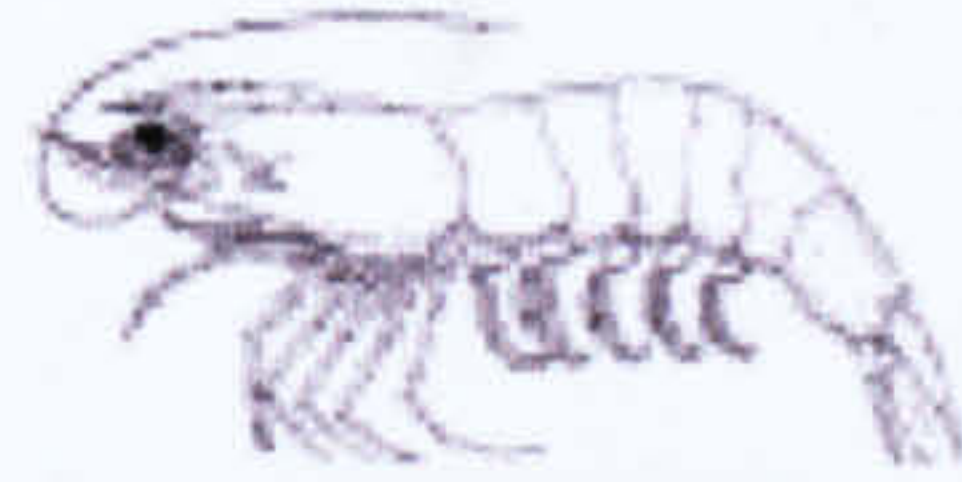


Thesis
3989



A study of semi-intensive shrimp culture in Ecuador in relation to physical, chemical and biological conditions in the production ponds during El Niño and La Niña events (1996 to 1999)



Thesis presented for the degree of Doctor of Philosophy
University of Stirling

by

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SCOTLAND

December - 2002

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Dedication

to Maria Laura...

for the strength she gave me

to my daughters... Maria Laura, Carolina and Sofia

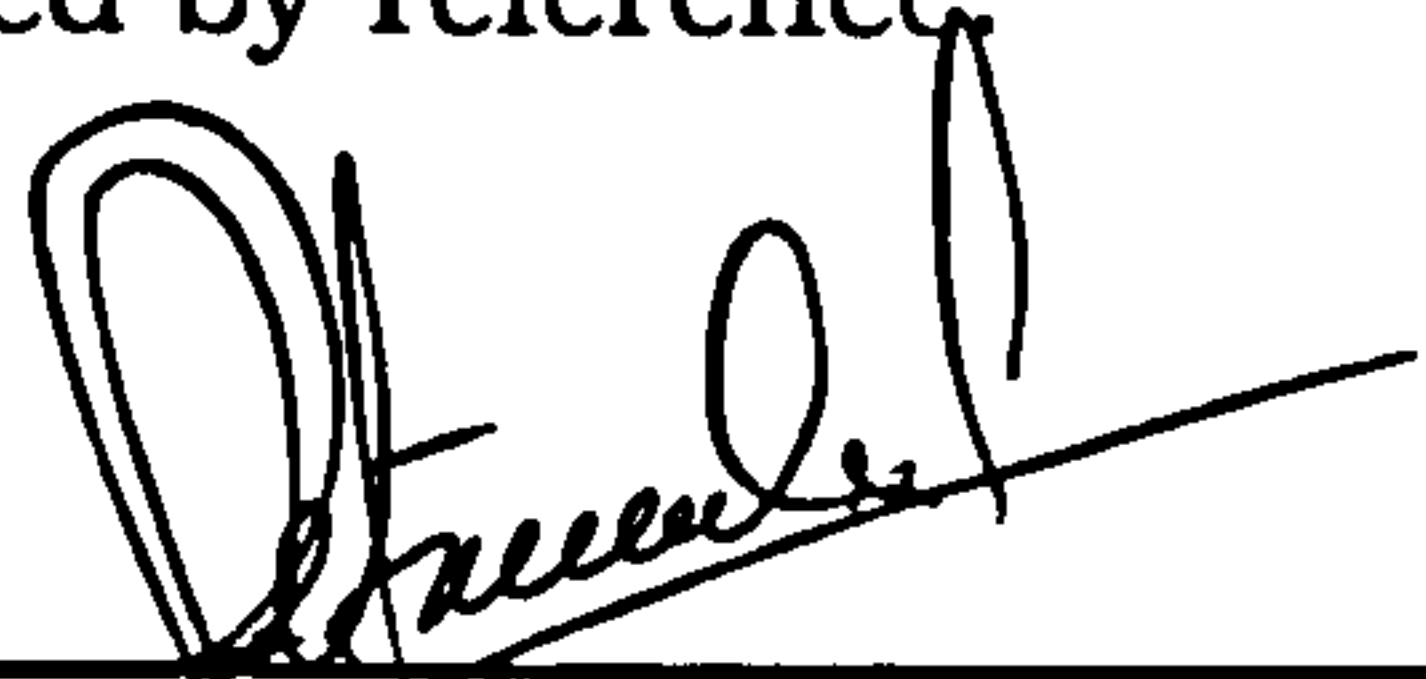
in return the sacrificed time during these years

and as a symbol of amendment for their lives.

DECLARATION

I hereby declare that this thesis has been composed by myself and has not been submitted in any previous application for a degree.

The work of which it is a record has been carried by myself. The nature and extent of any work carried out by, or in conjunction with others, has been specifically acknowledged by reference.



Roberto Retamales

*Professor Donald Macintosh
Principal Supervisor*

ACKNOWLEDGEMENTS

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Finally I want to thank María Laura, who has been an essential stimulus during this study and provided me with her love and kindness and sacrificed many desires for the sake of my study.

LIST OF PUBLICATIONS

Retamales, R. 2000. Phytoplankton community composition in a semi intensive commercial shrimp pond. *International Simposium El evento La Niña y su impacto biologico, and Workshop.* OEA. IAI. IAW. RIBEN. 9-10 Nov. Lima, Perú.

[http://www.concytec.gob.pe/investigacion/biologia/riben/org
.htm](http://www.concytec.gob.pe/investigacion/biologia/riben/org.htm)

Retamales, R. 2001. Bacterial community composition in a semiintensive shrimp culture of *L.vannamei* during El Niño 1997-1998 and La Niña 1999. *XXV Jornadas Nacionales de Biología and Workshop.* 22-23 Nov. FCN Universidad de Guayaquil. Ecuador.

ABSTRACT

Once every three, four or even seven years, the Southeast trade winds which blow west-ward across the tropical zone of the Pacific Ocean, from the shores of South America towards the Asian land mass, weaken and sometimes even reverse their direction. This phenomenon is known as El Niño. Conversely, the climatic condition known as La Niña is characterised by unusually cold ocean temperatures in the equatorial Pacific, as compared to El Niño. Global climate anomalies associated with La Niña tend to be opposite to those of El Niño.

A study of shrimp culture in Ecuador was carried out to analyse the temporal changes in pond water quality, phytoplankton composition and diversity, and bacterial composition and diversity in the intestines of cultured *Litopenaeus vannamei* in Ecuador. These parameters were studied in relation to their impact on the growth, survival and production in a semi-intensive shrimp culture farm situated in the Chone River Estuary, Ecuador. Five culture periods were studied during the climatic events of El Niño, La Niña and transition periods (1996 to 1999).

Shrimp were stocked in ponds at 10 PL/m². Pond management included pond drying, inorganic fertilisation, and feeding with a commercial pellet twice a day.

The physicochemical characteristics of the pond water and the phytoplankton and bacterial counts were estimated at intervals of 7-15 days of culture during each period of study.

The concentrations of nitrite, nitrate, sulphide, ammonia, pH and suspended solids in the pond water in all ponds during the five culture periods fluctuated within ranges considered compatible with shrimp

farming. Phosphorus, silica, temperature and salinity, however, showed significant differences during the five periods of shrimp culture, reaching sub-optimal levels during some culture periods.

The species composition and diversity of phytoplankton was different during El Niño, La Niña events and transition periods, with a decrease in the diatom community and an increase in the cyanophytes algae community associated with changes in nutrients and nutrient ratios, and temperature, salinity is discussed.

A low diversity of bacterial genera with a predominance of *Vibrio spp.*, particularly *V. harveyi* and *V. parahaemolyticus*, was observed in shrimp intestines during disease outbreaks in the transition and La Niña periods associated with significant environmental changes in temperature and salinity.

Shrimp performance was significantly different between El Niño, La Niña and transition climatic periods. Survival, feed conversion ratio and yield were better during El Niño periods because of the positive effects of higher pond water temperature and salinity (29°C and 28 psu) on the shrimp stocks.

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1. INTRODUCTION

1.1 History of world shrimp farming

Shrimp farming traces its origins to Southeast Asia where for centuries farmers raised incidental crops of wild shrimp in tidally operated fishponds, as described by Schuster (1952) in Indonesia.

Modern shrimp farming was born in the 1930s when Motosaku Fujinaga (Hudinaga), a graduate of Tokyo University, succeeded in spawning the kuruma shrimp (*Marsupenaeus japonicus*), a species native to the East China Sea region. He cultured larvae through to market size in the laboratory and succeeded in mass producing them on a commercial scale. For more than 40 years, he published papers on his work e.g. Hudinaga (1935, 1942 and 1962). However a cool climate, a rugged coast and high costs mitigate against shrimp farming in Japan, so Fujinaga's achievements had limited impact on commercial aquaculture in his own country.

During the late 1960s and early 1970s, researchers in France, China and Taiwan, witnessing the decline of commercial fisheries, began to investigate the potential of shrimp farming. In the South Pacific, French researchers at the Centre Oceanologique Pacifique in Tahiti, working with several penaeid species, including *Marsupenaeus japonicus*, *Penaeus monodon* and eventually

Litopenaeus stylirostris and *Litopenaeus vannamei* (species indigenous to the western hemisphere), developed successful techniques for breeding and raising shrimp intensively in ponds.

In China, researchers at the Yellow Seas Fishery Research Station discovered ways to grow large stocks of *Fenneropenaeus chinensis*, mainly in semi-intensive ponds. whilst at the same time in Taiwan, researchers at the Tungkang Marine Laboratory, working primarily with *Penaeus monodon*, developed techniques for farming this species intensively in small ponds, (Tookwinas, 1991).

In the United States, the Department of Commerce (DOC) of the National Marine Fisheries Service assumed control of the Galveston Lab. DOC also funded the National Sea Grant College Program, which backed shrimp farming research at several coastal universities, including Texas A&M University, a leader in shrimp farming technology even today. The Sea Grant Program was also an early backer of shrimp virus research at the University of Arizona. The results from these investments in shrimp research have led to the development of an ultra-intensive growout system, as reviewed by Fast (1992), both within USA and in other countries, such as in Puerto Peñasco, Sonora, Mexico.

As elements of the new shrimp farming technology emerged one by one, consultants, large corporations, feed companies and investors carried them to Latin America, particularly Honduras, Panama and Ecuador, where they teamed up with local entrepreneurs to build shrimp farms, hatcheries, feed mills and processing plants (reviewed by Lester, 1992).

To summarize the evolution of shrimp culture from the global perspective, during the 1970s researchers and farmers tested dozens of penaeid species worldwide for their farming potential. In the process, they worked out breeding and spawning techniques for most of the preferred farmed species. Other research concentrated on growout technology, nutrition, disease identifications and health management. These early efforts laid the groundwork for an industry that expanded spectacularly for two decades before reaching a brief plateau by the early 1990s (FAO, 2001), (Fig.1.1).

1.2 Shrimp farming in Ecuador

By the mid 1970s, there were three large semi-intensive shrimp farms in Central America: Sea Farms, Maricultura and Agromarina de Panama. Shrimp farming also started up in Ecuador about the same time (Sidall, Atchue and Murray, 1985), followed by Perú, but there were no shrimp farms in Venezuela or Colombia until the early 1990s.

In the mid-1970s, when fishermen and hatchery producers began supplying large quantities of juvenile shrimp to farmers, the production of farm-raised shrimp really took off. Shrimp farmers in Ecuador and over a dozen countries discovered that stocking, feeding and a pumped water supply were the keys to profit-making. A new industry was born. In some cases, the results were astounding. Large extensive farms in Ecuador recaptured their entire investment in the first year and sometimes from the first crop (Olsen and Arriaga, 1989); small-scale intensive farms in Taiwan produced dozens of shrimp millionaires; and even semi-intensive government farms in China reaped untold profits from formerly unused land around the Gulf of Bohai, (Rosenberry, 1990).

By mid-1975, even before the infusion of shrimp farming technology from USA, Ecuador was well on its way to becoming the leading producer of farm-raised shrimp in the western hemisphere. According to Hirono and van Eys (1990), the rapid expansion of the Ecuadorian shrimp industry could be attributed to many factors, including a favourable climate, availability of land, water rich in nutrients, an abundant supply of wild seed, cheap labour and high demand for shrimp in the world market. They defined three phases in the shrimp industry's development.

1. A first decade, based on rudimentary extensive systems.

2. A " boom " era with expansion from 3,000 ha in 1977 to 100,000 ha of ponds in 1988 (Olsen and Arriaga, 1989; Rosenberry, 1990, 1991, 1992; FAO, 1992; Hirono and Leslie, 1992).

3. A " survival phase ", of economic difficulties coupled with environmental problems (1989), which have increased production costs, thereby causing a reduction in revenue.

From 1978 onwards, the increased availability of material inputs for the industry such as formulated shrimp feed, abundant shrimp seed brought about by the occurrence of favourable warmer oceanographic conditions (El Niño 1982-1983), as well as the ready availability of financial support provided by the oil boom in Ecuador, encouraged the investment in shrimp pond operations during the first years of the 1980s. By 1983, cultured shrimp came to be the second most important export product in the country (Arellano, 1983), making Ecuador one of the world's leading producer of cultured shrimp (The World Bank, 1991).

Olsen and Arriaga (1989) suggested that the slight decline in shrimp production between 1984-1985 could be attributed not only to the interruption of wild larvae supply caused by cooler post-El Niño conditions, but also to the non-declaration or smuggling carried out by shrimp processing plants over the southern Peruvian frontier. This was due to the

difference in the exchange rate between the official and the free market dollar imposed by government regulations which lasted until 1986.

Ecuador exported eight to ten million pounds (3,636 – 4,545 mt) of shrimp per year, 99% of it being boat-caught. In comparison shrimp farms produced only 50,000 to 100,000 pounds per year (22,727 – 45,454 mt).

1.3 Global Production Trends

In 1985, world production of farm-raised shrimp (whole weight) hovered around 200,000 mt, which was about 10% of total world supply of shrimp of around two million metric tons per annum. About 75% of it was produced in Southeast Asia. In 1987, the United States National Marine Fisheries Service released a new estimate of farmed shrimp production for 1986, suggesting a much higher production that year of about 300,000 mt. It was obvious that the industry was going through a rapid growth phase in the mid 1980s, but statistical recording at that time could not keep up with the rate of change.

In 1988, the world's shrimp farmers produced an estimated 576,000 mt of shrimp. China (199,418 mt), Indonesia (77,785 mt), Ecuador (74,480) and Thailand (55,633 mt) were the leading producing countries, followed by the Philippines, Taiwan and Vietnam (FAO, 2001)..

The industry also witnessed its first major production crash in the period 1987-90. Hundreds of small intensive shrimp farms on Taiwan's west coast suddenly experienced unexplained stock mortalities. In a single year, production dropped from roughly 80,000 mt to only 20,000 mt, (Lin, 1989).

After the collapse of the Taiwanese intensive shrimp farming in 1987-88, Taiwanese entrepreneurs carried their knowledge and technology to other parts of Asia, especially the Philippines, Indonesia and Thailand, and to several spots in the western hemisphere, including Brazil, the Dominican Republic and Texas, in the United States. In Thailand, which already had a long history of shrimp farming dating back to the 1930s (Teinsongrusmee, 1970; Macintosh and Phillips, 1992), the Taiwanese technology found fertile territory (Patmasiriwat *et al.*,1999). Suddenly, there were thousands of small, intensive shrimp farms in Thailand, and in 1992, Thailand became the world's leading producer of farm-raised shrimp, producing 139,884 mt that year (FAO, 2001).

In the early 1990s, several new countries developed sizable shrimp farming industries. In the eastern hemisphere, Vietnam, India and Bangladesh became major producers for the first time and in the western hemisphere, Honduras (3,269 mt), Mexico (4,371 mt) and Colombia (6,000 mt), became significant producing countries, (FAO 2001).

The more established shrimp producing countries began facing a new reality in the early 1990s. Production of farm-raised shrimp in China had grown

quickly from about 100,000 mt in 1986, to about 200,000 mt in 1991. Then in 1994, China's shrimp production crashed to about 63,000 mt. A virus appears to have been the culprit, but industrial and domestic pollution around the Gulf of Bohai probably also played a role. In addition, China's ponds were in low-lying areas, making it difficult to clean the pond bottoms between harvests. The Chinese practice of feeding live molluscs, insects, and agricultural and fishery wastes to the shrimp may also have encouraged the spread of pathogenic viruses (Primavera, 1998).

Other countries also experienced crashes in shrimp production at about this time, including Indonesia, Philippines, Thailand and Vietnam, when too many intensive farms became concentrated in one area. For example, the northern coast of Java, Indonesia's main island, supported thousands of shrimp farms during 1991, (Anon, 1996). Similarly, intensive shrimp farms on Negros Island, the Philippines, went through dramatic fluctuations in production until many decided to adopt less intensive farming strategies (Platon, 1999). In Thailand in the early 1990s, hundreds, if not thousands, of shrimp farmers went out of business just south of Bangkok (Flaherty, 1995; Dierberg and Kiattisimkul, 1996; Stevenson, 1997). The local waters became so polluted that they would no longer support cultured shrimp, even at much reduced stocking levels.

The effluent from intensive farms overwhelms the carrying capacity of the local receiving waters. With time, the effluent backs up into the farming

areas causing a phenomenon known in the industry as “self-pollution”, (Macintosh and Phillips, 1992). The farms have little choice but to utilise the inferior water. This stresses the shrimp, making them more susceptible to viruses. Viruses can also kill in high quality water, but they are much more likely to reach pathological levels in the rich organic waters of intensive shrimp ponds, (Yuan *et al.*, 2000; Corea and Jayasingue, 2001).

This phenomenal development of shrimp farming over the last 25 years is illustrated globally, and by the major producing countries, in Fig.1.1 and Table 1.1 respectively. Current annual production stands at around 1 million mt, which is equivalent to about one third of the total world shrimp supply. Farmed shrimp represents < 1% of global fisheries production by weight and accounts for 10% of the global fisheries export value.

The global geographical distribution of shrimp and prawn output is highly skewed towards Asia. Six of the top ten shrimp producing countries were Asian (Fig.1.2), accounting for 75% of global production, compared with 14% for Latin America.

Thailand the world’s largest producer in 1994, saw its production decrease from a peak of 265,000 mt that year to around 227,000 mt in 1997. India and the Philippines also reported similar decreases during this period. China's shrimp culture showed evidence of recovery and between 1994 and 1998 output increased from a low of 63,000 mt in 1994 to reach 143,000 mt in 1998. Ecuador, dominates farmed shrimp production in Latin America, and in 1998

accounted for 80% of the total Latin American production, or 144,000 mt, followed by Mexico (23,000 mt), Panama (10,000 mt), Honduras (8,000 mt) and Colombia (7,400 mt) (Table 1.1).

1.4 Shrimp species in aquaculture

By species, the giant tiger shrimp (*Penaeus monodon*) is the major farmed shrimp species contributing around 52% of total global farm production (Fig.1.3). This species dominates production in Thailand, Indonesia, India and Philippines. In contrast, China cultures the fleshy prawn, *Fenneropenaeus chinensis* due to its tolerance of lower water temperatures.

The Pacific white shrimp, *Litopenaeus vannamei* (*Penaeus vannamei* (Boone)) (Perez-Farfante and Kensley, 1997) is native to the Pacific coast of Central America and South America (from Mexico to Perú). This species is the leading farm-raised shrimp in Ecuador, and everywhere else in Latin America, accounting for 90 % of the cultured shrimp production in the country (Fig.1.3).

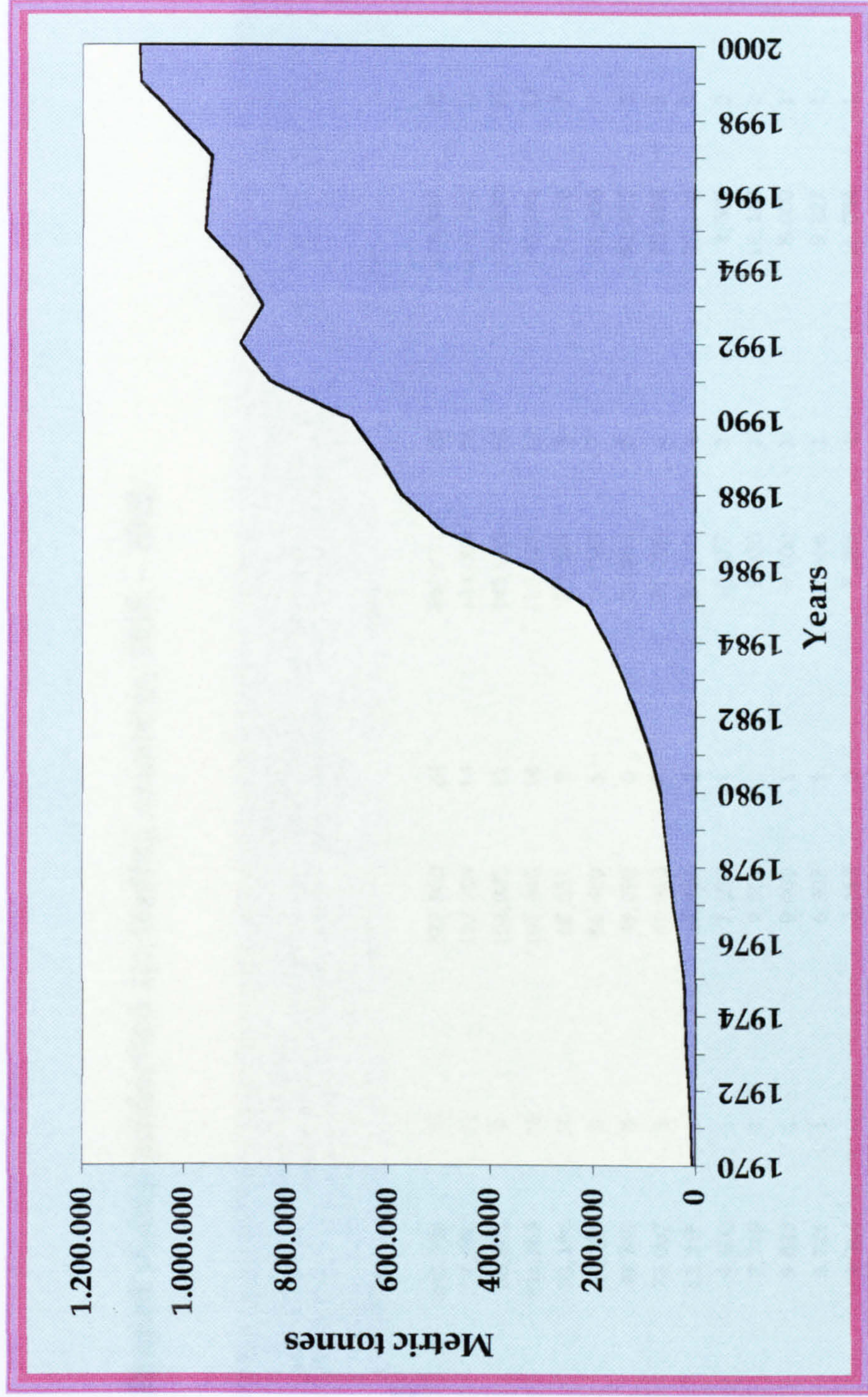


Fig.1. 1 World farmed shrimp production, 1970 –2000

Source: FAO, 2001.

Source: <http://www.fao.org/fi/statist/fisoft/fishplus.asp>

Table.1. 1 Farmed shrimp production for leading countries, 1996 – 2000.

Country	1996		1997		1998		1999		2000	
	Annual production (metric tonnes)	Proportion of global farmed shrimp production (%)	Annual production (metric tonnes)	Proportion of global farmed shrimp production (%)	Annual production (metric tonnes)	Proportion of global farmed shrimp production (%)	Annual production (metric tonnes)	Proportion of global farmed shrimp production (%)	Annual production (metric tonnes)	Proportion of global farmed shrimp production (%)
Thailand	240,339	25	227,560	24	252,731	25	275,544	25	299,700	
Ecuador	107,920	11	132,709	14	144,000	14	119,700	11	50,110	
China	88,851	9	102,923	11	143,086	14	170,830	16	217,994	
Indonesia	151,759	16	167,445	18	118,111	12	140,946	13	138,023	
India	95,152	10	65,581	7	78,709	8	71,072	7	52,771	
Bangladesh	49,260	5	56,480	6	66,080	7	81,068	7	58,183	
Viet Nam	49,749	5	49,298	5	54,853	5	57,433	5	69,433	
Philippines	78,067	8	41,610	4	37,798	4	35,898	3	41,811	
Mexico	13,315	1	17,422	2	23,749	2	29,120	3	33,480	
Panama	4,900	1	7,050	1	10,077	1	2,585	0	1,212	
Malaysia	7,749	1	9,514	1	9,835	1	12,188	1	15,894	
Honduras	9,833	1	9,000	1	8,000	1	8,000	1	8,500	
Colombia	5,221	1	6,907	1	7,466	1	9,227	1	11,390	
Brazil	3,364	0	3,613	0	7,254	1	16,054	1	25,000	
Other	47,539	5	47,957	5	51,314	5	55,210	5	63,610	
Total	953,018	100	945,069	100	1,013,063	100	1,084,875	100	1,087,111	

Source: FAO, 2001.
<http://www.fao.org/fi/statist/fisoft/fishplus.asp>

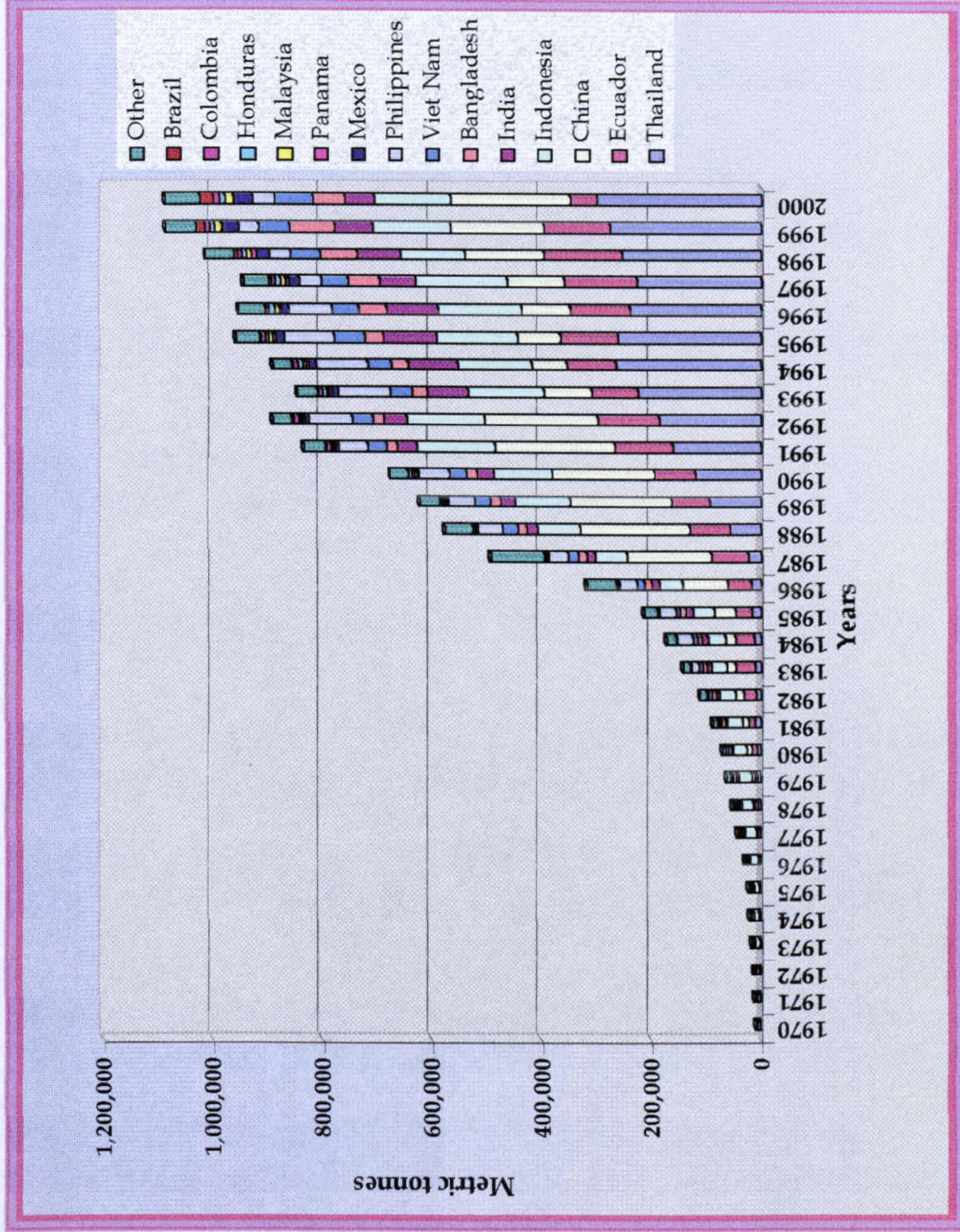


Fig.1. 2. Farmed shrimp production for leading countries, 1970 – 2000.

Source: FAO, 2001. <http://www.fao.org/fi/statist/fisoft/fishplus.asp>

1.5 Shrimp farming and sustainability

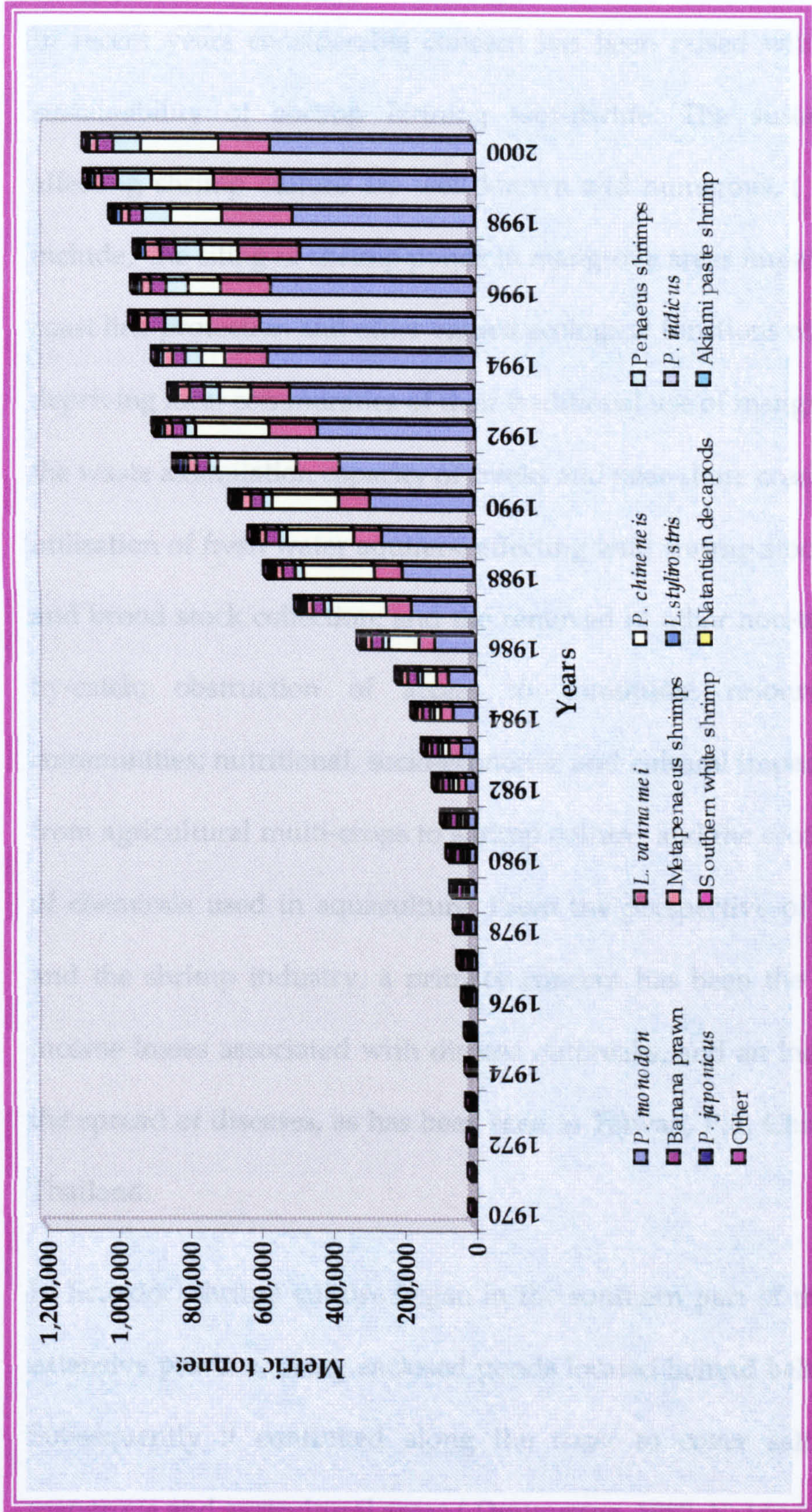


Fig.1. 3 World farmed shrimp species production, 1970 – 2000

Source: FAO, 2001 <http://www.fao.org/fi/statist/fisoft/fishplus.asp>

1.5 Shrimp farming and sustainability

In recent years considerable concern has been raised with respect to the sustainability of shrimp farming worldwide. The sustainability issues affecting shrimp culture are well known and numerous, (Table 1.2) and include, the siting of shrimp ponds in mangrove areas impairing the habitat, coast line protection and other valued ecological functions of mangroves and depriving local communities of their traditional use of mangroves; exceeding the waste assimilation capacity of creeks and near-shore coastal waters; over-utilisation of fresh water aquifers; affecting wild shrimp stocks through seed and brood stock collection, and the removal of other non-target stocks as a by-catch; obstruction of access to communal resources by coastal communities; nutritional, socio-economic and cultural impacts of conversion from agricultural multi-crops to shrimp culture; and the ecotoxicological risk of chemicals used in aquaculture. From the perspective of shrimp farmers and the shrimp industry, a primary concern has been the production and income losses associated with disease outbreaks, and an inability to control the spread of diseases, as has been seen in Taiwan, P.R. China, Ecuador and Thailand.

In Ecuador, shrimp culture began in the southern part of the country as an extensive practice, using enclosed ponds located behind belts of mangroves. Subsequently it continued along the coast to cover salt flats, clear-cut mangrove and agricultural areas (Doumenge, 1989; Valdivieso *et al.*, 1992),

but remained principally close to river mouths. With increasing development, there was a tendency towards semi-intensive systems of production. Weidner and Rosenberry (1992) report " harvests have increased from 5 mt in 1979 to over 70,000 mt by 1990 ". Shrimp farming is a year round activity but the warm, wet season is generally regarded as better for shrimp rearing due mainly to the fact that wild post larvae are more abundant and seem to be more resistant to disease than in the cool dry season.

Table.1. 2. Sustainability issues of shrimp farming worldwide

Issue (s)	Author (s)
Environmental aquaculture pollution	Chua <i>et al.</i> , 1989
Sustainability shrimp mariculture	Olsen and Arriaga, 1989
Environmental impact coastal	GESAMP, 1991
Culture pond critical review	Primavera, 1993
Chemical pollution	Primavera <i>et al.</i> , 1993
Natural resource degradation	Flaherty and Karnjanakesorn, 1995
Code of conduct, regulate use of chemicals	FAO, 1995
Water management regimes, abate the environmental impact.	Hopkins <i>et al.</i> , 1995
Ecotoxicological, chemicals	Redshaw, 1995
Mangroves as filters	Robertson and Phillips, 1995
Coastal mangroves	Macintosh, 1996
Ecological, social impacts	Barracough and Finger-Stich, 1996
Sustainability aquaculture	Pillay, 1996
Impacts shrimp aquaculture	Dierberg and Kiattisimkul, 1996
Design of sustainable pond-bases	Sandifer and Hopkins, 1996
Pollution, wetlands	Sansanayuth <i>et al.</i> , 1996
Environmental, antibacterial drugs	Weston DP, 1996
Environmental sustainability	Boyd, 1997
Environmental sustainability	Beveridge <i>et al.</i> , 1997
Ecotoxicological, pesticides	Castillo <i>et al.</i> , 1997
Environmental sustainability	Barg and Phillips, 1997
Sustainability shrimp aquaculture	Clay, 1997
Chemicals in coastal aquaculture	GESAMP, 1997
Policies for sustainability shrimp culture	FAO, 1998
Environmental shrimp aquaculture	Boyd and Clay, 1998
Environmental, chemicals	OECD, 1998
Coastal environmental	Phillips, 1998
Policies for sustainability shrimp culture	FAO, 1998
Sustainability tropical shrimp farming	Primavera, 1998
Sustainability shrimp farming	Phillips and Barg, 1999
Environmental, chemicals	Boyd and Massaut, 1999
Antimicrobial resistance	WHO, 1999
Aquaculture development	NACA, FAO, 2000
Aquaculture fisheries supplies	Naylor <i>et al.</i> , 2000

Shrimp farms are now commonly semi-intensive, (Fig.1.4) (use of fertilisers combined with supplemental feeding, intermediate stocking, occasional pumping of water and yields of 1-2 tons / ha / yr); and intensive systems are scarce (high stocking density, formulated complete feeds, aeration and water pumping with yields of more than 3 tons / ha / yr). A one-phase or two-phase production cycle is utilised. With the two-phase cycle, the juvenile shrimp are stocked in nursery ponds and then, several weeks later, transferred to growout ponds. With the one-phase cycle, the nursery ponds are eliminated, and the shrimp are stocked directly into growout ponds, after having spent a short period in an acclimation tank. Farms usually produce three crops a year.

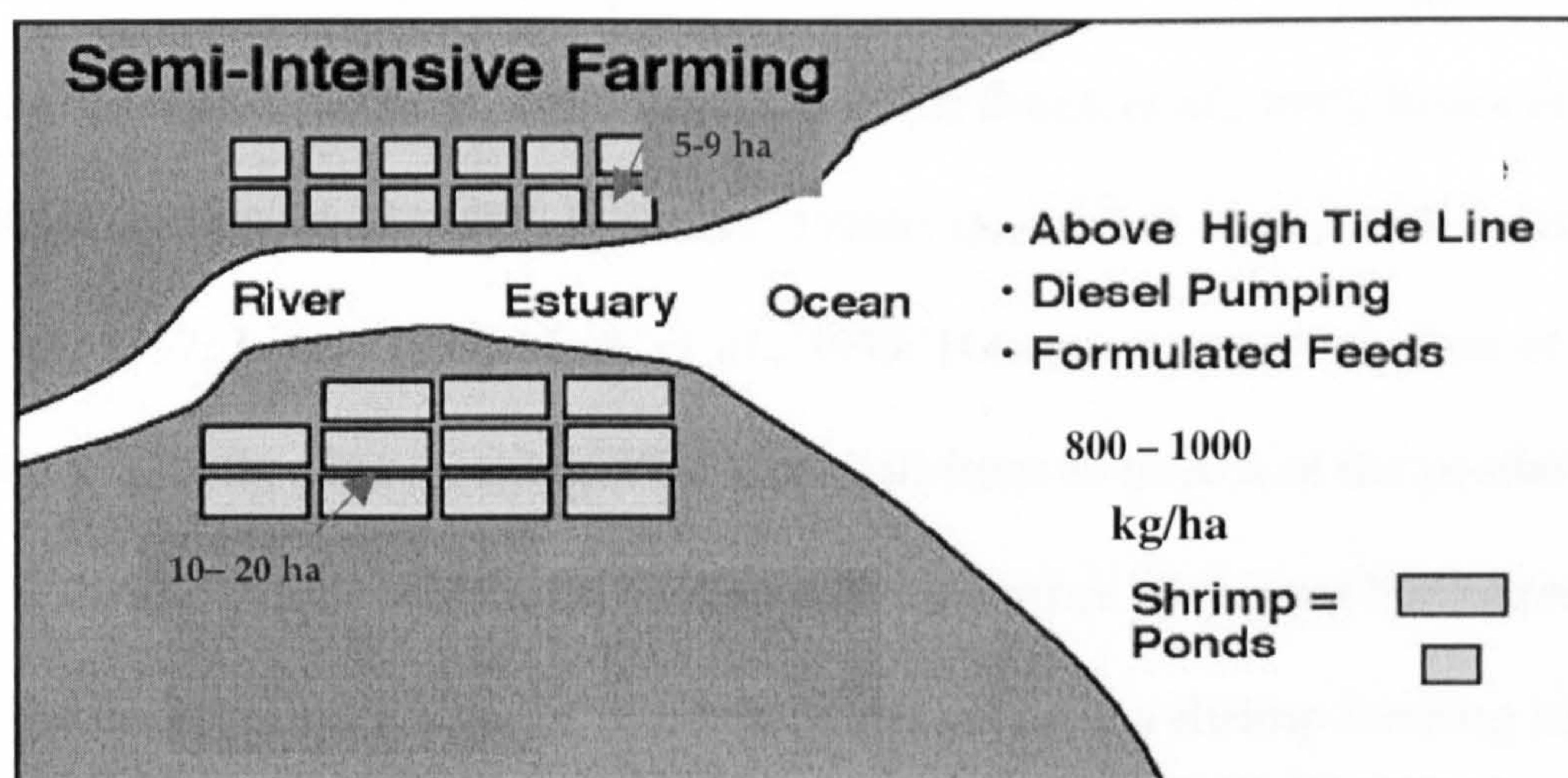


Fig.1. 4 Diagrammatic illustration of a typical semi-intensive shrimp farm in Ecuador.

Before 1997 and during recent years different diseases affecting shrimp production have been reported in Ecuador (Table 1.1, Fig.1.2). In this context, bacterial diseases due mainly to *Vibrio* species were often associated with low survival rates in hatchery or growout conditions. Larval mortalities

associated with the presence of *V. harveyi* have been reported in *L. vannamei* (Morales, 1992; Zherdmant, 1996; Robertson *et al.*, 1998). *Vibrio* species such as *V. alginolyticus*, *V. damsela*, *V. parahaemolyticus*, *V. vulnificus* and *V. penaeicida*, (Mohny *et al.*, 1994; Vandenberghe *et al.*, 1999), and other bacterial (Krol, *et al.*, 1991; Freiler, *et al.*, 1992; Siavichay, 1996; Solis, 1996; Jimenez *et al.*, 1997; Intriago, 1998; Peeters *et al.*, 1999) and viral diseases outbreaks (Browdy, *et al.*, 1993; Jimenez *et al.*, 1999, 2000) have been observed in nursery or growout ponds of *L. vannamei* (Lightner, 1983; Lightner, 1992; Lightner *et al.*, 1992).

Prior to the arrival of white spot syndrome virus (WSSV) in Ecuador in the second half of 1999, Taura syndrome virus (TSV) was the biggest killer (Lightner and Redman, 1992; Jimenez, 1992; Brock *et al.*, 1995; Brock *et al.*, 1995; Lightner *et al.*, 1995; Lightner, 1996b; Overstreet *et al.*, 1997; Intriago *et al.*, 1997; Lotz, 1997; Mari, *et al.*, 1998; Hasson *et al.*, 1999; Soto *et al.*, 2000). Shortly after stocking, WSSV can kill from 40 to 90% of the postlarvae in a shrimp pond. Although Taura syndrome virus may have been present in the background for years, it officially arrived on the shrimp farming scene in Ecuador in June 1992, near Guayaquil. It hit several farms and then disappeared until March 1993 when it returned as a major epidemic, killing farm-raised shrimp throughout the Gulf of Guayaquil. Dubbed " Taura Syndrome " because it was first reported on farms along the Taura River, an area about 25 kilometers southeast of Guayaquil, it is also called "Little Red

Tail" (La Colita Roja) because the tail fan and body of affected shrimp turn pale pink.

The main histopathological TSV lesions consist of multifocal areas of acute necrosis of the cuticular epithelium and subcutis of the exoskeleton, gills, appendages, foregut, and hindgut as revealed with hematoxylin and eosin-phloxine staining. The cuticular lesions contain karyorrhectic nuclei and numerous variably sized eosinophilic to basophilic cytoplasmic inclusion bodies, giving them a characteristic "peppered" or 'buckshot' appearance (Lightner and Redman, 1994; Lightner *et al.*, 1995; Lightner, 1996a; Hasson *et al.*, 1995; Hasson *et al.*, 1997).

Nowadays, Ecuadorian shrimp farms face another virus that arrived from Asia, the White spot syndrome virus (WSSV), (Nunan, *et al.*, 1998; Alday, 1999a; Alday, 1999b; Calderón, *et al.*, 1999; Boyd, *et al.*, 1999; Jory and Dixon, 1999).

White spot syndrome virus (WSSV) has caused high mortalities in many species of penaeid shrimp and other aquatic crustaceans (Lightner, 1996a; Lo *et al.*, 1996; Maeda *et al.*, 1998) throughout the world. Disease attributed to this virus was first observed in eastern Asia during 1992–1993 (Huang *et al.*, 1994; Inouye *et al.*, 1994; Nakano *et al.*, 1994; Chen, 1995). The disease spread very rapidly, and by 1996 it had had severe impact on most of the shrimp farming regions of Asia (Flegel, 1997), including Japan (Inouye *et al.*, 1994; Takahashi *et al.*, 1994), Thailand (Wongteerasupaya *et al.*, 1995),

China (Huang *et al.*, 1995), India (Hameed *et al.*, 1998) and Korea (Park *et al.*, 1998). Subsequently, WSSV spread to many countries of the western hemisphere. In the United States, WSSV was first recorded in Texas in 1995 (Lightner, 1996a); and later found in South Carolina in 1997 and 1998 (Lightner, 1999).

In many penaeids, the clinical signs of WSSV disease are the appearance of white spots inside the carapace and a reddish discolouration of the body. Histological analysis reveals distinct hypertrophied nuclei in the cuticular epithelial cells, connective tissue cells, and hemocytes (Lightner, 1996a). WSSV is an enveloped, rod-shaped virus, containing double-stranded DNA (Wang *et al.*, 1995; Wongteerasupaya *et al.*, 1995). It is large, 70–150 nm X 275–380 nm (Wang *et al.*, 1995; Durand *et al.*, 1996), and extremely virulent; mortalities in affected shrimp stocks can reach 100% within 3–10 days (Lightner, 1996 b).

At the present time there are no medications to treat shrimp viruses, but management techniques have evolved which lessen their impact. Diseases represent the biggest obstacle to the future of shrimp farming in Ecuador (personal observation).

During 1997 and 1998 (El Niño event), the production of pond reared shrimp reached high record of 109,090 and 114,993 mt respectively, (Fig.1.5). In contrast, no publications were found describing the conditions

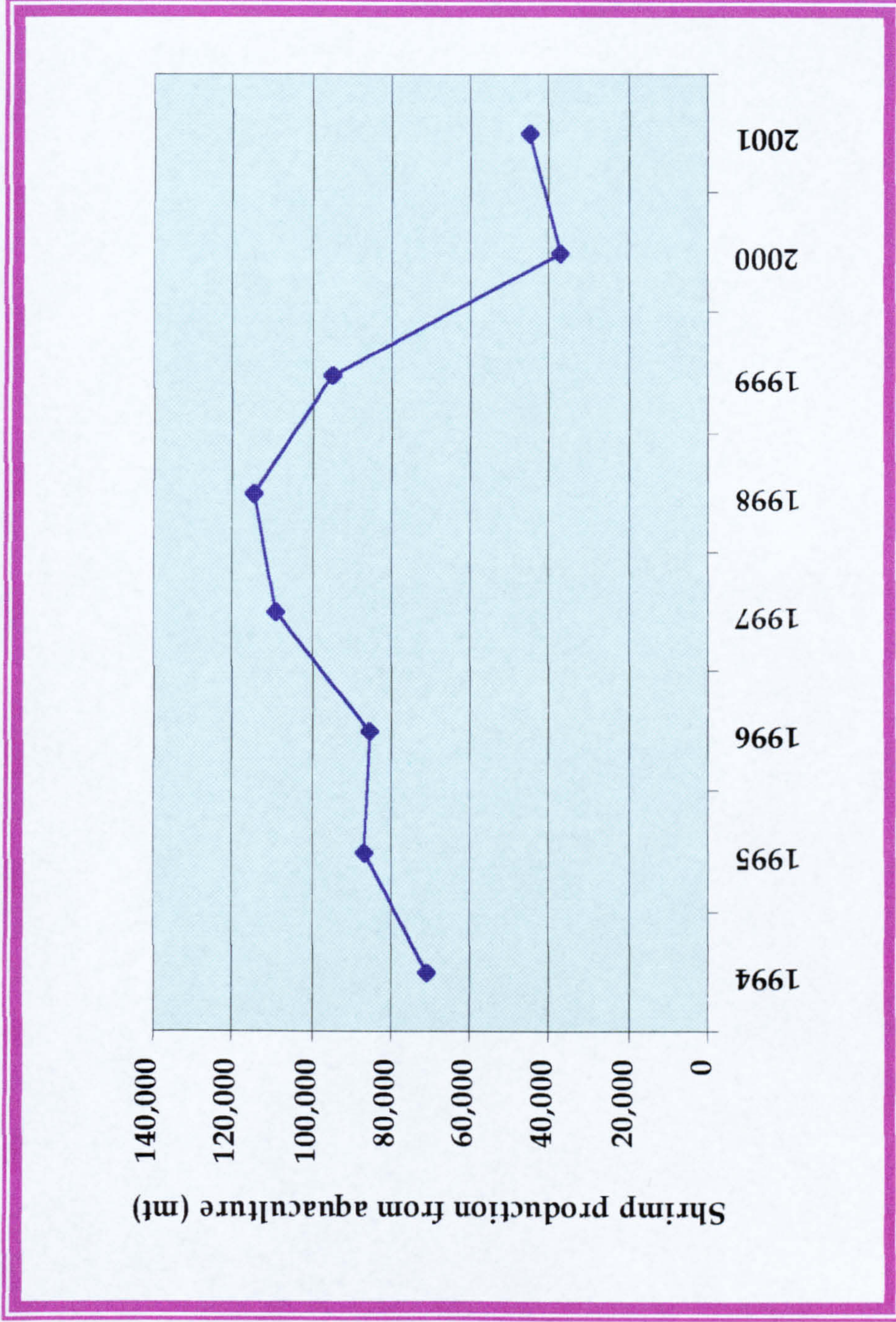


Fig.1. 5 Farmed shrimp production in Ecuador, 1994 – 2000

Taken from: Aquaculture National Chamber Ecuador
<http://www.cna-ecuador.com/>

in water quality or other parameters, such as phytoplankton or bacterial analysis during the shrimp production cycles in 1996-1997-1998-1999, (La Niña and El Niño events).

Considerable variation in shrimp pond water quality is evident in relation to a number of operational conditions associated with the husbandry of *Litopenaeus vannamei* (stocking density of shrimp larvae into the ponds and the start of feeding, water resource, water exchange and farm management) and climate factors (e.g. rainfall). Water quality requirements are based on the results of aquatic toxicity test. These test measure the responses of in aquatic organisms to defined quantities of specific compounds (APHA 1989). Maintaining water quality means keeping the concentration of harmful substances in water low enough so that they do not adversely affect behavior and physiology of the cultured organisms.

Some part of the nitrite-nitrogen in the rearing ponds came from the oxidation of ammonia by bacteria such as *Nitrosomonas spp.* Nitrite-nitrogen is toxic to shrimp at concentrations higher than 1.0 mg/L, (Chiu, 1988) while shrimp larvae (PL₁₀) are only resistant to nitrite-nitrogen concentration below 0.36 mg/L (Tookwinas, 1984). Crustaceans show decreased resistance to nitrite during their moulting periods. Chen and Lei (1990) have suggested a safe level of 3.8 mg/L nitrite-N for *P. monodon* juveniles. However, sub-acute or chronic exposure to nitrite levels as low as 2

to 3 mg/L can damage the gills, causing changes to the shrimp's normal gill colour (Lightner, 1988).

Nitrate is a primary nutrient in the shrimp pond environment. Diatoms are the dominant algae in brackish waters of moderate to high salinity. According to Boyd (1989), diatoms require large amounts of nitrogen and this contributes to nitrate being a more important limiting nutrient than phosphorus. Nitrate is generally regarded as non-toxic to shrimp, as it has been reported that even at concentrations of 200 mg/L of nitrate-N shrimp can grow normally (Wickins, 1981).

The ammoniacal nitrogen content in the shrimp pond can be divided into two forms: unionised ammonia (NH_3) and ionised ammonia (NH_4). The ammonium ion is not toxic to shrimp since it cannot penetrate through the cell membrane of shrimp. The ammonia content depends on the pH, the temperature and the salinity of the water in the ponds. The ammoniacal nitrogen in a shrimp pond is derived from the decomposition of organic matter, such as that from left over feed, the decomposition of dead plankton, and the excretion of shrimp.

Ammonia affects the growth rate of shrimp by decreasing the oxygen transportation capacity in the blood. The ammoniacal nitrogen content should no be more than 0.1 mg/L, (Mohanty and Mohanty, 2001). The growth rate of shrimp is lower if the ammoniacal nitrogen is high. At a concentration higher than 0.1 mg/L, the growth rate will decrease by 60-70%,

while a concentration of 0.4-2.0 mg/L is acutely toxic to shrimp (Hernandez-Herrera *et al.*, 1999).

Phosphorus is an important element for the growth of phytoplankton. The form of phosphorus that phytoplankton are able to utilize approximates to the inorganic orthophosphate form. Most of the orthophosphate is precipitated and absorbed by the bottom of the shrimp pond. Phosphorus in water can be precipitated easily at an acidic pH by reacting with ferric and aluminium to form ferric phosphate and aluminum phosphate. At a basic pH, it will react with calcium to form tricalcium phosphate. Therefore, the concentration of usable phosphate for phytoplankton depends on the decomposed rate of organic matter in the water, as well on the precipitation and photosynthetic rate of phytoplankton. According to Boyd (1992), the average concentration of total phosphorus in an unfertilised shrimp pond is around 0.026 mg/L, while those receiving applications of fertilizer show phosphate levels of around 0.175 mg/L.

Weathering of rocks and soil produces dissolved silica, either by direct dissolution from amorphous silica, or by reaction with other acids (CO_2). More important is the role of estuaries and river plumes in the biological part of the silica cycle. Many such systems are highly productive and, due to the high concentrations of silica, diatoms are a main component of the phytoplankton. The biological part of the silica in microalgae leads to interaction with other nutrient elements (N, P), (Yusoff, 2001).

Hydrogen sulphide is produced by bacteria in anoxic waters, silts and muds, especially where organic loadings are high, such as in heavily stocked shrimp ponds. Hydrogen sulphide accumulates on the pond bottom and turns the soil black. It exists in two forms in the water, HS⁻ (ionised sulphide ion) and H₂S (unionised hydrogen sulphide); the H₂S form is toxic to shrimp. In well oxygenated waters, sulphide is oxidised rapidly to sulphate. Thus, the best way to stop hydrogen sulphide being formed is to maintain a well oxygenated and mixed system, especially close to the sediments.

The water pH will also affect the sulphide content (Boyd, 1989, 1990; Chanratchakool *et al.*, 1995). According to Chiu (1988), the relationship between hydrogen sulphide levels safe for shrimp and pH in ponds under semi-intensive operation are < 0.004 mg/L (pH 6-7); < 0.007 mg/L (pH 7-8) and < 0.04 mg/L (pH 8-9). A hydrogen sulphide concentration of 1.3 mg/L can cause shock, paralysis and eventually the death of shrimp (Kongkeo, 1990).

Total suspended solids, the portion of total solids retained by a filter, include a wide variety of materials, such as silt and decaying plant and animal matter (Boyd, 1989). High TSS can reduce the amount of light passing through the water, causing a reduction in photosynthesis. Reduced rates of photosynthesis lead to less dissolved oxygen being released into the water by plants. Suspended sediments can also clog the gills of shrimp, reduce their growth rates and decrease their resistance to disease. Suspended clay particles are considered undesirable in shrimp ponds (Chiu, 1998).

Shrimp are sensitive to changes in salinity in their culture environment. The preferred salinity range is highly dependent on the species concerned. *Litopenaeus vannamei* survives and grows well in intermediate salinities (20 psu), (Huang, 1983; Barlett *et al.*, 1990; Ponce-Palafox *et al.*, 1997).

Temperature influences the distribution, abundance and development of aquatic organisms. Temperature also influences other parameters important in aquaculture, including the dissolved oxygen level and the unionised ammonia and sulphide concentrations (Boyd, 1990), as well as the nutritional requirements, feeding, growth and digestion rate of the cultured species (Hilton and Slinger, 1982; Fast, 1985).

Wyban *et al.* (1995) suggested that temperature optima for highest growth are size-specific and decrease as shrimp size increases. For small shrimp (<5 g), temperature optima may be greater than 30°C, while for large shrimp, the temperature optimum is about 28°C. Lester and Pante (1991) considered that penaeid shrimp pass through three stages: (1) larvae adapted to oceanic salinities and surface temperatures; (2) juveniles adapted to estuarine and coastal temperature patterns and; (3) adults which are adapted to oceanic salinities and bottom temperatures. The reported temperature ranges for the successful growth and survival of juveniles of *L. vannamei* under laboratory conditions are 28 – 30 °C (Ponce-Palafox *et al.*, 1997).

The optimal pH for shrimp in rearing ponds is 7.5 - 8.5 (Boyd, 1989, 1990). The growth rate of shrimp is low at pH 4 – 6 (acidic pH), or 9 – 11 (basic pH). If the pH is lower than 4 or higher than 11, shrimp will die. The pH will also affect the ammonia and sulphide content of the pond water. If the pH is high, the ammonia content will be high as well, and ammonia may be toxic to shrimp, (Boyd, 1989, 1990; Chanratchakool *et al.*, 1995).

Phytoplankton play an important role in maintaining water quality by affecting nutrient concentration and bacterial numbers (Chien, 1992) that are essential in shrimp farm management to ensure optimal growth and survival of shrimp. Farmers often stimulate phytoplankton blooms by adding organic or inorganic nitrogen and phosphorus. The blooms shade the shrimps, prevent the growth of benthic algae, maintain oxygen levels, reduce ammonia levels and provide a food source for zooplankton and other invertebrates which are eaten by shrimps. Algal blooms may benefit shrimp indirectly through their effects on other trophic levels. Manzi, *et al.*, (1977) suggested that algal production of bactericides may promote shrimp growth through the reduction of adverse bacteria. Dominant bacterial species have been shown to change in response to fluctuations in the algal community and the types of organic matter released from algae (Painting, 1989). Changes in algal populations have also been correlated with fluctuations in protozoans and larger zooplankton (Landry *et al.*, 1984; Roman *et al.*, 1988; Bjørnsen *et al.*, 1989). These complex interactions between algae and other components of the microbial community compound the difficulties in designing studies

to identify the relative importance of different microbial groups in relation to shrimp production, (Bratbak and Thingstad, 1985).

While the role of phytoplankton in maintaining water quality has been studied in shrimp ponds (Carpenter *et al.*, 1986; Lumare *et al.*, 1987; Ziemann *et al.*, 1992; Hopkins *et al.*, 1993; Cortes Altamirano *et al.*, 1995; Burford and Glibert, 1999), researchers have not sampled seasonally to determine how the phytoplankton biomass and community composition change, and their relationship to shrimp survival and production. Quantitative studies with a longer time horizon, which would yield insights into strategic aspects of water quality management, such as seasonal effects on pond dynamics, are scarce (Tucker and van der Ploeg, 1993; Seok *et al.*, 1995; Lorenzen *et al.*, 1997; Cowan *et al.*, 1999).

The microbial community have major roles in pond culture, particularly with respect to productivity, nutrient cycling (Moriarty *et al.*, 1983; Moriarty, 1986a, b; Anderson, 1987; Fry, 1987; Moriarty and Pullin, 1987; Moriarty, 1990a, b; Allan *et al.*, 1995; Moriarty, 1997). The microbial community can positively affect shrimp production through direct and indirect provision of nutrients for the target crop. Microorganisms can also benefit water quality and has been shown to significantly enhance shrimp growth (Leber and Pruder, 1988; Moss *et al.*, 1992, 1995; Moss and Pruder, 1995). The significance of these beneficial processes depends upon factors such as the pond management strategy, the shrimp and the pond age. In addition to a

direct nutritional benefit, pond water affects both the abundance and species composition of microflora in shrimp hindguts. Gut flora may enhance shrimp growth and survival by contributing exogenous enzymes out competing pathogenic bacteria and supplying essential compounds lacking in the shrimp's diet, (Moss *et al.*, 2000, 2001).

Descriptive studies are a necessary pre-cursor to sensible experimentally manipulative analyses of ecology. In addition, in many fields that have not developed manipulative experimental methodologies, descriptive studies have retained relatively high status. Properly designed, carefully analysed tests of hypotheses about patterns, therefore, deserve appropriate recognition as part of the spectrum of experimental ecology. Critical tests of logically derived hypotheses about ecological patterns are valid as experimental science, including studies that are observational (or mensurative) rather than manipulative. Well-planned, logically constructed observational, studies to test coherent *a priori* hypotheses have the same logical content as any experimental manipulation. There is no lack of agreement that the primary purpose of ecological investigation is to *understand* and *explain* natural phenomena, therefore, the resultant patterns of distribution, abundance, diversity and interactions of species (Underwood, 1996; Underwood *et al.*, 2000). Since aquaculture is basically a managed ecological process, it is essential to consider the underlying ecological principles involved in

aquaculture, as this may help to understand and contribute to the solution of problems facing shrimp farmers.

1.6 Study objectives

The present study was designed originally to monitor, during the different seasons around the year (the wet summer season and the cool dry season), the trends in water quality, phytoplankton and bacterial composition and diversity in commercial semi- intensive shrimp culture ponds and to assess how these fluctuations could affect the farmed shrimp performance in terms of the growth, survival and production of *Litopenaeus vannamei*. However unforeseen climatic events associated with El Niño and La Niña phenomena occurred in Ecuador during the study, which had a dominating influence on the conditions for shrimp culture.

1.6.1 Specific objectives

1.6.1.1 Obtain records of fluctuations in physicochemical water quality parameters from consecutive periods of shrimp culture.

1.6.1.2 Analyze the fluctuation in phytoplankton composition and diversity during the different culture periods.

1.6.1.3 Analyze the fluctuation in composition of bacterial diversity (e.g. total heterotrophic aerobic bacteria (THAB) and presumptive *Vibrio* (PV) from the intestines of *L. vannamei*, during the different culture periods.

1.6.1.4 Analyse statistically the fluctuations mentioned above in relation to shrimp performance and recommend more sustainable management practices especially to reduce disease outbreaks by opportunistic pathogens caused primarily by adverse environmental changes.

1.6.1.5 Overall, to gather a time series of pond environmental data that could be used for the later development of a primary simulation model, (Andrew and Mapstone, 1987), e.g. to examine the dynamics of selected nutrients in semi intensive shrimp culture (for example N:P ratio, silica, phosphorus, nitrogen) under different conditions of temperature, salinity, and the pond phytoplankton community (relative abundance of diatoms, cyanophytes, green algae).

2. STUDY SITE AND CLIMATIC FEATURES

2.1 Study site

The study site was a commercial shrimp mangrove forestry farm (owner Maria Laura) culturing *Litopenaeus vannamei* . It is located at the Chone River estuary, L. 0° 40.13 S , L 80° 20.25 S, Manabí Province, Ecuador, where a total shrimp farming area of 5,545 hectares has been developed,(Fig.2.1). Manabí Province, in which the project was located, is the third largest of Ecuador's 21 provinces in terms of population, containing 10% of the country's population of 11 million. About 40% of Ecuador's gross national product (GNP) is accounted for by fisheries, oil, agriculture, and forestry. The Province of Manabí is one of the main centres of fisheries industries, accounting for approximately 10% of Ecuador's total fisheries products. It is especially known for its production of shrimp for export.

2.2 Climatic features

The estuary of the Chone River (Fig.2.2) has a tropical climate defined by differences in rainfall throughout the year corresponding to 95 % of the annual precipitation during the wet summer season from December - January to April (Stevenson, 1981). In contrast, 5 % or less of the yearly precipitation occurs during the dry season, which extends from May through

November. In the wet season, the coastal seawater is characterised by low salinity and high temperatures, while the dry season features high salinity and low temperatures.

A coupled oceanic and atmospheric interaction, known as El Niño and La Niña events, can change this normal pattern by producing intervals of higher or lower than normal temperatures and salinities,(Figs. 2.3 and 2.4).

The ENSO (El Niño/ Southern Oscillation) cycle refers to the coherent, large-scale fluctuation of ocean temperatures, rainfall, atmospheric circulation, vertical motion and air pressure across the tropical Pacific. Thus, it represents a coupled ocean-atmosphere phenomenon centred in and over the tropical Pacific. The scale of these fluctuations is quite vast, with the changes in sea-surface temperatures typically spanning a distance of more than one-quarter the circumference of the globe, and the changes in tropical rainfall and winds spanning a distance of more than one-half the circumference of the earth.

During La Niña episodes, drier than normal conditions are generally observed along coastal Ecuador and north-western Perú. La Niña episodes also contribute to large-scale temperature departures throughout the world, with most of the affected regions experiencing abnormally cool conditions. Some of the most prominent temperature departures include cooler than

normal conditions along the west coast of South America and parts of central America.

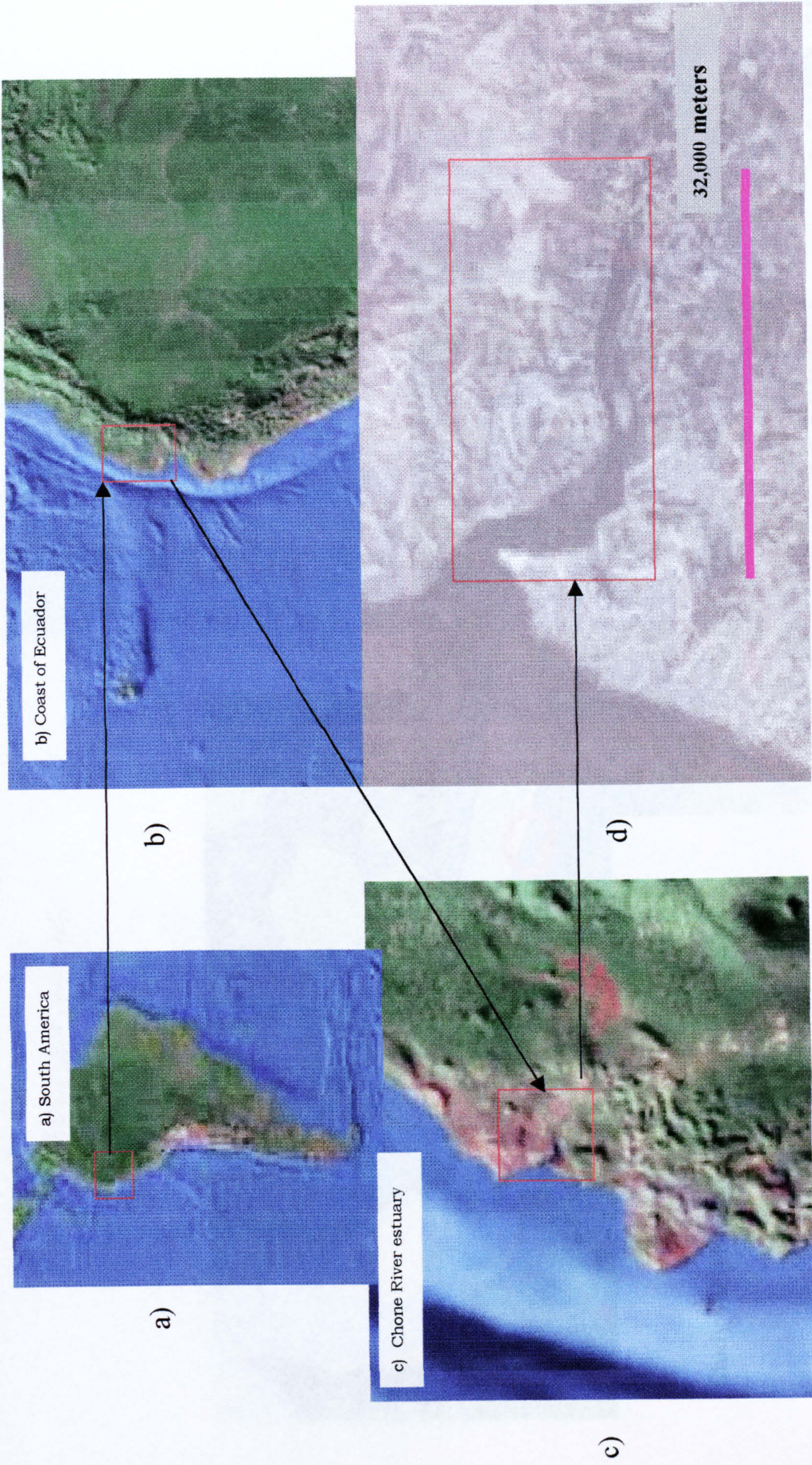
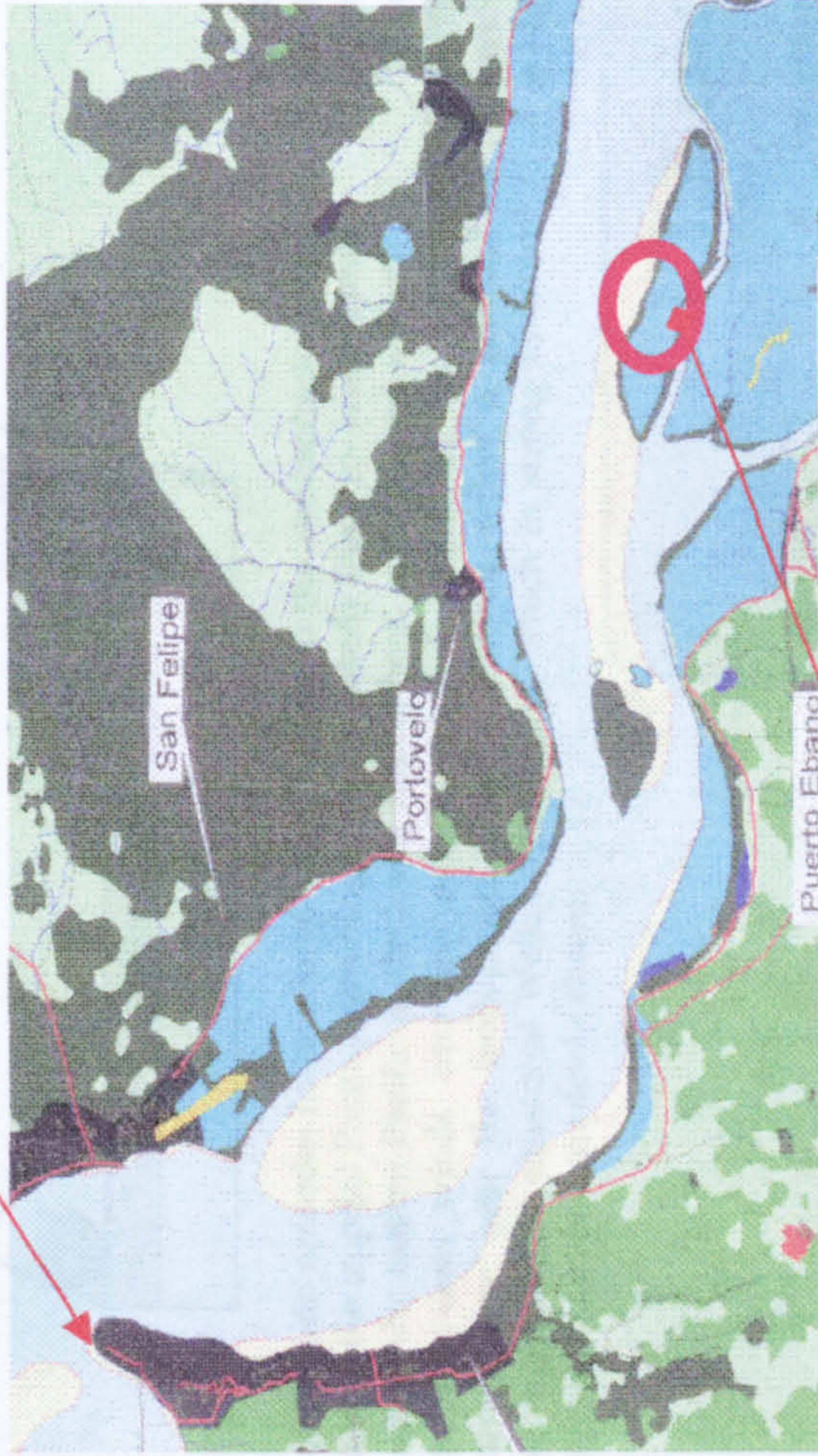


Fig.2. 1 a,b,c,d. Chone River estuary, Manabí Province, Ecuador.

Fig.2. 2. María Torres's study area. Chone River estuary, Manabí Ecuador.

