Latin American Journal of Aquatic Research, 54(2): xxx-xxx, 2026
DOI: 10.3856/vol54-issue2-fulltext-3434

Research Article

Feed particle size preference in white shrimp *Penaeus vannamei* larvae and early postlarvae

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ABSTRACT. Shrimp larval feed success depends not only on nutritional profile but also on its physical characteristics, and one of these properties is particle size. However, no clear criteria exist for selecting the appropriate size for each larval stage. Feed particle size significantly impacts shrimp growth performance and feed production costs. Therefore, the present study evaluates the effect of particle size on feed consumption in *Penaeus vannamei* larvae and early postlarvae. The extruded diet was crumbled and sieved into the following size ranges: <53, 53-106, 106-150, and 150-250 µm, and fed to larvae and early postlarvae for 30 min. Organismal feed particle size preference was determined using the feeding efficiency (FE) index (FE = feeding incidence × gut fullness). Feeding incidence values indicate that larvae from Protozoea III to Postlarvae 6 had the capacity to identify, capture, and ingest feed particles from <53 to 250 µm - no significant effect was detected by particle size. In contrast, particle size had a significant effect ($P < 0.05$) on feed intake, as indicated by gut fullness values. Larval stages had a greater ingestion capacity of particles smaller than 150 µm, while early postlarval stages showed increased gut fullness when fed on particles larger than 150 µm. In general, based on the FE results, feed particle sizes smaller than 150 µm are recommended for larval stages, and 150-250 µm for early postlarvae.

Keywords: *Penaeus vannamei;* shrimp; larvae feeding; microparticle; feed size; feed consumption; gut fullness

INTRODUCTION

In Mexico, total postlarvae (PL) for stocking grow-out shrimp ponds are supplied by hatcheries that rear larvae under controlled conditions. The high dependence of shrimp larvae on PL culture from commercial hatcheries represents a critical point in shrimp production systems (Gamboa-Delgado & Le Vay 2009). Hatcheries are responsible for producing and supplying high-quality PL, contributing significantly to subsequent performance during farm grow-out (Rowel et al. 2024). PL quality tests try to represent the organism's physiological condition (Racotta et al. 2003). To achieve the highest PL quality, optimal environmental conditions and management practices are needed during larviculture. Additionally, balanced feeds should provide all the nutrients shrimp require from protozoea (PZ) to PL stages, and adequate nutrition is a key factor in PL production, especially in intensive production systems. In fact, aside from administrative expenses, feed is the largest variable cost (Naegel 2010). The main nutritional sources still used in commercial shrimp

Associate Editor: Ingo Wehrtmann

hatcheries are live feeds. Traditional feeding techniques use microalgae during the PZ stage and newly hatched *Artemia* nauplii during later stages as main nutrient sources. However, there has been a rapid increase in the use of artificial feeds as partial replacements for live feed in co-feeding protocols (Wouters 2008).

Although artificial feeds are accepted by shrimp larvae and their nutritional composition is adequate, these feeds are still used mainly as partial *Artemia* nauplii replacement (Velu & Munuswamy 2008, Gamboa-Delgado & Le Vay 2009, O'Brien 2023), and recently, liquid micro-encapsulated diets have demonstrated success in totally replacing *Artemia* nauplii on larval and PL stages (Rowel et al. 2025). Shrimp larval feed success depends not only on their nutritional profile but also on their physical characteristics, which are very important. One of these physical properties is particle size, which has been considered in several studies (Teshima & Kanazawa 1983, De la Cruz 1989, Gelabert & Pacheco 2011, Castro et al. 2024). However, no clear criteria have been used to select the proper size for each larval stage. Some commercial shrimp larval feeds are available, and each company recommends different particle size ranges, starting at 5 µm and up to 500-800 µm, as a function of the larval stage (Table 1).

