
# Effect of Different Dietary Lipid Levels on Spawning Performance and Egg Quality of *Pangasianodon hypophthalmus* (Sauvage, 1878)

MUHAMMAD ANAMUL KABIR[1,4], MOHAMMAD BODRUL MUNIR[2,*], SHARIFAH LIA FARLIANA BINTI WAN ALIAS[2], ADRIANA LEJA[2], LEE SEONG WEI[1], ROSHADA HASHIM[3]
[1]Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Kampus Jeli, Jeli, Kelantan, Malaysia
[2]FRST Aquatic Resource Science and Management, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak 94300, Malaysia
[3]Universiti Sains Islam Malaysia, Nilai, Negeri Sembilan, Malaysia
[4]Faculty of Fisheries, Sylhet Agricultural University, Sylhet 3100, Bangladesh

*E-mail: hsjewel730@yahoo.com | Received: 10/04/2019; Accepted: 16/11/2019


ISSN: 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2019.32.4.001

## Abstract

Dietary lipid manipulation of broodstock diet offers a strategy for improving spawning performance and egg quality of broodstock fish. Although many studies were conducted on basic nutrient requirements for fingerlings and juveniles of *Pangasianodon hypophthalmus* (Sauvage, 1878); the artificial feed for *P. hypophthalmus* broodstock is still lacking. This study analysed the effect of different levels of lipid in the diet on spawning performance and egg quality of female *P. hypophthalmus* broodstock. Three test diets were formulated containing 1:1 ratio of palm oil and fish oil blends at the level of 60, 90 and 120 g.kg$^{-1}$ with a constant 300 g.kg$^{-1}$ protein and fed to triplicate groups of female broodstock for 150 days at the rate of 20 g.kg$^{-1}$ body weight twice daily. The ovary weight, gonadosomatic index, fecundity, egg weight and diameter and egg fertilisation rate were significantly ($P < 0.05$) increased for fish fed with 90 and 120 g.kg$^{-1}$ lipid diet compared to 60 g.kg$^{-1}$ lipid diet. However, there was no significant difference between fish fed with 90 and 120 g.kg$^{-1}$ lipid diet; thus an increase in dietary lipid above 90 g.kg$^{-1}$ would not make any significant contribution. The increased performance of female broodstock fed with higher dietary lipid and fish oil at the ratio of 1:1 for crude palm oil and fish oil up to 90 g.kg$^{-1}$ contributed in the enhancement of the reproductive performance and egg quality of *P. hypophthalmus*.

**Keywords:** broodstock nutrition, crude palm oil, fatty acids, fecundity, reproductive performance

## Introduction

Broodstock conditioning is one of the principal components in aquaculture, and this is mandatory if the fish species has a high market value. The omnivores *Pangasianodon hypophthalmus* (Sauvage, 1878) is a significant export commodity in Asia (Ahmed and Hasan, 2007; Asdari et al., 2011; Phumee et al., 2011). Biologically, the fish has an attribute of faster growth that requires lower management cost compared to other fish species. The availability of continued quality seed production is reported as one of the major constraints in the farming of *P. hypophthalmus*. Furthermore, the scarcity of information on this fish broodstock's diet that focuses on triggering faster fish maturation has become a key limiting factor for consistent supply of quality seed of this species (Kabir et al., 2015). Lipids and its fatty acids in the dietary component influence the reproductive development (Ghaedi et al., 2016; Nzohabonayo et al., 2017; Sotoudeh and Yeganeh, 2017), spawning performance (Zakeri et al., 2011) and quality eggs as well as larvae supply (Noori et al., 2019).

The broodstock nutrition plays an important role because every essential nutrient that is needed before the exogenous feeding begins are supplied maternally and incorporated during vitellogenesis into the oocyte (Brooks et al., 1997). The quality of fish egg and reproductive performance has been related to the existence of dietary docosahexaenoic acid (DHA), arachidonic acid (ARA) and eicosapentaenoic acid (EPA), highly unsaturated fatty acids (HUFA) and their ratios (Sargent, 1995; El-Sayed et al., 2005; Nguyen et al., 2010; Zakeri et al., 2011; Ghaedi et al., 2016; Nzohabonayo et al., 2017). Numerous studies have demonstrated that dietary lipid and its fatty acid profile provides a strategy for enhancing the performance of

the reproduction and the fish eggs quality as observed in the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (El-Sayed et al., 2005), channel catfish *Ictalurus punctatus* (Rafinesque, 1818) (Sink and Lochmann, 2008), and yellowfin sea bream *Acanthopagrus latus* (Houttuyn, 1782) (Zakeri et al., 2009). Furthermore, the reproductive performance and the quality of eggs in numerous marine and freshwater fish are affected by nutritional related factors such as the apportion of feeds, the levels of nutrient and its composition (Sink and Lochmann, 2008; Zakeri et al., 2009; Lanes et al., 2012; Ghaedi et al., 2016; Jiang et al., 2018).

To date, only the essential requirements of nutrients for fingerlings and juveniles of *P. hypophthalmus* are documented (Phumee et al., 2009; Asdari et al., 2011; Phumee et al., 2011). Most recently, Kabir et al. (2015) have emphasised on protein requirements for growth performance and reproductive development of *P. hypophthalmus* broodstock in captivity. With the absence of appropriate broodstock feed of *P. hypophthalmus*, the production of seeds in captivity is inefficient and highly variable, and hatchery owners have to maintain a high number of brood fish to meet the seed production target (Bui et al., 2010). Hence, there is a need to improve reproductive performance and egg quality with suitable dietary lipid levels for broodstock development.

The work presented here is the first report on the effects of different dietary lipid levels on spawning performance and egg quality of *P. hypophthalmus* broodstock maintained in captivity.

# Materials and Methods

## *Diet formulation*

The diet formulation was done at the Fisheries Research Institute, Pulau Sayak, Kedah, Malaysia. Three experimental diets containing the lipid levels of 60, 90 and 120 g.kg$^{-1}$ were prepared with a constant protein of 300 g.kg$^{-1}$. The feed ingredients and proximate composition analyses of experimental diets are presented in Table 1.

Fish oil (FO) and crude palm oil (CPO) were supplemented in equal proportion so that the residual lipid from soybean meal (plant based) and fish meal (animal base) were in equal proportion of 1:1. Table 2 represents the fatty acids composition of the three dietary treatments.