City of Bahía de Caráquez



Study site. Maria Laura's shrimp farm

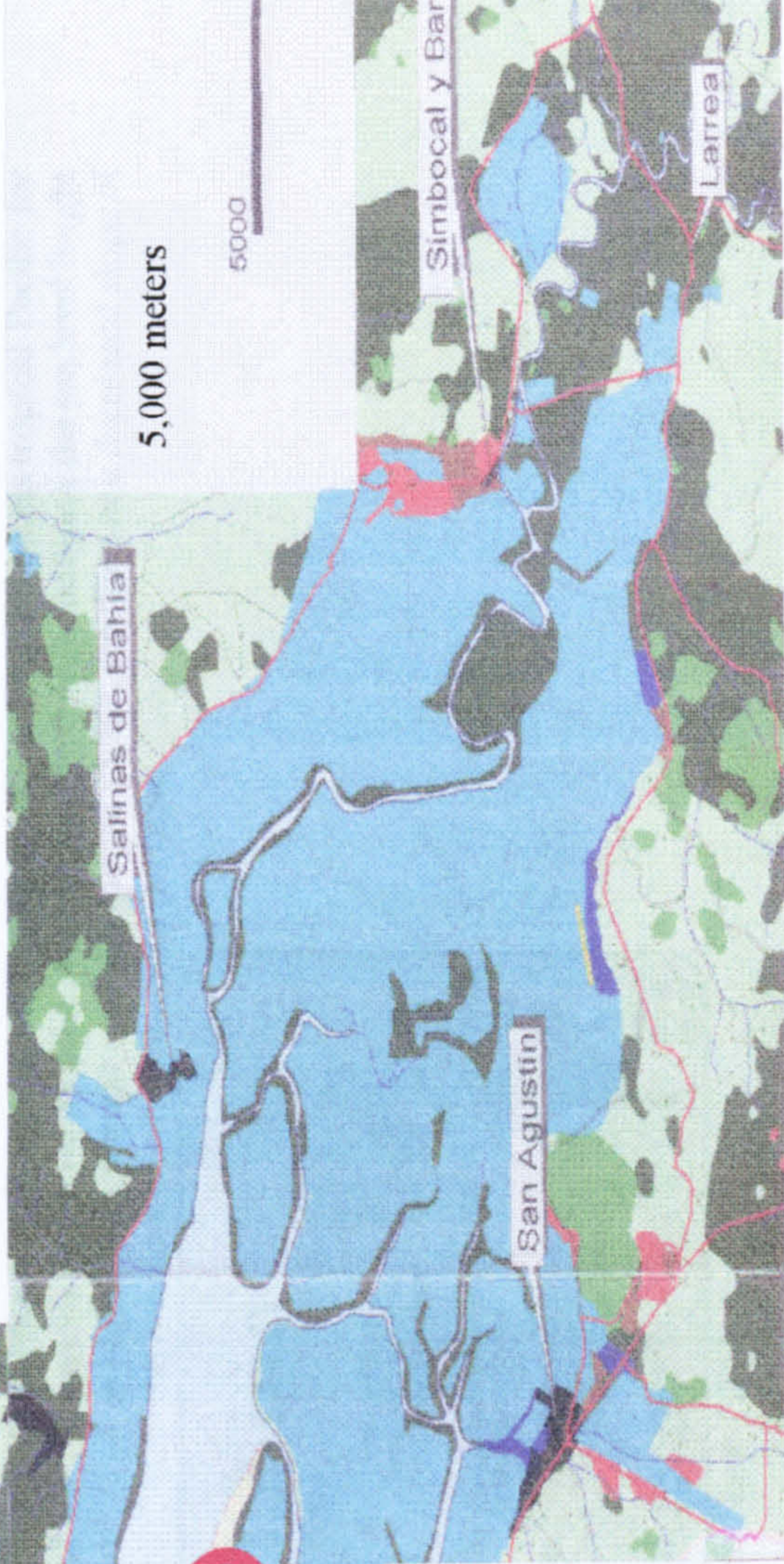
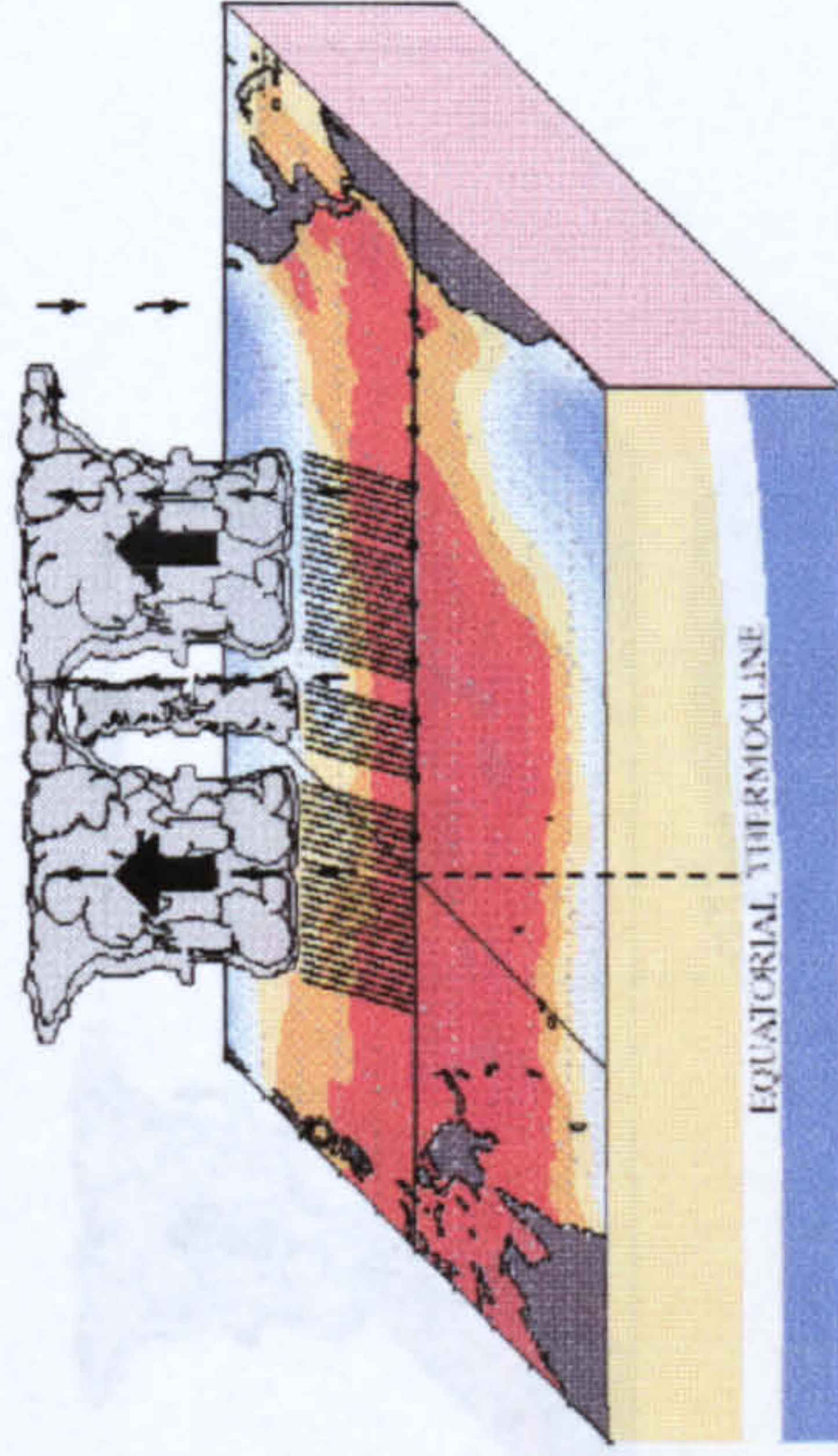
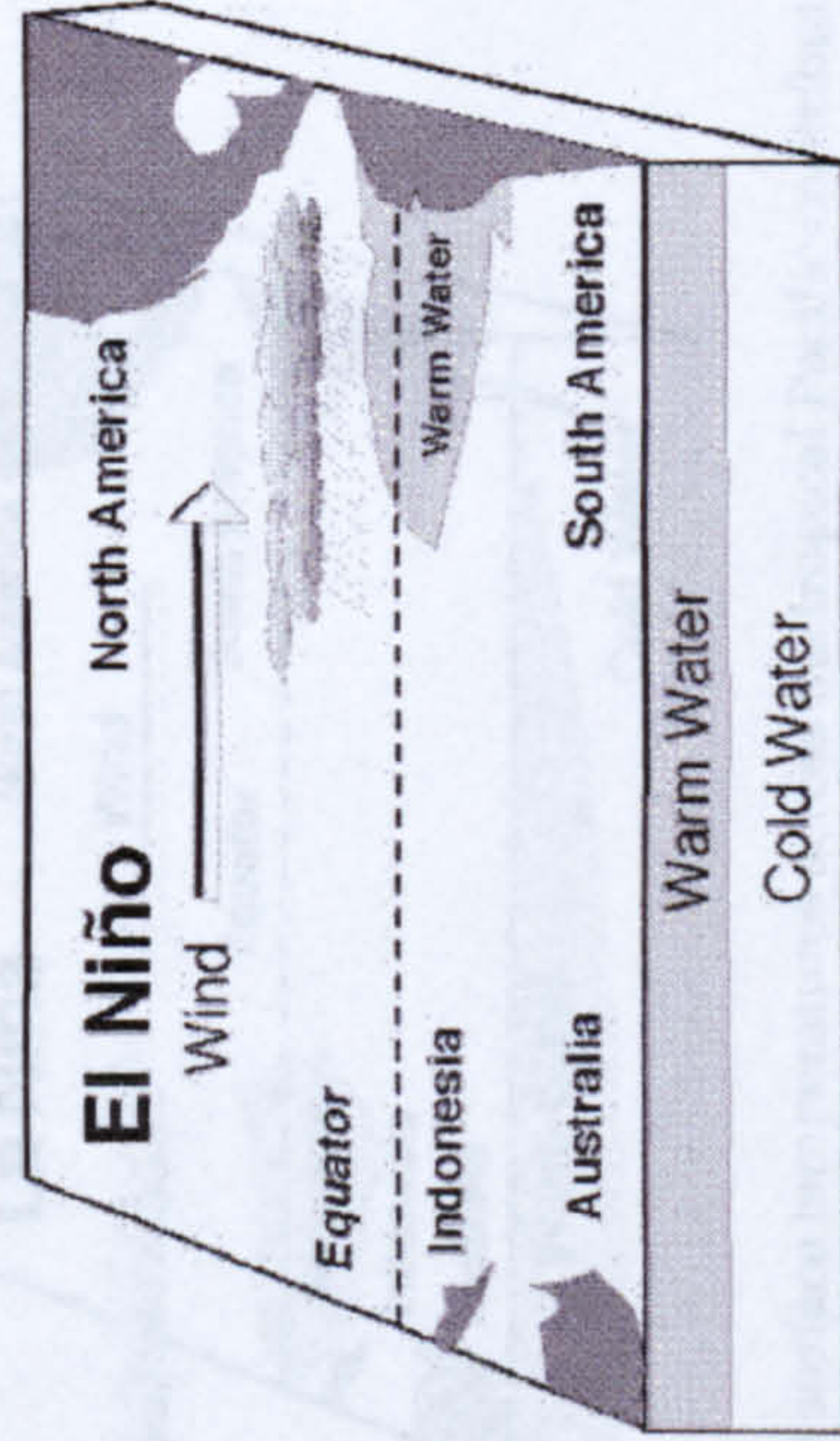


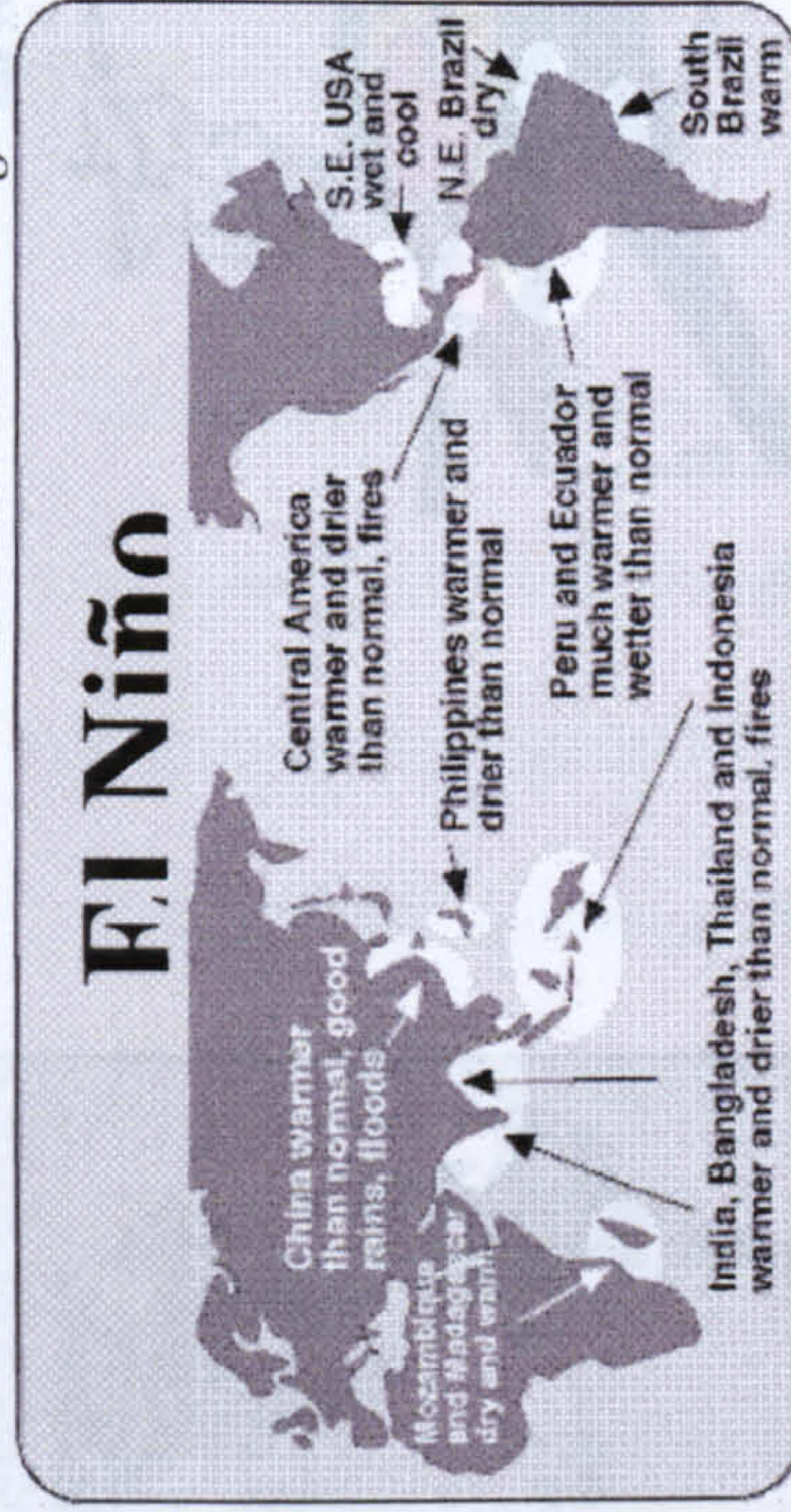
Fig.2. 2. Maria Laura's shrimp farm. Chone River estuary, Manabí Ecuador .



b) During the developing phase of the El Niño, the subsurface ocean structure is characterized by an abnormally deep layer of warm water and an increased depth of the thermocline across the eastern tropical Pacific. Thus, the slope of the thermocline is reduced across the basin. In very strong El Niño episodes, the thermocline can actually become flat across the entire tropical Pacific for periods of several months. Accompanying these conditions, the sea level height is higher than normal over the eastern Pacific, resulting in a decreased slope of the ocean surface height across the basin.



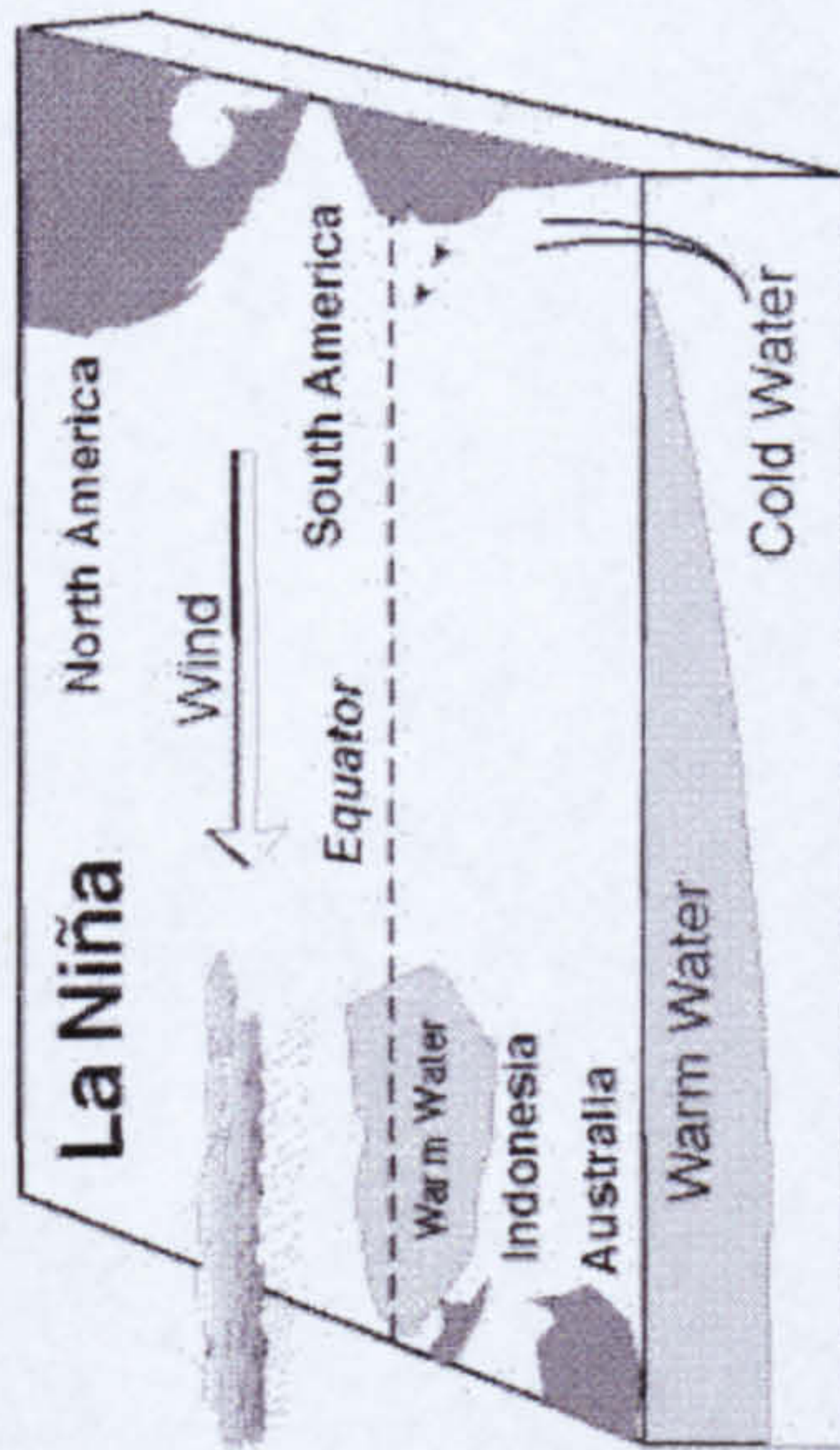
a) El Niño episodes feature large-scale changes in the atmospheric winds across the tropical Pacific, including reduced easterly (east-to-west) winds across the eastern Pacific in the lower atmosphere, and reduced westerly (west-to-east) winds over the eastern tropical Pacific in the upper atmosphere near the tropopause. These conditions reflect a reduced strength of the equatorial Walker Circulation, which in strong El Niño episodes can be completely absent.



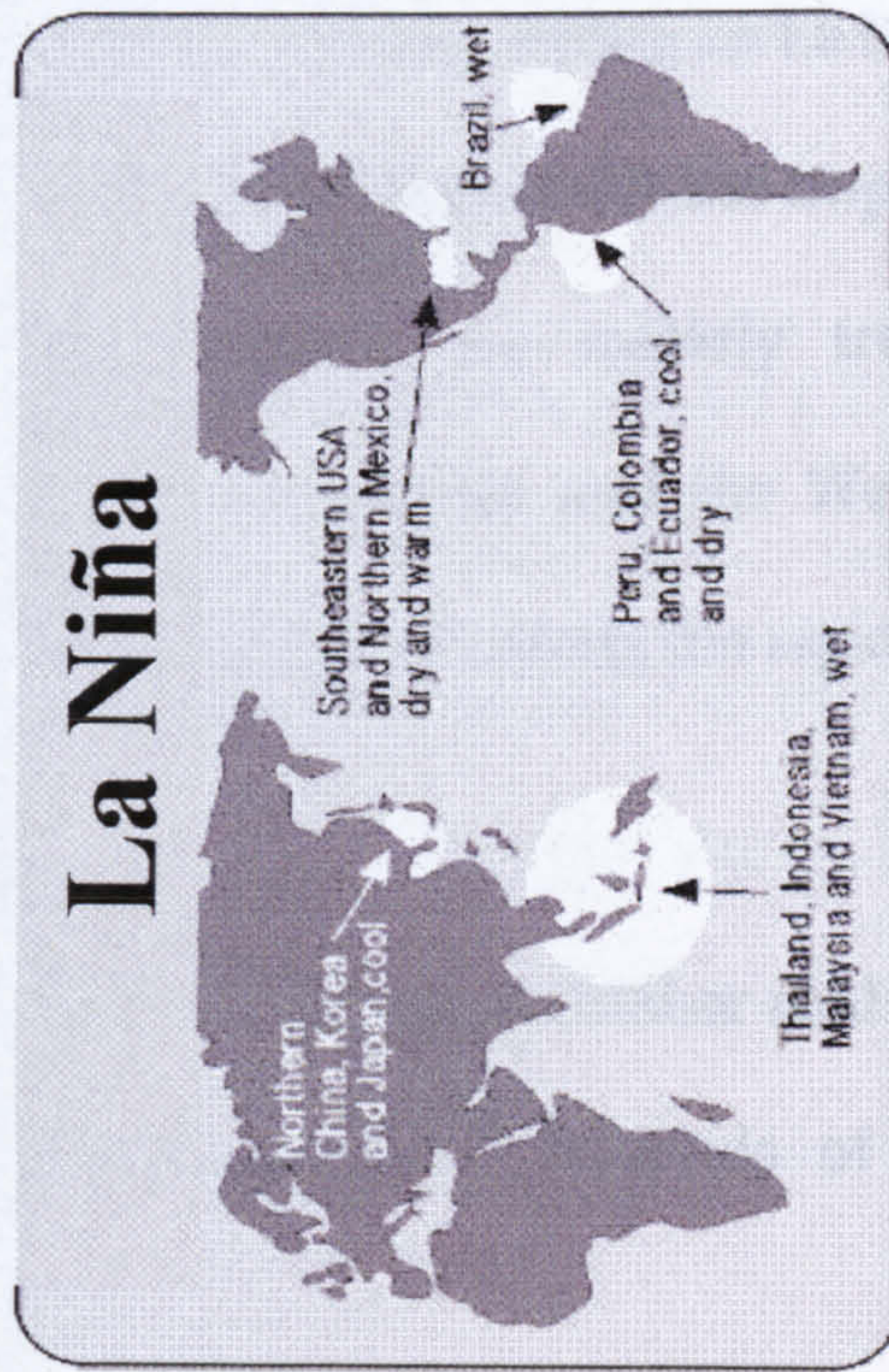
c) Taken from Nash: (2002).

Fig.2. 3 a,b,c. El Niño Current.

The western basin of the tropical Pacific slowly collects much of the excess heat from the sun along the earth's midline bulge. Then, almost like a lake overflowing an invisible dam, this huge reservoir of warm water is released as one long, sustained burst, known as an El Niño.

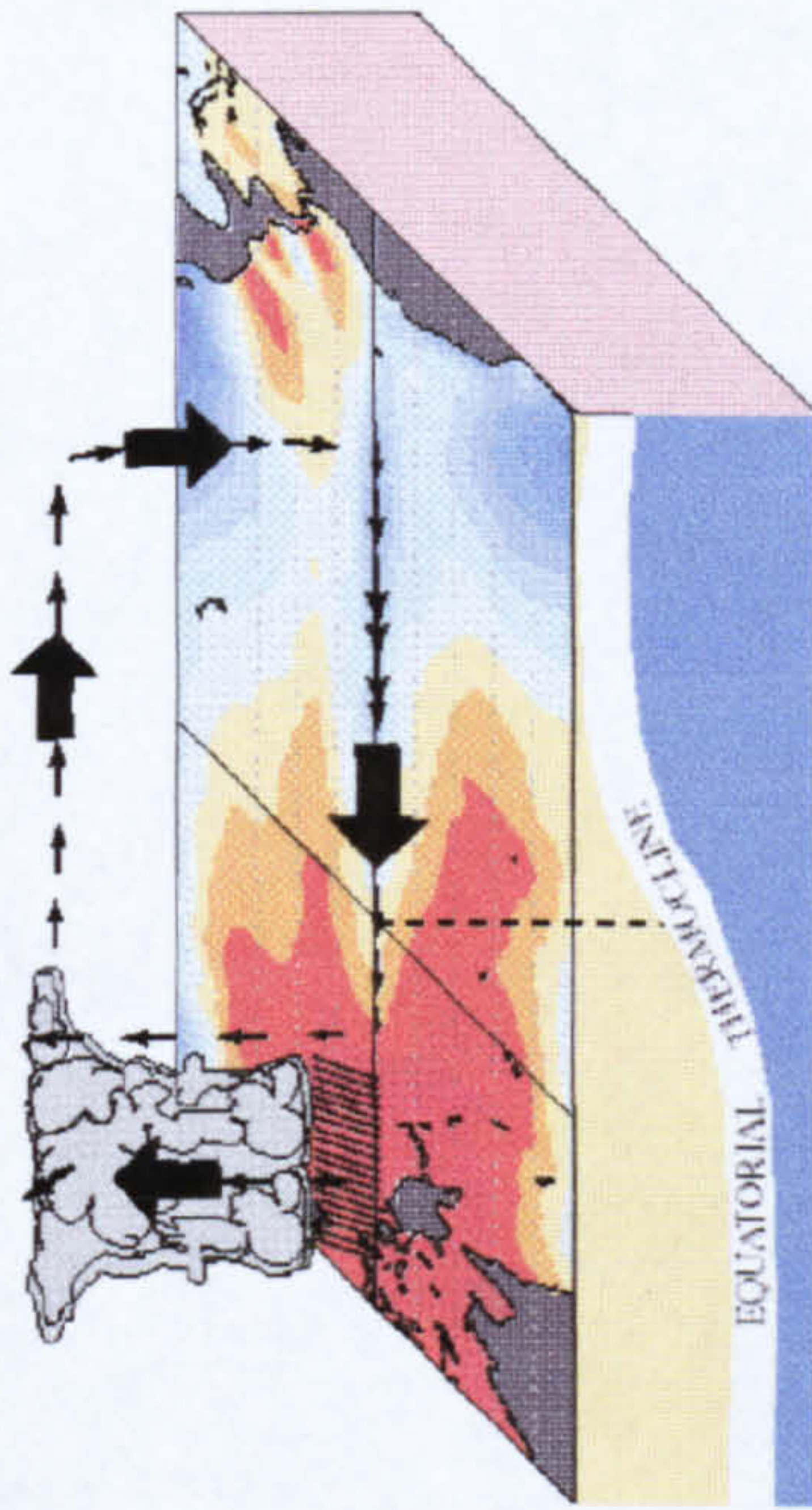


a) Ocean surface temperatures across the tropical Pacific contribute significantly to the observed patterns of tropical rainfall and tropical thunderstorm activity. The heaviest rainfall is typically observed across Indonesia and the western tropical Pacific, and least rainfall is normally found across the eastern equatorial Pacific.

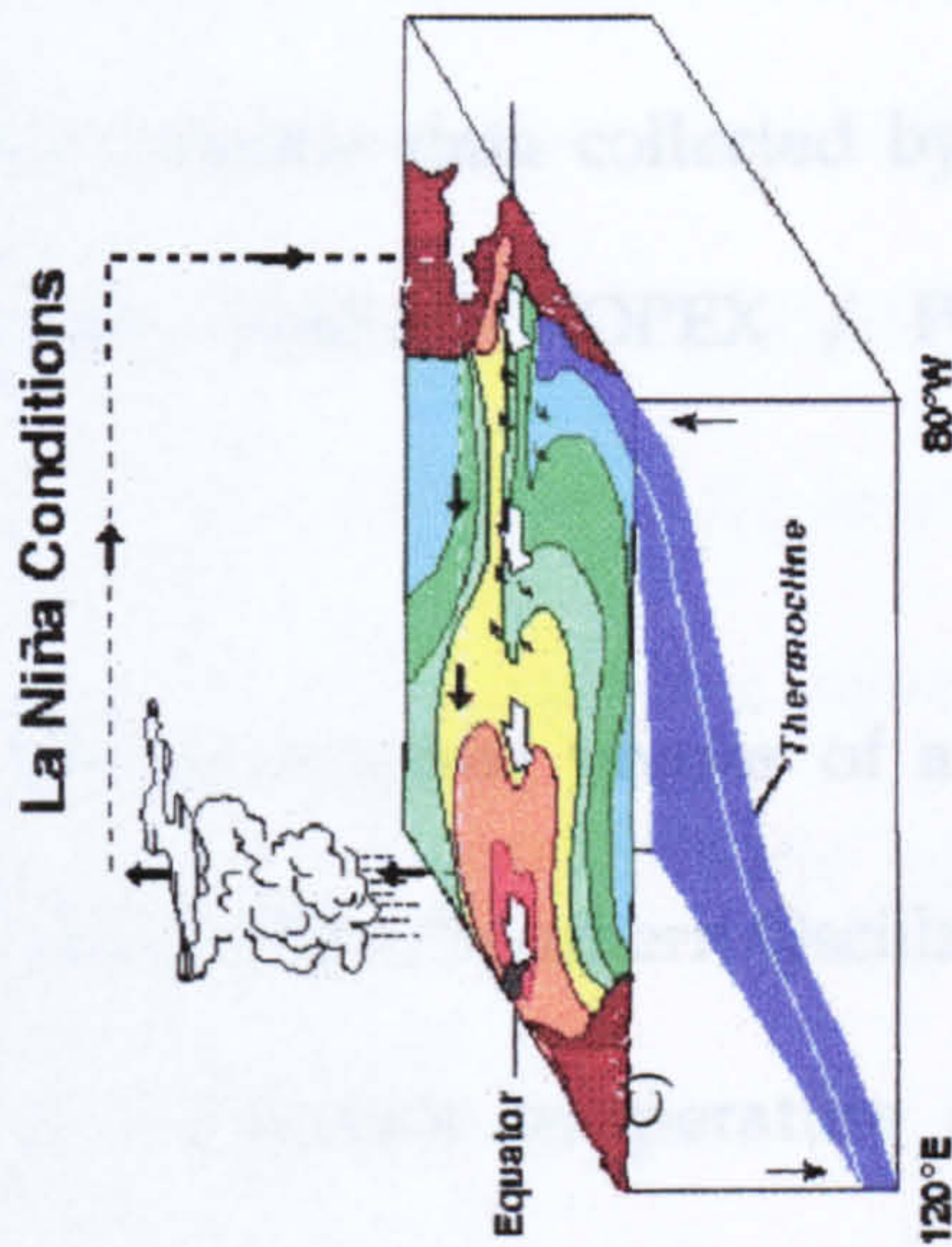


Taken from Nash (2002).

Fig. 2. 4 a,b,c. La Niña Current.



b) The subsurface ocean structure is characterised by a deep layer of warm water in the western tropical Pacific, and by a comparatively shallow layer of warm water in the eastern Pacific. This warm water is separated from the cold, deep ocean waters by the oceanic thermocline, which is normally deepest in the west and slopes upward toward the surface farther east. The resulting east-west variations in mean upper-ocean temperatures result in east-west variations in sea level height, which is higher in the west than in the east.



c) La Niña brings an influx of unusually cool water into the region. To the east of the date line is customarily cool so that La Niña represents an exceptional state of the ocean, an exaggeration of its normal condition.

Sea surface temperatures in the tropical Pacific Ocean are monitored by data buoys and satellites. The National Oceanic & Atmospheric Administration (NOAA) operates a network of 70 data buoys along the equatorial Pacific that provide important data about conditions at the ocean's surface. The data are complemented and calibrated with satellite data collected by NOAA's Polar Orbiting Environmental Satellites, NASA's TOPEX / POSEIDON satellite and others.

The El Niño and La Niña phenomena are extreme phases of a naturally occurring climate cycle referred to as The El Niño/Southern Oscillation. Both terms refer to large-scale changes in sea-surface temperature across the eastern tropical Pacific. Usually, sea-surface readings off South America's west coast range from the 16°C to 21°C, while they exceed 26°C in the "warm pool" located in the central and western Pacific. This warm pool expands to cover the tropics during an El Niño event; whereas during an La Niña phase, defined as cooler than normal sea-surface temperatures in the tropical Pacific ocean that impact global weather patterns, the easterly trade winds strengthen and cold upwellings along the equator and the West coast of South America intensify. Sea-surface temperatures along the equator can fall as much as 3°C below normal.

The system oscillates between warm (El Niño) to neutral or cold (La Niña) conditions. El Niño events occur irregularly at intervals of 2-7 years,

although the average is about once every 3-4 years. They typically last 12-18 months, and are accompanied by swings in the Southern Oscillation (SO), like an interannual “ see – saw ” in tropical sea level pressure between the eastern and western hemispheres. During El Niño, unusually high atmospheric sea level pressures develop in the western tropical Pacific and Indian Ocean regions and unusually low sea level pressures develop in the south-eastern tropical Pacific. SO tendencies for unusually low pressures west of the date line and high pressures east of the date line, have also been linked to periods of anomalously cold equatorial Pacific sea surface temperatures (SSTs) sometimes referred to as La Niña.

According to the National Centers for Environmental Prediction, NOAA, this century's previous La Niñas began in 1903, 1906, 1909, 1916, 1924, 1928, 1938, 1950, 1954, 1964, 1970, 1973, 1975, 1988 and 1995-1996. These events continued typically into the following spring. Since 1975, La Niñas have been only half as frequent as El Niños.

In Ecuador, the El Niño current brings warmer surface water (29 - 30 °C) to the eastern boundary of the Pacific Ocean (Cucalón, 1984). According to García, (1983), Ecuadorian coastal waters are influenced by tropical surface waters ($T > 23\text{ }^{\circ}\text{C}$, $S < 33.5\text{ }^{\circ}/_{\text{oo}}$) from the North and subtropical surface waters ($T < 21\text{ }^{\circ}\text{C}$, $S > 34.5\text{ }^{\circ}/_{\text{oo}}$) from the South, converging in a transitional front known as the Equatorial Front, whose circulation depends on the

seasonal wind regime. In this respect, Cucalón (1989) indicates that during the summer the front is weakly formed, or displaced to Perú, and in its place, a narrow southward coastal flow of warm (25 - 27 °C), low salinity (33 - 33.8 ‰) water from the Panama Bight persists along the Ecuadorian coast. In contrast, Cucalón notes that the front is clearly evident in Ecuadorian waters during winter, when the south-east winds are strong, bringing the cold upwelled saline waters from the " Humbolt " or " Peruvian current " toward the Ecuadorian coast.

La Niña events have occurred more intensively in the tropical Pacific after January 1996 (CDB 1993 - 2002), (Figs.2.5 and 2.6). Cooler than normal sea surface temperature (SST) – 1.5 °C anomaly, were recorded on the coast of Ecuador (Niño region 1+2), which also affected the Chone River estuary. From August to December 1996, cooler SST's were recorded in the Chone River estuary, coinciding with a cool episode for the eastern equatorial Pacific (CDB, 1995-1996), (Fig.2.7). During the years 1999 - 2000, this phenomenon was repeated and the anomaly temperature was on average – 1.3 °C (CDB, 1993 - 2002), (Figs.2.6 and 2.8).

In contrast, an El Niño event occurred during most of 1997 and the first half of 1998, (Figs.2.9 and 2.10). More than normal warm oceanographic conditions, +3.0 °C anomaly (CDB, 1997–1998) and very high rainfall increased the Chone River outflow and contributed to a decrease in salinity

in the inner part of the estuary, thereby affecting the shrimp farms located in this area.

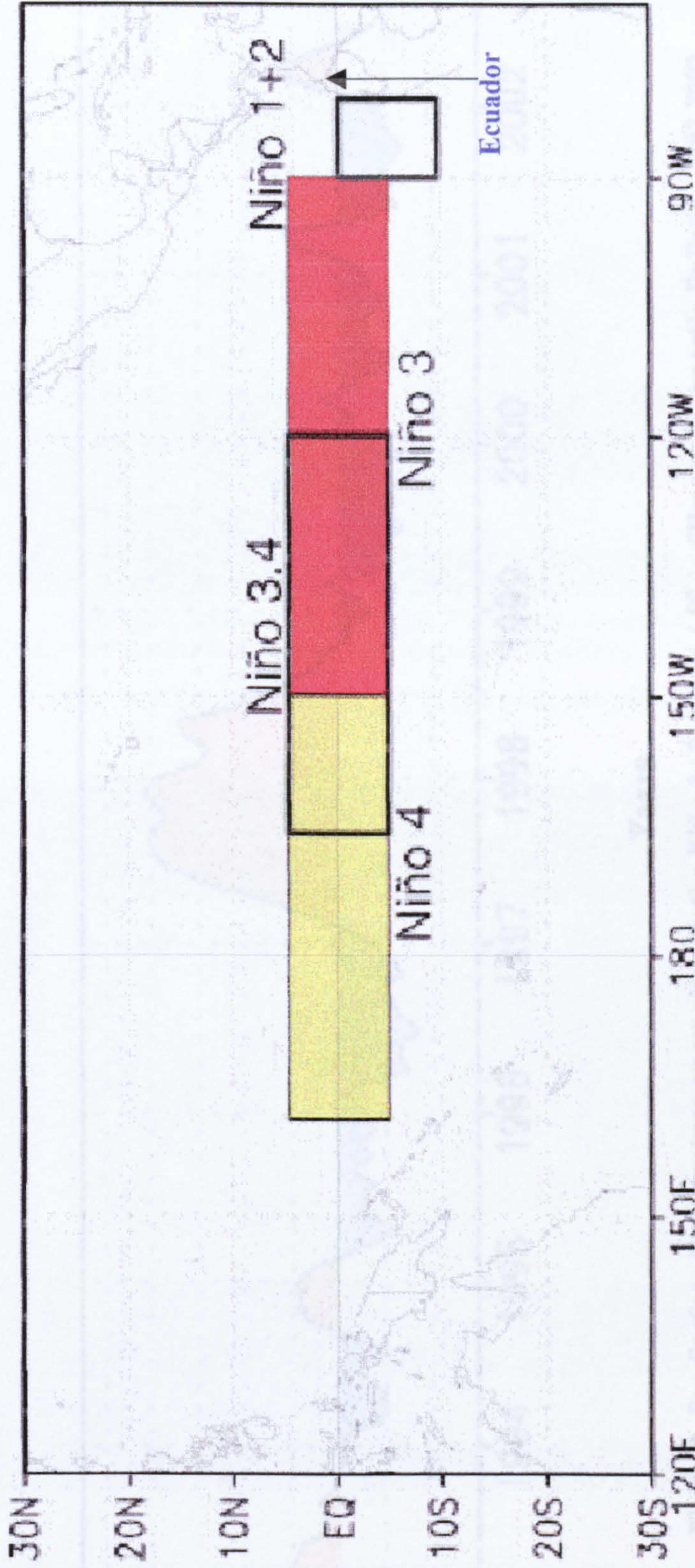


Fig.2. 5 Graphical depiction of the four Niño regions of the Pacific Ocean.

Niño 1+2 = From 80 W – 90 W ; Niño 3 = From 90 W – 150 W ; Niño 3.4= From 120 W – 165 W ; Niño 4= From 150 W – 160 E

Taken from: http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/nino_regions.html

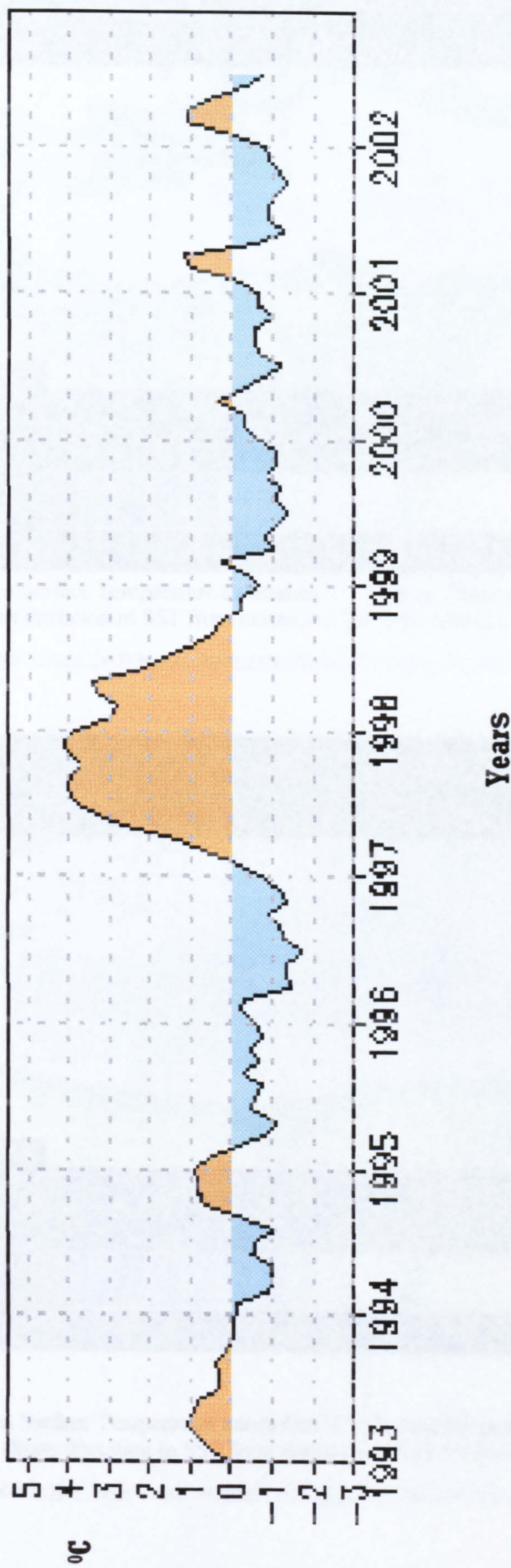


Fig.2. 6. Sea Surface Temperature anomalies in the Niño 1+2 region (°C). Climate Diagnostic Bulletin. 1993-2002

Anomaly = The deviation of a Sea Surface Temperature in the region over a thirty year mean long-term average.

Source:http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/quick_look/sst12.gif

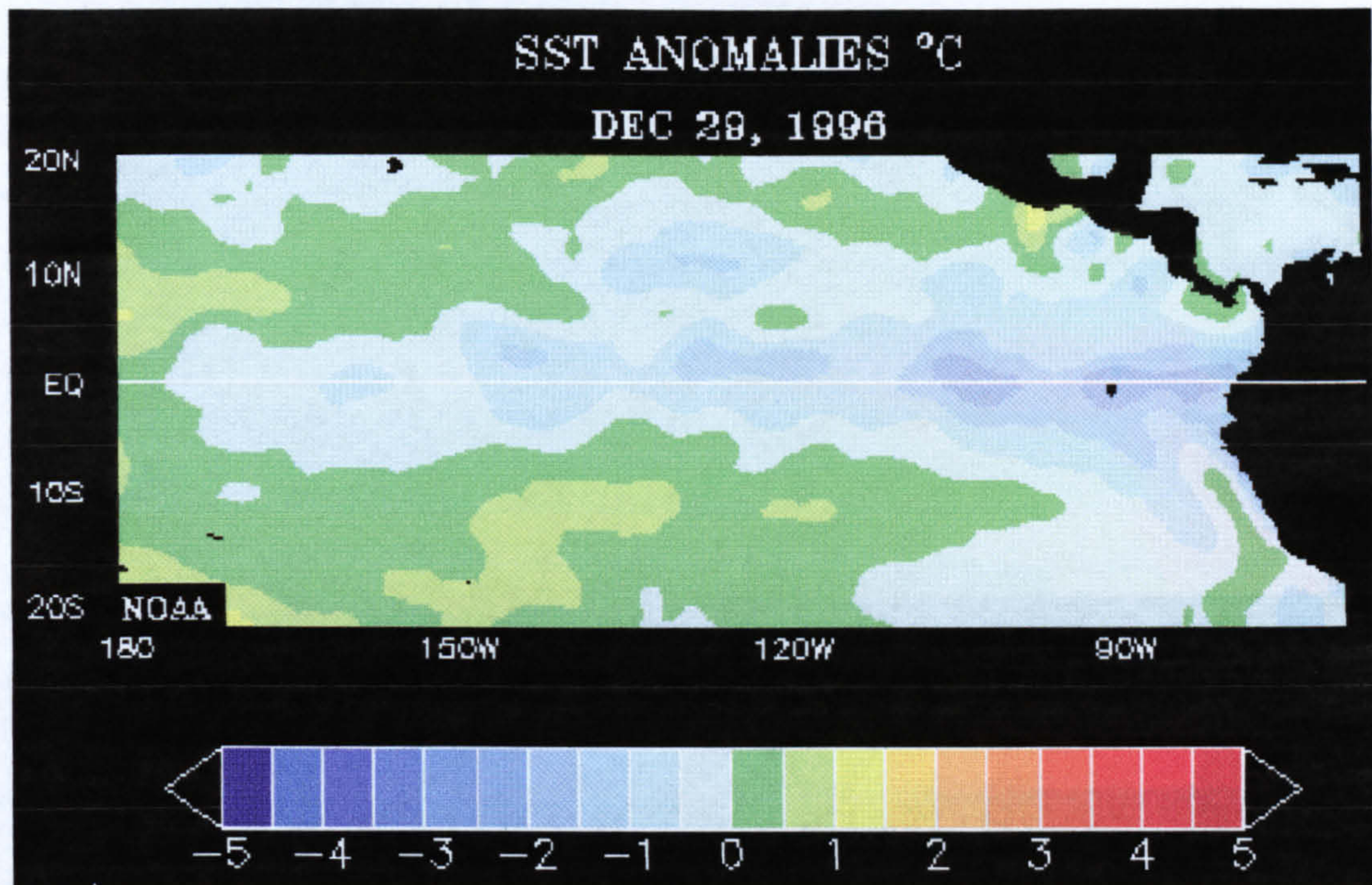


Fig.2. 7. Sea Surface Temperature anomalies $^{\circ}\text{C}$. Climate Diagnostic Bulletin 1995-1996. Colour scale shows deviation in SST from normal on 29/12/96 during a Transition Niña – Niño event.

Source: http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9596_anim.shtml

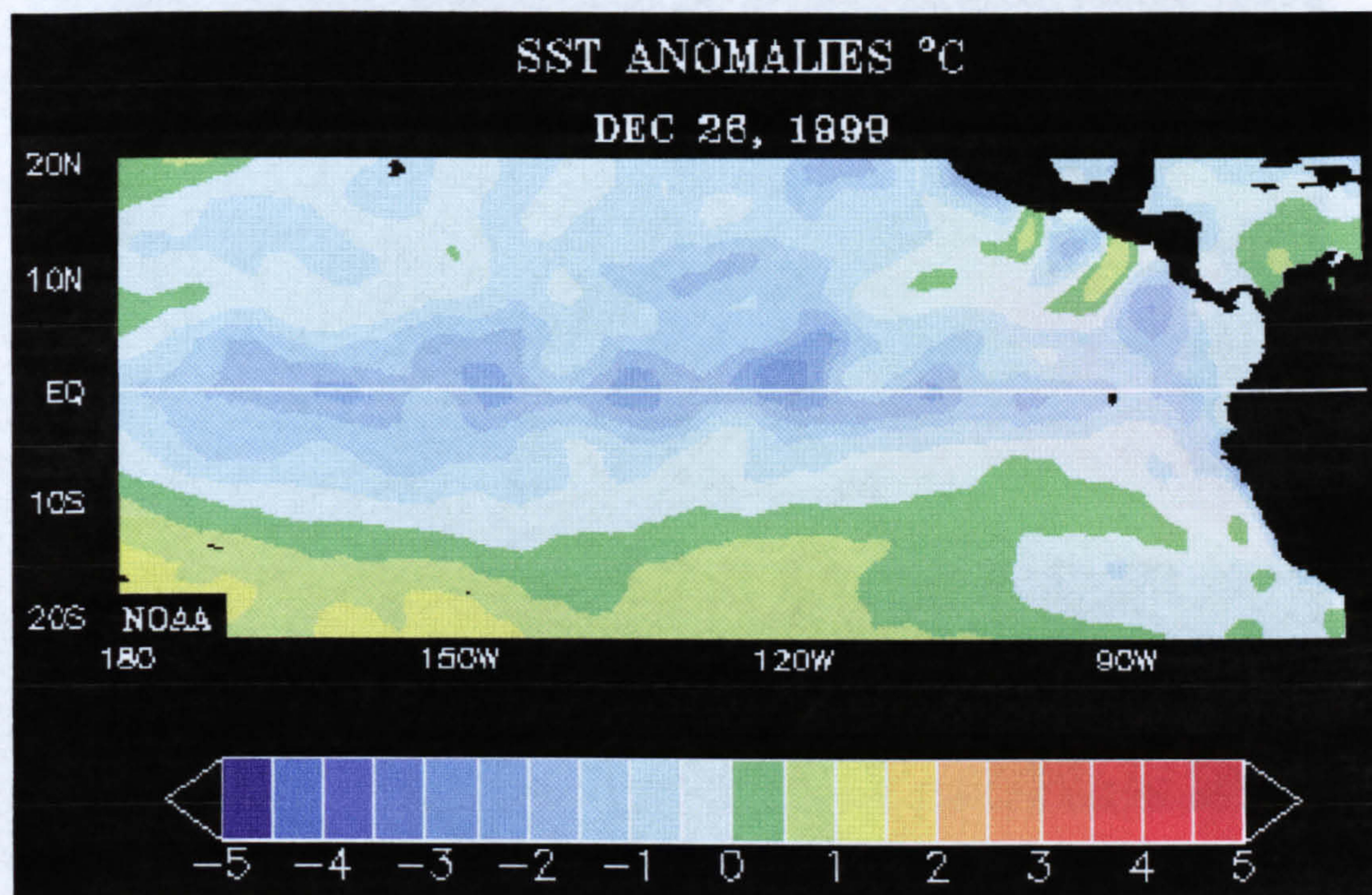


Fig.2. 8. Sea Surface Temperature anomalies $^{\circ}\text{C}$. Climate Diagnostic Bulletin 1999. Colour scale shows deviation in SST from normal on 26/12/99 during a La Niña event.

Source: http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9899_anim.shtml

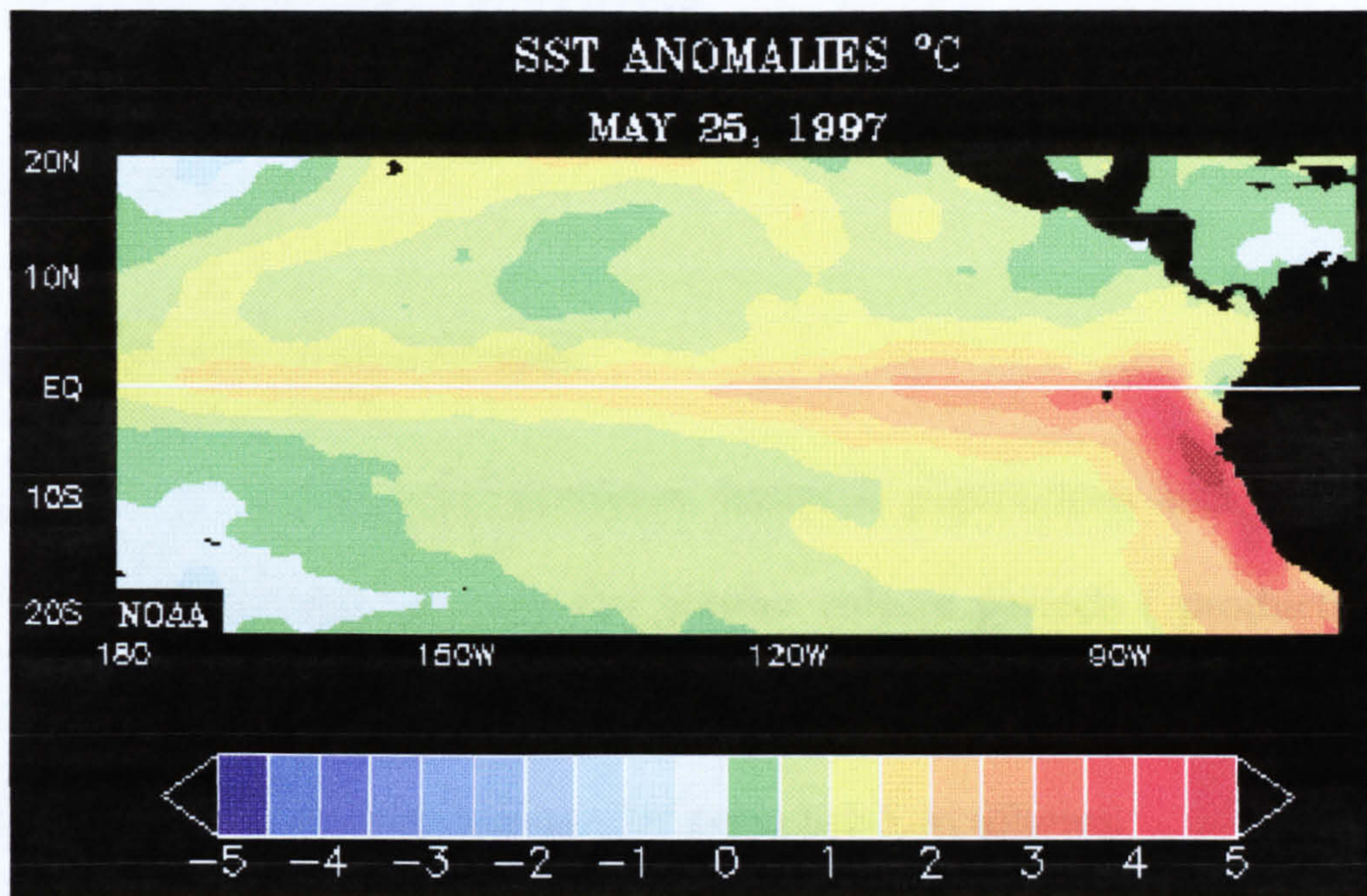


Fig.2. 9 Sea Surface Temperature anomalies $^{\circ}\text{C}$. Climate Diagnostic Bulletin 1997. Colour scale shows deviation in SST from normal on 25/05/97 during an El Niño event.

Source:http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9798_animated.gif

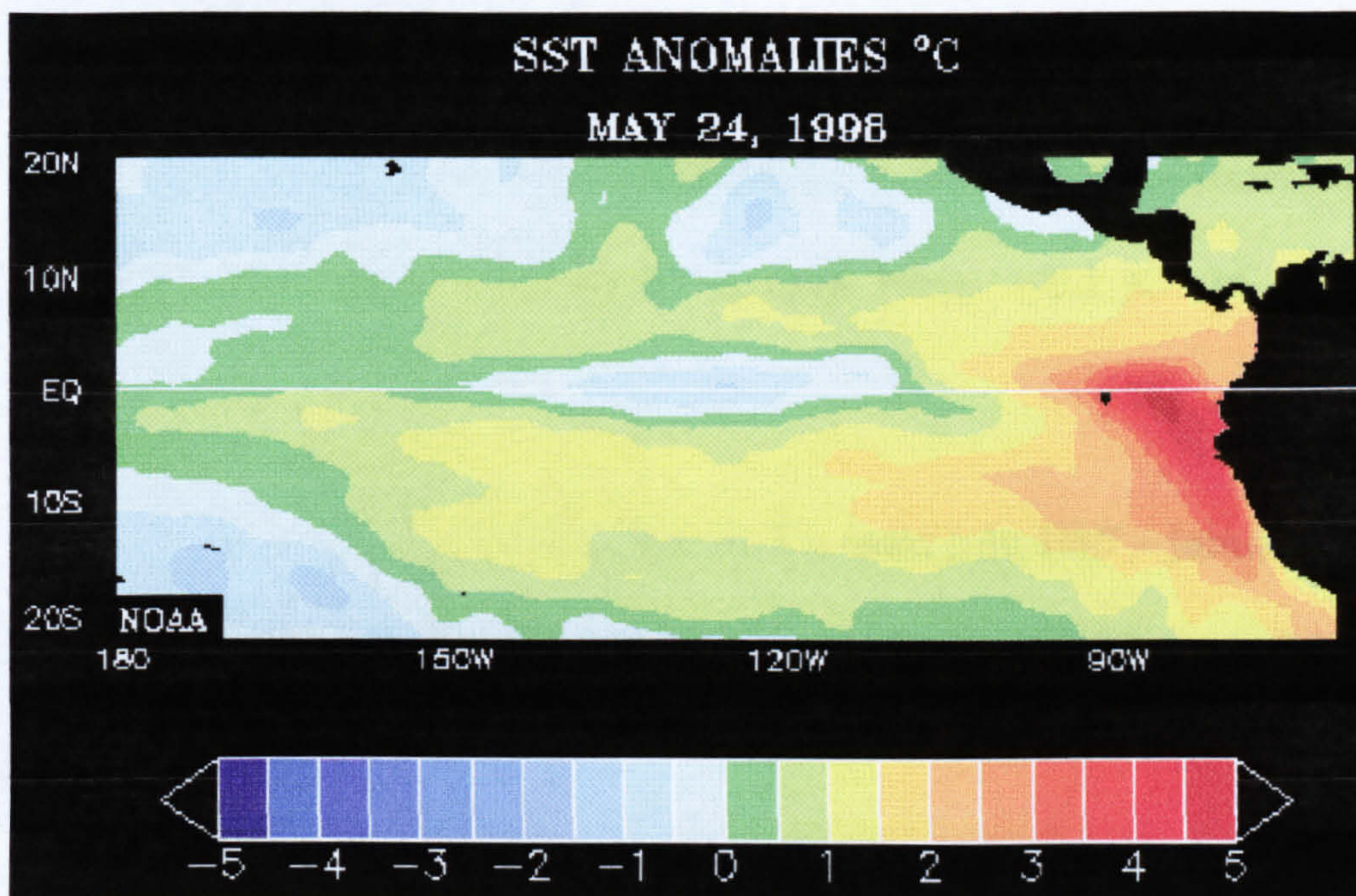


Fig.2. 10 Sea Surface Temperature anomalies $^{\circ}\text{C}$. Climate Diagnostic Bulletin 1998. Colour scale shows deviation in SST from normal on 24/05/98 during an El Niño event.

Source:http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9798_animated.gif

3. MATERIALS AND METHODS

3.1 Study Design

3.1.1 Selection of shrimp ponds

Data on water quality, phytoplankton, bacterial populations and shrimp production were collected from five shrimp culture periods (production cycles), during prevailing La Niña and El Niño events and the intervening transition periods. These are designated periods 1-5, as follows:

First period of shrimp culture (Period 1): from November 1996 – March 1997, transition La Niña- El Niño, where temperature anomalies were from - 1.5 °C to +1 °C (Fig.3.1).

Second period of shrimp culture (Period 2): from February – June 1997, El Niño, where temperatures anomalies ranged from - 0.5 °C to + 3 °C (Fig.3.2).

Third period of shrimp culture (Period 3): from July 1997 – January 1998, El Niño, where temperatures anomalies ranged from + 3 °C to + 3.5 °C , (Fig.3.3).

Fourth period of shrimp culture (Period 4): from September 1998 – February 1999, transition El Niño- La Niña. Temperatures anomalies ranged from + 0.5 °C to - 0.5 °C in this period (Fig.3.4).

Fifth period of shrimp culture (Period 5): from March 1999 – June 1999, La Niña. Temperature anomalies were from 0 °C to – 1.5 °C (Fig.3.5).

Temperature data were recorded in the selected shrimp ponds during the five shrimp culture periods. The measurements were taken from each pond *in situ* (see section 3.3.1).

For the first, second and third periods of shrimp culture (November 1996 to January 1998), sampling data were obtained from six shrimp ponds.

From September 1998 to June 1999, representing the fourth and fifth periods of shrimp culture, the data were collected only from five shrimp ponds.

General information on the temperature anomalies for Niño region 1+2, during the five shrimp culture periods, were taken from the NOAA, Climate Prediction Center (Maryland, USA), which provides monthly atmospheric and sea surface temperatures indices.

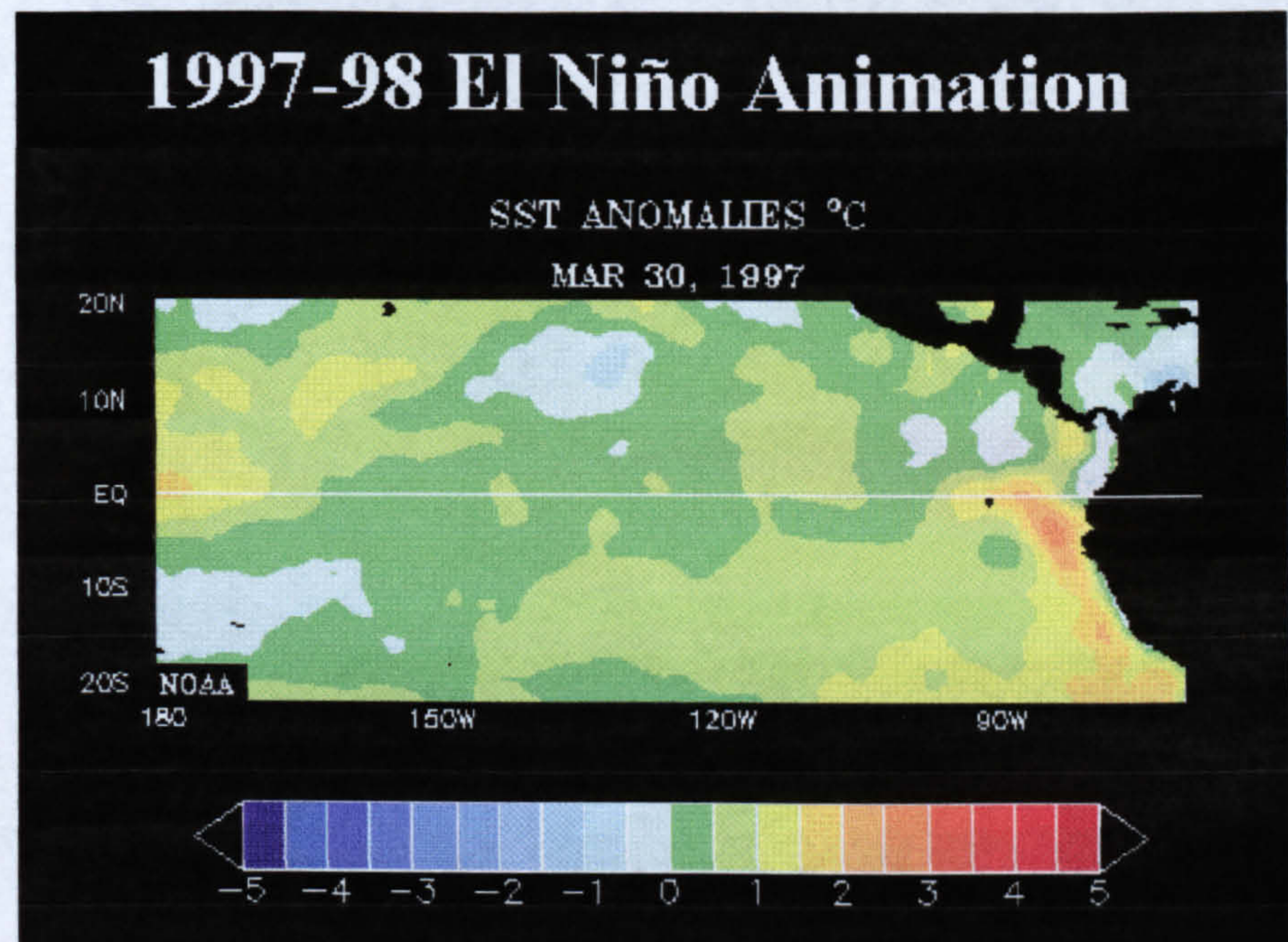
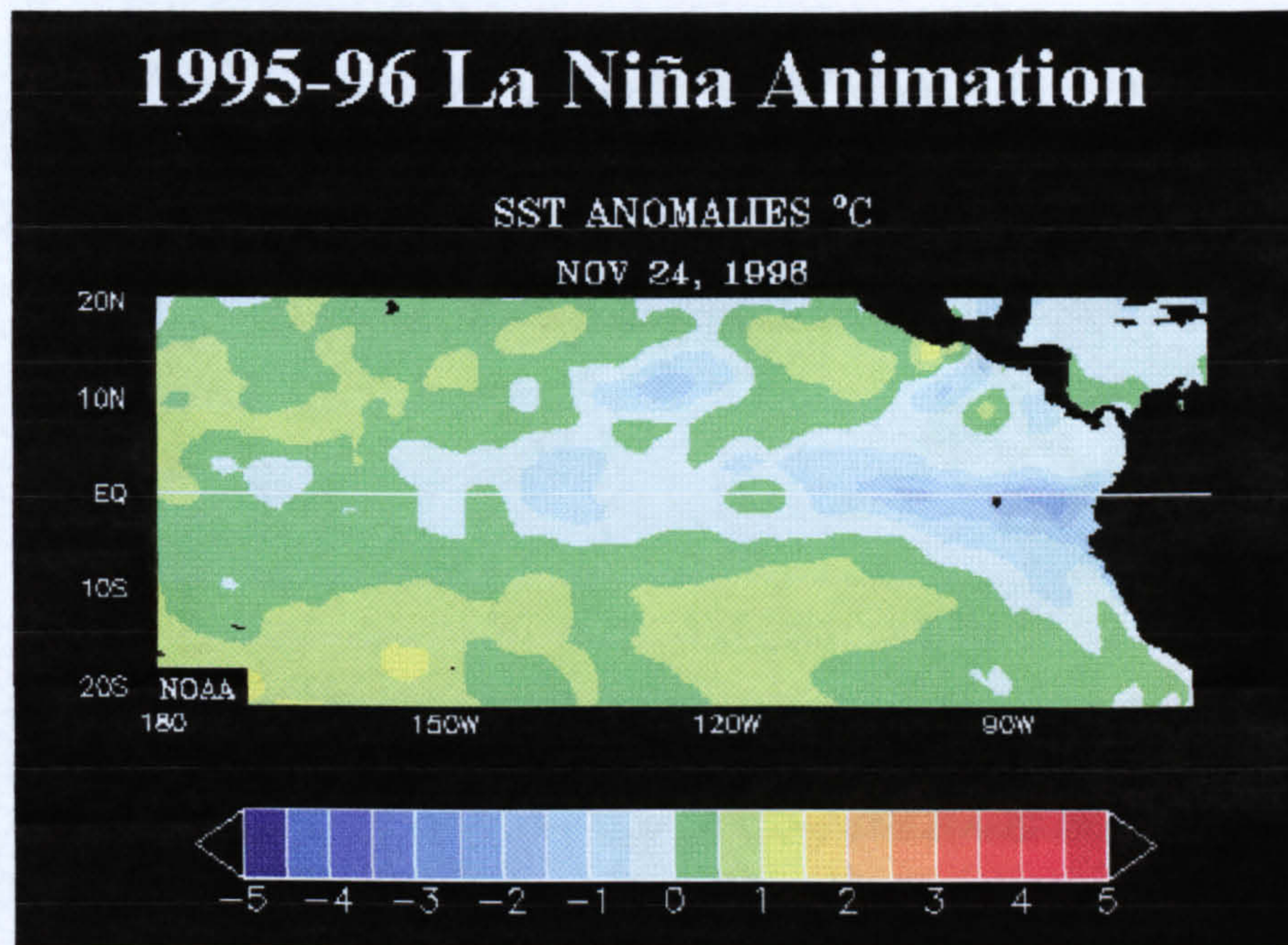


Fig.3.1. Sea Surface Temperature anomalies °C. Climate Diagnostic Bulletin. Colour scale shows deviation in SST from normal during shrimp culture Period 1:

November 1996 - March 1997.

Source: http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9798_animated.gif

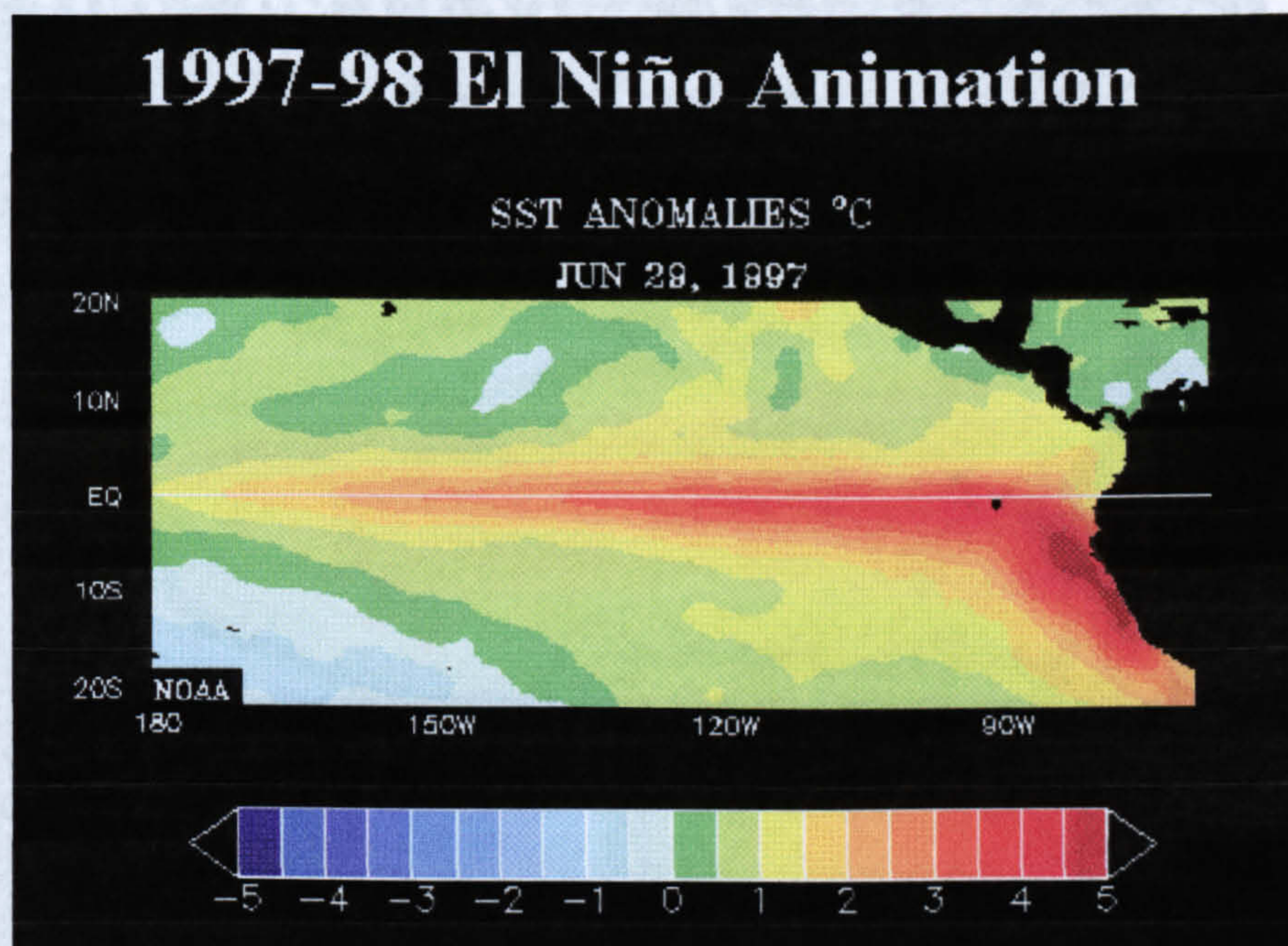
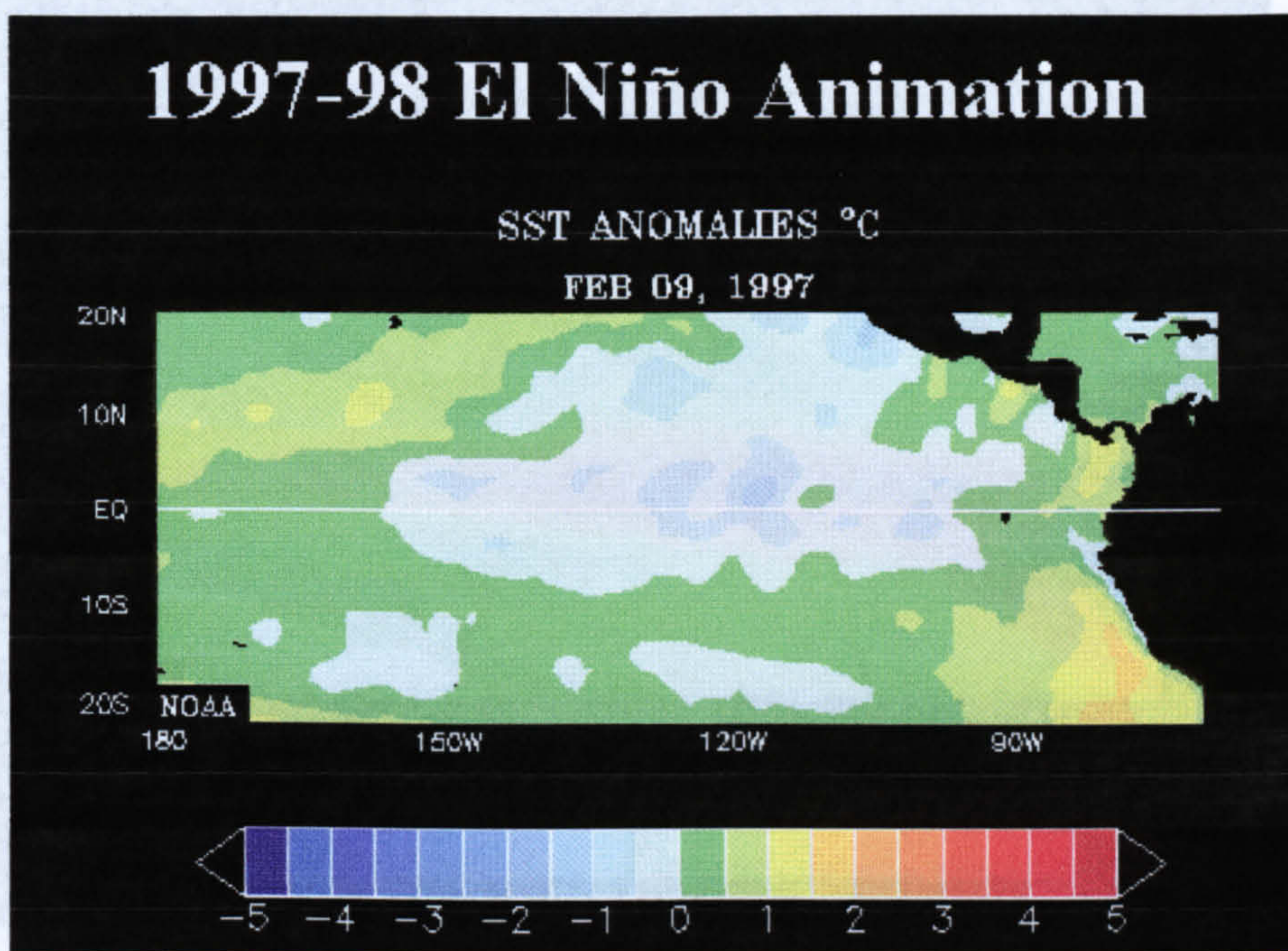


Fig.3. 2 Sea Surface Temperature anomalies °C. Climate Diagnostic Bulletin. Colour scale shows deviation in SST from normal during shrimp period Period 2:

February - June 1997.

