
Seasonal Variability in Trypsin and α -Amylase Activities Caused by the Molting Cycle and Feeding Habits of Juvenile Pink Shrimp *Farfantepenaeus duorarum* (Burkenroad, 1939)

Herón Aragón-Axomulco¹, Xavier Chiappa-Carrara², Luis Soto³, Gerard Cuzon⁴, Leticia Arena², Carlos Maldonado¹, René Cárdenas⁵ and Gabriela Gaxiola^{2,*}

¹ Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Puerto de Abrigo S/N, Sisal, Hunucmá, Yucatán 97355 México

² Unidad Multidisciplinaria de Docencia e Investigación de Sisal, Facultad de Ciencias, Universidad Nacional Autónoma de México, Puerto de Abrigo S/N, Sisal, Hunucmá, Yucatán 97355 México

³ Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Puerto de Abrigo S/N, Sisal, Hunucmá, Yucatán 97355 México

⁴ Ifremer, Centre du Pacifique, BP 7004, Taravao, 98719 Tahiti

⁵ Facultad de Ciencias, Universidad Nacional Autónoma de México, Puerto de Abrigo S/N, Sisal, Hunucmá, Yucatán 97355 México

*: Corresponding author : G. Gaxiola, email address : mggc@ciencias.unam.mx

Abstract:

In view of the relationship between shifts in diet composition and the activity of digestive enzymes in penaeid shrimp, the present study focused on the analysis of digestive trypsin and α -amylase activities of wild *Farfantepenaeus duorarum* (Burkenroad, 1939) juveniles and their changes in phenotypic expression, during the molt cycle as endogenous factor and their changes due to different feeding regimes (exogenous factor) in relation with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signature as an index of food assimilation induced by the seasonal availability of food items in the nursery area. Wild juveniles of *F. duorarum* were captured from April 2007 to February 2008, in the Celestun coastal lagoon, Yucatan, Mexico. Samplings were carried out considering all quarters of the lunar cycle and in each of the recognized seasons for this region: dry, rainy, and the Nortes (North Wind). Copepods and amphipods were the main source of food for juveniles of *F. duorarum*. Values of $\delta^{13}\text{C}$ in the muscular tissue were near -20‰ hence the feeding regime of *F. duorarum* in the lagoon was composed by material of marine origin. Isotopic signature differences were found between the three annual seasons. It is an opportunist generalist organism that is located in the 4th trophic level. The digestive enzymatic activities of both trypsin and α -amylase in fresh hepatopancreas tissue showed an interaction between season and molt stages ($p < 0.05$). Activity of the trypsin was highest during the Nortes at molt stage C (140 mU mg⁻¹ HP) and activity of α -amylase was higher in the Nortes at stage B2 (674 mU mg⁻¹ HP). The amylase/trypsin ratio also showed significant interaction between season and molt stages ($p < 0.05$), with higher values in premolt stages during the rainy and Nortes seasons. Isoforms of these digestive enzymes differed in expression according to the molt stage and also to the season with expression generally being greater at stage C.

Keywords: coastal lagoons, digestive enzymes, *Farfantepenaeus duorarum*, feeding habits, pink shrimp

1. Introduction

The postlarvae and juvenile penaeid shrimp distribution has been associated with estuarine vegetation due to decreased mortality rates from juvenile fishes and as well as to food availability, and *Farfantepenaeus duorarum* is not the exception (Sanchez, 1997). Juvenile shrimp recruited into tropical nursery grounds normally consume different food sources depending on their seasonal availability, location and the rate at which such materials are accumulated on the bottom, that change during the year (Pech *et al.*, 2007). The feeding habits of shrimp juveniles will match those of an omnivorous or a carnivorous regimen. Assimilated food produces a rapid growth rate around 1 mg day⁻¹ in *L. vannamei* (Gaxiola *et al.*, 2005).

In decapod crustaceans a close relationship between molt cycle and the lunar phases has been shown (Dall *et al.*, 1990). Molina-Poveda *et al.* (2002) observed a synchrony between molt cycle and moon cycle for *L. vannamei* growing in earth ponds, founding 50% of shrimp population in postmolt stages in waning moon, reaching the pick of molting in new moon phase, corresponding to 5 days of low and high tide.

Another relevant aspect related to shrimp growth is the digestive enzymatic capacity to break down nutrients, store energy reserves, and assimilate food items from both planktonic and benthic sources, which are available to shrimp at different life stages. The study of such enzymatic activity is essential to establish the functioning of the shrimp digestive system in relation to food requirements (Le Moullac *et al.*, 1996).

Digestive enzymes are substrate specific. In the carbohydrate digestion of both larvae and postlarvae of *Litopenaeus schmitti*, α -amylase and α -glucosidase maintained a linear correlation in their specific enzymatic activity (Arena *et al.*, 2003). In protein digestion, trypsin is active in nutrient assimilation throughout the shrimp life cycle (Sainz *et al.*, 2004; Cara *et al.*, 2004). However, the physiological conditions at different stages during the molt cycle influence significantly the digestive enzymatic activity of penaeid shrimp (Klein *et al.*, 1996). Just prior to ecdysis, decapod crustaceans cease their feeding activity causing a general reduction in their metabolic level. At this critical stage, the digestive enzymatic activity is almost shut down. Casillas-Hernández *et al.* (2002) indicated that enzymatic activity in the hepatopancreas during premolt and postmolt stages of *Litopenaeus stylirostris* was negligible. However, there is little data about digestive enzymatic process, particularly on individuals undergoing critical molt stages in the cycle. The pink shrimp *F. duorarum* is known for its omnivorous feeding behavior in the juvenile phase although it shifts to carnivory as adult. Even if data on the stomach contents of this native species are available (Schwamborn and Criales, 2000), their digestive capacity has never been used to identify the feeding habits of juveniles in the wild.

It is hypothesized that digestive enzymes activities will be modified as a result of transcription or translation according to food availability during seasons, and molt stages affected by lunar cycle.

In view of the relationship between shifts in diet composition and the activity of the digestive enzymes in penaeid shrimp, the present study focused on the analysis of digestive trypsin and α -amylase activities and their changes in phenotypic expression, during the molt cycle (as endogenous factor) of juveniles of *F. duorarum*, (Pérez-Farfante, 1970) and their changes due to different feeding regimes (exogenous factor) and their changes in the isotopic signature of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as an index of assimilation of food induced by the seasonal availability of food items in the nursery area.

