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Original Article

Utilization of thraustochytrid, *Schizochytrium* sp. as live food enrichment enhances growth performance, digestive enzyme activities, and stress tolerance of silver pompano (*Trachinotus blochii*) larvae

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Abstract: One of the primary challenges in larval fish production lies in ensuring the nutritional quality of live feed. Most marine fish hatcheries commonly rely on rotifers and *Artemia* as live prey for the larvae. However, these live food sources often lack essential fatty acids crucial for normal growth. An encouraging solution to this nutritional deficiency is the utilization of thraustochytrids. This group of microorganisms exhibits a remarkable capacity to accumulate substantial amounts of omega-3 fatty acids, particularly DHA (docosahexaenoic acid). Thus, the present study explores the potential of utilizing *Schizochytrium* sp. as an enrichment source for *Artemia* and its effect on the development of Pompano (*Trachinotus blochii*) larvae. This study compares the larval performance of pompano-fed three different diets: *Schizochytrium*-enriched *Artemia, Artemia* enriched with commercial enrichment, and unenriched *Artemia* as a control group. Several key parameters were evaluated, including growth, survival rates, fatty acid levels, digestive enzyme activities, and the larvae's response to stress. The results revealed significantly higher survival rates and increased stress resistance in larvae that were fed *Artemia* enriched with *Schizochytrium* sp. and commercial enrichment compared to those fed unenriched live feed. The larvae fed with *Schizochytrium*-enriched *Artemia* exhibited the highest levels of DHA, body weight, and body length in comparison to the other treatments. Additionally, the enrichment with *Schizochytrium* demonstrated the capacity to enhance the digestive enzyme activities of the larvae, potentially leading to improved larval digestion and, consequently, enhanced growth, survival, and stress resilience. This study highlights the promising potential of *Schizochytrium* sp. as an effective enrichment source for *Artemia*, leading to remarkable improvements in the performance and nutritional quality of pompano larvae.

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Introduction

Thraustochytrids, are heterotrophic marine protists within the kingdom Stramenipila bearing striking resemblances to diatoms and brown algae (Cavalier-Smith et al., 1994; Partfrey et al., 2006). These microorganisms are prevalent in marine and estuarine environments (Barr, 1992) and thrive abundantly in decomposing plant materials, such as mangrove leaves (Leaño, 2001), and algae (Miller and Jones, 1983). In the detrital food web, thraustochytrids play a crucial role as decomposers and actively contribute to nutrient recycling (Ulken, 1981). Their decomposing capabilities extend to the production of a diverse range of hydrolytic enzymes that efficiently break down organic components within leaf materials into substances readily accessible to detritus-feeding organisms (Raghukumar et al., 1994). Notably, thraustochytrids possess a remarkable ability to synthesize substantial quantities of polyunsaturated fatty acids (PUFAs), including the valuable omega-3 fatty acid, such as DHA, thereby enriching the nutrient composition of detrital organic matter (Leaño et al., 2003; Leaño and Liao, 2004; Raghukumar, 2008).

PUFAs hold an important role in numerous physiological processes within organisms, serving not only as essential cellular fuel sources but also as integral components of cell membranes (Stillwell and Wassal, 2003). Furthermore, PUFAs are key players in the regulation of gene expression for enzymes involved in lipid and carbohydrate metabolism

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(Sessler and Ntambi, 1998), making them essential for animal nutrition. Extensive research underscores the significance of PUFAs, particularly the omega-3 fatty acid docosahexaenoic acid (DHA), in the development of marine fish larvae. Elevated dietary levels of DHA have been shown to enhance metamorphosis, improve growth rates, increase survival rates, enhance pigmentation, and bolster stress tolerance in fish larvae (Ostrowski and Divakaran, 1990; Watanabe, 1993; Ozkizilcik and Chu, 1994; Dhert et al., 1990; Hamre et al., 2005). However, fish possess limited capabilities to synthesize omega-3 fatty acids, primarily due to low enzyme activity or the absence of enzymes responsible for fatty acid chain elongation and desaturation (Sargent et al., 1993; Ganuza et al., 2008; Nowosad et al., 2017). Consequently, fish must obtain omega-3 fatty acids from their diets.

