247	7286	5905	4694
Glycine	3600	3460	4626	4488	4056	4231
Histidine*	1743	1672	2365	1767	1475	1041
Hydroxylysine	139	113	213	151	133	125
Isoleucine*	4938	4621	4572	4674	3630	2010
Leucine*	7951	7615	8307	7438	5814	3617
Lysine*	6237	5993	6127	5571	4337	2907
Methionine eq.*	1577	1704	1242	1274	918	886
Phenylalanine*	3227	3167	3504	3269	2573	1593
Proline	4351	4426	4979	3651	2875	1794
Serine	4974	4833	5620	5112	3678	2172
Threonine*	4309	4037	4235	3961	3168	2072
Tryptophan	474	444	368	349	283	182
Tyrosine*	2328	2295	2549	2268	1743	1111
Valine*	7023	6339	6162	6158	4673	2656
Essential	42808	40893	42788	39797	31064	19870
Non-Essential	38238	37276	41687	36931	29182	21443
Total	81046	78169	84475	76727	60246	41313

Figure 10: Changes in the serine and valine content (nmoles / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.

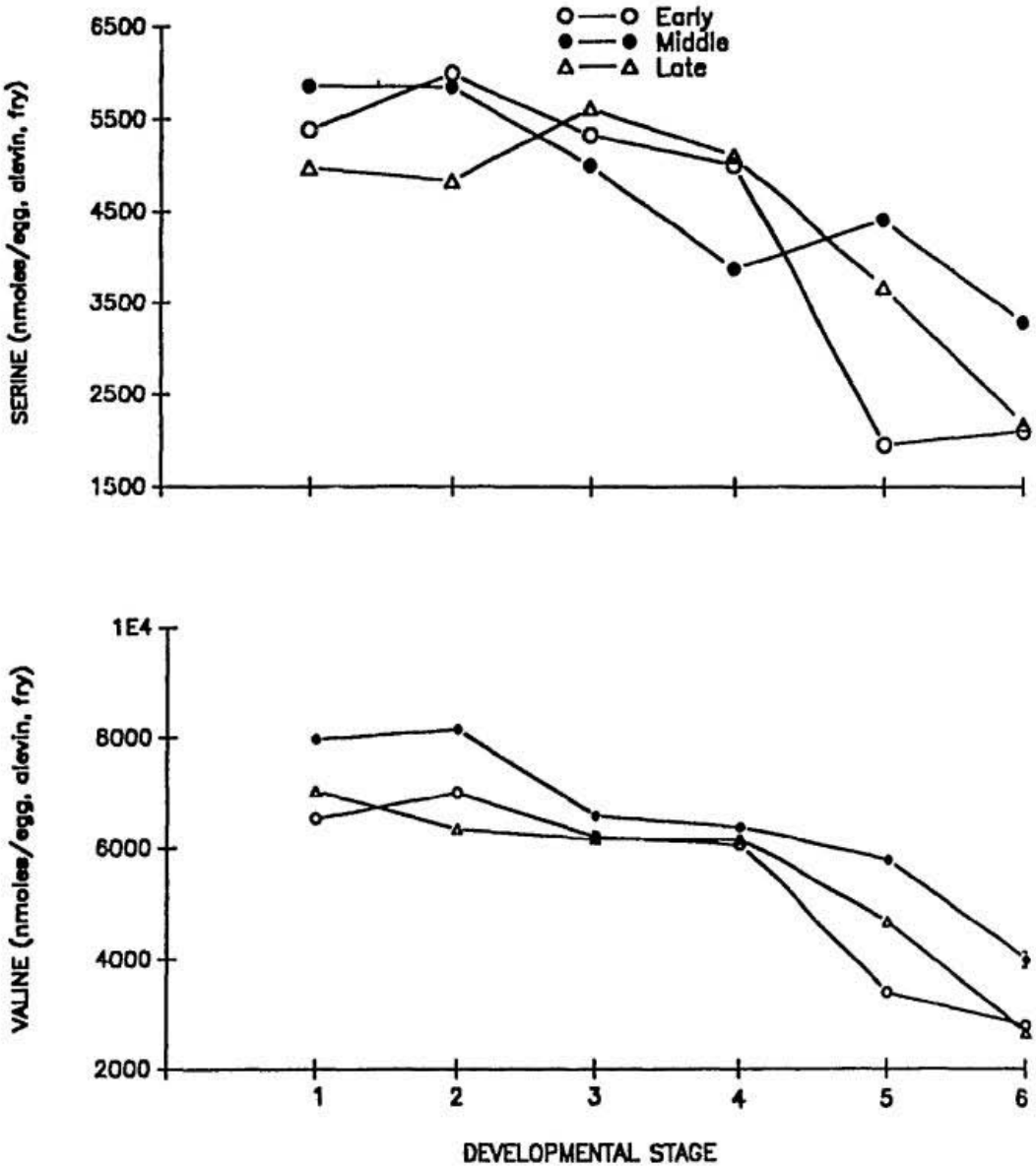

Early
Middle
Late
SERINE (nmoles/egg, alevin, fry)
6500
5500
4500
3500
2500
1500
VALINE (nmoles/egg, alevin, fry)
1E4
8000
6000
4000
2000
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 11: Changes in the tryptophan and lysine content (nmoles / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.

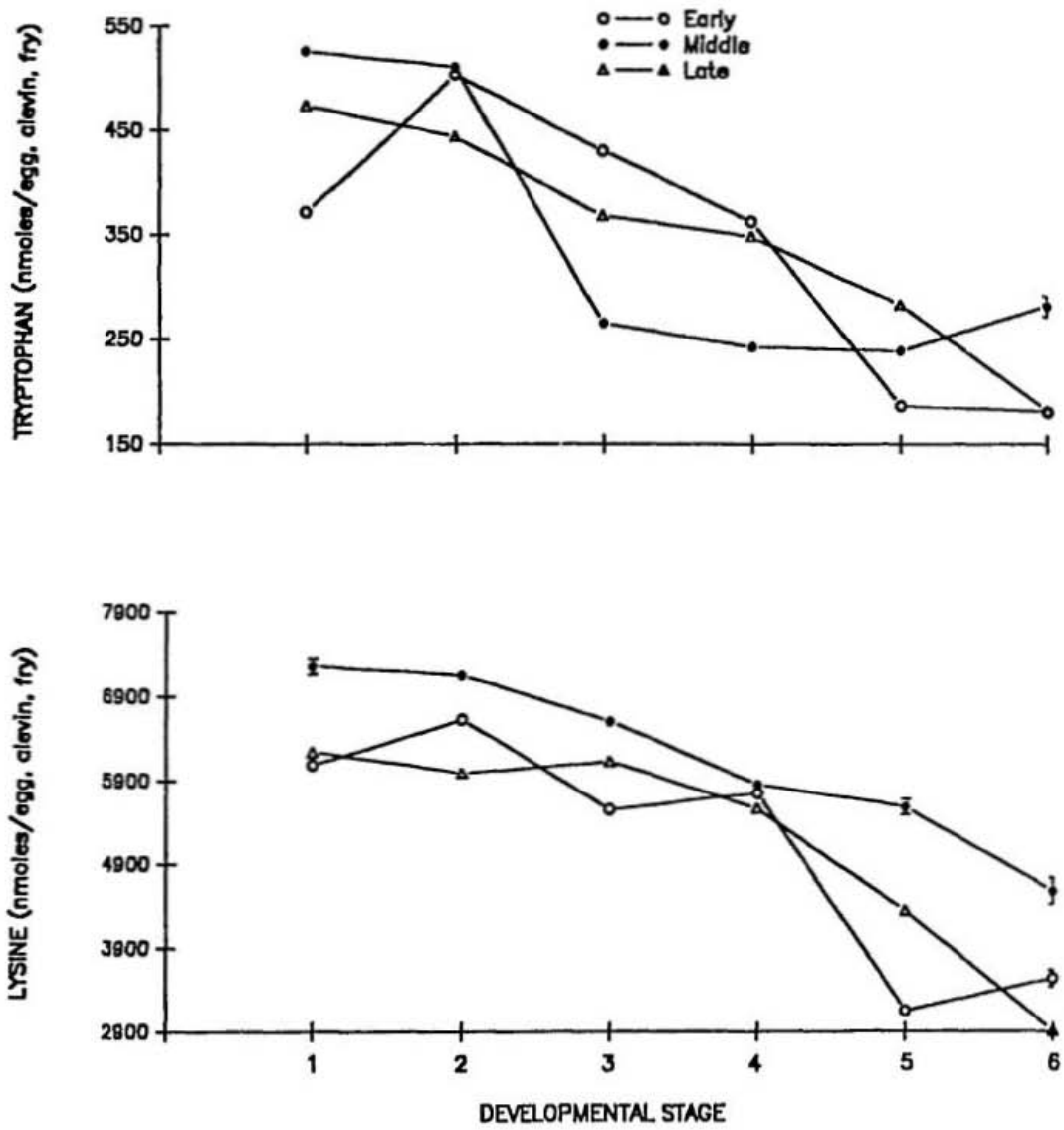

Early
Middle
Late
TRYPTOPHAN (nmoles/egg, alevin, fry)
550
450
350
250
150
LYSINE (nmoles/egg, alevin, fry)
7900
6900
5900
4900
3900
2900
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 12: Changes in the isoleucine and threonine content (nmoles / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.

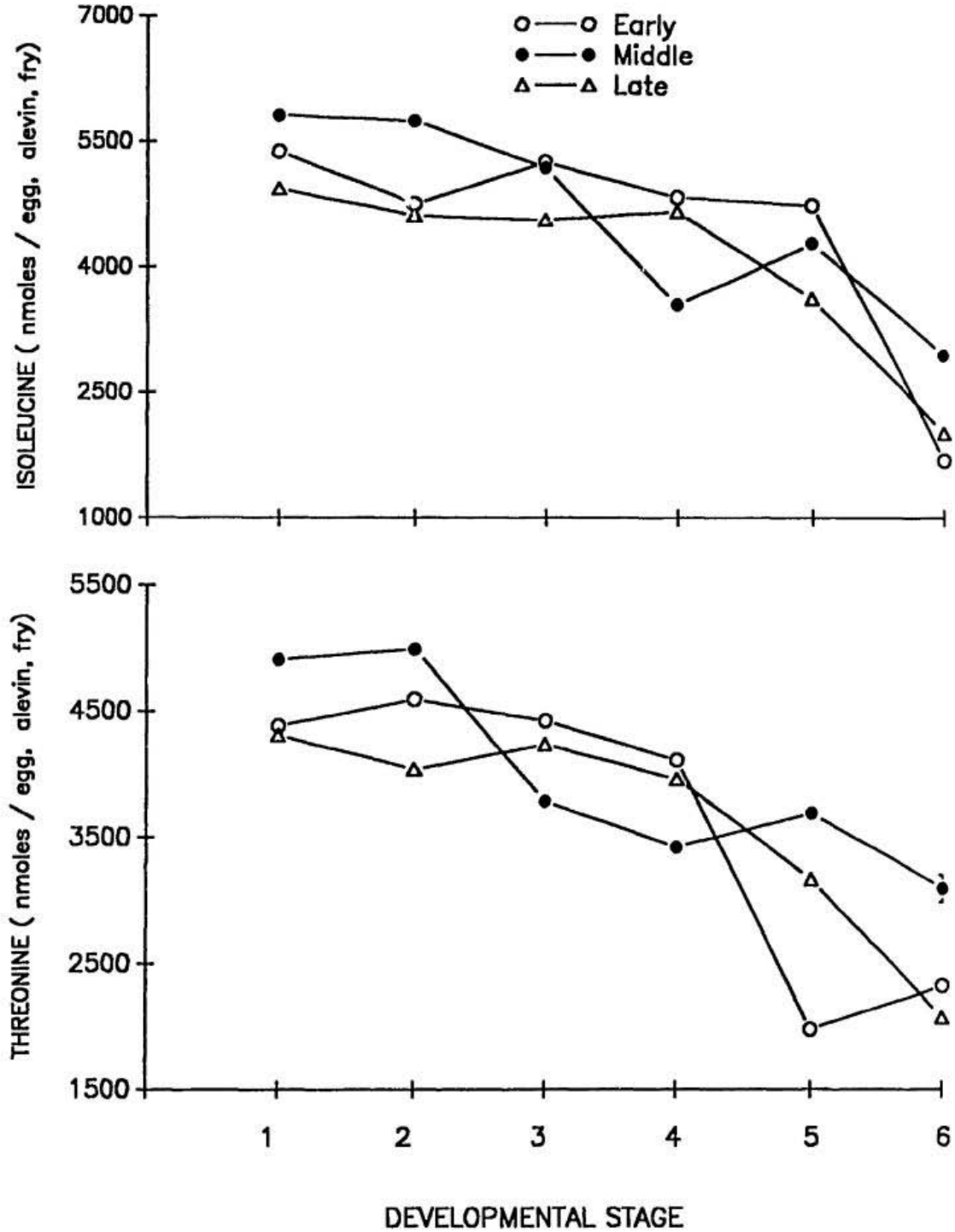

Early
Middle
Late
ISOLEUCINE (nmoles / egg, alevin, fry)
7000
5500
4000
2500
1000
THREONINE (nmoles / egg, alevin, fry)
5500
4500
3500
2500
1500
1
2
3
4
5
6
DEVELOPMENTAL STAGE

TABLE 7.

Levels of free amino acid (nmoles / egg, alevin, fry) at different developmental stages of Arctic charr stripped in the early spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding, * = essential amino acids. Stages 3 and 4 were not determined.

Amino Acid	Developmental Stages 1	2	5	6
Alanine	17	30	603	1150
Arginine*	9	15	184	217
Asparagine	8	16	2	0
Aspartic acid	27	42	218	293
Cysteine eq.	176	311	231	612
Glutamic acid	28	48	389	1003
Glutamine	26	47	162	6
Glycine	13	21	479	1039
Histidine*	2	4	152	195
Isoleucine*	3	10	189	337
Leucine*	13	24	412	623
Lysine*	7	13	101	554
Methionine eq.*	4	7	149	294
Phenylalanine*	10	15	155	249
Proline	13	17	244	357
Serine	10	18	389	205
Threonine*	7	13	258	393
Tryptophan	16	0	18	44
Tyrosine*	4	7	116	31
Valine*	8	30	314	506
Essential	68	136	2031	3399
Non-Essential	335	550	2733	4709
Total	403	686	4764	8108

TABLE 8.

Levels of free amino acid (nmoles / egg, alevin, fry) at different developmental stages of Arctic charr stripped in the middle of spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding, * = essential amino acids.

