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Semi-Synoptic Sampling of Phytoplankton in Florida Keys National Marine Sanctuary

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INTRODUCTION

It is generally agreed that ecological changes are occurring in the FKNMS (EPA, 1992; Ogden et al., 1994). One of the suspected causes of some of these changes, increased nutrients, is well known to alter ecosystems. For example, increased nutrients can cause macroalgae to overgrow corals (Smith et al., 1981; Maragos et al., 1985) and for epiphytes to overgrow seagrasses (Tomasko and Lapointe, 1991; Lapointe et al, 1994). It has been estimated that there are 25,000 cesspools and septic tanks, 281 injection wells, 4 active and 10 inactive landfills, 182 marinas with 2707 wet slips, and 1410 live-aboard boats in the Florida Keys (EPA, 1992). These sources along with several sewage outfalls, high nutrient water in Florida Bay, and other human activities are thought to be injecting nutrients into FKNMS waters, but in most cases are not yet proven to be significant. Our goal was to determine if any of these potential sources are actually causing eutrophication in FKNMS.

The basic objective was to detect nutrient inputs that may be highly localized or ephemeral. To do this, we measured a large number of samples, by using a rapid, inexpensive method of detecting nutrient eutrophication that can be carried out within a reasonable budget. The method is based on the fact that most nutrients are quickly taken up by plants in shallow tropical waters and phytoplankton increase their biomass quickly in response to nutrients, so that the measurement of chlorophyll as an indicator of plant biomass is a better and more sensitive indicator of nutrient eutrophication than is the measurement of the residual nutrients.

METHODS

Water samples were collected from small boats. A total of 883 samples were taken in this study. At each station, determined with a GPS instrument, one liter of water was taken at a depth of 0.5 meters, and temperature, salinity and turbidity were measured with a YSI-CTS meter and a LaMotte 2008 Turbidimeter. In vivo chlorophyll fluorescence was measured with a Turner Designs 10-000R or 10-AU fluorometer, 10^{-5}M DCMU was added and fluorescence once again measured. 100 ml of water was preserved with 5%

formalin buffered with sodium tetraborate and another 100 ml was frozen so that other analyses can be conducted in the future.

Three 100 ml replicate water samples were filtered (after adding 1 mg of $MgCO_3$) through GF/F glass fiber filters and the filters were frozen until extracted (within a few days). These filters were extracted for 30 minutes with 10 ml of dimethyl sulfoxide and then with an added 15 ml of 90% acetone at 5°C overnight and measured fluorometrically before and after acidification for the measurement of chlorophyll and phaeopigment concentrations (Burnison, 1980; Parsons et al., 1984). Fluorescence measurements were made with a Turner Designs 10-000R or 10-AU fluorometer equipped with an infrared-sensitive photomultiplier and calibrated using pure chlorophyll a.

On November 22, 1996, we participated in the inter-laboratory calibration study on the measurement of chlorophyll sponsored by the Florida Bay Program Management Committee. Our measurements compared favorably with 6 of the 7 laboratories that participated.

We also conducted an internal intercomparison of different methods in our own laboratory for the measurement of chlorophyll. For this, on February 6, 1997, water was collected at 5 stations in Florida Bay:

#1	Rabbit Key Pass	24° 58.474'N 80° 49.594'W
#2	Rabbit Key Basin	24° 59.459'N 80° 52.522'W
#3	Oxfoot Bank	25° 00.944'N 81° 00.284'W
#4	south of Cape Sable	25° 05.678'N 80° 59.694'W
#5	between Rankin Key and Tin Can Channel	25° 07.046'N 80° 48.442'W

That evening the water was filtered and extracted overnight in 100% acetone. The following instruments were compared:

3 Turner 10-000R fluorometers equipped with infrared sensitive photomultipliers, D-daylight bulbs, 3-66 reference filters, 5-60 excitation fiters, and 2-64 emission filters.

2 Turner 10-AU fluorometers equipped with infrared sensitive photomultipliers, D-daylight bulbs, 10-032 reference filters, 10-AU-045 excitation filters, and 10-AU-050 emission filters.

Turner 10-AU fluorometer equipped with an infrared sensitive photomultiplier, 10-089 bulb, 10-032 reference filter, 10-113 excitation filter, and 10-115 emission filter (Welschmeyer technique).

Hitachi F-4500 Fluorescence Spectrophotometer with 2.5 nm bandwidths set at 432 nm excitation and 667 nm emission

Shimadzu UV 2100U UV-VIS Recording Spectrophotometer.

Gilson HPLC system with 2 model 302 pumps, 811B mixer, 802B manometric module, 621 data module and an Rheodine injector (100ul sampling loop) with

a Shimadzu SPD-6AV UV-VIS detector set at 452 nm, and with an 8 microliter flow through cell and a Shimadzu CTO-6A column oven modified for cooling as well as heating. A Viadac 201TP C18 reverse phase column was run at 34°C using a binary solvent system (80:20 methanol:0.5M ammonium acetate and 80:20 methanol:acetone).

Results are shown in Table 1. Although there is more variability than we would like, we see no evidence of any systematic difference between the methods. The high spectrophotometric measurement on sample 5 is not surprising, as we know that method is inaccurate at low concentrations of chlorophyll. We concluded that our measurements of chlorophyll are accurate.

RESULTS

We found increasing chlorophyll in Hawk Channel and over the reefs as we move from the upper Keys to the lower Keys (Fig. 1). It is fairly clear that water from Florida Bay causes at least some of the increase in the middle and lower Keys, but sewage input from the Keys may also be a factor. The net flow of water in Hawk Channel is from the northeast to the southwest (Pitts, 1994), and chlorophyll increases as it moves along the Keys. We found an increase in chlorophyll along the transect from Elliot Key to Plantation Key (and Pacific Reef to Crocker Reef) where virtually no Florida Bay water would be expected to be getting into Hawk Channel.

In a transect from the Gulf of Mexico over the Key West sewage outfall and out through Hawk Channel to Sand Key we observed higher chlorophyll around the Key West sewage outfall (Fig. 2).

We observed higher chlorophyll in the various canals in the Keys, some as high as 17.7 μg/l. The canals are quite different from each other, with some having much higher chlorophyll concentrations than others. This may indicate that some septic systems are much "leakier" than others. Chlorophyll concentrations usually drop dramatically not too far outside the canal mouths (Fig. 3).

We observed plumes of low salinity, nutrient-rich, high chlorophyll water from the Keys out to the reefs on occasion, particularly when strong winds are from the north. Quite often, one observes plumes out into Hawk Channel but they do not make it out to the reefs and the water over the reefs appears quite clear. Sometimes, however, one can observe plumes of turbid water all the way out to the reefs and beyond. For example, on March 23 we observed a plume of very turbid, high chlorophyll water out over the reefs at Looe Key, which can be observed in the uncorrected satellite image of reflectance generated by Dr. Richard Stumph (Fig. 4). We actually observed increasing turbidity and chlorophyll as we went from Big Pine Key out to the reefs and beyond. We did not see declining turbidity and chlorophyll until well beyond the reefs. It can be seen in the satellite image generated by Dr. Richard Stumph that this water apparently originated in western Florida Bay or in the area west of Key West, perhaps somewhere around the Marquesas. It appears this water was pushed by the northerly winds into the Gulf Stream, which picked it up and carried it east out over the reefs. This plume appears to be squeezed between the Gulf Stream and the coastal countercurrent as a narrow front. There appears to be

considerable structure in the front with the salinity (Fig. 5) and phosphate (Fig. 6) minimum more inshore, the turbidity (Fig. 7) maximum more offshore, and a relatively broad chlorophyll (Fig. 8) maximum.

We generally observe lower concentrations of chlorophyll in shallow waters dominated by seagrasses and/or macroalgae, suggesting that grazing by animals attached to these macrophytes are facilitating the transfer of nutrients from the water column to the benthic community. For example, in a transect through Pine Channel, one sees lower chlorophyll in the channel than in Hawk Channel or in the open water to the north (Fig. 9). In this particular instance, larger scale events that generate higher chlorophyll both north and south of the keys can be seen by satellite imagery. There may well be nutrients coming from land, but we cannot detect it on this scale because of the overwhelming signal from outside the keys. We must then ask why the high chlorophyll water is not coming through Pine Channel from one side or the other. We suspect it is, but the phytoplankton are being filtered out of the water by animals attached to the various macrophytes found in the shallow waters of Pine Channel. This was also observed in an earlier study of Biscayne Bay (Brand et al., 1991). There are much higher concentrations of chlorophyll in the north bay than in the south, except next to the only large seagrass meadow remaining in north Biscayne Bay. We think chlorophyll concentrations were consistently lower there because of grazing by animals attached to the seagrass blades.

