International Journal of Fisheries and Aquaculture Vol. 3(5), pp. 89-95, May 2011
Available online at http://www.academicjournals.org/IJFA

Full Length Research Paper

Differences in fatty acid composition of egg capsules from broodstock spotted babylon, *Babylonia areolata*, fed a local trash fish and formulated diet under hatchery conditions

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Accepted 11 April, 2011

This study was the first attempt to condition broodstock *Babylonia areolata* using the formulated diets under hatchery conditions. Samples of spotted babylon egg capsules from broodstock, which had been fed either a formulated diet or a local trash fish, carangid fish (*Seleroides leptolepis*) for 120 days were analyzed for proximate composition and fatty acid composition. The formulated diet contained significantly higher levels of arachinodic acid (ARA) (20:4n-6), eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) than those of the local trash fish. The formulated diet also had significantly higher ratios of DHA/EPA and (n-3)/(n-6) polyunsaturated fatty acids (PUFA) than those of the local trash fish but not for ARA/EPA ratio. The fatty acid compositions of egg capsules produced from broodstock fed formulated diet contained significantly more ARA, EPA and DHA compared to the broodstock fed the local trash fish. The ARA/EPA and DHA/EPA ratios in egg capsules were significantly higher in the trash fish–fed group compared to those fed the formulated diet. However, (n – 3)/(n – 6) PUFA ratios in egg capsules produced from broodstock fed the formulated diet did not significantly differ compared to those from broodstock fed the local trash fish. The relatively low DHA/EPA, ARA/EPA and (n – 3)/(n – 6) ratios in the egg capsules produced from the formulated diet – fed broodstock of *B. areolata* suggested that this diet was inferior, when compared to the traditional food of trash fish.

Keywords: *Babylonia areolata*, broodstock diet, egg capsules, fatty acid composition.

INTRODUCTION

A major constraint to the development of the spotted Babylon "*Babylonia areolata"* aquaculture in Thailand is the insufficient supply of seed and high cost of production. Successful conditioning of broodstock *B. areolata* is still a crucial step for selective breeding programs to produce a large quantity of eggs and larvae of good quality for the growing industrial importance of this species in Thailand because large variability in spawning events, hatchability, and larval and juvenile survival rates of the spotted babylon has been observed during the same season between batches and hatcheries. This variability remained high despite each batch of larvae being reared in a standardized manner which included the control of larval density, water management and the use of selected microalgal species. Production of good quality larvae is very inconsistent (Chaitanawisuti and Kritsanapuntu, 1997). In teleosts, nutrients such as protein, fatty acids, vitamin E, ascorbic acids and carotenoids have been implicated in various reproductive-related processes such as gonadal maturation, gamete quality and spawning performances. Interaction between nutrients and reproductive

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Abbreviations: ARA, Arachinodic acid; **DHA,** docosahexaenoic acid; **PUFA,** polyunsaturated fatty acids; **EPA,** eicosapentaenoic acid; **LCFA,** laboratory center for food and agricultural product; **FAME,** fatty acid methyl esters; **MUFA,** monounsaturated fatty acid.

Table 1. Ingredients and proximate composition of local trash fish and formulated diet for *B. areolata* broodstock

Ingredient (%)	Trash fish	Formulated diet
Fish meal	-	20.0
Shrimp meal	-	20.0
Squid meal	-	10.0
Soybean meal	-	31.0
Tuna oil	-	5.0
Wheat flour	-	8.0
Polymethylocarbamide	-	2.0
Vitamin mix[1]	-	2.0
Mineral mix[2]	-	2.0
Proximate composition (g /100 g diet)		
Crude Protein	19.81+0.01	28.73+0.1[a]
Total fat	1.31+0.01	14.97+0.05[a]
Carbohydrate	0	13.82±0.4[a]
Moisture	78.20±0.01	40.17±0.2[a]
Ash	1.31±0.03	12.31±0.3[a]

[1]Vitamin A 150,000,000 IU; vitamin D, 3,000,000 IU; vitamin E, 27.5 g; vitamin K, 4.67 g; vitamin B_1, 25 g; vitamin B_2, 26 g; vitamin B_6, 5,000 µg; nicotinamide, 20 g; folic acid, 0.4 g; vitamin C, 143 g; calcium D pantothenate 5 g. [2]1 kg of mineral mix consisted of calcium 147 g, iron 2,010 mg, 147 g phosphorus, 3,621 mg copper, 6,424 mg zinc, 10,062 mg manganese, 105 mg cobalt, 1,000 mg iodine and 60 mg selenium. Trash fish = fresh meat of carangid fish (*Seleroides leptolepis*).

