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Conceptive execution, egg and larval quality and egg unsaturated fat structure of incubation facility raised Spotted Babylon (Babylonia areolata) broodstock took care of regular and detailed weight control plans under incubator conditions

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Abstract

A 120 day feeding trial was conducted to evaluate the reproductive performance, egg and larval quality and egg fatty acid composition in Spotted Babylon (*Babylonia areolata*) broodstock fed with natural food (fresh meat of carangid fish, *Seleroides leptolepis*,) and one of four experimental formulated diets containing 5 or 15% of dietary lipid from either tuna oil or a mixture of tuna oil and soybean oil (6:4) labeled as 5% TO, 15% TO, 5% MO and 15% MO respectively. Using trash fish as a control food resulted is the lowest levels of in 20:5 n - 3, 22:6 n - 3 and 20:4 n - 6 fatty acids compared to those of all experimental diets, while the highest contents of those fatty acids were found in the 5% TO diet. Statistically significant differences in reproductive performance between dietary treatments with the best reproductive performance were found only for females fed the 5% TO diets, but egg and larval quality showed no variability among females fed trash fish and all experimental diets. No significant differences were observed in the survival duration in the starvation tolerance test for females fed trash fish or any of the experimental diets. However, the fatty acid profile of egg capsules was significantly affected by the dietary treatments. The levels of major fatty acids (20:5 n - 3, 22:6 n - 3 and 20:4 n - 6) in egg capsules produced from females fed diets containing 5% tuna oil (5% TO) was significantly higher than those from females fed trash fish or other experimental diets. We therefore conclude that formulated diets resulted in successful reproduction and high essential fatty acids in egg capsules comparable to the use of trash fish.

Keywords: *Babylonia areolata*, broodstock diet, reproductive performance, egg and larvae quality.

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 production. Successful conditioning of broodstock *Babylonia 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 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). One of the main reasons for unpredictable larvae culture outputs is the variable quality

Table 1. Experimental diets for *B. areolata* broodstock used in this work: Ingredients and their proximate composition (%).

Ingredients (g kg^{-1} diet)	Control[1]	5% TO[1]	15% TO[1]	5% MO[1]	15% MO[1]
Fish meal	-	20.0	20.0	20.0	20.0
Shrimp meal	-	20.0	20.0	20.0	20.0
Squid meal	-	10.0	10.0	10.0	10.0
Soybean meal	-	31.0	21.0	31.0	21.0
Tuna oil	-	5.0	15.0	-	-
Mixed tuna oil and soybean oil[1]	-	-	-	5.0	15.0
Wheat flour	-	8.0	8.0	8.0	8.0
Polymethylocarbamide	-	2.0	2.0	2.0	2.0
Vitamin mix[1]	-	2.0	2.0	2.0	2.0
Mineral mix[2]	-	2.0	2.0	2.0	2.0
Proximate composition (%)					
Crude protein	19.81 ± 0.01	36.24 ± 0.1[a]	35.93 ± 0.6[a]	37.71 ± 0.3[a]	37.61 ± 0.1[a]
Total fat	1.31 ± 0.01	18.64 ± 0.05[a]	26.54 ± 0.1[a]	16.76 ± 0.3[a]	25.37 ± 0.3[a]
Carbohydrate	0	19.75 ± 0.4[a]	12.78 ± 0.4[a]	20.49 ± 0.6[a]	11.47 ± 0.4[a]
Moisture	77.6 ± 0.01	8.17 ± 0.2[a]	7.68 ± 0.8[a]	8.41 ± 0.2[a]	8.52 ± 0.3[a]
Ash	1.31 ± 0.03	12.21 ± 0.3[a]	12.01 ± 1.2[a]	11.96 ± 0.7[a]	11.83 ± 0.1[a]

[1]1 kg of mineral mix consisted of calcium 147 g, iron 2,010 mg, phosphorus 147 g, copper 3,621 mg, zinc 6,424 mg, manganese 10,062 mg, cobalt 105 mg, iodine 1,000 mg, selenium 60 mg. [2] 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.[1] Diet abbreviations are as follows: Control = fresh meat of carangid fish (*Seleroides leptolepis*); TO = tuna oil; MO = mixture of tuna oil and soybean oil (6:4).

of eggs and larvae. Several factors affect egg and larvae quality in fish and shellfish species. These are either endogenous (genotype, age, and size of broodstock, egg size) or exogenous (egg management, broodstock feeding, bacterial colonisation of egg surface (Ballestrazzi et al., 2003). 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 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). Teruel, Millamena and Fermin (2001) reported that a higher amount of essential nutrients such as protein, lipid and the highly unsaturated fatty acid, e.g. 20:4 n - 6, 20:5 n - 3, 22:6 n - 3 in the artificial diet influenced the increased reproductive performance for abalone, *Haliotis asinina*. Utting and Millican (1997) reported that the number of eggs produced and polyunsaturated fatty acid (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. Under optimal hatchery rearing conditions, differences in initial egg lipid reserves may not necessary affect subsequent larval growth and survival. In addition, the importance of lipid and PUFA reserves, in particular, eicosapentaenoic acid, on 20:5n3, during the development of embryos and larvae can, however, be clearly demonstrated under more stressful rearing conditions. It remains unclear which constituents are responsible for triggering maturation, egg laying of brood-stock, 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. Thus, there is a need to develop a reliable technique for Spotted Babylon broodstock development through dietary manipulation. This study aimed at determining the effects of dietary lipid sources on reproductive performance, the egg and larval quality and egg fatty acid composition in Spotted Babylon (*B. areolata*) and at providing information as a guideline for development of appropriate practical diets for broodstock of this species.

