LIPID UTILIZATION AND FEEDING OF JUVENILE YELLOWTAIL FLOUNDER (Pleuronectes ferrugineus)

KAREN S. WHALEN

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

LIPID UTILIZATION AND FEEDING OF JUVENILE YELLOWTAIL FLOUNDER (*Pleuronectes ferrugineus*)

by

Karen S. Whalen

A thesis submitted to the School of Graduate Studies in partial fulfilment of the requirements for the degree of Master of Science (Aquaculture)

Ocean Sciences Centre
Memorial University of Newfoundland

St.John's May 1999 Newfoundland

ABSTRACT

Interest in the culture of small flounder has increased in recent years due to their fast growth, low metabolic activity and good quality white flesh. Egg production and larval rearing of yellowtail flounder (*Pleuronectes ferrugineus*) has improved and large numbers of juveniles are capable of being produced. However, protocols for grow-out of juveniles have not been developed and little is known of the nutritional requirements and feeding of this species. This study was undertaken as a preliminary investigation into feeding strategies for yellowtail flounder, in order to increase growth during the juvenile stage and to provide recommendations for the development of a species-specific diet.

It is known that growth and food conversion are influenced by feeding frequency (number of meals per day) and experiments were set up to determine the best feeding regimes for optimal growth and food conversion of 0+ fish (under one year of age). Yellowtail flounder responded well to differing feeding regimes, and displayed good growth rates and low food conversion ratios (FCRs) when fed one, two, or four meals a day, and twice every other day. However, feeding frequency was shown to affect growth rates and food consumption. Behavioural observations showed that juveniles fed fewer meals per day ingested more pellets per feeding but were not as accurate at hitting the pellets as fish fed more often. Juveniles fed twice daily had the highest growth rates and lowest FCR, and it is recommended that fish at this stage of grow-out be fed twice per day.

Body composition, condition factors and hepatosomatic indices (HSI) of wild and cultured yellowtail flounder were examined to make estimates for the possible formulation

of diets for yellowtail, as well as to determine if present diets are adequate. Levels of storage fat were higher in the muscle and liver of cultured flounder and HSI was significantly higher ($p < 0.05$) in these fish, demonstrating an accumulation of fat. This, combined with higher condition indices in these fish, may suggest obesity, or a surplus in body fat due to caloric intake exceeding the amount of energy required, relative to wild counterparts. Total proportions of *n*-3 polyunsaturated fatty acids (PUFA) were higher in wild fish. Body composition of wild and cultured fish closely resembled the respective diets, and it is recommended that a diet be formulated for juvenile on-growing with high levels of protein, low levels of lipid and, within this lipid, high levels of PUFA.

The effect of *n*-3 PUFA on the growth and body composition of cultured 0+ juvenile yellowtail flounder was examined in a third experiment. Yellowtail flounder did not display typical essential fatty acid (EFA) deficiency symptoms observed in other marine fish when fed levels of *n*-3 PUFA as low as 0.4% for twelve weeks. However, they show poor growth after four weeks and preferentially conserve PUFA in phospholipid of liver and muscle and accumulate triacylglycerol in the liver, suggesting the commencement of a deficiency. The increase in the ratio of DHA/EPA in polar tissues of yellowtail flounder was related to good growth. Neutral fatty acid composition in both liver and muscle was affected by diet. Results suggest that yellowtail flounder require 2.5% *n*-3 PUFA as a percentage of dry diet, with 10% lipid, for optimal growth and development. This level is higher than has been seen in the literature, and may be due to its cold natural climate and wild diet of invertebrates, such as polychaete worms or amphipods.

ACKNOWLEDGMENTS

There are many, many people who helped make this thesis possible. First, I would like to thank the Canadian Centre for Fisheries Innovation and Fishery Products International for helping to provide me with the funding to complete my research.

I must also thank my committee, including former member Dr. Steve Goddard for getting me started, and my present supervisors, Dr. Joe Brown and Dr. Santosh Lall. In addition I wish to thank the other member of my committee, Dr. Chris Parrish, without whom I would not have completed this thesis. I give special thanks to Dr. Lall, who took me on (sight unseen) and who has been unwaveringly patient.

I am also grateful to the staff at the Ocean Sciences Centre who gave me so much of their time (willing or not!). This includes faculty, technicians, office and workshop staff. I am especially grateful to Danny Boyce and Sue Budge for their professional support.

Thanks to Dr. Jay Parsons, Keith Rideout and Laura Halfyard for help along the way, and countless other people who gave me their time. Thanks to all of Joe's students who made the two years more fun!

Finally I must be most appreciative of my fiancé, Grant, who I think owns half of this thesis. He has been involved from the very beginning, from measuring fish, to putting together

posters, to painstakingly adding superscripts to Excel worksheets. I am eternally grateful. Also to some of my dearest friends, Corina Rice, Dena Wiseman (who warned me that this is how it would be!), Jennifer Hann and Martha Hiscock. I also wish to thank Dukes, a new Boxer puppy, who spurred me to finish up the last details, so I could play with him.

To my parents a special thanks mostly for their commiseration and emotional support. I always remember how lucky I am.

I can't believe it's done!!!!!

TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGMENTS iv

LIST OF TABLES ix

LIST OF FIGURES xi

LIST OF TABLES IN APPENDICES xiii

LIST OF ABBREVIATIONS xvii

CHAPTER 1.0 General Introduction 1

CHAPTER 2.0 Optimum Feeding Frequency in Juvenile Yellowtail Flounder .. 11

2.1 Introduction 11

2.2 Materials And Methods 15
2.2.1. Fish 15
2.2.2 Feed And Feeding 15
2.2.3 Experimental Design And Conditions 16
2.2.4 Measurement 19
2.2.5 Statistical Analysis 21

2.3 Results 22
2.3.1 Growth And Survival 22
2.3.2 Specific Growth Rate 24
2.3.3 Food Consumption 24
2.3.4 Food Conversion Ratio 27
2.3.5 Behaviour 30

2.4 Discussion 33

CHAPTER 3.0 Body Composition of Wild Versus Cultured Yellowtail Flounder 39

3.1 Introduction 39

3.2 Materials And Methods 42
3.2.1 Fish 42
3.2.2 Feed And Feeding 43
3.2.3 Experimental Conditions 43
3.2.4 Measurement 44
3.2.5 Dissection 45
3.2.6 Biochemical Analyses 45
3.2.7 Statistical Analyses 48

3.3 Results 49
3.3.1 Diet Analysis 49
3.3.2 Hepatosomatic Indices (Hsi) And Condition Indices (Ci) 49
3.3.3 Nutrient Analysis 49
3.3.4 Lipid Class Analysis 57
3.3.5 Fatty Acid Analysis 60

3.4 Discussion 64

CHAPTER 4.0 Essential Fatty Acid Requirements of Juvenile Yellowtail Flounder 71

4.1 Introduction 71

4.2 Materials And Methods 76
4.2.1 Fish 76
4.2.2 Feed And Feeding 76
4.2.3 Experimental Design And Conditions 77
4.2.4 Measurement 78
4.2.5 Biochemical Analyses 79
4.2.6 Statistical Analysis 79

4.3 Results 80
4.3.1 Diet Analysis 80
4.3.2 Growth And Survival 80
4.3.3 Specific Growth Rate (Sgr) 85
4.3.4 Hepatosomatic Index 85
4.3.5 Nutrient Analysis 89
4.3.6 Lipid Analysis 92
4.3.7 Fatty Acid Analysis 95

4.4 Discussion 104

CHAPTER 5.0 Conclusions and Recommendations 113

5.1 Feeding in Wild and Captive Yellowtail Flounder 113

5.2 Feeding Frequency of Juvenile Yellowtail Flounder 114

5.3 Body Composition of Wild And Cultured Yellowtail Flounder 115

5.4 Essential Fatty Acid Requirements of Juvenile Yellowtail Flounder .. 116

LITERATURE CITED 119

APPENDIX A 127

APPENDIX B 145

APPENDIX C 150

APPENDIX D 159

LIST OF TABLES

		Page
Table 2.1:	Comparison of initial and final weights (g), lengths (cm), condition indices and survival by treatment in yellowtail flounder (mean ± standard error, $n = 3$).	23
Table 3.1:	Nutrient, lipid and fatty acid analysis of diets (Moore-Clark Nutra Fry, 1.5 and 2.0 mm) fed to cultured yellowtail flounder.	50
Table 3.2:	Nutrient analysis results of wild (inshore and offshore) and cultured juvenile yellowtail flounder whole body (entire fish).	52
Table 3.3:	Fatty acid composition of total, polar and neutral lipid fractions of the liver of wild and cultured yellowtail flounder.	61
Table 3.4:	Fatty acid composition of total, polar and neutral lipid fractions of the muscle of wild and cultured yellowtail flounder.	62
Table 4.1:	Nutrient and lipid class analysis of diets (C-1000 Biokyowa and ICES) fed to cultured yellowtail flounder.	81
Table 4.2:	Fatty acid composition of total, polar and neutral lipid fractions of liver of yellowtail flounder fed control diet Biokyowa C-1000.	82
Table 4.3:	Fatty acid composition of total, polar and neutral lipid fractions of muscle of yellowtail flounder fed control diet Biokyowa C-1000.	96
Table 4.4:	Fatty acid analysis of diets (C-1000 Biokyowa and ICES) fed to cultured yellowtail flounder.	97
Table 4.5:	Fatty acid composition of the total lipid fraction of the liver of yellowtail flounder fed varying amounts of n-3 PUFA.	98

Table 4.6: Fatty acid composition of polar and neutral lipid fractions of the liver of yellowtail flounder fed varying amounts of *n*-3 PUFA. 99

Table 4.7: Fatty acid composition of the total lipid fraction of the muscle of yellowtail flounder fed varying amounts of *n*-3 PUFA. 100

Table 4.8: Fatty acid composition of polar and neutral lipid fractions of the muscle of yellowtail flounder fed varying amounts of *n*-3 PUFA. 101

Table 4.9: Comparison of essential fatty acid requirements among fish species. (Partly from Takeuchi, 1997). 107

LIST OF FIGURES

Figure 2.1: Temperature profile of ambient water in a flow-through system in Logy Bay, Newfoundland. Starting May 24, 1997. 18

Figure 2.2: Mean coefficients of variation (%) for 0+ yellowtail flounder fed according to four feeding schedules. 25

Figure 2.3: Mean specific growth rate (SGR) (% body weight/day) of 0+ yellowtail flounder fed according to four feeding schedules. 26

Figure 2.4: Consumption parameters of 0+ yellowtail flounder fed according to four feeding schedules (A: meal size (mg feed/g fish); B: consumption per day; C: total consumption over experimental period (70 days)). 28

Figure 2.5: Mean food conversion ratios (FCR) (g feed/ g weight gain) of 0+ yellowtail flounder fed according to four feeding schedules. 29

Figure 2.6: Behavioural observations on activity (A), foraging frequency (B), and aggression (C) of juvenile yellowtail flounder fed according to four feeding regimes. 31

Figure 2.7: Mean ingestion rate (# pellets/minute) (A) (n = 170, 131, 65 and 90 respectively) and capture success (# bites/# ingested) (B) (n = 62, 75, 47 and 54 respectively) for 0+ yellowtail flounder fed according to four feeding regimes. 32

Figure 3.1: Hepatosomatic indices (HSI) (%) of wild inshore and cultured yellowtail flounder. 51

Figure 3.2: Moisture (A) and protein (B) content (% dry weight) in the muscle of wild inshore and cultured yellowtail flounder. 54

Figure 3.3: Protein content (%) in the muscle by sex of wild (A) (n = 11 males, 1 female) and cultured (B) (n = 2 males, 4 females) yellowtail flounder. 55

Figure 3.4: Moisture content (%) in the liver of wild and cultured yellowtail flounder. 56

Figure 3.5: Total lipid content (μg/g) in liver (A) and muscle (B) of wild inshore ($n = 6$) and cultured ($n = 6$) yellowtail flounder. 58

Figure 3.6: Lipid class composition (μg/g) in wild ($n = 6$) and cultured ($n = 6$) yellowtail flounder liver (A) and muscle (B). 59

Figure 4.1: Average growth over time of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA. 83

Figure 4.2: Average condition index for 0+ yellowtail flounder fed varying levels of *n*-3 PUFA over the course of the 12 week experiment. 86

Figure 4.3: Average specific growth rate (SGR) (% BW/day) for 0+ yellowtail flounder fed varying levels of *n*-3 PUFA over the course of the 12 week experiment. 87

Figure 4.4: Average specific growth rate (SGR) (% BW/day) for 0+ yellowtail flounder fed varying levels of *n*-3 PUFA. 88

Figure 4.5: Average hepatosomatic indices (% liver weight/BW) of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA at the beginning ($n = 5$) and end ($n = 6$) of the experiment. 90

Figure 4.6: Moisture (A), protein (B), and lipid (C) content (%) of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA. Vertical bars represent standard error. $n = 3$. 91

Figure 4.7: Total lipid (μg/g) in muscle (A) and liver (B) of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA. 93

Figure 4.8: Average levels (μg/g) of triacylglycerol and phospholipid in muscle (A) and liver (B) of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA. 94

LIST OF TABLES IN APPENDICES

APPENDIX A - STATISTICS

Table A.1: Randomized block three-way ANOVA for the model relating logweight of juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 128

Table A.2: Randomized block three-way ANOVA for the model relating loglength of juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 129

Table A.3: Randomized block three-way ANOVA for the model relating logweight gain of juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 130

Table A.4: Randomized block three-way ANOVA for the model relating condition index of juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 131

Table A.5: Randomized block three-way ANOVA for the model relating coefficient of variation of weight in juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 132

Table A.6: Randomized block three-way ANOVA for the model relating specific growth rate of juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 133

Table A.7: Randomized block three-way ANOVA and one-way ANOVA for the models relating consumption parameters of juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 134

Table A.8: Randomized block three-way ANOVA for the model relating food conversion ratios of juvenile yellowtail flounder to time (over ten weeks[1]), treatment (1 to 4[2]), replicate (3), as well as interactions. 135

Table A.9: One-way ANOVA for the model relating behaviour of juvenile yellowtail flounder to treatment (1 to 4[1]). 136

Table A.10: One-way ANOVAs for the model relating hepatosomatic index and condition index of juvenile yellowtail flounder to treatment (1 to 4[1]). 137

Table A.11: Two-way ANOVA for the model relating levels of protein in the muscle of juvenile yellowtail flounder to sex[1] and treatment (1 to 4[2]). 138

Table A.12: Three-way ANCOVA for the model relating the logweight gain of juvenile yellowtail flounder to time (over 12 weeks[1]), treatment (diets 1 to 4[2]), replicate (3), as well as interactions. 139

Table A.13: Three-way ANCOVA for the model relating the loglength gain of juvenile yellowtail flounder to time (over 12 weeks[1]), treatment (diets 1 to 4[2]), replicate (3), as well as interactions. 140

Table A.14: Three-way ANCOVA for the model relating condition index of juvenile yellowtail flounder to time (over 12 weeks[1]), treatment (diets 1 to 4[2]), replicate (3), as well as interactions. 141

Table A.15: Three-way ANCOVA for the model relating the logweight gain of juvenile yellowtail flounder to time (over 12 weeks[1]), treatment (diets 1 to 4[2]), replicate (3), as well as interactions. 142

Table A.16: Three-way ANOVA and one-way ANOVA for the model relating the hepatosomatic index in juvenile yellowtail flounder to time (beginning and end), treatment (diets 1 to 4[1]), replicate (3), as well as interactions. 143

Table A.17: One-way ANOVAs for the model relating levels of lipid classes in the liver of juvenile yellowtail flounder to treatment (diets 1 to 4[1]). 144

APPENDIX B - DIET FORMULATIONS

Table B.1: Manufacturer's specifications (Moore-Clark) of fish fry feed1,2 used in Experiment 2 (Body Composition of Wild and Cultured Yellowtail Flounder). 146

Table B.2: Formulation of standard ICES diets (from the Aquaculture and *Artemia* Reference Centre, University of Ghent, Belgium). 147

Table B.3: Crude fat content and fatty acid composition according to manufacturer's specifications (ICES/INVE Group) for ICES diets greater than 1200 μm. 148

Table B.4: Crude fat content and fatty acid composition according to manufacturer's specifications (Miyako Kagaku Co., Ltd.) for Fry Feed Kyowa (Biokyowa) C-1000. 149

APPENDIX C - INTERNAL STANDARD AND % AREA DATA

Table C.1: Fatty acid composition of total, polar and neutral lipid fractions of the liver of wild and cultured yellowtail flounder. 151

Table C.2: Fatty acid composition of total, polar and neutral lipid fractions of the muscle of Wild and cultured yellowtail flounder. 152

Table C.3: Fatty acid composition of polar lipid fractions of the liver of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks. 153

Table C.4: Fatty acid composition of neutral lipid fractions of the liver of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks. 154

Table C.5: Fatty acid composition of polar lipid fractions of the muscle of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks. 155

Table C.6: Fatty acid composition of neutral lipid fractions of the muscle of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks. 156

Table C.7: Fatty acid composition of total, polar and neutral lipid fractions of liver of initial yellowtail flounder samples and those fed Biokyowa C-1000. 157

Table C.8: Fatty acid composition of total, polar and neutral lipid fractions of muscle of initial yellowtail flounder samples and those fed Biokyowa C-1000. 158

APPENDIX D - ESTIMATION OF FATTY ACID CONCENTRATIONS USING ACYL LIPID DATA

Table D.1: Results of calculations to produce fatty acid concentrations from fatty acid methyl ester proportions and lipid class concentrations in a sample of the neutral portion of lipid from wild yellowtail flounder liver. 161

LIST OF ABBREVIATIONS

AA = Arachidonic acid
ALC = Alcohols
AMPL = Acetone-mobile polar lipids
BW = Body weight
DAG = Diacylglycerols
DHA = Docosahexaenoic acid
EFA = Essential fatty acid
EPA = Eicosapentaenoic acid
FCR = Food conversion ratio
FFA = Free fatty acids
HC = Hydrocarbons
HSI = Hepatosomatic index
KET/EE = Ketones/ Ethyl esters
LNA = Linolenic acid
ME = Methyl esters
PL = Phospholipids
PUFA = Polyunsaturated fatty acid
SE/WE = Steryl ester/wax ester
SGR = Specific growth rate
ST = Sterols
TAG = Triacylglycerols

NOTE ON TABLES:

Saturated fatty acids (SFA) include 14:0, 15:0, 16:0, 17:0, 18:0; monounsaturated fatty acids (MUFA) include 16:1, 18:1*n*-9, 18:1*n*-7, 20:1*n*-11, 20:1*n*-9, 20:1*n*-7, 22:1*n*-11, and 22:1*n*-9; polyunsaturated fatty acids (PUFA) or those with 2 or more double bonds and include 18:2*n*-6, 18:3*n*-3, 18:4*n*-3, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, 21:5*n* -3, 22:5*n*-6, 22:5*n*-3 and 22:6*n*-3; the sum of fatty acids of the *n*-6 and *n*-9 series includes 18:2*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6 and 18:1*n*-9, 20:1*n*-9 and 22:1*n*-9, respectively; the sum of polyunsaturated fatty acids of the *n*-3 series includes 20:5*n*-3, 21:5*n*-3, 22:5*n*-3 and 22:6*n*-3; the ratio of *n*-3 to *n*-6 fatty acids include the sum of *n*-3 fatty acids to the sum of *n*-6 fatty acids expressed as a ratio. Other fatty acids were present in trace quantities or could not be identified.

CHAPTER 1.0 GENERAL INTRODUCTION

With a decline in natural fisheries and a need to diversify the aquaculture industry, there has been growing interest in the culture of marine fish. Interest in flatfish aquaculture has focused mainly on the culture of Japanese flounder (*Paralichthys olivaceus*) in Japan and Korea, turbot (*Scophthalmus maximus*) and Atlantic halibut (*Hippoglossus hippoglossus*) in Norway, Chile and the United Kingdom, all of which are now cultured commercially. Research into the potential aquaculture of other small flatfish species has commenced in North and South America, and include summer (*Paralichthys dentatus*), witch (*Glyptocephalus cynoglossus*), southern (*Paralichthys lethostigma*), winter (*Pleuronectes americanus*) and yellowtail flounders (*Pleuronectes ferrugineus*) (World Aquaculture Society Proceedings, 1998).

Flatfish are laterally compressed and, as adults, have both eyes on the same side of the body. Usually this ocular side is pigmented, while the ventral side (blind) is white, allowing for a camouflage effect (Robins and Ray, 1986). Flatfish are an important commercial catch in worldwide fisheries, and command high market prices. They grow relatively quickly, have low metabolic activity, have low incidences of aggression and can therefore be stocked at high densities (Liewes, 1984). In addition, flounder are highly prolific and, although they do not produce large, well-developed eggs, they are extremely fecund (Liewes, 1984). As wild fisheries decline, flatfish are gaining importance in

aquaculture as a tasty, lean, alternative white-fleshed fish (Liewes, 1984). One such flatfish is the small, cold-water yellowtail flounder (*Pleuronectes ferrugineus*).

MORPHOLOGY

The yellowtail flounder is a right-eyed flounder, in which the eyes migrate to the right side of the body, from the family Pleuronectidae and is commonly known as rusty dab or sole (Scott, 1947; Scott and Scott, 1988). Yellowtail possess a small head and mouth with one row of teeth. They have a distinctively arched lateral line above the pectoral fin, grey to brown coloration with distinguishing rusty brown spots on the eyed or ocular side of the body, a rounded caudal fin and a yellow stripe running alongside the posterior two-thirds of the underside of the anal and dorsal fins (Scott and Scott, 1988), which gives it its name.

In the wild, growth of yellowtail flounder is relatively fast (Lux, 1964; Scott and Scott, 1988), with southern stocks having a higher growth rate likely due to higher temperatures. During the first year of life, yellowtail grow only about 3-5 cm, but reach up to 30 cm during the second year (Royce *et al.*, 1959). Yellowtail have reached lengths of up to 62.7 cm (Scott and Scott, 1988). Male fish grow faster and mature at 4 years of age, while females mature at 5 years (Scott and Scott, 1988).

HABITAT

Yellowtail flounder are sedentary, and settle on coarse substrates, such as sand and gravel (Royce *et al.*, 1959; Scott, 1982). Their preferred temperature range is 3 to 4°C, but they have been found at temperatures ranging from -1 to 15°C, at depths of 38 to 110 m (Langton, 1979) and at a salinity less than 32.9 ‰ (parts per thousand) (Perry and Smith, 1994). They do not appear to migrate as they have been found in the same area, regardless of season and temperature. Their preference to remain at shallow depth prompted Perry and Smith (1994) to describe them as "depth-keepers" and Scott (1982) to assume they select a specific bottom type. Choice of substrate is most likely related to prey preference (Scott, 1982).

REPRODUCTION, EGGS AND LARVAE

Female yellowtail are highly fecund; a 42 cm fish may produce up to 1 456 000 eggs in 7 ± 1 batches per spawning season (Zamarro, 1991); Pitt (1971) places this number as high as 4 570 000 eggs. In captivity, this number is lower, at approximately 100 000 eggs per batch with a mean batch number between fourteen to twenty-two (Manning and Crim, 1998). Spawning begins in May and may continue into late July (Scott, 1947), with peak spawning occurring in the latter half of June in the wild (Pitt, 1970), and late July to August in captive broodstock (Manning and Crim, 1998). They are serial batch spawners and once they begin spawning, will do so daily for a period of 7 ± 1 days (Zamarro, 1991). This one day interval for production of eggs was confirmed by Manning and Crim (1998) for captive females. The

eggs are buoyant, have no oil globule and have an average diameter of 0.9 mm (Scott, 1947). Hatching time is temperature-dependent and the pelagic larvae are small and unpigmented, with an undeveloped digestive system. At night the larvae rise to the surface, presumably to feed (Langton, 1979). They settle on sandy bottoms when they are approximately 12 to 14 mm in length (Smith *et al.*, 1978; Van Guelphen, 1980).

DISTRIBUTION

Yellowtail flounder are distributed in the western North Atlantic from Labrador to Chesapeake Bay (Scott and Scott, 1988); however, commercially important stocks are found in five areas: the New England Bank, Georges Bank, Cape Cod, Scotian Shelf and Grand Banks. On the Grand Banks, the largest concentration is found on and around the Southeast Shoal (NAFO Div. 3N and 3O) and because this area straddles the Canadian 200-mile limit, are subject to heavy fishing mortalities by foreign countries (Walsh, 1992). Since yellowtail flounder are not migratory, they spawn, settle and go through much of their life cycle in one area, which means that increasing numbers of younger fish are being captured annually (Morgan and Walsh, 1996; Walsh, 1991).

Fish distributed in the more southern areas usually grow faster, but mature earlier and reach a smaller final size than those distributed in northern areas, such as the Grand Banks (Beacham, 1983; Pitt, 1974).

COMMERCIAL IMPORTANCE

Yellowtail flounder became an important commercial fish in the 1930s off New England and in the 1960s on the Grand Banks, probably because of a decline in winter flounder and haddock respectively, causing fish harvesters to pursue replacement species (Lux, 1964; Pitt, 1970). In 1969, 10 000 metric tonnes were taken by otter trawl in the Grand Bank area by Canadian trawlers (Pitt, 1970) and by 1989, this had been reduced to 5000 metric tonnes (Walsh, 1991). DFO reports a decline in abundance of 1+ fish (between one and two years old) from the 1970s (from 450 000 000 in 1972 to 150 000 000 in 1990) onwards (Brodie and Walsh, 1994). Heavy exploitation outside the 200-mile limit in the spawning and nursery areas is seen as a possible explanation (Morgan and Walsh, 1996). In 1992, a moratorium was enforced on all groundfish species within the 200-mile limit. Although reopened in 1998, it is difficult to determine the effect this had on population numbers because, unfortunately, many of the stocks lie outside this limit. However, it has been postulated that yellowtail flounder have high potential to "bounce back" after a decline in stocks (Goff, 1993).

The price of yellowtail fluctuates over time but has been fairly constant over the last few years. It is a marginally expensive species, and costs less than Atlantic halibut and witch flounder. However, it is a species that is constantly in demand and therefore shows less price elasticity than other flatfish (Goff, 1993).

DIET

Yellowtail flounder, like most flatfish, are benthivores; that is, benthic animals form the majority of their diet as juveniles and adults. Larval yellowtail ingest algae and zooplankton and as juveniles, eat a diet mainly comprising amphipods and polychaete worms; however, as adults, polychaetes take on greater importance (Collie,1987; Langton and Bowman, 1981; Libey and Cole, 1979; Martell and McClelland, 1994; Walsh, 1992). Some of the important genera of polychaete worms found in the guts of yellowtail are *Eunice*, *Polydora*, *Lumbrineris*, *Nephthys* and *Aphrodita* (Langton and Bowman, 1981). Their choice of diet is restricted by their small mouth size. The fish ingest the upper layers of sandy bottom, taking in the infauna and epifauna, and the gills sort out waste from food. Nonetheless, many studies have shown that sand and pebbles are found in the stomach (Libey and Cole, 1979).

It was assumed that yellowtail are visual feeders, because Langton (1979) found that stomach weight was heaviest in late afternoon and early evening, revealing they feed in the daytime. However, Pitt (1976) and Beamish (1966) caught more yellowtail in the nighttime, and thus concluded that they were actively feeding in the dark, which implies yellowtail feed by sensory cues not limited to vision.

WHY CULTURE YELLOWTAIL?

Yellowtail flounder fetches a marginally high price in fish markets (ranges from $5.00/kg (Canadian dollars) in October 1998 to $7.50/kg in February 1998; Fulton's Fish

Market, New York City, USA), it has established markets in Asia and Europe, and the potential exists for co-culture with halibut, making it an attractive candidate for aquaculture in Newfoundland (Brown *et al.*, 1995). In addition, it is a hardy animal and can withstand wide changes in temperature and salinity (Larraneta, 1986; Perry and Smith, 1994; Walsh, 1992), which may be necessary for the grow-out phase of commercial production. Yellowtail are a very thin flounder (Scott, 1947), with a high fillet to body ratio and have good quality white flesh.

HISTORY OF RESEARCH

The first efforts to propagate yellowtail flounder were by Smigielski in 1979, who spawned yellowtail broodstock under ambient conditions. Since then, research has been done at the Huntsman Marine Sciences Centre by Dr. Greg Goff, the University of New Hampshire under Dr. W. Hunt Howell, and at the Ocean Sciences Centre (OSC) under Drs. Joe Brown and Larry Crim. Research from these groups has greatly improved the ability to culture this species. As with any new species, the protocols developed for yellowtail flounder benefitted from information published on other flounder, such as Japanese flounder (Anon., 1981) and winter flounder (Howell and Litvak, in press).

Research into the culture of yellowtail flounder at the OSC began in 1993, and has focused on broodstock management, egg incubation and larval rearing. Broodstock collected from the wild are kept at the OSC and prior to spawning, undergo gametogenesis spontaneously in captivity. Eggs are collected after manually stripping the males and females

and then incubated. Broodstock can be induced to spawn earlier than their normal spawning season by hormone injections and larvae from both methods are thought to be of good quality and have high survival rates (Copeman, 1996; Manning and Crim, 1998).

Eggs are incubated at approximately 10°C in upwelling systems and are reared by protocols which give survival rates of up to 40% (Puvenendran, unpub. data). Research has been done on the growth and behaviour of larvae (French, 1995; Morris, 1997), prey densities (Puvenendran and Brown, 1995), stocking density, and light levels (Puvenendran, unpub. data). By the second year of research, over 10, 000 juveniles were produced and this number has been increasing steadily. There has been some work done on photoperiod and light intensity in juvenile rearing of yellowtail flounder (Purchase *et al.*, submitted), but to date, a definitive on-growing protocol for juveniles has not been developed. This will be the next step for the successful cultivation of this species.

The proper nutrition and feeding practices are vitally important factors that influence the ability of fish to attain genetic potential for growth, reproduction and longevity. Nutrition is the sum of the processes by which an animal consumes and utilizes food, including ingestion, digestion, absorption and utilization of various nutrients. The nutrients fish need include water, proteins, lipids, carbohydrates, vitamins and minerals. In order to develop successful artificial feeds for aquaculture, basic information is required on the quantitative nutrient requirements, chemical composition and organoleptic properties of feed ingredients in relation to their acceptability and the ability of fish to digest and utilize nutrients from various sources.

The cost of feed may represent more than 50% of fish production costs (Merola and Cantelmo, 1987) and feeding practices directly affect growth, feed utilization and water quality. Feeding is also linked to the health and well-being of fish. Proper feeding practices lead to lower food conversion ratios in fish, and FCRs are important in production estimates. Thus, not only is the type of diet important, but also the feed management, since nutrient requirements and feeding habit vary among different species of fish. Generally, consumers seem to desire a product that is as similar to the wild product as possible. Thus, body composition of the fish at market size is important and this can be manipulated with diet. Diets with an inappropriate level of lipid, in conjunction with other components, may lead to early maturity (Herbinger and Friars, 1992) and impairment of growth, which is a problem when culturing fish.

Cold-water marine flatfish, such as yellowtail flounder, are inactive fish that eat a diet of crustaceans and worms in the wild (Libey and Cole, 1979). They are a lean fish and probably require higher levels of protein and lower levels of lipid in diets than are currently produced commercially for salmonid fishes (Exler and Weihrauch, 1976). The development of an efficient grower diet is essential to economically culture yellowtail, so exact feed requirements must be determined. Presently, yellowtail are fed salmonid diets, but it is unclear whether these diets produce optimal growth of flounders.

Currently the bottleneck in the production of yellowtail flounder is juvenile growth, which has typically been slow. Our knowledge of the nutrition and feeding requirements of yellowtail has been inadequate and until now there had been no studies conducted on the

nutrition and feeding of juvenile yellowtail flounder in captivity. Therefore, the preliminary steps were taken here to formulate a species-specific, well-balanced, cost-effective diet. The next step in the domestication of yellowtail flounder was to identify and design a grow-out protocol for metamorphosed juveniles. Both nutrition and feeding strategies had to be examined to determine how this diet should be administered to the fish. Knowledge of natural conditions may aid in developing a feeding regime and diet that is suitable for yellowtail flounder and may enable the researcher or farmer to reduce costs, improve water quality, health, food conversion and increase growth.

The research in this thesis had four main objectives:

1) To determine the feeding frequency which produced the best growth and lowest food conversion ratio in juvenile yellowtail flounder.
2) To determine and compare the body composition of wild and cultured yellowtail.
3) To determine the *n* - 3 polyunsaturated fatty acid (PUFA) requirement for juvenile yellowtail.
4) To compare the results of this work with commercial diets presently available to determine if they are suitable for yellowtail.

CHAPTER 2.0 OPTIMUM FEEDING FREQUENCY IN JUVENILE YELLOWTAIL FLOUNDER

2.1 INTRODUCTION

Feed management is an issue that demands attention when advancing into the grow-out phase of aquaculture (Goddard, 1996). Areas of importance include feeding rate (amount fed in a set period of time), ration (daily food allotment, usually based on percent body weight), type of diet and feeding frequency, which refers to the number of meals fed to the fish daily. Optimal feeding frequency results in maximum growth, condition factors, survival and food conversion ratios. Additionally, identifying an optimum feeding frequency schedule assists in minimizing food wastage, reducing size variation of the population, and ultimately, decreasing costs. Feeding frequency is also an important consideration in maintaining water quality in aquatic systems (Ekanem, 1996; Lovell, 1989; Phillips *et al.*, 1998).

Each species has its own optimum feeding frequency and this is related to size and metabolism, age, environmental factors and food quality (Goddard, 1996). Feeding frequency may also affect metabolism and body composition. In rats, infrequent feeding has been known to increase fat synthesis (Grayton and Beamish, 1977). The optimum feeding frequency for a species can also be affected by stomach size and rate of gastric emptying. Appetite in fish is known to be controlled by stomach fullness (Talbot, 1994). Growth is affected both by the amount of food consumed and the efficiency of assimilation (Buurma and Diana, 1994), that is, if fish are fed in excess of their requirements, they might not digest

food efficiently, or they may not eat at all, resulting in food wastage. Alternatively, if fish are not fed enough, growth is lost and dominant hierarchies may be created, whereby larger, more aggressive fish obtain more food than the smaller fish, causing size variations, known as depensatory growth (Buurma and Diana, 1994).

It has been shown that increasing the number of meals a fish is fed per day increases growth and lowers food conversion rates by improving food intake (Buurma and Diana, 1994). However, Jobling (1982) has found that fish under restricted feeding regimes display compensatory growth, which is growth that occurs immediately after a period of starvation or malnutrition, and is faster than normal. When restricted feeding regimes are imposed on fish, they tend to eat more per meal, a phenomenon known as hyperphagia, which may result in a larger gut capacity. This has been illustrated in plaice (*Hippoglossoides platessa*) and other animals after periods of food deprivation (Jobling, 1982).

Recently, many studies have focused on the effect of feeding frequency of a fixed ration. That is, the ration, a specified amount of food, is divided among meals and fed to fish, ensuring that fish in different treatments get the same amount of food per day. Under this type of arrangement, channel catfish (*Ictalurus punctatus*) did not display any differences in growth, survival or body composition when fed on four different regimes (Jarboe and Grant, 1996). Groups of estuarine catfish (*Chrysichthys nigrodigitatus*), fed 5% body weight per day, gained more weight when fed once daily but had lower food conversion rates when fed once every other day (Ekanem, 1996). Walking catfish (*Clarias fuscus*) fed 3% body weight per day in three meals grew better than those fed the same ration in one or two meals

(Buurma and Diana, 1994). The optimum feeding frequency for juvenile red-spotted grouper (*Ephinephelus akaara*) is between four and six times daily when the same ration was fed at different meal frequencies (Kayano *et al.*, 1993). Thus, by using a set ration, it can be demonstrated that there is much variation in feeding frequency among offspring.

Studies that use fixed ration feeding assume fish eat all the food presented and that they receive an equal amount. However, appetite in fish is not the same every day and food might be wasted under this regime. Talbot (1994) reported studies stating that if a constant ration is divided into too many meals, there are actually less pellets distributed than is necessary to satiate the population. Feeding in rations does not examine the voluntary intake of fish, which may change daily based on feeding regime, fish size and temperature.

Research that employs satiation feeding is more relevant to preliminary investigations into feeding patterns of new species. The significance of feeding frequency using satiation feeding has been examined in catfish (*Ictalurus punctatus*) (Andrews and Page, 1975), grouper (*E. tauvina*) (Chua and Teng, 1978), plaice (Jobling, 1982), wolffish (*Anarhichus lupus*) (Fam, 1997) and others. Groups of channel catfish fed twice daily grew faster and used food more efficiently than fish fed 24 times a day (Andrews and Page, 1975), whereas for estuary grouper, optimum feeding frequency is once every 48 hours (Chua and Teng, 1978).

An important first step in examining feeding frequency for a particular species, is to consider feeding patterns in wild populations. Yellowtail flounder feed primarily on polychaete worms and small arthropods. Flatfish such as this, which have small stomachs

and relatively long intestines, have a limited capacity for food storage (Liewes, L984). This means that gastric emptying time is rapid in these fish and they probably feed more frequently in nature. No information exists on the frequency of feeding or feed intake in cultured yellowtail flounder; however, it is hypothesized that yellowtail will perform better when fed smaller meals more frequently, as is thought to occur in the wild. To date, juvenile yellowtail flounder have exhibited large size variations within the same age class, low levels of voluntary food consumption and slow growth. It is likely, therefore, that better feed management could help to alleviate this problem. It was hypothesized that because of the presumed natural feeding habits of yellowtail flounder, fish would perform better when fed smaller, more frequent meals. The following objectives were examined in this study:

1) To determine the optimal feeding frequency for this species which results in maximum growth and the lowest FCR.

2) To examine if feeding frequency affects size variation and contributes to the development of social hierarchies.

2.2 MATERIALS AND METHODS

2.2.1. FISH

The experimental fish for this study were 0+ juveniles (under one year old) that had been cultured from captive broodstock held at Memorial University's Ocean Sciences Centre in Logy Bay, Newfoundland. Eggs were stripped from females, fertilized and incubated until hatch in aerated tanks. Larvae hatched in July and August 1996 and were reared under standard laboratory protocols; first, being fed microalgae (*Isochrysis* sp.), and rotifers (*Branchionus plicatilis*), and finally *Artemia franciscana* nauplii until they were weaned onto dry food. Metamorphosed juveniles were placed in flow-through, shallow, circular tanks and kept on a continuous light photoperiod until about 6 months old. On May 24, 1997, one hundred and eighty juveniles weighing 6.8 ± 0.2 g (mean ± standard error) at a standard length of 7.3 ± 0.1 cm, were randomly distributed among 12 experimental buckets.

2.2.2 FEED AND FEEDING

During the experiment, fish were fed a commercial diet of C-2000 FryFeedKyowa (Kyowa Hakko Kogyo Co., Ltd.; lot number 025056) which contained a crude protein level of not less than 55%, fat not less than 10%, fibre not more than 4% and ash not more than 17%, according to manufacturer's specifications. At each meal, fish were fed to satiation, as judged by the point at which pellets, introduced to the feeding area, remained on the bottom and were not approached for more than 2 minutes. Food consumption was

determined by weight difference of the food container before and after feeding. Pellets remaining using this method of feeding were deemed negligible (under 0.001 g), and tanks were siphoned daily to remove waste.

2.2.3 EXPERIMENTAL DESIGN AND CONDITIONS

Black, 13 L buckets with a bottom surface area of 490 cm^2, were placed within three large square (91.5 x 91.5 (sides) x 39.5 (height) cm) tanks which served as an external water bath for the individual units. Four aerated buckets were placed in each tank and gravity-fed water was supplied individually through plastic tubes leading from the header tank, which received ambient sea water through a degassing system. Water outflows were located at the opposite top side of each bucket and covered with mesh screen. The experimental design consisted of four treatments triplicated in these buckets within each tank. Lids were placed over the top of each bucket so that fish could not jump out.

Fifteen juvenile yellowtail flounder were randomly assigned to each bucket, and this resulted in about a 70% coverage of the bottom of the buckets and a stocking density of approximately 8 g/L. Within each large tank, buckets were assigned one of four treatments:

1) one meal per day (0900 h);
2) two meals per day (0900 and 1500 h);
3) four meals per day (0900, 1200, 1500 and 1800 h) ;
4) two meals every other day (0900 and 1500 h).

These feeding frequencies allowed for 24, 6, 3, and 42 hours between meals, respectively.

Animal care protocols were followed in the manner of the Canadian Council of Animal Care guidelines (CCAC Guidelines, 1993) and under protocols developed at the Ocean Sciences Centre (Protocol 99-20-JB - "Behavioural and Phenotypic Strategies of Fish").

The system was flow-through (water velocity 1.1 L/min) at ambient temperature, with an average of 7.1 ± 0.1 °C, ranging from 5.2 to 10.7 °C (see Figure 2.1). Water quality parameters were monitored periodically throughout the experiment with a total dissolved gas monitor (Model TBO-F, Common Sensing, Inc., Idaho, USA), to ensure appropriate rearing levels were maintained. Water saturation was approximately 98% oxygen and 101% nitrogen. Ammonia levels were negligible, and the total gas reading was approximately 99.9%. Salinity was also ambient, at about 34‰, at a pH of 8.0. Fish were kept on an 18L:6D photoperiod, maintained by automatic timers, which came on at 0700 h and went off at 0100 h. Lighting was from a 40W incandescent light bulb located 60 cm above the tanks and surface intensity was approximately 40 lux.

The experiment lasted for ten weeks, after which fish were beginning to outgrow the buckets. Behavioural observations commenced about six weeks into the trial and lasted for the remainder of the experiment. This allowed for an acclimation period to the different feeding schedules before observations began. Observations consisted of observing five fish

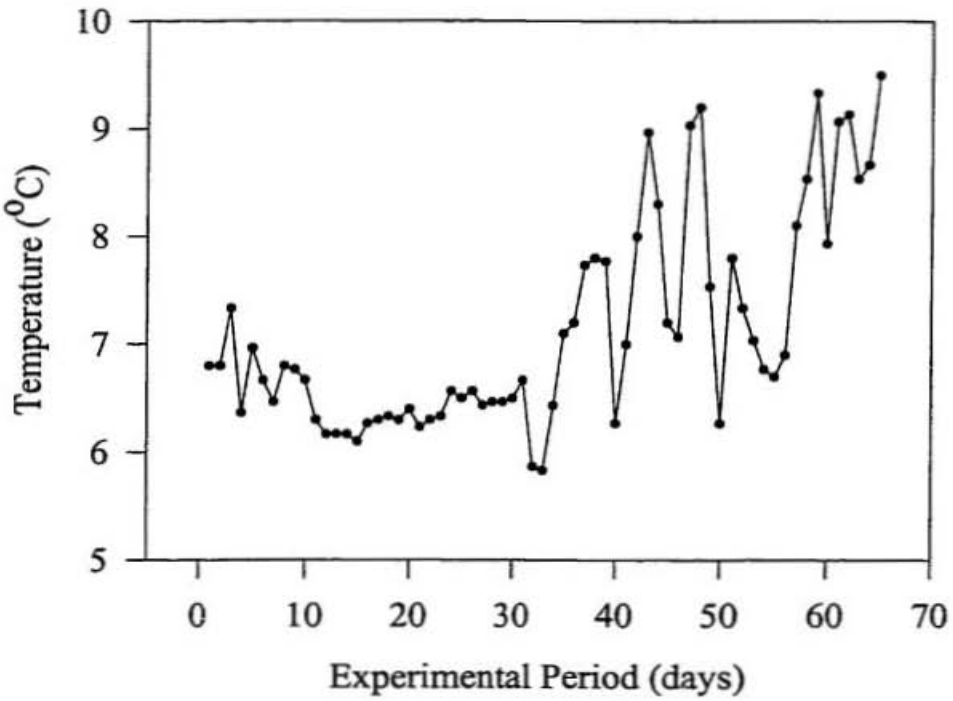

Figure 2.1 Temperature profile of ambient water in a flow-through system in Logy Bay, Newfoundland. Starting May 24, 1997.

in each tank for a period of one minute each at every meal and recording behaviours. The behaviours consisted of:

1) focus - fish turns and orients towards a pellet;

2) bite - fish moves towards a pellet and attempts to ingest it;

3) ingest - fish eats the pellet;

4) reject - fish spits the pellet out;

5) aggression - any display of antagonism towards another fish.

