EFFECTS OF SUSTAINABLE OFFSHORE CAGE CULTURE OF *Rachycentron canadum* AND *Lutjanus analis* ON WATER QUALITY AND SEDIMENTS IN PUERTO RICO

By

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ABSTRACT

This work was part of environmental impact study conducted from August 2002 until October 2003 related to two 3000m^3-Ocean Spar submerged open-ocean growout cages stocked with *Rachycentron canadum* and *Lutjanus analis* south of the island of Culebra, Puerto Rico. The principal objectives were to determine the local environmental effects of open-ocean submerged cage culture on water and sediment quality, as well as changes over time of some environmental quality parameters, including the feasibility of these operations on tropical marine waters. Nutrient concentration (ammonia-N, nitrite-N, nitrate-N, and phosphate) were evaluated bimonthly in the column water and interstitial water at fifteen stations around the cage and three depths for the water samples; likewise, several water and sediment quality parameters were analyzed (dissolved oxygen, water temperature, turbidity, chlorophyll-*a*, salinity and organic matter). Water analyses indicated that, in general, both cages and the control site showed similar nutrients concentrations throughout the months analyzed. Ammonia was the nutrient with the highest concentration; however, these values were relatively low and normal for these waters. Results of the first year indicate that this operation did not impact the quality of the water column, or the sediments even though large quantities of feed were introduced into the system. This was probably due to the large amounts of water flowing through the cages. The information obtained from this study provides a basis to evaluate the feasibility of this operation, encourages the open-ocean aquaculture industry.

RESUMEN

Este trabajo fue parte de un estudio de impacto ambiental desarrollado desde agosto del 2002 hasta octubre del 2003 en un área donde se instalaron dos jaulas sumergidas de 3000 m^3, diseñadas para el cultivo de peces en mar abierto. El objetivo principal del estudio fue determinar el efecto ambiental del cultivo en la calidad del agua y sedimentos, incluyendo cambios temporales de algunos parámetros de calidad ambiental, así como la viabilidad de estas operaciones en aguas marinas tropicales. Las jaulas se instalaron hacia el sur de la Isla de Culebra, Puerto Rico y se cultivaron las especies *Rachycentron canadum* y *Lutjanus analis*. Se evaluaron bimensualmente quince estaciones alrededor de las jaulas; se midieron las concentraciones de nutrientes (amonio, nitrito, nitrato, y fosfato) a lo largo de tres profundidades de la columna de agua, así como en muestras de agua intersticial. También se evaluaron algunos parámetros de calidad ambiental sobre el agua y sedimento (oxígeno disuelto, temperatura del agua, turbulencia, clorofila-*a*, salinidad y materia orgánica). Los análisis de aguas indicaron que en general, tanto las jaulas como el punto control tuvieron concentraciones similares de nutrientes a través de todo el estudio. El amonio fue el nutriente con las concentraciones más altas, pero los valores en general fueron relativamente bajos y son normales para estas aguas. Los resultados obtenidos durante el primer año sugieren que este proyecto no produjo impacto ambiental en la calidad de la columna de agua, aunque se introdujo una gran cantidad de comida al sistema; en parte esto quizás se debe al gran flujo de agua que pasa constantemente por las jaulas. La información obtenida a partir de este estudio proporciona una base para evaluar la viabilidad de estas operaciones y promoverlas en la industria de la acuicultura.

Dedicated with love to my parents:

Hernando Mejia and Elvira Niño for giving me their love, understanding, and unconditional support during my life.

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I. INTRODUCTION

During the last decade, development of aquaculture has increased along coastal zones and has progressively played a more important role as a viable industry. However, this practice is a potential source of impact on the marine environment (Karakassis et al., 2000). The primary effects of aquaculture on the nutrient levels in the sediments, the benthic biota, and on the surrounding water quality is due to the possible deposition of organic wastes. The load of nutrients originating from a cage-culture system can be divided into a dissolved fraction and a particulate fraction; these nutrients can precipitate and accumulate in the sediments. Nevertheless, in most studies, the quantity of nutrients released to the environment from aquaculture activities has been theoretically calculated, with only a few of them based on field studies (Molina – Domínguez et al., 2001).

Open-ocean aquaculture is one of the emerging aquaculture technologies in this decade (FAO 2001), but its long-term success depends on developing farming techniques with minimal impacts on the surrounding environment, and with each area requiring distinct monitoring requirements. Intensively cultured marine aquaculture organisms are concentrated in small areas while constantly receiving large amounts of feed; unconsumed feed and wastes are directly or indirectly released to the surrounding environment. Thus, some agencies are concerned about the environmental impact of large-scale fish production systems. Local environmental impacts from the discharges originating from open-ocean aquaculture systems can be reduced significantly in systems where wastes are diluted by currents, moving them away from the culture area (Goldburg et al., 1996).

For cage culture of fish, the principal environmental impacts arise from the release of dissolved organic material into the water column and deposition of organic solids to the benthos. Fish farms produce wastes; in particular N and P are released in dissolved form into the water column (Holby and Hall, 1991; Hall et al., 1992). The pattern of nutrient release presents a significant deviation from the natural fluctuation of nutrient concentrations in the water column (Pitta et al., 1999). This information is relevant for the development of monitoring programs for open-ocean aquaculture because it provides a basis for incorporating suitable management procedures (Grizzle et al., 2003). New technologies have lower environmental impact than traditional aquaculture methods and their implementation should be encouraged. For instance, quality feeds result in fast and efficient fish growth and less release of wastes to the environment.

Commercial development of United States open-ocean aquaculture has been impeded by the lack of demonstrated feasibility in critical areas, such as engineering of containment structures to withstand open–ocean conditions, adequate information on the growth rate and survival in cages, and efficient production management and harvesting methods (Helsley, 2000). Fisheries operations in Puerto Rico have exceeded maximum sustainable yield due to ocean pollution, over-fishing, and destruction of suitable habitat for native species. The development of a strong aquaculture industry will help meet the needs of the Puerto Rican market, reduce imports of fishery products and benefit from the improved balance of trade. Snapperfarm, Inc. sought to test such a new technology to culture fish in open-ocean conditions and to provide new alternatives for the local fisheries industry.

Snapperfarm installed two 3000-m^3 Ocean Spar submerged grow-out Sea Station cages during July 2002 and stocked them the following month with 12000 cobia (*Rachycentron canadum)* and 4000 mutton snapper (*Lutjanus analis)*. The site was located 3 km southwest of Culebra Island, Puerto Rico.

The Department of Marine Sciences, University of Puerto Rico at Mayagüez campus received funding to determine the environmental impact and feasibility of these operations. The project focused on the chemical, physical, and biological variables in the area surrounding the operation to evaluate possible impacts generated by the culture system. This thesis study monitored the most important water and sediment quality variables and their effects on the surrounding environment. Environmental monitoring started in August 2002 before the first feed input into the cages and ended in October 2003.

The overall goal of this study was to determine the environmental effects of open-ocean submerged cage culture of *Rachycentron canadum* (cobia) and *Lutjanus analis* (mutton snapper) on water and sediment quality. The specific objectives were to determine the:

- Possible impact of inorganic nitrogen (ammonia-N, nitrate-N, and nitrite-N) and phosphate near an open-ocean submerged cage culture operation versus a control site
- Variations over time in the ammonia-N, nitrate-N, nitrite-N, and phosphate concentrations
- Variations in nutrient concentrations at different depths of the water column

- Accumulation over time of organic matter in sediments
- Relations of nutrient concentration (ammonia-N, nitrate-N, nitrite-N, and phosphate) with physical and chemical variables of dissolved oxygen, water temperature, chlorophyll-*a* concentration, turbidity, and salinity
- Probable success of sustainability of open-ocean submerged cage culture in reference to water and sediment quality

Information obtained from this study provides data for the aquaculture industry, investors, and regulatory agencies so that they can evaluate the feasibility of developing sustainable open-ocean cage operations on a large scale. The results address ecological issues, such as the impact of organic wastes on the environment and perturbations or effects in the marine habitat. Because this new industry could alleviate the impact on fishery resources by providing fresh fish to the community, regulatory agencies will use the information to establish guidelines for granting permits to new open-ocean aquaculture industries, especially in areas using ecosystem based management plans. For instance, open-ocean aquaculture operations in suitable areas (not impacting reefs) could provide fresh fish for communities that decide to minimize impacts on their reefs or to allow reefs to recover from heavy fishery activities.

Ocean Spar Sea Station™ submerged grow cages are designed to withstand strong water currents and are suitable for the depth (28 m) at the open-ocean site selected by Snapperfarm. Site selection was based on water depth and currents to sufficiently dissipate organic and inorganic pollutants that would have accumulated in shallow inshore areas with little current.

The culture species *Rachycentron canadum* (cobia) and *Lutjanus analis* (mutton snapper) are native to Puerto Rico and the Caribbean. *Lutjanus analis* has a high commercial value, while *R. canadum* is a pelagic fish selected for its fast growth, as demonstrated by several fish culture cage studies in Taiwan (Liao 2003), and also has a worldwide distribution. Even though few *R. canadum* are sold in Puerto Rico, this species has commercial value in some areas of the United States. The technology for fingerling production has been developed in several hatcheries in the United States. Because *R. canadum* and *L. analis* are native fish, problems with exotic introductions are avoided. If these fish escape, problems with dilution of the gene pool are minimized.

II. PREVIOUS WORKS

The development and proliferation of aquaculture in the coastal zone during the last decade has been considered as an alternative to over-exploitation of natural stocks, destruction of habitats, and mortality in populations of non-commercial species induced by the fisheries industry. Nevertheless, coastal zone aquaculture operations have caused concern due to impacts on critical environmental variables studied during the last 10 years (Karakassis, 1998; Karakassis et al., 1998). Aquaculture operations located in open-water conditions avoid the impacts of operations located near the coastline.

The effects of cage-culture fish farming on benthic ecosystems and seasonal changes of environmental variables related to aquaculture have been demonstrated in terms of both the benthic fauna and the water column. Changes in sediment characteristics could be detected over the short term, or during weeks or months, depending on production levels. However, for the water column, the excretion of soluble wastes might induce significant changes in water quality for only a few hours after feeding (Karakassis et al., 2001).

Most studies of environmental impacts of cage aquaculture have been developed for inshore waters in temperate regions. Some have shown an increase in suspended solids and nutrients (ammonia, organic nitrogen, and carbon), and a decrease in dissolved oxygen concentrations and pH in sediments near the cages (Chen et al., 2000). Environmental impact of open-ocean aquaculture in tropical and subtropical regions may be different from temperate inshore areas. The higher water temperatures in the tropics and the strong current conditions predominating in the open-ocean environment should allow

for increased metabolic processes and dispersal of released nutrients, which in turn should substantially minimize the potential effect of marine fish farms.

Karakassis et al. (1998), in their study concerning chemical and physical changes in sediment profiles beneath and around fish farm cages in the Mediterranean, reported a fluctuation of the farm sediment accumulation thickness throughout the year for stations directly beneath the cages, presumably to be due to the seasonal fluctuation in food inputs. They also found that surface concentrations and the vertical distribution of benthic chemical and physical variables changed with distance from the cages and with season.

Karakassis et al. (2000) reported that impacts of fish farming on the benthos in the Mediterranean vary considerably, depending on site characteristics and seasonal variability in geochemical and macrofaunal variables. Impacts were always more pronounced at stations closer to the farm (0-10 m) than at the control site or at stations 25 m distant from the cages. This may be attributed to seasonal differences in food supplied to the farmed fish and/or to the increased oxygen supply to the sediments during winter.

Karakassis et al. (2001) studied the impact of dissolved wastes from sea bream and sea bass farmed in sea cages. Their results showed that a large amount of soluble nutrients was released to the environment. The water column was homogeneous in terms of temperature and salinity, with differences of 0.5 ºC and 0.39 psu respectively. Nutrient concentrations presented some fluctuations, with ammonium (NH_4) decreasing with increased depth, and with the bottom layer having the lowest value. Fluctuations of phosphate were less conspicuous with increased depth. Concentrations of phosphate were highest (0.2 - 0.34 μM), showing an increase at noon. Concentrations of nitrates and

nitrites showed some fluctuations during the day, with peaks during the first hours after feeding and after the maximal ammonium concentration. Silicate concentrations intensified during the day for different water layers.

Pitta et al. (1999) determined the concentrations of nutrients at three fish farms, noting a significant increase ($P<0.01$) in phosphate and ammonium concentrations for stations in areas between the cages compared to the control site in one farm, but without a significant effect on chlorophyll-*a* concentration. Other farms showed no significant differences ($P>0.05$) among samples taken from stations in areas between the cages and the respective control stations, in concentration of nutrients and chlorophyll-*a,* among others.

Hesley (2000) indicated that a Hawaiian open-ocean aquaculture project to culture *Polydactylus sexfilis* (moi) at depths of 15 to 30 m did not impact the water column, the sea bottom, or the nearby coral reefs, and concluded that this could be a viable large-scale aquaculture system. In Hawaii, ByBee and Bailey–Brock (2003) determined that an open-ocean fish culture system did not affect the species richness of the benthic invertebrate community, and that populations of *Capitella capitata* returned to pre-stocking levels in less than two months after feeding ended. This polychaete worm is considered as an indicator of organic loading. The Hawaiian study indicated that increased abundance of *C. capitata* beneath the cage was related to food input at the cage.

A study of sediments beneath a marine cage farm at Grand Canary Island (Molina-Dominguez, et al. 2001) determined that the physical and chemical characteristics of sediments studied were hardly affected by the operations of the farm over the year of study; however, this site is characterized by strong water currents.

Grizzle et al. (2003) reported no significant trends for open-ocean aquaculture of shellfish sites in the Gulf of Maine. Likewise, they determined that nutrient concentrations were low and did not present consistent seasonal trends due to the relative low biomass of the cultured organisms in relation to water depth and the hydrodynamic conditions at the site.

Rachycentron canadum is an ideal species for open-ocean aquaculture (Liao, 2003) and is intensively cultured in Taiwan because of its fast growth rate compared to other marine fish. This species tolerates high stocking densities, resists ectoparasites, and is desired by consumers. Liao (2003) suggests that "cobia aquaculture in tropical or subtropical regions may become as competitive as salmon aquaculture in the temperate region." *Rachycentron canadum* is cultured in offshore cages for the final production stage until they reach the size of 6-10 kg for export or domestic market. The culture period usually last 6- 8 months, depending of the required size. Pellets for feeding have a 42 – 45 % crude protein content and 15 – 16 % fish oil; for this reason, this feed is more expensive than the feeds for other fish (Liao and Leaño, 2005).

III. METHODOLOGY

Monitoring was done bimonthly near the Snapperfarm site. The cages were located 3 km south of the island of Culebra, Puerto Rico (Fig. 1), where the prevailing currents are southeast to northwest. One cage was stocked with 12,000 *Rachycentron canadum* and the other cage with 4, 000 *Lutjanus analis* and both were fed twice daily during the morning and afternoon with commercial pellets containing 51 % crude protein. Feed was introduced through pipes connected from the boat to each cage (Fig. 2). Pellet size and the daily feeding rate were adjusted according to fish weight during the culture period of about one year. By the end of the culture period, the daily amount of the feed for the *R. canadum* cage was 200 Kg.

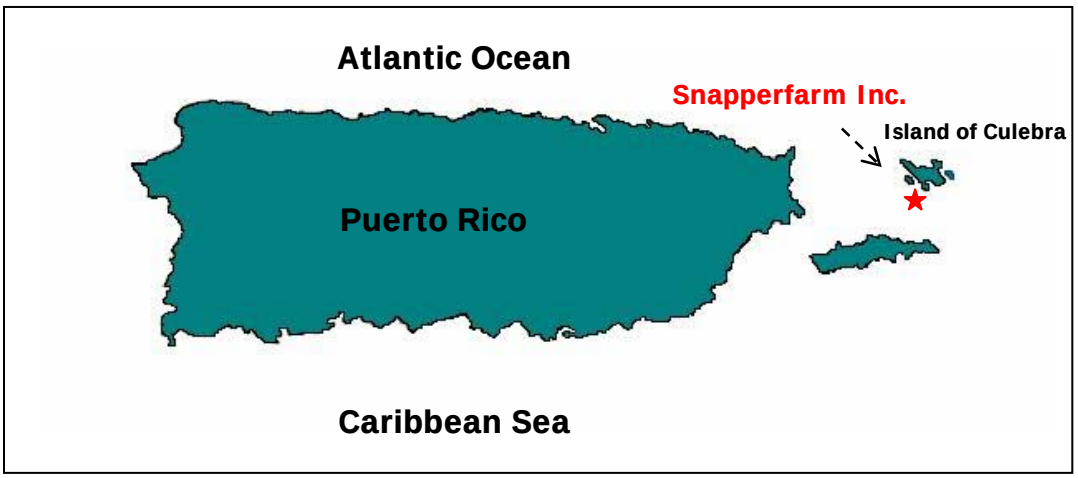

Figure 1. Location of the Snapperfarm site, 3 Km south of Culebra.

