UNIVERSITEIT GENT

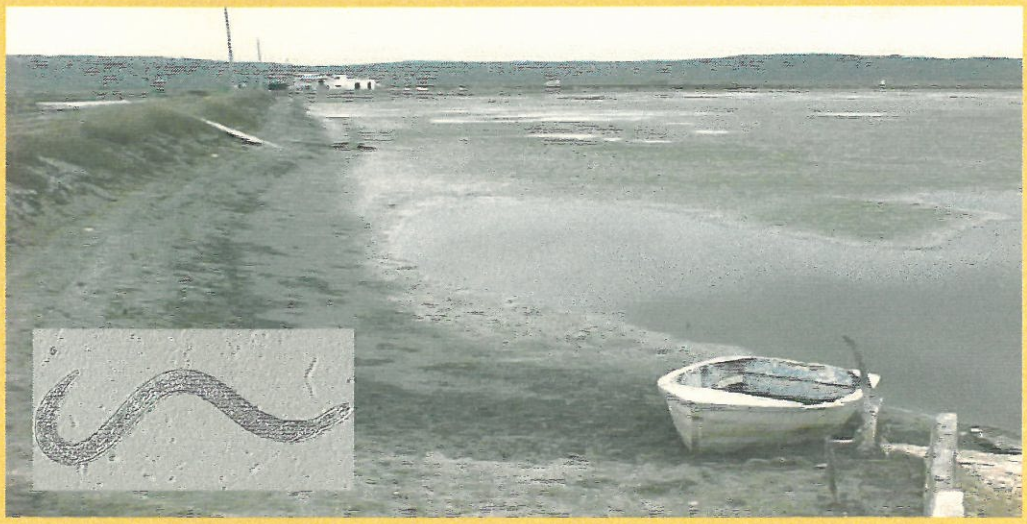
FACULTY OF SCIENCES

CENAIM - ESPOL

Vakgroep Biologie

Krijgslaan 281-S8, B-9000 Gent, BELGIUM

Meiobenthic communities in shrimp production ponds (Ecuador)



María Herminia Cornejo Rodríguez

Promoter: Prof. Dr. Magda Vincx

Co- promoter: Dr. Stanislaus Sonnenholzner

Academic year 2005 – 2006

Thesis submitted in partial fulfillment of the requirements for the degree of
Doctor in Science (Biology)

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The closer one gets to being motivated by altruism, the more fearless one becomes in the face of even extremely anxiety-provoking circumstances

Dalai Lama, 1998

To César Aníbal, César Andrés, Ana María and Manuel Eduardo the persons who enjoy my life every day and who "let me fly"

A César Aníbal, mi esposo; a mis hijos, César Andrés, Ana María y Manuel Eduardo, las personas que llenan mi vida cada dia y quienes me permitieron llegar adonde estoy

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SUMMARY

The white shrimp, *Litopenaeus vannamei,* is one of the most important commercial natural products of Ecuador. Therefore, studies that help to improve the knowledge about the shrimp pond ecosystem are important for a sustainable production. Studies about the bottom dynamics of shrimp ponds and about the characterization of the benthos of these ponds in general are very scarce. The dominant benthic components are the free-living nematodes, which constitute more than 90 % of the benthos.

The nematode community of the shrimp pond bottoms of five different shrimp farms in the coastal region of the Guayas province (Ecuador) were investigated under field and mesocosm conditions. Emphasis was given on the temporal fluctuation of the nematode density and diversity throughout one year, based on monthly measurements. The effects of the presence of shrimp as well as the effects of the different additives that the shrimp farmers use in their production systems (fertilizer, lime) on the nematode community were also studied. Management practices and some environmental variables were co-monitored in the analysis. The study sites were located near the Ecuadorian coast (marine environment) as well as along the Guayas river estuary (estuarine water environment).

In total 159362 nematodes were counted and 20436 specimens identified at the species level. 32 nematode species belonging to 12 families were identified (5 species still remain unclassified). 9 species are associated to the seawater environment, 14 species are associated to the estuarine environment and only 3 species were observed to both locations. A density range between 13 and 634 ind.10cm^{-2} was found through the different systems.

Terschellingia longicaudata (1A) and *Spilophorella papillata* (2A) were the most abundant species with abundances between 31 % and 81% of the total nematode community. The highest abundances of *T. longicaudata* correspond to the

seawater environment while *S. papillata* was abundant in the estuarine environment. *Daptonema* spp (1B), *Gomphionema* spp (2A) and *Theristus* spp (1B) were also important species inside the ponds. In spite of the abundance of the major species, the non-selective deposit-feeders species (1B) were the most abundant in number of genera. Shrimp pond environments are characterised by a very low density and diversity of the nematode community, compared with other sediments in the coastal region (beaches, mangrove sediments).

The management practices, which aim to improve water quality, have not a significant influence ($p>0.05$) at the "common doses" on the nematode community. But some effects of additives were observed on the different species; these species are candidates as bio-indicator of the chemicals conditions of the pond.

The drained period of the pond, between two shrimp production cycles, has an effect on the benthic community although no clear benthic cycles could be detected in subsequent shrimp production cycles. Copepods are the initial colonizers of the shrimp pond bottom, followed by nematodes, which dominate the bottom very rapidly. A possible effect of the presence of shrimp on the nematode community was also observed, probably associated with soil disturbance (molting and feeding activities).

Resumen

El camarón Blanco, *Litopenaeus vannamei,* es uno de los mas importantes productos comerciales del Ecuador. Por este motivo los estudios que ayudan al mejoramiento del conocimiento acerca del ecosistema de las piscinas camaroneras son importantes para el desarrollo de una producción sostenible. Los estudios acerca de la dinámica de los suelos de las piscinas camaroneras y acerca de la caracterización del bentos de estas piscinas en general son muy escasos. Los componentes dominantes del bentos son los nemátodos de vida libre, los cuales llegan a constituir mas del 90% del total del bentos.

Se estudiaron las comunidades de nemaáodos de cinco camaroneras de la región costera de la provincia del Guayas, Ecuador, tanto a nivel de campo como en tanques experimentales. Se dió énfasis a la fluctuación temporal de nemátodos, tanto en densidad como en diversidad a través de un año de estudio. El efecto de la presencia de camarón, asi como también el efecto de algunos aditivos que se utilizan en las camaroneras (fertilizantes y cal), sobre la comunidad de nemátodos fue también estudiada. Se tomó información de la camaronea sobre las variables de producción. También se midió la temperatura, salinidad, pH y niveles de oxígeno en cada experimento.

En total 159362 nemátodos fueron contados y 20436 especímenes fueron identificados hasta nivel de especie cuando fue posible. Se registraron 32 especies de nematodos, pertenecientes a 12 familias (5 especies no pudieron ser identificadas). De estas, 9 especies estuvieron asociadas a condiciones salinas y 14 a condiciones estuarinas. Unicamente 3 especies se registraron en ambos ambientes (*T. longicaudata, S. papillata* y *Daptonema* sp). La densidad de nemátodos se registró entre 13 y 634 Ind.10cm^{-2} para todos los sistemas estudiados.

Terschellingia longicaudata (1A) y *Spilophorella papillata* (2A) fueron las especies mas abundantes con rangos entre 31 % y 81% del total de la comunidad de nemátodos registrada. La mayor abundancia de *T. longicaudata* correspondió a un ambiente salino, mientras que la mayor abundancia de *S. papillata* se registró

en ambiente estuarino. *Daptonema* spp (1B), *Gomphionema* spp (2A) y *Theristus* spp (1B) fueron tambien importantes en densidad dentro de los sistemas investigados. Sin embargo, la mayor abundancia de especies se la registró dentro del grupo de los consumidores depositivoros no selectivos (1B).

Las piscinas camaroneras se caracterizan por ser ambientes con una baja densidad y diversidad de nemátodos, comparado con otros ambientes en la regiòn costera de la provincia del Guayas y en las zonas externas de manglares, aledaños a las granjas camaroneras, donde se lllevaron a cabo estudios de la meiofauna bentònica.

Las prácticas de manejo que implican un mejoramiento de la calidad de agua, no tuvieron una influencia significativa en las poblaciones de nemátodos (p>0,05). No obstante, algunos efectos de los aditivos se observan sobre las especies. Esto permite considerar a estas especies como posibles bio-indicadores de las condiciones quìmicas de las piscinas de camarón.

Se observó que el proceso de secado de las piscinas que se realiza entre ciclos de producción tiene un efecto sobre la comunidad meiobentónica. Los copépodos son los colonizadores iniciales de las piscinas de camarón, seguido de los nemátodos, pero estos últimos dominan rápidamente los sedimentos. Un posible efecto de la presencia de camarón sobre la comunidad meiobentónica fue observado probablemente asociado a la perturbación del suelo que realiza el camarón durante sus actividades de muda y de alimentación.

CHAPTER 1

Introduction

1. Introduction

1.1 Shrimp farming: General overview

Shrimp farming traces its origins to Southeast Asia where for centuries farmers raised incidental crops of wild shrimp in tidal fishponds. Modern shrimp farming was born in the 1930s when Motosaku Fujinaga, a graduate of Tokyo University, succeeded in spawning the Kuruña shrimp (*Litopenaeus japonicus*). He cultured larvae in the laboratory and succeeded in mass-producing them through to market size in a commercial scale. In early 1960s, a small shrimp farming industry sprang up along Japan's Island Sea on the southern side of Kyushu Island, near the cities of Amakusa and Kagoshima. Production of 3000 metric tons (live weight) was obtained, from 150 semi-intensive and intensive farms (400 hectares of ponds in total).

In 1950, the Department of Interior's Bureau of Commercial Fisheries (later to be named U.S. Fish and Wildlife Service) established a laboratory in Galveston, Texas (U.S.A.) to investigate the red tides that were killing larvae populations of commercially valuable marine life. These investigations led to the development of techniques for the culture of marine phytoplankton. In 1958 when the lab started with the larval shrimp rearing, marine phytoplankton was used to feed the larvae stages of shrimp, and the famous Galveston Hatchery Technology was born. In the eastern hemisphere during the late 1960s and early 1970s, researchers in France, China and Taiwan witnessing the decline of commercial fisheries, began to investigate in of shrimp farming.

In the South Pacific, French researchers at the Centre Oceanologique Pacifique in Tahiti, working with several Penaeid species, including *L. japonicus, L. monodon* and eventually *L. stylirostris* and *L. vannamei* indigenous species to the western hemisphere), developed successful techniques for breeding and raising shrimp in intensive ponds. In China, unknown much of the world until the mid-1980s, researchers at the Yellow Seas Fishery Research Station discovered means to culture huge crops of *L. chinensis* in large, semi-intensive ponds in northern China. In Taiwan, researchers at the Tungkang Marine Laboratory, working primarily with *L. monodon*, in small intensive ponds, developed techniques for farming shrimp.

In the United States, the department of Commerce's (DOC) National Marine Fisheries Service assumed control of the Galveston Lab and DOC also funded the National Sea Grant College Program, which backed shrimp farming research of several coastal universities, including Texas A & M University, a leader in shrimp farming technology. Sea Grant was also an early backer of shrimp virus research at the University of Arizona.

Later, consultants, large corporations, feed companies and investors transformed the technology to Latin America, particularly Honduras, Panama and Ecuador, where they teamed up with local entrepreneurs to build farms, hatcheries, feed mills and processing plants.

Worldwide, researchers and farmers tested dozens of Penaeid species for their faming potential. In the process, they worked out breeding and spawning technology for most of the farming species. Other research concentrated on grow outs technology, nutrition and disease. This early effort laid the bqsis for an industry, which expanded over two decades.

In the mid-1970s, *fishermen* and *hatchery technicians* began to supply large quantities of juveniles shrimp to the farmers, producers in over a dozen countries discovered that stocking densities, feeding regimes and pumping were the keys to making profits. For example, large extensive farms in Ecuador recaptured the entire investment in the first year and sometimes with the first crop.

In the mid-1975, before the infusion of United State shrimp farming technology, Ecuador was well on its way to becoming a leading producer of farm-raised shrimp in the western hemisphere. The salt flats around the Gulf of Guayaquil provided an almost perfect habitat for shrimp farming and in Ecuador the shrimp industry grew steadily until 1999.

In the eastern hemisphere, Taiwan and China were the leaders. Meanwhile the tidal fish farms in Thailand, Indonesia and Philippines, were experimenting with shrimp monoculture and semi-intensive shrimp farming, practices which added to the increasing volume of farmed shrimp. From 1975 to 1985, production grew from 50,000 to nearly 200,000 metric tons in the world, which was about 10% of total world supplies of around 2 million metric tons. About 75% of it was produced in

Southeast Asia. In 1987, the United States National marine Fisheries Service released a new estimate of world production for 1986 of approximately 300,000 metric tons.

In general, the 1980s witnessed a remarkable growth in shrimp farming, particularly in tropical regions of the world (Landesman, 1994). The practice of culturing shrimp in ponds with artificial stocking of shrimp seed (post-larvae), feeding with specially formulated feeds and harvest for export to foreign markets is expanded both in Latin America and Asia. As of 1991, 750,000 tons of cultured shrimp were produced worldwide making up 30% of all shrimp consumed. It is projected that this will increase to 50% of world shrimp consumption by the year 2000 (Weidner 1992). A long tradition exists of coastal aquaculture in Southeast Asia, based primarily on the culture of milkfish (*Chanos chanos*) in hand excavated coastal ponds (Chua, 1987). Farmers in India and China have cultured shrimp in tidal impoundments on an extensive basis. This tradition of extensive mariculture depended on natural recruitment of shrimp and fish larvae, little or no fertilization or feeding, and low production costs. Yields were also low, typically 50 to 500 kg per hectare per year (Bailey, 1992, Chamberlain, 1991)

In 1988, the world's shrimp farmers produced about 450,000 metric tons of shrimp. China, Ecuador, Taiwan and Indonesia were the leaders. The Philippines, Indonesia and Thailand were also major contributors. The industry witnessed its first major crash in 1987-88. Hundreds of small intensive shrimp farms on Taiwan's west coasts suddenly experienced unexplained mortalities. In one year, the production dropped from roughly 100,000 metric tons to 20,000 metric tons. The industrial and domestic pollution combined with the rich effluent from too many intensive shrimp farms overwhelmed the carrying capacity. Farmers didn't know what to do with the sludge that built up on the bottoms of their ponds, so they piled it on the pond banks, creating an ideal home for pathogens and toxins. As the water quality deteriorated, the stressed out shrimp became susceptible to ever-present pathogens. After the collapse of Taiwanese intensive farming, the industry of Philippines, Indonesia and Thailand filled the gap, as well as production of Brazil, Dominican Republic and Texas in the United States. In 1992, Thailand became the world leading producer of farm-raised shrimp and remains in this position.

At the final of 1992, production of farm-raised shrimp reached 700,000 metric tons, which represented about 25% of world shrimp supplies. In mid-1990s, commercial fisheries produced around 2 million metric tons of shrimp per year.

In China, the production of farm-raised shrimp quickly grew from about 100 metric tons in 1988, to about 2,000 metric tons in 1992. Then in 1993 and 1994, it crashed due to viral disease. The Chinese practice of feeding living molluscs, insects, and agricultural and fishery wastes to the shrimp most likely probably encouraged the spread of viruses.

Worldwide, more than a million metric tons of farm-raised shrimp are produced each year; Rosenberry (2003) found in 2003 that shrimp farming is growing at 12% to 15% per year. Cascorbi (2004) have noted that in 2000, farmed shrimp production topped 700,000 metric tons; about one-quarter of world shrimp production is farm-raised.

Since 1995, viral and bacterial diseases slowed the growth of shrimp farming in both the eastern and western hemispheres. Costs went up as well as the industry adjusted to international pressures of product quality and environmental impacts. In 1999 the shrimp industry in the western hemisphere experienced one of its greatest viral epidemiological diseases, White Spot Syndrome Virus (WSSV).

1.2. Shrimp farming in Ecuador

The white shrimp, *Litopenaeus vannamei (Boone, 1931),* is one of the most important commercial products in Ecuador (figure 1.1; CNA, 2004; Exportaciones Ecuatorianas, 2005), Although with a dastric decline from 2000 awards.

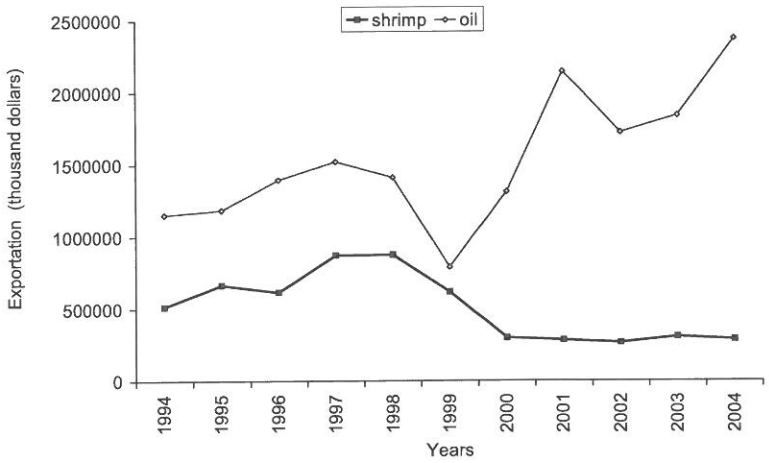


Figure 1.1 Major commercial products for export in Ecuador (shrimp and oil export value in thousand of dollars; source: Exportaciones Ecuatorianas, 2004).

The Ecuadorian shrimp farming industry started around 1970 (Rosenberry, 2001). In it's beginning, shrimp production was based on natural productivity of the environment the shrimp ponds had a water exchange cycle that was dependent on the tidal exchange of water (either with the sea or with the estuary), allowing shrimp larvae and food organisms, such as small crustaceans and phytoplankton, to enter the pond. Since the 1980's, the Ecuadorian shrimp industry experienced a significant growth, mainly due to the use of post-larval seed cultivated in hatcheries (hatchery seeds), improvement in commercial feeds and the high profit of shrimp sales (Leung, 2000). The intensification of the shrimp farming systems, with higher densities and production, increased the need for improving the of feeding systems, not only with supplemental commercial feeds, but also with the application of fertilizers to increase plankton blooms. During this 80's period Ecuador faced different diseases, such as the *Sea Gull Syndrome* in 1989, the *Taura Syndrome* in 1992 (Loth *et al.,* 1997) and others (CNA, 2000a). The shrimp production dropped 70% from its 1998 (Alava, 2004).

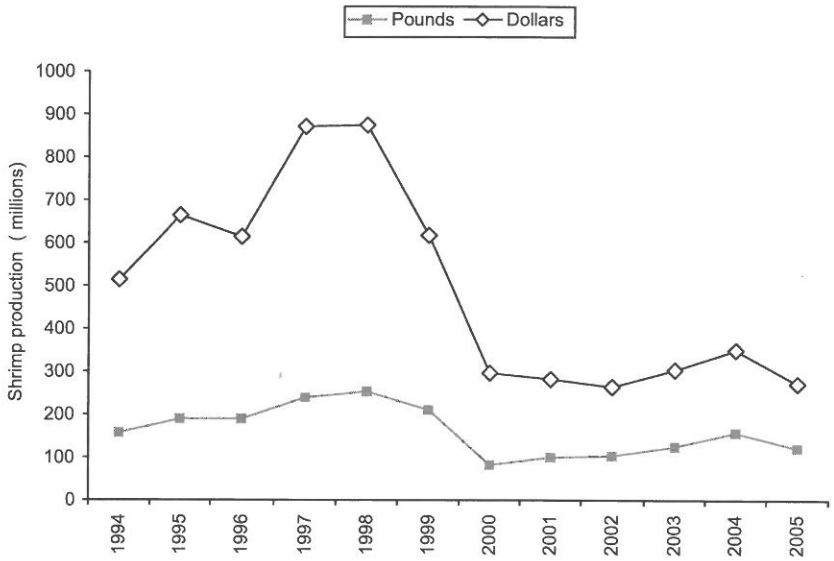


Figure 1.2 Shrimp production (pounds) and exports (dollars) in Ecuador, just until August 2005 (source: CNA, 2005).

Late 1999, the production declined as a result of the WSSV (Jiménez *et al.*, 2000; CNA, 2001b) (figure 1.2). Today, diseases are considered the most limiting factor in shrimp aquaculture as they affect shrimp production and its development (Subasinghe *et al.*, 1997; Browdy & Jory, 2002). Losses due to viral diseases are the most significant, in both Eastern and Western Hemispheres. Ecuador is still the leading producer in the western hemisphere in spite of the decline of shrimp production in Ecuador because of WSSV. Before the WSSV outbreak, Ecuador was the second largest producer of cultured shrimp, after Thailand (Bayot, 1999). The Ecuadorian industry generates employment for about 600,000 people, from workers in hatcheries to the people in the export.

The shrimp production has also had a strong dependence on climate variability both at the seasonal and inter annual scales. Inter-annual changes mostly occur in response to *El Niño* and *La Niña* events (Regueira-Linares 2001).

Table 1.1 World trends to aquaculture shrimp commercialization: 2001-2005 (source: Panorama Acuícola, 2004; CNA, 2005).

Major producers	2001			2005		
	Production surface (ha)	Production ($kg.ha^{-1}.Year^{-1}$)	Production (Tones. $Year^{-1}$)	Production surface (ha)	Production ($kg.ha^{-1}.Year^{-1}$)	Production (Tones. $Year^{-1}$)
Thailand	80000	3750	300000	100000	3500	350000
China	220000	1136	249920	320000	1094	350080
Indonesia	151000	1113	168063	396375	1113	441165
Vietnam	240000	500	120000	350000	571	199850
India	150000	667	100050	170000	1176	199920
Bangladesh	140000	450	63000	200000	450	90000
Ecuador	80000	563	45040	150000	600	90000
Brazil	85000	4706	400010	25000	6000	150000
Mexico	28000	929	26012	40000	1000	40000
Honduras	14000	1071	14994	16000	1000	16000
Others	170711	819	139812	278,185	900	250366
Total	1282211	968	1241180	2045560	1064	2176476

1.3 Biology and general ecology of shrimp

In its natural environment *Litopenaeus vannamei* (figure 1.3) prefers muddy bottoms at depths from the shallow shoreline to about 72 m (235 feet) (Dore & Frimodt, 1987). *Litopenaeus vannamei* has a translucent carapace, which permits the color of the ovaries to be seen. In females, the gonad, which is first whitish, turns golden brown or greenish brown on the day of spawning (Brown & Patlan, 1974). The spawning process occurs in the open sea and begins by sudden jumps and active swimming of the female and the whole process lasts about one minute. The males deposit the spermatophores only on hard-shelled females, which will spawn a few hours later. The courtship and mating behavior begins, at the end of daylight (GSMFC, 2005). Regression of developing ovaries is very rare and development of the ovaries leads almost every time to spawning.

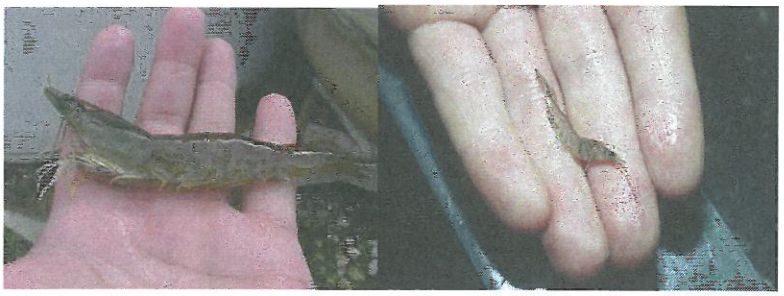


Figure 1.3 *Litopenaeus vannamei* juvenile (left), and post-larva, (right).

The cortical reaction is very rapid and first segmentation occurs in a few minutes (Ogle, 1992). The numbers of eggs vary according to female size. For *L. vannamei* of 30 to 45 g size; egg numbers are 100,000 to 250,000. The eggs are approximately 0.22 mm in diameter. Cleavage to the first nauplius stage occurs approximately 14 hours after spawning (Aquacop, 1979). Within the life cycle six nauplii stages, three (proto)zoeal stages, three mysis stages, several post-larvae, and juvenile stages and finally adults are recognized (figure 1.4) (Kitani, 1986).

The carapace length (CL) of *L. vannamei* post-larvae (PL= post-larvae) ranges from 0.88 to 3.00 mm (Kitani, 1993). The larval stages (1.95 - 2.73 mm CL) can be recognized by the lack of a thoracic spine on the 7th sternite, and the ratio of rostral length against the length of eye plus eye stalk ranges from 2/5 - 3/5, rarely 4/5 (Kitani, 1994). The most distinguishable morphological character is the development of supraorbital spines in the second and third (proto) zoea (Kitani, 1986). The coloration is "translucent white', therefore the species is known as the "white shrimp". The body of the species often has a bluish hue that is due to a predominance of blue chromatophores, which are concentrated near the margins of the telson and uropods (Eldred & Hutton, 1960).

The post-larva, reach estuarine areas and change there to a benthic mode of life. After 30 days the gills are fully development and they reach the juvenile stage. Fifteen days later the sexes can be identified. After 4 months the onset of sexual maturity marks the sub-adult stage. The animals migrate initially to inner littoral areas, later they migrate to outer littoral areas. The sexual maturity is obtained 10

months after reaching the adults' stage (figure 1.4). The adults can reach up to 230 mm in length (Dore & Frimodt, 1987). In the case of *L. monodon*, a related species (include *L. vannamei*) the longevity is estimated to be about one and half years for males and two years for females (Groth, 1997). The adults inhabit the benthic zones of the outer littoral areas (Kurata, 1978 *in* Groth, 1997; Motoh, 1981; Staples & Vance, 1985; Turner, 1989; Dall *et al.*, 1990).

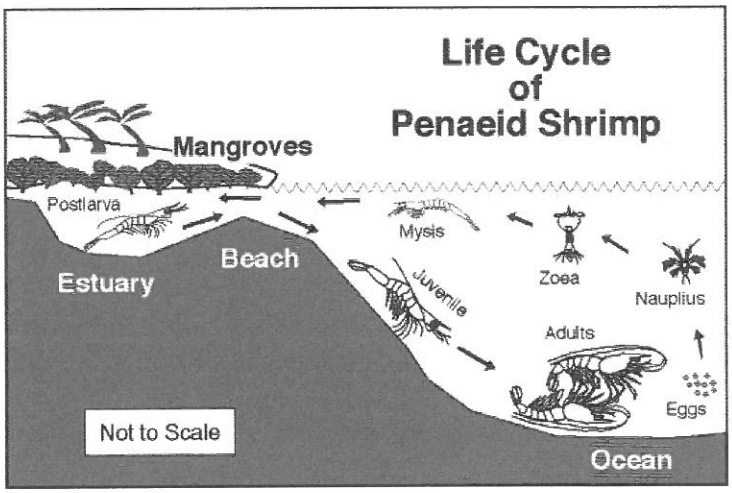


Figure 1.4 Natural cycles of shrimp (source: Shrimp News International, 2005).

Once in the estuarine environment, juvenile shrimp find ideal nursery conditions for growth and survival. Vegetation in estuaries, such as mangrove roots, offers young shrimp protection against predators (Minello & Zimmerman, 1984). These estuarine ecosystems are very productive environments (Gleason & Zimmerman, 1984; Leber, 1985). Estuarine muddy bottoms, without vegetation, are also valuable and, deters predation because the sediments are favorable for burrowing, while the turbid water obstructs the visibility for predators (Minello *et al.*, 1987). Muddy bottoms may also provide large numbers of infaunal organisms, mainly polychaete and nematode worms, on which shrimp feed (Flint, 1985).

While *L. vannamei* tends to be omnivores, *L. stylirostris*, another indigenous species, is notably more carnivorous. Mixed populations of phytoplankton and zooplankton

stimulate growth of newly stocked *L. vannamei*, whereas a predominance of zooplankton and benthic fauna is preferred for maximum growth of newly stocked of *L. stylirostris* (Shrimp News International, 1998).

Several factors regulate shrimp activities such as soil composition, oxygen level, light intensity, temperature, and salinity as well as feed availability. The Penaeid distribution into the sediment depend of the particle size distribution, porosity, water content, organic matter level and the presence of prey organisms. *L. japonicus* go into the sediment until 3 cm and *L. duorarum can get almost* 4 cm depth (Dall *et al.*, 1990). Personal observations revealed that *L. vannamei* can penetrate within the surface layers of the sediment as well.

Shrimp are benthic omnivores, and ingest small organisms and organic detritus (Rubright *et al.* 1981). During the larvae period, diatoms are an important part of the diet of the shrimps. After this period the shrimp remains on the bottom and consumes bacteria and benthic diatoms (Moriarty, 1997) as well, but some meiofauna organisms as well including nematodes (Dall *et al.,* 1990; Romano & Caraballo, 1996; Hoffman, 1980; Heip *et al.*, 1984).

These meiobenthic organisms are also prey to some commercial crustaceans such as *Cragnon cragnon* (Gerlach & Schrage, 1969), Penaeid prawns (Martínez-Córdova *et al.,* 2002; Moriarty *et al.*, 2005). Tidwell *et al.* (1997) pointed out that prawns preferred nematodes amongst other meiobenthic organisms. These prey organisms increase in abundance as result of the bio-perturbation by shrimps (Escaravage & Castel, 1990). Stoner & Zimmerman (1988 *in* Dall, *et al.*, 1990) mentioned that epiphytic algae are also a nutritional source for Penaeids. *L. semisulcatus* eat bivalves, crustaceans and foraminiferous while *L. setiferos* consume *Artemia.* Dall *et al.* (1990) added that shrimp prefer living prey as opposed to dead ones. Changes in prey diet have been attributed to seasonality of prey availability (Dall *et al.,* 1990).

According to Day *et al.* (1990) most of the Penaeids remain inside the sediment during the day (protection for predation) and they eat late in the afternoon . The Penaeid graze while looking for the food. If we assume a search area of 60 mm (double size of shrimp cephalotorax), these species will walk around 11 m^2 per night searching food. Some species such as *L. merguiensis,* can have stronger

movements when they walk and with the periopods they can burrow until 1 cm deeper into the sediment (Dall *et al.*, 1990). *L. setiferus* attack and feed on fish and others crustaceans, mainly during molting time. On feeding activities the food can be easily eaten in 20 seconds by *L. merguiensis* or 1 minute in the case of *Metapenaeus bennettae* when these shrimps have been in previous ignition period (Dall *et al.*, 1990).

1.4 Shrimp management practices

1.4.1 Shrimp development under aquaculture conditions

Shrimp farms either operate their own hatcheries or purchase seed stock from independent hatcheries. They utilize one or two-phase production cycles. With the two-phase cycle, they first stock the seed in nursery ponds and then, transfer it later to grow out ponds. With the one-phase cycle, the nursery ponds are eliminated, and the seed is stocked directly into grow out ponds, after having spent a short period in an acclimation tank. Farms usually produce two crops a year, although farms within 10 degrees of the equator sometimes get 2.5 crops a year.

The development of shrimp production starts often with post-larvae (see above), obtained directly from the sea by fisherman with a "Scissors Net" (figure 1.5). This practice stopped in 2000 because of a ban imposed by Ministry of Agriculture, in order to protect the natural shrimp seeds and also in order to manage the virus infection (WSSV) which was also present in the crustacean at the sea (Chapman *et al.*, 2004)

Gravid (ready to spawn) shrimp captured either in the wild or matured in the hatchery, spawn at night. Sometimes the adult males and females are sourced from shrimp ponds. Depending on a number of variables (temperature, species, size wild/captive and number of time previously spawned), they produce between 50,000 and 1,000,000 eggs. After one day, the eggs hatch into nauplii, the first larval stage. Nauplii, looking more like tiny aquatic spiders than shrimp, feed on their reserves for a couple of days. The nauplii metamorphose into (proto)zoea, the second larval stage, which have "feathery" appendages and elongated bodies but few adult shrimp

characteristics. Zoea feeds on algae for three to five days and then, metamorphose into mysis, the third and final larval stage. Mysis have many of the characteristics of adult shrimp, like segmented bodies, eyestalks and shrimp-like tails. They are kept in special tanks where they are fed first with phytoplankton (*Skeletonema, Chaetoceros, Tetraselmis, Chorella, Isochrysis*) and later with zooplankton (copepods) and *Artemia.* Some artificial food is periodically added. This stage takes another three or four days, and then the mysis metamorphose into post-larvae (PL).

Figure 1.5 A fisherman holds a "scissors net", which is used to catch shrimp larva at the beach and the sea behind the surf zone.

Post-larvae, looking like adult shrimp feed on zooplankton, detritus and commercial feeds. From hatching, it takes about 20 days to produce PL10. There, the animals are acclimatized to the salinity and temperature condition of the shrimp pond (figure 1.6). The aclimatation period lasts from a half-day to four days, and the animals may be fed special diets to prepare them for pond life. The most important consideration during acclimation is that the water quality parameters adjusted slowly. Two weeks before stocking, the pond is filled with water coming from the sea or from the estuary; fertilizers are added to increase the primary productivity. Later the pond is stocked with shrimp at different densities and artificial feeds (with different composition) are added immediately (Villalòn, 1991).

The nursery phase of the shrimp farming is when the post-larvae are cultured at high densities in small earthen ponds (and occasionally in intensive raceways or tanks, or in net cages within a shrimp pond), and occurs prior to the grow out phase.

Proponents of nursery ponds argue that they improve inventory, predator and competitors controls; increase size uniformity at final harvest; better utilization of farm infrastructure; permits more crops per year; improve risk management; produce stronger post-larvae; and decrease feed waste.

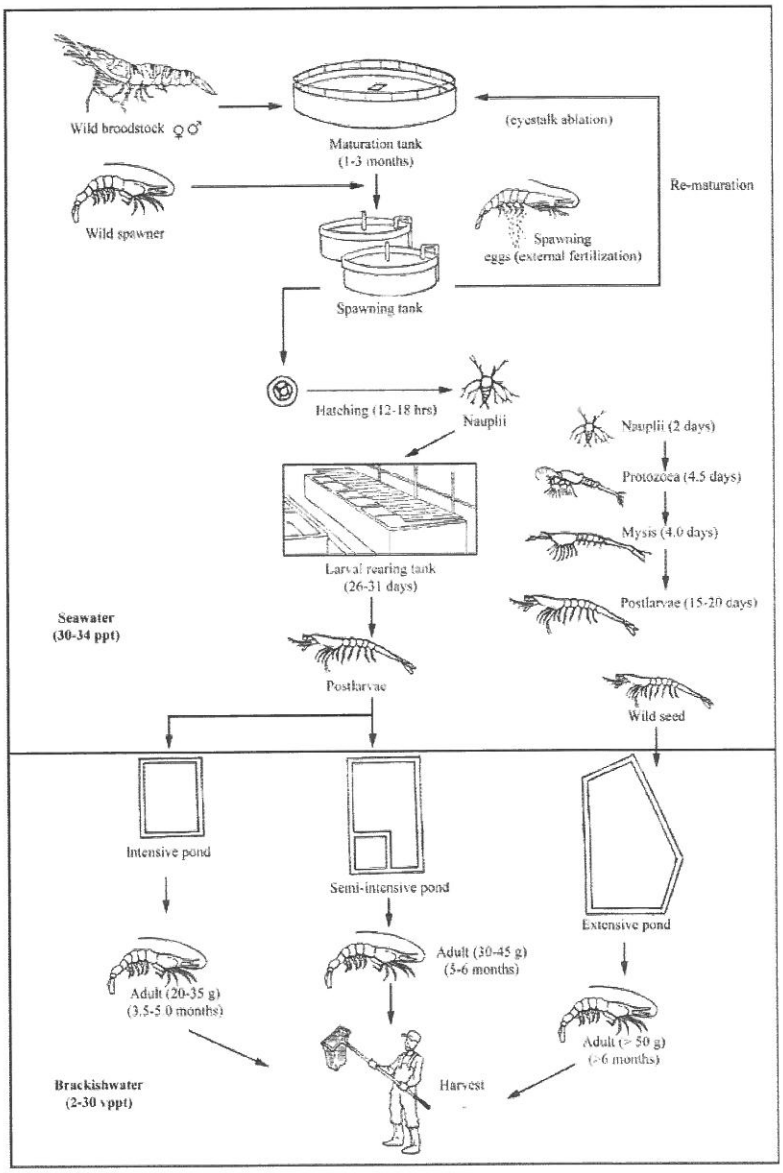


Figure 1.6 General description of shrimp aquaculture (source: FAO, 2005).

During three to four months of grow out, there is usually limited water exchange with the environment; water is only added to compensate losses due to percolation and evaporation. This results in *a stable* pond water column during the culturing period (Calderón *et al.,* 1999). Fertilizers are added (Boyd, 1990; Villalón, 1991; Calderón *et al.,* 1991; Marcillo, 2001) and also commercial feed at different levels according to shrimp growth (general practice is 10% of the body biomass per day, which decline to below 2% in the final month) (Villalón, 1991). The shrimps are grown until they reach a commercial weight of 12-15 g. Finally, they are harvested by drained the pond of all the water and then the animals are transported to a shrimp processing facility, mainly for export to the United States and to Europe (CNA, 2004). After harvest, the shrimp pond is dried out until filled once again to start a new cycle.

1.4.2 Shrimp pond structure

A shrimp pond is a shallow basin filled with water from the ecosystem. These ponds are built on soil surfaces where the fertilized surface layer disappeared and the vertical profile of the soil was destroyed (figure 1.7). Boyd (1995) mentioned that aquaculture ponds usually are constructed of mineral soils (low level of organic matter), but there are places where ponds are built on organic soils (high levels or organic matter). He added that the major factors affecting the development of soils are the composition of organic original rocks, climate, topography, biological activity and time.

Ponds are usually built in places where soils have a discernible profile. However, the upper horizons often are removed or covered with material during construction. A new profile tends to occur in the ponds (Boyd, 1995); organic and mineral sediments accumulate over the harder original pond bottom. Boyd (1995) also commented that as in native soils, the concentration of organic matter in pond bottoms decreases with the soil depth. Shrimp ponds in general have an average depth of 1.5 m.

Ponds have different shapes but most of them are rectangular, with a size between 1 and 15 hectares. The walls are made with the sediment (mainly clay and silt), taken out of the basin. This material is compacted to avoid wind and water erosion.

Most of the shrimp ponds in Ecuador have been constructed over saline areas, few in mangrove areas (around 25% according to CLIRSEN, 2005).

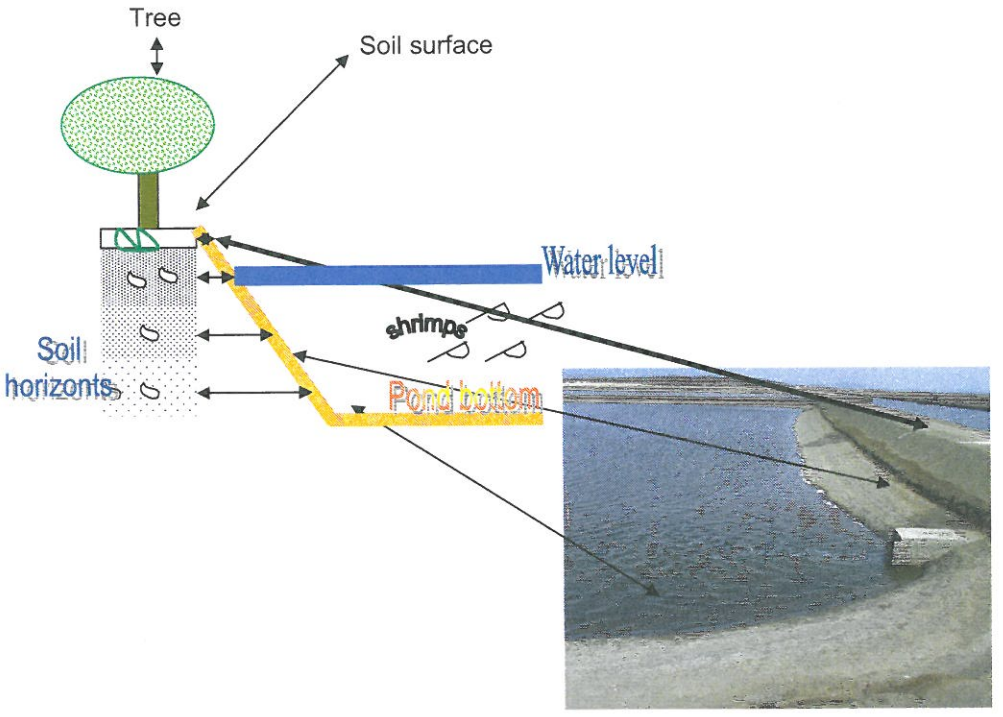


Figure 1.7 Natural vertical profiles of soil in relation with shrimp pond vertical profile (walls).

The mangrove soils have large quantities of pyrite (FeS_2), which has an effect on bacteria, plankton composition and biomass, meiobenthos composition and biomass, fish and shrimps and on macrophytes (submerged, rooted vegetation and grasses planted on pond dykes) (Simpson & Pedini, 1985).

1.4.3 Stocking density of shrimp

Farmers stock post-larvae in nursery ponds (0.5 to 10 ha) at densities of 30 to 500 per square meter. Most shrimp farmers think nursery phase should not exceed 25 days.

Different levels of stocking densities commonly determine several cultured systems: extensive systems (low density), 2-8 shrimp.m^{-2}, semi-intensive systems 10-20 shrimp.m^{-2} and intensive systems 30-60 shrimp.m^{-2} (Aiken, 1990; Rosenberry, 1999; 2001 and figure 1.6) Extensive system in the tropics are conducted in low-lying impoundments along bays and tidal rives. Construction and operating costs are low but so are the yields. The application of feed and fertilizers is not used or just in low quantities. Normal tidal flux is relied upon to bring larvae and feed into the ponds. Shrimp feed on naturally occurring organisms, which may be encouraged with organic or chemical fertilizers. This process results in low and variable stocking densities and variable growth. Whetstone *et al.* (2002) mentioned that when the stocking density is about 1 to 10 shrimp.m^{-2} obtaining yields of 100 – 1,000 kg,ha^{-1}.$crop^{-1}$. Extensive farms have little effect on the environment.

Under semi-intensive conditions, different artificial feeds and fertilizers (nitrogen, phosphorous and silicate) are used and exchange of water is considered important. Shrimp are cultured in ponds from 2 to 20 hectares. The shrimp farm is constructed above the high tide level. Larvae are obtained from hatcheries and held in nursery ponds for several weeks. The shrimp farm is equipped with pumps, which exchange water in a rate of 1% to 25% per day. The stocking rates range from 100,000 to 300,000 post-larvae per hectare. Shrimp farmers argument natural productivity with shrimp feeds. Under these conditions shrimps are 1,000 – 3,000 kg,ha^{-1}.$crop^{-1}$ (Whetstone *et al.*, 2002). In this system the farmers harvest by draining the pond through a net or harvest monk, or by using a harvest pump.

The intensive system is the most sophisticated and most expensive practice used in Ecuador. The ponds are small, usually between 1-10 hectares, and are stocked with 80,000 to 1,000,000 post-larvae.$hesctares^{-1}$. Aeration is necessary to keep oxygen level above 3 mg.l^{-1} (Sonnenholzner, 2000). Intensive farming is also practiced in raceways and tanks, which may be covered or indoor. Sophisticated technology

such as aeration systems, automatic feeders, elaborated feeds and selection of "a high quality larvae", is implemented and production costs are correspondingly high (Aiken, 1990; Shrimp News International, 1998). The yield in this system is over 3,000 to 6,000 kg.hectare^{-1}.crop^{-1} (Whetstone *et al.*, 2002).

1.4.4 Added nutrients in shrimp ponds

During the shrimp production cycle (3-4 months), fertilizers may be applied every fifteen days while commercial feeds are provided daily. As farms evolved from low to high stocking densities, the quality of feed becomes very important. Most extensive farms (low stocking densities) don't feed at all; shrimp feed on naturally occurring food in the pond. Other extensive farms use small amounts of feed and fertilizer to stimulate the natural food chain. On semi-intensive farms, with many more shrimp scouring the bottom of the ponds, the shrimp consumes most of the feed and, less is available to serve as stimulant to the natural food web. Therefore, the quality of the feed is more important because the shrimp get most of their nutrition from it. On intensive farms, shrimp depend on commercial diets for most of their nutrition, so intensive farms require the very best feeds.

The productivity in shrimp aquaculture (hence also the profit) depends on feed quality (CENAIM, 2000b,c; Molina, 2003). The feed is applied by spreading, from the dikes of shrimp pond or by boat or by adding to feeding trays (rounded or rectangular dishes made of plastic, with almost 1/2 square meter of surface, mesh-bottomed baskets) into the pond to monitor consumption (Shrimp news International, 1998; Molina & Piña, 1999; figure 1.8).

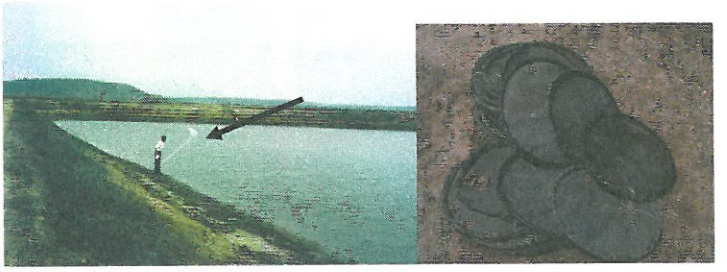


Figure 1.8 Feeding systems in shrimp ponds: spreading of artificial food (left) and feeding tray (right)

The difference between spread feeding and tray-feeding systems has been also evaluated (Molina & Piña, 1999; Martínez-Córdoba *et al.*, 2002a). The feeding trays are supported on wooden stakes and are installed at 30 tray.hectare^{-1}. The labour cost is high with this technique. At least, two employees are required for every 10 hectares of ponds. However, because feed conversion ratios are so much lower when feeding trays are used, the cost of labour and equipment costs are easily covered by reduced feed costs. In addition, feeding trays produce less pollution and offer a cleaner pond bottoms. With the feeding trays an invaluable source of data on what is going on in the pond and early detection of disease are considered. Also more control of cost feed and reduced pumping and aeration costs. Less pond maintenance between harvest and betters harvest estimation are also denoted with the use of feeding trays.

The behavior of shrimp is different from one species to the other; juveniles of *L. stylirostris* exhibit a much aggressive feeding behavior compound than *L. vannamei.* They will migrate considerable distances within a pond in search of food and also possess a voracious appetite. Cliford (1998) noted that in an experimental feeding trial in which the total feed ration was offered on feeding trays (six 0.42 m trays.hectare^{-1}) the shrimp consumed as much as 7 kg of pellets from each tray in less than 12 hours. This voracious feeding behavior leads itself to the practice of applying 100% of the daily ration on feeding trays and requires fewer trays per hectare to dispense the feed to the shrimp. Administering 100% of the feed on trays not only improves feed conversion and mitigates pond bottom deterioration; it also provides a mechanism for instantly adjusting feeding rates in response to sudden changes in the physiological state of shrimp as increasing molting activity or changes in population density.

Feed is added most of times in two doses, generally 30% of the daily dose is applied in the morning and the other 70% later in the afternoon. The exact doses of feed vary from farm to farm; depending on the shrimp farmer (Villalón, 1991). Ideally, shrimp in semi-intensive and intensive farms should be fed four to five times daily, with at least three hours between feedings.

The commercial feed composition varies (mainly protein levels), depending on the shrimp species as well as on the seasonality (22 and 40 % in average, in the cold and warm season, respectively). High-quality feeds offer several advantages over lower quality feeds: better feed conversion, faster growth, lower mortalities and improved water quality.

The nutrients required by cultured species can be broadly classified as proteins, carbohydrates, lipids, vitamins and minerals. The optimum levels of these nutrients vary from one species to the next (van Wyk, 2005).

Protein makes up 65 to 70% of the dry weight of shrimp, and is a major component of the muscles. The protein diet is the source of 20 amino acids, but only 10 of these are considered to be essential in the diet. The minimal requirements for each of the ten essential amino acids are indicated in the table 1.2.

Most commercial shrimp feeds formulated for intensive culture systems contain between 35 and 50% protein. If the level of protein in the feed is too low, growth rates will be reduced (van Wyk, 2005). Excess protein in the diet may also inhibit growth (Lim & Persyn, 1989). The shrimp as a source of energy will metabolize this excess, and nitrogen will be excreted as ammonia. Protein requirements are fairly high for post larvae and small juveniles, but decline as the shrimp grow. In table 1.2, the recommended protein levels for different sizes of shrimp in high-intensity culture systems are indicated.

Table 1.2 Recommended amino acid levels in commercial shrimp feeds, in an as fed basis (source: Akiyama & Tan, 1991).

Amino acid	Protein (%)	Feed			
		36% protein	38% protein	40% protein	45% protein
Arginine	5.8	2.09	2.20	2.32	2.61
Histidine	2.1	0.76	0.80	0.84	0.95
Isoleucine	3.5	1.26	1.33	1.40	1.58
Leucine	5.4	1.94	2.05	2.16	2.43
Lysine	5.3	1.01	2.01	2.12	2.39
Methionine	2.4	0.86	0.91	0.96	1.08
Phenylalanine	4.0	1.44	1.52	1.60	1.80
Threonine	3.6	1.30	1.37	1.44	1.62
Trytophan	0.8	0.29	0.30	0.32	0.36
Valine	4.0	1.44	1.52	1.60	1.80

Most producers initiate the production cycle with 40% protein "crumble" starter diet and shift to a pelleted 35% protein formulation when juveniles reach 3-5 g (to the case of the blue shrimp *L. stylirostris).* These levels are higher than in the case of *L. vannamei* due to the faster growth rates and the fact that *L. stylirostris* can reach larger, more lucrative commercial sizes, 31-35 cm of this shrimp against 26-30 cm for *L. vannamei.*

Under ideal culture conditions and with a properly managed feeding program, accumulative growth rates of shrimp range from 1.0 – 1.3 g.week-1 in *L. stylirostris* (Clifford, 1998), while in *L. vannamei* the rates ranges from 0.88 – 1.02g.week-1. (Martínez-Córdova *et al.,* 2000). The rate growth is affected by temperature (López-Martínez *et al.,* 2003), because it controls the rates of metabolism (Ocampo *et al.,* 2000; Mugniez & Soyez, 2005). And, also by changes in oxygen level (Mugniez & Soyez, 2005), population density, water quality, feed quality and other factors. Feed formulators mix and match different sources of protein, each with different amino acids profiles are also relevant. Fishmeal is generally considered to be the highest quality protein source because the amino acid composition of fishmeal closely matches that of shrimp muscle. The lipid or fats in the diet correspond the free fatty acids, phospholipids, triglycerides, oils, waxes and sterols (Kanazawa & Teschima, 1981), which are also an important energy source for shrimp. The most important fatty acids are linolenic acid (18:3n3), Linoleic acid (18:2n6), eicosapentaenoic acid (20:5n3) and decosahexaenoic acid (22:6n3) (Kanazawa & Teschima, 1981).

The phospholipids (glycerol, fatty acid and phosphoric acid composition) are important components of the cell membrane and play an important role in lipid metabolism. Crustaceans also require sterols for maturation and molting. Carbohydrates (starches, sugars and fiber) serve as an in-expensive energy source in shrimp diets. Vitamins (soluble and insoluble) are also important for normal growth and development of the shrimp. The requirement of vitamins depends on shrimp size, age, growth rates and environmental factors (Akiyama *et al.,* 1991). Young juvenile shrimp may require 50% higher vitamin levels in their diets compared with adult shrimp. The minerals, inorganic elements required for various metabolic processes include calcium, phosphorous, magnesium, sodium, potassium, chloride and sulfur (major minerals). Calcium and phosphorous are required for the

exoskeleton of shrimp and the last is also important for the ATP activities. Other minor minerals are also considered important as iron, iodine, manganese, copper, zinc cobalt, selenium, molybdenum, fluorine, aluminum, nickel, vanadium, silicon, tin and chromium.

The shrimp's habit of slowly nibbling feed particles cause substantial nutrient losses even if the pellets are of good quality. Increasing the water stability of the feed beyond a couple of hours does not help. Because leaching of the nutrients will continue, even from pellets showing excellent physical stability. Within an hour, shrimp feed can lose more than 20% of its crude protein, about 50% of its carbohydrates and 85% to 95% of its vitamin content. As much as 77% of the nitrogen and 86% of the phosphorous compounds in shrimp feed is wasted. The waste either accumulates on the pond bottom, or is discharged into the environment. Instead of increasing pellets stability beyond a couple of hours, feed should include attractants, so they are consumed within 20 or 30 minutes, after being administrated.

Natural feed is also important to aquacultured animals (Martínez-Córdova *et al.,* 2002). Clifford (1998) mentioned that in shrimp ponds zooplankton densities (excluding protozoans) favor fast initial growth of the juvenile blue shrimp, *L. stylirostris*. The benthic organisms are important in recycling the nutrients within the pond (Rhoads, 1974; Wolfe *et al.,* 1982; Hartley 1982, 1984; Bilyard, 1987).

Nunes *et al.* (1997) indicated that the availability of prey organism is related to the stocking density of the consumer organism and is also related to the population dynamics inherent to each individual species. On the other hand, Gamboa *et al.* (2003) commented that in semi-intensive systems artificial feed makes a low contribution to the shrimp diets. In their studies the feed in the stomach contents reached a maximum of 20% in 6g-shrimp. This may be related to the observation of Molina & Piña (1999) who noted that the consumption of feed increases each week until it stabilizer between 8 and 11g. After attaining this weight, a decrease in consumption was registered, but shrimp growth rate kept increasing. Focken *et al.* (1998) and Molina & Piña (*op. cit.*) also commented a change in the feeding preferences of *L. vannamei*. The growth rate is mainly supported by nutrients found in different components of the natural productivity.

Farmers in the western hemisphere depend almost entirely on dry, commercial feeds, while 50% of those in the eastern hemisphere utilize farm-made feeds and natural foods, such as trash fish, seafood processing by-products and various molluscs and crustaceans. This practice can enhance the spread of disease and adds to the organic load of the pond. Feeds can represent over 50% of the production costs on intensive shrimp farms.

Shrimp feeds that are uneaten contribute to the sludge in the pond (Shrimp News International, 1998; Van Wyk, 2005). To obtain better profits in production, the natural feeding behavior of shrimp, i.e. timing, type, frequency and quantity of feed should be investigated. In the absence of the abundance, naturally occurring populations of zooplankton, controlled applications of organic and inorganic fertilizer will generally stimulate natural productivity. Clifford (1998) found that 500-1000 kg.hectare^{-1} of pesticide-free chicken manure applied to the pond bottom before filling, produced good results to enhance the natural productivity. He added that if the process is followed by 3 - 4 applications of a fertilizer at 48-hour intervals of 10-15 kg.hectare^{-1} of nitrogen-fertilizer during the initial pond filling process, it should be better to enhance the primary production

Intensive culture systems do not require much fertilization since the heavy feeding provides sufficient nutrients to maintain an algae bloom in the pond. Both extensive and intensive ponds are treated with calcium carbonate (in the form of agricultural limestone) to help neutralize the pond bottom, which tends to become acidic because of anaerobic conditions (Boyd 1989). Among fertilizers there are organic and inorganic ones. The major fertilizers used are inorganic (summarized in the table 1.3) composed mainly of phosphorous and nitrogen.

Table 1.3 Composition of inorganic fertilizers used in shrimp pond farming Relative abundance (%).

Substance	N	P_2O_5	K_2O
Ammonium Nitrate ($N0_3NH_4$)	33-35		-
Ammonium sulphate (SO_4NH_4)	20-21	-	-
Calcium Nitrate (NO_3Ca)	15.5	-	-
Potassium nitrate (NO_3K)	13		44
Sodium nitrate (NO_3Na)	16		
Ammonium phosphate (PO_4NH_4)	11-16	20-48	-
Calcium Metaphosphate PO_4Ca	-	65-64	-
Superphosphate	-	18-20	-
Triple or double super phosphate Ca $(H_2PO_4)_2$	-	32-54	-
Potassium sulphate (SO_4K)	-	-	50
Potase Muriate	-	-	50-62

Source: Boyd, 1995

In the shrimp ponds plants take up nitrogen primarily as nitrate (NO_3^-) (Stickney, 1994). Animals satisfy their nitrogen requirements through the intake of food and nitrogen is eliminated in the form of ammonia, creatine, creatinine, free-aminoacids, urea and uric acids (Stickney, 1994). Nitrogenous compounds are also released during bacteriological decomposition of plants and from animal matter, while phosphorous is often present in only minute concentrations in natural waters (from about 0.01 to 200 $mg.l^{-1}$) (Stickney,1994). Ponds are excellent reservoirs for nutrient accumulation, which are elevated when is necessary through fertilization.

The shrimp pond environment can be described as follows: nutrients (nitrogen and phosphorous mainly) entering the pond as fertilizers and artificial feed. These nutrients are incorporated in the water column as well as in the soil, by primary producers (phytoplankton and phytobenthos, bacteria and aquatic plants). These primary producers are consumed by zooplankton, zoo-benthos and shrimp, which in turn introduce other nutrients to the environment through the various processes such as uneaten algae, faecal pellets, ammonia excretion (Gómez-Jiménez *et al.,* 2001) (see figure 1.9, as visual description of shrimp pond).

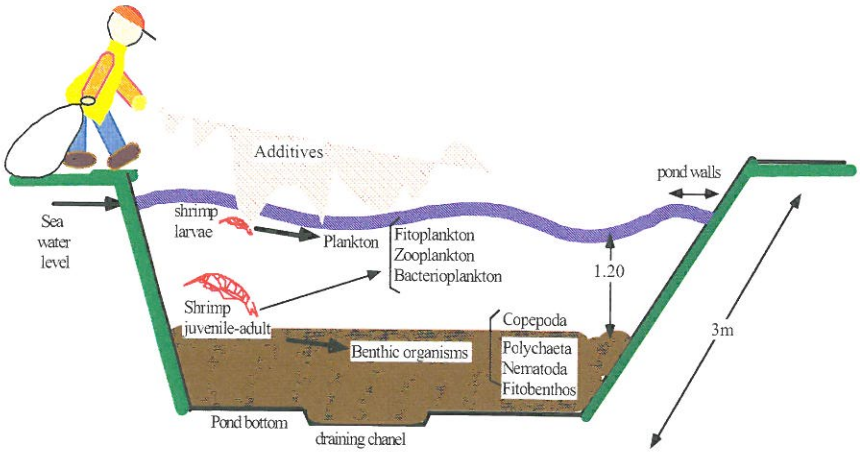


Figure 1.9 General description of the shrimp pond.

1.4.5 Draining of the shrimp ponds

After the shrimp is harvested, the pond is drained, some times completely, but most of the time some parts of the pond remain moist (figure 1.10). In this part the bottom fauna (polychaetes, nematodes, small crustaceans, etc.) are kept alive as we observed in preliminary samples collected inside a pond.

Figure 1.10 Drained shrimp pond.

The common procedure of draining the pond after harvest is a practical way to decrease the risk of virus development on the surface of the sediment. The bottom is kept dry for 1 to 3 weeks to enhance oxidation of organic matter and other reduced substances. In other cases the soil is ploughed up to 5 to 10 cm depth to increase the oxidation (Boyd, 2003; Gopakumar, 2003).

Several methods for the so-called "disinfections procedure" have been used too. Lime is added to the pond soil to produce an alkaline environment that helps to inactivate viruses and kill any organism in the first centimeters of the soil. The WSSV is also inactivated by exposure to ozone in residual oxidant concentrations at a range of 0.5 $\mu g.ml^{-1}$ to 0.8 $\mu g.ml^{-1}$ for 10 minutes (Chou *et al.*, 1998). The use of quick lime (CaO) to disinfect ponds has been recommended by Juang (1996) as a common practice of disinfections in aquaculture ponds. Francis-Floyd (2003) mentioned that ponds could be treated with hydrated lime (also called calcium hydroxide, $Ca(OH)_2$), applied to damp mud at 1000-2000 $kg.ha^{-1}$ as a sterilizer product. Hydrated lime will rapidly cause the pH in treated areas to rise above the level, which will be lethal to parasites and bacteria and as in the elimination of ammonia tied up in the mud. The last author added that ponds treated with quicklime at the recommended rates, could be refilled after 10-14 days for another production cycle.

