NOAA Technical Memorandum NMFS-NE-253

A Pilot Study Using Graded Yellow Mealworm (*Tenebrio molitor*) Meal in Formulated Diets for Growth Performance of Black Sea Bass (*Centropristis striata*)

US DEPARTMENT OF COMMERCE
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ABSTRACT

In a pilot study, juvenile black sea bass (*Centropristis striata*) (mean initial wet weight (± SE) 29.0 g ± 0.33) were fed experimental diets containing graded amounts of yellow mealworm meal, (*Tenebrio molitor*) as a protein replacement for fishmeal. Formulated diets (0, 25%, 50%, 75%, and 100% fishmeal replacement) were fed 3 times per week to triplicate tanks of fish (N = 14) over a 121-day period. Formulations provided a gradual gradation in nutritional profile, with the highest protein in the 0% *T. molitor* meal (100% fishmeal) diet and the lowest in the 100% *T. molitor* feed. Diets were evaluated by weight and length-based growth metrics including weight gain (g), length gain (mm), weight-based specific growth rate (%/day), length-based specific growth rate (% /day), final weight (g), final length (mm), feed consumed (g), dry weight of feed consumed adjusted for survival (%), and % survival. Black sea bass fed the 25% replacement diet showed significantly greater weight-based specific growth than did fish fed the other experimental diets. Fish consuming the 0% and 25% replacement diets had significantly higher weight and length-based specific growth than did those fed the 75% and 100% diets. Use of *T. molitor* meal offers a promising partial substitute to fishmeal as a nutritional component of formulated finfish diets.

INTRODUCTION

The black sea bass (*Centropristis striata*) is a marine serranid inhabiting Atlantic coastal waters from the Gulf of Maine to Florida (Bigelow and Schroeder 1953). Black sea bass are recreationally and commercially valued, prized for a mild flavor and firm texture. Market demand for this species has risen sharply with wholesale prices ranging from $11 - $24 kg, live weight (Berlinsky et al. 2000; Watanabe et al. 2007). Declining US commercial landings from 10 million kg in 1952 to just under 1 million kg in 2014 (Atlantic States Marine Fisheries Commission 2017) suggest consumer demand for this species may eventually exceed supply.

Black sea bass show promise as a potential aquaculture species (Cotton et al. 2003; Perry et al. 2007). Adults can be spawned under hatchery conditions and successfully cultured from egg through the larval and juvenile stages (Berlinsky et al. 2000; Watanabe et al. 2003; Berlinsky et al. 2004, 2005; Copeland et al. 2002; Perry et al. 2007). Juveniles have a large gape size relative to many marine fish and are easily weaned from live feed to a pellet diet. Pilot studies are underway to refine culture methods for black sea bass (University of Maine Center for Cooperative Aquaculture Research 2017), but little is known about their nutritional requirements. Traditionally, researchers have relied on commercially available trout, salmon, and flounder chows for hatchery feeding (Kupfer et al. 2000; Power et al. 2002; Cotton and Walker 2005; Carroll and Watanabe 2005; Alam et al. 2009).

Protein and lipid represent key macronutrients in fishmeal that provide energy and raw material for growth of carnivorous fish. Fishmeal represents the gold standard protein source for feed formulation, and commercial diets typically contain 32% - 45% protein and 4% - 20% lipid (Miles and Chapman 2005). High demand for fishmeal has limited supply with corresponding price increases from $800 per metric ton in 2009 to more than $1605 per metric ton in 2018 (World Bank 2018). To ensure a consistent supply of feed and reduce production costs, aquaculturists are investigating alternative protein sources to substitute for traditional fishmeal in pellet diets (Ng et al. 2001; Kikuchi 2002; Ajani et al. 2004; Bai et al. 2005). Recent studies have investigated diets for black sea bass which replace fishmeal with other protein sources such as soybean meal (Alam et al. 2012) and low-gossypol cottonseed flour (Anderson et al. 2016).