We have found the biomass of benthic microalgae per area to be generally 20 to 40 times higher than planktonic microalgae. We found from 5 to 72 mg chlorophyll a/m^2 in the sediments, with most values from 20 to 40 mg/m^2. Typical concentrations in the water column above are around 1 mg/m^2. As a result, we also found that wind-driven resuspension events lead to higher chlorophyll concentrations in the photic zone. Waters with higher turbidity tend to have higher chlorophyll concentrations (Figs. 10-15), although there is considerable scatter in the data. The field data and laboratory experiments however demonstrate that resuspension of sediments does not inject sufficient amounts of nutrients to allow the phytoplankton to bloom. The increase in chlorophyll concentration appears to be primarily simply the result of the transfer of benthic microalgae into the water column.

DISCUSSION

It appears that chlorophyll increases as the coastal current moves from the northeast to the southwest in Hawk Channel. The two most likely sources of nutrients to generate this pattern are sewage from the Florida Keys and algal blooms and nutrients from Florida Bay. At this time, it is impossible to separate the two potential causes, but the most likely scenario can be hypothesized for further testing. In the Upper Keys, which are heavily populated, there are only a few small channels where Florida Bay water could get into Hawk Channel. Furthermore, water in northeast Florida Bay is low in chlorophyll and phosphate, so what Florida Bay water might get into Hawk Channel probably would not have much effect in the Upper Keys. The Middle Keys, however, have a much smaller human population, but large passes that are well

documented to allow large amounts of water from Florida Bay to reach Hawk Channel. Furthermore, the Long Key area is known to be downstream of the largest blooms in western Florida Bay. It is hypothesized that sewage is the cause for the increase in chlorophyll in the Upper Keys, and Florida Bay influx is the dominant cause for the increase in the Middle Keys. In the Key West area, the sewage outfall appears to have a large impact.

The most likely source of the nutrients that generate the high chlorophyll concentrations observed in many of the canals in the Florida Keys is the cesspools and septic tanks connected to the homes along the sides of the canals. While high concentrations of chlorophyll are observed in many of the canals, concentrations usually drop off dramatically in the shallow waters outside the canals. One hypothesis for this of course is simple dilution as the canal waters enter the coastal waters. An alternative hypothesis is also plausible.

Many times, we have observed much lower chlorophyll concentrations in shallow waters with beds of macroalgae or seagrasses than in nearby waters without benthic vegetation or waters that are deeper, both in Biscayne Bay (Brand, 1988; Brand et al., 1991) and in FKNMS (Brand, unpublished data). We hypothesize this is the result of animals attached to the macroalgae and seagrasses grazing on the phytoplankton as water passes by, as others (Lemmens et al., 1996) have observed. This is a mechanism by which phytoplankton (and ultimately nutrients) can be rapidly transported from the water column to the benthic community. Continued flux of elevated concentrations of phytoplankton by this mechanism would be expected to ultimately alter the ecological structure of the benthic community. Transects in and out of seagrass meadows and drogue studies following tidal flow through seagrass meadows can be used to test this hypothesis.

We have found that in the shallow waters of FKNMS, benthic microalgal biomass is typically 20 to 40 times as high per m^2 as phytoplankton biomass in the overlying water column (Brand, unpublished data). This reflects the greater availability of nutrients in the shallow sediments where light is still available. Just as in the water column, we find in the surface sediments much more of the nutrients are in the algal biomass than as dissolved nutrients in the porewaters (Brand and Szmant, unpublished data). Dissolved nutrients are the residual nutrients after the microalgae have increased their biomass as much as possible. Therefore, just as with water column phytoplankton, benthic microalgal biomass can be expected to be a much better indicator of eutrophication than the residual nutrients. Benthic microalgal biomass along transects from canals to adjacent coastal waters should provide an indication of the long term impacts of eutrophication and its spatial extent.

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Table 1. μg/l chlorophyll a

Method	#1	#2	#3	#4	#5
Turner 10-000R fluorometer A	0.66	3.98	4.06	6.59	0.57
Turner 10-000R fluorometer B	0.78	3.83	3.99	7.07	0.61
Turner 10-000R fluorometer D	0.70	3.81	3.87	6.65	0.56
Turner 10-AU fluorometer C	0.73	3.64	over	6.61	0.59
Turner 10-AU fluorometer E	0.74	3.86	3.88	6.53	0.56
Turner 10-AU fluorometer F (Welschmeyer)	0.65	3.47	3.60	5.83	0.57
Hitachi spectrofluorometer	0.74*	3.18	3.42	6.31	0.52
Shimadzu spectrophotometer				7.06	1.17
Gilson HPLC				5.52	0.44

* Because of very low fluorescence in the spectrofluorometer of a diluted sample #1 , one anomalously high data point was thrown out.

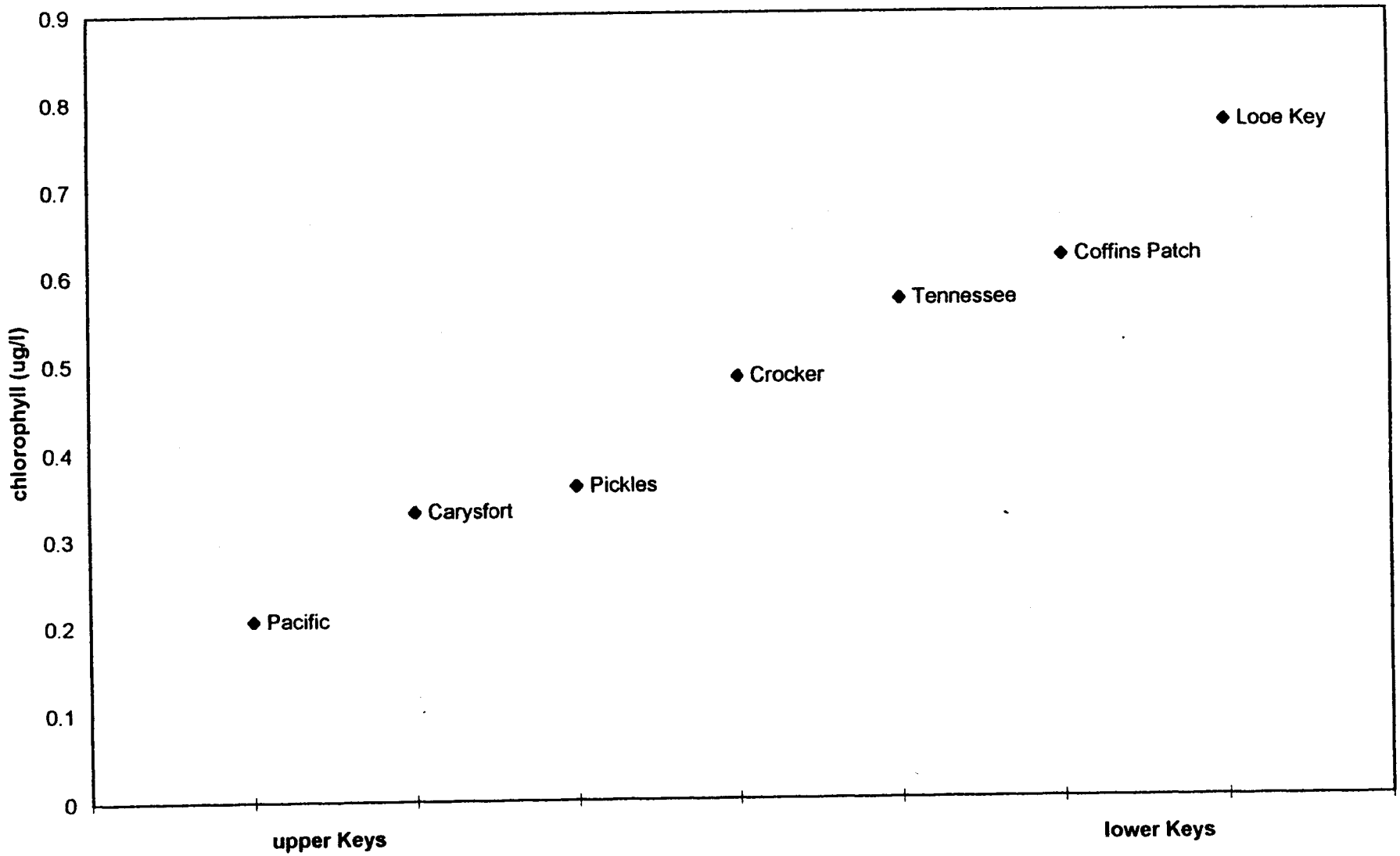
Average Chlorophyll - Hawk Channel and Reefs
chlorophyll (ug/l)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Looe Key
Coffins Patch
Tennessee
Crocker
Pickles
Carysfort
Pacific
upper Keys
lower Keys