These behaviours (Stradmeyer, 1989) served to determine capture success, ingestion rate and overall level of activity within a tank.

2.2.4 MEASUREMENT

Initially and every two weeks afterwards, fish were measured for wet weight and standard length. Fish were removed with a net, blotted dry with paper towels and placed in a tared beaker of seawater on an electronic balance and weighed to the nearest 0.01 g. Standard length was then taken by placing the fish on a ruler, closing its mouth and taking the length from tip of snout to the end of the caudal peduncle to the nearest 0.1 cm. Fish were not fed the afternoon in any treatments before sampling days or at all on the day of sampling. Mortalities were recorded and were replaced in the first two weeks of the experiment. Other parameters measured were:

Condition Index (CI) = $(W/L^{3.09}) * 100$,

where W is wet weight (grams) and L is standard length (cm); 3.09 is the estimated slope of weight versus length in yellowtail flounder, based on the work by Tuene and Nordvedt (1995), using Atlantic halibut.

Specific Growth Rate (SGR) = $((\ln W_f - \ln W_i)/(t_f - t_i)) * 100$,

where W_f and W_i are the total wet weights (g) of fish in buckets at times t_f and t_i, final and initial times, respectively (Goddard, 1996).

Feed Conversion Ratio (FCR) = feed ingested (mg)/$TW_2 - TW_1$ (g),

where TW_2 and TW_1 are the sum weights of fish at the beginning and end of a sampling period (Goddard, 1996).

% Coefficient of Variation (CV) = $(100 * SD_{WT})/W$,

where SD_{WT} is the standard deviation of the wet weight of all fish in a treatment at harvest and W is the average wet weight of that group (Buurma and Diana, 1994).

Behavioural calculations:

Capture Success = number of pellets ingested/number of bites

Ingestion Rate = number of pellets ingested/ minute

Activity = number of behaviours observed/minute

Foraging = number of times fish focused + number of bites

2.2.5 STATISTICAL ANALYSIS

All statistical tests were performed using the statistical package Minitab (Minitab Inc., Version 9.2). Data was analysed using analysis of variance (ANOVA) in a randomized block model to test for differences among treatments as replicates were separated in compartments and therefore no replicate*treatment interaction terms were considered (Zar, 1974). Significance level was set at $\alpha = 0.05$. Explanatory variables included time, treatment (feeding frequency) and replicate. Means for weight, length, and condition indices were based on individual measurements of each fish per tank, as fish were assigned randomly to tanks (and gave a better indication of variation than tank means alone) but specific growth rates, coefficients of variances, food conversion ratios and consumption data were calculated on a per-bucket basis. Individual fish were not tagged because they had been seen biting the tags of other individuals, and it was thought they would interfere with feeding and behaviour. Only in cases where there was a significant effect of treatment*time interaction was the significance at different times examined. Tukey's pairwise comparisons with a family error rate of 0.05 were used to determine where significant differences lay. Residuals were

examined for normality, independence and homogeneity using histograms of residuals, normal probability plots, and plots of residuals versus the lag of residuals at 1 and residuals versus fits. Data that showed non-normal residuals were log-transformed. If residuals were still not normally distributed after this transformation and α lay within 0.01 of 0.05, a randomization was performed 500 times and a more accurate p-value derived. Fish were randomly distributed to tanks at the beginning of the experiment.

2.3 RESULTS

2.3.1 GROWTH AND SURVIVAL

Fish increased in weight and length over the course of the experiment; however, both weight and length were independent of treatment ($p = 0.106$; $p = 0.262$, three-way randomized block ANOVA respectively; Appendix Table A.1, A.2 and Table 2.1). Weight gain, however, was significant ($p = 0.007$, Appendix Table A.3). Survival was also not dependent on feeding frequency ($p = 0.311$, three-way randomized block ANOVA; Table 2.1). Other than the removal of one bucket of fish (fed four times daily) in the latter part of the experiment, due to possible disease, there was only one other mortality.

There was no bucket effect detected for initial condition index of 0+ yellowtail flounder ($p = 0.302$, one-way ANOVA; Table 2.1). There was no significant increase nor differences among treatments at the end of the trial ($p = 0.474$, two-way ANOVA). Throughout the experiment, however, there was a significant effect of feeding frequency on condition index ($p = 0.010$, three-way randomized block ANOVA; Appendix Table A.4) but

Table 2.1 Comparison of initial and final weights (g), lengths (cm), condition indices and survival (%) in yellowtail flounder fed at different feeding frequencies.

	Weight		Length		Condition Index		
Feeding Frequency	**Initial (g)**	**Final (g)**	**Initial (cm)**	**Final (cm)**	**Initial**	**Final**	**Survival (%)**
4 meals/day	6.8 ± 0.4*	11.9 ± 0.7	7.3 ± 0.1	8.7 ± 0.2	1.5 ± 0.0	1.5 ± 0.0	66.7 ± 57.7
2 meals/day	6.8 ± 0.4	12.7 ± 0.8	7.3 ± 0.1	8.9 ± 0.2	1.5 ± 0.0	1.5 ± 0.0	100.0 ± 0.0
1 meal/day	6.9 ± 0.4	11.3 ± 0.6	7.4 ± 0.1	8.7 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	100.0 ± 0.0
2 meals/2 days	6.6 ± 0.3	11.2 ± 0.6	7.3 ± 0.1	8.7 ± 0.1	1.4 ± 0.0	1.5 ± 0.0	95.6 ± 7.7

* mean ± standard error, $n = 3$.

this was masked by a replicate effect and could not be explored further. Overall, fish fed four times daily had a significantly higher condition index than fish fed once daily ($p = 0.011$, one-way ANOVA; Tukey's).

The coefficient of variation of fish weight over the course of the experiment was not significantly affected by replicate, treatment or time ($p = 0.559$; $p = 0.989$; $p = 0.101$, three-way randomized block ANOVA; Figure 2.2; Appendix Table A.5).

2.3.2 SPECIFIC GROWTH RATE

Specific growth rate was dependent on feeding schedule ($p = 0.018$, three-way randomized block ANOVA; Figure 2.3; Appendix Table A.6). Growth rate increased from weeks 1-4, began to plateau after this time, and then began to increase again towards the end of the experiment (see Figure 2.3). This might be related to temperature fluctuations around the same time. When treatments were divided into high feeding frequencies (fed four times/day and two times/day) and lower feeding frequencies (fed once daily and twice every other day), differences became more significant ($p = 0.001$, one-way ANOVA; Tukey's). Average specific growth rate for 0+ yellowtail in all treatments was 0.80 ± 0.03 % body weight/day ($n = 59$).

2.3.3 FOOD CONSUMPTION

Mean food intake (g feed) of juvenile yellowtail flounder increased significantly ($p < 0.0001$, three-way randomized ANOVA) over time. This might have been a result of fish

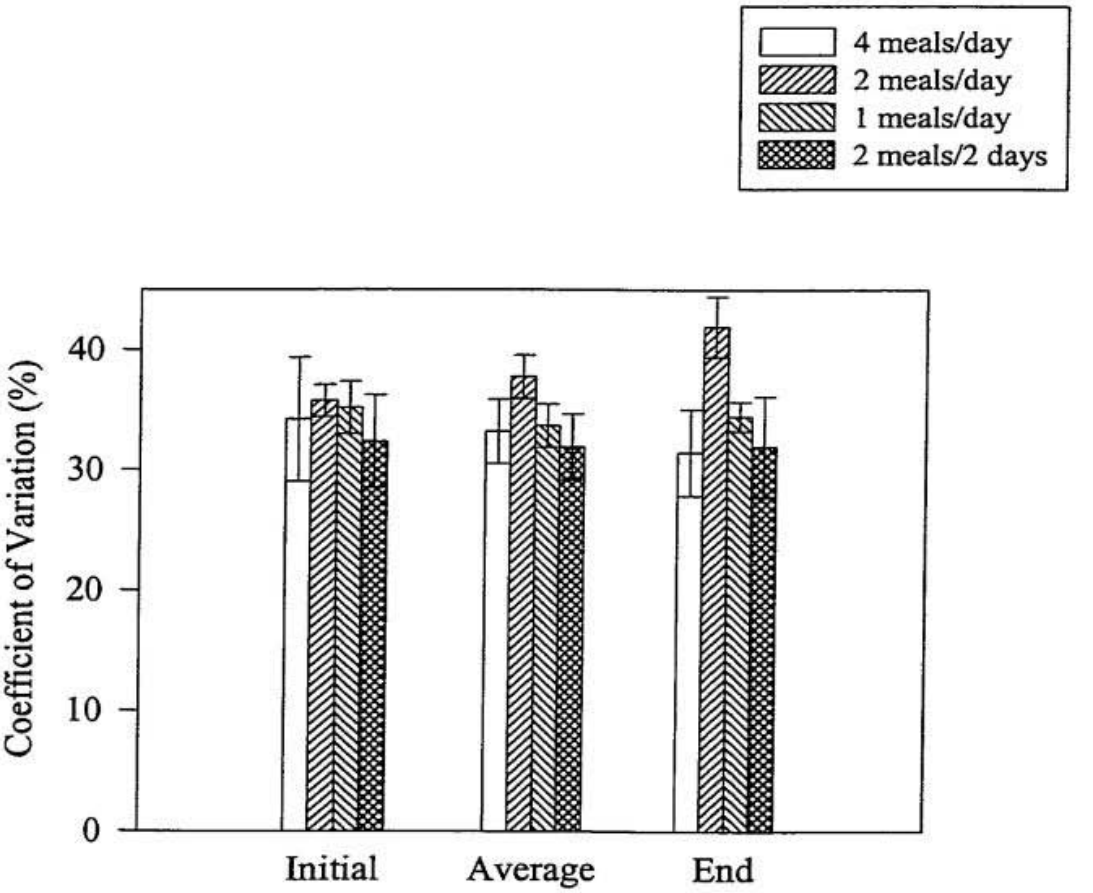

Figure 2.2 Mean coefficients of variation (%) in fish weight for 0+ yellowtail flounder fed according to four feeding schedules throughout the experiment. Vertical bars represent standard error. $n = 17$, 18, 18 and 18, respectively.

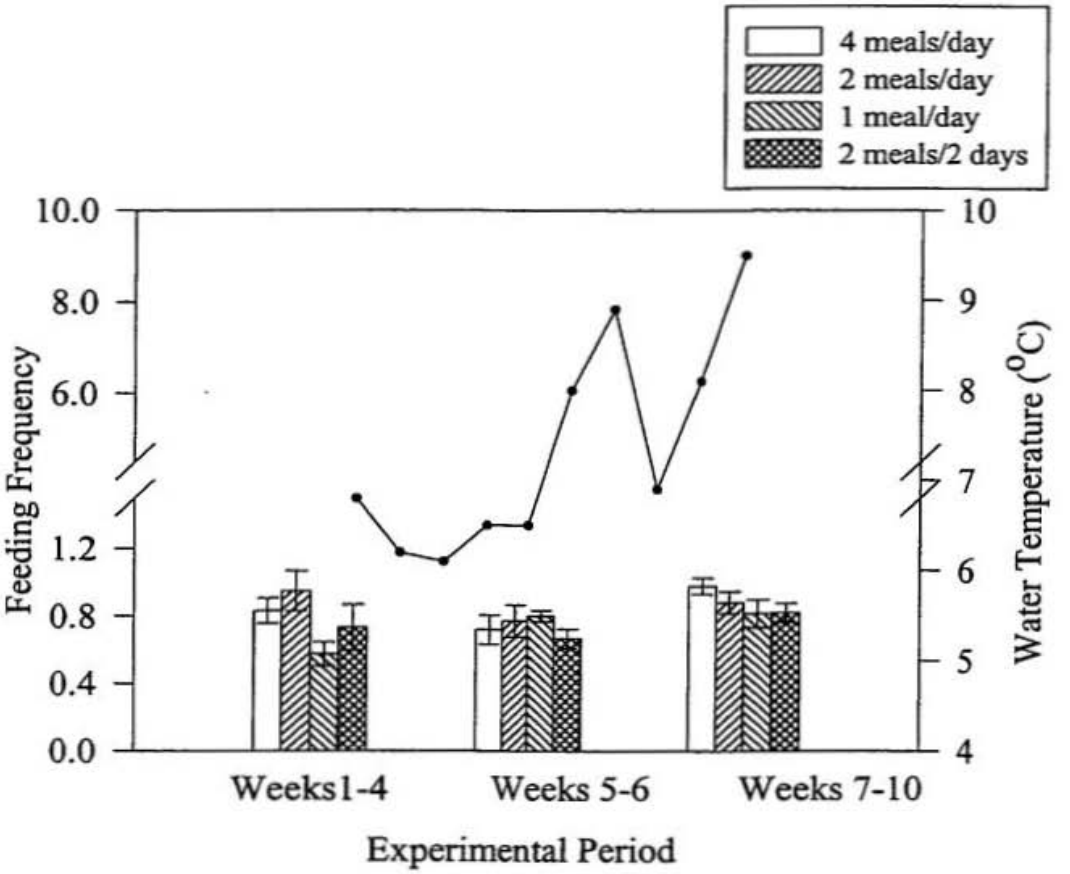

Figure 2.3 Mean specific growth rate (SGR) (% body weight/day) of 0+ yellowtail flounder fed according to four feeding schedules. Vertical bars represent standard error. $n = 14, 15, 15,$ and 15, respectively.

becoming acclimated to the different feeding schedules. Average meal size was significantly ($p < 0.0001$, three-way randomized ANOVA) and inversely proportional to feeding frequency; that is, as number of meals fed to yellowtail flounder per day increased, the size of the meal voluntarily ingested by the fish was smaller. Fish fed four times per day ate significantly ($p < 0.0001$, one-way ANOVA; Tukey's) less than fish fed twice daily, and fish fed twice daily in turn, ate significantly ($p < 0.0001$, one-way ANOVA; Tukey's) less per meal than both of the lower treatments (Figure 2.4a). Average consumption per day (daily feed intake) was significantly higher in fish fed every other day ($p<0.001$, one-way ANOVA; Tukey's; Appendix Table A.7), with the three other treatments not differing significantly ($p>0.05$), but in decreasing order from four meals daily to one meal per day (14.2 ± 0.6, 9.8 ± 0.5, 8.9 ± 0.5, and 7.1 ± 0.4 mg feed/g fish, respectively; Figure 2.4b). Total food consumed over 10 weeks was significantly ($p = 0.034$, one-way ANOVA; Tukey's; Figure 2.4c; Appendix Table A.7) higher in fish fed higher frequencies than by fish fed lower frequencies.

2.3.4 FOOD CONVERSION RATIO

There was no significant effect of replication on food conversion of 0+ yellowtail flounder in this experiment ($p = 0.258$, three-way randomized ANOVA; Appendix Table A.8). There was no significant effect of treatment ($p = 0.065$, three-way randomized block ANOVA); however there was an effect of time ($p = 0.002$, three-way randomized block ANOVA), and may have been affected by temperature (see Figure 2.5). Although not

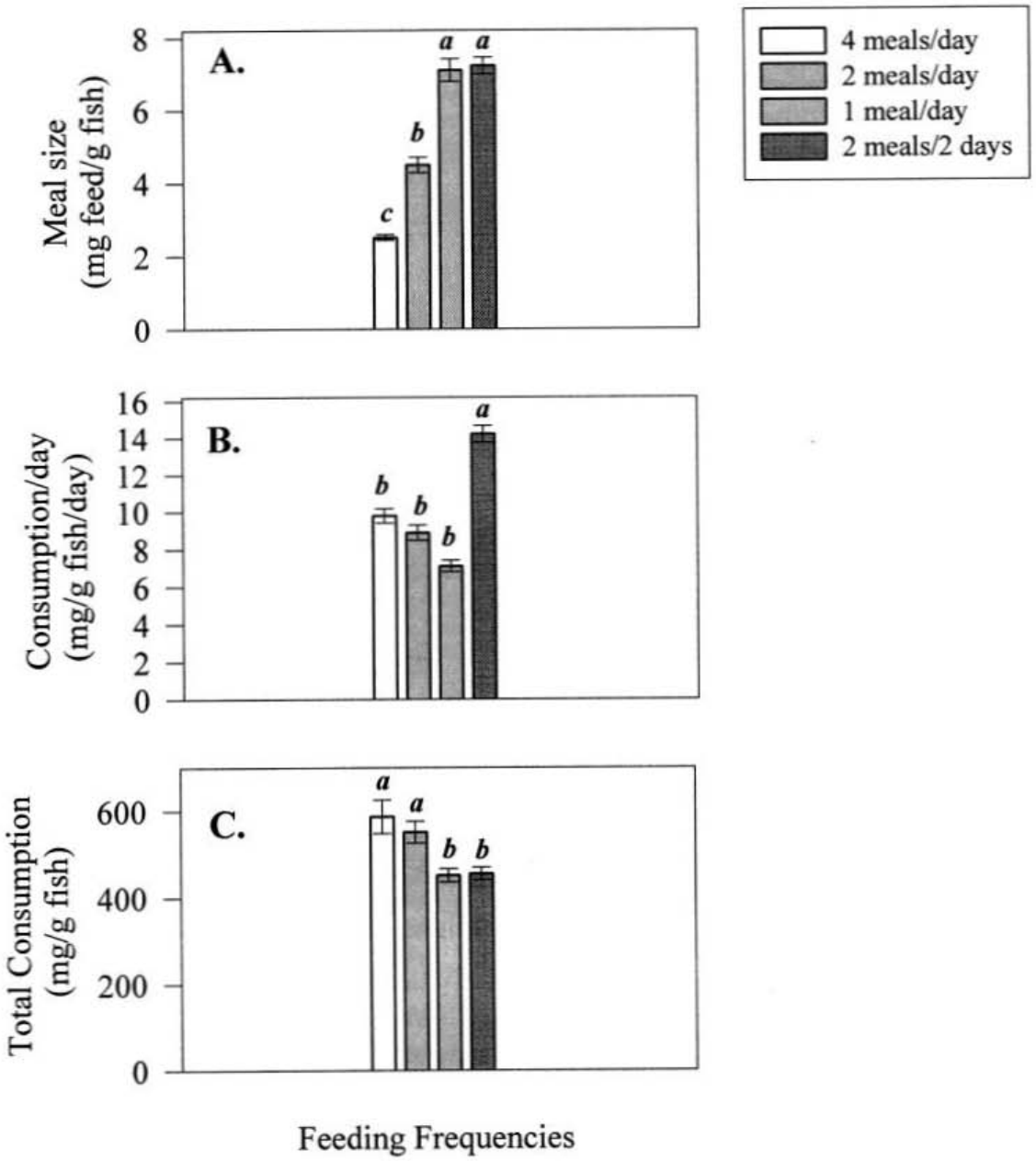

Figure 2.4 Consumption of 0+ yellowtail flounder fed four feeding schedules (A: meal size (mg feed/g fish); B: consumption per day (mg feed/g fish/day); C: total consumption over experimental period (mg feed/g fish/70 days)). Vertical bars represent standard error. $n = 3, 3, 3,$ and 3, respectively. Letters indicate significance.

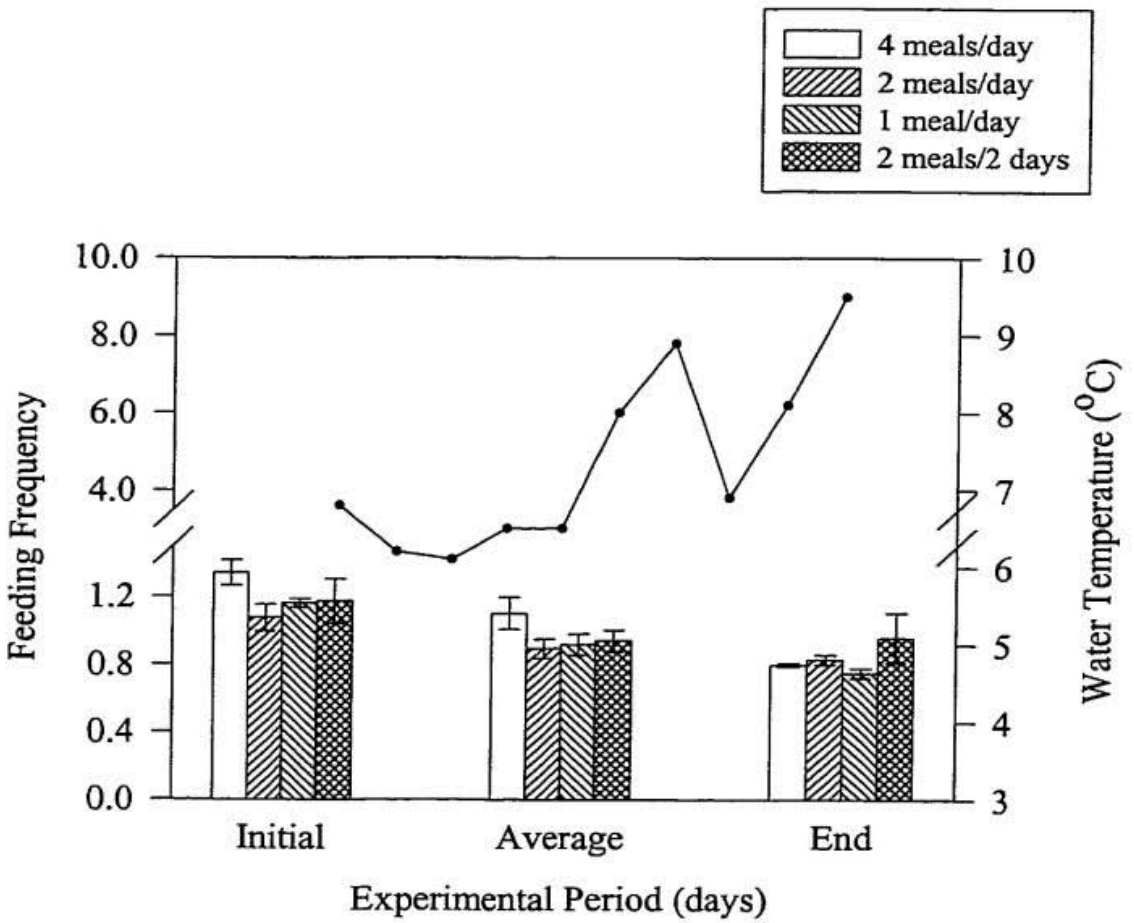

Figure 2.5 Mean food conversion ratios (FCR) (g feed/ g weight gain) of 0+ yellowtail flounder fed according to four feeding schedules. Vertical bars represent standard error. n = 14, 15, 15 and 15, respectively.

significant ($p = 0.051$, one-way ANOVA; Tukey's), fish fed four times daily had the highest overall FCR and fish fed twice daily had the lowest (Figure 2.5).

2.3.5 BEHAVIOUR

Feeding frequency significantly ($p<0.001$, one-way ANOVA; Tukey's; Appendix Table A.9) affected total activity, foraging and ingestion rate of yellowtail flounder. Fish fed four times daily were less active, foraged to a lesser extent, and had a depressed ingestion rate (number of pellets per minute during the time fish were exposed to food) compared with fish fed the other regimes. However, there were no significant differences between treatments for levels of aggression ($p = 0.095$, one-way ANOVA) or capture success ($p = 0.172$), although incidences of aggression were observed to be lower in fish fed the highest feeding frequency, and capture success was slightly higher, although not significant, in this group (Figure 2.6a, b, c and Figure 2.7a and b).

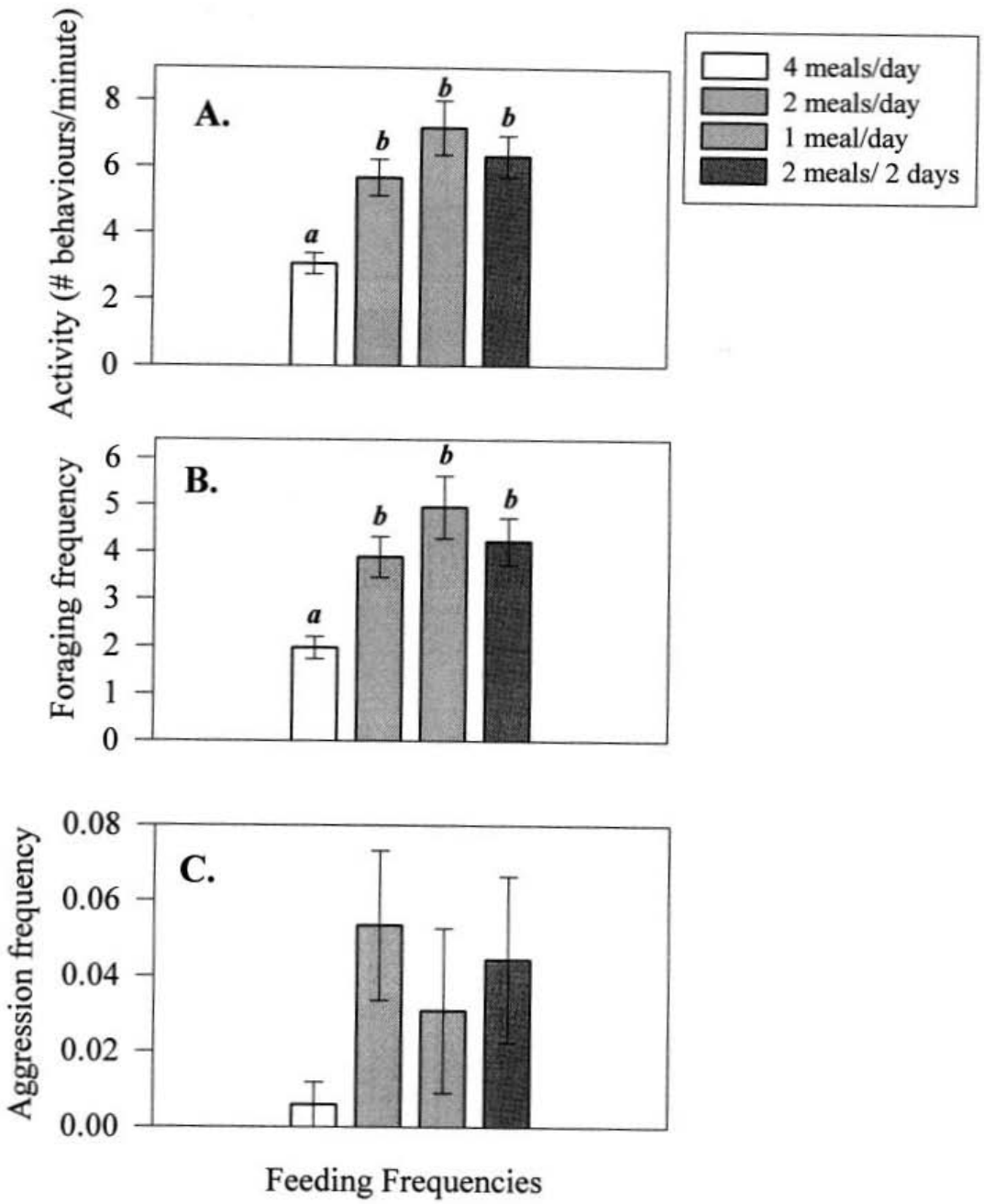

Figure 2.6 Behavioural observations on activity (A), foraging frequency (B), and aggression (C) of juvenile yellowtail flounder fed according to four feeding regimes. n = 170, 131, 65 and 90, respectively. Letters indicate significance.

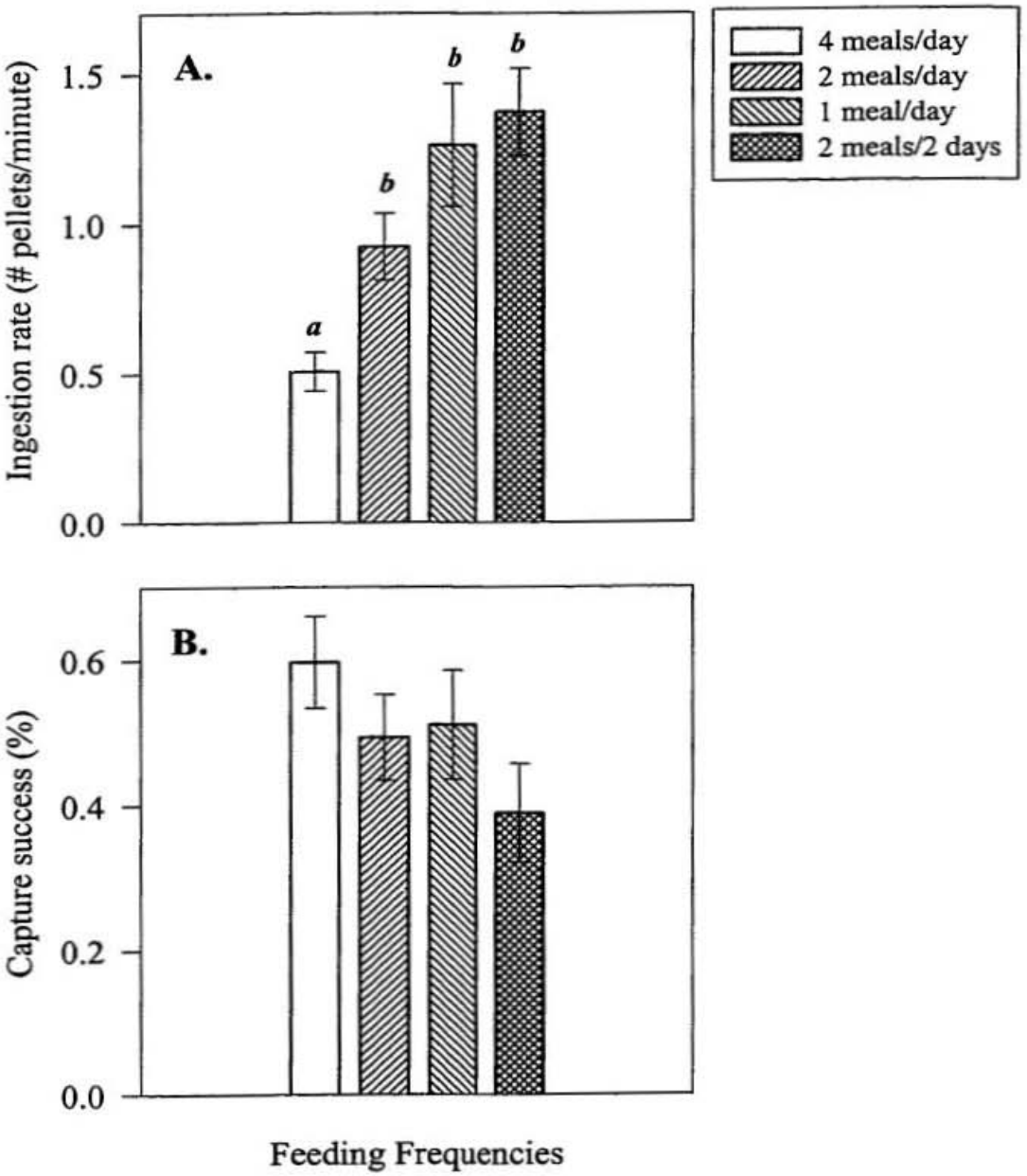

Figure 2.7 Mean ingestion rate (# pellets/minute) (A)(n = 170, 131, 65, 90) and capture success (# bites/# ingested) (B) (n = 62, 75, 47, 54) for 0+ yellowtail flounder fed according to four feeding regimes. Vertical bars represent standard error. Letters indicate significance.

2.4 DISCUSSION

Relatively good growth rates and food conversion efficiencies were obtained in fish from all treatments, suggesting that fish were not negatively affected by the more restricted feeding regimes. Yellowtail flounder, like salmon, seem capable of adapting to a variety of feeding regimes (Talbot, 1994). Juvenile wolffish obtained a growth rate of 0.4% body weight/day in a study by Fam (1997) and Atlantic halibut were shown to have an SGR of 0.3% body weight/day (Tuene and Nortvedt, 1995). Yellowtail flounder in this study had a mean specific growth rate of 0.8 % body weight/day. Wolffish were also shown to have a FCR of 1.3 to 2.7, while the FCR of Atlantic halibut ranged from 0.9 to 1.1; yellowtail, with an average FCR of 1.0, apparently convert food as efficiently as halibut.

Feeding frequency did not seem to affect water quality in this experiment but water quality parameters were not measured on a strict regime. In flow-through systems, this is not as important an issue as in recirculation systems. One bucket (fed four times daily) had to be removed from the experiment after fish in the tank began to show decreased food intake and mortality. The cause of the mortality in fish in this study is unknown, but Chua and Teng (1978) suggested that high mortality in feeding frequency studies may be due to the "result of physiological stress caused by intense feeding".

Grayton and Beamish (1977) found that large variation in appetite among individual fish in feeding experiments may result in biological differences in growth between treatments but not show statistical significance, especially when fish are fed to satiation. Preliminary observations of older yellowtail flounder (2+ fish) suggest that individual variation in

appetite of fish is exceptional (personal observation) and is verified by Tuene and Nordvedt (1995) in Atlantic halibut. Such variation may be a contributing factor to the lack of statistical significance seen in growth between treatments and also may be the cause of slightly different growing patterns within replicates. Although the coefficient of variation in body weight did not change from the beginning to the end of the experiment, all treatments showed a high coefficient, and therefore high variation.

Feeding frequency was positively related to growth and food conversion ratios (Table 2.1 and Figure 2.5). Chua and Teng (1978) found that both parameters improved with increasing numbers of meals when fed to satiation, but after an optimum point, which is species-specific, start to level off and decline. Although there were no significant differences in weight gain or standard length of yellowtail flounder fed higher feeding frequencies, reducing the number of meals fed to one per day significantly decreased the specific growth rate. If the experiment had continued for a longer period (ie. greater than ten weeks), it is likely that weight gain would have been noticeably higher in fish fed higher meal frequencies. Increasing the number of feedings from two to four meals daily did not increase the SGR or weight gain in yellowtail flounder. Kayano *et al.* (1993) found that fish fed an increasing number of meals per day tend to accumulate lipid in the liver and intraperitoneal fat body ; therefore it is not always desirable to increase feeding frequency beyond an optimum point.

Fish fed four times daily had a higher food conversion ratio than fish fed the other frequencies. This suggests that although food intake overall is higher in this group, they do

not seem to be utilizing the food properly. That is, the food is being ingested but is not put towards growth. Fish fed two times daily had a slightly lower FCR than fish fed the other treatments, meaning that the food was utilized efficiently; in fact, fish from all treatments showed low FCRs.

Fish fed at higher frequencies consumed higher quantities of food than the lower frequencies but individual meal size was smaller (Figure 2.4). This is consistent with studies conducted on other species (Ishiwata, 1969). Alternatively, fish fed more restricted feeding regimes became hyperphagic (Grayton and Beamish, 1977; Jobling, 1982) and the fish in this experiment (fish fed the lowest feeding frequencies) appeared to cope in this way. Other coping mechanisms may include modifying metabolic pathways, accumulating fat, and improving growth efficiency by reducing activity levels in order to deal with periods of food reduction (Jobling, 1982). Yellowtail flounder fed fewer meals in this experiment were able to eat more food per day, utilize this food more efficiently and prevent loss of weight. This is somewhat akin to compensatory growth described by Jobling (1993) as a period of rapid growth when fish are returned to adequate feeding after a period of malnutrition. It cannot be determined, though, whether this trend would continue over a long period of time.

In this study, there was a trend towards increased daily food intake (Figure 2.3) and growth (Figure 2.2) as number of meals per day increased, with the exception of fish fed twice every other day. Fish fed according to this schedule ate significantly ($p < 0.05$) more food on the days they were fed than other groups. The fact that they did not grow more may

be because the food put towards growth has to be averaged over two days, and growth rates were reduced in comparison to groups fed the highest frequencies.

Behavioural observations were useful in assessing other responses of fish to differing feeding regimes. The low ingestion rate obtained with fish fed the highest frequency suggests that the return of appetite after a meal was longer than three hours. Other small flatfish such as lemon sole (*Microstomus kitt*) retained food in their guts for 72 hours, while appetite in Atlantic halibut did not return for 120 hours in an experiment by Davenport *et al.* (1990) in which fish were held at a temperature of 9.5 to 10.5°C. Observations of lemon sole and halibut showed that when fish are fed to satiation, they are not interested in eating again until food has almost completely cleared from the gut. Alternatively, yellowtail flounder seem to feed more frequently, as fish fed every second day fed ravenously, and fish fed only once daily were active and foraged often. Only in treatments of 2 and 4 meals/day did fish regularly show no interest when food was offered. However, when these fish did attempt to eat a pellet, capture success was higher. This may be due to the fact that fish fed once daily or once every two days are more "eager", and make more attempts at pellets, regardless of the accuracy in obtaining them. Other studies have shown that fish fed lower feeding frequencies actually showed a decline in activity and metabolic rate, apparently to conserve energy (Buurma and Diana, 1994; Grayton and Beamish 1977) and fish fed continuously are disturbed more often and eat intermittently, causing increased activity (Andrews and Page, 1975). This was obviously not the case in this experiment, and may be attributable to flounder naturally having low metabolic rates (due to a benthic and inactive

way of life) or that the regimes imposed were not extreme enough to produce this effect. Because yellowtail fed the lower frequencies in this experiment were more active, it may be that growth was lost due to maintenance during non-feeding.

Dominant hierarchies did not seem to form in any groups and the coefficient of variation was higher in fish fed twice daily. Thus increased numbers of meals did not seem to decrease size variation. This result was unexpected, but may be related to the fact that yellowtail flounder do not seem to be an aggressive species, may be stocked densely and exhibit low rates of cannibalism (personal observation; Puvanendran and Brown, 1995).

Using ambient temperatures to rear yellowtail flounder, as used in this experiment, demonstrates the level of growth which would be obtained by juveniles in a grow-out situation under these feeding regimes. However, fish reared in an intensive setting in tanks would be reared at temperatures higher than those used in this study, and it is important to note that although growth might be higher in that situation, the results seen here would probably be the same. The changes in specific growth rate and condition index throughout are apparently due to increases and decreases in temperature over the duration of the study and explain why there are no significant differences in growth at the end of the experiment.

An improvement on this experiment would have been to lengthen the duration of the study because although there were differences in specific growth rate, this is not reflected in weight gain or length increase, which may be due to differences in temperature. Over time, such effects may have become more pronounced and a more definitive regime would have emerged. Other types of restricted feeding might be examined, as there is some suggestion

that feeding fish even less than in this experiment may actually increase growth and food utilization over time (Grayton and Beamish, 1977). It is predicted that as yellowtail increase in size, they will require less feedings per day, and this should be investigated. It is important to note that the type of diet fed to fish also has an effect on the number of times daily a fish will voluntarily eat (Lovell, 1989). If the energy level of the diet is low, fish will eat larger satiation meals and if the lipid levels are high, fish will eat less.

In terms of grow-out, the feeding frequencies used in this study are in the range that should be used for captive rearing of this species. These were not seen to affect growth, but did affect feed intake in yellowtail flounder. Because there were no significant differences in growth, it appears that feeding juvenile yellowtail flounder once daily or twice every other day may be sufficient for grow-out. These regimes also represent the least labour-intensive methods of feeding yellowtail flounder. However, the highest specific growth rate and lowest food conversion ratios were obtained with fish fed twice daily which implies that yellowtail may reach market size faster when raised under this feeding regime. Therefore feeding twice daily must be further studied, and at this time is recommended as these parameters may significantly affect growth over longer time periods than used in this study.

CHAPTER 3.0 BODY COMPOSITION OF WILD VERSUS CULTURED YELLOWTAIL FLOUNDER

3.1 INTRODUCTION

The biochemical composition of fish flesh is known to be affected mainly by diet (Shearer, 1994). In the wild, animals feed voluntarily on natural foods and the constitution of their organs and whole-body (entire fish) is thought to reflect the utilization of their natural diet and may provide some idea of how to formulate a diet for these fish in captivity. In addition, comparisons between wild and captive-reared fish may help to inform aquaculturists whether diets fed to fish in hatcheries are adequate. Such a comparison will give insights into how stress caused by aquaculture conditions affect the physiology and biochemistry of fish (Blaxter, 1975). Nutrient analysis, which gives an indication of levels of water, protein, fat, ash, carbohydrate and energy, may enable nutritionists to modify the composition of the carcass in intensive rearing situations to meet consumer demands (Shearer, 1994). Lipid analysis provides a benchmark for understanding the utilization of fats from the diet, the level of fitness of the fish (Jobling *et al.*, 1998), and the type and quantity of fatty acids supplied to the fish for functioning and growth.

Many of the components of commercial diets are very expensive, such as protein supplements and certain fish oils. Feed may account for up to 50% of production costs (Higgs *et al.*, 1994). This means that knowledge of the nutritional requirements of fish is critical for the economic feasibility of culture. For these reasons the whole body was

examined, and, in addition, the fillet and liver of cultured and wild fish separately analyzed to ascertain how dietary components were utilized.

Yellowtail flounder in the wild eat primarily polychaete worms and crustaceans (Libey and Cole, 1979), both of which are relatively low in fat but high in *n*-3 highly unsaturated fatty acids. This fat is mostly composed of phospholipid, so that benthic fish, such as flounder, usually have lower storage fat (neutral lipid) than other marine fish (Sargent *et al.*, 1989).

At present, young yellowtail flounder raised in hatcheries have been fed high lipid, low protein diets, which were originally formulated for salmon or oily marine fish. It is not known how these diets affect flounder in terms of health and survival, but it is thought that they accumulate excess lipid in the liver and flesh, which is undesirable for growth and for producing a lean product. The lipid composition of commercial fish diets does not correspond to the natural diet of marine fish. As well, *n*-3 PUFAs are often provided in commercial diets in the form of methyl or ethyl esters and in the wild these are provided in naturally occurring triglycerides (Greene and Selivonchick, 1987). In recent years, the importance and benefits of marine fish in health of humans has been noted (Sargent *et al.*, 1989). Fish are known to provide a good dietary source of protein and polyunsaturated fats, which have been implicated in reducing the incidence of heart disease (Ackman, 1996) and other ailments. By incorporating lipid supplements containing high concentrations of *n*-3 PUFA in fish feeds, it is possible to produce fish for human consumption that supplies a high amount of these fatty acids.