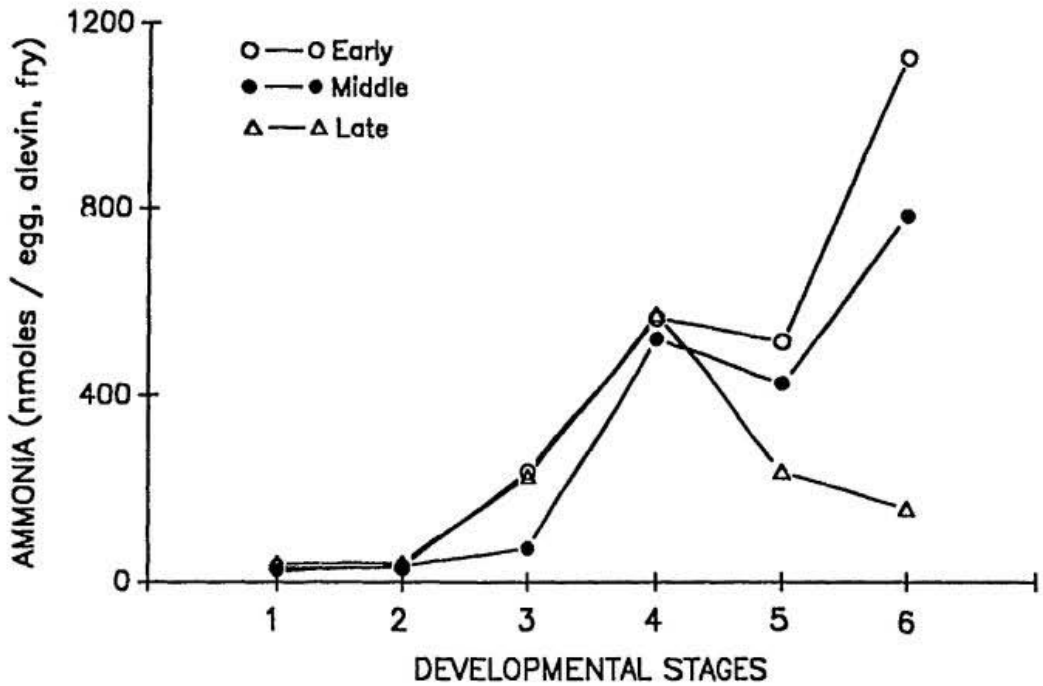
	Developmental Stages					
Amino Acid	1	2	3	4	5	6
Alanine	29	12	49	588	413	774
Arginine*	10	5	73	153	167	127
Asparagine	21	16	22	12	7	0
Aspartic acid	19	11	33	333	219	35
Cysteine eq.	267	167	123	71	59	19
Glutamic acid	62	32	86	438	357	676
Glutamine	71	43	36	165	144	23
Glycine	19	10	29	447	433	614
Histidine*	10	4	31	146	122	156
Isoleucine*	13	6	30	246	130	225
Leucine*	24	10	39	419	227	430
Lysine*	16	7	89	451	350	292
Methionine eq.*	9	4	22	170	86	180
Phenylalanine*	10	7	50	212	135	179
Proline	15	6	18	397	199	250
Serine	18	10	49	517	362	279
Threonine*	11	6	21	238	172	274
Tryptophan	2	1	14	38	31	30
Tyrosine*	9	4	29	147	83	18
Valine*	17	9	39	352	206	340
Essential	130	62	423	2534	1677	2221
Non-Essential	521	307	459	3007	2224	2700
Total	651	369	882	5541	3901	4921

TABLE 9.

Levels of free amino acid (nmoles / egg, alevin, fry) at different developmental stages of Arctic charr stripped late in the spawning season. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding, * = essential amino acids.

Amino Acid	Developmental Stages					
	1	2	3	4	5	6
Alanine	56	16	1140	433	276	149
Arginine*	17	7	34	48	164	42
Asparagine	21	20	0	0	12	0
Aspartic acid	21	17	19	194	209	38
Cysteine eq.	184	181	117	56	54	6
Glutamic acid	70	50	733	303	262	142
Glutamine	67	66	5	43	98	52
Glycine	23	16	692	212	274	230
Histidine*	12	14	190	79	101	54
Isoleucine*	18	12	308	144	109	34
Leucine*	26	16	451	180	203	67
Lysine*	26	13	553	277	325	76
Methionine eq*	14	10	204	73	75	25
Phenylalanine*	17	11	255	126	111	33
Proline	23	12	417	286	135	44
Serine	30	17	493	346	227	91
Threonine*	11	13	318	121	118	58
Tryptophan	2	6	50	31	24	8
Tyrosine*	13	10	40	56	73	24
Valine*	23	17	414	165	171	73
Essential	178	121	2767	1269	1451	486
Non-Essential	496	400	3666	1905	1572	760
Total	674	521	6433	3174	3023	1246

Figure 13: Changes in the ammonia content (nmoles / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.


1200
800
400
0
AMMONIA (nmoles / egg, alevin, fry)
O—O Early
●—● Middle
△—△ Late
1
2
3
4
5
6
DEVELOPMENTAL STAGES

3.4 Discussion

Eggs collected in the middle of the spawning season had significantly higher egg weight, egg diameter, protein, lipid, carbohydrate and amino acids content, which were associated with greater growth and survival than eggs collected early or late in the spawning season. The egg weight and egg diameter was not correlated to the alevin weight and alevin length at hatching, respectively. Although, Wallace and Aasjord (1984a) showed that larger charr eggs produced larger alevins which grew faster. Eggs collected in the middle of the spawning season had a higher energy content and faster embryonic development; as a result they took less time to reach the eyed-stage, hatched earlier, and produced better (heavier alevins with large yolk-sac volume) and fewer deformed alevins at hatching than those collected early or late. Adequate amounts of amino acids, nucleic acids, protein and lipid are necessary for successful fertilization and mitosis, without which embryonic development will halt or slow down (Fraser, 1989; Metcoff, 1986; Yafei and Noble, 1990). This may in turn result in production of less viable fry. Lower amounts of protein. lipid and amino acids in eggs of the early and late groups might have been the reason for the reduced fertilization success, survival to eyed-stage, hatching success, survival to first feeding and one month after first

feeding.

Eggs collected in the early and late spawning seasons had a significantly higher water content and relatively lower amounts of high density solutes such as proteins which contributed to the reduction in egg weight compared to those collected in the middle of the spawning season. The percent increase in body length of fry from first feeding to one month after first feeding was highest for the early spawned group compared to fry from the middle or late spawned group, but percent increase in wet weight of fry from the early spawned group was lower than fry from the middle spawned group during the same period. Because of this differential growth, fry from the early spawned group were thinner and longer but lighter compared to fry from the middle spawned group. When the dry weight of fry was considered, there was a negative growth in all the groups during the same period. This was probably due to low water temperature which has considerable affects on metabolism, as a result, fry would not have fed properly.

In the present study, protein, lipid, carbohydrate and total amino acids decreased simultaneously during development due to metabolic utilization of energy reserves by embryos, alevins and fry. These findings are contrary to the work of Deuchar (1965) who found that "first carbohydrate, then protein and, last of all, fat are used as energy sources during the development of teleost fish." On

the other hand, a more important use of protein energy for metabolism during egg development and a predominant utilization of lipids after hatching was noticed in other fish species by Takahashi et al. (1978), Kaushik et al. (1982), Davenport et al. (1983) and Dabrowski et al. (1984). The reason for this is unknown. The energy content ccntinuously decreased until first feeding, reflecting the more intensive metabolism of the eleutheroembryo. Such a trend was also noticed by several authors in salmonids (Hays, 1949; Rice and Stokes, 1974; Zeiton et al., 1977) as well in other fishes (Kamler, 1976; Davenport and Lonning, 1980).

The free amino acid pool increased during development, because protein breakdown rate would have been higher than the anabolic and catabolic losses of developing embryos. On this basis, it can be concluded that the developing embryo needs increasing amounts of free amino acid as a buffer as it grows to meet an increasing and immediate energy requirement. The increase in the free amino acid pool during embryogenesis of Arctic charr is contrary to that of developing Atlantic cod (Gadus morhua) embryos, where the free amino acid pool declined during development (Fyhn, 1989). The decline in free amino acid pool of Atlantic cod embryos may be either because of less energy reserves initially present in cod eggs to support metabolic requirement of embryos or protein breakdown rate is slower

than anabolic and catabolic requirement of embryos. Serine, valine, tryptophan, lysine, isoleucine, and threonine declined during development, suggesting that they are important for growth, development and survival of Arctic charr embryos, alevins and fry. In this experiment, four essential amino acids (valine, lysine, isoleucine and threonine) declined during development. Since fish can not synthesize essential amino acids (Walton, 1985), it can be suggested that these amino acids should be present in the diet of broodstock in order to meet embryonic requirement.

Knowledge of the nitrogen excretion rates during the early development of fish is very scarce. In salmonids, such studies were done in Salmo gairdneri (Smith, 1974; Rice and Stokes, 1974; Oliva-Teles and Kaushik, 1987) and Oncorhynchus gorbuscha (Bailey et al., 1980). It is still controversial whether nitrogen is excreted only in the form of ammonia or urea also. Rice and Stokes (1974) could not detect urea as a waste product in rainbow trout eggs during development, but Depeche et al. (1979) detected urea formation in the early stages of development (up to hatching) of rainbow trout eggs via urea cycle pathways. In this study nitrogen excretion was measured in the form of ammonia excretion. Ammonia accumulated gradually during the egg stage and was quickly excreted after hatching. The increase in ammonia content in eggs was accompanied by a decrease in total amino acid content in eggs, indicated that

amino acids were catabolized by embryos for energy production. During this experiment nitrogen excretion patterns were similar to those observed by Smith (1974), Rice and Stokes (1974) and Bailey et al. (1980) in other salmonids.

The reason for the lower quality of eggs collected in the early spawning season may be because the fish did not mobilize enough glycolipophosphoprotein (VTG, vitellogenin) and amino acids in oocytes. These compounds play a significant role in embryonic development and survival. On the other hand, for the eggs collected late in the spawning season, over-ripening might have occurred which was accompanied by significant changes such as: (i) an increase in water content, (ii) a decrease in protein, dry matter, egg weight, lipid and total amino acids. These results agreed with the findings of Craik and Harvey (1984), who found that for over-ripened eggs of rainbow trout (Oncorhynchus mykiss), there were significant increases in water content, free lipid, iron, calcium and decreases in bound lipid, precipitable protein, protein phosphorus, lipid phosphorus, total lipid and chorion weight. Craik and Harvey (1984) suggested that in over-ripening, complex molecules such as proteins and peptides would be broken down into simpler molecules such as amino acids which would be lost through the egg membrane. This may explain why eggs collected late in the spawning season were of poor quality

and had a slow embryonic growth and development and a poor survival rate. It has already been reported that in the teleost oocyte, ageing could lead to: (a) a decrease in fertilization success, (b) an increase in embryonic mortality or an increased number of deformed embryos and alevins (Suzuki, 1975; Hirose et al., 1979; Statova et al., 1982; McEvoy-Barton, 1984).

These data imply that an adequate amount of amino acids is necessary for successful embryonic, alevin or fry development of Arctic charr. A reduced content of total amino acids could halt the energy production and, thus, the growth rate of the fish embryo; which would result in smaller and less viable fry. The above-specified amino acids should be present in sufficient amounts in the diet of broodstock so that they can be transported from mother to developing oocytes during oogenesis, which would support the specific energy metabolism of the embryos, alevins or fry. Other biochemical factors such as fatty acids, carotenoid pigments and micronutrients (vitamins and trace elements) are also important for good growth and survival. However it was beyond the scope of this study to consider these factors.

It can be concluded that in order to obtain better quality eggs, time of stripping is very important. If eggs are left in the abdominal cavity of broodstock, over-ripening will occur. Identification of optimum time of

stripping has been a recognized problem in salmonid culture. The data have shown that the time of stripping is an important determinant of egg quality, which in turn is related to higher growth and survival. Therefore, emphasis should be given on better quality eggs rather than obtaining maximum number of eggs. Other biochemical factors such as

In addition, egg weights and egg diameters were not correlated to alevin weights and alevin lengths at hatching, respectively. Therefore, these traits will not be appropriate to use as indices of egg quality. The amino acid and/or energy content of eggs will be more appropriate to use as egg quality criteria (traits) for growth, development and survival of embryos, alevins and fry.

CHAPTER 4. A COMPARATIVE STUDY OF EGG QUALITY OF CULTURED AND WILD ATLANTIC SALMON (*Salmo salar*).

4.1. Introduction

The Atlantic salmon is native to the basin of the North Atlantic Ocean, from the Arctic Circle to Portugal in the eastern Atlantic, from Iceland and southern Greenland, and from the Ungava region of northern Quebec south to the Connecticut River (Scott and Crossman, 1973; Scott and Scott, 1988). In Canada, it is found throughout Newfoundland and Labrador, the Maritime Provinces and eastern Quebec and the Ungava region of northern Quebec (Scott and Crossman, 1973).

The Atlantic salmon is found in two forms, anadromous and landlocked. The anadromous fish lives and grows in salt water and return to fresh water streams to spawn, returning again to saltwater. Migration across the ocean-freshwater boundary provides a gain to individual fitness (lifetime reproductive success) that exceeds the costs of this behaviour (Gross et al., 1988). These costs may include adjustments to physiology, allocation of energy for swimming, and increased probability of mortality during migration (Gross, 1987). The factors which may favour

migration are decreased predation, decreased disease, decreased physiological stress, or increased food availability (Gross and Sargent, 1985; Gross, 1987; Gross et al., 1988). Anadromous salmon average about 2 to 6 kg in weight rarely over 12 kg, while landlocked salmon are estimated to average about 0.8 to 1.2 kg in weight. On average, a female deposits 523 to 1385 eggs per pound of body weight but number of eggs per female varies greatly (Baum and Meister, 1971). Spawning occurs in the late fall. When they are 10 or 12 cm long (usually 2 or 3 years old), the young salmon (smolt) commence their seaward migration. When mature they often return to the same stream in which they were hatched.

For salmonids held in captivity, it has been found that nutrition influences reproduction by affecting egg size, fecundity, egg hatchability, fry viability and chemical composition of eggs (Hardy, 1985; Springate et al., 1985).

It is well known that alevins vary in sizes at hatching and at yolk absorption in accordance with their developmental conditions, including variation in temperatures, dissolved oxygen, dissolved solids, and ambient water exchange (Hamor and Garside, 1977b; Peterson et al., 1977; Thorpe, et al., 1984). In general, higher temperature causes accelerated differentiation and maturation of tissues and organs, but retards the growth of such structures relative to the action of lower temperature

and alevins.

4.2 Materials and Methods

4.2.1 Origin of Broodstock

Wild broodstock (weight = 2.54 ± 0.09 kg, length = 57.2 ± 0.62 cm) were collected from the Northeast Placentia River (47^0,48'N, 53^0,52'W; Fig. 14), Newfoundland, during their upstream migration in November, and were held in a tank at the Marine Sciences Research Laboratory until stripping. Cultured broodstock (weight = 2.52 ± 0.11 kg, length = 57.8 ± 0.42 cm) were raised (from fry) at St. Mary's Bay (46^0,48'N, 53^0,39'W; Fig. 14), Newfoundland. They were spawned for the second time. The cultured broodstock were fed pelleted feed obtained from Corey Feed Mills Ltd., Fredericton, New Brunswick.

