FINAL REPORT

CM - 194

IMPOUNDMENT MANAGEMENT

FINAL REPORT

CM-194

(SEPT. 1987-SEPT. 1988)

March 29, 1989

****

ABOVE-GROUND PRIMARY PRODUCTION IN DITCHED IMPOUNDED AND NATURAL MARSHES ALONG THE INDIAN RIVER LAGOON

(J.R. Rey, Florida Medical Entomology Laboratory)

and

FISH ADAPTATIONS TO METABOLIC STRESS

(R.G. Gilmore & M. Peterson, Harbor Branch Oceanographic
Institution, Inc.),

SPONSORING AGENCY: INDIAN RIVER MOSQUITO CONTROL DISTRICT

****

[FUNDS FOR THIS PROJECT WERE PROVIDED BY THE DEPARTMENT OF ENVIRONMENTAL REGULATION, OFFICE OF COASTAL MANAGEMENT USING FUNDS MADE AVAILABLE THROUGH THE NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION UNDER THE COASTAL ZONE MANAGEMENT ACT OF 1972, AS AMENDED.]

ABOVE-GROUND PRIMARY PRODUCTION IN DITCHED IMPOUNDED AND NATURAL MARSHES ALONG THE INDIAN RIVER LAGOON

and

FISH ADAPTATIONS TO METABOLIC STRESS

****

Enclosed are the final reports of the two principal investigators for CM 194. Dr. Rey's vegetation study compared above-ground primary production in impounded, ditched and open Indian River lagoon marshes while Dr. Gilmore/Dr. Peterson's work examined hypoxia induced physiologic stress in sheepshead minnows, sailfin mollies and juvenile common snook, both in laboratory and field experiments.

Dr. Rey's final report explains that when the impoundment was left open throughout the year, only minor differences in above-ground vegetation production were found between impounded and non-impounded stations. However, when the impoundment was closed and flooded for mosquito control, production decreased considerably. Dr. Rey's data indicates that marsh flooding decreased above-ground primary production but the overall impact on marsh and estuarine energy dynamics was not so apparent. For instance, even with artificial flooding, there was still significant production at the flooded impoundment stations. Also, the vegetative community appears to be resilient; production during the following year was back to levels comparable with at least one of the two un-impounded stations. Other important factors are the nutritional quality of the material being exported to the estuary and the possible self-stabilizing effect of marsh floor litter accumulation. Interestingly, they hypothesize that because microbial enrichment of litter was greatest at impoundment stations, litter from there may represent a more nutritious food source for detritivores than litter from non-impounded sites.

Drs. Gilmore and Peterson's lab work determined how sheepshead minnows, sailfin mollies and juvenile common snook exhibit stress under hypoxic conditions. Secondary stress reponse characteristics were studied by measuring hematocrit, plasma osmolality, plasma chloride ion concentrations, and oxygen consumption rates. All three fish species showed significantly increased hematocrits in 40 torr (30 ppt and 30 degrees C); however, only sheepshead minnows exhibited increased plasma osmolality. Plasma chloride ion concentration decreased significantly in juvenile common snook. Oxygen consumption rates decreased in the three species when exposed to 40 torr for 2.5 hours and there was a weight-dependent respiratory metabolism in snook. Additionally, there was no mortality in sheepshead minnows and sailfin mollies when exposed to either 60 or 40 torr over 24 hours but juvenile snook (<50-60 g) were able to survive 40 torr media whereas large juveniles (>50-60 g) could not survive. Field observations verified these lab findings where artifically induced stressful conditions resulted in snook >145 mm dying. Younger ones, although stressed, managed to survive.

The ability of fish to tolerate hypoxic conditions depends on physiological and behavioral mechanisms which differ between species. For instance, sailfin mollies are morphologically adapted to use aquatic surface respiration (ASR) whereas sheepshead minnows are not; however, sheepshead minnows possess physiological adaptations that sailfin mollies do not possess. There is some evidence that juvenile common snook may also use ASR.

These findings aid impoundment managers who are aware that environmental conditions commonly deteriorate in impounded marshes during the closure period. This study showed that resident fish can generally tolerate sublethal hypoxia for certain periods of time; however, water quality does deteriorate to levels lower than those considered in this study. Such untested lower levels may be lethal to resident as well as transient fish.

RECOMMENDATIONS:

This study has verified several observations marsh managers have made over the past few years and lends scientific credibility to the increased use of certain impoundment management manipulations. As always, as research answers some questions new ones are generated.

Dr Rey's work suggests that periodically (perhaps once every several years if possible), it seems advantageous to leave impoundment culverts open year-round to allow for some vegetative recovery and to maximize detrital flushing to the estuary. Caution must be taken to avoid adverse lagoonal impacts when releasing impounded water, laden with large litter accumulations. However, suspending flooding for a summer may require increased insecticide treatment on and near the open marsh.

Most important for long-term vegetation production is to avoid widespread vegetation kills since this sets back production much more so than transient declines due to routine seasonal flooding. It appears that this can be accomplished by carefully managed minimal flooding elevations and emergency water releases in the event of large, unforseen storm events.

One area highlighted by this work which requires further research is a study of both dissolved and particulate material flux between the perimeter ditch and lagoon. Possibly, increases in the number and size of tidal connections (culverts) will help in marsh production export to the estuary but the full impact of such improved connection is unclear.

Very importantly, Drs. Gilmore/Peterson's study documented the ontogenetic physiological patterns of snook (i.e., the vigor of smaller snook compared to larger ones). This verifies that whenever possible, impoundment managers should try to induce fish migration out of impoundments during the late fall and spring. This will allow the more sensitive snook (i.e., those >100 mm and other transient fish especially susceptible to hypoxia) to migrate from the impoundment prior to closure thus escaping the typically stressful summer management period. There are management variations that may allow for this migration. Dr. Gilmore's HRS funded experiments to induce fish migration through subcells has shown promise in this regard. Other possibilities include overpumping, inverted flapgates, reduced closure periods, staged reopenings at appropriate locations, increased tidal access through additional culverts, and increased export of fine sediments and detrital material. These techniques deserve further study for combating anoxic/hypoxic perimeter ditch waters and enhancing fish habitat and survival.

PUBLICATIONS DURING THE CONTRACT PERIOD DRAWING ON CZM WORK.

A paper entitled "Mosquito production in a rotationally managed impoundment compared to other management techniques" by Douglas Carlson and Peter O'Bryan (IRMCD) was published in the June 1988 volume of the Journal of the American Mosquito Control Association. This paper drew on mosquito sampling work from previous CZM funded grants. A reprint is enclosed.

Mark Peterson and Grant Gilmore authored a paper published in the Journal of Experimental Marine Biology and Ecology. The paper is entitled: "Hematocrit, osmolality and ion concentration in fishes: consideration of circadian patterns in the experimental design". A reprint is enclosed.

PRESENTATIONS DURING THE CONTRACT PERIOD DRAWING ON CZM WORK.

AMERICAN MOSQUITO CONTROL ASSOCIATION. At their annual meeting in Denver, Colorado (Feb. 1988), Doug Carlson presented a paper co-authored by Peter O'Bryan and Grant Gilmore entitled: "Salt marsh mitigation in Florida: an example of balancing mosquito control, natural resource and development interests".

FLORIDA ANTI-MOSQUITO ASSOCIATION. At their annual meeting in Pensacola (March 1988), Peter O'Bryan made a somewhat longer version of the above-mentioned paper. Co-authored with Doug Carlson and Grant Gilmore it too was entitled: "Salt marsh mitigation: an example of balancing mosquito control, natural resource and development interests".

Doug Carlson presented "Progress during 1987-88 by the Subcommittee on Managed Marshes in addressing Florida's salt marsh management issues".

AMERICAN SOCIETY OF ICHTHYOLOGISTS AND HERPETOLOGISTS. Dr. Peterson presented a paper on circadian patterns at this annual meeting held at the University of Michigan, Ann Arbor (June 1988).

FIT/MARINE RESOURCES COUNCIL PRESENTATIONS. CZM sponsored research provided the basis for two papers presented at the Second Indian River Research Symposium (Sept. 1988, Florida Institute of Technology, Melbourne),

"Salt marsh mitigation: an example of balancing mosquito control, natural resource and development interests", Peter O'Bryan, Doug Carlson and Grant Gilmore.

"Salt marsh and mangrove swamp fish communities of the Indian River Lagoon", Grant Gilmore, Ron Brockmeyer and Douglas Scheidt.

FLORIDA COORDINATING COUNCIL ON MOSQUITO CONTROL/SUBCOMMITTEE ON MANAGED MARSHES. CZM sponsored impoundment research proved critical at the October 1987 meeting of the Florida Coordinating Council on Mosquito Control/Subcommittee on Managed Marshes. This was the first time that the Coordinating Council was requested to make a recommendation on an impoundment management issue following the guidelines for resolution of conflicts set out in the Florida Statutes.

St. Lucie County Mosquito Control District was requesting permission to close a dike breach in St. Lucie Impoundment #19B and implement a Rotational Impoundment Management (RIM) plan there but this request has been opposed by the Fla. Dept. of Natural Resources. After considerable technical input by the Subcommittee and discussion by the Coordinating Council, the Council recommended that St. Lucie be allowed to implement the RIM plan at #19B but permission to close dike breaches in other impoundments be withheld unless receiving a favorable reccomendation by the Subcommittee. Research findings from CZM work at Impoundment #12, in particular fish movement through culverts, were essential to these deliberations.

ABOVE-GROUND PRIMARY PRODUCTION IN DITCHED IMPOUNDED AND NATURAL MARSHES ALONG THE INDIAN RIVER LAGOON

Jorge R. Rey, John Shaeffer,
Roy Crossman and Derek Tremain

University of Florida - IFAS
Florida Medical Entomology Laboratory
200 9th Street S.E.
Vero Beach, FL 32962

March, 1989

INTRODUCTION

Impounding salt marshes for the control of the salt marsh mosquitoes _Aedes taeniorhynchus_ and _A. sollicitans_ is an effective and commonly-used technique along the Indian River Lagoon, in east-central Florida. Although impounding significantly reduces the need to use potentially harmful pesticides in these fragile habitats, there is concern about possible deleterious effects of salt marsh impoundments upon the lagoon system. One of the most frequently voiced concerns is the effects of impoundments upon the productivity of the affected marshes.

It is well known, that salt marshes are extremely productive systems when compared to other terrestrial communities (Odum 1959, Schelske and Odum 1961). A variety of factors have been implicated in the high primary productivity of coastal marshes. Among these are:

(1) A significant energy subsidy from flowing tidal waters.

(2) Vertical orientation of leaves of most marsh plant species (reduces heating, results in maximum exposure to sunlight, reduces shading).

(3) Abundant soil water.

(4) High nutrient content of many marsh soils.

(5) High concentration of organic matter in the soil leads to colloid formation. The latter absorb exchangeable ions which are necessary for plant growth (Gorham 1953).

It is also known that the correlation between high fish and shellfish productivity and proximity to extensive coastal wetlands is not a spurious one. Some factors that have been shown to be important in this respect are (de la Cruz 1973):

(1) High net primary production by marsh plants.

(2) Only a small fraction of the marsh organic production is consumed in situ.

(3) Most of the dead organic material is transported to neighboring estuarine waters either directly, or after it decomposes to particulate organic matter in the marsh floor.

(4) Organic detritus is protein-enriched by microbial colonization.

(5) The ample supply of protein-rich detritus allows the existence of large detritivore populations, which form the base of most estuarine food webs.

There is an extensive literature dealing with the production dynamics of salt marsh plants. The overall net above-ground production reported span a wide range of values, from less than 500 g(dry weight)/m^2/yr (i.e. Morgan 1961) to more than 3000 g/m^2/yr (i.e. Odum 1959, 1961). Keefe (1972), de la Cruz (1973) and Turner (1976) present reviews of the marsh production literature, while Kirby and Gosselink (1976), Turner (1976), Linthurst and Reimold (1978), and Dickerman et al. (1986) review the various techniques used to measure marsh macrophyte production.

The effects of differing hydroperiods are usually prominent in most published discussions of physical factors affecting marsh production (Wierke 1935, Jervis 1964, Binet 1965, Langlois 1971, Steever et al. 1976, Odum et al. 1983). Below we report on the results of studies on vegetation production in salt marshes with natural and modified tidal regimes (due to impounding or ditching) along the Indian River Lagoon, in east-central Florida.

METHODS

Production by herbaceous halophytes was studies in three sites (Fig. 1) representing an open natural marsh (Oslo Road Marsh), a ditched marsh (North Marsh), and an impounded marsh (IRC #12). The vegetation composition of each sampling station was similar, comprising a mix of approximately 90% _B_. _maritima_ and 10% _Salicornia_ spp., except at North Marsh were _Salicornia_ cover was less than 5%.

Study Sites.

Oslo Road Marsh is a 15 ha salt marsh located on the mainland side of the lagoon, east of the Florida Medical Entomology Laboratory near the city of Vero Beach. The vegetation at this site is a mixed stand of _Batis_ _maritima_, _Salicornia_ _virginica_, and _S_. _bigelovii_ with black mangroves (_Avicennia_ _germinans_) along the upland edge of the marsh, and red mangroves (_Rhizophora_ _mangle_) and _Spartina_ _alterniflora_ fringing the marsh on the lagoon side. A sampling station (OR) was established in the middle of the _Batis_ - _Salicornia_ stand, approximately 150 m west of the lagoon border.

North Marsh, with an area of approximately 10 ha, is located on the barrier island side of the lagoon, almost directly across Oslo Road Marsh. Its vegetation also consists of mixed _Batis_-_Salicornia_ stands, but black mangroves are more prevalent in the interior of the marsh than at Oslo. A series of ditches were cut in the marsh for mosquito control during the 1950's, and are still operational. A natural berm approximately 45 cm high borders the marsh on the lagoon side. A station (NM) was established in a large Batis meadow 18.5 m away from the nearest ditch and 7.0 m from the nearest black mangrove stand.

IRC #12 is a 20.4 ha marsh that was impounded for mosquito control in 1965. This involved building a dike on the lagoon side of the marsh with the concomitant creation of a perimeter ditch running along the inside of the dike. A 46 cm diameter culvert connected the marsh with the lagoon. In 1978, management of the impoundment for mosquito control was discontinued, the culvert was sealed and the marsh was allowed to dry through seepage and evaporation. This resulted in salinities exceeding 100 ppt (Gilmore et al. 1981) and in complete elimination of the marsh vegetation, which originally consisted of extensive Batis-Salicornia meadows with interspersed black mangroves (Harrington and Harrington 1982). The culvert at this site was re-opened in early 1980 and in 1983 an additional 46 cm diameter culvert was installed. During 1983, 1984, 1986,and 1988 the culverts were closed only during the May-October mosquito-producing season, whereas the culverts were left open during the entire year in 1985 and 1987. Pumping for mosquito control was only done in 1986 and 1988.

There was significant recovery of herbaceous halophytes at this site after the culverts were re-opened and management resumed in 1980, but very little recovery by mangroves has been observed (Rey 1986). Additionally, some vegetation damage was recorded in 1986 after the marsh was artificially flooded for mosquito control, but most of the damaged vegetation recovered by the following summer (Rey et al. in prep.).

Two stations were established at this site. The first (PD), was located in a large Batis- Salicornia stand near the perimeter ditch on the west side of the impoundment; the second (RG) was located in a similar stand near the upland edge, on the northeast side.

Standing Crop.

Above-ground standing crop was measured using the clip-quadrat method (Keefe 1972). During each monthly sampling, all the above-ground vegetation falling within a 0.25 m^2 area in 5 locations at each station was clipped at ground level and placed into plastic bags for transport to the laboratory. In addition, all litter within the sample quadrat was also collected and stored in separate bags. To establish the sampling quadrats we placed a 61 cm high - 0.25 m^2 aluminum frame in the center of each randomly selected point (5 per station X 4 stations / month) and considered all materials inside the frame as being part of the sample. Sampled areas were marked to avoid re-sampling the same areas.

In the laboratory, we separated the contents of the bags containing the standing vegetation clippings into species and further separated live from dead fractions. We then dried the live, dead and litter fractions separately at 104°C to constant weight (24-48 hrs). The final weight was recorded as the dry weight of each biomass component (live, standing dead, and litter). Each fraction was then ground in a Wiley mill, inserted into a muffle furnace, and its ash-free weight determined after combustion at 550° C. The difference between the last two weights estimates the amount of organic matter present in the sample.

Material Lost.

The litter bag method was used to estimate the amount of material lost between samples (White 1978, 1982). For the first year, we placed 20 grams of oven-dried material in each of 104 litter bags (2 mm^2 mesh). At each station, two rows of 13 bags each were set on the ground, the bags in each row were tied together approximately 40 cm from each other, and the ends of the string holding the bags were tied to stakes driven into the ground. During each monthly sampling one bag from each row at each station was chosen at random and was placed in a plastic bag for transport to the laboratory. In the laboratory, the bag contents were lightly washed, dried, and weighed to determine the amount of litter lost during the interval. In March of each year, an additional set of 56 bags were prepared, laid out in two rows of seven at each station, and processed as above. Comparison of the loss rates of the first set of bags, which was set out in October of each year, and the second set provided an estimate of season-dependent differences in the rate of litter decomposition and loss from the marsh floor.

The procedures during the second year were the same as above except that fresh litter was used in the litter bags. Prior to bag preparation, we filled 25 bags with fresh material and then immediately removed the materials from each bag, dried each to constant weight, and calculated a regression of fresh weight on dry weight. The bags were then weighed, filled with fresh material and weighed again. The dry weight equivalent of each bag was calculated from the (highly significant, $R^2 > 0.935$) regression equation after subtracting the weight of the bag from the total weight.

Production.

A variety of methods have been used to calculate net primary production (Net Production = Gross Production/Respiration) from field biomass data (see Kirby and Gosselink 1976 for a review). The Milner and Hughes (1968) method involves simple calculation of production by changes in standing crop through time. If B_n is the weight recorded at time n, then net production is given by

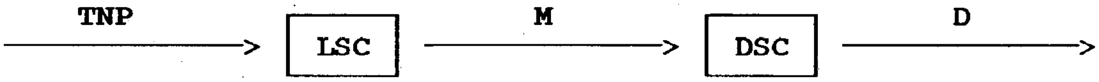
$$\Sigma(B_n - B_{n-1})$$

which reduces to $B_n - B_1$ or maximum minus minimum biomass.

Smalley (1959), corrected for losses during the sampling intervals by considering changes in dead biomass. If the live standing crop change for a period is positive then production is the sum of changes in live and changes in dead biomass; if changes in live biomass are negative, then production is calculated as the algebraic sum of live and dead biomass, or zero, whichever is greatest. Although this method accounts for a certain (unknown) amount of the losses of live vegetation between samples, it ignores apparently negative production, thus biasing sampling errors upward (Kirby and Gosselink 1976).

Wiegert and Evans (1964) used a method that accounts for all losses (except herbivory) between sampling intervals by using data from losses in paired plots or from litterbags. The paired plot method involves the removal and weighing of all vegetation (live and dead) from a sample plot, and the removal of only live vegetation from an "identical" paired plot. The second plot is re-sampled after a predetermined interval, and the amount of material lost is calculated as the difference between the weight of dead biomass in the first sample and the weight in the second. The litter bag method is as explained above.

The basis for the Wiegert and Evans calculations can be visualized as follows: Let **TNP** be the true net production, **M** the vegetation mortality, D the disappearance of dead vegetation, and **LSC** & **DSC** live standing crop and dead standing crop, respectively. Then:



An instantaneous loss rate (g lost/g dead/time) can be calculated from the paired plot or litterbag data and multiplied by the average dead standing crop or litter biomass (if available) to yield **D**. **M** can be calculated from **M** = D + DSC, and TNP from **TNP** = **M** + **LSC**.

The question remains as to which is the best method for calculating the instantaneous loss rate, litter bag or paired plot. Wiegert and Evans (1964) found that losses from a Michigan old field estimated from litterbags were lower than those estimated from paired plots. They attributed this result to the fact that litter bags restricted access to the litter by scavengers and detritivores. Kirby and Gosselink, on the other hand, indicate that on salt marshes, litterbags may result in higher estimates because the bags may provide a more suitable microenvironment for small scavenging invertebrates and microorganisms. We chose the literbag method for the following reasons: (1) We had litter biomass data available, (2) paired plots would have been too destructive, (3) Vegetation removal from paired plots can significantly affect microclimate and thus influence the results (Wiegert and Evans 1964). (4) Our sampling intervals were relatively short, therefore the major component of inter-sample losses were from the ground litter not from the standing dead biomass; furthermore, an unknown proportion of the conversion of standing dead biomass to ground litter was accounted for by our ground litter data. We used our field data to calculate the three production estimates described above Milner and Hughes, Smalley, Wiegert and Evans).

To minimize the effects of litter bag handling and transport to the field upon the initial measurements of material lost from the bags, we substituted the mean of the losses during the first and second months for the losses during the first month in all the production calculations. T-tests of production by <u>Batis</u> and <u>Salicornia</u> indicated that there were no significant differences in production between species at any of the stations (PD: t =

1.02, p = 0.33; RG: t = 1.152, p = 0.276; NM: <u>Salicornia</u> too scarce for analysis; OR: t = 1.16, p = 0.275) so the data for both species were pooled.

Physical Variables.

We measured the following variables at each station during each sampling: Salinity (A.O. temperature-compensated refractometer), D.O. and water temperature (YSI D.O - Temp meter), air temperature (mercury thermometer), and pH (Gallenkamp pH Probe). Two water level recorders were installed, one in the lagoon, and one inside IRC #12. The flooding elevation of each station was then determined by direct observation, and the number of days per month during which each station received at least one flooding "tide" (flood-days) was calculated from the above data. We also installed soil temperature recorders with sensors on the marsh surface at the PD, NM, and OR stations.

Soils.

Prior to the start of this study we obtained soil cores from each site (since several concurrent studies were being carried out in the same marshes, the samples for RG were taken in an adjoining location - designated as HB in this report - that was also representative of RG). Cores were taken from 0-10 cm and from 11-20 cm deep. Large rocks and vegetation debris were removed from each core and the remaining materials were dried, and shipped to the University of Florida's IFAS Soil Analysis Laboratory where the following determinations were made on each core: organic carbon, soluble salts, pH, Na, P, NH_4^+, NO_3^-, Cl^-, and % organic matter. A portion of each core was also sent to the University of Florida'a Soil Characterization Laboratory, where complete particle size distribution analyses were performed.

RESULTS

Physical Data.

Soil temperatures recorded by the thermographs at PD, NM, and OR exhibited similar seasonal patterns, with peaks during July and August, and minima during December - February (Fig 2). Temperatures of over 40°C were recorded at all three stations during the summer of 1987, with the highest soil temperature observed during July at OR (52°C). Summer high temperatures were lower during 1988 with the highest recorded temperature being 43.9 °C at OR. Winter minima were never below 0°C, and were lower during 1987-1988 than during 1986-1987 (Table 1). The lowest temperature recorded during the study was 1.1°C at OR during the winter of the second year. Average monthly temperatures did not differ significantly between any of the sites (Fig. 2).

Water temperature, salinity, and pH measured at each station give only a rough estimation of conditions at each site, since they represent single observations made at sampling time, and there are many gaps in the data because these measurements could only be taken if the site happened to be flooded during our regular sampling. A summary of these data are shown in Table 1.

Flooding Frequency.

Peaks in flood-days occurred during March and during Oct-Nov of 1987 (Fig. 3). A third peak observed at PD and RG during the summer of 1988 was due to the artificial flooding for mosquito control undertaken that year.

The Perimeter Ditch station had the most flood-days, followed by RG and OR, and then by NM (Fig. 4). Most of the natural flooding tides took place from November to April, which is the period with highest water levels in the lagoon. The large number of days with flooding tides evident at PD and RG from May to October of 1988 again reflects the artificial water management for mosquito control during the summer of that year (Fig. 4).

Water levels were higher overall during 1986-1987 than during 1987-1988 but management practices at the impoundment stations resulted in more "flood-days" at those sites during the latter interval (Fig. 4).

Soils.

The major differences found in the particle size distribution of the various marsh soils was in the relative proportions of sand and silt. Soils from the top 10 cm of HB (representing RG) and the bottom 10 of OR had less sand than most others, but whereas the former offset the dearth of sand with a greater proportion of silt, the latter had a greater proportion of clay than most other stations (Fig. 5). The PD sites and the top cores from NM had relatively less silt and more sand than the others.

The lowest values of total phosphorus, sodium, NH_4, NO_3, Cl, and organic carbon were recorded at NM, whereas HB had higher values of sodium, NH_4, soluble salts, Cl, and organic carbon. Oslo Road had the highest concentrations of phosphorus and NO_3 and the highest nutrient:salinity ratios of all the stations (Figs. 6 - 11).

Biomass.

The two impoundment sites exhibited similar temporal patterns of above-ground biomass. Live biomass was generally low from November 1986 to May 1987; it then increased and remained fairly constant until the summer of 1988, when levels decreased at both stations; a live biomass peak at RG during December 1986-January 1987 was nor observed at PD (Figure 12). Dead biomass showed the opposite pattern, starting at close to 300 g/¼ m^2 in November of 1986, declining through the spring and summer of 1987, and remaining close to 0 until July of 1988, when increases at both stations were again evident (Fig.13). Litter biomass fluctuated between 40 - 110 g/¼ m^2 from November 1986 to June 1987. At PD, litter biomass increased during the summer of 1987 to near 150 g/¼ m^2 and declined to about 50 g/¼ m^2 in October, while at RG levels stayed near 50 g/¼ m^2 during the same period. Further decreases were evident at both sites during January-February of 1988, with litter biomass dropping to near 0 and remaining close to that level during the rest of the study (Fig 14).

Biomass patterns at NM and OR were somewhat different from the above. At both sites live biomass increased from the winter of 1986 to the spring of 1987. Levels fluctuated around 300 g/¼ m² from late spring to early summer of 1987, increased during August - September, and declined slightly in October. The patterns the following year were similar except for the lack of a late-summer rise, with increases evident from December of 1987 to the spring of 1988 and slight declines during early summer (Fig. 16).

Dead biomass at both unimpounded sites was low throughout the study. There were peaks (20 - 40 g/¼ m²) during November of 1986 at both sites, during August of 1987 and February-March of 1988 at OR, and in April of 1988 at NM (Fig 17). Litter biomass was also low, remaining close to 0 during much of 1986-1987, and increasing to maxima during May-June 1988 (Fig.18).

The relationships between the different biomass fractions at each site are summarized in Table 2. At PD and RG there were significant ($p \leq 0.056$) negative correlations between live biomass and both dead and litter biomass, and a positive correlation between dead biomass and litter. At NM dead biomass was positively correlated with litter biomass, and at OR live biomass was negatively correlated with litter biomass.

Analysis of variance of the biomass data indicate that there were significant differences in live, dead, litter and total biomass between sites and between years (Table 3). Live biomass was greater at the open stations than at the impoundment stations, and during 1987-1988 than during 1986-1987, whereas total biomass was greater at OR than at the other three stations, during both years. There were significant site X year interactions in the analyses for dead and litter biomass. The interaction evident after analysis of the dead biomass data is due to the fact that during year 1 dead biomass was greatest at PD, followed by RG and then by NM and OR, but during year 2 there were no significant differences between any of the stations (Table 3). Likewise, during 1986-1987, litter biomass was higher at PD and RG than at NM and OR but there were no significant differences between stations during 1987-1988 (Table 3).

The relative proportions of live, dead and litter biomass at PD and RG also varied considerably between years. During 1986-1987, standing dead vegetation and litter comprised the major part of the mean total biomass at these sites, but during the following year live vegetation was more prevalent (Fig. 20). At NM and OR, live biomass predominated and standing dead and litter made up a small proportion of the total biomass during both years.

Organic Matter.

As expected, the seasonal patterns of organic matter in the different biomass components roughly followed the patterns for their respective biomass fractions (Figs. 21 - 24). The relative percentage of organic content (Figs. 25 - 26) however, exhibited significant variation between sites and between years.

At NM, the live fraction had higher organic content than the dead and litter fractions, whereas at OR the live and litter fractions contained more organic matter that the dead. There were no significant differences in organic matter content between years at the above sites (Table 5). Significant effects of year and fraction, and significant year X fraction interactions were evident after analysis of the data for PD and RG vegetation; similar results were obtained when all four stations were considered together (Table 5).

During 1986-1987, the litter and live fractions at PD were not significantly different and had higher organic content than the dead fraction, but during 1987-1988 live was again highest, but litter organic content was not significantly different from that of live or dead (Table 6). At RG there was no difference in organic content between the live and litter fractions during 1986-1987, but the live fraction had higher organic content than the litter fraction during 1987-1988; dead biomass had the lowest organic content during both years. When the data for the four sites are considered together, the results are similar to those at RG (Table 6).

Live vegetation at RG had higher organic content than at OR and NM, but PD was not significantly different from the other three stations. There were no between-site differences in organic content of dead vegetation, but litter at the impoundment stations had a greater organic content than at NM and OR (Table 7).

Litter Bag Losses.

Yearly loss rates from litter bags ranged from close to 82% at OR during 1987-1988 to 60.8% at PD during the previous year (Table 8), but comparison of the slopes of the cumulative percent litter bag loss curves (Fig. 27) by the Gabriel Confidence Interval Method (Sokal and Rohlf, 1981), however, did not reveal any significant difference ($p \leq 0.05$) between sites during either year, nor were there significant differences in loss rates between the bags set in October and those set in May ($T = 0.599$, $p = 0.559$, 14 df).

Production.

During 1986-1987, the seasonal production patterns were different at the various stations (Fig. 28). At PD, production was highest in the winter and lowest in late summer and early fall while RG had peak production in the spring and minima in late summer. Production at NM was highest during early summer and lowest during late spring and late fall but at OR production peaked in the winter and then decreased to its lowest values in summer.

The patterns for 1987-1988, on the other hand, were similar at all four stations, with the highest production observed from January to May, and the lowest during the summer (Fig. 29).

Yearly production was highest at OR during both years regardless of the computation method used (Fig. 30). Production at this site ranged from 1028.0 $g/m^2/yr$ for 1987-1988 using the Milner and Hughes method, to 2316.5 $g/m^2/yr$ for 1986-1987 computed with the Wiegert and Evans method (Table 9). Production at NM was second highest, except for the Wiegert and Evans estimates for 1986-1987 which ranked this site in last place. Of the two impounded sites, the estimates for PD were consistently higher than those for RG.

Comparison of the production values obtained using the different methods indicates that, as expected, the Milner and Hughes (M-H) method always yielded much lower production figures that the other two methods. There was a good correspondence between the Smalley (SMA) and Wiegert and Evans (W-E) method for NM and OR, but the similarity was less for PD and RG, particularly for 1986-1987, when Smalley's method produced much lower estimates than Wiegert and Evans' (Table 9). It is evident that the correspondence between SMA and W-E decreases as the relative importance of litter in a station's production dynamics increases; thus the correlation between the two methods was best for the unimpounded stations, and worst for PD and RG during 1986-1987, when a large proportion of the biomass at those stations was in the form of litter. A decrease in the relative proportion of litter at the impoundment stations during the following year (Fig. 20) resulted in higher similarity between the two measures.

Total yearly production was not correlated with mean soil temperature ($r = 0.515$, $p = 0.191$, $N = 8$), but it was negatively correlated with the total number of flood-days per year ($r =-0.789$, $p = 0.0199$, $N = 8$). No significant correlations were evident between the monthly production values and mean monthly soil temperature nor between monthly production and total number of flood-days per month, even when lags of 1 to 3 months were introduced into the data.