2. Material and methods

The tropical coastal lagoon of Celestún belongs to the biosphere reserve “Ría de Celestún” in Yucatan, which extends along the Gulf of Mexico at 20°51'21" N and 90°23'02" W (Pech *et al.*, 2007). Herrera-Silveira *et al.* (1998) described this lagoon as a karstic site in which the input of freshwater from groundwater discharges varies according to the rain regime (Fig.1). According to the hydrological conditions, this lagoon has three zones: seaward, middle and inner (Herrera-Silveira, 1993).

A total of 1189 juvenile shrimp of *F. duorarum* (mean cephalothoracic length; CL 16.4 mm \pm 0.1) were collected from April 2007 to February 2008 during the dry (March-June), rainy (June-September), and the Nortes (October-February) seasons. Within each sampling period, organisms were collected weekly in each phase of the lunar cycle. Samplings were made in the middle zone of the lagoon during night hours (between 19 and 23 hours), and the temperature, salinity, and dissolved oxygen concentrations were measured with a YSI-556 multiparameter (YSI Incorporated, 1700/1725 Brannum Lane, Yellow Springs, OH 45387 USA). Sediment was obtained with a Van Veen dredge. Two samples of 100 cm³ of the surface material of the lagoon bottom were collected. One of the samples was placed in 150 mL plastic bottle and 0.6 mg L⁻¹ of Mg Cl₂ diluted in the water obtained from the lagoon was added. After one hour, a solution of 10% formalin neutralized with sodium tetraborate was added to preserve the meiobenthos samples. The other sample was placed on ice until preparation for isotope analysis.

Circular surface plankton tows were taken at each sampling station with a 0.5 m diameter net with 500 μ m mesh, equipped with a flowmeter to measure the volume of water sampled. Samples were stored in 4% buffered formalin. Samples were divided and one preserved on ice without any chemical treatment until preparation for isotope analysis. The other was preserved in 10% formalin solution and used for species identification.

STOMACH CONTENTS ANALYSIS

The digestive tract was dissected from individuals while in the field. The foregut was extracted and kept in a 0.6 mg MgCl₂ L⁻¹ solution and preserved in 10 % formalin solution for further stomach contents analysis. Prey items from each stomach were examined under the microscope and identified according to prey types found in plankton and sediment samples collected at the same time. Data were expressed as frequency of occurrence of prey types.

STABLES ISOTOPE ¹³C AND ¹⁵N ANALYSIS

Isotope analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was performed according to Coplen *et al.* (2006) on muscle of *F. duorarum*, estuarine bottom sediment, plankton (>500 μ m) and *Halodule wrightii*. The samples were prepared as follow: (i) only muscle tissue of *F. duorarum* in intermolt stage C was used to avoid water content variations. The exoskeleton was removed and the muscular tissue washed with distilled water, then dried for 24 hours in an oven at 60°C. (ii) Sediment: 10 g of the frozen preserved sample were screened through a 270 μ m mesh to remove inorganic detritus. Four grams of wet sediment were obtained. Ten more grams of the sample, without sifting, remained in a 0.5N HCl solution for one hour to remove carbonates. Both samples were washed 2 times with distilled water then placed in an oven to be dried at 60 °C for 24 hours.(iii) plankton and *Halodulewrightii*: The material collected was treated with 0.5M HCl solution for one hour. Then it was washed twice with distilled water and left to dry in an oven at 60°C for 24 hours and cooled in a

excicator. Subsequently, each sample of muscle, sediment, plankton, and *H. wrightii*, was crushed into a fine powder in a mortar previously washed with water and Ingrain, rinsed with distilled water and acetone, and dried for 24 hours at 60 °C. Samples were stored in 2 ml Eppendorf tubes previously labeled and kept in a foil bag. Elemental analysis was carried out in the Laboratory of Soil Science, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were obtained in the Mass Spectrometry Laboratory, both at the Institute of Geology (UNAM), using a Dumas combustion elemental analyzer coupled to a Delta Plus Mass Spectrophotometer XL, which has an accuracy of 0.2 ‰.

ANALYSIS OF ENZYMATIC ACTIVITY.

Immediately after capture, shrimp hepatopancreas (HP) were dissected and individually, kept in small vials initially preserved in liquid nitrogen, and later maintained in the laboratory at -80° C until analysis. The HP were individually homogenized in 500 μL of distilled water, with a tissue homogenizer and were centrifuged at 14000 rpm at 4°C during 20 minutes and the supernatant was removed for further enzyme analysis.

Trypsin activity was determined according to Gieger and Fritz (1988), with 100 mM BAPNA (benzoyl-arginin-paranitro-anilide, Sigma B7632) as substrate in TRIS 0.1 M pH 8 buffer at 4° C. The hydrolysis rate of substrate was measured as absorbance increment using a spectrophotometer (Spectronic model 21D) at 405 nm during two minutes, with the extinction coefficient $\epsilon_{405}=1.02 \text{ L mol}^{-1}\text{cm}^{-1}$. A unit was defined as 1mM of p-nitroanilidine released in one minute.

The α -amylase activity was measured according to a modification of Bernfeld's method (1955), using 1.5% glycogen (Fluka, 50573) as substrate diluted in a 2.5 mM MnCl_2 , 10 mM NaCl, 10 mM phosphate buffer, at pH 7. Enzymatic activity was expressed as milligrams of maltose liberated per min at 37°C, according to van Wormhoudt (1980).

The specific isozymes of trypsin and chymotrypsin were determined by electrophoresis polyacrylamide gel with sodium dodecil-sulfate (SDS-PAGE) (García-Carreño *et al.*, 1993). The α -amylase isozymes were determined by a method previously described by Arena *et al.* (2003). HPs from each sampling were homogenized in 500 μl of Tris-phosphoric acid buffer (0.06 Mol L^{-1} , pH 7) and centrifuged at 14000 rpm (4°C, 20 min). Conventional 10% vertical polyacrylamide gel electrophoresis was used with Tris-glycine as the running buffer and separation was carried out for 4 h at a constant voltage of 250 V. Gels were then incubated in 3%boric acid for 10 minutes. Subsequently, gels were placed in a 1% starch solution-with buffer phosphate (pH 6) and incubated for 30 min at 37° C. Development of activity in gels was achieved by retiring the starch solution to agar and adding lugol diluted in ultra-pure water in a 1:5 proportion. They were maintained in this state until bands became visible, at which point lugol was retired. The gels were fixed with a 7.5% acetic acid solution and washed with ethanol at 10%.

