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METABOLIC PROFILES OF PENAEID SHRIMP: DIETARY LIPIDS AND OVARIAN MATURATION*

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SUMMARY

The major impediment to the culture of penaeid shrimp in captivity in the United States has been an inability to obtain ovarian maturation and spawning. Lipid profiles of tissues (gonads, hepatopancreas, and tail muscle) of *Penaeus setiferus* caught at sea have shown that cholesterol is the dominant sterol and that polyunsaturated fatty acids known to be essential in man comprise a significant portion of the fatty acid fraction. A proprietary marine ration contains cholesterol, but is devoid of polyunsaturated fatty acids. Ovarian maturation and spawning were obtained when the shrimp diet was supplemented with an annelid rich in lipids containing these compounds. The biochemical significance of these findings is discussed.

INTRODUCTION

Wild stocks of shrimp are being depleted, their natural habitat is threatened by pollution, and increasing energy costs are adversely affecting the economics of shrimping. These factors alone are sufficient to stimulate an interest in the breeding of shrimp in captivity. Of greater long-term significance, however, is that the achievement of this objective would open the door to selective breeding to obtain animals with superior characteristics. Previous attempts to obtain spawning of

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penaeid shrimp in captivity in the United States have been less than successful: ovarian maturation has not been obtained except in isolated instances[1,2].

The study of metabolic profiles has provided insight to many human biochemical disorders[3]. Notable among these are the discovery of new inborn errors of amino acid metabolism[4–6], the diagnosis of diabetes mellitus by analysis of urinary volatiles[7], and the investigation of rheumatoid arthritis[8] and breast lesions[9]. We have used a similar approach to the investigation of the maturation of female penaeid shrimp.

The classes of compounds which we selected for study were steroids and fatty acids[10]. Shrimp are unable to biosynthesize steroids[11], which are used by these animals as molting hormones and membrane components. The essential fatty acids also feature as membrane components and, in some animals, are precursors of prostaglandins which are important in reproduction[12].

The species of shrimp studied was the white shrimp *Penaeus setiferus*, native to the Gulf of Mexico. Steroid and fatty acid profiles for the gonads, hepatopancreas (digestive gland), and tail muscle of male and female specimens were determined, and compared with the corresponding dietary profiles.

EXPERIMENTAL

Animals

P. setiferus were obtained from the Gulf of Mexico off the Texas coast. All collections were made during the natural spawning season, which runs from May to September. The stages of gonadal development were estimated using gonadal indices[13]. Appropriate tissues were removed as soon as possible after capture and were frozen until analyzed.

Materials

All solvents were Mallinckrodt Nanograde (Mallinckrodt, St. Louis, MO, U.S.A.) except for anhydrous diethyl ether (U.S.P. grade) and hydrochloric acid and sodium hydroxide (reagent grade). Samples were exposed only to Teflon, aluminium, or glass rinsed with chloroform.

Extraction

The samples were homogenized in 20 ml of water and were saponified with 4 ml of 4 *M* sodium hydroxide by heating at 110°C for 2 h. Basic and neutral components (including sterols) were extracted with 2 × 15 ml of diethyl ether (extract A). After acidification with 2 ml of concentrated hydrochloric acid, acidic components (including fatty acids) were extracted with 2 × 15 ml of diethyl ether (extract B). Each extract (A and B) was fractionated by chromatography on separate 20 × 1 cm glass columns containing activated silica gel (80–200 mesh). In each case, a 40-ml benzene eluate was discarded and the components of interest were eluted with 40 ml of ethyl acetate. Thus, a sterol and a fatty acid fraction were obtained.

