LIPID COMPOSITION AND FATTY ACID PROFILES OF EGGS FROM WILD AND CULTURED CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) BROODSTOCK

by

Heather Ashton

B.Sc. University of Victoria, 1976.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Animal Science)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October 1991

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Department of ANIMAL SCIENCE

The University of British Columbia
Vancouver, Canada

Date 11 OCTOBER 1991

ABSTRACT

In Experiment 1, eggs of Big Qualicum (BQ) and Robertson Creek (RC) wild and cultured Chinook salmon (*Oncorhynchus tschawytcha*) were analyzed for lipid composition and fatty acid profiles. Each of the cultured broodstocks had been fed two formulated diets designated as COMM and WV33.

Significantly higher concentrations of saturated and n3 fatty acids and lower concentrations of n6 and n9 fatty acids were found in both the total and polar lipids of the eggs from the wild fish than in those from either group of cultured fish. Highly unsaturated fatty acids (HUFAs), primarily 20:5n3 and 22:6n3, were the major contributors to the n3 series in both the wild and cultured eggs. The n3:n6 ratios of both the total and polar lipids were up to 5.3 times greater in the wild than in the cultured eggs. The monounsaturate concentration was significantly greater in the cultured eggs than in the wild eggs.

The fatty acid composition of the eggs generally reflected the fatty acid profiles of the diets fed to the broodstock. Condition factors for the wild fish were significantly lower than for either group of cultured fish, reflecting the significantly greater body length of the wild fish than the

cultured fish. There were no significant differences in body weights. Survival to eyeing among the cultured eggs was lower than that reported by the respective federal government hatcheries for the wild eggs.

The quality of eggs was poor in both stocks fed the WV33 diet, with high incidences of abnormal and retained eggs and low fertility, particularly in the RC fish. Survival to eyeing was poorest in the RC-WV33 eggs. Some factor other than lipid and fatty acid composition (possibily Vitamin C) appeared to be responsible, as the fatty acid profiles in the WV33 eggs were generally closer to those of the wild fish than to those of the COMM fish. There was no explanation for the inferior performance of the RC-WV33 fish compared to the BQ-WV33 fish.

In experiment 2, feed was withdrawn from a group of Chinook broodstock for 7 days beyond the usual 7 day starvation period prior to transport to freshwater for final maturation. There were no differences in lipid composition and few differences in fatty acid profiles. Fecundity and survival to eyeing were not compromised by this treatment.

Experiment 3 was carried out to determine whether a formulated diet (COMM) augmented with krill (designated as the BROOD diet) for six weeks prior to the pre-transport starvation period

would affect lipid composition, fatty acid profiles and incubation success. The control diet was the COMM formulation. The BROOD eggs were significantly lower in total lipid concentration due to the presence of significantly less neutral lipid than in the COMM eggs. Small significant differences in individual fatty acids were found between the two groups. While some of these differences reflected the dietary fatty acids (eg. 22:1n11), others appeared to be due to retention (ie. n3 fatty acids) or to elongation and desaturation of fatty acids (eg. 18:1n9). No differences in incubation success were found between the groups.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
TABLE OF CONTENTS	v
LIST OF FIGURES	viii
LIST OF TABLES	ix
ACKNOWLEDGEMENTS	xiv
INTRODUCTION	1
LITERATURE REVIEW	7
1 Lipids of physiological importance for fish	7
2 Dietary fatty acids and requirements for growth	9
3 Digestion of dietary lipids	13
4 Role of lipids in fish	15
5 Fatty acid synthesis and mobilization	23
6 Vitellogenesis	28
7 Embryonic development	32
8 Feeding regime for cultured maturing salmonids	34
SECTION 1 - A comparison of the lipid composition and fatty acid profiles of the eggs of two stocks of wild and cultured Chinook salmon.	
1.1 Materials and Methods	37
1.1.1 Experimental design and conditions	37
1.1.2 Sample collection	41
1.1.3 Egg composition	42
1.1.4 Diet composition	49
1.1.5 Egg size	50
1.1.6 Fertilization and eyeing success	51
1.1.7 Statistical procedures	51
1.2 Results and Discussion	53
1.2.1 Morphometric measurements	53
1.2.2 Spawning and incubation success	55
1.2.3 Composition of the eggs	63
1.2.4 Fatty acid composition of the total lipids	68
1.2.5 Fatty acid composition of the polar lipids	81
1.2.6 Diets	93
1.3 Conclusions	101

SECTION 2 - Feed withdrawal from cultured Chinook broodstock prior to transfer to freshwater for maturation.

2.1 Materials and Methods 109

2.2 Results and Discussion 111
2.2.1 Morphometric measurements 111
2.2.2 Spawning and incubation success 111
2.2.3 Composition of the eggs 115
2.2.4 Fatty acid composition of the total lipids 118
2.2.5 Fatty acid composition of the polar lipids 123

2.3 Conclusions 130

SECTION 3 - Alteration of lipid composition and fatty acid profiles in eggs from Chinook broodstock.

3.1 Materials and Methods 131

3.2 Results and Discussion 133
3.2.1 Morphometric measurements 133
3.2.2 Spawning and incubation success 133
3.2.3 Composition of the eggs 136
3.2.4 Fatty acid composition of the total lipids 138
3.2.5 Fatty acid composition of the polar lipids 140
3.2.6 Diets 147

3.3 Conclusions 152

CONCLUDING REMARKS 153

REFERENCES 155

APPENDIX 1 - Preliminary analyses of selected nutrient parameters in the eggs of wild and cultured Chinook salmon - 1985 & 1986. 164

APPENDIX 2 - Diet Data.

Table A2.1: Manufacturer's feed label data for commercial (COMM) and brood (BROOD) diets. 165
Table A2.2: Formulation of the West Vancouver 33 (WV33) diet. 166
Table A2.3: Dry matter and lipid composition of the experimental diets used in Sections 1, 2 and 3. 167
Table A2.4: Fatty acid profiles of diets. 168
Table A2.5: Dry matter and lipid composition and fatty acid profile of a euphausiid (krill) sample. 169

APPENDIX 3 - Formulae for calculating composition parameters. 170

APPENDIX 4 - Fatty acid profiles of eggs.

Table A4.1: Fatty acid profiles of TOTAL LIPIDS in the eggs of BIG QUALICUM broodstock on three diets. 171

Table A4.2: Fatty acid profiles of TOTAL LIPIDS in the eggs of ROBERTSON CREEK broodstock on three diets. 172

Table A4.3: Fatty acid profiles of POLAR LIPIDS in the eggs of BIG QUALICUM broodstock on three diets. 173

Table A4.4: Fatty acid profiles of POLAR LIPIDS in the eggs of ROBERTSON CREEK broodstock on three diets. 174

Table A4.5: Fatty acid profiles of TOTAL LIPIDS in the eggs of 4-year old BIG QUALICUM broodstock. Feed was withdrawn from these fish for 7 days or 14 days prior to transfer to freshwater for maturation. 175

Table A4.6: Fatty acid profiles of POLAR LIPIDS in the eggs of 4-year old BIG QUALICUM broodstock. Feed was withdrawn from these fish for 7 days or 14 days prior to transfer to freshwater for maturation. 176

Table A4.7: Fatty acid profiles of TOTAL LIPIDS in the eggs of 4-year old broodstock on a commercial grower diet (COMM) or on a brood (BROOD) diet prior to transfer to freshwater for maturation. 177

Table A4.8: Fatty acid profiles of POLAR LIPIDS in the eggs of 4-year old broodstock on a commercial grower diet (COMM) or on a brood (BROOD) diet prior to transfer to freshwater for maturation. 178

LIST OF FIGURES

Figure		Page
1	Conversion of arachidonic acid (20:4n6) to eicosanoids.	20
2	Fatty acid synthesis in fish.	25
3	Flowchart of procedures for lipid analysis and fatty acid determinations.	43
4	Egg abnormalities in Chinook broodstock on the WV33 diet.	60
5	A comparison of saturated, n3, n6 and n9 fatty acids and n3:n6 ratios in total and polar lipids of eggs from cultured and wild Big Qualicum and Robertson Creek broodstock with the COMM and WV33 diets.	95
6	A comparison of selected n3 fatty acids, n3 PUFAs and HUFAs in total and polar lipids of eggs from cultured and wild Big Qualicum and Robertson Creek broodstock with the COMM and WV33 diets.	97
7	A comparison of selected fatty acids and n6 PUFAs in total and polar lipids of eggs from cultured and wild Big Qualicum and Robertson Creek broodstock with the COMM and WV33 diets.	98
8	A comparison of selected fatty acids in the total and polar lipids of eggs of 4-year old broodstock with those in their diets (COMM and BROOD).	150

LIST OF TABLES

Table		Page
1	Morphometric data for wild and cultured Big Qualicum and Robertson Creek female broodstock.	54
2	Spawning data for wild and cultured Big Qualicum and Robertson Creek broodstock.	56
3	Egg retention and other reproductive abnormalities observed in Big Qualicum and Robertson Creek cultured females on two diets.	58
4	Incubation data for wild and cultured Big Qualicum and Robertson Creek broodstock.	62
5a	Composition of the eggs of BIG QUALICUM broodstock on three diets.	64
5b	Composition of the eggs of ROBERTSON CREEK broodstock on three diets.	65
6a	Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in TOTAL LIPIDS of eggs from BIG QUALICUM broodstock on three diets.	70
6b	Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in TOTAL LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets.	71
7a	Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in TOTAL LIPIDS of eggs from BIG QUALICUM broodstock on three diets.	73
7b	Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in TOTAL LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets.	74
8a	Selected n6 fatty acids and total n6 PUFAs in TOTAL LIPIDS of eggs from BIG QUALICUM broodstock on three diets.	76
8b	Selected n6 fatty acids and total n6 PUFAs in TOTAL LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets.	77
9a	Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in TOTAL LIPIDS of eggs from BIG QUALICUM broodstock on three diets.	79

9b Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in TOTAL LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets. 80

10a Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in POLAR LIPIDS of eggs from BIG QUALICUM broodstock on three diets. 82

10b Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in POLAR LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets. 83

11a Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in POLAR LIPIDS of eggs from BIG QUALICUM broodstock on three diets. 85

11b Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in POLAR LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets. 86

12a Selected n6 fatty acids and total n6 PUFAs in POLAR LIPIDS of eggs from BIG QUALICUM broodstock on three diets. 88

12b Selected n6 fatty acids and total n6 PUFAs in POLAR LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets. 89

13a Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in POLAR LIPIDS of eggs from BIG QUALICUM broodstock on three diets. 91

13b Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in POLAR LIPIDS of eggs from ROBERTSON CREEK broodstock on three diets. 92

14 Morphometric data for Big Qualicum 4-year old broodstock. Feed was withdrawn from these fish for 7 or 14 days prior to freshwater transfer. 112

15 Spawning and incubation data for Big Qualicum 4-year old broodstock. Feed was withdrawn from these fish for 7 or 14 days prior to freshwater transfer. 113

16 Composition of the eggs of 4-year old Big Qualicum broodstock. Feed was withdrawn from these fish for 7 or 14 days prior to freshwater transfer. 116

17 Absolute volumes of total and polar lipid per egg calculated from mean egg volumes and percentages of these parameters. 117

18 Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 119

19 Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 120

20 Selected n6 fatty acids and total n6 PUFAs in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 121

21 Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in TOTAL LIPIDS of eggs from 4-year Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 122

22 Absolute volumes of total saturates, n3 fatty acids, n3 HUFAs and 22:6n3 in the total and polar lipids per egg calculated from the absolute volumes of total and polar lipid per egg and the percentage of these parameters, as reported in previous tables. 124

23 Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 126

24 Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 127

25 Selected n6 fatty acids and total n6 PUFAs in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 128

26 Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in POLAR LIPIDS of eggs from 4-year Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. 129

27 Morphometric data for 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 134

28 Spawning and incubation data for Big Qualicum 4-year old broodstock on a commercial diet (COMM) or a brood diet (BROOD) prior to transfer to freshwater for maturation. 135

29 Composition of the eggs of 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial (COMM) diet prior to transfer to freshwater for maturation. 137

30 Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 139

31 Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 141

32 Selected n6 fatty acids and total n6 PUFAs in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 142

33 Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in TOTAL LIPIDS on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 143

34 Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 144

35 Selected n3 fatty acids, total n3 PUFAs and total n3 HUFAs in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 145

36 Selected n6 fatty acids and total n6 PUFAs in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 146

37 Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids in POLAR LIPIDS of eggs from 4-year Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation. 148

ACKNOWLEDGEMENTS

My sincere appreciation goes to all who provided support and encouragement to me throughout my studies. In particular I wish to thank Dr. David Groves for so willingly accepting me as a student at Sea Spring Salmon Farm and for his generous sharing of knowledge and practical experience in the culture and nutrition of salmon. I will never forget his unfailing good humour and ready tales of his years as a student and university professor.

I wish also to thank Dr. Beryl March for her guidance and knowledge and for bringing to my attention the grammatical *faux pas* of the split infinitive. Her questioning nature, fascination with the biology of all animals and insights into nutrition were an inspiration.

Thank you to Dr. David Higgs who provided useful suggestions and encouragement throughout. His positive attitude toward research problems and life in general and his sunny disposition were greatly appreciated.

Thanks also to Doug Harpham and the staff (especially Peter and Cathy) of Sea Spring Salmon Farm Ltd; Gilles Galzy, Andy Hickman and Leslie Hart of the Department of Animal Science, UBC; Gordon Miller, Susan Keller, Henrik Krieberg and Iain Whyte of the Pacific Biological Station in Nanaimo and West Vancouver; Yvonne Yole of the Scale Ageing Lab at DFO, Vancouver; and Don Lawseth and Grant Ladocoeur, managers of Robertson Creek and Big Qualicum Hatcheries.

Thanks also to my family - Eric, Merrell, Mum and Dad, Laurie, Brian, John, Elma and Margaret for their love, patience and encouragement throughout my years as a student.

This work was supported by Sea Spring Salmon Farm Ltd., Chemainus, BC and by the BC Science Council through a GREAT award.

Finally I would like to dedicate this thesis to my 17 month old daughter, Merrell, who will 'learn much more than I'll ever know.'[1]

"I hear babies cry; I watch them grow,
They'll learn much more than I'll ever know,
And I think to myself: What a Wonderful World."

Louis Armstrong, "What a Wonderful World."

INTRODUCTION

The survival of cultured Chinook salmon (*Oncorhynchus tschawytcha*) from fertilization to hatch and through to ponding has, until recently, been much lower than that of wild Chinook taken from their natal streams and spawned and incubated using the same hatchery procedures. Until 1987, the best survival to ponding reported by a private hatchery was approximately 70%. This figure was unusually high for the industry and survival to the eyed stage at some private hatcheries had been in the range of 13 to 35%. Survival to ponding had been even lower. Wild Chinook, spawned in the federal government hatchery system and reared to release as smolts, routinely have survival rates to eyeing of 90% or more and to ponding of approximately 85% (D. Lawseth, Department of Fisheries and Oceans, Big Qualicum Hatchery, pers. comm.).

Sea Spring Salmon Farm Ltd., a company that rears smolts for the salmon farming industry, has held a federal government contract with the Department of Fisheries and Oceans for twelve years to take and spawn maturing Chinook from the Chemainus River on Vancouver Island and to rear the smolts for release back to the wild. Survival rates to eyeing for eggs from the wild Chemainus fish have been 20 to 60% better than the survival rates of eggs from cultured Big Qualicum broodstock, spawned and reared under identical conditions. In 1986, the inability of the private hatcheries to produce enough eggs

consistently for the growing salmon farming industry in British Columbia called into question the viability of salmon farming as a whole.

Poor survival rates also hampered the development of domestic strains of fish selected for tolerance to stress, disease resistance, enhanced growth rates, improved reproductive performance, etc. while avoiding the negative impacts of inbreeding. Without adequate numbers of progeny, hatcheries could ill afford to undertake genetic experiments.

In 1985 and 1986, preliminary investigations into the problem of poor egg survival in cultured Chinook salmon were initiated by Dr. David Groves (Sea Spring Salmon Farm Ltd.) in conjunction with the British Columbia Salmon Farmers Association and the Government of Canada, Department of Fisheries and Oceans (IRAP (PILP) #CA910-5-0042/866). Broodstock handling and spawning techniques were examined but the major emphasis of the investigation was directed to broodstock nutrition. With the participation of several private hatcheries, a survey of selected nutrient parameters in cultured eggs and in the eggs from three wild stocks was carried out. Results from the 1985 and 1986 analyses, presented in Appendix 1, revealed important differences in fatty acid composition between cultured and wild eggs.

The objectives of this thesis were set out in view of these

results. The first goal was the accurate determination of the lipid profiles in the eggs of wild and cultured fish. For this purpose, the eggs of two stocks (Big Qualicum and Robertson Creek) of Chinook salmon were sampled. Two groups of cultured fish of each stock were fed either a commercial diet in wide use in the industry or the open formulation, West Vancouver 33 diet (D.A. Higgs, Department of Fisheries and Oceans, West Vancouver Laboratory, unpub. data). These diets were manufactured by two independent commercial feed companies in British Columbia. Wild Chinook eggs were sampled from the returns to the Big Qualicum and Robertson Creek Hatcheries, two Government of Canada facilities. The results of this study, documenting lipid concentrations and fatty acid profiles of the total and polar lipids in the eggs of Chinook salmon for the first time, are presented in Section 1.

In the early studies (D. Groves, 1987 unpub. report), it was found that the muscle of cultured fish had higher levels of lipid than wild fish and that cultured fish produced eggs with higher lipid concentrations (Appendix 1). A general observation was also made that condition factors were lower in the wild than in the cultured broodstock. It was postulated that 'fat fish do not tend to be productive broodstock' and that ingested energy in cultured fish was being deposited as fat rather than as protein. Reasons proposed for this were that cultured fish expend less energy than their wild counterparts in their daily lives, that they are on a higher

plane of nutrition than wild fish or that some nutritional imbalance may exist which promotes the deposition of fat rather than protein.

Wild Chinook returning to their natal streams from the ocean cease feeding upon or prior to entry to freshwater. Many stocks then undergo long arduous migrations prior to reaching the spawning grounds. Greene (1919) and others have reported the extensive depletion of body reserves in migrating Chinook during the period when gonad tissues are proliferating and maturing. This lends credence to the suggestion that cultured fish are on a higher plane of nutrition than is necessary and that this higher feeding regime may be detrimental to the production of high quality eggs.

To test the hypothesis that the plane of nutrition in cultured fish towards the end of vitellogenesis is excessive, feed was withdrawn from a group of cultured fish for 14 days prior to transfer to freshwater for maturation and spawning. A second group, the control, was starved for one week prior to transfer, following the usual protocol for broodstock transport. Lipid composition and survival rates of the eggs of both groups were monitored.

The third objective was aimed at modifying the fatty acid profile of the eggs of cultured broodfish by feeding a commercially manufactured brood diet containing krill for six

weeks prior to transport to the hatchery. It was thought that enrichment with krill would provide a more favourable balance of fatty acids, ie. closer to what may be found in eggs from fish feeding in the wild. This work is presented in Section 3.

Hatchery facilities and all cultured fish for this work were provided by Sea Spring Salmon Farm Ltd., Chemainus, BC. The Big Qualicum and Robertson Creek fish fed the West Vancouver 33 diet were part of a larger study conducted by the Government of Canada, Department of Fisheries and Oceans; the Government of British Columbia, Ministry of Agriculture and Fish; BC Research; and the BC Salmon Farmers' Association. This study was undertaken to examine the potential for rearing these and four other stocks at environmentally diverse sites along the Pacific coast. The net pen facility of Sea Spring Salmon Farm Ltd. at Genoa Bay near Duncan on Vancouver Island was one of these sites. Fish from each stock, reared at each site, were from the same genetic mix and all were fed the West Vancouver 33 diet to eliminate genetic and diet factors from the study (Withler *et al.*, 1986). Some fish matured in 1987 at three years of age when the sampling for the present study was undertaken.

Since the preliminary studies in 1985 and 1986 (D. Groves, 1987 unpub. report), the commercial feed companies have made modifications to their formulations including the addition of higher levels of Vitamins C and E and selenium, demonstrated as

essential for the reproductive success of many species (Watanabe, 1985). Because the diets are closed formulations and because no controlled experiments were performed to test their effects, it is impossible to ascertain precisely which modifications have been responsible for the significant increase in survival of cultured eggs seen since 1986. While perhaps not as consistently successful as their wild counterparts, cultured Chinook salmon typically now have survival rates to ponding of 75-85%.

LITERATURE REVIEW

1 Lipids of physiological importance for fish

Lipids include fats, oils, waxes and related compounds. They are relatively insoluble in water but soluble in nonpolar solvents such as chloroform, ether and benzene.

Fatty acids are constituents of all lipids. They consist of a hydrocarbon chain with a terminal carboxylate group. Fatty acids can be divided into two broad categories, the saturates and the unsaturates. Saturated fatty acids have no double bonds. Unsaturated fatty acids have double bonds between some of the carbon atoms in the hydrocarbon chain and may be called monoenoic, dienoic, trienoic, ... hexaenoic if one, two, three, ... six double bonds are present. Consecutive double bonds are methylene interrupted so that a $-CH_2-$ group occurs between two carbons carrying double bonds. The position of the first double bond from the methyl end of the molecule in unsaturates is of physiological significance and determines the omega (n) number of the fatty acid [1].

The fats and oils are usually considered insoluble in water due

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[1] Fatty acid nomenclature follows the notation C:xnp, where C = the number of carbon atoms in the hydrocarbon chain, x = the number of double bonds, n is an abbreviation for omega and p = the position of the first double bond from the methyl end of the molecule. Therefore 16:1n7 is a monoenoic unsaturated fatty acid with 16 carbon atoms, 1 double bond and the position of the double bond is on carbon 7 counting from the methyl end.

to the presence of nonpolar hydrocarbon groups. However polar groups in association with fatty acids, forming phospholipids, sphingolipids and others, impart various degrees of water solubility to the molecule. Lipids can therefore be classified on the basis of their polarity, ie the neutral and the polar lipids.

Neutral lipids include the triacylglycerides (TAGs), which form the major lipid energy depot. TAGs consist of three long-chain fatty acids esterified to the alcohol, glycerol. Saturates, particularly 14:0 and 16:0, and the monounsaturates, 16:1n7 and 18:1n9, typically occupy positions on the first (α) and third (τ) carbon of glycerol in TAG molecules. The second (β) carbon of glycerol may be attached to another saturate or to a monounsaturate or, as is common in fish, to a PUFA or HUFA (Christie, 1986). In the second position, these long chain fatty acids are less susceptible to oxidation for energy (Murray, 1988). Other common neutral lipids are the mono and diacylglycerides (with one or two fatty acids esterified to glycerol); cholesterol and cholesteryl esters (cholesterol esterified to a fatty acid), and free fatty acids.

The polar lipids include the phosphoglycerides, glycolipids, sphingomyelins and plasmolagens. These compounds have either a glycerol or sphingosine (an amino alcohol) backbone, one or two fatty acid residues and a polar head group. With the exception of the glycolipids all of these polar lipids contain a

phosphate group and are often referred to as phospholipids. Glycolipids contain sphingosine, a fatty acid and one or more sugar groups. Phosphoglycerides and glycolipids are important constituents of biomembranes. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and diphosphatidylglycerol (cardiolipin) are all phosphoglycerides. Cerebrosides and gangliosides are glycolipids, important throughout the body but especially in neural tissue and the brain. Sphingomyelins are also found in brain and nerve tissue while plasmolagens comprise as much as 10% of the phospholipids in brain and muscle tissue (Murray *et al.*, 1988).

Saturated and monounsaturated fatty acids are frequently present in the α-position of the glycerol backbone in polar lipids with an n3 PUFA or HUFA in the β-position. In this position, the unsaturate is protected if the saturate or monoene is oxidized (Greene and Selivonchick, 1987).

2 Dietary fatty acids and requirements for growth

Saturated fatty acids with even numbers of carbon atoms can be synthesized *de novo* from acetate (Castell, 1979). Some dietary fatty acids can be elongated and desaturated and incorporated into compounds as required. Other fatty acids cannot be synthesized, have specific metabolic functions and must be provided in the diet. These are the essential fatty acids.

Fish cannot synthesize any member of the linoleic (n6) or the linolenic (n3) series unless a precursor with the same omega structure is present. In addition, there is competitive inhibition between the n3, n6 and n9 series such that elongation and desaturation of one series is inhibited by the members of another series. The n3 series is the most potent inhibitor, followed by the n6 and n9 series (Halver, 1980).

Dietary studies have demonstrated the essentiality of the n3 (linolenic series) fatty acids for rainbow trout. Castell *et al.* (1972 a,b,c) established the requirement for 18:3n3 at 1% of the diet by weight or approximately 2.7% of the dietary calories. This was based on achieving the best growth rates and feed conversions and on alleviating several signs of essential fatty acid deficiency, eg. fin erosion, heart myopathy and syncope that occurred during their studies.

Takeuchi and Watanabe (1977b) found that as the total level of lipid increased in the diet of rainbow trout, the requirement for 18:3n3 also increased. For example 1% 18:3n3 was sufficient in diets containing relatively low lipid levels (up to 5%) but in diets containing 14% lipid, the requirement for 18:3n3 was more than 2% of the diet for maximum growth. Hence,

it is suggested that the essential fatty acid requirement should be expressed as a percentage of the dietary lipid and for rainbow trout the requisite level was set at approximately

20% as 18:3n3.

Some workers believe that n6 fatty acids may also be essential for salmonids. Nicolaides and Woodall (1962) reported 70-80% of juvenile Chinook salmon exhibited dermal depigmentation when they were fed diets containing either no lipid, 18:0 as triolein at 1% of the diet, or 0.1% of the diet as 18:3n3. When the fish were fed 1% of the diet as 18:2n6 (as trilinolein), 86% displayed normal pigmentation. When 0.1% 18:3n3 and 1% trilinolein were included in the diet, 69% of the fish were normal. Growth rates were also studied and found to be highest in those fish receiving 18:3n3 alone at 0.1% of the diet and these were not improved even when 3% 18:2n6 (as trilinolein) was added to the diet.

Ackman and Takeuchi (1986) reported levels of 18:3n3 and 20:4n6 that were three and ten times higher, respectively, in wild than in hatchery-reared Atlantic pre-smolts overwintering at 0-0.5° C. The hatchery smolts experienced severe fin erosion which often led to mortality. This prompted an investigation into the possibility of an essential fatty acid deficiency. Although the hatchery reared fish had higher levels of 18:2n6 than the wild fish, they did not elongate and desaturate 18:2n6

to 20:4n6. It was suggested that high levels of n3 fatty acids may have inhibited the elongation/ desaturation of 18:2n6 in the hatchery fish. Moreover, it was noted that the aquatic

insect diet of the wild fish included high levels of pre-formed 20:4n6. There was no conclusive proof that high levels of 20:4n6 were preventing fin erosion in the wild fish. However Ackman and Takeuchi suggested that 20:4n6, being a precursor to prostaglandins (see below), may have been important in a defense mechanism against the lesions.

It has also been shown that dietary inclusion of 18:2n6 prevents the accumulation of 20:3n9, an indicator of essential fatty acid deficiency (Greene and Selivonchick, 1987).

It is generally believed that the n6 fatty acids (linoleic series) are not essential for salmonids although they are for other species, particularly the homeothermic land-dwelling animals. Castell (1972a) demonstrated that no combination of 18:2n6 and 18:3n3 resulted in as good a growth rate or feed conversion as 18:3n3 alone at 1% of the diet. Yu *et al.* (1979) also showed that the best growth rates occurred when trout were fed 1% 18:3n3 as the sole dietary essential fatty acid.

Yu and Sinnhuber (1979b) found that coho salmon (*Oncorhynchus kisutch*) fry required 1 to 1.5% of a diet containing 10% lipid as n3 fatty acids. The presence of more than 1% n6 fatty acids or extremely low or high dietary levels of n3 fatty acids depressed growth. The optimum level of polyunsaturated fatty acids (n3+n6) in the diet was approximately 2.5% or less.