STATISTICAL ANALYSIS.

For digestive enzymes activity a bifactorial ANOVA of 3 X 6 (3 for seasons and 6 for molt stages) of Log_{10} transformed data was used to analyze the interactions among factors. For all cases a probability level of 0.05 was used (Zar, 1996).

3. Results

The effect of season on surface water temperature was notable between the rainy season (mean 30.7 °C) and Nortes season (mean 26.8 °C). Salinity values were highest during dry season (27 psu) and were lowered by the input of fresh water in the rainy season (22 psu) (Table 1).

Juvenile *F. duorarum* in premolt stage D₀ (35.5%) comprised the majority of the captured shrimp, followed by those in intermolt stage C (20%) , and premolt stage D₁' (19.6%). Molt stages C and D₀ are the ones of the largest duration and while in these stages, shrimp display an active feeding behavior. During the last premolt (D₁'', 11.2%; D₁'''', 3.1%) and early postmolt stages (A, 1.6%; B₁, 4.25%) organisms burrow and do not feed and therefore they are more difficult to capture (Fig. 2).

STOMACH CONTENTS

During dry, rainy, and Nortes season, 17.3%, 46.6%, and 63.9% of the 124 stomachs analyzed were full. A total of 16 taxa were identified in the stomach contents. The number of taxa identified increased between the dry (7 taxa), rainy (11 taxa) and Nortes (12 taxa) seasons. The amorphous animal tissue percentage diminished in the rainy and Nortes seasons (Table 2).

Plant material in the stomach contents represented between 7% and 8% in the three seasons and filamentous algae, diatoms, and the seagrass *Halodule wrightii*, could be identified. The latter represented 2.5% in the dry season, 27.5% in the rainy season, and 13.6% in the Nortes season (Table 2, Fig 3).

STABLE ISOTOPES ($\delta^{13}\text{C}$ AND $\delta^{15}\text{N}$)

Isotopic signatures of shrimp tissue showed seasonal differences. Higher values of $\delta^{13}\text{C}$ were found during dry and Nortes seasons and were similar to those found for the *H. wrightii* samples. The $\delta^{13}\text{C}$ isotopic signature of the shrimp tissue captured in the rainy season was similar to the zooplankton values. The lowest $\delta^{13}\text{C}$ value was observed in sediment (Table 3). The $\delta^{15}\text{N}$ values found in shrimp tissue were highest in the dry season and lowest in the Nortes season. Values from sediment, plankton and *H. wrightii* were lower than shrimp tissue values (Table 3).

DIGESTIVE ENZYME ACTIVITY

A significant interaction ($p < 0.05$) between season and molt stage was found for trypsin activity in juvenile shrimp's HPs (Fig. 4). In the dry and rainy seasons, trypsin activity did not change during the various molt stages. In contrast, in the Nortes season trypsin activity was significantly lower while organisms were in molt stage D₀ increasing steadily until ecdysis ($p < 0.05$).

The α -amylase activity also exhibited a significant seasonal variability ($p < 0.05$), particularly in the dry season. During the Nortes season, the α -amylase activity reached values of 674 and 457 mU mg⁻¹ HP at molt stages B₂ and C, respectively (Fig. 5).

The amylase:trypsin ratio (A/T) calculated for the three seasons and 6 molt stages showed the lower value in the dry season and reached the higher values in the Nortes season, especially while organisms were in late premolt stages (Table 4).

TRYPsin AND α -AMYLASE ISOFORMS.

Polyacrylamid gels revealed 3 isoforms for trypsin during a molt cycle. The gel that contained trypsin from shrimp captured in the rainy season showed the main expression during a molt cycle. Three genes were identified. From the molecular weight viewpoint, the first isoform was localized at 19.4 kDa, the second one at 20.7 kDa and the third one at 22 kDa. In the gel showing the expression of trypsin in the rainy season, only a functional gene, at 19.4 kDa, was observed in postmolt stages A and B₁. The rest of the molt cycle displayed all three of the identified isoforms. During the Nortes season, the presence of two isoforms of trypsin, at 19.4 kDa and 20.7 kDa, was observed throughout the molt cycle (Fig.6).

Another protease, chymotrypsin, was observed in bands located between 24 kDa and 36 kDa. Although its presence could be identified in the three seasons, it was more noticeable during the dry season (Fig 6).

The expression of α -amylase changed with each season. This enzyme is a complex of two systems that are differentially expressed. System I can be expressed by three alleles while system II can be expressed by 5 or 6 alleles. In the juvenile shrimp evaluated in all seasons, system I was represented by only one allele. System II, in the dry season, was expressed by 3-4 alleles and in the rainy season it was expressed by 5-6 alleles throughout the molt cycle. In the Nortes season, system II was expressed by fewer alleles, except in premolt stage D₁ (6 alleles) and postmolt stage A which was not represented (Fig.7).

4. Discussion

The results obtained in the present study showed that *F. duorarum* juveniles display different biochemical adaptations for the digestion and assimilation of food in the wild. The environmental conditions of Celestún are strongly affected by the seasonal cycle, especially considering the wind, temperature, and rainfall regimes, which in turn modifies the nutrient input that is correlated with changes in benthic diversity (Pech *et al.*, 2007). During the dry season, Celestún lagoon displays low primary and secondary productivity (Tapia-Gonzalez *et al.*, 2008), which are reflected in the stomach content of *F. duorarum* sampled at this time of the year, when low prey occurrence and low stomach fullness were observed, and coinciding with low trypsin and α -amylase activity in the HPs as well as the highest isozyme expression of both digestive enzymes. As the lagoon productivity increased in the rainy season (Tapia-Gonzalez *et al.*, 2008), the percentage of full stomachs also increased, but digestive enzymes activities remained low. A peak of activity appeared during the Nortes season, corresponding to the lowest isozyme expression of the digestive enzymes. Within this season, activity of both enzymes reaches a peak in organisms in post molt and intermolt stages, when high feeding activities are usually found.

