SPATIO-TEMPORAL VARIATION IN ORGANIC NITROGEN AND CARBON IN SEDIMENTS ASSOCIATED WITH TROPICAL SUBMERGED-CAGE AQUACULTURE

by

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ABSTRACT

The diminution of marine fish populations due to over-fishing has stimulated the increase of mariculture activities, including in cages located near the coast. However, these activities may be detrimental influence to marine sediments near the culture sites in coastal sites. The first open-ocean mariculture operation began during 2002 south of Culebra Island, Puerto Rico to culture the fish *Rachycentron canadum* and *Lutjanus analis*. The purpose of this study was to evaluate the concentrations of total organic nitrogen (TON), total carbon (TC), and organic matter (OM) in marine sediments near the mariculture site to determine the spatial and temporal dynamics of these nutrients during the first culture period. Results indicate significant differences in the concentration of TON between the cage and control sites. The *L. analis* cage had a higher mean concentration of TON (0.442 mg N/g) than the *R. canadum* cage (0.380 mg N/g) and control site (0.300 mg N/g). TC and OM mean concentrations were not significantly different; however, mean TC concentrations had fluctuations similar to those of the mean TON concentrations. TON and TC mean concentrations were significantly different over time, with an increase in the mean TON (0.66 mg N/g) and TC (199 mg C/g) concentrations from April 2003 to August 2003, with a peak in June 2003, which agreed with the increase in the mean monthly feed input at the culture site (12,947 kg) and the increase of wastes because the fish had reached a commercial weight (4.5 kg). Harvesting began in June 2003, so numbers of fish decreased during subsequent months. Organic matter decreased during June, but peaked during October 2003. Although the increase of the nutrient concentration is relatively low compared with other studies, data represent only the first year of mariculture activity. As the company increases the number of cages, this site should be monitored to determine possible increases in nutrient concentrations in the sediments.

RESUMEN

La disminución de las poblaciones naturales de peces comerciales en el mar por causa de la sobrepesca, ha estimulado el incremento de actividades de maricultura, especialmente en jaulas ubicadas en lugares cercanos a la costa. Se ha reconocido que estas actividades presentan una influencia perjudicial sobre los sedimentos marinos alrededor de los cultivos. Al Noreste de Puerto Rico, se instalo la primera operación de maricultura ubicada en la Isla Culebra y tiene como objetivo el cultivo de peces de las especies *Rachycentron canadum* y *Lutjanus analis*. El propósito de esta investigación fue evaluar las concentraciones de nitrógeno orgánico total (TON), carbono total (TC) y el contenido de materia orgánica (OM) en los sedimentos marinos alrededor del lugar donde se desarrollan estas actividades, a fin de conocer la dinámica espacial y temporal de estos nutrientes durante el desarrollo del cultivo. Los resultados indican que existen diferencias significativas en la concentración de TON entre las jaulas. La jaula de *L. analis* presento una concentración mayor (0.442 mg N/ g) que *R. canadum* (0.380 mg N/ g) y el punto de control (0.300 mg N/ g). Por el contrario, TC y OM no reflejaron diferencias significativas, Sin embargo, el TC presentó un comportamiento similar al de TON. Al comparar las concentraciones de TON y TC durante los meses de desarrollo del cultivo, se encontraron diferencias significativas. La diferencia esta dada por un aumento en la concentración TON (0.66 mg N/g) y TC (199 mg C/g) durante el período comprendido entre Abril 2003 y Agosto 2003, con un pico en el mes de Junio 2003. Este incremento en la concentración de TON y TC coincide con la mayor entrada mensual de alimento al cultivo (12947 kg) y la mayor descarga de desperdicios, ya que los peces se encontraban en su peso promedio de venta (4.5 kg) y había un mayor número de ellos entre las jaulas. Por el contrario el contenido de materia orgánica presenta una disminución en el mes de Junio, mientras que en el mes de octubre 2003 alcanza su pico máximo en los sedimentos. Aunque el aumento de la concentración de los nutrientes es relativamente bajo comparado con otros estudios, es importante tener en cuenta que este es el primer año de actividades de la empresa. También, Snapperfarm esta ubicada en un sitio de alta flujo de agua, totalmente abierta y adyacente a una reserva natural, lo que sugiere que el incremento de concentración de los nutrientes aquí reportados, requieren de un muestreo mas prolongado que pueda reafirmar la tendencia de incremento en las concentraciones de los nutrientes en los sedimentos cercanos a las jaulas y visualizar cambios en el sedimento a largo plazo.

To my parents …

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INTRODUCTION

Increasing human population has resulted in several different problems, especially to provide an adequate supply of high quality protein as food. In the past, humanity obtained some of their protein by fishing on a subsistence level. Fisheries now use technologically advanced methods on a world-wide basis, and, as such, exploitation of marine species has increased rapidly with these sophisticated fishing fleets and intensive harvesting technologies, resulting in over-fishing. As a result, the catch-per-unit-of-effort of commercial species has increased and most aquatic resources have been heavily impacted during the last decade, resulting in the collapse of the populations of several fished species (Pillay 1997). Other species are now being exploited, but capture fisheries have not increased their ability to meet demand since 1996, with a mean catch of 92.3 million metric tons, ranging from 87.3 to 94.8 million mt (FAO 2004).

Consequently, other methods such as marine aquaculture or mariculture have become important to fill the need for seafood products. Mariculture has steadily increased its ability to supply high quality products, provide nutritional and economic benefits, and decreases the intensity of exploitation on declining wild fish resources. Moreover, mariculture seems to be an excellent system to augment seafood production. Asia, as a whole, produces 84% of the global production; animal cultivation has become an important economic pillar of the Chinese mariculture industry (Feng et al. 2004).Mariculture activities are helping communities to

become self-sufficient by increasing jobs in rural areas, as well as improving the nutrition in these areas (Pillay 1997).

Marine fish culture in coastal waters across many parts of the world has grown dramatically in recent years and further growth is expected in the upcoming decade (Leung et al. 1999). Expansion of marine fish farming, rapid development, and introductions of exotic mariculture species have aroused increased concern from the impact on coastal environments and has resulted in the degradation of environmental conditions in many areas (Phillips et al. 1985; Feng et al. 2004). For instance, Chinese mariculture production increased each year-to-year from 1961-2001. Annual production was less than 800,000 mt during the 1960s-1970s. From the mid-1980s to present, intensive mariculture activities increased to meet the demand for aquatic products. The annual production was 1,246,500 mt in 1985, 7,215,100 in 1995, and 11,315,000 in 2001. The production during 2001 was 71 times that of 1961, and 9.1 times that of 1985. Feng et al. (2004) indicated that cage culture of marine fish began in China during the 1970s; now, there are more than one million cages deployed in Chinese coastal waters. Similar to Asia, comparable trends are occurring on other continents such as North and South America. However, North and South America account for only 3.5% of the global aquaculture production (FAO 2007).

One relatively innovative option to intensively culture fish is open-ocean aquaculture in submerged cages to rear fish in an enclosed mesh or netting structure held within a larger body of water. The cages are designed to withstand energetic oceanic conditions. In Taiwan, marine cage aquaculture has gained popularity because of

limited land and freshwater resources (Liao et al. 2004) and investors have been attracted to new technologies that can be utilized in open-ocean conditions.

Consequently, Snapperfarm, Inc., initiated a marine aquaculture industry to culture *Lutjanus analis* (mutton snapper) and *Rachycentron canadum* (cobia) in submerged cages about 3 km south of the Puerto Rican Island. Cobia is a pelagic fish widely distributed throughout most tropical and subtropical waters and warm temperate seas, although they are rare or uncommon in Puerto Rican waters. They are carnivorous, aggressive feeders that opportunistically consume fish, squid, and crustaceans. Mariculture of cobia began in the early 1990s when the technology to mass-produce cobia fry developed; several marine fish hatcheries now produce cobia fingerlings for stocking into inshore cages. The increased success in Taiwan and other Asian Pacific countries after artificial propagation and larval production of cobia appears to have the greatest potential among of the species for offshore cage culture in Taiwan and other topical waters. Cobia have a fast growth rate and comparatively low production costs with good feed conversion efficiency in off-shore net cages, and high quality flesh (Chou et al. 2001; Chou et al. 2004; Liao et al. 2004). It has also been cultured as a recreational fish species.

Open-ocean aquaculture sites can affect both offshore and onshore environments, with severity related to the size and intensity of the farming operation. Impacts include short- and long-term alteration of the local ecosystem; near-field and far-field effects of eutrophication; contamination by xenobiotics; cross-transmission of parasites and pathogens; and deterioration from eutrophication along coastal areas (Black 2001).

Repercussions are not only due to the presence of cages, which take up previously unoccupied space, but may include variations in current flow patterns due to the proliferation of these culture structures and changes in the esthetic appearance of a scenic area (Phillips et al. 1985; Feng et al. 2004). Problems may also develop as a result of massive fish mortalities, which may contaminate the area from released mucus and decomposition. Wild fish may be harmed through ingestion of contaminated waste. Fish escapes may dilute the gene pool of native fish of the same species or disturb the ecological balance by competing for resources with wild fish (Phillips et al. 1985; Leung et al. 1999; Chen et al. 2000; Black 2001; Read and Fernandes 2003).

The degree of impact from effluent wastes is dependent on husbandry parameters, including species, culture method, stocking density, feed type, and the nature of the receiving environment. Because the cages are basically open systems, effluents generated from marine aquaculture sites are inevitably released into the surrounding environment. These effluents can have undesirable impacts on the local environment, depending on amounts released, the time-scale over which the releases take place, and the flushing ability and assimilative capacity of the local recipient water body (Carroll et al. 2003). The effluents from intensive aquaculture systems originate from a variety of sources, including uneaten feed particles and excretions as metabolic waste (feces and urine). Waste particles are deposited on the seabed near the cage while dissolved chemical wastes are discharged directly into the water column from marine cage systems.

Environmental factors also influence the dispersion of particulate waste and uneaten feed from the cages, including hydrographic conditions (tidal flow, depth, salinity), topography, geography, temperature, and the particulates characteristics, such as composition, size and settling behavior (Read and Fernandes 2003). In shallow waters with little current, particulate waste products from aquaculture installations settle to the bottom close to the discharge point. Effluent released into deeper waters, or where the bottom is swept by strong currents, will be dispersed over a large area (Read and Fernandes 2003).

For each culture species, it is essential to understand fundamental differences in feeding habits and growth rates, which influence the type of feed. Water temperatures of temperate and tropical/sub-tropical countries may affect nitrogen requirement, metabolism, and excretion. Management techniques for intensive open-ocean tropical or sub-tropical culture systems (e.g. Hong Kong, Thailand, Japan and Singapore) may include feeding poor-quality commercial pellets or trash fish, thereby increasing the leaching rate by two to four times, resulting in the release of large amounts of dissolved nutrients and suspended solids. Increased eutrophication resulting in poor water quality and dense phytoplankton blooms is a threat to the aquaculture sites themselves, especially if toxic algal blooms develop or if sudden algal die-offs deplete oxygen in the water column from rapid decomposition. If trash fish are used and culture conditions are poor, dissolved oxygen content may be affected up to one km away from the fish farm (Feng et al. 2004). Total organic matter may be two to six

times higher than normal; total nitrogen may be four to ten times higher; and inorganic phosphate may be five times higher (Wu et al. 1994; Leung et al. 1999; Chu 2000; Feng et al. 2004).

Thus, nutrient loading in tropical or sub-tropical areas may be much higher compared with temperate countries (e.g., Scotland, Norway and Canada) where high-quality pellet feed is the norm. A higher water temperature regimen in topical and subtropical zones may lead to increased fish metabolic rate and hence higher nitrogen utilization from the food. Limited data of tropical and sub-tropical species and of culture systems does not permit an accurate estimation of nitrogen loading in open-sea cage farms (Leung et al. 1999). In general, eutrophication related to fish farming has not been sufficiently studied in tropical waters.

Research in temperate coastal regions has been intense relating to eutrophication from marine aquaculture activities and strict regulations have been developed in many countries, such as, Denmark, Norway, New Zealand, Australia, Great Britain, and Canada (Wu et al. 1994; Leung et al. 1999). Several studies in temperate zones indicate mariculture effects are localized, despite high pollution loading from fish farms. These effects are generally restricted to areas within the immediate vicinity of the farms, probably due to the low dispersion of waste feed and fecal materials (Wu et al. 1994). Nevertheless, marine eutrophication has become one of the major issues of our time because of increasing numbers of intensive aquaculture systems which attempt to optimize profits by harvesting greater biomass per cubic meter (Zbigniew et al. 2003; Feng et al. 2004). However, other authors suggest that the effects of

dissolved nutrients in the marine environment from mariculture waste are negligible in relation to other inputs, such as, from industry or agriculture (Carroll et al. 2003).

Organic input into the sediment, whether from natural or artificial sources impact the benthos. The extent of environmental impact on the sea bottom is a local function of the amount of organic waste and the assimilative capacity of the sediment (Carroll et al. 2003), where assimilative capacity is defined as the ability of an area to maintain a "healthy" environment and "accommodate" waste by GESAMP Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection (Read and Fernandes 2003).

With high feed inputs, accumulation of organic sediment material produces increased oxygen demand, anoxic sediments, production of toxic gases, and results in decreased benthic diversity directly beneath the culture cage (Wu 1995). Geochemical changes include negative redox values, increased organic content, and buildup of nitrogenous and phosphorous compounds (Karakassis et al. 2002). Anoxic conditions result in an anaerobic layer of the sediment and bottom water depleted in oxygen. These conditions result in the buildup and release of toxic products, including ammonia, hydrogen sulfide, and methane from the sediment, thus posing a threat to fish, shellfish, as well as other marine organisms. Upwelling of oxygen-depleted bottom water can kill cultivated animals (Feng et al. 2004).However, little information exists relating biogeochemical and biological processes in culture period and the recovery time of a site following production. Recovery of sediments may require several months (Pearson and Rosenberg 1978).

Pereira et al. (2004) suggested that the changes in macrofaunal structure community of pulp mill waste are similar across both in spatial and temporal gradients related to nutrient accumulation.

Furthermore, Gowen et al. (1988) and Lumb (1989), found no significant benthic macrofaunal recolonization after one year of salmon production. Ritz (1989), reported the recovery of moderately disturbed sediments beneath salmon cages after a seven-week period in southeast Tasmania. However, the macrobenthic community in Norway was still dominated by *Capitella capitata* one year after the removal of a salmon farm, thus indicating a slow recovery rate (Johannessen et al. 1994). After six years of cage culture in two areas with intensive salmon net-pen mariculture, sediments had not recovered 23 months after the cages were removed in Cephalonia Bay, Greece (Karakassis et al. 1999). Pohle et al. (2001) indicated that the benthic macrofauna had not recovered within one year of the cessation of net-pen salmon farming activities in two areas in Canada (Lime Kiln Bay and Bliss Harbour).

Based on recovery times of mariculture cage operations, best management practices suggest re-locating cages after 6 to 12 months of operation to facilitate the recovery of enriched sediments to minimize near- and far-field environmental effects. More research is needed to determine the recovery processes and relate results to the climate and management practices for a variety of culture sites. With more data, appropriate cage re-location or the length of fallowing periods could be recommended (Karakassis et al. 1999; Feng et al. 2004; Pereira et al. 2004).

Corredor et al. (1999) indicated the importance of maintaining a balance in coupled nitrification and denitrification and the depurative capacity to ameliorate eutrophication because coastal tropical marine ecosystems function differently from their temperate counterparts where coupled nitrification and denitrification serve an important mechanism for nitrogen depuration. Conversely, coastal tropical ecosystems are more susceptible to nitrogen loading because depurative capacity of the microbial communities is limited by the fragility of the microbial sequential oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-) in nitrification. Aerobic chemolithotrophic bacteria have an affinity for environments with lower concentrations of oxygen than in air. The activity of chemolitotrophic nitrifying bacteria, whose activity peaks in microareophilous environments, is inhibited by light and by oxygen depletion. Thus, subsurface sediments usually pose a lower limit for active nitrification, possibly explaining the fragility of tropical sediments. Nitrification represents an important link in the nitrogen cycle between the mineralization of organic matter (NH_4^+ production) and the loss of fixed nitrogen by denitrification (Caffrey et al. 2003). In the absence of the depurative system for nitrogen and phosphorus in the tropical marine environment, an excess of either of these nutrients results in the proliferation of algal blooms because algae preferentially use dissolved organic nitrogen (i.e., ammonium) from the sediments as a nitrogen source in marine waters. Thus, benthic metabolism plays an important role in the regulation of water column nitrogen concentration and consequently, the productivity of coastal marine systems. Sediments can be a source of nitrogen as well as a major sink in the cycling of this element (Mosquera et al. 1998; Mortazavi et al. 2001).

The overall objectives of this study were to assemble temporal variation of nitrogen, carbon, and organic matter content in marine sediments in different sample sites near an open-ocean tropical submerged-cage fish culture system of cobia (*R. canadum*) and snapper (*L. analis*). Based on the information from this research, the aquaculture industry can optimize their best management practices to improve sustainable tropical aquaculture activities and decrease environmental effects in the sediments or in surrounding areas. The specific objectives are to:

- Quantify total organic nitrogen concentration and total organic carbon in marine sediments at different stations at the cage culture site during the first year of production of cobia (*R. canadum*) and *(L. analis)*.

- Elucidate temporal variation of total organic nitrogen, total organic carbon concentrations, and organic matter content in marine sediments during the first year of production.

- Compare total organic nitrogen and carbon concentration in marine sediments affected by the aquaculture activities and the control ("unaffected") site. The stations are in a distance gradient pattern from the center of the cages.

- Describe the temporal pattern of total organic nitrogen, total organic carbon, and organic matter content in marine sediments at the cage site and at the control site.

MATERIALS AND METHODS

STUDY AREA

This study was conducted at an open-ocean submerged-cage fish-culture farm situated 3 km south of the Puerto Rican island of Culebra (Figure 1), where Snapperfarm Inc., installed two Ocean Spar Sea Stations™, each with a volume of 3000 m^3. Each cage was anchored over a sandy flat bottom at a depth of 28 m of water with each cage submerged 8 m below the surface.

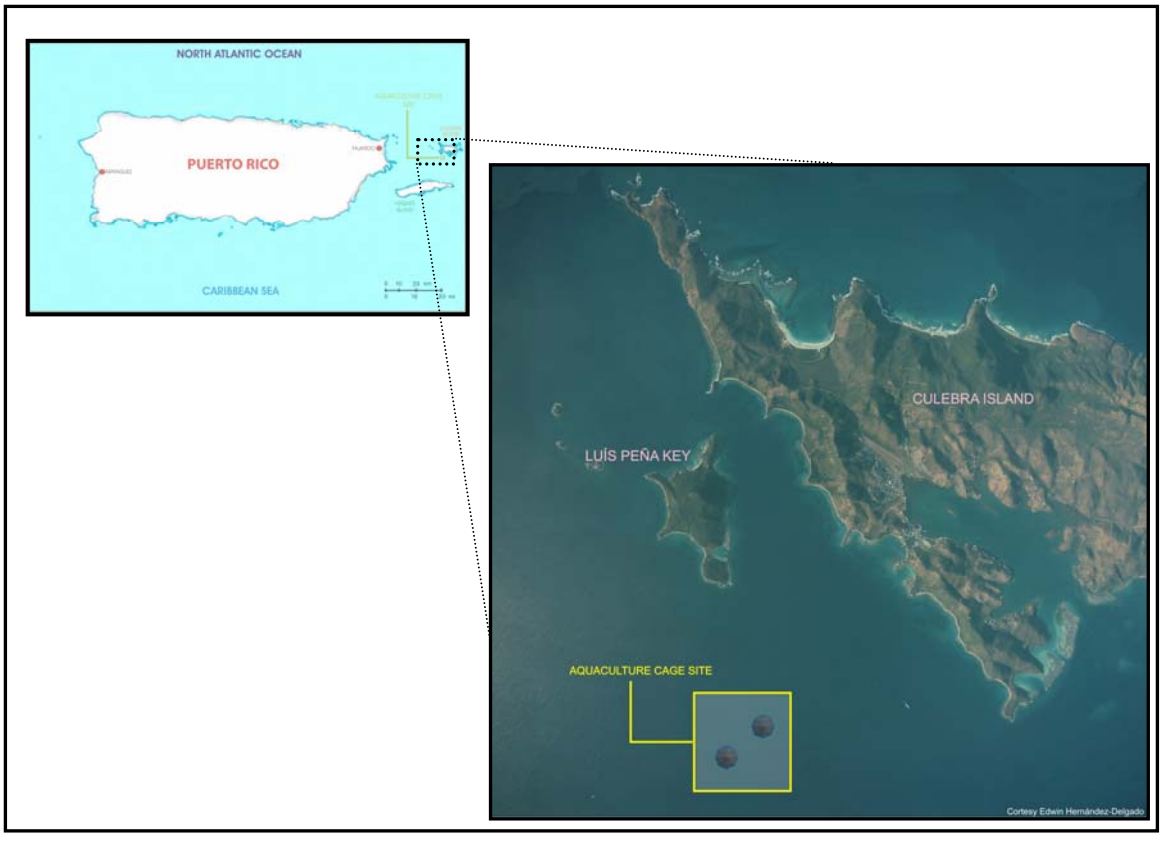

Figure 1. Location submerged aquaculture cage, Snapperfarm, Inc.

Fry of mutton snapper (*Lutjanus analis*) and cobia (*Rachycentron canadum*) fingerlings were cultured at the Aquaculture Center of the Florida Keys Marine Fish Hatchery in Marathon, Florida and air-shipped in plastic bags to San Juan, Puerto Rico, transported by truck to the city of Fajardo, and subsequently loaded onto a boat for transport to the Snapperfarm cage site (Figure 2).

Figure 2. *Rachycentron canadum* fingerlings transported from Florida to Puerto Rico to be stocked in a nursery cage (within the growout cage).

Each cage was stocked with fingerlings of either 3,000 *L. analis* or 5,000 *R. canadum* during August 2002. They were stocked by gravity flow into nursery nets via a flexible hose (Figure 3). During this period, the fish were fed until satiation twice daily as observed by divers. After two months, the fish were released from the nursery nets into the in respective growout cage. Harvest of the *R. canadum* began in July 2003. Due to slow growth, the *L. analis* were not harvested.

Figure 3. Fingerling stocking process in the nursery cage.

Commercial high quality pellets were purchased by the Snapperfarm operation, stored in dry conditions, and transported by boat to the cages where they were fed via a flexible hose. Refer to Table 1 for feed contents. The water-stable high-quality feed had a protein level of 53.0% (Figure 4).

Table 1. Proximate analysis of the commercial pelleted feed, Burris AquaXcelTM 5310.