Intensively managed ponds depend on diesel or electrically driven pumps to exchange water during the production cycle. Aeration by means of paddlewheel aerators is essential to maintaining sufficient dissolved oxygen in the ponds. In very intensive systems, pond water is replaced by using fresh brackish or salt water. After draining the ponds for harvest they are allowed to dry completely and sometimes the bottom sediment is pumped out or shrimps are removed manually (Chamberlain, 1991).

1.4.6 Abiotic conditions inside the ponds

Oxygen, temperature, salinity, pH, organic matter and nutrient levels are important inside the shrimp ponds. Changes in the levels affect shrimp growth and shrimp

health (Olsen, 1989; Villalon, 1991; Ponce-Pelafox *et al.*, 1997; Mugnier & Soyez, 2005).

The oxygen level is an environmental factor that depends on management practices, but could also vary due to "natural factors" (phytoplankton production, bacterial activities, etc). Inside the ponds the oxygen level can vary from less than 2 $mg.l^{-1}$ up to 12 $mg.l^{-1}$ (CENAIM, 2004). According to Spanopoulos-Hernández *et al.* (2005) oxygen availability for organism under culture is a critical feature. Oxygen is essential in different oxidative reactions releasing the energy necessary for biological work. Mikulski (2000) mentioned that estuarine organisms routinely encounter fluctuations in dissolved oxygen, carbon dioxide and pH, which can vary both seasonally and diurnally. On the other hand low oxygen (hypoxia) and elevated CO_2 (hypercapnia) are characteristic for soft estuarine environments and decrease the resistance of shrimp to bacterial pathogens (Burgens *et al.*, 2005).

Groth (1997) indicate a range of <0.5 to 1 $mgO_2.l^{-1}$ as critical for *L. monodon,* with and optimum at 4 mg $O_2.l^{-1}$; a minimum of 2.4 mg $O_2.l^{-1}$ and a maximum of 12.4 mg $O_2.l^{-1}$. Repeated exposure to lower than optimal levels could produce stress, reducing growth and feed conversion efficiency (Groth, *op. cit.*). The same author mentioned that during the rearing period, animal wastes, plant detritus and also un eaten feed accumulate on the pond bottom and can produce a depletion in oxygen levels, because of increase in bacterial activity. The pond sediments contribute 75 to 84% of the total oxygen demand in shrimp ponds (Fast *et al.*, 1988), therefore sediments can become a *silent killer* for shrimps as it may cause high mortality, due to anaerobic metabolites such as ammonia and hydrogen sulphide (Avnimelech & Ritvo, 2003; Fish, Depart. Sabah, 2004; Aquafarmer, 2005).

Changes in temperature and salinity affect the oxygen levels and the feed ingestion by organisms (Martínez-Palacios & Ross, 1994; Rosas *et al.*, 1996). Most aquaculture facilities are operated without salinity adjustment except in the case where environment salinity of incoming water is excessive or when the evaporation will lead to and increase in salinity. Adding seawater to recuperate the losses will reduce the salinity (Stickney, 1994). *L. vannamei* is found in waters with a wide salinity range from 1 to 40 PSU (Davis *et al.*, 2004).

Inside the ponds, the presence of mangrove vegetation often resulted in acid-sulphate problems during the first few years of operation of the shrimp ponds (FAO, 2004). This reduces feeding, producing slower growth, higher mortality and possibly higher sensitivity of shrimp to diseases (Avnimelech & Ritvo, 2003).

Temperature is an environmental variable that can affect *Litopenaeus* spp growth (Mugnier & Soyez, 2005). For small shrimp (< 5g) the optimum temperature is above 30°C, while for large shrimp the optimum temperature is about 27°C in the case of the Pacific White Shrimp (*Litopenaeus vannamei*) (Wyban *et al.*, 1995). Reduced growth and feeding were observed when the pond temperature is 30°C or higher (Wyban *et al., 1995.*). Ponce-Palafox *et al.* (1997) found the best survival between 20° and 30°C and best growth between 25°C and 35°C at salinity above 20 PSU.

One important aspect to be considered in the shrimp life cycle is molting. During this period the shrimps do not eat and are susceptible to changes in the environmental conditions (Echeverría *et al.*, 2001). Laboratory observations indicate that the pathogenetic effect of some bacteria increases at 35°C with a negative effect on shrimp survival. This is due to the fact that an increase in bacteria results in more energy used for the immune system. Under these conditions the immunological system could have a "focus deviation" making the shrimp more susceptible to virus infection (Cedeño *pers. comm.*). Several laboratory experiments have shown that survival increases in WSSV infected shrimp when water temperature is kept at 33°C (CENAIM, 2002); so better production should be obtained when the temperature is kept at this level.

The pH will vary depending on the aquatic life within the pond. Carbon dioxide produced by aquatic organisms has an acidic reaction in the water. pH in ponds will rise during the day as phytoplankton and other aquatic plants remove CO_2 from the water during photosynthesis. The pH will decrease at night because of respiration and production of CO_2 by all organisms. The fluctuation of water pH will depend the density plants in the shrimp pond (Aquaculture S.A, 2005). Sonnenholzner (2000) analysed the soil content of 74 ponds in Ecuador and found an average pH of 6.8 ± 0.5 and total carbon of 2.38% in the upper 5 cm of the sediment.

The soil is an important component inside the pond. Bayot (1999) observed that apart from geographical location conditions, such as texture, chemical composition, age of the pond, the fertilization and feeding practices are important to the shrimp production.

In the case of the fish farms, deposition results in the organic matter accumulation on bottom sediment, causing strong modifications of the physical characteristics of the benthic environment (Holmer, 1991; Wu, 1995 *in* Mirto *et al.*, 2002; Hargrave, 2004). Solid inorganic compounds in the water, will hamper light penetration into the pond and may reduce primary production per unit area, and affect the organisms (Zeitzschel, 1978), because of the "aquaculture waste" (overload of feed resulting in an overload of nutrients) (Guerrero, 2000), which in turn could be beneficial to benthic organisms (Janssens, 1999).

There are few ecological studies on shrimp pond dynamics (Romano & Caraballo, 1996; Moriarty, 1997; Mazola *et al.,* 1999; Orellana *et al.*, 2001; Mirto *et al.,* 2002). Most of them have been performed under experimental conditions and are related to the effects of temperature, salinity and oxygen levels on shrimp production (Boyd, 1995). In order to understand the feeding ecology of shrimp, studies on the relationships of the food uptake and, circadian rhythms have been performed (Molina *et al.,* 2000; Molina, 2003; CENAIM, 2000c).

1.4.7 Biotic conditions inside the ponds

It is important to understand the trophic relationships inside the pond to obtain good shrimp production. Before 1999, the Ecuadorian shrimp farmers considered that the natural productivity as a good feeding source for shrimp was important. The general practice was to fertilize the pond and to obtain a high density of planktonic organisms. Every production cycle the inlet water would "introduces" phytoplankton and zooplankton together with different stages of benthic organisms and some fish larvae.

Before WSSV appeared in 1999, the common practice was to exchange water during each shrimp production cycle (10% daily; Cornejo-Rodríguez, 1999). The

shrimp farmers stopped this practice, placing nets of 300µm at the pond inlet to avoid zooplankton, some crustacean's virus vectors, from entering the pond (CENAIM, 2000a). This process contributes to the reduction of the development of planktonic and benthic communities inside the pond. Shrimp farmers control dragonfly (Odonata, Anisoptera) nymphs too, as they are presumed to be competitors of shrimp (Whitis, 2001) and according to Marcillo (2001) predators for shrimp larvae, but also vectors of WSSV (Bayot, 1999).

Phytoplankton biomass has been used as an indicator of water quality (Ferguson, 2005). However, the presence of phytoplankton colonies may result in overestimation of size class contribution to the total biomass. These organisms are affected by changes in nitrogen-to-phosphorus ratios and ammonia concentrations (Budford, 1997).

The zooplankton inside the aquaculture ponds includes mainly rotifers, cladocerans, and copepods, mainly (Morris, 2005). These organisms have also proven useful as water quality bio-indicators. Their species composition and abundance are influenced by water quality changes. The zooplankton community itself responds directly or indirectly to changes in the physical and chemical variables and to the availability of phytoplankton as food (Raymont, 1980).

In the shrimp ponds, planktonic diatoms are part of the shrimp diet, but benhic fauna is also important. Juvenile shrimp stay on the bottom and consume bacteria and benthic diatoms (Villalón, 1991; Moriarty, 1997) as well as some meiobenthic organisms (Villalón, 1991; Romano & Caraballo, 1996). *L. duorarum* eat polychaetes, ostracods, copepods and malacostraca during juvenile stages and squid, octopus and different kinds of annelids when adults (Fwie, 1996). These benthic organisms occupied the first 20 cm of soil (sometimes deeper depending on the soil characteristics). Preliminary investigations in shrimp pond soils (CENAIM, 1999, Cornejo-Rodríguez, 1999), revealed the presence of polychaetes, foraminifers, nematodes, bivalves, gastropods and copepods. Like in other environments, water quality, oxygen, and nutrients levels mainly govern the presence of these organisms. The species composition depends on the adaptation of the species to the specific conditions in the habitat.

It is hypothesized that the benthos colonizes shrimp ponds through transport by birds (at least 15 different kinds of bird species were inventoried moving from one pond to another at shrimp farms in the Gulf of Guayaquil; CENAIM, 1999). Gerlach (1977b) shows that part of the meiobenthos with pelagic larval stage, has a better year-to-year chance to establish populations in other areas than other type of meiobenthos, which allow them to colonize different habitats. But also crabs, such as *Uca* sp. and several insects move between the water reservoir and the shrimp pond or between ponds (*personal observations*). Moreover, the boats used to distribute feed and fertilizers in the pond, can be carriers for organisms from one pond to another.

The natural diet of *L. monodon* consists of small crustaceans, polychaetes, mollusks, fishes, organic detritus and algal material (Thomas, 1973). It has been suggested that bacterial colonies attached to decaying organic material may be important for the nutrition of penaeids (Cam *et al.*, 1991 *in* Groth, 1997). Cam *et al. (op. cit)* estimated the contribution of natural food to shrimp biomass gain to be about 13 to 87%, depending of the stocking density, rearing stage and feeding scheme. The same authors added that knowledge of natural food uptake by shrimp in ponds is necessary to adjust feeding rates and feeding time. The type of natural food present in the ponds affects shrimp survival and production (Bombeo-Tuburán *et al.,* 1993).

Very little information is available about benthic communities in aquaculture ponds. A general description of benthic groups had been performed, (De Paiva & Chuna da Silva, 1998) with an emphasis on the relationships in management practices (Martínez-Córdova *et al.*, 1998a, 2002b, 2003, 2005). Most of the researchs was done on the influence of the aquaculture system on the surrounded environment with the meiobenthos as one group in the analysis (Mirto, 1998; Mazzola *et al.,* 1999, 2000; La Rosa *et al.,* 2001; Mirto *et al.,* 1999, 2002). Few studies refer to free-living nematodes species (Abu Henna, 2004). Mirto *et al.* (2002) found nematode abundances in Mediterranean fish farm sediments ranging from 223±29 ind.10cm^{-2} to 519±188 ind.10cm^{-2}. The control site with no fish culture had nematode densities from 436±131 to 1328±349 ind.10cm^{-2}. La Rosa *et al. (op. cit.)* found a similar relationship. In these systems no physical contact occurred between

the fish and the sediment, as the animals were reared in suspended cages. In these cages the farmer uses an automatic feeder, where part of the feed is lost as it falls down under the cages.

1.5 Aquaculture and the environment

The concern of environmental impacts of shrimp aquaculture arises from the consumption of resources (land, water, seed and feed), their transformation into products valued by the society and the subsequent release of wastes into the environment (Ronnback, 2001). The direct impact includes release of eutrophicating substances and toxic chemicals, the transfer of diseases and parasites to wild stock and the introduction of exotic and "alien" genetic material into the environment. The environmental impact can also be indirect through the loss of habitat (e.g. mangroves) and niches and changes in food webs.

The loss of mangrove habitat eliminates nursery grounds for larval shrimp and fish. Mangrove forests are critically important habitats for the reproduction and growth of shrimp post larvae and juveniles (Turner, 1986). Their replacement by shrimp ponds will adversely affect the recruitment of larval fish and shrimp (Zimmerman *et al.,* 1989). If shrimp farming is to expand, there must be a trade-off between reclaiming new mangrove areas for shrimp ponds or intensifying existing shrimp ponds, with concomitant increased pumping and nutrient discharge. The present policy of many countries is to protect mangrove environments and intensify production from existing ponds (Villalon *et al.*, 1989).

Bangladesh and Ecuador used to be dependent on collecting wild shrimp post-larvae to stock shrimp ponds (Olsen 1989). Depletion of local populations of shrimp post larvae can occur due to this activity (Bashirullah 1989, Turner 1986). This practice stopped in Ecuador in 2000 as mentioned above. In Bangladesh as well in Ecuador collectors of shrimp post-larvae used to catch fish larvae and small invertebrates as a by-catch. These by-catches died on the beach. Practices such as this may adversely affect populations of fish and invertebrate (Meltzoff & LiPuma, 1986), natural populations.

As was mentioned above, the extensive aquaculture system uses the natural production in the ponds or of the incoming waters, semi-intensive and intensive production system are heavily dependent on formulated feed on fish meal and fish oils (Ronnback, 2001). Most aquaculture systems are so-called *throughput* systems (Daly & Cobb, 1989), which means that resources, collected over large areas, are introduced and used in the aquaculture production site, and released back into the environment in concentrated form as nutrient and pollutants, causing various environmental problems (Folke & Kautsky, 1992). Uneaten food, faecal and other physiological products may lead to eutrophication and oxygen depletion, in the surrounding environment the magnitude of which is depend in the type and size of operation as well as the nature of the site, topography and water retention time (Kautsky *et al.*, 2000).

Eutrophication of surrounding coastal areas from nutrients discharged in shrimp pond effluents can affect receiving waters (Landesman, 1994). This is especially true for intensive shrimp culture systems where the high feeding, fertilization and water exchange rates require frequent discharge of pond effluents. Chemicals used for predator and pest control, and for pond soil sterilization may kill non-target organisms after discharge of pond effluents. Copper compounds for instance, used for algae control in shrimp ponds can be toxic to crustaceans and benthic fauna (Clifford 1992). In semi-intensive and intensive farms, artificial feeds provide most of the nitrogen (N), phosphorus (P) and organic matter inputs to the pond system. Only 17% (by dry weight) of the total amount of feeds applied to the pond is converted into shrimp as faeces or eliminated as metabolites. Outlet water during regular flushing and at harvest, account for 45% of nitrogen and 22% of organic matter both suspended and dissolved (Boyd & Musig, 1992; Briggs and Funge-Smith, 1994). This high biological oxygen demand can cause oxygen depletion in receiving waters, especially since these estuaries already receive organic wastes from nearby urban and agricultural areas. If all the ponds are pumping out effluents during periods of low water, problems can arise due to this surplus organic matter and increased salinity (Twilley, 1989). Consequently, pond sediments is the major sink of N, P and organic matter, and accumulates in intensive shrimp ponds at the rate of almost 200 tons (dry weight) per hectare and production cycle (Briggs & Funge-Smith, 1994). During pond preparation between

cropping, the top sediment is removed and usually placed on pond dikes, from where it continuously leaks nutrients to the environment (Ronnback, 2001).

On the other hand, intensive and semi-intensive shrimp culture involving discharging large amounts of pond water can affect estuary or other receiving waters. Since ponds are shallow, evaporation is greater than in neighboring mangroves or estuaries. Effluents discharged from these ponds will be more saline and during periods of low flow can affect the salinity of receiving waters.

As shrimp biomass and feed input grow, the water quality in high–density ponds deteriorates over the cropping cycle. Total N and P, silicate, dissolved oxygen and biological oxygen demand increase and water visibility decrease in intensive Thailand's ponds thought the grow out period (Macintosh & Phillips, 1992). Quality of receiving waters may deteriorate if the assimilative capacity of the environment is exceeded. The enormous amount of wastes released into the environment has great potential to cause pollution and collapses in shrimp production (through negative feedback). It is well established that the re-use of waste-laden pond water discharge so-called self-pollution, is a major triggering factor behind disease susceptibility for cultured shrimp. Lin (1989) reported that self-pollution was a main causative factor behind the mass mortalities in Taiwanese shrimp crop in 1988. In addition to nutrients discharged from shrimp culture ponds sediments removed from pond bottoms are often discharged into receiving waters (Boyd & Musig, 1992). These sediments can increase turbidity in receiving waters.

If an intensively cultured shrimp pond is abandoned, the bottom soil is usually saline making it unavailable for agriculture or other uses. Therefore conversion of land to shrimp farming may for practical purposes be irreversible (Meltzoff & LiPuma, 1986). Salt-water intrusion into the water table of nearby agricultural land can occur when shrimp ponds discharge effluents into the irrigation systems supplying farmlands. This is a serious concern in Indonesia where the same canals supply both fresh and brackish water, depending on the season (Chamberlain 1991).

Another consequence of using saline waters to raise shrimp is the need to maintain a particular salinity in the pond. Since the ideal salinity for *L. monodon,* is 15 to 25 parts per thousand, freshwater is needed for pond dilution if full-strength seawater is

used. In Taiwan land subsidence has occurred due to well water extraction to dilute coastal shrimp ponds (Avault 1993).

Shrimp farming may make use of exotic species or varieties in areas where these species are not native. In oceanic islands such as Hawaii, Seychelles, Tahiti, etc. where shrimp farming has been introduced, the species cultured are all foreign to their environments. What effects this will have on the local ecosystem are unknown. Even if the presence of an exotic species of shrimp is innocuous, diseases and parasites can spread to local penaeid species from the exotic cultured shrimp. Cultured shrimp are vulnerable to a wide assortment of parasitic fungi and virulent bacteria and viruses (Brock *et al.,* 1992). If these pathogens spread to a local shrimp or invertebrate fishery it could have serious economic consequences (Hoffman, 1970).

Chemicals used in shrimp culture may be classified as therapeutants, disinfectants and soil treatment compounds, algaecides and pesticides, plankton growth inducers (fertilizers and minerals) and feed additives. Excessive and unwanted use of such chemicals results in problems related to toxicity to non-target species (cultured species, human consumers and wild biota), development of antibiotic resistance and accumulation of residues (Primavera, 1998). Antibiotic use reduces natural microbial activity, which leads to waste accumulation and reduced degradation and nutrient recycling (Ronnback, 2001). The use of antibiotics in shrimp feed has led to the occurrence of antibiotics in shrimp tissue (Weidner 1992). This may conceivably lead to the spread of antibiotic resistance in humans. Since shrimp ponds are downstream from agricultural lands, pesticides may accumulate in shrimp tissue as well. Harmful pollutants present in estuaries as radioactive isotopes, heavy metals, etc. can also occur in shrimp tissue.

All these impacts described above may occur in addition to the impacts coastal areas already get from industrialization, urbanization, increased use of agricultural chemicals, recreational development, petroleum exploitation, etc. Coastal areas are especially affected by these impacts because they are downstream from sources of urban (sewage) and agricultural pollution (pesticides). In addition large urban centres are often on or near coasts (Lima, Jakarta, Manilla, Bangkok, etc.). These

environmental stresses all reduce the capacity of the coastal environment to absorb the effects of mariculture (Bailey, 1992).

If shrimp ponds are built close together they share their water supply. If the wastes from one pond are discharged close to the water supply intake of another pond, that pond's effluent may enter the adjacent farms. The recycling of pond water between ponds or farms increases the incidence of diseases and parasites. This recycling of water between heavily stocked ponds contributed to the collapse of the shrimp farming industry in Taiwan (Avault, 1993). Dense algal growth followed by an algal population collapse can lead to die offs of shrimp and fish in an affected area. Hatcheries can also be affected if they pump from waters polluted by pond discharges (Chamberlain, 1991).

1.6 Benthos

Within this doctoral thesis, emphasis will be given to the study of the benthos living in commercial shrimp pond bottoms. Benthos refers to all the organisms, plants and animals (figure 1.11), which live in relationship with sediment, temporally or permanently. Some benthic organisms have a temporal planktonic life as it is the case for some polychaetes (Boltovskoy, 1981), and some copepods (Shimek, 1997).

Benthic organisms can be classified in two general groups: phytobenthos, which refers to macro and microalgae and zoobenthos, animals living mainly in and upon the bottom. Bacteria are also part of the benthic communities (microbenthios) together with protozoa, ciliates and fungi. The zoobenthos (often called 'benthos') can be divided into: the epibenthos that includes fish and larger invertebrates such as crustaceans and starfish living on or near the bottom. The hyperbenthos include all organisms larger than 1 mm, which live in the lowest layer of the water column, just above the bottom and include mysids, amphipods and larvae of epibenthos. The organisms can be permanent or temporal hyperbenthic. To the first group belong the mysids, amphipods, isopods, cumaceans and pycnogonids. The second group consists mainly of post-larval stages of shrimp, crab and fish (Ghent University, 1997; Mees & Jones, 1997).

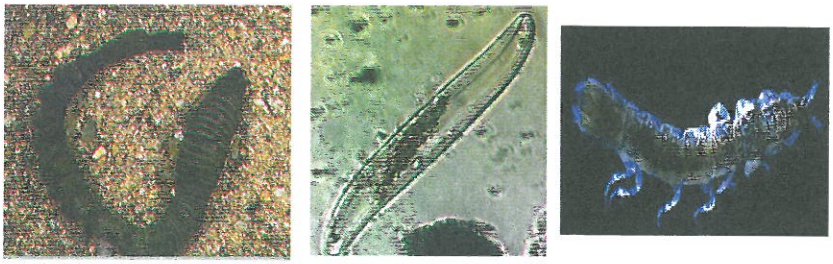


Figure 1.11 Benthic organisms, Annelida, benthic algae and Crustacea, (sources: Ghent University, 1997; Cenaim, 2004).

Sizes are also used as classification criterion. Macrobenthos living in or on the bottom includes all organisms larger than 1 mm. The most common macrobenthic groups are mollusks, crustaceans, annelids and echinoderms. They are cosmopolitan from the beach to the deep sea and from Polar Regions to tropical ones. These groups play an important role in the ecosystems of the sea, on the one hand as consumer of dead organic matter, grazing on small algae or predating on small animals, and on the other hand as feed to benthic/demersal fish, crabs and birds (Ghent University, 1997).

The meiobenthos is smaller than 1 mm and is retained on a sieve of 38 µm; these include Nematoda, Copepoda, Turbellaria and small Polychaeta, among others (Coull, 1973; Giere, 1993; Ghent University, 1997). In total, 26 higher phyla are recognized within the meiobenthos. In shrimp pond bottoms, meiobenthic organisms are dominant over the macrobenthos, and in many cases only meiobenthos is present (Quevedo, *in press*). The smallest size class in the benthos is the microbenthos mainly composed of bacteria, Ciliata and Foraminifera, which are also important inside the soils of shrimp ponds. Concentrations of nutrients, organic matter and microorganism's density in the ponds bottoms are several orders of magnitude greater than in the water (Avnimelech & Ritvo, *2003*). In this doctoral thesis emphasis will be focused on the meiobenthos of the shrimp pond bottoms.

1.6.1 Meiobenthos

Meiobenthos is found in a wide diversity of habitats. They occur both in fresh water and marine habitats, from high on the beach to the deepest water body (Higgins & Thiel, 1988; Austen & Widbom, 1991; Traunspurger & Drews, 1996; Adao, 2003). Meiobenthos also occupies several "above sediment" habitats, depending on their body size and the space between sand and mud grain, including rooted vegetation moss, salt marsh (Vickova *et al.*, 2002; Adao, 2003), macroalgae fronds (Higgins & Thiel, 1988; Arlt, 2005), sea ice (Schnack-Schiel *et al.,* 2001; Funch *et al.*, 2004; Fradinger *et al.,* 2005) and various animal structures like coral crevices (Guzman *et al.*, 1987) and worm tubes (burrowing) (Higgins & Thiel, 1988; Funch *et al.,* 2004). They feed on small unicellular algae, bacteria and suspended organic matter, and are prey to larger benthic animals, macrobenthos, and small fish. Some meiobenthos are symbionts living commensally in animal tubes, with bivalves in association with woodborers or hydrozoan colonies (Higgins & Thiel, 1988). Meiobenthic animals are very active in the regeneration and mobilization (re-suspension) of nutrients present in or on the bottom (Tietjen, 1980; Alkemade *et al.*, 1992; Adao, 2003).

On shallow sea bottoms (<100m), meiobenthos densities range between 10^4 and 10^7 ind.m^{-2}. Several trophic groups are represented within the meiobenthos including predators, herbivores, bacterivores and microvores. Most meiobenthos communities exhibit patchy spatial distributions both vertically and horizontally and involve a variety of biological, physical and chemical variables, including granulometry, salinity, oxygen tension, food availability and chemical compounds in the water (Giere, 1993).

Other factors affecting the distribution of the meiobenthos is the granulometry of the sediment. The size and shape of the sediment particles determine the area for the establishment of the biotic conditions of the sediment: bacteria, fungi, diatom and mucus secretions. Fine estuarine sediments are characterized by low permeability and reducing conditions (Dye, 1983). Physical disturbance can cause sediment re-suspension and instability and it also affects sediment chemistry (Austen *et al.*, 1998, Steyaert & Vincx, 1996).

The meiobenthos is mainly concentrated to the top five centimeters of the sediment (McIntyre, 1969; Adao, 2003) (figure 1.12). Observations throughout a year, or over several years, are not common and in most studies seasonal peaks have been noted (McIntyre, *op. cit.*; Juario, 1975; Dye, Coull, 1985, 1986; 1977; Vincx, 1989; Alongi, 1990c; Santos *et al.,* 1996; Olafsson & Elmgren, 1997; Hashimoto *et al.,* 2004). On the other hand Li & Vincx (1993) who performed a study of temporal variability of intertidal nematodes in an estuarine area (Westerschelde, Netherlands), have noted that an unstable habitat influences the stability of the nematode communities.

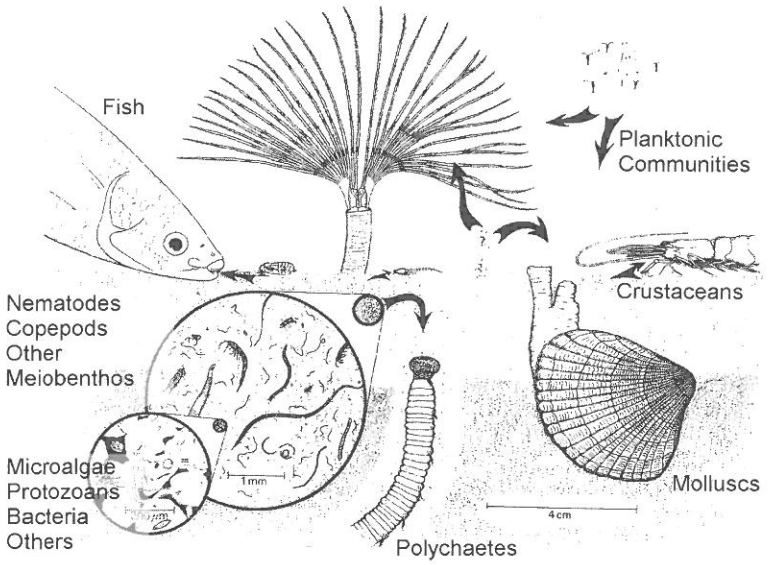


Figure 1.12 Benthic distribution within and upon the sediment (source: Higgins & Thiel, 1988).

Riemann & Schrage (1978), Tietjen (1980) and Jensen (1996) found that nematodes may stimulate bacterial growth in different ways due to the mechanical break down of detrital particles. As a result, the detritus becomes more susceptible to increased bacterial colonization. Moens *et al.* (2005) have noted that nematodes may transport specific bacteria to resource patches. Meiobenthos also directly excrete nutrients into the medium such as nitrogen and phosphorous. Through the secretion of mucus, meiobenthos may produce slime trails that attract and sustain bacterial

growth (Riemann & Schrage, 1978). The burrowing and swimming activities of meiobenthic animals may act as vertical conveyors of nutrients and oxygen within the sediments, and between the sediments and the overlying water column (Gerlach, 1978). However, Montagna *et al.* (1983) reported an absence of spatial correlations between bacterial and meiobenthos abundances. Montagna (1984) showed that predominant grazing pressure could be attributed to polychaetes as well. Epstein & Shiaris (1992) have evidence that on muddy tidal flats by grazing the micro and meiobenthos may not influence bacterial numbers.

The density of meiobenthos decreases with depth in natural sediments (Dye, 1977, Platt, 1977; McLachlan *et al.*, 1979; Steyaert & Vincx, 1996). Around 40 % of the meiobenthos was found in the top 5 cm of the sediment. Platt (1977), Teal & Wieser (1966) and Moore & Bett (1989) speculated that oxygen and food are factors involved with the decrease in number with depth. The progressive mineralization and the decrease of organic matter (and available food) with depth (McLachan *et al.,* 1981) may be an important factor structuring nematode communities (Dye, 1983). Dye (*op. cit.*) also showed that this fits with the finding that the redox potential Eh (and to a lesser extend pH) correlates with vertical distribution as well. As organic matter is degraded, the concentration of H_2S increases, which is toxic for aerobic animals. However, Steyaert *et al.* (2005) reported that for estuarine zones most nematodes are tolerant to short-term anoxia. The extension of the oxidized layer is reflected in the depth to which copepods and nematodes penetrate (Heip *et al.,* 1977).

Within marine meiobenthos the free-living **nematodes** are the most abundant metazoan (Warwick *et al.,* 1979; Warwick, 1981a; Bouwman, 1983; Adao, 2003) representing 50-100% of the total meiobenthos (figure 1.13). Harpacticoid copepods represent the second most abundant metazoan group (McIntyre, 1969; Coull, 1973; Tietjen, 1980; Epstein & Shiaris, 1992; Kim *et al.,* 1998).

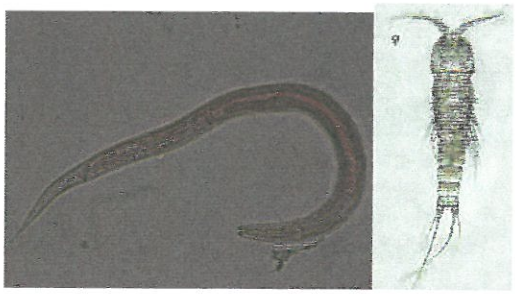


Figure 1.13 The most abundant meiobenthic organisms (left: nematode Mohystera; right, copepod Harpacticoidea) (source: Stutervant, 2004).

The nematodes are widely distributed and occur in nearly all benthic biotopes, e.g. in coarse sands (Adao, 2003), fine sands (Gerlach, 1978; Alongi, 1986; Gheskiere, 2005), mud (Nicholas *et al.*, 1991; Thinphanga, 2004) and on the surface of littoral macrophytes (De Casablanca, 1997). In terms of biomass, nematodes are also the dominant group and the number of species in estuarine habitats is not equaled or exceeded by any other taxon (Bouwman, 1983). Although nematodes and copepods have both their largest density at the surface, nematodes penetrate much deeper into the sediment (Heip *et al.*, 1977; Guotong, 1999).

In general, the composition of the nematode community depends on the adaptation of the species to the specific conditions of the biotope, such as microstructure of the substratum, organic flux and salinity (Kinne, 1964; Lambshead *et al.,* 2003). In mud, which is the main substratum in shrimp ponds the interstitial salinities are closely related to those of the overlying water (Smith, 1956). Other important structuring environmental factors for the meiobenthic communities are temperature (Gunter, 1957; Kinne, 1963; Bouwman, 1983; Jensen, 1984), light (Friedrich, 1961; Bouwman, 1983; Jensen, 1984) water oxygen saturation, concentration of hydrogen sulphide and dissolved organic components. But also the quality and the quantity of feed (Schrijvers & Vincx, 1999; Steyaert *et al.*, 2001) and nutrients (Barnes, 1957) are included as factors affecting benthic communities.

When the natural conditions of a particular area are altered and some organisms die, the decaying animals greatly affect the meiobenthos community (Jorgensen, 1977). In oxic sediments, bacterial sulphate reduction can occur within fecal pellets

and detrital particles. This could occur near dead animals, producing an accumulation of H_2S and a bacterial community similar to that of the Redox Potential Discontinuity layer (RPD) (Jorgensen, 1977). For nematodes, Olafsson (1992) found a concurrent decrease in number of the most abundant species and an increase in an opportunistic species in black spot areas. Generally nematodes are considered to be the meiobenthos taxa most resistant to low oxygen concentrations and sulphide exposure (Hendelberg & Jensen, 1993). But, the diversity of nematodes may decrease after an hypoxic event (Austen & Widbom, 1991).

Rudnick (1989) suggested that there might be two groups of meiobenthos present in sediments: one utilizing fresh phytodetritus on the sediment surface and ones utilizing the large reservoir of old detritus. Meiobenthic crustaceans dominate the first group, mainly, harpacticoid copepods, but also ostracods and small nematodes. The group living deeper in the sediment is mainly dominated by large nematodes but also contains the slender interstitial harpacticoids, turbellarians and kinorhyncha. As well as moving around within sediments, the meiobenthos build tubes, constructs burrows and feeding pits, transports sediment and also changes the structures of the sediment (Heip, 1995).

The bio-perturbation activity of nematodes, but also other meiobenthos may influence sediment diffusion coefficients for a variety of solutes, including O_2 (Alkemade *et al.,* 1992). The construction of tubes around decomposing organic matter, may enhance the surface area available for microbial degradation process, through their mucus secretions (Riemann & Marion, 1978; Jensen, 1996). These mucus secretions may help in strengthening burrows or attaching eggs to the substratum and also serve as a substrate for algal and bacterial growth; which in turn may be exploited by nematodes. This phenomenon is called gardening (Riemann & Schrage, 1978; Epstein & Shiaris, 1992; Montagna, 1995). In the same sense, Nehring *et al.* (1990) pointed out that the nematodes might play a significant role at the interfaces by increasing pore water exchanges and stabilizing newly sediment detritus with excreted mucus.

Benthic infauna is an important mediator of nutrient recycling from the sediments into the water column (Rhoads, 1974; Wolfe *et al.,* 1982; Hartley 1982, 1984; Bilyard, 1987). The re-suspension of nutrient-rich bottom mud into the water column

provides a potential feed source for suspension feeders. Mann (1976), Escalona (1983), Adao (2003) and Forja *et al.* (2003) concluded that, in general in coastal waters and in coastal lagoons, the nutrient regeneration from the sediment is one of the main factors influencing primary production. In a very rough calculation; the meiobenthos and macrofauna are responsible for about 20% of the regenerated nutrients, while microbenthos (microflora and ciliates) is responsible for the remaining 80%. Escalona (1983) mentioned that macro-organisms, fish and crabs could contribute with around 53%, while sediment with 31% and suspended organisms with 13% to the nitrogen flux in the water column.

In general, it is expected that benthic assemblages respond to organic disturbance in terms of decreased species diversity, the selection of a few opportunistic species (Ritz & Lewis, 1989; Weston, 1990); creation of reduced conditions into the sediment by depletion of oxygen penetration (Mazola *et al.*, 1999), reduced density and biomass (Frid & Mercer, 1989; Weston, 1990) and partial offset of the increasing opportunistic species.

1.6.2 Meiobenthos as bio-indicator of environmental conditions

Benthic infauna clearly provides important quantitative, site-specific information that addresses the most common objectives of marine monitoring programs. Benthic animals are mainly sedentary (Hartley, 1982; Bilyard, 1987). Mann (1976) concluded that benthic infauna is of great economic importance as prey for commercially valuable species of demersal fishes or large epibenthic invertebrates (e.g. shrimp, crab). Platt & Warwick (1980) also commented that any general assessment of the ecology of intertidal habitats is incomplete if the nematodes are "not taken into consideration".

Benthic organisms are very sensitive to habitat disturbances, including organic enrichment of the sediments and contamination of the sediment by toxic substances (Hartley, 1982; Wolfe *et al.*, 1982) and because of their variable sensibility; benthic communities undergo dramatic changes in species composition and abundance. Through a careful survey design, spatial gradients of benthic community structure may be related to known and suspected sources of pollution. The sedentary habits

of benthic infauna also facilitate the development of models that describe cause-effects relationships (Hartley, 1982).

Within the benthic infauna, the free-living nematodes are considered indicators of environmental quality of sediments (Zullini, 1976; Heip *et al.,* 1985, Sandulli & Nicolla, 1991; Schratzberger *et al.*, 2000; Gheskiere *et al.,* 2005a). They have the advantage of being the most abundant metazoans present in the sediments (so a small sediment sample yield enough animals to make scientifically sound statements) and are proven to be important in organic decomposition and nutrient regeneration (Tenore *et al.,* 1977; Tietjen, 1980; Moens, 1999; Thinphanga, 2004). Overall, they are permanent members of the benthos and therefore are unable to physically escape from bottom pollution effects. Gheskiere *et al.* (2005a) add that the diversity is high, resulting in a range from very tolerant to very sensitive species.

Nematodes also have a short generation time; most (estuarine) species have life history of generally less than one month (Tietjen & Lee, 1977; Ferris & Ferris, 1979; Alongi & Tietjen, 1980; Gheskiere *et al.,* 2005c). Therefore, changes in the environment that changes the tolerance of the species, can be quickly detected because of the rapid response of the species. Because of their wide range of adaptations, nematodes have exploited all littoral habitats, including the biologically hostile sandy beaches. So, the composition of nematode assemblages may reflect the general health of the benthos (Kennedy & Jacoby, 1999).

Most estuarine nematode species are relatively easy to establish and maintain in laboratory culture (Lee *et al.*, 1970; Tietjen & Lee, 1984). Small mesocosms (microcosms) are required to perform experiments. Especially bacterial feeders and diatom feeders are suitable for experimental work since food can be provided under well-controlled conditions. Smaller nematodes, can be cultured and experimentation with predators is also possible (Moens, 1999). The small size of nematodes also makes them especially useful in conducting experiments on the effect of toxicants in populations.

But, the study of free-living nematodes also has disadvantages. They are difficult to determine at the species level for non-experts. Most nematodes are 1 to 3 mm in length (Funch *et al.,* 2004) and weigh less than 5 µg. Information of life history is

available just for a few species (Tietjen & Lee, 1984; Herman & Vranken, 1988; Vranken *et al.,* 1988; Moens *et al.,* 1996b).

1.7 Bio-monitoring of the shrimp pond soils

Soil analysis as a tool in aquaculture management in shrimp ponds has been studied considering mainly chemical and physical conditions (Boyd & Musig, 1981; Boyd, 1976, 1995, 2003; Stickney, 1994,). Nevertheless, poor soil conditions are suspected when pond managers cannot explain poor growth or survival during disease outbreaks and/or parasite problems. The quality of the soil is very much influenced by artificial feed quality and quantity, weather conditions, water quality and management practices. The reason why the soil of the ponds is less understood is mainly because of methodological problems. It is more difficult to sample soil than water, and few managers tried to relate production problems with soil analysis. Today soil-testing laboratories can quickly provide data on several chemical properties of soil at a reasonable cost, but the interpretation of data in relation with shrimp production is a problem.

Normal concentration ranges of chemical properties in pond soil are poorly defined, and few correlations have been made between pond soil properties and aquaculture production (Boyd *et al.*, 1994).

Shrimp use the soil for different activities during their life cycle, such as feeding and shelter during molting (FAO, 2005). Good growth results were noted when shrimp are cultured in floating cages, to isolate them from the bottom to avoid transmission of viruses by vectors. On the other hand, good production results were also obtained without this floating system (Calderón, 2001). Some studies have demonstrated the use of benthic organisms, as indicators of health conditions mainly to fish aquaculture systems (Mazzola *et al.*, 1999, 2000: Mirto *et al.*, 2002). *Panagrellus redivivus* has been used as biomonitor to detect toxin concentrations that affect molting (Neher, 2001). Also in the 1970s the use of a nematode: copepod ratio (Rafaelli & Mason, 1981) was popular in monitoring condition of aquatic ecosystem.

2. Aim of the thesis

The shrimp production cycle in artificial ponds is mainly supported by empirical findings and based on *trial and error* managing activities. Therefore, the production of the shrimps is highly variable, even in apparently similar systems. Many explanations are available for the observed phenomena (examples given in the introduction) although few of them are supported by strong scientific results. The way to a sustainable shrimp aquaculture is still long and a better insight into the ecological characteristics of the ponds will help in the development of sustainable management practices. In the past, the characteristics of the food web within the water column (where the shrimp is living) is rather well documented. The effects of the nature and the amount of artificial nutrients on the shrimp life cycle are clear, but again, sustainability for the shrimp pond is a problem. One cause of this, might be the accumulation of organic material in the soil of the pond creating a changing environment which is not understood yet. It has been frequently observed that two neighbouring shrimp ponds, apparently with the same soil characteristics, same intensity and nature of the aquaculture activity, can have very different harvest of shrimp.

Only a few studies are available about the benthos in artificial shrimp ponds (study in Mexico, Martinez-Cordova, 1998a, 2202b, 2003, 2005; study in Mexico. Rubright *et al.*, 1998). The macrobenthic community in Ecuadorian shrimp ponds is very poor, and therefore we have put emphasis in this study on the smaller dominant benthic representatives, i.e. the meiobenthos.

In this doctoral thesis the benthic characteristics of the soil of Ecuadorian shrimp ponds will be investigated both in the field and under experimental conditions. Some of the different management practices used in shrimp aquaculture (e.g. increase in artificial nutrients) will be registered and analyzed in relation to changes in the structure of the benthic communities with emphasis on the free-living nematode communities.

In order to evaluate the importance of free-living nematodes in the ecology of artificial shrimp ponds, the 'natural' variability of the structural characteristics such as

density and biomass, will be described. Experiments were conducted in order to test the effects of nutrients and lime on the nematode community characteristics.

2.1 Outline of the thesis

Chapter 1

General characteristics of Ecuadorian shrimp aquaculture and general benthic characteristics, are illustrated within this chapter.

Chapter 2

Field and laboratory methodology is described together with the list of statistical analysis applied to the data. Four shrimp ponds (A, B, C, D) were chosen in the saline, coastal area of Ecuador and in the estuarine zone of the Gulf of Guayaquil. A fifth shrimp pond (Pond E), was chosen to take out sediment for use in the experiment. These ponds are located within 4 commercial shrimp farms. Collection of environmental data and information on management practices was also performed.

Chapter 3

The annual variability of the nematode communities under the "common" management conditions of shrimp ponds (four production cycles within 1 year) is investigated. During one year, soil samples of one pond in the saline, coastal environment; were taken on a monthly basis. The abundance and species diversity of the nematode communities are considered in the analysis and the relationships of theses variables with environmental and management practices are studied.

Chapter 4

The colonization process of the nematodes and the copepods in four shrimp ponds from different environments (one saline and three estuarine ponds) are studied. With regular intervals, several shrimp production cycles are monitored within the ponds and the colonization at the higher taxon level (nematodes versus copepods) is evaluated.

Chapter 5

The characterization of the nematode community of three shrimp ponds during the same season was performed. Management practices and some environmental variables are integrated in the analysis. In this study, the evolution at the nematode species level is performed in order to detect the indicator value of some of the species. In this way, the characteristics of the nematode species composition as tools for bio monitoring are evaluated.

Chapter 6

The effects of some nutrients over nematode communities are studied in an experimental design. A mesocom infrastructure with 12 tanks of 1000l each filled with sediment brought from a shrimp pond in the Gulf of Guayaquil was used. The nutrient sources tested corresponded to commercial fertilizers (SPT; 4.96 $g.tank^{-1}$; 400 $k.ha^{-1}$ and NO_3NH_4; $1g.tk^{-1}$; $200k.ha^{-1}$). Chemical and physical analysis of the water and the soil are also performed.

Chapter 7

The effects of lime (in the form of $Ca(OH)_2$, $200kg.ha^{-1}$) added as an additive during a shrimp production cycle, was tested on free-living nematodes communities within the same mesocosm experimental design. The mesocosms tanks were filled with sediments from an artificial shrimp pond, originating from an estuarine environment.

Chapter 8

The general discussion of the actual research will emphasize about the shrimp pond bottom characteristics.

Chapter 9

The conclusions and ideas for future work are presented.

Chapter 10

The general literature is cited here.

CHAPTER 2

Material and methods

2 Material and methods

In this chapter we explain the materials and methods used throughout the whole study. When needed a complementary description is presented in the following chapters.

2.1 Research sites

The field monitoring and experiments were conducted in three different shrimp farms located near the Pacific coast (Pond A) and along the Guayas estuary in the Gulf of Guayaquil (Ecuador) (Ponds B-E). The shrimp farms Naturisa (floodgates area, Guayaquil) and Veronesi (Chongon) are located near the Guayas estuary and the third shrimp farm Opumarsa (Palmar) is located along the west coast of Ecuador (figure 2.1; table 2.1).

The Fincacua shrimp farm, Chongon, is not shown in the map because only its sediment was used in the experimental design

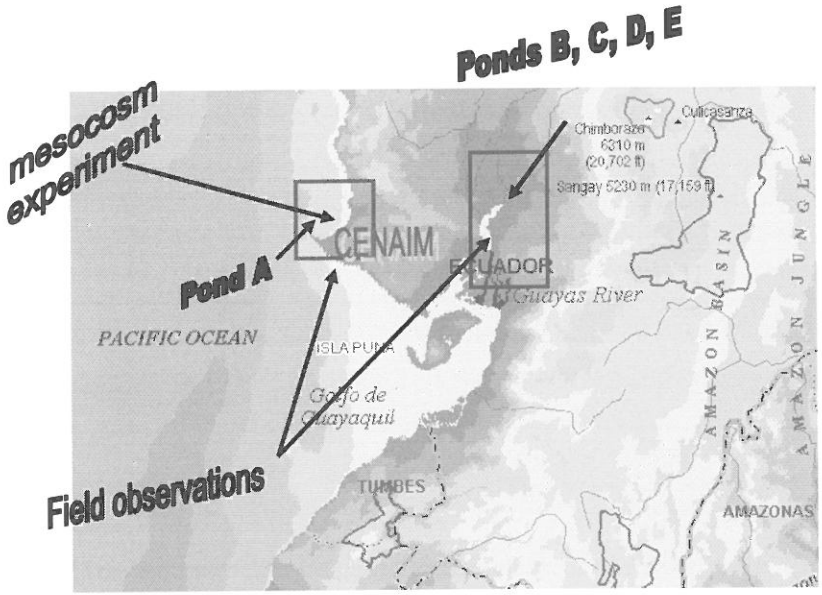


Figure 2.1 Location of the study sites.

Five shrimp ponds were used for the current research, and one of them was used only to extract sediment to perform the experiments (Pond E). The laboratory (mesocosm) experiments were performed at the National Aquaculture and Marine Research Center, CENAIM in San Pedro de Manglaralto (www.cenaim.espol.edu.ec) (figures 2.1 and 2.2).

Table 2.1 Location of the study ponds.

Shrimp farm	Location	Shrimp pond name	Chapter where information was used
Opumarsa	Palmar	Pond A	Chapters 3 , 4 and 5
Veronesi	Guayaquil Gulf	Pond B	Chapter 4
Naturisa	Guayaquil Gulf	Ponds C, D	Chapters 4 and 5
Fincacua (*)	Guayaquil Gulf	Pond E	Chapters 6 and 7

(*)We used only sediment from this pond.

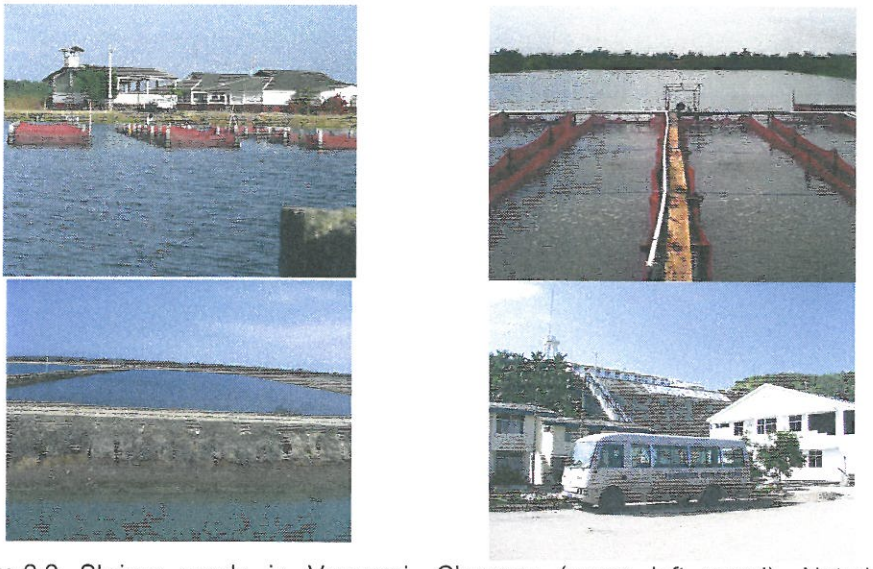


Figure 2.2 Shrimp ponds in Veronesi, Chongon (upper left panel), Naturisa-Guayaquil flooded gates area (upper right panel), Opumarsa, Palmar (bottom left panel) shrimp farms and National Aquaculture and Marine Research Center, CENAIM (bottom right panel).

The coastal morphology along the Guayas province coast is characterised as sandy, alternated with rocky parts and hyper saline lagoons. Aeon (1988) mentioned that the hyper-saline conditions reduce the vegetation in this zone. Only at places where the seawater enters the coastal lagoon during spring tide periods, mangrove vegetation is present.

A marine environment, with no mangrove and few of the vegetation present, characterizes the coastal shrimp ponds. Mangrove and other vegetation surround the ponds located inside the Guayas estuary. The shrimp ponds receive water from the sea through artificial channels and through these channels from the open sea (figure 2.3).

The largest estuary of the West Coast of South America surrounds the research sites in the Gulf of Guayaquil (Cucalón, 1976; Cucalón 1984 in Twilley *et al.*, 1998). It is the most important area of the Ecuadorian coast for fishery and shrimp farming due to the richness of commercial fish such as tuna and herrings and also shrimp production (Jimenez, 1981).

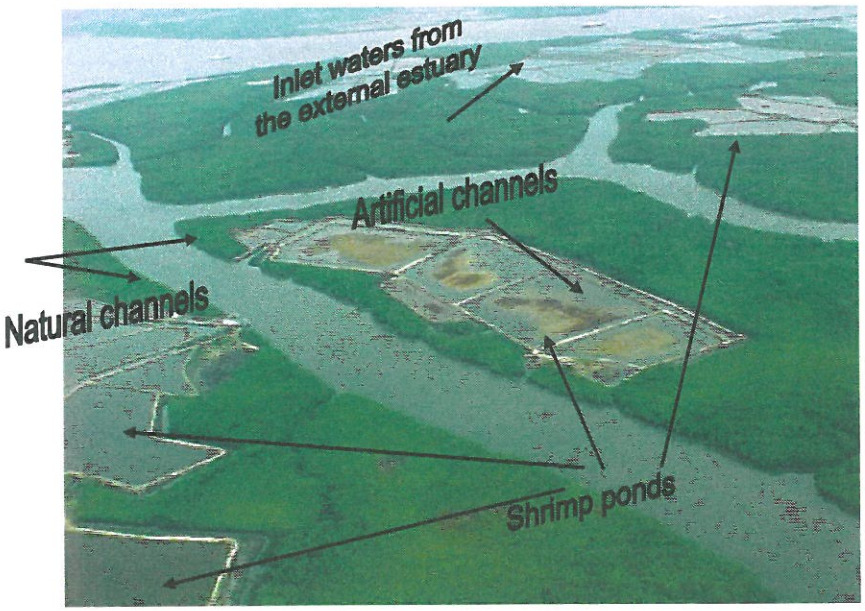


Figure 2.3 Shrimp ponds with artificial and natural water channels.

The Gulf of Guayaquil receives run off from some 20 rivers with a watershed of 51,230 km^2 (Twilley, 1989). The Guayas River is the major source of fresh water for the Guayaquil Gulf, which forms 60 km inland at he confluence of the Daule and the Babahoyo rivers. This fresh water enters into the Guayas estuary, and to a lesser extent into the Estero Salado, around the city of Guayaquil and flows 55 km to the

Gulf of Guayaquil. The mean discharge of Guayas River is 1,143.7$m^3.s^{-1}$. The mean precipitation in the Guayas river drainage system, north of Guayaquil, is 885 $mm.yr^{-1}$ (Twilley, 1989). Eighty percent of the total area of shrimp ponds along the coast of Ecuador is located in the Gulf of Guayaquil (Twilley, 1989).

2.2 Ecuadorian coastal climate

Ecuador is divided into three regions: the Coast, the highland (Sierra) and the Amazonas region (Oriente). The coastal Ecuador climate is influenced primarily by the proximity of warm or cool ocean currents. By contrast, climate in the Andes varies more as a function of altitude. The eastern area has a fairly uniform climate that varies only slightly between the two subregions. Climate in the Galapagos Islands is both modulated by the ocean currents and affected by altitude. Throughout Ecuador variation in rainfall primarily determines the seasons. Temperature is determined by altitude. With each ascent of 200 meters in altitude, temperature drops 1° C.

The Coast has a tropical climate. Temperatures for the region as a whole remain fairly constant, ranging from 23° C in the south to 26° C in the north. Although seasonal changes in temperature are not pronounced, the hottest period occurs during the rainy-warm season, especially from February to April. Near Guayaquil, the coolest (driest) months are August and September. Rainfall in the Coast decreases from north to south, with vegetation changing from tropical rainforest in the north to tropical savannah to dry tropical forest in the south. Differences in temperature and rainfall in the coast are caused by the Peruvian Current and periodic appearances of El Niño. The Peruvian Current, also formerly known as the Humboldt current, is a cold oceanic current that flows north along the coasts of Chile and Peru. At Cabo Blanco, where the Gulf of Guayaquil begins, the main current veers to the west; a branch continues northward to Cabo Pasado, in Manabí Province, where it also turns westward to merge with the main current near the Galapagos Islands. The cold water and air temperatures associated with the Peruvian Current inhibit rainfall along the coast, creating dry to arid conditions. This effect is greatest along the southern coast of Ecuador.

The El Niño occurs periodically every six or seven years. In the past it was tight to the warm season, starting in December. A simplified overview follows. An ocean wide change in atmospheric pressure shifts ocean currents so that warm waters come closer to shore and displace the cold waters. During this time, air and water temperatures, tides, sea levels and wave heights, and relative humidity all are higher than usual. These conditions produce heavy rainfall that generally lasts until May in an area that normally experiences nothing more than a drizzle. The resulting flooding and landslides can be devastating.

When the Peruvian Current is dominant, the amount of precipitation along the coast varies from north to south, with levels ranging from 300 centimeters to 30 centimeters, respectively. Two rainy seasons in the northernmost part of the coast become a single season (December through June) not far south. Near Esmeraldas (northeast province of Ecuador), average annual rainfall is 250 centimeters. The rainy season shortens farther south, lasting only from January to May at Guayaquil. Very little rainfall occurs at the tip of the Santa Elena Peninsula west of Guayaquil. Arid conditions prevail on the border with Peru south of the Gulf of Guayaquil.

Separated from the effects of oceanic currents by the coastal range, the inner coastal area has a hot and humid climate. Temperatures can surpass 26° C, and the vegetation and cloud cover tend to retain and augment the heat. Rain is constant during the winter months of December through May, with the heaviest rainfall occurring in February and March.

2.3 General description of the field monitoring and experiments

The "natural" temporal variability of the nematode communities under the "common" management conditions of shrimp pond was studied in Opumarsa shrimp farm (Pond A), situated near the Guayas province coast. The abundance and species biodiversity of the nematode communities were considered in the analysis and the relationships of these variables with environmental and management practices were studied. We took monthly benthic samples from an 8 has shrimp pond during one

year (2000-2001), covering four shrimp production cycles. These cycles were between the following periods October, 2000-January 2001; January-April, 2001; April-June, 2001; June–October, 2001. The management practices (feeding, lime, fertilizers) and environmental conditions of the shrimp pond were monitored as well and the results are shown in Chapter 3.

The colonization process of meiobenthic organisms (nematodes and copepods) in four ponds (Ponds A, B, C and D) was analysed. The environmental conditions and management practices were considered for data interpretation results in Chapter 4.

A comparison of the three ponds during the same season was performed. The structure of the nematode communities of Opumarsa, saline area (Pond A) was compared with ponds inside Guayaquil Gulf, estuarine area (Naturisa, Ponds C and D). The structural characteristics of nematode community under field conditions were studied (results are presented in Chapter 5).

Mesocosm conditions were used to study the effect of some nutrients (nitrogen and phosphorous), over nematode community (figure 2.4) (results in Chapter six). And, also the effect of lime (used to improve soil condition) was also investigated (results in Chapter seven).

Figure 2.4 Mesocosm at Cenaim (1,000 l tanks capacity).

2.4 Samples collection

The following general methodology was applied to all field observations as well as to the mesocosm experiments.

2.4.1 Benthic samples

The samples for the field experiment (Pond A, Opumarsa; Ponds C and D, Naturisa) were collected from 5 stations inside the pond with three replicates each, for a total of fifteen samples per pond. The selection of these fives sites and the number of replica were based on the number of samples necessary to evaluate the nematode community according to Ramírez-González, (1999).

Simple random sampling was performed. In this way, the same probability to be collected is given to all the organisms in a determined environment. An increase in the number of collected samples, will decrease the confidence intervals.

Hence

$$n=t^2s^2 / E^2 \qquad 2.1$$

Where,

n= sample size

t= *t*-statistic with n-1 degrees of freedom

s= standard deviation

$$E= \mu \times r \qquad 2.2$$

Where

μ = mean

r= error of the mean (10%) then r=0.1

At Pond B, the samples were obtained from 25 cages constructed inside one shrimp pond. In mesocosm experiment one replicate per tank was considered.

A modified sampling core of 5 cm diameter (19.635 cm^2; figure 2.5) was used in all cases for the benthic sampling, with a floating system, to avoid losses (Fadeeva & Demchenko, 2004; modified by Yagual, 2004). Sediment samples were taken at 5 cm in depth. For the environmental factors one measurement was taken in each of the five sampling points.

Figure 2.5 Corer used for benthic sampling. The small ones for mesocosm experiments and the larger one for field experiment (with floating system (re-designed by Yagual, G., 2004)

Samples were fixed with formaline (CH_2O–NH_2) 4%, neutralised with sodium tetra borate ($B_4Na_2O_7$).

2.4.2 Environmental measurements

The environmental variables considered during the different experiments and measured directly in the field, in the water column, were: temperature (°C), oxygen ($mg.l^{-1}$) and salinity PSU) and were measured just above the sediment with an oxygen-meter YSI 85, Yellow Springs Instrument Co. The pH was measured with a pH 320/SET –1, pH meter WTW, at the laboratory. The data were collected at the surface of the water in the pond and just above the bottom.

2.4.3 Shrimp data

Since shrimp are considered as one of the structuring elements in the food web of the ponds, they were monitored as well in order to evaluate their possible effect on

the benthos. The amount of food added to the shrimp ponds depends on the stocking shrimp densities (numbers and biomass). The wet weight of the shrimp, in grams (as a measurement of stock) was determined with a Sartorius balance with two decimals accuracy. Thirty animals were considered to represent the total population (same number use at field and mesocoms experiment). Ten percent of the total weight of the organisms per day was always added as food.

2.5 Laboratory processing

2.5.1 Benthic samples

All the analyses were performed in both the CENAIM Laboratory Ecuador and in the Marine Biology Laboratory in Ghent University, Belgium.

The meiofauna was selected and counted using a microscope (Olympus TH2) and stereomicroscope BH2. The biological samples were processed according to Vincx (1996), and Vincx & Heip (1996) with some modifications.

The meiofauna was extracted from the sediment by using various methods: washing, decantation and centrifugation (Vincx, 1996). First, the fauna was washed out of the sediment over a sieve mesh size of 1mm to remove the macrofauna and other larger particles. The samples were collected into jars of 5 litres. Using a gentle jet of water, the samples were washed and decanted over sieves of mesh size 40μ (not 38μ sieve was available at CENAIM laboratory) about 10 times to separate sediment from the meiofauna. After decantation the residues were centrifuged using Ludox 40HS at a specific gravity of 1,18 (2500 rpm), for 10 minutes. The supernatant was centrifuged two times again using the same Ludox. This process enabled the separation of the meiofauna from the sediment and detritus.