Source:http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9798_animated.gif

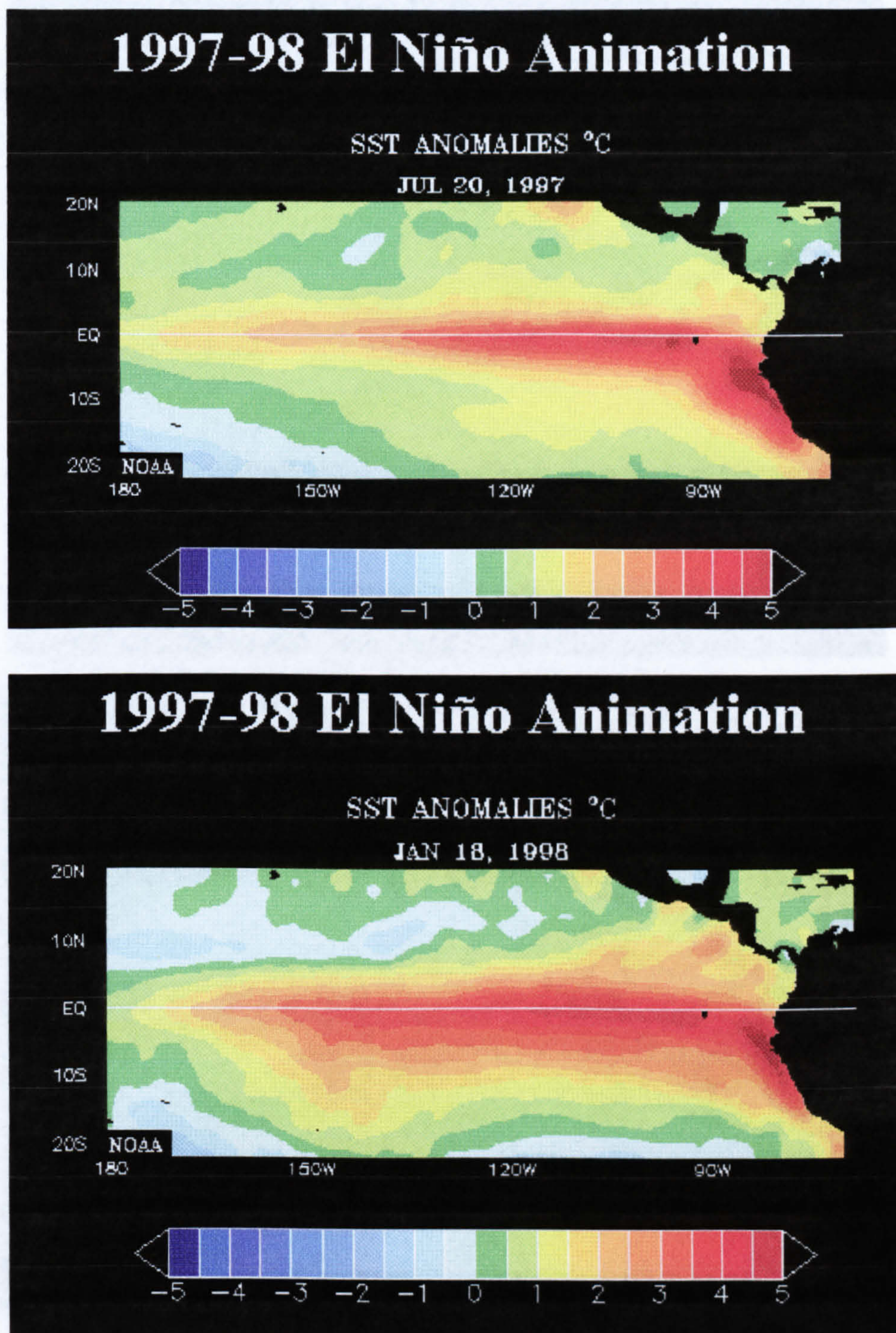


Fig.3.3 Sea Surface Temperature anomalies °C. Climate Diagnostic Bulletin. Colour scale shows deviation in SST from normal during shrimp period Period 3:

July 1997- January 1998.

Source:http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9798_animated.gif

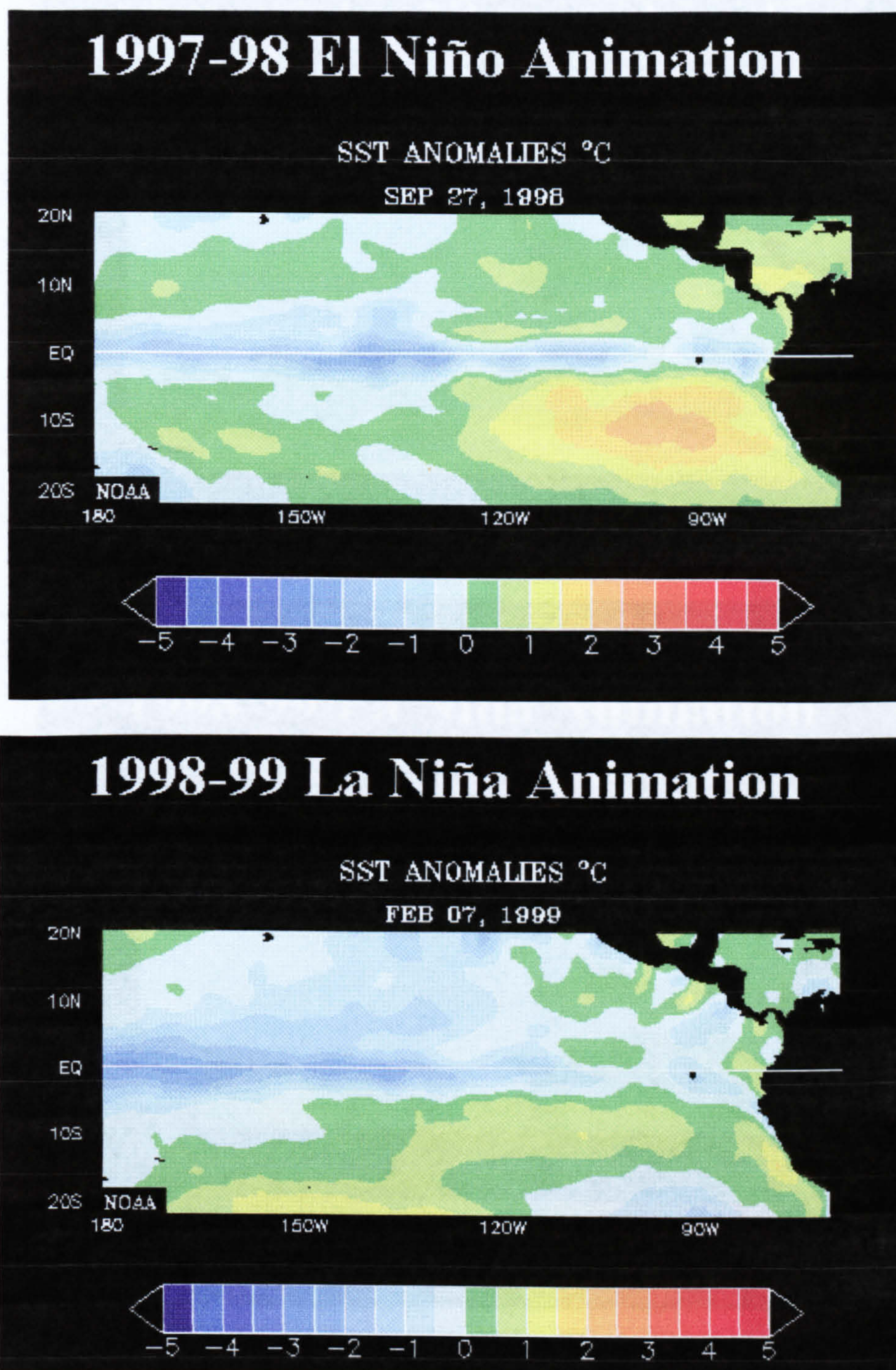


Fig.3.4 Sea Surface Temperature anomalies °C. Climate Diagnostic Bulletin. Colour scale shows deviation in SST from normal during shrimp culture Period 4:

September 1998 – February 1999.

Source: http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9899_anim.shtml

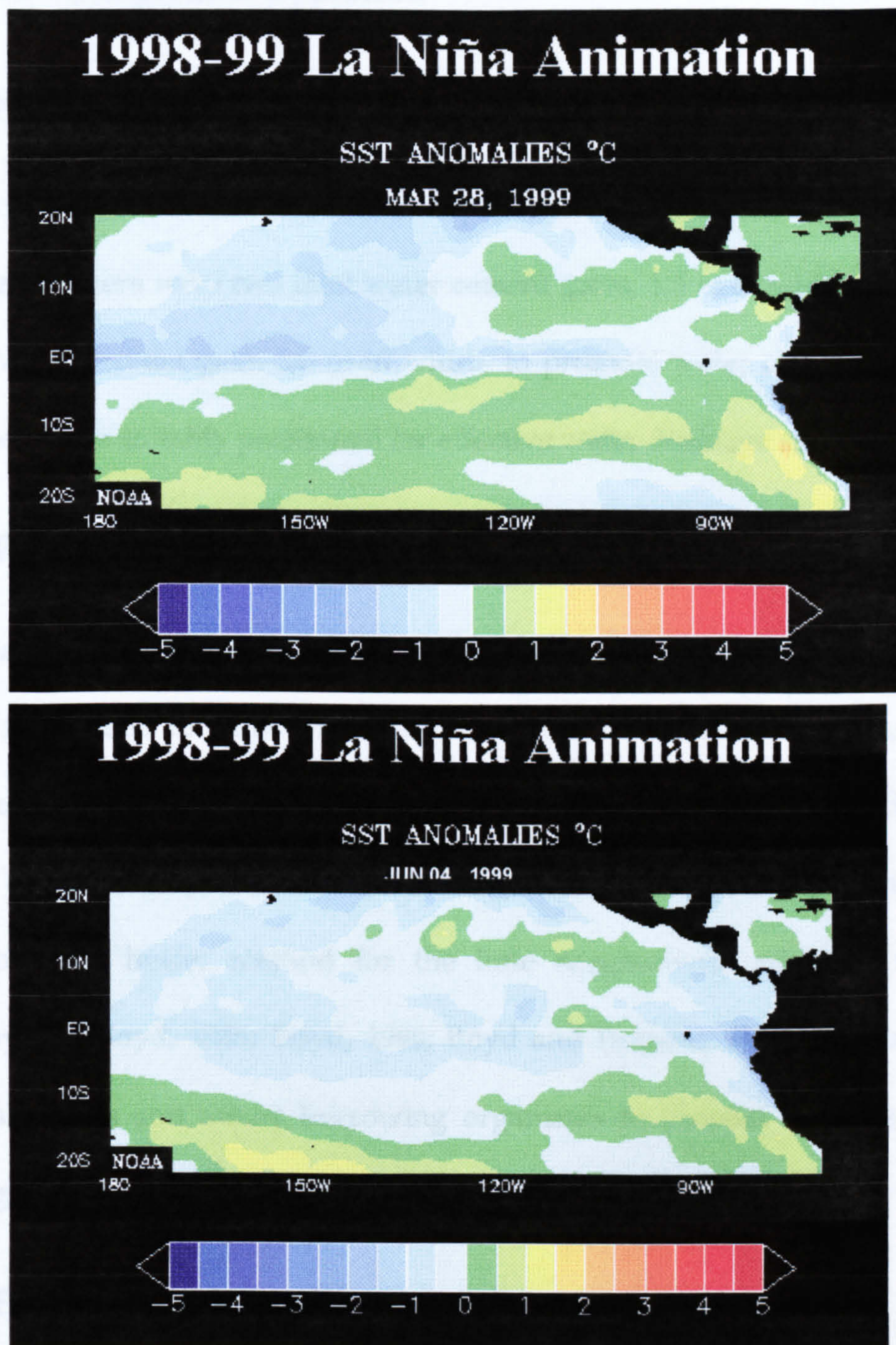


Fig.3.5 Sea Surface Temperature anomalies °C. Climate Diagnostic Bulletin. Colour scale shows deviation in SST from normal during shrimp period Period 5:

March 1999 - June 1999.

Source: http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9899_anim.shtml

3.2 Pond design and Operation

3.2.1 Pond design

The ponds were 0.9 – 1.2 m in water depth with a surface area of 6.0 ha (150 x 400 m). There were two inlet water cement gates, 1.5 m in width, and one outlet water cement gate, 2.0 m in width, to promote water circulation. The channels were suitably positioned for effective water drainage and flushing.

3.2.2 Pond bottom treatment

Between crops, the pond bottoms were dried and the upper 10 cm of substratum tilled to allow for further oxidation of reduced substances and mineralization of organic matter by bacteria. When the soil pH was below 7.0, agriculture limestone was added to increase pH, following the p-nitrophenol buffer method for the lime requirements of pond muds, (Pillay and Boyd, 1985; Boyd, 1990; Boyd and Daniels, 1993), and also to kill fish, crabs and others burrowing organisms to prevent water leakage through holes.

3.2.3 Pond water preparation

Seven days before the beginning of the culture cycle, the ponds were filled with water and fertilized with Nutrilake 20TM (NO₃ 21%, Na 20%, B 0.035%, Mg 0.15%, K 0.37%) at a rate of 13 kg/ha and superphosphate at 20 kg/ha. During the culture period, additional fertilization was applied at a rate of 7 kg/ha of Nutrilake 20TM and 2 kg/ha of superphosphate twice a week when

the water transparency was higher than 40 cm (as determined by Secchi disk).

3.2.4 Shrimp stocking

Each pond was stocked with white shrimp (*Litopennaeus vannamei*) postlarvae (reared from wild naupli) at a density of 10 PL /m², fifteen days old. The postlarvae were hatched in the facilities of LABGAR at Bahía de Caráquez, a commercial shrimp hatchery, and transported to the shrimp farm.

3.2.5 Pond management

After 15 days of culture, a 4 – 5% daily water exchange was made. Ponds were not aerated during the shrimp culture cycles. Shrimp were fed twice a day (at 07:00 h and 16:00 h) with a formulated shrimp diet (protein content 26 % by weight) using 6 feed trays/ha. Feeding began during week 3 at a rate of 10 % of shrimp biomass per day. In weeks 4 and 5, the feeding rate was reduced to 5%, and then further reduced to 3% for the rest of the culture period.

For growth estimation, ten random samples were taken from each pond with a knotted monofilament cast net (1.8 m radius) every week and one hundred shrimp were weighed individually to the nearest 0.1 g in a CASBEE™ balance (Model MW120), and their mean weight calculated.

3.2.6 Shrimp harvesting

At harvest, ponds were drained and the shrimp captured in a net bag attached to the outside of the outlet gate as water drained from the pond on the outgoing tide. The entire crop was weighed *en masse* and subsamples of individual shrimp were weighed to determine their mean final weight, instantaneous growth rate, feed conversion ratio, yield and survival.

Instantaneous growth rate (IGR) was calculated for each pond from the following equation (Cushing, 1968) :

$$\text{IGR} = [100 \times \ln (\text{Final weight} / \text{Initial weight})] / \text{Days of culture.}$$

Feed conversion ratio (FCR), a measure of the weight of shrimp produced per kg of dry food supplied, was calculated as:

$$\text{FCR} = \text{Total dry feed consumed} / \text{Total wet weight gained.}$$

The FCR varies depending on the stocking density, quality of the feed and the size at which the shrimp are harvested, but ideally it should not be higher than 2.0

Yield was calculated as the total biomass of shrimp harvested (kilograms) per hectare of pond water surface.

Survival percent was calculated as:

$$\text{Survival (\%)} = \frac{\text{Mean number of stocked shrimp} \times 100}{\text{Mean number of shrimp harvested (MNSH)}}$$

where MNSH was calculated as total biomass shrimp / mean final shrimp weight .

3.3 Water Sampling and Analysis

3.3.1 Water sampling

Water sampling and measurements were conducted every week between 08:00 and 10:00 hours. Temperature and salinity was measured *in situ* at a depth of 20 cm below the water surface using a mercury thermometer and a refractometer respectively.

Four, 1 litre subsamples at a depth of 30 cm below the water surface from near the inlet gate, the middle of the pond and near the outlet gate, were taken from each pond. A 3 litre pooled water sample was transported in polypropylene bottles on ice to the laboratory and analysed one hour after the collection time.

3.3.2 Water analysis

Laboratory determinations of pH were made with a standardised WTW pH meter; total ammonia ($\text{NH}_3 + \text{NH}_4^+$) by the phenol-hypochlorite method (Strickland and Parsons, 1972; Golterman *et al*, 1978); nitrate (NO_3) by the

cadmium reduction method (Grasshoff, 1964 ; Wood, Armstrong and Richards, 1967, Strickland and Parsons, 1972); nitrite (NO_2) with sulphanilamide (Shinn, 1941 ; Strickland and Parsons, 1972 ; Mackereth *et al.* 1978); dissolved reactive phosphorus (DRP) by molybdate reaction in an acid medium (Strickland and Parsons, 1972); silica (SiO_2) using a silicomolibic complex (Strickland and Parsons, 1972; Koroleff and Grasso, 1983); total suspended solid (TSS) by the photometric method (Hach, 1992: method 8006) after filtration through a standard GF/F glass fibre filter; and total sulphide ($\text{H}_2\text{S}+\text{HS}+\text{S}^{2-}$) using the methylene blue method (Mackereth *et al.*, 1978 ; Golterman *et al.*, 1978 ; Frevert, 1980; Fonselius, 1983; Parson *et al.*, 1984).

The data resulting from the analysis of total ammonia were corrected for to convert un-ionised ammonia which varied according to the pond's water pH, temperature and salinity (Trussell, 1972; Bower and Bidwell, 1978). The data obtained for total sulphide were converted similarly to un-ionized sulphide according to the pond's water pH and temperature (Boyd, 1990).

3.4 Phytoplankton analysis

Four 1 litre subsamples from the inlet gate, the middle of the pond and the outlet gate were taken weekly from each pond using a sterile plastic bottle, 20 cm immersed below the water surface. A final 1 litre pooled sample was fixed with Lugol (Uthermohl, 1958) and the organisms counted in a

sedimentation chamber. Table 3.1 lists the taxonomic references used to identify each phytoplankton group.

Table 3. 1 A lists of the taxonomic references used to identify each phytoplankton group.

Plankton group	Area covered	Author(s)
Algae	Perú.	Aldave, 1989
Diatoms	Argentina.	Bastida and Stupak, 1979
Algae	Worldwide	Bourelly, 1970
Cyanophita	Worldwide	Desikachary, 1959
Diatoms	Australian & New Zealand	Ferguson, 1961
Phytoplankton, general	Guayas River, Ecuador	Guzman, 1993.
Phytoplankton, general	Ecuador	Jimenez and Pesantes, 1978
Diatoms	Guayas River, Ecuador	Jimenez, 1983
Diatoms	Chone River, Ecuador	Maridueña, 1991
Phytoplankton, general	Ecuador and Gulf Panama.	Marshall, 1970
Phytoplankton, general	Chile	Parra <i>et al.</i> , 1982
Dinoflagelates	Ecuador	Pesantes, 1983 a, b
Algae	Worldwide	Prescot, 1968
Phytoplankton general	Chile	Rivera <i>et al.</i> , 1973
Microalgae	Venezuela	Varela <i>et al.</i> , 1983, 1985
Diatoms	Worldwide	Weber, 1971

3.5 Bacteriological analysis

Every 20 days during each period of the shrimp culture study, five subsamples of 30 shrimp in their intermoult stage from each pond were removed, pooled and a final sample of 30 shrimp was weighed. To reduce the risk of external contamination while removing their intestines, the shrimp were washed and sterilized with 0.002% (w/v) iodine. The intestines of the 30 shrimp were removed, pooled, weighed and one gram of the intestinal material homogenized aseptically. A series of ten-fold dilutions of pooled intestines sample was made using sterile saline (0.5% NaCl solution, w/v for samples taken between 4 – 20 psu and 2.0% NaCl for samples > 20 psu) as dilutions blanks, and 0.1 ml from each dilution was plated in triplicate on agar by the spread method.

The commercial brands of “marine agar” developed from the work of Oppenheimer and Zobell (1952) are now considered too high in nutrients despite their continued widespread use for the isolation of bacteria from estuarine invertebrates (West, 1988; Austin, 1988, 1989, 1993). As an alternative a variety of low-nutrient media, mostly of which are not currently available as commercial preparation, have been developed for use in estuarine and coastal marine environments (Goulder, 1976 ; Väättänen, 1977 ; Weiner *et al.*, 1980; Austin, 1993).

Total heterotrophic aerobic bacterial counts were made using the estuarine agar preparation devised by Weiner *et al.* (1980). The medium was prepared by the addition of 112.22 g marine broth (Difco®) and 15 g bacteriological agar to 1000 ml of filtered water collected at the site of sample collection . The mixture was boiled for 1 min then autoclaved for 15 min at 121°C.

Viable counts of *Vibrio* were enumerated using thiosulfate-citrate-bile salt sucrose (TCBS), (Difco®). For the enumeration of total bacteria and presumptive *Vibrio*, the inoculated plates were incubated at 27 °C in the dark for 4 days and 48 h, respectively. The colonies were then counted and recorded as colony-forming units per gram (CFU/g).

To analyze the composition of the heterotrophic aerobic bacterial population from each sample, 30 bacterial isolates were selected from Weiner agar containing 30 – 200 colonies. A grid with 30 cross lines was placed at the bottom of the Petri dish, and the colonies located on the crossing, or on the one nearest to it in two random quadrants, were selected for identification. The isolates were then purified and stored on plates on Weiner agar at room temperature in the dark.

All the isolates were then identified using the Biolog MicroPlates® system (BIOLOG, Hayward, CA, USA). In the Biolog® test, bacteria are precultured on a Biolog Universal Growth® agar plate, (Fig.3.6).

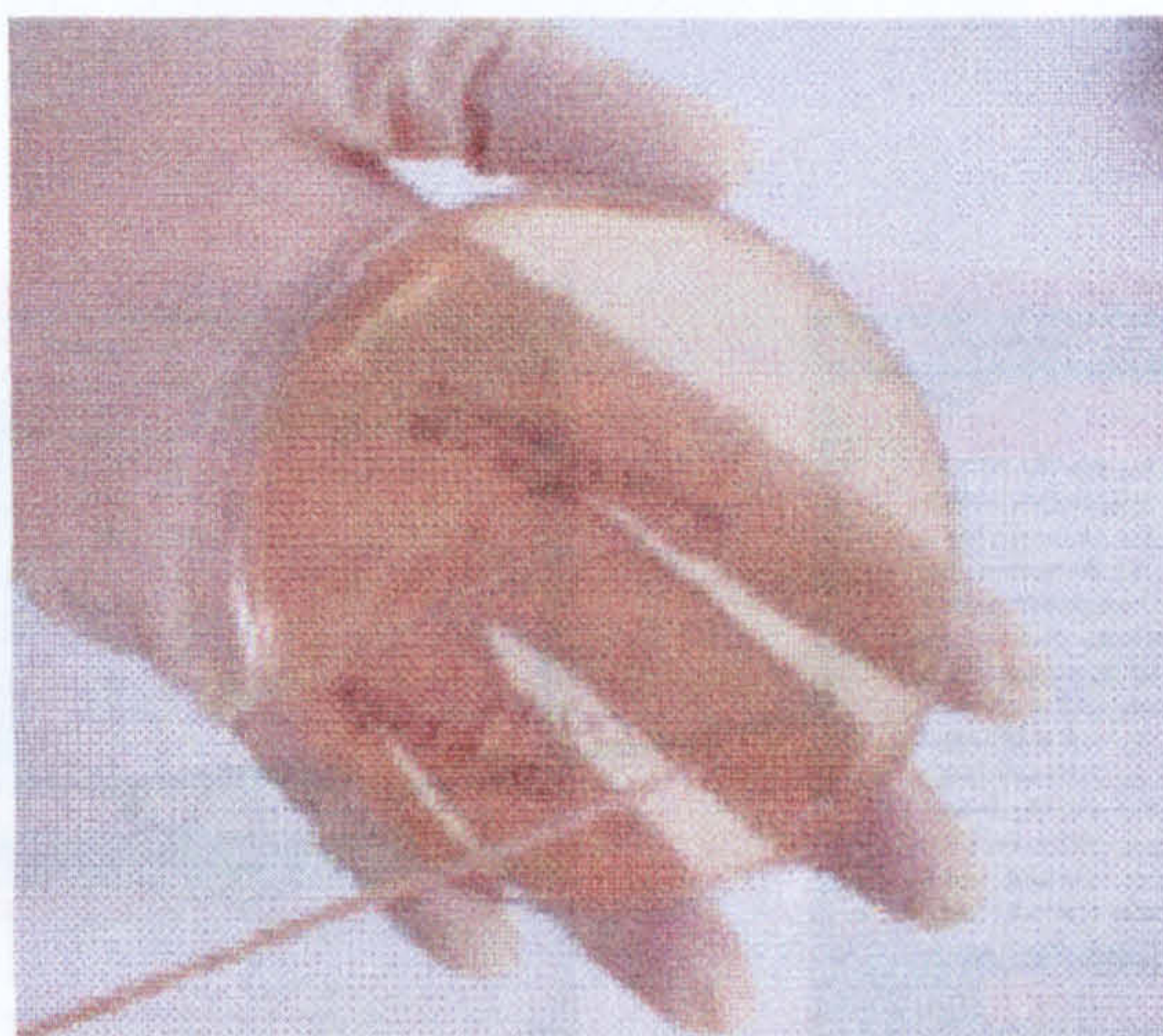


Fig.3.6 Selected bacteria isolated from Weigner agar are precultured in Biolog Universal Growth agar.

The bacteria were swabbed from the surface of the agar plate and suspended to a specified density in inoculating fluid.

One hundred and fifty μl of the bacterial suspension was then pipetted into 96 wells of the MicroPlate and incubated at 30°C for 4 - 24 hours (Fig.3.7).

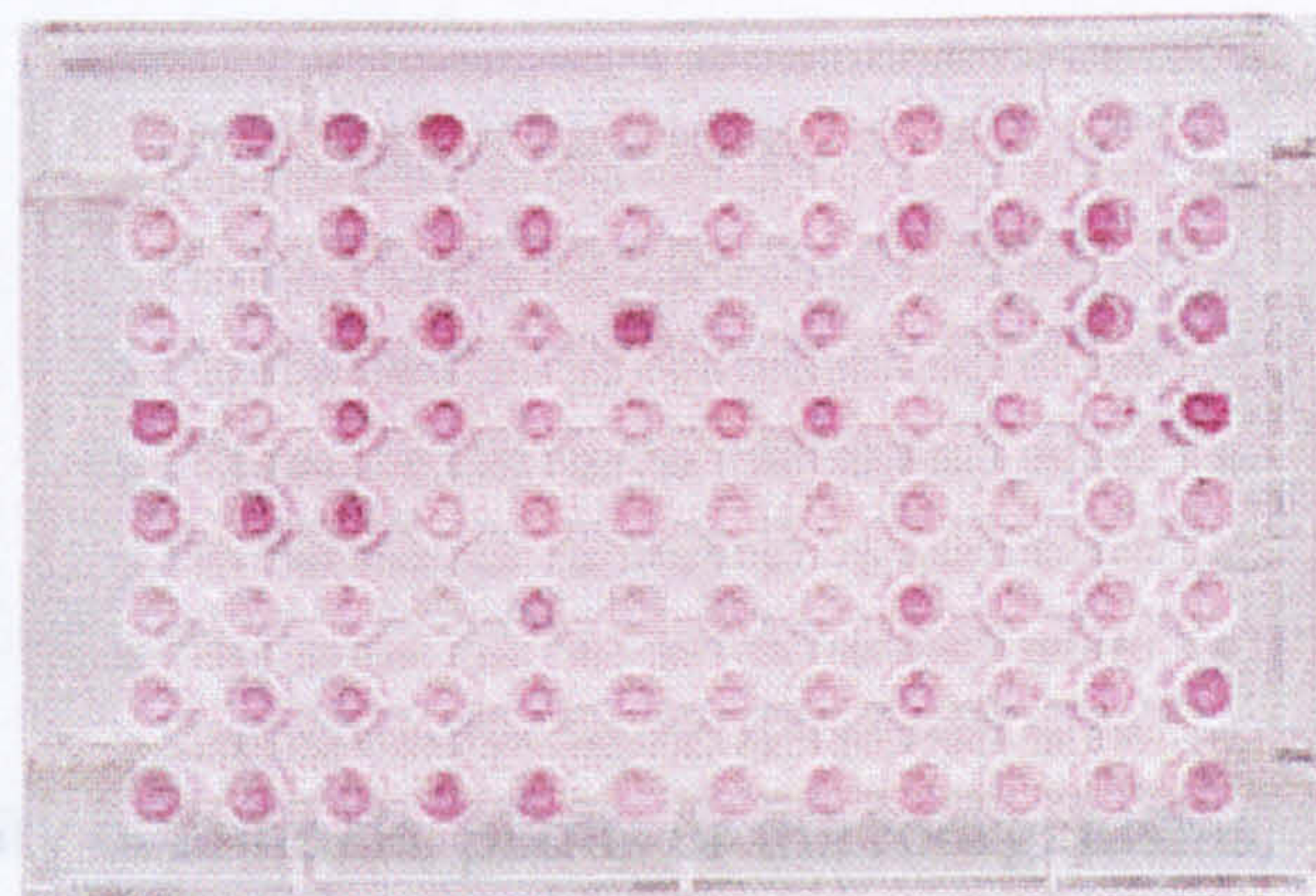
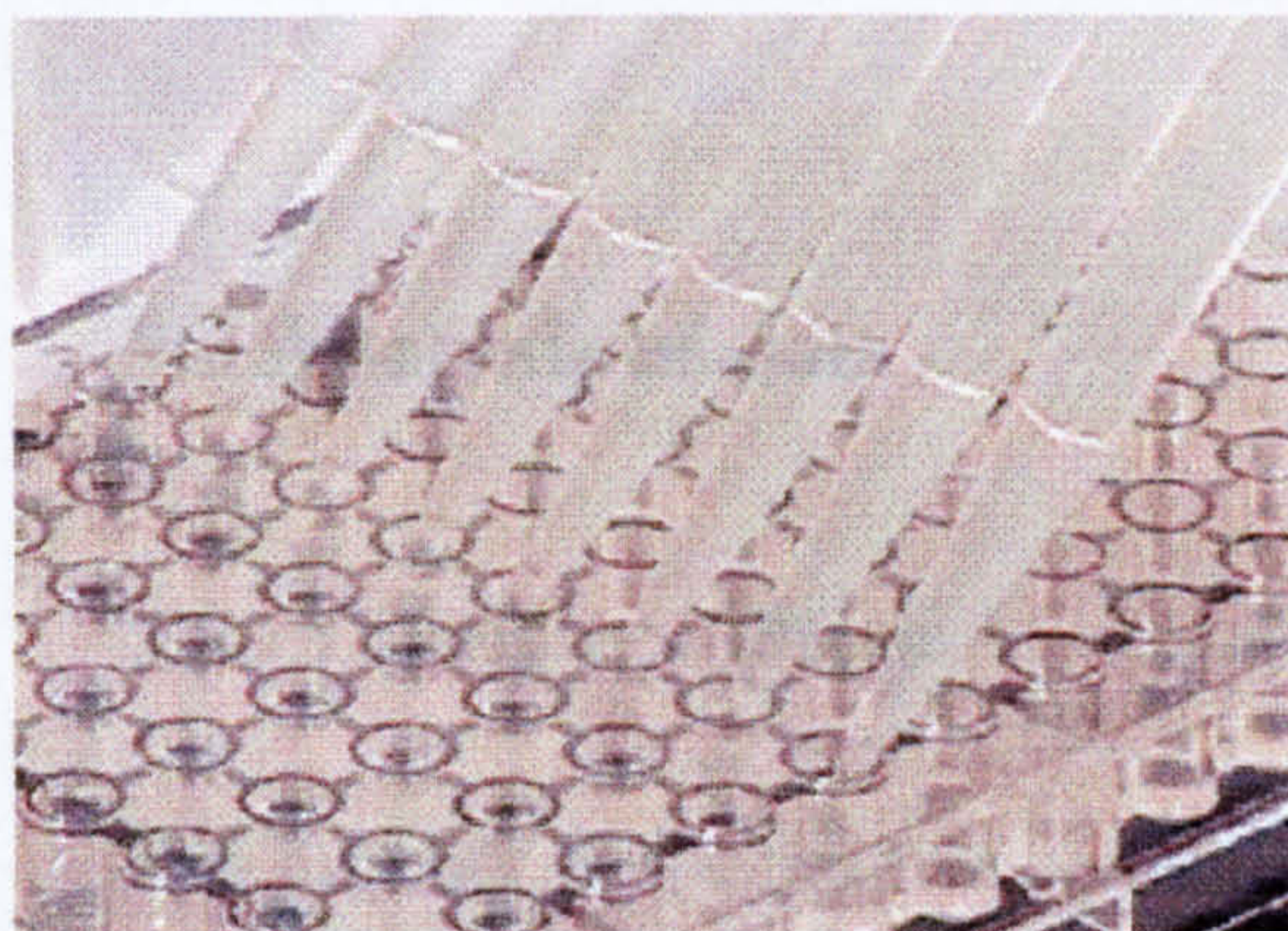


Fig.3.7 The inoculation of bacterial suspension into the wide variety of pre-selected carbon sources in the MicroPlate, produces a characteristic pattern called a metabolic "fingerprint".

of the individuals among the species i.e. how abundance is partitioned among the species. This aspect has been called equitability (evenness).

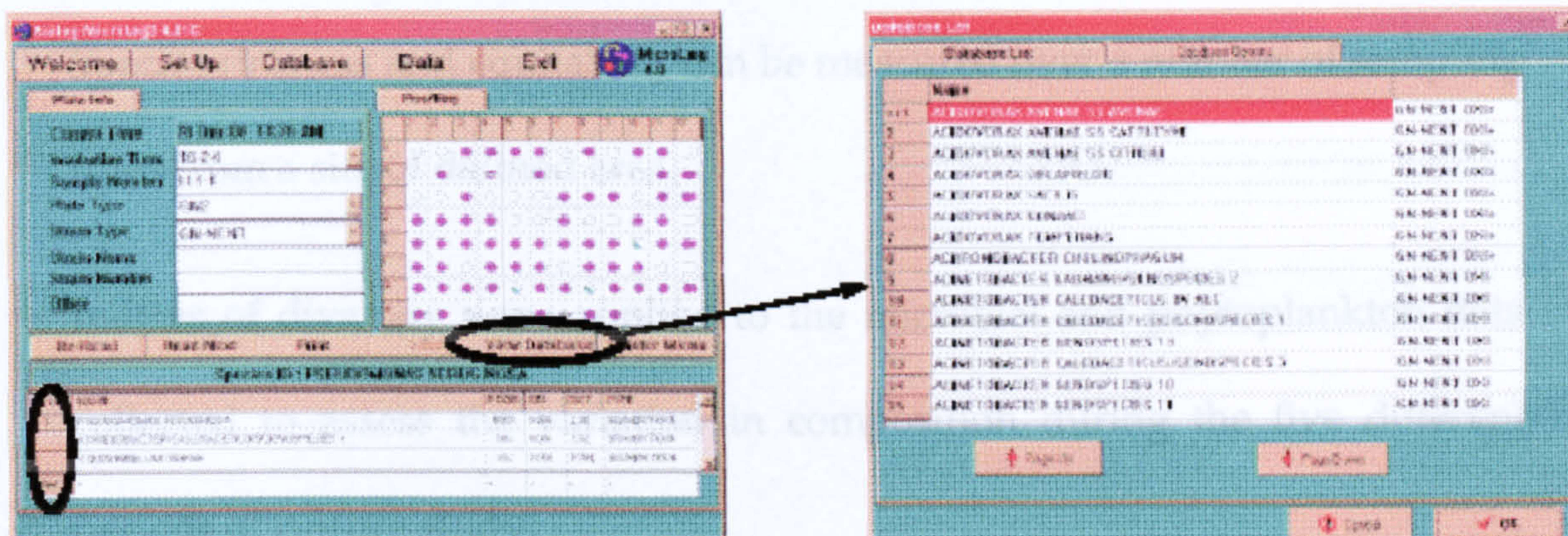


Fig.3.8 The metabolic fingerprint patterns were compared and identified using Microlog 2[®] database software.

3.6.1 Indicators of information content

Based on the Biolog[®] identification guidelines, the similarity index must be at least 0.5 to be considered an acceptable identification, (Fig.3.8). Some isolates could not be identified by this system and these were analyzed further according to Bergey’s manual of determinative bacteriology, (Holt *et al.*, 1994).

3.6 Analysis of data

3.6.1 Diversity indices

The number of species in a community of animals, plants or microorganisms, and their relative abundances are the basis of describing the community as “simple”, “complex” or “dominated by one or a few species”, each of which refers to the general diversity term. It is a function of two elements, one being the number of species (richness) and the other the proportional distribution of the individuals among the species i.e. how abundance is partitioned

of the individuals among the species i.e. how abundance is partitioned among the species. This aspect has been called equitability (evenness). Species richness and equitability can be measured over a number of sampling units from a site of defined area.

Indices of diversity were applied to the microbial and phytoplankton data collected to assess the variation in composition during the five different periods of culture study.

3.6.1.1 Indices of information content

The mathematical theory of information provides an index that is useful as a measure of the possibilities of choice. Margalef (1957, 1958) expressed diversity as the information content per individual (H); the units of H are bits.

$$H = 1 / N (\log_2 (N! / n_1! n_2! \dots n_s!))$$

$$= 3.321928 / N (\log_{10} N! - \sum_{i=1}^S \log_{10} n_i!)$$

where N is the total number of individuals and n_1, n_2, \dots, n_s are the respective number of individuals of each species.

When N and n_i are sufficiently large, the information content per individual can be approximated from the equation of Shannon & Weaver (1949)

$$H' = \sum_{i=1}^S P_i \log_2 P_i$$

where S is the total number of species and P_i is the proportion of the number of individuals of species i to the total number of individuals, that is, $P_i = n_i / N$.

3.6.1.2 Indices of the number of species

Simpson (1949) examined the probability that two specimens taken at random belong to the same species. Such probability (λ) is expressed by:

$$\lambda = \sum_{i=1}^S n_i(n_i-1) / N(N-1)$$

where S is the total number of species, N is the total number of individuals and n_i is the number of individuals of the species i . The numerical value of this expression is low for a high diversity and its maximum value is 1 for a community of one species.

Wittaker (1952, 1960, 1965, 1972, 1975, 1999) suggests the reciprocal of Simpson's index, (which is called HD_2 by Gray (2000))

$$HD_2 = 1 / (p_1^2 + p_1^2 \dots \dots + p_n^2)$$

where p_1 is the proportional abundance of the first species compared to the total number of individuals in the samples.

3.6.1.3 Indices of the abundance of species

The measurement of equitability may be expressed as the difference between the actual diversity and the hypothetical diversity resulting from all species being either equally frequent, or distributed, according to a given hypothesis. The indices J' , Pielou (1966, 1976), and E (Sheldon, 1969) are often used for this measurement.

J' (evenness component diversity), is expressed as

$$J' = H' / \log_2 S$$

Where H' is the Shannon – Weaver diversity index and S is the number of species. When the value of J' is great, the difference in the number of individuals among species is small.

On the other hand Sheldon's E (equitability),(Sheldon, 1969; Hill, 1973), is expressed as:

$$E = e^H / S$$

Where e^H is a diversity index in which the base of natural logarithms is used, the units of e^H are *nits*, H is the Margalef diversity, and S is the total number of species.

3.6.2 Statistical analyses

All the data collected were analysed using the statistical software StatMost® version 3.2 (DataMost Corporation, Salt Lake City, UT), SYSTAT® version 5.0 (SYSTAT, Inc.Ili. USA), Minitab version 13 (Minitab® Inc. U.S.A).

Analyses of significant differences in physicochemical parameters and nutrients associated with culture days and among culture periods, were performed by ANOVA techniques, (Sokal and Rolf, 1995; Zar, 1984; Snedecor and Cochran, 1980) and by the Kolmogorov - Smirnov goodness - of - fit test when data exhibited normality. If significant differences were indicated at $\alpha = 0.05$, then Duncan's multiple range test with Kramer's adjustment for unequal sample size, was used to resolve significant differences.

Mean final weight, survival, instantaneous growth rate and feed conversion ratio were analysed using a one-way ANOVA. Percent survival and feed conversion ratio data were transformed prior to conducting the analysis to satisfy the assumption of normality and /or homoscedasticity of variance. The same significance level of $P < 0.05$ was used for all tests.

4. RESULTS AND DISCUSSION

The results of this study are presented in four sections (4.1 – 4.4) dealing with the quality of pond water, phytoplankton, shrimp gut bacteria, and shrimp production. There is also a short discussion of each results section.

4.1 Water quality

A summary of water quality parameters recorded during the five periods of shrimp culture studied is provided in Table 4.1. Each water quality parameter is compared between culture periods in the following sections, dealing with nitrite-nitrogen, nitrate-nitrogen, unionised ammonia, phosphorus, silica, sulphide, TSS, salinity, temperature and pH.

4.1.1 Nitrite nitrogen (NO₂-N)

4.1.1.1 Period 1. Transition Niña – Niño

During this period there were no significant differences in the concentration of nitrites throughout the shrimp culture cycle ($P > 0.05$); the nitrite content remained very low throughout from 0.000 – 0.001 mg/L (Fig.4.1.1).

4.1.1.2 Period 2. Niño

The nitrite concentration for this period ranged from 0.000 to 0.008 mg/L, with a significant difference in the concentration of nitrite through the period of culture ($P < 0.05$), (Fig.4.1.2). In this period, it was observed that the

nitrite concentration fluctuated from forty days of culture onwards, probably due the oxidation of unionised ammonia produced during the same period (Fig.4.3.2).

4.1.1.3. Period 3. Niño

There was a significant difference in the concentration of nitrite throughout the period of culture ($P < 0.05$) ; recorded values ranged from 0.000 to 0.002 mg/L (Fig.4.1.3).

4.1.1.4 Period 4. Transition Niño - Niña

The levels of nitrite during this period ranged from 0.000 to 0.005 mg/L; the differences in the concentration of nitrite throughout the period of culture were significant ($P < 0.05$) (Fig.4.1.4).

4.1.1.5. Period 5. Niña

During this period of La Niña, the pond nitrite level ranged from 0.000 - 0.003 mg/L. The change in the concentration of nitrite throughout this period of culture was not significant ($P > 0.05$) (Fig.4.1.5).

Although, there were significant differences in the concentration of nitrites throughout the shrimp culture cycle during periods 2, 3 and 4 ($P < 0.05$), when the five culture periods were compared together, the variation in nitrite was not significant ($P > 0.05$) and, overall, the nitrite concentration remained acceptable for shrimp culture.

4.1.2 Nitrate nitrogen (NO₃-N)

4.1.2.1 Period 1. Transition Niña – Niño

The nitrate concentration during this period ranged from 0.000 - 0.030 mg/L, and there was a significant difference in concentration throughout the days of culture ($P < 0.05$), (Fig.4.2.1).

4.1.2.2 Period 2. Niño

During this period there was a significant difference in the concentration of nitrate throughout the culture cycle ($P < 0.05$), with a range from 0.01 - 0.13 mg/L (Fig.4.2.2). A high average concentration of nitrate, 0.033 mg/L, occurred during this period, probably due to the oxidation of nitrite (Fig.4.1.2).

4.1.2.3 Period 3. Niño

There was no significant difference in the concentration of nitrate during the culture cycle ($P > 0.05$) ; the nitrate level ranged from 0.00 - 0.03 mg/L (Fig.4.2.3).

4.1.2.4 Period 4. Transition Niño – Niña

The level of nitrate ranged from 0.007 - 0.023 mg/L. There was no significant difference in the concentration of nitrate in relation to the days of culture during this period ($P > 0.05$) (Fig.4.2.4).

4.1.2.5. Period 5. Niña

During this period of culture, the nitrate content ranged from 0.007 - 0.027 mg/L, but the difference in concentration of nitrate during the days of culture in this period was not significant ($P > 0.05$) (Fig.4.2.5).

When all five periods of culture were compared, no significant differences were found between the nitrate concentrations ($P > 0.05$). Overall, nitrate levels were well below the suggested safe level of 300 mg/L (Anon, 1994).

4.1.3 Unionised ammonia ($\text{NH}_3\text{-N}$)

4.1.3.1. Period 1. Transition Niña – Niño

During this period, the ammonia concentration increased significantly from 0.00 to 0.06 mg/L throughout the days of culture ($P < 0.05$) (Fig.4.3.1). However, even the highest value of 0.06 mg/L is within the safe range for cultured shrimp.

4.1.3.2 Period 2. Niño

No significant difference ($P > 0.05$) was found in the ammonia concentration throughout the days of culture; the ammonia level ranged from 0.02-0.06 mg/L (Fig.4.3.2), with an average of 0.033 mg/L.

4.1.3.3 Period 3. Niño

The ammonia level in this period varied from 0.01 - 0.05 mg/L , and there was a significant difference in the concentration of ammonia throughout the culture cycle ($P < 0.05$) (Fig.4.3.3). A level of 0.05 mg/L was found on day one and again after 130 days of culture; there was a decrease from the first week of culture and an increase after hundred days of culture.

4.1.3.4 Period 4. Transition Niño - Niña

There was an increase in ammonia to 0.07 mg/L after 100 days of culture, or twice the concentration observed on the first day of culture when it was found to be 0.04 mg/L. During this period, there was a significant difference in the concentration of ammonia throughout the culture cycle ($P < 0.05$), with a range from 0.00 - 0.07 mg/L, (Fig.4.3.4).

4.1.3.4 Period 5. Niña

The ammonia concentration for this period ranged from 0.00 – 0.08 mg/L, with a significant difference in the concentration of ammonia throughout the days of culture ($P < 0.05$) (Fig.4.3.5). In this period, the trend in the ammonia concentration was similar to that in culture periods 3 and 4 period, with ammonia increasing through the final days of culture.

In summary, there was an increase in the concentration of ammonia during the final days of shrimp culture in periods 1, 3, 5. Despite this, the levels

reached remained below the reported toxic level for shrimp of 0.1 mg/L (Frias-Espericueta *et al.*, 2000). Overall, there was no significant difference in ammonia concentration ($P < 0.05$) between the five periods of culture.

4.1.4 Phosphorus (P)

4.1.4.1 Period 1. Transition Niño – Niña

There was a significant difference in the concentration of phosphorus through the days of the culture cycle ($P < 0.05$). The concentration of dissolved reactive phosphorus was on average of 0.05 mg/L, with a range from 0.02 – 0.1 mg/L; phosphorus increased from day 40 of the culture cycle onwards (Fig.4.4.1).

4.1.4.2 Period 2. Niño

During this period of culture, the concentration of phosphorus was on average 0.12 mg/L, with a range from 0.04 – 0.20 mg/L, and increasing after four weeks of culture. There was a significant difference in the concentration of phosphorus throughout the days of culture ($P < 0.05$) (Fig.4.4.2).

4.1.4.3 Period 3. Niño

There was a significant difference in the concentration of phosphorus throughout the days of culture ($P < 0.05$), with a range from 0.10- 0.29 mg/L, (Fig.4.4.3). The average value for phosphorus was 0.16 mg/L with no specific trend during the days of culture.

4.1.4.4 Period 4. Niño

The phosphorus concentration during this period ranged from 0.11- 0.30 mg/L. The highest average phosphorus concentration in the study was found in this period (0.19 mg/L). Phosphorus showed an increase from the second week onwards with a significant difference in the concentration of phosphorus throughout the days of culture ($P < 0.05$) (Fig.4.4.4).

4.1.4.5 Period 5. Niña

The concentration of phosphorus was from 0.10- 0.28 mg/L (Fig.4.4.5). In this period of culture, there was a significant difference in the concentration of phosphorus throughout the days of culture cycle ($P < 0.05$). The average concentration of phosphorus was at 0.15 mg/L, with a trend of decrease in the final month of culture.

In summary, from the first to the fourth periods of culture there was an increase in the mean phosphorus concentration (0.05 to 0.19 mg/L) and, overall, there was a significant difference in phosphorus between the five periods of culture ($P < 0.05$).

4.1.5 Silica (SiO_2)

4.1.5.1 Period 1. Transition Niña – Niño

During this period of culture, the concentration of silica ranged from 4.7 – 13.1 mg/L, with an average of 7.2 mg/L. There was no trend in silica content

during the culture cycle, but the concentration of silica did vary significantly in this period ($P < 0.05$) (Fig.4.5.1).

4.1.5.2 Period 2. Niño

There was significant difference in the concentration of silica throughout the days of culture ($P < 0.05$); the concentration of silica during this period increased to an average of 19.2 mg/L, with a range from 12.7 – 27.0 mg/L, (Fig.4.5.2) with a trend to increase from the first month of shrimp culture.

4.1.5.3 Period 3. Niño

Silica concentration for this period ranged from 23.0 – 35.0 mg/L. The average concentration was high (27.7 mg/L) and no trend was observed in this period. There was significant difference in the concentration of silica throughout the culture cycle ($P < 0.05$) (Fig.4.5.3).

4.1.5.4 Period 4. Transition Niño - Niña

In this period, the silica content was from 18.6 – 38.4 mg/L, with a significant difference in the concentration of silica throughout the days of culture ($P < 0.05$) (Fig.4.5.4). The average was 26.0 mg/L, with a trend to decrease in silica from the first month of culture.

4.1.5.4 Period 5. Niña

A trend of decrease in the silica concentration from 25.2 mg/L to 12.5 mg/L was observed after 70 days of culture. There was a significant difference in

the concentration of silica throughout the days of culture ($P < 0.05$); the silica level ranged from 11.8 – 26.5 mg/L, with an average of 20 mg/L (Fig.4.5.5).

In summary, from the second period of culture onwards, there was an increase in the mean silica concentration; the level fell in the last 30 days of the fifth period of culture (Niña) almost to the same value found during the first period. Overall, there was a significant difference in silica concentration between the five periods of culture monitored ($P < 0.05$).

4.1.6 Sulphide (S=)

4.1.6.1 Period 1. Transition Niña – Niño

The pond sulphide concentration during this period ranged from 0.017 – 0.042 mg/L and there was a significant difference in the concentration of sulphide throughout the days of culture ($P < 0.05$) (Fig.4.6.1). The average sulphide level during this period was 0.028 mg/L, with an increase from 0.017 mg/L on the initial day of culture to 0.042 mg/L by the end of the culture cycle.

4.1.6.2 Period 2. Niño

During this period of shrimp culture, there was a significant difference in the concentration of sulphide throughout the days of culture ($P < 0.05$). An average of 0.026 mg/L was recorded in this period, with no trend for the sulphide concentration to increase with time. The sulphide concentration ranged from 0.014 – 0.042 mg/L (Fig.4.6.2).

4.1.6.3 Period 3. Niño

The concentration of sulphide was from 0.070 – 0.035 mg/L, an average of 0.025 mg/L, and there was a significant difference in the concentration of sulphide throughout the of culture cycle ($P < 0.05$), but with no clear apparent trend (Fig.4.6.3).

4.1.6.4 Period 4. Transition Niño – Niña

There was a significant difference in the concentration of sulphide through the days of culture ($P < 0.05$). The concentration of sulphide ranged from 0.014 – 0.047 mg/L, with an average of 0.028 mg/L (Fig.4.6.4). An increase from 0.012 mg/L on the initial day of culture, to 0.047 mg/L by the end of the culture cycle was observed.

4.1.6.5 Period 5. Niña

The concentration of sulphide ranged from 0.024 – 0.043 mg/L, and the highest average value for the study of 0.035 mg/L was recorded in this period. There was no significant difference in the concentration of sulphide throughout the culture cycle ($P < 0.05$) (Fig.4.6.5). An increase from 0.024 mg/L on the initial day of culture, to 0.043 mg/L by the end of culture, was recorded; a similar trend to that shown during culture period 4.

In summary, there was a trend of increase in the sulphide content of the shrimp pond water towards the end of the production cycle in culture

periods 1, 4 and 5, but no significant difference in concentration was found between these periods.

4.1.7 Total suspended solids (TSS)

4.1.7.1 Period 1. Transition Niña - Niño

In this period the mean concentration of TSS was 31.4 mg/L, with a range of 26.0 – 42.5 mg/L. The concentration of total suspended solids varied significantly during the culture cycle ($P < 0.05$) (Fig.4.7.1).

4.1.7.2 Period 2. Niño

There was significant difference in the concentration of total suspended solids with the days of culture ($P < 0.05$). The concentration of TSS ranged from 15.3 – 38.0 mg/L (Fig.4.7.2).

4.1.7.3 Period 3. Niño

The concentration of TSS during this period was from 20.0 – 51.5 mg/L, with an average of 32.1 mg/L. There was a significant difference in the concentration of total suspended solids throughout the days of culture ($P < 0.05$) (Fig.4.7.3).

4.1.7.4 Period 4. Transition Niño – Niña

A significant difference in the concentration of total suspended solids was found throughout the culture period ($P < 0.05$). The concentration of TSS ranged from 22 – 36 mg/L (Fig.4.7.4).

4.1.7.5 Period 5. Niña

During this period, TSS ranged from 19.7 – 35 mg/L. The TSS values varied significantly ($P < 0.05$) throughout the days of culture (Fig.4.7.5).

An analysis of variance between all periods of culture revealed no significant differences ($P > 0.05$). No trends, increase or decrease, in the concentration of total suspended solids were found during any of the culture periods.

4.1.8 Salinity

4.1.8.1 Period 1. Transition Niña - Niño

During this period of shrimp culture, the mean pond water salinity value was 38.1 psu, with a range of 35 – 39 psu. This was above the normal seawater salinity level because of the dry season, the use of seawater as a water supply, and from evaporation of water in the ponds. There was a significant difference in salinity throughout the days of culture ($P < 0.05$) (Fig.4.8.1).

4.1.8.2 Period 2. Niño

The mean salinity value was 21.9 psu and ranged from 11.3 – 28 psu. There was a significant difference in salinity throughout the days of culture ($P < 0.05$) (Fig.4.8.2).

4.1.8.3 Period 3. Niño

The change in salinity during this culture period of culture was also significant ($P < 0.05$). The mean salinity value was 15.2 psu, i.e. less than half the salinity concentration range recorded during period 1, with a salinity of 12.0 – 18.7 psu (Fig.4.8.3).

4.1.8.4 Period 4. Transition Niño - Niña

In this culture period the salinity value remained stable with intermediate values and observed from 18.7 – 22.0 psu, with a mean of 20.2 psu. There was a significant difference in salinity throughout the days of the culture cycle ($P < 0.05$) (Fig.4.8.4).

4.1.8.5 Period 5. Niña

During this period, salinity fell to less than one third of that during period 1. The mean salinity value was 10.2 psu and ranged from 4.3 – 15.7 psu. There was a significant difference ($P < 0.05$) in salinity through the culture cycle (Fig.4.8.5).

In summary, the salinity pattern revealed above is one of a strong decline throughout the study. In culture period 1, salinity values were stable with a mean of 38 ± 1.7 psu (\pm SD). From the second month in the second shrimp culture period (Niño), there was a decrease in salinity from 28 to 11psu. In the third and fourth culture periods, salinity remained more stable before falling considerably again during period 5 to a minimum of 4 psu, because normally during these months is the rainy season.

4.1.9 Temperature

4.1.9.1 Period 1. Transition Niña - Niño

In this period of shrimp culture, the pond water temperature ranged from 28.0 – 31.0 °C; there was a small but significant difference in temperature throughout the culture cycle ($P < 0.05$) (Fig.4.9.1).

4.1.9.2 Period 2. Niño

There was also a significant difference in temperature throughout the culture cycle of period 2 ($P < 0.05$), even through the temperature range was only 28.7 – 30.0 °C, (Fig.4.9.2).

4.1.9.3 Period 3. Niño

Temperature conditions remained similar to those in the second period of shrimp culture, with a temperature range of 28.3 – 29.7 °C and a significant difference in temperature throughout the culture cycle ($P < 0.05$) (Fig.4.9.3).

4.1.9.4 Period 4. Transition Niño - Niña

In this period, the temperature range decreased to 28.0 – 26.3 °C and there was a significant difference in temperature throughout the days of culture ($P < 0.05$) (Fig.4.9.4).

4.1.9.5 Period 5. Niña

Conditions during period 5 remained similar to those during period 4. There was a significant difference in temperature throughout the days of culture ($P < 0.05$). Temperature ranged from 26.0 – 28.0 °C (Fig.4.9.5).

In summary, the temperature in the shrimp ponds rose during the first period of culture, from about an initial 28.0 °C to 31.0 °C in the last month of culture. Higher temperatures prevailed in the second and third periods of culture (28.3 – 30.0 °C). In the second half of the fourth period of culture, the temperature decreased sharply (26.3 °C). The fifth period of culture (Niña) started with the lowest temperature, increasing 2.0 °C in the second half of this period of shrimp culture.

4.1.10. pH

There was a significant difference between the periods of culture ($P < 0.05$), despite this, the pH values during the five periods varied only from 7.32 – 7.63, which an acceptable range for shrimp culture, (Fig.4.10. 1- 5).

The following points summarise the main findings with respect to the pond water quality study:

- 1) There were significant differences in the concentration of nitrites, nitrates, unionised ammonia, sulphide and total suspended solids through the culture cycle during the same period of shrimp culture ($P < 0.05$); however, no significant differences were found between the periods of culture ($P > 0.05$).
- 2) Phosphorus, silica, salinity and temperature showed significant differences throughout the days of culture in the same period ($P < 0.05$) and also significant differences between the periods of culture ($P < 0.05$).
- 3) The temperature in ponds, in the first period of culture (Transition Niña - Niño) was around 28 °C (November 1996) and rose to 31 °C in the last month of culture (March 1997) (Fig.4.9.1). The average salinity was 38 psu (Fig.4.8.1), because of the dry season and the water evaporation processes in the ponds.
- 4) The higher temperatures in the second period of culture (El Niño) (30 - 29 °C, Feb - Jun 97) coincided with intermediate salinities between 28 - 11 psu during heavy rains of the strong El Niño event throughout most of 1997 (Figs 4.8.2 and 4.9.2).

5) The temperature remained at 28-29 °C during the third period of culture (El Niño) (Jul 97 – Jan 98) and salinities continued in the range 12-18 psu because of heavy rainfalls, (Figs.4.8.3 and 4.9.3).

6) In the second half of 1998 (Transition Niño – Niña), during the fourth period of culture, salinities were in the range 18 – 22 psu, but the temperature began to decrease sharply at the end of this period (26 °C) (Figs. 4.8.4 and 4.9.4).

7) The last period of culture (March - Jun 1999) began with the lowest temperature and salinity recorded (26 °C and 4 – 15 psu respectively) due to the cool - wet season associated with a La Niña event, (Figs. 4.9.5, 4.8.5 and 4.11).

As the concentration of phosphorus and silica showed an increase, on average, from the second period of culture Feb 97 - Jun 97 through Sep98 - Feb 99, (Fig.4.4 (2,3,4); Fig.4.5 (2,3,4); Figs. 4.12 and 4.13), the data for all five periods were pooled and a regression analysis was carried out to determine whether there was any statistical correlation between phosphorus or silica versus salinity or temperature. A significant negative correlation was obtained between phosphorus versus salinity ($r^2 = 0.364$) and silica versus salinity ($r^2 = 0.4959$) because of the warm rainy season (El Niño) and the cool-wet season in the early months (1999) associated with La Niña (Figs. 4.14, 4.15, 4.16, 4.17). As expected, no significant correlation was found

between phosphorus and temperature, or between silica and temperature (Figs. 4.18, 4.19, 4.20, 4.21).

Table 4. 1 Mean, standard deviation and range of water quality data from the five consecutive shrimp culture periods studied.

Parameter	TIME PERIOD				
	1 Transition Niña-Niño Nov 96 - Mar 97	2 Niño Feb - Jun 97	3 Niño Jul 97 - Jan 98	4 Transition Niño-Niña Sep 98 - Feb 99	5 Niña Mar - Jun 99
NO₂-N (mg/L)					
Mean	0.000	0.002	0.001	0.002	0.001
Standard deviation	0.000	0.003	0.001	0.002	0.001
Minimum	0.000	0.000	0.000	0.000	0.000
Maximum	0.001	0.008	0.002	0.005	0.003
NO₃-N (mg/L)					
Mean	0.015	0.033	0.016	0.016	0.015
Standard deviation	0.009	0.031	0.009	0.005	0.008
Minimum	0.000	0.010	0.000	0.007	0.007
Maximum	0.030	0.130	0.030	0.023	0.027
NH₃-N (mg/L)					
Mean	0.03	0.04	0.03	0.03	0.03
Standard deviation	0.02	0.01	0.01	0.03	0.03
Minimum	0.00	0.02	0.01	0.00	0.00
Maximum	0.06	0.06	0.05	0.06	0.08
Each mean is an average for all the weekly sample points					

P (mg/L)			
Mean	0.05	0.12	0.16
Standard deviation	0.03	0.04	0.06
Minimum	0.02	0.04	0.10
Maximum	0.10	0.20	0.29
		0.19	0.15
		0.06	0.06
		0.11	0.10
		0.30	0.28

SiO ₂ (mg/L)			
Mean	7.2	19.2	27.7
Standard deviation	2.6	4.3	3.7
Minimum	4.7	12.7	23.0
Maximum	13.1	27.0	35.3
		26.0	20.0
		6.5	6.0
		18.6	11.8
		38.4	26.5

S ⁻ (mg/L)			
Mean	0.028	0.026	0.025
Standard deviation	0.008	0.009	0.008
Minimum	0.017	0.014	0.007
Maximum	0.042	0.042	0.035
		0.026	0.035
		0.010	0.007
		0.012	0.024
		0.046	0.043

TSS (mg/L)			
Mean	31.4	27.5	32.1
Standard deviation	10.0	7.1	6.0
Minimum	25.5	15.3	20.0
Maximum	42.0	38.0	51.5
		22.0	28.6
		6.3	6.7
		20.0	19.7
		36.0	35.0

Each mean is an average for all the weekly sample points

Salinity (psu)						
Mean	38.1	21.9	15.2	20.2	10.2	
Standard deviation	1.7	7.0	2.8	1.4	4.7	
Minimum	35.0	11.3	12.0	18.7	4.3	
Maximum	39.0	28.0	18.7	22.0	15.7	

Temperature (°C)						
Mean	29.1	29.3	29.1	27.2	27.3	
Standard deviation	1.37	0.45	0.43	0.82	0.80	
Minimum	28.0	28.7	28.3	26.3	26.0	
Maximum	31.0	30.0	29.7	28.0	28.0	

PH						
Mean	7.42	7.50	7.46	7.45	7.56	
Standard deviation	0.06	0.06	0.06	0.07	0.07	
Minimum	7.32	7.40	7.37	7.30	7.43	
Maximum	7.50	7.60	7.57	7.50	7.63	

Each mean is an average for all the weekly sample points

RATIO						
Inorganic N : inorganic P	0.90	0.64	0.27	0.24	0.30	

Nitrite

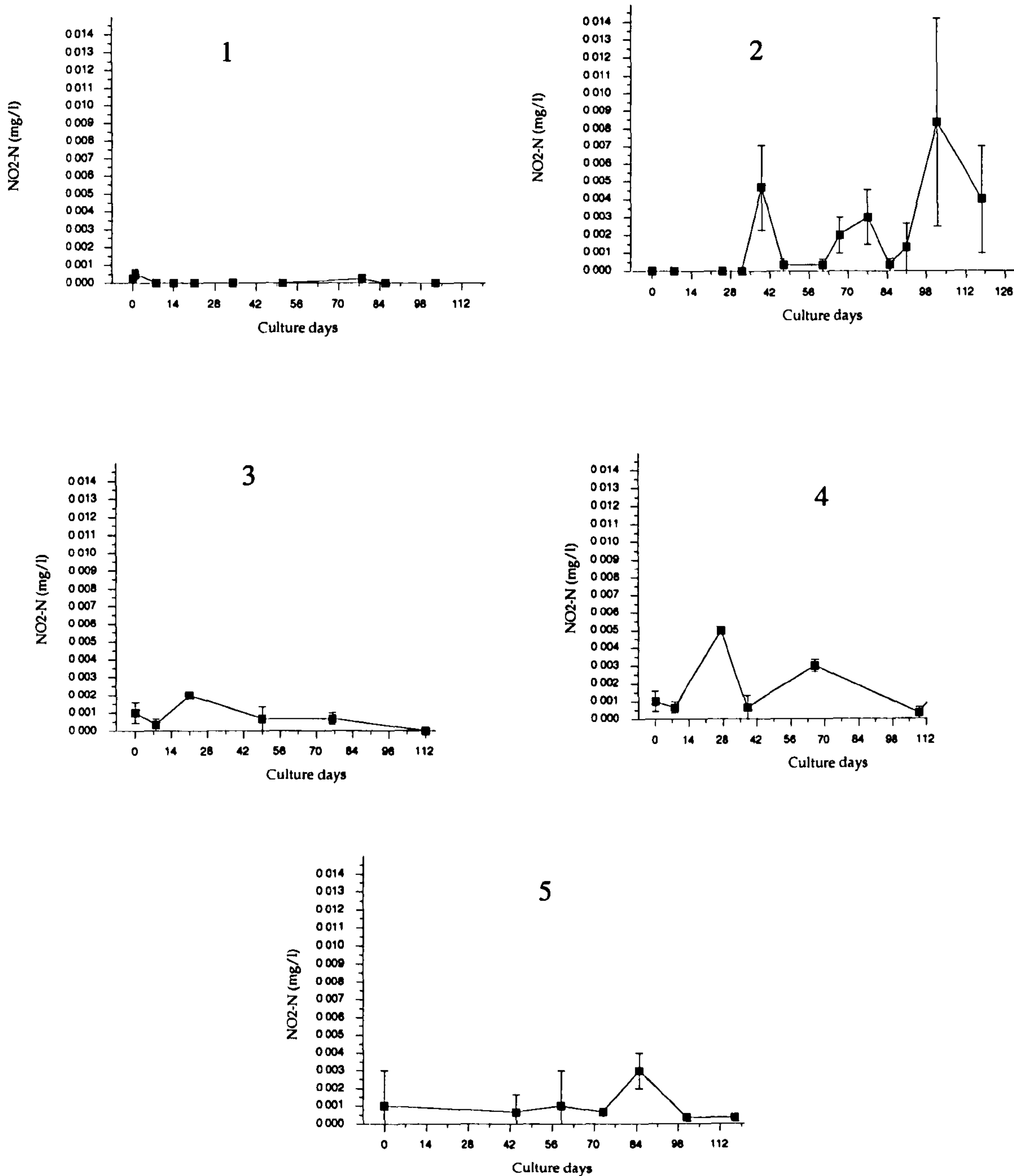


Fig.4. 1 Mean nitrite concentration (mg/L) in ponds from the five different culture periods with standard deviation bars.

1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

Nitrate

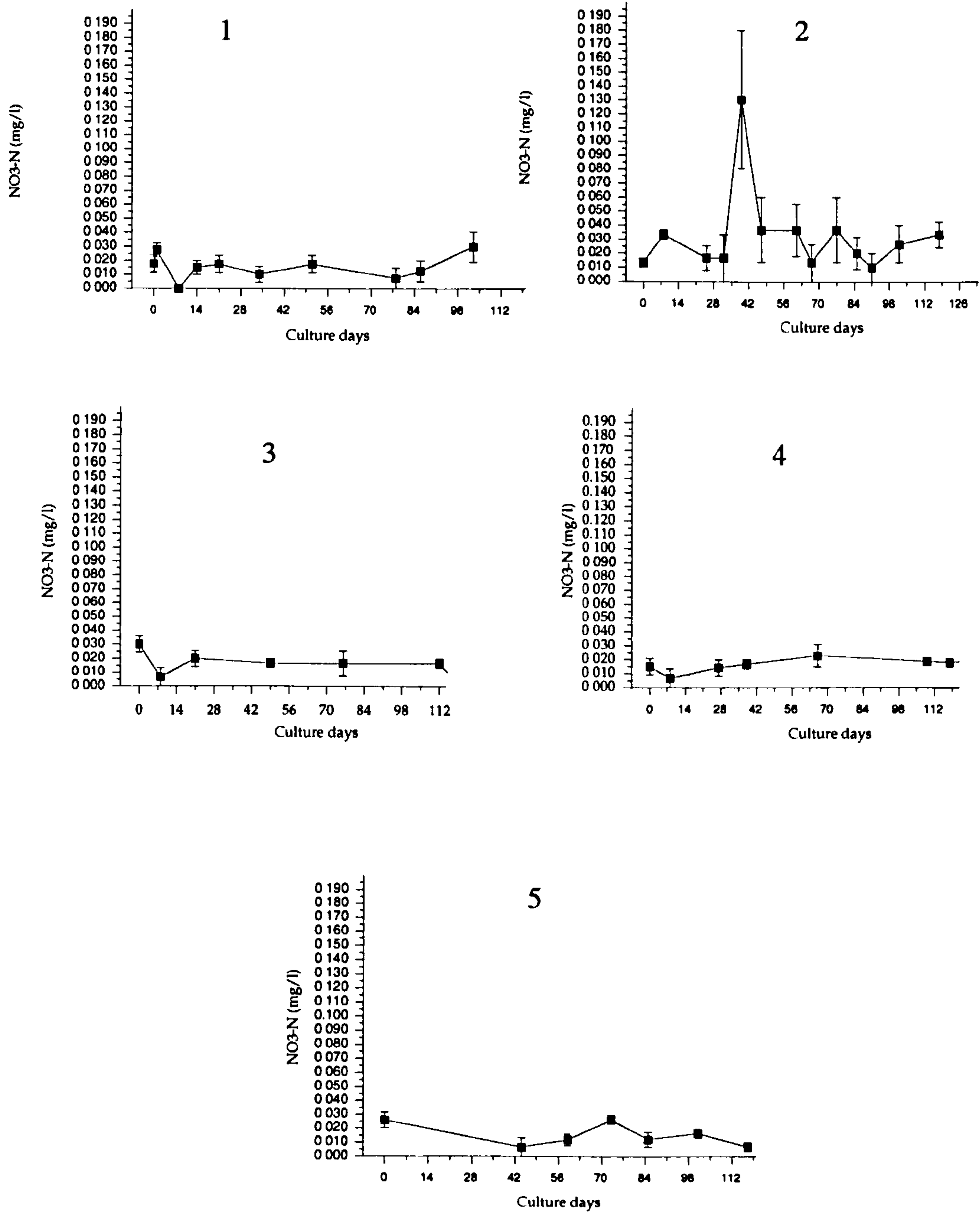


Fig.4. 2 Mean nitrate concentration (mg/L) in ponds from the five different culture periods with standard deviation bars.
 1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

Unionised ammonia

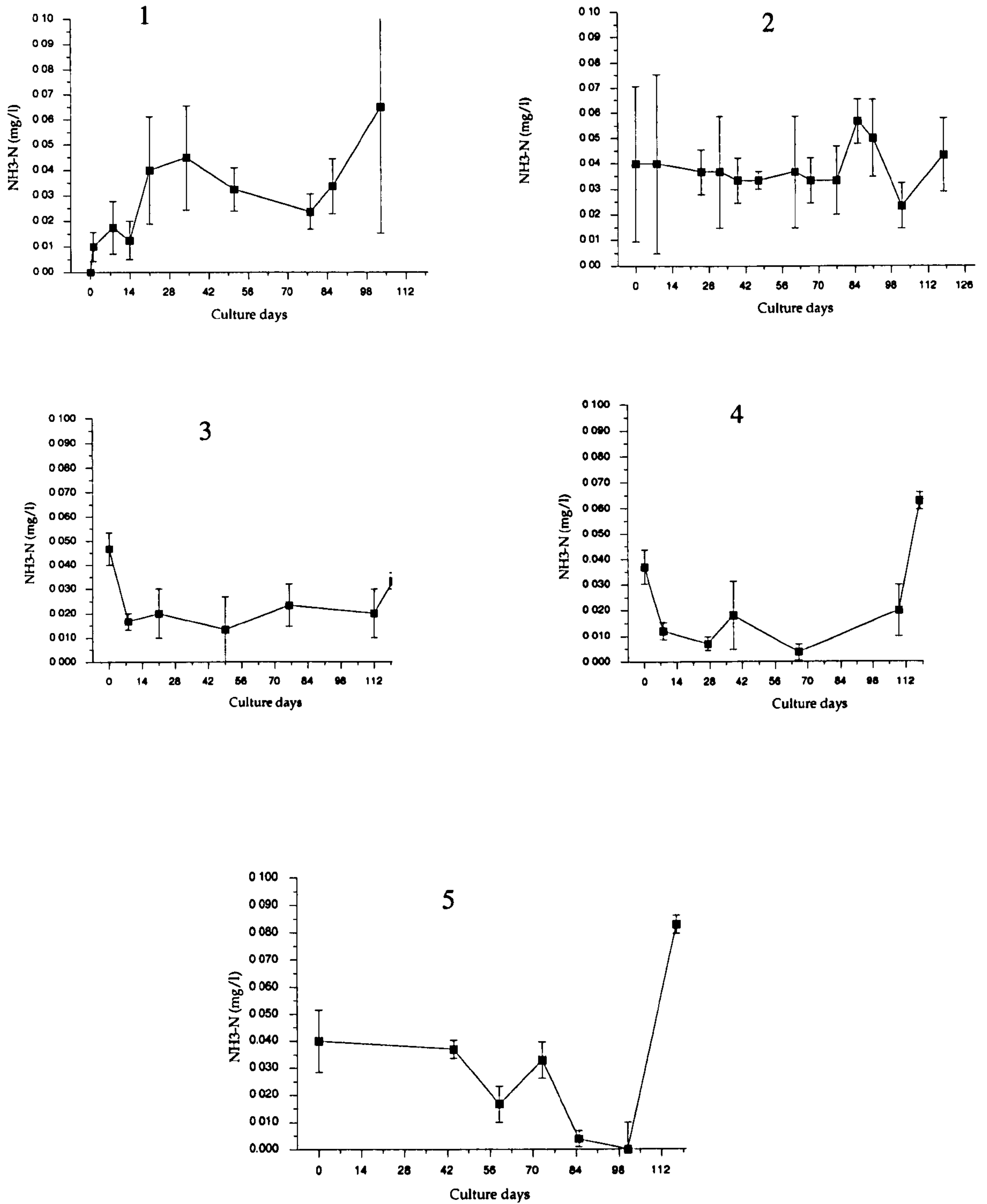


Fig.4. 3 Mean unionised ammonia concentration (mg/L) in ponds from the five different culture periods with standard deviation bars.
 1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

Phosphorus

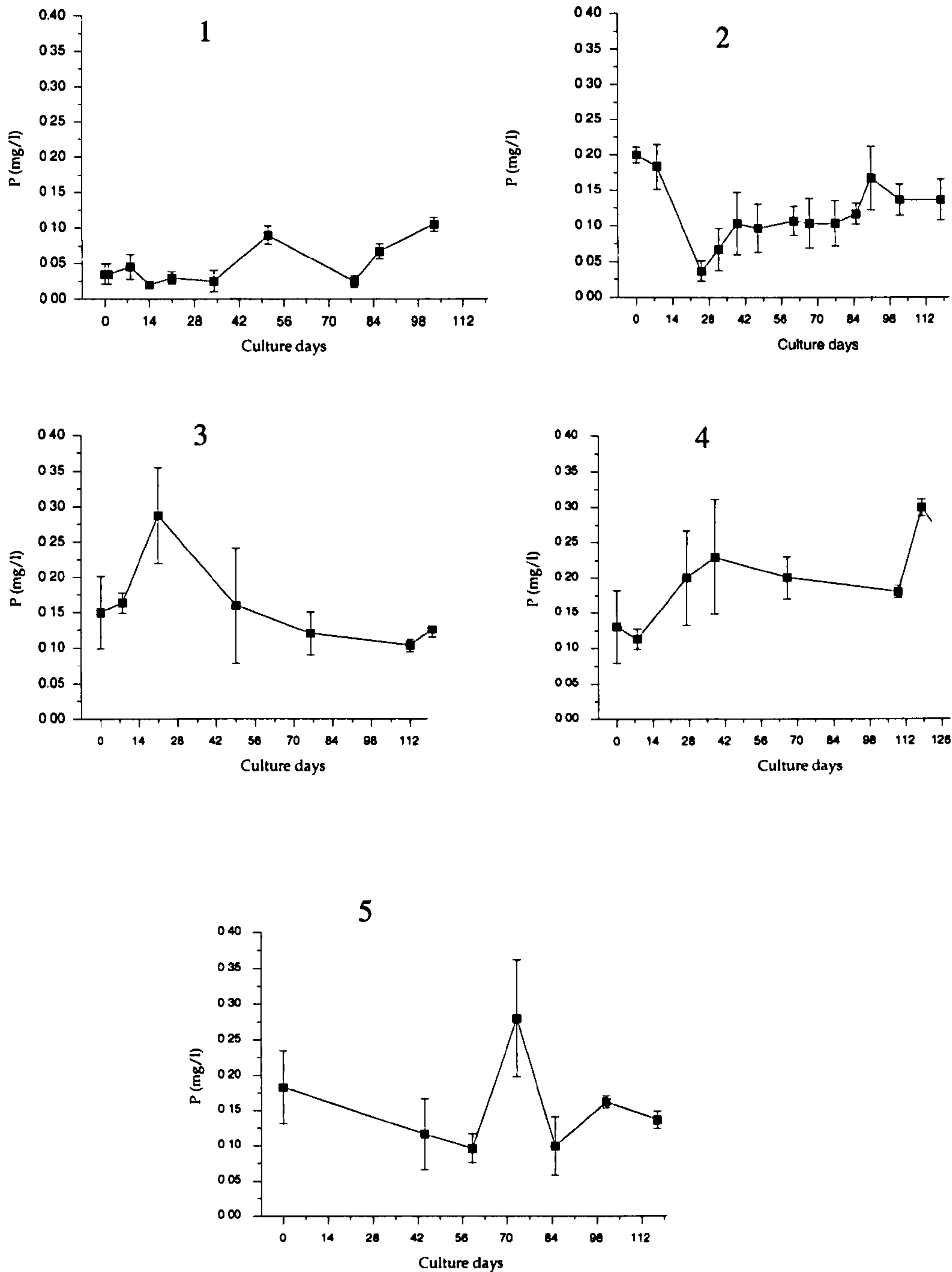


Fig.4. 4 Mean of dissolved reactive phosphorus concentration (mg/L) in ponds from the five different culture periods with standard deviation bars.
 1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

Silica

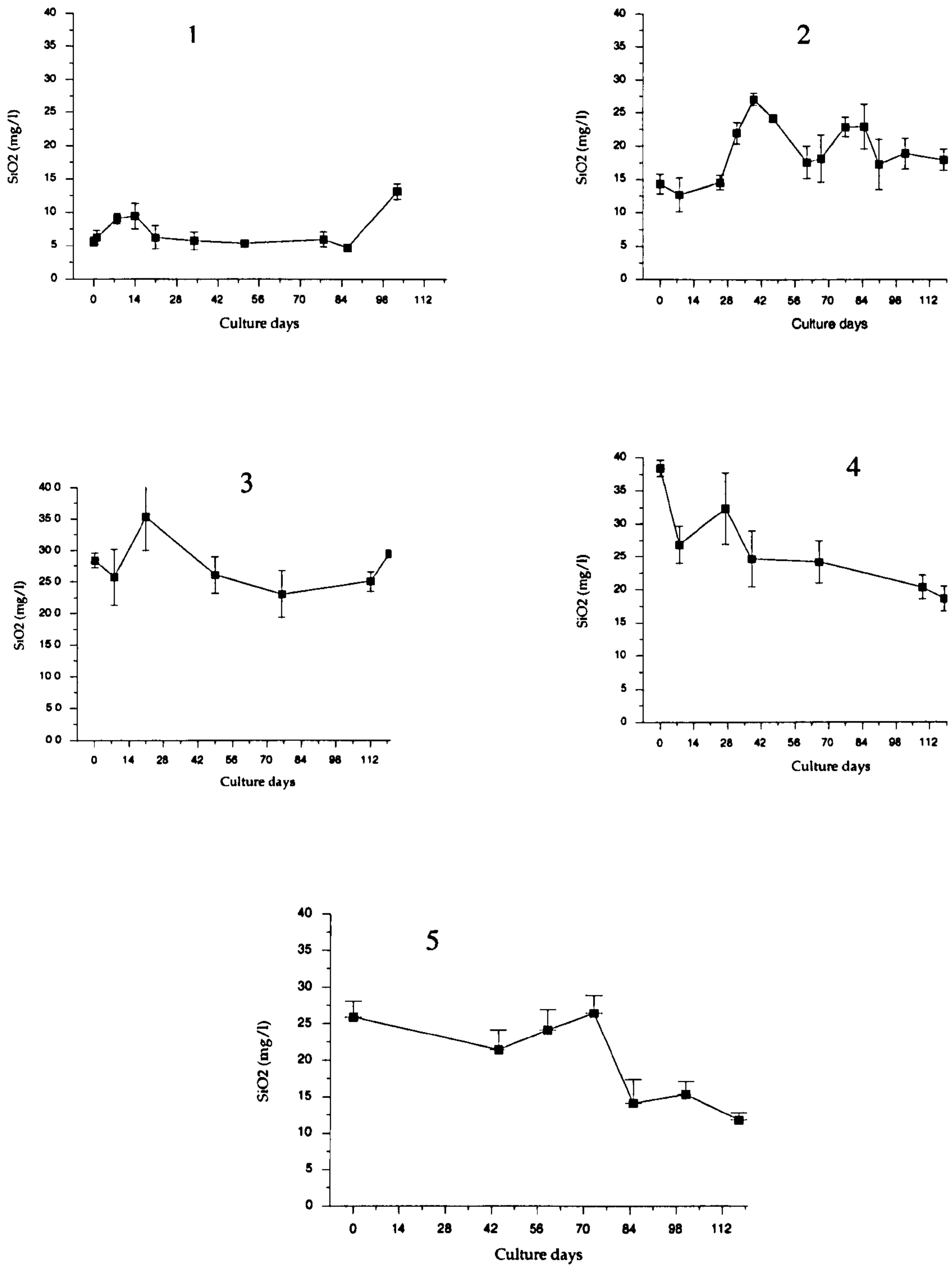


Fig.4. 5 Mean silica concentration (mg/L) in ponds from the five different culture periods with standard deviation bars.

