Journal of Aquaculture Research & Development

Suloma and Ogata. J Aquac Res Development 2011, 2:2
DOI: 10.4172/2155-9546.1000111

Research Article Open Access

Arachidonic acid is a Major Component in Gonadal Fatty acids of Tropical Coral Reef fish in the Philippines and Japan

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Abstract

The aim of the present study was to investigate the characteristics of gonadal fatty acid composition in 19 species of wild coral reef fish (*Serranidae, Lutjanidae, Lethridae, Siganidae and Labridae*) from Philippine (11 species of female) and Japanese (8 species of female and 5 species of male) waters with special attention to arachidonic acid (ArA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) levels and their ratios. ArA levels were always higher than EPA levels in polar lipids of all the species and in neutral lipids in 17 of the 19 species. In ovarian polar lipids of the 19 species, ArA level ranged from 6.0% to 19.4%, while EPA level ranged from 0.9% to 6.2%. Ovarian DHA level was also always higher than EPA in all the species analyzed. Consequently, ArA/EPA ratios of these species were high, unlike cold- and temperate-water species. ArA was the top fatty acid component in testis polar lipids of three Lethrinus species (21.4% to 22.9%). Thus, ArA is not a minor component, that is, the major highly unsaturated fatty acids (HUFAs) of polar lipids in all coral reef fish gonads are DHA and ArA (not EPA). The present information on gonadal fatty acid composition can be used as a guideline for advancing appropriate broodstock diets of tropical coral reef fish, emerging aquaculture commodities in developing countries.

Keywords: Arachidonic acid; EPA; DHA; Coral reef; Gonads; Fatty acids; Broodstock diets

Introduction

Coral reef fishes are commercially very important. Some species such as groupers and snappers are high-valued food fish and are emerging as aquaculture commodities in tropical and subtropical countries due to increasing demand [1]. Fry availability is an essential component in the development of the culture systems for new species and in further increasing production of established culture species [2]. However, the expansion of aquaculture of coral reef fish is hindered by unstable and limited supply of fry for aquaculture. At present, supply of the fry is mostly dependent on natural resources, and the reduction of the natural stock is a concern [3]. In responding to this situation, there has been considerable interest in developing reliable methods for spawning and rearing larvae and fry. Spawning and egg/larval quality are greatly affected by the quantity and quality of feed, and nutritional deficiency could be one of the reasons for inconsistent quality of spawns and fry. However, formulated broodstock diets for coral reef fish are still under development because of the lack of information on nutritional traits of these species.

Marine fish have low or no capacity to synthesize highly unsaturated fatty acids (HUFAs) from C18 fatty acids. HUFAs are important components of cell membranes and are thought to play important roles in membrane fluidity, modulation of enzyme activity, neural development and regulation of stress resistance. Especially, eicosapentaenoic acid (EPA: 20:5*n*-3) and docosahexaenoic acid (DHA: 22:6*n*-3) are considered as dietary essential fatty acids for normal growth and survival in most marine fish. Most of the studies have focused on the effects of dietary supplementation of EPA and DHA on broodstock performance. Indeed, dietary EPA and DHA have successfully improved reproductive performance and egg/larvae quality such as fecundity, embryo development, hatchability and survival in several species [4,5]. Thus, the importance of EPA and DHA in broodstock nutrition has been emphasized [6]. Little attention was given to the importance of dietary *n*-6 HUFA, especially arachidonic acid (ArA: 20:4*n*-6) in marine fish, because ArA is found in only small quantities in cold and temperate water fish, and dietary ArA was presumed to be unimportant in these species. Since Castell et al. [7] found the dietary essentiality of ArA in juvenile turbot (*Scophthalmus maximus*), the importance of ArA in fry production technologies of marine fish has been re-considered [8].

There have been some papers reporting that Australian or tropical marine fish contain ArA levels equivalent to or higher than those of EPA in muscle or edible parts [9-12] unlike cold and temperate water fish in the Northern Hemisphere. However, this fact has been overlooked in fry production of tropical or coral reef fish species. On the other hand, Ogata et al. found that ArA is not a minor component in the ovaries of several tropical species such as mangrove red snapper (*Lutjanus argentimaculatus*), rabbitfish (*Siganus guttatus and S. canaliculatus*), striped jack (*Caranx fulvoguttatus*) and coral trout (*Plectropomus leopardus*), suggesting that this fatty acid may be nutritionally much more important for egg development and larvae growth in the tropical species than in cold and temperate water species [13]. The degree of the preferential retention of ArA in gonadal polar lipids appears larger in tropical species than in cold and temperate water species. The degree of the physiological importance of ArA in reproduction and larvae/fry performance of tropical or coral reef fish might result in this difference in HUFA profile between tropical and cold/temperate species. When we consider the importance of dietary HUFA including ArA in fry production, as described above, we should pay more attention to HUFA characteristics in tropical fish.