The samples collected after centrifugation were stained overnight using Bengal Rose. This allowed easy observation and identification during counting. Under a binocular, the meiofauna was counted and identified to taxon level (nematodes, copepods, others). The density of total meiofauna and main taxa was expressed as ind.10cm^{-2}. About 120 individual nematodes were selected from the upper slice of each replicate for further identification to genus level. Vincx (1996) indicated that for

most monitoring programs, 100 nematodes from each sample were sufficient for the species identification in order to evaluate the relative abundance of the different species.

The nematodes were isolated from the sample with a very fine needle and put into a cavity block (recipient) under stereoscopic microscope into a solution, which contains 99% formaldehyde (4%) and 1 % glycerol. The organisms were maintained inside the recipient into a vial containing a bottom of 95% (v/v) ethanol at 35°C for about 24 hours (this process permits the ethanol to evaporate into the solution of formaldehyde and glycerol). Later, drops of a second solution (95 % ethanol and 5% glycerol) were added inside the cavity block; this stays in an oven at 35°C for one hour. After that, the same solution was added again and left for 8 hours. After 8 hours, we took out the samples and a third solution, which consisted of 50% ethanol (96%) and 50% glycerol was added. The cavity block stayed partly open at 35°C inside the oven until all the ethanol was evaporated and the organisms remained in pure glycerol. Generally after 12 hours we took out the samples from the oven and put them in the desiccation chamber (with silica). In this way, the nematodes were kept in a (anhydrous) glycerine solution, which has the advantage that the nematodes become transparent (necessary for species identification with a light microscope).

Organisms were mounted on glass slides after being transferred to glycerol. First the glass slide and the cover were cleaned with ethanol to avoid particles interrupting the identification of the organisms. Two rings of paraffin were put on the middle of the slide together with a drop of glycerine each one; 5 to 20 nematodes were mounted per slide. The glycerine drop with the organisms were covered; put over a thermo plate (ERMA INC) in order to let the paraffin melt and be attached to the slide. For permanent slides the cover glass was sealed with "Glycerol" (Vincx, 1996). The name and/or number and date of the sample were written on the slide.

All nematodes were identified at the genus level using a light microscope (Type Olympus BH2, under 10 times 100 magnification). The pictorial keys of Platt & Warwick (1988), Andrassy (1984a,b) and Bongers (1988) were used for the identification of the nematodes, as well as the genus files compiled at the Marine

Biology Section of the Ghent University. The Wieser's feeding types (1953b) were used for the nematode trophic groups (Annex 1).

2.5.2 Physical-chemical analysis

The granulometric analysis of the bottom samples was performed at Marine Biology Laboratory in Ghent University. The grain size of the sediment was catalogued on the basis of the Wentworth scale (Buchanan, 1984; Annex 2).

The analysis of the nutrients in the water such as nitrogen (nitrite ($N\text{-}NO_2$); nitrate ($N\text{-}NO_3$), total ammonia (TAN)) and phosphorous (as reactive phosphate ($P\text{-}PO_4$), of the soil), was performed with Standard Methods (Clesceri *et al.,* 1998).

2.6. Statistical Analysis

General statistical analyses have been used for the investigation of the results obtained from the different surveys and experiments. The specific statistical analyses are presented in each chapter separately.

Differences in densities between dates, treatments and ponds were investigated by means of one-way analyses of variance and multivariate techniques. A log+1 transformation was used prior to the analysis. A preliminary exploration of the data was performed to determine homogeneity of variances (Statistica 4.1. 1995, 1999; Statistica 6.0, 2000; Zar, 1999). Further comparisons of density estimates were carried out with the post hoc Scheffe test, using 95% confidence limits. (When conditions for the use of parametric test were not fulfilled, Kruskal-Wallis, Spearman ranks test (Statistica 4.1, 1995,1999; 6.0, 2000,), was employed.

Analysis of variance (ANOVA)

ANOVA statistic was used to test and evaluate the effect of abiotic and biotic factors, within and between the different treatments and field observations. Contrast analysis in the Statistica 4.1, 6.0 programs, permitted to statistically test specific differences in certain areas of the design used in the study. In one-way ANOVA the groups whose

means are compared are usually thought of as different categories of a single factor. The degrees of freedom (df) hold the df based on the number of observations found in the variables associates with each row of the table.

A one-way ANOVA, tests differences between groups that are only classified on one independent variable. You can also use multiple independent variables and test for interactions using factorial ANOVA (see below). The advantage of using ANOVA rather than multiple t-tests is that it reduces the probability of a type-I error. Making multiple comparisons increases the likelihood of finding something by chance—making a type-I error. One potential drawback to an ANOVA is that one looses specificity: all an *F*-test tells you is that there is a significant difference between groups, not which groups are significantly different from each other. To test for this, one uses a post-hoc comparison to find out where the differences are, which groups are significantly different from each other and, which are not. Some commonly used post-hoc comparisons are Scheffe's and Tukey's.

The F-test or F-ratio is the ratio of the treatment mean square to the Error mean square. When the null hypothesis is true, both mean square values estimate sigma2, the population variance, so the F-ratio will tend to be near 1.0. The mean square for Error estimates sigma2 even when the treatment means differ, but the mean squares for treatments will grow as the treatment means vary. Thus when the treatment means are different, the F-ratio will tend to be larger than 1.0. The probabibility (Prob) value is the probability of observing an F-ratio as large as the one computed or larger, if the null hypothesis were true. The null hypothesis of equal treatment means can be rejected when the Prob value is smaller than the alpha-level for the test.

Two-way ANOVA

A two-way ANOVA (two factor ANOVA) is used when two factors are considered simultaneously. It determines the interaction effects between independent variables in a set of data. The Interaction effects occur when the impact of one independent variable depends on the level of the second independent variable. And, it can measure both the difference among treatments and among age of participants simultaneously. A factorial ANOVA can show whether there are significant main

effects of the independent variables and whether there are significant interaction effects between independent variables in a set of data. Interaction effects occur when the impact of one independent variable depends on the level of the second independent variable.

Multiway ANOVA

Multi-way ANOVA introduces more than two factors, each specified by its own variables. The factors might affect the response variable both individually and jointly trough some interaction. The assumptions underlying multiway ANOVA are the same as those for two way ANOVA.

Levene's Test for equality of Variances

Levene's test (Levene, 1960) is used to test if *k* samples have equal variances. Equal variances across samples are called homogeneity of variance. Some statistical tests, for example the analysis of variance, assume that variances are equal across groups or samples. The Levene's test can be used to verify that assumption.

Scheffes test

It is a log-ANOVA test for homogeneity of variances. To carry out this test one forms sub samples of the variables in each group of an ANOVA and separately calculates the variance of each sub sample. These resulting variances are transformed to their natural logarithms and a single classification ANOVA is carried out on these logarithmic transforms. If the resulting ANOVA is significant it means that the variances among groups are significantly greater than would be expected on the basis of the average variances within groups (heteroscedasticity) (Sokalf & Rohlf, 1981). (Post Hoc test Scheffe, **highly significant=99%; *significant=95%).

Non-parametric Spearman Ranks Correlations coefficient test

Non-parametric Spearman Rank Correlation was used to establish the relation between two sets of variables (Conover, 1971; Sokal & Rohlf, 1995). We used them here to determine the correlation between biotic and abiotic factors (nematodes species, shrimp variables and environmental variables). The assumption of normality

is not required. The correlation is represented by a linear regression line (least squares). The variables need first to be ranked in size. Next the difference is calculated between every pair of ranked values. The Spearman-rank correlation is calculated on the basis of the following formula:

$$R = 1-(6 \times \sum D^2 / n^3-n) \qquad 2.3$$

Where D = A' – B' (A'=first variable range and B'=second variable range).

Kruskal-Wallis one-way analysis by ranks

This is also a non-parametric test (Kruskal & Wallis, 1952). The null hypothesis of this test states that all samples are derived from the same population and that there are no differences in the mean densities between the various samples. The amount of observations in every sample may differ. k is partitioned according to a X^2 distribution with i-1 degrees of freedom. The null hypothesis is rejected when $p < 0,05$.

$$H= 12 / n(n+1) \times \sum R_1^2 / n_j – (3(n+1)) \qquad 2.4$$

Where n_j (j = 1, 2,..., k) represent the sample sizes for each of the k groups (i.e., samples) in the data. Next, rank the combined sample. The compute Ri is equals to the sum of the ranks for group i.

This statistic approximates a chi-square distribution with k-1 degrees of freedom if the null hypothesis of equal populations is true.

Principal Component Analysis (PCA)

The starting point for a PCA is the original data matrix rather than a derived similarity matrix (though there is an implicit dissimilarity matrix underlaying PCA, that of Euclidean distance). The data array is though of as defining the positions of samples in relation to axes representing the full set of species, one axis for each species. There are many species so the samples are points in a very high-dimensional space. In the PCA, the PC1 is the axis, which maximises the variances of points projected perpendicularly onto it (the biggest differences between samples take place along this axis). This axis is a sum of roughly equal (and positive) contributions from each

of the species; it is essentially ordering the samples from low to high total abundance. The PC2 is constrained to be perpendicular to axis PC1, but is then again chosen as the direction in which the variance of points projected perpendicularly onto it is maximised (the differences between samples changes few in this direction). PC3 is the axis perpendicular to both PC1 and PC2 (Clarke & Warwick,1994).

Multi-dimensional scaling (MDS)

The MDS refers to the Kruskall; non-metric procedure. The MDS construct a configuration of samples, in specified number of dimensions, which attempts to satisfy all the conditions imposed by the rank (dis)similarity matrix. The inter-point distances have the same rank order as the corresponding dissimilarities between samples. It is base in relevant samples information; not work with the original data array, so there is complete freedom of choice to define similarity of community composition in whatever terms are biologically most meaningful. And the number of species on which it was based is largely irrelevant to the amount of calculation required (Clarke & Warwick, 1994).

Diversity index

The different diversity patterns within the nematode communities were investigated by interpreting the k-dominance plots (Lambshead *et al.,* 1983). Univariate measures of diversity are species richness (S), the exponential of the Shannon-Wiener index (exp H') (log base 2) and the reciprocal of Simpson's index (1/simpson) (Whittaker, 1972, 1977; Magurran, 1991). Hill (1973) labeled these diversity measures N_0, N_1 and N_2, respectively.

$$H' = \sum piLog\ Pi \qquad 2.5$$

Where Pi= proportional abundance of a species in a sample

The Shannon-Wiener index (Platt *et al.*, 1984; Pielou, 1975) is often accompanied by the evenness, a measure for the dispersion of the different species. The evenness is important for interpreting H', because otherwise it is difficult to determine whether the difference in diversity is the result of a difference in species richness or an evenly

dispersion of the number of individuals per species. The evenness varies between 0 and 1. When the evenness is 1, all species are equally represented in the sample.

Production index

The Production Index, IPM (formula 3.1) (Bayot, 2004) of the shrimp pond was calculated as well and used as one of the possible structuring factors of the benthic communities. This index was developed to compare the production levels between shrimp production cycles. The Production index (IPM) standardizes management variables and includes culture time (in days, CT); initial shrimp density (in ind.$10m^{-2}$; D); final average weight of shrimp (W) and Production/ha/cycle ($kg.ha^{-1}.cycle^{-1}$), P.

IPM=P/D / CT/W

CHAPTER 3

Temporal variability in nematode communities of a shrimp pond bottom in Palmar, (Guayas province, Ecuador)

3 Temporal variability in nematode communities of a shrimp pond bottom in Palmar, (Guayas province, Ecuador)

3.1 Abstract

The nematode community of a pond bottom of an Ecuadorian shrimp farm was investigated. The temporal fluctuation in diversity and density of this community was followed throughout one year, and with four shrimp production cycles. Management practices and some relevant environmental variables were integrated in the analysis. The nematode density was established between 0 and 80 ind.10cm^{-2}. Nine different species were identified belonging to eight families, dominated by *Terschellingia longicaudata, Daptonema* sp and *Spilophorella papillata.* There were no seasonal patterns within the nematode community, although differences among sampling dates were obtained. A positive correlation was found with the total numbers of nematodes in the pond bottom. No differences in nematode community structured were observed related to shrimp production cycles. A positive correlation was found with the total number of nematodes in the pond bottom and the temperature. The densities of *Spilophorella papillata* were positively correlated with temperature and negatively correlated with oxygen; *Daptonema* sp was also positively correlated with temperature. Last two species have increased reproduction (highest abundance of juveniles).

3.2 Introduction

The white shrimp, *Litopenaeus vannamei,* is one of the most important commercial natural products in Ecuador (Exportaciones Ecuatorianas 2004; CNA, 2005). Moreover, Ecuador is the leading producer in the western hemisphere in spite of the evolution of the shrimp production in Brazil in the last years and even with the White Spot Syndrome Virus (WSSV) presence that affected the shrimp industry since 1999 (Figure 1.1; Chapter 1).

Since the eighties, the Ecuadorian shrimp industry experimented a quick growth, mainly due to the use of cultured larvae (hatchery seeds), improved feeds and the high profit of shrimp farms, which made its expansion attractive (Leung, 2000). The shrimp aquaculture activities increased from a system with low shrimp densities (2-8 thousand shrimp.ha^{-1}) in the eighties to high shrimp densities (100 - 150 thousand shrimp.ha^{-1}) in 2000 (Rosenberry, 1999; 2001). Therefore, the requirements of artificial food also increased, which caused not only an increase in the total production cost but in the nutrient load on the pond ecosystem. An excess of nutrients application resulted in eutrophication conditions inside the shrimp pond. This eutrophication is often at the basis of anoxic conditions in the shrimp pond bottoms (Buford & Longmore, 2001; Boyd, 2003). Therefore, emphasis is put recently on the search for alternatives of artificial food in order to sustain aquaculture activities for longer periods.

One of the problems to enhance a 'natural' sustainability in aquaculture is the lack of basic information on shrimp pond ecosystem structural characteristics and dynamics. The organisms of the water column are rather well documented (Burford, 1997; Guerrero-Galván *et al.,* 1998; Alongi *et al.,* 1999; Johnson *et al.*, 2002; Coman *et al.,* 2003) but the life in the sediments is very much unknown (Rubright *et al.*, 1981; Ordner *et al.,* 1990; Tidwell *et al.* 1997). Nevertheless, some benthic organisms seem to be an important part of the diet of 'wild' shrimp living in the sea (Rubright *et al., op cit.*; Hunter *et al.,* 1987; Hedqvist-johnson & Andre, 1991; Bombeotuburan *et al.,* 1993; Nilsson *et al.,* 1993; Tidwell *et al.,* 1997; Nicovita, 1997; Nunes *et al.,* 1997; Hill, 2005).

It seems that the age of the pond influences the characteristics of the benthic communities in the shrimp ponds; new ponds have lower concentrations of soil organic matter than older ponds (Munsiri *et al.,* 1995, 1996). There is also influence from the pond's geographic position and, of course of the different management practices (fertilizers and food, mainly). Preliminary field observations have indicated the presence of Polychaeta, Nematoda, Copepoda and Mollusca within the benthos community of the shrimp pond (Cornejo-Rodríguez, 1999). There are few studies about biological

communities in shrimp ponds. There are mainly related with macrofauna (Rubright *et al.*, 1998), general benthic organisms (Martínez-Córdova *et al.*, 2002) and a few of nematode description (Kito, 1998). Most of the studies are related with the influence of aquaculture on the surrounding ecosystem (Mazola *et al.*, 1999; Orellana *et al.*, 2001; Mirto *et al.*, 2002). This study emphasizes on the variability of the dominant benthic group, the Nematoda.

Several studies have demonstrated that shrimp eats nematodes under natural conditions (Phil & Rosenberg, 1984; Smith & Coull, 1987; Jonsson *et al.*, 1993; Nilsson *et al.*, 1993), or under experimental conditions (Gerlach & Schrage, 1969; Bell & Coull, 1978), and few under aquaculture systems (Nicovita, 1997; Feller, 2004). Otherwise, nematode community structure can be used as a tool for bio-monitoring in order to assess the quality of the environment, such as nutrient load leading to oxygen stress, and so on (Atkinson 1973a, b; Wieser *et al.*, 1974; Wieser & Schiemer, 1977; Warwick, 1981b; Moens, 1999).

We have studied the temporal variability of the nematode community inside a shrimp pond, considering four production cycles (each of an average of three months duration) and to determine whether this community is affected by management practices. The management practices under consideration are the application of fertilizers, feeds, antibiotics (known as "additives"), as well as the pond drained periods, shrimp stocking and shrimp harvest period.

3.3 Material and methods

The samples were collected at shrimp Pond A (8 has) in a farm located in Palmar (Ecuadorian coast at 2°11´S, 80°45´W) (figure 3.1). The sampling campaigns were performed between September 2000 and October 2001, during the coastal dry and rainy seasons. The rainy (warm) season runs from December through April, while the

dry (cold) one from June through October, with May and November considered as transition periods. The rainiest months are usually February and March.

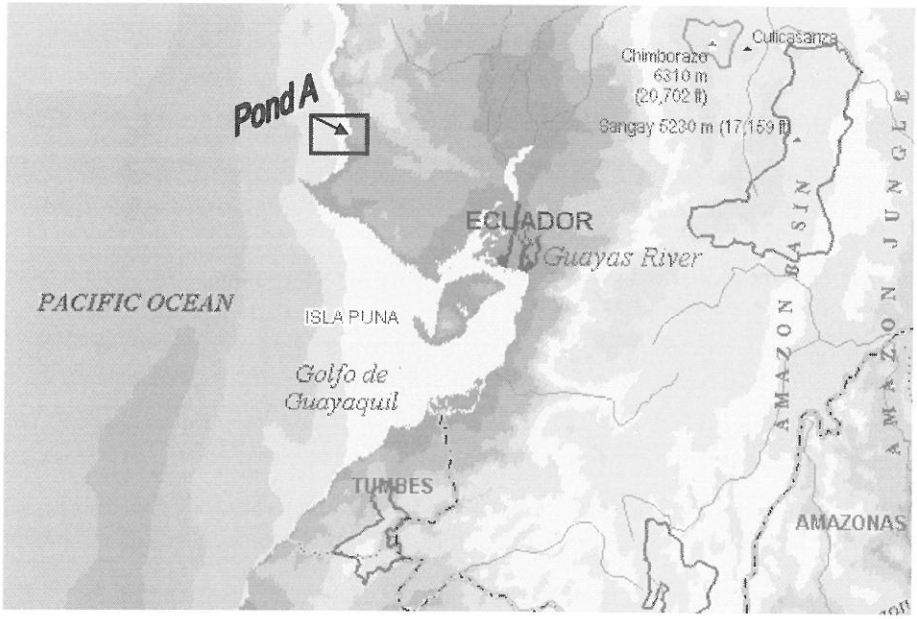


Figure 3.1 Location of monitoring programs.

There were four shrimp production cycles during the study period (September 2000 – October 2001) (Table 3.1). The samples were taken monthly on five sites (A through E) inside the shrimp pond with three replicates at each site (see Chapter 2). The selection of these fives sites and the number of replica were based on the number of samples necessary to evaluate the nematode community according to Ramírez-González, (1999).

The meiobenthic samples for nematode investigations were collected with a 5cm

diameter sample core, pushed into the sediment through a depth of 5cm. The samples were preserved with 4% neutralized formaline. We followed Vincx & Heip (1996) for sample preparation. The identification of the organisms was done with a Olympus BH2 microscope. The nematode community abundance per date is expressed as ind.$10cm^{-2}$.

The temperature (^{o}C), oxygen ($mg.l^{-1}$), salinity (PSU) and pH of the water column, were monitored weekly inside the pond. Temperature and oxygen were measured with an oxygen-meter YSI 55, Yellow Springs Instrument Co; pH was measured with 320/SET-1, pH meter WYW. Salinity data was excluded because of inconsistency and missing points within the data.

Table 3.1 Sampling dates during the four shrimp production cycles. The shrimp pond drained periods are marked with an "*".

Shrimp Production Cycle	Management data		Sampling dates
	Stocking date	Harvest day	
First	9 October 2000	24 January 2001	21 September (*) 20 October 4 November 5 December 4 January
Second	30 January 2001	2 April 2001	30 January 1 March
Third	9 April 2001	5 June 2001	12 April 14 May 12 June (*)
Fourth	25 June 2001	2 October 2001	12 July 13 August 14 September 12 October (*)

The seasonality of the nematode densities for all species as well as by each one was investigated considering the dry and rainy seasons and their transition periods. Initially, the production cycles were analyzed separately from the shrimp pond drained periods. The population structure for all species and for each individual one was also analysed.

Information about the local shrimp management practices such as the amount and frequency of the application of artificial food and other additives, and the resulting

shrimp biomass and survival rate were also obtained.

An ANOVA was applied when assumption of homogeneity of variances and independency of mean and variances were fulfilled. Data were Log+1 transformed. When assumptions for normality were not fulfilled after Log+1 transformation, non-parametric Kruskall-Wallis test was applied. The average number of replicas per point was used to evaluate the relationships among sample dates, seasons and shrimp production cycles (Statistica 6.0, 2000). Spearman Rank Correlation (Sokal & Rohlf, 1981, 1995) was used to analyse the relationship between oxygen and temperature, and nematode communities' distribution plus the relationship inside these communities (Statistica 6.0, 2000).

The Production Index, IPM (formula 3.1) (Bayot, 2004) of the shrimp pond was calculated as well and used as one of the possible structuring factors of the benthic communities. This index was developed to compare the production levels between shrimp production cycles. The Production index (IPM) standardizes management variables and includes culture time (in days, CT); initial shrimp density (in ind.$10m^{-2}$; D); final average weight of shrimp (W) and Production/ha/cycle (kg.$hectares^{-1}$.$cycle^{-1}$) P.

$$IPM=P/D \,/\, CT/W \qquad (3.1)$$

A Multi-dimensional scaling (MDS) (Clarke & Warwick, 1994) was performed to evaluated the relationship of sampling points inside the ponds.

3.4 Results

3.4.1 Environmental variables

In general, the lowest oxygen values were registered between December 2000 and March 2001 ($\leq 3mg.l^{-1}$), at the end of the first production cycle and, during the second shrimp production cycle. The rest of the time, the oxygen levels were higher than 3

mg.l-^{1} (figure 3.2, upper panel). For detailed information about the temperature and oxygen levels see Annex 3.

A temperature increase was registered from October 2000 to March 2001 (figure 3.2). A rapid decrease of 6 – 7 °C was measured at the end of April- early May. Notice that the horizontal axis is not at regular intervals. The lowest temperature period corresponded to the dry season (July - October 2001) and the highest temperature period to the warm one (December 2000 – March 2001) (figure 3.3).

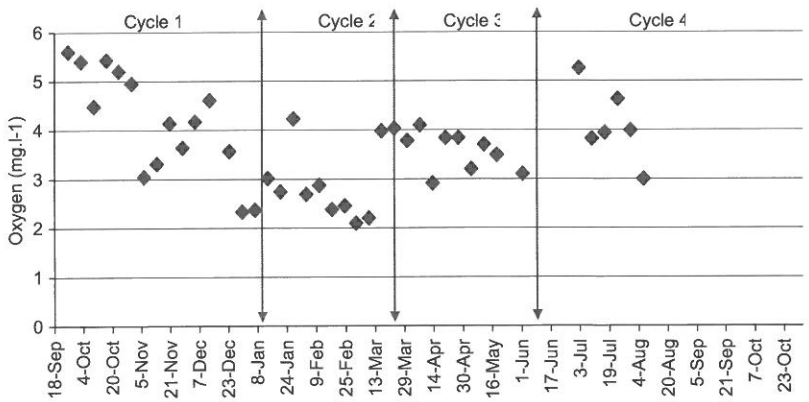


Figure 3.2 Oxygen during the time series in Pond A, Palmar shrimp farm. Notice that the horizontal axis is not at a regular interval.

The seasonal cycle of the sea surface temperature in the El Pelado station near Palmar, indicate that observed temperature pattern of 2000-2001 is comparable with the cycles in the former years (figure 3.4). Period 1997-1998 indicates an El Nino event, in El Pelado station (1°55'53"S, 80°46'55"W), near Palmar; the sampled shrimp pond.

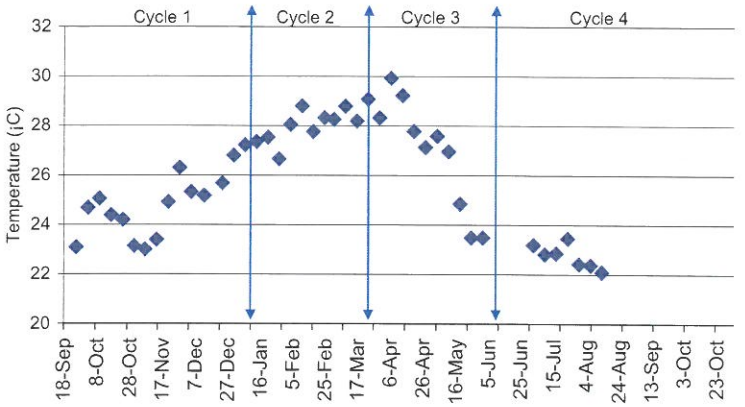


Figure 3.3 Temperature during the time series in Pond A, Palmar shrimp farm. Notice that the horizontal axis is not at a regular interval.

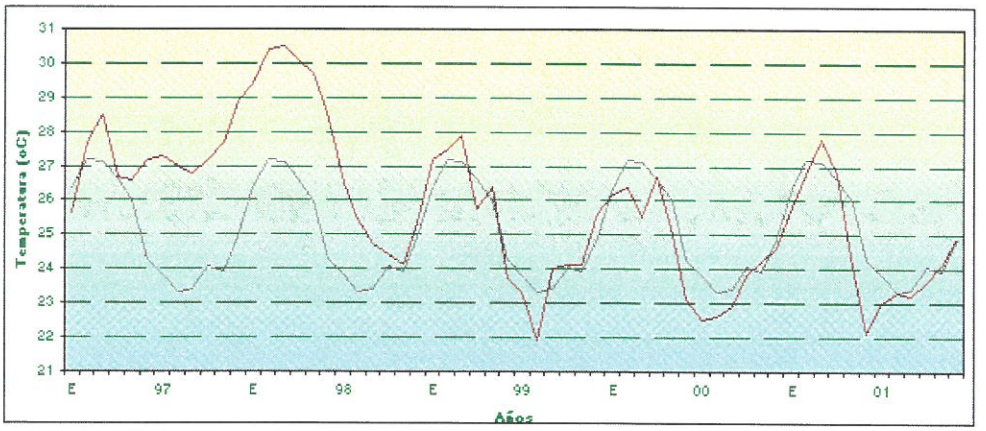


Figure 3.4 Sea surface temperature at El Pelado –FUNDACION CENAIM-ESPOL. The grey line is the seasonal cycle and the red one the observed value. From http://www.cenaim.espol.edu.ec/acuiclim/hist.html

3.4.2 Description of the nematode community

Throughout the whole sampling period, nematodes dominated the benthic environment with an average relative abundance, varying between 85% and 100%. Copepods were also found in the samples and are analysed in next chapter. Other groups were not present in this pond. From the 210 replicates, we counted 13,675 nematodes and identified 3,642 individuals to the species level. The nematodes average density was 52 ind.10cm^{-2} with a minimum value of 1 ind.10cm^{-2} in October 2000 and a maximum value of 80 ind.10cm^{-2} in November 2000 (Figure 3.5). The nematode density fluctuations were calculated for the four shrimp production cycles shown in table 3.1.

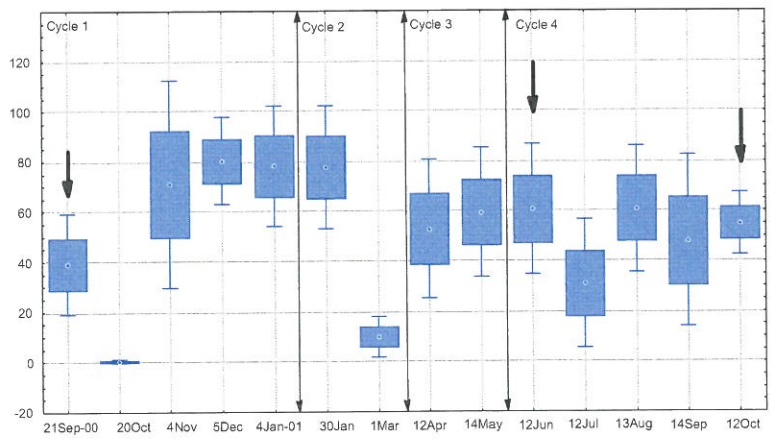


Figure 3.5 Temporal fluctuations of nematode densities. Double-headed arrow separate the four shrimp production cycles. (Data:mean/SE/1.96SE).

No assumptions to ANOVA were fulfilled. A Kruskal-Wallis test was applied to find if there were significant differences for total nematode densities between sampling dates

(H=23.60; df=13; p<0.05). There were no significant differences between the rainy and dry seasons and the transition periods in the total density of nematode species (H=15.98; df=13; p>0.05). There were neither significant differences among the four shrimp production cycles and the pond drained periods (H=3.30; df=4; p>0.05). No trend could be detected in nematode densities. The highest number of species corresponded to Cycle four (nine species), where the highest shrimp biomass was obtained.

The MDS analysis indicated an association of sampling stations inside the pond for most of the months; exceptions were May, July, September and October of 2001 (figure 3.6).

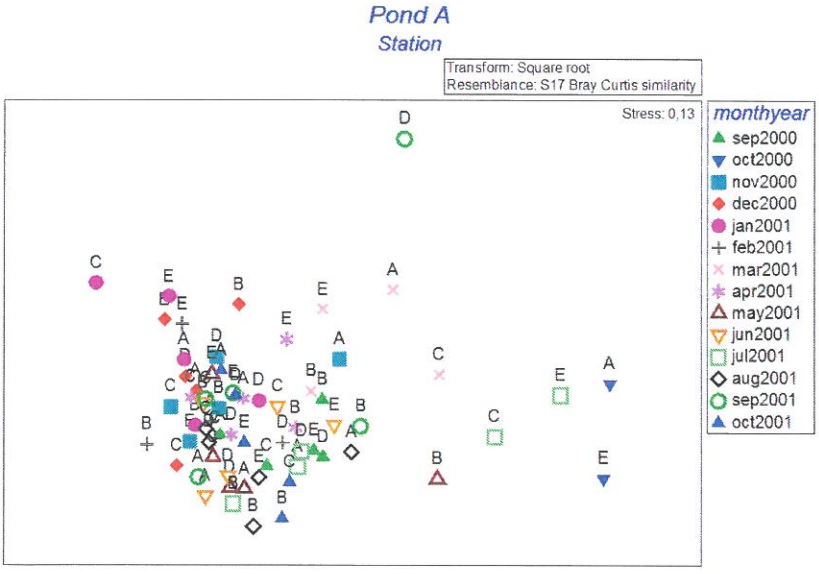


Figure 3.6 MDS analysis to sampling stations inside the Pond A. A-E letters means replicas inside the stations

The most abundant species were *Terschellingia longicaudata* (52.8%) and *Spilophorella*

papillata (35.9%). Other species like *Sabatieria* sp, *Daptonema* sp, *Theristus* sp, and *Gomphionema* sp were less abundant aff *Chromaspirina* sp, aff *Sphaerolaimus* sp and one species of the Oncholaimidae family were also present. So, in total only 9 nematode species were identified (Table 3.2). The detailed species composition per sampling time is given in Annex 3.

Table 3.2. Relative abundance (%) of the nematode species in Pond A, together with their feeding types according to Wieser, 1953a,b: 1A Selective detritus feerders, 2A Epistratum feeders, 1B Non-selective detritus feeders, 2B Predators & omnivores.

Nematodes Species	Abundance (%)	Feeding type
Terschellingia longicaudata	52.83	1A
Spilophorella papillata	35.91	2A
Gomphionema sp	0.58	2A
Sabatieria sp	1.40	1B
Theristus sp	0.49	1B
Daptonema sp	8.57	1B
Oncholaimidae	0.05	2A
aff. Chromaspirina	0.08	2A
aff. Sphaerolaimus	0.08	2A

The densities of the different species did not follow the same temporal distribution pattern. No assumptions of ANOVA to species were fulfilled. Kruskall-Wallis test was applied for the three most abundant species. *Terschellingia longicaudata* densities do not register significant differences among dates ($H=20.40$; $df=13$; $p>0.05$), seasons ($H=16.57$; $df=2$ $p>0.05$) or shrimp production cycles ($H=7.47$; $df=4$; $p>0.05$) (figure 3.7). *Spilophorella papillata* densities were statistically different among dates ($H=26.76$; $df=13$; $p<0.05$), but not seasons ($H=5.76$; $df=2$; $p>0.05$), nor shrimp production cycles ($H=5.48$; $df=4$; $p>0.05$). This specie registered the highest densities at the first shrimp

production cycle (figure 3.8).

Daptonema sp (figure 3.9) has a similar fluctuation that *S. papillata*. There were significant differences among dates for this specie (H=23.56; df=13; p<0.05), but no statistical differences were obtained when the seasonality was considered (H=1.10; df=2; p>0.05) or among the four shrimp production cycles (H=1.08; df=4; p>0.05). The other species densities were analysed together but no significant differences were observed among dates (H=15.80; df=13; p>0.05), seasons (H=0.06; df=2; p>0.05) or shrimp production cycles (H=8.73; df=4; p>0.05) (Figure 3.10). The shrimp drained periods have not influence in the density of these nematode species.

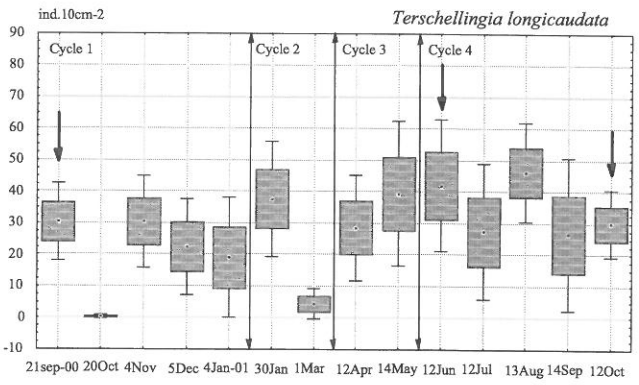


Figure 3.7 Temporal fluctuations of *Terschellingia longicaudata* densities. Double-headed arrow separated the four shrimp production cycles. The thick arrows show the pond-drained period. (Data: mean/SE/ ±1.96SE).

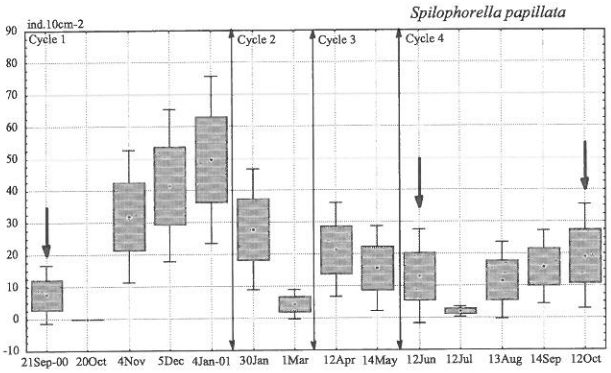


Figure 3.8 Temporal fluctuations of *Spilophorella papillata* densities. Double-headed arrow separated the four shrimp production cycles. The thick arrows show the pond-drained period. (Data: mean/SE/ ±1.96SE).

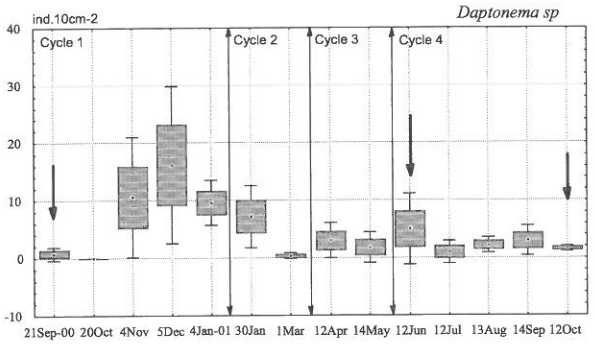


Figure 3.9 Temporal fluctuations of *Daptonema* sp densities. Double-headed arrow separated the four shrimp production cycles. The thick arrows show the pond-drained period. (Data: mean/SE/ ±1.96SE).

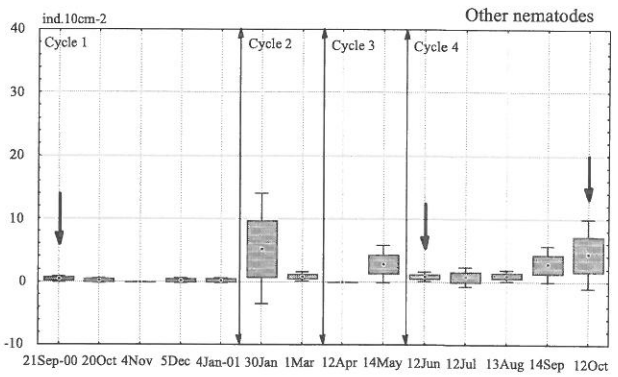


Figure 3.10 Temporal fluctuations of other nematodes species densities. Double-headed arrow separated the four shrimp production cycles. The thick small arrows show the pond-drained period. (Data: mean/SE/ ±1.96SE).

The Spearman ranks correlation test applied to the total density and species densities of nematodes confirmed that the nematode density was determined by the presence of *T. longicaudata* and *S. papillata* (r=0.78 and 0.69, respectively; $p<0.01$).

3.4.3. Population structure

The population structure (relative abundance of juveniles, females and males) of the total nematode community indicated that juveniles were present throughout the year ranging between 20 – 60 % (Figure 3.11) of the community. No clear seasonal trends could be detected in the recruitment process.

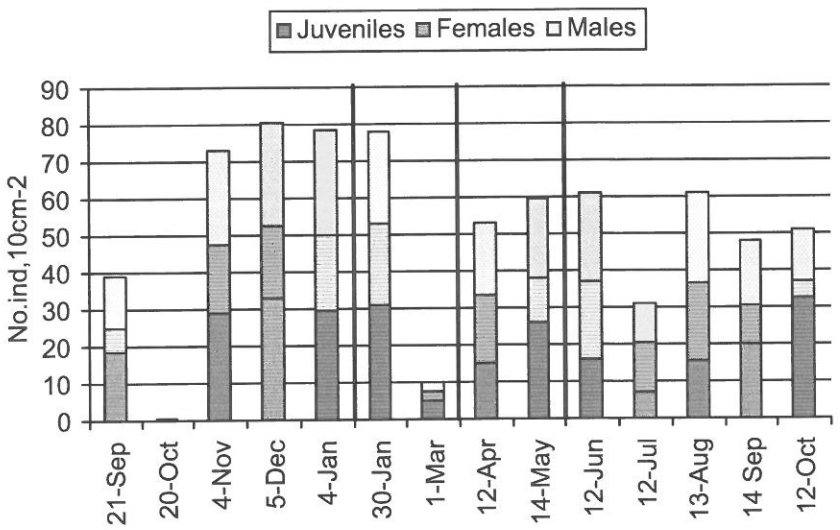


Figure 3.11 Nematode population structure at Pond A (vertical lines separate the shrimp production cycles).

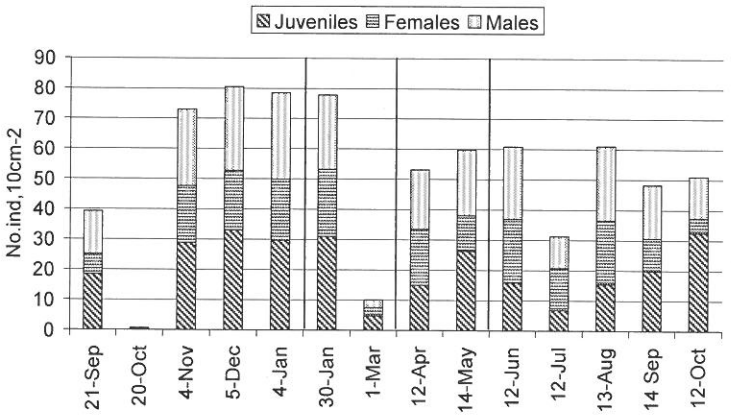
The population structure by species is indicated at figures 3.12, 3.13 and 3.14. We only analyzed the three most abundant species. There were periods where low numbers of nematodes were found and the presence of juveniles look overestimated as is the case of *Daptonema* sp, I March 1, when only 2 juveniles were obtained, and October 12, when just 7 juveniles and 1 nematode adult were registered.

Errata for Chapter 3

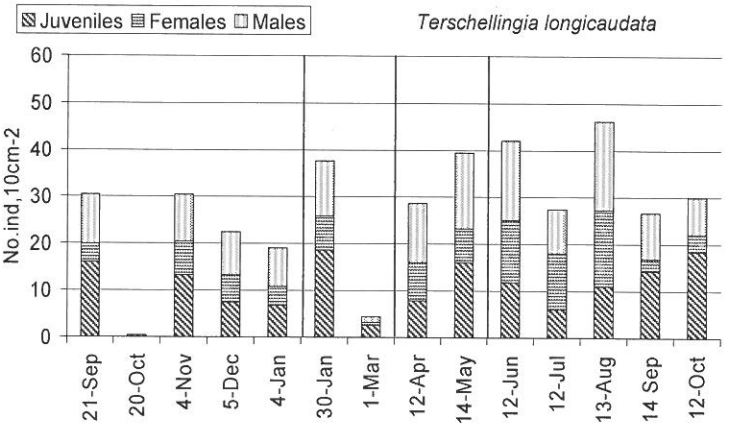
Some figures were not properly printed in the textbook.

The corrected version is given below.

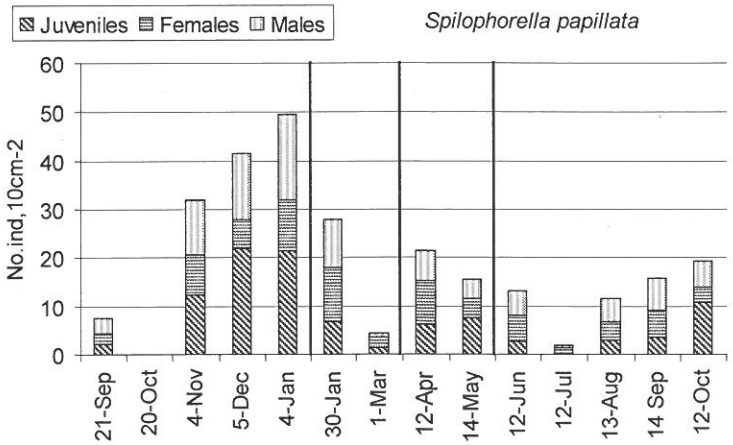
Pag.82, Figure 3.11



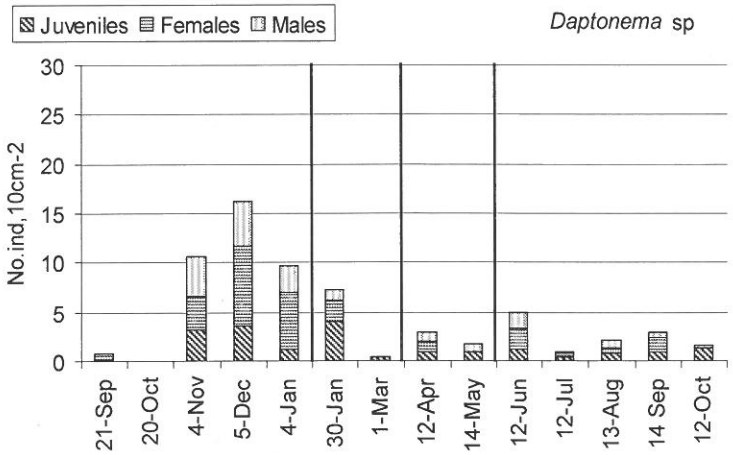
Pag.83, Figure 3.12



Pag.83, Figure 3.13



Pag.84, Figure 3.14



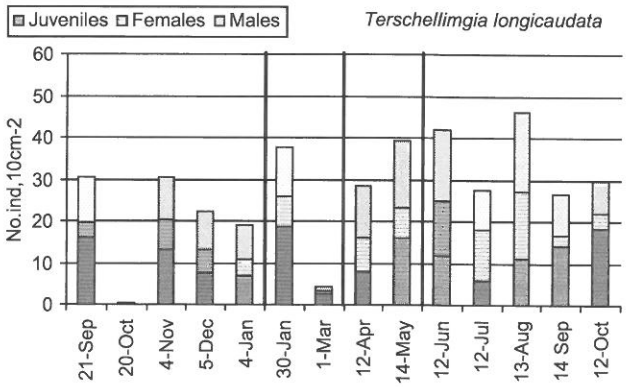


Figure 3.12 Temporal fluctuation in population structure of *Terschellingia longicaudata* (vertical lines separate the shrimp production cycles).

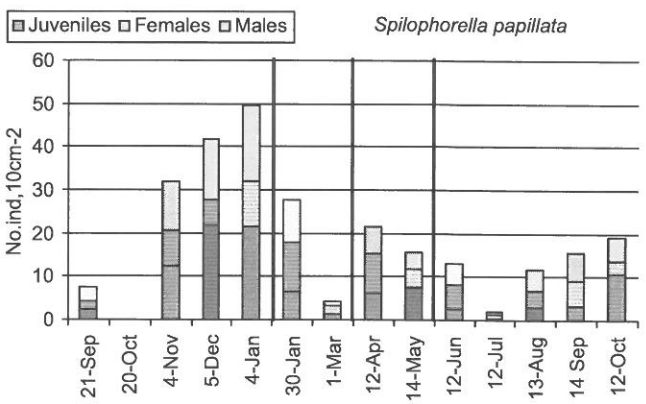


Figure 3.13 Temporal fluctuation in population structure of *Spilophorella papillata* (vertical lines separate the shrimp production cycles).

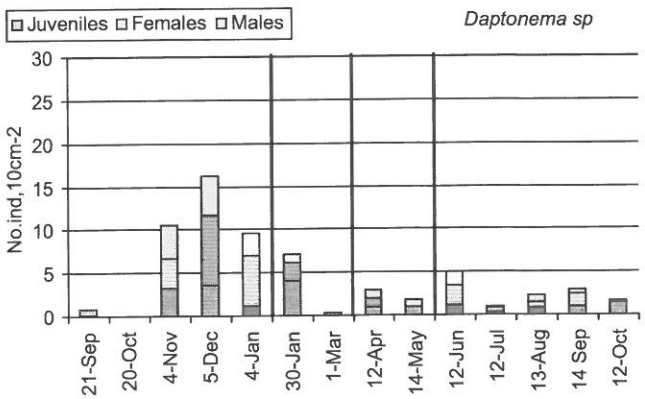


Figure 3.14 Temporal fluctuation in population structure of *Daptonema* sp. (Vertical lines separate the shrimp production cycles; the scales is different from figures 3.12 and 3.13.

3.4.4. Relationship among environmental variables and nematode communities

We did a Spearman rank correlation analysis between the nematode species densities and the environmental variables. *Spilophorella papillata was* negatively correlated with oxygen level. Other species did not show any correlation with oxygen. A statistically significant correlation was observed between total density (sum of all species, averaged of the sampling period) of the nematodes and the temperature; but individually only *S. papillata* and *Daptonema* sp showed a positive correlation with this variable (Table 3.3).

In spite of the correlations (or lack of it) there are seasonal trends in the relative abundance of the different species of nematodes. The highest relative abundance of *Spilophorella papillata, Sabatieria* sp, *Daptonema* sp, *aff Sphaerolaimus* and *aff Chromaspirina* occur during the rainy (warm) period while *Terschellingia longicaudata,*

Gomphionema sp and *Theristus* sp have the highest relative abundance during the dry (cold) season (Figure 3.15).

Table 3.3 Spearman rank correlations (r) between environmental factors and the nematode species densities. The significance is shown in the superscript as a=statistically significant and b= not statistically significant

	Species	r
Oxygen	Total density	$-0.28^{(b)}$
	Terschellingia longicaudata	$0.12^{(b)}$
	Spilophorella papillata	$-0.33^{(a)}$
	Daptonema sp	$-0.28^{(b)}$
	Others	$0.10^{(b)}$
Temperature	Total density	$0.42^{(a)}$
	Terschellingia longicaudata	$0.07^{(b)}$
	Spilophorella papillata	$0.42^{(a)}$
	Daptonema sp	$0.51^{(a)}$
	Others	$0.08^{(b)}$

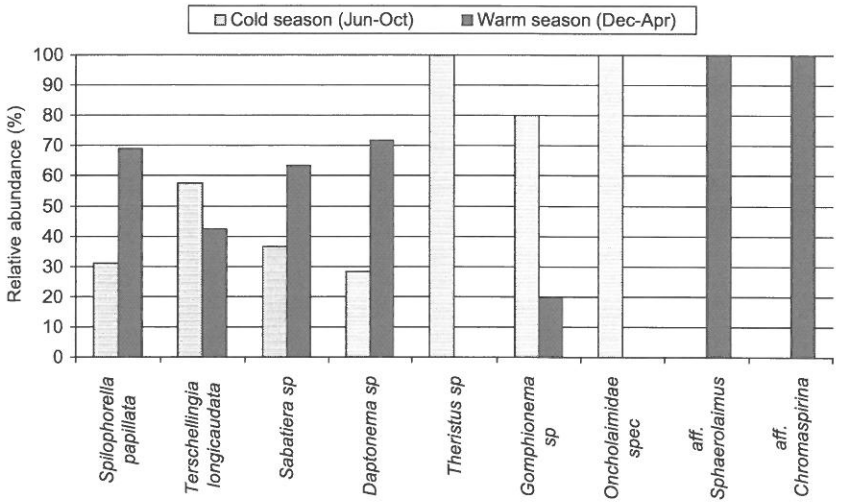


Figure 3.15 Seasonal distributions of species shown as relative abundance (%).

3.4.5 Management practices

Four shrimp production cycles occurred during the sampling period: October 2000–January 2001; January–April 2001; April–June 2001 and June–October 2001 (table 3.1). The shrimp weight and survival rate were highest in the first two cycles (table 3.4). The highest nematode densities were registered on the first two production cycles (Figure 3.5).

Table 3.4 Management information of Pond A. Palmar shrimp farm. The feed conversion rate (FCR) has been computed as equal to feed ingested (g)/ weight won by shrimp (g), where the weight won by shrimp is equal to the final minus initial weight over the whole period.

	Shrimp Cycle production			
	October 2000 -January 2001	January-April 2001	April-June 2001	July-October 2001
Initial density of shrimp larvae (ind.m^{-2})	16.9	15.0	5.6	9.4
Harvest pounds (pounds.ha^{-1})	545	540	130	789
Survival of shrimp (%)	11.80	12.12	9.69	62.48
Average weight of shrimp (g)	14.00	13.50	12.12	8.20
Feed conversion factor	0.86	0.46	0.10	0.20
Culture days	170	91	43	105
Production index	1.21	2.43	2.96	3.73
No nematode species	4	5	6	9
Densities of the nematodes (ind.$10m^{-2}$),	30	24	31	28
Time to get the highest nematode densities (days)	22	1	35	49

The production index increased from the first to the fourth shrimp production cycle. During the four shrimp production cycles several additives (feed, fertilizer, lime, antibiotics) were added at irregular intervals. The lowest period in nematode densities corresponded to October, March and July dates (figure 3.4). The fertilizer and the antibiotic were applied at the same period from October 2000 to March 2001 (table 3.5). It is important to notice that the feed is applied every day since the stocking day

Table 3.5 Application dates for the additives in Pond A, Palmar shrimp farm

Type of product	Application period (*)
Feed	October 1 – November 11 (2000)
	January 28 – February 25 (2001)
	April 8 – March 3 (2001)
Fertilizer	October 1 – November 19 (2000)
	February 11 – March 18 (2001)
Antibiotic	February 11 – March 4 (2001)
Lime	April 8 – March 3 (2001)

(*) Other dates data were not available

3.5 Discussion

The shrimp pond temperature followed the seasonal cycle observed in the adjacent coastal ocean, with higher temperatures during the rainy (warm season) and lower during the dry (cold) season. The oxygen level was over the level considered healthy to aquatic organisms (Boyd, 1990), with the exception of the second shrimp production cycle when the oxygen levels were almost always under 3 $mg.l^{-1}$. Most studies of shrimp pond conditions were often limited to the evaluation of physical-chemical conditions of the water in the pond (sometimes including information about the phytoplankton or zooplankton communities) (Boyd, 1990; Villalón, 1991; Spanopoulos-Hernández, *et al.,* 1991 *in* Martínez-Córdova & Peña-Messina, 2005; Burford & Lorenzen, 2004; Lutz, 2005). The characteristics of the benthos were not taken into account, mainly because the benthic dynamics was considered as 'not-important'. However, all processes occurring in the water phase do influence benthic activities and vice-versa (Martínez-Córdova & Peña-Messina, *2005*). The so-called bentho-pelagic coupling is especially important in shallow coastal environments (Graff, 1992; Orejas *et*

al., 2000; Konsulov & Konsulova, 2005; López *et al.*, 2005) and is also important in shrimp ponds. Within artificial shrimp ponds, the importance of the benthos and the interaction between production cycles of shrimp and the characteristics of the bottom have only been occasionally investigated (Martínez-Córdova, 1998b; Martínez-Córdova *et al.*, 2002; Martínez-Córdova & Peña-Messina, 2005) .

A poor benthic community consisting of free-living nematodes (higher than 80% of all organisms) and a few harpacticoid copepods characterize the bottom of an artificial shrimp pond (pond A) along the Ecuadorian coast in the Palmar shrimp farm. There were no other organisms in this environment. The high abundance of nematodes in relation to other meiobenthic organisms is probably caused by the tolerance of these organisms to a variety of environmental stress factors (Hartley, 1982; Bouwman, 1983; Bilyard, 1987). Especially, in artificial shrimp ponds, there is a continuous change from dry to wet periods. The shrimp pond receives fertilizers, feed, antibiotics, probiotics, among others additives, which change the level of the different compounds, including organic matter inside the ponds. Just nine nematode species were found. During the shrimp production cycle, the shrimp farmer added different kind of additives, which we could not identify in an isolated way. Nevertheless, the organic input of these additives produces an important organic enrichment in shrimp pond bottom (Boyd, 1990; Boyd & Tucker, 1998). As a consequence, the biomass of benthic animals in the ponds generally increased (Boyd & Tucker, 1998.).

In spite of the highest abundance of nematodes over other groups, the nematode densities compared to other studies (Olafsson, 1992; Okondo, 1995; Janssens, 1999 with a record of about 500 ind.10 cm^{-2}) were low in the Palmar shrimp pond bottom (densities up to 80 ind.10cm^{-2} maximum).

Li & Vincx (1993) mentioned that the seasonal cycle of nematodes can be very different from one site to another, and varied according to different local environmental conditions.. During our research a temporal variability was observed in nematodes densities, but the cause of this variability was not easy to identify because of the

multiple factors acting inside the shrimp pond bottom: physical, chemicals as well as management practices. The temporal variability was significant among sampling dates, but not seasonality of total density or species density were observed.

Changes in temperature and oxygen should be important in the density variations of the nematode species. Olafsson & Elmgren (1997) mentioned that temperature and food availability are the main factors affecting the temporal distribution of benthic populations. The highest density of nematodes was found between November 2000 and January 2001, which correspond to the warm season period in coastal Ecuador. Dye & Furstenberg (1978) showed that an increase in temperature reduces the density of the organisms; Coull (1985) showed that nematode densities had a positive correlation with temperature in a sandy site in the North inlet of South Carolina (USA) (11 years data; no statistical differences in trend in the nematode densities could be found over 7 years). In our study area, we found a positive relationship between temperature and total nematode density but mainly related to two species *Spilophorella papillata* and *Daptonema* sp (table 3.3), which are two of the most abundant species. Bouwman *et al.* (1984b) and Li *et al.* (1996) mentioned that changes in the temperature cause changes in the primary production and therefore changes in food quality for the meiobenthos. Steyaert *et al.* (2003) have shown clear responses to the changes in primary production in subtidal sediment in the North Sea and by Adao (2003) in sediments from seagrass meadows (Portuguese coast). For tropical areas, little information is available about the seasonal signals present meiobenthic communities (Hopper *et al.,* 1973; Alongi, 1987b, 1990c; Okondo, 1995; Schrijvers, 1996) and the current study did not show significant trends.

No correlation could be detected between the levels of oxygen and the nematode densities. This is contrary to what Steyaert *et al.* (1999) found in shallow subtidal water, where they demonstrated that oxygen level is of prime importance for nematodes. However, Cook *et al.* (2000), Wettzel *et al.* (2001), and Steyaert *et al.* (2005) mentioned that nematodes are more tolerant than other meiofauna taxa to anoxic conditions

(confirmed by other authors as well, e.g. Giere, 1993; Moodley *et al.,* 2000) while, crustacean meiofauna such as harpactocoid copepods are much less resistant (Nilsson & Rosenberg, 2003).

The continuous changes in water levels (stocking and harvest of the shrimp by drying out the shrimp ponds) can contribute to a continuous change in nematode communities' species composition. Nevertheless there were three species, which remain in high density over the sampled year. The presence of the relatively high densities of *Spilophorella papillata* and *Terschellingia longicaudata* indicate a high tolerance of these species to environmental changes, as salinity in the shrimp pond, which is known to vary between 35 to 50 PSU (Boyd, 1990; Green *et al.*, 1999), mainly due to the evaporation (Boyd, 1995). Coull (1985) registered a negative correlation between nematode densities and salinity in sand site. Before the start of the shrimp production cycle, natural seawater (35 PSU) is pumped into the shrimp pond and one could expect that the sea organisms were introduced in the ponds in this way and disappear later with the increase in salinity. However, we did not observe species richness in nematode communities in the pond bottom with the start of a new shrimp production cycle. *Spilophorella* spp and *Daptonema* spp have been found at the beach close to the seawater source of the shrimp farm in Palmar, Pond A (Calles, 2001, 2002). Hence, the conditions of the shrimp pond were not suitable to all nematode species. Only *Spilophorella papillata* and *Terschellingia longicaudata* could survive in this environment, with a continuous input of organic matter. There are different opinions in relationship to the input of organic matter. Soares (2000) had commented that the nematode community shows a quick response to the addition of organic matter. Sandulli & Giodic (1989) have also observed that this addition results in faunal enrichment of opportunistic species as we observed here with the three most abundant species. However, Schratzberg & Warwick, (1998) commented about a negative response of most nematode species to an increase level of organic enrichment. Essink & Romery (1994) also talked about the decrease in diversity with the increase of organic matter, which should be the case inside the shrimp ponds. During the current research, nine

nematode species were identified in the shrimp ponds while Calles (2001) colleted 12 species in the nearby sandy beach -close to the water supplied inlet that provides water to Pond A.

We do not find an abundance peak of dominant species determined by seasonality or by shrimp management practices. *Terschellingia* spp has a cosmopolitan distribution and is especially abundant under a certain disturbance stress (e.g. high adaptability to low oxygen levels, <3 $mg.l^{-1}$) (Vincx *et al.*, 1990; Soetaert *et al.*, 1995)). The three dominant species that we have found are also present in different kinds of environment such as estuarine areas, *Spilophorella* spp (Muthumbi, 1994; Rzeznik-Orignac *et al.*, 2003), *Terschellingia* spp (Austen, 1989; Li & Vincx,1993; Muthumbi, 1994; Netto & Galluci, 2002, 2003; Rzeznik-Orignac *et al.*, 2003; Soares, 2003) and *Daptonema* spp (Li & Vincx, 1993; Muthumbi, 1994; Olaffson & Elmgren, 1997; Steyaert *et al.*, 2001; Netto & Galluci, 2002, 2003); and sandy environments *Terschellingia* spp (Alongi, 1986; Calles, 2002; Gheskiere, 2005); *Daptonema* spp (Gheskiere, 2005) and *Spilophorella* spp (Burges *et al.*, 2005) . However, there is no clear inter-specific relationship between these three dominant species that one could draw from the collected data.

