THE REPRODUCTIVE CONDITION OF THE WHITE SHRIMP *LITOPENAEUS SETIFERUS* (CRUSTACEA; PENAEIDAE): EVIDENCE OF ENVIRONMENTAL DETERIORATION IN THE SOUTHERN GULF OF MEXICO

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INTRODUCTION

The effects of constant environmental changes in the oceans and coastal systems due to human activities require different tools and strategies for the evaluation of ecosystems health. Penaeid shrimp are aquatic organisms abundant in the tropical coastal zones throughout the world, and the Gulf of Mexico is not an exception. The white shrimp *Litopenaeus setiferus* is one of the shrimp species that inhabit the Gulf of Mexico and the North American Atlantic Ocean coastal zone. The northern limit of its distribution is southern New York, and the southern limit is the continental shelf adjacent to Laguna de Términos Lagoon, Campeche (Williams 1984).

The life cycle of the white shrimp in the Gulf of Mexico starts when the adults reproduce on the continental shelf between the 4 and 40 m isobars (Williams 1984). The fertilized eggs are at the mercy of the marine currents, and the nauplius larvae hatch within 12 to 14 hours and do not feed. The planktonic larvae develop over the next 12 to 15 days and move towards the coast in search of refuge in the coastal lagoons and estuaries, where they arrive as post larvae. There the shrimp gather in the brackish areas with submerged aquatic vegetation, which constitute the growth areas. The shrimp remain in these areas for seven to nine months before migrating back to the sea as adult recruits (King 1948; Renaud 1986; Zein-Eldin 1986; Chow *et al.* 1993; Misamore and Browdy 1996; Rosas *et al.* 1999).

As the white shrimp *L. setiferus* inhabits estuaries and marine zones, and plays an important environmental role because of its abundance, it is a suitable species to monitor the health of both ecosystems. In the southern Gulf of Mexico *L. setiferus* is exposed to several environmental pressures including overfishing, contamination of the bays, estuaries, and coastal lagoons by pesticides that originate from agricultural activities, sewage discharge from coastal cities, and by oil industry operations in Campeche Bay. As a consequence of the synergy of these multiple environmental pressures the structure of benthic communities has changed, affecting, among others, the shrimp populations that inhabit this area (Fucik *et al*. 1994; Benítez and Bárcenas 1996; Botello *et al.* 1996; Gold-Bouchot and Herrera-Rodriguez 1996).

In general terms, establishing the health of living organisms is controversial due to the lack of specific criteria that precisely define their tolerance to environmental variations in a multi-factorial context. Numerous assays have been performed with aquatic organisms in an attempt to establish the tolerance thresholds of environmental factors and contaminants on the performance and survival of several species of fish, crustaceans and molluscs (Vernberg 1983; Schmidt-Nielsen 1990; Rosas *et al.* 1992; Hanke 1993). However, it has been demonstrated that the results obtained in the laboratory are limited to speculation regarding the ability of aquatic organisms to compensate the joint action of environmental variables in the aquatic environment.

The interaction between environmental variables and the physiological ability of organisms to maintain homeostasis needs to be considered in the evaluation of their health. At the same time it has been recognized that the majority of environmental factors that determine the distribution of organisms, including contaminants, act in a time scale that can hardly be

reproduced under laboratory conditions. This limits the extrapolation of results to wild populations (Krebs 1998).

Several studies have been carried out in recent years seeking to apply existing methods to assess the health of aquatic organisms. Using penaeid shrimp as a model (Pascual *et al.* 1998, 2003b; Sánchez *et al.* 2001; Rosas *et al.* 2002) it has been possible to establish a close relationship between the nutritional condition, immune system and reproductive ability of shrimp maintained under controlled conditions, as well as collected in the wild. These studies have led to the establishment of variation intervals of blood metabolites and several immune responses of several species of crustaceans, including the white shrimp of the Gulf of Mexico, *L. setiferus*, relative to the multiple interactions of environmental factors (Pascual *et al.* 2003a; Pascual *et al.* 2003b).

This study presents the results of more than ten years of research with populations of the white shrimp, *L. setiferus*, in the Gulf of Mexico and demonstrates how environmental conditions modulate and can affect their health and reproductive ability. Using shrimp as a model and based on research results, and considering that penaeid shrimp are ecologically important species with broad distribution, it can be established that the deterioration of their populations may be a reflection of the deterioration of the benthic community at the coastal zones where they occur.

BACKGROUND

The white shrimp of the Gulf of Mexico, *L. setiferus* (formerly *Penaeus setiferus*), is among the most important shrimp species in the American Atlantic. It is distributed from the south of New York to the Yucatán Peninsula (Muncy 1984). Although the description of the reproductive biology of *L. setiferus* was done over 50 years ago (King 1948), the mechanisms related to their maturation and reproductive processes have only been investigated recently. The male reproductive system is composed of two parallel structures: the testicle and the vas deferens. The latter is divided into four regions: proximal, middle and distal portions, and a highly dilated muscular region called the terminal ampulla, where the final stage of the spermatophore formation takes place (Fig. 27.1).

The thelycum of the females is open in *L. setiferus* and, therefore, the egg fertilization is closely related to the adequate transfer and adhesion of the spermatophore, as the spermatozoids are transported and protected by this structure. The spermatophore is expelled from the terminal ampulla through the gonopore. During the process of ejection both halves are assembled outside the body, forming a complete spermatophore with structures that facilitate anchorage on the surface of the female's thelycum (King 1948; Ro *et al.* 1990; Chow *et al.* 1991; Krol *et al.* 1992). Therefore, the reproductive success of the species is directly related to the characteristics of the spermatophore, which are determined by the integrity of the structures, the adherence and quality of the sperm cells.