MATERIALS AND METHODS

Experimental diets

Four practical diets were formulated to contain different levels of tuna oil and soybean oil based on the ingredient composition outlined in Table 1. Treatments were arranged in a completely randomized experimental design. The natural food of fresh meat of carangid fish (*Seleroides leptolepis*) was used as control diet. Experimental diets were formulated with 5 and 15% lipids originated from tuna oil and mixed oils (6:4 of tuna oil and soybean oil) and four dietary treatments were labeled as 5% TO, 15% TO, 5% MO

and 15% MO respectively. The diets were prepared by weighing the dry ingredients and mixing thoroughly in a mixer. The lipid sources were added 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 using electric fan at room temperature for 48 h. All experimental diets were then stored at -20°C until use. The proximate compositions and major fatty acid composition of the experimental diets were analyzed according to standard methods (AOAC, 1990) . While feeding, the feeds were formed into small pieces of 1.5 cm diameter to facilitate sucking by the snails. Uneaten diets in each tank were removed immediately to prevent contamination of seawater. Spotted Babylon broodstock were initially fed fresh meat of the carangid fish, *Seleroides leptolepis,* and gradually switched to the experimental diets by the second week of culture. The broodstock were fed the experimental diet once daily at 10:00 hours 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. Mortalities were recorded daily. 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. areolata*, broodstock used in this study were already used in the commercial private hatchery for 4 - 6 months. They were graded to the same size with an average individual wet weight of 46.5 - 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. Three hundred broodstock were randomly distributed with a female: male ratio of 10:10 into 15 units. Each plastic tank was 0.5 × 0.5 × 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 day 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 - 32.0°C, 29 - 30 ppt, 4.5 - 7.0, 0 - 0.17 and 0 - 0.04 mgl^{-1}, respectively. The rearing tanks were kept under a natural photoperiod. The Spotted Babylon broodstock were checked for spawning each day in the early morning.

Reproductive performance

Reproductive performance was expressed in terms of total number of spawning, monthly spawning frequency, number of eggs/embryos per capsules, total egg capsule production, total egg/ embryo production, sizes of egg capsules, incubation time and hatching rate. Egg capsules produced naturally by female broodstock given each experimental diet were collected every day during the experimental period of 3 months. For each spawn, egg capsules were collected from each tank by gently scooping them with a net or by hand collection. The number of spawning animals and number of egg capsules spawned were recorded for each feeding trial, thereafter, the total number of egg capsules produced and monthly spawning frequency (average spawning number per month) were estimated at 30 day intervals. The total mean egg production was estimated from total egg capsule production throughout the experimental period multiplied by the average number of eggs / embryos per capsule.

Egg quality

Egg quality was expressed in terms of length and width of egg capsules, number of fertilized eggs per egg capsule, diameter of fertilized eggs and hatching rate. For each spawn, thirty egg capsules were sampled from each tank and measured (length and width) and the number of fertilized eggs / embryos within each egg capsule were counted. Thereafter, the diameter of 20 fertilized eggs in 30 egg capsules from each spawn was measured under an inverted microscope at ×400 magnification and averaged for each batch. To determine the hatching rate, egg capsules of each batch were placed in separate hatching jars of 1 L capacity. All jars were set up with low water flow and low aeration. The water was turned off ca. 2 h before hatching began. The hatching duration of each batch was recorded. The hatching rate of eggs (expressed as percentage) was determined by counting the number of unhatched eggs in three 1 mL samples, calculating the total of unhatched eggs, and subtracting these from the total number of successfully fertilized eggs.

Larval quality

Larval quality was expressed in terms of the initial shell length of newly hatched larvae, starvation tolerance test and final shell length at the end of starvation tests. The quality of larvae was determined by observing their phototaxic response. After switching off aeration, weak and dead larvae concentrated at the bottom of the tank were siphoned out and triplicate samples were counted. The newly-hatched larvae from each spawn were sampled (n = 50) and the initial shell length (SL) was measured microscopically. Starvation tolerance tests were conducted with larvae to check the quality of larvae in the stress condition of no food supply. From each batch, three replicate groups of 100 larvae were placed in 1 L plastic beakers in order to detect the time of 100% mortality under starvation conditions and standardized larvae culture methods at 30 ± 1°C and 29 ± 1 ppt (Chaitanawisuti and Kritsanapuntu, 1997). The starvation period was recorded at 100% mortality and final shell length of larvae were measured (n = 30).

