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Research Article

Ontogenetic development of digestive enzymes in the crayfish *Procambarus acanthophorus*

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ABSTRACT. Mexico boasts a great diversity of decapod crustaceans, including crayfish known locally as acociles. Within this group, *Procambarus acanthophorus* stands out as an important species for regional fisheries and has high aquaculture potential because it adapts quickly to captive conditions and develops directly without larval stages, giving it a significant advantage in growth and survival over other crayfish species. However, its digestive and physiological capabilities, which could inform the development of aquaculture technologies and enhance its aquaculture potential, remain largely unknown. This study aims to identify the digestive enzymes present during ontogeny and characterize the acocil crayfish. Adult crayfish were collected and processed in the Aquatic Resources Physiology Laboratory, where the spawn and fry of the crayfish were obtained. At the end of the culture period, we collected five organisms daily to determine the crayfish enzymatic profile. We determined protease, amylase, lipase, and phosphatase activities through biochemical analysis. The results show pepsin-like enzyme activity from day 1 after hatching, while alkaline proteases are active during the first few days, with fluctuations throughout development. It is concluded that the ontogenetic development of the crayfish *P. acanthophorus* exhibits, from its first days of life, a primary enzymatic machinery that allows it to hydrolyze vitelline components and improves as it grows, enabling it to digest different nutrients from exogenous feed and demonstrating its strong nutritional adaptation for aquaculture.

Keywords: *Procambarus acanthophorus*; amylase; crayfish; digestive physiology; enzymes; lipase; ontogeny; phosphatases; proteases

INTRODUCTION

Aquaculture has become a vital industry for meeting the growing global demand for seafood, particularly as wild fish stocks continue to decline due to overexploitation and environmental pressures (FAO 2024). Among the species of greatest interest to consumers and aquaculture producers are crustaceans, particularly shrimp such as *Penaeus vannamei*, a species cultivated in virtually all tropical and subtropical environments

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worldwide. In addition to shrimp, other crustacean species suitable for cultivation include prawns, crabs, lobsters, and blue crabs. Specifically, crayfish are freshwater decapod crustaceans that inhabit lakes, rivers, and wetlands and play a crucial ecological role in the proper functioning of freshwater ecosystems. In Mexico, crayfish are highly diverse; some species, such as *Procambarus acanthophorus*, are important in artisanal fisheries and are traded regionally, forming part of the daily diet of communities near freshwater bodies (Palacino-Rodríguez et al. 2026). Advances in knowledge of the genus *Procambarus* show high aquaculture potential because it adapts quickly to captive conditions and tolerates environmental fluctuations and varying water quality (Palacino-Rodríguez et al. 2026).

Research on enzymatic ontogeny in crustaceans has emerged as a vital field of study, given its essential contribution to understanding developmental physiology, nutritional ecology, and process optimization in aquaculture. Investigations into digestive enzyme activity during larval and embryonic stages have progressed from preliminary biochemical characterizations to comprehensive assessments that correlate enzyme profiles with morphological and ecological adaptations (Martínez-Salazar et al. 2023). This field has become increasingly important because crustaceans like penaeid shrimp, crabs, and crayfish are economically important and make up a large share of global aquaculture production (Rojo-Arreola et al. 2020). For instance, understanding how enzymes evolve helps develop feeding strategies that help young fish survive and flourish during their most vulnerable life stages, a problem that arises when producing large numbers of fry (Chen et al. 2018, Khoa et al. 2019). Moreover, enzyme development reflects physiological changes that facilitate metamorphosis and habitat transitions, with consequences for species conservation and ecosystem dynamics (Sacristán et al. 2020). Although many studies have examined digestive enzymes in crustaceans, we still know little about how they vary over time, how they are regulated, or what those changes mean for their function. Research shows that enzyme activity varies in species like *P. vannamei* and *Macrobrachium* spp., but the timing of peak enzyme expression and activity, and how these changes relate to feeding, vary (Musin et al. 2018, Rojo-Arreola et al. 2020). Disagreements also remain about how important endogenous and exogenous enzymes are during feeding (Serrano 2012). Moreover, these studies need to be strengthened with molecular data, such as mRNA expression profiles, because enzyme activity constrains developmental patterns at both molecular and morphological levels, hindering a thorough understanding of gene-enzyme interactions (Hernández-Cortés et al. 2017). This knowledge gap obstructs the development of standardized feeding programs and limits understanding of species-specific developmental physiology (Ziaeinejad et al. 2018). Addressing these challenges is critical because insufficient knowledge can lead to poor culture conditions and reduced aquaculture output (Ribeiro 2000). Understanding these correlations helps pinpoint critical periods for feeding interventions and improves larval rearing methods (Chamchuen et al. 2014). Studying crayfish digestive capacity from early development to adulthood helps researchers understand changes that influence feeding and growth during captive rearing. Currently, alternative protein sources are being sought to enable the sustainable development of aquaculture, taking into account the reduction of fishmeal and the search for new feed sources, as well as the ability of aquatic organisms to digest feed depending on the number of digestive enzymes present in their intestines (Fu et al. 2025). It should be noted that incorporating digestive enzymes into crustacean feed aims to increase animal growth, especially in stages where the activity of natural enzymes restricts feed use (García-Galano & Carrillo-Farnes 2015). This research aims to elucidate the enzymatic developmental role of *P. acanthophorus* during ontogeny by synthesizing biochemical data to understand its physiology and develop more suitable and sustainable techniques for cultivating this species for aquaculture.