Alternatively, insects offer an inexpensive, high-quality protein for potential incorporation into fish feeds (Barroso et al. 2014; Makkar et al. 2014; Henry et al. 2015; Riddick et al. 2014). Numerous studies with freshwater fish have successfully used meal from various insects as a replacement for fishmeal (Habib et al. 1994; Ng et al. 2001; Belforti et al. 2015; Su et al. 2017). Yellow mealworm (*T. molitor*) meal made from larvae has a favorable nutritional profile relative to fishmeal, and is composed of roughly 46% crude protein, 33% crude fat, and 5% dietary fiber on a dry weight basis (Ravanaadii et al. 2012). Black sea bass juveniles consume chitinous, epibenthic crustaceans (e.g., amphipods, isopods, sand shrimp [*Crangon septemspinosa*], copepods, small crabs, mysids) as part of their natural diet (Drohan et al. 2007) and may therefore be a good candidate species for testing *T. molitor* meal diets.

In this study, graded amounts of *T. molitor* meal used as protein replacement for fish meal in formulated diets (0%, 25%, 50%, 75%, and 100% replacement) were fed to juvenile black sea bass over a 121-day period.

MATERIALS AND METHODS

Diet Formulation

Since little is known about the nutrient requirements of black sea bass, diets were formulated to match the proximate composition of a commercial diet (Bio-Oregon® BioBrood® has a minimum of 48% protein and 20% fish oil) successfully used to rear black sea bass in the laboratory. To identify possible limiting amino acids in *T. molitor* meal, amino acid data were compared to similar data for fishmeal. However, since requirements for black sea bass are not well understood and availability of amino acids in *T. molitor* meal is unknown, we chose not to supplement potentially limiting amino acids during this initial feeding trial. This preliminary study was therefore designed to acertain growth over the widest possible range of fishmeal replacement. Prior to diet formulation, amino acid content in herring-based fishmeal and *T. molitor* meal was analyzed by the Experiment Station Chemical Laboratories (ESCL) at the University of Missouri-Columbia (AOAC 1980) (Table 1). The *T. molitor* meal, obtained from a supplier in China, was purchased from the Exotic Nutrition Pet Company in Newport News, Virginia. Herring-based fishmeal was a product of West Coast Reduction LTD.

Five experimental diets were formulated by using graded levels of *T. molitor* meal substituted for fishmeal (Table 2). The 0% diet was prepared with fishmeal and fish oil and contained no *T. molitor* meal. The replacement diets substituted 25%, 50%, 75%, and 100% *T. molitor* meal for fish-based ingredients and contained sufficient lipids from the mealworm meal to substitute for fish oil. The 100% diet was supplemented with 3% fish oil to meet assumed requirements for n-3 fatty acids. Diets were made by mixing the 0% batch and the 100% batch separately and then blending the 2 batches with the following proportions 25:75, 50:50, and 75:25 to make the intermediate diets. Formulations for the 25%, 50%, and 75% batches were calculated from proportions of the 0% and 100% values. Feeds were formulated to be isocaloric and isonitrogenous and were otherwise similar in composition to the (0% *T. molitor* meal) fishmeal-based diet.

T. molitor meal presented challenges during feed formulation and was difficult to bind with other ingredients, therefore a 1% alginate binder was added. Once heated, the insect meal had a plastic-like consistency. Feed pellets were produced with a 4 mm die compaction machine

(California Pellet Mill Co., Crawfordsville, IN) and tray dried overnight in a forced-air, ambient cabinet.

Proximate analysis and energy content were determined for each experimental feed (Table 3). Protein was measured by determining nitrogen concentration in a 0.25g sample by Dumas combustion methodology with a LECO FP-2000 nitrogen analyzer (LECO Corp., St. Joseph, MI) and then multiplying results by 6.25. Moisture samples were dried to a constant weight in a convection oven at 105°C, and ash samples were determined by ashing at 550°C in a muffle furnace (AOAC 1980). Lipids were determined by extraction with methylene chloride by using a Büchi 810 Soxlet fat extraction apparatus (Büchi Laboratories, Flawil, Switzerland).

