Université de Montréal

Time and space distribution of *Pontodrilus litoralis* and *Tylos wegeneri* in inter-tidal ecosystems of northern Venezuela, and their use as food for penaeid shrimp aquaculture

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Sommaire

Cette étude a déterminé la variation spatiale et temporelle de l'oligochète intertidale *Pontodrilus litoralis* et l'isopode *Tylos wegeneri*, et étudié les effets de variables de l'environnement sur leur présence et distribution le long de la côte Nord-Est de Venezuela.

À Guayacán, un écosystème intertidale de la côte orientale de Venezuela, nous avons étudié la distribution temporelle et spatiale de *P. litoralis* et *T. wegeneri* quant à densité, biomasse, et dimension pendant une période de 13 mois. Nous avons aussi utilisé distribution de la dimension de la particule, matière organique, pigments totaux, température, salinité, et pH pour déterminer l'importance relative de ces variables dans déterminer la distribution de les deux invertébrés intertidale. Également, pendant août 98 à mai 99, nous avons inclus six plages le long de 293 km de la côte vénézuélienne qui a été divisée dans deux groupes (Garrapata, San Luis et Guayacán dans région de l'ouest) et (Saucedo, Manzanillo et Grande dans région de l'est). Nos résultats ont montré que *P. litoralis* était abondant dans les écosystèmes intertidales protégeai où l'action de les houles, pendant que *T. wegeneri* tait abondant dans les écosystèmes intertidales ouverts où l'action de les houles. Nous discutons la capacité pour les deux de ces espèces être utilisé comme indicateurs de qualité de l'environnement de plages côtières vénézuéliennes, qui vues pour être sensible aux impacts anthropogéniques.

Nous avons aussi déterminé la variation mensuelle dans les substrats énergiques, et classes de lipides pour l'oligochète intertidal *Pontodrilus litoralis*, ainsi que ses composantes énergiques le plus important de ce *P. litoralis* lequel était lipide, a suivi par protéine et hydrate de carbone. Nous avons trouvé la grande variation mensuelle dans esters du cholestérol, phospholipides, triglycérides, portions rares de cholestérol et autres lipides, acides gras saturés et acides gras insaturés dans phospholipides, aussi bien qu'acides gras saturés et acides gras insaturés dans triglycérides.

Sous conditions expérimentales contrôlées, nous avons évalué l'alimentation, assimilation, taux de la conversion de la nourriture, et croître de postlarve de *Penaeus vannamei* qui a été nourri les diètes expérimentales suivantes; OL, 100% oligochètes (*Pontodrilus litoralis*); ISO, 100% isopodes (*Tylos wegeneri*); OLI, un mélange de 50% *P. litoralis* et 50% *T. wegeneri*; et K, 100% de Zeigler (une alimentation commerciale comme contrôle). Nous avons trouvé des différences très considérables dans la croître des postlarves de *P. vannamei* nourrie avec les quatre diètes différentes. Un testent *Post hoc* de Bonferroni montre que trois groupes pourraient être formés: K, OLI et OL-ISO. Une relation linéaire a été obtenue entre masse du corps (mg) et longueur du corps (mm) de postlarve de *P. vannamei* nourri avec les quatre diètes différentes. Les animaux nourris avec OLI sont devenus plus lourd dans un court montant de temps, alors que ce ont nourri avec K, a grandi dans longueur pendant la même période du temps mieux.

Mots clés: *Tylos*, isopode, *Pontodrilus*, oligochète, écologie côtier, zone intertidale, écologie trophique, lipides, énergie, acides gras, *Penaeus*, aquaculture, nutrition.

Abstract

This study determined the spatial and temporal variation of the inter-tidal oligochaete *Pontodrilus litoralis* and the isopod *Tylos wegeneri*, and studied the effects of environmental variables on their presence and distribution along the north-eastern coast of Venezuela.

At Guayacán, an inter-tidal ecosystem on the eastern coast of Venezuela, we studied the temporal and spatial distribution of *P. litoralis* and *T. wegeneri* in terms of density, biomass, and size during a 13-month period. We also used particle size distribution, organic matter, total pigments, temperature, salinity, and pH, in order to determine the relative importance of these variables in determining the distribution of both these inter-tidal invertebrates. Likewise, during August-98 to May-99, we included six beaches along 293 Km of the Venezuelan coast, which were divided in two groups (Garrapata, San Luis and Guayacán in western region) and (Saucedo, Manzanillo and Grande in eastern region). Our results showed that *P. litoralis* was abundant in the sheltered inter-tidal ecosystems, while *T. wegeneri* was abundant in the open inter-tidal ecosystems. We discuss the potential for both of these species to be used as indicators of environmental quality of coastal Venezuelan beaches, which seen to be sensitive to anthropogenic impacts.

We also determined the monthly variation in energy substrata, and lipid classes for the intertidal oligochaete *Pontodrilus litoralis*, as well as the most important energetic component, which was lipid, followed by protein and carbohydrates. We found large monthly variation in cholesterol esters, phospholipids, triglycerides, scarce portions of cholesterol and other lipids, saturated fatty acids and unsaturated fatty acids in phospholipids, as well as saturated fatty acids and unsaturated fatty acids in triglycerides.

Under controlled experimental conditions, we evaluated the feeding, assimilation, food conversion rate, and growth of *Penaeus vannamei* postlarvae that were fed the following experimental diets; OL, 100% oligochaetes (*Pontodritus litoralis*); ISO, 100% isopods (*Tylos wegeneri*); OLI, a mixture of 50 % *P. litoralis* and 50 % *T. wegeneri*; and K, 100 % of Zeigler (a commercial diet as control). We found highly significant differences in the growth of *P. vannamei* postlarvae fed with the four different diets. A *Post hoc* Bonferroni test show that three groups could be formed: K, OLI and OL-ISO. A linear relationship was obtained between body mass (mg) and body length (mm) of *P. vannamei* postlarvae fed with the four different diets. Animals fed with OLI grew heavier in a short amount of time, whereas those fed with K, grew better in length during the same time period.

Keywords: *Tylos*, isopod, *Pontodrilus*, oligochaete, coastal ecology, inter-tidal zone, trophic ecology, lipids, energy, fatty acids, *Penaeus*, aquaculture, nutrition.

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Liste des sigles et abréviations

°C	Degree (s) Celsius
µm	Micrometer (s)
cm	Centimeter (s)
g	Gram (s)
$gdw.m^{-2}$	Gram (s) dry weight per meter square
$ind.m^{-2}$	Individual per meter square
ISO	Isopod (experimental diet)
K	Control diet.
m	Meter (s)
m^2	Meter (s) square
mg	Milligrams
mg/gdw	Milligram (s) per gram dry weight
mg/gww	Milligram (s) per gram wet weight
mL/L	Milliliter (s) per liter
mm	Millimeter (s)
OL	Oligochaete (experimental diet)
OLI	Oligochaete and isopod mixture (experimental diet)
rpm	Revolution (s) per minute
SE	Standard error
SOM	Sedimentary organic matter

Dédicace

Pour ma famille, raison de ma vie

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Chapter 1

General introduction

Inter-tidal ecosystems are critically important habitats for both ecological and economic aspects. Inter-tidal ecosystems are facing serious worldwide threats due to anthropogenic input. In addition, changing oceanic conditions may have significant impact on the productivity of coastal invertebrates. Given the concurrent changes in coastal as well as oceanic conditions, the organisms of inter-tidal zones may be the best integrators of both terrestrial and oceanic changes. This study carried out to evaluate the impact of anthropogenic and environmental factors on the ecology and population dynamics of coastal invertebrates will contribute to the understanding of stressors shaping coastal ecosystem. This study is particularly important because no similar studies have been conducted on coastal ecosystems of Venezuela. Furthermore, this study may provide some insights into using coastal invertebrates as natural diets for aquaculture. The following section describes the biology of *Pontodrilus litoralis* (oligochaete) and, *Tylos wegeneri* (isopod), their nutritional values and their potential use as a food source for shrimp aquaculture.

1. Biology of *Pontodrilus litoralis* and other oligochaetes

Studies on *Pontodrilus litoralis* are scarce and the knowledge of this species is restricted to the first description by Grube in 1855 (*fide* Gates, 1972), the gender revision of *Pontodrilus* by Michaelsen in 1910, a new revision by Easton in 1984 which synonymies three species in *P. litoralis*, and their definition as a circuntropical species distributed in the temperate and warm regions of the world by Cantú - Marínez in 1987. However, studies on the biology, ecology and population dynamics have been conducted on other oligochaetes species.

1.1 Spatial and temporal distribution

Some species of Tubificidae and Enchytraeidae inhabit brackish water coastlines. The genus *Pontodrilus* is a common inhabitant of inter-tidal zones and has a wide distribution, occupying many tropical and subtropical coasts of the Indian and Atlantic oceans (Giere & Pfannkuche, 1982; Cantú - Marínez, 1987). The presence of *P. litoralis* has been reported for Venezuela on Isla de Aves and in the Mochima National Park (Drachember *et al.* 1989).

1.2 Nutrition

Oligochaetes are saprophagous animals. Their diet comprises mainly organic detritus in various stages of decay and incorporation into the soil. Although the bulk of their ingested food is dead plant tissue, living microorganisms, fungi, microfauna and mesofauna and their dead tissues also form an important part of their diet. Most of the information on the food preferences of oligochaetes has been derived from examination of the contents of their alimentary canal (Lee, 1985).

The early conception derived from freshwater studies is that aquatic oligochaetes are non-selective deposit feeders or diatom grazers. This is established in general textbooks (Lee, 1985), and although generally unexamined, is widely adopted for marine species. A closer look at food demands of aquatic oligochaetes leads, however, to a far more accurate view of their food selection and preference (Giere & Pfannkuche, 1982). The trophic spectrum of marine oligochaetes may be grouped into five main categories: 1) live microalgae (diatoms, flagellates), 2) micro organisms (bacteria, fungi, ciliates), 3) particulate organic matter, 4) fresh plant material (seaweeds) washed ashore, and 5) dissolved organic matter (Giere & Pfannkuche, 1982).

The food uptake of most oligochaetes, however, overlaps these clear-cut divisions which are often difficult to separate. For example, the muddy deposits that are the main food of large tubificids populating tidal flats and estuarine bottoms contain both organic particles from decaying plants and animals (detritus) and diatoms, as well as rich bacteria colonies. Enchytraeids which abundantly colonize the upper shore not only feed on plant cells from algal thalli or *Zostera* leaves, but also ingest bacterial slimes and diatoms (Fenchel, 1970; Harrison & Harrison, 1980 *in cita* Giere & Pfannkuche, 1982).

1.3 Longevity

Studies on the life history of oligochaetes are difficult due to a number of complicating factors which may partly explain the scarcity of data on *Pontodrilus litoralis*: 1) absence of discrete age classes, 2) neither body size nor number of segments are specific features for the determination of maturity stages (they can only be grouped statistically by division into length frequency or width frequency classes), 3) examination of maturity stages involves time consuming microscopical examination of each specimen, 4) in mixed oligochaete populations the cocoons of different species are not identifiable by morphological features, and 5) culturing aquatic oligochaetes, especially tubificids and naidids, often fails because of the length of time needed for life history studies. The failure of culturing oligochaetes is also partly due to nutrient problems and to chemical changes in the culture water (Giere & Pfannkuche, 1982).

1.4 Reproduction, abundance, biomass and population dynamics

Appreciable quantities of sexually mature *Pontodrilus litoralis* have been reported for Indian waters at the end of the monsoon (Subba Frays & Ganapati, 1975 *fide* Giere & Pfannkuche, 1982). Cocoon deposition begins in November and continues until May, with a peak in February and March, although peak deposition of cocoons has also been observed at the end of winter and beginning of spring (Stephenson, 1915).

The abundance of the ubiquitous marine and brackish water species *Paranais litoralis*, *Amphichaeta sannio*, and *Nais elinguis* is <1000 ind.m^{-2} from the end of November until the beginning of February. Towards spring, the numbers increase, due to a rise in *A. sannio* which attains its maximum of 21000 ind.m^{-2} in April and then drops again to 1000 ind. m^{-2} in July and August. The increase in this species coincides with the presence of benthic diatoms on which they feed. The numbers of *Paranais litoralis* and *N. elinguis* increase continuously from the middle of March, reaching their maximum in June (17500 *Paranais litoralis*. m^{-2} and 4500 *N. elinguis*. m^{-2}), resulting in a total of 34500. m^{-2} naidids in June (Giere & Pfannkuche, 1982).

In general, the population dynamic in enchytraeids can only be estimated from fragmentary observations. It seems likely that eulitoral and supralitoral enchytraeid populations, due to astatic and unpredictable habitats conditions fluctuate strongly in abundance and do not show regular seasonal peaks (Giere & Pfannkuche, 1982).

1.5 Oligochaetes consumed by predators

Polychaeta are the second macrobenthic group reported as regular oligochaete consumers. According to Rees (1940) and Yablonskaya (1953, *fide* Giere & Pfannkuche, 1982) the omnivorous and eurytopic *Nereis diversicolor*, which often reaches numbers of 1000 ind./m^2 on mud flats, feed on the oligochaete *Peloscolex benedeni*. As consequence of this interaction *P. benedeni* shows strong fluctuations in population density and distribution (both in space and time), inversely correlated with population sizes of *N. diversicolor*.

There is good evidence from the Baltic and North seas (Plagmann, 1939; Muus, 1967; Scherer & Reise, 1981, *fide* Giere & Pfannkuche, 1982) for predatory activity of small crustaceans, such as young shore crabs (*Carcinus maenas*) and shrimps (*Crangon crangon*), on oligochaetes such as *Paranais litoralis*, *Peloscolex benedeni* and *Tubifex* spp. which are easy prey due to their epibenthic life. Certainly, oligochaetes represent food of high importance for the macrofauna of coastal areas, especially of estuaries and lagoons. Many of these oligochaetes are ingested by a large number of shorebirds (Evans, 1987).

2. Biology of *Tylos wegeneri* and other isopods

Tylos wegeneri (Oniscidea) initially described by Vandel in 1952, was registered for the first time on the beaches of Trinidad, Tobago and Venezuela, under layers of organic material brought on the shore by waves and tidal action (Kensley & Schotte, 1989). There is complete lack of information on the biology of *T. wegeneri* for Venezuela. However, there is information for a few species in the genus.

2.1 Behaviour

The genus *Tylos* is essentially nocturnal (Ondo, 1953; Pardi, 1955; Hamner *et al.* 1968, 1969; Hayes, 1969, 1974). During high tide, most *Tylos* populations remain inactive in sandy sediments (Hamner *et al.* 1968, 1969; Kensley, 1974; Hayes, 1977). At dusk, individuals start to emerge from their burrows to feed on algae and dead organisms brought ashore by the waves. After 2 to 3 hours they return to the approximate position of high tide and move deep in the sediment again (Ondo, 1952; Hamner *et al* 1968, 1969; Hayes, 1970, 1974; Kensley, 1974; Brown & Odendaal, 1994).

2.2 Nutrition

The genus *Tylos* is considered as essentially herbivorous; however, it should be classified better as an omnivorous scavenger which, in some cases, prefers to feed on animal instead of vegetable matter (Kensley, 1974; Newell, 1979; Brown & Odendaal, 1994). *T. punctatus* prefers marine algae rather than fresh zooplankton (Hayes, 1974) while, on Mediterranean beaches, *T. latreillei* feeds on decomposed matter (Arcangeli, 1953), live amphipods (Matsakis, 1956) and dead fishes (Vandel, 1960). Other species feed on fishes, cnidarians, seals and dead cats (Hamner *et al* 1969).

Adults of *T. granulatus* were reported at densities of 300/m^2 on thalli of algae (*Ecklonia* and *Laminaria*), consuming up to 110 mg of algae per individual, the equivalent of 20 to 25% of their corporal weight (Kensley, 1974; Brown & Odendaal, 1994). The digestive tract of *T. granulatus* is similar to that of *Ligia* sp. (Kensley, 1974; Brown & Odendaal, 1994). The species appears to be adapted to eat large quantities of food in a short time. Its the digestive tract contains powerful gastric juices allowing rapid digestion of large quantities of food in short time (Brown & Odendaal, 1994).

2.3 Growth and density

In most isopods, and indeed most Crustaceans, size is measured in terms of length. Because *Tylos* tends to roll up into a ball, maximum width results the most convenient measurement. Width provides adequate indication of size since length is directly proportional to width in all species of *Tylos*, as well as for other oniscid species (Paris & Pitelka, 1962; Hamner et al.1969; McLachlan & Sieben, 1991; Brown & Odendaal, 1994). Individuals of *T. capensis* (McLachlan & Sieben, 1991) and *T. punctatus* (Hayes, 1974), aged between 4 and 5 years, have a maximum width of 14 mm. They moult approximately every month, with a growth rate of 0.46 mm per moult, and need about 20 to 30 moults to reach their maximum size (McLachlan & Sieben, 1991).

Tylos individuals are gregarious and can be found in high densities, up to 20,000 ind.m^2 in Carlsbad, Torrey Pines and Punta Banda (California and Mexico, respectively) (Hayes, 1969, 1974). Their distribution is homogeneous in silt substrata. However, the spatial distribution in sandy sediments is unknown.

In *Cyathura carinata*, seasonal variations in densities seem to be determined by the size and reproductive cycle of the population (Amanieu, 1969). Studies were also conducted on the feeding, nutrition and energy transfer, life cycle and growth of *Ligia pallasii* (Carefoot, 1973). Other studies dealt with some aspects of the biology of some isopod species: 1) rhythmic swimming of *Pseudoega punctata* in sandy beaches of New Zealand (Finchan, 1973); 2) social behavior and orientation of *Ligia* sp. (Farr, 1978); 3) use of energy and growth of *Idotea* sp. in Baltic regions (Strong & Daborn, 1979); 4) life cycle and population dynamics of *Idotea pelagica* and *I. granulosa* (Heally & O´Neil, 1984); 5) ecology of *Idotea* and the amphipod *Gammarus locusta* in the bay of Galway, Ireland (Tully & Céeleligh, 1986). No such extensive studies were conducted to document population dynamics, food web ecology and nutrition of *Tylos wegeneri*.

3. Aquaculture importance

Due to the rapid demographic growth of human populations around the world and the consequent increase on food demand, the production of food derived from aquaculture is object of a growing interest. Aquaculture is primarily concerned with the food production necessary to satisfy the worldwide human nutritional needs and to generate employments and economic benefits for the communities. To achieve these goals, aquaculture sectors need to develop new technologies and update existing ones to allow the appropriate maintenance, management, nutrition, reproduction, growth and development of commercially important species. The main objective of this study is to find natural products, which could serve as food sources and in the treatments of illnesses for cultivated organisms. New focuses in aquaculture research make use of results obtained in other scientific areas (ecology, biology and population dynamics, biochemistry, physiology, sociological aspects, etc.) to develop new and more efficient technological packages (Sevilla, 1984; Barnabé *et al.*, 1996).

In Venezuela, aquaculture is a relatively recent activity, basically characterized by the development of technological packages for exotic species, adapted and applied in the country. For example, *Penaeus vannamei*, *Penaeus stylirrostris* and *Oreochromis* *mozambicus* are now commercially important and highly productive species in Venezuela (Weidner *et al.*, 1992; Giménez *et al.*, 1995). *Penaeus vannamei* is one of the most common and successfully cultivated penaeid species in Latin America (Panamá, Ecuador, Colombia, Brazil and Venezuela). This shrimp is excellent for commercial cultivation because it adapts well to intensive and semi-intensive culture techniques, tolerates high temperatures, resists to a wide range of salinity, and reproduces during the whole year with high fecundity (Sandifer & Stokes, 1987; Liao & Liu, 1989; Molina, 1999).

During the aquaculture process of these organisms, it is important to provide them with energy-balanced diets. As the composition of nutritious compound in aquacultured organisms can only be derived from their food, a critical point is to develop diets containing essential compounds not only for the shrimp, but also for human health. Studies have been conducted to produce diets that guarantee good yields (New, 1976). It is well known that an unbalanced food can cause extensive mortalities on *Penaeus* spp. larvae and, if they survive, their development can be strongly affected (Sánchez, 1986). However, very little research effort has been done to find diets that provide balanced nutrition for humans.

During the last decade, the main problem encountered by Latin American commercial shrimp producers has been high prices and the scarcity of natural diets for cultivated species. Several studies dealing with the nutritional and feeding profiles of shrimps and the evaluation of new non-conventional foods have been conducted in order to satisfy regional necessities and produce new food sources for shrimp production (Weidner, 1992, García, 1998; Ricque-Marie, *et al.*, 1998).

There is still necessary to find new food sources. Many of the new food sources are organisms that show biological and genetic adaptations to support harsh environmental conditions. In this sense, the organisms living in supra-tidal and inter-tidal habitats possess such adaptations which involve the presence of certain natural substances such as long chain lipids (terpenoids), polyunsaturated fatty acids, specific proteins, carotenoids, lectins, saponins and phytic acids (Sudaryono, 1999; Hárvardsson & Imsland, 1999). Nevertheless, some species suffer adverse effects while feeding on organisms that contain some of these substances (De Silva & Anderson, 1995).

The coastal areas of the northeastern Venezuela involve a great variety of rocky, sandy and muddy beaches, along with the presence of coastal lagoons and estuaries. In these habitats the average tide level does not exceed 30 cm. However, in Venezuela, lacks of information have been found on the ecology and ecochemistry of the organisms inhabiting sandy beaches. Most the studies were restricted to organism ecology on rocky coastlines (Rodríguez, 1959, 1972; Urosa, 1972; Quintana, 1980; Velásquez & De Grado, 1997).

The study of intertidal regions to be of critical importance due to continuing worldwide degradation of coastal habitats. The highly fluctuating physical and chemical conditions make these areas susceptible of severe impact by man, and thus need to be better understood. Furthermore, inter-tidal ecosystems support a very diversified infauna which might constitute important resources for non-conventional diet in aquaculture.

Along the northeastern coast of Venezuela, two abundant and dominant invertebrate species the oligochaete *Pontodrilus litoralis* and the isopod *Tylos wegeneri*, inhabit the inter-tidal areas of sandy beaches,.

4. Objectives

One main objectives were to investigate the population and bioenergetic aspects of both invertebrate species, and to evaluate their potential use as food in for producing penaeid shrimps. More specifically, our objectives were: a) to characterize the ecology of *Pontodrilus litoralis* and *Tylos wegeneri*; b) to determine the relationships between habitat, feeding and trophic interactions in both species; c) to characterize energy substrata (proteins, lipids and carbohydrates), lipidic contents (cholesterol esters, triglycerids, cholesterol, phospholipids, and fatty acids) and their seasonal variations in *P. litoralis* and d) to test the efficiency of concentrated foods prepared from *P. litoralis* and *T. wegeneri* for growing shrimp larvae of *Penaeus vannamei.*

To achieve these objectives, it has been necessary to elaborate sub-projects to get specific ecological knowledge of species as well as of their bioenergetics, and possible use as diet for other organisms. In this sense, the first sub-projects (chapters 2 and 3) deal with the spatial and temporal variations of *Pontodrilus litoralis* and *Tylos wegeneri*, and environment effects on their occurrence and distribution on the coast north-eastern Venezuela.

The second sub-project (chapter 4) was designed to characterize and quantify the energetic content (energy substrata, lipid classes and their monthly variation) of *Pontodrilus litoralis*.

The third sub-project (chapter 5) was designed to test the efficiency of both the oligochaete and the isopod for producing penaeid shrimp larvae under controlled conditions.

We hope that this study will not only provide fundamental knowledge on the importance of diet quality in determining the growth efficiency of larval shrimps, but will also lead to the development of nutritionally balanced diets for shrimp aquaculture and to the possibility of using both these inter-tidal organisms as potential indicators of pollution and environmental impacts on inter-tidal regions. Note that some of the same figures have been presented in all of the chapters, especially those showing sampling sites and general environmental characteristics. As the individual chapters were prepared as manuscripts to be submitted to different journals, it seemed appropriate to include figures for general characteristics in each of the 4 chapters.

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Chapter 2

Temporal and Spatial Distribution of *Pontodrilus litoralis* (Annelida, Oligochaeta) in Inter-tidal Ecosystems of North-Eastern Venezuela

Andrade, José & Asit Mazumder

ABSTRACT

We determined the spatial and temporal variation of inter-tidal oligochaete *Pontodrilus litoralis*, and evaluated the effects of environmental variables on its presence and distribution along the north-eastern coast of Venezuela. *P. litoralis* is an abundant and dominant species in the inter-tidal ecosystems of Venezuelan coastal beaches. At Guayacán, a beach on the eastern coast of Venezuela, we determined monthly variation in size distribution and density of this annelid during June-96 to June-97. During the same time we quantified the monthly variations of granulometry, organic matter, total pigments, temperature, salinity and pH in the inter-tidal sediment of Guayacán beach. In order to characterize the influence of environmental variables on the distribution of this annelid, and environmental factors that determine its distribution in the north-eastern coast of Venezuela, we continued our sampling from August-98 to May-99. We sampled six sites along 293 km of the Venezuelan coast, which were divided in two groups (Garrapata, San Luis and Guayacán in western region), and (Saucedo, Manzanillo and Grande in eastern region). *P. litoralis* was not observed in Garrapata, San Luis or Saucedo sites.

Our results showed that this oligochaete is abundant in the sheltered inter-tidal ecosystems where low wave actions, normal salinity, small sizes of sediments particles and important concentrations of total pigments are available. High densities of small individuals were observed during the whole year indicating a constant reproduction and a potentially high metabolic activity. It is important to note that high pH and organic matter seem to produce a negative impact on *P. litoralis*. It seems to prefer temperature ranging from 28 to 29 °C, salinity in the order the 35-37‰, between 500-700 µm of sediment particle size, and total pigments of 0.5-1 mg/gww. These specific conditions are found in sheltered sites like Guayacán. In addition, we think that this oligochaete could be an important component of coastal food webs and a nutritional source for aquaculture. We also believe that this annelid could be used as an excellent indicator of environmental changes due to anthropogenic impacts on coastal ecosystems.

Keywords: *Pontodrilus litoralis*, inter-tidal oligochaetes, tropical ecology, foodweb, temporal and spatial variations.

INTRODUCTION

Pontodrilus litoralis, a benthic oligochaete, is common in the inter-tidal ecosystems of north-eastern coast of Venezuela, and is commonly found in sediments with high concentrations of organic matter. Our preliminary studies in the Guayacán region, north-eastern Venezuela (10° 39′N – 63° 48′W), showed densities as high as 1000 ind.m^{-2}. Very little is known on the ecology and distribution of this species. To develop such high densities this annelid must be able to respond to rapid changes in environmental conditions of inter-tidal zones. Our objective was to determine what environmental conditions and processes govern the temporal and spatial distribution of this oligochaete.

We wanted to test whether or not the temporal and spatial distributions of *P. litoralis* are regulated by environmental factors like temperature, salinity, sizes of sediment particles and food availability. Some oligochaete species have been found to feed on plant tissue, living microorganisms, fungi, meiofauna (Giere and Pfannkuche, 1982; Lee, 1985). Our preliminary data from the Guayacán region shows that *Pontodrilus* individuals range from 10.5 to 73.0 mm in size and have a consistent presence in inter-tidal zones. It is well known that appreciable quantities of sexually mature *Pontodrilus* individuals have been observed in inter-tidal zones in India at the end of the monsoon season, with a large frequency of cocoon deposition during November to May (Giere, Pfannkuche, 1982). Although several studies dealt with population biology, as well as with spatial and temporal distributions of other eulitoral and supralitoral oligochaetes, none has been conducted on *Pontodrilus litoralis*.

The consistent presence of this inter-tidal annelid along the north-eastern coast of Venezuela, specifically in sites close to the fishermen and urban communities, may be associated with the input of organic materials associated with human activities. It is possible that this species might serve as a good indicator of environmental quality associated with anthropogenic impacts. Others have considered *P. litoralis* as a potential indicator species of organic pollution (Subba Rao & Venkateswara Rao, 1980; Giere & Pfannkuche, 1982), but no systematic research has been done on the relationships between environmental factors and the population ecology of this oligochaete along gradients of natural and anthropogenic characteristics. On the north-eastern coast of Venezuela, fishermen communities, fish canning industries, and petroleum industries have been producing severe impacts on the inter-tidal ecosystems. Our specific objectives were first to describe the horizontal and vertical distribution of *Pontodrilus litoralis* at several coastal sites presenting different environmental and anthropogenic conditions, and secondly, to model the relative importance of various environmental factors and processes related with the distribution of this annelid.

