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Effects of dietary lipid and environmental salinity on growth, body composition, and cold tolerance of juvenile red drum (*Sciaenops ocellatus*)

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Abstract

Simultaneous, 6-week feeding trials were conducted in which diets containing menhaden, corn, coconut and hydrogenated menhaden oil at 7.0%, plus a diet containing 14% menhaden oil, were fed to triplicate groups of juvenile red drum (*Sciaenops ocellatus*) at two different salinities (5 and 32‰). Weight gain was significantly ($p < 0.05$) affected by diet and salinity. Fish fed the diet containing 14% menhaden oil had the greatest weight gain; whereas, fish fed the diet containing coconut oil gained the least weight. Fish in brackish water had significantly greater weight gain than fish in full-strength seawater over the 6-week period, although fish fed coconut and saturated menhaden oil in brackish water had reduced survival. Dietary lipid also significantly affected muscle and liver total lipid, hepatosomatic index (HSI), and intraperitoneal fat (IPF) ratio, as fish fed the diets containing 14% menhaden oil had higher values for all of these body condition indices.

After the feeding trial, fish were subjected to a chronic cold tolerance assay. In the chronic trial, where temperature was gradually reduced over a 3-week period, fish fed the diets containing menhaden oil had significantly lower median lethal temperatures (MLT) than those fish fed the diets containing coconut, corn and saturated menhaden oils. No significant effects of cold exposure were observed on muscle and liver total lipid. Cold exposure prompted a modification in lipid metabolism by lowering total saturated fatty acids and raising (n – 3) highly unsaturated fatty acids (HUFA) in the neutral lipid of liver. Fish with the lowest MLT in the chronic assay exhibited signs of conserving (n – 3) HUFA and depleting (n – 6) fatty acids [primarily 18:2 (n – 6)], resulting in higher (n – 3)/(n – 6) ratios in the polar lipid of liver. These data suggest that the lower lethal temperature of juvenile red drum can be reduced through dietary manipulation involving the inclusion of high levels of dietary lipid rich in (n – 3) HUFA.

Introduction

Lipids obtained predominantly from aquatic organisms are unique in that they contain high levels of highly unsaturated fatty acids (HUFA) of the (n – 3) series, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These long-chain HUFA have been determined to be essential fatty acids (EFA) for many marine fishes (Fujii and Yone 1976; Cowey *et al.* 1976; Kanazawa *et al.* 1979; Bell *et al.* 1985; Lochmann and Gatlin 1993), and have been incorporated as fish oils into

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diets of many cultured species to meet their requirements. These HUFA are essential for fish to maintain various body functions, including cell membrane function (Skuladottir *et al.* 1990).

Poikilotherms typically react to lowered environmental temperature by increasing the unsaturation of their body lipids (Hazel 1979). Alterations of unsaturated fatty acids in membranes can influence the physical properties of these membranes and impart a partial independence from acclimation or growth temperature, a process referred to as homeoviscous adaptation (Sinensky 1974; Hazel 1979, 1984). Several studies have demonstrated homeoviscous adaptation through increases in the content and kinds of HUFA in membrane phospholipids which, in turn, increased the membrane fluidity and lowered the phase transition temperature in fish (Bell *et al.* 1986). These changes in the composition of HUFA in membrane phospholipids are believed to increase the survival of fish at temperatures near 0°C (Farkas 1979). Changes in the composition of biological membranes in response to cold acclimation are achieved primarily through modifications in three components: acyl chain composition, fatty acid distribution within the phospholipid (PL) molecule, and relative PL composition (Hazel 1984).

Dietary manipulations are well known to affect the body composition of cultured animals (Lovell 1989). Because marine fish are relatively inefficient at the production of long-chain HUFA through elongation and desaturation reactions (Owen *et al.* 1975; Greene and Selivonchick 1987; Watanabe *et al.* 1989; Ostrowski and Divakaran 1990), the diet could influence the ability of these fish to withstand cold by supplying certain HUFA necessary to maintain membrane fluidity. Thus, in marine fish, mobilization of existing reserves of HUFA may play a more prominent role in the alteration of membrane composition than fatty acid elongation and desaturation reactions. Diets rich in long-chain HUFA may enable fish to conserve and build up a reserve of HUFA from which mobilization can occur in response to low environmental temperatures.

The red drum (*Sciaenops ocellatus*) has become a prominent candidate for aquaculture in the Gulf Coast states due to its ease of production in hatcheries and its tolerance of a wide range of salinities and other environmental conditions, coupled with high demand for the fresh product. Procarione (1986) showed that juvenile red drum can survive temperatures as low as 6.9°C in water of 5‰ salinity. Increases or decreases in salinity elevated the lower lethal temperature. Currently, most red drum aquaculture in Texas is conducted in outdoor earthen ponds with brackish (3–15‰) water. Because red drum is a relatively cold-intolerant species, water temperatures in south Texas have been low enough to cause several cold kills in recent years (1983, 1989, and 1990) which devastated fish production. A decrease in the lower lethal temperature as small as 0.5 to 1.0°C through dietary manipulation could significantly influence the survival of red drum in outdoor ponds during cold winters. The purpose of this study was to determine the effects of salinity and dietary lipids on growth and cold tolerance of red drum. In addition, the effects of salinity and dietary lipid on fat deposition and body composition were investigated.

Materials and methods

Two feeding trials were run concurrently in separate systems containing brackish (5‰) and full-strength seawater (32‰) produced by mixing well water with sodium chloride and synthetic sea salts according to manufacturer specifications (Fritz Aquaculture, Dallas, Texas). Each recirculating system consisted of fifteen 110 l glass aquaria connected in parallel with each other and in series with a packed-column biofilter for removal of nitrogenous wastes and a sand filter for mechanical filtration. Water temperature was maintained at 27 ± 2°C. Five diets were formulated with different kinds and levels of lipid, each chosen for specific characteristics (Table 1). Coconut oil at 7% of diet provided high levels of 12- and 14-carbon saturated fatty acids, corn oil at 7% of diet provided high levels of 18:2(n – 6), and menhaden oil at 7 and 14% of diet provided high levels of EPA and DHA. In addition, a portion of the menhaden oil was hydrogenated in a nickel-catalyzed reaction and added to a diet at 7% to compare with untreated menhaden oil and coconut oil, thus permitting in-

Table 1. Composition of the experimental diets (g 100 g^{-1} dry-weight) fed to red drum