Biochemical composition of the egg capsules

At the end of the experiment, 200 egg capsules from each replicate tank (n = 3) were pooled, and stored frozen at -20°C for subsequent biochemical analysis. All samples were analyzed at the Laboratory Center for Food and Agricultural Product (LCFA), Bangkok, Thailand. Egg capsules from each dietary treatment were analyzed for proximate analysis (crude protein, total fat, carbohydrate, ash and moisture) according to standard methods (AOAC, 1990). Fatty acid determination in experimental diets and egg capsules was performed by gas- liquid chromatography (GLC) based on AOAC (1990). Briefly, the total lipid was first extracted from samples of each diet. An aliquot of the liquid extract 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 using gas-liquid chromatography (Automatic System XL, Perkin Elmer) equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm fused silica capillary column (Omegawax 250, Supelco, Bellefonte, PA, USA).

Table 2. Fatty acid composition (mg/100 g wet weight) of experimental diets for *B. areolata* broodstock.

Composition (±SD)	Control[1]	5% TO[1]	15% TO[1]	5% MO[1]	15% MO[1]
C12:0	-	-	7.8±0.8	-	7.1 ± 0.3
C14:0	67.2± 0.4[a]	198.5 ± 0.1[b]	548.2 ± 0.1[c]	146.7 ± 1.0[d]	349.5 ± 0.9[e]
C15:0	14.0± 0.0[a]	63.6± 0.1[b]	192.8 ± 0.3[c]	56.2± 0.5[d]	113.3 ± 0.3[e]
C16:0	561.6 ± 0.1[a]	1,581.3 ± 0.4[b]	3,898.0 ± 0.1[c]	1,474.6 ± 0.3[d]	3,292.2 ± 0.6[e]
C17:0	42.7± 0.6[a]	155.9 ± 0.1[b]	630.3 ± 0.2[c]	138.0 ± 0.2[d]	257.4 ± 0.9[e]
C18:0	289.5 ± 0.5[a]	525.2 ± 0.2[b]	1,267.0 ± 0.1[c]	544.3 ± 0.8[d]	1,077.5 ± 0.1[e]
C20:0	23.9 ± 0.04[a]	26.2± 0.3[b]	81.2± 0.1[c]	30.1± 0.1[d]	74.5± 0.5[e]
C21:0	-	10.4± 0.2[a]	37.0± 0.2[b]	7.2 ± 0.3[c]	19.4± 0.6[d]
C22:0	20.9± 0.5[a]	21.7± 0.1[b]	59.6± 0.6[c]	34.9± 0.1[d]	81.2± 0.3[e]
C23:0	-	12.1± 0.1[a]	29.3± 0.0[b]	11.0 ± 0.08[a]	20.6 ± 0.2[c]
C24:0	14.9± 0.1[a]	30.7± 0.4[b]	54.1± 0.7[c]	30.2 ± 0.09[b]	61.4± 0.0[d]
C16:1n7	75.6± 0.5[a]	269.7 ± 0.3[b]	746.6± 0.10[c]	200.8 ± 1.0[d]	454.3 ± 0.3[e]
C18:1n9t	17.3± 1.4[a]	51.2± 0.2[b]	473.1 ± 0.1[c]	29.6± 0.5[d]	111.9 ± 0.7[e]
C18:1n9c	50.2± 1.3[a]	814.9 ± 0.8[b]	1,969.0 ± 0.4[c]	897.6 ± 0.3[d]	2,429.5 ± 0.1[e]
C20:1n11	-	22.2± 0.2[a]	101.8 ± 0.6[b]	13.8 ± 0.1[c]	44.4± 0.7[d]
C22:1n9	-	39.3± 0.5[a]	166.1 ± 0.1[b]	22.9 ± 1.3[c]	91.8± 0.8[d]
C24:1n9	8.5 ± 0.0[a]	40.3± 0.1[b]	106.6± 0.08[c]	31.2± 0.7[d]	56.3± 0.3[e]
C18:2n6	10.0± 0.2[a]	403.3 ± 0.2[b]	477.0 ± 0.7[c]	1,036.3 ± 0.3[d]	1,796.7 ± 0.1[e]
C18:3n3	-	120.3 ± 0.3[a]	224.1 ± 0.3[b]	139.1 ± 0.09[c]	253.2 ± 0.3[d]
C20:2	-	20.6± 0.5[a]	71.5± 0.1[b]	19.5± 0.2[a]	43.8 ± 0.8[c]
C20:3n6	-	11.1 ± 0.2	-	-	-
C20:4n6 (ARA)[1]	13.3± 0.4[a]	71.1± 0.1[b]	56.2± 0.3[c]	61.8± 0.3[d]	49.4± 0.6[e]
C20:5n3 (EPA)[1]	6.3 ± 0.3[a]	99.1± 0.1[b]	34.9± 0.7[c]	59.2± 0.1[d]	38.1± 0.2[e]
C22:6n3 (DHA)[1]	10.9± 0.7[a]	376.4 ± 1.3[b]	164.8 ± 0.6[c]	217.7 ± 0.3[d]	108.0 ± 0.8[e]
SFA[1]	1,034.9 ± 0.2[a]	2,625.7 ± 0.2[b]	6,805.3 ± 0.5[c]	2,473.2 ± 0.09[d]	5,354.1 ± 0.3[e]
MUFA[1]	151.6 ± 0.1[a]	1,237.6 ± 0.03[b]	3,585.4 ± 0.3[c]	1,195.9 ± 0.3[d]	3,188.2 ± 0.5[e]
PUFA[1]	40.5± 0.4[a]	1,101.9 ± 0.2[b]	1,028.5 ± 0.4[c]	1,533.6 ± 0.7[d]	2,291.2 ± 0.1[e]
Total unsaturated fatty acid	192.1 ± 0.3[a]	2,339.4 ± 0.1[b]	4,593.9 ± 0.9[c]	2,729.5 ± 0.4[d]	5,479.4 ± 0.3[e]
n - 3 PUFA	17.2 ± 0.04[a]	595.7 ± 0.0[b]	423.8 ± 0.2[c]	416.0 ± 0.4[d]	146.1 ± 0.8[e]
n - 3 HUFA[1]	17.2± 0.1[a]	475.5 ± 0.3[b]	199.7 ± 0.6[c]	276.9 ± 0.3[d]	399.3 ± 0.2[e]
n - 6 PUFA	23.3± 0.3[a]	485.5 ± 0.3[b]	533.2 ± 0.1[c]	1,098.1 ± 0.5[d]	1,846.1 ± 0.1[e]
n - 9 PUFA	76.0± 0.6[a]	945.7 ± 0.1[b]	2,714.8 ± 0.2[c]	981.3 ± 0.6[d]	2,689.5 ± 0.8[e]