MATERIALS AND METHODS

Organism management

The study was conducted at the Laboratory of Physiology and Aquatic Resources (LAFIRA, by its Spanish acronym) at the Juárez Autonomous University of Tabasco. Crayfish fry for the study were obtained from the controlled reproduction of reproductive organisms, which were maintained in a recirculating system consisting of a 2 m^2 rectangular fiberglass tank, 0.15 m deep, with 50% water exchange every two days, at a 1:3 sex ratio (male:female) (Cervantes-Santiago et al. 2010). Twelve adult reproductive organisms were used at a 3:1 female-to-male ratio and placed in three experimental units connected to an open recirculation system featuring a biological filter with bio-spheres. The reproductive organisms were fed a commercial shrimp feed containing 35% protein, 15% lipids, and 10% carbohy-

drates (Camaronina 0.5 mm, 35 H.P. Purina®) three times daily to satiation. During the reproductive phase, the crabs were kept under a natural 12 h photoperiod. System water quality was monitored daily over the 30 days; temperature (28.6 ± 2°C) and dissolved oxygen (5.6 ± 0.3 mg L^{-1}) were recorded using an oximeter (YSI 85; OH, USA), and pH (7.1 ± 0.2) was measured with a pH meter (HANNA HI 991001, Romania). We removed organic matter by siphoning to maintain water quality. Once ovigerous females were observed, males were removed, and females were kept in the system until offspring release to obtain juveniles of uniform size. After observing the first free-swimming juveniles, the female was removed, and the juveniles were kept in the units until the end of the study. During this period, the juveniles were provided with an initial ration of *Artemia* equivalent to 5% of their biomass, three times daily, for the first 10 days; subsequently, they were fed a commercial shrimp feed (Camaronina 0.1 mm, 35 H.P. Purina®) containing 40% protein, 10% lipids, and 10% carbohydrates, rations were provided three times daily (09:00, 12:00, and 16:00 h) to apparent satiation until the adult stage.

For the study, 225 crayfish fry (0.05 g) were used for the biochemical analyses, collecting 5 samples per tank (15 ind) at 0 (egg), 1 day after hatching (DAH), 2, 3, 4, 5, 10, 12, 16, 18, 21, 41, 50, 70, 90, and 122 days, randomly distributed among the experimental units. At the start of the experiment, we performed initial biometry on all organisms to ensure homogeneity within each day's groups. Subsequent biometry measurements were taken daily. Weight (g) was recorded using an analytical balance (OHAUS, Scout Pro, CS200, USA; ± 0.01 g), and total length (mm) was recorded using a digital caliper (Steren, USA; ± 0.01 mm). Samples for biometry were collected using a fine-mesh spoon net; airflow was stopped beforehand to facilitate capture.

Biochemical and enzymatic analyses

We used the methodology proposed by Frías-Quintana et al. (2016), which involves randomly collecting a sample of five organisms per day at the end of the culture period to determine the enzyme profile in the crayfish. Sampling was carried out in the morning before the first feeding, and the organisms were euthanized by thermal shock at -20°C until subsequent analysis.

For the preparation of the multienzyme extract, a complete homogenate of the organisms was prepared, placed in 1.5 mL microtubes, and homogenized in a 200 mg mL^{-1} solution with glycine-HCl buffer (100 µmol L^{-1}, pH 2) to determine acid activity (pepsin) and with Tris-HCl buffer (30 µmol L^{-1} + $CaCl_2$(12.5 µmol L^{-1}, pH 7.5) for alkaline enzyme activity. The homogenate was then centrifuged at 14,000 RFC (relative centrifugal force) for 30 min at 4°C, and the supernatant containing the multienzyme extract was extracted and stored at -20°C until analysis.

Biochemical analyses of the multienzyme extract were performed at the LAFIRA of the Juárez Autonomous University of Tabasco. The concentration of soluble protein in the multienzyme extracts of hepatopancreas and intestine was determined using the technique described by Bradford (1976), by preparing a calibration curve with bovine albumin at diluted concentrations ranging from 0 to 40 mg mL^{-1}. The method involved adding 8 µL of the previously obtained multienzyme extract to a 1.5 mL microtube, followed by 792 µL of distilled water and 200 µL of Bradford reagent (Supelco® B6916), then incubating for 7 min at room temperature in a test tube rack. Protein concentration was determined by reading the absorbance at 595 nm on a Thermo Scientific spectrophotometer (NanoDrop 2000c).

We determined the enzymatic activity of acid proteases using the technique described by Anson (1938), with 0.5% bovine hemoglobin (Millipore®, 08449) in 100 µmol L^{-1} glycine-HCl buffer at pH 2. We determined alkaline protease activity using the technique described by Kunitz (1947) and modified by Walter (1984), with 1% casein (Sigma-Aldrich®, E0789) in 100 µmol L^{-1} Tris-HCl buffer + µmol L^{-1} $CaCl_2$ at pH 9. We performed the analyses as follows: incubate the enzyme extract mixtures at room temperature (37°C) for 60 min, then stop the reaction by adding 0.5 mL of trichloroacetic acid (TCA, 20%). The mixture was then centrifuged at 14,000 RFC for 15 min. We measured the absorbance of the reaction products at 280 nm. Enzyme activity was defined as 1 µg of tyrosine released per minute, based on the molar extinction coefficient (0.005).

