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Optimizing nursery diets for post-metamorphic stage black sea bass: Growth performance, body composition, and feed utilization on open-formulated and commercial starter diets

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Abstract

The objective of this study was to optimize nursery diets for post-metamorphic stage black sea bass by evaluating growth performance, whole-body proximate and fatty acid composition, and utilization of University of North Carolina Wilmington (UNCW)-formulated and commercial diets under laboratory conditions. A feeding trial was conducted to compare two UNCW-formulated diets (D1 and D2) for black sea bass (54% crude protein = CP and 14% crude lipid = CL) and two premium, commercial marine finfish fry diets, Otohime (Reed Mariculture Inc., Campbell, CA, CP = 48% and CL = 14%, CD3) and Gemma Diamond (Skretting, Nutreco, Canada, CP = 57%, CL = 15%, CD4). The UNCW-formulated diet 1 (D1) contained high fish meal (FM, 40% of diet), whereas UNCW-formulated diet 2 (D2) replaced 50% FM protein by high-quality poultry by-product meal (PBM) protein. Post-metamorphic stage black sea bass (~0.60 g, d40ph) were stocked in each of sixteen 75-L tanks at a density of 1 fish per L (75 per tank), with four replicate aquaria per treatment. Fish were fed four times per day (0800, 1100, 1400, and 1600 h) to apparent satiation for 30 days. Final body weight (5.70–5.74), specific

growth rate (7.40–7.45%/d), and percent body weight gain (834–848%) of fish fed the UNCW-formulated D1 (FM-based) and D2 (FM + PBM-based) were higher ($p < .05$) than in fish fed the commercial diets CD3 and CD4 (4.66–5.21 g, 6.80–7.15%/d, and 668–756%, respectively). Feed intake (% body weight/d was significantly lower for fish fed commercial diet (CD4) (3.94) compared with fish fed CD3 (4.20), but feed intake for CD3 was not significantly different compared with the UNCW-formulated diets D1 and D2 (4.10–4.12). Feed conversion ratios (0.76–0.82) were significantly higher for fish fed CD3 (0.82) than for fish fed D1 and CD4 (0.76). Survival was high (99–100%) in all diet treatments. Final whole-body crude protein content (15.2 to 15.9% wet basis), moisture (68.9–69.6%), and ash (4.31–4.77%) showed no significant differences; however, whole-body crude lipid was lower in fish fed CD3 (9.67%) than in fish fed the other diets (9.96–10.22%). Final whole-body fatty acid composition reflected the diet composition. Higher feed consumption and growth of fish fed the UNCW-formulated diets were attributed to a more optimal combination of marine (fish, squid, and krill meals), terrestrial plant (soybean meal) protein sources, and the addition of chemo-attractants, which provided both higher nutritional quality and palatability. The study suggests that the species-specific starter diets may improve growth performance and fingerling quality and may therefore lower production costs under intensive nursery conditions.

KEYWORDS
black sea bass, body composition, *Centropristis striata*, growth, nursery culture, palatability, post-metamorphic stage, starter diets

1 | BACKGROUND

Black sea bass, *Centropristis striata* (family Serrranidae) is a high-value marine finfish found in the continental shelf waters of the US East coast from the Florida Keys to Maine and an important candidate for commercial aquaculture because of high market demand and strict quotas on harvests of wild populations that limit future supplies. Broodstock husbandry techniques for maturation and spawning of black sea bass adults in captivity (Berlinsky, King, & Smith, 2005; Watanabe, et al., 2003, 2021), hatchery methods for rearing larvae through juvenile stages (Berlinsky, Watson, Nardi, & Bradley, 2000; Carrier III et al., 2011; Copeland & Watanabe, 2006; Rezek, Watanabe, Harel, & Seaton, 2010; Russo, Watanabe, Kinsey, & Seaton, 2017), and technology for grow out of juveniles to

marketable stages in recirculating aquaculture systems (RAS) (Copeland, Watanabe, Carroll, Wheatley, & Losordo, 2003; Copeland, Watanabe, & Dumas, 2005; Watanabe, 2011; Watanabe et al., 2021) have been established in the eastern United States. Research in our laboratory at UNCW has shown that black sea bass can be raised from juvenile to adult stages in RAS using alternative protein-based diets to fish meal protein, and that cultured product can command high-value niche market prices (Alam, Watanabe, & Carroll, 2008; Alam, Watanabe, Sullivan, Rezek, & Seaton, 2012; Watanabe, 2011; Watanabe et al., 2003; Watanabe et al., 2021; Wilde, Dumas, & Watanabe, 2008).

The availability and price of high-quality fingerlings to support commercial black sea bass growout operations is a critical variable cost (Copeland et al., 2005; Ibarra-Castro, Martinez Cordero, & Alvarez-Lajonchere, 2013; Kam, Leung, Ostrowski, & Molnar, 2002; Lee, Leung, & Su, 1997; Stephanis, 1995; Watanabe, Dumas, Carroll, & Resimius, 2015). Based on an earlier economic analysis of a hypothetical commercial hatchery for black sea bass, it was hypothesized that fingerling production costs could be substantially lowered by reducing age and size at harvest as well as by maximizing stocking densities in larval and nursery tanks (Watanabe et al., 2015). A prerequisite to this goal is to identify the optimum starter diets that maximize post-larval and early juvenile growth, survival, and fingerling quality (i.e., tolerance to handling and environmental stressors) and allow transport-ready fingerlings to be raised at higher densities and harvested at younger ages and smaller sizes to lower hatchery production costs (Watanabe et al., 2015**).**

Weaning diets have been studied for several species including cod *Gadus morhua*, Florida pompano *Trachinotus carolinus*, and Haddock *Melanogrammus aeglefinus* (Hamre, 2006; Hauville, Zambonino-Infante, Bell, Migaud & Main, 2014; Lall, Lewis-McCrea1, & Tibbetts, 2018). Although macronutrient requirements and utilization of alternative protein sources in advanced juvenile stage black sea bass diets are well established (Alam et al., 2008; Alam et al., 2012; Alam, Watanabe, & Daniels, 2009), experimental data regarding the optimal formulated starter diets for rearing the post-larval and early juvenile stages of black sea bass are limited. The goal of this study was to identify and develop the optimum starter diets for black sea bass as a basis for improving juvenile production efficiency in hatcheries/nurseries, and hence availability and costs of fingerlings for startup commercial growers. The objectives were to develop a species-specific microparticulate diet for black sea bass containing alternative protein sources to fish meal and to benchmark post-larval and early juvenile fish growth performance, survival, and body composition against the premium commercial diets under controlled laboratory conditions.

