**Journal of Crustacean Biology**
July 2012, Vol. 32 (4) : Pages 597-606
http://dx.doi.org/10.1163/193724012X630679




# Physiological and Biochemical Changes of Wild and Cultivated Juvenile Pink Shrimp *Farfantepenaeus duorarum* (Crustacea: Penaeidae) during Molt Cycle

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**Abstract:**

Changes in metabolite levels in hemolymph and hepatopancreas were used as indicators of physiological status of juvenile wild and cultivated *Farfantepenaeus duorarum* (Burkenroad, 1939), during hyper-osmoregulatory conditions (salinity 22 ppt, 726 mOsm/kg). We analyzed the relationship between biochemical changes of wild and cultivated shrimp at different molt stages by measurement of osmotic capacity, hemocyanin, acylglycerides, cholesterol, glucose, glycogen and total protein. Biochemical analyses of hemolymph (blood) and hepatopancreas showed a decreasing trend in stages closer to molt and an increasing trend before intermolt. Osmotic capacity and blood proteins indicated a significant effect of the molt stage. Stages A, D1′ and D1‴ showed the greatest effect on the biochemical variables. Higher hemocyanin, cholesterol and glycogen concentrations were observed in wild shrimp than in cultivated shrimp. The effect of the molt cycle was associated with changes in cyclic ions and water balance that produced chemical changes in the hemolymph in both wild and cultivated.

**Keywords:** biochemical analysis, *F. duorarum*, molt stages, osmoregulation

In crustacean biology, the molting process constitutes a physiological crisis that shrimp undergo at intervals (Passano 1960). Moreover, there are cyclic changes that will affect the structure of the integument, as well as morphological and physiological variations that are controlled hormonally (Bliss 1985; Anger 2001). This physiological crisis is resolved through many events that condition its success. Hence, the molting process, which is a means of development and growth for an animal with an exoskeleton, dominates the life of the crustacean, as well as most of its physiological processes. Metabolism, behavior, reproduction, and osmoregulation will be involved. These processes are influenced either directly or indirectly by the periodic replacement of the integument and underlying cycles of metabolite accumulation (Kuo and Lin 1996). The shrimp molt cycle is generally divided into the following phases: postmolt (A, B1, and B2), intermolt (C), premolt (D0, D1´, D1´´, D1´´´, and D2) and molt (ecdysis) phases as described for Crustacea (Drach and Tchernigovtzeff 1967) and adapted to shrimp (Aquacop et al. 1975).

In recent years, blood metabolites, and hemocyanin in particular, have been used as indicators to monitor the changes in physiological condition of pink shrimp, Farfantepenaeus duorarum, when exposed to different environmental conditions (Rosas et al. 2007). In addition, biochemical changes in hemocyanin, glucose and glycogen have been studied throughout the molt cycle in white shrimp, Litopenaeus vannamei, and have been related to variations in osmotic capacity (Galindo et al., 2009). Hemocyanin is an important protein in shrimp, and its levels are affected both by the molting process (Bursey and Lane 1971a; Cheng et al. 2002) and by dietary protein levels (Rosas et al. 2006). The digestive gland, the hepatopancreas, is an important gland for storage of glycogen (Loret 1993), which is used in the formation of the exoskeleton (Rosas and Carrillo 2006). Variations in osmoregulatory capacity and blood glucose concentrations have also been studied in order to detect physiological stress (Charmantier et al. 1989, 1994; Mercier et al. 2009; Aparicio-Simon et al. 2010) or the effect of toxic compounds (Lignot et al. 2000), or to relate these variations to molt stages (Galindo et al. 2009).

Studies have shown that there is a linear relationship between hemolymph and medium osmolality at a certain temperature (Chen and Lin 1998). An osmolality of 768 mOsm/kg, at which the hemolymph is isosmotic to the ambient seawater, is reported for F. duorarum in intermolt stage is around salinity 26 ppt (Williams 1960; Castille and Lawrence 1981) and 29 ppt (Bursey and Lane 1971b). However, it is difficult to express the isosmotic conditions experimentally, especially when the osmoregulatory capacity varies with the molt stage in an experimental medium (Charmantier et al. 1994), and in natural conditions.

Cholesterol is an important cell constituent in crustaceans, and is a precursor of steroid hormones and molt hormones. However, the crustacean cell is unable to synthesize cholesterol or other sterols *de novo*, so these can only be obtained from the diet (Teshima 1972).

Higher levels of acylglycerides have been found in the hepatopancreas and hemolymph of wild-caught L. vannamei spawners than in pond-reared individuals (Palacios et al. 2000). Cultured shrimp, or shrimp in the process of domestication, have had altered protein requirement and a different capacity to use nutrients such as glucose from their diet (Cuzon et al. 2004).

The aim of the present research was to determine metabolite changes in blood and digestive gland (hepatopancreas) throughout the molt cycle of wild and cultivated juvenile pink shrimp, fed 40% animal-based protein diet in hyper-osmoregulatory capacity (hyper-OC), or salinity 22 ppt, and constant temperature (28 C). The physiological and biochemical status of F. duorarum juveniles in wild and cultivated populations was monitored in order to identify the main differences.

## Material and Methods

### Wild-caught shrimp

Wild pink shrimp of about 4 g individual wet weight were collected during the night in a lagoon (salinity around 30 ppt) in Sisal, Yucatan (21°09´48´´ N, 90°01´65´´ W), on the Gulf of Mexico. Animals were transported to the laboratory of UMDI-Sisal and acclimated to culture condition and fed with artificial diet (Table 1) for 15 days before the start of the experiment.

### Cultivated shrimp

Second generation (F2) juvenile pink shrimp of 4 g were sampled in the laboratory of shrimp production at UMDI-Sisal, from wild broodstock captured offshore in the Gulf of Mexico. We maintained the water at salinity 22 ppt, temperature at 28 C, and fed the shrimp with a 40% animal protein based diet (Table 1) until the start of the experiment.

### Experimental design

There were two treatments: wild and cultivated. We developed a recirculation water system with ten 40 L tanks, each with 9 divisions. One of the tanks was used for aeration while the others were used to isolate individuals, at 80 individuals per treatment. To obtain the isosmotic point for juvenile F. duoraurm, a previous experiment measured the osmotic pressure of the hemolymph and water in different salinities (Table 2). Water temperature (28 C ± 1 C) and salinity (22 ppt corresponding to a mean osmolality of 726 mOsm/kg which is hyper-OC of F. duorarum) were controlled in the system. The daily photoperiod was 12 hours light: 12 hours dark. Shrimp were fed in three daily portions (at 0800, 1400, and 2000) with a 40% animal protein diet (Table 2). These conditions were maintained for 60 days until biochemical analysis. Molt stage of each individual was determined by the setal development of the uropod (Drach and Tchernigovtzeff 1967; Aquacop et al. 1975).