The aim of the present study was to investigate the characteristic of

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Received February 14, 2011; **Accepted** May 04, 2011; **Published** May 09 2011

Citation: Suloma A, Ogata HY (2011) Arachidonic acid is a Major Component in Gonadal Fatty acids of Tropical Coral Reef fish in the Philippines and Japan. J Aquac Res Development 2:111. doi:10.4172/2155-9546.1000111

fatty acid composition of gonads in wild coral reef fish from Philippine (11 species of female) and Japanese (8 species of female and 5 species of male) waters, and special attention was paid to ArA, DHA and EPA levels and their ratios. Gonadal fatty acid composition in fish is affected by various biotic and abiotic factors, diets, species, size, age, maturity, temperature, seasons, salinity etc. Nevertheless, HUFA profile of gonads would be useful to develop broodstock diets for coral reef species where their requirement data are not yet available. Although the present information on gonadal fatty acid composition can be used as a guideline for developing appropriate broodstocks diets for coral reef fish, it should be noted that the conclusion of the present study is based solely on limited data. Absolute comparison of HUFA profile in coral reefs fish gonads would require much larger sample sizes and more systematic sampling from different locations, seasons, species, habitats and maturity.

Materials and Methods

Fish species

Nineteen species of wild coral reef fish were obtained from local dealers of three sites: Ishigaki (Okinawa, Japan), Puerto Princesa (Palawan, Philippines) and Igang (Guimaras, Philippines). Fish were immediately killed with iced water, transferred to local laboratories with ice and directly dissected to take gonad samples. The sampled gonads in Ishigaki were directly stored at -80°C for a night and sent to our laboratory (Japan International Research Center for Agricultural Sciences, Tsukuba, Japan) with dry ice by air, and thus fatty acid composition in wet tissue was determined. Samples collected in Philippines were temporarily stored at -20°C, being freeze-dried, pulverized and sent by air. Fatty acid composition in dried tissue was determined for the samples collected in Philippines. Species name, body weight (g), body length (cm) and gonadosomatic index (GSI) were listed in Table 1. Of the samples, species names were not able to be identified in two species of *Epinephelus* that were collected at Puerto Princesa. The present paper gives them temporarily the names of *Epinephelus sp*. 1 and *Epinephelus sp*. 2 (Table 1).

Analysis

All the wet and dried samples were stored at -80°C until lipid extraction. Lipid extraction was carried out with a mixture of chloroform and methanol (2:1, v/v) [14] containing 0.01% butylhydroxytoluene (BHT). Total lipids were separated into polar (PL) and neutral lipids (NL) with a silica cartridge (Sep-pak plus, Waters, Milford, MA, USA) as described by [15] (Table 2). Fatty acid methyl esters (FAME) were prepared by transesterification with borontrifluoride in methanol according to the procedure of [16] and were purified with thin-layer chromatography (Silicagel 70 Plate, Wako, Osaka, Japan; solvent system: petroleum ether/diethyl ether/ acetic acid = 90:10:1, v/v). FAME were analyzed using gas liquid chromatography (GC- 17A; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector (FID) and an Omegawax 320 fused silica capillary column (30 m x 0.32 mm i.d.; Supelco, Bellefonte, PA, USA). The column temperature was initially held at 160 C for 5 min, followed by an increase at a rate of 4 C/min to a final temperature of 210°C. The carrier gas was helium and the pressure was 80 kPa. Individual FAME was identified using a reference standard (Funakoshi, Tokyo, Japan) and known fish meal FAME and was quantified with an integrator (C-R7A plus; Shimadzu).

Results

Table 2 shows composition of total lipid (%, wet basis for Ishigaki samples and dry basis for Philippine samples), neutral lipid (NL, % of total lipid) and polar lipid (PL, % of total lipid). The data of *Lethrinus ornatus* were not recorded due to a laboratory mistake. Total lipid levels

Name			Body		Body		No. of sample
Family name	Scientific name	English name	Gonad	weight (g)	length (cm)	GSI*	
Ishigaki, Japan							
Serranidae	*Plectropomus leopardus*	leopard coral grouper	ovary	2265±215	56.3±1.3	0.2±0.1	2
Lutjanidae	*Pristipomoides argyrogram-micus*	ornate jobfish	ovary	535±35	34.2±0.9	0.4±0.1	2
	Lutjanus gibbus	humpback snapper	ovary	523±73	32.0±1.5	0.3±0	2
Lethridae	*Lethrinus miniatus*	sweetlip emperor	ovary	305±5	25.3±0.3	1.4±0.1	2
	Lethrinus atkinsoni	Pacific yellowtail empero	testis	458±3	27.0±0.4	2.6±0.1	2
			ovary	472±31	27.8±0.4	3.0±0.5	2
	Lethrinus neblosus	spangled emperor	testis	418±3	28.0±0	0.1±0	3
	Lethrinus ornatus	ornate emperor	testis	228±3	20.0±0.1	0.1±0	3
Siganidae	*Siganus guttatus*	orange-spotted rab-bitfish	testis	525±15	28.2±0.2	5.1±0.1	3
			ovary	605±156	29.8±1.9	7.1±1.9	4
	Siganus canaliculatus	white-spotted rabbitfish	ovary	537±61	30.8±0.9	15.4±2.0	3
	Siganus virgatus	virgate rabbitfish	testis	128±12.2	17.4±0.3	2.3±0.3	3
			ovary	143±13	18.2±0.2	1.8±0.0	2
Puerto Princesa, Philippines							
Serranidae	*Cephalopholis argus*	peacock grouper	ovary	575±150		2.7±0.3	2
	Cephalopholis cyanostigma	blue-spottedgrouper	ovary	358±65		3.8±0.6	3
	Epinephelus areolatus	areolate grouper	ovary	350±53		1.8±0.4	3
	Epinephelus quoyanus	longfin grouper	ovary	500±100		0.3±0	2
	Epinephelus sp. 1		ovary	513±163		1.8±1.4	2
	Epinephelus sp. 2		ovary	550		0.3	1
	Plectropomus leopardus	leopard coral grouper	ovary	205±25	25.0±0.6	1.7±0.4	3
Lutjanidae	*Lutjanus decussatus*	checkered snapper	ovary				2
	Lutjanus erythropterus	crimson snapper	ovary	308±25	28.0±0.6	3.2±0.4	3
Labridae	*Cheilio inermis*	cigar wrasse	ovary	150±25		5.2±1.7	3
Igang, Philippines							
Siganidae	*Siganus canaliculatus*	white-spotted rabbitfish	ovary	225±25	28.5±1.5	8.6±0.5	2