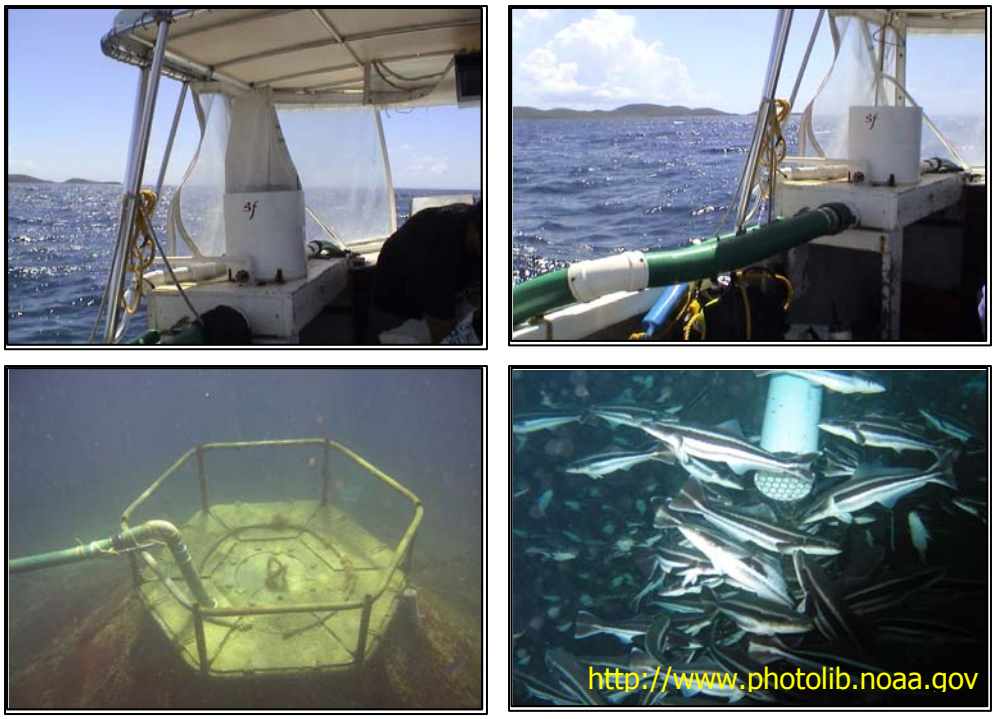

Figure 2. Photographs of the feeding process through pipes connected from the boat to each cage.

Each culture cage was secured to the bottom with an 11,000-kg ballast and five 1,300-kg Danforth anchors in 28 m of water, with three anchors on the southeast side and two on the northwest side. The three mooring systems on the southeast side added strength for protection from hurricanes that normally maintain a southeast to northwest track in this area. The frame of the cages consisted of a vertical 14-m central spar, surrounded by a 25-m diameter steel rim. The cage was covered with Spectra™ mesh netting attached to spoke lines forming the Sea Station's shape. Nursery cages were installed inside the growout cages (attached to the central spar) and stocked with fingerlings. Zippered doors in the net provide easy diver access (Fig. 3). The cages were placed 30 m apart (from rim to rim), perpendicular to the prevailing southeast to northwest currents, with their tops submerged 8 m below the water surface (Fig. 4). The cage system could be easily lowered or raised in less than 5 minutes by varying the buoyancy of the spar.

Figure 3. Ocean Spar submerged grow-out Sea Station cage; cages were stocked with either *Rachycentron canadum* or *Lutjanus analis.*

Samples were taken before cage installation and continued until harvest. Bimonthly samples were taken and evaluated for the most environmentally important water column and sediment quality variables. Fifteen stations were selected 20 and 40 m north, south, east, and west from the center of the each cages, and beneath of cages (Fig. 4). A control station was selected 375 m south of the cages to determine if possible changes of the nutrient concentrations are random seasonal variations or effects of the culture activities on the environment, and to compare effects of the cage site versus the control site.

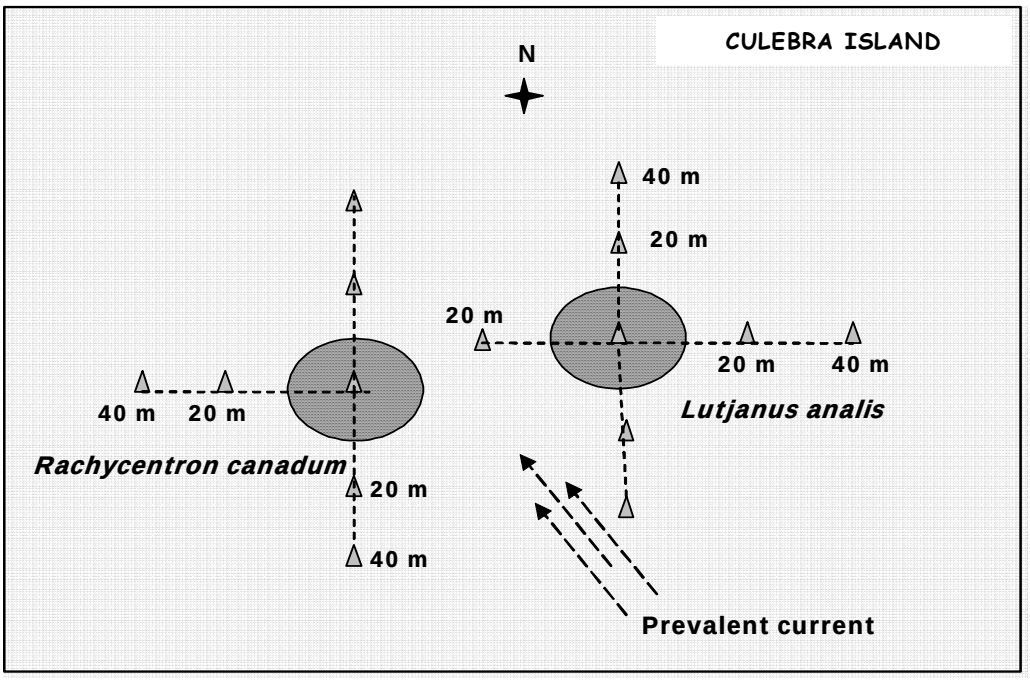

Figure 4. Sampling locations for water and sediment quality variables near the submerged culture cages.

Nutrient analyses at water column

Duplicate samples were collected from the water column with an alpha bottle sampler, lowered with a rope from the side of a boat at each sampling site at three depths at 8, 16, and 26 m, referring to the top, mid-depth, and near-bottom depths of the cages respectively (Fig. 5). Samples located beneath the center of each cage were taken by divers with opaque plastic bottles. Each sample was preserved with 2-3 drops of H_2SO_4, placed on ice to control bacterial activity, and transported to the Department of Marine Sciences, Magueyes Island laboratory facilities and frozen until analyzed (after 2 or 3 days). The variables monitored in the water and sediments at the cage site were dissolved oxygen, water temperature, turbidity, chlorophyll-*a*, salinity, organic matter, ammonia-N, nitrate-N, nitrite-N, and phosphate.

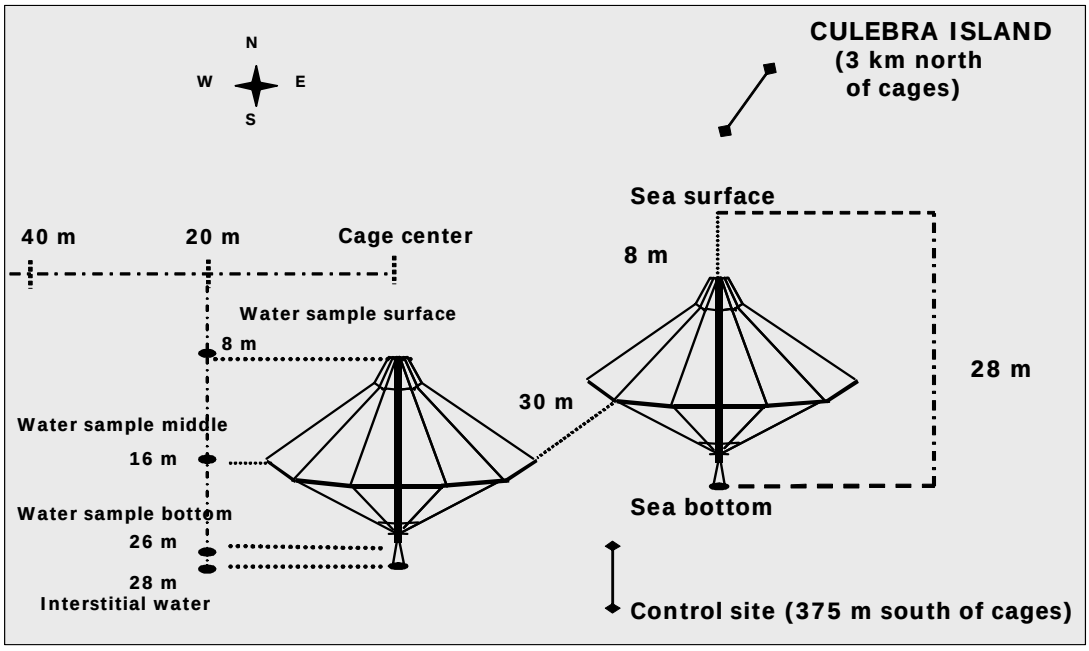

Figure 5. Diagram of the Sea Station™ culture cages and sampling locations in the water column.

Duplicate water samples were analyzed by colorimetric analysis according to analytical procedures followed by Strickland and Parsons (1972) and described in Standard Methods (Clesceri et al., 1998). For nitrite-N, the samples were analyzed in 15-ml assay tubes following the nitroprusiade method. The determination of nitrate-N was also by the nitroprusiade method, after to reduced nitrate-N to nitrite-N with a packed cadmium column. Ammonia-N determination was analyzed following the indophenol method. The Molibdate method was followed for the phosphate analysis described in Standard Methods (Clesceri et al., 1998).

Nutrient analysis at interstitial water

Each duplicate benthic interstitial water sample was taken with a PVC core sampler (5-cm diameter, 10-cm length) by a diver at each of the 16 sampling

locations. The samples were preserved with 2-3 drops of H_2SO_4, stored on ice to control bacterial activity, transported to the Magueyes Island laboratory, and frozen until analyzed. Duplicate samples were analyzed by colorimetric analysis following the method for each nutrient as listed in Standard Methods (Clesceri et al., 1998) and described by Strickland and Parsons (1972).

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.

Organic matter:

Each duplicate benthic interstitial water sample was taken and processed as described for nutrient analysis at interstitial water. Duplicate samples were analyzed by placing thawed sediment samples into cleaned and dried crucibles to determine organic matter of the sample by using the gravimetric method (Holme and McIntyre 1984; Páez-Osuna et al., 1984; Clesceri et al., 1998).

Statistical analyses:

Statistical analyses were made with InfoStat Software, version 3.0 (2003). The data for all variables were analyzed using analysis of variance (ANOVA) with

contrasts for a factorial design and Tukey's Test (α= 0.05). Each nutrient concentration was compared for each station and between the sampling locations at the cage and control site, significant differences were at the 95% probability level ($P<0.05$). Contrast analyses for each nutrient in the water column were made between sampling locations at the cages and control site by months and by depths (surface, middle, and bottom) as follows: Contrast 1 (between sampling locations of the R*. canadum* cage versus *L. analis* cage), Contrast 2 (between *L. analis* cage versus the control site), Contrast 3 (between *R. canadum* cage versus the control site), Contrast 4 (between control site versus intermediate station located between each of the cages), Contrast 5 (between beneath both the cages versus the control site), Contrast 6 (between upstream versus downstream), Contrast 7 (between surface versus middle depths), Contrast 8 (between surface versus bottom depths), and Contrast 9 (between middle versus bottom depth).

Sediment variables analyses compared the cage and control sites by months and sampling locations, as follows: Contrast 1 (between sampling stations at *L. analis* cage versus the control site), Contrast 2 (between sampling locations at *R. canadum* cage versus the control site), and Contrast 3 (between upstream versus downstream). Pearson correlations compared data among nutrient concentrations in the column water with the nutrients in the interstitial water in sediments, oxygen concentration, water temperature, salinity, chlorophyll-*a* concentration, turbidity, and organic matter.

IV. RESULTS

Nutrient concentration at water column

Water analyses indicated that nutrient concentrations in the water column for the cages and control site were low during each sampling period. The highest values of ammonia-N, nitrate-N and phosphate were found in December 2002 during the initial phase of culture (Fig. 6). For ammonia-N concentration (32.27 µg/L ± 3.00 s.d) was higher than other nutrients, and had significant differences ($P<0.05$) among months (Table 1). Nitrate-N concentrations were significantly different among months ($P<0.05$), although concentrations were low for all months analyzed, usually less than 2.29 µg/L with maximum values of 13.00 µg/L (± 2.00 s.d) (Table 2).

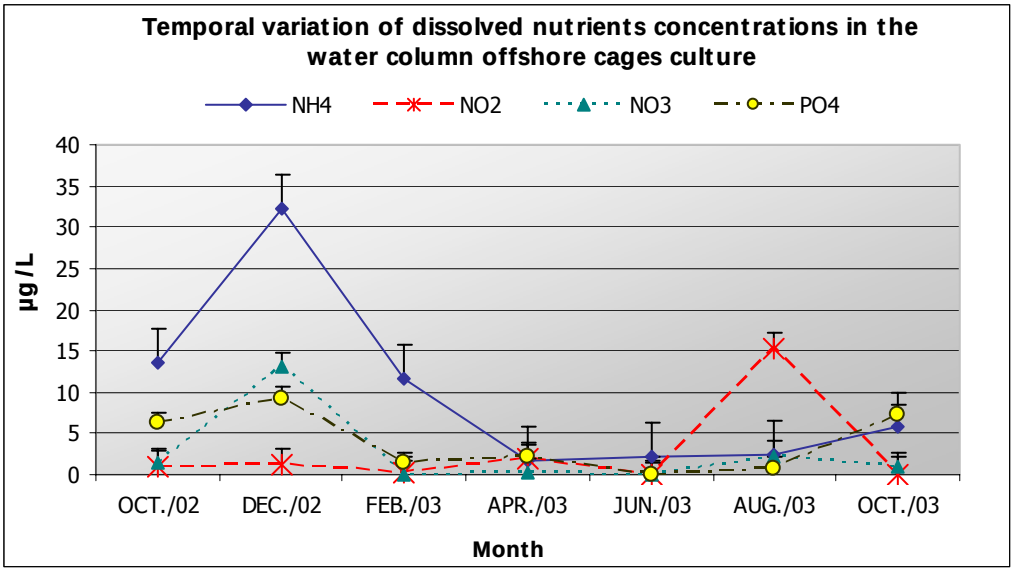

Figure 6. Temporal variation of dissolved nutrients concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column of the cage site during all sampling periods.

Table 1. ANOVA for dissolved nutrient concentrations of ammonia-N (NH_4) among months, sampling locations, and depth and their interactions. Values with * represent significant differences (P< 0.05).

Source	*df*	MS	*F*	p
Model	321	0.00038	7.22	<0.0001 *
Months	6	0.00063	44.55	<0.0001 *
Sampling locations	15	0.00013	2.42	0.0023 *
Months x Sampling locations	90	0.00012	2.24	<0.0001 *
Depth	2	0.00190	36.37	<0.0001 *
Months x Depth	12	0.00160	30.71	<0.0001 *
Depth x Sampling locations	29	0.00010	1.95	0.0030 *
Months x Depth x S. locations	167	0.00011	2.02	<0.0001 *
Error	320	0.00005		
Total	641			

Table 2. ANOVA for dissolved nutrient concentrations of nitrate-N (NO_3) among months, sampling locations, and depth and their interactions. Values with * represent significant differences (P<0.05).

Source	*Df*	MS	*F*	p
Model	322	0.000150	10.94	<0.0001 *
Months	6	0.002000	31.05	<0.0001 *
Sampling locations	15	0.000027	1.91	0.0215 *
Months x Sampling locations	90	0.000026	1.84	0.0001 *
Depth	2	0.002000	146.30	<0.0001 *
Months x Depth	12	0.002100	151.41	<0.0001 *
Depth x Sampling locations	29	0.000025	1.81	0.0076 *
Months x Depth x S. locations	168	0.000024	1.75	<0.0001 *
Error	318	0.000014		
Total	640			

Phosphate concentrations were low during most months (less than 2.18 µg/L), with higher values found during the initial culture period. There were significant differences among months (P<0.05) (Table 3). Nitrite-N concentration was highest in August 2003, but in general concentrations were low throughout the study, normally less than 1.89 µg/L (Fig. 6); there was a significant difference among months (P<0.05) (Table 4).

Table 3. ANOVA for dissolved nutrient concentrations of phosphate (PO_4) among months, sampling locations, and depth and their interactions. Values with * represent significant different (P<0.05).

Source	*Df*	MS	*F*	p
Model	322	0.000140	2.96	<0.0001 *
Months	6	0.001200	17.14	<0.0001 *
Sampling locations	15	0.000130	2.87	0.0003 *
Months x Sampling locations	90	0.000095	2.03	<0.0001 *
Depth	2	0.000065	1.38	0.2539
Months x Depth	12	0.000530	11.32	<0.0001 *
Depth x Sampling locations	29	0.000120	2.50	0.0001 *
Months x Depth x S. locations	168	0.000100	2.19	<0.0001 *
Error	318	0.000047		
Total	640			

Table 4. ANOVA for dissolved nutrient concentrations of nitrite-N (NO_2) among months, sampling locations, and depth and their interactions. Values with * represent significant different (P<0.05).