Some authors mentioned a strong seasonal signal in the reproduction cycle of nematodes (McIntyre & Murison, 1973; Bouwman, 1983; Palacin, 1990). This is not the case in the nematode populations of the shrimp pond bottom when the proportions of juveniles were considered. However, we observed differences in individual species occurrence depending on the season (see figure 3.14). Some species were with their highest density during the warm period (*Spilophorella papillata* and *Daptonema* sp), and even disappear completely during the cold period. Steyaert *et al.* (2001) commented that the temperature could impact reproductive and/or metabolic activity of nematodes and serve as stimuli for the nematode species to migrate to deeper layers (Alongi *et al.*, 1983; Olafsson & Moore, 1990). In spite of this, it was not possible to establish a clear time series in the population densities. In tropical areas, we have little information about the natural temporal variability of nematode communities (compared with the clear

patterns found in the temperate areas; (Heip *et al.,* 1985; Nicholas *et al.,* 1991; Epstein & Shiaris, 1992; Boucher & Lambshead, 1995; Hashimoto *et al.*, 2004).

During the shrimp production cycles, which take in average 3 months, the management practices (e.g. lime, food, fertilizers) produce strong changes in the pond bottom dynamics as well (Boyd, 1990; Villalón, 1991). Due to the fertilizers and continuous artificial feed applications, the sediment of the shrimp pond some times becomes black and some times a strong sulphur smell is present due to the soil decomposition (Boyd, & Tucker, 1992). The feeds are not evenly sprayed over the shrimp ponds. Some of the remaining feed accumulate in specific areas due to wind action, areas usually low in oxygen (personal observations), and thus avoided by the shrimps. This in turns generates different habitats within a shrimp pond, which are colonized by different nematode species. Moreover, the shrimps would only prey on those nematodes living under suitable conditions (adequate oxygen levels).

On the other hand, the shrimp activities mechanically disturb the sediment, so the oxygen and organic compounds are mixed with deepest sediment layers (Tahey *et al.*, 1996). That in turn increases the activity of deep-living bacteria and enlarges the capacity of the sediment community to deal with an enhanced supply of organic matter (van Duyl *et al.*, 1992 *in* Tahey *et al.*, *op cit.*). Steyaert *et al.* (2003) added that in the fine sediment, the nematodes are confined to the surface layers, only a few could occasionally penetrate into deeper layers, which mean declination of diversity and density with the depth in the sediment.

The microalgae are the primary food sources of a wide variety of meiofauna (Olaffson *et al,* 1999; Sandulli & Pinckney, 1999), and contribute to the growth of bacteria (Montagna & Yoon, 1991; La Rosa *et al.,* 2001), which in turn are also part of the diet of shrimp (Boyd & Tucker, 1998; Moens, 1999; Moriarty *et al.,* 2005), and of nematode diet too (Nicholas, 1984;). Moriarty *et al.* (2005) reported that bacterial productivity is high in the water column and in the sediment of shrimp ponds and these bacteria depend of various sources of organic matter inside the ponds; such as feed and fertilizer, which

become a substrate to them. Moriarty *et al.* (2005) added that the bacteria production in the sediments was higher than in the water column, these bacteria utilized the most organic matter. Then, the oxygen demand of these bacteria can lead to low dissolved oxygen levels in the system, inhibiting the shrimp growth (Van Wyk, 2005). Bacteria is part of the shrimp diet, but Moriarty *et al.* (2005) have noted that meiofauna is the link between bacteria and shrimp, and that copepods are one of the preferred preys.

A few authors have reported that large species of nematodes dominate organic enriched environments (Lorenzen *et al,* 1987; Moore & Bett, 1989; Porter *et al.*, 1996; Tsujino, 1998). But, in the shrimp pond we investigated, no large nematode species were found; the two dominants species, *Terschellingia longicaudata* en *Spilophorella papillata*, are rather small.

The presence of a crustacean predator can reduce the density of meiobenthic groups (Reise, 1979; Holland *et al.*, 1980; Mattila *et al.*, 1990; Hedqvistjohnson & Andre, 1991; Nilsson & Rosenberg, 2003); including nematodes (Bell & Coull, 1978; Smith & Coull, 1987). During the four shrimp production cycles monitored we observed a decreased in shrimp density as well as in the weight of harvested shrimp, altogether with a lower feed conversion factor. The highest number of nematode species was registered when the highest yield in shrimp was registered. It should be possible that some effects of bio-perturbation or an increase in feed density favoured the diversity of nematodes during this last period. The density of *Spilophorella papillata* decreased while *Terschellingia longicaudata* increased. Feller (2004) had demonstrated that shrimp eats nematodes; we assume that the decrease of *Spilophorella papillata* was due to an increase of shrimp predation because shrimp survival and density was higher than during the other shrimp production cycles. *Teschellingia longicaudata* situation was different; this nematode has the capacity to dwell in deeper layer or to growth under anoxic conditions inside the ponds, where shrimp does not used to be. It has been observed that shrimps are not found where the oxygen conditions are depleted. However, last findings have to

be tested in an experimental design in order to unravel the food web of the shrimp pond bottom.

T. longicaudata showed similar distribution of juveniles during all the study period but *Daptonema* sp and *S. papillata* showed the highest densities of nematode juveniles between November 2000 and January 2001; these two species were positively related with temperature. *T. longicaudata* is well adapted to shrimp pond environment, and its temporal distribution is independent of the general changes in environmental conditions inside the shrimp pond.

Aarnio & Bonsdorff (1992) argued that nematode densities increased in 7 weeks in around 40%, which was explained by the fact that nematodes are not a favoured prey to other animals. During our research the increase in nematode densities occurred after two months. While the changes in copepod densities did not follow a spatial pattern.

Heip *et al.* (1977) and Chen (1999) commented that nematodes penetrate deepest into the sediment or some times live in anoxic zones, where not all the nematode have the capacity to be; which should be the case of *T. longicaudata*; which avoid the shrimp predator activities since shrimp avoid anoxic zones inside the ponds. *Spilophorella papillata*, an epistratum feeder and *Daptonema* sp, a non-selective feeders probably remain at the surface where they could be preyed by shrimps. And *S. papillata* was negatively affected by oxygen level, there is probably that this nematode species remain just in the pond zones where the general conditions are also suitable to shrimps.

The effects of environmental variables over *S. papillata* and *Daptonema* sp, population need to be elucidated more in detail, preferably in a mesocosm experimental approach.

3.6 Conclusions

- A very poor nematode community characterizes the shrimp pond bottom of the Palmar shrimp farm, with low-density values compared to natural bottoms in comparable environments.

- *Terschellingia longicaudata*, *Spilophorella papillata*, and *Daptonema* sp are the three dominant species in the shrimp pond investigated, which made around 85% of the benthos.

- *Spilophorella papillata, Daptonema* sp, *aff Sphaerolaimus* and *aff Chromaspirina* were more abundant during the rainy (warm) season while *Terschellingia longicaudata*, *Theristus* sp and Oncholaimidae spp were more abundant during the dry (cold) season.

- Temporal fluctuation in nematode densities was observed but changes in season conditions or management practices could not be attributed as the cause of these changes.

- A positive correlation was found with the total number of nematodes in the pond bottom and the temperature. *Spilophorella papillata* and *Daptonema* sp were affected by environmental conditions inside the ponds. The first species is negatively correlated with oxygen and positively with temperature. The second species is positively correlated with temperature.

CHAPTER 4

Colonization of shrimp pond sediments: how fast can meiobenthic communities develop?

4. Colonization of shrimp pond sediments: how fast can meiobenthic communities develop?

4.1 Abstract

The colonization process of the shrimp pond bottom is analysed in different field situation. Four shrimp ponds (one coastal and 3 estuarine ponds) were investigated after drained period, during the water filling period and after stocking with shrimp larvae. Initial colonization was observed at the discharge of water into the pond. Environmental data of temperature and oxygen are also considered in the analysis. The colonization and the survival of the benthic animals within the shrimp pond during a shrimp production cycle do not follow a clear pattern. No clear effect of pond drained is observed, neither the water filled nor the stocking period. Copepod seems to be the initial colonizers, but decrease rather quickly in numbers; nematodes remained during the full shrimp production cycle. Indications are found that copepod communities are probably preyed upon by the shrimp and, that the nematodes are better competitors to survive the harsh shrimp pond environment.

4.2 Introduction

Aquaculture is the main industrial activity along the Ecuadorian coast. The shrimp ponds occupy around 1,700 km of coast. The construction of shrimp ponds for aquaculture purposes implicates the creation of a particular 'ecosystem' in a terrestrial environment (coastal sands, mangrove and saline areas; Twiley, 1989, 1998). The shrimp pond is a seawater filled ecosystem with its own characteristics. Especially, the shrimp pond bottom contains a particular mineral chemical composition (Egna & Boyd, 1997); and new flora and fauna is present, both in the water and in the sediments of the ponds with each shrimp production cycle (Martínez-Córdova *et al.*, 2002b; Martínez-Córdova & Peña-Messina, 2005).

The colonisation of the newly created ponds occurs with animals from the natural environment (Martínez-Córdova *et al.*, 2002b). The main source of organisms inside the shrimp ponds in the Gulf of Guayaquil, is the Guayas River where several groups of benthic organisms have been reported (Gualancañay, 1983; Villamar, 1983; Cruz, 1998; Tapia, 2002).

The productivity of these natural ecosystem outside the shrimp ponds is mainly kept by the nutrients coming from mangrove forest (Dittel, 1998). Alongi & Christoffersen, (1992), Boto (1992) and Netto & Galluci (2003) have mentioned that the estuarine areas with heterogeneous systems are highly productive and usually have richcommunities of fringing, benthic and pelagic biota. The management practices inside the pond (feed and fertilizers application, mainly) contribute highly to increase the level of nutrients outside (Guerrero, 2000) and inside the ponds (Villalón, 1991; Martínez-Córdova, 2003). This extra input of nutrients has an effect on the composition of the flora and fauna inside the pond, mainly polychaete communities (Martínez-Córdova, 2002). But, the composition of the shrimp pond communities also depends on the colonisation capacity of the organisms, which enter the pond with the filled incoming water from the natural environment.

One of the shrimp pond management practices, which should affect these biological communities is the drained of the pond. This practice is performed in order to 'restore' the soil properties and 'to improve soil quality' (see also Chapter 1). This process is repeated between shrimp production cycles, which are three to four times a year. The pond bottom is drained for one to three weeks in between the production cycles. Most times some parts of the pond remain wet. In this part the bottom fauna (polychaetes, nematodes, small crustaceans, etc.) are kept alive as we observed in preliminary samples collection inside a pond. Some times lime is added to the pond soil to produce an alkaline environment that helps to inactivate viruses and lime is likely kills any organism in the first centimetres of the soil (Boyd, 1998).

After this period, the pond is filled again with water and the shrimp post-larvae are added one or two weeks later. The shrimps remain there during three to four months

until they have an average weight of 12 g. During this time, the shrimp use beside the artificial feed also the natural feed inside the pond (Martínez-Córdova *et al.,* 2002b; Gamboa-Delgado *et al.,* 2003; Feller, 2004).

In this study, the meiobenthic colonization process of the shrimp pond bottom including drained period and shrimp larvae stocking period are evaluated. Focus will be put on nematodes and copepods, the dominant benthic organisms. Information of the nematode species level will be given in Chapter 5 (with the exception of data from Pond B, which is not included in this thesis the production cycle was not completed in this pond).

4.3 Material and methods

Datasets from four different shrimp ponds were chosen to evaluate the colonization process of the two more abundant groups within the benthos of the shrimp pond: the free-living nematodes and the copepods. The data set comes from Ponds A, B, C and D (figure 4.1; see also table 2.3 in Chapter 2).

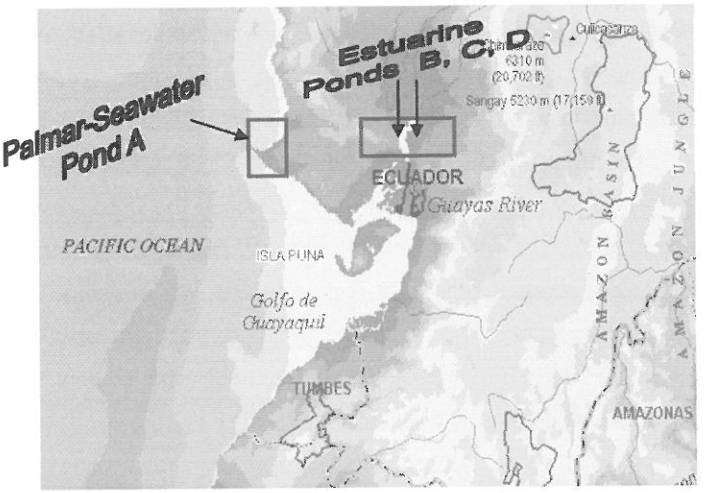


Figure 4.1 Localization of the shrimp experimental ponds.

The Pond A data set originates from the annual cycle of the nematode communities study (see Chapter 3 for details). The Pond B was monitored just during the first month of shrimp production cycle. And the Ponds C and D correspond to another shrimp farm, where the total shrimp production cycle was followed. The sampling design is indicated in table 4.1.

Table 4.1 Data set of shrimp ponds, where sampling characteristics are indicated.

Ponds	A	B	C	D
Localization	Palmar	Chongon, Guayaquil Gulf	floodgate Guayaquil Gulf	floodgate Guayaquil Gulf
Sampling period	September 2000 – July 2001	June – August 2002	June – September 2000	June – September 2000
Pond size (hectares)	8	1	4.5	1.5
Sampling frequency	monthly	once a week	Every 15 days	Every 15 days

Temperature and oxygen data were measured as relevant environmental factors. Details of sampling activity were listed in Chapter 2. The field and laboratory methodology were also indicated in Chapter 2. At the ponds C and D, the information of July 30 was eliminated from the analysis since sampling problems occurred them. Management practices data (stocking density, harvest time, etc.) from the four shrimp ponds were also collected.

An ANOVA was applied when the assumption of homogeneity of variances and independency of mean and variances were fulfilled. The data was log + 1 transformed. A Post Hoc test Scheffe was applied (only significant data are considered to the tables). When assumptions for normality were not fulfilled after log+1 transformation, non-parametric Kruskall-Wallis test was applied. The total number of replicas was used to evaluate the relationships among sample dates, seasons and shrimp production cycles (Statistica 6.0, 2000). Spearman Rank Correlation (Sokal & Rohlf, 1995) was used to analyse the relationship between oxygen and temperature and nematode community's distribution and the relationship inside these communities (Statistica 6.0, 2000).

4.4 Results

4.4.1 Environmental variables

The environmental factors of the four ponds are indicated in figures 4.2 and 4.3 (Extended data in Annex 4). The water temperature was on the average between 25-26°C in all ponds, even though B, C and D were all sampled during the dry-cold season (June - September). However, Pond A showed an increase in temperature up to 30°C mainly due to the rainy-warm season sampling (September 21st, 2000 –June 12, 2001).

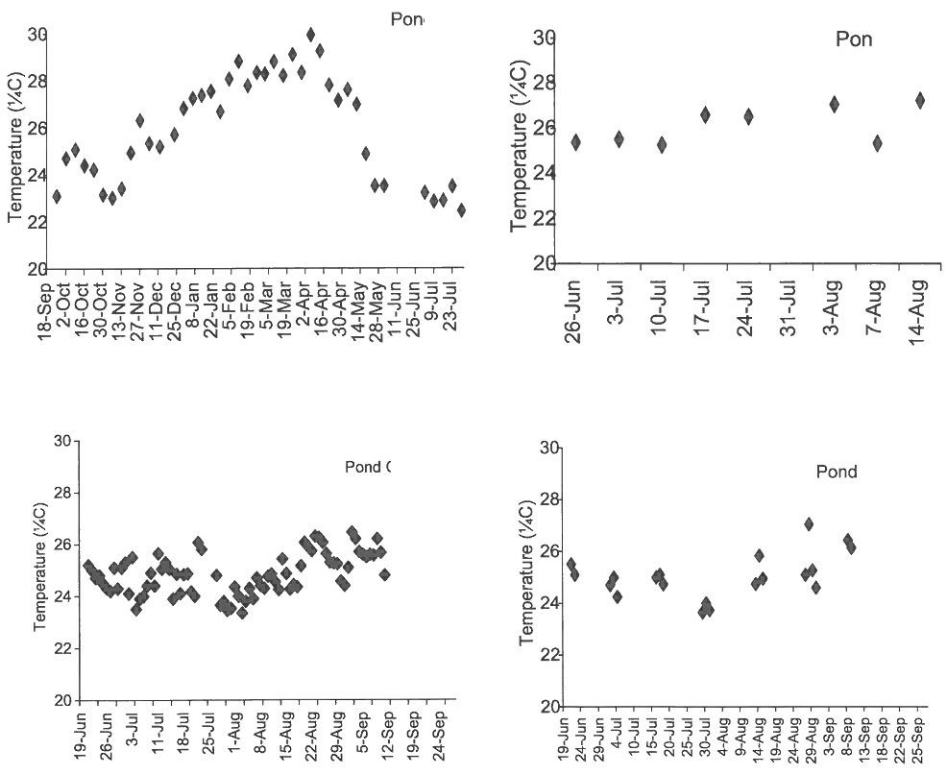


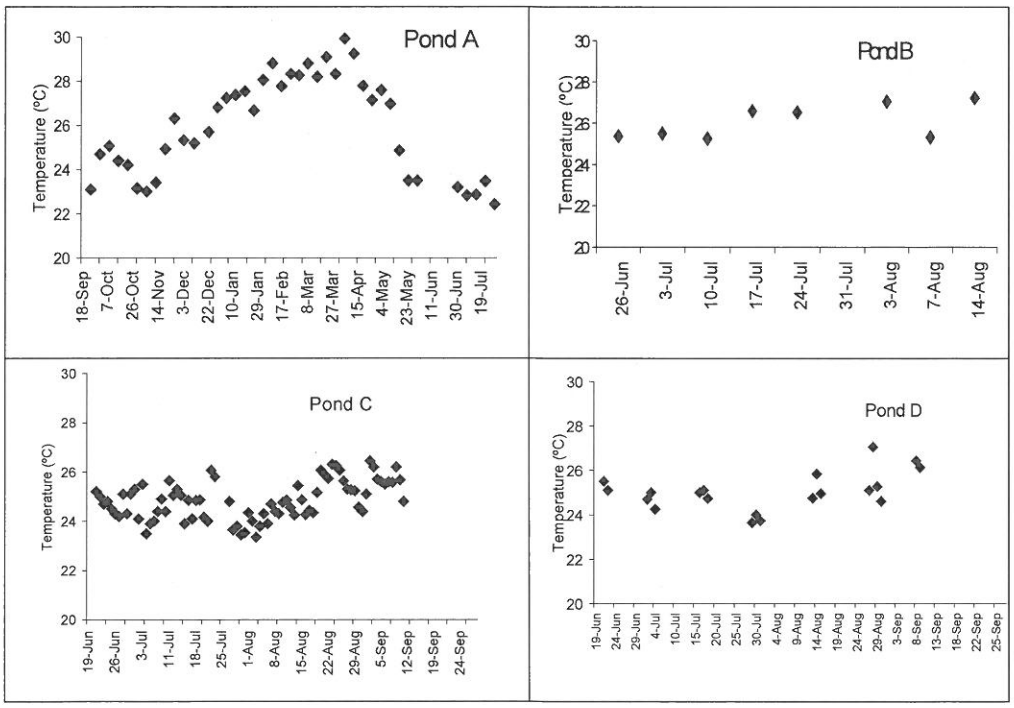
Figure 4.2 Temperature (°C) fluctuations in the four shrimp ponds.

Errata for Chapter 4

Some figures were not properly printed in the textbook.

The corrected version is given below.

Pag.102, Figure 4.2

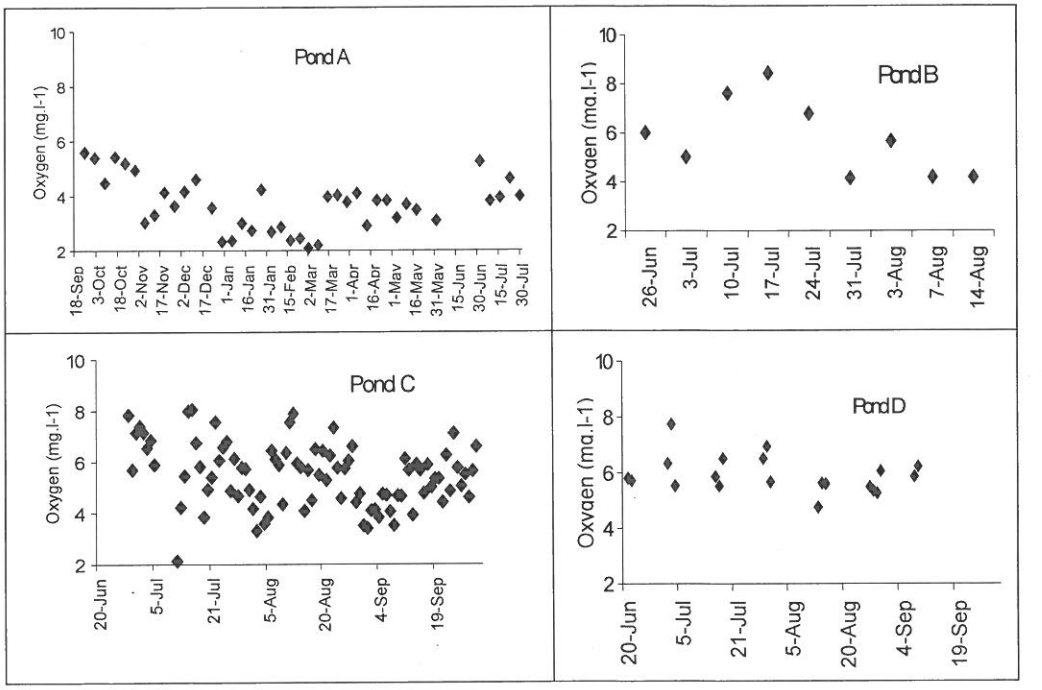


Errata for Chapter 4

Some figures were not properly printed in the textbook.

The corrected version is given below.

Pag. 103 Figure 4.3



Errata for Chapter 4

Pag.107, paragraph 2, line 5 must be (H=41; df=2; $p<0.01$)

The oxygen level was always higher than 3 mg.l-[1] (except during the rainy-warm season in Pond A), which is considered the survival level for aquatic organisms (Boyd, 1995). However, there were higher fluctuations of oxygen level in Ponds B and C (figure 4.3), but all above the 3 mg.l^{-1} value.

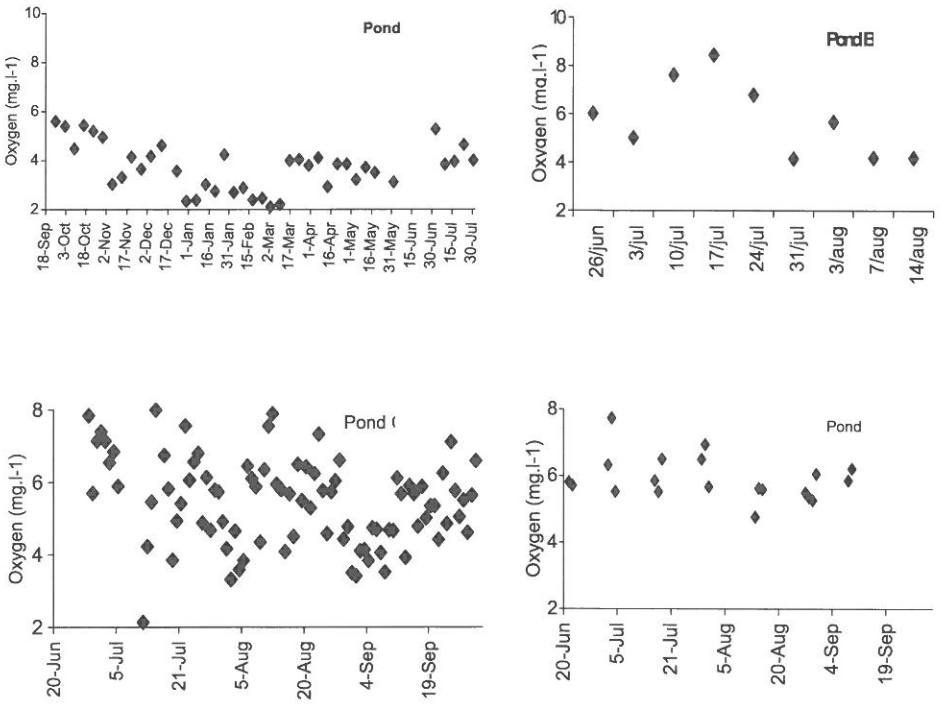


Figure 4.3 The oxygen (mg.l^{-1}) fluctuations in the four shrimp ponds.

4.4.2 Description of the meiobenthic communities

Pond A

The meiobenthic community of shrimp pond A, was investigated considering nematode and copepod groups between September 21st, 2000 and July 12th, 2001. The extended data are indicated in Annex 4. Four shrimp production cycles and two drained periods were considered in the analysis. There were 35 nematodes.10cm^{-2} and five copepods.10cm^{-2} in average. During the first cycle, there were seven mematodes.10cm^{-2} and five copepods.10cm^{-2}; during the second one 44 nematodes.10cm^{-2} and 0 copepods.10cm^{-2}; and during the third cycle 53 nematodes.10cm^{-2} and 11 copepods.10cm^{-2} were registered. There was a low but significant correlation between the nematode density and the copepod densities ($r=0.07$; $p<0.05$). These two meiobenthic groups registered similar behaviour, just to the first and fourth shrimp cycle production (figures 4.4 and 4.5). At the first cycle both groups increase in abundance while at the last shrimp cycle production both groups decrease after the drained period.

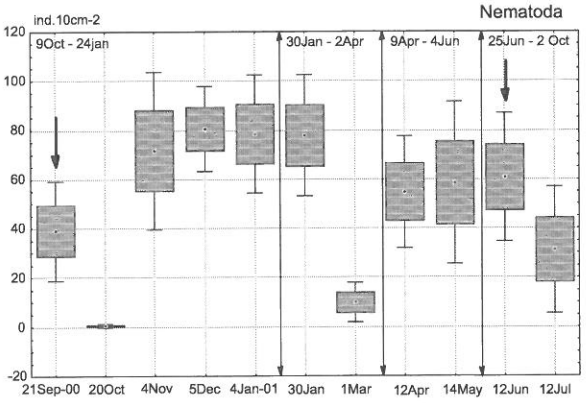


Figure 4.4 Temporal fluctuation of nematode densities at Pond A during 2000-2001. The double-headed arrows separated the production cycles, while the thick arrows show the drained periods. (Data: mean/SE/ ±1.96SE).

No assumptions to ANOVA were fulfilled. A Kruskal-Wallis test was applied to find significant differences for total nematode densities (figure 4.5) between sampling dates to both groups, nematodes ($H=22.99$; $df=10$; $p<0.05$) and copepods ($H=22.99$; $df=10$; $p<0.05$). But, while copepods showed significant differences among shrimp cycle productions ($H=13.99$; $df=4$; $p<0.01$), there were no significant differences in nematode densities among shrimp cycles productions ($H=3.79$; $df=4$; $p>0.05$).

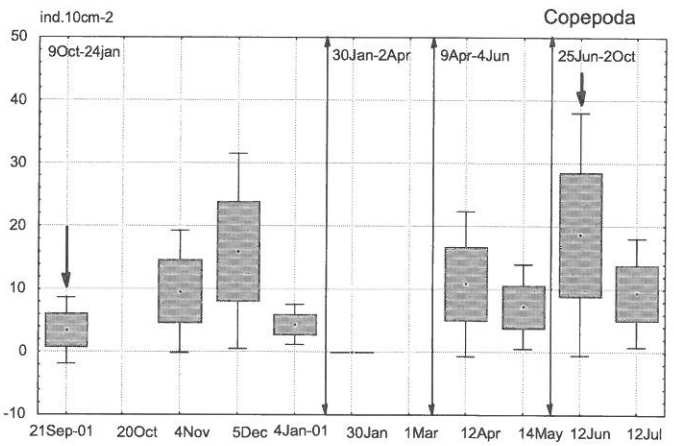


Figure 4.5 Temporal fluctuation of copepod densities in Pond A during 2000-2001. The double-headed arrows separated the production cycles, while the thick arrows show the drained periods. (Data: mean/SE/ ±1.96SE).

Pond B

The fluctuations in nematode and copepod densities were studied between the June 12 and August 20, 2002. Extended data are shown in Annex 4. We investigated a previous drained period, a water filled period and a shrimp larvae stocking period. A low density of nematodes and copepods were registered in the sediments for the whole

period. The first date (June 12) corresponded to the drained period previous to the beginning of the shrimp production cycle. During the second period the pond was filled with 10cm of water. Later the water level was increased up to a depth of 1.2 m. There was a density of 36 nematodes.10cm^{-2} and 29 copepods.10cm^{-2}. There was no significant correlation between both groups ($p > 0.05$).

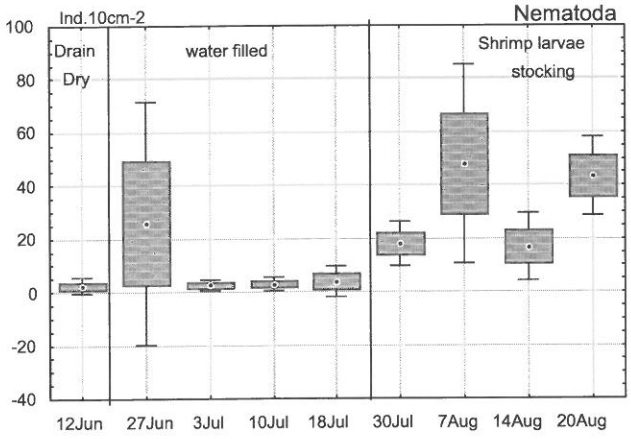


Figure 4.6 Temporal fluctuations of nematode densities at the shrimp pond B during 2002. (Data: mean/SE/ ±1.96SE).

The assumptions of ANOVA were fulfilled just for nematode densities (figure 4.6). An ANOVA showed that nematode densities (log transformed) where significantly different between different sampling dates ($F=27.75$; $df=8$; $p<0.01$). The results of post hoc comparisons, Scheffe test are showed in table 4.2.

Table 4.2 Differences of nematode densities for Pond B between the sampling dates during 2002. (Post Hoc test Scheffe; **highly significant; *significant).

Dates (2002)	31 July	7 August	14 August	20 August
12 June	**	**	**	**
27 June	**	**	**	**
3 July	**	**	**	**
10 July	**	**	*	**
18 July	**	**	*	**
14 August				*

An ANOVA showed that nematode densities (log transformed) where also significantly different between different production cycles, drained period, water filled period and shrimp stocking period ($F=93.19$; $df=2$; $p<0.01$). The results of post hoc comparisons, Scheffe test are showed in table 4.3.

Table 4.3 Differences of nematode densities between shrimp production cycle periods for Pond B during 2002. (Post Hoc test Scheffe, **highly significant; *significant).

Period	Shrimp larvae stocking
Drained	**
Water filled	**

No assumptions to ANOVA were fulfilled for copepods densities (figure 4.7). A Kruskal-Wallis test was applied. A significant difference for copepod densities (log+1 transformed) between sampling dates was observed ($H=90.44$; $df=8$; $p<0.01$). There were also significant differences in copepod densities between shrimp production cycles ($H=41.44$; $df=2$; $p>0.01$).

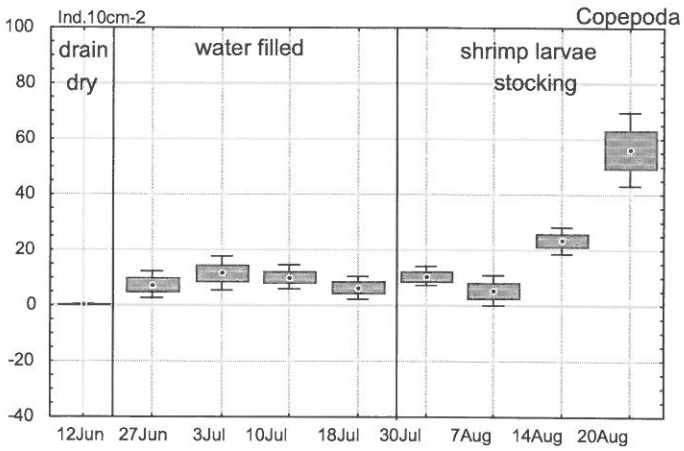


Figure 4.7 Temporal fluctuations of copepod densities at the shrimp Pond B during 2002. (Data: mean/SE/ ±1.96SE).

Pond C

The benthos of pond C in the Guayaquil Gulf was monitored from the stocking date (June 20, 2000) of the shrimp until one week after harvest (September, 2000). Densities of 121 nematodes.10cm^{-2} and 11 copepods.10cm^{-2} were registered (figures 4.8 and 4.9). Extended data are shown in Annex 4.

The density of nematodes and copepods were low. From the first day of shrimp larvae stocking there was a decrease in density for both groups. An increase in nematode numbers but also for copepods was observed until the harvest date with the highest increase during the drained period (September, 19). However, there was no significant correlation between both groups ($p > 0.05$).

The assumptions of ANOVA were fulfilled for nematode densities (figure 4.8). An ANOVA showed that nematode densities (log transformed) were not significantly different between different sampling dates ($F=0.39$; $df=6$; $p>0.05$).

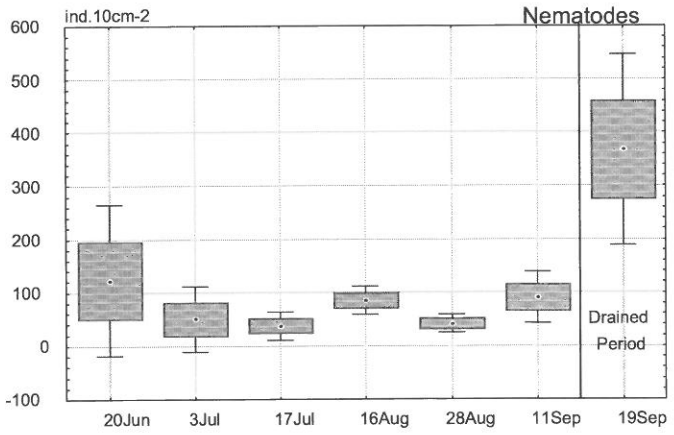


Figure 4.8 Temporal fluctuations of nematode densities at shrimp Pond C during 2000. .(Data: mean/SE/ ±1.96SE).

The assumptions to ANOVA were fulfilled for copepods (figure 4.9). Copepod densities (log transformed) were not significantly different between different sampling dates ($F=1.75$; $df=6$; $p>0.05$).

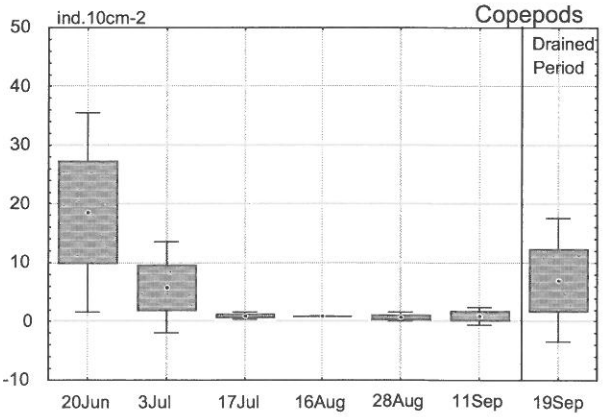


Figure 4.9 Temporal fluctuations of copepod densities at shrimp Pond C during 2000. (Data: mean/SE/ ±1.96SE).

Pond D

The benthos of the pond D in the Guayaquil Gulf was also monitored from the shrimp larvae stocking date (June 20, 2000) until one week after harvest (September 11, 2000). There were 252 nematodes.10cm^{-2} and 8 copepods.10cm^{-2} of the average. Extended data is shown in Annex 4. Nematodes showed a higher density than copepods. Initially, at pond there was a low density of nematodes and a high density of copepods. However, during the next period, an increase in density was observed for nematodes and a decrease to copepods, with a decrease at the end of the shrimp production cycle. There was not significant correlation between both groups ($p>0.05$) (figures 4.10, and 4.11).

The assumptions of ANOVA were fulfilled for nematode densities. Significant differences for nematode densities (log transformed) between sampling dates were

observed (F=0.34; df=6; <0.01). The results of post hoc comparisons, Scheffe test are showed in table 4.4.

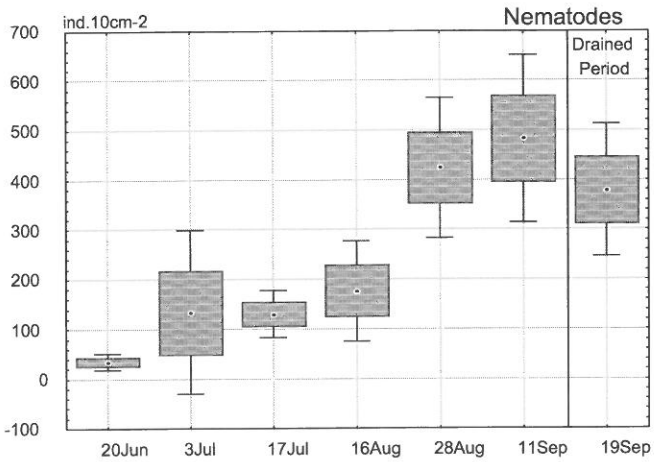


Figure 4.10 Temporal fluctuations of nematode densities at shrimp Pond D during 2000. (Data: mean/SE/ ±1.96SE).

Table 4.4 Differences of nematode densities among dates for Pond D during 2000. (Post Hoc test Scheffe; **highly significant; * significant).

Dates	June 20	July 3
August 28	**	**
September 11	**	**

The assumptions of ANOVA were fulfilled to copepods densities (figure 4.11). An ANOVA showed that copepod densities (log transformed) were significantly different between different sampling dates (F=0.39; df=6; p<0.01). The results of post hoc comparisons, Scheffe test are showed in table 4.5.

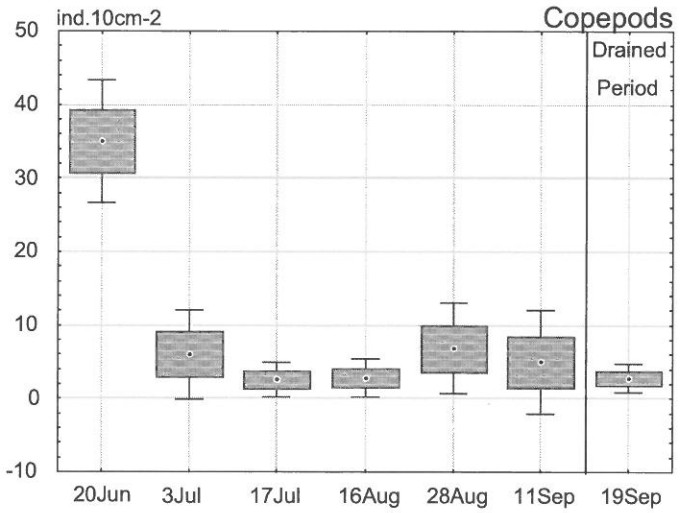


Figure 4.11 Temporal fluctuations of copepod densities at shrimp Pond D. (Data: mean/SE/ ±1.96SE).

Table 4.5 Differences of copepods densities among dates for Pond D during 2000. (Post Hoc test Scheffe; **highly significant; * significant). Other dates did not show significant values

Dates	June 20	July 3
June 20		**
July 3	**	
July 17	*	**
August 16	**	**
August 28	**	**
September 11		**
September 19	**	**

4.4.3 Relationship among environmental variables and meiobenthic communities

There was not significant correlation between temperature and nematode densities at ponds A, C and D ($p>0.05$). At Pond A, a significant correlation between temperature and copepod density was obtained ($r=0.07$; $p<0.05$). At Pond B a significant correlation was showed between temperature and nematode densities ($r=0.37$; $p<0.01$); and between temperature and copepods densities ($r=0.19$ $p<0.01$). There were no

significant correlations among oxygen levels and nematode or copepod densities in any of the ponds ($p>0.05$).

4.4.4 Management practices.

No relationship was found between the meiobenthic organisms and the management practices. No pattern was observed. The general data of shrimp farms management along with the nematode and copepod data are shown in table 4.6.

Table 4.6. Management practices information of the four shrimp ponds

	POND A (three shrimp production cycles were considered only)		
Area (ha)	8	8	8
Stocking dates	9-Oct-00	30-Jan-01	9-Apr-01
Initial weight of shrimp			
Shrimp quantity	1200000	910000	450000
Initial density (ind.m^{-2})	15	11	6
Harvest date	24-Jan-01	2-Apr-01	5-Jun-01
Harvest weight of shrimp (g)	14	13.5	12.2
Harvest density (ind.m^{-2})			
Survival (%)	11.8	12.12	9.69
Culture time (days)	100	62	57
Shrimp pounds harvested (pounds.ha^{-1}a	545	540	130
Interval between production cycle (days)	9	12	6
Nematode density (ind.$10cm^{-2}$)	7	44	53
Copepods density (ind.$10cm^{-2}$)	5	0	11
	POND B	**POND C**	**POND D**
Area (ha)	1	4.5	1.5
Stocking date	26-Jul-02	19-June-00	20-June-00
Initial weight of shrimp	0.03	0.02	0.19
Shrimp quantity	918	448600	181700
Initial density (Ind.m^{-2})	17	10	12
Harvest date	20-Aug-02	11-Sep-00	11-Sep-00
Harvest weight of shrimp (g)	0.96	8.85	9.30
Harvest density (ind.m^{-2})	9	1.2	1,7
Survival (%)	50	11.8	14.3
Culture time (days)	24	84	83
Shrimp pounds harvested (pounds.ha^{-1})	0.11	230	354
Interval between production cycle (days)	90	unknown	unknown
Nematode density (ind.$10cm^{-2}$)	36	121	252
Copepods density (ind.$10cm^{-2}$)	29	11	8

4.5 Discussion

The two most abundant meiobenthic groups in marine and estuarine environments are nematodes and copepods (Rafaelli & Masson, 1981; Coull, 1999; Funch *et al.*, 2004; Ullberg, 2004) as were registered during our research. At the start of the shrimp production cycle, there were always a substantial number of nematodes present in the bottom. Riemann (1979), Funch *et al.* (2002) and Ullberg & Olafsson (2003) mentioned that nematodes could colonize virtually every habitat that can sustain metazoan life (from almost dry dune sand to beach sand, coarse shell sub-littoral grounds, down to hadal trenches). These organisms are be very tightly associated with the sediments and are sometimes found in zooplankton (Grainger *et al.*, 1985). The meiofauna occurrence in the water column is generally caused by passive suspension of individuals (Palmer & Brandt, 1981; Palmer 1983); this is especially the case for copepods (Alldredge & King, 1977). The densities were fluctuating a lot and we assume that this depends on the activities performed inside the pond (long drained period or lime soil process) prior to the shrimp stocking. Larvae or adults of meiobenthic organisms should arrive simultaneously in large numbers to establish a successful recruitment but inside the pond the conditions are not always suitable for them.

The planktonic larval period of copepods give them an advantage over nematodes, but the disadvantages of planktonic dispersal can be that the stress experienced during dispersal can decrease general fitness after the recruitment (Pechenik, 1999) as could happen inside the shrimp ponds. Other causes might be dispersal away from suitable habitats and less time for local adaptation (Pechenik, 1999). Gerlach (1977b) shows that most of the meiobenthos that has pelagic larval stage has a better year-to-year chance to establish populations in other areas than other type of meiobenthos. As we mentioned in Chapter 1, rafting is a method by which organisms are transported by attaching themselves (free or accidentally) to different kinds of objects (Jokiel, 1989). Small-scale dispersal in meiofauna is also achieved by sediment film rafting (Ullberg, 2004). Sediment rafts are made up of sand particles; and only a few sand grains are held together by microbial mucopolysaccharides (Hicks, 1988). And numerous studies indicate that nematodes can be dispersed via the water column (Palmer & Gust, 1985;

Bertelsen, 1997; Powers, 1998). But, Gerlach (1977) commented that most of meiofauna lack of pelagic larvae that allow their dispersion. It is hypothesized that the benthos colonizes shrimp ponds through transport by birds, crab (Ullberg, 2004) or by the boats used in the shrimp ponds during feed and fertilizer application activities. The colonization of the shrimp ponds should proceed by the inlet water transport of detritus from the outlet channels. These mats of detritus can harbour several taxa as nematodes, copepods, crustacean larvae and ciliates, in quite high number.

The difference in nematode species densities could be due to the different scales of dispersal. They have different structures, which should give different fall down speeds (Ullberg, 2004). Also, nematodes have been considered as poor swimmers (Palmer, 1984; Fegley, 1985) and they lack the circular muscle in the body wall muscle (Giere, 1993), which is the principal muscle in nematode locomotion (Barstead *et al.,* 1991). Therefore many nematodes will have the capacity to take out the effect of settling due to gravitational forces by swimming. Only the smallest nematodes will be able to swim freely in water, because for larger nematodes, the viscosity will be insufficient for the sinusoidal wave propagation that nematodes utilize for swimming (Crofton, 1966 in Ullberg, 2004).

Whether meiofaunal organisms are likely to be suspended or not depends on life history traits as well as physical factors such as light, exposure to temperature or available oxygen (Hicks, 1984, Armonies 1988, Hicks, 1988a; Walters, 1991). Meiofauna is also commonly associated with naturally occurring transient aggregation called marine snow, which can have substantial influence on their residence time in the water column. Inside the pond this "snow" of detritus is frequently due to the feed applications. The artificial feed has binding characteristics that allow for pellet stability of about four to six hours. The average size is about 3/32-inch diameter.

After drained periods, new organisms can enter the pond with the incoming water. The only surviving organisms are those well adapted to the local shrimp pond conditions. D'Ambramo & Conklin (1996) and Martínez-Córdova *et al.* (1998c,d; 2002b) mentioned that most of the benthic organisms are grazed upon during the first weeks after the

stocking of the shrimp and their abundance declines over time. This decline in benthic density during the first phase of the shrimp production cycle does not always occur. Moriarty *et al.* (2005) commented that meiofauna is eaten by Penaeid prawns and added that copepods together with polychaetes were particularly preferred while few nematodes remained uneaten. These authors found that in the presence of prawns, the meiofauna decreased and the variability registered among densities of the meiofauna depended on the different levels of predation. They also registered a lower biomass and production of meiofauna in ponds containing prawns.

The initial conditions within the four ponds were different. The densities were not always comparable and we assume that this depends on the activities prior to shrimp stocking inside Ponds A and C, where there were the highest density of nematodes and copepods during the first sampling dates and later the densities decrease when the shrimp larvae was stocked. There was an increase in nematode density with the beginning of the new shrimp production cycle. At Pond B, when the pond was water filled, the nematodes and copepods increased and in Pond D, in the case of nematodes was the same, to nematodes; nematodes increased in time but copepods remained almost at the same density, and even lowest, which could be the result of the nematodes capacity to colonize. Nematodes have no pelagic larvae but they have a swimming ability coupled to its size and water viscosity (Ullberg & Olafsson, 2003) that allow them to move in the water column. By this kind of activity, nematodes can enter the shrimp pond when the water inflow is coming from the natural saline or estuarine environment. At pond C and D copepods enter in high densities into the pond, but their density declines very rapidly. Begon *et al.* (1999) commented that early species are often good colonizers (like copepods), which inhabit all available benthic habitats in the sea, freshwater and inland saline waters (Funch *et al.,* 2002). The dramatic decline in copepods can also be due to the stress offered by the shrimp pond bottom characteristics or by the predatory activity of the shrimp. At the drained period, without shrimp, there were higher densities in both ponds and for both groups. Some "wet spots" remain at shrimp pond bottoms and it is possible to observe some humidity under

five to ten cm of depth into the sediment, where we assume the meiobenthic community is supported.

Inside the pond, the artificial or natural food, along with fertilizers, are the most probable factors determining the colonization of the substrate and the patchiness of benthic organisms (Fleeger *et al.*, 1995; Thinphanga, 2004). Also the presence of shrimp produces changes in benthic communities caused by predatory activities or disturbance of the sediments (Bell & Coull, 1978; Hedqvist-Johnson & Andre, 1991; Martínez-Córdova *et al.*, 2002b; McNeill, 2001). However, this predator effect is not clear in all the ponds we sampled. The presence of shrimp can have an effect at Pond A, which means a removal of soil particles, followed by an increase of fixation surface for bacteria (Kemp, 1987; Jönsson *et al.,* 1993). The sudden decline in copepods can be due to the presence of shrimp, since many authors have demonstrated the predation pressure of shrimp over them (Rubright *et al.*, 1981; Bombeo-tuburan *et al.,* 1993; Nilsson *et al.*, 1993; Nicovita, 1997; Martínez-Córdova & Peña-Messina, 2005).

Under natural conditions the benthic habitats are subjected to a variety of disturbances (hydrodynamics, temperature, salinity and oxygen fluctuation). The same factors can influence the benthic community within the shrimp pond. The effects of environmental factors on nematode densities and diversity have been demonstrated by many authors: Hopper *et al*, 1973; Tietjen & Lee, 1972, 1977; Warwick, 1981b; Herman & Vranken, 1988; Vranken *et al.*, 1988; Foster, 1998; Moens & Vincx, 2000. However, during our study we did not find a clear relationship between the meiobenthic organisms and temperature and oxygen. Inside the shrimp pond the nematodes have a sufficiently long period of calm to settled at the bottom shrimp pond. Wetzel *et al.* (2001) found increased abundance in the water column during a period of severe hypoxia. They added that high values of nematode abundance later returned to normal levels when the oxygen levels in the sediment returned to normal.

Harpacticoid copepods colonize new habitats fast and, are considered as an opportunistic group (bell, 1980; Widbom, 1983). Sherman é& Coull (1983) mentioned that these copepods live in the uppermost centimeter or millimeter of the sediment and

can be suspended easily than the nematodes, with shrimp surface movement. And copepods are active swimmers, which allow them to be transported from one place to another (Armonies, 1988).

Salinity and temperature changes with time and the community composition are forced to change to resist this osmotic stress. The early occupants of a newly created ecosystem (just as a shrimp pond at the start of a shrimp production cycle) changed the abiotic environment in a way that makes it comparatively less suitable for themselves and more suitable for the recruitment of others (Begon *et al.*, 1999). As it was mentioned in Chapter one, Cook *et al.* (2000); Wettzel *et al.* (2001) and Steyaert *et al.* (2005) had mentioned that nematodes are more tolerant than other meiofauna as harpactocoid copepods, which are much less resistant (Nilsson & Rosenberg, 2003). Alongi *et al.* (1983) had mentioned that the colonization of meiofauna is a rapid process, which occurs within few weeks or even in hours or days (Billheimer & Coull, 1988). The lowest initial nematode density (1 ind.10cm^{-2}) was found at Pond B, where there were three months of drained previous to the experiment. At the other ponds, the shrimp farmer took two to three weeks to dry the pond. When the time between shrimp cycle production is shortest and the pond is "empty of shrimp" some "spot" of water remain inside so that little wet pools (of about 10 to 20 cm depth) remain. We assume that these "wet spots" are enough to keep a benthic community alive until the next shrimp production cycle. But after this period we thought to find an increase in nematode or copepods densities. However, no clear trends were observed inside the studied ponds.

On the other hand, Resh *et al.* (1988) observed that during the shrimp harvest, the flow of the water run off is high and it could exert a strong influence on the benthic organisms as well as washing them away from the pond. This might cause a substantial decline in abundance and diversity of benthic organisms. But, there is also a transport of sediment and organisms within the shrimp pond, from the low parts of the pond to the higher parts, which causes a high internal dispersal within the pond. Guerra-García *et al.* (2003) commented that the manipulation of the natural environment produces changes in it and we had observed that during the harvest of shrimps at least the first

five centimetres of the sediment are flushed. Even more during this process the shrimp farm workers go into the shrimp pond, walk around and they remove the sediment to get the last shrimp out of the pond.

The colonization and the survival of the benthic animals within the shrimp pond during a shrimp production cycle did not follow a clear pattern as we mentioned above. Indications are found that copepod communities, which decline strongly after colonisation are probably preyed upon by the shrimp and that the nematodes are better competitors to survive the harsh shrimp pond environment. However the mechanism behind this correlation among copepods and nematode communities should be investigated though an experiment approach.

4.6 Conclusions

- Nematodes and copepods are continuous residents of the soils of the shrimp ponds (even after drained periods).

- There are no clear effects of drained-periods over nematodes and copepods densities but an increase in densities is observed with the new water entering the pond.

- The colonization and the survival of the benthic animals within the shrimp pond during a shrimp production cycle do not follow a clear pattern.

- Nematodes are better competitors to survive the harsh shrimp pond environment compared to other meiobenthic organisms.

- Experimental studies are necessary to know the direct relationship among nematodes-copepods and shrimps.

CHAPTER 5

Characterization of nematode communities in three shrimp pond bottoms (Guayas province, Ecuador)

5 Characterization of nematode communities in three shrimp pond bottoms (Guayas province, Ecuador)

5.1 Abstract

The Nematode community of three shrimp ponds in the Guayas province, Ecuador was investigated. The temporal fluctuation in diversity and density of this community was followed during one shrimp production cycle. The average densities in the three ponds were 49 ind.10cm^{-2}, 121 ind.10cm^{-2}and 252 ind.10cm^{-2}, for the ponds A, C and D, respectively. Nineteen different species were identified. *Terschellingia longicaudata* and *Spilophorella papillata* and *Daptonema* sp were the most abundant species. The temporal fluctuation in nematode communities could not be explained by environmental variables. Also no relation was found between the nematode fluctuations and management treatments within the shrimp ponds.

5.2 Introduction

The white shrimp, *Litopenaeus vannamei,* is one of the most important commercial natural products in Ecuador after oil (CNA, 2005; Exportaciones ecuatorianas 2004). The Ecuadorian shrimp farming industry started around 1970 (Rosenberry, 2001). In the beginning, shrimp production was based on natural productivity; the shrimp ponds had a water exchange cycle that was dependent from the natural tide (either with the sea or with the estuarine water). In this way the shrimp larvae and other organisms, which are part of the shrimp diet (small crustaceans, phytoplankton, among others), enter the pond. The shrimp aquaculture activities increased from an extensive system with low shrimp densities (2-8 thousand shrimp.hectares^{-1}) in the 80's to a semi-intensive system with high shrimp densities (30-60 thousand shrimp.hectares^{-1}) (Aiken, 1990; Ronseberry, 1999). Nowadays, some of the intensive systems reach up to 500 thousand shrimp.hectares^{-1}. Therefore, the requirements for artificial food, which is also very costly, increased around 40 % of the total production cost (Calderón &

Sonnenholzner, 2003). Hence, emphasis is put recently on the search for alternatives, e.g. the improvement of natural food, in order to get a more sustainable aquaculture.

One of the problems to enhance 'natural' sustainability in aquaculture is the lack of basic information on a shrimp pond ecosystem, its structural characteristics and dynamics. *Litopenaeus vannamei* spends part of its life cycle close to the bottom as protection during moulting (Moller & Jones, 1975; Molina-Poveda *et al.*, 2002) but also for searching for food (McNeill, 2001; Molina & Orellana, 2001; Gamboa-Delgado *et al.,* 2003).

The organisms living in the water column of the shrimp ponds are rather well documented (Burford, 1997; Guerrero-Galván *et al.,* 1998; Alongi *et al.,* 1999; Johnson *et al.*, 2002; Coman *et al.,* 2003) but, the life in the sediments is hardly known (Rubright *et al.*, 1981; Tidwell *et al.* 1993). Nevertheless, some benthic organisms seem to be an important part of the diet of 'wild' shrimp living in the sea (Rubright *et al.,* 1981; Hunter *et al.,* 1987; Hedqvist-Johnson & Andre, 1991; Bombeo-tuburan *et al.,* 1993; Nilsson *et al.,* 1993; Nicovita, 1997; Nunes *et al.,* 1997) and in aquaculture conditions too (Feller, 2004). Preliminary field observations have indicated the presence of Polychaeta, Nematoda, Copepoda and Mollusca inside the shrimp pond bottom (Cornejo-Rodríguez, 1999; McNeill, 2001; Quevedo, *in press*). However, the studies about these pond ecosystems are mainly about macrobenthic communities (Rubright, *et al.*, 1981; Tidwell *et al.,* 1993) and the effects of aquaculture activities over surrounded benthos (Mazola *et al.*, 1999; Orellana *et al.,* 2001; Mirto *et al.,* 2002*s*). Very few information is available about the meiobenthic communities in aquaculture ponds (Somsak, 1995).

Several studies have demonstrated that shrimp eat nematodes under natural conditions (Phil & Rosenberg, 1984; Smith & Coull, 1987; Jonsson *et al.,* 1993; Nilsson *et al.,* 1993), and little research has been performed under aquaculture systems (Nicovita, 1997; McNeill, 2001; Feller, 2004). Otherwise, nematodes community structure can be used as a tool for bio-monitoring, in order to assess the quality of the environment, such as nutrient load leading to oxygen stress (Wieser *et al.,* 1974; Wieser & Schiemer, 1977; Warwick, 1981b; Moens, 1999).

This study will emphasize on the variation in density and diversity of the nematode community inside three shrimp pond bottoms. Details about the copepod communities were not considered due to the time consuming nematode. We have considered three ponds located one in a saline zone, Pond A and the other two in an estuarine environment, Pons C and D. A general estimation of relationships among biological and environmental variables is considered and management practices are also taken into account.

5.3 Material and methods

The samples were collected in three shrimp ponds in the Guayas province. The first (Pond A), has a surface of 8 hectares and it is within a shrimp farm located in Palmar-Guayas province, Ecuador (2°11´S, 80°45´W). The other two shrimp ponds (Pond C), are 4.5 hectares and (Pond D), 1.5 hectares) are in a shrimp farms inside the Guayaquil Gulf system (80°0´W; 2°15´S) (figure 5.1).

The benthos was monitored during the shrimp production cycle in the three ponds. Pond A (35 PSU), was monitored between July and October 2001. Ponds C and D are (15 PSU in average) were monitored between June and September 2000.

The samples were taken monthly on 5 sites (A-E, see figures 5.3, 5.4, 5.5) inside the three shrimp ponds with three replicas at each site (see Chapter 2, for details).

The meiobenthic samples for nematode investigation were collected with a corer of 5 cm diameter until a sediment depth of 5 cm. The samples were preserved with 4% neutral formaline. For the preparation of the samples, we followed Vincx & Heip (1996). The identification of the organisms was done with a microscope Olympus BH2. The abundance per date of the nematode community is expressed in ind.10cm^{-2} (see also Chapter 2).

In Pond A the temperature and the oxygen levels, were monitored in the water column in the morning of the sampling day. Temperature and oxygen were measured with an

oxygen-meter YSI 85, Yellow Springs Instrument Co. In Ponds C and D, daily average of temperature and oxygen were considered. Information about shrimp management practices such as shrimp growth, and survival were registered.

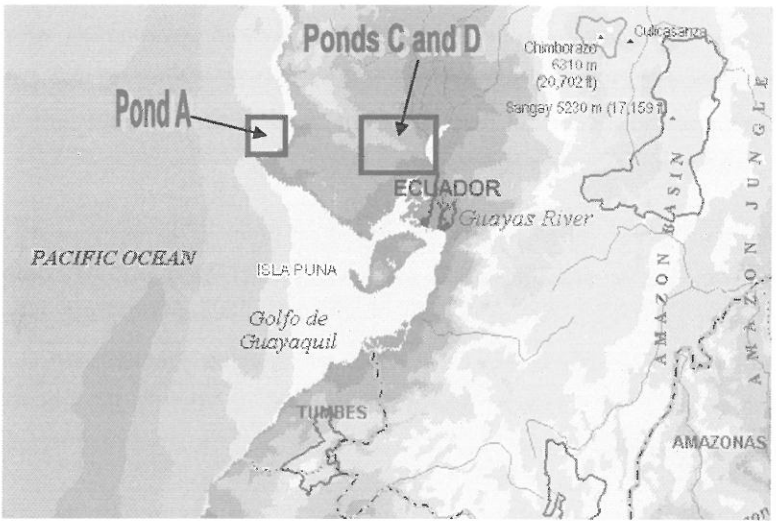


Figure 5.1 Localization of shrimp farm, where Ponds A, C and D were selected.

An ANOVA was applied when assumption of homogeneity of variances and independency of mean and variances were fulfilled. Data were log + 1 transformed. When assumptions for normality were not fulfilled after log+1 transformation, non-parametric Kruskall-Wallis test was applied. The total number of replicas was used to evaluate the relationships among sample dates, and shrimp production cycles (Statistica 6.0, 2000). Spearman Rank Correlation (Sokal & Rohlf, 1995) was used to analyse the relationship between oxygen and temperature and nematode community's distribution and the relationship inside these communities (Statistica 4.1, 1999; Statistica 6.0, 2000).