Takeuchi *et al.* (1979c) investigated fatty acid requirements in chum salmon (*Oncorhynchus keta*) fry held in both fresh and saltwater. The best weight gain and feed conversion was obtained when both 1% 18:3n3 and 1% 18:2n6 were present.

Fatty acid requirements for Chinook salmon (*Oncorhynchus tshawytscha*) have not been reported for any stage of their life cycle. The presence of long chain highly unsaturated n3 fatty acids (n3 HUFAs) are a notable feature of the body composition of all Pacific salmon (Plotnikoff *et al.*, 1984), and juvenile Chinook salmon are able to convert 18:3n3 to n3 HUFAs readily (Mugrditchian *et al.*, 1982 and Dosanjh *et al.*, 1988). This suggests the importance of the linolenic (n3) series for this species.

Signs of essential fatty acid deficiency in salmonids include: poor growth; elevated levels of 20:3n9; necrosis of the caudal fin; fatty, pale liver; dermal depigmentation; increased water content of the muscle; syncope in response to shock; increased mitochondrial swelling; heart myopathy; increased respiration of liver homogenates; and low hemoglobin levels (Sinnhuber, 1969).

3 Digestion of dietary lipids

Hydrolysis of triacylglycerides, the major component of dietary lipids, is catalysed by pancreatic lipase. This results in the

liberation of fatty acids and monoacylglycerides. If the fatty acids esterified to the TAG are all saturated, then the rate of hydrolysis for all three positions will be the same. If position two is occupied by an unsaturate then it may not be hydrolysed, leaving a monoacylglyceride. If the unsaturate is in position one or three, it will be hydrolysed at the same rate as a saturated fatty acid (Leger, 1985).

Hydrolysis of dietary phospholipids is thought to result in the formation of free fatty acids and lysophospholipids as in mammals (Henderson and Tocher, 1987). Typically polar lipids have an essential fatty acid esterified to the β position and a saturate or monoene at the α position. This arrangement probably makes the essential fatty acid less susceptible to hydrolysis and removal than fatty acids in the α position (Dave *et al.*, 1976, cited in Castledine and Buckley, 1982).

Liberated fatty acids, monoacylglycerides and lysophospholipids are absorbed by the proximal intestine and the pyloric caecae. The rate of absorption is much slower than in mammals and appears to increase with the degree of unsaturation and the water temperature (Henderson and Tocher, 1987). All dietary fatty acids, except those that are rapidly incorporated into phospholipids, are either catabolized for energy or stored (Yu *et al.*, 1977). Sire *et al.* (1981) has demonstrated that fatty acids are re-esterified to TAGs within the intestinal epithelium and it is thought that lysophospholipids are re-

esterified with fatty acids to phospholipids as occurs in mammals (Henderson and Tocher, 1987).

After crossing the intestinal wall, most dietary lipids are packaged into chylomicrons and some into lipoproteins (VLDL) for transport by the lymph (Leger, 1985). Most lipoprotein (eg. VLDL, LDL, HDL) synthesis takes place in the liver in salmonids, including the lipoprotein, vitellogenin, which is produced by maturing female fish (Henderson and Tocher, 1987). Uptake and storage of TAGs occurs in adipose tissue (Henderson and Sargent, 1985) and in muscle, particularly in the red muscle in salmonids (Hepher, 1988).

4 Role of lipids in fish

Energy is required for maintenance, growth and reproduction in all animals. While fish preferentially catabolize protein to meet their energy requirements, diets for farmed fish are formulated to minimize protein consumption for energy. This spares costly protein for growth and tissue repair. Salmonids have a limited ability to utilize carbohydrate as an energy source. Excess (>20%) dietary carbohydrate fed to rainbow trout resulted in enlarged livers with glycogen-filled vacuoles in the hepatocytes, depressed growth rates and increased mortality (Hilton and Slinger, 1981). The nutritionist therefore incorporates lipids into the diet as the major energy source. The metabolizable energy value of lipids is 9.45

kcal/g lipid, more than double that available from carbohydrate (4.0 kcal/g for dextrin and glucose) or protein (4.5 kcal/g for crude protein) (McCallum and Higgs, 1989).

Aside from the provision of energy, lipids function in the transport of the fat soluble vitamins, A, D, E and K, to all tissues. They improve the palatability of formulated diets and provide essential fatty acids as discussed previously.

Fatty acids are integral parts of many vital components of the body of all animals. The amphipathic nature (having both polar and nonpolar components) of polar lipid molecules is central to their function in cell membranes. Orientation of these lipids at water-oil interfaces is such that the polar group is in the water phase and the nonpolar portion is in the oil phase. A bilayer of these polar lipids is the basic structure of biological membranes, both cellular and subcellular.

Naturally occurring unsaturated fatty acids are almost entirely of the cis configuration and the molecule is bent 120° at a double bond. As the level of unsaturation increases in membranes, the molecules pack together less tightly due to greater numbers of these bonds and the concomitant reduction in van der Waal attractions. High levels of unsaturation promote membrane fluidity and permeability. This is especially important in cold acclimated species such as the salmonids where it has been shown that as temperature decreases a

restructuring of membranes with greater unsaturation occurs (Hazel, 1979; Lie *et al.*, 1989).

Without a shift to a more unsaturated state, membrane associated functions such as carbohydrate transport, rates of mitochondrial oxidation, ATP production, catalytic activity of membrane-bound enzymes, nuclear to cytoplasmic transport of RNA, and nonelectrolyte permeability would be compromised. Conversely, as temperatures increase, a shift toward less unsaturation occurs. Without this shift an increase in membrane permeability would occur with a dissipation of ion gradients, increased neural excitability and ultimately heat death (Hazel, 1979).

The dynamic composition of membranes also reflects salinity changes. Leray *et al.* (1984) reported a transformation in the fatty acid composition of PC in the intestinal mucosa of rainbow trout, ie from 22.4% to 11.9% for 18:0 and from 10.6% to 28.0% for 22:6n3 within one day of transfer from fresh to salt water. Similar changes were found in PE and to a lesser extent in PS and PI. These changes were related to a measured increase in membrane fluidity and it was suggested that important ion transport mechanisms may require an increase in fluidity to function in the more saline environment.

Sheridan *et al.* (1985) found an increase in 20:5n3, 22:5n3 and 22:6n3 in both the triacylglycerides and phospholipids in

hatchery reared steelhead trout during smoltification. At the same time they noted the reduction in total body lipids typical of the parr-smolt transformation. This lipid depletion was due to a decrease in monoenes and saturated fatty acids rather than PUFA and illustrates selective retention of PUFA over other fatty acids by salmonids.

Bell *et al.* (1985) determined that 22:6n3 was essential for the maintenance of healthy gill epithelium in turbot, *Scophthalmus maximus*. Four diets were fed that contained n6 fatty acids only or that had 20:5n3 to 22:6n3 ratios of 1.8, 2.2 or 13.8. Juvenile turbot were able to survive with 20:5n3 to 22:6n3 ratios of 1.8 or 2.2 but could not survive on the diet containing only n6 fatty acids. Those on the high 20:5n3 diet (13.8 ratio) also experienced high mortality. These fish were apparently unable to convert 20:5n3 to 22:6n3. When dietary 22:6n3 was low or absent, chloride cells disappeared and the epithelium of the primary and secondary lamellae sloughed off leaving a skeleton of connective tissue with masses of debris in the interlamellar spaces.

The rapid turnover of gill epithelium, and particularly the chloride cells with their convoluted plasma membranes and numerous mitochondria, makes the gills a sensitive indicator of the essential fatty acid status of fish and illustrates the absolute requirement for 22:6n3 as a structural component of biomembranes. Langdon and Thorpe (1984) demonstrated a

proliferation and enlargement of chloride cells on the gill filaments of Atlantic salmon (*Salmo salar*) smolts on transfer to saltwater. These cells produce Na^+-K^+ ATPase and gill succinic dehydrogenase, key enzymes in osmoregulation and ion transport.

Fatty acids have an important role as substrates for eicosanoid synthesis. Eicosanoids comprise the prostaglandins (PG), prostacyclins (PGI), thromboxanes (TX) and leukotrienes (LT). PG, PGI and TX together are often referred to as prostanoids. All are physiologically active compounds derived from 20 carbon (eicosa-) fatty acids and containing a 5-carbon ring. Eicosanoids are extremely potent and a very small amount has a pronounced effect. As little as 1 ng PG/ml causes the contraction of smooth muscle in animals (Murray *et al.*, 1988). They are synthesized within membranes, rapidly taken up, used and rapidly de-activated.

In mammals, the predominant pathway is from arachidonic acid, 20:4n6, as shown in Figure 1 (adapted from Murray *et al.*, 1988) giving rise to the group 2 eicosanoids. Groups 1 and 3 are derived from 20:3n6 and 20:5n3, respectively, in analogous pathways (Fischer and Weber, 1984). The subscript of the eicosanoid indicates the number of double bonds in the molecule and the group to which it belongs.
The functions of eicosanoids are many and varied. TXA_2 (a group 2 thromboxane synthesized in platelets from 20:4n6) is a

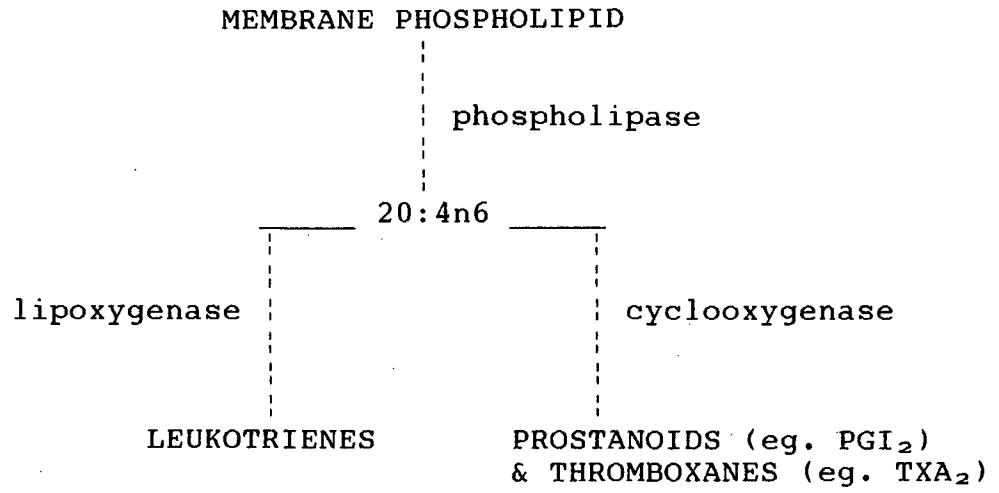


Figure 1: Conversion of arachidonic acid (20:4n6) to group 2 eicosanoids. By analogous pathways, eicosatrienoic acid (20:3n6) and eicosapentaenoic acid (20:5n3) are substrates for the synthesis of groups 1 and 3 eicosanoids, respectively.
(after Murray *et al.*, 1988)

potent stimulator of platelet aggregation and vasoconstriction. PGI_2 is produced in the walls of blood vessels and is antagonistic to TXA_2. TXA_3 (a group 3 thromboxane, also synthesized in platelets but from 20:5n3) also stimulates platelet aggregation but it has much weaker clotting effects than TXA_2. PGI_3 equals PGI_2 in anti-clotting potency but because TXA_3 is weaker, the balance shifts toward anti-aggregation. In addition, group 3 prostanoids block the synthesis of group 2 prostanoids by inhibiting the release of 20:4n6 from membrane phospholipids. This is thought to be the basis of the low incidence of ischemic heart disease, reduced platelet aggregation and prolonged clotting times in Eskimos whose diet is high in fish oils with high concentrations of 20:5n3 and low in cholesterol, triacylglycerides and very low density lipoproteins (Murray *et al.*, 1988).

Leukotrienes are synthesized in leukocytes, platelets and macrophages. They act to promote the inflammation response in infections and the hypersensitivity reaction in allergies. In mammals eicosanoids also modulate the action of hormones, regulate blood flow to particular organs, control ion transport across some membranes, and modulate synaptic transmission along nerve cells (Murray *et al.*, 1988).

Although 20:5n3 appears to be the the main prostaglandin precursor in fish, there is little evidence for the production of the PG_3 series or their effects in fish tissue (Greene and

Selivonchick, 1987). $PGF_{2\alpha}$ is a potent *in vitro* stimulator of ovulation in brook trout (Stacey and Goetz, 1982); however it is not clear what natural role the PGF prostaglandins may have at ovulation (Goetz, 1983). Kayama *et al.* (1986) demonstrated the production of TXA_3, from 20:5n3 in rainbow trout. Studies on plaice skin indicated that 20:5n3 (or its products) may inhibit the transformation of 20:4n6 to a muscle and vaso-contracting PG (presumably TXA_2) (Anderson *et al.*, 1979, 1981) and that 20:5n3 may be a precursor to PGI_3, the potent vaso-dilating compound, as in man (Fischer and Weber, 1984). Greene and Selivonchick (1987) suggest that PGI_3 may be important in temperature acclimation in fish.

There is some speculation that 22:6n3, often the predominant HUFA in fish, including salmonids, may be retroconverted to 20:5n3 for PG synthesis (Tinoco, 1982; Yu and Sinnhuber, 1972; Greene and Sevilonchick, 1987). The presence of 22:6n3 in membrane phospholipids may be more than a mechanism to promote fluidity at low temperatures and may, in fact, be a local storage depot for PG precursors (Greene and Selivonchick, 1987). Other workers have demonstrated the synthesis of leukotrienes directly from 22:6n3 (German *et al.*, 1986a, 1986b).

The lipids in the retinal membrane of the human eye contain 30% or more of the fatty acid, 22:6n3. Rhodopsin molecules in the eye change shape in response to light, enabling images to be

perceived. It is thought that the flexibility of the retinal membrane, due to the presence of 22:6n3, facilitates the response of rhodopsin (Castell, 1988).

Tocher and Harvie (1988) found high levels of 22:6n3 in the phosphoglycerides (PC, PE, PS, and PI) of the retinas of rainbow trout and cod (*Gadus morhua*). They postulated that 22:6n3 may be retroconverted to 20:5n3 before prostaglandin synthesis. No potential function for prostaglandins in the eye was suggested. PI was found to have higher levels of 20:5n3 than the other phosphoglycerides in both retinas and brains of the fish in this study leading to speculation that PI may serve as a reservoir for prostaglandin precursors.

5 Fatty acid synthesis and mobilization

The liver is the principal site of *de novo* fatty acid synthesis in fish. Little synthesis takes place in the adipose tissue in contrast to the situation in mammals. Fatty acid synthesis has also been reported *in vitro* in the ovary of rainbow trout (Weigand and Idler, 1982).

De novo synthesis of fatty acids from precursor acetate results in the production of saturated and monoenoic fatty acids with even numbers of carbon atoms. Fish cannot synthesize any members of the n3 or n6 fatty acid series but, when provided in the diet, salmonids can elongate and desaturate n3 and n6 fatty

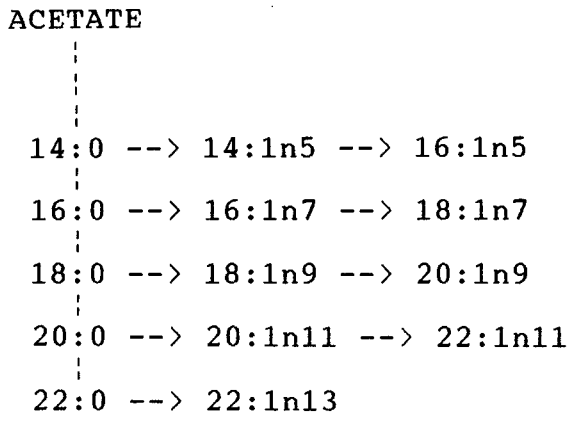
acids to PUFAs and HUFAs as shown in Figure 2 (Castell, 1979).

Rahm and Holman (1964) found that by increasing the amounts of dietary 18:2n6, the elongation and desaturation of 18:3n3 to 20:5n3, 22:5n3 and 22:6n3 was suppressed in weanling rats. This suppression could be displaced in the other direction when a high level of 18:3n3 was fed, ie the elongation and desaturation of 18:2n6 to 20:3n6, 20:4n6 and 22:5n6 was inhibited.

This inhibitory effect is apparently concentration dependent and, in salmonids, Yu and Sinnhuber (1976, 1979a) determined that n3 fatty acids are more potent inhibitors of n6 metabolism than the reverse. The mechanism of competitive inhibition is related to substrate specificity of the enzyme, δ6-desaturase, which desaturates 18:2n6 to 18:3n6 and 18:3n3 to 18:4n3 (Leger *et al.*, 1981). It is generally accepted that the preferential affinity of this enzyme is for the more unsaturated fatty acid, ie desaturation occurs in the order n3 > n6 > n9 except when there is a large preponderance of n6 or n9 fatty acids (Henderson and Tocher, 1987).

Inhibition of n3 elongation and desaturation may also occur when the level of saturates in the diet is raised. Takeuchi and Watanabe (1977b) found that an increase in dietary laurate from 4 to 14% increased the requirement for 18:3n3 from 1 to 2% in rainbow trout.

SATURATED AND MONOENOIC FATTY ACIDS



POLYUNSATURATED FATTY ACIDS

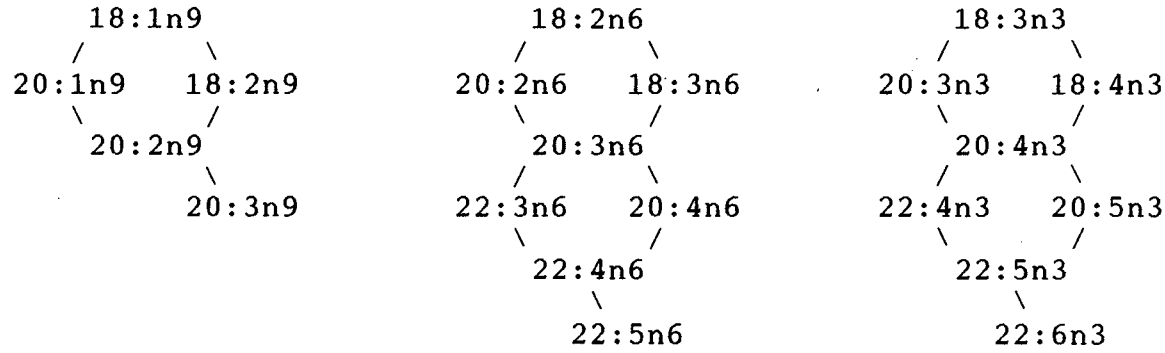


Figure 2: Fatty acid synthesis in fish.
(from Castell, 1979)

Competition between substrates for δ6-desaturase explains the results of many of the early feeding studies. Growth rates in trout fed diets deficient in n3 fatty acids were improved by the addition of 18:2n6. However, if the diet contained adequate levels of n3 fatty acids, the addition of 18:2n6 at 0.5 or 1% depressed the growth rate. Furthermore, when 18:3n3 was held at 0.5 or 1% and 18:2n6 was increased from 0 to 5%, the 22:6n3 and total n3 fatty acids in the phospholipid fraction decreased while the 18:2n6 level increased (Yu and Sinnhuber, 1981). This is an example of concentration dependent inhibition of n3 elongation and desaturation by high levels of n6 fatty acids.

Leger *et al.* (1981) showed that a high level of n3 HUFAs such as 22:6n3 could exert a negative feedback on the desaturation of both 18:3n3 and 18:2n6.

Mobilization and catabolism of lipids occur during starvation, when the food supply is scarce and during the spawning migration, when wild Pacific salmon cease feeding. When an energy deficit is pending TAGs are preferentially catabolized over phospholipids (Yu *et al.*, 1977; Castledine and Buckley, 1980, 1982). When phospholipids are catabolized, it is likely that the PUFA or HUFA in the β position are protected from oxidation and it is the monoenoic fatty acid in the α position that is broken down. Recycling of long chain unsaturates between phosphoglycerides to maintain vital physiological

function has been postulated by Castledine and Buckley (1982).

Castledine and Buckley (1980) starved juvenile rainbow trout to induce mobilization of fatty acids and to determine whether there was any crossover of PUFAs and HUFAs from the neutral lipid pool to phospholipids. Changes in fatty acid composition in the neutral lipid fraction were slight and no preferential catabolism of fatty acids was evident. However in the phospholipid component, there was a general decrease in the saturates and monoenes but no catabolism of 18:2n6 or 22:6n3 was evident. No transfer of the PUFAs and HUFAs from neutral to phospholipids occurred.

Mobilization also occurs when nutritionally unbalanced or deficient diets are fed as is the intention in some experimental designs. The fatty acid content of the neutral lipid fraction of the body largely reflects the composition of dietary lipid. Phospholipids are buffered to some extent from dietary changes and fatty acids with specific metabolic functions are conserved. Yu *et al.* (1977) found that trout retained disproportionately high levels of PUFAs and HUFAs in the phospholipid fraction in comparison with the levels employed in their experimental diets. In particular the concentration of 22:6n3 was high, indicating the importance of this fatty acid for trout. Similarly when Castledine and Buckley (1980, 1982) fed diets deficient in essential fatty acids, nearly complete retention of n3 fatty acids in the

phospholipids was found. An increase in n9 fatty acids, particularly 18:1n9 and 20:3n9, was seen as the experiment progressed.

Mechanisms may also exist to modulate other lipids. Yu *et al.* (1977) fed isocaloric diets containing various levels of saturated fatty acids to three groups of rainbow trout. Regardless of the saturate levels used in their diets, they found that the concentration in the total body lipids remained fairly constant (approximately 24%). This suggests regulation and maintainance of a specific degree of saturation. As the level of saturates increased in the diet, the concentration of monoenes, particularly 18:1n9 increased, indicating elongation and desaturation of the saturates.

Mobilization of lipids also occurs during maturation and in the process of vitellogenesis.

6 Vitellogenesis

Vitellogenesis is the synthesis of yolk materials, particularly vitellogenin, and their accumulation by the developing oocytes. Weigand and Idler (1982) demonstrated the ability of oocytes to synthesize lipids early in their development, in the period termed 'endogenous vitellogenesis'. This terminology may be considered a misnomer as it is the liver where vitellogenin is synthesized, as discussed below, not the ovary. Endogenous

synthesis of yolk lipids precedes and overlaps exogenous vitellogenesis in time but its contribution in quantitative terms is relatively minor (Wiegand and Idler, 1982; Mommsen and Walsh, 1988). The importance of endogenous synthesis seems to be related to the provision of a structural framework for the subsequent accumulation of yolk materials (Fremont *et al.*, 1984). It is during exogenous vitellogenesis that the ovary gains most of its mass.

The onset of vitellogenesis is under hormonal control. In response to the production of a gonadotropin by the pituitary, the ovarian follicles synthesize estrogen hormones which are secreted into the systemic circulation. The liver is the primary target for 17 β-estradiol, and it responds with the synthesis (exogenous vitellogenesis) and export of vitellogenin (Mommsen and Walsh, 1988).

Vitellogenin is a very high density, female specific lipophosphoprotein complex which contains ca. 80% protein and is rich in phospholipids and essential fatty acids (Leger, 1985). It also carries carbohydrates, phosphate groups and mineral salts (Mommsen and Walsh, 1988). Vitellogenin is selectively taken up from the circulation by the developing oocytes by micropinocytosis and is cleaved into its components, including phosvitin and lipovitellin. According to Wiegand (1982), vitellogenin is the major source of ovarian polar lipids. Up to 87% of the lipid in the vitellogenin of the

goldfish, *Carassius auratus*, is polar lipid and 58% of the lipovitellin lipid in coho salmon, *Oncorhynchus kisutch*, is phospholipid. Early in vitellogenesis, polar lipids predominate in the ovary. Later, during the exogenous stage, TAGs start to increase and finally predominate (Wiegand, 1982).

Circulating lipoproteins, the VLDL, LDL and HDL are macromolecular components of the blood plasma of all vertebrates. They transport lipids from the site of absorption in the intestinal mucosa and from the site of biosynthesis in the liver into the circulation and to the sites of conversion, storage or utilization. Lipoproteins consist of a hydrophobic core of TAGs and cholesterol surrounded by a hydrophilic envelope of polar constituents such as phospholipids and apoproteins (Murray *et al.*, 1988) and provide another source of materials for oogenesis. Dietary lipids are thought to be deposited directly into the eggs during this period, transported by lipoproteins and bypassing the adipose tissue (Fremont *et al.*, 1984; Luquet and Watanabe, 1986).

The exact partitioning of vitellogenin and lipoprotein, for the movement of yolk precursors into the oocyte is not well understood. It is thought that both are involved perhaps at different times (Wiegand, 1982). Leger *et al.* (1981) report that egg lipids in trout are found in two separate fractions of the egg and that they can be separated by centrifugation. The oil globule was found to contain primarily TAGs with traces of

cholesterol and cholesteryl esters; the yolk globule contained lipovitellin and phosvitin. The n3 fatty acid concentration, and particularly 22:6n3, was highest in the lipovitellin and it was suggested that the major source of structural components, both lipid and protein, for the developing embryo is the yolk globule. The oil globule is the major energy reservoir through incubation.

A variety of other compounds is also accumulated by the growing oocytes, including glycogen, carotenoids, lectins, wax and sterol esters, and sialoglycoproteins. While some of these are energy sources, the functions of others is not clear (Mommsen and Walsh, 1988).

Maximum growth and the alleviation of essential fatty acid deficiency signs are generally used as the criteria for establishing dietary requirements for lipid levels and essential fatty acids. Little attention has been paid in setting these requirements to physiological changes such as vitellogenesis and maturation. However, recent information suggests that in general nutritional requirements of broodstock may be quite different from fish in the somatic stages of their lifecycles. For example Luquet and Watanabe (1986) report work showing that maturing salmonids may be better able to utilize carbohydrate than younger fish due to the presence of an amylase in their pyloric caecae. Takeuchi *et al.* (1981) demonstrated that diets relatively low in protein (33-35%) had

no adverse effects on reproduction compared with a commercial diet high in protein (43-47%) when fed to rainbow trout for 3 years as long as the diet was also high in energy (390 kcal/100 g., including 5-7% beef tallow). A similar result was reported by Roley (1983) who found that maximum growth rate was achieved with a diet containing between 37 and 47% protein and 3.8 kcal/g metabolizable energy but that fecundity, egg size and embryo survival were unaffected by a protein level as low as 27% at the same energy level.

Fremont *et al.* (1984) demonstrated that rainbow trout were able to maintain high levels of 22:6n3 in vitellogenin and lipoprotein when fed an n3 deficient diet for 6 months prior to spawning. Levels of all other n3 fatty acids, including 20:5n3, were reduced drastically during this period. The tenacious retention of 22:6n3 over all other n3 fatty acids indicates its probable importance for reproductive success and a possible difference in requirements for growth versus reproduction in this species.

7 Embryonic development

From the foregoing, it is clear that salmon embryos require a supply of fatty acids and preformed compound lipids (triacylglycerides, phospholipids, sphingolipids, cholesterol etc.) to develop successfully through the incubation and alevin stages. These lipids will form the major energy source as well

as structural components for growth and precursors for compounds of physiological importance such as steroids and prostaglandins. Deficiency of essential fatty acids in the yolk supply has dire consequences for the health and survival of the developing fish.

When EFA deficient diets were fed to broodstock of rainbow trout, carp and red sea bream, low fecundity and fertilization rates and poor hatchability resulted (Watanabe, 1982). In the case of red sea bream, EFA deficient diets resulted in egg abnormalities and embryo deformities.

Leray *et al.* (1985) observed deformities in trout embryos when broodstock were fed n3-deficient diets with concomitant high levels of n6 fatty acids. Anomalies occurred as early as the 16- and 32-cell stage, when the arrangement of cells was disturbed. Later, the bodies of the alevins were curved or curled into helical shapes. Leray *et al.* (1985) speculated that these deformities were related to the absence of n3 HUFAs, the precursors of hydroxy fatty acids, thought to act as modulators or mediators in processes of cellular recognition occurring in embryonic development (Aveldano and Sprecher, 1983; Boukhchache and Lagarde, 1982, cited in Leray *et al.*, 1985). The role of 20:5n3 as a prostaglandin precursor was also implicated.