## *Broodstock fish and feeding trial*

A group of sexually matured *P. hypophthalmus* broodstock female fish (average weight 3.29 ± 0.02 kg) maintained in captivity for 3 years at a private fish farm in Perak Malaysia were moved to the Aquaculture Research Complex, Universiti Sains Malaysia, Penang, for this study. The *P. hypophthalmus* were stocked in canvas tanks (4 m length × 1 m width × 1 m depth) equipped with a flow-through system and aeration. Fish were acclimatised for 2 months and fed once daily at 20 g.kg$^{-1}$ of the body weight on a commercial feed containing 320 g.kg$^{-1}$ protein, 40 g.kg$^{-1}$ lipid (Cargill Feed Sdn. Bhd., Malaysia). After acclimatisation, the fish were randomly distributed into 9 cages (3.5 m length × 3 m width × 2 m depth) provided with continuous aeration. There were three treatments, and each treatment consisted of three replicates. The stocking density was five fish per cubic meter with a male: female sex ratio of 2:8 with average weight of 2.8 ± 0.04 kg for the males and 3.32 ± 0.03 kg for the females. Before the feeding trial, the broodfish were starved for 2 weeks to normalise the status of their nutritional condition. The brood fish were fed with the test diets at the rate of 20 g.kg$^{-1}$ body weight in two portions twice daily (09:00 and 17:00 h) for 150 days. During the experiment, the water temperature remained at 29 ± 2 °C, dissolved oxygen concentration at 4.72 ± 1.12 mg.L$^{-1}$ and pH ranged between 6.29 and 6.90. Tanks were cleaned fortnightly by replacing 50 % of water in the system to remove the sediments and to reduce the water impurities.

Table 1. Feed ingredients and proximate composition of the experimental diets (g.kg$^{-1}$ dry matter).

| | Lipid in experimental diets (g.kg$^{-1}$) | | |
|---|---|---|---|
| | 60 | 90 | 120 |
| Ingredients (g.kg$^{-1}$) | | | |
| Fish meal | 338.20 | 338.20 | 338.20 |
| Soybean meal | 159.30 | 159.30 | 159.30 |
| Fish oil | 0.00 | 13.50 | 28.50 |
| Crude palm oil | 24.70 | 41.20 | 56.20 |
| Corn starch | 447.80 | 417.80 | 387.80 |
| Carboxymethyl cellulose | 10.00 | 10.00 | 10.00 |
| Vitamin mix | 10.00 | 10.00 | 10.00 |
| Mineral mix | 10.00 | 10.00 | 10.00 |
| Proximate composition (g.kg$^{-1}$) | | | |
| Moisture | 53.10 | 45.60 | 34.20 |
| Protein | 303.30 | 306.20 | 302.30 |
| Lipid | 60.30 | 93.00 | 121.30 |
| Ash | 73.00 | 73.00 | 72.00 |
| Fibre | 10.20 | 18.90 | 19.60 |
| NFE* | 553.20 | 508.90 | 484.80 |
| GE (MJ kg$^{-1}$)** | 14.92 | 15.65 | 16.34 |

*NFE = Nitrogen free extract (1000 - {Moisture + Protein + Lipid + Ash + Fiber}).
**GE = Gross energy measured using bomb calorimeter, Parr 1356 bomb calorie.

Table 2. Fatty acids composition (g.kg$^{-1}$ of total fatty acids detected) of different lipid dietary treatments.

| Fatty acids | Lipid in experimental diets (g.kg$^{-1}$) | | |
|---|---|---|---|
| | 60 | 90 | 120 |
| 14:00 | 4.17 ± 0.12 | 3.83 ± 0.04 | 3.21 ± 0.66 |
| 16:00 | 38.49 ± 1.08 | 38.34 ± 0.73 | 37.49 ± 3.13 |
| 18:00 | 7.62 ± 0.30 | 7.86 ± 0.32 | 6.75 ± 0.60 |
| ∑ SFA[1] | 50.29 ± 1.51 | 50.02 ± 1.10 | 47.44 ± 1.95 |
| 16:1n7 | 3.34 ± 0.01 | 2.60 ± 0.01 | 4.03 ± 0.01 |
| 18:1n9 | 30.66 ± 1.83 | 30.22 ± 0.60 | 28.73 ± 1.05 |
| 18:1n7 | 1.50 ± 0.24 | 1.56 ± 0.11 | 1.13 ± 0.47 |
| 20:1n9 | 2.17 ± 0.06 | 2.89 ± 0.12 | 1.81 ± 0.14 |
| 22:1n11 | nd | nd | nd |
| ∑ MUFA[2] | 37.66 ± 2.02 | 37.30 ± 0.81 | 35.69 ± 0.27 |
| 18:2n6 | 7.63 ± 0.23 | 7.88 ± 1.13 | 8.80 ± 0.73 |
| 18:3n3 | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.15 ± 0.07 |
| 20:3n6 | nd | nd | 0.39 ± 0.22 |
| 20:4n6 (ARA)[3] | 0.07 ± 0.01 | 0.13 ± 0.02 | 0.62 ± 0.08 |
| 20:5n3 (EPA)[4] | 0.80 ± 0.05 | 0.93 ± 0.03 | 1.64 ± 0.16 |
| 22:5n3 | nd | nd | 0.06 ± 0.03 |
| 22:6n3 (DHA)[5] | 0.26 ± 0.02 | 0.27 ± 0.02 | 1.31 ± 0.37 |
| ∑ PUFA[6] | 8.86 ± 0.31 | 9.31 ± 1.20 | 12.96 ± 1.68 |
| ∑n-3 HUFA[7] | 1.06 ± 0.03 | 1.2 ± 0.05 | 3.01 ± 0.58 |
| ∑n-6 HUFA[7] | 0.07 ± 0.01 | 0.13 ± 0.02 | 0.62 ± 0.08 |

[1]SFA: Saturated fatty acid, [2]MUFA: Monounsaturated fatty acid. [3]ARA: Arachidonic acid, [4]EPA: Eicosapentaenoic acid, [5]DHA: Docosahexaenoic acid, [6]PUFA: Polyunsaturated fatty acid, [7]HUFA: Highly unsaturated fatty acid. Data are expressed as mean ± SD.

## Evaluation of spawning and reproductive performances

At the end of the feeding period, the female fish were intra-muscularly injected dorsolateral with two successive doses of Ovaprim hormone (Syndel, Canada) with an initial dose of 1 mL.kg$^{-1}$ body weight followed by a second injection at a dose of 2 mL.kg$^{-1}$ after 6 to 7 h. The timing and the doses of hormone injections were based on what is being practised in the local *P. hypophthalmus* hatcheries. A single injection of Ovaprim was applied to the males at a dose of 1 mL.kg$^{-1}$ of body weight at the same time when the first dose was given to the females. Sperms were collected from male by stripping them directly into a tube containing a 9 g.kg$^{-1}$ NaCl solution (dilution rate of 1/5) and kept at 4 °C for a maximum period of 2 h before being used for fertilisation. Eggs from females were also collected by stripping, weighed and immediately fertilised. Sample collection of eggs, ovary weight, fecundity, egg weight and diameter and fertilisation rate were determined as previously described by Kabir et al. (2012 and 2015). A subsample of 50 g of eggs was immediately frozen at –20 °C for proximate and fatty acid analyses. At the end of the study period, three fish were taken from every cage to be sacrificed to measure the gonadosomatic index (GSI). The reproductive performance was determined using the following formulae:

$$Spawning\ response\ (\%) = 100 \times \frac{Number\ of\ hormone\ injected\ fish\ spawned}{Total\ number\ of\ hormone\ injected\ females}$$

$$Ovulation\ time\ (h) = Total\ time\ until\ ovulation - Time\ at\ first\ injection$$

$$Gonadosomatic\ index\ (GSI) = 100 \times \frac{Gonad\ weight}{Body\ weight}$$

$$Relative\ fecundity\ (eggs.kg^{-1}) = \frac{Total\ number\ of\ eggs\ in\ female\ ovary}{Total\ weight\ of\ female}$$

$$Egg\ fertilization\ rate\ (\%) = 100 \times \frac{Number\ of\ fertilised\ egg\ in\ sub\ sample}{Total\ number\ of\ eggs\ in\ sub\ sample}$$