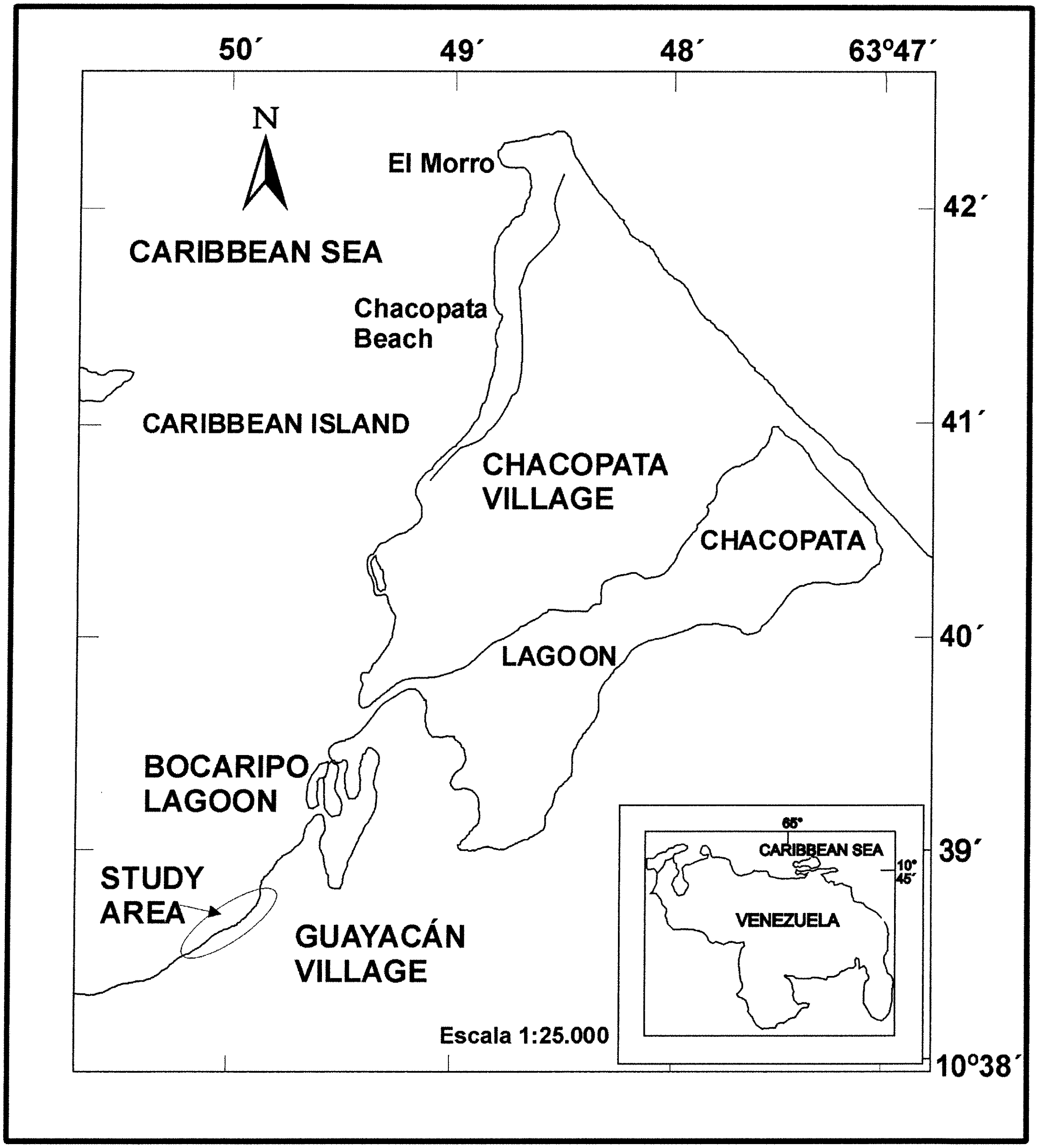
MATERIALS AND METHODS

During June-1996 to June-1997, in order to determine the monthly size distribution and population density of *P. litoralis*, as well as environmental patterns such as temperature, salinity, pH, organic matter, size-distribution of sediment particles and total pigments, we performed, triplicate samples from 4 plots of 0.25 m^2 at the Guayacán research site (Figure 1), each in transect of 108 m^2 (9x12 m). Sediment samples were collected by driving a core (15.25 cm in diameter) 30 cm deep into the sediments. The specimens were washed and preserved in 40% alcohol and their wet weight (± 0.01mg) and total length (± 0.01mm) were taken. Size distribution was developed from frequency histograms (Zar, 1984; SPSS, 1999).

Environmental parameters such as temperature, salinity (refractometer ± 1‰) and pH were determined on site. The size-distribution of sediment particles, was obtained by drying samples in an oven at 70 °C for 24 hours. Subsamples (100 g) were separated into different size-categories (0.063, 0.125, 0.250, 0.500, 1.000, 2.000 and 4.000 mm) using mechanical sieves (Holme and McIntyre, 1971; Gray, 1981). Four replicates of 1g from each sediment core were weighed, digested with concentrate chlorhidric acid and washed with double distilled water (to remove carbonated materials). After drying at 40 °C for 24 hours, the dry weight was obtained and the ashed samples were collected after burning at 450 °C for 24 hours. The difference between the dry weight and the ashed weight provided an estimate of organic matter contents (mg/gdw) (Holme and McIntyre, 1971).

Total content and variation of total pigments (mg/gww) in sediments was determined. Four replicas of 1g of sediments from each sediment core were placed in a beaker with 15 mL of 90% acetone. They were agitated for15 minutes at 40 °C, cooled in darkness to room temperature and finally, placed in the refrigerator for 24 hours with 10 mg of sulphate of magnesium. The samples were then filtered and centrifuged to 4000 rpm during 10 minutes and the concentrations of total pigments were measured using a spectrophotometer (Strickland and Parsons, 1972).

Figure 1. Geographic location of the Bocaripo-Chacopata lagoon complex and study area on the northern side of the Araya peninsula in north-eastern Venezuela.


50´
49´
48´
63°47´
N
El Morro
42´
CARIBBEAN SEA
Chacopata
Beach
CARIBBEAN ISLAND
41´
CHACOPATA
VILLAGE
CHACOPATA
LAGOON
40´
BOCARIPO
LAGOON
STUDY
AREA
39´
GUAYACÁN
VILLAGE
65°
CARIBBEAN SEA
10°
45´
VENEZUELA
Escala 1:25.000
10°38´

In August 1998, a field survey was conducted at 32 sites distributed along 293 km of coastline of north-eastern Venezuela (Figure 2). *P. litoralis* was present everywhere except at Garrapata, San Luis and Saucedo sites. San Luis and Saucedo are located close to large urban settlements, while Garrapata is located in the National Park of Mochima. From the 32 sites, based on accessibility, 6 sites were retained for intensive seasonal sampling. Sampling would have been too difficult and expensive at other sites due to difficult access (no nearby road). The general environmental characteristics of the six sites are given in Table 1. These sites (Garrapata, San Luis, Guayacán, Saucedo, Manzanillo and Grande) were sampled every three months between August 1998 and May 1999. The horizontal distribution of *Pontodrilus* was indicated by the densities of organisms at four stations of 0 (corresponding to the high-tide zone), 3, 6 and 9 meters (low-tide zones), and the vertical distribution of *Pontodrilus* was indicated by the densities of organisms at four depths (5, 10, 15 and 20 cm from surface). The sampling protocols for physical, chemical and biological variables were similar to those described above.

One-way ANOVA and a Bonferroni *post hoc* tests were performed in order to quantify the variations of physical, chemical and biological parameters as a function of time and space among and within sites. Multiple regression analysis was conducted to determine the relative importance of environmental factors in determining the distribution of *P. litoralis* (Sokal and Rohlf, 1980; Zar, 1984).

Figure 2. Map showing the distribution of six regional sampling sites.

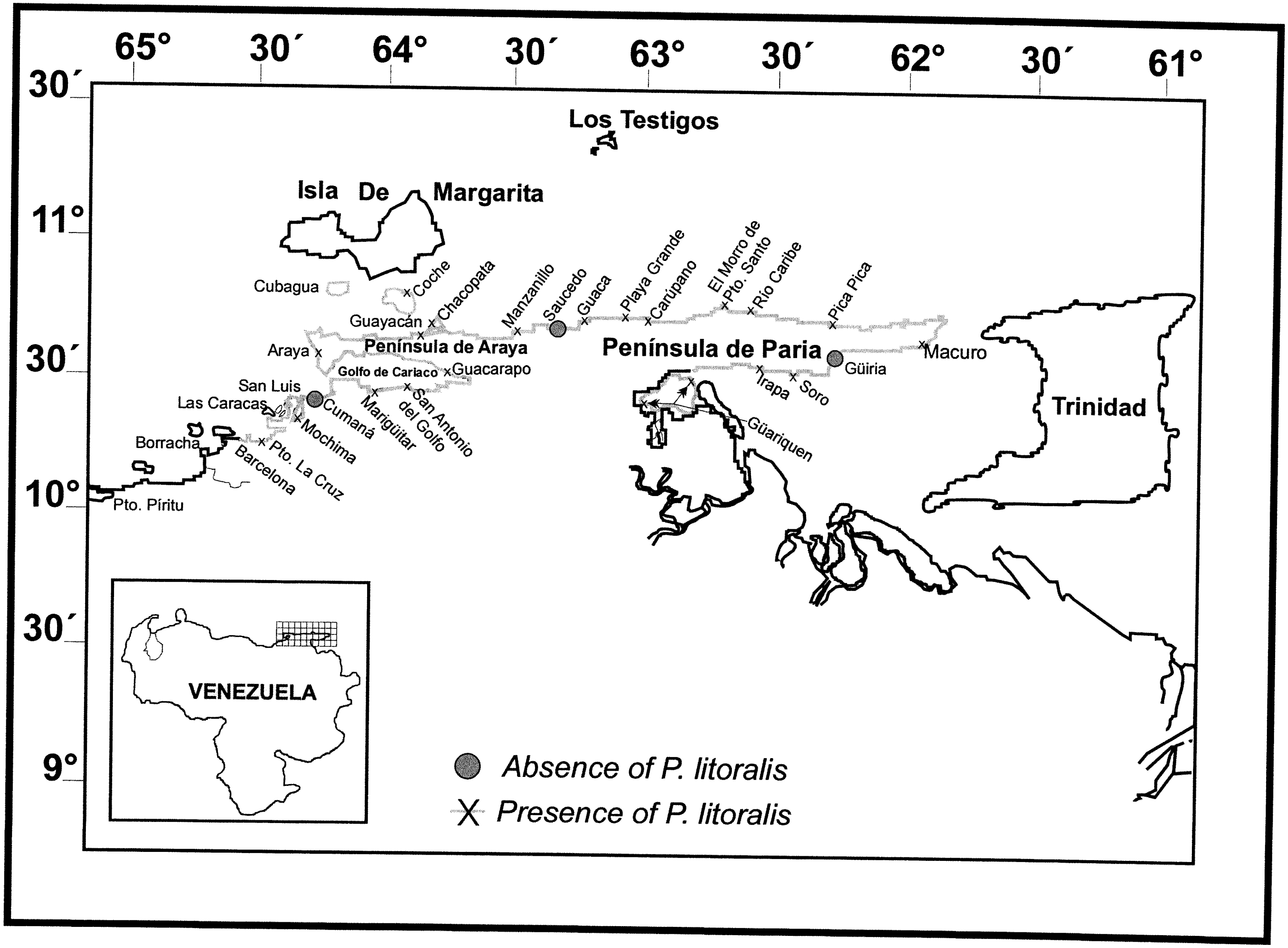

65°
30´
64°
30´
63°
30´
62°
30´
61°
30´
11°
30´
10°
30´
9°
Los Testigos
Isla De Margarita
Cubagua
Coche
Chacopata
Guayacán
Manzanillo
Saucedo
Guaca
Playa Grande
Carúpano
El Morro de Pto. Santo
Río Caribe
Pica Pica
Península de Araya
Península de Paria
Macuro
Güiria
Araya
Golfo de Cariaco
Guacarapo
San Luis
Las Caracas
Cumaná
Marigüitar
San Antonio del Golfo
Irapa
Soro
Güariquen
Trinidad
Borracha
Mochima
Pto. La Cruz
Barcelona
Pto. Píritu
VENEZUELA
Absence of P. litoralis
Presence of P. litoralis

Table 1. Present status of different sites considered in this study.

Regions	Places	Habitants	Status*	Characteristic	Enterprises	Impact
Western	Garrapata	500	a	Sheltered and low energy beach	National Park	(+) on the inter-tidal zone.
	San Luis	280,000	a	High energy inter-tidal zone	Presence of nearly salt processing and fish canning industries, jetties, port and navigation, plus diverse agricultural cultivations	(-) on the inter-tidal zone.
	Guayacán	1,000	b	Sheltered and low energy inter-tidal zone	Fishermen beach	(±) on the inter-tidal zone.
Eastern	Saucedo	3,000	b	High energy inter-tidal zone	Fishermen beach	(±) on the inter-tidal zone.
	Manzanillo	800	a	Sheltered and low energy inter-tidal zone	Nearly fish canning industry	(-) on the inter-tidal zone.
	Grande	85,000	a	Sheltered area of high energy inter-tidal zone	Presence of nearly salt processing and fish canning industries, jetties, port and navigation, plus diverse agricultural cultivations	(-) on the inter-tidal zone.

***a: Recreational activities**
b: Absence of recreational or industrial activities

RESULTS

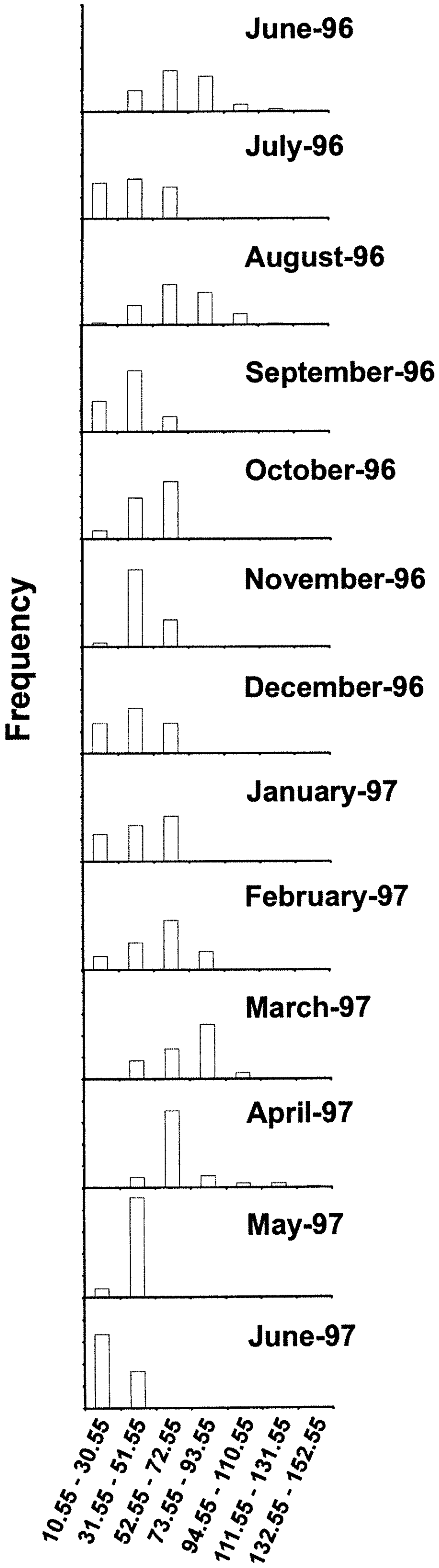
Size structure

The temporal (monthly) variation in the size structure of *P. litoralis* was determined using the 13-month data from the Guayacán site (Figure 2). Sizes of *Pontodrilus* varied significantly during the 13-month period. The mean length varied from 10.6 to 152.6 mm, with the most dominant sizes ranging from 30.0 to 73.0 mm. During July-96, September-96 through January-97, and May-97 through June-97, none of the individuals were larger than 73.0 mm. Smallest size classes were observed in May-97 and June-97, when over 90% of individuals were within the two size classes of 10.35-30.55 mm and 31.55-51.55 mm (Figure 3). The larger sizes were dominant during June and August of 1996 and during March and April of 1997 when 75% of individuals were as long as 132 mm.

Seasonal variation in population density and biomass

During June-96 to June-97 at Guayacán site, the population density of *P. litoralis* ranged from as low as 10 ind.m^{-2} in September, October, December, January and May, to as high as 500 to 2500 ind.m^{-2} from June to August, November, and from February to April (Figure 4A). The highest biomass, 190 gdw.m^{-2}, was observed in August when more than 55% of individuals were larger than 73 mm. The biomass of *Pontodrilus* was less than 5 gdw.m^{-2} during October, December, January, May and June.

Figure 3. Size frequency distribution of *Pontodrilus litoralis* between June 1996 and June 1997 at the Guayacán site.


June-96
July-96
August-96
September-96
October-96
November-96
December-96
January-97
February-97
March-97
April-97
May-97
June-97
Frequency
10.55 - 30.55
31.55 - 51.55
52.55 - 72.55
73.55 - 93.55
94.55 - 110.55
111.55 - 131.55
132.55 - 152.55

The trimestrial sampling of the six sites also showed strong temporal variations in the population density and biomass of *P. litoralis* (Figure 4B). As mentioned above, this annelid was absent from the Garrapata, San Luis and Saucedo sites. Manzanillo and Grande sites exhibited very low biomass and density during the entire sampling period. Density ranged from 20 to 40 and 50 to 140 ind.m^{-2}, and biomass ranged from <5 to 20 and 10 to 22 gdw.m^{-2} Manzanillo and Grande, respectively. Contrarily, the Guayacán site showed higher density (ranging from 550 to 780 ind.m^{-2}) and biomass (ranging from 20 to 185 gdw.m^{-2}) for the four sampling dates.

Horizontal and vertical distribution

The horizontal (from high-tide to low-tide zone) and vertical (at four depths in each zone) distributions of *P. litoralis* were highly variable among the different sites (Figure 5). As previously mentioned, at the Garrapata, San Luis and Saucedo sites, no organisms were found in any of the horizontal and vertical areas. The three other sites showed strong horizontal and vertical variation (Figure 5). At Guayacán, *P. litoralis* was distributed at the three high-tide zones (0, 3 and 6 m) and no individuals were found at the low-tide zone (9 m). At this site, *Pontodrilus* was abundant in the deeper sediment (10, 15 and 20 cm). At the Grande site, individuals *P. litoralis* were found at all four horizontal zones and were most abundant in the deeper sediment strata (10, 15 and 20 cm). No

Figure 4. Seasonal variation of density (ind.m^{-2}) and biomass (gdw/m^{-2}) during June 1996 to June 1997 in Guayacán beach (A) and during August 1998 to May 1999 in six different beaches of north-eastern coast of Venezuela (B).

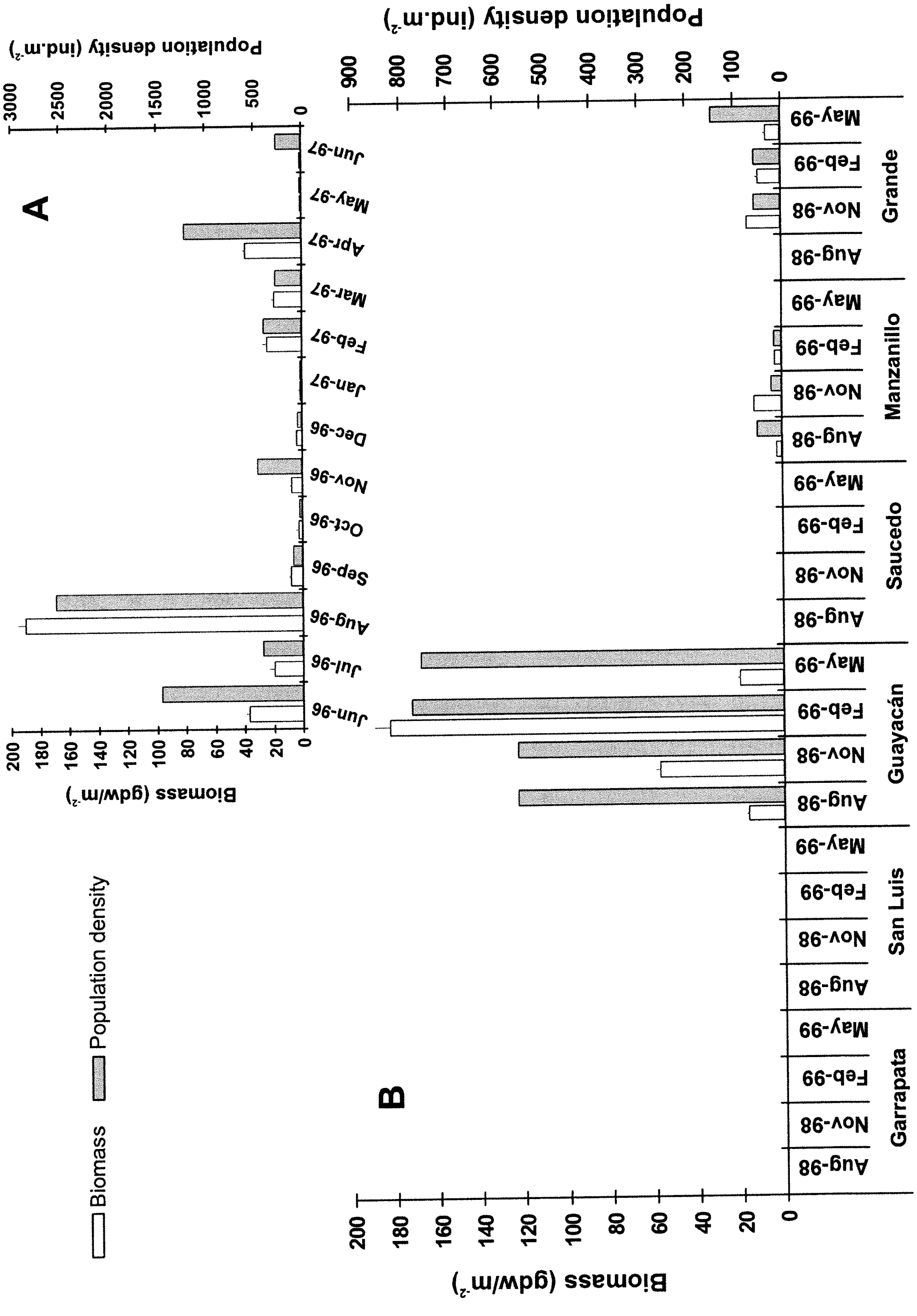
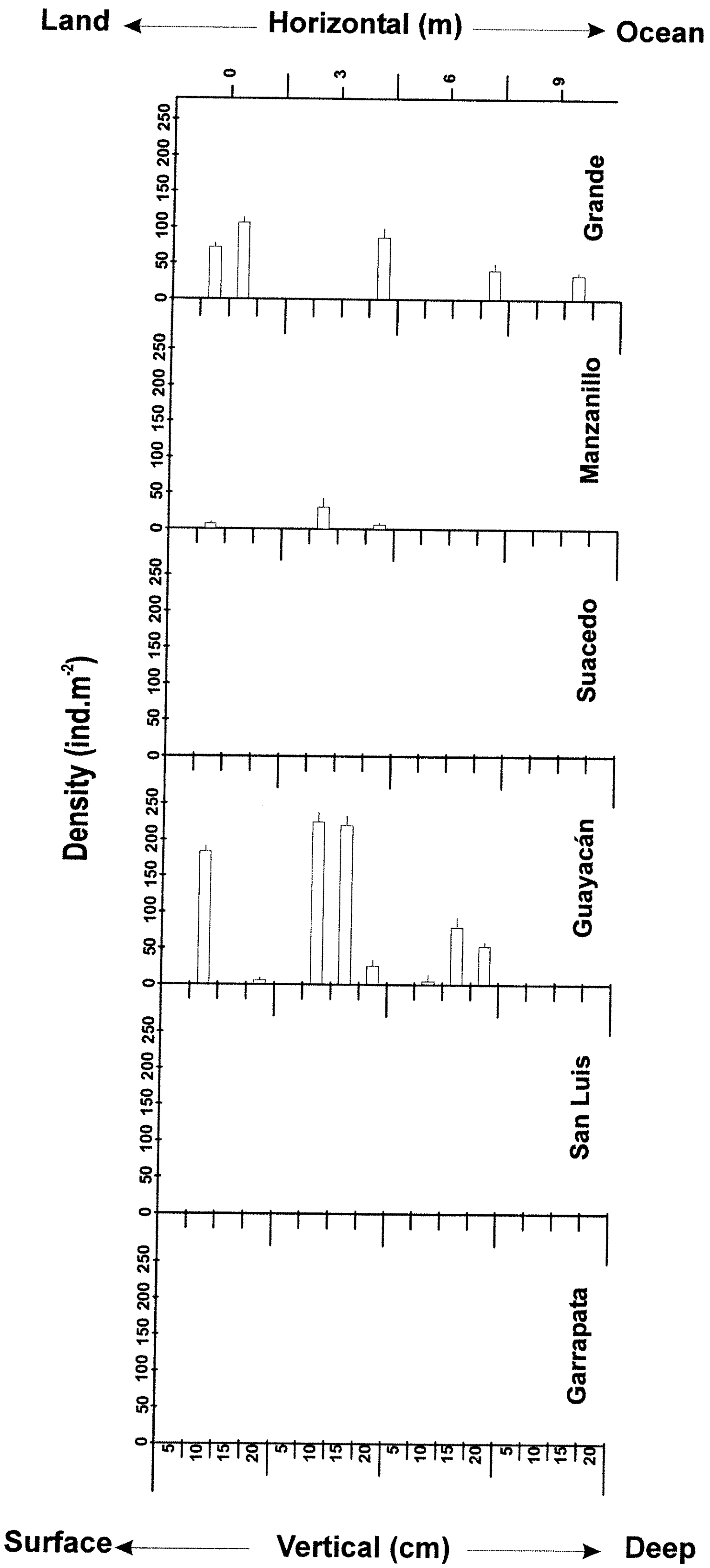

A
Population density (ind.m-2)
0
500
1000
1500
2000
2500
3000
Biomass (gdw/m-2)
0
20
40
60
80
100
120
140
160
180
200
Jun-96
Jul-96
Aug-96
Sep-96
Oct-96
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
B
Population density (ind.m-2)
0
100
200
300
400
500
600
700
800
900
Biomass (gdw/m-2)
0
20
40
60
80
100
120
140
160
180
200
Garrapata
San Luis
Guayacán
Saucedo
Manzanillo
Grande
Aug-98
Nov-98
Feb-99
May-99
Biomass
Population density

Figure 5. Horizontal (m) and vertical (cm) distribution of *Pontodrilus litoralis* in the inter-tidal region of six different beaches on the coast of north-eastern Venezuela.


Land
Horizontal (m)
Ocean
0
3
6
9
Density (ind.m^{-2})
0
50
100
150
200
250
Grande
Manzanillo
Suacedo
Guayacán
San Luis
Garrapata
5
10
15
20
Surface
Vertical (cm)
Deep

individuals were found in the surface sediments (5 cm). Contrarily, at the Manzanillo site, this annelid was restricted to the surface sediments of the 0 to 3 m zones.

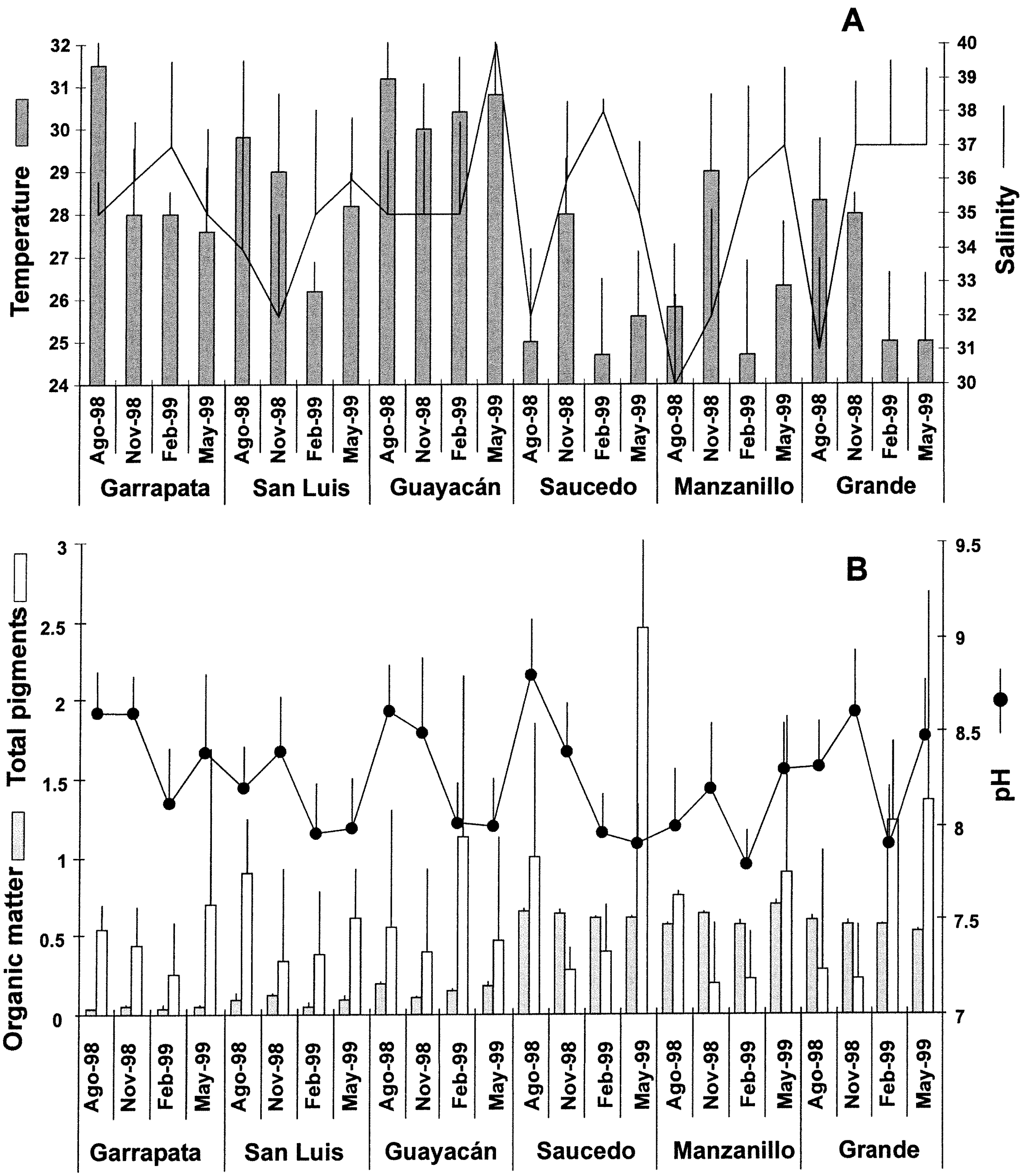
Abiotic and biotic characteristics

Among the six sites, temperature showed a consistent regional pattern (Figure 6A). The three western sites (Garrapata, San Luis and Guayacán) were on average 3 °C warmer than the three eastern ones (Saucedo, Manzanillo and Grande). Among the western sites, Guayacán was the warmest (~30 °C). Temperature averaged 26 °C in the three eastern sites. Salinity ranged from 30 ‰ to 37 ‰ among both western and eastern sites. The western sites seemed to show a more stable salinity than did the eastern ones (Figure 6) and pH ranged from 7 to 8 among the six sites.

The concentrations of total pigments were highly variable not only among the six sites, but also according to dates within each of the six sites (Figure 6B). Among the three western sites, the average concentrations of total pigments ranged from 0.48 ± 0.19 $mg.gww^{-1}$ at Garrapata, to 0.55 ± 0.26 $mg.gww^{-1}$ at San Luis, and 0.62 ± 0.33 $mg.gww^{-1}$ at Guayacán. Among the three eastern sites, the average concentrations of total pigments ranged from 0.51 ± 0.36 $mg.gww^{-1}$ at Manzanillo, to 0.77 ± 0.61 $mg.gww^{-1}$ at Grande, and 1.03 ± 1.00 $mg.gww^{-1}$ at Saucedo. The data on total pigments show that the eastern sites had the higher concentrations of total pigments than the three western ones.

The concentration of sedimentary organic matter (SOM) was also highly variable among the six study sites (Figure 6B). The three western sites (Garrapata, San Luis and

Figure 6. A) Seasonal variation (columns) and standard error (vertical bars) of temperature (°C) and salinity (‰) at the six regional sites. B) Seasonal variation (columns) and standard error (vertical bars) of organic matter (mg/gdw), total pigments (mg/gww) and pH (units) at the six regional sites.


