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Supplementation of Dietary Fungal Oil Containing Arachidonic Acid Improves Fatty Acid Composition and Promotes Ovarian Development in Giant Freshwater Prawn, *Macrobrachium rosenbergii* (De Man, 1879)

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Abstract

Arachidonic acid (ARA) has been used as a feed additive to improve ovarian development in crustaceans. Therefore, in this study, ARA was supplemented at various levels to observe the induced development of ovaries in the broodstock of *Macrobrachium rosenbergii*. Four experimental maturation diets were formulated with different inclusion levels of ARA (0, 1, 2, and 2.9 %), sourced from *Mortierella* sp., a fungal oil which contains 45% ARA. The graded inclusion level of fungal oil resulted in 0.03, 0.35, 0.70, and 1.17% ARA in the diets. The results showed that broodstock fed with 0.70 % ARA diet, exhibited the highest hepatopancreas somatic index (HSI) value compared to broodstock fed with other diets. The histological evaluation of the ovaries revealed that broodstock fed a 0.70 % ARA diet resulted in significantly bigger oocytes ($P < 0.05$) among all diets. Thus, higher dietary ARA resulted in higher ARA content in the tail muscle, hepatopancreas, and ovary of prawn broodstock. Therefore, a dietary level of 0.70 % ARA promoted the reproductive performance and development of ovary in prawns broodstock.

Keywords: Arachidonic acid, gonadosomatic index, *Macrobrachium rosenbergii*, maturation diet, reproductive performance

Introduction

The Malaysian giant freshwater prawn, also known as the giant freshwater prawn (GFP), *Macrobrachium rosenbergii* (de Man, 1879), is a widely cultured species in Asia and other regions. GFP is a native species in Malaysia; exhibits a notable growth rate and a high-profit margin, contributing to its popularity in the aquaculture industry. However, a critical bottleneck in its development lies in the insufficient supply of high-quality post larvae (PL). Moreover, the availability of wild broodstock is declining over time. The inadequacy of high-quality broodstock supply remains a significant challenge for GFP production in Asia, particularly in Malaysia. The majority of GFP PL producers rely mainly on wild broodstock for PL production in hatchery, which are then grown in pond culture.

Domesticated broodstock is an alternative to utilizing wild broodstock. However, according to discussion with local hatchery managers in Malaysia, domesticated broodstock is often regarded as being inferior quality. Thus, the quality of domesticated broodstock needs to be improved genetically and with other important contributing factors, such as using high-quality maturation feed. Maturation feed should consist of all the essential nutrients for improving the quality of broodstock, especially for better gonad development and larval quality.

In crustaceans, the reproductive performance, such as ovarian maturation is usually under the control of prostaglandins (PGs) (Sarojini et al., 1989) or eicosanoids with hormone-like functions (Mustafa and Srivastava, 1989; Sargent, 1995; Sargent et al., 1999; Stacey & Goetz, 1982; Tocher, 2003). PGs have been reported to play a role in stimulating ovarian maturation, including vitellogenesis and spawning, in many decapod crustaceans (Tahara & Yano, 2004; Rowley et al., 2005; Sumpownon et al., 2015). Arachidonic acid (ARA, C20:4n-6) is the major prostanoid precursor. Prostanoids are a class of hormonally active fatty acids composed primarily of oxygenated C18, C20, and C22 acids, known as eicosanoids, which are derived from n-3 and n-6 polyunsaturated fatty acids ($C \geq 20$; LC-PUFA) such as arachidonic acid (ARA), docosahexaenoic acid (DHA; C22:6n-3), eicosapentaenoic acid (EPA; C20:5n-3), dihomo-gamma-linolenic acids (DGLA, 20:3n-6), and gamma-linolenic acid (GLA, 18:3n-6) through the cyclooxygenase pathways (Smith et al., 2000).

ARA is known to act as a catalyst for the development of reproductive gonads (Wouters et al., 2001; Glencross, 2009; Coman et al., 2011; Xu, Cao, et al., 2017; Xu, Zhang, et al., 2017). Coman et al. (2011) reported that ARA supplementation of a semi-moist pelleted diet increases the cumulative number of females spawning and the number of eggs produced by *Penaeus monodon* broodstock. In addition, supplementation of ARA in the diets affected the fatty acid compositions in the hepatopancreas, ovaries, and fertilised eggs of female Pacific white shrimp (*Litopenaeus vannamei*) broodstock, which enhanced the final reproductive performances (Xu,

Zhang, et al., 2017).

The objectives of the present study were to determine the effect of different inclusion levels of ARA in the formulated diet on the gonad development of female GFP broodstock, *M. rosenbergii*, and the impact of dietary ARA on the fatty acid composition of broodstock tissues.

Materials and Methods

Experimental diets and chemical analysis

Four isonitrogenous and isoenergetic experimental diets were formulated with 45 % crude protein and 9 % crude lipid following the nutrient level by Cavalli et al. (1999, 2000), with an energy level of 400 kcal per 100 g diet (Nik Sin et al., 2016). Fish, shrimp, and soybean meals were used as protein sources. The inclusion of fungal emulsion ARA oil from *Mortierella* sp. was added at 0, 1, 2, or 2.9 % across the four diets. The diets were thoroughly mixed using an industrial food mixer and distilled water was added until a stiff dough was formed. This dough was screw-pressed through a 3 mm die using a locally assembled Pellet Mill and the feed pellets formed were fan-dried and stored frozen at 0 °C until use.

Four experimental diets with different ARA percentage levels of 0.03, 0.35, 0.70, and 1.17 were prepared and named as T1, T2, T3, and T4, respectively. The formulation and proximate composition of the experimental diets are presented in Table 1. The moisture, crude protein, crude lipid, and ash contents of the ingredients and experimental diets were determined using the standard method (AOAC, 1990). Meanwhile, the lipid content of the experimental diet and prawn samples were extracted using a modified Folch et al. (1957) protocol and used for fatty acid (FA) analysis. The fatty acid analysis methodology followed the same method as explained by Chee et al. (2020). Briefly, the lipid of the weighed samples was extracted with chloroform: methanol at a ratio of 2: 1 (v / v) containing potassium chloride in a separatory funnel. For separation, gravitation was applied to collect the bottom layer containing chloroform and extracted lipids with a round bottom flask. The chloroform was evaporated by using a rotary evaporator at 75 °C. The extracted lipids were then esterified into methyl esters with methanolic boron trifluoride (BF₃), followed by the saponification-esterification method (AOAC, 1990).

The fatty acid composition of the experimental diets is presented in Table 2. Specifically, fatty acid methyl esters (FAME) were resolved and analysed by a gas-liquid chromatograph (Hewlett-Packard, Model 6890) equipped with a flame ionisation detector (GC-FID), GC Autosampler, and SP™-2560 fused silica capillary column (100 m × 0.25 mm ID, 0.2 µm film thickness, Supelco, Bellefonte, PA, USA). Initially, the column temperature was programmed to hold at 140 °C for the first 2 min, then increased to 225 °C at a rate of 2 °C min⁻¹ and held for 5 min, then raised again to 240 °C at a rate of 2 °C min⁻¹ and held for 3 minutes. The carrier gas was nitrogen and

the pressure was 80 kPa. Fatty acid peaks were identified by comparing the retention time with known standards (Supelco 37 Component FAME Mix, PUFA No.2, and PUFA No. 3: Supelco, Bellafonte, PA, USA). The resulting peak areas were corrected by theoretical relative flame ionisation detector response factors. The response factors were calculated by the ratios between the peak area of the individual FAME. The FAMES were expressed as weight percentages of the total FA content (Ackman, 2002; Trbović et al., 2017).

Table 1. Formulation and proximate composition of the experimental diets (g/kg dry weight)

Ingredients (g / kg)	Dietary arachidonic acid level (% of diet)			
	0.03	0.35	0.70	1.17
	ARA1	ARA2	ARA3	ARA4
Shrimp meal ¹	111.4	111.4	111.4	111.4
Fish meal ²	238.7	238.7	238.7	238.7
Soybean meal ³	335.7	335.7	335.7	335.7
Soybean oil	29.1	19.1	9.1	0.0
Fungal oil (45% ARA) ⁴	0.0	10.0	20.0	29.1
Fish oil	19.7	19.7	19.7	19.7
Corn starch	34.8	34.8	34.8	34.8
Soybean lecithin	10.0	10.0	10.0	10.0
Cholesterol ⁵	6.0	6.0	6.0	6.0
Vitamin mix ⁶	30.0	30.0	30.0	30.0
Mineral mix ⁷	40.0	40.0	40.0	40.0
L-ascorbic acid ⁸	7.0	7.0	7.0	7.0
Choline chloride ⁹	10.0	10.0	10.0	10.0
Inositol ¹⁰	0.1	0.1	0.1	0.1
Binder ¹¹	8.0	8.0	8.0	8.0
BHT ¹²	5.0	5.0	5.0	5.0
Astaxanthin ¹³	20.0	20.0	20.0	20.0
α-cellulose	94.6	94.6	94.5	94.6
Proximate composition (mg / g dry basis)				
Moisture	107	121	111	126
Crude protein	449	445	449	443
Crude lipid	101	101	101	100
Ash	131	129	130	127
Crude fibre	26	26	25	25
NFE ¹⁴	293	299	295	305

1 Shrimp meal (g / kg dry weight): crude protein 808.0, crude lipid 35.2, ash 133.4.

2 Fish meal (g / kg dry weight): crude protein 754.0, crude lipid 89.5, ash 147.0.

3 Soybean meal (g / kg dry weight): crude protein 536.2, crude lipid 17.7, ash 69.4, crude fibre 25.6.

4 Fungal oil (% of total fatty acids): oil from fungi (*Mortierella alpina*) with ARA content ≥40%; Cargill, USA

5 Cholesterol BP (Feed grade), NK Ingredients Pte Ltd, Singapore.

6 Vitamin premix (g/kg of dry weight) contains ascorbic acid, 45; choline chloride, 75; inositol, 5; niacin, 4.5; riboflavin, 1; pyridoxine HCL, 1; thiamine mononitrate, 0.92; d-calcium pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; menadione, 1.67; DL-α-tocopheryl acetate (500 IU/g), 8; d-biotin, 0.02; folic acid, 0.09; Vitamin B12, 0.00135; cellulose, 854.11

7 Mineral premix (g/kg of dry weight) contains calcium phosphate monobasic, 397.65; calcium lactate, 327; ferrous sulphate, 25; magnesium sulphate 7H₂O, 137; potassium chloride, 50; sodium chloride, 60; potassium iodide, 0.15; copper sulphate 5H₂O, 0.785; manganese oxide, 0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite 5H₂O, 0.2

8 Rovimix C, DSM

9, 10 Sigma, USA

11 Binder: Pegabind® dry from Bentoli AgriNutrition Co., Ltd., Thailand

12 BHT: Butylated hydroxytoluene is used as an antioxidant

13 Astaxanthin (*Haematococcus* sp)-Algatche Malaysia

14 Nitrogen-free extract = 100 – (protein + lipid + ash + fiber)

Table 2. Fatty acid composition (% of total fatty acid) of the experimental diets

Fatty acid	Dietary ARA level (% of diet)			
	0.03 ARA1	0.35 ARA2	0.70 ARA3	1.17 ARA4
C14:0	3.53	4.04	3.56	4.25
C15:0	0.37	0.40	0.41	ND
C16:0	25.28	22.24	19.39	19.32
C17:0	0.32	0.34	0.35	0.38
C18:0	4.09	4.75	5.52	7.25
C20:0	0.29	0.36	0.44	0.61
C22:0	ND	0.48	0.78	1.2
16:1n-7	3.44	3.53	3.56	4.31
17:1n-7	ND	ND	ND	ND
18:1n-7	2.01	2.00	1.99	2.37
18:1n-9	23.90	20.29	16.98	0.54
20:1n-9	3.10	3.20	3.32	3.89
22:1n-9	0.30	0.31	0.32	4.93
24:1n-9	0.39	0.43	0.47	0.58
18:3n-3	1.68	1.70	1.93	3.89
18:4n-3	ND	ND	ND	ND
20:3n-3	ND	2.77	0.96	0.38
20:4n-3	ND	ND	ND	ND
20:5n-3 (EPA)	4.55	4.68	4.78	5.74
22:5n-3	ND	ND	ND	ND
C22:6n-3 (DHA)	6.96	7.26	7.53	9.05
18:2n-6	13.54	13.21	12.91	14.54
C18:3n-6	0.52	0.53	0.55	1.15
C20:2n-6	ND	ND	ND	ND
C20:3n-6	4.09	2.23	4.29	1.58
C20:4n-6 (ARA)	<u>0.31</u>	<u>3.51</u>	<u>6.96</u>	<u>11.72</u>
22:4n-6	ND	ND	ND	ND
Σ SFA ^a	33.87	32.60	30.45	33.01
Σ MUFA ^b	33.12	29.75	26.64	16.62
Σ n-3 PUFA ^c	13.18	16.40	15.20	19.07
Σ n-6 PUFA ^d	18.44	19.48	24.71	28.98
(n-3)/(n-6)	0.71	0.84	0.62	0.66
ΣPUFA (total)	31.62	35.88	39.91	48.05
Σ n-3 LC-PUFA ^f	11.51	14.70	13.27	15.18
Σ n-6 LC-PUFA ^g	4.39	5.74	11.25	13.29
ARA/EPA	0.07	0.75	1.46	2.04
ARA/DHA	0.04	0.48	0.92	1.29
EPA+DHA	11.51	11.93	12.31	14.80
EPA/DHA	0.65	0.64	0.63	0.63
DHA/EPA	1.53	1.55	1.58	1.58

^a Σ SFA : sum of all saturated fatty acids

^b Σ MUFA : sum of all monounsaturated fatty acids

^c Σ PUFA : sum of all polyunsaturated fatty acids

^d Σ n-3 PUFA : sum of all omega-3 polyunsaturated fatty acids

^e Σ n-6 PUFA : sum of all omega-6 polyunsaturated fatty acids

^f Σ n-3 LC-PUFA : sum of all omega-3 long chain polyunsaturated fatty acids

^g Σ n-6 LC-PUFA : sum of all omega-6 long chain polyunsaturated fatty acids

ND: Not detected

Experimental diets and chemical analysis

Rectangular fiberglass tanks with a holding capacity of 1700 L and a working capacity of 1200 L (2.4 m length × 1.2 m width × 0.6 m height) were used in this experiment with four replicate tanks for each diet treatment. The individual samples were treated as replicate samples for chemical analysis based on their ovarian stages since the development of the broodstock ovarian stages varied within the same treatment due to many factors (Chang & Shih, 1995). Each tank was supplied with ten air stones (50 mm in height and 17 mm in diameter) for aeration.

The berried female GFPs were purchased from local prawn farmers at Jeneri, Sik, Kedah, Malaysia (5°53'29.2"N 100°40'26.2"E). Before bringing them into the hatchery, the prawns were screened for *Macrobrachium rosenbergii* nodavirus (MrNV). The berried female GFP of six months old was kept in a circular fiberglass tank (1.2 m in diameter × 0.5 m depth) until they hatched all the larvae. During this incubation period, a commercial shrimp pellet (2.5 mm in size, 38 % crude protein) was used to feed the broodstock and the management of broodstock was according to Daniels et al. (2010) and New (2002).

Initial samples of spent female broodstock were taken for histology analysis. Prior to the commencement of the feeding trial, the individual weight and standard length of spent female broodstock were measured, recorded, and assigned to the experimental tanks using a completely randomised design. Twenty-one pieces of GFP broodstock in the range of 18 – 35 g were randomly distributed in each experimental tank. For each experimental tank, one adult male prawn was placed in a small cage (40 cm in diameter) to trigger the maturation of the females (Nagabhushanam et al., 1989). The experimental broodstock was fed up to satiation (approximately 3 % of body weight) and two times daily (0900 and 1630 h). Every morning, feces and uneaten feed were siphoned out of the tank. The salinity of the water was in the range of 0 – 6 ppt as suggested by Yen & Bart (2008).

The water quality parameters of the experiment tanks were recorded daily for temperature, dissolved oxygen, and salinity. Ammonia, nitrate, nitrite, pH, and alkalinity were measured weekly. The water quality was monitored throughout the experimental period and remedial actions were taken to ensure the water quality parameters were in the acceptable range. Sodium bicarbonate and commercial mineral premixes were used and added weekly to maintain water alkalinity higher than 60 mgL⁻¹ as suggested by Coyle et al. (2010) and Shofiquzzoha et al. (2016). The dissolved oxygen was measured with a YSI Model 550 polarographic oxygen meter (Yellow Springs Instruments Company, Yellow Springs, OH, USA). Meanwhile, ammonia, nitrite, nitrate, and alkalinity were determined according to the methods of Solorzano (1969), using a Hach Model DR-2000 spectrophotometer (Hatch Company, Ames, IA, USA).

After 50 days of feeding trials using the experimental diets, the experiment was terminated and all prawn broodstock from each tank were sampled for physical, histological, and chemical examination. The physical evaluation was carried out by measuring the individual weight and total length. Then, the treated broodstock were dissected, the hepatopancreas and the gonads were weighed to estimate the hepatopancreas somatic index (HSI) and gonadosomatic index (GSI).

The cephalothorax or head section of prawn broodstock was taken for histological samples as shown in Figure 1. Freshly dissected sample was put into Davidson's solution for further processing. Additionally, other parts of prawn broodstock such as the tail muscle, hepatopancreas, and gonad from each prawn broodstock were individually packed, labeled, and directly frozen at 0 °C for fatty acid analysis. The ovarian stages (OS) were determined based on GSI values (Chang & Shih, 1995). The individual samples of tail muscle, hepatopancreas, and gonad were then grouped according to their OS for each treatment diet.

The histological slide for the treated prawn broodstock samples was classified according to OS and analysed accordingly. The number of hepatopancreas and gonad samples from each diet treatment was limited for a particular OS, since the development of OS varies individually within the same diet treatment. The histological slide samples of hepatopancreas and ovary of OS III were available for all treated broodstock groups (n=3 of treated prawn broodstock samples for each diet). Hence, these samples were analysed and compared accordingly.

Sample collection and reproductive indices

Samples of both spent broodstock (at the beginning of the experiment) and the ARA treated broodstock (at the end of the experiment) were sacrificed and subjected to histological analysis. The standard formulae used to estimate HSI, GSI, and gonad stage index are listed below (Fatima et al., 2013; Kangpanich et al., 2016; Xu, Zhang, et al., 2017).