DISCUSSION

Although the salt marsh literature is brimming with data on production by marsh halophytes, a great majority of those data deal with production by only a few species, particularly Spartina alterniflora. There is very little information on production by Batis-Salicornia marshes, and almost none on production in "stressed" and/or impacted marshes (Turner 1976). The range of values for annual above-ground production reported in the literature is so broad that stating that certain values fall within that range does not carry very much meaning anymore.

Production from our sites (Table 9) does compare favorably with that reported from Batis - Salicornia marshes in California, which ranged from 400 - 1450 g/M^2/yr (Winfield 1980, Zedler et al 1980), and that from Salicornia europae marshes in Massachusetts which produced an average of 240 g/M^2 (Ruber et al 1981). The California data, however, come mainly from hypersaline marshes that receive very little precipitation during the year, and the Massachusetts data come from a higher latitude area exposed to a temperate (vs our sub-tropical) climate. Soil salinity (Zedler 1963, Chalmers, 1982), soil temperature (Young 1974), and latitude (Turner 1976) have been shown to have significant effects upon salt marsh production.

The unimpounded marshes in our studies had higher production than the impoundment stations over the whole two-year period, regardless of the method used to calculate production (Table 9). The Milner & Hughes and the Smalley methods, however underestimate production because they do not consider material losses between sampling intervals (Linthurst and Reimold 1978). This underestimation will be greatest for stations such as PD and RG that maintain a large proportion of their biomass as litter. If we consider only production calculated using the Wiegert & Evans method, then the patterns are less clear-cut. During 1986-1987, the production rankings were OR > PD > RG > NM; but during 1987-1988, and when both years are combined the rankings were OR > NM > PD > RG.

Several factors may be responsible for the above differences. Most obvious is the effect of summer closure and flooding upon the vegetation. Temporary, but significant damage to both Batis and Salicornia has been measured at IRC #12 after summer management for mosquito control (Rey et al in prep.). Although the immediate effect of summer flooding is the conversion of live biomass into dead and litter biomass, the differences in production by the impoundment station between 1986-1987 (impoundment open all year) and 1987-1988 (impoundment closed and flooded from May to October) suggest that production was adversely affected. There is no information on the effects of flooding regime on production by Batis maritima or Salicornia virginica, but studies on other species of Salicornia (S. europea-

Wiebe 1935, S. stricta - Langlois 1971, - S. stricta and S. ramosissima - Langlois and Ungar 1976) produced conflicting results.

If we examine the production patterns at PD and RG, it becomes apparent that in 1987, the summer decline in production did not start until August in RG and September in PD (Fig. 28). In 1988, however, the decline started much earlier and summer, production dropped to lower levels than in 1987, particularly at PD (Fig. 29) and stayed low until October. Although a sharp drop in production was also evident in March at NM and OR, production at those stations increased during the summer. During May-October of 1987, total production at PD and RG was 577.8 g/m^2 and 661.0 g/m^2, respectively while during the same interval in 1988 the figures were 242.1 g/m^2 and 190.3 g/m^2. These differences yield production deficits of 314.9 and 470.7 g/m^2 for PD and RG, respectively. If we add the deficits to the PD and RG production figures for 1987-1988, then the yearly production totals increase to 1252.2 g/m^2 at PD and 1305.6 g/m^2 at RG, which are close to the production estimates for 1986-1987. It is apparent, then, that the drop in production during the 1988 artificial flooding period was responsible for most of the decline in production evident at PD and RG during 1987-1988. The flooded, anaerobic conditions existing at these sites during the summer of 1988 probably caused a considerable slow-down in plant metabolism and resulted in much lower production than during the same period in 1987. Similar results were reported by Conner and

Day (1976) for riverine swamps in Louisiana, and Odum _et al_. (1983) for giant cutgrass (_Zizaniopsis miliacea_) marshes in Georgia.

Thus, although regularly-pulsed tidal subsidies tend to enhance salt marsh production, the summer flooding for mosquito control apparently pushed these systems over the hump of the "subsidy-stress gradient" (Odum _et al_. 1979), eliciting a negative response in the form of reduced production. Whether, as a result, the stability of these systems is also reduced (Beddington & May 1977, Odum _et al_. 1979) remains to be seen.

Differences in soil composition may play a part in the higher production observed at OR, the site with consistently highest production. Soils at this site had higher nutrient and lower salt concentrations than soils at the other stations (Figs. 6 - 11). Variation in the growth of different salt marsh species has been attributed to differences in nutrient availability (Jeffries _et al_. 1972) and to the interaction of nutrient concentrations and salinity (Stewart _et al_. 1973). Mendelssohn and Marcellus (1976) found that high soil salinities and low phosphorus and nitrogen levels tended to limit production in Virginia salt marshes, but the amount of variation explained by single soil factors was low, and varied significantly from site to site. Nixon and Oviatt (1973), reported that ammonia in the marsh water could explain 49% of the variation in _Spartina_ standing crop of Rhode Island salt marshes, while Lanthwell _et al_ (1969) could not rank individual soil variables with regard to their importance to plant growth in wild rice marshes. Mendelssohn and Marcellus (1976) conclude that those nutrient forms that happen to be immediately available for absorption by plants may be most important in determining their production. Although the concentrations of some nutrients may have been higher at the most productive sites in this study, (i.e. NO_3 (Fig. 7)), others were not (i.e NH_4 (Fig. 7)); additionally, the soil nutrient measurements reported here represent single (replicated) observations, that may change seasonally. Thus, definite conclusions are not warranted from the data.

The flooding patterns at the various stations (Figs. 3 - 4) were more a reflection of marsh physiognomy than of relative elevation. The proximity of PD to the impoundment perimeter ditch resulted in a greater number of flood days than at the other stations, whereas the natural berm surrounding NM impeded tidal flooding so that NM received the least number of floods of all stations. RG and OR, on the other hand, may offer a better representation of the "natural" flooding patterns of high marshes in this region even though the number of flood days at RG during 1987-1988 was artificially elevated due to mosquito control activities.

Although tidal energy has been proposed as one of the factors responsible for the high primary productivity of salt marshes, total annual production at the study stations was negatively correlated with number of flood-days per year. A large proportion of this correlation, however, was due to the

1987-1988 patterns, when PD and RG had the highest number of flood-days (because of the summer closure and flooding, Fig. 3), but the lowest yearly production. This observation points to the non-trivial difference between frequent tidal **flushing**, and prolonged **flooding**; the former tends to enhance production, whereas the latter, as discussed above, may significantly reduce it. The observation that on a month-by-month basis no correlations existed between production and flooding reinforces the importance of the summer management scheme to production dynamics at the study sites.

Studies in non-tropical marshes usually report highest production during the summer (i.e., Stroud _et_ _al_. 1968, Hopkinson _et_ _al_. 1978) but our data indicate that production actually declined in the summer (Figs. 28 & 29). The local decline in summer production is probably a result of the high temperatures evident at all the stations during the summer (Fig. 2), and of the fact that summer is the period when water levels in the lagoon are at their lowest (Provost 1973) and, therefore, tidal energy and nutrient subsidies to the marsh are at their minimum.

Given the differences in their flooding regimes it is surprising that there were no significant differences in the rates of loss from the litterbags at the various stations. Once again, the difference between flooding and flushing may be important here. For example, White and Trapani (1982) report that in Louisiana _Spartina_ _alterniflora_ marshes, the loss rates from litterbags were positively correlated with numbers of tidal flushing, but negatively correlated with hours of flooding. Prolonged flooding may reduce the rates of litter decomposition by modifying the ambient salt, mineral, and nutrient concentrations which, in turn, may adversely affect detritivore metabolism (Bradshaw 1968). The anaerobic conditions usually associated with long-term flooding may also reduce the rates of litter decomposition by limiting the activity of aerobic decomposers (White and Trapani 1982, see also Howarth et al. 1982).

Perhaps the most striking difference between the impounded and unimpounded marshes was the difference in the relative contributions of live, dead, and litter biomass to their total biomass stock (Fig. 20). Of particular interest is the fact that since there were no between-site differences in the rates of litter decomposition, the large litter input to the impounded sites after flooding in 1986 signifies that the average residence time of litter particles was greater there than at NM and OR. Since the degree of litter enrichment is often positively correlated with residence time (Turner 1976), litter from the impounded sites may actually be a better energy source for detritivores than that from NM and OR.

Additional evidence for the above assertion comes from the comparison of organic matter content at the various stations. Litter at the impoundment stations did have significantly higher organic content than litter at NM and OR, whereas there were no significant differences in organic content in the dead fractions, and the patterns for the live fractions did not separate the impoundment stations from the unimpounded ones (Table 7). Furthermore, the only significant difference in organic content between years was for litter biomass, and indicated that litter organic content during 1986-1987, when PD and RG had large litter stocks resting on the marsh floor, was significantly greater than during 1987-1988, when litter stocks at the impoundment stations were smaller and fresher.

The large stock of litter at PD and RG may also function as a nutrient reservoir (Mendelssohn and Marcellus 1976) and may be partially responsible for the increased production of these stations during the second year. At this time, however, we have no data on the nutrient status at any of the stations, nor do we know if nutrient limitation is important to plant production at these sites.

CONCLUSION

Although the data presented above indicate that marsh flooding for mosquito control decreased above-ground primary production in the study sites, the overall impact on the marsh and estuary energy dynamics is not as clear-cut. For one thing, even with artificial flooding, there was still significant production at the impoundment stations. Second, the system appears to be resilient; production during the following year was back to levels comparable with at least one of the two unimpounded stations. Further complicating factors that must be considered are the quality of the materials being exported to lagoon, and the possible self-stabilizing effect of litter accumulation in the marsh floor.

All this discussion is academic if impounding wipes out the marsh vegetation and/or if a large proportion of the production is sequestered in the perimeter ditches and never makes it into the estuary; the flux of materials (both dissolved and particulate) between perimeter ditches and the lagoon is a subject in dire need of investigation.

It seems prudent at this time to recommend that impoundments be left open during one whole year on a regular basis (perhaps every 2-3 years) to give vegetation a chance for recovery and to flush some of the litter that accumulates on the marsh floor. Caution should be exercised, however, when releasing water from impoundments with large litter accumulation, or with very stagnant water to prevent deleterious impacts upon the lagoon. Most important, however, is the need to avoid widespread vegetation kills, since this would probably have a greater impact on whole marsh production (as opposed to production per unit area) than the observed declines due to seasonal flooding for mosquito control. Increase in the number and size of tidal connections (culverts) will probably help in the export of marsh production to the estuary.

ACKNOWLEDGEMENTS

The authors wish to thank the Indian River Mosquito Control District personnel, particularly Doug Carlson and Pete O'Bryan for their help and cooperation during this study. We also wish to thank Jim Stoutamire of the Florida Department of Environmental Regulation for his help with administrative details, and Robert Stahl for assistance with numerous aspects of the project.

LITERATURE CITED

Beddington, J. R. and R. M. May. 1977. Harvesting natural populations in a randomly-fluctuating environment. Science 197: 463-465.

Binet, P. 1965. Etudes d'ecologie experimentale et physiologique sur Cochlearia anglica L. Oecol. Plant. 1:7-38.

Bradshaw, J. S. 1968. Environmental parameters and marsh Foraminifera. Limn.and Oceanogr. 13: 26-38.

Chalmers, A. G. 1982. Soil dynamics and the productivity of Spartina alterniflora. Pp. 231-242 In: V. S. Kennedy (ed.). Estuarine Comparisons. Academic Press, N.Y.

Conner, W. H. and J. W. Day. 1976. Productivity and decomposition of a bald cypress-water tupelo site and a bottomland hardwood site in a Louisiana swamp. Am J. Bot 63: 1354-1364.

de la Cruz, A. A. 1973. The role of tidal marshes in the productivity of coastal waters. Assoc. Southeastern Biologists Bull. 20: 147-156.

Dickerman, J. A., A. J. Stewart, and R. G. Wetzel. 1986. Estimates of net annual aboveground production: Sensitivity to sampling frequency. Ecology 67: 650-659.

Gilmore, R. G., D. W. Cooke, and C. J. Donohoe. 1981. A comparison of fish populations and habitat in open and closed salt marsh impoundments in east-central Florida. N.E. Gulf Sci. 5: 25-37.

Gorham, E. 1953. Chemical studies on the soils and vegetation of water-logged habitats in the English Lake District. J. Ecology 41: 345-360

Harrington, R. W. & E. S. Harrington. 1982. Effects on fishes and their forage organisms of impounding a Florida salt marsh to prevent breeding by salt marsh mosquitoes. Bull. Mar. Sci. 32: 646-666.

Hopkinson, C. S., J. G. Gosselink, and R. T Parrondo. 1978. Aboveground production of seven marsh plant species in coastal Louisiana. Ecology 59: 760-769.

Howarth, R. W. and J. E. Hobbie. 1982. The regulation of decomposition and heterotrophic microbial activity in salt marsh soils: A review. Pp. 183-206 **In:** V. S. Kennedy (ed.). Estuarine Comparisons. Academic Press, N.Y.

Jeffries, R. L., A. J. Davy, and T. Rudmik. 1972. The growth strategies of coastal halophytes, Pp. 243-268 **In:** R. L. Jeffries and A. J. Davy (eds.). Ecological Processes in Coastal Environments. Blackwell, London.

Jervis, R. A. 1964. Primary production in a freshwater marsh ecosystem. Ph.D. Dissertation, Rutgers University.

Keefe, C. W. 1972. Marsh production: A summary of the literature. Contr. Mar. Sci. 16: 163-181.

Kirby, C. J. and J. G. Gosselink. 1976. Primary production in a Louisiana Gulf Coast Spartina alterniflora marsh. Ecology 57: 1052-1059.

Langlois, J. 1971. Influence du rythme dimmersion sur la croissance et la metabolisma proteique de Salicornia stricta Dumort. Oecol. Plant 6:227-245.

Langlois, J. and I. A. Ungar. 1976. A comparison of the effect off artificial tidal action on the growth and protein nitrogen content of Salicornia stricta Dumort and Salicornia ramossisima Woods. Aquat. Biol. 2: 43-50.

Lanthwell, D. J., H. F. Mulligan, and D. R. Bouldin. 1969. Chemical properties, physical properties, and plant growth in twenty artificial wildlife marshes. N. Y. Fish and Game J. 16: 158-183.

Linthurst, R. A. and R. J. Reimold. 1978. An evaluation of methods for estimating the net aerial primary productivity of estuarine angiosperms. J. Applied Ecol. 15: 919-931.

Mendelssohn, I. A. and K. L. Marcellus. 1978. Angiosperm production of three Virginia marshes in various salinity and soil nutrient regimes. Chesapeake Sci. 17: 15-23.

Milner, C & R. E. Hughes, R. E. 1968. Methods for the Measurement of the Primary Production of Grasslands. IBP Handbook No. 6. Blackwell Scientific Publications, Oxford.

Morgan, M. H. 1961. Annual angiosperm production on a salt marsh. M.S. Thesis, University of Delaware.

Nixon, S. W. and C. A Oviatt. 1973. Analysis of local variation in the standing crop of Spartina alterniflora. Botanica Marina 16: 103-109.

Odum, E. P. 1959. Fundamentals of Ecology. W. P. Saunders Co., N. Y.

Odum, E. P. 1961. The role of tidal marshes in estuarine production. N. Y. St. Conserv. 15: 12-15.

Odum, E. P., J. T. Finn, and E. H. Franz. 1979. Perturbation theory and the subsidy-stress gradient. BioScience 29: 349-352.

Odum, E. P., J. B. Birch, and J. L. Cooley. 1983. Comparison of giant cutgrass productivity in tidal and impounded marshes with special reference to tidal subsidy and waste assimilation. Estuaries 6: 88-94.

Provost, M. W. 1973. Mean high water mark and use of tidelands in Florida. Fl. Scientist 36: 50-66.

Rey, J. R., R. A. Crossman, T. R. Kain, and D. S. Taylor. 1986. An overview of impounded mangrove forests along a subtropical lagoon in east-central Florida, U.S.A. Pp. 341-350 In: L. Bhosale (ed.). The Mangrove. Proc Nat. Symp. Biol. Util. Cons. Mangroves. Shivaji University Press, Kolhapur, India.

Schelske, C. L. and E. P. Odum. 1961. Mechanisms maintaining high productivity in Georgia estuaries. Proc. Gulf Carib. Fish Inst. 14: 75-80.

Smalley, A. E. 1959. The role of two invertebrate populations, Littorina irrorata and Orchelimum fidicinum in the energy flow of a salt marsh ecosystem. PH. Dissertation, University of Georgia.

Sokal, R. R and F. J. Rohlf. 1981 Biometry. W. H. Freeman, San Francisco.

Steever, E. Z., R. s. Warren, and W. A. Niering. 1976. Tidal energy subsidy and standing crop production of Spartina alterniflora. Est. Coast. Mar. Sci. 4:473-478.

Stewart, G. R., J. A. Lee, and T. O. Orebamjo. 1973. Nitrogen Metabolism in halophytes. New Phytol. 72: 539-546.

Stroud, L. M. and A. W. Cooper. 1969. Color infrared aerial photographic interpretation and net primary productivity of a regularly-flooded North Carolina salt marsh. Univ. of North Carolina Water Resources Institute Report #14, 86 pp.

Turner, R. E. 1976. Geographic variation in salt marsh macrophyte production: A review. Contr. Mar. Sci. 20: 47-68.

White, D. A. and J. M. Trapani. 1982. Factors influencing the disappearance of Spartina alterniflora from litter bags. Ecology 63: 242-245.

White, D. A. T. E. Weiss, J. M. Trapani, and L. B. Thien. 1978. Productivity and decomposition of the dominant salt marsh plants in Louisiana. Ecology 59: 751-759.

Wiebe, P. O. 1935. A quantitative study of the influence of tide upon populations of Salicornia europaea J. Ecol. 23: 323-333.

Wiegert, R and F. Evans. 1964. Primary production and the disappearance of dead vegetation from an old field. Ecology 45: 49-63.

Winfield, T. P. 1980. Dynamics of carbon and nitrogen in a Southern California salt marsh. Ph.D dissertation, University of California - Riverside.

Young, D. L. 1974. Studies of Florida Gulf coast salt marshes receiving thermal discharges. Pp. 532-550 In: J. W. Gibbons and R. R. Sharitz (eds.) Thermal Ecology. U.S. AEC Sym. CPNF-730505.

Zedler, J. B. 1963. Freshwater impacts on normally hypersaline marshes. Estuaries 6: 346-355.

Zedler, J. B., T. P. Winfield, and P. Williams. 1980. Salt marsh productivity with natural and altered tidal circulation. Oecologia 44: 236-240.

TABLE LEGENDS

Table 1. Summary of the physical data measured at the four stations. Salinity, water temperature and pH were measured on the water flooding the marsh, soil temperature with electrodes on the marsh surface.

Table 2. Pearson correlations among the different biomass fractions from 1986 to 1988. Upper value is the correlation coefficient, lower value the probability level.

Table 3. Results of analyses of variance for differences in biomass at the four sites during 1986-1988. DMR indicates the rankings produced by Duncan's Multiple Range Test; SI is entered under DMR if the interaction term for the comparison is significant. NS = main effect probability > 0.05, PD = Perimeter Ditch, RG = Rain Gauge, NM = North Marsh, OR = Oslo Road.

Table 4. Results of one-way analyses of variance for differences in dead biomass and litter biomass during 1986-1987 and 1987-1988. Symbols are as in the previous table.

Table 5. Results of analyses of variance for differences in percent organic matter between biomass fractions during 1986-1988. All symbols as in Table 3.

Table 6. Results of one-way analyses of variance for differences in percent organic carbon between biomass fractions at the Perimeter Ditch and Rain Gauge sites, and at all sites combined. Data were subjected to an arcsine-square root transformation prior to analysis.

Table 7. Results of analyses of variance for differences in percent organic matter between sites and between years after angular transformation of the data.

Table 8. Percent losses from litter bags at all stations during 1986 - 1988. Set indicates the month in which the litter bags were placed in the marsh; L-190 = percent loss after 190 days, L-YR = percent loss after a year.

Table 9. Yearly production estimates (g/M^2) at the four stations for 1986-1987 and 1987-1988. Centered titles indicate the method used to calculate the estimates that follow them.

FIGURE LEGENDS

Figure 1. Map of the study area showing the sampling sites.

Figure 2. Soil temperatures during the study. A - NM, B - OR, C - PD, D - Mean soil temperatures.

Figure 3. Number of flood-days per month at the study stations.

Figure 4. Flooding frequency during 1986-1988 at the four stations. N-A = November to April, M-O = March to October.

Figure 5. Particle size distribution at the study sites. First two letters of the site captions identify the stations (NM = North Marsh, PD = Perimeter Ditch, OR = Oslo Road; HB is a nearby site that represent Rain Gauge); third letter represents depth of cores (T = 0-10 cm, B = 11 - 20 cm).

Figure 6. Soil Phosphorus (top) and Sodium (bottom) concentrations at the study sites. Symbols as in Fig. 5.

Figure 7. Soil Ammonia (top) and Nitrate (bottom) concentrations at the study sites. Symbols as in Fig. 5.

Figure 8. Soil Cl (top) and organic Carbon (bottom) concentrations at the study sites. Symbols as in Fig. 5.

Figure 9. Soil soluble salt (top) concentration and pH (bottom) concentrations at the study sites. Symbols as in Fig. 5.

Figure 10. Nitrate:soluble salts ratios at the study sites.

Figure 11. Phosphorus:soluble salts ratios at the study sites.

Figure 12. Seasonal patterns of live biomass at PD and RG.

Figure 13. Seasonal patterns of dead biomass at PD and RG.

Figure 14. Seasonal patterns of litter biomass at PD and RG.

Figure 15. Seasonal patterns of total biomass at PD and RG.

Figure 16. Seasonal patterns of live biomass at NM and OR.

Figure 17. Seasonal patterns of dead biomass at NM and OR.

Figure 18. Seasonal patterns of litter biomass at NM and OR.

Figure 19. Seasonal patterns of total biomass at NM and OR.

Figure 20. Relative distribution of biomass components at the four sites during both years.

Figure 21. Organic matter content of the live fraction at the four stations.

Figure 22. Organic matter content of the dead fraction at the four stations.

Figure 23. Organic matter content of the litter fraction at the four stations.

Figure 24. Total organic matter content at the four stations.

Figure 25. Percent organic matter content at PD (top) and RG (bottom).

Figure 26. Percent organic matter content at NM (top) and OR (bottom).

Figure 27. Cumulative percent losses from the litterbags at the four stations.

Figure 28. Three-point moving average of the monthly production data for 1986-1987.

Figure 29. Three-point moving average of the monthly production data for 1987-1988.

Figure 30. Comparison of annual production estimates at the four stations using the Milner and Hughes (M-H), Smalley (SMA) and Wiegert & Evans (W-E). 1 = 1986-1987, 2 = 1987-1988.

Table 1. Summary of the physical data measured at the four stations. Salinity, water temperature and pH were measured on the water flooding the marsh, soil temperature with electrodes on the marsh surface.

	1986-1987			1987-1988		
VAR/STA	HIGH	LOW	MEAN	HIGH	LOW	MEAN
Salinity (PPT)						
PD	29.0	15.0	24.3	40.0	21.0	32.8
RG	45.0	13.0	29.0	30.0	25.0	28.3
NM	22.0	17.0	19.5	-	-	-
OR	28.0	6.0	20.2	27.0	20.0	23.5
Water Temperature (°C)						
PD	28.0	20.0	23.0	28.0	5.0	22.9
RG	34.0	21.0	27.5	29.0	5.0	19.7
NM	24.0	21.0	22.5	-	-	-
OR	25.0	19.0	21.7	20.0	12.0	16.0
pH						
PD	8.0	7.1	7.5	7.2	6.8	7.1
RG	7.7	7.6	7.7	8.0	6.9	7.5
NM	7.1	7.0	7.0	-	-	-
OR	7.9	6.9	7.5	6.6	6.7	6.6
Soil Temperature (°C)						
PD	47.8	12.2	-	43.3	3.9	-
NM	43.3	13.3	-	38.3	12.8	-
OR	52.8	11.1	-	43.9	1.1	-

Table 2. Pearson correlations among the different biomass fractions from 1986 to 1988. Upper value is the correlation coefficient, lower value the probability level.

	Perimeter Ditch			Rain Gauge		
	LIVE	DEAD	LITTER	LIVE	DEAD	LITTER
LIVE	1.0000	-0.8695	-0.5177	1.000	-0.8460	-0.3907
	-	0.0001	0.0080	-	0.0001	0.0535
DEAD		1.0000	0.3858		1.0000	0.5229
		-	0.0568		-	0.0073
LITTER			1.0000			1.0000
			-			-

	North Marsh			Oslo Road		
	LIVE	DEAD	LITTER	LIVE	DEAD	LITTER
LIVE	1.0000	0.0057	-0.3137	1.00001	-0.0304	-0.5276
	-	0.9784	0.1268	-	0.8854	0.0067
DEAD		1.0000	0.7301		1.0000	-0.0774
		-	0.0001		-	0.7133
LITTER			1.0000			1.0000
			-			-

Table 3. Results of analyses of variance for differences in biomass at the four sites during 1986-1988. DMR indicates the rankings produced by Duncan's Multiple Range Test; SI is entered under DMR if the interaction term for the comparison is significant. NS = main effect probability > 0.05, PD = Perimeter Ditch, RG = Rain Gauge, NM = North Marsh, OR = Oslo Road.

SOURCE	DF	F	p	DMR
			<u>LIVE BIOMASS</u>	
SITE	3	79.00	0.0001	OR > NM > PD = RG
YEAR	1	37.66	0.0001	87-88 > 86-87
S x Y	3	2.22	0.0905	-
			<u>DEAD BIOMASS</u>	
SITE	3	17.16	0.0001	SI
YEAR	1	19.17	0.0001	SI
S x Y	3	9.54	0.0001	-
			<u>LITTER BIOMASS</u>	
SITE	3	25.12	0.0001	SI
YEAR	1	61.87	0.0001	SI
S x Y	3	15.08	0.0001	-
			<u>TOTAL BIOMASS</u>	
SITE	3	4.75	0.0040	OR > NM = PD = RG
YEAR	1	2.79	0.0985	NS
S x Y	3	0.63	0.5944	-

Table 4. Results of one-way analyses of variance for differences in dead biomass and litter biomass during 1986-1987 and 1987-1988. Symbols are as in the previous table.

FRACTION	DF	F	p	DMR
<u>DEAD</u>				
86-87	3	17.22	0.0010	PD > RG > OR = NM
87-88	3	2.01	0.1269	NS
<u>LITTER</u>				
86-87	3	24.45	0.0001	PD = RG > OR = NM
87-88	3	1.56	0.2118	NS

Table 5. Results of analyses of variance for differences in percent organic matter between biomass fractions during 1986-1988. All symbols as in Table 3.

SOURCE	DF	F	p	DMR
		All Sites		
YEAR	1	10.97	0.0011	SI
FRACTION	2	35.23	0.0001	SI
Y x F	2	6.52	0.0017	-
		Perimeter Ditch		
YEAR	1	0.72	0.3990	NS
FRACTION	2	21.94	0.0001	SI
Y x F	2	3.32	0.0428	-
		Rain Gauge		
YEAR	1	6.19	0.0155	SI
FRACTION	2	21.94	0.0001	SI
Y x F	2	7.13	0.0016	-
		North Marsh		
YEAR	1	3.34	0.0700	NS
FRACTION	2	10.61	0.0002	LIVE > LITT = DEAD
Y x F	2	0.10	0.9050	-
		Oslo Road		
YEAR	1	2.07	0.1560	NS
FRACTION	2	7.22	0.0017	LIVE = LITT > DEAD
Y x F	2	1.56	0.2189	-

Table 6. Results of one-way analyses of variance for differences in percent organic matter between biomass fractions at the Perimeter Ditch and Rain Gauge sites, and at all sites combined. Data were subjected to an arcsine-square root transformation prior to analysis.

SOURCE	DF	F	p	DMR
		All Sites		
FRACT 86-87	2	13.13	0.0001	LIVE = LITT > DEAD
FRACT 87-88	2	28.23	0.0001	LIVE > LITT > DEAD
		Perimeter Ditch		
FRACT 86-87	2	12.46	0.0001	LITT = LIVE > DEAD
FRACT 87-88	2	5.78	0.0860	LIVE > DEAD
		Rain Gauge		
FRACT 86-87	2	9.25	0.0006	LITT = LIVE > DEAD
FRACT 87-88	2	18.21	0.0001	LIVE > LITT > DEAD

Table 7. Results of [illegible] analyses of variance for differences in percent organic matter between sites and between years after angular transformation of the data.

SOURCE	DF	F	p	DMR
		LIVE		
SITE	3	4.32	0.007	RG > OR = NM
YEAR	1	1.10	0.297	NS
S x Y	3	1.61	0.198	-
		DEAD		
SITE	3	0.25	0.864	NS
YEAR	1	2.71	0.106	NS
S x Y	3	1.61	0.198	-
		LITTER		
SITE	3	6.23	0.001	PD = RG > NM = OR
YEAR	1	14.90	0.001	86-87 > 87-88
S x Y	3	0.99	0.400	-

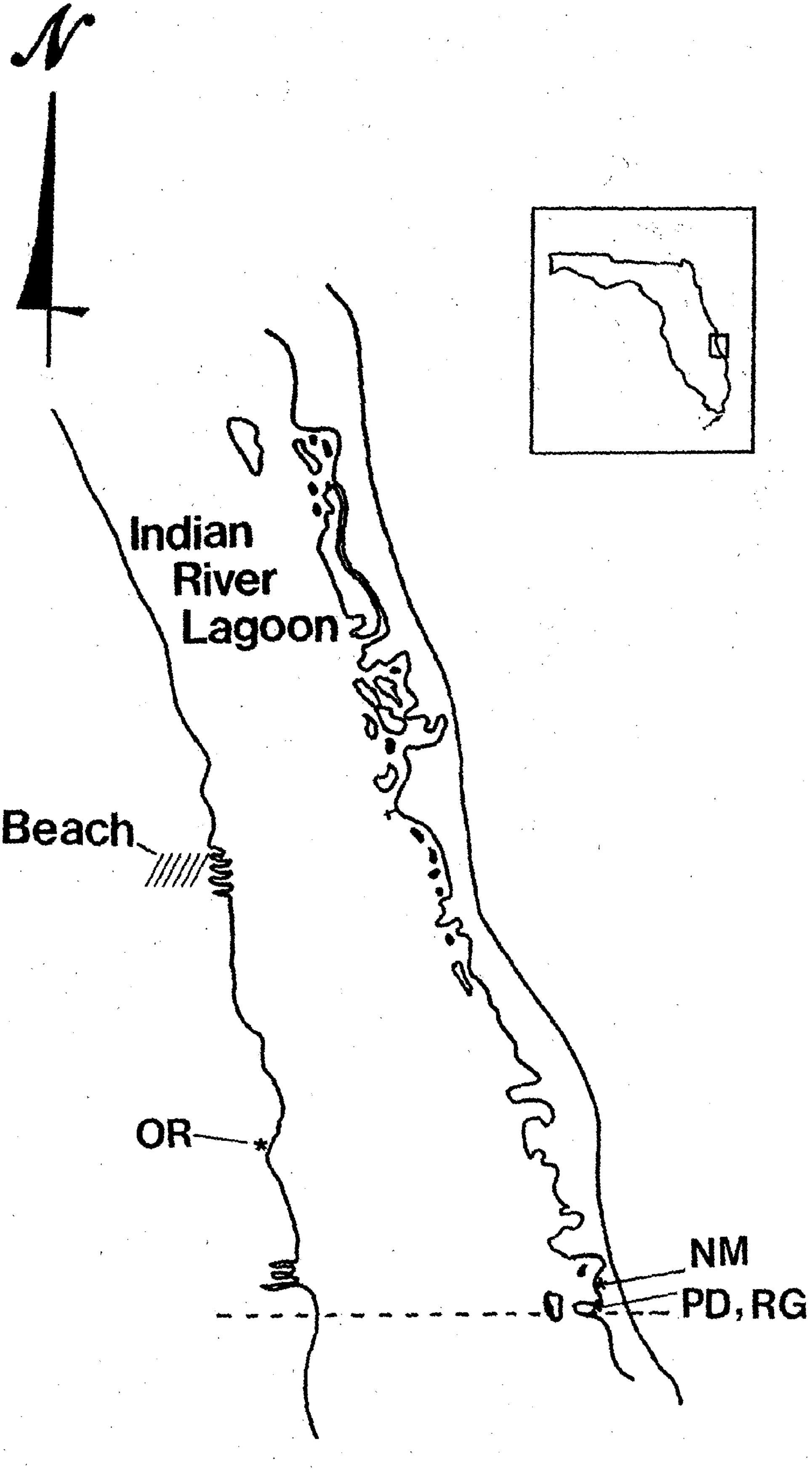
Table 8. Percent losses from litter bags at all stations during 1986 - 1988. Set indicates the month in which the litter bags were placed in the marsh; L-190 = percent loss after 190 days, L-YR = percent loss after a year.