Feed particle size (FPS) has a significant impact on shrimp performance and feed production cost. Feed manufacturing process efficiency for microparticles depends on the initial grinding of the ingredients. In fact, pelleting or extrusion operations impose maximum limits on the largest particle that can be allowed by the grinding step. For good results, particle-size ingredients should be reduced by one-third of the final FPS (Sorensen & Phillips 1992), as this is the most time-consuming feed-processing step and can account for up to 60% of feed production costs. Thus, given that smaller particle sizes are more costly (Sorensen & Phillips 1992), the largest particle size that larvae can manipulate and ingest is important for meeting their feeding and nutritional requirements at different developmental stages. Therefore, the present study was conducted to evaluate the effect of FPS on feed consumption in *P. vannamei* larvae and early PL under controlled culture conditions.

MATERIALS AND METHODS

A nutritional trial was conducted at the Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR, La Paz, Baja California Sur, Mexico). *P. vannamei* larvae were obtained from a commercial hatchery (Acuacultura Mahr, La Paz, B.C.S., Mexico) at the nauplii IV stage and then stocked in a 500-L plastic tank at a density of 120 nauplii L^{-1}. When larvae reached the postlarval 1 (PL1) stage, density decreased to 30 PL1 L^{-1}. The tank was equipped with a 300-W submersible heater, an air stone, filtered seawater through a sand filter, cartridge filters (10, 5, and 1 µm), and ultraviolet (UV) light. Temperature, dissolved oxygen, and salinity were recorded once daily (28 ± 0.5°C, 6 ± 0.5 mg L^{-1}, and 37 ± 0.1, respectively). Larvae were cultured until they reached the PL6 stage, and a sample was taken from each stage from PZIII onwards. Feeding was based on *Chaetoceros calcitrans* (PZI-PZIII 100,000 cells mL^{-1}; PZIII-PL1 120,000 cells mL^{-1}) diatoms and freshly hatched *Artemia* nauplii (PZIII 0.05 nauplii mL^{-1}; mysis (M) I-II 1 nauplii mL^{-1}; MIII-PL6 1.5 nauplii mL^{-1}), twice a day.

A microparticulate diet was formulated to contain 50% crude protein and 12% lipids (Table 2). Before preparing the experimental diet, all dry ingredients were finely ground using an ultra-centrifugal mill (Type ZM 200, Retsch GmbH, Haan, Germany) and sieved through a 36-µm-screen. Then they were mixed thoroughly in a food processor before being added to an oil mixture (fish oil, sunflower oil, and soy lecithin). After the oil mix was dispersed, water was added (approximately 40% of the total "as-is" ingredient weight), and the mixture was finally mixed. The resulting mixture was pressure-extruded through a 2-mm-diameter hole die in a meat grinder, as described by Civera & Guillaume (1989). The pellets obtained were dried in a forced-air oven at 40°C for 12 h, crumbled using an electric coffee grinder, and sieved to the desired particle size ranges: <53 µm (<270 mesh), 53-106 µm (140-270 mesh), 106-150 µm (100-140 mesh), and 150-250 µm (60-100 mesh).

The experiment started when larvae reached the PZIII stage. At each stage (MI-MIII, PL1-PL6), volumetric samples of 30 larvae or 20 PL were gently rinsed with seawater on a 360-µm-mesh sieve to remove microalgae and *Artemia* nauplii. Then, the samples were transferred to two experimental units (2-L transparent plastic containers with smooth walls and flat bottoms) per treatment and allowed to go without food for 2 h before they were fed (100 mg per container) with the different particle sizes. To maintain the temperature, the flasks were placed in a water bath at 28°C, with submersible heaters, and each flask was aerated with air stones. After 30 min of feeding, the organisms from the two containers were rinsed through a 360-µm-mesh sieve to remove uneaten feed and feces, then fixed in formalin solution (4% in seawater) and stored at -2°C. Using the transparency of the organisms, they were observed using a stereoscopic microscope with a digital camera (National Model DC3-420TH, National Optical & Scientific Instrument Inc., Texas,

Table 1. Feed particle sizes (µm) recommended for larval and postlarval shrimp stages by some commercial feed brands. PZ: protozoea, M: mysis, PL: postlarvae.