	Burris AquaXcelTM 5310
Crude Protein, not less than	53.0%
Crude fat, not less than	13.0%
Crude fiber, not more than	2%
Ash, no more than	13.3%
Moisture, no more than	12%

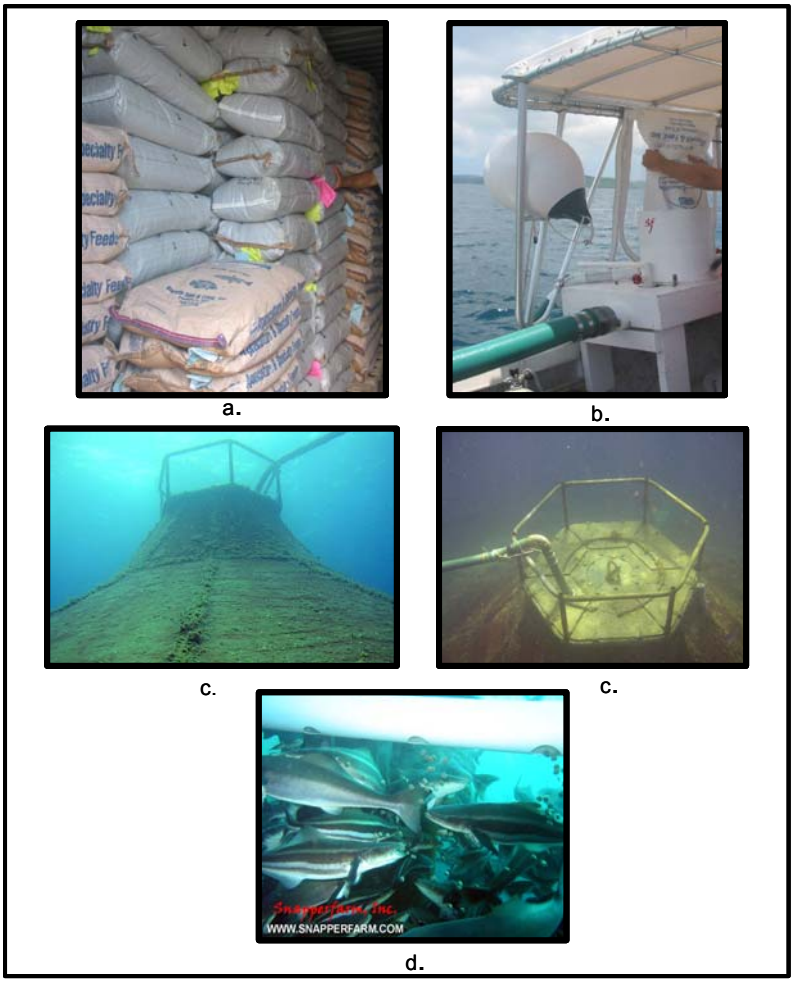

Figure 4. Feeding method: (a) feed storage; (b) adding feed to hopper on boat; (c) delivery of feed via a flexible hose (d) *R. canadum* feeding on pellets.

SAMPLING STATIONS

Sampling stations were selected at 20 and 40 m north (RN), south (RS), and west (RW) from the center of the *R. canadum* cage; and north (LN), south (LS), and east (LE) of the *L. analis* cage. The west (W) station of the *L. analis* cage was shared with the east (E) station of the *R. canadum* cage, equidistant (about 15 m) from the rim of each cage and designated as RE/LW. Other stations were located beneath each cage (LB

beneath *L. analis* cage; RB beneath *R. canadum* cage) next to the cage ballast and at a control site (control) located 375 m south of the cage site. The control site was selected to compare values with the cage sites. The 15 sample station abbreviations, plus the control site is shown in Figure 5. The prevailing current was from southeast to northwest with an approximate velocity of 17 cm/s, however due to the ebb and flows of tides, the current frequently change directions (Figure 6).

SEDIMENTS SAMPLES

At bimonthly intervals, each duplicate benthic sample for chemical analysis was taken with a PVC core sampler at each of the 15 sampling stations, plus at the control site (figure 6). Each core sampler was 10 cm long with a diameter of 5 cm with a sample area of 0.00196 m^2. The length of 10 cm for the core sampler represented the sample depth because research indicates few organisms are collected deeper than this depth (Morrisey et al. 1992). The distance from the cage center to 20 or to 40 m was measured using a tape attached to the center of the cage. Divers swam either north, south, east, or west from the cage center until they reached either the 20 or 40 m station where random core samples were taken (Figure. 7). Field samples were stored in plastic bottles and preserved with ice to reduce bacterial activity. The samples were frozen until they were analyzed. Two sub-samples from thawed sediment samples were dried in an oven at 60˚C for 24 to 48 hours until a constant weight was obtained for each sample, pulverized with a mortar and pestle, and placed in tin capsules to determine sediment total organic nitrogen (TON) and sediment total carbon (TC) using the gas chromatography method (Clesceri et al. 1998) with an elementary analyzer (CNH-O Euro 3000 series).

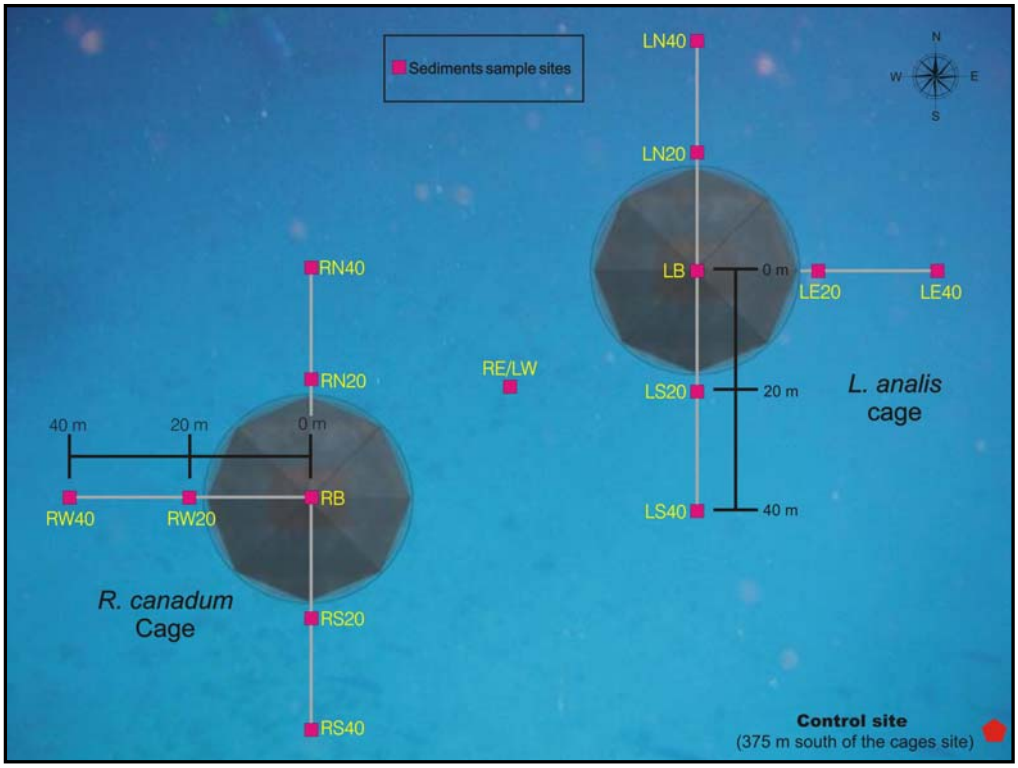

Figure 5. Sample station diagram. RN20: *R. canadum* north, 20 m; RN40: *R. canadum* north, 40 m; RS20: *R. canadum* south, 20 m; RS40: *R. canadum* south, 40 m; RW20: *R. canadum* west, 20 m; RW40: *R. canadum* west, 40 m; RB: *R. canadum* beneath, 0 m; LN20: *L. analis* north, 20 m; LN40: *L. analis* north, 40 m; LS20: *L. analis* south, 20 m; LS40: *L. analis* south, 40 m; LE20: *L. analis* east, 20 m; LE40: *L. analis* east, 40 m; LB: *L. analis* beneath, 0 m; RE/LW: *R. canadum*/*L. analis* shared; Control: control.

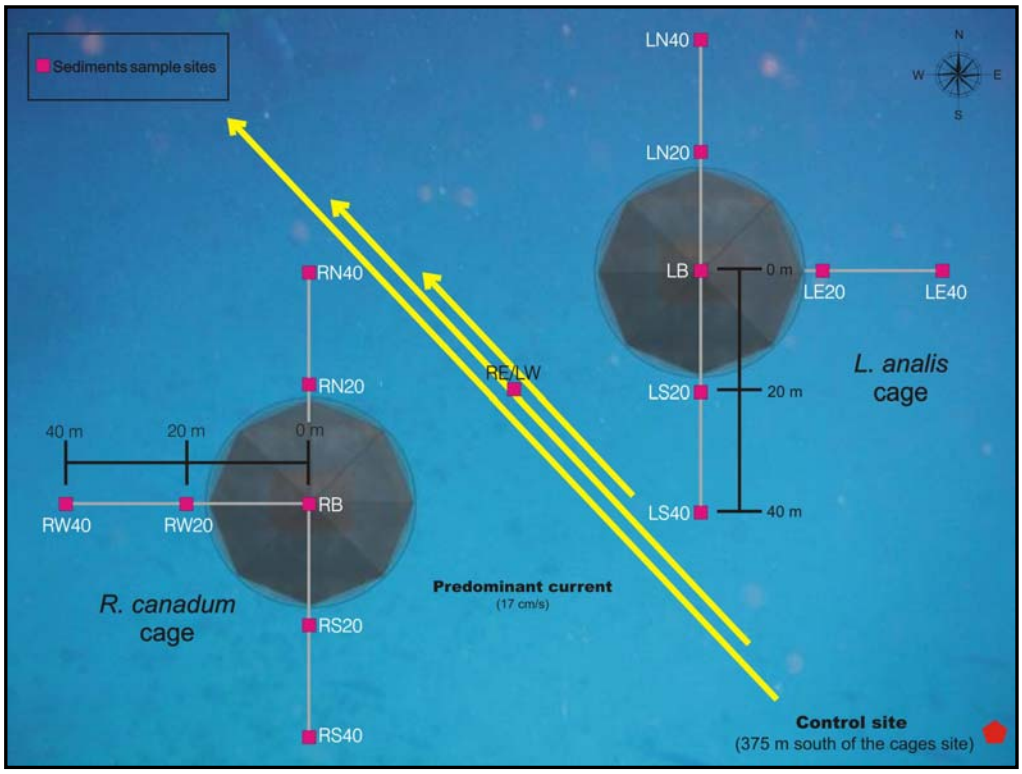

Figure 6. Sediment sampling stations.

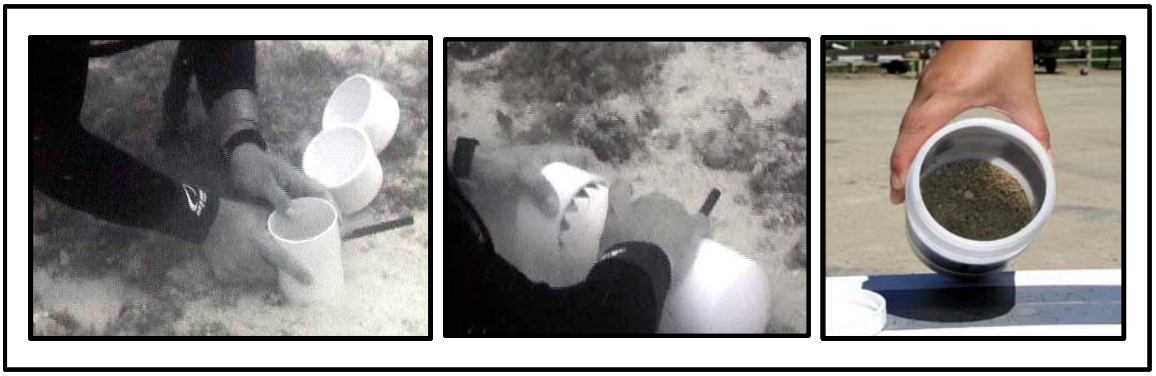

Figure 7. Process for taking core samples sediments. Note: the core sampler in this photo is larger than the actual core samplers used to take the sediment samples in this paper.

FEED SAMPLES

Because the pelleted feed size increased according to increasing fish size, feed samples were obtained for each different feed size. Thus, the commercial feed was analyzed to determinate the total organic nitrogen content by Kjeldhal analysis and an elementary analyzer (CHNO Euro EA 3000 series).

ORGANIC MATTER

The content of organic matter in the sediments was estimated by using the gravimetric method described by (Holme and McIntyre 1984; Páez-Osuna et al. 1984; Clesceri et al. 1998). Duplicate samples were analyzed by placing thawed sediment samples into crucibles; they were dried at room temperature to remove excess water and then placed into an oven at 75ºC until a constant weight was obtained for each sample.

CONTINUOUS MONITORING

Dissolved oxygen concentration, water temperature, and salinity were continuously monitored at 15-min intervals with two data-logging monitoring systems (Data Sonde 4a™ from Hydrolab). One system was attached to the *R. canadum* cage rim and the other was placed at the control site (above the current meter). Each month, information was collected and downloaded to a portable computer. After the data was collected, the Hydrolabs were recalibrated, reprogrammed, and reinstalled to continue the data-logging process at the cages.

STATISTICAL ANALYSES

Statistical analyses were made with InfoStat Software, version 3.0 (2003). Total organic nitrogen (TON), organic matter (OM), and total organic carbon (TC) differences were evaluated with non-parametric statistics because one of the variables did not meet the assumptions (normality, homogeneity of variance) for analysis via parametric statistics. In cases where data did meet the assumptions, a nested ANOVA test was utilized to evaluate results to avoid a type 1 error (α error) which does not detect a difference when it exists (Sokal and Rohlf 1994).

In general, data for all variables were analyzed using the Krustal Wallis test for TON and TC concentrations, and the percentage of organic nitrogen and carbon present in OM in marine sediments. Comparisons were made among cages and control site, among months, and among sampling sites. Significant differences were established at the 95% probability level ($P<0.05$). Dunn's pair comparisons were also used to determine differences among variables.

RESULTS

CONTINUOUS MONITORING

Water temperature was collected during 190 days at the cage site (December 2002 to October 2003). Maximum and minimum values were 29.3ºC in September 2003 and 26.8ºC in February 2003 respectively, with a mean of 28.0ºC. The mean water temperatures declined modestly from December 2002 to the first part of April 2003 by approximately 1ºC and then increased to a maximum of 29.3ºC by October 2003 (Figure 8). Changes throughout the year were less than 3ºC (from 26.8 - 29.3ºC); during most of the culture period, temperatures were above 26ºC. Water temperatures recorded during 120 days at the control site was similar to those at the cage site.

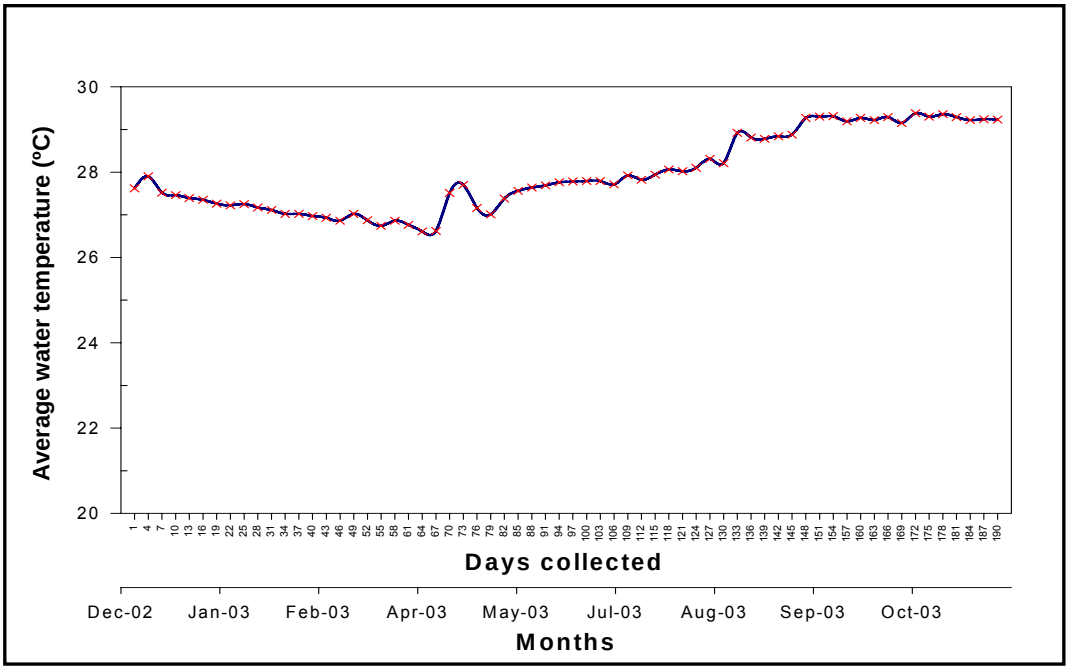

Figure 8. Temporal variation of average water temperature during the sampling period for the cage site.

Dissolved oxygen concentrations were collected during 175 days at the cage site, ranged from minimum of 4.66 in January 2003 to maximum value of 6.85 mg/L in July 2003, from December 2002 to September 2003 (Figure 9). The mean dissolved oxygen concentration in water was 5.32 mg/L. Dissolved oxygen for the control site was recorded for the last 175 days and generally remained in the same range as those of the cage site.

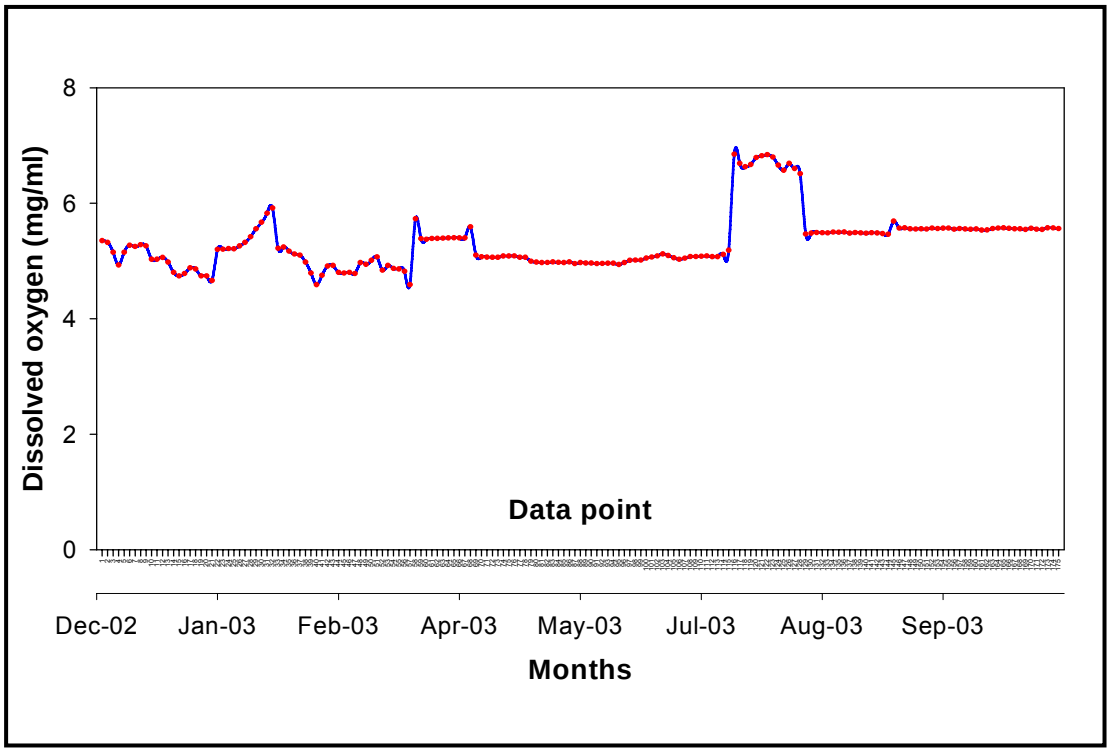

Figure 9. Temporal variation of water dissolved oxygen concentration during the sampling period for the cage site.

Practical salinity values were collected during 188 days at the cage site, with mean values of 34.6, with a minimum value of 31.3 and maximum value of 35.8 in October 2003 and February 2003, respectively (Figure 10). No differences in salinity were detected between the cage and control sites. Values for the control site were recorded for the last 120 days and remained in the same range as those of the cage site.

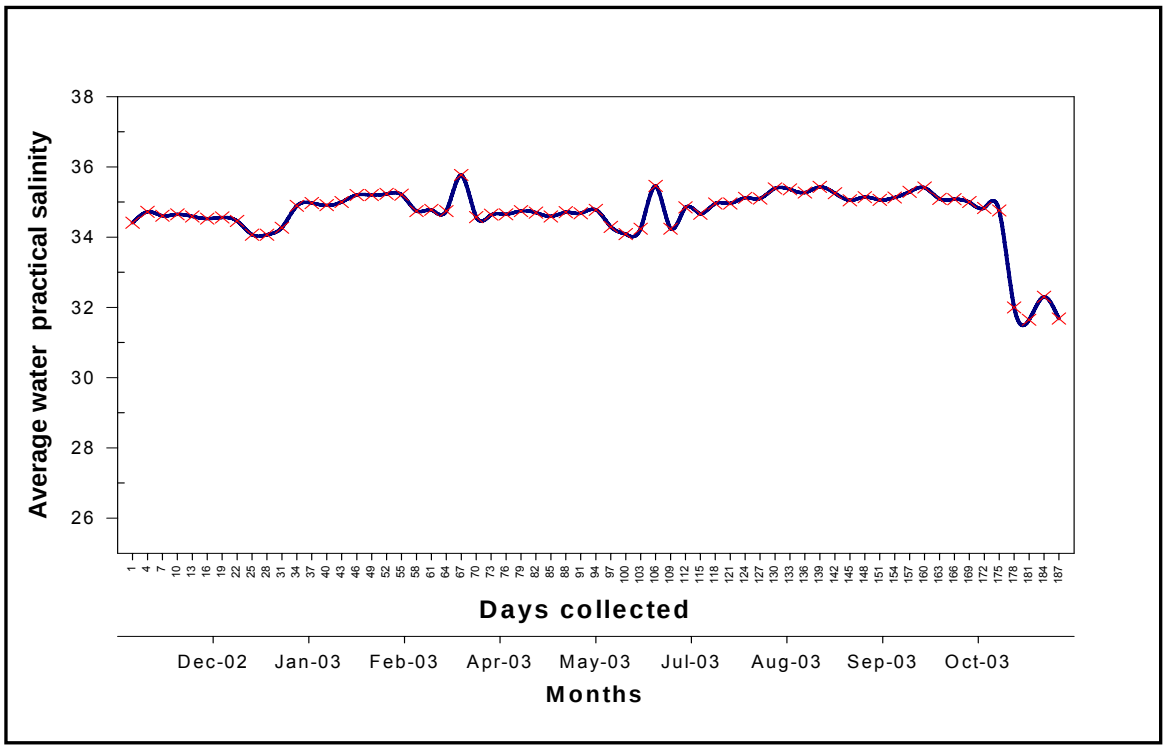

Figure 10. Temporal variation of average water practical salinity during the sampling period for the cage site.

The wind velocity data were collected daily throughout of culture period. Maximum and minimum values of wind velocity were found in February 2003 with 20.2 km/h, and June 2003 with 20.0 km/h; and November 2003 with 10.3 km/h respectively. The mean wind velocity was 16.1 km/h from June 2002 to April 2003 (Figure 11).