*Gonadosomatic index: (weight of gonad)/(body weight)

Table 1: Fish name, body weight, body length and gonadosomatic index of sample fish (average±S.E.) and number of sample.

Source	Fish name	TL (%)	PL (%)	NL (%)
Japan ovary	*Plectropomus leopardus*	2.0±0.3	38.7±5.6	61.3±5.6
	Pristipomoides argyrogrammicus	2.6±0.1	61.4±1.4	38.6±1.4
	Lutjanus gibbus	4.6±0.3	58.9±4.1	41.1±4.1
	Lethrinus miniatus	5.0±0.3	33.5±2.1	66.5±2.1
	Lethrinus atkinsoni	4.9±0.6	63.9±1.2	36.1±1.2
	Siganus guttatus	8.4±0.9	28.5±0.8	71.5±0.8
	Siganus canaliculatus	11.0±0.6	31.4±0.4	68.6±0.4
	Siganus virgatus	3.6±0.1	12.3±1.2	87.7±1.2
Philippines ovary	*Cephalopholis argus*	17.2±2.5	53.3±19.4	46.7±19.4
	Cephalopholis cyanostigma	20.4±1.4	34.5±0.7	65.5±0.7
	Epinephelus areolatus	20.6±1.0	44.2±1.9	55.8±1.9
	Epinephelus quoyanus	12.4±0.6	83.6±2.1	16.4±2.1
	*Epinephelus sp.*1	18.4±1.4	86.7±0.4	13.3±0.4
	*Epinephelus sp.*2	4.2	41.4	58.6
	Plectropomus leopardus	17.8±3.3	37.2±0.2	62.8±0.2
	Lutjanus decussatus	28.0±16.2	28.2±15.4	71.8±15.4
	Lutjanus erythropterus	23.5±0.9	36.3±1.8	63.8±1.8
	Cheilio inermis	22.7±1.2	35.1±1.4	64.9±1.4
	Siganus canaliculatus	24.6±2.5	31.5±4.4	68.5±4.4
Average			44.8±4.6	55.2±4.6
Japan testis				
	Lethrinus atkinsoni	2.4±0.0	74.8±2.3	25.2±2.3
	Lethrinus nebulosus	1.2±0.1	57.5±0.8	42.6±0.8
	Lethrinus ornatus	nd	nd	nd
	Siganus guttatus	2.6±0.3	43.7±3.3	56.3±3.3
	Siganus virgatus	3.4±0.5	53.5±6.1	46.5±6.1
Average			57.4±6.5	42.7±6.5

* Wet basis for Ishigaki samples and dry basis for Philippine samples.

Table 2: Composition of total lipid (%)*, neutral lipid (NL, % of total lipid) and polar lipid (PL, % of total lipid) (mean value±S.E.).

ranged from 2.0% to 11.0% in ovaries (wet basis, Ishigaki), from 4.2% to 28.0% in ovaries (dry basis, Philippines) and from 1.2% to 3.4% in testes (wet basis, Ishigaki), respectively. The ranges of polar lipid levels in ovaries and testes were respectively from 12.3% to 86.7% and from 43.7% to 74.8%, those of neutral lipid levels in ovaries and testes being respectively from 13.3% to 87.7% and from 25.2% to 56.3%.