[1]Diet abbreviations are as follows: Control = fresh meat of carangid fish (*Seleroides leptolepis*); TO = tuna oil; MO = mixture of tuna oil and soybean oil (6:4), SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, ARA = arachinodic acid. Values are means ±SD (n = 3) from three replicate tanks per diet. Means in the same row with different superscript letters are significantly different (P < 0.05).

Helium was used as the carrier gas and temperature programming was from 50 - 220°C at 4C/min, and then held at 220°C for 35 min. The injector and detector temperatures were 250 and 260°C respectively. Individual FAME was identified by comparing their retention times with those of authentic standards (Sigma Chemical Company, St. Louis, Missouri, USA).

Statistical analyses

Data are 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

Biochemical composition of experimental diets

The proximate composition and fatty acid composition of the experimental formulated diets are presented in Table 2. The levels of protein content did not differ significantly among the different experimental diets and ranged 35.93-37.71%, but the lipid content of diets of 15%TO (26.54%) and 15%MO (25.37%) were significantly higher than those of 5%TO (18.64%) and 5%MO (16.76%). Table 3 shows the fatty acid composition of the diets. All formulated diets contained higher total unsaturated fatty acids for

Table 3. Reproductive performance and egg and larval quality of *B. areolata* broodstock fed different experimental diets for 120 days.

Parameters	5% TO	15% TO	5% MO	15% MO	Control
Broodstock performance					
Initial body weight of females (g/snail)	46.51 ± 6.34	46.69 ± 5.87	45.53 ± 3.21	47.50 ± 8.43	50.39 ± 9.50
Final body weight of females (g/snail)	47.35 ± 4.82	47.41 ± 4.67	46.57 ± 6.16	48.40 ± 5.93	51.52 ± 10.13
Average weight gain (g/snail)	0.89 ± 0.06^{a}	0.92 ± 0.14^{a}	0.99 ± 0.08^{a}	0.96 ± 0.85^{a}	1.03 ± 0.13^{a}
Mean survival of females (%)	95.0 ± 7.07^{a}	85.0 ± 7.07^{a}	85.0 ± 7.07^{a}	80.0 ± 14.14^{a}	90.0 ± 14.14^{a}
Reproductive performance					
Total number of spawning (times)	20.6 ± 5.69^{a}	8.9 ± 4.59^{b}	11.7 ± 5.03^{c}	6.6 ± 5.03^{d}	17.7 ± 2.52^{e}
Mean monthly frequency of spawning	5.2 ± 2.04^{a}	2.7 ± 1.58^{b}	4.4 ± 2.44^{c}	2.9 ± 2.34^{b}	4.4 ± 1.72^{c}
Average total egg capsule production	1,985^{a}	861^{b}	1,021^{c}	604^{d}	2,128^{e}
Average total egg production	815,739^{a}	334,469^{b}	383,064^{c}	212930^{d}	880,993^{e}
Egg quality					
Mean fertilised eggs per capsule (eggs)	415 ± 52.19^{a}	387 ± 36.11^{b}	375 ± 34.33^{c}	390 ± 23.40^{d}	403 ± 69.03^{e}
Length of egg capsules (cm)	1.80 ± 0.07^{a}	1.73 ± 0.09^{a}	1.75 ± 0.11^{a}	1.71 ± 0.21^{a}	1.76 ± 0.11^{a}
Width of egg capsules (cm)	0.80 ± 0.04^{a}	0.78 ± 0.05^{a}	0.77 ± 0.08^{a}	0.79 ± 0.02^{a}	0.83 ± 0.06^{a}
Egg incubation time (day)	5.6 ± 0.82^{a}	5.2 ± 0.44^{a}	5.4 ± 0.52^{a}	5.3 ± 0.52^{a}	5.4 ± 0.14^{a}
Egg hatching rate (%)	100	100	100	100	100
Larval quality					
Initial shell length of larvae (µm)	422.00 ± 3.61^{a}	424.66 ± 12.14^{a}	427.58 ± 16.49^{a}	425.16 ± 4.11^{a}	433.00 ± 6.55^{a}
Survival duration at starvation test (day)	4.4 ± 0.59^{a}	4.5 ± 0.87^{a}	4.3 ± 0.58^{a}	4.5 ± 0.55^{a}	4.6 ± 0.58^{a}
Final shell length of larvae (µm)	431.66 ± 7.34^{a}	433.22 ± 9.20^{a}	435.25 ± 11.33^{a}	434.16 ± 7.44^{a}	442.41 ± 9.15^{a}