Figure 1

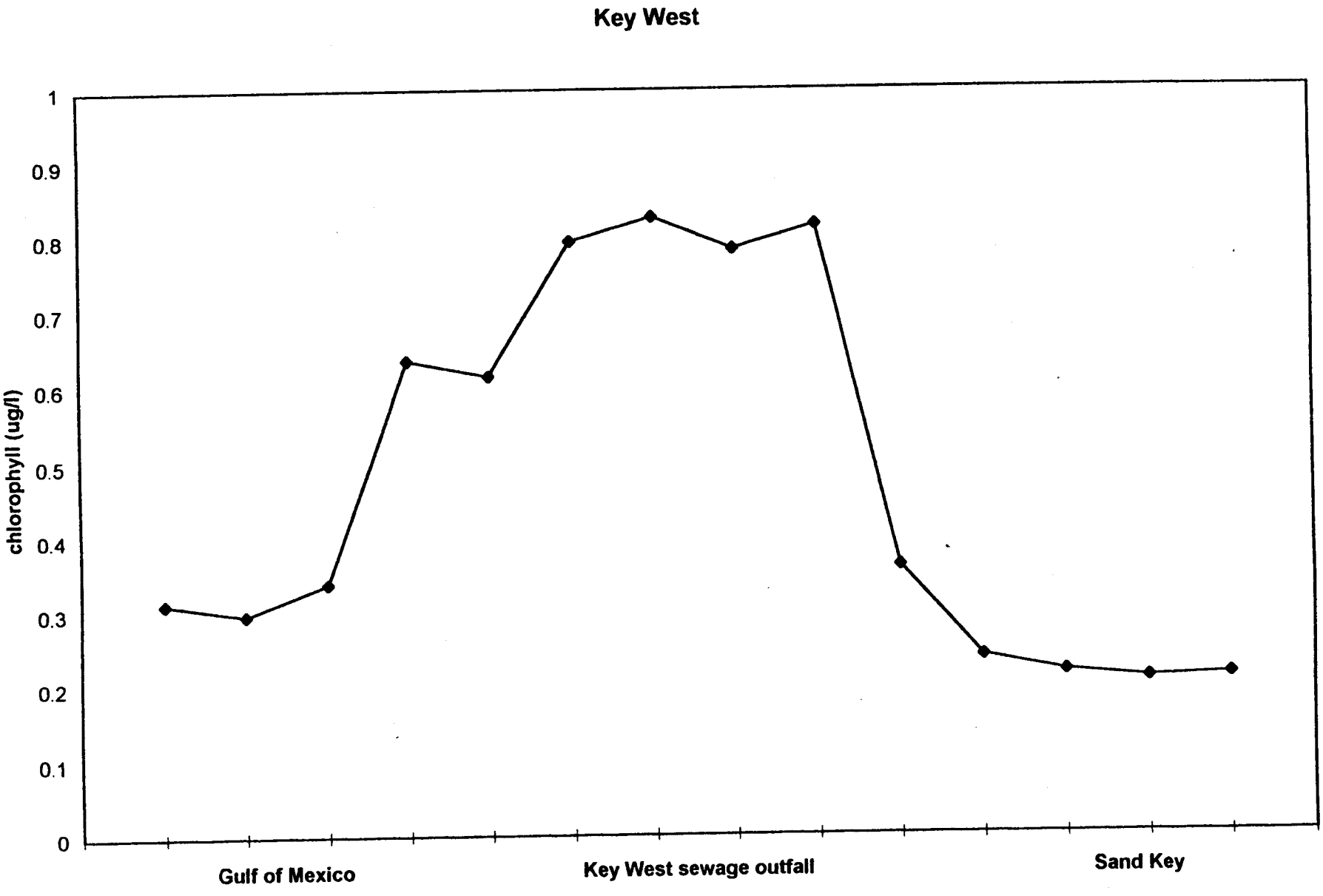
Key West
chlorophyll (ug/l)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Gulf of Mexico
Key West sewage outfall
Sand Key

Figure 2

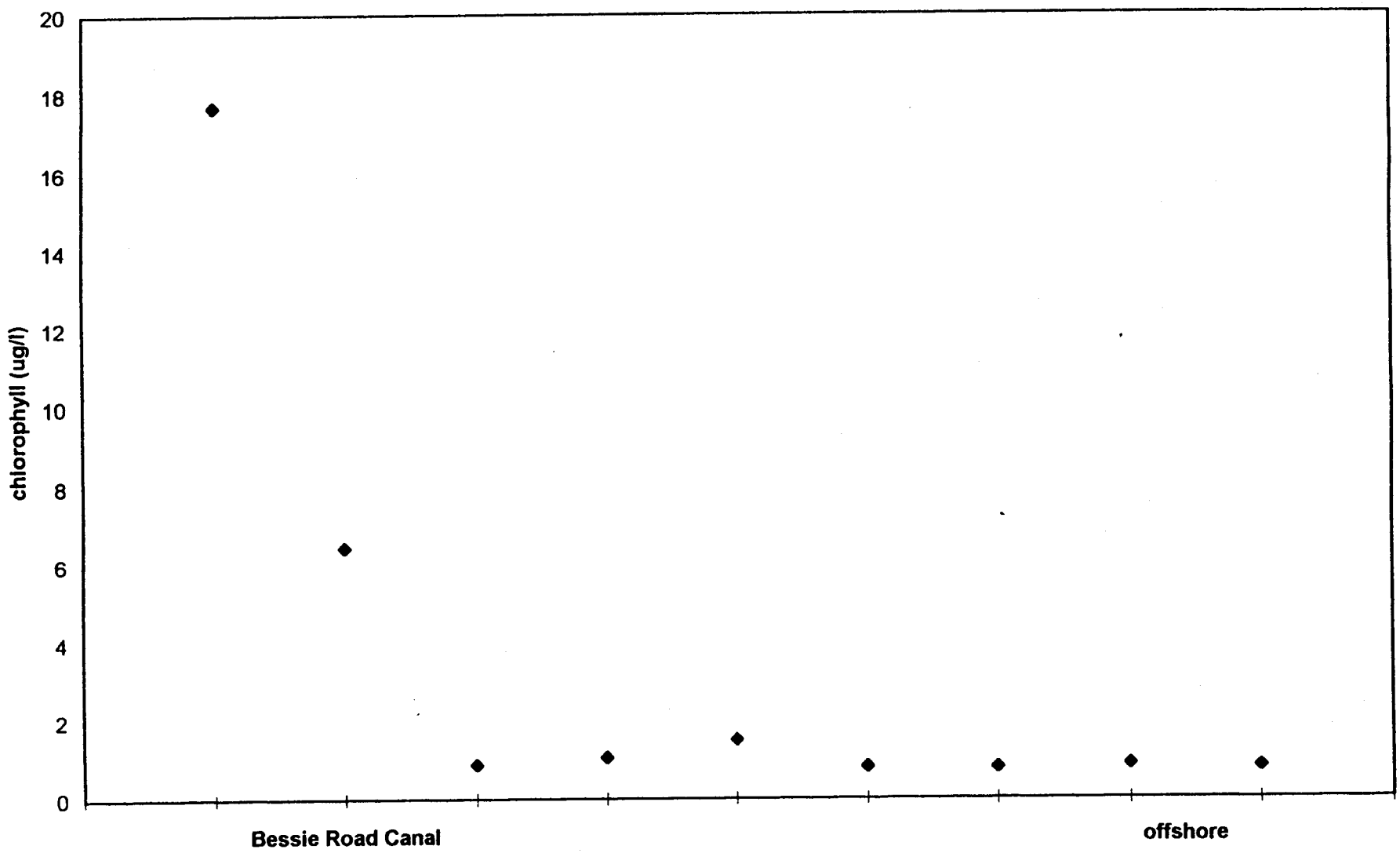
Key Largo - October 9, 1996
chlorophyll (ug/l)
20
18
16
14
12
10
8
6
4
2
0
Bessie Road Canal
offshore