### Extraction of blood

Hemolymph (100 µL) was sampled in 80 wild and 80 cultivated pink shrimp (8 individuals per molt stage) with a new syringe previously rinsed with an anticoagulant solution (SIC- EDTA (SIC: 450 mM NaCl, 10 mM CaCl, 10 mM KCl, 10 mM Hepes, 10 mM EDTA-Na2, 1033 mOsm/kg, pH 7 at 2-8 C). This solution was discarded immediately before the syringe was used to puncture the ventrolateral sinus of the first pleomere to obtain hemolymph (Vargas-Albores et al. 1993). Hemolymph was placed on a piece of parafilm® above cooling gel to keep the sample cold. Subsamples were taken to assess hemocyanin and blood analysis of metabolites (glucose, cholesterol, acylglycerides, and total protein) (Pascual et al. 2007). Immediately after obtaining the hemolymph, osmotic pressure was measured with a micro osmometer (American Advance Instruments). Osmotic capacity (mOsm/kg) was calculated as the difference between the osmotic pressure in hemolymph and the external medium (Charmantier et al. 1989; Lignot et al. 2000).

## Extraction of hepatopancreas

The hepatopancreas (HP) of wild and cultivated shrimp was removed through an incision on the back of the cephalothorax and weighed. Two portions of HP were placed in a 1.5 mL Eppendorf tube and preserved in liquid nitrogen. Samples were maintained at -80 C until further analysis of glycogen and HP metabolites (glucose, cholesterol, acylglycerides, and total protein).

## Biochemical analysis

Hemocyanin (mmol/L) concentrations were determined from 10 µL of hemolymph diluted in 990 µl of distilled water in a quartz cuvette. Absorbance at 350 nm was measured with a spectrophotometer (Bio-Rad), manually calibrated with distilled water. The final concentration was determined according to Chen and Cheng (1993) using the coefficient of extinction of hemocyanin (17.26) and factor of dilution.

To obtain glucose, cholesterol, acylglycerides and total protein concentrations in blood, plasma was extracted that had been previously diluted (1:20) in an isotonic solution with a complex developed to avoid coagulation of the hemolymph of shrimp (SIC-EDTA) according to Vargas-Albores et al. (1993) considering a hemolymph-anticoagulant ratio equal to 1:2. The hemolymph diluted in an anticoagulant was centrifuged at 2500 revolutions per minute (rpm) for 3 minutes at 4ºC and the supernatant was collected in Eppendorf tubes. Glucose, cholesterol, acylglycerides, and total protein (Bradford 1976) determinations were made in plasma aliquots of 20 µL with 200 µL of reactive solution (Kits ELITech GPSL-5505; ELITech CHSL-0507; ELITech TGML-0427; Bio-Rad cat. 500-0006 respectively), which were placed in microplates and read in an ELSA lector (Bio-Rad Benchmark Plus) at 500 nm and 595 nm for total protein. SIC-EDTA was used as a blank. The final concentrations (µg/mL) were calculated from a calibration curve, in which the standard was the substrate that acts as the reagent in the kit.

We homogenized the HP in 500 ml of distilled water for 2 minutes. Aliquots of 20 µL were taken to determine cholesterol and acylglycerides with 200 µL of reactive solution (Kits ELITech CHSL-0507; ELITech TGML-0427 respectively). The rest of the sample was centrifuged at 2500 rpm for 3 minutes at 4 C, and 20 µL of the supernatant was collected in Eppendorf tubes to determine glucose and total protein (Bradford, 1976) with 200 µL of reactive solution (Kits ELITech GPSL-5505; Bio-Rad cat. 500-0006 respectively). A blank was considered with distilled water and the final concentrations (µg/mg) were calculated from a calibration curve.

Glycogen in hepatopancreas (HP) was extracted in trichloroacetic acid (TCA 5%), homogenized for 2 minutes, and determined through the reaction with sulfuric acid and phenol (Dubois et al. 1965), after was centrifuged for 6 minutes at 7000 rpm. The supernatant was quantified and 200 µL were pipetted into a tube and mixed with 500 µL of 95% ethanol. Tubes were placed in an oven at 37 C for 3 hours. After precipitation, the tubes were centrifuged at 7000 rpm for 15 minutes. The glycogen pellets were dissolved adding 0.5 mL of boiling water. One mL of concentrated sulfuric acid and 200µL phenol (5%) was added and mixed. The contents of the tubes were transferred to microplates in triplicate and read at 490 nm with a microplate reader (Bio-Rad Benchmark Plus). A blank and a standard with glucose were considered, and the final concentration of glycogen (µg/mg) was determined according to the equation of Carroll et al. (1955).

## Statistical analysis

Differences in osmotic capacity, hemocyanin, glucose, cholesterol, acylglycerides, total protein, and glycogen among the molt stages were averaged and assessed with two-way ANOVA after normality was checked. To analyze effects of biochemical variables among molt stages in blood

and HP a model using empiric orthogonal functions analysis (EOF) or principal component analysis (Gómez-Valdés and Jeronimo 2009), through MATLAB version 2007a modeling, was used. EOF is a multivariate analysis technique that is to derive the dominant patterns of variability from a statistical field (a random vector) (Von Storch and Zwiers, 2001). We analyzed a matrix with blood and HP biochemical data, grouped by molt stages and compared between wild and cultivated shrimp. It was possible to extract quantitative information about the source of variation and correlation to compare effects of molt stages in biochemical and physiological status of F. duorarum.

## Results

Some wild F. duorarum in stage $D_2$ died from stress prior to biochemical analysis; therefore blood measurements failed and HP removed were not taken into account.

### Osmotic capacity

A previous experiment with juvenile F. duorarum with a major range of salinities and under the same conditions showed that the hemolymph is isosmotic at salinity of 26 ppt (853 mOsm/kg) (Table 2, Fig.1). In both wild and cultivated shrimp, osmotic capacity differed significantly ($P < 0.05$) and independently of the molt stages. The mean values were highest in intermolt stages D2 and A. Values were higher in cultivated shrimp than in wild shrimp (Fig. 2), except in stage A, where values were lower in cultivated than in wild shrimp.

### Hemocyanin

Concentration levels of hemocyanin in hemolymph were higher in wild shrimp than in cultivated shrimp. In wild shrimp, concentrations differed significantly among molt stages ($P < 0.05$) and were lowest in late postmolt (B1), intermolt (C), and early premolt (D0) stages. In cultivated shrimp, no significant difference was observed in relation to molt stage, although there was a trend towards an increase before ecdysis (Fig. 3).

### Acylglycerides

Acylglyceride concentrations did not differ significantly with molt stage in both wild and cultivated shrimp ($P > 0.05$). In wild shrimp, concentrations in HP differed significantly among molt stages ($P < 0.05$); there was an inverse relationship between concentrations in blood and HP at times when ecdysis occurred. The highest concentration in blood occurred in the early postmolt (B1) stage, the same stage at which the lowest concentration in the HP occurred (Fig. 4). In cultivated shrimp, there was a trend towards an increase in concentration in intermolt stage in HP with the exception of the premolt D1´´ stage, where a decrease was observed.