The quality of live feed presents a significant bottleneck in fish larvae production. Commonly used live prey in marine fish hatcheries are rotifers and *Artemia*, however, these microorganisms lack sufficient levels of omega-3 fatty acids necessary for the optimal growth and development of fish at the early stages (Watanabe et al., 1978). Enriching live feed with omega-3 fatty acids has been demonstrated to enhance its dietary value (Lewis et al., 1998; Estudillo-del Castillo et al., 2009). Thraustochytrids, including species like *Schizochytrium*, have emerged as promising sources of essential fatty acids for live food enrichment in fish larval culture (Lewis et al., 1998; Estudillo-del Castillo et al., 2009). Research has shown that *Schizochytrium*, efficiently produces omega-3 fatty acids (Yaguchi et al., 1997) and can significantly enhance the nutritional profile of rotifers and *Artemia* (Estudillo-del Castillo et al., 2009). In light of these findings, the present study aims to explore the potential application of a locally isolated *Schizochytrium* sp. in the larval culture of snubnose pompano (*Trachinotus blochii*), an economically significant aquaculture species in the Asia-Pacific region. Furthermore, the study evaluates the impact of a *Schizochytrium* sp.-enriched diet on the growth, survival, digestive enzyme activities, and osmotic-stress tolerance of pompano larvae.

Materials and Methods

Thraustochytrid culture: The thraustochytrid, *Schizochytrium* sp. used in this study was locally isolated from fallen senescent leaves of mangrove, *Avecinia rumphiana* in Baybay, Leyte, Philippines. The culture was maintained in the Larval Food Laboratory of the Aquaculture Department of Southeast Asian Fisheries Development Centre, Aquaculture Department (SEAFDEC/AQD), Tigbauan, Iloilo, Philippines. The mass production process for *Schizochytrium* sp. used by de la Pena et al. (2016) was followed. *Schizochytrium* sp. cultures were grown and maintained in solid glucose yeast extract peptone seawater (GYPS) medium containing 10 g glucose, 1 g peptone, 1 g yeast extract, and 15 g agar in 1 L of 25 g L^{-1} natural seawater (NSW) adjusted to pH = 6.0 using lactic acid. Then after 3 days of culture, the cells on the agar medium were scraped and inoculated to a 250 mL flask with 50 mL culture medium under 200 rpm shaking condition at 25^{o}C. The flask cultures were maintained in axenic condition to prevent contamination. After 3 days of incubation, the cells were harvested through centrifugation, freeze-dried, and stored at -80^{o}C until use for enrichment.

Rearing conditions and feeding treatments: A 10-day old larvae pompano larvae were obtained from the Marine Fish Hatchery of the Southeast Asian Fisheries Development Centre/Aquaculture Department (SEAFDEC/AQD) (Tigbauan, Iloilo Philippines). Nine hundred ninety larvae were randomly transferred equally to 9 fiberglass cylindrical tanks (50 L). Rearing was done under a natural photoperiod. Every morning and before feeding, the tanks were cleaned by siphoning the fecal waste and excess feeds, then, 80% of the total water volume was changed. The ambient temperature and salinity in the rearing water were 29^{o}C and 32 g L^{-1}, respectively. The larvae were acclimated for 5 days before the experiment. During the acclimation period, the larvae were weaned to feed *Artemia* nauplii obtained at the Larval Food Laboratory of SEAFDEC/AQD. After the acclimation

period, 10 larvae were sampled from each tank for initial body measurements. The initial stocking density was 100 larvae/ tank. The feeding experiment included three dietary treatments, with three replicates each. *Artemia* nauplii (instar II) were enriched with (i) *Schizochytrium* sp., (ii) commercial enrichment (Selco), and (iii) *Artemia* nauplii without enrichment as control. The feeding density for the pompano larvae is 2 to 5 nuplii ml^{-1}. The live feed was given twice a day at 0800 h and 0400 h. The experiment was terminated after 20 days. Survival, growth performance, digestive enzyme activities, and tolerance to salinity stress of the larvae were assessed at the end of the experiment. Further, fatty acid analysis was also determined in both the feeds and the larvae.