Major fatty acids (% of total fatty acids) of the gonads in wild coral reef fish are shown in Tables 3 for polar lipids and 4 for neutral lipids. The most striking result in the present study was that ArA levels were always higher than EPA levels in polar lipids of all the gonad samples analyzed, and that in 17 of the 19 species, ArA levels were also higher than EPA levels in neutral lipids of their gonads. In ovarian polar lipids, ArA levels ranged from 6.0% (*Epinephelus areolatus*) to 19.4% (*Lethrinus atkinsoni*), while EPA levels ranged from 0.9% (Siganus virgatus) to 6.2% (*Pristipomoide argyrogrammicus*). The minimum and the maximum ArA/EPA ratios in ovarian polar lipids were 1.3 in E. areolatus and 15.6 in *Plectropomus leopardus* (Japan). ArA in ovarian polar lipids was one of the top three fatty acid components in *Cephalopholis argus* (12.6% with an ArA/EPA ratio of 3.3), *Epinephelus quoyanus* (15.2% with a ratio of 9.1), *Epinephelus. sp*.-1 (11.4% with a ratio of 5.3), *Epinephelus sp*.-2 (11.7% with a ratio of 3.4), *Lutjanus decussatus* (16.5% with a ratio of 5.3), *Lutjanus erythropterus* (17.5% with a ratio of 4.1), *P. leopardus* (Japan) (19.2% with a ratio of 15.6), *Lutjanus gibbus* (12.3% with a ratio of 4.3), *Lethrinus miniatus* (17.3% with a ratio of 3.6), *Siganus canaliculatus* (Japan) (9.9% with a ratio of 4.7), *L. atkinsoni* (19.4% with a ratio of 4.1), *Siganus guttatus* (12.7% with a ratio of 5.3) and *S. virgatus* (8.9% with a ratio of 10.0). Irrespective of the maturity, thus, ovarian polar lipids had higher levels of ArA than EPA levels, for instance in *P. leopardus* (Japan) (immature, Tables 1 and 3) and *S. canaliculatus* (mature, Tables 1 and 3). DHA was also one of the top three fatty acid components in ovarian polar lipids in 15 of the 19 species. Ovarian DHA level in polar lipids was always higher than EPA in all the species analyzed, ranging from 7.9% with DHA/EPA ratio of 8.9 (S. virgatus) to 27.8% with a ratio of 18.3 (S. canaliculatus). DHA levels in ovarian polar lipids were lower in the six species and higher in the remaining species than ArA levels, respectively. The variation of DHA/ArA ratios with the range from 0.5 (*P. leopardas*, Japan) to 4.2 (*E. areolatus*) was relatively small among species, compared to those of ArA/EPA (1.3—15.6) and DHA/EPA (3.0—18.3) ratios. Surprisingly, ArA was the top fatty acid component in testis polar lipids of *Lethrinus ornatus* (22.9%), *Lethrinus nebulosus* (22.5%) and *L. atokinsoni* (21.4%). Consequently, ArA/EPA ratios of these species were extremely high with the range from 8.4 to 20.2. ArA levels in testis polar lipids of two Siganus species were intermediate but higher than EPA levels with the ArA/EPA ratios of 10.0 and 6.1.

ArA, EPA and DHA levels in neutral lipids (Table 4) were lower than those in polar lipids due to relatively high levels of 16:0, 18:0 and 18:1*n*-9 fatty acids in neutral lipids in gonad tissues. Yet, in neutral lipids, ArA levels were entirely higher than EPA levels throughout all the species with the exception of *E. areolatus* ovary and *P. leopardus* (Philippines) ovary. ArA, EPA and DHA levels in neutral lipids of ovaries ranged from 1.3% (*Cephalopholis cyanostigma* and *E. areolatus*) to 10.1% (S. virgatus), from 0.5% (*C. argus*) to 4.9% (*P. argyrogrammicus*) and from 0.7% (*C. argus*) to 16.7% (*P. argyrogrammicus*), respectively. These HUFA levels in testes ranged from 4.6% (*L. ornatus*) to 14.3% (*L. atokinsoni*), from 1.3% (*L. atokinsoni*) to 2.8% (*L. ornatus*) and from 1.6% (*L. ornatus*) to 10.4% (*S. guttatus*), respectively.

Discussion

It is obvious that ArA is not a minor HUFA but an essential component in gonads of wild coral reef fish, judging from the results of the present study and our previous paper that intermediate or high ArA and DHA levels with relatively low EPA level were found in ovaries, eggs, fry and/or muscle of mangrove red snapper (*L. argentimaculatus*), two species of rabbitfish (S. guttatus and S. canaliculatus), striped jack (*C. fulvoguttatus*) and coral trout (*P. leopardus*) sampled in Central Philippines [13]. Earlier papers reported that muscle tissue or edible part of Australian [9, 11], Malaysian [10] and Arabian Gulf [12] marine fish were rich in ArA whose level was equivalent to or higher than EPA level, unlike cold and temperate water species in the Northern

