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The utilization of maggot meal and earthworm meal ratios for reproductive improvement of kurumoi rainbowfish (*Melanotaenia parva*) Broodstock

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Abstract
Earthworms have known as one of the live food which can provide the good reproductive performance on fish broodstock, whereas there are many other types of live food which is also considered potentially include maggot. Research about the use of maggot meal and earthworm meal ratio to improve reproductive capacity on Kurumoi rainbowfish broodstock was conducted. The research was conducted in male and female broodstock and were paired as two pairs in an aquarium (3-5 g, 6-7 cm, ± 5 months of age) during 4 months of observation. Tests carried out with the use of maggot meal (MM) and earthworm meal (EM) ratio with treatments as follows: A (0% and 100% EM MM), B (25% and 75% EM MM), C (50% MM and 50% EM), D (75% MM and 25% EM) and E (100% and 0% EM MM). Each treatment was four replicates. Fishes were fed 3 times daily at 08.00, 12.00 and 16.00 in satiation. Sampling was done every month to measure of weight and length. Other parameters such as the spawning intensity, the number of larvae performed incidental (direct current events) with daily observation, larvae were observed and separated in another container after hatching. The results showed that there was no significant difference as statistically between each treatment using analysis of variance and Tukey test on weight, length and survival rate parameters both in females and males ($P > 0.05$) except the final weight of males ($P < 0.05$). The intensity of spawning that occurs typically 4 to 6 times per month, which means that one or less than one week occurs of continuous spawning (partial). The average of larvae number in each replication showed a significant difference on each treatments where the use of 100% maggot meal ratio (E) was the highest, while the use of 50% maggot meal and 50% earthworm meal ratio (C) was the lowest.

Keywords: broodstock, earthworm meal, maggot meal, reproduction, *Melanotaenia parva*

Introduction
The parameters that influence gametogenesis and fecundity such as feed ratio, temperature, and photoperiod have been studied extensively [1]. However, selecting the relatively important ones from these factors is very difficult [2]. Reproduction approaches via the role of feed have often been conducted because these approaches are considered safer and quite important in improving the reproductive ability in fish. It is known that the growth and maturation of gonads occur when there is a surplus of energy obtained from food [3]. The spawning quality, gonad development, and fish fecundity can be improved through improvements in the broodstock feed's nutritional quality as proven in various species [4-6]. Moreover, the quality and quantity of feed also have an important role in gonad maturation in order to produce high-quality eggs.

Deficiency in essential nutrients, especially amino acids, fatty acids, vitamins, and minerals, impede the development of eggs and cause failure to ovulate [7]. Feed quantity also has an influence; if the feed fed to the broodstock is deficient in energy content, the number of atretic oocytes could increase [8]. During the last decade, the role of various broodstock feed components such as protein [9], essential fatty acids [10, 11], vitamin E [12], vitamin C [13], astaxanthin [6] and phosphoglyceride [14] have been discovered. Among the broodstock feed components, fat is the component that influences egg composition the most [15, 16]. Egg and sperm quality can be improved through the improvement of the quality of nutrients in the broodstock's feed [17].

Fat plays the main role as a membrane element and as an energy reserve in embryo development [18]. During oogenesis, oocytes receive a fat reserve directly from food [19] through transfer from the fat reserve in muscles (glycogen) and digestive glands for gonads [20]. Therefore, fat and the broodstock feed's composition is the main factor in supporting the success of the brood stock's reproduction and the survival rate of the seed produced [21].

The source of feed fat could be from various ingredients, but in general, the main fat and protein components in the fish feed industry are fish meal and oil which has increased the demand and is predicted to become difficult to obtain resource in a few decades [22]. Replacement of some or all the fish-based feed components with plant-based meals and oils is very important for the future development of aquaculture [23-25].

One of the natural feeds that is a source of fatty acids that are known to be beneficial for fish reproduction fish is earthworms, but other natural feeds have not yet been studied extensively, especially maggots which have long been developed as an ingredient alternative for feed to replace fish meal. The study by Caruso *et al.* [26] noted that fatty acids in maggots in palm kernel cake had a saturated fatty acid value of 43.78, mono-unsaturated fatty acid of 19.27, and polyunsaturated fatty acid of 4.09. Palm kernel cake itself already has a saturated fatty acid value of 63.59, mono-unsaturated fatty acid of 10.01 and poly-unsaturated fatty acid of 1.90 [26]. This shows that maggots are believed to be able to influence the reproductive ability of fish through the fatty acids they contain.

It is well-known that all fish species require three chains of highly unsaturated fatty acids (HUFA) for optimum growth and development, including for reproduction, which consist of docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) [27]. Usually freshwater fish need linoleic acid in their feed (18:2n-6) or linolenic acid (18:3n-3) or both, while marine fish need eicosapentaenoic acid (20:5n-3) and/or docosahexaenoic acid (22:6n-3) [28]. However, the presence of two types of unsaturated fatty acid, omega 6 and omega 3, is very much needed by both freshwater and marine fish [28, 27].