### Glucose

Glucose concentrations in the blood and HP did not differ significantly with molt stage in either wild or cultivated shrimp. In wild shrimp there was a trend towards high values in the early premolt (D1´), and decreasing values in the late postmolt (B1 and B2) and intermolt (C) stages. In cultivated shrimp, concentrations in the blood were higher at the D1´´ stage and in the HP at the D1´´´ stage (Fig. 4).

## Cholesterol

Cholesterol concentrations in the blood and HP did not differ significantly with molt stage in either wild or cultivated shrimp. In wild shrimp there was a trend to decrease the cholesterol concentrations in blood before ecdysis. Cholesterol levels were also higher in wild than cultivated shrimp (Fig. 4).

## Total Protein

In wild shrimp, protein concentration differ significantly with molt stage ($P < 0.05$); in HP, lowest values were observed in the intermolt (C) and early premolt (D0) stages; in blood, values increased from early postmolt (A) to premolt (D1´´) stages, but decreased in the late premolt (D1´´´). In cultivated shrimp there was no significant change between molt stages. An increasing trend was observed between postmolt and premolt stages, decreasing in blood in D1´´ and in HP in D0 stage (Fig. 4).

## Glycogen in the Hepatopancreas

Glycogen concentrations of wild shrimp showed significant differences throughout molt stages ($P < 0.05$); however these differences were not observed in cultivated shrimp with exception in $D_2$ stage. Glycogen content was higher in wild shrimp than cultivated ones and both groups showed higher values before ecdysis. Only wild shrimp showed a lower content in the postmolt stage $B_1$ (Fig. 5).

## Multivariate Analysis

EOFs analysis was carried out on the anomaly of each data set. Because most of the variability was captured by the first EOF, we restrict our discussion to the pattern of this mode.
We obtained through matrix data a Pearson correlation coefficient *(r)* product of the whole array of all the evidence that the molt stages have an inverse correlation ($r = -0.78$) with biochemical variables. This means that concentrations of most variables tended to fall as the molt cycle progressed. We also calculated the correlation coefficient between biochemical variables, where we found the relationship between the values in the wild and cultivated shrimp for each biochemical variable in turn. We obtained a high correlation value ($r = 0.92$). These matrices were taken in mode 1 (85%) of the matrix representing the highest confidence in the data.
In the first model based on empiric orthogonal functions, we found effects of molt stages on biochemical variables, especially in the late premolt (D1´ and D1´´´) and early postmolt (A) stages (Fig. 6). From a second model, we obtained a highest pattern of variability in mode 1 within osmotic capacity (85%, Wild Amplitude = 65; Cultivated Amplitude = 91) and in mode 2 with blood total protein (15%, Wild Amplitude = -27; Cultivated Amplitude = 34) between wild and cultivated shrimp for all biochemical variables and molt stages. In mode 1, the osmotic capacity had a marked effect, followed by total protein in blood and HP, with a small effect of acylglycerides (Fig. 7). In mode 2, total protein in the blood depends on molt stage in both wild and cultivated shrimp, and acylglyceride concentrations are also affected although to a lesser extent (Fig. 8).

## Discussion

Biochemical Analysis

Molting produces cyclic changes such as physiological and biochemical changes in the structure of the integument (Anger 2001), which are controlled hormonally (Molina and Cadena 2001). In F. duorarum only one report linked a physiological and biochemical monitoring for wild populations (Rosas et al. 2007). However, similar studies have been conducted on other penaeid species such as L. vannamei for osmoregulation (Charmantier et al. 1989; Chan 1988; Betancourt et al. 1993; Palacios et al. 1999; Pascual et al. 2003; Gaxiola et al. 2005; Pascual et al. 2006, and Galindo 2009) Penaeus monodon for effects of salinity on total protein and calcium concentrations of hemolymph (Ferraris et al. 1986), P. japonicus for the relation of protein concentrations to sex, size and molt cycle (Chen and Cheng. 1993), L. stylirostris for carbohydrate metabolism and osmoregulation (Rosas et al. 2000; Lemaire et al. 2002), and L. setiferus for characterization of lectin in hemolymph (Alpuche et al. 2005).

Blood metabolites reflect physiological adaptations in crustaceans, such as those related to the specific energy demand of shrimp. Blood metabolites can also provide markers for nutritional status (Carrillo et al. 2006). They have been used to record the status of HP in crustaceans, during the intermolt and as a reserve of inorganic substances absorbed from the decalcification of the old exoskeleton (Passano 1960). According to Rosas et al. (2007), extensive studies of tropical crustacean species are needed in order to determine whether the observed differences among species are related to environmental characteristics (temperature or salinity or dissolved oxygen, or their combination) or to morphological and physiological adaptations independently of environmental conditions.

In the present study of F. duorarum, the metabolite concentrations in blood and HP were lower than in other penaeids. Low metabolic activity could be the rule with F. duorarum, indicating that low metabolism in this species could be a factor indicator of changes according to molt stages. This is in agreement with previous studies in L. vannamei (Pascual et al. 2003; Galindo et al. 2009), L. setiferus (Rosas et al. 2007), and *P. monodon* (Kuo and Lin 1996). Bioenergetics and nutritional variables did not vary during an intermolt cycle in F. duorarum. The higher concentrations of metabolites in wild than in cultivated population possibly reflect a difference depending on their previous diets before entering the experiment.

The results of this experiment clarified that the isosmotic point is found at salinity of 26‰ and an osmolality of 853 mOsm/kg. This is confirmed in previous reports (Williams 1960; Castille and Lawrence 1981). In this work the salinity of 22 ppt is considered a condition of hyper-osmotic regulation. Wild and cultivated F. duorarum during different molt stages showed an osmoregulatory capacity around 100 and 250 mOsm/kg at salinity 22 ppt. Changes in osmoregulatory values have been associated with changes in the balance of water and ions, producing a cyclical pattern in hemolymph (Anger, 2001). But compare such values with values given by previous authors: Rosas et al. (2007) reported an average in wild F. duorarum juveniles and adults from the Campeche Sound of around 971 mOsm/kg. Lignot et al. (1999) found in cultivated juveniles of L. stylirostris a value of 735 mOsm/kg. There are reports on osmoregulatory capacity in relation to molt stages in penaeid species such as L. vannamei (Mantel and Farmer 1983; Charmantier et al. 1994; Mugnier and Justou 2004; Galindo et al. 2009) and L. stylirostris (Lignot et al. 1999), which reported that this variable increased in premolt stage (A), but we found a decreasing of osmoregulatory capacity in postmolt stage (D2) in both wild and cultivated species. According to Anger (2001), the osmolarity in hemolymph is usually minimal in the early postmolt stage (A) but we found a increasing of osmoregulatory capacity in postmolt stage (D2) and lower value in premolt stage (A) in cultivated species.