A
Temperature
Salinity
32
31
30
29
28
27
26
25
24
40
39
38
37
36
35
34
33
32
31
30
Ago-98
Nov-98
Feb-99
May-99
Garrapata
San Luis
Guayacán
Saucedo
Manzanillo
Grande
B
Total pigments
Organic matter
pH
3
2.5
2
1.5
1
0.5
0
9.5
9
8.5
8
7.5
7

Guayacán) had substantially lower concentrations (one-way ANOVA; Fs: 1335; P <0.001) than the three eastern sites (Saucedo, Manzanillo and Grande). Among the three western sites, Garrapata concentrations (0.040 ± 0.031 $mg.gdw^{-1}$) tended to be the lowest, while slightly higher concentrations were found at San Luis (0.080 ± 0.060 $mg.gdw^{-1}$) and Guayacán (0.15 ± 0.080 $mg.gdw^{-1}$). The concentration of SOM at the three eastern sites ranged from 0.56 ± 0.062 $mg.gdw^{-1}$ at Grande, to 0.62 ± 0.04 $mg.gdw^{-1}$ at Saucedo, and 0.62 ± 0.09 $mg.gdw^{-1}$ at Manzanillo.

The size-distribution of sediment particles was highly variable among the six sites (Figure 7). The three western sites had similar ranges of particle sizes. The 0.250-mm size-class was dominant at San Luis, while the 0.500-mm one was dominant at Garrapata and Guayacán. Among the three eastern sites, particle sizes ranged from 0.125 to 4 mm, with peak concentrations in the 0.250-mm size-class for Saucedo and 0.500-mm one for Manzanillo and Grande.

Relationships of *Pontodrilus litoralis* with environmental parameters

The multiple regressions analysis indicated that the six environmental variables explained 65 % of the variability in *P. litoralis* density (Table 2). It is important to note that, in this multiple regression model, temperature, salinity, granulometry and total pigments were the significant parameters in predicting the density of *P. litoralis*. The other environmental parameters such pH and organic matter showed no significant relationship (Table 2; Figure 8a to e). The addition of salinity, particle-size of sediments and total pigments to the density-temperature relationship explained 60, 57 and 54 %, respectively,

Figure 7. Average size distribution of sediment particles (mean±SE) on the coast of north-eastern Venezuela.

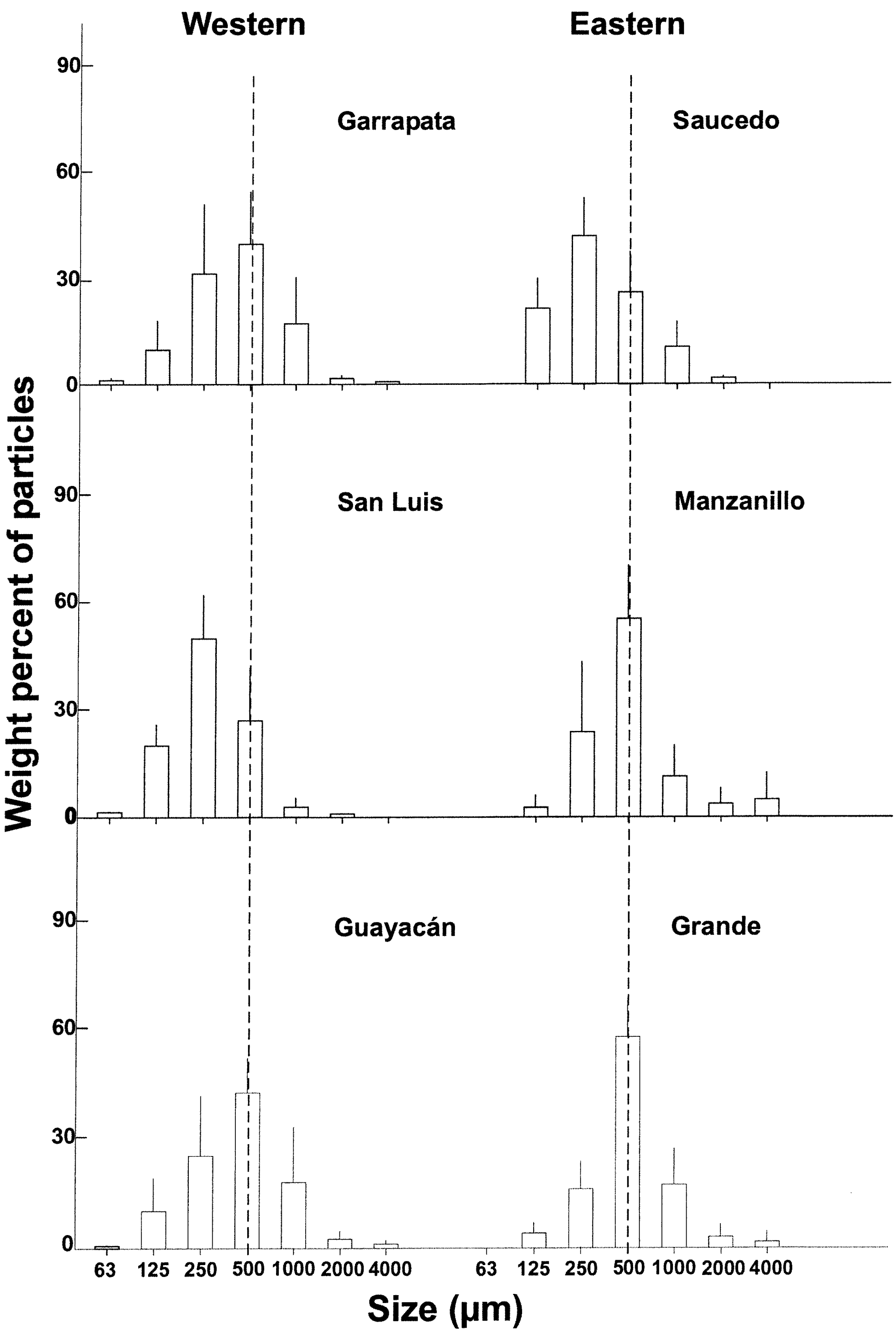

Western
Eastern
Garrapata
Saucedo
San Luis
Manzanillo
Guayacán
Grande
Weight percent of particles
90
60
30
0
63
125
250
500
1000
2000
4000
Size (µm)

Table 2.- Parameters describing the linear relationships between the population density of *P. litoralis* (ind.m^{-2}) and temperature (Temp.; °C), salinity (Sal.; ‰), particle size (Par. Size; µm), total pigments (T. P.; mg/dww), pH (units), and organic matter (Org. Mat.; mg/gdw). K= constant, R^2 = determination coefficient, t1, t2, t3, t4, t5 and t6 = student t for temperature, salinity, granulometry, total pigments, pH and organic matter respectively and their respective significance P level.

Relationships	K	Temp.	Sal.	Par. Size	T. P.	pH	Org. Mat.	R^2 deter.	t. 1 (P.)	t. 2 (P.)	t. 3 (P.)	t. 4 (P.)	t. 5 (P.)	t. 6 (P.)
I	-2729.1	80.0**	33.4*	496.2*	140.9*	-111.5	94.0	55	3.3 (0.004)	2.0 (0.07)	1.9 (0.07)	1.7 (0.10)	-0.8 (0.50)	0.5 (0.60)
II	-2427.6	52.1**	31.0*	-	-	-	-	60	4.8 (<0.0001)	3.1(0.006)	-	-	-	-
III	-1657.8	56.3**	-	421.9*	-	-	-	57	4.9 (<0.0001)	-	2.7 (0.013)	-	-	-
IV	-1654.3	60.6**	-	-	124.1*	-	-	54	4.9 (<0.0001)	-	-	2.4(0.028)	-	-
V	187.8	60.0**	-	-	-	-211.5	-	53	4.8 (<0.0001)	-	-	-	-2.3 (0.036)	-
VI	-1575.5	59.4*	-	-	-	-	106.3	44	3.6 (0.002)	-	-	-	-	0.8 (0.44)

*I: Population density vs. (temperature, salinity, size distribution of sediment, total pigments, pH and organic matter); II: Population density vs. (temperature and salinity); III: Population density vs. (temperature and size distribution of sediment); IV: Population density vs. (temperature and total pigments); V: Population density vs. (temperature, pH); VI: Population density vs. (temperature and organic matter).

*P <0.05

**P <0.01

***P <0.001

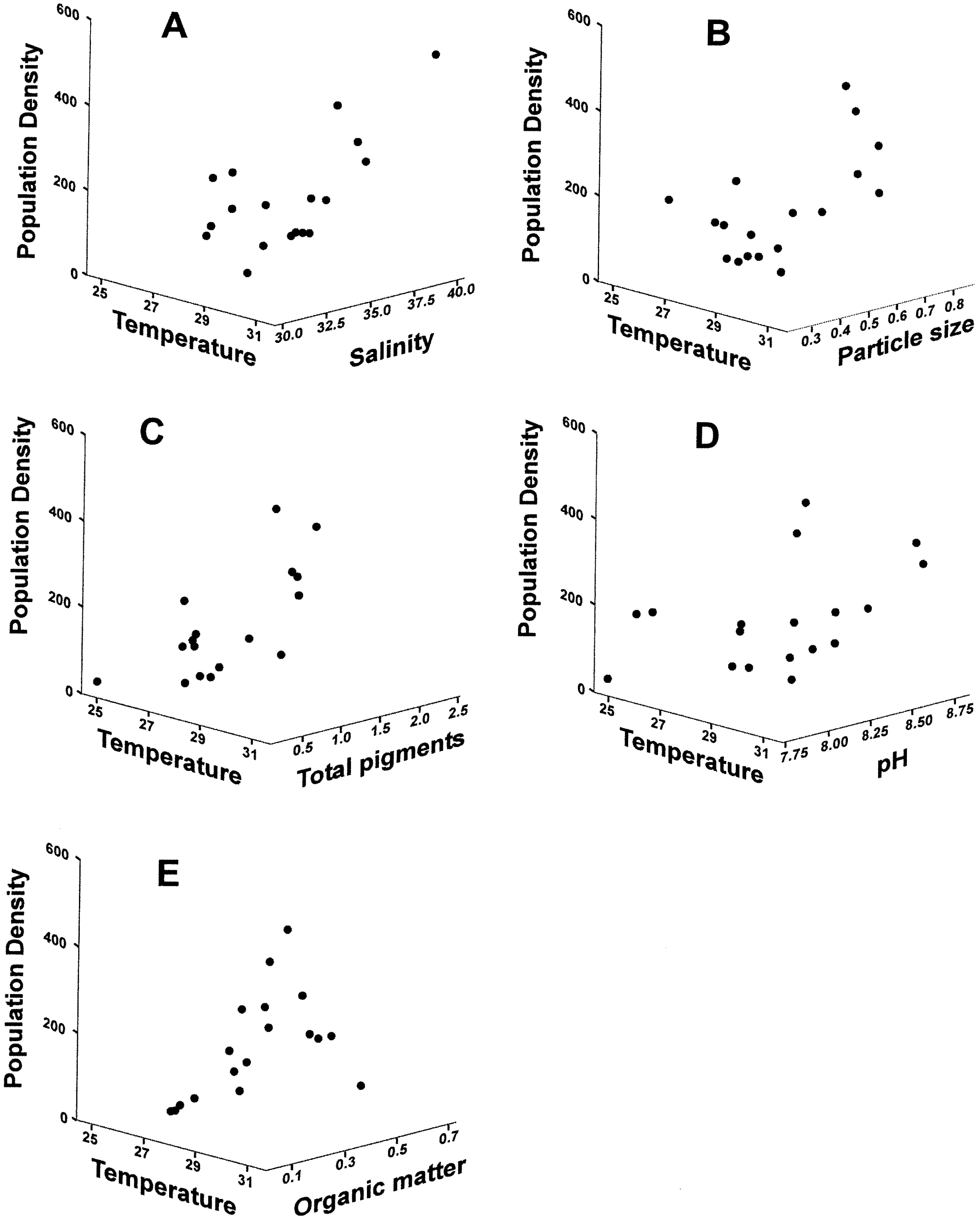
of the variability in density. PH had a negative impact (Figure 8d; Table 2) with a *P. litoralis* density declining at a pH values of 7.75 and organic matter concentration of 0.5 mg.gdw^{-1}.

DISCUSSION

There is a significant temporal pattern in the size and density distribution of *Pontodrilus litoralis*. Based on the high density of small size-classes in July-96, September-96, October-96, November-96, December-96, January-97, May-97 and June-97, this annelid appears to have extensive reproduction during the whole year. These results agree with Stephenson (1915, *fide* Giere and Pfannkuche 1982). Panikar and Aiyar (1939) observed that cocoon deposition in Madras-India begins in November and continues until May with peaks in February-March and a late winter or early spring. On the other hand, the consistent presence of smaller organisms in colder as well as warmer months could be explained by the hermaphroditic behaviour of this annelid.

Exogenous factors and processes such as high variations in temperature, salinity and pH and low concentrations of available food may also limit *P. litoralis* to smaller sizes. During the winter (dry season), high biomass in the coastal waters (Figure 9) may offer greater food resource. However, at the same time, there is an important increase in the number of shorebirds overwintering on the inter-tidal habitats of north-eastern Venezuela. The migratory patterns of Nearctic shorebirds to our study region have been described by McNeil (1970); McNeil and Burton (1973) and McNeil, *et al.* (1985). The decrease in

Figure 8. Linear multiple regressions between the population density (ind.m^{-2}) of *Pontodrilus litoralis* and temperature and salinity (A), temperature and particle size (B), temperature and total pigments (C), temperature and pH (D), and temperature and organic matter (E).

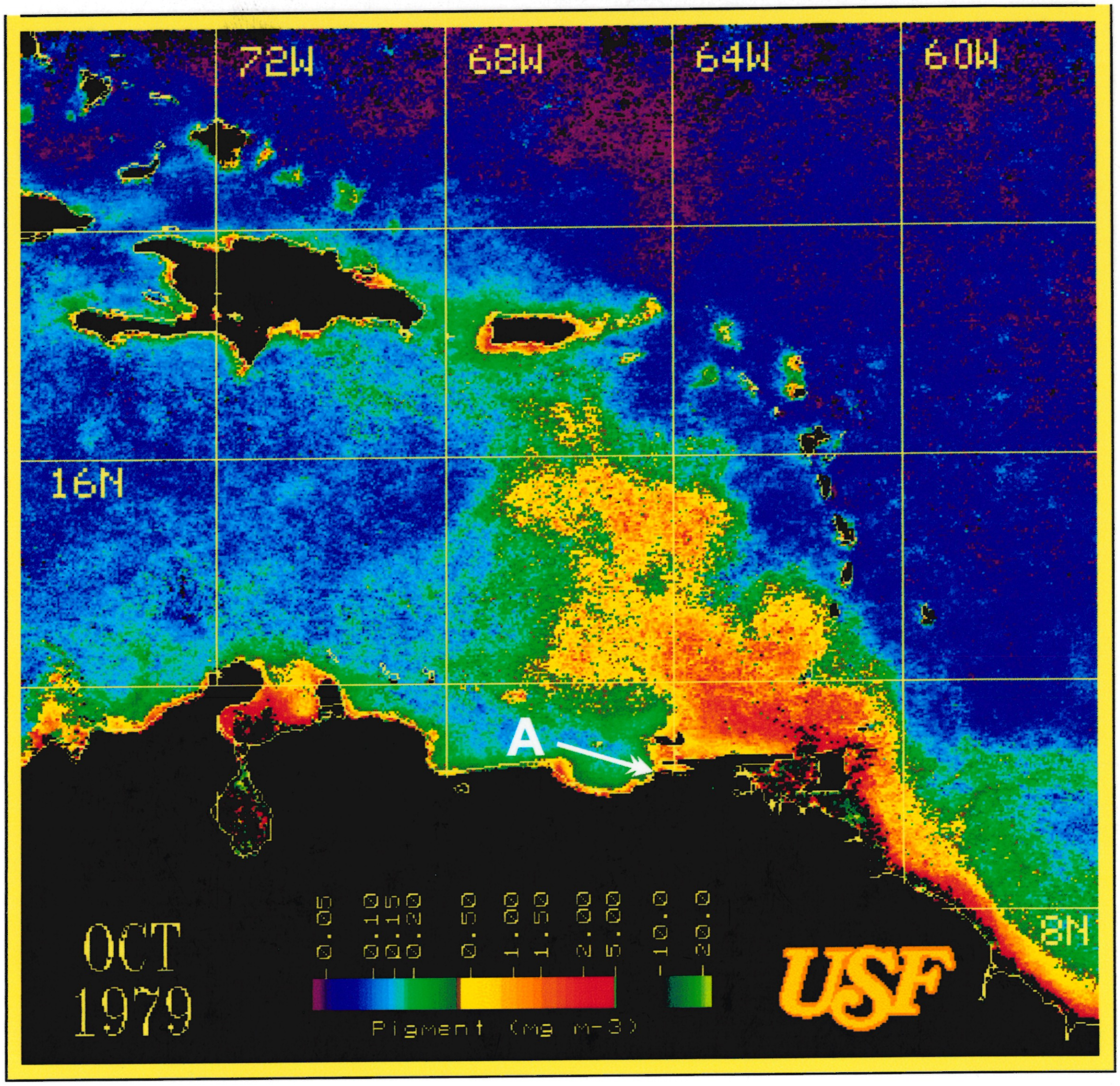

A
Population Density
600
400
200
0
25
27
29
31
Temperature
30.0
32.5
35.0
37.5
40.0
Salinity
B
Population Density
600
400
200
0
25
27
29
31
Temperature
0.3
0.4
0.5
0.6
0.7
0.8
Particle size
C
Population Density
600
400
200
0
25
27
29
31
Temperature
0.5
1.0
1.5
2.0
2.5
Total pigments
D
Population Density
600
400
200
0
25
27
29
31
Temperature
7.75
8.00
8.25
8.50
8.75
pH
E
Population Density
600
400
200
0
25
27
29
31
Temperature
0.1
0.3
0.5
0.7
Organic matter

density of larger size-classes in winter may be probably due to selective predation by migratory birds on large-size *Pontodrilus*. This assumption agrees with our field observations. At dawn and dusk we observed individuals of *Calidris pusilla*, *Tringa melanoleuca*, *Charadrius semipalmatus*, *Cataptrophorus semipalmatus*, and *Numenius phaeopus* feeding on *Pontodrilus litoralis*. On the other hand, the decline of density of larger size-classes from August could be related to increased harsh conditions associated with the dry season. A similar explanation has been offered by Brown and McLachlan (1990) for various inter-tidal invertebrate species of coastal areas of California and Mexico.

Temporally and regionally variable densities and biomass

P. litoralis was totally absent from three sites (two western sites and one eastern site). Environmental and anthropogenic conditions may be responsible for their complete absence at these sites. For example, the three sites that show high densities and biomasses of this annelid have low tidal energy and sheltered beaches with large inputs of organic matter from industrial and fishing activities. It seems that eutrophic conditions are ideal the for the recruitment and growth of these worms (Giere & Pfannkuche, 1982) which, in this specific case, may be created by fishermen when they leave unwanted invertebrates and fish on tidal areas to decompose. There are two potential reasons for the absence of *P. litoralis* at Garrapata, San Luis and Saucedo. Garrapata is the most pristine site of our study sites, with the lowest concentrations of organic matters and pigments (Table 1). Alternatively, wastewater inputs in San Luis and Saucedo may be responsible for the absence of this

Figure 9. Primary productivity (mg.m^{-3}) in the coast of Venezuela during October-79. "A" corresponds to the Araya Peninsula (see Figure 1) (Courtesy of Dr. Frank Müller-Krager, University of South Florida, Remote Sensing Laboratory, Global Change Data Centre, NASA Goddard Space).


72W
68W
64W
60W
16N
8N
A
OCT
1979
0.05
0.10
0.15
0.20
0.50
1.00
1.50
2.00
5.00
10.0
20.0
Pigment (mg m-3)
USF

annelid. In addition, all of these sites are high-energy inter-tidal zones, with large size sediment particles that may be inadequate for *P. litoralis*. We suggest that toxic inputs and high-wave energy may have negative impacts on *Pontodrilus* populations. When the inputs of industrial effluents are high, *Pontodrilus* is not abundant. While the organic matter enrichment by fishermen seems to support high densities of this annelid, toxic wastes seem to inhibit its growth and survival. Pearson and Rosenberg (1978) mentioned that anthropogenic activities can produce negative effects on benthic communities. However, more extensive research is be needed to verify the impact of eutrophication versus toxic waste inputs on coastal oligochaetes.

Our data show a highly variable spatial distribution at the six sites more intensively surveyed. The total absence of *P. litoralis* at Garrapata, San Luis and Saucedo can be explained by drastic alteration of sedimentary environments due to anthropogenic effects discussed earlier. Both the vertical and horizontal distribution patterns of *P. litoralis* seem to be strongly site dependent, probably because strong variations in the physical, chemical and biological characteristics of sediments. The aggregation of *Pontodrilus* within both the high-tide and low-tide zones and the deepest sediment strata observed at Guayacán and Grande contrasts with the aggregation within the high-tide zones and superficial strata at Manzanillo. These results suggest that *P. litoralis* needs appropriate environmental conditions associated with different sedimentary strata in the inter-tidal zone. Our results indicate that a temperature of 27-31 °C, a salinity of 35-37‰, sedimentary size particles of 500-700 µm, and total pigment content from 0.5 to 1 mg/dww represent the best environmental conditions for *Pontodrilus litoralis*.

Temperature and salinity have been considered as important factors regulating the distribution of (littoral) oligochaetes (Lassèrre, 1971; Edwards & Lofty, 1977 Giere & Pfannkuche, 1982). Temperature has been suggested to regulate growth, metabolism, respiration and reproduction (Edwards & Lofty, 1977), but very little field research has been done to demonstrate the relative importance of environmental factors in regulating the distribution and growth of marine oligochaetes. Certain enchytraeids and tubificids can endure high temperatures (35 °C); for exampled, the interstitial tubificid *Aktedrilus monospermathecus* can tolerate 35 °C and 40 ‰ (Giere & Pfannkuche, 1982). Such conditions can certainly contribute to reducing population density in oligochaetes when the anoxic conditions of the sediments inhibit vertical migrations. Finally, burrowing deeper into the sediments could be, for *P. litoralis*, a strategy to avoid their predators.

The environmental conditions of Venezuelan coastal ecosystems depend largely on the Inter–Tropical Convergence Zone (ITCZ) which produces a bi-seasonal environmental regime in the equatorial South American coast. The coast of north-eastern Venezuela is also influenced by up-welling phenomena (Fukuoka, 1965, 1966; Mandeli and Ferráz-Reyes, 1982; Margalef, 1980). Both phenomena (ITCZ and up-welling) result in seasonal changes in temperature, salinity, density, concentration of oxygen, pH, and wave energy (Fukuoka, 1966; Margalef, 1980; Mandeli and Ferráz-Reyes, 1982; Del Cano and Velásquez, 1992), which directly influence the inter-tidal flora and fauna. The coast of north-eastern Venezuela is characterized by the presence of bays, gulfs, cliffs, sheltered beaches, coastal lagoons and high-energy beaches. In addition, the Venezuelan coastal ecosystems are influenced by the Orinoco River plume (Figure 9).

Based on our multiple regression models, temperature seems to play the most important role in determining the presence and distribution of *P. litoralis*. Other significant environmental factors related with the density of this oligochaete were salinity, size-distribution of sedimentary particles and total pigments. Our results, although they constitute the first comprehensive study of Venezuelan coastal ecosystems, seem to agree with other studies on oligochaetes from tropical and temperate zones. Complete disappearance of *Lumbricillus lineatus*, *Enchytraeus albidus*, *Marionina subterranean*, *Aktedrilus monospermathecus* populations were explained by strong variations of sedimentary environments (Gates, 1961; Edwards and Lofty, 1977; Giere and Pfannkuche, 1982; Lee, 1985). Microphytobentos organisms are severely affected by sedimentary environments and are a source of food for benthic invertebrates (Montagna, 1984; Colijn and De Jonge, 1984; Plante-Cuny and Plante, 1986 *fide* Sundbäck and Jönsson, 1988), and more specifically for oligochaetes (Giere and Pfannkuche, 1982).

It is important to note that *P. litoralis* had a poor response to organic matter and pH, which would suggest that *Pontodrilus* feeds predominantly on microorganisms that inhabit in sedimentary interstices. Likewise, this annelid may be adapted to wide pH variations that commonly occur in sedimentary environments with anoxic conditions.

CONCLUSION

In this paper, we present the first detailed study of *Pontodrilus litoralis* from inter-tidal ecosystems of north-eastern Venezuela, and discuss the synergistic effects of environmental parameters (temperature, salinity, size-distribution of sedimentary particles and total pigments) and anthropogenic factors on the presence and distribution of this oligochaete. The species is abundant in sheltered inter-tidal ecosystems where low wave actions, normal salinity (35-37‰), small-sizes of sediment particles, and important concentrations of total pigments are available. High densities of small individuals were observed during the whole year indicating a constant reproduction and a potentially high metabolic activity. High concentrations of pH and organic matter seem to produce a negative impact on *P. litoralis*. The species seems to prefer conditions of temperature ranging from 28 to 29 °C, salinity in the order the 35-37 ‰, sediment particle sizes ranging between 500-700 µm, and total pigments of 0.5-1 mg/dww. These specific conditions are found in sheltered sites like Guayacán. We also believe that this annelid could be used as an excellent indicator of environmental changes due to anthropogenic impacts on coastal ecosystems.

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Chapter 3

Temporal and Spatial Distribution of *Tylos wegeneri* (Crustacea, Isopoda) in Intertidal Ecosystems of the Coast of North-Eastern Venezuela

Andrade, José & Asit Mazumder

ABSTRACT

The spatial and temporal variation of the inter-tidal isopod *Tylos wegeneri* was determined in order to evaluate the effects of environmental variables on its presence and distribution along on the coast of north-eastern Venezuela, where it is a dominant species. At Guayacán, on the coast of north-eastern Venezuela, we studied the temporal and spatial distribution of *T. wegeneri* in terms of density, biomass, and size during a 13-month period. Granulometry, organic matter, total pigments, temperature, salinity and pH were measured to determine the relative importance of these variables in determining the distribution of *T. wegeneri*.

In order to determine the distribution of the species over a broad range of environmental factors, we sampled six sites along 293 km of the Venezuelan coast. Three of these six sites were located in the western region (Garrapata, San Luis and Guayacán) and three were in the eastern region (Saucedo, Manzanillo and Grande).

We show the combined synergistic effect of environmental factors such as temperature, salinity, granulometry and total pigments in determining the presence and distribution of this isopod in coastal Venezuela. We also discuss the potential for this species to be used as an indicator of the environmental quality of Venezuelan coastal beaches, which may be sensitive to anthropogenic impacts.

Keywords: *Tylos wegeneri*, isopod, coastal ecology, inter-tidal zones, tropical ecology, food web, nutrition.

INTRODUCTION

The benthic isopod, *Tylos wegeneri,* inhabits the sandy coastal beaches of north-eastern Venezuela, and is commonly observed along the higher levels of inter-tidal zones (sand dunes). They are found in large numbers on high-tide zones, but their density is highly variable. There is a lack of information on which factors regulate their distribution and how they adapt to a wide range of physical and chemical conditions of the tidal ecosystems (Brown and Odendal, 1994). A preliminary sampling conducted at the Guayacán in north-eastern Venezuela (10º39′N–63º 48'W) revealed densities of up to 1000 ind.m^{-2}. To survive at such high densities, this species must respond to rapid changes in the dry and highly variable environmental conditions. Our objective was to determine the environmental factors and processes that determine the temporal and spatial distribution, of this isopod. We also wanted to determine the ecological role of this isopod in the inter-tidal ecosystems.

We suspected that seasonal and spatial distributions of *T. wegeneri* are controlled by the availability of food, temperature, salinity, sediment moisture and size of sediment particles. Some *Tylos* species have been reported to feed on macroalgae in the sediments and on dead organisms such fish and macro-invertebrates (Brown and Odendal, 1994). Our preliminary data from Guayacán show that *Tylos* individuals range from 0.35 cm to 3.5 cm in size. With such a great range in size distribution, they may switch from herbivory to omnivory or carnivory with increasing size (ontogenetic niche shift). Such patterns have been reported for *Mysis relicta*, a freshwater crustacean commonly known as the opposum shrimp (Branstrator *et al.* 1999).

Although *Tylos*, as a genus, is largely nocturnal (Ondo, 1953; Pardi, 1955; Hamner *et al.*, 1968 and 1969; Hayes, 1969), some *Tylos* species seem to show diurnal rhythms in their distribution and feeding activities. *T. europaeus* shows diurnal activity in damp, shady and rocky crevices, but not in sandy environments (Pardi, 1955). *T. europaeus* and *T. sardous* show diurnal activity following the tidal flooding of their burrows (Mead and Mead, 1974). Nocturnal activity in such invertebrates has been proposed to be associated with the diurnal activity of predators such as birds (Hamner, *et al.*, 1969; Kensley, 1974), and also with the maintenance of favourable tidal conditions (Holanov and Hendrickson, 1980). Members of the genus *Tylos* generally surface at night or at low tide (Ondo, 1952; Hamner *et al.*, 1969) and feed on a variety of organisms brought to them by wave action (Hamner *et al.*, 1969; Brown and Odendal, 1994). Although various studies have dealt with the spatial and temporal distributions of *Tylos* species, none has been conducted on *Tylos wegeneri*, the most dominant species on the sandy beaches of tropical latitudes.

