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Does the Gradual Substitution of Fishmeal by Dried Zooplankton in the Diet of Gilthead Seabream *Sparus aurata* Fries Improve their Growth Performance and Survival Rates?

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ABSTRACT

Finding suitable alternatives to animal protein used in the manufacture of fish feed is one of the major challenges at present since these alternatives must be sustainable, with an abundance in nature, available and easy to obtain, and economically inexpensive. The goal of this study was to investigate how the gradual replacement of fishmeal with dried zooplankton (DZ) affected gilthead seabream (*Sparus aurata*) larvae over a four-month experiment. Nine hundred fish were allocated into five groups with an initial average weight of 0.227± 0.030g. The fish were fed five isonitrogenous and isocaloric diets in which fish meal was replaced by dried zooplankton (DZ) at different levels: 0 (G1), 25 (G2), 50 (G3), 75 (G4), and 100% (G5). The effects of diets on growth performance, feed utilization, survival, and body composition were tested. The results showed that substituting dried zooplankton for fishmeal had a strong relationship with fish weight gain, length gain, and feed conversion ratio, with R^2 values of 0.99, 0.94, and 0.97, respectively. The polynomial regression model, which exhibited the maximum response at G4 and G5, was determined to be the most suitable regression model to reflect the fish's reaction to the replacement of fish meal with dried zooplankton (DZ). Using dried zooplankton (DZ) instead of fish meal (FM) in the early stages of gilthead seabream feeding proved to be an excellent strategy for enhancing growth and reducing mortality rates by 50%. The 75% replacement rate showed the best results in terms of lowering mortality, while both the 75 and 100% replacement rates were optimal for growth criteria. In summary, this study found that a complete substitution of fish meal with dry zooplankton meal in gilthead seabream diets was achievable without adverse impacts on weight development, length growth, or feed utilization.

INTRODUCTION

Plankton, both phyto and zoo types, are organisms found in all water bodies around the world, and their diversity, abundance, and densities vary according to the nature and type of the water body **(Ashour *et al.*, 2018; Farrag *et al.*, 2019)**. The richness of primary productivity in the Egyptian waters is attributed to the ample availability of fertile agricultural land. Agricultural fertilizers, draining into water channels that eventually reach the Red Sea and the Mediterranean Sea, as well as various lakes, contribute to this phenomenon. Consequently, phytoplankton growth is promoted, followed by zooplankton proliferation **(Abdel-Aziz *et al.*, 2011; Abou Zaid *et al.*, 2014; Abo-Taleb *et al.*, 2016a; El-feky; Abo-Taleb, 2020)**. The proliferation of these organisms, as highlighted in studies by **Abo-Taleb *et al.* (2015, 2016b)** and **Abdelsalam *et al.* (2020)**, is detrimental to the natural characteristics of water bodies. Consequently, efforts have been directed toward devising a mechanism to harvest plankton from their natural habitats, aiming to achieve two objectives: firstly, to utilize them as feed for aquatic organisms, and secondly, to provide an opportunity for ecosystem recovery.

Several studies have focused their attention on the distribution, diversity, and abundance of plankton in different Egyptian waters such as the Red Sea **(El-Sherbiny *et al*., 2007; Abu El-Regal *et al*., 2018; Abo-Taleb, 2019, 2020b)**, the inland waters **(Abdel-Aziz *et al*., 2011; El-Feky *et al*., 2019)** as well as the Mediterranean **(Zakaria *et al*., 2016; Abo-Taleb & Gharib, 2018; Abo-Taleb, *et al*., 2020a)**.

The analysis of the chemical composition of these organisms proved that they contain high percentages of crude animal protein as well as fats, in addition to containing many vitamins necessary for the growth of fish in the early stages of life. It is also worth noting that these organisms contain good percentages of antioxidants such as β-carotene and Tannic acid, which works to raise the immunity of living organisms and increase their ability to resist diseases **(Abo-Taleb *et al*., 2020, 2021a; El-feky & Abo-Taleb, 2020)**. Therefore, adding these organisms to the food in order to raise the resistance of fish larvae to pathogens and control fish diseases is a necessity at the present time to reduce the use of synthetic antimicrobial materials since their excessive use affects the environment and the public health of humans and animals severely, besides creating new bacterial species more resistant and deadlier **(Ashour *et al*., 2021)**.

Gilthead seabream, *Sparus aurata*, is the only species of seabream that is currently cultivated on a large scale. It is common throughout the Mediterranean coasts **(Mona *et al*., 2019)**. It can live in marine waters as well as in the brackish waters of coastal lagoons **(Uçal, 2002)**. Since there are only a few verified protein sources as a reliable potential alternative to fishmeal **(Luthada-Raswiswi *et al*., 2021; Suhaimi *et al*., 2022)**, the search for such few alternatives is an urgent necessity. Indeed, only a handful of studies have delved into the utilization of plankton as a substitute for fishmeal. For instance, **Hassan *et al.* (2020)** investigated the impact of substituting fish meal with zooplankton meal on the growth and survival of seabass (*Dicentrarchus labrax*) larvae. **Abo-Taleb *et al*. (2021a, b)** studied the effect of replacing the powder of one zooplankton species, *Daphnia magna*, as well as a mixture of zooplankton on the nutritional and histological status of larvae of gray mullet, *Mugil cephalus*.

The importance of the current study is due to the fact that plankton is the main food source for the early stages of most aquatic organisms in the wild, it works to build up the basic structure of fish during its early life, as well as enhancing their immune system and resistance to diseases.

Therefore, attempting to harvest plankton from natural habitats and incorporating it into captive-bred fish may reduce mortality and enhance the immunity and growth rates of these fish. This is particularly crucial considering the risks associated with rearing larvae of economically valuable species like seabream, including high mortality rates and slow growth rates. Addressing these challenges requires intensive research into the nutrients used and the natural properties of the water utilized. Moreover, finding alternative sources of fishmeal, which serves as a crucial source of animal protein in fish larval diets, is a significant challenge that warrants more attention.