Molting is one of the intrinsic factors that affect the concentration of hemocyanin in F. duorarum (Bursey and Lane 1971a), Marsupenaeus japonicus (Chen and Cheng 1993), and L. vannamei (Galindo et al. 2009), as a result of hemolymph dilution due to water uptake. Hemocyanin is a multifunctional protein, because it transports oxygen and other metabolites, such as proteins between blood, HP and integument (Passano 1960). It tends to increase before ecdysis and decrease in the intermolt stage in F. duorarum (Bursey and Lane 1971a) and in M. japonicus (Chen and Cheng 1993). This is associated with the energy demands for ecdysis, when shrimp use the aerobic route. Also, the metabolites transported serve to build a new exoskeleton. An average of 0.77 mmol/L hemocyanin has been reported for adults of F. duorarum (Carrillo et al. 2006; Rosas et al. 2007). The hemocyanin concentrations found through the molt cycle in the present study (wild, 1.33-1.83 mmol/L; cultivated, 0.86-1.49 mmol/L) are similar to those found in cultivated juvenile L. vannamei by Cheng et al. (2002) at a salinity of 25 ppt (1.03-1.74 mmol/L) and Galindo et al. (2009) at a salinity of 36 ppt (1.63-2.51 mmol/L). Adult shrimp in those studies, with few changes of molting, had similar concentrations; this is associated with the longer intervals to ecdysis, and to the use of energy for reproduction.

The low hemocyanin concentrations observed here in intermolt might be related to a change in function performed by hepatopancreatic F-cells because they produce digestive enzymes during this molt stage (Lehnert and Johnson 2002; Galindo et al. 2009).

Glucose is used primarily as a direct source of metabolic energy; its function in the diet has been tested in L. vannamei, in a study of starch digestion and glucose metabolism at different stages of the molt cycle (Gaxiola et al. 2005), but the molt cycle had no effect on glucose or hemocyanin concentration in P. monodon (Ferraris et al. 1986) or L. vannamei (Chen and Cheng 1993). Circulating glucose has been reported in a wild F. duorarum population at an average of 0.18 mg/mL (Rosas et al. 2007). Variations in glucose concentrations in hemolymph are related to quantity and quality of carbohydrates in the diet (Rosas et al. 2001), but glucose can derive from glyconeogenesis from amino acids (Wiglesworth and Griffith 1994). In this study glucose decreased in premolt stages in both wild and cultivated shrimp, as a result of the energy demands of ecdysis. Also, in which glucose concentrations were higher in cultivated than in wild shrimp, they declined just before ecdysis. Here the cultivated shrimp were the outcome of several generations maintained on an artificial diet. A low blood glucose level in premolt stages is compensated by glycogen store in the HP of shrimp (Passano 1960).

Acylglycerides are a source of energy in L. vannamei (Fenucci and Haran, 2006). An increase in acylglycerides in the HP is due mainly to an accumulation of reserve lipids in the crustacean HP. During stage C these then yield fatty acids and glycerol after hydrolysis with lipase (Passano 1960). Many of these reserves are also converted to glycogen and glucose during the formation of the hard chitinous shell in Cancer pagurus (Renaud 1949). Xu et al. (1994) for Penaeus chinensis and Lim et al. (1997) for L. vannamei suggest that the composition of dietary fatty acids for different penaeid species is reflected in the composition of different tissues such as muscle and especially in the HP. Concentrations of acylglycerides in blood have been recorded at around 0.28 mg $mL^{-1}$ (Rosas et al. 2007); a value similar to those found in the wild (0.25-0.52 mg/mL) and cultivated (0.28-0.40 mg/mL) shrimp of the present study.

Cholesterol in crustaceans is used as constituent of cellular membranes, sub-cellular structures, and as a precursor of steroid hormones and molting hormones such as ecdysone. It is an essential nutrient because, as shown in P. japonicus, crustaceans can not synthesize cholesterol (Teshima 1972), and must be supplied by either natural or artificial diets. Here, blood cholesterol concentrations were directly related to cholesterol present in the food.

The diet used for maturation in shrimp normally contains between 11% and 17% lipids, i.e. some 3% more total lipids than those used in growth (Bray and Lawrence 1990).

The decrease in cholesterol concentrations in the blood before ecdysis in the present study was due to construction of cell membranes and to cellular growth, whereas the higher concentrations

before ecdysis and lower in intermolt stage in the HP of cultivated shrimp (0.08-0.16 µg/mg) agreed with the findings of Renaud (1949) for C. pagurus.
Protein requirement may be influenced by domestication, and changes in the ability to use nutrients have been found in L. vannamei (Cuzon et al. 2004). The 40% animal protein diet was efficient enough for growth, but we found that protein concentrations in blood were higher in wild shrimp than in cultivated ones. This might be associated with captivity and with the unvarying diet of 40% animal protein in experimental conditions.
Glycogen reserves are used for exoskeleton formation and their synthesis is essential for shrimp growth (Loret 1993). Glycogen is converted to glucose; this may be converted to glucosamine and acetylglucosamine, which can then be polymerized to form chitin (Passano 1960).
In the present study, blood glucose decreased in stage D1´´´, and glycogen in HP increased at stage D2 close to ecdysis in cultivated shrimp. Passano (1960), Anger (2001), Cuzon et al. (2003), and Galindo et al. (2009) reported that glycogen continues to decline steadily in HP. During late postmolt, peak concentrations of glycogen reserves are related to increase feeding activity of shrimp (Galindo et al., 2009); during intermolt stage they decrease, because the glycogen is used for glucosamine formation, which is then transported to the hypodermis (Santos and Keller, 1993). A relationship between low blood glucose concentrations and high mean of glycogen content in HP during late premolt was noted in wild and cultivated shrimp. Galindo et al. (2009) found a similar tendency in cultivated L. vannamei at a salinity of 36 ppt. Carbohydrates are acting on glycogen reserves just before molt, with activation of the glycolytic route before ecdysis, and whereas the hexose monophosphate cycle is active in the intermolt stage (Santos and Keller 1993).
Variation in glycogen content was greater in wild shrimp (between 3.81-7.85 µg $mg^{-1}$) than in cultivated shrimp (between 2.01-3.17 µg/mg). In juvenile wild F. duorarum population values around 0.09 mg/mg (90 µg/mg) have been reported (Rosas et al. 2007), which were very high compared with the present study. We might attribute such low values to the fact that an organism kept alone in a given space remains quieter. There, energy expenditure remains low compared to the freedom of movement in their natural environment (Fuss and Ogren 1966).