The consistent presence of this inter-tidal crustacean along the coast of north-eastern Venezuela, especially in sites close to fishing and urban communities, suggests that this organism could be an excellent indicator of environmental quality and of anthropogenic environmental changes. There is a lack of information on how *Tylos wegeneri* responds to organic pollution, habitat degradation and human invasion of beaches and dunes. Along the coast of north-eastern Venezuela, dense fishing communities, fish canning industries, and petroleum exploitation resulted in severe negative effects on the quality of inter-tidal ecosystems (Brown and Odendal, 1994). Our specific objectives were

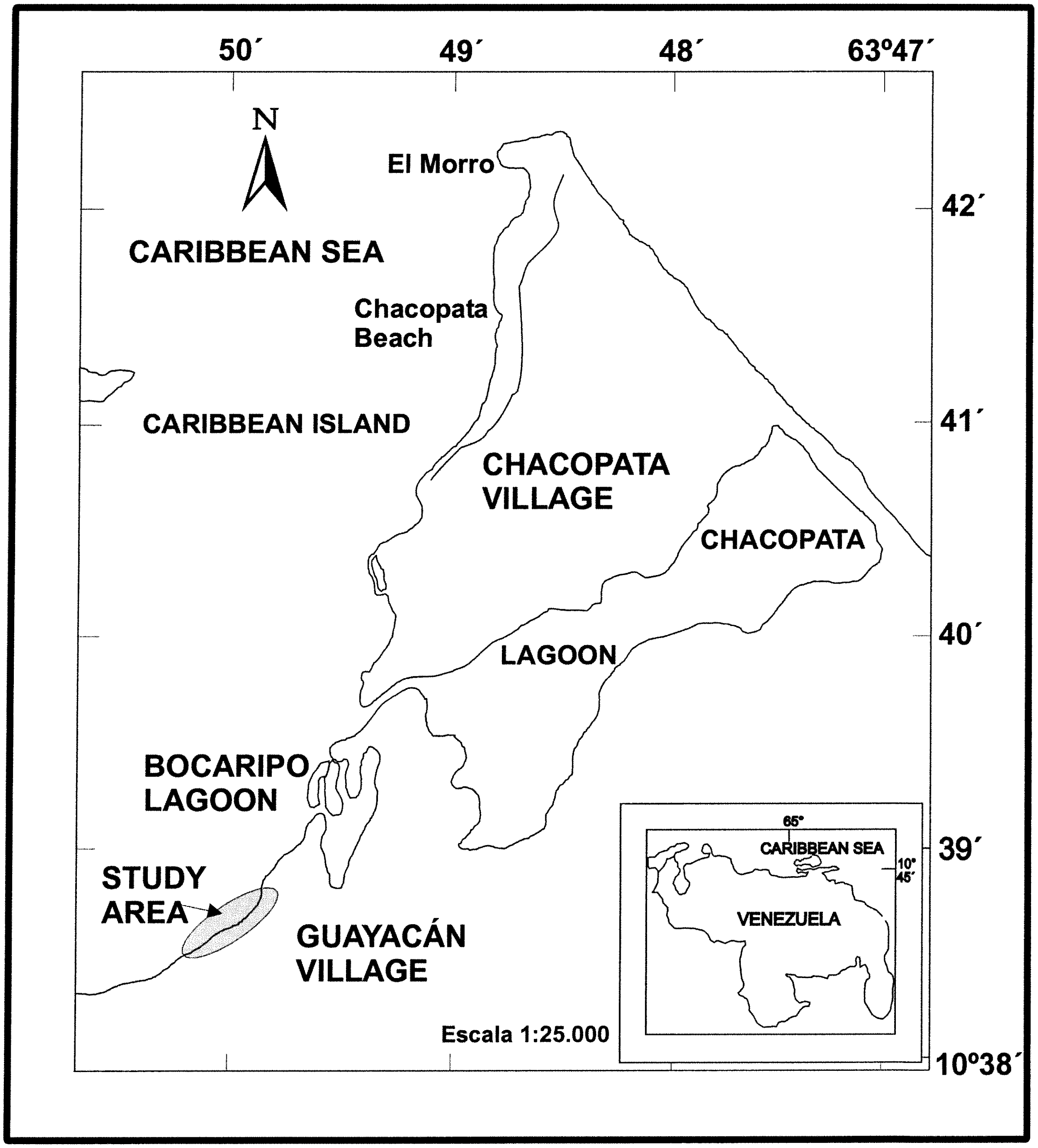
1) to study the horizontal and vertical distributions of *Tylos wegeneri* along a section of the coast of north-eastern Venezuela covering a wide range of environmental and anthropogenic conditions, and 2) to determine what environmental factors and processes are governing the temporal and spatial distributions of this isopod. Although we evaluated the pattern of diet shift in *Tylos* with increasing body size, this will be addressed in a separate paper.

MATERIALS AND METHODS

Once a month between June 1996 and June 1997, triplicate samples were collected from 4 plots of 0.25 m^2 at the Guayacán research site (Figure 1), in each transect of 108 m2 (9x12-m) in order to determine the monthly size distribution and population density of *T. wegeneri*, as well as the environmental patterns of temperature, salinity, pH, organic matter, size of sediment particles and total pigments. Sampling was done by driving a core of 15.25 cm in diameter 30 cm deep into the sediments. Collected specimens were washed and preserved in 40% alcohol and their wet weight (± 0.01mg) and total length (± 0.01mm) were estimated. Size distribution was developed using frequency histograms (Zar, 1984; SPSS, 1999).

Temperature, salinity (refractometer ±1‰) and pH were measured on the site. The size-distribution of sediment particles was obtained by drying samples in an oven at 70°C for 24 hours. Subsamples (100 g) were separated into different size-categories (0.063, 0.125, 0.250, 0.500, 1.000, 2.000 and 4.000 mm) using mechanical sieves (Holme and

Figure 1. Geographic location of the Bocaripo-Chacopata lagoon complex and study area on the northern side of the Araya peninsula in north-eastern Venezuela.


50´
49´
48´
63°47´
N
El Morro
42´
CARIBBEAN SEA
Chacopata Beach
CARIBBEAN ISLAND
41´
CHACOPATA VILLAGE
CHACOPATA
40´
LAGOON
BOCARIPO LAGOON
39´
STUDY AREA
GUAYACÁN VILLAGE
65°
CARIBBEAN SEA
10° 45´
VENEZUELA
Escala 1:25.000
10°38´

McIntyre, 1971; Gray, 1981). Four replicates of 1g from each sediment core were weighed, digested in concentrate chlorhidric acid and washed with double distilled water (to remove carbonated materials). After drying at 40ºC for 24 hours, the dry weight was obtained, and thereafter the ashes were collected after burning at 450ºC for 24 hours. The difference between dry weight and ashed weight provided an estimate of organic matter contents (mg/gdw) (Holme and McIntyre, 1971).

Total pigment concentration (mg/gww) was determined from four 1 gww of sediment by extracting pigments in 15 mL of 90% acetone. Samples were agitated for 15 minutes at 40ºC, and cooled to room temperature. Thereafter, 10 mg of magnesium sulphate were added, and samples were placed in a refrigerator for 24 hours. Finally, the samples were filtered and centrifuged, and the filtrates were analysed for pigment concentrations using a spectrophotometer (Strickland and Parsons, 1972).

A preliminary survey was conducted in August 1998 at 32 sites distributed along the coast of Sucre State in north-eastern Venezuela in order to search for the presence or absence of *Tylos wegeneri* (Figure 2). The species was present everywhere except at Garrapata and Saucedo sites. Saucedo is located close to large urban settlements while Garrapata is located in the relatively pristine National Park of Mochima. From the 32 sites, based on accessibility, 6 sites were retained for intensive seasonal sampling. Sampling would have been too difficult and expensive at other sites due to difficult access (no nearby road). The general environmental characteristics of these six sites are given in Table 1. These six sites (Garrapata, San Luis, Guayacán, Saucedo, Manzanillo and Grande) were

Figure 2. Map showing the distribution of six regional sampling sites.

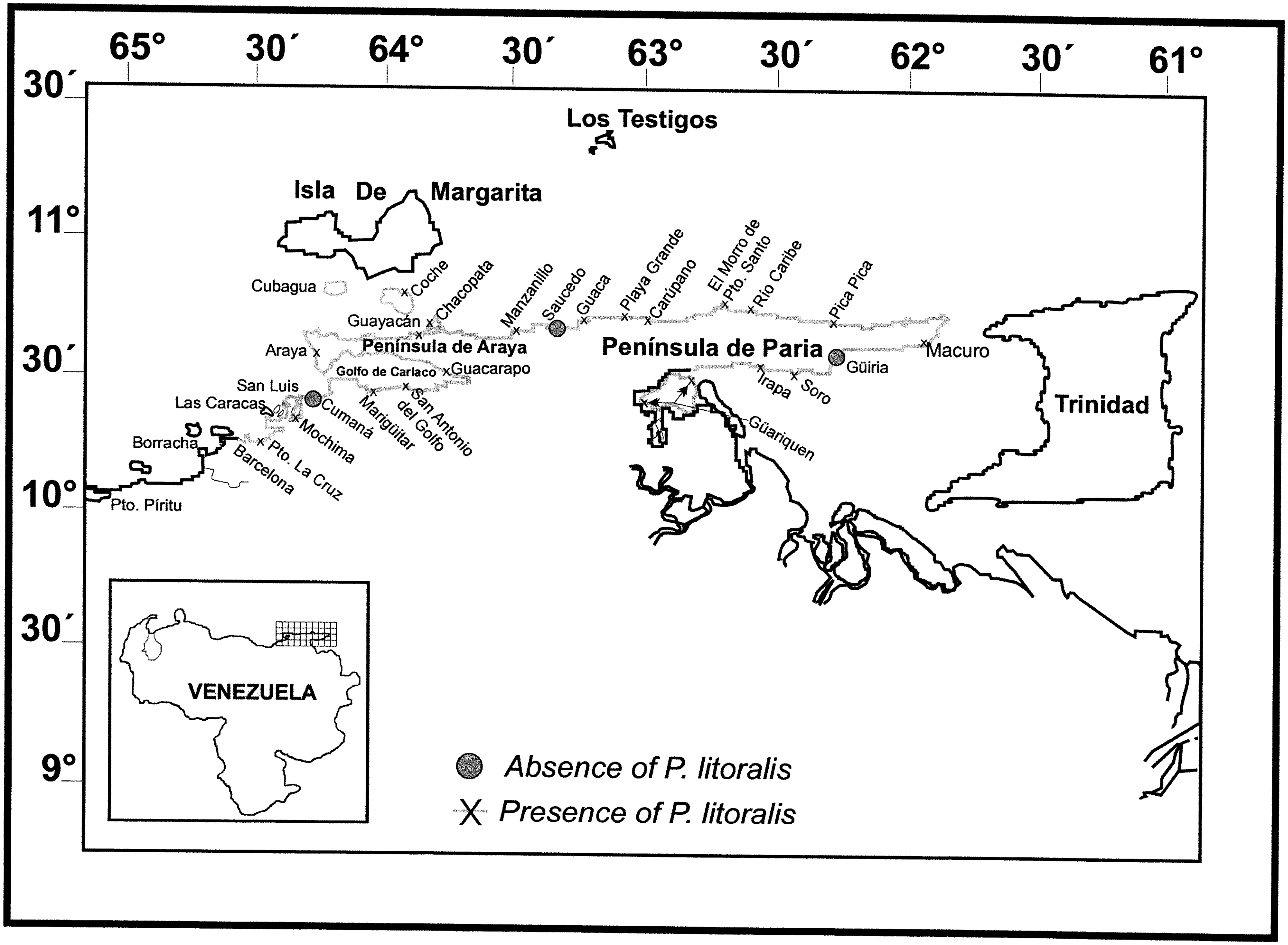

65°
30´
64°
30´
63°
30´
62°
30´
61°
30´
11°
30´
10°
30´
9°
Los Testigos
Isla De Margarita
Cubagua
Coche
Chacopata
Guayacán
Manzanillo
Saucedo
Guaca
Playa Grande
Carúpano
El Morro de Pto. Santo
Río Caribe
Pica Pica
Macuro
Araya
Península de Araya
Golfo de Cariaco
Guacarapo
Península de Paria
Güiria
Irapa
Soro
Güariquen
Trinidad
San Luis
Las Caracas
Cumaná
Mochima
Marigüitar
San Antonio del Golfo
Borracha
Pto. La Cruz
Barcelona
Pto. Píritu
VENEZUELA
● Absence of P. litoralis
X Presence of P. litoralis

Table 1. Present status of different sites considered in this study.

Regions	Places	Habitants	Status*	Characteristic	Enterprises	Impact
Western	Garrapata	500	a	Sheltered and low energy beach	National Park	(+) on the inter-tidal zone.
	San Luis	280,000	a	High energy inter-tidal zone	Presence of nearly salt processing and fish canning industries, jetties, port and navigation, plus diverse agricultural cultivations	(-) on the inter-tidal zone.
	Guayacán	1,000	b	Sheltered and low energy inter-tidal zone	Fishermen beach	(±) on the inter-tidal zone.
Eastern	Saucedo	3,000	b	High energy inter-tidal zone	Fishermen beach	(±) on the inter-tidal zone.
	Manzanillo	800	a	Sheltered and low energy inter-tidal zone	Nearly fish canning industry	(-) on the inter-tidal zone.
	Grande	85,000	a	Sheltered area of high energy inter-tidal zone	Presence of nearly salt processing and fish canning industries, jetties, port and navigation, plus diverse agricultural cultivations	(-) on the inter-tidal zone.

***a: Recreational activities**
b: Absence of recreational or industrial activities

sampled every three month between August 1998 and May 1999 in order to study the temporal and spatial distribution of *T. wegeneri* at both horizontal and vertical scales. Horizontal distribution was indicated by the densities of organisms at four stations of 0 (corresponds to the high-tide zone), 3, 6 and 9 meters (low-tide zone), while vertical distributions was indicated by the densities of organisms at four depths (5, 10, 15 and 20 cm from surface) at each horizontal location. These six sites were distributed along 293 km of coastline of north-eastern Venezuela. The sampling protocols for physical, chemical and biological variables were similar to those described above.

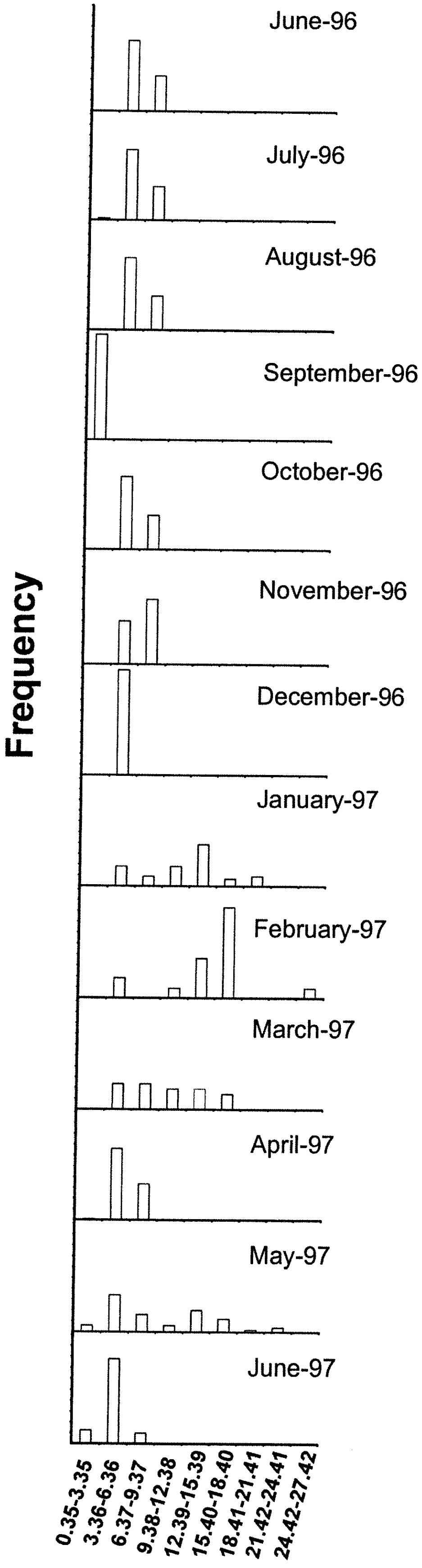
One-way ANOVA and a Bonferroni *post hoc* tests were performed in order to establish spatial and temporal variations of physical, chemical and biological parameters. Multiple regression analyses were conducted in order to determine the relative importance of environmental factors in determining the distribution of *T. wegeneri* (Sokal and Rohlf, 1980; Zar, 1984).

RESULTS

Size structure

The monthly variations in the size structure of *T. wegeneri* were examined using the 13-month data obtained from the Guayacán site (Figure 3). During this period, mean length varied from 0.49 to 24.95 mm, with the most dominant sizes ranging from 2.0 to 9.0 mm. Sizes observed during June through December were smaller than those observed during the drier months of January through March. During June through December, none of

Figure 3. Size frequency distribution of *Tylos wegeneri* between June 1996 and June 1997 at the Guayacán site.


June-96
July-96
August-96
September-96
October-96
November-96
December-96
January-97
February-97
March-97
April-97
May-97
June-97
Frequency
0.35-3.35
3.36-6.36
6.37-9.37
9.38-12.38
12.39-15.39
15.40-18.40
18.41-21.41
21.42-24.41
24.42-27.42

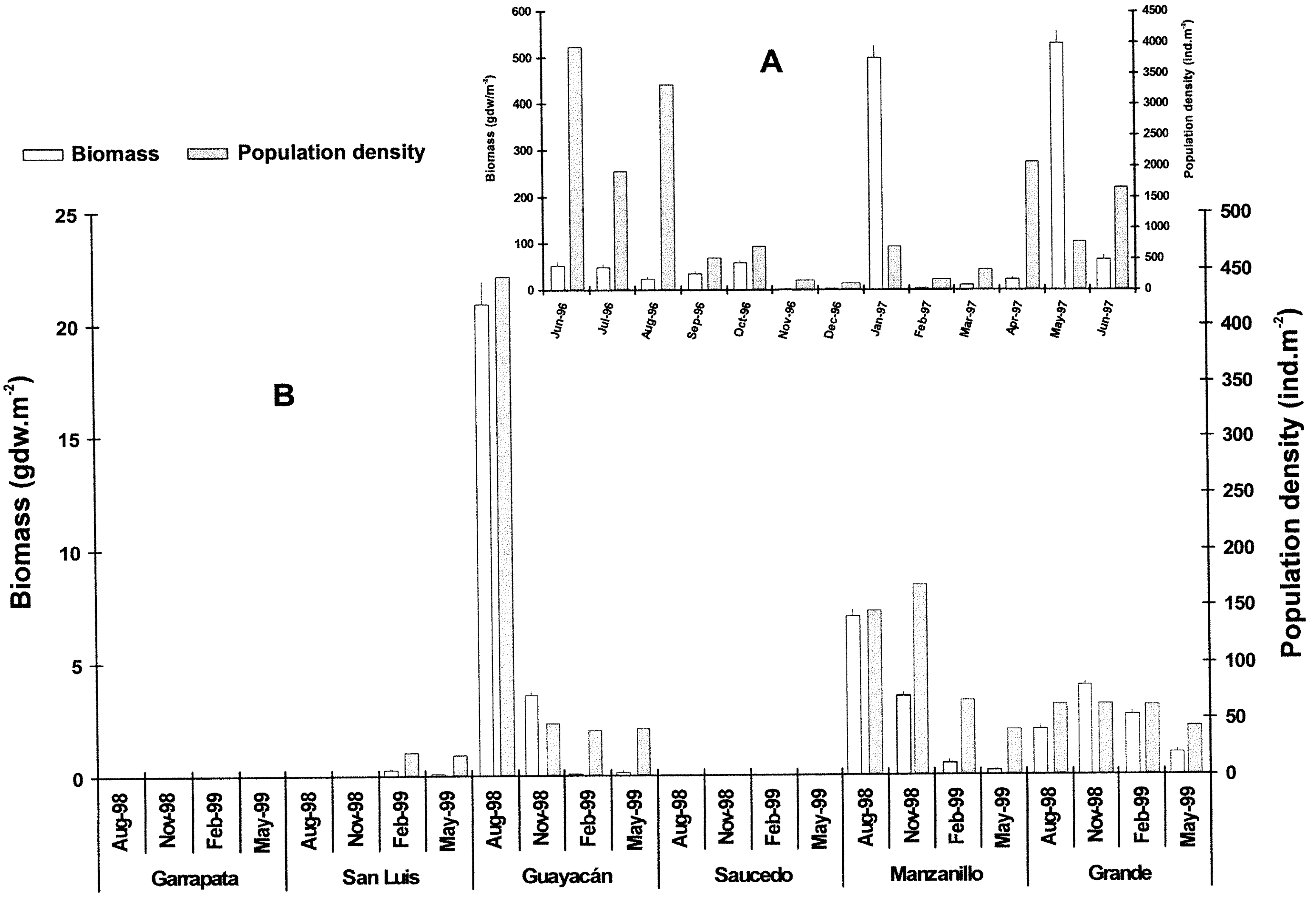
the individuals exceeded 9.3 mm in length, while between January and May 83% of the population reached 18.4 mm. However, in September and December, only two size-classes (0.35-3.35 mm and 3.35-6.35 mm, respectively) represented the entire populations of *Tylos* (Figure 3).

Seasonal variation in population density and biomass

During the 13-month period (June 1996 to June 1997), the density of *T. wegeneri* at the Guayacán ranged from as low as 50 ind.m^{-2} during November-March to as high as 1000 to 4000 ind.m^{-2} during April to August (Figure 4A). However, biomass did not follow the same monthly pattern because the size-distribution of organisms was highly variable (Figure 3). The highest biomass of 500 gdw.m^{-2} was observed in January and May when more than 50% of the individuals exceeded 9 mm in length. Biomass was less than 10 gdw.m^{-2} during November, December, February and March, and varied between 20 to 75 gdw.m^{-2} in other months.

The trimestrial sampling of six sites along the Sucre coast also showed strong seasonal variation in the population density and biomass of *T. wegeneri* (Figure 4B). The species was absent from the Garrapata and Saucedo sites. The San Luis site exhibited very low biomass and density of *T. wegeneri* in February and May and total absence in August and November. Among the Guayacán, Manzanillo and Grande sites where the species was consistently present, population densities ranged from 50 to 500, 40 to 200, and 100 to 200 ind.m^{-2}, while biomass ranged from < 2 to 20, 2 to 7, and 2 to 6 gdw.m^{-2} respectively. A comparison of population density and biomass of *Tylos* in Guayacán for 1996 to 1999

Figure 4. Seasonal variation in density (ind.m^{-2}) and biomass (gdw/m^{-2}) of *Tylos wegeneri* during June 19967 to June 1997 in Guayacán beach (A) and during August 1998 to May 1999 in six different beaches of north-eastern coast of Venezuela (B).


Biomass
Population density
A
Biomass (gdw/m²)
600
500
400
300
200
100
0
Population density (ind.m⁻²)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Jun-96
Jul-96
Aug-96
Sep-96
Oct-96
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
B
Biomass (gdw.m⁻²)
25
20
15
10
5
0
Population density (ind.m⁻²)
500
450
400
350
300
250
200
150
100
50
0
Aug-98
Nov-98
Feb-99
May-99
Garrapata
San Luis
Guayacán
Saucedo
Manzanillo
Grande

showed large inter-annual variability.

Horizontal and vertical distribution

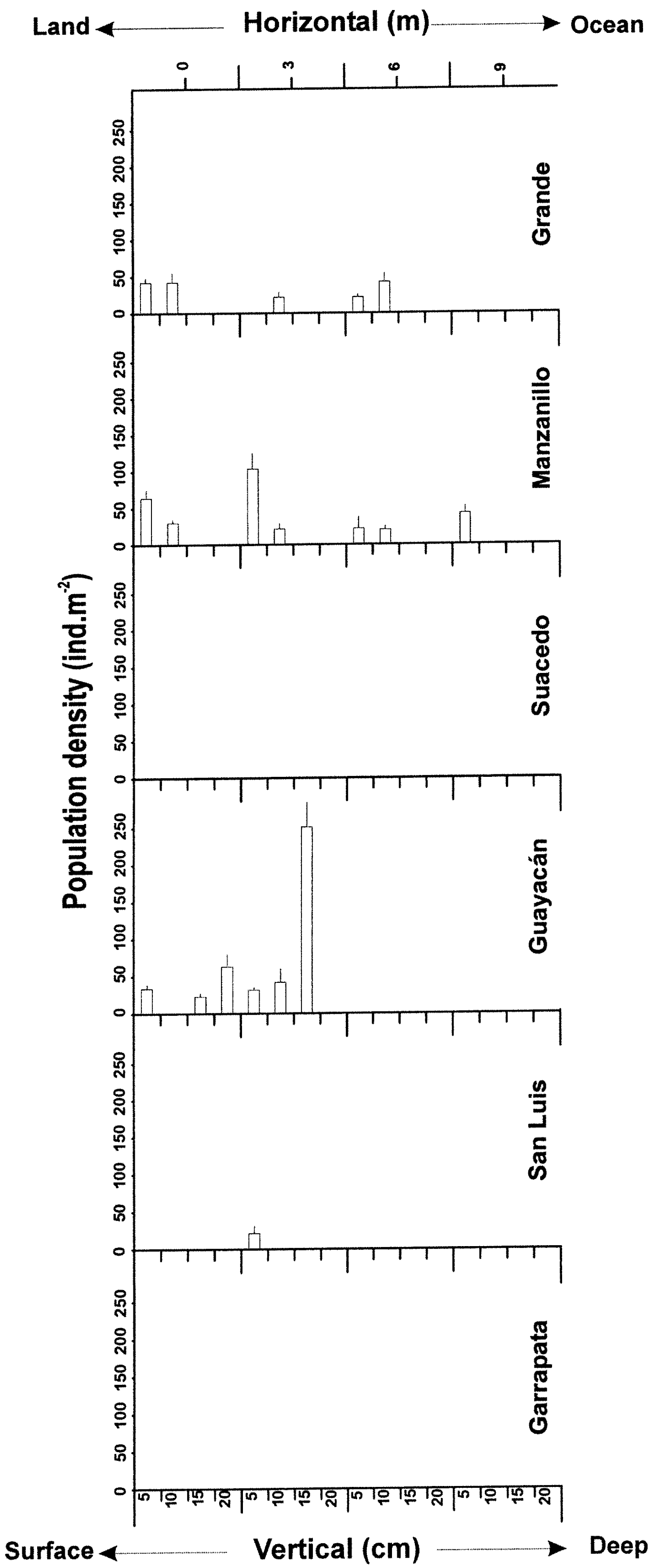
Figure 5 shows the trimestrial variations in horizontal (from high-tide to low-tide zone) and vertical (at four depth levels) distribution of *T. wegeneri* at the six study sites. There was a strong variation in both the horizontal and vertical distribution patterns. As mentioned above, at Garrapata and Saucedo, no organisms were found in any of the horizontal or vertical zones. At San Luis, only a few organisms (42 ind.m^{-2}) were found at the 3-m location from the high-tide line and at the depth of 5 cm (Figure 5).

The three other sites with a higher abundance of *T. wegeneri* showed pronounced horizontal and vertical variations in distribution. At Guayacán, this isopod was distributed only at the two high-tide zones (0 and 3 m), and no individuals were found in the low-tide one. In the two high-tide zones, *Tylos* was more abundant in the deeper sediments (15 and 20 cm). At Manzanillo and Grande, *T. wegeneri* showed a broader horizontal distribution but was limited to the top strata (5 to 10 cm). At Manzanillo, *T. wegeneri* was observed at all horizontal locations from the high- (0 m) to the low-tide (9 m) zones.

Abiotic and biotic factors

Among the six sites, salinity ranged from 30 ‰ to 37 ‰, but appeared to be more stable in the western sites, (Garrapata, San Luis and Guayacán) (Figure 6A). Temperature showed slightly wider regional variability than salinity (Figure 6A). The three western sites were on average 3°C warmer than the three eastern ones (Saucedo, Manzanillo, and Grande). Among the western sites, Guayacán was the warmest (~ 30°C). Temperature

Figure 5. Horizontal (m) and vertical (cm) distribution of *Tylos wegeneri* in the intertidal region of six different beaches on the coast of north-eastern Venezuela.


Land
Horizontal (m)
Ocean
0
3
6
9
Population density (ind.m^{-2})
0
50
100
150
200
250
Grande
Manzanillo
Suacedo
Guayacán
San Luis
Garrapata
5
10
15
20
Surface
Vertical (cm)
Deep

averaged 26°C in the three eastern sites, and pH varied from 7 to 8 among the six sites.

The three western sites had substantially lower concentrations of sedimentary organic matter (SOM) (one-way ANOVA; Fs = 1335, $P < 0.001$) than the three eastern ones. Among the three western sites, Garrapata showed the lowest concentration (0.040 ± 0.031 $mg.gdw^{-1}$), while slightly higher concentrations were observed at San Luis (0.080 ± 0.060 $mg.gdw^{-1}$) and Guayacán (0.15 ± 0.080 $mg.gdw^{-1}$). The concentration of SOM at the three eastern sites ranged from 0.56 ± 0.062 $mg.gdw^{-1}$ at Grande, to 0.62 ± 0.04 $mg.gdw^{-1}$ at Saucedo, and 0.62 ± 0.09 $mg.gdw^{-1}$ at Manzanillo.

The concentration of total pigments was highly variable not only among the six sites, but also on different dates within each of the six sites (Figure 6B). Among the three western sites, the average concentrations of total pigments ranged from 0.48 ± 0.19 $mg.gww^{-1}$ at Garrapata, to 0.55 ± 0.26 $mg.gww^{-1}$ at San Luis, and to 0.62 ± 0.33 $mg.gww^{-1}$ at Guayacán. Among the three eastern sites, total pigment concentrations ranged from 0.51 ± 0.36 $mg.gww^{-1}$ at Manzanillo to 0.77 ± 0.61 $mg.gww^{-1}$ at Grande, and 1.03 ± 1.00 $mg.gww^{-1}$ at Saucedo. Again, as for SOM, two of three eastern sites tended to have the highest concentrations of total pigments.

Size-distribution of sediment particles varied among the six sites (Figure 7). Among the three eastern ones, particles sizes ranged from 0.125 to 4 mm, with peaks in the 0.250 mm size-class for Saucedo, and the 0.500 mm size-class for Manzanillo and Grande. The three western sites had similar ranges of particle sizes. The 0.250 mm size-class was dominant at San Luis, compared to that of 0.500 mm for Garrapata and Guayacán.

Figure 6. Seasonal variation (columns) and standard error (vertical bars) of temperature (°C) and salinity (‰) at the six regional sites. B) Seasonal variation (columns) and standard error (vertical bars) of organic matter (mg/gdw), total pigments (mg/gww) and pH (units) at the six regional sites.