Together these factors permit an adequate fertilization of the oocytes at the moment of spawning. The transfer of the spermatophore is the final stage of a series of events that comprise the copulatory behavior of decapods, which is influenced by environmental factors such as temperature, salinity, depth of the water column, light intensity, photoperiod, contamination and stress, among others (Adiyodi 1985; Yano *et al.* 1988; Perez and Ramos 1992). Since the first report on the successful maturation and spawning of *L. setiferus* under laboratory conditions, difficulties encountered by males in transferring the spermatophore were noted and attributed to

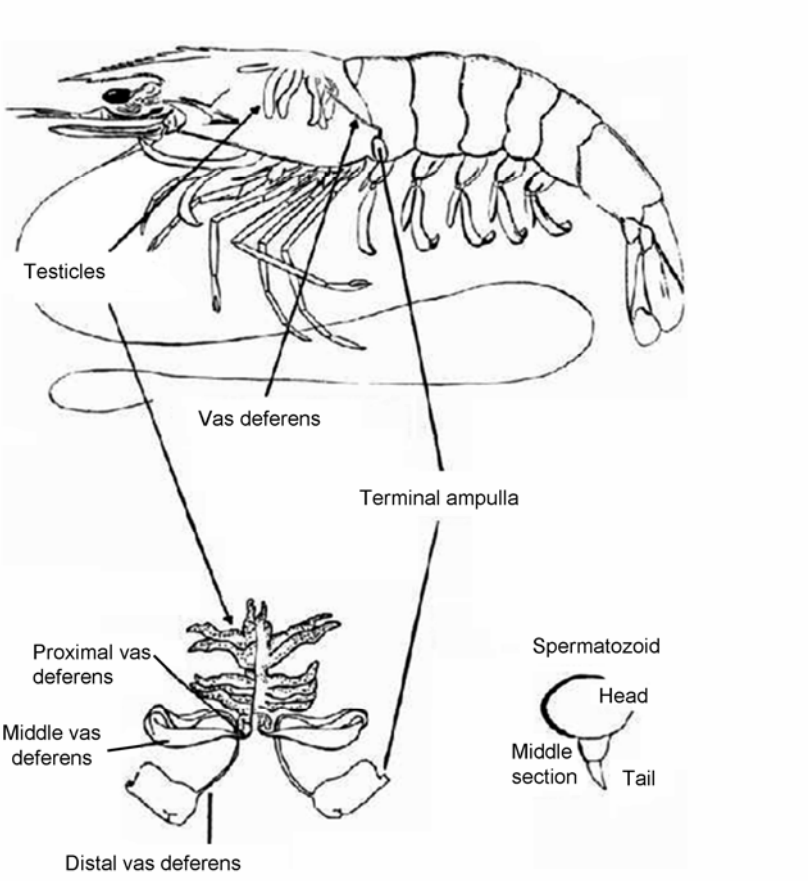

Fig. 27.1. Reproductive apparatus of adult males of *L. setiferus.*

bacterial infections (Brown *et al.* 1979). Several studies have been carried out with the objective of explaining the possible causes of the deterioration of the reproductive capacity of male shrimp. The phenomenon has been defined as the "male reproductive tract degenerative syndrome" (MRTDS) and is characterized by the progressive reduction in the number of sperm cells and the increase in the percentage of abnormal and dead cells (Chamberlain *et al.* 1983). This syndrome and the male reproductive melanization (or darkening of the reproductive structures) syndrome (MRMS), were associated with the same disease (Bray *et al.* 1985; Talbot *et al.* 1989). Currently, melanization and degeneration of the spermatophore are considered as two different processes (Alfaro and Lozano 1993). The darkening of the genital apparatus is due to the production of melanin that is generated by one of the most important defense mechanisms of shrimp, the prophenoloxidase (ProPo) system, which is activated by the presence of pathogenic agents (Ashida and Söderhäll 1984; Talbot *et al.* 1989; Alfaro and Lozano 1993). The degeneration syndrome has been correlated with problems of endocrine nature which, in turn, are associated with environmental stress (Alfaro and Lozano 1993; Rosas *et al.* 1993). Laboratory studies provided the basis for the precise determination of how environmental stress modulates the reproductive capacity of shrimp populations. Recent studies have shown that the reproductive capacity of *L. setiferus* in Campeche Bay is significantly lower than that of populations of the same species located in other parts of the Gulf of Mexico. The melanization and low number of

viable sperm cells have been a frequent characteristic of the *L. setiferus* population inhabiting waters of the state of Campeche (Pascual *et al.* 2003b). There are a number of hypotheses to explain how changes in the structure of the benthic community on which shrimp feed due to environmental perturbations affect the shrimp populations. Some of the most important hypotheses include: marine contamination; adverse effects of high summer temperatures, which affect the reproductive quality of the organisms; genotoxic damage resulting from the accumulation of contaminants coming from estuaries; increase in inbreeding as a consequence of population reduction due to overfishing.

This chapter presents the results of the last ten years of research on the variation in the sperm quality of adult male white shrimp *L. setiferus* in the Gulf of Mexico, using sperm quality as a model to explain the state of the reproductive health of the population. At the same time, the baseline of the nutritional state and immune response of juvenile and adult shrimp of this species is proposed as a tool to identify possible future environmental changes in the southern Gulf of Mexico, assuming that this baseline represents the biological variability of each of the evaluated response.

REPRODUCTIVE HEALTH

The study of the reproductive quality of the adult male population of *L. setiferus* began with investigations performed by several authors on the populations of South Carolina and Texas in the U.S., and Tuxpan, Veracruz and Ciudad del Carmen, Campeche in Mexico (Bray *et al.* 1982; Leung-Trujillo and Lawrence 1987, 1991; Browdy *et al.* 1991; Rosas *et al.* 1993; Pascual *et al.* 1998, 2003b). From these studies it was established that the amount of cells per spermatophore decreases along the latitudinal gradient of the species' distribution (Fig. 27.2).

These results brought attention to two aspects: the first is that the reproductive capacity of *L. setiferus* responds directly to the gradient imposed by environmental conditions throughout its distribution interval; and the other is that the reproductive condition varies not only throughout the latitudinal gradient, but also on an yearly basis. Although both observations can be of great practical value it has been necessary to first undertake several studies to establish how the environmental conditions govern reproductive ability, so that the results can be used to explain how possible environmental or human disturbances affect the distribution of the species.

EFFECT OF TEMPERATURE

According to Muncy (1984) the populations that are found to the south of New York reproduce only during the summer, when the temperature reaches 25°C, whereas the populations in the southern Gulf of Mexico reproduce year-round, as temperatures exceeding 28°C favor the development of the female ovaries, which in turn stimulate male reproductive activity between March and November. The effect of the temperature gradient is dramatic. The populations found in the extremes of the distribution interval have large differences in sperm quality, with 20 times fewer cells in the population from the southern Gulf of Mexico than in the population from the American North Atlantic (Fig. 27.2). Therefore, there is an inverse relationship between temperature, reproductive activity and sperm quality (Pascual *et al.* 2003b).