The occurrence of prey in the stomach contents of shrimp changed according to the seasonal variations in the benthic community of the Celestún lagoon (Pech *et al.*, 2007). An increment of crustacean abundance was observed between the Nortes and the dry season for both in the benthic community and in the stomach contents of juvenile pink shrimp. Copepods and amphipods, together with other groups such as ostracods and nematodes, dominated the stomach contents of shrimp (Table 2). Schwamborn *et al.* (2000) and Sánchez *et al.* (2002) also found that the main groups upon which *F. duorarum* feeds are copepods and amphipods allow classifying this species as a carnivore. But the

feeding habits of this species change according to variations in prey availability, which is controlled by changes in the abiotic factors such as temperature and light intensity, which modifies both the productivity of the system and the activity of the digestive enzymes in shrimp's HPs (Alpuche *et al.*, 2005). Molting, controlled by the lunar phases, is one of the biotic factors that affects the feeding habits of juvenile *F. duorarum* as previously reported for *Parapenaeus longirostris* (de Coursey, 1983). In the present study, we sampled organisms every 7 days, and the distribution of molt stages in all phases of lunar cycle shows the predominance of intermolt stages, particularly stage C which has the longest time span (Fig. 2). Organisms in molt stages closed to ecdysis had empty stomachs since shrimp are unable to use the mouth structures formed by chitin, hence restraining the possibilities of food ingestion while the exoskeleton has not hardened up (Le Moullac *et al.*, 1996, Vega-Villasante *et al.*, 2000; Molina-Poveda, 2002). At these molt stages there is a drop of the enzymatic activity, especially in late premolt (D₁'') and in postmolt (A and B). During the phase of tegument stability, in intermolt C, the enzymatic activity of trypsin and α -amylase increased especially during dry and Nortes seasons (Fig. 4 y 5). As seasonal patterns in the physicochemical parameters of the environment modify prey availability, organisms change their feeding habits to maximize ingestion. During the rainy season, we found the lowest values of the enzyme activity, in correspondence with the stressful characteristics of the habitat such as high water temperature and that leads to a scanty mobility of shrimp. The highest mean temperature (30.7 °C) was measured during this season when trypsin activity should also be the highest activity reaches the maximum values at temperatures between 40 and 70°C (Le Moullac *et al.*, 1994; Sainz *et al.*, 2004). Nevertheless, salinity was below the optimum for the species (22 psu). Trypsin activity reached a maximum during Nortes season, with a 148% increase compared to the value obtained during the dry season (Fig 4). Changes in the environment are mirrored by variations in the enzyme activity that enhances survival in such a habitat. When a higher load of nutrients is present in the lagoon, we found the highest α -amylase activity. Trypsin and α -amylase are complementary and account for 60% of the digestive processes (Lovett y Felder, 1990 van Wormhoudt *et al.*, 1998).

Animals reduce their food intake at low temperatures due to a decrease in the metabolic activity in the cold season as described, for example, for *L. stylirostris* (Wabete, 2006). α -amylase is expected to decrease in a lack-of-food event and, accordingly, low values (120mU mg⁻¹) were found during the dry season while trypsin activity showed an opposite trend.

A/P or A/T ratios give an indication on the level of herbivory or cannivory at each time of the year (Rodriguez *et al.*, 1994 and Jones *et al.*, 1997, Gaxiola *et al.*, 2009). This ratio was used to classify *Homarus americanus* as more herbivorous than peneids (Jones *et al.*, 1997). It was possible to identify the sequence of the carnivore - herbivore shift during the molt cycle both in the rainy and Nortes seasons, since annual changes in the values of $\delta^{15}\text{N}$ allow to classify *F. duorarum* as a carnivore in the dry season (trophic level 5), and as an opportunistic generalist during rains and Nortes (trophic level 4; Fry, 1988). During these seasons there is an increase in the ingestion of vegetal materials (Table 2) accompanied by an increase in the A/P ratio. In intermolt stage D₀ cells of HP produce hemocyanin and the increase in ratio A/P at premolt stages prefigured a decrease in trypsin rather than an increase in amylase activity. The variations of the expression of the enzymatic activity examined by polyacrylamide gels (figs. 6, 7) showed three bands next to 20 kDa in the specific case of trypsin that can be referred to the forms a, b and c of trypsin *F. duorarum*, in contrast to the five isoforms reported for *L. vannamei* (Klein *et al.*, 1996). The presence of chymotrypsin as indicated by the band located between 24 and 29 kDa. This enzyme is needed to complete the hydrolysis processes of a variety of materials that are usually consumed by crustaceans (Lovett and Felder, 1990; Le Moullac *et al.*, 1996).

Band patterns of α -amylase are similar to those reported by Van Wormhoudt *et al.* (2003), showing system I formed by three alleles and system II formed by five. Low values of α -amylase expression, with five isozymes, were found in the Nortes season. At this time, a low value of the A/P ratio (2.8 ± 0.8) was also found. On the other hand, high values of α -amylase activity, with a high A/P ratio (10.7 ± 0.7), were found during Nortes when gastric repletion reached a maximum with high occurrence of copepods and members of Malacostraca. Vegetal detritus was present in low percentages, especially when compared to the dry season. The increment in copepod occurrence in the Nortes season could be related to the increase of phytoplankton, which is their principal source of food (Hernández-Guevara *et al.*, 2008).

Variation of $\delta^{13}\text{C}$ values from -18‰ (rainy season and carnivory), -21‰ (in the dry season and omnivory) to -24‰ (Nortes), were observed in the shrimp muscle. The shift towards herbivory can be inferred based on the stomach contents analysis that show greater values of occurrences of *Halodule wrightii* (which $\delta^{13}\text{C}$ value was -23‰). Even if remains of other phytobenthic materials were found in the stomachs, $\delta^{13}\text{C}$ values of sediment and plankton were -7‰ and -18‰ , respectively. In the Nortes season, winds produce water currents that facilitate the permanence of shrimp into the lagoon enhancing the importance of the seagrass habitat for shrimp during this time of the year. Marine or continental influences (Pech *et al.*, 2007; Herrera Silveira *et al.*, 1999) have an impact on the isotopic signature of shrimp tissue according to the origin of food sources at each time of the year. The understanding of the dynamics of the shrimp's habitat would contribute to a better protection of this resource in the future, which is crucial for this native species.

Conclusion

The results obtained in the present study showed that *F. duorarum* juveniles display different biochemical adaptations for the digestion and assimilation of food in the wild. A synergistic effect of molt stages and seasonal variation of food was observed on trypsin and α -amylase activities of *F. duorarum* wild juveniles. However changes were only observed on α -amylase enzyme through phenotypic expression due to these factors.