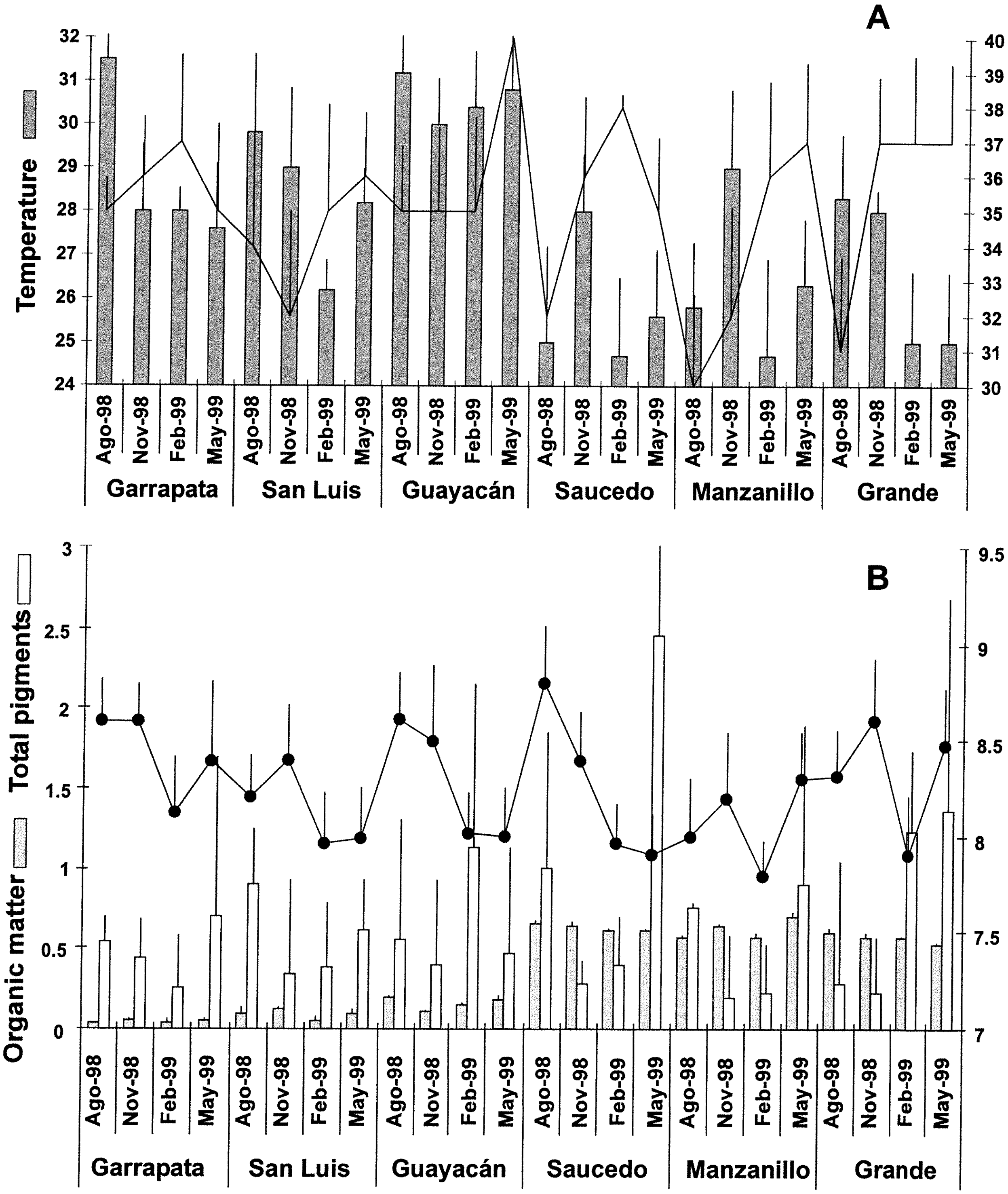
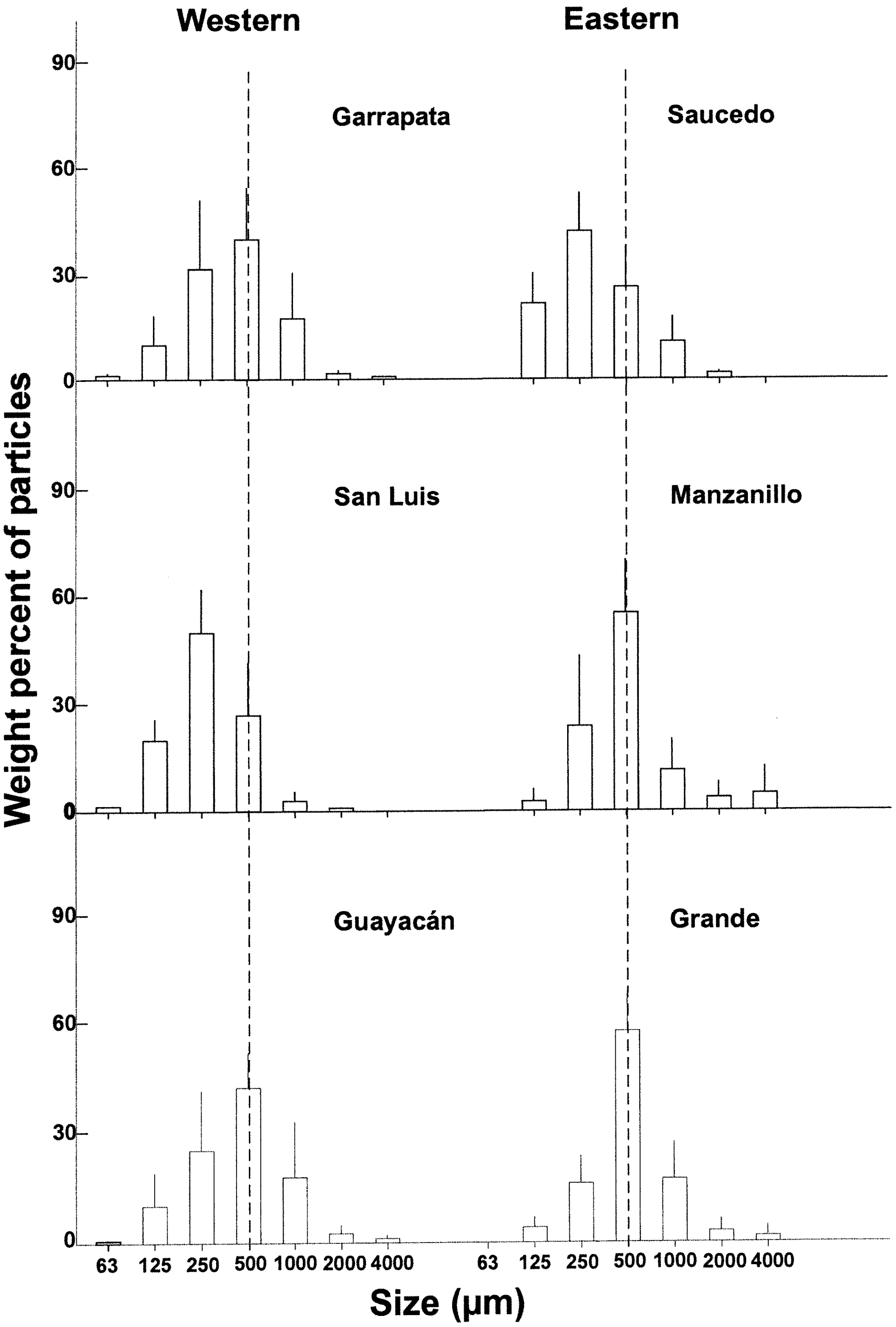

A
Temperature
Salinity
32
31
30
29
28
27
26
25
24
40
39
38
37
36
35
34
33
32
31
30
Ago-98
Nov-98
Feb-99
May-99
Garrapata
San Luis
Guayacán
Saucedo
Manzanillo
Grande
B
Organic matter
Total pigments
pH
3
2.5
2
1.5
1
0.5
0
9.5
9
8.5
8
7.5
7

Figure 7. Average size distribution of sediment particles (mean±SE) on the coast of north-eastern Venezuela.


Western
Eastern
Garrapata
Saucedo
San Luis
Manzanillo
Guayacán
Grande
Weight percent of particles
0
30
60
90
63
125
250
500
1000
2000
4000
Size (µm)

Relationships of *Tylos wegeneri* with environmental variables

Linear multiple regressions indicated that six environmental variables, temperature, sediment particle size, concentration of organic matter, salinity, pH and total pigments, explained 60% of the variability in the *T. wegeneri* population density (Table 2). However, in this multiple regression model, the first four were the significant variables predicting the abundance of *T. wegeneri*. The two other variables, pH and total pigments, had no significant impact (Figures 8 a to e; Table 2). Note that in the multiple regression model the impact of salinity on *T. wegeneri* was significantly negative. The addition of sediment particle size to the density-temperature relationship explained 50% of the variability, while the addition of organic matter to the temperature-density relationship explained 42% of *T. wegeneri* density. The density of *T. wegeneri* increased positively as a function of both temperature and sediment particle size (Figure 8a), and as a function temperature and concentration of organic matter (Figure 8b). However, the scatter plot showed a tendency of *T. wegeneri* to decline in density in the presence of organic matter concentrations above 0.5 mg.gdw^{-1}.

DISCUSSION

Based on 13-month data, there is a significant temporal pattern in the size distribution of *Tylos wegeneri*. This seasonal pattern could be related to the timing of growth and reproductive cycles. During our study, gravid females were present throughout the year and individuals larger than 3 mm were found to carry eggs. We suggest that female

Figure 8. Linear multiple regressions between the population density (ind.m^{-2}) of *Tylos wegeneri* and temperature and salinity (A), temperature and sediment size distribution (B), temperature and total pigments (C), temperature and pH (D), and temperature and organic matter (E).

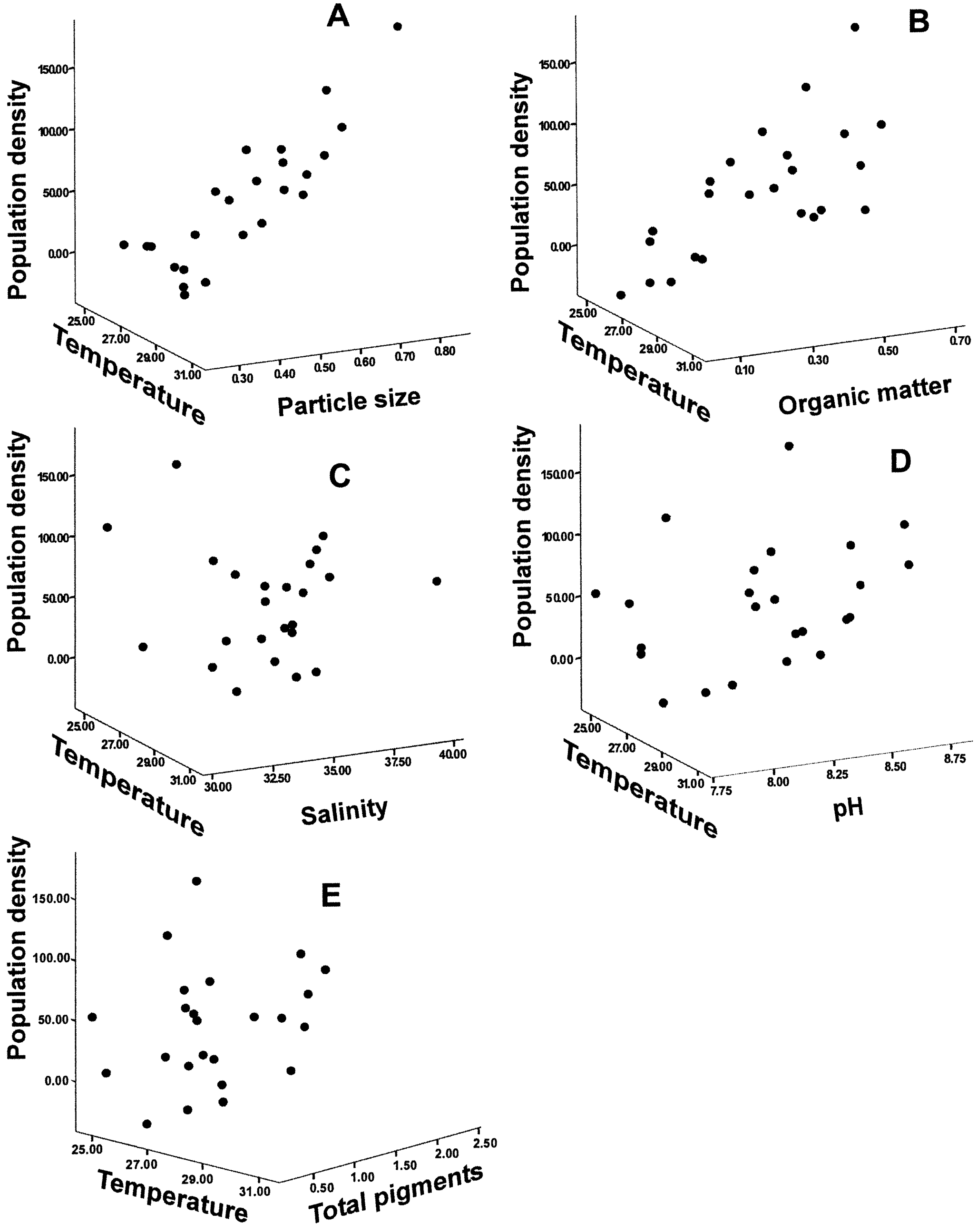

A
Population density
150.00
100.00
50.00
0.00
Temperature
25.00
27.00
29.00
31.00
Particle size
0.30
0.40
0.50
0.60
0.70
0.80
B
Population density
150.00
100.00
50.00
0.00
Temperature
25.00
27.00
29.00
31.00
Organic matter
0.10
0.30
0.50
0.70
C
Population density
150.00
100.00
50.00
0.00
Temperature
25.00
27.00
29.00
31.00
Salinity
30.00
32.50
35.00
37.50
40.00
D
Population density
150.00
100.00
50.00
0.00
Temperature
25.00
27.00
29.00
31.00
pH
7.75
8.00
8.25
8.50
8.75
E
Population density
150.00
100.00
50.00
0.00
Temperature
25.00
27.00
29.00
31.00
Total pigments
0.50
1.00
1.50
2.00
2.50

Table 2.- Parameters describing the linear relationships between the population density of *Tylos wegeneri* (ind.m^{-2}) and temperature (Temp.;°C), particle size (Par. Size; µm), organic matter (Org. Mat.; mg/gdw), salinity (Sal.; ‰), pH (units), and total pigments (T. P.; mg/dww). K= constant, R^2 deter.= percentage determination coefficient, t1, t2, t3, t4, t5 and t6= student t for temperature, granulometry, organic matter, salinity, pH and total pigments respectively and their respective P level .

Relationships*	K	Temp.	Par. Size	Org. Mat.	Sal.	pH	T. P.	R^2 deter.	ts. 1 (P)	ts. 2 (P)	ts. 3 (P)	ts. 4 (P.)	ts. 5 (P)	ts. 6 (P)
I	-632.5	21.0***	219.2***	98.2***	-3.8*	11.0	4.0	0.60	1.8 (0.095)	1.7 (0.10)	1.0 (0.32)	-0.5 (0.66)	0.2 (0.90)	0.1 (0.92)
II	-466.1	14.3**	252.2*	-	-	-	-	0.50	1.8 (0.069)	2.3(0.035)	-	-	-	-
III	-618.9	22.4**	-	146.7*	-	-	-	0.42	2.1 (0.051)	-	1.7 (0.113)	-	-	-
IV	-4.8	11.2**	-	-	-7.2*	-	-	0.32	1.3 (0.22)	-	-	-0.9(0.40)	-	-
V	-350.6	10.9	-	-	-	12.4	-	0.27	1.2 (0.27)	-	-	-	0.2 (0.90)	-
VI	-242.7	10.8	-	-	-	-	-7.3	0.27	1.1 (0.30)	-	-	-	-	-0.2 (0.84)

*I: Population density vs. temperature, particle size, organic matter and salinity; II: Population density vs. temperature and size distribution of sediment particles, III: Population density vs. temperature and organic matter; IV: Population density vs. temperature and salinity; V: Population density vs. temperature, pH; VI: Population density vs. temperature and total pigments.

*P < 0.05

**P < 0.01

***P < 0.001

Tylos lay eggs as soon as they reach 3 mm in width, a size-class which is present throughout the year. However, based on the high density of small-size individuals in September, December and June, there may be three periods of extensive reproduction. Based on size frequency analyses of *T. punctatus* from five sites located in California and Mexico, Hayes (1977) suggested that this species breeds only once a year, and that reproduction that exceeds once a year was mainly observed in older females. These results agree with those of Hamner *et al.* (1969) who suggested that the contribution of 2-year old females to recruitment was significantly less than that of 3-year old ones. It has been reported by Mead (1965) as well as Brown and Odendaal (1994) reported that gravid females of *T. sardous* results seem to suggest that *T. wegeneri* can start breeding at less than 2-years old.

Another interesting pattern of the seasonal size-distribution of *T. wegeneri* is the consistent small size of individuals found at Guayacán during June and December. Energy expenditure by *T. wegeneri* might be substantially higher during the rain season than during the dry season. In addition, harsh environmental conditions such as high temperature, high salinity and low food concentrations may limit them to smaller sizes. During the dry season, high productivity in the coastal waters, which provides greater food resources, and lower temperatures may allow them to grow larger and reproduce at large sizes.

Alternatively, between July and March, numerous Nearctic shorebirds (Charadridae and Scolopacidae) overwintering in the Guayacán region increase the predation pressure on *T. wegeneri*. The migratory patterns of Nearctic shorebirds in north-eastern Venezuela have been documented by McNeil (1970), McNeil and Burton (1973) and McNeil *et al.* (1985). The decline in density of larger size-classes from January to April may be due to selective predation pressure by these migratory shorebirds on larger sizes of *T. wegeneri*. It is important to note that this assumption agrees with our field observations. At dawn and dusk we observed individuals of *Calidris pusilla*, *Tringa melanoleuca*, *Charadrius semipalmatus*, *Cataptrophorus semipalmatus*, and *Numenius phaeopus* feeding on *T. wegeneri*. A similar explanation has been provided by Brown and McLachlan (1990) for coastal sites of California and Mexico.

Temporal and regional variation in population density and biomass

On an annual basis, while the average size of *T. wegeneri* was smaller during the rain season (during June-October) (Figure 3) than during the dry season (during November-March), its density was higher during the rain season. This is consistent with the existing theory of body-size and abundance of organisms (Peters 1986). According to this theory, the abundance of organisms decreases logarithmically with increasing body size. On the other hand, the density and biomass of organisms should have been higher during the rain season when the environmental conditions such as temperature, food and salinity were more favourable. However, a significantly lower density and biomass during the dry season could also be due to intense predation pressure from shorebirds.

Regionally, three sites (two western and one eastern sites) showed total absence of this isopods. We think that environmental and anthropogenic factors were responsible for such observed regional differences. For example, the three sites that showed high densities and biomasses of *Tylos wegeneri* are low energy tidal areas with large input of organic matter from fishing activities (Brown and McLachlan (1990). Fishermen leave unwanted fish and invertebrates on the tidal zone to decompose, which may provide a substantial food source for inter-tidal organisms like isopods. On several occasions during our nighttime sampling, we observed a large number of isopods feeding on dead fish left by the fishermen. There are two potential reasons for the absence or very low numbers of this isopod at Garrapata, San Luis and Saucedo. Wastewaters may be responsible for the absence of *Tylos wegeneri* at San Luis and Saucedo. However, these sites are also high-energy inter-tidal zones, where the large sizes of sediment particles may also be unsuitable for the species. Garrapata is the most pristine site of all of our study sites, with the lowest concentration of pigments and organic matters. Thus, it thus appears that anthropogenic impacts may have negative impacts on *T. wegeneri*. When natural organic matter is enriched through the fishermen activities, there are high numbers of *T. wegeneri*. On the other hand, when the input of industrial pollutants such as wastewaters is high, the species is not abundant.

The vertical and horizontal distribution patterns of *T. wegeneri* varied among Guayacán, Manzanillo and Grande sites. The aggregation of *T. wegeneri* within the high-tide zones, and the deepest sediment strata observed at Guayacán contrasts with the aggregation within both low-tide and high-tide zones and the surface strata of Manzanillo and Grande. This suggests that *T. wegeneri* prefers temperatures of 27-31 °C, sediment particle sizes of 500 µm, and between 0.1 and 0.50 mg/gdw of organic matter.

The temperature of 30°C has been found appropriate for the survival of *T. granulatus* (Imafuku, 1976), which agrees with our results (Table 2). Hayes (1977) demonstrated that *T. punctatus* burrows at greater depth with sand particles of 0.7-1.4 mm in size and found that grain size influences the depth of burrowing. However, Hayes (1977) and Kensley (1974) concluded that sediment particle size is not important in determining the horizontal distribution of isopods within the tidal zone. Nevertheless, our results suggest that *T. wegeneri* prefers a specific particle size (500 μm). This particle size was found at the Garrapata, Guayacán, Manzanillo and Grande. However, *T. wegeneri* was not found at Garrapata, apparently due to other environmental factors. In particular, the Garrapata site had very low concentration of organic matter compared to the three other sites, which may limit the growth and reproduction of *T. wegeneri*.

We also suggest that behavioural strategies can also influence the vertical distribution of this isopod. *T. wegeneri* might avoid predators by burrowing deeper into the sediments. Brown and Odendaal (1994) have signalled that *Tylos* is entirely dependent upon its behavioural plasticity to survive and that, because of behavioural differences between species and populations, generalizations about the biology of the genus is difficult.

The environmental conditions of the Venezuelan coast depend largely on the Inter–Tropical Convergence Zone, which produces a bi-seasonal environmental regime in the equatorial South American coast. The coast of north-eastern Venezuela is also influenced by up-welling phenomena which result in changes in temperature, salinity, density, concentration of oxygen, pH, and current (Fukuoka, 1965, 1966; Margalef, 1980; Mandeli and Ferráz-Reyes, 1982; Del Cano and Velásquez, 1992). Such changes directly influence inter-tidal flora and fauna. Other studies suggested that salinity and temperature act as primary factors in determining the distribution and abundance of isopods from other parts of the world (Neale, 1964; Puri *et al.*, 1964; Maddocks, 1966; Puri, 1966 *fide* McIntyre, 1969). They also suggested that the nature of the bottom, depth, shelter, food supply and predators act as secondary factors in determining the abundance of isopods. At our study sites, significant changes in temperature, salinity, sizes of sediment particles, concentration of organic matter and pigments seem to be strongly correlated with the density of *T. wegeneri*.

Based on multiple regression models, temperature seems to play the most significant role in determining the presence and distribution of *T. wegeneri.* Other important environmental factors related with the density of this isopod are particle size, organic matter, salinity and total pigments. The relationships we observed for the inter-tidal beaches of north-eastern Venezuela seem to agree with previous studies on isopods and other invertebrates from other tropical and temperate zones (McIntyre, 1969; Vernberg and Vernberg, 1972; Edwards and Lofty, 1977; Margalef, 1980; Giere and Pfannkuche, 1982; Healy and O'Neill, 1984; Lee, 1985). Inter-tidal zone animals tend to tolerate wider ranges in salinity than do sub-tidal and open ocean organisms (Vernberg and Vernberg, 1972).

It is interesting to note that population density of *T. wegeneri* is apparently not affected by total pigments. This could indicate that *T. wegeneri* feeds predominantly on animals. This question is being addressed in a separate paper based stable isotopes signatures to describe the ontogenetic niche shift in *Tylos*. This isopod may be herbivorous only during the early stages of its life. In Mediterranean inter-tidal ecosystems, *T. latreillei* feeds on decomposed matter (Arcangeli, 1953), on living amphipods (Matsakis, 1956) and dead fish (Vandel, 1960).

CONCLUSION

Our results show the potential effects of environmental factors (temperature, size of sediment particles, organic matter and salinity) and anthropogenic factors on the presence and distribution of *T. wegeneri*. This is the first detailed study of this isopod for the inter-tidal ecosystems of north-eastern Venezuela. *T. wegeneri* is abundant in inter-tidal ecosystems with low wave actions, small-size sediment particles, moderate temperature and moderate concentration of organic matter. Larger individuals are observed during colder months (January and February), when energetic costs for growth are lower and the availability of food resources is higher. However, an extensively high concentration of either organic matter or plant pigment seems to produce a negative impact on this isopod. Our results allow to conclude that the preferences of *T. wegeneri* are characterized by temperatures ranging from 27 to 31°C, sediment particle sizes in the order of 250-500 µm, between 0.1-0.50 mg/gdw of organic matter, and salinity of 30-35‰. Such conditions were found in sheltered sites like Guayacán. We also consider that this isopod could be used as an excellent species for fundamental research on coastal foodwebs, energy transfers and ontogenetic niche shifts. We believe that this species could be used as an indicator of anthropogenic impacts on coastal environments.

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Chapter 4

Variations of energy content, lipid classes and fatty acids in the intertidal oligochaete *Pontodrilus litoralis*

Andrade, José & Asit Mazumder

ABSTRACT

There is a great deal of interest in the study of energetic compounds of annelid worms (polychaetes and oligochaetes), especially since some of these annelids are used as natural and processed food for invertebrate and vertebrate aquaculture. For this reason, we wanted to determine the energy substrata, lipid classes and their monthly variations in the intertidal oligochaete *Pontodrilus litoralis*. This allowed us to establish the basic knowledge requered to use this oligochaete as a non conventional and alternative food by shrimp farms.

Important energy components were lipids ($\overline{X}$ = 95.32 ± 40.04 S.D. mg/gdw), followed by proteins ($\overline{X}$ = 33.08 ± 19.07 S.D. mg/gdw) and carbohydrates ($\overline{X}$ = 1.69 ± 1.33 S.D. mg/gdw). Strong monthly variations were found in cholesterol esters ($\overline{X}$ = 40.89 ± 19.50 S.D. mg/gdw), phospholipids ($\overline{X}$ = 34.80 ± 12.15 S.D. mg/gdw), triglycerids ($\overline{X}$ = 18.41 ± 10.56 S.D. mg/gdw), scarce portions of cholesterol and other lipids, saturated fatty acids (32.34%) and unsaturated fatty acids (27.23 %) in phospholipids, as well as saturated fatty acids (52.32%) and unsaturated fatty acids (23.90%) in triglycerids.

Keywords: Oligochaeta, Acanthodrilidae, ***Pontodrilus litoralis***, lipids, fatty acids.

INTRODUCTION

Oligochaetes are relatively small, thin and cylindrical annelids that can live on land or in water. Those that live in water can live either in fresh or seawater, and are distributed mainly in coastal and intertidal areas (Storer *et al.*, 1975; Barnes, 1989). They constitute an important link in the bentonic trophic chain since they serve as food for insect larvae, nematodes, leeches, turbelarids and benthic fishes (Gaviria, 1993). In addition, some species of land oligochaetes are used as proteinic supplement for domestic animals (Bouguenec, 1992). These annelids also have a great ecological importance since they allow soil ventilation, penetration of water in the substrata, and constant mixing of organic residues, thus contributing to the fertility of the ecosystem and its energy budget (Drachenberg, 1990; Drachenberg, 1992; Mason, 1994)

Pontodrilus litoralis is a circumtropical (Cantú-Martínez, 1987) oligochaete belonging to the Acantodrilidae family. It usually inhabits sediments with high salinity, abundance of organic matter, and pieces of floating trees that arrive to the beach due to wave action (Gates, 1972; Drachenberg, 1989). This oligochaete support wide oxygen variations (0 to 45 mL/L), and thus is considered as an hyperionic organism (Subba & Venkateswara, 1980; Olav & Olaf, 1982). Deposition of cocoons takes place in November and continues until May (Olav & Olaf, 1982).

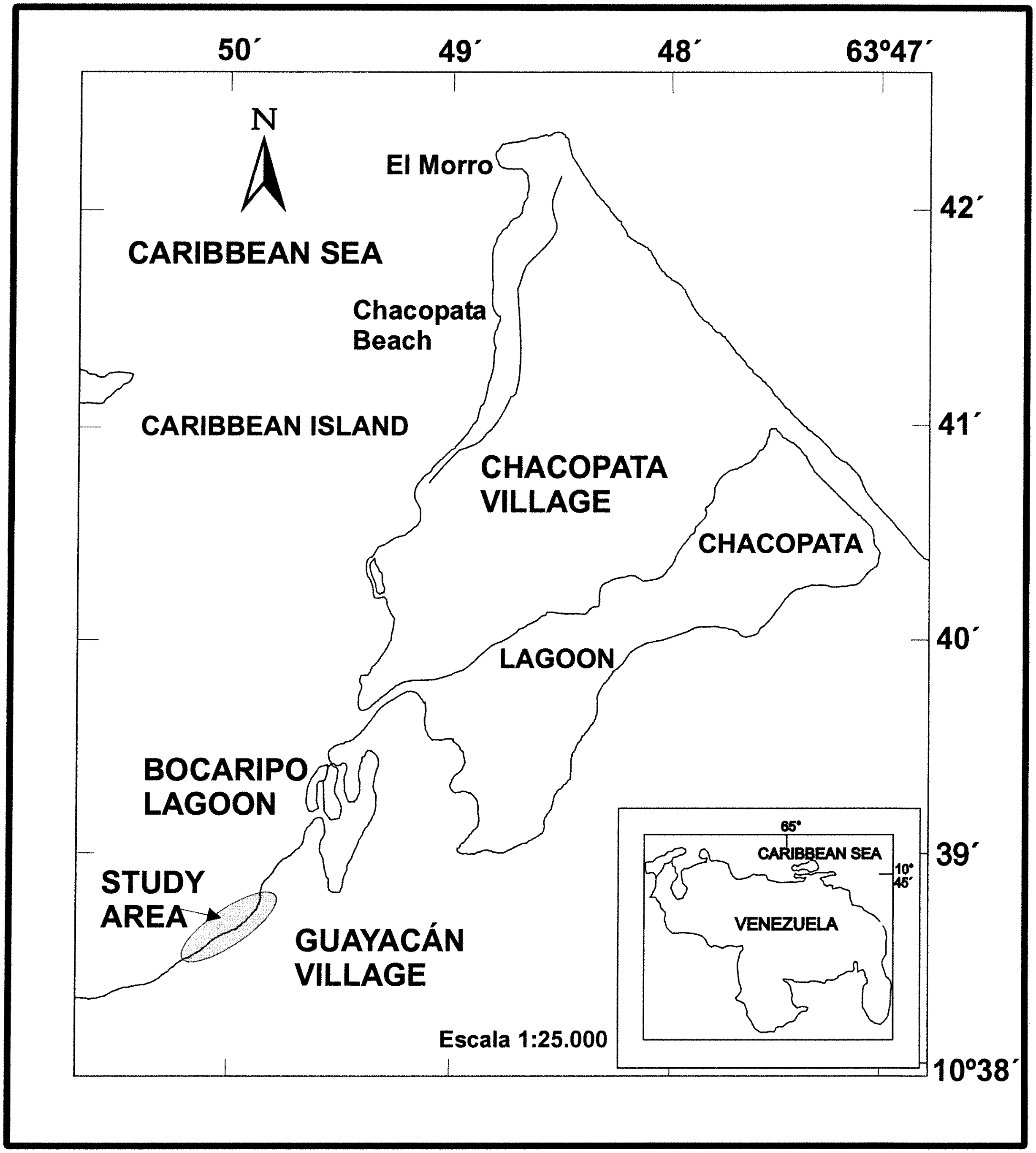
Like other oligochaetes, *P. litoralis* may have great ecological importance (Cantú-Martínez, 1987; Drachenberg, 1989), and could be used as a non-conventional and alternative food for shrimp aquaculture. However, at this time, there is a lack of information on the nutritional value of this species and the natural variability of its essential energy contents. Thus our objectives were first to characterize the nutritional value of *P. litoralis* in inter-tidal ecosystems of north-eastern Venezuela, and secondly to characterize the seasonal variability in lipid and energy contents of the species. Results of this study will allow us to develop or formulate the environmental conditions for culturing *P. litoralis* with the most desirable nutritional values.