## Proximate and fatty acid analyses

The proximate composition of the ingredients of the experimental diets, body muscle and eggs were performed according to AOAC (1997) and were analysed in triplicate. The fatty acid composition was evaluated according to Indarti et al. (2005). For fatty acid analysis, the muscle and egg were frozen at -80 °C. Then, the tissues were freeze-dried (LABCONCO 117, Labconco, USA). These freeze-dried tissues were then grounded finely, packed separately in the screw-capped bottles and stored at -80 °C until used for fatty acid analysis in less than 3 days. All experimental diets and pooled samples of fish muscle and egg were analysed for fatty acid composition according to Indarti et al. (2005) in triplicate. Using one step extraction transesterification method, fatty acid methyl esters (FAME) were synthesized. Briefly, 100 mg samples were weighed into clean 10 mL screw-top glass bottles and mixed with 2 mL of a mixture of methanol and sulphuric acid and (85:15, v:v) and 2 mL of chloroform. The bottles were vortexed for 30 s, and nitrogen gas was bubbled in, closed with Teflon cap to avoid leakage. Samples were heated at 100 °C for 30 min and cooled down to room temperature in desiccators. Then, 1 mL of distilled water was added into the mixture and thoroughly vortexed for 1 min. After the formation of two phases, the lower phase containing FAME was transferred and dried with sodium sulphate anhydrous ($Na_2SO_4$). Samples were stored in the freezer (-20 °C) until gas chromatography (GC) analysis. For injecting the sample into GC, 1 µL caproic acid (6:0) methyl ester, diluted in chloroform (1: 499, v:v) as an internal standard was added to 3 µL dried sample solution in a vial and vortexed, 1 µL of this mixture was injected.

Fatty acid methyl esters (FAME) were separated and quantified by gas chromatography (Automatic System XL, Perkin Elmer, USA) equipped with a flame ionisation detector and a 30 m × 0.25 mm fused silica capillary column (Megawax 250, Supelco, USA). Helium was used as the carrier gas and hydrogen and

compressed air were used for flame ionisation detection (FID). The oven temperature programming was set to rise at a rate of 4 °C.min$^{-1}$ from 50 °C to 220 °C, and then kept at 220 °C for 35 min. The injector and detector temperatures were set at 250 °C and 260 °C, respectively. Chromatographic data were recorded and integrated into a personal computer (Optiplex GX 110, Dell Computers, Malaysia) using Turbochrom software (Perkin Elmer, USA). Fatty acids were identified by comparing the retention times of FAME with the standard component FAME mixture and menhaden oil. Individual FA content was quantified as percentage of total fatty acids detected.

### *Statistical analysis*

The performances of reproduction, quality of eggs, body muscle and the biochemical data were statistically analysed by using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS 20 for Windows. Significant differences were based on the $P < 0.05$ level.

## Results

### *Spawning performance and egg quality*

The parameters of spawning performance and egg quality of *P. hypophthalmus* broodstock female fish are summarised in Table 3.

Table 3. Spawning performance and egg biometry (mean ± SD) from *Pangasianodon hypophthalmus* broodstock fed with diets containing different dietary lipid levels.

| Reproductive variables | Lipid in experimental diets (g.kg$^{-1}$) | | |
|---|---|---|---|
| | 60 | 90 | 120 |
| Spawning response (%) | 58.33 ± 14.43 | 66.66 ± 14.43 | 63.88 ± 12.72 |
| Ovary weight (kg) | 0.25 ± 0.02$^{a}$ | 0.33 ± 0.06$^{ab}$ | 0.42 ± 0.06$^{b}$ |
| GSI[1] | 7.05 ± 1.30$^{ab}$ | 8.81 ± 1.22$^{bc}$ | 10.48 ± 1.00$^{c}$ |
| Ovulation time (h) | 15.94 ± 0.63 | 16.52 ± 0.42 | 16.18 ± 0.05 |
| Fecundity (eggs.kg$^{-1}$) × 10$^{4}$ | 15.32 ± 0.43$^{a}$ | 16.90 ± 0.16$^{b}$ | 17.18 ± 0.40$^{b}$ |
| Egg weight (mg) | 1.00 ± 0.02$^{a}$ | 1.06 ± 0.02$^{b}$ | 1.06 ± 0.03$^{b}$ |
| Egg diameter (mm) | 1.00 ± 0.01$^{a}$ | 1.12 ± 0.02$^{b}$ | 1.13 ± 0.01$^{b}$ |
| Egg fertilization rate (%) | 58.33 ± 3.26$^{a}$ | 77.21 ± 3.03$^{b}$ | 80.28 ± 1.95$^{b}$ |

[1]GSI= Gonado somatic index; different superscripts in each row denotes significantly different results ($P < 0.05$)

The reproductive performance in terms of mean ovary weight, GSI, fecundity, egg weight and diameter and fertilization rate were significantly higher ($P < 0.05$) for fish fed with 120 g.kg$^{-1}$ lipid levels compared to the fish fed with 60 g.kg$^{-1}$ lipid level but not significantly different in fish fed with 90 g.kg$^{-1}$ lipid. The reproductive performance in terms of mean ovary weight, GSI and fecundity were significantly the highest ($P < 0.05$) for fish fed with 90 g.kg$^{-1}$ and 120 g.kg$^{-1}$ lipid. However, the spawning response and total ovulation time were not significantly ($P > 0.05$) different among the dietary lipid levels. Numerically, the broodfish fed with 60 g.kg$^{-1}$ lipid diet had lower spawning success than the fish fed with 90 g.kg$^{-1}$ or 120 g.kg$^{-1}$ diets. Fecundity was significantly elevated in fish fed on 90 g.kg$^{-1}$ and 120 g.kg$^{-1}$ dietary lipid compared with the remaining treatments. The quality of eggs in terms of the egg diameter, the weight of individual egg and the rate of fertilisation were significantly ($P < 0.05$) different among the treatments, whereby the values were high in fish fed the 90 g.kg$^{-1}$ and 120 g.kg$^{-1}$ dietary lipid levels compared with the remaining treatments. The mean of individual egg diameters, weights and fertilisation rates were lower for the broodstock fed with 60 g.kg$^{-1}$ diet than with the other treatments.