Leucine aminopeptidase activity was determined using the method of Maraux et al. (1973) with leucine p-nitroanilide (Sigma-Aldrich®, L9125) in 0.1 µmol L^{-1} DMSO as the substrate, in 50 µmol L^{-1} sodium phosphate buffer, pH 7.2, 37%. The concentration was then determined by absorbance at 410 nm. Enzyme activity was determined as 1 µmol L^{-1} of nitroanilide released per minute, using the molar extinction coefficients of trypsin, chymotrypsin, and leucine aminopeptidase. Trypsin activity was performed by the method of Erlanger et al. (1961), where the sample was incubated at 37ºC with the substrate BAPNA (Nα-benzoyl-DL-arginine-p-nitroanilide) (Sigma-Aldrich®,

B4875), at a concentration of 3.5 mmol dissolved in a buffer solution of Tris-HCl 100 mmol^{-1}, $CaCl_2$ 10 mmol L^{-1} at pH 8.2. The absorbance was determined at 410 nm. Chymotrypsin activity was determined by the method of Del Mar et al. (1979) at 37°C, using 0.1 mM SAAPNA (N-succinyl-ala-ala-pro-phe p-nitroanilide) (Sigma-Aldrich®, S7388) dissolved in dimethyl sulfoxide (DMSO), 5 mmol $L^{-1,}$ and Tris-HCl buffer (100 mmol L^{-1}, $CaCl_2$ 100 mmol L^{-1}) at pH 7.8. The absorbance was determined at 405 nm. We evaluated leucine-aminopeptidase activity using the protocol of Maroux et al. (1973), with 0.1 mmol L^{-1} leucine p-nitroanilide (Sigma-Aldrich®, L5006) dissolved in DMSO as the substrate and a sodium phosphate 50 mmol L^{-1}, pH 7.2 buffer solution. Enzyme activity was defined as 1 µmol of nitroanilide released per minute, using a molar extinction coefficient of 8.8 for trypsin and chymotrypsin and 8.2 for leucine aminopeptidase.

For the identification of α-amylase enzyme activity, 2% soluble starch (Sigma-Aldrich®, S9765) in 0.1 µmol L^{-1} phosphate-citrate buffer with 0.005 µmol L^{-1} NaCl at pH 7.5 was used as a substrate, and the absorbance was measured at 600 nm. One unit was defined as the amount of enzyme that releases 1 µg of maltose per minute using a 0.0034 molar extinction coefficient (Robyt & Whelan 1968). Lipase activity was determined using the technique of Versaw et al. (1989), using 200 µmol L^{-1} of β-naphthyl caprylate (Santa Cruz Biotecnology® sc-280282) dissolved in 50 µmol L^{-1} Tris-HCl buffer at pH 7.2 with a sodium taurocholate solution (100 µmol L^{-1}) as a substrate, and the absorbance was measured at 540 nm. Lipolytic activity was defined as 1 µg of naphthol released per minute using a coefficient of molar extinction of 0.02.

Alkaline phosphatase activity was estimated using 4-nitrophenyl phosphate (Sigma-Aldrich®, 71768) at 2.04% as substrate in citric acid/citrate buffer at pH 5.5 under acidic conditions or in NaOH-glycine buffer at pH 10.1 under alkaline conditions; it was incubated for 30 min, and 0.05 N NaOH was used to stop the reaction in both techniques. We defined enzymatic activity as 1 µg of naphthyl released per minute, using a 0.0185 molar extinction coefficient at 405 nm, according to Bergmeyer (1974). The values obtained were expressed in international units (U), which represent the amount of enzyme that catalyzes the formation of 1 µmol of product per minute at 25°C.

Enzyme activity was calculated in triplicate, following this equation: total activity (U mL^{-1}) = [Δabs × reaction final volume (mL)] / [MEC × time (min) × extract volume (mL)]. Specific activity (U mg proten^{-1}) = total activity/soluble protein (mg), where Δabs represents the increase in absorbance, and MEC represents the respective molar extinction coefficient.

To evaluate growth in weight and length, we tested the data for normality (KS) and homoscedasticity (Levene) and applied analysis of variance (ANOVA). Larval growth was modeled as Y = aebX, with data log-transformed (base 10), and model parameters were estimated using least squares. Because enzymatic activity values were not normally distributed, we compared the data using the nonparametric Kruskal-Wallis test. We used the nonparametric Nemenyi test when we detected significant differences between treatments, with a significance level of 0.05. All tests were performed with software StatisticaTM v.8.0 (Analytical Software, AZ, USA). Data are presented as mean ± standard error (SE). All tests were performed in triplicate.

RESULTS

Growth

The crayfish *P. acanthophorus* exhibits direct ontogeny (without larval stages or metamorphosis). An exponential regression yielded an R^2 of 0.97, indicating that total length has an intercept of 5.37 mm and a slope of 0.548 mm per day. The exponential regression slope (0.548) suggests constant growth in total length over time (Fig. 1).