	Diet designation[1]				
Ingredient[2]	Fish	Sat.fish	Corn	Coconut	14Fish
Red drum muscle	20.7	20.7	20.7	20.7	20.7
Amino acid premix[3]	15.0	15.0	15.0	15.0	15.0
Dextrin	38.6	38.6	38.6	38.6	22.8
Menhaden fish oil (MFO)	7.0	–	–	–	14.0
Saturated MFO	–	7.0	–	–	–
Corn oil	–	–	7.0	–	–
Coconut oil	–	–	–	7.0	–
Mineral premix[4]	4.0	4.0	4.0	4.0	4.0
Vitamin premix[4]	3.0	3.0	3.0	3.0	3.0
Carboxymethyl cellulose	2.0	2.0	2.0	2.0	2.0
Calcium phosphate (dibasic)	1.0	1.0	1.0	1.0	1.0
Cellulose	8.7	8.7	8.7	8.7	17.5

[1]Fish = menhaden fish oil; Sat.fish = saturated menhaden fish oil; 14Fish = high level menhaden fish oil; [2]sources of ingredients: red drum muscle, obtained from wild fish and solvent extracted to contain 96.6% crude protein (dry) and 1.7% lipid (dry); amino acids provided by Nutriquest, Inc., Chesterfield, Missouri; dextrin, corn oil, coconut oil, vitamins, carboxymethyl cellulose and cellulose purchased from United States Biochemical Corp., Cleveland, Ohio; winterized menhaden fish oil provided by Zapata Haynie Corp., Reedville, Virginia; [3]the amino acid premix, consisting of the L-isomers of crystalline amino acids, had the following composition (%): Arginine HCl, 8.54; Glycine, 5.74; Histidine, 2.94; Isoleucine, 5.87; Leucine, 9.60; Lysine HCl, 14.67; Methionine, 3.87; Cystine, 3.60; Phenylalanine, 4.80; Tyrosine, 4.27; Serine, 4.60; Threonine, 5.47; Tryptophan, 1.00; Valine, 6.40; Proline, 11.14; Alanine, 11.14; [4]same as Moon and Gatlin (1991).

vestigation of the effects of fatty acid saturation and chain length. Diets were formulated to provide 35% crude protein on a dry-matter basis from a combination of lyophilized red drum muscle and an amino acid premix. This combination resulted in dietary amino acid levels that equaled or exceeded those found in whole chicken egg or red drum muscle (Moon and Gatlin 1991). The red drum muscle was extracted with 5 volumes of a hexane: ethanol mixture (4:1 v/v) to reduce endogenous lipid levels; the muscle provided approximately 0.35% (n – 3) HUFA when added to the diet at 20.7% (dry). Therefore, the diets other than those containing untreated menhaden oil were marginally adequate in EFA (Lochmann and Gatlin 1993). Dietary lipid and dextrin levels were adjusted to provide 3.60 kcal available energy/g (Serrano *et al.* 1992).

At each salinity, 15 aquaria were stocked with 32 juvenile red drum initially weighing 104.0 ± 2.6 g $group^{-1}$ (mean ± SD). Prior to stocking, fish were fed a conditioning diet containing coconut oil for 10 days. During the feeding trial, fish in three replicate aquaria were fed each diet at a fixed percentage of body weight per day divided into two equal allotments, one for the morning and one for the evening. Fish in each aquarium were collectively weighed each week and feed allowances adjusted for growth and mortalities. Feeding rate was progressively reduced equally among treatments from 7 to 3% of body weight over the course of the experiment as the fish grew so that a level close to satiation was maintained without overfeeding. Adjustments in biomass among treatments were not made because the culture systems provided adequate water quality regardless of biomass in each aquarium. The feeding trials lasted 6 weeks after which weight gain, feed efficiency and survival data were subjected to factorial analysis of variance (ANOVA) and Duncan's multiple range test using SAS (1985). Differences were considered significant at $p \leq 0.05$. Weight gain was calculated based on initial and final average weight of fish in each aquarium and expressed as a percentage, while feed efficiency was computed as the ratio of total wet body weight gain to total dry feed given. At the end of the 6-week feeding trials, three fish from each aquarium (nine per dietary treatment per salinity) were randomly

Table 2. Weight gain, feed efficiency and survival of red drum fed diets containing different kinds and levels of lipid in brackish (B) and full-strength artificial seawater (S) for 6 weeks[1]

Diet designation	Water	Weight gain (% of initial wt)	Feed efficiency (g gain g dry feed^{-1})	Survival (%)
Coconut	B	246 } ^{c}	0.76^{b}	74^{b}
	S	228 }	0.67^{c}	97^{a}
Sat.fish	B	369 } ^{b}	0.82^{a}	71^{b}
	S	299 }	0.71^{bc}	91^{a}
Corn	B	341 } ^{b}	0.75^{b}	97^{a}
	S	337 }	0.76^{b}	97^{a}
Fish	B	496 } ^{a}	0.88^{a}	95^{a}
	S	461 }	0.83^{a}	95^{a}
14Fish	B	463 } ^{a}	0.85^{a}	95^{a}
	S	443 }	0.84^{a}	97^{a}
Analysis of variance, Pr > F				
Diet		0.0001	0.0001	0.0001
Water		0.0215	0.0002	0.0001
Diet × water		0.4652	0.0097	0.0001
Pooled SEM		10.77	0.01	0.01

[1]Means of triplicate groups. A significant ($p < 0.05$) main effect and no interaction allowed data to be pooled prior to mean separation by Duncan's multiple range test; whereas, a significant main effect and interaction precluded pooling of data.

selected for measurement of body tissue indices and composition. The remaining fish were exposed to acute and chronic cold tolerance assays. Results of the acute assays were highly variable and therefore inconclusive (Craig 1994); only the chronic assay will be described here.

Fish destined for the chronic cold tolerance assay continued to be fed their respective diets at 3% of body weight per day. For the chronic cold tolerance assay, fish were maintained in the original culture systems and the water temperature was lowered 1°C per day with in-line 0.5-hp chilling units. When water temperature reached 19°C (from 26°C), fish from triplicate aquaria were pooled and then two groups of 10 fish for each diet and salinity treatment were transferred into 20 l containers in an environmental chamber, also at 19°C. Each container was equipped with an internal biological filter to aid in water quality maintenance and an airstone for supplemental aeration. The temperature was then lowered 1°C per day by ambient-air cooling until 12°C, then 0.5°C per day until all fish had died. Fish were fed their respective diets until water temperature reached 9°C when the fish began to refuse food. Total ammonia, nitrite, and nitrate levels were monitored every other day and, if needed, water exchanges were performed with water at the same temperature and salinity to maintain adequate water quality. However, water exchanges were only necessary twice during the chronic assay and were not necessary after the water temperature fell below 9°C. Water temperature and activity of the fish were monitored daily, and when fish began to die, they were monitored constantly so that at death, temperature was recorded and fish were immediately frozen for future analysis. A median lethal temperature for each dietary treatment at each salinity was determined and subjected to factorial ANOVA and Duncan's multiple range test to determine effects of diet and salinity.