2 | MATERIALS AND METHODS

2.1 | Experimental animals: Induced spawning and larval rearing

Ten wild-caught female black sea bass with mean oocyte diameters ranging from 394 to 477 μm were each implanted with a fast-release cholesterol-cellulose (80–20) pellet containing luteinizing hormone releasing hormone analogue (LHRHa) to induce final oocyte maturation and ovulation (Watanabe et al., 2003, 2021). Females were strip-spawned at 36 hr post-implantation, and eggs were fertilized with sperm from four wild-caught black sea bass males. The eggs were incubated in 300-L conical tanks supplied with flow-through seawater at 32 g/L salinity and at 19°C for 48 hr. Sinking (non-viable) eggs were siphoned from the incubation tanks two times per day until late embryos were stocked into larval rearing tanks (LRTs).

Larval rearing protocols followed those developed at UNCW for black sea bass (Carrier III et al., 2011; Copeland & Watanabe, 2006; Rezek et al., 2010; Russo et al., 2017; Watanabe, 2011; Watanabe et al., 2021). To stock LRTs, salinity in the incubators was increased to 39 g/L to increase the buoyancy of the embryos. Floating viable embryos were stocked into two 2,000-L LRTs. Water quality in LRTs at stocking was as follows: temperature 19°C, dissolved oxygen 7.78 mg/L, salinity 34.0 ppt, and pH 7.2. Light intensity was 100 lx, while photoperiod was 18 hr light: 6 hr dark. Aeration was maintained at a level sufficient to keep larvae in suspension.

Beginning on d2ph, nonviable algae paste *Nannochloropsis oculata* ("greenwater") was added to the LRTs daily at a density of 1,000,000 cells/ml. One-third of the algae was added at 08:00 hr, whereas the remaining two-thirds were added in equal portions at 13:30, 18:30, and 23:30 hr using an automatic peristaltic pump. Enriched rotifers *Brachionus rotundiformis* were added to the LRTs on d3ph starting at a density of 6 rotifers/ml and increasing to 40 rotifers/ml by d11ph. Rotifers were enriched with Algamac 3,600 (0.3 g/million rotifers), *N. oculata* (0.3 g/million rotifers), and taurine (8 g/million rotifers). One-fourth of the rotifer ration was fed by hand at 09:00 hr each morning, and three-fourths of the ration was cold-banked at 4°C and fed with the microalgae via peristaltic pump. Rotifer densities were decreased from 40 rotifers/ml on d16ph to 10 rotifers/ml on d22ph. On d23ph, the addition of both algae and rotifers was ceased and water exchange was increased to 400%/d. *Artemia* nauplii were fed from d16ph at a density of 0.3 ind./ml and was increased to 2 ind./ml by d22ph. On d23ph, enriched *Artemia* metanauplii were fed at density of 2.5 ind./ml, increasing to a maximum of 6 ind./ml from d29-d30ph, with complete weaning to artificial microdiets by d33ph. *Artemia* were enriched with Inve S.presso (0.6 g/L/100,000 metanauplii) (INVE Aquaculture, Salt Lake City, UT). Dry microdiets (Otohime, Marubeni Nisshin Feed Co., Tokyo, Japan, 51–53% CP, 8–12% CL) were introduced on d18ph and co-fed by hand with live feeds until d33ph. From d33ph, microdiet was fed using automatic feeders six times a day until fingerlings (~0.6 g) were harvested from the LRTs on d40ph and transferred to the controlled-environment laboratory for nursery diet experiments.

2.2 | Experimental system

The feeding trial experimental system located in a controlled-environment laboratory consisted of sixteen 75-L rectangular (76 × 32 × 43 cm) glass tanks supported by a seawater (32 g/L) RAS. Water quality was maintained by liquid oxygen backup, diffused aeration, UV sterilizer, a bead filter, a foam fractionator, and heat pump. A timer-controlled fluorescent lamp was used to supply the illumination. A 12 L:8 D photoperiod was maintained throughout the experiment.

2.3 | Experimental design

A laboratory-controlled feeding trial was conducted to determine the optimum starter diets for black sea bass, to compare two UNCW-formulated diets for black sea bass and two premium commercial marine fish starter diets. Both UNCW diets (particle size 1,000 μm) contained 54% crude protein (CP) and 14% crude lipid (CL) (Table 1). The UNCW diet formulas were based on previous studies on juvenile black sea bass in our laboratory (Alam, Watanabe, & Copeland, 2006; Alam et al., 2008, Alam et al., 2012; Dawson, Alam, Watanabe, & Carroll, 2018). Both diets incorporated a combination of high-quality protein sources, including menhaden fish meal (FM) and other practical sources (e.g., soybean meal, soy protein concentrate, squid meal, poultry by-product meal (PBM), and krill meal). UNCW Diet 1 (D1) contained high FM (40% of diet), whereas UNCW Diet 2 (D2) replaced 50% FM protein by PBM protein. All other ingredients were added in the UNCW-formulated test diets based on recent nutrient requirements studies on black sea bass (Alam et al., 2008, 2012), including supplemental amino acids (0.5% methionine, 0.5% lysine), 1% attractants (alanine, glycine, taurine, and betaine), 4% vitamins, and 2.25% mineral premix. Soybean lecithin and menhaden fish oil were added as lipid sources. UNCW-formulated diets D1 and D2 (1.2 mm size) were prepared at UNCW-CMS Aquaculture Research Lab using a Kitchen Aid mixer, meat grinder, and a drying oven (Alam et al., 2008, 2009). After drying, feed was crushed, passed through 1,000 μm mesh sieve, and the particles retained on a 750 μm sieve were collected. The particles that passed through the 750 μm mesh size sieve were not used for feeding trial. The particle size range for D1 and D2 was between 750 and 1,000 μm. All diets were stored at −20 C.