Leray *et al.* (1985) also reported prolonged embryo development times and more rapid (premature) yolk resorption than in control fish when n3-deficient diets were fed to broodstock.

8 Feeding regime for cultured maturing salmonids

Depletion of a major portion of the somatic reserves of wild salmonids occurs during vitellogenesis, particularly in the latter stages when feeding ceases and the fish migrate to the spawning grounds. This journey is long and arduous for stocks that spawn hundreds of miles from the ocean. In Chinook salmon, a loss of ca. 85% of the neutral lipids, 60-70% of the phospholipids and 30% of the protein from muscle, the major storage depot in this species, was documented after a 4-5 month journey of more than 800 miles from the mouth of the Columbia River to their spawning grounds (Greene, 1919).

Kato (1975) observed that cultured maturing trout naturally reduce their feed intake several months prior to spawning, but to a lesser extent than the wild fish do. Cultured Chinook salmon also reduce their feed intake over several months prior to spawning but they will continue to take some feed right up until they are moved to freshwater (personal observation).

Gutsell (1940) fed three lots of 3-year old rainbow trout either to satiation, approximately 50% of satiation, or about 25% of satiation for eight months before spawning. No

definition was given for satiation but reference was made to the fish fed to satiation as being "overfed". Group 1 (satiation) had the best fecundity on an individual basis followed by group 2 (50% satiation) and 3 (25% satiation). However 10% of group 1 females failed to spawn. Egg size decreased in the order: group 1 > group 2 > group 3. Percent survival to eyeing was greatest in group 3 (80.7%) followed by group 2 (78.7%) and then by group 1 (70.4%). It was concluded that egg quality suffered from overfeeding the broodstock.

Ridelman *et al.* (1984) starved a group of rainbow trout broodstock for ca. 40 days prior to spawning. No differences in egg quality, quantity or viability were found. Vitellogenesis was apparently complete, or nearly so, by 40 days prior to spawning and no negative effects of the treatment were evident.

Springate *et al.* (1985) fed rainbow trout either full or half rations (0.7% or 0.35% of body weight per day) for a year prior to spawning. The first fish to be stripped were those fed full ration. This occurred approximately 2-3 weeks before the half ration fish started to spawn. While all of the full-fed fish spawned, ca. 11% of the restricted fish failed to mature. Fecundity was greater (by 22%) in the former group but when fish of similar sizes from both groups were compared, fecundities were similar. The eggs of the full-fed fish were larger in both diameter and weight and produced larger fry.

Atresia (egg resorption) levels were ca. 7% in the full ration group versus ca. 22% in the half ration group. There were no differences in the total lipid or protein levels and the amino acid and mineral profiles were similar in the two groups. Fertilization success and survival to eyeing and to 6 months of age were not different. Initial differences in fry size were obscured by variances in growth in the two groups after 4 months.

Scott (1962) reported that the degree of follicular atresia increased with the level of starvation in cultured trout. Egg size was not affected by a reduced feeding regime, but the proportion of fish within a particular age class in a population that matured was reduced. Similar results were obtained by Bagenal (1969) working with brown trout.

The results of many of the preceding studies are conflicting. At present there appears to be no clearcut benefit to fasting on the reproductive processes of Pacific salmon. Differences between studies in experimental conditions, particularly in the nutritional history of the fish and in the level of food deprivation, may account for the dissimilar findings. However if broodfish can be maintained without feeding for some period prior to spawning with no detriment, a significant saving in feed and labour costs would accrue to the farmer. If fecundity or survival can be improved by optimizing feeding protocol before spawning, an additional advantage is obtained.

SECTION 1 - A comparison of the lipid composition and fatty acid profiles of the eggs of two stocks of wild and cultured Chinook salmon.

1.1 MATERIALS AND METHODS

1.1.1 Experimental design and conditions

Cultured 3-year old Chinook salmon were held in saltwater net pens at the broodstock rearing site of Sea Spring Salmon Farm Ltd. in Genoa Bay, near Duncan on Vancouver Island. Two stocks, Big Qualicum (BQ) and Robertson Creek (RC), were each fed two formulated diets (designated as COMM or WV33 and described later). The experimental design consisted of four pens of fish, each containing one stock/ diet combination.

This facility is a commercial operation and it was not possible to assign fish randomly to treatment groups because the broodstock were already in their third year at the start of this project. However, subsampled fish from each pen were sampled randomly, as described later. No replication of pens was possible.

The numbers of fish in the pens varied widely. In the two pens fed the COMM diet there were 149 (RC) and 811 (BQ) and in the pens fed the WV33 diet there were 189 (BQ) and 283 (RC). Two different sizes of pens were also used, 680 m^3 and 340 m^3, the

smaller pens holding the fish fed the WV33 diet. Pen densities were. All were below 6 kg/ m^3.

Not all of the fish in the pens matured in 1987. During September of their third summer, those fish maturing as 3-year olds (1987 broodstock) were identified and transported to the hatchery where they were held for several weeks for final maturation, prior to spawning. Fish not maturing in 1987 were held for spawning in 1988.

When the fish were brought to the hatchery they were tagged with consecutively numbered plastic spaghetti tags (Floy Tag & Manufacturing, Inc., Seattle, Wash., USA), inserted in the dorsal musculature anterior to the dorsal fin. All numbers were recorded and used to generate the random selection of fish to be sampled for eggs. Ten fish from each group were randomly sampled. Subsequently five fish were randomly selected from these ten for complete lipid analysis of their eggs. A deviation from this procedure resulted when only three of the Robertson Creek females on the COMM diet matured. In this case all three were sampled.

The fish were fed to satiation twice a day during the third year of their rearing period at Genoa Bay and received ca. 1% of their body weight per day. During their final summer, they cut back naturally to ca. 0.5% of their body weight per day. Towards the end of the summer they were taking even less feed.

Because the pens contained some fish that did not mature, it is impossible to state more precisely the level of feeding in the 1987 broodstock. They were not fed at all during the freshwater holding period (ca. 2-4 weeks) prior to spawning.

One pen of each stock was fed a commercial diet (COMM); the other pen of each stock was fed the open formulation West Vancouver 33 diet (WV33). The formulation for the commercial diet was proprietary and not available, other than the data provided on the feed label. Feed label information for the commercial diet is given in Appendix 2 Table A2.1. The formulation of the West Vancouver 33 diet is presented in Appendix 2 Table A2.2.

Males and females were held separately and checked regularly for ovulation or the start of spermiation. When ovulation was evident, a female was killed with a sharp blow to the head and the eggs were stripped into a collecting bucket. Milt was collected and divided among the egg buckets so that each bucket of eggs received the milt from two different males of the same stock. Milt was taken without killing the fish and an individual male could potentially be used to fertilize many females.

The eggs of each female were incubated in separate Heath trays (Flex-a-Lite Consolidated, Tacoma, Wash., USA). After eyeing and the first egg pick, small lots of eggs were often combined.

Consequently data on the incubation success of many individuals were not available beyond the eyed stage.

Wild fish from each stock returning to their natal streams to spawn were sampled at Big Qualicum Hatchery and Robertson Creek Hatchery, by arrangement with the Department of Fisheries and Oceans, Government of Canada. When the returning wild fish reached the hatchery, they were held in freshwater raceways and checked regularly for maturity before spawning. Ovulating females and spermiating males were both killed and stripped of their eggs or milt. Eggs from several females were combined in an egg bucket prior to fertilization by several males. After fertilization, the egg buckets were emptied into Heath trays. No attempt was made to keep eggs from different females separate at these hatcheries. It was therefore impossible to track the incubation success of individual wild fish. Data on the success of the entire run (all age groups together) were, however, available.

Female Chinook salmon returning to the hatcheries are 3, 4, and 5-years old. In order to obtain 3-year old fish for comparison with the cultured 3-year olds, sampling was carried out during that portion of the run when the peak of 3-year old fish is known to return. This was determined in consultation with the hatchery managers, Mr. Grant LaDocoeur at Big Qualicum and Mr. Don Lawseth at Robertson Creek. In addition only those fish falling within the size range of 3-year old fish were sampled.

Scales were taken coincidentally with egg samples for subsequent confirmation of age. Scales were aged by Ms Yvonne Yole of the Ageing Unit of the Department of Fisheries and Oceans.

After the fish were aged, five 3-year old fish of each stock were randomly selected from all 3-year old fish sampled for complete lipid analysis.

1.1.2 Sample collection

Approximately 50-60 grams of unfertilized eggs were taken within 10 minutes of stripping. They were drained of most of the ovarian fluid, placed into plastic bags and immediately frozen. All samples were re-packaged, without thawing, into oxygen barrier bags (W. Grace & Co. Canada Ltd., Missisauga, Ontario) within a week of collection for storage at -35°C. Samples were analysed within 10 months of collection.

Fork length and weight data were taken from all females, cultured and wild, that were spawned.

Approximately 12 hours after fertilization, ten eggs were taken from the Heath tray of each cultured female that had been sampled. These eggs were put into Stockard's clearing solution (Velsen, 1980) and later examined under a dissecting microscope to determine whether fertilization had taken place. Because

the eggs of several females at Big Qualicum and Robertson Creek hatcheries are pooled at spawning, fertilization success could not be assessed for the wild fish.

1.1.3 Egg composition

A flowchart outlining all analytical procedures is presented in Figure 3.

Dry weight determination

Samples of ca. 5-10 grams of frozen eggs were accurately weighed into each of three dried and tared aluminum pans. The pans were dried overnight to constant weight at 70° C. The dry weight of the eggs was determined by subtracting the pan weight. Dry weight was calculated as a percent of the original wet weight of the eggs. Percent moisture was calculated as the difference between 100% and the percent dry weight. The means of the wet and dry weights were used in calculating percent lipid in the samples. All formulae used for calculating composition parameters are presented in Appendix 3.

Lipid extraction

Lipid was extracted from the eggs according to the procedure of Bligh and Dyer (1959) with modifications by Christie (1982). A sample of ca. 20 grams of frozen eggs was accurately weighed

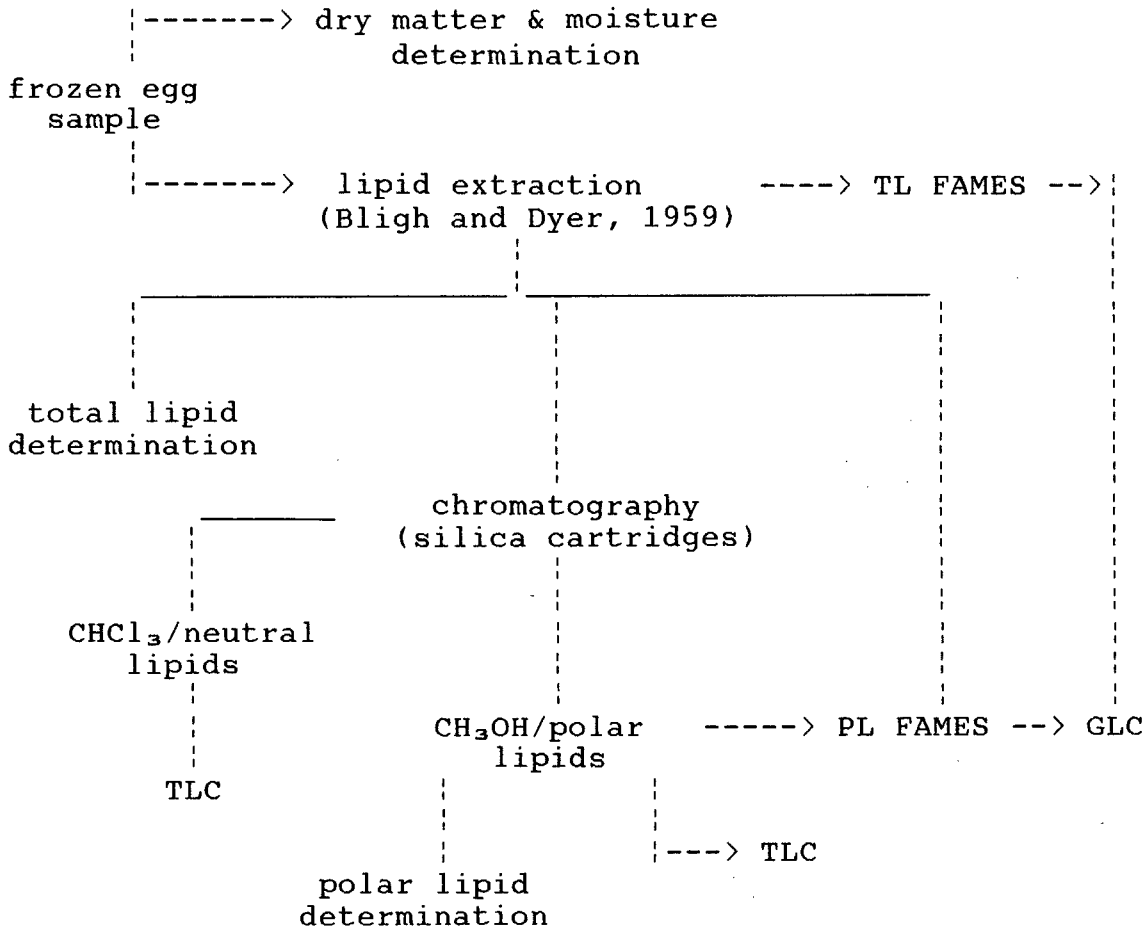


Figure 3: Flowchart of procedures for lipid analysis and fatty acid determinations (see text for full description).

TL = total lipids
PL = polar lipids
FAMES = fatty acid methyl esters
TLC = thin layer chromatography
GLC = gas liquid chromatography

and homogenized for 2 minutes with 50 ml of chloroform and 100 ml of methanol. An additional 50 ml of chloroform were added and homogenization continued for a further 30 seconds. The homogenate was filtered under pressure through a Buchner apparatus fitted with a Whatman No.1 filter paper. The contents of the funnel were returned to the homogenizer and a second extraction was carried out using the same procedure. The two filtrates were combined and 100 ml of 2.6% KCl solution was added. The solution was poured into a 500 ml graduated cylinder. The addition of the KCl caused the separation of the solution into an aqueous phase and a chloroform phase, the latter containing the extracted lipid. After separation was complete, the volume of the chloroform layer was measured and the aqueous layer removed by aspiration. This procedure was repeated on a second 20 gram sample of eggs from each fish analysed.

The amount of lipid in triplicate subsamples of the chloroform layer of each extraction was determined by pipetting 10 ml aliquots into dried and weighed aluminum pans. The chloroform was evaporated by gentle warming on a hot plate. The pans were then dried overnight at 70° C. and weighed to a constant weight. The mean amount of dried lipid in 10 ml was determined for each extraction and used to calculate the total amount of lipid in each sample, based on the volume of the chloroform layers (Appendix 3). Lipid in the original egg sample was calculated as a percent of wet and dry weight for each

extraction and the means were determined.

Separation of polar lipids

An aliquot of the chloroform phase, containing ca. 200-250 mg of lipid was evaporated under reduced pressure on a Rotovapor (Büchi Rotovapor-R, Switzerland). The remaining oil was applied to two Sep Pak silica cartridges (Waters Associates, Milford, Massachusetts) joined by plastic tubing and attached to the Luer lock of the barrel of a glass syringe which was mounted vertically on a retort stand. A small stream of nitrogen gas was applied through a plastic tubing and rubber cork assembly in the open end of the syringe barrel to force the lipid into the silica cartridges.

After the lipid was loaded onto the top of the first silica cartridge, 60 ml of a solution of 15% hexane in chloroform was poured into the top of the syringe, and the cork and tubing was attached allowing a slight flow of nitrogen to force the solvent slowly through the cartridges. The eluent contained the non-polar or neutral lipid fraction of the sample.

After the neutral lipid was eluted, 60 ml of methanol was poured into the syringe and the procedure repeated, eluting the polar lipids from the sample.

The separation of neutral and polar lipids was checked

frequently by thin layer chromatography (TLC) using Silica G Plates (A. G. Merck, Darmstad, W. Germany) and a solvent system consisting of hexane-diethyl ether-formic acid (80:20:2 by volume), according to the methods of Christie (1982). Bands were visualized with iodine vapor and separation was found to be very satisfactory.

The polar lipid eluent was made up to 80 ml with methanol and three 10 ml aliquots were pipeted into dry, pre-weighed aluminum pans; evaporated on a warm hot plate; dried overnight (70° C); and weighed to a constant weight. The weight of polar lipid in each aliquot was calculated by subtracting the weight of the dry aluminum pan. The mean was used to calculate the total amount of polar lipid in the sample (Appendix 3). The remaining polar lipid was used for the preparation of polar lipid fatty acid methyl esters.

The entire first eluent, containing the neutral lipids, was evaporated and dried overnight to a constant weight. The neutral plus polar lipid weight was used as a check on the calculated amount of lipid originally applied to the silica cartridges.

Neutral and polar lipid concentrations were calculated as percentages of the dry weight of the eggs. Polar lipid concentration was also calculated as a percentage of the total lipid and as a percentage of the fat free dry weight of the

eggs. These calculations are presented in Appendix 3.

Polar lipid determinations were done in duplicate initially but this became expensive. Since the precision of these determinations was satisfactory, it was decided that one determination per sample was sufficient.

Fatty acid profiles

Fatty acid methyl esters (FAMEs) were prepared following the base catalyzed methyl esterification procedure of Christie (1982), using sodium methoxide. An aliquot of the chloroform phase (of the original extraction), containing ca. 50 mg of lipid was used to produce total lipid FAMEs. The balance of the polar lipid eluent, after determination of percent polar lipid, was used to make polar lipid FAMEs. FAMEs were stored in hexane in 2 ml septum vials under nitrogen below 0° C. until they could be analysed by gas liquid chromatography.

FAME solutions (1 µl) were separated into their component fatty acid methyl esters on a Varian 3700 gas liquid chromatograph (GLC) equipped with a flame ionization detector. The column was a Supelcowax 10 (Supelco Canada Ltd., Oakville, Ontario) fused silica capillary column (30 m x 0.32 mm ID, 0.25 µm film). Peak areas were integrated by a CDS microprocessor (Varian Instrument Group, Palo Alto, CA, USA).

The GLC was temperature programmed at 170° C. for 10 minutes then increased 2° C. per minute to 230° C. and held at 230° C. for 20 minutes. The injection and detection temperatures were 220° C. The split ratio was 50:1. Helium carrier gas was purified through a heated furnace (Supelco Canada Ltd.) to remove all traces of oxygen and water. The linear velocity was 22 cm/sec.

Fatty acid peaks were compared with those obtained by running PUFA-1 and Rapeseed standards (Supelco Canada Ltd.) and identified according to Ackman (1982). Peaks representing less than 0.20 % of the total area of the chromatogram were not included in the profiles.

Unidentifiable peaks were assigned a number or number and letter code. These codes were used consistently for any peak occurring in the same position in the chromatograms of all samples and are included in Appendix 2, Tables A2.4 and A2.5 and Appendix 4, Tables A4.1-A4.8 in the order that they were eluted. The unidentifiable fatty acids were generally present at levels less than 0.50 %.

Fatty acid profiles in the total and polar lipids were initially carried out in duplicate. However, since little was gained by the duplication of effort, a single determination of the fatty acid profile was deemed sufficient as the analyses proceeded.

To avoid fatty acid oxidation during analysis, the following precautions were taken:

> Egg samples were kept in the freezer until immediately prior to analysis. Frozen eggs were used in the extraction procedure and the bucket of the homogenizer was placed in an ice bath during the grinding step.

> BHT, butylated hydroxy-toluene (50-100 mg/litre), was added to all solvents used in the extraction and separation procedures and in the preparation of methyl esters. Solvents were flushed with nitrogen prior to use.

> After evaporation of solvents under reduced pressure, the vacuum was broken with nitrogen ensuring that exposure of lipid to air was minimal.

> Nitrogen was used to force lipid and solvents through the silica cartridges. As the eluent left the cartridges, the solvent carrying the lipid was run down the side of the collection flask to avoid splashing with the possible incorporation of air into the sample.

1.1.4 Diet composition

A sample of each of the two formulated diets was taken and stored in oxygen barrier bags, as for the egg samples.

To determine dry weight ca. 5-10 grams of frozen pellets were accurately weighed into each of three dried and tared aluminum pans. Because the pellets were relatively hard, 5-10 ml of distilled water were added to the pans to break them down. The slurry in the pans was then dried to a constant weight at 70° C and mean dry weight and moisture in the pellets was calculated.

Lipid was extracted from the diets following the same procedure applied to the eggs, with the exception that it was necessary to soak the still frozen pellets in the first solvent system for several minutes to break them down prior to grinding. Total lipid and fatty acid profiles in the total lipid were determined following the procedures for the eggs. All analyses on the diets were carried out in duplicate and the means were calculated.

1.1.5 Egg size

Egg size was measured in two ways. A minimum of ten eggs from the samples preserved in Stockard's solution (Velsen, 1980), taken to monitor fertilization success, were placed in a trough calibrated in millimeters. Mean egg diameter was determined from the total length divided by the number of eggs measured. Egg volume was measured by displacement of water in a buret. Again a minimum of ten eggs was measured and the mean volume determined by dividing total volume displaced by the number of eggs. Egg diameter was then calculated from this mean. Egg

diameters determined by each method were compared for consistency but only diameters determined by direct measurement (first method) were reported.

1.1.6 Fertilization and eyeing success

Fertilization success was monitored by taking a minimum of ten eggs from all fish sampled 12-24 hours after fertilization. These eggs were placed in Stockard's solution (Velsen, 1980) for clearing. Egg cleavage, indicating that fertilization had occurred, was easily observed by eye or under the low power objective of a dissecting microscope.

Fecundity was approximated at spawning by estimating the volume of eggs spawned by each female. Eggs are too fragile at this time to count and it is not advisable to drain the ovarian fluid so that an accurate weight can be obtained. At eyeing the eggs are much more robust and a count of the live and the dead eggs was made for each fish. The total of live plus dead eggs equals the number of eggs spawned or the fecundity. Eyeing success was calculated as the percentage of viable eggs at the eyed stage divided by the fecundity.

1.1.7 Statistical procedures

All data reported as percentages were transformed by the arcsine transformation before analyses. Morphometric data

(condition factors, egg diameters, etc.); incubation results (fertilization rates, eyeing success, etc.); composition parameters (dry matter; moisture; total lipid; polar lipid; polar lipid, fat free dry weight basis, etc.), individual fatty acids, and fatty acid series (saturates, n3, n6, n9, etc.) were all analysed for differences among the three diets for each stock and for differences between stocks for each diet using the general linear model procedure (PROC GLM) for the analysis of variance (1x6 ANOVA), recommended for unbalanced data sets (SAS, 1985). Differences between means were determined by least square means (LSMEANS) with the PDIFF option (SAS, 1985) to identify which means were different. The significance level was set at 95% for all tests.

1.2 RESULTS AND DISCUSSION

1.2.1 Morphometric measurements

Fork length and weight data were recorded by the hatchery staff from all cultured females spawned at Sea Spring Salmon Farm. This information is not routinely taken at Big Qualicum or Robertson Creek Hatcheries but all fish sampled for eggs at these hatcheries during this study were weighed and measured. Condition factor (CF) was calculated for each fish according to the formula of Vanstone and Markert (1968):

$$CF = (W \times 1000) / FL^{3.25}$$

where W = weight (g) and FL = fork length (cm)

All morphometric data are presented in Table 1. Unfortunately only 5 of the 10 fish sampled at Big Qualicum were 3 years old as indicated by scale analysis and only three females in the RC-COMM group matured. An ANOVA was carried out on all three diet groups of the BQ stock, including BQ-WILD. Because the sample size was so low, the results of this test risked a Type 1 error in the case of the BQ-WILD fish. For the RC groups an ANOVA was performed excluding the RC-COMM fish as the power of this test would have been very low (ie the possibility of a Type 2 error would have been high).

The wild fish of both stocks were significantly longer (and

Table 1. Morphometric data (mean and standard error of the means) for wild and cultured Big Qualicum (BQ) and Robertson Creek (RC) female Chinook broodstock. Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet

	BQ WILD		BQ WV33		BQ COMM	
	mean	sem (n)	mean	sem (n)	mean	sem (n)
Fork length, cm	77.8[a]	0.903 (5)	70.1[b]■	0.678 (22)	69.8[b]	0.447 (46)
Weight, kg	5.1■	0.258 (5)	5.1■	0.179 (22)	5.0	0.124 (46)
Condition factor	3.7[a]	0.126 (5)	5.1[b]	0.097 (22)	5.1[b]	0.134 (46)

	RC WILD		RC WV33		RC* COMM	
	mean	sem (n)	mean	sem (n)	mean	sem (n)
Fork length, cm	80.3[a]	0.547 (12)	67.2[b]■	0.838 (17)	70.7*	1.764 (3)
Weight, kg	6.3[a]■	0.124 (12)	4.1[b]■	0.166 (17)	4.8*	0.252 (3)
Condition factor	4.1[a]	0.067 (12)	4.8[b]	0.167 (17)	4.7*	0.152 (3)

* = The RC-COMM fish were excluded from the ANOVA as the sample size was inadequate. See text for discussion.

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated.

heavier in the case of the RC fish) than the BQ cultured groups or the RC-WV33 group. However the mean condition factor was significantly lower for the BQ-WILD fish than for the BQ cultured fish. Condition factor was also significantly lower in the RC-WILD fish than in the RC-WV33 fish.

For simplicity, significant differences in a parameter between the stocks for each diet group have been indicated by '■' in Table 1 and in any of the tables that follow.

The RC-WILD fish were significantly heavier than the BQ-WILD fish but there was no difference in length or condition factor. By contrast, the RC-WV33 fish were smaller in both length and weight than the BQ-WV33 fish. There were no significant differences in length or weight between stocks on the COMM diet. There were no significant differences in condition factors between the stocks for each diet group.

1.2.2 Spawning and incubation success

The volume of eggs stripped from each female was measured at spawning but fecundity could not be determined until the eyed stage as eggs are too fragile to count until then (Table 2). Eggs from one or more females were combined at spawning if there were too few eggs to fill an incubation tray or at eyeing if the number of surviving eggs was low. As a result, individual fecundity data were not available for all the fish

Table 2. Spawning data (mean and standard error of the means) for wild and cultured Big Qualicum (BQ) and Robertson Creek (RC) Chinook broodstock. Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet

	BQ WILD		BQ WV33		BQ COMM	
	mean	sem (n)	mean	sem (n)	mean	sem (n)
Volume of eggs spawned, ml	no data*		597.6■	44.8 (21)	605.4	23.9 (46)
Fecundity (# eggs spawned)	no data*		3019.9■	249.2 (12)	3204.0	92.9 (40)
Egg diameter, mm	7.1[a]■	0.271 (5)	7.7[b]	0.044 (9)	7.9[b]	0.038 (17)

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

	RC WILD		RC WV33		RC COMM	
	mean	sem (n)	mean	sem (n)	mean	sem (n)
Volume of eggs spawned, ml**	no data*		311.5■	39.7 (13)	666.7	44.1 (3)
Fecundity** (# eggs spawned)	no data*		1595.6■	413.6 (5)	2527.5	294.5 (2)
Egg diameter, mm**	7.7■	0.061 (12)	7.7	0.125 (15)	8.3	0.133 (3)

* = These data are not recorded at the government hatcheries

** = ANOVA was not conducted for the RC fish as there was no data for the WILD fish and sample size was inadequate for the COMM fish.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated.

that were spawned. This explains why the number of observations (n) was lower for fecundity compared with the n values reported for the volume of eggs spawned (Table 2).

Combined egg lots, representing the fish with the lowest fecundities, were excluded from the calculation of the means. Consequently the figures given for mean fecundity in Table 2 are over-estimates, particularly in the case of the BQ and RC fish fed the WV33 diet, where combining eggs from more than one female was most prevalent (see also Table 3).