Hepatopancreas-somatic index (%), HSI= $(\text{Hepatopancreas weight} / \text{body weight}) \times 100$

Gonadosomatic index (%), GSI = $(\text{Gonad weight} / \text{body weight}) \times 100$

Gonad stage index = $(\sum Si \times ni) \times N^{-1}$,

Where,

Si: stage of gonad (i=1-5),

ni: number of samples in stage *Si* and

N is the total number of samples counted

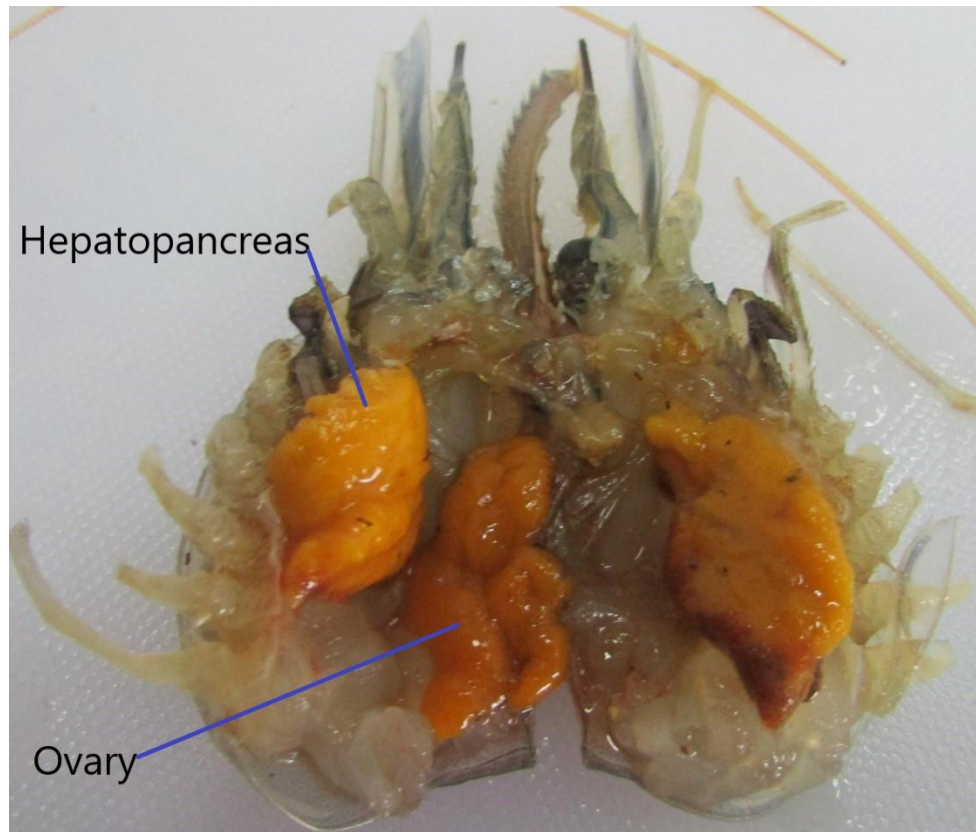


Figure 1. The head part with organs of the broodstock sample were used for histology analysis.

For histology evaluation, half of the prawn broodstock's cephalothorax section (Fig. 1) is used which consists of the hepatopancreas and ovary. The fresh sample was fixed in Davidson's solution for 24 hours before replacing with 75 % ethanol until further processing. The samples were processed (Leica ASP 300) and embedded in paraffin wax. The embedded specimens were sectioned at 5 micrometers (Leica RM2245), stained with haematoxylin and eosin (H&E), and finally mounted with DPX before examined under a microscope (Leica DM5000B) connected to a digital camera (Leica DFC 320) associated with computer software (Leica QWin). The histological techniques were performed according to Humason (1979). Histological analysis of both the ovary and hepatopancreas was performed using standard histological procedures. The cells of the hepatopancreas tubules and oocytes were chosen randomly from the histology slides for diameter measurement of both the ovary and hepatopancreas, respectively.

Statistical analysis

Results are expressed as the mean $\pm$ standard error of the mean (SE). The data was analysed using IBM SPSS Statistics 25 program (SPSS Inc., Chicago, IL, USA). Data were subjected to one-way analysis of variance (ANOVA) and Duncan multiple comparison tests were used to determine significant differences among dietary treatments at $P < 0.05$. Before subjecting the data to ANOVA analysis, the data were checked for normal distribution and variance homogeneity under

descriptive statistics and explored for normality plots to a test using the SPSS program. Whenever these assumptions were violated, non-normally distributed continuous variables were transformed using a two-step approach described by Templeton (2011). Step one involves transforming the variable into a percentile rank, which results in uniformly distributed probabilities. Step two applies the inverse normal transformation to the first step results to form a variable consisting of normally distributed z-scores.

Results

The water quality parameters recorded were in the acceptable range for freshwater prawn culture in tanks (Roustaian et al., 1999; Habashy, 2013). The parameters recorded were in the range of 26.5 – 32.5 °C for temperature, 34.0 – 136.0 mg L⁻¹ for alkalinity, 0.0 – 0.58 mg L⁻¹ of nitrite, 0.50 – 20.30 mg L⁻¹ of nitrate, 0.0 – 0.50 mg L⁻¹ of ammonia, 7.14 – 8.24 pH, and 5.10 – 8.70 mg L⁻¹ for dissolved oxygen.

Experimental diets

The crude protein content of the experimental diets ranged from 443 to 449 mg g⁻¹ dry weight, while the crude lipid ranged from 100 to 101 mg g⁻¹ dry weight (Table 1). The fatty acid profile showed that the primary source of ARA was derived from the fungal oil *Mortierella sp.* with a concentration of 45.78 % of the total fatty acid (TFA). Other ingredients contributed to a small percentage of ARA, such as fishmeal (0.48 %), fish oil (0.21 %), and shrimp meal (1.08 %).

The fatty acid composition of the experimental diets is shown in Table 2. The diets showed gradually increased ARA by 0.31, 3.51, 6.96, and 11.72 % of TFA which were incorporated with 0.03, 0.35, 0.70, and 1.17 levels of ARA, respectively. The fatty acid composition of ARA in the experimental diets reflected the inclusion level of fungal oil used. However, a higher level of ARA was detected for the T4 diet with a 2.9 % inclusion level of fungal oil, which was higher than the calculated value of 1.05 % ARA. A similar pattern of increasing value was observed for stearic acid (C18:0), eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) with increasing dietary ARA levels (Table 2). Fish meal (18.0 % of TFA), shrimp meal (8.5 % of TFA), and fish oil (11.3 % of TFA) were found to contain a large amount of total n-3 PUFA compared to vegetable oils such as soybean meal residual oil (7.3 % of TFA) and soybean oil (0.01 % of TFA). As for vegetable oil, 50.45 % of TFA was contributed by linoleic acid (LA) in the soybean meal.

Evaluation of the treated broodstock

Physical evaluation

The physical evaluations are summarised in Table 3. The final weight of the broodstock showed no significant difference ($P > 0.05$) among the groups, and the value increased compared to the initial weight. No significant differences ($P > 0.05$) were observed for the survival of all treatment, with the value ranging from 60 – 73 % across the treatment. The HSI value of treated broodstock increased as the ARA content in the diets increased from 4.42, 4.69, and 5.25 for treated broodstock fed with T1, T2, and T3 diets, respectively. However, the HSI value decreased to 5.07 for treated broodstock fed with T4 diet. The highest HSI was recorded for the treated broodstock fed with T3 diet, which showed no significant difference compared to treated broodstock fed with other diets ($P > 0.05$). Further analysis of HSI was carried out by plotting the graph as shown in Figure 2. As Figure 2 illustrates, a second-order polynomial regression relationship between HSI value and dietary ARA for the treated GFP female broodstock. By applying the dose-response concept or broken line analysis, the optimal level of ARA in the diet can be extrapolated and estimated as shown in the red circle in Figure 2.

The GSI value showed an increasing pattern from treated broodstock fed with T2 and T3 diets, with a value of 3.20 and 3.52, respectively. However, the GSI value for treated broodstock fed with T4 diet showed a similar value as T3 diet of 3.51. This indicated that the ARA level in treated broodstock fed with T3 and T4 diets resulted in similar GSI value. The total HSI and GSI values of treated broodstock showed an increasing trend for treated broodstock fed with T2 and T3 diets at 7.90 and 8.77, respectively (Table 3). However, an opposite trend was observed with treated broodstock fed with T4 diet (8.58 ± 1.41). Although no significant differences were observed among treated broodstock ($P > 0.05$) as seen in Table 3, the value increased as higher dietary ARA was used. Further analysis of the HSI and GSI combination values, including GSI and HSI values, was carried out as illustrated in Figure 3.

Matured OS production, which is the cumulative value of OS III, IV, and V, were calculated. The values fluctuated between treatments. The maximum value of 68.89 % was recorded in treated broodstock fed with T3 diet, followed by T1, T2, and T4 diets, as shown in Figure 4.

are expressed as the mean $\pm$ standard error of the mean (SE). The data was analysed using IBM SPSS Statistics 25 program (SPSS Inc., Chicago, IL, USA). Data were subjected to one-way analysis of variance (ANOVA) and Duncan multiple comparison tests were used to determine significant differences among dietary treatments at $P < 0.05$. Before subjecting the data to ANOVA analysis, the data were checked for normal distribution and variance homogeneity under descriptive statistics and explored for normality plots to a test using the SPSS program. Whenever these assumptions were violated, non-normally distributed continuous variables were

transformed using a two-step approach described by Templeton (2011). Step one involves transforming the variable into a percentile rank, which results in uniformly distributed probabilities. Step two applies the inverse normal transformation to the first step results to form a variable consisting of normally distributed z-scores.

Table 3. The final weight, gonadosomatic index and hepatosomatic index of the treated broodstock fed with increasing ARA levels diet⁵

	<u>Dietary arachidonic acid level (% of diet)</u>			
	0.03	0.35	0.7	1.17
Reproductive performance	ARA1	ARA2	ARA3	ARA4
Initial weight (g)	24.05 ± 1.16	26.07 ± 1.62	24.31 ± 0.36	24.80 ± 1.09
Final weight (g)	25.44 ± 0.55 ^a	31.92 ± 2.68 ^b	30.53 ± 0.80 ^{ab}	25.88 ± 0.26 ^a
Survival rate	60.79 ± 4.32	73.49 ± 6.54	69.84 ± 11.11	72.86 ± 8.55
GSI ¹	3.72 ± 1.15	3.20 ± 1.00	3.52 ± 0.82	3.51 ± 1.30
HSI ²	4.42 ± 0.22	4.69 ± 0.23	5.25 ± 0.58	5.07 ± 0.23
Sum of (HSI + GSI)	8.14 ± 1.35	7.90 ± 0.85	8.77 ± 1.30	8.58 ± 1.41
Cumulative matured OS (%)	49.16 ± 14.13	42.01 ± 12.38	68.89 ± 17.35	34.47 ± 6.13
Gonad stages index ³	2.28 ± 0.24	2.63 ± 0.69	2.76 ± 0.46	1.86 ± 0.07
Mature OS production (%) ⁴	17.22 ± 1.47	15.56 ± 2.94	25.00 ± 5.36	13.33 ± 5.09

Note:

¹: gonadosomatic index (%): $GSI = (\text{gonad weight} / \text{body weight}) \times 100$

²: Hepatopancreatic index (%): $HSI = (\text{hepatopancreas weight} / \text{body weight}) \times 100$

³: Gonad stages index = $(\sum Si \times ni) \cdot N^{-1}$, where Si : stage of gonad (i=1-5), ni : number of samples in stage Si and N is the total number of samples counted.

⁴: Mature OS production = Cumulative number of broodstock with OS III, IV & V / Initial number of broodstock used (initial stock) $\times 100$; The number of samples, $n = 31 - 40$ / diet

⁵Values are the mean $\pm$ SE of the samples.

Different letters in rows indicate significant differences at $P < 0.05$

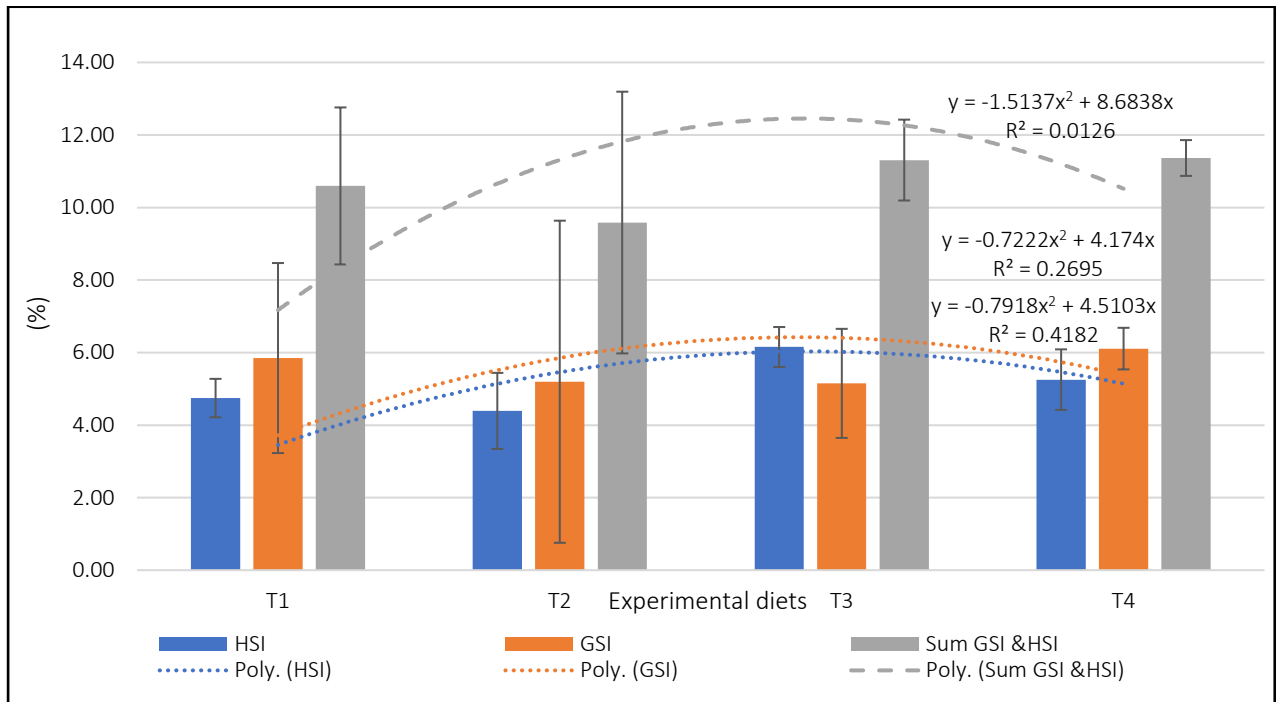


Figure 1. The relationship of GSI, HSI and sum GSI & HSI value with dietary ARA for treated GFP female broodstock as described by second-order polynomial regression

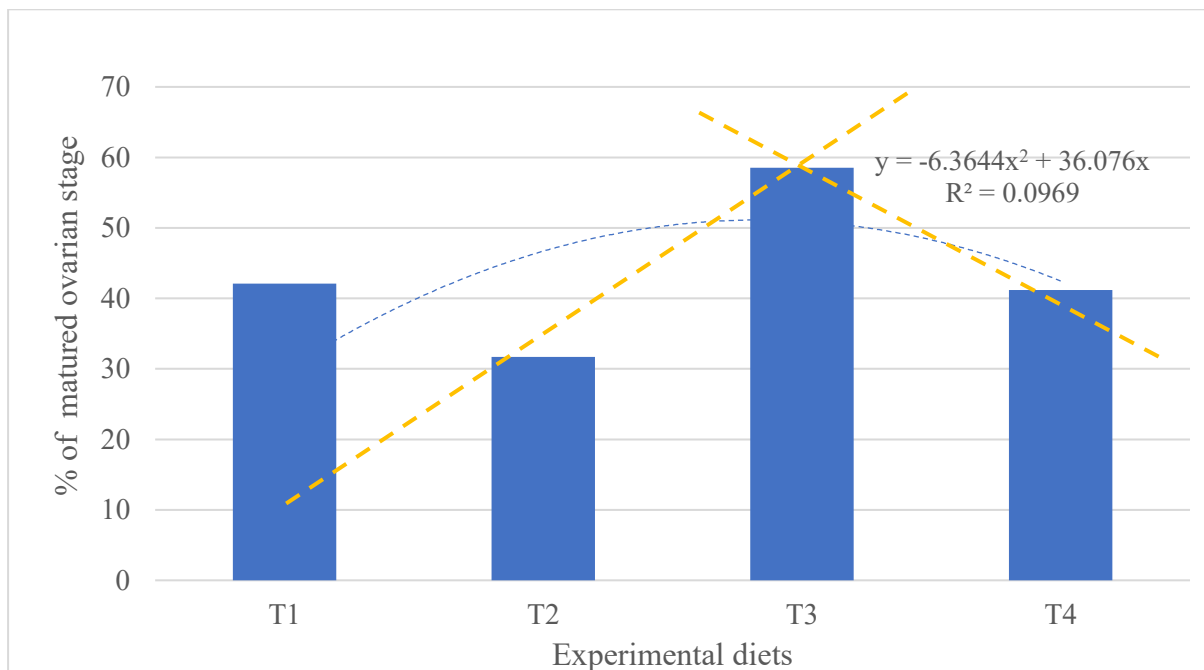


Figure 3. Relationship between production of matured ovarian stage (matured OS are OS III, IV & V) of treated GFP female broodstock and ARA content in diets as described by broken-line analysis as indicated by the dotted yellow line. The red circle is the range of optimal level of ARA that resulted the highest percentage of matured OS