Compared with information on freshwater and marine fish, studies pertaining to the need for fatty acids in ornamental fish, especially in broodstock fish until the optimal growth of larvae are still very limited [28, 29]. The use of natural feed such as earthworms and maggots and the combination of the two in the form of meal to simplify the process and storage through the manufacture of feed for ornamental fish broodstock has never been conducted. This study was aimed to discover the reproductive ability of Kurumoi rainbowfish ornamental broodstock through the maggot and earthworm meal ratio in feed.

Materials and Methods

This study was conducted at the Research and Development Institute for Ornamental Fish Culture using Kurumoi rainbowfish fish (*Melanotaenia parva*) broodstock aged ≥ 5 months in aquaria with a stocking density of 4 individuals (2 pairs) consisting of 2 male parents and 2 female parents. The broodstock used was the fourth generation (F4) of parents originating from Kurumoi Lake, Papua. Before the fish for the experiment were put in the aquaria, the water in the containers was cleaned and disinfected using methylene blue (MB) for 24 hours. The water was then replaced with well water that had been filtered and aerated for 2 days as the broodstock maintenance medium. Coral was also placed in the medium to raise the pH to match the rainbow fish's natural habitat (>7.00). A shelter or a nest for the eggs in the form of shredded raffia rope was floated on the surface of the water.

Before the experiment was conducted, the feed given to the broodstock was bloodworms or tubifex worms. A week before plotting, the fish were acclimatized to the manufactured feed according to the treatments to be tested. The nutritional value of the manufactured feed tested for each treatment was according to the proximate analysis presented in Table 1.

The treatments tested on the Kurumoi rainbowfish broodstock were feeds with maggot meal (MM) and earthworm meal (EM) ratios of A (0% MM and 100% EM); B (25% MM and 75% EM); C (50% MM and 50% EM); D (75% MM and 25% EM); and E (100% MM and 0% EM). Each treatment was repeated 4 times. The treatment feeds were given 3 times a day at 08.00, 12.00 and 16.00 *ad satiation*.

Table 1: Nutritional value of feed of the various treatments of maggot meal and earthworm meal ratios.

Nutrition value (g/kg dry matter)	Maggot meal (MM) and earthworm meal (EM) ratio				
	A	**B**	**C**	**D**	**E**
Moisture	44.0	22.3	89.5	85.1	83.6
Protein	357.4	373.8	356.8	364.9	387.6
Fat	120.2	119.9	157.1	178.5	182.5
Ash	112.6	150.2	180.0	196.1	233.9
Crude fiber	122.4	154.0	154.7	119.9	87.2
Nitrogen Free Extract	287.4	202.1	151.4	140.7	108.8
Gross Energy (MJ kg^{-1})	18.13	17.03	17.23	18.08	18.23
Protein energy ratio	19.71	21.94	20.71	20.18	21.26

Note: A (0% MM and 100% EM), B (25% MM and 75% EM), C (50% MM and 50% EM), D (75% MM and 25% EM), and E (100% MM and 0% EM)

The fish plotting was done by first sexing the broodstock according to the known specific morphological characteristics. The specific characteristics in males are they have generally longer and wider bodies with a brighter and more striking coloration (yellow to orange) and a head that tapers towards the mouth and a dorsal fin which extends from the head to the tail. In females, the specific characteristics are a generally shorter and narrower body, a distended abdomen (if gonad mature), having a greyish green coloration, and a short dorsal fin.

Observations during the plotting and sampling were measuring the broodstock's weight using an AND brand digital scale (0.01 g) and length using a millimeter block (0.1 cm) and taking photographs of the fish. Sampling was done monthly for the weight and length parameters. Other parameters were spawning intensity, the number of larvae produced incidentally (immediately during the event), by conducting daily observations during the four months of the study. Larvae were observed and separated in another container after hatching and then fed initial feed, infusoria, followed by artemia and natural feed for an advanced seed phase such as bloodworms and tubifex worms. The parameters for water quality such as temperature, DO, pH, conductivity, ammonia, and nitrite were also recorded as supporting data.

Results and Discussions

During the observations, the water quality physical and chemical parameters showed an oxygen range between 4.00-7.7 mg L^{-1}, temperature 26-28 °C, pH 6.0-7.5, ammonia (NH_3-N) 0.000-0.009 mg L^{-1}, and nitrite (NO_2^-) 0.0020-14.000 mg L^{-1}. These ranges are still considered good for growth and development in Kurumoi rainbowfish broodstock rearing.

There were no significant differences between each treatment of maggot meal and earthworm meal ratios using the analysis of variance and the follow-up Tukey's test for the survival, initial weight, and initial and final length parameters for both the females and males ($P>0.05$). A significant difference was found in the final weight of the females and males ($P<0.05$). The spawning intensity was generally 4 to 6 times per month, which means that in one week or less there was a continuous (partial) spawning. The average total larvae in each repeat of each treatment revealed a significant difference. The 100% ratio maggot meal (treatment E) had the highest average, while the 50% maggot meal and 50% earthworm meal ratio (treatment C) had the lowest (Table 2).

Table 2: Parameters of observation on the various treatments of maggot meal and earthworm meal ratios.