Growth Trial

Black sea bass were spawned and reared for 8 months at the NEFSC Milford Laboratory, in Milford, CT. Fish were transported to The Sound School Regional Vocational Aquaculture Center in New Haven, CT, for the feeding trial and held in 3 independent, closed, recirculating, seawater systems. Each system contained six 144 L tanks, and 14 fish were placed randomly into 5 of the 6 tanks. The 5 experimental diets were randomly distributed among each of the 3 systems, for 3 replicate tanks per diet (N = 3).

Water quality parameters were measured weekly. Seawater temperature, salinity, and dissolved oxygen were monitored with a YSI® 85. Mean temperature and salinity (± standard deviation) measured 21°C ± 1.8 and 24°C ± 2.1 PPT, respectively. Dissolved oxygen remained close to saturation, ranging from 6 to 8 mg/L. Ammonia, nitrate, and nitrite concentrations were measured with a LaMotte® water quality test kit and were maintained at < 1 ppm with 50% water changes in the recirculating systems as needed. Flow rates were set at 5 liters per minute, and tanks were aerated. Fluorescent lights above the tanks and controlled by timers provided a photoperiod of 13:11 hours (light:dark). The tanks were covered with clear plastic lids to retain fish.

Mean initial wet weight and total length (± SE) of individual fish at the beginning of the study measured 29.0 g ± 0.33 and 119.6 mm ± 0.43, respectively. During alternate weeks, fish were carefully net-collected from each tank and bulk-weighed. A modified, pair-feeding method was used to determine feeding rations. To account for differences in moisture content between feeds, a dry weight equivalent of the ration was offered to each tank. Moisture content of the diets ranged from 77.3% - 83.3% dry weight and increased with proportion of *T. molitor* meal. Fish were fed until apparent satiation, and remaining uneaten food was subtracted from the original ration to determine the amount of feed consumed. Fish were fed 3 times per week to ensure active feed consumption. Feed ration for each diet formulation and tank was recalculated at 2-week intervals to account for changes in biomass. At the end of the experiment, final wet weight (g) and total length (mm) measurements were obtained for each individual fish.

Calculations and Statistical Analysis

Formulas used to calculate growth efficiencies are shown in Table 4. Final mean growth parameters included final weight (g), weight gain (g), weight-based specific growth rate (SGR) (%/day), final length (mm), length gain (mm), length-based SGR (%/day), feed consumed (g), percent dry weight of feed consumed adjusted for survival (FCR_{adj}), and survival (%). All pairwise comparison of fish growth response to experimental diets (N = 3 tanks) was conducted at the conclusion of the experiment with a percentile bootstrap-based ANOVA and trimmed means, by using the Hochberg method to control familywise error across multiple comparisons (Wilcox

2016). Statistical analysis was completed with the R statistical software program, version 3.3.0 (www.r-project.org).

RESULTS AND DISCUSSION

Diet Formulation

Amino acid composition for *T. molitor* meal and herring based-fishmeal showed differences in key amino acids (Table 1), notably a lack of taurine and low levels of the sulfur amino acids. Lysine levels in *T. molitor* meal were less than half the concentration found in fishmeal, while several other amino acids were present at 50-75% of fishmeal content. Although no attempt was made to supplement amino acids, it is likely that the diets containing low percentages of fishmeal were deficient in one or more amino acids or taurine. Black sea bass were able to utilize higher concentrations of low-gossypol cottonseed flour protein (CSM) provided in place of fishmeal protein in experimental diets with supplemental amino acids (Anderson et al. 2016). Information on specific dietary requirements for this species are not yet well understood.