TABLE 1 Composition (g/100 g) of experimental diets

Diet number Diet source description	D1 UNCW high FM-based	D2 UNCW 50% FM replaced by PBM	CD3 Otohime-EP2[a]	CD4 Gemma-Diamond[b]
Ingredients				
Menhaden meal[c]	40	20		
Squid meal[d]	20	20		
Krill meal[d]	10	10		
Poultry meal[d]	0	20		
Soy protein[e]	5	5		
Fish oil[f]	4	4		
Soybean lecithin[g]	2.5	2.5		
Methionine	0.5	0.5		
Lysine	0.5	0.5		
Wheat gluten[h]	5	5		
n-HUFA	0.5	0.5		
Vitamin premix[i]	4	4		
Mineral premix[j]	2.25	2.25		
Taurine	0.5	0.5		
Alanine	0.25	0.25		
Glycine	0.25	0.25		
Betaine	0.25	0.25		
Wheat starch	4	4		
Cellulose	0.5	0.5		
Total	100	100		
Protein %	54.2	54.0	48	57
Lipid %	14.4	14.6	14	15
Energy kj/g diet[k]	15	15		

Note: Diets 1 and 2 were formulated and prepared at UNCW. Diets CD3 and CD4 were sourced commercially.
Abbreviations: FM, fishmeal; PBM, poultry by-product meal.

[a]Diet formulation is proprietary. Labeled ingredients: krill meal, fish meal, squid meal, potato starch, wheat flour, fish oil, brewer's yeast, calcium phosphate, guar gum, soy lecithin, betaine, licorice plant, apple extract, wheat germ.

[b]Diet formulation is proprietary. Labeled ingredients: fish meal, algae, fish oils, lecithin, betaine, wheat gluten, vitamins and minerals.

[c]Omega protein Corporation, Houston, TX, USA (crude protein 59%, lipid 11%).

[d]Scoular Company Inc, (CP 65%, CL12%).

[e]SPC soy protein concentrate (CP 65%, CL).

[f]Virginia Prime Silver, Omega Protein, Hammond, LA, USA.

[g]ADM, IL, USA.

[h]VWR International, Radnor, PA, USA (crude protein 78%).

[i]DSM, Canada.

[j]Alam et al. (2008) (g/kg diet) $MgSO_4$, 3.17; Na_2HPO_4, 2.02; K_2HPO_4, 5.54; $Ca(HPO_4)$,3.14; Fe-citrate, 0.68; Ca-lactate, 7.56; $Al(OH)_3$, 0.01; $ZnSO_4$, 0.08; $Cu(SO_4)$, 0.002, $MnSO_4$, 0.02; $Ca(IO_3)_2$, 0.003 and $CoSO_4$, 0.02.

[k]Calculated based on carbohydrates, proteins and lipids are 17.2, 23.6, and 39.5 kJ/g, respectively (Blaxter, 1989).

One commercial diet tested was Otohime C (CD3, CP = 48% and CL = 14%, particle size 750 μm −1 mm, Reed Mariculture Inc., Campbell, CA) (Table 1). The other commercial diet was Gemma Diamond (CD4, CP = 57%, CL = 15%, particle size 1 mm, Skretting, Nutreco, Canada) (Table 1).

2.4 | Feeding trial

At the beginning of the experiment, 75 metamorphic stage black sea bass (~0.60 g, d40ph) were stocked in each of sixteen 75-L tanks at a density of 1 fish per L ($N = 1{,}200$ total). Four replicate aquaria were assigned to each diet treatment. Otohime © (particle diameter 1.0 mm) was initially fed for 2 days to fish in all groups before their respective treatment diets were fed. Fish were fed four times per day (0800, 1100, 1400, and 1600 hr) to apparent satiation (i.e., as much as they could consume during a 20-min period without wastage) and the amount of diet consumed was recorded.

2.5 | Experimental conditions

Fish were reared under 34 g/L salinity, 18 L: 6D photoperiod, 23°C, and 8 mg/L DO and ammonia, nitrite, and nitrate concentrations were maintained within suitable ranges documented for black sea bass (Watanabe, 2011; Watanabe et al., 2021). To monitor growth, fish were weighed on days 0, 15, and 30 post-stocking. Water quality parameters (dissolved oxygen, pH, temperature, ammonia, and nitrate concentrations) were recorded twice weekly and maintained within optimal ranges for rearing black sea bass juveniles (Watanabe, 2011; Watanabe et al., 2021). At the end of the 30-day feeding trial, all fish from each tank were collected, sacrificed, and freeze-dried and stored at −30°C for whole-body proximate composition and fatty acid analysis.

2.6 | Whole-body proximate analysis

Part of the proximate composition analysis was conducted at the UNCW Aquaculture Research Laboratory (AOAC, 2000) and the remainder was conducted at a commercial laboratory (New Jersey Feed Lab, Trenton, NJ) because of renovation of the UNCW Aquaculture Research Lab after Hurricane Florence. Fish whole bodies were freeze-dried to determine moisture content (Labconco Freeze Dryer, Kansas City, MO), and ash content was determined using a muffle furnace (BARNSTAD Thermolyne Muffle Furnace, IA). CP and total lipid of all experimental diets and body tissues were analyzed at New Jersey Feed Lab (Trenton, NJ).

2.7 | Fatty acid analysis of diets and fish whole bodies

Fatty acid composition of the UNCW-formulated diets (D1 and D2) and black sea bass whole fish bodies was determined as stated by Anderson et al. (2016) by extracting total lipids into chloroform–methanol (Folch, Lees, & Sloane-Stanley, 1957). In each sample, nonadecanoic acid (19:0) was added as an internal standard (1 ml of a 0.001 g/ml solution). As described by Rezek et al. (2010), lipid fatty acids were converted to their methyl esters (FAMEs) for GC analysis. At UNCW department of Chemistry and Biochemistry lab, Gas chromatography analysis was performed on a HP-6890 GC using a 30 m length × 0.25 μm film thickness × 0.25 mm internal diameter column.

2.8 | Statistical analysis

All statistical analysis was conducted using the JMP Pro 13.0 statistical software (SAS Institute Inc., Cary, NC). Treatment mean values were compared using a one-way ANOVA, and Tukey Kramer test (Kramer, 1956) was used to determine significant ($p < .05$) differences among test diets.