4.2.2 Mating Design

Both broodstocks (cultured and wild) were stripped in November. The cultured fish were stripped at St. Mary's Bay, and eggs and sperm were brought to the Marine Sciences Research Laboratory (MSRL). The wild fish were stripped at the MSRL.

(Hamor and Garside, 1977b; Crisp, 1988). The optimum temperature from fertilization to eye pigmentation has been found near 6^0C for Atlantic salmon (Peterson et al., 1977).

Studies on the chemical composition of Atlantic salmon eggs and its significance for embryonic and fry development have not been properly investigated in spite of its high commercial importance. Investigators have studied morphological (eg. Hansen and Moller, 1985), biological (eg. Hamor and Garside, 1977b), and biochemical (eg. Hamor and Garside, 1977a) egg quality measures individually or in combination, but no attempt has been made to investigate all three measures and correlate them to embryonic and fry growth, development and survival. For example, Hamor and Garside (1977a) analyzed the protein, phospholipid, nonpolar lipids, carbohydrate, ash, nonprotein nitrogen, nucleic acids, and water in the total ovum, yolk, and zona radiata of freshly fertilized Atlantic salmon eggs. They did not correlate these levels with embryonic and fry development.

The purpose of this study were: (1) to determine morphological, biological and biochemical composition of cultured and wild (anadromous) Atlantic salmon (Salmo salar) eggs and correlate these to their embryonic and fry development, growth and survival, and (2) to determine which egg quality traits would be most appropriate as condition indices for the development, growth and survival of embryos

Figure 14: Origins of Atlantic salmon broodstocks. Wild (Anadromous) and cultured broodstocks were from the Northeast Placentia river and St. Mary's Bay, respectively.


NEWFOUNDLAND AND LABRADOR
♥ St. John's
O St. Mary's Bay
■ Northeast Placentia River

At each stripping, 2000 eggs from four females were fertilised with the milt of three males and divided in half (two replicates). After water hardening, the eggs were transferred to an incubator where water temperature varied from 6-8^0 C (Peterson et al., 1977).

4.2.3 Incubation and Sampling

Incubation and sampling technique are described as in Chapter 2.

4.2.4 Rearing of Alevins and Fry

Yolk-sac fry were transferred to 12 litre buckets when most (>75%) of the yolk-sac was absorbed. Water temperature was maintained between 7-9^0 C with a light intensity of 50 lux and a 12h L: 12h D photoperiod (Jobling, 1987; Wallace et al., 1988). Fry were fed (granulated feed obtained from Corey Feed Mills Ltd.) to satiation throughout the experiment. Dead alevins / fry were removed regularly.

4.2.5 Biochemical Analyses

Biochemical analyses (protein, lipid, carbohydrate, free and total amino acids, dry matter and ash content) of eggs, alevins or fry were carried out as described in chapter two.

4.3 Results

Data were not normally distributed (Shapiro-Wilk statistic), therefore nonparametric statistic (Mann-Whitney tests) was used. There were no significant differences between replicates of all the variables studied ($P > 0.05$, Appendices 7, 8, 9 and 10), so replicates were pooled.

Eggs collected from the wild Atlantic salmon stock were significantly higher in mean wet weight than eggs collected from the cultured stock (Table 10; Fig. 15). Egg wet weight were also significantly different after fertilization and at eyed-stage (Table 10; Fig. 15). Eggs collected from the wild stock produced significantly heavier alevins (wet weight) at hatching compared to eggs collected from the cultured fish (Table 11; Fig. 15). The mean fry weight variation was also significant at first feeding and one month after first feeding (Table 11; Fig. 15). The percent increase in wet

TABLE 10.

Results of Kruskal-Wallis analysis (X^2) on egg variables of cultured and wild Atlantic salmon at three developmental stages (1 = before fertilization, 2 = after fertilization, 3 = eyed-stage). N = 40, S = Significance difference at $P < 0.05$, NS = No significance difference.

VARIABLES	STAGES					
	1		2		3	
	X^2	P	X^2	P	X^2	P
Egg weight	59.28	S	59.27	S	59.26	S
Egg diameter	3.87	S	13.67	S	29.82	S
Protein	59.28	S	59.27	S	59.26	S
Lipid	59.28	S	59.27	S	59.26	S
Carbohydrate	59.28	S	59.27	S	59.26	S
Energy	59.28	S	59.27	S	59.26	S
Dry matter	59.28	S	59.27	S	59.26	S
Ash	59.28	S	59.27	S	59.26	S

Figure 15: Mean wet weight (mg) of egg, alevin or fry at different stages of development for eggs collected from the cultured and wild Atlantic salmon stocks. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

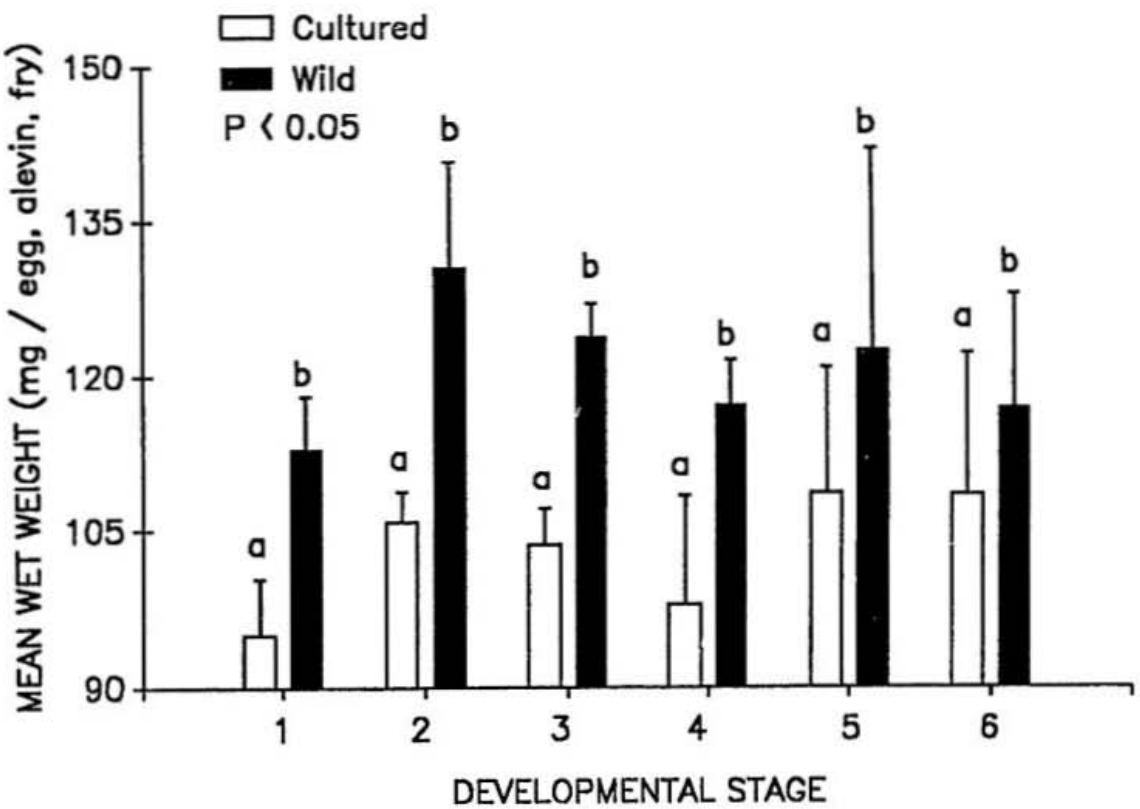

Cultured
Wild
P < 0.05
MEAN WET WEIGHT (mg / egg, alevin, fry)
150
135
120
105
90
a
b
a
b
a
b
a
b
a
b
a
b
1
2
3
4
5
6
DEVELOPMENTAL STAGE

TABLE 11.

Results of Kruskal-Wallis analysis (X^2) on fry variables of cultured and wild Atlantic salmon at three developmental stages (4 = hatching, 5 = first feeding, 6 = one month after first feeding). N = 40, S = Significance difference at $P < 0.05$, NS = No significance difference.

VARIABLES	STAGES					
	4		5		6	
	X^2	P	X^2	P	X^2	P
Fry weight	54.12	S	16.69	S	7.87	S
Fry length	24.17	S	28.85	S	36.23	S
Yolk-Sac volume	45.98	S	-	-	-	-
Protein	55.83	S	34.46	S	10.58	S
Lipid	59.34	S	59.26	S	59.26	S
Carbohydrate	59.34	S	53.35	S	43.96	S
Energy	59.19	S	59.26	S	44.86	S
Dry matter	57.28	S	50.71	S	15.14	S
Ash	59.19	S	48.27	S	26.50	S

weight of alevins from hatching to first feeding from the cultured stock was higher (10.83%) compared to alevins from the wild stock (4.56%). Eggs collected from the wild stock had a significantly greater diameter compared to eggs collected from the cultured stock (Table 10; Fig. 16). The same trend was noticed after fertilization and at eyed-stage (Table 10; Fig. 16). Eggs collected from the wild stock produced significantly larger alevins with greater yolk-sac volume at hatching compared to eggs collected from the cultured stock (Figs. 17 and 18). The fry length variation was also significantly different at first feeding and one month after first feeding (Table 11; Fig. 17). The percent increase in alevin length from hatching to first feeding (cultured = 35.5%, wild = 39.11%), and fry length from first feeding to one month after first feeding (cultured = 8.30%, wild = 8.84%) were higher for the wild stock compared to the cultured stock.

There were positive correlations between egg weights and egg diameters for both stocks (cultured: $r = 0.36$, $df = 1, 38$, $P < 0.05$; wild: $r = 0.72$, $df = 1, 38$, $P < 0.05$). Alevin weight and alevin length at hatching were also positively correlated (cultured: $r = 0.52$, $df = 1, 38$, $P < 0.05$; wild: $r = 0.59$, $df = 1, 38$, $P < 0.05$). There was no correlation between egg weight and alevin weight at hatching (cultured: $r = 0.08$, $df = 1, 38$, $P > 0.05$; wild: $r = 0.07$, $df = 1, 38$, $P > 0.05$). The correlation between egg diameter and alevin

Figure 16: Mean egg diameter (mm) at different stages of development for eggs collected from the cultured and wild Atlantic salmon stocks. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

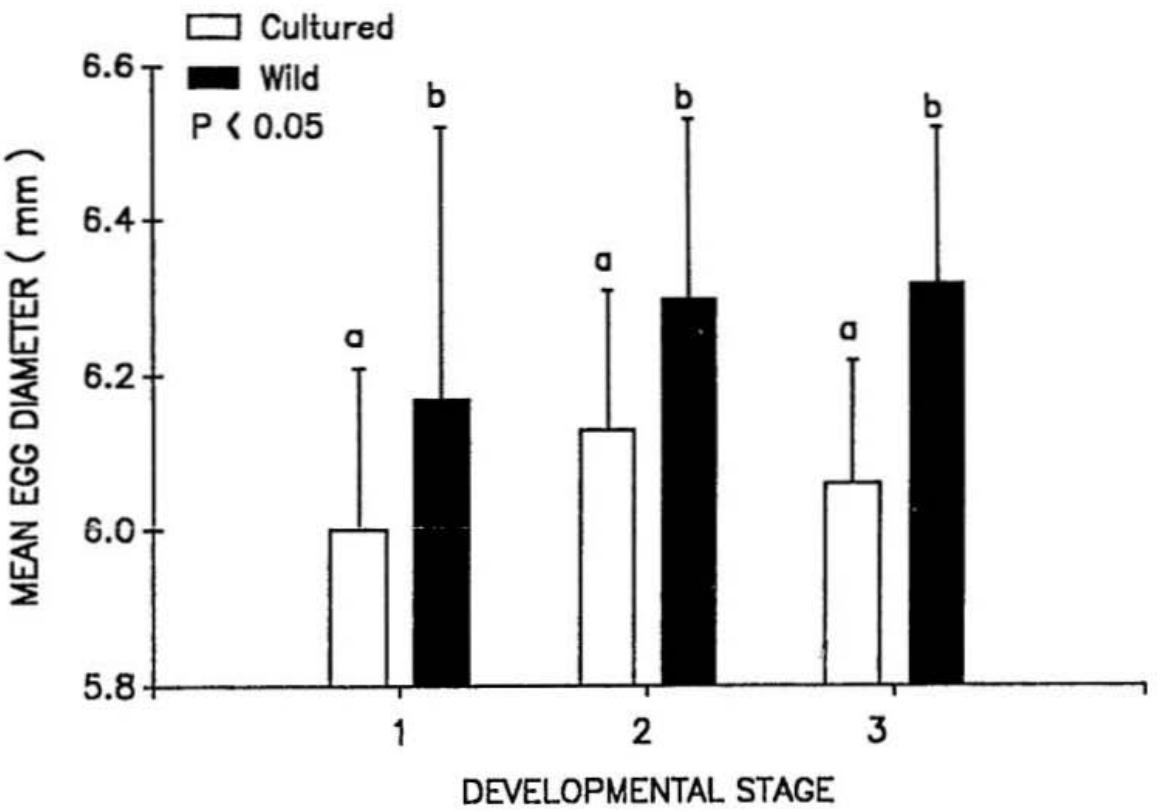

Cultured
Wild
P < 0.05
MEAN EGG DIAMETER (mm)
6.6
6.4
6.2
6.0
5.8
a
b
a
b
a
b
1
2
3
DEVELOPMENTAL STAGE

Figure 17: Mean body length (cm) of alevins or fry at different stages of development from eggs collected from the cultured and wild Atlantic salmon stocks. 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