Diet formulations, shown in Table 2, provided a gradual gradation in nutritional profile. Proximate composition of diets (Table 3) indicate feeds had similar protein levels (54% - 56% dry weight) but varied slightly in energy from 505 to 551 Kcal/100g. Lipid concentrations ranged from 32% - 36% (dry weight) among diets.

Growth Trial

Mean wet weight (g) (±SE) of black sea bass increased among all treatments over the 121-day experiment; however, growth performance differed significantly among formulations (Figure 1, Table 4) and was more apparent in weight versus length-based metrics. Replacement of *T. molitor* meal at 25% substitution for fishmeal outperformed the 0% (*T. molitor* meal) fishmeal-based diet and 50%, 75%, and 100% replacement diets for all weight-based growth metrics (Table 4). In fusiform fish, weight-based measures are generally more sensitive indicators of growth and condition than are length-based metrics, since changes in body weight reflect storage or depletion of fat and energy reserves. Similarly, Berlinsky et al. (2000) found weight-based growth of black sea bass ≤100 g to respond more quickly to differences in diet formulations than did length-based measurements.

Black sea bass consumed a larger quantity of 25% diet pellets than any other *T. molitor* feed, which may indicate superior palatability of this formulation. Dietary fiber, primarily in the form of chitin, was proportionally lower in the 25% feed and may have resulted in greater digestability and feed assimilation compared to other *T. molitor* diets (Ng et al. 2001). In a study with African catfish (*Clarias gariepinus*) Ng et al. (2001) observed the highest growth with a 20% replacement of *T. molitor* meal for fishmeal. Gilthead sea bream (*Sparus aurata*) fed a 25% substitution of *T. molitor* larvae in place of fishmeal experienced no negative effects on weight gain after 163 days (Piccolo et al. 2017). In European seabass (*Dicentrarchus labrax*) a replacement of 25% *T. molitor* meal was used without affecting growth performance (Gasco et al. 2016). A study of yellow catfish (*Pelteobagrus fulvidraco*) found that the addition of 18 grams of worm meal per 100 gram diet may boost immune response and bacterial resistence of fish without negative impacts on growth performance (Su et al. 2017). In our study, substitution rates of up to 25% *T. molitor* meal for fishmeal supported growth rates of fish equivilent to or exceeding those produced by the fishmeal-based diet (0% *T. molitor* meal).

Efficient conversion of feed to biomass is important in determining suitability of fishmeal substitutions. Black sea bass consuming the 25% diet demonstrated the best feed conversion ratio (FCR_{adj}) of all diets (1.61), similar to the range of FCR values (1.49 - 1.62) previously documented for black sea bass fed commercial fishmeal-based feeds (Copeland et al. 2002). Other studies, using soybean meal and low-gossypol cottonseed flour-based meal for protein in black sea bass diets, measured FCR values of 0.96 – 1.70 (Alam et al. 2012) and 1.05 – 1.33 (Anderson et al. 2016). In a study which varied protein content in formulated fishmeal diets, Alam et al. (2009) reported a wider range of FCR values (1.36 - 2.39) for black sea bass.

Weight-based specific growth rate (SGR) was highest (0.66%$^{d-1}$) in fish consuming the 25% diet. Similar growth rates of 0.5%$^{d-1}$ have been previously reported in black sea bass fed fishmeal-based diets (Perry et al. 2007; Alam et al. 2009). Black sea bass fed soybean meal and low gossypol cottonseed meal-based diets demonstrated higher SGR values (range 1.10 - 2.8 %$^{d-1}$) (Anderson et al. 2016; Alam et al. 2012). During our study, fish fed 75% and 100% diets grew slowly with an SGR of 0.29%$^{d-1}$ and 0.21%$^{d-1}$, respectively. Slower weight-based growth and lower weight gain of fish fed the highest replacement diets could indicate that *T. molitor* meal lacks certain essential dietary components provided by fishmeal. Concentrations of many essential amino acids and taurine were low in *T. molitor* meal relative to herring meal (Table 1).