processes, however, remains poorly understood. Several studies have highlighted the importance of both quantity and quality of dietary lipid on reproductive performances of broodstock (Ling et al., 2006). Under optimal hatchery rearing conditions, differences in initial egg lipid reserves may not necessarily affect subsequent larval growth and survival. Similarly, the importance of lipid and polyunsaturated fatty acids (PUFA) reserves, in particular eicosapentaenoic acid (EPA; 20:5n3), during the development of embryos have also been studied. However, it remains unclear which constituents are responsible for triggering maturation, egg laying of broodstock, therefore, more detailed research on reproductive performance is needed. There are no published studies on the influence of nutrition on the reproductive performance of spotted babylon broodstock, despite their importance in commercial aquaculture. Teruel et al. (2001) reported that a higher amount of essential nutrients such as protein, lipid and the highly unsaturated fatty acid, such as 20:4n-6, 20:5n-3, 22:6n-3 in the artificial diet influenced the increased reproductive performance for abalone, *Haliotis asinina*. In addition, Utting and Millican (1997) reported that the PUFA composition of the eggs of marine bivalves (scallops, oysters and clams) are influenced by the quantity and quality of lipid in microalgae diet supplements. Thus, there is a need to develop a reliable technique for spotted babylon broodstock development through dietary manipulation. This study aimed to assess the differences in biochemical composition and fatty acid composition of egg capsules from broodstock spotted babylon, *B. areolata*, fed a local trash fish and formulated diet under hatchery conditions at providing information as a guideline for the development of appropriate practical diets for broodstock of this species.

MATERIALS AND METHODS

Experimental diets and feeding

The basal diet was formulated by adding different supplements to the diet (Table 1). The basal diet utilized fish meal as the protein source, wheat flour as the carbohydrate source and tuna oil as the lipid source. Mineral and vitamin mixes were added to the diets. Wheat gluten was used as binders. The diets were prepared by weighing the dry ingredients and mixing thoroughly in a mixer. Lipid source that originated from tuna oil (5%) was added to the basal diets drop by drop while the mixture was further blended to ensure homogeneity. Approximately 200 ml hot water was then added for each kg of this mixture. The diets were extruded and dried with the use of electric fan at room temperature for 48 h. All experiment diets were then stored in plastic bag at -20°C until use. All diets were analyzed in duplicate for the proximate compositions and fatty acid composition according to standard methods (AOAC, 1990). During feeding, the feeds were formed into small pieces of 1.5 cm diameter to facilitate sucking by the snails. The traditional natural food (fresh meat of the carangid fish (*Seleroides leptolepis*) commonly used in commercial hatchery was used as the control diet. The broodstock were fed, each diet once daily at 10:00 h with the daily amount calculated as 15% of total broodstock biomass per tank. Excess diet was removed and the feeding rate was adjusted based on weight gain after each sampling, which was done every 2 weeks. The feeding trials were conducted for 120 days.

Broodstock and rearing system

This experiment was carried out during the spawning season from March to June 2008 (Chaitanawisuti and Kritsanapuntu, 1997). Female and male, *B. areolat*a, broodstock used in this study were already used in the commercial private hatchery for 4 to 6 months. They were graded to the same size with an average individual wet weight of 46.5 to 50.3 g and transferred to the hatchery of the Research Unit for Commercial Aquaculture of the Spotted Babylon, Aquatic Resources Research Institute, Chulalongkorn University, Petchaburi Province, Thailand. 300 broodstock were randomly distributed with a female:male ratio of 10:10 into 15 units. Each plastic tank was 1.5 m x 0.5 m x 0.5 m, with three replicate tanks per dietary treatment. The tank bottoms were covered with a 5 cm layer of coarse sand as substratum for burying of the broodstock. Unfiltered natural seawater was supplied in a flow-through system at a constant flow rate of 16 l/min for 12 h daily and adequate aeration was provided throughout the experimental period. A constant water depth of 30 cm was maintained. Feeding was carried out by hand to apparent visual satiety at 10:00 h. Sufficient food as could be consumed by the snails was provided over 60 min. To prevent degradation of the seawater, uneaten diets in each tank were removed immediately after the snails stopped eating. Tanks and sand substrate were cleaned of faeces at 15 days intervals by flushing it with a jet of water. Thereafter, the tanks were refilled with new ambient natural seawater. Water temperature, salinity, dissolved oxygen, nitrite nitrogen and ammonia nitrogen during feeding experiment ranged between 30.0 to 32.0°C, 29 to 30 ppt, 4.5 to 7.0 mg L^{-1}, 0 to 0.17 mg L^{-1} and 0 to 0.04 mg L^{-1}, respectively. The rearing tanks were kept under a natural photoperiod. The egg capsules from each replicate treatment were collected every day, and were then stored in plastic bag at -20°C for further biochemical analysis.