Source	*df*	MS	*F*	p
Model	322	0.000063	37.10	<0.0001 *
Months	6	0.002700	37.37	<0.0001 *
Sampling locations	15	0.000005	2.73	0.0006 *
Months x Sampling locations	90	0.000012	6.99	<0.0001 *
Depth	2	0.000004	2.51	0.0829
Months x Depth	12	0.000023	13.22	<0.0001 *
Depth x Sampling locations	29	0.000015	8.87	<0.0001 *
Months x Depth x S. locations	168	0.000013	7.55	<0.0001 *
Error	318	0.000002		
Total	640			

When comparing nutrient concentrations among depths for both cages, it is observed that means of ammonia-N and nitrate-N in the bottom water samples were higher than the middle and surface water samples (Fig. 7). Mean nitrite-N concentrations ranging from 2.40 – 2.86 µg/L (± 0.10 s.d) were not significantly different among the three depths (Table 4). On the other hand, nitrate-N significantly increased with depth (P<0.05), with a high mean concentration of 5.96 µg/L (±2.00 s.d). Ammonia-N had the highest mean concentration at each

depth, with significant differences among their ($P<0.05$ s.d) means ranging from 6.71 – 12.26 µg/L (± 2.00 s.d) (Fig. 7). Phosphate concentrations were the most stable with depth, with no significant differences.

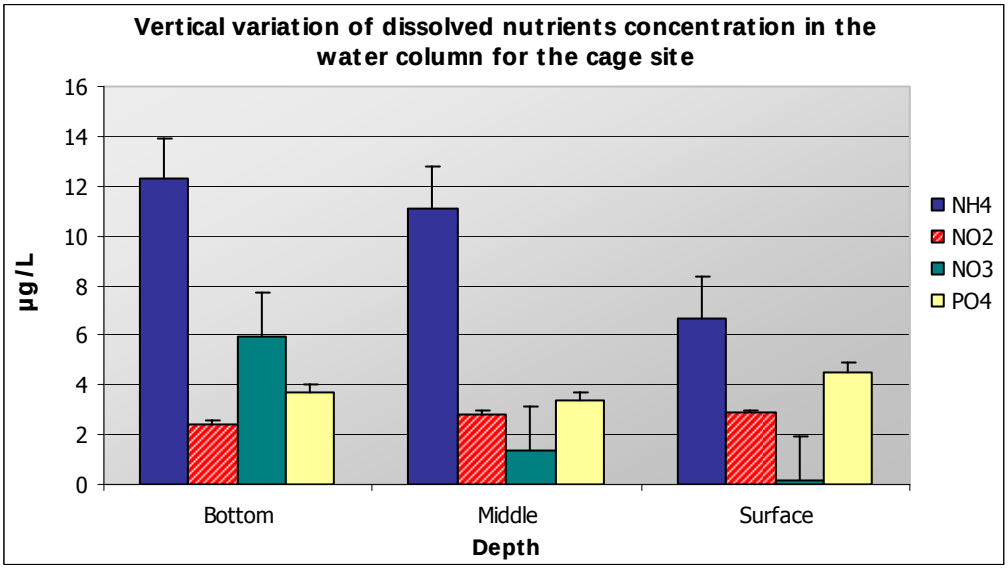

Figure 7. Vertical variation of dissolved nutrients concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column for the cage site at the three depths sampled.

There were no significant differences ($P>0.05$, for interactions among cages, months, and depths) for ammonia-N, nitrite-N, nitrate-N, or phosphate concentrations among *R. canadum* and *L. analis* cages and the control site (Table 5). The snapper cage had mean for ammonia-N (10.59 µg/L ± 1.00 s.d) while the control site had mean concentrations (8.44 µg/L ± 1.00 s.d). The mean nutrient concentrations for nitrate-N and nitrite-N were low (less than 3.00 µg/L), with similar concentrations for both- cages. The control site had the lowest mean concentration for nitrite-N and the highest value for nitrate-N (Fig. 8).

Phosphate concentrations were more lower than ammonia-N, and although there were no significant differences, the highest mean value was in the snapper cage at 4.45 µg/L (± 0.50 s.d) (Fig. 8).

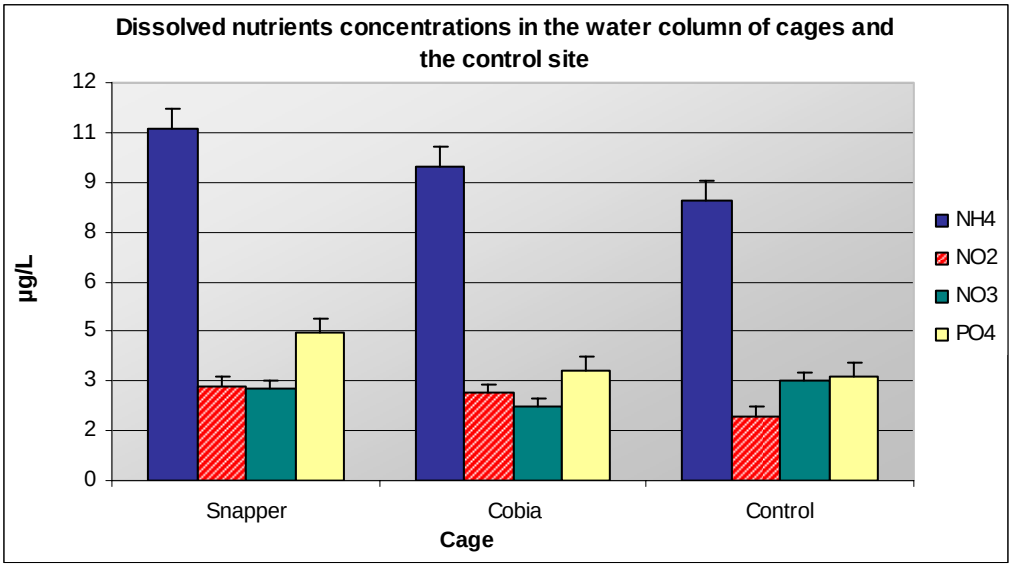

Figure 8. Dissolved nutrients concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column for each cage and control sites.

Significant differences were found when comparing nutrient concentrations at sampling locations for each cage (Tables 1 - 4). Although there were differences among the sampling locations, the general responses were similar for each sampling location for each cage (Figs. 9, 10). The highest concentrations at sampling locations at each cage and control site were for ammonia-N; nevertheless, these values always were below 13.32 µg/L (Fig. 9 and 10). For the *R. canadum* cage, the highest concentrations for ammonia-N and phosphate were beneath the cage (CE), with values of 13.32 and 8.83 µg/L, respectively (Fig. 9).

In the *L. analis* cage, nitrite-N concentrations were similar at each sampling location, while phosphate concentrations were significantly higher south at 40–m (S4) and north at 20-m (N2) of the center cage (Fig. 10). The control site (CO) had the lowest concentrations for nitrite-N and phosphate (Figs. 9, 10); however, these values were not statistically different from the cage site (Table 5).

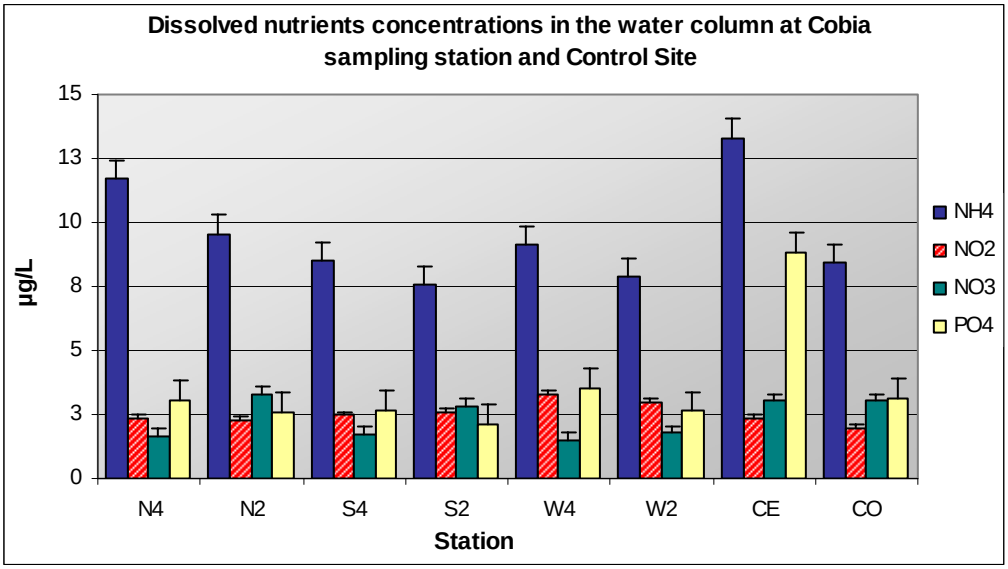

Figure 9 Dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column at each *R. canadum* cage sampling location and at the control site.

The temporal variation of nutrients for the *R. canadum* cage showed that, in general, ammonia-N, nitrate-N, and phosphate concentrations were significantly higher during the first months although the feeding rate was lower; however, nitrite-N concentration showed a maximum concentration during August 2003 (Fig. 11).

Table 5. Contrast analysis for dissolved nutrient concentrations of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) among each cage and control site.

Source	*Df*	MS	*F*	p
	Ammonia-N			
Cages	2	0.000071	1.34	0.2624
Error	320	0.000053		
Total	641			
	Nitrite-N			
Cages	2	0.00000021	0.12	0.8864
Error	318	0.00000170		
Total	640			
	Nitrate-N			
Cages	2	0.000035	2.48	0.0857
Error	318	0.000014		
Total	640			
	Phosphate-N			
Cages	2	0.000059	1.25	0.2867
Error	318	0.000047		
Total	640			

The *L. analis* cage exhibited a similar pattern (Fig. 12), but in general ammonia-N, nitrite-N and phosphate concentrations were somewhat higher than at the *R. canadum* cage sampling locations. The control site had concentrations similar to the cages sites for each nutrient (Fig. 13) indicating that these changes are seasonal and independent on feeding rate.

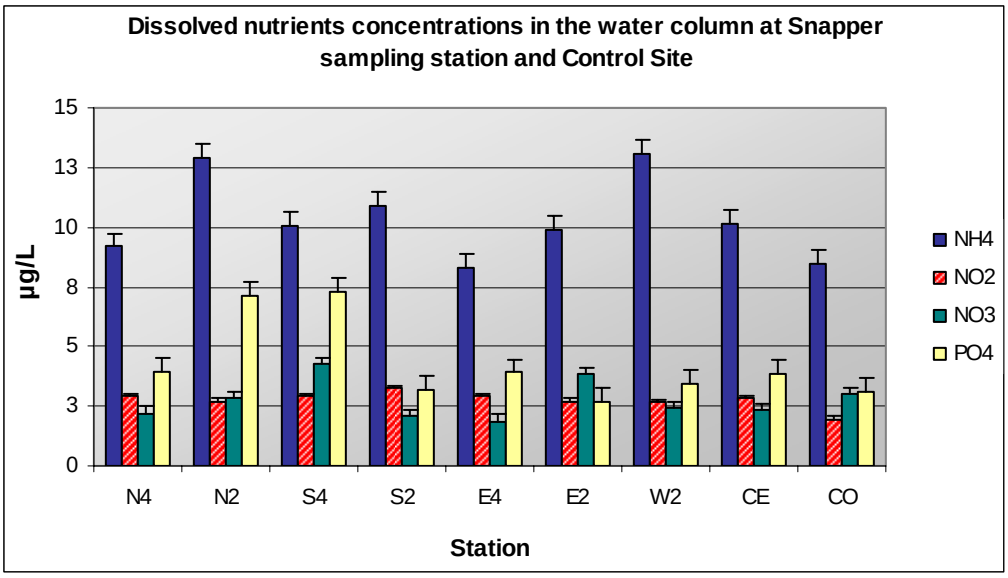

Figure 10 Dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column at each *L. analis* cage sampling location and at the control site.

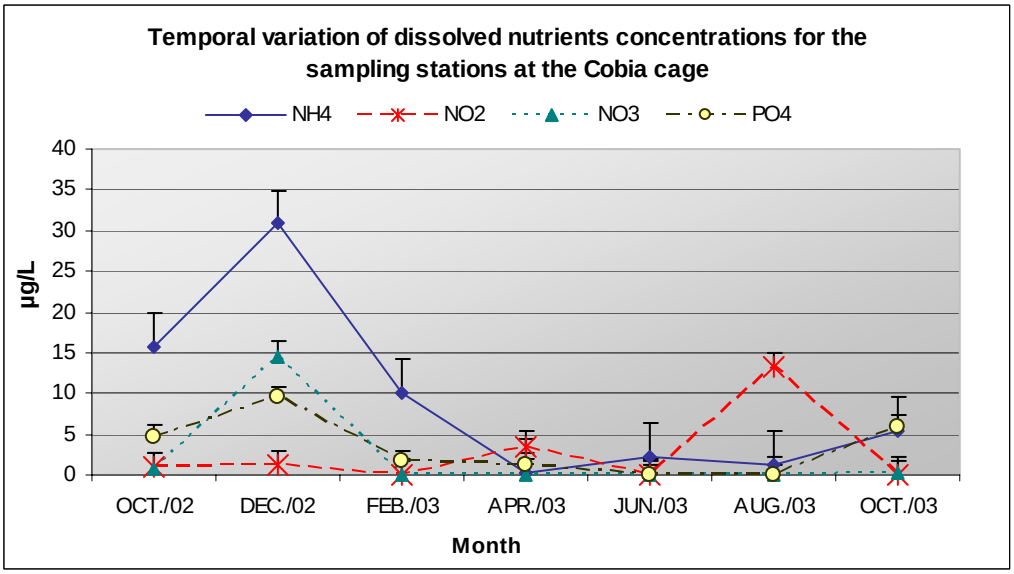

Figure 11. Temporal variation of dissolved nutrient concentration (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) for the sampling locations for the *R. canadum* cage.

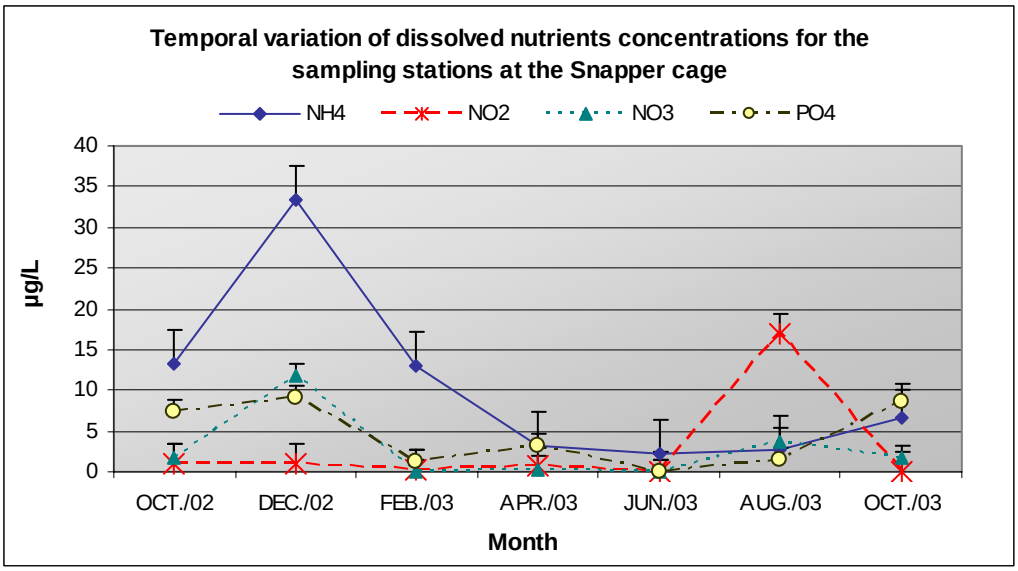

Figure 12. Temporal variation of dissolved nutrient concentration (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) for the sampling locations for the *L. analis cage.*

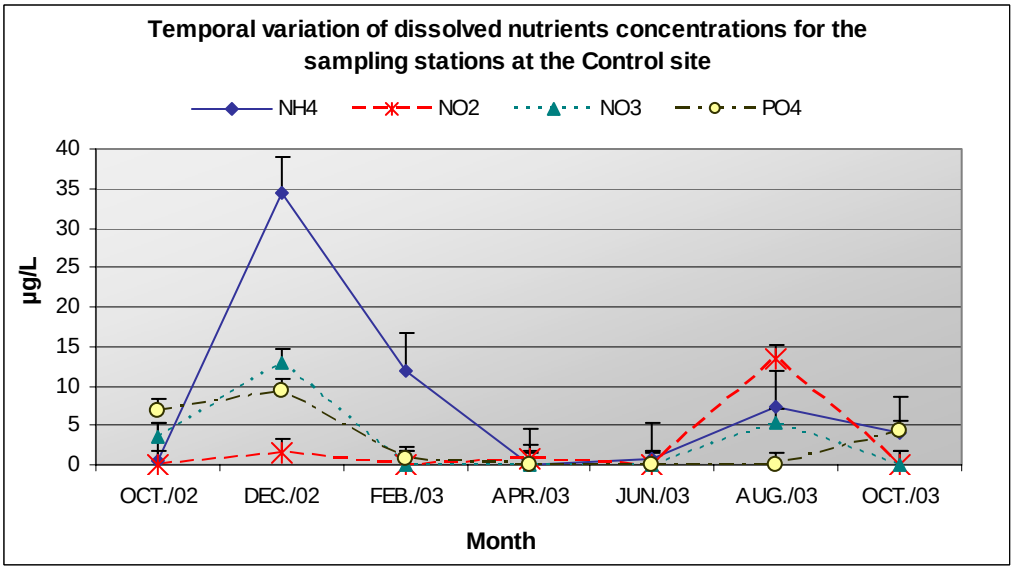

Figure 13. Temporal variation of dissolved nutrient concentration (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) for the control site.