3 | RESULTS

3.1 | Growth performance and feed efficiency

The growth performance of fish fed the test diets and feed efficiency data are presented in Table 2. Initial body weights ranged from 0.60 to 0.61 g and were not significantly different among treatment groups. Final body weights (g) between fish fed the UNCW-formulated diets D1 (5.74) and D2 (5.70) were not significantly different but were higher than in fish fed commercial diets CD3 (4.66) and CD4 (5.21). Specific growth rate (SGR, %/d)) showed the same trend as final body weights with significantly higher SGR for fish fed the UNCW-formulated D1 and D2 (7.45–7.50) than those fed CD3 (6.80) and CD4 (7.15), with lower SGR in CD3 than in CD4. Body weight gain (%) also showed the same trend, with higher BWG for UNCW-formulated diets D1 and D2 (834–848%) than in CD3 (668%) and CD4 (756%), with lower BWG in CD3 than in CD4 (Table 2). Feed intake (% body weight/day) was not significantly different among fish fed both UNCW-formulated diets D1 and D2 (4.10–4.12) and in fish fed CD3 (4.20). Feed intake was higher in fish fed CD3 (4.20) than in fish fed CD4 (3.94). FCRs were excellent across all diet treatments and were not significantly different among fish fed UNCW-formulated diets D1 (0.76) and D2 (0.78) and commercial diet CD4 (0.76). FCR was higher for fish fed CD3 (0.82) than for fish fed CD4 (0.76). Survival was high (99.2–100%) in all treatments and not significantly different (Table 2).

3.2 | Whole-body proximate composition

The range of final whole-body moisture was 68.9–69.6%, with no significant differences among treatment groups (Table 3). The CP (%wet basis) content in final fish whole bodies was 15.2–15.9% with no significant differences (Table 2). Whole-body CL was not significantly different among fish fed and commercial diet D4 (10.22%) and UNCW-formulated diets D1 (9.96%), D2 (10.68%). However, whole-body CL in fish fed CD3 (9.67%) was lower than in fish fed D2 (10.68%). Ash content in fish whole bodies ranged from 4.31 to 4.77% with no significant differences.

3.3 | Fatty acid composition of diets and whole bodies

The fatty acid profiles of the UNCW-formulated diets D1 and D2 are presented in Table 4. The fatty acid profiles were not analyzed in our laboratory; however, data published by the respective companies are presented in Table 5 as a basis for comparison. Total saturated fatty acids (SFA) ranged from 32.18–34.91 mg/g^{-} diet among the UNCW experimental diets (Table 4). The UNCW-formulated D2 (FM + PBM-based l) showed higher oleic acid (18:1n-9) (18.79 mg/g) than D1 (high FM-based) (10.72 mg/g). The concentration of linoleic acid (18:2n-6) also a showed similar pattern with higher levels in D2 (17.42 mg/g) than in D1 (12.92 mg/g). n-3 polyunsaturated fatty acids (PUFA) were higher in D1 (26.50 mg/g) than in D2 (20.39 mg/g) (Table 4). Arachidonic acid (20:4n-6) was lower in D1 (1.12 mg/g) than in D2 diet (1.33/mgg); however, eicosapentaenoic acid (EPA, 20:5n-3) (11.46 mg/g) and docosahexaenoic acid (DHA, 22:6n-3) (10.49 mg/g) concentrations were higher in D1 than in D2 (8.99 and 7.83 mg/g, respectively). DHA/EPA ratios in D1 and D2 ranged from 0.91 to 0.87 (Table 4).

Fish whole-body fatty acid profiles after the feeding trial are presented in Table 6. Whole-body fatty acid profiles of fish fed the UNCW-formulated D1 and D2 reflected dietary levels and lipid sources. Total SFAs ranged from 59.62 to 79.72 mg/g^{-} dry weight (dw), with 16:0 representing a major component. Here 16:0 was significantly lower in whole bodies of fish fed CD3 (41.71 mg/g) compared with fish fed the other diets (51.62–56.28 mg/g). Total MUFAs in whole bodies ranged from 64.85 to 82.06 mg/g dw, with 16: 1n-7 and 18: 1n-9, representing major components. The 16:1n-7 content in the whole bodies of fish fed the UNCW D1 and D2 (21.61–22.32 mg/g) were significantly higher than in fish fed CD3 (14.09 mg/g) and CD4 (17.52 mg/g). The 18:1n-9 content in fish fed UNCW-

TABLE 2 Growth performance and feed efficiency of juvenile black sea bass on UNCW-formulated starter diets (D1 and D2) and commercially-sourced diets (CD3 and CD4)[a]

Diets[a]	Initial wt. (g)	Final wt. (g)	SGR (%/d)	BWG (%)	FI (% body wt./d)	FCR	Survival (%)
D1	0.61 ± 0.002	5.74 ± 0.11a	7.50 ± 0.05a	848 ±14.5a	4.10 ± 0.07ab	0.76 ± 0.01b	99.7 ± 0.25a
D2	0.61 ± 0.004	5.70 ± 0.07a	7.45 ± 0.03a	834 ± 7.9a	4.12 ± 0.03ab	0.78 ± 0.01ab	99.2 ± 0.25a
CD3	0.60 ± 0.006	4.66 ± 0.08c	6.80 ± 0.04c	668 ± 14.5c	4.20 ± 0.09a	0.82 ± 0.01a	100 ± 0a
CD4	0.61 ± 0.001	5.21 ± 0.04b	7.15 ± 0.04b	756 ± 9.2b	3.94 ± 0.08b	0.76 ± 0.01b	99.2 ± 1.1a

Note: Data are means ± *SEM* ($N = 4$).

[a]D1 (FM-based), D2 (FM+PBM-based), CD3 (Otohime), CD4 (Gemma-Skretting).

[a]BWG (body weight gain) (%): {(Final weight-Initial fish wet)/Initial weight} × 100.

[b]SGR (specific growth rate) (%/d): [ln (mean final weight)−ln (mean initial weight)/30 d] × 100.

[c]FCR (feed conversion ratio): Feed intake (g)/wet weight gain (g).

[d]FI (feed intake): % body weight/day.