## Multivariate Analysis

The most effective analysis to evaluate all biochemical variables present in blood and HP was using empirical orthogonal functions, where found an inverse correlation between molt stages on biochemical variables. The first model showed that in late premolt (D1´ and D1´´´) and early postmolt (A), of wild and cultivated shrimp, i.e. moments before and after ecdysis, extreme physiological changes occurred (Fig. 6); this corroborates the findings of other studies (Bursey and Lane 1971; Chen and Cheng 1993; and Rosas et al. 2007).
The second model indicated that osmotic capacity and blood protein concentrations reflect the physiological status of wild and cultivated shrimp and as proposed by Charmantier et al. (1994) and Galindo et al. (2009) can be considered the best indicators of adaptation. The effect of proteins can result from a good diet administered in experiments, because proteins (amino acids) are certainly a component of the reserves, which are accumulated over during the intermolt HP to be used in all the cyclical process of growth in wild and cultivated shrimp. This supports the view of Rosas and Carrillo (2006) that cultivated shrimp are well adapted to use protein as an energy source, and as a molecule for growth.
In this initial phase of culture under laboratory condition, there is evidence for a change in biochemical adaptation with a compounded feed containing 40%CP, 9%lipid and 20%cbh Response in relation with a trend to increase storage of lipids in case of cultivated shrimp contrasted with a glycogen reserves in wild animals after 50 days feeding. Long term trials

would allow to follow weight gain or reduction in intermolt period. Then, further work will be needed to address the potential of this species in a whole process of domestication

## Acknowledgements

This work was supported by research grants PAPIIT-DGAPA UNAM (IX219404-1), CONACYT-Basico 44602-Q, and CONACYT-Basico 60824 to X. Chiappa and G. Gaxiola. We are grateful to Miguel Arévalo, Ariadna Sánchez, Gabriela Palomino, Adriana Paredes, Gema Martínez, Gabriel Taboada, Luis Jiménez Yaan, and Maribel Badillo for technical support. We also express our gratitude to Ann Grant, Chelsea Combest-Friedman and the anonymous reviewers whose comments improved and enriched this manuscript. J. L. Bonilla-Gómez thanks to the Organization for American State (OAS) for a scholarship to carry out M.Sc. studies at the Universidad Nacional Autónoma de México (UNAM).

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## Tables

Table 1. Diet formulation used in the experiment in percentages (%) of dry matter.

| Ingredient | Total (%) | Protein (%) | Lipids (%) | Carbohydrates (%) |
|---|---|---|---|---|
| Chilean fishmeal[a] | 46.0 | 29.4 | 3.7 | |
| Squid meal | 10.0 | 7.1 | 0.5 | 1.1 |
| Soluble fish proteic concentrate[b] | 5.0 | 3.5 | 1.0 | |
| Cod liver oil | 3.0 | | 2.0 | |
| Wheat starch | 20.0 | | | 20.0 |
| Soy lecithin | 1.0 | | 1.0 | |
| Cholesterol | 0.5 | | 0.5 | |
| Vit premix[c] | 1.5 | | | |
| Robimix C[d] | 0.5 | | | |
| Carboxymethyl Cellulose | 1.0 | | | |
| Filler (Talc) | 12.0 | | | |
| Total (%) | 100 | 40 | 8.7 | 21 |

[a] 65% protein, Apligen S.A. Mexico.

[b] 70% protein.

[c] DSM Nutritional Products, Mexico.

[d] Sodium ascorbate (stay C)

Table 2. Average hemolymph osmolality of juvenile Farfantapenaeus duorarum in different salinities during premolt stage (D0).

| Salinity (ppt) | Sea water osmotic pressure (mOsm/kg) | Hemolymph osmotic pressure (mOsm/kg) ± S.E. |
|---|---|---|
| 14 | 491 | 759 ± 6 |
| 16 | 536 | 783 ± 2 |
| 18 | 624 | 798 ± 2 |
| 20 | 677 | 825 ± 6 |
| 22 | 726 | 817 ± 4 |
| 24 | 787 | 835 ± 4 |
| 26 | 853 | 853 ± 5 |
| 28 | 881 | 872 ± 5 |
| 30 | 973 | 874 ± 6 |
| 36 | 1095 | 873 ± 4 |

## Figures

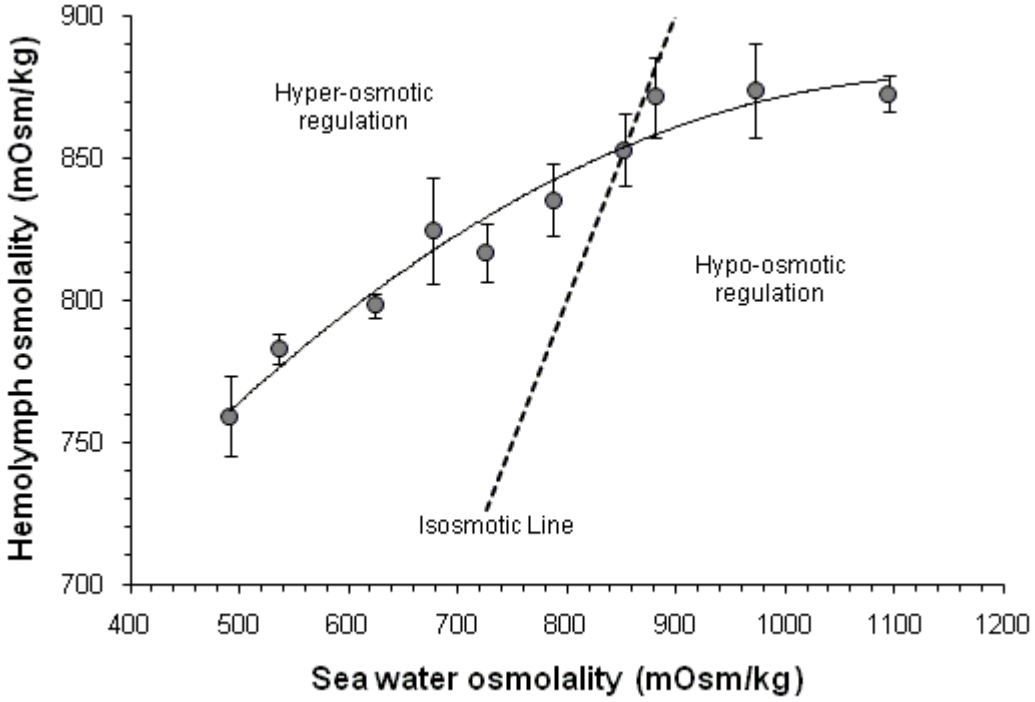


Figure 1. Hemolymph osmolality (solid line) as a function of seawater osmolality (corresponding to salinities from 14 to 36 ppt) in juvenile Farfantapenaeus duorarum reared at 28 C and measured at premolt stage D0, showing hypo-osmotic and hyper-osmotic regulation. The dotted line is the isosmotic line. The intersection between the isosmotic line and the hyper-hypo regulation line corresponds to the isosmotic point. Values are mean ± standard deviation.