Temporal variation of mean ammonia-N concentration for the *R. canadum* and *L. analis* cages, and the control site, showed that the highest values ranged from 30.81 to 34.45 µg/L during December 2002; the lowest concentrations for the *R. canadum* cage were found during April 2003 with mean values of 0.22 µg/L, and for the *L. analis* cage in June 2003 with mean values of 2.23 µg/L. Likewise, the control site showed the lowest concentrations in these months (Fig. 14).

The pattern for these nutrient values was similar for both cages and for the control site except for two months: in October 2002, when this concentration was lower at the control site compared to each cage, while in August 2003, the control site had the highest value. These results also suggest seasonal fluctuations and not differences due to culture.

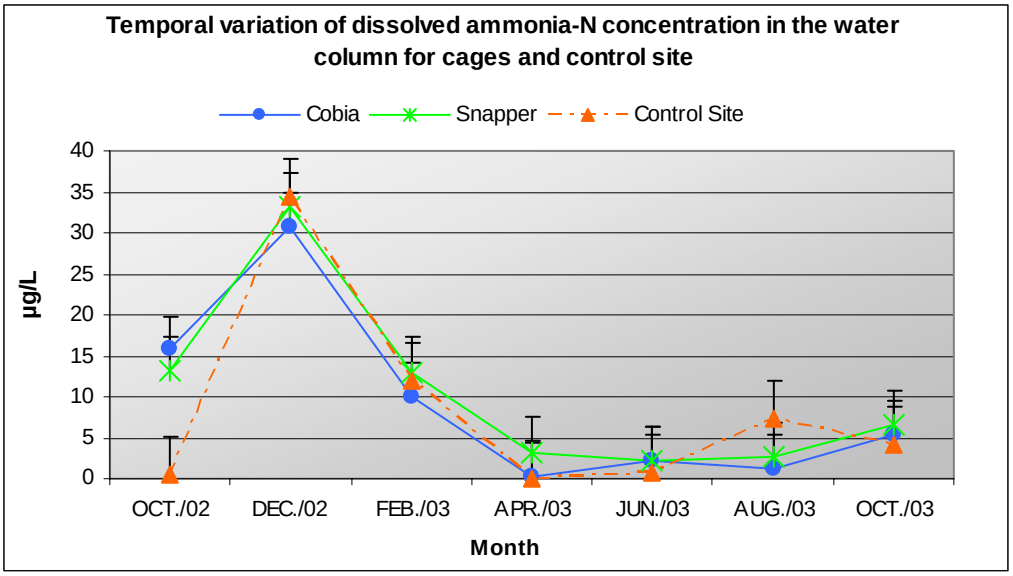

Figure 14. Temporal variation of dissolved ammonia-N (NH_4) concentration (µg/L) in the water column for *R. canadum* and *L. analis* cages and for the control site.

Contrast analyses for means for ammonia-N showed significant differences only among the control site versus the intermediate station located between each of the cages, and between the sample taken beneath both the cages versus the control site, contrasts 4 and 5, respectively. For the other stations, means for ammonia-N indicated a similar response among each cage and the control site (Table 6).

Table 6. Contrast analysis for dissolved nutrient ammonia-N (NH_4) concentrations among sampling locations. Values with ***** represent significant differences (P< .05).

Source - Sites	*df*	MS	*F*	p
cobia vs. snapper	1	0.000048	0.92	0.3385
snapper vs. control site	1	0.000180	3.41	0.0659
cobia vs. control site	1	0.000052	0.98	0.3239
control site vs. intermediate station	1	0.000480	9.03	0.0029 *
beneath cages vs. control site	1	0.000220	4.15	0.0423 *
upstream vs. downstream	1	0.000091	1.72	0.1905

Nitrite-N concentration was different from other nutrients, with the highest recorded values for each cage site and for the control site obtained during August 2003, with mean values from 13.18 – 16.97 µg/L (±1.3 s.d); during the remaining sampling periods the concentration remained below 3.55 µg/L (Fig. 15).

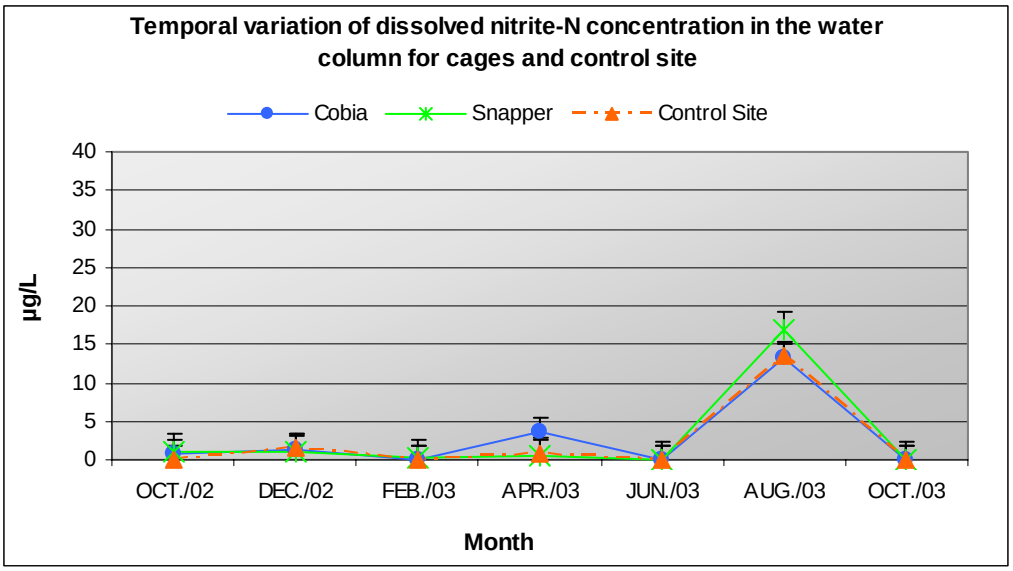

Figure 15. Temporal variation of dissolved nitrite-N (NO_2) concentration (µg/L) in the water column at for *R. canadum* and *L. analis* cages and for the control site.

Contrast analysis showed significant differences among *L. analis* cage versus the control site, *R. canadum* cage versus the control site; and under both cages versus the control site; contrast 2, 3, and 5, respectively (Table 7).

Table 7. Contrast analysis for dissolved nutrient nitrite-N (NO_2) concentrations among sampling locations. Values with * represent significant differences (P<0.05).

Source - Sites	*df*	MS	*F*	p
cobia vs. snapper	1	0.000001	0.82	0.3645
snapper vs. control site	1	0.000032	18.72	<0.0001 *
cobia vs. control site	1	0.000021	12.57	0.0005 *
control site vs. intermediate station	1	0.0000060	3.66	0.0566
beneath cages vs. control site	1	0.0000540	31.74	<0.0001 *
upstream vs. downstream	1	0.0000004	0.23	0.6336

Temporal variation of nitrate–N concentrations showed two peaks throughout the study period. The maximum value for both cages and the control site was found in December 2002, with concentrations ranging from 11.75 (± 2.00 s.d)

and 14.46 (± 2.00 s.d) µg/L. The other peak occurred for the *L. analis* cage and the control site, with concentrations less than 5.28 µg/L during August 2003 (Fig. 16). Both cages and the control site showed similar pattern, there were no significant differences among any sampling locations or among both cages and control site (Table 8).

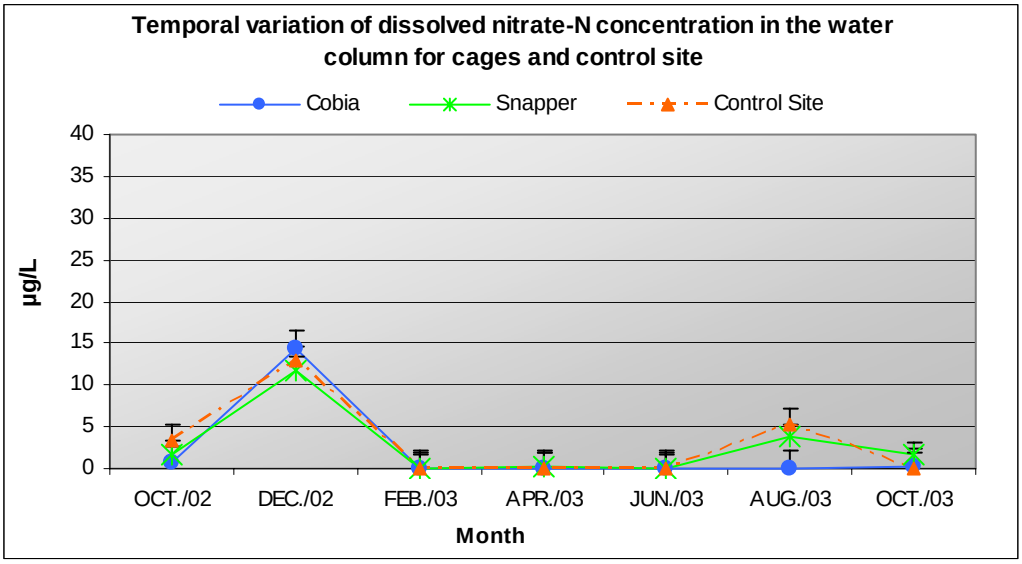

Figure 16. Temporal variation of dissolved nitrate-N (NO_3) concentration (µg/L) in the water column at for *R. canadum* and *L. analis* cages and for the control site.

Table 8. Contrast analysis for dissolved nutrient nitrate-N (NO_3) concentrations among sampling locations. Values with * represent significant differences ($P<0.05$).

Source - Sites	*df*	MS	*F*	P
cobia vs. snapper	1	0.000039	2.77	0.0971
snapper vs. control site	1	0.000007	0.49	0.4838
cobia vs. control site	1	0.000034	2.44	0.1196
control site vs. intermediate station	1	0.000010	0.70	0.3899
beneath cages vs. control site	1	0.000007	0.52	0.4710
upstream vs. downstream	1	0.000040	2.87	0.0913

Phosphate concentrations were uniform throughout all months, and were always less than 9.47 µg/L (±1.00 s.d) (Fig. 17). There were no significant differences among sampling locations, but significant differences occurred beneath each cage versus the control site (Table 9).

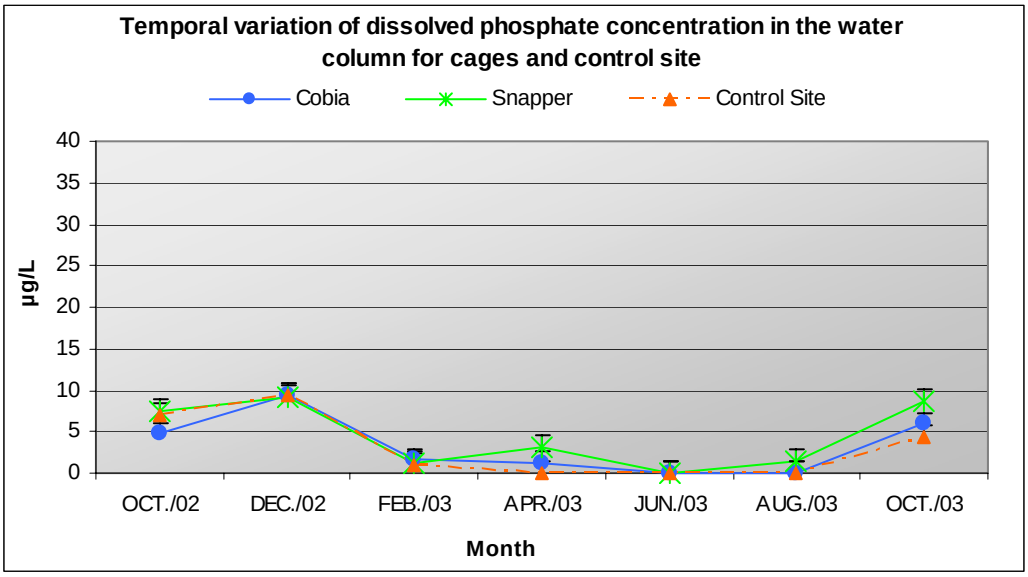

Figure 17. Temporal variation of dissolved phosphate (PO_4) concentration (µg/L) in the water column for *R. canadum* and *L. analis* cages and for the control site.

Table 9. Contrast analysis for dissolved nutrient phosphate (PO_4) concentrations among sample locations. Values with ***** represent significant differences ($P < 0.05$).

Source - Sites	*df*	MS	*F*	p
cobia vs. snapper	1	0.000180	3.81	0.0518
snapper vs. control site	1	0.000120	2.64	0.1049
cobia vs. control site	1	0.000009	0.20	0.6590
control site vs. intermediate station	1	0.000004	0.09	0.7677
beneath cages vs. control site	1	0.000280	5.99	0.0149 *
upstream vs. downstream	1	0.000003	0.07	0.7949

Ammonia-N and nitrate-N concentrations for the *R. canadum* cage indicated increased values at 26-m at the bottom of the water column ((12.92 ± 2.00 s.d and 6.34 ± 3.00 µg/L, respectively); otherwise, phosphate and nitrite-N had increased concentrations at the 8-m and at the surface of the water column (4.97 µg/L ± 1.00 s.d and 3.59 µg/L ± 1.00 s.d, respectively). Nitrate-N was only above detectable at the lowest depth (Fig. 18).

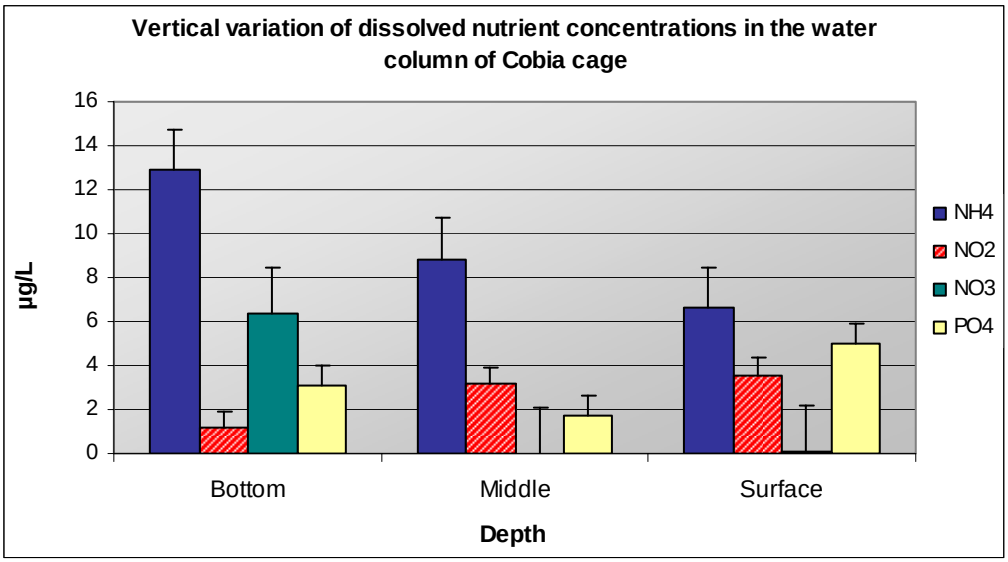

Figure 18. Vertical variation of dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column for the *R. canadum* cage.

Ammonia-N concentration in the water column was higher for the *L. analis* cage at 16 (middle) and 26-m (bottom) depths in the water column. The surface (8-m) depth was characterized by having the lowest concentrations of nitrate-N (0.26 µg/L), while phosphate was more stable at the three depths with regard to other cage and the control site (Fig. 19).

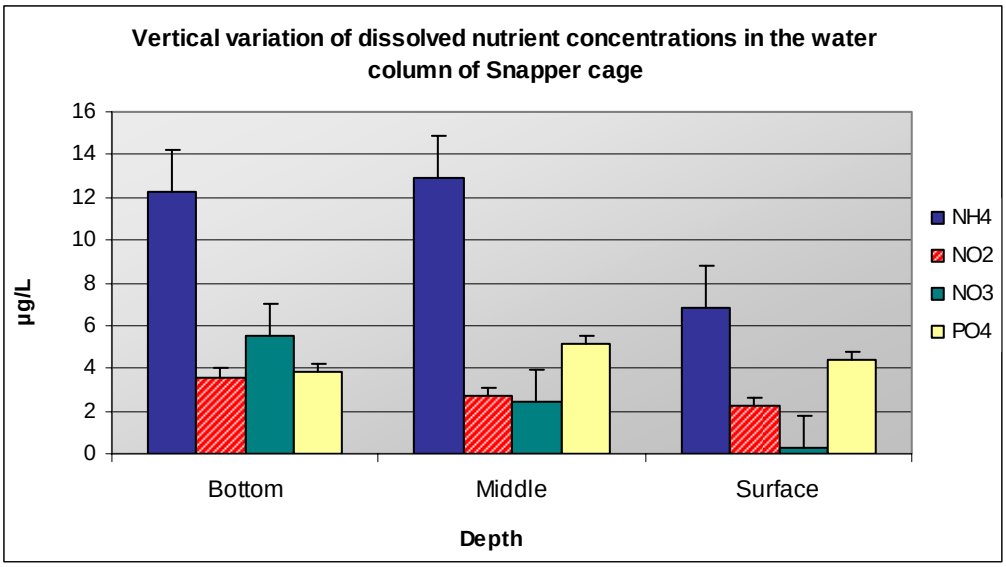

Figure 19. Vertical variation of dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column for the *L. analis* cage.

The control site had lower ammonia-N concentrations than the cages, while phosphate and nitrate-N were higher than the cages. Ammonia-N was higher at 16-m (middle depth) in the water column, with mean value of 11.29 µg/L, while the other nutrients were lower (less than 1.98 µg/L) at 16-m (middle) than at 8-m (surface) and 26-m (bottom) depths (Fig. 20).