According to Haug *et al.* (1988), there is also a difference in body composition between sexes in fish; with females having higher levels of lipid than males but this was measured in sexually mature fish. It is unknown whether there are differences in body composition between sexes before maturity. The body composition of fish is also affected by season, condition index (the "fatness" index, or ratio of body length to weight) and the reproductive status of the individual (Blaxter, 1975).

It was hypothesized that wild and cultured yellowtail flounder would have different body compositions based on different diets. The objectives of this experiment were:

1) To compare the nutrient, lipid and fatty acid composition of wild and cultured yellowtail flounder.

2) To compare indices (condition and hepatosomatic) between wild and cultured fish.

3) To use the above information to determine whether commercial diets are adequate and to estimate EFA and lipid requirements based on the composition of the whole body (entire fish) and individual tissues of fish.

3.2 MATERIALS AND METHODS

3.2.1 FISH

Twelve wild, small yellowtail flounders (in the range of 37 to 92 g; average weight 60.2 ± 5.5 g) were live-collected during late August by SCUBA divers in an area off Chapel's Cove, Newfoundland (47°26.20' N, 53°07.60'W) at a depth of 18 metres. They were captured by hand in an area that was covered in coarse sand amongst outcrops of rock. They were brought to the Ocean Sciences Centre and held at ambient temperature for a period of three days, during which time they were not fed. These fish were then killed with an overdose of MS-222, and six fish allotted to whole-body nutrient analysis and another six to lipid and fatty acid analysis. The six fish removed for whole-body nutrient analysis were frozen for a three-week period, and then thawed. Six more fish were captured in early September from the same area and these were used for tissue nutrient analysis. Fish were presumed to be about 1.5 to 2 years old, according to the growth curves of Pitt (1974) but may have been slightly younger based on inshore temperatures. Their small size was comparable to that of the cultured fish. A number of offshore fish (twelve fish, approximately 60g each) were also collected by DFO boats on the southern Grand Banks, in 60 m or less, and immediately frozen at -50°C until use in whole-body nutrient analysis.

Cultured fish were reared at the Ocean Sciences Centre in Logy Bay, Newfoundland, from eggs collected from broodstock and raised by standard hatchery protocols. They were hatched in July and August of 1997, and were 1+ year class fish during the experiment in August-September 1998 . These fish weighed 36.5 ± 1.6 g, with a range from 29 to 46 g and

were sampled for analysis in the same manner as wild fish; that is, they were held for three days without food and then six were removed and frozen for whole-body nutrient analysis, while six more were immediately dissected for lipid analysis. Six fish were killed for tissue nutrient analysis two weeks later.

3.2.2 FEED AND FEEDING

It was unknown if the yellowtail collected from the wild had been actively feeding at the time of capture. However, upon dissection, some of the fish had food in the lower intestine. Contents were unidentifiable. It was assumed that most of the food had been voided from the gut of yellowtail after the three days in which they were held without feed based on results from the previous experiment.

Cultured fish had been fed a mixture of 1.5 and 2.0 mm Moore-Clarke diet twice a day to satiation for at least four months (manufacturer's specifications are shown in Appendix Table B.1). Nutrient (proximate and gross energy), lipid, and fatty acid analysis is shown in the results section.

3.2.3 EXPERIMENTAL CONDITIONS

Wild and cultured fish were selected so that sizes were as similar as possible for comparison. All fish were starved for three days prior to slaughter. Cultured fish were reared in square black tanks (91.5 x 91.5 x 39.5 cm subdivided into four compartments), and water flow was approximately 1 l/min. They had been held at 6°C from May until August,

at which time, temperatures rose to 10 -11 °C and was beginning to decrease again. Lighting was 12L:12D at approximately 35 lux. Fish were frozen for a one week before nutrient analysis and for other analyses were dissected immediately.

3.2.4 MEASUREMENT

All fish were weighed to the nearest gram and standard length quantified by placing the fish on a ruler, closing its mouth, and taking the length from the snout to the end of the vertebral column (to the nearest 0.1 cm). For nutrient analysis, whole-body fish were pooled and a triplicate sample taken. When muscle and liver were examined, fish were analysed individually.

At the time of dissection (see below), the sex of the fish was determined visually; immature males being identified as having thread-like testes along the visceral cavity, maturing males having white to cream colored elongate testes and spermiating males having milky white enlarged anteriorly-directed testes. Females had pinkish distinctively shaped ovaries but none were found to be mature. However, a small number of males were spermiating.

Calculations were the same as for the previous experiments for SGR and CI, with the addition of:

Hepatosomatic Index (HSI) = W_L/W_T,

where W_L is wet liver weight (g) and W_T is total wet weight of the fish (g).

3.2.5 DISSECTION

Fish were dissected by making a ventral cut from under the gills to the midbody and a transverse cut to expose the gut. The liver was then removed by teasing away mesentaries which attached it to the gut. To obtain HSI data, the whole fish was weighed and then reweighed once the liver was dissected out. To obtain muscle samples, a cut was made across the body from the gut cavity, along the inside edge of the fin to behind the head. A piece of muscle, approximately 7.5% of the body weight, was removed. Tissues of liver and muscle were weighed to the 0.0001 g before processing.

3.2.6 BIOCHEMICAL ANALYSES

Nutrient analysis was performed on diets and whole-body fish. Thirteen whole-body fish were pooled per treatment of cultured fish (35 g average weight); 3 fish were pooled per inshore wild treatment (85 g average weight), and 8 wild offshore fish were pooled (60 g average weight). Triplicate samples were analysed from these fish for crude moisture, protein, lipid and ash. The moisture content was obtained by placing pre-weighed samples of tissue in a 105°C oven for 24 hours. At all times before samples were weighed, they were kept in desiccators. Crude protein was determined using the Kjeldahl method (Tectator Digestion System 20, 1015 digestor, Sweden; Tectator Kjeltec System 1028 Distilling Unit, Sweden). Crude lipid values were obtained using a hexane-based Soxhlet apparatus (Tectator Soxtec System HT 1043 Extraction Unit, Sweden) and ash weight was obtained by pre-weighing crucibles and placing these and the dried sample in a muffle furnace

(Thermolyne, Sybron Corporation, Dubuque, Iowa, USA) set at 450°C for 24 hours, and then cooling the sample in a desiccator and re-weighing. Carbohydrate was obtained by subtracting the sum of the other nutrients, moisture, ash, crude protein, crude fat and fibre from 100 (Goddard, 1992). The gross energy was determined by multiplying the percent protein by 5.6 kcal/gram, percent lipid by 9.5 kcal/gram and percent carbohydrate by 4.1 kcal/gram (Goddard, 1992). These values are the gross energy per calorie for each of protein, lipid and carbohydrate, respectively (Goddard, 1992). The sum of these values is equal to the gross dietary energy per 100 grams. Diets were analysed using the same methods.

When individual tissues were analysed, nutritional analysis was done somewhat differently. Crude moisture content was obtained by placing livers and muscles in pre-weighed aluminum trays, weighing them and putting them in an oven (Stabil-Therm Gravity Oven, Blue-M Electric Co., Blue Island, Illinois, USA) at 105°C for 24 hours. They were kept in a desiccator and re-weighed. Thus these previously dried samples were then placed in a muffle furnace (Thermolyne 1500 Furnace, Sybron Corporation, Dubuque, Iowa, USA) for another 24 hours at 450°C to obtain ash weight values. These were again re-weighed. Crude protein (N* 6.25) values were determined only for muscle and duplicate samples (weighing approximately 0.05 g) were measured by the Dumas method (Ebling, 1968) using an FP-228 Nitrogen Determinator (Leco Corp., St. Joseph, Michigan, USA).

Lipids were extracted from diets, liver and muscle tissues following a simplification of the method of Folch *et al.* (1957), using a 2:1 (v/v) ratio of chloroform to methanol. Samples were stored at -20°C under nitrogen until analysis. Total lipid and lipid classes were

analysed using the TLC-FID Chromarod Iatroscan (Iatroscan MK V, Chromarods-SIII; RSS Inc., Bemis, TN) system which separates the lipid into hydrocarbon (HC), sterol ester or wax ester (SE/WE), ketone or ethyl ester (KET/EE), sterol (ST), triacylglycerol (TAG), free fatty acid (FFA), alcohol (ALC), diacylglycerol (DAG), acetone-mobile polar lipid (AMPL), and phospholipid (PL). Total lipid was determined by summing these individual lipid classes. The extract was separated into polar and neutral fractions by silicic acid gel column chromatography. Columns were eluted with chloroform:methanol:formic acid (98:1:1, v/v/v) to collect the neutral portion and subsequently with methanol (5 ml) to remove the polar portion. More than 80% of total lipids were recovered from the column using this method. At this point, an internal standard was added to the portions. Tricosanoic acid methyl ester (23:0) (0.5 ml) at a concentration of 50 mg/100 ml was added to the wild fish samples and 1 ml was added to the cultured fish samples. These portions were transesterified with boron trifluoride in order to transform fatty acids into fatty acid methyl esters, and then separated and quantified by gas chromatography. A Varian 3400 gas chromatograph equipped with an autoinjector was used to perform fatty acid analyses; the instrument contained an Omegawax 320 column (30 m, 0.32 mm i.d., 0.25 μm film thickness: Supelco, Inc.). Hydrogen was used as a carrier gas. Peaks were identified by using a Supelco polyunsaturated fatty acid mixture as a standard and were expressed as fatty acids as a percent by weight of total fatty acids, calculated from actual values as well as the actual values themselves in mg fatty acid/100 grams of fish tissue and are found in Appendix C, Tables C.1 and C.2.

This internal standard method of quantification is highly accurate for freshwater samples but not generally for marine samples, due to complex fatty acid compositions (Budge, 1999). In addition, because the internal standard was added after extraction and column separation, fatty acid values provided from this method were underestimated and inaccurate, although proportional data was found to be accurate. Thus estimations of fatty acid methyl ester concentrations are provided using acyl lipid data provided by TLC-FID, in the manner of Budge (1999) and provided in the forms of % total weight of lipid class and mg/100 g wet weight See Appendix D for the explanation of the method.

3.2.7 STATISTICAL ANALYSES

Biochemical values including crude protein, lipid, moisture, ash, fibre and carbohydrate were compared using a one-way ANOVA (Zar, 1974) for wild and cultured fish. This was also done with HSI, CI, lipid class and fatty acid values for the tissues. In cases where sex differences were compared, a 2-way ANOVA (with sex and wild/cultured as the explanatory variables) was used.

3.3 RESULTS

3.3.1 DIET ANALYSIS

Diet assessment of the Moore-Clark pellets showed that our nutrient analysis was similar to the crude nutrient analysis specified by the manufacturer (Table 3.1 and Appendix B, Table B.1). Both particle sizes were composed primarily of triacylglycerol, and had ratios of DHA/EPA of 0.8 ± 0.0 for 1.5 mm pellets and 1.3 ± 0.0 for 2.0 mm pellets.

3.3.2 HEPATOSOMATIC INDICES (HSI) AND CONDITION INDICES (CI)

Wild fish displayed significantly lower hepatosomatic indices (HSI) than cultured fish ($p < 0.001$, one-way ANOVA; Figure 3.1; Appendix Table A.10). In addition, it was noted that wild fish livers were redder in colour, and seemed more vascularized. Cultured yellowtail had pale livers.

The condition index of wild yellowtail flounder, 1.1 ± 0.03, was significantly lower than the condition index of cultured yellowtail, 1.4 ± 0.04 ($p < 0.0001$, one-way ANOVA; Appendix Table A.10).

3.3.3 NUTRIENT ANALYSIS

There were significant differences in whole-body nutrient composition between wild and cultured yellowtail nutrient composition. Moisture content was higher ($p < 0.001$, one-way ANOVA; Table 3.2) in wild inshore flounder than both offshore flounder and cultured flounder, and cultured yellowtail had lower whole-body moisture content than offshore

Table 3.1 Nutrient, lipid and fatty acid analysis of diet (Moore-Clark NutraFry) fed to cultured yellowtail flounder.

	Food Size	
	1.5 mm	2.0 mm
Nutrient (%)		
Moisture	6.3±0.0[1]	7.8±0.0
Crude Protein	52.8±0.3	51.0±0.3
Crude Lipid	25.1±0.1	21.5±0.6
Crude Ash	7.9±0.0	7.0±0.0
Carbohydrate	7.9±0.3	12.8±0.8
Gross Energy (kcal/g)	5.6±0.0	5.3±0.0

	Food Size			
	1.5 mm		2.0 mm	
Lipid Classes	%	mg/100 g	%	mg/100 g
Hydrocarbons	0.2	23.4±1.2	0.3	63.1±4.2
Steryl Ester/Wax Ester	0	0.0±0.0	0	0.0±0.0
Ketones/Ethyl Ester	0.1	15.8±2.9	0.5	104.5±4.9
Triacylglycerols	84.3	10350.0±405.5	88.2	17685.8±416.6
Free Fatty Acids	2.0	242.4±14.0	1	198.9±67.3
Alcohols	0	0.0±0.0	0	0.0±0.0
Sterols	1.5	186.2±3.2	1.63	326.2±9.4
Diacylglycerols	3.0	358.3±50.2	0.7	146.5±1.0
Acetone-Mobile Polar Lipid	4.1	505.4±46.3	1.9	385.6±59.7
Phospholipid	4.8	593.4±327.2	5.7	1142.8±17.5
Total Lipid	12.3	12274.7±327.2	20.1	20053.2±580.6

	Food Size			
	1.5 mm		2.0 mm	
Fatty Acid	% wt	mg/100 g	% wt	mg/100 g
14:0	8.1±0.0	868.7±34.2	5.4±0.0	978.6±35.2
15:0	0.3±0.0	37.5±1.6	0.3±0.0	45.8±8.6
16:0	20.3±0.3	2173.0±117.7	20.9±0.6	3775.8±9.3
16:1	8.5±0.3	911.9±4.4	7.7±0.2	1393.5±5.3
16:2	1.4±0.0	148.9±6.3	0.7±0.0	135.7±5.4
17:0	0.7±0.0	80.3±0.3	0.5±0.0	94.9±1.8
18:0	3.4±0.1	359.0±7.5	2.4±0.3	438.0±65.8
18:1	16.8±0.1	1806.0±86.5	24.3±0.3	4391.3±85.2
18:2*n*-6	5.0±0.0	537.3±16.2	6.9±0.4	1256.3±106.2
18:3*n*-3	1.3±0.0	142.4±5.4	1.4±0.0	245.4±14.6
18:4*n*-3	2.1±0.1	219.3±3.1	1.5±0.1	269.9±1.8
20:1	1.7±0.1	182.2±19.4	2.6±0.5	464.9±107.6
20:2*n*-6	0.2±0.0	17.0±0.1	0.2±0.1	35.4±15.0
20:3*n*-6	0.0±0.0	4.9±4.9	0.1±0.0	24.4±5.8
20:4*n*-6	1.0±0.0	105.4±0.9	1.3±0.0	230.5±29.5
20:5*n*-3	14.9±0.1	1591.6±52.9	8.9±0.1	1611.0±73.9
22:1	0.2±0.0	25.9±3.4	1.8±0.3	316.7±40.6
21:5*n*-3	0.6±0.0	67.4±2.2	0.4±0.0	67.8±2.7
22:5*n*-3	1.9±0.0	204.0±8.6	1.6±0.0	287.5±2.8
22:6*n*-3	11.5±0.0	1235.8±53.1	11.3±0.0	2038.8±75.5
≤SFA	32.8±0.0	3518.6±164.9	29.5±0.3	5333.2±120.8
≤MUFA	27.3±0.0	2926.0±113.7	36.3±0.3	6566.4±157.4
≤PUFA	25.1±0.3	2685.2±79.5	20.6±0.7	3740.7±246.0
n-3	15.5±0.1	1658.9±55.1	9.3±0.1	1678.9±76.6
n-6	6.2±0.0	664.5±22.1	8.5±0.6	1546.6±156.5
n-9	15.8±0.2	1696.8±86.1	24.2±0.5	4385.1±63.0
n-3/*n*-6	2.5±0.0		1.1±0.1	
DHA/EPA*	0.8±0.0		1.3±0.0	

[1] mean ± standard error; $n = 3$

*Docasahexanoic acid to eicosapentanoic acid ratio

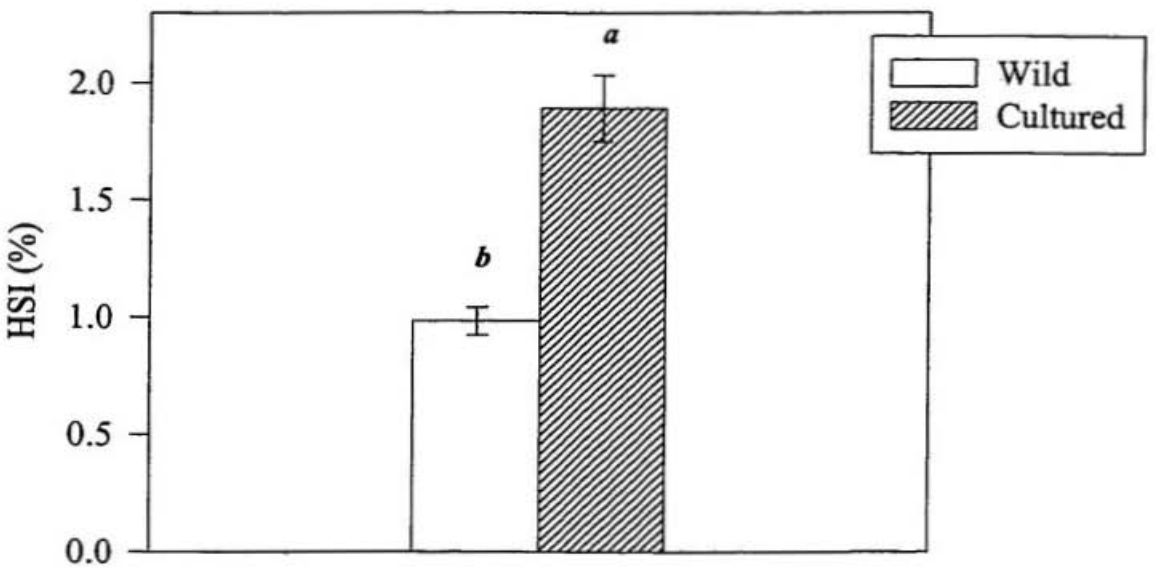

Figure 3.1 Hepatosomatic indices (%) of wild inshore and cultured yellowtail flounder. Vertical bars represent standard error. $n = 12$. Letters indicate significance.

Table 3.2 Nutrient analysis results of wild (inshore and offshore) and cultured yellowtail flounder.

	Wild		Cultured
	Inshore	Offshore	
Moisture (%)	80.7 ± 0.12*	78.96 ± 0.15	76.95 ± 0.19
Protein (%)	74.7 ± 2.18	75.83 ± 1.94	50.2 ± 2.54
Lipid (%)	3.67 ± 0.54	4.58 ± 0.99	24.63 ± 1.01
Ash (%)	15.94 ± 0.05	15.57 ± 0.08	12.36 ± 0.32
Carbohydrate (%)	1.11 ± 0.5	0.87 ± 0.36	2.96 ± 0.73
Gross Energy (kcal/g)	4.43 ± 0.14	4.57 ± 0.08	5.17 ± 0.17

* indicates mean ± the standard error; $n = 3$.

flounder. Protein content was lower in cultured fish than in wild fish ($p = 0.001$, one-way ANOVA) and cultured whole-body fish were higher in lipid content ($p < 0.001$, one-way ANOVA). There were also significant differences in ash content, with cultured fish again containing lower ash ($p < 0.001$, one-way ANOVA) than the two wild groups. There was no significant differences in carbohydrate content between groups but energy content (kcal/g) was significantly higher in the cultured fish when compared to the inshore wild group, which was expected because of their high lipid content.

Sex differences were examined for moisture and protein content of the muscle and liver Moisture content of the muscle was significantly higher in wild fish ($p < 0.001$, two-way ANOVA; Figure 3.2a) but there were no differences between male and female moisture content ($p = 0.770$, $n = 4$ ♂ and $n = 8$ ♀, two-way ANOVA; Appendix Table A.11). Protein content was significantly higher ($p < 0.001$, two-way ANOVA; Figure 3.2b) in the muscle of wild fish but because there was only one female from the wild population, sex differences could not be tested statistically. However, the protein content of this wild female fish was much higher than the male flounders. When cultured fish were examined separately, female fish had significantly higher protein content in the muscle than male fish ($p = 0.01$, one-way ANOVA; $n = 2$ ♂ and $n = 4$ ♀; Appendix Table A.11 and Figure 3.3). Moisture content of the liver was significantly higher in wild fish ($p = 0.001$, two-way ANOVA; Figure 3.4) than in cultured fish, but there were no significant differences in liver moisture content between the sexes ($p = 0.06$, two-way ANOVA). Significant differences were also seen in ash content

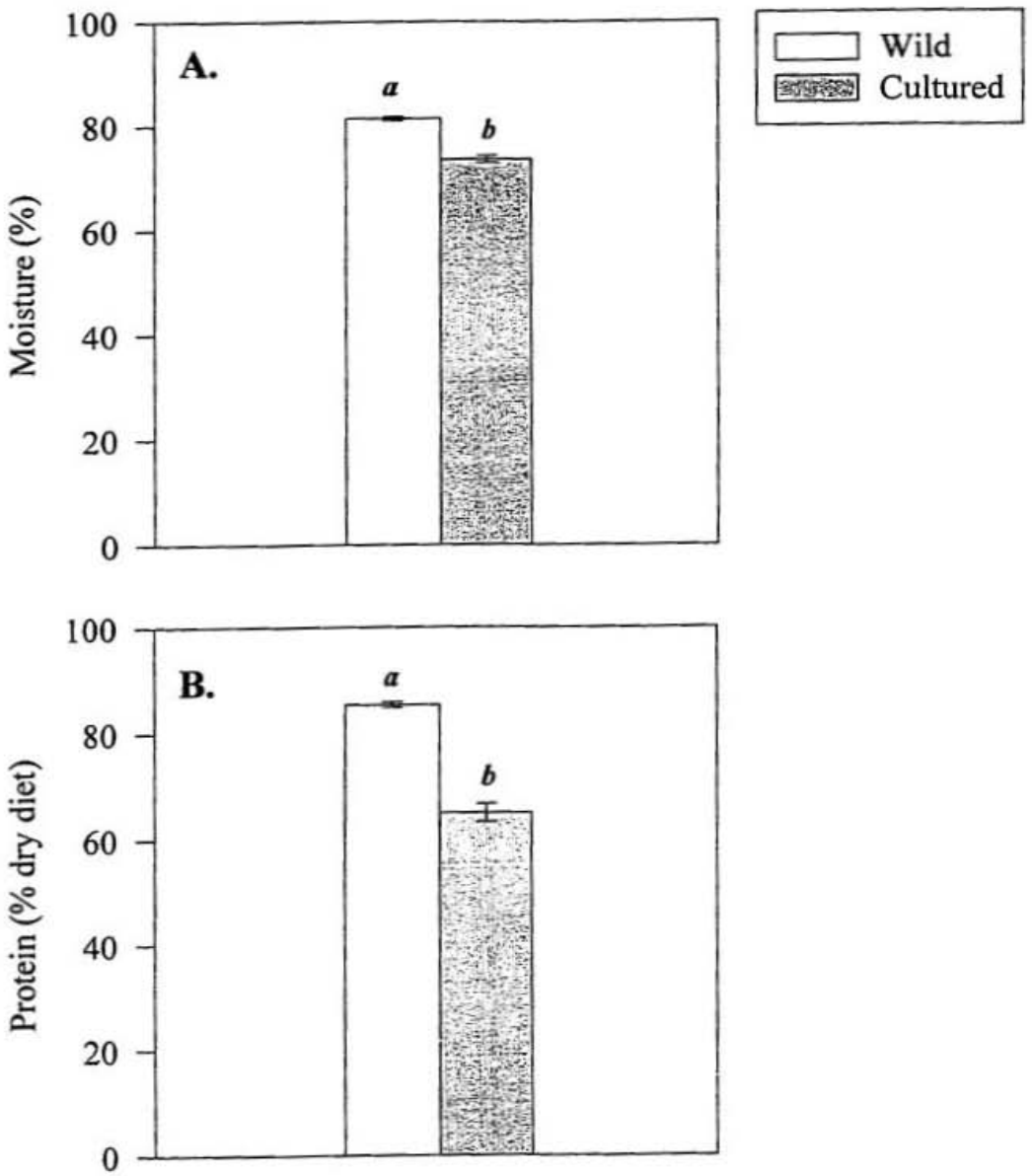

Figure 3.2 Moisture (A) and protein (B) content (%) in the muscle of wild and cultured yellowtail flounder. Vertical bars represent standard error. $n = 12$. Letters indicate significance.

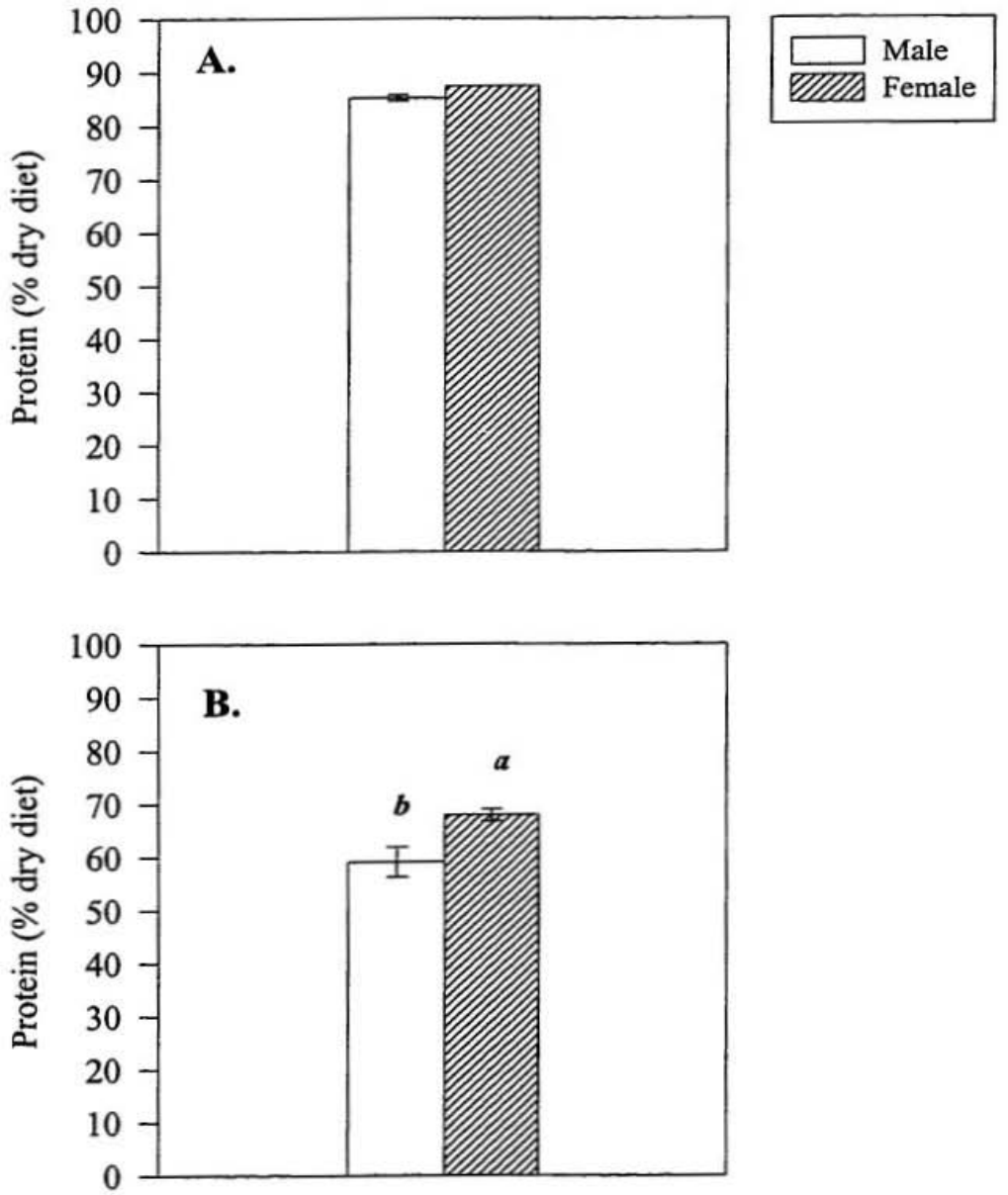

Figure 3.3 Protein content (%) in the muscle by sex of wild (A) (n = 11 males, 1 female) and cultured (B) (n = 2 males, 4 females) yellowtail flounder. Vertical bars represent standard error. Letters indicate significance.

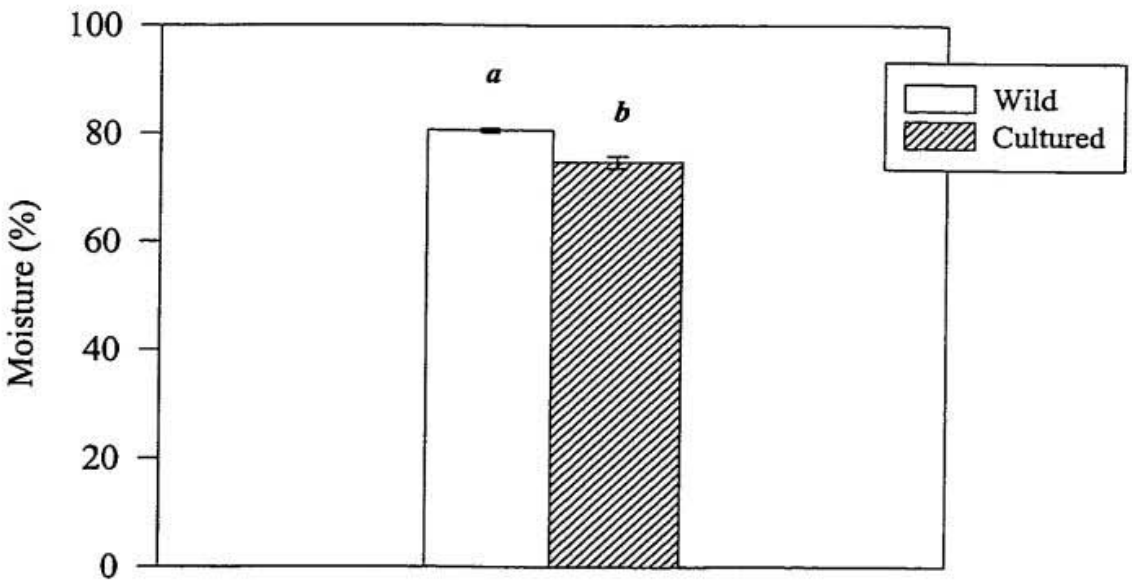

Figure 3.4 Moisture content (%) in the liver of wild and cultured yellowtail flounder. Vertical bars represent standard error. $n = 6$. Letters indicate significance.

of the muscle, with cultured fish having a higher ash weight than wild fish ($p = 0.011$, one-way ANOVA).

3.3.4 LIPID CLASS ANALYSIS

The sum of the total lipid classes in the livers of wild yellowtail flounder was lower than cultured yellowtail flounder ($p = 0.052$, one-way ANOVA), and significantly lower in the muscle (Figure 3.5a and b, $p = 0.004$, one-way ANOVA). In wild fish muscle and liver, respectively, lipid was composed mainly of triacylglycerols (60%, 21%), phospholipid (34%, 65%) and sterols (2%, 6%) whereas in cultured fish, lipid was composed of triacylglycerols (87%, 75%), phospholipids (5%, 12%) and acetone-mobile polar lipid (5%, 4%). In terms of absolute amounts, cultured fish contained significantly higher levels of TAG in their liver ($p = 0.047$, one-way ANOVA; Figure 3.6a) and also in their muscle ($p = 0.004$, one-way ANOVA; Figure 3.6b). Phospholipids in both the muscle and liver showed the opposite trend, with wild fish showing higher absolute amounts ($p = 0.007$ and $p < 0.001$, respectively, one-way ANOVA; Figure 3.6).

There was no relationship between sex and lipid content of either the liver ($p = 0.490$, one-way ANOVA) or muscle ($p = 0.367$, one-way ANOVA) in wild and cultured fish. Wild yellowtail flounder had higher levels of lipid in their liver ($3.6 \pm 0.7\%$) than in their muscle ($1.3 \pm 0.3\%$), as did cultured fish ($14.0 \pm 4.3\%$ liver, $8.4 \pm 1.7\%$ muscle, Figure 3.5a and b). Wild fish had higher levels of structural lipids, such as sterol and phospholipid, while cultured fish contained higher levels of intermediate and by-product lipids, such as free fatty

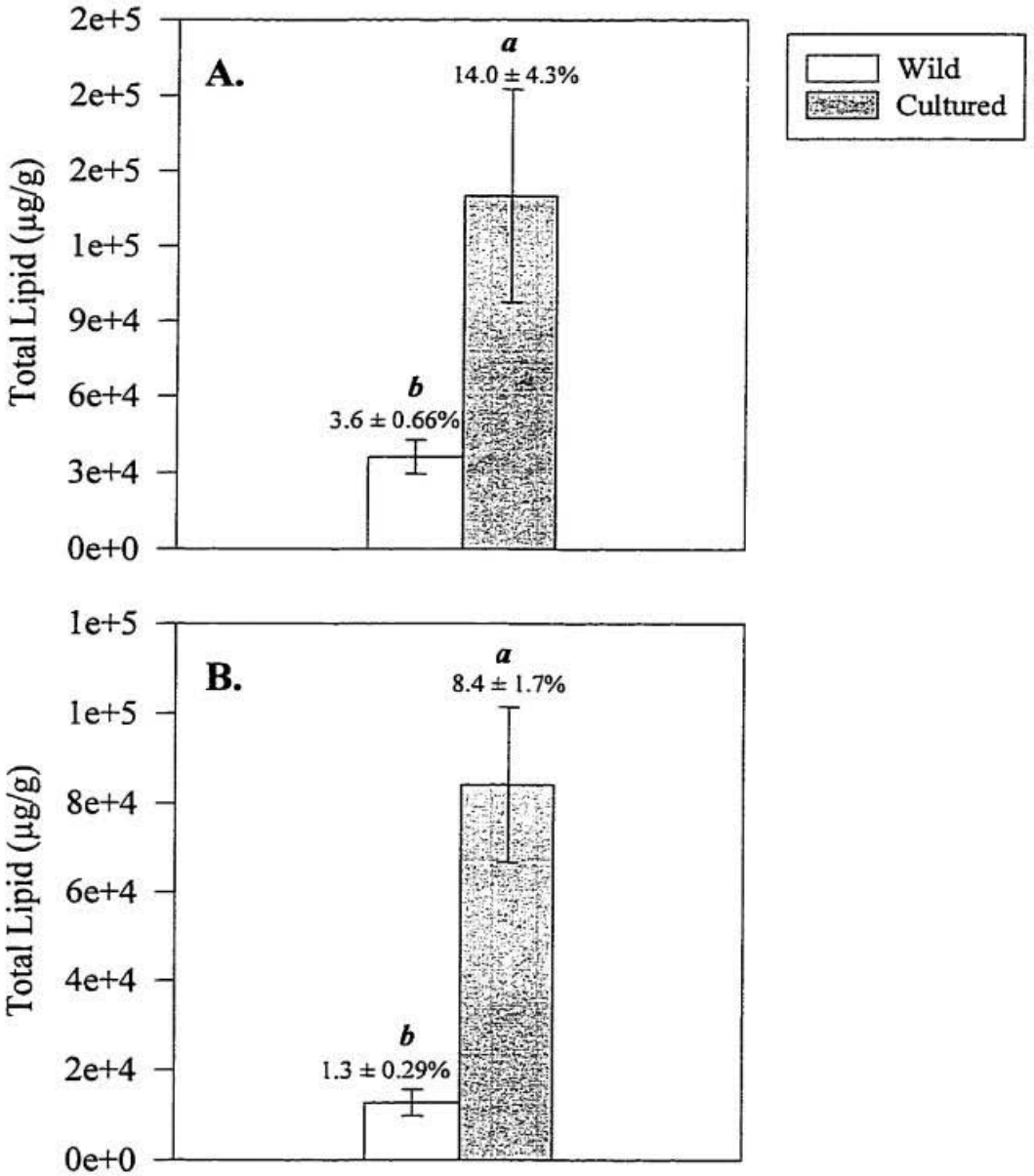

Figure 3.5 Total lipid content (µg/g) in liver (A) and muscle (B) of wild and cultured yellowtail flounder. Vertical bars represent standard error. $n = 6$. Values on bars represent % dry weight ± se. Letters indicate significance.

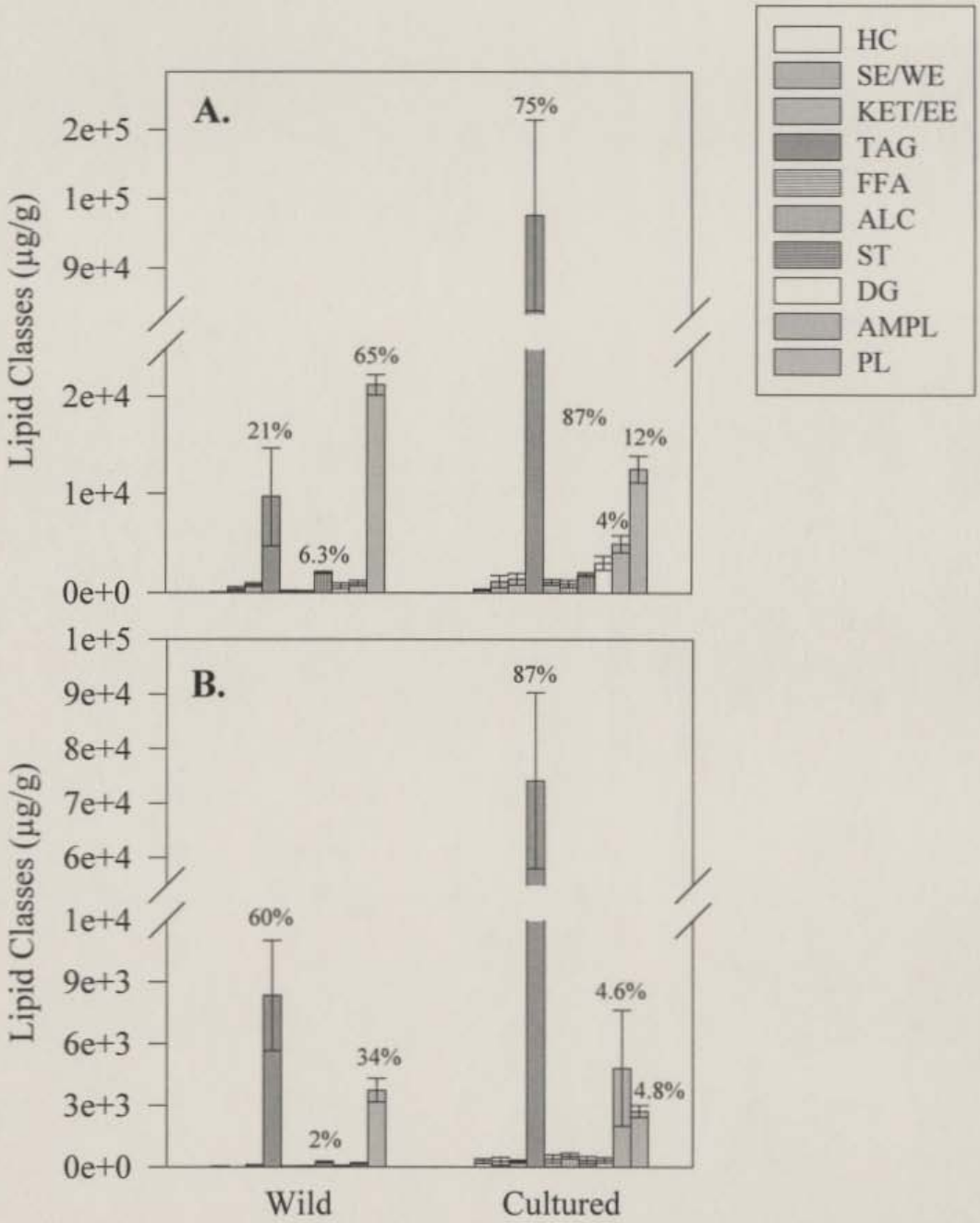

Figure 3.6 Lipid class composition (µg/g) for wild ($n = 6$) and cultured ($n = 6$) yellowtail flounder liver (A) and muscle (B). Class percentages of total lipid written above the bars. Vertical bars represent standard error. $n = 6$. Abbreviation list: HC - hydrocarbons, SE/WE - steryl and wax esters, KET/EE - ketones and ethyl esters, TAG - triacylglycerol, FFA - free fatty acids, ALC - alcohols, ST - sterols, DAG - diacylglycerol, AMPL - acetone-mobile polar lipids, PL - phospholipid.

acids, diacylglycerols and acetone-mobile polar lipids, which contain monoglycerols. Cultured fish also have the highest levels of storage lipids and hydrocarbons.

3.3.5 FATTY ACID ANALYSIS

The analysis of the Moore-Clark diets indicates that the dietary fatty acid analysis of the neutral muscle portion in cultured fish resembled the fatty acid composition of the diets. Of the total fatty acids in the muscle and liver, respectively, 16:0 constituted the major saturated fatty acid (12.6%, 14.6%); 18:1 the major monounsaturated fatty acid (15.1%, 13.8%) and DHA the major PUFA (18.2%, 27.7%; Table 3.3 and Table 3.4). Livers contained lower 16:1 than muscle and higher 20:4*n*-6. Cultured fish followed the same trends in their muscles and livers, but had higher percentages of 18:1 and lower percentages of PUFA.

Levels of almost all fatty acids (mg/100 g) were higher in cultured fish, which was not unexpected, since levels of total lipid were much higher in these fish. However, the differences between wild and cultured levels of fatty acids were much smaller when comparing polar fractions of muscle and liver; in fact, the level of EPA in the polar liver fraction is significantly higher in wild fish ($p = 0.027$, one-way ANOVA). The ratio of *n*-3/*n*-6 was significantly higher in wild yellowtail in all fractions ($p < 0.05$, one-way ANOVAs) and the DHA/EPA ratio varied with the separated fraction.

Table 3.3 Fatty acid composition of total, polar and neutral lipid fractions of the liver of wild and cultured yellowtail flounder.