Stage	BERNAQUA	GOLDEN PEARLS	ZEIGLER	FRiPPACK	CP
PZI	5 - 50	5 - 50	< 50	5 - 30	< 80
PZII	5 - 50	5 - 50	< 50	5 - 30	< 80
PZII/PZIII	50 - 100	5 - 50	< 50	5 - 30	< 80
PZIII	50 - 100	5 - 50 + 50 - 100	< 100	5 - 30	< 80
PZIII/MI	50 - 100	50 - 100	< 100	5 - 30	< 80
MI	50 - 100	50 - 100	< 100	30 - 90	< 80 - 200
MII	50 - 100	50 - 100 + 100 - 200	< 100 + 100 - 150	30 - 90	< 80 - 200
MIII	100 - 200	100 - 200	< 100 + 100 - 150	30 - 90	< 80 - 200
MIII/PL1	100 - 200	100 - 200	< 100 + 100 - 150	30 - 90	< 80 - 200
PL1	100 - 200	100 - 200	100 - 150 + 150 - 250	90 - 200	< 200 - 400
PL2	100 - 200	100 - 200 + 200 - 300	100 - 150 + 150 - 250	90 - 200	< 200 - 400
PL3	100 - 200	200 - 300	100 - 150 + 150 - 250	90 - 200	< 200 - 400
PL4	100 - 200	200 - 300	150 - 250 + 250 - 450	90 - 200	< 200 - 400
PL5	100 - 200	200 - 300	150 - 250 + 250 - 450	200 - 400	< 200 - 400
PL6	200 - 300	300 - 500 + 500 - 800	150 - 250 + 250 - 450	200 - 400	< 200 - 400

USA) and an image analyzer computer program (Motic Image Plus 2.0 ML, Motic China Group Co., LTD). FPS preference was determined using the following formulae:

Feeding efficiency (FE) = (feeding incidence × gut fullness) / 100

where feeding incidence (FInc) = number of larvae or PL with feed particles in the foregut/total number of organisms × 100, and gut fullness (GF) is the foregut length (mm) containing feed particles/foregut total length (mm) × 100.

Data are presented as mean ± standard error (SE). All data were evaluated for normality (Lilliefors test) and homogeneity of variances (Bartlett test) prior to analyses. Statistical significance of data was determined by one-way analysis of variance (ANOVA) and Least Significant Difference (Fisher LSD) for parametric data, or by the Kruskal-Wallis test and Multiple comparisons (two-tailed *P*-values) for nonparametric data, with a 95% confidence interval (Sokal 1995, Ott 1992). All computations were performed using the software package Statistica 6.0 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

A total of 2,133 organisms were analyzed and measured, of which 943 were larvae and 1,190 PL (Table 3). On average, 89.5% of the organisms presented fed in the digestive tract (FInc) after 30 min, with no significant differences among development stages, whereas GF and FE were significantly affected by development stage. Both criteria, GF and FE, showed a decreasing trend with the development stage. FPS had no significant effect ($P > 0.05$) on FInc values (Fig. 1).

In the case of smaller size feed (< 53 µm), a tendency to diminish FInc values is observed as the organisms grow; for larval stages PZIII to MIII, lower FInc values were observed with higher FPS (150-250 µm) and larvae tended to consume smaller FPS, while in PL (from PL1 to PL6) lower Finc values were observed with FPS smaller than 53 µm. GF values were affected by the FPS ($P < 0.05$) (Fig. 2).

In larvae, higher GF values were presented in feed with FPS smaller than 106 µm, while for PL, the highest rates occurred with FPS greater than 106 µm. The lowest GF rates for larval stages were observed with FPS 150 to 250 µm, and in the case of PL, with FPS smaller than 53 µm.