MATERIALS AND METHODS

1. Collection, estimation and identification of zooplankton

During April 2020, zooplankton samples were collected from Mariout Lake, Alexandria, Egypt; using a pump system (Bio-condenser) and plankton net with a mesh size of 55µm. The filtration of $10m^3$ of water yielded 385.7g wet-weight of zooplankton (we noticed temporal changes in zooplankton biomass). For species identification, samples were transferred into a small glass bottle and preserved in 5% neutralized formalin solution, and the sample volume was then concentrated and adjusted to 100ml. The samples were examined under a binocular research microscope. The identification was undertaken to species levels according to **Sars (1927)**, **Gurney (1931)** and **Abou Zaid *et al*. (2014)**.

For the estimation of standing crop, subsamples of 5ml were transferred to a counting chamber (Bogorov chamber) using a plunger pipette; this operation was performed three times, and the average of the three counts was taken. The standing crop was calculated and estimated as individuals per m^3 using **Santhanam and Srinivasan (1994)** formula (Table 1):
N= (n*v) / (V*S)

Where, **N** is zooplankton number/ m^3; **n** is the average counts in 5ml; **v** is the concentrated volume = 100ml; **S** is subsample volume= 5ml, and **V** is the volume of all filtered water in m^3= 100L/ 1000= 0.1m^3.

Table 1. Diversity and abundance of zooplankton groups recoded during the current study

Group	Individual/ m^3	(%)	No. of species
Rotifera	11306	42.63	24
Copepoda	13854	52.25	10
Protozoa	710	2.68	5
Cladocera	371	1.4	7
Insecta	237	0.89	2
Nematoda	10	0.04	2
Annelida	9	0.03	1
Fish eggs	20	0.08	1

2. Preparation of dried zooplankton and diets

The collected zooplankton biomass underwent drying for 48 hours at a temperature ranging from 50 to 60°C. From an initial wet-weight of 385.7 grams of zooplankton, the drying process yielded 80.3 grams of dry-weight zooplankton. Table (2) shows the nutritional profile of the DZ. Five isonitrogenous (420g kg^{-1}) and isolipid (100g kg^{-1}) diets (G_1, G_2, G_3, G_4 and G_5) were formulated (Table 3). The diets were formulated with varying degrees of replacement of fishmeal by dried zooplankton (DZ), ranging from 0 to 100% replacement in increments of 25%. In the feed formula, FM, DZ and soybean meal were used as a main protein source. Carbohydrate was obtained from the whole wheat and yellow corn meal, and fish oil was used as a main lipid source. All dry ingredients were ground into powder to pass through an 80-mesh sieve, and then mixed with fish oil. Finally, worm distilled water was added to the mixture to make a dough. The dough was pelleted with a meat grinder (Tornado meat grinder, model MG-2000, Egypt) with a diameter of 1.0mm. Subsequently, the experimental diets were broken up and sieved into a proper size. The diets were dried at 55°C until the moisture content was <10%, after which they were stored in a freezer at -20°C.

Table 2. Chemical composition of dried zooplankton and fishmeal (% on dry matter basis)

Item	Dry matter	Crude protein	Crude lipid	Ash
Dried zooplankton (DZ)	90.04±0.05	65.00±1.18	11.82±1.13	7.01±0.09
Fishmeal (FM)	91.12±0.34	62.23±1.12	9.01±0.45	12.56±0.67

Table 3. Formulation and proximate composition of the experimental diets (g/ 100 g)

Ingredient (g /100g)	Group				
	G_1	G_2	G_3	G_4	G_5
Fishmeal[1]	35	26.25	17.5	8.75	0
Dried zooplankton	0	8.75	17.5	26.25	35
Soybean meal[2]	35	35	35	35	35
Corn meal[2]	15	15	15	15	15
Whole wheat[2]	10	10	10	10	10
Fish oil[3]	3	3	3	3	3
Minerals and vitamins mix[4]	2	2	2	2	2
Total of ingredients	100	100	100	100	100
Proximate composition					
Dry matter (%)	94.47±0.09	94.34±0.07	94.20±0.19	94.84±0.08	94.30±0.03
Crude protein (%)	41.82±0.08	41.90±0.12	41.85±0.05	41.81±0.02	41.80±0.04
Crude fiber (%)	6.90±0.04	6.83±0.03	6.88±0.06	6.91±0.11	6.82±0.12
Crude lipid (%)	10.84±0.33	9.02±0.13	9.04±0.03	9.19±0.06	9.04±0.05
Ash (%)	8.31±0.04	6.16±0.06	6.28±0.08	6.11±0.04	6.09±0.04
Carbohydrates (%)	32.13±0.31	35.60±0.19	35.11±0.11	35.58±0.15	34.85±0.13

[1]Fish meal (62.0% crude protein, 9.0% crude lipid, and 12.8% ash) was purchased from Zhanjiang Haibao Feed.
[2]Ingredients were sourced from local feed ingredients' market, Kafr elsheikh, Egypt.
[3]Sardine fish oil was purchased from Bawa Fishmeal and Oil, Karnataka, India.
[4]Minerals and Vitamins mix (g/kg diet): KCl 200mg, KI (1%) 60mg, $COCl_2 \cdot 6H_2O$ (1%) 50mg, $CuSO_4 \cdot 5H_2O$ 30mg, $FeSO_4 \cdot H_2O$ 400mg, $ZnSO_4 \cdot H_2O$ 400mg, $MnSO_4 \cdot H_2O$ 150mg, $Na_2SeO_3 \cdot 5H_2O$ 65mg, $MgSO_4 \cdot H_2O$ 2,000mg, zeolite powder 645.85mg. A, 10.00g; D3, 50.00g; E, 99.00g; K3, 5.00g; B1, 25.50g; B2, 25.00g; B6, 50.00g; B12, 0.10g; pantothenic acid 40.00g; nicotinic acid, 101.00g; biotin, 2.50g; inositol, 153.06g; folic acid, 6.25g; cellulose, 411.59g.