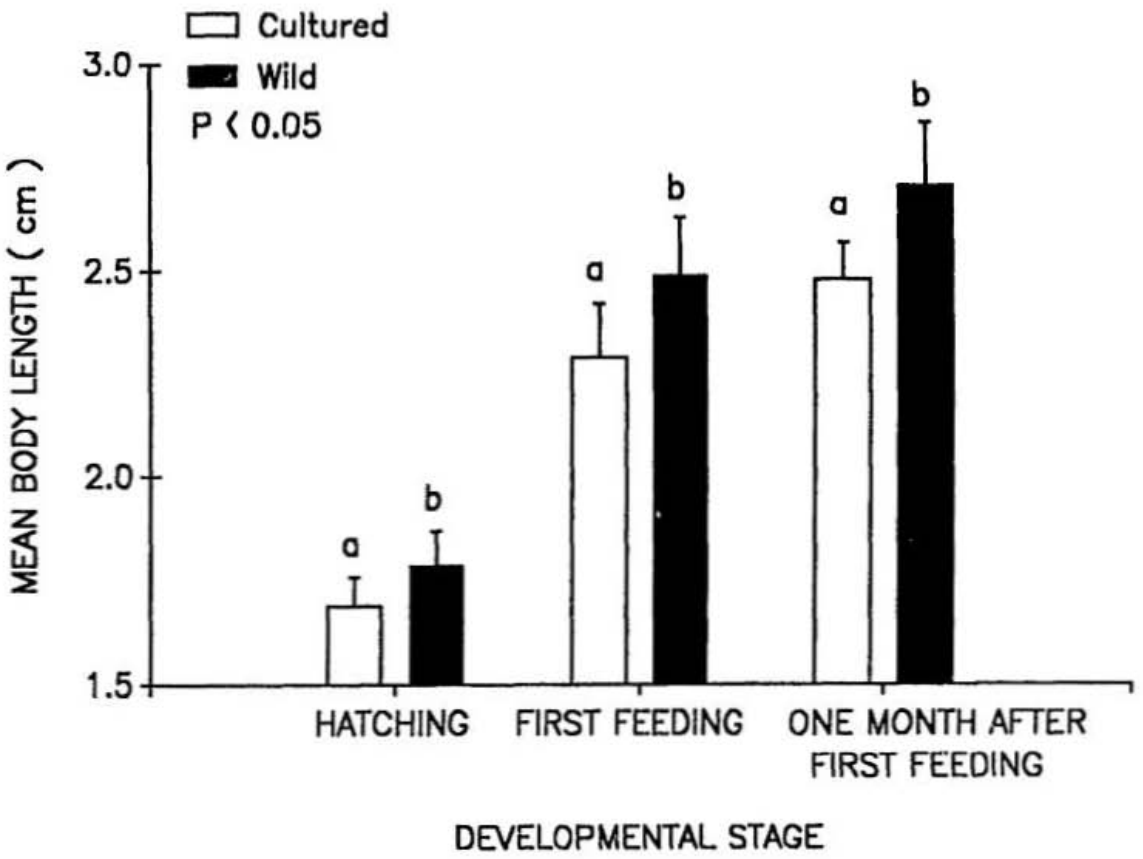
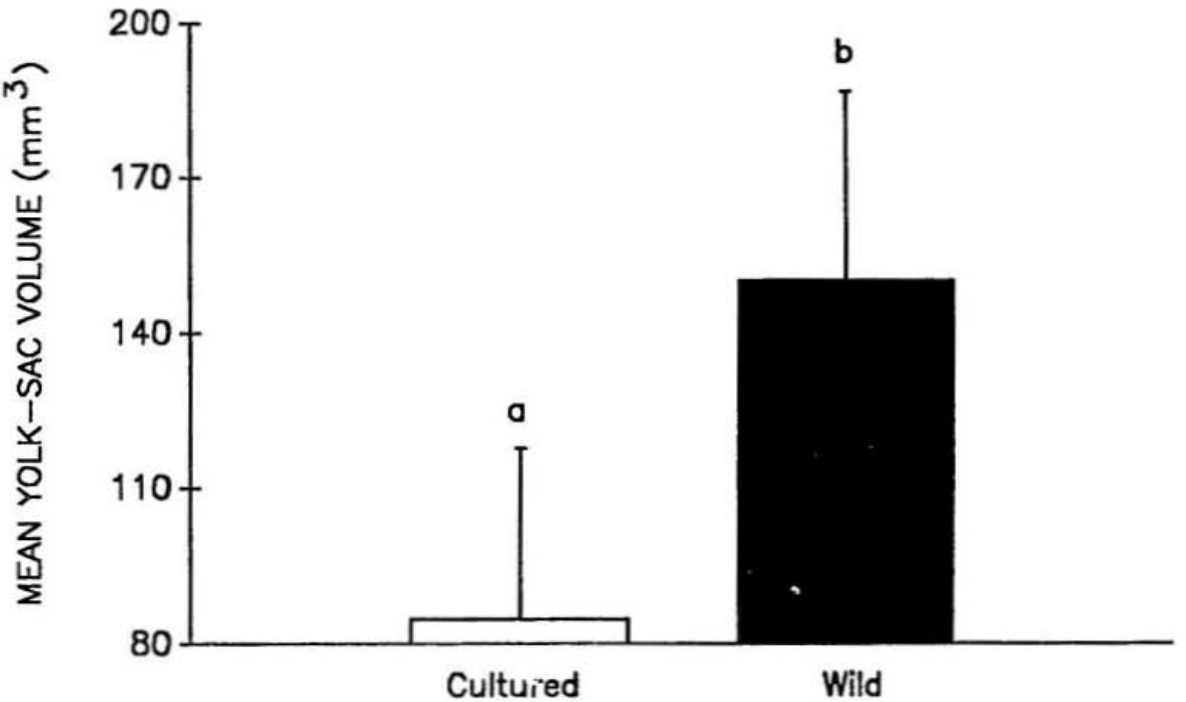

Cultured
Wild
P < 0.05
MEAN BODY LENGTH (cm)
3.0
2.5
2.0
1.5
a
b
a
b
a
b
HATCHING
FIRST FEEDING
ONE MONTH AFTER FIRST FEEDING
DEVELOPMENTAL STAGE

Figure 18: Mean yolk-sac volume (mm^3) of alevins hatched from eggs of the cultured and wild Atlantic salmon stocks. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.


MEAN YOLK-SAC VOLUME (mm3)
200
170
140
110
80
a
b
Cultured
Wild

length was also not significant (culture: $r = 0.07$, $df = 1, 38$, $P > 0.05$; wild: $r = 0.07$, $df = 1, 38$, $P > 0.05$).

The wild salmon stock had greater fertilization success, survival to eyed-stage, hatching success, survival to first feeding and one month after first feeding compared to the cultured stock (Fig. 19). Eggs collected from the wild stock produced fewer deformed alevins at hatching than the cultured stock (cultured: deformed alevins (%) = 5.77 ± 0.17; wild: deformed alevins (%) = 3.29 ± 0.17). The development rate of embryos and fry of the wild stock was faster compared to the cultured stock (Table 12).

The wild stock had significantly greater amounts of protein, lipid and carbohydrate per egg, alevin or fry than the cultured stock (Tables 10 and 11; Figs. 20, 21 and 22). The amount of protein and lipid per egg, alevin or fry declined through development in both stocks. The amount of carbohydrate per egg, alevin or fry declined up to first feeding, after that it increased in both stocks (Tables 10 and 11; Fig. 22).

The amount of dry matter per egg, alevin or fry from the wild stock was always significantly higher than the cultured stock and continuously declined during development (Tables 10 and 11; Fig. 23). The percent decrease in dry weight of alevins from hatching to first feeding from the cultured stock was higher (22.65%) compared to alevins from the wild stock (19.42%), but percent decrease in dry weight

Figure 19: Survival (percent) at various stages (from previous stage to present stage) of development for eggs collected from the cultured and wild Atlantic salmon stocks. 1 = fertilization success, 2 = survival to eyed-stage, 3 = survival to hatching, 4 = survival to first feeding, 5 = survival to one month after first feeding. Values are means ± SD.

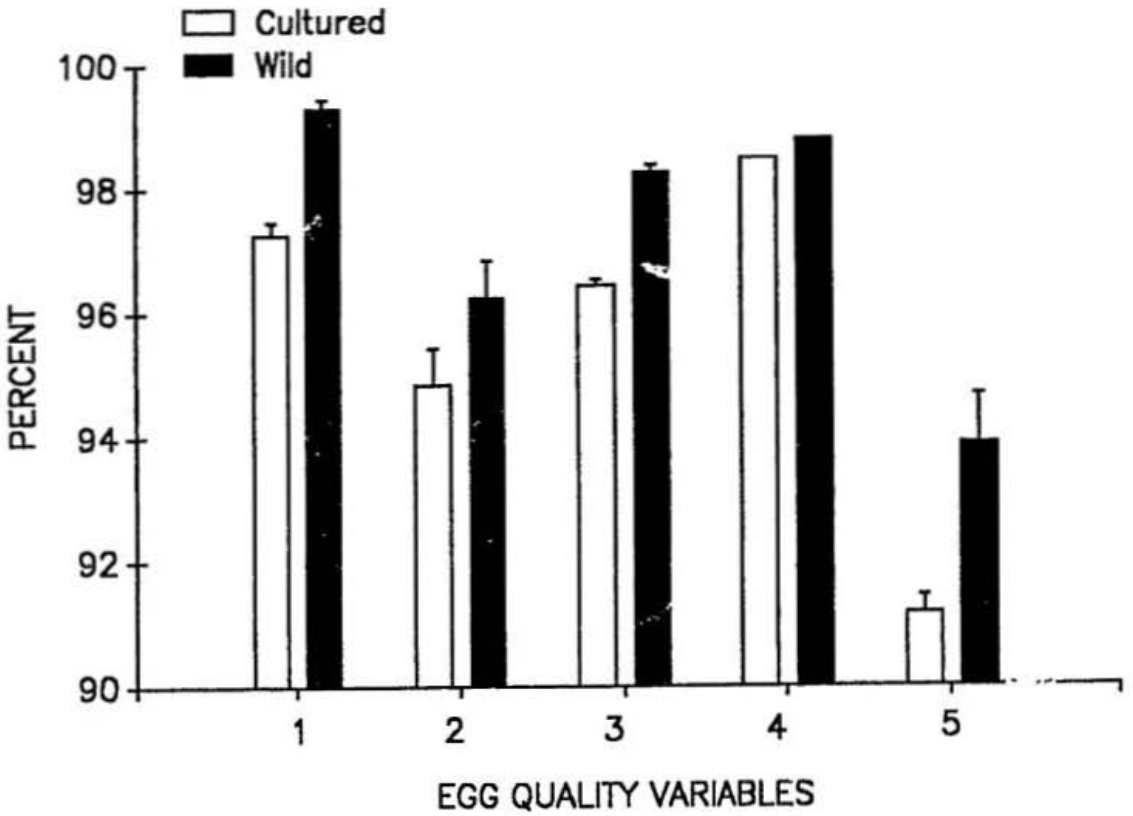

Cultured
Wild
100
98
96
94
92
90
PERCENT
1
2
3
4
5
EGG QUALITY VARIABLES

TABLE 12.

Number of degree-days from fertilization to eyed-stage, hatching and first feeding for cultured and wild Atlantic salmon.

	Degree-Days	
Stage	Cultured Atlantic salmon	Wild Atlantic salmon
Eyed-stage	457.0	424.0
Hatching	574.5	520.0
First Feeding	841.0	753.0

Figure 20: Changes in the protein content (mg / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed- stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

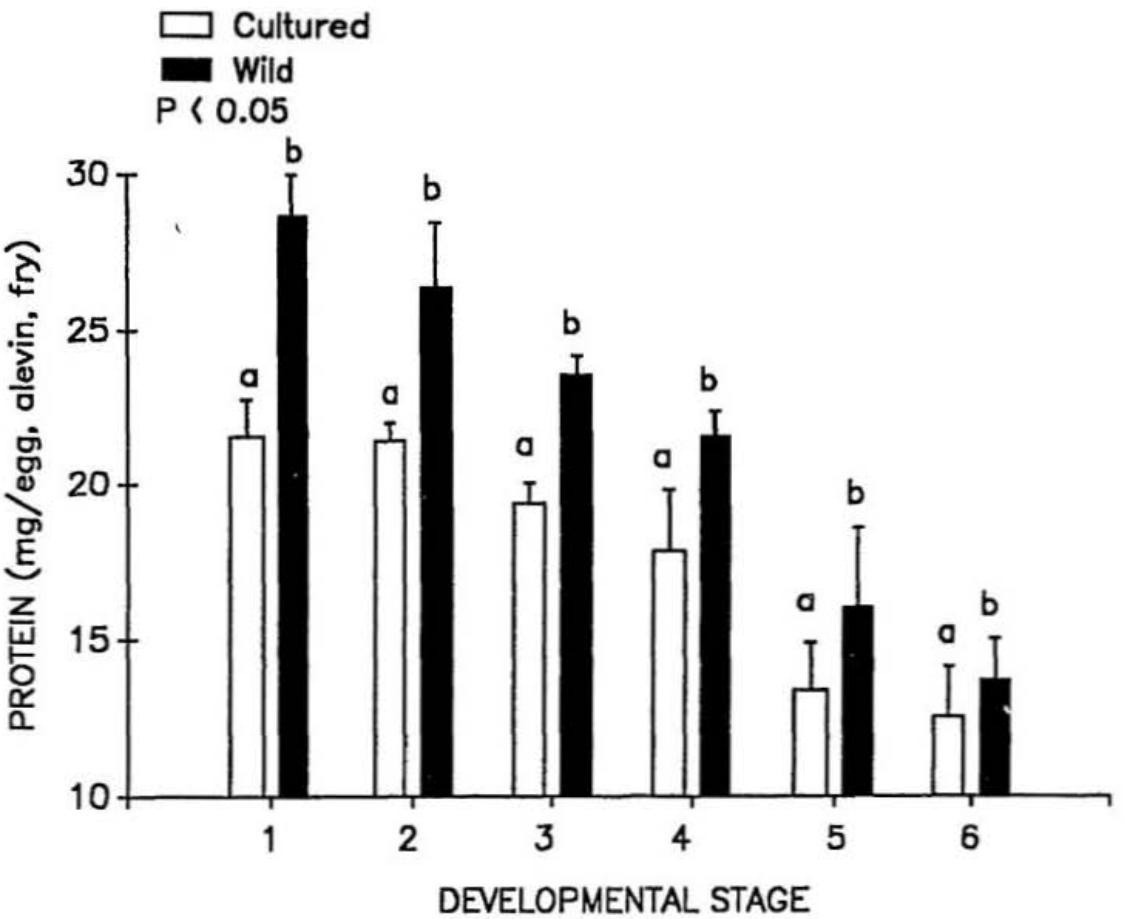

Cultured
Wild
P < 0.05
PROTEIN (mg/egg, alevin, fry)
30
25
20
15
10
a
b
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 21: Changes in the lipid content (mg / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