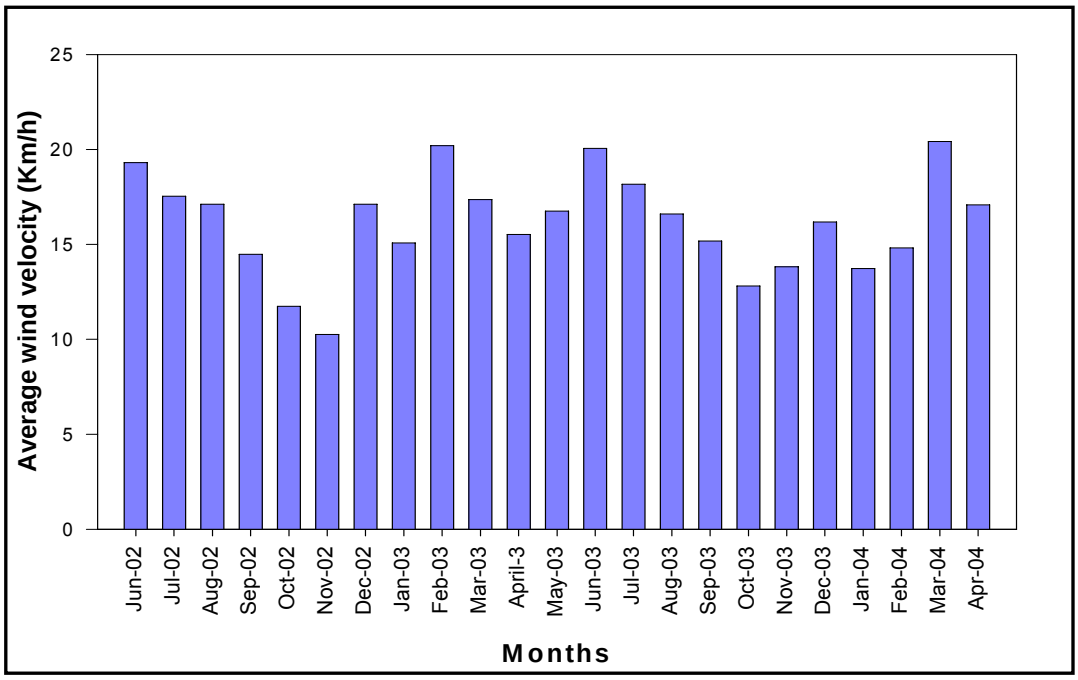

Figure 11. Temporal variation of wind velocity (Km/h) collected by the Roosevelt Road Station, Puerto Rico.

Comparison were made among the sample sites of the *L. analis*, the *R. canadum* cages and control site for mean TON, TC, concentrations, OM content and proportion of TON and TC in sediments organic matter. Temporal variations of TON, TC concentration and OM content comparisons included each sample site through six months during of the study period.

SEDIMENT TOTAL ORGANIC NITROGEN (TON) CONCENTRATION

The Krustal Wallis ANOVA test indicated no differences for mean TON concentration among cages and control site (Table 2). However, the p-value was 0.053, which is close to the established probability level (P<0.05). The Krustall Wallis ANOVA test uses rank or score-type data, but is a less powerful test. Thus, a nested ANOVA test was utilized to possibly avoid a type-1 error. The nested ANOVA test did indicate significant differences among cages for mean sediment TON (Table 3). Table 4 indicated pair-wise comparisons using a Tukey test to determine differences among the two cages and control site. The control site had the lowest mean nitrogen concentration with 0.300 mg N/g ± 0.102 SD. The next highest mean nitrogen concentrations were found for the *R. canadum* cage with 0.380 mg N/g ± 0.208 SD, which had values more similar to the control site than to the *L. analis* cage. The highest mean concentrations (Figure 12) were found for the *L. analis* cage with 0.442 mg N/g ± 0.37 SD.

Table 2. Krustal Wallis ANOVA for TON concentration in sediments among cages and the control site. Values with * represent significant difference (P‹0.05).

Cage	n	Mean	Std. Dev	Median	H	P-value
Control	10	0.30	0.10	0.30	5.87	0.0532
R. canadum	79	0.38	0.21	0.35		
L. analis	89	0.44	0.24	0.39		

Table 3. One way ANOVA for TON concentration in sediments among cages and the control site. Values with * represent significant difference (P<0.05).

Source	D.F.	M.S.	F	P-value
Model	2	0.14	0.81	0.0455
Cages	2	0.14	0.81	< 0.05*
Error	175	0.05	0.26	
Total	177			

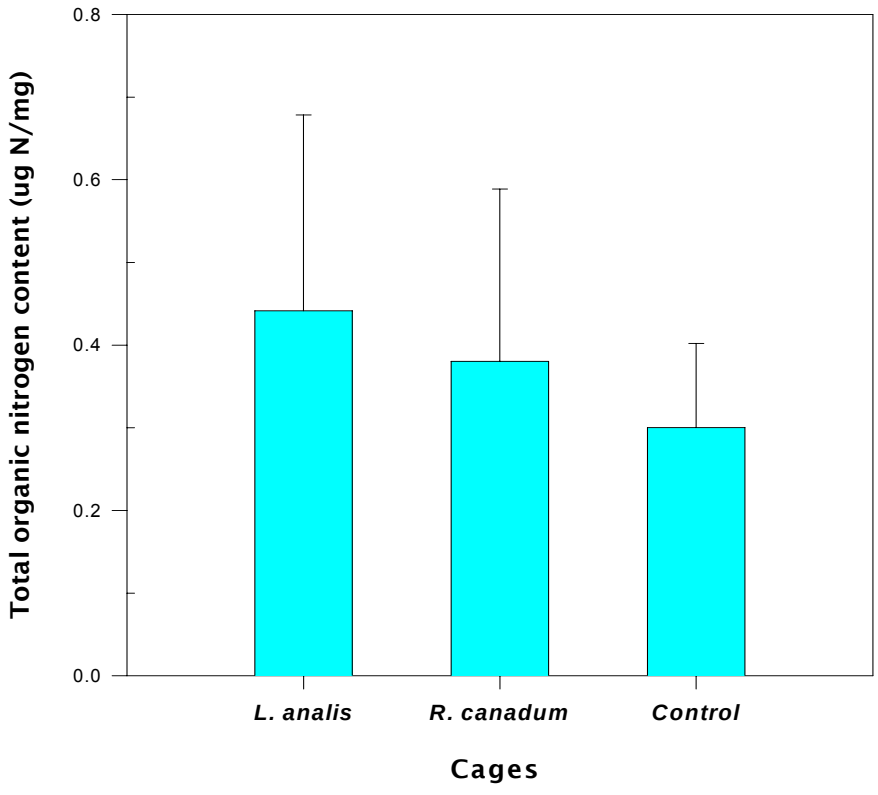

Figure 12. Variation of TON concentration (mg N/g) in sediments located at the cage and control site.

Table 4. Tukey test for TON in sediments among cages and control site. Different letters represent significant difference (P<=0.05). Tukey α= 0.05.

Cages	Means	n		
Control site	0.30	10	A	
R. canadum	0.38	79	A	B
L. analis	0.44	89		C

Table 5 indicated significant differences (P<0.0001) among months for sediment TON concentrations. The Dunn's pair-wise comparison established differences with the formation of three groups (Table 6). The first grouping contained only one month, October 2003, with the lowest mean TON concentrations of 0.234 mg N/g ± 0.122 SD. The next grouping indicated similarities among several months with intermediate mean TON concentrations: October 2002 (0.35 mg N/g ± 0.05 SD), December 2002 (0.36 ± 0.06 SD), February 2003 (0.38 ± 0.06 SD), April 2003 (0.45 ± 0.29 SD), and August 2003 (0. 396 ± 0.28 SD). The grouping with the highest concentration also contained only one month, June 2003 with 0.66 mg N/g ± 0.22 SD (Figure 13).

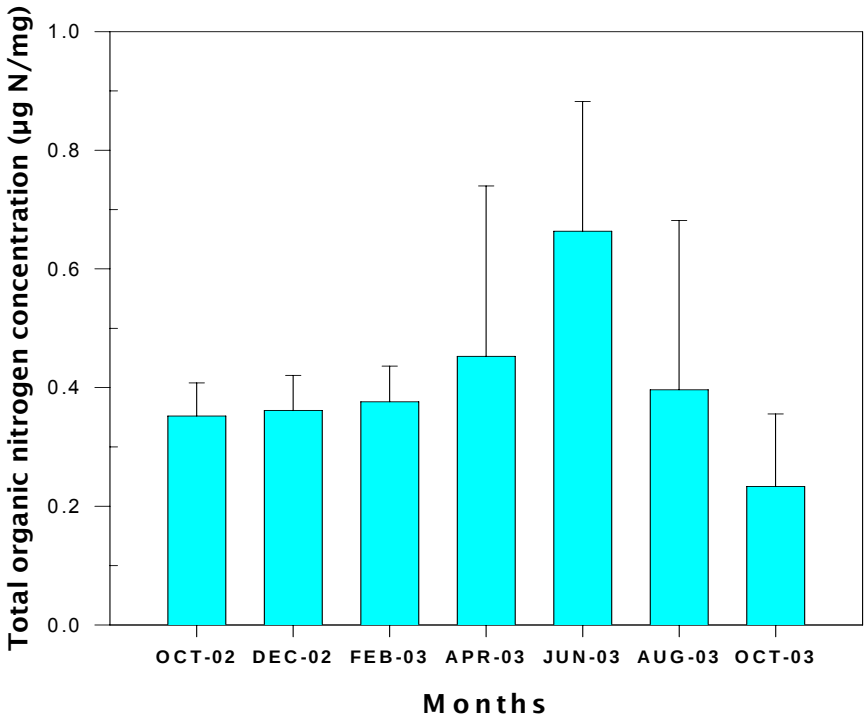

Figure 13. Temporal variation of TON concentration (mg N/g) in sediments through of period studied.

Table 5. Krustal Wallis ANOVA for TON concentrations in sediments among months. Values with * represent significant difference (P<0.05).

Month	n	Mean	Sts. Dev.	H	P-value
October-03	28	0.23	0.12	62.71	<0.0001*
October-02	20	0.35	0.05		
December-02	21	0.36	0.06		
February-03	31	0.38	0.06		
August-03	27	0.40	0.29		
April-03	24	0.45	0.29		
June-03	27	0.66	0.22		

Table 6. Dunn's pairs comparison for TON in sediments among months. Different letters represent significant difference. ($p \leq 0.05$)

Month	Ranks			
October 03	36.96	A		
August 03	77.44		B	
October 02	89.00		B	
December 02	89.02		B	
April 03	93.40		B	
February 03	96.69		B	
June 03	145.06			C

The Krustal Wallis ANOVA indicated no significant differences among sample sites with p- value of p=0.5296 (Appendix 1). Because of natural fluctuations in the environment, the control site TON concentrations fluctuated during the year. The mean TON concentration at the control site (Figure 16) did not fluctuate much during the study period, yet during June 2003 the values increased slightly. Assuming the control site was unaffected by the mariculture activities, changes in TON concentrations probably were due to environmental or seasonal fluctuations. However, when such variations were subtracted from the respective values of each sample site, the peak in June was still present and the mean values for that month in most of the sample sites were still three times those of the control value.

Appendix 10, indicates the sequential patterns of mean TON sediment concentrations for the *R. canadum* cage in which the respective control site mean concentrations were subtracted from each *R. canadum* mean value. The reason for subtracting the control values was to eliminate environmental fluctuations to present changes occurring at each site during each sampling period. The mean TON sediment concentrations were highest from April to August 2003 for the *R. canadum* cage (Figure 14). Two sampling sites with the highest mean TON concentrations occurred in April 2003 (RE/LW with of 0.26 mg N/g; and RN40 with 0.05 mg N/g); three sampling sites for June 2003 (RS40 with of 0.12 mg N/g; RW40 with 0.47 mg N/g, and RB with 0.14 mg N/g); and three sampling sites in August 2003 (RN20 with value of 0.41 mg N/g, RS20 with 0.51 mg N/g, and RW20 with 0.26 mg N/g).

Appendix 11 and 15 indicated the sequential patterns of TON sediment concentrations for the *L. analis* cage by subtracting the respective control site mean concentration from each *L. analis* mean value. The TON sediment concentrations were highest from April to August 2003 for the *L. analis* cage. Three sampling sites with the highest mean TON concentrations occurred in April 2003 (LE40 with 0.83 mg N/g; LS40 with of 0.30 mg N/g; and RE/LW with 0.26 mg N/g); four sample sites for June 2003 (LN40 with 0.20 mg N/g; LE20 with 0.45 mg N/g; LB with 0.36 N/mg; and LN20 with 0.45 mg N/g); and one for August (LS20 with 0.0.44 mg N/g).

In all sample sites, a comparison of "start" (October 2002) and "final" (October 2003) were graphically evaluated to determine the spatial pattern of TON concentration. Values of TON were higher for the October 2003 at sample sites LS40, LS20, RE/LW, and RS20 (Figure 17).

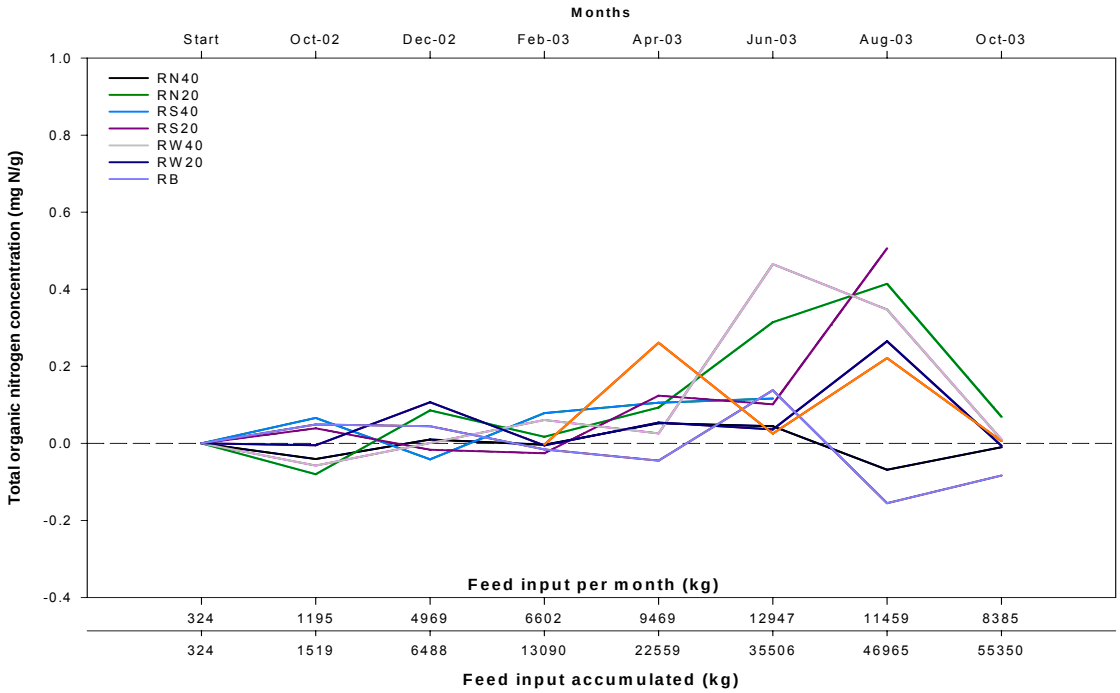

Figure 14. Temporal variation of TON concentration per sample site minus control site in *R. canadum* cage.

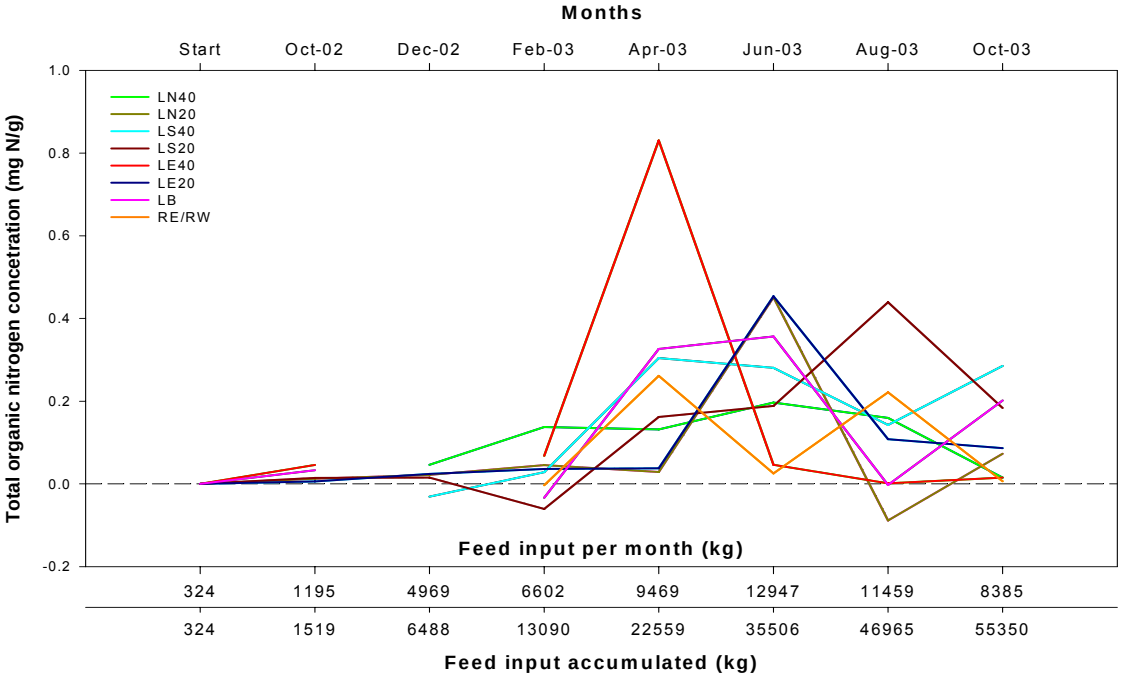

Figure 15. Temporal variation of TON concentration per sample site minus control site in *L. analis* cage.

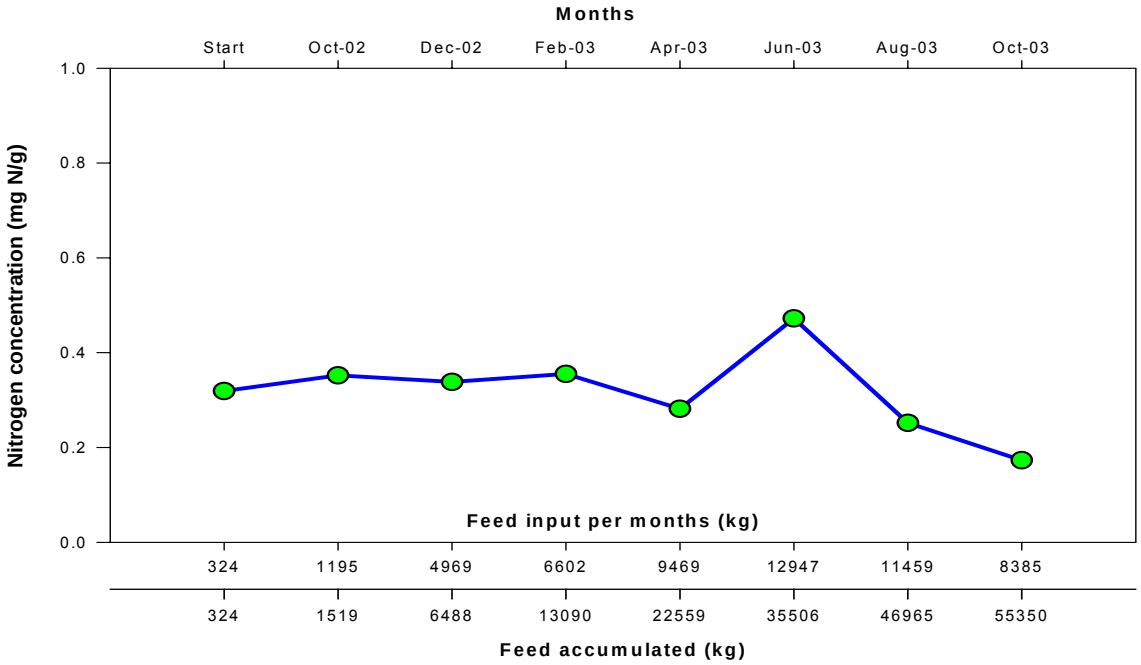

Figure 16. Temporal variation of TON concentration in control site

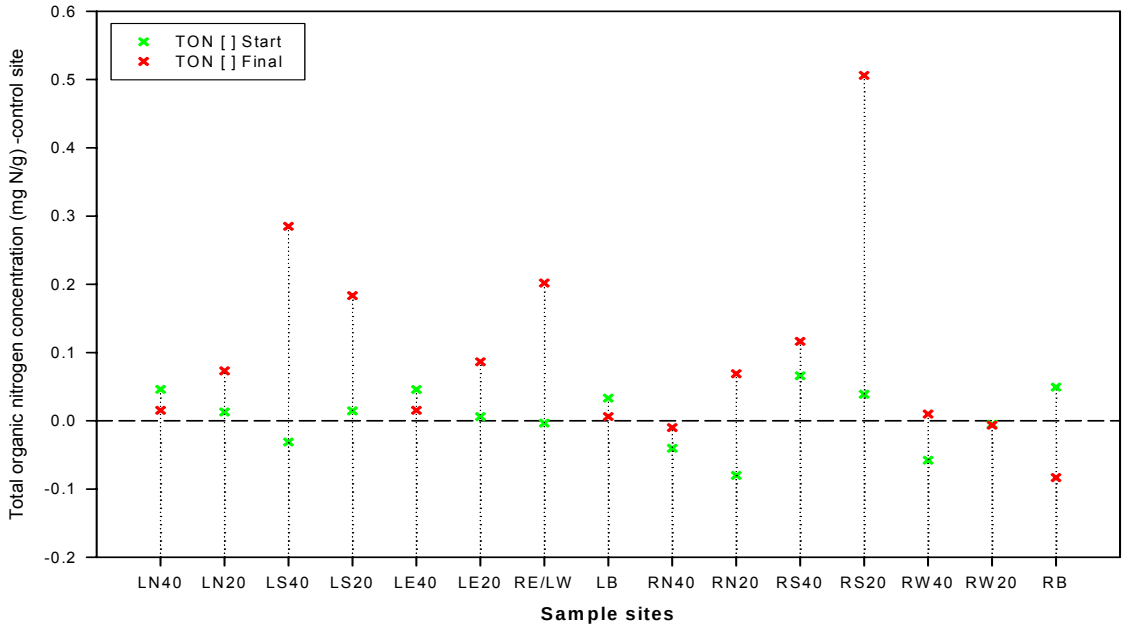

Figure 17. Variation of mean TON concentrations for each sample site by comparing initial data (Start-February 2003) with final data (final-October 2003).

TOTAL CARBON (TC) CONCENTRATION

The Krustal Wallis ANOVA test indicated no significant differences for sediments TC concentration among cages or control site (Appendix 2). Figure 18 showed the *L. analis* cage presented the highest concentration (139.1 µg C/mg ± 55.7 SD), followed by the *R. canadum* cage with a mean value of (128.4 µg C/mg ± 41.0 SD), and followed by the control site with (117.4 µg C/mg ± 3.4 SD).