### *Biochemical content and fatty acid composition of muscle and eggs*

The proximate compositions of body muscle and eggs are shown in Table 4. Lipid contents of body muscle and eggs were significantly influenced ($P < 0.05$) by the different levels of dietary lipid. An increasing trend in the muscle lipid content in fish fed on the 120 g.kg$^{-1}$ dietary lipid showed significantly higher ($P < 0.05$) values compared to the fish fed with 60 g.kg$^{-1}$ lipid. Consequently, the lipid levels in the eggs were significantly higher ($P < 0.05$) from fish fed with 90 g.kg$^{-1}$ and 120 g.kg$^{-1}$ dietary lipids. Egg and muscle protein were not significantly ($P > 0.05$) influenced by the lipid diets. However, ash content in the muscle varied significantly among the test diets and no significant differences were seen in the ash content of the egg.

Table 4. Proximate compositions (g.kg$^{-1}$ dry weight basis) of muscle and eggs from *Pangasianodon hypophthalmus* broodstock fed diets containing different dietary lipid levels.

| | Lipid in experimental diets (g.kg$^{-1}$) | | |
|---|---|---|---|
| | 60 | 90 | 120 |
| Muscle | | | |
| Protein | 86.25 ± 1.21 | 84.46 ± 1.27 | 85.81 ± 1.68 |
| Lipid | 3.45 ± 0.43$^{a}$ | 4.56 ± 0.62$^{ab}$ | 5.15 ± 0.67$^{b}$ |
| Ash | 8.96 ± 0.49$^{a}$ | 11.40 ± 1.41$^{b}$ | 7.76 ± 0.67$^{a}$ |
| Egg | | | |
| Protein | 65.49 ± 1.95 | 64.46 ± 0.44 | 62.69 ± 1.69 |
| Lipid | 28.99 ± 0.67$^{a}$ | 30.86 ± 0.86$^{b}$ | 30.59 ± 0.16$^{b}$ |
| Ash | 5.82 ± 0.32 | 5.44 ± 0.59 | 6.11 ± 0.81 |

Data are expressed as mean ± SD. Different superscripts in each row denote significantly different results ($P < 0.05$).

The fatty acid composition of the body muscle of fish is presented in Table 5. The most dominant fatty acids in the muscle for all treatments were 16:0 and 18:1n9. However, the level of 18:0 in fish muscles across the treatments was significantly ($P < 0.05$) elevated. There was significantly ($P < 0.05$) lower level of 18:2n6 in fish muscle from groups fed with 120

g.kg$^{-1}$ lipid diet. The fish fed with 60 g.kg$^{-1}$ dietary lipid levels contained the significantly lowest ($P < 0.05$) level of 16:0 fatty acid and total saturated fatty acids (SFAs) (44.68) in muscle, compared with the fish that received the remaining lipid diets. *P. hypophthalmus* broodstock fed with 90 g.kg$^{-1}$ lipid level contained the significantly ($P < 0.05$) highest MUFA in the muscle.

Table 5. Fatty acid composition (g.kg$^{-1}$ of total fatty acids detected) of body muscle from *Pangasianodon hypophthalmus* broodstock fed diets containing different dietary lipid levels of total monounsaturated fatty.

| Fatty acids | Lipid in experimental diets (g.kg$^{-1}$) | | |
|---|---|---|---|
| | 60 | 90 | 120 |
| 14:0 | 2.32 ± 0.17$^{b}$ | 2.11 ± 0.08$^{ab}$ | 2.71 ± 0.03$^{c}$ |
| 16:0 | 30.96 ± 0.16$^{a}$ | 36.80 ± 0.96$^{c}$ | 34.20 ± 0.62$^{b}$ |
| 18:0 | 11.39 ± 0.28$^{a}$ | 11.65 ± 0.02$^{a}$ | 13.17 ± 0.19$^{b}$ |
| ∑SFA[1] | 44.68 ± 0.27$^{a}$ | 50.52 ± 1.03$^{b}$ | 50.08 ± 0.85$^{b}$ |
| 16:1n7 | 4.43 ± 0.04$^{a}$ | 7.42± 0.18$^{b}$ | 4.75 ± 0.31$^{a}$ |
| 18:1n9 | 25.30 ± 0.57 | 27.08 ± 0.98 | 25.69 ± 0.41 |
| 18:1n7 | 2.22 ± 0.61$^{b}$ | 0.40 ± 0.04$^{a}$ | 2.93 ± 0.31$^{b}$ |
| 20:1n9 | 0.74 ± 0.31$^{b}$ | 0.88 ± 0.00$^{b}$ | 0.18 ± 0.04$^{a}$ |
| 22:1n11 | 1.87 ± 1.59 | 0.57 ± 0.01 | 1.04 ± 0.02 |
| ∑MUFA[2] | 34.56 ± 0.03$^{a}$ | 36.36 ± 0.83$^{b}$ | 34.59 ± 0.39$^{a}$ |
| 18:2n6 | 6.55 ± 0.13$^{c}$ | 6.07 ± 0.04$^{b}$ | 4.58 ± 0.14$^{a}$ |
| 18:3n3 | 0.02 ± 0.02$^{a}$ | 0.06 ± 0.02$^{a}$ | 0.15 ± 0.02$^{b}$ |
| 20:3n6 | 0.46 ± 0.36 | 0.12 ± 0.02 | 0.18 ± 0.01 |
| 20:4n6 (ARA)[3] | 0.75 ± 0.04$^{b}$ | 0.58 ± 0.02$^{a}$ | 0.67 ± 0.06$^{ab}$ |
| 20:5n3 (EPA)[4] | 0.72 ± 0.66 | 0.22 ± 0.00 | 0.24 ± 0.04 |
| 22:5n3 | 1.47 ± 0.07 | 0.93 ± 0.00 | 1.40 ± 0.07 |
| 22:6n3 (DHA)[5] | 4.06 ± 0.02$^{b}$ | 2.43 ± 0.24$^{a}$ | 4.88 ± 0.77$^{b}$ |
| ∑ PUFA[6] | 14.05 ± 0.29$^{c}$ | 10.42 ±0.35$^{a}$ | 12.11 ± 0.57$^{b}$ |
| ∑ n-3 HUFA[7] | 6.26 ± 0.72$^{b}$ | 3.58 ± 0.24$^{a}$ | 6.53 ± 0.65$^{b}$ |
| ∑ n-6 HUFA[7] | 0.75 ± 0.04$^{b}$ | 0.58 ± 0.02$^{a}$ | 0.67 ± 0.06$^{ab}$ |

[1]SFA: Saturated fatty acid, [2]MUFA: Mono unsaturated fatty acid, [3]ARA: Arachidonic acid, [4]EPA: Eicosapentaenoic acid, [5]DHA: Docosahexaenoic acid, [6]PUFA: Polyunsaturated fatty acid, [7]HUFA: Highly unsaturated fatty acid. Data are expressed as mean ± SD; different superscripts in each row denote significantly different results ($P < 0.05$).