Proximate analysis of the feeds (Table 3) showed a minor decline in lipid levels with increasing proportions of mealworm. Low concentrations of fish oil (3% of the diet) in the 100% replacement diet, relative to the other formulations, may have provided a less favorable fatty acid profile (Ng et al. 2001). Survival of cultured black sea bass ranged from 54.8% - 69% and did not differ significantly among feed formulations. These rates are low relative to studies where sources of plant-based protein were used in place of fishmeal in alternative diets for black sea bass (Anderson et al. 2016; Alam et al. 2012). Further research is needed to optimize insect-based feed formulations and to refine culture conditions for improved growth and survival of hatchery-reared black sea bass.

Reduced fish growth might relate to the high proportion of chitin, a nitrogen-containing polysaccharide forming the exoskeleton of mealworms (Ng et al. 2001; Mohanta et al. 2016). Certain polysaccharides act as antinutritional factors that limit availability of one or more nutrients. Mealworm diets high in chitin may have an antinutritional effect inhibiting fish growth, although chitin content was not measured here. Lower growth was also documented in African catfish fed 60% or more *T. molitor* meal in place of fishmeal (Ng et al. 2001). In our study, black sea bass receiving the 100% replacement diet appeared disinterested in feeding and consumed significantly less feed. Lacking a pathological effect, this might indicate a palatability issue with *T. molitor* meal. The high fiber content of chitin-rich diets may negatively impact acceptability of feed by fish. Poor palatability, coupled with antinutritional compounds in diets with high mealworm content, may have contributed to lower growth rates in black sea bass. Higher growth rates on *T. molitor* meal-based diets might have been achieved by a daily feeding regime; our preliminary trial distributed feed 3 times per week. Although our study evaluated performance of diets based on the inherent nutritional value of *T. molitor*, growth of fish might have been enhanced by supplementing the feeds to include potentially limiting nutrients such as amino acids and essential fatty acids.

Our study found that juvenile black sea bass fed 25% *T. molitor* meal in place of fishmeal exhibited the highest weight-based growth rates among all diets tested, including a formulated fish meal-based diet containing no mealworm. With additional research to facilitate the manufacturing process, methods to reduce chitin content, and identification of potentially limiting nutrients, *T.*

molitor meal may prove an economical replacement for a portion of the fishmeal component in future finfish diets. Replacement of protein with insect-based sources may reduce the high demand on fishmeal and increase affordability of aquaculture operations.

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Table 1. Analysis of amino acid content and proximate analysis for *Tenebrio molitor* meal (g/100g), herring based fish meal (g/100g), and relative differences (mealworm/fishmeal) between them.

Amino Acid (% dry weight)	Worm meal[a]	Fish meal[b]	Relative Difference
Arginine	2.78	3.73	75%
Histidine	1.74	1.53	114%
Isoleucine	2.34	3.64	64%
Leucine	3.86	4.69	82%
Lysine	3.18	7.30	44%
Methionine + Cysteine	1.15	3.80	30%
Phenylalanine + Tyrosine	6.16	4.78	129%
Threonine	1.90	2.49	76%
Tryptophan	0.73	0.67	109%
Valine	3.59	3.26	110%
Taurine	0.02	4.90	0%
Proximate Analysis (%)			
Moisture	8.0	6.0	133.3%
Crude Protein	72.0	70.0	102.9%
Crude Lipid	9.0	10	90.0%
Crude Ash	10	15	66.7%

[a]Actual analysis of *T. molitor* meal used in the experiment (mean of N = 4 samples)

[b]Typical analysis of herring-based fishmeal (West Coast Reduction LTD.)

TABLE 2. Ingredients in *Tenebrio molitor* diet formulations.