3. Fish and experimental design

The feeding experiment was carried out at the National Institute of Oceanography and Fisheries (NIOF). Prior to the experiment, a total of 900 gilthead seabream *S. aurata* fries at the age of 28 days after hatching with initial length of 3.03± 0.10cm and an initial weight of 0.23± 0.03g, were placed in 15 hapa net-cages with a water volume of 0.6m^3, in triplicates. Before stocking, the *S. aurata* fries were firstly acclimatized to the experimental conditions at a temperature of 25- 27˚C, with a pH value of 7.2 and provided with a continuous aeration for 24hr. The experiment lasted for 120 days to assess the effect of the feed given to the fish. The fry in each dietary treatment tank was fed four times per day with a ratio of 7% at 09:00, 11:00, 13:00 and 15:00. Partial water exchange was daily conducted at 6:00pm during waste removal. Water parameters such as temperature, dissolved oxygen, and pH were maintained and monitored twice a week using YSI meter.

4. Sample collection

After the feeding trial, fish samples were fasted for 24hr. Then, fish in each cage were counted and weighed to calculate the growth indices. Ten fish per cage were randomly selected and anaesthetized with MS-222 (Sigma, USA) to obtain total length and weight. For the analysis of proximate composition, six whole fish per cage were pooled & homogenized, and samples were frozen on dry ice (n= 3).

5. Growth performance calculations

The weight growth and length growth parameters were calculated using the formulae outlined by **Castell and Tiews (1980)**, as follows:

- Total weight gain (g) = final fish weight (g) - initial fish weight (g)
- Weight gain (%) = total weight gain (g)/ initial fish weight (g) ×100.
- Average daily weight gain (mg/day) = final fish weight (g) - initial fish weight (g)/ days
- Weight specific growth rate (%/day) = {(ln final fish weight) – (ln initial fish weight) /days} × 100
- Feed intake (g) = feed consumption (g) ⁄ total weight gain (g)
- Feed conversion ratio (FCR) = total feed intake in dry basis (g) ⁄ total weight gain (g)
- Survival rate (%) = (final fish number) ⁄ (initial fish number) × 100
- Total length gain (cm) = final fish length (cm) - initial fish length (cm)
- Length gain (%) = Total length gain (cm)/ initial fish length (cm) × 100
- Length average daily gain (mm/day) = final fish length (cm) - initial fish length (cm)/days
- Length specific growth rate (%) = {(ln final fish length) - (ln initial fish length) /days} × 100

6. Proximate composition analysis

For fish, dried zooplankton and experimental diets, all chemical tests were performed in triplicates according to the **AOAC (1995)** standard methodology. Drying samples to a constant weight at 70°C in an oven was used to assess moisture content. For each dietary replicate, the whole fish were ground and pooled. The crude protein was analyzed by using the Kjeldahl method with a protein conversion factor (F= 6.25) to transform total nitrogen to crude protein **(Sawyer & Kirk, 1991)**. The lipid content was measured using the Soxhlet method following ether extraction (VELP Scientifica, SER 148, Usmate Velate, Italy). The ash concentration was determined by burning the samples for 5 hours at 550°C in a muffle furnace (Nabertherm B150, Bremen, Germany).

7. Statistical analysis

The experimental data were analyzed by one-way ANOVA with SPSS 20.0 (SPSS Company, New York, USA), and multiple comparisons were made with Duncan method. The significant difference level was $P< 0.05$, and the results were expressed as mean ± SD (standard deviation).

RESULTS

1. Zooplankton diversity and abundance

A total of eight zooplankton groups in addition to fish eggs and free-living nematodes were addressed. The data in Table (1) indicate that the highest mean abundant group was copepods with an average of 13854 individuals/ m^3. While the highest diversity was recorded for the rotifers group (24 recorded species). Copepods were the most abundant group and represented 52.25% of the total zooplankton abundance, followed by rotifers 42.64%, however the lowest group in abundance and diversity was Annelida (only 0.03%).

2. Dried zooplankton chemical analysis

The dried zooplankton powder had an approximate chemical composition of 90.04% dry matter, 65.00% crude protein, 11.82% crude lipid, and 7.01% ash (On a dry matter basis), as shown in Table (2).

3. Weight growth parameters and survival

The growth performance, feed utilization and survival of gilthead seabream are presented in Table (4). With DZ levels increasing, weight gain rate, weight average daily gain and weight specific growth rate initially decreased and then increased, reaching a significant improvement in G_5 group (P< 0.05). Fish fed the DZ inclusion diets had higher feed intake than that of fish fed the G_1. Feed conversion ratio showed the trend of increasing first and then decreasing, reaching the lowest level in G_5 group. However, the increase of FM replacement up to 100% showed a significant increase in the survival rate. The relation among weight gain, survival rate, and increasing fishmeal substitution levels with DZ showed polynomial regression trends with a very strong correlation R^2= 0.99 and 0.80, respectively, which noted that the best replacement level of fishmeal with DZ could be between 75 and 100% replacement level. Fish fed diets G_4 and G_5 showed a significantly (P< 0.05) higher feed intake, as well as the best FCR among all fish groups. The polynomial regression is the best regression model to present the relation of FCR among different treatments, and dried zooplankton replacement levels with a very strong correlation coefficient R^2= 0.97 (Fig. 1).

Table 4. Effect of fishmeal replacement with dried zooplankton on weight growth parameters of gilthead seabream, *Sparus aurata*, fry (Mean ± SD)