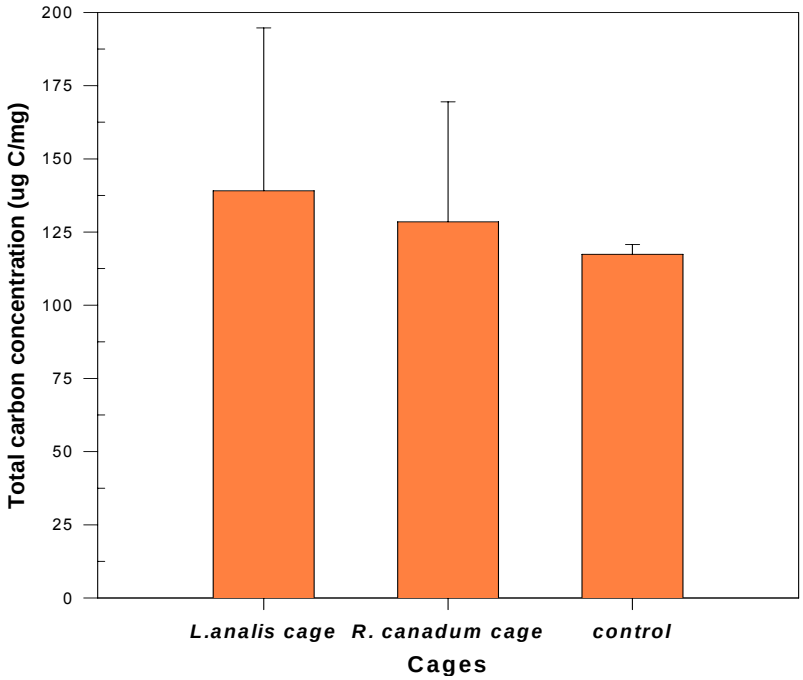

Figure 18.. Variation of TC concentration in *L. analis*, *R. canadum* cages, and control site.

Table 7 indicated significant differences with a p-value ($p<0.0001$) for TC concentrations among months. The Dunn's pair-wise comparison established differences with the formation of three groups (Table 8 and Figure 19). The first grouping contained only one month, February 2003, with the lowest mean TC concentrations (115.7 µg C/mg ± 3.5 SD). The next grouping indicated similarities among several months with intermediate TC concentrations: August 2003 (117.8 µg C/mg ± 3.4 SD), and April 2003 (115. 6 µg C/mg ± 10.1 SD). The grouping with the highest concentration contained two months: October 2003 (121.6 µg C/mg ± 1.4 SD) and June 2003 (199.2 µg C/mg ± 82.2 SD).

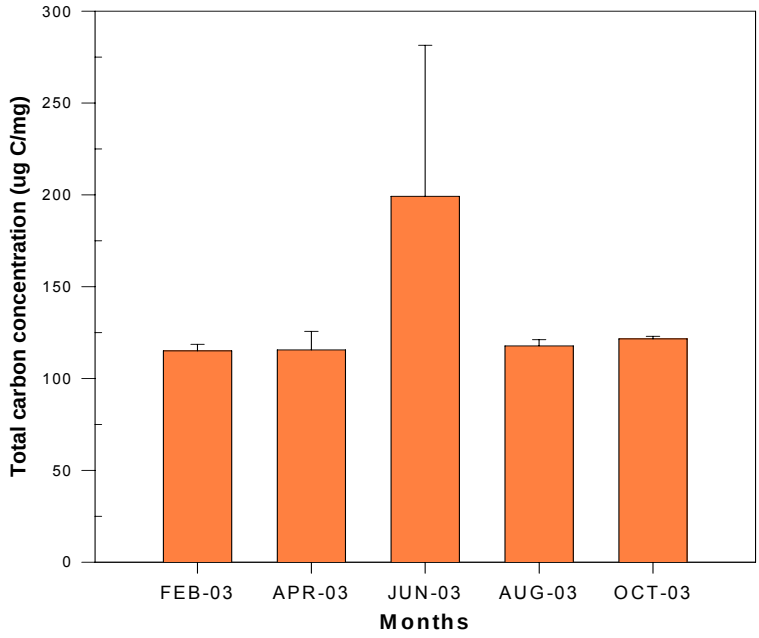

Figure 19. Temporal variation of TC concentration in sediments during the study period.

Table 7. Krustal Wallis ANOVA for TC concentration in sediments among months. Values with * represent significant difference (P≤0.05).

Months	N	Mean	Std. Dev.	Median	D F	H	P-value
February-03	31	115.1	3.46	115.5	4	72.08	<0.0001*
April-03	24	115.6	10.09	118.4			
August-03	27	117.8	3.38	116.2			
October-03	28	121.6	1.37	1.4			
June-03	27	199.2	82.25	223.0			

Table 8. Dunn's pairs comparison for TC concentrations in sediments among months. Different letters represent significant difference (p≤ 0.05).

Month	Ranks			
February-03	30.8	A		
August-03	53.3		B	
April-03	60.7		B	
October-03	98.5			C
June-03	105.4			C

The Krustal Wallis ANOVA indicated no significant differences for sediment TC concentration among sample sites with p-value of 0.9960 (Appendix 3). Figures 39, Appendix 12 and Figure 20 showed the tendency of TOC concentration in each sample site for the *R. canadum* cage by subtracting the respective control site mean concentration from each *R. canadum* mean value. The TOC sediment concentrations were highest in June 2003 for the *R. canadum* cage. Two samples sites presented the

highest TOC concentrations for April 2003 (RS40 with 6.93 µg C/mg, and RB with 5.83 µg C/mg); four sample sites for June 2003 (RN40 with 67.88 µg C/mg; RN20 with 134.40 µg C/mg; RS20 with 79.86 µg C/mg; RW40 with 192.6 µg C/mg); and one sample site in August 2003 (RW20 with 8.04 µg C/mg). The control site had the highest TOC concentration for October 2003 (121.4 µg C/mg).

The TOC sediment concentrations were highest in June 2003 for the *L. analis* cage. Appendix 13 and Figure 21 illustrated the pattern for each of the sampling sites for the *L. analis* cage by subtracting the respective control site mean concentration from each *L. analis* mean value. Only one sample site presented a maximum TOC concentration in April 2003 (RE/LW with 7.82 µg C/mg). Seven sample sites with the highest TC concentrations for June 2003 (LE20 with 188.2 µg C/mg; LE40 with 52.0 µg C/mg; LB with 168.5 µg C/mg; LN40 with 102.7 µg C/mg; LN20 with 162.0 µg C/mg; LS40 with 59.0 µg C/mg; and LS20 with 133.0 µg C/mg).

The mean TC concentration at the control site (Figure 22) did not fluctuate much during the study period, yet during June 2003 the values increased slightly. Assuming the control site was unaffected by the mariculture activities, changes in TC concentrations probably were due to environmental or seasonal fluctuations. However, when such variations were subtracted from the respective values of each sample site, the peak in June was still present and the mean values for that month in most of the sample sites were still twice the control value.

Nonparametric ANOVA was used to determine homogeneity of variances, although the data was not normally distributed. Nonparametric ANOVA provides differences in

extreme cases with less information; therefore, it is a less powerful test. However, an inspection for outliers (Grubb test) was performed to decrease distortion of the data from unusually high values (e.g., for the month of June). Results showed five points that can be considered outliers during June 2003. However, month comparisons (Krustal Wallis and Dunn's tests) using this new data set produced the same results that were obtained with the full data set with June 2003 having the highest TC concentration. Moreover, the same tests were performed with the data from June arbitrarily taken out from the analyses to inspect for time variations without this month with the highest concentrations (Figure 24); however, the tendency for increase in TC concentration was still observed. In all sample sites, a comparison of "start" (February 2003) and "final" (October 2003) were graphically evaluated to determine the spatial pattern of TOC concentration. Values of TOC were higher for the October at sample sites RN40, RS20, LS20, and RS40 (Figure 23).

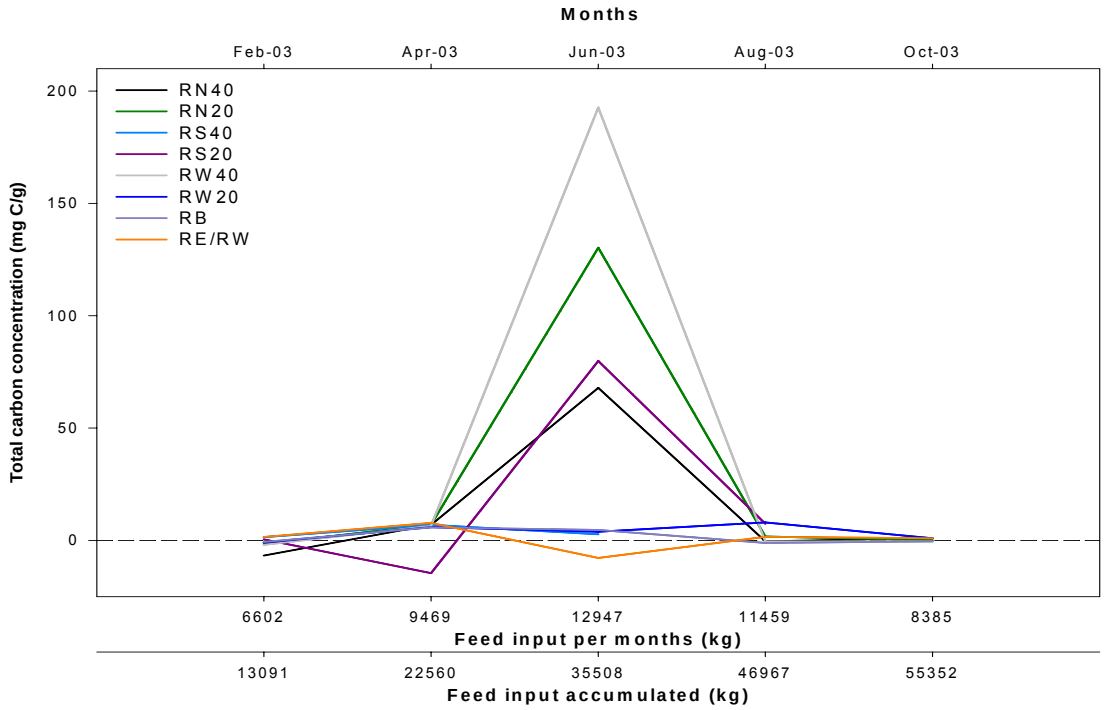

Figure 20.Temporal variation of TC concentration per sample site in the *R. canadum* cage minus value concentration in control site.

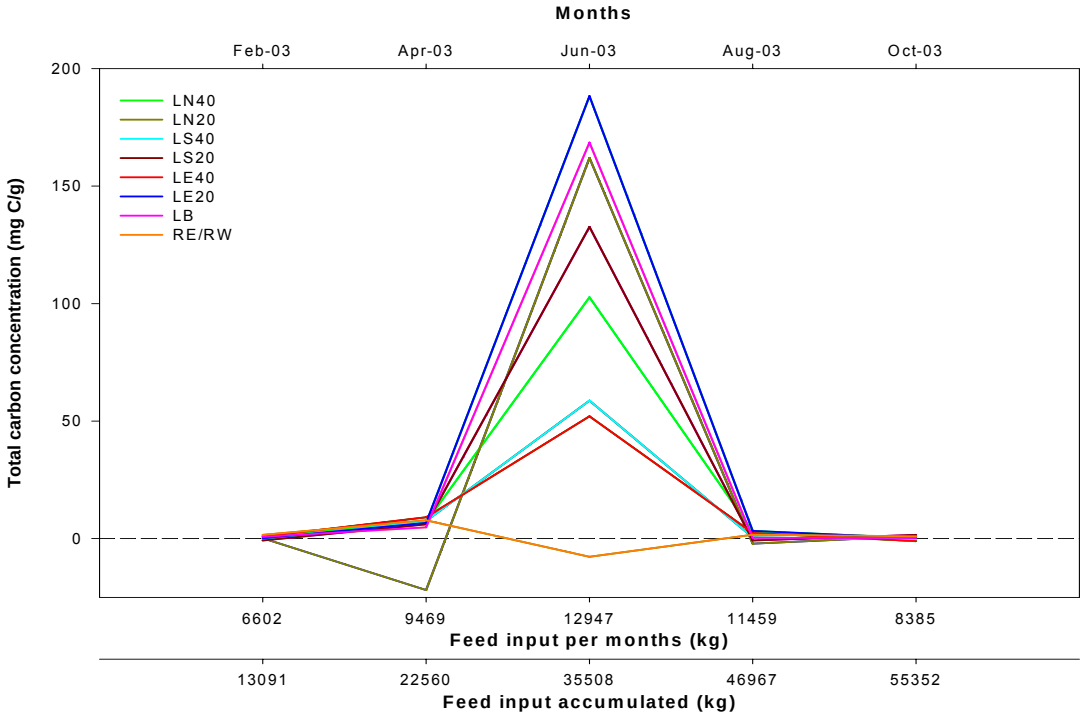

Figure 21.Temporal variation of TC concentration per sample site, and control site in *L. analis* cage minus value concentration in control site.

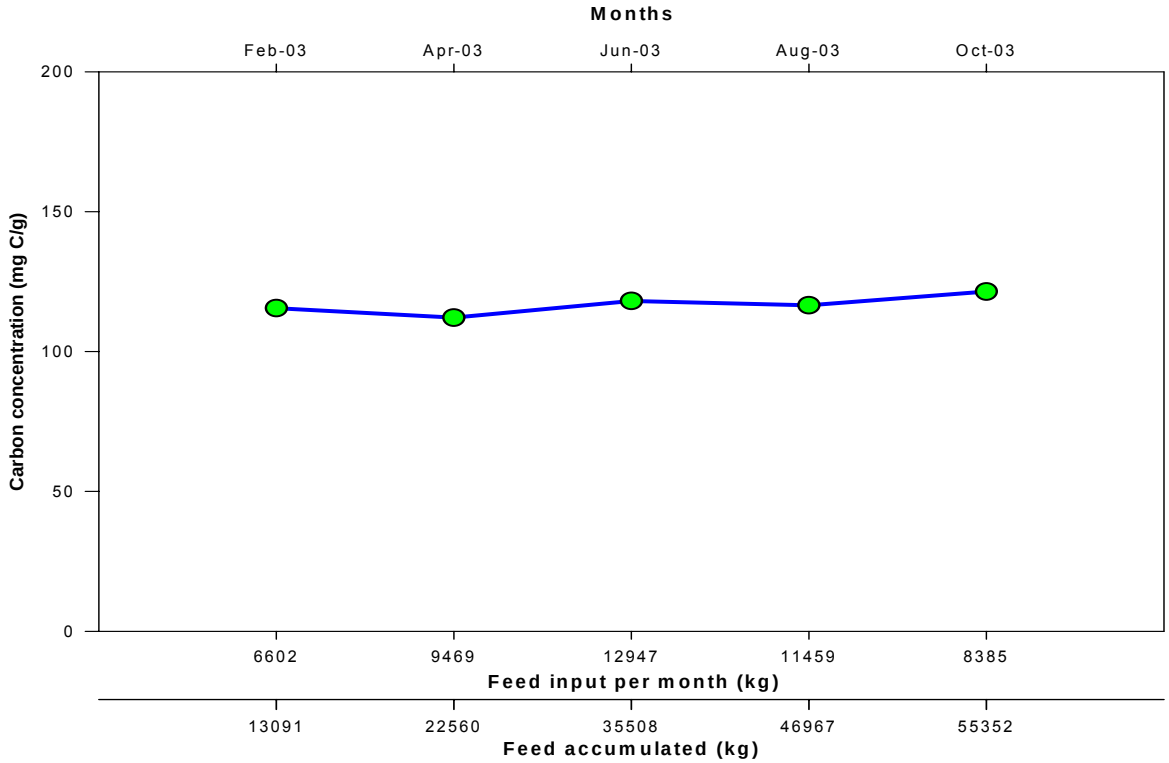

Figure 22. Temporal variation of TC concentration in control site.

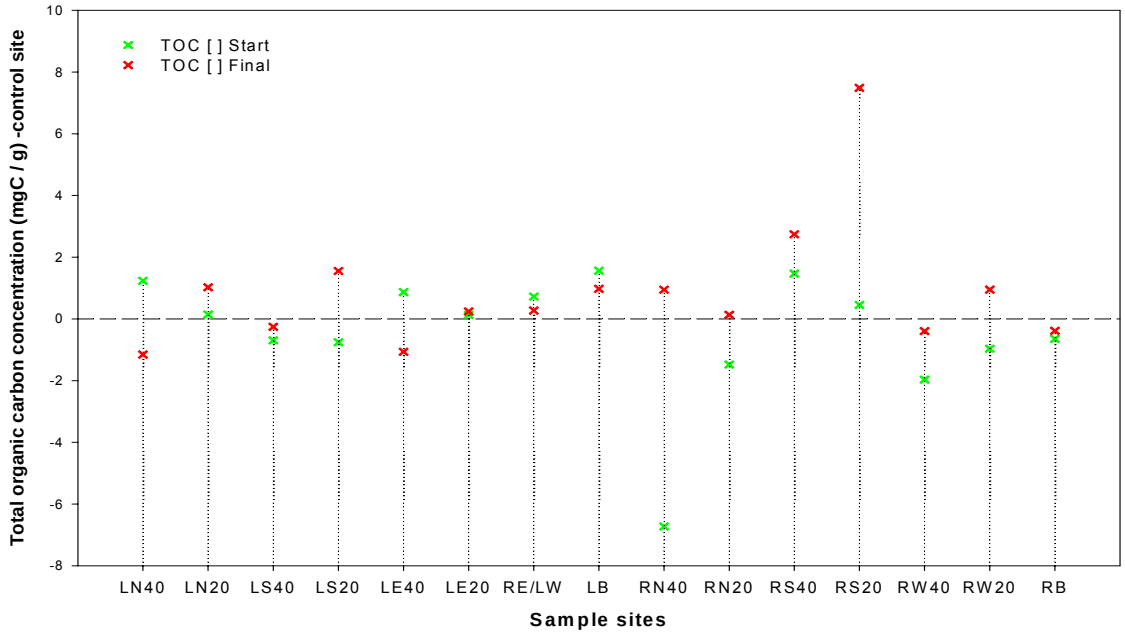

Figure 23. Variation of mean TOC concentrations for each sample site by comparing initial data (start-February 2003) with final data (final-October 2003).

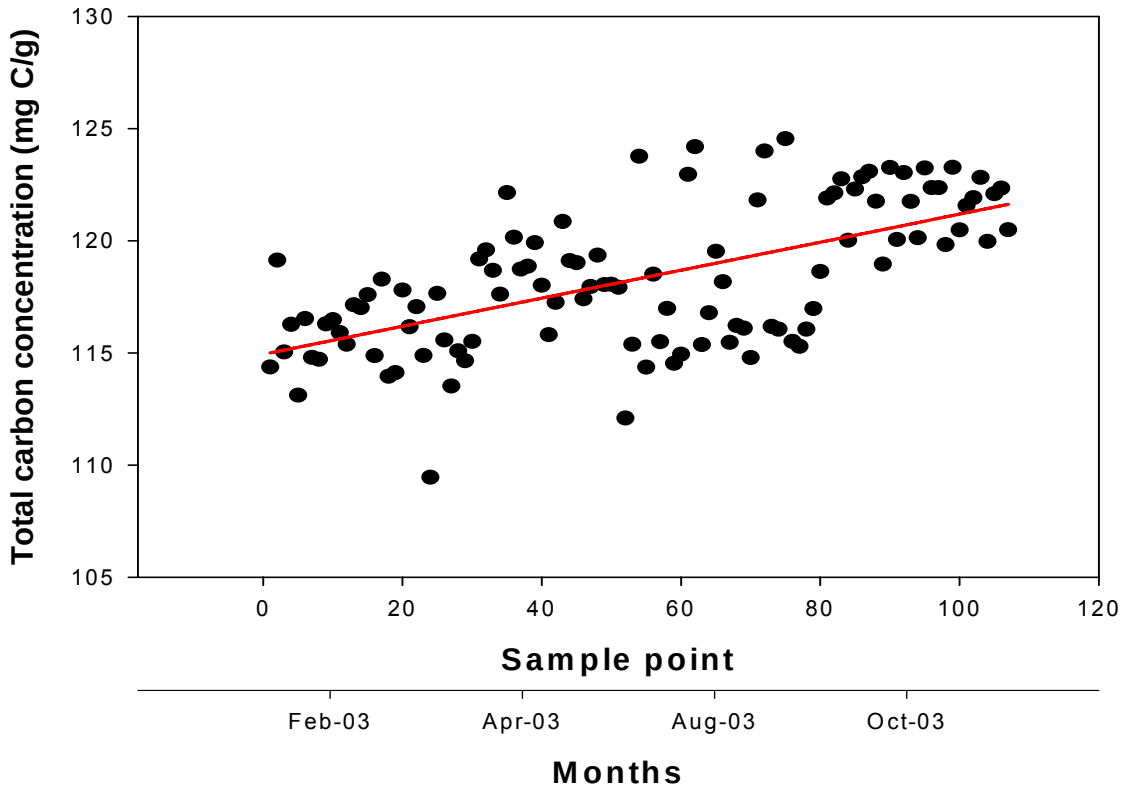

Figure 24. Temporal variation of TC concentrations not including June 2003.

ORGANIC MATTER CONTENT

The Krustal Wallis ANOVA test indicated no significant differences for sediment OM content among cages or control site with a p-value of 0.5211 (Appendix 4). Figure 25 indicated that the *R. canadum* cage presents the highest OM content with a mean value of 5.02% ± 1.26 SD, followed by the control site with 4.96% ± 1.10 DS and *L. analis* cage with 4.74% ± 1.08 SD.

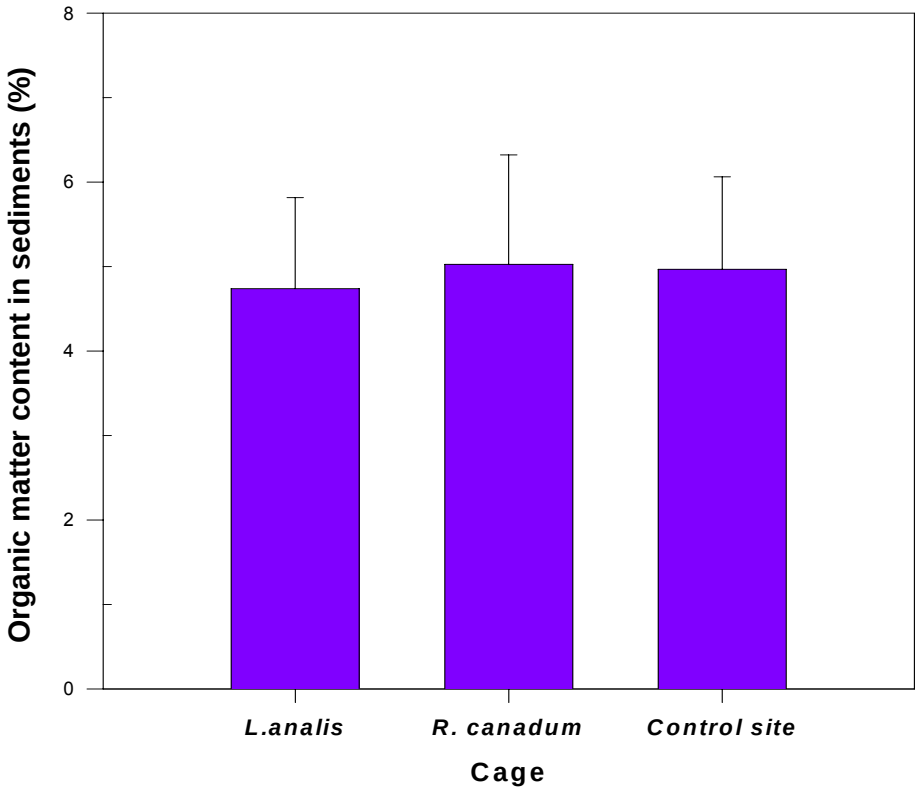

Figure 25. Variation of percentage OM content in the *L. analis* and *R. canadum* cages and for the control site.