Enrichment of *Artemia:* *Artemia* cysts (INVE Aquaculture, Thailand) were allowed to hatched in a 30-L conical tank at 29°C. The cyst were incubated at 30 g L^{-1} of seawater and decapsulation of *Artemia* cyst were carried out for 24 hours. The incubation tanks were provided with strong aeration and illumination. The newly hatched nauplii were collected and washed to remove empty shells. After 36 hours, *Artemia* instar II were inoculated into a tank containing 10 L of seawater at 100 individuals ml^{-1}. Enrichment with *Schizochytrium* sp. or commercial enrichment was carried out for 24 h under strong aeration at 29°C following the drip method of Ludevese-Pascual (2018). Enrichment products were given at 0.30 g L^{-1}.

Fatty acid analysis: The analysis of total lipid content in the samples involved a chloroform-methanol extraction method, which is a modified version of Bligh and Dyer (1959). For analysis of the fatty acid profile, fatty acid methyl esters (FAME) were prepared from the chloroform-methanol extracts through transesterification with boron trifluoride (BF3) methanol, following the AOAC (1996). These FAME compounds were then reconstituted using isooctane at a concentration of 50 mg FAME per mL of isooctane before injection into the Shimadzu gas chromatograph e17A. The cod liver oil (CLO) from Supelco, PA, USA, was used as a standard for comparison. The total lipid content in live prey and pompano larvae was determined by pooling samples and conducting two measurements per pool. Subsequently, the fatty acid profiling was performed on the same samples used for total lipid determination, where the corresponding lipid extracts were trans-esterified to FAME and reconstituted before injection.

Enzyme assay: At the end of the enrichment experiment, 15 larvae were taken from each treatment group. The mid-portion of the larvae which includes the digestive organs was used in the preparation of crude enzyme extract. Samples were weighed and stored in a bio-freezer at -80°C before the preparation of crude enzyme extracts. Samples (1 g) were homogenized in 25 ml of 50 mM Tris-HCl buffer, pH 7.5, centrifuged (12,500 × G, 30 min at 4°C), filtered through a Sephadex G-25 M column (1×10 cm.), centrifuged (2000 × G, 5 min at 4°C) and then decanted. The supernatant (crude enzyme extract) was used for different enzyme assays. The soluble protein of crude enzyme extracts was quantified following Bradford's method using bovine serum albumin as the standard. Enzyme assays were done on pooled samples with three determinations per pool.

The alpha-amylase activity was quantified using soluble starch as a substrate, defining one unit as the amount of enzyme capable of producing one μmole of reducing groups (calculated as maltose) per minute at 25°C, following the protocol outlined by Worthington Biochemical Corporation in 1993. Lipase activity was assessed using p-nitrophenyl laurate as the substrate, with one unit of activity corresponding to one μmole of acid produced per minute at 25°C under specified conditions, as described by Pinsirodom and Parkin (2001). Pepsin activity was determined using haemoglobin as a substrate, and one unit of pepsin activity expressed in tyrosine was equivalent to 0.001 of TCA soluble hydrolysis products per minute under standard conditions, as outlined in Worthington Biochemical Corporation's 1993 protocol. The activity of trypsin, chymotrypsin, and leucine aminopeptidase was quantified according to the methods also described by Worthington Biochemical Corporation in 1993. Specifically, trypsin activity corresponded to one μmole of N α-p-Tosyl-L-arginine

Table 1. Growth performance and survival pompano larvae fed with different enrichment diets.