	Total				Polar				Neutral			
	Wild (n = 6)		Cultured (n = 6)		Wild (n = 6)		Cultured (n = 6)		Wild (n = 6)		Cultured (n = 6)	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100 g	% wt	mg/100 g	% wt	mg/100 g	% wt	mg/100 g
14:0	1.6±0.3	47.2±21.1	3.8±0.2*	501.2±211.7	1.1±0.1	16.7±1.7	1.3±0.1	13.5±1.5	2.3±0.4	30.5±20.9	4.1±0.2*	487.8±210.8
15:0	0.2±0.0	7.5±3.4	0.3±0.0	42.0±21.7	0.2±0.0	2.8±0.2*	0.2±0.0	1.5±0.4	0.3±0.1	4.7±3.4	0.3±0.0	40.5±21.4
16:0	14.6±0.8	353.5±53.8	13.3±0.2	1635.5±602.3	15.6±1.1	243.2±18.5*	14.9±0.3	150.3±17.4	11.9±1.2	110.2±54.8	13.1±0.3	1485.2±604.2*
16:1	5.5±1.1	172.6±86.1	9.4±0.4*	1171.1±442.3	3.3±0.2	51.0±2.8*	3.1±0.3	31.3±3.8	8.5±1.7	121.5±86.0	10.2±0.3	1139.8±440.1*
16:2	1.1±0.1	30.6±10.0	1.4±0.2	138.7±25.8*	1.1±0.1*	16.5±1.4*	0.6±0.1	6.2±0.8	1.0±0.2	14.1±10.1	1.5±0.2	132.6±25.7*
17:0	1.2±0.1*	34.7±13.2	0.8±0.1	86.5±22.7	1.2±0.2*	19.1±3.4*	0.6±0.0	6.0±0.6	1.3±0.1*	15.7±10.2	0.8±0.1	80.5±22.5*
18:0	5.8±0.6*	138.9±22.7	2.1±0.4	200.8±29.6	6.5±0.8	103.5±17.4	6.9±0.5	69.7±8.5	6.3±1.5*	35.4±6.3	1.5±0.3	131.1±26.3*
18:1	13.8±1.3	390.9±145.4	25.1±1.0*	3093.0±1127.4*	11.7±0.4	183.7±14.2	15.2±0.5*	151.7±13.9	16.8±1.7	207.1±133.5	26.3±0.9*	2941.4±1129.3*
18:2n-6	1.1±0.2	33.6±15.4	5.1±0.2*	576.1±167.5*	0.8±0.1	12.5±1.4	2.6±0.1*	25.5±2.2*	1.6±0.2	21.1±14.3	5.4±0.3*	550.6±167.5*
18:3n-3	0.3±0.0	9.1±3.7	0.7±0.0*	92.3±39.5	0.2±0.0	3.7±0.4	0.3±0.0	3.2±0.6	0.4±0.1	5.3±3.6	0.7±0.0*	89.1±39.6
18:4n-3	0.1±0.0	3.6±0.8	0.5±0.0*	64.6±24.8*	0.1±0.0	1.3±0.3	0.1±0.0	1.0±0.2	0.3±0.1	2.4±1.1	0.6±0.0*	63.6±24.8*
20:1	3.3±0.5	99.4±44.0	3.6±1.0	641.1±430.7	2.7±0.3	42.0±6.9*	2.0±0.6	19.3±5.5	4.5±0.7	57.3±38.0	3.7±1.0	621.8±425.4
20:2n-6	0.4±0.1	12.5±6.2	0.6±0.0	63.3±19.6*	0.3±0.0	5.2±1.0	0.3±0.0	3.0±0.4	0.5±0.1	7.3±5.3	0.6±0.0	60.3±19.5*
20:3n-6	0.2±0.1	5.3±3.1	0.2±0.0	16.8±2.9*	0.2±1.1	2.7±1.1	0.1±0.0	1.2±0.2	0.1±0.1	2.6±2.1	0.2±0.0	15.6±2.8*
20:4n-6	3.8±0.2*	93.9±17.5	1.8±0.4	155.8±12.1*	4.2±0.6	67.3±13.9	5.5±0.6	54.8±7.7	4.6±1.0*	26.7±4.8	1.3±0.3	100.9±6.2*
20:5n-3	15.4±0.5*	387.4±78.5	10.9±0.8	1164.3±259.6*	15.4±0.6*	240.2±12.9*	10.7±0.3	107.9±13.0	15.2±0.7*	147.2±74.8	11.1±0.9	1056.4±261.1*
22:1	0.4±0.1	14.0±7.9	2.0±1.1	458.2±381.5	0.2±0.0	3.1±0.4	0.4±0.3	4.3±3.4	0.8±0.2	10.9±7.7	2.1±1.1	453.9±378.1
21:5n-3	0.3±0.1	8.4±3.9	0.7±0.1*	76.6±19.2*	0.2±0.0	2.8±0.2	0.2±0.0*	2.1±0.3	0.4±0.1	5.6±3.8	0.8±0.1*	74.5±19.1*
22:5n-3	3.3±0.2	82.5±17.3	3.9±0.4	397.6±74.2*	3.0±0.2	46.1±1.6*	2.8±0.2	27.5±3.1	4.0±0.4	36.5±16.8	4.0±0.4	370.0±75.7*
22:6n-3	27.7±2.4*	637.1±50.7	14.0±1.3	1601.4±510.1	32.4±0.5	508.1±20.9*	32.3±0.7	328.3±40.6	19.1±2.5*	129.0±34.3	11.6±0.9	1273.1±511.1
≤SFA	23.4±0.7*	581.9±110.5	20.3±0.4	2466.1±880.1	24.5±0.6	385.3±24.0*	23.8±0.5	241.0±26.5	22.1±1.9	196.6±94.4	19.9±0.3	2225.0±881.2*
≤MUFA	23.0±3.0	676.8±283.1	40.0±2.9*	5363.5±2377.3	17.8±0.6	279.9±20.8*	20.8±1.2	206.6±19.9	30.6±4.0	369.9±265.0	42.3±2.5*	5156.9±2368.2
≤PUFA	52.5±2.5*	1273.5±194.0	38.3±2.6	4208.8±1105.1*	56.7±0.3	889.9±43.5*	54.8±1.1	554.6±63.9	46.2±2.9*	383.6±158.7	36.3±2.3	3654.2±1114.1*
n-3	46.6±2.7*	1115.5±148.6	29.5±2.2	3240.0±858.4*	51.0±1.0*	797.3±29.8*	45.9±0.9	465.9±56.2	38.7±2.5*	318.2±128.5	27.5±1.9	2774.1±862.5*
n-6	5.5±0.3	145.3±42.0	7.6±0.6*	811.9±184.4*	5.4±0.7	87.6±17.1	8.5±0.6*	84.5±8.9	6.8±0.7	57.6±25.8	7.5±0.6	727.4±188.4*
n-9	13.6±1.4	390.7±149.7	25.4±1.8*	3402.2±1500.8*	11.4±0.5	179±14.5	14.6±0.6*	145.6±13.3	17.0±1.8	211.7±137.3	26.7±1.6*	3256.6±1497.7*
n-3/n-6	8.8±0.8*		5.0±1.2		10.3±1.4*		5.6±0.4		5.8±0.5*		3.7±0.2	
DHA/EPA*	1.8±0.2		1.8±0.6		2.1±0.1		3.0±0.1*		1.3±0.2		1.1±0.1	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on estimates from TLC-FID. Significantly higher levels of fatty acids (*) are indicated.

Table 3.4 Fatty acid composition of total, polar and neutral lipid fractions of the muscle of wild and cultured yellowtail flounder.

	Total				Polar				Neutral			
	Wild (n = 6)		Cultured (n = 6)		Wild (n = 6)		Cultured (n = 6)		Wild (n = 6)		Cultured (n = 6)	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100 g	% wt	mg/100 g	% wt	mg/100 g	% wt	mg/100 g
14:0	3.8±0.3	42.2±13.4	6.1±0.2*	450.1±106.9*	1.4±0.2	3.8±0.7	2.2±0.3*	6.0±1.0	4.8±0.1	38.4±13.5	6.3±0.1*	444.0±106.8*
15:0	0.5±0.1	5.7±2.4	0.4±0.1	26.6±5.5*	0.2±0.1	0.5±0.2	0.2±0.1	0.7±0.3	0.6±0.1	5.2±2.3	0.4±0.1	25.9±5.5*
16:0	12.6±0.4	133.2±34.9	11.6±0.3	845.3±196.3*	14.0±1.3	39.6±8.1	12.6±0.7	35.2±6.8	11.7±0.3	93.6±33.9	11.5±0.3	810.2±197.6*
16:1	11.3±1.1	127.7±43.7	11.9±0.3	878.3±205.6*	3.3±0.3	8.4±1.3	4.2±0.4	11.7±2.4	15.3±0.6*	119.3±43.2	12.3±0.3	866.7±205.2*
16:2	1.0±0.1	11.8±3.8	1.5±0.1*	108.1±22.6*	0.7±0.1	1.9±0.4	0.6±0.1	1.9±0.6	1.1±0.1	9.9±3.8	1.5±0.1*	106.2±22.5*
17:0	0.8±0.1	8.7±2.6	0.6±0.0	46.9±10.5*	0.5±0.0	1.4±0.3	0.5±0.1	1.5±0.5	0.9±0.1*	7.3±2.5	0.7±0.0	45.5±10.4*
18:0	3.0±0.4*	29.5±5.7	1.6±0.2	111.3±21.5*	5.6±0.3	15.7±2.8	5.6±0.8	17.8±6.7	1.9±0.2*	13.8±3.9	1.4±0.1	93.5±19.6*
18:1	15.1±0.5	163.9±47.0	20.0±0.6*	1490.5±358.5*	12.8±0.4	35.7±6.1	15.4±0.6*	42.6±7.7	16.1±0.6	128.2±44.9	20.3±0.6*	1447.8±358.4*
18:2n-6	1.6±0.1	18.0±5.6	6.1±0.3*	447.8±98.5*	0.7±0.1	2.1±0.4	3.2±0.1*	9.0±2.0*	2.0±0.1	15.9±5.5	6.3±0.3*	438.8±98.2*
18:3n-3	0.6±0.1	6.9±2.3	1.4±0.1*	99.0±22.3*	0.4±0.1	1.2±0.3	0.3±0.1	1.0±0.2	0.6±0.1	5.7±2.1	1.4±0.1*	98.1±22.3*
18:4n-3	1.3±0.2	16.3±6.3	2.0±0.1*	146.2±35.4*	0.1±0.0	0.2±0.1	0.8±0.4	2.0±0.8	1.8±0.2	16.1±6.3	2.0±0.1	144.2±35.4*
20:1	6.2±0.8	63.6±15.4	4.6±1.3	346.3±144.1	3.6±0.3	9.8±1.8	3.7±0.8	9.7±1.8	7.8±1.1	53.8±14.4	4.6±1.3	336.6±143.6
20:2n-6	0.4±0.1	4.4±1.2	0.3±0.0	26.1±7.5*	0.2±0.0	0.5±0.1	0.2±0.1	0.7±0.3	0.5±0.1	3.9±1.2	0.3±0.0	25.4±7.5*
20:3n-6	0.2±0.0	2.1±0.7	0.2±0.0	12.4±4.2*	0.1±0.0	0.3±0.1	0.3±0.1	1.2±0.9	0.2±0.0	18.±0.7	0.2±0.0	11.2±3.6*
20:4n-6	1.7±0.2*	17.1±3.6	1.0±0.1	67.5±14.2*	3.0±0.2*	8.3±1.4	2.4±0.3	7.1±2.1	1.2±0.1	8.8±2.7	0.9±0.1	60.3±13.3*
20:5n-3	16.2±1.0*	178.7±60.3	12.5±1.0	899.3±194.9*	15.5±0.9*	40.9±6.4	12.4±0.7	34.4±6.5	16.6±1.2*	137.9±59.0	12.5±1.1	864.9±194.6*
22:1	0.8±0.1	8.5±1.9	2.5±0.9	192.7±92.7	0.4±0.3	0.2±0.2	1.1±0.4	3.1±1.3	1.2±0.1	8.2±2.0	2.5±0.9	189.6±92.3
21:5n-3	0.5±0.0	5.6±1.6	0.7±0.1	50.2±10.4*	0.4±0.1	1.0±0.3	0.3±0.1	0.9±0.3	0.6±0.0	4.6±1.5	0.7±0.1	49.3±10.3*
22:5n-3	4.1±0.3*	43.9±13.3	3.0±0.3	216.2±48.2*	5.1±0.8	13.2±2.8	3.6±0.3	3.6±0.3	3.7±0.2	30.7±11.9	3.0±0.3	205.6±46.9*
22:6n-3	18.2±2.3*	178.9±37.2	12.1±0.9	850.1±176.8*	32.0±1.6	91.7±16.9	30.3±1.5	30.3±1.5	11.2±0.8	87.2±31.1	11.1±0.7	762.9±175.9*
≤SFA	20.6±0.5	219.3±58.2	20.3±0.5*	1480.3±335.9	21.7±1.1	60.9±11.1	21.1±0.6	21.1±0.6	20.0±0.4	158.4±55.9	20.2±0.6	1419.0±337.0*
≤MUFA	33.5±1.9	363.7±105.0	39.0±2.3	2907.8±767.7	20.0±0.7	54.2±8.9	24.5±1.8*	24.5±1.8	40.4±1.5	309.5±101.6	39.7±2.3	2840.7±767.2*
≤PUFA	44.9±2.2	471.9±127.9	39.2±2.5	2814.8±600.8*	57.5±1.0	159.4±27.1	53.8±1.7	154.1±34.6	38.5±1.7	312.5±120.4	38.5±2.5	2660.8±597.1*
n-3	39.0±2.4*	407.2±109.3	7.6±0.4*	2015.8±427.9*	52.9±0.9*	146.8±25.1	46.6±1.4	133.0±29.3	32.1±1.8	260.4±102.9	27.4±2.1	1882.8±424.6*
n-6	15.1±0.6	41.5±10.8	19.2±0.6*	553.8±118.7*	4.1±0.1	11.2±1.9	6.1±0.3*	18.0±5.1	3.9±0.2	30.3±9.9	7.7±0.4*	535.7±117.7*
n-9	16.0±0.6	171.9±47.4	21.6±1.3*	1620.7±432.7*	12.8±0.3	34.8±5.8	15.9±0.8	43.9±8.2*	17.7±0.8	137.0±45.2	21.9±1.3*	1576.8±432.7*
n-3/n-6	10.1±0.7*		3.7±0.2		13.1±0.4*		7.8±0.4		8.4±0.8*		3.6±0.2	
DHA/EPA	1.2±0.2		1.0±0.1		2.1±0.2		2.5±0.2		0.7±0.0		0.9±0.1*	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on estimates from TLC-FID. Significantly higher levels of fatty acids (*) are indicated.

Polar fractions of liver and muscle contained the most PUFA, with liver containing more than muscle. The saturated fatty acid content of polar lipid in the liver was dominated by 16:0 in both wild and cultured fish, while 18:1 was the dominant monounsaturate. DHA was the major PUFA, and EPA the second-largest contributor, in a ratio of about 2:1. The fatty acids 18:1and 18:2*n*-6 were significantly higher on a percentage basis in the polar fraction of cultured fish liver, but 20:5*n*-3 was significantly higher in wild fish. These trends were also true for the muscle polar portion.

The neutral portion of the liver showed the same trends, except 14:0 and 20:1, took on greater importance and 18:0 was reduced. The fatty acids 14:0, 18:1, and 18:2*n*-6 were significantly higher on a percentage basis in cultured yellowtail neutral liver, but 18:0, 20:4*n*-6, 20:5*n*-3 and 22:6*n*-3 were present in significantly higher proportions in the wild fish. Again, the fatty acid profile of neutral muscle was similar to liver, but there were no significant differences between 20:4*n*-6 and 22:6*n*-3 as there was between wild and cultured fish in the neutral liver portion. The total fatty acid composition tended to follow the same trends as the neutral portion, with the same significance attributed to the same fatty acids. The only exception was that in the total fatty acids of the muscle, there was also a significantly higher proportion of 20:4*n*-6 in the wild fish.

3.4 DISCUSSION

Yellowtail flounder is considered a "lean" fish, with total lipid content reportedly under 5% of fillet weight (Exler and Weihrauch, 1976). According to Sargent *et al.* (1989), marine flatfish contain less storage fat than pelagic fish. The analyses presented here are in agreement with this for wild yellowtail, although the total fat for cultured fish was much higher, placing it at the same level as halibut (Haug *et al.*, 1988). Yellowtail flounder in this study showed a different fatty acid composition than that of plaice (Owen *et al.*, 1972), indicating that these flatfish species possibly utilize lipids differently. Values were similar to those found for wild halibut (Haug *et al.*, 1987) for fatty acid composition of the polar lipid of muscle and liver, but were higher in EFA of the neutral lipid of these. As well, the composition of white muscle was similar between halibut and yellowtail flounder; however, the composition of liver in halibut showed higher levels of neutral lipid than wild yellowtail.

Lipid values found for wild yellowtail flounder agree with values from the literature for other analyses done on yellowtail fillet (1.2% lipid of tissue weight; Exler, 1975). Fatty acid values in this study are comparable with those published by Exler (1975) and even closer to the values reported by Kinsella (1987), such as the 423 mg/100g from Kinsella's data, and the 471.9 mg/100 g reported here for total PUFA in the fillet (see Table 3.4). The findings of the research herein is also very similar, albeit higher, than the value of 0.35 WW reported by Krzynowek and Murphy (1987). However, it is not known which methods (of extraction, methylation, etc.) were employed and which section of muscle was used in the analyses, if all the fillet was used, or at what time of year the analysis was performed.

Because only a small section of the upper left side of the top fillet was used in the analysis herein, it may have a slightly different fatty acid composition than that used by Kinsella (1987). In addition, the wild yellowtail flounder used in this study were captured during late summer-early fall, at a time when feeding may have been somewhat elevated after the spring and early summer spawning. Tissue analyses of fish taken during the fall and winter months have been shown to be lower in phospholipids than other times of the year, but higher in triacylglycerols (Lapin, 1977). Sexual status is another factor to be taken into consideration, but that is probably not a factor with yellowtail in this study, as most fish analysed were not mature. Finally differences in geographical area may influence the amount of unsaturation in the tissues of fish (Lapin, 1977).

There were no differences in lipid composition between males and females at this age, as was seen in other studies (Haug *et al.*, 1988), but there were differences in the protein content. There were some early maturing males present among the wild and cultured fish, but differences between males and females are probably not due to sexual status at this point, as mentioned, because there were no statistical differences found. Instead it might be, although this should be investigated further, that females grow faster than males, as they seem to be laying down more protein in their muscles at this time. Apparently in mature halibut, females grow faster than males (Haug *et al.*, 1988) but little is known of sexual growth differences between juvenile fish. Yellowtail were chosen for this experiment on the basis of size and sexual status, so that they would be comparable, however, it is worth noting that different life cycles influence the proximate composition of fish (Shearer, 1994).

Yellowtail flounder juveniles and young adults raised in captivity have different body compositions than those captured from the wild. This has been seen in other studies of halibut (Haug *et al.*, 1988), turbot (Sheenan *et al.*, 1994) and plaice (Owen *et al.*, 1972). Blaxter (1975) reported that hatchery-reared brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) had lower levels of protein and ash but higher levels of fat than wild fish. It is commonly known that diet can cause differences in tissue composition of fish.

High levels of neutral lipid in the muscle and low levels in the liver suggest that yellowtail flounder liver, like that of salmon, does not accumulate lipid, but metabolizes it. The HSI of cultured yellowtail was significantly higher than wild fish, and this may be indicative of problems metabolizing excess lipid from the diet of these fish. In addition, livers of cultured fish were higher in lipid and lower in moisture than livers in wild yellowtail. Levels of moisture are known to have an inverse relationship with levels of fat (Shearer, 1994). Although it does not seem that yellowtail normally store energy in the form of lipid in their livers, domestic yellowtail, which are fed a high lipid diet, seem to be doing this. In addition, captive reared flatfish get much less exercise than pelagic fish, or wild flatfish thus they respond by accumulating fat (Shearer, 1994). This is not favoured in the culture of yellowtail, or in aquaculture in general, as growth energy is diverted from lengthening the fish (Grant *et al.*, 1998). The condition index, or measure of "fatness" was significantly higher for cultured fish, indicating these fish may be obese with respect to the yellowtail captured from the wild, as condition index is a measure of how fast weight is put on relative to length. Grant *et al.* (1998) suggest that the largest fish in domestic cod

populations will probably have the fattiest livers due to social hierarchies within the population. It is difficult to say whether this is the case here, but it implies that diet is not the only factor which may have an effect on the well-being and growth of cultured fish. It is also suggested that future studies should focus on the effect of feed formulation on energy deposition, especially total protein energy to total energy, in order to reduce the accumulation of fat in the liver (Grant *et al.*, 1998).

Wild yellowtail flounder consume benthic invertebrates, such as polychaete worms and crustaceans (Langton and Bowman, 1981; Libey and Cole, 1979; Sargent *et al.* 1989). These invertebrates are not rich in body oils but have high quantities of phospholipids rich in *n*-3 PUFA, which they obtain by eating *n*-3 PUFA-rich phytoplankton landing on the sand (Sargent *et al.*, 1989). Parrish *et al.* (1996) described the lipid and fatty acid composition of a species of polychaete worm (*Nephthys ciliata*) from Conception Bay, Newfoundland (same location as the wild inshore yellowtail were captured) living at low temperatures. Although it cannot be said with certainty whether yellowtail eat this particular species of worm, they are known to consume members of the same genus (Langton and Bowman, 1981) and it is of interest that the lipid composition of yellowtail flounder liver closely resembles the lipid composition of *Nephthys ciliata*. These polychaetes have high levels of sterols, which are also present in high levels in wild yellowtail flounder. Sterols are lipids which have a structural function in the modulation of saturation in the membrane, and may be related to high levels of PUFA (Parrish *et al.*, 1996). In addition, the polar fatty acid portion of the same species of polychaete worm closely resembles the neutral portion of the muscle and

liver of the wild yellowtail flounder, with *Nephthys ciliata* having an EPA level of 26.7%, a DHA level of 9.2%, and an AA level of 1.3%. This corresponds with the yellowtail flounder, which has 16.6% (EPA), 11.2% (DHA), and 1.2% (AA) in the neutral portion of the muscle, and gives a similar ratio of DHA/EPA of under 1.0. In agreement with this, Parrish *et al.* (1996) reported that the fatty acid composition of the neutral portion of an animal reflects its diet.

Wild fish had significantly higher proportions of the essential fatty acids, AA, DHA and EPA, in total and neutral fatty acids in the liver and significantly higher levels of EPA in all portions of the muscle. It seems that EPA may have a more important function in metabolism of fish than has previously been thought. As well, a level of AA such as is seen in wild yellowtail flounder might correspond to the fact that AA is abundant in benthic invertebrates found in shallow coastal waters (Greene and Selivonchick, 1987) . In addition, the *n*-3/*n*-6 ratio was consistently and significantly higher in wild yellowtail at about 10:1, whereas cultured fish had a lower ratio, at about 4:1.

Similarly, the fatty acid composition of the neutral portion of the liver and muscle of cultured yellowtail flounder resembles its commercial diet (Table 3.1 and Appendix Table B.1). Subsequently, hatchery-reared flounder have higher levels of TAG than their wild counterparts, as well as lower levels of structural components such as PL and sterols, implying that much of the energy from their diet is going into storage and not growth. Cultured fish have higher levels of intermediate and by-product lipids such as FFA, DAG, and AMPL. It is unknown why these are higher in cultured fish but may be a result of an

inclusion of "unnatural" plant and/or animal fat, or pigments in commercial diets. Because the proportion of EFA in cultured flounder was lower than that of the wild fish, it is assumed that the commercial diet does not contain sufficient levels of these fatty acids. If, in the wild, most PUFA is obtained from the phospholipid of invertebrates, more attention should be paid to feeding PUFA in a different form than the methyl or ethyl ester form currently used in any commercial diets (Greene and Selivonchick, 1987; Lochmann and Gatlin, 1993b).

As well as moisture, lipid and protein differences, there were also differences seen in the ash content, with wild fish having significantly higher levels of whole-body ash. This may be due to abnormal or impaired ossification of the skeleton, often seen in hatchery-reared fish (Blaxter, 1975). It may also be due to the fact that yellowtail flounder in the wild ingest a portion of sand when they consume benthic prey (Libey and Cole, 1979) and may have higher levels of minerals in their whole body. The wild fish may have been malnourished, but this is unlikely as all nutritional analyses are comparable to values found in the literature. There were no differences in whole-body composition between wild inshore yellowtail and offshore groups, except for moisture content, which was higher in offshore fish, and the reason for this is unknown.

Because of this, it seems that the commercial diets commonly fed to cultured yellowtail flounder are inadequate to meet their needs. Inappropriate levels of protein and lipid have been associated with slow growth, high food conversion ratios, health problems such as fat cell necrosis syndrome, enlarged fatty livers (fatty liver syndrome) and excessive obesity (Anon., 1997; Bricknell *et al.* 1996; Grant *et al.*, 1998; Post, 1987). In some

yellowtail fed the Moore-Clark diet, large yellowish globules of fat were observed in the portion of muscle under the dorsal and anal fin, and this may be an accumulation of fatty tissue filling the spaces between the muscle to the fin rays (personal observation). Excessive lipid deposition is known to cause necrosis of tissue in other finfish, particularly when the tocopherol concentration (Vitamin E) is low (Bricknell *et al.*, 1996). It seems that, in general, flatfish have similar body compositions, and therefore a diet should be formulated based on the natural diet of these animals. Otherwise, feeding yellowtail diets such as ones specifically formulated for salmonids or other fatty fish may not only change the lipid metabolism and excessive fat deposition in this species, but, in addition, the consumer will not get a lean, high-protein, white-fleshed fish.

Since the body composition of wild yellowtail neutral lipid is similar to the wild diet, it seems obvious that feeding these flatfish commercial diets which are based on fish oils, such as the mixture of anchovy and mackerel oils used by Moore-Clark to make this diet (Keith Weir, pers. obs.), is inappropriate. Based on these results, it is suggested that a diet for yellowtail flounder should have the following characteristics: higher protein (greater than 45%) and lower fat (less than 20%), with higher levels of *n*-3 PUFA, specifically EPA. This might be partly accomplished by using different marine fish oils in the formulation such as Menhaden or a mixture of these and cod liver oil. Studies would have to be done to find the optimum levels of each of these nutrients and to determine whether this type of diet would promote the growth necessary to warrant some of the expensive components.

CHAPTER 4.0 ESSENTIAL FATTY ACID REQUIREMENTS OF JUVENILE YELLOWTAIL FLOUNDER

4.1 INTRODUCTION

Lipids are important macronutrients in the diets of all animals and are especially significant in fish nutrition. Dietary lipid supplements supply energy and essential fatty acids (EFA) for growth and development of fish. EFA are vital in the physiological functioning of fish and other animals but, in marine fish, EFA cannot be synthesized *de novo*, and therefore must be obtained in the diet. In recent years, certain EFA have gained importance in human nutrition, where they have been credited with lowering serum lipids such as cholesterol, and are therefore beneficial in reducing or preventing ischemic heart disease and tissue inflammation disorders (Sargent *et al.*, 1989).

In nature, the marine food chain is rich in long-chain polyunsaturated fatty acids (PUFA), as microalgae contain up to 50% of their dry weight as *n*-3 PUFA (Sargent *et al.*, 1989). This allows the zooplankton that ingest phytoplankton to biosynthesize large quantities of wax-esters (may be up to 92% in some algal species (Virtue *et al.*, 1993)), which, in turn, are high in long chain fatty alcohols and fatty acids. As the food chain levels increase, the amount of *n*-3 PUFA present decreases slightly but still remains a large source of these fatty acids. Benthic invertebrates, which are consumed by flatfish such as yellowtail flounder, are not as substantial in levels of *n*-3 PUFA, but because these invertebrates tend to ingest phytoplankton which settle on the bottom, they are still a high source of *n*-3 PUFA.

However, flatfishes do not contain as high levels of neutral lipid as pelagic fish (Sargent *et al.*, 1989).

It is now accepted that fish require certain long-chain polyunsaturated fatty acids (PUFA) as EFA, with freshwater and marine fishes differing somewhat in their requirements for EFA. Freshwater fish require more fatty acids of the *n*-6 origin, while marine fish require primarily *n*-3 fatty acids (Ackman and Kean-Howie, 1994). Linolenic acid, or 18:3*n*-3, is required by all fish, but marine fish are particularly susceptible to reductions in docosahexaenoic acid (DHA; 22:6*n*-3) and eicosapentaenoic acid (EPA; 20:5*n*-3). The quantitative *n*-6 fatty acid requirement of marine fish, specifically 20:4*n*-6 (arachidonic acid), is not established, but it is generally agreed they are required in some fish diets (Castell, 1979).

Freshwater fish are able to convert quantities of shorter chain fatty acids (eg. 18:3*n*-3) into long chain fatty acids (eg. DHA and EPA) by processes of desaturation and elongation. Most marine species are unable, or at least are very limited in their capacity to do this, and therefore diets must contain an adequate amount of PUFA. It has been suggested that flatfish may be able to elongate and desaturate shorter chain fatty acids to some degree (Takeuchi, 1997), but this has not been proven. Unfortunately, this equates to higher costs because of the rising expense of obtaining fish oils.

EFA play a vital role in the metabolic functioning of fish. They are structurally significant in maintaining fluid membranes of the cells, especially in poikilothermic animals, which have to tolerate changing temperature. Animals which live at lower temperatures

usually display higher levels of *n*-3 PUFA in polar membranes, or phospholipids. They may also be used to a lesser extent in the neutral lipids or triacylglycerols for storage. The *n*-6 fatty acids are the precursors of eicosanoids, which are hormone-like substances employed in tissue inflammation and blood clotting (Sargent *et al.*, 1989). For these reasons, and because EFA are closely related to growth and food efficiency, it is of increasing interest to expand our understanding of the subject.

Another important aspect of fatty acid nutrition is the ratio of DHA, EPA and AA within the diet. Because EFAs play such a complex role in environmental adaptation and it is necessary to maintain a balance of saturated and unsaturated fatty acids for temperature acclimation (Greene and Selivonchick, 1987), and because there is competition between the enzymes for fatty acid substrates, a balance of the correct fatty acids is essential.

Diets that are deficient in EFA may affect performance and the overall health of fish, such as decreased growth, high food conversion ratios, increased mortalities and high hepatosomatic indices (Ibeas *et al.*, 1994). Bell *et al.* (1985) found that fish fed a diet deficient in *n*-3 PUFA developed gill abnormalities and the authors believed this was due to a loss of chloride cells. EFA-deficient fish have displayed "shock syndrome", or loss of consciousness, when exposed to a stressor (Sargent *et al.*, 1989). Other symptoms of EFA deficiencies include fin and jaw erosion, pale, swollen livers or livers which have high neutral lipid levels, enlarged hearts (myocarditis), high moisture content of whole-body tissue, and a swelling of liver mitochondria. It is interesting to note that diets which contain

n-3 PUFA above the requirements of a species are likely also to show some of the same symptoms of deficiency including poor growth and conversion of food (Sargent *et al.*, 1989).

Most marine fish studied thus far have shown a quantitative requirement for PUFA of approximately 0.5 - 2.0% of their dry diet. Juvenile red seabream (*Pagrus major*) have a requirement for 1% EPA or 0.5% DHA in the diet; those fish fed only trace amounts of each showed mortality, poor appetite and fatty livers after one week of feeding (Takeuchi *et al.*, 1990). Juvenile gilthead seabream demonstrated a requirement of 1.9% dietary *n*-3 PUFA and at the lowest level of dietary *n*-3 PUFA, 0.76%, showed poor growth rates, food conversion efficiencies and a large increase in liver lipids (Ibeas *et al.*, 1994). Wantanabe *et al.* (1989) showed that juvenile striped jack (*Longirostris delicatissimus*) had a requirement for dietary *n*-3 PUFA of 1.7% of dry diet and diets low in *n*-3 PUFA caused an increase in 18:1 in the polar lipids of the body and liver. Finally, Owen *et al.* (1972) showed that plaice (*Pleuronectes platessa*) require *n*-3 PUFA and those fed diets low in PUFA accumulate triacylglycerols for storage. Turbot also lack the desaturase enzymes necessary to chain elongate oleic acid, linoleic acid or linolenic acid (Cowey *et al.*, 1976); however, they are able to grow just as well on 4% linolenic acid as with 0.57% *n*-3 PUFA of dry diet (Takeuchi, 1997). While these studies have been useful in pinpointing the range of requirements for marine fish in general, there have been few studies done on cold-water fish and no studies done on the requirements of yellowtail flounder for these essential fatty acids. As well, there has been no qualitative descriptions of deficiency signs in yellowtail flounder.

Finally in addition to improving growth, feed utilization and avoiding the EFA deficiency symptoms, farmers and researchers may use diets to manipulate the body composition of their fish for appropriate human nutrition. This is something that could be explored once it is known how much *n*-3 PUFA is required in the diet, as well as how long it takes to change the body composition of fish raised in cold waters.

Because of the cold waters yellowtail inhabit and their natural diet of invertebrates, it was hypothesized that juvenile yellowtail flounder would require a high level of dietary *n*-3 PUFA. The objectives of this study were to determine:

1) The optimum range of *n*-3 PUFA level required in the diet of yellowtail flounder.
2) How the different levels were utilized in yellowtail by examining body composition.
3) If any EFA-deficiency symptoms caused by a diet low in *n*-3 PUFA.
4) If a commercial diet, Biokyowa (Miyako Kagaku Co., Ltd.), and standard formulated ICES (INVE, Ghent, Belgium) diets were acceptable in the above respects for raising yellowtail flounder.

4.2 MATERIALS AND METHODS

4.2.1 FISH

Yellowtail flounder eggs were collected from broodstock at the Ocean Sciences Centre in Logy Bay, Newfoundland during summer 1996. Larvae were fed algae, rotifers, *Artemia* and finally dry food (Biokyowa Fry Feed B-250 to C-700) until January 1997. Immediately before the experiment, they were fed dry food and a small daily supplement of *Artemia*. They were kept in large round black flow-through tanks with a light intensity of about 600 lux and photoperiod constant at 24 hours. At the time of the experiment, juveniles were weighed, measured, and transferred to the experimental set-up described below.

4.2.2 FEED AND FEEDING

Fish were fed three times (4% body weight) at 0900 h, 1500 h and 2100 h every two days and buckets were siphoned every other day. This was assumed to be the least stressful method and best food ration for fish of this size. It was noted that this quantity was more than needed for the appetites of the fish. After every sampling period (every two weeks), the food ration was changed based on bulk weight of juveniles per bucket. Standard diets, developed by the International Council of Exploration of the Sea (ICES/INVE; Ghent, Belgium; diet formulations, Appendix Table B.2) were formulated to contain three different levels of *n*-3 PUFA. These were 0.4% PUFA per dry weight of diet, 1% and 2.5% (lot numbers 00113, 00114 and 00115, respectively). A commercial diet, Fry Feed Biokyowa, served as a benchmark for the growth performance of other diets (lot number 16104/20101; see

Appendix Table B.4 for manufacturer's specifications). Over the course of the experiment, the fish gape size changed and particle size was changed accordingly, from 800-1200 μm to >1200 μm by about halfway through the experiment.

4.2.3 EXPERIMENTAL DESIGN AND CONDITIONS

A total of one hundred eighty 0+ juveniles (with an initial weight of 1.9 ± 0.0 g) were placed randomly in 12 buckets (13 L containers with a bottom area of 490 cm^2), so that there were 15 fish per bucket and 45 fish per treatment. These buckets were placed in three larger tanks which served as a water bath for the buckets. Stocking density was about 50% coverage of the bottom of the tanks. Total gases were monitored throughout the experiment with a total dissolved gas monitor (Model TBO-F, Common Sensing, Inc., Idaho, USA) and kept at acceptable levels. Water flow ranged from about 500 to 800 ml/min. Individual air and water tubing ran to each bucket in a flow-through system. Fish were kept at an 18L:6D photoperiod (altered by automatic timers which came on at 1900 h and went off at 0100 h) and light intensity (fluorescent lighting from above) was about 600 lux and did not vary more than 100 lux between buckets at any one time. Temperature was 7.3 ± 0.01°C throughout the course of the experiment and salinity was about 32 ‰.

Tanks were randomly assigned to one of the four diets, which served as the treatments. After the tenth week of experimentation, the experiment had to be moved to another location. Buckets were transferred to another area; other than the physical move, the

experimental conditions were kept the same. This posed stress on the fish, and was reflected in weight gain.

4.2.4 MEASUREMENT

Initially 10 fish were taken for biochemical analyses (5 for proximate analysis, 5 for fatty acid analysis and measurement of hepatosomatic indices). Every two weeks, fish were removed from buckets and wet weights and standard lengths recorded. Fish were removed with a net, patted dry on a paper towel and placed in a beaker of seawater on a tared electronic balance and weighed to the nearest 0.01 g. The fish was then taken out and standard length quantified by placing it on a ruler, closing its mouth, and taking the length of the fish from the snout to the end of the vertebral column (to the nearest 0.1 cm). Fish were not fed the night before sampling. The experiment lasted for 12 weeks, at which point the fish in control buckets (fed Biokyowa) had at least doubled their body weight. There were no mortalities over the course of the experiment. At the end of the experiment, a sample of fish was removed for biochemical analyses and two fish per bucket were removed, frozen on dry ice and dissected later for HSI and fatty acid analysis (see Section 3.2.5 for details of dissections). Fish were killed with an overdose of MS-222.

4.2.5 BIOCHEMICAL ANALYSES

Proximate analysis was performed on diets and whole-body fish; because of the small numbers, six fish were pooled per treatment and triplicate samples performed for crude moisture, protein, lipid and ash of each. Thus, one sample was analysed in triplicate.

Details of the biochemical analysis are the same as those in Section 3.4.6, except there was no internal standard added to the extractions in this experiment. Therefore, all values are expressed as a percentage of total weight of lipid class and mg/100 g wet weight based on acyl lipid data from TLC-FID estimates (see Appendix D). Data is also provided as % area in Appendix Tables C.3 to C.6. As well, a number of the initial samples were found to be low in PUFA, high in free fatty acids and have Iatroscan chromatograms which showed a large amount of debris at the origin. It was assumed that these had oxidized, or undergone lipolysis due to a long storage time and may have dried out during this time. Values can be found in the Appendix (Tables C.3 to C.8).

4.2.6 STATISTICAL ANALYSIS

Wet weight, standard length, specific growth rate and hepatosomatic index over time were analysed using a three-way analysis of covariance (ANCOVA) to test differences between treatments. Explanatory variables were time, treatment and replicate, with time as the covariant. Biochemical differences were compared using a one-way analysis of variance (ANOVA). To determine at which point in the experiment differences became significant, ANOVA was used at each time; as well, differences among specific diets were

tested at the times that were significant (a time*treatment interaction) using Tukey's pairwise comparisons with a family error rate of 0.05. Minitab was used to test differences and significance level was set at $\alpha = 0.05$.

4.3 RESULTS

4.3.1 DIET ANALYSIS

Diets were re-analysed at the end of the experiment for nutrient analysis and fatty acid analysis (see Table 4.1 and 4.2). Re-analysis showed that actual composition of diets was similar to manufacturer's analysis, with slightly higher levels of total PUFA than described by the manufacturer. Diets were adequate for the experiment in that levels varied as per specifications, but were somewhat higher in *n*-3 and PUFA levels. This might have been due to some oxidation of fatty acids over the course of the experiment. It was noted that Bioyowa contained approximately 2.3% *n*-3 PUFA, which fell between the two highest ICES diets (1.5% and 3.8%, respectively, according to re-analysis).

4.3.2 GROWTH AND SURVIVAL

There were no mortalities in any of the treatments over the course of the experiment. All fish increased their weight and standard length over time, and by week 12, fish from the control group had doubled their weight and length had significantly increased (see Figure 4.1). Log weight gain was examined, rather than actual weight at each time, due to replicate effects. Overall, dietary level had a significant ($p = 0.016$, three-way ANCOVA; Appendix

Table 4.1 Nutrient and lipid class analysis of diets (Biokyowa and ICES) fed to cultured yellowtail flounder.

	Biokyowa		ICES	
	1.0 mm (2.2% PUFA)	0.4% PUFA	1% PUFA	2.5% PUFA
Nutrient (%)				
Moisture	0.5*	5.9	5.4	5.0
Crude Protein	55.7	58.5	58.0	58.8
Crude Lipid	13.5	10.1	9.6	8.6
Crude ash	14.2	6.4	6.2	6.3
Carbohydrate	16.1	19.1	20.8	21.3
Energy (kcal/g)	5.0	4.9	4.9	4.9

Lipid Classes	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Hydrocarbons	0.2	26.3±9.4	0.1	7.8±6.4	0	0	0	0
Steryl Ester/Wax Ester	0.8	138.2±1.6	0	0	0	0	4.6	546.2±26.1
Ketones/Ethyl Ester	8.7	1415.1±331.9	0.3	36.2±30.0	8.1	657.1±162.4	25.2	2994.2±75.6
Triacylglycerols	48.8	8030.0±419.0	74.5	7766.0±224.1	66.9	5349.3±1226.2	45.9	5440.8±86.6
Free Fatty Acids	1.5	253.2±12.9	0.3	35.3±7.5	0.3	24.0±7.5	0.1	15.4±1.0
Alcohols	0	0	0	0	0	0	0	0
Sterols	4.8	788.1±18.7	2.1	260.1±23.8	2.6	202.4±39.6	2.1	251.9±17.0
Diacylglycerols	2.3	370.0±11.2	1.5	157.4±53.2	1.0	83.0±25.5	2.5	291.8±28.2
Acetone-Mobile Polar Lipid	5.6	926.2±104.2	2.1	222.4±14.8	1.8	148.5±49.3	3.5	418.6±29.6
Phospholipid	27.3	448.2±35.8	27.3	1967.2±158.5	19.3	1546.6±358.6	16.1	1902.2±32.3
Total Lipid	16.4	16429.1±157.0	10.5	10452.4±460.8	8.0	8011.0±1869.1	11.9	11861.1±229.9

* $n = 3$

Table 4.2 Fatty acid analysis of diets (Biokyowa and ICES) fed to cultured yellowtail flounder.