Larvae weight growth parameter	Group				
	G1	**G2**	**G3**	**G4**	**G5**
Initial weight (g)	0.228± 0.014 ^{NS}	0.227±0.015^{NS}	0.229± 0.016 ^{NS}	0.228± 0.012 ^{NS}	0.226± 0.017 ^{NS}
Final weight (g)	3.144±0.323	2.968±0.435 ^{NS}	2.929±0.489 ^{NS}	3.283±0.936**	3.912±0.404**
Weight gain (g)	2.917±0.283	2.741±0.173 ^{NS}	2.691±0.217 ^{NS}	3.056±0.534**	3.687±0.007**
Average daily weight gain (mg/day)	24.31±2.36	22.84±1.44 ^{NS}	22.43±1.81 ^{NS}	25.46±4.45 ^{NS}	30.72±0.89 ^{NS}
Weight specific growth rate %/day)	2.19± 0.07	2.14± 0.07 ^{NS}	2.12± 0.05 ^{NS}	2.22± 0.12 ^{NS}	2.38± 0.04 ^{NS}
Weight gain (%)	1280.6±122.81	1211.3±109.67 ^{NS}	1175.3±80.84 ^{NS}	1339.6±217.10 ^{NS}	1636.2±89.02**
Feed intake (g)	5.583±0.003	5.36±0.001**	5.29±0.002*	5.44±0.002 ^{NS}	6.14±0.003**
Feed conversion ratio	1.93±0.24	1.96 ±0.13 ^{NS}	1.98 ±0.17 ^{NS}	1.82 ±0.05 ^{NS}	1.67 ±0.18 ^{NS}
Survival rate (%)	92.00 ±1.00	93.00±0.58 ^{NS}	94.00±0.58 ^{NS}	96.00±0.58 ^{NS}	93.00±1.53 ^{NS}

:** Statistically significant, *:** Statistically high significant, NS: Statistically non-significant.

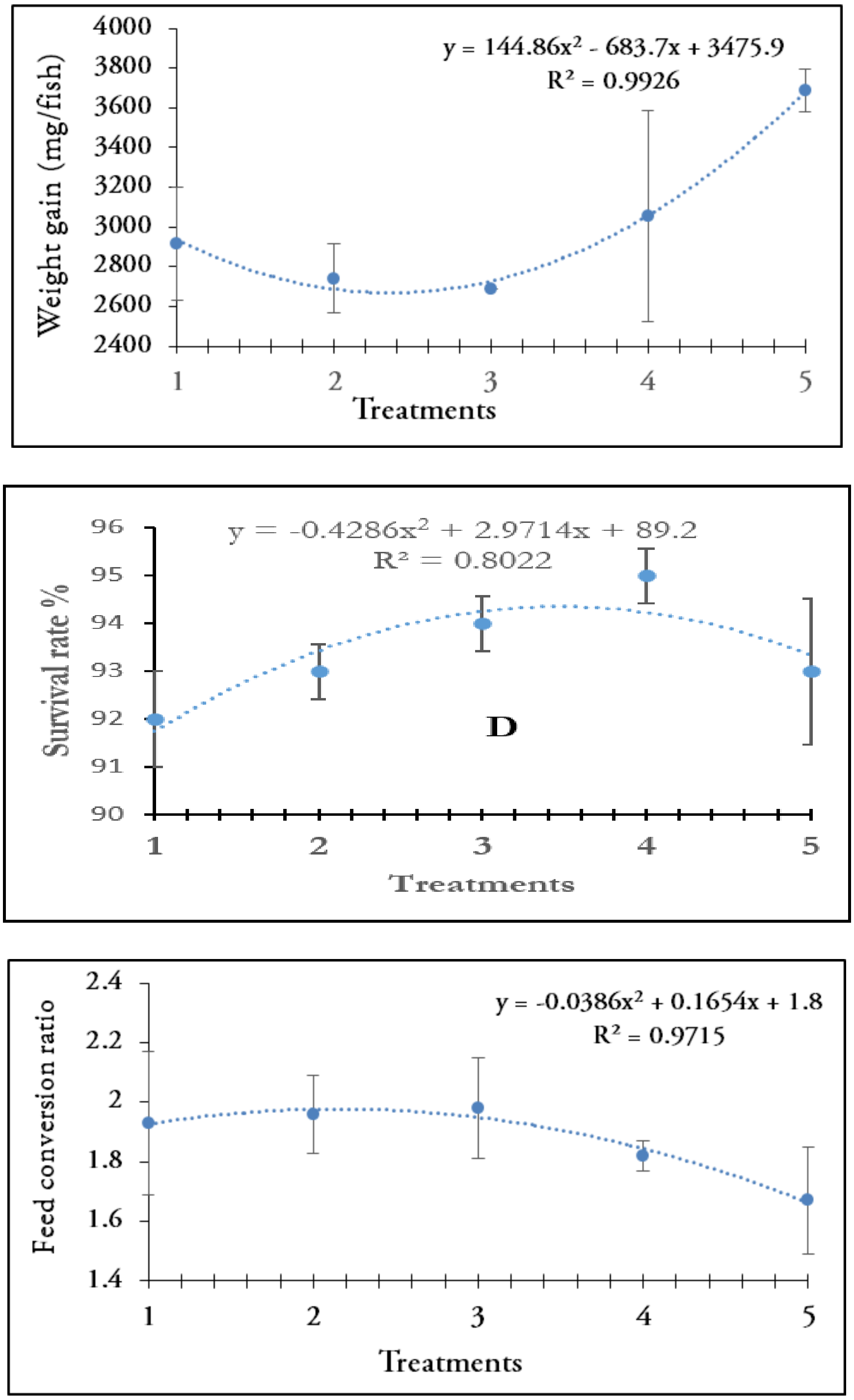

Fig. 1. Polynomial regression analysis of weight gain, survival rate, feed conversion ratio and fishmeal replacement levels with DZ in the diets of gilthead seabream fries, *Sparus aurata* (1: G_1, 2: G_2, 3: G_3, 4: G_4 and 5: G_5)

4. Length growth parameters

The length growth parameters of gilthead seabream are illustrated in Table (5). Length gain and length average daily gain in G_4 and G_5 were significantly higher than in G_1 ($P < 0.05$). Length gain in G_2, was, however, lower than in G_1 ($P < 0.05$). Furthermore, replacing fishmeal with DZ resulted in a substantial increase in length growth indices ($P < 0.05$).