Figure 3

Figure 4

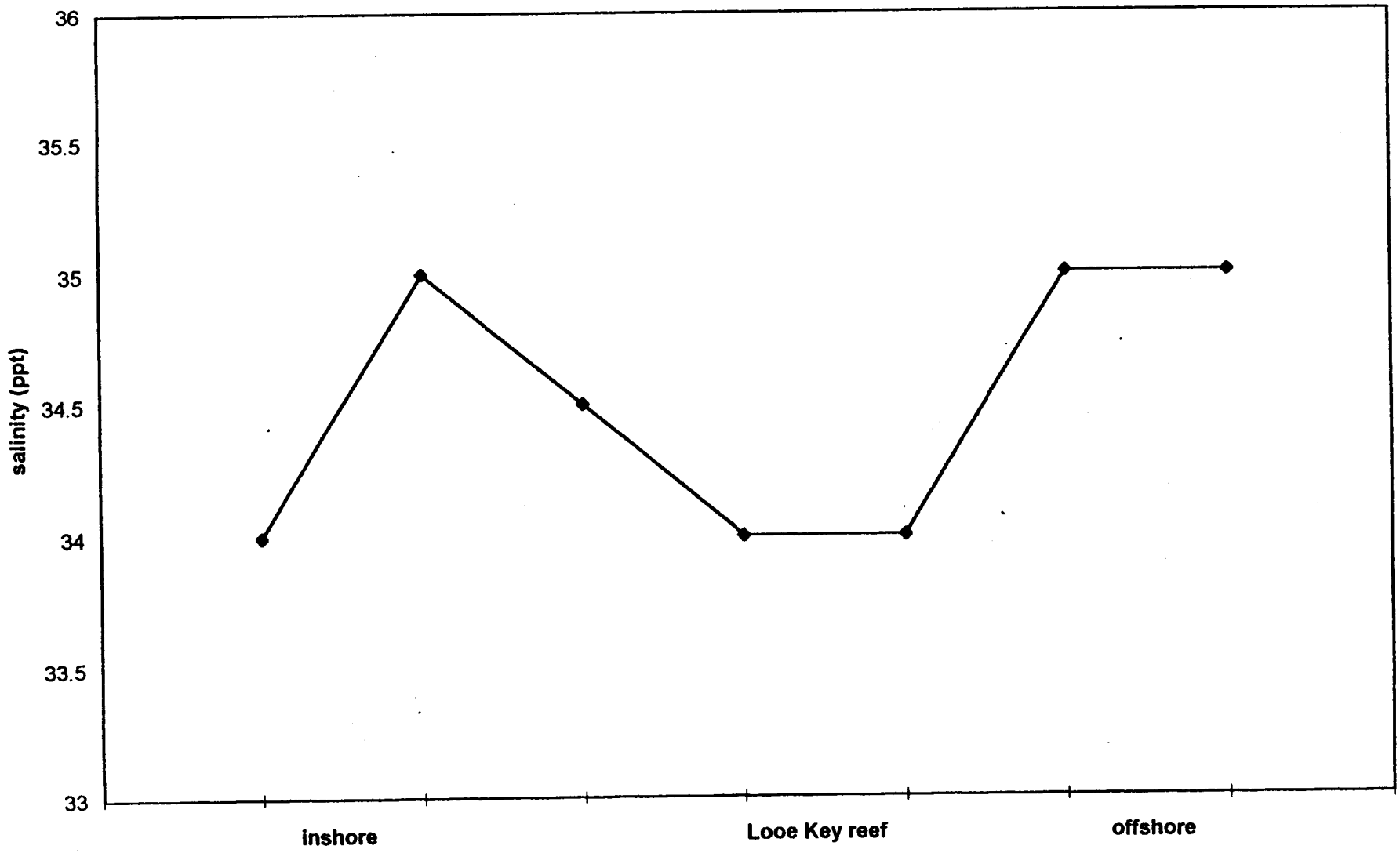
36
35.5
35
34.5
34
33.5
33
salinity (ppt)
inshore
Looe Key reef
offshore

Figure 5

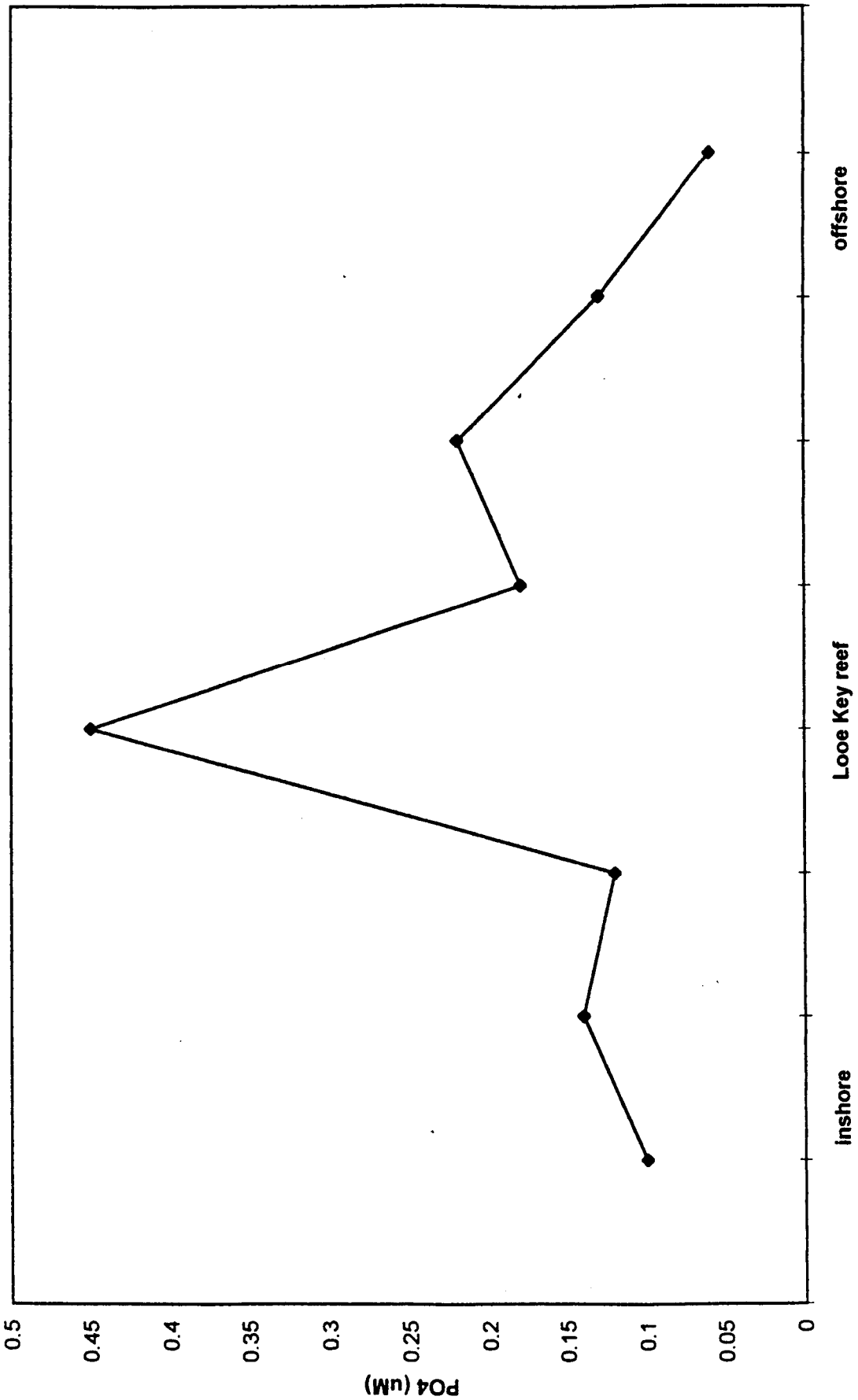
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
PO4 (uM)
inshore
Looe Key reef
offshore