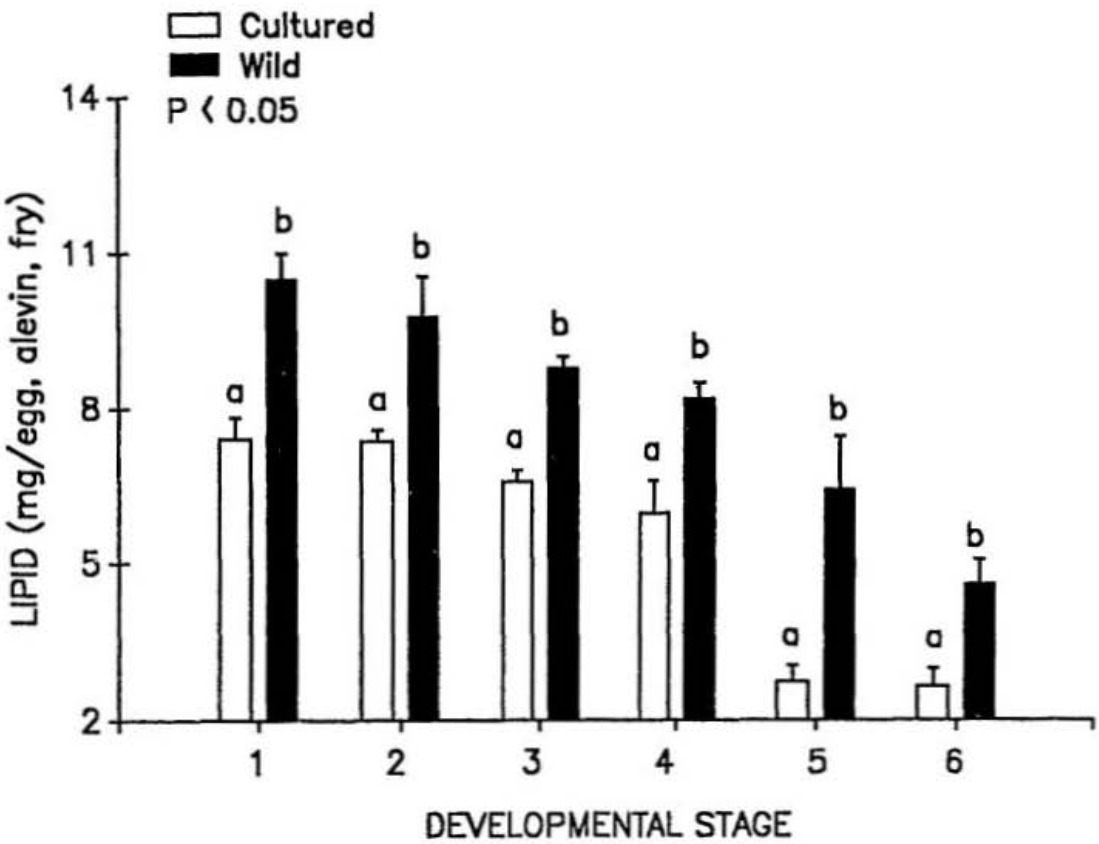

Cultured
Wild
P < 0.05
LIPID (mg/egg, alevin, fry)
14
11
8
5
2
a
b
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 22: Changes in the carbohydrate content (mg / egg, alevin, fry) at different stages of development.1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

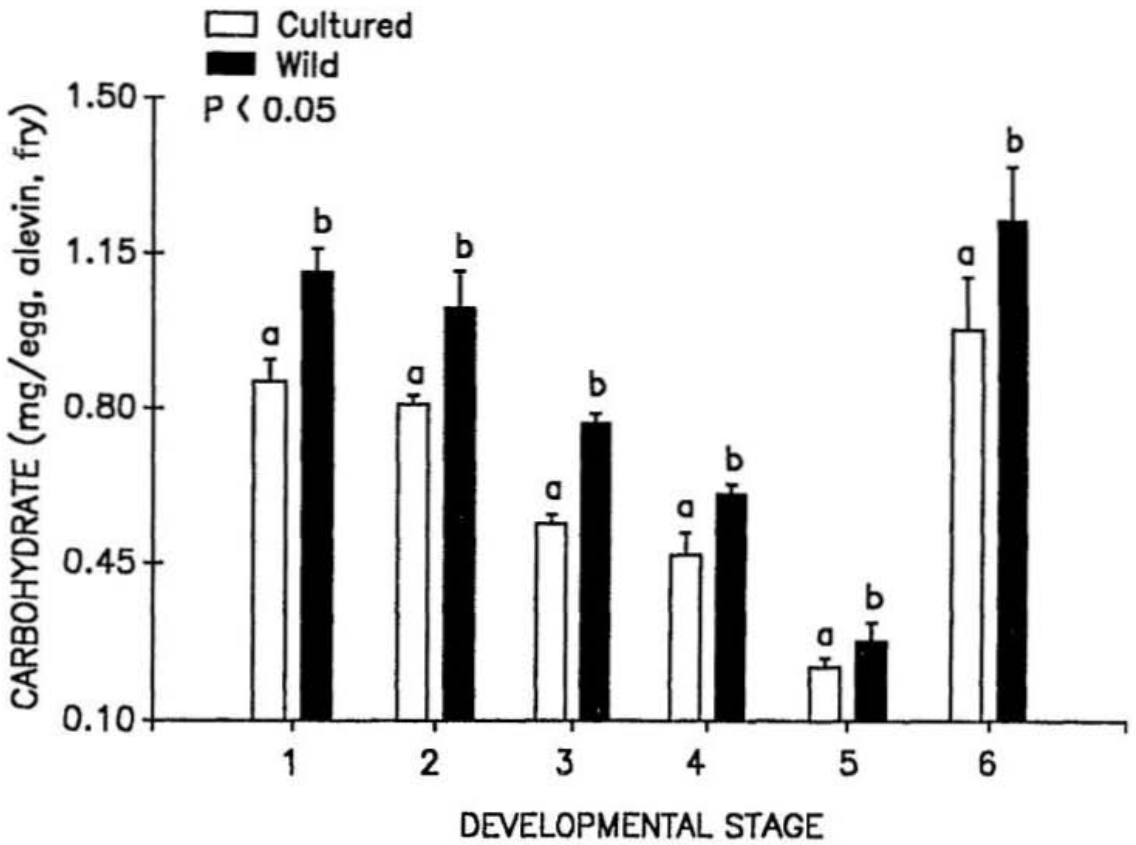
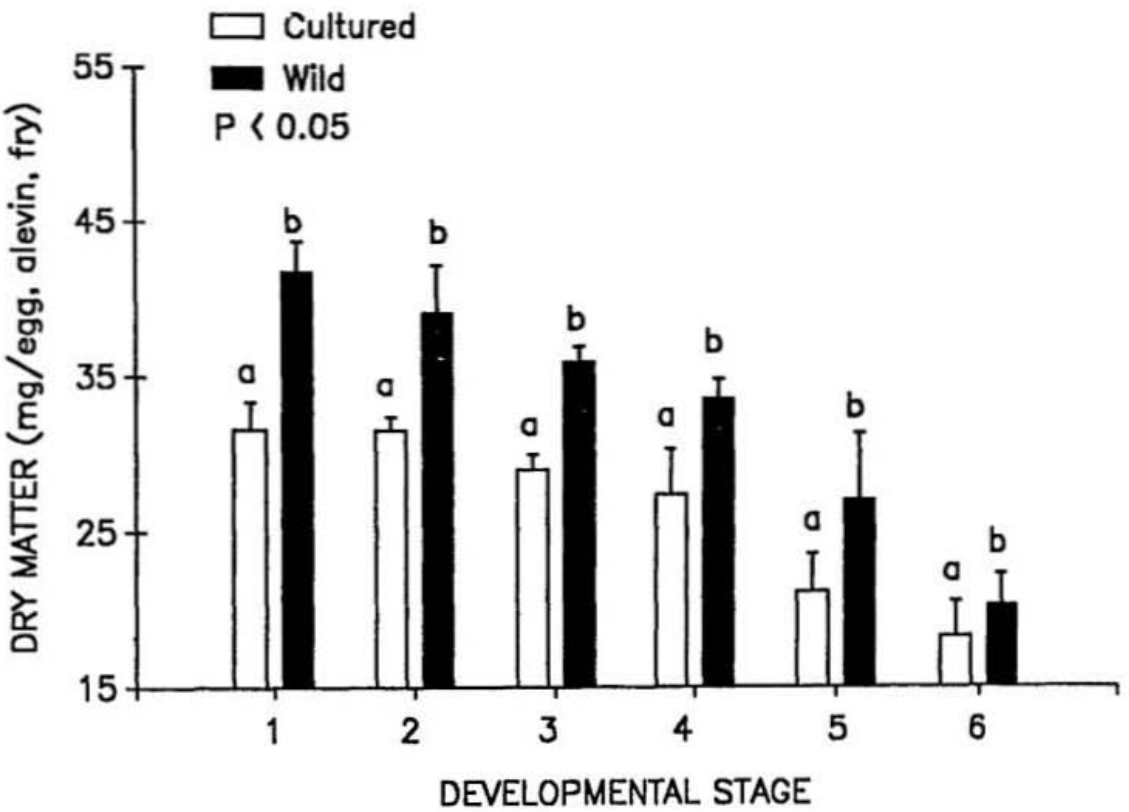

Cultured
Wild
P < 0.05
CARBOHYDRATE (mg/egg, alevin, fry)
1.50
1.15
0.80
0.45
0.10
a
b
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 23: Changes in the dry matter content (mg / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.


Cultured
Wild
P < 0.05
DRY MATTER (mg/egg, alevin, fry)
55
45
35
25
15
a
b
1
2
3
4
5
6
DEVELOPMENTAL STAGE

of fry from first feeding to one month after first feeding from the cultured stock was lower (13.93%) compared to fry from the wild stock (24.97%). The ash content per egg, alevin or fry from the wild stock was always significantly higher than the cultured stock, and declined up to the first feeding in both stocks (Tables 10 and 11; Fig. 24).

The amount of protein, lipid and carbohydrate per egg, alevin or fry was converted into total caloric value in order to compare energy content of both stocks and its changes during development. The energy content per egg, alevin or fry for the wild stock was always significantly higher than the cultured stock and declined through development (Tables 10 and 11; Fig. 25). The decline in energy content from before fertilization to one month after first feeding of the cultured stock was very low (95.38 calories) compared to the wild stock (139.59 calories).

The total amino acid content per egg from the wild stock (213,064 nmoles / egg) was higher than eggs from the cultured stock (178,104 nmoles / egg) (Tables 13 and 14). The total amino acid content per egg, alevin or fry for the wild stock declined continuously through development with minimum (97,878 nmoles / fry) at one month after first feeding, but for the cultured stock a decline was noticed up to first feeding (113,295 nmoles / fry) and after that it increased (116,474 nmoles / fry). Eggs from wild stock had higher amounts of all protein amino acids than eggs from

Figure 24: Changes in the ash content (mg / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

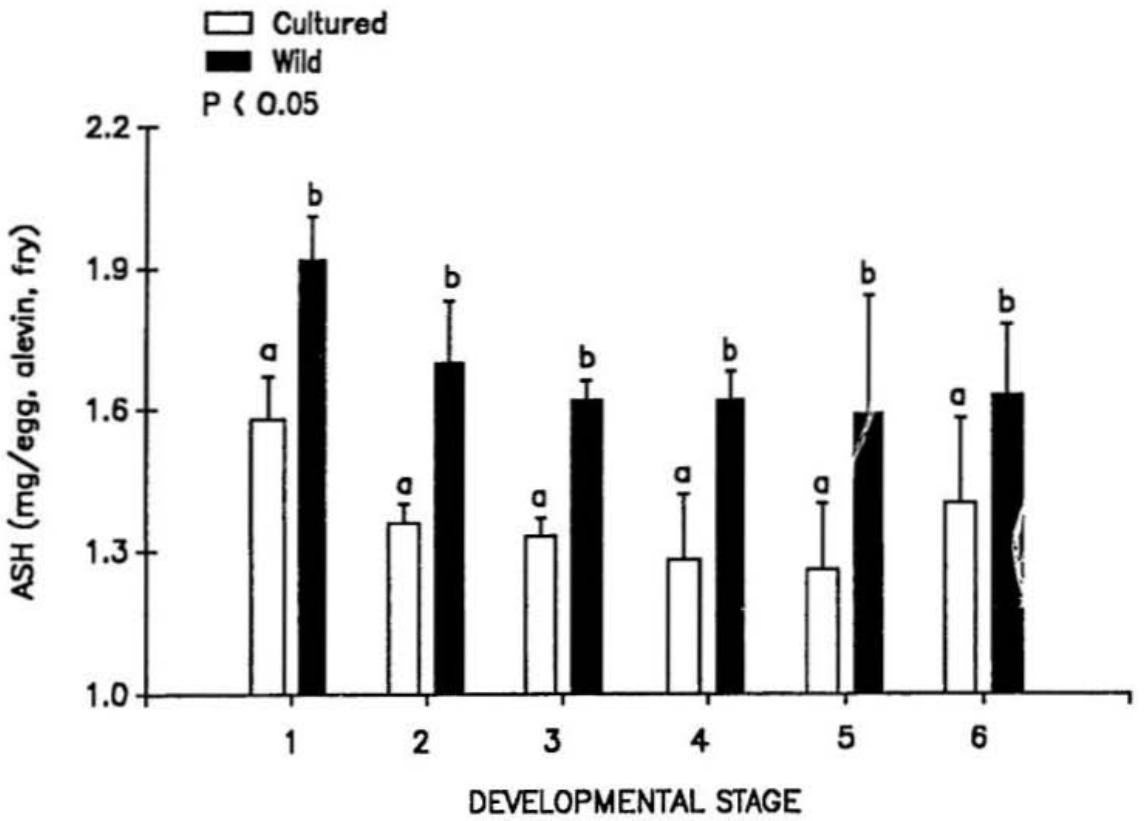

Cultured
Wild
P (0.05
ASH (mg/egg, alevin, fry)
2.2
1.9
1.6
1.3
1.0
a
b
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 25: Changes in the energy content (calories / egg, alevin, fry) at different stages of development. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. Values are means ± SD, n = 40. Bars with same letter are not significantly different, $P < 0.05$.