Diet	0%	25%	50%	75%	100%
Ingredient (g/100g)					
Fishmeal[a]	65.0	48.8	32.5	16.2	0
Mealworm[b]	0	18.8	37.5	56.3	74.9
Fish oil	19.5	15.3	11.2	7.1	3.0
Wheat flour	12.2	13.8	15.5	17.1	18.8
Vitamin Premix[c]	1.0	1.0	1.0	1.0	1.0
Kelgin HV[d]	1.0	1.0	1.0	1.0	1.0
Choline Cl[e]	0.6	0.6	0.6	0.6	0.6
Stay-C[f]	0.3	0.3	0.3	0.3	0.3
Yttrium oxide[g]	0.3	0.3	0.3	0.3	0.3
TM mix[h]	0.1	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0	100.0

[a]Exotic Nutrition Pet Supply Company, Newport News, Virginia
[b]West Coast Reduction LTD
[c]ARS 702 DSM Nutritional Products, Basel, Switzerland. Provides per kg diet before processing; vitamin A 19300 IU; vitamin D 13200 IU; vitamin E 264 IU; vitamin K3 2.2 gm: thiamin mononitrate 18.2 mg; riboflavin 19.2 mg; pyridoxine hydrochloride 27.4 mg; pantothenate DL-calcium 93; cyancobalamin 0.06 mg; nicotinic acid 43.6 mg; biotin 0.68 mg; folic acid 5.0; inostitol 1200
[d]Sodium Alginate added as a binder (FMC Corporation, Fresno CA)
[e]70% solution of Choline Chloride (Sigma Chemicals, St Louis, MO)
[f]Ascorbic acid Stay-C 35, DSM Nutritional Products, Basel, Switzerland
[g]Sigma Chemicals, St. Louis, MO
[h]USFWS #3 Trace mineral mix (Hardy 1989).

TABLE 3. Proximate analysis and energy composition for diets where *Tenebrio molitor* was substituted for fishmeal.

Proximate analysis	0%	25%	50%	75%	100%
Content (% dry weight)					
Protein	56.6	55.1	55.4	54.7	54.3
Lipid	36.1	35.1	34.1	33.1	32.0
Ash	7.3	8.8	10.6	12.3	13.7
Total	100.0	100.0	100.0	100.0	100.0
Calculated energy (Kcal/100g)	551.6	545.1	528.2	516.3	505.3

TABLE 4. Final mean growth parameters[a] for juvenile black sea bass (initial mean wet weight (± SE) 29.0 g ± 0.33) fed diets containing graded amounts of yellow mealworm, (*Tenebrio molitor*) meal, as a protein replacement for fishmeal over 121-days.

Feed	0%	25%	50%	75%	100%
Final weight (g)*	56.11±2.03 z	64.71±2.75 y	50.44±5.11 zx	39.95±0.82 xw	37.69±1.50 w
Weight gain (g)*	27.11±2.29 z	35.39±1.72 y	21.62±4.22 zx	11.67±0.57 w	8.52±0.94 v
SGR weight (%/day)*	0.549±0.04 z	0.659±0.01 y	0.458±0.06 z	0.288±0.01 x	0.213±0.02 w
Final length (mm)*	151.03±3.41 z	153.00±1.21 z	149.90±3.98 zy	140.30±1.94 yx	138.86±0.22 x
Length gain (mm)*	31.29±2.61 z	32.31±1.17 z	29.71±3.04 zy	22.11±1.12 yx	19.74±0.05 x
SGR length (%/day)*	0.192±0.01 z	0.196±0.01 z	0.182±0.02 zy	0.142±0.01 y	0.127±0.00 x
Feed consumed (g)*	450.2±29.87 z	477.3±40.02 z	397.7±49.46 zy	329.7±28.68 y	243.84±38.50 x
FCR_{adj}*	1.74±0.06 z	1.61±0.12 z	3.11±1.26 zy	3.08±0.21 y	3.61±0.28 y
Survival (%)	69.0±0.06	61.9±0.10	54.8±0.15	66.7±0.09	57.1±0.07

[a]Table includes final weight, weight gain, specific growth rate (SGR) weight = (final weight – initial weight)/ (time in days) X 100, SGR length = (final length – initial length)/ (time in days) X 100, amount of feed consumed (g/tank/day), FCR_{adj} = (dry weight of feed consumed) / [(final total biomass) – (number of remaining fish at the end of the experiment) X (initial mean weight)] and survival (%) = number of fish remaining at end of experiment /initial stocking density (14 fish) x 100. Values represent means of 3 replicate tanks (±SE). Values in the same row with different letters are significantly different (*P < 0.0001).