Tissue indices [muscle ratio (MR), hepatosomatic index (HSI), and intraperitoneal fat (IPF) ratio] were determined as indicators of the effects of the different dietary lipids on fat deposition. These indices were computed as follows: MR = total muscle (fillet) weight × 100/total fish weight; HSI = liver weight × 100/total fish weight; and IPF ratio = total IPF weight × 100/total fish weight. In addition, total lipid (Folch *et al.* 1957) and fatty acid composition of the neutral and polar lipid fractions (Satoh *et al.* 1989) of muscle and liver tissues from the fish before and after the chronic assays

Table 3. Muscle ratio, hepatosomatic index (HSI), and intraperitoneal fat (IPF) ratio of juvenile red drum fed diets containing different kinds and levels of lipid in brackish (B) and full-strength artificial seawater (S) for a period of 6 weeks before (BF)[1] and after (AF)[2] the chronic cold tolerance assay[3]

Diet designation	Water	Muscle ratio[4]		HSI[5]		IPF ratio[6]	
		BF	AF	BF	AF	BF	AF
Coconut	B	32.1^{bc}	37.2*^{b}	2.80*^{b}	2.13^{bc}	0.02^{c}	0.00^{c}
	S	31.5^{c}	35.7*^{b}	2.70*^{b}	2.60^{ab}	0.00^{c}	0.00^{c}
Sat.fish	B	37.8^{a}	37.5^{b}	2.17*^{c}	1.56^{d}	0.03^{c}	0.00^{c}
	S	33.1^{bc}	38.3*^{b}	2.02^{c}	1.79^{cd}	0.05^{c}	0.03^{c}
Corn	B	31.7^{c}	39.1*^{b}	2.46*^{b}	1.76^{cd}	0.05^{c}	0.00^{c}
	S	31.3^{c}	36.8*^{b}	2.46^{b}	2.78^{a}	0.07^{c}	0.00^{c}
Fish	B	37.7^{a}	38.2^{b}	3.18*^{a}	1.97^{cd}	0.33^{b}	0.23^{b}
	S	33.2^{bc}	38.3*^{ab}	3.47*^{a}	2.02^{cd}	0.37^{b}	0.20^{b}
14Fish	B	38.8^{a}	39.8^{b}	3.03*^{a}	2.26^{bc}	0.24^{b}	0.51*^{a}
	S	34.6^{b}	40.0*^{a}	3.51*^{a}	2.57^{ab}	0.54^{a}	0.52^{a}
Analysis of variance, Pr > F							
Diet		0.0001	0.0075	0.0001	0.0002	0.0001	0.0001
Water		0.0001	0.3806	0.2982	0.0002	0.0196	0.7840
Diet × water		0.0154	0.2887	0.2576	0.0418	0.0143	0.9103
Pooled SEM		0.86	0.72	0.16	0.14	0.05	0.04

[1]Means of three fish selected randomly from each of three replicate groups for each treatment and water type. Tissue samples were taken immediately following the feeding trial; [2]means of three fish selected randomly from the total number of fish which died at or below the median lethal temperature; [3]asterisks denote a significant ($p<0.05$) difference in values before and after cold exposure as determined by *t*-test. A significant ($p<0.05$) main effect and no interaction allowed data to be pooled prior to mean separation by Duncan's multiple range test; whereas, a significant main effect and interaction precluded pooling of data; [4]muscle weight × 100/total weight; [5]liver weight × 100/total weight; [6]IPF weight × 100/total weight.

were determined as described by Lochmann and Gatlin (1993). These measurements also were subjected to factorial ANOVA and Duncan's multiple range test.

Results

Performance

Growth of red drum was significantly affected by dietary lipid and water salinity (Table 2). In brackish water, fish fed the diets containing 7 and 14% menhaden oil had significantly greater weight gain over the 6-week feeding trial than fish fed the other dietary lipids. Fish fed the diet containing coconut oil had the least weight gain, while fish fed the corn oil and saturated menhaden oil diets had intermediate responses. Fish reared in full-strength seawater showed similar dietary trends, but with lower weight gain relative to fish reared in brackish water. Feed efficiency and survival were significantly affected by diet and water type with an interaction between these variables (Table 2). In brackish water, fish fed the diets containing menhaden oil (7 and 14%) and saturated menhaden oil had higher feed efficiency than fish fed the diets containing corn oil and coconut oil, while those fed the diets containing saturated menhaden oil and coconut oil had significantly lower survival than fish fed the other diets. In seawater, fish fed the diets containing menhaden oil had significantly higher feed efficiency than fish fed the remaining diets, but there were no significant differences in survival among treatments.

Body composition

Biological indices

Muscle ratio and IPF ratio values of red drum before cold exposure were significantly affected by dietary lipid and salinity (Table 3). The interaction

Table 4. Total lipid of muscle and liver, and median lethal temperature (MLT) of juvenile red drum fed diets containing different kinds and levels of lipid in brackish (B) and full-strength artificial seawater (S) for 6 weeks before (BF)[1] and after (AF)[2] the chronic cold tolerance assay[3]

Diet designation	Water	Muscle lipid (% wet wt)		Liver lipid (% wet wt)		MLT (°C)
		BF	AF	BF	AF	
Coconut	B	0.93^{de}	0.88	29.1^{cde}	23.0^{*}	8.6
	S	1.03^{cde}	0.83^{*} ^{b}	37.2^{ab}	37.3 ^{ab}	9.4 ^{a}
Sat.fish	B	0.87^{e}	0.98	25.0^{e}	21.0	6.6
	S	0.99^{cde}	0.86 ^{b}	26.8^{cde}	26.2 ^{b}	7.5 ^{b}
Corn	B	1.27^{ab}	0.84^{*}	24.8^{e}	13.5^{*}	6.8
	S	1.05^{cde}	1.01 ^{b}	31.2^{cd}	28.6 ^{b}	7.1 ^{b}
Fish	B	1.19^{abc}	1.29	33.3^{bc}	33.5	4.9
	S	1.10^{bcd}	0.91 ^{ab}	30.8^{cd}	35.3^{*} ^{a}	6.5 ^{bc}
14Fish	B	1.41^{a}	1.14	36.7^{ab}	34.9	5.1
	S	1.20^{abc}	1.37 ^{a}	38.7^{a}	40.7 ^{a}	3.9 ^{c}
Analysis of variance, Pr > F						
Diet		0.0001	0.0081	0.0001	0.0023	0.0001
Water		0.1743	0.4347	0.0045	0.0087	0.1581
Diet × water		0.0319	0.8900	0.0246	0.0701	0.4753
Pooled SEM		0.06	0.07	1.37	2.0	0.16