Results are means ±S.D. (n = 3). Control = fresh meat of carangid fish (*Seleroides leptolepis*); TO = Tuna oil; MO = Tuna oil and soybean oil (6: 4). Values in the same row with the same superscript are not statistically different. No statistical analysis was performed for hatching rate.

both monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) than the trash fish. The highest C20:5n -3 (EPA), C22:n6 - 3 (DHA), C20:4n - 6 (ARA), total n - 3 PUFA and n - 3 HUFA were found in the diet of 5% TO. The highest total saturated fatty acids (SFA) were found in the diet of 15% TO, followed by the diets 15% MO, 5% TO, 5% MO and trash fish, respectively.

Broodstock performance

This study was the first attempt to condition *B. areolata* broodstock using formulated diets under hatchery conditions over a period of 120 days. All broodstock groups accepted and readily consumed the experimental formulated diets. They showed no signs of stress as exhibited by active movement and feeding and protrusion of the siphon tube throughout the experiment. At the end of the experiment, the mean survival of female broodstock ranged from 80.0 - 95.0% for all dietary treatments. The weight gain of female broodstock increased slightly (0.89 - 1.04 g/snail) and no significant difference was found among dietary treatments (Table 3).

Reproductive performance

The reproductive performance of the Spotted Babylon *B. areolata* broodstock fed different experimental diets is presented in Table 3. The parameters studied to determine spawning quality (total number of spawning, monthly spawning frequency, total egg capsule production and number of fertilized eggs/embryos per capsule) differ significantly among the broodstocks fed either the experimental formulated diets or the natural food. The mean total number of spawning throughout the experiment differs significantly among dietary treatments ($P < 0.05$). Females fed 5%TO diet had the highest total number of spawning (20.6), followed by the control diet (17.6), 5% MO (11.7), 15% TO (8.9) and 15% MO (6.6), respectively. The monthly frequency of spawning for females fed diet 5% TO (5.2 times) was significantly higher ($P < 0.05$) than those of females fed control diet (4.4 times), 5% MO (4.4 times), 15% TO (2.7 times) and 15% MO (2.9% times).

Egg quality

The egg quality of the Spotted Babylon *B. areolata* brood-

stock fed different experimental diets is presented in Table 3. The total egg capsules production obtained from females fed the 5% TO diet (1,985 capsules) and control diet (2,128 capsules, respectively) was significantly higher ($P < 0.05$) than those from the females fed the diets of 5% MO (1,021 capsules), 15% TO (861 capsules) and 15% MO (604 capsules). No significant differences in mean number of the fertilised eggs in egg capsules were observed, ranging 375.0 - 415.0 eggs per capsule ($P > 0.05$). Total egg production obtained from females fed 5% TO diet (815,739 eggs) and control diet (880,993 eggs) were significantly higher ($P < 0.05$) than those from females fed diets with 5% MO diet (383,064 eggs), 15% TO (334,469 eggs) and 15% MO (212,930 eggs) (Table 3). The mean total length and width of egg capsules produced from females fed all experimental diets ranged from 1.71 - 1.80 cm and 0.77 - 0.83 cm, respectively, without significant differences among diets ($P > 0.05$).