The accumulation of organic reserves to meet the energy demand required by in the various stages of the molting cycle of juvenile *F. duorarum* correlates well with the expression of hepatopancreatic enzymes such as trypsin and α -amylase. Both enzymes displayed a significant variability throughout the annual period of observations of this study. However, their activity is apparently triggered by the nature of the food available in the shrimp's nursery area. Higher activity of trypsin was observed in the dry season, compared to rainy season, when values of gastric repletion reached a minimum but the occurrence of vegetal material in the stomachs reached its maximum (2%) in coincidence with the highest value of $\delta^{15}\text{N}$ (14‰). At this time of the year, shrimps can be situated in the fifth trophic level. The $\delta^{13}\text{C}$ value of -20.6‰ suggests that juvenile *F. duorarum* fed on zooplankton of marine origin, rather than material associated with the sediment.

In the rainy season, the isotopic signature of $\delta^{13}\text{C}$ indicates that shrimp consume materials of littoral or land origin, consistent with the drag of organic matter due to the rain.

During Nortes, the isotopic signature of nitrogen was lower enough (11‰) to position juvenile *F. duorarum* in the fourth trophic level, as a general opportunistic feeder. At this time of the year, values of $\delta^{13}\text{C}$ are close to the ones of *H. wrightii* that serve as a refuge habitat as currents in the lagoon increase due to the increase of northerly winds.

The limited composition of food resources for *F. duorarum* in the dry season, contrasting with the abundance was observed during the Nortes season. However, the activity of trypsin made it clear the use of proteins as the main nutrient in the three seasons. The α -amylase was expressed in digestive processes as part of a compensatory mechanism with

carbohydrates intake. Incidentally, they were scarce in the diet of *F. duorarum* during the three seasons studied, especially during the Nortes season. Copepods and Malacostracea predominate in the stomach content during Nortes season. It is therefore possible to state that the modulation of enzymatic expression in *F. duorarum* is subject to the type and abundance of food components available in each season of the year; physiological changes associated with cyclical periods of ecdysis acted upon enzymes expression as well.

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Tables

Table 1. Parameters monitored at the beginning of each sampling. (Moon phases: 1/4 = waning, 2/4 = new, 3/4 = crescent and 4/4 = full).

Annual season	Moon phases	Start hour	T (°C)	Salinity (ups)
Dry	1/4	19:36	29.5	33.0
Dry	2/4	20:02	25.0	18.0
Dry	3/4	20:15	29.0	29.0
Dry	4/4	19:00	29.5	28.0
	Mean =		28.2	27.0
Rain	1/4	20:10	30.3	17.6
Rain	2/4	20:45	30.9	19.6
Rain	3/4	20:30	30.0	32.8
Rain	4/4	20:00	31.7	18.0
	Mean =		30.7	22.0
N	1/4	20:22	26.2	13.1
N	2/4	19:48	27.1	20.6
N	3/4	19:38	26.1	31.3
N	4/4	19:25	27.8	33.5
	Mean =		26.8	24.6

Table 2. Food composition in stomach of juveniles *Farfantepenaeus duorarum* collected in annual season. Frequency of occurrence and Index of Importance (IIMP) of prey (N = 124). * (% of volume).

Food type	Occurrence (%)			IIMP (%)	References related
	Dry	Rainy	Nortes		
Malacostraca Amphipoda	7.50	47.50	65.90	19.69	a,b
Isopoda	0.00	22.50	9.00	1.47	
Decapoda	2.50	5.00	2.20	1.30	b
Brachiopoda Anostracoda	0.00	0.00	4.50	0.25	
Cladocera	7.50	2.50	22.70	5.14	
Copepoda	12.50	50.00	77.20	29.34	a,b
Ostracoda	5.00	32.50	6.80	7.83	a
Pterygota Diptera	0.00	32.50	22.70	10.26	
Opisthobranchia Gastropoda	2.50	0.00	0.00	0.74	a
Granuloreticulosa Foraminifera	0.00	52.50	0.00	5.65	a
Acari Halacaroida	0.00	0.00	4.50	0.37	
Nematoda	7.50	2.50	29.50	9.60	a
Annelida Oligochaeta	0.00	2.50	9.00	0.82	
Polychaeta	0.00	7.50	9.00	1.02	a,b
<i>Halodule wrightii</i>	2.50	27.50	13.60	6.52	
Amorphous animal tissue*	79.00	55.00	64.00		
Plant detritus*	8.00	7.00	7.00		
Sand*	11.00	19.00	13.00		

a: Schwamborn and Criales, 2000 (*F. duorarum*); b: Sánchez et al., 2002 (*L. schmitti*).

Table 3. Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), C and N values of juvenile *Farfantepenaeus duorarum*, sediment, plankton and *Halodule wrightii* from Celestun lagoon (N = 31). * (≥ 0.27 mm).

	$\text{d}^{15}\text{N}_{\text{AIR}}$ (‰)	$\text{d}^{13}\text{C}_{\text{VPDB}}$ (‰)	%N	%C
Season				
Dry	14.21 ± 1.99	-21.64 ± 1.17	14.92 ± 0.14	44.88 ± 0.4
Rainy	12.75 ± 2.05	-17.68 ± 1.58	9.69 ± 0.32	43.87 ± 0.99
Nortes	11.86 ± 1.16	-23.06 ± 0.73	14.32 ± 0.14	45.10 ± 0.39
Productivity				
Sediment *	8.86	-8.04	0.33	13.7
Dry				
Rainy	9.04	-7.58	0.37	13.57
Nortes	8.51	-7.22	0.30	13.28
Plankton *				
Rainy	8.22	-10.63	0.85	16.26
Nortes	7.68	-18.43	5.06	34.44
<i>Halodule wrightii</i>	5.58	-20.28	1.8	36.2

Table 4. Ratio amylase/trypsin for the identification of the level of herbivory. (Mean \pm Standard Error).

Annual season	B ₂	C	D ₀	D ₁ '	D ₁ ''	D ₁ '''
Dry	2.3 \pm 1.6	3.6 \pm 0.5	3.6 \pm 0.4	1.9 \pm 0.2	2.8 \pm 0.2	2.6 \pm 0.2
Rain	6.1 \pm 1	8.9 \pm 1.8	9.8 \pm 0.7	8.3 \pm 1.4	12 \pm 3	11 \pm 2
Nortes	7.9 \pm 2.5	4.3 \pm 1.4	9.9 \pm 1.1	8.6 \pm 1.6	19 \pm 5	16 \pm 2

Figures



Figure 1
Geographic position of Celestun lagoon and sampling area.