Biochemical analysis

At the beginning of the experiment (month 1) as well as at end of the experiment (month 4), the whole body tissues of female broodstock and spawned egg capsules from each samples group were used for analysis. These samples were frozen in liquid nitrogen at -90°C and then were freeze-dried and weighed. All samples were analyzed for proximate composition, fatty acid, and amino acid at the Laboratory Center for Food and Agricultural Product (LCFA), Bangkok, Thailand. Proximate analysis of the whole body of each snail (crude protein, total fat, carbohydrate ash and moisture) of all samples was performed at the Laboratory Center for Food and Agricultural Product (LCFA), Bangkok, Thailand, using the in house method based on AOAC official method (1990). Moisture content was determined by oven drying to constant weight at 150°C. Using freeze-dried material, crude protein was derived from Kjeldahl nitrogen analysis using copper and selenium as catalysts. Ash was determined as the residue after muffle furnace ignition at 600°C for 24 h. Total lipid content was determined by Soxhlet extraction with petroleum ether (bp 40 to 60°C for 6 h) (AOAC, 1990). Fatty acid composition in whole body of each snail was performed using the in house method based on the AOAC official method (1990). Total lipid was first extracted from samples of each diet. An aliquot of the liquid extract so obtained was separated by homogenization in chloroform/methanol (2:1, v/v), methylated and transesterified with boron trifluoride in methanol. Fatty acid methyl esters (FAME) were separated and quantified by gas-liquid chromatography equipped with a flame ionization detector and a 30 m x 0.25 mm fused silica capillary column. Helium was used as the carried gas and temperature programming was from 50 to 220°C at 4C/min, and then was held at 220°C for 35 min. The injector and detector temperature was set at 250 and 260°C, respectively. Individual methyl esters were identified by comparison to known standards and by reference to published data. Amino acid profile and cholesterol in whole body tissues were analyzed using the in house method based on AOAC official method (1990).

Statistical analysis

Data were presented as mean ± standard deviation (SD). The statistical significance of differences among treatments was determined using one-way analysis of variance (ANOVA), and Duncan's multiple range test (P<0.05) was applied to detect significant differences between means (P<0.05).

RESULTS

Proximate composition and fatty acid composition of the experimental diets

Two group of spotted babylon broodstock were fed formulated diet containing fish meal, which was rich in both EPA (20:5n-3) and DHA (22:n6-3), and a local trash fish. The proximate composition and fatty acid composition of the local trash fish and formulated diets are shown in Tables 1 and 2. The levels of protein content (28.73 g/100 g diet) of the formulated diet did not differ significantly among the local trash fish (19.81 g/100 g diet) but lipid content of the formulated diet (4.97 g/100 g diet) was significantly higher than those of the control food (1.31%). The formulated diets contained significantly higher levels of total unsaturated fatty acids (2,339.4 mg/100 g diet) for both monounsaturated fatty acid (1,237.6 mg/100 g diet) and polyunsaturated fatty acid (1,101.9 mg/100 g diet) than those of the local trash fish (192.1, 151.6 and 40.5 mg/100 g diet, respectively). The formulated diet contained significantly higher EPA (99.1 mg/100g diet), DHA (376.4 mg/100 g diet), C 20:4n – 6, ARA (71.1 mg/100 g diet), total n – 3 PUFA (595.7 mg/100 g diet) and total n – 3 HUFA (475.5 mg/100 g diet) than those in local trash fish (6.3, 10.9, 13.3, 17.2 and 17.2 mg/100 g diet, respectively).

Proximate composition and fatty acid composition of the egg capsules

The proximate composition and fatty acid composition of egg capsules produced from the *B. areoata* broodstock fed the local trash fish and formulated diet over 120 days are shown in Table 3. Analyses of the proximate composition and fatty acid composition of egg capsules revealed that there were no significant differences in the proximate composition of egg capsules produced from broodstock fed the formulated diet and the local trash fish, but significant differences in fatty acid composition of egg capsules produced from broodstock fed the formulated diet and the local trash fish were found. There were no significant differences (P>0.05) in protein and lipid contents in egg capsules produced from the

Table 2. Fatty acid composition (mg/100 g wet weight) of local trash fish and formulated diet for *B. areolata* broodstock.