Figure 6

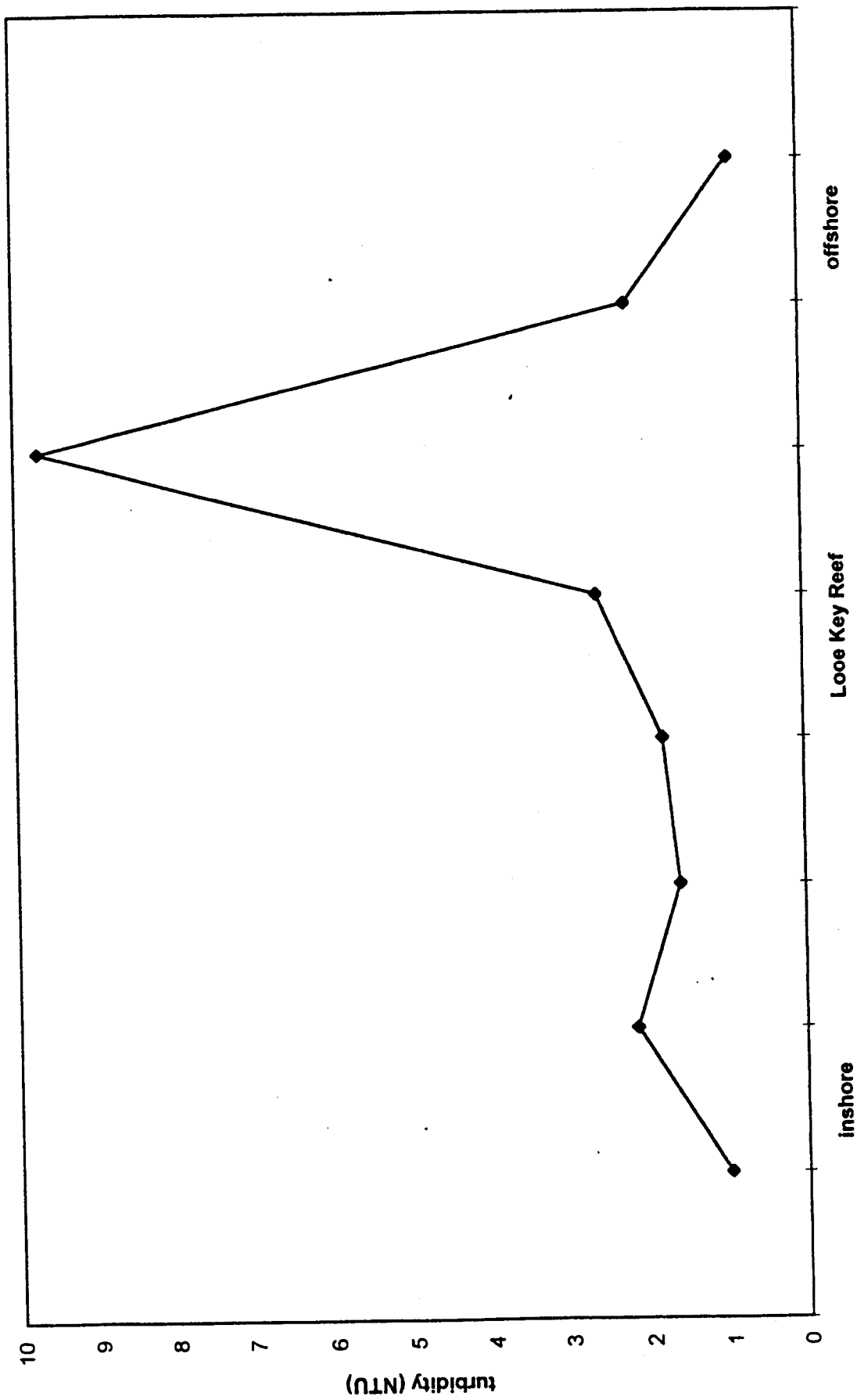
turbidity (NTU)
0
1
2
3
4
5
6
7
8
9
10
inshore
Looe Key Reef
offshore

Figure 7

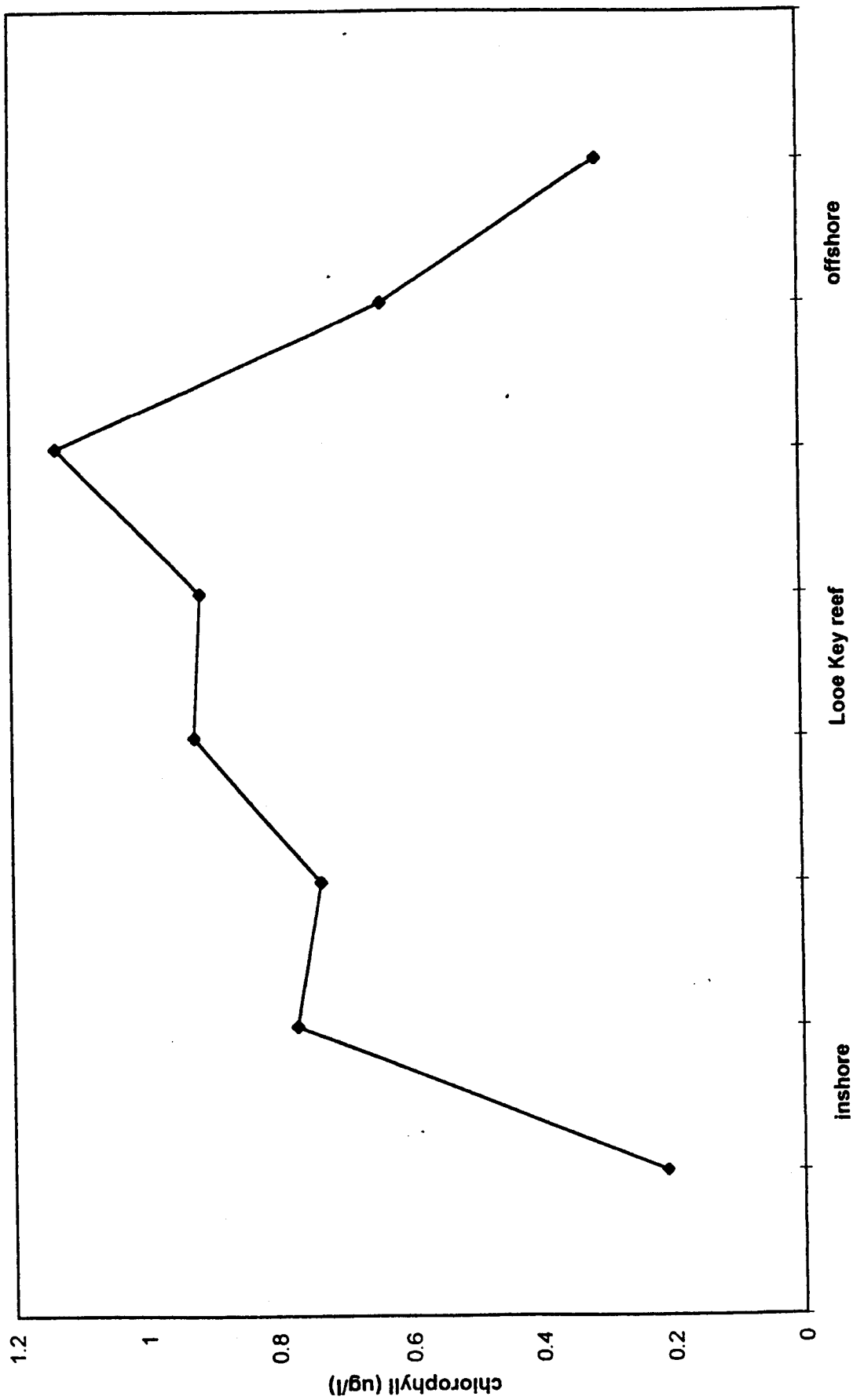
chlorophyll (ug/l)
1.2
1
0.8
0.6
0.4
0.2
0
inshore
Looe Key reef
offshore