Statistical analyses using contrast for a factorial design for each nutrient between depths consisted of: Contrast 7 (between surface versus middle depths), Contrast 8 (between surface versus bottom depths), and Contrast 9 (between middle versus bottom depth). Refer to the table 10 for ammonia-N, 11 for nitrite-N, 12 for Nitrate-N, and 13 for phosphate.

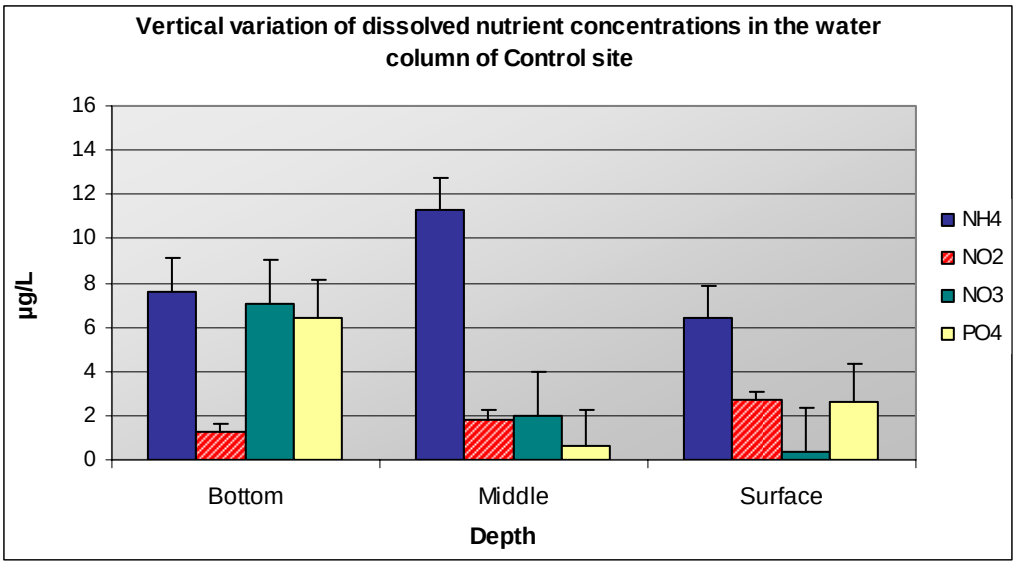

Figure 20. Vertical variation of dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column of the control site.

Temporal mean variations of ammonia-N in the water column showed similar patterns for the three depths. Higher concentrations were found during the first months of the culture period; from April 2003, these concentrations usually were less than 5.38 µg/L at every depth (Fig. 21); nevertheless, for contrasts 8 and 9, (Table 10) significant differences were found among surface (8-m) and middle (16-m) depths compared to the deepest (26-m) depth of the water column.

Table 10. Contrast analysis for dissolved nutrient ammonia-N (NH_4) concentrations among depths. Values with ***** represent significant differences ($P < 0.05$).

Source - Depth	*df*	MS	*F*	p
surface vs. middle depth	1	0.00014	2.67	0.1031
surface vs. bottom depth	1	0.00330	63.43	<0.0001 *
middle vs. bottom depth	1	0.00200	38.44	<0.0001 *

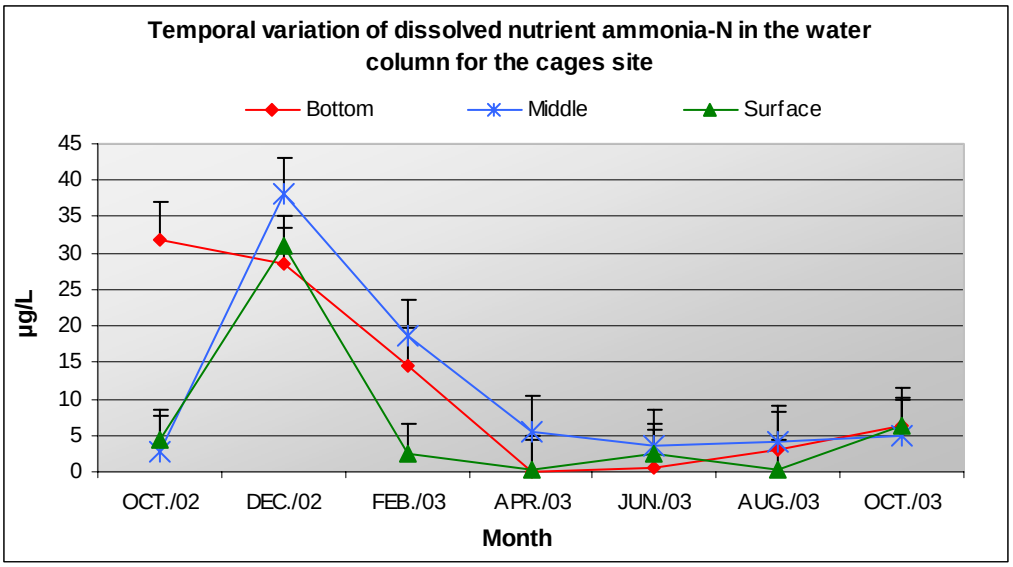

Figure 21. Temporal variation of dissolved nutrient ammonia-N (NH_4) in the water column among sampling locations for the cages site.

There were no temporal differences for the mean nitrite–N concentration for each depth for the cages sites. The maximum concentration was found during August 2003 for each of the three depths, with mean concentrations ranging from 14.14 (± 1.00 s.d) to 16.85 (± 2.00 s.d) µg/L (Fig. 22). There were no significant differences among 8-m (surface), 16-m (middle), and 26-m (bottom) depths of the water column (Table 11).

Table 11. Contrast analysis for dissolved nutrient nitrite-N (NO_2) concentrations among depths. Values with ***** represent significant differences (P< 0.05).

Source - Depth	*df*	MS	*F*	p
surface vs. middle depth	1	0.0000044	2.56	0.1104
surface vs. bottom depth	1	0.0000065	3.82	0.0516
middle vs. bottom depth	1	0.0000001	0.08	0.7802

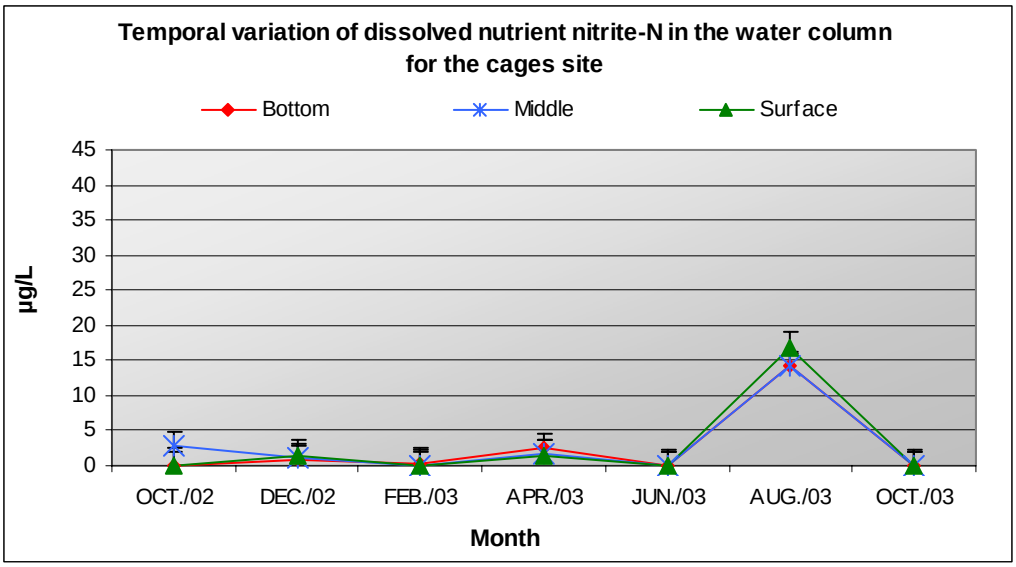

Figure 22. Temporal variation of dissolved nutrient nitrite-N (NO_2) in the water column among sampling locations for the cages site.

Nitrate-N concentrations were significantly higher (37.38 ± 2.00 µg/L) for the 26-m (bottom) depth during December 2002 (Fig. 23). Contrast analysis indicated significant differences between 8-m (surface) and 16-m (middle) depths, between 8-m (surface) and 26-m (bottom) depths, and between 16-m (middle) and 26-m (bottom) depths for contrast 7, 8, and 9 respectively (Table 12).

Table 12. Contrast analysis for dissolved nutrient nitrate-N (NO_3) concentrations among depths. Values with ***** represent significant differences ($p < 0.05$).

Source - Depth	*df*	MS	*F*	p
surface vs. middle depth	1	0.0021	151.42	<0.0001 *
surface vs. bottom depth	1	0.0036	257.52	<0.0001 *
middle vs. bottom depth	1	0.0001	9.82	0.0019 *

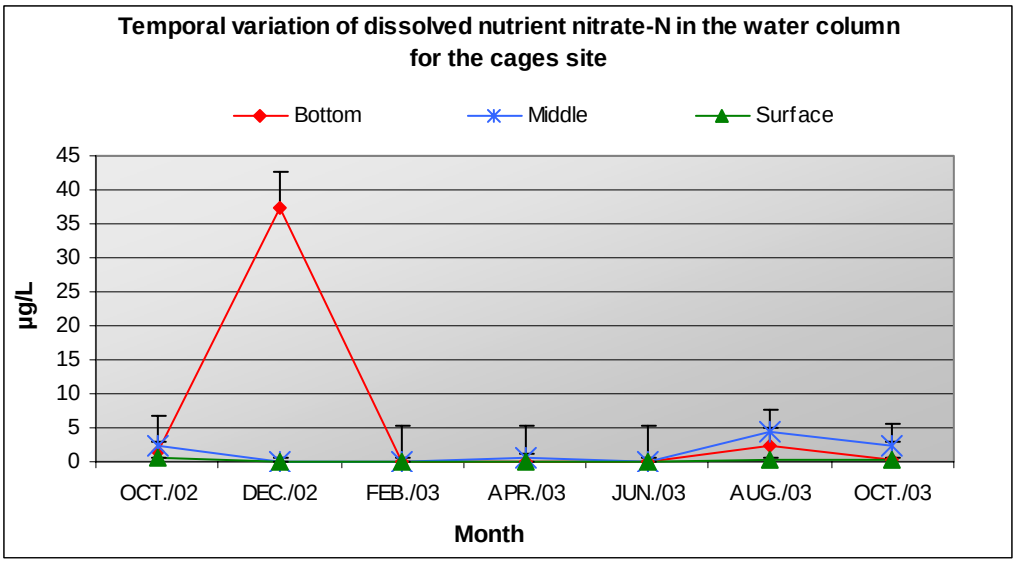

Figure 23. Temporal variation of dissolved nutrient nitrate-N (NO_3) in the water column among sampling locations for the cages site.

Phosphate concentration was more or less stable throughout the study, with means less than 13.89 µg/L for each depth; the maximum concentrations occurred during the first months of the study (Fig. 24). There were no significant differences in phosphate concentration for each depth to the cages site (Table 13).

Table 13. Contrast analysis for dissolved nutrient phosphate (PO_4) concentrations among depths. Values with ***** represent significant differences ($P < 0.05$).

Source - Depth	*df*	MS	*F*	p
surface vs. middle depth	1	0.000003	0.06	0.8115
surface vs. bottom depth	1	0.000120	2.58	0.1092
middle vs. bottom depth	1	0.000150	3.15	0.0767

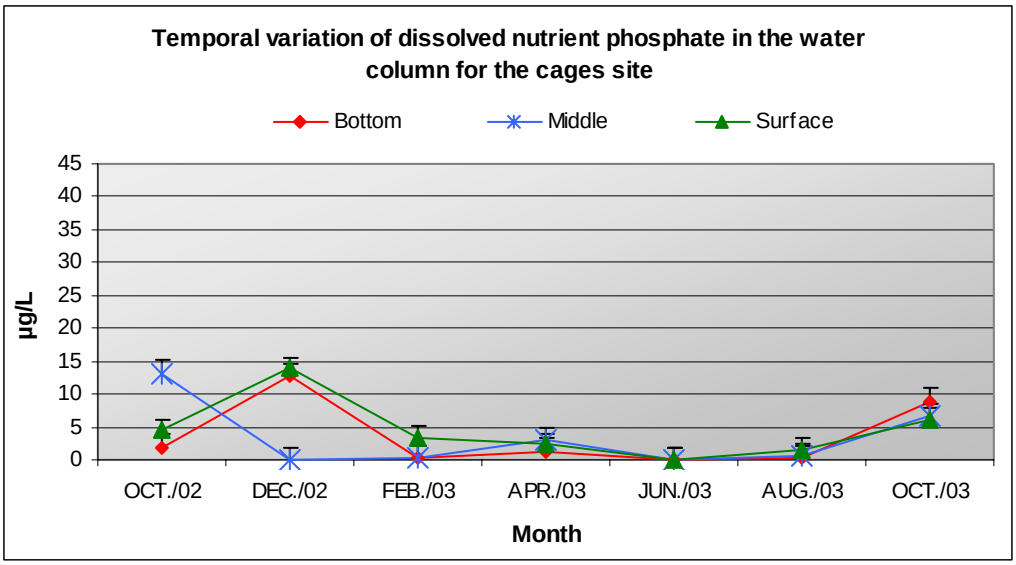

Figure 24. Temporal vertical variation of dissolved nutrient phosphate (PO_4) in the water column among sampling stations for the cages site.

Feeding rates were adjusted according to estimated fish growth; thus, *R. canadum* received more feed because more fish (4 fish/m^3) were stocked in the cage. Water analyses of dissolved nutrients in the water column indicated that when the amount feed increased, the nutrient concentrations decreased (Fig. 25). The *L. analis* cage was only stocked with 1.3 fish/ m^3, thus the feeding rates were lower and the fish were less crowded. However, water analyses of dissolved nutrients in the water column indicated no increase in the concentration when the amount of feed supplied was increased for the cage. An exception was that in both cages the mean nitrite-N concentration was highest when the feeding rate was highest at the end of the culture period (Fig. 26).

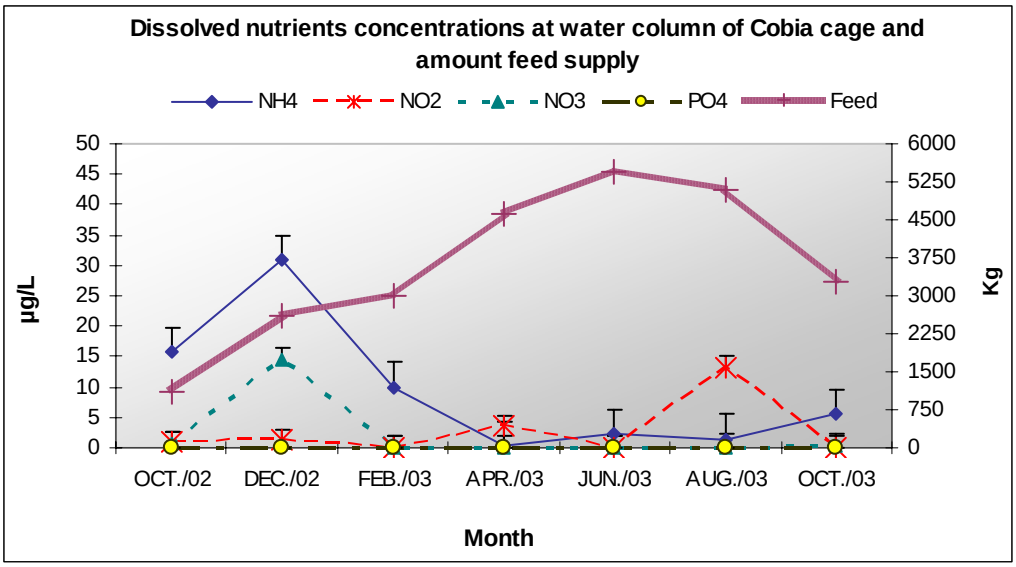

Figure 25. Temporal variation of dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column of the *R. canadum* (cobia) cage compared with feed supply (kg/month).

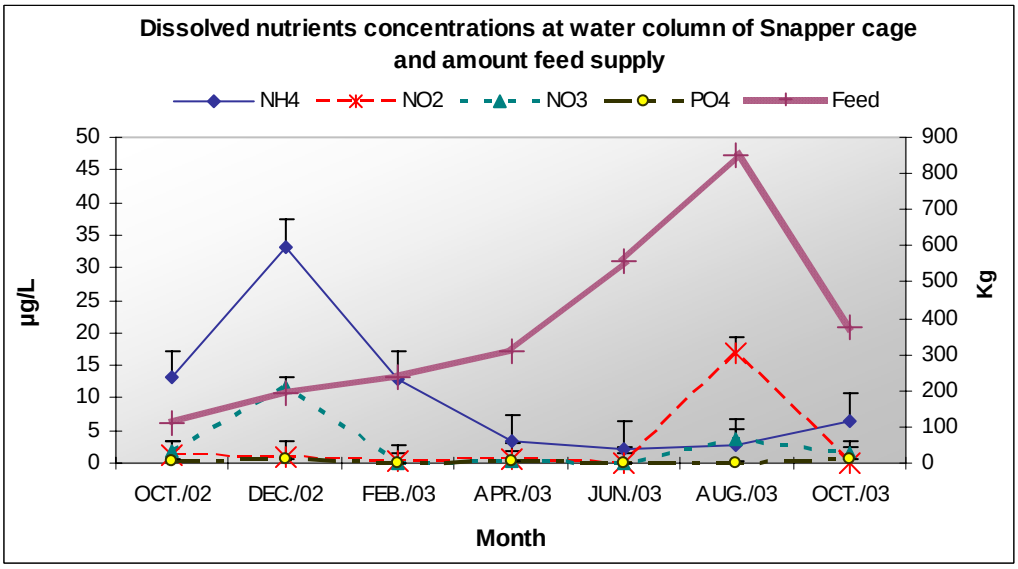

Figure 26. Temporal variation of dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column of the *L. analis* (snapper) cage compared with feed supply (kg/month).