	Biokyowa				ICES			
	1.0 mm (2.2% PUFA)		0.4% PUFA		1% PUFA		2.5% PUFA	
Fatty Acid	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
14:0	4.9±0.1*	3126.4±123.8	26.5±0.9	18670.0±6347.8	22.6±0.2	5078.5±1783.3	8.4±0.5	4143.5±328.3
15:0	0.2±0.0	115.4±26.7	0.1±0.0	94.1±25.1	0.2±0.0	37.0±11.7	0.1±0.0	54.8±1.5
16:0	20.6±0.1	13211.2±661.1	21.6±0.1	14976.3±4846.9	18.1±0.1	4066.4±1425.5	10.4±0.1	5139.9±157.1
16:1	4.9±0.1	3131.6±138.1	0.8±0.0	571.8±198.7	0.8±0.0	184.9±55.6	0.9±0.0	422.7±7.5
16:2	1.0±0.0	610.4±26.5	0.2±0.0	118.5±38.2	0.2±0.0	37.8±14.0	0.2±0.0	99.3±7.1
17:0	0.5±0.0	312.6±19.2	0.1±0.1	126.2±64.9	0.0±0.0	6.7±6.7	0.0±0.0	0.0±0.0
18:0	3.3±0.0	2114.6±111.3	16.7±0.6	11800.0±4039.9	13.4±0.0	2996.0±1040.6	7.2±0.2	3558.4±168.2
18:1	18.7±0.2	12024.9±579.7	10.0±0.3	6784.8±2094.8	10.0±0.2	2250.2±809.3	10.8±0.2	5304.8±45.5
18:2*n*-6	11.2±0.1	7190.8±399.4	17.9±0.9	12066.8±3635.5	15.8±0.2	3528.7±1177.7	13.4±0.3	6603.8±2.4
18:3*n*-3	1.9±0.0	1213.6±65.0	2.3±0.1	1574.3±472.7	2.2±0.0	484.8±166.6	2.1±0.1	1018.0±1.1
18:4*n*-3	0.1±0.0	89.7±5.1	0.0±0.0	0.0±0.0	0.3±0.0	57.4±21.7	0.1±0.0	54.7±6.8
20:1	4.1±0.0	2642.9±124.3	0.3±0.0	235.1±76.1	0.9±0.1	212.4±86.0	2.3±0.0	1117.8±32.1
20:2*n*-6	0.2±0.0	135.1±11.4	0.0±0.0	22.9±22.9	0.1±0.1	7.4±7.4	0.3±0.1	132.4±36.6
20:3*n*-6	0.1±0.0	76.4±7.6	0.0±0.0	0.0±0.0	0.1±0.1	19.8±19.8	0.3±0.1	132.6±26.1
20:4*n*-6	1.0±0.0	614.4±40.6	0.2±0.0	112.4±33.9	0.6±0.0	124.6±42.8	1.5±0.0	723.0±1.0
20:5*n*-3	12.8±0.2	8251.8±502.8	0.9±0.1	622.0±187.5	6.1±0.1	1369.1±460.5	18.3±0.3	9030.3±350.0
22:1	2.8±0.1	1770.6±50.9	0.2±0.0	152.5±35.1	1.0±0.1	227.4±89.2	3.5±0.3	1717.8±120.7
21:5*n*-3	0.4±0.0	251.4±10.5	0.0±0.0	0.0±0.0	0.4±0.0	77.7±19.7	0.9±0.0	456.9±6.3
22:5*n*-3	0.7±0.0	444.8±22.4	0.1±0.0	45.1±30.4	1.3±0.2	279.0±61.9	3.5±0.1	1739.8±70.3
22:6*n*-3	10.6±0.1	6853.2±418.6	1.9±0.1	1281.5±382.5	5.9±0.0	1315.5±444.1	15.5±0.0	7635.5±169.0
≤SFA	29.4±0.2	18880.2±886.2	65.1±1.6	45666.6±15312.5	54.4±0.3	12184.5±4254.4	26.1±0.7	12896.6±652.1
≤MUFA	30.5±0.3	19570.0±892.3	11.4±0.4	7754.2±2400.3	12.8±0.3	2874.9±1040.0	17.4±0.5	8563.1±50.5
≤PUFA	39.0±0.4	25151.1±1474.6	23.2±1.2	15725.3±4726.5	32.6±0.5	7264.0±2407.5	55.8±0.2	27527.0±523.5
n-3	24.7±0.3	15892.2±959.1	6.9±2.8	3604.4±918.7	16.1±1.9	3445.6±814.0	38.4±0.5	18972.2±708.8
n-6	12.5±0.1	8016.6±454.7	18.1±0.9	12202.1±3680.0	16.5±0.2	3680.5±1232.9	15.4±0.5	7591.8±64.2
n-9	3.8±0.1	2431.4±82.0	0.3±0.0	211.2±54.1	1.2±0.1	280.6±110.6	4.1±0.3	1997.3±112.7
n-3/*n*-6	2.0±0.0		0.4±0.1		1.0±0.1		2.5±0.1	
DHA/EPA	0.8±0.0		2.1±0.0		1.0±0.0		0.8±0.0	

* mean ± standard error; $n = 3$.

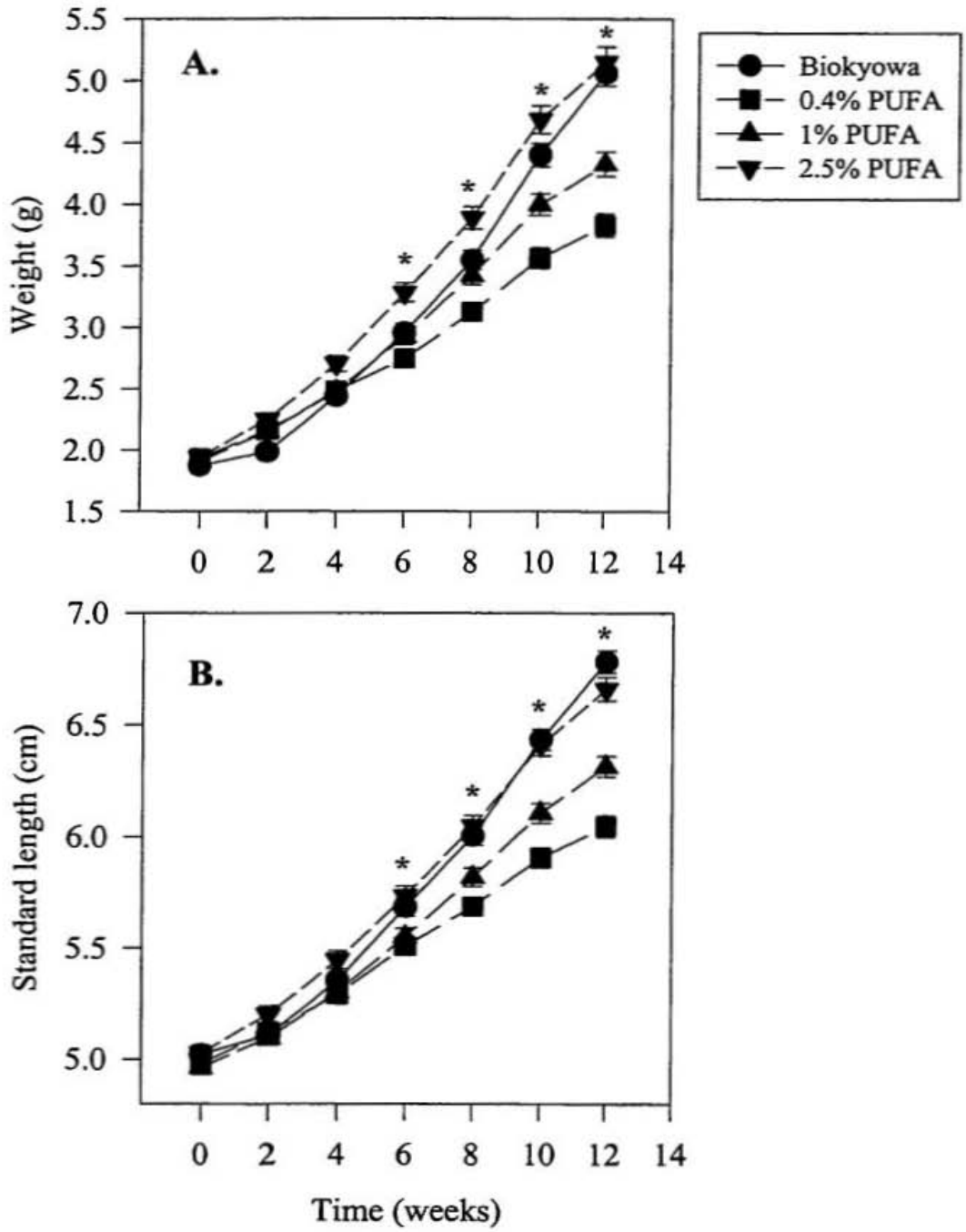

Figure 4.1 Average growth over time of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA. Vertical bars represent standard error. $n = 45$ per treatment. * indicates significance.

Table A.12) effect on wet weight gain but not on standard length gain ($p = 0.705$, three-way ANCOVA; Appendix A.13). There was a significant time*treatment interaction ($p < 0.003$ and $p = 0.032$ for weight and length, respectively), however, indicating that there were significant differences in growth at different times of the experiment. Thus, the slopes of the lines were significantly different. Fish fed the 2.5% PUFA diet grew as well as the control diet and there were no significant ($p > 0.05$, one-way ANOVA; Tukey's) differences between the two. Between weeks 10 and 12, weight gain was slightly depressed, and this is thought to be due to the transportation of the experiment locations. Differences became significant between weeks six and eight (Appendix Table A.12 and A.13), at which time, fish fed 0.4% PUFA had gained significantly less weight than the other diets, and fish fed 1% PUFA had gained significantly ($p = < 0.0001$, one-way ANOVA; Tukey's) less weight than fish fed the 2.5% PUFA or control diets. These differences increased over time, and by the end of the experiment, fish fed the 2.5% PUFA diet had gained significantly more weight than fish fed either the 0.4% or 1% PUFA diets ($p < 0.0001$, one-way ANOVA; Tukey's) but there were no differences between the 2.5% PUFA diet and the fish fed Biokyowa. Trends were similar for standard length as well ($p < 0.0001$, one-way ANOVA; Tukey's).

Replicate effects were significant ($p = 0.009$, three way ANCOVA) different, so differences in treatment for condition index ($p = 0.006$, three-way ANCOVA; Appendix A.14), could not be explored further. The cause of these replicate effects were unknown, but may have been due to inherent genetic or biological differences in the flounder or to some slight difference in lighting, noise or a combination of factors. Condition factors

increased for all treatments over the duration of the experiment, indicating all diets were acceptable (Figure 4.2). It is interesting to note that fish fed both the lowest PUFA diet, and the control diet had lower condition indices than fish fed the other diets.

4.3.3 SPECIFIC GROWTH RATE (SGR)

There were no replicate differences for specific growth rate ($p = 0.897$, three-way ANOVA; Appendix Table A.15). At most times, SGR of all groups tended to increase, except during the last two weeks; again this is thought to be caused by the moving of the experiment (Figure 4.3 and Figure 4.4). There were no significant ($p = 0.954$, three-way ANCOVA, Appendix Table A.15) differences in the specific growth rate of juveniles fed the different diets, but average SGR was lower for fish fed the 0.4% PUFA diet (0.82 % BW/day) and 1% diet (0.97% BW/day), while fish fed the control and 2.5% PUFA diets both had SGRs above 1.0 (1.19 and 1.20% BW/day, respectively). All fish decreased their SGR after the moving of the experiment, but it is interesting to note that after this time of stress, the groups fed the lowest levels of *n*-3 PUFA (also had lower levels of 20:4*n*-6), both had a lower SGR than fish fed the two higher levels of *n*-3 PUFA.

4.3.4 HEPATOSOMATIC INDEX

Hepatosomatic indices (HSI) did not change significantly ($p = 0.090$, three-way ANOVA, Appendix Table A.16) from the beginning to the end of the experiment, but at the end of the experiment, fish fed diets 1% and 2.5% PUFA had a significantly higher HSI than

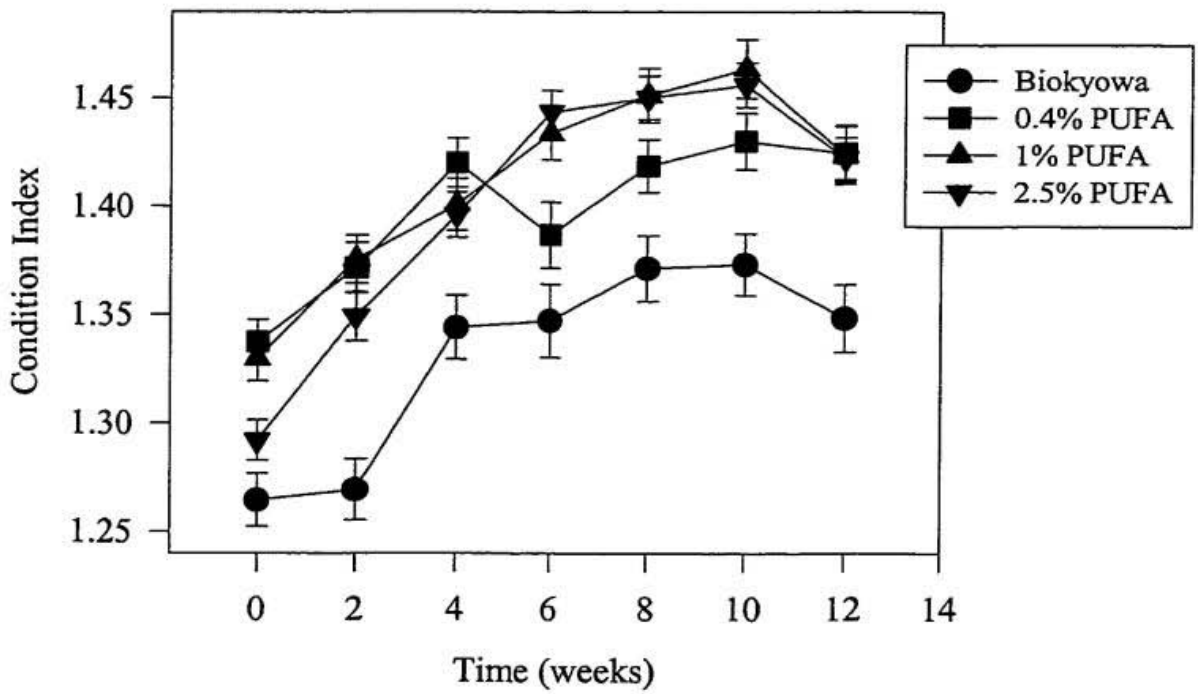

Figure 4.2 Average condition index for 0+ yellowtail flounder fed varying levels of dietary *n*-3 PUFA over the course of the 12 week experiment. Vertical bars represent standard error. $n = 45$ per treatment.

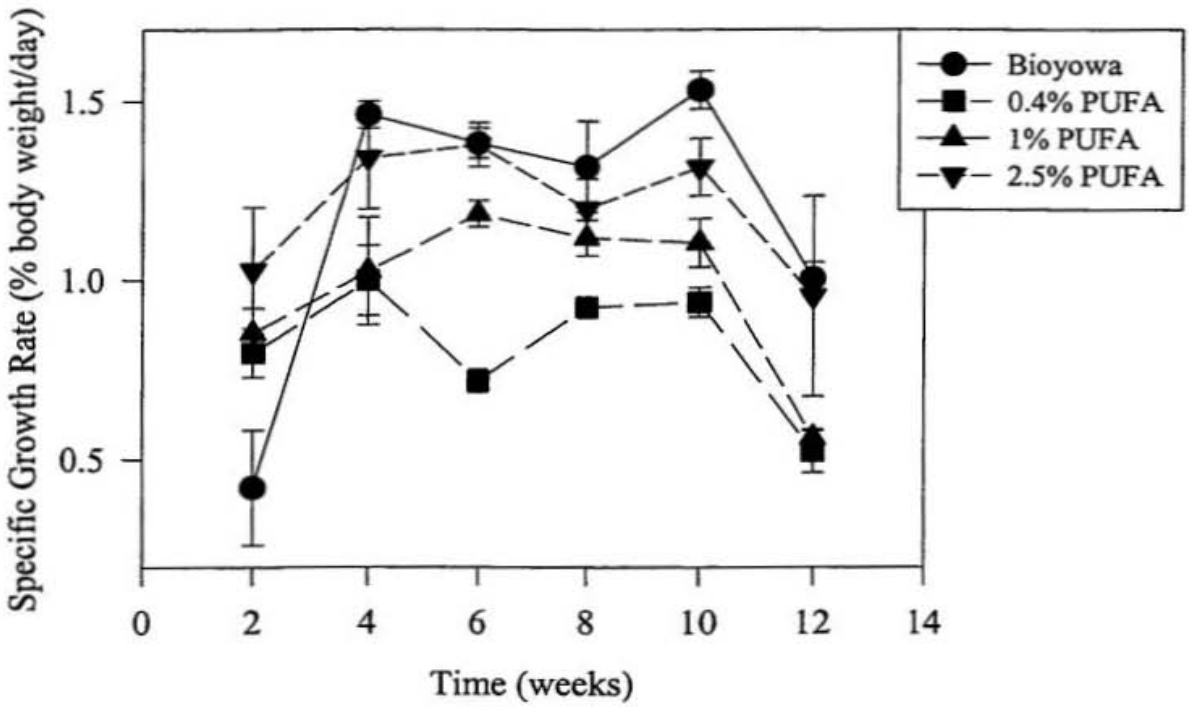

Figure 4.3 Average specific growth rate (SGR) for 0+ yellowtail flounder fed varying dietary levels of *n*-3 PUFA over the course of the experiment. Vertical bars represent standard error. $n = 45$ per treatment.

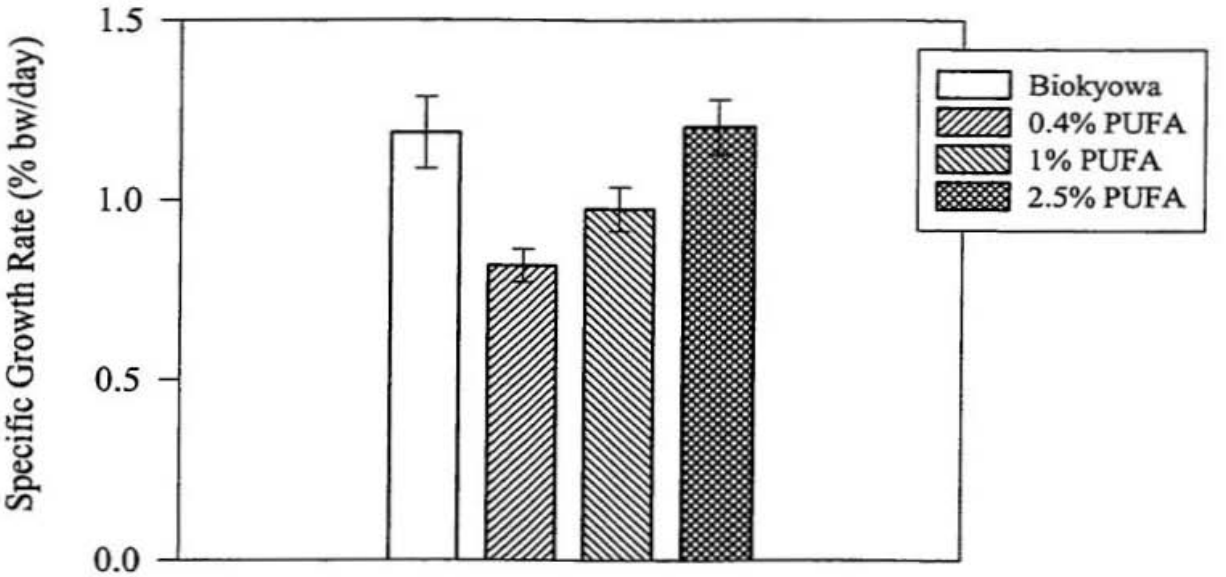

Figure 4.4 Average specific growth rate (SGR) for 0+ yellowtail flounder fed varying dietary levels of *n*-3 PUFA. Vertical bars represent standard error. $n = 45$ per treatment.

fish fed diets 0.4% and the control diet ($p = 0.018$, one-way ANOVA; see Figure 4.5). This result was unexpected.

4.3.5 NUTRIENT ANALYSIS

Body composition was affected by the level of *n*-3 PUFA in the diet. Whole-body moisture in fish fed 0.4% PUFA, 2.5% PUFA and the control diet, Biokyowa, increased significantly from the beginning of the experiment ($p = 0.009$, $p = 0.009$ and $p = 0.016$, one-way ANOVAs, respectively; Figure 4.6a). At the end of the experiment, however, there were no differences among the different groups in whole-body moisture content ($p<0.05$).

There were no significant differences either over time or among treatments in whole-body protein levels for juvenile yellowtail flounder. Fish fed the 1% and 2.5% *n*-3 PUFA diets had the highest levels of protein and the fish fed the control diet had the lowest level of protein (Figure 4.6b).

Fish fed the control diet and the diet highest in *n*-3 PUFA had significantly higher levels of whole-body lipid ($p = 0.004$, one-way ANOVA). There was no significant change over time in whole body lipid in fish fed the control diet, however, levels of lipids over time for fish fed the other diets were significantly lower ($p <0.05$, one way ANOVAs, see Figure 4.6c).

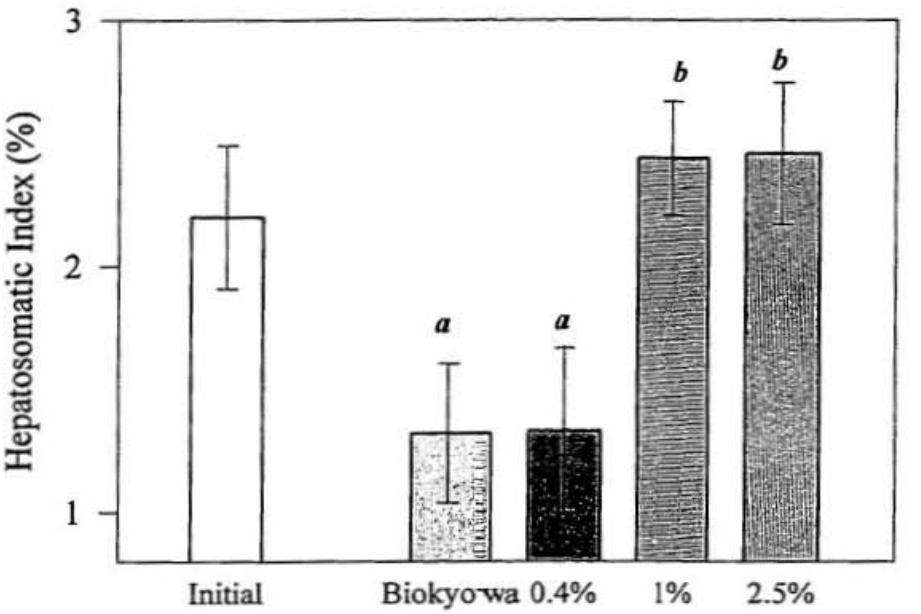

Figure 4.5 Average hepatosomatic indices (% liver weight/body weight) of 0+ yellowtail flounder fed varying levels of dietary *n*-3 PUFA at the beginning ($n = 5$) and end of the experiment ($n = 6$).Letters indicate significance.

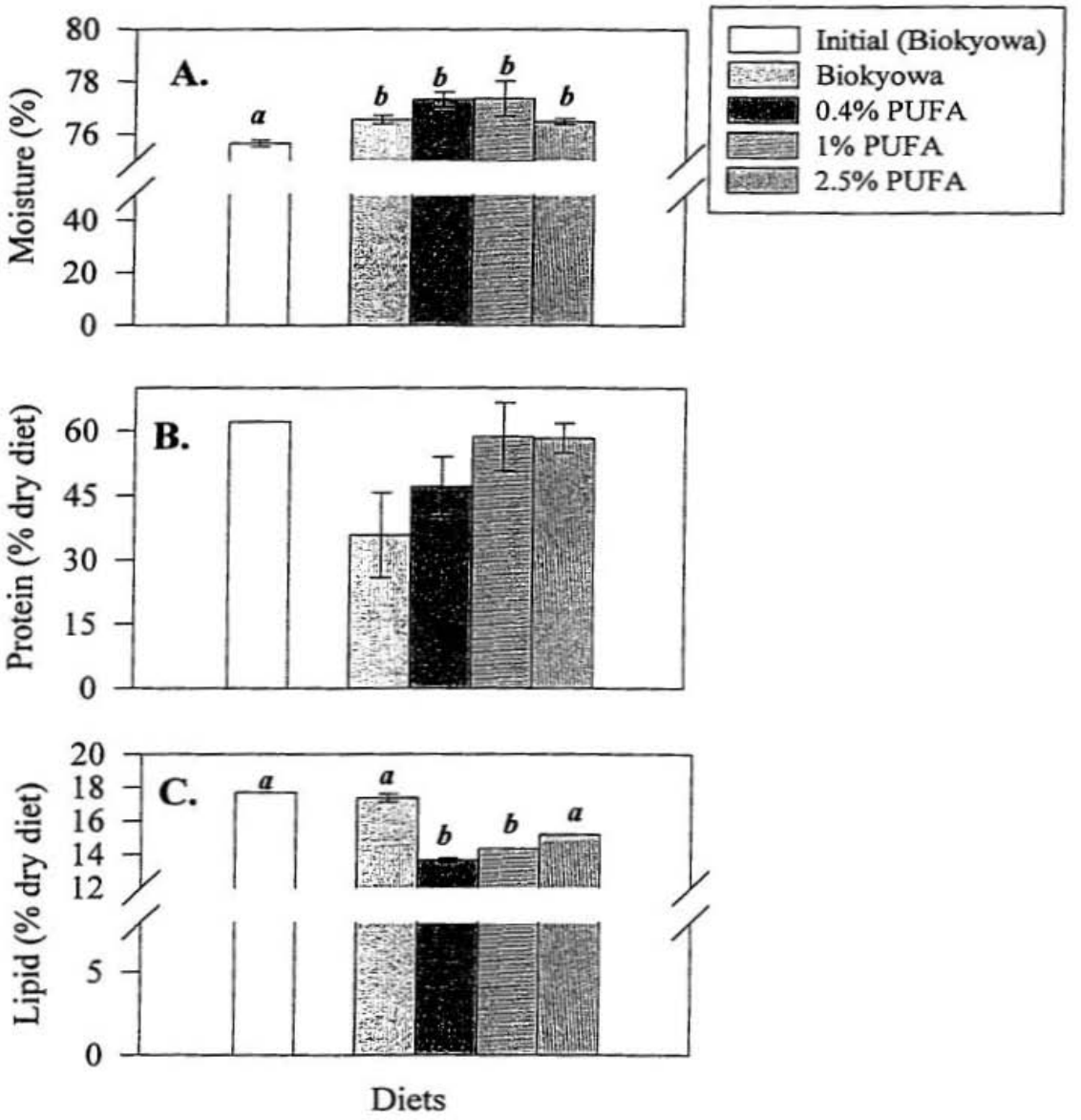

Figure 4.6 Moisture (A), protein (B), and lipid (C) content (%) of 0+ yellowtail flounder fed diets varying in *n*-3 PUFA. Vertical bars represent standard error. $n = 3$. Letters indicate significance.

4.3.6 LIPID ANALYSIS

Levels of *n*-3 PUFA in the diet also affected the composition of lipid in the muscle and liver of juvenile yellowtail flounder as well as the total lipid in these tissues (Figure 4.7). There were no significant differences in total lipid in the liver between fish sampled initially and fish sampled at the end of the experiment. There were significant increases, however, in fish fed the control diet, 1% and 2.5% diets, in total lipid in the muscle ($p = 0.03$, $p = 0.003$ and $p = 0.017$, one-way ANOVAs, respectively; Figure 4.7a). Yellowtail sampled initially from a population of hatchery-reared fish seem to have a relatively low lipid yield. Reasons for this may be that during storage time, some of the samples dried out, or that the initial fish were fed an *Artemia* supplement that may have become deficient in PUFA.

Although there were no significant differences ($p = 0.388$,one-way ANOVA, Appendix Table A.17) in total lipid in the liver in the fish fed different diets, Figure 4.7b shows that there may be a trend occurring, whereby fish fed 0.4% PUFA diet had higher levels of lipid in their livers at the end of the experiment. From Figure 4.8b, it can be seen that the highest proportion of this lipid is triacylglycerol ($p = 0.039$, one-way ANOVA, Appendix A.17) and that levels of this lipid class are higher in fish fed the lowest *n*-3 PUFA diet than in those fed the other diets. Conversely, the same group showed the same trends of low levels of total lipid in their muscle, and had lower levels of both triacylglycerols and phospholipids, although not significant (see Figure 4.8a).

Triacylglycerols accounted for up to 80% of total lipid in yellowtail in the anterior muscle region, indicating that this area is probably an energy storage area. From this

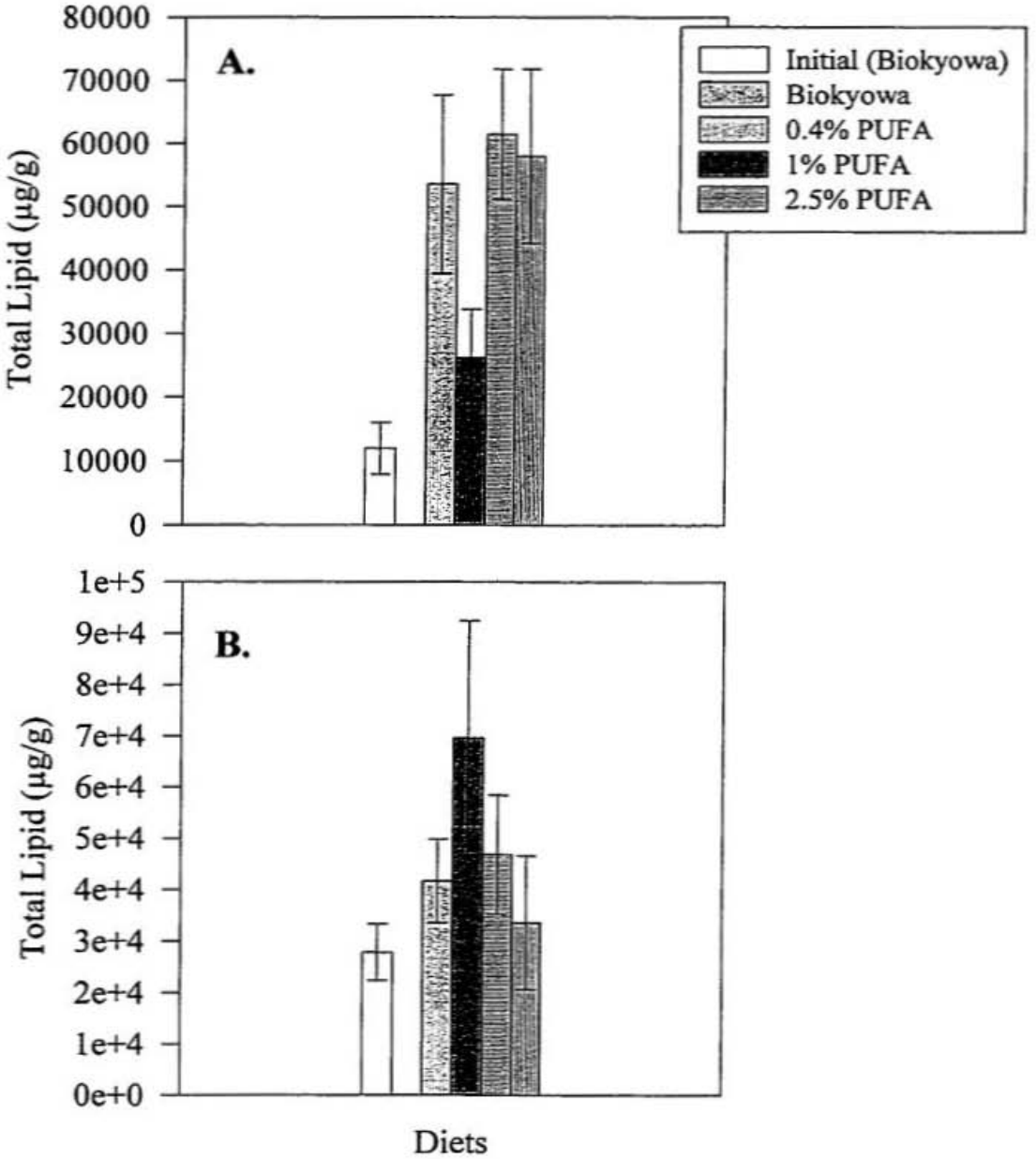

Figure 4.7 Total lipid (µg/g) in muscle (A) and liver (B) of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA at the beginning ($n = 5$) and end of the experiment ($n = 6$). Vertical bars represent std. error.

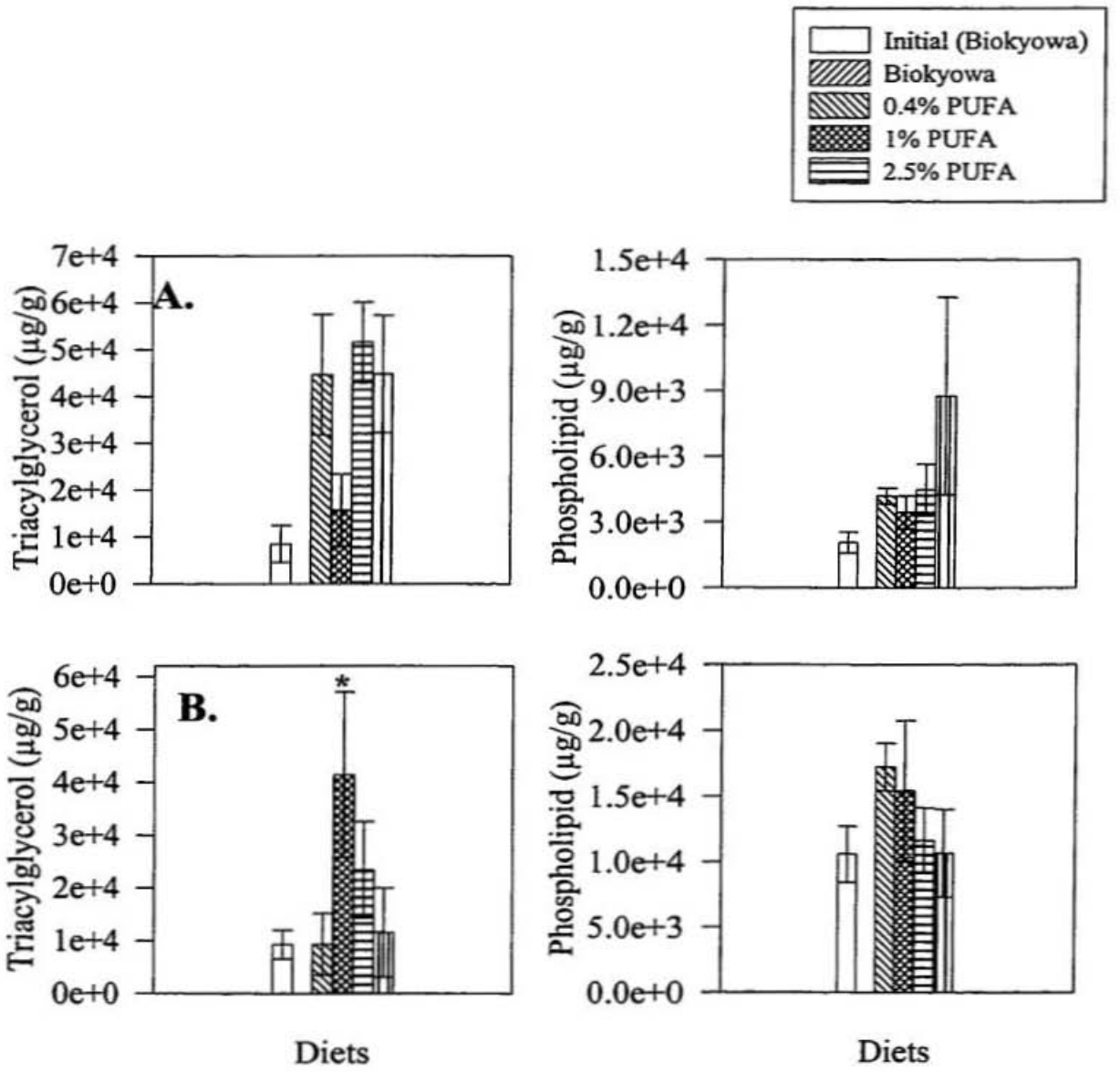

Figure 4.8 Average levels (µg/g) of triacylglycerol and phospholipid in muscle (A) and liver (B) of 0+ yellowtail flounder fed varying levels of *n*-3 PUFA. Vertical bars represent standard error. $n = 5$ for initial sample and $n = 6$ for others. * indicates significance.

observation it could be seen that the edge of the body wall closest to the fin was fatty when examined microscopically in this section of muscle. There seemed to be a trend also, whereby there were higher levels of phospholipid in the liver of yellowtail flounder, as well as intermediate products, such as diacylglycerols and free fatty acids, but this was not significant ($p > 0.05$).

4.3.7 FATTY ACID ANALYSIS

The initial sample, which was composed of fish sampled from a large population of post-metamorphosed yellowtail flounder, showed the lowest levels of all fatty acids, which was unexpected (see Appendix C, Tables C.3 to C.8). In the ICES diets, however, levels of *n*-3 PUFA increased with level of PUFA in diet for all fractions in both tissues (Tables 4.5 to 4.8). Fish fed the control diet, Biokyowa (Table 4.3 and 4.4), had a fatty acid composition most resembling the fish sampled initially, which had been fed another Biokyowa diet (B-750) and *Artemia*. Both of these samples had levels of fatty acids lower than the ICES diets. Because the levels of *n*-3 PUFA were low in these samples, it was assumed either fish were deficient at the beginning of the experiment, or some oxidation of PUFA had resulted from a necessary long storage time of these particular samples, which caused them to dry out. These are included in the Appendix (Tables C.3 to C.8) but are not discussed in this section.

In the neutral lipid fraction of the liver, 16:0 was the fatty acid that made up the greatest proportion of saturated fatty acids (SFA) (Table 4.6). Of the monounsaturated fatty acids (MUFA), 18:1 was the fatty acid present in greatest quantity, and 22:6*n*-3 (DHA) the

Table 4.3 Fatty acid composition of total, polar and neutral lipid fractions of liver of yellowtail flounder fed control diet Biokyowa C-1000.

	Total		Polar		Neutral	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	5.5±0.6	168.3±42.2	5.7±1.0	70.2±11.3	5.4±0.2	91.6±32.2
15:0	1.5±0.3	45.1±11.1	1.7±0.3	22.9±7.5	1.4±0.4	20.7±6.3
16:0	27.9±2.6	823.6±169.1	31.4±1.3	402.5±61.4	25.4±2.8	398.2±117.2
16:1	6.4±0.6	219.4±78.2	5.1±0.4	65.5±9.8	7.5±0.4	140.8±59.6
16:2	3.0±0.5	92.4±23.1	3.0±0.9	37.4±9.9	3.0±0.1	51.2±16.5
17:0	1.0±0.1	31.7±9.9	0.8±0.2	11.8±3.9	1.1±0.1	18.6±6.2
18:0	8.1±1.3	217.5±28.1	11.7±1.7	143.3±19.0	4.9±0.5	73.5±19.1
18:1	26.4±2.4	890.8±306.4	22.0±2.4	288.4±53.7	29.9±1.8	557.9±228.7
18:2*n*-6	5.4±1.3	199.9±93.0	4.0±1.0	51.6±12.7	6.8±1.2	139.3±69.1
18:3*n*-3	0.6±0.1	19.6±8.2	0.3±0.1	3.6±1.1	0.7±0.1	14.1±6.3
18:4*n*-3	0.5±0.2	14.9±4.7	0.6±0.4	6.8±3.3	0.4±0.1	7.0±3.2
20:1	4.8±0.2	147.4±37.0	5.1±0.5	62.4±5.7	4.4±0.4	79.4±30.1
20:2*n*-6	0.5±0.0	15.4±4.9	0.3±0.	3.9±0.9	0.6±0.0	10.5±3.9
20:3*n*-6	0.2±0.1	6.1±1.6	0.1±0.1	0.8±0.8	0.3±0.1	4.7±1.6
20:4*n*-6	0.7±0.2	20.1±5.7	0.8±0.3	10.4±3.0	0.6±0.2	9.6±4.0
20:5*n*-3	2.3±0.6	75.6±27.4	2.5±0.8	31.4±8.3	2.0±0.4	40.6±20.7
22:1	1.9±0.3	62.8±22.5	1.3±0.3	17.8±5.1	2.6±0.4	44.2±15.9
21:5*n*-3	0.2±0.1	4.4±1.7	0.1±0.1	1.0±0.5	0.2±0.1	3.1±1.2
22:5*n*-3	0.4±0.1	13.7±6.2	0.3±0.1	4.2±1.4	0.4±0.1	8.4±4.8
22:6*n*-3	2.7±0.5	80.8±20.0	3.2±1.0	39.1±10.0	2.1±0.2	37.7±16.0
≤SFA	44.0±4.6	1286.2±254.7	51.2±3.6	650.8±95.0	38.1±3.8	602.6±179.5
≤MUFA	39.5±3.1	1320.4±442.7	33.5±2.5	434.1±70.6	44.4±2.6	822.3±333.7
≤PUFA	13.4±2.3	450.5±165.0	12.3±2.8	152.9±30.4	14.1±1.6	274.9±128.1
n-3	5.5±1.1	174.5±53.8	6.2±1.8	75.7±19.2	4.8±0.6	89.8±42.2
n-6	6.7±1.4	241.5±103.0	5.2±1.2	66.8±14.7	8.3±1.2	164.0±77.1
n-9	25.0±2.2	838 6±285.0	21.1±2.1	275.2±49.1	28.0±1.7	522.0±213.3
n-3/*n*-6	0.9±0.2		1.3±0.3		0.7±0.1	
DHA/EPA	1.3±0.2		1.4±0.2		1.2±0.2	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on TLC-FID estimates. $n = 6$.

Table 4.4 Fatty acid composition of total, polar and neutral lipid fractions of muscle of yellowtail flounder fed control diet Biokyowa C-1000.

	Total		Polar		Neutral	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	5.2±0.3	270.0±87.8	2.5±0.6	9.1±3.1	5.5±0.3	260.9±85.6
15:0	0.8±0.1	41.5±18.1	0.8±0.2	2.9±1.0	0.8±0.1	38.6±17.2
16:0	16.5±0.5	800.0±235.5	17.1±1.7	57.9±9.0	16.5±0.5	742.0±229.8
16:1	6.9±0.4	354.1±116.1	3.1±0.8	11.5±4.2	7.3±0.3	342.6±113.0
16:2	0.4±0.1	17.2±4.3	0.6±0.2	1.7±0.5	0.4±0.1	15.4±4.4
17:0	0.0±0.1	26.9±8.8	0.8±0.1	2.6±0.5	0.5±0.0	24.2±8.7
18:0	2.6±0.2	119.4±31.0	6.7±0.6	22.0±1.8	2.2±0.1	97.5±30.7
18:1	23.8±0.9	1185.6±360.2	18.4±2.3	64.1±13.9	24.6±0.7	1121.5±350.5
18:2*n*-6	10.9±0.4	578.9±15.6	6.3±0.7	21.6±4.2	11.5±0.2	526.2±153.2
18:3*n*-3	1.8±0.1	93.7±27.1	0.7±0.1	2.3±0.5	2.0±0.1	91.5±26.7
18:4*n*-3	1.6±0.1	79.0±20.2	0.3±0.1	1.0±0.3	1.8±0.1	78.0±20.1
20:1	4.4±0.2	220.7±66.0	3.8±0.5	13.2±2.8	4.5±0.2	207.5±64.0
20:2*n*-6	0.5±0.0	23.5±6.2	0.5±0.0	1.6±0.1	0.5±0.0	21.9±6.2
20:3*n*-6	0.1±0.0	4.7±1.5	0.1±0.0	0.3±0.1	0.1±0.0	4.4±1.6
20:4*n*-6	0.8±0.1	33.5±6.5	2.2±0.4	6.9±1.0	0.6±0.0	26.6±6.5
20:5*n*-3	10.0±0.9	434.0±95.4	13.3±1.9	42.4±6.0	9.5±0.8	391.6±97.1
22:1	1.7±0.1	92.5±23.1	0.7±0.3	2.7±1.3	1.8±0.1	79.8±22.1
21:5*n*-3	0.4±0.0	17.8±4.3	0.2±0.1	0.7±0.2	0.4±0.0	17.1±4.3
22:5*n*-3	1.1±0.1	45.0±9.1	1.5±0.2	5.0±0.8	1.0±0.1	40.0±9.3
22:6*n*-3	9.7±1.4	385.4±76.5	20.0±3.4	64.0±12.5	8.2±1.0	321.4±78.3
≤SFA	25.6±0.9	1257.7±397.8	27.9±1.9	94.5±13.1	25.5±0.9	1163.2±370.6
≤MUFA	36.8±1.5	1842.7±564.6	26.1±3.8	91.4±21.9	38.3±1.2	1751.4±548.9
≤PUFA	37.0±2.2	1664.5±370.8	45.1±5.0	145.7±17.0	35.6±2.0	1518.7±379.3
n-3	21.2±2.4	882.2±182.8	35.1±5.5	112.1±19.3	19.1±1.9	770.1±187.7
n-6	12.4±0.4	609.6±168.3	9.1±0.4	30.4±3.6	12.8±0.2	579.1±166.0
n-9	22.5±0.8	1122.2±340.6	17.5±2.2	60.9±13.2	23.3±0.7	1061.2±331.3
n-3/*n*-6	1.7±0.2		4.0±0.7		1.5±0.1	
DHA/EPA	0.9±0.1		1.4±0.1		0.9±0.0	

Results are expressed in % weight and mg fatty acid/100 g wet weight. $n = 6$

Table 4.5 Fatty acid composition of total lipid fraction of liver of yellowtail flounder fed varying levels of *n* -3 PUFA.