Analysis

Sterols were analyzed as trimethylsilyl (TMS) ethers, prepared using bis-(trimethylsilyl)acetamide (Supelco, Bellefonte, PA, U.S.A.) and fatty acids as methyl

esters, prepared using dimethylformamide dimethylacetal (Pierce, Rockford, IL, U.S.A.). Gas chromatography was performed using Perkin-Elmer 3920B instruments equipped with flame ionization detectors and 2 m × 2 mm I.D. silanized glass columns containing 3% OV-1 on Gas-Chrom Q (100–120 mesh), programmed from 100 to 270°C (fatty acid methyl esters) or 200 to 270°C (sterol TMS ethers). The injection port and interface temperatures were, respectively, 250 and 280°C. Some analyses were performed using a similar column with SP-2330 as the stationary phase or a 10-m glass capillary column coated with OV-101. Combined gas chromatography–mass spectrometry (GC–MS) was performed using a Hewlett-Packard 5992A instrument equipped with a 2 m × 2 mm I.D. silanized glass column containing 3% OV-1 on Gas-Chrom Q, with appropriate temperature programs and an electron energy of 70 eV.

RESULTS AND DISCUSSION

Shrimp sterols

The relative concentrations of sterols in the three major tissues (gonad, hepatopancreas, tail muscle) of both sexes of *P. setiferus* are given in Table I. Cholesterol was always the predominant sterol, and 22-dehydrocholesterol was found in most samples. Desmosterol was also found in some samples. 24-Methylcholesterol and sitosterol were encountered only in the hepatopancreas. The identities of all of these compounds were verified by GC–MS.

TABLE I

RELATIVE CONCENTRATIONS OF STEROLS* IN TISSUES OF *P. SETIFERUS* FROM THE GULF OF MEXICO

*Specimen***	*Tissue****	*n*	*Cholesterol*	Δ^{22}	Δ^{24}	*24-Me*	*24-Et*
Male	G	6	98.7 ± 1.9[§]	0.7 ± 0.8			
	H	6	93.9 ± 7.4	2.7 ± 3.0	4.2 ± 1.4	2.5 ± 1.6	0.6 ± 1.3
	T	6	82.2 ± 40.3	0.7 ± 0.9	0.2 ± 0.6		
Female (stage 1)	G	2	100 ± 0.0				
	H	1	96.2	3.8			
	T	2	100 ± 0.0				
Female (stage 2)	G	4	99.0 ± 0.8	1.0 ± 0.8			
	H	4	84.0 ± 11.3	5.5 ± 3.8	5.1 ± 3.7	3.3 ± 2.6	2.2 ± 1.7
	T	4	99.5 ± 1.0	0.3 ± 0.5	0.3 ± 0.5		
Female (stage 3)	G	2	98.1 ± 2.7	1.0 ± 1.3			
	H	3	89.8 ± 9.0	4.6 ± 1.8	3.1 ± 3.2	1.6 ± 2.8	0.9 ± 1.6
	T	3	100 ± 0.0				
Female (stage 5)	G	8	92.5 ± 15.2	0.7 ± 0.8	0.3 ± 0.8		
	H	8	82.5 ± 4.7	6.4 ± 1.9	5.2 ± 1.2	4.0 ± 1.2	2.0 ± 1.1
	T	8	100 ± 0.0				

* Δ^{22} = 22-dehydrocholesterol; Δ^{24} = desmosterol; 24-Me = 24-methylcholesterol; 24-Et = sitosterol.

** For females, stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 4 animals were collected.

*** G = gonad; H = hepatopancreas; T = tail muscle.

§ Mean ± standard deviation.