The control site was a located 375 m south of the cages site, so it should not have been influenced by the cages due to physical characteristics of the site; i.e currents. Results indicate that during the monitoring period, the water analyses of nutrients dissolved in the water column at the control site did not increase with feeding at the cages site (Fig. 27).

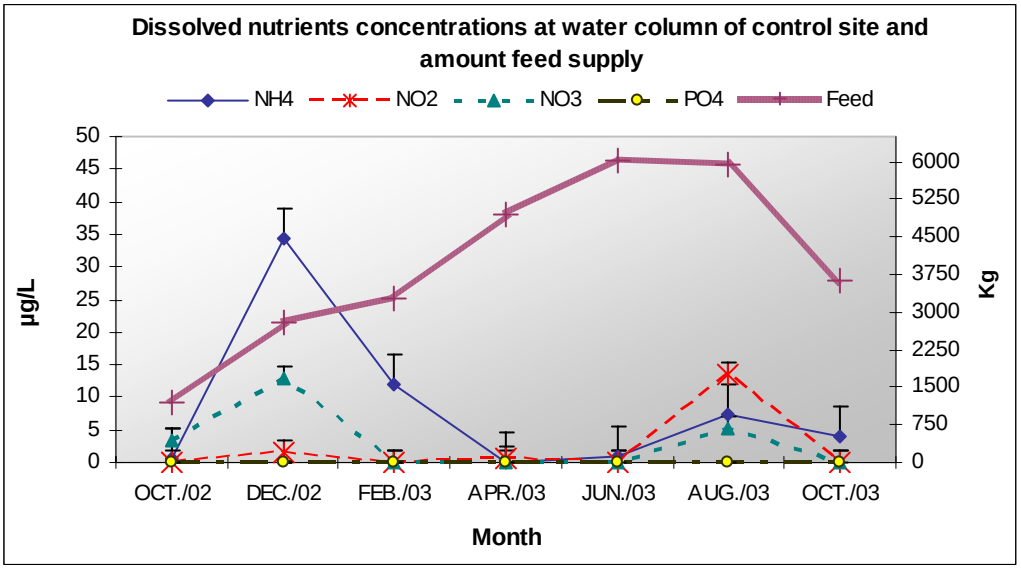

Figure 27. Temporal variation of dissolved nutrient concentrations (µg/L) of ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the water column of control site comparing with amount feed supply (kg/month) at the cages site.

Nutrients at interstitial water

Collection of interstitial water in sediments was difficult due to the consistency of the sand. Also, the method was not adequate to consistently obtain interstitial water. For instance, interstitial water sometimes "leaked" from the core samplers before analysis, or, the sand substrate itself was problematic because individual layers could not be kept distinct; thus, interstitial water represented water within 10 cm of sand collected during the sampling procedure.

In some cases, samples were not analyzed because they were lost due to the leakage and sufficient quantities of interstitial water were not obtainable to complete the analyses.

In general, mean ammonia-N had the highest nutrient concentration among the nutrients measured at the cage and control sites; the highest mean concentration occurred near the *L. analis* cage and was lowest at control site. Nitrite-N and nitrate-N concentrations were usually less than 0.003 mg/L at cage and the control site. Mean phosphate concentrations were higher at the cage site than at the control site (Fig. 28). Significance differences ($p<0.005$) were found for ammonia-N concentrations among months, nitrite-N concentrations among months and sampling locations, and nitrate-N concentrations among sampling locations. There were no significant differences in phosphate concentration for months and sampling locations (Table 14).

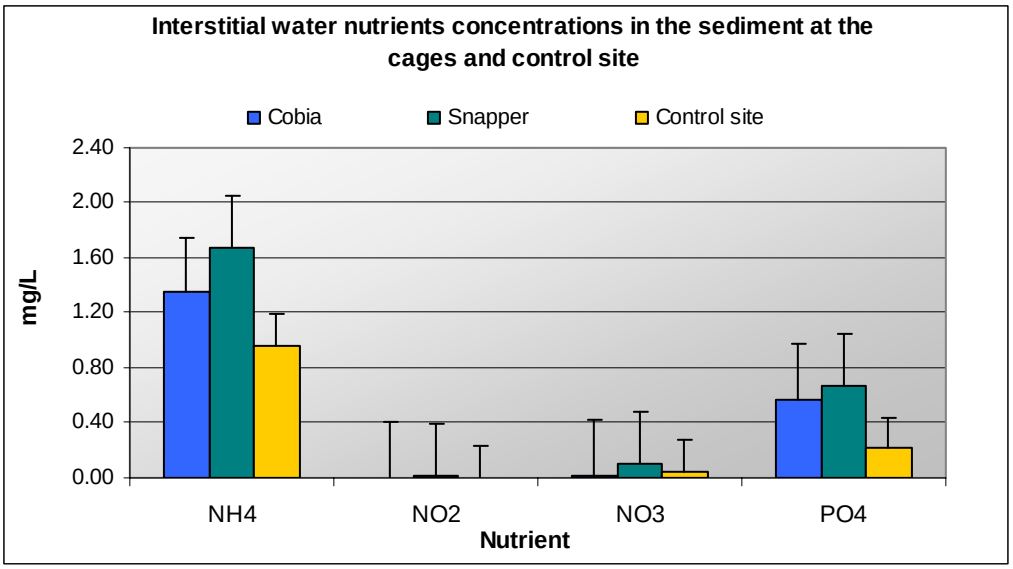

Figure 28. Interstitial water nutrients concentrations (mg/L) ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the sediment at the cage and control sites.

Table 14. ANOVA for dissolved nutrients concentrations comparing among months, and sampling locations. Values with * represent significant differences ($p<0.05$).

Source	*df*	MS	*F*	p
ammonia-N				
Months	6	4.18	4.51	0.0012 *
Sampling locations	15	1.60	1.27	0.2892
Months x S. locations	45	0.93	0.73	0.8219
Error	26	1.26		
Total	92			
nitrite-N				
Months	6	0.00073	2.81	0.0207 *
Sampling locations	15	0.00014	2.46	0.0213 *
Months x S. locations	45	0.00026	4.59	<0.0001 *
Error	26	0.00006		
Total	92			
nitrate-N				
Months	6	0.07	1.74	0.1326
Sampling locations	15	0.03	5.14	0.0001 *
Months x S. locations	45	0.04	6.75	<0.0001 *
Error	26	0.01		
Total	92			
phosphate				
Months	6	0.19	0.21	0.9726
Sampling locations	15	0.90	1.62	0.1373
Months x S. locations	45	0.93	1.67	0.0810
Error	26	0.56		
Total	92			

Temporal variation of nutrients at the *R. canadum* cage showed that none of the nutrients had seasonal abundance patterns. Phosphate obtained maximum mean values during June and August 2003. For ammonia-N, the minimum and maximum mean concentrations were obtained during April 2003 and August 2003, respectively. Nitrite-N was not detectable or had values lower than 0.003 mg/L, with the highest concentrations occurring in August 2003 (0.030 mg/L ± 0.005 s.d). Likewise, nitrate-N concentrations were usually less than 0.03 mg/L (± 0.01 s.d), with the highest mean value found in December 2002 (0.071 ± 0.02 mg/L) (Fig. 29).

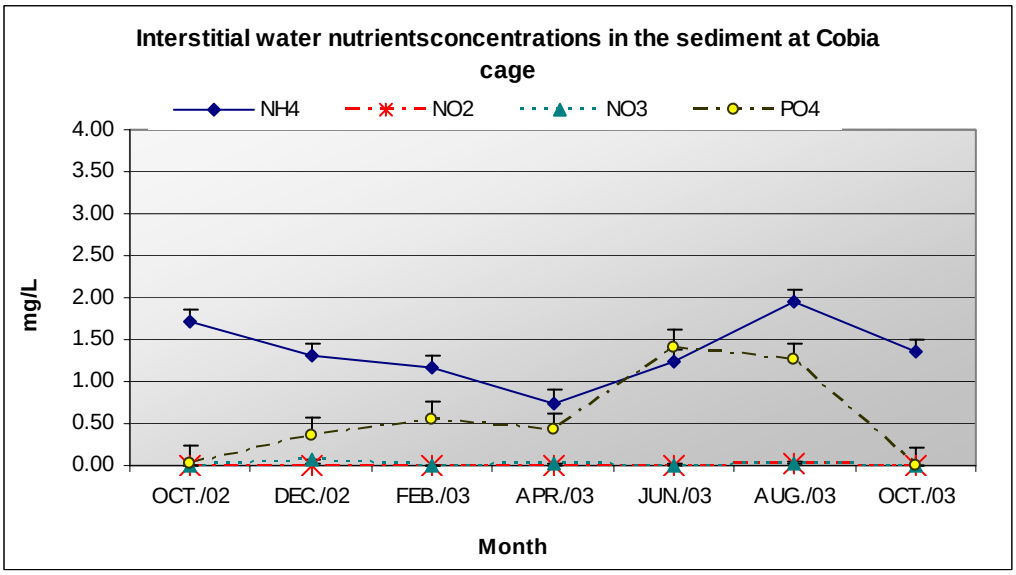

Figure 29. Interstitial water nutrients concentrations (mg/L) ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in sediment at the *R. canadum* cage.

The *L. analis* cage showed no seasonal patterns for the nutrient values. Ammonia-N again presented the highest means concentrations; its monthly, mean oscillated from 0.81 (± 0.10 s.d) to 3.16 (± 0.35 s.d) mg/L. Phosphate presented a very similar pattern for the *R. canadum* cage, with means fluctuating from 0.40 (± 0.005 s.d) to 0.93 (± 0.3 s.d) mg/L and the highest concentrations observed in April 2003. Mean nitrite-N and nitrate-N concentrations were either not detectable or had low values, with the highest concentrations occurring during December 2002 (0.043 mg/L and 0.51 mg/L, respectively) (Fig. 30).

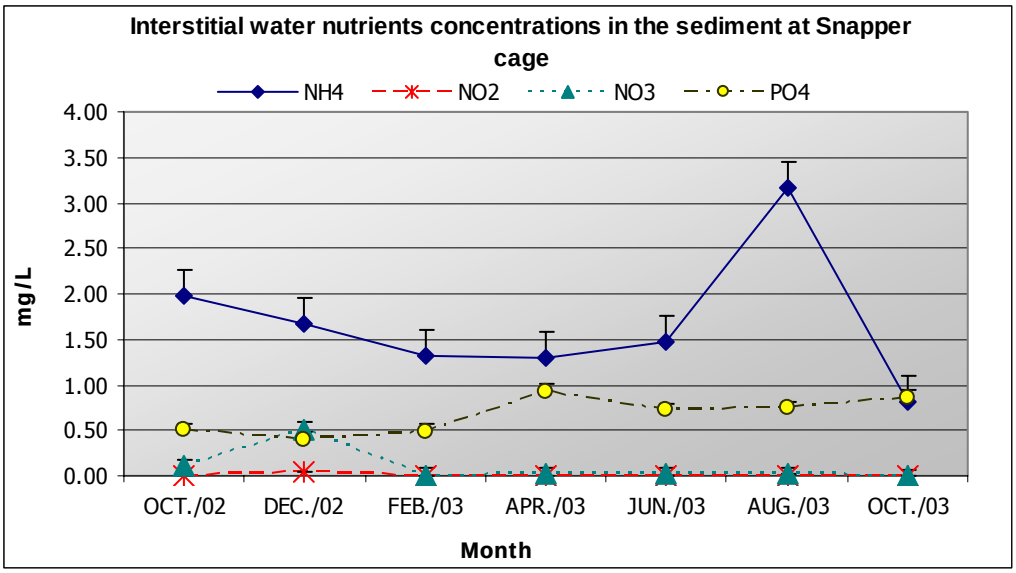

Figure 30. Interstitial water nutrients concentrations (mg/L) ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in sediment at the *L. analis* cage.

The control site had mean concentrations similar to or lowers than the nutrients at the cages site. The highest concentrations occurred mostly in December 2002 during the first sampling periods; however ammonia-N was also high during February 2003 (1.74 mg/L). Nitrate-N was only detectable during December 2002 (0.25 mg). Mean nitrite-N concentrations were less than 0.01 mg/L (Fig. 31).

Contrast analysis indicated significant differences for nitrite and phosphate between sampling stations at the *L. analis* cage versus the control site (Contrast 1), sampling locations at the *R. canadum* cage versus the control site (Contrast 2), and upstream versus downstream sampling locations (Contrast 3), (Table 15).

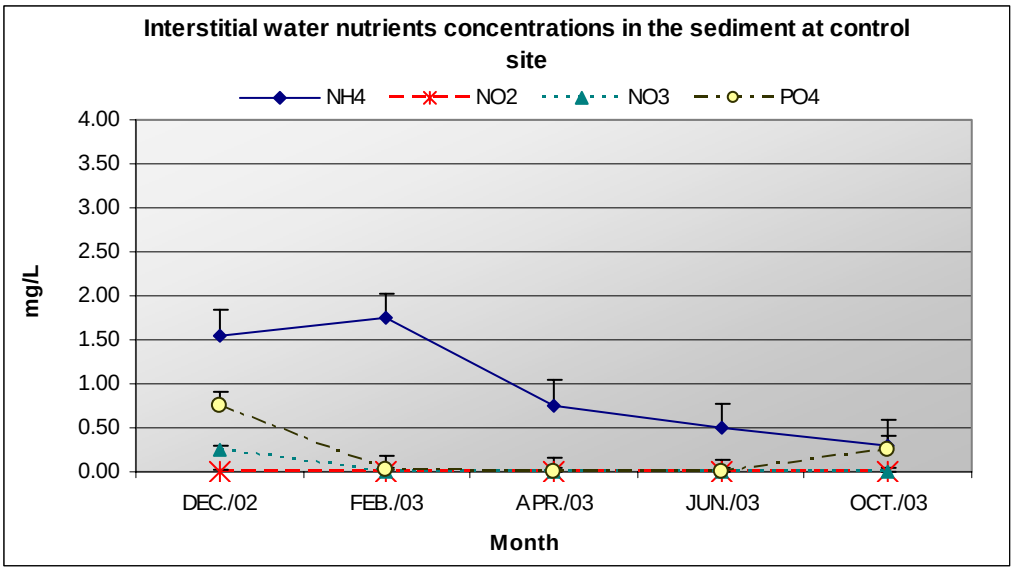

Figure 31. Interstitial water nutrient concentrations (mg/L) ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) in the sediment at the control site.

Table 15. Contrast analysis for dissolved nutrient concentrations among sampling locations. Values with * represent significant differences (P< 0.05).

Source - Depth	*df*	MS	*F*	p
ammonia-N				
snapper vs. control site	1	0.08	0.06	0.8023
cobia vs. control site	1	0.19	0.15	0.7038
upstream vs. downstream	1	2.39	1.89	0.1808
nitrite-N				
snapper vs. control site	1	0.00054	9.49	0.0048 *
cobia vs. control site	1	0.00047	8.32	0.0078 *
upstream vs. downstream	1	0.00048	8.49	0.0072 *
nitrate-N				
snapper vs. control site	1	0.00070	0.11	0.7383
cobia vs. control site	1	0.00067	0.11	0.7442
upstream vs. downstream	1	0.00220	0.36	0.5554
phosphate				
snapper vs. control site	1	13.14	23.57	<0.0001 *
cobia vs. control site	1	14.01	25.14	<0.0001 *
upstream vs. downstream	1	5.33	9.57	0.0047 *

A Pearson correlation was used to relate mean nutrient concentrations in the water column and in the interstitial water in the sediment. There was a positive correlation of mean ammonia-N concentrations in the sediment in relation to phosphate concentrations in the water column. There was also a positive correlation during the study for mean nitrate-N concentrations in the sediment compared with mean nitrate-N and nitrite-N concentrations in the water column (Table 16).

Table 16. Pearson correlation coefficients for mean dissolved nutrients ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) among the water column and interstitial water (Int) in the sediment. Values with ***** represent significant correlation.

	Int-NH4	**Int-NO2**	**Int-NO3**	**Int-PO4**
Water -NH4	0.42	0.04	0.0036	0.09
Water -NO2	0.00027	0.000012	0.99 *	0.19
Water -NO3	0.12	0.64	0.84 *	0.60
Water -PO4	0.88 *	0.18	0.01	0.12

Continuous monitoring

Water temperature was collected during 320 days at the cage site (December 2002 to October 2003). Maximum and minimum values were 29.4 and 26.6ºC, respectively, with a mean of 27.9ºC. The mean water temperatures declined slightly from December 2002 to February 2003 by approximately 1ºC and then increased to a maximum of 29.8ºC by October 2003 (Fig. 32). Changes throughout the year were less than 3.0ºC (from 26.5 - 29.5ºC); during most of the culture period temperatures were above 26.0ºC. Water temperatures were recorded during 120 days at the control site were similar to those at the cages sites.