The Krustal Wallis ANOVA test (Table 9) indicated significant differences with a p-value of <0.0001 for OM content among months. Dunn's pair-wise comparison for OM contents established differences with the formation of five groups (Table 10). Figure 26 shows the first grouping containing three months, February 2003 (4.1% ± 0.05 SD), April 2003 (4.5% ± 0.10 SD), and June 2003 (4.5% ± 0.07 SD); the next grouping indicates similarities among several months with intermediate organic matter content, April 2003 (4.5% ± 0.10 SD), June 2003 (4.5% ± 0.07 SD), and December 2003 (4.7% ± 0.09 SD). The third grouping included June 2003 (4.5% ± 0.07 SD), December 2003 (4.5% ± 0.09 SD), August 2003 (4.6% ± 0.03 SD), and October 2002 (5.3% ± 0.09 SD). The grouping with the highest concentration contained two months, October 2002 (5.3% ± 0.09 SD), and October 2003 (6.3% ± 0.10 SD).

Table 9. Krustal Wallis ANOVA for OM content in sediments among months. Values with * represent significant difference (P≤0.05).

Months	N	Mean	Std. Dev.	Median	D F	H	P-value
February-03	31	4.1	0.05	3.89	6	66.70	<0.0001*
June-03	30	4.5	0.07	0.62			
April-03	31	4.5	0.10	4.13			
August-03	28	4.6	0.03	4.53			
December-02	26	4.7	0.09	4.22			
October-02	19	5.3	0.09	4.85			
October-03	26	6.3	0.10	6.29			

Table 10. Dunn's pairs comparison for OM content in sediments among months. Different letters represent significant difference ($p \leq 0.05$).

Month	Ranks					
February-03	54.1	A				
April-03	75.1	A	B			
June-03	81.8	A	B	C		
December-02	88.4		B	C		
August-03	106.7			C	D	
October-02	129.0				D	E
October-03	159.4					E

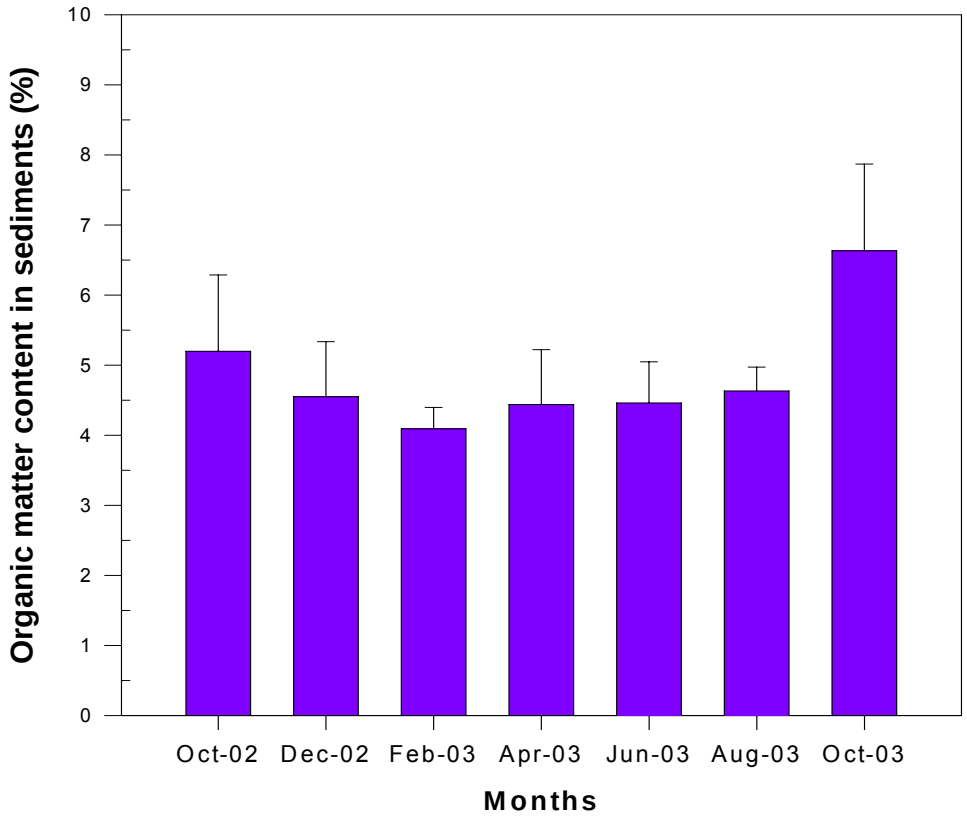

Figure 26.Temporal variation of organic matter content (%) in sediments during the study period.

The Krustal Wallis ANOVA test indicated no significant differences for sediment OM content among sampling sites with a p-value of 0.7072 (Appendix 5). Figures 41, Appendix 14 and Figure 28 show the tendency of OM content in each sample site for the *R. canadum* cage by subtracting the respective control site mean concentration from each *R. canadum* value. The sediment OM content was highest in October 2002 and 2003. One sample site had the maximum OM content in October 2002 (RS20 with value of 5.9%); one in June 2003 (RS40 with 4.5%); six in October 2003 (RN40 with 7.5%; RN20 with 7.5%; RW40 with 6.7%; RW20 with 6.6%; RB with 6.4%; and one at the control site with 4.7%).

Appendix 15 and Figure 27 show the tendency of OM content for each sample site for the *L. analis* cage by subtracting the respective control site mean concentration from each *L. analis* value. The OM content in sediments was highest in October 2002 and 2003. One sample site had the highest OM content in June 2003, (RE/LW with 4.65%); six samples sites in October 2003 (LN40 with 7.08%; LN20 with 6.54%; LS20 with 9.69%; LE40 with 6.11%; LE20 with 5.73%; LB with 6.69%; and control site with 4.65%); and one for December 2003 (LS40 with 6.01%).

The mean OM concentration at the control site (Figure 29) did not fluctuate much during the study period, yet during June 2003 the values decreased and during October 2003 the values increased. Assuming the control site was unaffected by the mariculture activities, changes in OM concentrations probably were due to environmental or seasonal fluctuations. However, when such variations were subtracted from the respective values of each sample site, the peak in October was

still present and the mean values for that month in most of the sample sites were still higher than the control value; however, the tendency for increase in OM concentration was still observed. In all sample sites, a comparison of “start” (October 2002) and “final” (October 2003) were graphically evaluated to determine the spatial pattern of OM content. Values of OM content were higher for the October at sample sites LN40, LN20, LS20, LB, and RW40 (Figure 30).

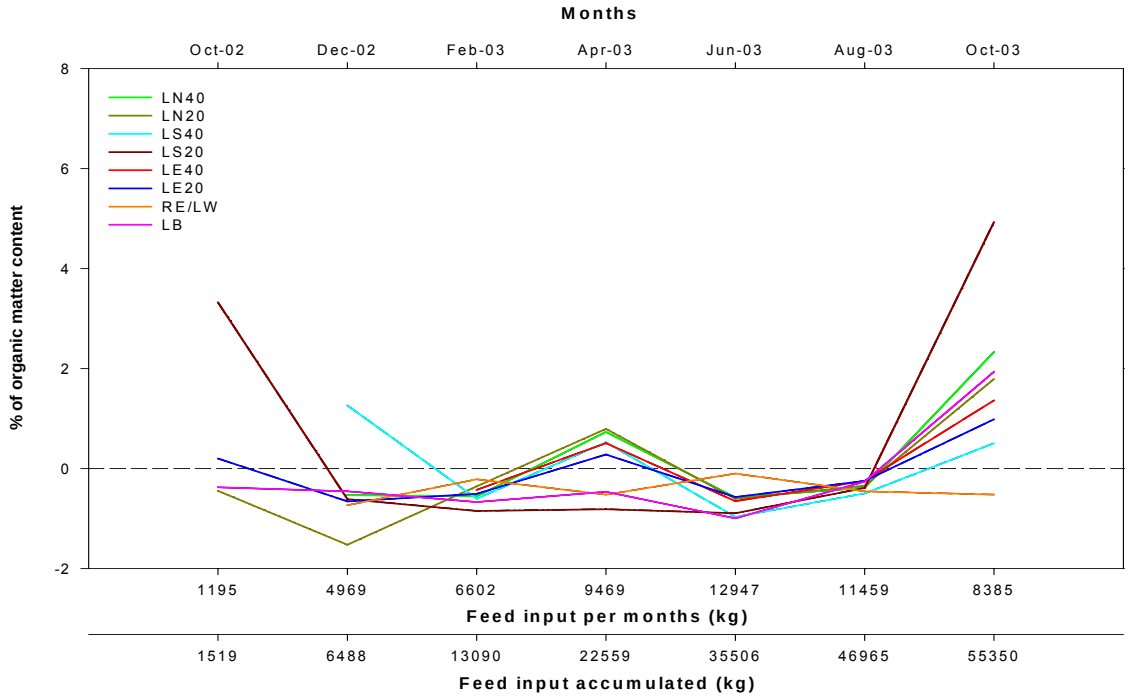

Figure 27. Temporal variation of percentage of organic matter minus control site per sampling site in *R. canadum* cage.

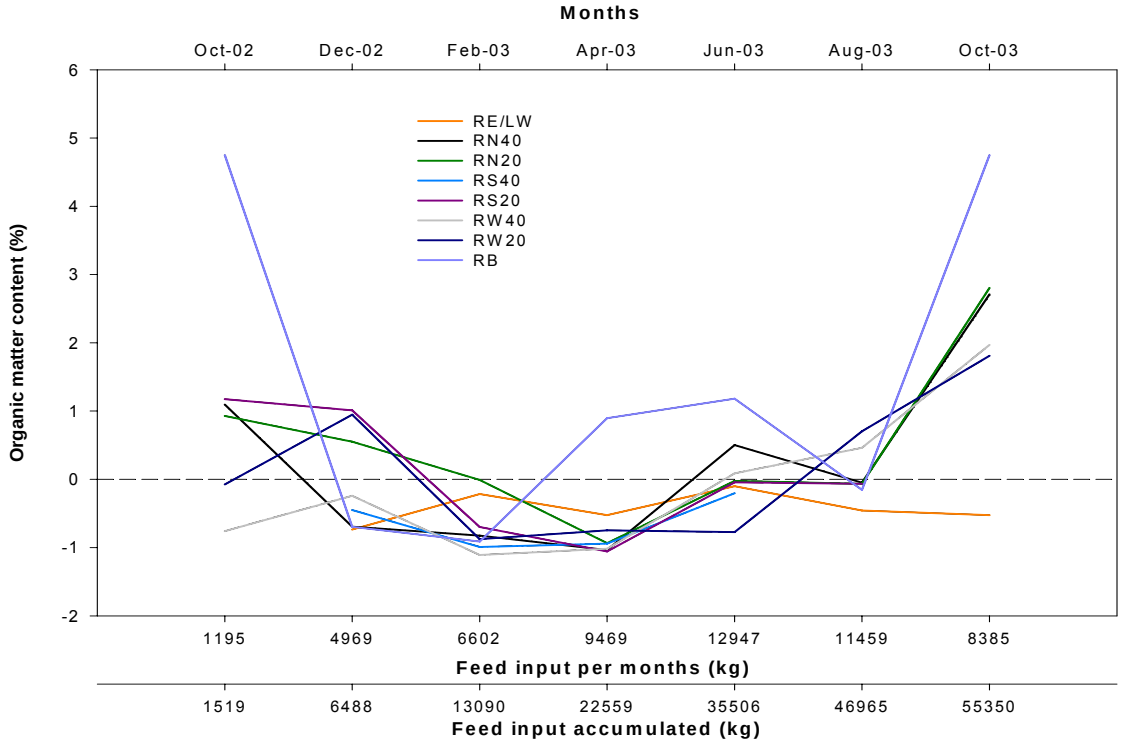

Figure 28. Temporal variation of percentage of organic matter minus control site per sampling site in *L. analis* cage.

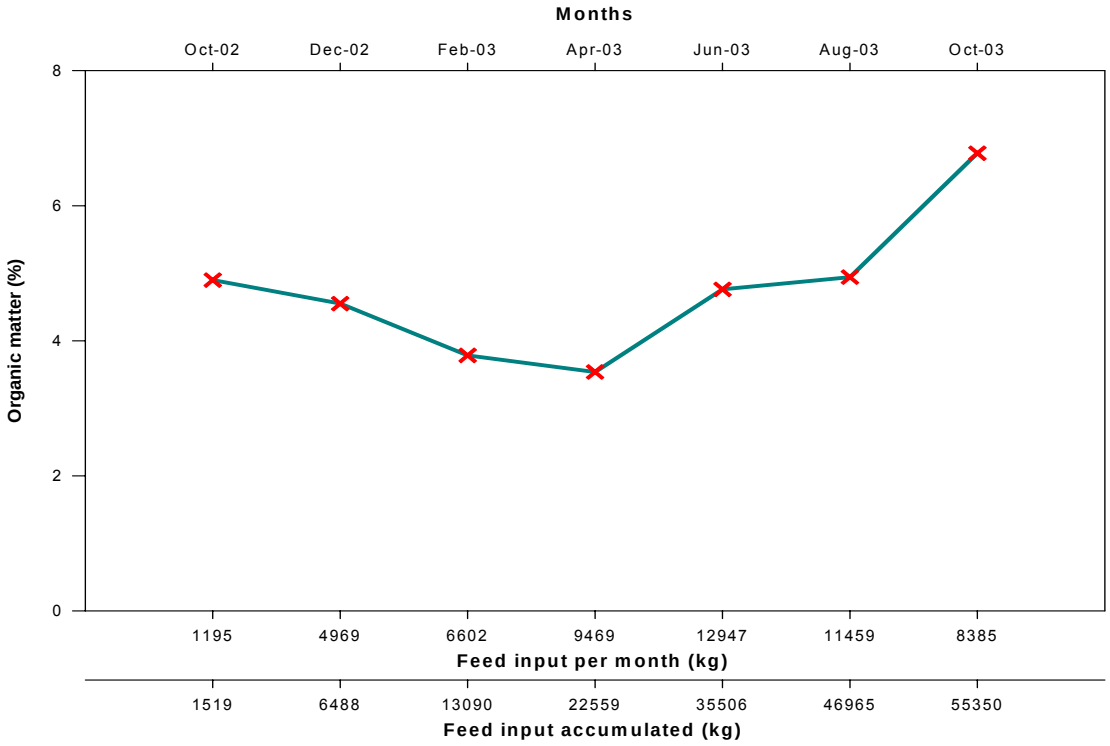

Figure 29. Temporal variation of percentage of organic matter minus control site per sampling site in *L. analis* cage.

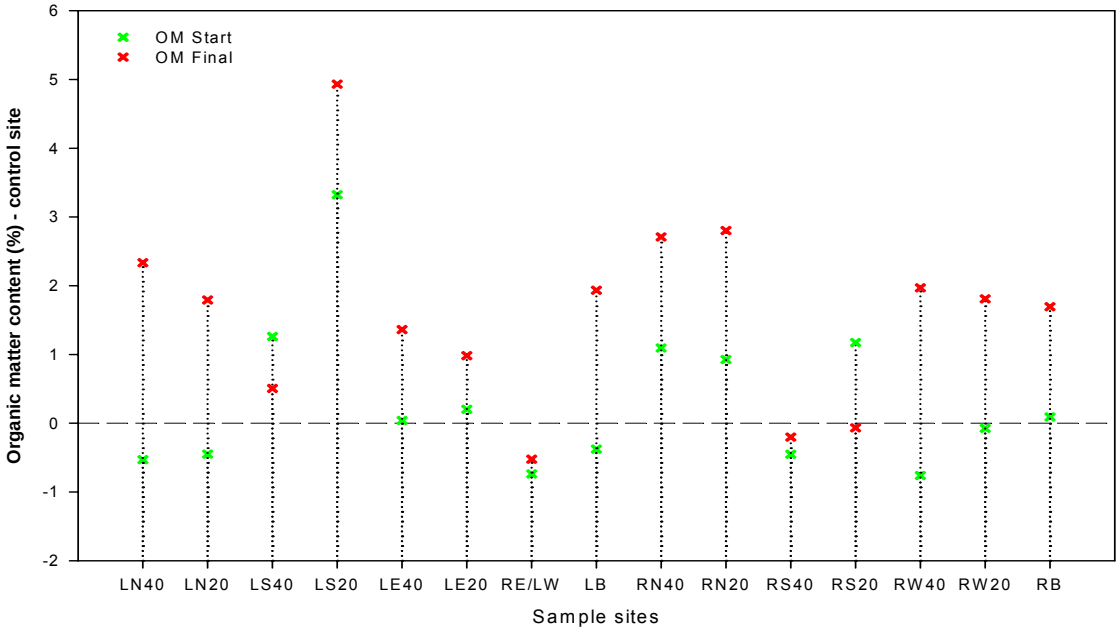

Figure 30. Variation of mean OM content for each sample site by comparing initial data (start-October 2002) with final data (final-October 2003).

PROPORTIONS OF TOTAL ORGANIC NITROGEN AND TOTAL ORGANIC CARBON CONTENT IN SEDIMENT ORGANIC MATTER

The test of Krustal Wallis ANOVA indicated no differences for proportions of TON and TC in organic matter among cages or control site with a p-value of p=0.1155 and p= 0.5070 respectively (Appendix 6 and 7). Figure 31 indicates the sequential patterns of proportion TON and TC in organic matter for the *R. canadum* cage, *L. analis* cage, and control site. The highest percentage of TON and TC in sediment OM content occurred in the *L. analis* cage with mean values of 0.02% N ± 0.009 SD and 6.52% C ± 2.21 SD respectively; *R. canadum* cage with 0.018% N± 0.008 SD and 6.42% C ± 2.56 SD respectively; and control site with 0.01% N± 0.004 SD and 5.49% C ± 1.67 SD respectively.

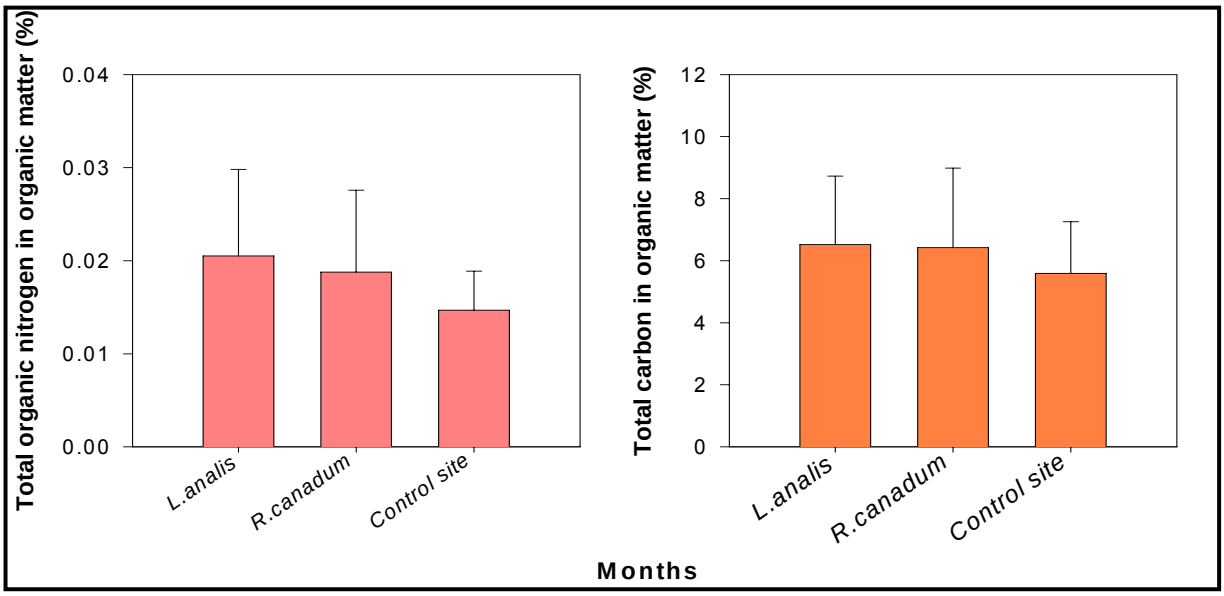

Figure 31. Variation of percentage TON and TC in organic matter sediments of *L. analis*, *R. canadum* cages and control site.

The Krustal Wallis ANOVA test (Table 11) indicates highly significant differences with a p-value of $p<0.0001$ for proportions of TON in sediment OM among months. Dunn's pairs-wise comparison for percentage of TON in sediments OM contents established differences with the formation of two groups (Table 12). Figure 32 indicates the first grouping contained six months (October 2002 with a mean value of 0.015% N ± 0.004 SD, February 2003 with 0.015% N ± 0.002 SD, April 2003 with 0.020% N ± 0.011 SD, December 2002 with 0.016% N ± 0.004 SD, August 2003 with 0.018% N ± 0.010 SD, and October 2003 with 0.018% N ± 0.007 SD); and one for June-03 (0.03% N ± 0.007 SD).

Table 11.. Krustal Wallis ANOVA for TON proportions (%) in sediment organic matter among months. Values with * represent significant difference ($P \leq 0.05$).

Months	N	Mean	Std. Dev.	Median	D F	H	P-value
April-03	16	0.019	0.011	0.015	6	25.50	<0.0001*
August-03	15	0.018	0.010	0.016			
December-02	13	0.016	0.004	0.015			
February-03	16	0.015	0.002	0.015			
June-03	16	0.029	0.007	0.027			
October-02	12	0.015	0.004	0.027			
October-03	14	0.018	0.008	0.013			

Table 12. Dunn's pairs-wise comparison for proportions of TON in sediment organic matter among months. Different letters represent significant difference (p≤0.05).

Month	Ranks		
October-03	37.3	A	
February-03	40.9	A	
April-03	45.0	A	
December-03	45.1	A	
August-03	50.4	A	
October-02	56.8	A	
June-03	83.3		B

The Krustal Wallis ANOVA test indicated significant differences with a p-value of $p<0.0001$ for proportions TC in sediment organic matter among months (Table 13). Dunn's pair-wise comparison for OM contents established differences with the formation of three groups (Table 14). Figure 32 shows the first grouping contained two months, February 2003 (4.7% C ± 0.38 SD) and April 2003 (5.1% C ± 0.96 SD); the next grouping with April 2003 (5.1% C ± 0.96 SD) and August 2003 (5.5% C ± 0.48 SD). The grouping with the highest percentage TC in OM contained two months, June 2003 (8.9% C ± 3.03 SD) and October 2003 (8.1% C ± 1.53 SD).

Table 13. Krustal Wallis ANOVA test for proportion of TC (%) in sediment organic matter among months. Values with * represent significant difference (P≤0.05).

Months	N	Mean	Std. Dev.	Median	D F	H	P-value
April-03	16	5.1	0.96	4.87	4	47.81	<0.0001*
August-03	15	5.5	0.48	5.36			
February-03	16	4.7	0.38	4.72			
June-03	16	8.9	3.03	9.27			
October-03	14	8.1	1.53	8.08			

Table 14. Dunn's pairs-wise comparison for TON (%) in sediment organic matter among months. Different letters represent significant difference (p≤0.05).