Table 5. Effect of fishmeal replacement with dried zooplankton on length growth parameters of gilthead seabream, *Sparus aurata* fry (Mean ± SD)

Length growth parameter	Treatment				
	G_1	G_2	G_3	G_4	G_5
Initial length (cm)	3.033± 0.07	3.044± 0.113 ^{NS}	3.044± 0.073 ^{NS}	3.033± 0.100 ^{NS}	3.033± 0.124 ^{NS}
Final length (cm)	7.422± 0.192 ^{NS}	7.411± 0.293 ^{NS}	7.500± 0.181 ^{NS}	7.544± 0.391 ^{NS}	8.111± 0.267 ^{NS}
Length gain (cm)	4.389± 0.096 ^{NS}	4.367± 0.145 ^{NS}	4.455± 0.051 ^{NS}	4.511± 0.186 ^{NS}	5.067± 0.167 ^{NS}
Length average daily gain (mm)	0.366± 0.008 ^{NS}	0.364± 0.012 ^{NS}	0.371± 0.004 ^{NS}	0.372 ±0.016 ^{NS}	0.422± 0.014**
Length gain (%)	144.72± 4.64 ^{NS}	143.51± 6.89 ^{NS}	146.33± 0.62 ^{NS}	145.28 ± 5.46 ^{NS}	166.58± 9.24^{NS}

:** Statistically significant, *:** Statistically high significant, NS: Statistically non-significant

5. Whole-body proximate composition

The effects of substituting fish meal with DZ on the proximate composition of gilthead seabream are shown in Table (6). Fish crude protein was substantially greater (P < 0.05) in G_5 and G_4 than in G_1. Moisture, ash, and crude lipid, on the other hand, showed no significant changes (P> 0.05) among the groups.

Table 6. Effect of fish meal replacement with dried zooplankton on whole-body proximate composition of gilthead seabream, *Sparus aurata*, fry (% on dry basis)

Item	Group				
	G1	**G2**	**G3**	**G4**	**G5**
Dry matter	32.47±0.29 ^{a}	38.64±0.37 ^{a}	35.11±0.21^{b}	32.77±0.15 ^{b}	32.70±0.14 ^{b}
Crude protein	58.45±0.41^{a}	56.40±0.26 ^{b}	58.35±0.27 ^{ab}	59.03±0.35 ^{c}	59.54±0.26 ^{c}
Crude lipid	26.97±0.28 ^{a}	26.91±0.18 ^{ab}	27.23±0.34 ^{b}	27.55±0.41 ^{b}	26.14±0.32 ^{b}
Ash	14.58±0.27 ^{a}	16.69±0.21^{a}	14.42±0.26 ^{b}	13.42±0.11^{b}	14.32±0.15 ^{b}

DISCUSSION

Dried zooplankton powder has high animal protein content, making it a viable component source to use as an animal protein source alternative to fishmeal in aquaculture, particularly in larval rearing **(Baeza-Rojano *et al.*, 2014; Abo-Taleb *et al.*, 2021b)**. The use of live food (plankton organisms) in the hatchery and raising of most fish and shellfish species remains critical and is predicted to undergo significant changes in the future. Unlike in nature, where larvae of various aquatic organisms can consume a wide range of plankton species, live food in hatcheries consists of only a few plankton species, including the rotifer, *Brachionus* spp., a few species of copepods, and the brine shrimp, *Artemia* spp., and these are the main live foods used in all aquaculture industries **(Dhont *et al.*, 2013)**. Therefore, the concept of this research arose from the need to collect plankton from their natural habitats, condense and dry them, in addition to analyzing their protein and fat content to leverage the combined nutritional benefits of these planktonic organisms. This was followed by the use of plankton powder as a substitute for fishmeal in fish diets.

Zooplankton has been considered a nutritiously outstanding live feed for fish early stages of cultivable species, proving that they have a higher nutritional value than any other live food used alone; consequently, it plays a vital role in initial feeding of aquatic organisms for their survival and growth **(Rajkumar *et al***., **2004; Abo-Taleb *et al*., 2021a)**. Collecting zooplankton from nature is considered a sustainable option if the necessary and appropriate capabilities and equipment are available to collect it from nature in an optimal way, and this is the main factor to help in reducing feed costs and increasing the economic viability of the fish farming industry.

Indeed, since the zooplankton was collected from its natural habitat in the current study, and considering that its chemical composition varies depending on the season, it is essential to analyze the approximate composition of each collected biomass before incorporating it into fish feed formulations to ensure accurate diet compositions **(Abo-Taleb *et al.,* 2021a)**. This necessity is underscored by the observed differences in the chemical composition of the plankton mixture collected in the current study compared to previous ones, particularly evident in the protein concentration, which was 65.00% in the plankton used in this study. Meanwhile in the study of **Ashour *et al*. (2018)**, the analysis of protein concentration in the zooplankton was 47.7, and 66.54% **(Hassan *et al*., 2020)**. On the other hand, **Abo-Taleb *et al*. (2021a)** mentioned that protein concentration in plankton collected from nature was 49.23%.

Gilthead seabream is considered one of the fish with a high economic value in production and a popular species for consumers. Seabream farming in Egypt is carried out using extensive and semi-intensive systems. In extensive aquaculture systems, farmers typically stock low densities to allow fish to benefit from natural food sources such as plankton **(Sadek, 2000)**. Utilizing natural plankton in this type of culture helps reduce feeding costs. In the semi-intensive system, which contains a slightly high density of fish, a large proportion of farmers depend on the feed used in raising tilapia with the addition of small shrimp caught from the northern Delta Lakes **(Mona *et al*., 2019)**. This happened in order to reduce the feeding costs needed for the cultivation of this type of very voracious fish, while other farmers use expensive feeds for their high protein content and high quality. Therefore, finding sustainable, low-cost, and reliable sources of animal protein for feeding this type of fish is an urgent necessity, especially if this type of food has proven effective in reducing mortality rates among larvae by half and increasing growth parameters, as in the case of the current results.