Higher FE values were observed in PZIII fed FPS smaller than 150 µm (Fig. 3). Larvae had higher FE values when they consumed feed with particle sizes greater than 53 µm and smaller than 150 µm, while PL showed higher FE when consuming feed with particle sizes greater than 106 µm, and lower values when they consumed feed smaller than 53 µm.

DISCUSSION

The success of a balanced feed used in the production of shrimp PL not only relies on its nutritional content, which is of vital importance, but also on other physical characteristics that must also be met (de Lima

Table 2. Experimental diet ingredients, proximate composition, and gross energy content. [1]Arancia Ingredientes Especiales, Monterrey, NL. Mexico. [2]*Pleuroncodes planipes*, prepared in our laboratory. [3]Proteínas Marinas y Agropecuarias, S.A. de C.V., Guadalajara, Jal., México. [4]Tron Hermanos, S.A de C.V., Morelia, Mich. México [5]ODONAJI Distribuidora de Alimentos Naturales y Nutricionales, S.A. de C.V., D.F., México. [6]Sigma S4126 St. Louis, MO, USA. [7]Farmacia Paris, S.A. de C.V., D.F. México. [8]Mineral premix (g kg^{-1} diet): KCl, 0.5; $MgSO_4.4H_2O$, 0.5; $ZnSO_4.7H_2O$, 0.09; $MnCl_2.4H_2O$, 0.0234; $CuCl_2.2H_2O$, 0.005; KI, 0.05; $CoCl_2.6H_2O$, 0.0025; Na_2HPO_4, 2.37. St. Louis, MO, USA. [9]Sigma C8503 St. Louis, MO, USA. [10]Vitamin premix (mg or IU kg^{-1} diet): A acetate, 15,000 IU; D_3, 7,500 IU; E, 400; K_3, 20; choline cloride (99%) 400 mg; tiamine HCl, 150; riboflavine, 100; piridoxine HCl, 50; pantothenic acid, 100; niacine, 300; biotin, 1; inositol, 500; folic acid, 20; cyanocobalamine, 0.1. ICN Biomedical Inc, Aurora, Ohio, USA. [11]Stay-C (L-ascobil-2-poliphosphate 35% activity), Roche Vitamins Inc., Parsippany, NJ, USA [12]Butylated hydroxytoluene (antioxidant), ICN 101162, ICN Biomedicals, Aurora, OH, USA.

Ingredients	g 100 g^{-1} feed
Wheat gluten[1]	35.00
Red crab meal[2]	25.00
Soybean paste[3]	21.59
Sunflower oil[4]	4.33
Soybean lecithin[5]	3.00
Corn starch[6]	6.00
Cod liver oil[7]	2.72
Mineral mix[8]	1.50
Cholesterol[9]	0.50
Vitamin mix[10]	0.26
Stay C[11]	0.09
BHT[12]	0.004
Proximate composition (g 100 g^{-1} dry matter, except moisture)	
Moisture	3.92
Protein	48.9
Ether extract	12.0
Crude fiber	4.4
Ash	11.3
Nitrogen Free Extract	17.4
Gross energy (Kcal g^{-1})	4.53

Table 3. Body length, feed incidence, gut fullness, and feed efficiency (means ± standard error) by development stage without regard to feed particle sizes. Different superscripts on values within each row indicate significant differences ($P < 0.05$).