Month	Ranks			
February-03	16.7	A		
April-03	24.8	A	B	
August-03	36.8		B	
June-03	59.3			C
October-03	59.8			C

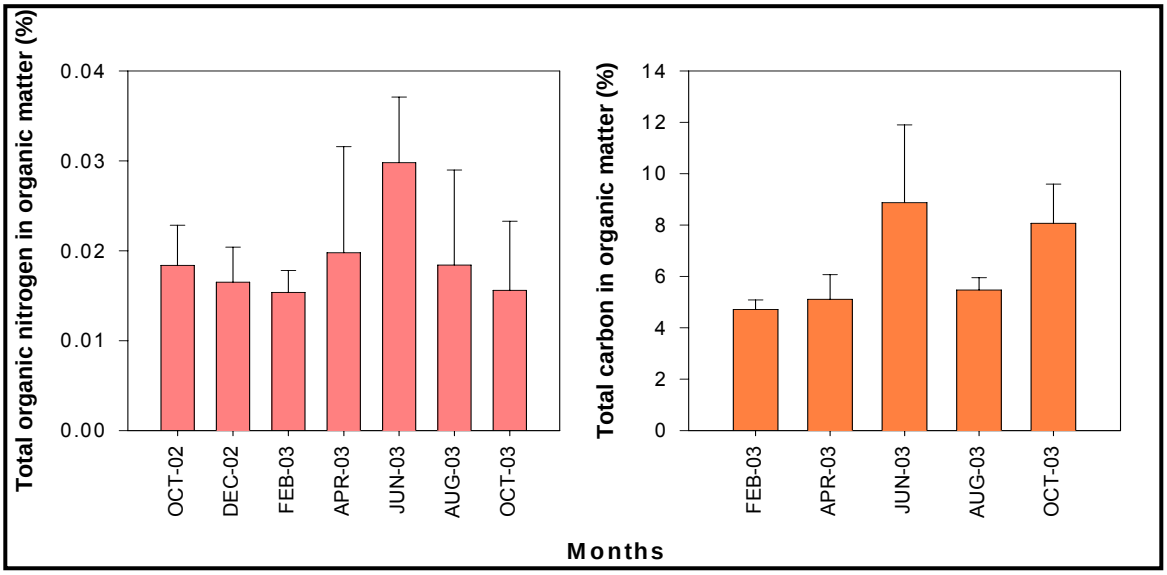

Figure 32. Temporal variation of percentage TON and TC in sediment organic matter *L. analis* cage, *R. canadum* cage and control site.

The Krustal Wallis ANOVA test indicated no significant differences for proportions of TON and TC in sediment organic matter among sampling sites with a p-value of 0.7363 and 0.9593, respectively (Appendix 8 and Appendix 9). Appendix 16, and Figure 33 indicates the proportions of TON in organic matter for the *R. canadum* cage by subtracting the respective control site mean concentrations from each *R. canadum* value. Six sampling sites presented the highest percentage TON in organic matter in June 2003 (RN40 with value of 0.012% N, RN20 with 0.023% N, RS40 with 0.012% N, RW40 with 0.031% N, RB with 0.022% N, and the control site with 0.022% N); and two sites in August 2003 (RS20 with 0.021% N and RW20 with 0.01% N).

Appendix 17, and Figure 34 indicates the pattern of proportions of TON in organic matter for the *L. analis* cage by subtracting the respective control site mean concentrations from each *L. analis* value. Two samples sites presented the highest percentage TON in organic matter in April 2003 (LS40 with value of 0.016% N; LE40 with 0.044% N); five samples sites in June 2003 (LN40 with 0.013% N; LN20 with 0.024% N; LE20 with 0.024% N; LB with 0.016% N; and RE/LW with 0.008% N); and one sample site in October 2003 (LS20 with 0.020% N).

Appendix 18, and Figure 35 indicates the tendency of proportion of TC in sediment organic matter for the *R. canadum* cage by subtracting the respective control site mean concentration from each *R. canadum* mean value. The percentages of TC in sediment organic matter were highest in June and October 2003 for the *R. canadum* cage. Four samples sites presented the highest percentage TC in organic matter in June 2003 (RN40 with value of 4.2% C; RN20 with 6.1% C; RS20 with 3.7% C; RW40 with 9.4% C); four samples site occurred in October 2003 (RS40 with of 0.8% C; RW20 with 2.4% C; RB with 2.4% C; and control site with 8.2% C).

Appendix 19, and Figure 36 indicates the tendency of TC in sediment organic matter for the *L. analis* cage by subtracting the respective control site mean concentration from each *L. analis* mean value. One sample site presented highest percentage TC in organic matter in February 2003 (RE/LW with value of 0.3% C); six samples occurred in June 2003 (LN40 with of 3.6% C; LN20 with 6.0% C; LS40 with 1.1% C; LS20 with 4.1% C; LE20 with 7.2% C; LB with 5.2% C); and one sample site in October 2003 (LE40 with 1.8% C).

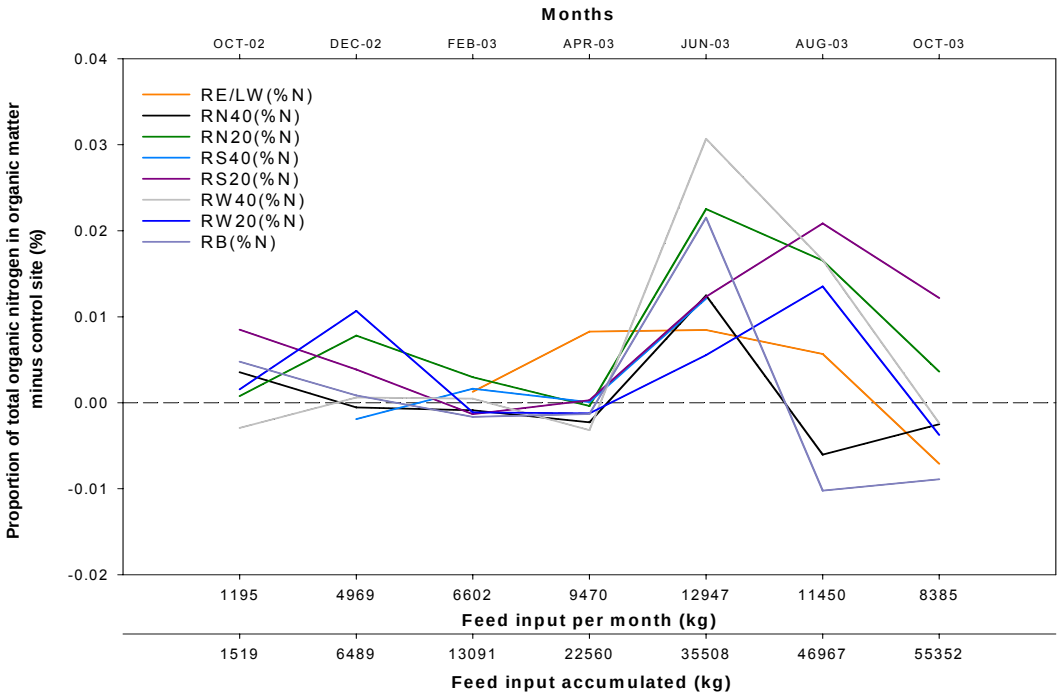

Figure 33.Temporal variation of proportion of TON in organic matter per sampling site in *R. canadum* cage.

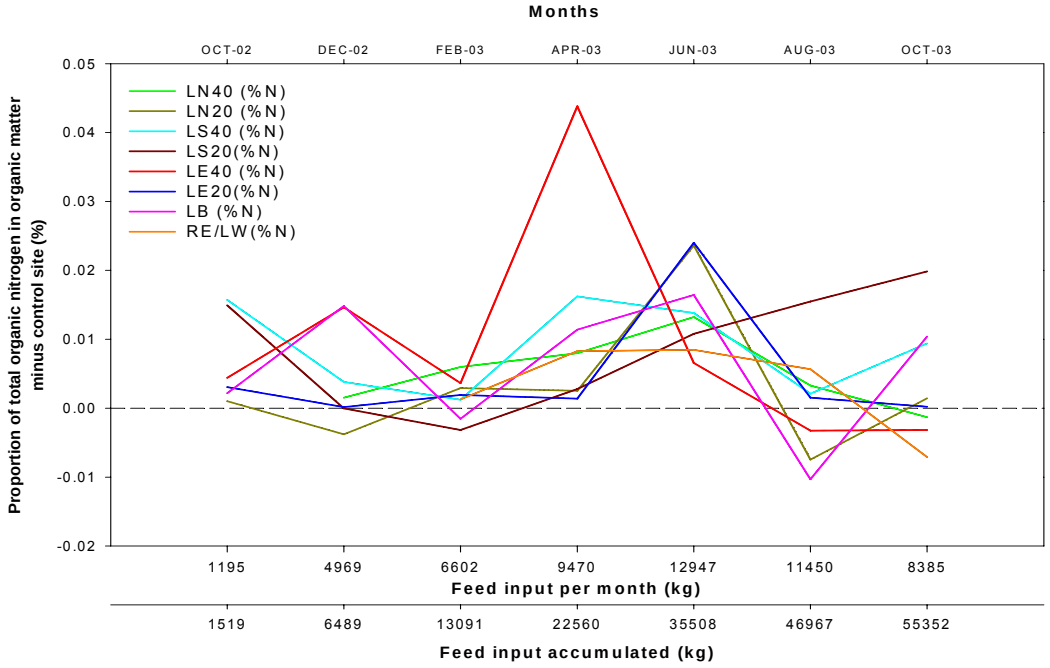

Figure 34. Temporal variation of proportion of TC in organic matter per sampling site in *L. analis* cage.

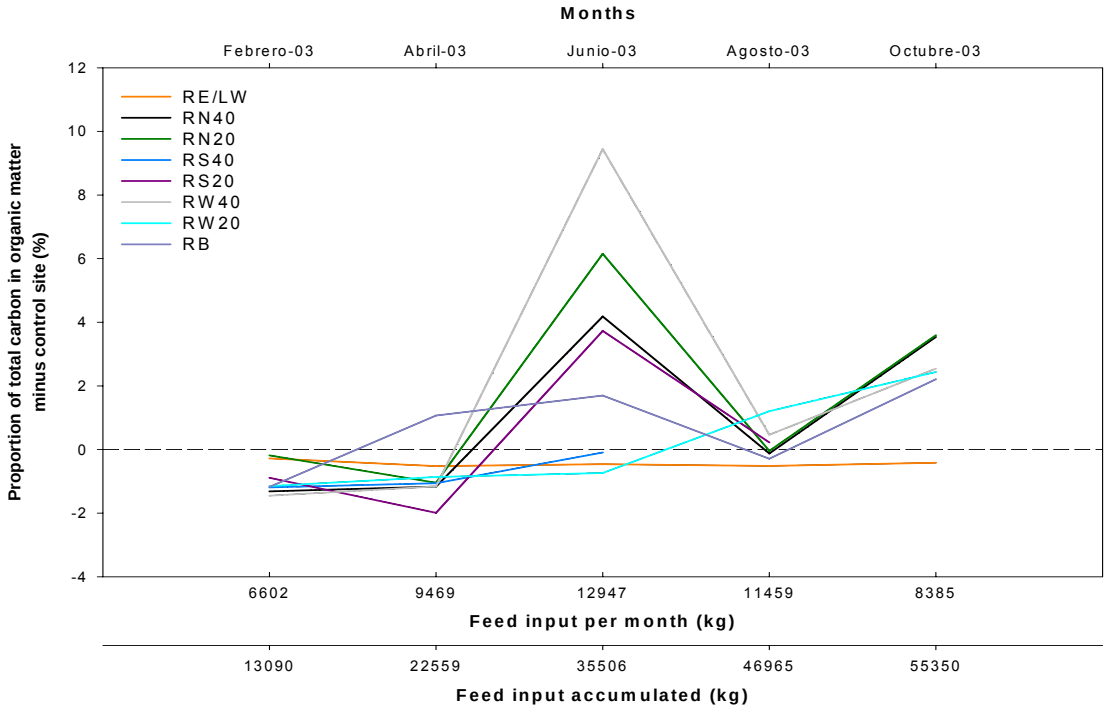

Figure 35. Temporal variation of proportion of TC in organic matter per sampling site in *R. canadum* cage.

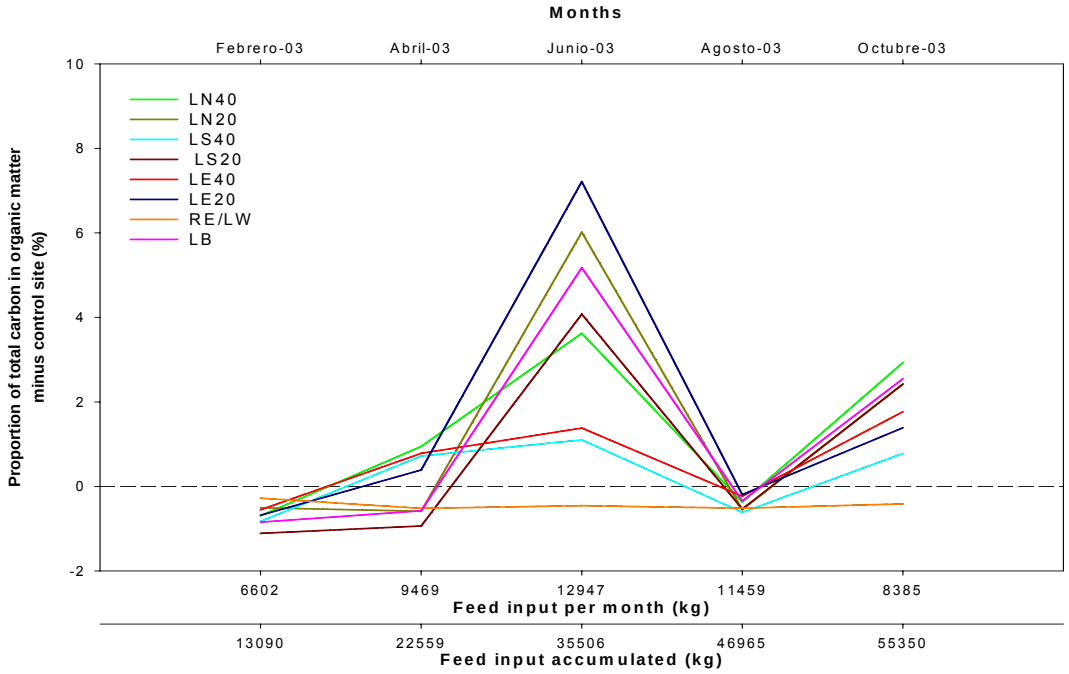

Figure 36.Temporal variation of proportion of TC in organic matter per sampling site in *L. analis* cage.

DISCUSSION

Aquaculture activities have inherent nutrient buildup in the sediment, resulting in the deterioration of ecosystems and the overall water and sediment quality. The intensive open-ocean aquaculture cage industry exacerbates this nutrient accumulation due to intense use of the space, which requires large amounts of feed to maintain and grow fishes. Feeding activities at Snapperfarm are associated with patterns of nutrient accumulation related to the cages (e.g., *L analis* and *R. canadum* cages, compared with the control site).

Spatial patterns developed at the cage and control sites indicate that significant differences of TON concentrations were highest for the *L. analis* cage, followed by the *R. canadum* cage, and then by the control site. TC concentration and TON and TC percentages showed similar trends as TON concentrations; the TON and TC concentrations of these nutrients were low at the control site, but are in agreement with several studies (Johannessen et al. 1994; McGhie et al. 2000; Aguado-Giménez and García-García 2004), indicating no organic enrichment at distances greater than 100 m. Thus, mariculture activity exerts a localized environmental impact.

Even though there were less *L. analis* stocked (3,000) than in the *R. canadum* cage, reports by divers indicated differences in feeding aggression between the two species. This probably accounted for the *L. analis* cage having higher nutrient levels because the *L. analis* were much less aggressive feeders, thus allowing uneaten pellets to fall though the nets. While the *L. analis* were still being cultured, an additional stocking was made with fingerling cobia within a nursery net placed within

the cage during June 2003. The consequent higher accumulation of nutrients below the *L. analis* cage was probably due to the species-specific utilization of the feed. This supports the assumption that resource utilization by species with dissimilar biological attributes would result in distinct feeding patterns, leading to different accumulation patterns of sediment nutrients.

Apart from spatial differences, among month comparisons of nutrient concentrations were significant. TC and TON concentrations, and percentages of TC and TON in organic matter were highest during the same period, with peaks in June 2003. Similarly, feed input accounted for 61% (~33,500 kg) of the total feed input from April to August 2003, with the highest feeding rate occurring during June 2003 with 12,947 kg. The increase in sediment nutrients (from April to August), apart from being related to feeding activities, is also concurrent with seasonal changes in the water column. The changes were due to freshwater inputs from the Amazon plume, which are rich in particulate organic matter (Corredor et al. 1999). Massive freshwater inputs enter the eastern Caribbean from precipitation and continental runoff, especially from the Amazon and Orinoco rivers. Peak flows of the Orinoco occur during August and September, while the Amazon peaks during May and June. Mean Orinoco River flow is 20% of the Amazon River flow, and together these rivers account for over 25% of the global riverine discharge (Capella et al. 1997). This transfer of particulate matter, including organic detritus and silicates, reflects the oligotrophic conditions in the water column. Over 80% of the organic matter is oxidized before reaching the bottom, which results in the low organic flux to the seafloor (Escobar-Briones 2004), so there is probably little effect from the Orinoco and Amazonas Rivers.

However, the results of this study support better *in situ* nutrient enrichment due to culture activities than from inputs from the Amazon River. Figures 12, 13, 18, 19, 30, 31, 34, and 35, show the net nutrient concentration (*in situ* mean values minus the mean control value). Assuming seasonal changes homogeneously affected the cage and control sites, by subtracting the mean values found at the control site, the resulting values indicate monthly increases in nutrients at the cage site due to feeding activities. The results indicated a two-fold net nutrient increase. Also, increased nutrient concentrations may have been enhanced via excretory activities as the fish increased in size.

The October 2003 organic matter content increase was correlated with fish number, size, and seasonal environmental variations (e.g. wind, Orinoco River discharges). Seasonal variation was accounted by subtracting the organic matter values of the control site from the value of the cage site. Therefore the remaining concentrations are assumed to be due to Snapperfarm activities. By October 2003, the remaining fish were still being harvested, but the standing crop was still high (about 6,000 fish) and the feed input, even though it was reduced from 12,947 kg in June 2003 to 8,385 kg in October, feeding activities was substantial at the cage site. A total of 1,497 fish were harvested from July 2003 to October 2003, each with a mean biomass of 5.45 kg (O'Hanlon personal communication). In October 2003, approximately 6,000 adult fishes remained in the cages (until January 2004). Thus the biomass remaining in the cages was still substantial as of October 2003, even though harvesting had begun during the previous July. The approximate amount of feed fed to individual fish was 1.73 kg in June and had been reduced to about 1.45 kg in October. Therefore wastes

continued to accumulate beneath the cages. The percentage protein in the feed remained high throughout the entire period, even though larger fish may not have the same protein requirements as younger fish. Feeding behavior also plays a role in the amount of nutrients lost to the environment. For instance, divers noted that *R. canadum* were more aggressive feeders than *L. analis*, thus resulting in more feed loss in the *L. analis* cage.

This aggressive feeding behavior enhances inefficacy in feeding resulting in suspended detritus composed of uneaten feed and fecal materials which settles on the seabed under the cages (Mazzola and Sara 2001; Aguado-Giménez and García-García 2004; Boyra et al. 2004). Several other studies reported increased ammonia and nitrite concentrations when sampling the water column above, at the same level, or below submerged cages. This suggests that some food particles were not consumed or completely dissolved (Farías 2003; Mejia Niño 2005). In this case, such wastes as feed particles, leached nutrients, and excretory products would exit near the middle or bottom of the cage.

Apart from increased nutrients in the sediments, some authors (Carroll et al. 2003; Pereira et al. 2004) suggest that the sediment community could be a more sensitive indicator than measuring chemical properties of the sediments. In a sister-study in the same cage and control site at the Snapperfarm operation, Morales-Nuñez (2005) reported an increased in the Tanaidaceae (Crustacea) abundances with a decrease in overall diversity during June 2003. Deterioration of sediment quality may have been involved in changes in the meiofaunal community. Although common pollution-tolerant Polychaeta (Annelida) were not found in high numbers (i.e., *Capitella*

capitata), the decrease in species richness may suggest degradation of the natural habitat. Brown et al. (1987) indicated that diversity of benthic animals served as a good indicator for organic pollution because diversity was reduced when the organisms were subjected to organic pollution derived from marine fish farming.

The overall accumulation of nitrogen, carbon and organic matter in the sediment found in this study may influence the microbial processes, which modulate eutrophication of nearshore marine environments (Mosquera et al. 1998). These costal systems like the one in Culebra are susceptible to nitrogen input as microbial communities are limited by the vulnerability of the nitrification process (Mosquera et al. 1998; Corredor et al. 1999). Besides, organic matter accretion in carbonate sediments provokes phosphorus immobilization by the sediment. By breaking depurative mechanisms for either phosphorus or nitrogen, algae growth is enhanced as a result of nutrient accumulation because these nutrients are no longer limiting (Corredor et al. 1999). This eutrophication in tropical marine systems is problematic given their limited capacity for depuration of excess nitrogen (Mosquera et al. 1998). In a case in Southwest Puerto Rico, Mosquera et al. (1998), showed input of heavy organic loads in nearshore sediments. The benthic fluxes of dissolved organic nitrogen suggest that these sediments act as a sink for organic nitrogen and that ammonification is a key process in the release of nitrogen from sediments to the water column with low nitrification and denitrification rates (Escobar-Briones 2004).

An alternative way to decrease such accumulation and break chemical process associated with microbial communities in the sediments is by the reduction of excess nutrients as a result of feeding activities by wild fish attracted to the cage site. The

physical structure of the cages also served as fish aggregating devices (FADS); this fact was corroborated at the same Snapperfarm cage site. Alston et al. (2005), reported wild fish of the family Carangidae associated with the aquaculture system. Wild fish and other associated organisms gather near cages and fed on wastes (i.e., uneaten feed and fecal materials) emanating from the cage, therefore decreasing or diluting the amount of nutrients entering nearby environments. Earlier reports indicated that wild fish populations can consumed up to 40% to 60% of the cage-derived nutrients, resulting in an important nutrient removal or redistribution in certain marine environments (Felsing et al. 2005; Fernandez-Jover et al. 2007). Fernandez-Jovier et al. (2007) reported up to 80% reduction in total organic wastes by wild fish populations at one Mediterranean farm. Therefore, wild fish associated with cage culture sites may decrease the benthic environmental impacts of particulate aquaculture wastes. However, introducing nitrogen and carbon through defecation and excretion into the pelagic environment disperses nutrients over a wide area, thereby possibly resulting in ecological impact of other ecosystems distant from the aquaculture site.