	Control	*Schizochytrium*-enriched treatment	Selco-enriched treatment
Initial body weight (mg)	9.80±1.30	9.80±1.30	9.80 ±1.30
Initial body length (mm)	5.71±1.01	5.71±1.01	5.71±1.01
Final body weight (mg)	144.77±5.25[a]	201.98 +4.60[b]	143.57±4.98[a]
Final body length (mm)	15.28±0.41[a]	17.65±0.08[b]	15.67±0.26[a]
Specific growth rate (% day $^{-1}$)	13.46±0.04 [a]	15.13±0.03 [b]	13.42±0.04 [a]
Survival (%)	77.67±1.76[a]	92.33±1.20[b]	97.33±1.33[b]

Values are expressed as mean ± standard error of the mean (n = 3). Treatment means with the same superscript in the same row are not significantly different (P<0.05).

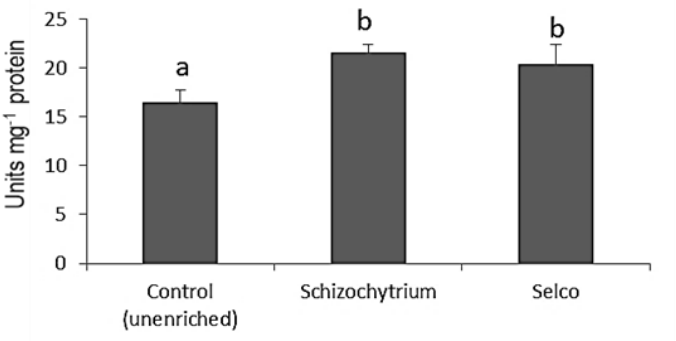

Figure 1. Lipase activity of pompano larvae fed different enriched diets. Values are expressed as mean + standard error of the mean (n = 3). Treatment means with the same superscript in the same row are not significantly different (P<0.05).

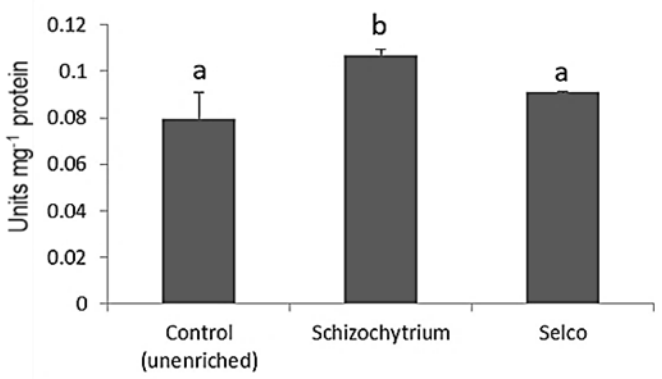

Figure 2. Pepsin activity of pompano larvae fed different enriched diets. Values are expressed as mean + standard error of the mean (n = 3). Treatment means with the same superscript in the same row are not significantly different (P<0.05).

Methyl Ester (TAME) hydrolyzed per minute at 25°C and pH 8.1, chymotrypsin activity to one micromole of N-Benzoyl-2-monophosphate-Na-Ca (BTEE) hydrolyzed per minute at 25°C and pH 7.8, while leucine aminopeptidase activity was defined as one µmole of leucinamide hydrolyzed per minute at 25°C and pH 8.5. The activities of acid and alkaline phosphatases were determined at pH 4.8 and pH 9.8, respectively, using nitrophenyl phosphate as the substrate, according to Bergmeyer (1974). The amount of 4-nitrophenol liberated per unit of time in an acidic solution served as a measure of acid phosphatase activity, while the amount of 4-nitrophenol liberated per unit of time in an alkaline solution provided a measure of alkaline phosphatase activity.

Osmotic stress tolerance: After 20 days of rearing, an osmotic stress test was conducted by exposing the larvae to very high saline conditions. Twenty larvae from each treatment tank were abruptly transferred to a 10 L container with a salinity of 65 g L^{-1}. Mortality was assessed at 15-minute intervals. Average cumulative mortalities of the different treatments at different time intervals were reported.

Statistical analysis: Data on growth parameters and digestive enzyme activities were subjected to statistical analysis using the software SPSS. The statistical significance of differences among treatments was determined using one-way ANOVA. Duncan's multiple range test was applied to detect significant differences.