A: Nov96 – Mar 1997; B: Feb – Jun 1997; C: Jul 97 – Jan 98; D: Sep 98 – Feb 99 ; E: Mar – Jul 99

Sulphide

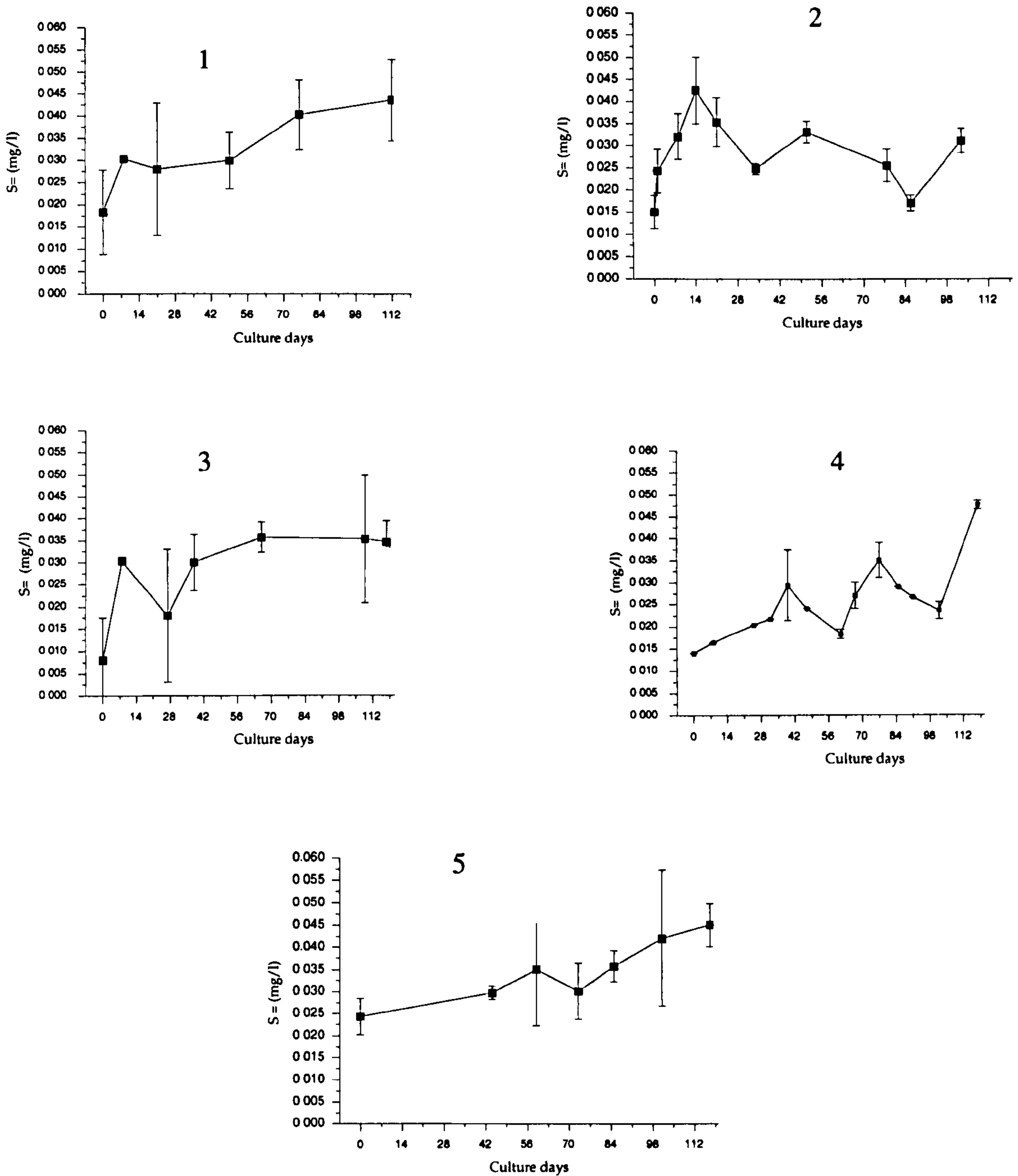


Fig.4. 6 Mean sulphide concentration (mg/L) in ponds from the five different culture periods with standard deviation bars.

1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

Total suspended solids

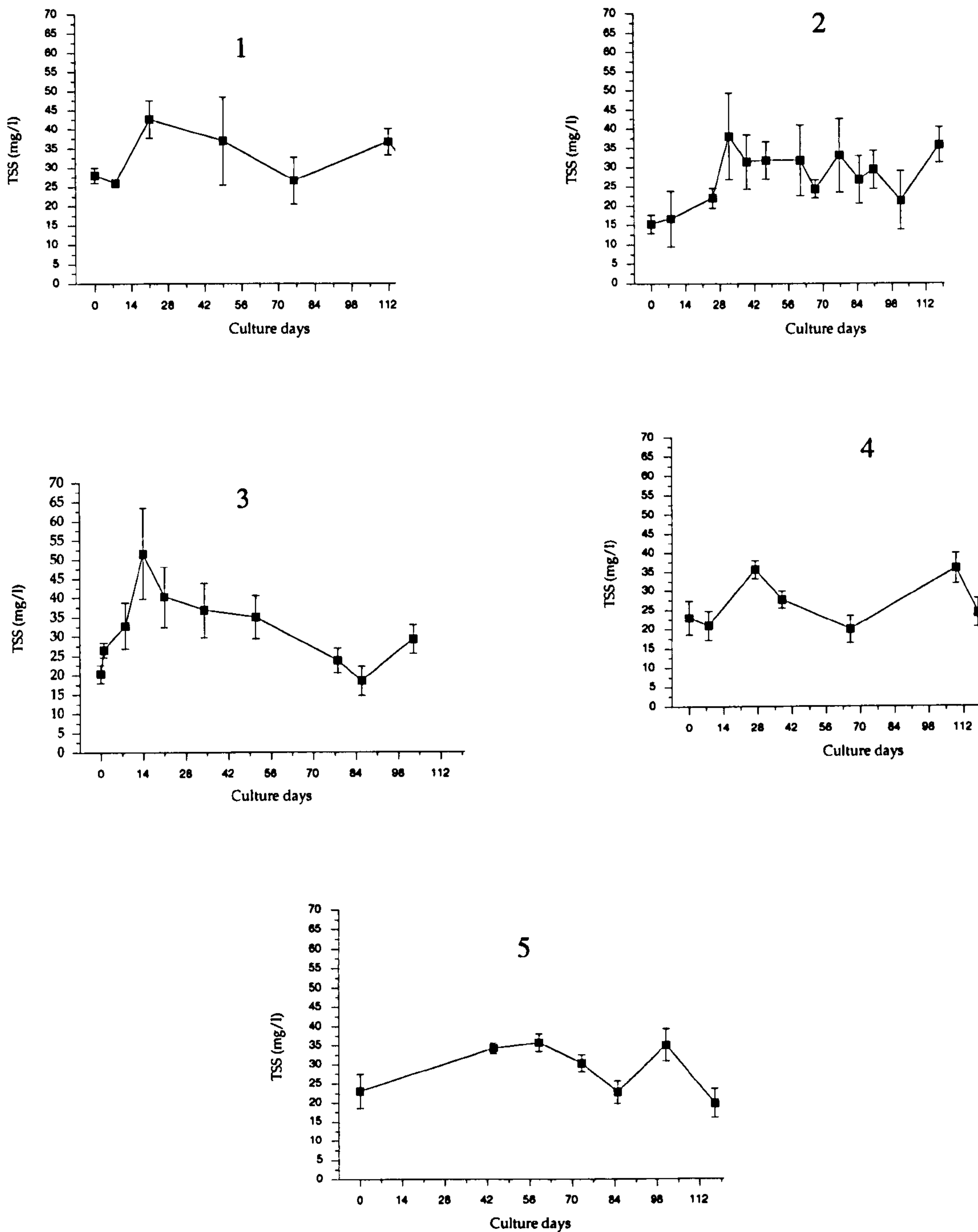


Fig.4. 7 Mean total suspended solids (mg/L) in ponds from the five different culture periods with standard deviation bars.

1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

Salinity

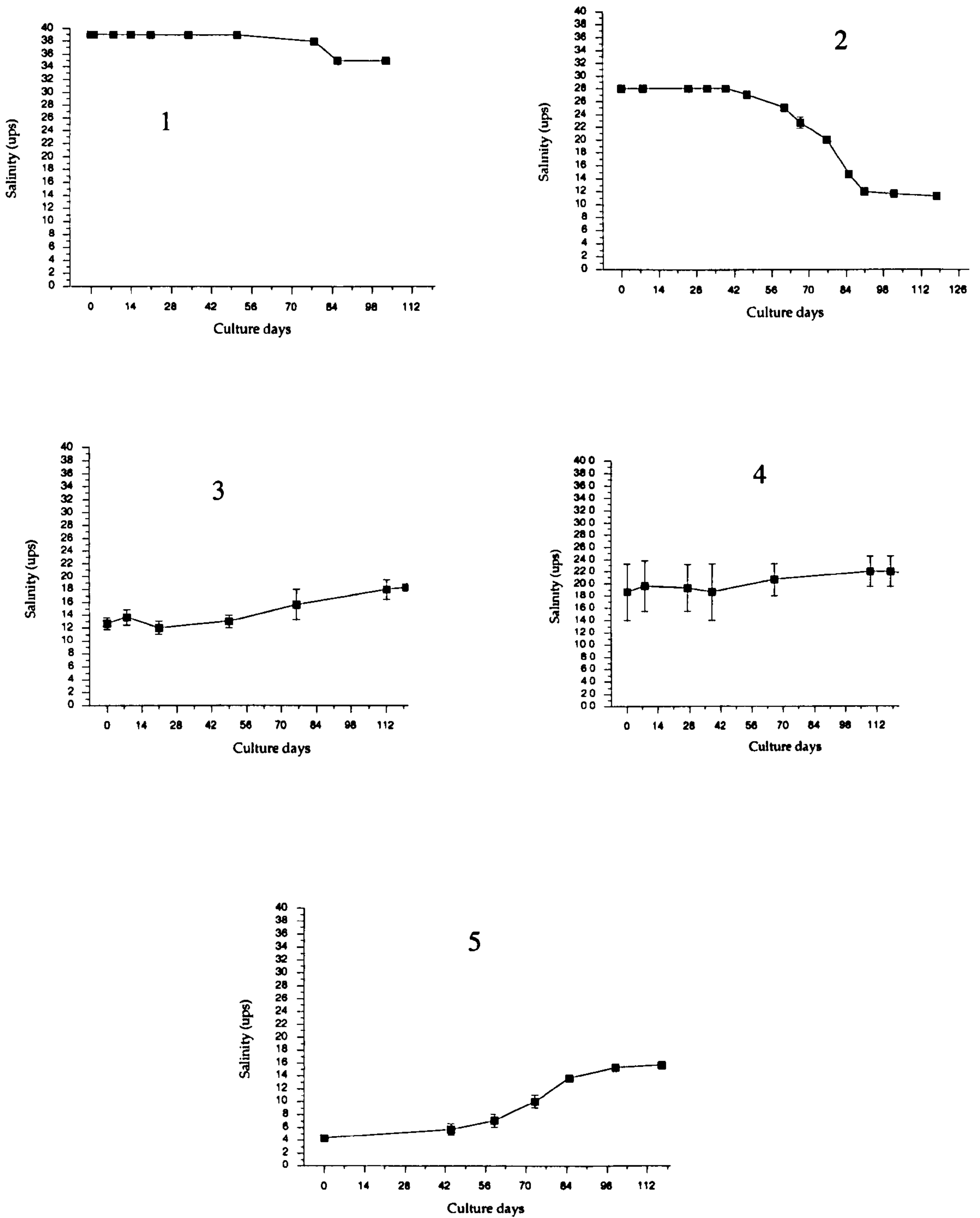


Fig.4. 8 Mean salinity (psu) in ponds from the five different culture periods with standard deviation bars.

1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

Temperature

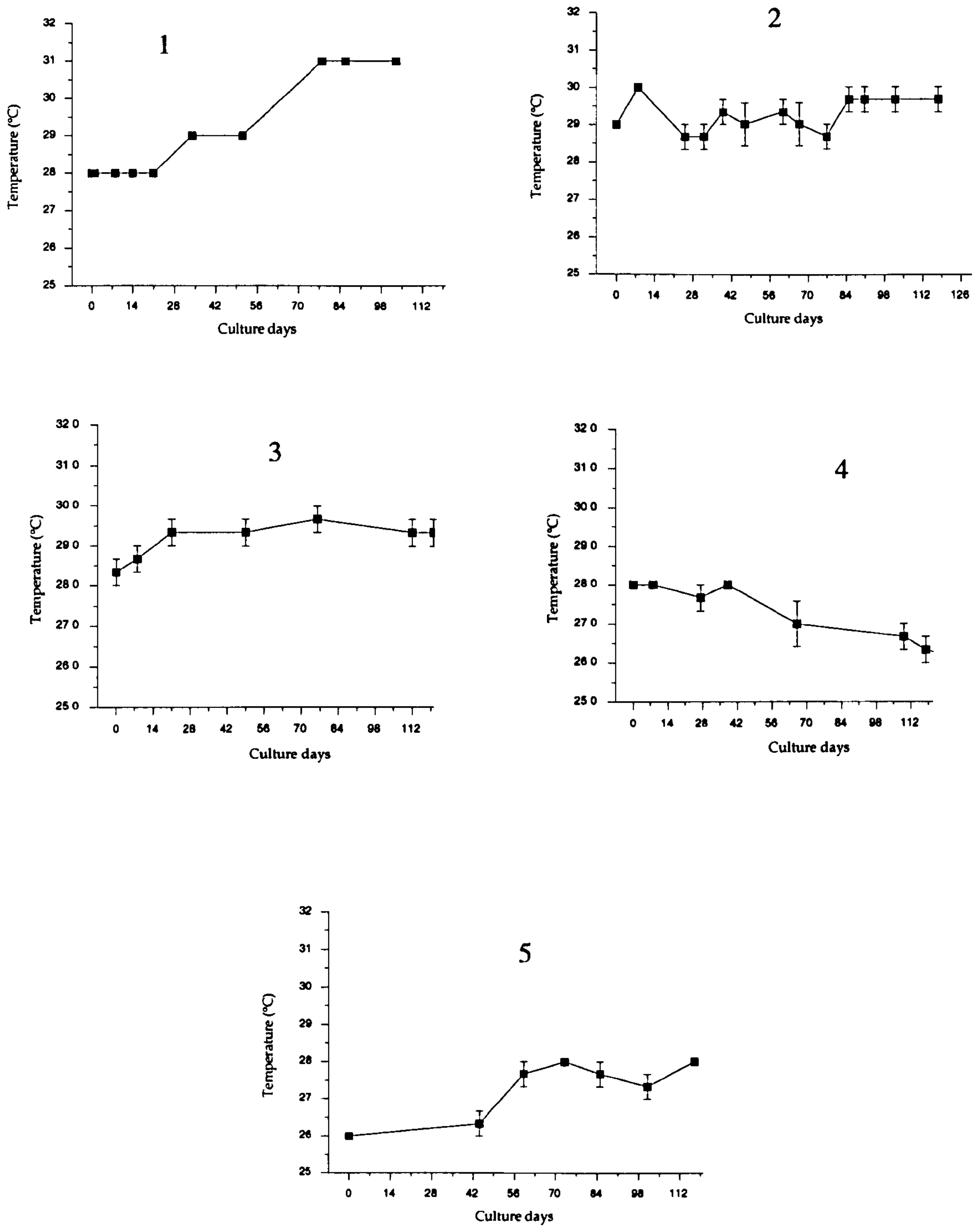


Fig.4. 9 Mean temperature (°C) in ponds from the five different culture periods with standard deviation bars.

1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

pH

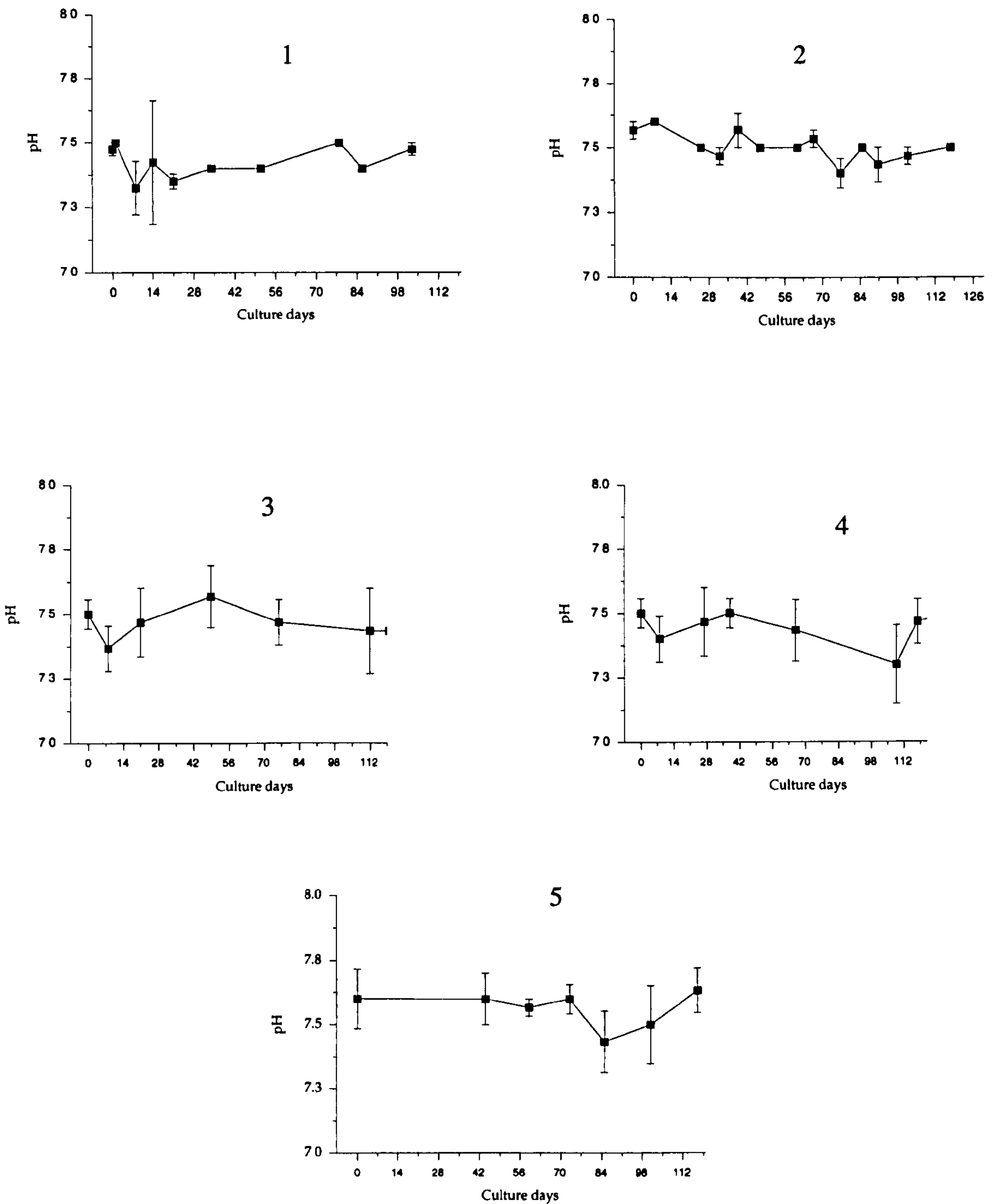


Fig.4. 10 Mean pH variation in ponds from the five different culture periods with standard deviation bars.

1: Nov96 – Mar 1997; 2: Feb – Jun 1997; 3: Jul 97 – Jan 98; 4: Sep 98 – Feb 99 ; 5: Mar – Jul 99

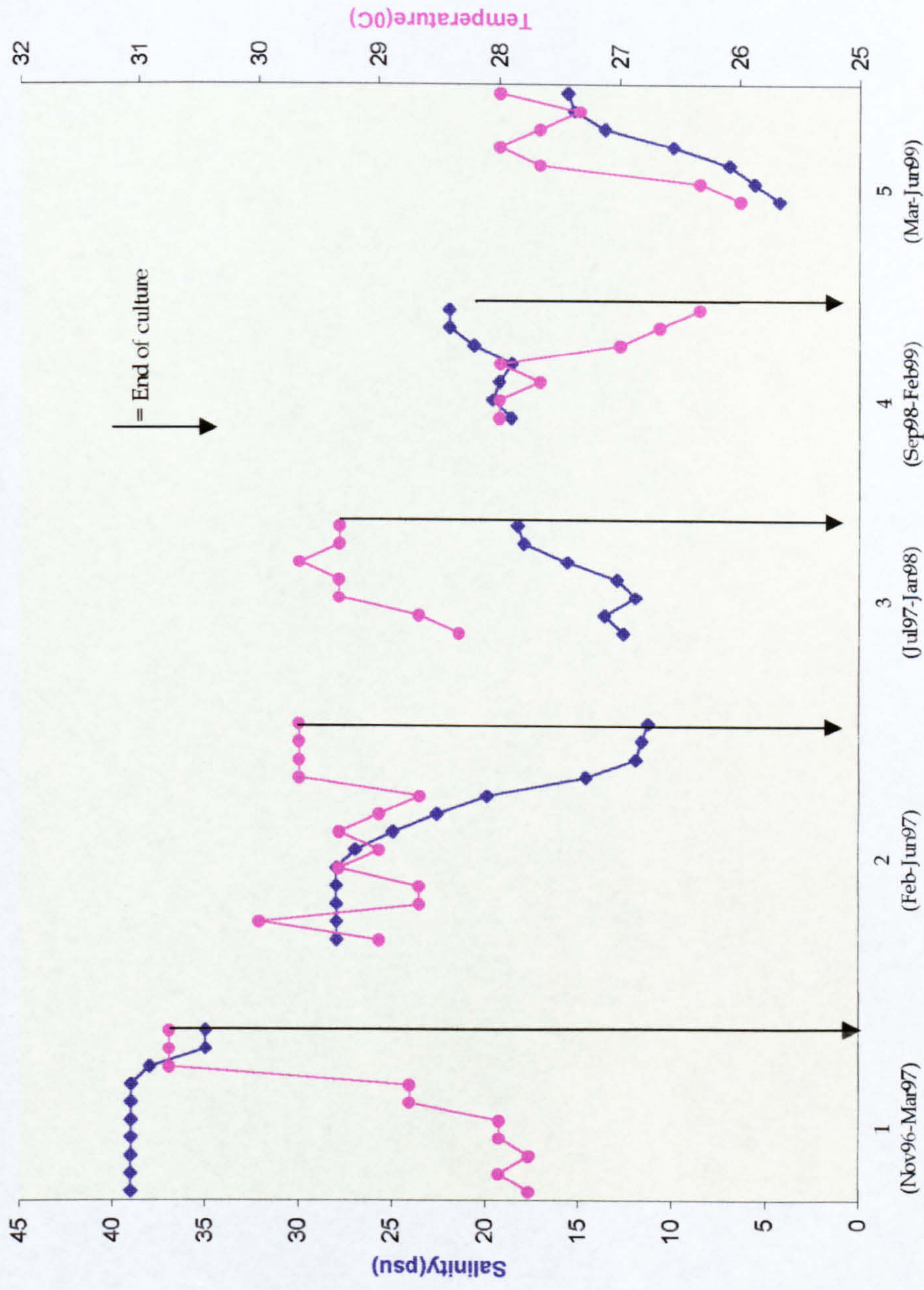


Fig.4. 11 Mean salinity and temperature in shrimp culture ponds during each culture period (1-5).

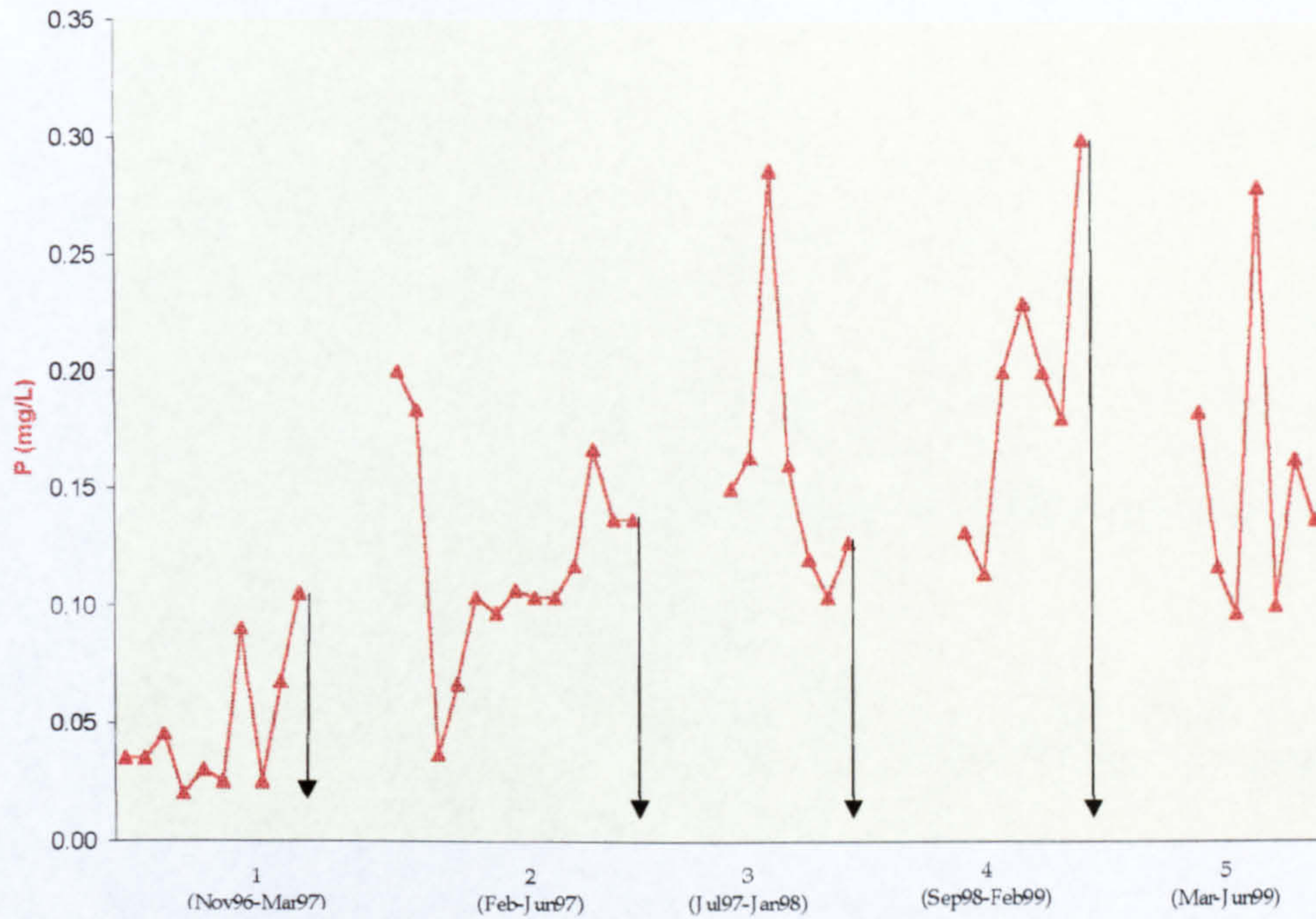


Fig.4. 12 Mean phosphorus in shrimp culture ponds during each culture period (1-5).

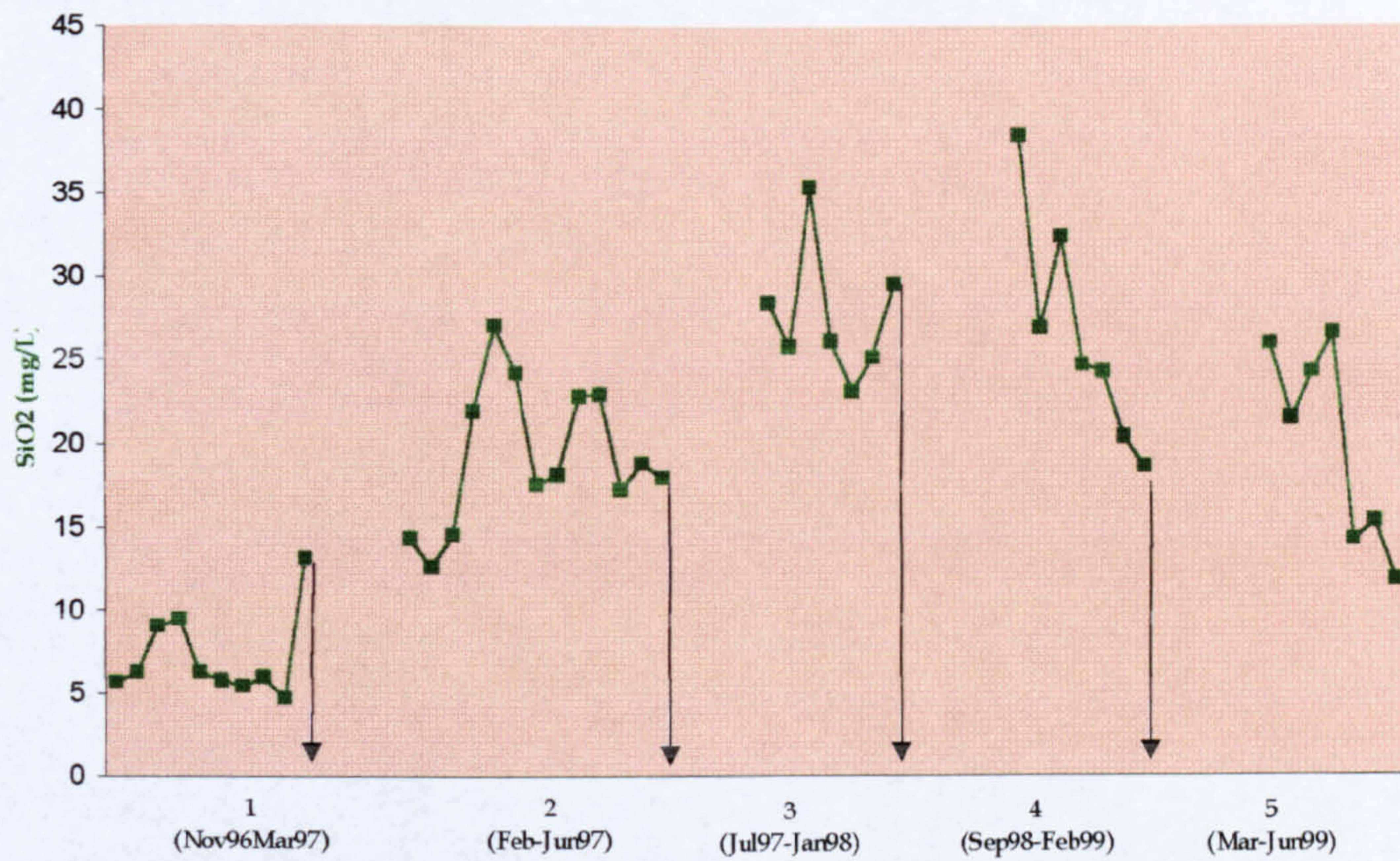


Fig.4. 13 Mean silica in shrimp culture ponds during each culture period (1-5).

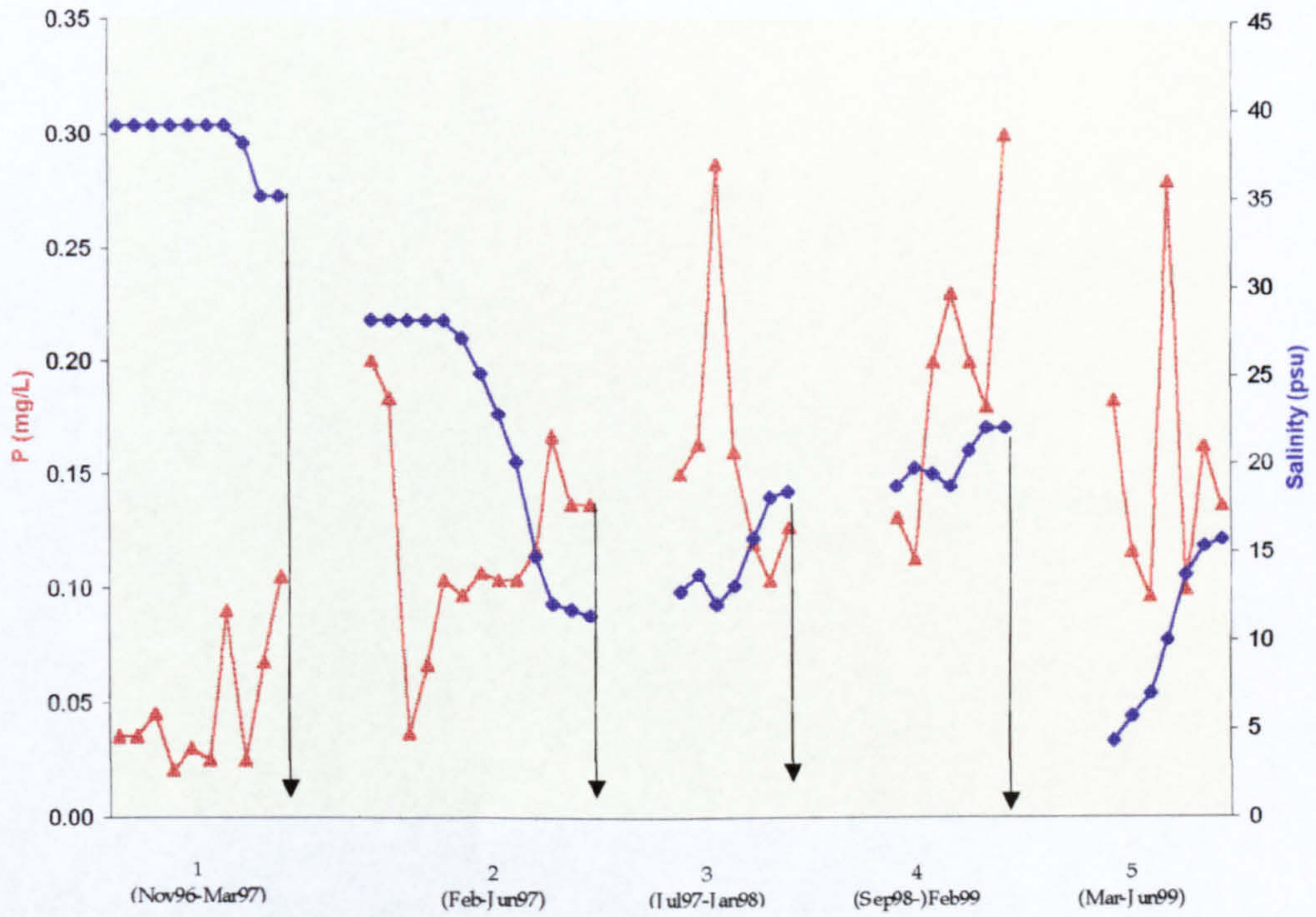


Fig.4. 14 Mean phosphorus and salinity in shrimp culture ponds during each culture period (1-5).

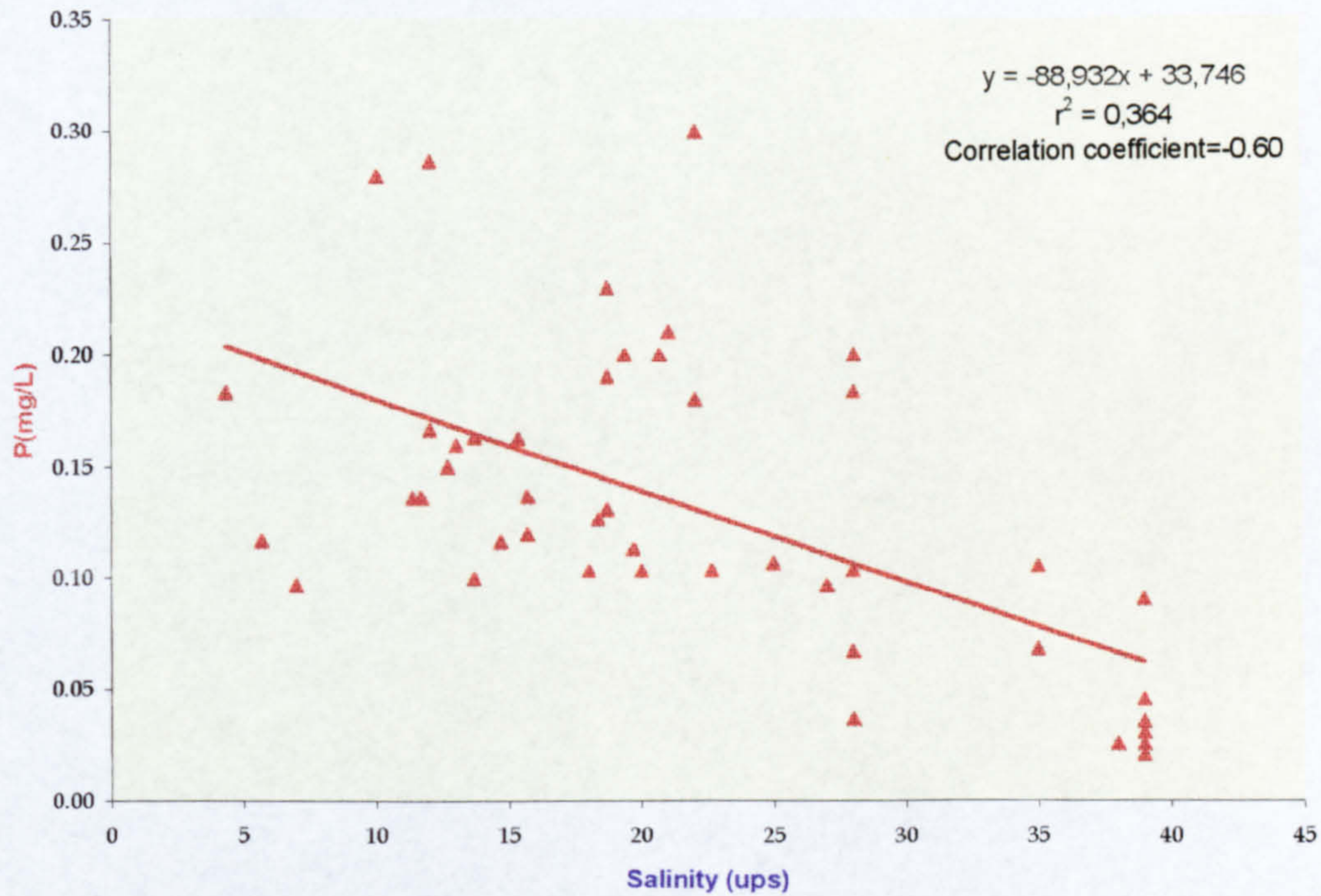


Fig.4. 15 Relation between mean phosphorus and salinity. Pearson product moment correlation coefficient was - 0.60 ($r^2=0.364$) indicating a statistically significant negative correlation ($P<0.05$).

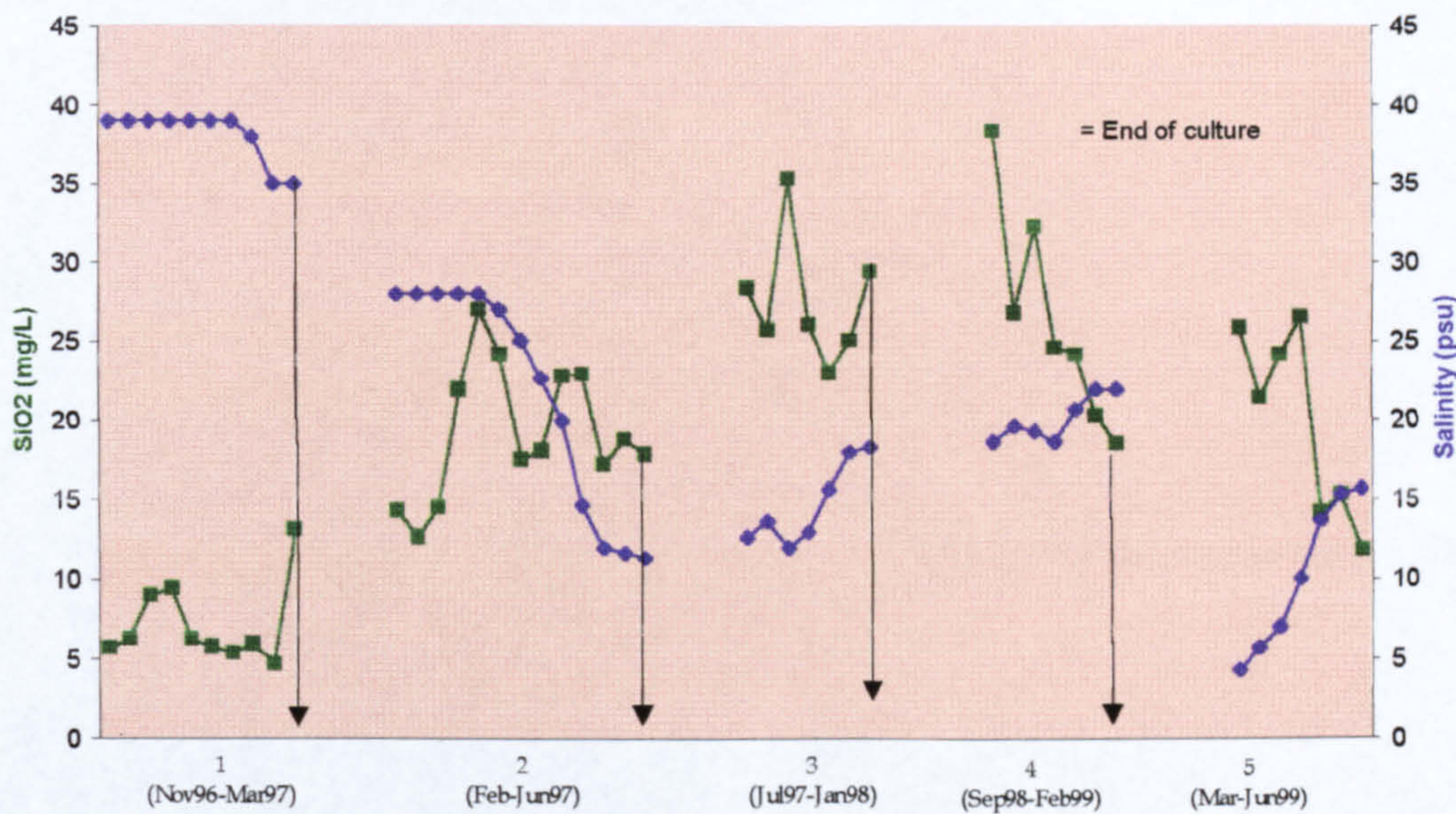


Fig.4. 16 Mean silica and salinity in shrimp culture ponds during each culture period (1-5).

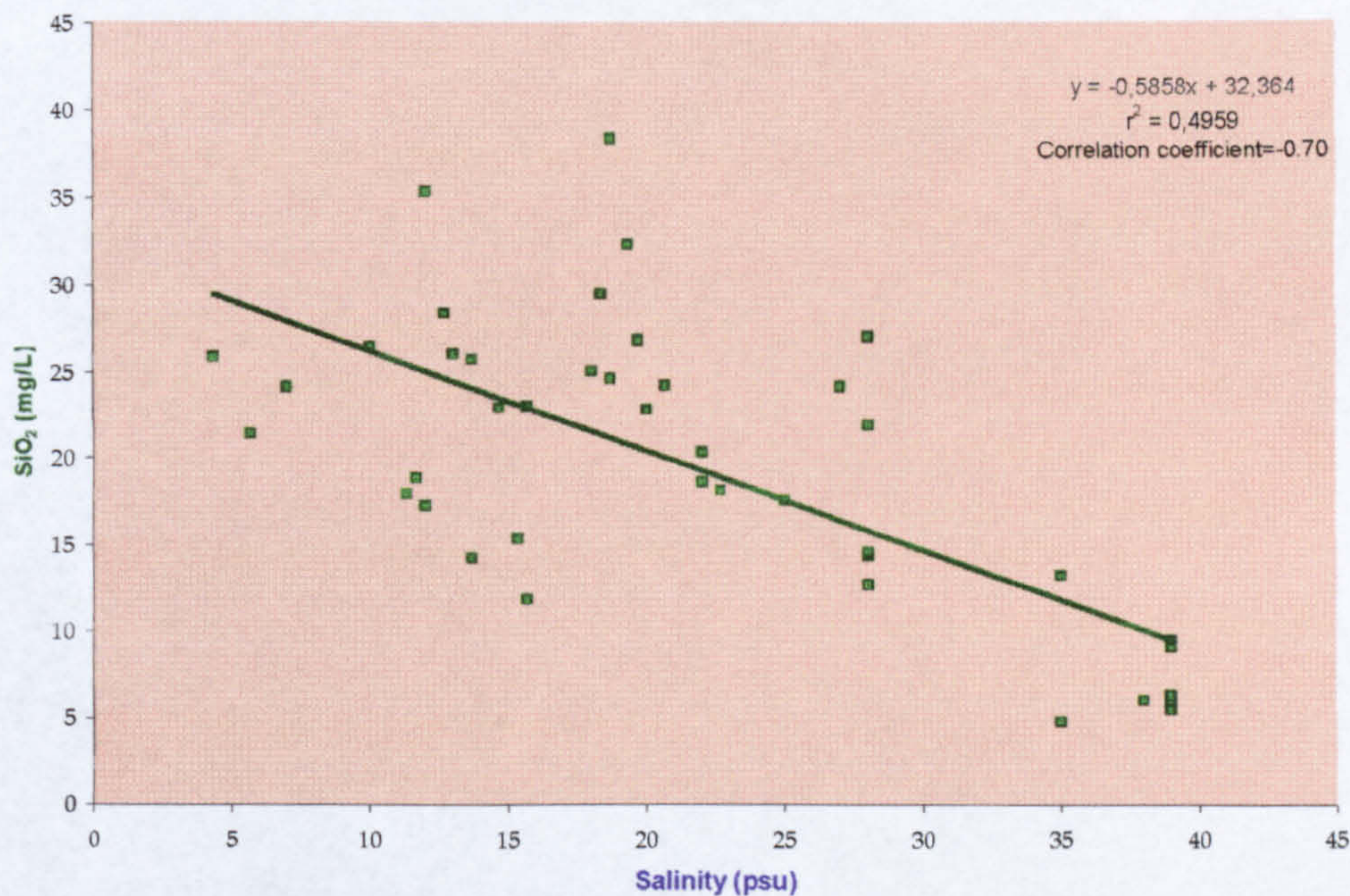


Fig.4. 17 Relation between mean silica and salinity. Pearson product moment correlation coefficient was - 0.70 ($r^2=0.49$) indicating a statistically significant negative correlation ($P<0.05$).

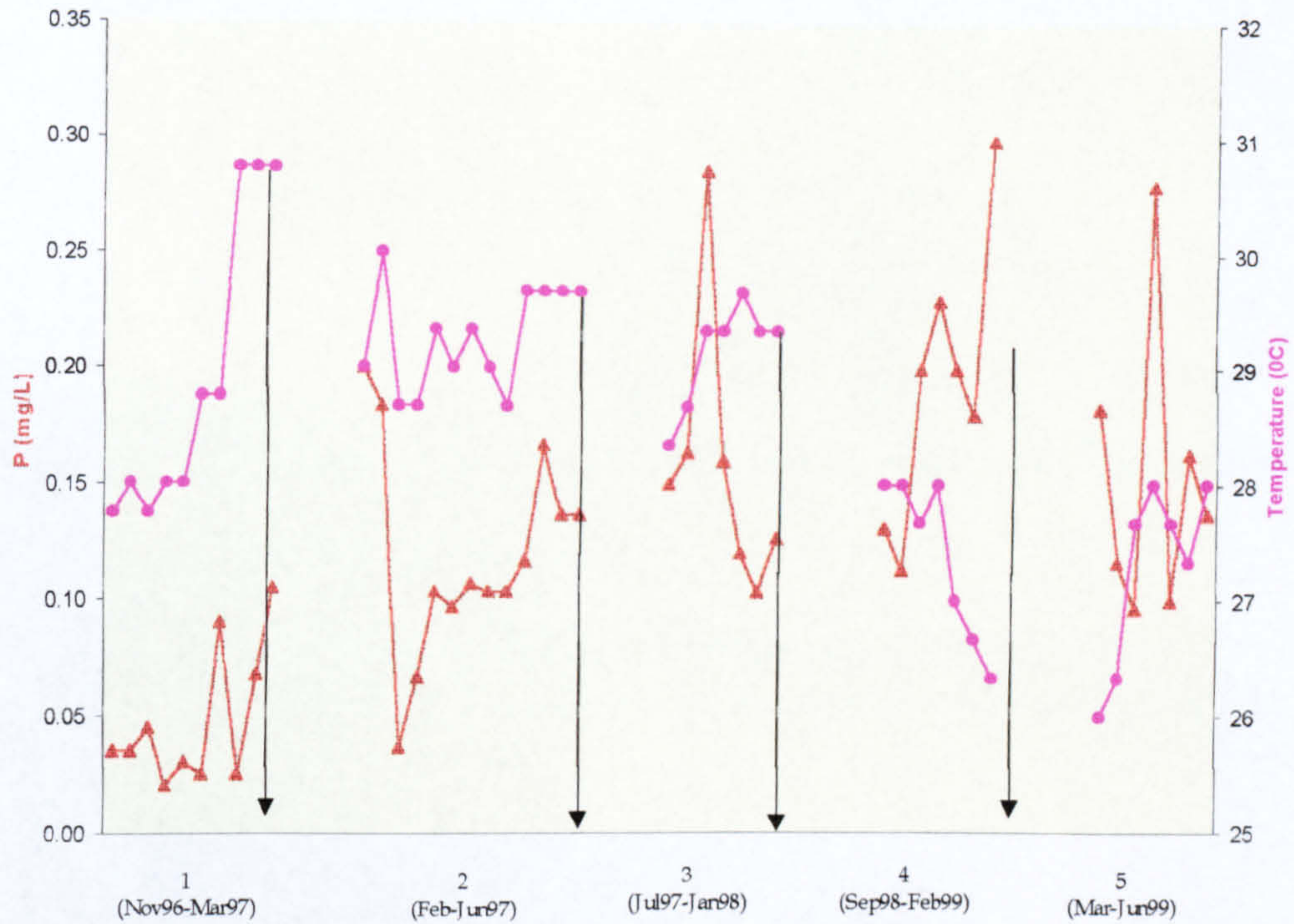


Fig.4. 18 Mean phosphorus and temperature in shrimp culture ponds during each culture period (1-5).

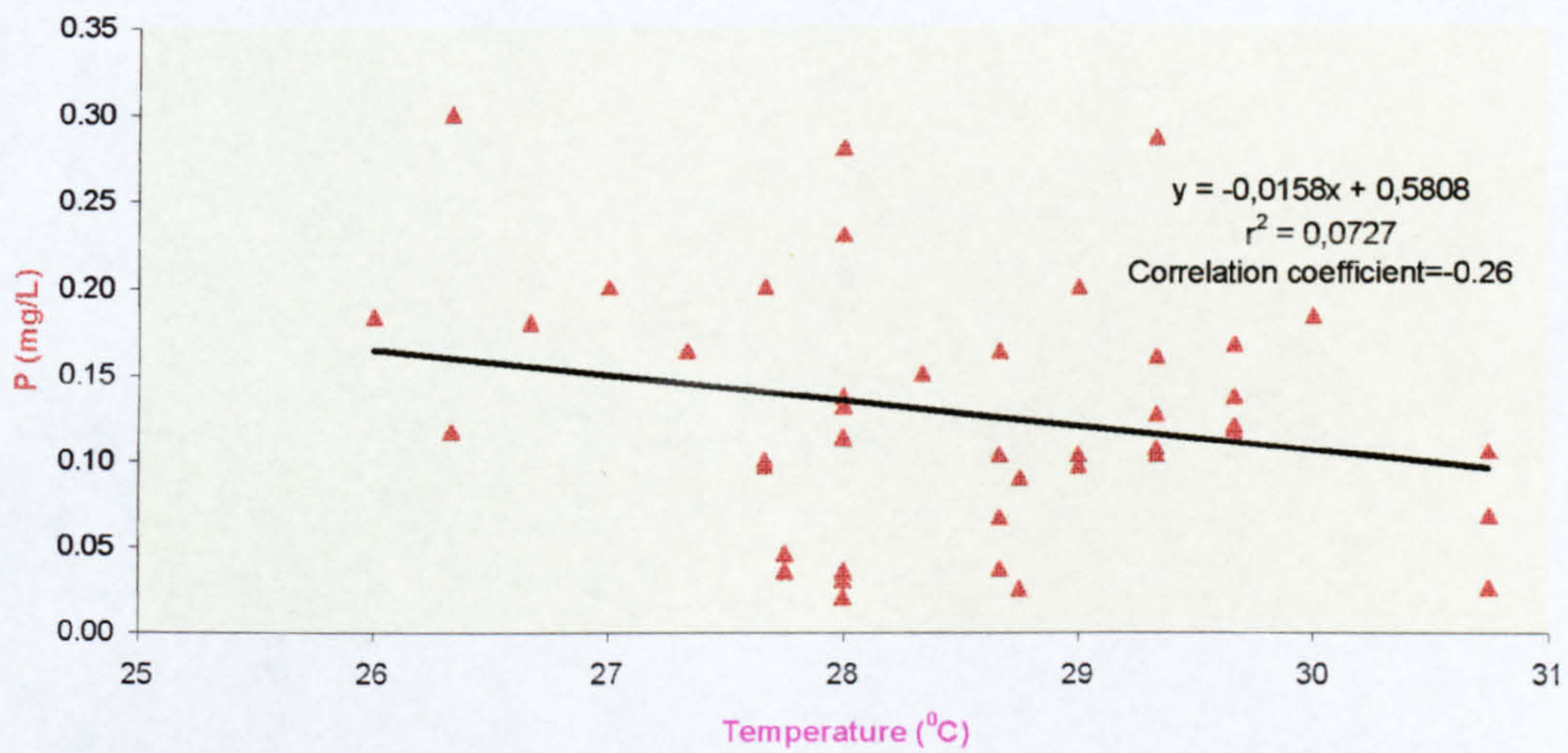


Fig.4. 19 Relation between mean phosphorus and temperature. Pearson product moment correlation coefficient was - 0.26 ($r^2=0.07$) indicating a non statistically significant correlation.

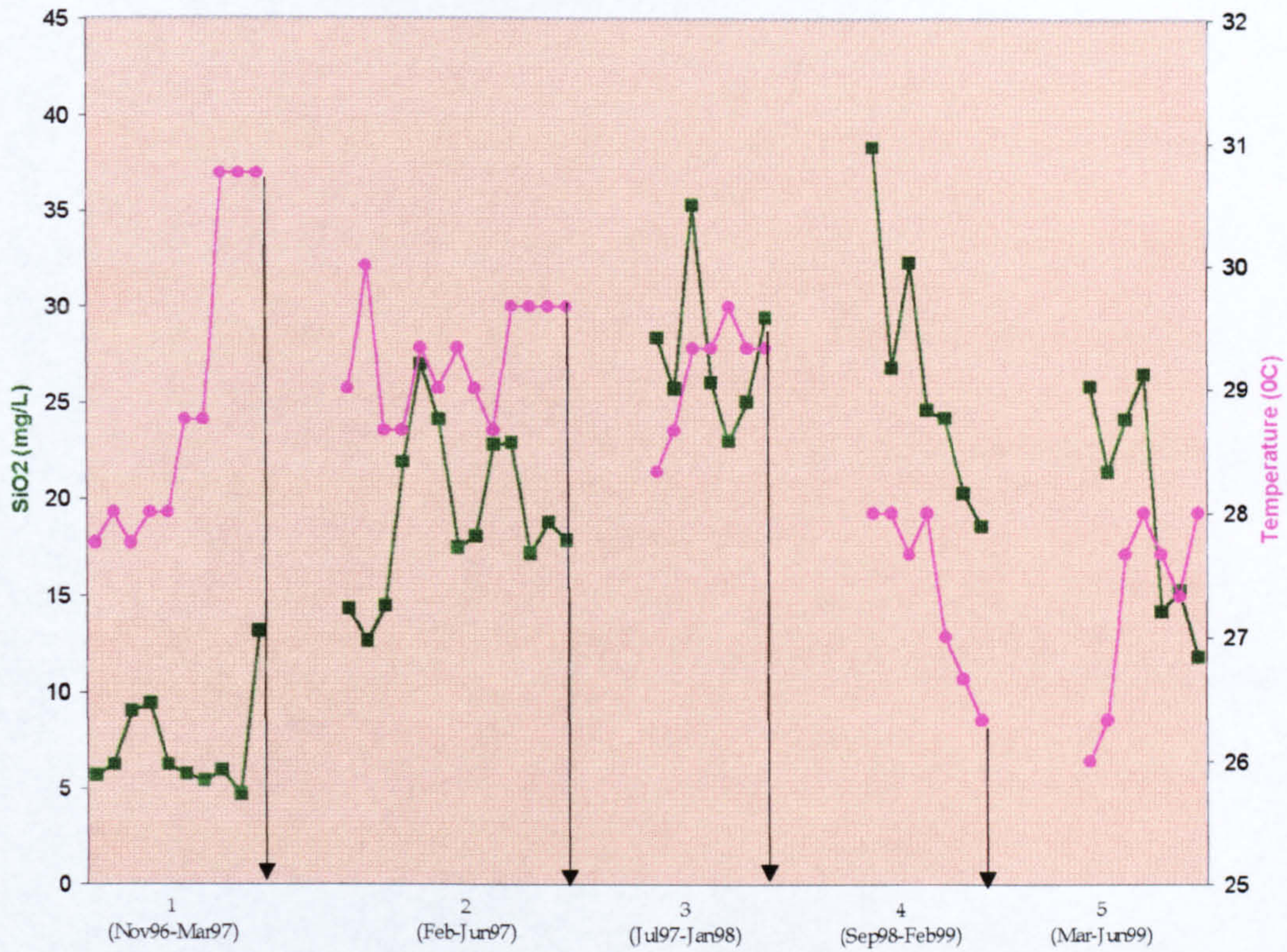


Fig.4. 20 Mean silica and temperature in shrimp culture ponds during each culture period (1-5).

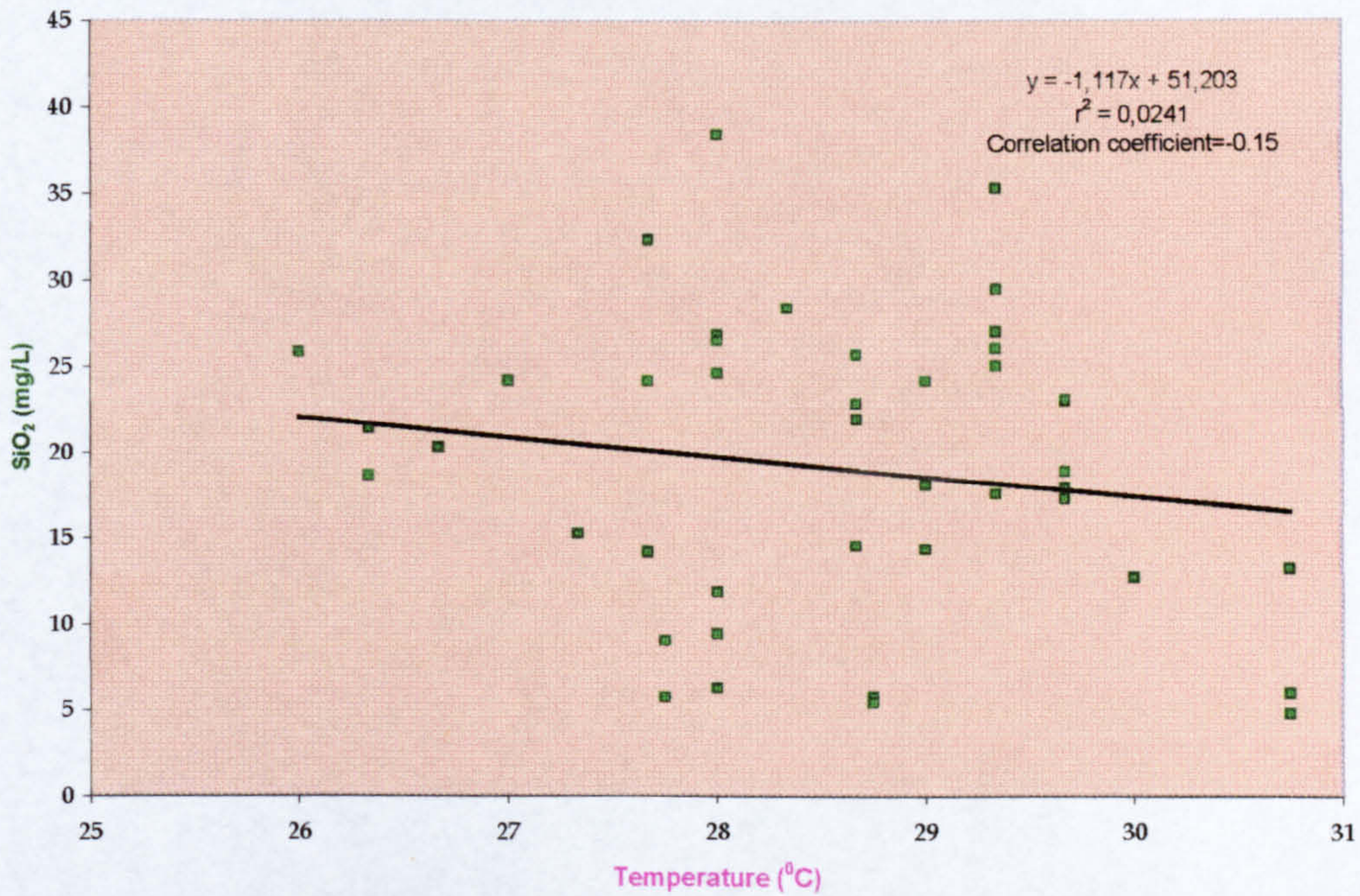


Fig.4. 21 Relation between mean silica and temperature. Pearson product moment correlation coefficient was - 0.15 ($r^2=0.02$) indicating a non statistically significant correlation.

Water quality discussion

The culture ponds selected for this study were about the same size, and, they were managed similarly, in terms of pond preparation, stocking density, type of feed used, feeding schedule and water exchange. Because of their close physical proximity, they were also subject to the same climatic conditions.

Water quality parameters recorded during the different periods of culture indicated acceptable levels for the culture of *Litopenaeus vannamei* and other species in semi-intensive culture conditions as nitrite, unionised ammonia, sulphide, nitrate, (Table 4.2), pH and total suspended solids were within those given by a number of authors (Shigueno, 1972; Chen, 1985; Boyd, 1989, 1990; Tsai, 1990; Boyd and Fast, 1992; Boyd *et al.*, 1994; Boyd and Tucker, 1998).

Significant temporal differences ($P < 0.05$) over the El Niño, La Niña and transition periods were found for the pond phosphorus and silica concentrations and for the temperature and salinity values.

During the first period of culture (Transition Niña - Niño), the temperature in ponds was around 28 °C and rose to 31 °C in the last month of culture. This coincided with the highest mean value of salinity, 38 psu, due to the dry season and the water evaporation processes in the ponds.

Higher temperatures during the second period of shrimp culture (30 - 29 °C, Feb - Jun 97) coincided with intermediate salinities generated by heavy rains of the strong El Niño event developing in most of 1997.

Table 4.2. Water quality ranges (nitrite, unionised-ammonia, sulphide and nitrate) by shrimp species under different culture conditions.

Nitrite (NO ₂ -N)	Specie (s)	Culture conditions	Water quality range	Author (s)
	<i>Penaeus monodon</i> (post-larvae)	Bioassay	0.36 mg/L	Tookwinas, 1984
	<i>Penaeus penicillatus</i>	Super intensive	0.013 –0.844 mg/L	Chen <i>et al.</i> , 1988
	<i>Penaeus monodon</i>	Semi intensive	0.48 mg/L	Kuo and Ting, 1989
	<i>Penaeus monodon</i> (juvenile)	Bioassay	3.8 mg/L	Chen and Lei, 1990
	<i>Penaeus penicillatus</i> (juvenile)	Bioassay	2.04 – 2.28 mg/L	Chen and Lin, 1991
	<i>Penaeus monodon</i>	Intensive	0.0 – 0.409 mg/L	Yont <i>et al.</i> , 1995
	<i>Penaeus penicillatus</i>	Bioassay	0.16 mg/L	Gao <i>et al.</i> , 1994
	<i>Penaeus paulensis</i>	Bioassay	MLC %H 109.4 mg/L	Cavalli <i>et al.</i> , 1996
	<i>Penaeus indicus</i>	Semi-intensive	0.56 – 8.67 µgat/L	Gopalakrishnan <i>et al.</i> , 1997
	<i>Penaeus monodon</i>	Semi-intensive	0.002 – 0.046 mg/L	Mohanty and Mohanty 2001
	<i>Penaeus monodon</i> and <i>Penaeus indicus</i>	Semi-intensive	0.05 – 0.25 mg/L	Mitra and Patra, 2001

Unionized ammonia (NH₃-N)

Specie (s)	Culture conditions	Water quality range	Author (s)
<i>Penaeus monodon</i> (post-larvae)	Bioassay	0.13 mg/L	Chin and Chen, 1987
<i>Penaeus monodon</i>	Super-intensive	0.022- 46.110 mg/L	Chen <i>et al.</i> , 1988
<i>Penaeus monodon</i> (juvenile)	Bioassay	0.21 mg/L	Allan <i>et al.</i> , 1990
<i>Penaeus monodon</i> (juvenile)	Bioassay	0.1 mg/L	Chen and Lei, 1990
<i>Penaeus monodon</i> (post-larvae)	Bioassay	0.060 – 0.183 mg/L	Chen <i>et al.</i> , 1990
<i>Penaeus penicillatus</i> (juvenile)	Bioassay	0.08 – 0.09	Chen and Lin, 1991
<i>Penaeus monodon</i> (post-larvae)	Bioassay	0.032 mg/L	Chen and Tu, 1991
<i>Penaeus chinensis</i> (juvenile)	Bioassay	0.16 – 0.22 mg/L	Chen and Lin, 1992 a
<i>Penaeus penicillatus</i> (juvenile)	Bioassay	LC ₅₀ 0.61 mg/L	Chen and Lin, 1992 b
<i>Penaeus monodon</i>	Intensive	0.0021 – 0.8676 mg/L	Yont <i>et al.</i> , 1995
<i>Penaeus paulensis</i>	Bioassay	0.03 mg/L	Ostrensky and Waisielesky, 1995
<i>Penaeus paulensis</i> (juvenile)	Bioassay	MLC %H 34.360 mg/L	Cavalli <i>et al.</i> , 1996
<i>Penaeus indicus</i>	Semi-intensive	1.2 – 9.6 µgat/L	Gopalakrishnan <i>et al.</i> , 1997
<i>Penaeus setiferus</i>	Bioassay	LC ₅₀ 1.12 – 1.49 mg/L	Alcaraz <i>et al.</i> , 1999
<i>Penaeus indicus</i>	Semi-extensive and semi-intensive	0.3 – 85.0 µgat/L	Gopalakrishnan and Ramadhas, 1999
<i>Litopenaeus vannamei</i> (post-larvae)	Bioassay	0.0 - 0.02 mg/L	Frias-Espeticueta <i>et al.</i> , 2000
<i>Litopenaeus vannamei</i>	Bioassay	0.0 - 0.05 mg/L	Argue <i>et al.</i> , 2001

<i>Penaeus monodon</i> and <i>Penaeus indicus</i>	Semi-intensive	0.0 – 0.5 mg/L	Mitra and Patra, 2001
<i>Litopenaeus vannamei</i>	Bioassay	0.16 – 0.16 mg/L	Lin and Chen, 2001
<i>Penaeus monodon</i>	Semi-intensive	0.002 – 0.12 mg/L	Mohanty and Mohanty, 2001
Sulphide (S⁼)			
Specie (s)			
<i>Penaeus monodon</i>	Semi-intensive	0.02 – 0.16 mg/L	Mitra and Patra, 2001
<i>Penaeus monodon</i>	Semi-intensive	0.01 - 0.25 mg/L	Mohanty and Mohanty 2001
Nitrate (NO₃-N)			
Specie (s)			
<i>Penaeus monodon</i>	Intensive	0.0 – 0.0263 mg/L	Yont <i>et al.</i> , 1995
<i>Penaeus paulensis</i>	Bioassay	MLC % H 2171.1 mg/L	Cavalli <i>et al.</i> , 1996
<i>Penaeus indicus</i>	Semi-intensive	10.4 – 65.90 µgat/L	Gopalakrishnan <i>et al.</i> , 1997
<i>Penaeus penicillatus</i>	Super-intensive	0.021 – 1.795 mg/L	Chen et al, 1988
<i>Penaeus monodon</i>	Semi-intensive	0.0	Mitra and Patra, 2001

The temperature during the third period of culture (Jul 97 – Jan 98) remained at 28-29 °C, but salinities fell to 12-18 psu because of heavy rainfalls.