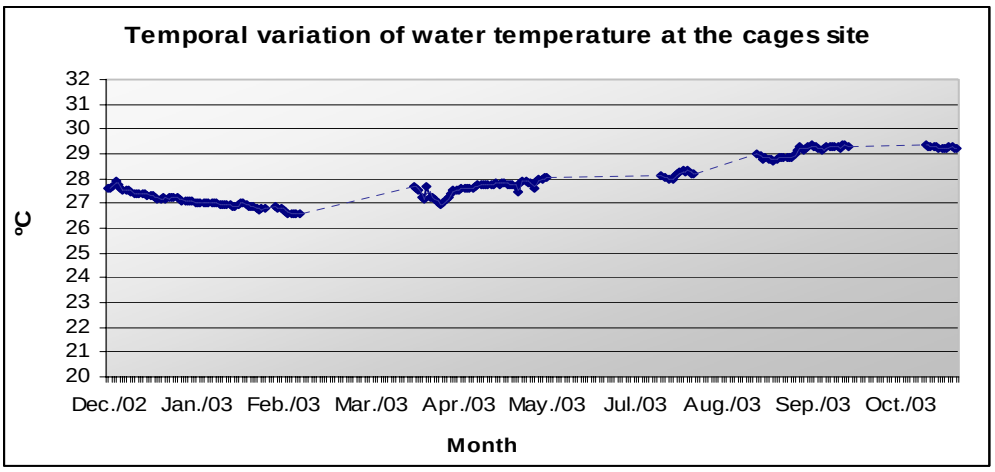

Figure 32. Temporal variation of water temperature at the cage site from December 2002 to October 2003; the dashed line indicates periods when temperatures were not measured.

Dissolved oxygen concentrations ranged from 2.7 to 6.8 mg/L from December 2002 to October 2003 (Fig. 33). The mean dissolved oxygen saturation in water was 5.6 mg/L. Dissolved oxygen for the control site was recorded for the last 120 days and generally remained in the same range as those of the cages site.

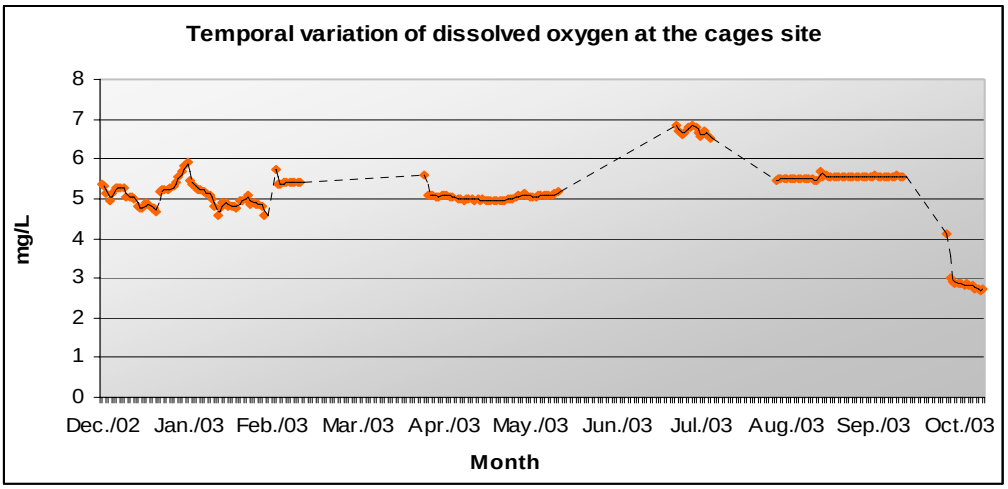

Figure 33. Temporal variation of dissolved oxygen concentrations at the cages sites from December 2002 to October 2003.

Salinity was homogenous from December 2002 to September 2003, with a mean of 34.6 and minimum and maximum values of 31.3 and 35.7, respectively (Fig. 34). Salinity decreased during October 2003. No differences in salinity were detected between the cage and the control sites. Values for the control site were recorded for the last 120 days and remained in the same range as those of the cages site.

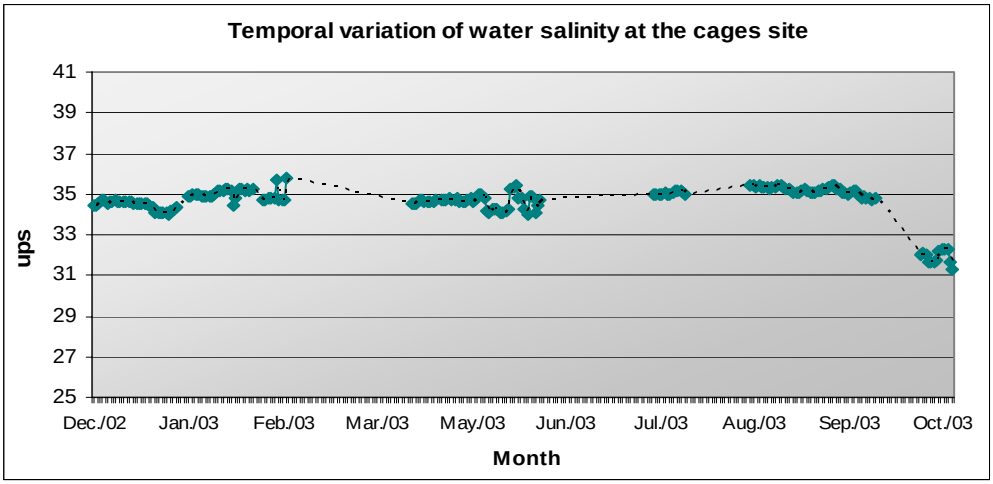

Figure 34. Temporal variation of salinity at the cages sites during December 2002 to October 2003.

Turbidity was recorded during 4 months (December 2002 to March 2003) and values were usually less that 1 NTU. The major fluctuations occurred in the last month (Fig. 35). Turbidity at the control site was registered during the last 120 days and showed the same pattern of the cages.

Chlorophyll-*a* concentrations at the cages site were recorded from December 2002 to March 2003 and oscillated throughout the months, with mean values of 7.37 µg/L (Fig. 36). The highest concentrations were found during the first months monitored. At the control site, chlorophyll-*a* concentration was monitored

during the last 120 days and exhibited values and fluctuations similar to those at the cages site.

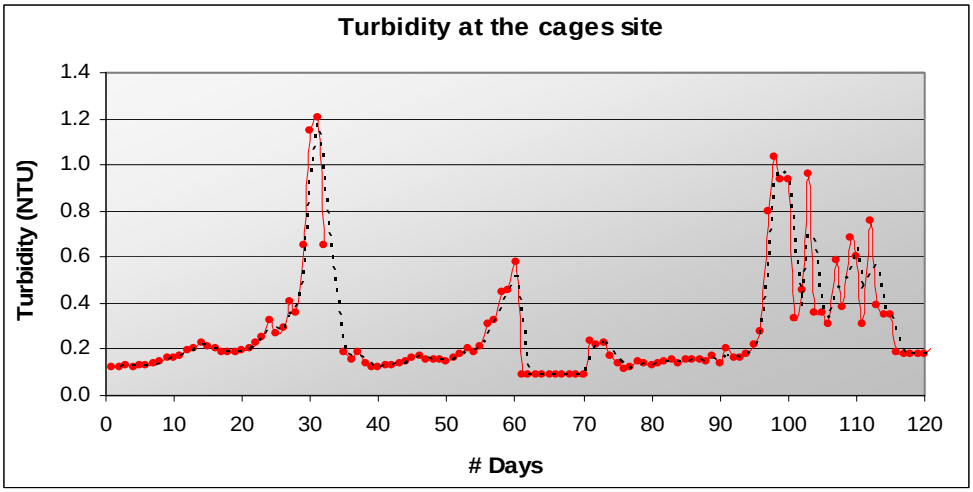

Figure 35. Temporal variation of turbidity at the cages site during December 2002 to March 2003.

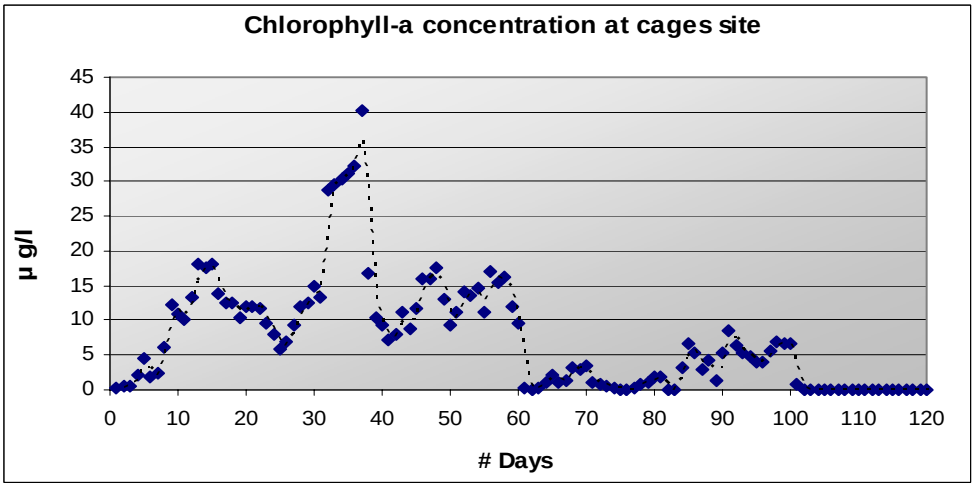

Figure 36. Temporal variation of chlorophyll-*a* at the cages sites during December 2002 to March 2003.

A Pearson correlation was used to relate mean nutrient concentrations in the water column with water temperature, dissolved oxygen concentration, salinity, turbidity, and chlorophyll-a concentration. There were no significant correlations

of mean ammonia-N, nitrite-N, nitrate-N, or phosphate concentrations in the water column in relation to water quality parameters (Table 17).

Table 17. Pearson correlation coefficients for mean dissolved nutrients ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) at the water column and water quality parameters (temperature, dissolved oxygen, salinity, turbidity, chlorophyll-a). Values with ***** represent significant correlation.

	Water -NH4	Water -NO3	Water -NO2	Water -PO4
Temperature	-0.15	0.36	0.46	-0.02
D. oxygen	0.56	0.44	0.39	0.62
Salinity	0.29	0.16	0.66	0.39
Turbidity	-0.15	0.52	-0.33	-0.39
Chlorophyll-a	0.34	0.12	0.71	0.29

Organic matter

There were no significant differences for organic matter percentages among the sampling stations for each cage and for the control site, with mean percentages fluctuating from 4.0 to 6.2%. Mean organic matter concentrations for the *R. canadum* and *L. analis* cages and at the control site were 4.9, 4.9, and 4.7%, respectively. Temporal variations showed the highest percentages during October 2003 at each cage and at the control site, while the months with the lowest percentage of organic matter for the *R. canadum* cage and for the control site were February and April 2003. The lowest percentage of organic matter for the *L. analis* cage was found in June 2003 (Fig. 37).

Stations of the *L. analis* cage generally, showed similar organic matter percentages. During October 2002 the south side of the cage had higher values and, while during October 2003 the values were lower for the sampling station west of the cage (Fig. 38).

The *Rachycentron canadum* cage showed a similar pattern among stations, and generally the percentage of organic matter was homogenous at all directions from the cage (Fig. 39).

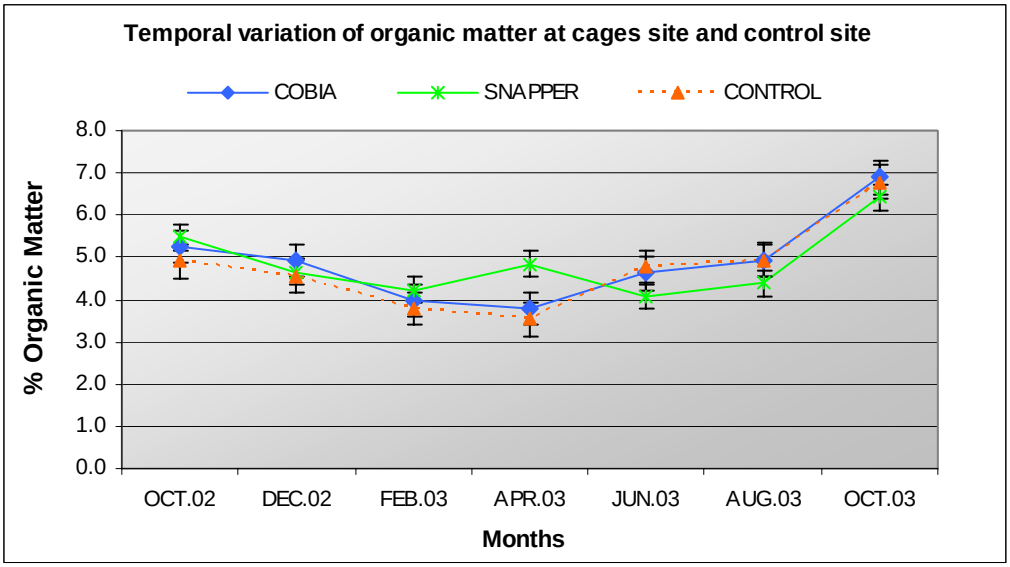

Figure 37. Temporal variation of organic matter at the *R. canadum* and *L. analis* cage, and control site.

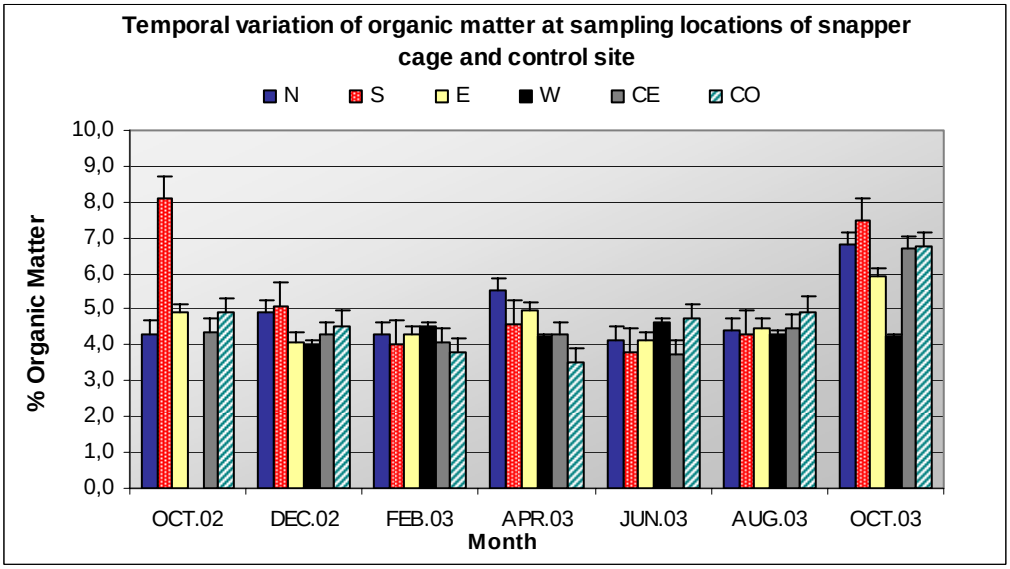

Figure 38. Temporal variation of organic matter at sampling locations of *L. analis* cage and control site.

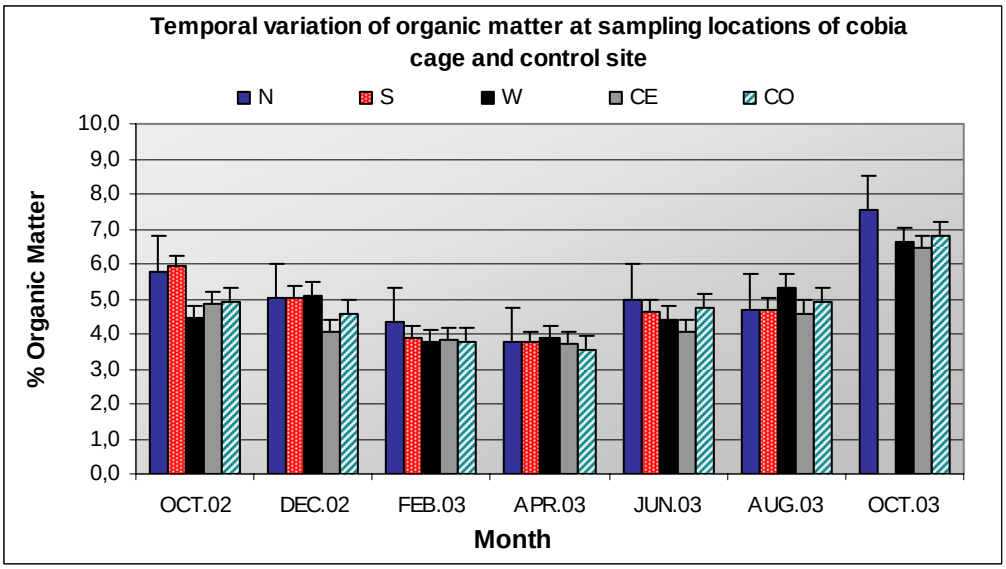

Figure 39. Temporal variation of organic matter at sampling locations of *R. canadum* cage and control site.

A Pearson correlation was used to relate mean nutrients concentrations in the water column and in sediment at interstitial water with percentage of organic matter. There were no significant correlations of mean ammonia-N, nitrite-N, nitrate-N, or phosphate concentrations in the water column, or in interstitial waters in relation to organic matter (Table 18).

Table 18. Pearson correlation coefficients for mean dissolved nutrients ammonia-N (NH_4), nitrate-N (NH_3), nitrite-N (NH_2) and phosphate (PO_4) at water column and at interstitial water in sediment and organic matter. Values with ***** represent significant correlation.