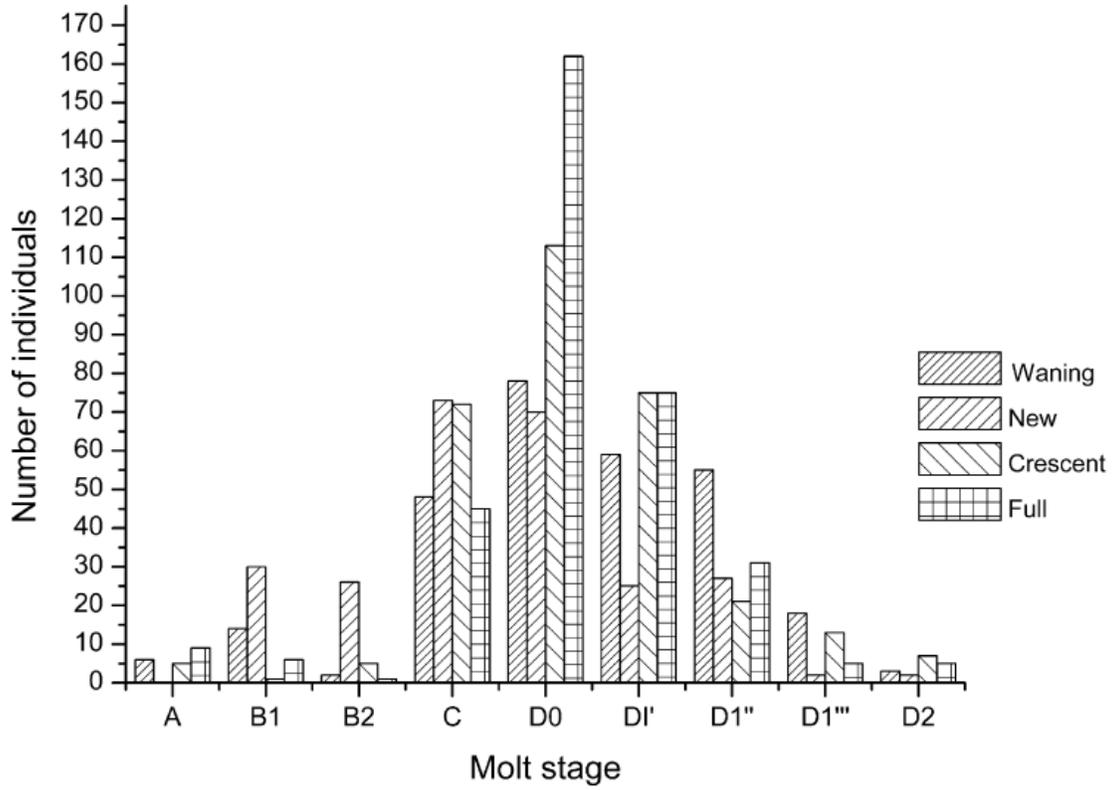


Figure 2
 Distribution of the number of juvenile *Farfantepenaeus duorarum* per molt stage and moon phase (N = 1189).

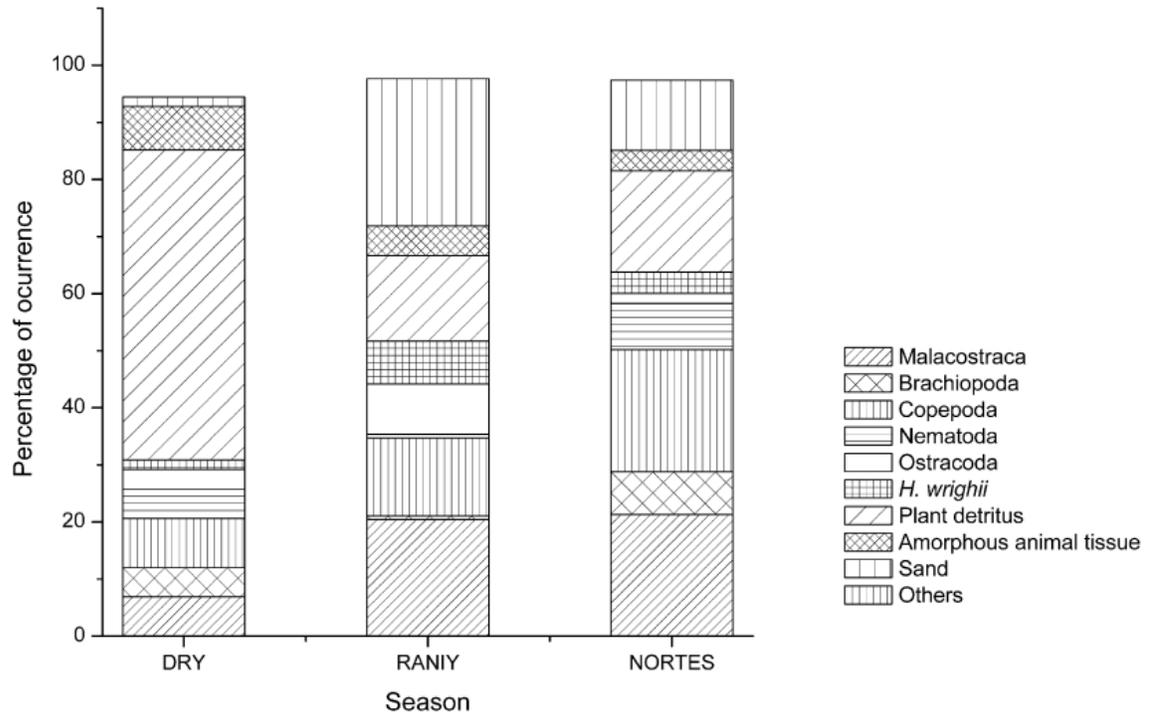


Figure 3
Composition of the diet of *Farfantepenaeus duorarum* in dry, rainy and Nortes seasons (N = 124).