The present work clearly confirmed that zooplankton can completely replace fishmeal in the actual diet for gilthead seabream larvae. When compared to fish larvae fed the fishmeal-contained diet without zooplankton, fish fed increasing levels of zooplankton-contained diets (25, 50, 75, and 100%) showed a trend toward better feed consumption, growth performance, and carcass composition. The current study found that fish larvae fed feed containing zooplankton at a substitution rate of 100% to fishmeal had the highest weight gain, length gain, and feed efficiency ratio, while achieving the lowest significant feed conversion ratio value, when compared to the control diet (G_1) and other treatments. On the other hand, fish fed a diet containing 75% replacement of zooplankton had the highest survival rate. Hence, these results clearly confirmed that fishmeal substitution (75 and 100%) by zooplankton meal is the ideal replacement of fishmeal for gilthead seabream larvae. The result is relatively similar to the observation of **Hassan *et al*. (2020)** who found that a 100% replacement level of fishmeal by zooplankton meal significantly increased the weight gain, specific growth rate, and protein efficiency ratio of the European seabass *Dicentrarchus labrax* fingerlings. While, **Mona *et al*. (2019)** mentioned that feeding gilthead seabream, *Sparus aurata*, fry with copepods, as a zooplankton group, at a 20% of commercial diet resulted in a significant increase in protein, carbohydrates, and lipids percentage in fish fry.

Hongxia *et al*. (2019) found that a 60% substitution level of fishmeal by *Daphnia magna* powder significantly increased the specific growth rate, weight gain, and protein efficiency of yellowhead catfish *Pelteobagrus fulvidraco* fingerlings. Similarly, **Abo-Taleb *et al*. (2021a)** found that replacing fishmeal in the feed of grey mullet, *Mugil cephalus* larvae with zooplankton meal up to 100% significantly improved the values of feed conversion ratio, protein efficiency ratio, and lipid efficiency ratio. In addition, *Dahpnia magna* meal as a fishmeal alternative in the diet (control diet contained 18% fishmeal) of grey mullet larvae could replace up to 75% of FM, which recorded the best growth and feed utilization parameters according to **Abo-Taleb *et al*. (2021b)**. Whereas, **Chiu *et al*. (2015)** noticed that using diets containing 5- 10% *Daphnia* powder to feed the fingerlings of the barramundi, *Lates calcarifer,* resulted in fish with high immunity and resistance to disease compared to fish fed the control diet.

The study of **Sharahi *et al*. (2016)** postulated that the replacement of fishmeal with *Gammarus* powder up to 20% substitution rate significantly promoted different growth performance parameters and feed utilization of Siberian sturgeon, *Acipenser baerii* juveniles.

In the current study, length gain throughout the entire experiment (120 days) for all treatments ranged from 43.67 to 50.67mm, which is considered higher than what was recorded by **Labib and El Sagheer (2012)** who mentioned that, the *Sparus aurata* raised on meal containing of rotifers and *Artemia*, as a live food, for 24 days gained about 4.20-8.35mm (21- 42mm/ 120 days).

The study of the regression trend of the present results showed a significant polynomial regression among increasing levels of zooplankton powder and different growth, feed utilization, and body composition parameters. Therefore, zooplankton meal could totally replace fishmeal without harmful impacts which was previously confirmed by **Abo-Taleb *et al*. (2021a, b)** for another fish species. In the current study, the obtained results of fish whole-body composition revealed that feeding increasing zooplankton meal content as fish meal substitution significantly increased crude protein and decreased lipid and ash content of gilthead seabream. The current findings are similar with those of **Aman and Altaff (2004)**, **Manickam *et al*. (2019)** and **Abo-Taleb *et al*. (2021b)** who found a substantial increase in protein, lipid, and carbohydrate content in freshwater prawn, *Macrobrachium rosenbergii*, and grey mullet, *Mugil cephalus* fed with wild single or mixed zooplankton species.

Finally, the food conversion ratios for seabream *Sparus aurata* larvae in the current study ranged from 1.67 to 1.98, a result higher than that achieved by **Olsen *et al*. (2006)** in his study feeding Atlantic salmon, *Salmo salar*, on fishmeal containing Antarctic krill, *Euphausia superba,* where the conversion ratio fluctuated between only 0.94– 1.26, which reflects the usefulness and effect of adding a mixture of plankton in the diet of fish larvae.

CONCLUSION

Dried zooplankton powder can be regarded as a worthy protein source for gilthead seabream larvae. Replacement up to 75 to 100% of dietary fishmeal with dried zooplankton meal led to significant reduction in larval mortality. These results could guide the industrial application of this alternative protein source in the manufacture of fish larval food. This aquatic trophic state is a characteristic of all types of the Egyptian water bodies. Therefore, much attention should be given to ensuring that zooplankton are harvested (for larval feed production) from the water bodies that have high densities of these organisms in nature.

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REFERENCES

Abdel-Aziz, N.; Aboul Ezz, S.; Abou Zaid, M. and **Abo-Taleb, H. (2011).** Temporal and spatial dynamics of rotifers in the Rosetta Estuary, Egypt. Egyptian Journal of Aquatic Research, **37**(1): 59-70.

Abdelsalam, A.A.; Saber, S.A.; El-Kafrawy, S.B. and **Abo-Taleb, H.H. (2020).** Long-term evaluation of eutrophication problem using multi-sensor satellite data along El-Max Bay, Alexandria coast and Abu-Qir Bay, Egypt. Egyptian Journal of Aquatic Biology and Fisheries, **24**(7-Special issue): 233-258. https://doi.org/10.21608/EJABF.2020.120320

Abo-Taleb, H. (2019). Key to the Red Sea Labidocera (Crustacea; Calanoida: Pontellidae) copepods, the distribution of the species in various habitats, with special reference to two new records, and a historical correction. Egyptian Journal of Aquatic Research, **45**(4): 367-374. https://doi.org/https://doi.org/10.1016/j.ejar.2019.11.004

Abo-Taleb, H. and **Gharib, S.M. (2018).** Seasonal distribution of epipelagic copepods at the different habitats in the North-Western Red Sea, Egypt. Egyptian Journal of Aquatic Biology and Fisheries, **22**(3): 139-157. https://doi.org/10.21608/EJABF.2018.9456

Abo-Taleb, H.; Abdel-Aziz, N.; Aboul-Ezz, S.; El Raey, M. and **Abou Zaid, M. (2016a).** Study of chromista and protozoa in a hotspot area at the Mediterranean coast with special reference to the potentiality to use it as bio-indicators. International Journal of Marine Science, **6**. https://doi.org/10.5376/ijms.2016.06.0053