		1986-1987		1987-1988	
SITE	SET	L-190	L-YR	L-190	L-YR
Perimeter Ditch	OCT	47.8	60.8	59.2	72.1
	MAY	43.1	-	58.6	-
Rain Gauge	OCT	58.4	80.7	59.8	80.3
	MAY	46.3	-	60.3	-
North Marsh	OCT	40.6	67.7	77.8	66.8
	MAY	49.8	-	54.7	-
Oslo Marsh	OCT	57.7	72.7	47.3	81.6
	MAY	51.9	-	62.4	-

Table 9. Yearly production estimates (g/M^2) at the four stations for 1986-1987 and 1987-1988. Centered titles indicate the method used to calculate the estimates that follow them.

STATION	1986-1987	1987-1988	1986-1988
		Milner and Hughes (1968)	
PD	579.0	692.1	1271.1
RG	492.6	605.0	1097.6
NM	770.4	893.0	1663.4
OR	1196.1	1028.0	2224.1
		Smalley (1959)	
PD	840.1	1060.2	1900.3
RG	762.7	976.1	1738.8
NM	1131.3	1437.7	2569.0
OR	2425.6	1990.0	4415.6
		Wiegert & Evans (1964)	
PD	1413.0	937.3	2350.3
RG	1542.8	834.9	2377.7
NM	1138.6	1419.2	2557.8
OR	2316.5	1984.2	4300.7

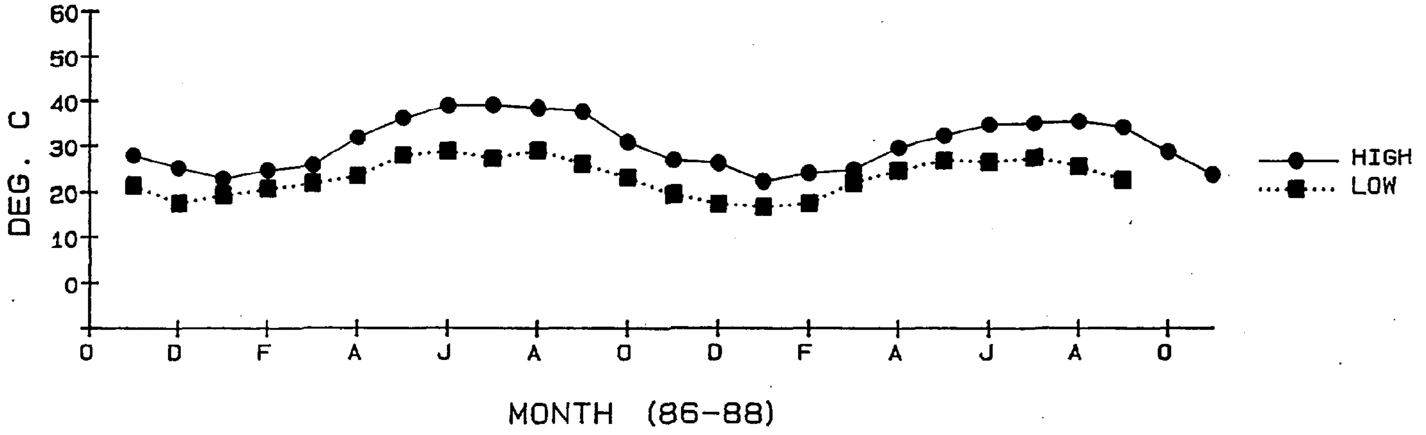
1



2

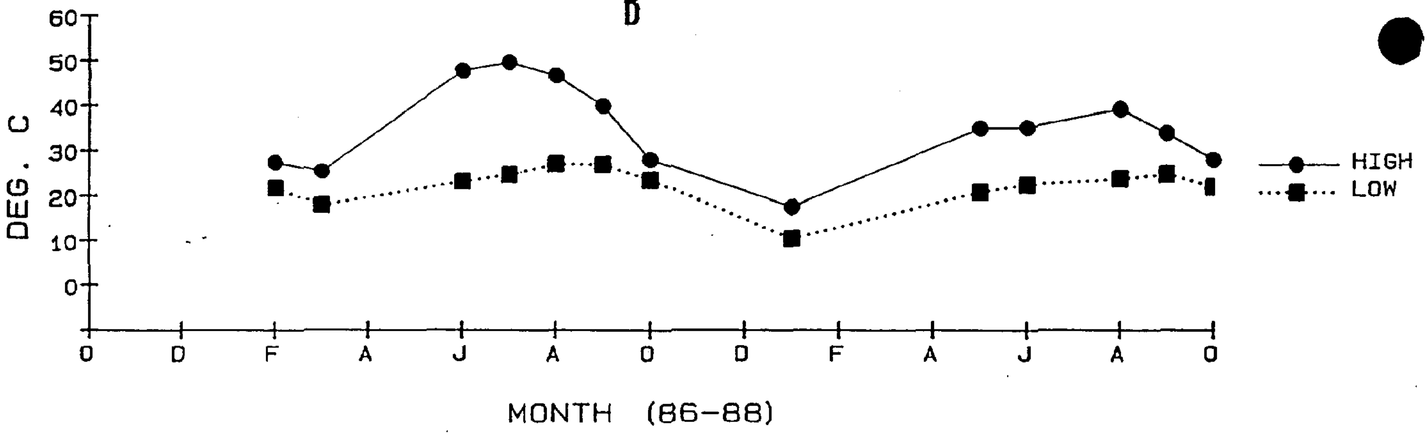
A

SOIL TEMPERATURE (NM)



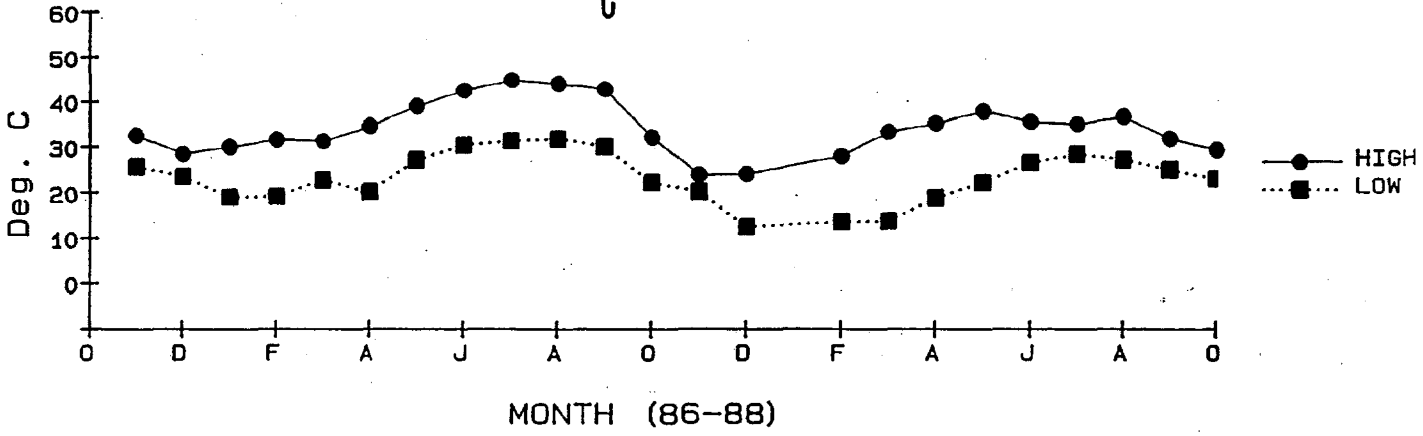
SOIL TEMPERATURE (OR)

B



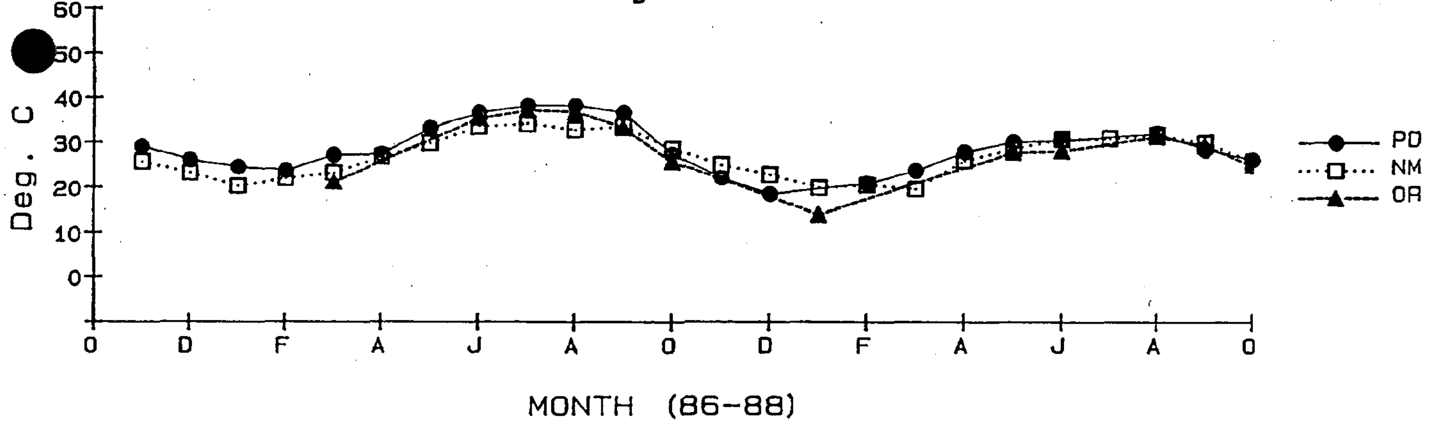
SOIL TEMPERATURE (PD)