What could be the reason why the temperature determines the quantity of sperm cells in the adult males of this species? What are the annual temperature variations in the area where adult *L. setiferus* occurs? With the objective of answering these questions a series of experiments was performed to measure sperm quality in male shrimp exposed to 26, 30 and 33°C

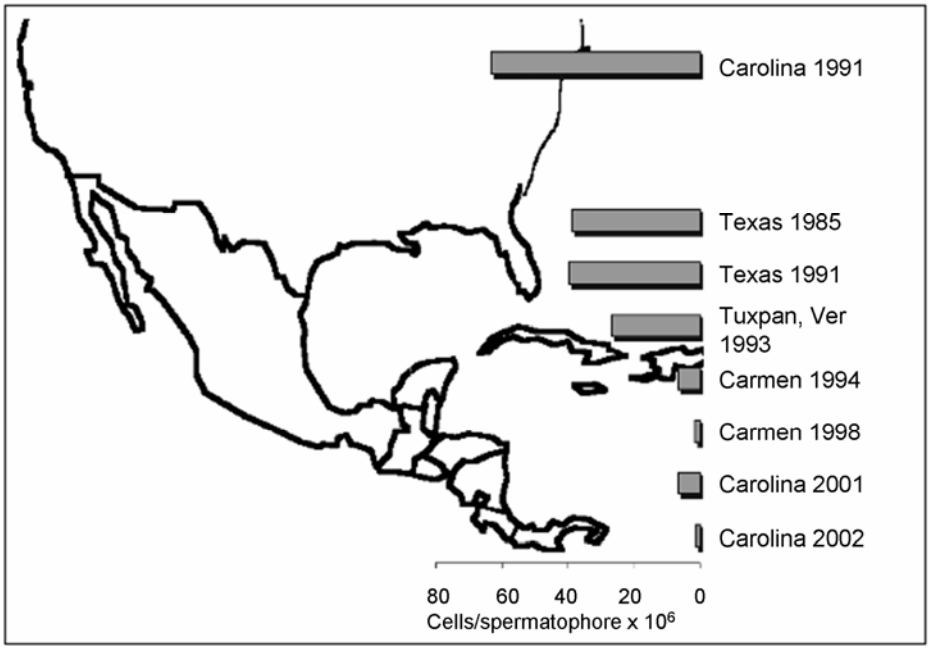

Fig. 27.2. Variations in the number of sperm cells per spermatophore of adult males of *L. setiferus.* Data taken from Bray *et al.* (1982); Leung-Trujillo and Lawrence (1987, 1991); Browdy *et al*. (1991); Rosas *et al*. (1993); Pascual *et al*. (1998, 2003b).

temperatures (Pascual *et al.* 1998; Sanchez *et al.* 2002). Concurrently to these experiments, automatic recording digital thermometers were placed at an 8 meter depth, where reproductive shrimp of this species are found, to register daily temperature variations.

The results of the laboratory tests showed a marked effect of temperature on sperm quality of adult male *L. setiferus* (Fig. 27.3). An increase in the proportion of abnormal cells was observed at temperatures above 26°C, indicating a decrease in the reproductive potential of the organisms. Although shrimp have a broad metabolic ability to tolerate the different experimental temperatures (Sanchez *et al.* 2002), the effect on sperm quality showed that the reproductive system is much more sensitive to temperature changes than the energetic metabolism.

Why does the temperature affect sperm quality? In another study Pascual *et al.* (2003b) used a 33°C temperature to trigger reproductive deterioration, and demonstrated that the effect of temperature is extraordinarily rapid and can affect the metabolism of lipids, carbohydrates and proteins, as well sperm quality, within 5 days of the start of the experiment (Fig. 27.4). These results are particularly important if it is considered that regeneration of the spermatophores of adult male *L. setiferus* under natural conditions takes 5 to 7 days, during which adverse temperatures can lead to an important loss of reproductive ability of the population (Rosas *et al.* 1993). In order to explain how temperature can affect sperm quality, the immune response at 33 °C was also examined. This was carried out in light of evidence that the shrimp immune system itself could be affecting the sperm cells through the loss of the mechanisms that regulate melanin production (Dougherty and Dougherty 1989; Dougherty 1998). The results of this study

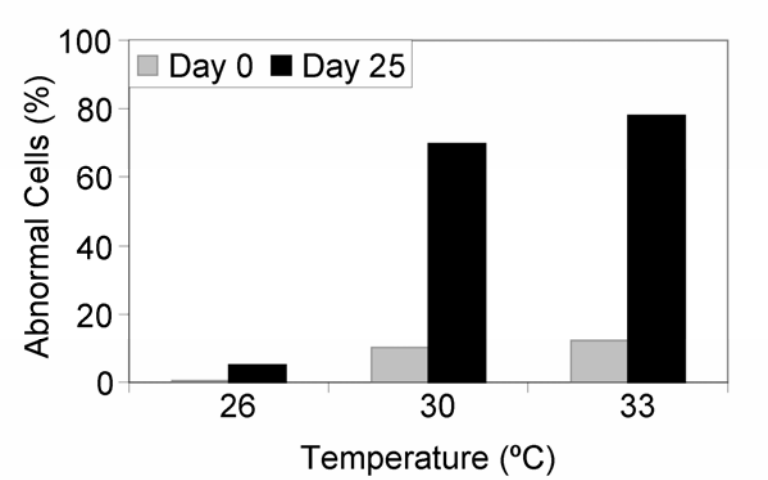

Fig. 27.3. Sperm quality, expressed as percent abnormal cells in male *L. setiferus* exposed to different temperatures.