The ARA significantly differs ($P < 0.05$) in muscle for fish fed with 90 g kg$^{-1}$ lipid diet than those fed with lower lipid diet (60 g.kg$^{-1}$). Conversely, lipid diets did not influence the EPA content in the muscle. A level of 22:6n3 (DHA), PUFA and total HUFA showed a remarkably higher trend in fish muscle for those groups that received 60 and 120 g.kg$^{-1}$ lipid diet. In particular, the body muscle of the fish fed with the 90 g.kg$^{-1}$ dietary lipid level contained the significantly ($P < 0.05$) lowest levels of DHA, total n-3 HUFA and total n-6 HUFA compared with the other feeding treatments which included 60 and 120 g.kg$^{-1}$ lipid level respectively.

Table 6 presents the fatty acid composition of eggs treated with test diets. From the results, it is depicted that there was a considerable amount of variation in fatty acid profile within feeding treatments. The fatty acids 16:0, 18:0 and 18:1n9 were dominant in the egg of fish.

Table 6. Fatty acid composition (g.kg$^{-1}$ of total fatty acids detected) of eggs from *Pangasianodon hypophthalmus* broodstock fed diets containing different dietary lipid levels.

| Fatty acids | Lipid in experimental diets (g.kg$^{-1}$) | | |
|---|---|---|---|
| | 60 | 90 | 120 |
| 14:00 | 3.89 ± 0.15$^{a}$ | 2.97 ± 0.01$^{b}$ | 3.20 ± 0.15$^{b}$ |
| 16:00 | 42.16 ± 0.62 | 42.45 ± 0.31 | 46.15 ± 2.73 |
| 18:00 | 10.34 ± 0.18$^{a}$ | 10.72 ± 0.10$^{a}$ | 12.26 ± 0.82$^{b}$ |
| ∑ SFA[1] | 56.39 ± 0.59$^{ab}$ | 56.15 ± 0.41$^{a}$ | 61.61 ± 3.71$^{b}$ |
| 16:1n7 | 0.06 ± 0.01 | 0.46 ± 0.54 | 0.11 ± 0.01 |
| 18:1n9 | 32.72 ± 0.66 | 32.35 ± 0.45 | 30.75 ± 4.25 |
| 18:1n7 | 1.61 ± 0.01 | 1.81 ± 0.12 | 0.97 ± 0.45 |
| 20:1n9 | 0.06 ± 0.01 | 0.27 ± 0.29 | 0.07 ± 0.00 |
| 22:1n11 | 0.23 ± 0.00 | 0.61 ± 0.45 | 0.38 ± 0.07 |
| ∑ MUFA[2] | 34.69 ± 0.67 | 35.50 ± 0.95 | 32.28 ± 4.61 |
| 18:2n6 | 1.86 ± 0.01$^{a}$ | 2.54 ± 0.02$^{b}$ | 2.57 ± 0.17$^{b}$ |
| 18:3n3 | 0.02 ± 0.00$^{a}$ | 0.04 ± 0.00$^{ab}$ | 0.06 ± 0.00$^{b}$ |
| 20:3n6 | 0.19 ± 0.01 | 0.25 ± 0.00 | 0.26 ± 0.04 |
| 20:4n6 (ARA)[3] | 0.46 ± 0.02$^{a}$ | 0.33 ± 0.02$^{a}$ | 0.83 ± 0.14$^{b}$ |
| 20:5n3 (EPA)[4] | 0.03 ± 0.00 | 0.05 ± 0.02 | 0.05 ± 0.00 |
| 22:5n3 | 0.61 ± 0.00 | 0.60 ± 0.22 | 0.48 ± 0.04 |
| 22:6n3 (DHA)[5] | 0.98 ± 0.17$^{a}$ | 3.90 ± 0.22$^{b}$ | 1.34 ± 0.63$^{a}$ |
| ∑ PUFA[6] | 4.16 ± 0.0.14$^{a}$ | 7.73 ± 0.52$^{c}$ | 5.60 ± 0.77$^{b}$ |
| ∑ n-3 HUFA[7] | 1.62 ± 0.17$^{a}$ | 4.55 ± 0.48$^{b}$ | 1.87 ± 0.69$^{a}$ |
| ∑ n-6 HUFA[7] | 0.46 ± 0.02$^{a}$ | 0.33 ± 0.02$^{a}$ | 0.83 ± 0.14$^{b}$ |

[1]SFA: Saturated fatty acid, [2]MUFA: Monounsaturated fatty acid, [3]ARA: Arachidonic acid, [4]EPA: Eicosapentaenoic acid, [5]DHA: Docosahexaenoic acid, [6]PUFA: Polyunsaturated fatty acid, [7]HUFA: Highly unsaturated fatty acid. Data are expressed as mean ± SD; different superscripts in each row denote significantly different results ($P < 0.05$).

The eggs from the fish fed with the 120 g.kg$^{-1}$ lipid diet contained the highest total SFA (61.61 %) contributed mainly by 16:0 compared to fish fed with the 60 g.kg$^{-1}$ diet (56.39 %) and 90 g.kg$^{-1}$ lipid diet. However, the total MUFA level in the eggs of the fish was not significantly affected by the different diets tested. In comparison to other fed groups, ARA was significantly ($P < 0.05$) higher in eggs of fish that were fed the highest level 120 g.kg$^{-1}$ lipid diet. The level of EPA did not differ in all treatments. Moreover, DHA, total PUFA and total n-3 HUFA were significantly ($P < 0.05$) higher in eggs of fish fed with 90 g.kg$^{-1}$ lipid diet. However, n-6 HUFA levels significantly ($P < 0.05$) increased in eggs of fish fed with 120 g.kg$^{-1}$ lipid diet over the remaining fed groups. The level of PUFA was significantly ($P < 0.05$) higher in eggs from the fish fed 90 g.kg$^{-1}$ lipid diet followed by fish fed 120 g.kg$^{-1}$ lipid diet. Conversely the high lipid dietary doses of 120 g.kg$^{-1}$ lipid did significantly ($P < 0.05$) influence n-6 HUFA of egg.

## Discussion

This is the first attempt to report so far on the effect of dietary lipid on the reproductive performance of *P. hypophthalmus* broodstock raised in captivity. To get reliable data, the experiment was done in earthen pond cage to mimic the natural condition. The control of fish reproduction is a crucial issue in aquaculture and is one of the limiting factors for reproductive success. Thus, all the morphological, biochemical composition and fatty acid profile of fish muscle and egg were observed to better understand the reproductive performance and egg quality in the female fish *P. hypophthalmus*. The success of spawning obtained in this study was much higher than the average success (22 to 42 %) of *P. hypophthalmus* reported in Vietnam (Bui et al., 2010). Hatchery producers typically use low-fat (40 to 60 $g.kg^{-1}$ lipid) diets for broodstock reproductive development.