	16:0	18:0	18:1 *n*-9	18:2 *n*-6	18:3 *n*-3	20:4 *n*-6	20:5 *n*-3	22:6 *n*-3	Σsatu-rates	Σmono-enes	Σ *n*-6	Σ *n*-3	ArA /EPA	DHA /EPA	DHA /ArA
Japan, ovary															
Plectropomus leopardus	19.0	12.6	7.3	0.9	0.1	19.2	1.2	9.3	34.5	14.8	29.3	13.3	15.6	7.6	0.5
Pristipomoides argyrogram-micus	17.6	8.8	13.6	1.1	0.2	10.7	6.2	18.4	27.9	19.1	19.3	28.4	1.7	3.0	1.7
Lutjanus gibbus	22.4	9.8	9.5	1.6	0.2	12.3	2.9	16.1	34.1	13.4	24.6	23.5	4.3	5.7	1.3
Lethrinus miniatus	21.4	10.7	6.7	1.4	0.2	17.3	4.8	14.8	35.0	11.2	24.6	24.5	3.6	3.1	0.9
Lethrinus atkinsoni	19.7	11.1	8.0	1.5	0.3	19.4	4.8	14.4	33.3	11.2	26.8	25.2	4.1	3.1	0.7
Siganus guttatus	27.0	7.3	7.4	0.9	0.2	12.7	2.7	19.9	37.0	11.1	19.5	28.4	5.3	10.3	1.6
Siganus canaliculatus	31.1	5.6	5.8	0.7	0.2	9.9	2.3	27.0	39.3	8.4	14.8	35.1	4.7	13.1	2.7
Siganus virgatus	23.4	11.4	6.4	0.7	tr	8.9	0.9	7.9	45.4	10.5	15.6	13.5	10.0	8.9	0.9
Philippines, ovary															
Cephalopholis argus	23.9	11.3	11.0	1.0	0.1	12.6	3.8	12.3	37.8	16.4	19.0	20.4	3.3	3.2	1.0
Cephalopholis cyanostigma	20.0	12.4	8.4	0.7	0.1	7.8	5.2	25.1	34.6	13.3	14.5	33.6	1.5	4.8	3.2
Epinephelus areolatus	22.0	11.7	8.1	0.7	tr	6.0	4.7	24.6	36.4	13.8	11.4	32.2	1.3	5.3	4.2
Epinephelus quoyanus	18.1	12.3	14.0	1.0	0.2	15.2	1.7	8.8	33.1	22.1	23.9	13.5	9.1	5.3	0.6
Epinephelus sp.1	23.4	11.2	11.2	0.9	0.3	11.4	2.2	9.6	36.5	16.5	20.6	14.5	5.3	4.4	0.8
Epinephelus sp.2	22.5	11.2	10.0	0.7	0.2	11.7	3.4	16.3	36.3	15.0	20.4	23.3	3.4	4.8	1.4
Plectropomus leopardus	25.6	10.2	7.6	0.8	tr	7.8	5.1	20.9	38.7	14.0	13.1	27.8	1.6	4.1	2.8
Lutjanus decussatus	8.6	12.5	7.1	0.6	0.3	16.5	3.3	23.4	22.8	11.8	27.5	30.7	5.3	7.3	1.4
Lutjanus erythropterus	14.4	15.7	8.2	1.0	0.1	17.5	4.3	18.3	31.4	11.8	25.3	26.9	4.1	4.2	1.0
Cheilio inermis	27.9	9.3	10.8	0.9	0.3	9.4	3.5	12.2	41.1	17.3	16.7	20.1	2.7	3.6	1.3
Siganus canaliculatus	30.6	6.7	7.7	2.5	0.2	6.8	1.5	27.8	40.0	10.8	13.5	32.4	4.5	18.3	4.1
Japan, testis															
Lethrinus atkinsoni	19.1	9.2	12.0	1.4	0.4	21.4	1.1	14.4	31.3	16.1	30.3	18.1	20.2	13.6	0.7
Lethrinus nebulosus	17.5	13.2	6.8	1.6	tr	22.5	2.2	4.9	35.5	13.0	30.9	10.8	10.2	2.2	0.2
Lethrinus ornatus	21.2	12.1	9.3	1.5	tr	22.9	2.7	4.5	36.8	14.7	30.2	10.2	8.4	1.6	0.2
Siganus guttatus	27.0	11.8	8.3	3.2	1.5	6.7	1.7	11.9	41.7	11.8	15.6	20.7	3.9	6.8	1.8
Siganus virgatus	28.1	12.6	9.0	1.0	0.3	7.9	1.3	9.6	47.0	12.8	15.0	15.8	6.1	8.2	1.2

Table 3: Major fatty acids (% of total fatty acids) in polar lipids of gonads of 19 species of coral reef fish from Philippines and Japanese water.