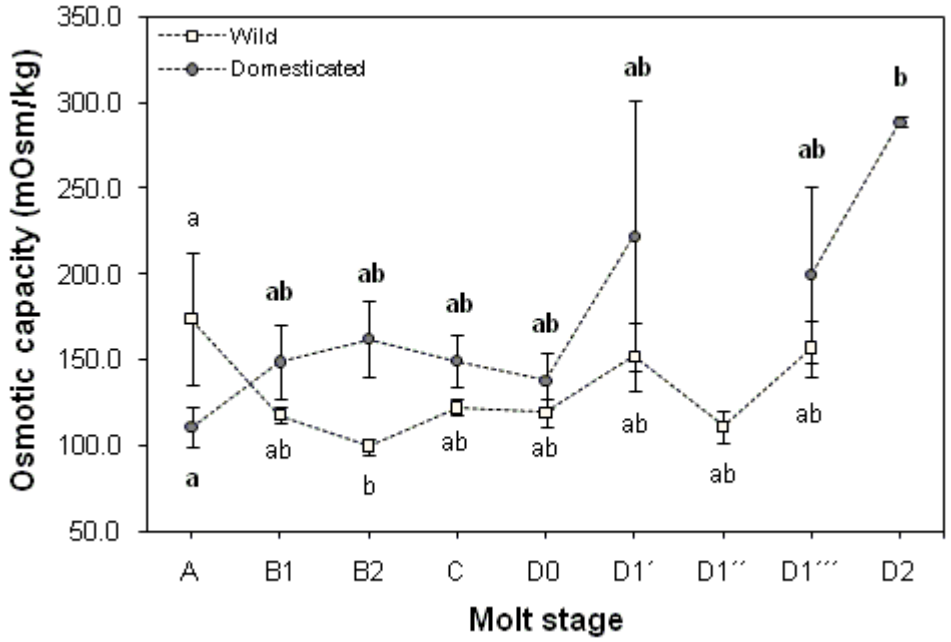


Figure 2. Osmotic capacity (mOsm/kg) of the blood of juvenile wild and cultivated Farfantapenaeus duorarum in relation to molt stage at salinity 22 ppt. Values are mean ± standard error. Different letters (normal= wild; bold = cultivated) denote significant differences ($P < 0.05$.)

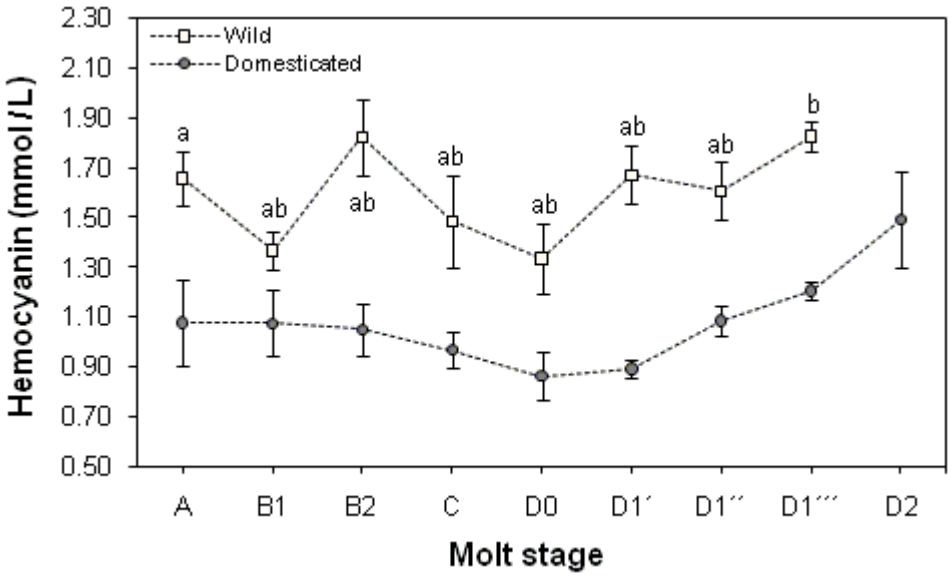


Figure 3. Hemocyanin concentration (mmol/L) in the blood of juvenile wild and cultivated Farfantapenaeus duorarum in relation to molt stage. Values are mean ± standard error.