[1]Means of pooled samples from three individual fish in each of three replicate groups for each treatment and water type. Tissue samples were taken immediately following the feeding trial; [2]means of pooled samples from three individual fish in each of two replicate groups for each treatment and water type. Tissues were taken from fish which died at or below the median lethal temperature; [3]asterisks denote significant ($p<0.05$) differences between values before and after cold exposure as determined by *t*-test. A significant ($p<0.05$) main effect and no interaction allowed data to be pooled prior to mean separation by Duncan's multiple range test; whereas, a significant main effect and interaction precluded pooling of data.

between main effects also was significant for these indices. The HSI was significantly affected by dietary lipid only. At both water salinities, fish fed the diets containing menhaden oil had significantly higher HSI and IPF ratio values. Fish fed the diet containing 14% menhaden oil in seawater showed the highest IPF ratio, while fish fed the diets containing coconut oil had the lowest IPF ratio. Fish fed the diets containing menhaden oil and saturated menhaden oil in brackish water had significantly higher muscle ratio values than fish fed the remaining diets at either water salinity. Fish reared in brackish water had significantly higher muscle ratio values when compared to fish fed the same diet in seawater.

Total lipid

Dietary lipid had significant effects on total lipid of muscle and liver before and after cold exposure (Table 4). At both water salinities, fish fed the diet containing 14% menhaden oil had the highest muscle and liver lipid content. Water salinity had a significant effect on liver lipid, but not muscle lipid, while the interaction between the two variables was significant for both muscle and liver lipid.

Fatty acid composition

Alterations in fatty acid composition in response to dietary lipids as well as the chronic cold tolerance assay were similar in muscle and liver tissues. Additionally, there appeared to be no influence of salinity on fatty acid composition. Therefore, only the fatty acid composition data for liver tissue from fish in brackish water are presented in tabular form.

Fatty acid groups

Dietary lipid effects on tissue fatty acid composition were most pronounced in the neutral fraction of liver (Table 5). Fish fed the diet containing coconut oil had significantly higher levels of saturates

Table 5. Fatty acid composition of liver neutral lipid of juvenile red drum fed diets containing different kinds and levels of lipid in brackish water for 6 weeks before (B) and after (A) the chronic cold tolerance assay. Values represent percent of total fatty acids. Tissue samples were taken from fish that had died at or below the median lethal temperature for each respective diet

	Coconut		Sat.fish		Corn		Fish		14Fish	
Fatty acid	B	A	B	A	B	A	B	A	B	A
12:0	12.7*,[1]	4.0	0.1	–	0.1*	–	0.1	–	–	–
14:0	17.4*	14.2	2.9*	1.9	1.1*	0.4	5.1*	3.7	6.7	4.5
16:0	21.2*	15.5	21.4*	15.8	14.5*	10.7	27.3*	21.9	22.1*	19.1
16:1	17.6*	23.0	23.2	24.5	5.2	5.0	17.7	17.9	18.9	18.3
18:0	3.1*	3.8	8.1	7.1	6.2*	3.8	8.4*	6.5	5.1	4.5
18:1	18.6*	27.7	31.3	35.1	26.4	26.3	21.3	21.5	17.3	18.5
18:2(n – 6)	2.3	3.7	4.1	5.7	36.0*	41.7	3.1	3.5	2.1	2.1
18:3(n – 6)	0.5*	0.8	0.5	0.8	6.2	5.8	0.8	0.7	1.8	0.6
18:3(n – 3)	0.1	0.2	0.1	0.1	0.4*	0.3	0.7	0.7	2.6	1.0
18:4(n – 3)	0.1	0.1	0.2	–	0.1	–	1.7	1.5	–*	2.4
20:0	0.1	0.2	0.6	0.5	0.2	0.2	0.1	0.2	0.8	–
20:3(n – 6)	0.1	0.2	0.1	0.4	0.7	1.3	0.1	0.3	–*	0.4
20:4(n – 6)	0.1	0.2	0.2	0.3	–	0.2	0.4*	0.8	0.7	0.9
20:3(n – 3)	–	–	–	–	–	–	–	0.1	–	0.1
20:5(n – 3)	0.3	0.3	0.6	0.5	–	0.2	5.1*	7.8	8.3	11.4
22:5(n – 3)	0.2	0.3	0.4	0.6	0.1*	0.3	1.2*	2.1	2.8	2.8
22:6(n – 3)	0.6*	1.5	1.0	2.0	0.5*	1.4	2.5*	5.3	4.5	6.4
Σ SAT[2]	55.2*	37.8	33.3*	25.6	22.0*	15.1	42.0*	33.8	35.4*	30.0
Σ MON[2]	38.7*	53.0	55.8	60.8	32.6	32.1	40.1	40.4	37.1	38.0
Σ PUFA[2]	6.2	9.3	11.0*	13.6	45.4*	52.9	18.1	25.9	27.5	32.0
Σ (n – 3)	1.3	2.4	2.2	8.3	1.1*	2.3	11.1*	17.5	18.2*	24.1
Σ (n – 6)	3.0	4.9	4.9*	7.2	42.8*	48.9	4.4*	5.3	4.7	4.0
(n – 3)/(n – 6)	0.4	0.5	0.5	1.2	0.0	0.1	2.5	3.3	3.8	6.0

[1]Asterisks denote significant ($p < 0.05$) differences between values for that particular fatty acid group before and after the chronic cold tolerance assay as determined by *t*-test; [2]SAT = saturates; MON = monoenes; PUFA = polyenes.

than fish fed the remaining diets. Fish fed the diet containing saturated menhaden oil had significantly higher levels of monoene, while fish fed the diet containing corn oil had significantly higher levels of polyenes. Additionally, fish fed the diets containing 7 and 14% menhaden oil had significantly higher levels of (n – 3) HUFA than fish fed the other diets. Similar responses were observed in liver polar lipid (Table 6).