Analysis of digestive enzyme activity

The specific activity (U mg protein^{-1}) of acid protease was detectable from 1 DAH (2.135 ± 0.39 mm), showing greater activity on days 4 and 16 (9.135 ± 0.122 and 8.859 ± 0.336 mm, respectively), remaining constant since then until day 122 (7.586 ± 0.276 mm) in the adult stage. Alkaline protease enzyme activity appears from day 0 (egg), increasing during the initial days, peaking on day 4 (5.333 ± 0.687 mm) and subsequently maintaining constant activity until the adult stage at 122 DAH (Fig. 2).

Trypsin-like activity showed variations during the crayfish's ontogenetic development, increasing from day 0 (egg), showing peak activity on day 4 (0.025 ± 0.02 mm) and decreasing on days 10, 12, 41, and 50 DAH, increasing and maintaining the same pattern until day 122 DAH (Fig. 3a). Chymotrypsin exhibits an increase in activity starting from day 0 (egg), showing peak activity levels on day 4 (0.254 ± 0.01 mm) and 18 DAH (0.276 ± 0.01 mm) and subsequently decreasing throughout development on day 122 DAH (Fig. 3b). Leucine aminopeptidase activity increased after vitelline

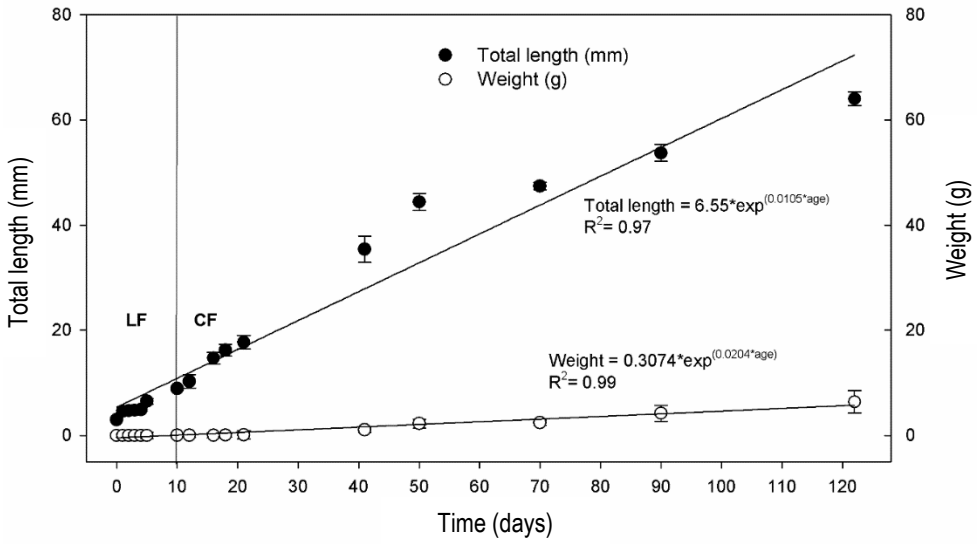


Figure 1. Total length (mm) and weight (g) obtained from the organisms during sampling (n = 5). Feeding with live food (LF) and commercial food (CF); $R^2 = 0.97$.

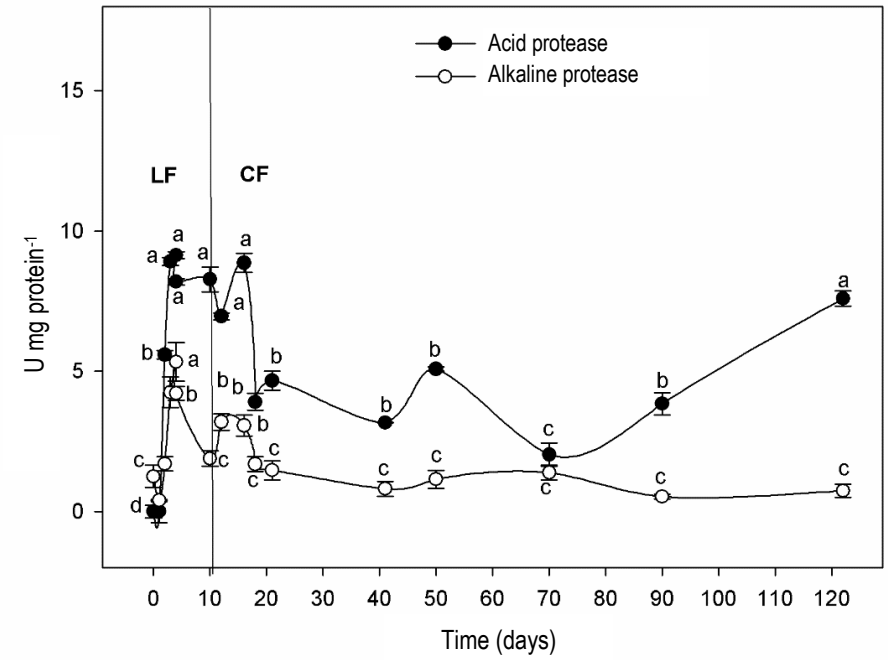


Figure 2. Specific activity (U mg protein^{-1}) of acid proteases (black circle) and alkaline proteases (white circle) during the ontogeny of the crayfish (*P. acanthophorus*) (mean ± standard deviation, SD, n = 3). Feeding with live food (LF) and commercial food (CF). Specific activity (U×10^{-3} mg protein^{-1} ± SD, n = 3). Mean values denoted with different letters (a, b, c, d) are statistically different, $P < 0.05$.

absorption (day 0) and after hatching on 1 DAH (0.536 ± 0.01 mm) and 16 DAH (0.704 ± 0.01 mm), decreasing on day 10 DAH until remaining constant on day 122 DAH (Fig. 3b).