In the second half of 1998, (Transition Niño – Niña) salinities were in the intermediate range 18 – 22 psu and the temperature began to decrease sharply at the end of this period.

The last period of culture, during a La Niña event, represented the lowest temperature and salinity recorded (26 °C and 4 – 15 psu respectively) due to the cool - wet season at the time.

In this study, the results obtained from natural conditions in ponds with respect to the growth and survival of shrimp during the period of the El Niño were in accordance with data obtained from experimental laboratory procedures showing that *L. vannamei* survive and grow well in intermediate salinities (20 psu) (Barlett *et al.*, 1990; Wyban *et al.*, 1995; Ponce-Palafox *et al.*, 1997). As noted early, the recorded temperature ranges for successful growth and survival of *L. vannamei* in laboratory conditions are 28 – 30 °C (Ponce-Palafox *et al.*, 1997).

Phosphorus and silica showed an increase on average from the second period of culture Feb 97 - Jun 97 through Sep98 - Feb 99, (0.12 mg/L – 0.19 mg/L, P ; 19.2 – 27.7 mg/L , SiO₂), because of the warm rainy season at that time and the increase in the water level from the Chone River.

Erosion caused by rainfall and the runoff of streams removes phosphorus and silica from rock substrates. This results in a phosphorus and silica supply in the soil which is available to plants. The phosphorus and silica are absorbed by phytoplankton or plant roots and are used to make organic compounds. As animals eat the plants, the phosphorus is passed on to them. Decomposing plant or animal tissue and animal droppings return organic forms of phosphorus or silica to the water and soil.

Silica is the major skeletal constituent of diatoms. Before an El Niño, the shrimp ponds were found to have a higher concentration of diatoms, with reduced free silica levels of 7.16 mg/L. During El Niño and even after the transition period Niño – Niña , however they had fewer numbers of diatoms and higher levels of free silica (19 – 27 mg/ l), and a high number of blue-green algae because the relation between inorganic N: inorganic P was low (described further in section 4.2).

4.2 Phytoplankton

Phytoplankton depend upon sunlight, water and nutrients to survive. Physical or chemical changes in any of these requirements over time for a given location will affect the phytoplankton concentrations there. Thus, populations of these marine plants will grow or diminish rapidly in response to changes in their environment. Conversely, changes in the trends for a given phytoplankton population, such as its density, areal distribution, and rate of population growth or diminishment, indicate that environmental conditions are changing there. Then, by comparing these phytoplankton trends to other measurements, such as temperature or salinity, we can learn more about how phytoplankton may be contributing to, and affected by environmental change, including climatic conditions.

Twenty eight genera of phytoplankton were identified from the shrimp culture pond water during the study of the five culture periods.

Chrysophytes (brown algae) : *Chaetoceros, Cocconeis, Cyclotella, Diploneis, Leptocylindrus, Melosira, Navicula, Nitzschia, Pleurosigma, Rhizosolenia, Thalassiosira, Thalassiotrix.*

Cyanophytes (blue-green algae) : *Anabaena, Anabaenopsis, Chroococcus, Oscillatoria, Merismopedia, Raphidiopsis, Romeria, Spirulina.*

Chlorophytes (green algae) : *Ankistrodesmus, Cosmarium, Chlorella,*

Closterium, Coclastrum, Netrium, Phytelios, Spondylosium.

4.2.1 Period 1. Transition Niña- Niño

The phytoplankton composition during the first grow-out season November 1996 – March 1997 (Transition Niña – Niño) showed, on average, similar quantities of Chrysophytes, (*Chaetoceros spp., Navicula spp., Melosira spp.*), and Cyanophytes (*Chroococcus sp., Oscillatoria spp., Anabaena spp.*) (Fig. 4.22; 4.23). The salinity in this period ranged from 39 – 35 psu and the temperature from 28-31 °C, (Figs 4.8.1 and 4.9.1). Diversity indices and abundance of genera in this period showed no dominance of any particular genus and also showed an equitable presence of genera (Figs 4.32, 4.33 and 4.34) .

4.2.2 Period 2. Niño

In the second period , February 1997 – June 1997 , the dominant algal classes were Cyanophytes, mainly *Chroococcus sp., Oscillatoria spp., Spirulina sp., Anabaena sp. and Merismopdeia sp.* (Figs 4.24 and 4.25). The salinity was between 28 – 11 psu, and the temperature was 28 – 30°C, (Figs 4.8.2 and 4.9.2). The diversity indices were high during this period of shrimp cultivation (El Niño), because there was an increase in the number of genera and their abundance showed good evenness (no dominance of any specific genus) (Figs 4.32, 4.33 and 4.34).

4.2.3 Period 3. Niño

During this period, July 1997 – January 1998, the diversity indices were highest because there were more phytoplankton genera and their abundance was equitable (high evenness), (Figs 4.32, 4.33 and 4.34). The main algal classes were Cyanophytes, mainly *Oscillatoria spp.*, *Chroococcus sp.*, *Spirulina sp.*, *Anabaena sp.*, *Merismopdeia sp.*, (Figs 4.26 and 4.27). The salinity range was between 12 – 19 psu, and temperature was in the range 28 – 30°C (Figs 4.8.3 and 4.9.3).

4.2.4 Period 4. Transition Niño – Niña

During the fourth growth-out season, September 1998 – February 1999 (transition Niño – Niña) the temperature was 26 – 28 °C and the salinity was 19 – 22 psu (Figs 4.9.4 and 4.8.4). The phytoplankton community was mainly constituted by *Oscillatoria spp.* (Figs 4.28 and 4.29). A reduced genera diversity index and a low number of genera with low evenness were found during this period of transition, (Figs 4.32, 4.33 and 4.34).

4.2.5 Period 5. Niña

In the fifth growth-out season, La Niña, March 1999 – July 1999, the salinity was 4 - 16 psu, and the temperature was 26 - 28 °C (Figs 4.8.5 and 4.9.5). During 1999 Cyanophytes, especially *Chroococcus sp.*, *Oscillatoria spp.*, and *Spirulina sp.*, were dominant (Figs 4.30 and 4.31). The diversity indices in

this period were similar to the first growth-out season transition Niña - Niño, associated with the number of genera present, but with a low evenness due to the dominance of *Oscillatoria spp.* (Figs 4.32, 4.33 and 4.34).

In summary, during the first period of shrimp culture there were similar quantities of Chrysophytes and Cyanophytes. Associated with a salinity average of 38 psu and temperature of 29 °C, there was no predominance of any particular genus of phytoplankton and also the abundance of the occurrence genera was equitable.

In culture periods 2 and 3, the pond phytoplankton were mainly Cyanophytes associated with low to medium salinity (11 – 28 psu) and high temperature (28 - 30°C) which resulting from the heavy rains of the strong El Niño which developed during most of 1997. Diversity indices for the phytoplankton were high during this period (El Niño), because there was an increase in the number of genera and their abundance was very even (no dominance of a specific genus).

During the fourth culture period in the second half of 1998, (transition Niño – Niña), pond salinities remained in the range 19 – 22 psu, but the temperature began to decrease sharply at the end of this period (to 26°C). The main phytoplankton members were still Cyanophytes, but their diversity indice was low in this period because of a decreased number of genera present and low evenness.

In the fifth period, the temperature stayed low (26°C) and salinity fell to 10 psu due to the cool – wet season (Niña). The diversity indices were similar to those in the first period (transition Niña – Niño) with a low evenness because of the dominance of one specific group of blue-green algae, *Oscillatoria spp.*

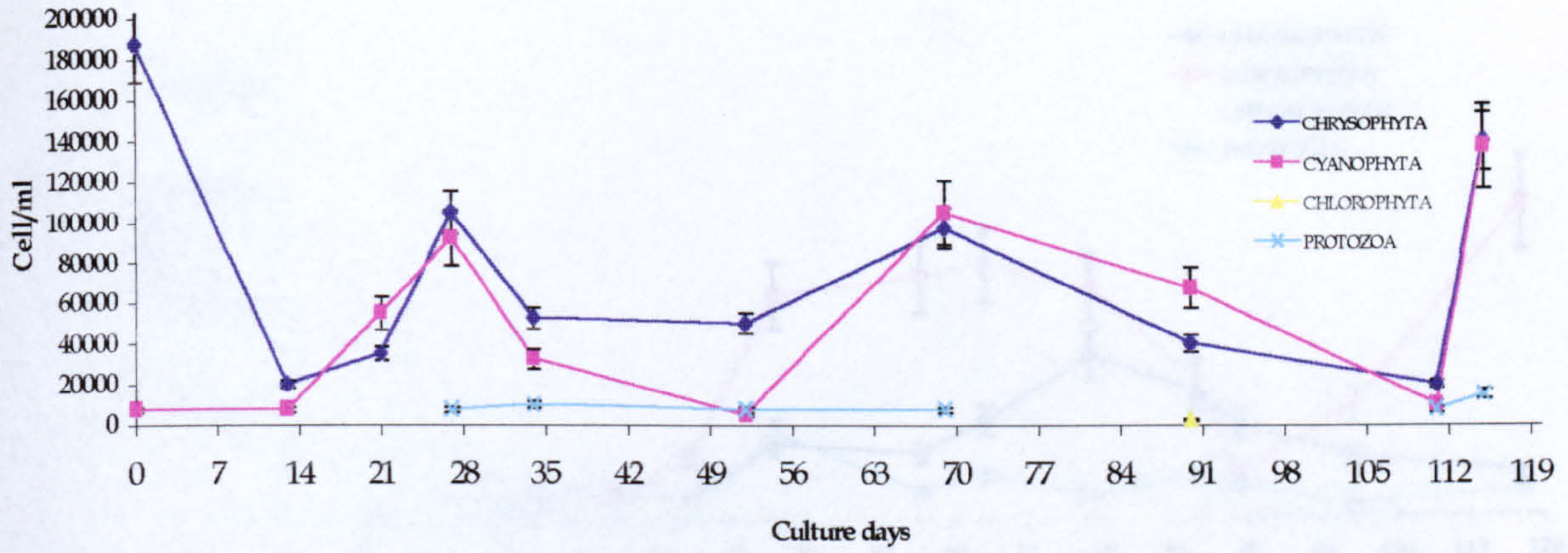


Fig.4. 22 Variation in the density (cell/ml) of the major groups of phytoplankton during the shrimp culture cycle. November 1996-March 1997.

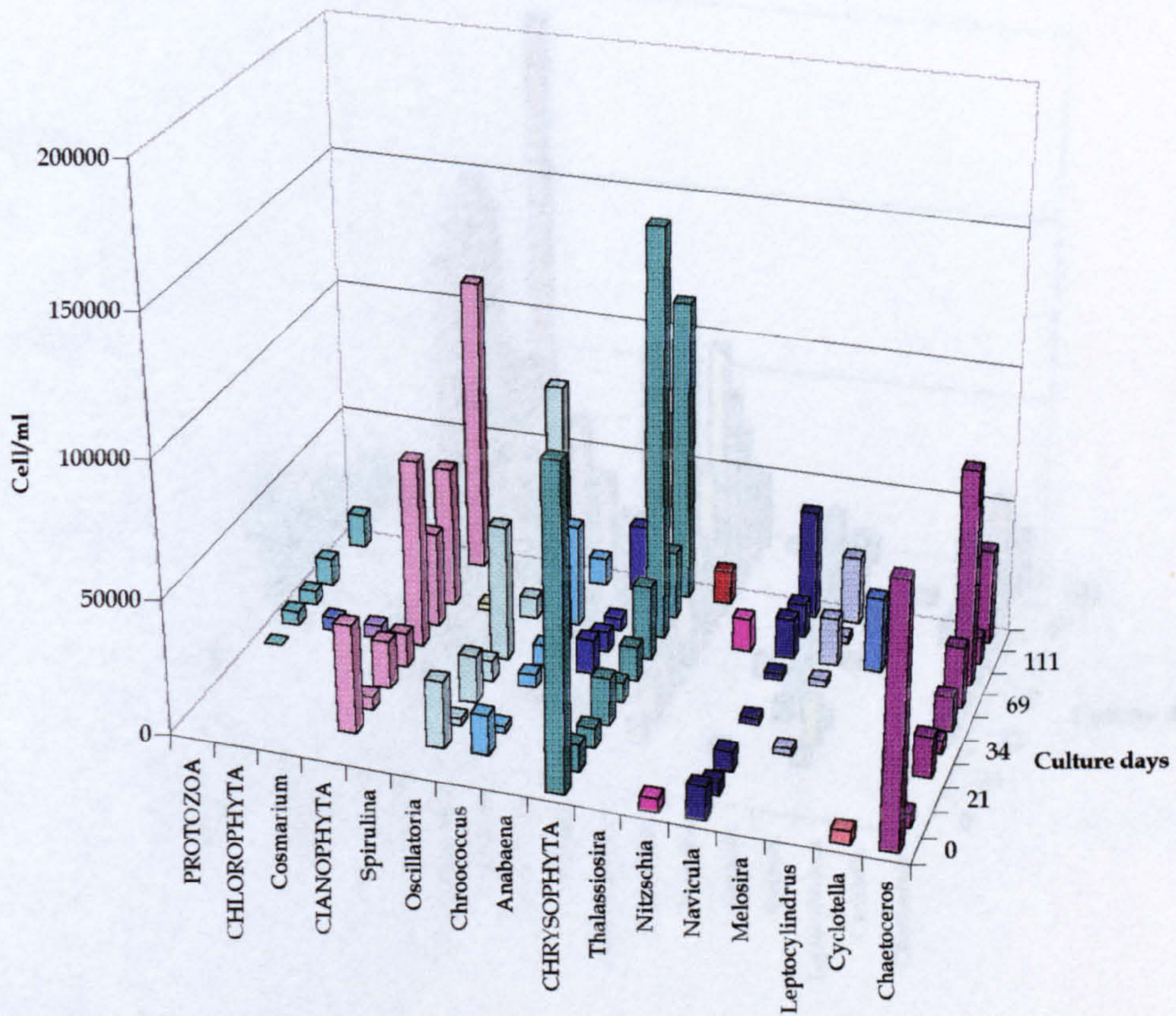


Fig.4. 23 Analysis of the main genera of phytoplankton present in the shrimp ponds November 1996 to March 1997.

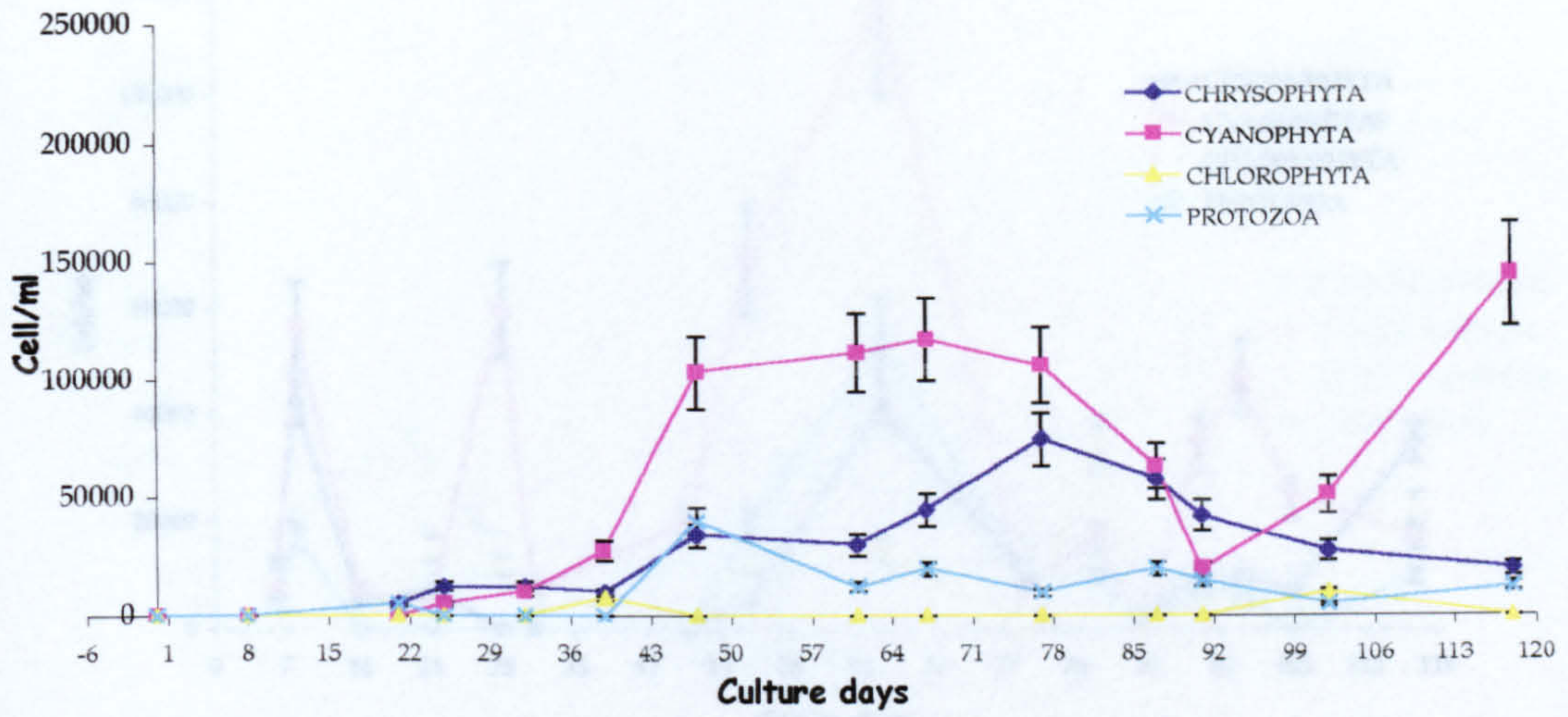


Fig.4. 24 Variation in the density (cell/ml) of the major groups of phytoplankton during the shrimp culture cycle February-June 1997.

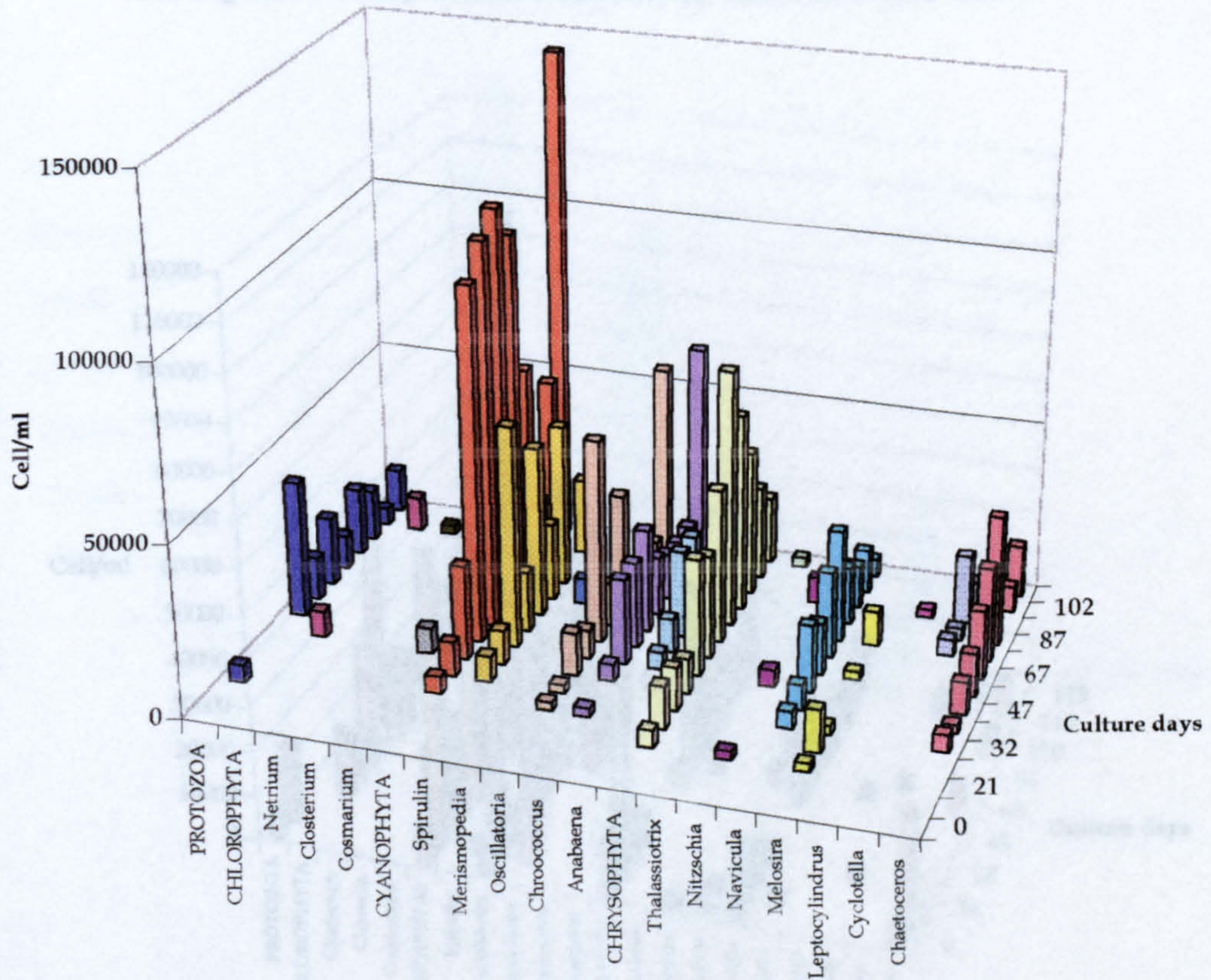


Fig.4. 25 Analysis of the main genera of phytoplankton present in the shrimp ponds February-June 1997.

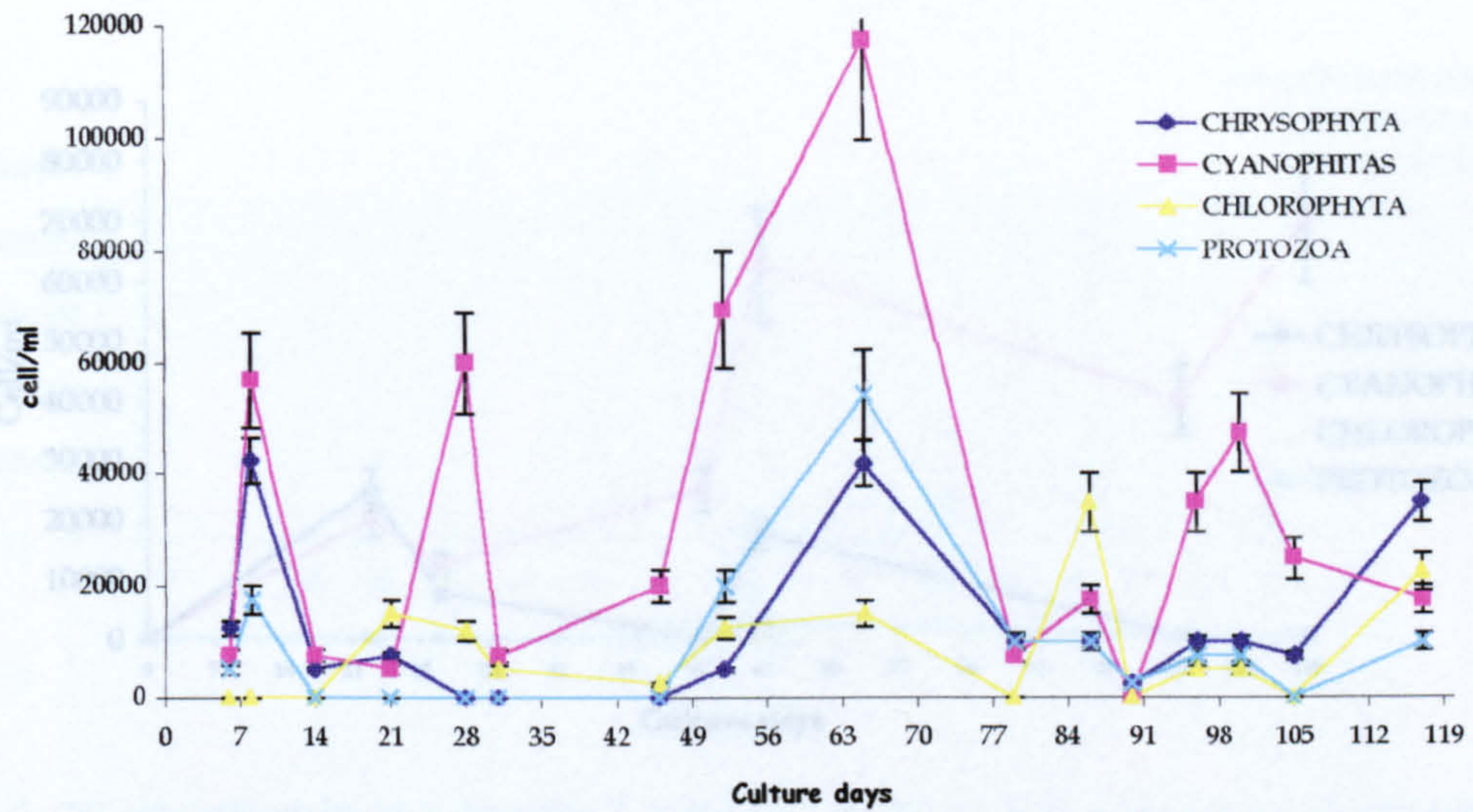


Fig.4. 26 Variation in the density (cell/ml) of the major groups of phytoplankton during the shrimp culture cycle December 1997-February 1998.

Fig.4. 26 Variation in the density (cell/ml) of the major groups of phytoplankton during the shrimp culture cycle July 1997-January 1998.

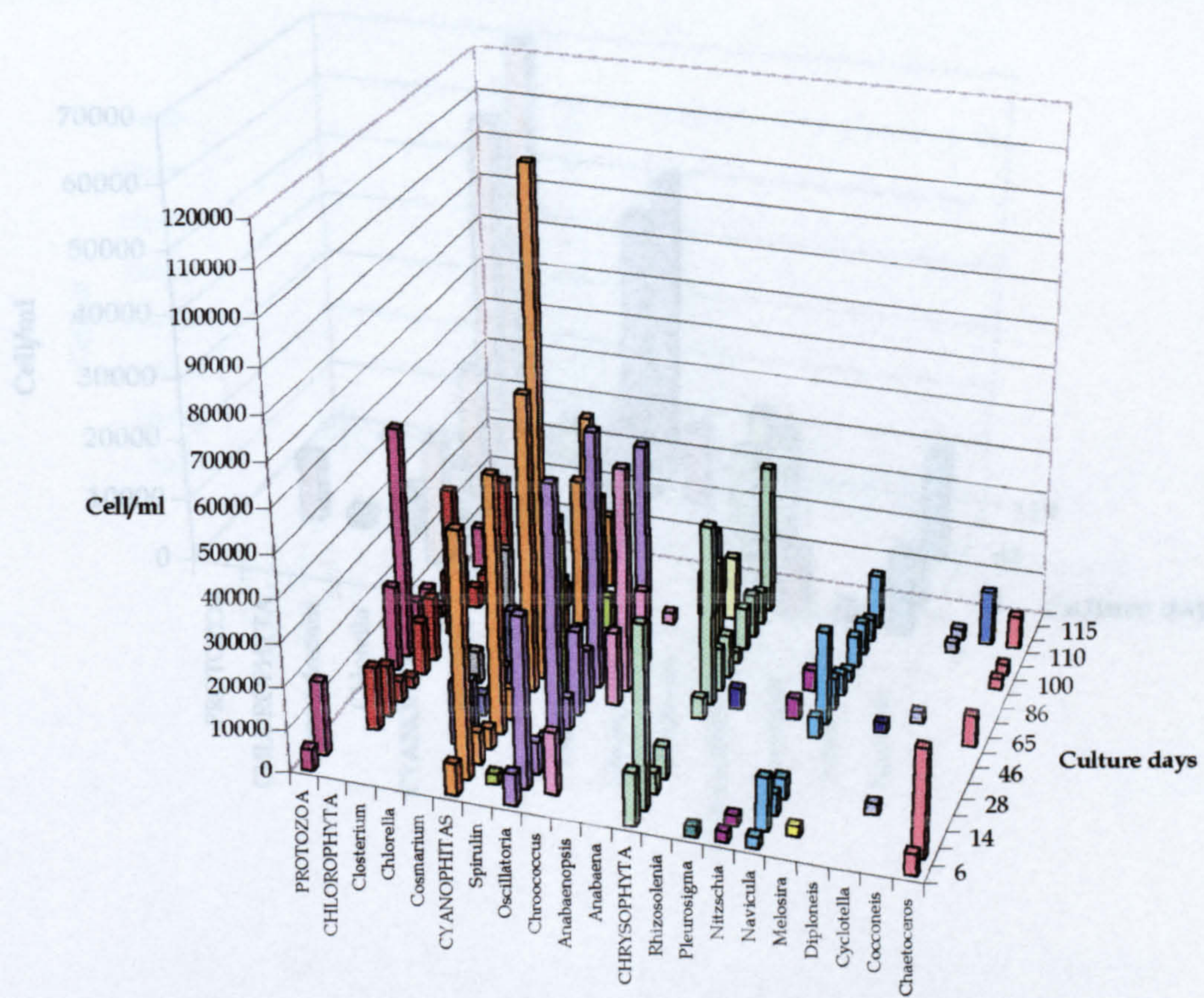


Fig.4. 27 Analysis of the main genera of phytoplankton present in the shrimp ponds July 1997-January 1998.

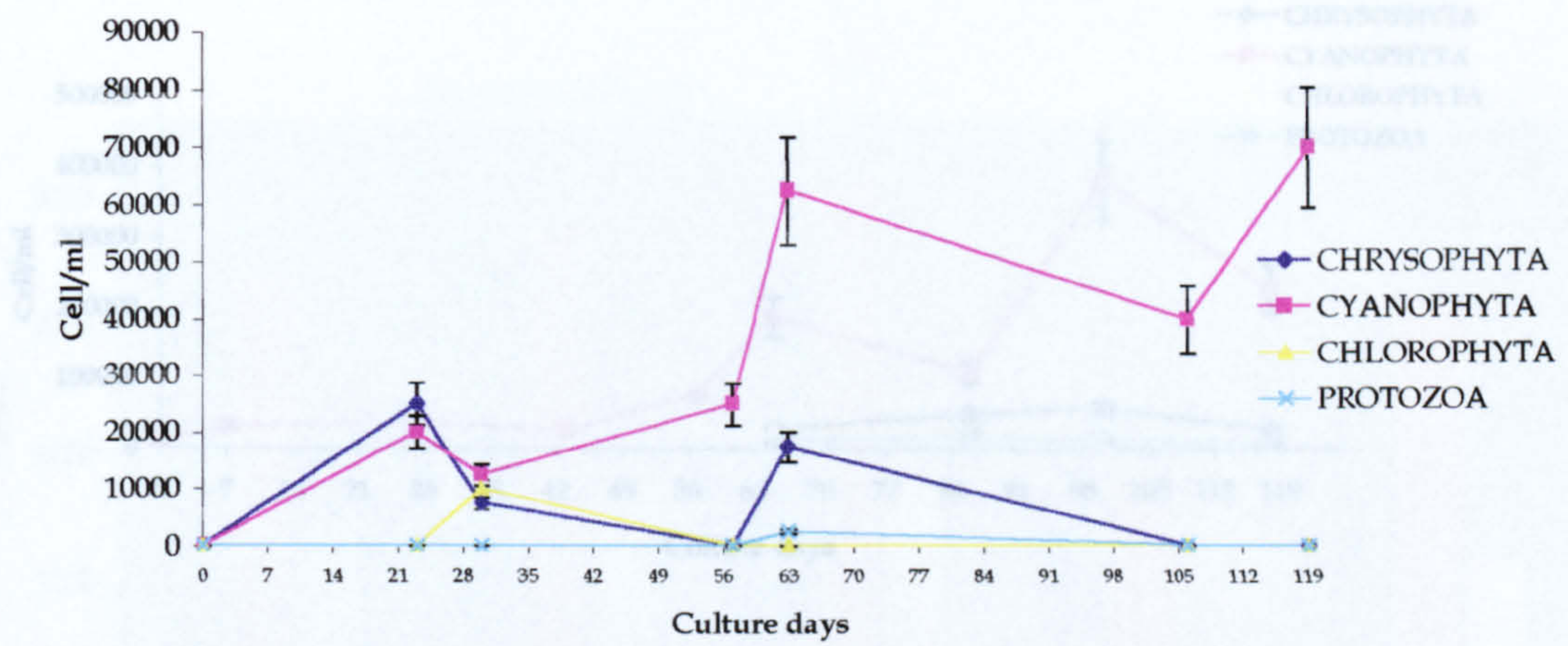


Fig.4. 28 Variation in the density (cell/ml) of the major groups of phytoplankton

Fig.4. 28 Variation in the density (cell/ml) of the major groups of phytoplankton during the shrimp culture cycle September 1998-February 1999.

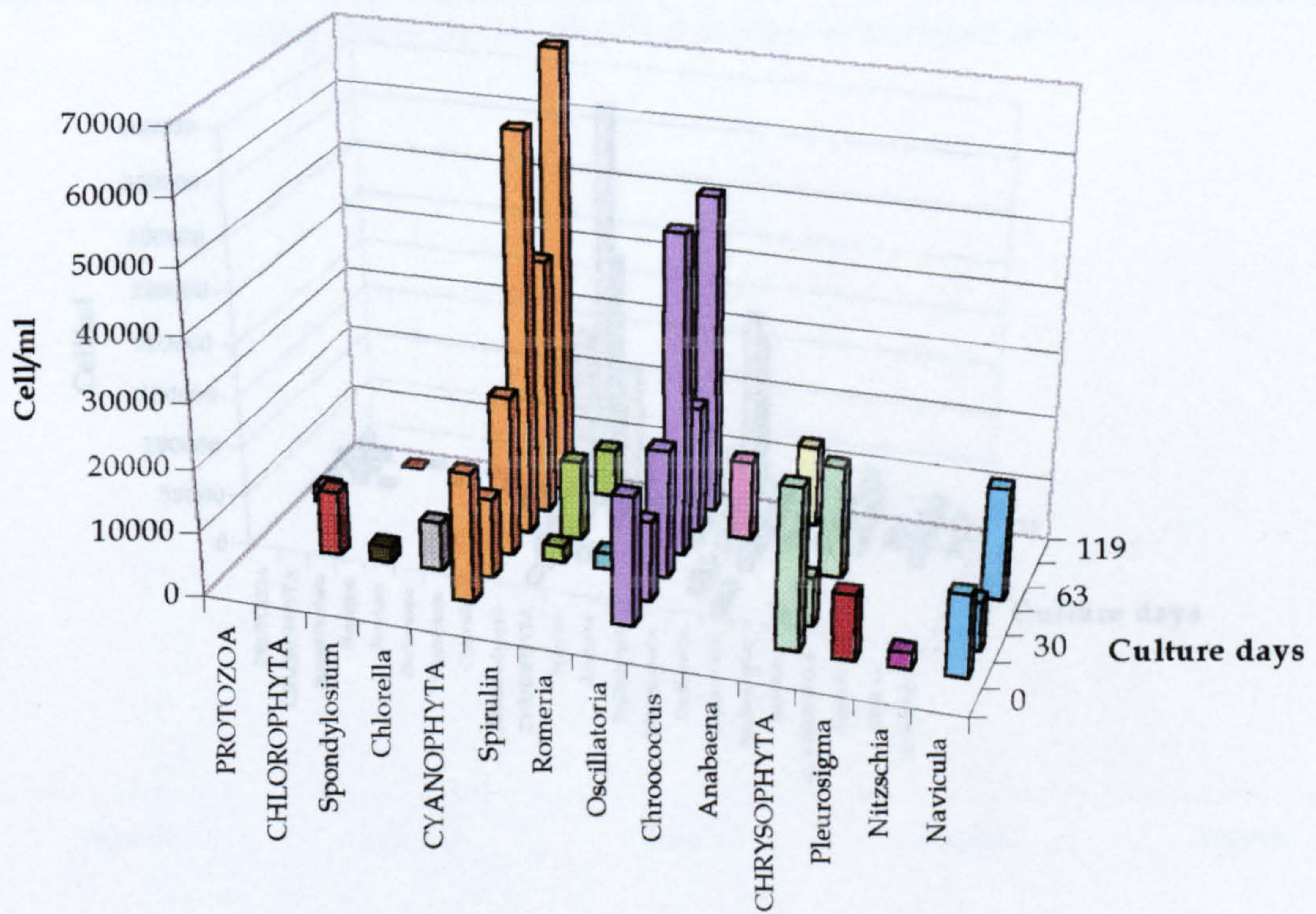


Fig.4. 29 Analysis of the main genera of phytoplankton present in the shrimp ponds September 1998-February 1999.

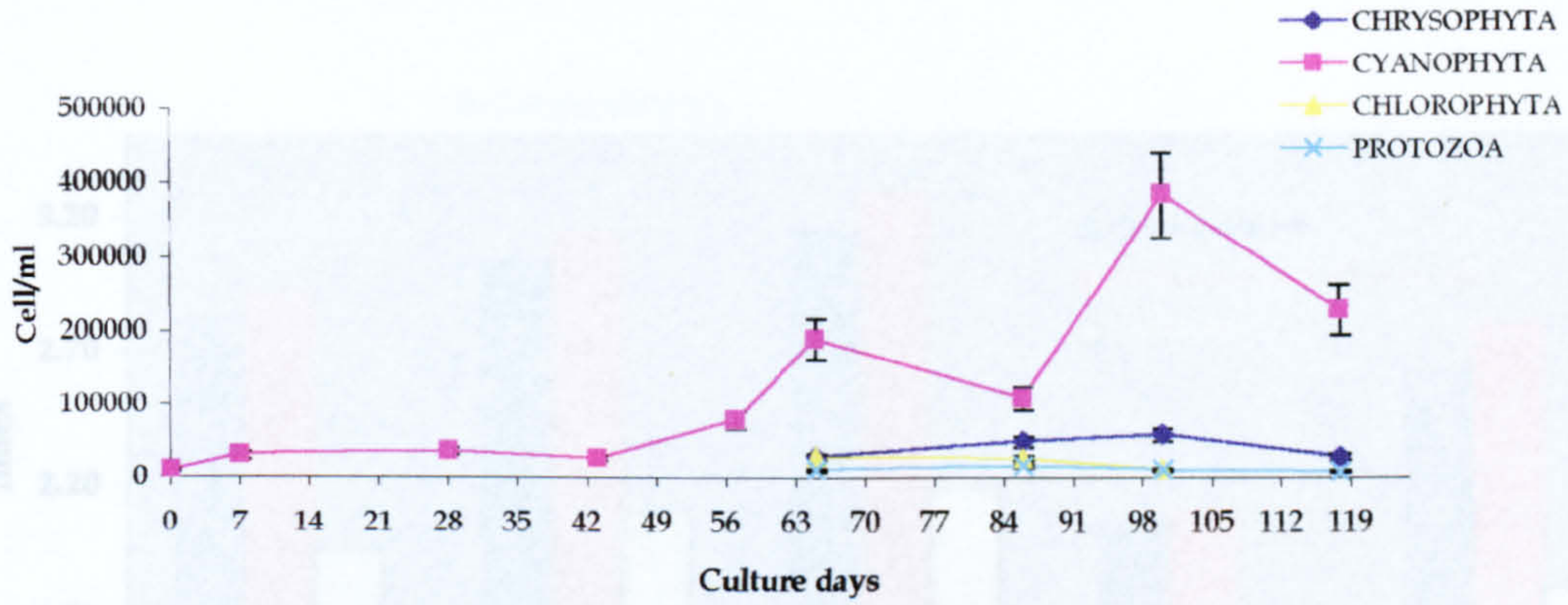


Fig.4. 30 Variation in the density (cell/ml) of the major groups of phytoplankton during the shrimp culture cycle March 1999-July 1999.

Fig.4. 32 Indices of information content (H') for phytoplankton in pond water during the 119 shrimp culture periods.

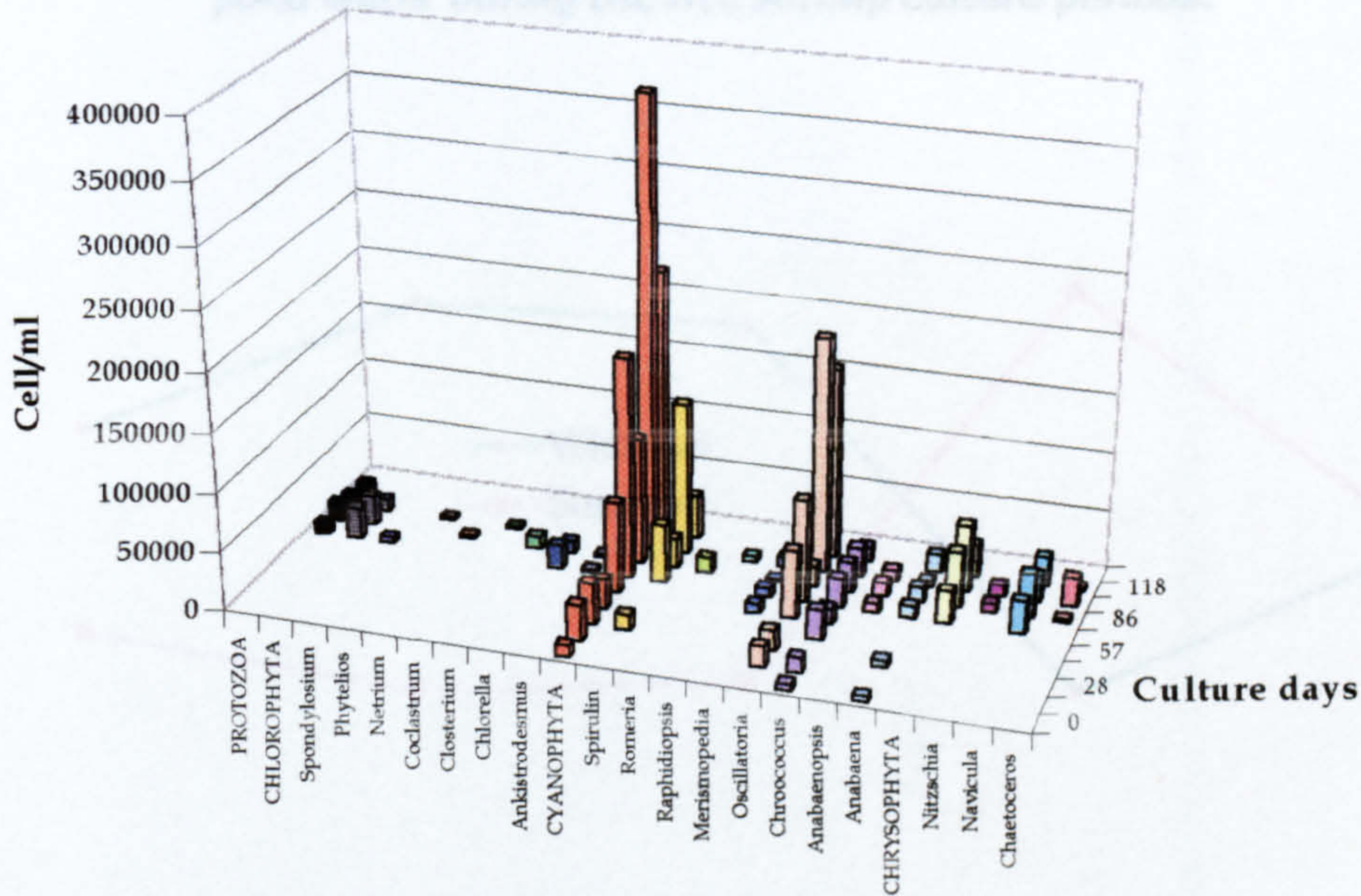


Fig.4. 31 Analysis of the main genera of phytoplankton present in the shrimp ponds March 1999-July 1999.

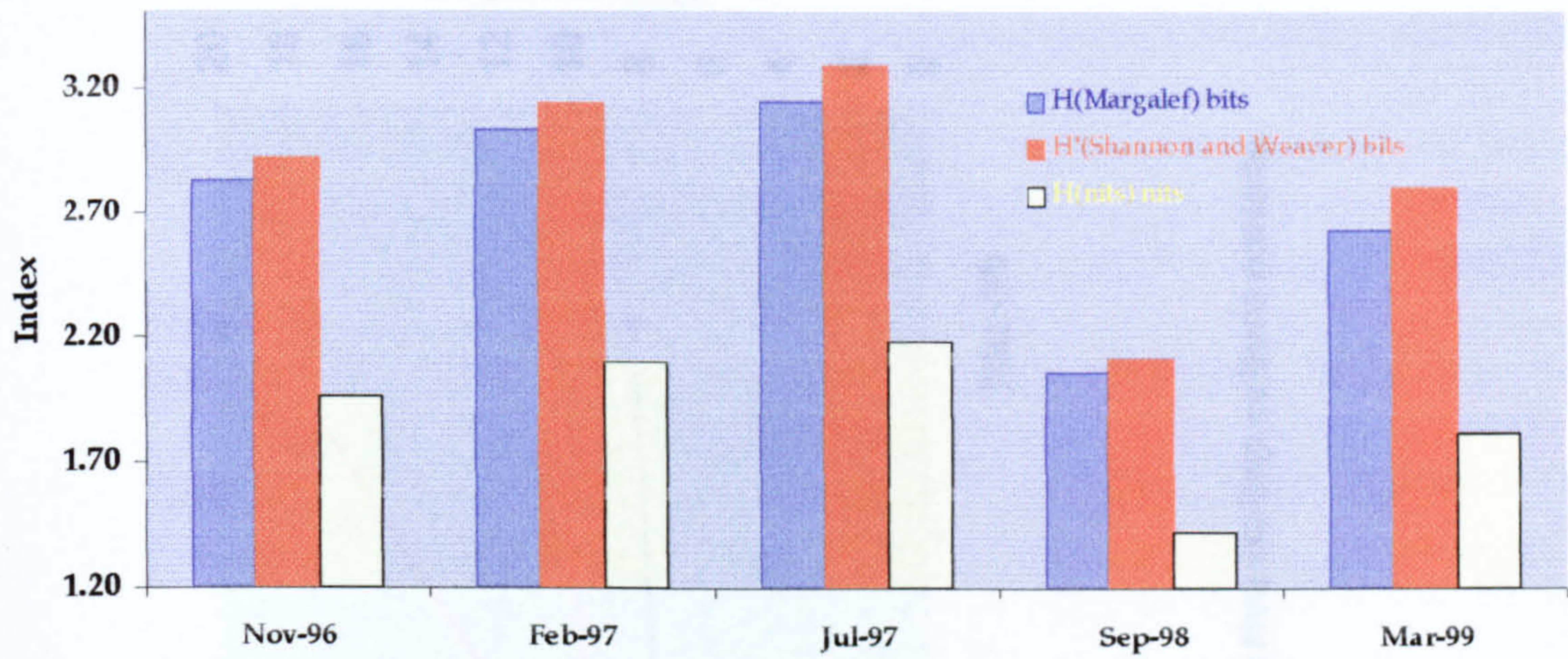


Fig.4. 32 Indices of information content (see section 3.6.1.1) for phytoplankton in pond water during the five shrimp culture periods.

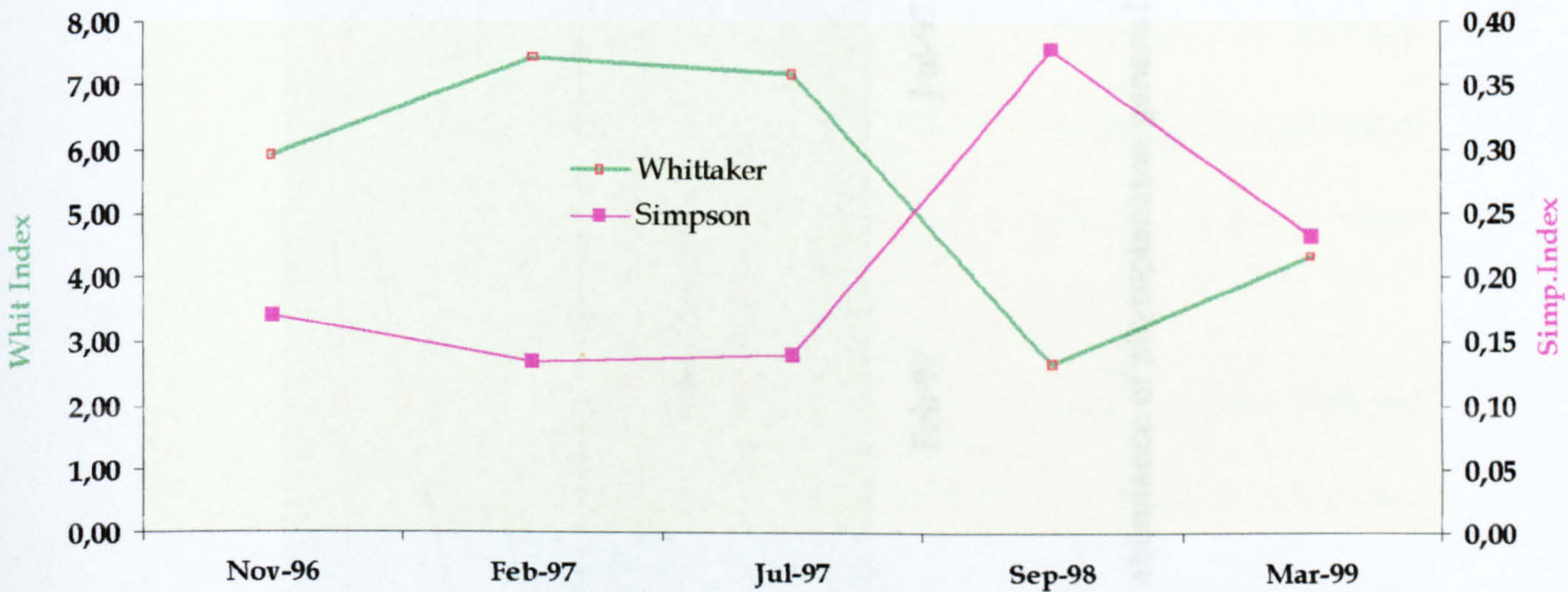


Fig.4. 33 Indices of number (see section 3.6.1.2) genera diversity for phytoplankton in pond water during the five shrimp culture periods.

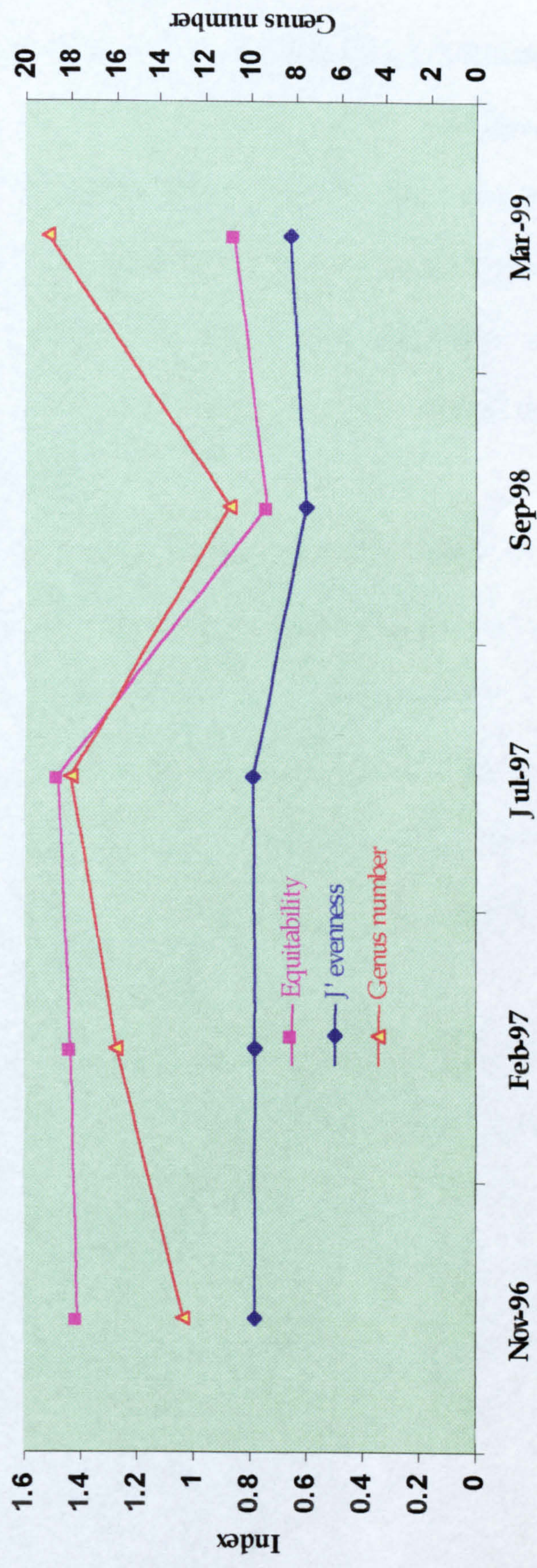


Fig.4. 34 Indices of abundance of phytoplankton genera in pond water during the five shrimp culture periods.

Phytoplankton discussion

Phytoplankton play a significant role in shrimp aquaculture by stabilising the whole pond ecosystem and by minimising the fluctuations of water quality. Phytoplankton enrich the system with oxygen through photosynthesis during daylight hours and lower the levels of CO₂, NH₃, NO₂ and H₂S. Moreover, a healthy phytoplankton community can reduce toxic substances in the pond since phytoplankton can consume NH₄ and tie up heavy metals.

A stable and manageable algal bloom is more readily achieved if the growth of particular algal classes is encouraged. Green algae provide a more stable bloom than brown water consisting mainly of diatoms (Chien, 1992). However, other shrimp farmers may stimulate diatom blooms through preference (Boyd, 1995). Cyanophytes, in contrast, are thought to decrease the diversity of other phytoplankton species, resulting in a poor water quality (Burford, 1997).

The species composition of phytoplankton in the shrimp ponds differed between the El Niño, La Niña events and transition periods, with a decrease in the diatom community and an increase in the green algae community. This was related to changes in the nutrients, e.g. the ratio of nutrients in the pond water affects the proportion of different phytoplankton groups present. In this study, due to the increase in phosphorus concentration during the

culture periods, the ratio of inorganic N: inorganic P were lower than 1.0 (Table 4.1). The dominance of Cyanophytes is mainly attributed to high nutrient loading, especially when nitrogen is limited in relation to phosphorus (Schindler 1977; Barica, Klin and Gibson, 1980; Smith 1983). A low N:P ratio encourages the growth of Cyanophytes (Rhee and Gotham, 1980; Seymor, 1980; Yusoff and McNabb, 1989; Paerl and Tucker, 1995), whereas high nitrate concentrations encourage diatom growth (Andreoli and Tolomio, 1988; Clifford, 1992; Yusoff *et al.*, 2001). When the nitrogen concentration in culture ponds is high (the ratio of total N to total P exceeds 29 in the water column), Cyanophytes are rare or absent (Smith, 1983, Yusoff and McNabb, 1997). Other environmental factors that control cyanobacterial dominance are water column stability and the buoyancy ability of blue green algae (Reynolds, 1987; Paerl, 1988; Paerl and Tucker, 1995), low light availability (Smith, 1986), high pH and low carbon dioxide concentrations (King, 1970; Shapiro, 1984), warm water temperatures (Tilman *et.al*, 1986; McQueen and Lean, 1987; Paerl and Tucker, 1995) and salinity. Lowell and Broce (1985) notice a musty odour to shrimp takes from culture pond and found these to be high concentrations of geosmin. The occurrence of the blue-green algae, which produced the off flavor in the shrimp, was due to the reduction in salinity in the coastal culture ponds.

The toxic effects of blooms of *Oscillatoria* species on farmed tiger shrimp (*P. monodon*) and Japanese kuruma shrimp (*P. japonicus*), have been described by Smith (1995, 1996). A similar toxicity by *Spirulina subsalsa* on the blue shrimp *P. stylirostris* has been reported by Lightner (1978). Despite these findings, during the El Niño period in the present study, *Oscillatoria limnetica* and *Oscillatoria amphigranulata* occurred at a concentration of 35000 - 60000 cell /ml and *Spirulina sp.* (35000-60000 cell/ml), but were associated with better yields and survival of *L. vannamei* (Retamales, 2000). Some publications suggest that cyanobacteria may produce substances toxic to bacteria (Davis and Gloyna, 1972; Mezrioui *et al.*, 1994; Oufdou *et al.*, 1998), hence the effect of *Oscillatoria spp.* and *Spirulina sp.* on cultured shrimp may be positive under certain conditions.

4.3 Bacterial results

Various types of association between aquatic invertebrates and their gut microflora have been reported (Moss *et al.*, 1992, 1995; Moss and Pruder, 1995; Moss *et al.*, 2000, 2001). Microorganism species may either be ingested transients or residents, and their presence has different implications for the host. Activities of the host, such as bioturbation or production of bacteriocidal substances, may enhance or inhibit the growth of microorganisms while, conversely, the same microbes have been reported to inhibit some activities of their invertebrate host, e.g., pathogenic bacteria (Harris, 1993). The most commonly reported association between aquatic invertebrates and gut microbes is that of ingestion of bacteria (Harris, 1993). Thus, bacteria present in the environment can enter a shrimp host through the ingestion of food items or rearing in water loaded with high numbers of microbes.

Twelve genera of heterotrophic aerobic bacteria were identified from the intestines of *Litopenaeus vannamei* sampled during the study of the five shrimp culture periods: these were *Xanthomonas*, *Vibrio*, *Pseudomonas*, *Providencia*, *Moraxella*, *Klebsiella*, *Flavobacterium*, *Cytophaga*, *Citrobacter*, *Alcaligenes*, *Aeromonas* and *Acinetobacter*. In some periods of shrimp culture, a disease outbreak was observed after 30 to 90 days. In the initial stage of the disease (2 – 3 days), affected shrimp showed a yellowish body discolouration,

which became reddish after about 6 – 8 days. The affected shrimp were anorexic, lethargic, swam near to the surface water, and were found dying near to the dikes of the ponds, suggesting that the culture system was under severe stress. The *Vibrio* bacteria isolated from these periods were dominated (90 % presence) by the species *V. harveyi* and *V. parahaemolyticus*, with a density range from 10^5 – 10^7 cfu/g. On average these *Vibrio* species represent only a 20% of total *Vibrio* during El Niño conditions.

4.3.1 Period 1. Transition Niña - Niño

Eleven genera of bacteria were identified in this period, represented mainly (on average) by *Vibrio spp.* (33.75%), *Pseudomonas spp.* (18.75%) and *Moraxella spp.* (13.75 %) (see Table 4.3; Fig.4.35). There was a distinct increase in temperature from 29 to 31 °C (Fig. 4.9.1) around sixty days of culture and a disease outbreak occurred as described above, which affected the final survival of the shrimp (33.9 %) and their yield (444 kg/ha) (Table 4.5). Moreover, there was a high ratio recorded between the number of presumptive *Vibrio* (PV) and total heterotrophic aerobic bacteria (THAB) (from 0.73 – 0.96, average 0.86), (Fig.4.36; Table 4.4), particularly in the final phase of the culture. The diversity indices were low in this period (Fig.4.37, 4.38), associated with a low evenness because of the predominance of *Vibrio* species in the intestine of the shrimp's bacterial flora (Fig.4.39).

4.3.2 Period 2. Niño

Twelve genera of bacteria were identified in this period, represented mainly by *Moraxella spp.* (17.5%), *Pseudomonas spp.* (12.5%) and *Cytophaga spp.* (10.0 %) and *Vibrio spp.* (8.75 %), (Table 4.3; Fig.4.40). There was a stable, high temperature from 29 to 31 °C (Fig.4.9.2) and no disease outbreak was observed. A low percentage of *Vibrio* bacteria (average 8.75 %) (Table 4.3), and a low ratio between the number of PV and THAB (0.58) (Fig.4.41; Table 4.4) were recorded. These findings were associated with high diversity indices (Figs 4.42 and 4.43,) and a high proportional abundance of the different bacteria genera (evenness) (Fig.4.44). The survival rate of *L. vannamei* at harvest was 69 % and the yield was 919 kg/ha (Table 4.5).

4.3.3 Period 3. Niño

Twelve genera of bacteria were identified during this period, represented mainly (on average) by *Vibrio spp.* (18.33%), *Moraxella spp.* (16.67%), *Pseudomonas spp.* (14.17%) and *Flavobacterium spp.* (8.33 %),(Table 4.3, Fig.4.45). There was a stable pond temperature of 28 to 29 °C (Fig.4.9.3) and a salinity range from 12 – 19 psu (Fig.4.8.3). A short disease outbreak was observed after 90 days of culture. The average ratio between the number of PV and THAB was 0.71 (Fig.4.46, Table 4.4). This was associated with low diversity indices after 90 days of culture (Figs 4.47 and 4.48) because of the decrease in the number of bacteria genera present (Fig.4.49). The final

survival of shrimp at harvest was 45 % and their yield was 725 kg/ha, (Table 4.5).

4.3.4 Period 4. Transition Niño – Niña

Seven genera of bacteria were identified in this period, mainly constituted by *Vibrio spp.* (23.0%), *Pseudomonas spp.* (21.0%), *Moraxella spp.* (10 %) and *Aeromonas spp.* (10.0 %), (Table 4.3; Fig.4.50). There was a decrease in the average pond temperature from 28 to 26 °C (Fig.4.9.4) and salinities ranged from 19 – 22 psu (Fig. 4.8.4). A disease outbreak was observed after 50 days of culture. The average ratio between the number of PV and THAB was 0.79 with a range of 0.85 – 0.65 from fifty days onwards in the culture cycle (Fig.4.51; Table 4.4). This was also associated with low diversity indices (Figs 4.52 and 4.53) after 50 days of culture because of the predominance of *Vibrio* and *Pseudomonas* bacteria (Figs 4.50 and 4.54). The final survival rate of the cultured shrimp stock was 34.5 % and their yield was 499 kg/ha (Table 4.5).

4.3.5 Period 5. Niña

Seven genera of bacteria were identified in this period, mainly represented by *Vibrio spp.* (36.25%), *Pseudomonas spp.* (16.25%) and *Aeromonas spp.* (8.75 %) as shown in Table 4.3 and Fig.4.55. There was a decrease in pond salinity from 14 – 4 psu, (Fig.4.8.5), and also in the temperature 28 – 26 °C

(Fig.4.9.5) during this culture cycle. A disease outbreak was observed after 35 days of culture. The average ratio between the number of PV and THAB was 1.03, with a range of 0.82 – 1.2 (Fig.4.56; Table 4.4). This was associated with low diversity indices for the gut bacteria (Figs 4.57 and 4.58) due the predominance of *Vibrio* and *Pseudomonas* species (Figs 4.55 and 4.60). The final survival of the shrimp stock was 32.5 %, with a yield of 443 kg/ha (Table 4.5).

Based on an analysis of the bacterial diversity indices between the five shrimp culture periods (the combined data are shown in Figs 4.60 and 4.61), high indices of diversity can be seen during February'97 (El Niño event) when pond temperatures were 29°C (on average) and salinity 28 psu, i.e. near to the optimal temperature and salinity conditions for *L. vannamei* (Barlett *et al.*, 1990; Wyban *et al.*, 1995; Ponce-Palafox *et al.*, 1997), and there was no predominance of any particular genus of bacteria during the February 97 (El Niño) period (Fig.4.62). During the second period, from July 97 (El Niño), despite the presence of optimal temperature conditions (on average 29°C), the mean salinity was only 15 psu, making it necessary for the shrimp to compensate osmotically, making them more susceptible to disease.

Low bacterial diversity indices during the transition and La Niña periods (Figs 4.60 and 4.61) were observed because of a decrease in the number of

bacteria genera and predominance of *Vibrio* species. Moreover, low salinity and or low temperature increase the occurrence of more virulent species of *Vibrio*, (Prayitno *et al.*, 1995; Liu *et al.*, 1996b; Shivappa, 1997). The mechanism of increase virulence is unknown but Farghaly (1950) demonstrated that low salinity reduced the growth rate of luminous bacteria. These stresses may induce or enhance the expression of virulence genes.

Table 4. 3 Average percentage composition of aerobic heterotrophic bacteria in shrimp intestines from five different culture periods, November 1996 to June 1999.

Genus of bacteria	Period of Culture				
	1	2	3	4	5
	Transition Niña - Niño Nov 96 - Mar 97	Niño Feb - Jun 97	Niño Jul 97 - Jan 98	Transition Niño - Niña Sep 98 - Feb 99	Niña Mar - Jun 99
<i>Acinetobacter spp.</i>	1.25	5.00	3.33	0.00	1.25
<i>Aeromonas spp.</i>	7.50	7.50	5.83	10.00	8.75
<i>Alcaligenes spp.</i>	1.25	3.75	2.50	0.00	0.00
<i>Citrobacter spp.</i>	1.25	1.25	0.83	0.00	0.00
<i>Cytophaga spp.</i>	2.50	10.00	6.67	5.00	5.00
<i>Flavobacterium spp.</i>	3.75	8.75	8.33	9.00	7.50
<i>Klebsiella spp.</i>	1.25	1.25	1.67	0.00	0.00
<i>Moraxella spp.</i>	13.75	17.50	16.67	10.00	7.50
<i>Providencia spp.</i>	1.25	1.25	1.67	2.00	0.00
<i>Pseudomonas spp.</i>	18.75	12.50	14.17	21.00	16.25
<i>Vibrio spp.</i>	33.75	8.75	18.33	23.00	36.25
<i>Xanthomonas spp.</i>	0.00	3.75	0.83	0.00	0.00
Others	13.75	18.75	19.17	20.00	17.50
Total percent	100.00	100.00	100.00	100.00	100.00

Table 4. 4 Average ratio of presumptive *Vibrio* bacteria, versus the total heterotrophic aerobic bacteria in shrimp intestines, from five different culture periods, November 1996 to June 1999.

	Period of Culture				
	1	2	3	4	5
	Nov 96 - Mar 97	Feb - Jun 97	Jul 97 - Jan 98	Sep 98 - Feb 99	Mar - Jun 99
	Transition Niña-Niño	Niño	Niño	Transition Niño-Niña	Niña
Ratio PV : THAB	0.88	0.61	0.64	0.85	0.95
	0.73	0.49	0.84	0.84	0.82
	0.96	0.51	0.59	0.91	1.16
	0.87	0.71	0.72	0.72	1.20
	3.44	2.31	4.26	3.97	4.12
Average Ratio PV:THAB	0.86	0.58	0.71	0.79	1.03

PV = Presumptive vibrio
 THAB = Total heterotrophic aerobic bacteria



Fig.4. 35 Change in the percentage composition of total heterotrophic aerobic bacteria during the shrimp culture cycle. Nov 96 - Mar 97 (Period 1).

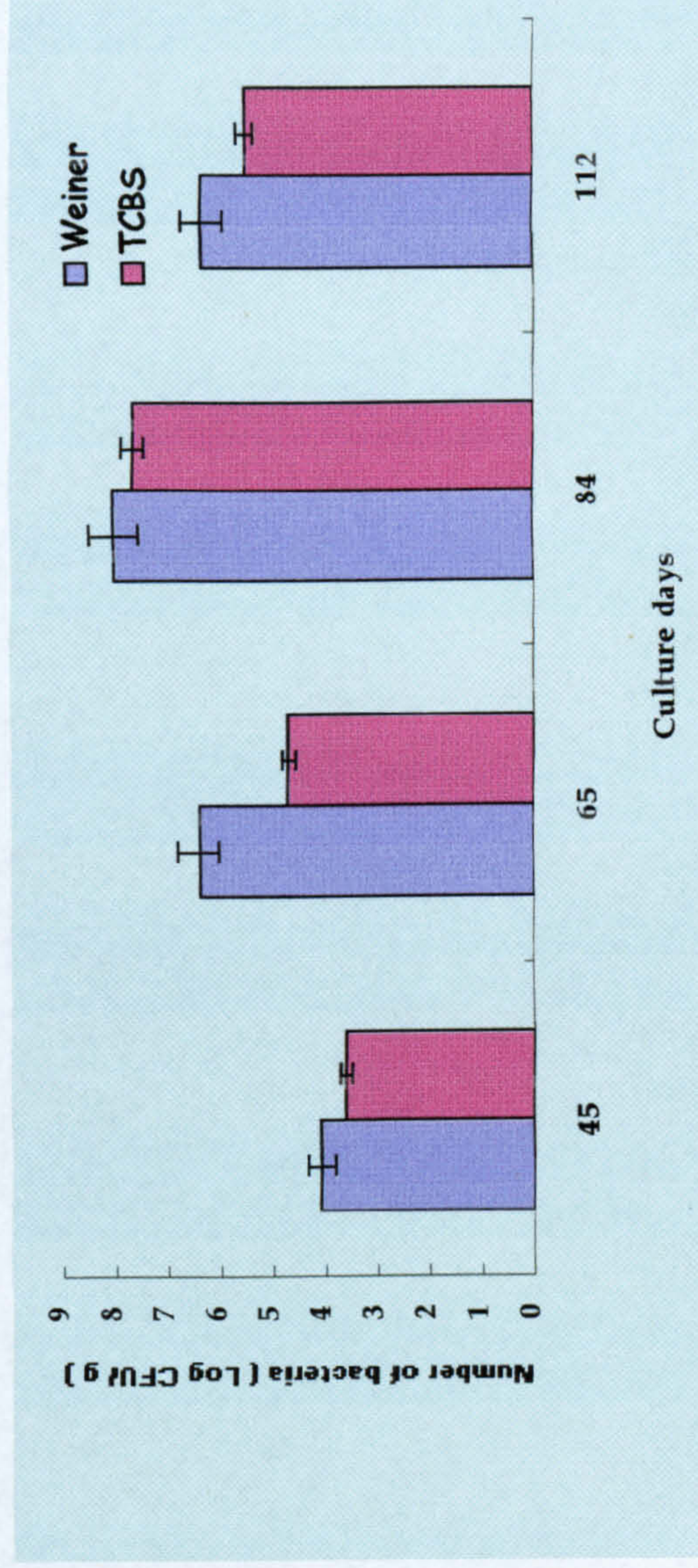


Fig.4. 36 Changes in the abundance (\pm SD) of total heterotrophic aerobic bacteria (Weiner agar) and presumptive vibrios (TCBS agar) during the shrimp culture cycle Nov 96 - Mar 97 (Period 1).

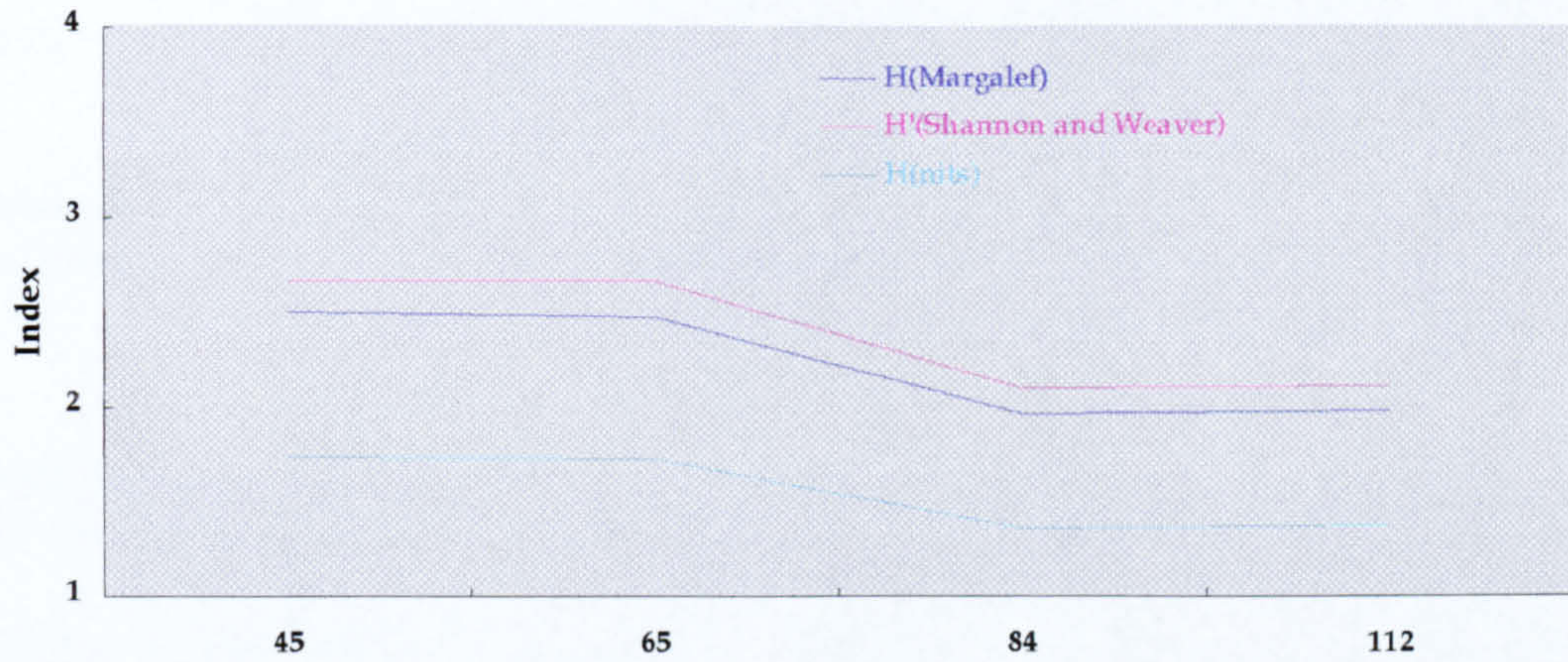


Fig.4. 37 Indices of diversity (information content, see section 3.6.1.1) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Nov 96- Mar 97 (Period 1).