Figure 8

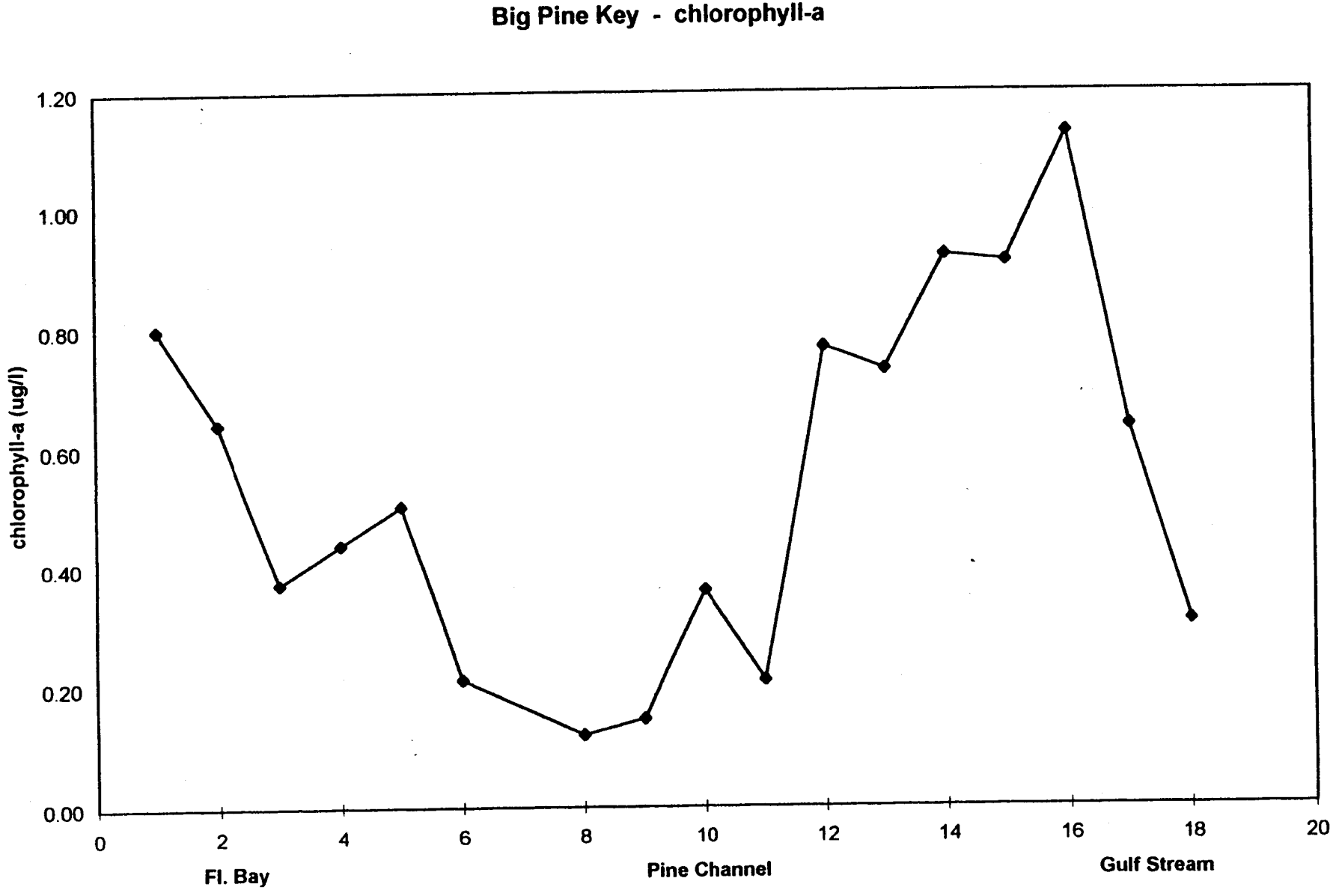
Big Pine Key - chlorophyll-a
chlorophyll-a (ug/l)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
2
4
6
8
10
12
14
16
18
20
Fl. Bay
Pine Channel
Gulf Stream

Figure 9

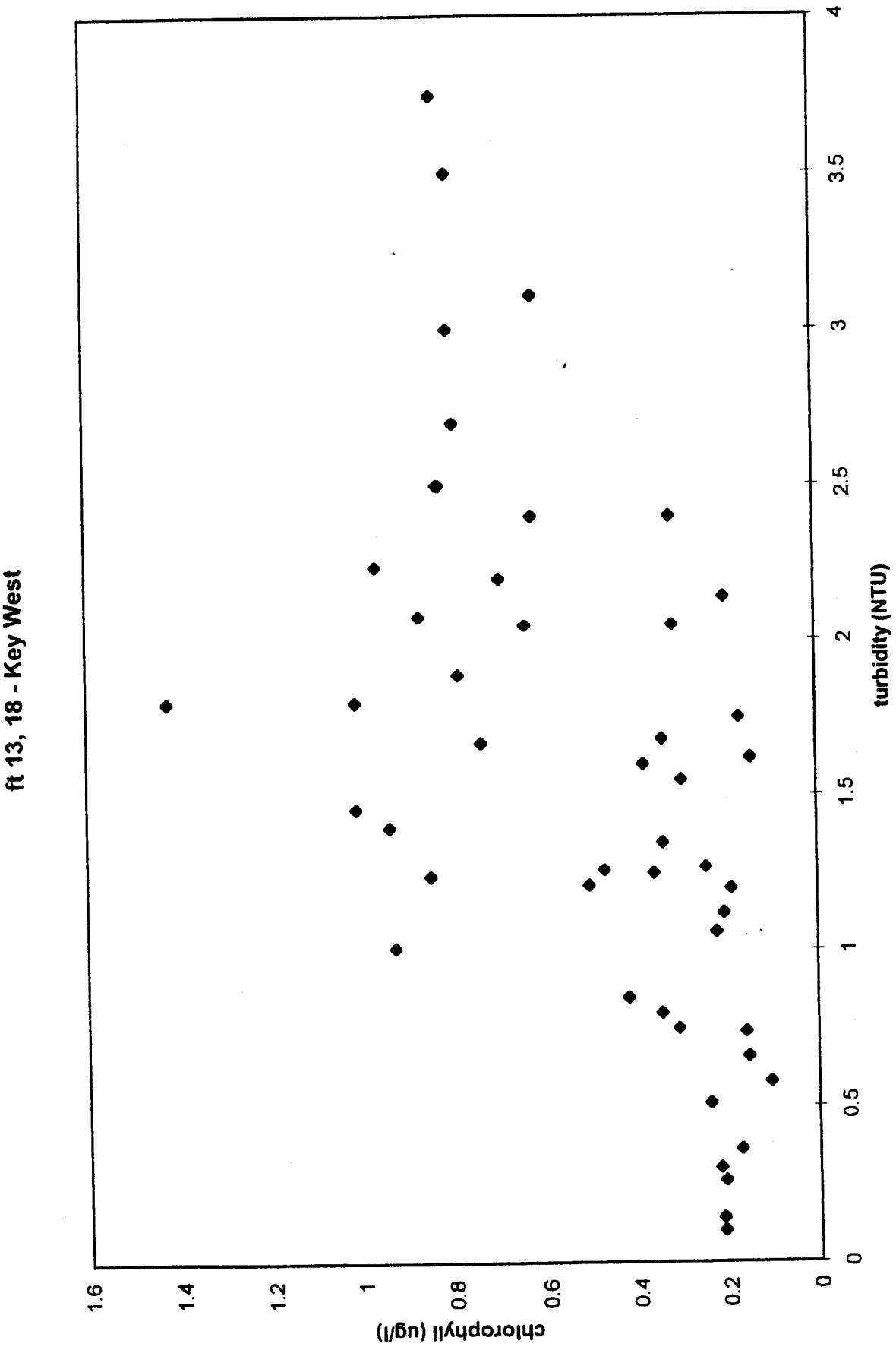
ft 13, 18 - Key West
chlorophyll (ug/l)
turbidity (NTU)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
3
3.5
4

Figure 10

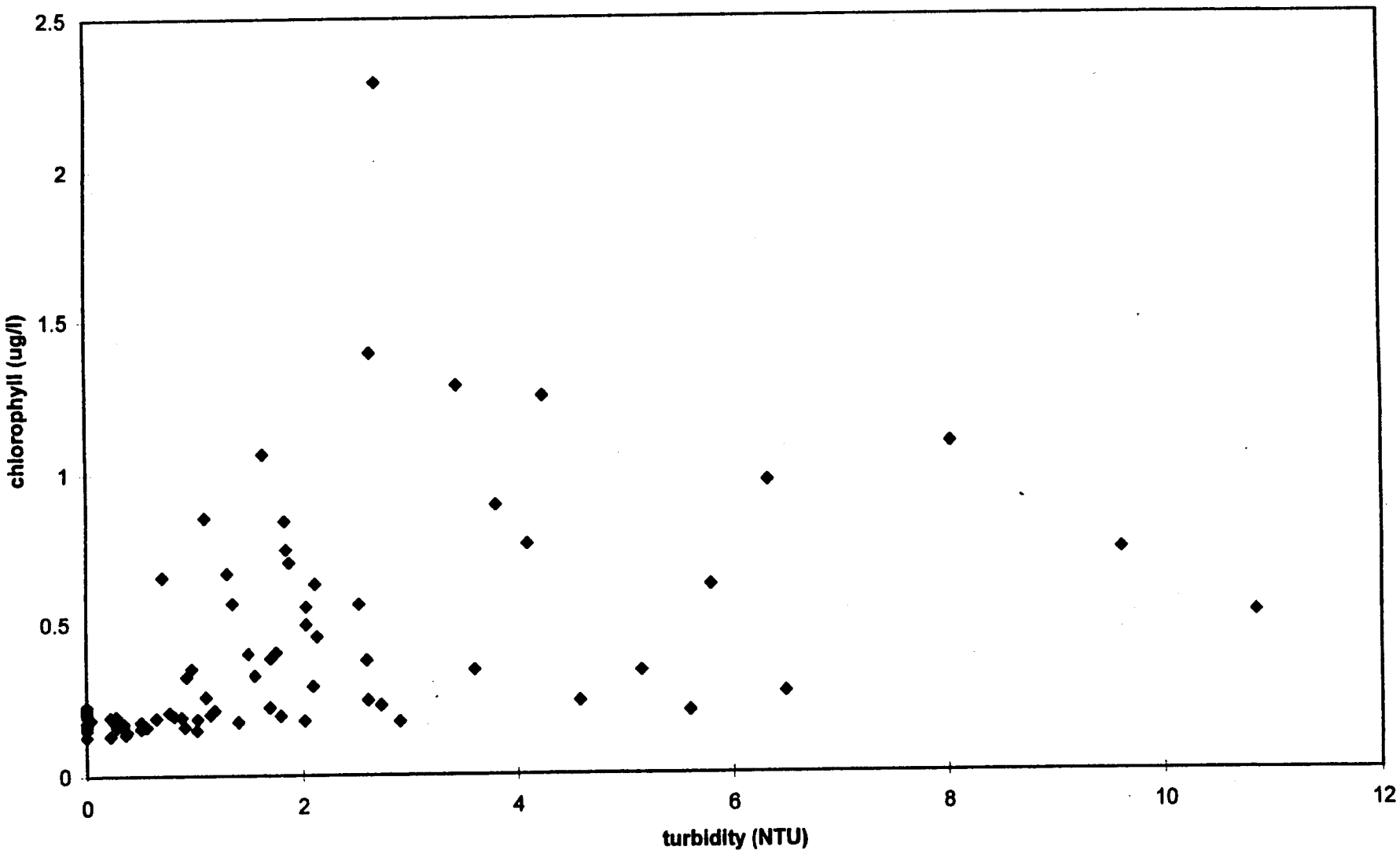
ft 2, 3, 4, 8, 9, 10, 26
chlorophyll (ug/l)
turbidity (NTU)
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12