Individual fatty acids in muscle and liver also reflected the dietary lipid component (Craig 1994). Fish fed the diets containing coconut oil had higher levels of 12- and 14-carbon fatty acids; those fed the diet containing corn oil had higher levels of 18:2(n – 6) and fish fed the diets containing 7 and 14% menhaden oil had the highest levels of 20:5(n – 3), 22:5(n – 3), and 22:6(n – 3) in the neutral and polar lipid of liver and muscle. Fish fed the diets containing 7 and 14% menhaden oil had the highest (n – 3)/(n – 6) ratio, while fish fed the diets containing corn oil had the lowest (n – 3)/(n – 6) ratio in both neutral and polar lipid of muscle and liver.

Cold tolerance assay

In the chronic cold tolerance assay, median lethal temperatures ranged from 3.9 to 9.4°C (Table 4). There was a significant effect of dietary lipid but not water salinity on the median lower lethal temperature. Fish fed the diets containing menhaden oil at 7 and 14% had significantly lower median lethal temperatures than those fed the remaining

Table 6. Fatty acid composition of liver polar lipid of juvenile red drum fed diets containing different kinds and levels of lipid in brackish water for 6 weeks before (B) and after (A) the chronic cold tolerance assay. Values represent percent of total fatty acids. Tissue samples were taken from fish that had died at or below the median lethal temperature for each respective diet

	Coconut		Sat.fish		Corn		Fish		14Fish	
Fatty acid	B	A	B	A	B	A	B	A	B	A
12:0	4.1	1.3	–	–	–	–	–	–	–	–
14:0	10.9	10.8	3.4	0.9	1.8	1.2	4.3	3.2	5.2	5.4
16:0	20.6	21.5	25.7	18.9	17.8	14.8	22.9	27.4	26.4	31.1
16:1	16.7	19.4	16.0	20.2	7.1	4.8	13.2	14.7	14.1	15.2
18:0	4.4	8.7	9.8	7.3	7.7	8.5	9.1	9.0	9.2	11.3
18:1	19.5	23.1	25.5	33.3	19.2	23.6	28.8	16.1	16.6	10.3
18:2(n – 6)	11.8	7.0	5.0	4.8	32.6	25.7	3.6	2.6	6.2	0.8
18:3(n – 6)	0.6	0.5	–	–	5.9	3.6	0.2	–	–	–
18:3(n – 3)	0.5	–	1.0	0.7	0.7	1.9	1.6	0.7	0.8	–
18:4(n – 3)	0.6	–	1.1	–	–	–	1.1	1.4	0.6	1.2
20:0	0.1	–	–	–	–	–	0.2	–	–	–
20:3(n – 6)	0.1	–	–	–	–	0.7	–	–	–	–
20:4(n – 6)	0.3	–	–	–	–	–	0.2	0.5	0.5	0.8
20:3(n – 3)	–	–	–	–	1.0	1.4	–	–	–	–
20:5(n – 3)	2.3	–	–	0.6	– *,[1]	1.6	4.1	8.4	2.6	7.1
22:5(n – 3)	0.8	–	1.1	–	1.0	0.8	0.4	1.8	0.9	1.3
22:6(n – 3)	1.9	3.0	2.5	4.9	1.1	5.7	5.7	8.6	9.0	11.1
Σ SAT[2]	40.4	42.3	38.9*	27.2	27.2	25.0	36.8	41.6	41.2	48.7
Σ MON[2]	32.1*	43.1	42.6	53.5	28.6	29.6	42.6	30.9	31.5	27.8
Σ PUFA[2]	22.6	12.9	18.7	19.5	44.3	45.4	20.7	27.6	27.4	23.6
Σ (n – 3)	6.1	3.0	5.8	6.2	3.9	11.5	12.9	20.9	19.3	20.6
Σ (n – 6)	12.8	7.5	5.1	4.8	38.5	30.0	3.9	3.1	6.6	1.6
(n – 3)/(n – 6)	0.5	0.4	1.1	1.3	0.1	0.4	3.3	6.7	2.9	12.9

[1]Asterisks denote significant ($p < 0.05$) differences between values for that particular fatty acid group before and after the chronic cold tolerance assay as determined by *t*-test; [2]SAT = saturates; MON = monoenes; PUFA = polyenes.

diets. Fish fed the diet containing coconut oil had the highest median lethal temperature in the assay, while fish fed the diets containing corn and saturated menhaden oils had intermediate median lethal temperatures.

Effects of cold exposure

Biological indices

Of the biological indices, the HSI was most markedly affected by cold exposure. In brackish water, fish fed all diets experienced a significant decrease in HSI after cold exposure (Table 3). Additionally, muscle ratio was significantly increased by cold exposure in fish fed the diets containing corn oil and coconut oil in brackish water. The IPF ratio was significantly higher in fish fed the diet containing 14% menhaden oil in brackish water after cold exposure.

In seawater, the effects of cold exposure on HSI were not as pronounced. Although HSI decreased after the chronic assay in fish fed all diets except corn oil, the only significant decreases in HSI were found in fish fed the diets containing 7 and 14% menhaden oil and 7% coconut oil. Significant increases in muscle ratio were found in fish fed all diets in seawater, while no significant effect on IPF ratio was observed.

Total lipid

Total lipid levels in muscle and liver after cold exposure did not change consistently (Table 4). In brackish water, the only significant differences were

found in muscle and liver lipid of fish fed the diet containing corn oil and in liver lipid of fish fed the diet containing coconut oil. In seawater, the only significant differences in total lipid levels were found in liver of fish fed the diet containing 7% menhaden oil and in muscle of fish fed the diet containing coconut oil.

Fatty acid composition

Fatty acid data after the chronic cold tolerance assay revealed several significant alterations in fatty acid group composition. The neutral fraction of liver samples revealed the most significant differences before and after cold exposure (Table 5). In brackish water, liver samples from fish fed all diets showed significant decreases in the level of total saturated fatty acids in the neutral fraction after cold exposure. In seawater, only liver samples from fish fed the diets containing corn oil and coconut oil did not show significant decreases in total saturates after cold exposure. In addition, fish fed the diets containing 7 and 14% menhaden oil in brackish water and seawater showed significant increases in the levels of (n – 3) HUFA in liver neutral lipid. Other significant increases in fatty acid groups in liver neutral lipid after cold exposure were total monoenes in fish fed the diet containing coconut oil and polyunsaturated fatty acids (PUFA) in fish fed diets containing saturated menhaden oil in brackish water. Liver neutral lipid of fish fed diets containing 7% menhaden oil, saturated menhaden oil and corn oil had significantly higher levels of (n – 6) fatty acids after cold exposure. Effects of cold exposure in liver polar lipid were limited to a significant increase in total monoenes in fish fed the diet containing coconut oil and a significant decrease in total saturates in fish fed the diet containing saturated menhaden oil.