	16:0	18:0	18:1 *n*-9	18:2 *n*-6	18:3 *n*-3	20:4 *n*-6	20:5 *n*-3	22:6 *n*-3	Σsatu-rates	Σmono-enes	Σ *n*-6	Σ *n*-3	ArA /EPA	DHA /EPA	DHA /ArA
Japan, ovary															
Plectropomus leopardus	31.4	8.2	9.4	1.6	0.5	7.0	2.1	5.1	46.1	21.3	15.0	11.4	3.4	2.5	0.7
Pristipomoides argyrogrammicus	16.2	11.2	12.4	1.2	0.3	9.1	4.9	16.7	29.5	19.6	16.8	25.4	1.8	3.4	1.8
Lutjanus gibbus	24.3	10.5	9.7	1.3	0.4	4.3	1.5	3.7	45.2	26.1	10.7	8.2	3.0	2.5	0.8
Lethrinus miniatus	27.3	8.9	15.7	1.6	0.7	3.7	1.7	2.6	44.2	36.1	8.2	6.6	2.2	1.5	0.7
Lethrinus atkinsoni	30.9	9.3	13.4	3.2	1.3	7.4	3.1	3.3	47.0	24.5	14.6	10.8	2.4	1.1	0.4
Siganus guttatus	29.0	3.1	12.6	2.2	1.3	4.3	2.6	7.0	38.9	30.5	9.6	16.4	1.9	3.2	1.6
Siganus canaliculatus	32.2	4.3	15.4	2.5	1.3	3.9	1.6	6.9	41.3	27.8	10.5	16.2	3.2	5.5	1.8
Siganus virgatus	29.1	7.9	16.6	1.3	0.5	10.1	1.7	6.0	41.3	24.6	16.3	14.9	5.8	3.4	0.6
Philippines, ovary															
Cephalopholis argus	41.1	16.7	10.3	1.1	0.1	1.8	0.5	0.7	63.6	22.5	5.8	2.3	3.7	1.5	0.4
Cephalopholis cyanostigma	38.7	8.7	16.2	0.8	0.2	1.3	1.0	4.9	54.0	29.8	4.5	7.4	1.5	4.3	4.1
Epinephelus areolatus	31.0	7.7	14.8	1.0	0.3	1.3	2.0	11.4	45.3	29.5	4.2	15.9	0.6	5.7	8.8
Epinephelus quoyanus	29.3	11.5	18.2	0.6	0.7	2.6	1.0	7.3	45.6	27.0	10.4	10.5	3.3	8.1	3.8
Epinephelus sp.1	32.1	11.5	16.2	0.5	0.6	1.8	1.1	6.0	47.6	27.0	7.8	2.7	1.8	5.3	7.1
Epinephelus sp.2	36.1	6.6	17.0	0.9	0.4	2.4	0.9	2.4	48.2	34.1	6.0	4.5	2.8	2.8	1.0
Plectropomus leopardus	28.5	8.4	16.8	0.9	0.2	2.2	2.4	12.0	43.2	28.6	5.3	15.7	0.9	5.0	5.7
Lutjanus decussatus	31.9	14.3	10.3	0.8	0.7	1.7	1.5	4.0	55.4	22.6	5.1	8.0	1.0	2.2	2.2
Lutjanus erythropterus	26.4	12.8	12.2	1.9	0.2	4.8	2.0	3.2	46.7	26.1	12.3	7.2	2.4	1.6	0.7
Cheilio inermis	38.9	10.3	11.3	1.1	0.4	1.9	1.0	1.8	60.1	25.1	6.2	5.0	2.2	2.0	0.9
Siganus canaliculatus	27.8	6.6	23.7	5.1	0.6	1.7	0.7	6.8	38.5	37.0	9.8	10.6	2.4	9.2	3.9
Japan, testi															
Lethrinus atkinsoni	26.2	11.9	8.2	1.7	tr	14.3	1.3	7.6	43.8	15.7	24.0	11.3	10.8	5.7	0.5
Lethrinus nebulosus	24.8	15.3	12.0	2.0	0.6	10.9	2.4	3.8	45.9	22.0	17.5	9.1	4.5	1.6	0.4
Lethrinus ornatus	30.2	11.3	20.9	2.1	0.8	4.6	2.8	1.6	47.6	31.7	9.8	7.3	1.6	0.6	0.3
Siganus guttatus	26.2	8.8	10.4	5.5	2.9	10.0	2.5	10.4	37.0	19.0	21.0	22.2	3.9	4.1	1.0
Siganus virgatus	26.0	10.1	12.5	1.7	0.5	13.2	2.1	9.7	39.0	18.1	22.4	18.5	6.7	5.2	0.7

Table 4: Major fatty acids (% of total fatty acids) in neutral lipids of gonads of 19 species of coral reef fish from Philippines and Japanese water.

Hemisphere. Mitochondrial membranes (liver and heart) of three coral reef species had a significantly higher proportion of ArA than those of cold water species, excluding *Mugil cephalus* (coastal and estuarine species) [17]. On the other hand, few papers have been available on gonadal fatty acid composition of wild coral reef fish, especially in commercially important species which are emerging as new aquaculture commodities. Alava et al. [18] reported a relatively high ArA level in wild-sourced grouper (*Epinephelus coioides*) broodstock (GSI: 0.73) that the levels of HUFA were: DHA (13.1%) > ArA (5.1%) > EPA (1.9%) with a DHA/EPA ratio of 6.8 and a DHA/ArA ratio of 2.5 in the ovarian total lipids. Fatty acid compositions of ovaries, eggs and fry from not wild but reared broodstock at tropical and subtropical hatcheries have been reported in striped mullet (*Mugil cephalus*) (coastal, estuarine and bethopelagic species)[19], milkfish (*Chanos chanos*) (coastal, estuarine and bethopelagic species) [20], Asian seabass (*Lates calcarifer*) (coastal, estuarine and demersal species) [21], white sea bream (*Diplodus sargus*) (*demersal and oceanodromous species*) [22] and cobia (*Rachycentron canadum*) (reef-associated pelagic species) [23]. Since gonadal fatty acid profile is greatly affected by feeds that have been fed during rearing period, the results of the hatchery-raised broodstock are not directly compared with the present ones based on wild coral reef fish. In general, gonads and eggs of high and temperate latitude species in the northern hemisphere have high levels of EPA and DHA, and consequently most of these species show extremely low ArA/EPA ratios [24, 25]. The average