Results

The data on growth are shown in Table 1. The body weight and body length of larvae fed *Schizochytrium*-enriched *Artemia* were significantly higher compared to other treatments. The lowest value was observed in the control treatment (without enrichment). The highest survival was observed in treatment fed with Selco-enriched *Artemia* (Table 1). However, the results did not significantly differ with treatment-fed *Schizochytrium*-enriched *Artemia*. Significantly lowest survival was recorded in the control group.

Based on the results of different digestive enzymes' activities of the larvae in response to dietary

Table 2. Fatty acid profile (weight percent value) of *Artemia* given with different enrichments.

Fatty Acids	Control (unenriched)	*Schizochytrium*	SELCO
Myristic, C14:0	*nd	nd*	0.82
Palmitic, C16:0	9.98	6.53	7.62
Palmitoleic, C16:1n-7	2.71	2.24	2.33
Stearic, C18:0	15.71	11.46	12.13
Oliec, C18:1n-9	25.93	24.90	24.82
Vaccenic, C18:1n-7	10.77	7.76	7.91
Linoleic, C18:2n-6	12.95	13.87	13.72
Linolenic, C18:3n-6	2.14	3.31	3.39
Linolenic, C18:3n-3	0.59	1.47	1.63
Eicosenoic, C20:1n-9	3.79	3.49	3.15
Arachidonic, C20:4n-6	1.94	1.45	1.55
Eicosapentanoic (EPA), C20:5n-3	12.60	12.38	12.73
Docosapentanoic (DPA), C22:5n-3	0.57	3.39	2.24
Docohexapentanoic (DHA) C22:6n-3	0.32	7.75	5.96
DHA:EPA	0.02	0.63	0.47
EPA:ARA	6.5	8.53	8.21
DHA:ARA	0.16	5.34	3.85
TOTAL n-6 FAs	19.02	18.63	20.26
TOTAL n-3 FAs	14.08	24.99	22.50
n-3:n-6	0.74	1.34	1.11

*nd - not detected

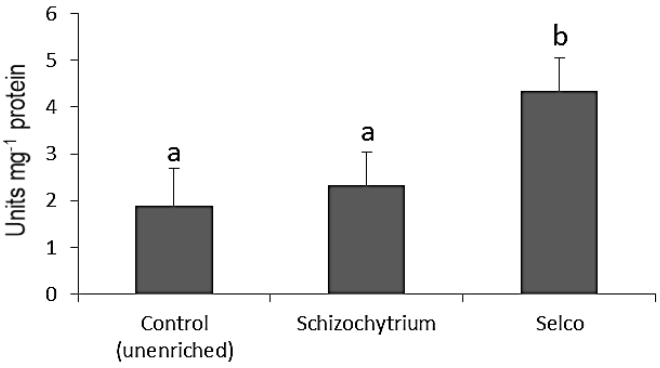

Figure 3. Trypsin activity of pompano larvae fed different enriched diets. Values are expressed as mean + standard error of the mean (n = 3). Treatment means with the same superscript in the same row are not significantly different (P<0.05).

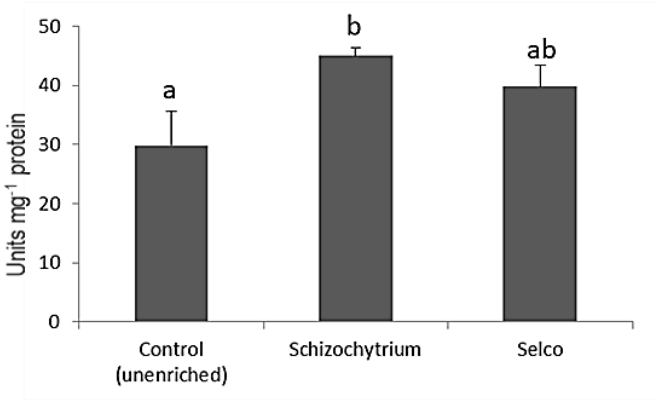

Figure 4. Chymotrypsin activity of pompano larvae fed different enriched diets. Treatment means with the same superscript in the same row are not significantly different (P<0.05).

treatments (Figs. 1-6), significantly higher lipase, amylase, pepsin, and chymotrypsin activities were observed in the group fed *Schizochytrium*-enriched *Artemia*. The results were not significantly different from the treatment fed *Artemia*-enriched with Selco. Trypsin activity was significantly highest in larvae fed with *Artemia*-enriched with Selco. The control treatment had the lowest digestive enzyme activities.