	0.4% PUFA (*n* = 4)		1% PUFA (*n* = 5)		2.5% PUFA (*n* = 3)	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	9.4±0.6[b]	551.8±92.2	8.9±0.6[b]	394.8±92.2	5.5±1.2[a]	85.7±48.3
15:0	1.9±0.9	55.2±9.8	0.7±0.1	32.3±9.8	0.6±0.1	8.1±2.8
16:0	20.1±0.4	918.1±188.2	16.2±0.4	735.3±188.2	13.2±0.2	196.0±80.5
16:1	12.6±1.7	961.1±207.1	13.3±1.7	648.2±207.1	8.0±0.3	121.8±51.8
16:2	1.0±0.0[b]	19.1±1.5	0.2±0.0[a]	6.5±1.5	0.2±0.0[a]	3.4±1.6
17:0	1.0±0.1	31.3±3.0	0.5±0.1	19.8±3.0	0.6±0.1	10.3±5.1
18:0	3.8±0.5	127.2±18.5	2.8±0.5	108.4±18.5	3.7±0.4	52.4±17.5
18:1	23.2±2.3	1861.6±472.1	32.0±2.3	1539.3±472.1	26.3±1.0	401.0±177.2
18:2*n* -6	10.7±0.8	782.9±65.6	8.8±0.8	361.8±65.6	9.7±0.4	139.0±51.2
18:3*n* -3	1.1±0.0	66.3± 8.0	0.8±0.0	34.7±8.0	0.9±0.0	13.3±5.4
18:4*n* -3	0.2±0.0	3.7± 1.1	0.0±0.0	1.9±1.1	0.1±0.0	1.8±0.6
20:1	2.6±0.3	172.7±41.8	3.1± 0.3	145.0±41.8	3.0±0.3	41.5±14.4
20:2*n* -6	2.0±0.1	69.1±7.5	0.8±0.1	33.5±7.5	1.2±0.2	16.3±5.0
20:3*n* -6	0.0±0.0	3.2±0.3	0.0±0.0	1.5±0.3	0.2±0.1	1.2±0.6
20:4*n* -6	0.5±0.3[a]	34.5±3.4	0.5±0.3[a]	27.2±3.4	2.2±0.3[b]	30.2±9.1
20:5*n* -3	2.8±0.8[a]	140.6±19.0	3.4±0.8[a]	130.8±19.0	8.4±0.5[b]	119.2±42.0
22:1	0.4±0.1	29.4±8.6	0.4±0.1	22.08±8.6	0.8±0.9	10.6±3.2
21:5*n* -3	1.3±0.0	7.9±1.5	0.1±0.0	4.0±1.6	0.5±0.1	7.6±3.1
22:5*n* -3	0.6±0.3[a]	32.3±7.6	1.5±0.3[a]	53.7±7.6	4.3±0.5[b]	67.9±31.0
22:6*n* -3	5.2±1.6	344.1±27.9	5.8±1.6	209.2±27.8	10.3±1.2	159.5±66.3
≤SFA	35.7±0.8	1683.7±307.2	29.0±0.8	1290.6±307.2	23.6±1.0	352.5±151.0
≤MUFA	38.7±3.9	3024.8±726.4	33.6±1.9	2354.5±726.4	38.1±0.9	574.9±246.4
≤PUFA	24.4±3.8	1484.5±130.4	21.7±1.0	858.3±130.4	37.8±1.7	556.1±212.1
n -3	9.8±2.7[a]	524.9±53.4	11.6±1.7[a]	397.8±53.4	23.5±1.5[b]	354.2±141.9
n -6	13.3±1.1	889.7±74.6	10.4±1.1	423.9±74.6	13.3±0.1	186.8±64.3
n -9	21.5±2.1	1718.7±434.8	29.5±2.1	1421.6±434.8	24.4±0.8	371.3±163.0
n -3/*n* -6	0.7±0.1[a]		1.0±0.1[a]		1.8±0.2[b]	
DHA/EPA	1.8±0.1		1.6±0.1		1.2±0.2	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on estimates from TLC-FID. Significantly different levels of fatty acids are indicated by letters.

Table 4.6 Fatty acid composition of polar and neutral lipid fractions of liver of yellowtail flounder fed varying levels of *n*-3 PUFA.

	Polar						Neutral					
	0.4% PUFA (*n* = 6)		1% PUFA (*n* = 5)		2.5% PUFA (*n* = 4)		0.4% PUFA (*n* = 4)		1% PUFA (*n* = 5)		2.5% PUFA (*n* = 5)	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	6.2±0.9	72.5±27.1	6.3±0.5	67.4±7.4	5.6±1.0	53.3±19.2	11.5±1.4^{b}	476.9±240.1	9.9±0.7^{ab}	327.3±95.0	6.4±1.1^{a}	64.0±24.8
15:0	1.8±1.4	8.1±3.4	0.3±0.1	2.6±0.3	0.3±0.0	3.1±1.2	1.0±0.2	45.6±23.8	0.9±0.1	29.7±9.7	0.8±0.2	7.9±2.7
16:0	19.6±5.0	177.1±53.2	17.1±0.8	183.0±20.4	14.4±1.4	136.1±49.3	18.1±3.4	722.7±352.1	15.5±0.6	552.4±186.9	12.5±1.0	118.1±35.5
16:1	7.3±1.6	89.0±36.7	6.9±0.6	72.42±5.2	5.2±0.4	49.1±17.1	14.5±2.5	858.7±463.4	15.8±1.5	575.8±205.2	11.8±1.8	118.7±44.0
16:2	1.3±1.0	5.1±2.4	0.1±0.0	1.3±0.4	0.2±0.0	1.8±0.7	0.3±0.1	12.7±6.9	0.2±0.1	5.3±1.8	0.3±0.0	2.6±0.8
17:0	0.8±0.3	6.3±1.4	0.4±0.1	4.1±1.0	0.5±0.0	4.6±1.6	0.6±0.1	24.7±12.8	0.5±0.1	15.7±3.8	0.7±0.1	6.8±2.3
18:0	5.7±1.0	54.5±15.0	4.8±0.4	52.0±8.1	5.4±0.3	50.2±17.3	2.7±0.9	75.3±36.8	1.9±0.3	56.4±15.6	2.0±0.1	18.1±5.2
18:1	19.4±3.3	248.7±92.6	22.3±0.6	237.2±18.6	20.9±0.9	193.8±67.5	25.1±6.8	1616.7±862.3	35.6±1.8	1302.1±468.9	31.0±2.0	305.0±100.2
18:2*n*-6	13.5±3.0	196.2±87.8	11.5±0.3	121.9±8.0	8.5±0.5	79.8±29.6	11.3±2.3	588.6±393.4	7.9±0.9	239.8±63.9	9.7±0.9	81.5±21.0
18:3*n*-3	1.29±0.2	13.8±5.3	0.8±0.0	8.1±0.7	0.7±0.0	6.3±2.4	0.9±0.3	51.8±32.7	0.8±0.1	26.6±7.7	0.9±0.1	7.7±2.5
18:4*n*-3	0.1±0.0	0.6±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.3±0.2	3.3±1.7	0.1±0.0	1.9±1.1	0.2±0.0	1.2±0.5
20:1	3.4±0.4	36.3±12.6	3.2±0.6	36.1±10.1	2.8±0.3	26.3±10.4	2.1±0.5	136.3±73.2	3.0±0.2	108.9±39.2	3.0±0.2	27.1±8.0
20:2*n*-6	1.1±0.2	15.2±6.8	0.9±0.1	10.1±1.9	1.2±0.1	11.2±4.7	3.0±2.1	53.0±23.9	0.7±0.1	23.4±7.1	1.1±0.1	9.0±2.3
20:3*n*-6	0.1±0.0	1.3±0.6	0.1±0.0	1.1±0.4	0.2±0.1	1.5±0.9	0.1±0.0	2.0±1.7	0.1±0.1	0.4±0.4	0.2±0.1	1.5±0.7
20:4*n*-6	1.4±0.3^{a}	18.0±6.7^{a}	1.7±0.2^{a}	17.9±2.3^{a}	3.3±0.3^{b}	30.4±10.9^{b}	0.3±0.2	18.0±16.2	0.5±0.2	9.3±1.6	0.9±0.2	7.5±3.3
20:5*n*-3	5.1±1.3^{a}	61.8±21.5	7.2±0.6^{ab}	77.3±8.2	9.7±0.6^{b}	90.4±32.8	1.9±0.8^{a}	80.0±55.8	2.1±0.7^{a}	53.5±12.4	6.1±1.3^{b}	47.3±17.2
22:1	0.1±0.0^{a}	1.2±1.0	0.1±0.0^{a}	1.4±0.8	0.5±0.2^{b}	4.1±1.5	0.5±0.1^{a}	27.9±14.9	0.5±0.1^{a}	20.6±8.4	1.0±0.1^{b}	8.3±2.1
21:5*n*-3	0.7±0.6	2.2±1.6	0.7±0.6	2.8±0.3	0.4±0.1	3.5±1.8	1.5±1.4	5.2±4.8	0.1±0.0	1.2±0.9	0.6±0.2	4.4±1.9
22:5*n*-3	1.07±0.2^{a}	213.5±4.5	1.1±0.2^{ab}	31.8±3.2	4.7±1.5^{b}	59.0±32.0	0.5±0.1^{a}	20.1±13.8	0.9±0.2^{a}	21.9±5.5	4.4±1.0^{b}	32.1±11.4
22:6*n*-3	10.1±2.3	130.8±49.8	10.1±2.3	135.4±17.3	15.2±2.1	161.3±68.3	3.5±1.6	215.8±173.4	3.2±1.2	73.8±16.2	6.2±1.4	48.8±20.0
ΣSFA	34.1±7.3	318.4±94.6	34.1±7.3	309.1±34.4	26.2±2.1	247.2±87.2	33.9±5.6^{b}	1345.2±657.1	28.6±0.9^{ab}	981.5±309.4	22.4±1.5^{a}	214.9±68.2
ΣMUFA	30.2±4.2	375.2±142.9	32.6±1.4	347.1±32.4	29.4±1.4	273.2±95.6	42.2±8.7	2639.6±1413.0	54.9±3.1	2007.4±720.7	46.9±3.6	459.0±151.4
ΣPUFA	34.4±6.1	453.3±179.9	38.1±2.2	406.5±38.1	43.8±2.8	443.6±182.7	23.4±4.8	1037.8±702.0	16.2±3.4	451.7±110.7	30.3±5.0	241.1±76.2
n-3	17.0±3.4^{a}	208.2±74.8	23.1±2.0^{ab}	247.4±27.8	29.9±3.1^{b}	314.2±134.7	7.4±2.1	321.0±240.7	6.2±2.2	150.4±34.0	17.3±3.8	132.7±50.5
n-6	16.1±3.5	230.7±101.6	14.2±0.3	151.0±11.4	13.2±1.0	123.0±45.8	14.7±2.7	661.7±431.9	9.1±1.2	272.9±72.0	12.0±1.2	99.5±25.8
n-9	18.3±2.9	232.9±86.4	20.9±0.6	222.5±19.0	19.5±0.9	181.0±63.3	23.1±6.3	1489.1±794.4	32.8±1.6	1199.1±431.7	28.7±1.8	281.2±92.2
n-3/*n*-6	1.0±0.2^{a}		1.6±0.1^{b}		2.3±0.3^{c}		0.5±0.1^{a}		0.6±0.1^{a}		1.3±0.3^{b}	
DHA/EPA	2.0±0.1		1.7±0.1		1.6±0.3		1.8±0.5		1.4±0.1		1.0±0.1	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on estimates from TLC-FID. Significantly different levels of fatty acids are indicated by letters.

Table 4.7 Fatty acid composition of the total lipid fraction of muscle of yellowtail flounder fed varying levels of *n*-3 PUFA.

	0.4% PUFA (*n* = 4)		1% PUFA (*n* = 5)		2.5% PUFA (*n* = 3)	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	6.7±0.9[a]	136.3±49.6[a]	10.3±0.5[b]	554.7±117.5[b]	7.1±0.3[ab]	414.2±87.6[b]
15:0	0.7±0.1	12.5±5.0	0.7±0.0	37.9±8.0	0.4±0.1	27.7±8.2
16:0	15.3±0.3[b]	295.0±118.6	14.7±0.2[b]	787.8±161.3	12.8±0.4[a]	770.5±165.1
16:1	6.1±0.8	128.1±49.9	7.1±0.2	369.5±68.6	4.9±0.3	304.8±74.8
16:2	0.4±0.1	6.1±2.9	0.2±0.0	8.6±1.5	0.3±0.0	18.3±5.7
17:0	0.4±0.1	9.8±4.3	0.5±0.0	25.1±5.4	0.4±0.0	23.2±5.6
18:0	4.2±0.6	67.1±27.7	2.5±0.1	130.8±24.7	3.8±0.5	228.6±52.6
18:1	22.8±1.0	452.0±182.3	23.9±0.4	1267.0±248.7	20.1±0.5	1220.1±274.8
18:2*n*-6	12.3±0.6	242.0±96.1	14.2±0.2	759.7±156.4	13.1±0.5	774.7±166.2
18:3*n*-3	1.7±0.2	36.0±15.2	2.2±0.0	116.2±24.4	1.9±0.1	113.5±25.2
18:4*n*-3	0.9±0.2	21.6±10.5	1.0±0.1	57.0±13.8	1.0±0.1	58.8±14.4
20:1	4.2±0.4	94.0±47.6	3.3±0.2	181.8±40.3	3.3±0.2	201.0±45.9
20:2*n*-6	0.8±0.1	13.2±4.4	0.7±0.0	39.2±8.4	0.7±0.0	42.3±8.4
20:3*n*-6	0.2±0.1	1.0±0.4[a]	0.1±0.0	6.0±1.0[b]	0.2±0.0	8.8±2.1[b]
20:4*n*-6	1.0±0.2	13.9±5.2	0.7±0.0	34.4±6.6	1.0±0.1	58.1±11.7
20:5*n*-3	8.9±0.9	153.5±65.6	7.6±0.3	405.3±81.3	12.1±0.4	705.3±139.9
22:1	1.2±0.3	32.4±19.1	1.2±0.2	68.8±18.6	1.4±0.1	87.2±21.1
21:5*n*-3	0.3±0.0	6.2±2.9[a]	0.3±0.0	17.5±3.5[ab]	0.7±0.0	40.1±8.3[b]
22:5*n*-3	1.0±0.1[a]	18.3±7.8[a]	1.3±0.1[ab]	69.2±13.3[ab]	3.3±0.2[b]	189.9±37.6[b]
22:6*n*-3	10.6±2.0	157.0±60.9	7.4±0.3	391.3±77.2	11.3±0.6	654.4±126.1
≤SFA	27.2±0.2	520.6±202.9	28.7±0.4	1536.2±313.8	24.4±0.7	1464.1±308.8
≤MUFA	34.3±2.1	706.4±297.2	35.5±0.7	1887.2±374.4	29.7±1.0	1813.1±415.5
≤PUFA	37.7±2.4	662.8±266.7	35.5±0.5	1895.8±381.9	45.3±1.4	2646.0±531.5
n-3	20.9±2.9	335.0±137.1	16.7±0.6	883.2±174.7	27.4±1.2	1589.8±310.7
n-6	14.3±0.4	270.1±105.2	15.7±0.3	839.3±172.0	15.0±0.5	883.9±185.6
n-9	21.6±0.9	430.3±175.7	22.3±0.4	1185.8±233.8	18.9±0.5	1148.4±258.7
n-3/*n*-6	1.5±0.2		1.1±0.0		1.8±0.1	
DHA/EPA	1.2±0.1[b]		1.0±0.0[ab]		0.9±0.0[a]	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on estimates from TLC-FID. Significantly different levels of fatty acids are indicated by letters.

Table 4.8 Fatty acid composition of polar and neutral lipid fractions of muscle of yellowtail flounder fed varying levels of *n*-3 PUFA.

	Polar						Neutral					
	0.4% PUFA (*n* = 6)		1% PUFA (*n* = 5)		2.5% PUFA (*n* = 4)		0.4% PUFA (*n* = 4)		1% PUFA (*n* = 5)		2.5% PUFA (*n* = 5)	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	3.6±0.6	9.2±2.4	3.9±0.2	12.9±3.1	3.5±0.1	24.5±14.2	8.0±0.7^{a}	127.0±49.7^{a}	10.9±0.6^{b}	541.5±115.1^{b}	7.6±0.3^{a}	336.0±89.9^{ab}
15:0	0.5±0.2	0.9±0.2	0.2±0.0	0.7±0.2	0.1±0.0	0.4±0.1	0.7±0.0^{b}	11.5±4.9	0.7±0.0^{b}	37.1±7.9	0.5±0.0^{a}	23.5±7.6
16:0	13.7±1.0	34.5±7.7	13.5±0.5	47.4±13.3	13.6±1.0	103.2±60.7	15.4±0.4^{b}	260.5±118.6	14.8±0.2^{b}	738.4±155.6	12.4±0.3^{a}	573.0±167.5
16:1	3.9±0.8	10.7±3.5	3.6±0.4	13.2±4.6	2.5±0.1	16.4±9.0	7.2±0.2^{b}	117.4±50.5	7.3±0.2	355.0±66.0	5.4±0.2	249.1±73.5
16:2	0.5±0.1	1.2±0.2	0.4±0.1	1.1±0.2	0.3±0.0	2.1±1.1	0.3±0.1	4.9±2.8	0.2±0.0^{b}	7.6±1.5	0.2±0.0^{a}	13.7±5.6
17:0	0.5±0.2	1.3±0.4	0.5±0.1	1.4±0.4	0.4±0.1	2.0±0.6	0.5±0.0^{b}	8.5±4.0	0.5±0.0^{b}	23.7±5.1	0.4±0.0^{a}	18.3±5.5
18:0	7.1±0.5	18.1±3.9	7.7±0.7	25.0±5.5	7.7±0.5	59.2±37.2	2.7±0.2	49.0±25.0	2.1±0.0	105.3±21.3	2.9±0.4	142.1±49.3
18:1	20.2±1.4^{b}	53.2±12.9	21.0±0.6^{b}	71.6±19.2	16.5±0.8^{a}	116.7±67.6	24.0±0.5^{b}	398.8±180.8	24.1±0.4^{b}	1193.4±240.8	20.5±0.4^{a}	948.5±275.3
18:2*n*-6	10.2±1.0	25.8±5.2	9.0±0.3	30.7±7.7	8.0±0.2	52.2±27.5	13.3±0.3	216.1±95.6	14.5±0.3	727.7±152.9	13.6±0.4	621.1±172.3
18:3*n*-3	0.8±0.1	2.3±0.7	0.8±0.0	2.7±0.6	0.6±0.1	4.3±2.7	2.0±0.1^{a}	33.7±15.2	2.3±0.1^{b}	113.5±24.0	2.0±0.1^{a}	93.7±26.2
18:4*n*-3	0.1±0.1	0.3±0.3	0.1±0.0	0.1±0.1	0.1±0.0	0.2±0.1	1.2±0.1	21.3±10.6	1.1±0.1	56.9±13.8	1.0±0.1	50.0±14.5
20:1	3.3±0.2	8.6±1.9	3.7±0.3	12.1±2.9	2.9±0.2	19.7±11.5	4.7±0.4^{b}	85.3±47.1	3.3±0.2^{a}	169.8±39.2	3.2±0.2^{a}	154.7±47.2
20:2*n*-6	1.0±0.1	2.6±0.6	1.1±0.1	3.8±1.1	0.9±0.0	6.2±3.5	0.7±0.0	10.6±4.2	0.7±0.0	35.3±7.5	0.7±0.0	31.0±8.3
20:3*n*-6	0.1±0.1	0.4±0.3	0.1±0.0	0.4±0.1	0.1±0.0	0.4±0.1	0.4±0.3	0.7±0.4^{a}	0.1±0.0	5.7±1.0^{ab}	0.2±0.0	7.3±2.0^{b}
20:4*n*-6	1.9±0.2	4.9±1.2	1.9±0.1	6.2±1.3	2.3±0.1	14.0±6.6	0.6±0.1^{a}	9.1±4.2^{a}	0.6±0.0^{a}	28.1±6.1^{ab}	0.9±0.0^{b}	37.9±10.0^{b}
20:5*n*-3	11.7±1.1	29.4±6.9	11.5±0.6	38.8±9.0	14.4±0.5	86.5±40.7	7.6±0.4^{a}	124.1±60.1^{a}	7.3±0.2^{a}	366.7±80.2^{ab}	12.1±0.4^{b}	534.5±142.7^{b}
22:1	0.0±0.0^{a}	0.0±0.0	0.2±0.0^{ab}	0.7±0.3	0.5±0.2^{b}	7.2±6.3	1.7±0.2	32.4±19.1	1.3±0.1	68.1±18.5	1.4±0.1	67.8±21.7
21:5*n*-3	0.2±0.1^{a}	0.7±0.2	0.4±0.0^{ab}	1.2±0.3	0.5±0.0^{b}	2.8±1.4	0.6±0.3	5.5±2.8^{a}	0.3±0.0	16.3±3.4^{ab}	0.7±0.0	32.2±8.6^{b}
22:5*n*-3	1.5±0.1^{a}	3.9±1.0	2.4±0.1^{b}	8.4±2.2	4.2±0.2^{c}	24.4±11.1	0.8±0.1^{a}	14.3±7.0^{a}	1.2±0.0^{a}	60.5±12.3^{ab}	3.3±0.2^{b}	143.6±37.7^{b}
22:6*n*-3	18.4±2.3	46.7±12.3	17.6±0.9	62.2±16.2	20.5±1.1	119.2±52.2	7.2±0.8^{a}	110.3±50.1^{a}	6.5±0.3^{a}	327.6±72.7^{ab}	10.6±0.3^{b}	464.5±122.9^{b}
≤SFA	25.4±1.2	64.0±12.8	25.9±0.7	87.4±22.1	25.2±1.3	189.3±112.5	27.3±1.0^{b}	456.6±200.5	28.9±0.5^{b}	1446.0±300.5	23.8±0.5^{a}	1092.9±315.4
≤MUFA	27.4±2.4^{b}	72.5±18.0	28.5±0.9^{b}	97.6±26.7	22.5±1.2^{a}	160.0±94.3	37.7±0.6^{b}	633.9±295.8	36.1±0.6^{b}	1786.4±363.0	30.5±0.7^{a}	1420.0±417.4
≤PUFA	46.0±2.9	471.9±127.9	44.9±1.5	154.6±38.2	51.5±1.9	310.1±145.4	34.5±1.2^{a}	545.8±248.8	34.7±0.4^{a}	1420.0±417.4	45.2±1.1^{b}	2016.0±544.7
n-3	31.8±3.5	407.2±109.3	31.9±1.4	110.7±27.6	39.5±1.8	232.9±105.3	16.2±1.2^{a}	254.3±119.9^{a}	15.4±0.5^{a}	771.1±168.4^{ab}	26.8±0.9^{b}	1174.9±311.8^{b}
n-6	13.3±1.0	41.5±10.8	12.1±0.3	41.1±10.0	11.3±0.2	72.8±37.5	15.1±0.4	236.5±103.8	15.9±0.3	796.8±167.1	15.4±0.4	697.4±192.6
n-9	19.0±1.3^{b}	171.9±47.4	19.8±0.6^{b}	67.4±17.9	15.6±0.7^{a}	110.0±63.7	22.8±0.4^{b}	380.3±174.2	22.5±0.4^{b}	1116.5±226.4	19.3±0.4^{a}	892.3±259.5
n-3/*n*-6	2.5±0.4		2.6±0.1		3.5±0.1		1.1±0.1^{a}		1.0±0.1^{a}		1.7±0.1^{b}	
DHA/EPA	1.6±0.0		1.5±0.1		1.4±0.0		0.9±0.1		0.9±0.0		0.9±0.0	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on estimates from TLC-FID. Significantly different levels of fatty acids are indicated by letters.

highest level of the polyunsaturated fatty acids (PUFA) (Table 4.6). The same was true of the neutral muscle portion, except 20:5*n*-3 (EPA) was the fatty acid in greatest quantity amongst the PUFA. Levels of neutral PUFA in the muscle of juvenile yellowtail flounder were higher than in the livers of these fish (Table 4.7 and 4.8).

In the polar portions of the liver and muscle (Tables 4.6 and 4.8, respectively), the same trends were seen as in the neutral portion, with 16:0 being the major SFA, 18:1 the major MUFA and with 22:6*n*-3 being the major PUFA . However, levels of polar SFA were higher in the liver, and MUFA and PUFA higher in the muscle.

The fatty acid 16:0 was generally higher in the polar fraction of the liver than the neutral fraction, but about the same for the muscle. The 18:1 content was much higher in the neutral fraction of both liver and muscle. The *n*-3 PUFA was present in greater abundance in the polar fraction of muscle and liver than neutral fraction and DHA/EPA ratio was generally higher in the polar fraction. In the polar fraction, this ratio ranged from about 1.4 to 2.0, depending on the tissue, indicating that DHA may be as important as EPA, or that they are utilized differently. In the neutral lipid of the muscle this ratio was consistently below 1 (Table 4.8).

When the level of *n*-3 PUFA in the diet was increased to 1% there was no significant change in saturates or *n*-3 PUFA in the liver or muscle from fish fed the 0.4% PUFA diet. However, when fish were fed a diet that was 2.5% PUFA, levels of saturates decreased and levels of *n*-3 PUFA increased significantly ($p < 0.05$, one-way ANOVA) in all portions of tissues examined. Analysis of DHA/EPA ratio in both lipid fractions revealed small

increases or decreases, which reflected the loss of one fatty acid with respect to another. The dietary DHA/EPA composition was altered quite noticeably in the neutral lipid of the muscle (Table 4.8), but reflected the dietary ratio in 1% PUFA and 2.5% PUFA diets.

In fish fed the 0.4% PUFA diet, levels of *n*-6 series fatty acids increased proportionally compared to the other diets, as can be seen as well in the *n*-3/*n*-6 fatty acid ratio. The ratio of saturates/unsaturates also increased in these fish.

The tissues reflected dietary trends, especially in the neutral lipid fractions. Levels of *n*-3 PUFA in the tissues increased substantially in these fractions as levels of PUFA rose in the diet from 1% to 2.5% and levels of n-3 PUFA increased in polar liver. Differences between levels of *n*-3 PUFA were not as noticeable in the polar fractions of the muscle and liver. Levels of 18:1 in all lipid portions (except the polar fraction of lipid) were higher in the fish fed the lowest PUFA diets, the converse trend of *n*-3 PUFA levels. The ratio of DHA/EPA in the tissues increased in polar fractions in fish fed the 1% and 2.5% *n*-3 PUFA diets, whereas in the neutral portions, this ratio decreased in the tissues of fish fed the 0.4% PUFA diet and reflected the dietary ratio in fish fed 1% and 2.5% diets (Tables 4.3 to 4.8).

4.4 DISCUSSION

The experiment demonstrated that yellowtail flounder show a requirement for *n*-3 PUFA to some extent for maximum growth, and maintenance of biomembranes. Yellowtail flounder seem to utilize a wide range of *n*-3 PUFA levels. Over the course of the experiment, there were no mortalities and fish appeared to be healthy. However, fish fed the lowest level of n-3 PUFA showed poor growth and increased levels of lipid in the form of triacylgylcerol in their liver. This indicates that juvenile yellowtail flounder have a requirement for *n*-3 PUFA in their diets in order to maximize growth and for physiological functioning. However, other than the above symptoms, there were no chronic pathologies due to EFA deficiency seen in post-metamorphic yellowtail flounder as have been associated with other fish such as fin erosion, gill abnormalities, red mouths, enlarged livers, high body moisture or "shock" syndrome (Castell, 1979; Sargent *et al.*, 1989). Thus, the possibility exists that the levels of *n*-3 PUFA used in this experiment were simply not low enough to cause a deficiency in yellowtail flounder, as other authors used lower levels of *n*-3 PUFA in feeding experiments. For example, Takeuchi *et al.* (1990) used a level of 0% PUFA (*Pagrus major*) and Ibeas *et al.* used 0.19%PUFA (*Sparus aurata*) to assess deficiency symptoms, although Watanabe *et al.* (1989) used a level of 0.7% PUFA and produced deficiency symptoms in juvenile striped jack (*Longirostris delicatissimus*).

The accumulation of neutral lipid which occurred in the livers of yellowtail flounder has been observed in other animals, including rats and EFA-deficient fish (Fukuzawa, 1971; Greene and Selivonchick, 1987; Ibeas *et al.*, 1996; Owen *et al.*, 1972). This accumulation

of neutral lipid has been attributed to low dietary levels of *n*-3 PUFA, specifically DHA. It is possible that this accumulation is caused by an impairment in lipoprotein synthesis, preventing lipids from being transported out of the liver (Sargent *et al.*, 1989). Castell (1979) has proposed that movement of protein-lipid complexes are stabilized by phospholipids. If the balance of *n*-3 PUFA is affected by a diet low in *n*-3 PUFA, then these phospholipids probably are not able to function properly. In this study, accumulation appeared to have begun, but was not large enough to be significant by the end of the experiment. This may mean that they may be conserving as much *n*-3 PUFA as possible in their phospholipids.

Other studies have shown that the fatty acid composition of the tissues reflect dietary patterns (Kalogeropoulos *et al.*, 1991; Lochmann and Gatlin, 1993a), and results from this study are in agreement. However, fatty acids in liver of yellowtail flounder seem to be under a more strict control than in other fish and dietary utilization patterns are reflected more in the muscle. Castell (1979) reported that not only are EFA requirements species-specific, but they are specific to tissue type as well. Muscle generally reflects the whole-body condition of fish, whereas the liver is an area of more dynamic metabolism. In agreement with this, diet did not affect hepatosomatic index in yellowtail flounder, although enlarged livers have been seen in EFA-deficient animals (Castell, 1979).

Increasing the level of *n*-3 PUFA from 0.4 to 1% did not cause a large increase in levels of *n*-3 PUFA in any of the fractions of tissues examined and it is not surprising that growth was not significantly different between these two groups. Only when the content of

n-3 PUFA in the diet was increased to a level of 2.5% was there a significant increase in *n*-3 PUFA in the neutral portion of the muscle. This level of PUFA is higher than has been seen in any comparable studies to date (see Table 4.9). In fact, levels of *n*-3 PUFA (>1.5% *n*-3 PUFA) such as this one, caused reduced growth, mortality and decreased levels of *n*-3 PUFA in juvenile gilthead bream (Ibeas *et al.*, 1996) and juvenile red drum (Lochmann and Gatlin, 1993a and b). This difference in response may be attributed to the cold temperatures that yellowtail flounder are raised in or it may be that because of this low temperature (<15°C), the duration of the experiment would have to be extended in order to see changes in the tissue associated with dietary patterns. Another possibility is that in the wild, yellowtail flounder have extremely small mouth sizes (Morris, 1997; Scott and Scott, 1988) and juveniles are probably still consuming a large quantity of their diet as zooplankton, which are known to be high in *n*-3 PUFA (Sargent *et al.*, 1989), whereas other fish may be consuming larger prey with reduced levels of *n*-3 PUFA. Results from Chapter 3 support this suggestion, as wild flounder show body compositions consistent with diets of prey high in *n*-3 PUFA, but low in total lipid.

Phospholipids of both liver and muscle contained higher levels of PUFA, as was expected due to the importance of PUFA to biological (cellular) membranes (Kalogeropoulos *et al.*, 1991). Muscle contained higher proportions of PUFA than liver, however, which was unexpected, as livers are known to contain high levels of biomembranes (Bell *et al.*, 1985). This suggests that the liver is important in this species metabolically and takes longer to

Table 4.9 Comparison of Essential Fatty Acid Requirements among fish species. (Partly from Takeuchi, 1997).

Species	Requirement	Optimum temp. (C)	Effectiveness	Reference
Sea bream				
Red (*Pagrus major*) < 10 g	0.5% DHA or 1% EPA	20-28	*n*-3 HUFA = DHA>EPA	Takeuchi *et al*., 1990
Gilthead (*Sparus aurata*) Larvae (fed rotifers)	5.5% *n*-3 HUFA dry weight	15-20	EPA>DHA	Rodriguez *et al*., 1994
<30 g	1% *n*-3 HUFA	20-25	EPA>DHA	Ibeas *et al*., 1996
>40 g	1.9% *n*-3 HUFA	20-25	EPA>DHA	Ibeas *et al*., 1994
Sea bass (*Centropristis striata*) <10 g	1% *n*-3 HUFA	20-25	DHA>EPA	Wanakowat *et al*., 1991
Red Drum (*Sciaenops ocellatus*) <10 g	0.5% n-3 HUFA	25 - 27	DHA>EPA	Lochmann and Gatlin, 1993
Yellowtail tuna (*Seriola quinqueradiata*) <10 g	2.1 to 3.1% *n*-3 HUFA	20-29	DHA>EPA	Takeuchi *et al*., 1992
Striped jack (*Pseudocaranx dentex*) < 10 g	1.7% *n*-3 HUFA 1.5% DHA	18-26	DHA>EPA	Watanabe *et al*., 1989 Takeuchi *et al*., 1992
Flatfish (*Paralichthys olivaceus*) Larvae (fed *Artemia*)	1.8-3.8% *n*-3 HUFA	15-25	DHA=EPA	Izquierdo *et al*., 1992
(*Scophthalmus maximus*) 2 yrs old	0.57% *n*-3 PUFA or 4% LNA		DHA>EPA	Leger *et al*., 1979
(*Pleuronectes ferrugineus*) < 10 g	**2.5% *n* - 3 PUFA**	**7-12 (?)**	**DHA>EPA**	**Present Study**
Cod (*Gadus macrocephalus*) Larvae (fed *Artemia*)	1.6-2.1% DHA	8-12	DHA	Zheng *et al*., 1996

show the effects of dietary *n*-3 PUFA content and that PUFA has a different function in these areas.

Generally, levels of monounsaturated fatty acids (containing 18:1) were higher in the fish fed the 0.4 and 1% PUFA diets and *n*-3 PUFA content lower. Conversely levels of *n*-3 PUFA were higher in fish fed the 2.5% *n*-3 PUFA diet. Neutral lipids generally reflect dietary changes more than polar lipids, where the compositions tend to be preferentially conserved when dietary *n*-3 PUFA is low (Greene and Selivonchick, 1987) and has been seen in juvenile gilthead seabream (Ibeas *et al.*, 1996) as well as juvenile red drum (Lochmann and Gatlin, 1993a), but the PUFA found in neutral lipids is depleted along with other fatty acids during EFA deficiency (Lochmann and Gatlin, 1993a). This was the case with juvenile yellowtail flounder in this experiment.

In most marine fish, and especially in larvae, DHA is considered to be the EFA that is most important relative to EPA (Takeuchi, 1997). In almost all portions of lipid, yellowtail flounder deposited more DHA than EPA regardless of diet, except in the neutral lipid fraction of muscle. This closely mimics the case in wild yellowtail flounder. The ratio of dietary DHA/EPA is thought to have an effect on growth and other parameters in fish (Ibeas *et al.*, 1997) and the increase of the DHA/EPA ratio of fish, specifically through the incorporation of 22:6*n*-3 into phospholipid, is related to the improvement of growth (Kanazawa, 1997; Izquierdo *et al.*, 1992) and may increase tolerance to stress, which is important during grow-out (Koven, 1993). The DHA/EPA ratio differed somewhat among ICES diets and it was interesting to note that in the polar fraction of lipids of tissues, the

DHA/EPA ratio was almost the same as the diet in fish fed the lowest PUFA diet but increased in fish fed the 1% and 2.5% PUFA diets, which also grew well. Thus, there is, as mentioned, preferential conservation of PUFA in the polar lipid portion of fish fed the lowest PUFA diet. This was seen in both the muscle and liver, but in the neutral lipid fraction of the lipid, the DHA/EPA ratio mirrored the ratio in the diet except in the fish fed the lowest PUFA diet. In those fish, the DHA/EPA ratio decreased a great deal, indicating that they were beginning to deplete selected PUFA in the storage area.

Levels of actual fatty acids (mg/100g) showed similar trends to the proportional data (%), but because the fish fed 0.4% PUFA were higher in fat, not as many individual fatty acid levels were significantly different. All fatty acid profiles of fish examined in this study showed lower PUFA levels and actual values than the wild fish, but DHA/EPA ratios in the separate portions reflected the same trends, indicating that lipid metabolism is similar in wild and hatchery-reared fish fed these particular diets, however, it must be noted that the ICES diets use cod liver oil in the formulation, rather than a higher fish oil level. It was also expected from the wild yellowtail compositions that these fish require a high level of DHA and EPA, and the results were indicative of that.

Comparison of ICES and Biokyowa diets should be done carefully, keeping in mind a number of things. First, pellet sizes, shapes and colours were different for Biokyowa and ICES diets. The largest sized particles from the ICES diets, a crumble diet, were greater than 1200 μm, while the pellets from the Biokyowa diet, an agglomeration, were 1000 μm. Stradmeyer (1989) showed that fish indicating a preference for a particular sized pellet will

increase food intake. This, perhaps, could explain why fish fed the Biokyowa diet grew well, but had lower levels of *n*-3 PUFA in their tissues than expected and also explains why fish fed the Biokyowa during the first two weeks grew slowly but afterwards increased their weight. They had been fed a smaller particle first, but then were switched to the larger 1000 µm pellets. Obviously the initial pellets were too small. The colours of the pellets were different also, with ICES diets being rusty red and the Biokyowa pellets a light brown colour. Biokyowa was a drier diet and had lower protein than the ICES diets.

Another problem with the experimental diets in this study was that PUFA was incorporated into the diets in different ways. For example, the 0.4% PUFA diet contained 5% triacylglycerol from hydrogenated coconut oil, and the PUFA was delivered through this. The 2.5% PUFA diet contained PUFA in an ethyl ester portion, while the 1% diet was a mixture of these. Some authors have seen improved growth in fish fed PUFA through intact triacylglycerol rather than methyl or ethyl esters (Greene and Selivonchick, 1987; Lochmann and Gatlin, 1993b). It must also be noted that the digestibility of hydrogenated oils by yellowtail flounder is not known so there may be differences based on this. For these reasons, comparisons between diets have to be carefully considered. This might explain the differences seen in the nutrient analysis of fish fed the two different diets. As well, the condition index of fish fed Biokyowa was as low as the fish fed the 0.4% PUFA and is due to the fact that condition index is often low when animals increase in length faster than they increase in weight.

Castell *et al.* (1994) and Takeuchi (1997) have also discussed whether arachidonic acid (20:4*n*-6) had growth-promoting activity. The ICES diets used in this experiment differed in *n*-3 PUFA but also very slightly in AA (see Table 4.1 and 4.2 and Appendix Table B.3) and although it cannot be determined here with any certainty whether AA influenced growth in yellowtail, it should be examined in the future.

The fatty acid 20:3*n*-9 was not seen in any of the tissue profiles examined in this experiment. This fatty acid has been termed the "universal indicator of fatty acid deficiency" (Greene and Selivonchick, 1987), but has not been seen in many species fed EFA-deficient diets (Lochmann and Gatlin, 1993a). As well, the index 18:1/total *n*-3 PUFA, which also has been used as an indicator of EFA deficiency (Castell, 1979; Sargent *et al.*, 1989), was not useful in determining EFA-deficient status in yellowtail flounder.

From this experiment, it cannot be conclusively determined whether yellowtail flounder elongate and desaturate 18:3*n*-3 or 18:2*n*-6 into 20:5*n*-3, 22:6*n*-3 or 20:4*n*-6 because each diet contained a level of the final products, DHA, EPA and AA. Because levels of intermediate products such as 20:2*n*-6, 20:3*n*-6, 22:5*n*-6 or 20:3*n*-3, 20:4*n*-3 or 22:5*n*-3 were low or present in trace quantities, and levels of 18:2*n*-6 remained the same, it can be tentatively proposed that yellowtail flounder cannot modify linoleic or linolenic acid, which agrees with studies done on other marine fish (Castell, 1979; Kanazawa *et al.*, 1979; Sargent *et al.* 1989). It is hypothesized that highly carnivorous animals, such as marine cold-water fish, do not need to alter shorter chain fatty acids because they have available to them an abundance of PUFA in their natural diets (Sargent *et al.*, 1989), and over time have lost the

use of the Δ4- and Δ5-desaturase enzymes necessary for this process (Takeuchi, 1997). In the fish fed the 0.4% PUFA there did not appear to be an elongation of 20:5*n*-3 to 22:6*n*-3 as the level of 22:5*n*-3 was not increased in these fish. However, the level of DHA in the diets of these fish may have been sufficient so that there was no need to elongate EPA.

Many studies at the present time focus on the importance of polyunsaturated fatty acids in larval fish but this study shows that PUFA requirements and utilization are as important in juvenile fish as they influence growth, functioning and the incorporation of *n*-3 PUFA into tissues.

Thus, it is concluded that at 7°C, post-metamorphic yellowtail flounder require 2.5% *n-3* PUFA as a percentage of dry diet, with 10% lipid, for optimal growth and physiological functions. This level is higher than has been seen in other marine fish in the literature and it is suggested that cold water fish such as yellowtail flounder require a high level of *n-3* PUFA for optimum growth. Fish do not display chronic deficiency symptoms when fed levels as low as 0.4% PUFA for twelve weeks, but they display poor growth after four weeks. Commercial feeds such as Biokyowa which contain high levels of *n*-3 PUFA, are adequate for feeding young juveniles in captivity and ICES diets were also satisfactory for obtaining information on *n*-3 PUFA requirements. There is, however, a need for more information in this area. More research must be done in order to determine the amount and type of lipid in the diet for yellowtail flounder which would provide the necessary quantity of EFA.