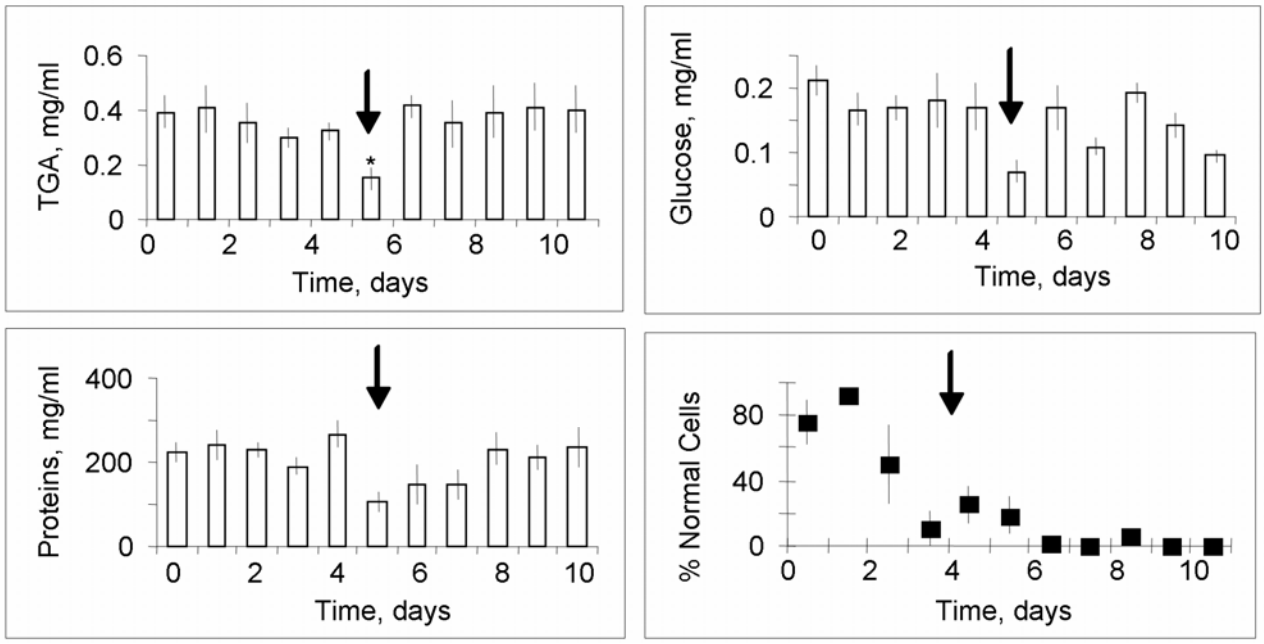

Fig. 27.4. Daily variations in lipids (TGA = triacylglycerols), carbohydrates (glucose), blood proteins and reproductive quality (% normal cells) in adult males of *L. setiferus* exposed to 33°C for 12 days. Values presented as mean ∀ S.E. From Pascual *et al.* (2003b).

demonstrated that when the temperature interferes with metabolic processes, it alters the regulatory mechanisms of immune responses, causing the defense mechanisms to turn against the organisms own sperm cells as if they were foreign bodies (Fig. 27.5).

Although these hypotheses partly explain the reason why high temperatures limit the quality and production of sperm cells in the southern Gulf of Mexico, it is still not known if temperature variations in nature really limit sperm quality of shrimp populations and if these variations could explain the oscillations in sperm quality that have been observed on the years in which these population were sampled (Fig. 27.2). To respond to these questions five digital thermometers were placed in the area where reproductive *L. setiferus* are normally captured, between 8 and 10 m depth, and temperature was registered every 15 minutes for 18 months, between April 2000 and October 2001. After this study and with the objective of corroborating the obtained information, temperature was monitored monthly in the same area and depth through April 2003. Given the large amount of collected data, a graph was plotted with the average daily values for one annual cycle in an attempt to establish the relationship between variations of this parameter and sperm quality obtained from male organisms captured in the same area (Fig. 27.6). The temperature on the continental shelf varied between 21.5°C in January and 30°C in September, with up to 4°C oscillations in one day during the months of July and September, demonstrating that shrimp are not only exposed to important annual temperature variations, but significant oscillations on a daily or weekly basis as well.

According to Primavera (1985), shrimp reproduction is most affected by temperature oscillations, since these can cause a delay in the development of female ovaries and loss of sperm quality in males. On a graph of annual variations of male reproductive quality of *L. setiferus* (Fig. 27.7) it is interesting to note the significant loss of sperm quality associated with the month of September, when the temperature is at its highest and oscillates most. These reproductive quality changes are completely justified by the experimental evidence demonstrating that adult males of the species are highly sensitive to temperatures greater than 28 °C, which, as seen in the data presented in Figure 27.6, usually occur in the natural habitat between July and September.

A broader analysis that takes into account the reported historic variation of temperature in the Gulf of Mexico is necessary to explain why sperm quality changes over time (Fig. 27.2). According to data from NOAA (http://www.ndbc.noaa.gov/images/climplot/42002_st.jpg) sea water temperatures 3°C above the annual average were observed between 1998 and 2002, which were linked to the El Niño phenomenon. Taking this data as reference and analyzing them in the context of the variations in sperm quality shown in Figure 27.2, it can be seen that the lowest levels of sperm quality in the male population of *L. setiferus* was registered precisely during these years. In contrast, between 1994 and 2001, when the average temperature of the sea was below the normal average (http://www.ndbc.noaa.gov/images/climplot/42002_st.jpg), higher sperm quality values were registered, indicating that it is linked to global variations of ocean temperature such as those caused by the El Niño phenomenon. Despite the lack of longer time series data to establish a cause and effect relationship with respect to these global variations in the temperature of the ocean, the results presented here show that the white shrimp *L. setiferus* is sensitive enough to temperature changes to be considered as an indicator species for changes caused by global warming or the El Niño phenomenon in the Gulf of Mexico region.

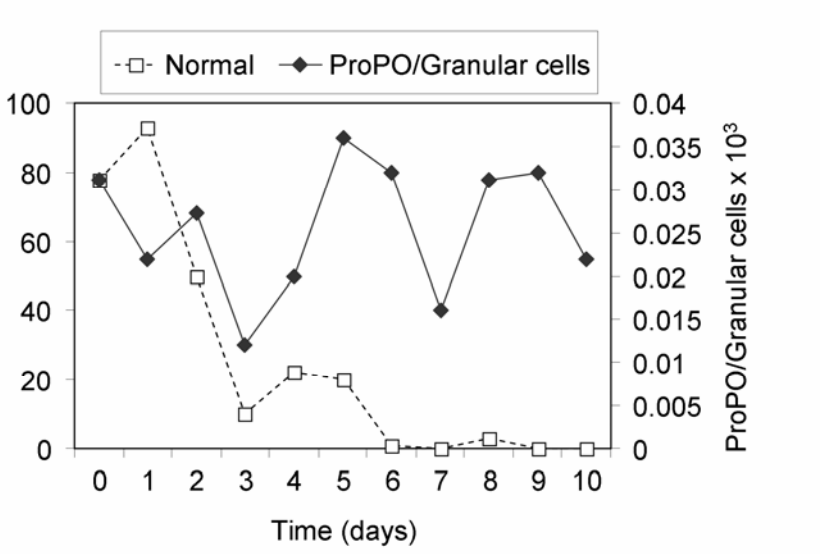

Fig. 27.5. Relationship between sperm quality and immune system (represented as the relationship between prophenoloxidase activity and concentration of granular blood cells) in adult males of *L. setiferus* exposed to 33°C for 12 days.