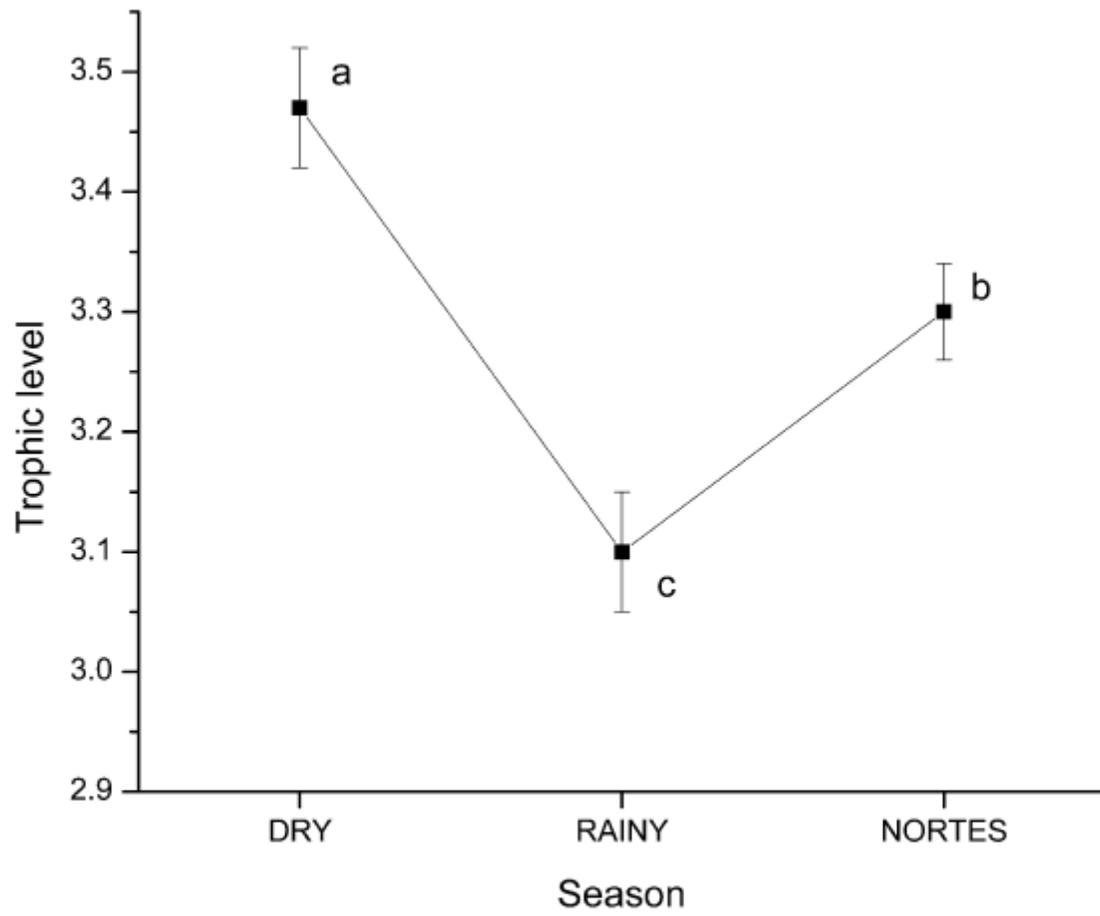


Fig. 4. Variation of trophic level calculated in *Farfantepenaeus duorarum* (N = 124).

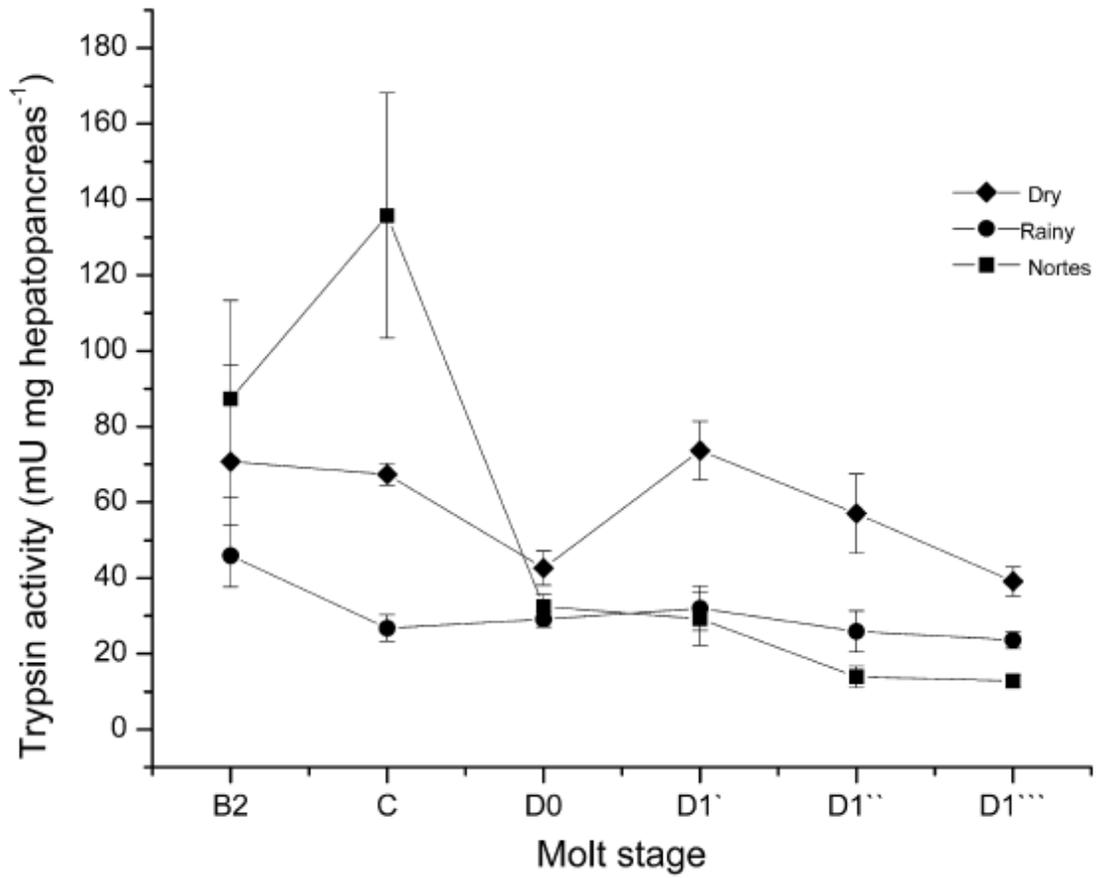


Fig. 5. Trypsin activity (mU mg^{-1} hepatopancreas) of juvenile *Farfantepenaeus duorarum* during an annual cycle. Interaction between molt stages and annual seasons ($N = 89$).