Development stage	Body length (mm)	Feed incidence (%)	Gut fullness (%)	Feed efficiency (%)
Protozoea III	2.35 ± 0.10	93.4^{a} ± 2.14	69.9^{a} ± 1.71	65.8^{a} ± 1.65
Mysis I	2.79 ± 0.12	93.4^{a} ± 2.36	59.1^{b} ± 1.52	55.5^{b} ± 1.48
Mysis II	3.18 ± 0.14	87.7^{a} ± 2.37	60.9^{b} ± 1.68	54.0^{b} ± 1.56
Mysis III	3.46 ± 0.14	93.4^{a} ± 1.83	57.9^{b} ± 1.57	54.3^{b} ± 1.50
Postlarvae 1	4.49 ± 0.19	89.9^{a} ± 3.19	49.0^{c} ± 1.79	44.7^{c} ± 1.73
Postlarvae 2	4.84 ± 0.22	87.5^{a} ± 3.63	37.1^{e} ± 1.96	32.9^{e} ± 1.80
Postlarvae 3	5.30 ± 0.28	92.6^{a} ± 1.78	48.7^{c} ± 1.43	45.7^{c} ± 1.39
Postlarvae 4	5.39 ± 0.19	88.6^{a} ± 3.16	43.8^{d} ± 1.39	39.1^{d} ± 1.26
Postlarvae 5	5.68 ± 0.36	93.4^{a} ± 2.17	42.0^{d} ± 2.03	39.5^{d} ± 1.96
Postlarvae 6	5.99 ± 0.34	75.2^{a} ± 2.94	34.1^{e} ± 1.74	26.7^{f} ± 1.45
Average		89.5 ± 2.56	51.9 ± 0.58	47.5 ± 0.55

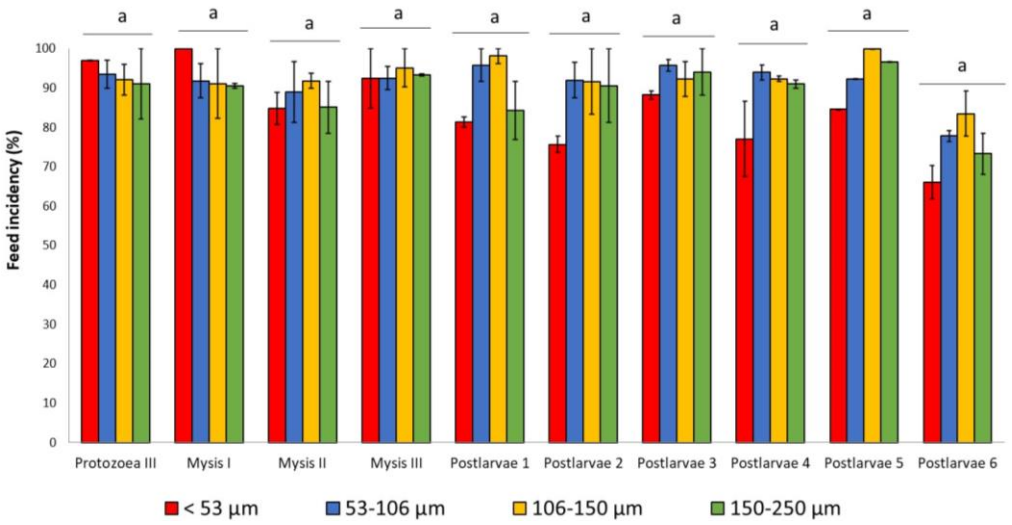

Figure 1. Feed incidence by development stage using different feed particle sizes (mean ± standard error). Different superscripts on bars within each development stage indicate differences among treatments ($P < 0.05$).

& Souza-Santos 2007, Gelabert & Pacheco 2011); one of the physical characteristics to be met is particle size feed (Baron 2024). The FInc values obtained in the present study show that in the worst case (PL6 with particle size less than 53 µm) 7 out of 10 organisms presented food in their digestive tract, indicating that both, larvae and PL, have the capacity to identify, capture, and ingest feed particles independently of feed size (from less than 53 to 250 µm), since no differences among treatments were detected ($P < 0.05$). This capacity is not surprising since shrimp larvae -after metamorphosis to PZ- already present functional feeding appendages (maxilla, maxillula, and maxillipeds) located in the cephalic region, which allow them to catch and manipulate prey (McVey & Fox 1983, Lovett & Felder 1989, de Lima & Souza-Santos 2007). Although larvae and PL can capture feed regardless of its size, the GF values obtained show that the amount of feed ingested is affected by particle size, with sizes smaller than 150 µm being the most consumed by larval stages, and larger than 150 µm being the most consumed by PL. Possibly, this behavior is a function of the development/maturity of the masticatory dentition degree of the mandibles.