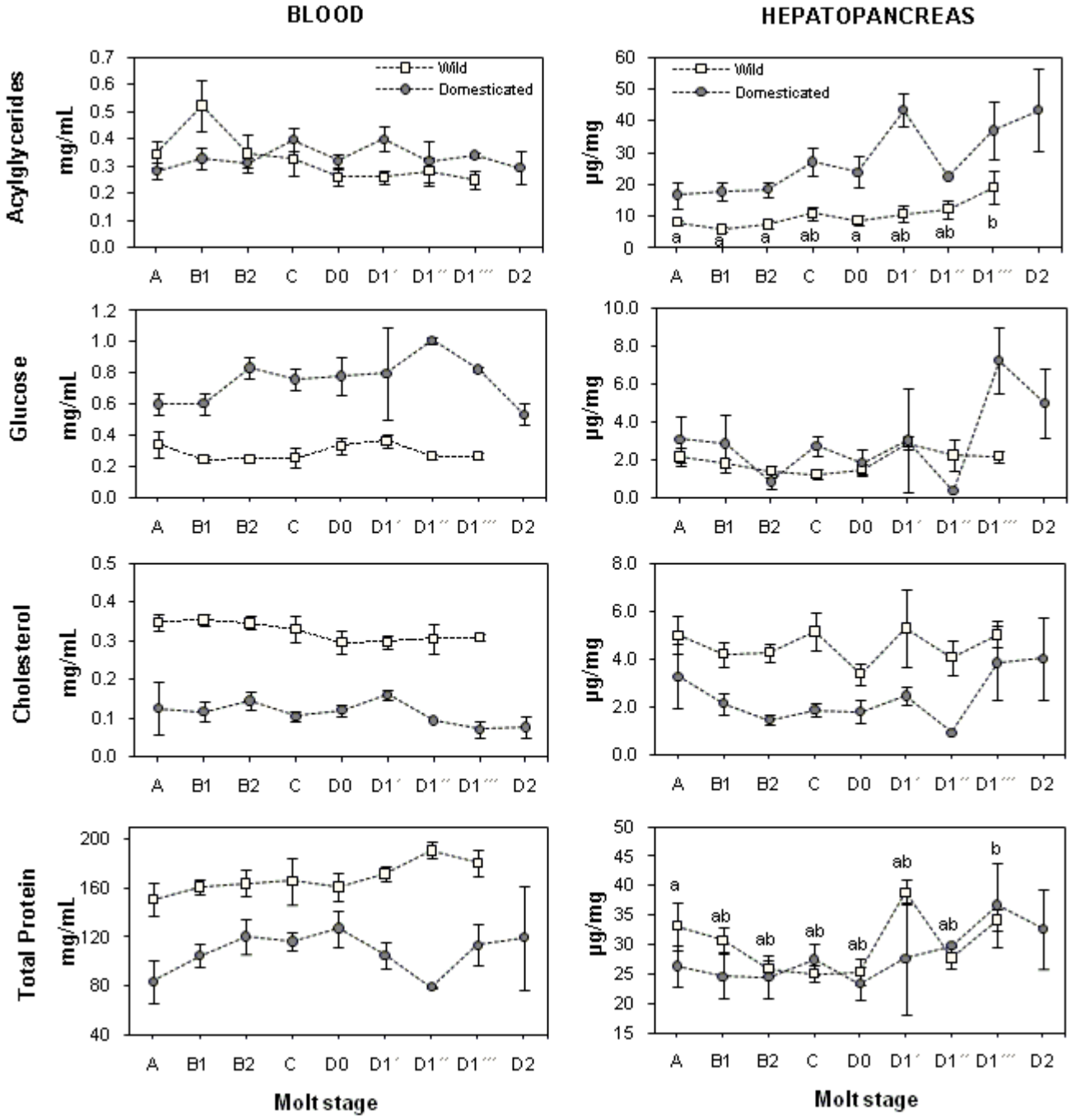


Figure 4. Concentrations of acylglycerides, glucose, cholesterol, and total proteins in blood (mg/mL) and hepatopancreas (µg/mg) of juvenile wild and cultivated Farfantapenaeus duorarum in relation to molt stage. Values are mean ± standard error. Different letters denote significant differences ($P < 0.05$).

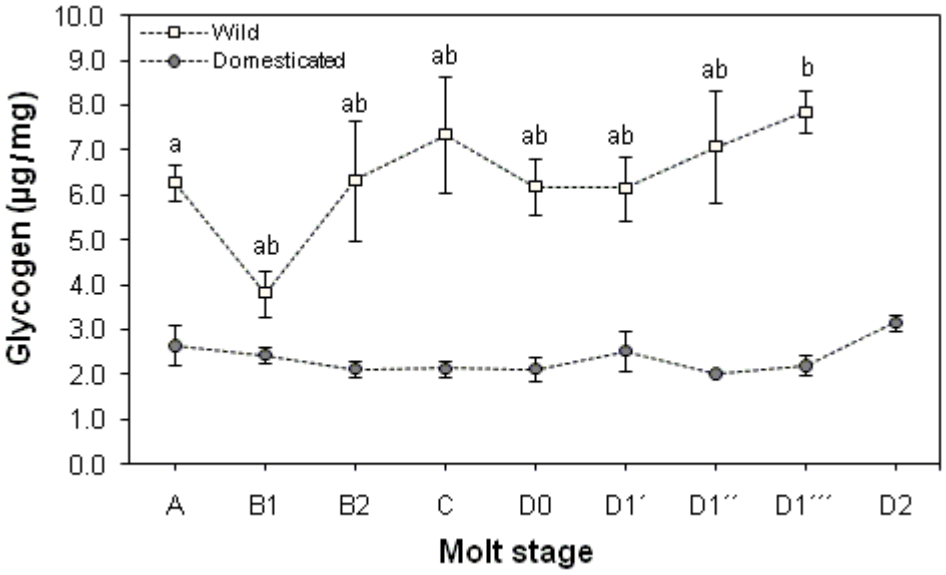


Figure 5. Glycogen concentration (µg/mg) in hepatopancreas of juvenile wild and cultivated Farfantapenaeus duorarum in relation to molt stages. Values are mean ± standard error. Different letters denote significant differences ($P < 0.05$).

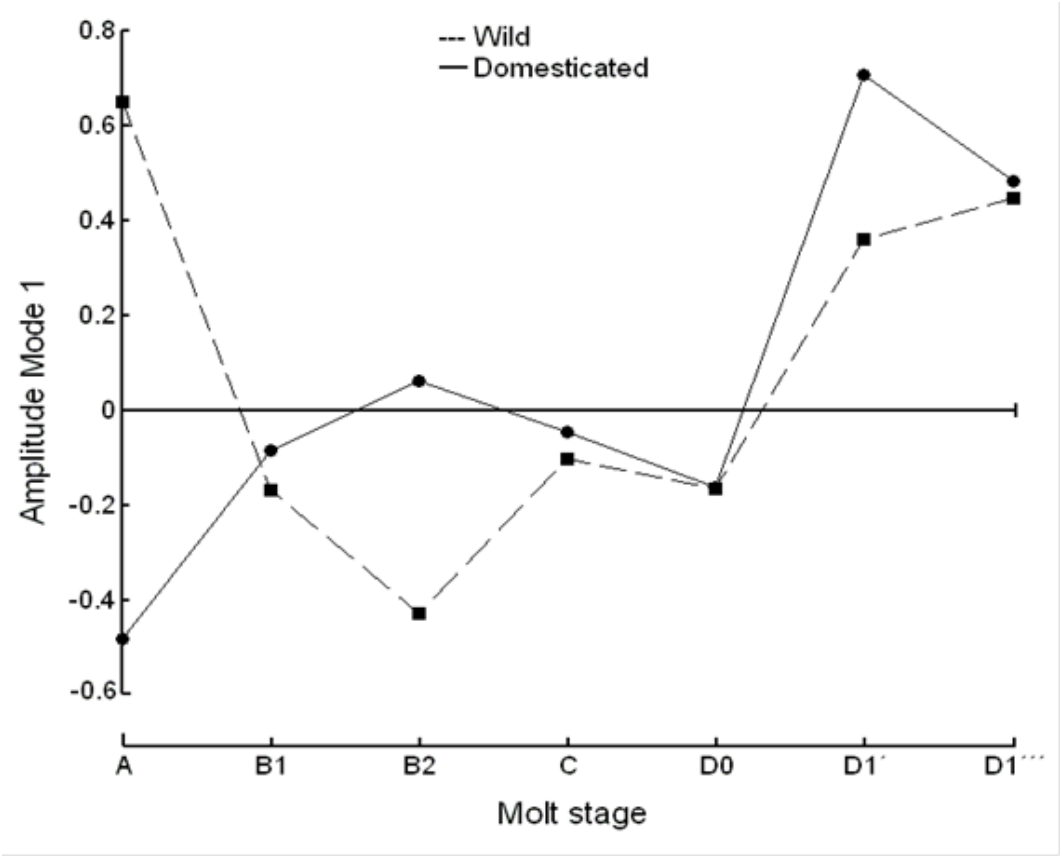


Figure 6. Patterns of variability (mode 1 = 85%) in relation to molt stage in wild and cultivated Farfantapenaeus duorarum. Graphics showed data from matrix including all biochemical variables.