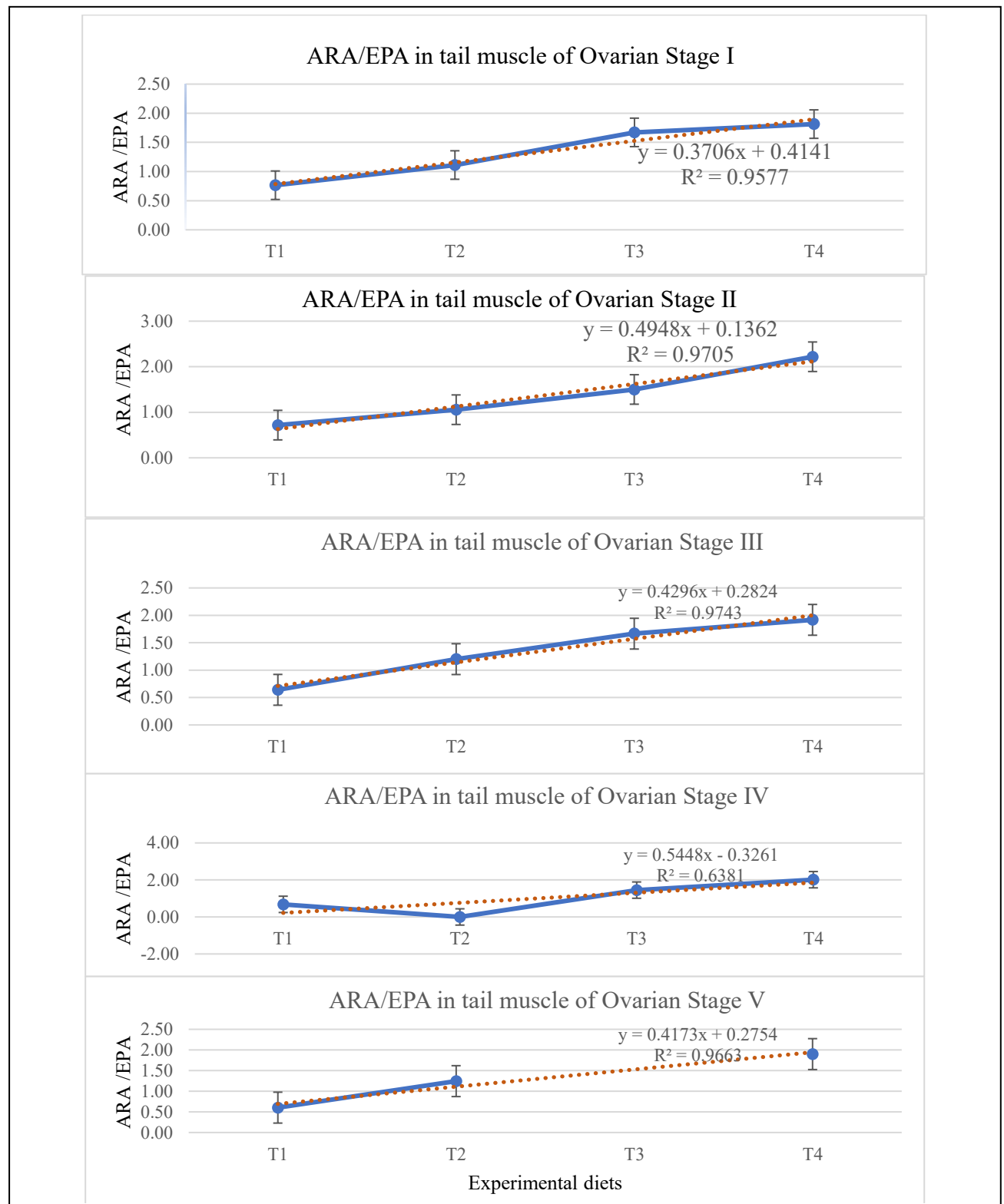


Figure 1. The relation of ARA/EPA for tail muscle of treated broodstock with increasing dietary ARA level at each ovarian stage

Tail Muscle Fatty Acid Composition

The fatty acid composition of treated broodstock tail muscle reflected the fatty acid profile of the diets used, as shown in Table 4. The significant level of fatty acid composition was tested within the same OS group with different diet treatments ($P < 0.05$). The concentrations of saturated fatty acids (SFA) in the treated broodstock of the ovarian stage (OS) I group showed no significant differences ($P > 0.05$) except for C18:0, which increased significantly ($P < 0.05$) by 10.22 %, 11.48 %, 12.02 %, and 12.63 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. Likewise, the C18:0 of the treated broodstock from other OS groups showed a similar trend of increasing across the experimental diets.

Meanwhile, the monounsaturated fatty acids (MUFA) in the tail muscle of treated broodstock for the OS I group were inversely proportioned with the dietary ARA content, whereby C18:1n9 content gradually declined with the value of 26.85, 24.04, 19.05, and 17.30 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. The same pattern was also observed for the C18:1n9 content for different OS groups when moving from T1 to T4 diet (Table 4).

The ARA content in the treated broodstock tail muscle at OS I significantly increased ($P < 0.05$) with the increasing dietary ARA content by 7.90, 10.30, 14.10, and 16.96 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. Moreover, at each ovarian stage, the highest ARA content was recorded from the treated broodstock fed with T4 diet, which was significantly higher ($P < 0.05$) compared to other treated broodstock groups. Furthermore, the ratio of ARA to EPA in the tail muscle showed an increasing trend for all OS across the experimental diets, with a range of 0.77 – 1.81 %, 0.72 – 2.22 %, 0.64 – 1.92 %, 0.68 – 2.01 %, and 0.60 – 1.90 % when moving from OS I to OS V, respectively (Figure 5).

Table 1. Tail muscle fatty acid composition (% of total fatty acid) of female freshwater broodstock fed the diets with increasing ARA levels for ovarian stage I, II, III, IV and V¹

Fatty acid	Ovarian stage I				Ovarian stage II				Ovarian stage III				One way ANOVA
	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	P-value OS I, II & III
C16:0	18.38	18.64	18.57	17.26	16.98 ^a	18.68 ^c	17.80 ^{ab}	18.41 ^{bc}	17.48	18.87	17.78	17.59	0.632, 0.023, 0.485
C17:0	7.50	7.48	6.02	8.53	8.35 ^b	7.00 ^{ab}	6.40 ^a	6.73 ^{ab}	7.52	7.24	7.50	7.34	0.573, 0.076, 0.492
C18:0	10.22 ^a	11.48 ^b	12.02 ^c	12.63 ^d	10.17 ^a	11.04 ^{ab}	11.50 ^{bc}	12.22 ^c	10.41	11.01	12.32	13.33	0.055, 0.031, 0.062
C18:1n9	26.85 ^a	24.04 ^b	19.05 ^c	17.30 ^c	25.01 ^{ab}	23.42 ^b	21.86 ^{ab}	19.75 ^a	24.02	24.07	19.72	18.59	0.002, 0.00, 0.121
C18:3n3 ALA	0.49	ND	0.78	ND	ND	ND	0.69	ND	0.57	ND	0.67	ND	NA
C20:5n3 EPA	10.33	9.27	8.44	9.35	10.57 ^b	9.52 ^b	8.30 ^a	7.86 ^a	10.90	8.13	8.38	9.03	0.446, 0.006, 0.072
C22:6n3 DHA	3.69	3.96	4.05	4.11	4.44 ^a	4.41 ^a	3.97 ^b	4.01 ^b	3.99 ^a	4.30	4.12	4.31	0.621, 0.023, 0.479
C18:2n6 LA	9.82	8.64	9.35	7.29	8.39 ^b	9.51 ^b	12.03 ^c	8.16 ^a	7.67 ^a	9.31 ^b	7.54 ^b	7.36 ^b	0.343, 0.001, 0.053
C20:4n6 ARA	<u>7.90^a</u>	<u>10.30^b</u>	<u>14.10^c</u>	<u>16.96^d</u>	7.59 ^a	10.05 ^b	12.45 ^c	17.43 ^d	<u>6.98^a</u>	<u>9.76^{ab}</u>	<u>13.96^b</u>	<u>17.32^c</u>	0.003, 0.00, 0.004
Σ saturated	38.33	38.91	38.61	40.71	37.70	38.09	37.68	38.55	37.34	38.43	39.86	38.25	
Σ MUFA	28.64	28.91	23.85	21.58	27.79	27.75	24.13	24.60	29.66	29.40	25.42	23.74	
Σ PUFA(n-3)	14.59	13.23	13.26	13.46	15.41	13.94	12.95	11.86	15.64	12.43	13.17	13.34	
Σ PUFA(n-6)	18.36	18.94	23.45	24.26	17.07	19.56	24.48	25.59	15.06	19.07	21.94	24.67	
Σ PUFA (total)	32.95	32.18	36.72	37.72	32.48	33.50	37.44	37.45	30.71	31.49	35.12	38.01	
Σ LC-HUFA(n-3)	14.02	13.23	12.49	13.46	15.21	13.94	12.27	11.86	15.07	12.43	12.50	13.34	
ARA/EPA	0.77	1.11	1.67	1.81	0.72	1.06	1.50	2.22	0.64	1.20	1.67	1.92	
ARA/DHA	2.14	2.60	3.48	4.13	1.71	2.28	3.14	4.35	1.75	2.27	3.39	4.02	
EPA+DHA	14.02	13.23	12.49	13.46	15.01	13.94	12.27	11.86	14.89	12.43	12.50	13.34	
EPA/DHA	2.80	2.34	2.08	2.28	2.38	2.16	2.09	1.96	2.73	1.89	2.04	2.10	
DHA/EPA	0.36	0.43	0.48	0.44	0.42	0.46	0.48	0.51	0.37	0.53	0.49	0.48	

Note:

¹Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05., ND: not detected, ARA1 = 0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Table 4 (continued)

Fatty acid	Ovarian stage IV				One way ANOVA P-value OS IV	Ovarian stage V				One way ANOVA P-value OS V
	ARA1	ARA2	ARA3	ARA4		ARA1	ARA2	ARA3	ARA4	
C16:0	17.51 ^a	NA	17.94 ^{ab}	18.87 ^{ab}	0.003	19.50 ^c	18.72 ^{bc}	NA	17.39 ^{ab}	0.024
C17:0	7.00	NA	6.63	6.67	0.301	7.03	7.11	NA	6.96	0.242
C18:0	9.85 ^a	NA	11.22 ^a	12.47 ^b	0.003	10.62 ^{ab}	12.85 ^c	NA	12.42 ^{bc}	0.026
C18:1n9	26.41 ^a	NA	20.52 ^b	21.31 ^c	0.003	30.41 ^a	21.97 ^b	NA	19.17 ^c	0.003
C18:3n3 ALA	0.84	NA	0.86	ND	NA	ND	ND	NA	ND	NA
C20:5n3 EPA	9.30 ^{ab}	NA	9.18 ^{ab}	7.84 ^a	0.041	10.08 ^a	8.92 ^{ab}	NA	8.55 ^{ab}	0.032
C22:6n3 DHA	3.96 ^b	NA	4.23 ^b	3.27 ^a	0.009	3.45	3.61	NA	3.58	0.359
C18:2n6 LA	9.31 ^a	NA	ND	9.36 ^b	0.003	8.89 ^a	7.55 ^b	NA	8.06 ^c	0.003
C20:4n6 ARA	6.33 ^a	NA	13.31 ^b	15.78 ^c	0.003	6.07 ^a	11.11 ^b	NA	16.24 ^c	0.003
Σ saturated ^a	36.91	NA	37.76	37.55		38.60	40.94	NA	38.89	
Σ MUFA ^b	29.84	NA	25.90	26.20		32.90	27.16	NA	24.01	
Σ PUFA(n-3) ^c	14.19	NA	14.26	11.11		13.53	12.53	NA	12.13	
Σ PUFA(n-6) ^d	16.06	NA	22.08	25.14		14.97	18.66	NA	24.30	
Σ PUFA (total)	30.25	NA	36.34	36.25		28.50	31.19	NA	36.43	
Σ LC-HUFA(n-3) ^e	13.35	NA	13.40	11.11		13.53	12.53	NA	12.13	
ARA/EPA	0.68	NA	1.45	2.01		0.60	1.24	NA	1.90	
ARA/DHA	1.60	NA	3.15	4.82		1.76	3.08	NA	4.53	
EPA+DHA	13.25	NA	13.40	11.11		13.53	12.53	NA	12.13	
EPA/DHA	2.35	NA	2.17	2.39		2.92	2.47	NA	2.39	
DHA/EPA	0.43	NA	0.46	0.42		0.34	0.40	NA	0.42	

Note:

- ^a Σ SFA : sum of all saturated fatty acids
^b Σ MUFA : sum of all monounsaturated fatty acids
^c Σ PUFA(n-3) : sum of all omega-3 polyunsaturated fatty acids
^d Σ PUFA(n-6) : sum of all omega-6 polyunsaturated fatty acids
^e Σ n-3 LC-PUFA : sum of all omega-3 long chain polyunsaturated fatty acids

¹Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05. ND: not detected.
 ARA1 =0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Hepatopancreas fatty acid composition

The fatty acid composition of treated broodstock hepatopancreas samples of five OS reflected the fatty acid profile of the experimental diets (Table 5). For all OS of treated broodstock samples analysed, the SFA and MUFA contents in the hepatopancreas decreased with increasing dietary ARA. The highest value of C16:0 was recorded in the treated broodstock group fed with T1 diet compared to other diets, with a value of 26.46 %, 25.05 %, 24.18%, 23.36 %, and 22.82 % for OS I, II, III, IV, and V, respectively. Moreover, the C16:0 content of the same OS decreased as the dietary ARA content increased (Table 5).

The n-3 PUFA, particularly C20:5n3 and C22:6n3, showed a significantly higher ($P < 0.05$) value for treated broodstock fed with T1 diet compared to treated broodstock fed with other diets for OS I. However, no significant differences ($P > 0.05$) were observed for n-3 PUFA for treated broodstock groups fed with T2, T3, and T4 diets within the same OS, moving from OS I until OS V (Table 5).

The n-6 PUFA content of treated broodstock, particularly for arachidonic acid (ARA, C20:4n6), increased with increasing dietary ARA levels and was significantly higher ($P < 0.05$) in the treated broodstock groups fed with T4 diet for all OS (Table 5). Moreover, the ARA to EPA ratio recorded for each OS increased with increasing dietary ARA levels from T1 to T4 as demonstrated in Figure 6. As shown in Figure 5, the ARA to EPA ratio increased for OS 1 from 0.45, 1.79, 2.88, and 4.92 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. The OS II, OS III, OS IV, and OS V groups recorded a similar trend of increasing ARA to EPA ratio with increasing dietary ARA levels for all treated broodstock (Figure 5).

Table 2. Hepatopancreas fatty acid composition (% of total fatty acid) of female freshwater prawn broodstock fed the diets with increasing ARA levels for ovarian stage I, II, III, IV and V¹

Fatty acid compounds	Ovarian stage I				Ovarian stage II				Ovarian stage III				One way ANOVA
	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	P-value of OS I, II & III
C16:0	26.62 ^c	22.83 ^b	23.18 ^b	19.85 ^a	25.05 ^a	23.58 ^b	23.09 ^b	21.86 ^c	24.18 ^b	23.15 ^b	22.40 ^b	20.02 ^a	0.007, 0.002, 0.012
C17:0	1.90	0.55	0.55	0.58	0.52	0.73	0.50	1.20	2.55	0.72	0.91	0.69	0.180, 0.236, 0.071
C18:0	7.29	5.77	6.81	6.80	5.91 ^b	5.74 ^b	6.71 ^b	8.15 ^a	8.02 ^{bc}	6.81 ^a	7.71 ^b	8.67 ^c	0.690, 0.011, 0.016
C18:1n9	28.89 ^b	25.57 ^b	28.15 ^b	19.55 ^a	30.52 ^a	27.08 ^{ab}	26.45 ^{ab}	19.13 ^b	34.37 ^c	27.34 ^b	25.86 ^{ab}	20.80 ^a	0.015, 0.093, 0.014
C20:1n9	2.57	3.22	2.01	3.22	2.74	2.63	2.60	3.54	1.46 ^a	3.18 ^b	2.91 ^b	3.93 ^c	0.296, 0.283, 0.002
C18:3n3 ALA	1.23	1.23	1.00	1.34	1.08	1.25	1.08	1.19	ND	1.21 ^b	1.04 ^a	1.04 ^a	0.203, 0.508, 0.032
C20:5n3 EPA	4.22 ^a	1.92 ^b	1.48 ^b	1.85 ^b	2.01 ^b	1.87 ^b	1.23 ^a	1.76 ^{ab}	4.87 ^a	1.71 ^c	1.32 ^{ab}	1.44 ^b	0.033, 0.50, 0.008
C22:6n3 DHA	3.31 ^a	2.18 ^b	1.94 ^b	2.34 ^b	2.17	2.70	1.61	2.08	3.28 ^a	1.98 ^{ab}	1.61 ^b	1.71 ^b	0.026, 0.060, 0.040
C18:2n6 LA	10.90	11.25	10.52	11.62	10.22 ^c	11.36 ^b	10.90 ^a	9.04 ^a	8.51	10.68	9.78	8.99	0.698, 0.004, 0.210
C20:4n6 ARA	<u>1.90^a</u>	<u>3.44^{ab}</u>	<u>4.26^b</u>	<u>9.10^c</u>	0.76 ^a	3.48 ^b	4.19 ^b	7.66 ^c	2.03 ^a	3.19 ^b	4.69 ^c	7.74 ^d	0.001, 0.00, 0.001
Σ saturated	39.20	35.21	35.67	33.91	35.96	34.46	35.80	38.92	37.15	35.76	36.68	36.22	
Σ MUFA	39.85	38.81	40.48	30.77	44.94	41.24	40.09	34.76	44.16	43.41	40.53	37.53	
Σ PUFA(n-3)	8.76	6.13	4.93	6.69	5.50	5.82	4.11	5.31	8.16	4.90	4.19	4.47	
Σ PUFA (n-6)	12.80	17.30	16.05	25.54	11.51	18.06	16.57	18.91	10.53	14.95	16.04	18.94	
Σ PUFA (total)	21.56	23.43	20.99	32.23	17.01	23.88	20.68	24.22	18.69	19.86	20.23	23.41	
Σ LC-PUFA(n-3)	7.53	4.26	3.55	4.64	4.18	4.57	2.84	3.84	8.16	3.70	2.93	3.15	
ARA/EPA	<u>0.45</u>	<u>1.79</u>	<u>2.88</u>	<u>4.92</u>	0.38	1.86	3.42	4.36	<u>0.42</u>	<u>1.86</u>	<u>3.54</u>	<u>5.36</u>	
ARA/DHA	0.57	1.58	2.20	3.89	I	1.29	2.60	3.67	0.62	1.61	2.92	4.53	
EPA+DHA	7.53	4.10	3.42	4.19	4.18	4.57	2.84	3.84	8.16	3.70	2.93	3.15	
EPA/DHA	1.27	0.88	0.76	0.79	0.93	0.69	0.76	0.84	1.48	0.86	0.82	0.85	
DHA/EPA	0.79	1.13	1.31	1.26	1.08	1.44	1.32	1.19	0.67	1.16	1.21	1.18	

Note:

¹Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05. ND: not detected, ARA1 = 0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Table 5 (continued)