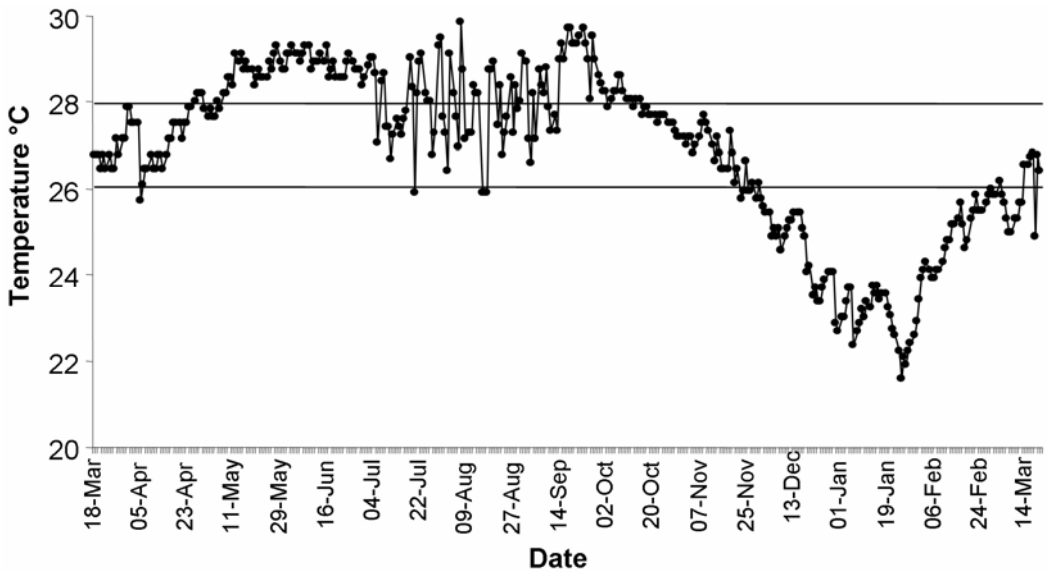

Fig. 27.6. Daily temperature variations in the continental shelf adjacent to Laguna de Términos Lagoon, Campeche. Values obtained with digital thermometers at 8 to 10 meters depth.

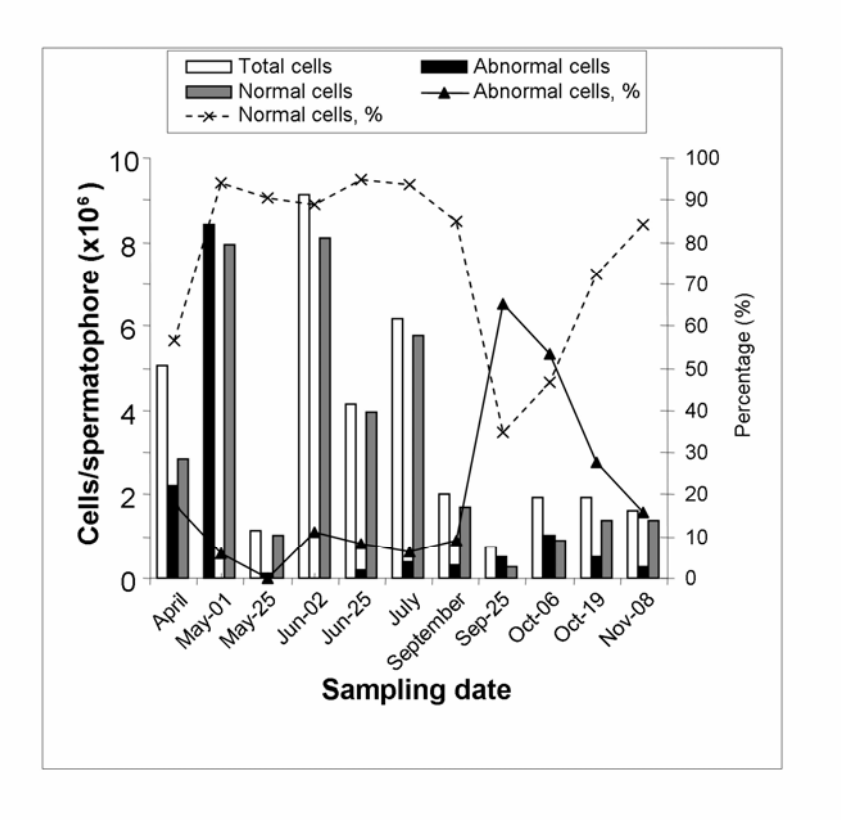

Fig. 27.7. Mean monthly variation of sperm quality in adult males of *L. setiferus* throughout an annual cycle. Values represent means of several samplings efforts between 1994 and 2003.

EFFECT OF DISSOLVED OXYGEN

Dissolved oxygen (DO), unlike temperature, is an environmental factor that acts as a metabolism regulator in shrimp (Martinez *et al.* 1998; Rosas *et al.* 1998, 1999). The regulatory role of DO is due to its direct mediation of the organism's ability to obtain energy through respiration, via phosphorylative oxydation.

In this process the oxygen is the last electron acceptor in the respiratory chain, allowing the shrimp and aerobic organisms in general to make maximum use of the energy contained in the bonds of the carbon molecules that are processed by the tricarboxylic acid cycle. The difference between using oxygen or not as the last electron acceptor indicates a large difference in the amount of energy available for work. In this context and depending on the species, metabolic processes can be more or less efficient, depending on the species tolerance to low DO levels. From the results obtained by Martínez *et al.* (1998) and Rosas *et al.* (1998, 1999) it is evident that a relatively small decrease in DO from 5 to 4 mg/L caused up to a 25% decrease in the energy channeled towards biomass production (Fig. 27.8). This limitation is important considering that low levels of oxygen are common in coastal lagoons and tropical estuaries, particularly when they receive organic matter as a result of summer rains. Given this situation, a 25% decrease in the energy channeled to production can affect not only the biomass *per se*, but also the amount of energy channeled to the production of gametes, which would directly affect

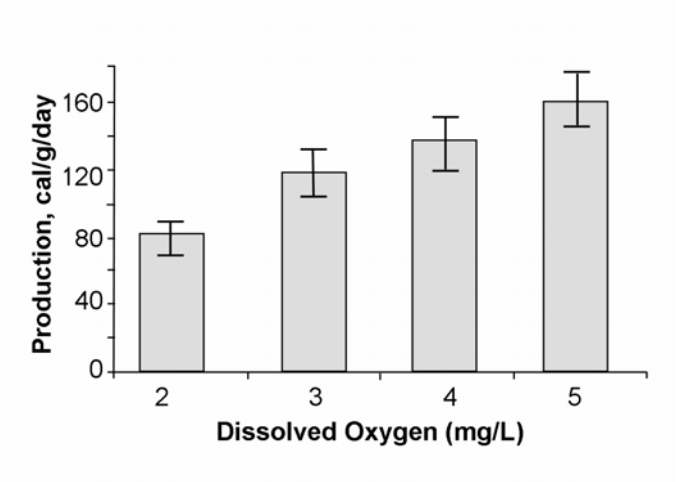

Fig. 27.8. Effect of dissolved oxygen on the energy channeled towards production in juvenile white shrimp *L. setiferus*. Average ∀ S.E. From Rosas *et al.* (1999).

reproductive success, with serious consequences to the population. Laboratory studies have demonstrated that shrimp can efficiently detect low levels of environmental oxygen and tend to avoid these areas by escape behavior (Renaud 1986; McMahon 1988; Martinez *et al.* 1998; Alcaraz *et al.* 1999; Muñoz *et al.* 2000). Considering this behavior it is possible to infer that data on the abundance of juvenile *L. setiferus* in tropical coastal environments and on DO levels could greatly aid in the establishment of the state of ecosystems health.