A MDS was performed to evaluate the nematode distribution in the stations inside the ponds (Clarke & Warwick, 1994).

5.4 Results

5.4.1 Description of the nematode communities

In the three ponds, nematodes dominated the benthic environment with an average relative abundance varying between 85 % and 100 %. Other groups (copepods; see Chapter 4), were present but they were not considered in the present research (see annex 3 and annex 5 for the database).

At Pond A, 3,451 nematodes were counted and 1,758 were used for species identification. At the estuarine ponds we found that in Pond C 11,070 nematodes were counted with 3,595 identified at species level; and, in Pond D, 21,483 nematodes were counted and 7,574 individuals were used for species identification. The average densities in the three ponds A, C and D, were 49 ind.10cm^{-2}, 121 ind.10cm^{-2}and 252 ind.10cm^{-2}, respectively.

Different fluctuations were observed in the three ponds (figure 5.2). No assumptions to ANOVA were fulfilled. A Kruskal-Wallis test shows no significant differences for nematode densities ($H=4.00$; $df=2$; $p>0.05$) between the three ponds. But, when only the most dominant species (*Terschellingia longicaudata, Spilophorella papillata* and *Daptonema* sp) were considered there was a significant difference among the ponds ($H=21.33$; $df=2$; $p<0.01$).

At Pond A, the assumptions to ANOVA were not fulfilled and a Kruskall-Wallis test was applied. The statistical differences were registered among months ($H=3.00$; $df=3$; $p>0.05$) at this pond. At Pond C, the assumptions to ANOVA were not fulfilled, and a Kruksall-Wallis test indicated statistical differences among months ($H=9.800$; $df=3$; $p<0.05$). While at Pond D, the assumptions to ANOVA were fulfilled and significant differences were registered when all the species were considered in the analysis ($F=5.375$; $df=3$; $p<0.01$); June 2000 was statistically different from September 2000 ($p<0.01$).

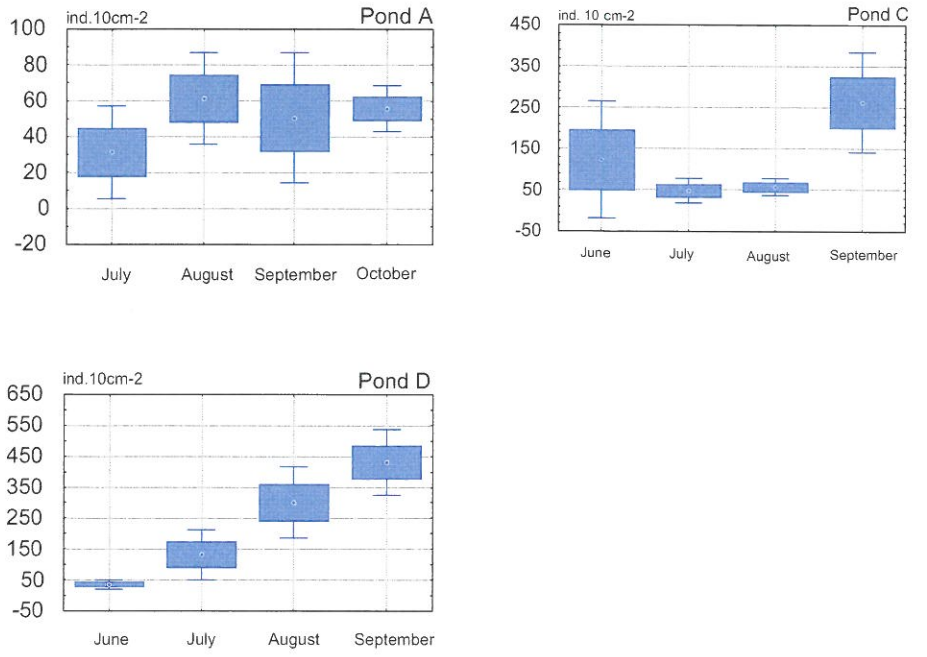


Figure 5.2 Temporal variation of the nematode densities in the three ponds A, C and D. (Data:mean/SE/1.96SE).

With the MDS test for the analysis of the nematode species densities of Pond A, it is shown that the variability between the 5 sites (A-E) within the pond is low during the months August and October (sampling points close together within the plot); while the variability in species composition between the 5 sites (A-E) within the pond is much higher in July and September (figure 5.3).

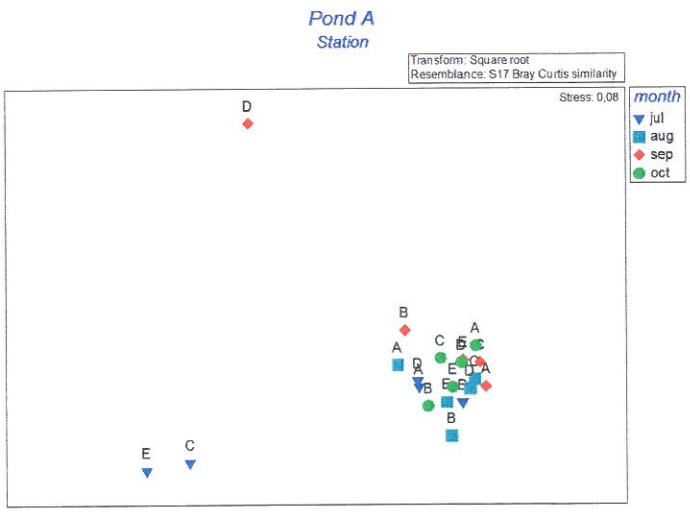


Figure 5.3 MDS Plot for the Pond A. A-E letters within the plot refer to the five sites sampled within each pond.

At Pond C, the nematode species composition (in terms of densities) was highly variable throughout the year, although with an indication of changes in species densities comparing June-July (lower part of the MDS –plot) with August-September (higher part of the MDS-plot) (figure 5.4).

In the case of Pond D, a gradual change in nematode species densities was observed from June to September (along the horizontal scale in figure 5.5). Similar situations were observed at figure 5.2 for the three shrimp ponds).

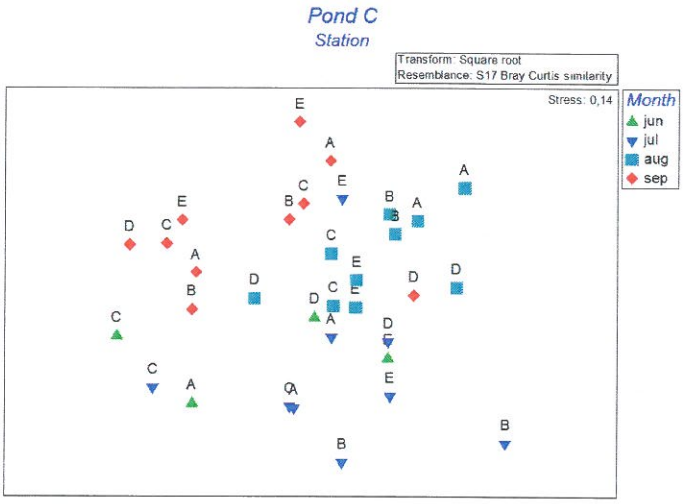


Figure 5.4 MDS Plot for the Pond C. A-E letters within the plot refer to the five sites sampled within each pond.

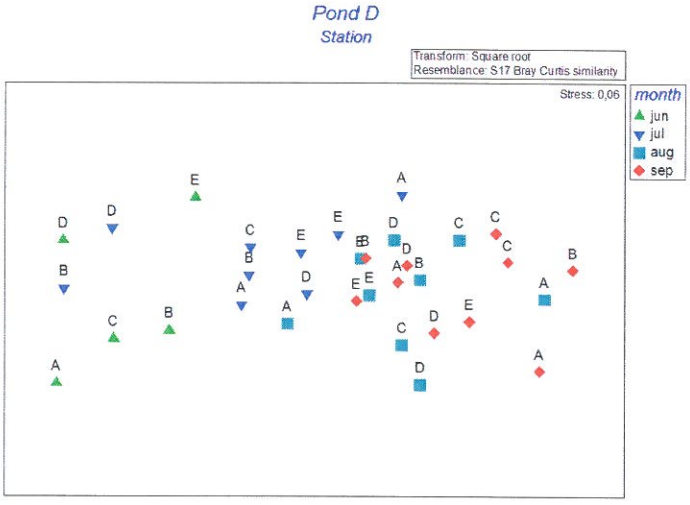


Figure 5.5 MDS Plot for the Pond D. A-E letters within the plot refer to the five sites sampled within each pond.

In total 7 nematode species were identified in Pond A for these period, whilewe identified 12 species in Pond C, and 11 species in Pond D (table 5.1). The most abundant species were *Spilophorella papillata. Terschellingia longicaudata* and *Daptonema* sp which, were also the only common species in the three shrimp ponds.

At Pond A the selective feeders were the most abundant, mainly due to the presence of *Terschellingia longicaudata*, but the density of this feeding group decreased on time as well as the epigrowth feeders increased (figure 5.6; Table 5.1). The most abundant nematodes were epigrowth feeders at Ponds C and D, (*Spilophorella papillata* mainly) (figure 5.6). It is important to observe that the predators and omnivorous nematodes were also present in both systems. *Daptonema* sp (1B; non-selective deposit feeder) was more dominant in the estuarine pond than in the saline ponds (figure 5.6).

Table 5.1 Relative abundance (%) of the nematode species in Pond A, together with the feeding type according to Wieser, 1953a,b. 1A Selective deposit feeders, 2A Epistratum feeders, 1B Non-selective deposit feeders; 2B Predators and omnivores.

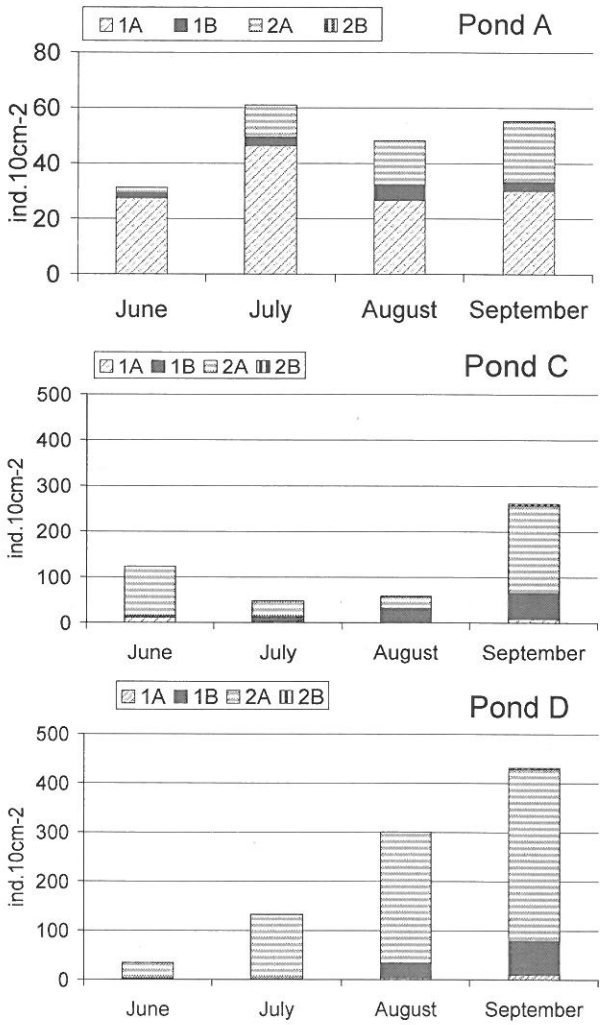
Species	Saline environment	Estuarine environment		Feeding type
	Pond A	Pond C	Pond D	
Spilophorella. papillata	23.10	69.82	84.63	2A
Daptonema sp	4.79	14.84	10.68	1B
Anoplostoma sp		8.35	0.76	1B
Gomphionema sp	4.86			2A
Oncholaimidae sp	0.37			2B
Terschellingia longicaudata	64.40	4.32	1.62	1A
Adoncholaimus sp		1.09	0.34	2B
Kraspedonema sp		0.66	1.76	2A
Sphaerolaimus sp		0.14	0.07	2B
Sabatieria sp	1.35			1B
Theristus sp	1.20			1B
Tubolaimoides sp		0.10		1B
Subsphaerolaimus sp		0.07		2B
Marylinia sp		0.04	0.13	2A
Chromadoridae		0.57	0.01	2A
Paracantholaimus sp		0.01		2A
Metadesmolaimus sp			0.01	1B
Theristus sp			0.01	1B

Errata for Chapter 5

Some figures were not properly printed in the textbook.

The corrected version is given below.

Pag.131, Figure 5.6



Errata for Chapter 5

Some figures and data were not properly printed in the textbook.

The corrected version is given below.

Pag.130, Table 5.1 must be "Relative abundance (%) of the nematode species in ponds A, B and C....."

Pag.132, paragraph 1, line 3 must be no significant differences.

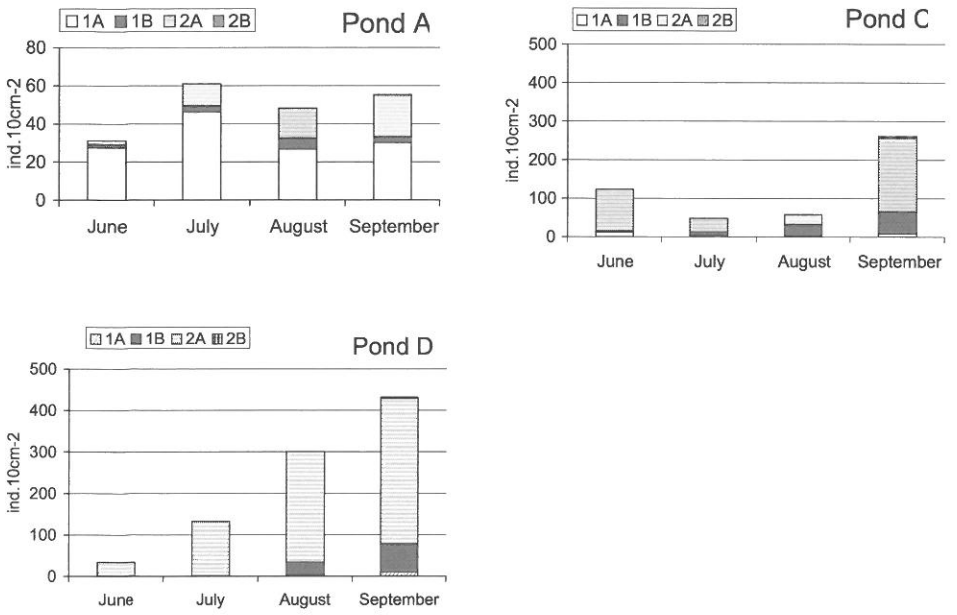


Figure 5.6 Temporal fluctuation of feeding types of nematodes in the three shrimp ponds: A (upper panel left), C (upper panel right) and D (lower panel left).

The three most abundant species were analysed for Pond A. A slight progressive increase was registered for *Spilophorella papillata*. But, statistical differences were not observed among months ($F=2.81$; $df=3$; $p>0.05$). While for the other species an initial increase was followed by a decrease at the end of the shrimp cycle production (figure 5.4). The density of *Terschellingia longicaudata* (figure 5.7), was not significantly different among months ($F=1.659$; $df=3$; $p>0.05$) and neither for *Daptonema* sp ($F=1.033$; $df=3$; $p>0.05$) (figure 5.7). Other species did not register significant differences ($F=1.08$; $df=3$; $p>0.05$).

S. papillata was correlated significantly with *Daptonema* sp while a significant correlation was found between *Terschellingia longicaudata* and *Daptonema* sp (Table 5.2).

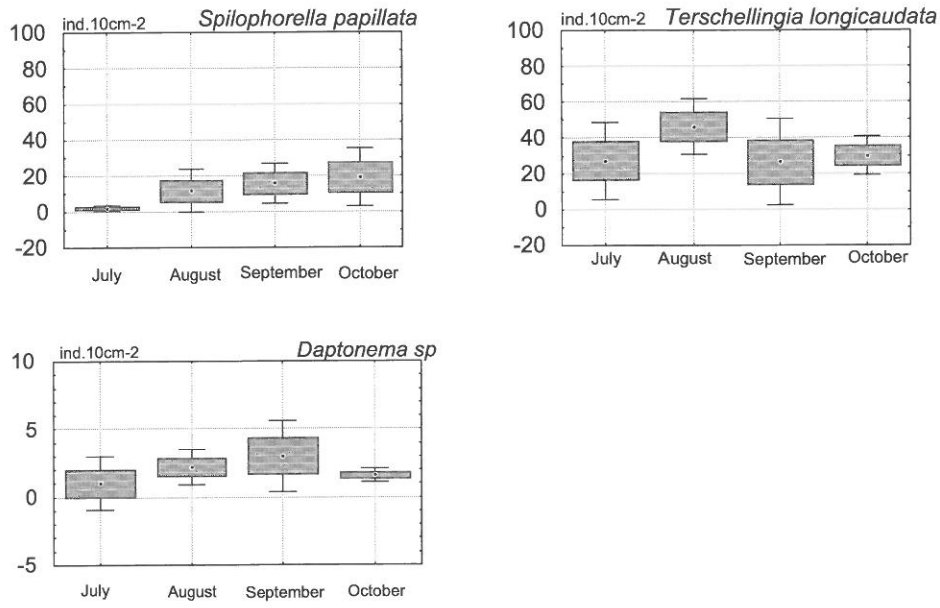


Figure 5.7 Temporal fluctuations of the most abundant nematode species in Pond A. (Data:mean/SE/1.96SE).

At Pond C, *S. papillata, T. longicaudata* and *Daptonema* sp showed an increase in density at the end of the shrimp production cycle, while *Anoplostoma* sp decreased (figure 5.8). No ANOVA assumption was fulfilled for *S. papillata* and significant differences were registered among sampling months through Kruskall-Wallis Test ($H=6.200$; $df=3$; $p>0.05$). For *Daptonema* sp and *Anoplostoma* sp ANOVA assumptions were fulfilled and significant differences were registered among months for the first species ($F=10.408$; $df=3$; $p<0.01$); June and July are significantly different from August ($p<0.05$). Meanwhile for *Anoplostoma* sp the main differences were between July and August. ($F=4.289$; $df=3$; $p<0.05$). For *T. longicaudata* and other species ANOVA assumptions were not fulfilled and Kruskall-Wallis test indicated that there were significantly different among months in the case of the first species ($H=12.780$; $df=3$; $p<0.01$) and not statistically different for the second species ($H=6.896$; $df=5$; $p>0.05$).

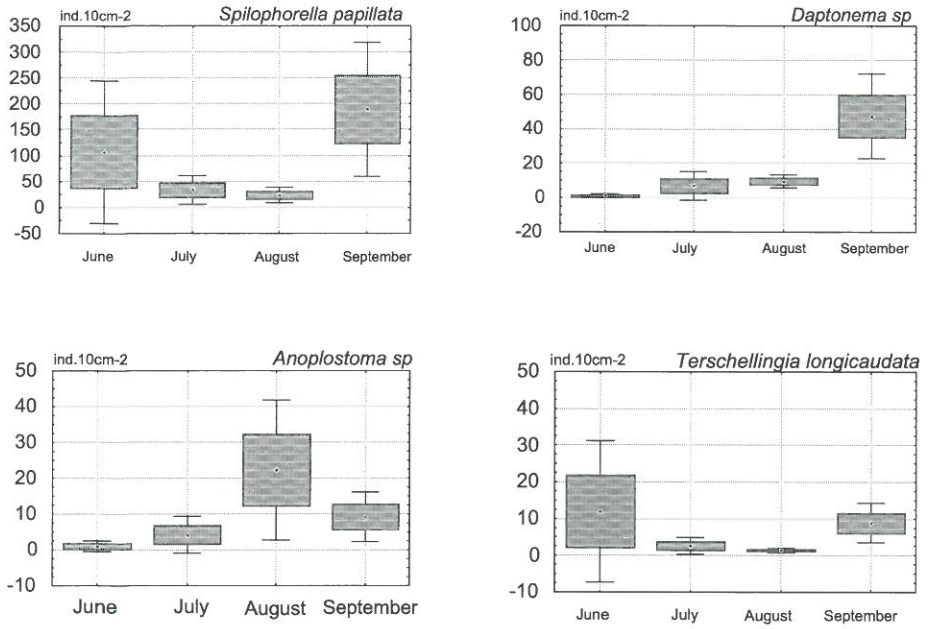


Figure 5.8 Temporal fluctuations of the most abundant nematode species in Pond C. (Data:mean/SE/1.96SE). Notice that different scales were used due to the data distribution.

At Pond D, the four most abundant species, *S. papillata, Daptonema* sp, *T. longicaudata* and *Kraspedonema* sp increased temporally in density (figure 5.9). Only for *S. papillata* the ANOVA assumption was fulfilled and statistical differences were observed among the months (F-4.743; df=3; $p<0.01$), July and August where the nematode densities were significant different from the October values ($p<0.05$). Kruskall-Wallis test indicated statistical differences in nematode densities for *Daptonema* sp (H=20.28; df=3; $p<0.01$), *Kraspedonema* sp (H=9.3857; df=3; $p<0.05$) and *T. longicaudata* (H=15.771; df=3; $p<0.01$). There were no significant difference between months for other species (H=4.301; df=3; $p>0.05$).

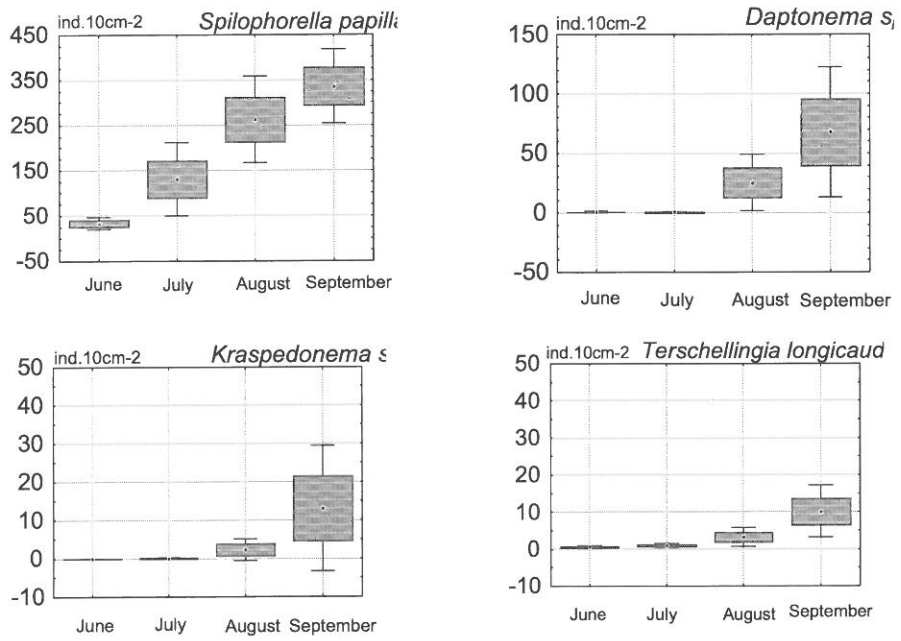


Figure 5.9 Temporal fluctuations of the most abundant nematode species in Pond D. (Data:mean/SE/1.96SE). Notice that the vertical scales are different due to data distribution

At Ponds A, C and D there were significant correlation between the total density of nematodes and the three most abundant species (tables 5.2; 5.3 and 5.4).

Table 5.2 Spearman rank correlation (r) for between the densities of the different nematode species in the shrimp pond A, Only significant correlations are registered. (**highly significant; *significant).

Ponds	Species	r	*p-value*
A	*Terschellingia longicaudata* vs *total*	0.82	**
	Terschellingia longicaudata vs Daptonema sp	0.54	**
	Spilophorella papillata vs total	0.66	**
	Spilophorella papillata vs. Daptonema sp	0.5	*
	Daptonema sp *vs* total	0.45	**

Table 5.3 Spearman rank correlation (r) for between the densities of the different nematode species in the shrimp pond C. Only significant correlations are registered. (**highly significant; *significant).

Species	r	*p-value*
Spilophorella papillata vs total	0.79	**
Daptonema sp *vs Anoplostoma* sp	0.59	**
Daptonema sp *vs* others	0.42	**
Daptonema sp *vs* total	0.43	**
Terschellingia longicaudata vs others	0.47	**
Terschellingia longicaudata vs total	0.39	*
Others *vs* total	0.40	*

Table 5.4 Spearman rank correlation (r) for between the densities of the different nematode species in the three shrimp pond D . Only significant correlations are registered. (**highly significant; *significant).

Species	r	*p-value*
Daptonema sp *vs. Kraspedonema* sp	0.56	**
Daptonema sp *vs* others	0.50	**
Daptonema sp *vs total*	0.70	**
Daptonema sp *vs Terschellingia longicaudata*	0.63	**
Spilophorella papillata v. Daptonema sp	0.67	**
Spilophorella papillata vs Kraspedonema sp	0.41	*
Spilophorella papillata vs Terschellingia longicaudata	0.70	**
Spilophorella papillata vs others	0.37	**
Spilophorella papillata vs total	0.97	**
Terschellingia longicaudata vs. Kraspedonema sp	0.60	**
Terschellingia longicaudata vs others	0.51	**
Terschellingia longicaudata vs total	0.75	**
Others *vs* total	0.46	**
Kraspedonema sp *vs* total	0.51	**
Kraspedonema sp *vs* others	0.51	**

5.4.2 Population structure

The population structure of the nematodes indicates that juveniles were present throughout the shrimp production cycles, ranging between 20 – 60 % (figure 5.10). At Pond A, we registered a temporal increase of juveniles. In the Ponds C and D an increase of juvenile nematodes was observed. However, this increase was with lower density than in Pond A (figure 5.10). Otherwise no clear seasonal trends could be detected.

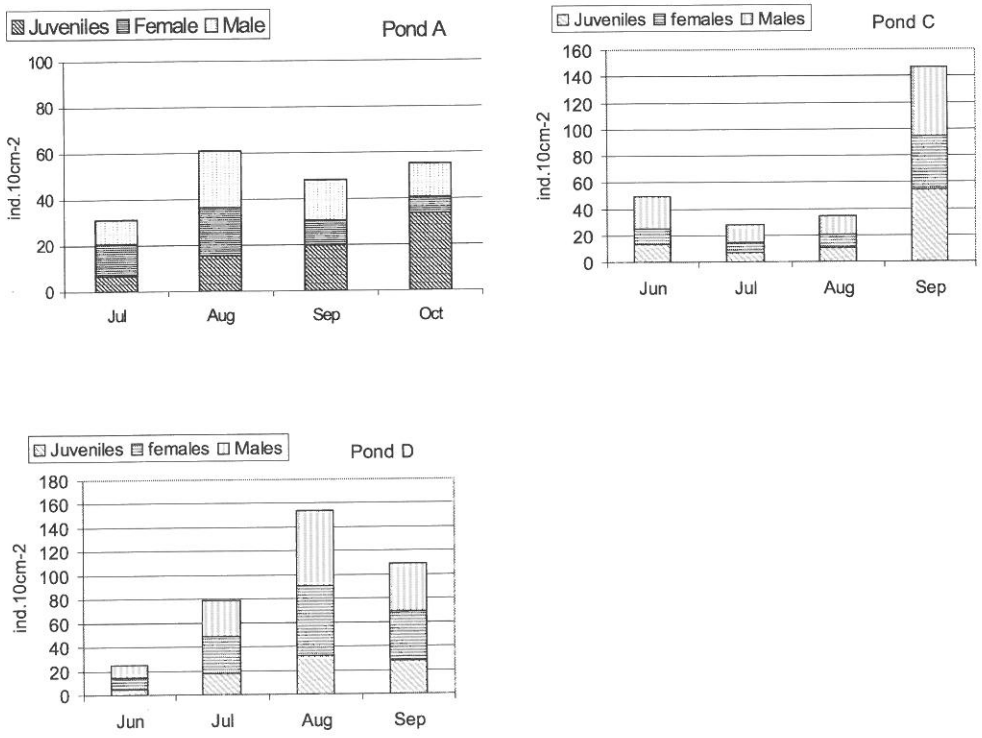


Figure 5.10 Nematode population structures in the three different shrimp ponds A, C and D. The scales for each graph have been adapted to the data distribution to facilitate the observation of the temporal fluctuation in the population structure.

5.4.3 Environmental variables

The research period (July - October) corresponds to the dry-cold season. The temporal fluctuation of temperature (figure 5.11) and oxygen (figure 5.12) in the water column was analyzed to determine possible effects on the nematode communities. At the saline environment in Pond A the average temperature for the Pond A was 22.9±0.73 °C. While at the estuarine environment, it was 24.8±0.79 °C in Pond C and 24.9±0.75

°C in Pond D. The fluctuation was similar for both environments even though there were lower temperatures in 2001 than in the 2000 (see figure 5.11).

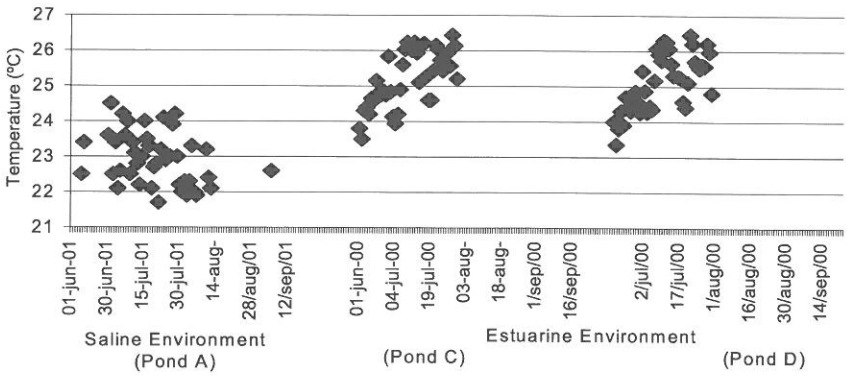


Figure 5.11 Temporal fluctuation of temperature in shrimp ponds A, C and D.

At the Pond A the oxygen level was 4.1±1.36 $mg.l^{-1}$, while to the other two Ponds , C and D, oxygen level were 5.4 ± 0.68 $mg.l^{-1}$ and 5.4± 1.3 $mg.l^{-1}$, respectively (figure 5.12). These values are within the tolerance limits given for aquatic organism (Boyd, 1995).

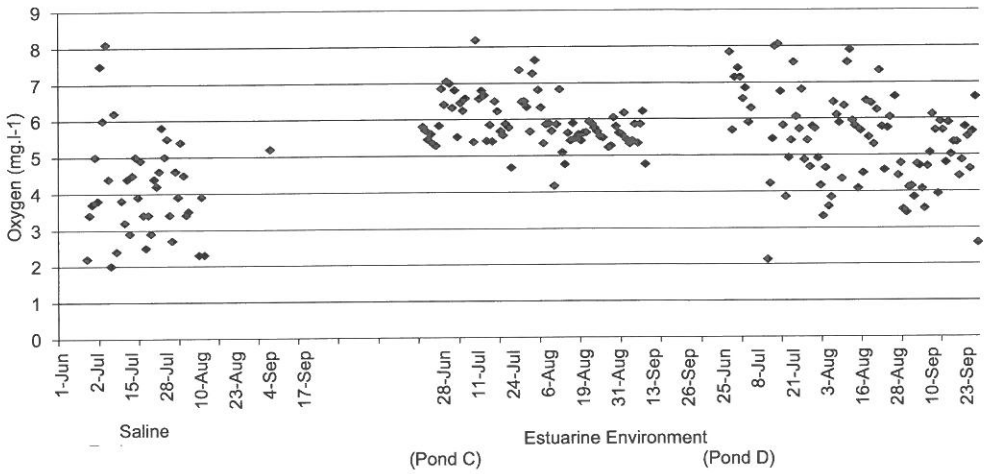


Figure 5.12 Temporal fluctuation of oxygen in shrimp ponds A,C and D.

There were no statistical significant correlation between the environmental variables and nematode density to the three ponds (table 5.3).

Table 5.5 Spearman rank correlation (r) for the nematode species in the three shrimp ponds A, C and D. Only significant correlations are registered; [(b)] means not statistical significant

	Variables	r
Pond A	Nematodes vs Temperature	-0.18[(b)]
	Nematode vs Oxygen	-0.20[(b)]
Pond C	Nematode vs Temperature	0.07[(b)]
	Nematode vs Oxygen	0.06[(b)]
Pond D	Nematode vs Temperature	0.00[(b)]
	Nematode vs Oxygen	-0.07[(b)]

5.4.4 Management practices

The sampling in each pond was during a shrimp production cycles. Shrimp weight and survival after the harvest was obtained. Different levels of survival and shrimp pounds produced were

registered (table 5.4). It was not possible to do a statistical analysis with management practice data, because we had a lot of missing values in the data set, but we observed that there was no relationship between nematode densities or diversities with production data.

Table 5.6 Management practices information of shrimp ponds A, C and D.

	Pond A	Pond C	Pond D
Initial density of shrimp larvae (ind.m^{-2})	9.4	10	12
Harvest pounds (pounds.ha^{1})	789	230	354
Survival (%)	62.48	11.8	14.3
Average weight of shrimp at harvest (g)	8.20	8.5	9.3
Stocking date	12 –July-01	19-Jun-00	20-Jun-00
Harvest date	12-Oct-01	11-Sep-00	11-Sep-00
Culture days	105	84	83
N° nematode species	7	12	11
Nematode densities No. ind.$10m^{-2}$	49	121	252
Time to get the highest nematode density (days)	28	70	90

5.5 Discussion

Very impoverished benthic communities characterized the shrimp pond bottoms. Nematodes represented about 90% of the total number of benthic organisms. The total densities of the nematodes were lower than recorded during benthic investigations of natural environments, where usually more than 1000 ind.$10cm^{-2}$ were found (Olafsson, 1992; Janssens, 1999; Netto & Galluci, 2003). The nematode densities had different fluctuations in the three shrimp ponds. However, at the end of the shrimp production cycle, an increase of nematode densities was registered for all of them.

It is important to consider that the water coming from the adjacent estuary has higher levels of nutrients than in oceanic or in river ones, because estuaries trap and re-suspends nutrients; also the primary producers are often not limited by nutrients as they are in the open ocean or in rivers (where they are flushed away; King & Garling, 1983; Drjoe, 2005). Moreover, the exchange of water is not continue within the shrimp pond and the initial species composition of the incoming water, from saline and estuarine environment, can also establish the differences found over the different shrimp ponds.

Inside the shrimp pond the artificial input of organic matter (fertilization and artificial food application; Villalón, 1991) should improve the nematode densities, even though we could not identify which additives were the most relevant for these benthic communities. The combination of these inputs of organic matter with the lack of water movement within the pond reduce the exchange of organic material. Hence, an anaerobic food web is originated based on the excess of added organics. Indeed, low values of oxygen were measured near the bottom of the pond and it is possible to create different microhabitat inside, with zones with high or low levels of organic matter and in consequence with high or low oxygen levels.

Nehring *et al.* (1990), Olaffson (1992) and Netto & Galluci (2003) commented that the free-living nematodes normally present a high degree of specificity in the microhabitats and they have the capacity to exploit those niches. On the other hand, within the shrimp pond, the ecosystem is changed on the average every three months (period of one shrimp production cycle); which means a continue periods of dessication-wet period cycles, where nematodes seem to be the only benthic organisms, which can survive these drastic changes.

In general, the species richness is very low: 9 to 13 nematode species are recorded in the ponds investigated. Schrijvers (1996), Janssens (1999), Gheskiere *et al.* (2002), Netto & Gallucci (2003) found up to 90 species in natural mangrove or beach ecosystems under natural conditions.

Tita *et al.* (2001) mentioned that the diversity is inversely proportional to the environmental disturbance. The presence of shrimp on the pond bottom cannot only cause effects on bioturbation creating favorable environments for microbial activities; the predation activities of the shrimp on the other hand, can reduce the densities and diversity of the nematodes as well. Vanreusel (1990) commented that a mechanical disturbance, associated with predictable or un-predictable environmental fluctuations, is one of the most important factors determining the structure of the nematode communities (Bouwman, 1983; Alongi, 1986; Tita *et al.*, 2002).

Li *et al.* (1996) mentioned that the nematode communities in the brackish zone of the Westerschelde Estuary (The Netherlands) had a lower biomass and higher temporal variation than the nematode communities in the marine part of the estuary. They attributed these differences to food web characteristics with different food sources and biomass level in both areas. In the Ecuadorian shrimp ponds investigated differences were observed when the saline pond is compared with the estuarine ponds. In both ponds systems, comparable management practices were performed; nevertheless, different nematode characteristics can be observed. The highest abundance of feeding type 1A was found in the estuarine environment (represented by *T. longicaudata*). In lower density of nematodes in Pond A, the highest shrimp survival rate was observed (62%) while in the other two ponds C and D, the survival rate were 12% and 14%, respectively.

Nevertheless, the effects of management practices produce changes in the pond bottom, every three months at least (harvesting practices with mechanically disturbing the soil, lime application, fertilizer application, etc. Boyd & Tucker (1998) determined that the soil from new pond is slightly basic, high in clay content and low in concentration of organic matter, nitrogen, sulphur and phosphorous. The increase of these compounds during the shrimp production cycle and after the pond harvest resulted from residues of feed, shrimp faeces, dead plankton and possible manure and chemical fertilizer. So, it is likely that some nematode species can be the result of the increase of the input of these additives inside the pond (Weston, 1991) while others species are eliminated. We assume that several nematode species come with the entering water into the pond (as indicated earlier), but the conditions of the shrimp pond were not suitable to all of them. Only *S. papillata*, *Daptonema* sp, *Kraspedonema* sp and *T. longicaudata* can survive in this environment.

The nematode communities from the different environments (saline and estuarine) receive similar input of nutrients and are characterized by three species. We could consider the *Terschellingia longicaudata* - *Spilophorella papillata* – *Daptonema* sp community as a characteristic community for Ecuadorian shrimp pond bottoms. Other

nematode species differed from one pond to the other and even they differed within the same ecosystem (differences occur between the two estuarine shrimp ponds as well).

Conversely *T. longicaudata,* a selective deposit feeder (1A), was more abundant in the saline environment. The others feeding groups, as non-selective deposit feeders (1B) mainly *Daptonema* sp, and *Anoplostoma* sp, an epigrowth feeders (2A) and predators and omnivorous (2B), were also present. The non-selective deposit feeders had the highest number of species within their community. This could be related to the amount of artificial food, which is added into the system. Nevertheless, the high abundance of *Spilophorella papillata,* an epigrowth feeder (2A), in the estuarine environment (compared with the saline environment) can be due to the presence of higher densities of diatoms found in the sediment (data not published here).

The presence of the three species, mentioned above, *T. longicaudata, S. papillata and Daptonema* sp dominated the general distribution of the benthic communities. This indicates that the adaptation capacity of these species to shrimp pond bottom conditions were higher than for other nematode species. *Terschellingia* species had been often associated with silty sediments characterized by a low diversity of species (Warwick, 1971; Warwick & Gee, 1984). And Navarrete & Herrera (2004) found *T. longicaudata* in a reduced environment (Chetumal-Quintana, Mexico Bay), which was characterized by a low diversity of nematodes too. Netto & Galluci (2003) found 3 species of *Terschellingia* in salinities from 21 to 25 PSU in mangrove area. *Daptonema* sp was also found by these authors but in low density. *Spilophorella* sp has been also found in special environment such as the Salton Sea, California, in relation with sport fishery (Linsley & Carpelan, 1961). And, also by Netto & Gallucci (2003) in mangrove area in Ratones Estuary at Santa Catarina Island in Brazil, where salinity had in average 21 PSU.

Temperature and salinity had been considered as a stress factor, important in structuring nematodes communities (Schratzberger & Warwick, 1999). Some correlation has been found between nematode communities and environmental factors (Netto & Galluci, 2003). Although we registered low oxygen levels in the shrimp pond in the

saline environment, with *Terschellingia longicaudata* as a dominant species, we cannot demonstrate a statistical cause-effect relationship the survival of benthic organisms.

5.6. Conclusions

- The Ecuadorian shrimp pond bottoms had a poor nematode communities, both in densities and species richness.
- *Spilophorella papillata, Daptonema* sp and *Terschellingia longicaudata* characterize the shrimp pond bottom communities.
- The nematode species composition (in terms of densities) did follow some temporal variability in ponds C and D, but less obvious in Pond A (MDS-plots).
- Nematode communities consist mainly of deposit-feeders and epistratum-feeders.
- There where different nematode densities in the three ponds, which indicate that the nematode communities behave differently from one pond to other.
- No correlation could be detected between the management practices in the shrimp ponds and the nematode community characteristics.

CHAPTER 6

The effects of some nutrients upon nematode communities: An experimental approach

6. The effects of some nutrients upon nematode communities: An experimental approach

6.1 Abstract

The effects of some commercial products, upon nematode communities were studied. A mesocosm with 12 tanks of 500 litres, filled with sediment brought from a shrimp pond in The Gulf of Guayaquil (Pond E), was used. Nutrients sources Super phosphate Triple, SPT (4.96 $g.tank^{-1}$; 400 $k.hectares^{-1}$) and NO_3NH_4 (1$g.tk^{-1}$; 200$k.hectares^{-1}$) were used. The experiment was followed during 3 months and samples were taken every 15 days. Environmental variables such as temperature, oxygen and salinity, were monitored. Water and soil samples for chemical and physical analysis were also taken. An average nematode density of 634 ind.$10cm^{-2}$ was registered in the tanks. Five families divided over ten genera were found. *Terschellingia longicaudata, Spilophorella papillata, Gomphionema fellator* and *Theristus parambronensis,* were the most abundant species. There were no statistical differences neither in total density of nematodes, nor between treatments and dates inside the treatments. Environment variables were the same for all the treatments. Total density of nematode was negatively correlated with the pH and with the nitrate levels. *S. papillata* was correlated positively with temperature and negatively correlated with pH and positively with total ammonia nitrogen (TAN).

6.2 Introduction

Shrimp farmers use several additives such as fertilizers, artificial food, probiotics, antibiotics, cupper sulphate and lime in order to improve shrimp production (Boyd, 1998, Verschuere *et al.*, 2000; Sonnenholzner, 2003). The fertilization is used to enhance the development of natural productivity (mainly phytoplankton) at the pond and, to increase the inorganic nutrient concentrations (Tacon, 1988; Stickney, 1994; Boyd, 1995, 1998; Romano & Caraballo, 1996). Some commercial fertilizers, which include organic and

inorganic components, are directly applied to the soil to improve soil quality and to enhance benthic communities (Stickney, 1994; Boyd, 1995).

Some shrimp farmer uses a combination of these inorganic and organic fertilizers since the blends seem to promote a wide variety of both autotrophic and heterotrophic organisms (Geiger, 1983). Organisms such as nematodes and polychaetes process these materials transforming them into usable ones for other organisms in the soil as well as in the water column. Through their movements up and down in the sediment, organisms facilitate the oxygen penetration to deeper layers (Hopkins *et al.*, 1994; Boyd, 1995).

Uneaten food, faeces, decaying plankton, eroded soil and microorganisms are also sources of nutrients in shrimp ponds. When these feed items are not consumed by aquaculture animals, they are accumulated at the pond bottom as organic matter, which is transferred to the animals more efficiently than organic matter resulting from primary productivity in ponds (Boyd, 1995). The principal organic components in waste waters from shrimp ponds, include proteins (40-60%), carbohydrates (25-50%) and lipids (10%) and are composed of various combinations of carbon, hydrogen, oxygen, nitrogen, phosphorous and sulphur, which are used by organisms within the water column (Boyd, 1995) as well as by the organisms of the sediment.

Inside the pond, the organic and inorganic elements increase the primary productivity of the water column and act over the benthic communities (Parson *et al.,* 1984; Nicovita, 1998). The development of this phytoplankton bloom will support a concomitant zooplankton bloom (Boyd, 1990; Stickney, 1994). Nevertheless, phytoplankton blooms increase the turbidity in ponds and reduce light penetration causing a collapse in primary production and hence food for zooplankton (Stickney, 1994; Boyd & Tucker, 1998).

Larvae and juvenile stages of various aquaculture animals require this living food. Those species accept prepared feed at first feeding but soon they will be commonly

benefit from the availability of natural food sources in the water and soil (Stickney, 1994; Martínez-Córdova *et al.,* 2003). Bacteria, protozoa, diatoms, nematodes and small crustaceans, are part of those living food in the ponds (Pillay, 1997).

Incoming water especially run off water from land and from a surface source may also contain sufficient supply of nutrients to establish and support natural productivity. The rivers which serve as a water supply for ponds used for marine shrimp farming, often have high concentrations of nitrogen and phosphorous (Ormaza, 1993; Stickney, 1994; Guerrero, 2000; INP, 2004). In Ecuador and Thailand, Boyd (1995) observed dense phytoplankton development in shrimp ponds with only nutrients introduced from the water supply systems. These nutrients include nitrogen, phosphorous and organic matter and, are removed during shrimp harvest (Guerrero, 2000). Boyd (1995) also mentioned that the organic carbon and nitrogen appear to accumulate in some ponds.

Phosphorous is continuously added to the pond through the feed and the fertilizers (Stickney, 1994; Boyd, 1990; Ritvo *et al.*, 1998). The nutrients and organic residues tend to accumulate at the bottom and thus, to some extend, are removed from the water phase (Avnimelech & Ritvo, 2003). However, an excessive accumulation beyond what could be defined as carrying capacity of the sediment may result in deterioration of the pond system. Such development seems to be of special importance for shrimp culture, since shrimp live in the soil-water transition zone (Avnimelech & Ritvo, 2003) and to the organisms, which live there.

The aim of these experiments was to test the effects nutrients, nitrogen and phosphorous using commercial products as nutrient sources (commonly used in shrimp aquaculture), on nematode communities under mesocosm conditions.

6.3 Material and methods

The experiments were performed at the CENAIM Laboratory in the Guayas province, Ecuador (figure 6.1).

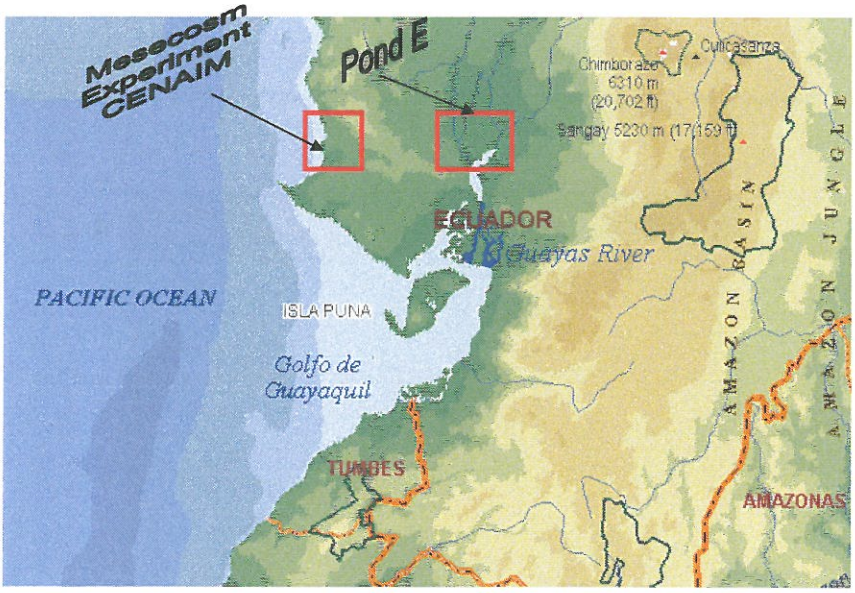


Figure 6.1 The location of the mesocosm experiment at CENAIM, Ecuador.

Experimental set up

A mesocosm system of 8 tanks of 1,000l capacity each (figure 6.2) with $1m^2$ in surface each was used.

Natural sediment from a commercial shrimp Pond E located in the estuarine environment at the Gulf of Guayaquil was 'inoculated' in each of the tanks (±20cm depth). This sediment was obtained with a bulldozer from a shrimp pond and the depth of the sediment was around 40 cm. The tanks were filled with un-filter seawater until

70cm (coming from the adjacent sea). Fresh un-filtered seawater was added to the tanks in order to keep the level, lost by the evaporation process.

The tanks remained without any treatment during one month before the start of the experiment, to allow the benthic community to reach a "stable state". From the beginning, aeration was used in all the tanks, in order to avoid anoxic conditions that might influence the experiment results. Shrimps were no present during this experiment.

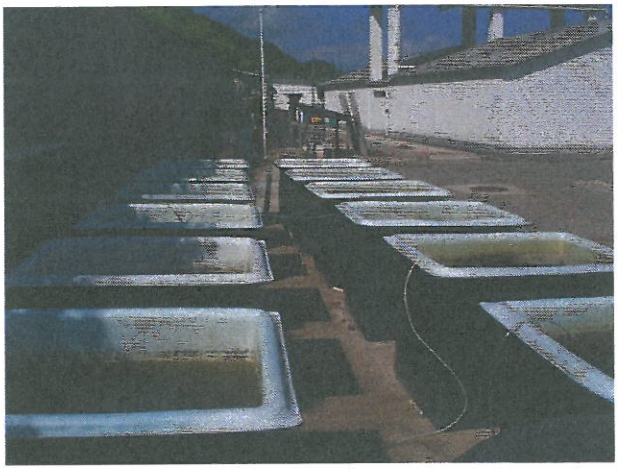


Figure 6.2 Mesocosm tanks 1,000 l capacity.

Experimental design

The effects of phosphorous and nitrogen were investigated using two commercial fertilizers $Ca(H_2PO_4)_2$ or SPT, called TR2-PH first treatment and NO_3NH_4, called TR3-NI second treatment and a combination of both products which is called TR4-PHNI, third treatment; the control situation had none of these products (Control). The doses that were used are indicated in table 6.1. These doses were obtained from literature

(Hepher, 1962; Boyd, 1976; Boyd, 1990; Brown *et al.*, 2001; Lin *et al.*, 2001; Yi *et al.*, 2001). The average of the lowest doses was chosen without considering the common doses used at shrimp farming in order to determine a concentration to be the first of a next experiments. These doses were initially discussed for usage in the experiment at CENAIM laboratories. For each treatment, 2 tanks were used as replicates and these were randomly chosen within the set of 8 tanks (table 6.1).

Table 6.1 Experiment treatments.

	Treatments	Tanks
Control	Control	2,9
TR2-PH	$Ca(H_2PO_4)_2$ or SPT; 4.96 tk^{-1} = 1.28g P_2O_5	3,5
TR3-NI	NO_3NH_4; 1gr = 0.34g.N.tk^{-1}	7,10
TR4-PHNI	$Ca(H_2PO_4)_2$ or SPT + NO_3NH_4; = 1.28 P_2O_5 g.tk^{-1} + 0.34 gN.tk^{-1}	6,12

1.28g $P\text{-}PO_4$ tk-1 = 75kg.ha-1

0.34gN.tk-1 = 15.2kg.ha-1

Tanks distributions:

	2	3		5	6
7		9	10		12

The sampling period for the experiment was from May 21- July 8th , 2002. The addition of the fertilizers started on June 6th, 2002 and continued on June 11, 18 and 25,2002.

According to the experiment requirements, water samples were taken once a week, with a plastic bottle to analyse reactive phosphate ($P\text{-}PO_4$), total ammonia (TAN), nitrite ($N\text{-}NO_2$) and nitrate ($N\text{-}NO_3$) in the laboratory (Clesceri *et al.*, 1998). Salinity, temperature

and oxygen level were monitored every sampling day with an YSI85 oxygen-meter and, pH with a TOA pH-meter HM-55 (Clesceri *et al.,* 1998). Soil samples were taken with a 5 cm diameter corer (see Chapter 2 for details), for nematode analysis. The samples for biological analysis were preserved with formaline 4% neutralized with sodium tetraborate.

Laboratory analysis

The biological samples were processed according to Vincx & Heip (1996) (see Chapter 2 for details). The density of organisms is express as ind.10cm^{-2}. These analyses were performed in CENAIM and at Marine Biology Laboratory of Ghent University.

Statistical analysis

An ANOVA was applied and a Two-way ANOVA was also performed in order to combine treatments and dates, when assumption of homogeneity of variances and independency of mean and variances were fulfilled. Data were Log + 1 transformed. When assumptions for normality were not fulfilled after log+1 transformation, non-parametric Kruskall-Wallis test was applied. The total number of replicas was used to evaluate the relationships among sample dates, seasons and shrimp production cycles (Statistica 6.0, 2000). Spearman Rank Correlation (Sokal & Rohlf, 1981, 1995) was used to analyse the relationship between oxygen and temperature and nematode communities' distribution and the relationship inside these communities (Statistica 6.0, 2000).

6.4 Results

6.4.1 Description of nematodes communities

In total 110,382 nematodes were counted over a period of 8 weeks sampling (May 20 to July 8, 2002) (full data set in annex 6). Because of sub-sampling for species identification, 4,988 nematode individuals were determined at the species level. Ten different species of nematodes belonging to 5 different families are reported (table 6.2). For the total of the treatments there were in average 634 nematodes.10cm^{-2}. The other meiofaunal organisms (such as copepods, small polychaetes), which represented together around 2% of the total of individuals, were not considered in the present research. For the Control 639 nematodes.10cm^{-2} were registered, while for TR2-PH, TR3-NI and TR4PHNI, 622 nematodes.10cm^{-2}, 613 nematodes.10cm^{-2} and 655 nematodes. 10cm^{-2}, were registered, respectively.

Table 6.2 Relative abundance (%) of the nematode species by treatment, together with the feeding type according to Wieser, 1953a,b. 1A Selective deposit feeders, 2A Epistratum feeders, 1B Non-selective deposit feeders; 2B Predators and omnivores.

	Control	TR2-PH	TR3-NI	TR4-PHNI	Feeding type
Spilophorella papillata	22.4	38.3	33.3	37.6	2A
Terschellingia longicaudata	51.2	28.5	41.8	33.6	1A
Daptonema sp	0.2	0.3	0.2	1.1	1B
Gomphionema fellator	7.8	8.8	8.5	8.7	2A
Theristus parambronensis	17.7	23.3	15.5	17.6	1B
Gnomoxyala sp	0.2	0.2	0.2	0.2	1B
Neochromadora sp	0.2	0.2	0.2	0.2	2A
Paracomesoma sp	0.2	0.2	0.2	0.2	1B
Prochromadorella sp	0.2	0.2	0.3	0.9	2A
Sabatieria sp	0.2	0.2	0.0	0.2	1B

The assumptions to ANOVA were not fulfilled and Kruskall-Wallis test was applied. No statistical differences were registered among treatments (H=4.800; df=5; p>0.5), nor among dates (H=1.00; df=4; p>0.05) (figures 6.3 and 6.4).

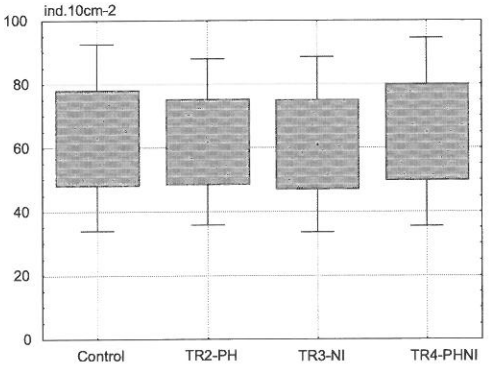


Figure 6.3 Nematode densities by treatment. (Data:mean/SE/1.96SE).

The assumption of ANOVA was fulfilled to all the treatments and no statistical differences in time were registered at Control (F=0.051; df=4; p>0.05), for TR2-PH (F=0.041; df=4; p>0.05), for TR3-NI (F=0.0528; df=4; p>0.05) and for TR4-PHNI (F=0.059; df=4; p>0.05).

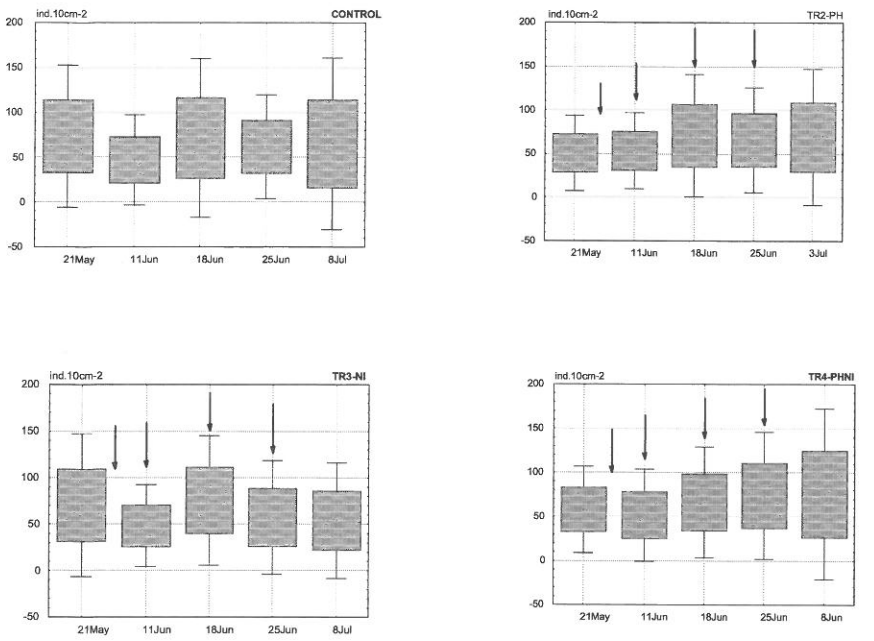


Figure 6.4 Temporal fluctuations of nematode densities by treatment. The arrows indicate the application of nutrients. (Data:mean/SE/1.96SE).

In all the graphs and statistical analysis, only the four most abundant species *Terschellingia longicaudata*, *Spilophorella papillata*, *Gomphionema fellator* and *Theristus parambronensis*, are discussed. These four species made up 90% of the community (table 6.3). The density fluctuations of the four dominant species were different within the four different treatments (figure 6.5). The highest relative abundance of *T. longicaudata* was registered at the Control followed by TR3-NI, with 52% and 42% respectively (table 6.2). For *S. papillata* the highest density was at TR2-PH and in TR4-PHNI (around 39%) and the lowest in Control (23%). *G. fellator* had a similar

abundance in all the treatments. Finally, *T. parambronensis* had the highest abundance in TR2-PH (24%). Other species were more abundant at the Control, but this group represented just 1% of the total density of nematodes.

ANOVA were fulfilled for all the treatments and there were statistical differences among specie densities to all of them. To all the treatments there were significant differences between the nematode species densities; Control ($F=5.327$; $df=3$; $p<0.01$), TR2-PH ($F=7.782$; $df=3$; $p<0.01$), TR3-NI ($F=7.782$; $df=3$; $p<0.01$), TR4-PHNI ($F=5.541$;$df=3$; $p<0.01$). Only significant differences for ANOVA and the Post Doc test Scheffe results are shown in table 6.3.

Table 6.3 Statistical differences between treatments at species level. Only highly significant "**" and significant differences "*" are shown.

	Control	TR2-PH	TR3-NI	TR4-PHNI
Terschellingia longicaudata vs Gomphionema fellator	**	**	**	*
Terschellingia longicaudata vs Theristus parambronensis	*			
Spilophorella papillata vs Gomphionema fellator		**	**	*
Gomphionema fellator vs Theristus parambronensis		**		

Statistical differences were found at the species level, between treatments (figure 6.5). For *Terschellingia longicaudata* and *Theristus parambronensis* no assumption of ANOVA were fulfilled and a Kruskall-Wallis test indicate no significantly differences among treatments ($H=4.444$; $df=3$; $p>0.05$) for *T. longicaudata* but statistically significant differences for *T. parambronensis* ($H=11.929$; $df=3$; $p<0.05$). For *Spilophorella papillata* and *Gomphionema fellator* the assumption of ANOVA were fulfilled and statistical differences were registered among treatments for the first specie ($F=2.685$; df;=3; $p<0.05$), but no for the second one ($F=0.127$; $df=3$; $p>0.05$).