TABLE 3 Whole-body proximate composition of fish after the feeding trial (% wet basis)

Diet	Moisture	Crude protein	Crude lipid	Ash
D1 (FM-based)	69.5 ± 0.37a	15.7 ± 0.02a	9.96 ± 0.23ab	4.31 ± 0.22
D2 (FM + PBM-based)	68.9 ± 0.24a	15.9 ± 0.42a	10.68 ± 0.17a	4.77 ± 0.41
CD3 (Otohime)	69.6 ± 0.30a	15.2 ± 0.22a	9.67 ± 0.24b	4.65 ± 0.14
CD4 (Gemma)	69.1 ± 0.11a	15.9 ± 0.14a	10.22 ± 0.19ab	4.51 ± 0.51

Note: Data are means ± *SEM* ($N = 4$). UNCW-formulated diets were based on fish meal protein (D1) or on a combination of fish meal protein and poultry by-product meal protein (D2 FM + PBM-based). Diets CD3 and CD4 were sourced commercially. Means in each column not followed by a letter in common are significantly different.

TABLE 4 Fatty acid profiles (mg/g dry sample weight) of UNCW-formulated diets D1 (FM-based) and D2 (FM-PBM-based)

Fatty acid	Diet 1 FM-based	Diet 2 FM + PBM-based
14:0	7.74	6.56
16:0	20.22	22.90
16:1n-7	11.06	10.70
18:0	4.03	5.24
18:1n-9	10.72	18.79
18:1n-11	3.62	3.31
18:2n-6	12.92	17.42
18:4n-3	2.71	2.13
20:0	0.19	0.21
20:1n-9	1.27	1.25
20:2n-6	0.34	0.31
20:4n-6 (ARA)	1.12	1.33
20:5n-3 (EPA)	11.46	8.99
22:5n-3	1.89	1.44
22:6n-3 (DHA)	10.44	7.83
Σ SFA	32.18	34.91
Σ MUFA	26.67	34.05
Σ n-3 PUFA	26.50	20.39
Σ n-6 PUFA	14.38	19.06
n-3/n-6 PUFA	1.84	1.07
DHA/EPA	0.91	0.87

Note: Values are means of duplicate analysis.

formulated diet replacing 50% FM by PBM (D2) (50.44 mg/g) was not significantly different than in fish fed CD4 (45.17 mg/g); however, UNCW-formulated D1 (high FM-based) and CD3 showed similar 18:1n-9 content (35.95–37.43 mg/g) that were significantly lower than the UNCW-formulated D2 and CD4. Total n-3 PUFAs ranged from 39.10 to 55.16 mg/g dw. Total n-3 PUFA content in whole bodies of fish fed the UNCW-formulated D1 (high FM-based) (49.12 mg/g) was comparable to fish fed CD3 and CD4 (41.91–55.16 mg/g), but was lower in fish fed D2

TABLE 5 Fatty acid composition of UNCW-formulated diets D1 (FM-based) and D2 (FM-PBM-based) and commercial diets CD3 and CD4) expressed as percent total fatty acid (%TFA)

Fatty acid	D1 (FM-based)	D2 (FM+PBM-based)	CD3[a]	CD4[a]
14:0	7.76	6.05	7.40	2.97
16:0	20.28	21.12	18.34	17.93
16:1n-7	11.09	9.87	5.26	2.19
18:0	4.04	4.83	3.09	3.16
18:1n-9	10.75	17.33	12.25	11.72
18:1n-11	3.63	3.05	Not available	
18:2n-6	12.95	16.06	4.54	30.46
18:4n-3	2.72	1.97	2.53	1.63
20:0	0.19	0.19	Not available	
20:1n-9	1.27	1.15	5.37	3.23
20:2n-6	0.34	0.29	0.15	0.15
20:4n-6 (ARA)	1.12	1.23	0.54	0.34
20:5n-3 (EPA)	11.49	8.29	10.79	4.12
22:5n-3	1.89	1.33	0.95	0.59
22:6n-3 (DHA)	10.46	7.22	9.57	7.32
Σ SFA	32.27	32.19	Not available	
Σ MUFA	26.74	31.41	Not available	
Σ n-3 PUFA	26.57	18.81	25.73	17.66
Σ n-6 PUFA	14.42	17.58	5.55	31.13
n-3/n-6 PUFA	1.84	1.07	4.63	0.57
DHA/EPA	0.91	0.87	0.89	1.78

Note: Values for D1 and D2 are means of duplicate analysis.
[a]Hauville et al. (2014).

(FM + PBM-based) (39.10 mg/g) (Table 6). EPA levels in fish fed the UNCW-formulated D2 (15.67 mg/g) and CD3 (13.86 mg/g) were significantly lower than in fish fed D1 (19.56 mg/g) and CD4 (22.11 mg/g). DHA content was higher in fish fed CD4 (24.04 mg/g) than in all other treatment groups and higher in fish fed CD3 (20.29 mg/g) and in D1 (20.79 mg/g) than in fish fed D2 (16.41 mg/g). The n-6 PUFA were lower in fish fed CD3 (12.18 mg/g dw) and CD4 (15.72 mg/g) compared with the UNCW-formulated D1 and D2 (25.95–34.55 mg/g dw), and these differences were primarily related to lower 18: 2n-6 linoleic in the commercial diets CD3 and CD4. The whole body 18:2n-6 was significantly higher in fish fed D2 (FM + PBM-based) (31.1 mg/g) compared with other diets; however, whole body18:2n-6 was significantly lower in both CD3 and CD4 (7.94–12.79 mg/g) than in fish fed the UNCW-formulated D1 and D2 (22.98–31.10 mg/g).