Diet composition (±SD)	Trash fish	Formulated diet
C14:0	67.2±0.4[a]	198.5±0.1[b]
C15:0	14.0±0.0[a]	63.6±0.1[b]
C16:0	561.6±0.1[a]	1,581.3±0.4[b]
C17:0	42.7±0.6[a]	155.9±0.1[b]
C18:0	289.5±0.5[a]	525.2±0.2[b]
C20:0	23.9±0.04[a]	26.2±0.3[b]
C21:0	-	10.4±0.2[a]
C22:0	20.9±0.5[a]	21.7±0.1[b]
C23:0	-	12.1±0.1[a]
C24:0	14.9±0.1[a]	30.7±0.4[b]
C16:1n7	75.6±0.5[a]	269.7±0.3[b]
C18:1n9t	17.3±1.4[a]	51.2±0.2[b]
C18:1n9c	50.2±1.3[a]	814.9±0.8[b]
C20:1n11	-	22.2±0.2[a]
C22:1n9	-	39.3±0.5[a]
C24:1n9	8.5±0.0[a]	40.3±0.1[b]
C18:2n6	10.0±0.2[a]	403.3±0.2[b]
C18:3n3	-	120.3±0.3[a]
C20:2	-	20.6±0.5[a]
C20:3n6	-	11.1±0.2
C20:4n6 (ARA)	13.3±0.4[a]	71.1±0.1[b]
C20:5n3 (EPA)	6.3±0.3[a]	99.1±0.1[b]
C22:6n3 (DHA)	10.9±0.7[a]	376.4±1.3[b]
Total unsaturated fatty acid	192.1±0.3[a]	2,339.4±0.1[b]
∑n - 3 PUFA	17.2±0.04[a]	595.7±0.0[b]
∑n - 3 HUFA	17.2±0.1[a]	475.5±0.3[b]
∑n - 6 PUFA	23.3±0.3[a]	485.5±0.3[b]
(n – 3) / (n -6)	0.7±0.1[a]	1.2±0.3[b]
DHA / EPA	1.7±0.3[a]	3.8±0.05[b]
ARA / EPA	2.1±0.3[a]	0.7±0.1[b]

HUFA = highly unsaturated fatty acid; PUFA = polyunsaturated fatty acids. Values are means ±SD (n = 3). Means in the same row with different superscript letters were significantly different (P<0.05).

broodstock fed the formulated diet (1.90 and 0.31 g/100 g diet, respectively) and the local trash fish (1.93 and 0.35 g/100 g diet, respectively). The total unsaturated fatty acids (237.7 mg/100 g diet) including monounsaturated fatty acid, (48.0 mg/100 g diet) and polyunsaturated fatty acid (189.7 mg/100 g diet) of egg capsules produced from the broodstock fed formulated diet was significantly higher (P<0.05) than those fed the local trash fish (149.7, 27.4 and 122.3 mg/100 g diet, respectively).

Egg capsules produced from the broodstock fed formulated diet had significantly higher levels of ARA (50.9 mg/100 g diet), EPA (48.6 mg/100 g diet) and DHA (54.3 mg/100 g diet) than those from broodstock fed the local trash fish (38.0, 27.0 and 49.6 mg/100 g diet respectively). Similarly, egg capsules produced from the broodstock fed the formulated diet had significantly higher levels of total n-3PUFA (113.1 mg/100 g diet), total n – 6 PUFA (70.4 mg/100 g diet) and total n – 3 HUFA (102.9 mg/100 g diet) than those from broodstock fed the local trash fish (76.6, 45.7 and 76.6 mg/100 g diet, respectively). The DHA/EPA and AA/EPA ratios of egg capsules differed significantly between each broodstock group. Egg capsules produced from the broodstock fed formulated diet had significantly lower levels of DHA/EPA (1.1) and AA/EPA (1.0) than those from broodstock fed the local trash fish (1.8 and 1.4, respectively). However, there were no significant differences in the (n – 3) /(n – 6) PUFA ratio of egg capsules produced from the broodstock fed formulated diet (1.6) and local trash fish (1.7).