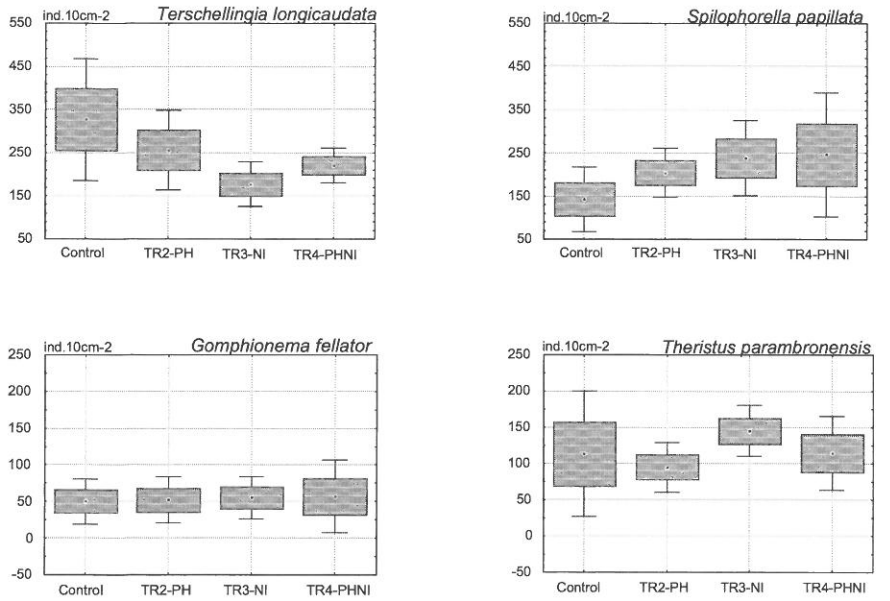


Figure 6.5 Fluctuation in nematode species densities by treatment. Notice that the vertical scales for the upper panels is different from the bottom ones for display reasons (Data:mean/SE/1.96SE).

Terschellingia longicaudata and *Theristus parambronesis* were statistically related (Spearman rank correlation, r=0.90, p<0.5). But, in the case of *T. longicaudata* in the treatments TR3-NI and TR4-PHNI there was an initial increase in density followed by a decrease throughout the time. At TR2-PH a decrease tendency was observed; while in the control there were not = clear effects. *Spilophorella papillata* registered a trend for increasing with time in all treatments but not in the Control (figures 6.6 and 6.7). *Gomphionema fellator* decreased in TR2-PH and TR3-NI, but there was not a clear answer for the treatment in the case of the TR4-PHNI. The same happened at the Control, where not clear pattern was observed.

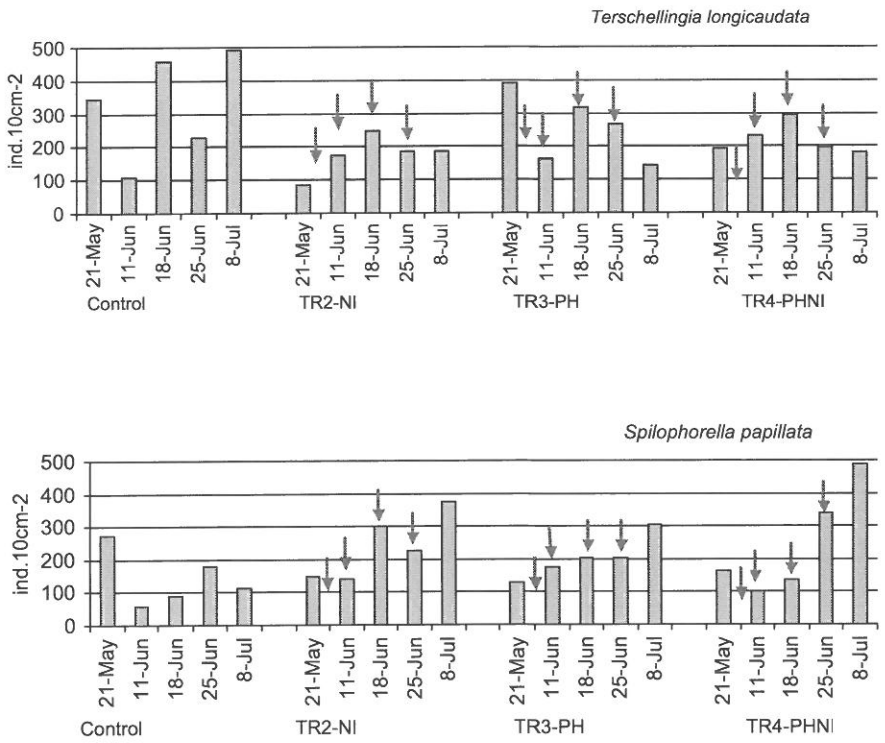


Figure 6.6 Temporal fluctuation of *Terschellingia longicaudata* (upper panel) and *Spilophorella papillata* (lower panel) for the different treatments. The arrows indicate the nutrient application dates.

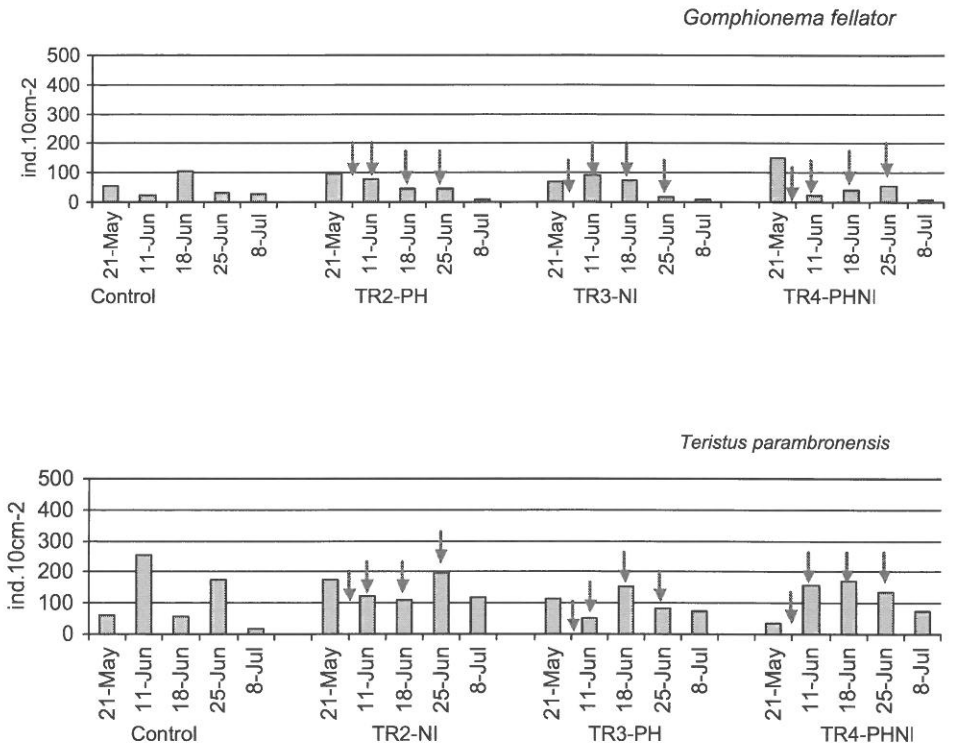


Figure 6.7 Temporal fluctuation of *Gomphionema fellator* (upper panel) and *Theristus parambronensis* (lower panel) for the different treatments. The arrows indicate the nutrient application dates.

6.4.2 Population structure

The presence of juveniles in all of the treatments was observed with an average of 19% of the total. Female represented 37%, while male registered the 44% of the population

(figure 6.8). The lowest density of juveniles was registered at the treatment with the phosphorous compounds (table 6.4).

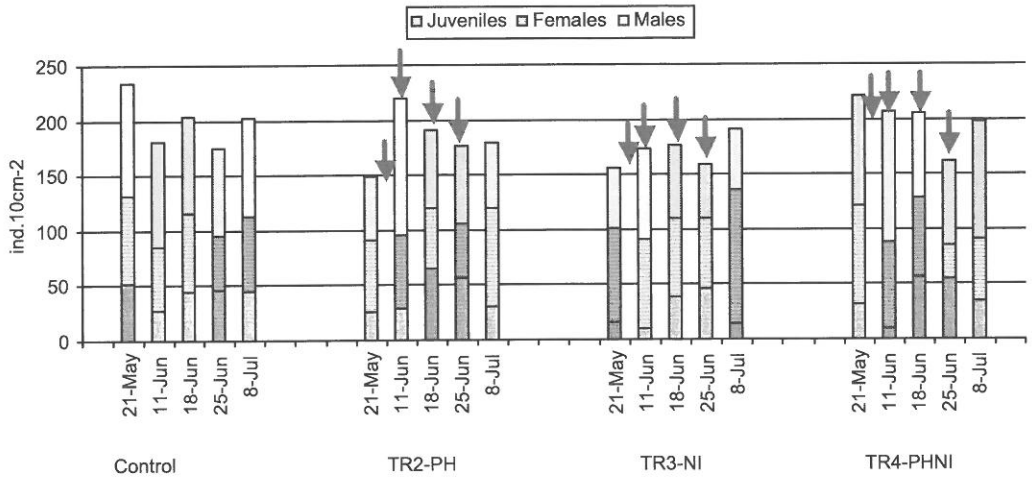


Figure 6.8 Temporal fluctuation of nematode population structure for the different treatments. The arrows indicate the nutrient application dates.

Table 6.4 Relative abundance (%) of juveniles, females and males for the different treatments

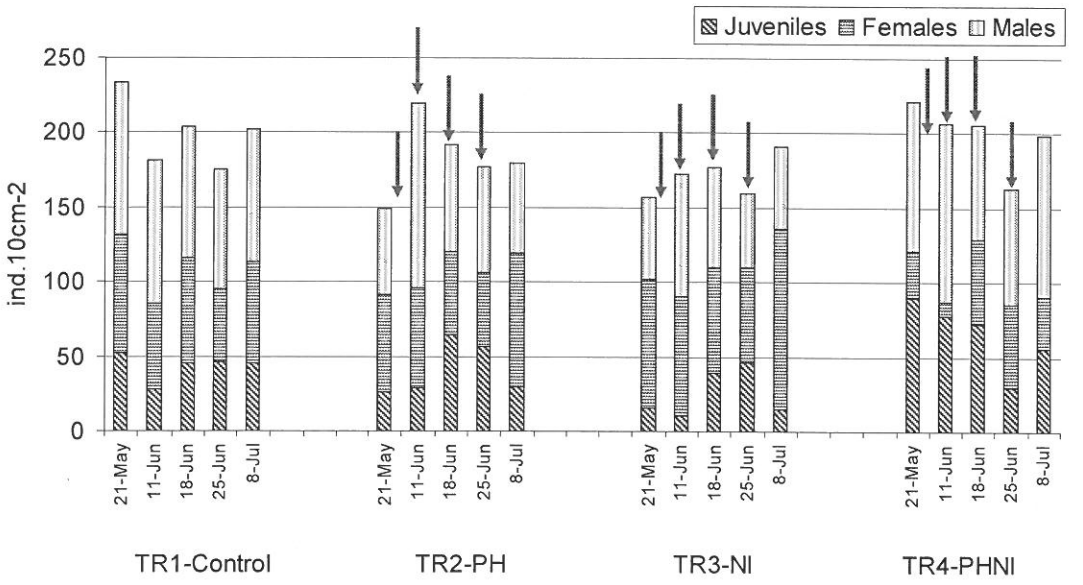
	Juveniles	Females	Males
Control	20	34	47
TR2-PH	15	46	39
TR3-NI	22	37	40
TR4-PHNI	18	34	48
Total	19	37	44

Errata for Chapter 6

Some figures were not properly printed in the textbook.

The corrected version is given below.

Pag.160, Figure 6.8



6.4.3 Environmental variables

In the table 6.5 the salinity, temperature, oxygen and pH data are summarized (see Annex 6 for the full data set). The salinity was high throughout the experiment (figure 6.9) because the tanks were filled one month prior to the experiment. New un-filtered seawater was added to the tanks in order to keep the level, lost by the evaporation process; no high variation in salinity levels was observed to none of the treatments (figure 6.9).

Table 6.5 Environmental variables data for the different treatments (averages plus standard deviation are indicated).

	Salinity (PSU)	Temperature (°C)	Oxygen (mg.l^{-1})	pH
Control	48.4±2.39	24.4±1.78	5.9±0.47	7.9±0.19
TR2-PH	48.7±2.43	24.3±1.78	5.8±0.52	8.0±0.21
TR3-NI	47.9±1.99	24.4±1.76	5.7±0.52	7.9±0.20
TR4-PHNI	49.0±2.67	24.3±1.82	6.1±0.65	7.9±0.18

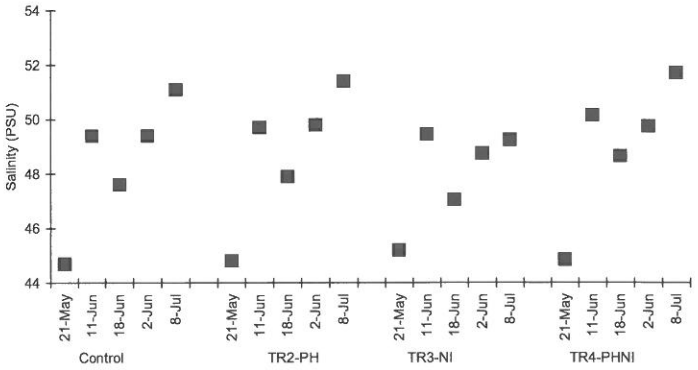


Figure 6.9 Temporal fluctuations in salinity (PSU) for all the treatments.

The water temperature registered was quite predictable for the dry-cold season in Ecuador, when the experiment was performed (figure 6.10; table 6.5).

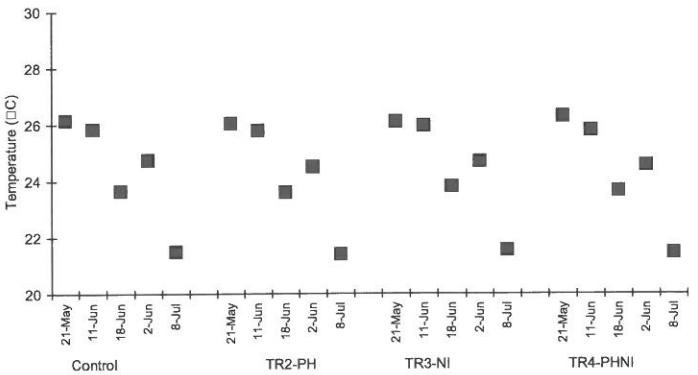


Figure 6.10 Temporal fluctuations in temperature (°C) for all the treatments.

The oxygen levels in the tanks varied probably because of the phytoplankton and bacterial activity inside the tanks (figure 6.11). A decrease in pH level up to the neutral value (figure 6.12) took place during the experiment, but always around the level indicated by Boyd (1990, 1998) as not harmful for aquatic organisms (table 6.5).

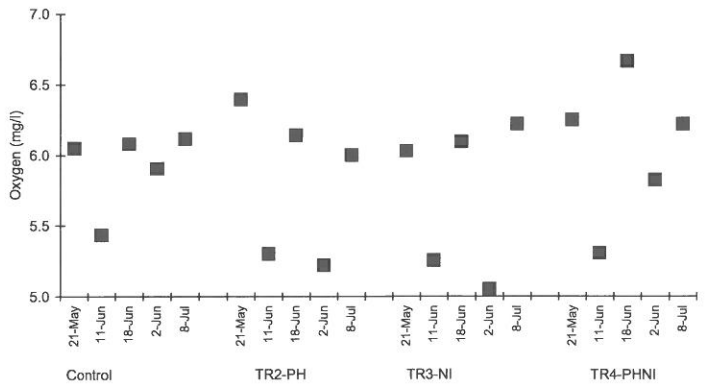


Figure 6.11 Temporal fluctuations of oxygen ($mg.l^{-1}$) for all the treatments.

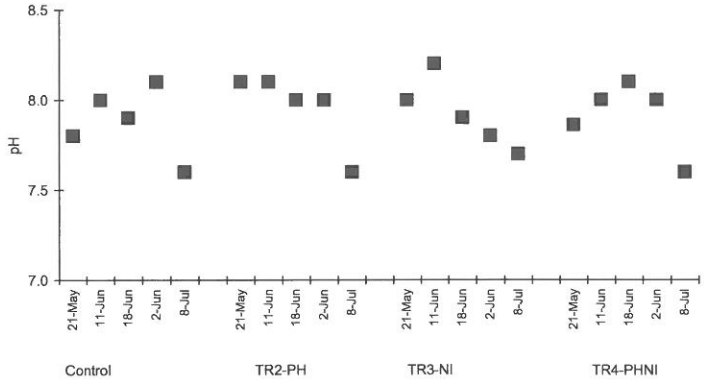


Figure 6.12 Temporal fluctuations in pH for all the treatments.

There was a high variability in the chemical variables between treatments (table 6.6). Different fluctuations were observed in the treatments. No assumptions to ANOVA were fulfilled for the phosphate ($P\text{-}PO_4$), ammonia (TAN) and nitrite (NO_2). A Kruskal-

Wallis test was applied for significant differences among the levels of the chemicals. No significant differences in phosphate level were registered (H=7.200; df=3; p>0.05). For ammonia levels no significant differences were registered among treatments (H=2.400; df=3; p>0.05) and for nitrite there were no statistical differences among treatments (H=0.800; df=3; p>0.05). For nitrate (NO_3) ANOVA assumptions were fulfilled and no significant differences were found in nitrate level among treatments (F=1.864; df=3; p>0.05).

Table 6.6 Chemicals variables during sampling period to the Control and the three treatments. (Averages plus standard deviation are indicated).

	Reactive Phosphate ($P\text{-}PO_5$) $mg.l^{-1}$	Ammonia (TAN) $mg.l^{-1}$	Nitrite ($N\text{-}NO_2$) $mg.l^{-1}$	Nitrate ($N\text{-}NO_3$) $mg.l^{-1}$
TR1-Control	0.47±0.20	0.47±0.57	0.07±0.13	0.07±0.06
TR2-PH	1.12±0.47	0.13±0.13	0.03±0.05	0.05±0.06
TR3-NI	0.69±0.41	0.33±0.39	0.06±0.09	0.12±-0.14
TR4-PHNI	0.92±0.39	0.13±0.14	0.03±0.04	0.07±0.06

As was expected, an increase in $P\text{-}PO_4$ level was observed with the application of the fertilizers on June 6th. The highest level of phosphorous was registered in the treatment TR2-PH where phosphorous fertilizer was applied. TR2-PH levels were followed by TR4-PHNI. In general, the phosphorous is absorbed by bacteria, phytoplankton and macrophytes (Rigler, 1964; Boyd & Musig, 1981), after the fertilization and also absorbed in the sediment. Phosphorus input also comes from metabolic wastes and from uneaten feed, but we did not perform our experiment in the presence of shrimp. We also observed an increase in phosphorous level in Treatment TR3-NI, where phosphorous was not applied, but in levels lower than in the other treatments. It is possible that some phytoplankton activity might be related to this increase in phosphorus, but we did not perform analysis of this ecological group during our research (figure 6.13).

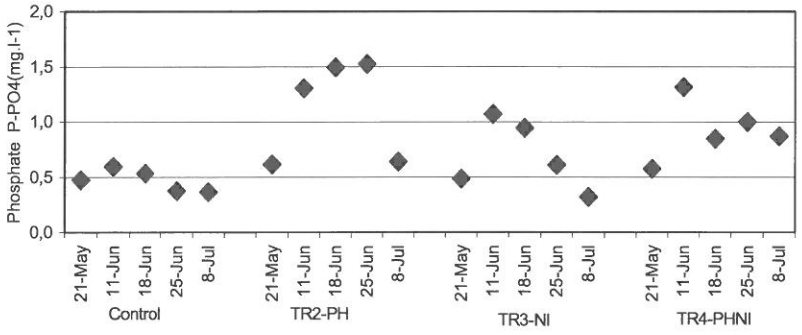


Figure 6.13 Temporal fluctuations in phosphate P-PO_4 (mg.l^{-1}) for all the treatments.

The levels of ammonia, nitrite and nitrate were comparable in all the treatments. The level of ammonia depends on the activity of the nitrifying and denitrifying bacteria and it is important as an excretory product from aquatic animal and also as per degradation of faecal matter and uneaten feed. But as we mentioned before, shrimp was not included during our experiment. Hence, we did not have an increase in TAN level beyond 0.05 mg.l-1 in treatments TR2-PH and TR4-PHNI. Also, the observed reduction in TAN (figure 6.14) with time in all treatments could be attributed to phytoplankton activity.

The nitrogen cycle involves ammonia fixing and nitrifying reactions and de-nitrification, which are the same process in reverse. The nitrification process involves oxidation of ammonia to nitrite and nitrite to nitrate, which is energy yielding process utilized by nitrifying bacteria. It means a continue change of these chemicals in the water column and sediment. Denitrifying bacteria does the reduction of nitrate to N_2. The nitrate (N-NO_3) followed the same fluctuation as the nitrite (N-NO_2) (figure 6.15).

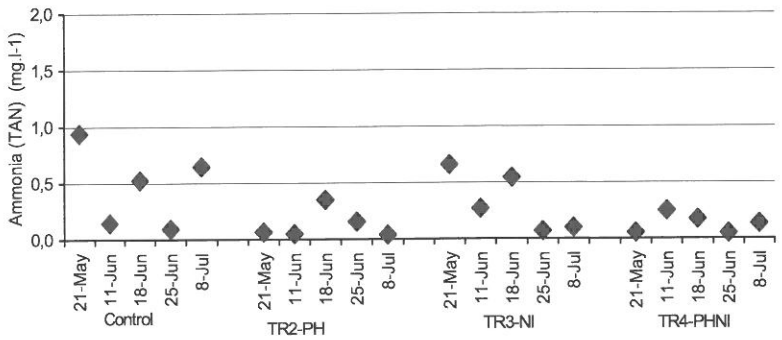


Figure 6.14 Temporal fluctuations in total ammonia as TAN ($mg.l^{-1}$) for all the treatments.

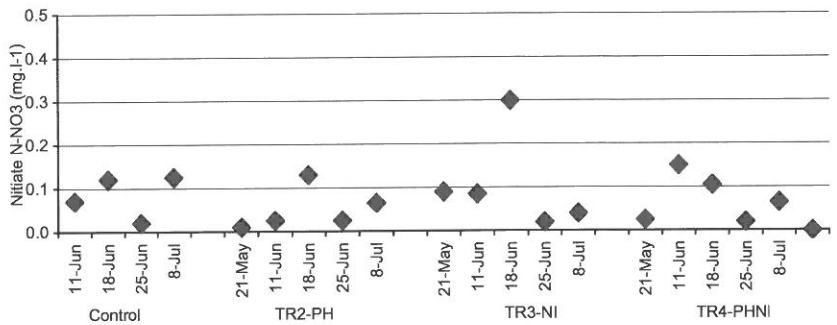


Figure 6.15 Temporal fluctuations in nitrate as $N\text{-}NO_3$ ($mg.l^{-1}$) for all the treatments.

The nitrite ($N\text{-}NO_2^-$) concentration had the same fluctuation in all treatments with the highest increase in TR3-NI treatment where nitrogen fertilizer was applied. In general the level of $N\text{-}NO_2$ remained lower than 0.1 $mg.l^{-1}$ until the end of the experiment, with exception of TR3-NI were the level increased at the end (figure 6.16).

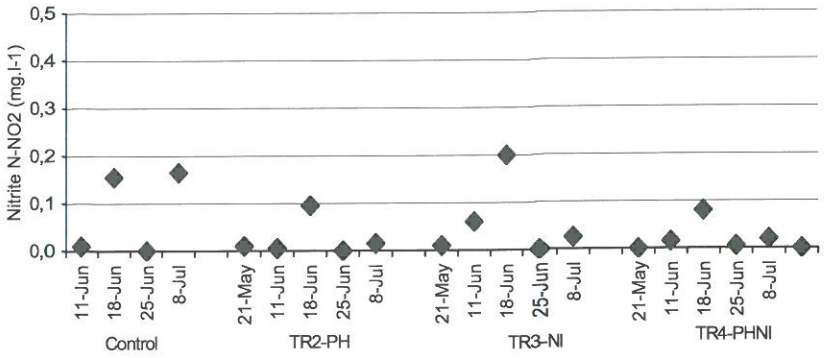


Figure 6.16 Temporal fluctuations in nitrite as $N\text{-}NO_2$ ($mg.l^{-1}$) for all the treatments.

Total nematode density were negatively correlated with pH level and with nitrate. At the species level only *Spilophorella papillata* show a relationship with the chemical variables (table 6.7). This species was positively correlated with the temperature and with the ammonia level and negatively correlated with pH level (table 6.7).

Table 6.7 Spearman rank correlation (r) between nematodes species and chemicals and physical variables inside the tanks. The highly significant "**" and significant "*"relationships are indicated.

Treatments		r	p-value
Control	Total nematode vs pH	-0.68	*
TR4-PHNI	*Spilophorella papillata* - pH	-0.80	**
	Spilophorella papillata- TAN	0.76	**
TR2-PH	Total nematode vs NO_3	-0.88	**
	Spilophorella papillata vs temperature	0.80	**

6.5 Discussion

From the ten nematode species found in the experimental mesocosm sediments, only four of them made up more than 90 % of the community: *Terschellingia longicaudata*, *Spilophorella papillata*, *Theristus parambronensis and Gomphionema fellator.* The non-selective deposit feeders *(T. parambronensis*) and epistratum feeders (*G. fellator, S. papillata*) were the most dominant. These nematodes, beside the detritus, feed also on diatoms, which are present in high densities in the soils coming from shrimp farms (Stickney, 1994; Boyd, 1995). *T. longicaudata*, belonging to selective deposit feeders, was one of the two most abundant species.

When the total density of nematodes was considered no statistical differences between treatments was found. It is known that phosphorous is perhaps the most important nutrient influencing the natural productivity of aquatic systems. Seawater (as we used in our experiments) has very low concentrations of phosphorous (as phosphate usually around 0.07 $mg.l^{-1}$) and productivity is generally low (Fish Farming, 2003). We registered concentrations above 0.20 $mg.l^{-1}$ in the Control and above 0.40 $mg.l^{-1}$ in the treatments. However, there was no a clear effect of the phosphorous over the total density of nematodes.

There was an increase of phosphorous in treatment TR3-NI when it was not applied. Then, it is probable that phytoplankton and bacterial activities have an influence in the nitrogen cycle inside the tanks, with highest level when nitrogen compounds are applied. There is not an explanation for the nitrogen level in the Control. The reduction in phosphorous level could be associated to the fact that it is rapidly adsorbed by the soil when it is applied to the shrimp pond (Boyd & Musig, 1981; Boyd, 1990; Egna & Boyd, 1997). Also, Stumm & Morgan (1970) mentioned that the transformation of phosphorous could take minutes to days inside the sediment by bacterial activity. It is also important to note that in water with high level of calcium, the phosphate, applied to the water column should be quickly absorbed by the precipitation as CaPO4 no soluble; in this way it is no available for the organisms (Boyd, 1982). A high level of pH was

registered for all the treatments (tables 6.5), and according to Boyd (1992) the level of phosphate should be the highest when the pH is over 6.5 than when the pH is the lowest. At TR2-PH the highest level of P-PO_5 was observed when the pH level was also high.

Rudnick (1989), Thiel (1983), Shiroyama *et al.* (1975) and Heip (1995) suggested that there might be meiobenthos in the sediment, that responded immediately to the increase of organic matter while other groups may respond later. Then, an "ecological classification", of nematodes species, resulted in a differential response to the different nutrients, considered as an association between nematode species and specific chemicals, in soil or even from the water column. There was a correlation between total nematodes and nitrogen compounds and specifically with *Spilophorella papillata* (table 6.6). Then, an association between these chemicals and nematode species directly or indirectly (through bacterial increase) should be investigated to know which chemicals/fertilizer will help to enhance the nematodes.

Spilophorella sp is a diatom feeder, hence its increase in response to nutrients probably reflects an increased microalgal production in the ponds (Moens 2005, person. comm.). Barsdate *et al.* (1974) commented that in a hot-water extractable pool of phosphorous, about 50% of it consists of poly-phosphate, which is assimilated by bacteria and P-PO_4. In our case, the level of phosphorous should be higher than the capacity of the microorganisms to process the fertilizer. For aquaculture systems it is an indicator of negative effect on shrimp production, because the phosphorous is lost to the environment, and not transformed by microorganisms. Then, the shrimp farmer can create, through fertilization, conditions with apparently high levels of phosphorous but in not digestible forms to be utilized, by the organisms in the ecosystems.

With a pH of 8.0, the majority of ammonia (TAN) is in the unionized form, and the sub-lethal effects of ammonia have not been well defined. Fish Farming Research (2003) have defined that reduction in growth rates of aquaculture organisms, may be the most important sub-lethal effect of high TAN levels in production. The same authors added

that, in general, the concentration of unionized ammonia-nitrogen should not exceed 0.05mg.l^{-1}. There were no statistical differences between treatments when the species level was considered. However, the lowest concentration of TAN we registered, was 0.13±0.12 mg.l^{-1}. *Spilophorella papillata* was positively correlated with ammonia level, and we observed the highest densities of this species at the treatments where nitrogen compound was applied. In turn *Terschellingia longicaudata* was in lowest density where nitrogen compound was applied as in treatment TR3-NI and TR4-PHNI. This means that this species density should be reduced when nitrogen fertilizer is used in shrimp ponds. Nevertheless, the highest density of this last species was in the Control. According to Boyd *et al.* (2002), the ammonia (TAN) may be absorbed by phytoplankton, converted to organic nitrogen and, eventually transformed into nitrogen of animal's protein via the food web. Primary production analysis should be considered for future researches.

We did not find references in the literature that could explain the relationship between these nutrients and nematode species. *Gomphionema fellator*, other abundant specie, "arrives" in the shrimp pond sediment, but does not increase in density as the other ones do, maybe due to the unsuitable conditions in the pond for this specie. Some competition for food should exist inside the tanks with *S. papillata* because both species are epistratum feeder (2A) nematodes.

It is known that, TAN is the excretory product of aquatic animals and in a high-density culture, high ammonia levels can develop. Also, NH_3 is excreted directly and by degradation of faecal matter and uneaten feed, and it is a result of decomposition of organic matter, too, through nitrifying and denitrifying bacterial activity (Boyd, 1989). In the presence of shrimp, Olivo (2002) registered a value of 0.005 mg.l^{-1} in the control system and values of 0.014 mg.l^{-1} of TAN. The current experiment was performed in the absence of shrimp and a difference of almost 10 times more (0.13-0.57mg.l^{-1}) than the levels found by Olivo (2002) were obtained. Free ammonium (NH_3) is highly toxic; whereas bound ammonia is much less so. In acidic water, most ammonia is in the

bound form while in alkaline water free ammonia may be more of a problem (Long, 2005). Ammonia in the water column is highly toxic at levels less than 0.1 $mg.l^{-1}$ (FAO, 2005). But, it is possible that this level did not affect the nematode densities in the soil. In relation to other compounds, Stocknet (1994) had pointed out that for invertebrate the tolerance level of nitrite is ranged from 0.11$mg.l^{-1}$ (minima safe level for crustaceans post-larvae and naupli) to 218 $mg.l^{-1}$ (for crustaceans of about 8-91 mm in size). During our research the highest level of TAN was over 0.15 $mg.l^{-1}$, at Control and in TR3-NI, which in turn means that there had not a particular effect on nematodes community. In relation to nitrite-nitrogen $(N\text{-}N0_2)^-$ – it is formed by the complete oxidation of ammonia. It is naturally present, sometimes in high concentration in surface waters of fish farms (Long, 2005). In seawater, its concentrations do not exceed 0.5 $mg.l^{-1}$ for long periods of time. But during our research the highest value was 0.07± 0.13 $mg.l^{-1}$

Changes in the population structure were also observed (figure 6.8). Temporal fluctuation in males, females and juveniles should be a consequence of the changes of the nutrients availability. The density of juveniles was only 19% of the total of nematodes, while the males were in highest density. No clear pattern in population structure was observed. At the previous chapters under field conditions e.g. juveniles were around 37% of the sample (Pond A) and around 33% (Pond C) and 23% (Pond D).

The environmental variables (temperature, salinity, pH and oxygen) were almost the same in all the treatments. Therefore we concluded that no direct effect of these variables was observed over nematode community. Nevertheless, *Spilophorella papillata* increase in density for all the treatments, and was positively correlated with temperature. During previous research we observed high level of *S.papillata* densities under estuarine conditions, but during these research when the salinity increased we did not registered a reduction in this species. Other factors should be acting over this nematode group that we did not consider under the current research.

6.6 Conclusions

- *Terschellingia longicaudata, Spilophorella. papillata*, *Theristus parambronensis* and *Gomphionema fellator* were the most abundant species occurring in the mesocosm experiment.
- Total density of nematode was affected negatively by the pH. *S. papillata* was correlated positively affected by temperature and negatively by the pH level.
- The concentrations of nutrients used in the experimental design do no affect clearly the nematode community. But a negative effect of nitrate concentration over total density was observed. Only *Spilophorella papillata* density was affected by the level of total ammonia at levels of 0.13±0.14 $mg.l^{-1}$.
- Further studies are necessary to analyse the effect of phosphorous and nitrogen over the nematode community, considering different levels of the nutrients.
- Future studies concerning the nematode-chemicals relationship must include shrimp presence, bacterial and phytoplankton activities as well.

CHAPTER 7

The effect of Lime used as an additive with and without shrimp presence, on nematode communities

7. The effect of Lime used as an additive with and without shrimp presence, on nematode communities

7.1 Abstract

The practice of adding lime ($Ca(OH)_2$), to the soil of the shrimp ponds is widely used in Ecuador in order to increase the pH and to produce an alkaline environment, which is thought to disinfect the shrimp pond from viruses. Effects of lime, $Ca(OH)_2$ on free-living nematodes communities were investigated at a mesocosm experiment. The mesocosm tanks were filled with sediment from a natural shrimp pond, with its natural life (consisting for more than 80 % of free-living nematodes). The effect of the combination of $Ca(OH)_2$ and the presence of shrimp was tested during a one-month experiment. We counted 2,752 nematodes and 637 nematodes were used for species identification; density was at an average of 24 ind.$10cm^{-2}$. *Terschellingia longicaudata* and *Spilophorella papillata* made up to 81% of the benthic nematodes in the mesocosm tanks. From the weekly controls it became clear that the lime did not influence the nematode abundance, nor its diversity in a direct way. Changes in water pH could be the cause of the decline in density of juvenile nematodes. The presence of the shrimp caused a decline in nematode abundance. If this was due to predation or to the adding of extra nutrients (food for shrimp) in the system, could not be detected.

7.2 Introduction

The commercial ponds are often fertilized to improve natural productivity. The fertilizers containing nitrogen, phosphorus and potassium (especially phosphorus) stimulate the growth of phytoplankton, zooplankton and benthic organisms, which, in turn, serve as food for animals in the aquatic food chain. But, Wurts & Masser (2005) commented that in ponds built on acidic soils and filled with fresh water of low mineral content, much of the phosphorus added in fertilizers becomes tightly bound in pond sediment where it is not available to support phytoplankton growth. The liming application can change the

phosphorus availability. Phosphate becomes unavailable when applied to high pH water with high calcium content, since insoluble calcium phosphate (Ca_3 (PO_4) $_2$) is formed. Boyd (1982) observes that with a Ca++ concentration of 20 $mg.l^{-1}$, more than 10 $mg.l^{-1}$ (as phosphorous), ortho-phosphate can exist in solutions at pH 8, but at pH 10 the orthophosphate concentration in water would not exceed 0.25 $mg.l^{-1}$. The nature of the precipitating compound is not exactly known in alkaline waters and the nature of fertilizer applied is really important and it is better to use fertilizers such as Ammonium phosphates, which would bring down pH, than calcium phosphates (Milington, 1995; FAO, 2005).

According to shrimp farmers the calcium is used to "sterilize the bottoms" of dry ponds (*personal communications of some shrimp farmers*). These applications reduce the presence of "pests", which include fish, snails, crabs, insects and vegetation. Pond preparation, drying, liming, levelling and gate repair all contribute to pest control. Despite pond preparation, some "pests" will still enter the ponds (FAO, 2005). Crabs and snails move over dikes and levees. Fish eggs and fry come through screens. Insects deposit eggs in the pond area, and some insect larvae feed on small fish or shrimp larvae and food/prey organisms. Many pests compete for food with the aquaculture species. Other pests compete with natural food production by either disturbing the pond mechanically, or interrupting the food chain. The following are some pest control measures. Apply hydrated lime $Ca(OH)_2$ to kill surviving animals (FAO, 2005). Lime application for soil conditioning will also serve to control pond pests. A layer of lime is spread over the bottom and worked into the sediment, manually or mechanically. Stickney (1994) mentioned that this technique is effective in killing "undesirable benthic animals" and may help for the control of pathogenic organisms (Boyd, 1998) as the cases of virus diseases vectors (Bayot, 2002).

In ponds with acidic soils filled with poorly mineralised water with low total alkalinity, liming will increase total alkalinity. Lime in the form of calcium hydroxide (Ca (OH) $_2$) (Slaked-lime) is often used in shrimp aquaculture to neutralize the pH of shrimp pond soils, because it has a higher neutralizing value (NV) (table 7.1) than other lime products (Wurts & Masser, 2005). Adding liming materials or gypsum increases

hardness. Most aquatic organisms can tolerate a broad range of calcium hardness concentrations, but a desirable range is 75 to 250 $mg.l^{-1}$ with a minimum concentration of 20 $mg.l^{-1}$. The commercial liming materials vary in their ability to neutralize soil acidity – their neutralizing value (NV). Slaked lime has an NV of 136%. The crushed agricultural limestone is composed of different sizes of particles. Small particles react faster and dissolve more rapidly and completely than large particles. Therefore, the neutralizing efficiency (NE) of agricultural limestone depends on the fineness of the mixture. But, it is possible to increase to levels that can be harmful to aquatic life (Wurts & Masser, 2005). The pH can swing widely from 6 to 10 during the day if total alkalinity is below 20 $mg.l^{-1}$. Large, daily changes in pH can stress aquatic animals, including aquaculture species.

Table 7.1 Common names, chemical names and neutralizing values (NV) of several liming materials. (source: Wurts, 2005)

Common Name	Chemical Name	NV (%)
Basic Slag		55-79
Calicitic Limestone	Calcium Carbonate, $CaCO_3$	85-100
Dolomitic Limestone	Calcium Magnesium Carbonate, $CaMg(CO_3)_2$	95-109
Slaked or Hydrated Lime	Calcium Hydroxide, $Ca(OH)_2$	136
Quick or Burnt Lime	Calcium Oxide, CaO	179

Most aquaculture species can live in a broad range of alkalinity concentrations, but the desired alkalinity for many animals is 50 $mg.l^{-1}$ or higher. Liming to increase total alkalinity for the required or preferred ranges buffers the water and reduces swings in pH (Boyd, 1990).

The lime increases the total alkalinity and the hardness of the pond water (Sonnenholzner & Medina, 2001). Hardness concentrations are important for aquatic animals. Calcium and magnesium are essential for carapace in shrimp during the moulting process (Perry *et al.*, 2001), and can affect the hardening of newly formed shells. It is also important for the bone and scale formation in fish. The most critical

component of total hardness, however, is the calcium concentration or "calcium hardness.

Environmental calcium is crucial for osmoregulation, and in low-calcium environments, animals can loose substantial quantities of these salts into the water. The lime application increases benthic production in fertilised ponds, apparently through increased nutrient availability rather than increased pH. And also increases certain microbial activity in mud through a favourable increase in pH. The increase in pH acts positively over the amount of carbon available for plants. It is a source of calcium, and reduces turbidity (but not as effectively as alum). Typically, liming at the rate of 2,00 kg/pond is the minimum quality expected to have an effect on the pH. An increase in total alkalinity for the required or preferred ranges buffers the water and reduces swings in pH. In ponds with mud containing heavy loams or clays, significantly higher levels of lime are required to raise the pH, as distinct for ponds with sand bottoms, which may only require half as much to achieve the same results (Millington, 1995).

Wurst & Masser (2005) commented that the liming materials should be effective if they are applied evenly over the bottom of the pond as some of the shrimp farmers performed without ploughing up the soil (carpet cover). Other farmers plough the soil in order to mix the lime with the soil particles as much as possible. In aquaculture, part of the management practice is to keep the shrimp pond dry for a period, in order to disinfect the bottom (Stickney, 1994; Boyd, 1995); during that process, lime is used as well in different doses and dispersed on the 'dry' soil. A liming truck or tractor-pulled liming wagon can be driven around in the dry pond to spread the lime evenly over the entire bottom. It is not necessary to mix the lime into the soil, but this will accelerate its neutralizing activity. However, the best and easiest, time to lime a pond is before it is filled with water. If the pond contains water, lime is applied eventually over the entire pond surface. Lime is loaded onto a boat or barge and then washed uniformly into the pond. The amount of lime needed depends on the chemical characteristics of the bottom sediment. In low-calcium environments, animals can loose (leak) substantial quantities of these salts into the water. In ponds built on acidic soils and filled with fresh water of low mineral content, much of the phosphorus added in fertilizers becomes

tightly bound in pond sediment where it is not available to support phytoplankton growth. Proper liming can improve phosphorus availability and greatly enhance pond productivity (Lazur et al, 1998; Wurts & Masser, 2005).

Liming of ponds to neutralize acidity of bottom soils and to increase the total alkalinity and total hardness of pond waters is a well-established practice (Boyd, 1974, 1982; Boyd and Tucker, 1998). Methods for determining lime requirement of pond soils are available and commonly used for determining liming rates (Boyd, 1995). However, there is still no consensus on whether it is more effective to apply liming materials to the bottoms of empty ponds or to wait and apply them over the water surface after ponds are filled. There is also little information on how deep lime reacts in pond sediment over time (Boyd & Cuenco, 1980), and whether the depth of reaction is different when liming materials are applied to the water or to the soil. Also, the influence of soil texture on depth of lime reaction has not been studied, and the possible benefit of tilling pond bottoms on the depth of lime reaction has not been evaluated.

The lime application helps to stabilize the pH of the water. It is known that pH are the result of interplay of photosynthesis and respiration, then during the night the increases of CO2 produces a decrease of pH, and during the day the pH increases. These changes in pH can stress the aquatic organisms. The shrimp farmer needs to evaluate the shrimp pond conditions to avoid these changes in pH, applying the lime and to know when the equilibrium is reached. The effect of $Ca(OH)_2$ on the benthic life has not been documented so far in literature. However, lime application has been associated with an increase of the natural productivity (by means phytoplankton and zooplankton increase), as a consequence of suspended solid precipitation (Boyd & Tucker, 1998). The increase of bicarbonates is also a carbon source for the photosynthesis (Boyd & Scarbrook, 1974). The bicarbonate in aquaculture is commonly used to remove the carbon dioxide, which is the end product of respiration and accumulates naturally in the ponds as part of the daily photosynthesis-respiration cycle (Tucker & Kingsbury, 2003). The impact of the addition of lime on the benthic dynamics in shrimp ponds is unknown.

The aim of this study is to investigate whether lime ($Ca(OH)_2$) with and without presence of shrimp, affects the meiobenthic community of the shrimp pond soils. The shrimp was considered in the current research because the calcium is essential for shrimp carapace structure and it is applied at shrimp ponds to improve shrimp health conditions, too. Liming adds calcium and magnesium, which are important in animal physiology (Wurts & Masser, 2005) by reduction of the pH variability range, and also for the reduction of pathogen organisms on the shrimp pond environment (Bayot, 2000).

7.3 Material and methods

In order to demonstrate the effects of lime on the dominant benthic organisms, the free-living nematodes, a mesocosm experiment was performed at CENAIM, external laboratory (figure 7.1).

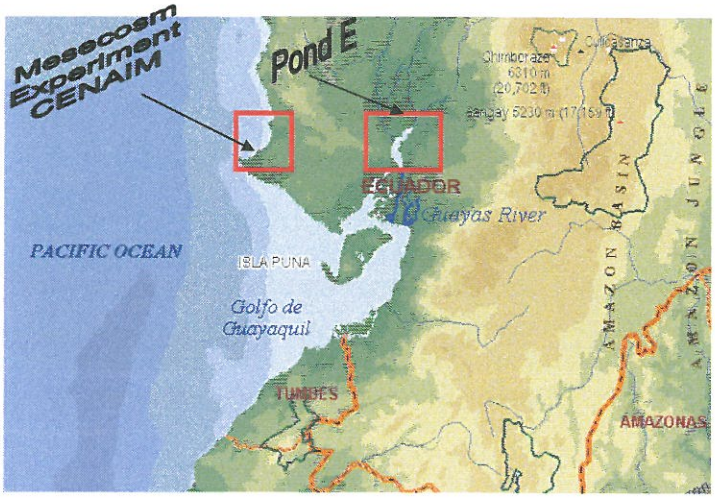


Figure 7.1 The location of the mesocoms experiment at CENAIM, Ecuador.

Experimental set up

The experiment was carried out in 12 square mesocosm tanks of 1,000l capacity. The sediment used in the mesocosm tanks was from the bottom of shrimp farm-Pond E (Chapter 2,table 2.2), in the Gulf of Guayaquil (2°10'S, 79°75'W; Figure 64). In the mesocosm tanks, 15 cm of sediment were covered with 65 cm of water. The tanks were aerated and protected from direct sunlight by a plastic black roof in order to reduce the temperature increase (figure 7.2). The stocking density of the shrimp was 30 shrimps/tank (semi-intensive systems; Rosenberry, 1999). The average weight of a shrimp was 1.4 g and came from a virus-free group (according to PCR analysis of CENAIM).

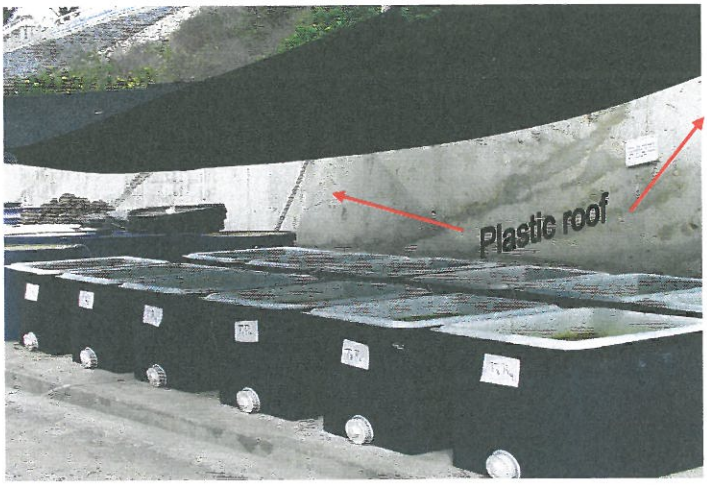


Figure 7.2 Experimental mesocosm tanks, 1000l capacity.

Experiment design

Three different treatments and one control treatment, with 3 replicas each, were performed. The treatments are indicated in table 7.2.

Table 7.2 Description of the mesocosm experiment.

Treatments	Shrimp (30 ind.500l^{-1})	Ca $(OH)_2$	Tanks No.
Control	NO	NO	3, 7, 11
TR1-LI	NO	200 kg/hectares	4, 8, 12
TR2-LISH	YES	200 kg/hectares	2, 6 , 10
TR3-SH	YES	NO	1, 5 , 9

LI= lime; SH=shrimp

The experiment was performed between March 17th and April 21st, 2000. The sediment and the water were added two weeks before the first experimental sampling in order to 'acclimatise' the sediment and the benthic community. The non-filtered water was taken directly from the sea. Two applications of $Ca(OH)_2$ were done on March 19th and March 29th, just before taking the samples. The doses of $Ca(OH)_2$ applied were the average used in Ecuadorian shrimp farms , i.e. 200 kg.ha^{-1}(Sonnenholzner & Medina, 2001; Sonnenholzner, 2003) which means 13.2 g.tk^{-1}, in the tanks with a 65 cm water column. The Ca $(OH)_2$ was diluted in the water (in suspension) in order to obtain a homogeneous sink to the bottom.

In each mesocosm tank, benthic samples were taken up to 5 cm depth, with a plastic corer of 19.635 cm^2 (surface area). The samples of sediment were preserved with formaline 4% neutralized with sodium tetraborate. (See Chapter 2 for details). Oxygen, salinity and temperature were monitored with an oxygen-meter YSI55. The pH was measured daily by a TOVA pH-meter.

Laboratory analysis

For the biological analysis, the techniques described by Vincx (1996) and Vincx & Heip (1996) were used (see Chapter 2 for details). Organism densities are expressed in ind.10cm^{-2}.

The weight of the shrimp was determined in a Sartorious balance. Growth of the shrimp was monitored weekly during the experiment. The shrimp was fed with *CENAIM 40* diets, considering the 10% of their body weight (Villalón, 1991). The feeding frequency

was twice a day: 08h00 and 18h00, after the measurement of the environmental variables.

Statistical analysis

An ANOVA was applied and a two way ANOVA was also performed in order to combine treatments and dates, when assumption of homogeneity of variances and independency of mean and variances were fulfilled. Data were log + 1 transformed. When assumptions for normality were not fulfilled after log+1 transformation, non-parametric Kruskall-Wallis test was applied. The total number of replicas was used to evaluate the relationships among sample dates, seasons and shrimp production cycles (Statistica 6.0, 2000). Spearman Rank Correlation (Sokal & Rohlf, 1981, 1995) was used to analyse the relationship between oxygen and temperature and nematode communities' distribution and the relationship inside these communities (Statistica 6.0, 2000).

7.4 Results

7.4.1 Description of the nematode communities

We counted 2,752 nematodes and 637 were used for taxa identification. The density was 24 ind.10cm^{-2}, but when only non-damaged organisms were counted a density of 13 ind.10cm^{-2} was registered (see annex 7 for the data set). In all treatments, nematodes made up at least 90 % of the benthos (copepods corresponded to the other 10%).

The density of the nematodes fluctuates between 0 and 244 ind.10cm^{-2}. The 30% of the nematodes were damaged and it was no possible to identify them. Figure 7.3 shows the densities registered for nematodes found in good and bad conditions for all treatments.

No assumptions of ANOVA were fulfilled. A Kruskall-Wallis test indicated no statistically significant differences in nematode densities among treatments when the total of nematodes were considered (H=1.224; df=4; p>0.05) neither when the damage organisms were excluded of the analysis (H=6.309; df=4; p>0.05).

The highest density was registered in the TR2-LISH, where the highest density of damage nematodes was also found. Nevertheless, the highest density corresponded to a single date data of April 17th (figure 7.3).

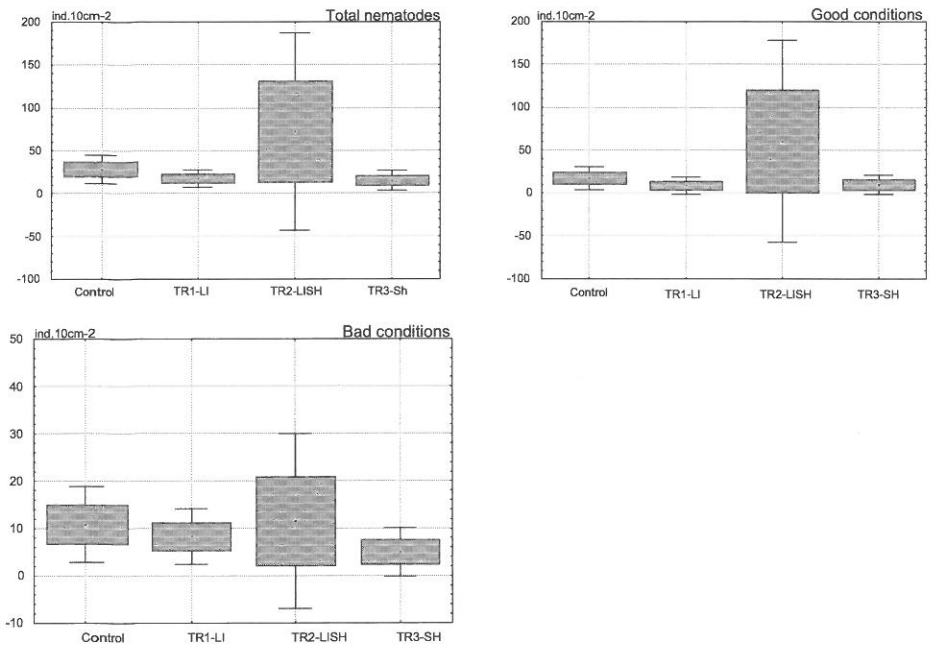


Figure 7.3 Nematode densities by treatment; total nematodes (upper left) in good conditions (upper right) and in bad conditions (bottom right). (Data:mean/SE/1.96SE). Nematodes in bad conditions registered the lowest density, and thus the scales must be different

There was a temporal fluctuation in the nematode communities, but there was no a specific pattern of distribution for the treatments (figure 7.4).

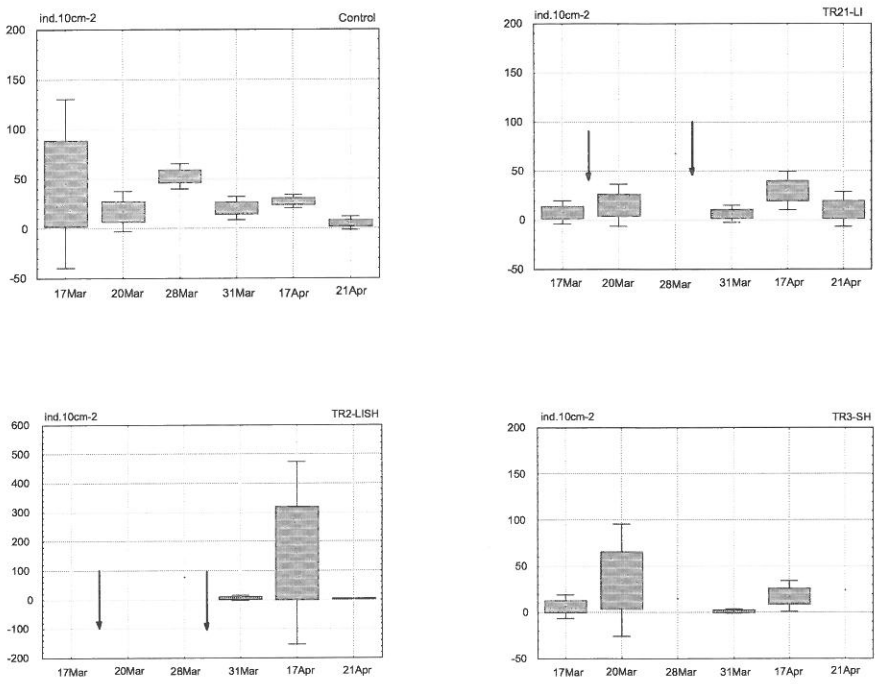


Figure 7.4 Temporal fluctuations on nematode densities by treatment. The arrows indicate the lime application dates. TR2-LISH registered the highest density and thus the scales must be different. (Data:mean/SE/1.96SE).

From the 443 nematodes identified, 16 species were found but five of them could not be identified properly because we did not have enough specimens (194 nematodes were damaged). The other 11 species belong to 7 families. Most species were non-selective deposit-feeders 1B and predators, 2B (Wieser, 1953). However, the most dominant species were selective deposit-feeders, 1A (*Terschellingia longicaudata*) and epistratum-feeder, 2A (*Spilophorella papillata)* (table 7.3).

Without considering the damaged nematodes, *Terschellingia longicaudata* had a dominance of 54%, followed by *Spilophorella papillata* with 33% and *Daptonema* sp 5%;

other species were lowest than 5% of the total. For the rest of the analysis only the most abundant species were considered separately; the other species were considered as "others".

There was a difference in density of nematode species by treatments (table 7.4). Differences in densities were found with and without lime and in combination with and without shrimp (figure 7.4). No assumptions of ANOVA were fulfilled for the three dominant nematode species nor for the "others" species. For *T. longicaudata* distribution the Kruksall-Wallis test indicated no statistical differences of this specie distribution among treatments ($H=7.229$; $df=4$; $p>0.05$). For *S. papillata* and *Daptonema* sp there were no statistical differences among treatments ($H=7.374$; $df=4$; $p>0.05$ and $H=6.774$; $df=4$; $p>0.05$ respectively). Neither for the others species there were statistical differences among treatments ($H=2.750$; $df=4$; $p>0.05$).

Table 7.3 Relative abundance (%) of the nematode species by treatment, together with the feeding type according to Wieser, 1953a,b. 1A Selective deposit feeders, 2A Epistratum feeders, 1B Non-selective deposit feeders; 2B Predators and omnivores.

	Control	TR1-LI	TR2-LISH	TR3-SH	Feeding type
Spilophorella papillata	11.0	26.3	47.5	17.9	2A
Terschellingia longicaudata	76.9	59.2	27.1	71.4	1A
Daptonema sp	5.5	0.0	6.8	0.0	1B
Sabatieria sp	0.0	2.6	0.9	0.0	1B
Theristus sp	0.0	0.0	0.9	0.0	1B
Anoplostoma sp	2.2	2.6	6.8	0.0	1B
Paramonhystera sp	2.2	0.0	0.0	0.0	1B
Prochromadorella sp	0.0	0.0	0.9	0.0	2A
Sphaerolaimus sp	0.0	0.0	4.5	0.0	2B
Adoncholaimus sp	0.0	6.6	2.3	3.6	2B
Viscosia sp	0.0	0.0	0.0	3.6	2B
Five species do not identified	2.2	2.6	2.3	3.6	

There were statistical correlations between *T. longicaudata* and *S. papillata* and also with "other" species in the Control. In the TR2-LISH treatment there was also a correlation between the two most abundant species and with *Daptonema* sp. In the

other treatments there were no correlations between the densities of the nematode species (table 7.4).

Table 7.4 Spearman rank correlation (r) between nematode species. Only highly significant "**" and significant "*" differences are shown.

Treatments	Variables	r	Significant level
Control	*Daptonema* sp vs others	0.65	**
	Terschillingia longicaudata vs *Spilophorella papillata*	0.56	*
	Terschellingia longicaudata vs Others	0.53	*
TR2-LISH	*Terschellingia longicaudata vs Spilophorella papillata*	0.76	*
	Terschellingia longicaudata vs *Daptonema* sp	0.76	*

The temporal fluctuation of nematode species was analysed. There was not a clear pattern in nematode distribution. Even if there was a fluctuation in the density of nematode species it was not due to the lime application because no statistical differences were found for species between dates.

In the Control, *T. longicaudata* densities were not different between dates ($H=2.946$;$df=5$;$p>0.05$) neither with the other treatments, TR1-LI ($H=5.288$; $df=5$; $p>0.05$), TR2-LISH ($H=3.427$; $df=4$; $p>0.05$) or TR3-SH ($H=0.216$; $df=6$; $p>0.05$) (figure 7.5).

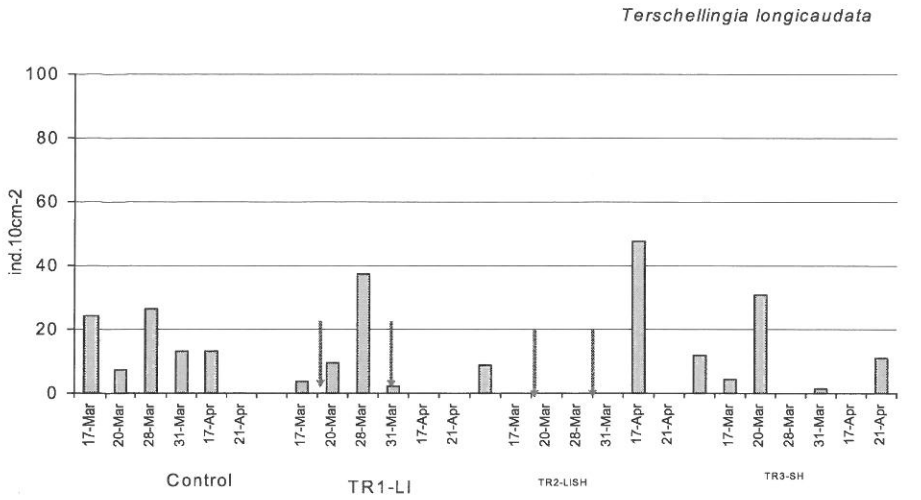


Figure 7.5 Temporal distribution of *Terschellingia longicaudata.* (Arrows indicate the lime application dates).