Parameters	Maggot meal (MM) and earthworm meal (EM) ratios				
	A	B	C	D	E
Initial weight of females (g)	3.70 ± 0.45[a]	3.67 ± 0.37[a]	3.79 ± 0.72[a]	3.93 ± 0.53[a]	3.70 ± 0.36[a]
Final weight of females (g)	2.62 ± 0.30[b]	2.76 ± 0.45[ab]	3.03 ± 0.95[a]	3.16 ± 0.62[a]	3.18 ± 0.48[a]
Initial weight of males (g)	4.24 ± 0.56[a]	4.15 ± 0.71[a]	4.22 ± 0.39[a]	4.10 ± 0.91[a]	4.56 ± 0.39[a]
Final weight of males (g)	3.38 ± 0.46[b]	3.55 ± 0.62[b]	3.35 ± 0.43[b]	3.87 ± 0.66[ab]	3.94 ± 0.90[a]
Initial length of females (cm)	6.3 ± 0.4[a]	6.4 ± 0.3[a]	6.4 ± 0.4[a]	6.5 ± 0.4[a]	6.3 ± 0.2[a]
Final length of females (cm)	6.3 ± 0.3[a]	6.4 ± 0.3[a]	6.5 ± 0.4[a]	6.5 ± 0.5[a]	6.5 ± 0.3[a]
Initial length of males (cm)	6.6 ± 0.3[a]	6.6 ± 0.4[a]	6. 6 ± 0.3[a]	6.5 ± 0.4[a]	6.5 ± 0.3[a]
Final length of males (cm)	6.7 ± 0.3[a]	6.7 ± 0.4[a]	6.7 ± 0.1[a]	6.7 ± 0.3[a]	6.9 ± 0.4[a]
Survival rate of broodstock (%)	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00± 0.00[a]
Spawning intensity (times/month)	5 – 6	5 – 6	4 – 5	4 – 5	5 – 6
Average number of larvae during research period (fish)	515[ab]	436[ab]	392[b]	443[ab]	692[a]

* The values under the same superscript in the same row indicate no significant difference ($P>0.05$).

Note: A (0% MM and 100% EM), B (25% MM and 75% EM), C (50% MM and 50% EM), D (75% MM and 25% EM), and E (100% MM and 0% EM)

The initial weight in females and males between each treatment indicated no significant difference ($P>0.05$), but there was a significant difference in the weight at the end of the experiment ($P<0.05$). The difference in the initial and final weight has been indicated by a negative weight gain each month, where the weight had a tendency to constantly decrease until the end of the study (Figures 1 and 2). The loss of weight when the initial weight was compared to the final weight in the females from treatment A to E respectively was 29.19%, 24.80%, 20.05%, 19.59%, and 14.05%. The loss of weight when the initial weight was compared to the final weight in the males from treatment A to E respectively was 20.28%, 14.46%, 20.62%, 5.61%, and 13.60%.

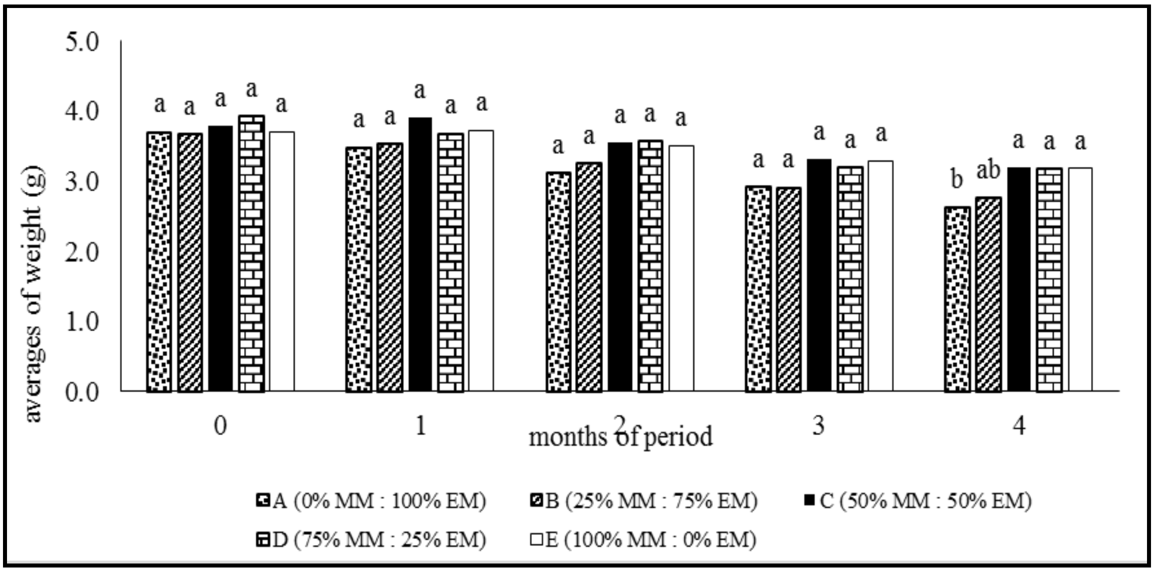


Fig 1: The average weight of females (g) during the research period.