No fecundity or incubation data for individual fish are routinely collected at Big Qualicum or Robertson Creek Hatcheries. And because there were insufficient data, ANOVA could not be conducted on the RC fish.

No significant difference was found in fecundity, whether determined by volume or by count, between the BQ-COMM and the BQ-WV33 fish (Table 2). An ANOVA could not be performed on the RC fish because the sample size for the RC-COMM group was too small and there were no fecundity data for the wild fish. However an ANOVA between stocks for the WV33 diet revealed that the BQ-WV33 fish were significantly more fecund than the RC-WV33 group.

These figures in Table 2 do not include the eggs from the combined egg lots. As shown in Table 3, the incidence of

Table 3. Egg retention and other reproductive abnormalities observed in Big Qualicum (BQ) and Robertson Creek (RC) cultured females on two diets, expressed as a percentage of all spawners in each stock and diet group. Actual numbers are reported in parentheses. Diet codes: COMM = commercial; WV33 = West Vancouver 33

Stock/Diet	BQ-WV33		RC-WV33		BQ-COMM		RC-COMM	
Number of spawners	22		17		46		3	
	%	(#)	%	(#)	%	(#)	%	(#)
Level of retention[1]								
low	31.8	(7)	17.6	(3)	6.5	(3)	0.0	(0)
moderate	4.5	(1)	29.4	(5)	0.0	(0)	0.0	(0)
high	4.5	(1)	29.4	(5)	0.0	(0)	0.0	(0)
Spawners not ripe	9.1	(2)	23.5	(4)	0.0	(0)	0.0	(0)
Spawners with only one ripe ovary	4.5	(1)	11.8	(2)	0.0	(0)	0.0	(0)
Spawners with glassy eggs	0.0	(0)	47.1	(8)	0.0	(0)	0.0	(0)
Spawners with no survivors at eyeing	18.2	(4)	41.2	(7)	2.2	(1)	33.3	(1)
Spawners with eyed survivors	68.2	(15)	35.3	(6)	97.3	(45)	66.7	(2)

1 Level of egg retention (unripe eggs; not ovulated):
low = < a count of 400 eggs;
moderate = > a count of 400 eggs or < a weight of 400 grams;
high = > a weight of 400 grams.

reproductive abnormalities was high, particularly among the WV33 groups of both stocks. Many females fed this diet had unripe eggs retained on the skeins at spawning. Unfortunately only rough estimates were made of this egg retention but it was clear that fecundities were reduced and that the RC-WV33 fish were the most severely affected. In several fish only one ovary (the left) ovulated, the second remained intact or nearly so and not ripe. Some fish did not mature at all. Glassy, apparently water-hardened, eggs were frequent in the RC-WV33 group. Some retained eggs were enlarged to double or triple normal size and were soft and flaccid, often pale in colour (Figure 4, photographs).

Egg diameter data are also given in Table 2. The eggs of the BQ cultured fish were significantly larger than those of the BQ-WILD fish. Interestingly the RC-WILD fish had significantly larger eggs than the BQ-WILD fish. This may reflect the lower fecundity found in the RC fish as an inverse relationship between egg size and fecundity has been reported (Blaxter, 1969; Roy and Higgs, 1987). This could not be tested for the wild fish as no fecundity data were available but correlation coefficients between egg diameter and fecundity were calculated for BQ-COMM and BQ-WV33. They were -0.1335 (n=15) and -0.2550 (n=6) respectively but were not significant at $\alpha=0.05$ (Zar, 1984). Correlation coefficients were not determined for the RC-COMM and RC-WV33 groups because fecundity data were available for only 2 and 4 fish, respectively.

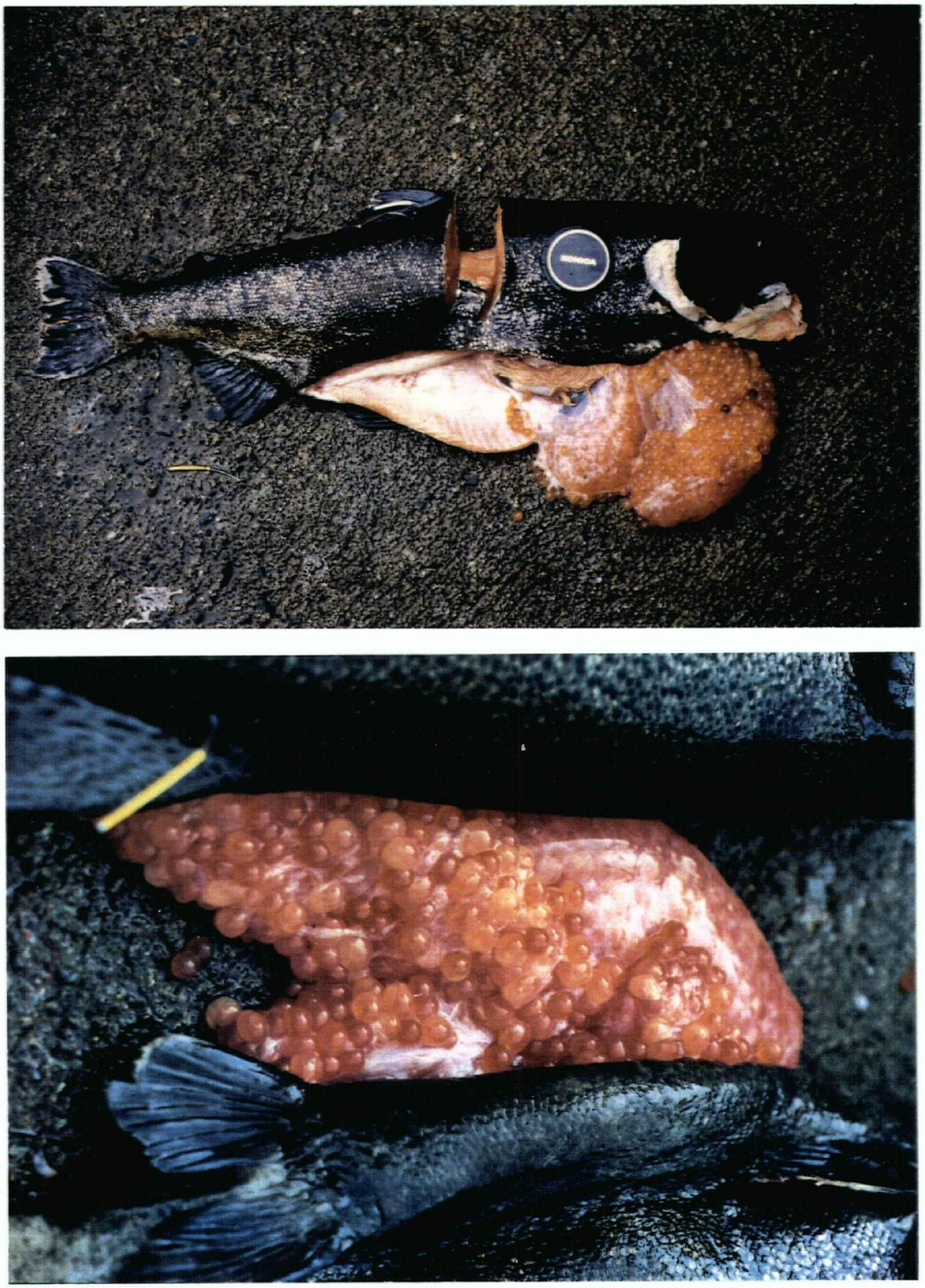


Figure 4: Egg abnormalities in Robertson Creek females on the WV33 diet. Top: Female with 660 grams of retained eggs. Bottom: Abnormal eggs are pale in colour, flaccid and enlarged 2-3 times normal.

No significant correlations (α=0.05) between fecundity and fork length, weight or condition factor were found in the BQ-COMM and BQ-WV33 groups as has been reported by Blaxter (1969). This may have been because these fish exhibited varying degrees of egg retention as discussed above.

No significant difference was found in fertility between the BQ-COMM and BQ-WV33 groups (Table 4). In the RC-WV33 fish, only 40% of the eggs from ten fish were fertile. This together with low fecundity (Table 2) and observations of spawning abnormalities (Table 3) suggest that poor performance was due to poor egg quality in the WV33 diet groups and particularly in the RC-WV33 fish.

Fertility was also low in the RC-COMM fish (69% of the eggs of 3 fish were fertile) but the sample size was too low to assess any difference between the RC-COMM and the RC-WV33 fish. Fertility was significantly greater in the BQ-WV33 than the RC-WV33 eggs and appeared (not tested) to be greater in the BQ-COMM than in the RC-COMM eggs.

There were no significant differences in the number of eyed eggs or in eyeing success (Table 4) between the BQ-COMM and BQ-WV33 eggs. Poor survival rates to the eyed stage occurred in both of the WV33 groups (Table 3), eg. 7 out of 17 RC-WV33 spawners and 4 out of 18 BQ-WV33 spawners had no surviving eggs at eyeing. As expected, the RC-WV33 group produced

Table 4. Incubation data (mean and standard error of the means) for wild and cultured Big Qualicum (BQ) and Robertson Creek (RC) Chinook broodstock. Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet

	BQ WILD		BQ WV33		BQ COMM	
	mean	sem (n)	mean	sem (n)	mean	sem (n)
Fertility, %	no data*		83.6[a]■	7.20 (8)	97.8[a]	2.20 (7)
Eyed eggs, #	no data*		1940.2[a]■	353.4 (16)	2406.3[a]	168.5 (44)
Eyed, %	90.87*		62.4[a]**	9.88 (16)	73.9[a]**	4.46 (44)

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

	RC WILD		RC WV33		RC COMM	
	mean	sem (n)	mean	sem (n)	mean	sem (n)
Fertility, %***	no data*		40.1■	10.7 (10)	68.9	26.3 (3)
Eyed eggs, #***	no data*		706.9■	272.1 (8)	2392.5	280.5 (2)
Eyed, %***	90.26*		36.3**	14.39 (8)	94.7**	0.070 (2)

* = Fertility data not available as the eggs of several fish are combined at spawning. Eyed figures for these fish are from the respective hatchery records and were not included in the ANOVA.

** = Data for individual fish were not available after eyeing as small egg lots were combined.

*** = ANOVA was not conducted for the RC fish as there was no data for the WILD fish and sample size was inadequate for the COMM fish.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated.

significantly fewer eyed eggs than the BQ-WV33 fish although there was no significant difference in the eyed percentage between these two groups. The figures for the RC-COMM fish in Table 4 are for 3 fish only (only 3 females matured in this group) and should not be regarded as an accurate assessment of the success of these fish.

At the time of eyeing, dead eggs were removed from the trays and more groups of surviving eggs were combined to keep the egg trays full. Data for individual fish were therefore lost beyond the eyed stage. This meant that survival to ponding could not be estimated accurately, even on a group basis. Beyond eyeing, however, mortality among all groups was low (D. Groves, pers. comm.).

The values for survival to eyeing for the BQ-WILD and RC-WILD fish in Table 4 are for all age groups spawned throughout the entire run. Three year old fish are generally somewhat less successful than four year olds and the majority of spawners at Big Qualicum and Robertson Creek Hatcheries are four year olds (G. Ladocoeur and D. Lawseth, pers. comm. and Blaxter, 1969).

1.2.3 Composition of the eggs

Composition data for the eggs of BQ and RC cultured and wild broodstock are presented in Tables 5a & 5b. The eggs of all groups of both stocks, except RC-WV33, contained ca. 40% dry

Table 5a. Composition of the eggs (mean and standard error of the means) of BIG QUALICUM (BQ) broodstock on three diets (n=5 for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet

	BQ WILD		BQ WV33		BQ COMM	
	mean	sem	mean	sem	mean	sem
Dry matter %	39.47	0.75	39.64■	0.24	39.32	0.49
Moisture %	60.54	0.75	60.38■	0.25	60.68	0.49
Lipid, % ww*	12.19	0.33	11.82	0.17	12.33	0.20
Lipid, % dw*	30.89	0.62	29.81	0.44	31.37	0.35
Polar lipid, % dw	12.78	0.17	12.84	0.27	13.24	0.30
Neutral lipid, % dw	18.83	0.56	17.51	0.56	18.95	0.43
Polar lipid, % of total lipid	41.33	0.40	43.14	0.41	42.18	0.59
Neutral lipid, % of total lipid	61.41■	0.66	58.79	0.58	60.38■	0.85
Polar lipid, % of fat free dw	19.35	0.55	18.58	0.45	19.75	0.51
Protein + ash, % ww	27.28	0.59	27.80■	0.27	26.99	0.37

*ww = wet weight; dw = dry weight

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 5b.

Table 5b: Composition of the eggs (mean and standard error of the means) of ROBERTSON CREEK (RC) broodstock on three diets (n=5 for each group except RC-COMM where n=3).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet

	RC WILD		RC WV33		RC COMM	
	mean	sem	mean	sem	mean	sem
Dry matter %	40.27[a]	0.26	35.89[b]■	1.70	39.59[a]	0.97
Moisture %	59.74[a]	0.26	64.11[b]■	1.70	60.41[a]	0.97
Lipid, % ww*	11.98	0.13	11.54	0.32	12.47	0.11
Lipid, % dw*	29.75[a]	0.30	32.32[b]	0.83	31.53[a]	0.69
Polar lipid, % dw	12.75	0.22	14.16	0.55	13.91	0.34
Neutral lipid, % dw	17.11	0.35	19.05	0.57	18.00	0.78
Polar lipid, % of total lipid	42.88	0.86	43.76	0.77	44.11	0.70
Neutral lipid, % of total lipid	57.50■	0.93	58.19	0.63	57.06■	1.73
Polar lipid, % of fat free dw	18.37	0.42	20.86	1.08	20.06	0.81
Protein + ash, % ww	28.01	0.33	24.36■	1.42	27.12	0.92

*ww = wet weight; dw = dry weight

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 5a.

matter and (by subtraction) 60% moisture. The concentration of dry matter was significantly lower in the eggs of the RC-WV33 broodstock than in either of the other RC groups or in the eggs of the BQ-WV33 broodstock.

Sinnhuber (1969) has reported an increase in water content in muscle of salmonids fed essential fatty acid deficient diets. However EFA deficiency seems not to have been a factor in the higher water levels in the RC-WV33 eggs. There was no significant difference in n3 fatty acids of the total lipids between the RC-WV33 and RC-COMM eggs (Table 6b) and the n3 fatty acid concentration of the polar lipids was significantly greater in the RC-WV33 eggs than in the RC-COMM eggs.

As shown in Table 3, the RC-WV33 group had a high level of glassy, apparently water hard eggs. Fecundity (Table 2), fertility and survival rate to eyeing (Table 4) were all lower in this group than in RC-COMM, although the sample sizes involved in these groups were too low to assess statistical significance.

The moisture values for the groups (other than RC-WV33) are intermediate between those given for cultured eggs (62.0 - 66.1%) and wild eggs (57.7%) by Groves (1987, unpub. report) and presented in Appendix 1. The difference in moisture content in the eggs of the wild fish between the previous study and this study may have been due to stock effects. The

previous study involved wild fish from the Chemainus River.

The dry matter in all groups was ca. 30% lipid of which ca. 41-44% was polar lipid and ca. 57-61% was neutral lipid. On a fat free dry weight basis, ca. 18-21% of the dry matter was polar lipid. There were no significant differences between diet groups of either stock in these parameters. However, there were significant differences in neutral lipid concentration between stocks on the same diet, ie. the BQ-WILD and BQ-COMM eggs had significantly greater neutral lipid levels and, although not statistically greater, BQ-WV33 eggs also contained more neutral lipid than the RC-WV33 eggs. Groves (1987, unpub. report) found that lipid values (wet weight basis) ranged from 13.5-19.0% in cultured eggs and 6.0-13.2% in wild eggs (Appendix 1) but in this study they ranged from 11.5-12.5% across all groups, cultured and wild (Tables 5a & 5b).

The higher lipid levels found in both the muscle and the eggs of cultured fish compared to the wild fish as reported by Groves (1987, unpub. report) coupled with the better reproductive performance of the wild fish lead to speculation that 'fat fish do not tend to be productive broodstock'. The lower condition factors in the wild fish and greater egg diameters in the cultured fish, reported here, lend some support to this suggestion. However in this study, there were no significant differences among any of the BQ groups, cultured or wild, in total egg lipid levels (Table 5a). The

significantly higher lipid level (dw) determined for the RC-WV33 eggs (Table 5b) appeared to be a reflection of the high moisture levels found in these eggs. The RC-WILD and RC-COMM lipid levels were statistically no different than those of the BQ eggs.

The data of Tables 5a and 5b are presented as percentages. Since the level of carbohydrate is likely to be very low in these eggs, % protein + % ash (composite value) can be calculated as:

$$100\ \% - [\%\ \text{moisture} + \%\ \text{lipid, wet wt}]$$

Coincident with the significantly higher moisture level in the RC-WV33 eggs was a significantly lower % protein + % ash value. There were no other differences in the protein + ash concentration between diet groups of either stock nor between the two stocks for each diet with the exception that RC-WV33 was significantly lower than BQ-WV33.

1.2.4 Fatty acid composition of the total lipids

The complete fatty acid profiles of the total lipids in the eggs for each stock by diet are presented in Appendix 4, Tables A4.1 and A4.2. Data for selected fatty acids and assemblages of fatty acids are included here.

Because the biosynthesis of polyunsaturated fatty acids in fish proceeds within the n3, n6 and n9 omega series (Figure 2), the fatty acids were grouped accordingly and statistically analysed (Tables 6-8).

As shown in Tables 6a & 6b, the wild fish of both stocks had significantly higher levels of n3 fatty acids in the total lipids of their eggs than the cultured fish, the difference between the wild and cultured groups being ca. 7-13% in the BQ eggs and 11-13% in the RC eggs. The wild eggs also had significantly higher levels of saturates. The n6 fatty acids were significantly lower (by 4-6%) in the wild eggs than in the cultured eggs. The n9 fatty acids were also significantly lower (by 6-9%) in the wild than in the cultured eggs.

The BQ eggs from the cultured fish on two diets were significantly different in n3, n6 and n9 series of fatty acids, with more n6 and n9 fatty acids in the BQ-COMM group and more n3 in the BQ-WV33 group. There were no significant differences in saturates, n3, n6, or n9 fatty acid series between the two diets in the cultured RC eggs. The BQ-COMM eggs had a statistically higher concentration of n6 fatty acids than the RC-COMM eggs. There were no other significant differences in these fatty acid series between stocks.

The ratio of n3 to n6 fatty acids in muscle lipids has frequently been used as an indication of the essential fatty

Table 6a. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in TOTAL LIPIDS of eggs from BIG QUALICUM (BQ) broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
Saturates	19.40[a]	0.38	18.00[b]	0.23	18.66[c]	0.21
n3	39.95[a]	0.87	32.30[b]	0.59	27.22[c]	0.35
n6	2.34[a]	0.07	6.61[b]	0.06	8.47[c]■	0.22
n9	21.71[a]	0.84	28.01[b]	0.75	30.33[c]	0.36

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 6b.

	BQ WILD		BQ WV33		BQ COMM	
	mean	sem	mean	sem	mean	sem
n3:n6 ratio*	17.07	0.58	4.89	0.07	3.21	0.12

* ANOVA was not conducted on the n3:n6 ratios.

Table 6b. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in TOTAL LIPIDS of eggs from ROBERTSON CREEK (RC) broodstock on three diets (n = 5 fish for each group except COMM where n=3). Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
Saturates	19.95[a]	0.32	18.69[b]	0.24	18.21[b]	0.01
n3	41.56[a]	0.59	30.38[b]	0.47	28.82[b]	0.18
n6	2.19[a]	0.13	6.97[b]	0.07	8.12[b]■	0.14
n9	21.88[a]	0.77	29.04[b]	0.43	29.87[b]	0.28

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 6a.

	RC WILD		RC WV33		RC COMM	
	mean	sem	mean	sem	mean	sem
n3:n6 ratio*	18.98	0.95	4.36	0.07	3.55	0.07

* ANOVA was not conducted on the n3:n6 ratios.

acid status of salmonids (eg. Castell, 1979). Gruger *et al.* (1964, cited in Castell, 1979) reported an n3:n6 ratio of 6.69 in the muscle of wild Chinook salmon in seawater. The difference in the n3:n6 ratios between Chinook muscle and Chinook eggs is striking. The n3:n6 ratio in the total lipids of the eggs of the wild fish was found to be ca. 2.6 to 2.8 times greater than Gruger's muscle ratio, ie. 17.07 in BQ eggs and 18.98 in RC eggs (Table 6a & 6b). In contrast, the n3:n6 ratio in the total lipids of the eggs of all cultured fish (both stocks) was ca. 27 - 52% lower than the level in the muscle of wild fish.

The n3:n6 ratio in the BQ eggs was ca. 3 to 5 times greater in the wild fish than in the cultured fish (Table 6a), eg. 17.07 for BQ-WILD eggs but only 3.21 for BQ-COMM and 4.89 for BQ-WV33 eggs. Similarly, it was 18.98 for the RC-WILD eggs but only 3.55 and 4.36 for RC-COMM and RC-WV33 eggs respectively (Table 6b).

The n3 series of fatty acids in the total lipids has been further broken down into n3 PUFAs (n3 fatty acids with 2 to 4 double bonds), HUFAs (n3 fatty acids with 5 or more double bonds) and the three fatty acids, 20:5n3, 22:5n3 and 22:6n3 in Tables 7a & 7b.

Over all groups in both stocks, the n3 PUFA present in the eggs was relatively low at 2-3.5% of all fatty acids in the total

Table 7a. Selected n3 fatty acids, total n3 PUFAs (polyunsaturated fatty acids) and total n3 HUFAs (highly unsaturated fatty acids) (mean and standard error of the means) in TOTAL LIPIDS of eggs from BIG QUALICUM (BQ) broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
20:5n3	13.70[a]■	0.36	8.88[b]	0.16	7.45[c]	0.14
22:5n3	5.49[a]■	0.23	3.47[b]	0.08	2.74[c]■	0.15
22:6n3	17.09[a]	0.74	17.73[a]	0.39	14.87[b]	0.45
Total n3 PUFAs[1]	2.92[a]	0.11	1.91[b]	0.05	1.91[b]	0.07
Total n3 HUFAs[2]	36.28[a]	0.73	30.08[b]■	0.56	25.06[c]■	0.38

1 n3 PUFAs = unsaturated n3 fatty acids with 2 to 4 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 5 or more double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 7b.

Table 7b. Selected n3 fatty acids, total n3 PUFAs (polyunsaturated fatty acids) and total n3 HUFAs (highly unsaturated fatty acids) (mean and standard error of the means) in TOTAL LIPIDS of eggs from ROBERTSON CREEK (RC) broodstock on three diets (n = 5 fish for each group except COMM where n = 3).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
20:5n3	15.06[a]■	0.46	8.36[b]	0.24	7.88[b]	0.09
22:5n3	4.81[a]■	0.20	3.10[b]	0.07	3.28[b]■	0.25
22:6n3	17.81[a]	0.51	16.70[ab]	0.25	15.63[b]	0.12
Total n3 PUFAs[1]	3.50[a]	0.42	1.89[b]	0.09	1.81[b]	0.07
Total n3 HUFAs[2]	37.68[a]	0.30	28.17[b]■	0.38	26.79[b]■	0.26

1 n3 PUFAs = unsaturated n3 fatty acids with 2 to 4 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 5 or more double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 7a.

lipid, compared with the total HUFA which represented 25-38% of the fatty acids in the total lipid. The levels of n3 PUFAs and HUFAs were significantly higher in the wild eggs than in the cultured eggs, regardless of stock. The BQ-WV33 eggs had a significantly higher concentration of HUFA than the BQ-COMM. There were no other significant differences in n3 PUFAs or HUFAs between the formulated diet groups of each stock. Testing between the stocks revealed significantly more HUFAs in the BQ-WV33 eggs than in the RC-WV33 eggs and significantly more HUFAs in the RC-COMM eggs than in the BQ-COMM eggs.

The 20:5n3 content of the total lipids was significantly higher (almost double) in the wild groups than in the cultured groups of either stock and the RC-WILD eggs contained significantly more 20:5n3 than the BQ-WILD eggs. The wild eggs of both stocks also contained significantly more 22:5n3 than the cultured eggs. The 22:6n3 concentration in the WILD and WV33 eggs was not significantly different regardless of stock but the COMM eggs contained significantly less 22:6n3 than the WILD eggs, again regardless of stock. Significant differences between stocks for the diets were found for 20:5n3 in the WILD groups and for 22:5n3 in the COMM and WILD groups.

Levels of the n6 fatty acids and the n6 PUFAs as shown in Tables 8a & 8b, were all significantly lower in the eggs of wild fish than in either group of cultured fish, regardless of stock. In both stocks, the eggs from the COMM groups had

Table 8a. Selected n6 fatty acids and total n6 PUFAs (polyunsaturated fatty acids) (mean and standard error of the means) in TOTAL LIPIDS of eggs from BIG QUALICUM (BQ) broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
18:2n6	0.79[a]	0.05	3.43[b]■	0.07	4.80[c]	0.14
20:2n6	0.00[a]	0.00	0.37[b]	0.02	0.39[b]	0.04
20:4n6	1.15[a]■	0.03	1.60[b]	0.04	1.69[b]	0.04
Total n6 PUFAs[1]	2.34[a]	0.07	6.61[b]	0.06	8.47[c]■	0.22

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 8b.

Table 8b. Selected n6 fatty acids and total n6 PUFAs (polyunsaturated fatty acids) (mean and standard error of the means) in TOTAL LIPIDS of eggs from ROBERTSON CREEK (RC) broodstock on three diets (n = 5 fish for each group except COMM where n = 3).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
18:2n6	0.85[a]	0.08	3.85[b]■	0.06	4.59[c]	0.18
20:2n6	0.00[a]	0.00	0.32[b]	0.02	0.46[c]	0.04
20:4n6	1.02[a]■	0.03	1.63[b]	0.03	1.73[b]	0.04
Total n6 PUFAs[1]	2.19[a]	0.13	6.97[b]	0.07	8.12[c]■	0.14

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 8a.

significantly higher levels of 18:2n6, the predominant n6 fatty acid, and higher total n6 PUFAs than the eggs from the WV33 groups. There were no differences in 20:4n6 in the eggs of either stock of cultured fish. Small but significant differences were found between the two stocks for 18:2n6 (RC-WV33 > BQ-WV33), 20:4n6 (BQ-WILD > RC-WILD) and total n6 PUFAs (BQ-COMM > RC-COMM).

Leray *et al.* (1985) found deformities in early rainbow trout embryos when the broodstock was fed an n3 deficient diet with a high n6 content. In this study most of the mortality in the cultured fish occurred before eyeing, during the period of highest mortality reported by Leray. However n3:n6 ratio was not directly related to the observed mortality. Fecundity (Table 2), fertility and survival to the eyed stage (Table 4) were better in the COMM than the WV33 groups in both stocks although the n3:n6 was lower in the former (Tables 6a & 6b).

The n9, n11 and total monounsaturated fatty acids (Tables 9a & 9b) were all significantly lower in the eggs of the wild fish than in the cultured fish, regardless of stock. The level of n7 monounsaturates in the eggs of the BQ-WILD fish was significantly higher than in the BQ cultured fish. The n7 monounsaturates were lower in the RC-WILD fish than in the RC cultured fish, though not significantly so in the case of the RC-WV33 eggs. There were no differences in n5 levels among the diet groups of either stock. Significant stock differences

Table 9a. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in TOTAL LIPIDS of eggs from BIG QUALICUM (BQ) broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
n5	0.42	0.03	0.45	0.00	0.43	0.00
n7	11.65[a]■	0.31	10.02[b]	0.11	10.30[b]	0.10
n9	21.71[a]	0.84	28.01[b]	0.75	30.33[c]	0.36
n11	0.53[a]	0.27	1.12[b]	0.05	1.19[b]	0.08
Total monounsaturates	34.31[a]■	0.98	39.60[b]	0.77	42.25[b]	0.33

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 9b.