CHAPTER 5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 FEEDING IN WILD AND CAPTIVE YELLOWTAIL FLOUNDER

Preliminary investigations of feeding strategies and nutritional requirements indicate that yellowtail flounder in captivity have different compositions and behaviours than their wild counterparts and that growth may be improved by further work in this area. In the wild, yellowtail flounder are presumed to be browsers because of the anatomy of their digestive system and behaviour. It is unlikely, however, that food is available to yellowtail whenever they want it in the wild, and therefore have learned to cope with small meals once a day or every second day. When food is available constantly, captive fish do not seem to perform substantially better, and experiments seem to suggest a feeding frequency of twice daily to maximize growth and lower food conversion ratio.

In the wild, yellowtail are known to eat a diet of invertebrates, and rely heavily on amphipods and polychaete worms into adulthood, even after similar flatfish species (such as winter flounder and American plaice) have begun to predate fish and larger prey. This was also suggested by data from this study, as wild yellowtail have body compositions that suggest a diet high that is low in total fat but high in PUFA, as are many crustaceans and polychaetes upon which they prey (Parrish *et al.*, 1996). This was also substantiated by the fact that yellowtail performed better when fed a diet that was 2.5% *n*-3 PUFA, which is higher than much of the comparable literature. Yellowtail also take longer to show EFA deficiencies when fed diets low in *n*-3 PUFA. It seems likely that a commercial diet modeled after the wild dietary items or body composition of wild fish would provide a good basis for

a preliminary diet, and might alleviate some of the problems with captive rearing of yellowtail flounder.

5.2 FEEDING FREQUENCY OF JUVENILE YELLOWTAIL FLOUNDER

1. 0+ yellowtail flounder had the highest growth rates and lowest food conversion ratios when fed two meals/day. Thus, it is recommended that at this life-cycle stage, yellowtail flounder are fed twice daily.
2. Meal size is inversely proportional to daily feeding frequency; fish fed lower frequencies were hyperphagic.
3. Condition index and FCR were not affected by feeding frequency.
4. Feeding frequency significantly affected food consumption in 0+ juvenile yellowtail flounder. Daily food intake was significantly higher in fish fed every other day (14.2 ± 0.6 mg/g).
5. Size variation was not significantly affected by feeding frequency.
6. SGR was significantly higher in fish fed increased feeding frequencies.
7. Fish fed increased feeding frequencies were less active, foraged to a lesser extent and were less aggressive.
8. Capture success in obtaining pellets was lower and ingestion rate (number of bites/minute) higher in fish fed more restricted feeding frequencies.

9. Yellowtail flounder demonstrated a wide tolerance for feeding frequencies and respond well to differing feeding regimes which will be important for grow-out.

10. Yellowtail flounder voluntarily eat about 0.01% body weight daily.

5.3 BODY COMPOSITION OF WILD AND CULTURED YELLOWTAIL FLOUNDER

11. HSI was significantly lower in wild yellowtail flounder than in cultured flounder.

12. Moisture and protein content was higher in whole-body wild flounder and lipid level lower. The same trend was also seen in muscle and liver.

13. Levels of TAG were significantly higher and PL lower in cultured yellowtail, indicating an accumulation of fat in muscle and liver.

14. Cultured fish have higher levels of intermediate and by-product lipid classes, and the highest levels of storage lipids.

15. Wild fish have a higher proportion of PUFA in their fatty acid composition of muscle and liver, but lower absolute levels due to a lower total lipid level.

16. Condition index of wild fish (1.1 ± 0.03) was significantly lower than cultured fish (1.4 ± 0.04), which may indicate obesity in cultured fish.

17. Differences in body composition are thought to be influenced by diet for yellowtail flounder.

5.4 ESSENTIAL FATTY ACID REQUIREMENTS OF JUVENILE YELLOWTAIL FLOUNDER

18. Yellowtail flounder require 2.5% *n*-3 PUFA as a percentage of dry diet, with 10% lipid, for optimal growth and functioning.

19. Yellowtail flounder do not display chronic deficiency symptoms when fed *n*-3 PUFA levels as low as 0.4% for 12 weeks, but display poor growth after only four weeks and accumulate TAG in the liver.

20. The increase of DHA/EPA in the polar portion of tissues in yellowtail flounder was related to an improvement in growth.

21. Yellowtail fed low levels of *n*-3 PUFA preferentially conserve *n*-3 PUFA in polar fractions of their tissues.

22. As levels of dietary *n*-3 PUFA increase from 1% - 2.5%, levels of *n*-3 PUFA in tissues increase significantly.

LITERATURE CITED

Ackman, R. G. and J. Kean-Howie. 1995. Fatty acids in aquaculture: are *n*-3 fatty acids always important? *Nutrition and utilization technology in aquaculture.* Ed. Cchlorn, L. and D. J. Sessa. AOCS Press, Champaign, IL: USA.

Anonymous. 1997. Diet protection against fatty liver syndrome. *Fish Farming International*: February. P. 13.

Anonymous. 1981. *Marine Aquaculture.* Flatfish (Chapter 16), Academic Press: New York, USA. pps. 144-149.

Andrews, J.W. and Page, J.W. (1975). The effects of frequency of feeding on culture of catfish. *Trans. Am. Fish. Soc.* 2: 317-321.

Beacham, T. D. 1983. Variability in size and age at sexual maturity of American plaice and yellowtail flounder in the Canadian Maritimes region of the Northwest Atlantic Ocean. *Can. Tech. Rep. Fish. Aquat. Sci.* 1196: iv + 11 p.

Beamish, F. W. H. 1966. Vertical migration by demersal fish in the Northwest Atlantic. *J. Fish. Res. Bd. Can.* 23(1): 109-139.

Bell, M. V., R. J. Henderson and J.R. Sargent. 1986. The role of polyunsaturated fatty acids in fish. *Comp. Biochem. Physiol.* 83B (4): 711-719.

Blaxter, J.H.S. 1975. Reared and wild fish- how do they compare? 10th European Symposium on Marine Biology. Ostend, Belgium, Sept. 1975. Vol. 1: 11-26.

Bricknell, I.R., D.W. Bruno, T.J. Bowden and P. Smith. 1996. Fat cell necrosis syndrome in Atlantic halibut, *Hippoglossus hippoglossus* L. *Aquaculture* 144: 65-69.

Brodie, W. B. and S. J. Walsh. 1994. Changes in distribution of yellowtail flounder on the Grand Bank during the late-1980s and early-1990s. NAFO SCR Doc. 94/46, June, 1994.

Brown, J., M. Helm and J. Moir. 1995. New-candidate species for aquaculture. In: *Cold-Water Aquaculture in Atlantic Canada.* Ed: Boghen, A. D. The Canadian Institute for Research on Regional Development, The Tribune Press: Sackville, NB, Canada.

Budge, S. M. 1999. Fatty Acid Biomarkers in a Cold Water Marine Environment. PhD. thesis, Department of Chemistry, Memorial University of Newfoundland.

Buurma, B. J. and J. S. Diana. 1994. Effects of feeding frequency and handling on growth and mortality of cultured walking catfish *Clarias fuscus*. *J. World Aqua. Soc.* 25 (2): 175-182.

Canadian Council on Animal Care. 1993. *Guide to the Care and Use of Experimental Animals*. Volumes 1 and 2. 2nd Edition. Olfert, E.D., Cross, B.M. and McWilliam A.A. (Eds.). Ontario, Canada.

Castell, J. D., J.G. Bell, D. R. Tocher and J.R. Sargent. 1994. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 128: 315-333.

Castell, J. D. 1979. Review of lipid requirements of finfish. From. *Proc. of World Symp. on Finfish Nutrition and Fishfeed Technology*: June 1978. Vol. I. Berlin, 1979.

Chua, T-E. and S-K. Teng. 1978. Effects of feeding frequency on the growth of young estuary grouper, *Epinephelus tauvina* (Forskål), cultured in floating net-cages. *Aquaculture* 14: 31-47.

Collie, J. S. 1987. Food selection by yellowtail flounder (*Limanda ferruginea*) on Georges Bank. *Can. J. Fish. Aquat. Sci.* 44: 357-367.

Copeman, L. A. 1996. Effects of induced ovulation on larval behaviour and size in the yellowtail flounder (*Pleuronectes ferrugineus*). BSc (Honours) thesis. Department of Biology, Memorial University of Newfoundland, St. John's, NF, Canada.

Cowey, C.B., J. M. Owen, J. W. Adron and C. Middleton. 1976. Studies on the nutrition of marine flatfish. The effect of different fatty acids on the growth and fatty acid composition of turbot (*Scophthalmus maximus*). *Br. J. Nutr.* 36: 479-484.

Davenport. J., E. Kjørsvik and T. Haug. 1990. Appetite, gut transit, oxygen uptake and nitrogen excretion in captive Atlantic halibut, *Hippoglossus hippoglossus* L., and lemon sole, *Microstomus kitt* (Walbaum). *Aquaculture* 90: 267-277.

Ebling, M.E. 1968. The Dumas method for nitrogen in feeds. *J. Assoc. Off. Anal. Chem.* 51:766-770.

Ekanem, S. B. 1996. Effects of feeding frequency, moist and dry feeds on the growth of *Chrysichthys nigrodigitatus* Lacépède on pond water quality. *Aquaculture Research* 27: 107-112.

Exler, J. and J. L. Weihrauch. 1976. Comprehensive evaluation of fatty acids in foods. *J. Am. Diet. Assoc.* 69: 213-248.

Exler, J. 1975. Lipids and fatty acids of important finfish: new data for nutrient tables. *J. Am. Oil Chem. Soc.* 52 (5): 154-159.

Fam, S. 1997. Food and Feeding Requirements of Juvenile Striped Wolffish (*Anarhichus lupus*). MSc thesis, Aquaculture Unit, Memorial University of Newfoundland, St. John's NF, Canada.

Folch, J., M. Lees and G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497-509.

French, K. J. 1995. The growth and behavioural development of yellowtail flounder *Pleuronectes ferrugineus*. BSc (Honours) thesis. Department of Biology, Memorial University of Newfoundland, St. John's, NF, Canada .

Fukuzawa, T., O.S. Privett, and Takahashi, Y. 1971. Effect of fatty acid deficiency on lipid transport from liver. *Lipids*, 6: 388-393.

Goddard, S. 1992. *Practical Laboratory Guide for Fish and Crustacean Nutrition.* Course Manual for Aquaculture Unit, School of Fisheries, Fisheries and Marine Institute, Memorial University of Newfoundland.

Goddard, S. 1996. *Feed Management in Intensive Aquaculture*. Chapman and Hall: London, U.K.

Goff, G. 1993. Report for Newfoundland Inshore Fisheries Development Agreement, March 1993, on "Flatfish Aquaculture Research". Fisheries Resource Development, Ltd.

Grant, S. M., J.A. Brown and D.L. Boyce. 1998. Enlarged fatty livers of small juvenile cod: a comparison of laboratory cultured and wild juveniles. *J. Fish Biol.* 52: 1105-1114.

Grayton, B. D. and F.W.H. Beamish. 1977. Effects of feeding frequency on food intake, growth and body composition of rainbow trout (*Salmo gairdneri*). *Aquaculture* 11: 159-172.

Greene, D. H. S. and D. P. Selivonchick. 1987. Lipid metabolism in fish. *Prog. Lipid Res.* 26: 53-85.

Haug, T., E. Ringø and G. W. Petersen. 1988. Total lipid and fatty acid composition of polar and neutral lipids in different tissues of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Sarsia* 73: 163-168.

Herbinger, C.M. and G.W. Friars. 1992. Effects of winter temperature and feeding regime on the rate of early maturation in Atlantic salmon (*Salmo salar*) male parr. *Aquaculture* 101: 147-162.

Higgs, D.A., B.S. Dosanjh, A.F.. Prendergast, R.M. Beames, R.W. Hardy, W. Riley, and G. Deacon. 1994. Use of Rapeseed/Canola Protein Products in Finfish Diets. In: Nutrition and Utilization Technology in Aquaculture. Ed(s): Lim, C. and D. J. Sessa. AOCS Press, Champaign, Illinois, USA. pp. 130-156.

Howell, W. H. and M. K. Litvak. 1999. Winter flounder (*Pleuronectes americanus*) culture. *Encyclopedia of Aquaculture* (in press).

Ibeas, C., J. R. Cejas, R. Fores, P. Badía, T. Gómez, and A.Lorenzo Hernández. 1997. Influence of eicosapentanoic and docosahexanoic acid ratio (EPA/DHA) of dietary lipids on growth and fatty acid composition of gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture* 150: 91-102.

Ibeas, C., J. Cejas, T. Gómez, S. Jerez and A. Lorenzo. 1996. Influence of dietary *n*-3 highly unsaturated fatty acids levels on juvenile gilthead seabream (*Sparus aurata*) growth and tissue fatty acid composition. *Aquaculture* 142: 221-235.

Ibeas, C., M. S. Izquierdo, A. Lorenzo. 1994. Effect of different levels of *n*-3 highly unsaturated fatty acids on growth and fatty acid composition of juvenile gilthead seabream (*Sparus aurata*). *Aquaculture* 127: 177-188.

Ishiwata, N. 1969. Ecological studies on the feeding of fishes-VIII. Frequency of feeding and satiation amount. *Bull. Jan. Soc. Sci. Fish.* 35(10): 979-984.

Izquierdo, M.S., T. Arakawa, T. Takeuchi, R. Haroun and T. Watanabe. 1992. Effect of *n*-3 HUFA levels in *Arte mia* on growth of larval Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 105: 73-82.

Jobling, M., S.J.S. Johansen, H. Foshaug, I.C. Burkow, and E. H. Jørgensen. 1998. Lipid dynamics in anadromous Arctic charr, *Salvelinus alpinus* (L.): seasonal variations in lipid storage depots and lipid class composition. *Fish Physiol. and Biochem.* 18: 225-240.

Jobling, M. 1982. Some observations on the effects of feeding frequency on the food intake and growth of plaice, *Pleuronectes platessa* L. *J. Fish Biol.* 20: 431-444.

Jarboe, H. H. and W. J. Grant. 1996. Effects of feeding time and frequency on growth of channel catfish *Ictalurus punctatus* in closed recirculating raceway systems. *J. World. Aqua. Soc.* 27 (2): 235-239.

Kalogeropoulos, N., M. N. Alexis and R. J. Henderson. 1991. Effect of dietary lipids on tissue fatty acid composition of gilthead bream (*Sparus aurata*). *Fish Nutrition in Practice*, June 1991. INRA: Paris, France.

Kanazawa, A. 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. *Aquaculture* 155: 129-134.

Kanazawa, A., S. Teshima and K. Ono. 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comp. Biochem. and Physiol.* 63B: 295-298.

Kayano, Y., S. Yao, S. Yamamoto, and H. Nakawaga. 1993. Effects of feeding frequency on the growth and body constituents of young red-spotted grouper, *Epinephelus akaara*. *Aquaculture* 110: 271-278.

Kinsella, J. E. 1987. *Seafoods and Fish Oils in Human Health and Disease*. Marcel Dekker, Inc.: New York, NY,USA. p.245.

Koven. 1993. The association of eicosapentaenoic and docosahexaenoic acids in the main phospholipids of different-age *Sparus aurata* larvae with growth. *Aquaculture* 116: 71-82.

Krzynowek, J. and J. Murphy. 1987. Proximate composition, energy, fatty acid, sodium, and cholesterol content of finfish, shellfish, and their products. NOAA Technical Report NMFS - 55, U.S. Department of Commerce, p. 10.

Langton, R.W. and R.E. Bowman. 1981. Food of eight Northwest Atlantic Pleuronectiform fishes. NOAA Technical Report NMFS SSRF - 749. U.S. Department of Commerce.

Langton, R. W. 1979. Food habits of yellowtail flounder, *Limanda ferruginea* (Storer), from off the Northeastern United States. *Fish. Bull.* 81 (1): 15-22.

Lapin, V. I. 1977. Seasonal dynamics in biochemical composition in different subspecies and forms of the flounder, *Platichthys flesus*. *J. Ich.* 17: 85-98.

Larraneta, M. G. 1986. Dynamics of yellowtail flounder and American plaice populations on the Grand Bank. *NAFO Sci. Coun. Studies* 10: 35-45.

Leger, C., F. Gatesoupe, R. Metailler, P. Luquet, and L. Fremont. 1979. Effect of dietary fatty acids differing by chain length and ω series on the growth and lipid composition of turbot *Scophthalmus maximus* L. *Comp. Biochem. Physiol.*, 64B: 1353-1456.

Libey, G. S. and C. F. Cole. 1979. Food habits of yellowtail flounder, *Limanda ferruginea* (Storer). *J. Fish Biol.* 15: 371-374.

Liewes, E.W. 1984. *Culture, Feeding and Diseases of Commercial Flatfish Species*. A.A. Balkema: Boston, MA, USA.

Lochmann, R.T. and D. M. Gatlin III. 1993a. Essential fatty acid requirement of juvenile red drum (*Sciaenops ocellatus*). *Fish Physiol. Biochem.* 12 (3): 221-235.

Lochmann, R. T. and D. M. Gatlin III. 1993b. Evaluation of different types and levels of triglycerides, singly and in combination with different levels of *n*-3 highly unsaturated fatty acid ethyl esters in diets of juveniles red drum, *Sciaenops ocellatus*. *Aquaculture* 114: 113-130.

Lovell, T. 1989. *Nutrition and Feeding of Fish*. Nelson Canada: Scarborough, ON, Canada.

Lux, F. E. 1964. Landings, fishing effort, and apparent abundance in the yellowtail Flounder Fishery. *ICNAF Res. Bull.* 1: 5-17.

Lux, F. E. 1969. Landings per unit of effort, age composition, and total mortality of yellowtail flounder, *Limanda ferruginea* (Storer), off New England. *ICNAF Res. Bull.* 6: 47-52.

Manning, A. J. and L.W. Crim. 1998. Maternal and inter-annual comparison of the ovulatory periodicity, egg production and egg quality of the batch-spawning yellowtail flounder. *J. Fish Biol.* 53: 954-972.

Martell, D. J. and G. McClelland. 1994. Diets of sympatric flatfishes, *Hippoglossoides platessoides*, *Pleuronectes ferrugineus*, *Pleuronectes americanus*, from Sable Island Bank, Canada. *J. Fish Biol.* 44: 821-848.

Merola, N. and O.A. Cantelmo. 1987. Growth, feed conversion and mortality of cage-reared tambaqui, *Colossoma macropomum*, fed various dietary feeding regimes and protein levels. *Aquaculture* 66: 223-233.

Morgan, M. J. and S. J. Walsh. 1996. Tracking movements of juvenile yellowtail flounder in the nursery area of the Southern Grand Bank, NAFO Divisions 3LNO. NAFO SCR Doc. 96/66.

Morris, N. J. 1997. Prey Size Selection in the Yellowtail Flounder (*Pleuronectes ferrugineus*): A Laboratory Study. BSc. (Honours) thesis. Department of Biology, Memorial University of Newfoundland, St. John's, NF, Canada.

Owen, J.M., J. W. Adron, J.R. Sargent and C.B. Cowey. 1972. Studies on the nutrition of marine flatfish. The effect of dietary fatty acids on the tissue fatty acids of the plaice *Pleuronectes platessa*. *Mar. Biol.* 13: 160-166.

Parrish, C.C., Z. Yang, A. Lau and R. J. Thompson. 1996. Lipid composition of *Yoldia hyperborea* (Protobranchia), *Nephthys ciliata* (Nephthyidae) and *Artacama proboscidea* (Terebellidae) living at sub-zero temperatures. *Comp. Biochem. Physiol.* 114B (1): 59-67.

Parrish, C.C. 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: *Lipids in Freshwater Ecosystems*. pp 4-20. Eds.: Arts, M.T. and Wainman, B.C. Springer-Verlag: New York, USA. 319 pp.

Perry, R. I. and Smith S. J. 1994. Identifying habitat associations of marine fishes using survey data: an application to the Northwest Atlantic. *Can. J. Fish. Aquat. Sci.* 51: 589-602.

Phillips, T. A., R. C. Summerfelt, and R.D. Clayton. 1998. Feeding frequency effects on water quality and growth of walleye fingerlings in intensive culture. *Prog. Fish Cult.* 60 (1): 1-8.

Pitt, T. K. 1970. Distribution, abundance, and spawning of yellowtail flounder, *Limanda ferruginea*, in the Newfoundland area of the Northwest Atlantic. *J. Fish. Res. Bd. Can.* 27 (12): 2261-2271.

Pitt, T. K. 1971. Fecundity of the yellowtail flounder (*Limanda ferruginea*) from the Grand Bank, Newfoundland. *J. Fish. Res. Bd. Can.* 28: 456-457.

Pitt, T.K. 1974. Age composition and growth of yellowtail flounder (*Limanda ferruginea*) from the Grand Bank. *J. Fish. Res. Bd. Can.* 31: 1800-1802.

Pitt, T. K. 1976. Food of yellowtail flounder on the Grand Bank and a comparison with American plaice. *ICNAF Res. Bull.* 12: 23-27.

Post, G. 1987. *Textbook of Fish Health*. T.F.H. Publications, Inc.: Montreal, PQ, Canada. pp. 233-234.

Purchase, C. F., D. Boyce and J.A. Brown 1999. Growth and survival of juvenile yellowtail flounder (*Pleuronectes ferrugineus*) under different photoperiods. Submitted to *Aqua. Res.*.

Puvenendran, V. and J. A. Brown. 1995. Preliminary experiments on the effect of prey concentration on the survival and growth of yellowtail flounder larvae (*Pleuronectes ferrugineus*). *Larvi '95 - Fish and Shellfish Larviculture Symposium*. Eds: P. Lavens, E. Jaspers, and I. Roelants. European Aquaculture Society, Special Pub. No. 24, Ghent, Belgium.

Robins, C. R. and G.C. Ray. 1986. *A Field Guide to Atlantic Coast Fishes of North America*. Houghton Mifflin Company: Boston, USA.

Rodriguez, C., J.A. Perez, A. Lorenzo, M.S. Izquierdo and J.R. Cejas. 1994. *n*-3 HUFA requirement of larval gilthead seabream *Sparus aurata* when using high levels of eicosapentaenoic acid. *Comp. Biochem. Physiol.* 107A: 693-698.

Royce, W. F., R. J. Buller and E. D. Premetz. 1959. Decline of the yellowtail flounder (*Limanda ferruginea*) off New England. *U.S. Fish. Wild. Ser. Fish. Bull.* 146: 169-267.

Sargent, J. , R. J. Henderson and D. R. Tocher. 1989. The lipids. In: *Fish Nutr.* Academic Press: New York, NY, USA.

Scott, D. M. 1947. The Biology of the Yellowtail Flounder (*Limanda ferruginea* - Storer). MSc thesis, McGill University, Montreal.

Scott, J. S. 1982. Selection of bottom type by groundfishes of the Scotian Shelf. *Can. J. Fish. Aquat. Sci.* 39: 943-947.

Scott, W.B. and Scott, M.G. 1988. Atlantic Fishes of Canada. *Can. Bull. Fish. and Aquat. Sci. No. 219.*

Shearer, K.D. 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture* 119: 63-88.

Sheehan, E. M., P.J.A. Sheehy, P.A. Morrissey and R. Fitzgerald. 1994. Compositional analysis on wild and farmed turbot, and fish feeds in Ireland. In: *Turbot Culture: Problems and Prospects*. European Aquaculture Society, Special Publication No. 22. Gent, Belgium. Eds.: Lavens, P. and R.A.M. Remmerswaal. pp. 302-311.

Smigielski, A.S. 1979. Induced spawning and larval rearing of the yellowtail flounder, *Limanda ferruginea. Fish. Bull.* 76(4): 931-936.

Smith, W. G., J. D. Sibunka and A. Wells. 1978. Diel movements of larval yellowtail flounder, *Limanda ferruginea*, determined from discrete depth sampling. *Fish. Bull.* 76(1): 167-178.

Stradmeyer, L. 1989. A behavioural method to test feeding responses of fish to pelleted diets. *Aquaculture* 79: 303-310.

Takeuchi, T., Toyota, M., Satoh, S. and Watanabe, T. 1990. Requirement of juvenile red seabream *Pagrus major* for eicosapentaenoic and docosahexaenoic acids. *Nippon Suisan Gakkaishi* 56 (8): 1263-1269.

Takeuchi, T. 1997. Essential fatty acid requirements of aquatic animals with emphasis on fish larvae and fingerlings. *Rev. Fish Sci.* 5(1): 1-25.

Takeuchi, T., T. Arakawa, S. Satoh and T. Watanabe. 1992a. Supplemental effect of phospholipids and requirement of eicosapentaenoic acid and docosahexaenoic acid of juvenile striped jack. *Nippon Suisan Gakkaishi* 58(4): 707-713.

Takeuchi, T., Y. Shiina, T. Watanabe, S. Sekiya and K. Imaizumi. 1992b. Suitable levels of *n*-3 highly unsaturated fatty acids in diet for fingerlings of yellowtail. *Nippon Suisan Gakkaishi* 58(7): 1341-1346.

Talbot, C. 1994. Frequency of feeding. *Fish Farmer*, May/June.

Tuene, S. and Nortvedt, R. 1995. Feed intake, growth and feed conversion efficiency of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Aqua. Nutr.* 1: 27-35.

Van Guelpen, L. 1980. Taxonomic variability and criteria for distinguishing metamorphosing larval and juvenile stages of *Limanda ferruginea* and *Hippoglossoides platessoides* (Pisces: Pleuronectidae) from the Scotian Shelf. *Can. J. Zool.* 58: 202-206.

Virtue, P. P.D. Nichols, S. Nicol, A. McMinn, and E.L. Sikes. 1993. The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerhelm. *Antarctic Sci. 5* (2): 169-177.

Walsh, S. J. 1991. Commercial fishing practices on offshore juvenile flatfish nursery grounds on the Grand Banks of Newfoundland. *Neth. J. Sea Res.* 27 (3/4): 423-432.

Walsh, S. J. 1992. Factors influencing distribution of juvenile yellowtail flounder (*Limanda ferruginea*) on the Grand Bank of Newfoundland. *Neth. J. Sea Res.* 42: 193-203.

Wanakowat, J., M. Boonyaratpalin and T. Watanabe. 1991. Essential fatty acid requirement of juvenile seabass. *Fish Nutrition in Practice*, June 1991. INRA: Paris, France.

Watanabe, T., T. Takeuchi, T. Arakawa, K. Imaizumi, S. Sekiya and C. Kitajima. 1989. Requirement of juvenile striped jack *Longirostris delicatissimus* for *n*-3 highly unsaturated fatty acids. *Nippon Suisan Gakkaishi* 55(6): 1111-1117.

World Aquaculture Society Proceedings, February1998 Conference. Las Vegas, NV, USA.

Zamarro, J. 1991. Batch fecundity and spawning frequency of yellowtail flounder (*Limanda ferruginea*) on the Grand Bank. NAFO S.C.S. 15: 43-51.

Zar, J. H. 1974. *Biostatistical Analysis*. Prentice-Hall: NJ, USA.

Zheng, F., T. Takeuchi, K. Yoseda, M. Kobayashi, J. Hirokawa, and T. Watanabe. 1996. Requirement of larval cod for arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid by using their enriched *Artemia* nauplii. *Nippon Suisan Gakkaishi*, 62: 669-676.

APPENDIX A

STATISTICS

Table A.1 Randomized block three-way ANOVA for the model relating weight of juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	5	60.05	<0.0001*
Treat	3	1.97	0.117
Replicate	2	0.22	0.8
Treat*Time	15	0.16	1.000
Rep*Time	10	0.13	0.99
Error	1026		
Total	1061		

[1] Refers to sampling at times 0, 2, 4, 6, 8 and 10.
[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.
* Significant with $\alpha = 0.05$.

Table A.2 Randomized block three-way ANOVA for the model relating length of juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	5	62.7	<0.0001*
Treat	3	1.34	0.261
Replicate	2	3.1	0.045*
Treat*Time	15	0.2	1.000
Rep*Time	10	0.07	1.000
Error	1026		
Total	1061		

[1] Refers to sampling at times 0, 2, 4, 6, 8 and 10.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.3 Randomized block three-way ANOVA for the model relating weight gain of juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	4	19.41	<0.0001*
Treat	3	4.96	0.007*
Replicate	2	0.21	0.808
Treat*Time	8	0.64	0.736
Rep*Time	12	0.68	0.757
Error	29		
Total	58		

[1] Refers to sampling at times 2, 4, 6, 8 and 10.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.4 Randomized block three-way ANOVA for the model relating condition index of juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	5	0.89	0.484
Treat	3	3.8	0.010*
Replicate	2	12.4	<0.0001*
Treat*Time	15	0.32	0.998
Rep*Time	10	0.25	0.975
Error	1026		
Total	1061		

[1] Refers to sampling at times 0, 2, 4, 6, 8 and 10.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.5 Randomized block three-way ANOVA for the model relating coefficient of variation of weight in juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	5	0.05	0.998
Treat	3	2.24	0.101
Replicate	2	0.59	0.559
Treat*Time	15	0.13	1.000
Rep*Time	10	0.1	1.000
Error	35		
Total	70		

[1] Refers to sampling at times 0, 2, 4, 6, 8 and 10.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.6 Randomized block three-way ANOVA for the model relating specific growth rate in juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	4	8.82	<0.0001*
Treat	3	3.92	0.018*
Replicate	2	0.35	0.706
Treat*Time	12	1.29	0.275
Rep*Time	8	0.37	0.928
Error	29		
Total	58		

[1] Refers to sampling at times 2, 4, 6, 8 and 10.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.7 Randomized block three-way ANOVA and one-way ANOVAs for the models relating consumption parameters of juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Consumption Parameter	Source of Variation	df	F	p
Daily Consumption	Time	4	0.79	0.542
	Treat	3	99.11	<0.0001*
	Replicate	2	3.4	0.55
	Time*Treat	12	2.57	0.018*
	Time*Rep	8	0.66	0.725
	Error	30		
	Total	59		
Daily Consumption	Treat	3	39.66	<0.0001*
	Error	8		
	Total	11		
Meal Size	Treat	3	66.9	<0.0001*
	Error	8		
	Total	11		
Total Consumption	Treat	3	4.77	0.034*
	Error	8		
	Total	11		

[1] Refers to sampling at times 2, 4, 6, 8 and 10.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.8 Randomized block three-way ANOVA for the model relating food conversion ratios of juvenile yellowtail flounder to to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	4	5.35	0.002*
Treat	3	2.68	0.065
Rep	2	1.42	0.258
Time*Rep	8	0.76	0.636
Time*Treat	12	0.71	0.73
Error	29		
Total	58		

[1] Refers to sampling at times 2, 4, 6, 8 and 10.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.9 One-way ANOVA for the model relating behaviour in juvenile yellowtail flounder to treatment (1 to 4[1]).

Behaviour	Source of Variation	df	F	p
Capture Success	Treat	3	1.68	0.172
	Error	452		
	Total	455		
Activity	Treat	3	12.79	<0.0001*
	Error	452		
	Total	455		
Forage	Treat	3	10.58	<0.0001*
	Error	452		
	Total	455		
Ingestion Rate	Treat	3	7.22	<0.0001*
	Error	452		
	Total	455		
Aggression	Treat	3	2.15	0.095
	Error	452		
	Total	455		

[1] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.
* Significant with a = 0.05.

Table A.10 One-way ANOVAs for the model relating hepatosomatic index and condition index of juvenile yellowtail flounder to treatment (1 to 4[1]).

Hepatosomatic Index:

Source of Variation	df	F	p
Treat	1	31.98	<0.0001*
Error	22		
Total	23		

Condition Index:

Source of Variation	df	F	p
Treat	1	37.29	<0.0001*
Error	22		
Total	23		

[1] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.
* Significant with $\alpha = 0.05$.

Table A.11 Two-way ANOVA for the model relating levels of protein in the muscle of juvenile yellowtail flounder to sex[1] and treatment (1 to 4[2]).

Source of Variation	df	F	p
Treat	1	177.69	<0.0001*
Sex	1	14.13	0.001*
Error	21		
Total	23		

[1] Refers to 1 = male, 2 = female.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.12 Three-way ANCOVA for the model relating weight gain of juvenile yellowtail flounder to time (over 12 weeks[1]), treatment (diets 1 to 4[2]), replicate (3) as well as interactions.

				p (by week)						
Source of Variation	**df**	**F**	**p**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Covariate (Time)	1	26.57	<0.0001*	0.929	0.241	0.435	0.077	0.014*	<0.0001*	<0.0001*
Treat	3	3.8	0.016*							
Replicate	2	1.46	0.243							
Treat*Time	**3**	**5.28**	**0.003***							
Rep*Time	2	1.97	0.15							
Treat*Rep	6	1.15	0.351							
Treat*Rep*Time	6	0.78	0.587							
Error	48									
Total	71									

[1] Refers to sampling at times 0, 2, 4, 6, 8, 10 and 12.

[2] Refers to 1 = Biokyowa, 2 = 0.4% PUFA, 3 = 1% PUFA and 4 = 2.5% PUFA.

* Significant with $\alpha = 0.05$.

Table A.13 Three-way ANCOVA for the model relating length gain of juvenile yellowtail flounder to time (over 12 weeks[1]), treatment (diets 1 to 4[2]), replicate (3) as well as interactions.

				p (by week)						
Source of Variation	**df**	**F**	**p**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Covariate (Time)	1	19.06	<0.0001*	0.884	0.708	0.886	0.165	0.013*	<0.0001*	<0.0001*
Treat	3	0.47	0.705							
Replicate	2	0.07	0.936							
Treat*Time	**3**	**3.19**	**0.032***							
Rep*Time	2	0.24	0.878							
Treat*Rep	6	0.13	0.962							
Treat*Rep*Time	6	0.35	0.907							
Error	48									
Total	71									

[1] Refers to sampling at times 0, 2, 4, 6, 8, 10 and 12.
[2] Refers to 1 = Biokyowa, 2 = 0.4% PUFA, 3 = 1% PUFA and 4 = 2.5% PUFA.
* Significant with $\alpha = 0.05$.

Table A.14 Three-way ANCOVA for the model relating specific growth rate in juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	1	0.73	0.397
Treat	3	0.11	0.954
Replicate	2	0.11	0.897
Treat*Time	3	0.73	0.541
Rep*Time	2	0.71	0.498
Treat*Rep	6	0.11	0.994
Rep*Time*Treat	6	0.72	0.638
Error	48		
Total	71		

[1] Refers to sampling at times 0, 2, 4, 6, 8, 10 and 12.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.15 Three-way ANCOVA for the model relating condition index in juvenile yellowtail flounder to time (over 10 weeks[1]), treatment (1 to 4[2]), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	1	74.85	<0.0001*
Treat	3	4.21	0.006*
Replicate	2	4.73	0.009*
Treat*Time	3	0.5	0.682
Rep*Time	2	1.27	0.268
Treat*Rep	6	0.46	0.628
Rep*Time*Treat	6	0.89	0.501
Error	1236		
Total	1259		

[1] Refers to sampling at times 2, 4, 6, 8, 10 and 12.

[2] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.

* Significant with $\alpha = 0.05$.

Table A.16 Three-way ANOVA and one-way ANOVA for the model relating hepatosomatic index in juvenile yellowtail flounder to time (beginning and end of experiment), treatment (diets 1 to 4^2), replicate (3) as well as interactions.

Source of Variation	df	F	p
Time	1	2.97	0.090
Treat	3	3.15	0.031*
Replicate	2	0.58	0.565
Treat*Time	3	3.18	0.03*
Rep*Time	2	0.56	0.575
Treat*Rep	6	0.28	0.944
Rep*Time*Treat	6	0.29	0.941
Error	61		
Total	84		
At end of experiment:			
Treat	3	4.25	0.018*
Error	20		
Total	23		

[1] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.
* Significant with $\alpha = 0.05$.

Table A.17 One-way ANOVAs for the model relating levels of lipid classes in the liver of juvenile yellowtail flounder to treatment (diets 1 to 4[1]).

Triacyglycerols (%):

Source of Variation	df	F	p
Treat	3	3.36	0.039*
Error	20		
Total	23		

Phospholipids (%):

Source of Variation	df	F	p
Treat	3	0.78	0.519
Error	20		
Total	23		

Total Lipid:

Source of Variation	df	F	p
Treat	3	1.06	0.388
Error	20		
Total	23		

[1] Refers to 1 = 4 meals/day, 2 = 2 meals/day, 3 = 1 meal/day and 4 = 2 meals/2 days.
* Significant with $\alpha = 0.05$.

APPENDIX B

DIET FORMULATIONS

Table B.1 Manufacturer's (Moore-Clark) specifications of fish fry feed[1, 2] used in Experiment 2 (Body Composition of Wild and Cultured Yellowtail Flounder).

	Feed Size	
Guaranteed Analysis	1.5 mm	2.0 mm
Crude Protein (Min.)	48 %	48 %
Crude Fat (Min.)	24 %	24 %
Crude Fibre (Max.)	2.1 %	2 %
Crude Ash (Max.)	7 %	7 %
Calcium	1.3 %	1.3 %
Phosphorous	1.3 %	1.2 %
Vitamin A (Min.)	2500 IU/kg	2500 IU/kg
Vitamin D (Min.)	2400 IU/kg	2400 IU/kg
Vitamin E (Min.)	200 IU/kg	200 IU/kg

[1] Salmonid starter feed, Nutra Fry.

[2] Ingredients: Fish meal, fish oil, whole wheat, krill meal, blood meal, betaine, lecithin, vitamin premix, mineral premix.

Table B.2 Formulation of standard ICES diets (from Aquaculture and *Artemia* Reference Centre, University of Ghent, Belgium).

Ingredients	% of diet
Extruded Basal Diet	92.465
Codfish powder	24
Egg white albumin	11
Whey protein concentrate	11
Isolated soy protein	11
Hemoglobin powder	4
Wheat gluten	3
α – celullose	2.255
Native corn starch	13
Hydrogenated coconut oil	4
Emulgator blend	0.4
Vitamin premix	2
Vitamin C	0.4
Choline chloride	1
Mineral premix	2
Attractant premix	3
Asthaxanthine	0.1
Calcium propionate	0.3
Butylated hydroxytoluene (BHT)	0.005
Butylated hydroxyanisole (BHA)	0.005
Additional coated fraction	*7.533*
De-oiled soya lecithin	2
Oil mixture	5
Emulgator blend	0.5
Ethoxyquin	0.015
Vitamin E	0.02

Table B.3 Crude fat content and fatty acid composition according to manufacturer's specifications (ICES/INVE) for ICES weaning diets >1200 µm.

	0.4% PUFA	1% PUFA	2.5% PUFA
Crude Fat (% in diet)	11.2	11.7	11.8
Fatty Acid Composition (% fatty acid):			
20:4*n*-3	-	0.2	0.5
20:5*n*-3 (EPA)	1.4	5	13.2
22:5*n*-3	0.2	0.9	2.5
22:6*n*-3 (DHA)	2.5	5.6	11.4
Total amount of *n*-3 (mg/g)	4.1	11.7	27.6
	0.4%	1.2%	2.8%
DHA/EPA Ratio	1.8	1.1	0.9

Table B.4 Crude fat content and fatty acid composition according to manufacturer's specifications (Miyako Kagaku Co., Ltd.) for Fry Feed Kyowa (Biokyowa) C-1000.

	C-1000
Crude Fat (% in diet)	12.9
Fatty Acid Composition (% fatty acid):	
20:4*n*-3	0.6
20:5*n*-3 (EPA)	10.9
22:5*n*-3	0.7
22:6*n*-3 (DHA)	10.1
Total amount of *n*-3 (mg/g)	22.3
	2.2%
DHA/EPA Ratio	0.9

APPENDIX C

INTERNAL STANDARD AND % AREA DATA

Table C.1 Fatty acid composition of total, polar and neutral lipid fractions of the liver of wild and cultured yellowtail flounder.

	Total		Polar				Neutral			
	Wild	Cultured	Wild		Cultured		Wild		Cultured	
Fatty Acid	mg/100g	mg/100g	mg/100 g	% area	mg/100 g	% area	mg/100 g	% area	mg/100 g	% area
14:0	19.4±6.2	90.5±21.0*	6.1±0.6	1.0±0.1	8.5±1.3	1.3±0.1	13.3±6.0	2.1±0.3	82.0±20.0*	2.5±0.4
15:0	3.2±1.0	7.1±2.1	1.0±0.1	0.2±0.0	1.0±0.3	0.1±0.0	2.2±1.0	0.3±0.0	6.1±1.8	0.2±0.0
16:0	143.9±17.8	347.0±64.7*	89.3±6.7	15.0±1.0	91.8±10.0	14.3±0.2	54.6±16.2	11.1±1.0	255.2±58.8*	11.8±1.0
16:1	70.4±25.0	218.5±48.6*	18.7±1.1	3.1±0.1	19.7±3.1	3.0±0.2	51.7±25.0	8.0±1.4	198.8±46.4	7.9±1.0
16:2	11.8±3.1	31.9±8.0	5.7±0.7	1.0±0.1	3.8±0.6	0.6±0.1	6.1±3.0	1.0±0.1	28.1±7.6	1.1±0.2
17:0	14.2±3.9	19.7±4.3	7.0±1.2	1.1±0.1	3.8±0.6	0.6±0.0	7.2±3.0	1.2±0.1	15.8±4.0	1.1±0.1
18:0	59.0±6.2	66.6±10.2	38.1±6.0	6.2±0.7	43.2±6.3	6.6±0.4	20.9±3.9	5.9±1.3	25.5±4.5	5.3±1.5
18:1	162.2±42.6	609.6±126.9*	67.6±5.4	11.2±0.4	94.5±7.7*	14.6±0.5	94.6±39.0	15.7±1.4	517.1±122.5*	18.0±2.4
18:2*n*-6	14.0±4.5	118.7±23.5*	4.6±0.5	0.8±0.0	15.8±1.7*	2.5±0.1	9.4±4.2	1.5±0.2	102.9±22.7*	2.7±0.8
18:3*n*-3	3.7±1.1	16.8±3.8	1.4±0.1	0.2±0.0	1.9±0.2	0.3±0.0	2.3±1.0	0.4±0.1	14.9±3.7*	0.4±0.1
18:4*n*-3	1.5±0.3	12.1±3.0*	0.5±0.1	0.1±0.0	0.6±0.1	0.1±0.0	1.1±0.3	0.3±0.1	11.5±2.9*	0.5±0.1
20:1	42.9±13.4	90.1±41.0	15.5±2.3	2.5±0.3	11.1±2.8	1.9±0.5	27.4±11.8	4.2±0.6	79.0±38.3	2.8±0.3
20:2*n*-6	5.0±1.9	12.9±2.5*	1.9±0.4	0.3±0.0	1.9±0.3	0.3±0.0	3.1±1.6	0.5±0.1	11.0±2.3*	0.4±0.1
20:3*n*-6	2.0±1.0	4.3±1.1	1.0±0.4	0.2±0.1	0.7±0.2	0.1±0.0	1.0±0.6	0.1±0.0	3.6±1.0	0.1±0.0
20:4*n*-6	41.2±6.0	54.9±9.0	24.8±4.8	4.0±0.5	33.3±4.7	5.3±0.6	16.4±3.9	4.3±0.8	21.7±4.8	3.8±1.0
20:5*n*-3	159.9±23.6	270.8±47.2	88.0±4.5*	14.8±0.6	65.5±6.6	10.2±0.3	71.9±21.5	14.2±0.7	205.3±45.2*	13.7±0.9
22:1	6.0±2.3	55.1±37.3*	1.2±0.1	0.2±0.0	3.2±2.4	0.4±0.3	4.9±2.2	0.7±0.2	51.9±34.9	0.6±0.2
21:5*n*-3	3.5±1.1	15.3±2.9*	1.1±0.1	0.2±0.0	1.3±0.1	2.9±2.5	2.4±1.1	0.4±0.1	14.0±2.8*	0.4±0.1
22:5*n*-3	35.0±4.9	90.0±17.1*	16.9±0.4	2.9±0.2	16.6±1.4	2.7±0.2	18.2±4.8	3.8±0.3	73.4±16.5*	4.1±0.3
22:6*n*-3	263.5±21.9	416.7±61.5	186.6±9.4	31.2±0.7	198.6±19.3	31.0±0.7	76.9±19.5	18.0±2.2	218.1±47.7*	16.5±2.1
≤SFA	239.6±33.2	532.9±98.8*	141.6±9.6	23.5±0.6	148.3±17.3	22.8±0.4	98.0±27.5	20.7±1.7	384.6±86.8*	18.7±0.3
≤MUFA	281.5±82.9	973.2±233.4*	102.9±7.9	17.1±0.5	126.5±14.5	19.9±1.0	178.6±77.4	28.6±3.2	846.8±222.5*	39.8±2.4
≤PUFA	501.6±60.8	944.7±155.3*	326.6±17.6	54.5±0.4	336.2±31.8	55.3±1.5	202.8±51.5	43.4±2.7	676.3±144.2*	34.6±1.5
n-3	462.0±48.4	792.8±122.1*	292.5±12.9	49.0±1.0	282.0±26.8	46.8±1.9	169.4±42.2	36.3±2.3	510.8±106.3*	26.3±1.3
n-6	62.3±12.9	190.8±33.5*	32.4±5.9	5.2±0.6	51.7±6.0	8.1±0.5	30.0±8.2	6.4±0.6	139.2±29.2*	7.0±0.4
n-9	156.7±41.6	571.1±121.4*	65.8±5.5	10.9±0.4	89.2±9.0	14.0±0.5	96.9±40.2	15.9±1.5	537.1±141.2*	25.1±1.5
n-3/*n*-6	8.2±0.7*	4.3±0.2	10.3±1.3*		5.6±0.4		5.9±0.5*		3.7±0.2	
DHA/EPA	1.8±0.1	1.6±0.1	2.1±0.1		3.0±0.1*		1.3±0.2		1.1±0.1	

Results are expressed in % total fatty acids and mg fatty acid/100 g wet weight using an internal standard of tricosanoic acid methyl ester (23:0). Significantly higher levels of fatty acids (*) are indicated. $n = 6$.