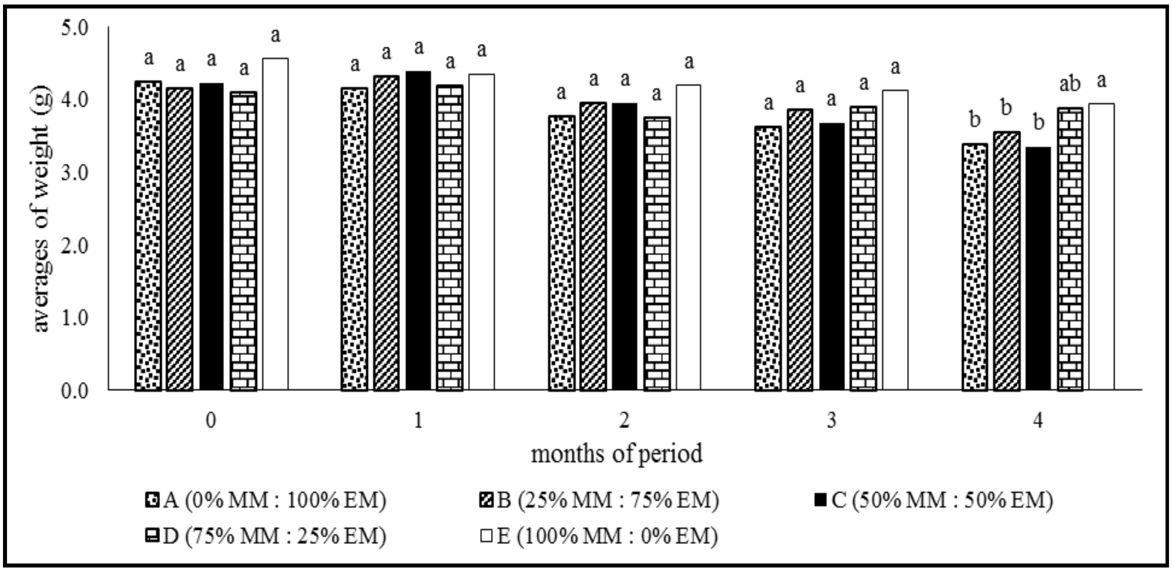


Fig 2: The average weight of males (g) during the research period.

The decrease in the weight of the females and males monthly, were caused by physiological and metabolic processes. These processes are for reproduction, continuously spawning during study had depleted their energy reserve for the growth and maintain. Another presumption was regarding the gonad weight (eggs and sperm) recorded at the initial weight at the beginning of the maintenance period. During the spawning, the gonads released in the form of eggs and sperm would automatically reduce the initial weight.

On the other hand, the initial and final length of both females and males between the treatments did not indicate a significant difference ($P>0.05$). At the end of the study, there was nearly no change in length; even if there was a change it was very insignificant compared to the initial length (approximately 0.1-0.3 cm). The female and male length tended to remain static every month until the end of the study (Figures 3 and 4).