Table 9b. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in TOTAL LIPIDS of eggs from ROBERTSON CREEK (RC) broodstock on three diets (n = 5 fish for each group except COMM where n = 3).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
n5	0.47	0.05	0.44	0.01	0.43	0.02
n7	9.97^{a}■	0.22	10.05^{ab}	0.16	10.62^{b}	0.11
n9	21.88^{a}	0.77	29.04^{b}	0.43	29.87^{b}	0.28
n11	0.52^{a}	0.26	1.03^{b}	0.41	0.81^{ab}	0.19
Total monounsaturates	32.83^{a}■	0.56	40.56^{b}	0.49	41.73^{b}	0.19

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 9a.

were found only in the wild eggs for n7 fatty acids and total monounsaturates (BQ-WILD > RC-WILD in both cases).

Differences in fatty acids between the eggs of the wild BQ and RC fish are probably a reflection of the differences in the diets that these fish consumed throughout their lives. An example of this is the n7 concentration in the total lipids which was 11.65 in the BQ-WILD eggs and 9.97 in the RC-WILD eggs. Differences in temperature (Hazel, 1979) and salinity (Leray *et al.*, 1984) in the marine environments of the two stocks may also contribute to differences between the stocks by affecting the level of unsaturation ultimately attained by the eggs.

1.2.5 Fatty acid composition of the polar lipids

The complete fatty acid profiles of the polar lipid fraction of total lipid are listed in Appendix 4, Tables A4.3 and A4.4, for each stock by diet. Data for selected fatty acids and for assemblages of fatty acids are presented here.

As for the total lipid, the saturated and n3 fatty acids were significantly higher and the n6 and n9 fatty acids were significantly lower in the polar lipids of the wild eggs than in those from the cultured fish (Tables 10a & 10b) in both stocks. This resulted in higher n3:n6 ratios in the wild than in the cultured eggs of both stocks. There were no significant

Table 10a. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in POLAR LIPIDS of eggs from BIG QUALICUM (BQ) Chinook broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
Saturates	27.80[a]	0.52	23.78[b]	0.53	■24.95[b]	0.30
n3	45.57[a]	0.86	39.66[b]	0.40	37.93[b]	0.51
n6	2.41[a]	0.15	■ 5.49[b]	0.08	7.04[c]	0.10
n9	13.73[a]	0.74	■20.68[b]	0.72	19.95[b]	0.26

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 10b.

	BQ WILD		BQ WV33		BQ COMM	
	mean	sem	mean	sem	mean	sem
n3:n6 ratio*	18.91	1.51	7.22	0.07	5.39	0.14

* ANOVA was not conducted on the n3:n6 ratios.

Table 10b. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in POLAR LIPIDS of eggs from ROBERTSON CREEK (RC) Chinook broodstock on three diets (n = 5 fish for each group except COMM where n=3). Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
Saturates	27.61[a]	0.67	24.80[b]	0.14	■25.86[c]	0.55
n3	46.81[a]	0.71	41.22[b]	0.87	37.99[c]	0.35
n6	2.54[a]	0.10	■ 6.11[b]	0.12	6.76[c]	0.21
n9	13.75[a]	0.55	■18.37[b]	1.62	18.61[b]	0.34

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 10a.

	RC WILD		RC WV33		RC COMM	
	mean	sem	mean	sem	mean	sem
n3:n6 ratio*	18.43	0.89	6.76	0.08	5.62	0.15

* ANOVA was not conducted on the n3:n6 ratios.

differences between the wild stocks in saturated, n3, n6 or n9 fatty acids but the RC-COMM eggs had significantly more saturates than the BQ-COMM eggs and the RC-WV33 eggs had significantly more n6 fatty acids and less n9 fatty acids than the BQ-WV33 eggs.

As shown in Tables 11a & 11b, significantly higher levels of 20:5n3, 22:5n3, n3 PUFAs and HUFAs were present in the polar lipids of the eggs of the wild fish than in the cultured fish of both stocks. The exception to this pattern was the fatty acid, 22:6n3. There were no significant differences in the levels of 22:6n3 among any of the diet groups, in either stock.

Castledine and Buckley (1980 and 1982) reported the presence of unfluctuating levels of 22:6n3 in the polar lipid of rainbow trout muscle during starvation and essential fatty acid deprivation. The physiological importance of this fatty acid as a membrane component and possible eicosanoid precursor is reflected by this tenacious retention. Fremont *et al.* (1984) found that rainbow trout fed an n3 deficient diet for 6 months before spawning maintained high levels of 22:6n3 in the vitellogenin and lipoprotein while levels of other n3 fatty acids, including 20:5n3, declined drastically. This fatty acid may therefore be essential for the reproductive success of salmonids.

A similar pattern of n6 fatty acids was seen in the polar

Table 11a. Selected n3 fatty acids, total n3 PUFAs (polyunsaturated fatty acids) and total n3 HUFAs (highly unsaturated fatty acids) (mean and standard error of the means) in POLAR LIPIDS of eggs from BIG QUALICUM (BQ) broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
20:5n3	14.97[a]	0.45	10.70[b]	0.25	10.59[b]	0.16
22:5n3	5.22[a]■	0.37	3.48[b]	0.04	3.19[b]	0.19
22:6n3	24.14	0.96	24.69	0.33	23.59	0.73
Total n3 PUFAs[1]	1.10[a]	0.24	0.44[b]	0.11	0.57[b]	0.09
Total n3 HUFAs[2]	44.47[a]	0.76	39.23[b]■	0.41	37.36[c]	0.50

1 n3 PUFAs = unsaturated n3 fatty acids with 2 or 4 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 5 or more double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 11b.

Table 11b. Selected n3 fatty acids, total n3 PUFAs (polyunsaturated fatty acids) and total n3 HUFAs (highly unsaturated fatty acids) (mean and standard error of the means) in POLAR LIPIDS of eggs from ROBERTSON CREEK (RC) broodstock on three diets (n = 5 fish for each group except COMM where n = 3).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
20:5n3	15.59[a]	0.76	11.05[b]	0.48	10.39[b]	0.03
22:5n3	4.42[a]■	0.38	3.35[b]	0.13	3.33[b]	0.36
22:6n3	25.16	0.42	25.56	0.25	23.93	0.41
Total n3 PUFAs[1]	1.41[a]	0.21	0.41[b]	0.14	0.34[b]	0.18
Total n3 HUFAs[2]	45.40[a]	0.82	40.81[b]■	0.85	37.65[c]	0.27

1 n3 PUFAs = unsaturated n3 fatty acids with 2 or 3 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 4 or more double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 11a.

lipids as was seen in the total lipids, namely, significantly lower levels of all n6 fatty acids in the wild than in the cultured eggs (Tables 12a & 12b). Within each stock, it was the COMM eggs that consistently had the highest, and usually significantly higher, levels of n6 fatty acids. Between stocks, the RC-COMM eggs contained significantly higher levels of 20:4n6 and total n6 PUFAs and the RC-WV33 eggs contained a significantly higher level of 20:2n6. This pattern was reflected in the polar lipid n3:n6 ratios (Tables 10a and 10b).

The absence of 20:2n6 (and 20:3n6, see Appendix 4) from both the total and the polar lipids of the wild eggs (both stocks) suggests that elongation and desaturation of 18:2n6 to 20:4n6 was not occurring. It is known that high levels of n3 fatty acids in the diet inhibit elongation and desaturation of n6 and n9 fatty acids (Halver, 1980). It was the wild eggs, with the highest n3 concentration, that had the lowest level of 20:4n6 in both the total and polar lipids.

The source of the 20:4n6 in all groups may have been dietary. Both 18:2n6 and 20:4n6 were present in the COMM and WV33 diets and a small amount (0.33%) of 18:3n6 was present in the WV33 diet. A dietary source could therefore account for the appearance of these fatty acids in the eggs of the cultured fish. If this was the case, it seems unlikely that 20:4n6, the primary precursor for eicosanoid synthesis in mammals, has much physiological significance for Chinook, particularly since the

Table 12a. Selected n6 fatty acids and total n6 PUFAs (polyunsaturated fatty acids) (mean and standard error of the means) in POLAR LIPIDS of eggs from BIG QUALICUM (BQ) broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
18:2n6	0.28[a]	0.04	1.40[b]	0.07	2.03[c]	0.04
20:2n6	0.00[a]	0.00	0.42[b]	0.02	0.45[b]■	0.03
20:4n6	1.68[a]	0.04	2.43[b]■	0.07	2.88[c]	0.12
Total n6 PUFAs[1]	2.41[a]	0.15	5.49[b]■	0.08	7.04[c]	0.10

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 12b.

Table 12b. Selected n6 fatty acids and total n6 PUFAs (polyunsaturated fatty acids) (mean and standard error of the means) in POLAR LIPIDS of eggs from ROBERTSON CREEK (RC) broodstock on three diets (n = 5 fish for each group except COMM where n = 3).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
18:2n6	0.34[a]	0.02	1.57[b]	0.05	1.83[c]	0.02
20:2n6	0.00[a]	0.00	0.40[b]	0.03	0.59[c]■	0.05
20:4n6	1.56[a]	0.04	2.68[b]■	0.05	2.84[b]	0.02
Total n6 PUFAs[1]	2.54[a]	0.10	6.11[b]■	0.12	6.76[c]	0.21

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 12a.

level of 20:4n6 was significantly lower in the wild than in the cultured eggs.

Another explanation for the absence of 20:2n6 in the wild eggs and its presence in the cultured eggs may lie in the use of the Supelcowax 10 (Supelco Canada Ltd., Oakville, Ontario) column employed on the GLC in these analyses. According to Ackman (1986), this column does not separate 20:3n9 and 20:2n6 very satisfactorily. Ackman (1986) suggests that normally this is not a problem in nutritional biochemistry unless there is a deficiency of essential fatty acids which leads to the formation of 20:3n9. In view of the measures which researchers must take in order to induce an EFA deficiency in fish (eg. Castledine and Buckley, 1980 and 1982), it is unlikely that the formation of 20:3n9 was occurring in the cultured fish in this study. Rather the fatty acid identified as 20:2n6, really was 20:2n6.

Monounsaturates, particularly 16:1n7 and 18:1n9, are important components of TAG and polar lipid molecules, as mentioned previously. These two fatty acids were the predominant monoenoic fatty acids in both the polar and the total lipids. As shown in Tables 13a & 13b, n9 fatty acids were significantly lower in the polar lipids of wild eggs than cultured eggs. The n7 fatty acids were not significantly different among the diet groups of the cultured BQ or RC eggs. This may indicate a requirement for a specific level of n7 fatty acids.

Table 13a. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in POLAR LIPIDS of eggs from BIG QUALICUM (BQ) broodstock on three diets (n = 5 fish for each group).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	BQ WILD		BQ WV33		BQ COMM	
	mean %	sem	mean %	sem	mean %	sem
n5	0.50	0.05	0.52	0.02	0.48	0.03
n7	6.59■	0.21	6.30■	0.27	5.98	0.17
n9	13.73[a]	0.74	20.68[b]■	0.72	19.95[b]	0.26
n11	0.37	0.34	0.71	0.36	0.37	0.04
Total monounsaturates	21.19[a]	0.91	28.21[b]■	0.63	26.78[b]■	0.14

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 13b.

Table 13b. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in POLAR LIPIDS of eggs from ROBERTSON CREEK (RC) broodstock on three diets (n = 5 fish for each group except COMM where n = 3).
Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet.

	RC WILD		RC WV33		RC COMM	
	mean %	sem	mean %	sem	mean %	sem
n5	0.59	0.07	0.48	0.01	0.50	0.04
n7	5.21■	0.21	5.75■	0.07	5.99	0.17
n9	13.75[a]	0.55	18.37[b]■	1.62	18.61[b]	0.34
n11	0.17	0.11	0.23	0.06	0.00	0.00
Total monounsaturates	19.72[a]	0.56	24.82[b]■	1.58	25.10[b]■	0.24

abc = a significant difference (α=0.05) was found between means with different superscripts within the same row.

■ = a significant difference (α=0.05) was found between stocks for the parameter indicated. See Table 13a.

Between the two stocks, where there were significant differences in monounsaturates, the BQ eggs consistently had the greater concentration, ie. for the n7 series, BQ-WILD > RC-WILD and BQ-WV33 > RC-WV33; for the n9 series, BQ-WV33 > RC-WV33; and for total monounsaturates, BQ-WV33 > RC-WV33 and BQ-COMM > RC-COMM.

1.2.6 Diets

All diet data are presented in Apppendix 2. This includes feed tag data for the COMM diet (Table A2.1) and the formulation of the WV33 diet (Table A2.2). Both diets were analysed for dry matter, moisture, lipid on a wet and dry weight basis (Table A2.3) and for total lipid fatty acids (Table A2.4). Dry matter, moisture and lipid levels were found to match specifications stated on the feed tags of both diets (Table A2.1).

For clarity, comparisons of individual fatty acids and fatty acid series in the total and polar lipids of the eggs are presented together with the same parameters in the diets in Figures 5, 6 and 7. Parallel egg data for the wild fish are shown at the bottom of each figure. Diet data were not available for the wild fish.

The saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios in the total and polar lipids of the eggs of both stocks on all

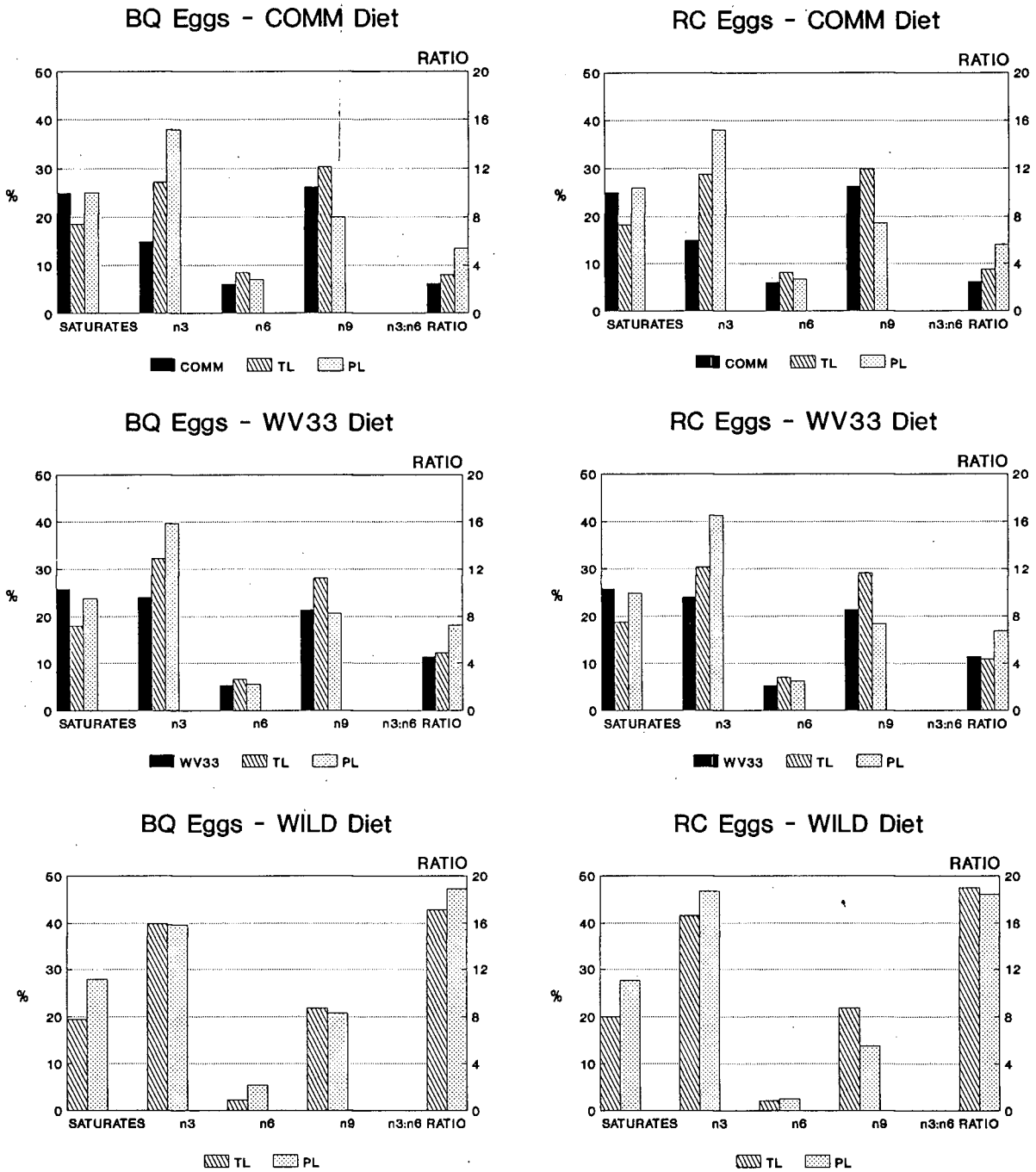
diets are displayed as histograms in Figure 5. Data for the diets (dark bars) are presented also. Except for the saturates, there was a strong tendency for fatty acids present in the diet to accumulate in the total lipids. Both the COMM and the WV33 diets were high in saturated fatty acids. It appears that some saturates were converted to monounsaturates (see Figure 7) or that they were oxidized. The fatty acid 16:0 was the major saturated fatty acid in the total and polar lipids of the eggs of both the cultured and wild fish. It is often present in TAGs and to a lesser extent in polar lipids.

The n9 fatty acids, predominant in the COMM diet, were the major series present in the total lipids of the COMM eggs. However it was the n3 series, the third largest component of the COMM diet and the second largest component in the WV33 diet, that formed the largest proportion of fatty acids in the polar lipids of the COMM eggs and in both the total and polar lipids of the WV33. The n3 series was also dominant in the total and polar lipids of the wild eggs of both stocks.

The levels of saturated and n3 fatty acids were within 2% of each other in the WV33 diet, followed by the n9 series. However, in the eggs, the n3 fatty acids were the major series in both the total and polar lipids, followed by the n9 fatty acids in the total lipids and the saturates in the polar lipids.

In this study, diet was a greater factor than stock affecting

Figure 5:
A comparison of saturated, n3, n6 and n9 fatty acids (%) andn3:n6 ratios in total (TL) and polar (PL) lipids of eggs from cultured and wild (WILD) Big Qualicum (BQ) and Robertson Creek (RC) broodstock with thecommercial (COMM) and West Vancouver (WV33) diets.

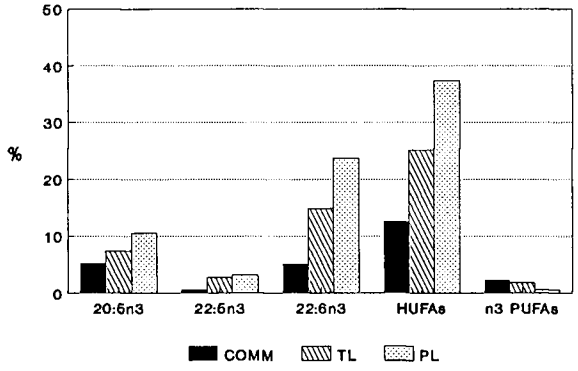


fatty acid profiles in the eggs of the cultured fish. Differences in fatty acid composition were greater between the COMM and WV33 diet groups than between the two stocks on the same diet, (Figure 5). This was also true for the other fatty acids, as described below and illustrated in Figures 6 and 7. The influence of temperature on the fatty acid composition of membranes and the ability of fish to alter levels of unsaturation in membranes in response to temperature changes has been documented (eg. Hazel, 1979). All cultured fish were reared at the same site and were exposed to the same temperature regime. Temperature was therefore not a factor.

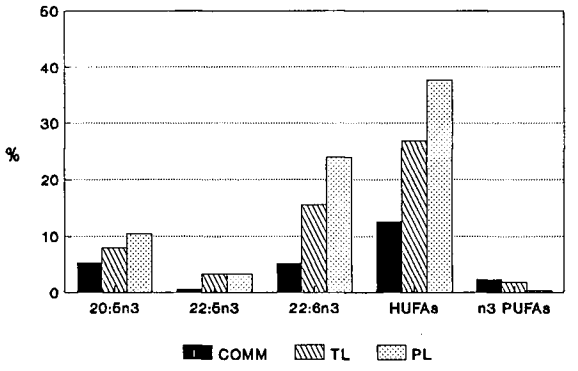
Although a wild diet would contain high levels of n3 fatty acids and particularly high levels of HUFAs, the effect of temperature cannot be ruled out as a major influence on the high levels of unsaturation in the wild eggs. During their ocean migration, the wild fish would have been exposed to lower water temperatures than the cultured fish in their near shore net pens. Since the Robertson Creek stock matures off the west coast of Vancouver Island while the Big Qualicum stock comes into the warmer Strait of Georgia, it may be expected that their temperature regimes and diets would both differ. The most apparent difference was the presence of higher levels of n3 and saturated fatty acids and lower levels of n9 fatty acids in the polar lipid fraction of the eggs of the RC-WILD fish than in the eggs of the BQ-WILD fish (Figure 5). These differences are consistent with their respective temperature

Figure 6:
A comparison of selected n3 fatty acids, n3 PUFAs and HUFAs in total (TL) and polar (PL) lipids of eggs from cultured and wild (WILD) Big Qualicum (BQ) and Robertson Creek (RC) broodstock with the commercial (COMM) and West Vancouver 33 (WV33) diets.

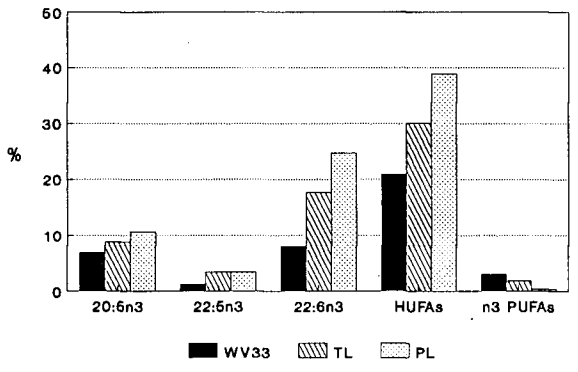
BQ Eggs - COMM Diet



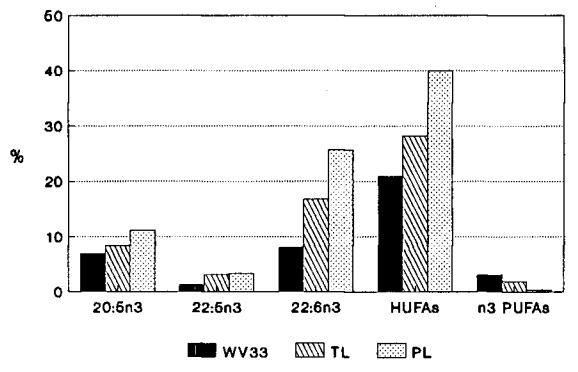
RC Eggs - COMM Diet



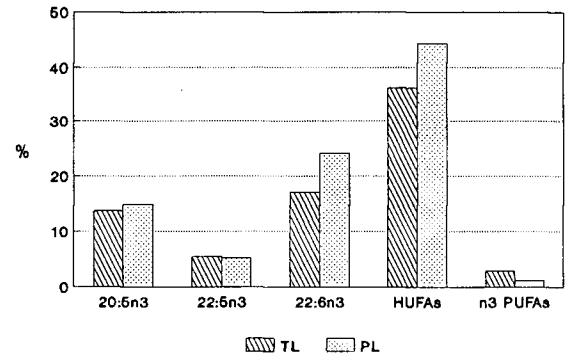
BQ Eggs - WV33 Diet



RC Eggs - WV33 Diet



BQ Eggs - WILD Diet



RC Eggs - WILD Diet

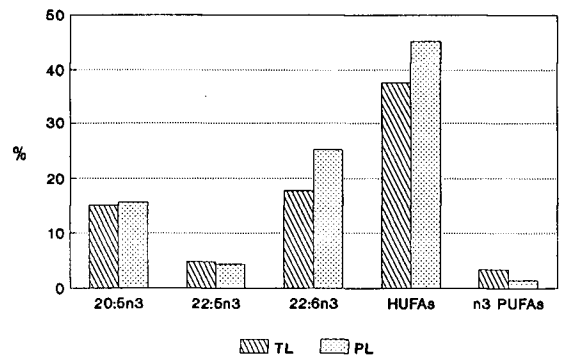
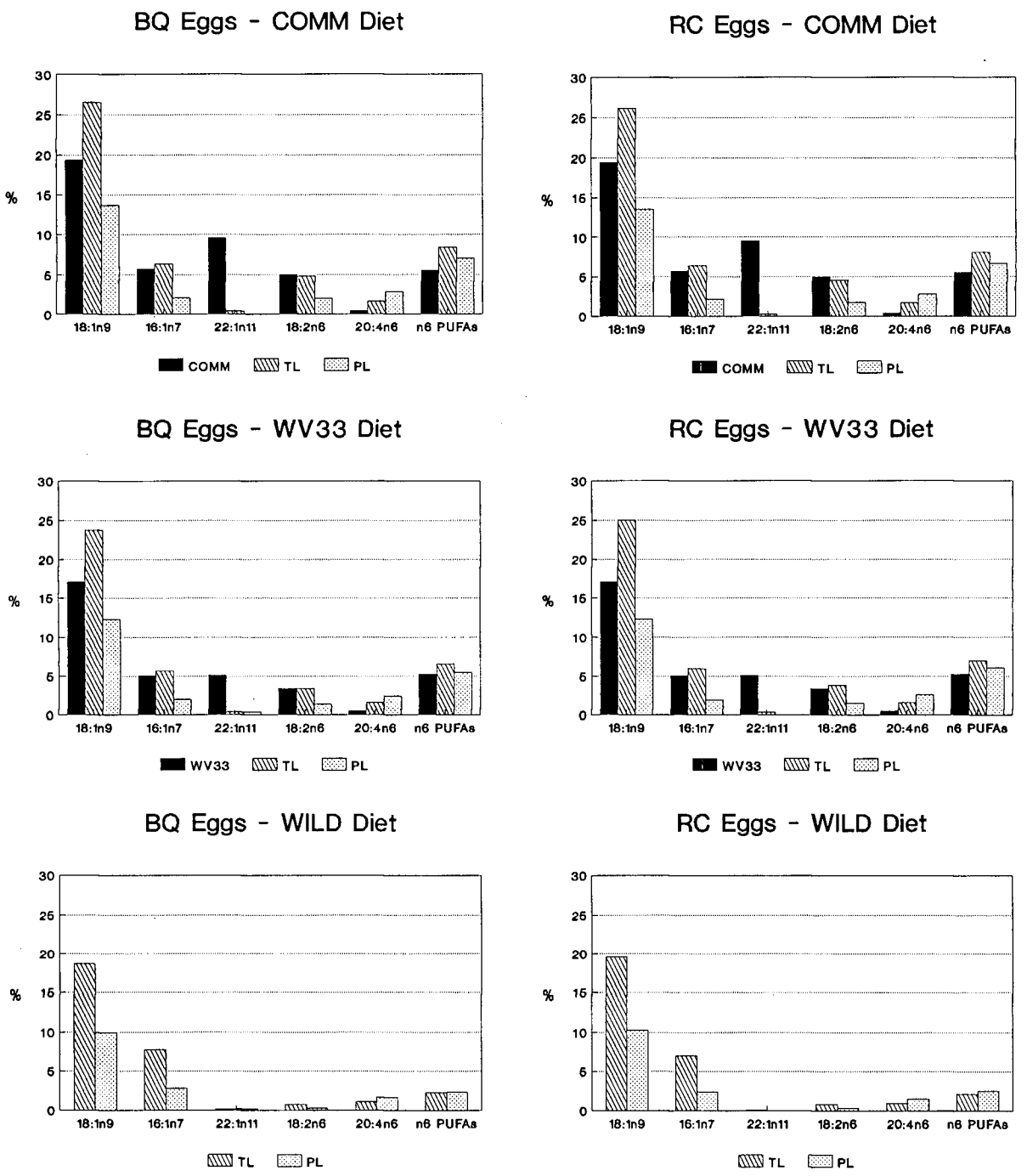


Figure 7:
A comparison of selected fatty acids and n6 PUFAs in total (TL) and polar (PL) lipids of eggs from cultured and wild (WILD) Big Qualicum (BQ) and Robertson Creek (RC) broodstock with the commercial (COMM) and West Vancouver (WV33) diets.



regimes. The two stocks were remarkably similar in composition otherwise (see also Figures 6 and 7).