The data from the present study proved that the spawning success improved with the increased dietary lipid level which is similar to the report of Sink and Lochman (2008) for channel catfish. Moreover, the total ovulation time was 15 to 17 h after two doses of the hormone Ovaprim with a 6-h gap between the two doses. This result is lower than the average total ovulation time of 24 to 72 h upon multiple injected doses observed in *P. hypophthalmus* hatcheries for artificial spawning in Vietnam (Bui et al., 2010). Legendre et al. (2000) noted that environmental and hormonal manipulation is been practised for spawning in *P. hypophthalmus* to alter the spawning time and to obtain continuous supplies of eggs and larvae. However, research on the effects of nutrition on the spawning time is very limited. Unfortunately, there is a gap in the knowledge of the mechanism of action of nutrition and environmental parameters, and they trigger the neuro-endocrine system to spawn. Ovary weight and GSI are considered as important morphological indices to evaluate the reproductive performance of broodfish. In the present study, the increased lipid level of 90 and 120 $g.kg^{-1}$ in the diet showed better ovary weight and GSI compared with the lower lipid treatment. Similar findings were observed (Cek and Yilmaz, 2009) in sharptooth catfish *Clarias gariepinus* (Burchell, 1822) in which the GSI and gonad weight of the female fish fed with 110 $g.kg^{-1}$ dietary lipid was significantly greater than those fed diets with 36, 70, 154 and 205 $g.kg^{-1}$ lipids.

Fecundity is an important parameter to assess the reproductive performance of fish and it is influenced by nutritional intake (Izquierdo et al., 2001; Nguyen et al., 2010; Nzohabonayo et al., 2017). The varied response to the increasing dietary lipid level resulting in a better fecundity is expected because levels as high as 200 $g.kg^{-1}$ lipid has been provided for greater successful fecundity in the yellowfin sea bream (Zakeri et al., 2009). The similar trend was observed in the present study, however, there were no significant difference found in between 90 $g.kg^{-1}$ and 120 $g.kg^{-1}$ lipid level broodstock feed diets; seemingly the dietary lipid 90 $g.kg^{-1}$ was appropriate, and an increase in dietary lipid (120 $g.kg^{-1}$) did not influence the fecundity markedly for *P. hypophthalmus* female brood.

In the present investigation, the egg diameter and individual weight were significantly affected by the tested dietary lipid intake levels which corresponded to other species such as *A. latus* (Zakeri et al., 2009), *Cyprinus carpio* (Linnaeus, 1758) (Manissery et al., 2001), *O. niloticus* (El-Sayed et al., 2003) and *Oreochromis karongae* (Trewavas, 1941) (Nzohabonayo et al., 2017). Contrary, Sink and Lochmann (2008) reported no change in egg diameters when channel catfish was maintained on different lipid diets. An increase in egg weight and diameter of *P. hypophthalmus* associated with lipid dietary intake could be attributed to higher yolk content resulting in larger-sized eggs, which allow hatched larvae to survive for a longer period without food.

Fertilisation success is one of the prime parameters to accurately estimate the egg quality. In the present study, the brood fish fed with the 90 and 120 $g.kg^{-1}$ lipid levels showed significantly similar but higher fertilisation rate compared to the fish fed with 60 $g.kg^{-1}$ diet lipid. Sink and Lochmann (2008) reported achieving higher hatching rates in channel catfish from eggs of fish fed with 100 $g.kg^{-1}$ lipid diet than those fed with a lower (40 $g.kg^{-1}$) lipid diet in captive condition. The lipid composition of eggs can be controlled via the maternal diet (Watanabe, 1982), while the biochemical composition of eggs can act as an indicator of egg quality for embryonic and larval development (Furuita et al., 2002; Ghaedi et al., 2016; Sotoudeh and Yeganeh, 2017). A high level of lipid in egg is deemed vital for larval survival, as lipids are the primary energy source used by fish from larvae to mature fish. The qualitative and quantitative lipid level in the diets was found to influence the spawning and egg quality (Watanabe et al., 1984). In the present study, egg lipid content was higher and significantly similar in the fish fed with 90 and 120 $g.kg^{-1}$ dietary lipid levels. Thus at 120 $g.kg^{-1}$ dietary lipid level did not show any increasing trend in term of reproductive performance. Indeed, the results appear to indicate that the concentration of lipid in the eggs of the fish fed with 90 $g.kg^{-1}$ dietary lipid levels seem to be sufficient to show better fecundity, mean gonad weight and GSI, egg diameter and individual egg weight as well as the fertilisation rate compared to other lipid levels.

Lipids are broken down into fatty acids for cellular growth, metabolism and body structural components for new tissues synthesis during embryogenesis process of fish larvae (Sargent et al., 2002; Sotoudeh and Yeganeh, 2017; Noori et al., 2019). Although the experimental diets in the present study were formulated to contain the same ratio (1:1) of crude

palm oil (CPO) and fish oil (FO), there was an obvious deposition of total PUFA in fish body muscle. However, total n-6 HUFA was visibly lower both in muscle and eggs fed with 90 g.kg$^{-1}$ lipid diet. In addition, dietary lipids significantly affect SFA levels in the muscle of higher fed groups, but it showed increased trend in eggs compared to muscle of the fish. Similarly, MUFA levels were not greatly affected both in muscle and eggs. Based on numerous studies (Sink and Lochmann, 2008; Nguyen et al., 2010; Asdari et al., 2011; Zakeri et al., 2011; Ghaedi et al., 2016), the observed differences in the fatty acid profiles of the muscle and egg in response to the fatty acid intake was anticipated. For example, the dynamics of total SFA and MUFA levels of muscle and eggs resulting from all tested groups were higher than the total PUFA, and these fatty acids in the muscle and egg corresponded to a favourable situation for this fish. Furthermore, it is important to note that this fatty acid profiling is a consequence of the modulation of many metabolic factors that are dependent on the initial fatty acid content, cumulative intake of dietary fatty acid, growth rate and duration of feeding (Robin et al., 2003). Studies revealed that SFA and MUFA are important sources of metabolic energy for broodstock (Sargent et al., 2002) and consequently become vital energy sources for the normal development of fish embryos and the development of fish larvae (Van der Meeren et al., 1993; Wing-Keong and Yan, 2011). The results of the present study suggest that the availability of SFA in eggs higher than the muscle exhibited higher reproductive performance of *P. hypophthalmus* because of lipid based nutritional quality of the test diet fed to broodstock.