Lipase activity showed a significant increase from hatching until day 18 DAH (9.65 ± 0.22 mm), decreasing on days 21 and 41 DAH, oscillating throughout the remaining growth, and stabilizing until

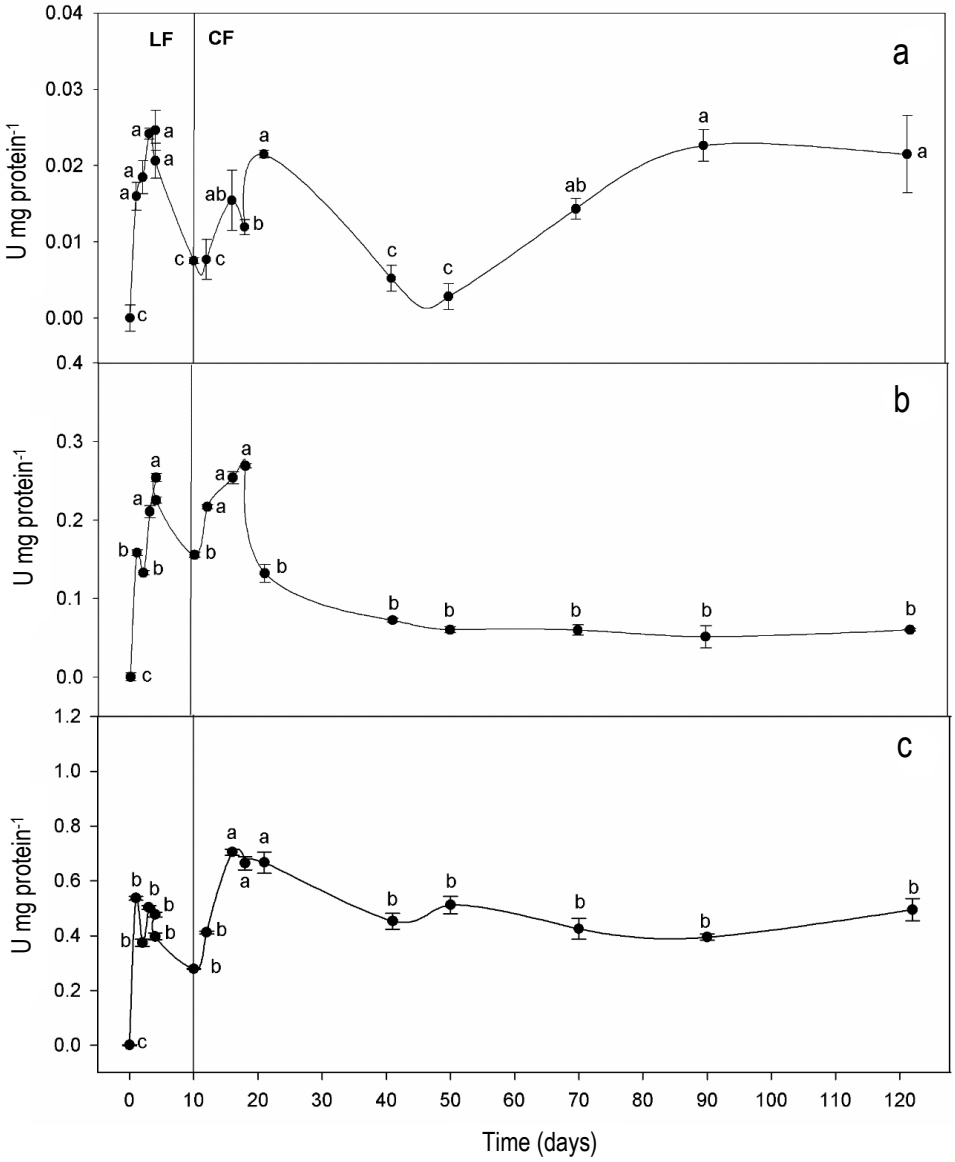


Figure 3. a) Trypsin, b) chymotrypsin, and c) leucine aminopeptidase activity during the ontogeny of the crayfish *P. acanthophorus*. Feeding with live food (LF) and commercial food (CF). Specific activity ($U \times 10^{-3}$ mg protein^{-1} ± standard deviation, n = 3). Mean values denoted with different letters (a, b, c) are statistically different, $P < 0.05$.

day 122 DAH (Fig. 4a). α-amylase activity increased from day 5 (67.82 ± 0.17 mm) and 16 DAH (69.02 ± 0.57 mm), and subsequently oscillating throughout development before stabilizing (Fig. 4b).

Acid phosphatase activity fluctuated during the first few days (0-5 DAH), increasing on day 10 (1.317 ± 0.045 mm), showing fluctuations throughout development, with 70 DAH (1.780 ± 0.098 mm) exhibiting the highest enzymatic activity. Alkaline phosphatase activity showed variations in enzymatic activity, with peak levels occurring on days 5 (0.065 ± 0.004 mm), 16 (0.076 ± 0.001 mm), and 70 (0.076 ± 0.001 mm) during development until day 122 DAH (Fig. 5).