Fatty acid compound	Ovarian stage IV				One way ANOVA P-value OS IV	Ovarian stage V				One way ANOVA P-value OS V
	ARA1	ARA2	ARA3	ARA4		ARA1	ARA2	ARA3	ARA4	
C16:0	23.36 ^b	NA	18.66 ^{ab}	24.00 ^b	0.035	22.82	20.20	23.43	20.88	0.390
C17:0	3.40	NA	3.26	0.82	0.193	2.17	3.23	4.24	1.34	0.386
C18:0	9.62	NA	9.66	9.34	0.317	7.72 ^a	9.44 ^a	14.39 ^b	10.28 ^a	0.029
C18:1n9	31.10 ^a	NA	22.32 ^c	28.27 ^b	0.003	31.06 ^b	28.42 ^{ab}	29.02 ^b	23.14 ^a	0.039
C20:1n9	1.91	NA	2.39	ND	0.860	3.00	2.25	ND	ND	0.269
C18:3n3 ALA	ND	NA	ND	ND	-	0.91	0.83	ND	0.96 ^a	0.995
C20:5n3 EPA	4.78	NA	3.11	ND	0.071	4.16	3.50	ND	1.43	0.170
C22:6n3 DHA	1.45	NA	2.79	ND	0.136	1.99	3.26	ND	1.79	0.170
C18:2n6 LA	9.05	NA	8.59	8.05	0.407	8.02	9.10	8.67	8.27	0.582
C20:4n6 ARA	1.04 ^a	NA	7.90 ^b	3.23 ^c	0.009	2.71 ^a	5.77 ^{ab}	10.01 ^b	7.20 ^b	0.030
Σ saturated	39.74	NA	36.31	42.07		35.74	35.74	42.06	39.29	
Σ MUFA	43.55	NA	37.78	41.89		44.20	41.26	39.72	35.63	
Σ PUFA(n-3)	6.67	NA	7.35	0.00		6.67	7.47	0.00	4.17	
Σ PUFA(n-6)	10.04	NA	17.99	9.57		10.57	15.54	18.22	17.68	
Σ PUFA (total)	16.71	NA	25.34	9.57		17.24	23.00	18.22	21.85	
Σ LC-PUFA(n-3)	6.00	NA	5.92	0.00		5.81	6.65	0.00	3.21	
ARA/EPA	<u>0.22</u>	NA	<u>2.54</u>	ND		<u>0.65</u>	<u>1.65</u>	ND	<u>5.05</u>	
ARA/DHA	0.69	NA	2.71	ND		1.36	1.77	ND	4.03	
EPA+DHA	6.00	NA	5.76	ND		5.81	6.65	ND	3.21	
EPA/DHA	3.14	NA	1.07	ND		2.10	1.07	ND	0.80	
DHA/EPA	0.32	NA	0.94	ND		0.48	0.93	ND	1.25	

Note:

¹Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05. ND: not detected, ARA1 =0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

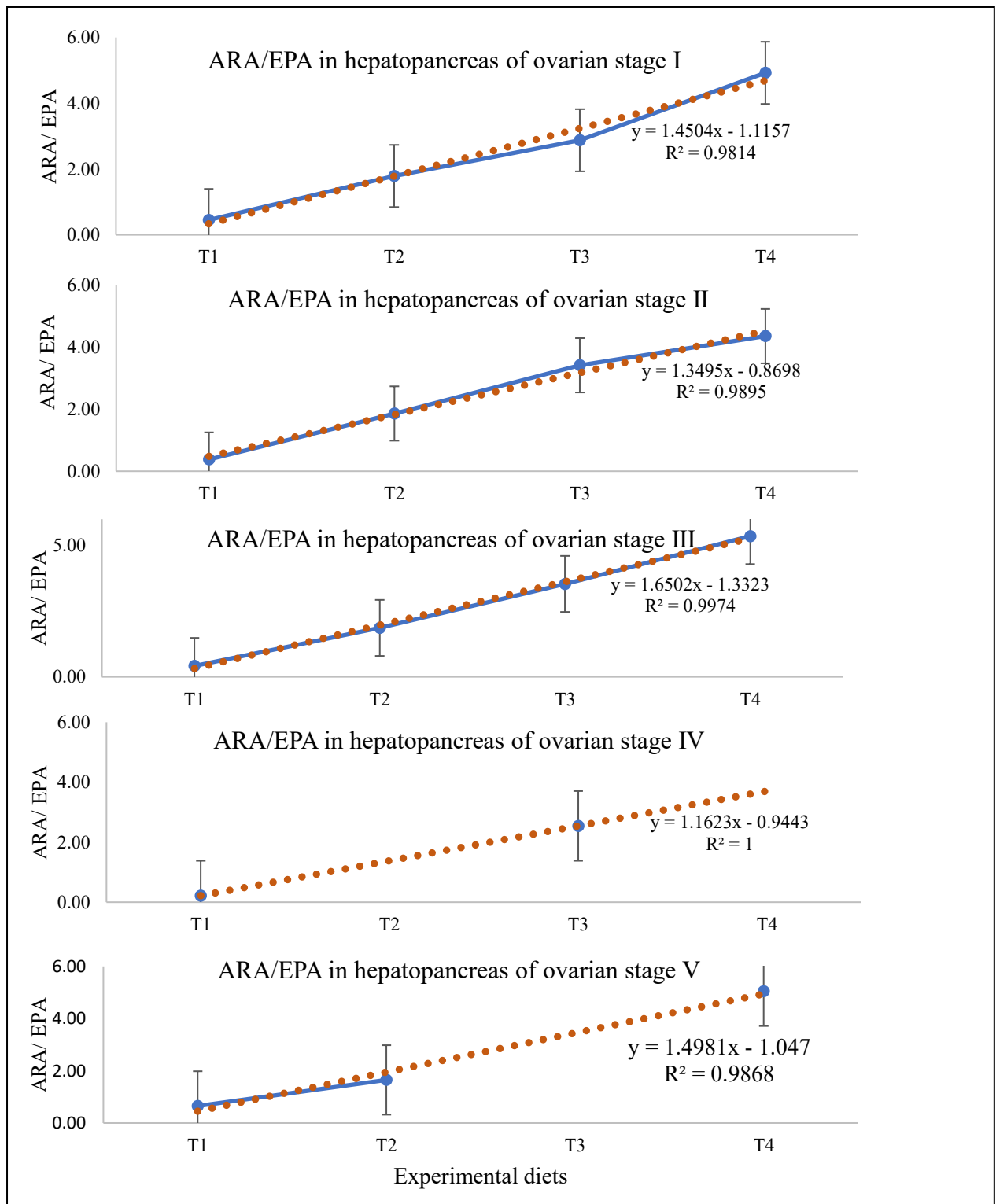


Figure 5. The relation of ARA/EPA for hepatopancreas of treated broodstock with increasing dietary ARA level at each ovarian stage

Gonad fatty acid composition

The gonad fatty acid composition of treated broodstock for all experimental groups are shown in Table 6. For each OS, the SFA content of the treated broodstock fluctuated across the experimental diets. For OS I, C16:0 content of 24.50 %, 21.25 %, 22.26 %, and 23.05 % were recorded for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. Meanwhile, the MUFA, C18:1n9 for OS I, decreased as treated broodstock dietary ARA increased, from 38.31 % to 31.89 %, 32.73 %, and 30.12 % for broodstock fed with T1, T2, T3, and T4 diets, respectively. The EPA value for OS I also decreased for treated broodstock with increasing ARA levels. Similar patterns were recorded for EPA content for all OS of the gonad samples within the same OS samples.

Like the ARA content in the tail muscle and hepatopancreas of treated broodstock, the ARA content in the gonad increased as the dietary ARA increased. The ARA values of OS I recorded were 5.92 %, 5.85 %, 8.06 %, and 9.93 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. In all other OS, the ARA contents increased in treated broodstock fed with T1 to T4 diets, as shown in Table 6. The highest ARA content was recorded in the gonad of the treated broodstock fed with T4 diet, with a value of 9.93 %, 10.75 %, 10.17 %, 9.25 %, and 8.91 % for OS I, II, III, IV, and V, respectively, as compared to other diets.

The ARA to EPA ratio recorded in the gonad for each OS was increased with increasing dietary ARA levels from T1 to T4 as demonstrated in Figure 7. The ARA to EPA ratios of tail muscle, hepatopancreas, and gonads of the treated broodstock fed with experimental diets for each OS are presented in Figures 4, 5, and 6, in which the ARA to EPA ratio shows an increasing trend as the dietary ARA level increased in the hepatopancreas, gonads, and tail muscles of treated broodstock for all OS. Although no samples were available for a certain OS, the prediction can still be made with the available data, as shown in Figure 5 for OS V. However, the ARA to EPA ratio in the gonads of the treated broodstock showed a different pattern for OS III, whereby the ARA to EPA ratio in the gonads increased for treated broodstock fed with T1, T2, and T3 diets, and then decreased sharply in the treated broodstock fed with T4 diets (Figure 7).

Table 3. Gonad fatty acid composition (% of total fatty acid) of female freshwater prawn broodstock fed the diets with increasing dietary ARA levels for ovarian stage I, II, III, IV and V¹

Fatty acid compound	Ovarian Stage I				Ovarian Stage II				Ovarian Stage III				One way ANOVA
	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	P-value OS I, II & III
C16:0	24.50 ^b	21.25 ^a	22.26 ^a	23.05 ^{ab}	22.80	20.37	21.89	22.08	22.89	20.97	21.12	20.47	0.037, 0.126, 0.333
C17:0	ND	1.48	0.69	1.36	0.89	1.53	1.26	2.40	1.17 ^a	1.18 ^a	0.68 ^a	1.34 ^b	0.043, 0.537, 0.036
C18:0	8.22 ^{bc}	6.03 ^a	7.80 ^b	8.69 ^c	5.84 ^a	7.12 ^b	7.35 ^b	8.98 ^c	5.98	6.38	8.02	8.23	0.00, 0.005, 0.393
C18:1n9	38.31	31.89	32.73	30.12	39.92 ^c	31.76 ^b	32.30 ^b	27.87 ^a	40.41 ^a	32.36 ^b	31.53 ^b	27.41 ^b	0.061, 0.003, 0.026
C20:1n9	ND	0.83	ND	ND	ND	1.03	ND	ND	ND	1.12 ^b	1.18 ^a	1.35 ^c	NA, NA, 0.001
C18:3n3 ALA	ND	1.22	1.34	1.31	1.30	1.31	1.28	1.44	0.95	1.29	1.14	1.28	0.010, 0.311, 0.19
C20:5n3 EPA	6.58	4.14	3.41	3.15	4.02	3.75	3.68	3.22	4.72	3.41	1.32	2.90	0.112, 0.339, 0.09
C22:6n3 DHA	ND	3.62	3.86	3.56	3.37	4.06	4.03	4.28	3.26 ^a	3.76 ^b	3.31 ^a	3.68 ^b	0.266, 0.521, 0.00
C18:2n6 LA	14.21	12.39	13.26	12.12	13.62	12.86	13.66	12.93	11.34	12.42	11.95	12.09	0.068, 0.171, 0.19
C20:4n6 ARA	5.92 ^a	5.85 ^a	8.06 ^{ab}	9.93 ^b	1.28 ^a	5.35 ^b	9.03 ^c	10.75 ^c	2.02 ^a	4.79 ^b	6.91 ^c	10.17 ^d	0.025, 0.001, 0.00
Σ saturated	32.57	31.14	32.26	34.90	30.96	30.97	32.05	35.12	31.65	30.47	31.95	32.58	
Σ MUFA	41.20	37.07	37.07	33.71	44.41	36.57	35.48	29.15	45.29	37.78	38.94	33.61	
Σ PUFA(n-3)	6.46	9.47	8.58	8.02	8.69	9.67	8.99	8.94	8.93	8.72	7.19	8.11	
Σ PUFA(n-6)	19.77	19.27	21.25	22.05	14.90	19.28	22.69	23.68	13.36	18.68	20.17	24.22	
Σ PUFA (total)	26.23	28.73	29.83	30.07	23.59	28.95	31.67	32.62	22.29	27.40	27.36	32.34	
Σ LC-HUFA(n-3)	6.46	8.11	7.24	6.71	7.39	8.22	7.71	7.50	7.98	7.27	6.05	6.83	
ARA/EPA	0.90	1.42	2.37	3.15	0.32	1.43	2.45	3.34	0.43	1.40	5.22	3.50	
ARA/DHA	NA	1.62	2.09	2.79	0.38	1.32	2.24	2.51	0.62	1.27	2.09	2.77	
EPA+DHA	6.46	7.75	7.24	6.71	7.39	7.81	7.71	7.50	7.98	7.17	4.63	6.58	
EPA/DHA	NA	1.14	0.88	0.89	1.19	0.92	0.91	0.75	1.45	0.91	0.40	0.79	
DHA/EPA	0.00	0.87	1.13	1.13	0.84	1.08	1.09	1.33	0.69	1.10	2.50	1.27	

Note:

¹Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05. P-value between group each OS. ND: not detected, ARA1 = 0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Table 6 (continued)

Fatty acid compound	Ovarian stage IV				One way ANOVA P-value OS IV	Ovarian stage V				One way ANOVA P-value OS V
	ARA1	ARA2	ARA3	ARA4		ARA1	ARA2	ARA3	ARA4	
C16:0	22.64 ^a	NA	21.53 ^b	20.40 ^c	0.003	21.93 ^b	19.87 ^a	21.09 ^b	19.75 ^a	0.009
C17:0	1.26	NA	1.38	1.31	0.375	0.34 ^a	1.30 ^b	0.58 ^a	1.42 ^b	0.008
C18:0	6.37 ^{ab}	NA	7.86 ^b	8.05 ^b	0.043	5.65 ^a	6.96 ^b	7.37 ^{bc}	7.93 ^c	0.010
C18:1n9	37.16 ^c	NA	31.79 ^{bc}	30.92 ^{ab}	0.008	38.16 ^c	11.65 ^a	31.18 ^b	30.63 ^a	0.004
C20:1n9	ND	NA	1.24	0.00	-	0.00 ^b	1.09 ^a	1.30 ^c	1.20 ^a	0.009
C18:3n3 ALA	1.13	NA	1.06	1.14	0.227	1.11 ^a	1.17 ^a	1.28 ^b	1.30 ^b	0.007
C20:5n3 EPA	3.54 ^a	NA	2.77 ^b	2.85 ^b	0.029	3.83 ^b	3.42 ^{ab}	3.00 ^a	2.81 ^a	0.034
C22:6n3 DHA	3.15	NA	2.99	3.46	0.279	3.15 ^{ab}	3.40 ^b	3.47 ^b	2.94 ^a	0.048
C18:2n6 LA	11.49	NA	10.88	11.93	0.184	11.46 ^a	11.50 ^a	12.23 ^b	10.84 ^c	0.006
C20:4n6 ARA	<u>1.37^a</u>	<u>NA</u>	<u>6.27^b</u>	<u>9.25^c</u>	<u>0.000</u>	<u>1.09^a</u>	<u>4.07^b</u>	<u>7.06^c</u>	<u>8.91^d</u>	<u>0.000</u>
Σsaturated	32.65	NA	33.80	32.07		30.21	30.48	31.90	31.27	
ΣMUFA	44.56	NA	38.30	35.43		45.55	40.03	38.30	36.90	
ΣPUFA(n-3)	7.82	NA	6.94	7.45		8.61	8.31	7.74	7.41	
ΣPUFA(n-6)	13.46	NA	18.65	22.81		13.29	16.70	21.05	21.58	
ΣPUFA (total)	21.28	NA	25.59	30.26		21.91	25.01	28.80	28.99	
ΣLC-HUFA(n-3)	6.69	NA	5.88	6.31		7.32	6.97	6.47	6.11	
ARA/EPA	0.39	NA	2.27	3.25		0.29	1.19	2.35	3.17	
ARA/DHA	0.43	NA	2.10	2.67		0.35	1.19	2.04	3.03	
EPA+DHA	6.69	NA	5.75	6.31		6.97	6.83	6.47	5.75	
EPA/DHA	1.12	NA	0.93	0.82		1.22	1.00	0.87	0.96	
DHA/EPA	0.89	NA	1.08	1.22		0.82	1.00	1.15	1.05	

Note:

¹Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stages indicate significant differences at P<0.05. ND: not detected, NA = Sample is not available, ARA1 = 0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

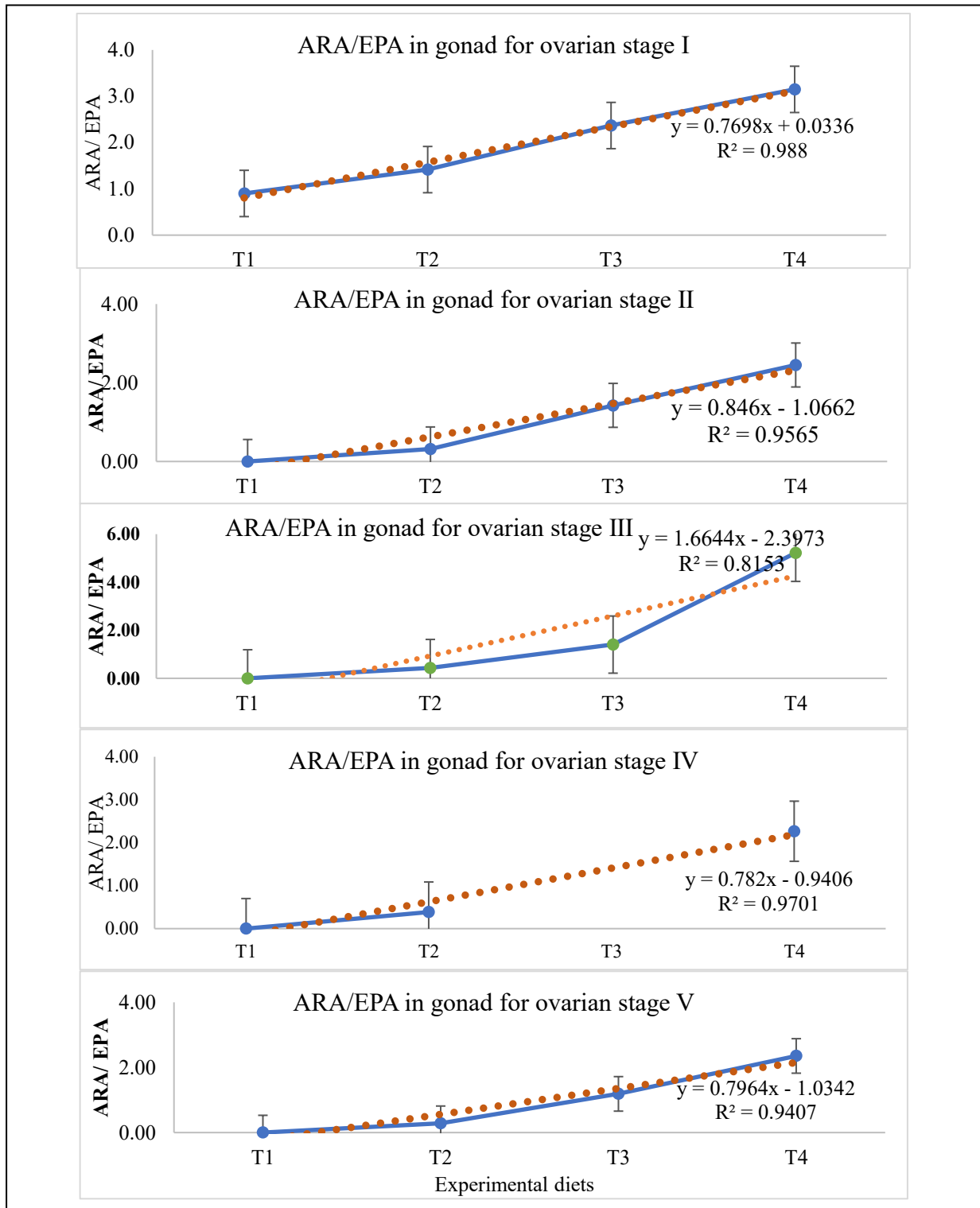


Figure 1. The relation of ARA/EPA for gonad of treated broodstock with increasing dietary ARA level at each ovarian stage

Histological evaluation

Figure 7 shows the representative histological cross-sections of the hepatopancreas and gonad of the initial sample. The cross-section of the initial sample indicated that the ovary was at OS I

and II, as described by Soonklang et al. (2012), Sumpownon et al. (2015), and Shen et al. (2020). The embryonic cell (E-cell) and fibrillenzellen cell (F-cell) for the initial sample of hepatopancreas and gonad are shown in Figure 7, and the identification of cells was referred to the work done by Silva et al. (2018). The histological slide images for the final samples are shown in Figure 8 for hepatopancreas and ovary of treated broodstock fed with T2 and T3 diets at OS III. Basically, the final cells of the ovary fed with T3 and T4 diets showed healthy and well-arranged cells. Figure 8 shows the representative cells in the hepatopancreas and ovary that were measured, and the results were as tabulated in Table 7.