DISCUSSION

This study was the first attempts to condition broodstock *B. areolata* using formulated diets under hatchery

Table 3. Proximate composition and fatty acid composition (mg fatty acid /100 g wet weight) of egg capsules produced from *B. areolata* broodstock fed of local trash fish and formulated diet (n = 3) for 120 days

Egg capsule composition (±SD)	Trash fish	Formulated diet
Crude protein	1.93±0.2[a]	1.9±0.4[a]
Total lipid	0.35±0.5[a]	0.31±0.7[a]
C14:0	-	6.1±0.5
C16:0	87.7±0.2[a]	129.1±0.1[b]
C17:0	10.9±0.3[a]	17.2±0.3[b]
C18:0	51.8±0.1[a]	74.2±0.5[b]
C24:0	9.4±0.1	-
C18:1n9c	19.4±0.08[a]	33.9±1.2[b]
C20:1n11	8.1±0.3[a]	14.1±0.0[b]
C18:2n6	7.7±0.3[a]	19.5±0.02[b]
C18:3n3	-	10.2±0.04[a]
C20:2	-	6.2±0.1[a]
C20:4n6 (ARA)	38.0±0.2[a]	50.9±0.1[b]
C20:5n3 (EPA)	27.0±0.2[a]	48.6±0.02[b]
C22:6n3 (DHA)	49.6±0.3[a]	54.3±0.3[b]
∑SFA	159.8±0.1[a]	226.6±0.3[b]
∑MUFA	27.4±0.3[a]	48.0±0.4[b]
∑PUFA	122.3±0.1[a]	189.7±0.5[b]
Total unsaturated fatty acid	149.7±0.1[a]	237.7±0.1[b]
∑n - 3 PUFA	76.6±0.02[a]	113.1±0.2[b]
∑n - 3 HUFA	76.6±0.1[a]	102.9±0.4[b]
∑n - 6 PUFA	45.7±0.3[a]	70.4±0.2[b]
(n - 3) / (n – 6) PUFA ratio	1.7±0.4[a]	1.6±0.1[a]
DHA / EPA ratio	1.8±0.01[a]	1.1±0.3[b]
ARA / EPA	1.4±0.03[a]	1.0±0.02[b]

HUFA = highly unsaturated fatty acid; PUFA = polyunsaturated fatty acids. Values are means ±SD (n = 3). Means in the same row with different superscript letters were significantly different ($P<0.05$).

conditions. This study found differences in biochemical composition of egg capsules between the two broodstock groups fed the local trash fish and the formulated diets. Broodstock fed the formulated diets produced egg capsules with higher levels of EPA, DHA and ARA than those of broodstock fed the trash fish, but was lower in desirable DHA/EPA, ARA/EPA and (n – 3) / (n – 6) ratios. This result suggested that this formulated diets may be inferior for sustained larval growth and survival. In an effort to improve egg quality and larval viability, effort should be directed towards establishing the best ratios of DHA/EPA/AA in formulated diets such that requirements for neutral function and visual performance are maximized and that production and efficacy of eicosanoids are adequate to permit physiological functions to operate efficiently. This result agrees with the study of Utting and Millican (1998) which also demonstrated the important factors for the production and viability of eggs and embryos of scallop (*Pecten maximus*), essential fatty acids particularly 20:5n-3, 22:6n-3 and 20:4n-6, which must be supplied in microalgae diets during broodstock conditioning. *P. maximus*, like most other bivalves, has limited ability to elongate or desaturate fatty acid precursors and has a dietary requirement for essential PUFA, in particular, 20:5n-3, and 22:6n-3. Using unialgal diets deficient in specific fatty acids, it can be shown that the essential fatty acid composition of *P. Maximus* gonad and egg lipids is related to the fatty acids in the microalgae fed to broodstock during hatchery conditioning. In addition, Utting and Millican (1998) also stated that the hatching success rate of *P. Maximus* is dependent on egg lipid reserves but not for subsequent larval growth rate. Endogenous reserves laid down in the oocyte are utilised by developing embryos and larvae until exogenous reserves became available as larvae begin to feed. Lipid, protein and carbohydrate reserves supply the energy needed for embryo development. Most of this energy requirement is for shell deposition. The total fatty acid content of egg capsules decreased during the first 5 days of embryonic development and all fatty acids 20:5n – 3 is preferentially utilised during embryogenesis. By contrast, there was no change in the level of 22:6n – 3 because this PUFA is conserved and is important for cell