Table C.2 Fatty acid composition of total, polar and neutral lipid fractions of the muscle of wild and cultured yellowtail flounder.

	Total		Polar				Neutral			
	Wild	Cultured	Wild		Cultured		Wild		Cultured	
Fatty Acid	mg/100g	mg/100g	mg/100 g	% area	mg/100 g	% area	mg/100 g	% area	mg/100 g	% area
14:0	19.4±5.3	107.0±15.1*	1.8±0.2	1.4±0.1	4.0±0.6*	2.1±0.2	17.6±5.4	4.5±0.1	103.0±15.2*	5.9±0.1
15:0	2.5±0.9	6.9±1.4*	0.2±0.1	0.2±0.0	0.4±0.1	0.2±0.1	2.3±0.9	0.6±0.1	6.5±1.5*	0.4±0.1
16:0	61.7±13.7	210.2±27.2*	18.4±4.0	13.4±1.1	22.2±3.1	11.9±0.6	43.3±13.8	10.8±0.2	188.0±28.2*	10.7±0.3
16:1	58.3±16.7	208.0±29.9*	3.9±0.5	3.1±0.2	7.5±1.0*	4.0±0.4	54.4±16.8	14.2±0.6	200.5±30.0*	11.5±0.2
16:2	5.4±1.5	25.8±4.3*	0.9±0.2	0.7±0.1	1.0±0.1	0.6±0.1	4.6±1.5	1.1±0.1	24.9±4.3*	1.4±0.1
17:0	4.1±1.1	11.5±1.7*	0.6±0.1	0.5±0.0	0.8±0.1	0.4±0.0	3.5±1.1	0.9±0.1	10.7±1.7*	0.6±0.0
18:0	13.5±2.1	31.8±4.0*	7.1±1.1	5.3±0.3	9.6±1.6	5.2±0.7	6.4±1.8	1.8±0.2	22.2±3.6*	1.3±0.1
18:1	77.4±20.0	360.0±50.0*	16.1±2.4	12.2±0.4	26.7±2.0*	14.6±0.5	61.3±19.9	14.9±0.5	333.3±51.0*	18.9±0.5
18:2*n*-6	8.4±2.4	107.6±15.8*	1.0±0.2	0.7±0.0	5.6±0.6*	3.0±0.1	7.4±2.4	1.9±0.1	102.0±16.0*	5.8±0.2
18:3*n*-3	3.2±1.0	23.1±3.0*	0.5±0.1	0.4±0.1	0.6±0.1	0.3±0.1	2.7±1.0	0.6±0.1	22.5±3.0*	1.3±0.0
18:4*n*-3	7.7±2.7	33.8±4.8*	0.1±0.1	0.1±0.0	0.8±0.1*	0.8±0.3	7.6±2.7	1.7±0.2	33.0±4.8*	1.9±0.1
20:1	28.5±6.1	82.6±22.5	4.5±0.7	3.5±0.3	6.4±1.7	3.5±0.7	24.0±5.9	7.2±1.0	76.2±21.1	4.3±1.2
20:2*n*-6	2.0±0.6	6.5±1.4*	0.2±0.1	0.2±0.0	0.4±0.1	0.2±0.1	1.8±0.6	0.5±0.1	6.1±1.4*	0.3±0.0
20:3*n*-6	1.0±0.3	3.6±4.8*	0.1±0.1	0.1±0.0	0.5±0.3	0.3±0.1	0.8±0.3	0.2±0.0	3.1±0.9	0.2±0.0
20:4*n*-6	7.7±1.4	18.1±2.9*	3.7±0.5	2.9±0.1	4.5±0.5	2.2±0.2	4.0±1.2	1.1±0.1	14.0±2.8*	0.8±0.1
20:5*n*-3	82.4±22.1	220.4±29.4*	18.7±2.3	14.8±0.7	22.1±3.0	11.7±0.6	63.7±22.6	15.4±1.1	198.4±30.7*	11.7±0.9
22:1	4.0±0.9	44.2±14.4*	0.2±0.2	0.3±0.3	2.1±0.8	1.0±0.4	3.7±0.9	1.2±0.1	42.1±13.7*	2.4±0.8
21:5*n*-3	2.5±0.7	12.0±2.0*	0.4±0.1	0.3±0.1	0.5±0.1	0.3±0.1	2.1±0.6	0.6±0.0	11.5±1.9*	0.7±0.1
22:5*n*-3	19.7±4.6	23.1±3.0*	5.7±0.5	4.8±0.7	6.2±0.7	3.4±0.3	14.0±4.5	3.5±0.2	47.5±9.0*	2.8±0.3
22:6*n*-3	83.1±14.8	33.8±4.8*	41.7±7.3	30.7±1.5	52.5±4.8	28.6±1.3	41.4±13.5	10.4±0.7	174.2±3.1*	10.4±0.6
≤SFA	101.2±22.6	6.5±1.4*	28.1±5.3	20.8±1.0	37.1±4.1	19.9±0.5	73.1±22.9	18.5±0.3	330.3±49.6*	18.9±0.5
≤MUFA	168.2±42.6	3.6±4.8*	24.7±3.4	19.2±0.5	42.6±5.0*	23.1±1.5	143.5±42.5	37.5±1.3	652.2±98.6*	37.1±2.2
≤PUFA	208.3±43.5	18.1±2.9*	72.2±10.6	55.1±0.9	93.2±8.7	50.9±1.5	145.5±48.7	35.7±1.5	574.7±81.3*	35.8±1.9
n-3	187.7±40.3	220.4±29.4*	66.3±9.9	50.7±0.9	81.3±7.7*	44.0±1.3	121.2±40.9	29.8±1.6	431.63.9*	25.5±1.7
n-6	19.1±4.5	44.2±14.4*	5.1±0.7	3.9±0.1	10.6±1.0*	5.7±0.2	14.1±4.4	3.6±0.2	125.1±20.3*	7.1±0.3
n-9	76.8±19.3	12.0±2.0*	15.8±2.2	12.2±0.3	27.6±2.7*	15.0±0.7	64.9±20.0	16.4±0.7	361.1±55.2*	20.5±1.2
n-3/*n*-6	10.3±0.5*	4.0±0.3	13.1±0.4*		7.8±0.3		8.4±0.8*		3.6±0.2	
DHA/EPA	1.2±0.2	1.1±0.1	2.1±0.2		2.5±0.2*		0.7±0.0		0.9±0.1*	

Results expressed in % total fatty acids and mg fatty acid/100 g wet weight using an internal standard of tricosanoic acid methyl ester (23:0). Significantly higher levels of fatty acids (*) are indicated. $n = 6$.

Table C.3 Fatty acid composition of polar lipid fractions of the liver of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks.

	Blokyowa (2.2% PUFA)		ICES		
	Initial	C-1000	0.4% PUFA	1% PUFA	2.5% PUFA
Fatty Acid	*n* = 3	*n* = 5	*n* = 6	*n* = 5	*n* = 4
14:0	4.1±0.6	5.3±0.8	5.8±0.8	6.2±0.4	5.2±0.8
15:0	2.7±1.0	1.6±0.3	1.4±0.9	0.3±0.1	0.3±0.0
16:0	26.1±3.0	29.5±1.1	17.1±2.8	16.7±0.7	13.4±1.1
16:1	2.6±0.5	4.8±0.3	6.9±1.5	6.8±0.6	4.9±0.3
16:2	1.9±0.4	2.8±0.7	1.0±0.7	0.1±0.0	0.2±0.0
17:0	1.0±0.1	0.8±0.2	0.7±0.2	0.4±0.3	0.5±0.0
18:0	10.0±4.2	10.9±1.3	5.1±0.6	4.6±0.3	5.0±0.3
18:1	18.1±2.9	20.9±2.2	18.6±3.1	21.8±0.6	19.4±0.4
18:2*n*-6	2.5±1.5	3.8±0.8	13.1±2.7	11.3±0.3	7.9±0.1
18:3*n*-3	0.4±0.0	0.3±0.1	1.2±0.1^{b}	0.8±0.0^{a}	0.6±0.0^{a}
18:4*n*-3	0.1±0.0	0.6±0.3	0.1±0.0	0.0±0.0	0.0±0.0
20:1	4.5±0.3	4.7±0.4	3.1±0.2^{b}	3.1±0.5^{b}	2.6±0.3^{a}
20:2*n*-6	0.9±0.6	0.3±0.1	1.0±0.2	0.9±0.1	1.1±0.1
20:3*n*-6	0.6±0.5	0.1±0.1	0.1±0.0	0.1±0.0	0.2±0.0
20:4*n*-6	0.9±0.3	0.8±0.2	1.3±0.3^{a}	1.6±0.1^{a}	3.1±0.3^{b}
20:5*n*-3	1.6±0.3	2.4±0.7	4.9±1.2^{a}	7.1±0.5^{ab}	9.0±0.3^{b}
22:1	1.1±0.1	1.3±0.3	0.1±0.0^{a}	0.1±0.1^{a}	0.5±0.1^{b}
21:5*n*-3	0.2±0.1	0.1±0.0	0.5±0.4	0.3±0.0	0.3±0.1
22:5*n*-6	0.3±0.3	0.0±0.0	0.2±0.1^{a}	0.3±0.0^{ab}	0.4±0.0^{b}
22:5*n*-3	0.2±0.1	0.3±0.1	1.0±0.2^{a}	2.9±0.2^{ab}	4.6±1.3^{b}
22:6*n*-3	1.7±0.5	3.0±0.8	9.8±2.2	12.4±1.2	14.4±2.1
≤SFA	44.0±7.8	48.0±2.6	30.1±3.8	28.2±1.1	24.4±1.8
≤MUFA	26.3±3.0	31.7±2.5	28.6±4.2	31.8±1.2	27.3±0.4
≤PUFA	9.3±1.8	11.6±2.4	33.3±5.8	37.5±2.0	41.6±3.5
n-3	3.7±0.7	5.8±1.6	16.3±3.2	22.6±0.8	28.3±3.4^{b}
n-6	4.9±1.5	4.9±1.0	15.6±3.1	13.9±0.3	12.2±0.5
n-9	14.2±2.1	17.4±1.3	16.5±2.6	20.4±0.8	17.3±0.3
n-3/*n*-6	1.2±0.5	1.4±0.3	1.0±0.2^{a}	1.6±0.1^{b}	2.3±0.3^{b}
DHA/EPA	1.1±0.3	1.4±0.2	2.0±0.1	1.7±0.1	1.6±0.2

Results are expressed in % total fatty acids (% area). Significantly higher levels of fatty acids between ICES groups are indicated by different letters.

Table C.4 Fatty acid composition of neutral lipid fractions of the liver of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks.

	Biokyowa (2.2% PUFA)		ICES		
Fatty Acid	**Initial** $n=3$	**C-1000** $n=6$	**0.4% PUFA** $n=4$	**1% PUFA** $n=5$	**2.5% PUFA** $n=5$
14:0	4.9±0.5	5.0±0.1	11.6±0.7^{b}	9.7±0.6^{b}	6.2±1.1^{a}
15:0	1.1±0.1	1.2±0.3	0.9±0.2	0.8±0.1	0.8±0.2
16:0	17.7±2.7	23.3±1.9	16.6±2.2	15.2±0.5	12.2±1.0
16:1	5.7±1.6	7.0±0.5	11.8±1.3	15.5±1.4	11.5±1.7
16:2	1.8±0.3	2.8±0.1	0.2±0.0	0.2±0.0	0.3±0.0
17:0	0.9±0.0	1.0±0.0	0.6±0.0	0.5±0.1	0.7±0.1
18:0	4.5±0.4	4.5±0.4	2.8±0.5	1.9±0.3	1.9±0.1
18:1	21.2±6.1	27.9±2.0	24.1±5.7	36.9±1.7	30.1±1.9
18:2*n*-6	5.5±2.0	6.4±1.1	12.1±1.5	7.7±0.8	9.4±0.9
18:3*n*-3	0.6±0.2	0.7±0.1	1.1±0.3	0.8±0.1	0.9±0.1
18:4*n*-3	0.3±0.2	0.3±0.1	0.3±0.2	0.1±0.0	0.2±0.0
20:1	2.7±0.7	4.1±0.3	2.1±0.5	2.9±0.2	2.9±0.2
20:2*n*-6	0.4±0.2	0.5±0.0	2.8±1.5	0.7±0.1	1.1±0.1
20:3*n*-6	0.6±0.2	0.3±0.1	0.0±0.0	0.1±0.1	0.2±0.1
20:4*n*-6	0.5±0.1	0.5±0.1	0.3±0.1	0.4±0.2	0.9±0.2
20:5*n*-3	1.8±0.9	1.9±0.3	1.9±0.7^{a}	2.1±0.6^{a}	6.0±1.2^{b}
22:1	4.6±2.4	2.4±0.3	0.5±0.1^{a}	0.5±0.1^{a}	1.0±0.1^{b}
21:5*n*-3	0.4±0.1	0.2±0.1	1.3±1.1	0.1±0.0	0.6±0.2
22:5*n*-6	0.4±0.1	0.2±0.0	0.3±0.2	0.1±0.0	0.2±0.0
22:5*n*-3	0.5±0.1	0.4±0.1	0.5±0.1^{a}	0.8±0.2^{a}	4.2±1.0^{b}
22:6*n*-3	2.1±0.4	1.9±0.2	2.4±1.4	3.1±1.1	6.0±1.4
≤SFA	29.1±3.6	35.0±2.6	32.5±3.3^{b}	28.1±0.8^{ab}	21.7±1.4^{a}
≤MUFA	34.2±6.1	41.4±2.8	38.5±6.5	53.9±2.9	45.5±3.4
≤PUFA	13.0±3.5	13.4±1.4	23.9±2.7	16.0±3.0	29.6±4.8
n-3	4.8±1.3	4.4±0.5	7.1±1.7^{ab}	8.9±1.0^{a}	16.8±3.6^{b}
n-6	6.9±2.0	7.7±1.1	15.2±1.0^{b}	31.4±2.1	11.2±1.1^{ab}
n-9	17.3±5.3	24.3±2.0	22.3±5.9	6.1±1.9^{a}	27.6±1.7
n-3/*n*-6	0.8±0.2	0.8±0.2	0.4±0.1	0.6±0.1	1.3±0.3
DHA/EPA	1.6±0.4	1.2±0.2	1.6±0.5	1.4±0.1	1.0±0.1

Results are expressed in % total fatty acids (% area). Significantly higher levels of fatty acids between the ICES groups are indicated by different letters.

Table C.5 Fatty acid composition of polar lipid fractions of the muscle of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks.

	Biokyowa (2.2% PUFA)		ICES		
Fatty Acid	Initial $n=3$	C-1000 $n=6$	0.4% PUFA $n=6$	1% PUFA $n=6$	2.5% PUFA $n=6$
14:0	5.1±1.1	2.3±0.2	3.4±0.5	3.7±0.2	3.4±0.1
15:0	2.2±0.8	0.8±0.1	0.5±0.2	0.2±0.0	0.1±0.0
16:0	21.2±1.6	16.4±0.5	13.2±0.9	12.8±0.5	13.1±0.9
16:1	6.8±1.7	3.0±0.2	3.7±0.7	3.4±0.4	2.4±0.1
16:2	0.5±0.2	0.5±0.1	0.5±0.1	0.4±0.1	0.3±0.0
17:0	0.6±0.1	0.7±0.0	0.5±0.2	0.4±0.1	0.4±0.1
18:0	7.3±0.2	6.4±0.2[a]	6.9±0.5	7.4±0.7	7.4±0.4
18:1	25.6±3.0	17.6±0.6	19.4±1.1[b]	19.9±0.5[b]	16.0±0.6[a]
18:2*n*-6	4.8±0.9	6.0±0.2	9.9±0.9	8.5±0.3	7.7±0.2
18:3*n*-3	0.3±0.1	0.6±0.0	0.8±0.1	0.8±0.0	0.5±0.1
18:4*n*-3	0.3±0.1	0.3±0.0	0.1±0.1	0.1±0.0	0.1±0.0
20:1	5.2±0.5	3.6±0.1	3.2±0.2	3.5±0.3	2.8±0.2
20:2*n*-6	0.4±0.1	0.5±0.0	1.0±0.1	1.1±0.1	0.9±0.0
20:3*n*-6	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0
20:4*n*-6	0.7±0.5	2.1±0.1	1.9±0.2	1.8±0.1	2.2±0.1
20:5*n*-3	4.7±3.3	12.7±0.5	11.3±1.1	10.9±0.6	13.9±0.5
22:1	1.0±0.4	0.7±0.1	0.0±0.0[a]	0.2±0.0[ab]	0.5±0.2[b]
21:5*n*-3	0.2±0.0	0.2±0.0	0.2±0.1[a]	0.4±0.0[ab]	0.5±0.0[b]
22:5*n*-6	0.2±0.1	0.4±0.0	0.7±0.3	0.3±0.1	0.5±0.1
22:5*n*-3	0.7±0.0	1.5±0.1	1.5±0.1[a]	2.3±0.1[b]	4.1±0.2[c]
22:6*n*-3	6.3±0.1	19.1±1.0	17.8±2.1	16.6±0.8	19.8±1.0
≤SFA	36.4±3.2	26.7±0.5	24.5±1.0	24.5±0.7	24.4±1.1
≤MUFA	38.6±5.3	24.9±1.0	26.3±1.9	27.1±0.8	21.7±1.0
≤PUFA	18.7±9.3	43.4±1.4	45.2±3.2	42.8±1.3	50.3±1.9
n-3	11.9±7.8	35.5±1.5	30.8±3.3	30.2±1.2	38.3±1.7
n-6	6.0±1.3	8.7±0.1	13.0±0.7[b]	11.5±0.3[ab]	11.0±0.3[a]
n-9	21.1±4.0	14.1±0.7	15.3±1.2	16.6±0.4	13.3±0.6
n-3/*n*-6	1.5±0.8	4.0±0.2	2.4±0.3[a]	2.6±0.1[b]	3.5±0.1[c]
DHA/EPA	1.7±0.2	1.4±0.0	1.6±0.0	1.5±0.1	1.41±0.0

Results are expressed in % total fatty acids (% area). Significantly higher levels of fatty acids between the ICES groups are indicated by different letters.

Table C.6 Fatty acid composition of neutral lipid fractions of the muscle of yellowtail flounder fed varying levels of *n*-3 PUFA after 12 weeks.

	Biokyowa (2.2% PUFA)		ICES		
Fatty Acid	Initial $n=3$	C-1000 $n=6$	0.4% PUFA $n=4$	1% PUFA $n=5$	2.5% PUFA $n=5$
14:0	6.8±0.5	5.3±0.2	7.6±0.6^{a}	10.6±0.5^{b}	7.4±0.3^{a}
15:0	1.2±0.3	0.7±0.1	0.7±0.1^{b}	0.7±0.0^{b}	0.5±0.0^{a}
16:0	21.0±1.9	15.8±0.4	14.7±0.5^{b}	14.4±0.1^{b}	12.1±0.3^{a}
16:1	8.0±0.1	7.0±0.3	6.9±0.2^{a}	7.1±0.2^{a}	5.2±0.2^{b}
16:2	1.0±0.0	0.4±0.1	0.3±0.1	0.2±0.0	0.2±0.0
17:0	0.6±0.1	0.5±0.0	0.5±0.0^{b}	0.5±0.0^{b}	0.4±0.0^{a}
18:0	3.1±0.5	2.1±0.1	2.6±0.2	2.1±0.0	2.8±0.4
18:1	26.5±1.2	23.6±0.6	23.0±0.6^{b}	23.5±0.4^{b}	20.0±0.3^{a}
18:2*n*-6	8.0±1.7	11.1±0.2	12.7±0.4^{a}	14.1±0.3^{b}	13.3±0.3^{ab}
18:3*n*-3	1.8±0.3	1.9±0.1	2.0±0.1^{a}	2.2±0.0^{b}	2.0±0.1^{ab}
18:4*n*-3	0.4±0.2	1.7±0.1	1.2±0.1	1.1±0.1	1.0±0.1
20:1	5.5±0.8	4.4±0.2	4.5±0.3^{b}	3.2±0.2^{a}	3.1±0.2^{a}
20:2*n*-6	0.5±0.2	0.5±0.0	0.7±0.0	0.7±0.0	0.7±0.0
20:3*n*-6	0.2±0.1	0.1±0.0	0.4±0.3	0.1±0.0	0.2±0.0
20:4*n*-6	0.4±0.2	0.6±0.0	0.6±0.1^{a}	0.5±0.0^{a}	0.8±0.0b
20:5*n*-3	3.7±1.6	9.1±0.7	7.2±0.3^{a}	7.1±0.2^{a}	11.8±0.3^{b}
22:1	2.6±0.6	1.7±0.1	1.6±0.2	1.3±0.1	1.4±0.1
21:5*n*-3	0.2±0.1	0.4±0.0	0.6±0.2	0.3±0.0	0.7±0.0
22:5*n*-6	0.0±0.0	0.2±0.0	0.2±0.0^{a}	0.1±0.0^{a}	0.3±0.0^{b}
22:5*n*-3	0.6±0.1	1.0±0.1	0.8±0.0^{a}	1.2±0.0^{a}	3.3±0.2^{b}
22:6*n*-3	3.0±1.3	7.9±0.8	6.8±0.6^{a}	6.4±0.2^{a}	10.3±0.3^{b}
ΣSFA	32.7±3.0	24.4±0.7	26.1±1.1^{b}	28.2±0.5^{b}	23.1±0.5^{a}
ΣMUFA	42.5±2.2	36.7±1.0	36.0±0.8^{b}	35.1±0.6^{b}	29.7±0.6^{a}
ΣPUFA	18.7±4.5	34.4±1.8	33.1±0.8^{a}	33.9±0.3^{a}	44.3±1.0^{b}
n-3	7.4±3.0	18.4±1.7	15.4±0.9^{a}	15.0±2.3^{a}	26.1±0.8^{b}
n-6	9.0±2.0	12.3±0.2	14.4±0.4	15.5±2.4	14.9±0.3
n-9	23.1±2.4	19.7±0.5	20.8±0.5^{a}	21.4±3.3^{b}	18.4±0.3^{a}
n-3/*n*-6	0.8±0.2	1.5±0.1	1.1±0.1^{b}	1.0±0.1^{a}	1.8±0.1^{b}
DHA/EPA	1.0±0.2	0.9±0.0	0.9±0.1	0.9±0.0	0.9±0.0

Results are expressed in % total fatty acids (% area). Significantly higher levels of fatty acids between the ICES groups are indicated by different letters.

Table C.7 Fatty acid composition of total, polar and neutral lipid fractions of liver of initial yellowtail flounder samples and those fed Biokyowa C-1000.

	Total				Polar				Neutral			
	Initial B-750		Biokyowa C-1000		Initial B-750		Biokyowa C-1000		Initial B-750		Biokyowa C-1000	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	5.8±1.4	101.1±62.8	5.5±0.6	168.3±42.2	5.3±1.2	31.4±6.2	5.7±1.0	70.2±11.3	6.5±0.7	76.3±32.1	5.4±0.2	91.6±32.2
15:0	1.9±0.9	26.4±11.6	1.5±0.3	45.1±11.1	3.4±1.3	18.1±4.1	1.7±0.3	22.9±7.5	1.5±0.4	14.8±5.5	1.4±0.4	20.7±6.3
16:0	26.6±1.8	529.6±378.6	27.9±2.6	823.6±169.1	31.9±1.0	236.1±109.4	31.4±1.3	402.5±61.4	22.9±1.2	289.3±126.4	25.4±2.8	398.2±117.2
16:1	4.5±1.8	123.5±109.0	6.4±0.6	219.4±78.2	3.0±0.4	25.5±15.2	5.1±0.4	65.5±9.8	6.6±1.6	103.5±50.7	7.5±0.4	140.8±59.6
16:2	2.5±0.9	38.1±20.2	3.0±0.5	92.4±23.1	2.6±0.8	13.8±1.2	3.0±0.9	37.4±9.9	2.4±0.2	29.2±12.5	3.0±0.1	51.2±16.5
17:0	1.3±0.3	22.7±14.6	1.0±0.1	31.7±9.9	1.3±0.0	9.3±4.3	0.8±0.2	11.8±3.9	1.3±0.3	13.1±5.0	1.1±0.1	18.6±6.2
18:0	5.6±3.2	167.8±155.2	8.1±1.3	217.5±28.1	11.0±5.7	94.1±58.4	11.7±1.7	143.3±19.0	6.0±0.6	71.4±31.8	4.9±0.5	73.5±19.1
18:1	25.3±2.8	575.3±455.4	26.4±2.4	890.8±306.4	22.6±3.9	178.6±98.8	22.0±2.4	288.4±53.7	24.9±6.0	388.1±189.2	29.9±1.8	557.9±228.7
18:2*n*-6	5.0±3.7	161.5±154.8	5.4±1.3	199.9±93.0	2.7±1.8	32.6±29.1	4.0±1.0	51.6±12.7	6.2±2.3	107.2±64.4	6.8±1.2	139.3±69.1
18:3*n*-3	0.8±0.1	17.7±14.1	0.6±0.1	19.6±8.2	0.5±0.1	3.4±1.3	0.3±0.1	3.6±1.1	0.8±0.2	12.0±7.3	0.7±0.1	14.1±6.3
18:4*n*-3	0.3±0.3	9.7±9.7	0.5±0.2	14.9±4.7	0.1±0.1	0.8±0.4	0.6±0.4	6.8±3.3	0.3±0.3	6.6±5.7	0.4±0.1	7.0±3.2
20:1	4.8±1.0	83.7±52.8	4.8±0.2	147.4±37.0	5.8±1.0	37.0±10.9	5.1±0.5	62.4±5.7	3.2±0.6	47.9±22.6	4.4±0.4	79.4±30.1
20:2*n*-6	1.6±1.1	17.5±3.2	0.5±0.0	15.4±4.9	1.5±1.3	6.7±4.2	0.3±0.	3.9±0.9	0.4±0.2	7.7±4.3	0.6±0.0	10.5±3.9
20:3*n*-6	1.3±1.2	8.4±5.3	0.2±0.1	6.1±1.6	0.9±0.9	3.2±3.2	0.1±0.1	0.8±0.8	1.0±0.7	4.7±1.2	0.3±0.1	4.7±1.6
20:4*n*-6	1.4±0.6	20.8±10.4	0.7±0.2	20.1±5.7	1.2±0.6	7.8±3.3	0.8±0.3	10.4±3.0	0.7±0.2	7.6±5.3	0.6±0.2	9.6±4.0
20:5*n*-3	2.8±0.7	68.8±57.6	2.3±0.6	75.6±27.4	2.0±0.4	16.2±9.7	2.5±0.8	31.4±8.3	2.3±1.0	35.4±27.8	2.0±0.4	40.6±20.7
22:1	4.8±3.5	45.7±1.4	1.9±0.3	62.8±22.5	1.4±0.2	9.1±2.9	1.3±0.3	17.8±5.1	8.5±6.8	33.8±2.4	2.6±0.4	44.2±15.9
21:5*n*-3	0.5±0.3	5.6±1.6	0.2±0.1	4.4±1.7	0.3±0.3	1.0±1.0	0.1±0.1	1.0±0.5	0.6±0.1	6.5±2.9	0.2±0.1	3.1±1.2
22:5*n*-3	0.6±0.1	11.5±7.9	0.4±0.1	13.7±6.2	0.3±0.1	2.2±1.5	0.3±0.1	4.2±1.4	0.7±0.2	6.7±3.9	0.4±0.1	8.4±4.8
22:6*n*-3	2.5±0.5	60.7±50.4	2.7±0.5	80.8±20.0	2.0±0.5	17.7±11.7	3.2±1.0	39.1±10.0	2.8±0.6	34.0±18.9	2.1±0.2	37.7±16.0
≤SFA	41.2±1.1	847.6±622.8	44.0±4.6	1286.2±254.7	52.9±7.0	389.0±177.2	51.2±3.6	650.8±95.0	38.2±2.7	464.9±200.0	38.1±3.8	602.6±179.5
≤MUFA	39.4±0.1	828.2±618.7	39.5±3.1	1320.4±442.7	32.9±4.1	250.1±127.6	33.5±2.5	434.1±70.6	43.2±1.8	573.3±260.3	44.4±2.6	822.3±333.7
≤PUFA	16.7±2.0	382.2±304.2	13.4±2.3	450.5±165.0	11.4±3.2	91.8±54.3	12.3±2.8	152.9±30.4	15.6±3.3	228.3±136.8	14.1±1.6	274.9±128.1
n-3	6.3±0.9	146.6±117.4	5.5±1.1	174.5±53.8	4.5±0.7	37.2±22.2	6.2±1.8	75.7±19.2	6.3±1.4	82.6±51.3	4.8±0.6	89.8±42.2
n-6	9.3±0.8	208.2±163.0	6.7±1.4	241.5±103.0	6.3±2.5	50.4±30.9	5.2±1.2	66.8±14.7	8.2±1.9	127.1±73.2	8.3±1.2	164.0±77.1
n-9	24.0±2.3	538.7±423.1	25.0±2.2	838.6±285.0	21.8±3.5	170.0±91.6	21.1±2.1	275.2±49.1	23.2±5.5	361.3±175.9	28.0±1.7	522.0±213.3
n-3/*n*-6	0.7±0.0		0.9±0.2		1.2±0.6		1.3±0.3		0.8±0.2		0.7±0.1	
DHA/EPA	0.9±0.0		1.3±0.2		1.1±0.3		1.4±0.2		1.6±0.4		1.2±0.2	

Results are expressed in % weight and mg fatty acid/100 g wet weight based on TLC-FID estimates, $n = 6$.

Table C.8 Fatty acid composition of total, polar and neutral lipid fractions of muscle of initial yellowtail flounder samples and those fed Biokyowa C-1000.

	Total				Polar				Neutral			
	Initial B-750		Biokyowa C-1000		Initial B-750		Biokyowa C-1000		Initial B-750		Biokyowa C-1000	
Fatty Acid	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g	% wt	mg/100g
14:0	6.2±0.2	60.0±6.0	5.2±0.3	270.0±87.8	5.5±1.5	10.2±3.4	2.5±0.6	9.1±3.1	7.8±0.5	37.7±16.2	5.5±0.3	260.9±85.6
15:0	1.0±0.0	9.2±1.3	0.8±0.1	41.5±18.1	2.4±1.0	4.7±2.7	0.8±0.2	2.9±1.0	1.2±0.4	5.3±2.1	0.8±0.1	38.6±17.2
16:0	20.1±1.7	193.1±9.6	16.5±0.5	800.0±235.5	22.6±2.4	43.2±9.6	17.1±1.7	57.9±9.0	22.1±2.2	111.9±45.9	16.5±0.5	742.0±229.8
16:1	7.8±0.6	74.9±3.8	6.9±0.4	354.1±116.1	7.3±2.2	14.0±6.2	3.1±0.8	11.5±4.2	8.4±0.0	46.8±20.3	7.3±0.3	342.6±113.0
16:2	0.9±0.1	8.9±0.5	0.4±0.1	17.2±4.3	0.5±0.2	0.9±0.3	0.6±0.2	1.7±0.5	1.0±0.0	5.5±2.3	0.4±0.1	15.4±4.4
17:0	0.8±0.0	7.3±0.6	0.6±0.1	26.9±8.8	0.6±0.1	1.2±0.3	0.8±0.1	2.6±0.5	0.6±0.1	4.1±1.9	0.5±0.0	24.2±8.7
18:0	3.6±0.1	34.4±3.4	2.6±0.2	119.4±31.0	7.7±0.3	15.1±3.5	6.7±0.6	22.0±1.8	3.2±0.7	14.8±5.9	2.2±0.1	97.5±30.7
18:1	26.2±2.5	251.1±9.4	23.8±0.9	1185.6±360.2	27.2±4.1	9.7±3.3	18.4±2.3	64.1±13.9	27.9±1.5	149.1±63.2	24.6±0.7	1121.5±350.5
18:2*n*-6	9.9±0.2	96.1±14.6	10.9±0.4	578.9±15.6	5.1±1.1	0.7±0.5	6.3±0.7	21.6±4.2	8.4±2.3	57.7±27.9	11.5±0.2	526.2±153.2
18:3*n*-3	1.4±0.3	14.3±4.3	1.8±0.1	93.7±27.1	0.4±0.2	0.5±0.1	0.7±0.1	2.3±0.5	1.9±0.3	9.5±4.3	2.0±0.1	91.5±26.7
18:4*n*-3	0.5±0.3	4.8±2.7	1.6±0.1	79.0±20.2	0.3±0.1	10.5±2.4	0.3±0.1	1.0±0.3	0.5±0.2	2.9±2.1	1.8±0.1	78.0±20.1
20:1	4.9±0.7	46.3±0.3	4.4±0.2	220.7±66.0	5.6±0.7	1.0±0.4	3.8±0.5	13.2±2.8	5.8±1.0	27.2±10.8	4.5±0.2	207.5±64.0
20:2*n*-6	0.7±0.2	6.4±0.9	0.5±0.0	23.5±6.2	0.5±0.1	0.3±0.2	0.5±0.0	1.6±0.1	0.6±0.2	3.7±2.0	0.5±0.0	21.9±6.2
20:3*n*-6	0.2±0.1	1.5±1.0	0.1±0.0	4.7±1.5	0.1±0.1	1.8±1.6	0.1±0.0	0.3±0.1	0.2±0.1	0.9±0.8	0.1±0.0	4.4±1.6
20:4*n*-6	0.7±0.1	7.2±2.0	0.8±0.1	33.5±6.5	0.7±0.6	17.1±3.6	2.2±0.4	6.9±1.0	0.4±0.2	3.2±1.6	0.6±0.0	26.6±6.5
20:5*n*-3	5.8±3.3	60.7±39.5	10.0±0.9	434.0±95.4	4.9±4.2	11.9±10.7	13.3±1.9	42.4±6.0	3.9±2.1	29.4±19.6	9.5±0.8	391.6±97.1
22:1	2.8±1.4	25.6±9.5	1.7±0.1	92.5±23.1	1.1±0.5	1.7±0.2	0.7±0.3	2.7±1.3	2.8±0.9	16.4±9.1	1.8±0.1	79.8±22.1
21:5*n*-3	0.3±0.1	2.9±0.9	0.4±0.0	17.8±4.3	0.5±0.0	0.4±0.1	0.2±0.1	0.7±0.2	0.2±0.1	1.6±0.9	0.4±0.0	17.1±4.3
22:5*n*-3	0.7±0.3	7.2±3.4	1.1±0.1	45.0±9.1	0.7±0.4	1.6±1.1	1.5±0.2	5.0±0.8	0.6±0.1	3.5±1.9	1.0±0.1	40.0±9.3
22:6*n*-3	5.3±3.5	55.9±41.1	9.7±1.4	385.4±76.5	6.5±5.3	15.8±13.5	20.0±3.4	64.0±12.5	3.2±1.6	22.9±16.1	8.2±1.0	321.4±78.3
≤SFA	31.6±2.0	304.0±20.9	25.6±0.9	1257.7±397.8	38.8±4.6	74.1±17.4	27.9±1.9	94.5±13.1	34.4±3.5	173.8±71.3	25.5±0.9	1163.2±370.6
≤MUFA	41.7±5.1	363.7±105.0	36.8±1.5	1842.7±564.6	41.2±7.2	79.3±23.4	26.1±3.8	91.4±21.9	44.8±2.7	239.6±101.6	38.3±1.2	1751.4±548.9
≤PUFA	25.6±7.1	256.9±101.2	37.0±2.2	1664.5±370.8	19.4±11.5	43.6±31.1	45.1±5.0	145.7±17.0	19.8±5.8	135.3±71.0	35.6±2.0	1518.7±379.3
n-3	12.1±7.2	126.6±84.9	21.2±2.4	882.2±182.8	12.4±9.9	29.6±25.4	35.1±5.5	112.1±19.3	7.8±3.9	57.5±38.4	19.1±1.9	770.1±187.7
n-6	11.5±0.0	111.2±14.7	12.4±0.4	609.6±168.3	6.4±1.6	12.8±5.2	9.1±0.4	30.4±3.6	9.5±2.6	65.5±31.3	12.8±0.2	579.1±166.0
n-9	24.8±2.4	237.5±84.1	22.5±0.8	1122.2±340.6	25.9±3.9	50.4±14.4	17.5±2.2	60.9±13.2	26.5±1.5	141.0±59.6	23.3±0.7	1061.2±331.3
n-3/*n*-6	1.1±0.6		1.7±0.2		1.5±1.0		4.0±0.7		0.8±0.3		1.5±0.1	
DHA/EPA	0.8±0.1		0.9±0.1		1.7±0.2		1.4±0.1		0.9±0.2		0.9±0.0	

Results are expressed in % weight and mg fatty acid/100 g wet weight. $n = 6$

APPENDIX D

ESTIMATION OF FATTY ACID CONCENTRATIONS USING ACYL LIPID DATA

EXPLANATION OF METHOD USED TO FIND FATTY ACID CONCENTRATIONS BY CALCULATION:

Estimation of Fatty Acid Concentrations Using Acyl Lipid Data

Budge (1999) used acyl lipid data from TLC-FID to make estimates on the FAME concentrations. This method involves converting the percent total fatty acids to mole percent data. The average number of double bonds and carbon atoms in that sample can then be calculated. Then the mole percent data is used to calculate the average fatty acid molecular weight, and a molar mass for each acyl lipid class. From the molar mass, the amount of fatty acids contributed to total mass from each acyl lipid is determined. Budge (1999) reports high accuracy from TAG, FFA and PL, and less reliability from SE/WE and AMPL due to non-acyl components of these lipid classes. An example is found on the next page.

Table D.1 Results of calculations to produce fatty acid concentrations from fatty acid methyl ester proportions and lipid class concentrations in a sample of the neutral portion of lipid from wild yellowtail flounder liver.

Fatty Acid	Molar Mass	Weight %	Moles (x 1000)	Mole %
14:0	242.40	1.35	5.56	1.77
15:0	256.43	0.22	0.88	0.28
16:0	270.45	18.47	68.30	21.80
16:1n-9	268.44		0.00	0.00
16:1n-7	268.44	3.51	13.06	4.17
16:1n-5	268.44		0.00	0.00
16:2n-4	266.42	1.12	4.21	1.34
17:0	284.48	1.00	3.53	1.13
16:3n-4	264.41		0.00	0.00
16:4n-3	262.39		0.00	0.00
16:4n-1	262.39		0.00	0.00
18:0	298.51	3.46	11.60	3.70
18:1n-11	296.49		0.00	0.00
18:1n-9	296.49	10.43	35.19	11.23
18:1n-7	296.49		0.00	0.00
18:1n-5	296.49		0.00	0.00
18:2n-6	294.48	0.82	2.80	0.89
18:2n-4	294.48		0.00	0.00
18:3n-6	292.46		0.00	0.00
18:3n-4	292.46		0.00	0.00
18:3n-3	292.46	0.31	1.06	0.34
18:4n-3	290.45	0.12	0.40	0.13
18:4n-1	290.45		0.00	0.00
20:1n-11	324.55		0.00	0.00
20:1n-9	324.55	1.68	5.17	1.65
20:1n-7	324.55		0.00	0.00
20:2n-6	322.53	0.32	0.99	0.32
20:3n-6	320.51	0.06	0.20	0.06
20:4n-6	318.50	3.48	10.94	3.49
20:4n-3	318.50		0.00	0.00
20:5n-3	316.48	16.93	53.50	17.07
22:1	352.60	0.10	0.27	0.09
21:5n-3	330.51	0.18	0.55	0.18
22:5n-6	344.54		0.00	0.00
22:5n-3	344.54	2.59	7.51	2.40
22:6n-3	342.52	30.02	87.63	27.97
Sum Moles			0.31	100.00
Avg Chain Length		19.04		
Avg Double Bonds		3.04		
AverageMolecular Weight		306.95		

Neutral portion of lipid in liver	Amount (g/g dry weight)
Lipid Class	FAME from
Hydrocarbons	Steryl/Wax Esters 0.00
Steryl Esters/Wax Esters	Methyl Esters 0.00
Methyl Esters	Ketones 0.00
Ethyl Ketones	Triacylglycerols 0.00
Triacylglycerols	Free Fatty Acids 0.00
Free Fatty Acids	Diacylglycerols 0.00
Alcohols	Acetone Mobile Polar 226.73
Pink Pigment	Phospholipids 17067.50
Sterols	
Diacylglycerols	Total Lipids 17294.23
Acetone Mobile Polar 1142.14	
Polar Lipids 22437.16	
Total 23579.30	