The individual fatty acid data were less clear, as they showed no consistent trends in terms of alteration of individual fatty acid composition in response to cold exposure. Samples of liver from fish fed diets containing 7 and 14% menhaden oil in both brackish and seawater generally had higher levels of (n – 3) HUFA and lower levels of 18:2(n – 6) in both the neutral and polar lipids. In liver, significant increases in DHA after cold exposure were observed in neutral lipid of fish fed all diets except saturated menhaden oil and 14% menhaden oil.

After the chronic cold tolerance assay, the (n – 3)/(n – 6) ratio increased dramatically in liver neutral and polar lipid in fish fed the diets containing 7 and 14% menhaden oil in brackish water and seawater, while this ratio decreased or remained the same in fish fed the remaining diets (Tables 5 and 6).

Discussion

Fish growth and body composition

In this study, the dietary lipid component was varied so that effects on body composition and cold tolerance could be examined. Significant effects of dietary lipid on weight gain and feed efficiency were observed, although all the diets marginally met the requirement of red drum for EFA, which has been estimated to be 0.3 to 0.6% (n – 3) HUFA in the diet or approximately 7.0% of total dietary lipid (Lochmann and Gatlin 1993). In the present study, the red drum muscle contributed approximately 0.35% (n – 3) HUFA in the diet, or 5.0% of total lipid. Best growth was achieved with menhaden fish oil containing a relatively high level of (n – 3) HUFA (25–30%). The growth-promoting effects of fish oil supplementation have been noted with many fish species, especially marine species (Lochmann and Gatlin 1993).

Weight gain and feed efficiency were significantly higher for fish fed all diets in brackish water. Although red drum is a marine species, it is euryhaline and most commercial production occurs at lower salinities. The isosmotic salinity of red drum is approximately 11‰ (Wakeman and Wohlschlag 1983). Presumably, when ambient salinity is isosmotic with the blood, physiological stresses and energy expenditures for osmoregulation are minimized, and thus the environment might be more conducive to growth. In studies with salmonids, Morgan and Iwama (1991) observed reduced growth at salinities above the isotonic salinity. Other studies have shown maximal growth of salmonids does not occur at the point of isotonic salinity, but

rather, below it (McKay and Gjerde 1985; McCormick *et al.* 1989). Moser and Hettler (1989) reported increased metabolic rates with increasing salinity for juvenile spot (*Leiostomas xanthurus*), a euryhaline sciaenid species found in many of the same estuarine habitats as juvenile red drum. Although fish fed two diets in brackish water had lower survival than in full-strength seawater, this could be the result of a combined effect between diet and salinity, and not solely salinity. These growth data are in agreement with a previous study in which juvenile red drum had better weight gain at lower salinities than in full-strength seawater (Gatlin *et al.* 1992).

Dietary lipid effects on tissue lipid deposition have been observed in many species (Tabachek 1986; Hanley 1991; Nematipour *et al.* 1992; Stowell and Gatlin 1992; Bazaz and Keshavanath 1993) including red drum, in which increases in dietary lipid resulted in increased lipid deposition in tissues (Williams and Robinson 1988; Ellis and Reigh 1991; Serrano *et al.* 1992). This response was observed in the present study, as the fish fed the diet containing 14% menhaden oil generally had higher total lipid in muscle and liver than fish fed the diets containing 7% lipid. Liver lipid generally was higher in red drum reared in seawater unlike the trend observed in milkfish (*Chanos chanos*) when reared in freshwater and seawater (Borlongan and Benitez 1992). Daikoku *et al.* (1982) reported total lipid increased in osmoregulatory tissues (gills, digestive tract and kidney) in the guppy upon seawater adaptation; whereas, it dropped in tissues with little or no osmoregulatory roles (eyes, liver and muscle). Additionally, increased levels of PUFA, especially DHA, were observed in all tissues assayed from seawater-adapted guppies. The role of increased total lipid in seawater adaptation is not well understood. Some theories suggest that membrane fluidity must be enhanced in seawater environments to increase membrane permeability for ionic transfer. Higher levels of total lipid in the liver, which is one of the main lipid depot sites in fish (Sheridan 1988), might provide a larger source of fatty acids which could be utilized to alter the composition of certain tissues. Different tissues may respond differently depending upon their osmoregulatory status and their exposure to the environment (Bell *et al.* 1986).

Cold tolerance

The chronic assay lasted for 22 days with daily reductions of temperature. The temperature decline in this study was more gradual and orderly than might be experienced in nature, but certainly it comprised a reasonable representation of progressive seasonal cold stress in outdoor ponds where red drum are grown. The data from the assay indicated that cold tolerance of juvenile red drum can be affected by the levels and kind of dietary lipid. Fish fed the diets rich in long-chain (n – 3) HUFA were able to survive temperatures 3.5 to 4.5°C lower than fish fed the diets containing coconut oil. Additionally, the increased level of (n – 3) HUFA from supplementing 14% menhaden oil to the diet also appeared to increase cold tolerancce. Fatty acid chain length also appeared to have effects on cold tolerance, as fish fed the diets containing saturated menhaden oil had lower median lethal temperatures than fish fed the diets containing coconut oil. It was not specifically determined how these longer chain fatty acids (as well as the monoenoic moieties observed in the chromatographic analysis of the dietary lipid) were able to increase cold tolerance. Certainly, fish fed the diets rich in (n – 3) HUFA had a greater reserve of these fatty acids with which to modify membrane fatty acid composition to cope with the lowered environmental temperatures. These fatty acids may have influenced the fluidity of membranes as well as having other functional properties. Salinity did not affect median lethal temperatures in the present study, which is in agreement with Stauffer (1986) who reported no salinity effect on the lower lethal temperatures of Mozambique tilapia (*Oreochromis mossambicus*).