TABLE II

RELATIVE CONCENTRATIONS OF SELECTED FATTY ACIDS IN TISSUES OF *P. SETIFERUS* FROM THE GULF OF MEXICO

*Specimen**	*Tissue***	*n*	*16:0*	*16:1*	*18:0*	*18:1*	*20:4*	*20:5*	*22:6*
Male	G	4	13.5 ± 4.6***	4.1 ± 0.6	10.1 ± 3.7	9.9 ± 1.3	9.5 ± 2.9	17.3 ± 2.5	17.5 ± 0.9
	H	5	20.7 ± 3.2	6.3 ± 0.7	9.9 ± 2.1	8.0 ± 1.0	4.7 ± 1.5	7.3 ± 1.4	5.1 ± 0.8
	T	5	15.7 ± 1.8	5.9 ± 0.8	12.7 ± 3.7	11.2 ± 1.8	6.9 ± 1.6	13.9 ± 5.4	12.8 ± 3.4
Female (stage 1)	G	1	19.1	7.6	8.3	19.8	8.3	10.1	6.9
	H	2	24.8 ± 1.1	7.5 ± 1.3	8.5 ± 0.1	9.6 ± 0.8	4.6 ± 0.6	8.0 ± 2.3	5.6 ± 0.6
	T	2	17.6 ± 1.4	8.3 ± 1.8	11.0 ± 0.8	12.8 ± 0.2	6.6 ± 0.9	14.6 ± 3.5	11.5 ± 1.2
Female (stage 2)	G	2	21.8 ± 3.3	11.0 ± 1.1	8.0 ± 1.1	16.1 ± 0.4	4.3 ± 0.6	9.4 ± 2.1	6.8 ± 2.1
	H	2	21.8 ± 2.1	10.9 ± 0.2	8.4 ± 0.3	10.9 ± 1.6	5.0 ± 0.1	7.7 ± 1.1	5.0 ± 0.6
	T	2	17.7 ± 1.9	8.5 ± 0.6	11.2 ± 1.2	12.0 ± 0.1	6.2 ± 1.8	13.1 ± 2.7	10.7 ± 2.5
Female (stage 3)	G	2	20.2 ± 2.8	10.9 ± 0.6	7.8 ± 0.4	15.2 ± 0.8	4.3 ± 0.7	12.8 ± 2.1	8.7 ± 1.6
	H	3	22.1 ± 2.3	10.7 ± 0.8	8.3 ± 0.3	9.4 ± 1.5	4.4 ± 0.4	8.6 ± 2.2	4.8 ± 0.1
	T	3	18.5 ± 1.1	8.7 ± 0.3	11.9 ± 0.9	11.8 ± 0.3	5.1 ± 1.2	16.2 ± 4.5	11.4 ± 3.3
Female (stage 5)	G	6	21.2 ± 2.8	10.5 ± 1.4	8.8 ± 2.3	15.2 ± 0.9	4.1 ± 1.0	9.9 ± 2.5	7.0 ± 2.0
	H	6	21.4 ± 1.2	9.6 ± 2.1	7.9 ± 0.6	9.8 ± 1.0	4.4 ± 0.5	8.8 ± 2.2	5.4 ± 1.1
	T	6	17.8 ± 1.4	7.9 ± 0.7	10.6 ± 1.9	12.3 ± 1.5	6.1 ± 1.4	14.1 ± 2.2	11.7 ± 1.7

* For females, stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 4 animals were collected.

** G = gonad; H = hepatopancreas; T = tail muscle.

*** Mean ± standard deviation.

Cholesterol has been known in marine invertebrates since the turn of the century[14], and is a common shrimp sterol[15]. It is derived from the diets of species which are unable to synthesize it[16–18].

22-Dehydrocholesterols (22-*cis* and 22-*trans*) are also widely distributed marine sterols[19–21]. The origin of this compound is not clear. In mammals, the formation of (22*R*)-22-hydroxycholesterol is the first step in the conversion of cholesterol into C_{21}, C_{19}, and C_{18} steroids[22]. Such steroids in crustaceans apparently do not share the significance of their counterparts in mammals[23]. It is tempting to speculate that (22*R*)-22-hydroxycholesterol is dehydrated to 22-dehydrocholesterols in some marine organisms and, like cholesterol, is transferred through the marine food web.

Desmosterol was first reported in a crustacean and has been found since in other crustaceans[24]. These animals can convert dietary desmosterol into cholesterol[25,26].

It is of interest that we found 24-methylcholesterol and sitosterol in the hepatopancreas but not in the gonads or tail muscle. Both of these phytosterols can be converted into cholesterol by crustaceans[15,27–31]. Our data indicate that the metabolism of these dietary sterols takes place in the hepatopancreas or, perhaps, some tissue other than the gonads and tail muscle.

Shrimp fatty acids

Analytical data for fatty acids in all three tissues of *P. setiferus* examined are given in Table II. Their identities were verified by GC–MS. Most samples contained the full range of saturated and unsaturated fatty acids from C_{14} to C_{22}. Compounds included in Table II are the major fatty acids: palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), arachidonic (20:4), 5,8,11,14,17-eicosapentaenoic (20:5), and 4,7,10,13,16,19-docosahexaenoic (22:6) acids. Their relative concentrations are expressed as percentages of the total fatty acid content.