Nutrients concentrations	Organic matter
Column water -NH4	0.06
Column water -NO2	- 0.08
Column water -NO3	0.08
Column water -PO4	0.15
Interstitial water -NH4	-0.03
Interstitial water -NO2	-0.01
Interstitial water -NO3	0.02
Interstitial water -PO4	-0.07

V. DISCUSSION

Nutrient concentration at water column

Caribbean surface waters have a well-defined seasonal pattern with stratified waters due to currents from different directions (Capella et al., 2003). The water around Puerto Rico there is a locally mixed-layered, seasonal thermocline reaching a maximum of 100 m in the spring (January-March) and a minimum of 25 m in the fall (September-October). Density, temperature, and salinity present the same seasonal pattern as the thermocline, with variations due to the northward advection-mixing of South American riverine outflow especially from the Orinoco River in the eastern Caribbean Sea. Other factors that affect the local waters are the Amazon River waters entering the Caribbean in eddies that arrive at the Windward Islands from the east, with high chlorophyll content and low salinity that in transit can lose their patchiness and may become a homogeneous water mass (Müller-Karger et al., 1988; Corredor and Morel, 2001; Capella et al., 2003). Physical and chemical conditions of the waters around the region should be ideal for offshore aquaculture enterprises; likewise, nutrient concentrations that are normally oligotrophic in Caribbean waters could be higher from occasional influx of Orinoco waters reaching the Snapperfarm cage site.

Concentrations of ammonia, nitrite, nitrate, and phosphate in the water column were more or less stable during the study, with no seasonal trends. However, there were significant differences among months. In general terms, concentrations for these nutrients were in the same general range or lower than values obtained in other studies. For example, Grizzle et al. (2003), in their 3-yr

environmental study to monitor an open ocean aquaculture site in the Gulf of Maine, found that nitrate and nitrite concentrations ranged from ~1 to 16 µmol/L (46 - 900 µg/L) and phosphate concentrations from <0.5 to ~1.5 µmol/L (47.5 – 142.5 µg/L). Values from our study were less than 2.2 µg/L for each of these nutrients. It is important to note that Caribbean waters are oligotrophic and that nitrogen is a limiting nutrient (Corredor et al., 1999).

Nutrients concentrations in the water column were generally higher during the initial months of our study, suggesting that changes in nutrient concentrations were not due to effects from this operation, even though feeding rates increased throughout the culture period. The most likely explanation is that the mean current speed dispersed the nutrients quickly from the site. Karakassis (2000) reported that impacts in the water column are relatively low even in conditions without significant tidal currents.

Nutrient concentrations of ammonia and nitrite increased with depth, suggesting that some food particles were not consumed or completely dissolved. In this case, particles of feed, nutrients leached from the feed, or excretory products would probably exit the cage from near the middle or bottom. Karakassis et al. (2001) reported the highest nutrients concentrations from the surface layer, but their results are not comparable with the open-ocean environment.

Since there were no clear patterns of nutrient fluctuations between the cage and control sites, differences appear to be random environmental events. Statistical differences among samples did not suggest effects on the environment by nutrients released to the water column. Again, because of the strong currents in the area, these nutrients were probably quickly dispersed throughout the

water column without a tendency for nutrients to accumulate. Rapid consumption of the food would also minimize leaching of nutrients from feeds. Apparently, *R. canadum* consumed feed at a voracious rate while *L. analis* consumed it at a slower rate.

Water analyses indicated that both cages and the control site had similar nutrient concentrations, although the *L. analis* cage received significantly less feed than the *R. canadum* cage. The control site was located 375 m south of the cages so it should not have been influenced by the cage site. Results indicate that during the first year this operation had no negative effect on the quality of the water column. Mazzola and Sarà (2001), and Ye et al. (1991), indicate that intensive culture in open–ocean cages generates particulate organic matter in the form of suspended detritus composed of uneaten feed and fecal material; these wastes are dispersed throughout the water column and serve as a food resource for organisms that live in the sediment (mollusks, oyster, and clams) or water column (zooplankton, other fish). This may partly explain that in this study there was no increase in nutrient concentration despite the feeding rates received at each cage.

Variation of nutrient concentration apparently caused no negative effects on water quality at the cages site. Variations of the dissolved nutrient concentrations may be attributed to several factors, including differences in sampling time. Since sampling occurred at different times of the day.

Due to logistic problems centering on boat transportation, feeding the fish in the cages, routine maintenance of the cages, and the period available for sampling, samples were not taken at the same time each day. Rainfall and the influx of waters from the Orinoco River could have contributed to changes in

temporal nutrient concentrations. The maximum influence of the Orinoco River occurs in October (Müller-Karger et al., 1988; Corredor and Morel, 2001), when maximum values for dissolved nutrients were observed, but it is unknown if these variations were directly influenced by the Orinoco river plume.

Ammonia-N was the nutrient with the highest concentration during the study, but values were below that 40.0 µg/L. These concentrations are normal for Puerto Rican waters. Ammonia-N concentrations oscillated from <0.02 to 0.7 µMol/L (0.34 – 11.9 µg/L) (OTEC 1980) in a project located southeast of Puerto Rico.

Pitta et al. (1999) reported that ammonia concentration increased significantly at two cage culture fish farms in the Mediterranean Sea, compared with others nutrients concentrations, but they did not find significant differences when comparing samples from near the cages with samples from the control station.

Helsley (2000) found a slight elevation in ammonia-N levels downstream of the cage, compared with the other nutrients. These concentrations were in the same range of nutrients for the bay. Similarly, Karakassis et al. (2001) determined that the highest concentrations at three stations with depths among 13 – 30m depth, were about 8.0 µMol/L (136.0 µg/L) for ammonia, <0.4 µMol/L (38 µg/L) for phosphate, < 1.6 µMol/L (73.6 µg/L) nitrite, and <6.0 µMol/L (372 µg/L) for nitrate.

Nitrite and nitrate had low concentrations during the study, with mean values below 30.0 µg/L and 20.0 µg/L, respectively. Data reported by OTEC (1980) for these nutrients were about 20.0 µg/L for the first 100 m of depth. Nitrite-N and

nitrate-N was similar between cage and control sites, although some nitrite-N and nitrate-N accumulated under the center of each cage.

Phosphate concentrations were fairly uniform throughout all months and depths; these values (less to 10.0 µgl/L) are normal ranges for this area. Data reported by OTEC (1980) found that phosphate concentrations oscillated from 14 to 40 µg/L. In general terms these concentrations were low and similar for cages and control sites. Pitta et al. (1999) detected a significant increase in concentrations of phosphate and ammonium within the cages of Mediterranean fish farms, compared with a control site; however most of these farms were inshore and were not directly comparable with the open-ocean environment.

The presence of biofouling at the net of the cages could be remove nutrients from the water column that originate in the cage. Otherwise, some of the nutrients will presumably be taken up by the phytoplankton. The response of chlorophyll-*a* will take time to develop within algae in response to increased concentrations of nutrients. It would take 1 to 2 days for an algal cell to divide, so even if all of its photosynthetic needs are met, it would takes 8-16 days (8-9 cell generations) to develop an algal bloom (Brooks, et. al., 2002). A phytoplankton community could travel about 14 km from the location during that time. Thus, it is difficult to conclude that the nutrient additions from the farm, generally undetectable 30 m downstream, would have any effect at all on primary production even if the water body is nutrient limited (Brooks, et al., 2002).

Nutrients at interstitial water

Nutrients concentration in the sediment samples was slightly lower at the control site. Generally, the nutrient concentrations ranged among normal values, suggesting no accumulation of nutrients near the cages. Several studies about the environmental impact of cage aquaculture have reported benthic enrichment with organic material and accumulation of nitrogenous and phosphorous compounds beneath cages (Hall et al. 1990; Holby and Hall 1991; Holmer 1991), but, this did not occur in Culebra.

Karakassis et al. (1998) reported phosphate concentrations significantly higher in sediments beneath the cages, and Molina-Domínguez et al. (2001) determined that sediment phosphorus content varied with the distance from the cages and showed a seasonal pattern; meanwhile, nitrogen content was low and responded to a similar seasonal pattern. During our monitoring, an accumulation of nutrients in the sand substrate was not demonstrated, probably due to strong currents and the assimilation of high quality feed by the cultured fish. Uneaten feed and waste from the cages may have been consumed by other fish, crustaceans, or collected by the biofouling organisms which may have acted as a screen as particles exited the cage (Mazzola and Sarà, 2001; Ye et al., 1991). As farms add cages, more work is needed to determine if the increased dissolved nutrients and total suspended solids from uneaten food can be assimilated by the environment. Biofouling organisms attached to the cage and wild fish populations may increase in response to the heavier nutrient loading released from submerged cages. Benthic populations of organisms, especially polychaetes will probably increase. It will be important to monitor these environmental responses, especially to make sure that anaerobic conditions do develop.

Ammonia-N accumulation beneath the cages, especially just before harvest, may lead to environmental effects, especially in the sediments, as more cages are added to areas and if stocking rates and subsequent feeding rates are increased. Waste food and fecal material could accumulate beneath the aquaculture cages. Although significant effects have been reported at distances up to 100 m from the cages, in general it seems that this impact is usually localized, not exceeding 20 – 50 m around the cages (Beveridge, 1996; Weston, 1990). In the future as, stocking rates and the number of cages increase, the area could experience an accumulation of nutrients.

Ammonia principally had trend toward to accumulated at bottom of water column and beneath of the cages; it is because the most important impact of mariculture is the sedimentation of wasted food and fecal material under the farm cages, as well as was determined in several studies concerning environmental impact of cage aquaculture (Hall et al. 1990; Holby and Hall 1991; Holmer 1991).

Continuous monitoring

Water temperature registered mean values of 27.9ºC, decreasing during December 2002 and February 2003, and increasing through October 2003, typical of tropical oceanic surface waters in Puerto Rico, where the coldest months are December to February. Data for the area range from between 25 to 30ºC (Capella et al., 2003; O'Hanlon et al., 2003; Corredor and morel, 2001; OTEC, 1980). Each species has its own optimum growing temperature; the temperature ranges in Puerto Rico are optimum for culturing *R. canadum* (Liao, 2003).

Mean dissolved oxygen concentration throughout the study site was 5.1 mg/L. No differences were detected between the cages and control sites. The concentrations were characteristic for these waters, values for the area range from 4 – 7 mg/L (O'Hanlon et al. 2003; OTEC, 1980). Oxygen levels were optimal for appropriate growth of the organisms cultured. Strong currents, waves, and winds of the area help to maintain the water well-aerated.

Mean salinity was 34.6 psu, and remained homogeneous among cages and control site. These values were similar to the ranges of 33 to 37 psu reported by others (Capella et al., 2003; O'Hanlon et al., 2003; Corredor and Morell, 2001; OTEC, 1980). A significant change in salinity was not expected because this open-ocean site is not directly influenced by rivers or freshwater discharge. The variations in offshore surface salinities, principally during the spring and summer, are due to the northward advection-mixing of South American riverine outflow in the eastern Caribbean Sea, especially from the Amazon and Orinoco Rivers. The seasonal surface salinity range is therefore narrower northwards into the North Atlantic (Corredor and Morel, 2001). The maximum rainfall in the Orinoco River basins occurs in July, and the highest influence of the Orinoco River happens in October (Corredor and Morel, 2001; Yoshioka et al., 1985); salinity around of Snapperfarm cages site was lowest during October.

Turbidity in the water column was less than 1 NTU and indicated a similar tendency between the cage and control sites; fluctuations may have resulted from increased turbidity while Snapperfarm cleaned the cages. Otherwise there was a natural fluctuation of flocculants in the water column, especially during days with stronger currents. The increased turbidity could not be related to feeding activities.

Mean chlorophyll-a values were 7.37 µg/L, with no significant differences between the cage and control sites, indicating no negative impacts on the environment. Pitta et al. (1999) found lower concentrations (usually <1.0 µg/L) in the Mediterranean near an aquaculture cage farm. Karakassis et al. (2000) reported concentrations of 2 to 5 µg/L. Caribbean waters are oligotrophic; research in these waters has reported concentrations for chlorophyll-a from 0.4 to 0.98 mg/m^3 for depths between 50 to 125 m (Corredor and Morel, 2001). Increases in the concentration of chlorophyll-a and zooplankton biomass are attributed to the influence of the Amazon and Orinoco Rivers; the Amazon River outflow becomes entrained in pools or eddies that, after a circuitous trajectory through the Tropical Atlantic, arrive at the Windward Islands as pools of green (high chlorophyll content, low salinity) water entering the Caribbean from the east (Capella et al., 2003; Corredor and Morel, 2001; Yoshioka et al., 1985).

Organic matter

Sediment organic matter concentration fluctuated from 4.0 - 6.2% with no significant differences among stations and the control site. Significant differences over time were found at both cage and control sites only during October 2003, the last month monitored, with concentrations greater than for previous months. Because organic matter increased at the control site, as well as at the cage site, the changes was seemingly a natural occurrence. Strong currents which are characteristic of the sites probably prevented accumulation of waste from the cages.

Similar concentrations of organic matter were found by Molina-Domínguez et al. (2001) in open-ocean cages, which fluctuated from 3.5% to 6.0%, indicating no accumulation of solid particulate wastes from a farm after the first year of operation. Likewise, Grizzle et al. (2003), during a 4-yr study of open-ocean aquaculture off the coast of New Hampshire, found that organic matter concentrations remained below 3 mg/L and no significant impact was detected. However, Karakassis et al. (1998) reported high concentrations of organic matter (7.0 – 20.0%) in the first 4 cm of sediment below cages in the Mediterranean Sea, probably due to high feeding rates. Procedures are being developed to till the soil in these cases to avoid accumulation of organic matter in the benthos. Aquaculturists should avoid the build-up of organic matter because under anoxic conditions. Anoxic conditions usually indicate that the organic matter will take longer to decompose than in aerobic conditions. Anoxic conditions also lead to the release of hydrogen sulfide (H2S), methane (CH4), and nitrous oxide (N2O).

Surface concentrations and the vertical distribution of the sedimentary parameters studied by Karakassis et al. (1998) included studies of organic matter, organic carbon/nitrogen, chlorophyll *a*, phaeopigments, water content, and total phosphorous, each of which varied substantially by season and according to distance from the cages. In that study farm sediment showed high concentrations of organic matter phaeopigments and total phosphorous, as well as high water content, while the compact subsurface layer had concentrations close to those at the control site. The thickness of the farm sediment layer under the cages varied with season, but in all seasons it decreased rapidly the further the distance from the cages (Karakassis et al., 1998).

Although no significant differences in organic matter accumulation occurred below or near of the cages during this study, future increases in the number of cages and in the stocking rate will produce higher feeding rates for each cage. The resulting addition of nutrients needs to be monitored to determine the carrying capacity on the benthic area. Several authors consider that scattering of waste food and fecal materials are generally restricted to areas in the immediate vicinity of fish farms (Gowen and Bradbury 1987; Hall et al. 1990; Holmer 1991; Lumb et al. 1989).

Results of the first year indicate that this operation had no detectable environmental impact on quality of the water column, even though feeding rates were high in the cages. This is probably due to the hydrodynamic conditions at the site (wind, waves, and tides), as the amounts of water flowing through the cages removed the nutrients from the site. The information obtained from this study provides a basis to evaluate the feasibility of this operation, as well as to encourage the open-ocean aquaculture industry. As more cages are added to a site, more work must be done to measure the impact of additional nutrients to the environment, including impacts such as algal blooms a considerable distance downstream from the site. Increased algal populations could impact coral reefs by covering the corals.

VI. CONCLUSIONS

- Concentrations of ammonia-N, nitrite-N, nitrate-N, and phosphate in the water column were stable during the study, with no seasonal trends. This operation had no detectable environmental impact on water quality of the cage site, even though feeding rates were high. This is due to the hydrodynamic conditions at the site and the amount of water flowing through the cages.

- The cages and the control site had similar nutrient concentrations in the water column, although the *L. analis* cage received significantly less feed than the *R. canadum* cage. During the monitoring period this operation had no significant negative effect on the quality of the water column, so there were no clear patterns of nutrient fluctuations when comparing the cage and control sites.

- Nutrient input was quickly dispersed throughout the water column due of the strong currents in the area. Rapid consumption of the food would also minimize the release of nutrients from feeds. Apparently, *R. canadum* consumed food at a voracious rate and *L. analis* consumed it at a slower rate.

- Nutrient concentration in the water column was generally higher during the initial months of our study, suggesting that changes in nutrient concentration were not caused by this operation, even though feeding rates increased throughout the culture period.

- Although ammonia-N was the most abundant nutrient in the water column and in sediment interstitial water, these values, as well as values of other nutrients, were low and normal for these waters. Nutrient concentration of ammonia and nitrite increased with depth, suggesting that some food particles were not consumed or completely dissolved.

- An accumulation of nutrients at interstitial water at sediment was not clearly demonstrated, probably due to strong currents and the assimilation of high quality feed by the cultured fish. Uneaten feed and waste from the cages may have been consumed by wild fish, crustaceans, or collected among the biofouling organisms that attached to the cage.

- Increases in organic matter at the cages and the control site suggest that the accumulation was a natural occurrence. Strong currents characteristic of the sites probably prevented accumulation of waste from the cages.

- This study provides a basis to evaluate the feasibility of this operation, as well as to encourage the open-ocean aquaculture industry. A modified environmental monitoring program may be proposed for the long term, when the stocking rates are increased, which would include another form of sampling at the sediment and interstitial water. As more cages are added to the site, more work needs to be done to determine the impact of additional nutrients to the environment.

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