In summary, despite the short duration of aquaculture during the first culture cycle and even though currents averaged 17 cm/s, TON, TC, and OM concentrations increased in the sediments. This buildup of nutrients was primarily due to wastes related to the Snapperfarm activities. Determining a clear cause and effect of the mariculture activity was not possible because there were no previous baseline studies for comparison. However, after one year of sampling, overall nutrient values were lower than the concentrations found in other studies, nonetheless, some considerations apply. First, most of the studies are performed in established

aquaculture systems after long culture periods of 3 to 7-yr where the accumulation of sediment nutrients and organic matter were greater. Second, mariculture activities have been traditionally associated with enclosed bays with restricted water flow and enhanced nutrient accumulations. Third, comparing tropical with temperate systems may be inappropriate because each area has its unique characteristics. For example, nitrification in tropical marine system may be more severely constrain by relatively low oxygen concentration in the warm water (Downing et al. 1999). Also, tropical areas are generally oligotrophic and its ecosystems (i.e., coral reefs) and nitrification-denitrification coupling are more susceptible to nutrient loading (Mosquera et al. 1998).

Finally, I suggest that new aquaculture systems in areas similar to this study consider implementing polyculture (i.e. algae, mollusk, polychaetes) to serve as buffers to ameliorate nutrient loading. Also, the results of this study are based on two cages with a specific number of stocked fish (approximately 8,000) with a specific feeding regimen. Therefore, if management techniques or the size of the operation increases, the subsequent larger-scale system would have to be closely monitored. For instance if stocking or feeding rates increased or changed (e.g. increased stocking rates, change in fish species, feeding with trash fish), nutrient accumulation would also increase.

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APPENDICES

Appendix 1. Table 15. Krustal Wallis ANOVA for TON concentration in sediments among sampling sites. Values with * represent significant difference (P<0.05).

Station	n	Mean	Std. Dev.	Median	H	P - value
Control	10	0.30	0.10	0.30	13.95	0.5296
LB	11	0.47	0.28	0.32		
LE20	14	0.43	0.22	0.36		
LE40	11	0.49	0.36	0.40		
LN20	11	0.38	0.20	0.34		
LN40	10	0.43	0.24	0.39		
LS20	12	0.47	0.24	0.42		
LS40	11	0.47	0.21	0.41		
RB	12	0.31	0.19	0.32		
RE/LW	9	0.39	0.14	0.46		
RN20	11	0.44	0.28	0.37		
RN40	14	0.32	0.17	0.30		
RS20	10	0.45	0.21	0.35		
RS40	8	0.43	0.12	0.40		
RW20	12	0.36	0.12	0.37		
RW40	12	0.41	0.29	0.31		

Appendix 2. Table 16. Krustal Wallis ANOVA for TC concentration in sediments among cages. Values with * represent significant difference (P≤0.05).

Cage	n	Mean	Std. Dev	Median	D F	H	P-value
Control	7	117.37	3.37	116.97	2	1.37	0.5049
L. analis	74	139.05	55.67	118.80			
R. canadum	56	128.45	41.01	118.59			

Appendix 3. Table 17. Krustal Wallis ANOVA for TC concentrations in sediments among sampling sites. Values with * represent significant difference (P<0.05).

Station	N	Mean	Std. Dev.	Median	D F	H	P-value
Control	7	117.37	3.37	116.97	15	4.43	0.9960
LB	10	151.66	72.31	118.09			
LE20	10	156.45	79.29	119.46			
LE40	10	129.40	32.96	119.55			
LN20	7	137.26	63.92	116.27			
LN40	9	141.55	66.88	119.19			
LS20	10	144.47	56.88	118.14			
LS40	9	131.02	39.33	118.50			
RB	9	118.45	3.56	117.92			
RE/LW	9	117.31	5.70	117.37			
RN20	8	134.40	46.18	119.47			
RN40	10	130.47	42.56	119.57			
RS20	7	135.27	63.35	117.40			
RS40	5	118.93	2.44	118.89			
RW20	8	119.56	3.85	119.98			
RW40	9	138.83	64.57	117.96			

Appendix 4. Table 18. Krustal Wallis ANOVA for OM content in sediments among cages. Values with * represent significant difference (P≤0.05).

Cage	n	Mean	Std. Dev	Median	D F	H	P-value
Control	11	4.96	1.10	4.89	2	1.30	0.5211
L. analis	99	4.74	1.08	4.36			
R. canadum	81	5.02	1.26	4.55			

Appendix 5. Table 19. Krustal Wallis ANOVA for OM content in sediments among sampling sites. Values with * represent significant difference (P<0.05).

Station	N	Mean	Std. Dev.	Median	D F	H	P-value
Control	11	4.97	1.10	4.89	15	11.62	0.7072
LB	12	4.61	1.02	4.32			
LE20	14	4.68	0.62	4.66			
LE40	11	4.85	0.86	4.69			
LN20	13	5.02	1.19	4.45			
LN40	12	4.92	1.18	4.36			
LS20	13	4.71	1.59	4.24			
LS40	12	4.79	1.21	4.45			
RB	13	5.07	1.62	4.55			
RE/LW	12	4.33	0.81	4.22			
RN20	11	5.21	1.40	4.93			
RN40	13	4.80	1.15	4.69			
RS20	11	4.81	1.37	4.55			
RS40	8	4.10	0.42	3.98			
RW20	13	4.96	1.26	4.67			
RW40	12	4.71	1.15	4.51			

Appendix 6. Table 20. Krustal Wallis ANOVA for % of TON in sediments organic matter among cages. Values with * represent significant difference (P≤0.05).

Cage	n	Mean	Std. Dev	Median	D F	H	P-value
Control	7	0.01	0.01	0.01	2	3.84	0.1155
L. analis	50	0.02	0.01	0.02			
R. canadum	54	0.02	0.01	0.01			

Appendix 7. Table 21. Krustal Wallis ANOVA for % of TC in sediments organic matter among cages. Values with * represent significant difference (P≤0.05).

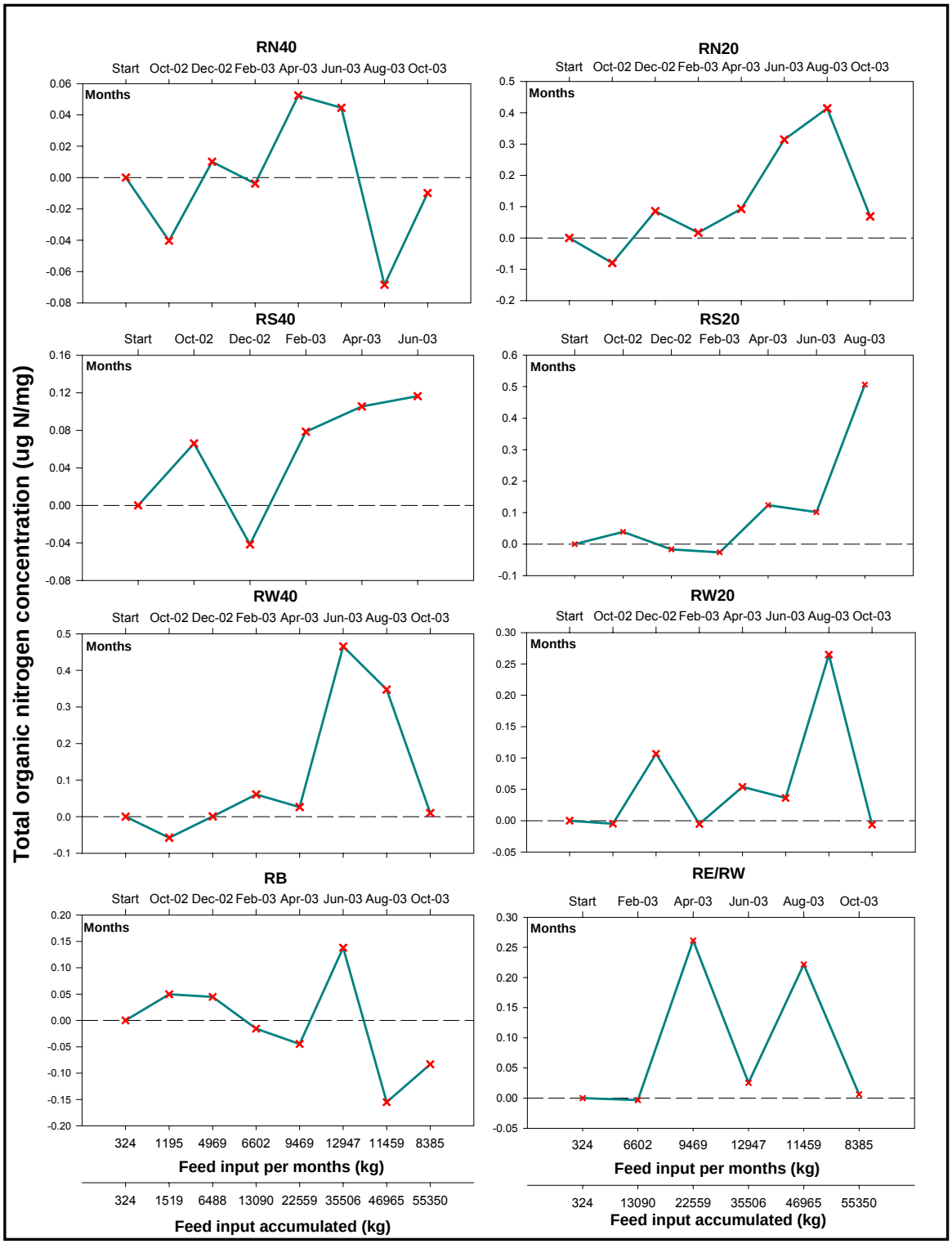
Cage	n	Mean	Std. Dev	Median	D F	H	P-value
Control	5	5.59	1.67	5.52	2	1.36	0.5070
L. analis	40	6.52	2.21	5.34			
R. canadum	32	6.42	2.56	5.48			

Appendix 8. Table 22. Krustal Wallis ANOVA test for % TON in sediments organic matter among sampling sites. Values with * represent significant difference (P<0.05).

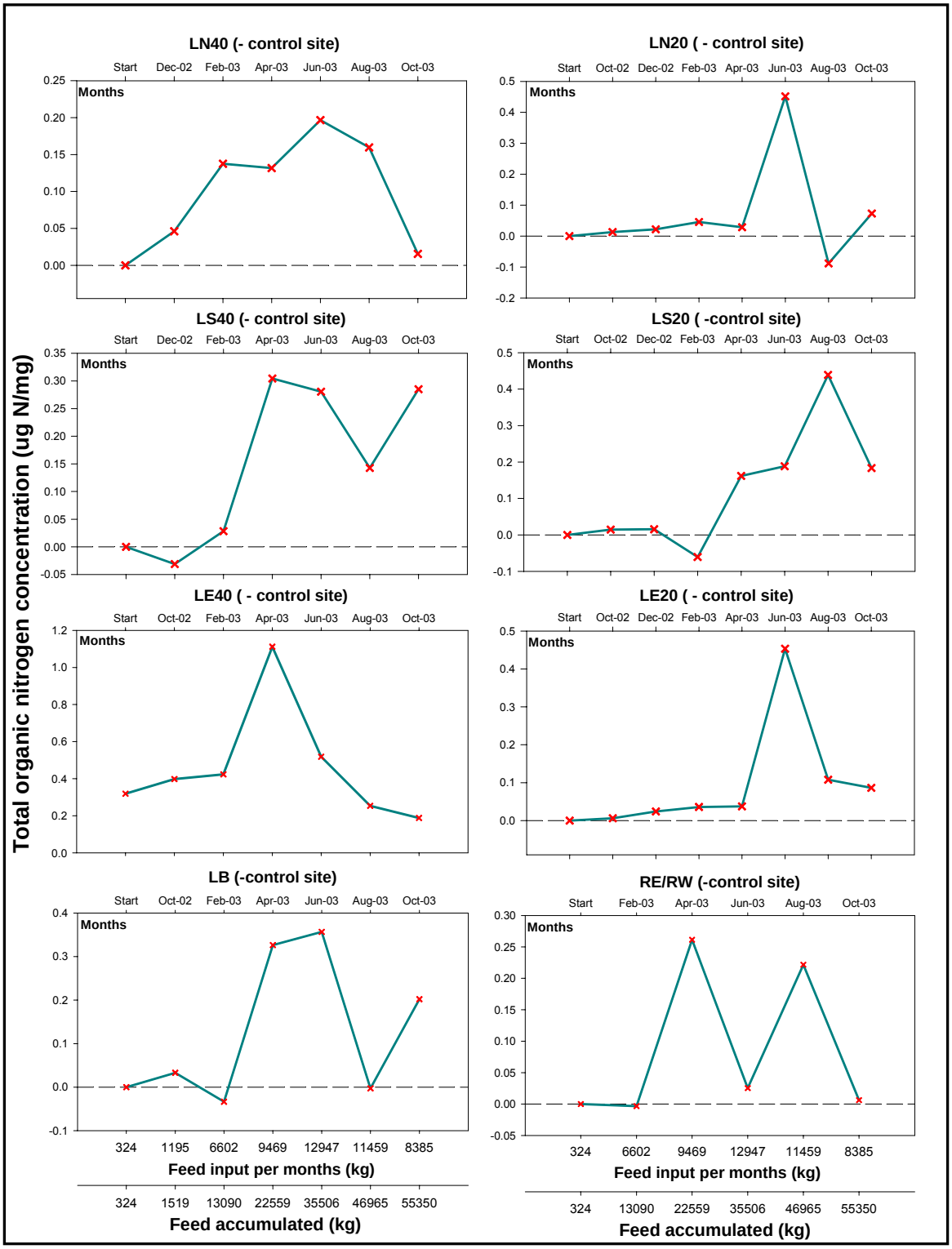
Station	N	Mean	Std. Dev.	Median	D F	H	P-value
Control	7	0.01	0.01	0.01	15	9.97	0.7363
LB	6	0.02	0.01	0.03			
LE20	7	0.02	0.01	0.02			
LE40	6	0.02	0.02	0.02			
LN20	7	0.02	0.01	0.02			
LN40	6	0.02	0.01	0.02			
LS20	7	0.02	0.01	0.03			
LS40	6	0.02	0.01	0.02			
RB	7	0.02	0.01	0.01			
RE/LW	5	0.02	4.5exp-03	0.02			
RN20	7	0.02	0.01	0.02			
RN40	7	0.01	0.01	0.01			
RS20	6	0.02	0.01	0.02			
RS40	4	0.02	0.01	0.02			
RW20	7	0.02	0.01	0.02			
RW40	7	0.02	0.01	0.02			

Appendix 9. Table 23. Krustal Wallis ANOVA test for % TC in sediments organic matter among sampling sites. Values with * represent significant difference (P<0.05).

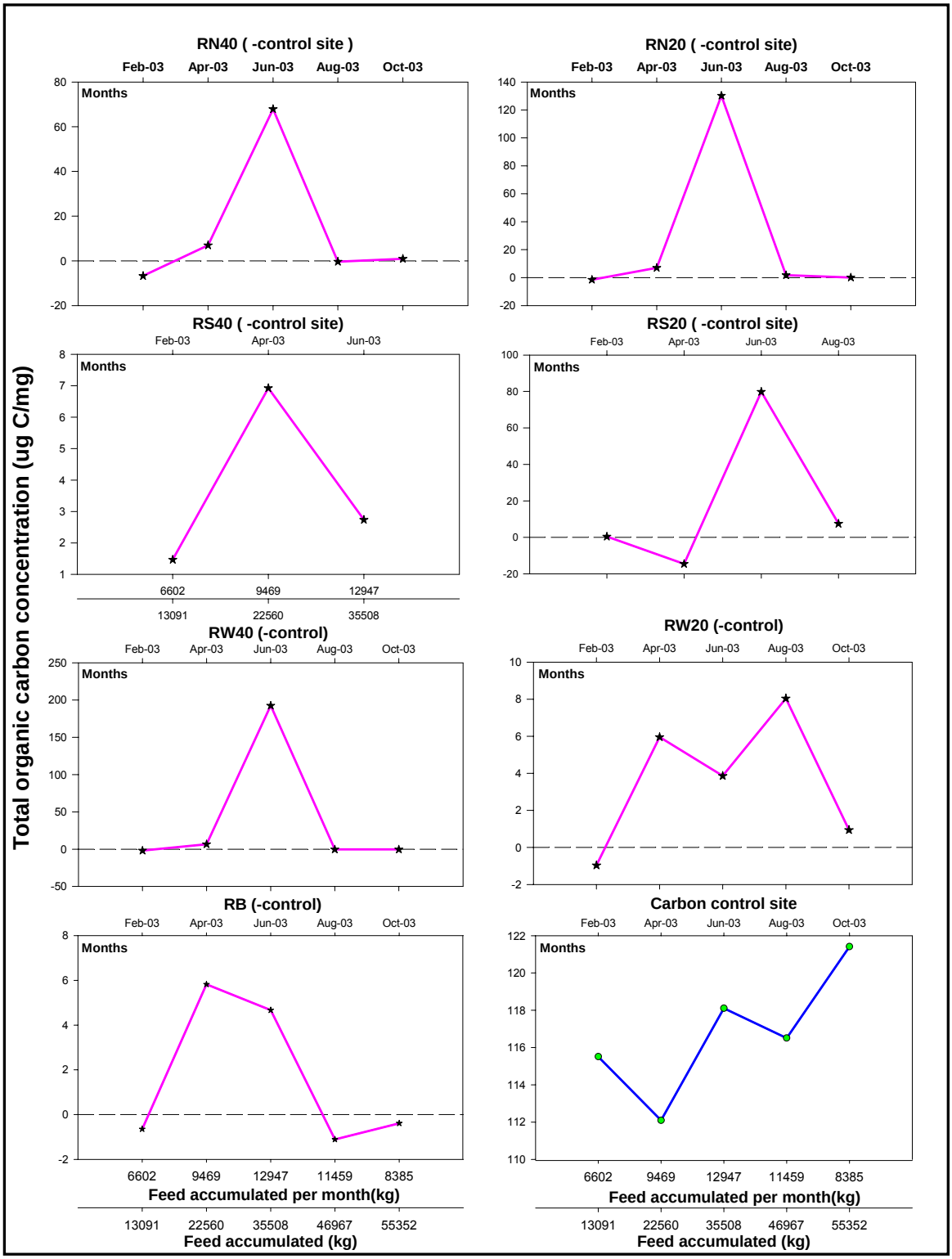
Station	N	Mean	Std. Dev.	Median	D F	H	P-value
Control	5	5.59	1.67	5.62	15	6.94	0.9593
LB	5	6.78	2.61	5.24			
LE20	5	7.21	3.22	5.98			
LE40	5	6.22	1.01	6.37			
LN20	5	6.95	2.91	5.08			
LN40	5	6.88	1.93	6.53			
LS20	5	7.15	3.42	5.05			
LS40	5	5.82	0.82	6.31			
RB	5	6.29	1.41	6.66			
RE/LW	5	5.15	0.10	5.13			
RN20	5	7.28	3.06	5.54			
RN40	5	6.61	2.64	5.46			
RS20	4	5.86	2.48	5.26			
RS40	3	4.81	0.60	4.53			
RW20	5	5.77	1.57	4.85			
RW40	5	7.56	4.47	6.05			



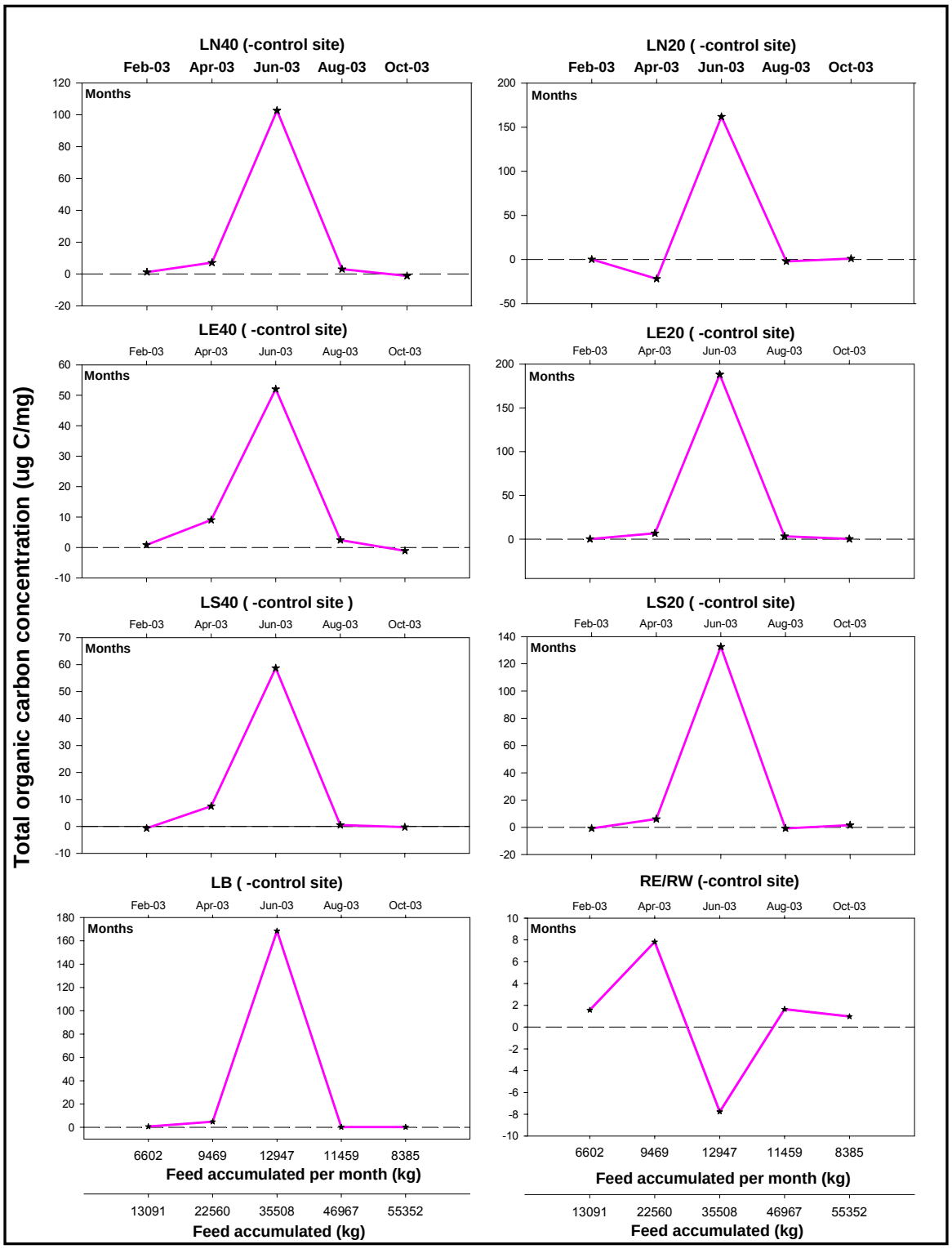
Appendix 10. Figure 37. Temporal variation of mean TON concentrations for each sample site for the *R. canadum* cage. Respective control site mean concentrations were subtracted from each *R. canadum* mean value.