DISCUSSION

Pepsin (an acid protease) is one of the digestive enzymes that allows for the detection of a functional stomach during early developmental stages; despite the influence of other digestive enzymes such as cathepsins, pepsin exhibits specific enzymatic activity at pH 2 using hemoglobin as a substrate, meaning its activity increases at this pH level (Martínez-Salazar et al. 2023). Research on crustaceans has identified this

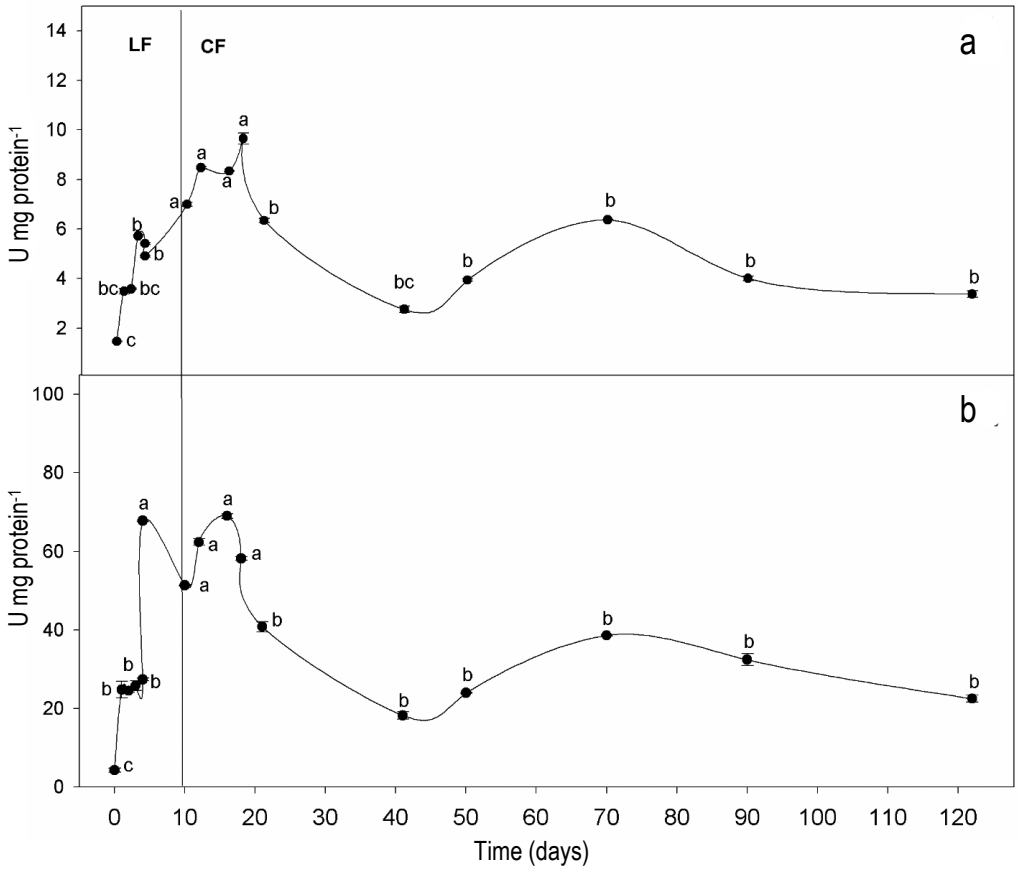


Figure 4. Lipase and α-amylase activity during the ontogeny of the crayfish *P. acanthophorus*. Feeding with *Artemia* live food (LF) and commercial food (FC). Specific activity (U mg protein^{-1} ± standard deviation; n = 3). Mean values denoted with different letters (a, b, c) are statistically different, $P < 0.05$.

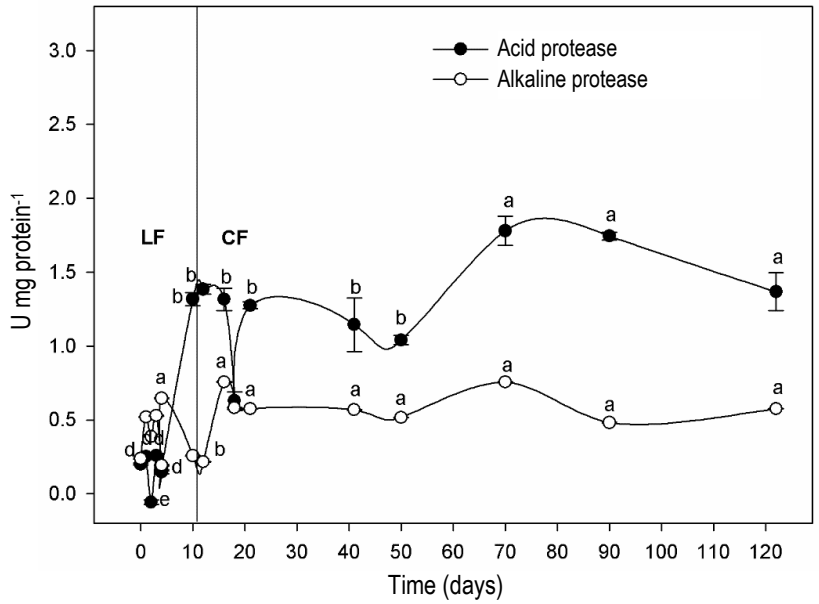


Figure 5. Specific acid and alkaline phosphatase activity during the ontogeny of the crayfish *P. acanthophorus* (U mg protein^{-1} ± standard deviation; n = 3). Feeding with live food (LF) and commercial food (CF). Mean values denoted with different letters (a, b, c, d) are statistically different, $P < 0.05$.