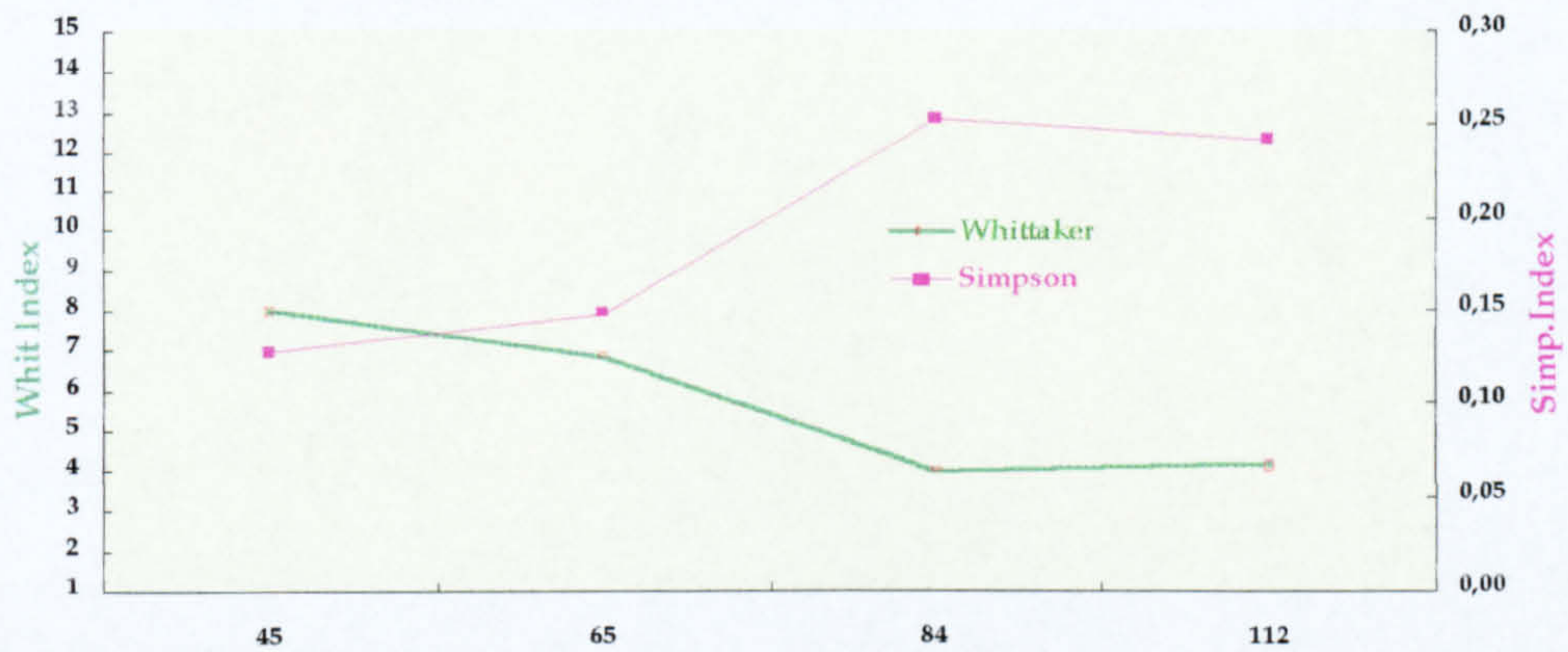


Fig.4. 38 Indices of diversity (number, see section 3.6.1.2) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Nov 96- Mar 97 (Period 1).

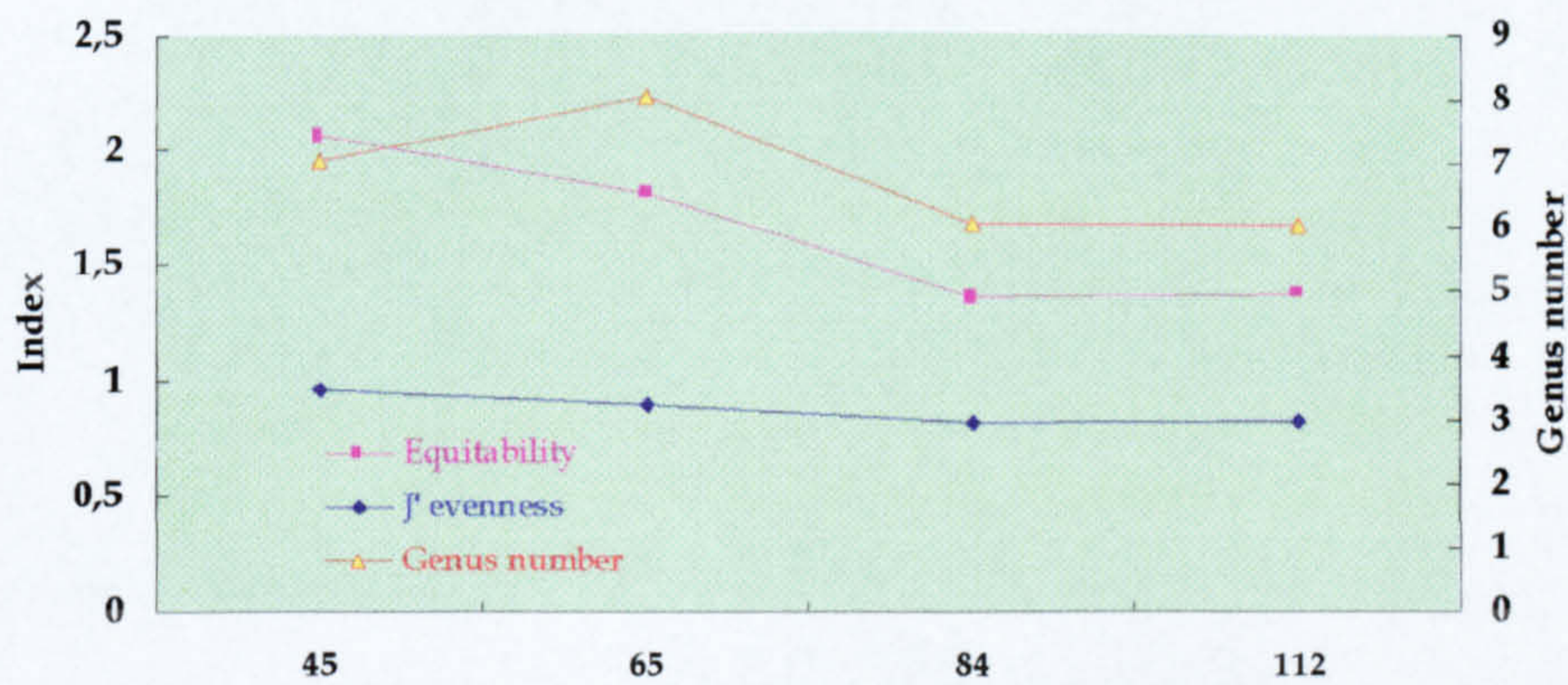


Fig.4. 39 Indices of abundance for genera of heterotrophic aerobic bacteria from different culture days Nov 96- Mar 97 (Period 1).

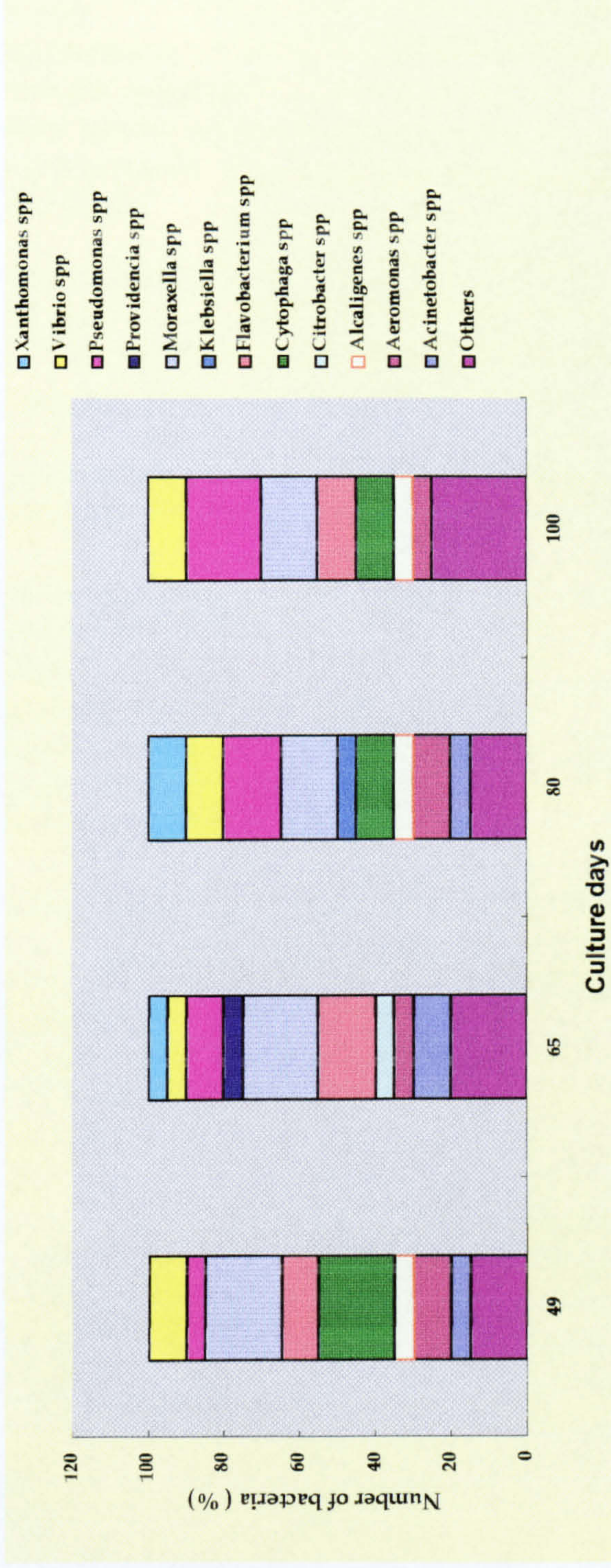


Fig.4. 40 Change in the percentage composition of total heterotrophic aerobic bacteria during the shrimp culture cycle. Feb 97 - Jun 97 (Period 2).

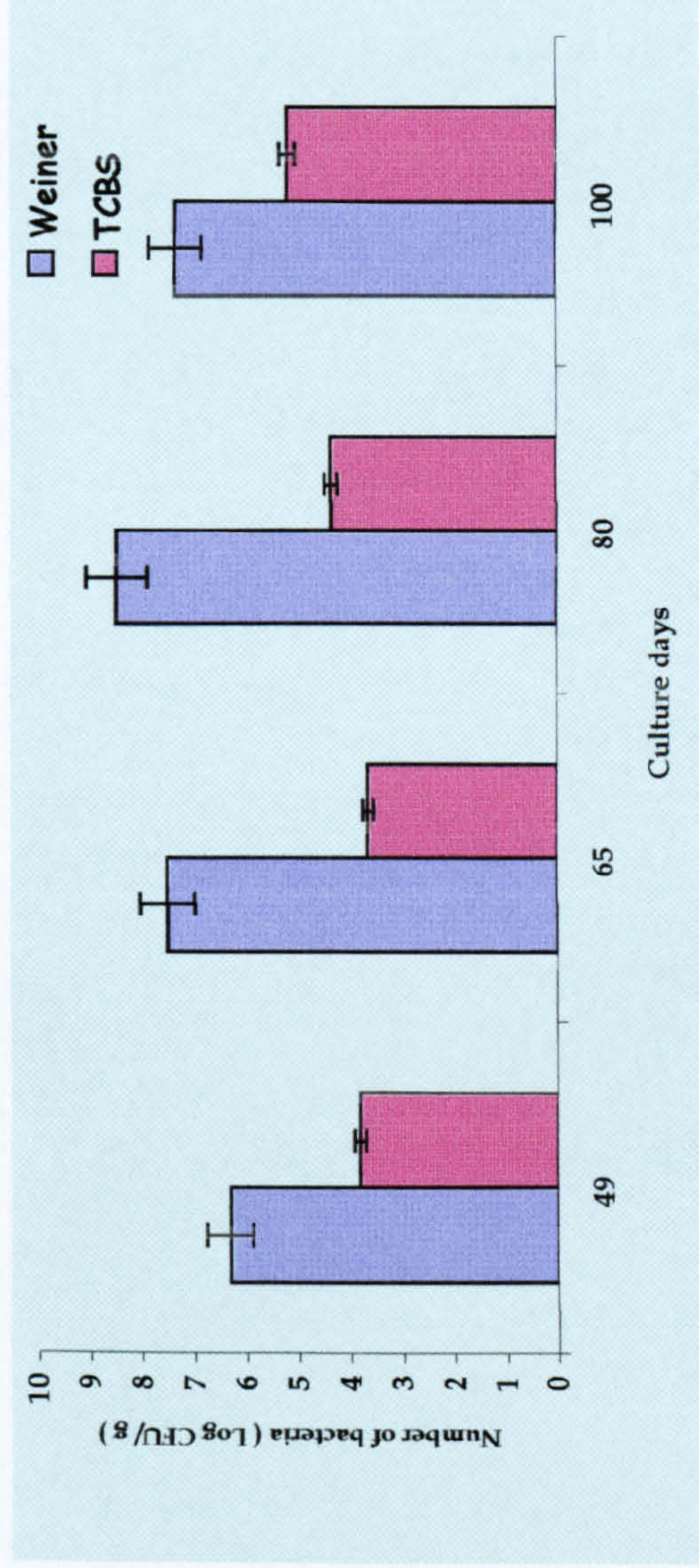


Fig.4. 41 Changes in the abundance (\pm SD) of total heterotrophic aerobic bacteria (Weiner agar) and presumptive vibrios (TCBS agar) during the shrimp culture cycle Feb 97 - Jun 97 (Period 2).

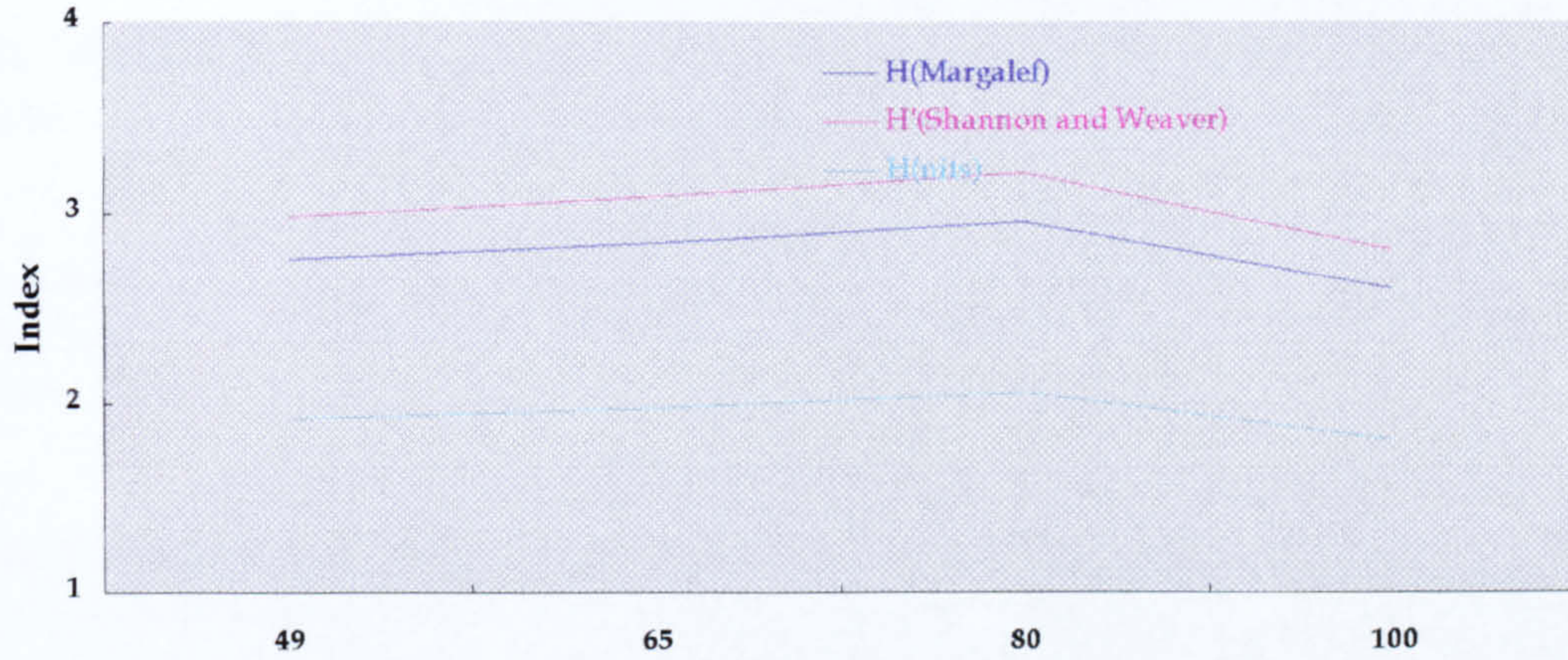


Fig.4. 42 Indices of diversity (information content, see section 3.6.1.1) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Feb 97- Jun 97 (Period 2).

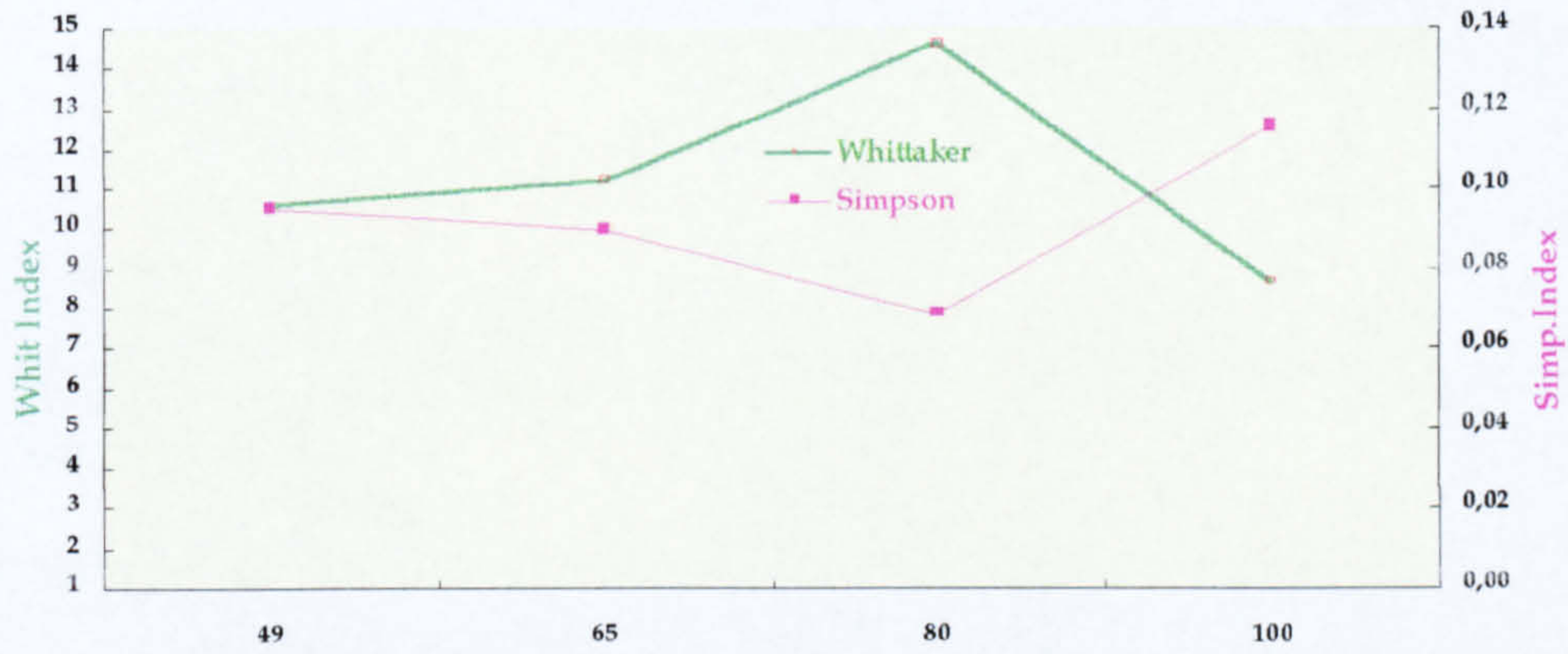


Fig.4. 43 Indices of diversity (number, see section 3.6.1.2) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Feb 97- Jun 97 (Period 2).

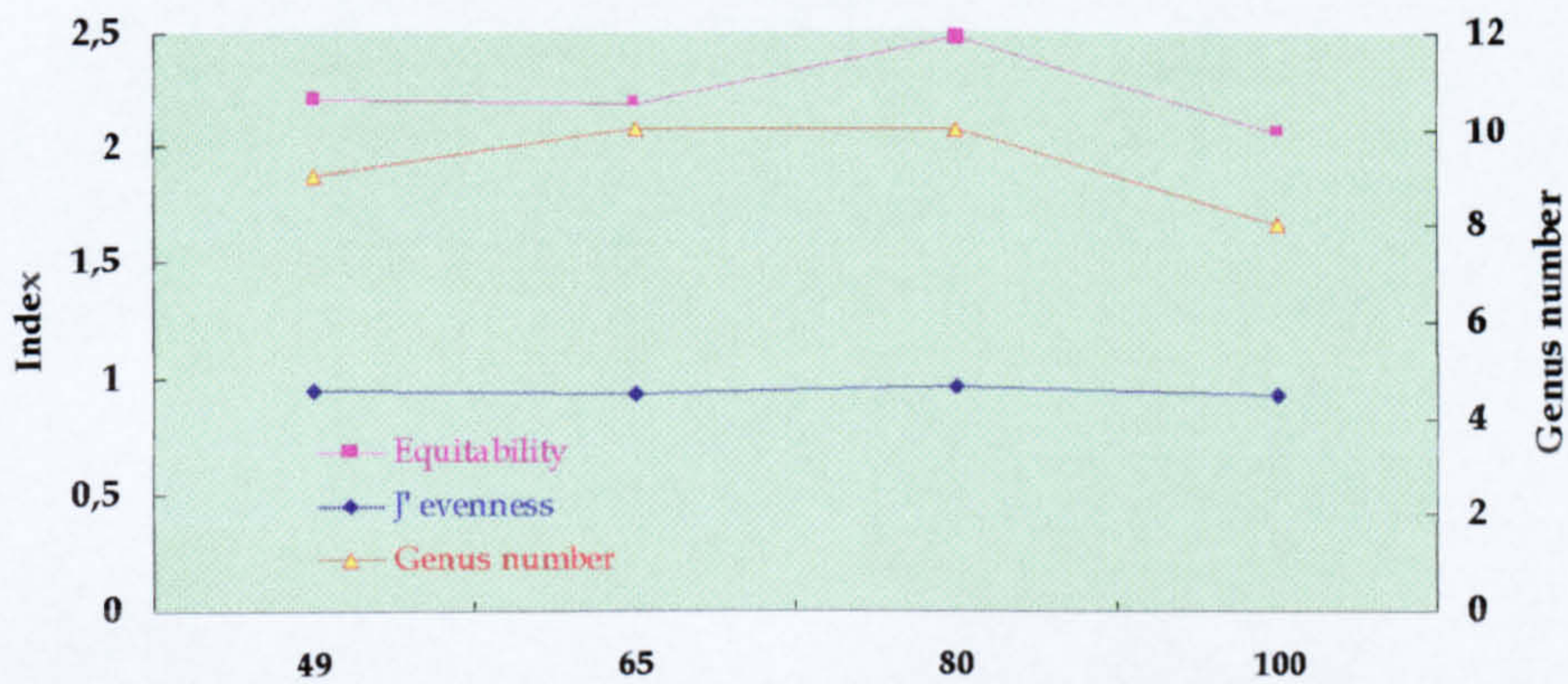


Fig.4. 44 Indices of abundance for genera of heterotrophic aerobic bacteria from different culture days Feb 97- Jun 97 (Period 2).

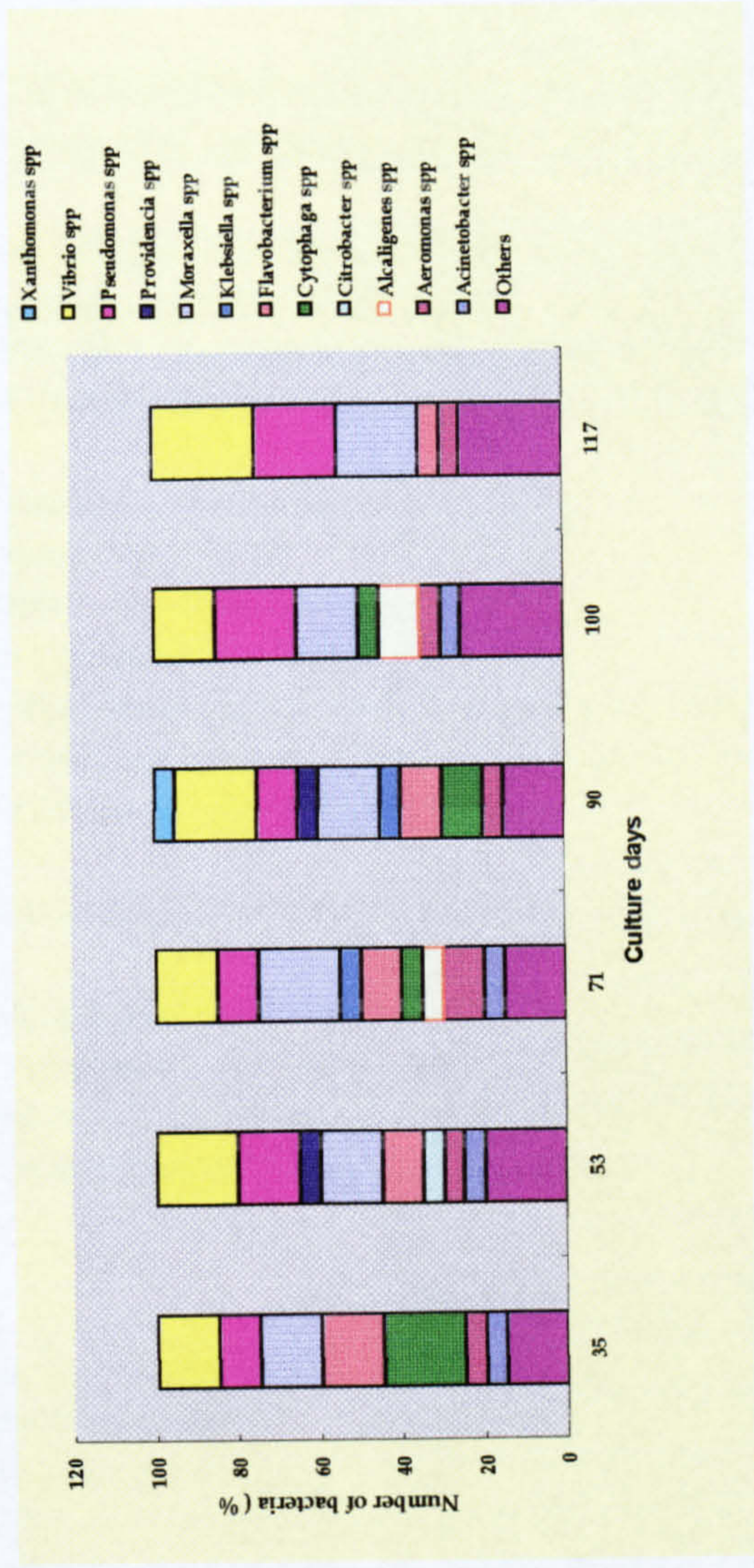


Fig.4. 45 Change in the percentage composition of total heterotrophic aerobic bacteria during the shrimp culture cycle July 97 - January 98 (Period 3).

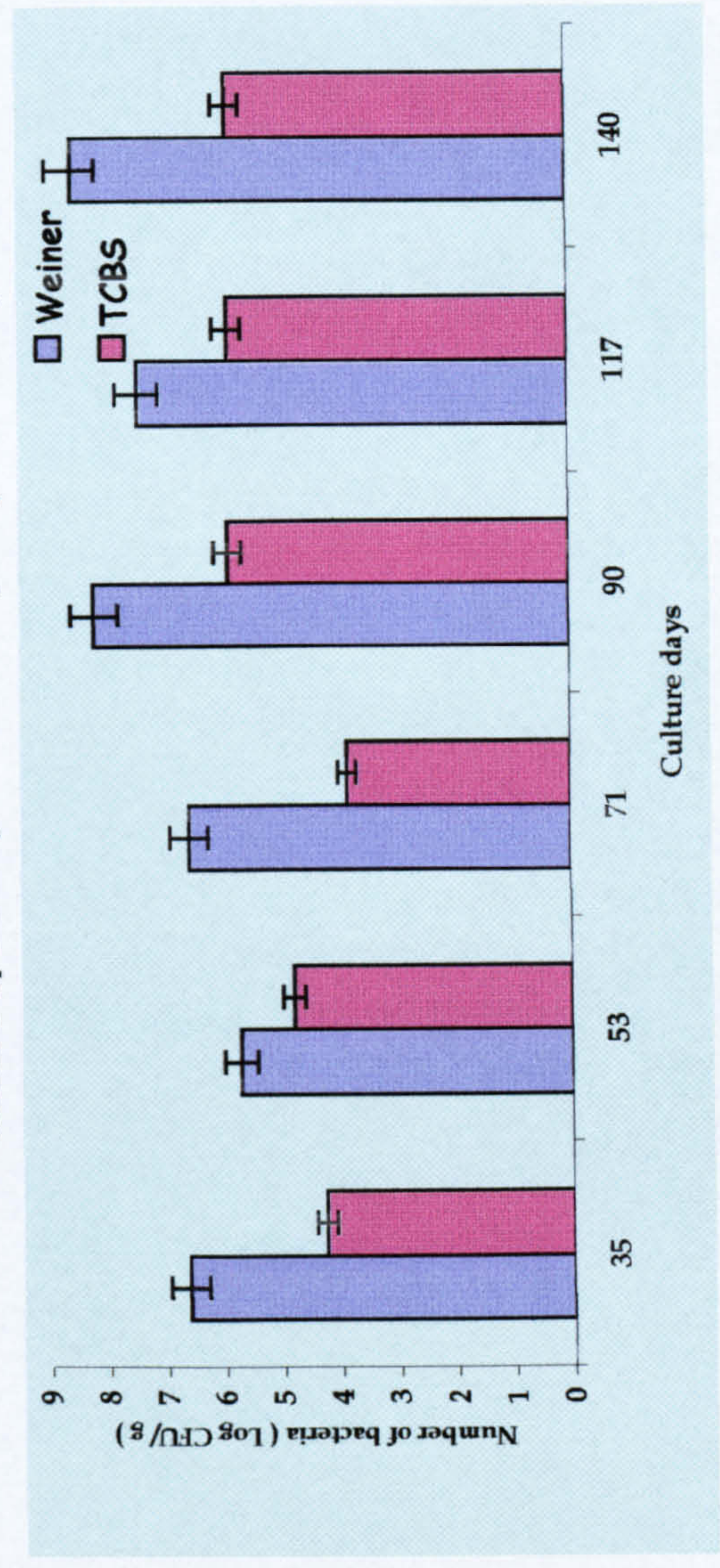


Fig.4. 46 Changes in the abundance (±SD) of total heterotrophic aerobic bacteria (Weiner agar) and presumptive vibrios (TCBS agar) during the shrimp culture cycle Jul 97 - Jan 98 (Period 3).

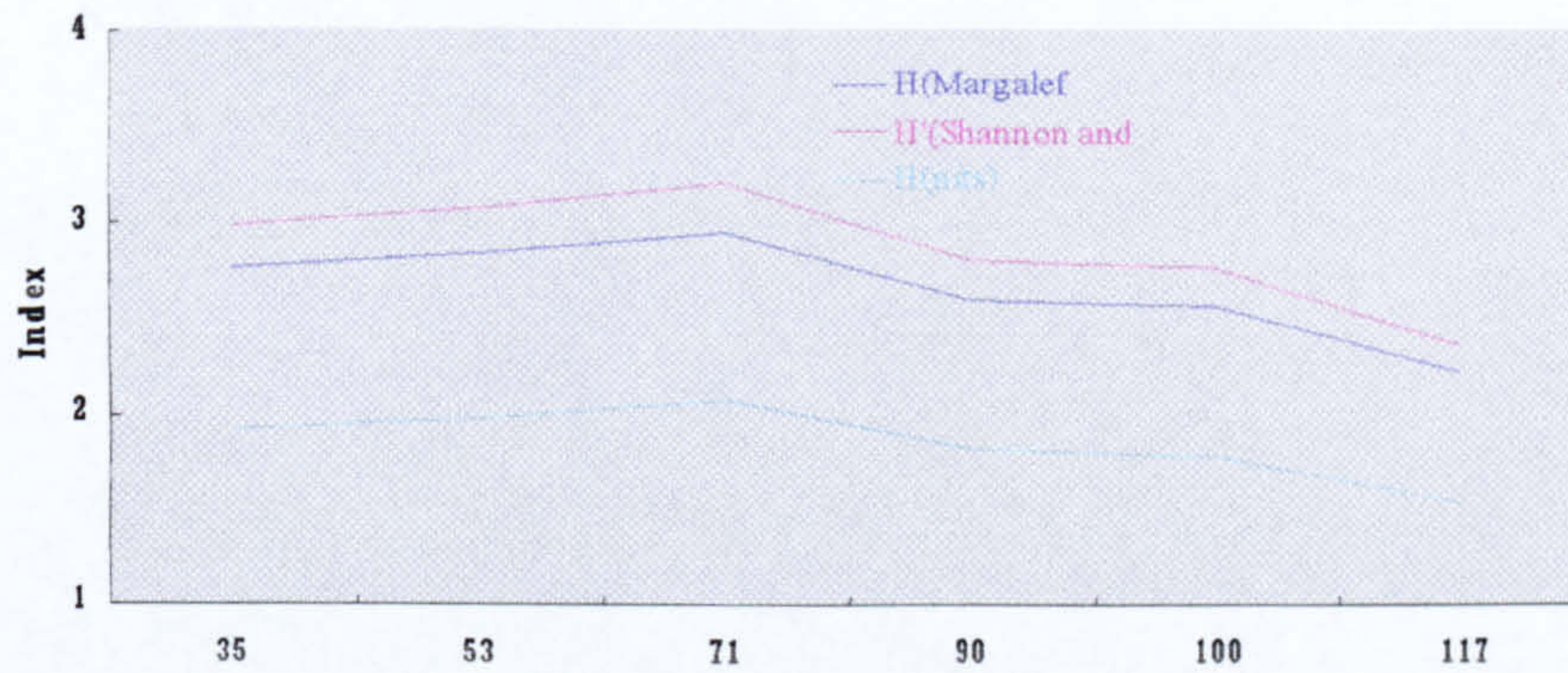


Fig.4. 47 Indices of diversity (information content, see section 3.6.1.1) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Jul 97- Jan 98 (Period 3).

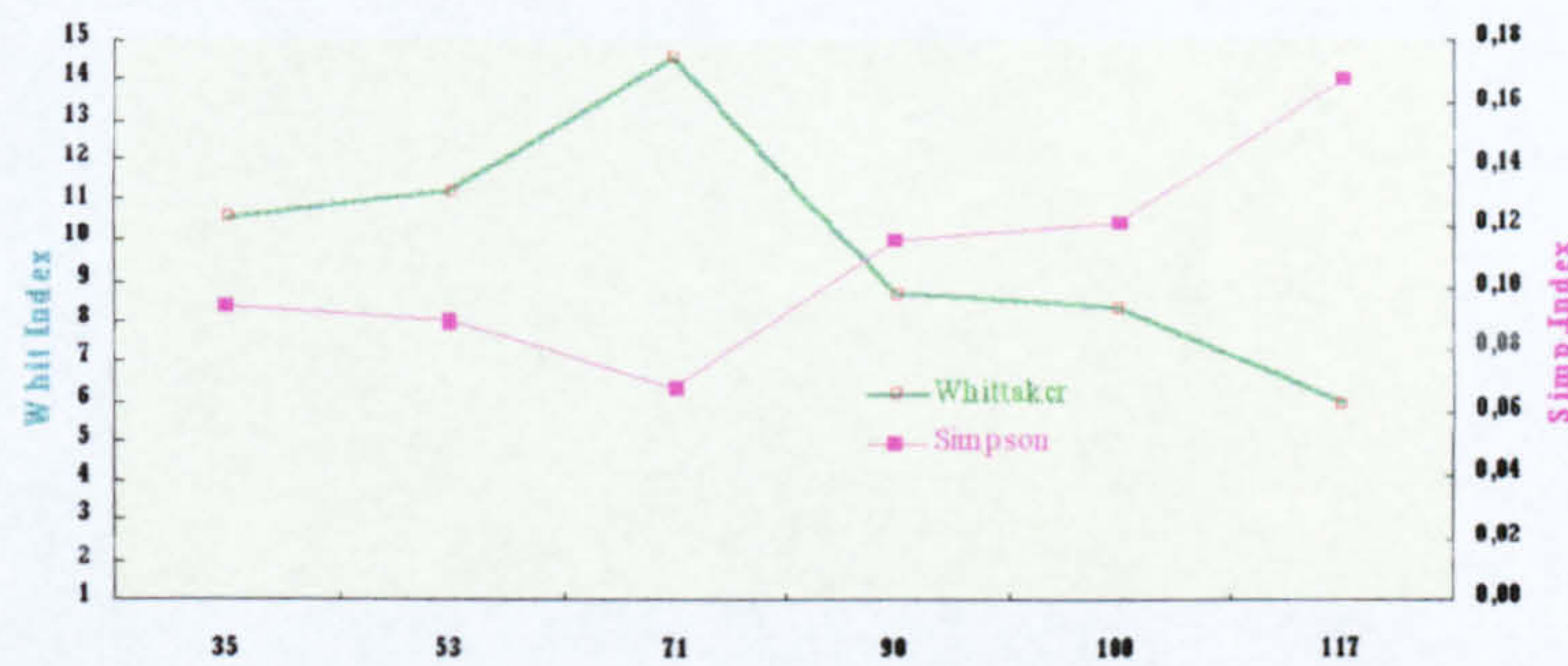


Fig.4. 48 Indices of diversity (number, see section 3.6.1.2) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Jul 97- Jan 98 (Period 3).



Fig.4. 49 Indices of abundance for genera of heterotrophic aerobic bacteria from different culture days Jul 97 – Jan 98 (Period 3).

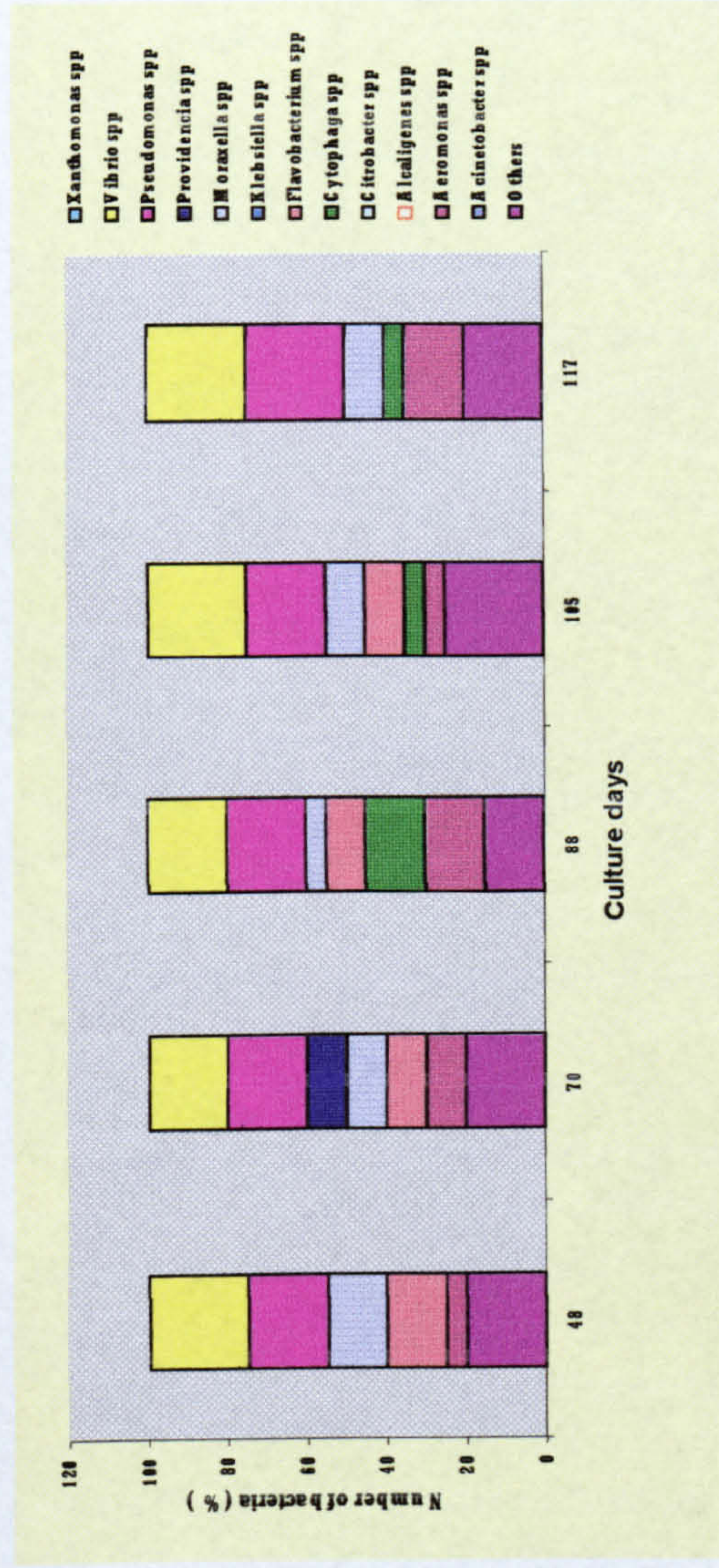


Fig.4. 50 Change in the percentage composition of total heterotrophic aerobic bacteria during the shrimp culture cycle Sep 98 – Feb 99 (Period 4).

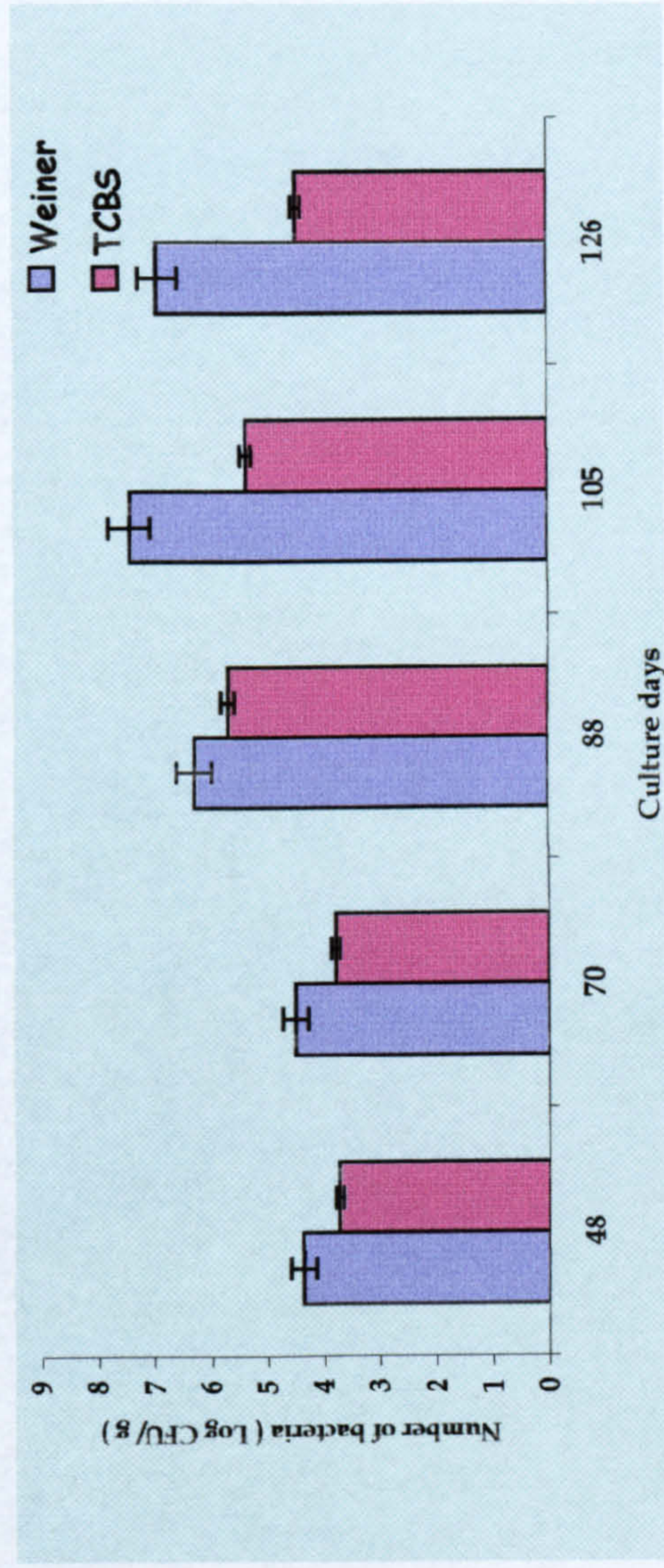


Fig.4. 51 Changes in the abundance (±SD) of total heterotrophic aerobic bacteria (Weiner agar) and presumptive vibrios (TCBS agar) during the shrimp culture cycle Sep 98 - Feb 99 (Period 4).

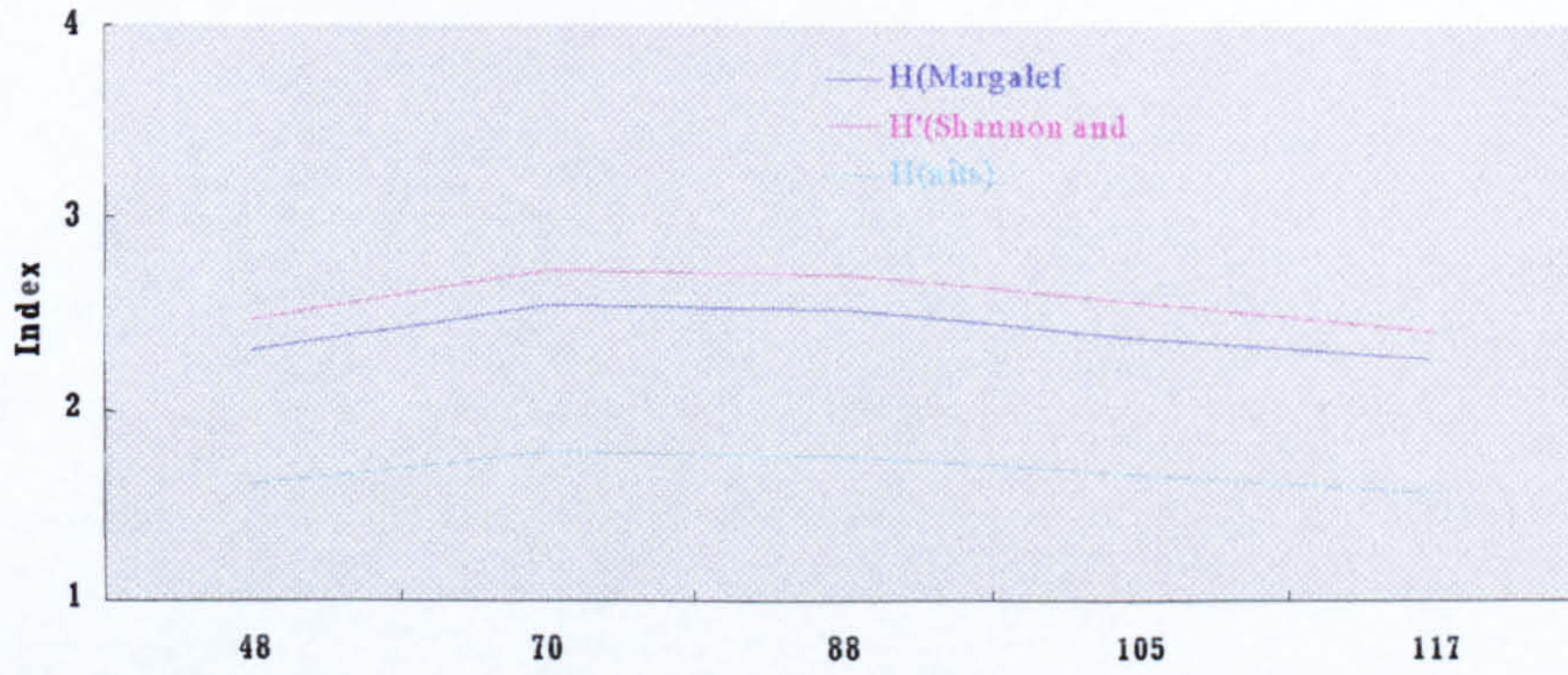


Fig.4. 52 Indices of diversity (information content, see section 3.6.1.1) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Sep 98 – Feb 99 (Period 4).

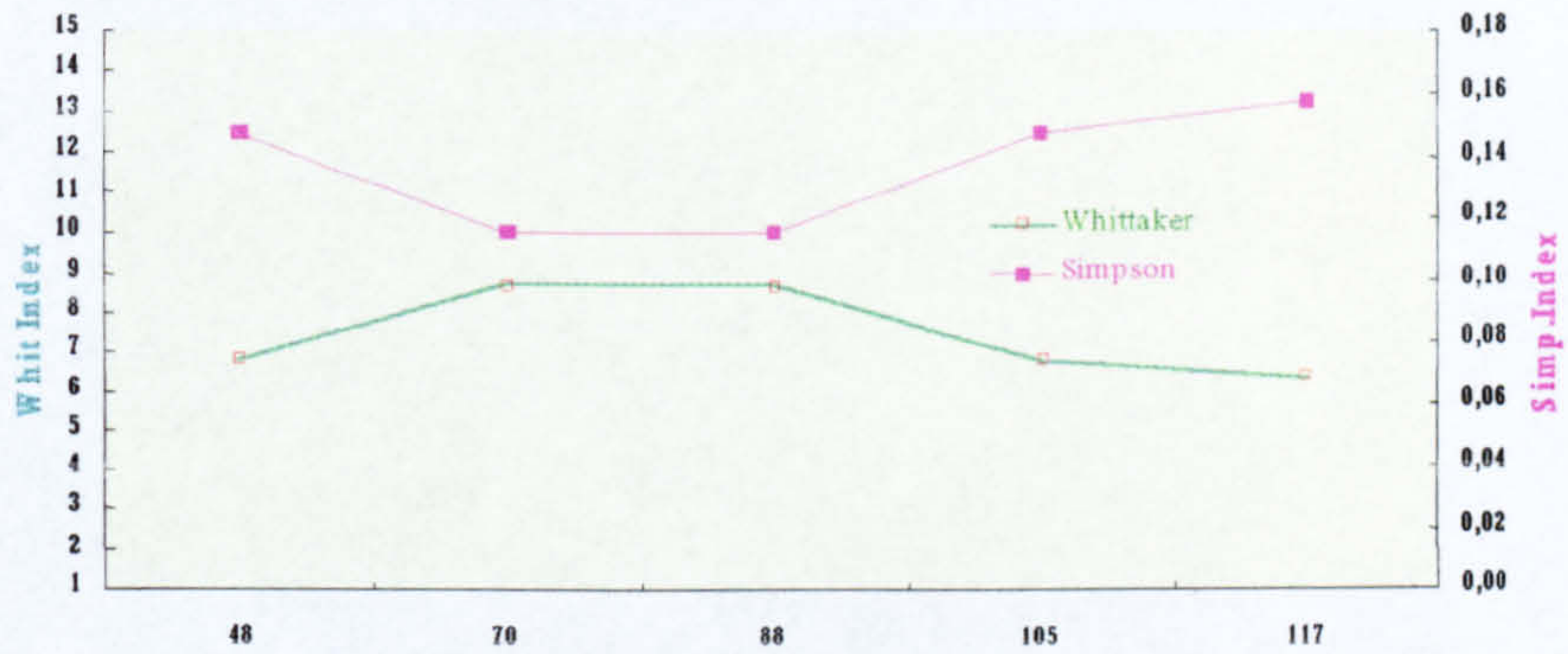


Fig.4. 53 Indices of diversity (number, see section 3.6.1.2) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Sep 98 – Feb 99 (Period 4).

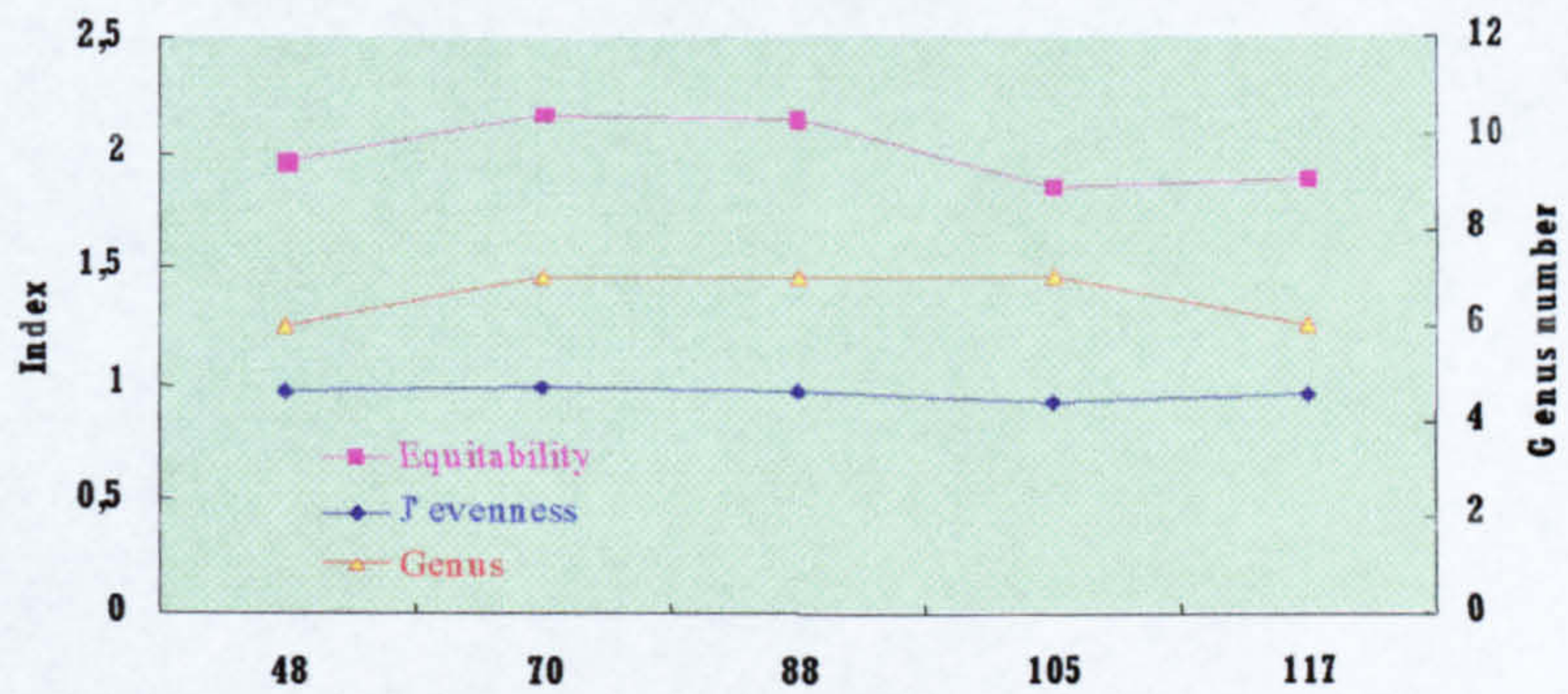


Fig.4. 54 Indices of abundance for genera of heterotrophic aerobic bacteria from different culture days Sep 98- Feb 99 (Period 4).

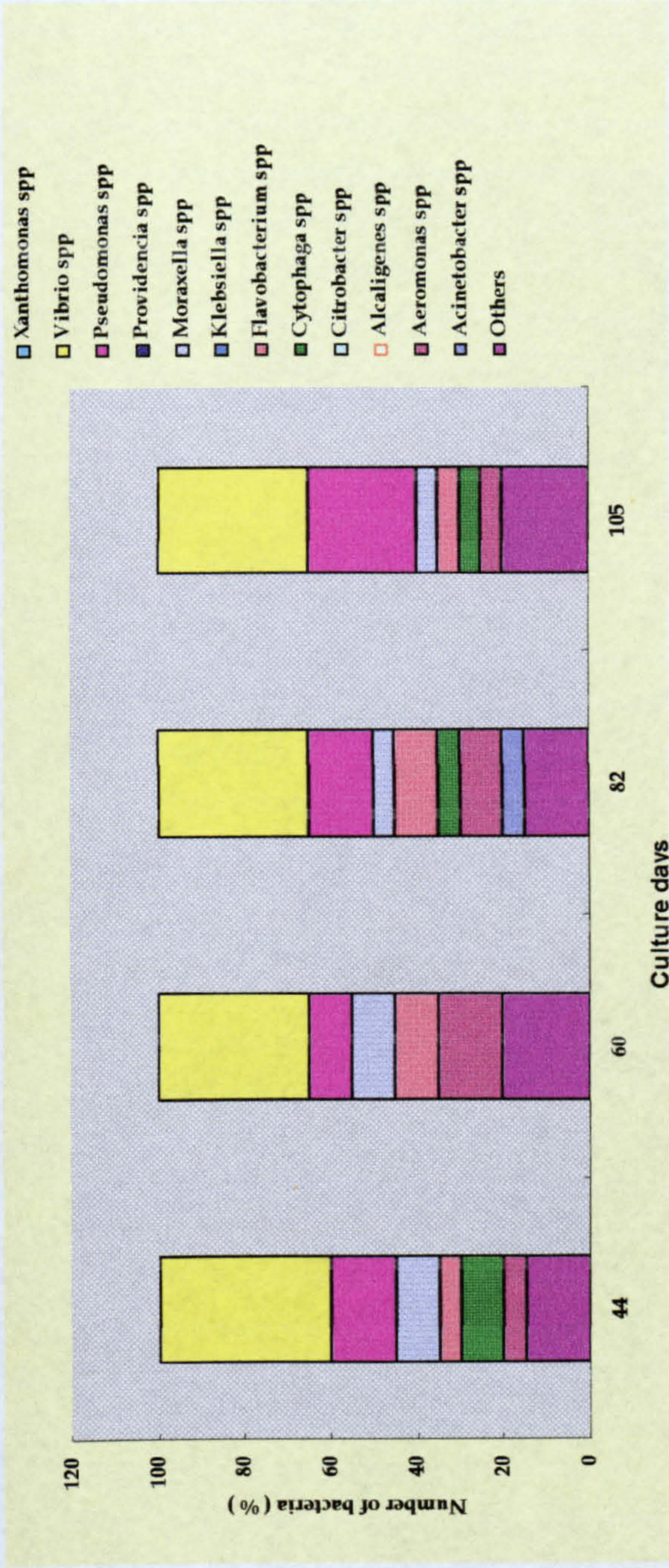


Fig.4. 55 Change in the percentage composition of total heterotrophic aerobic bacteria during the shrimp culture cycle Mar - Jul 99 (Period5).

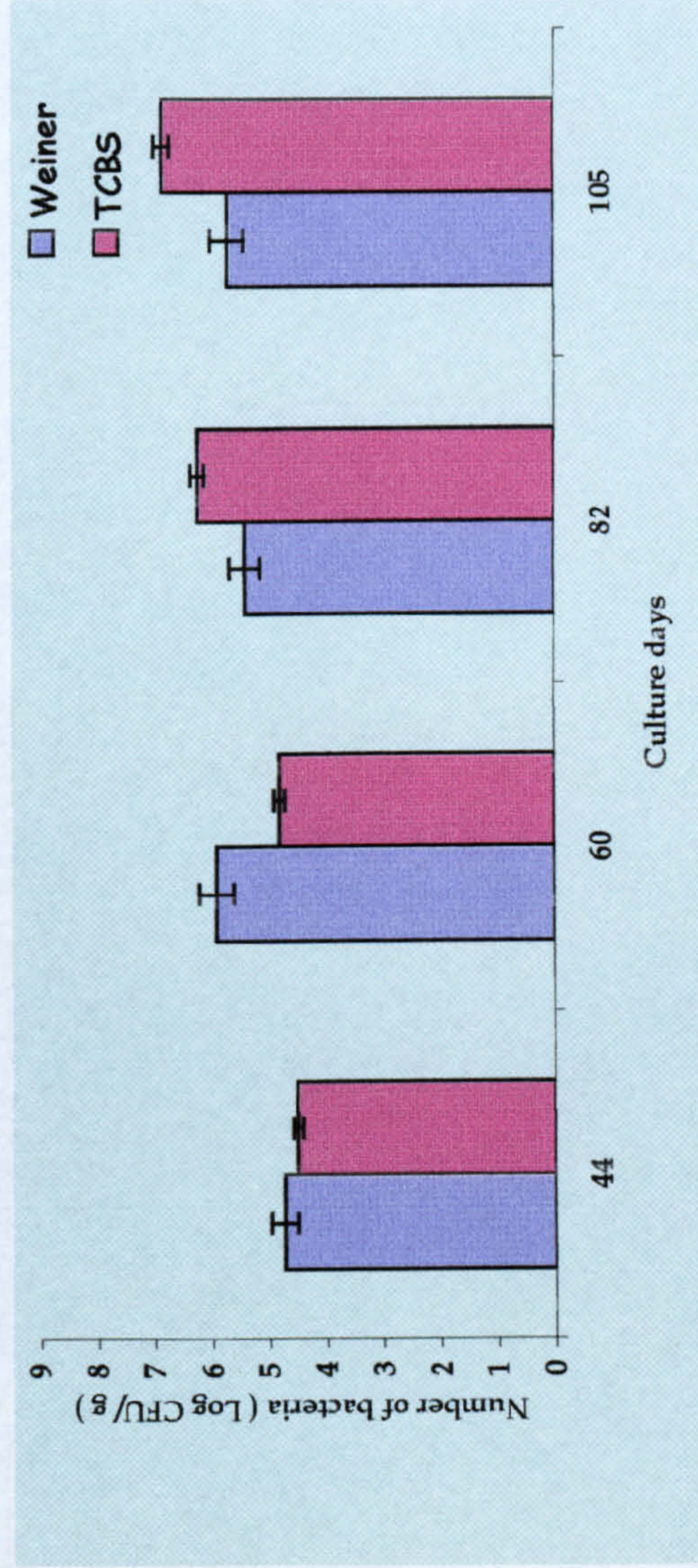


Fig.4. 56 Changes in the abundance (\pm SD) of total heterotrophic aerobic bacteria (Weiner agar) and presumptive vibrios (TCBS agar) during the shrimp culture cycle Mar 99 - Jul 99 (Period 5).

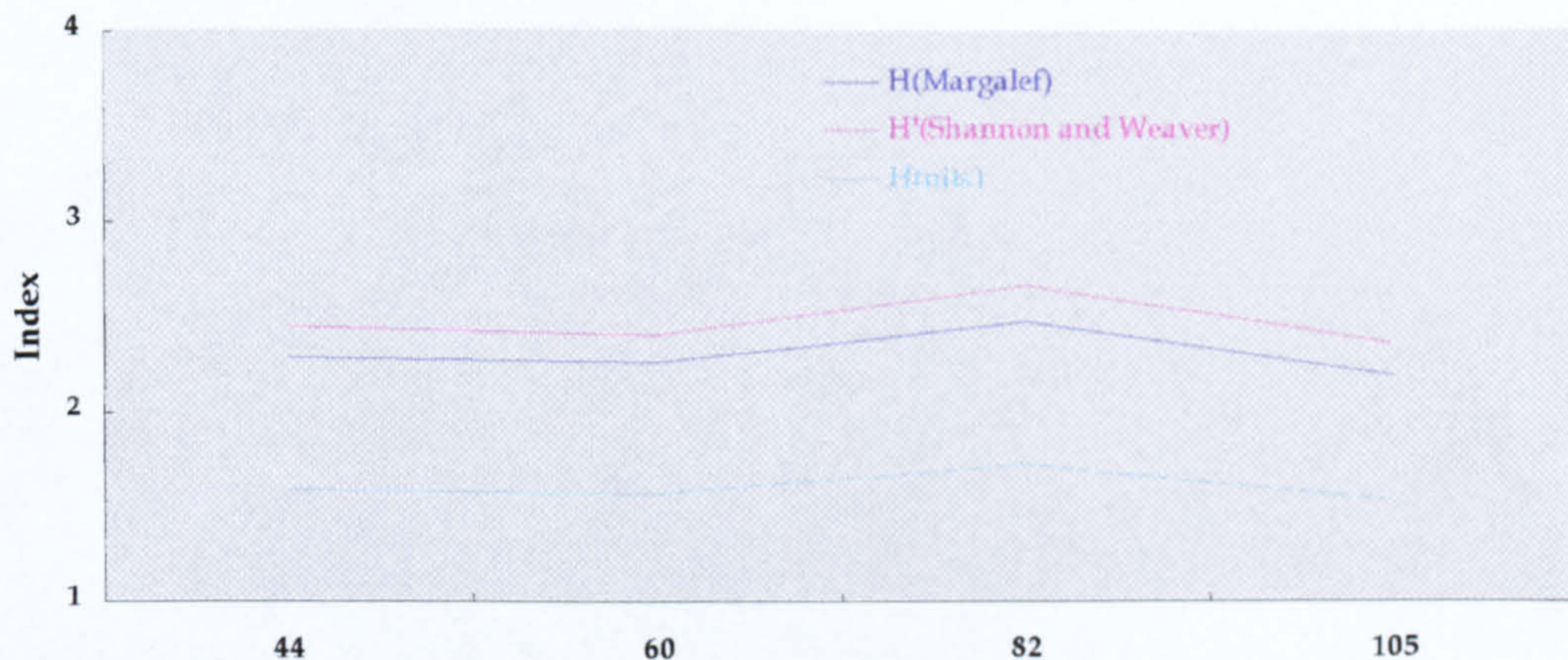


Fig.4. 57 Indices of diversity (information content, see section 3.6.1.1) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Mar 99 – Jul 99 (Period 5).

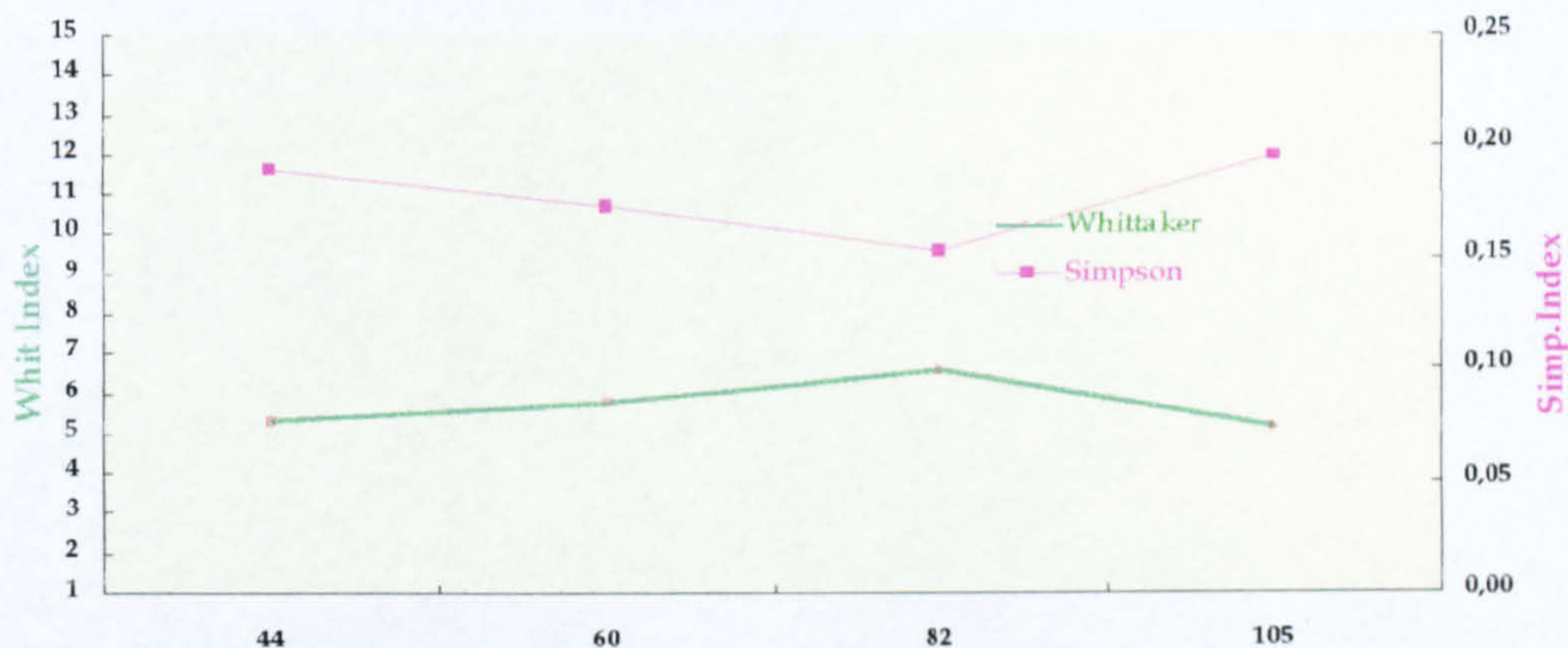


Fig.4. 58 Indices of diversity (number, see section 3.6.1.2) for genera of heterotrophic aerobic bacteria during the shrimp culture cycle Mar 99 – Jul 99 (Period 5).

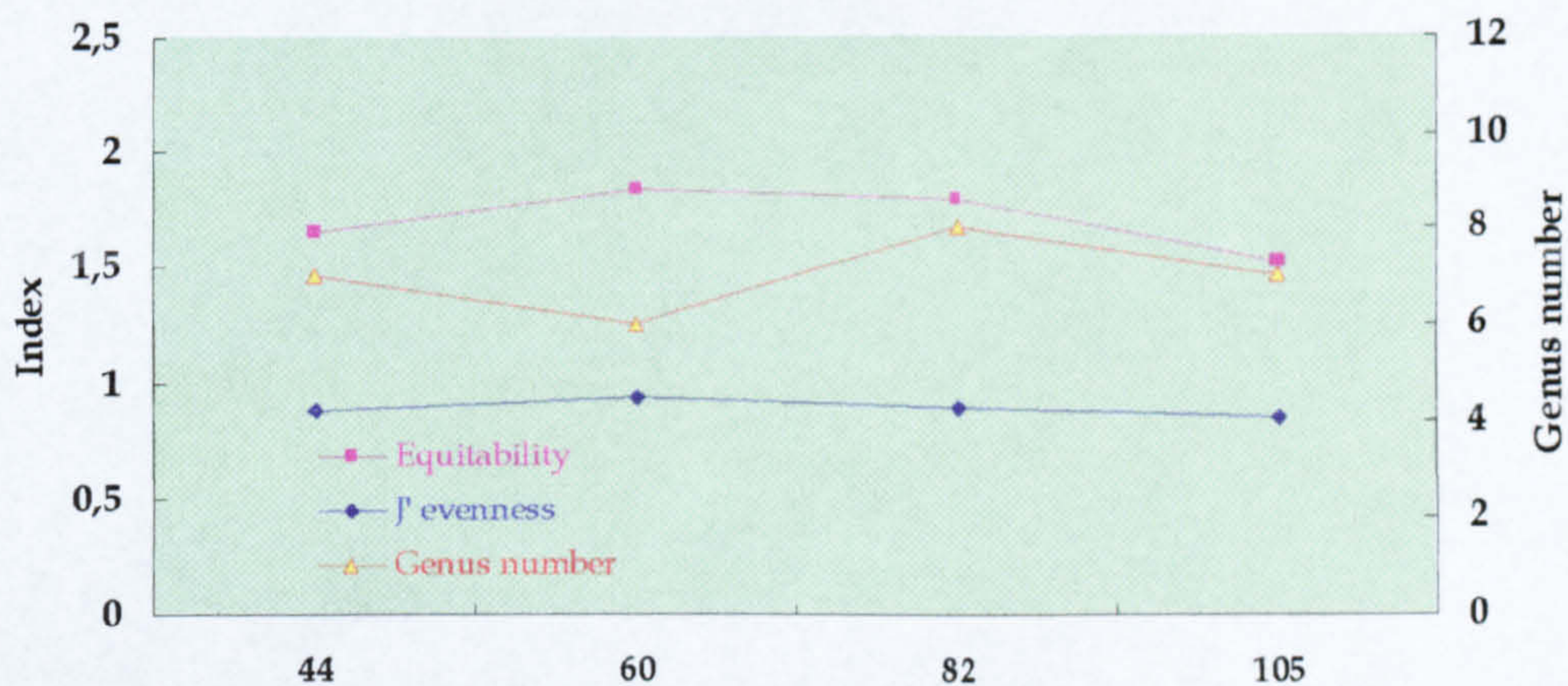


Fig.4. 59 Indices of abundance for genera of heterotrophic aerobic bacteria from different culture days Mar 99 – Jul 99 (Period 5).

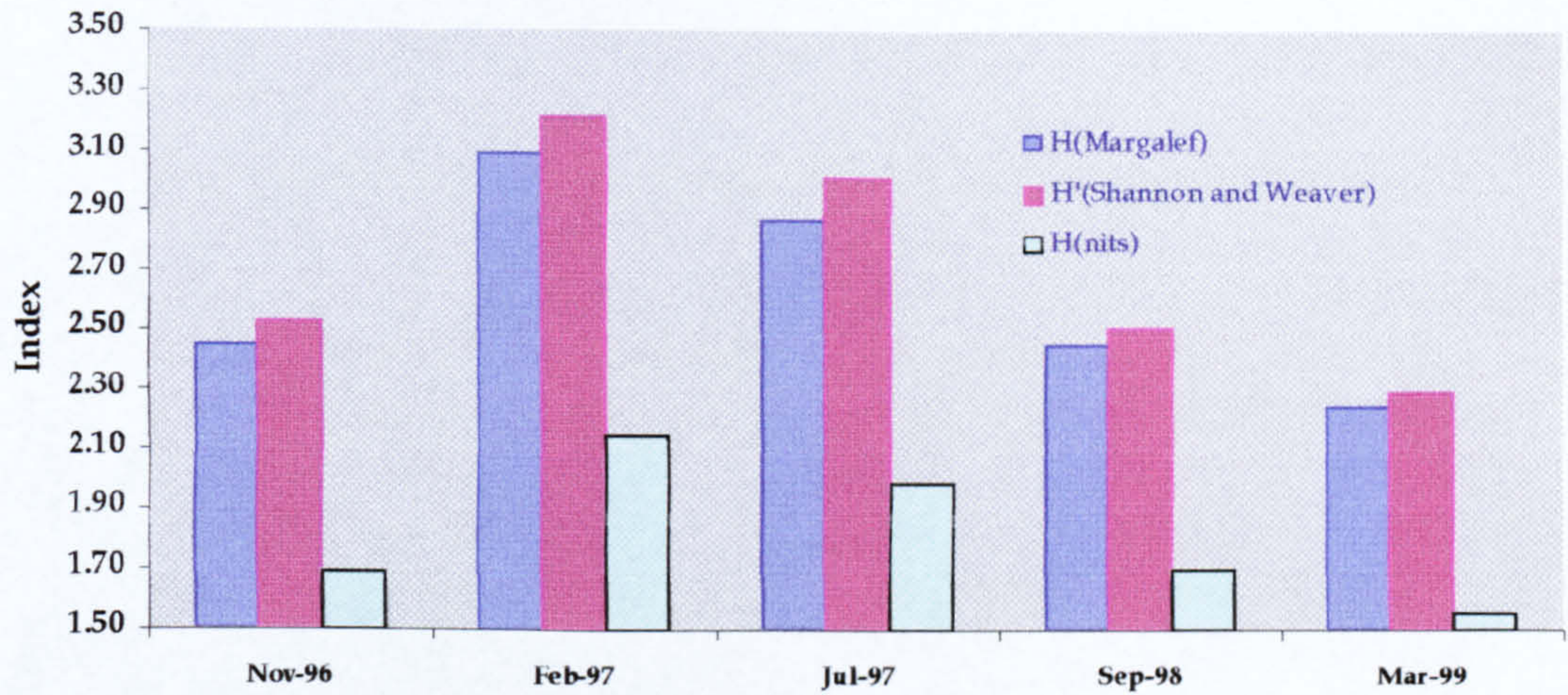


Fig.4. 60 Indices of diversity (information content, see section 3.6.1.1) for genera of heterotrophic aerobic bacteria from different culture periods.