levels of ArA, EPA and DHA in egg polar lipids of seven Northwest European species (wild) were 1.9%, 14.6% and 28.0%, respectively [24]. The average levels of ArA, EPA and DHA in ovarian polar lipids of eight cold and temperate water species (wild) sampled in the Pacific Ocean were 4.1%, 13.1% and 22.0%, respectively [25]. In these species, EPA levels were always higher than ArA levels with the exception of *Beryx splendens* (bentopelagic species) in which ArA and EPA levels were 5.4% and 4.6%, respectively (this specimen was sampled at 33°N and 139°E). In the present result, however, ArA level was always higher than EPA level in ovaries of all the species examined, irrespective of the species, sampling location and a wide range of gonadosomatic index. The average levels of ArA, EPA and DHA in ovaries of the coral reef species were 12.3%, 3.4% and 17.2% in polar lipids and 3.9%, 1.8% and 5.9% in neutral lipids, respectively. In this connection, eggs of six Australian but freshwater or brackish water species (wild) have high or middle DHA (8.2—29.3%) and ArA (1.8—15.3%) levels with low EPA level (0.2—2.2%) in their polar lipids [26] as did the coral reef species. We might say that coral reef fish appear to have a comparable ArA/EPA ratio with freshwater fish rather than cold and temperate water marine fish.

Not only the concentration but also the mutual ratio of DHA, EPA and ArA in diets may be important, and that the dietary optimum ratio appears to vary depending on species, stage, the geography and the species inhabits. In the present study, ovaries of 17 species of coral reef fish had average ratios of: ArA/EPA of 4.8, DHA/EPA of 6.2 and DHA/ArA of 1.7 in polar lipids and ArA/EPA of 2.5, DHA/EPA of 3.8 and DHA/ArA of 2.5 in neutral lipids, respectively. This result indicates that ovarian DHA/ArA ratio is about 2 in tropical coral reef fish, suggesting that the DHA/ArA (not EPA) ratio of around or more than 2 may be optimum for broodstock diets, especially for ovary development in coral reef fish. We, therefore, recommend a dietary ratio of DHA/ArA (not EPA) of about 2 or greater as an ideal for broodstock diets of tropical coral reef fish.

Information on testis fatty acid composition of wild fish has been relatively scarce compared to ovaries and eggs. However, there have been several papers reporting the effects of HUFA on male reproductive performance in cold and temperate water species [27, 28]. The present study showed that ArA was the major HUFA in testis as well as in ovary of the coral reef fish, especially in their polar lipids. Particularly, testis ArA levels in polar lipids of the three *Lethrinus* species were more than 20%. The average levels of ArA, EPA and DHA in testis polar lipids of nine species of cold and temperate water fish were 2.7%, 10.8% and 28.1%, respectively [25]. The range of ArA, EPA and DHA levels in testis polar lipids of three cold water marine species (*Clupea pallasi, Theragra chalcogramma* and *Osmerus eperlanus mordax*) were from 1.2% to 4.6%, from 9.9% to 17.0% and from 14.1% to 20.9%, respectively [29]. In skipjack tuna *Euthynnus pelamis* (sampled in the Southern fishing ground in the Pacific Ocean), testis polar lipids had ArA of 3.6%, EPA of 3.5% and DHA of 32.5%, respectively[30]. It is also clear that the testes of the coral reef fish analyzed here have higher ArA levels and ArA/EPA ratios than those of cold and temperate water species. This result suggests that ArA tends to be specifically much more concentrated in testis of wild coral reef fish, and that the degree of the essentiality of ArA during male reproductive process may be much greater in coral reef fish compared to cold and temperate water species.

Testes of the five species of coral reef fish had average ratios of: ArA/EPA of 9.8, DHA/EPA of 6.5 and DHA/ArA of 0.8 in polar lipids and ArA/EPA of 5.5, DHA/EPA of 3.4 and DHA/ArA of 0.6 in neutral lipids, respectively. This result suggest that since the average DHA/ArA ratio was smaller in the testis than in the ovary, the optimum dietary ratio of HUFA during gonadogenesis might be different between males and females, and moreover that the degree of the physiological essentiality of ArA might be greater in testis than in ovary.