The saturated and monounsaturated fatty acids are presumably most important as membrane components. Those which are likely to be most significant to reproduction are certain of the polyunsaturated fatty acids. The most prominent of the latter compounds in many samples are arachidonic acid, 5,8,11,14,17-eicosapentaenoic acid, and 4,7,10,13,16,19-docosahexaenoic acid.

The C_{20} acids are precursors of prostaglandins in many animals[12]. While there are no reports on endogenous prostaglandins in crustaceans, experiments performed *in vitro* have resulted in the conversion of the 20:3 acid into prostaglandin E_1 in low yield by lobster stomach and gill homogenates[32]. Since prostaglandin concentrations are particularly high in human seminal fluid[33–35] and since these compounds have been implicated in the stimulation of uterine contractions during labour[12], it has been suggested[10] that a role of the C_{20} acids in the reproduction of shrimp is mediated by prostaglandins.

Prostaglandins and prostaglandin analogues have been produced *in vitro* using sheep vesicular gland homogenates only from C_{19}, C_{20}, and C_{21} substrates[36,37]. There have been no reports of the formation of prostaglandin analogues from the 22:6 acid or other C_{22} acids. A correlation has been noted, however, between the sex ratio of zooplankton and the content of heneicosahexaene in algal feed at the nauplius stage[38], and it has been suggested[10] that the 22:6 acid is the active compound, while the hydrocarbon is merely a decarboxylation product.

There is strong circumstantial evidence to suggest that polyunsaturated fatty acids are involved in some capacity in the reproductive process, and attention to this possibility should be given in designing feedstocks for shrimp mariculture.

Dietary lipids

The fatty acid content of many crustaceans reflects that of their diets[39–41]. Of particular interest is the observation that the lipid content of the coconut crab is very similar to that of coconuts[42]. Thus, the foregoing discussion has shown that attention should be given to the sterol and fatty acid content of the diets of penaeid shrimp if they are to be expected to spawn in captivity.

The fatty acid composition of a commercially available marine chow is given in Table III. Cholesterol is the only sterol in this formulation. While the sterol content appears to be appropriate, there is clearly a deficiency of polyunsaturated fatty acids. If this feedstock is to be used for maturation, it should be supplemented with additives containing sufficient essential fatty acids.

TABLE III

RELATIVE CONCENTRATIONS OF SELECTED FATTY ACIDS IN COMMERCIALLY AVAILABLE MARINE CHOW AND THE BLOODWORM *G. DIBRANCHIATA*

Specimen	*16:0*	*16:1*	*18:0*	*18:1*	*20:4 + 5**	*22:6*
Marine chow	21.6	3.3	7.6	27.1	3.5	1.7
Bloodworm (live)	12.9	4.5	6.6	10.7	21.5	10.0
Bloodworm (frozen)	19.4	2.8	4.3	11.1	26.3	10.7

* 20:4 and 20:5 not resolved on OV-1.

Among the feedstock supplements which we have investigated is the bloodworm *Glycera dibranchiata*. The fatty acid profile (Table III) of this annelid appears to be appropriate for use with shrimp.

Feeding trials

P. setiferus were maintained in captivity with a diet supplemented with *G. dibranchiata*. The fatty acid profiles of these animals after three months on this diet are given in Table IV. These profiles are very similar to those for the wild shrimp in Table II. Ovarian development and spawning were obtained 3–4 weeks after the experiment was initiated: 63 egg collections yielded a total of 4.3 million eggs[43]. Fertilization did not take place since the male shrimp had a *Vibrio* infection. More recently, however, this protocol has resulted in the production of viable larvae of *P. stylirostris*[44].