The reproductive performance in fish has been linked to dietary n-3 HUFA concentrations (Sargent et al., 2002; Sink and Lochmann, 2008, Zakeri et al., 2011; Ghaedi et al., 2016). In the present study, n-3 HUFA in the eggs contributed mainly by DHA, showed significant effect on reproductive performance. Although the DHA levels in the muscle for fish fed with 90 g.kg$^{-1}$ lipid was significantly lower than those of 60 and 120 g.kg$^{-1}$ lipid fed groups. Contrary in eggs, the DHA level was significantly higher in fish fed with 90 g.kg$^{-1}$ lipid diet. The results obtained in this study show a clear variation in the deposition of n-6 and n-3 HUFA in muscle and eggs of the brood fish. In fish fed with 90 g.kg$^{-1}$ lipid diet, the total n-3 HUFA was significantly higher in eggs compared to the muscle, but the n-6 HUFA was relatively lower for both muscle and eggs of this group compared to the remaining lipid fed groups. Although, n-6 HUFA was comparatively higher in eggs of fish fed with 120 g.kg$^{-1}$ than the muscles. This variation in lipid contents in eggs in this fish may be species-specific and the increase in lipid levels did produce significantly similar effect on reproductive performance. The apparent increase of the DHA in the 90 g.kg$^{-1}$ diet was likely coupled with the better intake of diet by the fish. The n-3 HUFA, in particular DHA, has a crucial role in maintaining structural and functional integrity of cellular membranes, including neural cell membranes such as those in the brain and eyes of fish (Sargent, 1995). The concentration of DHA, due to its critical role may be a better indicator of egg quality than total n-3 HUFA in the reproductive development of fish. The investigation of the present study indicates that the higher deposition of DHA in eggs of *P. hypophthalmus* brood fish fed with 90 g.kg$^{-1}$ lipid level as a result of the combination of CPO and FO used in the diet was appropriate and reflected a good spawning performance and high egg quality compared to fish fed higher lipid level 120 g.kg$^{-1}$. In addition to DHA, ARA has also been reported to have an effect on reproductive performance whereby increasing dietary ARA levels improved fertilisation success in gilthead sea bream (Fernández-Palacios et al., 1995) and Atlantic halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758) (Mazorra et al., 2003). Arachidonic acid is known to be the primary precursor to eicosanoids (Patino et al., 2003), which control ovulation and most likely embryogenesis, egg hatching, and early larval performance (Bell et al., 1997; Ghaedi et al., 2016). Moreover, a lower content of ARA and EPA was found in the eggs of the fish fed with 90 g.kg$^{-1}$ lipid diet compared to muscle which suggests that level contained in the egg was apt for best reproductive performance compared to a higher level in fish fed with 120 g.kg$^{-1}$ lipid diet. In addition, the egg EPA concentration in *P. hypophthalmus* did not follow the same pattern as DHA and ARA. Identifying specific fatty acids with different functions may have low relevance for omnivorous fish such as *P. hypophthalmus* that can bioconvert 18-carbon fatty acids into HUFAs (El-Sayed et al., 2005; Sink and Lochmann, 2008).

The current study demonstrates that other than lipid levels, the quality of dietary lipid has a considerable effect on the eggs quality and fatty acid composition of eggs and the subsequent reproductive performance of *P. hypophthalmus* female broodstock. This study revealed that the best performance of spawning performance was achieved 90 g.kg$^{-1}$ dietary lipid with 300 g.kg$^{-1}$ protein. This result generally implies that using lipid sources with blended combinations of CPO and FO at 1:1 ratio, there was active elongation and desaturation of 18-carbon fatty acids into HUFAs. These FAs may be selectively transferred to and conserved in ovaries for better reproductive performance of this species.

## Conclusion

The present study exhibited that the nutritional value of lipid level at 90 g.kg$^{-1}$ with 300 g.kg$^{-1}$ protein in broodstock diet has a significant effect on the reproductive performance and quality of eggs that could contribute towards developing an improved diet for *P. hypophthalmus* broodstock. Furthermore, the impact of experimental diets in this study has a

crucial practical implication in whole *Pangasiidae* family nutrition and will draw attention for more research on broodstock nutrition of other potential aquaculture species.

## Acknowledgements

The authors would like to thank Universiti Sains Malaysia for the financial support to conduct this PhD research under the Postgraduate Research Grant Scheme-1001/PBIOLOGI/842068 and the USM-IPS fellowship.

## References

Ahmed, N., Hasan, M.R. 2007. Sustainable livelihoods of pangus farming in rural Bangladesh. Aquaculture Asia Magazine 12(4):5–11.

Association of Official Analytical Chemists (AOAC). 1997. Animal feed. Official methods of analysis, 16th ed. AOAC, USA. 30 pp.

Asdari, R., Aliyu-Paiko, M., Hashim, R., Ramachandran, S. 2011. Effects of different dietary lipid sources in the diet for *Pangasianodon hypophthalmus* (Sauvage, 1878) juvenile on growth performance, nutrient utilization, body indices and muscle and liver fatty acid composition. Aquaculture Nutrition 17:44–53. https://doi.org/10.1111/j.1365-2095.2009.00705.x

Bell, J.G., Farndale, B.M., Bruce, M.P., Navas, J.M., Carillo, M. 1997. Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture 149:107–119. https://doi.org/10.1016/S0044-8486(96)01436-6

Brooks, S., Tyler, C.R., Sumpter, J.P. 1997. Quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7:387–416. https://doi.org/10.1023/A:1018400130692

Bui, T.M., Phan, L.T., Ingram, B.A., Nguyen, T.T.T., Gooley, G.J., Nguyen, H.V., Nguyen, P.T., De Silva, S.S. 2010. Seed production practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta region, Vietnam. Aquaculture 306:92–100. https://doi.org/10.1016/j.aquaculture.2010.06.016

Cek, S., Yilmaz, E. 2009. The effect of varying dietary energy on gonad development at first sexual maturity of the Sharptooth catfish (*Clarias gariepinus* Burchell, 1822). Aquaculture International 17:553–563. https://doi.org/10.1007/s10499-008-9224-4

El-Sayed, A.F.M., Mansour, C.R., Ezzat, A.A. 2003. Effects of dietary protein levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture 220:619–632. https://doi.org/10.1016/S0044-8486(02)00221-1

El-Sayed, A.F.M., Mansour, C.R., Ezzat, A.A. 2005. Effects of dietary lipid source on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture 248:187–196. https://doi.org/10.1016/j.aquaculture.2005.04.024

Fernández-Palacios, H., Izquierdo, M.S., Robaina, L., Valencia, A., Salhi, M., Vergara, J. 1995. Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). Aquaculture 132:325–337. https://doi.org/10.1016/0044-8486(94)00345-O

Furuita, H., Tanaka, H., Yamamoto, T., Suzuki, N., Takeuchi, T. 2002. Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder. Aquaculture 210:323–333. https://doi.org/10.1016/S0044-8486(01)00855-9

Ghaedi, A., Kabir, M.A., Hashim, R. 2016. Effect of lipid levels on the reproductive performance of Snakehead murrel, *Channa striatus*. Aquaculture Research 47:983–991. https://doi.org/10.1111/are.12557

Indarti, E., Majid, M.I.A., Hashim, R., Chong, A. 2005. Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. Journal of Food Composition and Analysis 18:161–170. https://doi.org/10.1016/j.jfca.2003.12.007

Izquierdo, M.S., Fernandez-Palacios, H., Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197:25–42. https://doi.org/10.1016/S0044-8486(01)00581-6

Jiang, M., Peng, R., Wang, S., Zhou, S., Chen, Q., Huang, C., Han, Q., Jiang, X. 2018. Growth performance and nutritional composition of *Sepia pharaonis* under artificial culturing conditions. Aquaculture Research 49:2788–2798. https://doi.org/10.1111/are.13741

Kabir M.A., Ghaedi, A., Talpur, A.D., Hashim, R. 2015. Effect of dietary protein levels on reproductive development and distribution of amino acids in the body tissues of female *Pangasianodon hypophthalmus* (Sauvage, 1878) broodstock in captivity. Aquaculture Research 46:1736–1747. https://doi.org/10.1111/are.12326

Kabir, M.A., Ghaedi, A., Hashim, R. 2012. Ovarian development and sexual maturation of female striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) reared in captivity. Asian Fisheries Science 25:232–244.