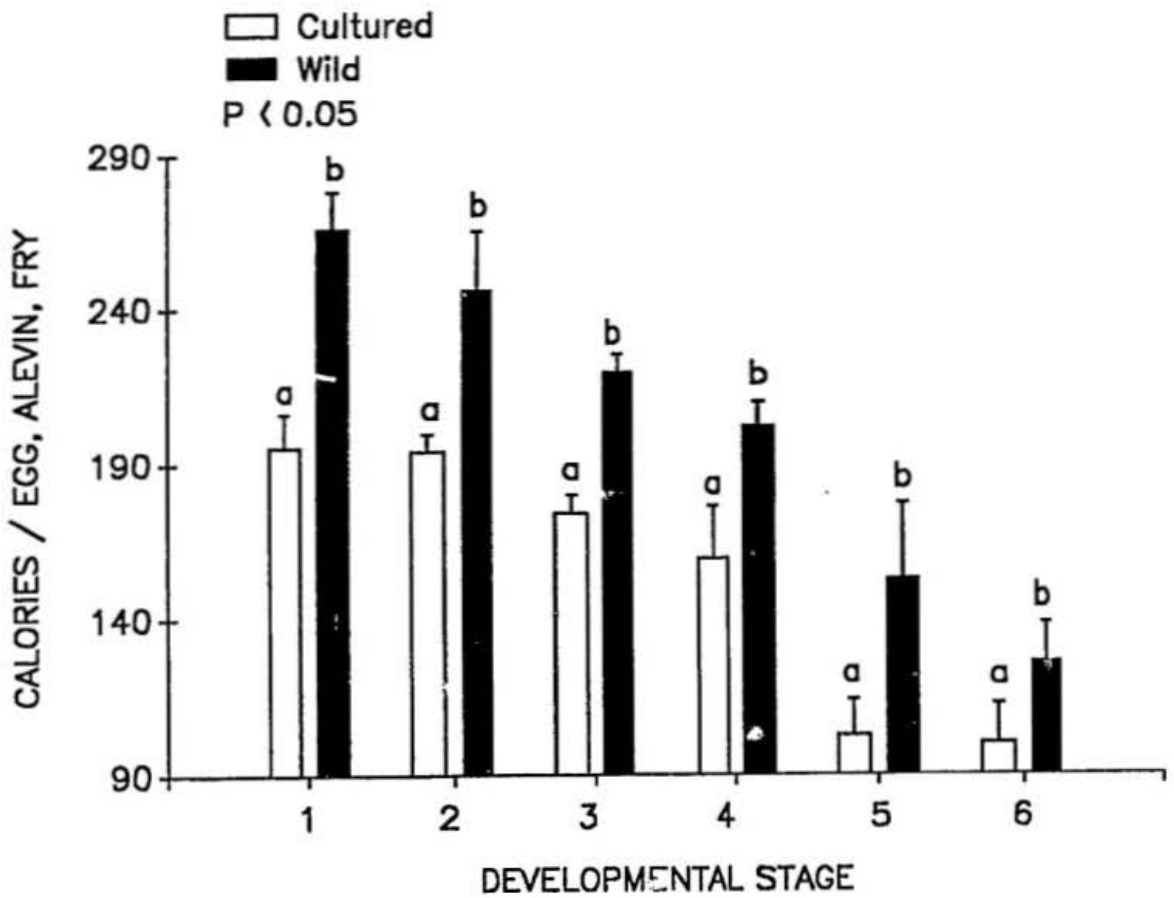

Cultured
Wild
P < 0.05
290
240
190
140
90
CALORIES / EGG, ALEVIN, FRY
a
b
1
2
3
4
5
6
DEVELOPMENTAL STAGE

TABLE 13.

Levels of amino acid (nmoles / egg, alevin, fry) at different developmental stages of cultured Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. * = essential amino acids.

	Developmental Stages					
Amino Acid	1	2	3	4	5	6
Alanine	20929	21081	18880	17669	11356	10068
Arginine*	8330	8390	7910	7198	5493	5693
Aspartic acid	14916	14882	13716	12210	9984	10979
Cysteine eq.	3017	2914	1641	2610	1706	1616
Glutamic acid	16062	16235	15703	13700	12078	13299
Glycine	7749	7673	8185	7842	10076	12678
Histidine*	3907	3900	3717	3490	2929	3702
Hydroxylysine	286	234	215	276	124	382
Isoleucine*	10606	10796	9316	8880	5860	5304
Leucine*	16470	16313	14978	13805	9873	9621
Lysine*	13262	13270	12349	11845	8715	8430
Methionine eq.*	3392	3260	1926	3085	1979	2675
Phenylalanine*	6814	6809	6341	5783	4375	4431
Proline	10128	9832	9607	7524	5263	4956
Serine	11358	11411	10669	9641	6400	6355
Threonine*	9781	9754	9031	8047	5717	5731
Tryptophan	1344	1195	1821	1086	406	667
Tyrosine*	5161	5099	4834	4383	3094	3119
Valine*	14594	14675	13075	12454	7867	6766
Essential	92316	92267	83476	78968	55901	55474
Non-Essential	85788	85457	80438	72559	57394	61000
Total	178104	177724	163914	151527	113295	116474

TABLE 14.

Levels of amino acid (nmoles / egg, alevin, fry) at different developmental stages of wild Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. * = essential amino acids.

	Developmental Stages					
Amino Acid	1	2	3	4	5	6
Alanine	25457	24959	21931	19324	14287	9719
Arginine*	9969	9938	8539	7580	6233	4628
Aspartic acid	17812	17697	17147	14301	9134	8549
Cysteine eq.	3815	4017	4272	2585	2183	1273
Glutamic acid	18409	19103	17166	13679	12086	11447
Glycine	9300	9337	8204	8087	9692	10466
Histidine*	4693	4668	4365	4195	3524	2350
Hydroxylysine	401	311	275	172	203	125
Isoleucine*	12808	12293	9108	7398	5252	4750
Leucine*	19622	19260	17066	13560	8126	8348
Lysine*	16080	15668	13456	11855	9089	6769
Methionine eq.*	4571	4678	3982	2991	2592	2145
Phenylalanine*	8118	8095	6679	5956	4867	3686
Proline	11[illegible]3	12048	9623	7819	6125	4433
Serine	14001	13697	10715	8794	6468	5195
Threonine*	11594	11486	8548	7233	6066	5095
Tryptophan	1219	1341	670	486	443	536
Tyrosine*	6194	5886	4603	3989	3149	2207
Valine*	17760	16760	15593	13554	9957	6156
Essential	111411	108732	91938	78310	58855	46135
Non-Essential	101654	102509	90002	75247	60622	51742
Total	213064	211240	181941	153557	119476	97878

cultured stock except tryptophan (Tables 13 and 14). However, alanine, aspartic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine were found to decrease during development in both stocks of Atlantic salmon (Figs. 26, 27, 28 and 29).

Total essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine; Walton, 1985) were higher in eggs, alevins or fry of wild stock than eggs, alevins or fry of cultured stock, and declined through development. Total non-essential amino acids in eggs collected from wild stock (101,654 nmoles / egg) were also higher than eggs collected from cultured stock (85,788 nmoles / egg), and declined during development up to first feeding. After first feeding, total non-essential amino acids in fry from the cultured stock increased from 57,394 nmoles to 61,000 nmoles as opposed to fry of wild stock where a declined was noticed from 60,622 nmoles to 51,742 nmoles.

The free amino acid pool was higher in eggs of the wild stock (4227 nmoles / egg) compared to the cultured stock (2506 nmoles / egg) (Tables 15 and 16). The free amino acid pool increased in both groups during development (Tables 15 and 16).

Figure 26: Changes in the alanine, aspartic acid and histidine content (nmoles / egg, alevin, fry) at different stages of development of Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.

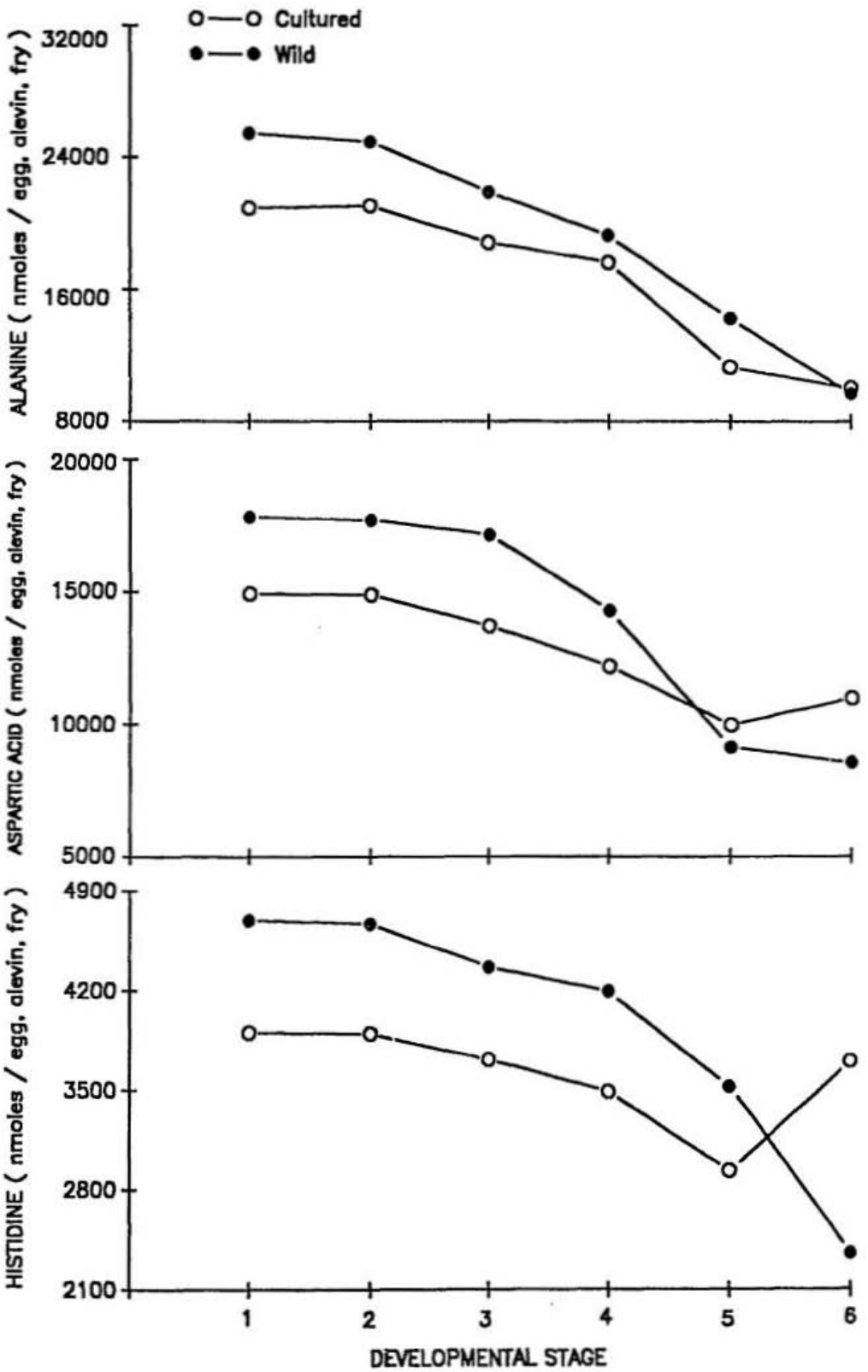

Cultured
Wild
ALANINE (nmoles / egg, alevin, fry)
32000
24000
16000
8000
ASPARTIC ACID (nmoles / egg, alevin, fry)
20000
15000
10000
5000
HISTIDINE (nmoles / egg, alevin, fry)
4900
4200
3500
2800
2100
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 27: Changes in the isoleucine, leucine and lysine content (nmoles / egg, alevin, fry) at different stages of development of Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.

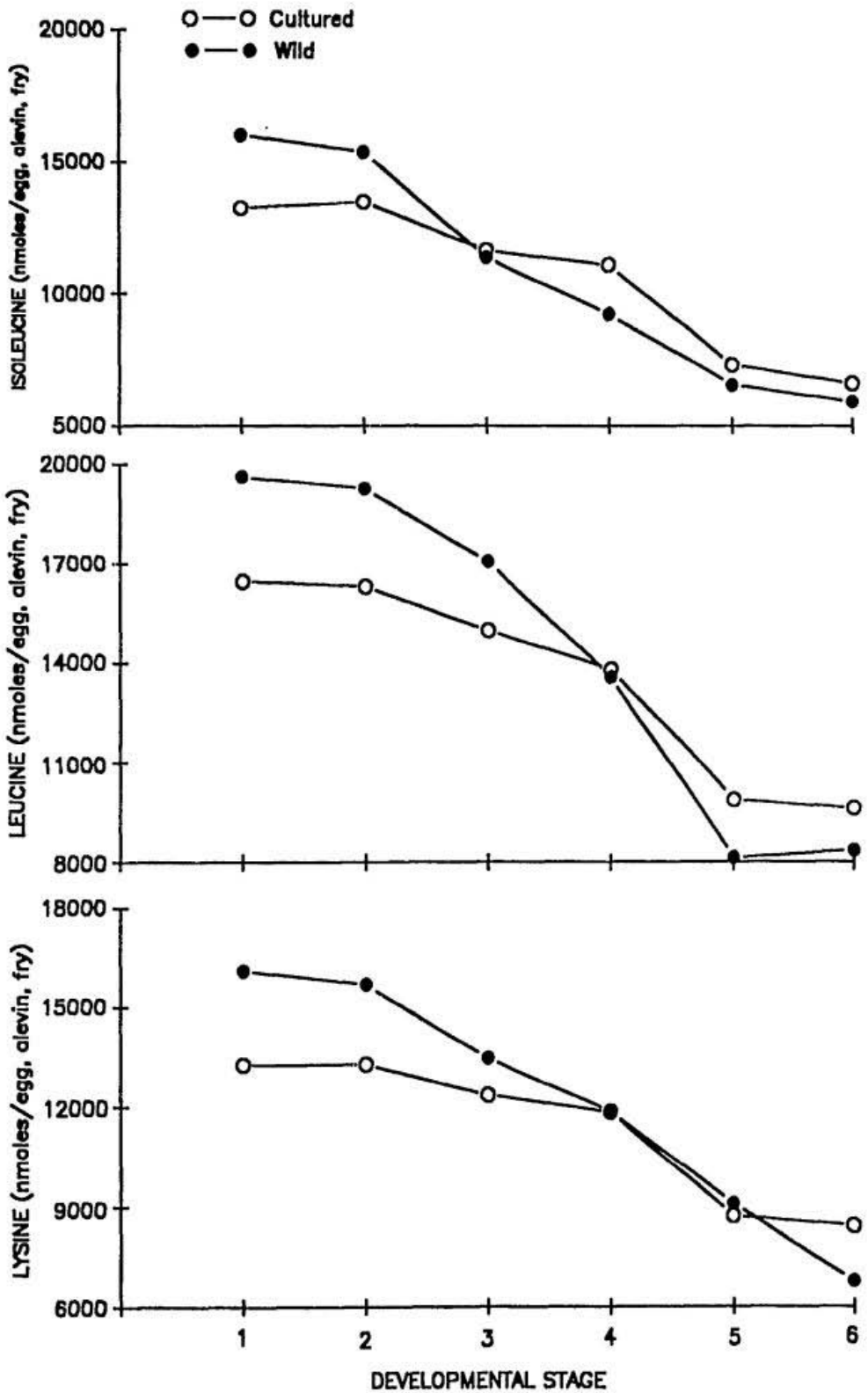

Cultured
Wild
ISOLEUCINE (nmoles/egg, alevin, fry)
20000
15000
10000
5000
LEUCINE (nmoles/egg, alevin, fry)
20000
17000
14000
11000
8000
LYSINE (nmoles/egg, alevin, fry)
18000
15000
12000
9000
6000
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 28: Changes in the phenylalanine, proline and serine content (nmoles / egg, alevin, fry) at different stages of development of Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.