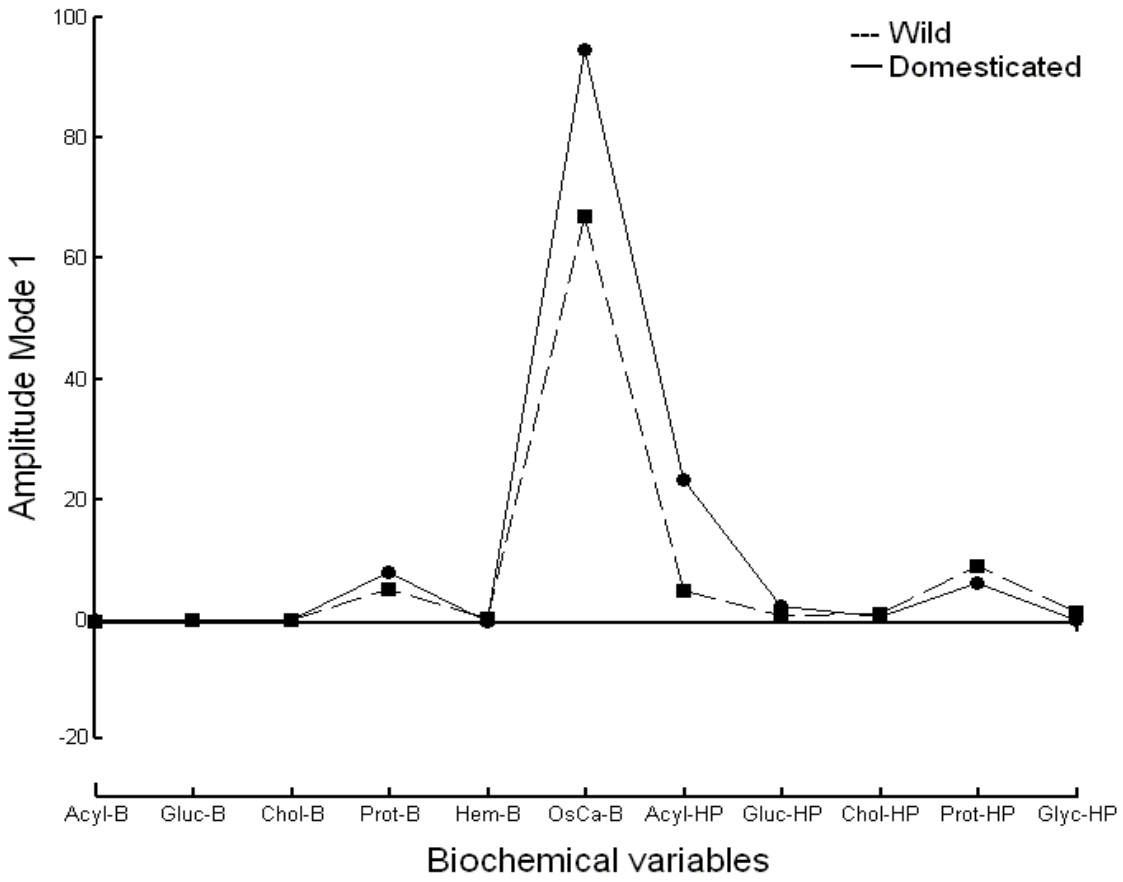


Figure 7. Patterns of variability (mode 1 = 85%) in all biochemical variables in wild and cultivated Farfantapenaeus duorarum. B= blood, HP= hepatopancreas.

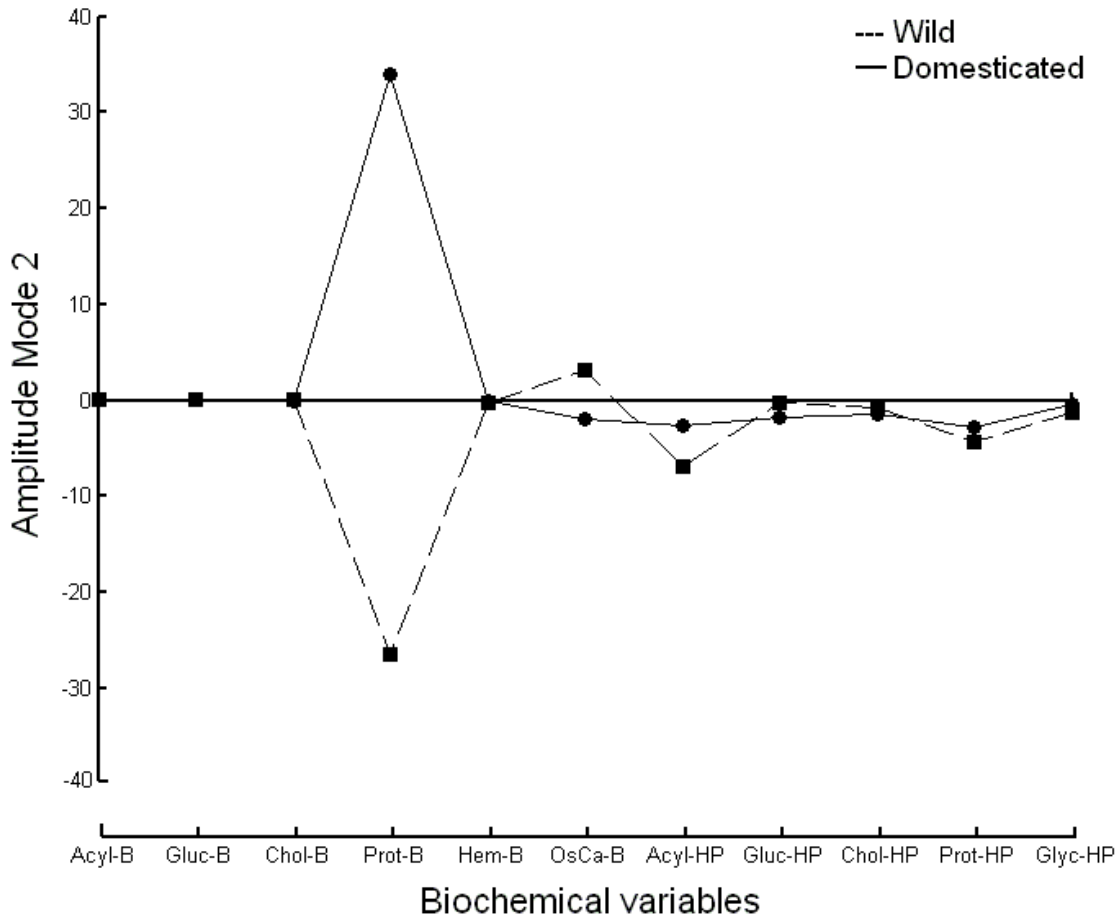


Figure 8. Patterns of variability (mode 2 = 15%) in all biochemical variables in wild and cultivated Farfantapenaeus duorarum. B= blood, HP= hepatopancreas.