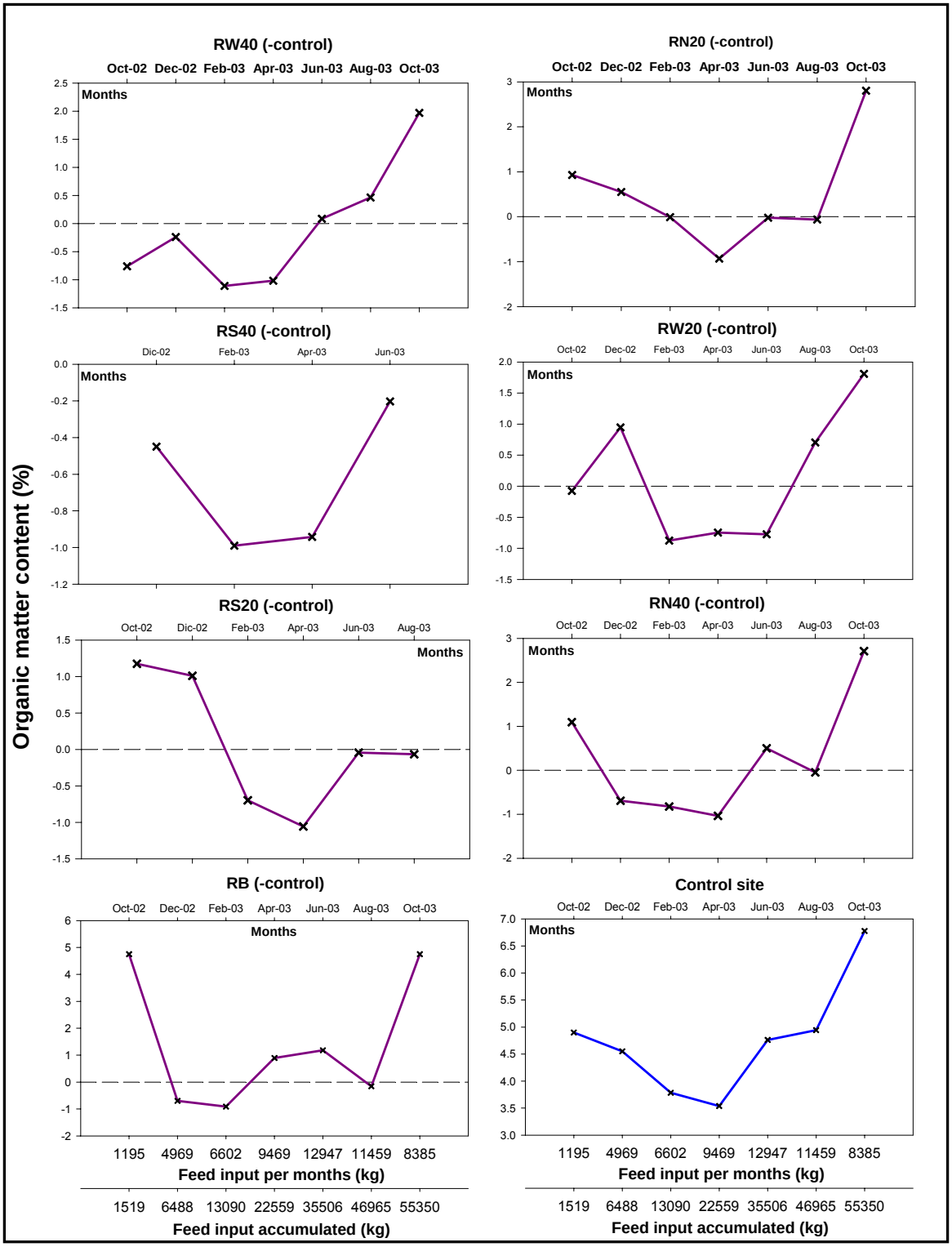
Appendix 11. Figure 38. Temporal variation of mean TON concentrations for each sample site for the *L. analis* cage. Respective control site mean concentrations were subtracted from each *L. analis* mean value.



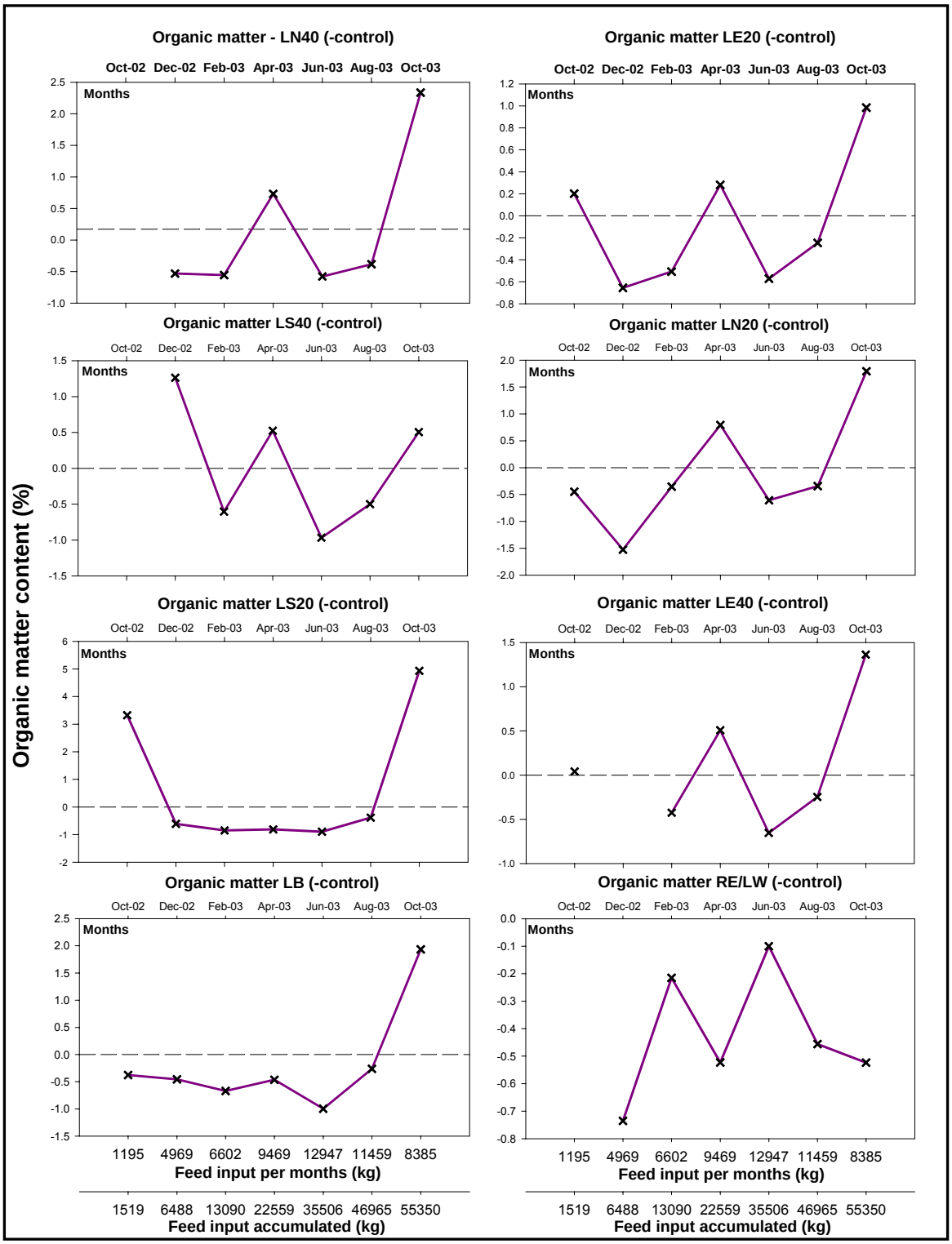
Appendix 12. Figure 39. Temporal variation of mean TC concentrations for each sample site for the *R. canadum* cage. The respective control site mean concentrations were subtracted from each *R. canadum* mean value.



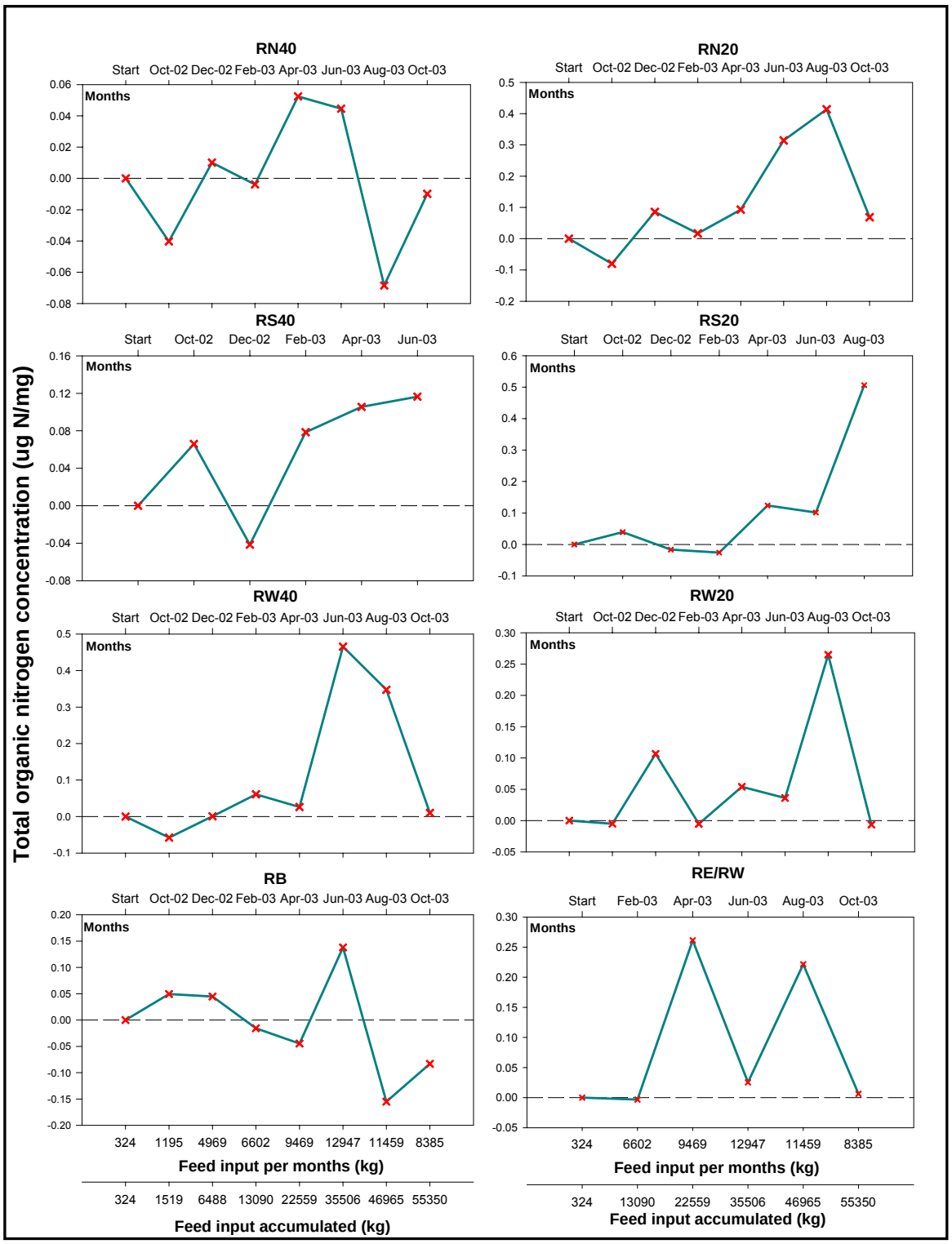
Appendix 13. Figure 40. Temporal variation of mean TC concentrations for each sample site for the *L. analis* cage. Respective control site mean concentrations were subtracted from each *L. analis* mean value.



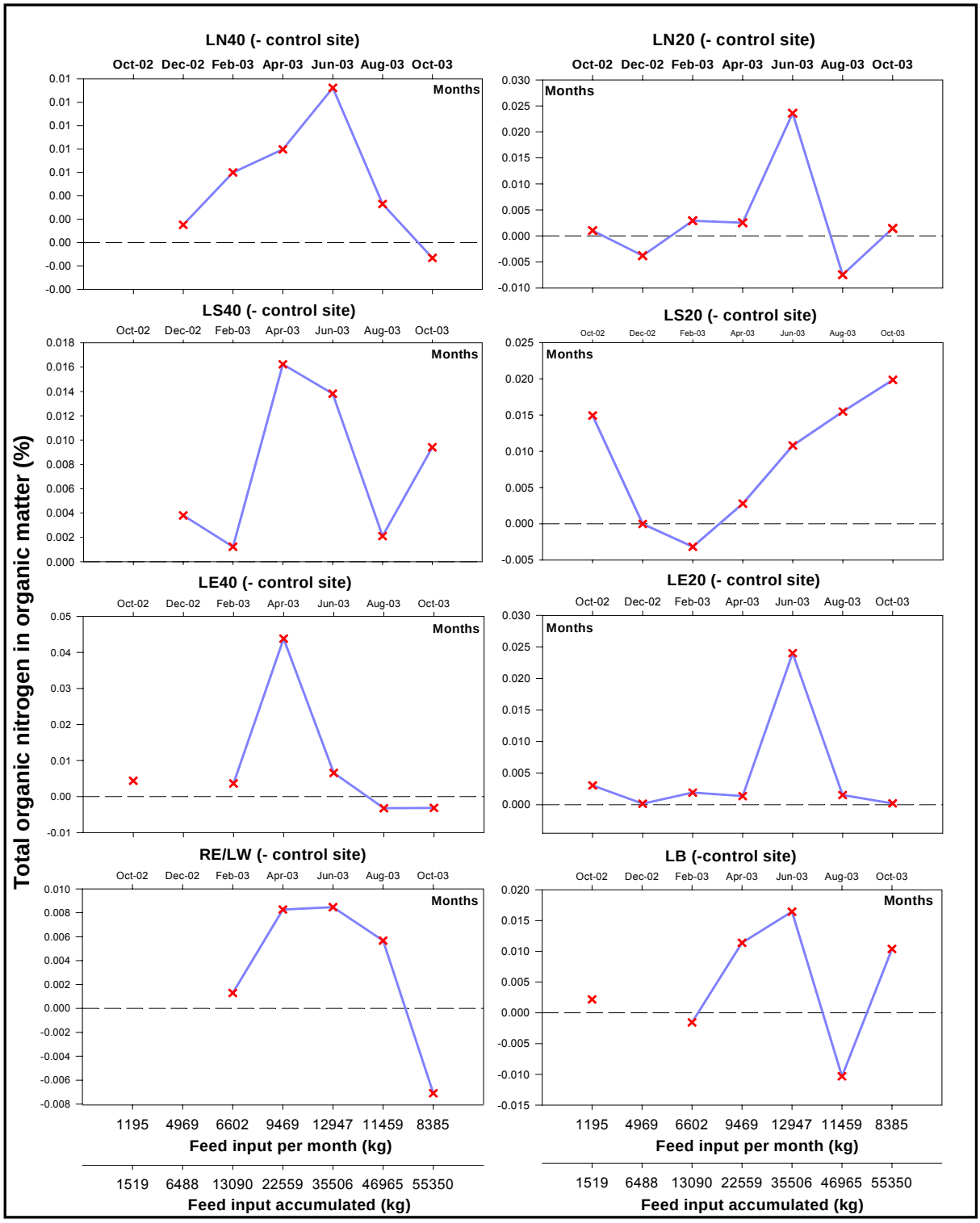
Appendix 14. Figure 41. Temporal variation of organic matter contents for each sample site for the *R. canadum* cage. Respective control site mean concentrations were subtracted from each *R. canadum* mean value.



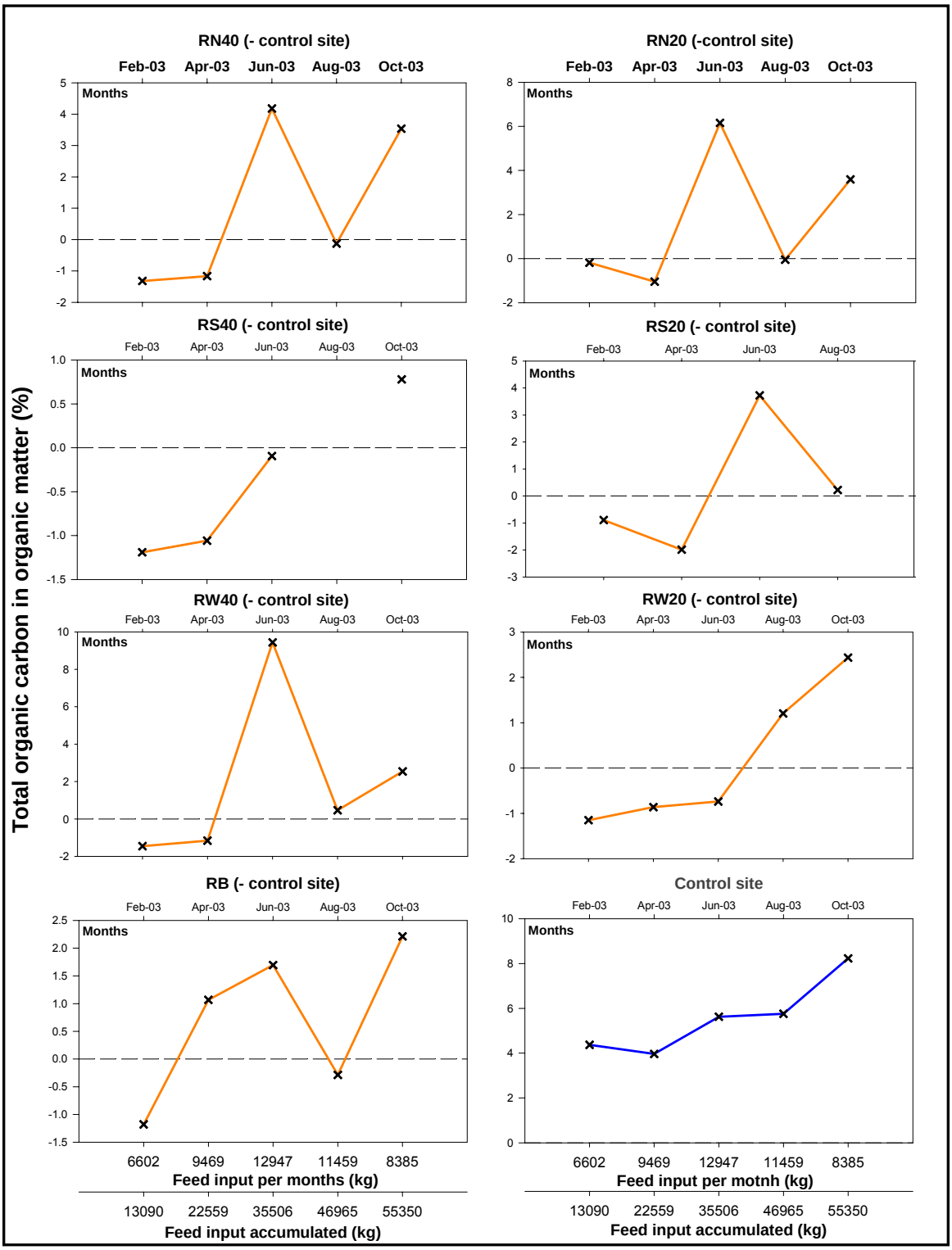
Appendix 15.Figure 42. Temporal variation of organic matter content for each sample site for the *L. analis* cage. Respective control site mean concentrations were subtracted from each *L. analis* mean value.



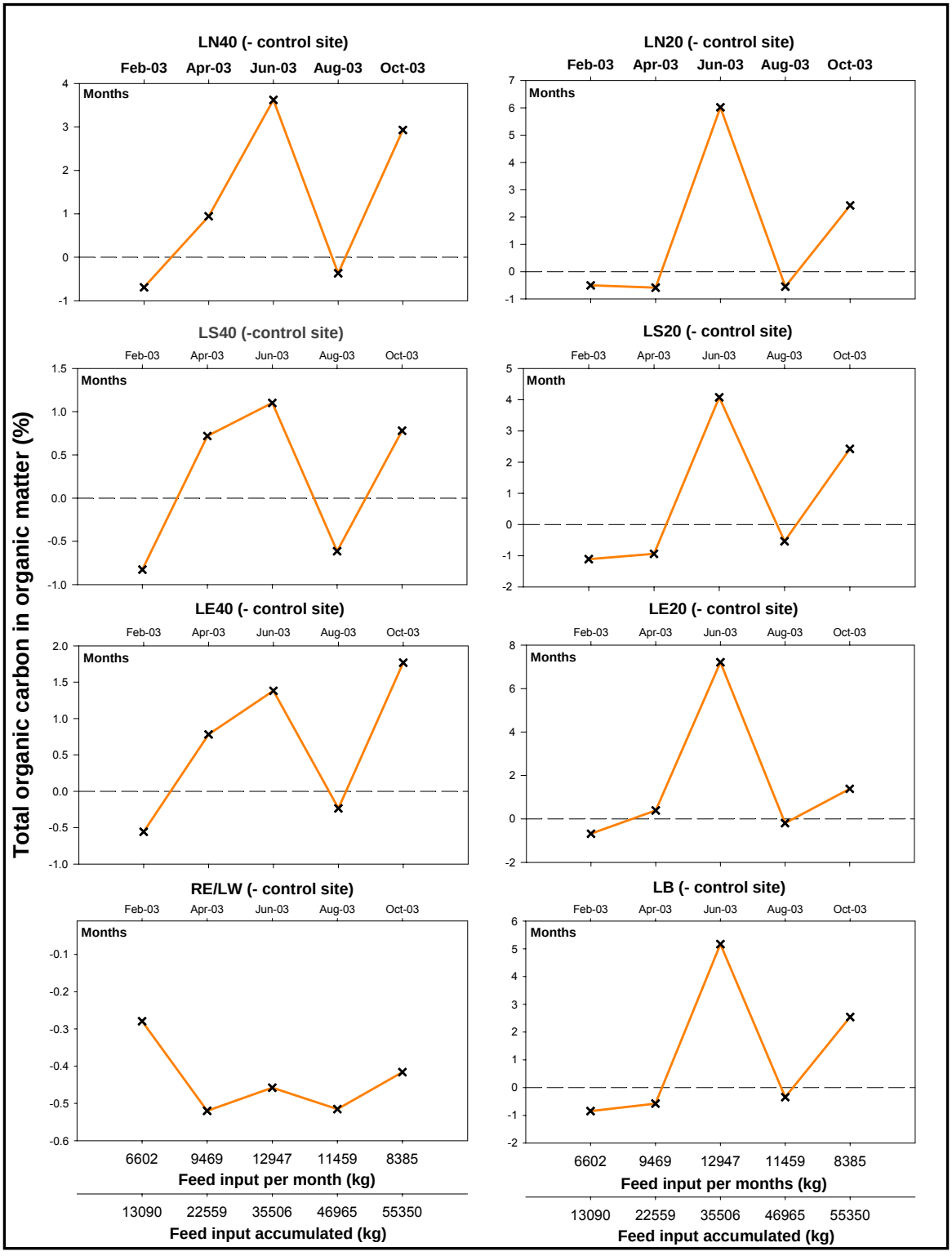
Appendix 16.Figure 43. Temporal variation of TON (%) in sediment organic matter for each sample site for the *R. canadum* cage. Respective control site mean concentrations were subtracted from each *R. canadum* mean value.



Appendix 17. Figure 44. Temporal variation of TON (%) in sediment organic matter for each sample site for the *L. analis* cage. Respective control site mean concentrations were subtracted from each *L. analis* mean value.



Appendix 18. Figure 45. Temporal variation of TC (%) in sediment organic matter in for each sample site for the *R. canadum* cage. Respective control site mean concentrations were subtracted from each *R. canadum* mean value



Appendix 19. Figure 46. Temporal variation of TC (%) in sediment organic matter for each sample site for the *L. analis* cage. Respective control site mean concentrations were subtracted from each *L. analis* mean value.