Fig.4. 61 Indices of diversity (number, see section 3.6.1.2) for genera of heterotrophic aerobic bacteria from different culture periods.

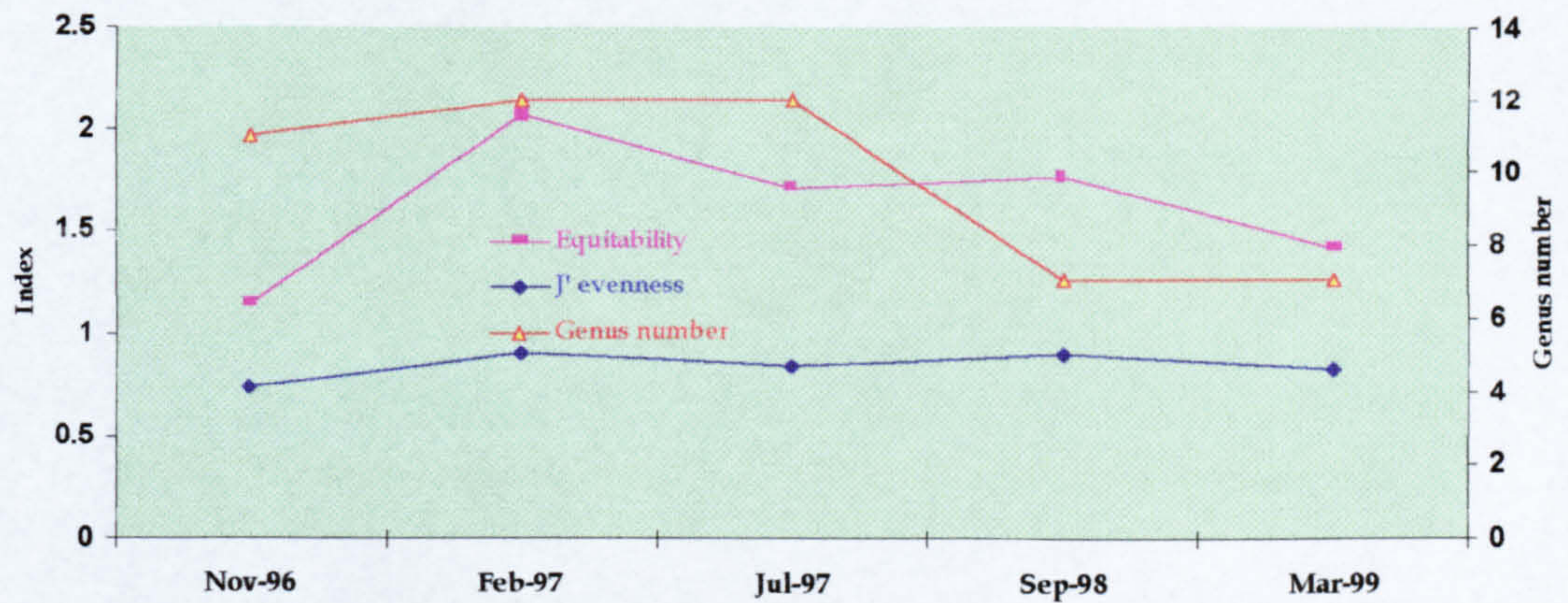


Fig.4. 62 Indices of abundance for genera of heterotrophic aerobic bacteria from different culture periods.

Bacterial discussion

Most of the genera of heterotrophic aerobic bacteria identified from the intestines of *Litopenaeus vannamei* in this study have already been identified as common gut flora in different species of *Penaeus*, both in the natural environment and in shrimp reared under experimental laboratory conditions, in hatcheries, and in culture ponds. These include: *P. aztecus* (Vanderzant *et al.*, 1970; Vanderzant *et al.*, 1971; Dempsey and Kitting, 1987; Dempsey and Kitting, 1989); *P. schmitti* (Pagnocca *et al.* 1991); *P. japonicus* (Yasuda and Kitao, 1980; Sano and Fukuda, 1987) ; *P. monodon* (Fonseka , 1990 ; Ruangan and Kitao, 1991; Sung *et al.*, 1999; Otta *et al.*, 2001; Sung *et al.*, 2001) ; juvenile *L. vannamei* (Gomez-Gil *et al.*, 1998); *P. indicus* (Kumar and Dube, 1990; Hameed, 1993); and *P. californiensis* (Hernandez López *et al.*, 1997).

Apparently healthy shrimp contain constant low levels of gut bacteria, especially *Vibrio spp.* (Lightner, 1988; Gomez-Gil *et al.*, 1998; Li *et al.*, 2000), although their mechanisms of defense seem capable of controlling these bacteria under normal circumstances (Lightner, 1988). Conversely, bacteria that may be part of the shrimp's normal microflora are found to cause disease in stressed shrimp (Lightner and Lewis, 1975; Liu and Chen, 1988; Lightner, 1996a; Li *et al.*, 2000). Vibriosis in penaeids is generally recognized as an infection influenced by factors such as stress, environmental failures,

and the presence of high numbers of potentially pathogenic bacteria (Chen, 1992; Nash *et al.*, 1992; Mohny *et al.*, 1994; Ruangpan *et al.*, 1995). In general, a stressed environment results in a decrease in the diversities of the microbial community and an increase in the numbers of functionally specific microbial groups (Dean-Ross and Mills, 1989; Atlas *et al.*, 1991; Geiselbrecht *et al.*, 1996).

The clinical signs of shrimp affected by pathogenic bacteria during the transition and La Niña periods of culture in the present study were similar to those observed by Liao *et al.*, (1977) and Alapide and Dureza, (1997). The shrimp body showed a yellowish discolouration and in the final stages of the disease became reddish, with the shrimp becoming lethargic and anorexic. The shrimp died near the pond dikes. The red discolouration is due to the distribution and deposition of hepatopancreatic carotenoids by the haemolymph into other tissues (Lightner and Redman, 1985).

The predominance of *Vibrios*, (23.0 – 36.5 %), especially *V. harveyi* and *V. parahaemolyticus*, observed in the intestines of shrimp during disease outbreaks of cultured *L. vannamei*, the low genera diversity, no toxic levels in physico-chemical parameters, namely unionised ammonia, nitrite or sulphide were recorded in these periods, suggested that vibriosis was associated with shrimp mortalities, brought about by strong changes in temperature and salinities associated with the climatic transition and La

Niña periods, (Retamales, 2001). In view of these results and in order to assess the absence or presence of other infectious agents, such as TSV or IHHN virus, shrimp samples fixed in Davidson's fixative (Humason, 1972) and stored in 70% ethanol, were sent to a laboratory (Acuatecnos, Guayaquil) for histological examination and DNA analysis using a ShrimProbe™, DiagXotics® test kit. The laboratory samples revealed the absence of these viruses and the presence of typical vibriosis tissue lesions.

Decreases in the diversity of the *Vibrio* community were observed in pond water and in the hepatopancreas from diseased and healthy *P. monodon* cultured in Taiwan (Sung *et al.*, 1999; Sung *et al.*, 2001), but there were no data offered to relate this reduction in diversity to any change in abiotic parameters of the pond water.

In a natural ecosystem, the microbial community exists in a dynamic state, and it is not only very susceptible to the stresses to which an ecosystem is subject, but also is strongly influenced by the seasons. Williams and LaRock (1985) showed that in an estuarine environment, similar to the *L. vannamei* culture pond, the prevalence of *Vibrio* species varied according to the season.

Vibrio harveyi, the causative organism of luminous vibriosis, is part of the normal flora of warm near-shore marine waters (Pizzuto and Hirst, 1995). They are commonly termed as the free-living bioluminescent bacteria which can attach to the surfaces of marine crustaceans (O'brien and Sizemore,

1979) or can be ingested by the host and establish residence in the gut. Shrimp mortalities associated with *V. harveyi* have been reported in *Penaeus monodon* and *Penaeus vannamei* in : Indonesia (Sunaryanto and Mariam, 1986) Thailand (Jiravanichpaisal *et al.*, 1994), India (Karunasagar *et al.*, 1994), the Philippines (Baticados *et al.*, 1990; Lavilla-Pitogo *et al.*, 1990; Lavilla-Pitogo, *et al.*, 1998), Australia (Pizzutto and Hirst, 1995), Taiwan (Song and Lee, 1993; Liu *et al.*, 1996a, 1996b) and Ecuador (Robertson *et al.*, 1998; Alvarez, *et al.*, 1998).

In the study of Lavilla-Pitogo *et al.* (1992), luminescent bacteria were found in the midgut contents of *P. monodon* spawners and pond-reared juveniles. Continuous exposure of shrimp postlarvae to high numbers of *V. harveyi* in the environment can easily cause primary vibriosis as the bacteria are able to multiply on larval surfaces in *P. monodon* (Lavilla-Pitogo *et al.*, 1990).

Environment factors such as low salinity and temperature (conditions found during the transition and La Niña periods) increase the virulence of *V. harveyi* to *P. monodon*, resulting in significant mortalities (Prayitno *et al.*, 1995; Liu *et al.*, 1996b; Shivappa, 1997).

Disease outbreaks attributed to *V. parahaemolyticus* have been observed in nursery or growout ponds of *L. vannamei*, *P. monodon*, *M. japonicus* and *L. stylirostris* in Ecuador (Lightner, 1992), Malaysia (Anderson *et al.*, 1988), Taiwan (Song *et al.*, 1993), the Philippines (Alapide-Tendencia and

Dureza, 1997), Japan (Takahashi *et al.*, 1985; de la Peña *et al.*, 1993) and New Caledonia (Costa *et al.*, 1998; Mermoud *et al.*, 1998) .

Vibrio parahaemolyticus is found frequently in surface sediments when the surrounding water column conditions are unfavourable, e.g. low temperature (Kancho and Colwell, 1973; Kancho and Colwell, 1978; Joseph *et al.*, 1983). This *Vibrio* species has been reported to produce septicemia in crustaceans (Thune *et al.*, 1991), including *P. monodon* (Anderson *et al.*, 1988). At 27 °C and 35 psu shrimp demonstrate an age susceptibility that depends on the *Vibrio* species and dose level ($10^5 - 10^7$ CFU/ml) (Aguirre-Guzmán *et al.*, 2001). The species *V. harveyi* and *V. parahaemolyticus*, in particular, induced significant mortality rates only at high doses in *L. vannamei* (Aguirre-Guzmán *et al.*, 2001).

All of the above suggests that a reduction in bacterial diversity in shrimp ponds may not only indicate environmental stress, but might also be a useful predictor for the occurrence of disease in cultured shrimps. Because of their short generation time, microorganisms are known to respond rapidly to such changes in their surrounding environment. Thus the composition of the community of microorganisms, or more strictly of a particular taxonomic group, can be used as an indicator of prevailing environmental quality, or of changes in that quality (Hellowell, 1986).

4.4 Harvest and shrimp performance results

The statistical analysis of shrimp growth data was performed using linear regression analysis and one-way analysis of variance at each sampling period to determine whether the growth rate of cultured *L. vannamei* differed significantly between the five periods of culture.

As expected, for all five culture periods there was a highly significant positive correlation between the size of *L. vannamei* and the duration of culture. In each case the Pearson product moment correlation coefficient was 0.99, $r^2 = 0.99$, (Figs 4.63 to 4.67).

4.4.1. Period 1. Transition Niña – Niño

The mean shrimp survival during period 1 was 33.8%, with a mean shrimp weight of 10.8 g., and a mean yield of 444 kg/ha. The mean instantaneous growth rate was 7.28 %, (% body weight gained per day), and the mean feed conversion ratio was 1.65 (total dry feed consumed/total wet weight gained) (Table 4.5).

4.4.2. Period 2. Niño

In period 2, the mean shrimp survival improved to 69.0%, with a mean weight of 12.1 g. The mean instantaneous growth rate was 7.38 %, the mean feed conversion ratio was 1.22 and the mean yield was 919 kg/ha (Table 4.5). Thus the survival and yield of cultured shrimp was more than double that

of period 1. The equivalent FCR represents only 73 % of the value in period 1, i.e., 27% less feed was consumed in period 2, while the shrimp were larger by 12%.

4.4.3. Period 3. Niño

Mean shrimp survival was 45.5% in period 3 with a mean weight of 12.1 g., leading to a mean yield of 725 kg/ha (Table 4.5). The mean instantaneous growth rate was 7.38 %, and the mean feed conversion ratio was 1.31. Survival was better than in period 1, but 35 % less than that of period 2 because of a short disease outbreak that occurred in this period. Overall, period 3 was characterised by a good performance in terms of shrimp yield, FCR and instantaneous growth rate.

4.4.4. Period 4. Transition Niño – Niña

Mean shrimp survival in period 4 was 34.5%, with a mean weight of 11.3 g. generating a mean yield of 499 kg/ha (Table 4.5). The instantaneous growth rate was 7.32 %, with a mean feed conversion ratio of 1.76. These performance data represent an approximate 50% decrease in survival and yield compared to the shrimp crop in period 2, and a higher FCR compared to that in period 2.

4.4.5. Period 5. Niña

Mean shrimp survival was 32.5%, with the surviving shrimp reaching a

mean weight of 12.4 g. The mean instantaneous growth rate was 7.4 %, mean feed conversion ratio was 1.98 and the mean yield was 443 kg/ha (Table 4.5). In this period, the significant difference observed in the final weight ($P < 0.05$) with respect to the other culture periods, was due the relationship between shrimp growth-density, because mortality, occurred mainly during a serious disease outbreak, which decreased the number of shrimp per ha. Despite this, and a poor FCR (39 % more feed consumed than in period 2) the yield obtained was almost half (48%) that obtained during period 2.

In summary, there were significant differences in the mean growth rate of shrimp between the five different culture periods ($P < 0.05$) (Fig.4.68). Despite this, it can also be seen that there was a convergence of individual shrimp weight to around 11- 13 g. at 115 - 125 days of culture.

The performance of cultured shrimp was significantly different ($P < 0.05$) between the El Niño and La Niña or transition climatic periods. Overall the mean survival, the feed conversion ratio and the yield were better during the El Niño culture periods, i.e. 45 - 69 %, 1.22 - 1.31 and 725- 919 kg/ha respectively.

Pooling the data from all the culture periods under these natural conditions, the interaction between the effects of temperature and salinity are clear from the surface response on shrimp survival and the yield over the days of culture in the ponds (Figs 4.69 and 4.70). The best survival (60 – 70 %) and yield (900 – 1000 kg/ha) coincided with a temperature range of 28 to 30 °C

and a salinity range of 20 – 30 psu (Table 4.5).

Although the mean instantaneous growth rate of *L. vannamei* was slightly improved during the La Niña phase, there was simultaneously a strongly negative effect on survival (32.5%), FCR (1.98) and yield (443 kg/ha) during this climatic period.

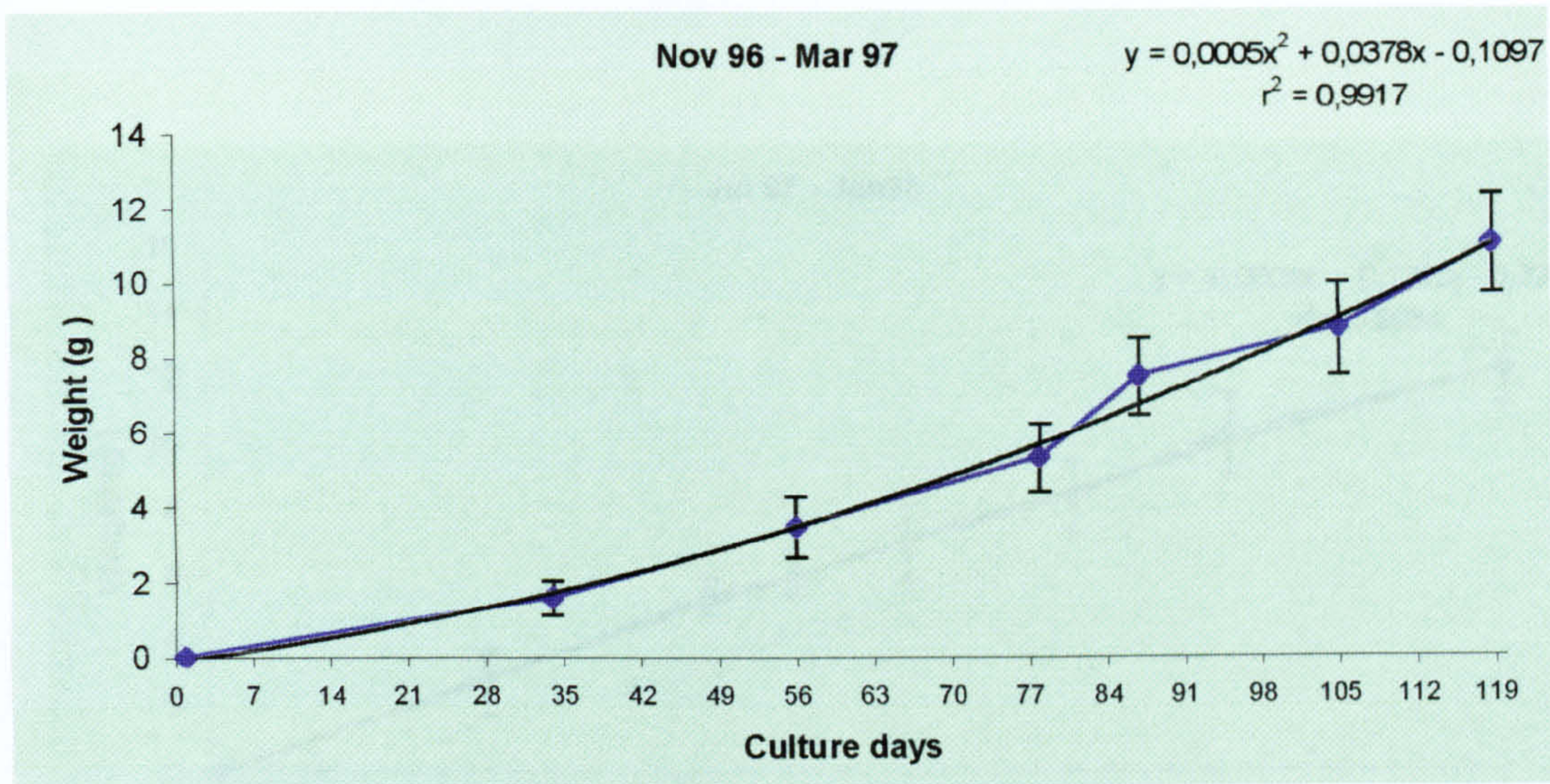


Fig.4. 1 Mean growth (±SD) of *L. vannamei* with time in a 6.0 ha pond during culture Period 1. November 96 – March 1997. Pearson product moment correlation coefficient = 0.99, (r2=0.99).

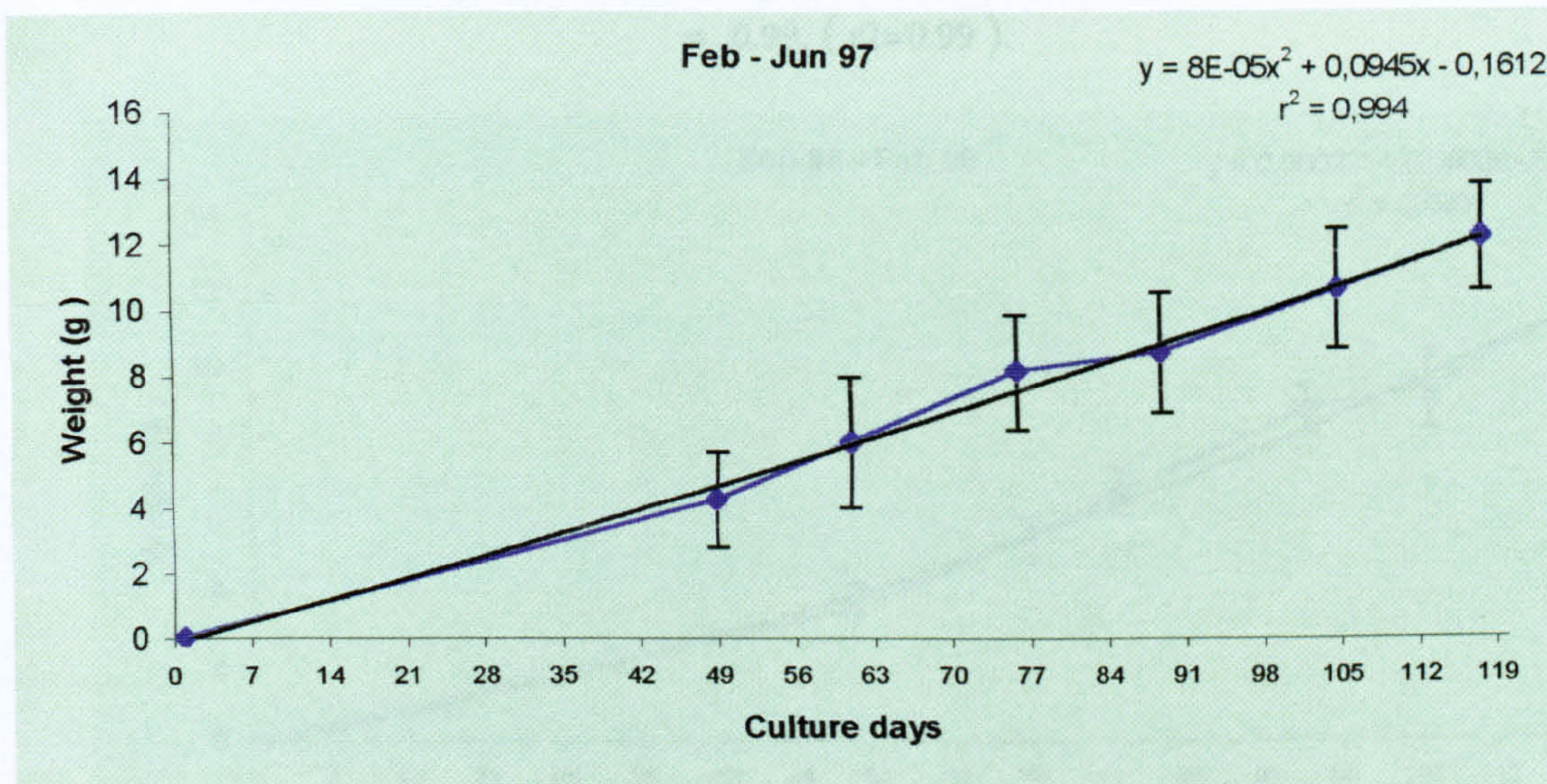


Fig.4. 2 Mean growth (±SD) of *L. vannamei* with time in a 6.0 ha pond during culture Period 2. February – June 1997. Pearson product moment correlation coefficient = 0.996, (r2=0.99).

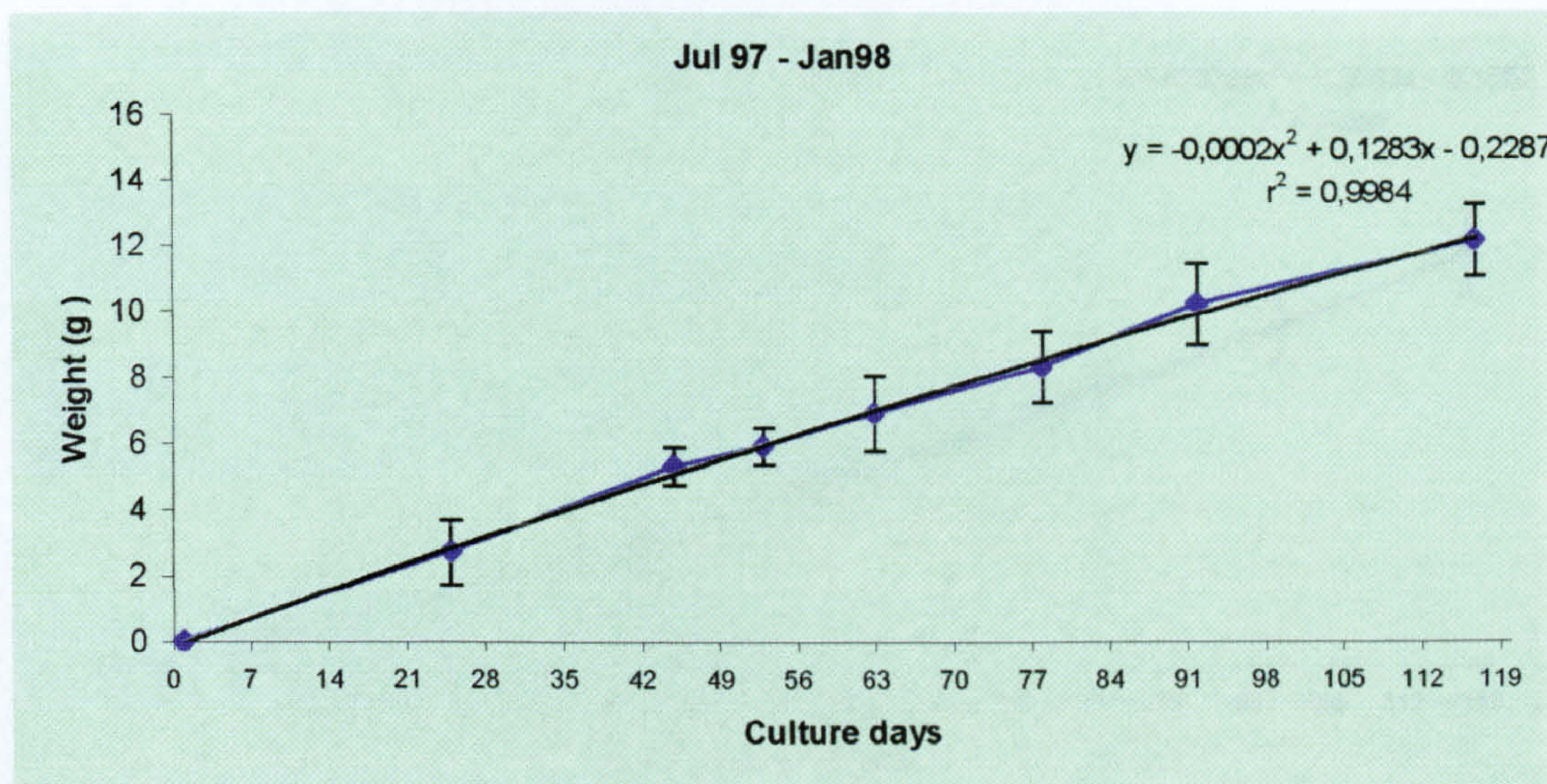


Fig.4. 3 Mean growth (±SD) of *L. vannamei* with time in a 6.0 ha pond during culture Period 3. July 1997 – January 1998. Pearson product moment correlation coefficient = 0.99, (r²=0.99).

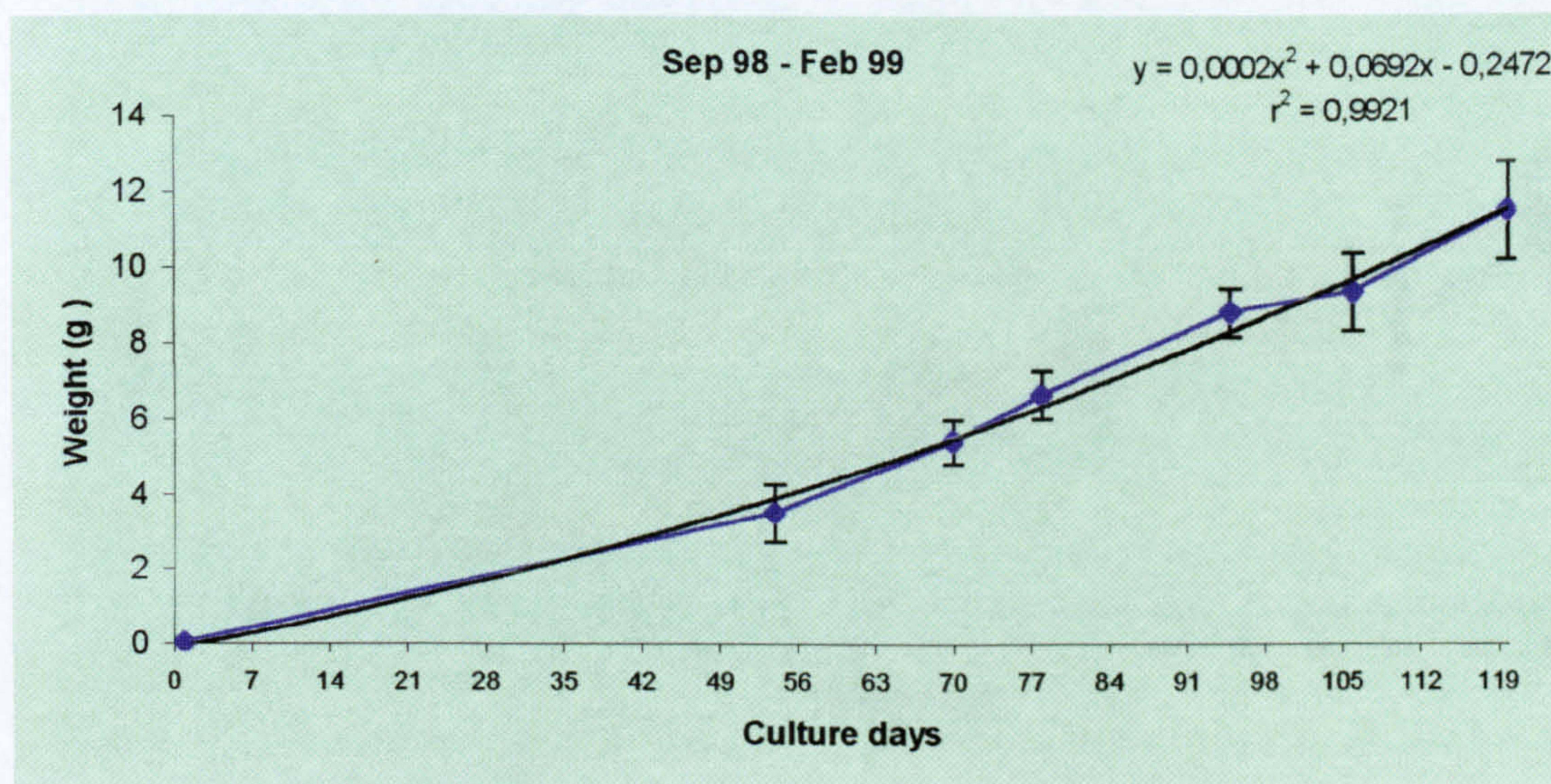


Fig.4. 4 Mean growth (±SD) of *L. vannamei* with time in a 6.0 ha pond during culture Period 4. September 1998 – February 1999. Pearson product moment correlation coefficient = 0.99, (r²=0.99).

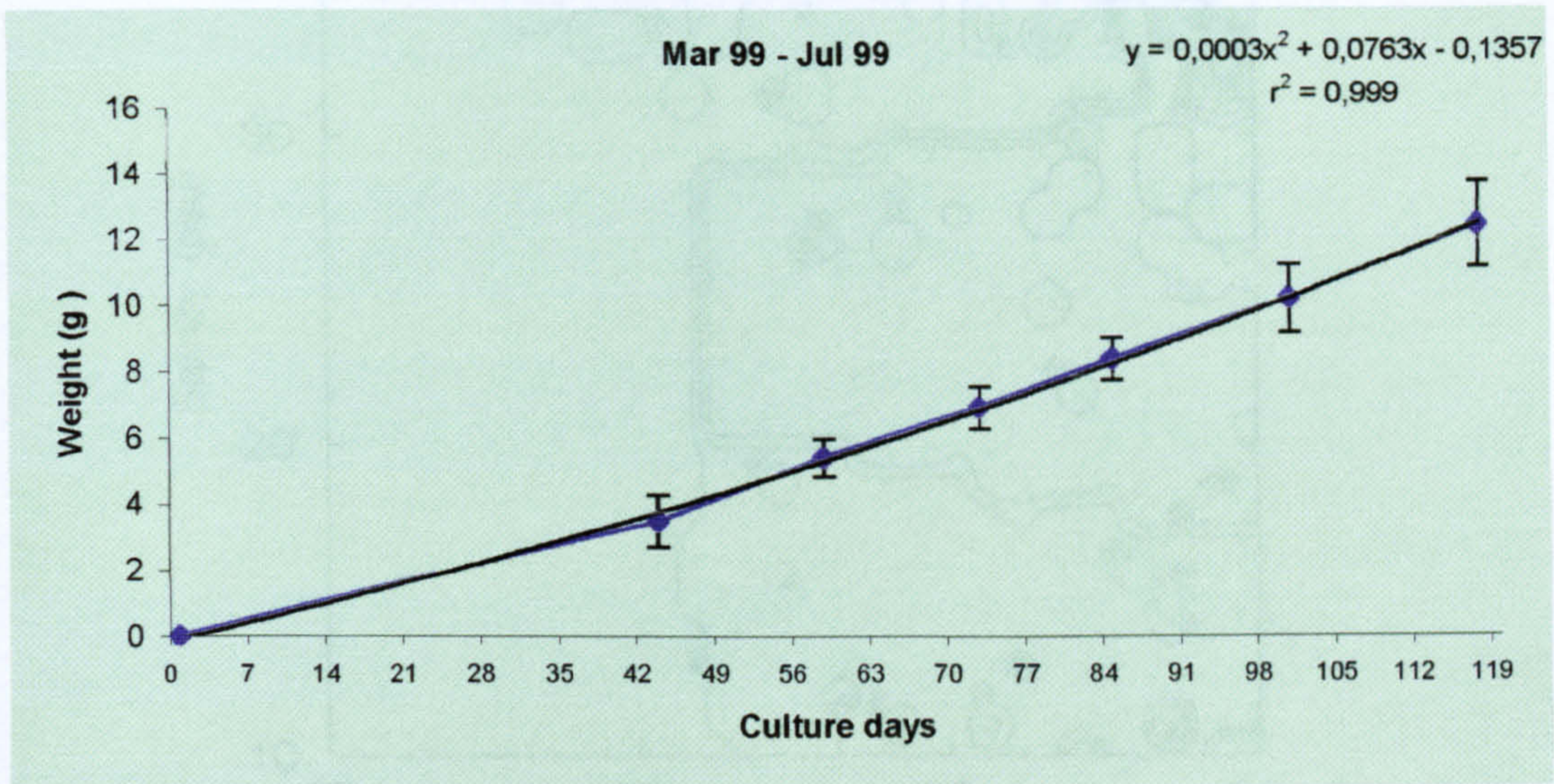


Fig.4. 5 Mean growth (±SD) of *L. vannamei* with time 6.0 ha pond during culture Period 5 . March – July 1999. Pearson product moment correlation coefficient = 0.999, (r²=0.999)

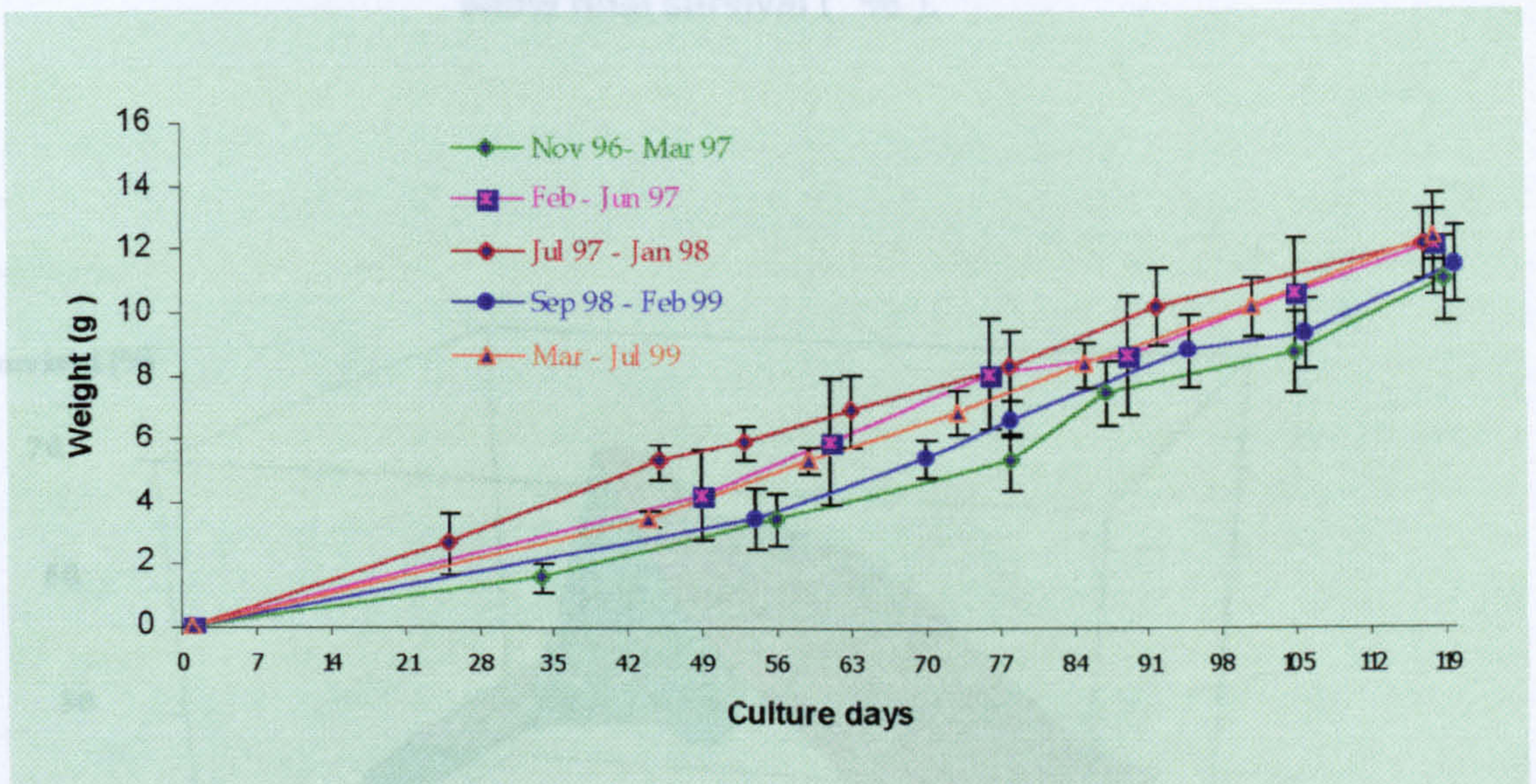


Fig.4. 6 Overall comparison of the mean growth (± SD) of *L. vannamei* in the five culture periods studied between November 1996 and March 1999.

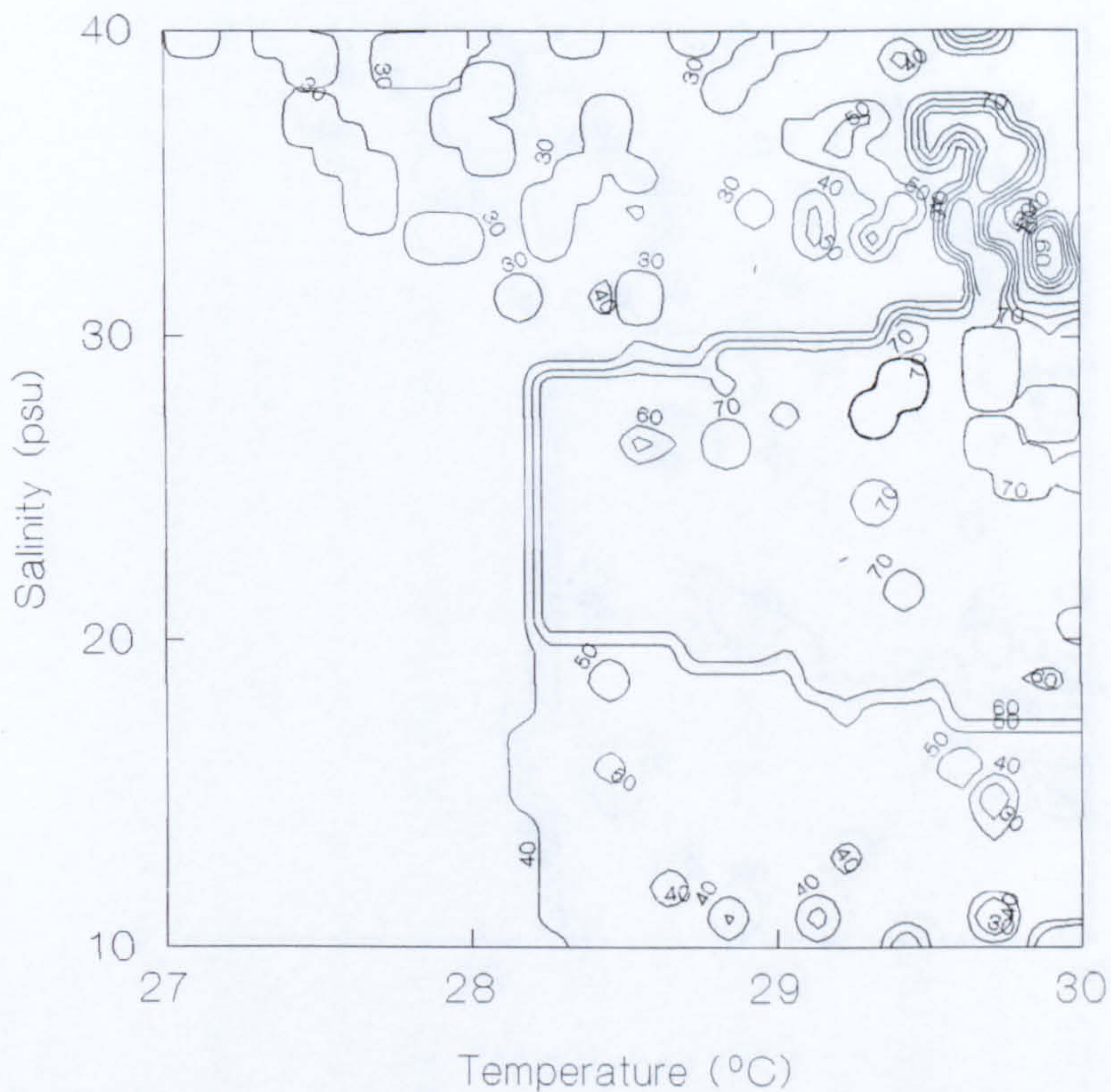
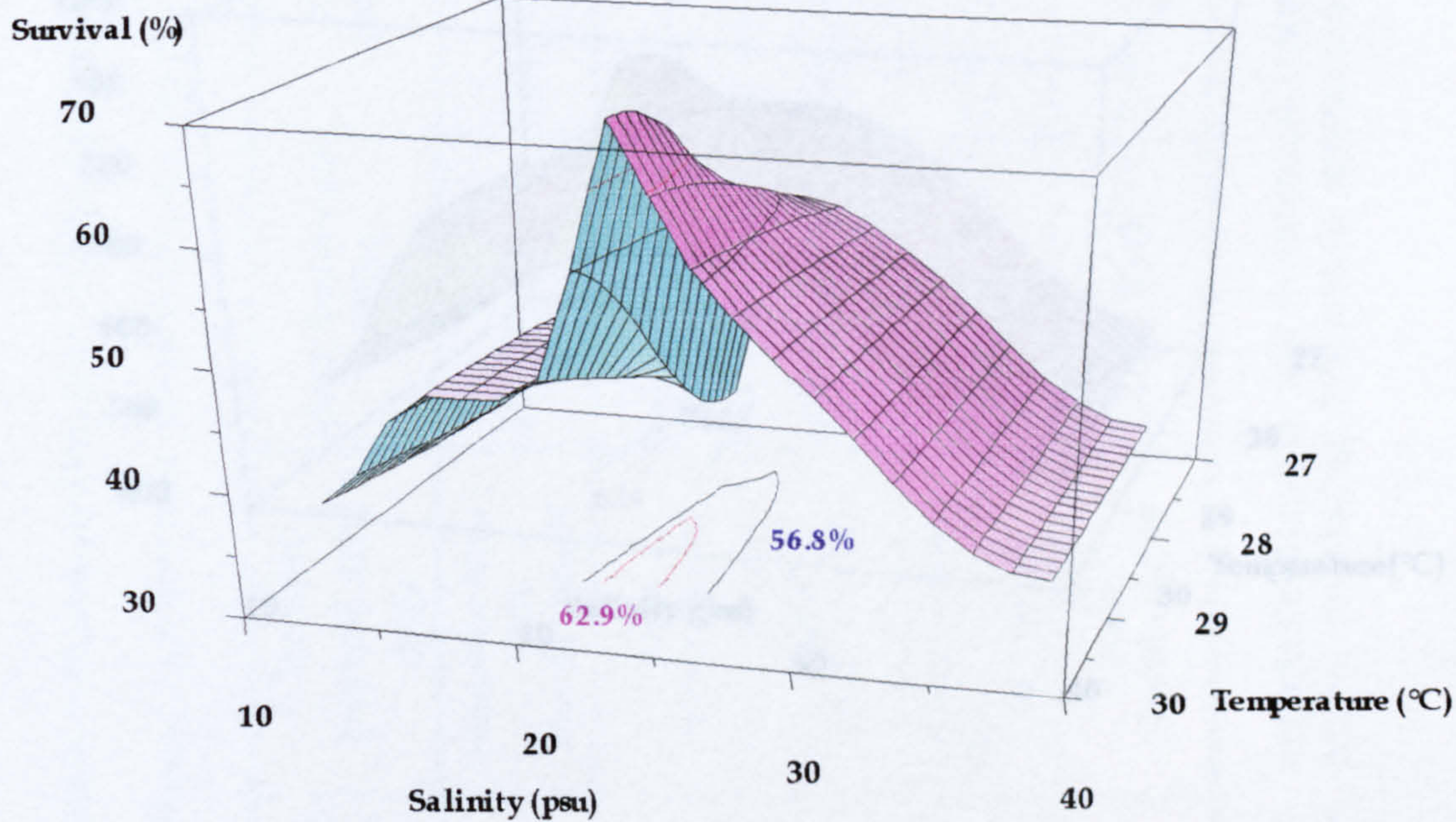


Fig.4. 7 Response surface showing final survival of *L.vannamei* at different temperatures and salinities over a culture period of 119 days. The contours lines show final survival (%).



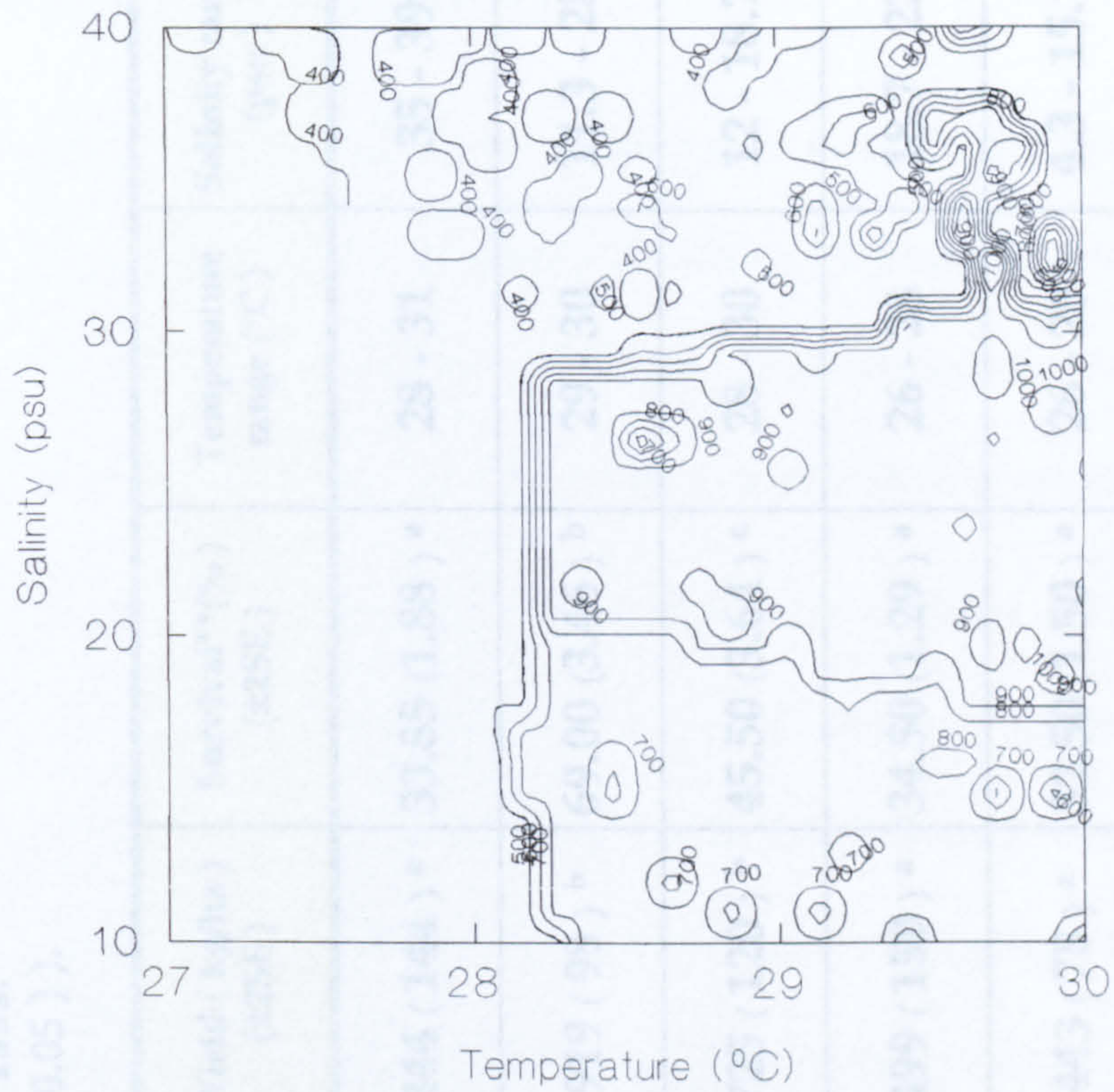


Fig.4. 8 Response surface showing final yield of *L.vannamei* at different temperatures and salinities over a culture period of 119 days. The contours lines show yield (kg/ha).

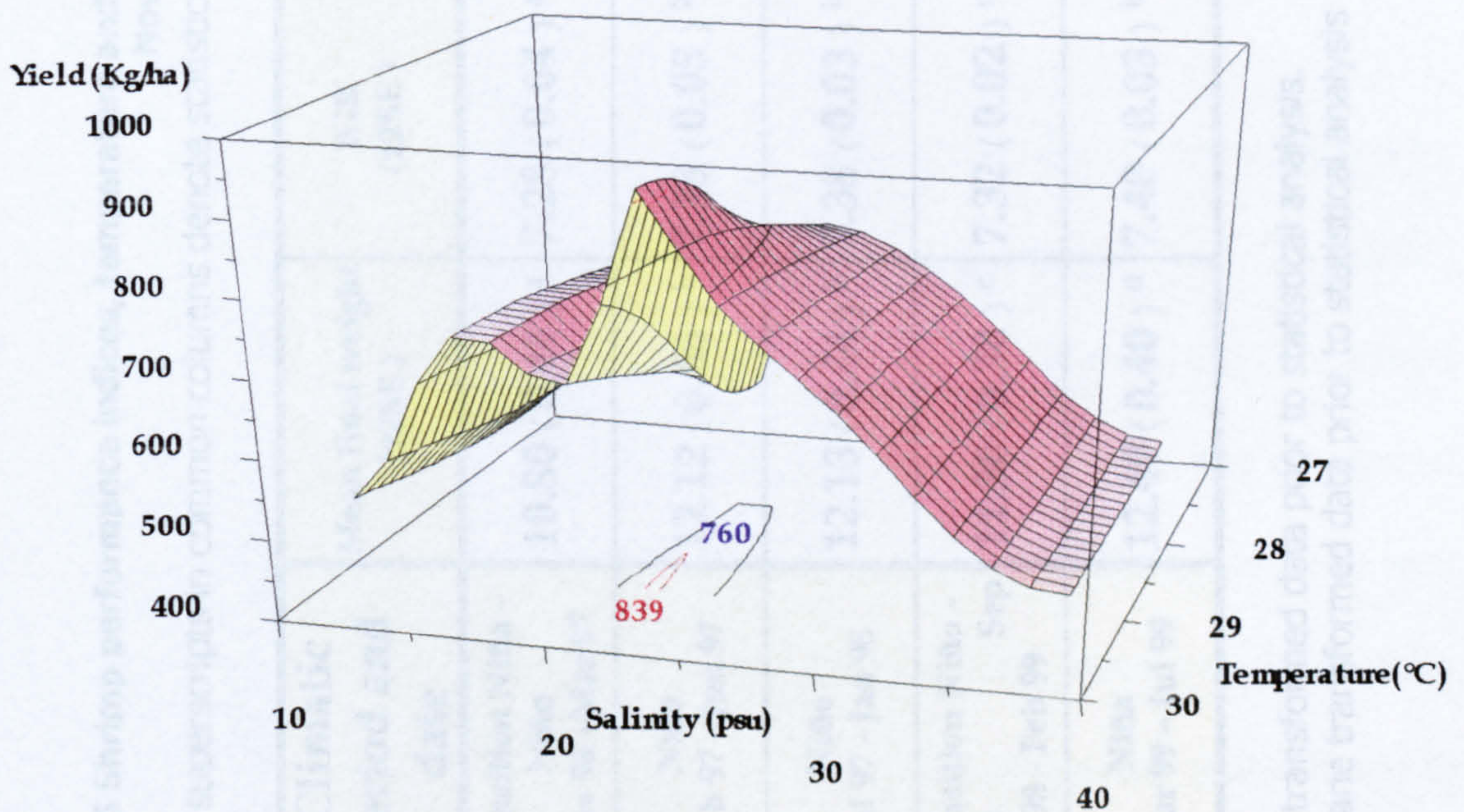


Table 4. 5 Shrimp performance indices, temperature and salinity range in pools stocked with *L.vannamei* from different culture periods, November 1996 to July 1999.

(Different superscripts in common columns denote statistical significance ($P < 0.05$)).

Culture Period	Climatic period and date	Mean final weight ($\pm 2SE$)	IGR ($\pm 2SE$)	FCR* ($\pm 2SE$)	Yield (kg/ha) ($\pm 2SE$)	Survival** (%) ($\pm 2SE$)	Temperature range ($^{\circ}C$)	Salinity range (psu)
1	Transition Niña - Niño Nov 96 - Mar 97	10.80 (0.46) ^a	7.28 (0.04) ^a	1.65 (0.02) ^a	444 (144) ^a	33.85 (1.88) ^a	28 - 31	35 - 39
2	Niño Feb 97 - Jun 97	12.12 (0.66) ^b	7.38 (0.05) ^b	1.22 (0.04) ^b	919 (98) ^b	69.00 (3.46) ^b	29 - 30	11.3 - 28
3	Niño Jul 97 - Jan 98	12.13 (0.44) ^b	7.38 (0.03) ^b	1.31 (0.02) ^c	725 (120) ^b	45.50 (3.64) ^c	28 - 30	12 - 18.7
4	Transition Niño - Niña Sep 98 - Feb 99	11.30 (0.30) ^c	7.32 (0.02) ^c	1.76 (0.1) ^{a, d}	499 (150) ^a	34.50 (1.29) ^a	26 - 28	18.7 - 22
5	Niña Mar 99 - Jul 99	12.40 (0.40) ^d	7.40 (0.03) ^b	1.98 (0.12) ^d	443 (78) ^a	32.50 (1.50) ^a	26 - 28	4.3 - 15.7

* log transformed data prior to statistical analysis.

** arcsine transformed data prior to statistical analysis

5. GENERAL DISCUSSION

Pond aquaculture production in Ecuador now exceeds 62,111 mt per annum, due principally to the production of white shrimp *Litopenaeus vannamei*, (FAO, 2001). This species is reared semi-intensively in earthen and mangrove forestry farms and its growth potential is year-round.

In Ecuador, shrimp farmers encounter two distinct growing seasons characterised as a “dry season” and a “rainy season”. Large differences in shrimp survival, and therefore in production, between these seasons are typical. Despite this, there is little information available from the literature in Ecuador on how these seasonal events affect shrimp culture. Under similar conditions of semi-intensive shrimp culture in Honduras, Teichert-Coddington *et al.* (1994) showed that temperature, was the major environmental factor determining seasonal production in Honduran shrimp farms.

Nowadays the extreme climatic events known as “El Niño”, characterised by warm ocean temperatures and an associated rainy season, and “La Niña”, featuring unusually cold ocean temperatures and dry season, are considered natural components of the ocean-atmosphere system within the equatorial Pacific. Although the general features of these climatic phenomena are well known, their affects on shrimp farming in Ecuador have not been fully explained until now. These events can change the properties (conditions) in

the natural environment, especially the natural water sources from which the water supply for shrimp culture ponds is obtained. Therefore, it was considered necessary to look critically at how some of the basic water quality and related environmental parameters in shrimp ponds affect the performance of cultured shrimp in Ecuador.

A sufficient supply of good quality water is essential to any aquaculture operation, as water quality affects both the growth and the survival of aquatic organisms (Tsai, 1990; Boyd, 1990; Boyd and Tucker, 1998). The criteria for good water quality vary with the kind of organism cultured and are established by safe levels, i.e. physical and chemical properties of water which have insignificant adverse effects on growth and survival. The factors controlling the composition of pond water are varied, and include physical, chemical and biological processes (Boyd and Tucker, 1998; Boyd and Massaut, 1999).

The changes in water quality in the shrimp ponds studied in the Chone River estuary showed no significant temporal differences in nitrite, nitrate, unionised ammonia, sulphide, pH and total suspended solids, (and these were within acceptable levels for *L. vannamei*). However, significant temporal differences in shrimp pond temperature and salinity values and in the phosphorus and silica concentration were revealed over the five shrimp production cycles which were monitored.

Diatoms (brown algae), cyanophytes (blue green algae), and chlorophytes (green algae) were the common groups of phytoplankton found in the ponds.

The changes in some pond nutrient concentrations found in the pond water during different periods of shrimp culture, affected the composition and diversity of the phytoplankton community in the water pond because cyanophytes dominated the phytoplankton community. In this study, the values of inorganic nitrogen: inorganic phosphorus were lower than 1. As many other research have indicated, low N:P ratios encourage the dominance of cyanophytes (Rhee and Gotham, 1980; Seymor, 1980; Yusoff and McNabb, 1989; Paerl and Tucker, 1995). Another environmental factor that seems to control the dominance of cyanophytes is salinity. Lowell and Broce, (1985) observed in pond-cultured shrimp, imported into the United States, high concentrations of geosmin, a musty odorous compound. The cause of this occurrence of off-flavour in the shrimp was the reduction in salinity in the coastal culture ponds, which allowed the growth of geosmin-producing blue-green algae.

The average survival and yield of *Litopenaeus vannamei* was 69% and 919 kg/ha respectively during the El Niño periods, even though cyanophytes were the dominant phytoplankton present in the ponds. However they did not develop into a bloom, which is usually characterised by the dominance of

one or two nuisance species. According to Paerl (1988), blooms exist as a massive accumulation of a single, or less often two coexisting species, with the species present accounting for as much as 90 % of the resident biomass of phytoplankton. Cyanophytes did not reach such critical levels during any of the five culture cycles studied in Ecuador.

Moreover, although cyanophytes are thought to decrease the diversity of phytoplankton species, resulting in poor water quality (Burford, 1997), an important point regarding the semi-intensive shrimp cultures operated in Ecuador, is that no negative effects on the main water quality parameters (nitrite, unionised ammonia, pH, total suspended solids and sulphide concentration) were recorded in the present study. However, this conclusion is based on rather limited information published on the lethal and sub-lethal affects of such water quality on culture shrimp (Table 4.2).

Water quality also plays a major role indirectly via its affects on shrimp health: any deterioration, or strong change in water quality causes stress to shrimp and brings about disease caused by the invasion of opportunistic pathogens such as *Vibrios* in shrimp culture. *Vibrios* are autochthonous bacteria of estuarine and marine waters, (Garay *et al.*, 1985). Their occurrence and distribution are seasonal, (Williams and La Rock, 1985) and they are temperature and nutrient dependent because of their physiological state (Baross and Liston, 1970).

The present study revealed (as discussed previously in the bacterial section) similar relationships between the temperature and salinity in shrimp ponds and the changes in the diversity of bacteria in the intestines of *L. vannamei*. Lower temperature and salinity promoted the predominance of *Vibrio* genera, particularly *V. harveyi* and *V. parahaemolyticus*. This suggests that these bacterial species were the cause of the disease outbreaks observed during some of the shrimp culture periods, particularly during the transition and La Niña seasons. Despite the absence of TSV and IHHN viruses, which were not detected in the laboratory by histology and shrimp probe testing, the moribund shrimp encountered in the culture ponds exhibited classical anatomopathologic septicemic vibriosis. This observation leaves open the question of whether these bacteria are only opportunistic pathogens responsible for a secondary infection and are there any other infectious or toxic agents involved ? Whatever the primary causes of the disease outbreaks observed during the shrimp culture cycles associated with the transition or La Niña climatic phases, disease affected the performance parameters of the shrimp farm, especially the survival and the final yield of the shrimp stock.

Higher shrimp yields were correlated positively with higher stock survival rates during the culture cycles in which no disease outbreaks were observed (El Niño) and environmental factors such as temperature - salinity interaction remained around 28 to 30 °C and 20 – 30 psu (i.e. near the

optimal ranges for *L. vannamei*).

Further studies need to be done to understand the species composition of the microbial community in relation to cultured shrimp and their pond environment. Such as, to what extent are pond microbial communities determined by chance (stochastic), and how are they influenced by predictable factors (deterministic) which allow one species to grow and divide more rapidly than others, thus becoming numerically dominant (Moriarty, 1998) ?

This study also provides a small contribution to the knowledge about the aerobic heterotrophic bacterial diversity observed in the gut of shrimp during periods where no disease outbreaks were observed. In theory it could be possible to manipulate, the bacterial species composition during disease outbreaks by seeding a number of desirable strains of bacteria into the pond. According to Smith (1993) the microbial species composition in aquaculture ponds can be changed by adding selected species to displace deleterious common bacteria. Success depends upon defining the ecological process or processes to be changed, the types of deleterious species that are dominant, the desirable alternative species or strains of bacteria that could be added (Havenaar *et al.*, 1992; Moriarty, 1998). The bacteria that are added must be selected for specific functions (e.g. colonization of digestive tract) and be

added (e.g. shrimp feed) at a high enough population density, to achieve the desired outcomes.

Climate cannot be controlled, but farm management can take its predictability into account. Shrimp farmers have to learn to live with a complex community of microbes and manage them. The use of beneficial bacteria (probiotics) to displace pathogenic bacteria by competitive processes is one of the possible tools that can be used rather than administering antibiotics (Queiroz and Boyd, 1998; Gomez-Gil *et al.*, 2000).

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The general environment of the Chone River estuary in Manabí Province, offers favourable conditions for shrimp culture during the El Niño climatic events, rather than during La Niña event or the transitional climatic periods of this region of Ecuador.
2. There were significant temporal differences in the shrimp pond temperature and salinity values, and in phosphorus and silica concentrations, between the El Niño, La Niña and transition periods. These changes affected the phytoplankton species composition and its

diversity, because the ratio of inorganic N: inorganic P, warm water temperature and low salinity encouraged the growth of cyanophytes.

3. Environmental stress, i.e. strong changes in temperature and salinity levels such as in the transition and La Niña periods resulted in a decrease in the diversity of the microbial community in the intestines of shrimp and an increase of specific microbial group. Due to the prevalence of *Vibrio* species (*V. parahaemolyticus* and *V. harveyi*) it is suggested that these species could have been the causative agents of the disease outbreaks experienced during these climatic periods.
4. Shrimp performance indices, i.e. survival, yield, harvest weight, feed conversion ratio, during El Niño were better, on average, than during the transition or La Niña periods, (Table.4.5). According to these findings, temperature, salinity and the interaction between temperature and salinity were correlated with disease outbreaks observed during the periods of shrimp culture associated with these climatic conditions.
5. The reduction in phytoplankton and microbial diversity observed in this study indicated not only environmental stress during certain shrimp culture periods, but also may serve as a useful predictive indicator for the occurrence (or risk) of disease outbreaks in shrimp culture.

6.2 Recommendations

Based on the findings of the present study, the following are tentative recommendations (guidelines) for management strategies to overcome, or minimize, the adverse impacts of unfavorable climatic events on shrimp culture in Ecuador

➤ **Shrimp stocking and shrimp age**

A reduction of shrimp stocking density to 6 - 7 post larvae/m², using PLs 20 days because of the relationship between the age of PL salinity end point and rate of salinity reduction during acclimation of *L. vannamei* postlarvae (e.g.gills are more developed) (Garza *et al.*, 2001; McGraw *et al.*, 2002).

➤ **Monitoring water quality**

More emphasis should be given to continuous monitoring programs during transitional periods for temperature, salinity and nutrients, such as inorganic nitrogen, inorganic phosphorus and silica, and their potential use as a indicators of the environmental changes.

➤ **Monitoring health conditions of shrimp during sampling.**

Regular monitoring of shrimp health condition such as external signs (colour of shrimp, soft shell, external fouling, changes in the gills, gut,

muscle) can also be used to identify the onset of problems in shrimp culture during these adverse climatic periods.

7. REFERENCES

- Aguirre-Guzmán, G., Vázquez-Juárez, R., Ascencio, F. 2001.** Differences in the susceptibility of American white shrimp larval substage (*Litopenaeus vannamei*) to four *Vibrio* Species. *Journal of Invertebrate Pathology* 78, 215 – 219.
- Alapide-Tendencia, E.V., Dureza, L.A. 1997.** Isolation of *Vibrio spp.* from *Penaeus monodon* Fabricius with red disease syndrome. *Aquaculture* 154, 107–114.
- Alcaraz, G; Chiappa-Carrara, X; Espinoza, V; Vanegas, C. 1999.** Acute Toxicity of Ammonia and Nitrite to White Shrimp *Penaeus setiferus* Postlarvae. *Journal of the World Aquaculture Society* Vol. 30, no. 1, 90 - 97.
- Aldave, A. 1989.** *Algas*. Universidad Nacional de Trujillo. Editorial Libertad E.I.R.L. Perú. 427 p.
- Alday, G.V., 1999a.** Diagnóstico de enfermedades del punto blanco y cabeza amarilla. In: Green, B.W., Clifford, H.C., MacNamara, M., Montaña, G.M. (Eds.), *V Central American Symposium on Aquaculture*, San Pedro Sula, Honduras. pp. 116–118.
- Alday, G.V., 1999b.** Diagnóstico y prevención de la enfermedad del punto blanco. *El Mundo Acuícola* Vol 5 N° 1. Fundación Cenaim Espol. Guayaquil. Ecuador.
- Allan, G., Moriarty, D.J.W., Maguire, G.B. 1995.** Effects of pond preparation of *Penaeus monodon* Fabricius, water quality, bacteria and benthos in model farming ponds. *Aquaculture* 130, 329 – 349.

- Allan, G.L., Maguire, G.B., Hopkins, S.J. 1990.** Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved-oxygen levels. *Aquaculture*, Vol. 91, N° 3-4, 265 - 280.
- Alvarez, J.D., Austin, B., Alvarez, A.M., Reyes, H., 1998.** *Vibrio harveyi*: a pathogen of penaeid shrimps and fish in Venezuela. *J. Fish Dis.* 21, 313–316.
- Anderson, I.G., Shamsudin, M.N., Shariff, M., Nash G. 1988.** Bacterial septicemia in juvenile tiger shrimp, *Penaeus monodon*, cultured in Malaysian brackishwater ponds. *Asian Fish.Sc* 2 , 93 – 108.
- Anderson, J.M. 1987.** Production and decomposition in aquatic ecosystem and implications for aquaculture. In: D.J. Moriarty and R.S.V. Pullin (Eds.). *Detritus and Microbial Ecology in Aquaculture*. ICLARM. Conference Proceedings 14, International Centre for Living Aquatic Resources Management. Manila, Philippines, pp 123 – 145.
- Andreoli, C., Tolomio, C. 1988.** Density and biomass of phytoplankton and picoplankton from fertilized fish ponds of "Valle Fosse" and "Valle Zappa" (Venice Lagoon – North Italy). *Archiv fuer Hydrobiologie supplement* 78, 505 – 528.
- Andrew, N.L., Mapstone, B.D. 1987.** Sampling and the description of spatial pattern in marine ecology. *Ann. Rev. Oceanogr. Mar. Biol.* 25, 39–90.
- Anon. 1994.** Final Report. *The environmental Management of Coastal Aquaculture. An assessment of Shrimp Culture in Southern Thailand*. NACA 167p
- Anon. 1996.** The status of shrimp culture, diseases and their impact on production in the province of Central Java. Makalah disajikan pada Pertemuan Teknis. Pengembangan Sistem Pengendalian penyakit Udang di BBAP, Jepara, 1-3 October. Jawa Tengah, Semarang, 22p.

- Arellano, E. 1983.** Extensive system of shrimp farming in Ecuador. pp 75-87. In: E. J. Vernberg, A.K. Vaniguchu, F.A. Russel, J.A. Chappell and A.B. Miller (Eds.). *Shrimp aquaculture in the Caribbean Basin:Prospects and Constraints* , South Carolina, U.S.A.
- Argue, B.J., Zogbi, P.R., Montgomery, A.D., Otoshi, C.A., Nagamine, K.T., Calderon, F.R.O., Moss, S.M. 2001.** Tolerance and heritability of Pacific white shrimp *Litopenaeus vannamei* to high concentrations of ammonia. Aquaculture 2001: Book of Abstracts, World Aquaculture Society, 143 J.M Parker Coliseum Louisiana State University Baton Rouge LA 70803 USA.
- Atlas, R.M., Horowitz, A., Krichevsky, M.K., Bej, A.K. 1991.** Response of microbial populations to environmental disturbance. *Microb. Ecol.* 22, 249–256.
- Austin, B. 1988.** *Methods in aquatic bacteriology.* Chichester; Willey.425p.
- Austin, B., Austin, D.A. 1989.** *Methods for the microbiological examination of fish and shellfish.*Chichester; Hellis Horwood.317p.
- Barg, U., Phillips, M.J. 1997.** Environment and Sustainability, p. 55-66. *In: Review of the State of World Aquaculture.* FAO Fisheries Circular N°. 886. (Revision1). Rome,FAO,163pp.
<http://www.fao.org/docrep/003/w7499e/w7499e00.htm>
- Barica, J., Kling, H., Gibson, J. 1980.** Experimental manipulation of algal bloom composition by nitrogen addition. *Canadian Journal of Fisheries and Aquatic Science* 37, 1175-1183.
- Barlett, P., Bonilla, P., Quiros, L., Takano, M. 1990.** Effects of high salinity on the survival and growth of juvenile *Penaeus vannamei*, *P. stylirostris*, and *P. monodon*. In Abstract, World Aquaculture, 90: 121/CP6. National Research Council. Ottawa, Ontario, Canada.

- Baross, J., Liston, J. 1970.** Occurrence of *Vibrio parahaemolyticus* and related haemolytic vibrio in marine environments of Washington State. *Applied Microbiology* 20 , 179 – 186.
- Barraclough, S., Finger-Stich, A. 1996.** *Some ecological and social implications of commercial shrimp farming in Asia.* Geneva: UNRISD, 62 pp.
- Bastida, R., Stupak, M.E. 1979.** Las diatomeas de las comunidades incrustantes del Puerto de Mar del Plata. Clave para su reconocimiento. CIDEPINT. *Anales*, 91-167 .
- Baticados, M.C.L., Lavilla-Pitogo, C.R., Cruz-Lacierda, E.R., de la Peña, L.D., Sunaz, N.A. 1990.** Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. *Dis. Aquat. Org.* 9, 133–139.
- Beveridge, M.C.M., Phillips, M.J., Macintosh D.J. 1997.** Aquaculture and the environment: the supply of and demand for environmental goods and services by Asian aquaculture and the implications for sustainability. *Aquacult Res* 28, 797 - 807.
- Bjørnsen, P.K., Riemann, B., Pock-Steen, J., Nielsen, T.G., and S.J. Horsted. 1989.** Regulation of bacterioplankton production and cell volume in a eutrophic estuary. *Applied Environmental Microbiology* 55,1512-1518.
- Bourelly, P. 1970.** *Les algues d'eau douce Initiation a la systématique. Tome III: Les algues bleues et rouges. Les Euglènes, Peridiniens et Cryptomonadines. Collection faunes et flores actualles* Edition N. Boubée & Cie. 512 pp.

- Bowell, C.E ., Bidwell, J.P. 1978.** Ionization of ammonia in seawater : effects of temperature, pH, and salinity. *J.Fish.Res.Board of Canada* 35 (7) , 1012 – 1016.
- Boyd, C., Fast, A. 1992.** Pond monitoring and management. In: A.W. Fast and J. Leebster editors. *Marine Shrimp Culture: Principles and Practices*. Elsevier Science Publishers B.V. 497 – 513.
- Boyd, C., Calderon, J., Sonnenholzner, S. 1999.** Sugerencias para combatir el WSSV en el Ecuador. *El Mundo Acuícola* Vol 5 N° 2. Fundación Cenaim Espol. Guayaquil. Ecuador.
- Boyd, C.E. 1982.** *Water Quality Management for Pond Fish Culture*. Elsevier Scientific Publication Company, New York, 319 pp.
- Boyd, C.E. 1989.** *Water quality management and aeration in shrimp farming*. Fish. Allied Aquacult. Dept.Serv. N° 2 Alabama Agricult. Exp. Sta., Auburn Univ., Auburn, AL., 83.
- Boyd, C.E. 1990.** *Water Quality in Ponds for Aquaculture*. Auburn University. Alabama.36849. U.S.A.
- Boyd, C.E. 1995.** Potential of sodium nitrate to improve environmental conditions in aquaculture ponds. *Journal of the World Aquaculture Society* 26, 38-40 .
- Boyd, C.E. 1997.** Environmental issues in shrimp farming. IV Simposio Centroamericano de Acuicultura: Cultivo Sostenible de Camarón y Tilapia, 22-24 de abril de 1997, Tegucigalpa, Honduras.
- Boyd, C.E., Clay, J.W. 1998.** Shrimp aquaculture and the environment. *Sci Am* 278, 42 - 49.