Larval quality

The larval quality of the Spotted Babylon *B. areolata* broodstock fed different experimental diets is presented in Table 3. Eggs needed a minimum of 5 days incubation to start hatching for all dietary treatments, and no remarkable differences were observed in the egg hatching rate (approximately 100%) for all feeding treatments. Likewise, larval quality was not affected by the dietary treatments. Table 3 shows that the initial shell length of the newly-hatched larvae at day 1 ranged 422.0 - 433.0 µm and no significant differences in the initial shell length of the newly-hatched larvae were observed among females fed any of the experimental diets ($P > 0.05$). The survival duration at starvation test of larvae produced from the broodstock fed all experimental diets and trash fish were not significantly different, ranging 4.3 - 4.6 days ($P > 0.05$). The mean final shell length of larvae at the end of incubation period was not significantly different ($P > 0.05$) in any of feeding treatments; it ranged from 431.6 - 442.4 µm.

Chemical composition and fatty acid compositions of the egg capsules

Table 4 shows the biochemical composition and fatty acid profile of egg capsules produced from *B. areoata* broodstock originating from snails exposed to different dietary treatments over 120 days. At the end of the experiment, a total of 13 fatty acids were identified in the egg capsules, with a considerable amount of variation in fatty acid profile within feeding treatments.

Protein content in egg capsules produced from the females fed the diet of 15% MO (2.34 / 100 g) had significantly higher ($P < 0.05$) protein content compared to those from the females fed the 5% MO (2.18 / 100 g), 15% TO (2.05 /100 g), control diet (0.35 /100 g) and 5% TO (1.90 /100 g) . The lipid content of egg capsules produced from the females fed the diet of 15% TO (0.42 / 100 g) had significantly higher ($P < 0.05$) lipid content than other diet groups, ranging from 0.30 - 0.35 /100 g).

The total saturated fatty acids (SFA) in egg capsules produced from the females fed diet 5% TO (226.6 mg/100 g) was significantly higher ($P < 0.05$) than those fed the 15% TO diet (162.9 mg/100 g), control diet (159.8 mg/100 g), 15% MO (131.9 mg/100 g) and 5% MO (117.2 mg/100 g). The total unsaturated fatty acids (mono-unsaturated fatty acid, MUFA and polyunsaturated fatty acid, MUFA) in egg capsules produced from the females fed diet 5% TO (237.7 mg/100 g) was significantly higher ($P < 0.05$) than those from the females fed the 15% TO (213.6 mg/100 g), 15% MO (171.4 mg/100 g), control diet (149.7 mg/100 g), and 5% MO (145.3 mg/100 g).

Levels of C20:5n3 (EPA) in egg capsules produced from the females fed the 5%TO diet (48.6 mg/100 g) were significantly higher than those of egg capsules produced from the 15% TO (38.2 mg/100 g), control diet (27.0 mg/100 g), 15% MO diet (19.7 mg/100 g) and 5% TO diet (16.5 mg/100 g), but the levels of C22:6n3 (DHA)) in egg capsules produced from 15% TO diet (68.2 mg/100 g) were significantly higher than those of egg capsules produced from the 5% TO (54.3 mg/100 g), control diet (49.6 mg/100 g), 15% MO diet (30.0 mg/100 g) and 5% MO diet (25.9 mg/100 g). Similarly, levels of C20:4n2 (ARA) in egg capsules produced from the females fed the 5% TO (50.9 mg/100 g) were significantly higher compared to those produced from the females fed 15%TO (39.6 mg/100 g), control diet (38.0 mg/100 g), 15% MO (27.1 mg/100 g) and 5% MO diet (21.8 mg/100 g).

The total n-3PUFA differed significantly between all feeding groups, with the highest total n-3PUFA level in egg capsules from 5 and 15%TO (113.1 and 113.8 mg/100 g, respectively) compared to those from the control diet (76.6 mg/100 g), 15% MO (49.7 mg/100 g) and 5% MO (42.4 mg/100 g). Egg capsules produced from each of the feeding groups differed significantly in the levels of total n - 3 HUFA, with the highest total n - 3 HUFA level obtained from the 15% TO diets (106.4 mg/100 g), followed by those produced from the 5% TO diet (102.9 mg/100 g), control diet (76.6 mg/100 g), 15% MO (49.7 mg/100 g) and 5% MO (42.4 mg/100 g). Similarly, the total n - 6 PUFA in egg capsules produced from the females fed the 5% TO (70.4 mg/100 g) was significantly higher compared to those produced from the females fed 15% MO (68.3 mg/100 g), 5% MO (60.2 mg/100 g), 15% TO (55.2 mg/100 g) and control diet (45.7 mg/100 g).

The ratio of DHA to EPA differed significantly between each broodstock group, with the greatest ratio in egg capsules from the control group, the 15% TO, 5% MO and 15% MO), ranged (1.5:1 - 1.8:1) . The ratio of ARA to EPA also differed significantly, with the greatest ratio in egg capsules from the control group, the 5% MO and

Table 4. Biochemical composition and fatty acid composition (mg fatty acid /100 g wet weight) of egg capsules produced from *B. areolata* broodstock fed different experimental diets (n = 3) for 120 days.