4 | DISCUSSION

Premium starter diets for marine finfish from Japan (Otohime) and Europe (Skretting) are used for early feeding of warm water marine fish in North America (Hauville et al., 2014); however, the availability of starter diets for marine finfish in the United States is currently limited, and such diets have relatively short shelf lives and are expensive to import. Furthermore, companies producing starter diets experience high costs with poor returns because the demand

TABLE 6 Whole-body fatty acid profiles (mg/g dry sample weight) of juvenile black sea bass on UNCW-formulated weaning diets (D1 and D2) and commercially-sourced diets (CD3 and CD4)

Fatty acid	UNCW-formulated D1 (FM-based)	UNCW-formulated D2 (FM + PBM-based)	Commercial CD3	Commercial CD4
14:0	12.10 ± 0.51a	10.27 ± 0.16b	8.87 ± 0.38b	10.03 ± 0.27b
16:0	52.09 ± 2.52a	56.28 ± 1.18a	41.71 ± 1.77b	51.62 ± 1.34a
16:1n-7	22.32 ± 0.96a	21.61 ± 0.49a	14.09 ± 0.63c	17.52 ± 0.43b
18:0	11.06 ± 0.52b	12.73 ± 0.25a	8.54 ± 0.38c	10.67 ± 0.25b
18:1n-9	37.43 ± 2.02b	50.44 ± 1.37a	35.95 ± 1.44b	45.17 ± 1.12a
18:1n-11	7.66 ± 0.18a	7.40 ± 0.12a	6.35 ± 0.24b	6.96 ± 0.21ab
18:2n-6	22.98 ± 0.79b	31.10 ± 0.47a	7.94 ± 0.69d	12.79 ± 0.55c
18:4n-3	4.04 ± 0.12a	3.16 ± 0.05b	4.10 ± 0.24a	4.40 ± 0.12a
20:0	0.40 ± 0.01b	0.44 ± 0.02b	0.40 ± 0.02b	0.52 ± 0.02a
20:1n-9	2.62 ± 0.17b	2.61 ± 0.09b	8.41 ± 0.52a	3.28 ± 0.11b
20:2n-6	0.89 ± 0.08b	0.99 ± 0.11b	2.27 ± 0.21a	1.00 ± 0.11a
20:4n-6 (ARA)	2.08 ± 0.06b	2.46 ± 0.05a	1.94 ± 0.09b	2.01 ± 0.05b
20:5n-3 (EPA)	19.56 ± 0.61b	15.67 ± 0.31c	13.86 ± 0.61c	22.11 ± 0.56a
22:5n-3	4.72 ± 0.16a	3.85 ± 0.08b	3.58 ± 0.21b	4.57 ± 0.10a
22:6n-3 (DHA)	20.79 ± 0.62b	16.41 ± 0.35c	20.29 ± 0.91b	24.04 ± 0.62a
Σ SFA	75.65	79.72	59.62	72.87
Σ MUFA	70.03	82.06	64.85	73.03
Σ n-3 PUFA	49.12	39.10	41.91	55.16
Σ n-6 PUFA	25.95	34.55	12.18	15.72
n-3/n-6 PUFA	1.89	1.13	3.44	3.51
DHA/EPA	1.06	1.05	1.46	1.09

Note: Diets are as follows: D1 (UNCW-formulated, high FM), D2 (UNCW-formulated, FM + PBM), CD3 (commercially-sourced, Otohime), and CD4 (commercially-sourced, Skretting). Data are means ± *SEM* ($N = 4$).

for such diets for marine finfish is currently limited. Therefore, it is important that marine fish growers in the United States develop high-quality species-specific starter diets that can potentially be produced locally at lower cost.

4.1 | Growth performance

All tested diets in the present study, including the UNCW-formulated diets D1 (containing high FM protein) and D2 (replacing 50% FM protein with PBM protein) and the premium commercial diets CD3 (Otohime) and CD4 (Gemma Diamond), produced excellent survival and rapid growth of post-metamorphic stage black sea bass. However, growth was higher on the UNCW-formulated diets D1 and D2 than on the commercial diets CD3 and CD4.High digestibility of ingredients, well-balanced nutrition profile, high water-stability, proper suspension in the water column, and palatability are prerequisites for high-quality starter diets for larval and early juvenile aquatic animals (Bengtson, 1993; Izquierdo, Fernandez-Palacios & Tacon, 2001; Lall et al., 2018; Teshima, Ishikawa, & Koshio, 2000). Survival of fish fed all of the test diets in the present study was 99–100% for the 30-day feeding period (from 40 to 70 dph), suggesting that irrespective of source (i.e., UNCW-formulated or commercially prepared), early post-metamorphic

stage black sea bass were able to efficiently utilize all of the starter diets tested to sustain high survival and good growth during the sensitive period just after transition from live to microparticulate diets was completed. In this study, higher SGR, final weight, and body weight gain were attained in fish fed UNCW-formulated diets D1 (FM-based) and D2 (FM + PBM-based) compared with commercial diets CD3 and CD4. This suggests that feed consumption (palatability), nutritional quality, and/or digestibility of the UNCW-formulated diets were higher than of the commercial diets CD3 and CD4.

The buoyancy, sinking speed, and leaching losses of nutrients for all tested diets were not measured or compared during the feeding trial; however, the chemical attractiveness, physical characteristics, color, particle size, shape and manufacturing techniques, and nutrient leaching of diets in water could influence feed intake and nutrient utilization by pre- and post-metamorphic stage fish (Lipscomb, Patterson, Wood, Watson, & DiMaggio, 2020; Teshima et al., 2000). Orihuela et al. (2018) also found that the particle-assisted rotational agglomeration (PARA) microdiets showed better performance for Fine flounder, *Paralichthys adspersus* as compared with Otohime diets because the manufacturing process produces less dense and smaller particle size PARA diets reducing sinking rates and increasing the probability of the larvae in detecting and ingesting the diet. We assume that our UNCW-formulated diets were comparable or better than the other two commercial diets in terms of chemical attractiveness, buoyancy in water, and physical characteristics of the particles as evidenced by the higher growth performance of fish fed D1 and D2 diets.