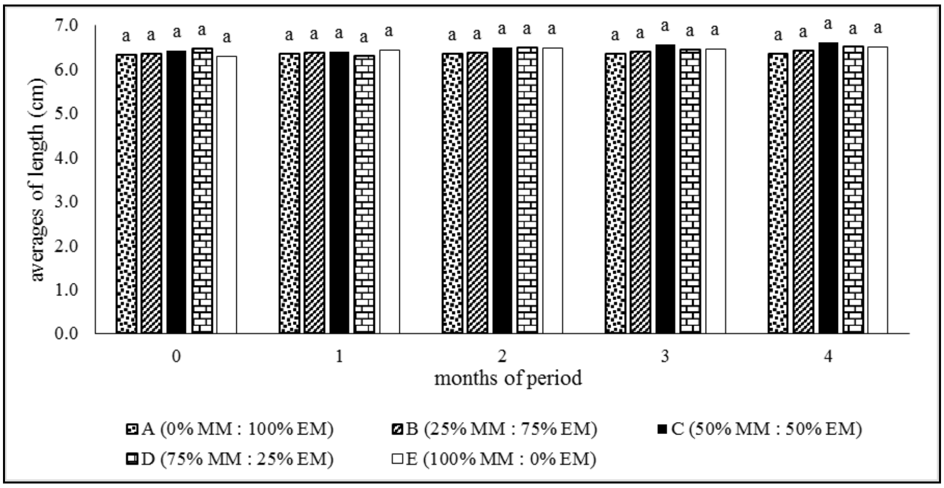


Fig 3: The average length of females (g) during the research period

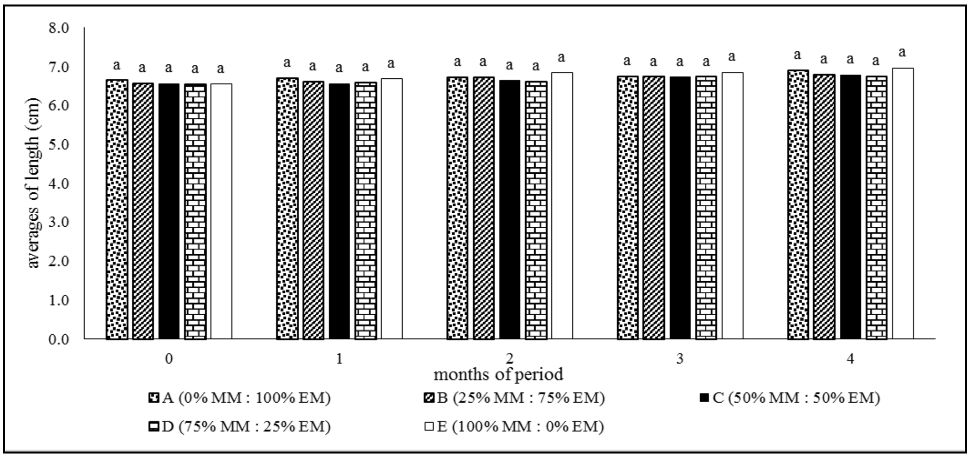


Fig 4: The average length of males (g) during the research period.

The use of earthworms and maggots in the form of meal was to simplify the processing and storage in the feed-making process. Earthworms and maggots were also used as a source of fatty acids from natural feed sources. As it has been mentioned, fat plays a very important role in fish nutrition, as an important energy and essential fatty acid source which is indispensable in the growth and survival of fish. Fat and fatty acids have a very significant role in regulating the biochemistry of the membrane and have a direct effect on the processes mediated by the membrane such as osmoregulation, nutrient assimilation, and physiological transport. Fat also plays a role as a medium for fat-soluble vitamins and sterols which are important in the biological structure of the membrane at both cellular and sub-cellular levels. Fat is also a component of hormones and a precursor for the synthesis of various metabolic functions such as prostaglandin and is clearly very important in providing palatability and texture to the feed consumed by fish [30].

However, the importance of feed fat does not mean that it can be used indiscriminately with no limit. As an important note, fish have a low energy requirement, making them prone to excessive fat deposition [31]. There are numerous studies pertaining to fish nutrition, among them on catfish's feed energy requirements for optimum growth [32, 33]. They concluded that the protein energy ratio in fish feed influenced the growth performance as well as the cost for feed because the feeding fish with low energy feed would make the fish consume feed protein for energy instead of for synthesizing tissues.

The proximate analysis to discover the nutritional value of the treatment feed revealed that the protein and fat content (%) respectively from treatment A to E was 35.74 and 12.02, 37.38 and 11.99, 35.68 and 15.71, 36.49 and 17.85, and 38.76 and 18.25, while the protein energy ratio was 19.71, 21.94, 20.71, 20.18, and 21.36 (Table 1). The nutritional value of each of the treatment feeds revealed that the 100% maggot meal ratio (treatment E) had the highest protein, fat, and protein-energy ratio and it tended to decrease as maggot meal ratio was decreased or as the earthworm meal ratio was increased. The female and male weight decrease tendency in each treatment followed the reduction in maggot meal ratio (Table 1, Figures 1 and 2).

An increased feed fat at similar protein levels increased the growth performance of swordtail fish [34], as the growth performance revealed by this study (low negative growth at higher feed fat). Theoretically, the utilization of energy which produces nutrients such as fat in feed could reduce oxidization of protein into energy and could, therefore, increase the utilization of feed protein for growth and tissue fulfillment [23, 24].

Because of the statement by Sales & Janssens [29] that fat is an important source of energy and fatty acids essential for the normal growth and survival of fish; therefore, the fatty acid content in this study needs to be stated clearly. In general, many fish need long-chain unsaturated fatty acids, even though the needs vary with the species. The most important essential fatty acids are n-3 and n-6 such as linoleic acid C18:2n6c, linolenic acid C18:3n3, arachidonic acid C20:4n6, eicosapentaenoic acid (EPA) C20:5n3, and docosahexaenoic acid (DHA) C22:6n3 [35]. The important fatty acid profile of each treatment is presented in Table 3.

Table 3: The profile of fatty acids in the various treatments of maggot meal and earthworm meal ratios