EFFECT OF CONTAMINANTS

Up to now there have been few studies evaluating the tolerance of juvenile or adult *L. setiferus* to specific contaminants. Alcaraz *et al.* (1999) measured the combined effect of ammonia, nitrite and DO on the oxygen consumption by post-larvae of *L. setiferus* and reported that this species is more sensitive than *Penaeus monodon* to the same contaminants. Nuñez-Garcia (2002) also reported that drilling fluids used in the oil industry affect the food intake rate in juvenile *L. setiferus* even at concentrations as low as 10,000 ppm in the suspended particles phase.

The genotoxic effect of hydrocarbon-derived contaminants has been studied in invertebrates (Cooper 1997). This model shows that the aryl hydrocarbon receptors (AhR) mediate many toxic responses induced by polyhalogenated hydrocarbons and polycyclic aromatic hydrocarbons (PAH). The AhR nuclear translocators (ARNT) are members of the super-family of heat shock proteins (bHLH-PAS). The ARNT dimerizes with some members of the PAH family, including the activated AhR ligand, forming a complex that alters the transcription by specific association with promoters associated to white genes involved with the immune system. Given that one of the populations of *L. setiferus* is distributed in the Sound of Campeche, where Mexico's most important oil exploitation zone is located, it is possible that a decrease in the reproductive quality of the adult males of this species could be associated with the effects of hydrocarbons on the regulation of the immune system through effects on gene transcription. Although this model needs to be established for shrimp, the evidences of loss of

immune control in this species led to the thought that a mechanism of this nature could be affecting the population of *L. setiferus* in the Gulf of Mexico, altering the reproductive capacity of males. Therefore, the environmental temperature changes associated with global phenomena such as the El Niño, as well as changes caused by the increase in organic matter in the rivers that flow into the coastal zone and reduce the amount of DO, by the presence of contaminants, by fisheries pressures, etc., are all reasons why the *L. setiferus* population is under great environmental pressure, which has evidently strongly affected its abundance. For this reason our team believes that this species could be used as a diagnostic tool to identify greater environmental changes in the area, which could be identified through the analysis of reproductive quality and physiological, nutritional and immune state of the species.

DIAGNOSTIC TOOL

In recent years several studies have demonstrated the usefulness of blood parameters as tools for monitoring the physiological condition of wild and cultivated shrimp exposed to a variety of environmental conditions. Palacios (2000) used triacylglycerols (TGA), glucose, cholesterol and proteins to monitor the physiological condition of wild and cultivated reproductive *L. vannamei* after various spawnings. In this study it was observed that wild shrimp had greater fecundity than cultivated shrimp, which was related to higher levels of triacylglycerols, cholesterol, proteins and glucose in the hemolymph. Sanchez *et al.* (2001) observed that a change in water temperature between 27 and 30°C produced variations in the levels of proteins, lactate, glucose, triacylglycerols and cholesterol, and of the immune response of wild adult *L. setiferus*. This study showed that the levels of proteins, triacylglycerols and cholesterol were lower in cultivated shrimp than in those captured in the wild.

The blood metabolites have also been used to monitor the effects of temperature on adult *L. setiferus* populations. Recent studies showed that there is a relationship among metabolic variables of the hemolymph, the immune response and the sperm quality of shrimp maintained under experimental conditions. The use of an extreme temperature as a stressor (33°C) promoted a loss of osmotic capacity, reduction in blood metabolites, concentration of the hemocytes, ProPo activity, and loss of sperm quality just five days after the beginning of the experiment, showing that there is a series of physiological and immune mechanisms that affect the reproductive capacity of the species under thermal stress (Pascual *et al.* 2003b). Another study by Rosas *et al.* (2002) showed that, in another species of shrimp (*L. vannamei*), salinity also affects blood metabolites due to physiological adjustments associated with the maintenance of the hydromineral equilibrium.

The levels of hemocyanin (OxyHc), blood proteins, glucose, triacylglycerols, cholesterol and lactate in juvenile *L. vannamei* have been studied with the objective of establishing a baseline for the nutritional state of shrimp under experimental or culture conditions in tanks that simulate production conditions (Pascual *et al.* 2003a). From this and other studies it has been established that food quality is the controlling factor of the level of blood metabolites, suggesting that these parameters could be used to establish whether a wild population is well nourished or if it is under nutritional pressure associated with any severe environmental changes (Rosas *et al.* 2001a, 2001b, 2002).

When blood components are compared with those obtained from other species of shrimp and crustaceans in general, it is observed that crabs and lobsters (species with low activity) present lower values than other more active species such as swimming crabs and shrimp (Pascual

et al. 2003a). For this reason it has been possible to establish that the blood components reflect physiological and morphological adaptations that depend on the ecosystem in which the organisms have evolved. In this context the present study presents biological intervals of different blood parameters that should be considered as baselines or reference points, and which could be used as diagnostic tools for the population of *L. setiferus* that inhabits both Terminos Lagoon and the adjacent coastal shelf. Specifically, the study was dedicated to obtaining information to define the baseline for osmotic pressure, oxyhemocyanin (OxyHc), glucose, proteins, cholesterol, lactate, and triacylglycerols as indicators of nutritional and physiological condition, as well as the quantification of the concentration of hemocytes, some of their components (ProPO), and phagocytic activity as indicators of the immune state of this species (Rosas *et al.* 2004).

Tables 27.1 and 27.2 present the values that describe the statistical behavior of a sample of more than 1,400 shrimp from Laguna de Términos and the adjacent continental shelf. In our opinion these values represent the biological range in which blood parameters have to operate in this population. Considering an average value (or median value in the case of non-normal distributions), ∀ a 95% confidence interval or quarter ranking, depending on the case, it is possible to obtain a reference parameter to establish if environmental changes which modify the nutritional or health conditions of the organisms are present, either in the juvenile shrimp or adults distribution area.