pepsin activity in *Macrobrachium americanum*; Laguna-Nataren et al. (2023) found that pepsin activity occurs at pH 2 during early larval stages and remains constant throughout the post-larval, juvenile, and adult phases. This pattern suggests that crustaceans' digestive capacity adapts as they mature to process more complex diets, enabling them to use a wider range of food sources. Sudtongkong et al. (2019) investigated protease activity in the sesarmid crab (*Episesarma singaporense*). They obtained similar results: enzymatic activity was lower in larvae, increased significantly in juveniles, and remained high in adults. These findings suggest that regulating pepsin activity is crucial for the dietary transition during crustacean ontogenetic development, although diet also plays a fundamental role, as higher dietary protein content correlates with increased enzymatic activity. Regarding feed during early growth stages, Fu et al. (2025) observed increased acid protease activity in the giant freshwater prawn (*Macrobrachium rosenbergii*) when evaluating diets enriched with high-quality proteins, thereby enhancing digestive capacity and, consequently, the growth and health of cultured crustaceans. In *P. acanthophorus*, an organism with direct ontogeny that bypasses larval stages upon hatching, the complete digestive apparatus (organs) is present to digest any food source while achieving optimal growth in both intensive and extensive culture systems.

Alkaline protease activity was present from day 0 (egg stage), before and after hatching, and fluctuated throughout growth. These fluctuations can be explained by adaptation to the type of exogenous feed in the environment and the shift from live to commercial feed. It should also be considered that as acid digestion (acid protease) increases, alkaline protease activity decreases, since the metabolism does not require maintaining a higher level of alkaline digestion because it does not depend on energy reserves (vitelline) and focuses on maintaining balanced digestion under both acidic and alkaline conditions. Hernández-Cortés et al. (2017) studied white shrimp (*P. vannamei*). They found that alkaline protease activity is low in early larval stages, increases significantly during postlarval and juvenile stages, and peaks in adults. This pattern suggests that crustaceans' digestive capacity adapts as they mature, allowing them to handle more complex and diverse diets, although factors such as survival and growth also influence it (Manríquez-Santos et al. 2018, Vargas-Ceballos et al. 2024).

Trypsin and chymotrypsin enzymatic activities were present from day 0 (pre-hatching), as digestion during the embryonic development phase within the egg is entirely alkaline; endopeptidases (trypsin and chymotrypsin) and exopeptidases (leucine aminopeptidase) are responsible for nourishing the embryo using the egg's vitelline sac until hatching (Luo et al. 2007). However, starting at 1 DAH, activity levels increase and fluctuate throughout development; peaks may reflect dietary shifts from *Artemia* to pelleted feed, adaptation to exogenous food, and the species' protein-hydrolysis capacity. This pattern has been observed in other crustacean species, such as *P. vannamei*, where trypsin activity is low during early larval stages, increases significantly during the post-larval and juvenile phases, and peaks in adults (Hernández & Murueta 2009); similar results regarding this increase have also been observed in species such as *Penaeus setiferus* (Sacristán et al. 2020). Generally, protease activity is high during early embryonic or larval stages to facilitate the hydrolysis of vitelline proteins, subsequently decreasing or changing as the larvae mature; conversely, amylase and lipase activities increase with age and the intake of exogenous food (Luo et al. 2007, Ziaeinejad et al. 2018, Sudtongkong et al. 2019, Rojo-Arreola et al. 2020). In various species, enzymatic activities peak during the larval growth phases and metamorphosis (Rotllant et al. 2012, Rojo-Arreola et al. 2020). Trypsin and chymotrypsin activities increase markedly at the onset of feeding or during the final larval stages (Hernández-Cortés et al. 2017, Khoa et al. 2019, Rojo-Arreola et al. 2020).

In *P. acanthophorus*, leucine aminopeptidase (LAP) activity increased during early growth stages and then remained stable, with fluctuations throughout development. Muhammad et al. (2012) observed in *P. vannamei* that LAP activity was relatively low during early larval stages and increased significantly during the post-larval and juvenile phases; given that this species undergoes indirect ontogeny, some of the observed changes in enzymatic activity were attributed to adaptation and the transition from a planktonic diet to one richer in protein, a shift requiring greater digestive capacity (Estrella-Soliz et al. 2025). The protease activity observed in our study was similar to results from studies evaluating diets with different protein levels in *Macrobrachium nipponense*, *P. vannamei*, and *Cherax quadricarinatus*, which observed a positive relationship between enzyme activity and increased dietary protein (Wang et al. 2018, 2024, Talukdar et al. 2021).