Figure 6 shows the tolerance of the larvae to extremely high saline conditions. When the larvae were exposed to extremely high salinity (65 g L^{-1}), higher mortalities were observed in the control treatment at an earlier time. Mass mortalities were observed in the control group after 45 minutes. Whereas, larvae fed enriched-*Artemia* survived longer compared to the control group. Mass mortalities in treatments fed enriched *Artemia* were observed after 2 hours.

Fatty acid values of *Artemia* are shown in Table 2. The values of total omega-3 fatty acid were highest in *Schizochytrium*-enriched *Artemia*. Both DHA and DPA were consistently highest in *Schizochytrium*-enriched, whereas the lowest value was observed in unenriched *Artemia*. The fatty acid ratios of DHA and

Table 3. Fatty acid composition (weight percent value) of pompano larvae fed with Artemia fed with different enrichment diets.

Fatty Acids	Control (unenriched)	*Schizochytrium*	SELCO
Myristic, C14:0	0.84	*nd	*nd
Palmitic, C16:0	10.93	5.61	5.92
Palmitoleic, C16:1n-7	3.37	1.05	0.67
Stearic, C18:0	13.79	9.84	10.62
Oliec, C18:1n-9	14.98	15.50	15.66
Vaccenic, C18:1n-7	7.72	5.60	5.45
Linoleic, C18:2n-6	10.63	9.50	10.17
Linolenic, C18:3n-6	1.98	1.21	1.38
Linolenic, C18:3n-3	0.46	0.98	0.61
Eicosenoic, C20:1n-9	3.89	2.93	3.13
Arachidonic, C20:4n-6	3.9	2.89	3.43
Eicosapentanoic (EPA) C20:5n-3	5.27	5.64	5.33
Docosapentanoic (DPA) C22:5n-3	7.25	10.32	12.19
Docohexapentanoic (DHA) C22:5n3	14.99	28.93	25.44
DHA:EPA	2.8	5.13	4.73
EPA:ARA	1.35	1.95	1.50
DHA:ARA	3.75	10.01	7.42
TOTAL n-3 FAs	27.97	45.87	43.57
TOTAL n-6 FAs	16.51	13.6	14.98
n-3:n-6	1.69	3.37	2.91

*nd – not detected

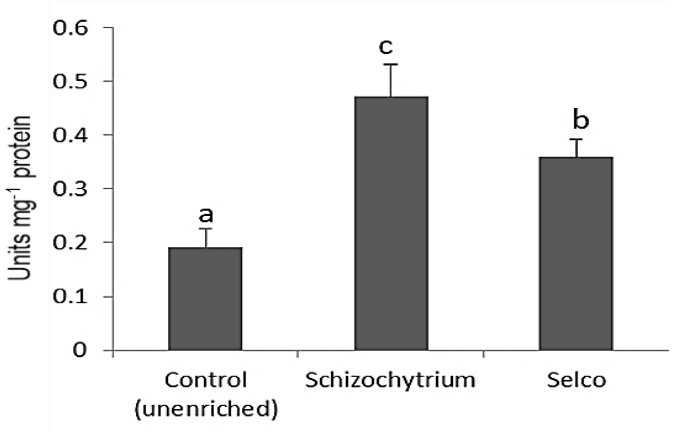

Figure 5. Amylase activity of pompano larvae fed different enriched diets. Treatment means with the same superscript in the same row are not significantly different (P<0.05).