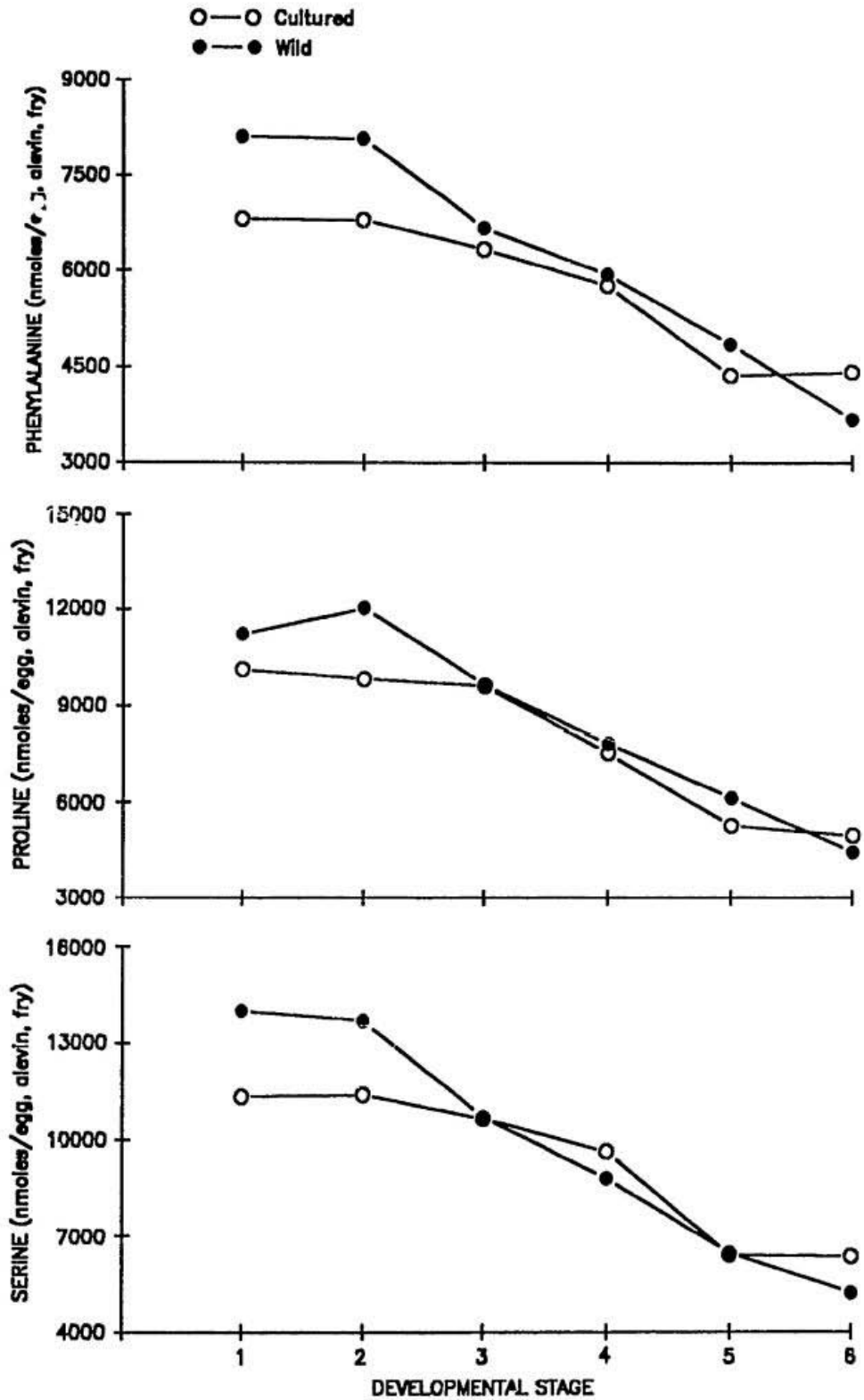

O—O Cultured
●—● Wild
PHENYLALANINE (nmoles/e, j, alevin, fry)
9000
7500
6000
4500
3000
PROLINE (nmoles/egg, alevin, fry)
15000
12000
9000
6000
3000
SERINE (nmoles/egg, alevin, fry)
16000
13000
10000
7000
4000
1
2
3
4
5
6
DEVELOPMENTAL STAGE

Figure 29: Changes in the threonine, tyrosine and valine content (nmoles / egg, alevin, fry) at different stages of development of Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding.

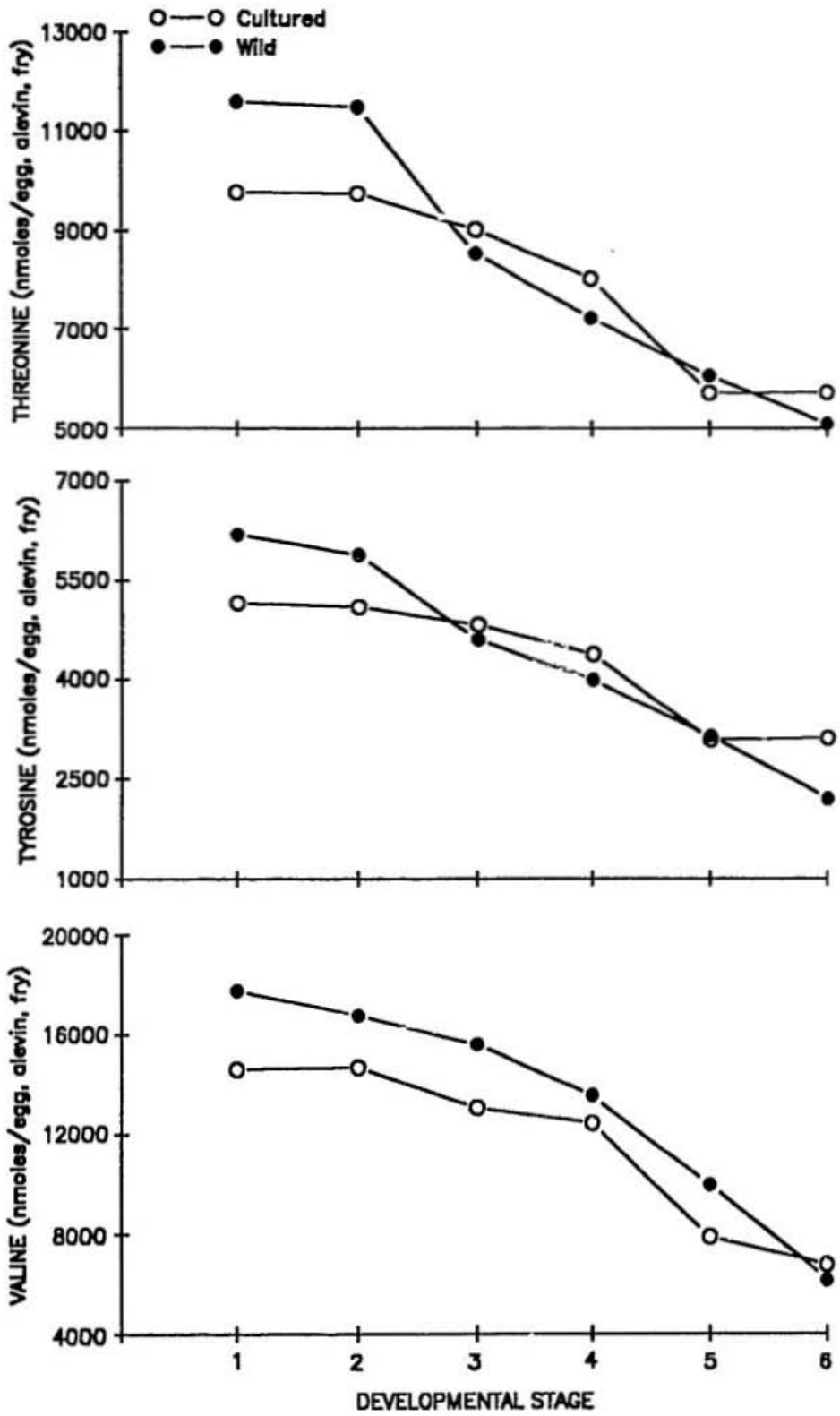

Cultured
Wild
THREONINE (nmoles/egg, alevin, fry)
13000
11000
9000
7000
5000
TYROSINE (nmoles/egg, alevin, fry)
7000
5500
4000
2500
1000
VALINE (nmoles/egg, alevin, fry)
20000
16000
12000
8000
4000
1
2
3
4
5
6
DEVELOPMENTAL STAGE

TABLE 16.

Levels of free amino acid (nmoles / egg, alevin, fry) at different developmental stages of wild Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. * = essential amino acids.

	Developmental Stages					
Amino Acid	1	2	3	4	5	6
Alanine	299	242	459	642	1384	1985
Arginine*	83	66	437	441	545	338
Asparagine	32	34	0	0	15	6
Aspartic acid	1113	845	243	194	573	323
Cysteine eq.	870	598	471	330	151	45
Glutamic acid	390	310	612	570	831	1645
Glutamine	274	195	157	176	473	14
Glycine	57	64	203	414	606	1718
Histidine*	54	48	185	276	405	543
Hydroxylysine	1	0	3	0	6	3
Isoleucine*	84	61	191	220	437	516
Leucine*	128	97	220	316	884	999
Lysine*	157	133	600	610	857	826
Methionine eq.*	48	36	164	199	397	484
Phenylalanine*	61	51	184	226	387	434
Proline	51	66	99	151	546	530
Serine	242	192	761	847	1139	810
Threonine*	77	67	197	223	460	664
Tryptophan	19	16	93	94	93	92
Tyrosine*	44	41	51	167	337	135
Valine*	142	107	300	349	700	791
Essential	879	707	2528	3028	5409	5729
Non-Essential	3348	2565	3102	3418	5817	7172
Total	4227	3272	5630	6446	11226	12901

TABLE 15.

Levels of free amino acid (nmoles / egg, alevin, fry) at different developmental stages of cultured Atlantic salmon. 1 = before fertilization, 2 = after fertilization, 3 = eyed-stage, 4 = hatching, 5 = first feeding, 6 = one month after first feeding. * = essential amino acids.

	Developmental Stages					
Amino Acid	1	2	3	4	5	6
Alanine	196	182	468	394	1409	787
Arginine*	49	40	438	375	602	193
Asparagine	14	29	0	0	18	9
Aspartic acid	528	737	264	167	549	291
Cysteine eq.	318	377	202	157	174	36
Glutamic acid	360	436	600	439	856	658
Glutamine	94	101	218	141	499	198
Glycine	84	73	196	226	1124	1386
Histidine*	37	36	178	184	449	304
Hydroxylysine	0	0	0	0	6	2
Isoleucine*	71	89	222	183	445	167
Leucine*	107	137	239	194	904	354
Lysine*	83	96	655	502	916	382
Methionine eq.*	31	33	138	128	417	204
Phenylalanine*	37	39	181	142	371	196
Proline	73	52	144	101	524	204
Serine	192	177	664	623	1203	539
Threonine*	64	73	233	182	445	248
Tryptophan	9	13	90	66	99	52
Tyrosine*	26	35	38	110	342	161
Valine*	132	158	335	260	713	294
Essential	638	736	2657	2260	5603	2502
Non-Essential	1868	2177	2847	2314	6461	4162
Total	2506	2913	5504	4574	12064	6664

4.4 Discussion

Eggs collected from wild Atlantic salmon had significantly higher egg weights, diameters, proteins, lipids, carbohydrates, and free and total amino acid content, which were correlated to greater growth and survival than eggs collected from the cultured stock. Egg weight variation between groups was significant through development. Eggs collected from the wild salmon produced heavier and larger alevins which had larger yolk-sac volume at hatching compared to eggs collected from the cultured stock.

Eggs collected from the wild Atlantic salmon had higher energy content and faster embryonic development (ie. took less time to reach the eyed-stage and hatched earlier); as a result, they produced better and fewer deformed alevins at hatching than those collected from the cultured stock. The energy content per egg, alevin or fry continuously declined through development in both stocks because nutrients were utilized for metabolism. Such a trend was also noticed by other investigators in salmonids (Rice and Stokes, 1974; Zeiton et al., 1977) as well in other fishes (Kamler, 1976; Davenport and Lonning, 1980). For successful fertilization and embryonic development, an adequate amount of amino acids, nucleic acids and lipid are necessary, otherwise the development will halt or slow down at a later stage (Monroy

and Maggio, 1964; Harper et al., 1970; Tews et al., 1979, 1980; Metcoff, 1986; Fraser, 1989; Yafei and Noble, 1990).

There are two potential sources for the differences observed, between cultured and wild eggs, genotype and diet. Effects of diet on the factors examined in this study are easier to explain. In salmonids held in captivity, nutrition has been found to influence reproduction by affecting fecundity, egg size, biochemical composition of eggs, embryos and fry (Shimma et al., 1978; Wootton, 1979; Harris, 1984; Hardy, 1985; Springate et al., 1985; Watanabe, 1985). Proteins obtained from the maternal circulation later serve as amino acid and energy sources for the growing embryo (Tyler et al., 1988). Dietary lipids also play important roles in the energy production processes of animal tissues and as the source of essential fatty acids which are found to affec. number and quality of eggs of rainbow trout (Watanabe et al., 1984e). Besides these functions, they have other important dietary roles as carriers of certain non-fat nutrients, notably the fat soluble vitamins A, D, and K. It has been suggested that wild (anadromous) fish feed on varieties of richer food resources which improve fecundity and egg quality compared to cultured fish (Gross and Sargent, 1985; Gross, 1987; Gross et al., 1988). This may explain why eggs collected from wild stock had higher levels of protein, lipid, carbohydrate, amino acids, and energy content which were correlated to greater growth and survival

of embryos and alevins than eggs collected from the cultured stock. More information is needed on how the energy, protein and fat levels of broodstock diets affect fecundity and egg viability. Potential effects of different genotypes on the differences observed can not be easily explained due to lack of studies.

The egg weight and fry weight were positively correlated to the egg diameter and alevin length respectively in both populations. The egg diameters and weights were not correlated to alevin lengths and weights, respectively. A similar result has been reported by Chambers et al. (1989) for capelin eggs, where egg diameter was not correlated to larva length. Although the commonly reported generalization is that larger eggs produce larger larvae (eg. Blaxter and Hempel, 1963). My results indicated that biochemical composition and energy level in eggs are better indicators for development, growth and survival of embryos and alevins than egg diameter. This is also supported by Chambers et al. (1989).

The dry matter and ash content per egg, alevin or fry were significantly greater for the wild stock than the cultured stock because of greater contents of cell solutes and carbon compounds in eggs, alevin and fry of the wild stock. A negative growth (decrease in dry matter) was observed in fry during first feeding to one month after first feeding in spite of their change in mode of feeding,

i.e. from endogenous to exogenous. This might have been due to low water temperature at first feeding or less than optimal rearing conditions. The decrease in dry matter and ash content reflects the metabolic loss during development.