There were no statistical differences in time for *Spilophorella papillata* densities in the Control (H=2.500; df=5; p>0.05), TR1-LI (H=9.916; df=5; p<0.05), neither for TR2-LISH (H=2.666; df=4; p >0.05) nor for TR3-SH (H=2.468; df=4; p>0.05) (figure 7.6).

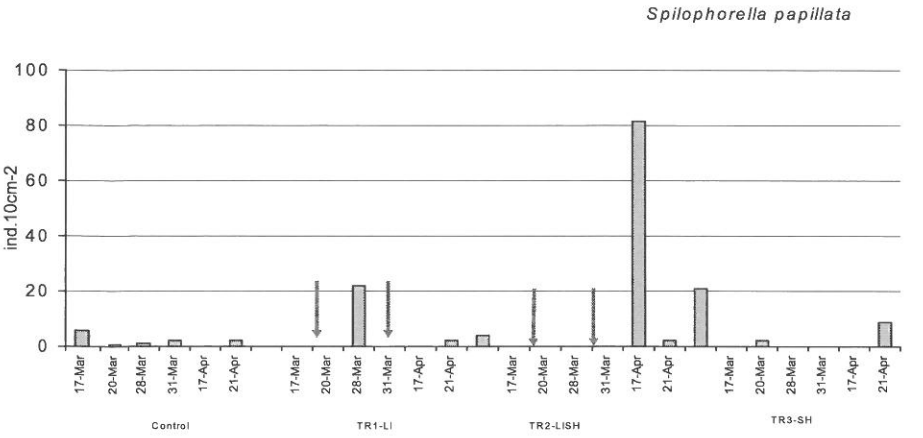


Figure 7.6 Temporal distribution of *Spilophorella papillata.* (Arrows indicate the lime application dates).

For *Daptonema* sp there were no statistical differences between dates for the Control (H=3.920; df=5; p>0.05) nor for TR1-LI (H=0.00; df=5; p>0.05). The same results were found for TR2-LISH (H=3.428; df=4; p>0.05) and TR3-SH (H=11.000; df=5; p>0.05) (figure 7.7).

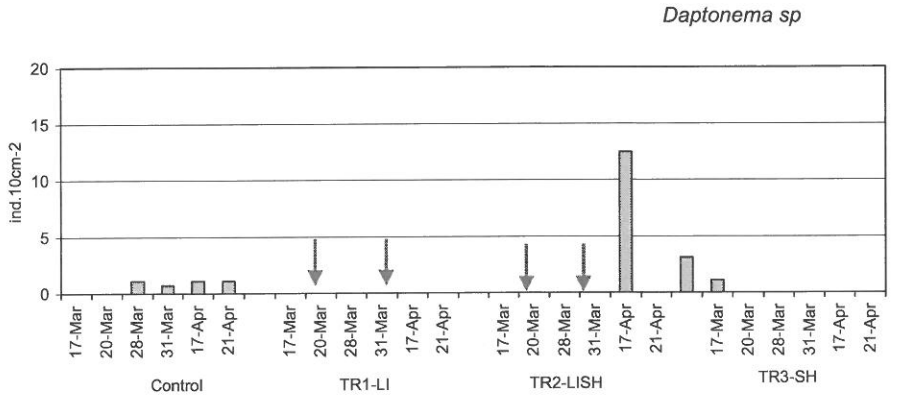


Figure 7.7 Temporal distribution of *Daptonema* sp. (Arrows indicate the lime application dates).

There were no statistical differences between dates in the case of other species. No assumptions of ANOVA were fulfilled. For the Control there were no statistical differences (H=6.346; df=5; p>0.05) and for TR1-LI (H=14.000; df=5; p<0.05); neither for TR2-LISH (H=6.00; df=5; p>0.05) nor TR3-SH (H=11.00; df=5; p>0.05) (figure 7.8).

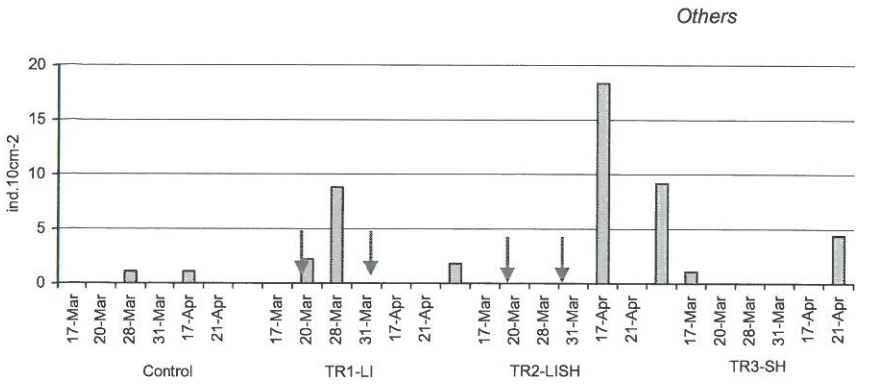


Figure 7.8 Temporal distribution of other nematodes. (Arrows indicate the lime application dates).

The temporal fluctuation of damages nematodes was also studied, but no pattern was found and no statistical differences were found between the treatments (ANOVA assumptions were fulfilled; F=0.712; df=3; p>0.05; figure 7.9).

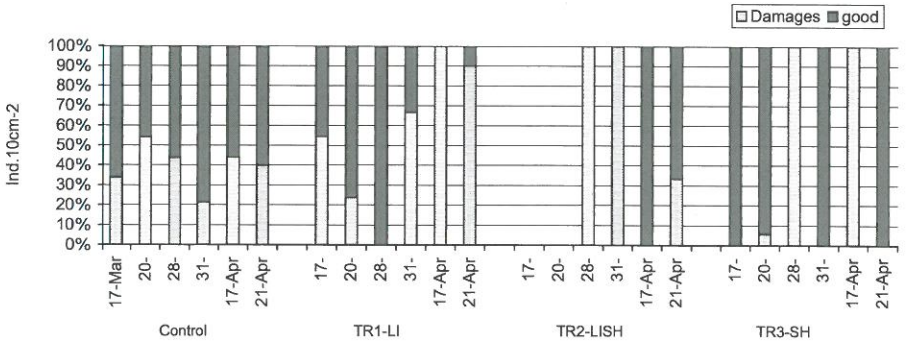


Figure 7.9 Temporal distributions of the damaged and "good" nematodes for all the treatments

The population structure was not considered due to the low number of nematodes.

7.4.2 Environmental variables

No assumptions of ANOVA were fulfilled for the environmental data. The temperature and salinity were not statistically different between the treatments (H=0.00; df=3; p>0.05 and H=0.952; df=3; P>0.05, respectively), ranging between 24 and 28°C with 34 and 40 PSU, respectively (figures 7.10, 7.11; table 7.5). The level of oxygen fluctuated between 4.8 $mg.l^{-1}$ and 8.3 $mg.l^{-1}$ with no statistical differences between treatments (H=7.200; df=3; p>0.05). The pH was between 7.8 and 8.9 units (table 7.4; figures 7.12, 7.13), and statistical differences were observed between the treatments (H=10.303; df=3; p<0.05). The pH registered levels were higher than the 'minimal' range found in the literature for aquatic organisms (Boyd, 1990).

Table 7.5 Environmental variables data by treatment (average plus standard deviation).

	Temperature (°C)	Salinity (PSU)	Oxygen ($mg.l^{-1}$)	pH
Control	26.2±1.26	36.9±2.11	6.1±1.52	8.2±0.21
TR1-LI	26.2±0.98	36.8±1.93	5.3±3.03	8.7±0.36
TR2-LISH	26.3±1.08	36.6±1.95	5.0±1.13	8.5±0.29
TR3-SH	26.1±0.87	36.6±1.71	5.3±1.10	8.0±0.17

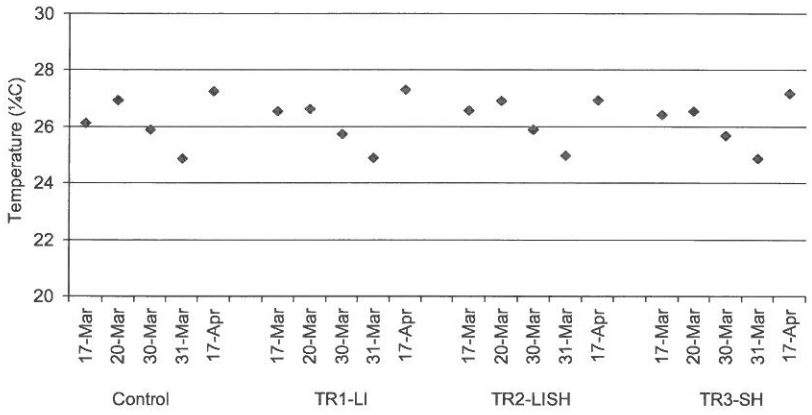


Figure 7.10 Temporal fluctuation of temperature for all the treatments.

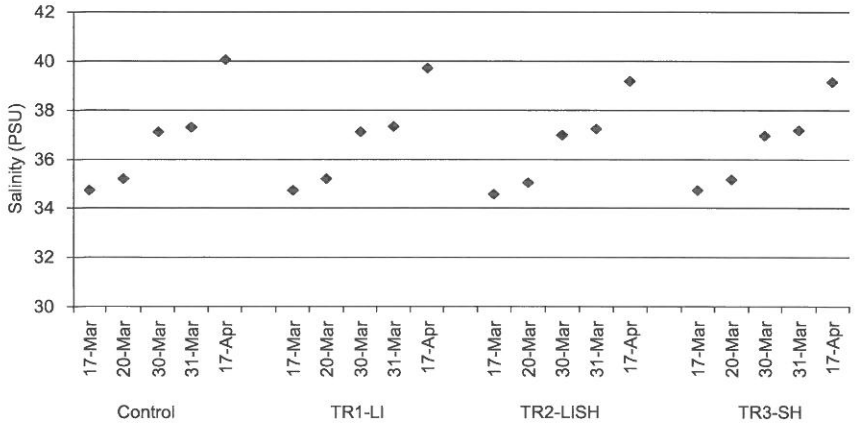


Figure 7.11 Temporal fluctuation of and salinity (lower panel) for all the treatments.

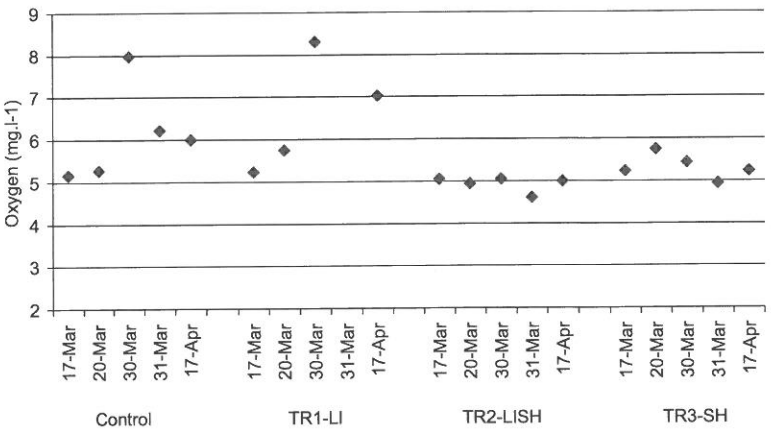


Figure 7.12 Temporal fluctuation of oxygen level for all the treatments.

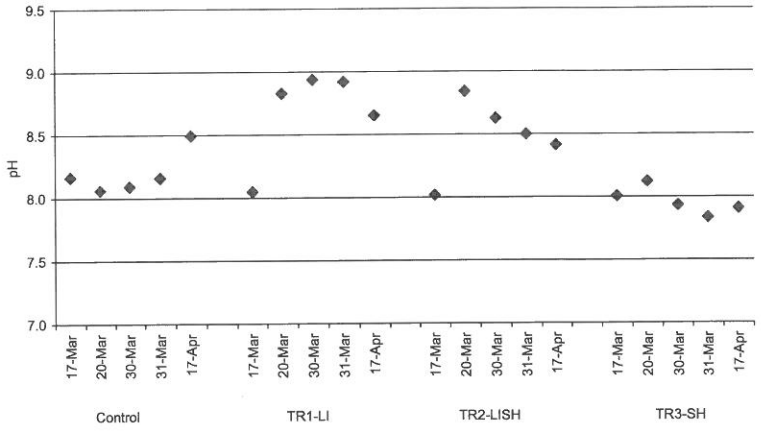


Figure 7.13 Temporal fluctuation of pH (lower panel) for all the treatments.

There were no statistical correlations between the nematode species and these environmental variables (p>0.05).

The shrimp growth was similar under both treatments. No statistical differences were observed between treatments (F=1.042; df=1; p>0.05) (figure 7.13). Survival rates were similar in both cases (figure 7.14).

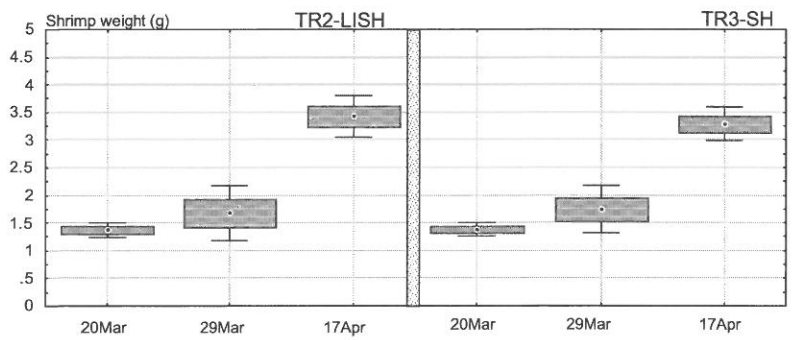


Figure 7.13 Temporal fluctuation of shrimp weight. (Data:mean/SE/1.96SE).

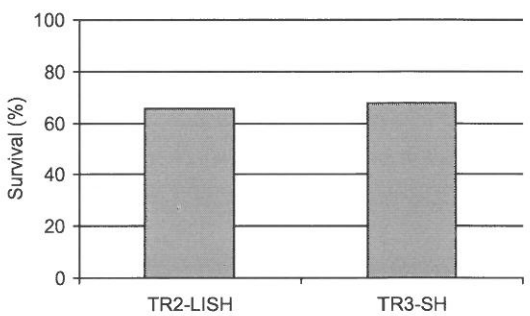


Figure 7.14 Shrimp survival rates (%).

7.5 Discussion

Very low densities of free-living nematodes characterized the soils of the mesocosm tanks. In the previous experiment (chapter 6), we registered less than 12 species, while during this experiment 16 species were found. However, the density here was lower (13 ind.10cm^{-2}) than in the previous mesocosm experiment (chapter 6) where 634 ind.10cm^{-2} were obtained.

The lower density in nematodes should be the result of the disturbance produced when the sediment was brought from the shrimp farm to the mesocosm. Coull & Palmer (1984) commented that there are a subsequently recovery after a perturbation which vary from hours to months. And, they preformed the observations in natural environment where the exchange of water, gave the possibility to introduce new species and new nutrients to the site. However, at mesocosm artificial food was added to feed the shrimp, but not changes were observed in nematode community. None recovery was observed.

At TR2-LISH, the highest nematode density was found but it ascribes just to the April 17th sample (see figures 7.3 and 7.4). We considered that this high variability was due to the high patchiness in nematode communities. It was also observed during the lab sampling campaign (high variability observed between the sampling dates especially in treatments). The nematode patchy distribution is also illustrated from field situations (Fleeger *et al.*, 1995, Moens *et al.*, 1999),and it has been attributed to the patchy distribution of microorganisms (Brown & Sibert, 1977; Rieper, 1982; Montagna *et al.*, 1983; Giere *et al.*, 1988; Danovaro, 1996, Moens & Vincx, 1997; Moens *et al.*, 1999).

Solano (2003) observed that weekly applications of lime, $Ca(OH)_2$ (100kg.ha^{-1}) have no effects on bacteria and *Vibrios* in sediment. We expected that the doubling of applications (concentration of 200 kg.ha^{-1}) might produce changes in microbiological conditions of the soil and as a consequence on the nematode bacteria feeders (Warwick, 1984; Heip *et al.*, 1985; Moens & Vincx, 1997), but no differences were observed. It is probable that lime application had an effect over shrimp weight more than over nematode communities because calcium is an important compound of the

shrimp body (Chen *et al.,* 2005; Van Wyk, 2005). And also that the feed applied to the shrimp was more consumed by shrimp than natural diet. Nevertheless, other changes in soil, as results of physiological activities of shrimp, should be considered for future studies.

Increase of pH was observed in the Control treatment but the opposite happened in the other treatments. The pH changes with time were always over 7.90. Dufour *et al.* (2003) mentioned that in the case of agricultural environment cyst nematodes do not hatch well in very acid soils (pH=4) or alkaline soils (pH=8). They do best in soil with a near-neutral pH of 6. So, the reduced density of nematode should be due to the high level of pH. Melakeberhan, *et al.* (2004) commented that the nematode numbers decreased with decreasing soil pH. Nevertheless, these authors are talking about terrestrial environments. None literature was found related to the effect of pH on marine nematodes. However, we can assume that the low level of nematode density was due to the increased pH.

It is known that meiobenthic organisms are part of the shrimp diet (Hedqvist-Jhonson & Andre, 1991; Dittel *et al.,* 1998) and nematodes are included (Feller, 1994). Escaravage & Castel (1990) found that nematodes, insect larvae and copepods increase in abundance in cage experiments under the presence of shrimp. They mentioned that the increase of abundance of some meiofaunal taxa could result from the bio-perturbation caused by the shrimp, which results in a stimulation of the potential food supply (diatoms and bacteria) and in an increased heterogeneity of the habitat. *Terschellingia longicaudata* was in higher density at the control than in the other treatments and the other species. *Spilophorella papillata* and *Daptonema* sp, were in higher density at TR2-LISH treatment than in the control. So, the presence of shrimp in general for both treatments where shrimps were introduced should affected nematode distribution through perturbation of their habitats, and predation over specific species. Through MDS test we observed an association of Control dates, which indicate a different behaviour of nematode densities, form the other treatments. The applications of lime and shrimp perturbation had an effect of this benthic community. However, further studies are necessary to be sure about this last statement.

Therefore, the scale of the mesocosm experiments or the experimental design had to be adapted in order to study the effect of lime on the characteristics of the dominant organisms in the shrimp pond soils.

7.6 Conclusions

- There was a low diversity and density of nematodes in the mesocosm as was observed in the previous chapter (chapter 6); in shrimp pond environment and mesocosm studies.
- The most abundant species in the mesoscom experiment were *Terschellingia Longicaudata*, a selective deposit-feeder and *Spilophorella papillata,* an epistratum feeder.
- Adding lime in the mesocosm experiment has not a significant effect on the nematode community in the doses applied (200kg.hectares^{-1}).
- There were differences between Control at the other treatments, but no clear effect of the presence of the shrimp was observed in the treatments.

CHAPTER 8

General Discussion

8. General Discussion

The knowledge of the bottom characteristics of Ecuadorian shrimp ponds provides a more complete insight in the dynamics of this "man made" ecosystems. Characteristics of the benthos can provide information about the health status of the shrimp pond (de Paiva & Machado Cunha da Silva, 1998; Martínez-Córdova *et al.*, 2002b; FAO, 2005). Among benthic organisms the meiofauna proved to be a sensitive tool for detecting bio deposition impact (Duplisea & Hargrave, 1996; Mazzola *et al.*, 2000). Heip, (1980b), Vincx & Heip (1987), Moore & Bett (1989) and Coull & Chandler (1992) had noted that meiofauna is important in pollution studies because of their facilities for studying changes of the community under meso or microcosm experiments. The free-living nematodes are very well suited as a bio-monitoring tool (Heip *et al.,* 1985, Sandulli & De Nicolla, 1991; Schratzberger *et al.*, 2000; Neher, 2001; Gheskiere, 2005. It was also emphasized in literature that benthos may be a potential food source for shrimp during some stages in their life cycle (Martínez-Córdova *et al.,* 2002a, b; 2003; Feller, 2004).

An aquaculture ecosystem heavily depends on well-functioning infrastructure and management, controlling the flows in and out, the water quality and the bottom. Artificial shrimp ponds are characterized by eutrophication phenomena produced by an overfeeding and over-fertilization (van Wyk & Scarpa, 1993; Boyd, 1995). For a sustainable management of shrimp ponds, the farmers shall develop a waste management system (Folke *et al.,* 1998); and an useful system for the evaluation of the shrimp pond bottom.

The data of the different species registered during the research period (3-years) in the different shrimp ponds are summarized in the table 8.1. Data of Pond B only report on the meiobenthic community and no identification of the species. In Chapters 3, 4 and 5, the research results were presented about nematode composition, both from the coastal area in Ecuador (Pond A) and from the estuarine environment in the Gulf of Guayaquil (Ponds B, C and D). Chapters 6 and 7 present the results of an experimental research to testing the management practices on the benthos characteristics.

8.1. Density and diversity of the nematodes

The lowest nematode density and diversity (32 species in total) were registered in the seawater environment in the presence of shrimp (Pond A, Palmar) with an average of 52 ind.10cm^{-2}. In the estuarine environment (Ponds C and D) we found a nematode density of 181 ind.10cm^{-2} (table 8.1). At the end of the shrimp production cycles at Ponds A, C and D, the highest shrimp biomass was at the Ponds C and D (see Chapter 5). Thirty-two nematode species were registered, belonging to 10 families; 16 species were found in the mesocosm experiment. Nine species were restricted to the seawater environment; 14 species to the estuarine environment and 3 species were found in both areas (no mesocosm considered) (table 8.1). The number of nematode species is comparable with the studies by Somsak (1995) who found 33 genera in 17 families at shrimp pond bottoms in Khung Kraben Bay, Thailand. But, the diversity on nematode species found was very low compared with the natural environment in the neighbourhood of the shrimp farms. Janssens (1999) found 52 nematode species divided over 26 families outside shrimp ponds in the Gulf of Guayaquil (Ecuador). Mazzola *et al.* (2000), La Rosa *et al.* (2001) and Mirto *et al.* (2002) have noted that nematodes have significant reduced density, diversity and species richness in sediments related with aquaculture systems. In these studies it was assumed that bio-deposition could be the cause for the low densities and diversity. However, Duplisea & Hargrave (1996) did not find differences in nematode densities comparing a fish-farm environment (under salmon cages) with a control, natural sediment:

Table 8.1 Nematode species list indicating the average relative abundance (%) in each system. The last column is the number of appearance of a species in a pond or experiment. For example *Adoncholaimus* sp appears three times: Pond C, Pond D and Mesocosm (lime), hence its frequency is 3. In bold the most abundant species are given.

	Pond A	Pond C	Pond D	Mesocosm (nutrients)	Mesocosm (lime)	Feeding types	Frequency
Chapters	3	5	5	6	7		
Adoncholaimus sp		1.09	0.34		2.08	2B	3
aff Sphaerolaimus	0.13					2B	1
aff. Chromaspirina	0.13					2B	1
Anoplostoma sp		8.35	0.76		3.13	1B	3
Chromadoridae spp		0.57	0.01				2
***Daptonema* sp**	**8.54**	**14.84**	**10.68**	**0.43**	**6.25**	**1B**	**5**
Gomphionema fellator				8.48		2A	1
Gnomoxyala sp				0.16		1B	1
Gomphionema sp	0.80					2A	1
Kraspedonema sp		0.62	1.76			2A	2
Marylinia sp		0.04	0.04			2B	2
Neochromadora sp				0.16		2A	1
NI 269(7)					0.36		1
NI SP1					0.17		1
NI SP11					0.17		1
NI SP3					0.17		1
NI SP4					0.17		1
Oncholaimidae sp	0.07						1
Paracantholaimus sp		0.01				1B	1
Paracomesoma sp				0.16		1B	1
Paramonohystera sp					0.001	1B	1
Prochromadorella sp				0.40	0.001	2A	2
Sabatieria sp	1.40			0.16	0.001	1B	3
Sphaerolaimus sp		0.14	0.07		2.08	2B	3
Spilophorella papillata	**35.79**	**69.82**	**84.63**	**32.86**	**37.50**	**2A**	**5**
Subsphaerolaimus sp		0.07				2B	1
Terschellingia longicaudata	**52.65**	**4.32**	**1.62**	**38.75**	**47.92**	**1A**	**5**
Theristus parambronensis				18.51		1B	1
Theristus sp	0.49		0.01		0.001	1B	3
Tubolaimoides sp		0.10				1B	1
Viscosia sp					0.001	2B	1
Metadesmolaimus sp			0.01			2B	1
Nº. species	9	12	11	10	16		
Density ind.10cm^{-2}	52	121	252	634	13		
Diversity index							
Shannon-Winner (bits)	0.43	0.62	0.30	0.58	0.49		

Terschellingia longicaudata, Spilophorella papillata and *Daptonema* sp were the most abundant species and the species with the highest frequency of occurrence. Heip *et a.l* (1985) commented that the families and dominant species of fine sediments are the same around the world. We have registered the same dominant species in all the systems investigated. The nematode community of the shrimp ponds we investigated can be defined as a *T. longicaudata – S. papillata*, community. *Terschellingia* sp had been found in tidal mud flats, in shallow coastal and estuarine environments (Heip *et al.*, 1985; Chen, 1999), with rather anoxic sediments and in general, it is a species with high tolerance to stressfull conditions (oxygen poor environments) (Vincx *et al.*, 1990; Soeaert *et al.*, 1995). The presence of this species in high density should be an indicator that "something bad" is happening inside the ponds; when the density of this species increases, the conditions of the soil would not be suitable anymore for other metazoan benthic life; and, as a consequence, neither for shrimp. Although this does not necessarily mean that shrimp production will decrease immediately, but the conditions of the pond would deteriorate progressively. Avnimelech & Ritvo (2003) added that shrimp do not eat when they live in an environment with reduced sediments.

When *T. longicaudata* (more abundant at the Pond A, saline environment), has its highest densities, *Spilophorella* sp density, which got the highest occurrence in the estuarine environment, was the lowest and vice versa. The most obvious example is during the field observations in the saline shrimp Pond A in Palmar, with the exception of October 20th 2000 and March 1^{rst} 2001 (figure 8.1).

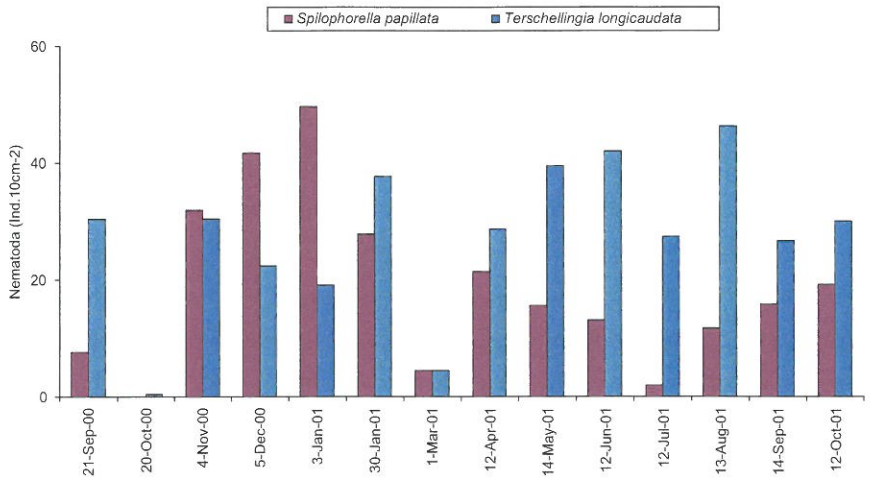


Figure 8.1 Temporal fluctuations of *Spilophorella papillata* and *Terschellingia longicaudata.*

Theristus parambronensis and *Gomphionema fellator* had in some cases, a densitiy higher than *Daptonema* sp, but the first species was less frequent. Nevertheless *Theristus* sp and, *Gomphionema* sp were only recorded from the marine environments (table 8.1). Other nematode species were very low in numbers, less than 10 ind.10cm^{-2}. It is interesting to observe that *Sabatieria* sp, which is considered a genus-indicator for muddy sediments with organic enrichment (Wieser, 1954; Warwick, 1971; Vanresusel, 1990; Vincx *et al.*, 1990; Lampadariou *et al.,* 1997; Chen, 1999), was not abundant in our environment. And even absent in the estuarine shrimp ponds. This species was reported in the North Sea, where temperatures are much lower than in tropical areas. Therefore, other specific indicators for bio-monitoring with special requirements need to be determined in the tropics.

8.2. Comparison with the surrounding environment

During our research we were locking for comparable nematode species from outside fromthe shrimp farms (Janssens, 1999; Calles 2002) (table 8.2). Only species for the Ecuadorian beaches (Calles, 2002) were recorded in this shrimp pond as well.

Table 8.2 Comparison between nematodes found inside the ponds and nematode registered by Calles (2002) and Janssens (1999) in nearby shrimp ponds area (only comparable species are mentioned).

	Pond A	Calles 2002	Pond C	Pond D	Janssens 1999
Adoncholaimus sp			√	√	√
aff. Sphaerolaimus	√				
aff. Chromaspirina	√				
Anoplostoma sp	√				√
Chromadoridae sp			√	√	
Daptonema sp	√	√	√	√	√
Gomphionema fellator				√	
Gomphionema sp	√				√
Kraspedonema sp	√				
Marylinia sp *p*			√	√	
Neochromadora sp *p*			√	√	
Oncholaimidae sp	√				
Paracantholaimus			√		
Paracomesoma sp	√				√
Paramonohystera sp		√	√		
Sabatieria sp	√				√
Sphaerolaimus sp			√	√	√
Spilophorella papillata	√		√	√	√
Subsphaerolaimus sp			√		
Terschellingia longicaudata	√		√	√	√
Theristus sp	√			√	√
Tubolaimoides sp			√		
Viscosia sp		√			√
Methadesmolaimus sp		√		√	

Janssens (1999) found 52 species in areas adjacent to the shrimp farms, in the Guayaquil Gulf. *Daptonema* sp, *Sphaerolaimus* sp, *Anoplostoma* sp, *Adoncholaimus*

sp, *Spilophorella papillata, Terschellingia longicaudata* and *Theristus* sp were registered at shrimp Ponds C and D and in areas surrounding the shrimp farm.

8.3 The physical and chemicals conditions inside the shrimp pond bottom

Snedaker (1978) and Mildward (1982) pointed out that mangrove areas are an enriched habitat with high primary productivity and shrimp farms are located close to them. But, we observed mainly nematodes and copepods inside the shrimp ponds; with higher nematode densities at the mangrove zone in the Guayaquil Gulf, than in saline area at the Guayas province coast.

Several studies are available from mangrove areas, the natural environment which surrounds the shrimp ponds (Warwick, 1971; Gunter, 1973; Dye, 198;, Alongi 1990 a, b; Nicholas *et al.*, 1991; Vanhove *et al.*, 1992; Okondo, 1995; Schrijves, 1996; Schrijves *et al.,* 1997; Schrijves & Vincx, 1999). Okondo (1995) found seven different groups of meiofauna inside *Avicennia marina* mangrove sediments and a density range of 9625 ind.10cm^{-2} to 929 ind.10cm^{-2}. Janssens (1999) found sixteen meiobenthic groups outside the shrimp farms in the Guayaquil Gulf. Calles (2002) also found a high number of meiobenthic organism in the saline area close to shrimp Pond A. Comparable low densities of nematodes are mainly found in sediments with high amounts of organic matter (Essink & Romeyn, 1994; Tita *et al.*, 1999, 2002). Palacin *et al.* (1991) also registered very low levels of nematodes in Els Alfacs Bay, Ebr Delta at the Mediterranean Sea (2 – 4 ind.10cm^{-2}). Alongi (1987a, b; 1990a, b) mentioned low-density values of nematodes in natural mangrove sediments (<150 ind.10cm^{-2}).

Boyd (1997) commented that too much organic matter in pond soils could be detrimental because microbial decomposition can lead to the development of anaerobic conditions at the soil-water interface. However, a small quantity of organic matter is beneficial, because it contributes to the cation-exchange capacity of the soil, chelates trace metals, providing food for benthic organisms, and releasing inorganic nutrients upon decomposition.

It is probable that the instability of the shrimp pond bottom ecosystem, with the regularly alternating drained and wet periods, results in low density of meiobenthic community. Furthermore, the input of freshwater to recover the shrimp pond water level, produce changes in salinity and in consequence benthic communities would be affected.

Many shrimp ponds are constructed close to or, in areas that have been before mangrove forests (at least 27% in Ecuador; CLIRSEN, 2005). Some mangrove soils contain high levels of iron, pyrite that leach sulphuric acid and toxic levels of heavy metals in aquaculture ponds (Simpson & Pedini, 1985 *in* Stickney, 1994). These conditions together with the application of artificial food, soil removal, and other products that shrimp farmers add to the shrimp ponds, can also change the conditions of the soil during the culture period and, in consequence, changes the environmental conditions of the pond creating a "special environment" inside the pond where only a few nematode species will survive..

The shrimp farmer "controls" the level of nutrients and water exchange. But they cannot totally control the environmental variables, such as temperature and salinity; inside the shrimp pond, these variables are different from the adjacent water (Guerrero, 2000). The meiofauna community, including nematode species, are regulated by the physical conditions of the environment (Decho *et al.,* 1985; Olafsson & Elmgren, 1997; Netto & Galluci, 2003). The management practices and their consequences, like low oxygen levels and pH decreases have also an effect on the aquaculture organisms (Boyd, 1990; Escaravage & Castel, 1990; Villalón, 1991) and on nematodes (Wieser *et al.,* 1974; Steyaert *et al.*, 2003).

When the organisms are entering the pond, they need to adapt to these conditions otherwise they will die or will be affected in the reproductive rates. Because of the management practices in shrimp ponds with a draining after each harvesting cycle, the pond soils will either be or completely dried or covered with water remains (the salinity in these areas increases to hypersaline conditions). This means that in most cases, at the beginning of a new shrimp production cycle, there will be animals colonising the

pond bottom originate from incoming water, either from the sea or from the estuarine channels, but also those which are originating from the previous shrimp production cycle.

Important environmental parameters like temperature, salinity and oxygen were monitored during the shrimp production cycles we investigated. Temperature is one of the major factors regulating animals' (and plants) distribution (Olafsson & Elmgren, 1997). It may act on any stage of the life cycle and affect survival, reproduction, or development. Temperature may also act indirectly to limit distributions through its effects on competitive ability, disease resistance, predation and parasitism (Krebs, 1972).

Wetzel *et al.* (1995) have drawn out that nematodes live in a complicated system around the chemocline where they are adapted to a set of chemical and ecological "microniches". Wetzel *et al.* (2001) in the Gulf of México, observed that aa general upward migration of nematodes toward the surface of the sediment and to the water column was due to more oxygen availability (Heip, 1995).

Experiments where the effects of these factors were considered were tested. But the direct effects of these factors on meiobenthic community composition were not studied. Nevertheless, it was possible to make some important observations. During our research in both the shrimp ponds and under mesocosm experiment some significant correlation was found between nematodes species and environmental variables. *Spilophorella papillata* and *Daptonema* sp were correlated positively with the temperature and *S. papillata* was negatively related with oxygen level, but these conditions were not consistent from one experiment to another.

Vernberg (1983) and Moens & Vincx (2000) commented that the effect of temperature and salinity on a given species is not the same for all life stages, physiological variations are age-dependent so metabolic responses can vary. Gerlach (1971a,b), Moens (1999) argued that in the case of nematodes, the temperature and salinity had an influence on the minimum generation time of small species: an increase in generation time from 30 days until 300 days is due to temperature influence. Low temperature is known to

increase the generation times of several marine nematodes. However, no clear trends were observed during our research, neither at field research, nor at mesocosm over the population structure of nematodes. Furthermore, Hopper *et al.* (1973) commented that in shallow water the density variations are always important and the generation times used to be short.

Salinity is one of the most conspicuously fluctuating environmental factors in the estuary and in the coastal waters. The number of marine species present in the estuary decreases with a decrease in salinity (Gunter 1957; Wells 1961; Vernberg & Vernberg, 1972; Vernberg, 1983). However we registered higher number of species and density in the estuarine environment (14 species and 181 ind.10cm^{-2} in average) than in the saline ones (9 species and 52 ind.10cm^{-2} in average). Under "natural conditions", inside the ponds when the oxygen level decreases or the level of nutrient increases, the best management practice is the exchange of water; otherwise the shrimp will die. However this means a drastic reduction in the salinity, which could affect the benthic organisms as well. The other key factors in the field for the changes in population structures are probably food availability (Vranken & Heip, 1985; Gerlach & Schrage, 1971, 1972). The application of food or fertilizers inside the shrimp ponds also contributes to the increase of the suspended solid levels in the water column. Olivo (2002) observed an increase in the size and survival of the shrimp when fertilizers were added. Stickney (1995) determines that these products "create" suspended solids, which are small pieces of particulate matter, made up of fine sand, silt, clay or organic material as detritus. Other suspended solids are bacteria, fungi, faeces, decaying plankton, airborne, debris, eroded soil and micro organisms and they should be also sources of nutrients for aquaculture animals (Hopkins *et al*, 1994; Avnimelech & Ritvo, 2003; Kiorboe *et al.*, 2003). Heip (1995) mentioned that the response of each ecological group is different; often there is an increase in nematode numbers and polychaetes while Kinorhyncha, ostracods and Harpaticoid copepods decreased.

Considering that the fertilizers serve to improve plankton community, Olafsson & Elmgren (1997) observed a decrease in nematode density when the primary production decreased in the sublittoral meiobenthos in Baltic Sea. During our research we found

different responses of the nematode communities to different nutrient sources. However, there was not a consistent pattern in the relationships between nematode densities and chemical variables and no immediate answer to the application of the chemicals was observed. Rudnick (1989) and Heip (1995) suggested that there might be two groups of meiobenthos in the sediment, one group that respond immediately to the increase of organic matter and the second, which reacts later and use the old detritus as food source first.

In addition to physical and chemical processes that result in instability of the sediment surface, intermittent biotic disturbances can contribute substantially to the unpredictability of the bottom (Nichols, 1979). The characteristics of the soil are also important for the organisms, living there. Coull & Bell (1979) observed that the meiofauna of the muddy sediment, mostly copepods, are one of the most important food sources for the higher trophic levels. By their feeding activities, the shrimp are stirring up the bottom mud; in this way settled nutrients become available again to the smaller organisms. It is also important to consider that shrimp distribution is not homogeneous. In general the shrimp avoid areas of the pond bottom with high level of organic matter and aggregate in *cleaner* areas (Corsin *et al.,* 2001). The shrimp *Litopenaeus vannamei* eats nematodes (Feller, 2004) and as we mentioned above, they avoid "bad patches" where anoxic sediment has developed, allowing in this way the development of only a few species. This could be the reason why some nematodes, which are characteristic for anoxic environment (as *Terschellingia longicaudata*), would not be eaten while the other nematodes were effectively predated (species we found in low densities).

8.4 Copepods versus nematodes

When we analysed the colonization of the shrimp ponds we registered a low density of copepods. The absence or low abundance of copepods could be due to their intolerance to hypoxia (Okondo, 1995) or also due to the predation by shrimp (Martínez-Córdova & Peña-Messina, 2005). Shiells & Anderson (1985) found that copepods decrease in density before other taxa. But nematodes (Moens, 1999; Moens & Vincx,

2000; Riemann, 2005) and copepods are bacteriovorus feeders (Rieper, 1978, 1982; Sochard *et al.,* 1979; Proctor, 1997) and a competition between these two groups should be present at the shrimp pond.

8.5 Population structure and feeding types

The results from the experimental approach, designed to evaluate the effect of fertilizers on the nematode communities indicated that no clear changes in the nematode population structure occur due to these manipulations. In general, the distribution of the males and the females did not change significantly with the annual cycle.

The information of the feeding types of the nematodes in our pond systems indicates that non-selective detritus feeders (1B) were the most abundant in number of species (including several species). Mirto *et al.* (2002) in a fish farm in the Mediterranean Sea, determined that non-selective detritus feeders (1B) strongly increased in organic enriched sediment after 225 days. The selective detritus feeders (1A), were less abundant in species number,.

The possible link nematodes provide between the microfauna and the macrofauna is probably of less importance in these systems since macrofauna is hardly present; only a few polychaete species survive in the shrimp pond bottoms (*personal observations*). Literature shows that the nematode epistratum feeders (2A) are most abundant in muddy and organic enriched sandy sediments (Wieser, 1952; Alongi & Tietjen, 1980; Chen, 1999; Olafsson *et al.*, 1995; Moens & Vincx, 1997) and predators and omnivores (2B) are more important in pure sands (Wieser, 1952; Chen, 1999). In figure 8.2 the average distribution of the nematode feeding types is given for the saline ponds and the estuarine ponds. Feeding type 2A (epistratum feeders) had the highest density at the estuarine environment and the feeding type 1A (represented by *T. longicaudata*) got the highest density in saline environment. The predators and omnivore nematodes (2B) were almost absent in both types of environments. The sediment of the shrimp pond bottom is mainly mud and clay and with high density of diatoms (Villalón, 1991;

Stickney, 1994; Boyd, 1995; Martínez-Córdova *et al.*, 2002a,b); this is a favourable environment for 2A–nematodes.

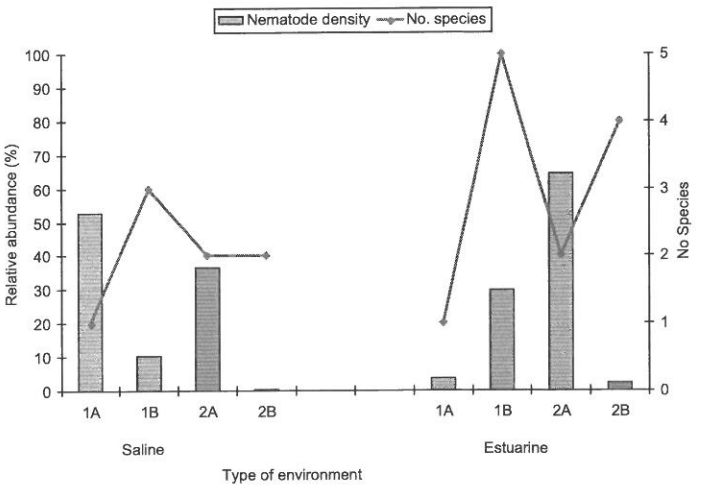


Figure 8.2. Average of feeding type distribution of nematodes for both environments. (Only the identified species were considered for the analysis).

The presence of nematodes with different food types, like we observed here, may reduce or eliminate competition in a particular habitat (Venekey, 2002). Hogue (1982) commented that the dominant species have not a spatial correlation. All, *Terschellingia longicaudata*, *S. papillata* and *Daptonema* sp registered a significant correlation among them (see Chapter 5). These organisms belong to two different feeding types; *T. longicaudata* is a selective deposit feeder (1A), which only feed on small particles. While *S. papillata* is an epistratum feeder (2A). So, they do not compete for food and the fluctuation of these two nematodes species should be based mainly on the adaptation to different salinity conditions.

The colonizers, opportunistic organisms with r-strategies (low generation times and high reproduction rates) have high colonization ability and also a high tolerance to disturbance, eutrohphication and oxygen changes. Bongers (1990) mentioned that these organisms are numerically important in the samples and that they show high fluctuation in densities, voluminous gonads which release large number of small eggs and often viviparous. He added that in general they live in ephemeral habitats, which should be the case of the shrimp pond (3-4 months for each cycle production). *Terschellingia longicaudata* and *Spilophorella papillata*, first and then *Daptonema* spp, *Theristus* spp and *Gomphionema* spp, can be considered as colonizesr inside the pond. While other species have the characteristic of persisters, which could be k-strategists (low reproduction rate, long life cycle) with low colonization ability and more sensible to disturbance.

Terschellingia longicaudata occur also in the deeper layers of the sediments and are often accumulated with high amounts of detritus (Warwick, 1971; Jensen, 1984). Then this confirm its classification as a persister inside shrimp pond bottoms. The other dominant species, *Spilophorella papillata* seems to disappear when the conditions of the soil change (it was negatively correlated with oxygen level and with high level of nitrogen). This species should be considered as colonizers and later persister in the estuarine environment, with a high level of primary production (Milward, 1982; as in Ponds C and D area). Both species are pioneers with rapid colonization of soils. However studies about their life histories are necessary to confirm these results.

8.5. A model to describe the shrimp pond bottom environment in relation to nematode densities

Davis (2004) and Fritz (2004), show a description of the biological condition gradient that could be adapted to the shrimp pond environment. They show the relationship between artificial disturbance and biological condition (figure 8.3). Biological condition of an aquatic resource is exhibited along a gradient, from natural/initial conditions, to severely affected ones First we have maintained the natural structure and function of

biotic community, no perturbation of human activities (1). Later there are minimal changes in structure and function of the ecosystem, some species decrease in density (2). Some evidences of changes in this structure and function begin to be observed, whith replacement of some species by others (3). Moderate changes in structure and function are observed; where more sensitive species disappear an others arrive to the ecosystem (4). The most tolerant species increase in density, becoming the dominant species (5). And finally severe changes in structure and function are registered (6).

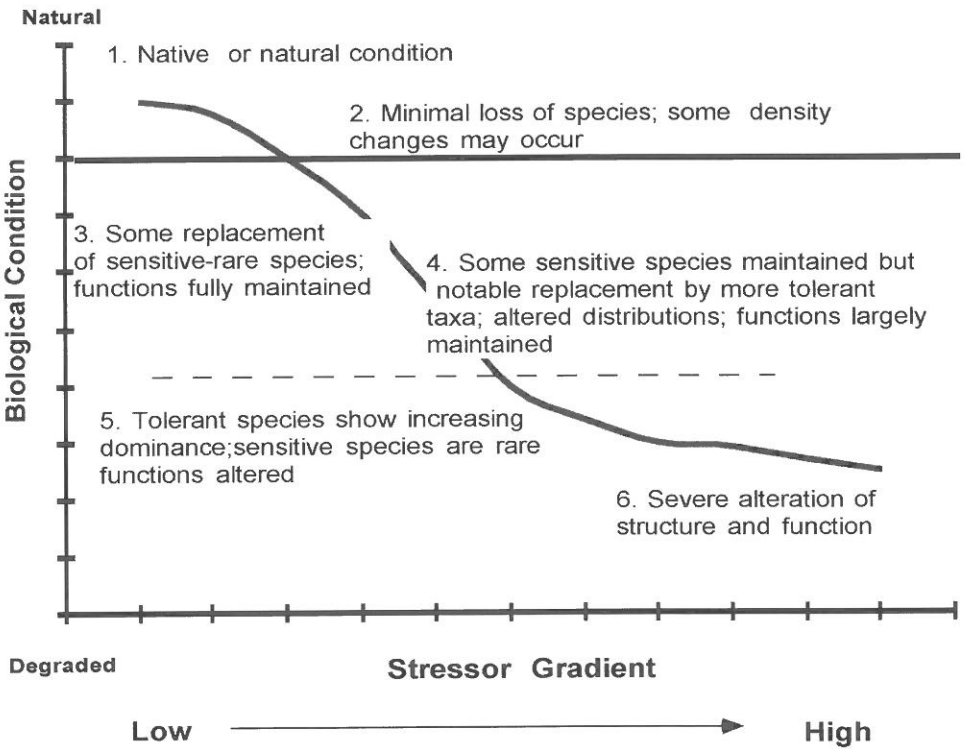


Figure 8.3. Biological conditions gradient (adapted from Davis, 2004).

Davis (2004) and Fritz (2004) talked about degraded ecosystems. However, this model can be used to explain the ecosystem in the shrimp pond bottom. Before the stocking of shrimp larvae, the shrimp pond is water filled, and several organisms such as planktonic and benthic ones enter the ponds. The initial conditions are a new community composed by the previous tolerant organisms (drain dry period) and the new organisms entering with the water (level 1, figure 8.3). During two weeks the conditions of the ponds change (level of salinity, oxygen, temperature, no water exchange) and several species may not be adapted to these changes. In this period some fertilizers are applied to the shrimp pond and the level of nutrients increase in the water and sediment (level 2, figure 8.3). With the stocking of shrimp larvae, the diet preys decreases in density and only a few species remain there (level 3, figure 8.3). The use of artificial feed, fertilizer and others additives (lime, antibiotic, etc.) changes the chemicals conditions of the water (level 4 and 5, figure 8.3) and at the end with the harvest of shrimp the condition of the pond is drastically changed (level 6, figure 8.3). Copepods and nematodes enter to the pond (and some others benthic organisms). But the density of the last rare organisms is low (Quevedo, *in press*) and they remain at the ponds no longer than at level 3.

The nematodes remain longest; species as *T. longicaudata* and *S. papillata* arrive to the pond and remain there until level 6. But most of the other nematode species remain at level 4 while some feed competition should be inside the pond. The physical condition is continuously changing and the competitive exclusion of the species by another is inevitable (Begon *et al.,* 1999). It is probable that the reduction in copepod is because of competitions with shrimp larvae inside the pond bottom. *T. longicaudata* together with *Sabatiera pulchra* were classified as indicators of polluted water by Vitiello & Aissa (1985) and belonging to organically enriched environment by Warwick & Buchanan (1970).

Mirto *et al.* (2002) reported that in fish farm zones, the recovery time to get the previous environment (before fish stocking) was 8 months. In a shrimp aquaculture ecosystem there is no time to recover to previous conditions. Some shrimp farmers work continuously in the shrimp ponds, with successive shrimp production cycles. Others

drained the pond for one to two weeks, apply lime or soil fertilizers and fill the pond with water for the next shrimp production cycle. In this way the farmers assume a "recovery" of the soil. In a certain way, the shrimp pond bottoms can be compared with a corn soil. The harvest should be good during the production cycle, but later the crop production decrease in quality and in density. Finally, the soil cannot produce any more. We assume that the same could be happening with the sediment of shrimp ponds; this could be also the reason why some shrimp farms have ponds with continuously low shrimp production which cannot be explained by virus diseases (Bayot, 1999).

The production of the shrimp ponds is not always the same. It has been suggested that organic matter increases in bottom soils with ponds age until an equilibrium organic matter concentration is attained (Avnimelech, 1984; Boyd, 1995; 2005). Studies have shown that new ponds have lower concentrations of soil organic matter than older ponds, but information on the rate of increase in organic matter over time is lacking (Munsiri *et al.,* 1995, 1996).

Like we explained in Chapter 1, a shrimp pond is a "basin" where all the natural soil horizons have been destroyed. Usually at 2 m depth, the composition of the soil is mainly mineral (low level of organic matter according to Boyd, 1995). The conditions of a new shrimp pond will depend of these original conditions of the original soil. Shrimp farmers add clay to the shrimp pond bottom in order to decrease the permeability of the soil; however part of the original sediment with their own chemical conditions remain there. Hence, the condition of the soil and specifically its pH will depend on these basic materials of the soil and, which are modified by the management practices and also by the rate of anaerobic and aerobic processes (locally and temporarily) there (Kuhnelt, 1995).

Kuhnelt (1955) mentioned that soil animals have some structural features in common; some animals have the capacity to adapt to changes in the soil structure and the moisture conditions of the substratum; but some animals adapt better than others. In the Ecuadorian shrimp pond bottoms, we assume that with the incoming water, benthos

from the natural environment enters the system and only a few species can survive under these conditions (the persister we mentioned before).

Organic matter is a major source of natural soil fertility. In many tropical soils (mainly coarse textural types) the soil fertility is based on the presence of the topsoil of organic matter, which was built up during several years where the land has been under natural vegetation. Once this vegetation is cleared and the land cropped without using fertilizers (extractive agriculture), the natural fertility disappears with the next seasons. Many years are necessary before the land will show some signs of recovery (Interconsults, 1989). When the shrimp farmer built a shrimp pond, he must add fertilisers continuously to improve and keep soil quality, otherwise the conditions of the soil decrease. However, the level of fertilizer and in general the management practices of shrimp ponds should be accompanied by an evaluation of biotic conditions not just in the water column but those of the pond bottom.

To conclude this discussion we can adopt the theory of Krebs (1972), Soetaert (1988) and Begon *et al.* (1999), about 6 factors, which regulate the diversity in meiofauna: time, heterogeneity, competition, predation, environmental stability and productivity. An environment, which remains more of the time under "stable conditions", has a more stable community with specialization of some species. However the shrimp pond is not a stable environment. The same authors also mentioned that soil heterogeneity (Heip *et al.,* 1985; Coull, 1999; Hashimoto *et al.*, 2004) is important in explaining the distribution of the meiofauna. Inside the pond, the heterogeneity of the bottom is due to the canal constructed for the pond drainage during the harvest and the plants which growth at the ponds walls. But also the heterogeneity of shrimp ponds is due to the diverse cement structures at the outlet of the pond, to the stick to feeding trays (with a higher level of organic matter under there), pallets (aeration system) among others (figure 8.4). All these elements induce habitat heterogeneity, which allow the colonization of different microorganisms, which are part of meiobenthic and macrobenthic organisms inside the shrimp pond.

Nematodes in general have a high capacity to colonize new ecological niches (Chen, 1999). It is probable that the heterogeneity of the shrimp pond bottom increases the competition for space between the two dominant nematode species *S. papillata* and *T. longicaudata*. These two species might be more specialists or best adapted to the shrimp environment than other nematodes (for estuarine and saline environment, respectively). Predation is the fourth factor regulating meiofauna distribution (Reise, 1979; Holland *et al.*, 1980; Evans, 1984; Mattila *et al.*, 1990; Beier *et al.*, 2004): more predators influence the abundance of prey. In these ponds, the predators are the shrimps and also the polychaetes, which sometimes are abundant in organically richness sediment, although with very low diversity even the nematodes (Alongi, 1987a; Palacin, 1990; Okondo, 1995).

Figure 8.4. General structures inside a shrimp pond. (Upper left: central drainage channel. Upper right: sticks for organism fixation. Bottom left: aerators. Bottom right: feeding tray).

The fifth factor is the environmental stability (Alongi, 1990c; Aller & Aller, 1992): in a more stable environment, more species can survive. In a more instable environment, more species with high capacity of adaptation show enough flexibility to be adapted to environmental changes. Definitely shrimp pond is not a stable environment. Giere (1993) and Coull (1999) mentioned that the productivity is the last factor. If we compare the different studies here, the highest density of nematode was in the mesocosm experiment with nutrients application. We did not study primary producers, but the fertilizer used in shrimp farm contribute to enhance this production (fertilizer are used two weeks before the stocking of shrimp larvae to enhance primary production). The increase in phytoplankton, phytobenthos or bacteria should increase nematode density, due to the increase of food resources. The flux of organic matter from surface productivity to the shrimp pond bottom can control the benthic standing stocks. The energy content of settling organic matter generally decreases with water depth in open sea due to degradation processes within the water column (Soltwedel, 2000). This processes is reduced inside the shrimp pond due to a depth no higher than 2 meters. Thus, meiobenthic densities and biomasses should show differences between areas with different primary productivity as a consequence of management practices together with environmental conditions. Sotwedel (2000) observed richer communities were generally found in areas with increased productivity and enhanced input of organic matter. Therefore, the changes of feed items as nematode (Feller, 2004; Beier *et al.*, 2004) should produce changes in shrimp biomass. The composition and density of meiofauna should be a tool to evaluate the general conditions of the shrimp ponds.

Steyaert *et al.* (1999) mentioned that the use of nematodes as ecological indicators is problematic. Nevertheless, we consider that the species identification, together with the temporal variation in nematode densities could be use as a tool to know the changes in shrimp pond that should affect shrimp densities. The presence and changes in density of *Terschellingia longicaudata* and *Spilophorella papillata* (the two most abundant species) likes competitors species seems to be important bio-indicators of environmental conditions inside the shrimp ponds to both environments. Neher (2001)

CHAPTER 9

Conclusions and Perspectives

9.1 General Conclusions

- Ecuadorian shrimp pond bottoms are investigated for the first time and are characterized by a benthos community which is very low in density and diversity.
- The benthos is dominated by free-living marine nematodes, which comprised more than 90% of its density. Thirty-two nematode species were identified, within the shrimp ponds.
- The non-selective deposit-feeders (1B-nematodes) had the higher number of species for both the estuarine and saline environments.
- The epistratum feeders (2A) were the most abundant feeding group within the benthos in the estuarine environment, while the selective deposit-feeders (1A) were the most abundant feeding group within the benthos in the saline environment.
- *Spilophorella papillata* (epistratum-feeding nematode) and *Terschellingia longicaudata* (non-selective deposit-feeding nematode) are the two most dominant species (ranging between 31 % and 81 % of the community) in the meiobenthos of all ponds. The density of these two dominant nematode species is probably related to the salinity within the pond.
- *Daptonema* spp, *Gomphionema* spp. and *Theristus* spp are the other important nematode species inside the Ecuadorian shrimp ponds.
- *Spilophorella papillata*, *Daptonema* sp, aff *Sphaerolaimus* and aff *Chromaspirina* are more abundant during rainy (warm) season, while *Terschellingia longicaudata*, *Theristus* spp and *Oncholaimidae* spp are more abundant during the dry (cold) season.
- The tested doses of nutrients and commercial products used as additives in shrimp pond cultures do not have a significant influence on the nematode

community; neither the environmental variables in a consistent way. Therefore the indicator value of some of the species could not be clearly detected.

- *Spilophorella papillata* is affected negatively by oxygen and ammonia and positively by temperature. While *Daptonema* sp is positively affected by temperature.
- Lime and shrimp presence have no a clear effect on nematode communities.
- The drained period of the pond, between two shrimp production cycles, have no clear effects over the benthic community.
- Copepods are the initial colonizers of the shrimp pond bottom after the drained dry period, and they enter the system with the incoming water from the environment.
- Nematode are better competitors to survive the harsh shrimp pond environment compared with other metazoan organisms.

9.2 New perspectives

- It is possible to have a differential response of nematode species to the different additives used in shrimp pond aquaculture. But further studies are necessarily to evaluate the effects of these products, using several concentration of these additives.

- The relationship between nematodes and shrimps should be evaluated in a more direct way; a possible approach is through immunological techniques.

- There is a relationship between the size of shrimps and their diets. It has been observed that a change in prey diet between 3 and 4 g of weight of shrimp, when the benthic density also changes. But there is no specific information about the prey items. It is important to know when exactly this switch occurs to optimize the fertilizer and feed application.

- The positive effects of nematodes on microorganisms have been documented under laboratory conditions. Actual management practices of shrimp ponds are oriented to improve the bacteria production and reduce the natural productivity called "phytoplankton-zooplankton". Studies about the relationship nematode-microorganisms, nematode-chlorophyll shall be established for better evaluating these management practices.

- The culturing of free-living nematodes would allow the realization of experiments under controlled conditions. In this way valuable information can be obtained about different aspects of nematode physiology like their life cycle, reproduction and feeding.

9.3 Conclusiones generales

- Los suelos de las piscinas camaroneras ecuatorianas fueron investigados por primeras vez y se caraterizan por una baja densidad y diversidad de organismos meiobentónicos

- Los nemátodos de vida libre son los organismos dominantes de los suelos de las piscinas de camarón, registrando mas del 90% del total de la densidad del meiobenthos. Se identificaron 32 especies de nemátodos

- Los nemátodos consumidores de particulas, no selectivos (1B) registraron el mayor número de especies en ambos sistemas (salino y estuarino).

- Los nemátodos consumidores en superficie (2A) constituyeron el grupo más abundante dentro del meiobentos en sistemas estuarino, mientras que los consumidores de particulas, selectivos (1A) fueron los mas abundantes en el ambiente salino.

- *Spilophorella papillata* (consumidor de superficie) y *Terschellingia longicaudata* (consumidor no selectivo de particulas), fueron las dos especies dominantes; registrándose en un rango entre 31 % y el 81 % del total de la comunidad de benthos. La densidad de estas dos species dominantes esta probablemente relacionada con la salinidad dentro de la piscina.

- En las piscinas de camaron *Daptonema* spp, *Gomphionema* spp, and *Theristus* spp son también importantes dentro del grupo de los nematodos.

- *Spilophorella papillata*, *Daptonema* sp, aff *Sphaerolaimus* y aff *Chromaspirina* son mas abundantes durante la época lluviosa (cálida). Mientras que *Terschellingia longicaudata*, *Theristus* spp y *Oncholaimidae* spp son más abundantes durante la época seca (fría).

- Las nutrientes y los productos comerciales en las dosis utilizadas no tienen una influencia sginificativa sobre la comunidad de nemátodos. Tampoco las variables ambientales registraron una influencia significativa en forma consistente. Sin embargo, es possible observar, aunque no claramente una influencia sobre las especies.