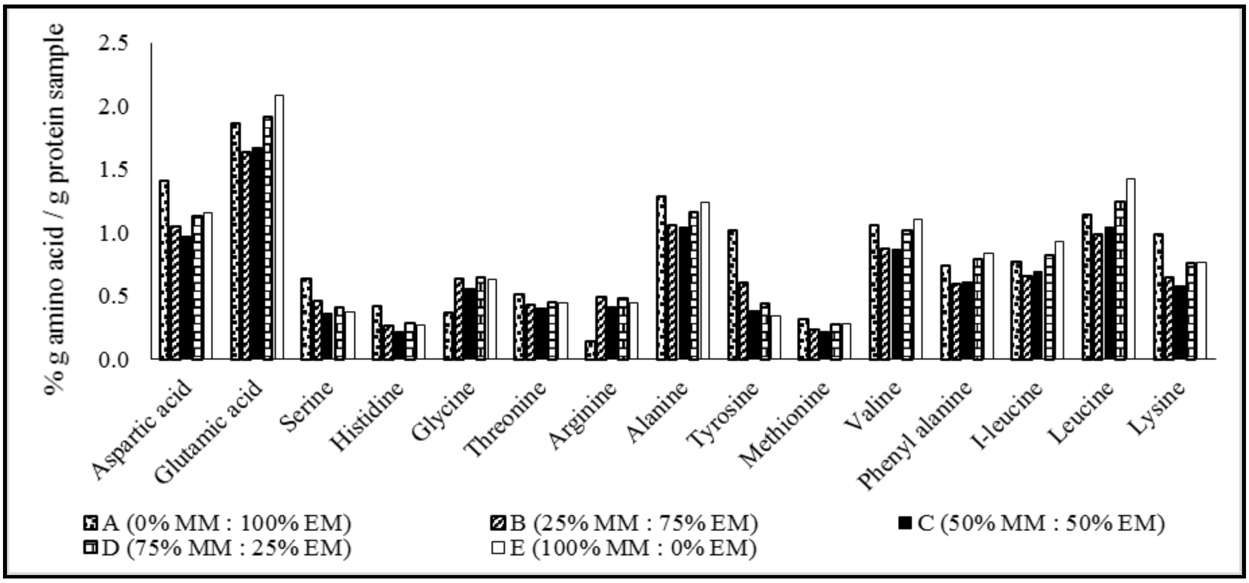
Fatty acid	**Maggot meal (MM) and earthworm meal (EM) ratios**				
	A	**B**	**C**	**D**	**E**
Fatty acid (wet)	10.19	13.59	19.41	25.96	24.3
Fatty acid (dry)	10.8	14.36	20.61	26.9	25.03
Fatty Acid value (% g fatty acid / g fat sample)					
Linoleic acid, C18:2n6c	1.44	2.38	2.71	3.16	3.15
Linolenic acid, C18:3n3	0.11	0.07	0.04	0.03	n.d
Arachidonic acid, C20:4n6	2.26	1.42	0.85	0.34	n.d
Eicosapentaenoic acid, C20:5n3	1.02	0.54	0.36	0.16	n.d
Docosahexaenoic acid, C22:6n3	0.05	0.03	n.d	n.d	n.d

Note: in% (w/w) Soxhlet method (Gravimetry), n.d: not detected.
A = (0% MM: 100% EM), B = (25% MM: 75% EM), C = (50% MM: 50% EM),
D = (75% MM: 25% EM), E = (100% MM: 0% EM)

The higher the earthworm meal ratio in the feed, the higher the linolenic acid, arachidonic acid, EPA and DHA profile is, while the linoleic acid decreases. This condition is the opposite with the maggot meal ratio in feed which means that the earthworm meal ratio is proportionate with linolenic acid, arachidonic acid, EPA, and DHA, whereas the maggot meal ratio is proportionate to linoleic acid (Table 3).

Omega 3 fatty acid has an important role in influencing the fish's physiology as a component of the phospholipid membrane and as an active biological precursor for eicosanoic acid [36]. Approximately 1% linolenic acid (18:3n-3) is required in feed for common carp to maintain the low level of lipogenesis and to protect against the over-production of oleic acid [37]. The negative weight gain demonstrated by the male and female parents was, among others, believed to be due to the linolenic acid profile in this study which did not fulfill the requirements.

Results in the field of fish nutrition revealed that omega 3 fatty acids such as linolenic acid 18:3n-3, eicosapentaenoic acid (EPA) C20:5n3, and docosahexaenoic acid (DHA) C22:6n3 have a very important role in fish health and improve the growth rate in many fish species such as rainbow trout [38], prawns [39], sea bream [40] and turbot [41]. The fairly complete fatty acid component in each treatment feed (Appendix 1) is presumed to be adequate for the needs during the maintenance period, proven by the broodstock survival in each treatment which was very good (approximately 100%).



Note: in% (w/w) method of HPLC (High-Performance Liquid Chromatography).

Fig 5: The amino acid profile in various treatments of maggot meal and earthworm meal ratios.

In addition, the natural needs and the quantity of fat depend on the species and habitat [42, 43]. In general, freshwater fish need linoleic acid in feed (18:2n-6) or linolenic acid (18:3n-3) or both, while marine fish need eicosapentaenoic acid (20:5n-3) and/or docosahexaenoic acid (22:6n-3) [28]. However, the high EPA and DHA levels shown in the fish cells, arachidonic acid C20:4n6 (ARA) as a component of the fatty acid omega 6, also has a vital role as the main precursor of eicosanoic acid [44], but in the present study it is presumed that ARA did not have an important role in reproduction and growth.

Even though the physiological importance of fatty acids is indispensable, in ornamental fish the information of the requirement is still difficult to find [29]. Therefore, in this study, the information of the requirements for each fatty acid component could not be discussed in detail and can only be compared between the treatments based on the measured values.

Unlike the fatty acid profile in Table 3 above which is proportionate to the earthworm meal and maggot meal ratio, the amino acid profile demonstrated a different performance. The amino acid profile for each treatment revealed that the glutamic acid, aspartate acid, alanine, leucine, and valine respectively were the highest of the fifteen amino acids detected in the feed using a high-performance liquid chromatography method. The amino acid value for the 100% ratio EM treatment (treatment A) and 100% MM (E) were always alternately in the highest position, while the combination 50% EM and 50% MM ratio (C) was usually at the lowest position (Figure 1). This indicated that the amino acid profile for the combination of the two different natural feed sources did not result in a balance, but in contrast reduced the value of amino acids contained.