Egg capsule composition (±SD)	Control[1]	5% TO[1]	15% TO[1]	5% MO[1]	15% MO[1]
Crude protein	1.93 ± 0.2[a]	1.9 ± 0.4[a]	2.05 ± 1.1[b]	2.18 ± 0.4[c]	2.34 ± 0.6[d]
Total lipid	0.35 ± 0.5[a]	0.31 ± 0.7[a]	0.42 ± 0.1[b]	0.30 ± 1.0[a]	0.34 ± 0.2[a]
Saturated fatty acids					
C14:0	*	6.1 ± 0.5	*	*	*
C16:0	87.7 ± 0.2[a]	129.1 ± 0.1[b]	95.6 ± 0.3[c]	70.1 ± 0.3[d]	77.9 ± 0.1[e]
C17:0	10.9 ± 0.3[a]	17.2 ± 0.3[b]	12.6 ± 0.1[c]	8.7 ± 0.1[d]	9.7 ± 0.4[e]
C18:0	51.8 ± 0.1[a]	74.2 ± 0.5[b]	54.7 ± 0.1[c]	38.4 ± 0.2[d]	44.3 ± 0[e]
C24:0	9.4 ± 0.1	*	*	*	*
Monounsaturated fatty acids					
C18:1n9c	19.4 ± 0.08[a]	33.9 ± 1.2[b]	30.0 ± 0.6[c]	30.6 ± 1.3[c]	34.2 ± 1.1[b]
C20:1n11	8.1 ± 0.3[a]	14.1 ± 0.0[b]	14.6 ± 0.9[b]	12.1 ± 0.1[c]	13.2 ± 0.4[d]
Polyunsaturated fatty acids					
C18:2n6	7.7 ± 0.3[a]	19.5 ± 0.02[b]	15.6 ± 0.6[c]	38.4 ± 0.2[d]	41.2 ± 0.6[e]
C18:3n3	*	10.2 ± 0.7[a]	7.4 ± 0.3[b]	*	*
C20:2	*	6.2 ± 0.8[a]	*	*	6.0 ± 0.2[a]
C20:4n6 (ARA)[1]	38.0 ± 0.2[a]	50.9 ± 0.1[b]	39.6 ± 0.4[c]	21.8 ± 0.3[d]	27.1 ± 0.2[e]
C20:5n3 (EPA)[1]	27.0 ± 0.2[a]	48.6 ± 0.02[b]	38.2 ± 1.6[c]	16.5 ± 0.3[d]	19.7 ± 0.06[e]
C22:6n3 (DHA)[1]	49.6 ± 0.3[a]	54.3 ± 0.3[b]	68.2 ± 0.5[c]	25.9 ± 1.2[d]	30.0 ± 0.2[e]
SFA[1]	159.8 ± 0.1[a]	226.6 ± 0.3[b]	162.9 ± 0.2[c]	117.2 ± 0.1[d]	131.9 ± 0.1[e]
MUFA[1]	27.40 ± 0.3[a]	48.0 ± 0.4[b]	44.6 ± 0.9[c]	42.7 ± 0.8[d]	47.4 ± 0.2[b]
PUFA[1]	122.3 ± 0.1[a]	189.7 ± 0.8[b]	169.0 ± 0.04[c]	102.6 ± 0.2[d]	124.0 ± 0.7[e]
Total unsaturated fatty acid	149.7 ± 0.6[a]	237.7 ± 0.7[b]	213.6 ± 0.2[c]	145.3 ± 0.8[d]	171.4 ± 0.3[e]
Saturated / Unsaturated fatty acid	1.07 ± 0.3[a]	0.95 ± 0.1[b]	0.76 ± 0.5[c]	0.81 ± 0.0[d]	0.77 ± 0.06[e]
MUFA / PUFA	0.2 ± 0.5[a]	0.25 ± 0.2[b]	0.3 ± 0.5[c]	0.4 ± 0.8[d]	0.4 ± 0.2[d]
n - 3 PUFA	76.6 ± 0.2[a]	113.1 ± 0.3[b]	113.8 ± 0.1[c]	42.4 ± 0.5[d]	49.7 ± 0.2[e]
n - 3 HUFA[1]	76.6 ± 0.1[a]	102.9 ± 0.4[b]	106.4 ± 0.7[c]	42.4 ± 0.3[d]	49.7 ± 0.4[e]
n - 6 PUFA	45.7 ± 0.3[a]	70.4 ± 0.8[b]	55.2 ± 0.2[c]	60.2 ± 0.2[d]	68.3 ± 0.7[a]
n - 9 PUFA	19.4 ± 0.08[a]	33.9 ± 1.2[b]	30.0 ± 0.6[c]	30.6 ± 2.3[c]	34.2 ± 0.1[b]
(n - 3) / (n – 6) PUFA ratio	1.7 ± 0.4[a]	1.6 ± 1.0[a]	2.1 ± 0.4[b]	0.7 ± 0.2[c]	0.7 ± 0.7[c]
DHA / EPA ratio	1.8 ± 0.6[a]	1.1 ± 0.03[b]	1.8 ± 0.2[a]	1.6 ± 0.7[a]	1.5 ± 0.2[c]
ARA / EPA ratio	1.4 ± 0.0[a]	1.0 ± 0.8[b]	1.0 ± 0.4[b]	1.3 ± 0.09[a]	1.4 ± 0.4[a]