Table 7 summarises the analysed parameters from the histological slides of the hepatopancreas and ovary. The hepatopancreas tubule diameter of treated broodstock fed with T2 diet was recorded at 96.54 μm , which was significantly lower than the treated broodstock fed with T4 diets ($P < 0.05$); but showed no significant different compared to treated broodstock fed with T1 and T3 diets ($P > 0.05$). The hepatopancreas tubule area of 6643.89 μm^2 was recorded for the treated broodstock fed with T1 diet, which showed no significant difference ($P < 0.05$) compared to other groups. Meanwhile, the hepatopancreas tubule diameter of 108.28 μm was recorded for treated broodstock fed with T4 diet and was significantly higher ($P < 0.05$) than the treated broodstock fed with T3 diet, but did not significantly differ ($P > 0.05$) with treated broodstock fed with T1 and T2 diets.

The oocyte diameter recorded for treated broodstock fed with T3 diets showed a significantly higher ($P < 0.05$) value of 207.64 μm compared to other treatment groups. Moreover, oocyte diameters of 126.57 μm , 169.54 μm , and 139.84 μm were recorded for treated broodstock fed with T1, T2, and T4 diets, respectively, yet no significant difference were found between these groups ($P > 0.05$) (Table 7). Based on the oocyte diameter, it showed a pattern of increasing in size as dietary ARA increased up to T3, and then decreased in T4 (Figure 9). In Figure 9, it illustrates a second-order polynomial regression relationship between oocyte diameter and dietary ARA for the treated GFP female broodstock. The optimal level of ARA in the diet, as represented by the red circle in Figure 9, can be estimated by applying the dose-response concept or broken line analysis to the diameter size of the oocyte.

Table 4. The diameter and area for tubule in hepatopancreas (tubule) and ovary (oocyte) of the experimental broodstock after feeding trial. (n=57, Mean $\pm$ S.E.)¹

Diet	Hepatopancreas		Ovary
	Tubule diameter (μm)	Tubule area (μm^2)	Oocyte diameter (μm)
Initial	113.35 $\pm$ 2.44	8605.51 $\pm$ 255.68	41.50 $\pm$ 1.12
ARA1	102.57 $\pm$ 2.85 ^{ab}	6643.89 $\pm$ 329.39 ^{ab}	126.57 $\pm$ 12.67 ^a
ARA2	96.54 $\pm$ 1.85 ^{ab}	6507.60 $\pm$ 322.12 ^{ab}	169.54 $\pm$ 3.28 ^a
ARA3	102.25 $\pm$ 2.54 ^a	6016.02 $\pm$ 222.26 ^a	207.64 $\pm$ 4.59 ^b
ARA4	108.27 $\pm$ 3.18 ^b	7307.58 $\pm$ 366.43 ^b	139.84 $\pm$ 6.07 ^a

¹Values are the mean $\pm$ SE of a group of representative histological slide samples (n=57). Different superscripts in the same column indicate significant differences at $P < 0.05$. Initial samples were excluded from statistical analysis.

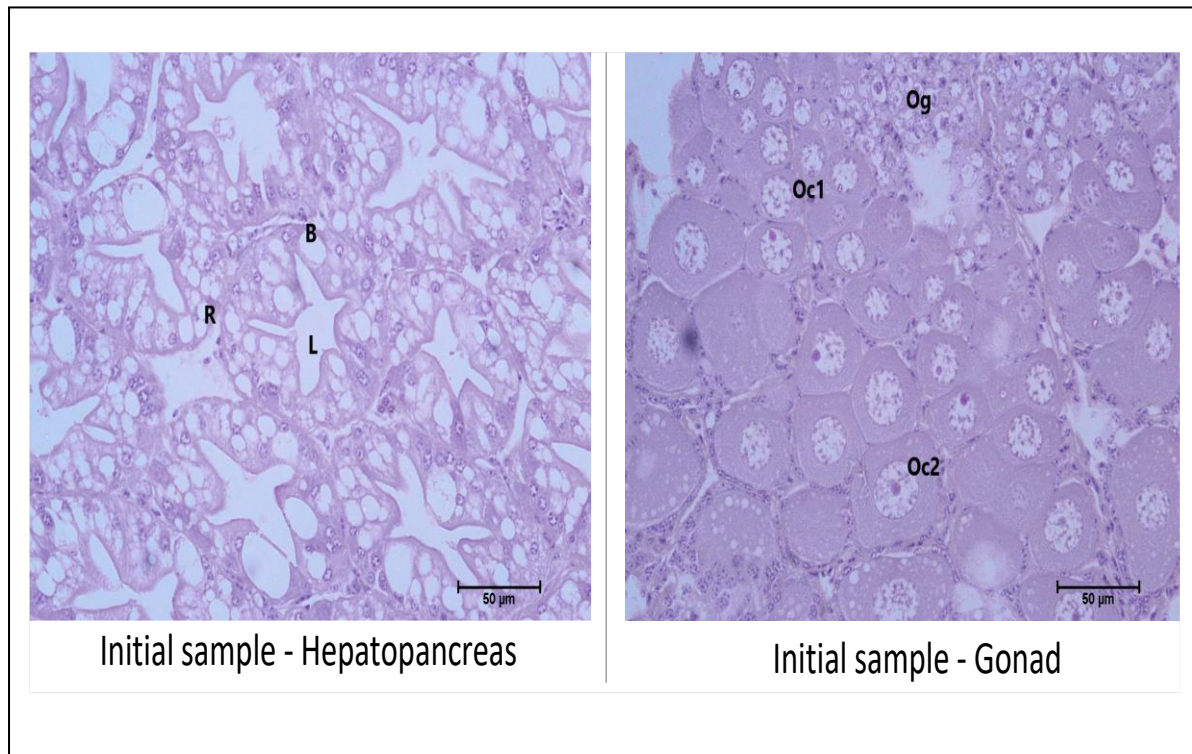


Figure 7. Representative histological cross-sections of the hepatopancreas of initial prawn showing the arrangement of hepatopancreatic tubules and cells (on the left) and gonad (on the right). (20 $\times$ Magnification) Og = oogonia, Oc1 & Oc2 = previtellogenic oocytes, L = Lumen of tubule, B = B- cell and R = R- cell

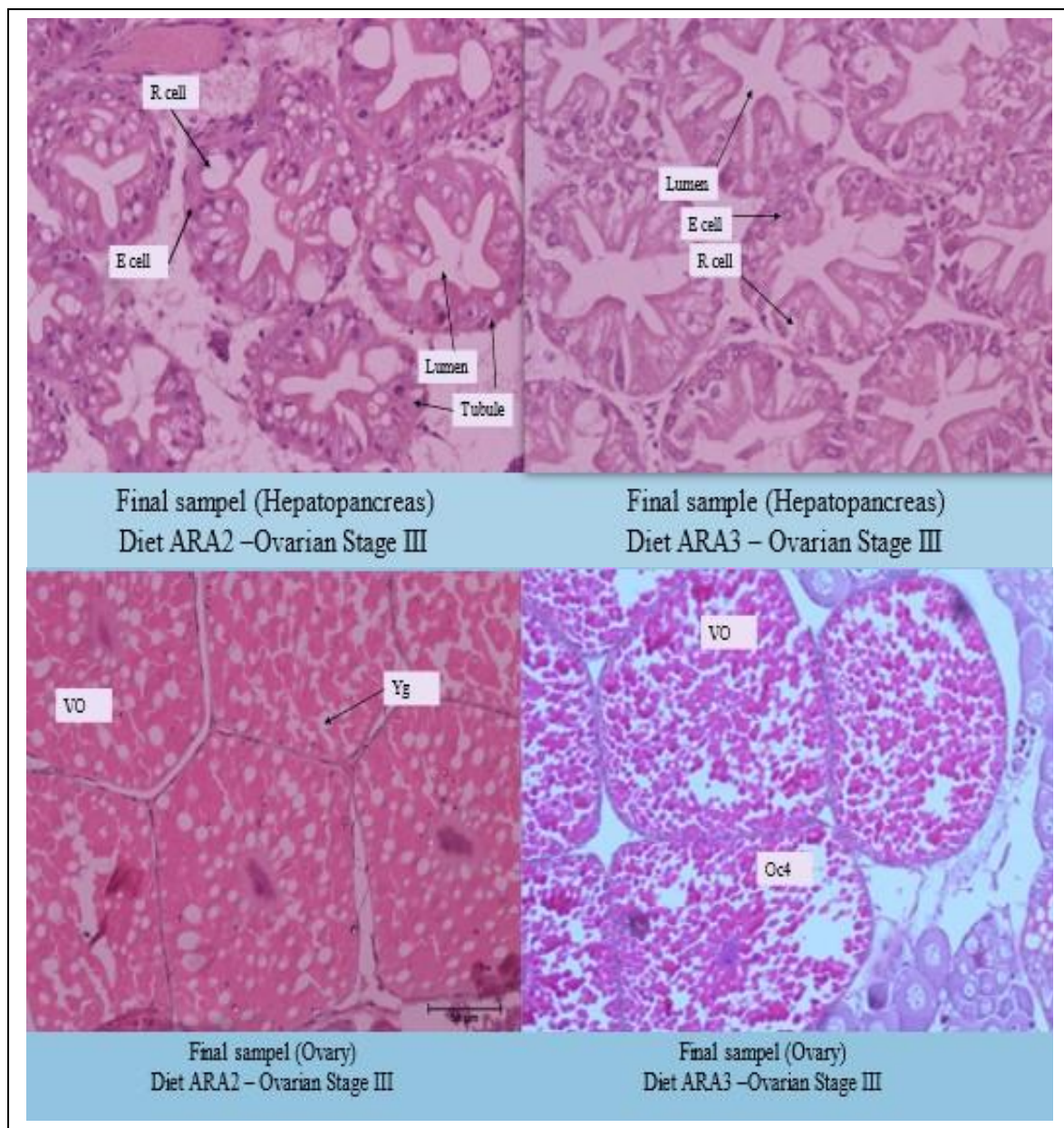


Figure 8. Representative final histological sample of ovary and hepatopancreas for treated female broodstock fed the diets T2 and T3 diets at ovarian stage III (20 x magnification) VO = vitellogenic oocyte, Yg = yolk granules, Oc4 = mature oocytes

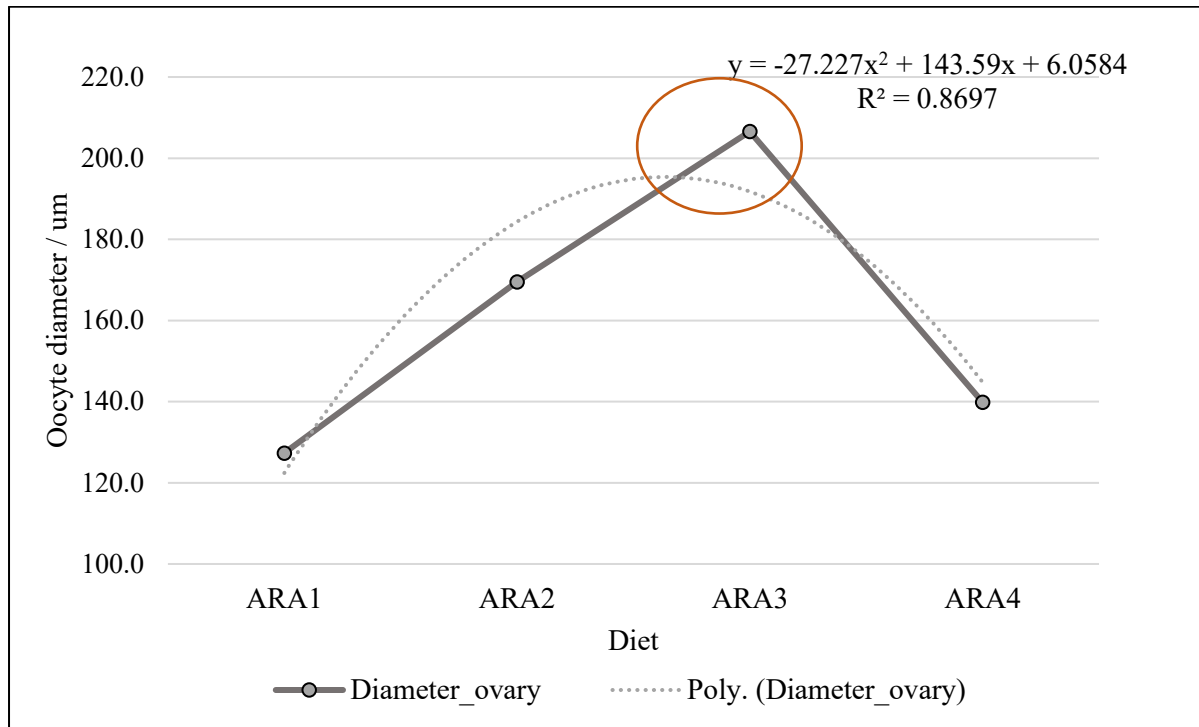


Figure 9. The relationship between oocytes diameter and ARA content in diets for GFP female broodstock was described as second-order polynomial regression

Discussion

The supplementation of fungal oil containing ARA at different levels (0.03, 0.35, 0.70, and 1.17 %) resulted in a significantly higher GSI, particularly at 0.70 % ARA. Nutrient storage in the hepatopancreas is transported to the muscle, gonads, and other tissues during the growth and reproductive stages of prawns (Jiang et al., 2009; Wang et al., 2014). Thus, the nutrients (particularly, fatty acids) stored in the hepatopancreas were found to be reduced at the last stage of ovarian development. The recorded total HSI and GSI reflected the ARA levels in the diets. This can be correlated with the gonad index stage and the oocyte size of the treated broodstock.

Similar results were reported by Kangpanich et al. (2016), where the increasing level of ARA in the diet resulted in increasing GSI and HSI values for *M. rosenbergii* broodstock. Other studies also reported the increasing value of GSI with increasing ARA in the diets of tiger shrimp (*P. monodon*) (Coman et al., 2011), Pacific white shrimp (*Litopenaeus vannamei*) (Xu, Zhang, et al., 2017), red claw crayfish (*Cherax quadricarinatus*) (Shehata et al., 2020), European sea bass juveniles (*Dicentrarchus labrax*) (Torrecillas et al., 2017), and Pacific oyster (*Crassostrea gigas*) (Seguineau et al., 2011). Several researchers also reported on fish and shrimp reproductive performance with different dietary ARA levels (Meunpol et al., 2005; Hoa et al., 2009;

Norambuena et al., 2012; Chimsung, 2014; Norambuena, Estévez, et al., 2013; Norambuena, Morais, et al., 2013; Xu et al., 2017a; Zuo et al., 2018). Like in the present study, the GSI value for sea urchin fed diets of 1 % ARA and 2 % ARA showed a constant value, meaning that the higher supply of ARA in diets beyond a certain level showed no beneficial effects and can have negative effects on reproduction (Zuo et al., 2018).

The fatty acid profile of tail muscle reflected the dietary ARA content in the present study, where the ARA content increased with dietary ARA levels for all OS. A similar result was reported for *M. rosenbergii* which was fed diets of increased dietary ARA levels for all OS (Kangpanich et al., 2016). Moreover, the marine shrimp *P. monodon* showed a higher value for the percentage of spawning and the number of eggs produced when fed with a higher level of ARA diet than a lower ARA (Coman et al., 2011).

This study indicates that the fatty acid profile of the hepatopancreas and ovary of treated broodstock showed increasing value of ARA content (% of total fatty acid) with increasing dietary ARA. Similar results were reported by Kangpanich et al. (2016) on *M. rosenbergii* for hepatopancreas and ovary for all OS. Similar results were also reported for the Pacific white shrimp, *Litopenaeus vannamei*, broodstock where increased ARA content was found in the hepatopancreas and ovary as dietary ARA increased (Xu, Zhang, et al., 2017).

This present study showed that supplementation of ARA in the diets affected the fatty acid deposition in tail muscle, hepatopancreas, and ovary of treated broodstock. Consequently, studies on the requirement of LC-PUFA in freshwater prawn maturation feed could provide more insights on the improvement of gonadal development and productivity of female broodstock.

In this present study, the highest ARA level used was 1.17 % in the diet (T4), whereas Kangpanich et al. (2016) used the highest level of 0.80 % ARA. Thus, based on the results obtained from this and Kangpanich et al. (2016) studies, we can suggest that the optimal ARA level in the diet is around the range of 0.70 – 0.80 % for better gonad development, reproductive performance, and larvae production. As exhibited by the present study, groups fed with the diet of 1.17 % ARA (T4) had significantly inferior HSI, GSI, and ovary size cells compared to treated broodstock fed with other diet groups ($P < 0.05$). The results of the present study indicated that there is no additional benefit in supplementing ARA beyond 0.70 % of TFA in terms of prawn reproductive performance. Eicosanoids derived from omega 6 fatty acids tend to be pro-inflammatory, while those derived from omega 3 fatty acids tend to be anti-inflammatory, as described by Davinelli et al. (2018) and Tsoukalas et al. (2019). Thus, a higher level of ARA may

impart negative results on reproductive performance since ARA synthesizes pro-inflammatory eicosanoids.