Even though the fatty acid profiles of the wild and captive shrimp were similar, there were significant ($P < 0.05$) differences between the total lipid contents of the two groups of animals, as shown in Table V. The hepatopancreas lipid content of the captive shrimp was 2.0–4.4 times that of the wild shrimp, the gonad lipid content of the captive shrimp was 1.2–2.0 times that of the wild shrimp, while the tail muscle lipid content of the captive shrimp was only 1.1–1.4 times that of the wild shrimp. The higher concentrations of the lipids in the captive shrimp indicate

TABLE IV

RELATIVE CONCENTRATIONS OF SELECTED FATTY ACIDS IN TISSUES OF *P. SETIFERUS* GROWN IN THE LABORATORY

*Specimen**	*Tissue***	*n*	*16:0*	*16:1*	*18:0*	*18:1*	*20:4*	*20:5*	*22:6*
Female (stage 1)	G	0	—	—	—	—	—	—	—
	H	1	21.0	4.4	5.5	7.4	2.1	12.5	17.2
	T	1	14.7	3.9	10.1	10.0	10.2	17.9	18.2
Female (stage 2)	G	3	19.6 ± 1.0***	5.1 ± 0.9	6.5 ± 0.0	15.9 ± 1.5	3.8 ± 0.8	16.8 ± 0.7	18.8 ± 0.8
	H	3	25.6 ± 2.5	5.8 ± 1.4	5.1 ± 0.7	9.5 ± 0.6	2.0 ± 0.6	10.4 ± 0.9	13.2 ± 2.2
	T	3	19.0 ± 1.0	4.9 ± 0.3	11.0 ± 0.5	13.1 ± 1.1	4.8 ± 0.3	16.5 ± 0.7	17.6 ± 0.9
Female (stage 3)	G	2	20.4 ± 0.5	6.8 ± 0.0	5.8 ± 0.2	14.8 ± 0.9	3.0 ± 0.1	15.5 ± 0.2	19.6 ± 0.1
	H	2	29.4 ± 1.8	8.1 ± 0.1	3.7 ± 0.4	8.3 ± 0.2	1.6 ± 0.1	8.9 ± 0.8	10.8 ± 2.3
	T	2	19.9 ± 1.4	6.2 ± 0.4	10.2 ± 0.4	12.6 ± 1.2	4.4 ± 0.1	16.7 ± 1.3	17.8 ± 0.8
Female (stage 4)	G	2	21.0 ± 1.0	6.9 ± 1.3	5.7 ± 0.0	16.1 ± 1.6	2.8 ± 0.3	13.5 ± 0.7	18:6 ± 1.8
	H	2	26.5 ± 2.3	8.5 ± 1.2	2.9 ± 0.1	9.3 ± 0.8	1.7 ± 0.0	11.2 ± 1.3	14.0 ± 0.4
	T	2	17.9 ± 1.1	5.6 ± 0.2	9.7 ± 0.1	13.6 ± 0.3	4.3 ± 0.6	15.0 ± 0.9	17.0 ± 1.8
Female (stage 5)	G	2	23.0 ± 0.8	7.1 ± 1.8	7.0 ± 0.1	14.7 ± 0.4	2.5 ± 0.1	12.6 ± 0.4	18.5 ± 1.4
	H	1	26.0	4.7	5.7	8.9	2.1	9.1	13.0
	T	2	18.5 ± 2.1	4.8 ± 1.1	10.8 ± 0.8	12.7 ± 0.2	4.5 ± 0.4	18.1 ± 1.3	19.6 ± 2.0

* Stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn.
** G = gonad; H = hepatopancreas; T = tail muscle.
*** Mean ± standard deviation.

TABLE V

TOTAL LIPID CONTENT (%) IN TISSUES OF *P. SETIFERUS* FROM THE GULF OF MEXICO AND GROWN IN THE LABORATORY

*Specimen**	*Tissue***	*Gulf shrimp*		*Captive shrimp*	
		n	*% lipid*	*n*	*% lipid*
Male	G	5	2.9 ± 0.7***	0	—
	H	5	24.1 ± 20.4	0	—
	T	5	4.0 ± 0.4	0	—
Female (stage 1)	G	2	6.0 ± 1.8	0	—
	H	2	22.6 ± 12.7	1	44.9
	T	2	4.2 ± 0.1	1	4.6
Female (stage 2)	G	2	7.7 ± 9.1	3	15.4 ± 1.9
	H	2	13.6 ± 0.1	4	32.9 ± 20.6
	T	2	3.5 ± 0.1	4	4.8 ± 0.4
Female (stage 3)	G	2	13.6 ± 0.3	5	19.4 ± 2.3
	H	3	15.1 ± 2.1	5	66.8 ± 9.8
	T	2	3.6 ± 0.2	5	5.1 ± 0.3
Female (stage 4)	G	0	—	3	20.1 ± 0.9
	H	0	—	3	51.4 ± 19.7
	T	0	—	3	4.6 ± 0.3
Female (stage 5)	G	6	16.1 ± 1.1	3	19.5 ± 1.1
	H	4	11.7 ± 5.9	3	23.6 ± 6.8
	T	6	3.8 ± 0.2	3	4.6 ± 0.2