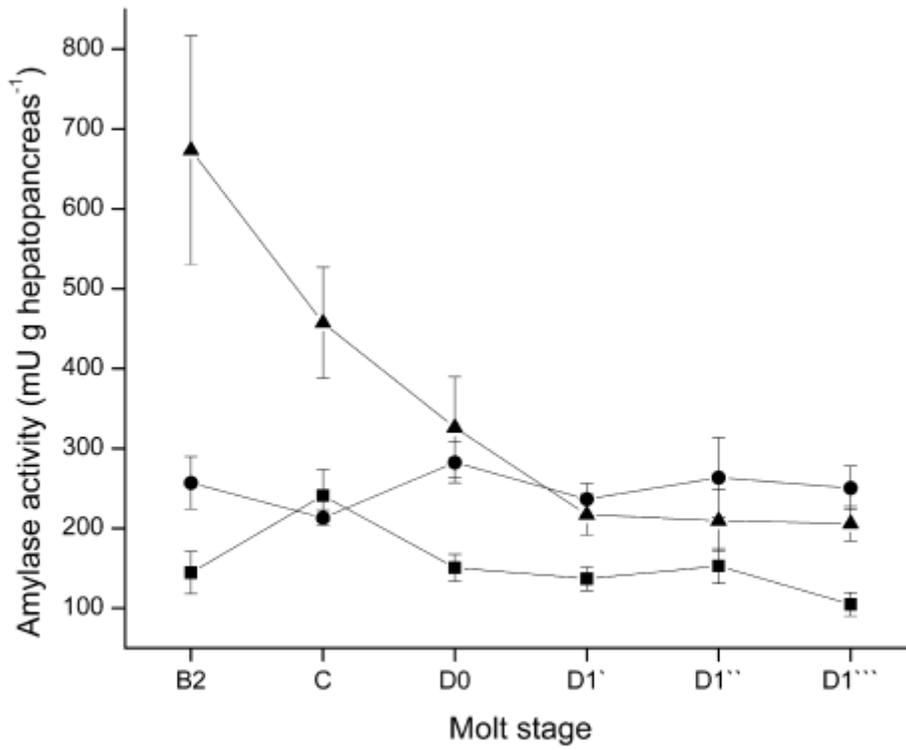


Fig. 6. α -amylase activity (mU mg^{-1} hepatopancreas) of *Farfantepenaeus duorarum* sampled during the three main seasons. Interaction between molt stages and annual seasons ($N = 89$).

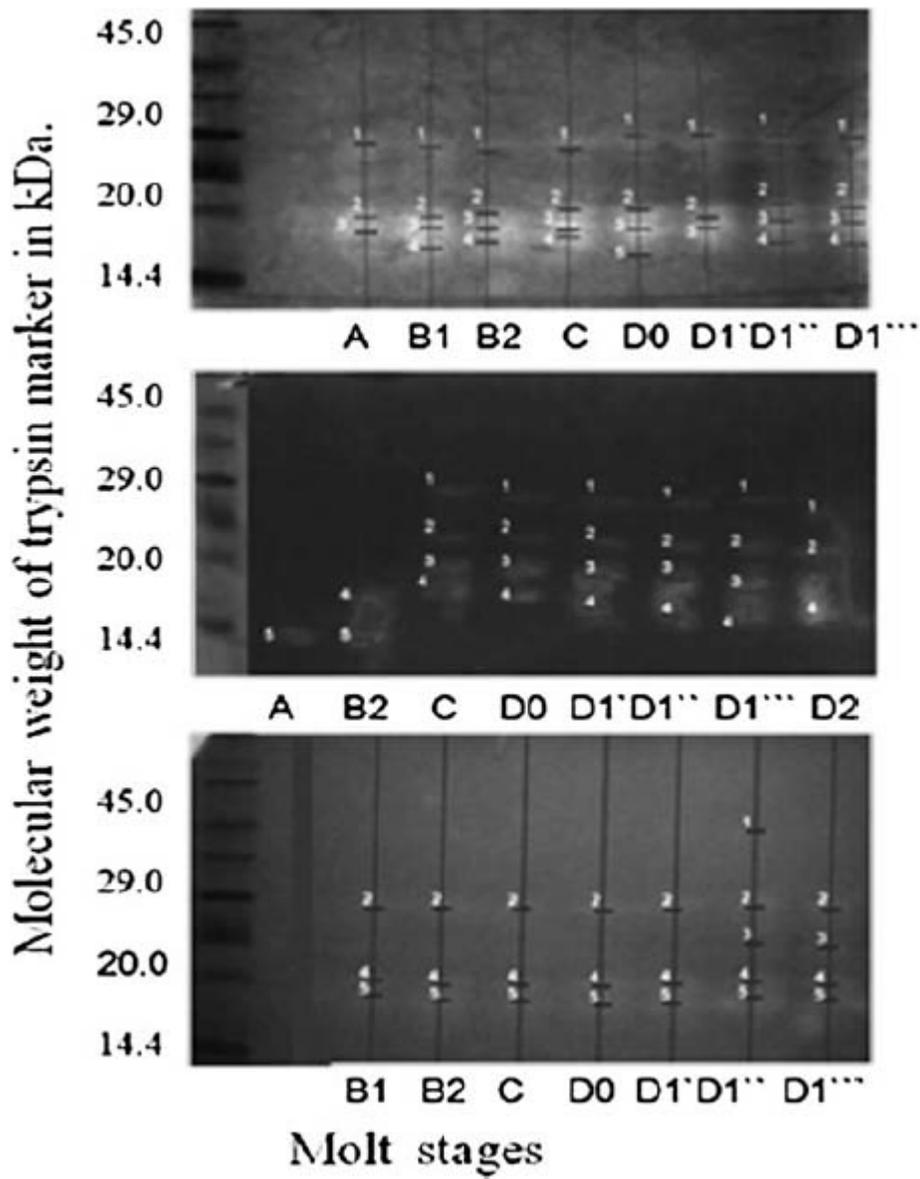


Figure 7. Trypsin isoforms of *Farfantepenaeus duorarum* during the three determined sampling period from April 2007 to February 2008. MWM: Molecular weight of marker that identify trypsin in 19.4, 20.7 and 22.0 kDa (N = 89).