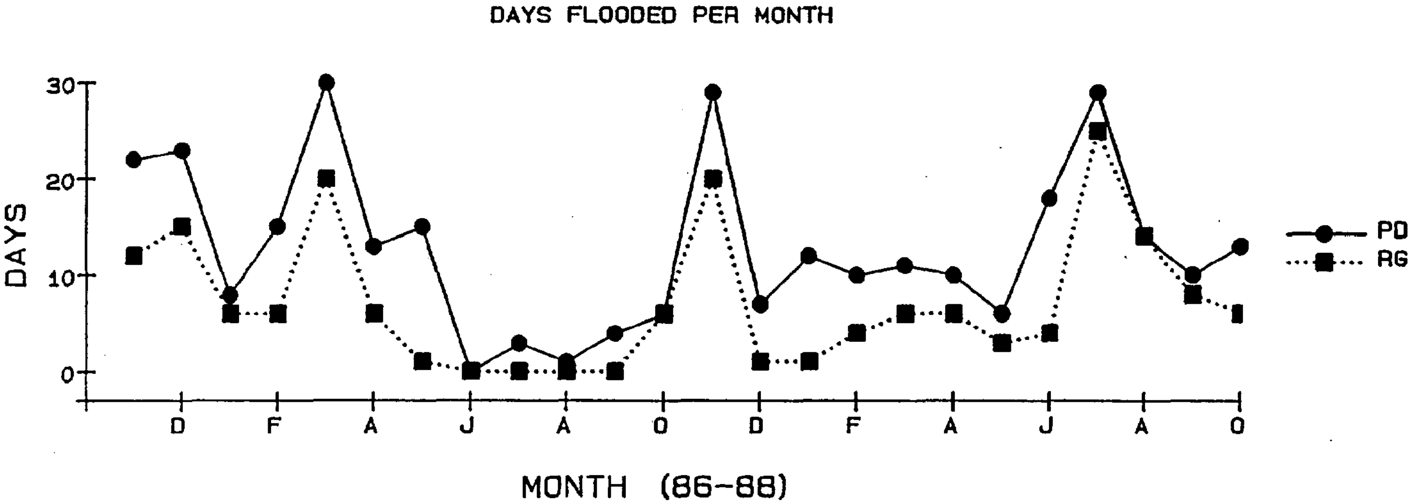
C

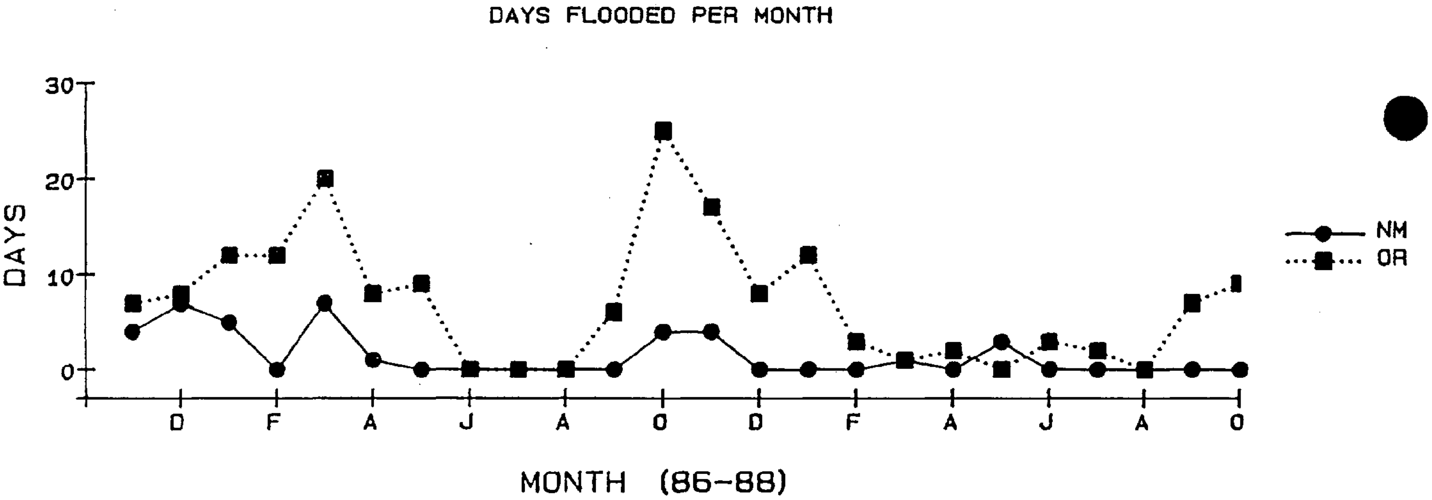


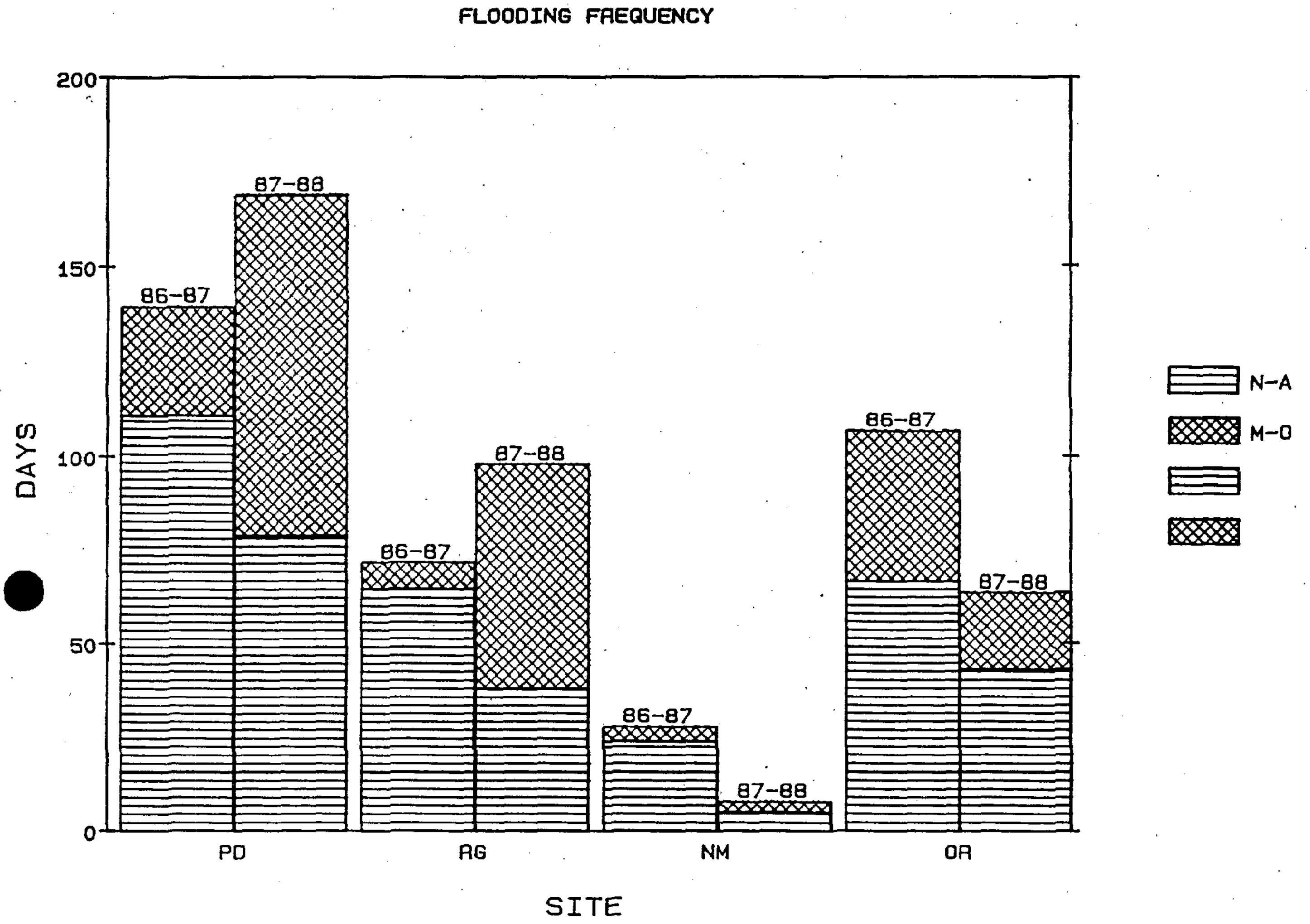
MEAN SOIL TEMPERATURE

D

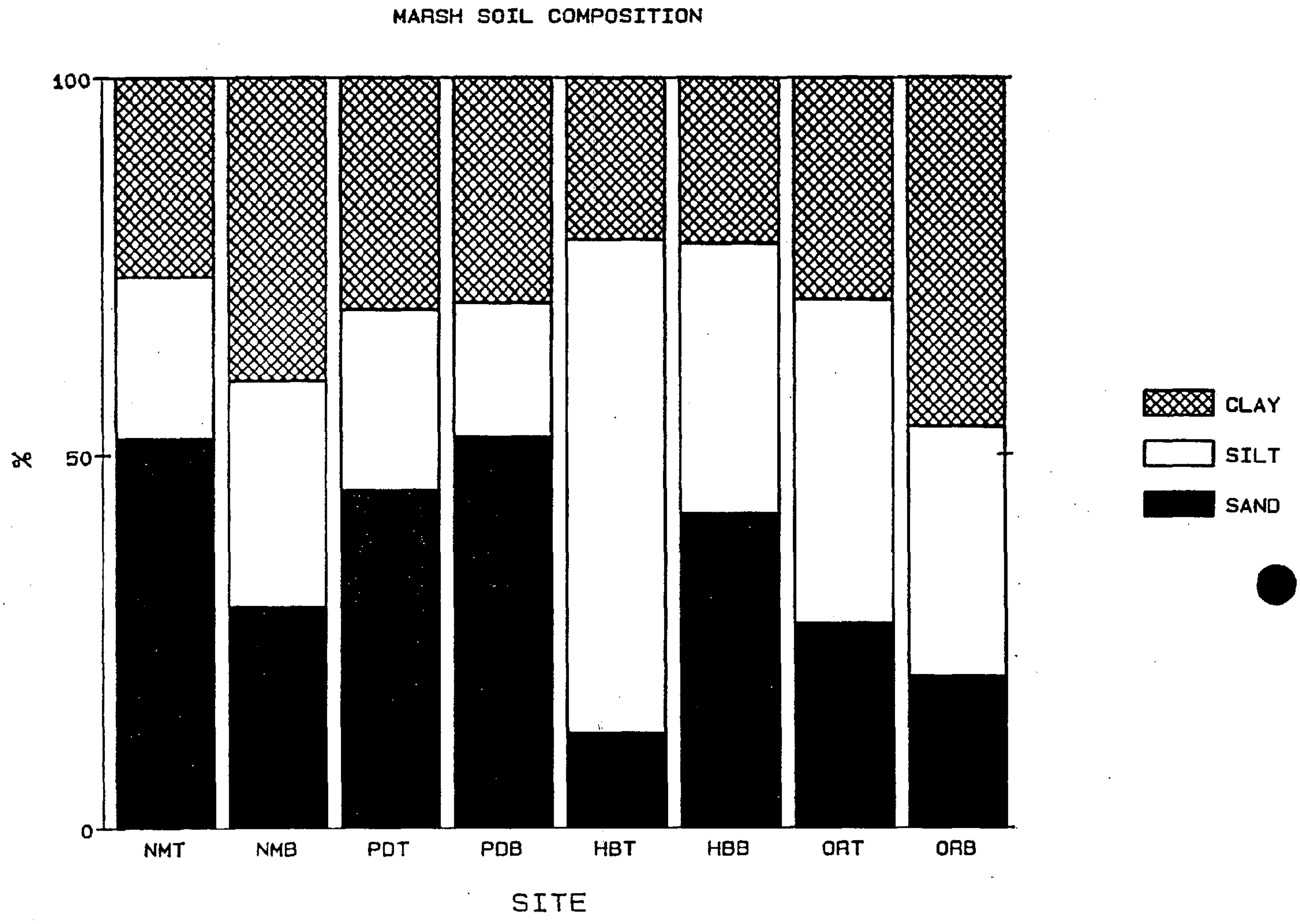


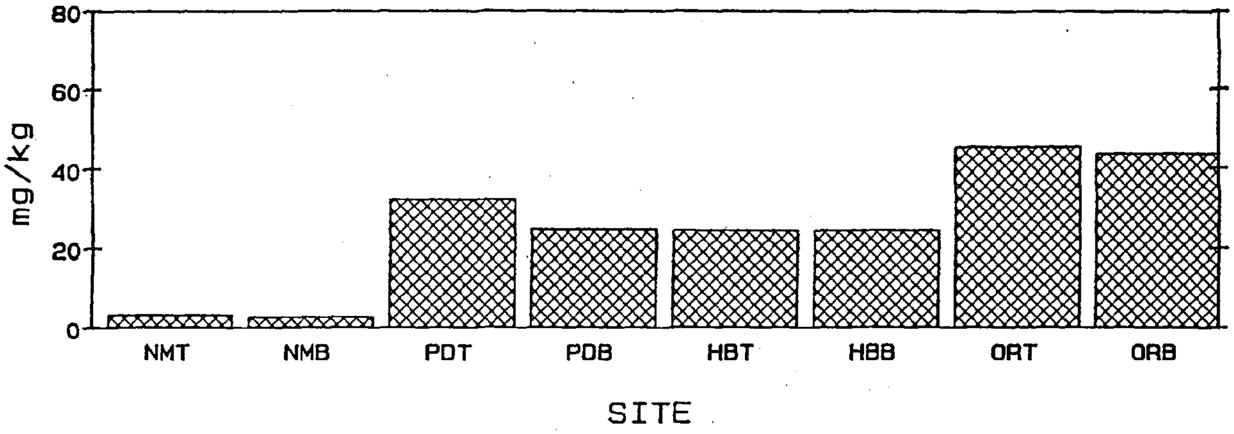

DAYS FLOODED PER MONTH
30
20
10
0
DAYS
D
F
A
J
A
O
D
F
A
J
A
O
MONTH (86-88)
PD
RG


DAYS FLOODED PER MONTH
30
20
10
0
DAYS
D
F
A
J
A
O
D
F
A
J
A
O
MONTH (86-88)
NM
OR

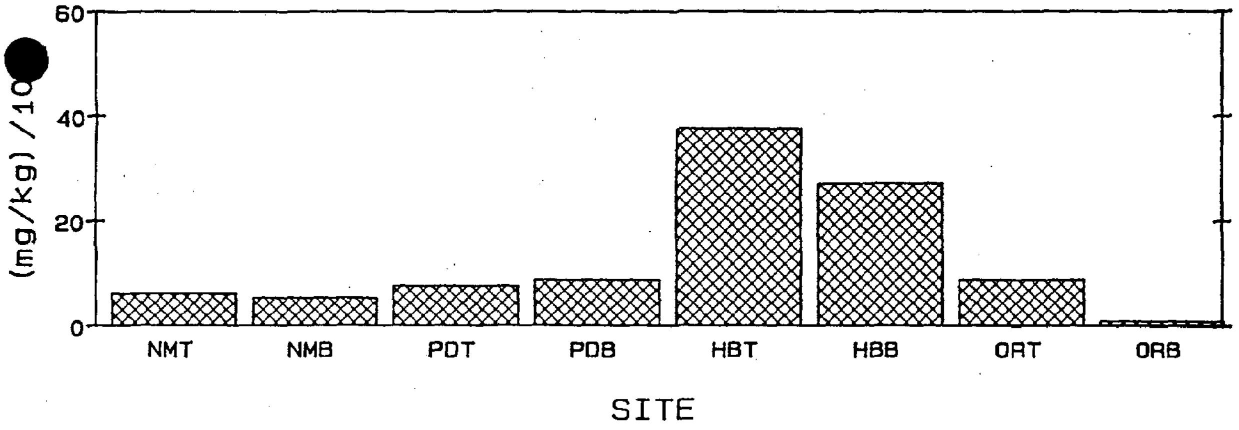

FLOODING FREQUENCY
DAYS
200
150
100
50
0
86-87
87-88
PD
AG
NM
OR
SITE
N-A
M-O

5

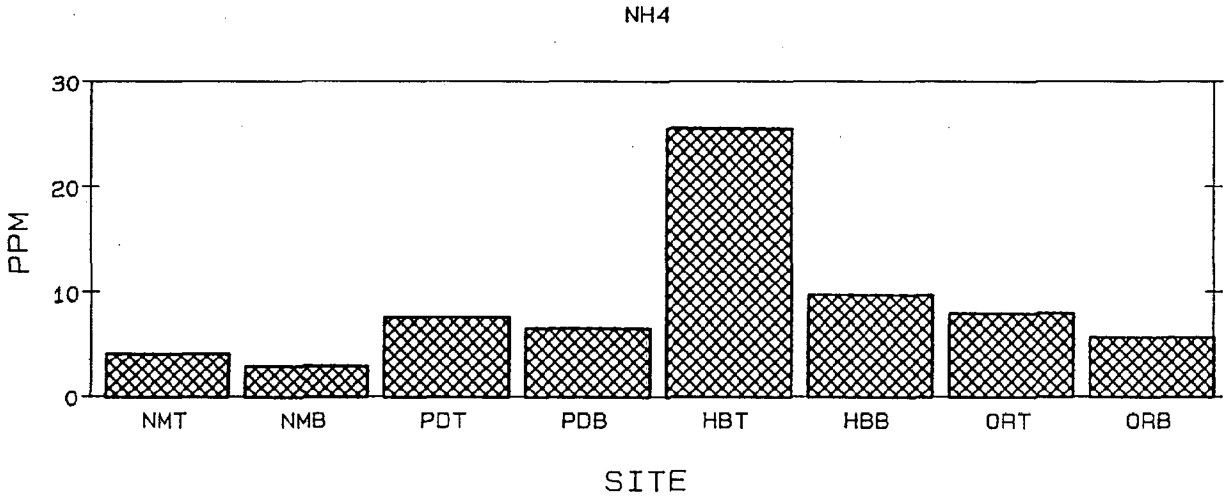

MARSH SOIL COMPOSITION
100
%
50
0
NMT
NMB
PDT
PDB
HBT
HBB
ORT
ORB
SITE
CLAY
SILT
SAND

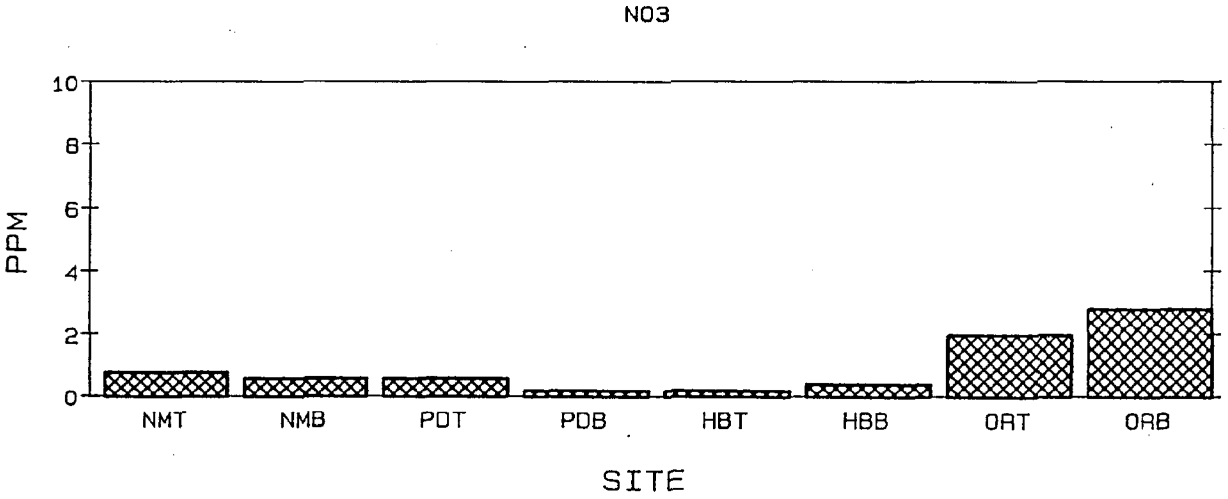


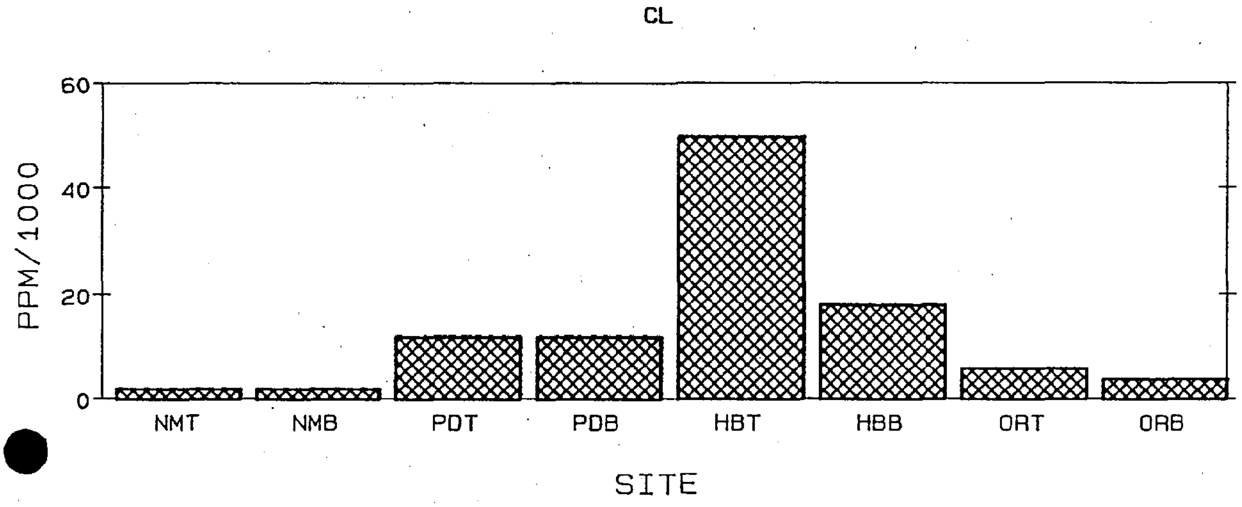
PHOSPHORUS

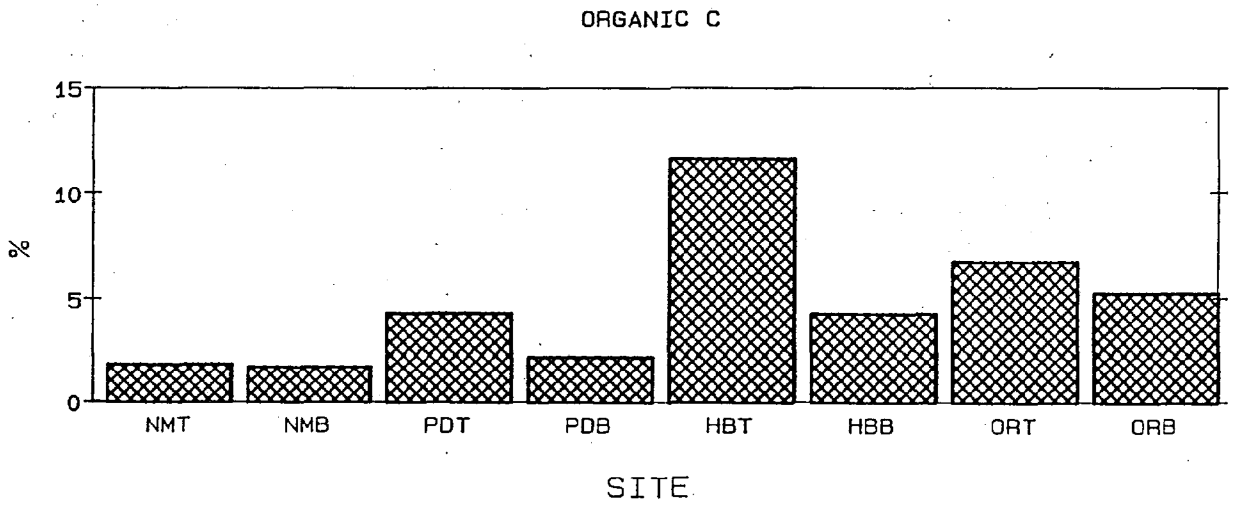


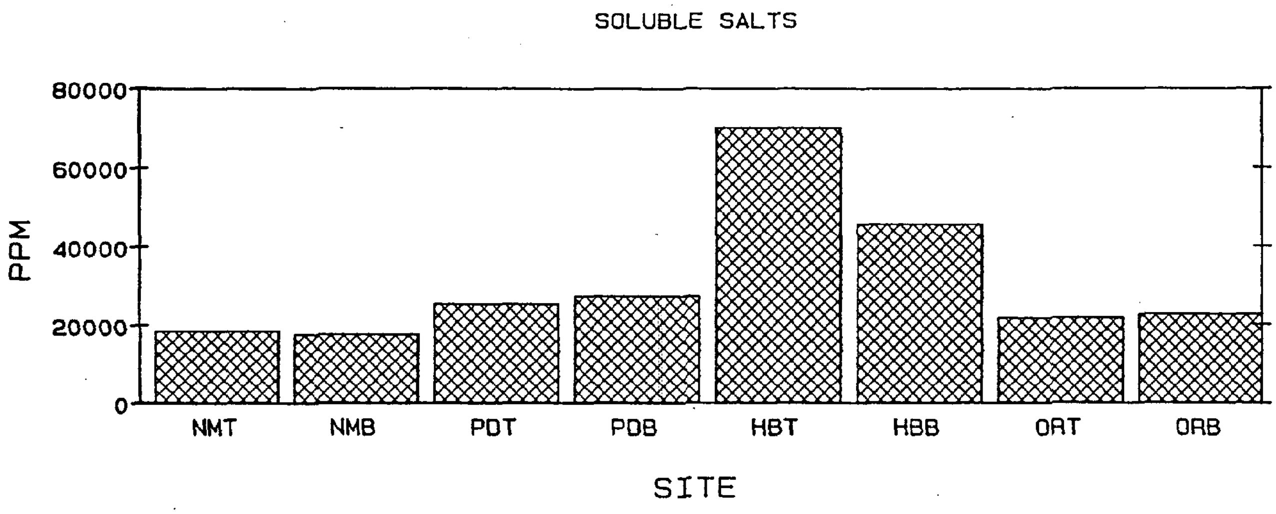
SODIUM

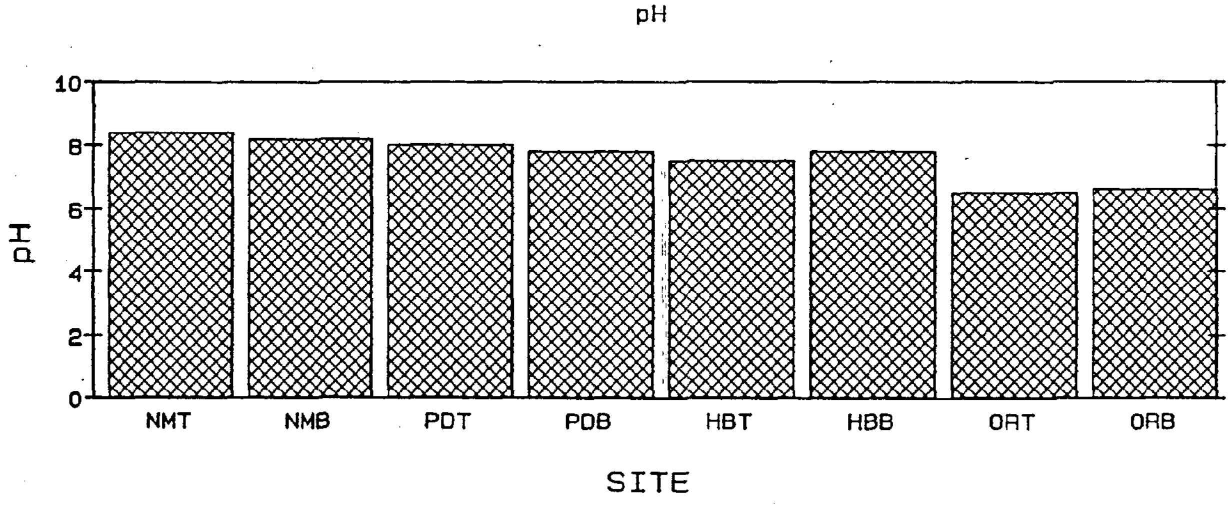

NH4
PPM
30
20
10
0
NMT
NMB
PDT
PDB
HBT
HBB
ORT
ORB
SITE

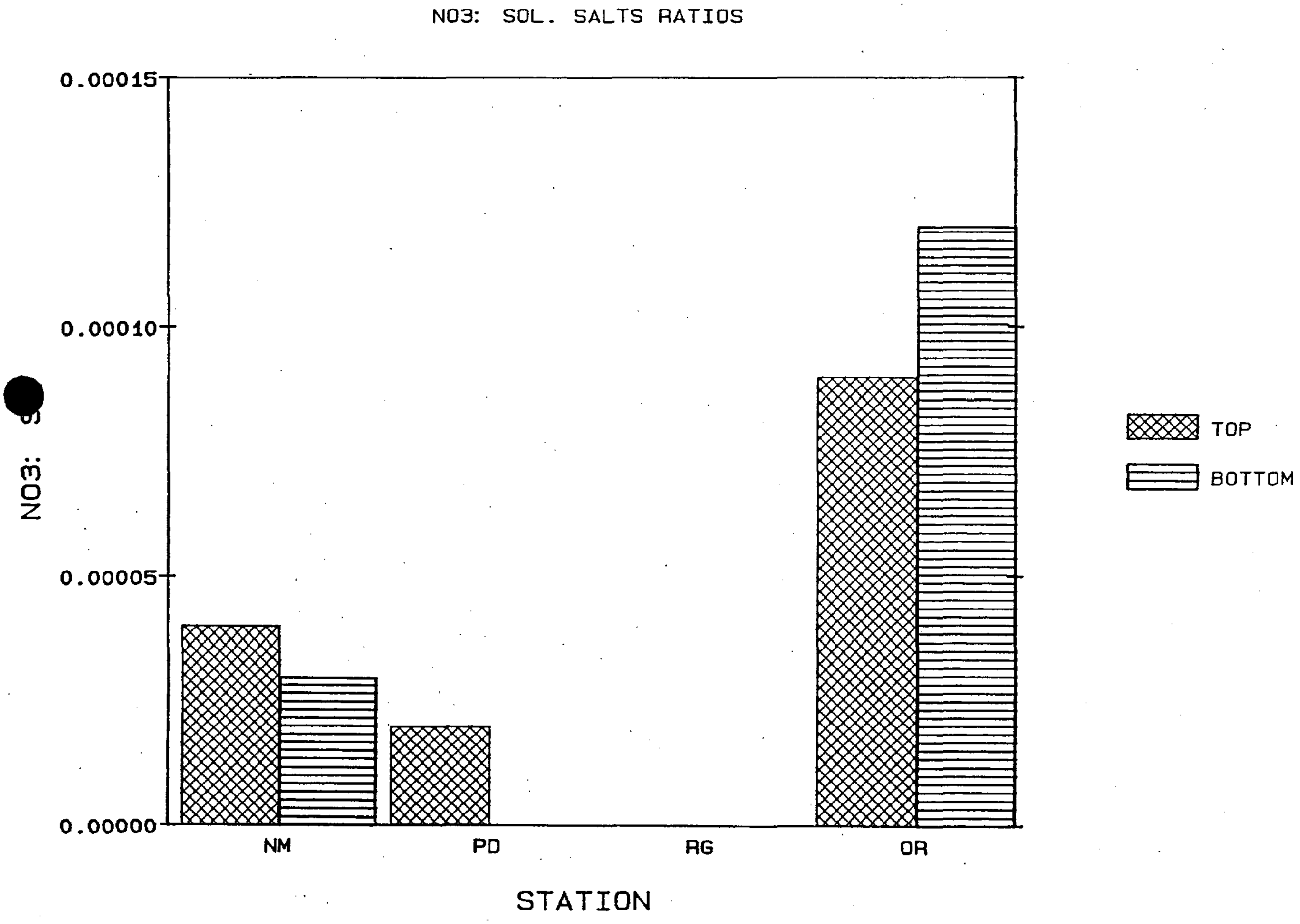

NO3
PPM
10
8
6
4
2
0
NMT
NMB
PDT
PDB
HBT
HBB
ORT
ORB
SITE

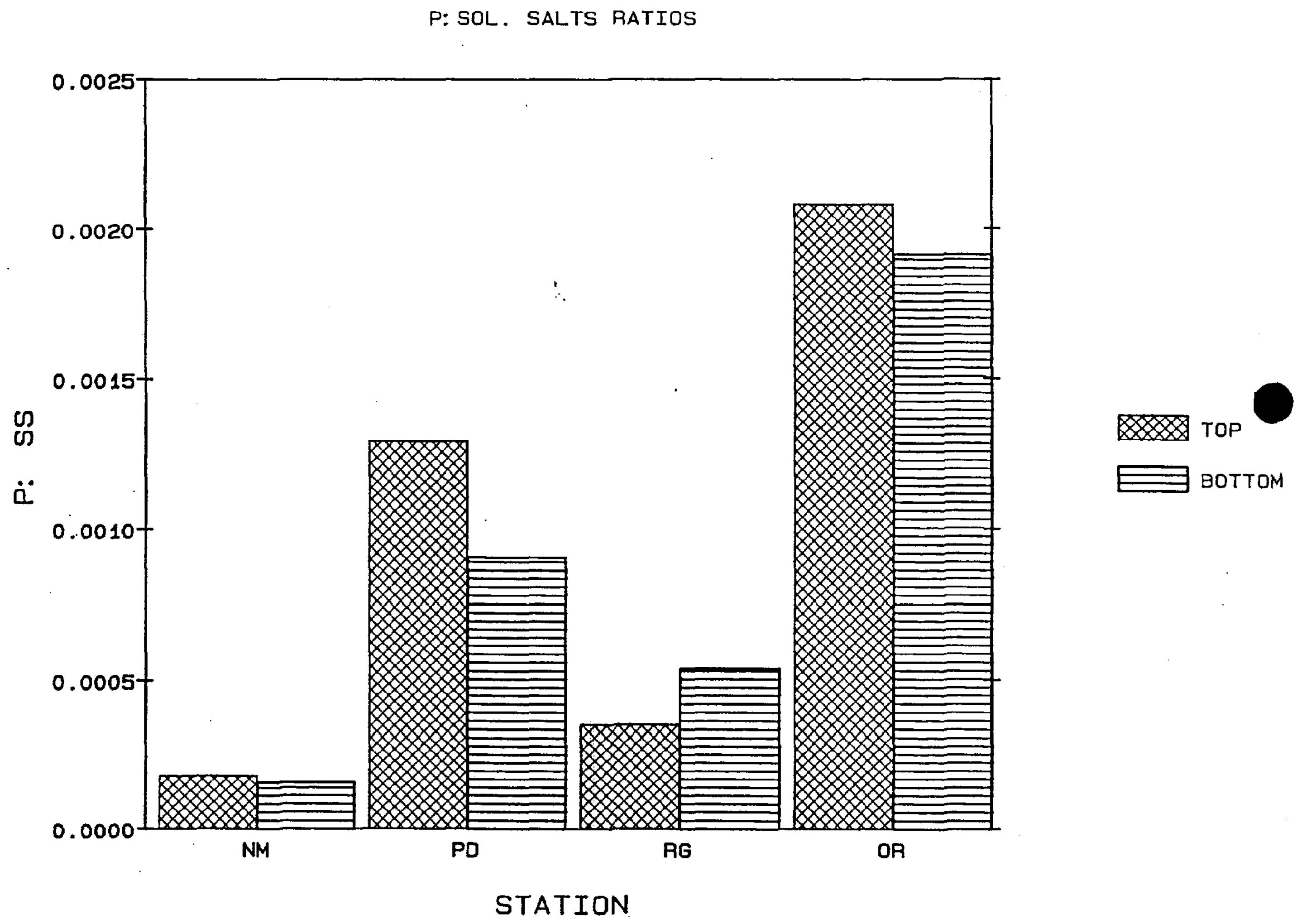

CL
PPM/1000
60
40
20
0
NMT
NMB
PDT
PDB
HBT
HBB
ORT
ORB
SITE


ORGANIC C
%
15
10
5
0
NMT
NMB
PDT
PDB
HBT
HBB
ORT
ORB
SITE

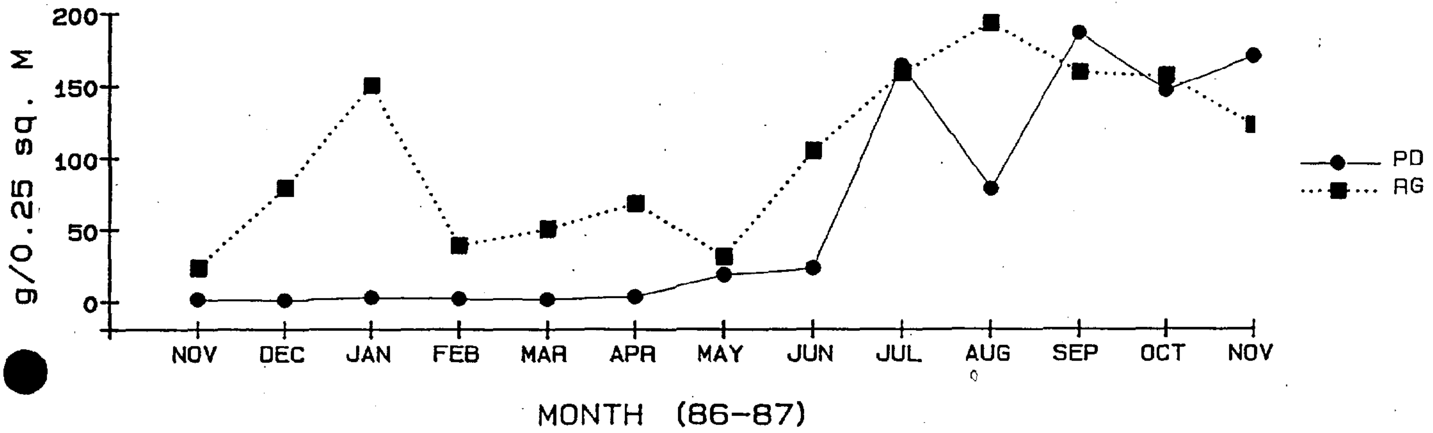

SOLUBLE SALTS
PPM
80000
60000
40000
20000
0
NMT
NMB
PDT
PDB
HBT
HBB
ORT
ORB
SITE


pH
pH
10
8
6
4
2
0
NMT
NMB
PDT
PDB
HBT
HBB
ORT
ORB
SITE

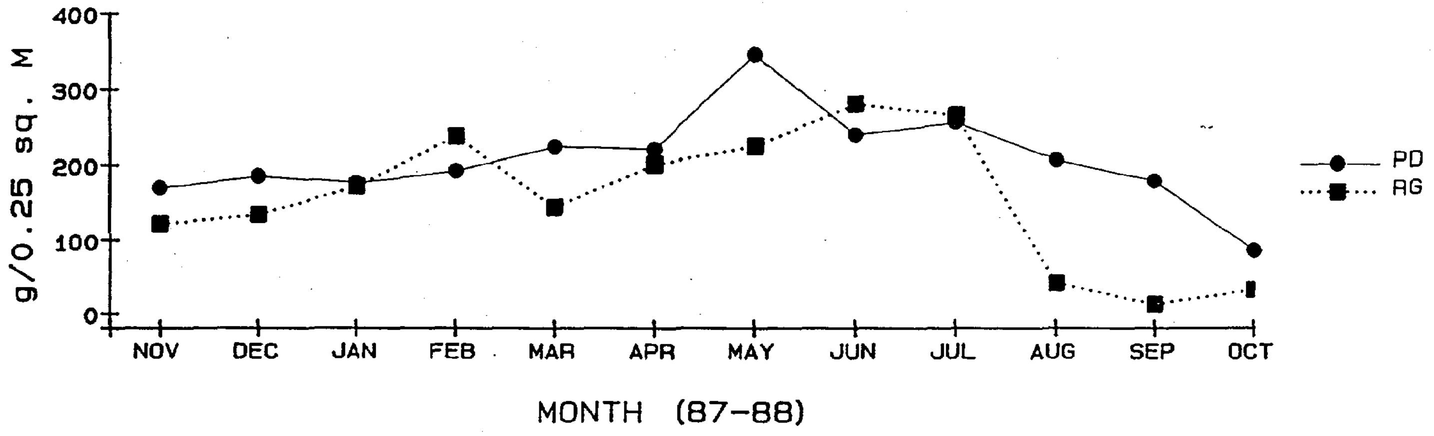

NO3: SOL. SALTS RATIOS
0.00015
0.00010
0.00005
0.00000
NO3: S
NM
PD
RG
OR
STATION
TOP
BOTTOM

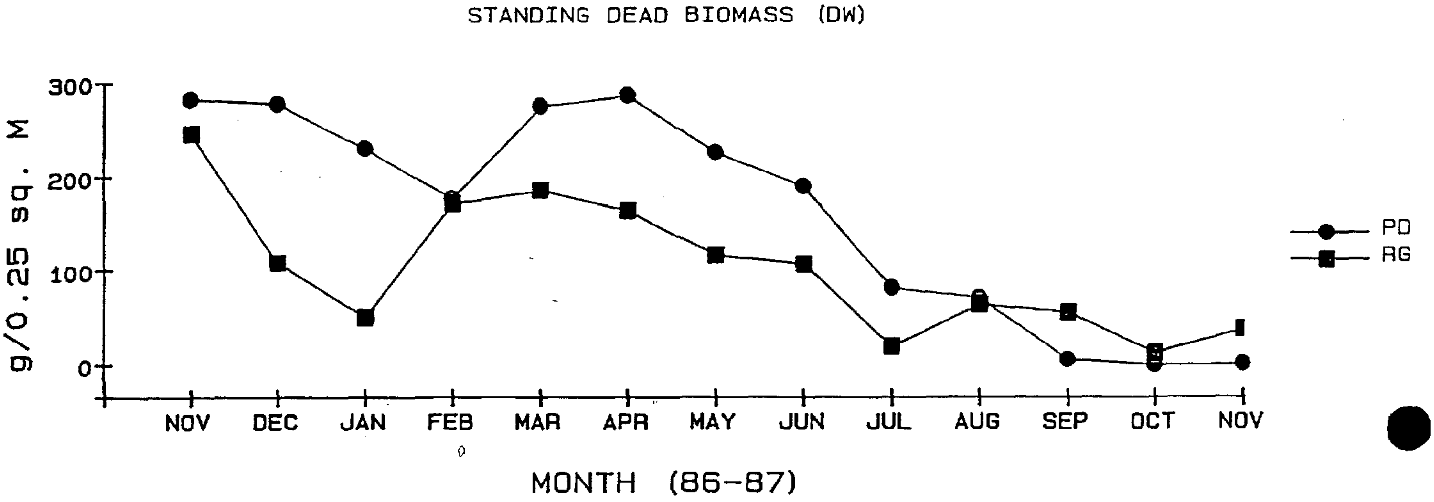

P: SOL. SALTS RATIOS
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
P: SS
NM
PD
RG
OR
STATION
TOP
BOTTOM

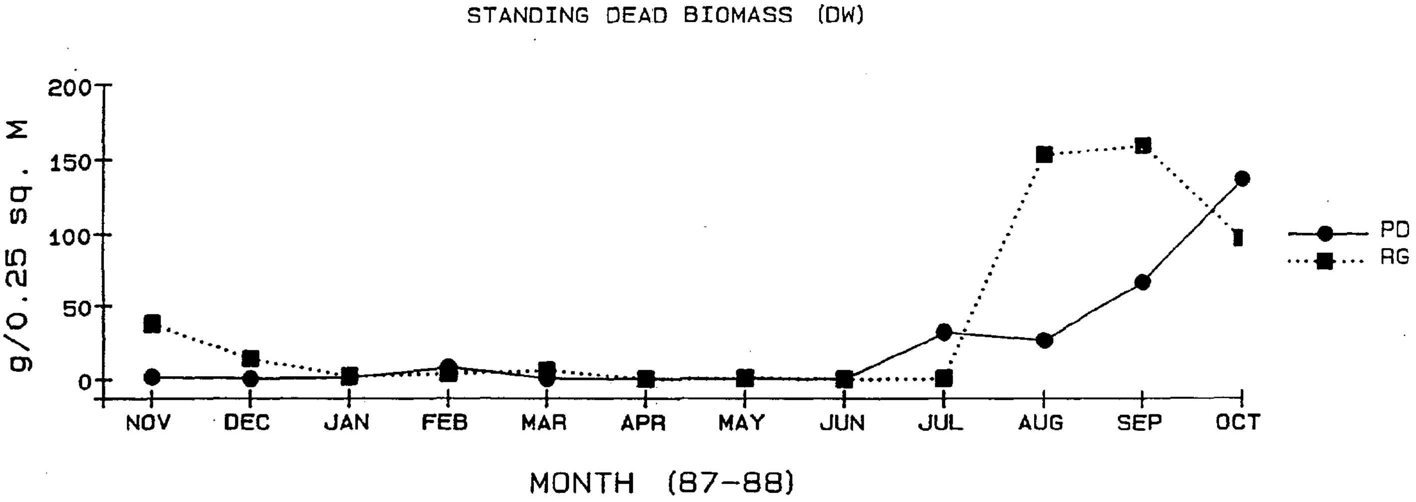
LIVE BIOMASS (DW)

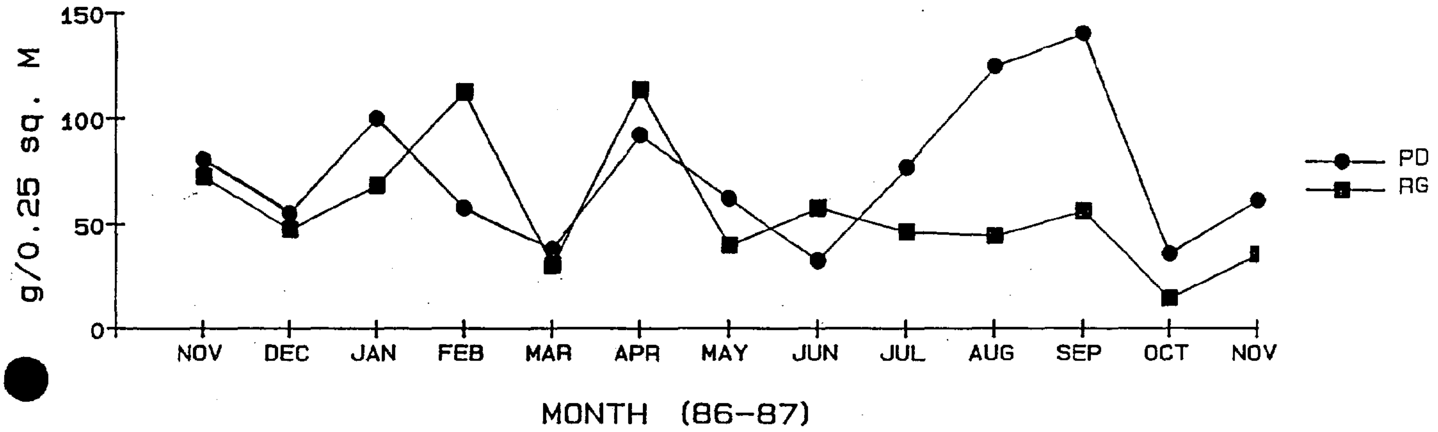


LIVE BIOMASS (DW)

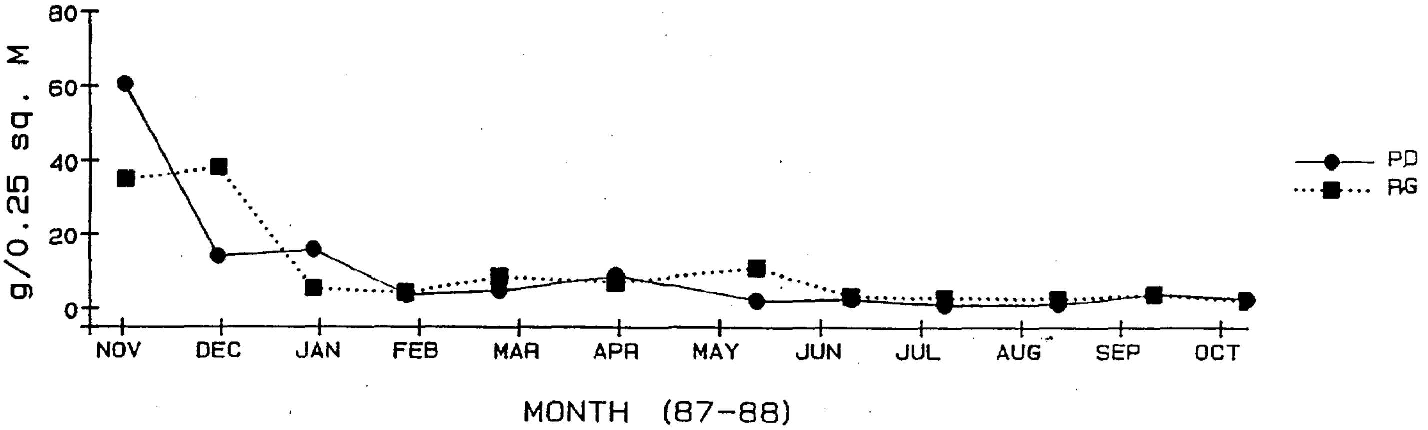



STANDING DEAD BIOMASS (DW)
g/0.25 sq. M
300
200
100
0
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
MONTH (86-87)
PD
RG

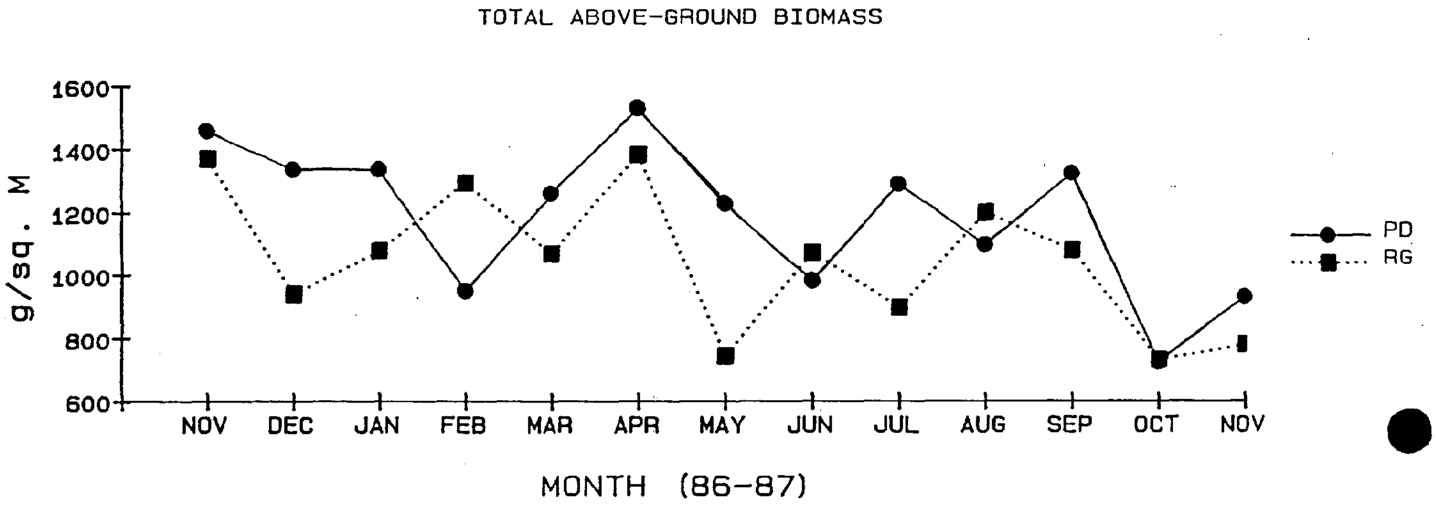

STANDING DEAD BIOMASS (DW)
g/0.25 sq. M
200
150
100
50
0
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
MONTH (87-88)
PD
RG