membrane structure. However, once larvae have reached the first feeding stage, their subsequent growth, survival and success at metamorphosis is dependent on a very fine balance between both quality and quantity of lipid in the diet provided, especially the 22:6n – 3 rather than on the initial oocyte reserves. Growth of larvae is very dependent on sufficient quantities of dietary polar lipids for incorporation into cell membranes as well as of neutral lipids for energy reserves (Delaunay et al., 1992). Bell et al. (1997) reported that one of the major roles of n-3 HUFA is as a component of membrane phospholipids and 22:6n-3 is especially abundant in the membranes of neural tissues such as eyes and brains. Adequate DHA supply is particularly important in rapidly growing and developing marine fish larvae which have a high percentage of neural tissues in their relatively small body mass. In addition, it is important that eggs contain the correct balance of DHA/EPA to ensure proper larval development on hatching. These considerations are especially important when it is considered that many of marine fish oils on which broodstock diets are based have DHA/EPA ratios of ≤ 1 and these may either provide insufficient DHA or a potentially harmful excess of EPA. In addition, fatty acids mobilized from the neutral lipid reserves of female broodstock adipose tissue during gonadogenesis are transferred via serum vitllogenin to developing eggs in the ovary. Thus, the essential fatty acids vital for early survival and development of newly hatched larvae are determined by the lipids derived directly from the dietary input of broodstock in the period preceding gonadogenesis.

Further, there have been several studies on broodstock conditioning of egg and larval quality of fish and shellfish with various diets supplemented with fatty acids. Bell and Sargent (2003) suggested that the dietary ARA/EPA/DHA ratio may be a critical factor in diets for broodstock and larvae of various fish and shellfish. The acclimation of essential nutrients such as essential fatty acids and vitamin C are dependent on the nutrient reserves in the mother animal, and consequently on the dietary input of broodstock in the period preceding gonadogenesis. In this regard, broodstock nutrition deserves special attention in order to guarantee optimal survival and development of the larvae during the period of endogenous feeding. It may be even advantageous to start feeding when there might only be a marginal uptake of essential nutrients. However, most of the studies on the essential fatty acids have focused on the qualitative and quantitative requirement of EPA and DHA and their optimum dietary ratio in broodstock and larval diets. Essential fatty acids are one of the nutritional factors which greatly affected egg and larval qualities. Variability in maturation, egg laying and larval and juvenile survival rates among batches may depend on many factors such as food, environmental factors and genetic background.

Moreover, variation in the nutritive composition of the larvae between broods may influence development of larvae in various molluscs (Berntsson et al., 1997; Marasigan and Laureta, 2001; Gallager and Mann, 1986; Soudant et al., 1996; Wilson et al., 1986). Teruel et al. (2001) reported that a higher amount of essential nutrients in the artificial diets such as protein, lipid and the highly unsaturated fatty acids, for example, 20:4n-6, 20:5n-3, 22:6n-3 in hatchery-bred donkey's ear abalone *Haliotis asinina* fed artificial diet alone and a combination of natural diet and artificial diet influenced the increased reproductive performance. Emata et al. (2003) reported that, for the mangrove red snapper, ARA may be nutritionally more important for egg and larvae development and survival and its supplementation in broodstock diets may enhance reproductive performance. Daume and Ryan (2004) reported that there is growing evidence that specific dietary lipids play an important role in gonadogenesis of abalone, *Haliotis fulgens*, and variations of the PUFA in the digestive gland and foot tissues over the year coincided with variation in their macroalgal diets. Furthermore, ARA is an essential fatty acid for the abalone and essential fatty acids are derived from the algal diet and are most likely important in cyclical gonad development. Variation in nutritive contents of the larvae between broods may arise during gametogenesis and influence the variation in the development of larvae in various molluscs. Unpredictable and variable egg quality is a major limiting factor for successful mass production of spotted babylon juveniles. It remains unclear which constituents are responsible for triggering maturation and egg laying of broodstock, therefore, more detailed research on maturation and reproductive performance is needed. Further research into hormonal control of *B. areolata* reproduction may help to explain the processes involved as well as the fatty acid composition of egg capsules, hatch-out larvae and quality of larvae. The spotted babylon broodstock will have to be successfully conditioned on farms to secure high egg and larvae quality for advanced and sustainable aquaculture, because only this will enable the optimal selection of breeding programs for further development of this species.

ACKNOWLEDGMENTS

This study was funded by the National Research Council of Thailand (NRCT) for the fiscal years 1996 to 2008. We are especially grateful to the associate Professor Dr. Somkiat Piyatiratitivorakul, of the Faculty of Science, Chulalongkorn University and Professor Yutaka Natsukari, of the Faculty of Fisheries, Nagasaki University for their encouragement and critical reading of the manuscript.

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