Some authors have reported that FPS should be specific to each developmental stage, which can vary by species (De la Cruz 1989, Jones 1998, Pereira de Barros & Cotroni 2003, Gelabert & Pacheco 2011, Baron 2024). Gelabert & Pacheco (2011) report that for *P. vannamei* larvae (PZI to PL1), the optimum feed size is 14.42 µm (range from 5.71 to 20.33 µm), and Gelabert & Brito (2013) for *Farfantepenaeus duorarum* report that the optimum sizes of the feed ingested from PZI to PL1 were between 6.17 and 12.02 µm, and the preferred sizes were from 2.68 to 18.65 µm. While commercial producers of shrimp larval feeds suggest, for stages evaluated in the present study (PZIII to PL6), the use of particle sizes from 5 to 200 µm for the larval period, while for the PL period, from 100 to 800 µm (Table 1).

Knowing the FPS at which organisms reach their maximum ingestion rate is important not only from a nutritional point of view but also for feed handling and manufacturing. Adequate FPS must allow maximum intake (Genodepa et al. 2004) and ensure that organisms receive a more complete "nutritional package" that satisfies their requirements with lower feeding effort (Waycott 2021, Spreitzenbarth & Jeffs 2024). Larger feed sizes imply a greater number of nutrients per particle trapped in the organisms, and lower energy expenditure to obtain the required nutrients. Adequate nutrition is essential for the proper development of organisms and should be reflected in production yields (Baron 2024).

Additionally, a larger particle size not only reduces nutrient leaching but also lowers feed production costs. The reason is that the grinding or size-reduction process is the costliest operation in aquafeed processing plants (Obaldo et al. 1998, Admasu & Wakjira 2021) and is a determining factor in the physical and nutritional characteristics of the final feed (Lyu et al. 2022). Obaldo et al. (1998) reported an exponential increase in energy consumption as the particle size of ingredients

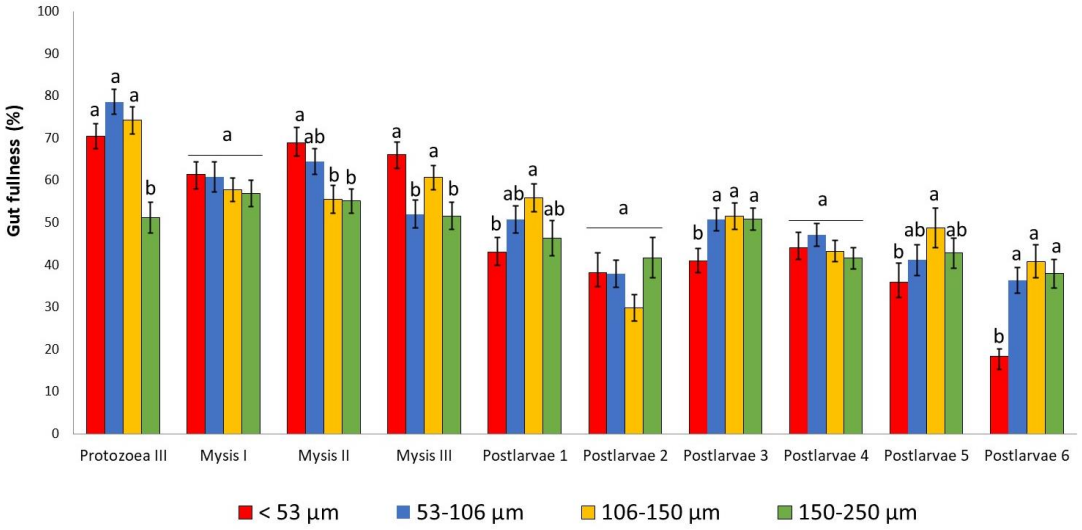

Figure 2. Gut fullness by development stage using different feed particle sizes (mean ± standard error). Different superscripts above bars within each development stage indicate differences among treatments ($P < 0.05$).