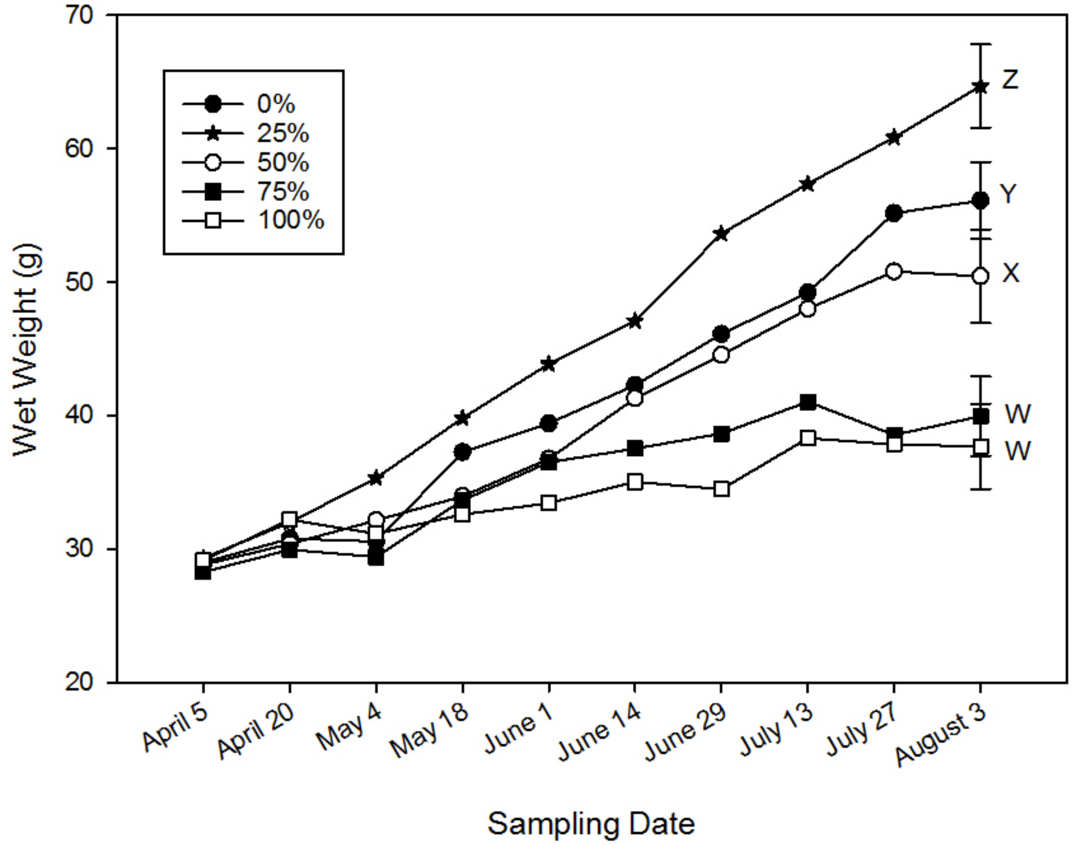

FIGURE 1. Mean wet weight (g) versus sampling date for black sea bass fed diets containing graded levels of *Tenebrio molitor* meal substituting for fish meal over 121-days. All-pairwise comparison of mean final weight (±SE) on the last day of the experiment was performed with a percentile bootstrap-based ANOVA and trimmed means by using the Hochberg method to control familywise error across multiple comparisons. Values with different letters are significantly different ($P < 0.005$).

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