The high ArA levels in gonads observed in the present study shows that coral reef fish species may require much more ArA for normal gonadogenesis and embryo development than cold and temperate water species, although dietary ArA tends to be concentrated in gonads, eggs and sperm even in cold and temperate water species. ArA as an eicosanoid precursor is well known to be multifunctional in fish reproduction including pheromonal activity in mating behavior [31, 32]: induction of ovulation [33], stimulation of steroidgenesis in ovary [34] and testes [35], interaction of gonadotropin and prostaglandin production [36]. Coral reef fish have, in general, a longer spawning period (from March through December) compared to cold and temperate water species, and they spawn once a month during the spawning period according to lunar phase. This mode of reproduction might increase the degree of the essentiality of ArA in the reproduction of coral reef fish compared with cold and temperate water fish. On the other hand, tropical species hatch-out in a short time (usually less than 24 h) during which the embryo has to build up many biological systems, while cold water species such as salmonids take a longer time, one or two months, to hatch out. Very fast embryo development in tropical species might increase the degree of the essentiality of ArA, prostaglandins and other eicosanoids. Heavy raining and typhoons bring critical changes of temperature, salinity, irregular currents and turbidity. The environmental stresses may further increase the degree of the necessity for the eicosanoids, although the effectiveness of dietary ArA appears different between the types of stress, acute (handling stress) and chronic (salinity change) [37].

It is widely accepted that fatty acid composition of fish tissues reflects dietary fatty acid composition. It is also obvious that ArA level in tissues including gonads is affected by dietary ArA level. When we consider the fact that most of marine fish lack the *de novo* ability to produce HUFA, the coral reef fish should also rely on a dietary supply of HUFA from the food web. EPA and DHA in fish have been known to be derived from the pelagic food chain from phytoplankton to accumulate in higher order carnivores, especially in pelagic species and deeper offshore demersal species [38]. All the species investigated in the present study were reef-associated species. *Serranidae, Lujanidae, Lethridae* and *Labridae* are carnivores, which feed on smaller fishes, crabs, shrimps, cephalopods, polychaeta worms, gastropods and urochordates. Sigaidae are herbivores, which feed mainly on benthic macroalgae. The coral reef fish perhaps depend heavily on their natural food as their ArA sources (without doubt), although there might be a special mechanism by which they store much more preferentially ArA in their gonads than cold and temperate water fish.

Renaud et al. [39] investigated fatty acid composition of 18 species of tropical Australian microalgae [39]. Only two pelagic species, *Nitzschia sp*. and *Fragilarias sp*. had relatively high ArA levels equivalent to EPA levels, but in the remaining 16 species, EPA was the major HUFA and ArA level was low. EPA was also the major HUFA in tropical phytoplankton sampled from coastal waters of the South China Sea during one year cycle [40]. In tropical paracalanid copepods, EPA level is higher than ArA level with DHA/EPA/ArA ratios of 14:3:1 for *Acartia sinjiensis*, 20:9:1 for *Parvocalanus crassirostris* and 25:6:1 for *Bestiolina similes*, respectively [41]. Thus, planktonic microorganisms do not appear to be the primary source of ArA even in tropical waters. High ArA levels are found in some species of marine red and brown seaweeds

from both temperate and tropical waters, although this phenomenon is not always limited to tropical areas [42-44]. Nevertheless, red and brown seaweeds may be at least one of the sources. Dunstan et al.[45] found that in temperate marine fish from Southern Australian coastal waters, demersal omnivore species (macroalgae consumers) have relatively high ArA/EPA ratio (0.9) compared to demersal carnivores (0.6) and pelagic carnivores (0.2) [45]. Thus, ArA appears to be provided primarily from some organisms existing in/on benthic substrate and benthic detritus rather than pelagic organisms. This speculation may be supported by a finding by Svetashev et al. [46] that ArA content is remarkably higher in tropical holothurians than in the temperate species [46]. Scarce data are available on fatty acid composition of benthic prokaryotes and eukaryotes, bacteria, fungi and protozoa towards the beginning of ArA source in tropical marine food chain. The present result, high ArA levels in coral reef fish, suggests that the existence of an ArA-rich food chain may be widespread in coral reef areas, and that the widespread existence of ArA-rich food chain may lead to comparatively higher ArA contents in the coral reef fish. However, the issue of ArA origin in the coral-reef food web is still unclear.

Effort is needed to establish formulated diets appropriate for broodstock of coral reef fish. Feeding locally available trash fish rich in ArA would be at least one of the measures for improving broodstock performance in coral reef fish, until appropriate formulated diets are established for coral reef broodstock. Nevertheless, the information in the present study can be used as a guideline (a dietary ratio of DHA/ArA of about 2 or greater) for development of appropriate broodstock and larval diets, to ensure high egg and larval quality of sustainable hatchery production in tropical and subtropical areas. We have already initiated a follow-up study on the effects of dietary ArA on reproductive performance and larval/fry quality in coral reef fish. Results of feeding trials will be published in separate papers.

Acknowledgements

We would like to thank Dr. K.D. Shearer, Northwest Fisheries Science Center, NMFS, Seattle, WA USA, for his critical reading of the manuscript. The authors are also grateful to Mr. D.R. Chavez, INVE Asia Services Ltd, Thailand, Mr. E.S. Garibay, the Aquaculture Department, SEAFDEC, Philippines and Dr. H. Furuita, National Research Institute of Aquaculture, FRA, Japan for their technical assistants for conducting the present study. This study was supported by the collaborative project titled “Studies on Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas” between Japan International Research Center for Agricultural Sciences, Japan and the Aquaculture Department, SEAFDEC, Philippines.

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