ARA is a precursor of signalling molecules necessary for crustacean reproduction and prostaglandins E and F of series II (PGE2 and PGF2 α) in the cyclooxygenase pathway. PGE2 production in the ovaries of *M. rosenbergii* was reported to be reflected by the retention of ARA in ovarian tissues throughout the maturation stages (Goodall, 2016; Kangpanich et al., 2016). Although the PGE2 level was not analysed in the present study, the retention level of ARA in ovaries increased for prawn broodstock fed with increasing dietary ARA diets for all OS. The ovarian ARA levels obtained from the present study were comparable to those detected in ovaries at different OS of *M. rosenbergii* (Kangpanich et al., 2016) and *L. vannamei* (Xu, Zhang, et al., 2017). The supplementation of ARA in diets did affect the prostaglandin synthesis (Goodall, 2016). The role of ARA in improving reproductive performance with the direct utilisation of ARA as the primary precursor for the synthesis of eicosanoids, namely series II prostaglandins, bioactive lipid mediators with an essential role in the pathway of reproduction in invertebrates (Wimuttisuk et al., 2013; Kangpanich, 2016; Thongbuakaew et al., 2021). The pathway of ARA serves as a precursor for the synthesis of eicosanoids and LC-PUFA is described by Wimuttisuk et al. (2013), Trevor et al. (2003), and Goodall (2016) in their studies.

Meanwhile, based on the histological analysis, the oocyte from the initial sample was at the primary stage with a diameter size of 41.50 μm , which is similar to that described by Martins et al. (2007) and Soonklang et al. (2012). The final samples showed the oocytes diameter of 127.3 – 206.5 μm with the biggest oocytes recorded in treated broodstock fed with T3 diet, and was significantly higher than other treatment groups ($P < 0.05$). All oocytes samples final size were in the development stage of vitellogenic oocytes (Martins et al., 2007). The final sample of oocyte diameter suggested an optimal ARA content in the diet is approximately 0.7 %, resulting in a bigger size compared to other diets. This finding can improve the size of newly hatched larvae generated by domesticated broodstock of *M. rosenbergii*, which were previously claimed to be smaller than those produced by wild broodstock (per comm with local hatchery managers). The smaller newly hatched larvae require special attention, and the artemia used must be small enough for these tiny larvae. By offering *M. rosenbergii* broodstock a maturation diet containing around 0.7 % ARA, the quality of larvae from domesticated broodstock can be improved and comparable to wild broodstock larvae.

The biological evaluation data showed that a dietary ARA level of 0.70 % exhibited better reproductive performance than other diets. Meanwhile, the fatty acid analyses showed that with higher dietary ARA level, the ARA content in tail muscle, hepatopancreas, and ovary of treated broodstock was also higher. Additionally, the histological evaluation revealed that dietary 0.70

% ARA resulted in significantly bigger oocytes in the ovary among treated broodstock fed with various experimental maturation diets ($P < 0.05$).

Conclusion

The results obtained from the present study suggest a dietary level of ARA that promotes the best reproductive performance, with the ovary development of female GFP at 0.70 %. A higher level of ARA (1.17 %) in the diet showed a counterproductive result for the reproductive performance of female GFP. Therefore, it is suggested that the optimal concentration of ARA in the diet should be maintained around 0.70 %.

Recommendation

The optimal inclusion level of ARA in the maturation diet for GFP broodstock has been determined. However, the price of *Mortierella* sp fungi, the source of ARA used in this experiment, is relatively high for the time being. Thus, it is imperative to look for cheaper sources of ARA, for example, alga (*Parietochloris incisa*), polychaeta and terrestrial animal liver, such as bovine liver meal.

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Conflict of Interest

The author(s) do not have any conflict of interest.

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Distribution of EDGE Coral Species and Community Perceptions of Reef Conservation in Semporna, Sabah, Malaysia

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Abstract

The Semporna Priority Conservation Area (PCA) in Sabah, Malaysia, is a Coral Triangle biodiversity hotspot increasingly threatened by destructive fishing, coastal development, and climate change. To address these challenges, this study assessed the distribution of Evolutionarily Distinct and Globally Endangered (EDGE) corals across ten islands alongside community conservation attitudes. Underwater surveys recorded six EDGE species, with *Heliofungia actiniformis* being the most abundant (n = 123 colonies), especially at Sebangkat, Larapan, and Sibuan. Conversely, *Nemanzophyllia turbida* and *Lithophyllon ranjithi* were entirely absent. Overall, EDGE corals were largely concentrated in the central PCA, an ecologically vital but heavily impacted zone. Concurrently, household surveys (n = 47) across four islands revealed significant variations in environmental awareness (p = 0.003), with Larapan scoring the lowest. While awareness of fish bombing was uniform across the sites, Bum Bum residents demonstrated a significantly higher willingness to combat the practice (p = 0.041). Synthesizing these results identifies Larapan as the most critical site for species-specific conservation, whereas Selakan and Bum Bum present strong opportunities for community-led engagement. These findings highlight the critical need to integrate ecological data with local social perceptions to prioritize conservation effectively. Establishing Locally Managed Marine Areas (LMMAs) in Semporna is recommended to safeguard threatened corals and empower local stewardship, directly contributing to Malaysia's biodiversity goals, the Global Biodiversity Framework's 30×30 target, and the Coral Triangle Initiative.

Keywords: Evolutionarily Distinct and Globally Endangered corals, community managed area, Coral Triangle, community awareness, coral reef conservation

Introduction

Coral reefs are among the most diverse and productive ecosystems on Earth. They provide goods and services to humans, including fishery resources, coastal protection, recreational opportunities, cultural benefits, and potential medical applications (Good & Bahr, 2021). However, reefs are increasingly threatened by local pressures such as overfishing and sedimentation (Andrello et al., 2022; Burke et al., 2012; Hughes et al., 2007), destructive fishing (Pet-Soede, 1998), coastal development and live coral harvesting (Bruckner, 2000; Wabnitz et al., 2003). On a global scale, climate change threatens reef health by intensifying coral bleaching and disease events (Hughes et al., 2004). Collectively, these stressors are driving reefs into degraded states (Bellwood et al., 2004; Hughes et al., 2007; Bruno et al., 2009), reducing coral species richness and associated fauna such as fish, echinoderms, and crustaceans. Consequently, conservation of tropical reef systems is a global priority (Roberts et al., 2002). To set priorities and guide conservation responses to these escalating threats, global assessment tools such as the IUCN Red List and the Evolutionarily Distinct and Globally Endangered (EDGE) framework have become essential.

The IUCN Red List of Threatened Species remains the global standard for assessing species extinction risk and setting conservation priorities (Rodrigues et al., 2006). However, its reliance on threat-based criteria does not account for evolutionary distinctiveness (Possingham et al., 2002; Redding & Mooers, 2006). To address this, the Evolutionarily Distinct and Globally Endangered (EDGE) framework integrates extinction risk with evolutionary uniqueness to identify species of high conservation importance (Isaac et al., 2007). Among reef-building corals (Scleractinia), one-third of species were previously assessed as threatened (Carpenter et al., 2008) but the most recent global reassessment by the IUCN, announced at COP29 in Baku, Azerbaijan (November 2024) indicates that 44% are now at risk of extinction (IUCN, 2024). EDGE analyses further highlight coral lineages facing the highest risk (Huang, 2012; Curnick et al., 2015). However, translating these global priorities into effective local conservation remains a challenge. Fine-scale mapping of EDGE coral species is still scarce, particularly in Southeast Asia, limiting the ability to align global assessments with site-specific management.

Malaysia harbours some of the most biodiverse coral reefs in the world, concentrated in Sabah within the Sulu-Sulawesi Marine Ecoregion (SSME) and the Coral Triangle (Veron, 2009; WWF-SSME, 2004). The Semporna Priority Conservation Area (PCA) is a globally significant hotspot, hosting diverse reef types and exceptional species richness, including several EDGE coral taxa (Waheed & Hoeksema, 2013; Choo et al., 2021). The reefs generate an estimated RM 185 million in gross annual value (Yeo, 2011). Yet they are under intense anthropogenic

pressure (Choo et al., 2025). Destructive fishing practices such as blast and cyanide fishing threaten 85% of reefs nationally (Burke et al., 2012), while land-based pollution, sedimentation, overharvesting, and rapid tourism growth have reduced coral cover and reef resilience (Ho & Kassem, 2009; Choo & Sumampouw, 2014). Mean live coral cover in Semporna declined from 35.7% in 2008 to 30.4% in 2013, with low abundances of commercially valuable reef fish and invertebrates (Ho & Kassem, 2009; Choo & Sumampouw, 2014). In addition, the distribution and status of EDGE coral species remain poorly documented. These findings underscore the urgency of targeted conservation measures in this region.

Marine Protected Areas (MPAs) remain the most widely applied conservation tool, offering ecological benefits such as safeguarding threatened species, protecting habitats, reducing overfishing and enhancing fisheries through spillover effects (Ban et al., 2011; Zupan et al., 2018). Large-scale protection is ideal, but full protection of all areas is unrealistic where millions of people rely directly on marine resources for subsistence and livelihoods (Foale et al., 2013). Complementary approaches such as Other Effective Area-Based Conservation Measures (OECMs) and Locally Managed Marine Areas (LMMAs) are increasingly recognised for their emphasis on local governance, cultural values, and socio-economic needs, often achieving higher compliance and cost-effectiveness (Govan et al., 2008; 2009; Jupiter et al., 2014; Estradivari et al., 2022). While similar to MPAs, LMMAs typically operate on a smaller spatial and temporal scale (Mills et al., 2010). Although the small size of LMMAs has raised concerns about effectiveness, particularly for critical habitats such as grouper spawning areas (Waldie et al., 2016), networks of LMMAs have demonstrated fisheries benefits (Rocliffe et al., 2014) as well as social and economic benefits (Syakur et al., 2012).

The longevity of conservation efforts depends strongly on local community support. Their perceptions of ecological effectiveness, social impacts, and good governance are positively correlated with their support for conservation (Bennett et al., 2019). However, local perceptions of ecological conditions may be inaccurate, depending on their level of awareness and knowledge (Christie, 2005). For instance, in the Philippines, communities perceived higher fish abundance inside and outside MPAs, while fish censuses showed only moderate improvements in quantity, size, and diversity (Yasué et al., 2010). Understanding community willingness to engage in conservation is therefore crucial for the success of MPAs and LMMAs (Jupiter et al., 2014).

The study aims to 1) investigate the distribution and abundance of EDGE coral species, 2) evaluate knowledge levels among nearby island populations, and 3) identify islands that should be prioritised for future LMMA establishment in the Semporna PCA. Such locally governed measures could contribute to a wider network of LMMAs in Sabah.

Materials and Methods

Study Site

The Semporna PCA is located in Malaysia, northeast of Borneo Island, spanning 7,680 km². The seascape encompasses diverse coral reef geomorphology (Kassem et al., 2011), including a barrier reef, fringing reefs, patch reefs, a pre-atoll, and an oceanic seamount (Sipadan Island). Semporna PCA harbours the highest concentration of coral reefs in Malaysia (Choo et al., 2021) and is recognised for its high biodiversity, with mushroom corals used as a proxy (Waheed & Hoeksema, 2013).

State jurisdiction covers Sabah's territorial waters (3 nautical miles), including the reefs of Semporna PCA. The Department of Fisheries Sabah regulates extraction, harvest, and export licences of corals (Section 43), while the Land Ordinance Sabah also governs coral extraction (Sections 23 and 23A). Two state parks exist within the Semporna PCA: Tun Sakaran Marine Park (340 km²) and Sipadan Island Park (168 km²), both managed under the Park Ordinance.

Target Species

The Zoological Society of London (ZSL) developed the EDGE corals list of threatened scleractinian species (Curnick et al., 2015). Based on the Semporna Marine Ecological Expedition in 2010, which focused on three coral families (see Kassem et al., 2012; Waheed & Hoeksema, 2013), six EDGE coral species have been reported in Semporna's waters (Table 1): *Heliofungia actiniformis*, *Lithophyllon ranjithi*, *Nemanzophyllia turbida*, *Physogyra lichtensteini*, *Catalaphyllia jardinei* and *Pachyseris rugosa*.

Underwater Surveys

Field surveys were conducted in April–May and June–July 2017 at sites where threatened coral species were previously reported in the SMEE report (Kassem et al., 2012). Prior to data collection, dive operators received two training dives and assigned specific supporting roles, including laying transect lines with bearings provided, acting as safety divers, and deploying surface buoys. Ecological data were collected by trained biologists using non-destructive techniques. Belt transects (50 m x 1 m) were laid to assess the abundance and distribution of target corals (Hoeksema & Borel-Best, 1984). Transects were conducted at three depths, 6, 9 and 12 m (lagoon, crest, and outer slope) where possible, up to three sites per island, with sites separated by at least 500 m. Surveys covered 10 islands: Adal, Batik, Timbun Mata, Larapan, Sebangkat, Kulapuan, Sibuan, Bodgaya, Bait, and Bum Bum (Figure 1).

Table 1. IUCN categories and EDGE scores of coral species identified in Semporna (summarised from previous studies) with notes on coral population and habitat from IUCN (2025).

Family	Species	Previous records and distribution in Malaysia	IUCN Category	EDGE Score	Notes
Fungiidae	<i>Heliofungia actiniformis</i> Quoy and Gaimard, 1833	Wood & Tan (1987), Wood (1994), Ditlev <i>et al.</i> (1991), Waheed & Hoeksema (2013) Found throughout Malaysia	Least Concern	3.81	• Common species • Free-living single polyp • Widely found on reef flats and reef slopes • Prefers soft or rubble substrates, especially in lagoons or shallow turbid environments • Depth range: 1–40 m • Average generation length: 10 years • Mature polyps may grow up to 21 cm
	<i>Lithophyllon ranjithi</i> Ditlev, 2003	Waheed & Hoeksema (2013) Only documented in Sabah, not in Peninsular Malaysia	Data Deficient	4.07	• Rare species • Very restricted range: northeast Borneo (Malaysia and Indonesia) and Spratly Islands • Found in nearshore coastal reefs and oceanic offshore reefs • In Darvel Bay: common below 10 m on steep hard substratum • Depth range: 10–20 m • Average generation length: 10 years

Table 1. continued.

Plerogyridae	<i>Nemanzophyllia turbida</i> Hodgson and Ross, 1982	Ditlev <i>et al.</i> (1991), Waheed & Hoeksema (2013) Only documented in Sabah, not in P. Malaysia	Least Concern	3.91	• Rare but conspicuous species • May form large carpets and can be locally abundant • Found in shallow, sheltered, turbid reefs • Depth range: 8–40 m • Average generation length: 10 years
	<i>Physogyra lichtensteini</i> (Milne Edwards and Haime, 1851)	Wood & Tan (1987), Wood (1994), Ditlev <i>et al.</i> (1991), Waheed & Hoeksema (2013) Found throughout Malaysia	Least Concern	3.98	• Occurs in shallow tropical reefs, often in turbid environments • Can be found across most reef habitats • Depth range: 1–38 m • Average generation length: 10 years • Mature colonies may reach 3 m diameter
Merulinidae	<i>Catalaphyllia jardinei</i> (Saville-Kent, 1893)	Ditlev <i>et al.</i> (1991), Waheed & Hoeksema (2013) Only documented in Sabah, not in Peninsular Malaysia	Least Concern	4.23	• Rare but widespread species • Found in shallow, sheltered, turbid reefs • Occurs in various reef types; prefers soft sandy substrates over dense coral growth • Depth range: 0–55 m • Average generation length: 10 years • Mature colonies may reach 100 cm diameter
Scleractinia <i>incertae sedis</i>	<i>Pachyseris rugosa</i> (Lamarck, 1801)	Wood & Tan (1987), Wood (1994), Ditlev <i>et al.</i> (1991), Waheed & Hoeksema (2013) Found throughout Malaysia	Least Concern	3.95	• Widespread and common species • Found in shallow waters • May forms large mound-shaped colonies • Smaller colonies distributed across a wide range of habitats, including strong current areas • Depth range: 2–75 m (most common at 9–20 m) • Average generation length: 10 years

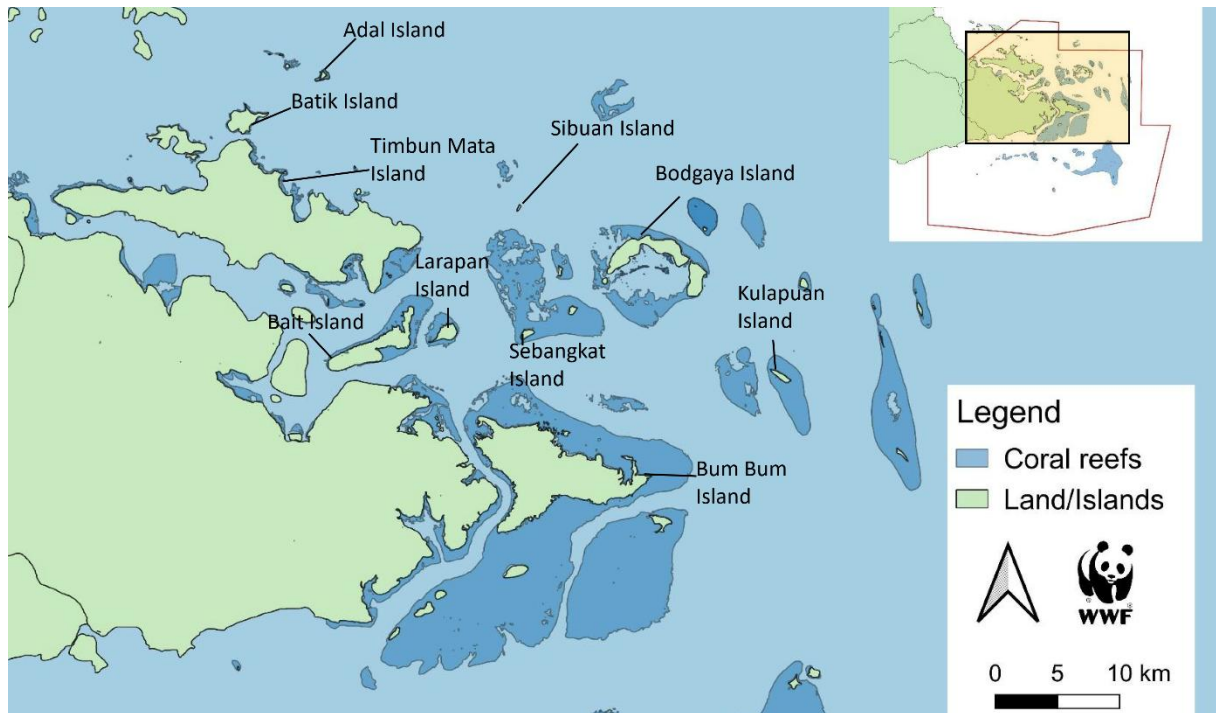


Figure 1. EDGE coral survey sites in the Semporna Priority Conservation Area (PCA)

GPS location, bearings, sea condition and tide levels at each site were recorded. In total, 69 belt transects were completed (Appendix A). Colonies of targeted species were photographed with a reference scale, and data on size, associated organism and coral condition were collected to quantify the health, size structure, and ecological relationships of the targeted species, as well as to detect early signs of stress or disease. Coral identification followed guides in situ records (Hoeksema, 1989; Veron, 2000), and where necessary, ex situ using photographs to verify species that were difficult to identify underwater. At each site, different transects were surveyed by two biologists, and species identifications were subsequently cross-checked to minimise observer bias and ensure taxonomic accuracy.