EPA, EPA and ARA, DHA and ARA were high in both enriched *Artemia* while unenriched *Artemia* had the lowest value. The fatty acid values in fish (Table 3) show a consistent trend, high omega-3 fatty acid values in treatments fed enriched *Artemia*, while the lowest value was recorded in control treatment fed unenriched *Artemia*.

Discussions

Proper nutrition is an essential factor for successful larval rearing in marine fishes. To improve the nutritional profile of the larval diet, enrichment of live food is a common practice so that the larvae will receive optimal nutrition. Heterotrophic microorganisms such as traustochytrid can be a potential live food enrichment, and indigenous species can also be a cheaper substitute for commercial enrichment. This study utilized a locally isolated thraustochytrid, *Schizochytrium* sp. from senescent fallen mangrove leaves. Several studies have confirmed that *Schizochytrium* has the capacity to accumulate large amounts of PUFAs particularly omega-3 fatty acids (Leano et al., 2003; Leano and Liao, 2004; Raghukumar, 2008; Atienza et al., 2012). Barclay and Zeller (1996) have also shown that freeze-dried *Schizochytrium* can be used as enrichment for *Brachionus plicatilis* and *Artemia*, and a significant increase in their PUFA especially the DHA levels was observed after enrichment. The same observation was also obtained by the present study, the *Artemia* given with *Schizochytrium*-enrichment showed improved PUFA, particularly the omega-3 fatty acid level. The DHA level of enriched *Artemia* was several times higher compared to unenriched treatment. Moreover, *Schizochytrium* enrichment also enhanced the DHA/EPA ratio of the live prey. The ideal level of DHA/EPA ratio is essential in the developing stages of farmed fish and may play a key role in the physiological and biochemical pathways responsible for the normal growth and development of the

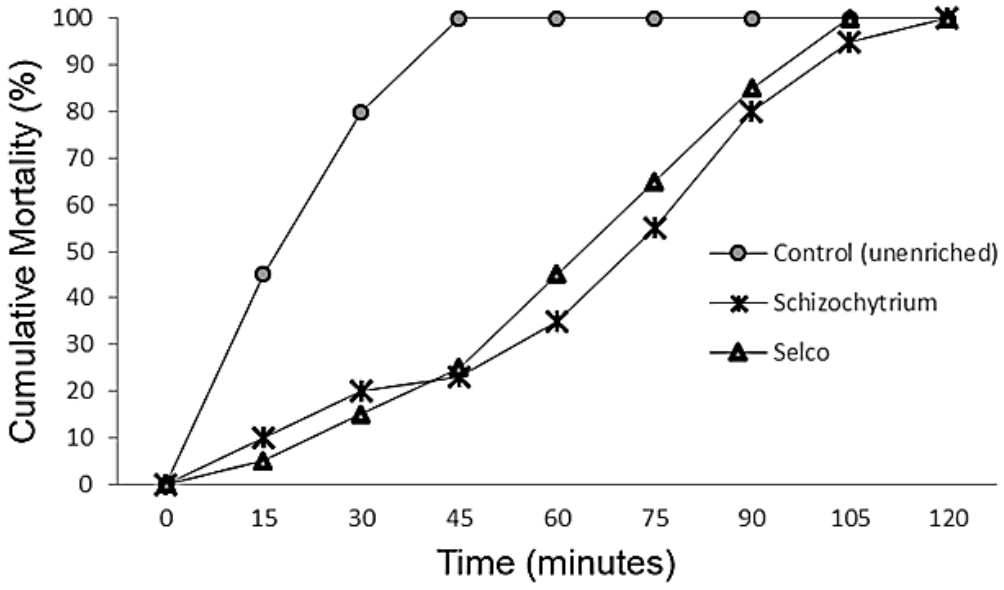

Figure 6. Cumulative mortalities of larvae exposed to 65 g L^{-1} salinity.