Abo-Taleb, H.; Ashour, M.; Elokaby, M.A.; Mabrouk, M.M.; El-feky, M.M.; Abdelzaher, O.F.; Gaber, A.; Alsanie, W.F. and **Mansour, A.T. (2021b).** Effect of a new feed *Daphnia magna* (Straus, 1820), as a fish meal substitute on growth, feed utilization, histological status, and economic revenue of grey mullet, *Mugil cephalus* (Linnaeus 1758). Sustainability, **13**(13): 7093. https://doi.org/https://doi.org/10.3390/su13137093

Abo-Taleb, H.; Ashour, M.; El-Shafei, A.; Alataway, A. and **Maaty, M.M. (2020b).** Biodiversity of Calanoida Copepoda in Different Habitats of the North-Western Red Sea (Hurghada Shelf). Water, **12**(3): 656. https://doi.org/https://doi.org/10.3390/w12030656

Abo-Taleb, H.; El Raey, M.; Abou Zaid, M.M.; Ezz, S.A. and **Aziz, N.A. (2015).** Study of the physico-chemical conditions and evaluation of the changes in eutrophication-related problems in El-Mex Bay. African Journal of Environmental Science and Technology, **9**(4): 354-364. https://doi.org/10.5897/AJEST2014.1823

Abo-Taleb, H.; El-feky, M.M.; El-Tabakh, M.A.M.; Hendy, D.M. and **Maaty, M.M., (2020a).** First record of Bryde's whale (*Balaenoptera brydei*, Olsen, 1913) in the southeastern Mediterranean Sea, Alexandria, Egypt. Egyptian Journal of Aquatic Biology Fisheries, **24**(1): 667-695. https://doi.org/10.21608/EJABF.2020.76557

Abo-Taleb, H.; El-feky, M.M.M.; Azab, A.M.; Mabrouk, M.M.; Elokaby, M.A.; Ashour, M.M.; Mansour, A.T.; Abdelzaher, O.F.; Abualnaja, K.M. and **Sallam, A.E. (2021a).** Growth performance, feed utilization, gut integrity, and economic revenue of grey mullet, *Mugil cephalus*, fed an increasing level of dried zooplankton biomass meal as fishmeal substitutions. Fishes, **6**(3): 38. https://doi.org/https://doi.org/10.3390/fishes6030038

Abo-Taleb, H.; Ezz, S.M.A.; Aziz, N.E.A.; Abou Zaid, M.M. and **El Raey, M. (2016b).** Detecting marine environmental pollution by biological beacons and GIS program. Journal of Fisheries Sciences, **10**(4): 69.

Abou Zaid, M.M.; El Raey, M.; Ezz, S.M.A.; Aziz, N.E.A. and **Abo-Taleb, H.A. (2014).** Diversity of Copepoda in a stressed eutrophic bay (El-Mex Bay), Alexandria, Egypt. Egyptian Journal of Aquatic Research, **40**(2): 143-162. https://doi.org/https://doi.org/10.1016/j.ejar.2014.05.001

Abu El-Regal, M.A.; El-Wazeer, A.; Abou Elnaga, Z.S. and **Amr, A.A. (2018).** Composition and spatio-temporal distribution of zooplankton community in the Egyptian Red Sea coast at Hurghada. Egyptian Journal of Aquatic Biology and Fisheries, **22**(3): 1-12. https://doi.org/10.21608/EJABF.2018.8761

Aman, S. and **Altaff, K. (2004).** Biochemical profile of *Heliodiaptomus viduus*, *Sinodiaptomus* (*Rhinediaptomus*) *indicus*, and *Mesocyclops aspericornis* and their dietary evaluation for postlarvae of *Macrobrachium rosenbergii*. Zoological Studies, **43**(2): 267-275.

AOAC (1995). Official Methods of Analysis of AOAC International: Agricultural Chemicals; Contaminants; Drugs, 16th Edition. Journal of AOAC International. Volume I. https://doi.org/http://lib3.dss.go.th/fulltext/scan_ebook/aoac_1995v78_n3.pdf..

Ashour, M.; Abo-Taleb, H.; Abou-Mahmoud, M. and **El-Feky, M.M. (2018).** Effect of the integration between plankton natural productivity and environmental assessment of irrigation water, El-Mahmoudia Canal, on aquaculture potential of *Oreochromis niloticus*. Turkish Journal of Fisheries Aquatic Sciences, **18**(10): 1163-1175. https://doi.org/10.4194/1303-2712-v18_10_03

Ashour, M.; Mabrouk, M.M.; Abo-Taleb, H.A.; Sharawy, Z.; Ayoub, H.F.; Van Doan, H.; Davies, S.J.; El-Haroun, E. and **Goda, A.M.-A. (2021).** A liquid seaweed extract (TAM®) improves aqueous rearing environment, diversity of zooplankton community, whilst enhancing growth and immune response of Nile tilapia, *Oreochromis niloticus*, challenged by *Aeromonas hydrophila*. Aquaculture, **543**: 736915. https://doi.org/https://doi.org/10.1016/j.aquaculture.2021.736915

Baeza-Rojano, E.; Hachero-Cruzado, I. and **Guerra-García, J.M. (2014).** Nutritional analysis of freshwater and marine amphipods from the Strait of Gibraltar and potential aquaculture applications. Journal of Sea Research, **85**: 29-36. https://doi.org/https://doi.org/10.1016/j.seares.2013.09.007

Castell, J. and **Tiews, K. (1980).** Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research, Hamburg, Federal Republic of Germany, 21-23 March 1979.

Chiu, S.T.; Shiu, Y.L.; Wu, T.M.; Lin, Y.S. and **Liu, C.H. (2015).** Improvement in non-specific immunity and disease resistance of barramundi, *Lates calcarifer* (Bloch), by diets containing *Daphnia similis* meal. Fish shellfish immunology, **44**(1): 172-179. https://doi.org/https://doi.org/10.1016/j.fsi.2015.02.002

Dhont, J.; Dierckens, K.; Støttrup, J.; Van Stappen, G.; Wille, M. and **Sorgeloos, P. (2013).** Rotifers, *Artemia* and copepods as live feeds for fish larvae in aquaculture, in: Advances In Aquaculture Hatchery Technology. Elsevier, pp. 157-202.