- Boyd, C.E., Massaut, L. 1999.** Risks associated with the use of chemicals in pond aquaculture. *Aquacult Eng* 20, 113 - 132.
- Boyd, C.E., Daniels, H.V . 1993.** Liming and fertilization of brackishwater shrimp ponds. *Journal of Applied Aquaculture* Vol. 2, no.3 - 4, 221 -234.
- Boyd, C.E., Tanner, M.E., Madkour, M., Masuda, K. 1994.** Chemical characteristics of bottom soils from freshwater and brackish water aquaculture ponds. *Journal of the World Aquaculture.Soc.* 25, 517 – 534.
- Boyd, C.E., Tucker, C.E. 1992.** *Water quality and Pond Soil Analysis for Aquaculture.* Alabama Experiment Station, Auburn University, Auburn, AL, USA.
- Boyd, C.E., Tucker, C.E. 1998.** *Pond Aquaculture Water Quality Management.* Kluwer, Norwell.MA, USA.
- Bratbak, G., Thingstad, T. F. 1985.** Phytoplankton-bacteria interactions: An apparent paradox? Analysis of a model system with both competition and commensalism. *Marine Ecology Progress Series* 25,23-30.
- Brock, J.A., Gose, R., Lightner, D.V., Hasson, K.W. 1995.** An overview on Taura syndrome, an important disease of farmed *Penaeus vannamei*. In: Browdy, C.L., Hopkins, J.S. (Eds.), *Swimming Through Troubled Water. Proceedings of the Special Session on Shrimp Farming, Aquaculture '95.* World Aquaculture Society, Baton Rouge, pp. 84–94.
- Browdy, C.L., Holloway J.D., King C.O., Stokes A.D., Hopkins J.S., Sandifer P.A. 1993.** IHNV virus and intensive culture of *Penaeus vannamei*: Effects of stocking density and water exchange rates. *Journal of Crustacean Biology* 13(1), 87-94.

- Burford, M., Glibert, P.M. 1999.** Short term nitrogen uptake and regeneration in early and late growth phase shrimp ponds. *Aquaculture Research*, 30, 215 – 227.
- Burford, M. 1997.** Phytoplankton dynamics in shrimp ponds. *Aquaculture Research*, 28, 351-360 .
- Calderón, J., Bayot, B., Betancourt, I., Alday, V. 1999.** Monitoreo de la mancha blanca en Ecuador. *El Mundo Acuícola* Vol 5 N° 2. Fundación Cenaim Espol. Guayaquil. Ecuador.
- Carpenter, K.E., Fast, A.W., Corre, V., Woessner, J.W., Janco, R.I. 1986.** The effects of water depth and circulation on the water quality and production of *Penaeus monodon* in earthen pond. In : *The First Asian Fisheries Forum* (ed. by J.L., Maclean, L.B. Dizon & I.V. Hosillos). 21-24 . Asian Fisheries Society. Manila. Philippines.
- Castillo, L.E., de la Cruz, E., Ruepert, C. 1997.** Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. *Environ Toxicol Chem* 16, 41 - 51.
- Cavalli, R.O., Wasielesky, W. Jr., Franco, C.S., Miranda Filho, K. 1996.** Evaluation of the short-term toxicity of ammonia, nitrite and nitrate to *Penaeus paulensis* (Crustacea, Decapoda) broodstock. *Arquivos de Biologia e Tecnologia*, Vol. 39, no. 3, 567 - 575.
- CDB (Climate Diagnostic Bulletin). 1993–2002.** Climate Prediction Center. US Department of Commerce, National Oceanic and Atmospheric Administration, Washington, DC.
http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/quick_look/sst12.gif

- CDB (Climate Diagnostic Bulletin). 1995–1996.** Climate Prediction Center. US Department of Commerce, National Oceanic and Atmospheric Administration, Washington,DC.
http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9596_animated.gif
- CDB (Climate Diagnostic Bulletin). 1997–1998.** Climate Prediction Center. US Department of Commerce, National Oceanic and Atmospheric Administration, Washington,DC.
http://www.cdc.noaa.gov/map/clim/sst_olr/old_sst/sst_9798_animated.gif
- CDB (Climate Diagnostic Bulletin). 1998–1999.** Climate Prediction Center. US Department of Commerce, National Oceanic and Atmospheric Administration, Washington,DC.
http://www.cdc.noaa.gov/map/clim/sst_olr/sst9899_animated.gif
- Chanratchakool, P., Turbull, J.F., Funge-Smith, S., Limsuwan, C. 1995.** *Health management in shrimp ponds.* Aquatic Animal Health Research Institute. Kasetsart. University. Campus. Bangkok. 111p
- Chen, H.C. 1985.** Water quality criteria for farming the grass shrimp, *Penaeus monodon*. *Proceedings of the first International Conference on the Culture of Penaeid prawns/Shrimps*, Iloilo City, Philippines, 4-7 December 1984., 1985, p. 165.
- Chen, J.C., Lei, S.C. 1990.** Toxicities of ammonia and nitrite to *Penaeus monodon* juveniles. *J. World Aquacult. Soc.* 21, 300–306.
- Chen, J-C, Lin, C-Y. 1991.** Lethal effects of ammonia and nitrite on *Penaeus penicillatus* juveniles at two salinity levels. *Comp. Biochem. Physiol.* 100C, 466–482.

- Chen, J-C, Lin, C-Y. 1992 a.** Lethal effects of ammonia on *Penaeus chinensis* Osbeck juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology* Vol .156, no.1, pp. ,139 - 148.
- Chen, J-C, Lin, C-Y. 1992 b .** Effect of ammonia on growth of *Penaeus penicillatus* juveniles. *Comparative Biochemistry and Physiology*, Vol. 101 C,no.3, 443 - 447.
- Chen, Jiann-Chu, Lei, Liu, Ping-Chung, Lin, Yao-Tsu, Lee, Chin-Kai. 1988.** Super intensive culture of red-tailed shrimp *Penaeus penicillatus*. *Journal of the World Aquaculture Society*, Vol 19, no3 ,127 – 131.
- Chen, Jiann-Chu, Liu, Ping-Chung, Tu, Chi-Ching. 1990.** Effect of ammonia on growth of *Penaeus monodon* postlarvae. *Journal of the Fisheries Society of Taiwan* Vol. 17, no. 3, 207 - 212.
- Chen, Jiann-Chu, Tu, Chi-Ching. 1991.** Influence of ammonia on growth of *Penaeus monodon* Fabricius post-larvae. *AQUACULT. FISH. MANAGE.*, Vol. 22, no. 4, 457 - 462.
- Chen, S.N. 1992.** Coping with diseases in shrimp farming. In: de Saram, H., Singh, T. (Eds.). , *Proceedings of the Global Conference on the Shrimp Industry*, 14–16 September 1992, Kuala Lumpur, Malaysia. Infofish, Kuala Lumpur.
- Chen, S.N., 1995.** Current status of shrimp aquaculture in Taiwan. In: Browdy, C.L., Hopkins, J.S. (Eds.), *Swimming Through Troubled Water*. Proceedings of the Special Session on Shrimp Farming, Aquaculture '95. World Aquaculture Society, Baton Rouge, LA, USA, pp. 29–34.
- Chien,Y.H. 1992.** Water quality requirements and management for marine shrimp culture. In :*Proceedings of the Special Session on Shrimp Farming* (Ed.) J. Wyban) 30-40 . World Aquaculture Society Baton Rouge. Louisiana.

- Chin, T.S., Chen, J.C. 1987.** Acute toxicity of ammonia to larvae of the tiger prawn *Penaeus monodon*. *Aquaculture* 66, 247–253.
- Chiu, Y.N. 1988.** Water quality management for intensive prawn ponds In:Y.N.Chiu, L.M.Santos and R.O. Juliano Ed. *Technical Considerations for the Management and Operation of Intensive Prawn Farms*. UP Aquaculture Society. The Philippines. 102 – 128
- Chua TE, Paw, J.N, Guarin, F.Y. 1989.** The environmental impact of aquaculture and the effects of pollution on coastal aquaculture development in South-east Asia. *Mar Pollut Bull* 20, 335 - 343.
- Clay, J. 1997.** Towards sustainable shrimp aquaculture. *World Aquaculture* 28 (3), 32 - 37.
- Clifford, H.C. 1992.** Marine shrimp pond management: a review. In: *Proceeding of the Special Session on Shrimp Farming* (ed. by J. Wyban) 100-137. World Aquaculture Society, Baton Rouge Louisiana.
- Corea, A., Jayasingue, J. 2001.** Environment and the major epizootics in shrimp culture – the Sri Lankan experience. 6th Asian Fisheries Forum Book of Abstracts. p. 72.
- Cortes Altamirano, R., Paez Osuna, F., Guerrero Galvan, S.R., Esparza Leal, H. 1995.** Diurnal variations of phytoplankton and the abiotic factors in a brackishwater shrimp pond in the southern of Sinaloa, Mexico. *Hidrobiológica (Iztapalapa)*, Vol. 5, no. 1-2, 45 - 55.
- Costa, R., Mermoud, I., Koblavi, S., Morlet, B., Haffner, P., Berthe, F., Legroumellec, M., Grimont, P. 1998.** Isolation and characterization of bacteria associated with a *Penaeus stylirostris* disease Syndrome 93 in New Caledonia. *Aquaculture* 164, 297–309.

- Cowan, V.J., Lorenzen, K., Funge-Smith, S.J. 1999.** Impact of culture intensity and monsoon season on water quality in Thai commercial shrimp ponds. *Aquaculture Research* 30, 123-133.
- Cucalón, E. 1984.** Temperature, Salinity and Water mass distribution of Ecuador during an El Niño event in 1976. *Revista Ciencias del Mar y Limnología* 2, 1- 5. Guayaquil. Ecuador.
- Cucalón, E. 1989.** Oceanographic characteristics off the Coast of Ecuador. pp 185-194. In: S. Olsen & L. Arriaga (Eds.). *A sustainable shrimp mariculture industry for Ecuador*. International Coastal Resources Management project. Technical Report Series TR-E-6. The Rhode Island University of Rhode Island, Government of Ecuador, U.S. Agency for International Development.
- Cushing, D.H. 1968.** *Fisheries Biology, a Study in Population Dynamics*. University of Wisconsin Press, Madison, WI.
- Davis, E. M. , Gloyna, E. F. 1972.** Bacterial die-off in ponds. *J. Environ. Eng. Div. ASCE* 98, 59 - 69.
- de la Peña, L.D., Tamaki, T., Momoyama, K., Nakai, T., Muroga, K. 1993.** Characteristics of the causative bacterium of vibriosis in the Kuruma prawn, *Penaeus japonicus*. *Aquaculture* 115, 1–12.
- DeanRoss, D., Mills, A.L. 1989.** Bacterial community structure and function along a heavy metal gradient *Appl. Environ. Microbiol.* 55, 2002–2009.
- Dempsey, A.C., Kitting, C. 1987.** Characteristics of bacteria isolated from penaeid shrimp. *Crustaceana* 52, 90 – 94.
- Dempsey, A.C., Kitting, C.L., Rosson, R.A. 1989.** Bacterial variability among individual penaeid shrimp digestive tracts. *Crustaceana* 56, 267 – 278.

- Desikachary, T.V. 1959.** *Cyanophita*. Botany Department University of Madras. Indian Council of Agricultural Research New Delhi. 686 pp.
- Dierberg F.E., Kiattisimkul W. 1996.** Issues, impacts and implications of shrimp aquaculture in Thailand. *Environ Manage* 20, 649-666.
- Doumenge, F. 1989.** Aquaculture in Ecuador. 1083 - 1094 pp. In: G. Barnabe (Ed.). *Aquaculture Vol 2*. Ellis Horwood Series in Aquaculture and Fisheries Support. Ellis Horwood. New York.
- Durand, S., Lightner, D.V., Nunan, L.M., Redman, R.M., Mari, J., Bonami, J.R. 1996.** Application of gene probes as diagnostic tools for white spot baculovirus (WSBV) of penaeid shrimp. *Dis. Aquat.Org.* 27, 59-66.
- FAO. 1992.** Aquaculture Production. Fishery Information. Data and Statistics Services. FAO Fisheries Circular 815 (4).
- FAO. 1995.** Code of conduct for responsible fisheries. Rome: FAO.
- FAO. 1998.** Report of the Bangkok FAO Technical Consultation on Policies for Sustainable Shrimp Culture. Bangkok, Thailand, 8-11 December 1997. Informe de la Consulta Técnica FAO / Bangkok sobre Políticas para el Cultivo Sostenible del Camarón. Bangkok, Tailandia, 8-11 de diciembre de 1997. FAO Fisheries Report /FAO Informe de Pesca No.572. Rome / Roma, FAO. 1998.31p.
<http://www.fao.org/FI/faocons/shrimp/bangk.asp>
- FAO.2001. Fishery Statistics.**
<http://www.fao.org/fi/statist/fisoft/fishplus.asp>
- Farghaly, A.H. 1950.** Factor influencing the growth and light production of luminous bacteria. *J.Cell.Comp. Physiol.* 36:165-184.

- Fast, A. 1992.** Penaeid ultra-intensive growout systems. In Fast, A and J. Lester (Eds.) *Marine Shrimp Culture: Principles and Practices*. Els, Sci. Pub.391-398.
- Fast, A.W. 1985.** Pond production systems. Water quality management practices. In : J.E.Lennan, R.O'neal-Smithern and G. Tchobanglous (Eds.). *Principles and Practices of Pond Aquaculture*. Oregon State University Publ. 141-161.
- Ferguson, E.J. 1961.** Description for Further sedentary species. Studies on Australian and New Zealand Diatoms. 88 (4), 669-698.
- Flaherty, M., Karnjanakesorn, C. 1995.** Marine shrimp aquaculture and natural resource degradation in Thailand. *Environ Manage*,19,27-37.
- Flegel, T.W. 1997.** Major viral diseases of the black tiger prawn *Penaeus monodon* in Thailand. In: NRIA International Workshop, *New Approaches to Viral Diseases of Aquatic Animals*. National Research Institute of Aquaculture, Nansei, Mie, Japan, 167–189.
- Fonseka, T.S.G. 1990.** Microbial flora of pond cultured prawn (*Penaeus monodon*). Indo Pacific Fishery Commission. Paper presented at the seventh session of the Indo Pacific Fishery Commission Working Party on Fish Technology and Marketing. Bangkok, Thailand, 19 - 22 April 1988. FAO Fisheries Report. Rome N 401,supp, 24 – 31.
- Fonselius, S.H. 1983.** Determination of hydrogen sulphide. In : Grasshoff,K. Ehrhardt,M. Kremling,K. (Eds.). *Methods of Seawater Analysis.*, 73 – 80.
- Freiler, P. F., Sis, R.F., Bell, T.A., Lewis D.H. 1992.** Microscopic and structural features of necrotizing hepatopancreatitis in Texas cultured shrimp (*Penaeus vannamei*). *Vet. Pathology* 29 , 269 – 277.

- Frevert, T. 1980.** Determination of Hidrogen Sulfide in Saline Solutions. *Swiss J. Hydrol.* , Vol 42 N° 42 , 255 – 267.
- Frias-Espericueta, M.G., Harfush-Melendez, M., Paez-Osuna, F. 2000.** Effects of Ammonia on Mortality and Feeding of Postlarvae Shrimp *Litopenaeus vannamei*. *Bulletin of Environmental Contamination and Toxicology* Vol. 65, no. 1, 98 – 103.
- Fry, J.C. 1987.** Functional roles of the masjor groups of bacteria associated with detritus. In: D.J. Moriarty and R.S.V. Pullin (Eds.). *Detritus and Microbial Ecology in Aquaculture*. ICLARM. Conference Proceedings 14, International Centre for Living Aquatic Resources Management. Manila, Philippines, pp 83 – 121.
- Gao, S., Zou, D. 1994.** Toxicity of nitrite to larvae of *Penaeus penicillatus*. *Journal of Oceanography in Taiwan Strait*, Vol 13 no 3, 236 – 239.
- Garay, E., Arnau, A., Maro, C. 1985.** Incidence of *Vibrio cholerae* and related vibrios in a coastal lagoon and sea water influenced by lake discharge along an annual cycle. *Applied Environmental Microbiology* 50, 426 – 430.
- García, M.L. 1983.** Variability in the distribution and abundance of eggs and larvae of *Scomber japonicus* (mackerel) and of some Clupeids in Ecuadorean waters. *Proceedings of expert consultation to examine the changes in the abundance and composition by species of the Neritic Fish Resources*. San Jose de Costa Rica. April, 1983. In: FAO Fisheries Report 291 (2).
- Garza, A., Rouse, D.B., Teichert-Coddington, D., Davis, A. 2001.** A. Effects of nurseries on the growth and survival of *Penaeus vannamei* in culture ponds. WAS. Aquaculture 2001: Book of Abstracts 246p.

Geiselbrecht, A.D., Herwig, R.P., Deming, J.W., Staley, J.T. 1996. Enumeration and phylogenetic analysis of polycyclic aromatic hydrocarbon-degrading marine bacteria from Puget Sound sediments. *Appl. Environ. Microbiol.* 62, 3344–3349.

GESAMP IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP.1991. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection , Reducing environmental impacts of coastal aquaculture. Rep. Stud. GESAMP, (47), 35 pp.
<http://www.fao.org/FI/publ/report/gesamp/r47/r47e.asp>

GESAMP IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP. 1997. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Towards safe and effective use of chemicals in coastal aquaculture. Reports and Studies, GESAMP. No 65, FAO, Rome, , 40 pp.

Golterman, H.L., Clymo, R.S., Ohnstad, M.A.M. 1978. *Methods for physical and chemical analysis of freshwaters.* IBP Handbook N 8, 2nd edition. Oxford. Blackwell, 213 pp.

Gomez-Gil, B., Trón-Mayén, L., Roque, A., Turnbull, J. Inglis, V., Guerra-Flores, A. 1998. Species of *Vibrio* isolated from hepatopancreas, haemolymph and digestive tract of a population of healthy juvenile *Penaeus vannamei*. *Aquaculture* 163, 1 – 9.

Gomez-Gil, B., Roque, A., Turnbull, JF. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* Vol. 191, no. 1-3, pp. 259-270.

- Gopalakrishnan, P., Ramadhas, V. 1999.** Distribution of nitrogenous nutrients in semi-extensive and semi-intensive culture systems. The Fourth Indian Fisheries Forum, Proceedings. 24-28 November, 1996, Kochi, Kerala., Asian Fisheries Society, Indian Branch, Mangalore (India), 175 – 177.
- Gopalakrishnan, P, Ramadhas, V, Sundararaj, V. 1997.** Nitrification in a semi-intensive shrimp culture system. Water quality issues in aquaculture. Proceedings of the National Seminar on Water Quality Issues in Aquaculture Systems, Dec. 18 and 19, 1996., Tamil Nadu Veterinary and Animal Sciences University, Tuticorin (India), 23 – 27.
- Goulder, R. 1976.** Evaluation of media for counting viable bacteria in estuaries. *Journal of Applied Bacteriology*, 41, 351-355 .
- Grasshoff, J. 1964.** Determination of nitrate. *Kiel. Meeresforsch*, Vol 20, 5-10 .
- Gray, J.S. 2000.** The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology* 250 23 – 49.
- Guzmán, R. 1993.** Catálogo de organismos fitoplanctónicos identificados en el río Guayas. *Boletín Científico y Técnico. Instituto. Nacional de Pesca Ecuador*. Vol XII, N 4. 100 pp.
- Hach. 1992.** *Water analysis handbook*. Hach Company. U.S.A.
- Hameed, A.S.S. 1993.** A study of the aerobic heterotrophic bacterial flora of hatchery-reared eggs, larvae and post-larvae of *Penaeus indicus*. *Aquaculture* 117, 195 – 204.

- Hameed, A.S.S., Anilkumar, M., Stephen Raj, M.L., Jayaraman, K. 1998.** Studies on the pathogenicity of systemic ectodermal and mesodermal baculovirus and its detection in shrimp by immunological methods. *Aquaculture* 160, 31–45.
- Harris, J.M. 1993.** The presence, nature and role of gut microflora in aquatic invertebrates: a synthesis. *Microb. Ecol.* 25, 195–231.
- Hasson, K.W., Hasson, J., Aubert, H., Redman, R.M., Lightner, D.V. 1997.** A new RNA-friendly fixative for the penaeid shrimp samples for virological detection using cDNA genomic probes. *J. Virol. Methods* 66,227–236.
- Hasson, K.W., Lightner, D.V., Poulos, B.T., Redman, R.M., White, B.M., Brock, J.A., Bonami, J.R. 1995.** Taura syndrome in *Penaeus vannamei*: demonstration of viral etiology. *Dis. Aquat. Org.* 23, 115–126.
- Hasson, K.W., Lightner, D.V., Redman, R.M., Poulos, B.T., White, B.M. 1999.** Taura syndrome virus TSV lesion development and the disease cycle in the Pacific white shrimp *Penaeus vannamei*. *Dis. Aquat. Org.*36, 81–93.
- Havenaar, R., Brink, T., Huis in't Veld, J. 1992.** Selection of strains for probiotics use. In: R. Fuller (Ed.), *Probiotics: the scientific basis*, Chapman and Hall, London. pp 209-224.
- Hellawell, J.M. 1986.** Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier Applied Science, London, pp. 45–77.

- Hernandez López, J., Gollas Galván, T., Magallón-Barajas, F., Vargas-Albores, F. 1997.** Isolation of *Vibrio* and *Pseudomonas* from brown shrimp (*Penaeus californiensis* Holmes) intestine. *Revista Latinoamericana de Microbiología* Vol 30, N° 3-4, 109 – 115.
- Hernandez-Herrera, R., Ramirez, J.L., Lavens, P., Racotta, I.S. 1999.** Survival in high ammonia concentration. Indication of the quality of larvae of *Litopenaeus vannamei*. *Acuicultura 99. Aquaculture 99*, Vol 1, 268 – 274.
- Hill, M.O. 1973.** Diversity and evenness: a unifying notation and its consequence. *Ecology* 54, 427–432.
- Hilton, J.W., Slinger, S.J. 1981.** Nutrition and feeding of rainbow trout. *Can.Spec.Publ.Fish.Aquat.Sc.* 55, 1 – 15.
- Hirono, Y. , Leslie, M. 1992.** Shrimp culture industry in Ecuador. In: A. W. Fast and L.J. Lester (Eds.). *Marine Shrimp Culture. Principles and Practices*. Development in Aquaculture and Fisheries Science. Vol 23. Elsevier, Science Publisher, N.Y.
- Hirono, Y., van Eys, S. 1990.** The Ecuadorean shrimp culture industry. *Infofish International* 4 / 90, pp 53-58 .
- Holt, J. G., Krieg, N. R., Sneath, P.H.A., Staley, J.T., Williams, S.T. 1994.** (Eds.) *Bergey's Manual of Determinative Bacteriology*. Ninth Edition.W & W. Balt.,M. U.S.A.
- Hood, M.A., Meyers, S.P., Colmer, A.R. 1971.** Bacteria of the digestive tract of the white shrimp, *Penaeus setiferus*. Abstract. *Bacteriological Proceedings*, 71 , G- 147.

- Hopkins, J.S., Sandifer, P.A., Browdy, C.L. 1995.** A review of water management regimes which abate the environmental impacts of shrimp farming. In: Browdy CL, Hopkins JS, editors. *Swimming through troubled water, Proceedings of the special session on shrimp farming, Aquaculture'95*. Baton Rouge, Louisiana, USA: World Aquaculture Society.
- Hopkins, J.S., Hamilton, R.D.H., Sandifer, P.A., Browdy, C.L. Stokes, A.D. 1993.** Effects of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp pond. *Journal of the World Aquaculture Society* 24, 304-320 .
- Huang, H.J. 1983.** Factors affecting the successful culture of *Penaeus stylirostris* and *Penaeus vannamei* at estuarine power plant site: temperature, salinity, inherent growth variability, damselfly nymph predation, population density and distribution, and polyculture. PhD dissertation. Texas A&M University. College Station, Tx, USA, 221pp.
- Huang, J., Song, X.L., Yu, J., Yang, C.H. 1994.** Baculoviral hypodermal and haematopoietic necrosis-pathology of the shrimp explosive epidermic disease. Abstract In: Abstract. Yellow Sea Fishery Research Institute, Qingdao, P.R. China.
- Huang, J., Song, X.L., Yu, J., Yang, C.H. 1995.** Baculoviral hypodermal and hematopoietic necrosis-study on the pathogen and pathology of the shrimp explosive epidemic disease of shrimp. *Marine Fisheries Research*.
- Hudinaga, M. 1935.** Studies on the development of *Penaeus japonicus* (Bate). Report. Hayatomo Fishery Institute. 1 (1).
- Hudinaga, M. 1942.** Reproduction, development and rearing of *P. japonicus*. *Jap. J. Zool.* 10(2), 305-393.

- Hudinaga, M., Miyaruma, M. 1962.** Breeding of the "Kuruma" prawn (*Penaeus japonicus* Bate). (In Japanese, English abstract.) J. Oceanogr. Soc.Jap., 20th Vol., p. 694-706.
- Humason, G.L. 1972.** *Animal Tissue Techniques*, 3 edn. W.H.Freeman, San Francisco.
- Inouye, K., Miwa, S., Oseko, N., Nakano, H., Kimura, T., Momoyama, K., Hiraoka, M. 1994.** Mass mortality of cultured kuruma shrimp *Penaeus japonicus* in Japan in 1993: electron microscopic evidence of the causative virus. Fish Pathol. 29, 149–158.
- Intriago, P. , Jimenez, R., Machuca, M., Barniol, R., Krauss, E., Salvador, X. 1997.** Experiments on toxicosis as the cause of Taura syndrome in *Penaeus vannamei* in Ecuador. In: *Diseases in Asian Aquaculture III* , T.W. Flegel and I.H. Mac Rae (Eds.) 365-379. Fish Health Section. Asian Fisheries Society. Manila. Philippines.
- Intriago, W. 1998.** Problemas de aislamiento y caracterización de bacterias asociadas al síndrome de la Zoea II y demostración experimental de su patogenicidad. Tesis de Acuicultor, ESPOL, Ingeniería Marítima y Ciencias del Mar. Guayaquil. Ecuador.
- Jimenez R, Barniol R, de Barniol L, Machuca M. 1999.** Infection of IHHN virus in two species of cultured penaeoid shrimp, *Litopenaeus vannamei* (Boone) and *Litopenaeus stylirostris* (Stimpson), in Ecuador during El Niño 1997–1998. Aquacult Res 30, 695–705 .
- Jimenez R, Barniol R, de Barniol L, Machuca M. 2000.** Periodic occurrence of epithelial viral necrosis outbreaks in *Penaeus vannamei* in Ecuador. Diseases of Aquatic Organisms Vol. 42, 91–99 .

- Jimenez R, Barniol R, Machuca M. 1997.** An epizootic of an intracellular bacterium in cultured penaeid shrimp (Crustacea: Decapoda) In the Gulf of Guayaquil. In: Flegel TW, MacRae IH (Eds.) *Diseases in Asian aquaculture III*. Fish Health Section, Asian Fisheries Society, Manila, 305–311 .
- Jiménez, R. 1983.** Diatomeas y silicoflagelados del Fitoplancton del Golfo de Guayaquil. *Acta Oceanográfica del Pacífico*, 2 (2), 193-281 .
- Jiménez, R. 1992.** Síndrome de Taura (Resumen). *Revista Acuicultura del Ecuador* Vol 1, 1-16. Camara Nacional de Acuicultura del Ecuador. Guayaquil. Ecuador.
- Jimenez, R., Pesantes, F. 1978.** Fitoplancton, producción primaria y pigmentos en aguas costeras ecuatorianas. *Publ. INOCAR.2* (1), 30 pp.
- Jiravanichpaisal, P., Miyazaki, T., Limsuwan, C. 1994.** Histopathology, biochemistry, and pathogenicity of *Vibrio harveyi* infecting black tiger prawn *Penaeus monodon*. *J. Aquat. Anim. Health* 6 1 , 27–35.
- Jory, D.E., Dixon, H.M. 1999.** Shrimp white spot virus in the western hemisphere. *Aquaculture Magazine* 25, 83–91.
- Joseph, S.W., Colwell, R.R., Kaper, J.B. 1983.** *Vibrio parahaemolyticus* and related halophilic vibrios. *Critical Reviews in Microbiology*, 10, 77 – 124.
- Kancho, T., Colwell, R.R. 1973.** Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *Journal of Bacteriology*, 113, 24 – 32.
- Kancho, T., Colwell, R.R. 1978.** The annual cycle of *Vibrio parahaemolyticus* in Chasepeake Bay. *Microbiology and Ecology*, 4 135 – 155.

- Karunasagar, I., Pai, R., Malathi, G.R., Karunasagar, I. 1994.** Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. *Aquaculture* 128, 203–209.
- King, D.L. 1970.** The role of carbon in eutrophication. *Journal of Water Pollution Control Federation* 42, 2035-2051.
- Kongkeo, H. 1990.** Pond management and operation. *Shrimp 90. Conference Proceedings*. Putra World Trade Centre. Malaysia. 56 – 65.
- Koroleff, F, Grasshoff, K. 1983.** Determination of nutrients. *Methods of seawater analysis*. pp. 125-188.
- Krol R.M., Hawkins, W.E., Overstreet R.M. 1991.** Rickettsial and mollicute infections in hepatopancreatic cells of cultured Pacific white shrimp (*Penaeus vannamei*). *Journal of Invertebrate Pathology* 57, 3622 – 3260.
- Kumar, A., Dube, H.C. 1990.** Aerobic bacteria flora of two commercial crustaceans from Bhavnagar muddy coast, Indian. *J. Microbiol.* 30, 197 – 200.
- Kuo, Shih-Pong, Ting, Yun-Yuan. 1989.** Studies on the water quality of tiger prawn, eel, and fish-cum duck ponds in southwestern Taiwan. Studies on the environmental improvement and the control of the off-flavor in fish. 2., 1989, pp. 113-141, COA fisheries series. Taipei [COA FISH. SER.], no. 16.
- Landry, M.R., Haas, L.W., Fagerness, V.L. 1984.** Dynamics of microbial plankton communities: Experiments in Kaneohe Bay, Hawaii. *Marine Ecology Progress Series* 16,127-133.

- Lavilla-Pitogo, C.R., Albright, L.J., Paner, M.G., Sunaz, N.A. 1992.** Studies on the sources of luminescent *Vibrio harveyi* in *Penaeus monodon* hatcheries. In: Shariff, I.M., Subasinghe, R.P., Arthur, J.R. (Eds.) *Diseases in Asian Aquaculture. Fish Health Section*, Asian Fisheries Soc., Manila, Philippines, pp.157–164.
- Lavilla-Pitogo, C.R., Baticados, M.C.L., Cruz-Lacierda, E.R., de la Peña, L.D. 1990.** Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture* 91, 1–13.
- Lavilla-Pitogo, C.R., Leano, E.M., Paner, M.G. 1998.** Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent *Vibrios* in the rearing environment. *Aquaculture* 164, 337–349.
- Leber, K.M., Pruder, G.D. 1988.** Using experimental microcosms in shrimp research: the growth enhancing effect of shrimp pond water. *Journal of the World Mariculture Society* 19, 197-203 .
- Lester, L.J. 1992.** Overview of shrimp farming in the western hemisphere. In Ed Fast, A & J.Lester *Marine Shrimp Culture: Principles and Practices*. Elsevier, Sci. Pub.771-782
- Lester, L.J., Pante, J.R. 1991.** Penaeid temperature and salinity responses. In: Fast, A.W., Lester, L.J. (Eds.) *Marine shrimp culture: Principles and Practices*. Vol 23, 515 – 534.
- Li, Y., Vanderberghe, J., Ji, W., Sorgeloss, P., Swings, J., Xu, H. 2000.** Comparison of vibrios isolated from different countries. *J.Fish.Sci. China / Zhongguo ShuichanKexue*, Vol 7, No 4, 52 – 55.
- Liao, I.C., Yang, F.R., Lou, S.W. 1977.** Preliminary report on some diseases of cultured prawn and their control methods. Report on fish disease research I. JCRR Fisheries Series 29, 28 – 33.

- Lightner, D.V., Bell, T.A., Redman, R.M., Mohny, L.L., Natividad, J.M., Rukyano, A, Poernomo, A. 1992.** A review of some major diseases of economic significance in penaeid prawns/shrimps of the Americas and Indopacific. In: Shariff M, Subashinghe RR, Arthur JR (Eds.). *Diseases in Asian aquaculture I*. Fish Health Section, Asian Fisheries Society, Manila, p 57–80.
- Lightner, D.V. 1978.** Possible toxic effects of marine blue-green algae, *Spirulina subsalsa*, on the blue shrimp *Penaeus stylirostris*. *J. Invertebrate Pathol.* 32 , 139 – 144.
- Lightner, D.V. 1983.** Diseases of cultured shrimp. In J.R. More (Ed.), *CRC Handbook of Mariculture*. P.P.Mc Vey (Ed.) *Crustacean Aquaculture*. CRC Press.Boca Raton, Florida. I, 289 – 320.
- Lightner, D.V. 1988.** *Vibrio* disease. In: Sinderman, C.J., Lightner, D.V. (Eds.). *Disease Diagnostic and Control in North American Marine Aquaculture. Developments in Aquaculture and Fisheries Science* vol. 17 Sci. Publishing, New York, USA, pp. 42 – 47.
- Lightner, D.V. 1992.** Shrimp pathology: major diseases of concern to the farming industry in the Americas. *Mem. Congr. Ecuat. Acuicult.*, 177–195.
- Lightner, D.V. 1996a.** *A handbook of shrimp pathology and diagnostic procedures for diseases of culture penaeid shrimp*. World Aquaculture Society, Baton Rouge. LA, USA,p.305
- Lightner, D.V. 1996b.** Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas. In: Hill, B.J., Hastein, T. (Eds.). *Preventing the spread of aquatic animal disease*. *Rev. Sci. Tech. Off. Int. Epiz.* 15, pp. 579–601.

- Lightner, D.V. 1999.** The Penaeid shrimp viruses TSV, IHNV, WSSV and YHV: current status in the Americas, available diagnostic methods and management strategies. *J. Appl. Aquacult.* 9, 27–52.
- Lightner, D.V., Lewis, D.H. 1975.** A septicemic bacterial disease syndrome of penaeid shrimp. *Mar. Fish Rev.* 37, 25–28.
- Lightner, D.V., Redman, K.M. 1985.** Necrosis of the hepatopancreas in *Penaeus monodon* and *P. stylirostris* (Arthropoda, Decapoda) with red disease. *Journal of Fish Disease* 8, 181 – 188.
- Lightner, D.V., Redman, R.M., 1992.** Penaeid virus disease of the shrimp culture industry of the Americas. In: Fast, A.W., Lester, L.J. (Eds.). *Culture of Marine Shrimp: Principles and Practices*. Elsevier, Amsterdam, The Netherland, pp. 569–588.
- Lightner, D.V., Redman, R.M. 1994.** Histopathology and ultrastructural studies of Taura syndrome, a putative toxicity syndrome of penaeid shrimp. *World Aquaculture, Book of Abstracts, Aquaculture '94*. World Aquaculture Society, Baton Rouge.
- Lightner, D.V., Redman, R.M., Hasson, K.W., Pantoja, C.R. 1995.** Taura syndrome in *Penaeus vannamei* Crustacea: Decapoda : gross signs, histopathology and ultrastructure. *Dis. Aquat. Org.* 21, 53–59.
- Lin, C.K. 1989.** Prawn culture in Taiwan. What went wrong?. *World Aquaculture*, 20 (2), 19-21.
- Lin, Y.C., Chen, J.C. 2001.** Acute ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology* 259, 109 – 119.

- Liu, C.I., Chen, M.S. 1988.** Experimental infection of *Vibrio sp.* to induce shell disease in grass shrimp (*Penaeus monodon* Fabricius). Int. Fish Health Conf., Fish Health Section, AFS, Vancouver, 215 pp.
- Liu, P.C., Lee, K.K., Yii, K.C., Kou, G.H., Chen, S.N. 1996a.** Isolation of *Vibrio harveyi* from diseased kuruma prawns *Penaeus japonicus*. Curr. Microbiol. 33, 129–132.
- Liu, P.C., Lee, K.K., Chen, S.N. 1996b.** Pathogenicity of different isolates of *Vibrio harveyi* in tiger prawn, *Penaeus monodon*. Lett. Appl. Microbiol. 22, 413–416.
- Lo, C.F., Ho, C.H., Peng, S.E., Chen, C.H., Hsu, H.C., Chiu, Y.L., Chang, C.F., Liu, K.F., Su, M.S., Wang, C.H., Kou, G.H. 1996.** White spot syndrome baculovirus (WSSV) detected in cultured and captured shrimp, crabs and other arthropods. Dis. Aquat. Org. 27, 215–225.
- Lorenzen, K., Struve, J., Cowan, V.J. 1997.** Impact farming intensity and water management on nitrogen dynamics in intensive pond culture: a mathematical model applied to Thai commercial shrimp farms. Aquaculture Research 28, 493-507.
- Lotz, J.M. 1997.** Effect of host size on virulence of Taura virus to the marine shrimp *Penaeus vannamei*. Crustacea: Penaeidae . Dis. Aquat. Org. 30, 45–51.
- Lowell, R.T., Broce, D. 1985.** Cause of musty flavor in pond-cultured penaeid shrimp. Aquaculture 50, 169 – 174.

- Lumare, F., Andreoli, C., Cuglielmo L., Masellu, M.A., Piscitelli, G., Scovacricchi, T., Tolomo, C. 1987.** Trophic community correlations in fertilised ponds for commercial culture of the kuruma prawn *Penaeus japonicus* Bate in the North Adriatic Sea (northeast coast of Italy). *Investigación Pesquera* 51 (Supl 1) 571-585.
- Macintosh, D.J., Phillips, M.J. 1992.** Environment issues in shrimp farming. In Saram, H and Sing, T ed., *Shrimp'92*. Hong Kong, Infofish and Eurofish, 118-145.
- Macintosh, D.J. 1996.** Mangroves and coastal aquaculture: doing something positive for the environment. *Aquaculture. Asia* 12, 3-8.
- Mackereth, F.J.H., Heron, J., Talling, J.F. 1978.** *Water analysis: some revised methods for limnologist*. Scientific Publication N 36. Ambleside, Cumbria LA 22OLP, U.K. Freshwater Biological Association, 120 pp.
- Maeda, M., Itami, T., Furumoto, A., Hennig, O., Imamura, T., Kondo, M., Hirono, I., Aoki, T., Takahashi, Y. 1998.** Detection of penaeid rod-shaped DNA virus PRDV in wild-caught shrimp and other crustaceans. *Fish Pathol.* 33, 373-380.
- Manzi, J.J., Maddox M.B., Sandifer P.A. 1977.** Algal supplement enhancement of *Macrobrachium rosenbergii* (De Man) larviculture. *Proceedings of the World Mariculture Society* 8, 207-223.
- Margalef, R. 1957.** La teoría de la información ecológica. *Mems. R. Acad. Cienc. Artes. Barcelona* 23, 373-449 .
- Margalef, R. 1958.** Temporal succession and spatial heterogeneity in phytoplankton. In: A. Buzzati-Traverso (Eds.). *Perspectives in Marine Biology*. University of California Press, Berkeley, 323-349 .

- Mari, J., Bonami, J.R., Lightner, D.V. 1998.** Taura syndrome of penaeid shrimp: cloning of the viral genome fragments and development of the gene probes. *Dis. Aquat. Org.* 33, 11–17.
- Maridueña, A. 1991.** Diatomeas del estuario del río Chone. Universidad Técnica de Manabí. Ecuador.
- Marshall, H. 1970.** Phytoplankton in Tropical surface waters between the coast of Ecuador and the Gulf of Panama. *J.Wash. Acad. Sci.*, 60 (1), 18-21.
- McGraw, W.J., Davis, D.A., Teichert-Coddington, D., Rouse, D.B. 2002.** Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *Journal of the World Aquaculture Society* Vol 33, N1, 78-84.
- McQueen, J.D., Lean, D.R.S. 1987.** Influence of water temperature and nitrogen to phosphorus ratios on the dominance of blue-green algae In Lake St George, Ontario. *Canadian Journal of Fisheries and Aquatic Science* 44, 598-604.
- Mermoud, I., Costa, R., Ferre, O., Goarant, C., Haffner, P. 1998.** 'Syndrome 93' in New Caledonia outdoor rearing ponds of *Penaeus stylirostris*: history and description of three major outbreaks. *Aquaculture* 164,323–335.
- Mezrioui, N., Oudra, B., Oufdou, K., Hassani, L., Loudiki, M., Darley, J. 1994.** Effect of microalgae growing on wastewater batch culture on *Escherichia coli* and *Vibrio cholerae* survival. *Water Sci. Technol.* 30, 295-302.
- Mitra, A, Patra, KC. 2001.** Semi-intensive culture of *Penaeus monodon* Fabricius under mono-and polyculture systems *Aquaculture 2001: Book of Abstracts, World Aquaculture Society, 143 J.M Parker Coliseum Louisiana State University Baton Rouge LA 70803 USA, 2001, 444.*

- Mohanty, S.K., Mohanty, R.K. 2001.** Studies on the management of biopond system in a semi-intensive shrimp farm. Perspectives in mariculture. The Marine Biological Association of India, Cochin (India), 77 – 84.
- Mohney, L.L., Lightner D.V., Bell, T.A. 1994.** An epizootic of vibriosis in Ecuadorian pond-reared *Penaeus vannamei*, Boone (Crustacea: Decapoda). Journal of the World Aquaculture Society 25, 116–125 .
- Morales, I. 1992.** Observaciones sobre el síndrome de descamación del epitelio digestivo “Bolitas” en larvas de camarón *Penaeus vannamei* en Ecuador. Memorias del Primer Congreso Ecuatoriano de Acuicultura-203-207.
- Moriarty, D.J.W. 1986a.** Bacterial productivity in ponds used for culture of penaeid prawns. Microb.Ecol., 12, 259 – 269.
- Moriarty, D.J.W. 1986b.** Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Adv. Microb. Ecol., 9, 245 – 292.
- Moriarty, D.J.W. 1990a.** Techniques for estimating bacterial growth rates and production of biomass in aquatic environments. Methods Microbiol., 22, 211 – 234.
- Moriarty, D.J.W. 1990b.** Interactions of microorganisms and aquatic animals, particularly the nutritional role of the gut flora. Microbiology in Poecilothersms, 217–222.
- Moriarty, D.J.W. 1997.** The role of microorganisms in aquaculture ponds. Aquaculture 151, 333 – 349.
- Moriarty, D.J.W. 1998.** Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164, 351 – 358.

- Moriarty, D.J.W., Cook, H.L., bin Hassan, R., Thanabal, M. 1983.** Primary production and meiofauna number in some penaeid prawn aquaculture ponds at Gelang Patah. *Malaysian Ag. J.* , 54, 37 – 51.
- Moriarty, D.J.W., Pullin, R.S.V. (Eds.). 1987.** *Detritus and microbial ecology in aquaculture*. ICLARM Conference Proceedings 13. International Center of Living Aquatic Resources Management. Manila, Philippines, 385 p.
- Moss, S.M. 1995.** Production of growth enhancing particles in a plastic lined shrimp pond. *Aquaculture* 132, 253-260.
- Moss, S.M., Divakaran, S., Kim, B.G. 2001.** Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp, *Litopenaeus vannamei* (Boone). *Aquaculture Research*, 32, 125-131 .
- Moss, S.M., Pruder G.D. 1995.** Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* Boone, reared under intensive culture conditions. *Journal of Experimental Marine Biology and Ecology* 187, 175-191.
- Moss, S.M., Pruder, G.D., Leber, K.M., Wyban, J.A. 1992.** The relative enhancement of *Penaeus vannamei* growth by selected fractions of shrimp pond water. *Aquaculture*, 101, 229-239.
- Moss, S.M., LeaMaster, B.R., Sweeney, J.N. 2000.** Relative abundance and species composition of gram-negative aerobic bacteria associated with the gut of juvenile white shrimp *Litopenaeus vannamei* reared in oligotrophic well water and eutrophic pond water. *Journal of the World Aquaculture Society* 31, 255-263.

- NACA/FAO. 2000.** Aquaculture Development Beyond 2000: the Bangkok Declaration and Strategy. Conference on Aqua-culture Development in the Third Millenium, 20-25 February 2000, Bangkok, Thailand. NACA, Bangkok and FAO, Rome, 27 pp.
- Nakano, H., Koube, H., Umezawa, S., Momoyama, K., Hiraoka, M., Inouye, K., Oseko, N. 1994.** Mass mortalities of cultured Kuruma shrimp, *P. japonicus*, in Japan in 1993: epizootiological survey and infection trails. *Fish Pathol.* 29, 135–139.
- Nash, G., Nithimathachoke, C., Tungmandi, C., Arkarjamorn, A., Prathanpipat, P., Ruamthaveesub, P. 1992.** Vibriosis and its control in pond-reared *Penaeus monodon* in Thailand. In: Shariff, M., Subasinghe, R.P., Arthur, J.R., (Eds.), *Diseases in Asian Aquaculture I, Fish Health Section*, Asian Fisheries Society, Manila, Philippines, pp. 143–155.
- Nash, J.M. 2002.** *El Niño: Unlocking the Secrets of the Master Weather-Maker.*
- Naylor R.L., Goldberg, R.J., Primavera, J.H., Kautsky, N., Beveridge M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. 2000.** Effect of aquaculture on world fisheries supplies. *Nature* 405, 1017-1024.
- Nunan, L.M., Poulos, B.T., Lightner, D.V. 1998.** The detection of white spot syndrome virus WSSV and yellow head virus YHV in imported commodity shrimp. *Aquaculture* 160, 19–30.
- O'brien, C.A., Sizemore, R.K. 1979.** Distribution of the luminous bacteria *Beneckeia harveyi* in a semitropical estuarine environment. *Appl. Environ. Microbiol.* 38, 928–933.

- OECD. 1998.** *Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical-Substances.* As endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals in November 1998. Paris, OECD 64 pp.
- Olsen, S., Arriaga, L. (Eds.). 1989.** *A sustainable shrimp mariculture industry for Ecuador.* International Coastal Resources Management Project. Technical Report Series TR-E-6. The University of Rhode Island, Government of Ecuador, U.S. Agency for International Development.
- Oppenheimer, C., Zobell, C.E. 1952.** The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. *Journal of Marine Research*, 11, 10-18
- Oseid, D.M., Smith, LL Jr. 1974.** Chronic toxicity of sulphide to *Gammarus pseudolimnaeus*. *Transaction of the American Fisheries Society*, Vol. 103, no 4, 819 – 822.
- Ostrensky, A., Wasielesky Jr. W. 1995.** Acute toxicity of ammonia to various life stages of the Sao Paulo shrimp, *Penaeus paulensis* Perez-Farfante, 1967. *Aquaculture* 132, 339–347.
- Otta, S.K., Karunasagar, J., Karunasagar, J. 2001.** Bacteriological study of shrimp, *Penaeus monodon* Fabricius, hatcheries in India. *J. Appl. Ichthyology*, 17, 59 – 63
- Oufdou, K., Mezrioui, N., Oudra, B., Barakate, M., Loudiki, M. 1998.** Effect of extracellular and endocellular products from cyanobacterium *Synechocystis sp.*, on the growth of some sanitation system bacteria. *Algol. Stud.* 90, 139-148.

- Overstreet, R.M., Lightner, D.V., Hasson, K.W., McIlwain, S., Lotz, J.M. 1997.** Susceptibility to Taura syndrome virus of some penaeid shrimp species native to the Gulf of Mexico and the southeastern United States. *J. Invertebr. Pathol.* 69, 65–176.
- Paerl, H.W. 1988.** Nuisance phytoplankton blooms in coastal, estuarine and inland waters. *Limnology and Oceanography* 33, 823-847 .
- Paerl, H.W., Tucker, C.S. 1995.** Ecology of blue-green algae in aquaculture ponds. *J. World Aquacult. Soc.* 26, 109–131.
- Pagnocca, F.C., Mendoca, L.C., Norton, A. 1991.** Heterotrophic bacteria associated with the shrimp *Penaeus schimitti*, sediment and water of Sepetiba Bay, Rio de Janeiro, Brazil. *Rev. Microbiol. Sao Paulo*, 22(3), 247-252.
- Painting, S.J., Lucas, M.I., Muir D.G. 1989.** Fluctuations in heterotrophic bacterial community structure, activity and production in response to development and decay of phytoplankton in a microcosm. *Mar. Ecol. Prog. Ser.* 53, 129-141.
- Park, J.H., Lee, Y.S., Lee, S., Lee, Y. 1998.** An infectious viral disease of penaeid shrimp newly found in Korea. *Dis. Aquat. Org.* 34, 71–75.
- Parra, O., González, M., Dellarosa, V., Rivera, P., Orellana, M. 1982.** *Manual taxonómico del fitoplancton de aguas continentales, con especial referencia al fitoplancton de Chile. III. Cryptophyceae, Dinophyceae, Euglenophyceae.* Editorial Universidad de Concepción. 99 pp
- Parsons, T.R., Maita, Y., Lalli, C.M. 1984.** *A manual of chemical and biological methods for seawater analysis.* Oxford. Pergamon Press. 173 pp.

- Patmasiriwat, D. 1999.** International Trade, Environmental Issues and the Impact on Sustainability of shrimp Culture in Thailand. in P. Smith (Ed.) *Towards Sustainable Shrimp Culture in Thailand and the Region*. ACIAR Proc N 90.
- Peeters, M., Rodríguez, J. 1999.** Problemas bacterianos en la industria camaronera ecuatoriana, practicas de manejo y alternativas de control. *El Mundo Acuícola* Vol 5 N° 1. Fundación Cenaim Espol. Guayaquil. Ecuador.
- Peragallo, M. 1965.** *Diatomées Marines de France. Et des Districts maritimes voisins. Anciens élèves de l'Ecole Polytechnique*. Reimpresion. A. Asher y Co. Amsterdam 493 pp.
- Perez-Farfante, I., Kensley, B . 1997.** *Penaeoid and Sergestoid Shrimps and Prawns of the World: Keys and Diagnoses for the Families and Genera. Mémoires du Museum National D'Histoire Naturelle. Tome 175*
- Pesantes, F. 1983a.** Dinoflagelados del fitoplancton del Golfo de Guayaquil. *Acta Oceanográfica del Pacífico*. INOCAR, Ecuador, 2 (2), 283-399 .
- Pesantes, F. 1983b.** Los dinoflagelados como indicadores de "El Niño" en el mar ecuatoriano. *Acta Oceanográfica del Pacífico*. INOCAR, Ecuador, 2 (1) , 85-117 .

- Phillips, M.J., Barg, U. 1999.** Experiences and opportunities in shrimp farming. Paper presented at the Second International Symposium on Sustainable Aquaculture. Food for the Future ? 2-5 November 1997, Oslo, Norway. Organised by the National Committee for Research Ethics in Science and Technology (NENT), Norwegian Academy of Technological Sciences (NTVA) and Centre for Technological Sciences (NTVA) and Centre for Technology and Culture (TMV) at University of Oslo.
- Phillips, M.J. 1998.** Tropical mariculture and coastal environmental integrity. In: S.S. De Silva (Ed.) *Tropical Mariculture*. Academic Press. 17-69 pp.
- Pielou, E.C. (Ed.). 1976.** *Population and Community Ecology*. Gordon & Breach, Chicago, pp. 1– 424.
- Pielou, E.C. 1966.** The measurement of diversity in different types of biological collections. *J. Theor.Biol.* 13, 131-144 .
- Pillay, T.V.R. 1996.** The challenges of sustainable aquaculture. *World Aquaculture*, 27 (2) , 7-9.
- Pillay, V.K., Boyd, C.E. 1985.** A simple method for calculating liming rates for fish ponds. *Aquaculture* 46, 157 – 162.
- Pizzutto, M., Hirst, R. G. 1995.** Classification of isolates of *Vibrio harveyi* virulent to *Penaeus monodon* larvae by protein profile analysis and M13 DNA fingerprinting. *Dis. Aquat. Organisms* 21,61-68.
- Platon, R. 1999.** Shrimp Aquaculture: the Philippine Experience. In P.Smith (Ed.) *Towards Sustainable Shrimp Culture in Thailand and the Region*. ACIAR Proc N 90.

- Ponce-Palafox, J., Martinez-Palacios, C.A., Ross, L.G. 1997.** The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. *Aquaculture* 157, 107 - 115.
- Prayitno, S.B., Latchford, J.W. 1995.** Experimental infections of crustaceans with luminous bacteria related to *Photobacterium* and *Vibrio*. Effect of salinity and pH on infectiosity. *Aquaculture* 132, 105 – 112.
- Prescot, G.W. 1968.** *The Algae. A review.* Michigan State University. Houghton Mifflin Company. Boston 300 pp.
- Primavera, J.H., Lavilla-Pitogo, C.R., Ladja, J.M., De la Pena, M.R. 1993.** A survey of chemical and biological products used in intensive shrimp farms in the Philippines. *Mar. Pollut. Bull.* 26, 35 - 40.
- Primavera, J. H. 1998.** Tropical shrimp farming and its sustainability. In: De Silva S, editor. *Tropical mariculture.* London: Academic Press, 487 pp.
- Primavera, J. H. 1993.** A critical review of shrimp pond culture in the Philippines. *Rev. Fish. Sci.* 1, 151 - 201.
- Queiroz, J.F., Boyd, C. E. 1998.** Effects of a bacterial inoculum in channel catfish ponds. *Journal of the World Aquaculture Society* [J. World Aquacult. Soc.]. Vol. 29, no. 1, pp. 67-73.
- Redshaw, C.J. 1995.** Ecotoxicological risk assessment of chemicals used in aquaculture: a regulatory viewpoint. *Aquacult Res* 26, 629 - 637.
- Retamales, R. 2000.** Phytoplankton community composition in a semi intensive commercial shrimp pond. *International Simposium El evento La Niña y su impacto biologico, and Workshop.* OEA. IAI. IAW. RIBEN. 9-10 Nov. Lima, Peru.
<http://www.concytec.gob.pe/investigacion/biologia/riben/org.htm>

- Retamales, R. 2001.** Bacterial community composition in a semiintensive shrimp culture of *L.vannamei* during El Niño 1997-1998 and La Niña 1999. *XXV Jornadas Nacionales de Biología and Workshop*. 22-23 Nov. FCN Universidad de Guayaquil. Ecuador.
- Reynolds, C.S. 1987.** Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments. *New Zealand Journal of Marine and Freshwater Research* 21, 379-390 .
- Rhee, G.Y., Gotham I.J. 1980.** Optimum N:P ratios and coexistence of planktonic algae. *Journal of Phycology* 16, 486 – 489.
- Rivera,P., Parra, O., González, M. 1973.** Fitoplancton del Estero Lengua, Chile. Gayana. Instituto Central de Biología. Universidad de Concepción.23, 1-65 p.
- Robertson A.I., Phillips, M.J. 1995.** Mangroves as filters of shrimp pond effluent: predictions and biogeochemical research needs. *Hydrobiologia* 295, 311 - 321.
- Robertson, P.A.W., Calderon, J., Carrera, L., Stark, J.R., Zherdmant, M., Austin, B. 1998.** Experimental *Vibrio harveyi* infections in *Penaeus vannamei* larvae. *Dis. Aquat. Org.* 32, 151–155.
- Roman, M.R., Ducklow, H.W., Fuhrman, J.A., Garside, C., Gilbert, P.M., Malone, T.C., McManus, G.B. 1988.** Production, consumption and nutrient cycling in a laboratory mesocosm. *Marine Ecology Progress Series* 42, 39-52.
- Rosenberry, B. (Ed.). 1986.** Ecuador Update. *Aquaculture Digest* (11), 8-12. San Diego, California, U.S.A.
- Rosenberry, B. (Ed.). 1990.** World shrimp farming. 1990. *Aquaculture Digest*. San Diego.U.S.A.

- Rosenberry, B. (Ed.). 1991.** World shrimp farming. 1991. Aquaculture Digest. San Diego.U.S.A.
- Rosenberry, B. (Ed.). 1992.** World shrimp farming.1992. Aquaculture Digest. San Diego.U.S.A.
- Ruangpan, L., Kitao, T. 1991.** *Vibrio* bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. J.Fish.Dis. 14 , 383 – 388.
- Ruangpan, L., Tabkaew, R., Sangrungruang, K. 1995.** Bacterial flora of ponds with different stocking densities of black tiger shrimp, *Penaeus monodon*. In: Shariff, M., Arthur, J.R., Subasinghe, R.P. (Eds.), *Diseases in Asian Aquaculture II, Fish Health Section*, Asian Fisheries Society, Manila, pp. 141–149.
- Sandifer, P., Hopkins, J. S. 1996.** Conceptual design of a sustainable pond-based culture system. Aquacultural Engineering 15, 41-52.
- Sano, T., Fukuda, H. 1987.** Principal microbial diseases of mariculture in Japan. Aquaculture 67 , 59 – 69.
- Sansanayuth, P., Phadungchep, S., Ngammontha, S., Ngdngam, S., Sukasem, P., Hoshino, H., Ttabucanon, M.S. 1996.** Shrimp pond effluent: pollution problems and treatment by constructed wetlands. Water Sci Technol 34, 93 - 98.
- Schindler, D.W. 1977.** Evolution of phosphorus limitation in lakes. Science 195, 260-262 .
- Schuster, W.H. 1952.** Brackish water ponds. Berita Perikanan – Jawatan Perikanan, Jakarta, Indonesia.

- Seok, K., Leonard, S., Boyd, C.F., Schwartz, M.F. 1995.** Water quality in annually drained and undrained channel catfish ponds over three-year period. *Progressive Fish Culturist* 57, 52-58.
- Seymor, E.A. 1980.** The effects and control of algal blooms in fish ponds. *Aquaculture* 19, 55 – 74.
- Shannon, C.E., Weaver, W. 1949.** *The Mathematical Theory of Communication*. University of Illinois Press, Champaign.
- Shapiro, J. 1984.** Blue-green dominance in lakes: the role and management significance of pH and CO₂. *Internationale Revue der Gesamten Hydrobiologie*, 69, 765-780.
- Sheldon, A.L. 1969.** Equitability indices: Dependence on the species count. *Ecology* 50, 466-467.
- Shigueno, K. 1972.** Problems of prawn culture in Japan. Coastal aquaculture in the Indo-Pacific region. pp. 282-312.
- Shinn, M. B. 1941** .The reaction of nitrite with a primary aromatic amine. *Ind. Eng. Anal. Ed.* 13, 33.
- Shivappa, R.B. 1997.** Efficacy of probiotics and disinfectants in controlling luminescent bacteria in shrimp post larvae under normal and stressed conditions. MSc Thesis. Asian Institute of Technology. Bangkok.
- Siaviachay, K. 1997.** Aplicación de nuevas técnicas para el seguimiento bacteriológico en un laboratorio de larvas de camarón. Tesis de Acuicultor, ESPOL, Ingeniería Marítima y Ciencias del Mar. Guayaquil. Ecuador.

- Sidall, S.E., Atchue, J.A., Murray, R.L. 1985.** Mariculture Development in Mangroves: A case study of Filipinas, Ecuador and Panama. In: J.R.Clark (Eds.). *Coastal Resources Management: Development case studies*. Renewable Resources Information Series. Coastal Management Publication N°3. Prepared for The National Park Service, U.S. Department of the Interior and the U.S. Agency for International Development Research Planning Institute, INC Columbia, South Carolina.
- Simpson, E.H. 1949.** *Measurement of diversity*. Nature, Lond. 163, 688 pp.
- Smith, P. 1995.** Mortalities of *Penaeus monodon* caused by toxic blooms of marine cyanobacteria, *Oscillatoria spp.* Abstract In: Aust Marine Science Association Annual Scientific conference. Sydney: AMSA.
- Smith, P. 1996.** Toxic effect of blooms of marine species of oscillatoriales on farmed prawns (*Penaeus monodon*, *Penaeus japonicus*) and brine shrimp (*Artemia salina*). Toxicon Vol. 34 N° 8, 857- 869.
- Smith, V.H. 1983.** Low nitrogen to phosphorus ratio favors dominance by blue-green algae in lake phytoplankton. Science 221, 669-671.
- Smith, V.H. 1986.** Predicting the proportion of blue-green algae in lake phytoplankton . Canadian Journal of Fisheries and Aquatic Science 43, 148-153.
- Smith, V.H. 1993.** Implications of resource-ratio theory for microbial ecology. Limnol Oceanogr. 38 , 239 – 249.
- Snedecor, G.W., Cochran W.G. 1980.** *Statistical methods*. 7th edn. The Iowa State University Press. Ames.
- Sokal, R., Rohlf F.J. 1995.** *Biometry*. San Francisco: W.H. Freeman.

- Solis, A. 1996.** Miniaturización y simplificación de pruebas bioquímicas de bacterias marinas asociadas al camarón. Tesis de Acuicultor, ESPOL, Ingeniería Marítima y Ciencias del Mar. Guayaquil. Ecuador.
- Song, Y.L., Cheng, W., Wang, C.H. 1993.** Isolation and characterization of *Vibrio damsela* infectious for cultured shrimp in Taiwan. *J. Invertebr. Pathol.* 61, 24–31.
- Song, Y.L., Lee, S.P. 1993.** Characterization and ecological implication of luminous *Vibrio harveyi* isolated from tiger shrimp *Penaeus monodon*. *Bull. Inst. Zool., Acad. Sin.* 32, 217–220.
- Soto, M. A., Lotz, J. M., Brel, V. M., Overstreet, R. M. 2000.** Studies on waterborne transmission of Taura Virus and White Spot Virus to penaeid shrimp of the Americas. *J. World Aquacult. Soc.* 309 A.
- Stevenson, M.R. 1981.** Seasonal variations in the Gulf of Guayaquil, a tropical estuary. *Bull Inst Nacional Pesca Ecuador* 4(1), 1–133.
- Stevenson, N.J. 1997.** Disused shrimp pond: option for redevelopment of mangroves. *Coastal Management* 25, 425-435.
- Strickland, J.D. H., Parsons, T.R. 1972.** *A practical handbook of seawater analysis*. Bulletin N 167, 2nd edition. Ottawa. Fisheries Research Board of Canada, 310 pp.
- Sunaryanto, A., Mariam, A. 1986.** Occurrence of pathogenic bacteria causing luminescence in penaeid larvae in Indonesia hatcheries. *Bull. Br. Aqua. Dev. Center* 8, 64–70.

- Sung, H.H., Li, H.Ch., Tsai, F.M., Ting, Y.Y, Chao, W.L. 1999.** Changes in the composition of *Vibrio* communities in pond water during tiger shrimp (*Penaeus monodon*) cultivation and the hepatopancreas of healthy and diseased shrimp. *Journal of Experimental Marine Biology and Ecology* 236, 261 – 271.
- Sung, H.H., Li, Hsu S.F., Chen, C.K., Ting, Y.Y., Chao, W.L. 2001.** Relationships between disease outbreak in cultured tiger shrimp (*Penaeus monodon*) and the composition of *Vibrio* communities in pond water and shrimp hepatopancreas during cultivation. *Aquaculture*, 192, 101 – 110.
- Takahashi, Y., Itami, T., Kondo, M., Maeda, M., Fujii, R., Tomonaga, S., Supamattaya, K., Boonyaratpalin, S. 1994.** Electron microscopic evidence of bacilliform virus infection in Kuruma shrimp *Penaeus japonicus*. *Fish Pathol.* 29, 121–125.
- Takahashi, Y., Shimoyama, Y., Momoyama, K. 1985.** Pathogenicity and characteristics of *Vibrio* sp. isolated from cultured kuruma prawn, *Penaeus japonicus* Bate. *Bull. Jpn. Soc. Sci. Fish.* 51, 721–730.
- Teichert-Coddington, D.R., Rodriguez, R., Toyofuku, W. 1994.** Causes of cyclical variation in Honduran shrimp production. *World Aquaculture* 25 (1), 57-61.
- The World Bank, United Nation, Development programme, Commission of the European Communities and Food and Agriculture Organization. 1991.** Research capabilities and needs in Latin America. *World Bank Technical Paper N° 148. Fisheries Series.*
- Thune, R.L., Hawke, J.P., Siebeling, R.J. 1991.** Vibriosis in the red swamp crawfish. *J. Aquat. Anim. Health* 3, 188–191.

- Tilman, D., Kiesling, R., Sterner, R., Kilham, S., Johnson, F.A. 1986.** Green, blue-green and diatom algae: taxonomic differences in ability for phosphorus, silica and nitrogen. *Archiv fur Hydrobiologie* 106, 473-485.
- Teinsongrusmee, B. 1970.** *A present status of shrimp farming in Thailand.* Invertebrates Fisheries Investigations. MFL.D. of R. and Invest. Bangkok, Thailand. Contribution N^o18.
- Tookwinas, S. 1984.** Nitrite-nitrogen and ammonia-nitrogen to mortality rate of shrimp larvae, *Penaeus monodon* and seabass larvae *Lates calcarifer*. National Institute of Coastal Aquaculture, Songkhla, Thailand. Department of Fisheries, Ministry of Agriculture, Technical Paper 6, 32p.
- Tookwinas, S. 1991.** Standard pattern for intensive marine shrimp culture techniques. Marine Shrimp Culture Development Office, Department of Fisheries, 8p.
- Trussell, R.P. 1972.** The percent un-ionised ammonia in aqueous ammonia solutions at different pH levels and temperature. *J. Fish. Res. Board Can.* 29, 1505 – 1507.
- Tsai, C-K. 1990.** Water quality management. *Proceedings of the Southeast Asia shrimp farm management workshop, Philippines, Indonesia, Thailand, july 26-august 11, 1989.*, American soybean assoc., Singapore (Singapore), 56 - 63.
- Tucker, C.S, van der Ploeg, M. 1993.** Seasonal changes in water quality in commercial channel catfish in ponds in Mississippi. *Journal of the World Aquaculture Society* 24, 473- 481.

- Underwood, A.J. 1996.** Detection, interpretation, prediction and management of environmental disturbances: some roles for experimental marine ecology. *J. Exp. Mar. Biol. Ecol.* 200, 1–27.
- Underwood, A.J., Chapman, M.G., Connell, S.D. 2000.** Observations in ecology: you can't make progress on processes without understanding the patterns. *Journal of Experimental Marine Biology and Ecology.* 250, 97 – 115.
- Utermohl, H. 1958.** Zur Vervollkommnung der quantitative Phytoplankton-Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* 9, 1-38.
- Väätänen, P. 1977.** Effects of composition of substrate and inoculation technique on plate counts of bacteria in the Northern Baltic Sea. *Journal of Applied Bacteriology* 42, 437-443 .
- Valdivieso, J.M., Vasconez, B., Icaza P. 1992.** Estudio multitemporal de manglares, camarónicas y áreas salinas de la costa Ecuatoriana, mediante el empleo de la información de sensores remotos actualizado a 1991. Convenio de Cooperación Técnica entre la Dirección General de la Marina Mercante y del Litoral (DIGMAR), Programa de Manejo de Recursos Costeros (PMRC), Subsecretaría de Recursos Pesqueros y el Centro de Levantamientos Integrados de Recursos Naturales por Sensores Remotos (CLIRSEN). *Memoria Técnica CLIRSEN 1992.*
- Vandenbergh, J., Verdonck, L., Robles-Arozarena, I.R., Rivera, G., Bolland, A., Balladares, M., Gomez-Gil, B., Calderon J., Sorgeloos, P., Swings, J. 1999.** Vibrios associated with *Litopenaeus vannamei* larvae, postlarvae, broodstock, and hatchery probionts. *Applied and environmental microbiology*, Vol.65, No.6, pp.2592-2597.

- Vanderzant, C., Mroz, D.E, Nickleson, R. 1970.** Microflora of Gulf of México and pond shrimp. *Journ. Milk Food Technol.* 33, 346-350.
- Vanderzant, C., Nickleson R., Judkins, P.W. 1971.** Microbial flora of pond-reared brown shrimp (*Penaeus aztecus*). *Appl. Microbiol.* 21, 916-921.
- Varela, M., Varela R. 1985.** Microalgas del Bajo Orinoco y Delta Amacuro, Venezuela. II. Bacillariophyceae, Dinophyceae. Fundación La Salle de Ciencias Naturales. Estación de Investigaciones Marinas de Margarita. Punta Piedras-Margarita-Venezuela. (126), 89-107 .
- Varela, R., Varela, M., Fariña, A.C. 1983.** Microalgas del Bajo Orinoco y Delta Amacuro, Venezuela. I. Cyanophyceae, Euglenophyceae, Chrysophyceae, Xantophyceae, Chlorophyceae, Zygothryxaceae. Fundación La Salle de Ciencias Naturales. Estación de Investigaciones Marinas de Margarita. Punta Piedras-Margarita-Venezuela (124) , 59 - 87 .
- Wang, C.H., Lo, C.F., Leu, J.H., Chou, C.M., Yeh, P.Y., Chou, H.Y., Tung, M.C., Chang, C.F., Su, M.S., Kou, G.H. 1995.** Purification and genomic analysis of baculovirus associated with white spot syndrome (WSBV) of *Penaeus monodon*. *Dis. Aquat. Org.* 23, 239–242.
- Weber, C.I. 1971.** *A guide to common diatoms at water pollution surveillance system stations.* U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory. Cincinnati, Ohio. , 96 pp.
- Weidner, D., Rosenberry, B. 1992.** World shrimp farming.1-21 pp. In: James Wyban (Ed.) *Proceeding of the special session on shrimp farming.* Paper presented at Aquaculture 92, May 22-25. Orlando Florida. Published by the World Aquaculture Society.

- Weiner, R.M., Hussong, D., Colwell, R.R. 1980.** An estuarine agar medium for enumeration of aerobic heterotrophic bacteria associated with water, sediment and shellfish. *Canadian Journal of Microbiology*, 26, 1366-1369.
- West, P.A. 1988.** Bacteria of Aquatic Invertebrates. In: *Methods in Aquatic Bacteriology*, B.Austin (Eds.), John Wiley & Sons Ltd, pp 143 – 170.
- Weston, D.P. 1996.** Environmental considerations in the use of antibacterial drugs in aquaculture. In: Baird D.J., (Ed.) . *Aquaculture and water resource management*. Oxford, UK: Blackwell Science, 219 pp.
- Whittaker, R.H. (Ed.). 1975.** *Communities and Ecosystems*, 2nd Edition. Macmillan, New York, pp. 1–385.
- Whittaker, R.H. 1952.** A study of summer foliage insect communities in the Great Smoky Mountains. *Ecol. Monogr.* 22, 1-44 .
- Whittaker, R.H. 1960.** Vegetation of the Siskiyou Mountains. Oregon and California. *Ecol. Monogr.* 30, 279–338.
- Whittaker, R.H. 1965.** Dominance and diversity in land plant communities. *Science* 147, 250–260.
- Whittaker, R.H. 1972.** Evolution and measurement of species diversity. *Taxon* 21, 213–251.
- Whittaker, R.J. 1999.** *Island Biogeography; Ecology, Evolution and Conservation*. Oxford. University Press, Oxford.
- WHO. 1999.** Containing antimicrobial resistance: review of the literature and report of a WHO workshop on the development of a global strategy for the containment of antimicrobial resistance. Geneva, Switzerland, 4_5 February 1999, WHO CDS CSR DRS 99.2.

- Wickins, J.F. 1981.** Water quality requirements for intensive aquaculture. A review. Proc. World Symp. on Aquaculture. Stavanger 1, 17 – 37.
- Williams, L.A., La Rock, P.A. 1985.** Temporal occurrence of *Vibrio* species and *Aeromonas hydrophila* in estuarine sediments. Appl. Environ. Microbiol. 50, 1490 – 1495.
- Wongteerasupaya, C., Vickers, J.E., Sriurairatana, S., Nash, G.L., Akarajamorn, A., Boonsaeng, V., Panyim, S., Tassanakajon, A., Withyachumnarnkul, B., Flegel, T.W. 1995.** A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn, *Penaeus monodon*. Dis. Aquat. Org. 21, 69–77.
- Woods, E.D., Armstrong, F.A., Richards, F.A. 1967.** Determination of nitrate in sea water by Cadmium-copper reduction to nitrite. J. Mar. Biol. Ass. U.K. Vol 47, 23 - 31 .
- Wyban, J., Walsh, W.A., Godin, D.M. 1995.** Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). Aquaculture 138, 267 – 279 .
- Yasuda ,K., Kitao, T. 1980.** Bacterial flora in the digestive tract of prawns, *Penaeus japonicus* Hata. Aquaculture 19, 229 – 234.
- Yont, M., Wanna, R., Suksri, S. 1995.** Effluents from intensive culture ponds of tiger prawn (*Penaeus monodon* Fabricius) Fish. Res. Bull. Kasetsart University, Bangkok, Thailand, no. 21, 17 – 24.
- Yuan, Y., Xin, F., Sun, Y., Qu, K., Cui, Yi., Yi, M., Song, Y., Chen, B., Chen, M. 2000.** TOC, TP, TN and pH in sediment environment of shrimp culture and quality assessment module. J. Fish. China/Shuichan Xuebao. Vol. 24, no. 3 , 247-253.

- Yusoff, F., Matias, H.B., Khalid, Z.A., Phang, S. 2001.** Culture of microalgae using interstitial water extracted from shrimp pond bottom sediments. *Aquaculture* 201 , 263 - 270.
- Yusoff, F.M., McNabb, C.D. 1997.** The effects of phosphorus and nitrogen addition on phytoplankton dominance in tropical ponds. *Aquaculture Research* 28, 591 - 597.
- Zar, J.H. 1984.** *Biostatistical Analysis*, 2nd edn. Prentice – Hall International.
- Zherdmant, M.T. 1996.** Caracterización de un cepa de *Vibrio harveyi* considerada agente causal del " Síndrome de Bolitas " en larvas de *Penaeus vannamei* y estudio de su interacción con una cepa de *Vibrio alginolyticus* utilizada como probiótico. Tesis de Acuicultor, ESPO, Ingeniería Marítima y Ciencias del Mar. Guayaquil. Ecuador.
- Zienman, D.A., Walsh, W.A., Saphore, E.G., Fulton-Bennett, K. 1992.** A survey water quality characteristics of effluent from Hawaiian aquaculture facilities. *Journal of the World Aquaculture Society* 23, 180 - 191.