The major fatty acids in the n3 series of both the cultured and wild eggs were 22:6n3 and 20:5n3. These fatty acids were present in the eggs in concentrations that were notably higher than in the diets and it is evident that they had been accumulated and conserved in the eggs (Figure 6). While the fatty acid, 20:5n3 was higher in both the total and polar lipids of the wild eggs than in the cultured eggs, 22:6n3 levels were remarkably similar, particularly in the polar fraction, in the cultured and wild eggs (Figure 6). In view of their physiological significance as membrane components and eicosanoid precursors and because n3 fatty acids have essential status for salmonids, it is reasonable to expect consistent high levels of these HUFAs in the egg where they would be required by the growing embryo.

As shown in Figure 7 both the COMM and WV33 diets contained high levels of monounsaturates, particularly 18:1n9, 16:1n7 and 22:1n11. The first two are the primary monoenes in fish egg lipids (Christie, 1986) and were reflected accordingly in the egg composition but 22:1n11 was apparently not deposited in the eggs or it was oxidized as it was present in only very small amounts. As previously mentioned, 18:1n9 and 16:1n7 are important constituents of both TAGs and of many polar lipids. Both were present in the wild eggs but not in as large amounts

as was found in the cultured eggs.

The major n6 fatty acid present in the COMM and WV33 diets was 18:2n6 followed by 20:4n6. The eggs of the cultured fish contained small amounts of these fatty acids and the COMM eggs of both stocks contained slightly more 18:2n6 than the WV33 eggs consistent with slightly higher levels of this fatty acid in the COMM diet. The low levels of 20:4n6, an important prostaglandin precursor for mammals, in the eggs of both cultured and wild fish leads to speculation that this fatty acid has little physiological significance for Chinook. Total n6 PUFAs were considerably higher in the cultured eggs than in the wild eggs. Dietary sources of these fatty acids are few in the marine environment (Ackman, 1986).

1.3 CONCLUSIONS

The intention of Section 1 was to compare lipid composition and fatty acid profiles in wild and cultured Chinook salmon. While not an objective of this experiment, it is tempting to speculate on possible reasons for the superior reproductive performance of the wild over the cultured broodstock (Table 4).

The most obvious differences found in the lipids between the cultured and wild fish were that the wild fish had much higher levels of n3 fatty acids, particularly the HUFAs, and lower levels of n6 and n9 fatty acids. The physiological importance of long chain fatty acids as membrane components and eicosanoid precursors has been discussed. Further, Leray *et al.* (1985) reported embryonic deformities leading to mortality as early as the 16-and 32-cell stages when rainbow trout broodstock were fed low levels of n3 and high levels of n6 fatty acids. It was suggested that the HUFAs (20:5n3 and 22:6n3) are the precursors for hydroxy fatty acids which are thought to be critical to the processes of cellular recognition. Most of the mortality in the cultured eggs in this study occurred before eyeing and, as shown in Table 6, the n3:n6 ratios in both total and polar lipids of the cultured eggs (both diets) were considerably lower than in the wild eggs.

The quantitative requirements of Chinook eggs for HUFAs during incubation are unknown. The eicosanoids are effective

in very low concentrations which suggests that a small pool of precursor fatty acids should satisfy the requirement for their synthesis. However as work proceeds in this area, more eicosanoids are being detected and more functions are being attributed to them. Eicosanoids are rapidly taken up and de-activated and in order to maintain a response for a continuous period, as may occur in a chronic infection, for example, a large pool of precursors may be necessary. Eicosanoids are synthesized from the membrane HUFAs and whether this reservoir has priorities for other functions is not clear. Also, little is known about the kinetics of the synthetic process. Future work in this area is bound to be very interesting.

The eggs of the cultured fish on the two formulated diets were more similar to each other than to the wild eggs, regardless of stock (Figures 5, 6 & 7). Too few fish were analysed for fecundity, fertility and eyeing success (Tables 2 & 4) relative to the standard error of the means, for reliable statistical significance to be assessed. However the COMM fish generally out-performed the WV33 fish (see also Table 3).

It is unlikely that the success of the COMM eggs relative to the WV33 eggs was due to fatty acid composition. The n3:n6 ratios in the COMM and WV33 diets were 2.73 and 5.68 (Appendix 2, Table A2.4), respectively, and were lower in the eggs of the COMM group than in the WV33 group in both the total and polar lipids of both stocks (Tables 6a & 6b and 10a & 10b).

The WV33 diet was manufactured by a commercial feed company and, during the year prior to this study, it was manufactured only twice. Product storage therefore exceeded the usual recommended shelf life of 3 months (NRC, 1981). While degradation to varying degrees of many of the nutrients in the diet probably occurred and nothing definitive can be concluded from this study, it is likely that losses of vitamin C were particularly critical to reproductive success.

The dietary requirement for vitamin C in coldwater fish is 100 mg/kg dry diet (NRC, 1981). The COMM diet was supplemented with a minimum of 700 mg/kg; the WV33 diet was supplemented with 1900 mg/kg (Appendix 2, Tables A2.1 and A2.2). Vitamin C is generally added because this vitamin is highly susceptible to degradation by heat and moisture during processing and storage. Despite high supplementation of the WV33 diet, prolonged storage times probably resulted in very little residual vitamin C. Slinger *et al.* (1979, cited in Poston, 1986) report losses of 67 to 83% due to storage at about 20°C for 6 months. Storage conditions at the net pen site may have further increased the rate of degradation. The feed for the week was stored in black plastic barrels in the sun. In the study by Slinger *et al.* (1979, cited in Poston, 1986), leeching during exposure of feed to water for up to 10 seconds caused further losses of 15 to 67% of the remaining vitamin C activity. New forms of vitamin C (eg. ascorbate-2-sulfate (C_2)) are now commonly used in formulated diets. They are heat

and water stable at pH 4-13 (Tucker and Halver, 1986).

Vitamin C has many important biochemical functions including roles in the prevention of scoliosis and lordosis (a scorbutic manifestation due to impaired synthesis of collagen and cartilage); lipid and amino acid catabolism; catecholamine synthesis; de-toxification of pollutants (eg. cadmium) and free radicals via the cytochrome P_{450} system; in the immune system and in steroidogenesis (Sandnes, 1988). Based on their poor spawning performance, it is possible that steroidogenesis was compromised in the fish fed the WV33 diet. Other functions may also have been impaired.

Several studies in various species (Hilton *et al.*,1979; Seymour, 1981 a,b; Tolbert, 1979; Lutwak-Mann, 1958; and Sandnes and Braekkan, 1981 - all cited in Sandnes, 1988) point to a relationship between the high levels of vitamin C found in fish ovaries with the synthesis of sex steroids. A rise in vitamin C concentration in the ovary during vitellogenesis followed by a decrease in concentration prior to ovulation parallels the synthesis of sex steroids by the ovarian follicle cells and suggests that vitamin C may be required in the hydroxylating reactions necessary to convert cholesterol to sex steroids such as the estrogens (Sandnes, 1988).

Waagbo *et al.* (1989) demonstrated a significantly higher level of 17-β-estradiol in fish fed adequate levels of vitamin C than

in those fed deficient diets. A higher level of circulating vitellogenin in a supplemented group was also found.

Sandnes *et al.* (1984) found that rainbow trout broodstock fed a diet deficient in vitamin C for 3 months prior to spawning tended to produce fewer eggs of smaller diameter than did supplemented broodstock fed a diet containing 1000 mg/kg. Their figures for fecundity and egg size were not significant and there was a high level of variability associated with the data as was the case for the fecundity data reported in this study (Table 2). Also reported was a significantly (α=0.05) greater survival rate to eyeing and hatching in the supplemented progeny than in the deficient group. A third group, used as a control against the experimental diets, was fed a commercial diet and produced eggs of similar quality to those of the supplemented group. Analysis of the eggs revealed significant differences in vitamin C levels between the deficient (15 µg/g wet weight ± 7, sd), supplemented (31 µg/g wet weight ± 9, sd) and commercial (20 µg/g wet weight ± 10, sd) groups.

In a study undertaken to determine the effects of broodstock diets varying in moisture, amount and composition of lipids and content of α-tocopherol and ascorbic acid for Atlantic salmon, Eskelinen (1989) reported that the highest total survival from fertilization to start feeding (92.7%) was achieved with a semi-moist feed which had a low fat and moderate energy content

that had been supplemented with ascorbic acid at 1050 mg/kg. The second highest survival rate (90.3%) was obtained with a dry diet which had higher fat and protein levels and ascorbic acid at the second highest level in the study - 230 mg/kg. No effect on survival was attributable to α-tocopherol levels in the diets.

Soliman *et al* (1986) demonstrated that a diet supplemented to 1250 mg/kg significantly improved hatchability in tilapia (*Oreochromis mossambicus*). They also showed that vitamin C deficient tilapia reached maturity two weeks later than the supplemented group. Waagbo *et al.* (1989) interpreted a similar finding in rainbow trout, fed a vitamin C deficient diet, to impaired steroidogenesis, affecting ovarian growth and maturation during vitellogenesis. In the present study, fish fed the WV33 diet exhibited high levels of egg retention and impaired maturation (one or both ovaries did not mature) (Table 3).

Rainbow trout fed a diet containing 1400 ppm vitamin C produced fewer 'blank' (unfertilized) eggs than trout fed a diet containing 800 ppm (Ridelman, 1981, cited by Hardy, 1985). In this study, the rate of fertilization was lower among the WV33 eggs than in the COMM eggs, although the difference was not significant in the case of the BQ group and there were too few fish to assess significance in the RC group (Table 4).

Vitamin C is also required for collagen synthesis by the embryo. The function of vitamin C in collagen synthesis is again as a reducing agent in hydroxylation reactions. The amino acids, proline and lysine, are hydroxylated promoting the formation of hydrogen bonds between protein chains.

Little is known about the metabolism of vitamin C after fertilization in fish eggs but in sea urchin eggs collagen synthesis started at gastrulation and increased many times before hatching (Golub *et al.*, 1974). Sandnes *et al.* (1988) demonstrated an increase in the percentage of hydroxyproline in protein in the eggs of rainbow trout through incubation which was probably coincident with increasing levels of collagen in the embryo.

Impaired collagen synthesis may lead to larval deformities, particularly to severe damage of the spinal column, and to poor hatching performance (Soliman *et al.*, 1986). Tilapia fry, hatched from broodstock fed a low level of vitamin C, grew slowly, had low feed conversion rates and poor survival. When the broodstock were fed supplemented diets, fry performance was improved (Soliman *et al.*, 1986).

From the foregoing, the influence of vitamin C in broodstock nutrition on ovarian growth and maturation and in the survival and growth of the progeny appears critical. Further work on establishing optimum levels for this vitamin in diets for

Chinook broodstock should be carried out.

Reasons for the apparent success of the BQ-WV33 fish over the RC-WV33 fish (Tables 2, 3 & 4) can only be guessed at. There is always some 'wild feed' in the net pens at Genoa Bay, the marine broodstock facility for Sea Spring Salmon Farm. However, the cultured fish generally do not utilize this feed. Perhaps some of the BQ-WV33 broodstock were more inclined to take advantage of this source than were the RC-WV33 fish. Otherwise, there may have been some stock effect in response to the low levels of vitamin C or to other factors in the diet or environment of these fish.

As indicated in Materials and Methods, there were 283 fish in the RC-WV33 pen and 189 in the BQ-WV33 pen for densities of ca. 3.41 kg/m^3 and 2.84 kg/m^3, respectively. There were 811 BQ fish and 149 RC fish reared on the COMM diet for densities of ca. 5.96 kg/m^3 and 1.05 kg/m^3, respectively. Pen densities below 6 kg/m^3 are considered appropriate in the industry (D. Groves, pers. comm.) and as the morphometric data presented in Table 1 indicates, growth was not compromised in the cultured fish at the highest pen densities. It is therefore unlikely that density was a significant factor in the poor performance of the RC-WV33 broodstock.

SECTION 2 - Feed withdrawal from cultured Chinook broodstock prior to transfer to fresh water for maturation.

2.1 MATERIALS AND METHODS

Two groups of cultured 4-year old Chinook salmon were held in 680 m^3 marine net pens at Genoa Bay. Pen densities were less than 2.5 kg/ m^3 in both pens. Both groups were fed the same commercial diet (COMM) at the same rate as was fed to the fish in Section 1. Again, not all of the fish in these pens matured in 1987 which made it impossible to accurately determine feed consumption by the broodstock. Feeding levels were approximately 1% of body weight per day at the start of their final summer but they cut back naturally to ca. 0.5% or less by the end of the summer.

The original intention of this experiment was to withdraw all feed from the fish in Pen 16 (the restricted group) for one full month before transfer to fresh water for maturation and spawning. The fish in Pen 4 (full ration group) were to act as controls and were to be starved for one week prior to transfer, the usual protocol for broodstock transport. The design was single factor with no replication.

The fish were progeny of Big Qualicum females and Chemainus males. The pure Chemainus stock typically spawns before the

pure Big Qualicum stock. Consequently, the fish matured earlier than expected. Feeding of the restricted fish was halted on September 2, 1987 but they were darkening rapidly ten days later and had to be transported on September 16 with only 14 days of saltwater starvation. Feeding of the control fish was stopped on September 28 and they were transported on October 5 for a total of 7 days of saltwater starvation.

Maturing fish were brought to the hatchery, held until fully mature (ca. 2 - 4 weeks) and spawned according to the methods outlined in Section 1. Sample collection, techniques for lipid and fatty acid analyses, collection of morphometric data, and procedures for measuring fertilization and eyeing success were identical to those employed in Section 1.

All data reported as percentages were transformed by the arc sine transformation before statistical analyses. One-way ANOVA (Proc GLM, SAS 1985) were carried out on the morphometric and incubation data, composition parameters, individual fatty acids and fatty acid series to detect any significant differences due to period of starvation. The significance level was set at 95%.

2.2 RESULTS AND DISCUSSION

2.2.1 Morphometric measurements

Morphometric measurements are presented in Table 14. There were no significant differences in fork length, weight or condition factor between the two groups of fish.

2.2.2 Spawning and incubation success

The restricted fish produced significantly smaller eggs than control fish (Table 14). If vitellogenesis was still occurring during the period of starvation, it is reasonable for the eggs of the fish starved for the longer period to be smaller. Fecundity, whether determined by volume or number of eggs spawned, was not adversely affected by starvation as no significant difference was found between the two groups (Table 15). It is unlikely that the short period of starvation would have an effect on fecundity because egg recruitment would have been determined much earlier in vitellogenesis (personal observation). Therefore the major effect of starvation was on egg size rather than on egg number. There was no apparent effect of smaller egg size on survival to eyeing in this study.

No significant difference due to period of starvation was found between groups in fertilization rate, number of eyed eggs produced, or rate of survival to eyeing (Table 15).

Table 14. Morphometric data (mean and standard error of the means) for Big Qualicum 4-year old broodstock. Feed was withdrawn from these fish for 7 or 14 days prior to freshwater transfer.

Period of Starvation, days	7	14
Fork length, cm	84.16	83.58
sem (n)	0.69 (56)	0.74 (57)
Weight, kg	8.44	8.41
sem (n)	0.19 (56)	0.21 (57)
Condition factor	4.69	4.78
sem (n)	0.10 (56)	0.12 (57)
Egg diameter, mm	8.6^{a}	8.3^{b}
sem (n)	0.06 (19)	0.11 (16)

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

Table 15. Spawning and incubation data (mean and standard error of the means) for Big Qualicum 4-year old broodstock. Feed was withdrawn from these fish for 7 or 14 days prior to freshwater transfer.

Period of Starvation, days	7	14
Volume of eggs spawned, ml	1266	1259
sem (n)	52.7 (55)	61.8 (57)
Fecundity (# eggs spawned)	4702	4831
sem (n)	155.3 (54)	149.6 (53)
Eyed eggs, #	4201	4474
sem (n)	164.8 (53)	166.5 (53)
Fertility, %	92.8	93.8
sem (n)	2.90 (18)	2.39 (16)
Eyed, %	89.05	91.96
sem (n)	1.91 (53)	1.41 (53)

A one-way ANOVA conducted on period of starvation indicated no significant difference (α=0.05) for any of the spawning and incubation parameters, above.

Ridelman *et al.* (1984) compared the reproductive performance of rainbow trout fed *ad libitum* or starved for 45 days prior to spawning. No significant differences in physical or chemical characteristics, including egg size, proximate composition, fecundity or egg viability, were found in the groups. It was suggested that vitellogenesis was complete or nearly so by 45 days prior to spawning. The period of starvation in this study was initiated 42 - 49 days prior to the peak of spawning.

Blaxter (1970) has reported that fecundity increases with length and weight in many species. Significant (α=0.05) positive correlations (Critical values of the correlation coefficient, r. Zar, 1984) for both groups were found between fecundity and fork length (r=0.465, n=60 in the restricted group and r=0.280, n=66 in the control group) and between fecundity and weight (r=0.371, n=60 in the restricted group; r=0.583, n=66 in the control group). Correlation analyses of fecundity and condition factor revealed a small significant positive correlation in the control fish (r=0.276, n=66) but a small negative correlation (not significant) in the restricted fish (r=-0.213, n=60).

Blaxter (1970) and Roy and Higgs (1987) reported that there is a strong tendency for egg size and fecundity to be inversely related. In the restricted fish there was a significant negative correlation (r=-0.628, n=14) between egg diameter and fecundity but there was no significant correlation between

these parameters in the control fish (r=-0.210, n=18).

Survival to eyeing in both groups was approximately 90%. The BQ fish of Section 1 on the COMM diet (Table 4) produced progeny with survival to eyeing of only 74%. Although the two stocks were different, ie. BQ in Section 1 and BQ crossed with Chemainus males in Section 2, the success of the Section 2 fish was probably due to their age at maturity. Incubation success in progeny from four year old broodstock is generally greater than that from three year olds (Groves, pers. comm. and Lawseth, pers. comm.). The high levels of egg retention and other abnormalities found in the three year old fish fed the WV33 diet were not present in the four year old fish regardless of treatment.

2.2.3 Composition of the eggs

There were no significant effects due to the treatment on dry matter; lipid concentration on a wet or dry weight basis; polar lipid concentration, as a percent of the total lipid or on a fat free dry weight basis; level of neutral lipids; or protein plus ash content in the two groups (Table 16).

Absolute volumes of total, polar and neutral lipid per egg were calculated (Table 17). Consistently, the restricted group had less of each of these lipid classes. This indicates that had the starvation period been longer, greater effects on

Table 16. Composition of the eggs (mean and standard error of the means) of 4-year old Big Qualicum broodstock. Feed was withdrawn from these fish for 7 or 14 days prior to freshwater transfer. (n=7 fish for those starved 7 days; n=5 fish for those starved 14 days)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
Dry matter	40.11	0.86	39.21	0.55
Moisture	59.89	0.86	60.77	0.55
Lipid, ww*	12.84	0.35	12.85	0.19
Lipid, dw*	32.01	0.40	32.78	0.48
Polar lipid, dw	12.41	0.03	12.70	0.22
Neutral lipid, dw	19.76	0.35	20.15	0.62
Polar lipid, of total lipid	38.79	0.46	38.75	0.39
Neutral lipid, of total lipid	61.73	0.42	61.42	1.29
Polar lipid, of fat free dw	18.25	0.13	18.93	0.48
Protein + ash, ww	27.27	0.56	26.39	0.49

* ww = wet weight; dw = dry weight

A one-way ANOVA conducted on period of starvation indicated no significant difference for any of the composition parameters, above (α=0.05).

Table 17. Absolute volumes of total and polar lipid per egg calculated from mean egg volumes and percentages of these parameters.
Eggs are from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Period of Starvation, days	7	14
Mean egg radius, mm	4.3 [a]	4.15 [b]
Mean egg volume, mm^3 $(4/3\ \pi\ r^3)$	333.0	299.4
Lipid concentration (ww*), %	12.84	12.85
Absolute volume lipid/egg, mm^3	42.8	38.5
Polar lipid of total lipid, %	38.79	38.75
Absolute volume polar lipid, mm^3	16.6	14.9
Neutral lipid of total lipid, %	61.73	61.42
Absolute volume neutral lipid, mm^3	26.4	23.6

ab = original ANOVA results, see Table 14
*ww = wet weight

composition may have been found and that even with this short period of restriction, a decline in lipid concentration was occurring.

2.2.4 Fatty acid composition of the total lipids

The complete fatty acid profiles of the total lipids in the eggs of both groups are presented in Appendix 4, Table A4.5.

The eggs from the restricted broodstock had significantly higher levels of the fatty acid, 14:0, in the total lipids than the control eggs. No other significant differences were present in the total lipid fatty acid profile (Table A4.5).

As shown in Table 18, there were no significant differences in total saturated, n3, n6 or n9 fatty acids in the total lipids between treatment groups. The n3:n6 ratios were not subjected to ANOVA but they were very close at 3.98 for the restricted group and 4.01 for the control group.

There were no significant differences in the long chain n3 (Table 19) or n6 (Table 20) fatty acids or in the n5, n7, n9 or n11 monounsaturates (Table 21) in the total lipids of the two groups of eggs.

The eggs from the restricted fish had lower absolute volumes of total saturated fatty acids, n3 fatty acids, n3 HUFAs and

Table 18. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. (n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
Saturates	16.83	0.23	17.54	0.36
n3	31.91	0.57	31.50	0.78
n6	8.02	0.35	7.93	0.19
n9	29.19	0.52	28.92	0.19

A one-way ANOVA conducted on period of starvation indicated no significant differences (α=0.05) for any of the series of fatty acids above.

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
n3:n6 ratio*	4.01	0.21	3.98	0.18

* ANOVA was not conducted on the n3:n6 ratios.

Table 19. Selected n3 fatty acids, total n3 polyunsaturated fatty acids (n3 PUFAs) and total highly unsaturated fatty acids (HUFAs) (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock prior to transfer to freshwater for maturation. (n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
20:5n3	8.76	0.24	8.41	0.26
22:5n3	3.89	0.13	3.73	0.14
22:6n3	16.64	0.43	16.88	0.48
n3 PUFAs[1]	2.23	0.05	2.17	0.06
n3 HUFAs[2]	29.68	0.62	29.33	0.69

1 n3 PUFAs = unsaturated n3 fatty acids with 2 to 4 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 5 or more double bonds

A one-way ANOVA conducted on period of starvation indicated no significant differences (α=0.05) for any of the fatty acids or series of fatty acids above.

Table 20. Selected n6 fatty acids, total n6 polyunsaturated fatty acids (n6 PUFAs) (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
18:2n6	4.56	0.16	4.51	0.18
20:2n6	0.42	0.02	0.40	0.02
20:4n6	1.62	0.06	1.64	0.07
n6 PUFAs[1]	8.02	0.29	7.93	0.32

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

A one-way ANOVA conducted on period of starvation indicated no significant differences (α=0.05) for any of the fatty acids or series of fatty acids above.

Table 21. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
n5	0.46	0.02	0.44	0.01
n7	9.66	0.12	9.82	0.13
n9	29.19	0.41	28.92	0.46
n11	0.77	0.07	0.95	0.08
Total monounsaturates	40.08	0.47	40.13	0.52

A one-way ANOVA conducted on period of starvation indicated no significant differences (α=0.05) for any of the fatty acids or series of fatty acids above.

22:6n3 per egg (Table 22) than the control group, probably indicating that a decline in these fatty acids had begun.

2.2.5 Fatty acid composition of the polar lipids

The complete fatty acid profiles of the polar lipids in the eggs of each group are presented in Appendix 4, Table A4.6.

As for the total lipid fatty acid profile, the eggs of the restricted fish also had significantly higher levels of the fatty acid 14:0 in the polar lipid fraction than was present in the eggs of the control fish (Table A4.6). Another saturate, 16:0, was also significantly higher in the polar fraction of these eggs. The only other significantly different fatty acid was 21:5n3, which was lower in the eggs of the restricted fish than in the eggs of the control fish.

The higher concentration of saturates in the eggs from the restricted broodstock was only apparent, an offset to the lower concentration of the n3 fatty acids (particularly 22:6n3) found in these eggs (Tables 23 and 24). This was further demonstrated when the absolute volumes of saturated and n3 fatty acids per egg were calculated (Table 22). In absolute terms, total saturated fatty acids, n3 fatty acids, n3 HUFAs and 22:6n3 were all lower in the restricted eggs than in the control group.

Table 22. Absolute volumes of total saturates, n3 fatty acids, n3 HUFAs and 22:6n3 in the total and polar lipids per egg calculated from the absolute volumes of total and polar lipid per egg and the percentages of these parameters, as reported in previous tables. Eggs were from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. (n=5 fish for each group)

Period of Starvation, days	7	14
Egg diameter, mm^3	8.6[a]	8.3[b]
Absolute volume total lipid, mm^3	42.8	38.5
Absolute volume polar lipid, mm^3	16.6	14.9
Composition of Total Lipid:		
Saturates, %	16.83	17.54
n3 fatty acids, %	31.91	31.50
n3 HUFAs, %	29.68	29.33
22:6n3, %	16.64	16.88
Composition of Polar Lipid:		
Saturates, %	24.69	25.26
n3 fatty acids, %	41.33 [a]	39.20 [b]
n3 HUFAs, %	40.94 [a]	38.81 [b]
22:6n3, %	25.10	23.92
Absolute volumes in Total Lipid of:		
Total saturates, mm^3	7.2	6.8
Total n3 fatty acids, mm^3	13.7	12.1
Total n3 HUFAs, mm^3	12.7	11.3
Total 22:6n3, mm^3	7.1	6.5
Absolute volumes in Polar Lipid of:		
Total saturates, mm^3	4.1	3.8
Total n3 fatty acids, mm^3	6.9	5.8
Total n3 HUFAs, mm^3	6.8	5.8
Total 22:6n3, mm^3	4.2	3.6

ab = original ANOVA results, see Tables 23 and 24

The n3:n6 ratios were not tested by ANOVA but there was only a small difference in the n3:n6 fatty acid ratios, 6.90 versus 6.62 for the control and restricted groups, respectively. There were no other significant differences between the two groups in total saturates, n6 or n9 fatty acids (Table 23) or in individual long chain fatty acids or the n3 PUFAs (Table 24).

There were no significant differences in individual n6 fatty acids, in total n6 PUFAs (Table 25), or in any of the series (n5, n7, n9, n11) of monounsaturates (Table 26) in the two treatment groups.

A loss of n3 fatty acids is contrary to the literature which suggests that n3 fatty acids are tenaciously retained particularly in polar lipids during starvation while saturates and others are oxidized (Castledine and Buckley, 1980; Castledine and Buckley, 1982; Fremont *et al.*, 1984; Bell *et al.* 1985a and Bell *et al.*, 1985b). However if vitellogenesis was still occurring, the lower level of n3 fatty acids in the eggs of the restricted fish may indicate that transfer of these fatty acids to the eggs was incomplete when feed was withdrawn.

Again, had the period of starvation been longer, more pronounced differences in fatty acid composition of both the total and polar lipids may have been seen.

Table 23. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation. (n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
Saturates	24.69	0.24	25.26	0.30
n3	41.33[a]	0.29	39.20[b]	0.68
n6	6.05	0.27	5.95	0.21
n9	18.64	0.57	20.23	0.79

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
n3:n6 ratio*	6.90	0.37	6.62	0.26

* ANOVA was not conducted on the n3:n6 ratios.

Table 24. Selected n3 fatty acids, total n3 polyunsaturated fatty acids (n3 PUFAs) and total highly unsaturated fatty acids (HUFAs) (mean and standard error of the means) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
20:5n3	11.55	0.26	11.08	0.29
22:5n3	3.94	0.14	3.72	0.15
22:6n3	25.10	0.53	23.92	0.59
n3 PUFAs[1]	0.39	0.03	0.39	0.01
n3 HUFAs[2]	40.94[a]	0.44	38.81[b]	0.50

1 n3 PUFAs = unsaturated n3 fatty acids with 2 to 4 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 5 or more double bonds

ab = a significant difference (α=0.05) was found between means with different superscripts within the same row.