Figure 11

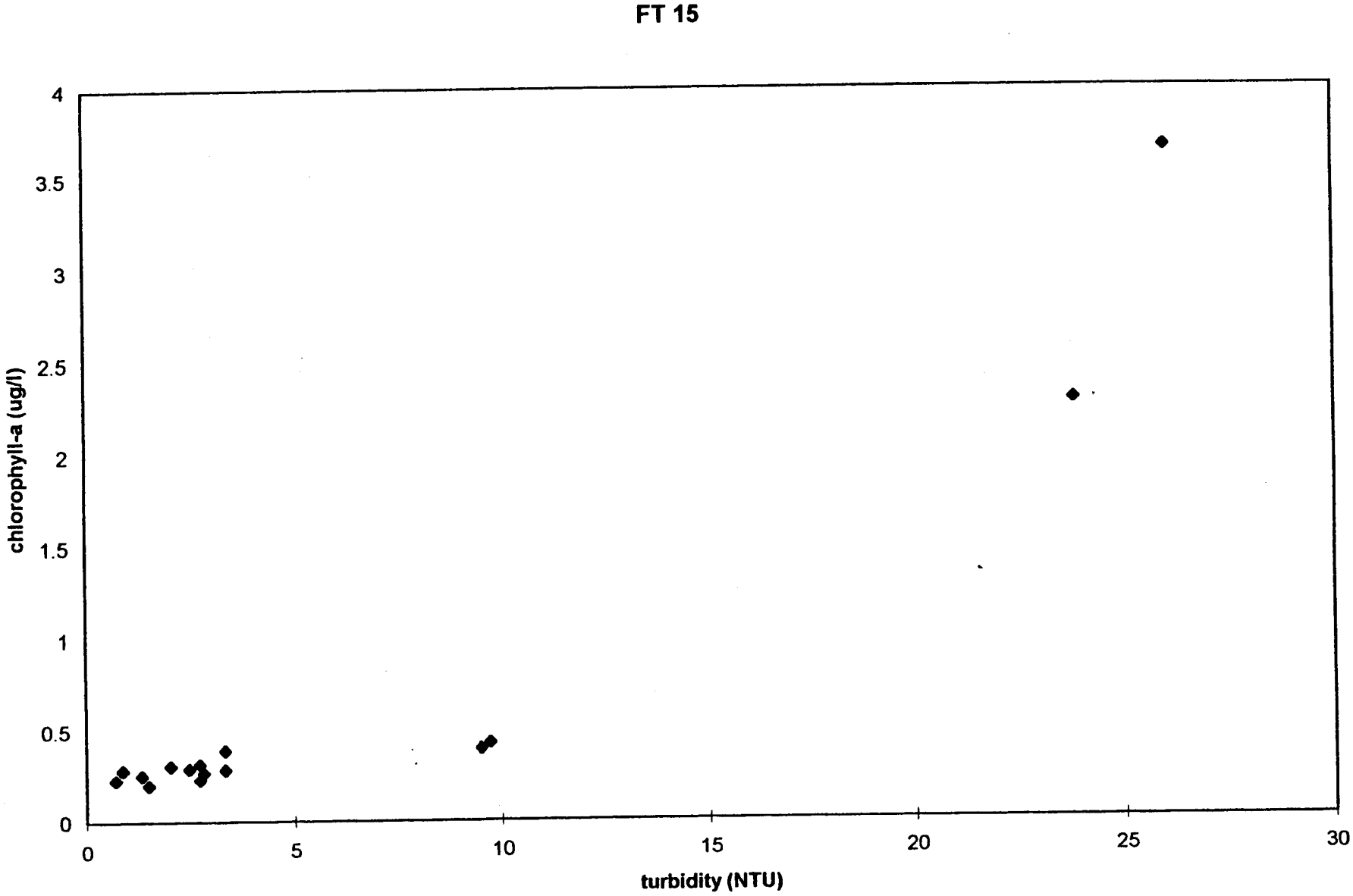
FT 15
chlorophyll-a (ug/l)
turbidity (NTU)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
5
10
15
20
25
30

Figure 12

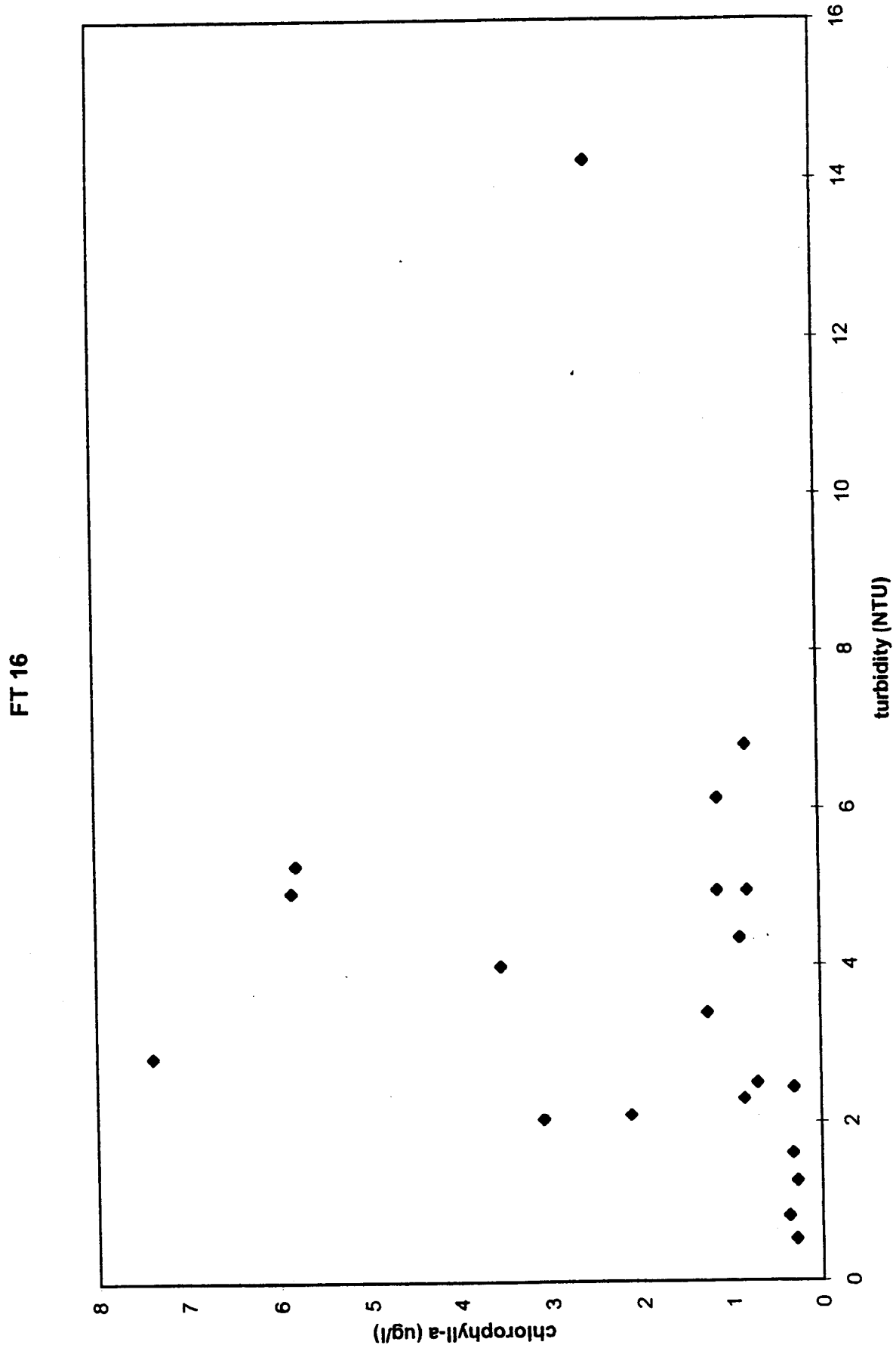
FT 16
chlorophyll-a (ug/l)
turbidity (NTU)
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14
16