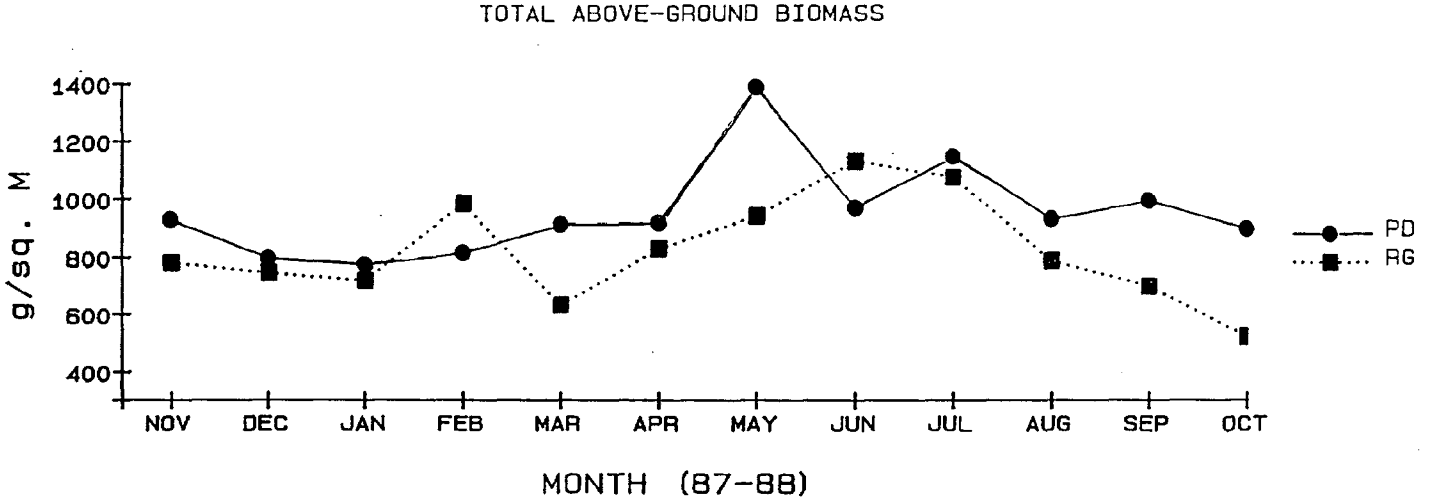
LITTER (DW)



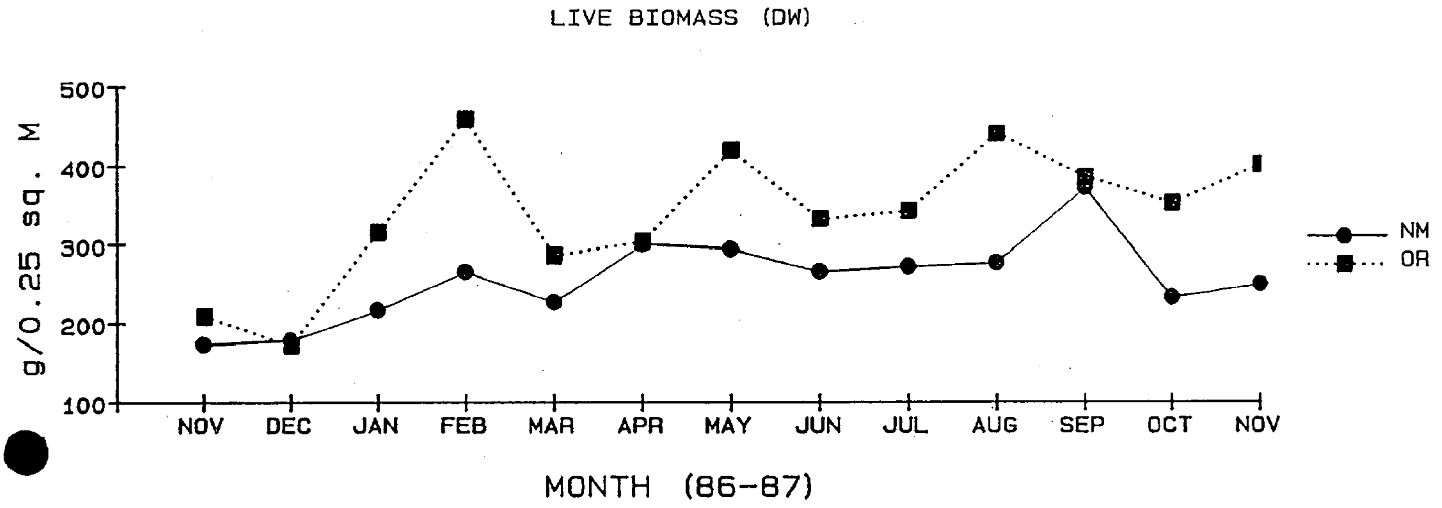
LITTER (DW)

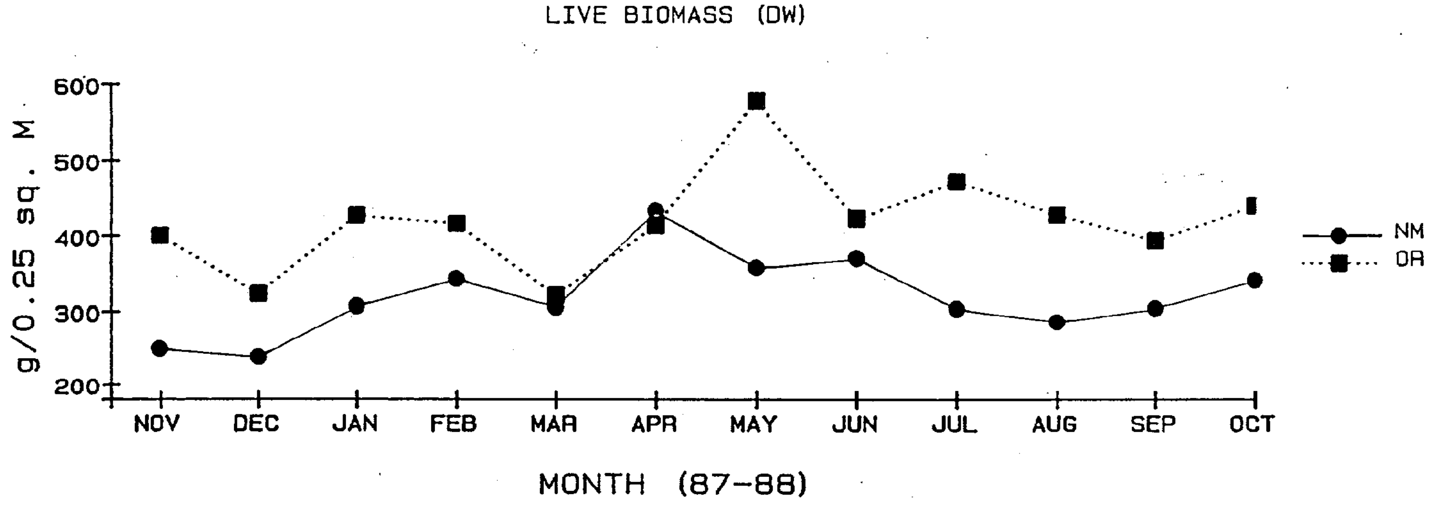


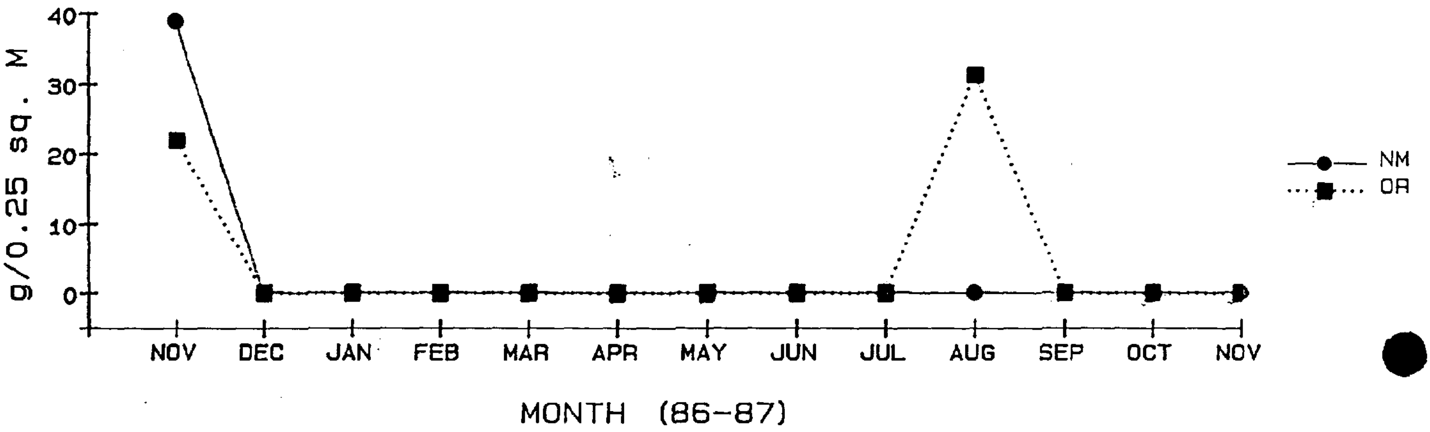
TOTAL ABOVE-GROUND BIOMASS



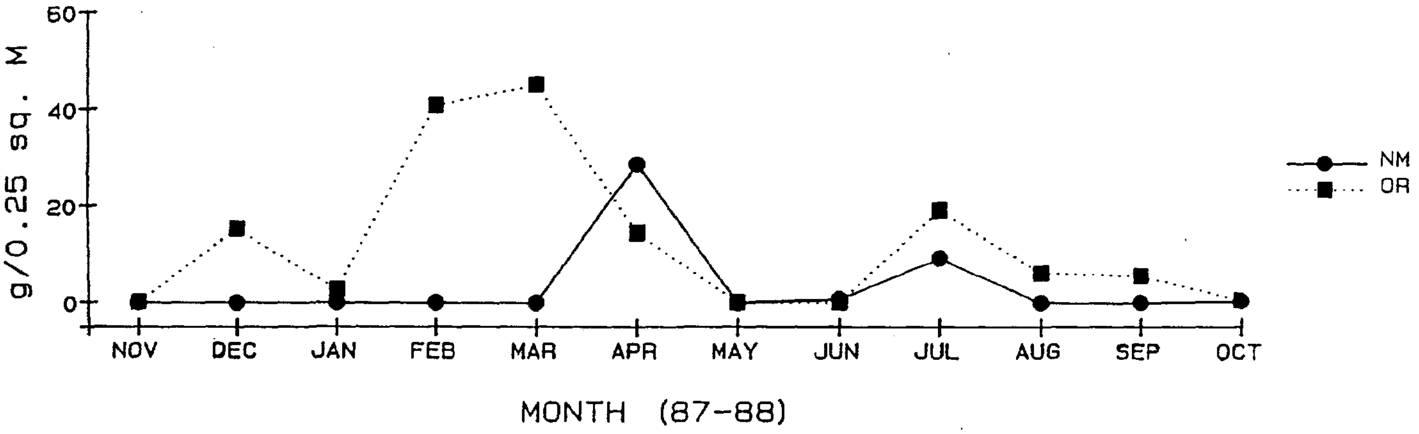
TOTAL ABOVE-GROUND BIOMASS


LIVE BIOMASS (DW)
g/0.25 sq. M
500
400
300
200
100
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
MONTH (86-87)
NM
OR

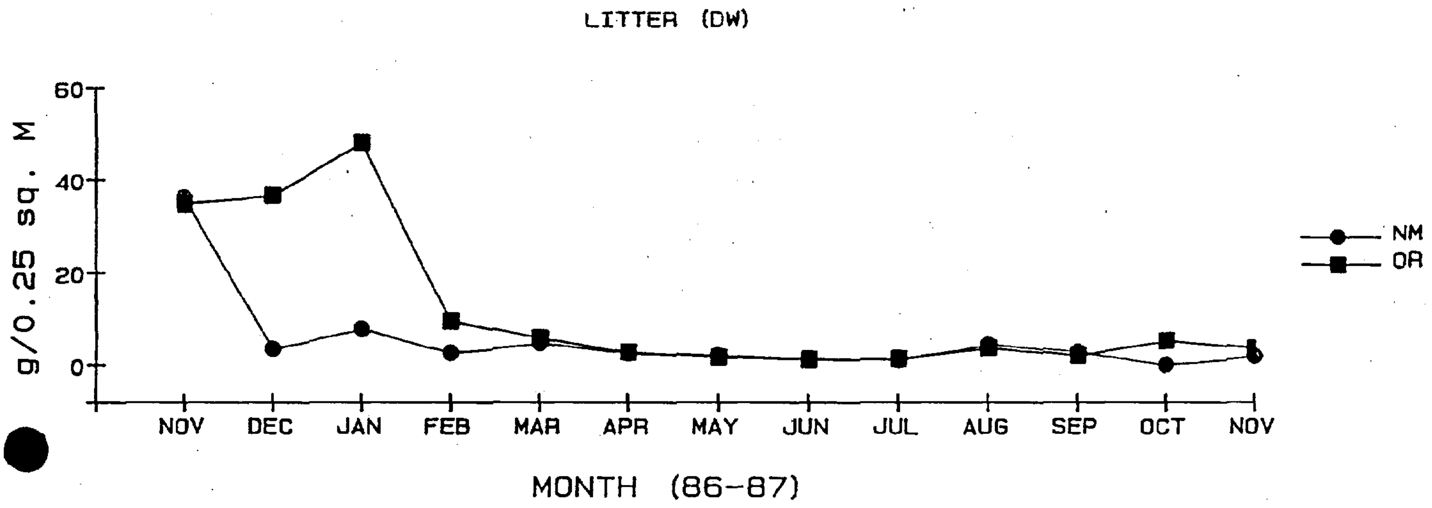

LIVE BIOMASS (DW)
g/0.25 sq. M
600
500
400
300
200
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
MONTH (87-88)
NM
OR

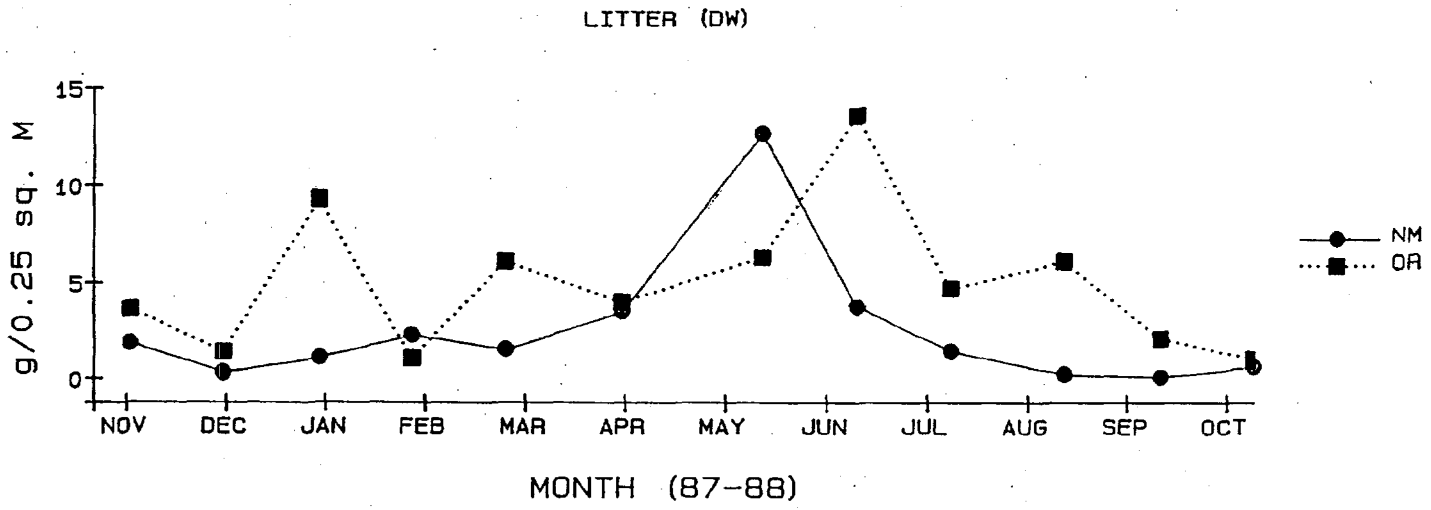


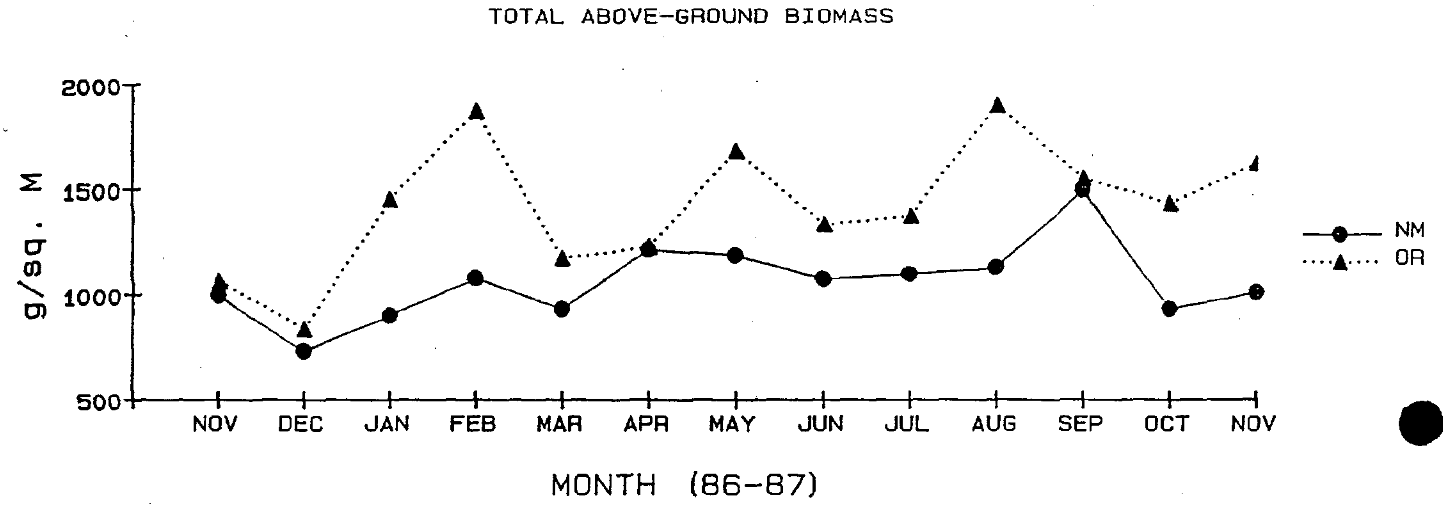
STANDING DEAD BIOMASS (DW)

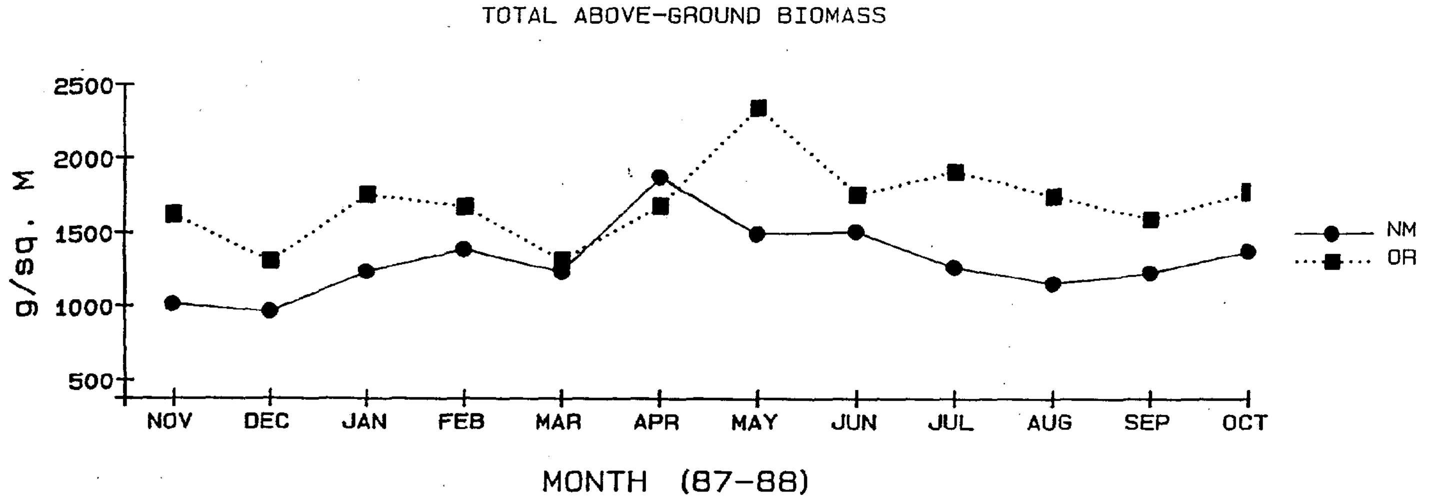


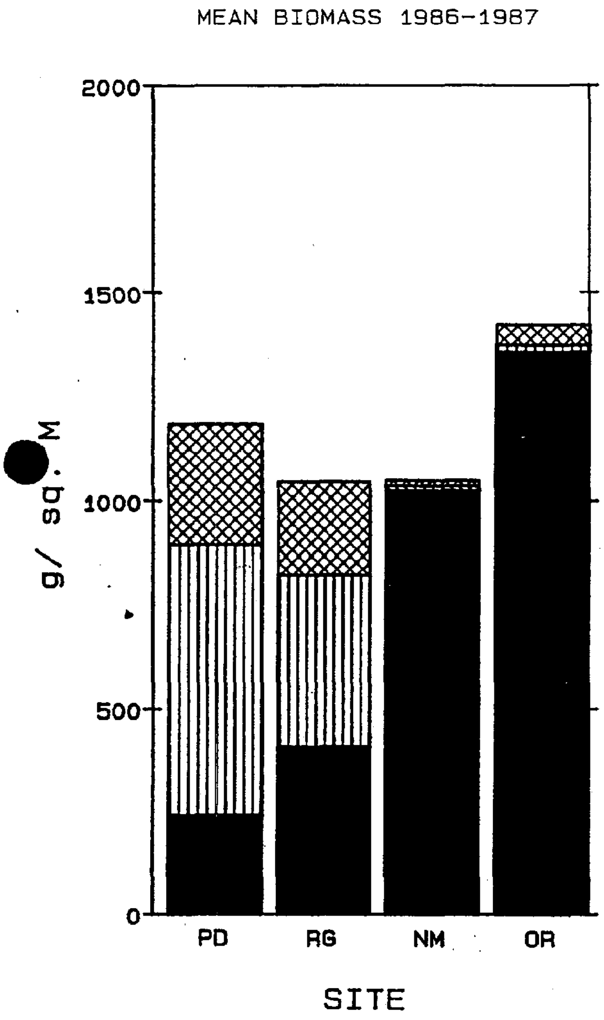
STANDING DEAD BIOMASS (DW)

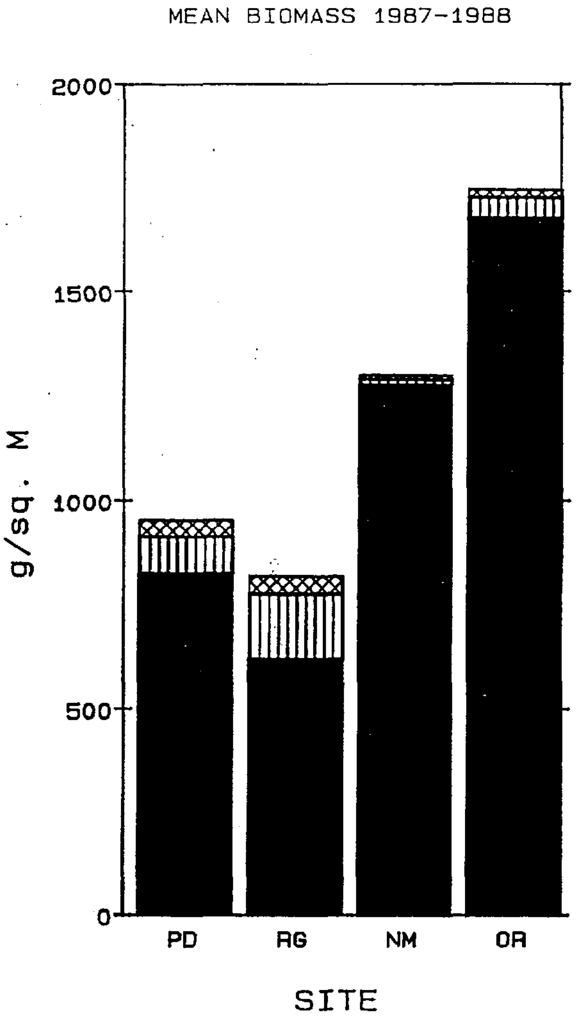

LITTER (DW)
g/0.25 sq. M
60
40
20
0
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
MONTH (86-87)
NM
OR


LITTER (DW)
g/0.25 sq. M
15
10
5
0
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
MONTH (87-88)
NM
OR

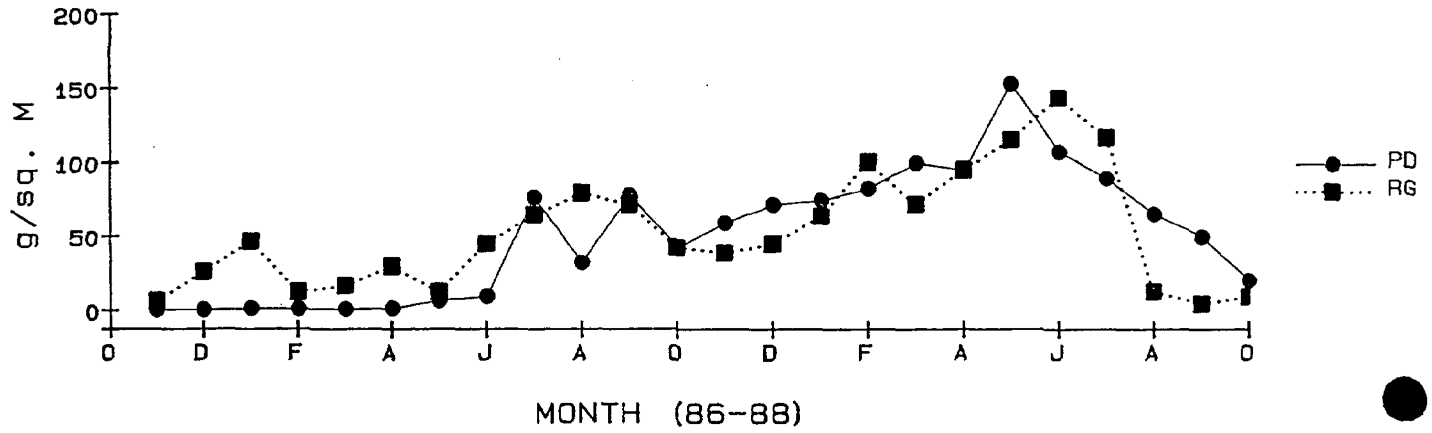

TOTAL ABOVE-GROUND BIOMASS
g/sq. M
2000
1500
1000
500
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
MONTH (86-87)
NM
OR


TOTAL ABOVE-GROUND BIOMASS
g/sq. M
2500
2000
1500
1000
500
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
MONTH (87-88)
NM
OR

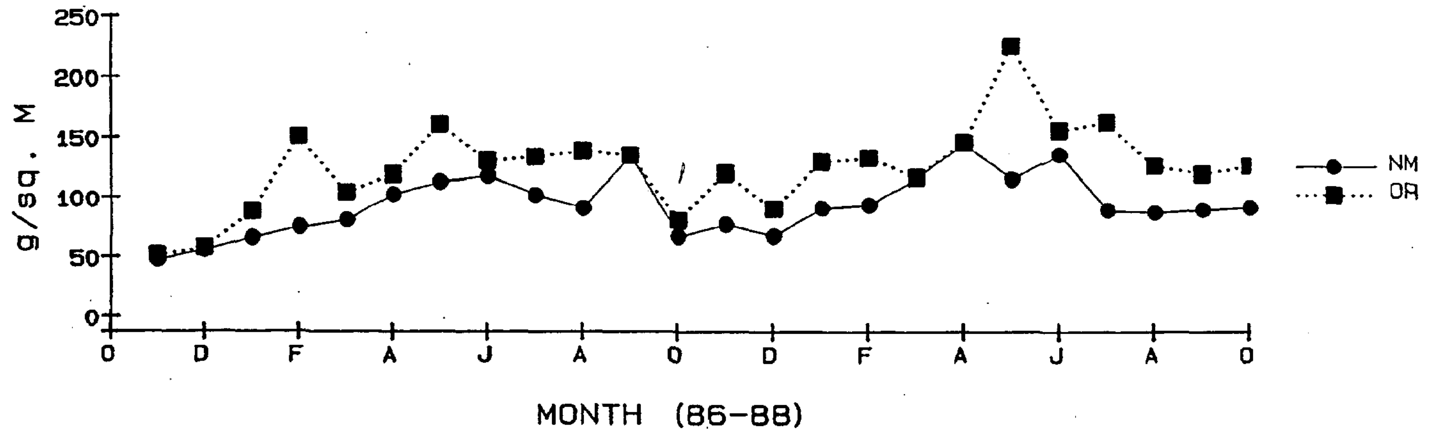

MEAN BIOMASS 1986-1987
2000
1500
1000
500
0
g/ sq. M
PD
RG
NM
OR
SITE

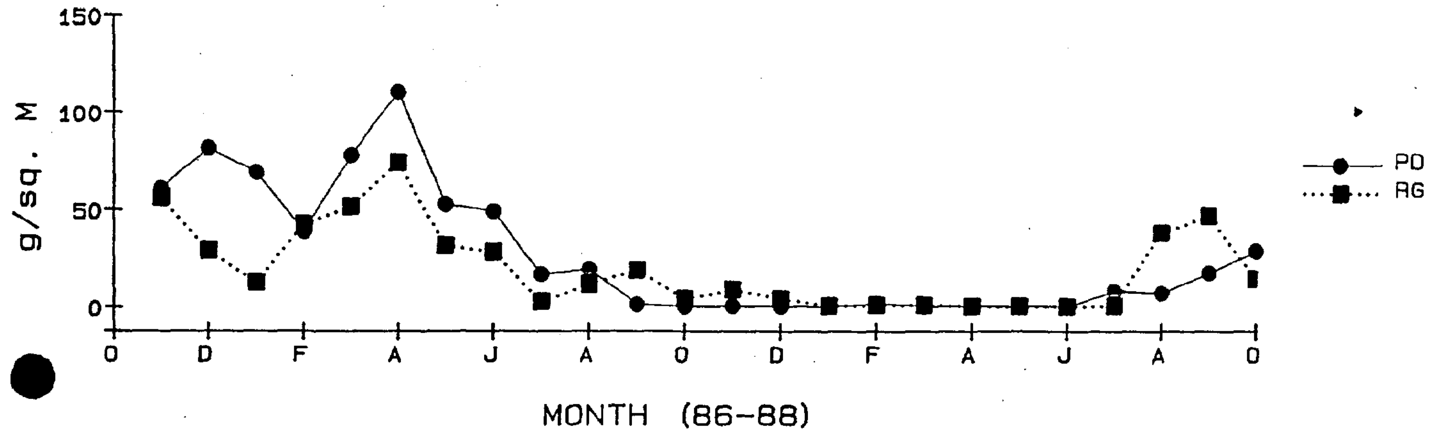

MEAN BIOMASS 1987-1988
2000
1500
1000
500
0
g/sq. M
PD
RG
NM
OR
SITE

ORGANIC CONTENT (LIVE)