*, **, *** Footnotes as in Table IV.

that the artificial diet of the captive shrimp, while adequate for inducing maturation and spawning, is not optimum. Further refinement of the artificial diet is required to reduce the accumulation of lipids in animals which are fed with it.

CONCLUSIONS

Fatty acid and sterol profiles were obtained for penaeid shrimp caught at sea. These profiles were duplicated in captive animals, and spawning resulted.

There have been many reports of seasonal changes in the lipid content and composition of crustaceans: *Astacus astacus*[45], *Astacus leptodactylus*[46], *Orconectes rusticus*[47], *Palaemon carcinus*[48], and *Panulirus polyphagus*[48]. These changes probably reflect differences in diet rather than changes in temperature. Minor variations in lipid content throughout the molt cycle, however, have been observed[49,50].

Our success in using *G. dibranchiata* as a feedstock additive is probably also related to the diet of the bloodworm. The essential fatty acids probably derive ultimately from phytoplankton, and other routes through the food web to shrimp could be envisioned.

Finally, it is not impossible that a factor other than dietary lipid content was responsible for the promotion of ovarian development. Now that spawning is possible in captivity on a reproducible basis, experiments can be performed to resolve some of these issues.

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REFERENCES

1 M. C. Johnston and J. R. Fielding, *Tulane Stud. Zool.*, 4 (1956) 175.
2 F. S. Conte, M. S. Duronslet, W. H. Clark and J. C. Parker, *Proc. 1978 World Mariculture Society Meeting*, in press.
3 O. A. Mamer, W. J. Mitchell and C. R. Scriver (Editors), *Application of Gas Chromatography–Mass Spectrometry to the Investigation of Human Disease*, McGill University, Montreal, 1974.
4 E. Jellum, O. Stokke and L. Eldjarn, *Clin. Chem.* ,18 (1972) 800.
5 E. Jellum, O. Stokke and L. Eldjarn, *Anal. Chem.*, 45 (1973) 1099.
6 K. Tanaka, M. A. Budd, M. L. Efron and K. J. Isselbacher, *Proc. Nat. Acad. Sci. U.S.*, 56 (1966) 236.
7 A. Zlatkis, W. Bertsch, H. A. Lichtenstein, A. Tishbee, F. Shunbo, H. M. Liebich, A. M. Coscia and N. Fleischer, *Anal. Chem.*, 45 (1973) 763.
8 J. Heininger, E. Munthe, J. Pahle and E. Jellum, *J. Chromatcgr.*, 158 (1978) 297.
9 C. D. Pfaffenberger, L. R. Malinak and E. C. Horning, *J. Chromatogr.*, 158 (1978) 313.
10 B. S. Middleditch, S. R. Missler, D. G. Ward, J. P. McVey, A. Brown and A. L. Lawrence, *Proc. 1979 World Mariculture Society Meeting*, in press.
11 D. I. Zandee, *Nature (London)*, 195 (1962) 814.
12 U. S. von Euler and R. Eliasson, *Prostaglandins*, Academic Press, New York, 1967.
13 A. L. Lawrence, D. Ward, S. Missler, A. Brown, J. P. McVey and B. S. Middleditch, *Proc. 1979 World Mariculture Society Meeting*, in press.