Lipase activity (LIP) increased after hatching. It remained elevated until day 20 DAH, with fluctuations throughout development, which may reflect the assimilation of fatty acids from both live food and the diet, and remained stable during the rest of the growth period (Torres-Ochoa 2020). The action of lipase on its

substrate is considerably enhanced by the presence of the water-lipid interface, which forms in the intestine thanks to the emulsifying action of bile salts or their analogs. Digestive LIP hydrolyzes triacylglycerols from dietary lipids to free fatty acids and is one of the main digestive enzymes in crustaceans (Silva et al. 2020). Recently, Goncalves et al. (2022) demonstrated that the activity of digestive enzymes (trypsin, amylase, and lipase) in juveniles of European lobster (*Homarus gammarus)* (stages VII-VIII) is modulated by diet composition and the energy contribution of fatty acids present in the food. These patterns may vary, as some species exhibit low or irregular activity during development. For example, some studies have shown low levels in embryos (Luo et al. 2007, Dai et al. 2009), while others show a constant increase in this activity (Frías-Gómez et al. 2023). Sarmiento-Cárdenas et al. (2026) reported that juvenile redclaw crayfish (*C. quadricarinatus*) fed a 25% protein diet showed lower lipase activity, increased hepatopancreatic lipid content, lower glycogen reserves, and a higher concentration of glucose in the hemolymph. However, studies such as Sacristán et al. (2016) noted that LIP activity remained relatively stable unless the animals were fasting.

For amylase (AMY), observed enzymatic activity was similar to that of lipase, showing peaks of heightened activity during feeding on *Artemia* and after feeding with particulate food. These increases often accompany dietary transitions (Martínez-Salazar et al. 2023). However, when dietary carbohydrates exceed optimal levels, excess substrate (glucose) in the hemolymph "overloads" the enzymatic system and, combined with prolonged hyperglycemia, reduces AMY expression; consequently, carbohydrates are excreted without being utilized (Wang et al. 2024). Therefore, excess dietary carbohydrates reduce the shrimp's ability to assimilate and use glucose as metabolic fuel (Ding et al. 2017). Conversely, Espinosa-Chaurand et al. (2019) reported higher amylase activity in juvenile *M. tenellum* fed a low-protein, high-nitrogen-free extract (NFE) diet compared to those fed a high-protein, low-NFE diet. The authors attributed this response to a greater metabolic reliance on dietary carbohydrates as an energy source. Similarly, Xiao et al. (2023) observed an increase in α-amylase activity in juvenile *Metapenaeus ensis* when dietary protein was reduced and NFE was increased; however, the rise in enzymatic activity did not translate into improved growth, which was observed only in shrimp fed high-protein diets. De Farias-Lima et al. (2014) state that prawns' digestive activity is determined by diet rather than phylogeny, as diet and digestibility are related; they note that carnivorous species exhibit higher levels of proteases, while omnivorous species show higher levels of amylases.

The enzymatic activities of acid and alkaline phosphatase fluctuated widely during the first few days, then stabilized after day 21, likely because of the availability of organic and inorganic phosphate in the diet and these enzymes' hydrolytic capacity to metabolize it. In fact, both phosphatases are associated with the active transport of glucose, proteins, and lipids, and, in the case of alkaline phosphatase, even water and ions (Phan et al. 2024). The fluctuations present in all the enzymes evaluated in this study appear to be strongly related to three aspects: 1) genetic processes, especially when certain specific activity is observed in starving larvae during vitelline absorption (for example, phospholipid hydrolysis); 2) a response to ingestion (feeding behavior) at the time of adding live food (rotifers and *Artemia*); and 3) the progressive disappearance of the metabolic function in which a certain enzyme is involved (lactase in the case of mammals), or a relative increase in soluble protein in the organism (Álvarez-González et al. 2008). Environmental factors such as food availability and starvation affect enzyme activity in variable ways (Chen et al. 2018). Understanding the timing and magnitude of digestive enzyme activities across developmental stages can inform optimized feeding regimes in aquaculture, ensuring diets match the enzymatic capabilities and nutritional requirements of larvae and juveniles and improving survival and growth rates (Chamchuen et al. 2014, Chen et al. 2018, Khoa et al. 2019).

CONCLUSIONS

The ontogenetic development of the crayfish *P. acanthophorus* reveals, from its earliest days of life, an enzymatic machinery primarily composed of acid and alkaline proteases, present from the first day of hatching. Similarly, enzymes such as amylases, lipases, and phosphatases show increasing activity during the first five days after hatching, allowing the organism to hydrolyze protein components in exogenous food and metabolize the remaining vitelline reserve after hatching, improving as growth progresses. These results allow us to conclude that the crayfish is a precocial organism with a complete enzymatic repertoire, and that as all the morphophysiological changes occur, its digestive capacity shifts to a monogastric type, where the stomach becomes the main

organ responsible for digestion and the intestine primarily performs the function of nutrient absorption from day 12 onwards, making it possible to replace live food with microdiets during the first few days after hatching, improving growth and survival compared with feeding live prey.

Credit author's contribution

K.A. Ferrer-Martinez: conceptualization, validation, methodology, formal analysis and writing-original draft; C.A. Frias-Quintana: supervision, review and editing; C.A. Álvarez-González: conceptualization, data curation, formal analysis, review and editing; L.D. Jiménez-Martínez: review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Ethics statement

This review presents research that, for all experiments and animal husbandry, we followed established guidelines for good management practices for aquatic animals, in accordance with the conduct standards of the University of Veracruz.

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