4.2 | Palatability

In this study, all treatment diets were offered to satiety without wastage, so availability of feed was not a limiting factor to consumption and growth performance. Data on feed intake (Table 2) indicate, therefore, that palatability of D1, D2, and CD3 was similar and slightly higher than of CD4. This strongly suggests that higher palatability and consumption (i.e., appetency) contributed to higher growth performance in fish fed D1 and D2 than in fish fed CD4. A reduction in diet palatability reducing feed intake has been shown to lower growth performance in European sea bass, *Dicentrarchus labrax*; Japanese sea bass, *Lateolabrax japonicas*, and starry flounder, *Platichthys stellatus* (Li et al.,2012; Song et al., 2014; Tibaldi et al., 2006). Several factors may have improved the palatability of D1 and D2 in this study. During the weaning period of marine fish larvae, supplementation of feed attractants plays an important role in the acceptance of dry diets or palatability in fish larvae (Alam et al., 2006; Kolkovski, 2001; Kolkovski, Koven, & Tandler, 1997). In this study, the UNCW-formulated diets D1 and D2 contained 20% squid meal and 5% krill meal, which are rich in taurine and betaine, chemo-attractants that are known to increase diet palatability in marine fish. The chemo-attractant properties of squid meal were reported for gilthead sea bream, *Sparus aurata* (Kolkovski & Tandler, 2000) and summer flounder, *Paralichthys dentatus* (Lian, Lee, & Bengtson, 2008). Because of favorable amino acid composition and high free amino acid contents, krill meal has been demonstrated to enhance food ingestion in some marine fish larvae and juveniles (Kolkovski et al., 1997; Shimizu, Ibrahim, Tokoro, & Shirakawa, 1990). Lall et al. (2018) also found a practical formulated diet with several practical ingredients such as krill, squid, and herring meal was a highly suitable starter diet compared with a premium commercial diet (Biokyowa; Kyowa Hakko Kogyo, Tokyo, Japan) for haddock postlarvae based on high feed acceptance, survival, and fish growth. Higher palatability and consumption of the UNCW-formulated diets D1 and D2 were also possibly related to the supplementation of crystalline amino acids (taurine, alanine, glycine, betaine, and taurine), which have been reported to increase attractability of weaning and starter diets in marine finfish larvae and early juveniles (Teshima et al., 2000).

In this study, the amino sulfonic acid taurine was also included in the UNCW-formulated diets D1 (FM-based) and D2 (PBM-based). The growth performance of Japanese flounder improved significantly with the dietary inclusion of taurine (Kim, et al., 2005). Most of the carnivorous species show a positive feeding response in the presence of alkaline and neutral substances, such as taurine, proline, glycine, valine, and betaine (Tonheim, Nordgreen, Høgøy,

Hamre, & Rønnestad, 2007). In this study, feed intake of CD3 was very similar to UNCW D1 and D2; however, CD3 produced the highest FCR and, therefore, the lowest growth performance among diet treatments (Table 2). This suggests that although palatability of CD3 was comparable to the UNCW-formulated D1 and D2, digestibility and nutrient utilization were lower in CD3. This may be related in part to the lower CP content found in CD3 (48% CP) compared with the other diets (54.0–57% CP). These results further suggest that although CD3 is a premium product and widely used as a weaning diet for marine finfish larval rearing (Hauville et al., 2014), it is not an optimized formulation for post-metamorphic stage black sea bass.

4.3 | Nutritional quality (diet protein sources)

The quality of dietary protein for post-metamorphic stage fish is dependent on the amino acid profile. A sufficient supply of dietary amino acids to fulfill the requirement is a prerequisite for high growth rates of marine fish (Alam et al., 2005). The growth performance of early black sea bass juveniles on the UNCW-formulated diets D1 (FM-based) and D2 (PBM-based) in this study indicate that the dietary proteins used as amino acid sources for these diets were of suitable quality and fulfilled the essential amino acid requirements of this species. The UNCW-formulated D1 (FM-based) contained a combination of protein sources of both marine (fish meal, squid meal, krill meal) and terrestrial (soybean meal), whereas UNCW-formulated D2 (FM + PM) contained these same protein sources, but replacing 50% FM protein by PBM protein. Growth and survival of larval black sea bass fed microdiets containing a combination of squid meal, fish meal, krill meal, and herring meal were higher compared with fish fed microdiets without squid meal (Alam et al., 2006). As protein sources, squid powder and fish meal were found to have high nutritional value for barramundi *Lates calcarifer* larvae, because of favorable amino acid profile and moderate to high digestibility (Nankervis & Southgate, 2006; Saen de Rodriganez, Gander, Alaiz, & Moyano, 2011). Soybean meal used in UNCW D1 and D2 in this study was also reported previously to be utilized efficiently by juvenile black sea bass (Alam et al., 2012).

The formulations of the commercial diets CD3 and CD4 are proprietary; however, some inferences may be made about the nutritional quality of these diets based on their ingredients as listed on the product labels. The commercial CD3 diet contained krill meal, fish meal, squid meal as main protein sources, whereas the commercial CD4 diet contained only fish meal and algae as protein sources. In general, both of the commercial diets CD3 and CD4 did not contain the variety of both aquatic (fish meal, squid meal, krill meal) and terrestrial animal-based protein sources (e.g., soybean meal and poultry by-product meal [PBM]) that were included in the UNCW-formulated diets D1 and D2 and may not have provided the balance of amino acids needed for optimal growth of post-metamorphic stage black sea bass.

The results of this study indicated that starter diets for black sea bass early juveniles may be formulated with high levels of FM replaced (50%) by the terrestrial protein poultry by-product meal. A blend of different protein sources such as fish meal, squid meal, krill meal, low-ash poultry meal, and soy protein concentrate supplemented with dietary crystalline L-lysine and DL-methionine used in D2 was of comparable nutritional quality to the high FM-based D1 in terms of growth performance and survival. Dawson et al. (2018) reported that up to 90% FM protein could be replaced by PBM protein without affecting the growth performance and survival of advanced juvenile black sea bass (grown from 1.2 to 17.8 g). The present findings demonstrate that early juvenile stage fish (grown from 0.6 to 5.7 g) are also able to utilize high levels of PBM protein without negative effects on survival and growth. PBM, therefore, appears to be a very beneficial ingredient to use in black sea bass juvenile diets. The commercial diets CD3 and CD4 notably lacked poultry meal. In addition, the apparent digestibility of protein and lipid in diets combining FM protein and PBM protein-based diets is high for black sea bass was high (Dawson et al., 2018). The results of the present study also suggest that black sea bass were able to utilize the PBM-based diet D2 as efficiently as the FM-based diet D1.

4.4 | Nutritional quality (fatty acids)

The dietary importance of n3-PUFAs for marine fish larvae and early juveniles has been widely studied and reviewed (Sargent, Tocher, & Bell, 2002). The analyzed values for n3-PUFAs in UNCW diets D1 (FM-based) and D2 (PBM-based) showed 26.57 and 18.81% total fatty acids, respectively. The fatty acid composition of the commercial diets (CD3 and CD4) were not analyzed in the present study; however, based on published information (Hauville et al., 2014), the n3-PUFA levels in diets CD3 and CD4 were 25.73 and 17.66% total fatty acids, respectively (Table 5), values very similar to the UNCW-formulated diets D1 and D2.