14 C. Doree, *Biochem. J.*, 4 (1909) 72.
15 S. Teshima, *Mem. Fac. Fish., Kagoshima Univ.*, 21 (1972) 69.
16 A. Kanazawa, N. Tanaka and S. Teshima, *Bull. Jap. Soc. Sci. Fish.*, 37 (1971) 211.
17 S. Teshima, A. Kanazawa and H. Okamoto, *Bull. Jap. Soc. Sci. Fish.*, 40 (1974) 1015.
18 O. Deshimaur and K. Kuroki, *Bull. Jap. Soc. Sci. Fish.*, 40 (1974) 421.
19 P. A. Voogt, *Comp. Biochem. Physiol.*, 39B (1971) 139.
20 P. A. Voogt, *Comp. Biochem. Physiol.*, 41B (1972) 831.
21 P. A. Voogt, *Comp. Biochem. Physiol.*, 50B (1975) 505.
22 S. Burstein, B. S. Middleditch and M. Gut, *J. Biol. Chem.*, 250 (1975) 9028.
23 M.-F. Blanchet, R. Ozon and J.-J. Meusy, *Comp. Biochem. Physiol.*, 41B (1972) 251.
24 D. R. Idler and P. Wiseman, *Comp. Biochem. Physiol.*, 26 (1968) 1113.
25 S. Teshima and A. Kanazawa, *Mem. Fac. Fish., Kagoshima Univ.*, 22 (1973) 15.
26 S. Teshima, H. J. Ceccaldi, J. Patrois and A. Kanazawa, *Comp. Biochem. Physiol.*, 50B (1975) 485.
27 A. Kanazawa and K. Kashiwada, *Bull. Jap. Soc. Sci. Fish.*, 37 (1971) 1015.
28 S. Teshima, *Comp. Biochem. Physiol.*, 39B (1971) 815.
29 S. Teshima and A. Kanazawa, *Bull. Jap. Soc. Sci. Fish.*, 37 (1971) 720.
30 S. Teshima and A. Kanazawa, *Bull. Jap. Soc. Sci. Fish.*, 38 (1972) 1305.
31 S. Teshima and A. Kanazawa, *Mem. Fac. Fish., Kagoshima Univ.*, 21 (1972) 91.
32 E. J. Christ and D. A. van Dorp, *Adv. Biosci.*, 9 (1973) 35.
33 S. Bergstrom and B. Samuelsson, *J. Biol. Chem.*, 237 (1962) 3005.
34 H. T. Jonsson, B. S. Middleditch and D. M. Desiderio, *Science*, 187 (1975) 1033.
35 H. T. Jonsson, B. S. Middleditch, M. A. Shexnayder and D. M. Desiderio, *J. Lipid Res.*, 17 (1976) 1.

36 D. A. van Dorp, *Fette, Seifen, Anstrichm.*, 68 (1966) 938.
37 D. A. van Dorp, *Mem. Soc. Endocrinol.*, 14 (1966) 39.
38 G. A. Paffenhofer, *Helgolander Wiss. Meeresunters.*, 20 (1970) 346.
39 S. P. Pathak C. V. Agarwal and S. S. Mathur, *J. Amer. Oil Chem. Soc.*, 29 (1952) 593.
40 J. A. Lovern, *Biochem. J.*, 54 (1953) 126.
41 F. Jezyk and A. J. Penicnak, *Lipids*, 1 (1966) 427.
42 E. Andre and M. T. Richert, *Oleagineux*, 15 (1960) 479.
43 A. Brown, Jr., J. B. McVey, B. S. Middleditch and A. L. Lawrence, *Proc. 1979 World Mariculture Society Meeting*, in press.
44 A. Brown, Jr., J. B. McVey, B. S. Middleditch, and A. L. Lawrence, unpublished results.
45 D. I. Zandee, *Arch. Intern. Physiol. Biochim.*, 74 (1966) 64.
46 S. Herodek and T. Farkas, *Acta Biol. Acad. Sci. Hung.*, 10 (1959) 53.
47 D. A. Wolfe, P. V. Rao and D. G. Cornwall, *J. Amer. Oil Chem. Soc.*, 42 (1965) 633.
48 J. C. George and B. S. Patel, *J. Animal Morphol. Physiol.*, 3 (1954) 49.
49 L. Renaud, *Ann. Inst. Oceanogr. (Paris)*, 24 (1949) 259.
50 S. Teshima, A. Kanazawa and H. Okamoto, *Mar. Biol.*, 39 (1977) 129.