The levels of protein, lipid, and carbohydrate per egg, alevin, or fry were always significantly greater for the wild stock than the cultured stock, and declined simultaneously during development. Therefore, it can be concluded that they were used for metabolism of developing embryos, alevins or fry. Contrary to these findings, an intensive utilization of protein energy for metabolism during egg development and a predominant utilization of lipids after hatching in other fish species was noticed by Takahashi et al. (1978), Kaushik et al. (1982), Davenport et al. (1983) and Dabrowski et al. (1984). Moreover, the present findings do not agree with those of Hays (1949) who suggested that salmonid eggs used fat as the primary source of energy while protein was predominant after hatching. Terner (1968b) reported that in trout eggs (rainbow trout, Salmo gairdneri, brook trout, Salvelinus fontinalis, brown trout, Salmo trutta, and cutthroat trout, Salmo clarki), glycogen and glucose were metabolized to lactate. Thus, those eggs were capable of generating energy by glycolysis. In another teleost, the loach (Misgurnus fossilis L.), Yurowitzky and Milman (1972a) found a complete absence of aerobic glycolysis in mature oocytes and embryos. Terner

(1968b) reported that the glycogen reserve of the eggs decreased during embryonic development until, at the eyed-up stage, it was one-half of the level in unfertilized eggs. He has calculated that the total carbohydrate reserve could not support the endogenous respiration of the embryos for more than a few hours of the period of development. Since carbohydrate remains in storage, other substrates, most probably lipids, are the principal energy reserves.

The total amino acid content per egg from the wild stock was higher than the eggs from the cultured stock and declined continuously through development. The decline in total amino acid content during development was much higher for the wild stock than the cultured stock, because developing embryos and alevins of wild stock had higher metabolic requirements and, as a result of that, they hatched earlier and produced larger alevins.

Amino acids (total), alanine, aspartic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine were found to decrease in both stocks of salmon because they were used for anabolism and catabolism of embryos, alevins and fry. For Arctic char, isoleucine, lysine, serine, threonine, tryptophan and valine were found to be important for embryonic and fry development. These difference in the types of amino acids is because of their species specific roles (Metcoff, 1986). Total essential and non-essential amino acids per egg,

alevin or fry were higher for the wild stock compared to cultured stock and declined through development. This may be due to the richer diets of wild fish as essential amino acids can not be synthesised by fish, therefore, whatever is present in eggs should come from maternal diets.

The free amino acid pool was higher in eggs of the wild stock compared to the cultured stock and increased during development, as protein breakdown would have been higher than the anabolic and catabolic losses of developing embryos. The same increasing trend in free amino acid pool has been noticed during the development of Arctic charr eggs and fry (chapter three). Changes in free amino acid pool during embryonic development of fresh water fishes have never been studied before, therefore this result can not be compared. However, contrary to this work, a decrease in the free amino acid pool of Atlantic cod (Gadus morhua) embryos has been noticed by Fyhn (1989). The decline in free amino acid pool of Atlantic cod embryos may be either because of less energy reserves initially present in cod eggs to support metabolic requirements of embryos or protein breakdown rate is slower than anabolic and catabolic requirement of embryos.

Although there are significant differences in biological, morphological and biochemical egg quality criteria (traits) between cultured and wild stocks, but it can not be concluded that these differences are due to

differences in diets or differences in genotypes of broodstocks because both broodstocks were from different origins. Further studies are needed to confirm the present findings in which both broodstocks should come from same origin.

On the basis of these results, it can be suggested that an adequate amount of amino acids, protein, lipid and carbohydrate are necessary for successful development, growth and survival of embryos, alevins or fry. A reduced content of these nutrients could halt the energy production and, thus, the growth rate of the fish embryo; which would result in smaller and less viable fry.

In addition, since egg weight and egg diameter were not correlated to alevin weight and alevin length at hatching, respectively, these traits should not be used as indices of egg quality. The amino acid and/or energy content of eggs will be more appropriate to use as egg quality criteria (traits) for growth, development and survival of embryos, alevins and fry.

SUMMARY

For Arctic charr, eggs collected in the middle of the spawning season had higher egg weight, egg diameter, protein, lipid and carbohydrate, and amino acids content, which were correlated with greater development, growth and survival than eggs collected early or late in the spawning season. Eggs collected in the middle of spawning had higher energy content and faster embryonic development; as a result they took less time to reach the eyed-stage, hatched earlier, and produced better (larger alevins with bigger yolk-sac volume) and fewer deformed alevins at hatching than those collected early or late spawning season. It can be concluded that the time of stripping is an important determinant for better egg quality.

Eggs collected from wild Atlantic salmon stock were larger and had higher biochemical nutrient composition than those collected from a cultured stock. The higher nutrient composition was correlated with higher fertilization and hatching success, and growth and survival of embryos, alevins and fry.

Egg weight and alevin weight were positively correlated with egg diameter and alevin length, respectively. There was no correlation between egg diameter and alevin length or egg weight and alevin weight at hatching in both species

studied. Therefore, it would not be appropriate to use egg weight or egg diameter as an indicator of egg quality.

The amount of protein, lipid, carbohydrate, total amino acids declined simultaneously, and used as substrates for energy production during embryonic and fry development of both Arctic charr and Atlantic salmon. The free amino acid pool increased during embryonic development of Arctic charr and Atlantic salmon, and it can be concluded that the developing embryo needs increasing amounts of free amino acids as a buffer as it grows to meet an increasing and immediate energy requirement.

The amino acids, serine, valine, tryptophan, lysine, isoleucine and threonine were important for growth and survival of Arctic charr embryos, alevins and fry. However, alanine, aspartic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine were important for embryos, alevins and fry of Atlantic salmon. This difference in the types of amino acids is probably related to some species specific role.

On the basis of these results, it can be suggested that for "egg quality" criteria, biological and biochemical composition of the eggs are most important. In order to avoid complications or repetitions for considering of egg quality criteria, selection of one or two criteria (traits) would be more logical. Therefore, energy level and/or amino acid content of eggs could be used as a condition index for

development, growth and survival of embryos and alevins of salmonids.

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APPENDIX 1.

Results of Kruskal-Wallis analysis (X^2) on egg variables on replicates of Arctic charr at 3 developmental stages (1 = before fertilization, 2 = after fertilization, 3 = eyed-stage) from eggs collected early in the spawning season. N = 20, NS = No significance difference at $P > 0.05$.

VARIABLES	STAGES					
	1		2		3	
	X^2	P	X^2	P	X^2	P
Egg weight	1.43	NS	0.33	NS	0.02	NS
Egg diameter	0.12	NS	0.01	NS	0.00	NS
Protein	1.43	NS	0.33	NS	0.02	NS
Lipid	1.43	NS	0.33	NS	0.02	NS
Carbohydrate	1.43	NS	0.33	NS	0.02	NS
Energy	1.43	NS	0.33	NS	0.02	NS
Dry matter	1.43	NS	0.33	NS	0.02	NS
Ash	1.43	NS	0.33	NS	0.02	NS

APPENDIX 2.

Results of Kruskal-Wallis analysis (X^2) on fry variables on replicates of Arctic charr at 3 developmental stages (4 = hatching, 5 = first feeding, 6 = one month after first feeding) from eggs collected early in the spawning season. N = 20, NS = No significance difference at P > 0.05.

VARIABLES	STAGES					
	4		5		6	
	X^2	P	X^2	P	X^2	P
Fry weight	0.02	NS	0.64	NS	0.003	NS
Fry length	0.50	NS	0.12	NS	0.010	NS
Yolk-sac volume	0.11	NS	-	-	-	-
Protein	0.02	NS	0.64	NS	0.003	NS
Lipid	0.02	NS	0.64	NS	0.003	NS
Carbohydrate	0.02	NS	0.64	NS	0.003	NS
Energy	0.02	NS	0.64	NS	0.003	NS
Dry matter	0.02	NS	0.64	NS	0.003	NS
Ash	0.02	NS	0.64	NS	0.003	NS

APPENDIX 3.

Results of Kruskal-Wallis analysis (X^2) on egg variables on replicates of Arctic charr at 3 developmental stages (1 = before fertilization, 2 = after fertilization, 3 = eyed-stage) from eggs collected in the middle of spawning season. N = 20, NS = No significance difference at P > 0.05.

VARIABLES	STAGES					
	1		2		3	
	X^2	P	X^2	P	X^2	P
Egg weight	0.09	NS	0.02	NS	0.74	NS
Egg diameter	0.13	NS	0.11	NS	0.06	NS
Protein	0.09	NS	0.02	NS	0.74	NS
Lipid	0.09	NS	0.02	NS	0.74	NS
Carbohydrate	0.09	NS	0.02	NS	0.74	NS
Energy	0.09	NS	0.02	NS	0.74	NS
Dry matter	0.09	NS	0.02	NS	0.74	NS
Ash	0.09	NS	0.02	NS	0.74	NS

APPENDIX 4.

Results of Kruskal-Wallis analysis (X^2) on fry variables on replicates of Arctic charr at 3 developmental stages (4 = hatching, 5 = first feeding, 6 = one month after first feeding) from eggs collected in the middle of spawning season. N = 20, NS = No significance difference at P > 0.05.

VARIABLES	STAGES					
	4		5		6	
	X^2	P	X^2	P	X^2	P
Fry weight	0.48	NS	1.06	NS	0.04	NS
Fry length	0.00	NS	0.00	NS	1.82	NS
Yolk-sac volume	0.39	NS	-	-	-	-
Protein	0.48	NS	1.06	NS	0.04	NS
Lipid	0.48	NS	1.06	NS	0.04	NS
Carbohydrate	0.48	NS	1.06	NS	0.04	NS
Energy	0.48	NS	1.06	NS	0.04	NS
Dry matter	0.48	NS	1.06	NS	0.04	NS
Ash	0.48	NS	1.06	NS	0.04	NS

APPENDIX 5.

Results of Kruskal-Wallis analysis (X^2) on egg variables on replicates of Arctic charr at 3 developmental stages (1 = before fertilization, 2 = after fertilization, 3 = eyed-stage) from eggs collected late in the spawning season. N = 20, NS = No significance difference at $P > 0.05$.

VARIABLES	STAGES					
	1		2		3	
	X^2	P	X^2	P	X^2	P
Egg weight	0.09	NS	0.10	NS	0.36	NS
Egg diameter	0.00	NS	0.01	NS	0.01	NS
Protein	0.09	NS	0.10	NS	0.36	NS
Lipid	0.09	NS	0.10	NS	0.36	NS
Carbohydrate	0.09	NS	0.10	NS	0.36	NS
Energy	0.09	NS	0.10	NS	0.36	NS
Dry matter	0.09	NS	0.10	NS	0.36	NS
Ash	0.09	NS	0.10	NS	0.36	NS

APPENDIX 6.

Results of Kruskal-Wallis analysis (X^2) on fry variables on replicates of Arctic charr at 3 developmental stages (4 = hatching, 5 = first feeding, 6 = one month after first feeding) from eggs collected late in the spawning season. N = 20, NS = No significance difference at $P > 0.05$.

VARIABLES	STAGES					
	4		5		6	
	X^2	P	X^2	P	X^2	P
Fry weight	0.32	NS	0.04	NS	1.00	NS
Fry length	0.98	NS	0.39	NS	0.17	NS
Yolk-sac volume	0.00	NS	-	-	-	-
Protein	0.32	NS	0.04	NS	1.00	NS
Lipid	0.32	NS	0.04	NS	1.00	NS
Carbohydrate	0.32	NS	0.04	NS	1.00	NS
Energy	0.32	NS	0.04	NS	1.00	NS
Dry matter	0.32	NS	0.04	NS	1.00	NS
Ash	0.32	NS	0.04	NS	1.00	NS

APPENDIX 7.

Results of Kruskal-Wallis analysis (X^2) on egg variables between replicates of cultured Atlantic salmon at three developmental stages (1 = before fertilization, 2 = after fertilization, 3 = eyed-stage). N = 20, NS = No significance difference at $P > 0.05$.

VARIABLES	STAGES					
	1		2		3	
	X^2	P	X^2	P	X^2	P
Egg weight	0.001	NS	0.066	NS	0.659	NS
Egg diameter	0.286	NS	0.005	NS	0.581	NS
Protein	0.001	NS	0.066	NS	0.659	NS
Lipid	0.001	NS	0.066	NS	0.659	NS
Carbohydrate	0.001	NS	0.066	NS	0.659	NS
Energy	0.001	NS	0.066	NS	0.659	NS
Dry matter	0.001	NS	0.066	NS	0.659	NS
Ash	0.001	NS	0.066	NS	0.659	NS

APPENDIX 8.

Results of Kruskal-Wallis analysis (X^2) on fry variables between replicates of cultured Atlantic salmon at three developmental stages (4 = hatching, 5 = first feeding, 6 = one month after first feeding). N = 20, NS = No significance difference at $P > 0.05$.

VARIABLES	STAGES					
	4		5		6	
	X^2	P	X^2	P	X^2	P
Fry weight	0.727	NS	0.165	NS	1.085	NS
Fry length	0.019	NS	0.001	NS	0.181	NS
Yolk-sac volume	2.845	NS	-	-	-	-
Protein	0.727	NS	0.165	NS	1.085	NS
Lipid	0.727	NS	0.165	NS	1.085	NS
Carbohydrate	0.727	NS	0.165	NS	1.085	NS
Energy	0.727	NS	0.165	NS	1.085	NS
Dry matter	0.727	NS	0.165	NS	1.085	NS
Ash	0.727	NS	0.165	NS	1.085	NS

APPENDIX 9.

Results of Kruskal-Wallis analysis (X^2) on egg variables between replicates of ·.ıd Atlantic salmon at three developmental stages (1 = before fertilization, 2 = after fertilization, 3 = eyed-stage). N = 20, NS = No significance difference at P > 0.05.

VARIABLES	STAGES					
	1		2		3	
	X^2	P	X^2	P	X^2	P
Egg weight	0.026	NS	0.047	NS	0.354	NS
Egg diameter	0.641	NS	0.099	NS	2.006	NS
Protein	0.026	NS	0.047	NS	0.354	NS
Lipid	0.026	NS	0.047	NS	0.354	NS
Carbohydrate	0.026	NS	0.047	NS	0.354	NS
Energy	0.026	NS	0.047	NS	0.354	NS
Dry matter	0.026	NS	0.047	NS	0.354	NS
Ash	0.026	NS	0.047	NS	0.354	NS

APPENDIX 10.

Results of Kruskal-Wallis analysis (X^2) on fry variables between replicates of wild Atlantic salmon at three developmental stages (4 = hatching, 5 = first feeding, 6 = one month after first feeding). N = 20, NS = No significance difference at P > 0.05.

VARIABLES	STAGES					
	4		5		6	
	X^2	P	X^2	P	X^2	P
Fry weight	0.116	NS	1.002	NS	0.212	NS
Fry length	0.003	NS	0.284	NS	0.619	NS
Yolk-sac volume	2.708	NS	-	-	-	-
Protein	0.116	NS	1.002	NS	0.212	NS
Lipid	0.116	NS	1.002	NS	0.212	NS
Carbohydrate	0.116	NS	1.002	NS	0.212	NS
Energy	0.116	NS	1.002	NS	0.212	NS
Dry matter	0.116	NS	1.002	NS	0.212	NS
Ash	0.116	NS	1.002	NS	0.212	NS