El-feky, M. and **Abo-Taleb, H. (2020).** Effect of feeding with different types of nutrients on intensive culture of the water flea, *Daphnia magna* Straus, 1820. Egyptian Journal of Aquatic Biology and Fisheries, **24**(1): 655-666. https://doi.org/10.21608/EJABF.2020.76554

El-Feky, M.M.; Alprol, A.E.; Heneash, M.M.A.; Abo-Taleb, H.A. and **Omer, Y.M. (2019).** Evaluation of water quality and plankton for Mahmoudia Canal in Northern West of Egypt. Egyptian Journal of Aquatic Biology and Fisheries, **22**(5 Special Issue): 461-474.

El-Sherbiny, M.M.; Hanafy, M.H. and **Aamer, M. (2007).** Monthly variations in abundance and species composition of the epipelagic zooplankton off Sharm El-Sheikh, Northern Red Sea. Research Journal of Environmental Sciences, **1**(5): 200-210.

Farrag, M.M.; El-Naggar, H.A.; Abou-Mahmoud, M.M.; Alabssawy, A.N.; Ahmed, H.O.; Abo-Taleb, H.A. and **Kostas, K. (2019).** Marine biodiversity patterns off Alexandria area, southeastern Mediterranean Sea, Egypt. Environmental Monitoring and Assessment, **191**(6): 1-28.

Gurney, R. (1931). British fresh-Water Copepoda. Ray Society.

Hassan, S.E.; Azab, A.M.; Abo-Taleb, H.A. and **El-Feky, M.M. (2020a).** Effect of replacing fish meal in the fish diet by zooplankton meal on growth performance of *Dicentrarchus labrax* (Linnaeus, 1758). Egyptian Journal of Aquatic Biology and Fisheries, **24**(6): 267-280. https://doi.org/10.21608/ejabf.2020.111756

Hongxia, Z.; Yang, W.; Xuan, W.; Xiangqian, Z.; Guang, Y.; Jianchao, Z.; Guoxia, Z. and **Dongqing, B. (2019).** Effects of *Daphnia magna* meal replacing fish meal on growth, biochemical indexes of *Pelteobagrus fulvidraco* and water quality indexes. Feed Ind, **2**: 212-221.

Labib, M. and **El Sagheer, H. (2012).** Effect of a rotifer and artemia on survival and growth performance of gilthead seabream *Sparus aurata* larvae. Egyptian Journal of Aquatic Biology and Fisheries, **16**(4): 1-8.

Luthada-Raswiswi, R.; Mukaratirwa, S. and **O'Brien, G. (2021).** Animal protein sources as a substitute for fishmeal in aquaculture diets: A Systematic review and meta-analysis. Applied Sciences, **11**(9): 3854. https://doi.org/https://doi.org/10.3390/app11093854

Manickam, N.; Santhanam, P. and **Bhavan, P.S. (2019).** Techniques in the collection, preservation and morphological identification of freshwater zooplankton, in: Santhanam, P., Begum, A., Pachiappan, P. (eds) (Ed.), Basic and Applied Zooplankton Biology. Springer, Singapore, pp. 139-195.

Mona, M.H.; Rizk, E.-S.T.; El-feky, M.M. and **Elawany, M.E. (2019).** Effect of nutritional quality of rotifers and copepods on sea bream (*Sparus aurata*) fry fish productivity. The Egyptian Journal of Experimental Biology, **15**: 135-142.

Olsen, R.; Suontama, J.; Langmyhr, E.; Mundheim, H.; Ringø, E.; Melle, W.; Malde, M. and **Hemre, G.I. (2006).** The replacement of fish meal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, *Salmo salar*. Aquaculture Nutrition, **12**(4): 280-290. https://doi.org/https://doi.org/10.1111/j.1365-2095.2006.00400.x

Rajkumar, M.; Santhanam, P. and **Perumal, P. (2004).** Laboratory culture of calanoid copepod, *Acartia clausi* Giesbrecht. Applled Fish. Aquac., **4**: 5-8.

Sadek, S. (2000). Sea bream culture in Egypt; status, constraints and potential. Fish Physiology and Biochemistry, **22**(2): 171-178. https://doi.org/10.1023/A:1007835126731

Santhanam, R. and **Srinivasan, A. (1994).** A manual of marine zooplankton. Oxford & IBH Publishing Company.

Sars, G. (1927). The fresh-water Entomostraca of the Cape Province (Union of South Africa). Part III: Copepoda. Annals of the South African Museum, **25**(1): 85-149.

Sawyer, R. and **Kirk, R.S. (1991).** Pearson's composition and analysis of foods. Longman, pp. 708.

Sharahi, A.R.; Falahatkar, B. and **Efatpanah, I. (2016).** Effect of fish meal replacement with *Gammarus* meal on growth and body composition of juvenile Siberian sturgeon, *Acipenser baerii* (Brandt, 1869). J. Aquat. Ecol., **6**: 102-113.

Suhaimi, H.; Yuslan, A.; Azani, N.; Habib, A.; Liew, H.J. and **Rasdi, N.W. (2022).** Effect of dietary enhanced *Moina macrocopa* (Straus, 1820) on the growth, survival and nutritional profiles of hybrid Nile tilapia fry. Egyptian Journal of Aquatic Research, **48**(1): 67-73.

Uçal, İ.O. (2002). Sea bream (*Sparus aurata* L., 1758) fry production in Turkey. Turkish Journal of Fisheries Aquatic Sciences, **2**(1). https://doi.org/https://dergipark.org.tr/en/pub/trjfas-ayrildi/issue/13294/160673

Zakaria, H.Y.; Hassan, A.-K.M.; Abo-Senna, F.M. and **El-Naggar, H.A. (2016).** Abundance, distribution, diversity and zoogeography of epipelagic copepods off the Egyptian Coast (Mediterranean Sea). Egyptian Journal of Aquatic Research, **42**(4): 459-473. https://doi.org/https://doi.org/10.1016/j.ejar.2016.11.001