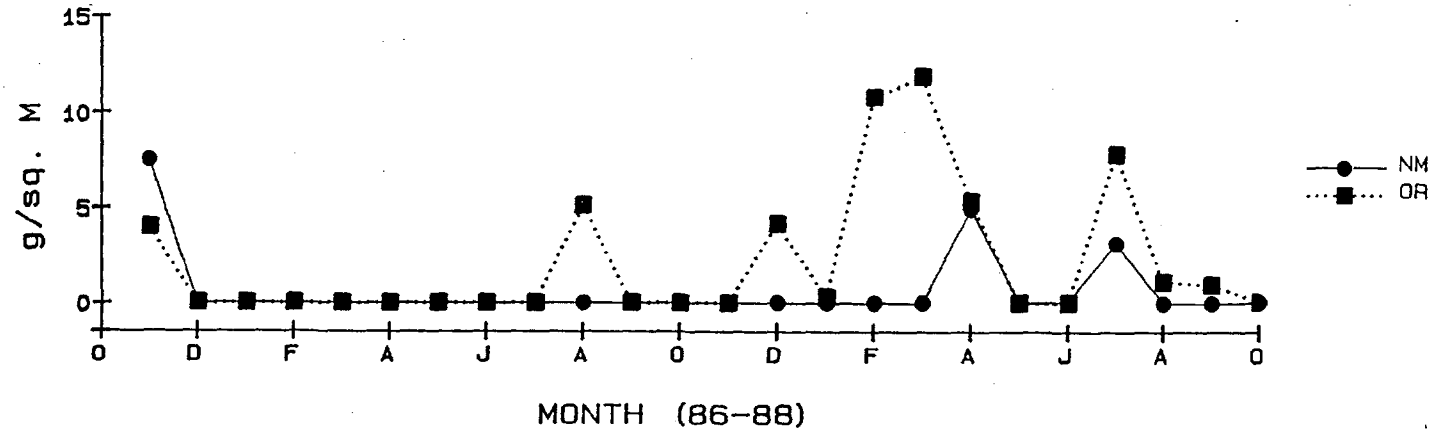


ORGANIC CONTENT (LIVE)

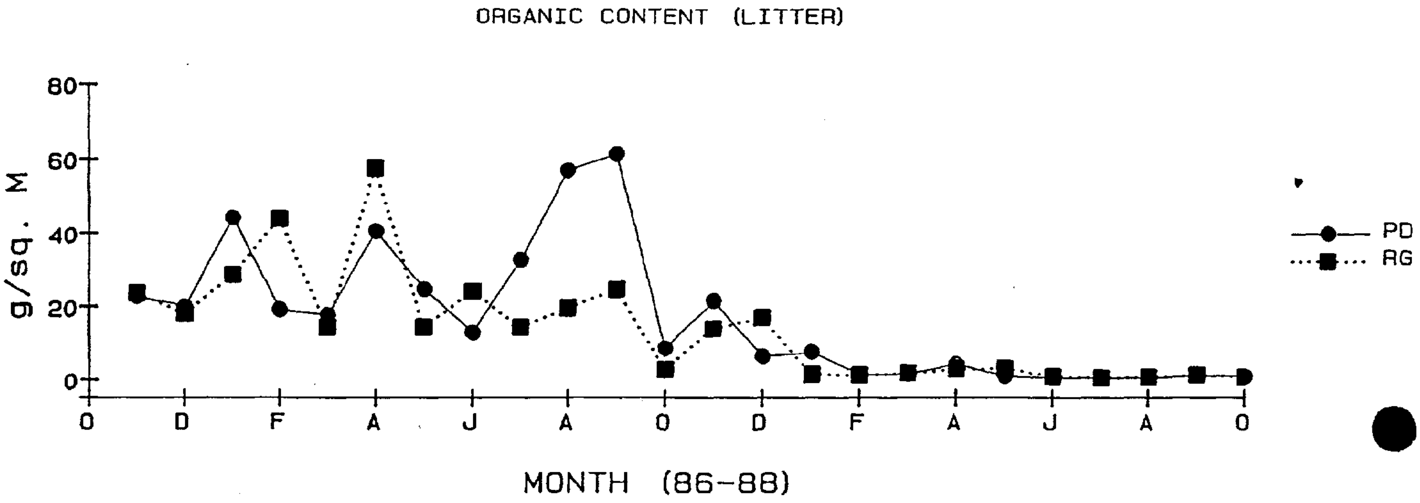


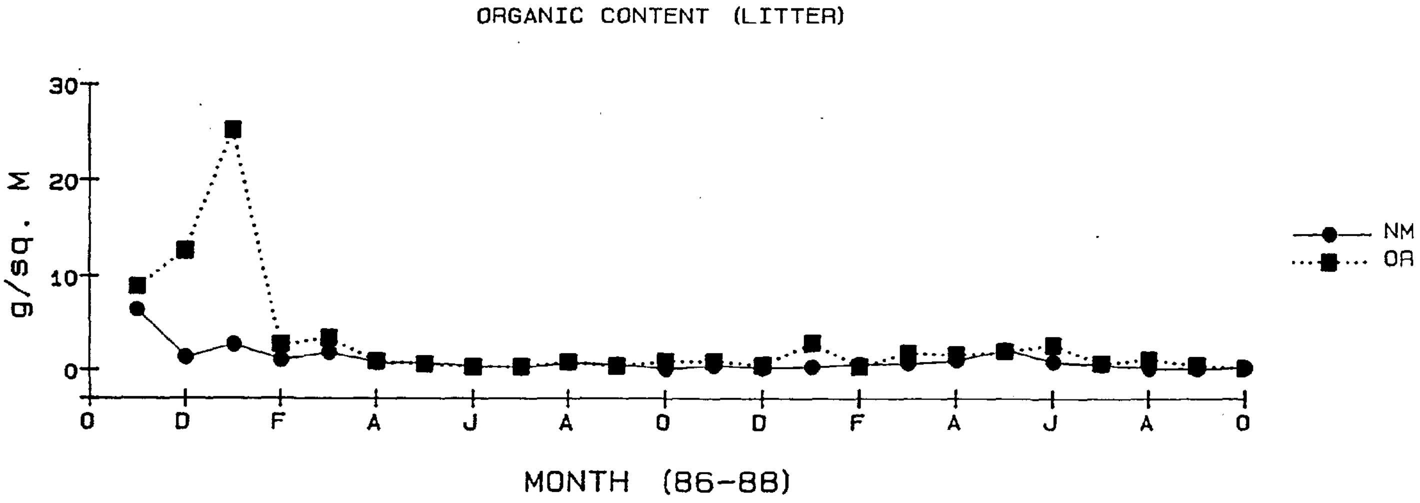


ORGANIC CONTENT (DEAD)

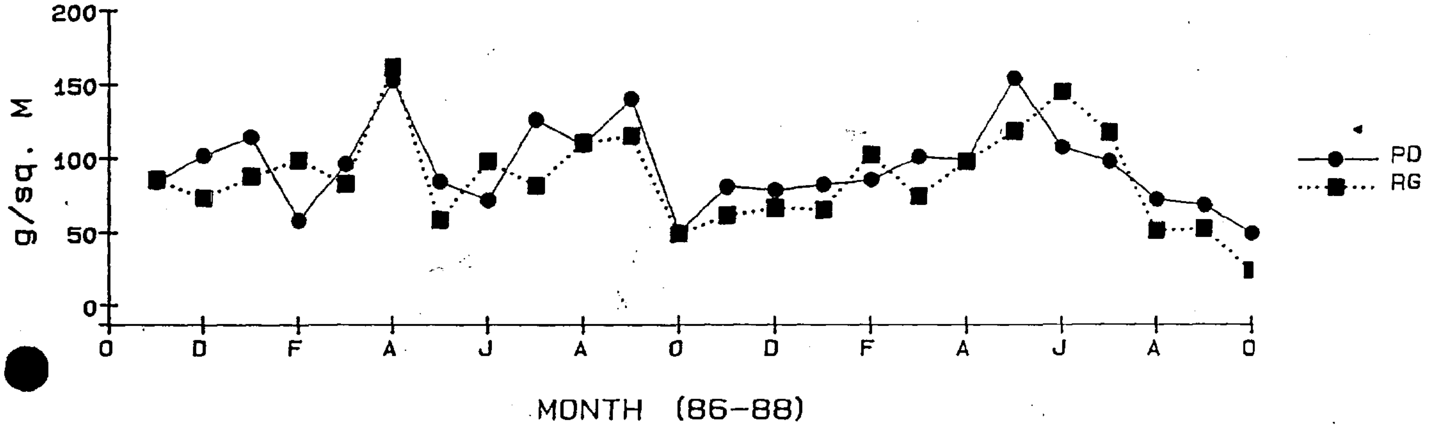


ORGANIC CONTENT (DEAD)

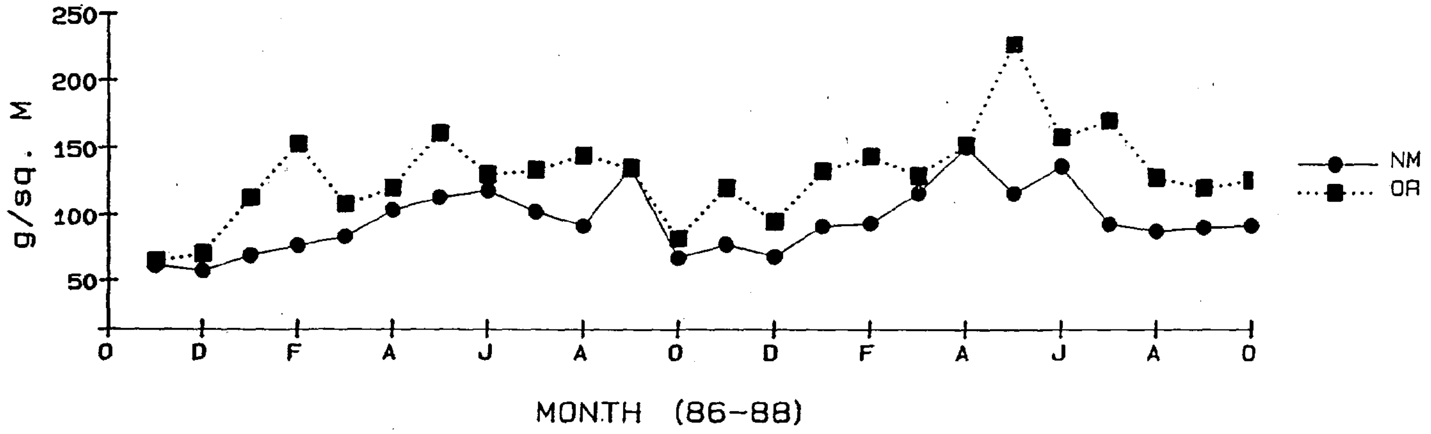

ORGANIC CONTENT (LITTER)
g/sq. M
80
60
40
20
0
O
D
F
A
J
A
O
D
F
A
J
A
O
MONTH (86-88)
PD
RG


ORGANIC CONTENT (LITTER)
g/sq. M
30
20
10
0
O
D
F
A
J
A
O
D
F
A
J
A
O
MONTH (86-88)
NM
OR

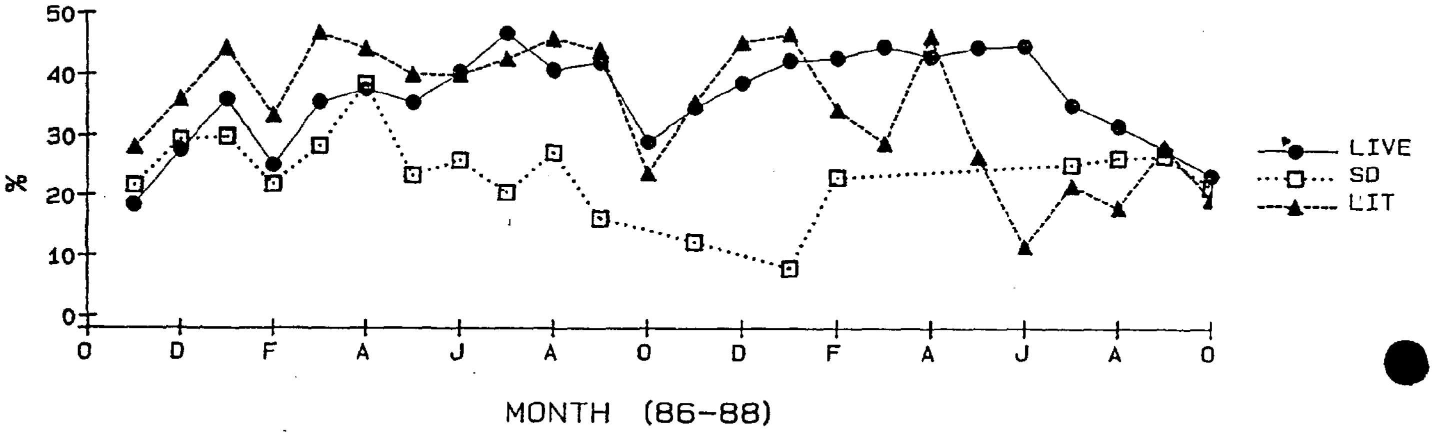
ORGANIC CONTENT (TOTAL)



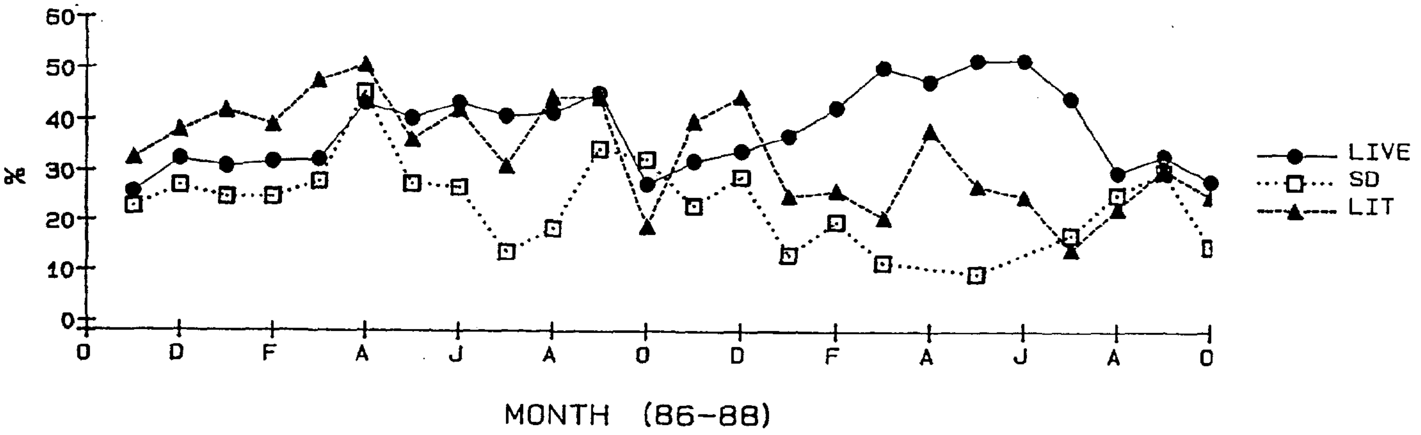
ORGANIC CONTENT (TOTAL)