In order to undertake this study, the *L. setiferus* population from estuarine and oceanic environments with large differences in water salinity was sampled: 11 and 32 ppt salinity in the environments where juvenile and adult shrimp were captured, respectively. Rosas *et al.* (2002) demonstrated that cultivated shrimp are well adapted to use proteins as a source of energy, for growth molecules, osmotic pressure maintenance, and glycogen and glucose production by gluconeogenesis. Moreover, the immune system, with a strong protein basis, depends to a significant degree on protein metabolism (Hall *et al.* 1999; Montaño-Pérez *et al.* 1999; Sritunyalucksana and Söderhall 2000; Vargas-Albores and Yepiz-Plascencia 2000). It has been found that hemocyanin is a multi-functional protein as it transports oxygen, stores proteins and osmolytes, transports ecdysone, is precursor to antifungal peptides, has a similar activity to phenol oxidase (PO) and is an important component of blood proteins. Taking this into account several authors agree that the blood proteins and protein metabolism in general are the central axis of shrimp metabolism (Fielder *et al.* 1971; Gellisen *et al.* 1991; Chen and Cheng 1993a, 1993b; Destoumieux *et al.* 2001; Adachi *et al.* 2003; Pascual *et al.* 2003a). This is why the evaluation of blood proteins and the development of reference values can be of great use for the identification of environmental changes and their effects on organisms.

Cholesterol and triacylglycerols are directly related to the type of food and the reproductive condition, for which reason they have been recognized as indicators of nutritional state (Palacios *et al.* 1999; Palacios 2000; Pascual *et al.* 2003a). As cholesterol is rapidly absorbed by the digestive gland, its dissolved level in the blood is consistently low, as has been observed in other species of shrimp (Pascual *et al.* 2003a). Laboratory studies have demonstrated that lipid absorption is higher than 90%, indicating that the processes of absorption and transport are very fast in shrimp. This behavior could be associated with the limited ability of the animals to synthesize polyunsaturated fatty acids, forcing a quick transport to the areas where they are stored or used (D'Abramo 1997; González- Félix *et al.* 2003a, 2003b). This feature can be used to monitor the nutritional state, since small variations in food abundance for wild populations

Table 27.1. Descriptive statistics of live weight, hemolymph and digestive gland parameters in juvenile and adult *L. setiferus.*

			Confidence Interval					Quartile				
	N	Average	-95.0%	+95.0%	Median	Min	Max	Lower	Upper	Range	S.D.	S.E.
Live weight, g												
Juveniles	112	10.54	9.48	11.61	9.25	0.80	27.81	6.67	13.68	7.02	5.67	0.54
Males	100	35.05	33.40	36.70	34.19	15.31	64.79	29.97	39.21	9.24	8.32	0.83
Females	60	40.33	38.17	42.50	40.04	25.33	64.79	33.64	44.68	39.46	8.38	1.08
Osmotic pressure, mOsm/kg												
Juveniles	102	718.02	705.10	730.94	719.00	573.00	914.00	668.00	761.00	341.00	93.00	65.80
Adults	159	918.55	909.28	927.81	925.00	715.00	1063.00	895.00	949.00	54.00	59.14	4.69
Osmotic Capacity, mOsm/kg												
Juveniles	100	224.19	206.18	242.20	242.00	26.00	417.00	147.50	295.00	391.00	147.50	90.75
Adults	159	-151.89	-162.52	-141.25	-175.00	-252.00	68.00	-200.00	-118.00	82.00	67.90	5.39
OxyHc, mmol/L												
Juveniles	102	1.28	1.21	1.36	1.30	0.31	1.96	1.07	1.53	1.65	0.46	0.39
Males	100	1.69	1.61	1.77	1.76	0.70	2.92	1.43	1.92	0.49	0.38	0.04
Females	60	1.53	1.44	1.61	1.52	0.65	2.16	1.30	1.82	0.51	0.33	0.04
Glucose, mg/ml												
Juveniles and Adults	248	0.20	0.19	0.22	0.19	0.04	0.70	0.14	0.26	0.13	0.10	0.01
Cholesterol, mg/ml												
Juveniles and Adults	248	0.37	0.34	0.40	0.32	0.06	1.20	0.14	0.58	0.44	0.24	0.02
Triacylglycerols, mg/ml												
Juveniles and Adults	247	0.44	0.40	0.48	0.39	0.06	2.20	0.21	0.60	0.40	0.32	0.02
Proteins, mg/ml												
Juveniles - Males	186	282.19	266.01	298.36	273.31	30.52	606.00	202.56	359.70	157.14	111.80	8.20
Females	58	320.92	295.52	346.33	332.52	122.32	538.79	244.83	386.72	141.89	96.62	12.69
Glycogen, mg/ml												
Juveniles	82	3.10	2.56	3.64	2.64	0.00	20.42	1.69	3.76	20.42	2.07	6.13
Adults	136	2.05	1.77	2.32	1.58	0.08	6.49	0.79	3.13	2.33	1.61	0.14
Lactate, mg/ml												
Juveniles	44	0.11	0.10	0.12	0.12	0.06	0.23	0.08	0.13	0.16	0.04	0.00
Males	57	0.19	0.12	0.25	0.09	0.02	1.62	0.05	0.23	1.60	0.17	0.07
Females	59	0.04	0.03	0.04	0.04	0.00	0.10	0.02	0.05	0.10	0.02	0.00

Table 27.2. Descriptive statistics of the immune response of juvenile males, adult males and females of *L. setiferus.*