Lanes, C.F.C., Bizuayehu, T.T., Bolla, S., Martins, C., Fernandes, J.M.O., Bianchini, A., Kiron, V., Babiak, I. 2012. Biochemical composition and performance of Atlantic cod (*Gadus morhua* L.) eggs and larvae obtained from farmed and wild broodstocks. Aquaculture 324–325:267–275. https://doi.org/10.1016/j.aquaculture.2011.10.036

Legendre, M., Slembrouck, J., Subagja, J., Kristanto, A.H. 2000. Ovulation rate, latency period and ova viability after GnRH- or hCG-induced breeding in the Asian catfish *Pangasius hypophthalmus* (Siluriformes, *Pangasiidae*). Aquaculture Living Resource 13:145–151. https://doi.org/10.1016/S0990-7440(00)00148-0

Manissery, J.K., Krishnamurthy, D., Gangadhara, B., Nandeesha, M.C. 2001. Effect of varied levels of dietary protein on the breeding performance of common carp *Cyprinus carpio*. Asian Fisheries Science 14:317–322.

Mazorra, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., Bromage, N. 2003. Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 227:21–33. https://doi.org/10.1016/S0044-8486(03)00493-9

Nguyen, H. Q., Tran, T.M., Reinertsen, H., Kjørsvik, E. 2010. Effects of dietary essential fatty acid levels on broodstock spawning performance and egg fatty acid composition of cobia, *Rachycentron canadum*. Journal of the World Aquaculture Society 41:687–699. https://doi.org/10.1111/j.1749-7345.2010.00411.x

Noori, F., Agh, N., Jafari, F., Jalili, R., Gisbert, E., Mozanzadeh, M.T. 2019. Dietary fatty acid profiling in plant protein-rich diets affects the reproductive performance, egg fatty acid profile and haematological parameters in female rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition 25:1050-1062. https://doi.org/10.1111/anu.12922

Nzohabonayo, E., Kassam, D., Ombe, J.K. 2017. Effect of lipid levels on reproductive performance of *Oreochromis karongae*. Aquaculture Research 48:1998–2003. https://doi.org/10.1111/are.13258

Patino, R., Yoshizaki, G., Bolamba, D., Thomas, P. 2003. Role of arachidonic acid and protein kinase C during maturation-inducing hormone-dependent meiotic resumption and ovulation in ovarian follicles of Atlantic croaker. Biology of Reproduction 68:516–523. https://doi.org/10.1095/biolreprod.102.009662

Phumee, P., Hashim, R., Aliyu-Paiko, M., Chong, A.S.C. 2009. Effects of dietary protein and lipid content on growth performance and

biological indices of iridescent Shark (*Pangasianodon hypophthalmus*, Sauvage 1878) fry. Aquaculture Research 40:456–463. https://doi.org/10.1111/j.1365-2109.2008.02116.x

Phumee, P., Wei, W.Y., Ramachandran, S., Hashim, R. 2011. Evaluation of soybean meal in the formulated diets for juvenile *Pangasianodon hypophthalmus* (Sauvage 1878). Aquaculture Nutrition 17:214–222. https://doi.org/10.1111/j.1365-2095.2009.00729.x

Robin, J. H., Regost, C., Arzel, J., Kaushik, S.J. 2003. Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. Aquaculture 225:283–293. https://doi.org/10.1016/S0044-8486(03)00296-5

Sargent, J.R. 1995. Origin and functions of egg lipids: Nutritional implications. In: Broodstock management and eggs and larval quality, Bromage, N.R. Roberts, R.J. (Eds.), Blackwell Science Ltd., London, England, pp. 352–372.

Sargent, J.R., Tocher, D.R., Bell, J.G. 2002. The lipids. In: Fish nutrition, 3rd edition, Halver, J.E. (Ed.), Academic Press Inc., San Diego, CA, USA, pp. 182–246.

Sink, T.D., Lochmann, R.T. 2008. Effects of dietary lipid source and concentration on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg and fry production, and egg and fry quality. Aquaculture 283:68–76. https://doi.org/10.1016/j.aquaculture.2008.07.024

Sotoudeh, A., Yeganeh, S. 2017. Effects of supplementary fennel (*Foeniculum vulgare*) essential oil in diet on growth and reproductive performance of the ornamental fish, Convict cichlid (*Cichlasoma nigrofasciatum*). Aquaculture Research 48:4284–4291. https://doi.org/10.1111/are.13249

Van der Meeren, T., Wilhelmsen, S., Klungsosyr, J., Kvenseth, P.G. 1993. Fatty acid composition of unfed cod larvae *Gadus morhua* L. and cod larvae feeding on natural plankton in large enclosures. Journal of the World Aquaculture Society 24:167–185. https://doi.org/10.1111/j.1749-7345.1993.tb00006.x

Watanabe, T., Arakawa, T., Kitajima, C., Fujita, S. 1984. Effect of nutritional quality of broodstock diets on reproduction of red seabream. Nippon Suisan Gakkaishi 50:495–501.

Watanabe, T. 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 73:3–15. https://doi.org/10.2331/suisan.50.495

Wing-Keong N., Yan W. 2011. Inclusion of crude palm oil in the broodstock diets of female Nile Tilapia, *Oreochromis niloticus*, resulted in enhanced reproductive performance compared to broodfish fed diets with added fish oil or linseed oil. Aquaculture 314:122–131. https://doi.org/10.1016/j.aquaculture.2011.01.034

Zakeri, M., Kochanian, P., Marammazi, J.G., Yavari, V., Savari, A., Haghi, M. 2011. Effects of dietary n-3 HUFA concentrations on spawning performance and fatty acids composition of broodstock, eggs and larvae in yellowfin sea bream, *Acanthopagrus latus*. Aquaculture 310:388–394. https://doi.org/10.1016/j.aquaculture.2010.11.009

Zakeri, M., Marammazi, J.G., Kochanian, P., Savari, A., Yavari, V., Haghi, M. 2009. Effects of protein and lipid concentrations in broodstock diets on growth, spawning performance and egg quality of yellowfin sea bream (*Acanthopagrus latus*). Aquaculture 295:99–105. https://doi.org/10.1016/j.aquaculture.2009.06.026