Figure 13

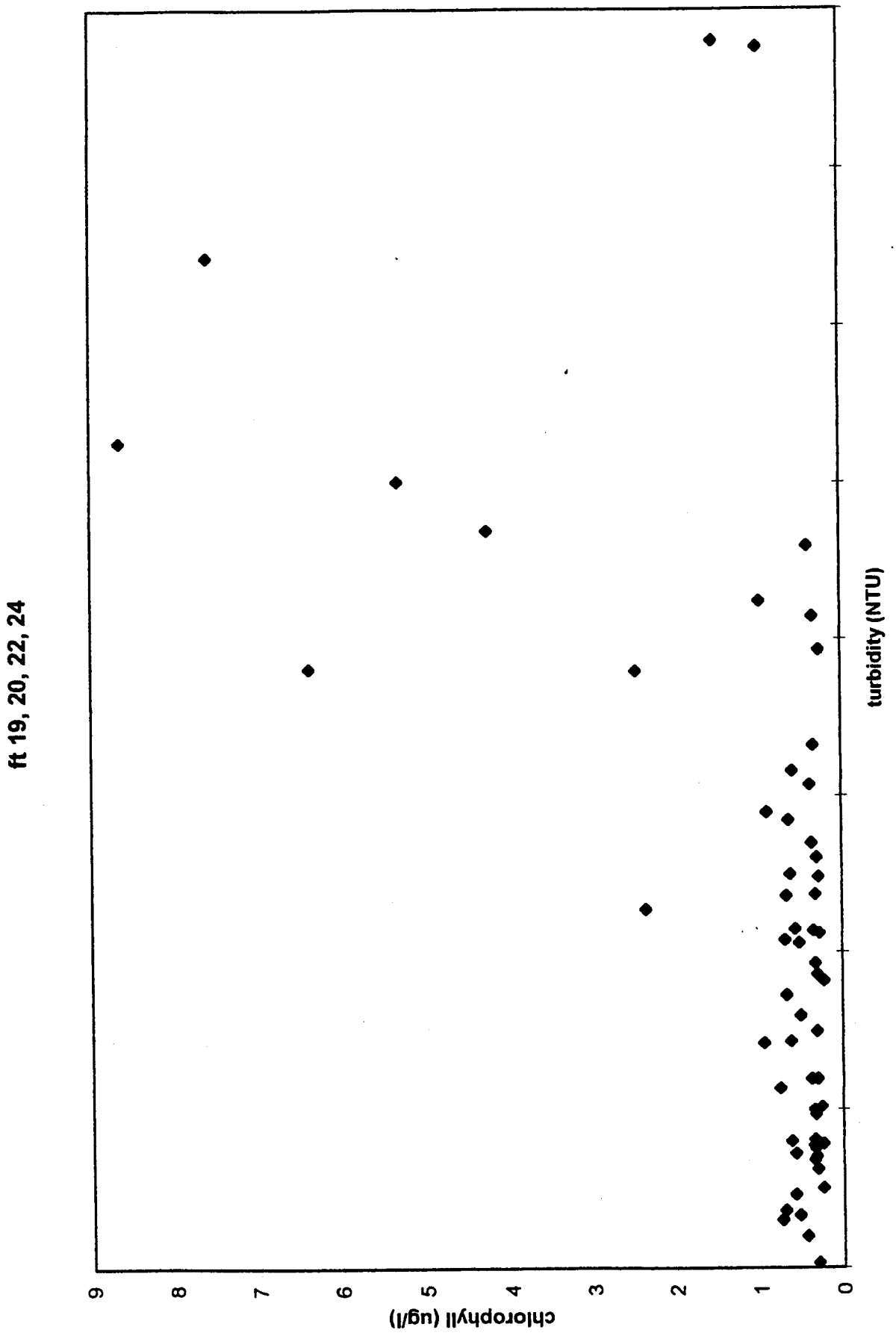

Figure 14

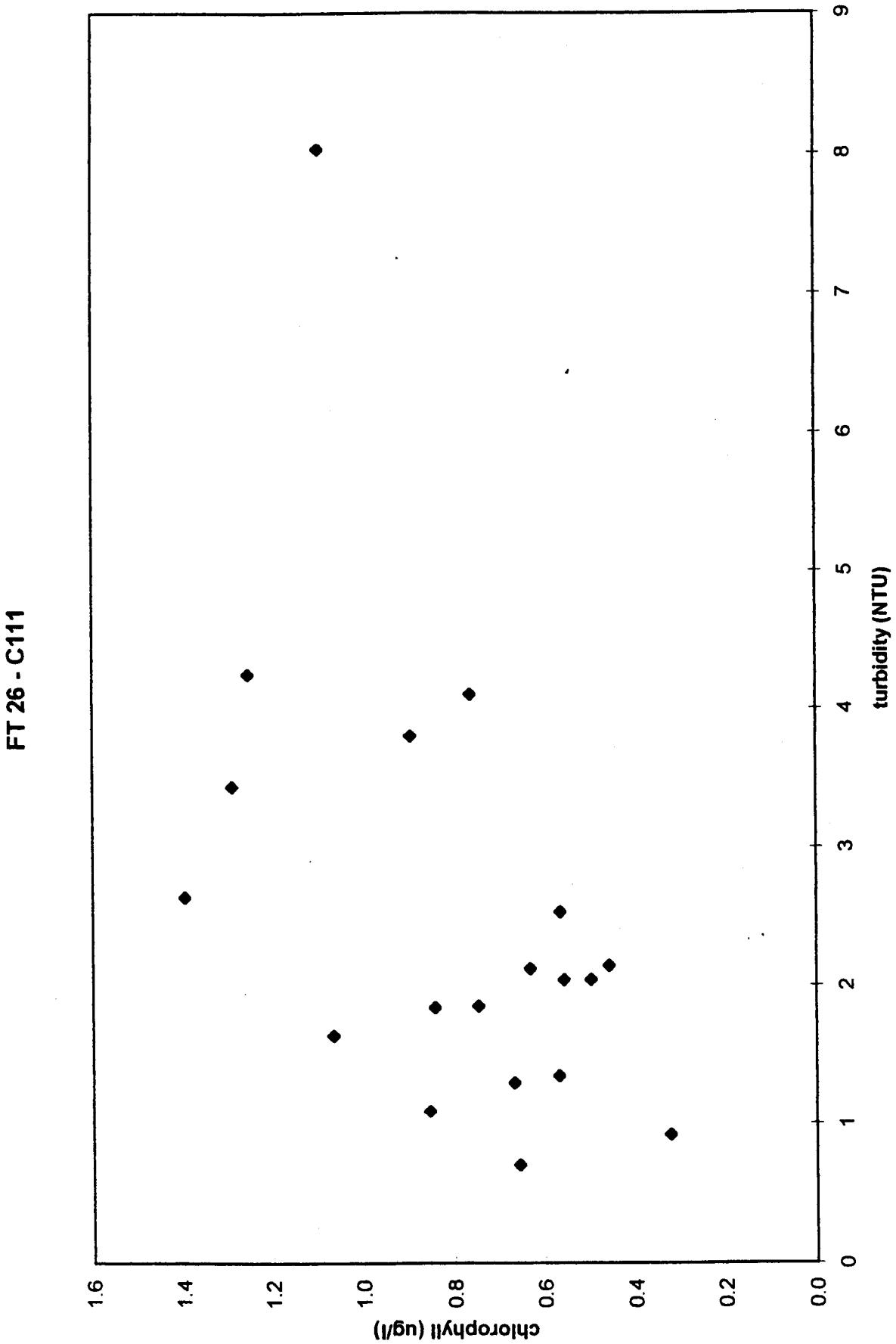
FT 26 - C111
chlorophyll (ug/l)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
turbidity (NTU)
0
1
2
3
4
5
6
7
8
9

Figure 15