- *Spilophorella papillata* es afectada negativamente por el oxígeno y el amonio y positivamente por la temperatura. *Daptonema* sp es afectada positivamente por la temperatura.

- La presencia de cal y de camarón no tiene un efecto claro, sobre las comunidades de nemátodos.

- Los periodos de secado de las piscinas entre ciclos de producción no registran un efecto claro sobre la comunidad bentónica.

- Los copépodos son los colonizadores iniciales de las suelos de las piscinas de camarón. Estos ingresan con el agua de llenado de las piscinas.

- Los nematodos son mejores para sobrevivir bajo las condiciones de las piscinas comparados con otros grupos meiobentónicos.

9.2. Nuevas perspectivas

- Conocemos que existe una respuesta diferencial de especies de nemátodos a los diferentes aditivos que se usan en acuicultura. Serán entonces necesarias nuevas investigaciones para fortalecer estos resultados.

- La relación entre nemátodos y camarón debe ser evaluada en una forma mas directa. Con el apoyo de técnicas inmunológicas.

- Existe una relación entre el tamaño del camarón y la dieta natural que éste consume. Ya ha sido observado por otros investigadores un cambio en la dieta, cuando el camarón alcanza entre 3 y 4 g, lo que corresponde a la época dentro del ciclo de producción en que la comunidad bentónica cambia. Pero no existe información específica sobre los items presa. Esta información sería útil para poder optimizar la aplicación de alimento y de fertilizantes.

- La positiva relación entre microorganismos y nemátodos ha sido documentada bajo condiciones de laboratorio. Las actuales practicas de manejo están orientadas a reducir la productividad natural (fitoplancton-zooplancton) dentro de las piscinas y a incrementar el uso de probióticos (bacterias) para mejorar la producción de camarón. Estudios acerca de la relación nemátodo-microorganismos y nemátodo-clorofila podrían ser establecidas para evaluar mejor las prácticas de manejo dentro de los sistemas de producción.

- El cultivo de nemátodos de vida libre permite realizar experimentos bajo condiciones controladas. De esta forma información valiosa sobre los ciclos de vida, alimentación y reproducción de estos organismos puede ser obtenida. Con estas mismas técnicas de cultivo también es possible conocer el efecto de los diversos químicos sobre las comunidades de nemátodos.

CHAPTER 10

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10. References

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Annex 1. Data base to taxa identification

Feeding types of the nematodes (cf. Wieser, 1953b)

GENUS	Feeding types	GENUS	Feeding types
Terschellingia longicaudata	1A	*Sabatieria* *Daptonema* sp *Anoplostoma* sp *Theristus parambronensis* *Theristus calx* *Theristus* sp *Paracomesoma* sp *Parodontophora* sp *Paramonhystera* sp *Gnomoxyala* sp *Tubolaimoides* sp	1B
Gomphionema fellator *Gomphionema* sp *Spilophorella papillata* *Prochromadorella* sp *Kraspedonema* sp *Neochromadora* sp *Paracantholaimus* sp	2A	*Subsphaerolaimus sp* *Sphaerolaimus* sp *aff. Sphaerolaimus* *Adoncholaimus* sp *Adoncholaimus papillatus* *Viscosia sp* *aff. Sphaerolaimus* *Marylynia* sp *aff. Chromaspirina* *Metadesmolaimus* sp	2B
		Chromadoridae sp *Oncholaimidae* sp	

Wieser (1953b) created 4 different feeding types for free-living aquatic nematodes on the basis of the morphology of their buccal cavities.

- 1A: selective deposit feeders. The representatives of this group do not have a buccal cavity (or a miniscule one). The feeding is carried out by suction, using the oesophagus. They only feed on soft particles. Big or hard particles were never found in their intestines.
- 1B: non-selective deposit feeders with cup, cone-like or cylindrical but un-armed buccal cavity. Feeding is performed by suction using the oesophagus and using the anterior parts of the buccal cavity. The food consists out of detritus but also larger objects (e.g. diatoms) can be digested.
- 2A: epistratum feeders have a lightly armed buccal cavity. The food is scraped off from larger surfaces or the object (e.g. diatoms) is pierced and the cell content sucked out.
- 2B: predators & omnivores have large and powerful armoury in their buccal cavity. The prey is either completely swallowed or can be pierced and eaten.

B. Systematic overview of the nematodes

The systematics of the free-living nematodes is based on the system of Platt & Warwick (1988a,b), Bongers, 1988 and Lorenzen (1994).

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Phylum Nematoda
Class Adenophorea
Subclass Chromadoria

Ordo Chromadorida
Subordo Chromadorina

Family Chromadoridae
Neochromadora sp
Prochromadorella sp
Spilophorella papillata Kreis, 1929

Family Neotonchidae/Ethomolaimidae
Gomphionema fellator Wieser & Hopper, 1966
Gomphionema sp1

Family Tubolaimoididae
Tubolaimoides sp

Family Comesomatidae
Subfamily Comesomatinae
Paracomesoma sp
Subfamily Sabatieriinae
Sabatieria sp

Family Cyatholaimidae
Marylynnia sp
Paracyatholaimus sp
Paracantholaimus sp

Subordo Desmodorina
Superfamily Desmodoroidea
Family Desmodoridae
aff. Chromaspirina

Ordo Monhysterida
Superfamily Monhysteroidea
Family Xyalidae
Daptonema sp
Gnomoxyala sp.
Methadesmolaimus sp
Theristus parambronensis (Timm, 1952)
Theristus sp
Theristus calx Wieser & Hopper, 1967

Paramonhystera sp

Family Sphaerolaimidae

Sphaerolaimus sp
aff. Sphaerolaimus
aff. Subsphaerolaimus

Family Cyatholaimidae

Subfamily Pomponematinae

Kraspedonema sp

Superfamily Siphonolaimoidea

Family Axonolaimidae

Parodontophora sp

Family Linhomoeidae

Subfamily Desmolaiminae

Terschellingia longicaudata
de Man, 1907

Subclassis Enoplia

Ordo Enoplida

Subordo Enoplina

Superfamily Enoploida

Family Anoplostomatinae

Anoplostoma sp

Superfamily Oncholaimoidea

Family Oncholaimidae

Subfamily Adoncholaiminae

Adoncholaimus papillatus,
Kreis, 1932
Adoncholaimus sp
Viscosia sp

ANNEX 1

Annex 2. Granulometry analysis

Granulometry was determined by using a Coulter LS particle size analyser which measure particles of size 4um to about 1 mm. Three size categories of particles were automatically determined: mud (4-63um), sand (63-800 um) and coarse sand (800-100um). The fraction >1 mm was regarded as gravel and was not considered in this work. From these measurements a size distribution was made. Other grain properties were also measured including median, kurtosis and skewness of the distribution. The median is an estimation of a general trend in the sediment; it is drawn from the cumulative distribution curve being the phi value corresponding with the 50-volume percentage line in the curve (Holme & McIntyre, 1984). The kurtosis is a measure for the height of the curve. A kurtosis of 0 corresponds to a height of q normal curve. The skewness gives an idea of the asymmetry of the cumulative distribution curve against a perfect symmetrical normal distribution (with 0) (Krumbein, 1938). A positive skewness indicates a dominance of grain sizes smaller than the median diameter. Sediment with a negative skewness contains more sandy fractions

Different categories of sediment fractions using the Wentworth scale :

Fraction	Median Grain size (µm)
Gravel	4000-2000
Very coarse sand	2000-1000
Coarse sand	1000-500
Medium sand	500-250
Fine sand	250-125
Very fine sand	125-63
Silt	<63

ANNEX 2

Annex 3 Shrimp pond at Coastal Area. Pond A

Table 3.1. Nematode density (ind.10cm^{-2}) 2000-2001 period

		2000				2001		
		21-Sep	20-Oct	4-Nov	5-Dec	4-Jan	30-Jan	1-Mar
Spilophorella. papillata	juveniles	2.20	0.00	12.40	22.00	21.40	6.60	1.40
	females	2.00	0.00	8.20	5.80	10.40	11.40	2.00
	males	3.40	0.00	11.40	13.80	17.80	9.80	1.00
	Total	7.60	0.00	32.00	41.60	49.60	27.80	4.40
Terschellingia longicaudata	juveniles	16.00	0.40	13.20	7.40	6.80	18.60	2.60
	females	3.80	0.00	7.20	5.80	4.00	7.20	0.60
	males	10.60	0.00	10.00	9.20	8.20	11.80	1.20
	Total	30.40	0.40	30.40	22.40	19.00	37.60	4.40
Sabatieria sp	juveniles	0.00	0.00	0.00	0.00	0.00	1.80	0.20
	females	0.20	0.00	0.00	0.00	0.00	1.20	0.00
	males	0.00	0.00	0.00	0.00	0.00	1.80	0.00
	Total	0.20	0.00	0.00	0.00	0.00	4.80	0.20
Daptonema sp	juveniles	0.20	0.00	3.20	3.60	1.20	4.00	0.40
	females	0.60	0.00	3.40	8.00	5.80	2.20	0.00
	males	0.00	0.00	4.00	4.60	2.60	1.00	0.00
	Total	0.80	0.00	10.60	16.20	9.60	7.20	0.40
Theristus sp	juveniles	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	females	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	males	0.00	0.20	0.00	0.00	0.00	0.00	0.00
	Total	0.00	0.20	0.00	0.00	0.00	0.00	0.00
Gomphionema sp	juveniles	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	females	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	males	0.00	0.00	0.00	0.00	0.00	0.00	0.40
	Total	0.00	0.00	0.00	0.00	0.00	0.00	0.40
Oncholaimidae spec	juveniles	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	females	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	males	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Cont. Annex 3 Table 3.1

		2000				2001		
		21-Sep	20-Oct	4-Nov	5-Dec	4-Jan	30-Jan	1-Mar
aff. Sphaerolaimus	juveniles	0.00	0.00	0.00	0.00	0.00	0.00	0.20
	females	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	males	0.20	0.00	0.00	0.20	0.00	0.00	0.00
	Total	0.20	0.00	0.00	0.20	0.00	0.00	0.20
aff. Chromaspirina	juveniles	0.00	0.00	0.00	0.00	0.20	0.00	0.00
	females	0.00	0.00	0.00	0.00	0.00	0.20	0.00
	males	0.00	0.00	0.00	0.00	0.00	0.20	0.00
	Total	0.00	0.00	0.00	0.00	0.20	0.40	0.00
	TOTAL	**39.2**	**0.6**	**73**	**80.4**	**78.4**	**77.8**	**10**

Cont. Annex 3 Table 3.1 2001 Period

		2001						
		12-Apr	14-May	12-Jun	12-Jul	12-aug	14-Sep	12-Oct
Spilophorella papillata	juveniles	6.20	7.40	2.60	0.40	3.00	3.40	10.60
	females	9.20	4.20	5.40	1.00	3.80	5.80	3.20
	males	6.00	4.00	5.00	0.60	4.80	6.60	5.40
	Total	21.40	15.60	13.00	2.00	11.60	15.80	19.20
Terschellingia longicaudata	juveniles	7.80	16.00	11.80	6.00	11.00	14.20	18.40
	females	8.20	7.20	13.20	12.00	16.20	2.60	3.60
	males	12.60	16.20	17.00	9.40	19.00	9.80	8.00
	Total	28.60	39.40	42.00	27.40	46.20	26.60	30.00
Sabatieria sp	juveniles	0.00	1.20	0.00	0.00	0.20	1.20	0.20
	females	0.00	0.00	0.00	0.20	0.00	1.00	0.00
	males	0.00	0.20	0.20	0.00	0.00	0.20	0.40
	Total	0.00	1.40	0.20	0.20	0.20	2.40	0.60
Daptonema sp	juveniles	1.00	1.00	1.20	0.40	0.80	1.00	1.40
	females	1.00	0.00	2.20	0.40	0.60	1.40	0.00
	males	1.00	0.80	1.60	0.20	0.80	0.60	0.20
	Total	3.00	1.80	5.00	1.00	2.20	3.00	1.60

Cont. Annex 3 Table 3.1

		2001						
		12-Apr	14-May	12-Jun	12-Jul	12-aug	14-Sep	12-Oct
Theristus sp	juveniles	0.00	0.00	0.00	0.00	0.40	0.00	0.60
	females	0.00	0.00	0.20	0.20	0.40	0.00	0.20
	males	0.00	0.20	0.20	0.40	0.00	0.40	0.20
	Total	0.00	0.20	0.40	0.60	0.80	0.40	1.00
Gomphionema sp	juveniles	0.00	0.60	0.00	0.00	0.00	0.00	2.20
	females	0.00	0.40	0.00	0.00	0.00	0.00	0.00
	males	0.00	0.20	0.00	0.00	0.00	0.00	0.40
	Total	0.00	1.20	0.00	0.00	0.00	0.00	2.60
Oncholaimidae spe	juveniles	0.00	0.00	0.20	0.00	0.00	0.00	0.20
	females	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	males	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.00	0.00	0.20	0.00	0.00	0.00	0.20
aAff. Sphaerolaimus	juveniles	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	females	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	males	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00
aff. Chromaspirina	juveniles	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	females	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	males	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	**53**	**59.6**	**60.8**	**31.2**	**61**	**48.2**	**55.2**

Cont. Annex 3 Table 3.2 Environmental data

	Oxygen mg.l^{-1}	Temperature °C		Oxygen l mg.l^{-1}	Temperature °C
Year 2000					
30-Aug	6.1	21.8	13-Nov	3.31	23.41
25-Sep	5.60	23.10	20-Nov	4.14	24.93
2-Oct	5.40	24.70	27-Nov	3.64	26.31
9-Oct	4.48	25.07	4-Dec	4.17	25.33
16-Oct	5.43	24.40	12-Dec	4.61	25.19
23-Oct	5.20	24.21	23-Dec	3.57	25.70
30-Oct	4.95	23.15	30-Dec	2.33	26.81
6-Nov	3.04	23.01			
Year 2001					
6-Jan	2.37	27.24	4-May	3.20	27.59
13-Jan	3.01	27.37	11-May	3.70	26.97
20-Jan	2.74	27.54	18-May	3.49	24.86
27-Jan	4.23	26.67	25-May		23.50
3-Feb	2.69	28.06	1-Jun	3.10	23.50
10-Feb	2.87	28.81	2-Jul	5.26	23.20
17-Feb	2.38	27.77	9-Jul	3.82	22.83
24-Feb	2.45	28.33	16-Jul	3.94	22.87
2-Mar	2.10	28.27	23-Jul	4.63	23.47
9-Mar	2.20	28.80	30-Jul	3.99	22.43
16-Mar	3.98	28.20	6-Aug	3.00	22.38
23-Mar	4.03	29.09	13-Aug		22.10
30-Mar	3.78	28.33	3-Sep	5.20	22.60
6-Apr	4.10	29.93			
13-Apr	2.91	29.24			
20-Apr	3.84	27.79			
27-Apr	3.84	27.14			

Cont. Annex 3 Table 3.3 Average of Shrimp weight

Year 2000		**Year 2001**	
	Weight (g)		**Weight (g)**
1-Sep		30-Jan	1.00
25-Oct	1.40	20-Feb	4.90
1-Nov	2.10	22-Feb	3.62
8-Nov	2.52	7-Mar	5.78
15-Nov	4.26	14-Mar	7.92
1-Dec		19-Apr	1.98
		24-Apr	5.00
		1-May	4.92
		7-May	6.50
		1-Jun	
		1-Oct	

ANNEX 3

Annex 4. Data of Ponds A, B, C and D.

Annex 4. Table 4.1 Data base Pond A

Pond A							
		2000				2001	
Nematoda		21-Sep	20-Oct	4-Nov	5-Dec	4-Jan	30-Jan
	A	77	1	15	98	105	88
	B	17	0	69	53	89	112
	C	43	0	116	78	93	74
	D	29	0	85	72	35	35
	E	30	2	73	101	70	80
Copepoda		21-Sep	20-Oct	4-Nov	5-Dec	4-Jan	30-Jan
	A	0	0	29	0	0	0
	B	14	0	2	17	9	0
	C	0	0	6	43	7	0
	D	1	0	3	0	4	0
	E	2	0	8	20	2	0
		2001					
Nematoda		1-Mar	12-Apr	14-May	12-Jun	12-Jul	
	A	4	46	64	74	41	
	B	23	28	10	85	73	
	C	6	95	70	42	5	
	D	1	75	88	85	35	
	E	16	21	66	60.8	2	
Copepoda		1-Mar	12-Apr	14-May	12-Jun	12-Jul	
	A	0	4	38	13	2	
	B	0	2	2	47	24	
	C	0	16	7	0	1	
	D	0	2	17	14	5	
	E	0	3	3	1	15	

Cont. Annex 4 Table 4.2 Database Ponds C and D

Pond C Year 2000									
Nematoda		20-Jun	3-Jul	17-Jul	30-Jul	15-Aug	28-Aug	11-Sep	19-Sep
	A	154	41	39	0	37	21	32	67
	B	0	24	5	0	24	11	47	15
	C	392	174	40	90	13	20	45	50
	D	51	0	18	0	36	5	10	66
	E	20	16	86	0	11	12	48	109
Copepoda		20-Jun	3-Jul	17-Jul	30-Jul	15-Aug	28-Aug	11-Sep	19-Sep
	A	3	0	0	0	1	2	0	4
	B	0	5	1	0	1	1	0	3
	C	13	21	1	0	1	1	0	282
	D	32	3	2	0	1	0	4	0
	E	45	0	1	0	1	0	1	0
Pond D Year 2000									
Nematoda		20-Jun	3-Jul	17-Jul	30-Jul	15-Aug	28-Aug	11-Sep	19-Sep
	A	16	0	108		214	529	598	307
	B	49	33	146		274	365	361	281
	C	29	163	220		269	236	205	317
	D	19	450	87		127	642	602	647
	E	60	26	92		0	351	649	347
Copepoda	20-Jun	3-Jul	17-Jul	30-Jul	15-Aug	28-Aug	11-Sep	19-Sep	20-Jun
	A	29	17	7		7	0	19	3
	B	51	8	0		3	3	1	0
	C	28	4	3		4	3	1	6
	D	30	1	2		0	11	0	2
	E	37	0	1		0	17	4	3

Cont. Annex 4 Table 4.3 Database Pond B

Year 2002	Cages									
Nematoda		12-Jun	27-Jun	3-Jul	10-Jul	18-Jul	31-Jul	7-Aug	14-Aug	20-Aug
	1	2	2	17	10	1	49	896	5	91
	2	0	0	2	16	65	22	49	56	40
	3	0	2	4	1	2	13	46	22	68
	4	16	2	6	7	0	4	0	3	30
	5	1	4	1	0	4	0	18	12	54
	6	0	2	1	2	0	16	9	5	135
	7	1	0	0	3	0	17	11	1	38
	8	1	6	7	2	0	7	53	3	49
	9	0	0	0	60	5	17	28	5	357
	10	70	3	1	1	0	18	3	4	35
	11	0	65	0	0	0	133	21	10	134
	12	1	1	13	2	0	10	151	22	73
	13	0	0	2	0	0	13	6	1	53
	14	8	0	0	1	0	17	1	22	87
	15	0	0	1	0	0	19	41	4	87
	16	1	4	3	8	0	10	114	20	220
	17	12	1	0	0	1	33	12	30	118
	18	0	9	53	4	0	172	38	25	105
	19	1	6		2	0	18	33	53	14
	20	0	5	0	1	0	32	359	66	15
	21	0	1	2	3	0	12	26	34	146
	22	1	9	1	2	0	39	98	73	46
	23	0	1145	2	1	0	84	29	317	57
	24	2	4	1	0	1	51	52	25	10
	25	0	2	1	5	0	39	260	4	71
Copepoda		12-Jun	27-Jun	3-Jul	10-Jul	18-Jul	31-Jul	7-Aug	14-Aug	20-Aug
	1	0	13	66	3	3	19	135	39	55
	2	0	0	4	3	28	30	0	16	28
	3	0	4	23	1	1	9	2	31	51
	4	0	0	23	22	0	6	3	11	21
	5	0	4	0	17	5	0	4	96	44
	6	0	0	0	22	15	21	4	42	44
	7	1	0	0	14	3	20	3	28	107
	8	1	20	52	33	0	15	14	40	101
	9	0	3	3	9	40	9	2	27	135
	10	0	37	1	35	0	16	0	19	62
	11	0	7	0	32	0	61	4	65	178
	12	2	12	3	21	0	22	0	64	146

Cont. Annex 4 Table 4.3 Database Pond B

		12-Jun	27-Jun	3-Jul	10-Jul	18-Jul	31-Jul	7-Aug	14-Aug	20-Aug
	13	1	12	11	2	0	10	3	33	85
	14	0	5	12	3	0	19	1	46	156
	15	1	1	20	15	0	10	0	91	156
	16	2	25	90	11	0	27	0	77	208
	17	0	8	0	0	32	25	2	47	95
	18	0	62	36	99	0	10	10	26	127
	19	1	8	0	10	0	3	4	51	58
	20	0	12	4	4	0	11	8	78	88
	21	1	0	28	31	0	16	1	55	66
	22	0	11	38	20	0	21	20	60	95
	23	0	106	30	20	0	16	6	59	151
	24	0	2	115	0	9	85	7	47	258
	25	2	10	8	47	0	17	31	7	254

Cont. Annex 4. Table 4.4 Environmental variables Ponds A, B, C and D

Pond A Year 2000	Oxygen (mg.l-1)	Temperature (ºC)	Pond A Year 2001	Oxygen (mg.l-1)	Temperature (ºC)
25-Sep	5.60	23.10	17-Feb	2.38	27.77
02-Oct	5.40	24.70	24-Feb	2.45	28.33
09-Oct	4.48	25.07	02-Mar	2.10	28.27
16-Oct	5.43	24.40	09-Mar	2.20	28.80
23-Oct	5.20	24.21	16-Mar	3.98	28.20
30-Oct	4.95	23.15	23-Mar	4.03	29.09
06-Nov	3.04	23.01	30-Mar	3.78	28.33
13-Nov	3.31	23.41	06-Apr	4.10	29.93
20-Nov	4.14	24.93	13-Apr	2.91	29.24
27-Nov	3.64	26.31	20-Apr	3.84	27.79
04-Dec	4.17	25.33	27-Apr	3.84	27.14
12-Dec	4.61	25.19	04-May	3.20	27.59
23-Dec	3.57	25.70	11-May	3.70	26.97
30-Dec	2.33	26.81	18-May	3.49	24.86
Year 2001			01-Jun	3.10	23.50
06-Jan	2.37	27.24	02-Jul	5.26	23.20
13-Jan	3.01	27.37	09-Jul	3.82	22.83
20-Jan	2.74	27.54	16-Jul	3.94	22.87
27-Jan	4.23	26.67	23-Jul	4.63	23.47
03-Feb	2.69	28.06	30-Jul	3.99	22.43
10-Feb	2.87	28.81			

Pond C Year 2000	Oxygen (mg.l-1)	Temperature (ºC)	Pond C Year 2000	Oxygen (mg.l-1)	Temperature (ºC)
21-Jun		25.20	8-Jul		24.90
22-Jun		25.00	10-Jul		24.40
23-Jun		24.70	11-Jul		25.65
24-Jun		24.80	12-Jul	2.14	25.05
25-Jun		24.50	13-Jul	4.23	25.30
26-Jun		24.30	14-Jul	5.46	25.05
27-Jun		24.20	15-Jul	8.00	23.90
28-Jun	7.85	25.10	16-Jul	8.06	24.85
29-Jun	5.70	24.30	17-Jul	6.75	24.10
30-Jun	7.15	25.10	18-Jul	5.82	24.83
1-Jul	7.40	25.30	19-Jul	3.86	24.87
2-Jul	7.15	24.10	20-Jul	4.93	24.17
3-Jul	6.55	25.50	21-Jul	5.41	24.00
4-Jul	6.85	23.50	22-Jul	7.56	26.07
5-Jul	5.90	23.90	23-Jul	6.07	25.80
6-Jul		24.00	24-Jul	6.57	
7-Jul		24.40	25-Jul	6.80	

Cont. Annex 4 Table 4.4

Pond C Year 2000	Oxygen (mg.l-1)	Temperature (°C)	Pond C Year 2000	Oxygen (mg.l-1)	Temperature (°C)
26-Jul	4.88		4-Sep	3.84	25.70
27-Jul	6.14	24.80	5-Sep	4.75	25.60
28-Jul	4.68	23.65	6-Sep	4.70	25.50
29-Jul	5.79	23.80	7-Sep	4.06	25.60
30-Jul	5.74	23.45	8-Sep	3.53	25.57
31-Jul	4.92	23.53	9-Sep	4.69	26.20
1-Aug	4.17	24.35	11-Sep	6.12	24.80
2-Aug	3.32	24.00	12-Sep	5.69	
3-Aug	4.65	23.35	13-Sep	3.93	
4-Aug	3.59	23.80	14-Sep	5.91	
5-Aug	3.84	24.30	15-Sep	5.69	
6-Aug	6.45	23.90	16-Sep	4.79	
7-Aug	6.11	24.70	17-Sep	5.89	
8-Aug	5.89	24.40	18-Sep	5.02	
9-Aug	4.35	24.30	19-Sep	5.35	
10-Aug	6.36	24.77	20-Sep	5.35	
11-Aug	7.55	24.85	21-Sep	4.43	
12-Aug	7.90	24.55	22-Sep	6.26	
13-Aug	5.95	24.25	22-Sep	4.86	
14-Aug	5.80	25.43	23-Sep	7.12	
15-Aug	4.09	24.87	23-Sep	5.77	
16-Aug	5.69	24.27	24-Sep	5.06	
17-Aug	4.51	24.43	24-Sep	5.51	
18-Aug	6.50	24.35	25-Sep	4.62	
19-Aug	5.50	25.17	26-Sep	5.65	
20-Aug	6.43	26.07	27-Sep	6.60	
21-Aug	5.30	25.90	10-Sep	4.67	25.68
22-Aug	6.25	25.73			
23-Aug	7.34	26.30			
24-Aug	5.78	26.25			
25-Aug	4.59	26.07			
26-Aug	5.74	25.63			
27-Aug	6.05	25.30			
28-Aug	6.61	25.27			
29-Aug	4.43	25.23			
30-Aug	4.77	24.57			
31-Aug	3.51	24.40			
1-Sep	3.42	25.10			
2-Sep	4.11	26.45			
3-Sep	4.14	26.20			

Cont. Annex 4 Table 4.4

Pond D Year 2000	Oxygen (mg.l-1)	Temperature (°C)	Pond B Year 2002	Oxygen (mg.l-1)	Temperature (°C)
21-Jun	5.81	25.5	26-Jun	6.02	25.38
22-Jun	5.71	25.1	3-Jul	5.02	25.51
2-Jul	6.3	24.7	10-Jul	7.62	25.26
3-Jul	7.74	25.0	17-Jul	8.44	26.60
4-Jul	5.5	24.3	24-Jul	6.79	26.52
16-Jul	5.9	25.0	31-Jul	4.15	
17-Jul	5.52	25.1	3-Aug	5.67	27.05
18-Jul	6.5	24.7	7-Aug	4.19	25.32
29-Jul	6.5	23.7	14-Aug	4.19	27.23
30-Jul	6.94	24.0			
31-Jul	5.7	23.7			
13-Aug	4.8	24.8			
14-Aug	5.6	25.8			
15-Aug	5.6	25.0			
27-Aug	5.5	25.1			
28-Aug	5.3	27.1			
29-Aug	5.3	25.3			
30-Aug	6.1	24.6			
9-Sep	5.9	26.4			
10-Sep	6.2	26.1			

ANNEX 4

Annex 5 Nematode densities (ind.10cm^{-2}) and Environmental variables to Ponds C and D.

Annex 5 Table 5.1 Nematode densities Pond C

Pond C Year 2000								
		20-Jun	3-Jul	17-Jul	15-Aug	28-Aug	11-Sep	19-Sep
Adoncholaimus sp	Juveniles	0,00	0,00	0,00	0,00	0,00	2,24	0,00
	Females	0,00	0,00	0,00	0,24	0,00	1,23	0,12
	Males	0,00	0,00	0,00	0,00	0,00	1,12	0,00
	No determine	0,00	0,00	0,00	0,36	0,12	1,12	0,36
	Total	0,00	0,00	0,00	0,60	0,12	5,71	0,85
Anoplostoma sp	Juveniles	0,00	0,00	0,26	4,34	1,08	3,70	1,69
	Females	0,00	0,00	1,18	4,82	2,29	1,57	1,81
	Males	0,00	0,00	0,78	8,93	3,62	4,59	2,89
	No determine	0,00	0,45	1,18	2,41	1,57	0,56	0,96
	Total	0,00	0,45	3,40	20,51	8,57	10,42	7,36
Chormadoridae	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Females	0,00	0,00	0,00	0,00	0,00	0,00	0,12
	Males	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	No determine	0,00	0,00	0,00	0,00	0,00	0,12	0,12
	Total	0,00	0,00	0,00	0,00	0,00	0,12	0,24
Daptonema sp	Juveniles	0,39	0,00	0,92	1,21	2,05	12,21	22,44
	Females	0,39	0,00	1,57	1,57	2,78	15,91	14,96
	Males	0,98	0,00	1,70	1,21	5,07	2,46	22,32
	No determine	0,00	0,45	0,65	0,72	0,48	1,90	8,68
	Total	1,76	0,45	4,83	4,71	10,37	32,49	68,40
Kraspedonema sp	Juveniles	0,00	0,00	0,00	0,00	0,00	0,22	0,48
	Females	0,00	0,22	0,00	0,12	0,12	0,45	0,36
	Males	0,00	0,00	0,00	0,00	0,12	1,01	0,48
	No determine	0,39	0,00	0,13	0,00	0,12	0,11	0,85
	Total	0,39	0,22	0,13	0,12	0,36	1,79	2,17
Marylinia sp	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Females	0,00	0,00	0,00	0,12	0,00	0,00	0,00
	Males	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	No determine	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Total	0,00	0,00	0,00	0,12	0,00	0,00	0,00
Paracantholaimus sp	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Females	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Males	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	No determine	0,00	0,22	0,00	0,00	0,00	0,00	0,00
	Total	0,00	0,22	0,00	0,00	0,00	0,00	0,00
Sphaerolaimus sp	Juveniles	0,00	0,00	0,00	0,12	0,00	0,00	0,36
	Females	0,00	0,00	0,00	0,00	0,00	0,00	0,24
	Males	0,00	0,00	0,00	0,12	0,00	0,00	0,00
	No determine	0,00	0,00	0,00	0,00	0,12	0,00	0,24
	Total	0,00	0,00	0,00	0,24	0,12	0,00	0,85

Cont.. Annex 5 Table 5.1 Pond C

		20-Jun	3-Jul	17-Jul	15-Aug	28-Aug	11-Sep	19-Sep
Spilophorella papillata	Juveniles	11,76	9,41	2,49	8,32	2,53	2,35	15,68
	Females	10,00	6,27	5,10	4,10	3,02	2,80	10,61
	Males	22,35	14,78	7,45	5,31	3,26	4,48	16,77
	No determine	6,47	2,69	2,22	2,53	1,57	1,57	5,07
	Total	50,58	33,16	17,25	20,26	10,37	11,20	48,13
Subsphaerolaimus sp	Juveniles	0,24	0,00	0,00	0,00	0,00	0,00	0,00
	Females	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Males	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	No determine	0,00	0,00	0,00	0,00	0,00	0,00	0,11
	Total	0,24	0,00	0,00	0,00	0,00	0,00	0,11
Terschellingia longicaudata	Juveniles	0,78	0,45	0,65	0,48	0,12	2,13	1,81
	Females	0,59	0,45	0,52	0,12	0,60	0,90	0,85
	Males	0,19	0,67	0,78	0,24	0,12	2,46	1,57
	No determine	0,39	0,22	0,65	0,00	0,36	1,34	1,08
	Total	1,96	1,79	2,61	0,85	1,21	6,83	5,31
Tubolaimoides sp	Juveniles	0,19	0,00	0,00	0,00	0,00	0,00	0,00
	Females	0,00	0,00	0,26	0,00	0,00	0,00	0,00
	Males	0,19	0,00	0,13	0,00	0,00	0,00	0,00
	No determine	0,00	0,45	0,13	0,00	0,00	0,00	0,00
	Total	0,39	0,45	0,52	0,00	0,00	0,00	0,00

Cont. Annex 5 Table 5.2 Nematode densities in Pond D

Pond D Year 2000		20-Jun	3-Jul	17-Jul	15-Aug	28-Aug	11-Sep	19-Sep
Adoncholaimus sp	Juveniles	0,00	0,00	0,00	0,00	0,12	0,00	0,00
	females	0,00	0,00	0,00	0,00	0,36	0,00	0,10
	Males	0,00	0,00	0,14	0,00	0,12	0,00	0,00
	No determine	0,00	0,00	0,29	0,00	0,36	0,00	0,10
	Total	0,00	0,00	0,43	0,00	0,96	0,00	0,19
Anoplostoma sp	Juveniles	0,00	0,00	0,29	0,48	0,12	0,00	0,00
	females	0,16	0,00	0,58	0,10	0,24	0,00	0,00
	Males	0,00	0,00	1,01	0,19	0,00	0,00	0,00
	No determine	0,32	0,00	0,43	0,48	0,00	0,00	0,00
	Total	0,48	0,00	2,31	1,25	0,36	0,00	0,00
Chromadoridae	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	females	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Males	0,00	0,00	0,14	0,00	0,00	0,00	0,00
	No determine	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Total	0,00	0,00	0,14	0,00	0,00	0,00	0,00
Daptonema sp	Juveniles	0,00	0,43	0,72	0,77	4,09	2,68	0,19
	females	0,32	2,60	2,31	4,53	4,57	4,54	0,29
	Males	0,16	3,18	3,61	3,57	1,32	2,27	0,10
	No determine	2,09	0,14	1,59	1,16	0,96	0,72	0,29
	Total	2,57	6,36	8,24	10,02	10,95	10,22	0,87

Cont.. Annex 5 Table 5.2 Pond D

		20-Jun	3-Jul	17-Jul	15-Aug	28-Aug	11-Sep	19-Sep
Kraspedonema sp	Juveniles	0,00	0,00	0,14	0,00	0,72	0,10	0,00
	females	0,00	0,00	0,72	0,39	1,32	0,31	0,10
	Males	0,00	0,00	0,72	0,10	1,81	0,51	0,00
	No determine	0,00	0,00	0,14	0,10	0,24	0,10	0,00
	Total	0,00	0,00	1,73	0,58	4,09	1,03	0,10
Marylinea sp	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	females	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Males	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	No determine	0,16	0,00	0,14	0,10	0,24	0,31	0,00
	Total	0,16	0,00	0,14	0,10	0,24	0,31	0,00
Methadesmolaimus sp	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	females	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Males	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	No determine	0,00	0,00	0,00	0,00	0,00	0,00	0,10
	Total	0,00	0,00	0,00	0,00	0,00	0,00	0,10
Sphaerolaimus sp	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	females	0,00	0,00	0,00	0,00	0,00	0,00	0,10
	Males	0,00	0,00	0,00	0,00	0,00	0,21	0,00
	No determine	0,00	0,00	0,00	0,10	0,00	0,00	0,00
	Total	0,00	0,00	0,00	0,10	0,00	0,21	0,10
Spilophorella papillata	Juveniles	4,33	16,32	33,80	27,16	16,01	40,45	18,68
	females	9,63	15,02	60,53	46,61	39,49	41,89	42,86
	Males	9,95	22,39	63,71	51,43	28,53	54,69	33,90
	No determine	6,42	0,58	19,50	12,32	8,43	22,39	9,05
	Total	30,33	54,32	177,54	137,52	92,45	159,42	104,49
Terschellingia longicaudata	Juveniles	0,00	0,00	0,43	0,58	0,60	0,41	0,19
	females	0,00	0,14	0,72	0,19	0,48	0,31	0,19
	Males	0,32	0,14	1,30	0,19	0,48	0,21	0,19
	No determine	0,32	0,00	0,87	0,19	0,24	0,41	0,10
	Total	0,64	0,29	3,32	1,16	1,81	1,34	0,67
Theristus sp	Juveniles	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	females	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Males	0,51	0,00	0,00	0,00	0,00	0,00	0,00
	No determine	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	Total	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Cont. Annex 5 Table 5.3 Environmental variables in Pond C

Pond C	Year 2000				
	Temperature (°C)	Oxygen (mg.l^{-1})		Temperature (°C)	Oxygen (mg.l^{-1})
20-Jun			4-Aug	23.8	3.6
21-Jun	25.2		5-Aug	24.3	3.8
22-Jun	25.0		6-Aug	23.9	6.5
23-Jun	24.7		7-Aug	24.7	6.1
24-Jun	24.8		8-Aug	24.4	5.9
25-Jun	24.5		9-Aug	24.3	4.4
26-Jun	24.3		10-Aug	24.8	6.4
27-Jun	24.2		11-Aug	24.9	7.6
28-Jun	25.1	7.8	12-Aug	24.6	7.9
29-Jun	24.3	5.7	13-Aug	24.3	6.0
30-Jun	25.1	7.2	14-Aug	25.4	5.8
1-Jul	25.3	7.4	15-Aug	24.9	4.1
2-Jul	24.1	7.2	16-Aug	24.3	5.7
3-Jul	25.5	6.6	17-Aug	24.4	4.5
4-Jul	23.5	6.9	18-Aug	24.4	6.5
5-Jul	23.9	5.9	19-Aug	25.2	5.5
6-Jul	23.9	6.3	20-Aug	26.1	6.4
7-Jul	24.0		21-Aug	25.9	5.3
8-Jul	24.4		22-Aug	25.7	6.2
9-Jul			23-Aug	26.3	7.3
10-Jul	24.9		24-Aug	26.3	5.8
11-Jul	25.7		25-Aug	26.1	4.6
12-Jul	25.1	2.1	26-Aug	25.6	5.7
13-Jul	25.3	4.2	27-Aug	25.3	6.0
14-Jul	25.1	5.5	28-Aug	25.3	6.6
15-Jul	23.9	8.0	29-Aug	25.2	4.4
16-Jul	24.9	8.1	30-Aug	24.6	4.8
17-Jul	24.1	6.8	31-Aug	24.4	3.5
18-Jul	24.8	5.8	1-Sep	25.1	3.4
19-Jul	24.9	3.9	2-Sep	26.5	4.1
20-Jul	24.2	4.9	3-Sep	26.2	4.1
21-Jul	24.0	5.4	4-Sep	25.7	3.8
22-Jul	26.1	7.6	5-Sep	25.6	4.8
23-Jul	25.8	6.1	6-Sep	25.5	4.7
24-Jul	23.8	5.7	7-Sep	25.6	4.1
25-Jul		6.8	8-Sep	25.6	3.5
26-Jul		4.9	9-Sep	26.2	4.7
27-Jul	24.5	5.4	10-Sep	26.0	5.1
28-Jul	23.7	4.7	11-Sep	24.8	6.1
29-Jul	23.8	5.8	12-Sep		5.7
30-Jul	23.5	5.7	13-Sep		3.9

Cont. Annex 5. Table 5.3 Pond C

	Temperature (°C)	Oxygen (mg.l^{-1})		Temperature (°C)	Oxygen (mg.l^{-1})
31-Jul	23.5	4.9	14-Sep		5.9
1-Aug	24.4	4.2	15-Sep		5.7
2-Aug	24.0	3.3	16-Sep		4.8
3-Aug	23.4	4.7	17-Sep		5.9
18-Sep		5.0			
19-Sep		5.4			
20-Sep		5.4			
21-Sep		4.4			
22-Sep		4.9			
23-Sep		5.8			
24-Sep		5.5			
25-Sep		4.6			
26-Sep		5.6			
27-Sep		6.6			
2-Sep		2.6			

Cont. Annex 5 Table 5.4 Environmental variables in Pond D

Pond D Year 2000					
	Temperature (°C)	Oxygen (mg.l^{-1})		Temperature (°C)	Oxygen (mg.l^{-1})
20-Jun			4-Aug	24.3	6.3
21-Jun	25.5	5.8	5-Aug	24.4	5.3
22-Jun	25.1	5.7	6-Aug	24.2	5.9
23-Jun	25.0	5.5	7-Aug	24.7	5.9
24-Jun	24.6	5.6	8-Aug	24.6	5.7
25-Jun	24.1	5.3	9-Aug	25.2	4.2
26-Jun	23.9	5.3	10-Aug	24.8	5.9
27-Jun	24.3	5.8	11-Aug	24.8	6.8
28-Jun	25.4	6.9	12-Aug	24.9	5.1
29-Jun	24.9	6.4	13-Aug	24.8	4.8
30-Jun	24.1	7.1	14-Aug	25.8	5.6
1-Jul	25.3	7.0	15-Aug	24.8	5.4
2-Jul	24.7	6.3	16-Aug	24.1	5.9
3-Jul	24.8	6.8	17-Aug	23.9	5.5
4-Jul	24.3	5.5	18-Aug	24.2	5.6
5-Jul	24.5	6.5	19-Aug	24.9	5.4
6-Jul	24.1	6.3	20-Aug	25.6	5.6
7-Jul	24.2	6.6	21-Aug	26.0	5.6
8-Jul	24.2		22-Aug	26.2	5.9
9-Jul			23-Aug	26.2	5.9
10-Jul	25.4	5.4	24-Aug	26.0	5.8

ANNEX 5

Annex 6 Database Mesocosm experiment: Nutrient effects

Annex 6 Table 6.1 Nematode densities (ind.10cm^{-2})

year 2002		TR-Control					TR2-PH				
		21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May	11-Jun	18-Jun	25-Jun	8-Jul
Daptonema sp	males		0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	5.7	5.7	0.0	0.0
Gnomoxyala sp	males	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gomphionema	males	90.7	28.3	34.0	11.3	22.7	34.0	102.0	22.7	39.7	22.7
fellator	females	56.7	22.7	73.7	28.3	28.3	39.7	68.0	17.0	22.7	11.3
	Juvenil	0.0	0.0	28.3	5.7	0.0	56.7	5.7	28.3	22.7	0.0
	no determine	0.0	0.0	5.7	0.0	0.0	0.0	5.7	0.0	0.0	0.0
	total	56.7	22.7	107.7	34.0	28.3	96.4	79.4	45.4	45.4	11.3
Neochromadora sp	males	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cont. Annex 6 Table 6.1

		TR-Control					TR2-PH				
		21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May	11-Jun	18-Jun	25-Jun	8-Jul
Paracomesona sp	males	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Prochromadorella sp	males	0.0	45.4	0.0	0.0	0.0	0.0	5.7	5.7	0.0	0.0
	females	0.0	28.3	0.0	0.0	0.0	0.0	5.7	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	28.3	0.0	0.0	0.0	0.0	5.7	0.0	0.0	0.0
Sabatiera sp	males	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spilophorella papillata	males	113.4	45.4	0.0	90.7	68.0	34.0	192.8	107.7	79.4	181.4
	females	107.7	56.7	51.0	79.4	73.7	107.7	102.0	158.7	136.1	311.8
	Juvenil	153.1	0.0	34.0	79.4	34.0	39.7	39.7	119.1	73.7	39.7
	no determine	11.3	0.0	5.7	22.7	5.7	0.0	0.0	22.7	17.0	22.7
	total	272.1	56.7	90.7	181.4	113.4	147.4	141.7	300.5	226.8	374.2

Cont. Annex 6 Table 6.1

		TR-Control					TR2-PH				
	21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May
Terschellingia	males	277.8	158.7	413.9	226.8	357.2	68.0	232.4	192.8	141.7	85.0
longicaudata	females	215.4	68.0	243.8	85.0	266.5	79.4	96.4	90.7	56.7	130.4
	Juvenil	130.4	39.7	170.1	96.4	204.1	5.7	79.4	136.1	107.7	56.7
	no determine	0.0	0.0	45.4	45.4	22.7	0.0	0.0	22.7	22.7	0.0
	total	345.8	107.7	459.2	226.8	493.2	85.0	175.7	249.5	187.1	187.1
Theristus	males	79.4	249.5	34.0	113.4	39.7	181.4	147.4	56.7	130.4	39.7
parambronensis	females	56.7	141.7	22.7	73.7	5.7	136.1	90.7	39.7	56.7	39.7
	Juvenil	5.7	113.4	17.0	73.7	11.3	39.7	34.0	68.0	107.7	68.0
	no determine	0.0	0.0	17.0	28.3	0.0	0.0	0.0	0.0	34.0	11.3
	total	62.4	255.1	56.7	175.7	17.0	175.7	124.7	107.7	198.4	119.1
Total		737.0	515.9	714.3	618.0	652.0	504.6	538.6	714.3	657.6	691.7

Cont. Annex 6 Table 6.1

		TR3-NI					TR4-PHNI				
		21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May	11-Jun	18-Jun	25-Jun	8-Jul
Daptonema sp	males	0.0	0.0	0.0	0.0	0.0	0.0	34.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	11.3	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	22.7	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	34.0	0.0	0.0	0.0	0.0

Cont. Annex 6 Table 6.1

		TR3-NI					TR4-PHNI				
		21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May	11-Jun	18-Jun	25-Jun	8-Jul
Gnomoxyala sp	males	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gomphionema fellator	males	22.7	153.1	107.7	11.3	28.3	96.4	22.7	34.0	39.7	11.3
	females	68.0	90.7	51.0	5.7	11.3	153.1	22.7	34.0	51.0	11.3
	Juvenil	0.0	0.0	22.7	11.3	0.0	0.0	0.0	5.7	5.7	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	68.0	90.7	73.7	17.0	11.3	153.1	22.7	39.7	56.7	11.3
Neochromadora sp	males	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Paracomesona sp	males	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cont. Annex 6 table 6.1

		TR3-NI					TR4-PHNI				
		21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May	11-Jun	18-Jun	25-Jun	8-Jul
Prochromadorella sp	males	0.0	5.7	11.3	5.7	5.7	0.0	34.0	11.3	0.0	0.0
	females	0.0	0.0	5.7	0.0	5.7	0.0	0.0	22.7	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0
	total	0.0	0.0	5.7	0.0	5.7	0.0	0.0	22.7	5.7	0.0
Sabatiera sp	males	0.0	0.0	0.0	0.0	0.0	5.7	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Juvenil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	no determine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spilophorella papillata	males	79.4	238.1	62.4	136.1	272.1	34.0	102.0	90.7	153.1	147.4
	females	102.0	164.4	79.4	68.0	204.1	130.4	96.4	85.0	192.8	464.9
	Juvenil	22.7	11.3	107.7	107.7	90.7	28.3	5.7	51.0	96.4	22.7
	no determine	5.7	0.0	17.0	28.3	11.3	5.7	0.0	0.0	51.0	0.0
	total	130.4	175.7	204.1	204.1	306.1	164.4	102.0	136.1	340.2	487.6
Terschellingia longicaudata	males	385.5	153.1	147.4	221.1	158.7	136.1	209.8	175.7	45.4	102.0
	females	289.1	124.7	175.7	79.4	56.7	158.7	181.4	136.1	90.7	130.4
	Juvenil	73.7	39.7	119.1	119.1	56.7	34.0	39.7	119.1	85.0	45.4
	no determine	28.3	0.0	22.7	68.0	28.3	0.0	11.3	39.7	22.7	5.7
	total	391.2	164.4	317.5	266.5	141.7	192.8	232.4	294.8	198.4	181.4

Cont. Annex 6 table 6.1

		TR3-NI					TR4-PHNI				
		21-May	11-Jun	18-Jun	25-Jun	8-Jul	21-May	11-Jun	18-Jun	25-Jun	8-Jul
Theristus parambronensis	males	62.4	107.7	96.4	51.0	130.4	28.3	85.0	56.7	34.0	39.7
	females	34.0	45.4	85.0	11.3	28.3	22.7	141.7	113.4	17.0	62.4
	Juvenil	79.4	5.7	62.4	68.0	45.4	11.3	11.3	39.7	68.0	11.3
	no determine	0.0	0.0	5.7	5.7	0.0	0.0	5.7	17.0	51.0	0.0
	total	113.4	51.0	153.1	85.0	73.7	34.0	158.7	170.1	136.1	73.7
Total		703.0	487.6	765.4	578.3	544.3	578.3	549.9	674.6	737.0	754.0

Cont. Annex 76 Table 6.2 Environmental data

		Salinity (PSU)	Temperature (°C)	Oxygen (mg.l^{-1})	pH		Salinity (PSU)	Temperature (°C)	Oxygen (mg.l^{-1})	pH
TR1-Control	20-May	44.7	26.2	6.1	7.8	TR3-NI	45.2	26.1	6.0	8.0
	10-Jun	49.4	25.9	5.4	8.0		49.5	26.0	5.3	8.2
	17-Jun	47.6	23.7	6.1	7.9		47.1	23.8	6.1	7.9
	24-Jun	49.4	24.8	5.9	8.1		48.8	24.7	5.1	7.8
	7-Jul	51.1	21.5	6.1	7.6		49.3	21.6	6.2	7.7
mean±stdev		48.4±2.39	24.4±1.78	5.9±0.47	7.9±0.19	mean±stdev	47.9±1.99	24.4±1.76	5.7±0.52	7.9±0.20
TR2-PH	20-May	44.8	26.1	6.4	8.1	TR4-PHNI	44.9	26.3	6.3	7.9
	10-Jun	49.7	25.8	5.3	8.1		50.2	25.8	5.3	8.0
	17-Jun	47.9	23.6	6.1	8.0		48.7	23.7	6.7	8.1
	24-Jun	49.8	24.5	5.2	8.0		49.8	24.6	5.8	8.0
	7-Jul	51.4	21.4	6.0	7.6		51.7	21.5	6.2	7.6
mean±stdev		48.7±2.43	24.3±1.78	5.8±0.52	8.0±0.21	mean±stdev	49.0±2.67	24.3±1.82	6.1±0.65	7.9±0.18

Cont. Annex 6 Table 6.3 Chemicals variables ($mg.l^{-1}$)

		Phosphate $P\text{-}PO_4$	Ammonia TAN	Nitrite $N\text{-}NO_2$	Nitrate $N\text{-}NO_3$		Phosphate $P\text{-}PO_4$	Ammonia TAN	Nitrite $N\text{-}NO_2$	Nitrate $N\text{-}NO_3$
TR1-Control	20-May	0.5	0.9	0.0	0.0	TR3-NI	0.5	0.7	0.0	0.1
	10-Jun	0.6	0.1	0.0	0.1		1.1	0.3	0.1	0.1
	17-Jun	0.5	0.5	0.2	0.1		0.9	0.5	0.2	0.3
	24-Jun	0.4	0.1	0.0	0.0		0.6	0.1	0.0	0.0
	7-Jul	0.4	0.6	0.2	0.1		0.3	0.1	0.0	0.0
mean±stdev		0.47±0.20	0.47±0.57	0.07±0.13	0.07±0.06	mean±stdev	0.69±0.41	0.33±0.39	0.06±0.09	0.12±-/14
TR2-PH	20-May	0.6	0.1	0.0	0.0	TR4-PHNI	0.6	0.1	0.0	0.0
	10-Jun	1.3	0.1	0.0	0.0		1.3	0.3	0.0	0.2
	17-Jun	1.5	0.4	0.1	0.1		0.8	0.2	0.1	0.1
	24-Jun	1.5	0.2	0.0	0.0		1.0	0.1	0.0	0.0
	7-Jul	0.6	0.0	0.0	0.1		0.9	0.1	0.0	0.1
mean±stdev		1.12±0.47	0.13±0.13	0.03±0.05	0.05±0.06	mean±stdev	0.92±0.39	0.13±0.14	0.03±0.04	0.07±0.06

Annex 7. Database Mesocom experiment: Lime effects

Annex 7 Table 7.1 Nematode densities (No. ind.10cm^{-2});

2000		Control					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Terschellingia	juveniles	39.6	3.9	13.2	2.9	11.0	0.0
longicaudata	females	24.2	1.1	28.6	5.1	11.0	0.0
	males	8.8	0.6	11.0	5.1	4.4	0.0
	total	72.6	5.5	52.8	13.2	26.4	0.0
Spilophorella	juveniles	4.4	0.0	0.0	0.0	0.0	0.0
papillata	females	11.0	0.0	0.0	1.5	0.0	4.4
	males	2.2	0.6	2.2	0.7	0.0	0.0
	total	17.6	0.6	2.2	2.2	0.0	4.4
Paramonohystera sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	2.2	0.0	0.0	0.0
	total	0.0	0.0	2.2	0.0	0.0	0.0
Prochromadorella sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Daptonema sp	juveniles	0.0	0.0	0.0	0.7	2.2	2.2
	females	0.0	0.0	2.2	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	2.2	0.7	2.2	2.2
Sabatieria sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Theristus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Adoncholaimus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0

Cont.Annex 7 Table 7.1

2000		Control					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Viscosia sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Anoplostoma sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	2.2	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	2.2	0.0
Sphaerolaimus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
269(7)	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp3	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp4	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp11	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp1	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0	0	0	0	0	0
	males	0	0	0	0	0	0
	total	0.0	0.0	0.0	0.0	0.0	0.0

Cont. Annex 7 Table 7.1

2000		TR1-LI					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Terschellingia longicaudata	juveniles	0.0	9.4	17.6	1.1	0.0	0.0
	females	2.2	5.5	13.2	1.1	0.0	0.0
	males	8.8	4.4	6.6	1.1	0.0	0.0
	total	11.0	19.3	37.4	3.3	0.0	0.0
Spilophorella papillata	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	3.9	15.4	0.0	0.0	0.0
	males	0.0	2.2	6.6	0.0	0.0	2.2
	total	0.0	6.1	22.0	0.0	0.0	2.2
Paramonohystera sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Prochromadorella sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Daptonema sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sabatieria sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.6	2.2	0.0	0.0	0.0
	total	0.0	0.6	2.2	0.0	0.0	0.0
Theristus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0	0	0	0	0	0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Adoncholaimus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.6	2.2	0.0	0.0	0.0
	males	0.0	1.1	4.4	0.0	0.0	0.0
	total	0.0	1.7	6.6	0.0	0.0	0.0
Viscosia sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0	0	0	0	0	0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Anoplostoma sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0

Cont. Annex 7 Table 7.1

Year 2000		TR1-LI					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Sphaerolaimus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp 269(7)	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp3	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp4	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp11	juveniles	0.0	0.6	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.6	0.0	0.0	0.0	0.0
Sp1	juveniles	0	0	0	0	0	0
	females	0	0	0	0	0	0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0

2000		TR2-LISH					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Terschellingia longicaudata	juveniles	0.0	0.0	0.0	0.0	35.2	0.0
	females	0.0	0.0	0.0	0.0	20.9	0.0
	males	0.0	0.0	0.0	0.0	15.4	0.0
	total	0.0	0.0	0.0	0.0	71.5	0.0
Spilophorella papillata	juveniles	0.0	0.0	0.0	0.0	19.8	0.0
	females	0.0	0.0	0.0	0.0	52.8	4.4
	males	0.0	0.0	0.0	0.0	49.5	0.0
	total	0.0	0.0	0.0	0.0	122.1	4.4
Paramonohystera sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0

Cont. Annex 7 Table 7.1.

2000		TR2-LISH					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Prochromadorella sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	2.2	0.0
	total	0.0	0.0	0.0	0.0	2.2	0.0
Daptonema sp	juveniles	0.0	0.0	0.0	0.0	6.6	0.0
	females	0.0	0.0	0.0	0.0	7.7	0.0
	males	0.0	0.0	0.0	0.0	4.4	0.0
	total	0.0	0.0	0.0	0.0	18.7	0.0
Sabatieria sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	1.1	0.0
	total	0.0	0.0	0.0	0.0	1.1	0.0
Theristus sp	juveniles	0.0	0.0	0.0	0.0	1.1	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	1.1	0.0
Adoncholaimus sp	juveniles	0.0	0.0	0.0	0.0	1.1	0.0
	females	0.0	0.0	0.0	0.0	1.1	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	2.2	0.0
Viscosia sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Anoplostoma sp	juveniles	0.0	0.0	0.0	0.0	4.4	0.0
	females	0.0	0.0	0.0	0.0	5.5	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	9.9	0.0
Sphaerolaimus sp	juveniles	0.0	0.0	0.0	0.0	1.1	0.0
	females	0.0	0.0	0.0	0.0	1.1	0.0
	males	0.0	0.0	0.0	0.0	4.4	0.0
	total	0.0	0.0	0.0	0.0	6.6	0.0
269(7)	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	1.1	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	1.1	0.0
Sp3	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	1.1	0.0
	total	0.0	0.0	0.0	0.0	1.1	0.0

Cont. Annex 7 Table 7.1

2000		TR2-LISH					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Sp4	juveniles	0.0	0.0	0.0	0.0	1.1	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	1.1	0.0
Sp11	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp1	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0	0	0	0	0	0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0

2000		TR3-SH					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Terschellingia longicaudata	juveniles	0.0	16.5	0.0	0.0	0.0	2.2
	females	2.2	11.0	0.0	0.0	0.0	4.4
	males	2.2	3.3	0.0	4.4	0.0	4.4
	total	4.4	30.8	0.0	4.4	0.0	11.0
Spilophorella papillata	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	1.1	0.0	0.0	0.0	0.0
	males	0.0	1.1	0.0	0.0	0.0	8.8
	total	0.0	2.2	0.0	0.0	0.0	8.8
Paramonohystera sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Prochromadorella sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Daptonema sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	1.1	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	1.1	0.0	0.0	0.0	0.0	0.0
Sabatieria sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0

Cont. Annex 7 Table 7.1

2000		TR3-SH					
		17-Mar	20-Mar	28-Mar	31-Mar	17-Apr	21-Apr
Theristus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Adoncholaimus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	1.1	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	1.1	0.0	0.0	0.0	0.0	0.0
Viscosia sp	juveniles	0	0	0	0	0	0
	females	0.0	0.0	0.0	0.0	0.0	2.2
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	2.2
Anoplostoma sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sphaerolaimus sp	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
269(7)	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	2.2
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	2.2
Sp3	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp4	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp11	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0
Sp1	juveniles	0.0	0.0	0.0	0.0	0.0	0.0
	females	0.0	0.0	0.0	0.0	0.0	0.0
	males	0.0	0.0	0.0	0.0	0.0	0.0
	total	0.0	0.0	0.0	0.0	0.0	0.0

Cont. Annex 7 Table 7.2 Environmental variables

2000		Temperature(°c)	Salinity (spu)	Oxygen (mg.l^{-1})	pH
Control	17-Mar	26.1	34.7	5.15	8.16
	20-Mar	26.9	35.2	5.28	8.06
	30-Mar	25.9	37.1	7.97	8.09
	31-Mar	24.9	37.3	6.22	8.16
	17-Apr	27.3	40.1	6.00	8.49
		26.2±1.26	36.9±2.11	561±1.52	8.2±0.21
TR1-LI	17-Mar	26.6	34.7	5.23	8.05
	20-Mar	26.6	35.2	5.75	8.83
	30-Mar	25.7	37.1	8.30	8.94
	31-Mar	24.9	37.3		8.92
	17-Apr	27.3	39.7	7.03	8.65
mean±sdev		26.2±0.98	36.8±1.93	5.3±3.03	8.7±0.36
TR2-LISH	17-Mar	26.6	34.6	5.04	8.02
	20-Mar	26.9	35.0	4.95	8.84
	30-Mar	25.9	37.0	5.05	8.63
	31-Mar	25.0	37.2	4.61	8.50
	17-Apr	27.0	39.2	5.00	8.42
mean±sdev		26.3±1.08	36.6±1.95	4.98±1.13	8.5±0.29
TR3-SH	17-Mar	26.4	34.7	5.23	8.01
	20-Mar	26.6	35.2	5.75	8.12
	30-Mar	25.7	37.0	5.44	7.93
	31-Mar	24.9	37.2	4.94	7.84
	17-Apr	27.2	39.1	5.24	7.91
mean±sdev		26.1±0.87	36.6±1.71	5.3±1.10	8.0±0.17

Cont. Annex 7 Table 7.3 Shrimp variables

	TR2-LISH	TR3-SH
Weight		
20-Mar	1.37	1.33
29-Mar	1.68	1.73
17-Apr	3.43	2.77
Rate growth		
20-Mar	0.00	0.00
29-Mar	0.31	0.40
17-Apr	1.75	1.03
	TR2-LISH	TR3-SH