Previously, it has been reported that marine fish larvae and post-larvae may utilize phospholipids more efficiently than triglycerides (Shields et al., 1999; Teshima et al., 2000) and dietary supplementation of phospholipid-rich ingredients such as soybean lecithin has been recommended for weaning feeds (Zambonino Infante et al., 2008). Hence, the excellent survival, growth performance, and feed utilization of early black sea bass juveniles fed the UNCW-formulated diets D1 and D2 compared with commercial diets CD3 and CD4 could also be attributed to the supplementation of soy lecithin as a phospholipid source in addition to the variety of marine and terrestrial protein and lipid (fish oil) sources used in these UNCW-formulated diets.

4.5 | Fish whole-body proximate composition

The percent CP content of commercial diet CD3 (48%) was lower compared with the other test diets (54–57%); however, the whole-body protein content of fish at the end of the feeding trial was not different among treatment groups (15.2–15.9%). The lack of significant differences in whole-body protein content of juvenile black sea bass fed D1 (replacing 0% fish meal with poultry meal) and D2 (replacing 50% fish meal with poultry meal) suggests that assimilation and digestion of PBM protein by these fish was comparable to FM protein. Similar results were previously reported for advanced juveniles of the black sea bass (Dawson et al., 2018). Whole-body protein did differ when greater amberjack, *Seriola dumerili* were fed diets replacing 20–60% of FM protein with PBM protein with or without supplemental amino acids (Takakuwa, Fukada, Hosokawa, & Masumoto, 2006).

Whole-body lipid content in fish fed UNCW-formulated diet D1 (FM-based) was not significantly different from D2 (FM + PBM-based), indicating that replacement of FM by PBM did not affect lipid utilization and whole-body lipid content. This is an agreement with a previous study in juvenile black sea bass in which whole-body lipid content was not significantly affected by the replacement of FM protein by PBM protein up to a level of 100% (Dawson et al., 2018). Similar findings have also been reported in other marine finfish, including greater amberjack (Takakuwa et al., 2006) and cobia (Zhou, Zhao, Li, Wang, & Wang, 2011) fed diets with up to 60% PBM protein and humpback grouper (Shapawi, Ng, & Mustafa, 2007) fed diets with up to 100% PBM protein in which whole-body lipid levels remained similar to fish fed control fish meal-based diets. On the other hand, the lower whole-body lipid in fish fed commercial diet CD3 could be because of lack of utilization/retention of lipids from the CD3 compared with the UNCW-formulated diet D2 (FM + PBM). This further suggests that dietary lipid as well as protein in CD3 may have been inadequate, causing some protein to be used for energy rather than for growth (i.e., lack of protein sparing) and thereby causing relatively high FCR and lower growth performance in fish fed CD3. Using commercial microdiet CD3 as a weaning diet prior to the experiment could have also contributed to lower growth performance and body fat composition in fish fed CD3.

4.6 | Fish whole-body fatty acid composition

Generally, fish whole-body fatty acid compositions reflected their dietary fatty acid profiles (Alam et al., 2012; Bell et al., 2002; Dawson et al., 2018). Fish fed UNCW-formulated D1 (FM-based) and D2 (FM + PBM-based) had higher

whole-body n-6 PUFA content compared with those fed CD3 and CD4. These differences were attributed to the higher levels of n–6 PUFA in D1and D2, especially linoleic acid (18:2 n-6) sourced from fish oil, squid meal, krill meal, and fish meal. High whole-body n-6 PUFA levels have been reported in other marine fish species fed diets with high levels of combined protein sources (De Francesco et al., 2007; Ribeiro et al., 2015; Valente et al., 2011).

The whole-body total n3 PUFA content (mg/g dw) was significantly higher in fish fed commercial diet CD4 (55.15) compared with fish fed the other diets (39.1–49.12) (Table 6). The importance of higher n-3 PUFAs (i.e., DHA and EPA) in whole bodies of fish fed CD4 is unclear because this was not associated with higher growth performance compared with fish fed the UNCW-formulated diets D1 and D2. Fish fed the commercial diet CD4 appeared to conserve EPA, which was available in relatively low concentrations compared with the other diets, possibly because of the lack of other potential sources of n-3 PUFAs (e.g., krill meal, squid meal) in CD4 that were available in all of the other diets.

5 | CONCLUSION

All diets tested in this study, including the UNCW-formulated diets D1 (containing high FM protein) and D2 (replacing 50% FM protein with PBM protein) and the premium commercial diets CD3 (Otohime) and CD4 (Gemma Diamond), produced excellent survival and rapid growth of post-metamorphic stage black sea bass. However, growth was higher on the UNCW-formulated diets D1 and D2 than on the commercial diets CD3 and CD4, and these differences were primarily related to greater feed intake of the UNCW-formulated D1 and D2. The higher palatability, feed consumption, and growth of fish fed the UNCW-formulated diets may be attributed to a more optimal combination of marine (fish, squid, and krill meals), terrestrial plant (soybean meal) protein sources in the UNCW-formulated diets and the addition of chemo-attractants such as taurine and crystalline amino acids, which provided higher nutritional quality as well as attractability of these diets. Replacement of fish meal protein with up to 50% PBM protein in the UNCW-formulated diets did not affect fish performance. The study demonstrated that the nutritionally balanced starter diets formulated to species-specific requirements improves the growth performance of post-metamorphic stage black sea bass compared with the available commercial starter diets. These findings for black sea bass could also assist commercial feed manufacturers to develop improved starter diet formulations for other marine finfish species.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Md Shah Alam: Methodology; resources; supervision; investigation; formal analysis; data curation; writing – original draft; funding acquisition (co-principal investigator). **Wade O. Watanabe**: Methodology; resources; supervision; writing – review and editing; project management and administration; funding acquisition (principal investigator). **Patrick M. Carroll**: Resources; investigation; supervision; funding acquisition (co-principal investigator). **Jennifer E. Gabel**: Biochemical and data analysis; assistance on feeding trial.

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