Distributions of the targeted species were mapped using QGIS, producing heat maps of occurrence based on data from Kassem et al. (2012). Abundance mapping was possible for *H. actiniformis* and *P. rugosa* only as other species were either absent or encountered very few times (≤ 6 individuals) to generate meaningful maps.

Local Community Perception of Reefs

Community surveys were conducted on four islands (Bum Bum, Denawan, Larapan, and Selakan-Sebangkat) with the permission and support of village heads. Site selection depended on village leaders' approval. Respondents were primarily household breadwinners, as they play a central role in livelihood decisions. Despite efforts to include women and youth, most declined participation.

Consequently, 46 respondents were adult men and one was an adult woman, all with primary responsibility for household support.

The interviews assessed local awareness of environmental condition of Semporna reefs, knowledge of fish bombing, and willingness to support efforts to curb fish bombing (Appendix B). Scoring matrix for the questionnaire is provided in Appendix C. Verbal consent was obtained from all participants and their respective village leaders.

Socio-demographic Characteristics of Respondents

Respondents belonged to Bajau, Bajau Sama, and Bajau Laut communities, groups often associated with illegal and destructive fishing practices such as blast and poison fishing (Clifton & Majors, 2012; Pet-Soede & Edrmann, 1998). Traditionally seafaring, some Bajau remain nomadic, travelling long distances across the Sulu-Celebes region to exploit remote reefs. Many now settle in permanent villages and abandon destructive practices once local reefs degrade.

Most respondents reported monthly incomes below RM500 (74.47%), similar to Yusah et al. (2018) but higher than values reported over a decade earlier Wood (2001). Fishing was the primary occupation (68.1%), with 23.4% combining fishing with other work such as house cleaning, woodworking, or security. A minority (8.5%) were homemakers, small business owners, 23.4% or government employees. The respondents were breadwinners for their families, with ages ranged from 21 to 72 years old, with men predominating. A copy of the questionnaire is provided in Appendix B.

Data Analysis

Responses were analysed using Pearson's chi-square test to evaluate general awareness, awareness of fish bombing, and attitudes toward fish bombing and conservation. Likelihood ratio tests were applied to examine response tendencies and potential biases by island towards fish bombing. Results of ranking of the awareness and altitude levels are presented in comparison tables.

Results

Distribution of EDGE Corals

The distribution and richness of the EDGE coral species were primarily concentrated in the centre region of the Semporna PCA (Figure 2, Appendix A). Diversity and abundance declined with increasing distance from the coastline, particularly the mushroom coral *H. actiniformis* (Figure 3).

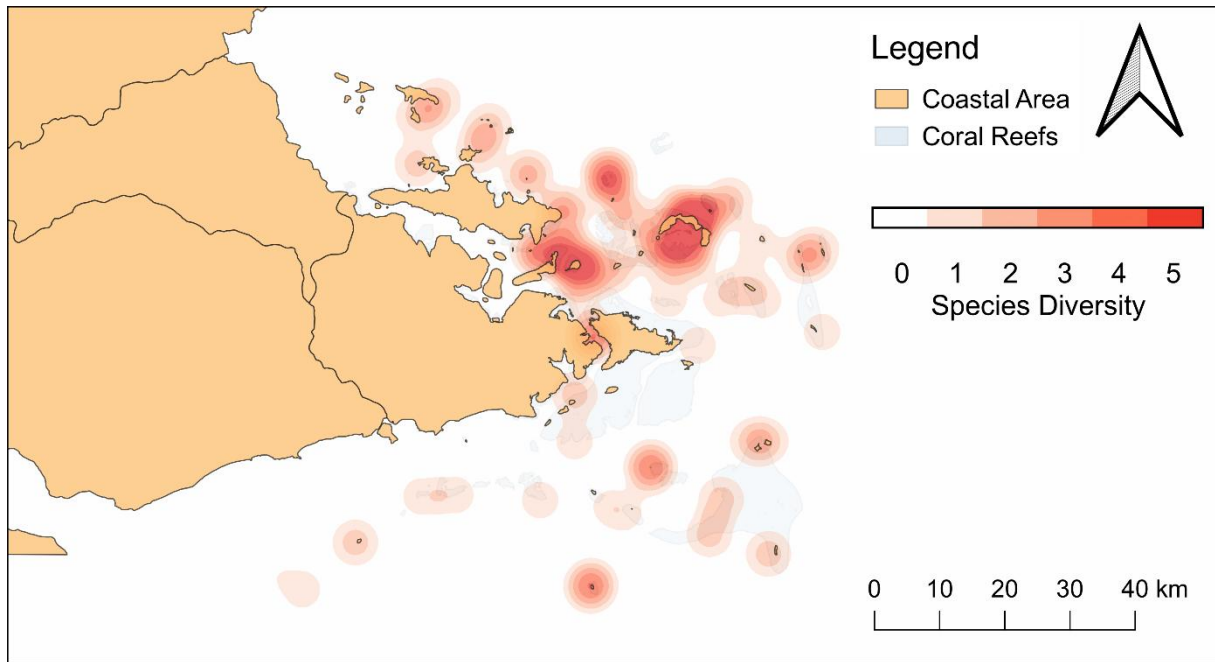


Figure 2. Heat map showing overall distribution of all six EDGE coral species in the Semporna PCA

Abundance of EDGE corals

A total of 123 colonies of *H. actiniformis* were recorded. Sebangkat Island (26.8%), Larapan Island (20.3%), and Sibuan Island (20.3%) hosted the highest number of colonies (Figure 3). No colonies were found at Adal Island. Colony size ranged from 3.2 cm to 26.4 cm, with a median size of 9.8 cm. Approximately 15.5% of colonies supported associated fauna such as small fish, shrimps, and sea stars, most frequently observed at Timbun Mata Island. Most coral colonies were in healthy condition, with less than 1% showing signs of disease.

For *P. rugosa*, 112 colonies were recorded, ranging from 7 cm to 128 cm in size (mean diameter 17.55 cm). Unlike *H. actiniformis*, this species was more evenly distributed across the surveyed islands (Figure 4). The highest proportion of colonies occurred at Timbun Mata (19.64%), followed by Sebangkat (16.07%), Batik (13.39%), and Sibuan (12.5%). As an encrusting coral, *P. rugosa* often occupies reef areas dominated by soft corals, which may explain its broader distribution and consistent presence across islands

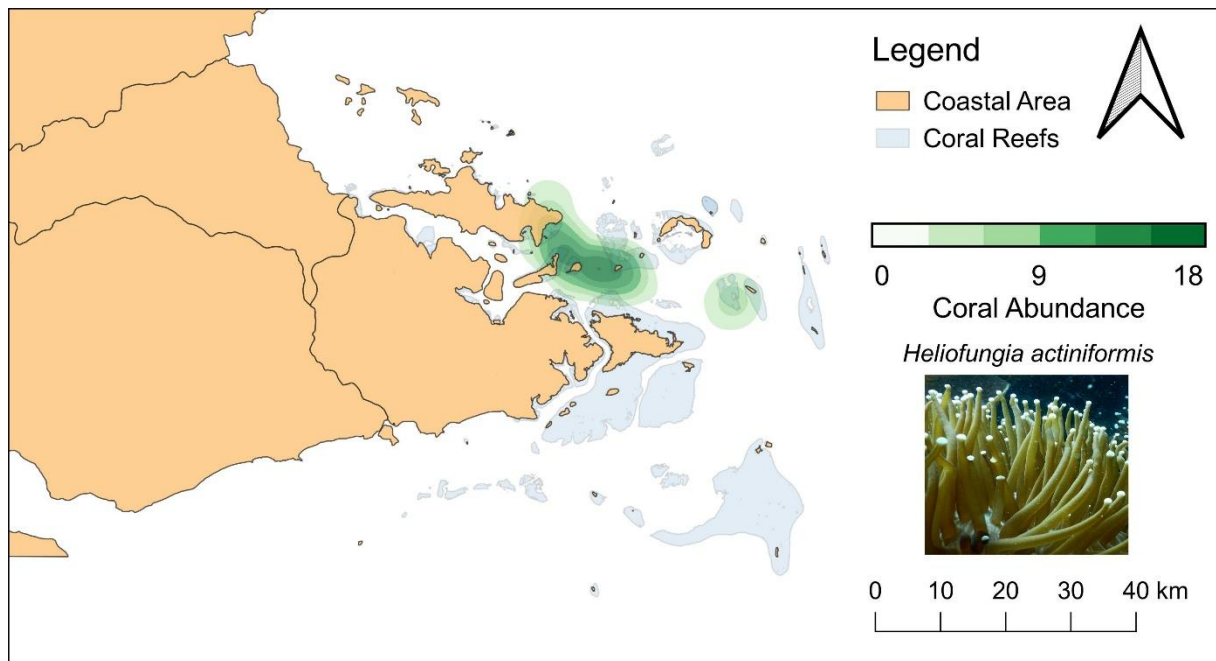


Figure 3. Heat map showing the abundance of *Heliofungia actiniformis* in the Semporna PCA

For other species of EDGE corals, only six colonies of *Physogyra lichtensteini* (mean diameter 17.22 cm), and four colonies of *Catalaphyllia jardinei* (mean diameter 15.75 cm) were recorded. No colonies of *Nemanzophyllia turbida* or *Lithophyllon ranjithi* were found.

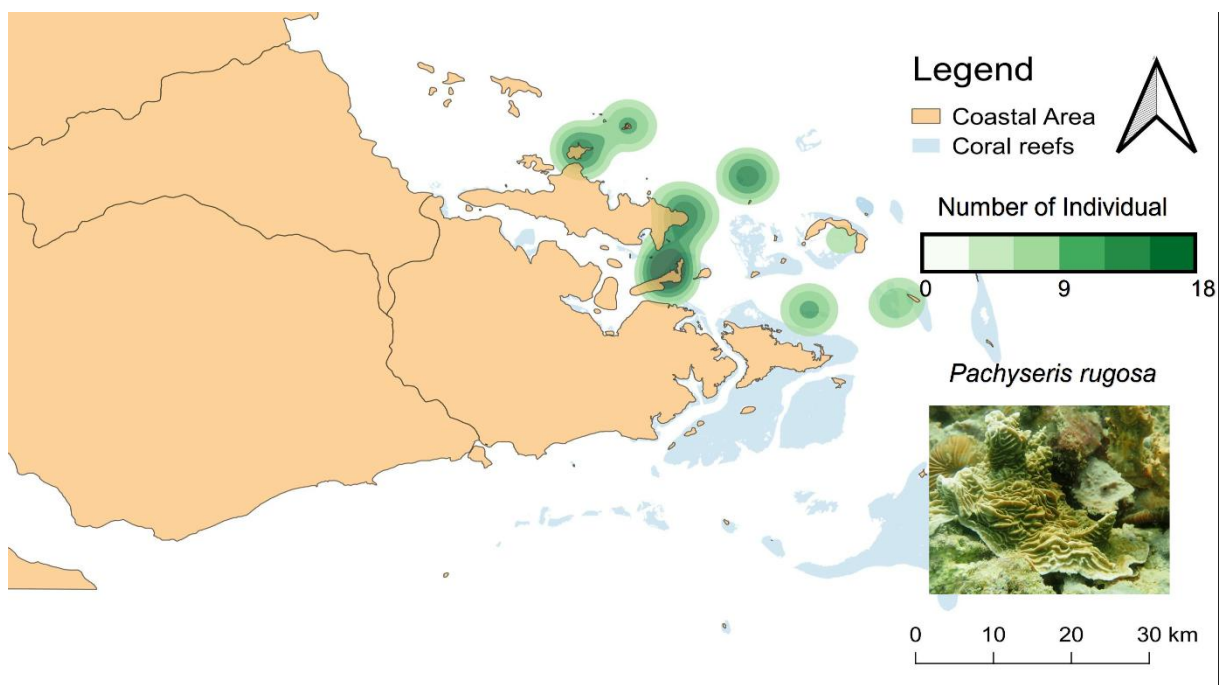


Figure 4. Heat map showing the abundance of *Pachyseris rugosa* in the Semporna PCA

Community Surveys

Environmental and Fish Bombing Awareness

Survey results revealed variation in environmental awareness and attitudes among islands (Table 2, Appendix C). Denawan had the highest level of environmental awareness with mean score of 9.67 (SE $\pm$ 0.667), followed by Bum Bum (9.30 $\pm$ 0.731), Selakan (9.00 $\pm$ 0.572), and Larapan (7.07 $\pm$ 0.579). These scores suggest that environmental changes might be more visible on Denawan Island compared to other islands. Conversely, Larapan scored highest for fish bombing awareness (16.07 $\pm$ 0.57), followed by Denawan (15.75 $\pm$ 0.63), Selakan (14.36 $\pm$ 0.78), and Bum Bum (13.80 $\pm$ 1.21).

Bum Bum respondents expressed the greatest willingness to cooperate against fish bombing (9.60 $\pm$ 0.37), exceeding Larapan (7.43 $\pm$ 0.80), Selakan (7.09 $\pm$ 0.53), and Denawan (7.08 $\pm$ 0.58). The higher mobility of the Bum Bum communities may make them more receptive to supporting new conservation initiatives.

Table 2. Summary of community survey scores for environmental awareness, fish bombing awareness, and attitudes

Island	Environmental Awareness Score	Average	Fish Bombing Awareness Score	Average	Total Attitude Score	Average
Denawan (n=12)	116	9.67	189	15.75	85	7.08
Larapan (n=14)	99	7.07	225	16.07	104	7.43
Selakan (n=11)	99	9.00	158	14.36	78	7.09
Bum Bum (n=10)	93	9.30	138	13.80	96	9.60

Perceived Environmental Change and Causes

Overall, 68% of respondents identified reef degradation as the primary environmental change observed, followed by marine resource depletion (62%), pollution (45%), beach erosion (45%), seagrass degradation (28%), and groundwater salinisation (4%). Respondents from Bum Bum diverged from this trend, identifying pollution as the dominant concern.

All respondents attributed reef degradation primarily to destructive fishing practices (fish bombing and poison fishing). Additional causes cited included anchor damage (43%), crown-of-thorns outbreaks (36%), marine debris (34%), and hook-and-line/net fishing (32%). A few respondents (15%) also attributed reef health decline to tourist-related impacts, including diving and snorkelling.

Fish Bombing Hotspots and Practices

Generally, island communities identified destructive fishing methods such as fish bombing (74%), poison fishing (57%), and gleaning (21%), as the main contributors to reef degradation on their respective islands. Fish bombing was reported widespread in the Semporna PCA. The central area of the PCA around Bum Bum and Sebangkat, as well as Ligitan reefs in the southeast recorded the highest reports of bombing (40% each), followed by the north around Larapan, Timbun Mata, Baik and Adal (26%).

Most respondents (91.5%) were aware that fish bombing is illegal. Incidents were most frequently reported in the morning (68%), evening (57%), and at noon (51%), with one respondent reporting night-time occurrences. Pump boats (81%) were the most commonly identified vessels, followed by engine boats (55%), sampans (34%), and outrigger canoes or 'bangka' (11%). Fish bombing was typically conducted in groups (74%) rather than individually (19%). More than 80% of respondents reported witnessing reef damage from fish bombing, with 32% hearing blasts almost daily, 21% monthly, 13% yearly, and 4% never.

Attitude Towards Combating Fish Bombing

Community support for anti-fish-bombing initiatives was high. All respondents agreed that bombing should cease, and 95.7% were willing to support prevention efforts. Many expressed willingness to join patrols (89.4%), cooperate or participate in training on fish bombing issues (85.1%), discourage friends and family from purchasing bombed fish (72%), report incidents to enforcement agencies (68%), or support anti-fish bombing campaigns (66%). In addition, 62% favoured the establishment of patrolling and monitoring teams, while 47% were interested in training to become patrollers. Fewer respondents were willing to provide financial support (9%).

Regarding enforcement agencies' presence, 79% of respondents observed marine police patrols on their island, followed by the army at 68%, the Malaysian Maritime Enforcement Agency at 51%, and the Department of Fisheries and Sabah Parks, each observed by only one respondent. When asked about potential solutions, 85% of respondents perceived increased enforcement as crucial, followed closely by strengthened regulations at 81%. Additionally, 70% emphasized the importance of awareness-raising, while 64% highlighted the significance of information sharing in combating fish bombing.

Priority Sites for Conservation

Statistical analysis of awareness indicators across 47 respondents (Table 3) produced three main outcomes. First, environmental awareness differed significantly among islands ($\chi^2 = 13.808$, $df = 3$, $p = 0.003$), with Larapan significantly lower than others. Second, fish bombing awareness showed no significant differences ($\chi^2 = 3.245$, $df = 3$, $p = 0.355$), suggesting that awareness of fish bombing is similar across all islands. Third, attitudes towards combating fish bombing varied ($\chi^2 = 8.254$, df

= 3, $p = 0.041$), with Bum Bum showing significantly more positive attitudes toward combating fish bombing. Overall, the findings suggest that while fish bombing awareness is fairly uniform across the islands, general environmental awareness and anti-fish-bombing attitudes differ, especially on Larapan and Bum Bum islands.

Table 3. Results of Pearson's chi-square tests comparing general environmental awareness, fish bombing awareness, and attitudes towards fish bombing

Awareness Score	N	Pearson Chi-Square	<i>df</i>	Asymptotic Significance (2-sided)	Remarks
General environmental awareness	47	13.808	3	0.003	Level of general awareness of Larapan community is significantly lower than other islands.
Awareness on fish bombing	47	3.245	3	0.355	Level of fish bombing awareness for all islands has no significantly differences.
Attitude toward combating fish bombing	47	8.254	3	0.041	Level of attitude towards fish bombing of Bum Bum island is significantly higher than other islands.

The ranking (Table 4) further highlights Larapan as the most ecologically significant site, hosting all six EDGE coral species and high abundances of *H. actiniformis*. Selakan and Bum Bum also support diverse species, though at lower abundances, while Denawan ranked lowest. Overall, the findings indicate that Larapan is the most significant site for species conservation, while Selakan and Bum Bum provide additional ecological support that can aid in the connection and resilience of coral populations in Semporna.

Table 4. Rankings of the four study islands based on EDGE coral distribution and abundance, and community survey scores for environmental awareness, fish bombing awareness, and attitudes (with statistical results in Table 3). Y=Yes and N=No

Island	Denawan	Larapan	Selakan	Bum Bum
Distribution				
H. actiniformis	Y	Y	Y	Y
P. rugosa	N	Y	Y	Y
P. lichtensteini	Y	Y	Y	Y
C. jardinei	N	Y	N	N
N. turbida	N	Y	N	N
L. ranjithi	N	Y	N	N
Abundance				
H. actiniformis	N	Y	Y	N
P. rugosa	N	N	N	N
P. lichtensteini	N	N	N	N
C. jardinei	N	N	N	N
N. turbida	N	N	N	N
L. ranjithi	N	N	N	N
Level of awareness (General)	Average	Low	Average	Average
Level of awareness (Fish bombing)	Average	Average	Average	Average
Attitude (Fish bombing)	Average	Average	Average	High

Discussion

This study identified areas of high EDGE coral diversity and abundance, revealing gaps in protection within the Semporna PCA. EDGE corals were concentrated in the central region, which lies partly outside Tun Sakaran Marine Park and is subject to intense pressures including fish bombing, marine debris, and coastal development (Choo et al., 2025). Strengthening conservation efforts in these areas, especially in the central region of Semporna PCA, is crucial to mitigating anthropogenic pressures and ensuring the effective preservation of coral biodiversity.