Several significant alterations in the fatty acid composition of neutral lipid in muscle and liver tissues of red drum indicative of thermal acclimation were apparent. Hazel (1979) investigated changes in the neutral lipid of liver and observed lower levels of 16:0 in cold-acclimated rainbow trout compared with warm-acclimated fish. Fish fed all diets in the present study experienced significant decreases in levels of this fatty acid in neutral lipid. Additionally, levels of (n – 3) HUFA also increased after cold exposure in the present study. There was a signifi-

cant drop in the level of total saturates in fish fed all diets in brackish water. This, in combination with the increases in (n – 3) HUFA effectively increased the ratio of unsaturated to saturated fatty acids in the liver of fish fed all diets, which also was seen by Hazel (1979) in trout acclimated to 5 and 20°C. Dietary input to red drum had ceased at 9.0°C when fish began to refuse food; therefore, modifications in the fatty acid composition below this temperature had to come from mobilization and preferential utilization of specific families of fatty acids (a reorganization of lipid metabolism) or biosynthesis.

Mobilization of existing fatty acid reserves may have played a role in the alterations of fatty acid composition observed in the present study. However, in fish which require (n – 3) fatty acids, their essentiality and preferential incorporation in the phospholipid fraction of tissues may limit the availability of long chain (n – 3) HUFA from the neutral fraction for mobilization (Hazel 1979). Several studies with fish under fasting conditions have shown no turnover in levels of 20:5(n – 3) and 22:6(n – 3) (Castledine and Buckley 1980; Jezierska *et al.* 1982), and while fatty acids of the (n – 9) family underwent mobilization, members of the (n – 3) family were probably conserved in the neutral fraction (Jezierska *et al.* 1982). Data from the present study are in agreement with these previous studies, as levels of (n – 3) HUFA in the neutral fraction of liver increased after cold exposure, indicating a possible conservation of these fatty acids. Because fish in this study had ceased feeding approximately 7–8 days before the conclusion of the chronic assay, they might have exhibited similar biochemical responses as would occur during starvation, even though their metabolic rate was severely depressed. Also, fish fed 14% menhaden oil which had the lowest median lethal temperature in the cold-tolerance assay exhibited signs of (n – 3) HUFA conservation and utilization of (n – 6) fatty acids, as the ratio of these two families increased most dramatically in the polar fraction of liver lipid. The predominant increases in the (n – 3)/(n – 6) ratio in polar lipid (phospholipids) after chronic cold exposure were due to increases in the total (n – 3) levels (primarily DHA) accompanied by decreases in the levels of (n – 6) fatty acids [primarily 18:2(n – 6)]. If preferential mobilization and utilization were occurring, presumably one group of fatty acids would be conserved at the expense of another, and thus one might expect a decrease in lipid levels in the depot tissue serving as the reserve. However, studies with rainbow trout indicated no significant temperature effect on liver lipid content (Dean 1969; Hazel 1979). In the present study, liver lipid generally increased or remained the same after cold exposure for fish fed all diets except those containing coconut oil and corn oil in brackish water, where significant decreases were observed. The only significant decreases in total lipid in muscle occurred in fish fed diets containing coconut oil in seawater and corn oil in brackish water.

A potential source of increased (n – 3) HUFA observed in the present study in response to lowered environmental temperatures is from an increase in desaturase and elongase enzyme activity. Red drum are thought not to be very efficient at elongation and desaturation of (n – 3) fatty acids, and thus require them in their diet (Lochmann and Gatlin 1993). Several studies have shown temperature effects on desaturase and elongase enzymes of fish (Sellner and Hazel 1982; Schünke and Wodtke 1983; Hazel 1984; Hagar and Hazel 1985), as well as the existence of a modulator protein found in bacteria which is only activated in response to low environmental temperatures (Fujii and Fulco 1977). However, studies on the ability of red drum to enzymatically elongate and desaturate fatty acids have not been carried out; thus, a mechanism whereby red drum could increase enzyme activities in response to lower environmental temperatures has not been demonstrated.

Preferential utilization of specific fatty acids (*i.e.*, saturates for energy, unsaturates for phospholipids) would result in increased percentages of the fatty acids being conserved [(n – 3) HUFA]. Based on data from the present study, modifications in fatty acid composition of red drum appeared to occur by this mechanism. However, preferential utilization would also result in decreased lipid levels unless lipid biosynthesis continued or increased during the cold exposure. If lipid biosynthesis continued,

then clearly there must have been increased desaturase activity, because the levels of saturates decreased although *de novo* fatty acid synthesis results in the production of saturated fatty acids.

In summary, weight gain of red drum was significantly affected by dietary lipid and by salinity of the medium during 6-week feeding trials. The effects of dietary lipid on body composition were significant as well, with fish fed diets rich in long-chain (n – 3) HUFA having higher HSI and IPF ratios and higher lipid levels in muscle and liver. In the chronic cold-tolerance assay, a readily interpretable dietary effect on the median lethal temperature was observed. Fish fed the diets rich in long chain (n – 3) HUFA had significantly lower median lethal temperatures than fish fed the diets containing lipid high in saturated and medium-chain fatty acids. The metabolic effects of chronic cold exposure amounted to a reorganization of lipid metabolism involving lowered levels of total saturates and increased levels of (n – 3) HUFA, mainly in the liver neutral lipid. These data suggest that it is possible to increase the cold tolerance of juvenile red drum through the inclusion of high levels of dietary lipid rich in (n – 3) HUFA.

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References cited

Bazaz, M.M. and Keshavanath, P. 1993. Effect of feeding different levels of sardine oil on growth, muscle composition and digestive enzyme activities of mahseer, *Tor khudree*. Aquaculture 115: 111–119.

Bell, M.V., Henderson, R.J., Pirie, B.J.S. and Sargent, J.R. 1985. Effects of dietary polyunsaturated fatty acid deficiencies on mortality, growth, and gill structure in the turbot *Scophthalmus maximus*. J. Fish Biol. 26: 181–191.

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

Borlongan, I.G. and Benitez, L.V. 1992. Lipid and fatty acid composition of milkfish (*Chanos chanos* Forsskal) grown in freshwater and seawater. Aquaculture 104: 79–89.

Castledine, A.J. and Buckley, J.T. 1980. Distribution and mobility of ω 3 fatty acids in rainbow trout fed varying levels and types of dietary lipid. J. Nutr. 110: 675–685.

Cowey, C.B., Adron, J.W., Owens, J.M. and Roberts, R.J. 1976. The effects of different dietary oils in tissue fatty acids and tissue pathology in turbot (*Scophthalmus maximus*). Comp. Biochem. Physiol. 53B: 399–403.

Craig, S.R. 1994. Dietary manipulation of lipid deposition and cold tolerance in juvenile red drum, *Sciaenops ocellatus*. Ph.D. Dissertation, Texas A&M University.

Daikoku, T., Yano, I. and Masui, M. 1982. Lipid and fatty acid composition and their changes in the different organs and tissues of guppy, *Poecilia reticulata* on seawater adaptation. Comp. Biochem. Physiol. 73A: 167–174.