MATERIALS AND METHODS

Once a month between November 1996 and November of 1997, individuals of *P. litoralis* were captured monthly from the inter-tidal zones of Guayacán (10º 39′N – 63º 48”W), located on the northern side of the Araya Peninsula (Figure 1). These annelids were wrapped in aluminium foil, put into a liquid nitrogen container and transferred to the Laboratorio de Nutrición y Productos Naturales en Acuicultura of the Universidad de Oriente.

Dry weight of the samples was estimated after drying at 50 °C and ash weight was determined after burning the samples at 450 °C. Total lipid (Overturf & Dryer, 1969), protein (Lowry *et. al.*, 1951) and carbohydrate (Dubois *et al.*, 1956) contents were determined and the results were expressed in milligrams per gram of dry weight (mg/gdw). In order to quantify and characterize lipids in *P. litoralis*, we used an Iatroscan MK-5 TLC-FID Analyser with a mixture of hexane and diethyl-ether (67:3 v/v) as system solvents. The identification of extracted lipids was made by means of comparison of a

Figure 1. Geographic location of the Bocaripo–Chacopata lagoon complex and study area on the northern side of the Araya Peninsula in north-eastern Venezuela.


50´
49´
48´
63°47´
42´
41´
40´
39´
10°38´
N
El Morro
CARIBBEAN SEA
Chacopata Beach
CARIBBEAN ISLAND
CHACOPATA VILLAGE
CHACOPATA
LAGOON
BOCARIPO LAGOON
STUDY AREA
GUAYACÁN VILLAGE
Escala 1:25.000
65°
CARIBBEAN SEA
10° 45´
VENEZUELA

mixture of commercial standards which contained phospholipids, triglycerids, cholesterol esters and free fatty acids, and their values were expressed as percentages of total lipids.

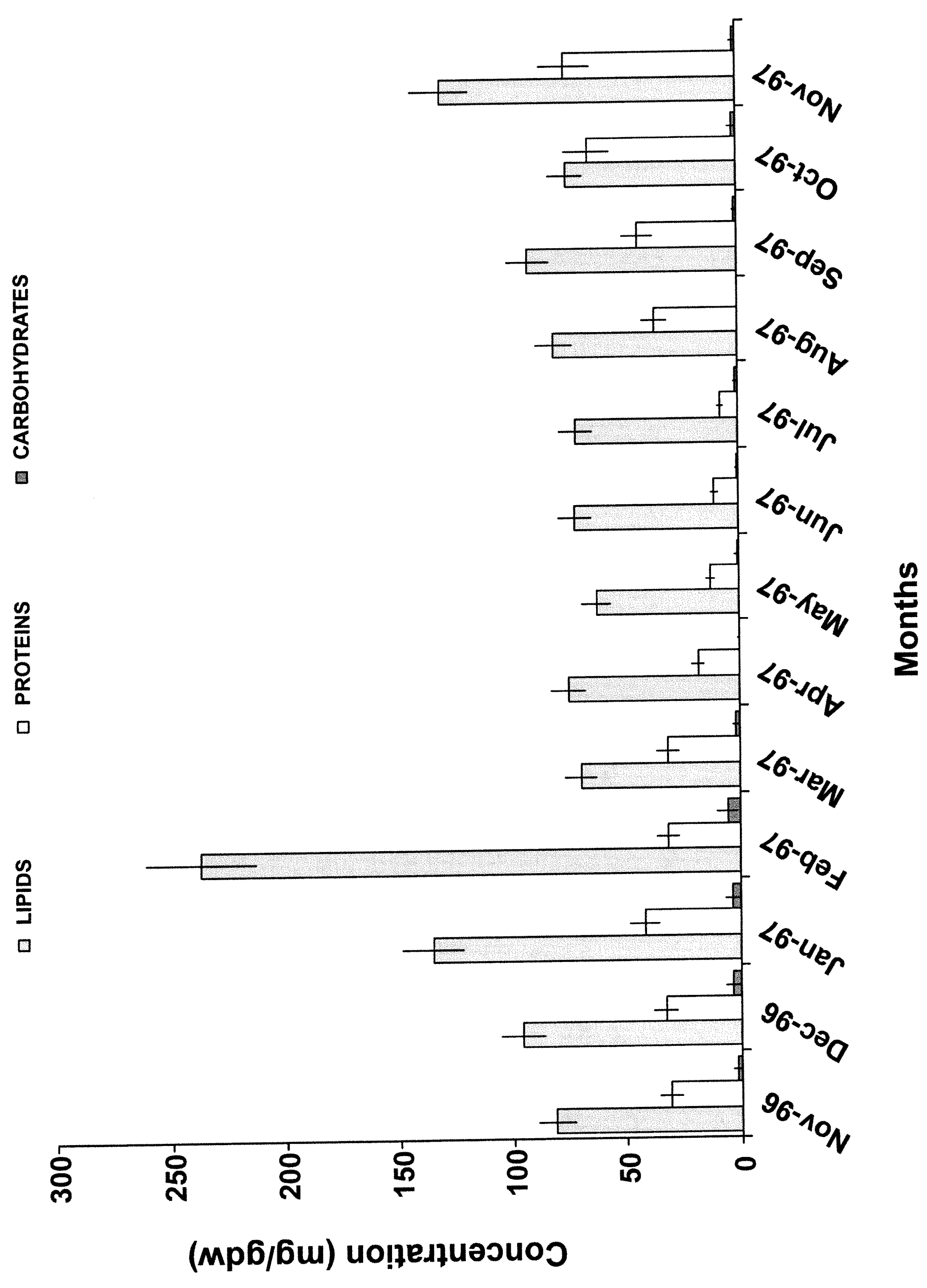
Following the separation of triglycerids and phospholipids by means of thin layer chromatography with a mixture of petroleum ether, ethylic ether and acetic acid (87:12:1) as system solvents, and trasmethylation (Litchfield, 1972), we characterized fatty acids by using a gas-chromatograph (Variam 3300 series coupled to Hewlett Packard integrator model 3390 A). Fatty acids and methylic esters were identified using the retention times of samples against commercial standards, and the results were shown as relative percentages of total fatty acids. Descriptive statistics (means and standard error of mean) were used to analyze the seasonal variations in energy contents (lipids, proteins and carbohydrates) and the results were ploted for concentrations expressed in mg/gdw. (Sokal and Rohlf, 1980; Zar, 1984; SPSS, 1999).

RESULTS

1. Energy substrata and lipid class variation

The lipid content of *P. litoralis* ranged from 62.4 to 237.3 mg/gdw ($\overline{X}$ = 98.2, S.D.= 47.5 mg/gdw) (Figure 2). The temporal variation of total lipids was characterized by peak concentrations in February (237.3 mg/gdw) and November (130.5 mg/gdw), and with lowest concentration in May (62.40 mg/g.d.w). A similar pattern was observed for total protein content (Figure 2), with peak concentrations in January (42.0 mg/gdw) and

Figure 2. Seasonal variation of the energy content (lipids, proteins and carbohydrates) of *Pontodrilus litoralis* (bars = means, and lines = standard error of mean).


LIPIDS
PROTEINS
CARBOHYDRATES
Concentration (mg/gdw)
300
250
200
150
100
50
0
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
Jul-97
Aug-97
Sep-97
Oct-97
Nov-97
Months

November (75.1 mg/gdw), and a strong decline in July (7.90 mg/gdw). Seasonally, the protein content showed strong variation (7.9 to 75.08 mg/gdw; $\overline{X}$ = 33.80, S.D.= 20.00 mg/gdw).

In contrast, total carbohydrates had the lowest values during the same period (Figure 2) with a mean value of 1.80 ± 1.59 S.D. mg/gdw. These reached the highest concentration (5.55 mg/gdw) in February, but fell to the lowest concentrations of 0.28 mg/gdw and 0.09 mg/gdw in April and August, respectively.

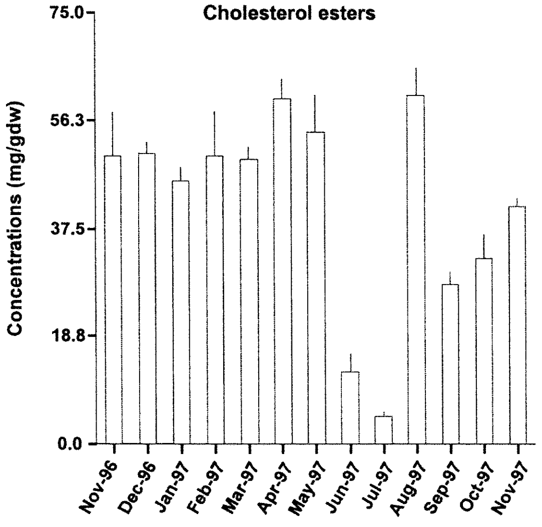
Compared to phospholipids ($\overline{X}$ = 34.7, S.D.= 9.7 %) and triglycerids ($\overline{X}$ = 17.5, S.D.= 9.7 %), cholesterol esters were the most abundant energy substrata ($\overline{X}$ = 41.6%, S.D.= 17.4%; Figure 3).

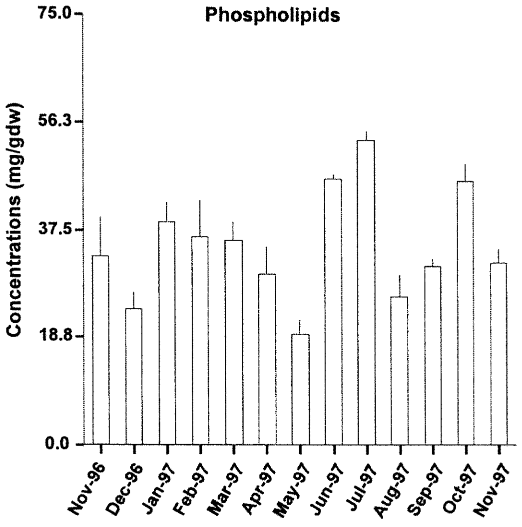
As shown in figure 3, cholesterol esters were highest (60.7%) in August and lowest (4.87%) in July, and phospholipids had their maximum value (53.2%) in July and minimum value (19.2%) in May. Triglycerid concentrations were at a maximum in July (36.9%) and minimum (7.6%) in August.

The phospholipids of *P. litoralis* were mainly constituted by saturated (32.4 %) and unsaturated (28.7 %) fatty acids. It is necessary to point out that 16-series (C16:0 and C16:1) and 18-series (C18:1, C18:2 and C16:3) fatty acids were not separated, and therefore only the total values are presented for these series (Figure 4).

Our results show that saturated fatty acids [C13:0 (1.6-16.2 %), C14:0 (1.6-9.4 %), C15:0 (1.5-14.8 %) and those with 16 and 18 carbon chain length] were the

Figure 3. Seasonal variation in the concentration of cholesterol esters, triglycerids and phospholipids in *Pontodrilus litoralis* (bars = means, and lines = standard error of mean).


Cholesterol esters
Concentrations (mg/gdw)
75.0
56.3
37.5
18.8
0.0
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
Jul-97
Aug-97
Sep-97
Oct-97
Nov-97


Phospholipids
Concentrations (mg/gdw)
75.0
56.3
37.5
18.8
0.0
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
Jul-97
Aug-97
Sep-97
Oct-97
Nov-97

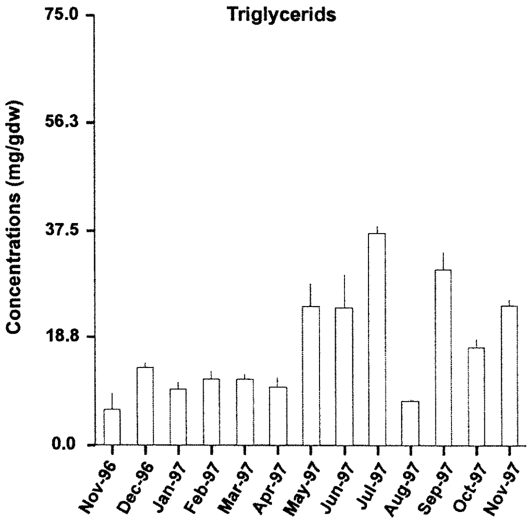
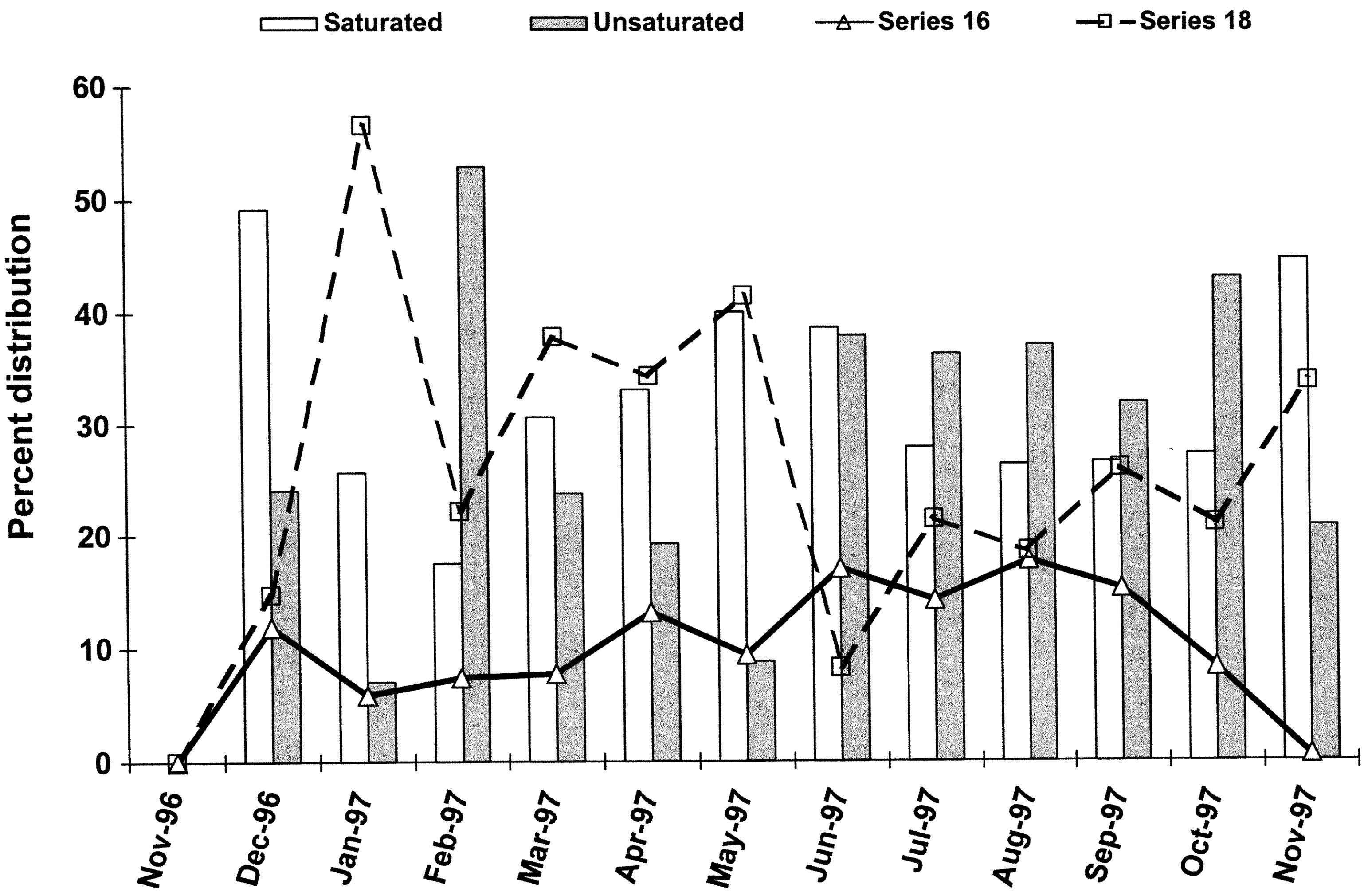

Triglycerids
Concentrations (mg/gdw)
75.0
56.3
37.5
18.8
0.0
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
Jul-97
Aug-97
Sep-97
Oct-97
Nov-97

Figure 4. Seasonal variation in the concentration of fatty acids of phospholipids in *Pontodrilus litoralis*.


Saturated
Unsaturated
Series 16
Series 18
Percent distribution
60
50
40
30
20
10
0
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
Jul-97
Aug-97
Sep-97
Oct-97
Nov-97

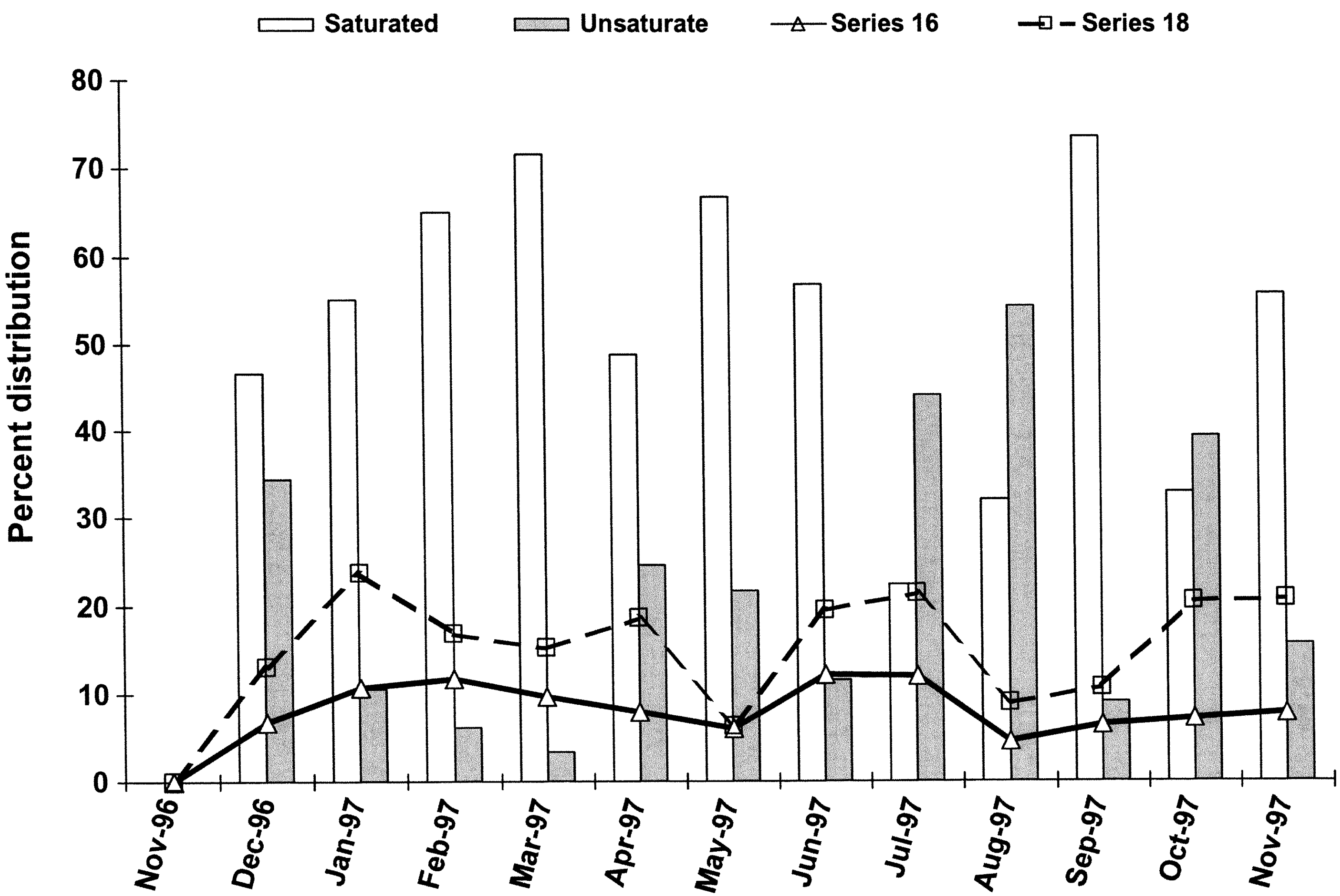
dominant fraction of fatty acids during the warmest months of the year (Figure 4). On the other hand, phospholipids of the series 18 (Figure 4) were lowest in June (8.2 %) and highest in January (56.6 %).

Monthly variations in fatty acids of triglycerid group are shown in the figure 5. These saturated fatty acids reached an average of 52.3 %, with the most dominant form of C12:0. Among the triglycerid fatty acids, the most common unsaturated fatty acid was C20:5, while the most abundant was C22:1. The series 16 (C16:0 + C16:1) showed the lowest value in August (4.5 %) and a highest value in June (12.1 %) while series 18 showed their lowest value in May (6.2 %) and their highest value in January (23.7 %). It is important to point out that, during the rainy season of July to October, the unsaturated fatty acids CI8:4, C20:4, C20:5 and C22:6 of phospholipids were the most abundant fatty acids.

The fatty acids in the triglycerids of *P. litoralis* (Figures 5), behave differently from the phospholipids. Saturated fatty acids C12:0, C13:0 and C15:0 were more abundant from January to June, but decreased in July and August, to increase again in September, November and December.

The fatty acids of the series 16 (C16:0 + C16:1) in the triglycerids of *P. litoralis* (Figure 5) counted for 8.6 % and maintained their values between 4.5 and 12.1 % during the period of study. Those of the series 18 (C18:0 + C18:1 + C18:2 were frequent and abundant during the whole sampling period.

Figure 5. Seasonal variation in the concentration of fatty acids of triglycerids in *Pontodrilus litoralis*.


Saturated
Unsaturate
Series 16
Series 18
Percent distribution
80
70
60
50
40
30
20
10
0
Nov-96
Dec-96
Jan-97
Feb-97
Mar-97
Apr-97
May-97
Jun-97
Jul-97
Aug-97
Sep-97
Oct-97
Nov-97

DISCUSSION

1. Energy substrata and lipid class variation

Our results show that *P. litoralis* has all the essential nutritional elements required to make an excellent non-conventional diet for shrimp aquaculture. The high lipid values in February and high protein ones in January were probably due to greater availability of microflora and microfauna living in sediment interstices (Andrade and Mazumder, unpublished data). Similarly, the low lipid and protein values in May and July may be a result of their use of energy reserves in reproduction.

Gardiner & Riley (1972) mentioned that monthly variations of energy content may be the result of seasonal fluctuations in endogenous and exogenous factors, including the availability of food with high nutritive value (Andrade, 1994; Andrade & Liñero, 1993). The catabolism of carbohydrates, proteins and lipids produces the energy required for the maintenance of essential physiological processes (Blackstock, 1984). Most marine invertebrates present recurrent changes in their energy demands due to variation in natural physiological processes (Giese, 1959), regulated by endogenous biochemical factors, which in turn are influenced by external environmental conditions. Reproduction of marine invertebrates is controlled by the regular cycles of energy reserves and the use of metabolic content that provides energy and intermediary metabolic components required for gamete production (Giese, 1966). The cycle of the energy reserves and the use of metabolic contents in association with the reproductive cycles of polychaetes (Blackstock, 1984) and other annelids have received little attention. Dales (1957) found monthly differences in the lipid concentration of polychaetes *Amphitrite johnstoni* and *Nereis diversicolor*. Andrade

(1994) found similar results for the lipids, proteins and total carbohydrates, as well as fatty acids, monoenics and polienics of *Americonuphis magna*.

The study site of Guayacán is subject to strong seasonal environmental changes, which probably influence food availability (microflora, microfauna and available organic matter) for *P. litoralis*. The biochemical composition and the reproductive periods of *P. litoralis* depend on the monthly changes in food availability. However, metabolic responses are potentially indicative of the effects of environmental changes on the catabolism of carbohydrates, which reflect the role of recurrent changes in the energy demand (Livingstone and Bayne, 1974; Chambers *et al.*, 1975; Gabbott, 1975, 1985; Livingstone, 1975; Gabbott *et al.*, 1979; Kluytmans *et al.*, 1979, 1980; Zandee *et al.*, 1980; Zaba *et al.*, 1981).

Monthly variations in total carbohydrates provide evidence for a continuous use of this energy sources joined with the possible use of lipids. In February, we observed an increase in both of these energy sources. During the early months of the year, up-wellings potentially contribute to an increase in available resources along the coast of north-eastern Venezuela (Margalef, 1980), which results is an increase in the variety of plant and animal species. This phenomenon appears to be intimately related with the maximum concentrations of lipids and carbohydrates observed in the present study. On the other hand, *P. litoralis* may use total carbohydrates as an alternative energy resource during the long and stressful dry periods of food scarcity and high temperatures.

The glycogen concentration of the other annelids such as polychaetes can vary considerably following changes in energy requirements which are imposed by periodic reproductive events (Vooys, 1975). It has been observed that *Owenia fusiformis* possesses high resistance to anoxia, showing in turn high glycogen values (21.0 mg per unit of fresh weight) (Dales, 1958, *fide* Blackstock, 1984). The quick decrease in the glycogen concentration of muscles and digestive glands of *Arenicola marina* has been related to gamete production, and the juveniles of this species have been noted to possess high glycogen concentrations (Vooys, 1975). De Jorge and Petersen (1969, *fide* Blackstock, 1984) pointed out that mature individuals of the polychaete *Telepsavus costarum* contain high glycogen concentrations but show the opposite for juveniles.

Lipids play an important role in the physiology of most animals in terms of energy and nutritive reserves during periods of food scarcity and negative energy balance (Giese, 1966; Pocock *et al.,* 1971; Bradshaw *et al.,* 1990). The presence, accumulation and variation of lipid contents in marine invertebrates are poorly known (Giese, 1966; Pocock *et al.,* 1971). Our results also show important concentrations of triglycerids, phospholipids, and cholesterol esters, the last being the most abundant energy substrata in *P. litoralis*. Triglycerids, phospholipids and free sterols were the main lipid classes reported for the polychaete *Nereis diversicolor* (Orlando & Passos, 1995). High values observed for *P. litoralis* agree with the results obtained by Benakker *et al.* (1985), who reported cholesterol esters as the main energy component in insects. Our results suggest that cholesterol esters, as well as the triglycerids, fulfil important functions as energy reserves in organisms (Lovern, 1964). Cholesterol esters and phospholipids play important roles in the composition and permeability of membranes, which seem to vary as a function of temperature (Laverack, 1963; Lawrence, 1967; Davidson, 1970; Fenical, 1982).

It is worthwhile to note that peak concentrations of phospholipids correspond with the period of maximum concentration of organic matter in the sediments. This maximunm concentration of organic matter may activate osmorregulatory mechanisms in *P. litoralis* and allow it to survive under adverse environmental conditions. Stefanov *et al.* (1993) pointed out that phospholipids are able to provide a double barrier between external and internal environments, thus allowing the phospholipids to sustain growth and reproduction under adverse conditions produced by pollution. Subba & Venkateswara (1980) suggested that *P. litoralis* could be used an indicator of environmental pollution.

Triglycerids seem to be the smallest component of energy reserves in *P. litoralis*, with relatively high values in July and September, and low values in August. Bell *et al.* (1994) found that triglycerids occur only in small quantities in oligochaetes, while opposite results were found for other invertebrate species. This pattern could be explained by the fact that triglycerids directly come from the diets, and their presence and proportions depend directly upon environmental conditions and nutrient availability (Carroll, 1965; Wolfe, *et al.* 1965; Lee *et al.*, 1971; Parrish *et al.*, 1987). The marked variation of triglycerid concentrations observed in this study is probably a result of variation in food availability, which seems to be regulated by diverse environmental factors such as tidal and surf action, changes in sediment structure, precipitation and eolian action.

2. Fatty acids in phospholipids and triglycerids

The low proportion of saturated fatty acids in the triglycerids in July and August may be associated with the use of these lipids as reserves, indicating environmental food scarcity and low proportion of saturated fatty acids in the phospholipids. We also believe that these results are probably related with lower temperatures recorded in February (Del Cano & Velásquez, 1991).

The degree of cell membrane permeability depends on the composition of fatty acids and associated free movement of molecules between external and internal environments (Stefanov *et al.*, 1993). An increase in the temperatures of inter-tidal zones could potentially accelerate the accumulation of saturated fatty acids, while the proportions of unsaturated fatty acids could increase with decreasing ambient temperatures (Hazel & Williams, 1990). Dominance of saturated fatty acids [C13:0 (1.6-16.2 %), C14:0 (1.6-9.4 %), C15:0 (1.5-14.8 %) and those with 16 and 18 carbon chain length] was demonstrate during the warmest months of the study period. The relative contribution of unsaturated fatty acids to total lipids is greater during the dry season when significantly lower air and water temperatures predominate. However, the higher concentration of unsaturated fatty acids during the dry season could also be due to greater availability of energy rich algae and macrophytes supplied by the upwellings.

Phospholipids of series 18 were lowest in June and highest in January. High quantities of long chain fatty acids allow the cellular membrane to maintain a greater selective permeability. However, low concentrations of fatty acids of series 18 in June can be related to the production or synthesis of lipidic compounds such as sexual hormones which are derived from C18:3 (De Moreno, 1976).

In August, the low proportion of unsaturated fatty acids of phospholipids, together with saturated fatty acids of phospholipids probably resulted from decreased salinity and increase temperatures. Such physico-chemical variations may have changed the permeability of cellular membranes. Hazel & Williams (1990) pointed out that phospholipids are polymorphic, with a variety of organizational phases (laminar, hexagonal and cubic) which depend on factors such as temperature, pressure, hydration and chemical composition. The transition among these phases can be derived from changes in temperature and thermotropic properties. Therefore, a particular phase or combination of phases that coexists in a membrane should be dependent upon temperature. Others authors have mentioned that the variations of fatty acids with high unsaturation usually reflect strong modifications in the fluidity of cellular membranes due to the influence of exogenous factors such as temperature and feeding, and endogenous factors such as gametogenesis (Stubbs, 1983; Orlando & Passos 1995).