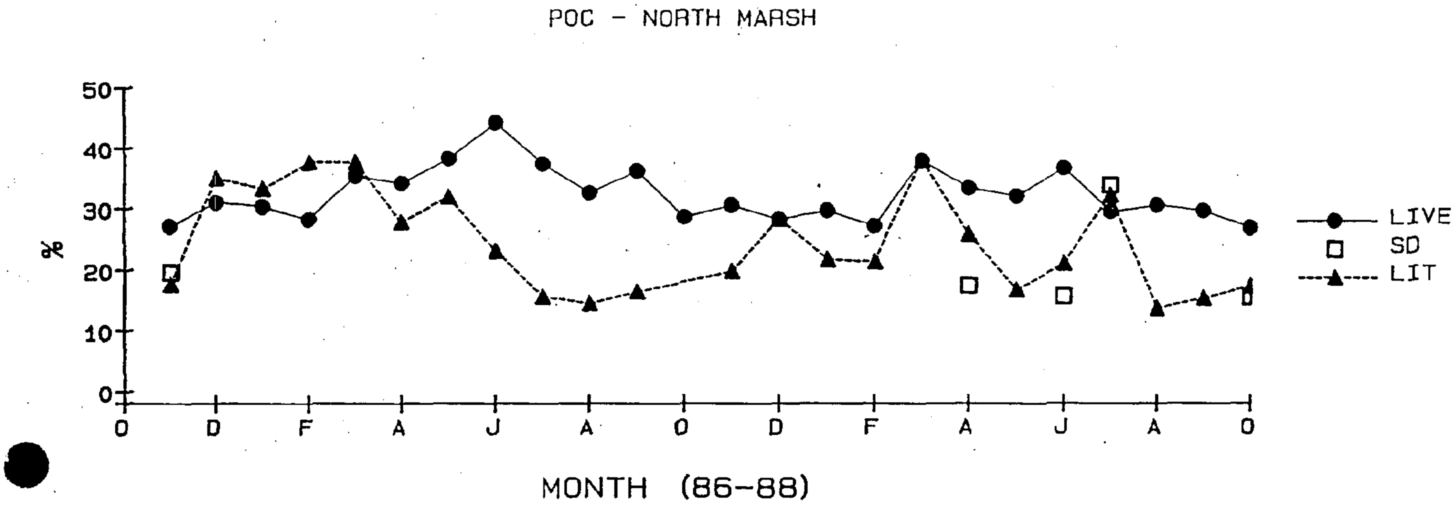


POC - PER. DITCH

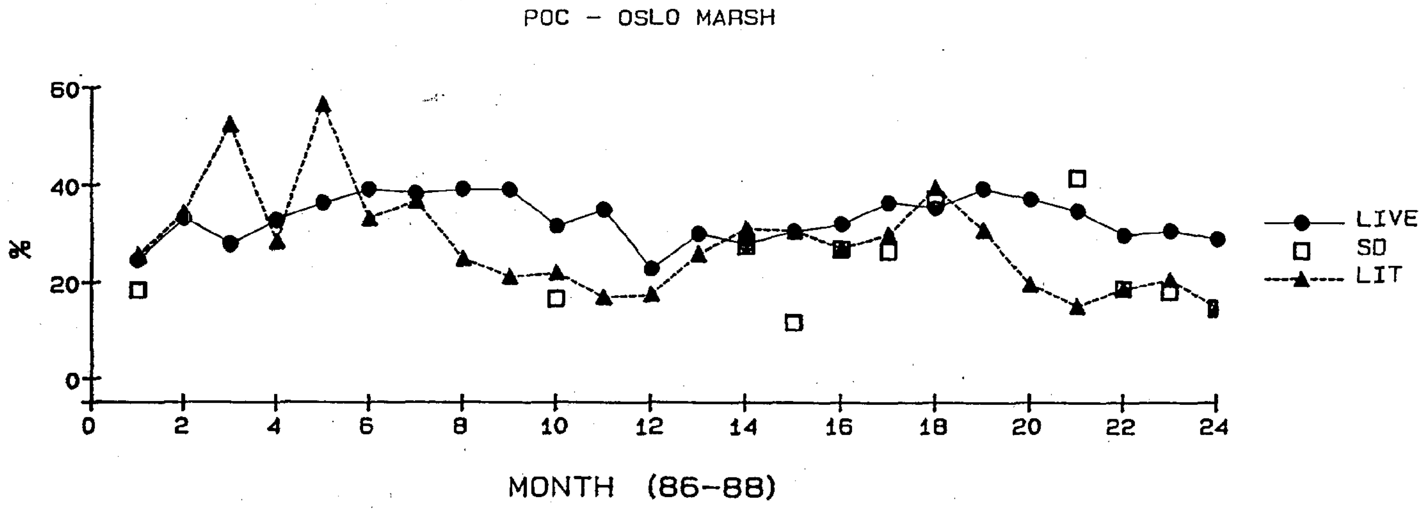


POC - RAIN GAUGE

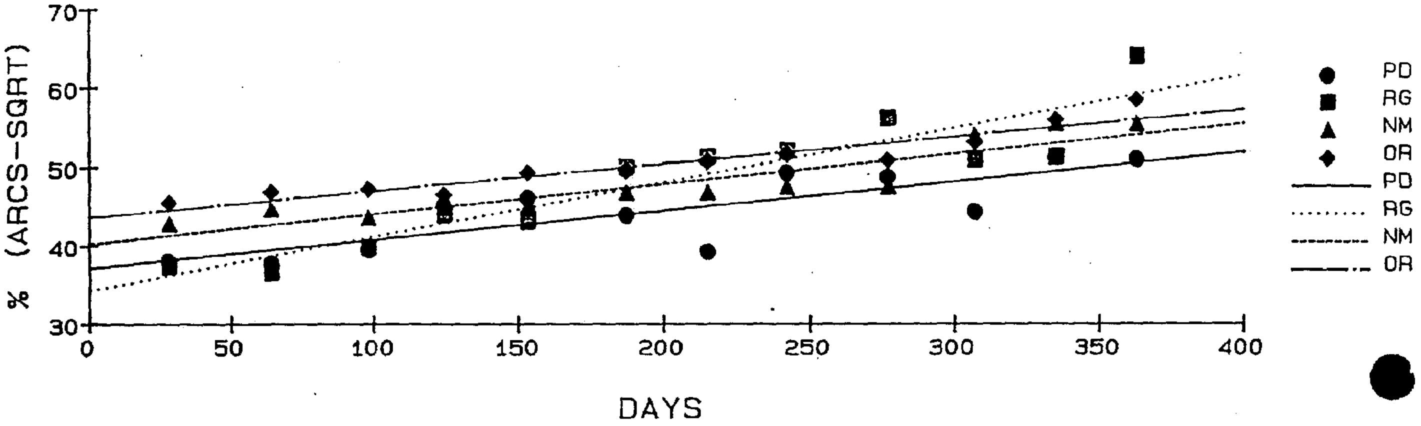




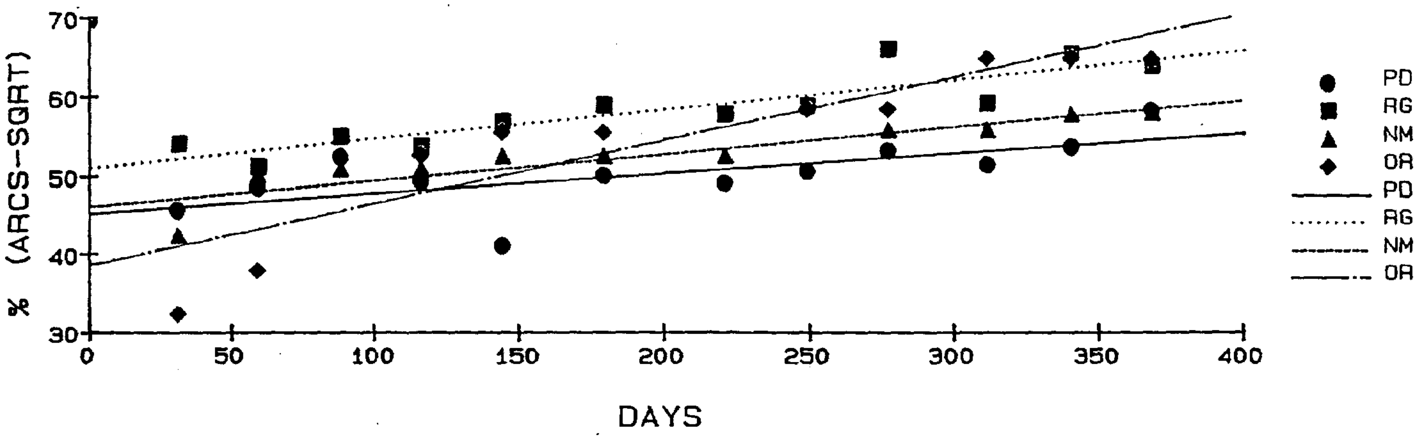
POC - NORTH MARSH



POC - OSLO MARSH

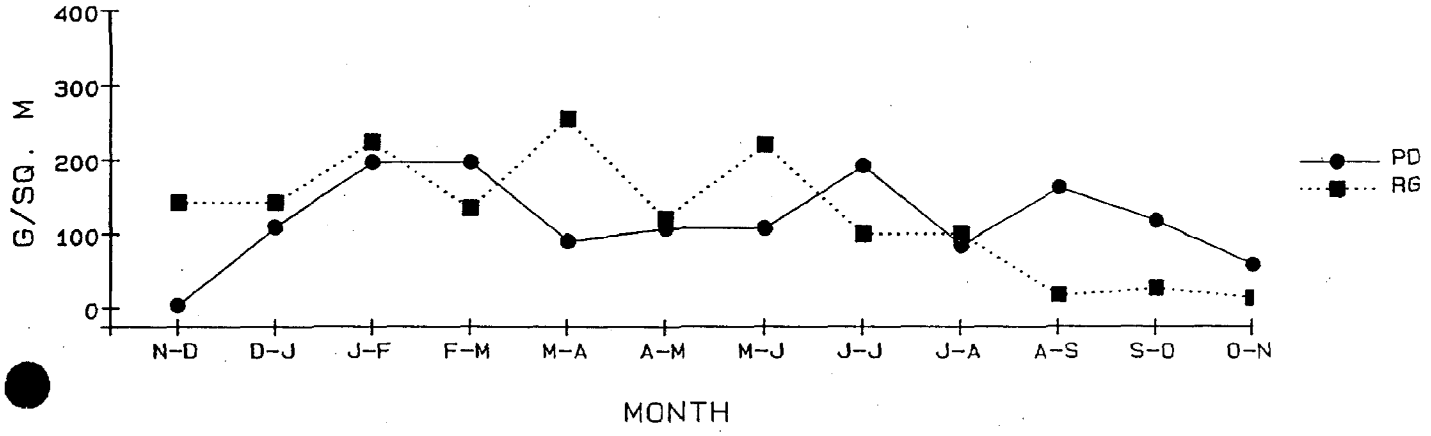


LITTER BAG LOSSES: 1986-1987

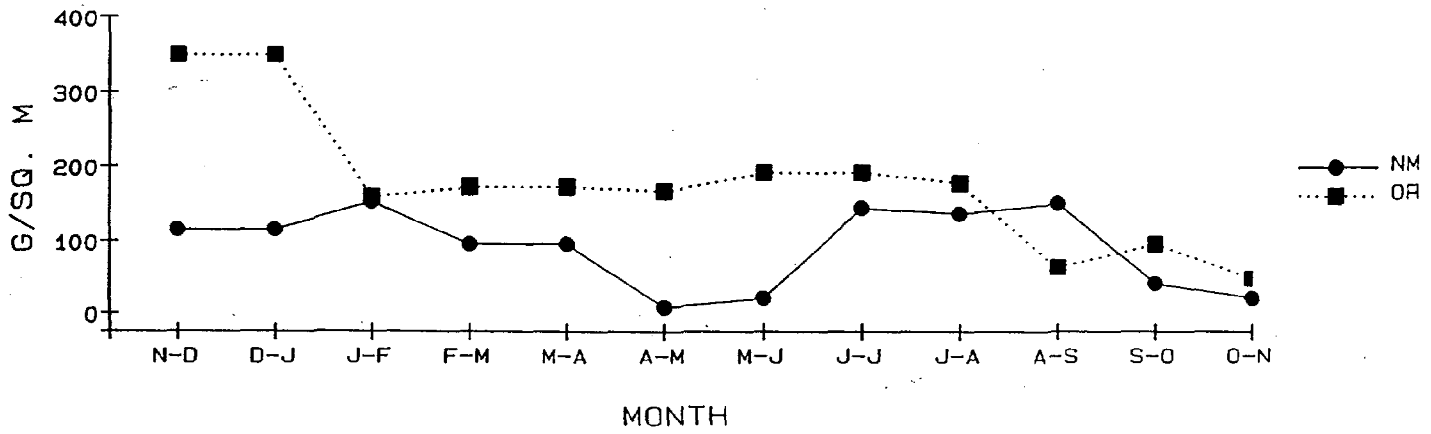


LITTER BAG LOSSES: 1987-1988

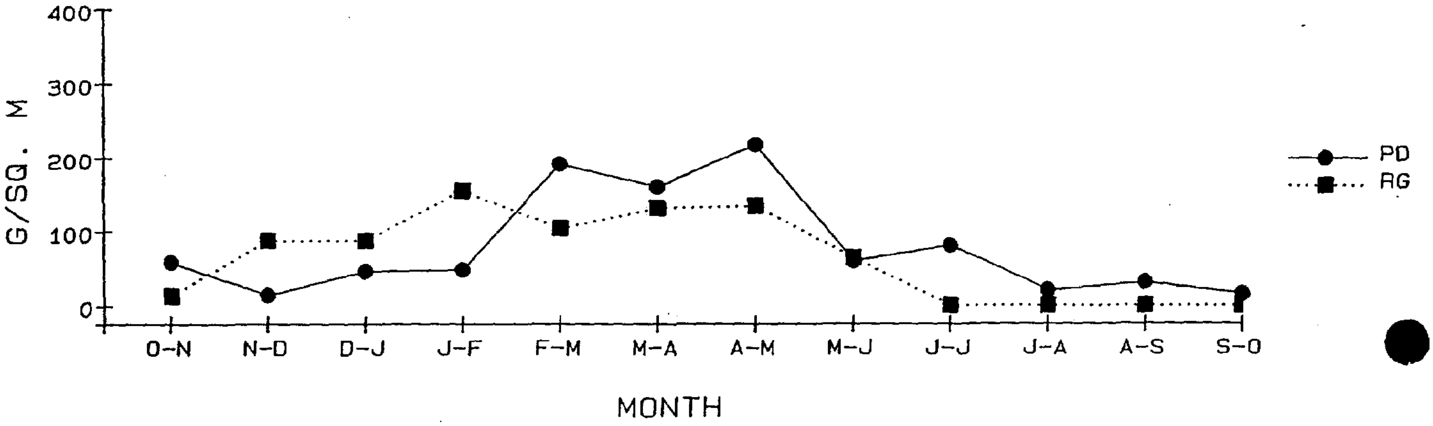
PRODUCTION (W & E) 1986-1987



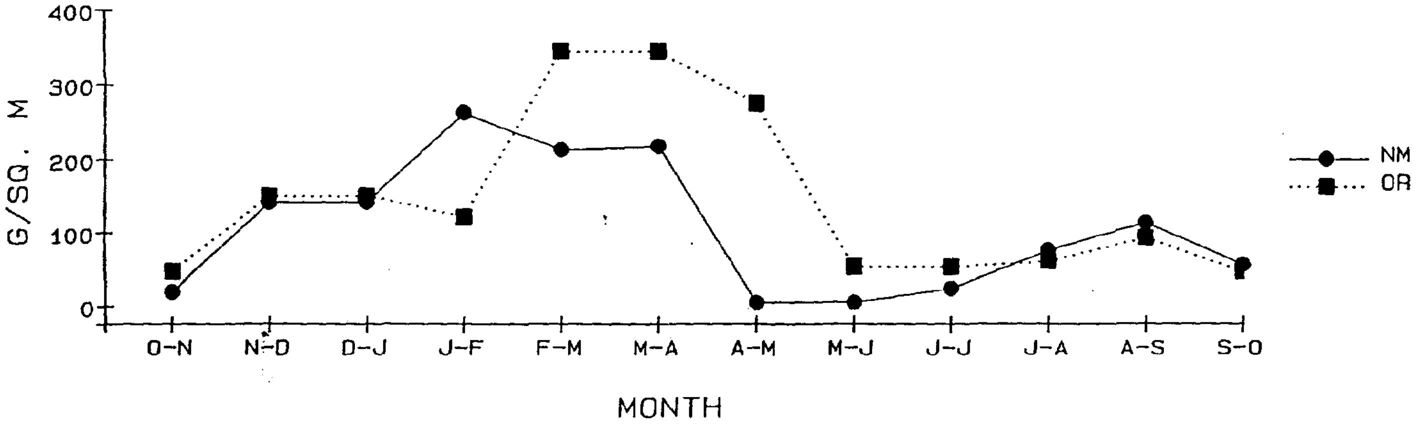
PRODUCTION (W & E) 1986-1987

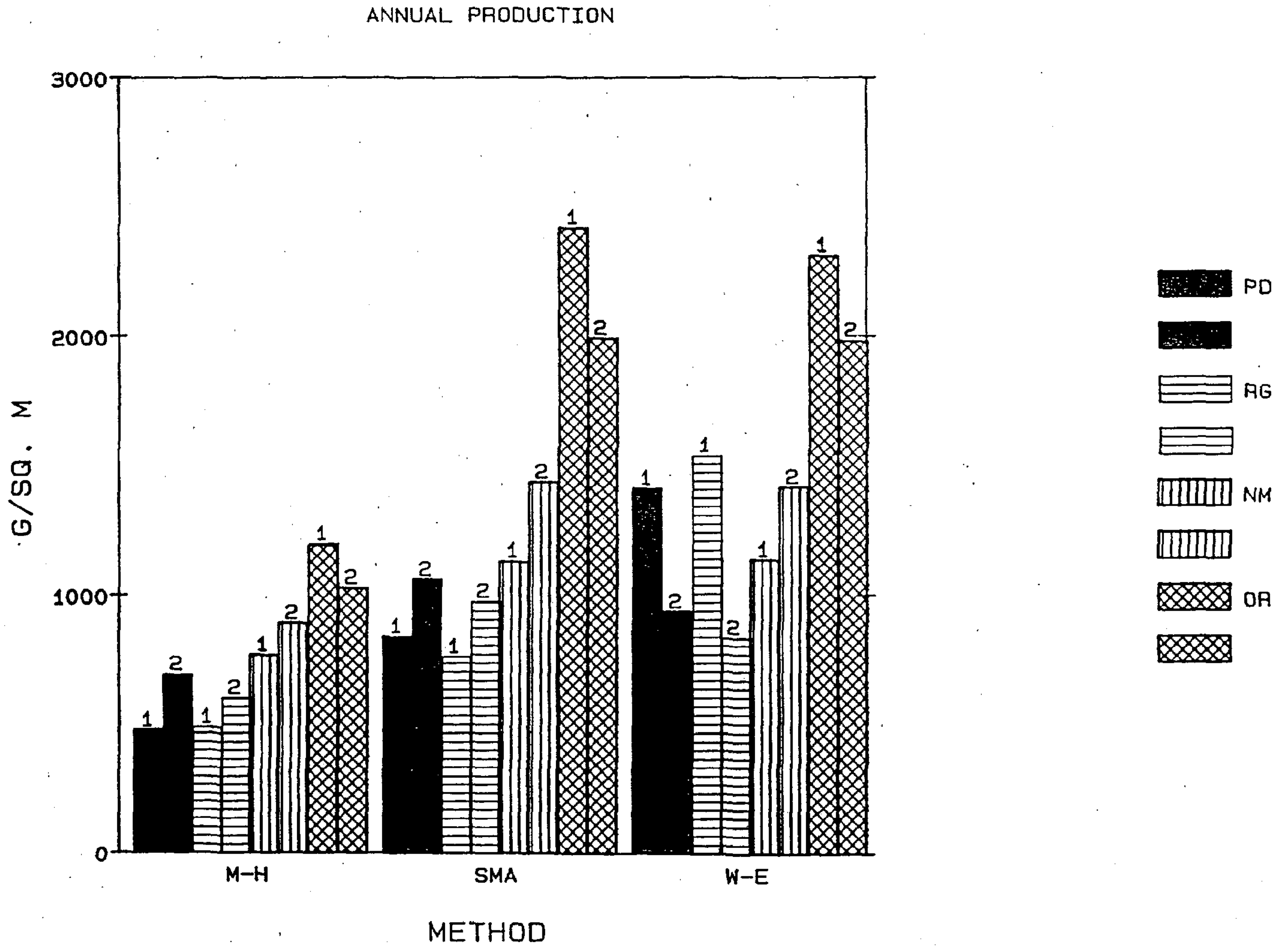


PRODUCTION (W & E) 1987-1988



PRODUCTION (W & E) 1987-1988




ANNUAL PRODUCTION
3000
2000
1000
0
G/SQ. M
1
2
1
2
1
2
1
2
1
2
1
2
1
2
1
2
1
2
1
2
1
2
1
2
M-H
SMA
W-E
METHOD
PD
RG
NM
OR

FINAL REPORT

22 March 1989

CZM-194

INTERSPECIFIC DIFFERENCES IN METABOLIC ADAPTATIONS TO HYPOXIA AND MASS MORTALITIES OF IMPOUNDED SALT MARSH AND MANGROVE SWAMP FISHES

R. GRANT GILMORE, JR.

MARK S. PETERSON

HARBOR BRANCH OCEANOGRAPHIC INSTITUTION, INC.

5600 OLD DIXIE HIGHWAY

FT. PIERCE, FLORIDA 34946

INTRODUCTION

During the 1950's and 1960's, the majority of the salt marsh mangrove swamp habitat along the Indian River lagoon was impounded for mosquito control. These impoundments were artifically flooded with water from the adjacent estuary or from artesian wells (Provost 1977), thus effectively reducing mosquito oviposition and population size. Initially, impounding was shown to be detrimental to marsh vegetation and fish species (Harrington and Harrington 1982; Gilmore et al. 1982; Carlson et al. 1985) and typically provided a habitat for resident and transient species that was stressful and at times lethal. To reduce these harsh environmental conditions, a new management practice was conceptualized and initiated in the mid to late 1970's (Provost 1974). This rotational impoundment management (RIM) practice closed off the impoundment during the main mosquito breeding season (May-October) and pumped water to a management height (each impoundment different). The impoundment is sequestered from the estuary during this time and upon the arrival of the fall high tides, they are opened to the estuary. This management practice has been rigorously studied since the early 1980's and has been found to be compatible with natural transient species migration and impoundment use patterns (Carlson et al. 1985; Gilmore 1987).

Although RIM has allowed increased use of wetland habitats by fishes, macrocrustaceans and zooplankton (Gilmore 1987; Rey et al. 1987), stressful abiotic conditions periodically prevail and at times cause extensive fish kills within the impoundment. These kills typically occur during the closed period when temperature and salinity are high and dissolved oxygen is low.

They occur: 1) when water quality deteriorates during the summer closed period; and 2) when pumps breakdown in impoundments where water is pumped over an extended period of the day, thus water quality deteriorates overnight.

During 1986-87, a number of significant fish kills occurred in managed mosquito impoundments in the southern half of the Indian River Lagoon. These mortalities consisted of a number of resident and transient fish species: sheepshead minnow, *Cyprinodon variegatus*, sailfin molly, *Poecilia latipinna*, mosquitofish, *Gambusia affinis*, ladyfish, *Elops saurus*, black drum, *Pogonias cromis*, common snook, *Centropomus undecimalis*, striped and white mullet, *Mugil* spp., spot, *Leiostomus xanthurus*, striped mojarra, *Eugerres plumeri*, yellowfin mojarra, *Gerres cinereus*, and the irish pompano, *Diapterus auratus*. Other species were documented but were infrequently encountered.

Fishes documented in the periodic mortalities reveal variable adaptation to the extremes of ambient environmental conditions. Many of the species occurring in the mortalities are residents and thus are adapted to stressed conditions for extended periods of time. For example, sheepshead minnows and sailfin mollies have been reported in hypersaline habitats (Simpson and Gunter 1956; Gilmore et al. 1982) and are strong osmoregulators (Gustafson 1981; Nordlie 1985, 1987). These two species and the mosquitofish, *G. affinis* are able to tolerate low oxygen levels (Subrahmanyam 1980; Cech et al. 1985) while mosquitofish (Cech et al. 1985), other mollies (Weber and Kramer 1983; Poulin et al. 1987), and striped mullet, *Mugil cephalus* (Cech and Wohlschlag 1973; Moore 1976) can behaviorally adjust their oxygen uptake by aquatic surface respiration. The transient striped mullet can also take atmospheric oxygen (Hoese 1985). The tarpon, Megalops atlanticus, is an obligate air breather and thus is well adapted to living in hypoxic habitats (Shlaifer and Breder 1940; Wade 1962), and has not been reported in fish kills.

Our objectives were to document: 1) hypoxia-induced changes in hematocrit, plasma osmolality, plasma chloride ion concentration and oxygen consumption and to investigate survival in low oxygen tensions in the common snook, Centropomus undecimalis, sheepshead minnow, Cyprinodon variegatus, and sailfin molly, Poecilia latipinna; 2) dissolved oxygen levels observed in the field which potentially cause fish kills; 3) the frequency and duration of hypoxic events; and 4) to develop management recommendations to reduce fish kills due to hypoxia.

MATERIALS AND METHODS

Field Collections and General Laboratory Protocol

Fishes were collected from impounded and non-impounded mangrove marshes in the Indian River Lagoon, Florida. They were transported to the laboratory in styrofoam coolers containing impoundment water where they were held at 25°C overnight under high aeration or transferred directly into exterior concrete vaults (900 ℓ) when environmental temperatures approached experimental temperatures. Experimental animals were transferred to 76 ℓ aquaria equipped with individual filters, aerators and heaters. They were held in 30 ± 1 0/00, 30 ± 1°C (increased from 25°C over a 24 hr period when held indoors) and under a 12L:12D photoperiod centered at 1230 h, for at least 7 days (see details within each sub-section). Light intensity at the waters surface was 0.65 X 10^{16}quanta/ sec/cm^2. *Cyprinodon variegatus* and *Poecilia latipinna* were fed flake food twice daily, whereas *C. undecimalis* were fed live fish daily. Snook were fed a number of natural prey species. These were: *C. variegatus*, *P. latipinna*, *Gambusia affinis*, *Lucania parva*, *Fundulus grandis*, *Lagodon rhomboides*, *Penaeus* spp., and *Palaemonetes* spp. In all experiments, we did not consider the sex of the fishes. Experimental salinities were produced using filtered (5 micron) Atlantic Ocean seawater diluted with aged reverse osmosis (RO) water. Salinities were checked daily with either a AO refractometer or a YSI S-C-T meter (Model 33). For all experiments except the survival portion, fish were fasted for 24 h prior to testing.

Twenty-four Hour Circadian Blood Constituent Patterns

Ten individuals were tested at 4-h intervals from 0400 through 2400. Fish tested at night (2000, 2400, 0400) remained in darkness until they were netted and tested under dim light (intensity at water surface= 0.01 X 10^{16} quanta/sec/cm^2). Blood of all individuals was collected and centrifuged within a 30-min period, centered at each previously mentioned test times. Subsequently, hematocrit (%), osmolality and chloride ion concentrations were determined within the following 30 min. Fish were netted from their experimental aquaria and immediately measured to the nearest mm standard length (SL). Blood samples were obtained by first blotting each individual dry and severing its caudal fin with a razor (Barton 1979). The incision was immediately blotted and blood from the caudal artery was drawn into a heparinized micro-capillary tube and centrifuged for 4 min at 11500 rpm in an International Micro-capillary Centrifuge (Model MB) for hematocrit (%) determination. Individual blood collection was completed within one minute to reduce handling effects on blood constituents (Chauvin and Young 1970; Robertson et al. 1987). All hypoxic individuals were sacrificed between 0800-0900. Plasma osmolality ($\mathrm{mmol/kg^{-1}}$) was determined on a 10 ul sample with a Wescor Vapor Pressure Osmometer (Model 5500). Plasma chloride ion concentration (meq/l) was determined from a 10 ul sample on a Buchler Digital Chloridometer (Model 4-2500). A total of 60 individual determinations per blood constituent and species (10 per time period) were made. All blood constituents were determined for each individual. Snook were held under the desired temperature and salinity conditions for 8.2 ± 1.5 days; mollies for 8.9 ± 0.2 days; and minnows for 8.6 ± 1.1 days prior to experimentation.

Normoxic/Hypoxic Blood Constituents Experimental Protocol

For the normoxic/hypoxic experiments, non-significantly different ($p<0.05$) individual responses documented for the 24-h studies were used for the normoxic portion of the experiments. These were compared to individual responses under hypoxic conditions. Sample sizes thus differed among the three species: _C_. _variegatus_, N=50; _P_. _latipinna_, N=30; and _C_. _undecimalis_, N= 40. Snook were held under the desired salinity and temperature conditions for 7.6 ± 0.5 days; minnows for 11.5 ± 0.5 days; and mollies for

7.0 days prior to experimentation. Hypoxic conditions were produced by bubbling nitrogen gas directly into the aquarium through an air-stone for 3.0 hours, thus gradually reducing the oxygen tension to hypoxic conditions (X_{torr}= 38.5 ± 2.6; approximately 26% saturation; 1.65 ppm). Experimental pO_2 concentrations were determined and monitored by injecting a single water sample into a calibrated Radiometer PHM-73/D616/E5046 oxygen analyzer system. The experimental aquaria had floating styrofoam slabs on the water in order to reduce surface breathing by sailfin mollies.

Oxygen Consumption Rates

For snook, we used a flow-through respirometer similar to the one described in Cech et al. (1979). It consisted of a acrylic tube (33.7 cm X 7.94 cm ID diameter or a 18.1 cm X 7.94 cm ID diameter) with PVC caps that were sealed by a rubber O-ring. The downend cap had a hole drilled into it in order to remove any bubbles. Water flowed into the chamber via vinyl tubing and was passed through a piece of fiberglass screen which was sandwiched between a set of plastic baffles (set at 45 degree angles) which were mounted in both ends of the tube. Inhalent water was supplied by a 456 ℓ headbox (six glass aquaria connected in series by PVC tubes and each equipped with a heater and aerator) via vinyl tubing through an small acrylic chamber (6.98 cm X 7.94 cm ID diameter; vol.= 300 ml) which fed into a PVC manifold and through PVC ball valves and tubing into the respirometers (Fig. 1). Exhalent water also entered a small acrylic chamber which enclosed a small stirring bar driven by a submersible stirrer. Water samples were taken from these chambers with a syringe and needle mounted through a rubber stopper. Exhalent water passed through vinyl tubing which enclosed a bypass valve used to measure flow rates (FR; ml/min over 30 sec period). Flow rates (59.1 ± 7.7 ml/min) flushed the large respirometers on average every 27.9 min and the small respirometers on average every 14.4 min. These flow rates and the size of the respirometer allowed us to reach steady state conditions rapidly (Propp et al. 1982). This design required that only three animals and a control could be run simultaneously. The entire system was enclosed in a large, heated water bath (244L X 122W X 27D cm) that had two water circulating pumps that aided in maintaining accurate and reproducible water temperatures. Additionally, the four respirometers were enclosed within a PVC and wood frame that had black plastic partitions between the tubes and an outer cover that reduced observer bias. The top of the frame was a one-way mirror which was used to observe the fish and count opercular movements. The chamber also had a small fluorescent light mounted above the tubes that was also set on the prescribed photoperiod.

Small flow-through respirometers (modified 125 ml Erlenmayer flasks) were used for sailfin molly and sheepshead minnow. They were equipped with two glass rods, one that allowed inflowing water to enter near the bottom of the flask whereas the other rod allowed water to pass out of the flask near the bottom of the stopper (top of flask). This allowed for complete mixing of the water. Fifteen respirometers were connected by vinyl tubing to a PVC manifold that possessed nylon values which controlled water flow rates (26.7 ± 12.4 ml/min for minnows; 21.6 ± 8.7 for mollies). Thus, these flow rates on average flushed the respirometers every 4.7 min for minnows and every 5.8

min for mollies, and achieved steady state rapidly (Propp et al. 1982). Water of the desired oxygen tension entered the manifold from a 456 ℓ headbox via an acrylic chamber (300 ml) which was used to obtain an initial water sample with a needle and syringe. Final water samples were taken with a needle and syringe from a piece of vinyl tubing attached to the glass rod leaving each respirometer. This design allowed us to run all 14 individuals and a control simultaneously.

Desired oxygen tensions (torr) were produced by passing water from the headbox through an in-line oxygen stripper (Cameron 1986) made of PVC (152.4 cm X 7.94 cm ID diameter) and filled with glass marbles to increase surface area for diffusion. Nitrogen gas was introduced (with a Dwyer flow meter) through a gas diffuser from the bottom, thus producing a counter-current flow. Regulation of the counter flows brought about the desired oxygen levels.

Oxygen tensions of inhalent (P_{IO2}) and exhalent (P_{EO2}) water samples were measured using the Radiometer oxygen analyzing system described above. Oxygen consumption rates (mg $O_2\ g^{-1}h^{-1}$) were calculated by the equation VO_2 = (P_{IO2}-P_{EO2}) SC (1.428) FR/ ww (g) (Lampert 1984), where SC= solubility coefficient (obtained from tables in Cameron 1986), the value 1.428 converts ml/l to mg/l oxygen, FR= flow rate (ml/h), and ww= wet weight (g). Results were consider routine metabolic rates (Brett and Groves 1979). For each experiment, fish were run over a 43.5 h period. An individual fish was placed within each of the respirometers at 1330 and allowed to acclimate to the chamber for 18.5 h in normoxic water (snook: X_{torr}= 177.1 ± 13.9; minnows: 150.1; mollies: 165.6). Between 0800-0900, initial and final pO_2 readings were taken as well as a flow rate for each respirometer. An additional respirometer was used to control for potential microbial respiration. The fish remained in the chamber for an additional 18.5 h and then nitrogen was used to gradually reduce the partial pressure of oxygen (over the next 3.0 h) flowing into the respirometers (snook: X_{torr}= 41.2 ± 2.1; minnows: 42.2; mollies: 42.8). Once the desired oxygen tension was obtained, the fish were allowed 2.5 h to adjust to the lower oxygen levels. The initial and final hypoxic readings were taken between 0800-0900 (Fig. 2). Animals (N=14 for each species) were used only once.

In order to remove some variability in our oxygen consumption rates, experiments were set up as follows: 1) to remove potential affects of diel patterns in osmoregulation (Peterson and Gilmore 1988), all individuals were run between 0800-0900; 2) the affect of the "lights on/off" phenomenon on oxygen consumption rates (Saint-Paul 1988) was mitigated by establishing the photoperiod to come on at 0630 (staggered over a 15 min period) which allowed at least 1.25 h before the determinations were initiated; and 3) the partial pressure of oxygen was gradually reduced (Burton et al. 1980) over a 3.0 h time period (instead of the more typical 0.5-1.0 h period). This gradual reduction allowed the fish to adjust to the low dissolved oxygen conditions for 2.5 h (typical time is 1.0 h) before beginning the oxygen consumption experiments. Although this procedure forced us to conduct these experiments over a longer time period than we would have liked, we feel the removal of these known problems associated with oxygen consumption rates make our values more accurate and realistic.

Ventilation Rates and Survival Experiments

In order to more adequately address the effects of hypoxia on the three target species, survival experiments were set-up at 60 and 40 torr. Individual fish were placed into either the 1.65 ℓ respirometer (*C. undecimalis*) or the 0.85 ℓ respirometer (*C. variegatus* and *P. latipinna*) and given 18.5 h to acclimate to the tubes under flowing, normoxic (X=162.3 ± 13.6) water. Subsequently, between 0730-0800, the oxygen tension was rapidly reduced to either 60 (X=59.6 ± 1.2) or 40 (X=39.6 ± 1.2) torr and death was monitored on 1, 2, 4, 6, 8, 10, 12 and 24 h intervals. Death was established when the fish did not move its operculum for 1 minute. Throughout this portion of the experiments, flow rates (121.1 ± 9.0 ml/min) flushed the 1.65 ℓ tubes every 13.6 minutes and the 0.85 ℓ tubes every 7.02 minutes, on average. Ventilation rates (VR) were taken during this phase of the experiments. Normoxic rates were taken immediately prior to decreasing the oxygen tension while hypoxic rates were taken after either 45 minutes (large snook) or 1 hour (small snook) after the tension was reduced. Ventilation rates were calculated by counting the number of times the opercular flap opened and closed over a one minute time period. Snook (N=5) were acclimated to the experimental conditions for 12.6 ± 2.5 days; sheepshead minnow (N=5) for 19.0 ± 1.0 days; and sailfin mollies (N=5) for 31.0 ± 2.1 days.

Historic Physical Conditions, Field Tests and Fish Kills

We conducted two field experiments in order to examine fish kills and some of the conditions that potentially cause these mortalities. Two impoundments were selected: Pine island, in Indian River county and Jack island, in St. Lucie county. Recording dissolved oxygen meters (YSI model 57 and a Rustrak recorder) were installed around 1600 in the impoundment and in the adjacent Indian River at Pine island on 16 September 1988. These meters were left overnight and the next morning we opened the culvert, releasing impoundment water into the Indian River lagoon. We did this a number of times over the next few hours and subsequently (that day and the following morning) collected and enumerated the dead fish. On 19 September, the same meters (after membrane changes and re-calibration) were installed in Jack island impoundment. The pump had been turned off at approximately 0900 on the 19th. One meter was located at the pump site while the other was located at site 130 (approx. 150-200 ft down the perimeter ditch). The meters were placed in the impoundment at 1900 on the 19th and allowed to record overnight. We returned at approximately 0600 the morning of the 20th and recorded and enumerated the dead fish. During both events, the meters were checked initially and at the end with a third meter.

Physical condition data was obtained from Jack Island between 1984-1986 during mid-June through mid-September. These data were from the closed period only and taken during four discrete time periods from impoundment and Indian River lagoon sites over a 24-h period. Historic fish kill data was obtained from Indian River and St. Lucie county mosquito control districts.

Statistical Treatments

Fish size, plasma osmolality, plasma chloride ion concentration, ventilation rates, oxygen consumption rates (all $\log_{10}$ transformed), and hematocrits (arcsine transformed) were analysed by analysis of variance (ANOVA; $\alpha=0.05$) using the SPSS/PC+ package (SPSS, Inc. 1988) on a CSI personal computer. Homogeneity of variance was examined using the Fmax test (Sokal and Rohlf 1969). The 24-h and normoxic/hypoxic experiments were analyzed using ANOVA and a Student-Newman-Keul (SNK) multiple comparison test (Klockars and Sax 1986). Oxygen consumption and ventilation rates were analysed by a paired t-test ($\alpha=0.05$) whereas (for snook) oxygen consumption rates vs wet weight were examined by linear regression (Jensen 1986). Mean 24-h survival (arcsine transformed) was analysed by a t-test.

RESULTS

Twenty-four Hour Circadian Blood Constituent Patterns

Individuals ranged between 24-41 mm SL for sheepshead minnow; between 32-56 mm SL for sailfin molly; and 54-120 mm SL for snook. There were no significant differences in size for either sailfin molly or snook ($p>0.05$; Table 1);however, the 2000 mean ($X=37.0$ mm SL) was marginally larger ($p=0.052$) than the 0800 mean ($X=33.2$ mm SL) for sheepshead minnow. There was no significant differences ($p>0.05$) in hematocrits across all time periods for all three species (Table 1).

Significant circadian patterns in osmolality and chloride were found for sailfin molly and sheepshead minnow (Table 1), whereas only chloride showed significant elevation for sheepshead minnow (Table 1). Osmolality during the 1200 and 1600 time period was significantly elevated for snook (Table 1; $p<0.05$). Values ranged from 327-407 mmol/kg. Chloride, however, was significantly only during the 1200 period and ranged from 112-191 meq/l (Table 1; $p<0.05$). For sailfin molly, the 1200 osmolality value was significantly higher than the 0400 and 0800 time periods whereas the 1600 value was higher that the 0400 value ($p<0.05$) and ranged from 301-401 mmol/kg. The 0400 chloride value, however, was significantly lower than all other time periods except 1600 ($p<0.05$). The chloride values ranged from 106-134 meq/l. There were no significant differences ($p>0.05$) in osmolality across all time periods for sheepshead minnow;however, the 0800 and 2000 chloride values were significantly lower than the 2400 value (Table 1). Osmolality values ranged from 312-368 mmol/kg whereas chloride values ranged from 109-145 meq/l. At all time periods for all species, hematocrit, osmolality, and chloride values among the 10 individuals sampled showed no effect of sampling order and, therefore, no indication of capture stress effects.

Normoxic/hypoxic Blood Constituents

Normoxic individuals of sheepshead minnow were processed between 12-9-87 and 1-11-88; common snook between 12-11-87 and 1-12-88; and sailfin molly between 12-11-87 and 1-20-88. Hypoxic individuals of sheepshead minnow were processed between 2-15-88 and 2-16-88; common snook between 3-8-88 and 4-7-88; and sailfin molly between 2-18-88 and 3-7-88.

Individual sheepshead minnow sizes ranged from 24-40 and 31-45 mm SL for the normoxic (N) and hypoxic (H) experiments, respectively. Common snook ranged from 54-107 (N) and 57-103 mm SL (H) whereas sailfin molly ranged from 36-54 (N) and 32-59 mm SL (H). There were no significant size differences between the normoxic and hypoxic individuals ($p>0.05$).

Significantly ($p<0.05$) elevated hematocrits were documented for all three species under hypoxic conditions (Fig. 3). Only sheepshead minnow exhibited significantly ($p<0.01$) elevated osmolality (Fig. 3) whereas common snook had significantly ($p<0.01$) reduced chloride values (Fig. 3).

Oxygen Consumption Rates

For the size range examined, there was no relationship between wet weight and oxygen consumption rates for sheepshead minnow (1.63-3.21 g wet weight (ww)) in either normoxic or hypoxic conditions ($p>0.05$); however, sailfin molly (0.75-1.84 g ww) had a significant ($p<0.05$) relationship in normoxic but not in hypoxic conditions ($p>0.05$). This normoxic relationship, however, did not explain much of the variance ($r^2=0.41$) and thus we pooled all sizes and analyses the data using a paired t-test. Significant reductions in weight specific oxygen consumption (for log10 transformed and non-transformed data) were documented for sheepshead minnow (paired $t=10.70$; $df=13$; $D= 0.8634$; $p<0.001$) and sailfin molly (paired $t= 6.56$; $df= 13$; $D= 0.5391$; $p<0.001$) under hypoxic conditions (Fig. 4). For snook, however, a significant relationship between log wet weight and log oxygen consumption was documented in normoxic and hypoxic conditions ($p<0.001$) (Fig. 5). For normoxia, the relationship can be explained by the equation: $\log Q= =0.4004127 - 0.4164017 * \log W$ ($r^2=0.82$); whereas, for hypoxia, the relationship can be explained by: $\log Q= -0.6130052 - 0.5924819$

log W (r^2=0.90). These data were also analyses by a paired t-test and were found to be significantly different (paired t= 9.67, df=13, D= 0.3270; $p<0.001$).

Ventilation Rates and Survival

Ventiliation rates (beats/min) were significantly elevated for small snook ($<\approx$ 50-60 g ww) under hypoxic (40 torr; paired t= -4.45, df= 4, D= -41.40, $p<0.05$) and moderately hypoxic (60 torr; paired t= -7.76, df= 4, D= -32.20, $p<0.01$) treatments (Fig. 6). Large snook ($>\approx$ 50-60 g ww) ventiliation rates were also significantly elevated in hypoxic (paired t= -15.61, df= 4, D= -26.40, $p<0.001$) and moderately hypoxic (paired t= -10.28, df= 4, D= -35.40, $p<0.01$) treatments (Fig. 6). Ventiliation rates could not be empirically determined for sailfin molly or sheepshead minnow.

For both sailfin molly and sheepshead minnow, oxygen levels of 60 and 40 torr (2.6 and 1.6 ppm, respectively) are not lethal over a 24-h period (Figs. 7 and 8). However, percent survival at both oxygen levels for juvenile snook are lethal and there is an ontogenetic difference in survival (Fig. 9; Table 3). For small snook ($<\approx$ 50-60 g ww), there was a 80 % survival in 60 torr after 24 h, whereas in 40 torr the survival decreased to 80 % after 3 h and 60 % after 9 h, which remained for the entire 24 h period (Fig. 9). For large snook ($>\approx$ 50-60 g ww), only 60 % survived the after 7 h, whereas in 40 torr, all died within 3 h (Fig. 9). There was on average significantly greater mortality in large snook for 40 torr (t= 7.05, df=88, $p<0.001$) and 60 torr (t= 3.09, df= 88, $p<0.01$) (Fig. 10) and there was significantly greater mortality in 40 than 60 torr ($t = 3.54$, $df = 88$, $p < 0.01$) for small snook and large snook ($t = 7.90$, $df = 88$, $p < 0.001$) (Fig. 10).

Historic Physical Conditions, Field Tests, and Fish Kills

Twenty-four h patterns of dissolved oxygen, salinity and temperature from Jack island impoundment and the adjacent Indian River lagoon suggests that there are significant differences between locations and times of day (Fig. 11). There are significant differences in dissolved oxygen between the impoundment and the river during the early morning and near midnight ($p < 0.05$), while the noon and late evening periods were not different ($p > 0.05$) (Fig. 11). There were no differences in temperature between the two locations ($p > 0.05$) but salinity was significantly lower within the impoundment ($p < 0.05$) during all periods except midnight.

Within a sampling location, dissolved oxygen was significantly higher in late afternoon and midnight within the river but within the impoundment the dissolved oxygen values were all significantly different ($p < 0.05$) and were elevated from noon to midnight, the lowest value was early morning (Fig. 11). For temperature, noon, late evening and midnight were significantly elevated ($p < 0.05$) over early morning but within the impoundment, only noon and late evening were elevated significantly ($p < 0.05$) over the two other periods. Salinity, did not vary significantly within the river but within the impoundment, midnight values were significantly higher that the other time periods ($p < 0.05$).

For the field tests, we documented reduction in dissolved oxygen overnight within Jack island (simulating a pump failure) and low dissolved oxygen at both locations in Pine island, both of which produced a fish kill (Fig. 12; Table 2). Visual observations of water quality indicated that these impoundments were different in terms of the apparent concentration of hydrogen sulfide. Pine island was reddish-colored while Jack island was clear. We tested the water for hydrogen sulfide (Van Handel 1987) within Jack island and in the adjacent river and got a positive reaction only within the river. We were not able to test water from Pine island, although earlier testing indicated the presence of hydrogen sulfide (pers. comm., Peter O'Brian, IRMCD). Further, during the fish kill at Jack island, we noticed that the vast majority of snook that were at the water's surface were small (<150 mm SL); however, those that actually died ranged between 145-305 mm SL.