Even though the role of the connection between amino acids and reproduction and the relationship between amino acids and the effectiveness of fatty acids are still unclear, from the parameters in Table 3 it can be assumed that there is a fairly significant effect of the amino acid profile produced in this study on reproduction. This was supported by the case study of Gilthead seabream fed feed with low essential amino acids, resulting in an increased number of egg fat droplets [44] as also found in seabream [15]. On the contrary, a higher fat content in eggs was supported by a high n-3 HUFA level in feed [45].

The rule that DHA plays a role in vitellogenesis [46] and larval development [47, 48] have been well documented. Some studies have informed the importance of providing a DHA/EPA ratio in feed for growth, survival, and egg quality [47].

One of the nutritional factors that have been known to have a strong influence on fish spawning quality is the fatty acid content [15]. Harel *et al.* [49] fed gilthead seabream fish broodstock with feed containing up to 1% n-3 HUFA (highly unsaturated fatty acid) and observed that the composition of the female organs related to reproduction which was modified by the feed's essential fatty acid content could affect the quality of the eggs for a short duration.

However, the other fatty acid levels in the eggs were not reported to be influenced by the fatty acid composition of the feed. Some studies [13, 50] suggest that the chemical composition of fish eggs must fulfill the embryonic nutritional requirement for growth and development if it is to be connected to spawning quality. On the other hand, the results of the study conducted by Fernández-Palacios *et al.* [45] demonstrated that the percentage of n-3 HUFA in eggs should not be used independently as a criterion to judge the quality of the eggs produced by gilthead sea bream broodstock. As it is already known, the high or low level of n-3 HUFA is associated with the low spawning quality using the base data of the total egg number and the survival rate of the larvae produced.

Based on this background, the fatty acid composition of the eggs is also influenced by the n-3 HUFA content of the broodstock's feed. The n-3 fatty acid and n-3 HUFA content of gilthead seabream eggs increased as the n-3 HUFA feed was elevated; the increase was usually the 18:3n-3, 18:4n-3 and EPA content of the eggs [45]. Therefore, a positive correlation was discovered between feed and the n-3 HUFA content of eggs where the EPA content was more influenced by the n-3 HUFA in feed compared to the DHA content in the eggs. Even though the fatty acid composition of the eggs was not analyzed in this study, the spawning quality could be judged from larva production of each treatment during the experiment.

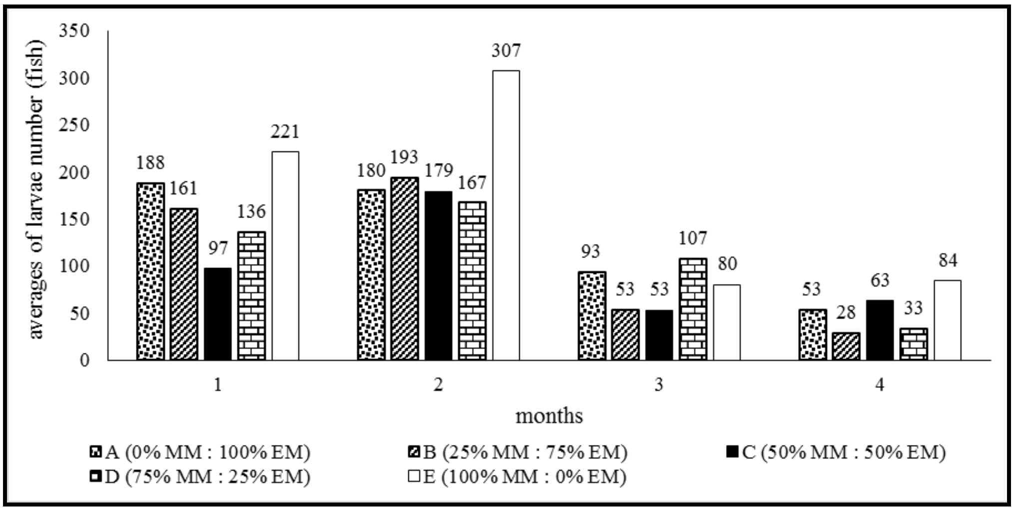


Fig 6: Average of larvae number (fish) during the research period.

The number of larvae produced every month and the total number of larvae during the course of the experiment tended to be the highest in treatment E (100%MM: 0%EM), followed by treatment A (0%MM: 100% EM) and was the lowest in treatment C (50%MM: 50% EM). In many animals, gonad maturity is a process that has many requirements which refer to the material and energy reserves. When food is abundant, the gonad maturation process can be gonad endured fully by the materials and energy absorbed, but when food is scarce, gonad maturity requires the mobilization of macromolecule components from other tissues to be used in gametogenesis [51].

Many studies have demonstrated that productive performance and egg and sperm quality are influenced by nutrient such as protein and fat and the fish size ratio such as in tilapia *Oreochromis niloticus* [52, 53], common carp [54], and rohu *Labeo rohita* [55]. Protein and fat, the main components of egg yolk, are believed to play a vital role in reproduction. Study results revealed that there is an optimal fat level for reproductive success and that the requirements must be associated with the growth of each species [55]. A correlation between the lower negative growth and higher larva production in treatment E answers this condition.