Fish bombing persists due to economic pressures, demand for rapid catches, limited enforcement, and lack of livelihood alternatives (Wood, 2006; Nina & Kassem, 2009; Kassem et al., 2012). Similarly, marine debris stems from inadequate waste management, population growth, and reliance on single-use plastics in tourism-heavy regions (MPMP, 2022; Tan et al., 2022). Both threats are exacerbated by gaps in education and awareness, limited community involvement in resource management, and the lack of effective enforcement mechanisms. Addressing these threats requires not only strengthening law enforcement but also fostering

community engagement, improving waste management systems, and exploring sustainable fishing practices to mitigate the pressures on marine ecosystems.

The relatively low prevalence of EDGE corals in surveys may reflect their natural rarity, specialised habitat preferences, occurrence outside the survey depth range (6–9 m), or anthropogenic pressures such as collection for the aquarium trade. For example, *C. jardinei* is often associated with sandy bottoms in high-light environments, whereas *L. ranjithi* is typically restricted to crevices in deeper or high-current areas. As limited baseline surveys exist in Semporna, further research is required to confirm long-term trends. *Lithophyllon ranjithi* illustrates this challenge. Previously assessed as Vulnerable under the Red List D2 criterion due to its highly restricted distribution, it is now listed as Data Deficient, reflecting uncertainty about its true status (Waheed et al., 2024). Knowledge of its population size, distribution and trends remains insufficient to make a definitive assessment. Species with limited ranges are particularly susceptible to localised impacts or unpredictable events (IUCN, 2001), and *L. ranjithi* may still face such risks. This highlights a broader challenge: limited population and distribution data for many coral species hampers systematic conservation planning and may mask the vulnerability of narrowly distributed taxa (Harvey et al., 2021). On the other hand, *H. actiniformis*, the most dominant EDGE species is a free-living coral and often abundant on reefs because it thrives on unconsolidated substrates such as rubble and sand, where many other corals cannot persist (Knittweis et al., 2009; Samimi-Namin et al., 2025). Its resilience in sediment-stressed habitats, combined with its capacity to support diverse associated fauna, further contributes to its ecological success and high local abundance (Hoeksema et al., 2019).

Community surveys indicated strong awareness of destructive fishing and willingness to support conservation through LMMAs in Semporna. Although these community initiatives may not always align perfectly with formal conservation objectives, they support the concept of sustainable resource management. Similar outcomes have been reported in Myanmar, where community support for LMMAs was high and dynamite fishing declined within managed areas (Thiha et al., 2023). Such findings highlight the value of community participation in conservation (Ferse et al., 2010). However, it should be noted that most respondents were men, which may have overlooked women's perspectives. In Bajau communities, gender roles shape environmental knowledge and management priorities, and women often contribute unique insights into resource use (Torell et al., 2020; Nessa et al., 2020). Future studies should therefore aim for a more gender-balanced approach to better reflect community-wide perceptions.

Larapan emerged as a priority site due to high coral diversity and community awareness. Its proximity to existing marine parks further enhances the feasibility of establishing an LMMA. Bum Bum, with its positive community attitudes, may also serve as a potential model site,

though it holds lower conservation priority than Larapan due to lower EDGE coral diversity. The development of LMMA networks requires strong local stewardship (Syakur et al., 2012; Jupiter et al., 2014), and Larapan could serve as a pilot for expanding community-based protection across Semporna.

International benchmarks such as the IUCN Red List can strengthen conservation by providing a standardised framework to guide national and local priorities. The case of *L. ranjithi* illustrates how global assessments can draw attention to species of concern, emphasising the need for complementary national protection measures. EDGE species with restricted ranges, in particular, should be formally recognised as threatened and afforded legal protection within Malaysia.

Conclusion

This study documented the distribution and abundance of EDGE coral species and assessed community awareness in the Semporna PCA. EDGE corals, including *H. actiniformis* and *P. rugosa*, were concentrated in the central region, partly outside existing protected areas, highlighting conservation gaps and the need for deeper, habitat-specific surveys. Community surveys showed strong awareness of destructive fishing and widespread support for LMMAs, with Larapan emerging as the priority site due to its high coral diversity and readiness for community-based management. Bum Bum also demonstrated potential as a complementary model site.

These findings underscore the value of integrating ecological data with community perspectives to guide conservation priorities. Strengthening LMMAs and other locally governed measures can help conserve threatened coral species, address anthropogenic pressures, and enhance governance through both formal and traditional systems. By combining ecological monitoring with active community stewardship, conservation in Semporna can support Malaysia's biodiversity targets, the Global Biodiversity Framework's 30 × 30 goal, and regional commitments under the Coral Triangle Initiative.

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Appendices

Appendix A. Survey sites across community islands in the Semporna PCA, Sabah, Malaysia, showing site name, geographic coordinates (latitude and longitude), abundance, and mean colony diameter (cm) of four EDGE coral species.

Island	Site Name	Latitude	Longitude	Total abundance				Mean Diameter (cm)			
				<i>H. actiniformis</i>	<i>P. rugosa</i>	<i>P. lichtensteini</i>	<i>C. Jardinei</i>	<i>H. actiniformis</i>	<i>P. rugosa</i>	<i>P. lichtensteini</i>	<i>C. Jardinei</i>
Adal	Adal 1	N 4.748417	E 118.520167	0	3	1	0	0	11.333	32	0
Adal	Adal 2	N 4.744444	E 118.520389	0	3	0	0	0	23.333	0	0
Adal	Adal 3	N 4.747306	E 118.513667	0	4	0	0	0	45.05	0	0
Batik	Batik 1	N 4.723861	E 118.463500	1	6	0	0	5	32.2	0	0
Batik	Batik 2	N 4.720750	E 118.450694	2	1	0	0	19	42	0	0
Batik	Batik 3	N 4.708889	E 118.465306	0	8	2	1	0	37.286	18	21
Timbun Mata	Timbun 1	N 4.621250	E 118.587472	0	5	0	0	0	55.58	0	0
Timbun Mata	Timbun 2	N 4.597194	E 118.568194	15	5	0	0	10.6	31.8	0	0
Timbun Mata	Timbun 3	N 4.640167	E 118.589056	2	8	0	0	8.4	44.5	0	0
Timbun Mata	Timbun 4	N 4.649806	E 118.563472	8	4	0	1	8.714	25.25	0	11
Larapan	Larapan 1	N 4.547528	E 118.608694	9	0	0	0	10.333	0	0	0
Larapan	Larapan 2	N 4.574306	E 118.604167	16	3	0	0	10.32	16.333	0	0
Larapan	Silawa, Bait	N 4.574944	E 118.566556	4	5	0	0	6.625	50.4	0	0
Sebangkat	Sebangkat 1	N 4.554225	E 118.667600	17	11	0	0	12.176	69.073	0	0
Sebangkat	Sebangkat 2	N 4.553144	E 118.655369	10	4	0	1	10.53	32.625	0	22.5
Sebangkat	Sebangkat 3	N 4.555528	E 118.654806	6	3	0	0	9.81	37.267	0	0
Sibuan	Church reef 1	N 4.681917	E 118.657889	6	11	0	0	17.567	41.545	0	0
Sibuan	Church reef 2	N 4.686250	E 118.649028	4	3	0	0	18.2	31.833	0	0
Bodgaya	Ribbon reef	N 4.602778	E 118.764889	2	4	0	0	9.85	57.9	0	0
Kulapuan	Batuara 1	N 4.513500	E 118.808667	5	0	0	0	13.94	0	0	0
Kulapuan	Batuara 2	N 4.525500	E 118.831917	5	7	0	0	9.9	70.214	0	0
Kulapuan	Batuara 3	N 4.510500	E 118.811167	4	2	0	0	15.825	37.433	0	0
Bum Bum	Pasalat Reef 1	N 4.514213	E 118.727238	1	4	2	0	4	17.35	24.6	0
Bum Bum	Pasalat Reef 2	N 4.514217	E 118.727092	4	6	1	1	7.625	32.017	28.7	8.5
Bum Bum	Palang - palang	N 4.446167	E 118.769317	2	2	0	0	12.95	42.6	0	0
Bum Bum	Channel	N 4.461306	E 118.635861	0	0	0	0	0	0	0	0
				123	112	6	4				

Appendix B. Questionnaire on coral reefs in Semporna. The survey was designed to assess local community perceptions of coral reefs, destructive fishing practices, environmental changes, and willingness to participate in conservation initiatives. Responses were voluntary and confidential.



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Borang Kaji Selidik Tentang Terumbu Karang di Semporna

Tujuan soal selidik ini adalah untuk mengetahui persepsi orang tempatan terhadap batu karang dan ancaman terumbu karang yang berada di kawasan masing-masing. Jawab soalan ini adalah secara sukarela dan informasi personal adalah terpelihara. Sila jawab soalan secara jujur dan ikhlas dan sila menambah sebarang maklumat yang tuan/puan rasa berkaitan

- 1) Apakah pendapat anda tentang terumbu karang berbanding dengan 10 tahun yang lepas?
 - a) Bertambah baik
 - b) Tidak berubah
 - c) Berkurang dan rosak
- 2) Apakah cara menangkap ikan yang merosakkan berleluasa di kawasan ini?
 - a) Tidak
 - b) Ya
 - c) Di kawasan: _____
- 3) Apakah pandangan anda tentang cara memancing secara merosakkan dalam jangka masa 10 tahun ini?
 - a) Bertambah
 - b) Tidak berubah
 - c) Berkurang
- 4) Apakah perubahan persekitaran yang anda dapat melihat di kawasan anda?
 - a) Hakisan pantai
 - b) Kerosakan terumbu karang
 - c) Air tawar menjadi masin
 - d) Air laut tercemar
 - e) Kekurangan kawasan rumput laut
 - f) Sumber laut semakin berkurang
 - g) Lain-lain: _____
- 5) Apakah cara penangkapan ikan yang merosakkan sering dilakukan di kawasan anda?
 - a) Bom ikan
 - b) Sujum
 - c) Memecahkan batu karang
 - d) Lain-lain: _____
- 6) Mengebom ikan adalah salah di sisi undang-undang. ()
Kenyataan atas adalah betul atau salah?
- 7) Pada pendapat anda, apakah aktiviti yang merosakkan batu karang?
 - a) Bom ikan dan Sujum
 - b) Memancing dan memukat
 - c) Sentuhan pelancong
 - d) Sampah sarap
 - e) Jangka dari boat
 - f) Mahkota laut/haiwan lain
 - g) Lain-lain: _____
- 8) Di manakah selalu menjadi kawasan bom ikan?
 - a) Pulau: _____
- 9) Bilakah masa yang selalu berlaku pengeboman ikan?
 - a) Pagi
 - b) Petang
 - c) Malam
- 10) Apakah jenis bot yang biasa dipakai oleh nelayan yang menjalankan aktiviti mengebom ikan?
 - a) Pam bot
 - b) Sampan (boggo')
 - c) Bot dengan enjin
 - d) Bangkero/Bangka/Katig
- 11) Aktiviti mengebom ikan yang pernah dilihat dijalankan secara individu atau berkumpulan?
 - a) Individu
 - b) Berkumpulan: Berapa bot?
Berapa orang ?
- 12) Pernahkah anda melihat terumbu karang yang telah dirosakkan akibat pengeboman ikan?
 - a) Tidak
 - b) Ya. di kawasan: _____

13) Apakah kekerapan anda mendengar bom ikan di kawasan anda?

- a) Sangat jarang (setahun sekali)
- b) Kadang-kala (sebulan sekali)
- c) Selalu (seminggu sekali)
- d) Sangat kerap (>5 kali seminggu)

14) Pernahkah anda melihat bot peronda pihak berkuasa meronda kawasan ini?

- a) Tidak
- b) Ya. Jika ya, sila pilih,
 - i) Tentera
 - ii) Polis marin
 - iii) APMR
 - iv) Lain-lain: _____

15) Adakah anda setuju untuk menghentikan aktiviti pengeboman ikan di Semporna?

- a) Setuju
- b) Tidak setuju Sebab : _____

16) Adakah anda sudi membantu dalam usaha menghentikan aktiviti pengeboman ikan di Semporna?

- a. Ya
- b. Tidak. Sebab: _____

17) Adakah anda pernah mengikuti latihan, meronda atau memantau?

- a) Tidak pernah
- b) Pernah. Agensi : _____

18) Adakah anda bersetuju untuk mengikuti latihan, pasukan meronda dan memantau bagi menghentikan bom ikan?

- a) Ya
- b) Tidak
- c) Tidak Pasti

19) Adakah anda bersetuju untuk menubuhkan pasukan pengawal-pengawal laut bertujuan memantau dan menghalang aktiviti pengeboman ikan di Semporna?

- a) Ya
- b) Tidak setuju Sebab : _____

20) Pada pendapat anda, manakah yang lebih penting dari segi pendidikan, perkongsian maklumat, undang-undang dan penguatkuasaan dalam

22) Adakah anda sudi memberikan kerjasama memberikan maklumat untuk dihubungi pada masa hadapan tentang latihan-latihan yang berkaitan usaha menghentikan bom ikan?

menangani masalah bom ikan? (Boleh pilih lebih dari satu).

- a) Pendidikan:
 - (1) Poster
 - (2) Risalah
 - (3) Bahan pembelajaran di sekolah
 - (4) Tayangan video
 - (5) Lain-lain: _____

- b) Perkongsian maklumat:
 - (1) Kajian tentang kesan bom ikan kepada terumbu karang
 - (2) Garis panduan untuk mengenalpasti kawasan bom dan sujud
 - (3) Lain-lain : _____

- c) Undang-undang:
 - (1) Denda setimpal terhadap orang yang melakukan kesalahan di sisi undang-undang
 - (2) Undang-undang perlu diketatkan terhadap penggunaan bom ikan dan sujud
 - (3) Lain-lain : _____

- d) Penguatkuasaan:
 - (1) Lebih ramai pegawai penguatkuasa
 - (2) Lebih banyak bot untuk rondaan
 - (3) Memberikan latihan
 - (4) Lain-lain : _____

21) Saya boleh memainkan peranan penting dalam usaha menghentikan pengeboman ikan melalui :

- a) Mengikuti latihan sebagai pengawal untuk meronda dan memantau
- b) Menyokong menubuhkan pasukan pengawal untuk meronda dan memantau
- c) Memberikan maklumat lokasi dan masa pengeboman ikan berlaku
- d) Memberitahu kepada kawan-kawan, ahli keluarga dan saudara mara supaya jangan membeli ikan yang telah dibom
- e) Menyumbang kepada pasukan meronda dan memantau (kos operasi)
- f) Menyertai dan menyokong Kempen Anti Bom Ikan
- g) Lain-lain : _____

- b) Ya. Nombor telefon dihubungi : _____

23) Nama : _____

Jantina :	Lelaki	Perempuan
-----------	--------	-----------

Umur : _____

Bangsa : Bajau Bajau Laut Suluk Lain - lain : _____

Pulau : _____

24) Pekerjaan

(c) Menyelam

a) Surirumah

(d) Menohor/Mengarang

b) Penternak ikan

(e) Lain – lain : _____

c) Pengusaha rumpai laut

d) Peniaga

e) Sektor kerajaan

f) Sektor pelancongan

g) Lain-lain: _____

h) Nelayan

(a) Memancing

(b) Memukat

25) Jumlah pendapatan bulanan :

a) RM 200 ke bawah

b) RM 250 – RM 500

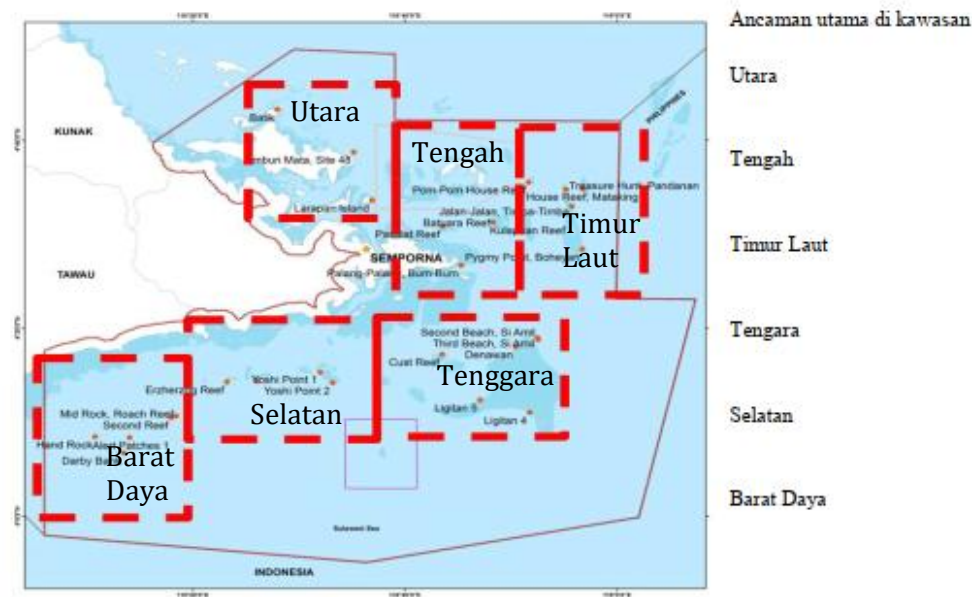
c) RM 500 – RM 1000

d) RM 1000 – RM 1500

e) RM 1500 – RM 2000

f) RM 2500 ke atas

26) Di manakah kawasan anda melakukan aktiviti perikanan? Sila nyatakan dalam peta di bawah



Appendix C. Scoring matrix for community survey responses in Semporna, showing component questions and maximum possible points for Environmental Awareness, Fish Bombing Awareness, and Fish Bombing Attitude scores.

Environmental Awareness Score									
Q1	Q2	Q3	Q4	Q5	Q6	Q7			Total
0/1	0/1	0-4	0/1	0-5	0/1	0-6			19
Fish Bombing Awareness Score									
Q6	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q20	Total
0/1	0-5	0/1	0/1	0/1	0/1	0-4	0/1	0-4	19
Fish Bombing Attitude Score									
Q15	Q16	Q18	Q19	Q21	Q22				Total
0/1	0/1	0/1	0/1	0-7	0/1				12