organism (Jin et al., 2017). The required dietary DHA/EPA ratios for marine fish range from 0.5 to 2.0, according to NRC (2011). The DHA/EPA ratios of enriched *Artemia* in this study were within the range recommended by NRC. Whereas, unenriched *Artemia* has DHA/EPA ratio that falls below the recommended value. Provision of diets with an ideal DHA/EPA ratio to farmed fish showed better growth (Hernadez-Cruz et al., 1999). This study supports this observation, when *Schizochytrium*-enriched *Artemia* were fed to pompano larvae, their growth and survival were significantly improved compared to the treatment-fed commercial enrichment. Similar results were also achieved when *Schizochytrium* sp. was incorporated into the diet of abalone (de la Peña et al., 2016) and feed-enrichment for seabass larvae (Ludevese-Pascual, 2018). Furthermore, feeding the larvae with *Schizochytrium* sp. enriched diet also improved their DHA content. Developing larvae need higher amount of PUFAs particularly DHA to sustain their rapid growth and critical development of their specialized cells and tissues (Atienza et al., 2012).

The enrichment of live prey has a significant impact on the digestive enzyme activities of pompano larvae. When larvae are fed the enriched *Artemia*, there is a noticeable increase in the activities of hydrolytic enzymes such as amylase, and lipase, as well as proteases like pepsin and chymotrypsin. This elevation in digestive enzyme activities is attributed to the positive influence of enrichment on the development of the fish larvae's digestive tract. A study conducted by Prusinska et al. (2020) demonstrated that feeding larvae with *Artemia* enriched with polyunsaturated fatty acids (PUFA) leads to the enhanced development of the active area of the intestine. This improved intestinal development ultimately results in more efficient feed utilization and, consequently, better growth rates. Furthermore, the use of microorganisms as a method for enriching live food or as a dietary supplement also plays a role in influencing the digestive enzyme activities of fish larvae, as observed in the work of Sankar et al. (2017). These microorganisms contribute to the digestion process of the cultured organisms by providing additional exogenous enzymes (Hortillosa et al., 2022).

The tolerance of pompano larvae to sudden exposure to high salinity levels (65 ppt) significantly improves when their diets are enriched with *Schizochytrium*. This finding aligns with the findings of de la Peña et al. (2016) regarding the effect of *Schizochytrium* incorporation in the diets of abalone. Their study demonstrated that abalone fed a *Schizochytrium*-enriched diet exhibited enhanced

resistance to osmotic shock. Additionally, other studies have consistently indicated that an increased presence of PUFAs in the diets of cultured organisms enhances their ability to withstand handling and environmental stressors (Furuita et al., 1996; Kanzawa, 1997). The increased levels of PUFAs in the diet can modify the functionality and composition of cell membranes by boosting permeability, viscosity, and fluidity. Furthermore, PUFAs may also play an important role in regulating several membrane functions, including transport, intercellular communication, and various enzymatic activities (Lund and Steenfeldt, 2011). Consequently, the augmented presence of PUFAs in cell membranes contributes significantly to an organism's capacity to adapt to adverse environmental changes (de la Peña et al., 2016).

The PUFA-rich diet seems to yield the most favorable results when subjected to stress tests, primarily because it stimulates increased membrane synthesis in gills (Palácios et al., 2004). The elevated membrane production leads to an expanded surface area with greater ramifications (Rees et al., 2004). Moreover, this diet can maintain an optimal fatty acid composition, counteracting the effects of salinity fluctuations that could otherwise affect permeability or the functioning of essential enzymes such as the Na+/K+ ATPase pump (Palácios et al., 2004). This ultimately makes the organisms more resilient to abrupt changes in salinity. The appropriate balance of the DHA/EPA ratio in the diet further enhances the larvae's ability to cope with environmental stress (Sui et al., 2007). Therefore, larvae fed a *Schizochytrium*-enriched diet gain a distinct nutritional advantage. Furthermore, the enrichment with *Schizochytrium* ensures that the larvae receive the essential fatty acids they need to shield themselves from adverse conditions.

In conclusion, this study has shown the potential of *Schizochytrium* sp. as live food enrichment for pompano larvae. Larvae fed *Schizochytrium*-enriched diet showed improved growth performance, digestive enzyme activities, and a better tolerance to osmotic stress.

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