The spawning quality in gilthead sea bream was observed in the increased amount of n-3 HUFA in the feed up to 1.6% [45]; similar results were found in other sparids [9, 15]. This feed level was higher than the findings in salmonids (approximately 1.0% n-3 HUFA) and was similar to the findings in red sea bream in Japan. However, the high amount of n-3 HUFA in feed reduced the total number of eggs produced and caused hypertrophy of the yolk in some of the larvae, resulting in a decline in the larval survival rate. In this study, this result was assumed to be because the high n-3 HUFA in the feed produced fewer larvae than the low n-3 HUFA in feed.

Because the egg composition and spawning quality in gilthead sea bream broodstock is influenced by the level of essential fatty acids in the feed [45], it suggested that essential fatty acids in feed are easily associated with eggs and spawning quality and that the spawning quality in this species can be improved by modifying the quality of the nutrients in the broodstock's feed during the period prior to the spawning season [45]. In broodstock that spawns during a short vitellogenetic period such as sparids, the spawning quality is apparently influenced by feed fat prior to [9] or during spawning.

In rainbow trout, deficiency of n-3 in feed was discovered to show a strong relationship between DHA and egg fat where the EPA content decreased by 50% [56]. However, the levels of other fatty acids in the eggs were not reported to be affected by the composition of the fatty acids in the feed. A number of studies [13, 50] suggested that the embryonic nutritional requirements for growth and development must be fulfilled since the beginning to have an effect on the egg composition, which in the end is related to the spawning quality. The maintenance period of more than four months in the present study is hoped to have fulfilled this requirement.

The broodstock feed's fat had a different effect on the fish egg's fatty acid profile;

the lack of essential fatty acids can alter the fecundity and egg hatching rate and would increase anomalies in the larvae developing in the eggs [9, 15, 16, 57]. In a number of freshwater carp, it has been known that the level of docosahexaenoic acid in feed significantly influences the egg hatching rate [57].

The eicosapentaenoic acid (EPA) and arachidonic acid (ARA) levels in feed have also been discovered to have a correlation with the fertilization rate of gilthead sea bream [45]. Arachidonic acid is also a PG precursor from the series II. Some PGs produced by female goldfish which are called PGFs play an important role as a pheromone, stimulating the male sexual behavior and synchronizing the male and female spawning, having a direct influence on the success of the fertilization [58].

The distribution of EPA in sperm could also be influenced by the fatty acids in feed as seen in rainbow trout [16, 59], and could also be responsible for sperm activity and fertilization rate as suggested by Watanabe *et al.* [16]. The percentage of morphologically normal eggs (the parameter often used to evaluate the viability of eggs) have been found to increase the elevation of the n-3 HUFA level in broodstock feed and the correlation between this fatty acid and egg development [45] indicated the importance of EPA for the development of normal eggs and embryos in gilthead sea bream.

In some species such as the halibut (*Hippoglossus hippoglossus*), n-3 HUFA (a polyunsaturated fatty acid) is also considered a main source of energy during the initial embryonic development phase [60]. A very low hatching rate or egg viability is associated with the total content which is higher in turbot, sole and sea bass eggs [61]. However, an elevated content of egg fat in gilthead sea bream up to 20% was not found to influence the hatching percentage or proportion of normal eggs [45].

Based on the data on male and female broodstock weight gain and spawning quality in the form of the number of larvae produced, it can be concluded that the fatty acids that play a role in the reproduction of Kurumoi rainbowfish fish are the n6 group, which in this case is the C18:2n6c linoleic acid more than the n3 HUFA fatty acid. These fatty acids which are commonly used as an alternative in feed such as 18:2 (n-6) and 20:4 (n-6) are involved in the physiology reproduction of because they are precursors of the prostaglandin important in the process of ovulation [62]. Even though the study by Sargent *et al.* [36] reflects the importance of DHA and EPA in fish nutrition, this is assumed to be only in marine fish and not the case in the present study.

Amino acids which are related or associated with fatty acids in feed in this study also influence the reproduction process, where the lower the amino acid profile, the worse the spawning, which is represented by the spawning intensity the number of larvae produced, is. The feed quality in the form of the content of nutrients such as protein, fat, and the protein energy ratio also influenced the results of this study. These results indicated that maggot meal was better than earthworm meal or a combination of the two for the reproduction process of Kurumoi rainbowfish fish.

Conclusion

The results of this study revealed that there was no significant difference between each treatment of maggot meal and earthworm meal ratio for the survival, initial weight, initial and final length parameters in both males and females. A significant difference was found in the final weight of the females and males. The spawning intensity was usually 4 to 6 times per month, which means there was one week or less than a week when there was continuous (partial) spawning. The average total number of larvae produced durcd/'ing the study demonstrated a significant difference between the treatments where the use of 100% maggot meal ratio (treatment E) resulted in the highest number and the 50% maggot meal and 50% earthworm meal ratio (treatment) the lowest.

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