Dean, J.M. 1969. The metabolism of tissues of thermally acclimated trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 29: 185–196.

Ellis, S.C. and Reigh, R.C. 1991. Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 97: 383–394.

Farkas, T. 1979. Adaption of fatty acid compositions to temperature – a study of planktonic crustaceans. Comp. Biochem. Physiol. 64B: 71–76.

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

Fujii, D.K. and Fulco, A.J. 1977. Biosynthesis of unsaturated fatty acids by *Bacilli* – hyperinduction and modulation of desaturase synthesis. J. Biol. Chem. 252: 3660–3670.

Fujii, M. and Yone, Y. 1976. Studies on the nutrition of red sea bream XIII. Effects of dietary linolenic and omega-3 polyunsaturated fatty acids on growth and feed efficiency. Bull. Jap. Soc. Sci. Fish. 42: 583–588.

Gatlin, D.M., III., MacKenzie, D.S., Craig, S.R. and Neill, W.H. 1992. Effects of dietary sodium chloride on red drum juveniles in waters of various salinities. Prog. Fish Cult. 54: 220–227.

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

Hanley, F. 1991. Effects of feeding supplementary diets containing varying levels of lipid on growth, food conversion, and body composition of Nile tilapia, *Oreochromis niloticus* (L). Aquaculture 93: 323–334.

Hagar, A.F. and Hazel, J.R. 1985. Changes in desaturase activity and the fatty acid composition of microsomal membranes from liver tissue of thermally-acclimating rainbow trout. J. Comp. Physiol. B. 156: 35–42.

Hazel, J.R. 1979. The influence of temperature adaptation on the composition of the neutral lipid fraction of rainbow trout (*Salmo gairdneri*) liver. J. Exp. Zool. 207: 33–42.

Hazel, J.R. 1984. Effects of temperature on the structure and metabolism of cell membranes in fish. Am. J. Physiol. 246: R460–470.

Jezierska, B., Hazel, J.R. and Gerkin, S.D. 1982. Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention to fatty acids. J. Fish Biol. 21: 681–692.

Kanazawa, A., Teshima, S. and Ono, K. 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. Physiol. 63B: 295–298.

Lochmann, R.T. and Gatlin, III. 1993. Essential fatty acid requirements of the red drum, *Sciaenops ocellatus*. Fish Physiol. Biochem. 12: 221–235.

Lovel, R.T. 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York.

McCormick, S.D., Saunders, R.L. and MacIntyre, A.D. 1989. The effect of salinity and ration level on growth rate and conversion efficiency of Atlantic salmon (*Salmo salar*) smolts. Aquaculture 82: 173–180.

McKay, L. and Gjerde, B. 1985. The effect of salinity on growth of rainbow trout. Aquaculture 49: 325–331.

Moon, H.Y. and Gatlin, D.M. III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95: 97–106.

Morgan, J.D. and Iwama, G.K. 1991. Effect of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48: 2083–2094.

Moser, M.L. and Hettler, W.F. 1989. Routine metabolism of juvenile spot, *Leiostomas xanthurus* (Lacèpède), as a function of temperature, salinity and weight. J. Fish Biol. 35: 703–707.

Nematipour, G.R., Brown, M.L. and Gatlin, D.M., III. 1992. Effects of dietary carbohydrate:lipid ratio on growth and body composition of hybrid striped bass. J. World Aquacult. Soc. 23: 128–132.

Ostrowski, A.C. and Divakaran, S. 1990. Survival and bioconversion of n-3 fatty acids during early development of dolphin (*Coryphaena hippurus*) larvae fed oil-enriched rotifers. Aquaculture 89: 273–285.

Owen, J.M., Adron, J.W., Middleton, C. and Cowey, C.B. 1975. Elongation and desaturation of dietary fatty acids in turbot, *Scophthalmus maximus* L, and rainbow trout, *Salmo gairdnerii* Rich. Lipids 10: 528–531.

Procarione, L.S. 1986. Ultimate lower lethal temperature of red drum *Sciaenops ocellatus* as a function of water hardness and salinity. M.S. Thesis, Texas A&M University, USA.

SAS Institute, Inc. 1985. SAS Users Guide: Statistics, Version 5 Edition. Cary.

Satoh, S., Poe, W.E. and Wilson, R.P. 1989. Effect of dietary n-3 fatty acids on weight gain and liver polar fatty acid composition of fingerling channel catfish. J. Nutr. 119: 23–28.

Schünke, M. and Wodtke, E. 1983. Cold-induced increase of Δ^9- and Δ^6-desaturase activities in endoplasmic membranes of carp liver. Biochim. Biophys. Acta 734: 70–75.

Sellner, P.A. and Hazel, J.R. 1982. Incorporation of polyunsaturated fatty acids into lipids of rainbow trout hepatocytes. Am. J. Physiol. 243: R223–R228.

Serrano, J.A., Nematipour, G.R. and Gatlin, D.M., III. 1992. Dietary protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. Aquaculture 101: 283–291.

Sheridan, M.A. 1988. Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comp. Biochem. Physiol. 90B: 679–690.

Sinensky, M. 1974. Homeoviscous adaptation – a homeostatic process that regulates viscosity of membrane lipids in *Escherichia coli*. Proc. Nat. Acad. Sci., U.S.A. 71: 522–525.

Skuladottir, G.V., Schiöth, H.B., Gudmundsdottir, E., Richards, B., Gardarsson, F. and Jonsson, L. 1990. Fatty acid composition of muscle, heart and liver lipids in Atlantic salmon, *Salmo salar*, at extremely low environmental temperature. Aquaculture 84: 71–80.

Stauffer, J.R. Jr. 1986. Effects of salinity on preferred and lethal temperatures of the Mozambique tilapia, *Oreochromis mossambicus* (Peters). Water Res. Bull. 22: 205–208.

Stowell, S. and Gatlin, D.M., III. 1992. Effects of dietary pantethine and lipid levels on growth and body composition of channel catfish *Ictalurus punctatus*. Aquaculture 108: 177–188.

Wakeman, J.M. and Wohlschlag, D.E. 1983. Time course of osmotic adaptation with respect to blood serum osmolality and oxygen uptake in the euryhaline teleost, *Sciaenops ocellatus* (red drum). Contrib. Mar. Sci. 26: 165–177.

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

Williams, C.D. and Robinson, E.H. 1988. Response of red drum to various dietary levels of menhaden oil. Aquaculture 70: 107–120.