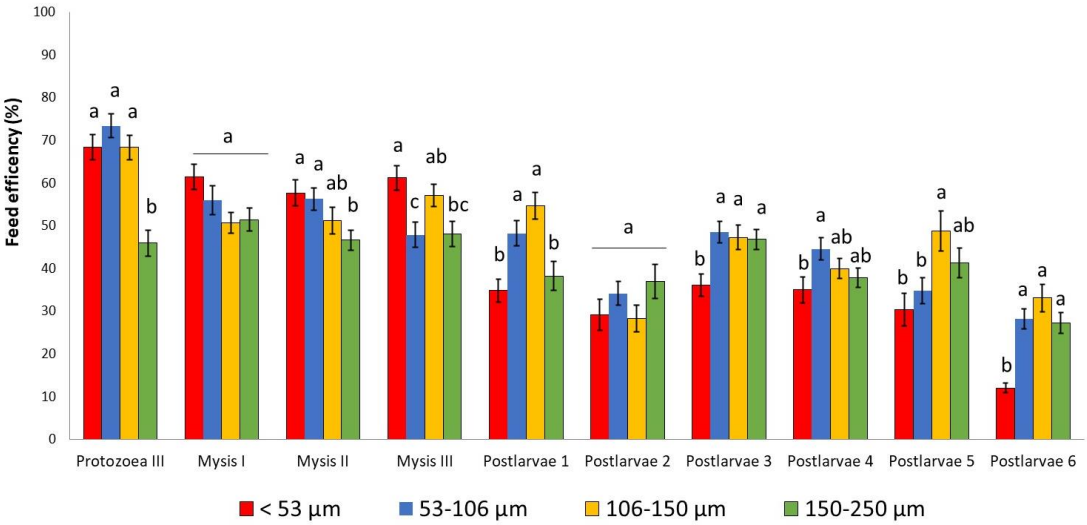

Figure 3. Feed efficiency by development stage using different feed particle sizes (mean ± standard error). Different superscripts on bars within each development stage indicate differences among treatments ($P < 0.05$).

was reduced during milling. Bortone & Kipfer (2016) commented that feed producers "use a rule of thumb" stating that the largest particle should be no larger than one-third of the die size". In addition, the larger the particle size, the greater the variety of ingredients present per particle; that is, theoretically, a 250 µm feed can "fit" 5 ingredients of 50 µm each. Nevertheless, the results in the present study demonstrate that particles from 150-250 µm do not produce good feed efficiencies in PZIII to MIII stages. Hence, particle size must be adapted to the developmental stage.

CONCLUSION

PZIII to PL6 had the capacity to ingest feed particles from <53 to 250 µm without a significant effect by particle size. Larval stages had a greater ingestion capacity of particles smaller than 150 µm, while early PL stages showed increased GF when fed on particles larger than 150 µm. Considering FE, FPS smaller than 150 µm are recommended for larval stages, and from 150 to 250 µm for early PL (up to PL6).

Credit author contribution

E. Goytortúa-Bores: conceptualization, validation, writing-original-draft, funding acquisition, project administration, methodology, formal analisis, investigation, supervision, writing-review and editing; R. Civera-Cerecedo: conceptualization, validation and writing-original-draft, funding acquisition, project administration, methodology, formal analisis, investigation, supervision, writing-review and editing; M.A. Cadena-Roa: conceptualization, validation and writing-original-draft and formal analisis; J.L. Ramírez-Arce: methodology and formal analisis; G. González-Gómez: methodology and formal analisis. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ACKNOWLEDGMENTS

All authors are grateful to Gustavo Pineda Mahr, Carlos A. Pineda Mahr, and Jesús Peiro López for their support and for allowing the use of the facilities of Acuacultura Mahr, S.A. de C.V., and to Diana Fischer for the English edition. The present study was conducted with the support of the SEP-CONACYT 79047 project and the CONACYT scholarship 213102 granted to the first author.

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Received: May 9, 2025; Accepted: February 10, 2026

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