Table 25. Selected n6 fatty acids, total n6 polyunsaturated fatty acids (n6 PUFAs) (mean and standard error of the means) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock starved 7 or 14 days prior to transfer to freshwater for maturation. (n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
18:2n6	1.74	0.08	1.68	0.10
20:2n6	0.43	0.03	0.50	0.03
20:4n6	2.65	0.12	2.71	0.14
n6 PUFAs[1]	6.05	0.24	5.95	0.27

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

A one-way ANOVA conducted on period of starvation indicated no significant differences (α=0.05) for any of the fatty acids or series of fatty acids above.

Table 26. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock starved for 7 or 14 days prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Period of Starvation, days	7		14	
	mean %	sem	mean %	sem
n5	0.58	0.02	0.52	0.03
n7	5.47	0.13	5.62	0.15
n9	18.64	0.63	20.22	0.71
n11	-	-	-	-
Total monounsaturates	24.70	0.57	26.37	0.64

A one-way ANOVA conducted on period of starvation indicated no significant differences (α=0.05) for any of the fatty acids or series of fatty acids above.

2.3 CONCLUSIONS

This study was confounded by the earlier than expected maturation of the broodstock. Had it been carried out as planned, greater differences in egg composition, fatty acid profiles and survival rates may have occurred.

The short term starvation treatment had no effect on the lipid content of the eggs or on the condition factor of the broodstock. However lipid concentration (as percent lipid, wet weight basis) was considerably lower in the eggs of both groups (12.84%; 12.85%) of fish compared with the levels reported by Groves (1987) for cultured eggs in 1985 (13.5-19.0%) and in 1986 (13.5-15.5%) (see Appendix 1).

It appears from this work that the effects of short term starvation of broodstock on fecundity and survival of Chinook salmon eggs are negligible. Not examined in this study was the potential effect of broodstock starvation on the growth of alevins and early fry. An experiment of longer duration, incorporating alevin and early fry growth, is needed to determine at what stage feed withdrawal has a negative effect. Prior to this a farmer may well cease feeding broodstock in order to save labour and feed costs.

SECTION 3 - Alteration of lipid composition and fatty acid profiles in eggs from Chinook broodstock.

3.1 MATERIALS AND METHODS

Two groups of cultured 4-year old Chinook salmon were held in 680 m^3 marine net pens at Genoa Bay. There were 245 fish in Pen 3 and 262 fish in Pen 11. Pen densities were less than 3 kg/ m^3 in both pens.

All fish were fed a commercial diet (COMM) during the year before this experiment. For six weeks prior to the pre-transport starvation period, Pen 11 was fed a brood diet (BROOD), commercially available to the industry. The formulation of the BROOD diet was proprietary but feed label information is presented in Appendix 2 Table A2.1. More vitamin E (200 mg), more vitamin C (500 mg) and less crude fat (2%) were present in the BROOD diet than in the COMM diet. Another difference between the two diets, not shown on the feed tag, was the addition of 4-5% of low temperature dried krill meal to the BROOD diet. Dry matter, lipid concentration and fatty acid profiles of both diets were determined. These data are shown in Tables A2.3 and A2.4 of Appendix 2.

The fish were fed at approximately the same rate as the fish described in Sections 1 and 2. Again, because not all the fish matured in 1987, it was impossible to calculate feed

consumption of the broodstock accurately. However feeding level was approximately 1% of body weight per day at the start of the summer before spawning and declined to ca. 0.5%, or less, prior to transport to the hatchery.

Maturing fish were transferred from the net pens and held in freshwater until fully mature and spawned according to the methods outlined in Section 1. Sample collection procedures, morphometric measurements, analytical techniques and calculations, determination of egg size and procedures for measuring fertilization and eyeing success were the same as those described in Section 1.

A one-way ANOVA (Proc GLM, SAS 1985) was carried out on the morphometric and incubation data; composition parameters; and individual fatty acids and fatty acid series to determine significant differences due to the diets. All data reported as percentages were transformed by the arcsine transformation before analyses. The significance level was set at 95%.

3.2 RESULTS AND DISCUSSION

3.2.1 Morphometric measurements

Fork length and weight were measured for each female spawned and condition factors were calculated. These data are presented in Table 27. There were no significant differences in fork length or weight between the two diet groups but the condition factor of the fish on the BROOD diet was significantly lower than those on the COMM diet.

Egg diameters were also measured and no significant difference was found between the two groups (Table 27).

3.2.2 Spawning and incubation success

As shown in Table 28 the fish on the BROOD diet produced significantly greater volumes of eggs than those on the COMM diet. However, there was no significant difference in fecundity or egg diameters between the two groups. The method of measuring the volume of eggs spawned is crude. The eggs are fragile at the time of spawning and handling must be minimized. Egg volumes were measured by comparison with lines marked off at 100ml intervals on the spawning bucket. Therefore the difference in egg volumes between the two groups was probably due to the inaccuracy of the method of measurement.
There were no significant differences in fertilization success,

Table 27. Morphometric data (mean and standard error of the means) for 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation.

Diet	COMM	BROOD
Fork length, cm	81.5	81.8
sem (n)	0.36 (136)	0.69 (140)
Weight, kg	7.6	7.5
sem (n)	0.07 (136)	0.10 (140)
Condition factor	4.74 [a]	4.49 [b]
sem (n)	0.05 (136)	0.05 (140)
Egg diameter, mm	8.41	8.30
sem (n)	0.10 (16)	0.06 (29)

ab = a significant difference (α=0.05) was found between means with a different superscript within the same row.

Table 28. Spawning and incubation data (mean and standard error of the means) for Big Qualicum 4-year old broodstock on a commercial diet (COMM) or a brood diet (BROOD) prior to transfer to freshwater for maturation.

Diet	COMM	BROOD
Volume of eggs spawned, ml	972 [a]	1033 [b]
sem (n)	21.2 (136)	22.9 (140)
Fecundity (# eggs spawned)	4220	4413
sem (n)	130.6 (51)	114.7 (67)
Eyed eggs, #	3866	3979
sem (n)	137.4 (51)	118.3 (67)
Fertility, %	94.8	93.9
sem (n)	2.08 (13)	3.96 (18)
Eyed, %	91.79	91.89
sem (n)	1.75 (51)	1.42 (67)

ab = a significant difference (α=0.05) was found between means with a different superscript within the same row.

number of eyed eggs produced or in the survival to eyeing between the two groups (Table 28).

Correlation analyses of both groups revealed significant correlations (α=0.05; Zar, 1984) between fork length and fecundity (COMM diet: r=0.403, n=51; BROOD diet: r=0.320, n=67) and between body weight and fecundity (COMM diet: r=0.290, n=51; BROOD diet: r=0.438, n=67) as predicted by Blaxter (1970) but no significant correlations were found between condition factor and fecundity (COMM diet: r=-0.207, n=51; BROOD diet: r=0.099, n=67) in either group. Roy and Higgs (1987) reported a strong tendency for egg size and fecundity to be inversely related but no significant correlation between egg diameter and fecundity (COMM diet: r=0.250, n=13; BROOD diet: r=-0.212, n=18) was found in either group in this study.

3.2.3 Composition of the eggs

As shown in Table 29, no significant difference in dry matter concentration was found between the two diet groups.

Significantly greater lipid levels (on a wet and dry weight basis) were evident in the eggs of fish fed the COMM diet than in those fed the BROOD diet. The polar lipid concentration (as a percent of total lipid) was significantly lower in the COMM group. However, on a fat-free dry weight basis, the polar lipid concentration was not significantly different between the

Table 29. Composition of the eggs (mean and standard error of the means) of 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial (COMM) diet prior to freshwater transfer.
(n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
Dry matter	41.36	0.40	40.75	0.26
Moisture	58.64	0.40	59.25	0.26
Lipid, ww*	13.58 [a]	0.14	12.39 [b]	0.15
Lipid, dw*	32.89 [a]	0.65	30.29 [b]	0.42
Polar lipid, dw	12.04	0.11	12.16	0.19
Neutral lipid, dw	21.20 [a]	0.19	18.38 [b]	0.16
Polar lipid, of total lipid	36.66 [a]	0.85	40.12 [b]	0.99
Neutral lipid, of total lipid	64.35 [a]	0.97	60.67 [b]	0.45
Polar lipid, of fat free dw	17.99	0.43	18.35	0.40
Protein + ash, ww	27.76	0.54	28.36	0.33

* ww = wet weight; dw = dry weight

ab = a significant difference (α=0.05) was found between means with a different superscript within the same row.

two groups. The lower polar lipid level found in the eggs from the COMM diet group was therefore thought to reflect the significantly higher neutral lipid concentration present in these eggs. Feed label information, presented in Appendix 2, Table A2.1, shows that the COMM diet contained up to 2% more crude fat than the BROOD diet and the same levels of moisture, crude protein, crude fibre and ash. The concentration of nitrogen free extract was not listed. A single proximate analysis of each diet revealed lipid concentrations (wet weight basis) of 16.31% and 15.85% for the COMM and BROOD diets, respectively. The higher lipid concentration in the eggs of fish on the COMM diet therefore appears to be directly related to the higher level of crude lipid in the diet.

3.2.4 Fatty acid composition of the total lipids

The complete fatty acid profiles of the total lipids in the eggs of both diet groups are presented in Appendix 4, Table A4.7. Significantly higher levels of 16:1n9, 18:4n3 and 20:1n11 and lower levels of 16:1n7 and 20:1n9 were present in the eggs of fish fed the COMM diet than in those fed the BROOD diet.

No significant differences in total saturates, n3, n6 or n9 fatty acids in the total lipid fatty acids were found (Table 30) in the two diet groups. The n3:n6 ratios were not statistically analyzed but were close in value at 3.62 and

Table 30. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
Saturates	17.03	0.36	18.02	0.36
n3	30.44	0.99	29.59	0.20
n6	7.85	0.17	8.17	0.15
n9	30.13	0.71	29.06	0.24

A one-way ANOVA conducted on diet indicated no significant differences (α=0.05) for any of the series of fatty acids above.

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
n3:n6 ratio*	3.88	0.07	3.62	0.05

* ANOVA was not conducted on the n3:n6 ratios.

3.88 for the BROOD and COMM eggs respectively. There were no significant differences in the n3 or n6 PUFAs or in the n3 HUFAs in the total lipids of the two groups as shown in Tables 31 and 32.

The eggs from the fish on the COMM diet had significantly greater levels of n11 monounsaturates in the total lipids than those from fish on the BROOD diet (Table 33). The COMM diet contained more 20:1n11 and 22:1n11 as shown in the diet analyses in Appendix 2, Table A2.4. Otherwise there were no significant differences between the two diet groups in n5, n7, n9 or total monounsaturates.

3.2.5 Fatty acid composition of the polar lipids

The complete fatty acid profiles of the polar lipids in the eggs of both groups are presented in Appendix 4, Table A4.8.

As shown in Table 34, there were no significant differences in total saturates, n3, n6 or n9 fatty acids in the polar lipid fraction of the eggs. The n3:n6 ratios were not subjected to ANOVA but it was slightly higher for the COMM eggs than for the BROOD eggs. No significant differences were found in the individual long chain n3 fatty acids (Table 35) or in the long chain n6 fatty acids (Table 36). However, the eggs from fish on the COMM diet contained significantly more n3 PUFA than did those on the BROOD diet (Table 35). The fatty acid analyses of

Table 31. Selected n3 fatty acids, total n3 polyunsaturated fatty acids (n3 PUFAs) and total highly unsaturated fatty acids (HUFAs) (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
20:5n3	8.12	0.45	8.29	0.15
22:5n3	3.32	0.22	3.48	0.08
22:6n3	16.38	0.33	15.58	0.14
n3 PUFAs[1]	2.27	0.08	2.00	0.10
n3 HUFAs[2]	28.16	0.92	27.58	0.25

1 n3 PUFAs = unsaturated n3 fatty acids with 2 to 4 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 5 or more double bonds

A one-way ANOVA conducted on diet indicated no significant differences (α=0.05) for any of the series of fatty acids above.

Table 32. Selected n6 fatty acids, total n6 polyunsaturated fatty acids (n6 PUFAs) (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a commercial diet (COMM) prior to transfer to freshwater for maturation. (n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
18:2n6	4.65	0.19	4.80	0.20
20:2n6	0.40	0.04	0.47	0.02
20:4n6	1.55	0.02	1.64	0.07
n6 PUFAs[1]	7.85	0.17	8.17	0.15

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

A one-way ANOVA conducted on diet indicated no significant differences (α=0.05) for any of the fatty acids or series of fatty acids above.

Table 33. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in TOTAL LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or a commercial diet (COMM) prior to transfer to freshwater for maturation. (n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
n5	0.45	0.01	0.44	0.01
n7	10.09	0.13	10.69	0.18
n9	30.13	0.71	29.06	0.24
n11	1.02[a]	0.07	0.68[b]	0.06
Total monounsaturates	41.69	0.63	40.88	0.26

ab = a significant difference (α=0.05) was found between means with a different superscript within the same row.

Table 34. Saturated, n3, n6 and n9 fatty acid series and n3:n6 ratios (mean and standard error of the means) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or a commercial diet (COMM) prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
Saturates	24.68	0.41	25.85	0.54
n3	39.87	0.52	38.88	0.53
n6	6.20	0.14	6.54	0.17
n9	18.31	0.35	17.93	0.79

A one-way ANOVA conducted on diet indicated no significant differences (α=0.05) for any of the series of fatty acids above.

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
n3:n6 ratio*	6.45	0.21	5.95	0.12

* ANOVA was not conducted on the n3:n6 ratios.

Table 35. Selected n3 fatty acids, total n3 polyunsaturated fatty acids (n3 PUFAs) and total highly unsaturated fatty acids (HUFAs) (mean and standard error of the mean) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a regular commercial diet (COMM) prior to transfer to freshwater for maturation.
(n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
20:5n3	10.77	0.28	11.00	0.33
22:5n3	3.50	0.18	3.64	0.64
22:6n3	24.27	0.40	23.89	0.38
n3 PUFAs[1]	1.33[a]	0.31	0.35[b]	0.04
n3 HUFAs[2]	38.54	0.31	38.53	0.53

1 n3 PUFAs = unsaturated n3 fatty acids with 2 to 4 double bonds

2 n3 HUFAs = unsaturated n3 fatty acids with 5 or more double bonds

ab = a significant difference (α=0.05) was found between means with a different superscript within the same row.

Table 36. Selected n6 fatty acids, total n6 polyunsaturated fatty acids (n6 PUFAs) (mean and standard error of the means) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or on a commercial diet (COMM) prior to transfer to freshwater for maturation. (n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
18:2n6	2.02	0.09	1.90	0.05
20:2n6	0.47	0.06	0.56	0.04
20:4n6	2.57	0.05	2.67	0.09
n6 PUFAs[1]	6.20	0.14	6.54	0.17

1 n6 PUFAs = unsaturated n6 fatty acids with 2 to 4 double bonds

A one-way ANOVA conducted on diet indicated no significant differences (α=0.05) for any of the fatty acids or series of fatty acids above.

the diets (Appendix 2, Table A2.4) did not reveal any large differences in individual n3 PUFAs. No significant differences were found in n3 HUFAs (Table 35) or in n6 PUFAs (Table 36) between eggs of the two groups.

As found in the total lipids, the eggs of the fish on the COMM diet contained significantly more n11 fatty acids in the polar lipids than did those on the BROOD diet (Table 37). There was ca. 1% more 22:1n11 in the COMM diet than in the BROOD diet that probably accounted for this difference in the eggs.

3.2.6 Diets

Feed tag data for the COMM and BROOD diets are provided in Appendix 2, Table A2.1. The level of crude fat in the BROOD diet was reported to be 14% (minimum) versus 16% (minimum) in the COMM diet. The BROOD diet contained 5% low temperature dried krill meal. This was expected to affect the fatty acid composition in the eggs of the fish on this diet. There were differences in the supplemented levels of Vitamin E and C in the two diets also. Otherwise the tag data was identical for the two diets.

Partial proximate analyses of the diets are presented in Table A2.3. Contrary to the feed tag data, the moisture level in the COMM diet was 8.50% and the lipid levels were ca. 16% for both diets. Therefore the major differences between the diets were

Table 37. Monounsaturates, reported as n5, n7, n9, n11 and total monounsaturated fatty acids (mean and standard error of the means) in POLAR LIPIDS of eggs from 4-year old Big Qualicum broodstock on a brood diet (BROOD) or a commercial diet (COMM) prior to transfer to freshwater for maturation. (n=5 fish for each group)

Diet	COMM		BROOD	
	mean %	sem	mean %	sem
n5	0.52	0.03	0.52	0.05
n7	5.73	0.14	6.07	0.13
n9	18.31	0.35	17.93	0.79
n11	0.45[a]	0.07	0.19[b]	0.05
Total monounsaturates	25.18	0.41	24.79	0.80

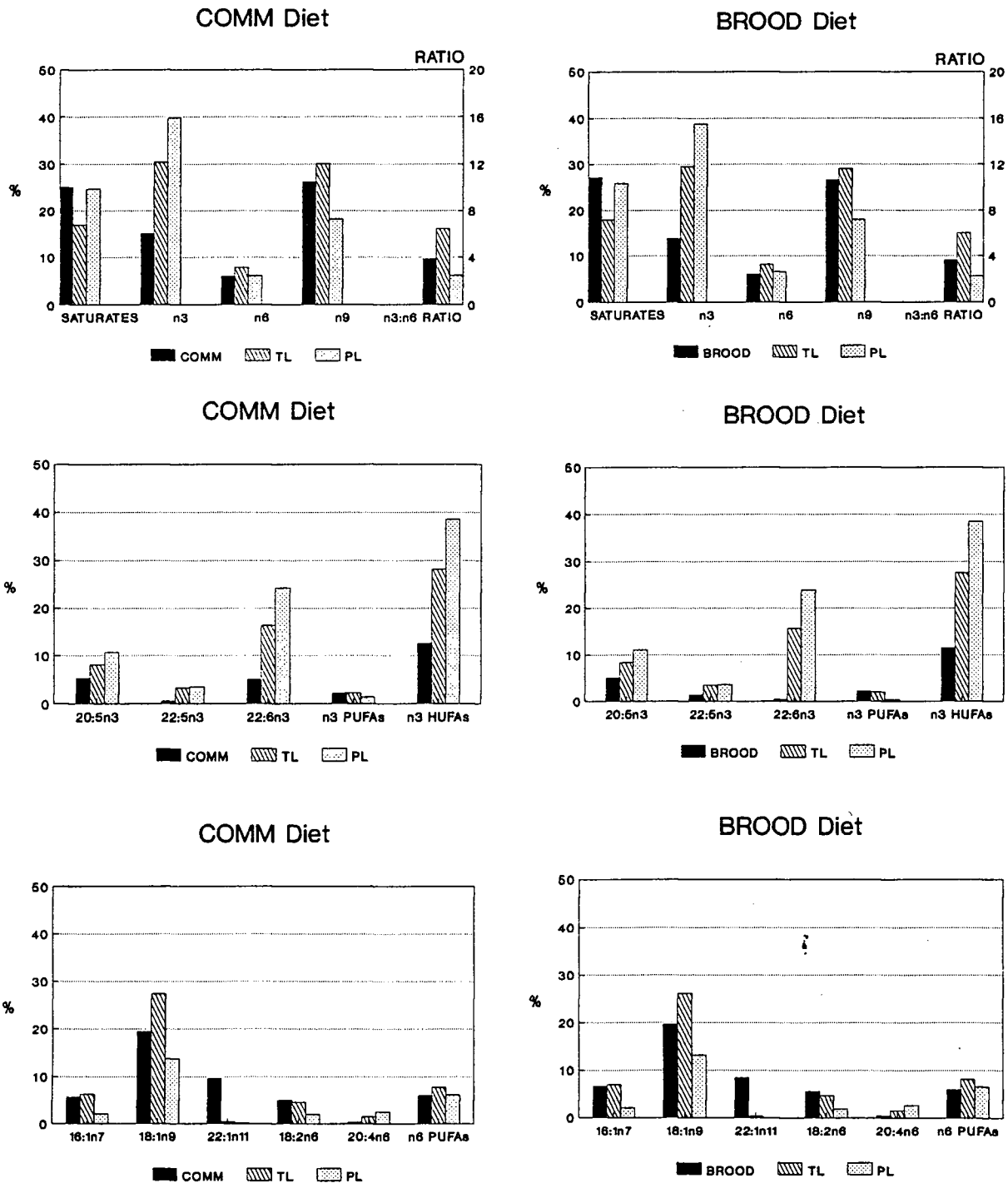
ab = a significant difference (α=0.05) was found between means with a different superscript within the same row.

the Vitamin C and E levels and the presence of krill meal in the BROOD diet.

The fatty acid profiles of the diets are listed in Table A2.4 and, for comparison with the BROOD diet, a euphausiid (krill) sample is presented in Table A2.5. Some of the differences in fatty acid concentration between the two diets seem to reflect the presence of krill in the BROOD diet; others do not. For example the krill and the BROOD diet had higher levels of 14:0 and 16:0 than the COMM diet but, based on the concentration of 20:5n3 and 22:6n3 in the krill sample, higher levels of these fatty acids might have been expected in the BROOD diet than was found in the COMM diet. This raises the possibility that the BROOD diet or the constituent krill meal had oxidized with the loss of some of the long chain fatty acids. When compared with the fatty acid profile of the WV33 diet (fed to the broodstock in Section 1), the COMM and BROOD diets were relatively similar to each other (Table A2.4).

Figure 8 displays selected fatty acids and series of fatty acids in the total and polar lipids of the COMM and the BROOD groups of eggs together with the same parameters for the respective diets. The individual graphs of this figure clearly show the similarity in fatty acid profiles between the diets themselves and between the eggs of the two diet groups in both the total and polar lipids.

Figure 8:
A comparison of selected fatty acids in the total (TL) and polar (PL) lipids of eggs of 4-year old broodstock with those in their diets (COMM and BROOD).



Egg composition was not consistently a reflection of the diet. Saturates and n9 fatty acids were the predominant series in the diets but the n3 fatty acids were found in the greatest concentration in the total lipids of both groups of eggs with the n9 fatty acids (primarily 18:1n9) a close second. The polar lipids of both groups of eggs contained n3 fatty acids in the highest concentration. The n3 HUFAs were the primary contributors to the high n3 levels in both the total and polar fatty acids.

The diets were not the sole source of fatty acids. Retention of essential fatty acids, particularly the n3 HUFAs, and elongation and desaturation of fatty acids also contributed to the final egg profiles. The source of the high 18:1n9 concentration in the egg total lipids appeared to be partially due to elongation and desaturation of saturated fatty acids as well as to the presence of high levels in the diets.

3.3 CONCLUSIONS

The BROOD and COMM diets were not sufficiently different in fatty acid composition to produce major differences in egg composition. Had the diets been fed over the entire period of vitellogenesis, greater differences may have resulted.

The higher lipid level in the COMM diet resulted in a greater proportion of neutral to polar lipids in the eggs of the broodstock fed this diet. No advantage or disadvantage could be directly attributed to higher lipid levels during the early incubation period. The egg mortality which has caused concern in the past typically occurred between fertilization and eyeing. There were no significant differences in eyeing rate between the two groups in this study. Beyond eyeing and up to first feeding, an extra store of lipid may be useful if lipid concentration became limiting to growth and maintenance.

The BROOD diet theoretically contained more (200 I.U./kg) vitamin E and more (500 mg/kg) vitamin C than the COMM diet. No effect of these differences was evident in this study.

No significant advantage in fecundity, fertilization rate or incubation success was conferred on the eggs of the fish fed the BROOD diet. Unless feeding this a diet over a longer period can be shown to result in some significant benefit, the additional cost of this diet is not warranted.

CONCLUDING REMARKS

Since 1986, the survival of eggs from cultured Chinook broodstock has improved markedly. Since 1988 an annual surplus of smolts has been produced by the industry and at present a high incidence of pre-eyeing egg mortality appears to be in the past.

The year 1987 was pivotal for hatchery production of smolts. The eyed survival rates observed in these studies reflected those obtained throughout the industry. Eyed survivals from broodstock fed the COMM diet reported here ranged from ca. 74% in the progeny of the three year old broodstock to 92% in the progeny of four year old broodstock - significantly better than in 1986 and prior years. Changes in diet formulation made by manufacturers appear to be the main reason for this success at Sea Spring Salmon Farm Ltd. Improved broodstock management and spawning and incubation techniques may have contributed to the success at some facilities.

Commercial diet formulations are closed. It is therefore impossible to determine which modifications promoted better survival. Groves (1987, unpub. report) suggested that combinations of selenium, vitamin E and/or vitamin C were low in the seven commercial diets that were in use and analyzed during 1986. These nutrients have been identified by numerous workers (eg. Watanabe, 1985; Halver, 1985; King, 1985; Bell and

Cowey, 1985; Soliman *et al.*, 1986) as being important for the production of high quality eggs. Diets manufactured since 1986 contain higher levels of vitamins E and C according to feed labels. Selenium is not routinely listed on feed labels. The differences in fatty acid composition of eggs between cultured and wild broodstock reported herein appear to be secondary in importance for survival in the light of the requirements for these nutrients.

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APPENDIX 1

Preliminary analyses of selected nutrient parameters in the eggs of wild and cultured Chinook salmon - 1985 & 1986. (Mean or range of values given)[1]

1985:		Cultured Eggs	Wild Eggs
% Moisture		66.1	57.7
% Lipid, wet weight basis		13.5-19.0	8.7-13.2
Fatty acids	%18:1	31.4	21.9
	%18:2	5.0	0.9
	%20:5	20.5	32.0
Selenium, ug/g dry weight		1.03	1.46
Vitamin A, ug/g dry weight		4.1	7.7
1986:			
% Moisture		62.0	57.7
% Lipid (eggs), wet weight basis		13.5-15.5	6.0
% Lipid (muscle), wet weight basis		2.4-8.0	1.0
Fatty acids	%18:1	26.4	23.7
	%18:2	3.8	1.0
	%20:5	10.1	17.4
Selenium, ug/g dry weight		2.16	3.24
Vitamin A, ug/g dry weight		3.2	3.9
Vitamin E, IU/kg dry weight		106.7	73.4

1 from Groves, 1987.

APPENDIX 2 Diet Data

Table A2.1:
Manufacturer's feed label data for commercial (COMM) and brood (BROOD) diets.

	Commercial (COMM)	Brood (BROOD)[1]
Moisture	10.0%	10.0%
Min. Crude Protein	47.0%	47.0%
Min. Crude Fat	16.0%	14.0%
Max. Crude Fibre	3.0%	3.0%
Maximum Ash	10.0%	10.0%
Actual Phosphorus	1.5%	1.5%
Actual Calcium	2.2%	2.2%
Actual Sodium	0.7%	0.7%
Min. I.U. Vitamin "A" per kg	10,000	10,000
Min. I.U. Vitamin "D" per kg	2,400	2,400
Min. I.U. Vitamin "E" per kg	300	500
Min. I.U. Vitamin "C" per kg	700	1,200

[1] Included in BROOD diet but not stated on the feed label was 5% low temperature dried krill meal.

APPENDIX 2 Diet Data

Table A2.2: Formulation of the West Vancouver 33 (WV33) diet.