Additionally, low temperatures could influence the cellular membrane so as to increase and redistribute the unsaturated fatty acids of series 18 (C18:0, C18:1, C18:2, C18:3), making the membrane more fluid to counteract existent adverse environmental conditions. A lack of oxygen in the environment can also induce elongation of unsaturated fatty acids. This is due to the fact that the lipidic composition of abundant unsaturated fatty acids in the cellular membranes confers them greater fluidity, allowing them to maintain a good exchange between external and internal environments (Marcel *et al.,* 1994).

The increase of fatty acids in January and February could result from the synthesis of other long chain fatty acids. De Moreno (1976) mentioned that many organisms have the capacity to synthesize C20:5 and C22:6, starting with C18:2 and C18:3. The variations of the main saturated fatty acids in the triglycerids could be related to the environmental temperature and nutritional readiness for the reproduction of *P. litoralis*. Carroll *et al.* (1965) emphasized that the fatty acids of triglycerids come mainly from the food.

The decrease in the unsaturated fatty acids observed during January to June may have been caused by variations in the food ingested by *P. litoralis*, but may also be a result of lower temperatures, strong winds and changes in the sediment structure that prevail during this period. The proportion of saturated fatty acids increases in a parallel with temperature, while the proportion of unsaturated fatty acids seems to be opposite. In this way, changes which stabilize the biological structures take place in the balance between the formation and rupture of non covalent interactions. (Hazel & Williams, 1990). Malavé (1995) has pointed out that the environmental variations generate changes in the functional characteristics and structures of-cellular membranes.

Variations of fatty acids in the triglycerids of *P. litoralis* showed a pattern similar to those observed in other marine invertebrates (oligochaetes and crustaceans). It is emphasized that saturated fatty acids in triglycerids and phospholipids are acquired in greater proportions. This is probably due to a high content of organic matter and the liberation of hydrogen sulphide on highly polluted sediments where organisms need more "rigid" cellular membrane.

On the other hand, it is important to point out the presence and abundance of C20:5. Organisms that present this fatty acid have been considered as a possible and efficient food source for shrimps. Brown *et al.* (1979) mentioned that the induction of ovarian maturation in ablated individuals of *Penaeus setiferus* was provoked by a natural food based on the use of polychaetes (which contain high levels of polyunsaturated fatty acids), bivalves and squids. D'Croz *et al.* (1988) mentioned the use of polychaete tubeworms *Americonuphis reesei* as a dietary supplement in order to accelerate gonad maturation in penaeid shrimp *Penaeus vannamei*. Lytle & Lytle (1990) mentioned that the presence of ω-6 fatty acids in shrimp diet migth be the key for the maturation of this species.

CONCLUSION

Pontodrilus litoralis contains relatively high lipid and protein concentrations, which seem to be highly variable due to variable availability of micro and meiofaunal organisms. Our results on the energy contents and nutritional values of *P. litoralis* are indications of the potential use of this species as a non-conventional diet for aquaculture.

The presence of saturated fatty acids in the phospholipids and unsaturated fatty acids in the triglycerids, and the high percentages of linoleic and eicosapentanoic acids in *P. litoralis* also allow us consider its use as a non conventional food for growing aquaculture shrimp larvae. Satisfying the nutritional requirements of these crustaceans with natural substances that are intimately bound to the appropriate funtion of the physiology and cellular membrane is ideal for their growth.

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Chapter 5

Coastal oligochaetes and isopods as food sources for penaeid shrimps

Andrade, José & Asit Mazumder

ABSTRACT

We evaluated the feeding, assimilation, food conversion rate, and growth of shrimp (*Penaeus vannamei*) postlarvae that were fed the following experimental diets: OL, 100% oligochaetes (*Pontodrilus litoralis*); ISO, 100% isopods (*Tylos wegeneri*); OLI, a mixture of 50% *P. litoralis* and 50% *T. wegeneri*; and K, 100 % Zeigler (a commercial diet as control). The lipid, protein and carbohydrate composition was analysed for all these diets. A random design experiment with three replicates for each of these diets was conducted using 390 postlarvae per aquarium. The larvae were fed with an equivalent portion of 15% of their biomass per day for two months. At the beginning of the experiment, the average mass and length of these postlarvae were of 0.6 mg and 0.7 mm, respectively.

At the end of the experiment (after 60 days), the postlarvae fed with the K diet reached an average mass and length of 55.41 mg and 21.90 mm, respectively. For postlarvae fed with the experimental OLI diet, the average mass and length were of 30.86 mg and 14.93 mm, respectively. Using one way ANOVA, we found highly significant differences in the growth of *P. vannamei* postlarvae fed with the four different diets. A *post hoc* Bonferroni test shows that three groups could be formed: K, OLI and OL-ISO. A linear relationship was obtained between body mass (mg) and body length (mm) of *P. vannamei* postlarvae fed with the four different diets. K and OLI diets had higher determination coefficients (99 and 96% respectively). A detailed analysis of slopes using ANOVA regression, allowed to see that the K diet better promotes growth in terms of length of *P.*

vannamei larvae, while the OLI diet better promotes growth in terms of weight. Animals fed with the OLI diet grow bigger in terms of weight in short time, while those fed with the K diet can grow bigger in terms of length, in a longer period of time.

Keywords: Isopods, Oligochaetes, Penaeidae, *Penaeus vannamei*, aquaculture, nutrition.

INTRODUCTION

The artificial production of aquatic organisms, or aquacultures is considered as one of the main alternatives to the over-exploitation of natural resources. Currently, there is a worldwide annual artificial production of 15.8 million tons of aquatic organisms (seaweeds excluded) including 53.4% from marine aquaculture, and 46.6% from freshwater aquaculture (Wheaton, 1982; FAO, 1995 *fide* Giménez *et al.*, 1995). Shrimp aquaculture has significant economic importance worldwide. Among the different types of shrimps produced in shrimp farms, penaeids are the most economically important crustaceans because they represent one of the main groups in the fisheries, with a high value on the international market. Penaeids also offer excellent prospects for foreign earnings for many developing countries (Meglitsch, 1978; Giménez *et al.*, 1995). For example, this activity has become a billion-dollar industry in Ecuador, with a production reaching over 100,000 metrics tons in 1997 for a total value of $871 million US (Molina, 1999).

One of the most common and successfully cultivated penaeid species in Latin American countries, including Panamá, Ecuador, Colombia, Brazil and Venezuela, is *Penaeus vannamei*. This shrimp is an excellent species for commercial cultivation because it adapts well to intensive and semi-intensive culture techniques, has a fast growth, tolerates high temperatures, is resistant to a wide range of salinity, and reproduces year around with high fecundity (Sandifer *et al.*, 1987; Liao & Liu, 1989; Molina, 1999).

When the shrimp larvae are cultivated, it is important to provide them with an energy-balanced diet. As the composition of nutritious compounds in aquacultured organisms can only be derived from their food sources, it is critical to develop diets that contain essential compounds not only for the shrimp, but also for human health. Studies have been conducted to produce diets that guarantee good yields (New, 1976). However, very little research effort has been devoted to diets that provide balanced nutrition for humans. It is well known that an unbalanced food can cause extensive mortalities on *Penaeus* spp. larvae and that, if they do survive, their development can be strongly negatively affected (Sánchez, 1986).

Proteins are essential nutrients for the growth and survival of shrimps (Kanazawa, 1989; Kucharski & Da Silva, 1991). Different levels of proteins in the diets of *Penaeus* have been observed. It has been determined that food with a 25-40% protein content promotes a good growth in *Penaeus japonicus* (Balazs *et al.*, 1973). *Penaeus indicus* fed with diets with a protein content of 21-53% showed good growth results (Colvin, 1976), while *Penaeus aztecus* required 23-31% protein to show good growth (Shewbart *et al.*, 1972). Different energy levels, temperatures, and food sources are responsible for the great variability in the adequate protein levels for shrimp production (Kanazawa 1989). Food should also contain appropriate levels of carbohydrates, the energy sources required for the metabolic processes necessary for the survival of shrimp larvae (Guillaume, 1989).

Shrimps, like most other invertebrates and fishes, also require adequate levels of lipids in their diets, their nutritional value depending on the daily balance of essential fatty acids. In addition, lipids also provide the necessary energy for optimum development and maintenance of metabolic processes (New, 1976; Liao & Liu, 1989; Kanazawa, 1989).

A problem that Latin American commercial shrimp producers have to face is the high price and scarcity of the natural diets for aquaculture. Several studies have dealt with the nutritional and feeding profiles of shrimps and the evaluation of new non-conventional foods. Our goal was to satisfy the regional necessities and to produce new food sources for shrimp production.

Organisms that live in marine supra-tidal and inter-tidal habitats possess adaptations that involve the presence of certain natural substances such as long chain lipids (terpenoids), polyunsaturated fatty acids, specific proteins, carotenoids, lectins, saponins, and phytic acids (Paul, 1992; Sudaryono, 1999; Hárvardsson & Imsland, 1999), allowing them to face drastic environmental changes. However, some species suffer adverse effects when feeding on organisms that contain some of these substances (De Silva & Anderson, 1995). On the north coast of the Araya Peninsula in north-eastern Venezuela, there are two dominant species of inter-tidal macroinvertebrates, the isopod *Tylos wegeneri* (with densities up to 4000 ind.m^{-2}) and the oligochaete *Pontodrilus litoralis* (with densities up to 2000 ind.m^{-2}). These two invertebrate species may provide the appropriate concentrations of energy substrata to serve as a non-conventional diet for shrimp aquaculture.

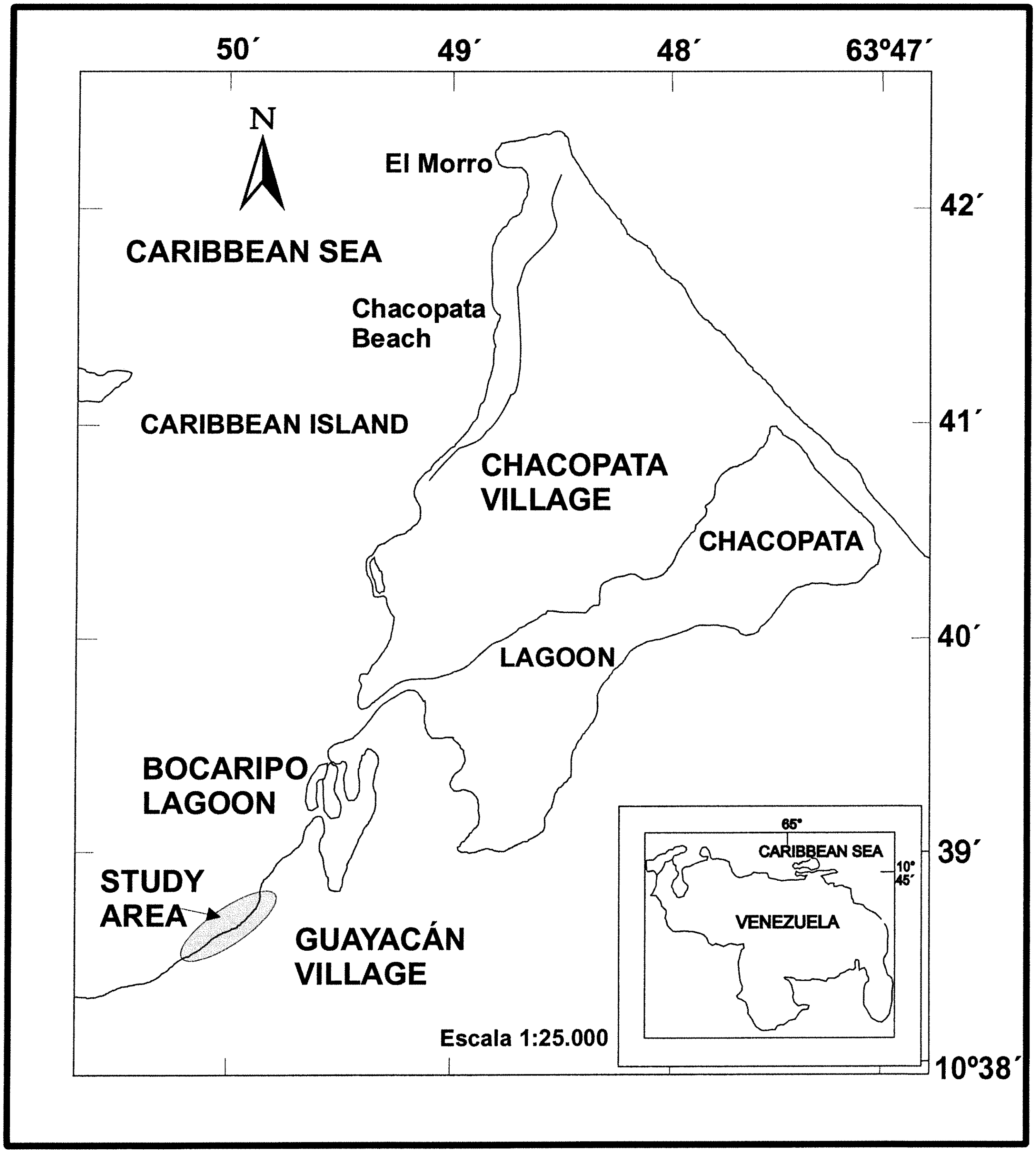
The goal of this study was to determine the efficiency of diets prepared from natural oligochaetes and isopods in order to produce penaeid shrimp larvae. We hope that this study will not only provide fundamental knowledge on the importance of diet quality in determining the growth efficiency of larval shrimp, but will also lead to the development of a nutritional balanced diet for shrimp aquaculture.

MATERIALS AND METHODS

Inter-tidal oligochaetes (*Pontodrilus litoralis*) and isopods (*Tylos wegeneri*) were collected by manual extraction from the sediments near the Guayacán village on the north coast of the Araya Peninsula, in north-eastern Venezuela (Figure 1). These organisms were immediately washed with clean seawater, placed in a cooler and transferred to the Laboratorio de Nutrición y Productos Naturales en Acuicultura (Department of Biology, Universidad de Oriente, Cumaná).

Both isopods and oligochaetes were freeze-dried, milled and sifted through 375 μm to obtain meals that could serve as diets for shrimp postlarvae. These diets were hermetically sealed in plastic bags and refrigerated to avoid deterioration. Subsequently, the following compounded meals were prepared: OL (100% oligochaetes), ISO (100% isopods) and OLI (50 oligochaetes and 50% isopods). The three experimental diets (OL, ISO and OLI) and a control diet, Ziegler (commercially available and thereafter called as "K"), were then offered to postlarvae of *Penaeus vannamei*, in triplicate containers per diet treatment, in order to evaluate their growth. K diet is a commercial food widely used for shrimp aquaculture.

Figure 1. Geographic location of the capture area of *Pontodrilus litoralis* and *Tylos wegeneri* in the Bocaripo-Chacopata lagoon complex on the north side of the Araya Peninsula in north-eastern Venezuela.


50′
49′
48′
63°47′
N
El Morro
42′
CARIBBEAN SEA
Chacopata Beach
CARIBBEAN ISLAND
41′
CHACOPATA VILLAGE
CHACOPATA
40′
LAGOON
BOCARIPO LAGOON
39′
STUDY AREA
GUAYACÁN VILLAGE
Escala 1:25.000
10°38′
65°
CARIBBEAN SEA
10° 45′
VENEZUELA

The shrimp aquaculture company Aquamarina de la Costa donated the postlarvae. These shrimps have been inbred for 15 years without crossbreeding with wild organisms. They have reached about 50% homozygosity (Aquamarina de la Costa, Pers. comm.), which allows us to minimize differences in growth results due the genetic vigour of the heterozygous organisms. Once transferred to the laboratory, the larvae were acclimatized for 72 hours and twelve groups of 30 postlarvae per litter (with mass and length of 0.60 mg and 0.70 mm, respectively) were placed in 13-l aquaria filled with 1-µm filtered sea water (salinity = 35 ± 1‰, temperature = 25 ± 1 °C). The aquaria were continuously aerated and exposed to natural photoperiod (10 hours of light and 14 hours of darkness). Seawater replacements were done (approximately 50% from total volume) by adding freshwater to maintain the initial values of salinity (37‰) and to avoid stress on the postlarvae.

Before the daily feeding of shrimps, the bottom and walls of aquaria were cleaned. Food remains and faeces were siphoned and dried at 40 °C for 24 hours in order to determine differences in the quantities of ingested food. It was assumed that 20% of remains were faeces (Silas, 1978). Finally, each of the 12 groups was fed with one of the three experimental diets in amounts equivalent to 15% their starting wet biomass for 60 days.

Every fifteen days, 30 larvae (90 per diet) were taken at random, measured from rostrum to telson with a millimetric slide (0.1 mm), dried with absorbent paper in order to reduce error, and weighed for wet mass (0.001 g). The larvae mass kept in the aquaria was used to adjust the quantity of experimental food to be offered for the following 15-day period.

The food conversion ratio was calculated according to the following equation Nascimento *et al.* (1987):

$CR = DWGF/GP$

where CR = conversion rate, DWGF = dry weigh of the offered food (mg), and GW = increase in weight (mg).

The percentage of ingested food (IF) was calculated using following equation Andrade (1988):

$\%IF = QIF/QOF \times 100$

where QIF = is the quantity of ingested food (mg), QOF = is the quantity of offered food (mg) and QIF is QOF – QRF or quantify of remaining food (mg).

IF values were quantified according to the following classes:

0 to 29% = poor, 30 to 59% = regular, 60 to 89% = good, 90 to 1 00% = excellent.

To determine the efficiency of the three different compounded diets for growing shrimp postlarvae, the following indexes were determined (Pereira *et al.* (1987):

Weight increase: $WI = FW\text{-}IW$, where FW was the final weight (mg) and IW is the initial weight (mg);

Instantaneous growth: IG = LnWI/T,

where LnWI is the natural logarithm of the weight increase in and T is time (days);

Length Increase: C = FL-IL,

where FL is the final length (mm) and IL is the initial length (mm).

The assimilation coefficient (A) of ingested food is equal to QIF-faeces determined according to Condrey *et.al.* (1972).

The total lipid, protein and carbohydrate contents of K, OL, ISO and OLI diets were analysed. One mg of each was homogenized in 1 mL of double distilled water (1:1) in order to determine the energy assimilation by *P. vannamei* postlarvae. Two replicates of homogenate (0.150 mL) were added to 0.5 mL of NaOH. Each aliquot was warmed to 100 °C for 10 min and filled to 1 mL with double distilled water. To extract proteins, 5 mL of cupro-alkaline solution was added (1 mL of $CuS0_4.5H_2O$ at 5% + 50 mL of Na_2CO_3 at 2% and 0.5 mL Folin-Ciocalteau's). Samples were centrifuged for 5 min and proteins were quantified using a Spectronic 20 at optical density of 750 nm, calibrated with bovine serum albumin as standard (Lowry *et al.*, 1951).

To determine total carbohydrates, two aliquots of 0.075 mL were added to 1 mL 5% phenol and 5 mL sulphuric acid; thereafter, samples were read in an Spectronic 20 at optical density of 490 nm (Dubois *et al.,* 1956) using glucose as standard. Two aliquots of

0.025 mL were taken from the same homogenized sample and dried in an oven at 110 °C for 1 h. After cooling at room temperature, 0.2 mL of CH_2Cl_2-MeOH (2:l) was added, and samples were dried at 80 °C. Then 1 mL of sulphuric acid and 2 mL of double distilled water were added, and total lipids were obtained by means of a Spectronic 20 at an optical density of 375 nm, calibrated with palmitic acid as a standard (Folch *et al.* 1957).

In order to determine differences between the lengths and the wet weights of the *P. vannamei* postlarvae that were removed every 15 days, one-way ANOVA and *post-hoc* Bonferroni tests were performed (Sokal & Rohlf, 1979; Zar, 1984). These tested differences in growth following the feeding on the four experimental diets. Regression analyses were also performed to evaluate length and weight increases following the feeding on each experimental diet.

RESULTS

Diet K, followed by OLI, had the highest protein, lipid and carbohydrate contents, while OL had the lowest value (Table 1). OL, ISO and OLI diets had higher ash and humidity contents than K (Table 1). The quantity of food ingested at 15-day intervals by *P. vannamei* postlarvae fed with K, OL, ISO, and OLI diets is shown in Figure 2. After 30 days, the food demand was larger and varied drastically depending on which diet the shrimp postlarvae were fed. The control diet (K) was the most heavily consumed, followed by OLI, OL and ISO, respectively. The similar consumption of the four diets during the first fifteen days may have been due to the time needed for individuals to adapt themsleves

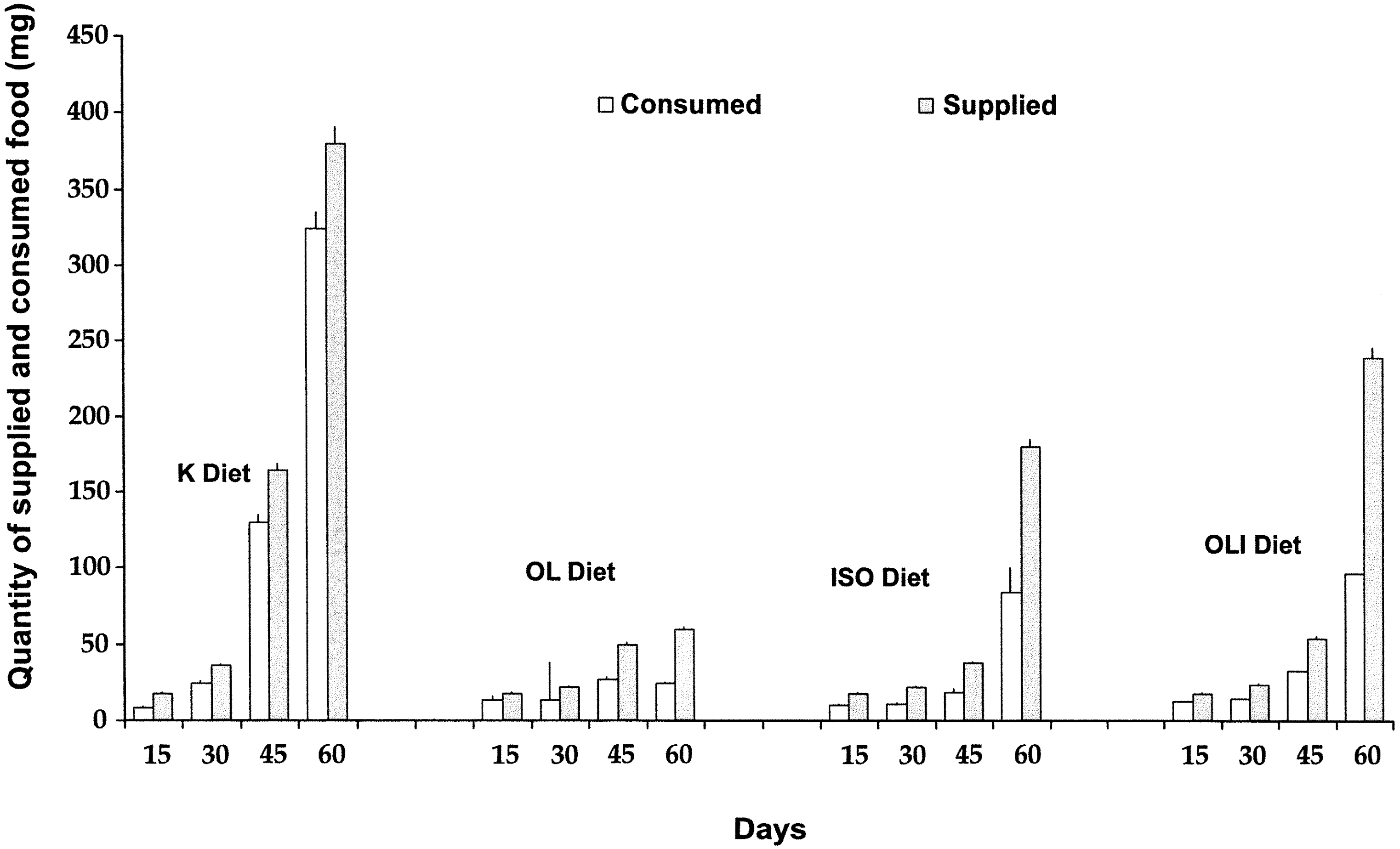
Table 1. Energy substrata, ash, humidity and other elements (%) of experimental diets.

	K	OL	ISO	OLI
Proteins	42.8	21.8	24.3	22.8
Lipids	12.3	8.0	9.5	8.6
Carbohydrates	8.9	4.0	4.2	4.5
Ash	11.0	21.0	24.6	23.8
Humidity	8.5	17.5	19.5	18.7
Other elements	16.5	27.7	17.9	21.6

Table 2. Final weight (Fw in mg), weight increase (WI in mg), and conversion rate (CR) of individuals of *P. vannamei* fed with K, OL, ISO, OLI experimental diets for 60 days.

Diets	FW	WI	CR
K	55.4 ± 1.6	54.8	3.1:1
OL	12.9 ± 0.5	12.3	3.0:1
ISO	14.0 ± 0.5	13.4	3.0:1
OLI	30.9 ± 1.2	30.3	2.5:1

Figure 2. Quantity (means ± SE) of supplied and consumed food during study period by *P. vannamei* postlarvae.


Quantity of supplied and consumed food (mg)
0
50
100
150
200
250
300
350
400
450
□ Consumed
▨ Supplied
K Diet
OL Diet
ISO Diet
OLI Diet
15
30
45
60
15
30
45
60
15
30
45
60
15
30
45
60
Days

to given foods. On the other hand, the great difference observed in the consumption of the diets starting after 30 days is closely related to their nutritional values. The average particle size of our experimental diets was of 375 µm, corresponding to the size-range recommended by various authors.

The final growth (mg) and the conversion ratio of *P. vannamei* postlarvae fed with K, OL, ISO and OLI diets was obtained after sixty days (Table 2). The largest final weights and weight increases were obtained with the K and OLI diets, with respective conversion rates of 3.1:1 and 2.5:1.

Postlarvae fed with the commercial K diet reached the largest average weekly length increment (2.0 mm) followed by those fed with the OLI (1.0 mm), and then by ISO (0.8 mm) and OL (0.7 mm).

The largest instantaneous growth (IG) of *P. vannamei* postlarvae was obtained with K (0.3 mg), followed by OLI (0.3 mg), ISO (0.2 mg) and OL (0.2 mg) (Figure 3). One-way ANOVA analysis showed significant differences (Fs = 79.4, $p < 0.001$) between the four diets after sixty days. *Post hoc* Bonferroni tests, also performed after sixty days, showed the formation of three groups, the first one consisting of K, the second of OL and third of ISO-OLI (Figure 3).

The relatively high contents of total lipids, proteins and carbohydrates found in *P. vannamei* larvae at the beginning of our study decreased during the first fifteen days (Figure 4). Consistent and increasing assimilation of proteins was observed with the K diet

Figure 3. Instantaneous growth (Log of means ± SE) of *Penaeus vannamei* individuals and groups obtained by *Post hoc* Bonferroni test.

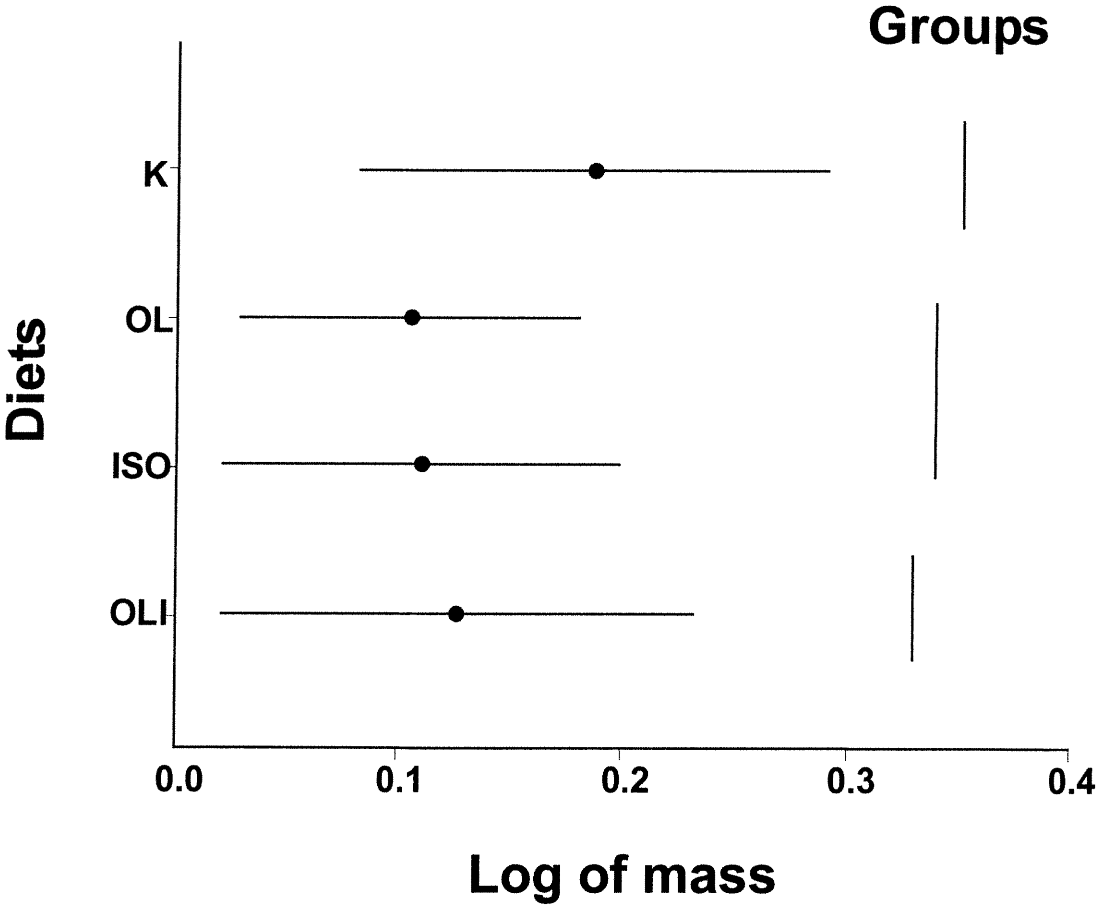
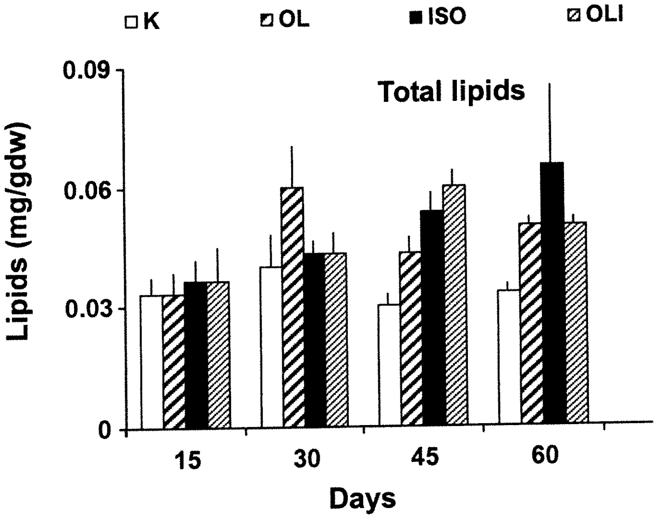
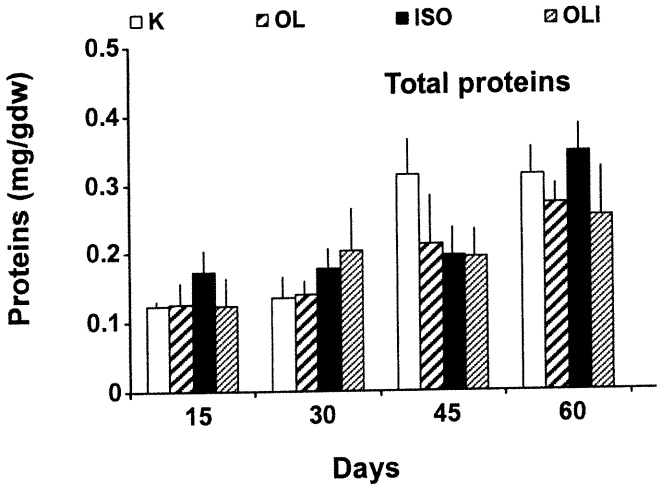
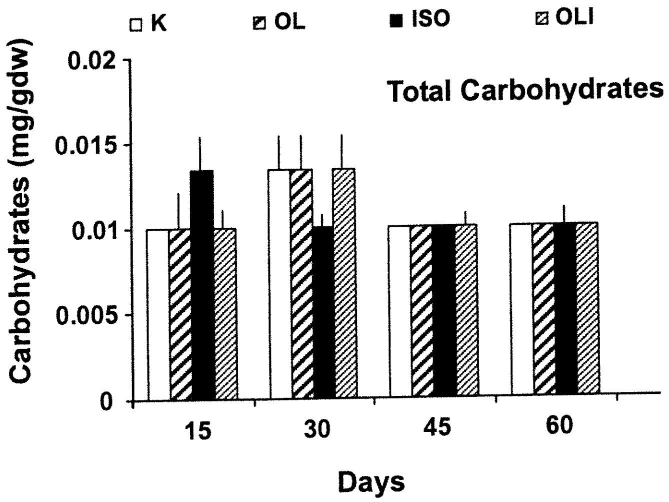

Groups
Diets
K
OL
ISO
OLI
0.0
0.1
0.2
0.3
0.4
Log of mass

Figure 4. Weekly changes (means ± SE) in assimilation of total lipids, proteins and carbohydrates in *P. vannamei* following feeding on four experimental diets.