[1] Diet abbreviations are as follows: Control = fresh meat of carangid fish (*Seleroides leptolepis*); TO = tuna oil; MO = mixture of tuna oil and soybean oil (6:4), SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = decosahexaenoic acid, ARA = arachinodic acid. Values are means ±SD (n = 3) from three replicate tanks per diet. Means in the same row with different superscript letters are significantly different (P < 0.05).

15% MO), ranged (1.3:1 - 1.4:1) . The (n - 3) /(n - 6) PUFA ratio also differed significantly, with egg capsules from the 15%TO group (2.1:1) having a higher ratio than egg capsules from the control group (1.7:1), 5% TO (1.6:1), 5% MO (0.7:1) and 15% MO (0.7:1).

DISCUSSION

The present study indicates that there were statistically significant differences in reproductive performance between the dietary treatments. The reproductive

performance in terms of total number of spawning, the monthly frequency of spawning and total egg capsule production obtained from the females fed 5%TO was significantly higher compared to those fed natural diet and the other experimental diets. However, egg quality (sizes of egg capsules, egg incubation time and egg hatching rate) and larval quality (initial shell length of newly-hatched larvae, survival duration at starvation tolerance test and final shell length of larvae) showed no variability among females fed the control diet or any of the experimental diets. The results of our study suggest that the higher reproductive performance of Spotted Babylon *B. areolata* fed the formulated diets would indicate that the nutritional quality of the broodstock diet influences reproduction. Although it is possible to mature and spawn Spotted Babylon with the natural diet, the provision of an effective formulated diet fed alone can achieve better reproductive performance of the Spotted Babylon broodstock. This observation could be used to enhance the production of quality seeds for Spotted Babylon hatcheries. The dietary nutrients, especially in terms of proteins, lipids, and fatty acids, e.g. 20:4n6 (ARA); 20:5n3 (EPA), 22:6n3 (DHA), which are essential in reproduction, and that may be insufficient in the natural diets, may have been compensated by feeding the formulated diets as a supplement or as total food for the Spotted Babylon. This result agrees with the study of Djunaidah et al. (2003) where artificial diets resulted in a reproductional success of mud crab (*Scylla paramamosain*) comparable to the use of fresh food and the nutritional composition of the artificial diets could be improved in order to produce larvae of optimal quality. In addition, 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, e.g. 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 may have influenced the increased reproductive performance. Further, this result also agrees with the study of Utting and Millican (1997) which showed that, in good environmental conditions, endogenous egg reserves, in particular PUFAs are important for survival through embryogenesis but not for subsequent larval growth and survival of marine bivalves. Utting and Millican (1998) 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 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 polyunsaturated fatty acids (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 comparison to this study, the formulated diets contain higher levels of 20:5n - 3, 22:6n - 3 and 20:4n - 6 than those fed trash fish and the essential fatty acids , particular 20:5n - 3, 22:6n - 3 and 20:4n - 6, in egg capsules obtained from *B. areolata* broodstock fed formulated diets were higher in those fed trash fish. This clearly demonstrates the dietary origin of these long - chain PUFAs. 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 decreases during the first 5 days of embryonic development and all fatty acids, 20:5n
- 3 is preferentially utilised during embryogenesis. By contrast, there is 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). Moreover, 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. Emata et al. (2003) reported that, for the mangrove red snapper, arachinodic acid (ARA) may be nutritionally more important for egg and larvae development and survival and its supplementation in broodstock diets may enhance reproductive performance. Lavens et al. (1999) stated that the nutritional status of the turbot *Scophthalamus maximus* broodstock can affect offspring quality. 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, Chaparro and Thompson, 1986). 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 polyunsaturated fatty acid (PUFA) in the digestive gland and foot tissues over the year coincided with variation in their macroalgal diets. Furthermore, arachidonic acid (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.

This study presented that broodstock diets containing different amounts of dietary lipid sources and levels influence reproductive performances and egg fatty acid composition in Spotted Babylon *B. areolata* but not for egg and larval quality. The data presented here indicate that other factors must be participating thus hampering conclusive detection of the nutritional components determining egg and larval quality in spotted babylon. Furthermore, variability in maturation, egg laying, and larval and juvenile survival rates among batches may depend on many factors such as food, environmental factors and stresses, uncontrolled genetic. Moreover, variation in nutritive contents of the larvae between broods may arise during gametogenesis and influence the variation in 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. Our preliminary results provide initial evidence that the biochemical compositions of broodstock and egg are influenced by the broodstock diets and these in turn may affect the spawning quality. 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.

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