Ingredient:	g/kg
steam dried herring meal	550
dried whey	80
corn dist. dried solubles	45
blood flour	50
whole frozen euphausiids	20
freeze-dried euphausiids	10
wheat middlings	81
vitamin supplement[1]	43
mineral supplement[2]	20.4
herring or salmon oil (with antioxidant)	75
permapel (lignin sulphonate binder)	18.9
ascorbic acid	1.9
choline chloride (60%)	4.7
canthaxanthin (10% potency)	0.4
	1000.3

1 The vitamin supplement supplied the following levels of nutrients/ kg diet: vitamin A acetate 15000 I.U.; cholecalciferol 3000 I.U.; DL-alpha-tocopherol acetate 567 I.U.; menadione (as Hetrazeen) 24.8 mg; D-calcium pantothenate 182.9 mg; pyridoxine HCl 42.2 mg; riboflavin 56.7 mg; niacin 284 mg; folic acid 18.9 mg; thiamine mononitrate 38.4 mg; biotin 2.84 mg; cyanocobalamin 0.057 mg; inositol 378 mg

2 The mineral supplement supplied the following levels of minerals/ kg diet: Mn (as $MnSO_4 * H_2O$) 69.2 mg; Zn (as $ZnSO_4 * 7H_2O$) 45.0 mg; Co (as $CoCl_2 * 6H_2O$) 0.94 mg; Cu (as $CuSO_4 * 5H_2O$) 4.50 mg; Fe (as $FeSO_4 * 7H_2O$) 47.3 mg; I (as KIO_3) 5.1 mg

APPENDIX 2 Diet Data

Table A2.3:
Dry matter and lipid composition of the experimental diets used in Sections 1, 2 and 3.

	COMM	WV33	BROOD
Dry matter %	91.50	90.33	89.91
Moisture %	8.50	9.67	10.09
Lipid, % wet weight	16.31	14.38	15.85
Lipid, % dry weight	17.83	15.92	17.63

COMM = commercial diet (Sections 1 & 2)
WV33 = West Vancouver 33 diet (Section 1)
BROOD = brood diet (Section 3)

APPENDIX 2 Diet Data

Table A2.4:
Fatty acid profiles of diets.
Diet codes: COMM = commercial, WV33 = West Vancouver 33, BROOD = commercial brood

Fatty Acid	COMM %	WV33 %	BROOD %
14:0	5.37	5.59	6.15
0 **	0.87	1.75	0.55
14:1n9	0.34	0.51	0.39
1a **	0.26	0.00	0.28
16:0	16.75	17.00	18.36
16:1n9	0.00	0.24	0.21
16:1n7	5.75	5.07	6.70
16:1n5	0.00	0.24	0.21
4 **	1.36	1.27	1.46
4a **	0.22	0.34	0.23
5 **	0.41	0.65	0.70
6 **	0.50	0.33	0.59
18:0	2.76	3.14	2.76
18:1n9	19.40	17.08	19.60
18:1n7	3.09	2.77	3.25
18:1n5	0.35	0.54	0.34
18:2n6	5.03	3.42	5.66
18:3n6	0.00	0.33	0.00
18:3n3	0.99	0.79	0.95
18:4n3	1.03	1.52	1.08
20:0?	0.26	0.00	0.00
20:1n11	2.70	4.28	2.39
20:1n9	5.78	2.76	5.79
20:1n7	0.28	0.36	0.28
20:4n6	0.44	0.48	0.40
20:4n3	0.29	0.83	0.22
20:5n3	5.32	6.96	5.11
22:1n11	9.58	5.11	8.47
22:1n9	0.65	0.79	0.66
21:5n3	1.56	4.59	1.46
22:4n6?	0.57	1.04	0.00
22:5n3	0.62	1.28	0.56
22:6n3	5.15	8.07	4.30
total	97.68	99.13	99.11
total n3	14.96	24.04	13.68
total n6	5.47	4.23	6.06
n3:n6 ratio	2.73	5.68	2.26

** = unknown/ unidentified fatty acids

APPENDIX 2 Diet Data

Table A2.5:
Dry matter, lipid composition and fatty acid profile of a euphausiid (krill) sample.

Fatty Acid	Krill %
14:0	7.11
0 **	0.43
14:1n9	1.71
1a **	0.89
16:0	21.25
16:1n9	8.05
16:1n7	0.60
2a **	0.46
3 **	0.28
4 **	1.47
4a **	2.94
5 **	1.55
? **	0.46
6 **	1.16
18:0	1.24
18:1n9	7.66
18:1n7	5.09
18:2n6	1.44
18:3n6	0.24
18:3n3	2.04
18:4n3	4.89
20:0?	1.47
20:1n7	0.25
? **	1.07
20:4n6	0.71
20:4n3	0.31
20:5n3	10.45
21:5n3	0.44
22:4n6	4.49
22:5n6	1.59
22:5n3	0.26
22:6n3	6.00
total	98.00
total n3	24.39
total n6	8.47
n3:n6 ratio	2.88

Dry matter %	21.54
Moisture %	78.47
Lipid, % wet weight	5.06
Lipid, % dry weight	23.47

** = unknown/ unidentified fatty acids

APPENDIX 3 Formulae for Calculating Composition Parameters.

Percent Lipid (wet weight basis):

[mean dry weight of lipid in 10 ml aliquot of lipid extract * (volume of lipid extract/ 10 ml)] * 100 % = total lipid in sample

(total lipid in sample/ wet weight of sample) * 100 % = Percent Lipid (wet weight basis)

Percent Lipid (dry weight basis):

(total lipid in sample/ dry weight of sample) * 100 % = Percent Lipid (dry weight basis)

Percent Polar Lipid of Total Lipid:

(total amount lipid in sample/ volume of chloroform layer) * volume of aliquot applied to silica cartridges = amount lipid in the aliquot applied to silica cartridges.

(polar lipid in aliquot applied to silica cartridges/ amount lipid in aliquot) * 100% = Percent Polar Lipid of Total Lipid

Percent Neutral Lipid of Total Lipid:

(neutral lipid in aliquot applied to silica cartridges/ amount lipid in aliquot) * 100% = Percent Neutral Lipid of Total Lipid

Percent Polar Lipid (dry weight basis):

percent polar lipid of total lipid * percent lipid (dry weight) = Percent Polar Lipid (dry weight basis)

Percent Neutral Lipid (dry weight basis):

percent neutral lipid of total lipid * percent lipid (dry weight) = percent neutral lipid (dry weight basis)

Percent Polar Lipid of Fat Free Dry Weight:

(polar lipid/ volume aliquot applied to silica cartridges) * volume of chloroform layer = total polar lipid in whole sample

total polar lipid/ (sample dry weight - total amount lipid in sample) * 100% = Percent Polar Lipid of Fat Free Dry Weight

Percent Protein + Percent Ash (wet weight basis):

100 % - [% moisture + % lipid] = Percent Protein + Percent Ash (wet weight basis)

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.1:
Fatty acid profiles (mean and standard error of the means) of TOTAL LIPID in the eggs of BIG QUALICUM broodstock on three diets. Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet. (n = 5 for all groups)

Fatty Acid	WV33 mean %	WV33 sem	COMM mean %	COMM sem	WILD mean %	WILD sem
14:0	2.08	0.04	2.39	0.12	2.43	0.23
14:1n9	1.73	0.59	1.11	0.19	1.71	0.46
1 **	0.25	0.01	0.25	0.01	0.26	0.03
16:0	11.71	0.22	11.90	0.16	11.85	0.41
16:1n9	0.60	0.06	0.57	0.08	0.42	0.10
16:1n7	5.72 a	0.13	6.42 b	0.07	7.76 c■	0.17
4 **	0.21 a■	0.00	0.10 b	0.06	0.56 c■	0.02
5 **	0.41	0.03	0.42	0.02	0.42	0.03
18:0	4.21 a	0.04	4.36 a■	0.09	5.12 b	0.04
18:1n9	23.73 a	0.26	26.55 b	0.25	18.71 c	0.53
18:1n7	4.30 a	0.05	3.87 b	0.08	3.89 b■	0.15
18:1n5	0.45	0.01	0.43	0.01	0.42	0.03
7 **	0.29 a	0.06	0.36 a	0.06	0.00 b	0.00
18:2n6	3.43 a■	0.07	4.79 b	0.14	0.79 c	0.05
18:2n4	0.00 a	0.00	0.00 a	0.00	0.32 b■	0.02
18:3n6	0.25 a	0.07	0.44 b	0.05	0.00 c	0.00
18:3n4	0.11 a	0.05	0.17 a	0.04	0.47 b	0.02
18:3n3	0.54	0.02	0.63	0.03	0.72	0.05
18:4n3	0.68	0.03	0.71	0.04	0.85	0.05
18:4n1	0.23 a	0.00	0.21 a	0.03	0.50 b	0.05
20:1n11	0.72	0.03	0.73	0.03	0.40	0.19
20:1n9	1.94 a	0.06	2.10 a	0.11	0.86 b	0.05
f **	0.32 a	0.03	0.34 a	0.04	0.00 b	0.00
20:2n6	0.36 a	0.01	0.39 a	0.04	0.00 b	0.00
20:3n6	0.70 a	0.03	0.91 b	0.05	0.00 c	0.00
20:4n6	1.60 a	0.04	1.68 a	0.07	1.15 b■	0.02
20:4n3	0.69 a	0.04	0.56 a	0.04	1.34 b■	0.04
20:5n3	8.88 a	0.16	7.45 b	0.14	13.70 c■	0.36
22:1n11	0.39 a	0.02	0.45 a	0.05	0.13 b	0.08
21:5n3	0.31	0.04	0.24	0.00	0.75	0.36
Y **	0.11	0.05	0.11	0.08	0.18	0.07
22:4n6	0.26 ■	0.02	0.23 ■	0.03	0.40	0.12
22:5n3	3.47 a	0.08	2.74 b■	0.15	5.49 c■	0.23
22:6n3	17.73 a	0.39	14.87 b	0.45	17.09 a	0.74
total	98.38		98.49		98.66	

** = unknown/ unidentified fatty acids
abc = a significant difference (α = 0.05) was found between means with different superscripts within the same row.
■ = a significant difference (α=0.05) was found between stocks for the fatty acid indicated. See Table A4.2.

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.2:
Fatty acid profiles (mean and standard error of the means) of TOTAL LIPID in the eggs of ROBERTSON CREEK broodstock on three diets. Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet. (n = 5 for WV33 and WILD; n = 3 for COMM)

	WV33		COMM		WILD	
Fatty Acid	mean %	sem	mean %	sem	mean %	sem
14:0	2.20	0.06	2.29	0.07	2.54	0.26
14:1n9	2.01	0.53	1.04	0.13	0.91	0.17
1 **	0.25	0.01	0.24	0.00	0.20	0.05
16:0	12.15	0.22	11.15	0.10	12.17	0.32
16:1n9	0.36	0.15	0.54	0.04	0.54	0.08
16:1n7	5.99 a■	0.13	6.47 b	0.10	7.01 c■	0.14
4 **	0.06	0.04	0.04	0.03	0.16 ■	0.05
5 **	0.40	0.02	0.33	0.02	0.35	0.01
18:0	4.34 a	0.12	4.77 b■	0.14	5.24 c	0.07
18:1n9	24.96 a	0.64	26.16 a	0.20	19.56 b	0.66
18:1n7	4.06 a	0.04	4.15 a	0.06	2.95 b■	0.09
18:1n5	0.44	0.01	0.42	0.01	0.47	0.05
7 **	0.32 a	0.02	0.31 a	0.01	0.00 b	0.00
18:2n6	3.85 a■	0.06	4.59 b	0.14	0.84 c	0.07
18:2n4	0.00 a	0.00	0.00 a	0.00	0.27 b■	0.03
18:3n6	0.30 a	0.03	0.29 a	0.02	0.00 b	0.00
18:3n4	0.17 ab	0.02	0.11 a	0.05	0.29 b	0.04
18:3n3	0.60	0.01	0.63	0.03	0.71	0.09
18:4n3	0.63 a	0.06	0.56 a	0.02	1.08 b	0.13
18:4n1	0.17 a	0.05	0.25 a	0.01	0.45 b	0.06
20:1n11	0.66	0.04	0.54	0.04	0.41	0.19
20:1n9	1.70 a	0.09	2.13 b	0.11	0.87 c	0.06
f **	0.31 a	0.05	0.37 a	0.04	0.00 b	0.00
20:2n6	0.32 a	0.01	0.46 b	0.03	0.00 c	0.00
20:3n6	0.78 a	0.05	0.98 a	0.03	0.03 b	0.03
20:4n6	1.62 a	0.03	1.73 a	0.03	1.02 b■	0.03
20:4n3	0.66 a	0.04	0.62 a	0.04	1.71 b■	0.21
20:5n3	8.36 a	0.24	7.88 a	0.07	15.06 b■	0.46
22:1n11	0.37 a	0.01	0.27 ab	0.10	0.11 b	0.08
21:5n3	0.33	0.03	0.21	0.03	0.37	0.05
Y **	0.27	0.06	0.20	0.15	0.25	0.11
22:4n6	0.10 a■	0.05	0.07 a■	0.05	0.30 b	0.02
22:5n3	3.10 a	0.07	3.28 a■	0.19	4.81 b■	0.20
22:6n3	16.70 ab	0.25	15.63 a	0.09	17.81 b	0.51
total	98.51		98.67		98.47	

** = unknown/ unidentified fatty acids
abc = a significant difference ($\alpha = 0.05$) was found between means with different superscripts within the same row.
■ = a significant difference ($\alpha = 0.05$) was found between stocks for the fatty acid indicated. See Table A4.1.

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.3:
Fatty acid profiles (mean and standard error of the means) of POLAR LIPID in the eggs of BIG QUALICUM broodstock on three diets. Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet. (n = 5 fish for all groups)

Fatty Acid	WV33 mean %	WV33 sem	COMM mean %	COMM sem	WILD mean %	WILD sem
14:0	1.09 ^{a}	0.04	0.92 ^{a}	0.03	1.32 ^{b}	0.10
14:1n9	5.64	0.79	3.40	0.42	2.44	0.69
1 **	0.21	0.01	0.13	0.05	0.23	0.06
16:0	15.22	0.37	15.55	0.27	16.41	0.38
16:1n9	0.45	0.06	0.35 ■	0.09	0.39	0.06
16:1n7	2.05 ^{a}	0.15	2.08 ^{a}	0.10	2.87 ^{b}■	0.19
4 **	0.44 ^{ab}	0.19	1.05 ^{b}■	0.39	0.22 ^{a}	0.17
4a **	0.28	0.03	0.36	0.02	0.24	0.06
18:0	7.46 ^{a}	0.25	8.48 ^{b}■	0.29	10.07 ^{c}	0.31
18:1n9	12.27 ^{a}	0.39	13.73 ^{b}	0.23	9.93 ^{c}	0.38
18:1n7	4.04	0.13	3.76	0.08	3.72 ■	0.19
18:1n5	0.51	0.02	0.48	0.03	0.49	0.05
18:2n6	1.39 ^{a}	0.07	2.03 ^{b}	0.04	0.28 ^{c}	0.04
18:3n3	0.05	0.05	0.18	0.05	0.25	0.07
18:4n3	0.09	0.04	0.14	0.06	0.19	0.10
18:4n1	0.00 ^{a}	0.00	0.00 ^{a}	0.00	0.20 ^{b}■	0.05
20:1n11	0.31 ^{a}	0.02	0.35 ^{a}■	0.02	0.12 ^{b}	0.09
20:1n9	2.31 ^{a}	0.10	2.46 ^{a}	0.14	0.96 ^{b}	0.15
20:1n7	0.20 ^{a}■	0.05	0.14 ^{a}	0.06	0.00 ^{b}	0.00
20:2n6	0.42 ^{a}	0.02	0.45 ^{a}■	0.03	0.00 ^{b}	0.00
20:3n6	0.77 ^{a}■	0.03	1.12 ^{b}	0.03	0.00 ^{c}	0.00
20:4n6	2.43 ^{a}■	0.07	2.88 ^{b}	0.12	1.68 ^{c}	0.04
20:4n3	0.29 ^{ab}	0.04	0.25 ^{a}	0.06	0.66 ^{b}	0.09
20:5n3	10.69 ^{a}	0.25	10.59 ^{a}	0.16	14.97 ^{b}	0.45
22:1n11	0.40	0.37	0.02	0.03	0.25	0.25
21:5n3	0.35 ^{a}■	0.14	0.00 ^{b}	0.00	0.14 ^{a}	0.07
Y **	0.11	0.08	0.25	0.12	0.29	0.13
22:4n6	0.47	0.01	0.56	0.02	0.45	0.09
22:5n3	3.48 ^{a}	0.04	3.19 ^{a}	0.19	5.22 ^{b}■	0.37
22:6n3	24.69	0.33	23.59	0.73	24.14	0.96
total	98.13		98.59		98.11	

** = unknown/ unidentified fatty acids
abc = a significant difference (α = 0.05) was found between means with different superscripts within the same row.
■ = a significant difference (α = 0.05) was found between stocks for the fatty acid indicated. See Table A4.4.

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.4:
Fatty acid profiles (mean and standard error of the means) of POLAR LIPID in the eggs of ROBERTSON CREEK broodstock on three diets. Diet codes: COMM = commercial; WV33 = West Vancouver 33; WILD = natural diet. (n = 5 for WV33 and WILD; n = 3 for COMM)

	WV33		COMM		WILD	
Fatty Acid	mean %	sem	mean %	sem	mean %	sem
14:0	0.99 [a]	0.06	1.09 [a]	0.06	1.33 [b]	0.09
14:1n9	3.83	1.66	2.30	0.35	2.23	0.50
1 **	0.18	0.06	0.24	0.01	0.24	0.01
16:0	15.73	0.25	15.37	0.49	16.28	0.64
16:1n9	0.32 [a]	0.12	0.00 [b■]	0.00	0.21 [a]	0.07
16:1n7	1.94 [a]	0.08	2.18 [ab]	0.12	2.43 [b■]	0.12
4 **	0.21	0.13	0.22 ■	0.22	0.05	0.03
4a **	0.17	0.07	0.08	0.08	0.26	0.04
18:0	8.08 [a]	0.27	9.39 [b■]	0.27	9.99 [b]	0.13
18:1n9	12.26 [a]	0.22	13.48 [b]	0.48	10.25 [c]	0.32
18:1n7	3.76 [a]	0.07	3.73 [a]	0.28	2.77 [b■]	0.12
18:1n5	0.47	0.01	0.50	0.04	0.59	0.07
18:2n6	1.57 [a]	0.05	1.83 [b]	0.02	0.34 [c]	0.02
18:3n3	0.02	0.02	0.07	0.07	0.17	0.06
18:4n3	0.09	0.05	0.00	0.00	0.31	0.05
18:4n1	0.00 [a]	0.00	0.00 [a]	0.00	0.12 [b■]	0.06
20:1n11	0.23 [a]	0.06	0.00 [b■]	0.00	0.17 [ab]	0.11
20:1n9	1.96 [a]	0.12	2.83 [b]	0.21	1.06 [c]	0.12
20:1n7	0.04 ■	0.04	0.08	0.08	0.00	0.00
20:2n6	0.40 [a]	0.03	0.59 [b■]	0.04	0.00 [c]	0.00
20:3n6	0.90 [a■]	0.05	1.10 [b]	0.07	0.00 [c]	0.00
20:4n6	2.68 [a■]	0.05	2.84 [a]	0.02	1.55 [b]	0.04
20:4n3	0.30 [a]	0.08	0.27 [a]	0.14	0.92 [b]	0.11
20:5n3	11.05 [a]	0.48	10.39 [a]	0.03	15.59 [b]	0.76
22:1n11	0.00	0.00	0.00	0.00	0.00	0.00
21:5n3	0.85 [a■]	0.20	0.00 [b]	0.00	0.23 [c]	0.03
Y **	0.00 [a]	0.00	0.33 [b]	0.07	0.38 [b]	0.09
22:4n6	0.55	0.09	0.41	0.09	0.64	0.05
22:5n3	3.35 [a]	0.13	3.33 [a]	0.36	4.41 [b■]	0.38
22:6n3	25.56	0.25	23.93	0.41	25.16	0.42
total	97.49		96.77		97.67	

** = unknown/ unidentified fatty acids
abc = a significant difference ($\alpha = 0.05$) was found between means with different superscripts within the same row.
■ = a significant difference ($\alpha = 0.05$) was found between stocks for the fatty acid indicated. See Table A4.3.

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.5:
Fatty acid profiles (mean and standard error of the means) of TOTAL LIPIDS in the eggs of 4-year old Big Qualicum broodstock. Feed was withdrawn from these fish for 7 days or 14 days prior to transfer to freshwater for maturation.
(n=5 for those starved 7 days; n=7 for those starved 14 days)

Period of Starvation, days	7		14	
Fatty Acid	mean %	sem	mean %	sem
14:0	1.70 [a]	0.04	1.95 [b]	0.06
14:1n9	0.37	0.05	0.53	0.08
1 **	0.19	0.05	0.26	0.00
16:0	10.65	0.13	11.27	0.31
16:1n9	0.77	0.05	0.71	0.03
16:1n7	6.00	0.20	6.10	0.04
5 **	0.38	0.10	0.44	0.02
18:0	4.48	0.10	4.33	0.04
18:1n9	26.67	0.55	26.08	0.18
18:1n7	3.66	0.11	3.72	0.10
18:1n5	0.46	0.02	0.44	0.01
7 **	0.34	0.03	0.35	0.02
18:2n6	4.56	0.16	4.51	0.18
18:3n6	0.36	0.03	0.40	0.03
18:3n3	0.68	0.02	0.66	0.01
18:4n3	0.77	0.04	0.78	0.03
18:4n1	0.20	0.02	0.23	0.01
20:1n11	0.53	0.03	0.61	0.04
20:1n9	1.38	0.07	1.60	0.08
20:2n6	0.42	0.02	0.40	0.02
20:3n6	0.84	0.06	0.78	0.05
20:4n6	1.62	0.06	1.64	0.07
20:4n3	0.78	0.05	0.73	0.05
20:5n3	8.76	0.24	8.41	0.26
22:1n11	0.24	0.06	0.34	0.03
21:5n3	0.38	0.16	0.30	0.05
22:4n6	0.23	0.11	0.19	0.04
22:5n3	3.89	0.13	3.73	0.14
22:6n3	16.64	0.43	16.88	0.48
Total	98.04		98.37	

** = unknown/ unidentified fatty acids
ab = a significant difference ($\alpha = 0.05$) was found between means with different superscripts within the same row.

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.6:
Fatty acid profiles (mean and standard error of the means) of POLAR LIPIDS in the eggs of 4-year old Big Qualicum broodstock. Feed was withdrawn from these fish for 7 or 14 days prior to transfer to freshwater for maturation.
(n=5 for those starved 7 days; n=7 for those starved 14 days)

Period of Starvation, days	7		14	
Fatty Acid	mean %	sem	mean %	sem
14:0	0.88 [a]	0.01	1.06 [b]	0.03
14:1n9	3.08	0.64	4.51	0.92
1 **	0.29	0.01	0.34	0.04
16:0	14.79 [a]	0.15	15.72 [b]	0.28
16:1n9	0.50	0.04	0.35	0.14
16:1n7	1.80	0.08	1.93	0.10
4 **	0.31	0.11	0.41	0.14
4a **	0.24	0.06	0.29	0.03
18:0	9.02	0.18	8.48	0.25
18:1n9	13.38	0.14	13.27	0.05
18:1n7	3.67	0.08	3.69	0.14
18:1n5	0.58	0.02	0.52	0.03
18:2n6	1.74	0.08	1.68	0.10
20:1n9	1.68	0.09	2.09	0.27
20:2n6	0.43	0.03	0.50	0.03
20:3n6	0.85	0.06	0.82	0.04
20:4n6	2.65	0.12	2.71	0.14
20:4n3	0.39	0.03	0.39	0.01
20:5n3	11.55	0.26	11.08	0.29
21:5n3	0.34 [a]	0.05	0.08 [b]	0.04
22:4n6	0.38	0.06	0.25	0.12
22:5n3	3.94	0.14	3.72	0.15
22:6n3	25.10	0.53	23.92	0.59
Total	97.57		97.80	

** = unknown/ unidentified fatty acids
ab = a significant difference ($\alpha = 0.05$) was found between means with different superscripts within the same row.

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.7:
Fatty acid profiles (mean and standard error of the means) of TOTAL LIPIDS in the eggs of 4-year old broodstock on a commercial grower diet (COMM) or on a brood (BROOD) diet prior to transfer to freshwater for spawning.
(n=5 fish for both groups)

Diet	COMM		BROOD	
Fatty Acid	mean %	sem	mean %	sem
14:0	1.95	0.10	2.12	0.08
14:1n9	0.38	0.07	0.64	0.12
1 **	0.25	0.01	0.25	0.01
16:0	11.11	0.22	11.72	0.32
16:1n9	0.75 [a]	0.08	0.39 [b]	0.12
16:1n7	6.35 [a]	0.06	7.02 [b]	0.18
5 **	0.50	0.03	0.49	0.03
18:0	3.96	0.07	4.17	0.11
18:1n9	27.42	0.74	26.17	0.21
18:1n7	3.73	0.08	3.66	0.18
18:1n5	0.45	0.01	0.44	0.01
7 **	0.38	0.10	0.23	0.04
18:2n6	4.65	0.19	4.80	0.20
18:3n6	0.43	0.09	0.29	0.02
18:3n4	0.16	0.05	0.13	0.05
18:3n3	0.70	0.05	0.70	0.04
18:4n3	0.84 [a]	0.08	0.60 [b]	0.05
18:4n1	0.20	0.05	0.20	0.05
20:1n11	0.68 [a]	0.05	0.40 [b]	0.07
20:1n9	1.58 [a]	0.09	1.85 [b]	0.06
20:2n6	0.40	0.04	0.47	0.02
20:3n6	0.69	0.02	0.78	0.04
20:4n6	1.54	0.02	1.64	0.07
20:4n3	0.73	0.06	0.70	0.03
20:5n3	8.11	0.45	8.29	0.15
22:1n11	0.34	0.03	0.28	0.03
21:5n3	0.35	0.04	0.24	0.03
22:4n6	0.13	0.06	0.19	0.04
22:5n3	3.32	0.22	3.47	0.08
22:6n3	16.38	0.33	15.58	0.14
Total	98.43		97.90	

** = unknown/ unidentified fatty acids
ab = a significant difference ($\alpha = 0.05$) was found between means with different superscripts within the same row.

APPENDIX 4 Fatty Acid Profiles of Eggs

Table A4.8:
Fatty acid profiles (mean and standard error of the means) of POLAR LIPIDS in the eggs of 4-year old broodstock on a commercial grower diet (COMM) or on a brood (BROOD) diet prior to transfer to freshwater for spawning.
(n=5 fish for both groups)

Diet	COMM		BROOD	
Fatty Acid	mean %	sem	mean %	sem
14:0	1.02	0.04	1.01	0.05
14:1n9	2.14	0.46	2.39	0.79
1 **	0.26	0.04	0.27	0.01
16:0	15.62	0.28	16.33	0.54
16:1n9	0.54 [a]	0.04	0.10 [b]	0.10
16:1n7	2.06	0.08	2.19	0.10
4 **	1.74 [a]	0.62	0.16 [b]	0.10
4a **	0.30	0.01	0.25	0.07
18:0	8.03	0.22	8.51	0.21
18:1n9	13.67	0.38	13.15	0.08
18:1n7	3.67	0.12	3.89	0.09
18:1n5	0.52	0.03	0.52	0.05
18:2n6	2.02	0.09	1.90	0.05
20:1n11	0.28	0.04	0.14	0.06
20:1n9	1.95	0.15	2.29	0.03
20:1n7	0.16	0.05	0.08	0.05
20:2n6	0.47	0.06	0.56	0.04
20:3n6	0.77	0.06	0.88	0.04
20:4n6	2.56	0.05	2.67	0.09
20:3n3	0.91 [a]	0.29	0.00 [b]	0.00
20:4n3	0.42	0.04	0.35	0.04
20:5n3	10.77	0.28	11.00	0.33
22:1n11	0.16	0.08	0.05	0.05
Y **	0.10 [a]	0.05	0.35 [b]	0.07
22:4n6	0.36 [a]	0.05	0.54 [b]	0.04
22:5n3	3.49	0.18	3.64	0.06
22:6n3	24.27	0.40	23.89	0.38
Total	98.27		97.10	

** = unknown/ unidentified fatty acids
ab = a significant difference (α = 0.05) was found between means with different superscripts within the same row.