			Confidence Interval					Quartile				
	N	Average	-95.0%	+95.0%	Median	Min	Max	Lower	Upper	Range	S.D.	S.E.
ProPO, DO 490nm												
Juveniles - Males	143	0.20	0.16	0.25	0.09	0.01	1.62	0.04	0.24	0.20	0.28	0.02
Females	45	0.39	0.23	0.55	0.11	0.00	1.89	0.06	0.40	1.89	0.53	0.08
PO, 490nm												
Juveniles	86	0.06	0.05	0.08	0.06	0.00	0.46	0.03	0.07	0.46	0.04	0.00
Adults	102	0.12	0.10	0.14	0.10	0.03	1.01	0.08	0.12	0.04	0.11	0.01
Granular cells, Cel/ml x 10^3												
Juveniles	74	6.80	6.17	7.44	6.80	1.20	13.80	4.80	8.60	12.60	3.80	0.44
Males	55	13.31	11.68	14.93	12.00	5.80	26.60	7.80	18.20	10.40	6.00	1.48
Females	30	9.85	7.87	11.84	8.90	2.60	24.20	6.20	12.20	21.60	5.31	0.97
Hyaline cells, cel/ml x 10^3												
Juveniles - Females	104	13.87	12.50	15.25	13.20	0.80	35.40	9.00	17.90	8.90	7.08	0.69
Males	40	18.50	15.55	21.45	16.20	8.00	48.80	12.20	21.60	9.40	9.21	1.46
Total cells, cel/ml x 10^3												
Juveniles	74	20.07	17.86	22.27	18.60	2.00	46.40	14.00	25.40	44.40	11.40	1.32
Males	55	31.63	27.89	35.36	27.40	14.60	75.40	21.80	37.60	15.80	13.80	1.86
Females	30	25.42	21.82	29.02	25.10	5.40	44.00	18.60	29.60	38.60	9.65	1.76
Basal respiratory outburst												
Young males	37	0.15	0.13	0.17	0.14	0.08	0.28	0.11	0.17	0.06	0.06	0.01
Females	15	0.21	0.18	0.25	0.20	0.13	0.37	0.17	0.23	0.23	0.06	0.02
Activated respiratory outburst												
Young males	37	0.22	0.19	0.25	0.20	0.10	0.43	0.16	0.28	0.12	0.09	0.02
Females	15	0.32	0.27	0.38	0.35	0.15	0.50	0.26	0.38	0.35	0.09	0.02

would be reflected immediately in the triglyceride and cholesterol levels in the shrimp blood, and thus allow a timely diagnosis of the environmental impact of anthropogenic activities.
The same is true of immune responses. The concentration of ProPO can increase or decrease dramatically during an infection or in times of stress (Sanchez *et al.* 2001; Lopez *et al.* 2003; Pascual *et al.* 2003b). The results presented in Table 27.2 show that the population of *L. setiferus* shows low immune activity, which can be a consequence of the absence of strong sanitary environmental pressures. Although this characteristic can be considered an indicator of good health, it is evident that, up to now, the immune system alone cannot be used as a reference for the interaction between the possible effects of contaminants and reproductive quality, as was mentioned earlier. The presence of contaminants and pathogenic organisms has to be correlated with variations in the immune response before one of these variables can be utilized by itself as a diagnostic tool for population health.

It is interesting to note that blood parameters change with the weight of the organisms, and to understand how the use and processing of nutrients change with age. This change is related to the change of habitat between coastal lagoons (juveniles) and the open sea (adults) (Table 27.3), for which reason these changes can also be interpreted as physiological adaptations developed by each stage in the life cycle according to the environment it inhabits. The OxyHc and total blood proteins of *L. setiferus* increase with its weight, indicating that proteins can be utilized in different manners depending on the environmental demands that each age group confronts. It is well documented that juvenile *L. setiferus* is subject to sudden changes in water salinity in the coastal lagoons it inhabits, where they are also exposed to daily temperature and DO variations (Rosas *et al.* 1997, 1998). Under such circumstances the juvenile shrimp need to adjust their osmotic pressure as a result of finding themselves in a more dilute environment (Gerard and Gilles 1972; Claybrook 1983). During the process of physiological adjustment to low salinity, the shrimp use the free amino acids pool as a way of reducing the increase in cell volume caused by water uptake, which reduces the general concentration of dissolved proteins in the hemolymph (Rosas *et al.* 2001b). Adult shrimp, in contrast, do not require proteins for osmotic adjustments, tend to accumulate them in the blood, and as a result have higher concentrations than those registered in juveniles.

The estuarine system offers protection and food for populations of juveniles. This is why estuarine systems have been classified as nursery areas for many species of crustaceans. A negative correlation between cholesterol, lactate, and glycogen in the digestive gland and body weight has been observed (Table 27.3), indicating that juveniles that inhabit the estuaries accumulate reserves in the blood, possibly as a consequence of a higher food intake rate. Cholesterol is the basis for membranes construction, takes part in collagen synthesis, and is the precursor of the hormone α-ecdysone (molting hormone). At the same time, lactate participates in carbohydrates metabolism by promoting the formation of glycogen, which is the basic element for the formation of N-acetylglucosamine, the main agent for the synthesis of chitin. These two molecules are more abundant in the blood of juvenile shrimp because this is the life stage when their greater mobilization is required for growth (Ceccaldi 1998; Teshima 1998).

The Gulf of Mexico has huge ecological importance due to its biological diversity. At the same time, the oil, fisheries and maritime transportation industries are growing and increasing the pressures on marine and coastal ecosystems (Ridgeway and Shimmieldb 2002). The results of the last decade of research presented here serve as a basis for the understanding of how environmental forces exert strong pressure on the valuable populations of the southern Gulf of Mexico, such as the white shrimp *L. setiferus*. With this information and the biological range of

Table 27.3. Analysis of the relationship between body weight and hemolymph parameters of juvenile and adult *L. setiferus* caught in Laguna de Términos and the adjacent continental shelf.

	Sum of Squares	GL	Mean Square	F	P
OxiHc: live weight	14,664.38	1	14,664.38	87.90	0.00
Glucose: live weight	114.56	1	114.56	0.50	0.48
Cholesterol: live weight	1,852.46	1	1,852.46	8.36	0.00
TGA: live weight	422.16	1	422.16	1.84	0.18
Proteins: live weight	1,968.92	1	1,968.92	8.80	0.00
Glycogen: live weight	4,548.74	1	4,548.74	23.39	0.00
Lactate: live weight	6,049.32	1	6,049.32	39.07	0.00
ProPO: live weight	34.17	1	34.17	0.13	0.72
PO: live weight	3,173.25	1	3,173.25	12.74	0.00
Granular Cells: live weight	6,231.16	1	6,231.16	27.46	0.00
Hyaline cells: live weight	2,335.41	1	2,335.41	8.63	0.00
Total cells: live weight	4,363.43	1	4,363.43	18.26	0.00
Basal respiratory burst: live weight	1,157.30	1	1,157.30	6.24	0.02
Cholesterol: live weight	1,207.76	1	1,207.76	6.55	0.01

blood parameters shown above it is possible to propose *L. setiferus* as a non-controversial indicator species, as well as a low cost model to evaluate the effects of potential changes caused by human activities on this important ecosystem. These results provide the scientific community and environmental planners with indicators of the nutritional state and immune response of juvenile and adult shrimp, which can aid the study of changes in the nutritional quality of the marine environment, or the presence of population health problems, including the integrity of the benthic ecosystem of the region.

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