□ K
▨ OL
■ ISO
▨ OLI
Total lipids
Lipids (mg/gdw)
0.09
0.06
0.03
0
15
30
45
60
Days


□ K
▨ OL
■ ISO
▨ OLI
Total proteins
Proteins (mg/gdw)
0.5
0.4
0.3
0.2
0.1
0
15
30
45
60
Days


□ K
▨ OL
■ ISO
▨ OLI
Total Carbohydrates
Carbohydrates (mg/gdw)
0.02
0.015
0.01
0.005
0
15
30
45
60
Days

(0.12- 0.32 mg/gdw) beginning at the 3rd and 4th weeks. The assimilation of lipids was observed to increase with OLI (0.03 to 0.07 mg/gdw). With OL, a high content of lipids (0.03-0.08 mg/gdw) was obtained after 30 days. Assimilation of carbohydrates showed a slight increase during the first 30 days with K, OL and OLI, but remained practically constant thereafter. These results suggest that growth observed with the K and OLI diets (Figure 3) was specifically due to the efficient assimilation of their energy contents.

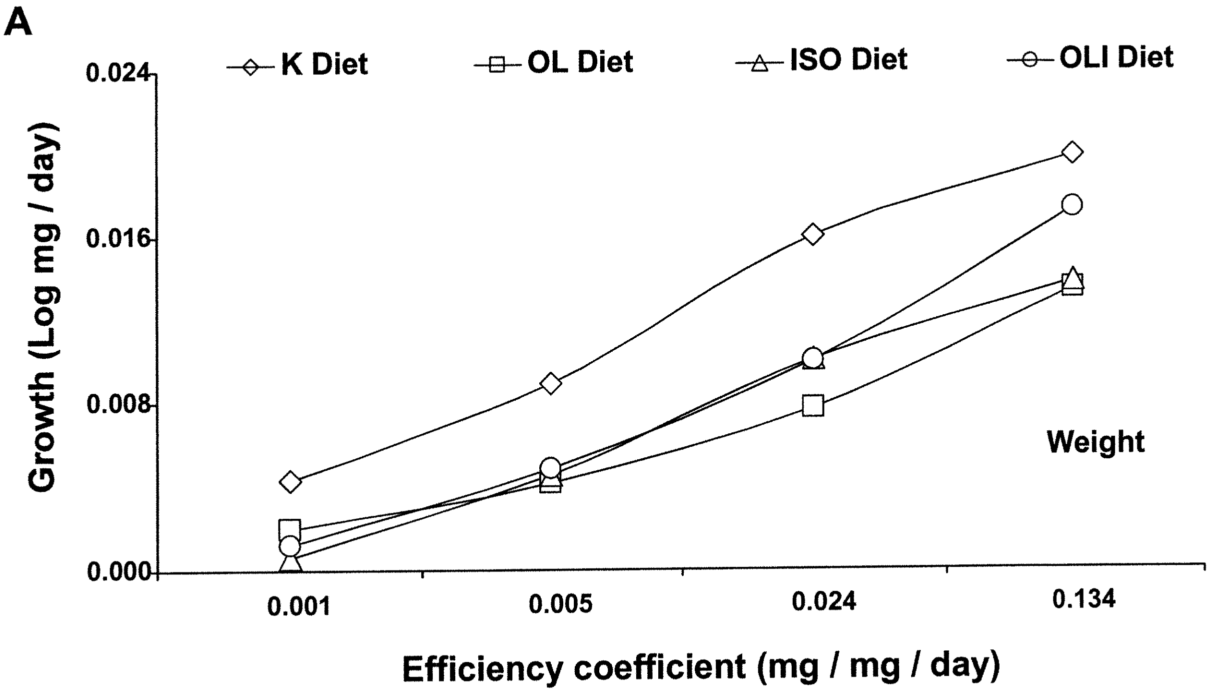
Based on the instantaneous growth in weight and length, it appears that all four diets had positive impacts on postlarvae growth (Figures 5 A, B). The highest increase in length was observed with the K diet (Figure 5B), while OLI produced a best increase in weight (Figure 5A). The higher growth observed with K and OLI were probably due to the greater protein and lipid concentrations in these diets (Table, 1).

A detailed ANOVA analysis showed that slopes of the regression lines between weight and length growth are significantly different (K with $Fs = 888.5 < 0.001$; OL with $Fs = 78.9 < 0.001$; ISO with $Fs = 70.8 < 0.001$ and OLI with $Fs = 225.2 < 0.001$). When fed with the K diet, postlarvae increased considerably their length while, when fed with OLI, they gained more weight in a short time (Figure 6).

DISCUSSION

Cultured organisms require specific concentrations of energy resources, macronutrients, and trace elements for their healthy maintenance, development and

Figure 5. Weekly changes in instantaneous growth expressed as A) mass (mg/day) and B) length (mm/day) increments as a function of assimilation efficiency (mg/g/day) for four experimental diets.


A
K Diet
OL Diet
ISO Diet
OLI Diet
Growth (Log mg / day)
0.024
0.016
0.008
0.000
Weight
0.001
0.005
0.024
0.134
Efficiency coefficient (mg / mg / day)

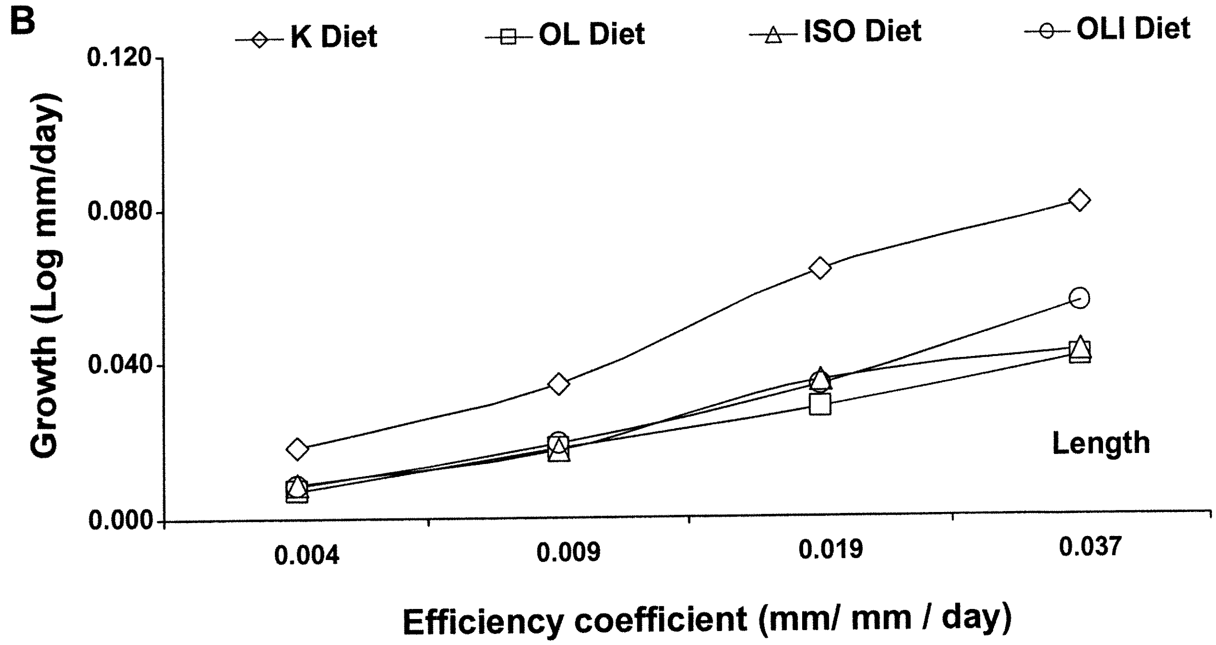
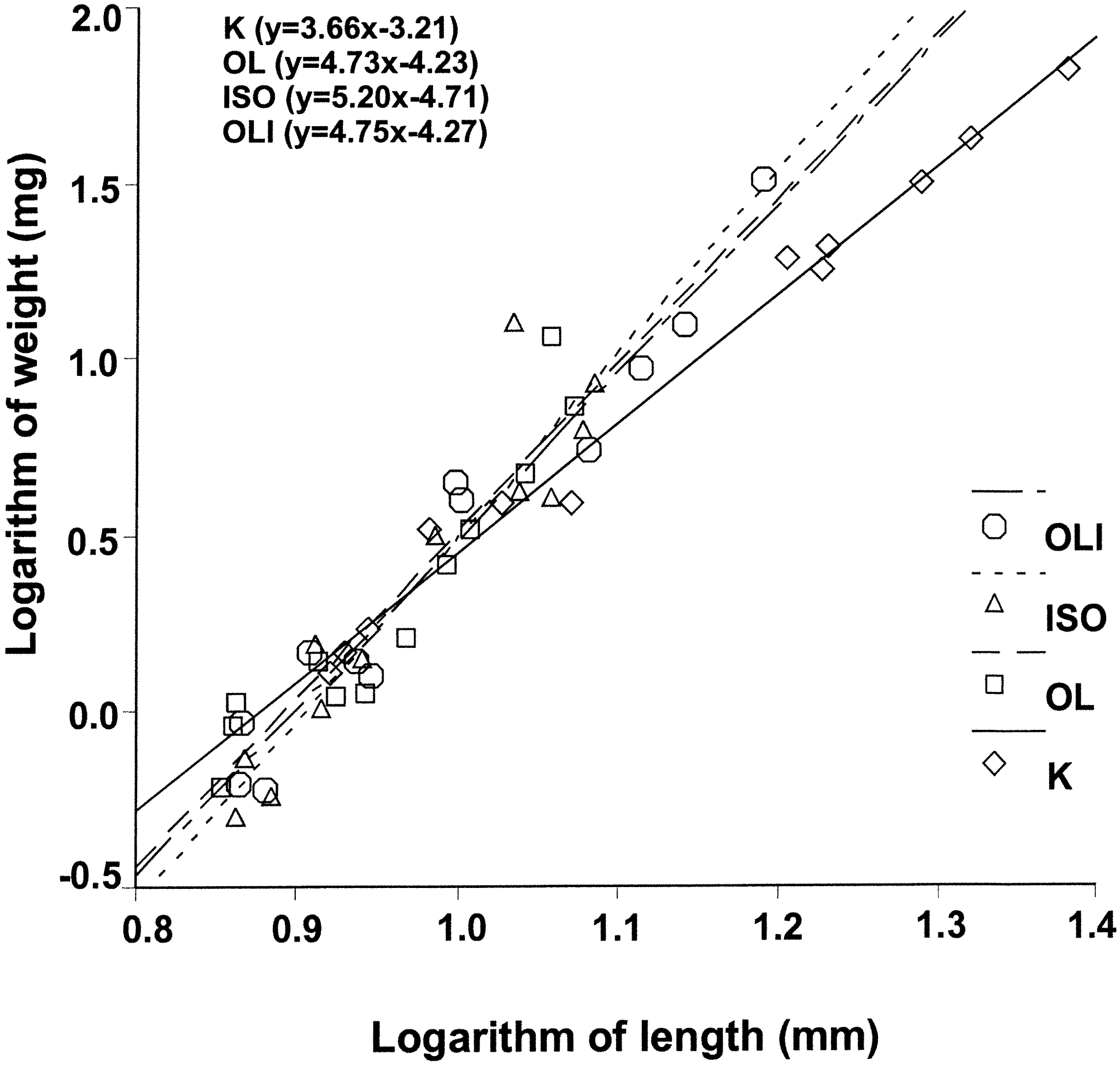

B
K Diet
OL Diet
ISO Diet
OLI Diet
Growth (Log mm/day)
0.120
0.080
0.040
0.000
Length
0.004
0.009
0.019
0.037
Efficiency coefficient (mm/ mm / day)

Figure 6. Relationships between body mass (mg) and body length (mm) of *P. vannamei* following feeding on each of the four experimental diets.


K (y=3.66x-3.21)
OL (y=4.73x-4.23)
ISO (y=5.20x-4.71)
OLI (y=4.75x-4.27)
Logarithm of weight (mg)
2.0
1.5
1.0
0.5
0.0
-0.5
0.8
0.9
1.0
1.1
1.2
1.3
1.4
Logarithm of length (mm)
OLI
ISO
OL
K

successful reproduction. Thus, it is important for the aquaculture nutritionists to develop compounded diets according to the specific needs of each species.

Fast growth was obtained with protein concentrations in the order of 23-31%, 43%, 30%, and 53% for *P. aztecus*, *P. indicus*, *P. vannamei*, and *P. japonicus*, respectively (Shewbart *et al.*, 1972; Colvin, 1976; Colvin & Brand, 1977; Deshimaru & Yone, 1978). Protein concentrations of 25-40% were required by *P. vannamei* under controlled conditions (Barbieri & Cuzon, 1980 *fide* Bados *et al.* 1994). Akiyama & Chwang (1994) concluded that penaeid shrimps require concentrations of 36% to reach body weight of 15 to 40 g. The ISO and OLI diets had protein values of 24.3% and 22.8, respectively (Table 1). The protein values of OLI compare well with those reported by Colvin & Brad (1977) as necessary for *P. vannamei*; ISO values better correspond to those recommended by Shewbart *et al.* (1972) for *P. aztecus*, and (Barbieri & Cuzon, 1980 *fide* Bados *et al.* 1994) for *P. vannamei*.

Lipids play a important role in the physiology of most animals because they supply the essential energy reserves during periods of food scarcity and negative energy balance such as hibernation (mammals), sexual reproduction (in invertebrates and many vertebrates) and migration (birds, fishes, insects and other invertebrates) (Giese, 1966; Pocock *et al.,* 1971; Bradshaw *et al.,* 1990). In addition, the degree of permeability of the cellular membrane depends on its lipid content which allows free passage to molecules between the external and internal environments (Stefanov *et al.*, 1993). Lipids also transport fat soluble vitamins and provide energy for other essential compounds necessary for the metabolic function of shrimp growth (Akiyama, *et al.*, 1989). However, the presence, accumulation and variation of lipids in marine invertebrates is not very well known (Giese, 1966; Pocock *et al.*, 1971).

According to Bautista (1988), lipid contents in the order of 6-7.5% are recommended for shrimps commercial diets and should not exceed 10% since the lipase activity is low in crustaceans. For this reason, diets with 10% or less lipid content are ideal for these invertebrates. In the present study, the lipid values of OL (8.0%), ISO (9.5%), OLI (8.6%) and K (12.3%) exceed the values recommended by Bautista (1988). Greatest growth was recorded for *P. vannamei* with the highest concentrations of lipids (K and OLI). Other studies showed a decrease in the growth of *Palaemon serratus* with diets containing 15% lipids (Forster & Beard, 1973). Best growth results were obtained for the lobster, *Homarus americanus*, with a diet of 5% lipids based on cod liver oil (Castell & Covey, 1976).

Read (1981) concluded that mixtures of marine organisms and vegetables provide the best growth results based on the fact that a mixture of cod liver and soya oils in a proportion of 3:1 gave the best growth for *Penaeus japonicus*. The organisms used for preparing OL, ISO and OLI diets (*Pontodrilus litoralis* and *Tylos wegeneri*) are intertidal organisms which ingest large quatities of sedimentary organic matter, incluiding meio- and microfaunal organisms (Edwards & Lofty, 1977; Lee, 1985) and macroalgae (Kensley, 1974; Newell, 1979; Brown & Odendaal, 1994).

Good growth results which concord with those of the present study were obtained for shrimps fed with diets containing 25-30% of the oligochaete *Eudrilus eugeniae* with a lipid content of 8.8% (García & Jaime, 1990). Nevertheless, other studies showed a reduction in the growth of shrimps *Palaemon serratus* with diets containing a 15% lipid content (Forster & Beard, 1973).

Carbohydrates, although not essential for shrimp growth, are considered useful energy sources and act in the metabolic process of growth (Akiyama *et al.*, 1989). Diet composition influences the metabolism of carbohydrates and lipids in some invertebrates. Diets rich in carbohydrates, offered to estuarine crabs *Chasmagnathus gratnulata*, were found to increased glucose and glycogen levels in blood, muscle and hepatopancreas, and to lower lipid levels in muscles. In contrast, diets with high concentration of proteins were found to reduce glycaemia and glycogen in the hepatopancreas and muscles (Kucharski & Da Silva, 1991).

Diets with carbohydrates values of 20% were considered as optimum for growing *Penaeus monodon* (Bautista, 1986). However, *P. japonicus* larvae showed better results with 35% carbohydrates, while juveniles preferred carbohydrates in the order of 15 and 20% (Guillaume, 1989) and a 30% (Maugle *et al.*, 1983). Table 1 shows that K (8.9), OL (4.0), ISO (4.2), and OLI (4.5) diets had lower percentages of carbohydrates than the above mentioned values and, as a consequence, could have affected the growth of *P. vannamei* postlarvae negatively. In this respect, it is important to point out that the shrimp larval and postlarval stages suffer daily ecdysis that induces a continuous energy expense, which could be compensated by larger food portions or by food with higher energy contents. The great variety of optima levels of these energy substrata reported in the literature is explained by differences among nutritious habits, water temperature, natural protein sources and energy levels in the diets during the cultivation operations (Kanazawa, 1989).

A given diet should not only be nutritionally well balanced, but should also have the appropriate particle size, adequate water stability, attractiveness and palatability (Akiyama & Chwang, 1994). The average particle size in our experimental diets corresponded to the size-range recommended by Bages & Sloane (1981) and offered to *P. shmitti* postlarvae by Jaime & García (1990).

Commercial foods are blended with several preservatives for improving their nutritive quality, attractiveness and palatability. Akiyama & Chwang (1994) suggested that attractive and palatable foods are essential for the optimum growth of shrimp larvae that feed on them continuously. This might to explain why the commercial K diet was the most consumed. However, OLI (combination of OL and ISO) seemed to be more atractive to shrimp larvae than OL and ISO separately. The stability of experimental diets offered to shrimp larvae was considered good since they maintained their integrity for more than 4 hours after being offered to the shrimp larvae. It is important to point out that the shrimp diets should be stable in the water so that the shrimp can feed slowly and continuously (Akiyama & Chwang, 1994).

The final growth (mg) and conversion ratio of *P. vannamei* postlarvae fed with K, OL, ISO and OLI showed good results and allow to consider them of good quality. A diet is considered of good quality when its conversion ratio ranges between 3:1 and 1:1 (Read, 1981; Young & Reinoso, 1982). Unbalanced and inadequate diets result in higher rates of food conversion (Martínez *et al.*, 1989). In addition, diets poor in calories generally result in high conversion rates (Liao & Liu, 1989). Although OLI had a lower conversion ratio than K, the latter resulted in better growth efficiency for *P. vannamei*. However, it is important to note that the weekly growth averages for OL, ISO and OLI were low compared to those reported for *P. setiferus* (4.7 mm, 14.7 mm and 3.4 mm) (Johnson & Fielding, 1956; Linder & Anderson, 1956; Pinto, 1971). Alvarado (1990) observed that the growth of *P. shmitti* and *P. vannamei* individuals produced in ponds was not influenced by any of two commercial diets. Nevertheless, the growth of *P. japonicus* was significantly influenced ($p < 0.001$) during 21 days of culture by different diets of marine origin (Nascimento, *et al.*, 1987)

As organisms grow and develop, their nutritive requirements change. During the period of fast growth, polyunsaturated fatty acids, specific proteins, and essential amino acids play a primary role (Watanabe *et al.*, 1983; Tacon 1987). It has been suggested that the content of amino acids in the diet should be equivalent to that in the shrimp tissues (Shigeno *et al.*, 1972; New, 1976). When *P. vannamei* postlarvae were growing, their requirements for other natural substances (carotenes, vitamins and flavonoids), energy substrates (cholesterol esters, phospholipids and triglycerids) and trace elements were probably supplied mainly by the K diet and partially by the OLI diet. Natural substances like carotenes, vitamins, flavonoids, cholesterol esters, phospholipids and triglycerids have strong influence on reproductive processes, healthy growth, pigmentation and immunity (Watanabe *et al.*, 1983; Tacon 1987; Håvardsson & Imsland, 1999; Cedeño *et al.*, 1999). Poor biochemical and physiological functions of cells during the growth of postlarvae, due to the absence of any of these energy compounds, might have negatively decreased the growth and food ratio conversion of *P. vannamei.* Triglycerids are considered as the best and largest energy source for animals and cholesterol esters and phospholipids play an important role in the composition and permeability of membranes and their structural modifications following environmental changes (Lim & Akiyama, 1992).

Postlarvae of *P. vannamei* fed with the K commercial diet reached the largest average weekly length increment (2.0 mm) followed by those fed with OLI (1.0 mm), and then by ISO (0.8 mm) and OL (0.7 mm). These weekly growth averages for OL, ISO and OLI are low compared to those of 4.7 mm, 14.7 mm and 3.5 mm reported for *P. setiferus* (Johnson & Fielding, 1956; Lindner & Anderson, 1956; Pinto, 1971).

The largest instantaneous growth (IG) of *P. vannamei* postlarvae was obtained with K (0.3 mg), followed by OLI (0.3 mg), ISO (0.2 mg) and OL (0.2 mg) (Figure 3). One-way ANOVA showed significant differences ($Fs = 79.4$, $p < 0.001$) between the four diets after sixty days. *Post hoc* Bonferroni tests showed the formation of three groups, the first one was constituted by K, the second by OL and ISO, and the third one by OLI after sixty days of experiment (Figure 3). Alvarado (1990) observed that the growth of *P. schmitti* and *P. vannamei* individuals produced in ponds was not influenced by any of two commercial diets. On the other hand, the growth of *P. japonicus* was significantly influenced ($p < 0.001$) during 21 days of culture by different diets of marine origin (Nascimento *et al.*, 1987).

The initial decrease in growth during the first fifteen days of our study was probably due to the initial adaptation of postlarvae to their new environmental conditions. Likewise, assimilation results suggest that growth observed with K and OLI diets was specifically due to the efficient assimilation of protein and lipid contents. Juvenile shrimp are known to possess a high nutritious efficiency (Condrey, *et al.*, 1972); for this reason, lipids and proteins are apparently assimilated more efficiently than carbohydrates.

Linoleic, linolenic, eicosapentanoic and docosahexanoic fatty acids are considered as essential for *P. japonicus* and *P. indicus* (Read, 1981; Lim & Akiyama, 1992). Oligochaetes generally contain all these essential fatty acids (Bell *et al.*, 1994). The observed results of instantaneous growth and assimilation are closely related with the concentrations of the oligochaete (*P. litoralis*) fatty acids, and additionally with the presence of the isopod (*T. wegeneri*) proteins such as chitin in the OLI combined diet. We argue that the variation in the assimilation of these energy resources was influenced by the ecdysis cycle, during which the energy consumption by the larvae is high, which requires a balanced and continuous supply of these energy compounds. Indeed, Ando *et al.*, (1977) and Patrois *et al.*, (1978) emphasized that growth and physiology of animals are related with their ecdysis. They based their conclusion on the fact that they found high lipid concentrations in the hepatopancreas and whole body of *P. japonicus* during early pre-ecdysis. Teshima & Kanazawa (1971) obtained an increase of the lipidic contain in the D2 stage in *Palaemon paucidens*, followed by an increase of C16 palmitic and C18:1ω9 oleic acids, and drastic decrease of eicosapentanoic C20:5ω3 and docosahexanoic C22:6ω3 polyunsaturated fatty acids in the C stage. Kanazawa & Teshima (1977) suggested that the fatty acids of $\omega3$ and $\omega6$ series were essential for *P. japonicus*, and they insist on the role of linolenic C18:3ω3 and linoleic C18:2ω6 during ecdysis.

The important increase in weight observed with OLI diet can be considered negatively by shrimp producers since, on the commercial scale, longer sizes are more attractive than shorter, heavier ones. Nevertheless, we argue that the increase in the total content of proteins and other nutritive substances (vitamins, amino acids, carotenoids etc.) in appropriate concentrations results in length increase of *P. vannamei* postlarvae.

The relationship between mass and length should remain constant in a population due to the influence of genetic factors (Le Cren, 1951; Rossi-Wongtschowski, 1977); however, it is important to point out that weight depends on several factors, such as gonadic maturation, population density, feeding, etc. (Braga, 1995; De Souza, 1997).

Despite the fact that additional nutritious substances (vitamins, amino acids, carotenoids etc.) were not added to our experimental diets to satisfy the nutritive requirements of *P. vannamei* during their growth, we obtained positive results under controlled conditions, specifically with the OLI diet. The differences observed between commercial and experimental diets may have resulted form the presence of antimetabolites, inhibiting the growth of *P. vannamei.* Indeed many organisms can synthesise a wide variety of chemical substances (antimetabolites) which cause deleterious effects such as inhibition of growth, decreasing of food efficiency, pancreatic hypertrophy, hypoglycaemia, liver damage and death when ingested by animals (De Silva & Anderson, 1995; Miralto, *et al.*, 1999). Cooking, usually destroys all these substances, but it is important to remember that many diets are lyophilised the same way as those used in the present study.

CONCLUSION

Decrease of food efficiency and inhibition of growth are factors that agree with our results. Although we consider that the combination of the two organisms (*P. litoralis* and *T. wegeneri*) offer interesting possibilities of non-conventional diets for growing penaeid shrimps, new experiments, combining the cooking of the raw matter and the addition of the other nutritious substances, are necessary to fully satisfy the nutritional requirements of cultivated shrimps.

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GENERAL CONCLUSIONS

1.- *Pontodrilus litoralis* was abundant in sheltered inter-tidal ecosystems where low wave action, normal salinity (35-37‰), small sizes of sediment particles and important concentrations of total pigments were observed. Important quantities of small individuals observed during a whole year indicate a constant reproduction and a potentially high metabolic activity. On the other hand, high concentrations of pH and organic matter seem to produce a negative impact on *P. litoralis*. In consequence, *Pontodrilus litoralis* prefers conditions of temperatures ranging from 28 to 29 °C, salinity in the order the 35-37 ‰, between 500-700 µm of sediment particle sizes, and total pigments of 0.5-1 mg/dww. These specific conditions were found in sheltered sites like Guayacán.

2.- *Tylos wegeneri* was abundant in inter-tidal ecosystems with low wave action, small-size sediment particles, moderate temperatures, and moderate concentrations of organic matter. Larger individuals were observed during colder months (January-February) when energetic costs for growth were lower and the availability of food resource was higher. However, an extremely high concentration of either organic matter or total pigments seems to produce a negative impact on this isopod. It appears that *T. wegeneri* prefers environmental conditions with temperatures ranging from 27 to 31°C, sediment particle sizes in the order the 250-500 µm, between 0.1-0.50 mg/gdw of organic matter, and salinity of 30-35‰. Such conditions were also found in sheltered sites like Guayacán.

3.- Both species *P. litoralis* and *T. wegeneri* can be used as excellent indicators of environmental changes caused by anthropogenic impacts on coastal ecosystems, since they are adapted to commonly inhabit inter-tidal zones with strong impacts from human activities.

4.- *Pontodrilus litoralis* contains relatively high lipid and protein concentrations, which are quite variable because of their direct dependence on micro and meiofaunal organisms used as food. Nevertheless, we think that this oligochaete can be used as a non-conventional food for growing penaeid shrimps.

Important concentrations of saturated fatty acids in the phospholipids, unsaturated in the triglycerids, and high percentages of linoleic and eicosapentanoic acids in *P. litoralis* allow to consider its use as a non-conventional food for shrimp larvae aquaculture. Satisfying the nutritional requirements of these crustaceans with natural substances that are intimately bound to the appropriate function of the physiology and cellular membrane is ideal for growth.

5.- The use of diets containing both *P. litoralis* and *T. wegeneri* had satisfactory results on the growth of shrimp postlarvae, even though additional nutritious substances (such as vitamins, amino acids, carotenoids etc.) were not added to our experimental diets. Differences observed between commercial and our experimental diets might be caused by the presence of antimetabolites inhibiting the growth of *P. vannamei* postlarvae.