DISCUSSION

Twenty-four Hour Circadian Blood Constituent Patterns

We documented distinct circadian patterns in osmolality in *P. latipinna* and *C. undecimalis* but not in *C. variegatus*. Significant chloride concentrations were documented for all three species. Hematocrit, however, was not significantly different for any species. Although there were variations within the 10 individuals sampled for each time period, there was never a trend indicating secondary stress responses resulting from the 30 min period of sampling in which aquarium captures were made. The range in chloride and osmolality observed in each of these species comprised a major portion of the values reported for other euryhaline fishes (Holmes and Donaldson 1969). To date, similar chloride and osmolality diel patterns have been documented in some euryaline cyprinodontids (Meier et. al. 1973; Spence et. al. 1977; Bulger 1986. Furthermore, Hannah and Pickford (1981) also documented significantly elevated hematocrits and sodium at 1600 in F. heteroclitus but no patterns were documented for chloride or potassium. The sodium cycle in F. heteroclitus seems to be in phase with the elevated values of osmolality documented by Bulger (1986) for the same species. Additionally, Ikeda et. al. (1976) detected diurnal peaks in chloride in the yellow-tail, Seriola quinqueradiata which varied depending upon the type of food fed to the fish. Leatherland et. al. (1974), however, failed to detect a significant pattern in hematocrit for juvenile kokanee salmon Oncorhynchus nerka. All of these studies suggest that there are species specific differences in diel patterns in osmolality, ion concentration and hematocrit. Hoewever, the apparent wide variation in diel or diurnal study responses within congeneric species (see Meier et. al. 1973; Figs. 1 and 2) may simply be due to juxtaposition of the time axis.

It is interesting to note that all of the species previously studied are euryhaline and inhabit estuarine habitats which present complex ambient physio-environmental conditions to the fish. Freshwater channel catfish Ictalurus punctatus and the hybrid I. punctatus X I. furcatus that occur in less fluctuating habitats, apparently do not have chloride cycles (Davis and Simco 1976). This suggests that the phenomena of circadian patterns in osmolality and at least chloride ion concentration may be most distinct in euryhaline fishes that inhabit biotopes that fluctuate in temperature (see Bulger 1986 for lengthy discussion).

Of major importance in ion- and osmoregulation studies is the time of day the blood samples were obtained, yet few studies indicate this important point. Comparison of treatment data within a study may show a significant effect simply due to differences in the time of day samples were collected. Additionally, comparison of data among studies almost never consider differences in time of collection, making comparisons suspect. Future studies of this nature should take into account the time of day samples will be collected or if patterns have already been established, collections should be made in light of this published data. Finally, we feel that future studies should state the time of day their animals were tested so as to facilitate easy comparison of data sets.

Normoxic/hypoxic Blood Constituents

We documented significant increases in hematocrit for all three species under hypoxic conditions. This secondary stress response (Mazeaud et al. 1977; Robertson et al. 1987) has also been documented in other species in similar hypoxic conditions and has been indicated as an initial response to hypoxia (Swift and Lloyd 1974). For example, Swift (1981) documented a significant increase in hematocrit for rainbow trout, Salmo gairdneri exposed to 2.3 mg/l dissolved oxygen or lower for periods longer than 3 h. Swift (1982) suggested that increased hematocrit might pre-adapt fishes to hypoxic conditions, thus allowing them to bind more available oxygen when it is environmentally low. It is though that elevated hematocrit is caused by an increased production of erythrocytes, swelling of the erythrocytes and/or fluid loss to the tissues with a subsequent decrease in the plasma volume

(Swift and Lloyd 1974; Kirk 1974; Swift 1981). Probably all are involved to some extent.

Although the values we obtained for sheepshead minnow and sailfin molly for osmolality and chloride ion concentration in 30 ppt and normoxic conditions were similar to those reported by Nordlie (1985, 1987) and Gustafson (1981), no data are available for these two species in hypoxic conditions. The significant increase in osmolality in sheepshead minnow suggests that as the pO_2 gradient across the gill epithelium decreased under hypoxia, sheepshead reduced the energy associated with osmoregulation and spent more energy maintaining a higher gradient by increasing its ventiliation rate. Unfortunately, we were not able to empirically document ventiliation rates in sheepshead or sailfin molly due to their small size but fish typically compensate for reduced gradients by increasing their ventiliation rates (Holeton 1980; Campagna and Cech 1981; Boese 1988; present study). There were no significant changes in either osmolality or chloride ion concentration for sailfin molly, suggesting that this species may have taken oxygen from the rich surface water within the experimental aquaria as has been documented in other mollies (Weber and Kramer 1983; Poulin et al. 1987).

Finally, the significant decrease in chloride ion concentration in common snook, suggests that as hypoxic conditions prevailed for three hours, diuresis occurred and subsequently a loss of chloride and possible other ions via the urine. Hunn (1969) documented significant loss of chloride, sodium, magnesium and potassium following hypoxia in rainbow trout, <u>Salmo</u>

gairdneri and suggested that it may be due to reduced tubular reabsorptive capacity when oxygen is reduced. Significant decreases in plasma chloride were documented by Swift (1981) for rainbow trout, S.gairdneri exposed to 1.7 mg/l oxygen but not in 2.3 or 3.0 mg/l. Significant decreases in plasma ion (chloride, sodium, potassium) concentrations were documented for S. gairdneri under salinity stress by Johnston and Cherverie (1985). Chloride has also been shown to be less tightly regulated than other ions in teleosts (Lutz 1972; Nordlie 1987).

Oxygen Consumption, Ventiliation and Survival Rates

We documented significant reductions in oxygen consumption rates for sheepshead minnow and sailfin molly in hypoxic conditions. This phenomena has been documented for sailfin mollies by Subrahmanyam (1980) but his sample sizes were small. A number of other studies with round bodied fishes (Cech and Wohlschlag 1973; Lomholt and Johansen 1979; Campagna and Cech 1981; Cech et al. 1985) and flatfishes (Watters and Smith 1973; Boese 1988) have documented reduced metabolic rates with increasing hypoxia. Moreover, we documented a significant decrease in metabolic rate with increased weight for sailfin mollies in normoxia; however, in hypoxia, there was no significant relationship. Subrahmanyam (1980) and Gustafson (1981) documented similar reductions in weight-specific oxygen consumption with increased weight in sailfin mollies but the relationships were not significant. We feel that the lack of significance under hypoxic conditions might be due to all individuals being effected similarily, regardless of weight. This would produce more variation in individual responses, compared to normoxic conditions, and thus no significant relationship. Considerable variation in oxygen consumption rates have been documented in a number of fish studies (Lomholt and Johansen 1979; Hughes et al. 1983; Eccles 1985; Boese 1988). Finally, although activity was markedly reduced, oxygen tensions as low as 40 torr were not lethal for sailfin mollies and thus this species is able to maintain basal metabolism. Since this species is morphologically well adapted to using the oxygen rich layer of water near the surface (Lewis 1970), they can survive even the lowest oxygen tensions.

We did not document significant size related differences in oxygen consumption rates in sheepshead minnow as did Barton and Barton (1987). This difference may have been due to differences in the size ranges examined among the studies (Table 4) and/or the techniques used. For example, Barton and Barton (1987) examined sheepshead minnow between 0.08-0.78 g ww, whereas, we examined sheepshead minnows between 1.63-3.21 g ww and suggest that at sizes above 1.0 g ww, minnows may not have significant size related oxygen consumption differences. Subrahmanyam (1980) also calculated metabolic rates for minnows (1.4-5.6 g) but his sample sizes were small and not conclusive in terms of size. As with sailfin mollies, activity decreased and oxygen tensions as low as 40 torr were not lethal to sheepshead minnow. Finally, Subrahmanyam (1980) and Barton and Barton (1987) calculated routine metabolic rates using a closed, oxygen depletion technique which has associated with it a series of problems not associated with flow through respirometry (Lampert 1984).

Juvenile common snook also exhibited significantly reduced metabolic rates, increased mortality, and concomitant increases in ventiliation rates in hypoxic conditions. Additionally, snook exhibited a weight dependent respiratory metabolism that follows its life-history habitat use pattern (Gilmore et al. 1983). Although we could determine normoxic rates for individuals larger than about 60 g ww (65.6 ± 19.5 g ww and 175.7 ± 17.1 mm SL), we were not able to keep the same individuals alive under hypoxic conditions. Survival of juvenile snook in low oxygen tensions also depends in part upon body weight. There was on average significantly greater mortality in large snook than small snook in 40 and 60 torr and significantly greater mortality in 40 than in 60 torr for small and large snook. Differences in oxygen consumption based upon weight and oxygen tension have been widely documented in fishes (Hughes et al. 1983; Eccles 1985; Castleberry and Cech 1986). Snook also had significantly increased ventiliation rates and had a more rhythmic opercular movement with decreased activity in hypoxic water. Increases in ventiliation rates have been suggested to increase energy expenditure for a number of fishes (Gee et al. 1978; Lomholt and Johansen 1979; Holeton 1980; Boese 1988). Indeed, Randall and Daxboeck (1984) have estimated that the cost of ventiliation is about 10 % of oxygen uptake in fishes, which also suggests that as oxygen tension decreases and ventiliation increases, the cost proportionally increases for snook. Decreasing activity under hypoxic conditions has been implicated as an energy conservation strategy in fishes (Lomholt and Johansen 1979; Subrahmanyam 1980). Obviously, the lower metabolic rates in low oxygen tensions cannot support snook basal metabolism and depending upon weight, death occurs in tensions as high as 60 torr.

These physiological data support the change in ambient environments experienced by snook and ontogenetic habitat use patterns demonstrated by Gilmore et al (1983) for the same east-central Florida population of common snook. Juvenile snook (< 30 mm SL) recruit into tidal freshwater and/or salt marsh habitats between mid-summer and winter where they grow 1 mm/day for 4-6 months (reaching 100-150 mm SL) and then in early spring leave these habitats for the adjacent seagrass beds. Juvenile snook in marsh habitats averaged 67 mm SL (10-174 mm SL; Gilmore et al. 1983), however, recent collections have produced larger snook from impounded marsh habitats but at a much reduced number. In general, we were not able to keep all large snook alive (40 % mortality) in 60 torr and in 40 torr, there was 100 % mortality in 3 h. For small snook, however, only 20 % died in 60 torr whereas 40 % died in 40 torr after 24 h.

Historical Physical Conditions, Field Tests, and Fish Kills

Twenty-four hour physicochemical data from Jack Island support our strategy of decreasing oxygen tension over an extended period of time, thus exposing the fish to a gradual, not acute decrease in oxygen tension. Because of this and discussions in Burton et al. (1980) concerning differences in survivibility due to the rate of decrease in oxygen, we feel our metabolic rates realistically reflect natural rates.

Although the field tests examined only two impoundments, they support the ontogenetic mortality pattern we documented for snook because only individuals >145 mm SL actually died while smaller snook which were obviously stressed, did not die.

Fish kill reports from Indian River county (Pine island and Impoundment #12) and St. Lucie county (Jack Island) indicate that fish kills are a regular summer (closed period) phenomena, but may be initiated by a combinations of different factors. Poor water quality (oxygen levels and hydrogen sulfide), overcast days (decreases light and thus photosynthetic oxygen) and pump failure work independently or synergistically to cause fish kills. These conditions differentially effect fish and macrocrustacean species which ultimately is based upon a species evolutionary history.

Impact of Observations on Impoundment Management

The practical application of these results to an impoundment management scenario under the present operating procedures needs further study, however, some recommendations can be made. The most obvious recommendation is to avoid producing the conditions which allow low oxygen levels (or high sulfides) to develop. This is not easily done considering the mangrove swamp is often naturally eutrophic and may become anoxic without the complication of impoundment construction. The faculative or obligatory air breathing abilities of a variety of fishes indigenous to this ecosystem are most likely an evolutionary adaptation to recurrent hypoxic conditions in this peripheral estuarine habitat. Therefore, periodic fish mortalities and hypoxic stress is predicted to occur in mangrove swamp habitats under natural conditions. However, that does not preclude the question: "Has management improved or deteriorated the carrying capacity of the mangrove swamp ecosystem by holding water and precluding tidal circulation during the warmer months of the year?" Normally under natural conditions the swamp would be periodically flooded and exposed during the summer and continuously flooded during the late summer and fall. These flooding patterns dictate the extent of fish use of shallow mangrove swamp habitats. Upper marsh flooding is increased during rotational management programs, therefore increasing habitat availability. However, the degree of flooding is not as important to survival as is the accessibility of tides and estuarine access to the deep water habitats within the swamp. When hypoxic conditions and fish mortalities develop, it typically occurs in the deep water habitats within the impoundment such as creeks and perimeter ditches. Mortality associated with hypoxic environmental conditions has not been documented from the high marsh which forms the majority of the impoundment and typically has high DO levels and lower detrital accumulation. Fish mortalities have been documented principally from June to September and most often just after the impoundment is closed or just before or after it is opened. Therefore, we now know the typical habitat and season for the anoxic conditions to develop.

The species most often occurring in these mortalities are transients. They typically spend their late larval/early juvenile developmental periods within the mangrove swamp and migrate to open estuarine habitats through tidal creeks as they increase in size and approach maturity. The snook falls into this category and was therefore examined in this study. Although the resident species are less likely to suffer hypoxic mortalities, the sheepshead minnow has been observed to die in numbers on occasion. The differential physiological and behavioral adaptations of all three species studied, sheepshead minnows, sailfin mollies and snook demonstrates the complexity of life history characteristics and differential microhabitat preference of the mangrove swamp fauna. This information gives the impoundment manager some idea of the complexity of the problem of fish survival in mangrove swamps.

Overpumping, inverted flapgates, reduced closure periods, reduced pump failure, staged reopenings at appropriate locations, induced fish migration through subcells, increased tidal access through additional culverts, increased export of fine sediments and detrital material are all management tools for combating hypoxic perimeter ditch waters and enhancing fish habitat. These techniques have been found to be variously successful in both Indian River and St. Lucie counties. The inducement of snook migration from impoundments during the late winter and spring when they have reached lengths over 100 mm SL should be considered as they have a greater suseptibility to low oxygen levels than the new recruits which enter the impoundment during the late summer and fall. As snook over 100 mm SL are positively rheotactic (Gilmore et al. 1988), periodic pumping with flapgates open (at high tide) near the pump site may induce snook migration out of the impoundment. Larger fishes trapped in the impoundment during the RIM closure period are more likely to suffer mortality and will need to be maintained by water quality enhancements such as overpumping. This research revealed the range of dissolved oxygen levels at which larger snook survive. This information should allow the impoundment manager to determine how effective his water quality management activities are and when to expect fish mortalities.

ACKNOWLEDGEMENTS

We wish to thank Drs. Kimon Bird and Roy Laughlin for the use of laboratory space and equipment, respectfully. Messers. John Holt and Roy Spor provided their usual expert engineering skills during this project. Finally, Doug Scheidt, Ron Brockmayer, Jr, Joe Luczkovich, Doug Carlson, Peter O'Brian, Derek Tremain and John Schaffer aided with field work and discussed various aspect's of this project. D. Scheidt and R. Brockmeyer, Jr. provided considerable assistance with figure production.

LITERATURE CITED

Barton, M. 1979. Serum osmoregulation in two species of estuarine blennoid fish, Anoplarchus purpurescens and Pholis ornata. Comp. Biochem. Physiol. 64A: 305-307.

Barton, M. and A.C. Barton. 1987. Effects of salinity on oxygen consumption of Cyprinodon variegatus. Copeia 1987(1): 230-232.

Boese, B.L. 1988. Hypoxia-induced respiratory changes in english sole (Parophrys vetulus Girard). Comp. Biochem. Physiol. 89A: 257-260.

Brett, J.R. and T.D.D. Groves. 1979. Physiological energetics. pp. 279-352. In: Hoar, W.S. and D.J. Randall, (eds.), Fish Physiology, Vol. VIII, Academic Press Inc., Orlando, Fla.

Burton, D.T., L.B. Richardson and C.J. Moore. 1980. Effect of oxygen reduction rate and constant low dissolved oxygen concentrations on two estuarine fish. Trans. Amer. Fish. Soc. 109: 552-557.

Bulger, A., 1986. Coincident peaks in serum osmolality and heat-tolerance rhythms in seawater-acclimated killifish (Fundulus heteroclitus). Physiol. Zool. 59: 169-174.

Carlson, D.B., R.G. Gilmore and J.R. Rey. 1985. Salt marsh impoundment management on Florida's central east coast: reintegrating isolated high marshes to the estuary, pp. 47-63. In: Webb, F.J.,Jr. (ed), Proc. 12th annual conf. on wetlands restoration and creation.

Cameron, J. 1986. Principles of physiological measurement. Academic Press, Inc., New York, N.Y. 278p.

Campagna, C.G. and J.J. Cech, Jr. 1981. Gill ventiliation and respiratory efficiency of Sacramento blackfish, Orthodon microlepidotus Ayres, in hypoxic environments. J. Fish Biol. 19: 581-591.

Castleberry, D.T. and J.J. Cech, Jr. 1986. Physiological responses of a native and an introduced desert fish to environmental stressors. Ecology 67(4): 912-918.

Cech, J.J. and D.E. Wohlschlag. 1973. Respiratory responses of the striped mullet, Mugil cephalus (L.) to hypoxic conditions. J. Fish Biol. 5: 421-428.

Cech, J.J., C.G. Campagna and S.J. Mitchell. 1979. Respiratory responses of largemouth bass (Micropterus salmoides) to environmental changes in temperature and dissolved oxygen. Trans. Amer. Fish. Soc. 108: 166-171.

Cech, J.J., M.J. Massingill, B. Vondracek and A. L. Linden. 1985. Respiratory metabolism of mosquitofish, Gambusia affinis: effects of temperature, dissolved oxygen, and sex difference. Envir. Biol. Fishes 13(4): 297-307.

Chauvin , W. and J.E. Young. 1970. Factors in the determination of normal serum glucose levels of goldfish, Carassius auratus L. Comp. Biochem. Physiol. 33: 629-653.

Davis, K.J.B. and B.A. Simco 1976. Diurnal measurements of plasma chloride in catfish. Copeia 1976: 608-609.

Eccles, D.H. 1985. The effect of temperature and mass on routine oxygen consumption in the South African cyprinid fish Barbus aeneus Burchell. J. Fish Biol. 27: 155-165.

Gee, J.H., R.F. Tallman and H.J. Smart. 1978. Reactions of some great plain fishes to progressive hypoxia. Can. J. Zool. 56: 1962-1966.

Gilmore, R.G. 1987. Fish, macrocrustacean and avian population dynamics and cohabitation in tidally influenced impounded subtropical wetlands, pp. 373-394. In: Whitman, W.R. and W.H. Meredith (eds.), Waterfowl and Wetlands symposium. Delaware coastal management program, Delaware dept. natural resources and environmental control, Dover, Delaware.

Gilmore, R.G., D.W. Cooke and C.J. Donohoe. 1982. A comparison of the fish populations and habitat in open and closed salt marsh impoundments in east-central Florida. Northeast Gulf Sci. 5(2): 25-37.

Gilmore, R.G., C.J. Donohoe and D.W. Cooke. 1983. Observations on the distribution and biology of east-central Florida populations of the common snook, *Centropomus undecimalis* (Bloch). Fla. Sci. 46: 313-336.

Gilmore, R.G., R.E. Brockmeyer and D.M. Scheidt. 1988. Final Report: Induced fish migration as a means of reducing hypoxic fish mortalities in impounded salt marsh and mangrove swamp habitats. Fla. Dept. of Health and Rehabilitative Service Contract No. LCNA6.

Gustafson, D.L. 1981. The influence of salinity on plasma osmolality and routine oxygen consumption in the sailfin molly, *Poecilia latipinna* (Leseur), from a freshwater and an estuarine population. M.S. Thesis, University of Florida, Gainsville, Fl. 24p.

Hannah, G.S. and G.E. Pickford. 1981. Diurnal peaks in the hematocrit and in serum sodium in the killifish, *Fundulus heteroclitus*, and their absence in serum potassium and chloride: a lack of correlation. Comp. Biochem. Physiol. 70A: 157-159.

Harrington, R.W., Jr. and E.S. Harrington. 1982. Effects on fishes and their forage organisms of impounding a Florida salt marsh to prevent breeding by salt marsh mosquitos. Bull. Mar. Sci. 32(2): 523-531.

Hoese, H.D. 1985. Jumping mullet: the internal diving bell hypothesis. Env. Biol. Fishes 13(4): 309-314.

Holeton, G.F. 1980. Oxygen as an environmental factor of fishes, pp. 7-31. In: Ali, M.A. (ed), Environmental physiology of fishes, NATO Adv. Study Inst. Series-A: Life sciences. Vol. 35, Plenum Press Co., N.Y., N.Y. 608p.

Holmes, W.N. and E.M. Donaldson. 1969. The body compartments and the distribution of electrolytes, pp. 1-90. In: W.S. Hoar and D.J. Randall (eds.), Fish physiology, Academic Press, New York and London.

Hughes, G.M., C. Albers, D. Muster and K.H. Gotz. 1983. Respiration of the carp, Cyprinus carpio L., at 10 and 20 °C and the effects of hypoxia. J. Fish Biol. 22: 613-628.

Hunn, J.B. 1969. Chemical composition of rainbow trout urine following acute hypoxic stress. Trans. Amer. Fish. Soc. 98: 20-22.

Ikeda, Y., H. Ozaki, S. Sawada, A. Fujii & I. Araki, 1976. Diurnal variations in serum levels oif various constituents in cultured yellowtail (Seriola quinqueradiata). Jap. J. Ichthyology 23(2): 93-99.

Jensen, A.L. 1986. Functional regression and correlation analysis. Can. J. Fish. Aquat. Sci. 43: 1742-1745.

Johnston, C.E. and J.C. Cheverie. 1985. Comparative analysis of ionoregulation in rainbow trout (Salmo gairdneri) of different sizes following rapid and slow salinity acclimation. Can. J. Fish. Aquat. Sci. 42: 1994-2003.

Kirk, W.L. 1974. The effects of hypoxia on certain blood and tissue electrolytes of channel catfish, Ictalurus punctatus (Rafinesque). Trans. Amer. Fish. Soc. 104(3): 593-600.

Klockars, A.J. and G. Sax. 1986. Multiple Comparisons. Sage University Paper series on Quantitative Applications in the Social Sciences, 07-061. Beverly Hills, CA., 87 pp.

Lampert, W. 1984. The measurement of respiration. Chapter 10, pp. 416-468. In: Downing, J.A. and F.H. Rigler (eds.), A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publs., Oxford.

Leatherland, J.F., B.A. McKeown and T.M. John. 1974. Circadian rhythm of plasma prolactin, growth hormone, glucose, and free fatty acid in juvenile kokanee salmon, Oncorhynchus nerka. Comp. Biochem. Physiol. 47A: 821-828.

Lewis, W.M, Jr. 1970. Morphological adaptations of cyprinodontoids for inhabiting oxygen deficient waters. Copeia 1970(2): 319-326.

Lomholt, J.P. and K. Johansen. 1979. Hypoxia acclimation in carp-How it affects O_2 uptake, ventiliation, and O_2 extraction from water. Physiol. Zool. 52: 38-49.

Lutz, P.L. 1972. Ionic patterns in the teleost. Comp. Biochem. Physiol. 42A: 719-773.

Mazeaud, M.M., F. Mazeaud and E.M. Donaldson. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Amer. Fish. Soc. 106(3): 201-212.

Meier, A.H., G.R. Lynch and L.E. Garcia. 1973. Daily rhythms of plasma chloride in two teleosts, Fundulus grandis and Fundulus chrysotus. Copeia 1973: 90-92.

Moore, R.H. 1976. Seasonal patterns in the respiratory metabolism of the mullets Mugil cephalus and Mugil curema. Contr. Mar. Sci. 20: 133-146.

Nordlie, F.G. 1985. Osmotic regulation in the sheepshead minnow Cyprinodon variegatus Lacepede. J. Fish Biol. 26: 161-170.

Nordlie, F.G. 1987. Plasma osmotic, Na and Cl regulation under euryhaline conditions in Cyprinodon variegatus Lacepede. Comp. Biochem. Physiol. 86A: 57-61.

Peterson, M.S. and R.G. Gilmore, Jr. 1988. Hematocrit, osmolality and ion concentration in fishes: consideration of circadian patterns in the experimental design. J. Exp. Mar. Biol. Ecol. 121 (1): 73-78.

Poulin, R., N.G. Wolf and D.L. Kramer. 1987. The effect of hypoxia on the vulnerability of guppies (Poecilia reticulata, Poeciliidae) to an aquatic predator (Astronotus ocellatus, Cichlidae). Envir. Biol. Fishes 20(4): 285-292.

Propp, M.V., M.R. Garber and V.I. Ryabuscko. 1982. Unstable processes in the metabolic rate measurements in flow-through systems. Mar. Biol. 67: 47-49.

Provost, M.W. 1974. Salt marsh management in Florida. Proc. Tall Timbers Conf. on Ecol. Anim. Control by Habitat Management (1973). 5: 5-17.

Provost, M.W. 1977. Source reduction in salt-marsh mosquito control: past and future. Mosq. News 37(4): 689-698.

Randall, D.J. and C. Daxboeck. 1984. Oxygen and carbon dioxide transfer across fish gills. pp. 263-314. In: Hoar, W.S. and D.J. Randall (eds.), Fish Physiology, Vol. 10A, Academic Press, Inc., Orlando, Fla. 456p.

Rey, J.R., R.A. Crossman, T.R. Kain, F.E. Vose and M.S. Peterson. 1987. Sampling zooplankton in shallow marsh and estuarine habitats: gear description and field tests. Estuaries 10(1): 61-67.

Robertson, L., P. Thomas, C.R. Arnold and J. M. Trant. 1987. Plasma cortisol and secondary stress responses of red drum to handling, transport, rearing density, and a disease outbreak. Prog. Fish-Cult. 49: 1-12.

Saint-Paul, U. 1988. Diurnal routine O_2 consumption at different O_2 concentrations by Colossoma macropomum and Colossoma brachypomum (Teleosti: Serrasalmidae). Comp. Biochem. Physiol. 89A: 675-682.

Shlaifer, A. and C.M. Breeder. 1940. Social and respiratory behavior of small tarpon. Zoologica 25(4): 493-512.

Simpson, D.G. and G. Gunter. 1956. Notes on habitats, systematic characteristics and life histories of Texas salt-water cyprinodontes. Tulane Stud. Zool. 4: 115-134.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Co., San Francisco, Ca. 776p.

Spence, B., A.H. Meier and T.H. Dietz. 1977. Circadian chloride rhythms in the Gulf killifish, Fundulus grandis. Copeia 1977: 557-560.

SPSS, INC. 1988. SPSS/PC + V 2.0, Base Manual. SPSS, Inc., 444 N. Michigan Ave., Chicago, Illinois.

Subrahmanyam, C.B. 1980. Oxygen consumption of estuarine fish in relation to external oxygen tension. Comp. Biochem. Physiol. 67A: 129-133.

Swift, D.J. 1981. Changes in selected blood component concentrations of rainbow trout, Salmo gairdneri, exposed to hypoxia or sublethal concentrations of phenol or ammonia. J. Fish Biol. 19: 45-61.

Swift, D.J. 1982. Changes in selected blood component concentrations of rainbow trout, Salmo gairdneri, following the blocking of the cortisol stress response with betwmethasone and subsequent exposure to phenol or hypoxia. J. Fish Biol. 21: 269-277.

Swift, D.J. and R. Lloyd. 1974. Changes in urine flow and haematocrit value of rainbow trout Salmo gairdneri (Richardson) exposed to hypoxia. J. Fish Biol. 6: 379-387.

Valentine, D.W. and R. Miller. 1969. Osmoregulation in the Califronia killifish, Fundulus parvipinnis. Calif. Fish Game 58: 20-25.

Van Handel, E. 1987. A sulfide detection test for field use. J. Amer. Mosq. Control Assoc. 3(6): 644-645.

Wade. R.A. 1962. The biology of the tarpon, Megalops atlanticus, and the ox-eye, Megalops cyprinoides, with notes on larval development. Bull. Mar. Sci. Gulf Carib. 12(4): 545-622.

Watters, K.W. and L.S. Smith. 1973. Respiratory dynamics of the starry flounder *Platichthys stellatus* in response to low oxygen and high temperature. Mar. Biol. 19: 133-148.

Weber, J.M. and D.L. Kramer. 1983. Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can. J. Fish. Aquat. Sci. 40(10): 1583-1588.

Table 1. Statistics for parameters examined at one of six times of day (X±SD). Each mean value is based upon 10 individuals. Entries within a parameter having a common superscript or no superscript are not different (P>0.05).

TIME OF DAY (h)	SIZE (mm SL)	HEMATOCRIT (%)	OSMOLALITY (mmol/kg)	CHLORIDE (meq/l)
		Cyprinodon variegatus		
0400	35.0±1.6^{1,2}	24.9±7.3	333.7±12.1	125.6±4.6^{1,2}
0800	33.2±4.5^{1}	28.7±3.4	340.4±9.5	119.3±.7^{1}
1200	36.0±2.0^{1,2}	23.3±4.2	337.5±8.8	125.6±3.9^{1,2}
1600	35.3±2.3^{1,2}	24.3±5.2	345.6±16.3	122.6±3.9^{1,2}
2000	37.0±1.5^{2}	27.2±3.5	344.3±5.5	118.0±7.9^{1}
2400	35.5±2.3^{1,2}	28.3±4.0	340.5±13.1	127.6±8.7^{2}
		Poecilia latipinna		
0400	41.8±7.5	26.9±3.8	316.8±4.1^{1}	116.1±5.9^{1}
0800	43.8±5.2	24.7±4.4	329.0±4.1^{1,2}	123.1±2.3^{2}
1200	42.1±7.1	30.2±5.0	350.9±18.8^{3}	124.0±3.2^{2}
1600	43.4±6.5	28.5±9.6	341.6±30.4^{2,3}	120.5±6.1^{1,2}
2000	44.2±6.9	27.4±4.6	335.3±5.8^{1,2,3}	124.0±6.5^{2}
2400	43.9±5.8	23.6±3.8	332.8±11.3^{1,2,3}	123.8±5.7^{2}
		Centropomus undecimalis		
0400	75.3± 8.3	32.1±3.5	342.2±6.9^{1}	121.6±5.1^{1}
0800	83.1±15.9	28.6±3.0	346.5±9.6^{1}	135.5±7.1^{1}
1200	81.1±15.9	32.9±2.9	382.1±15.9^{3}	154.7±22.7^{2}
1600	81.7±24.2	30.0±3.8	366.9±6.5^{2}	130.7±8.8^{1}
2000	74.4±3.1	32.5±5.8	337.8±8.9^{1}	126.0±5.4^{1}
2400	72.2±5.4	33.1±6.4	342.2±8.6^{1}	124.3±7.1^{1}

Table 2. Fish kill data from field experiments conducted at Pine Island and Jack Island impoundments. These data are considered minimal counts because a number of fish had settled to the bottom of the impoundments. Pine Island= P.I.; Jack Island= J.I.

SPECIES	P.I.(16 SEPT. 1988)	J.I.(19 SEPT. 1988)
Pogonias cromis		151
Elops saurus	4	110
Mugil cephalus	5	88
Centropomus undecimalis	35	32
Gerres cinereus		26
Leiostomus xanthurus		12
Archius lineatus		12
Eucinostomus spp.	100+	11
Lagodon rhomboides	15	6
Archosargus probatocephalus		3
Eugerres plumerii	6	2
Trinectes maculatus	7	2
Callinectes sapidus	30	1
Arius felis		1
Poecilia latipinna	1000+	1
Gambusia affinis	1000+	
Palaemonetes spp.	abundant	
Penaeus spp.	abundant	
Microgobius gulosus	2	
Gobionellus smaragdus	10	
Gobiosoma bosci	1	
Sciaenops ocellatus	1	
Lutjanus griesus	1	
Sphyrna barracuda	1	
Oligoplites saurus	1	
Anguilla rostrata	6	
Syngnathus spp.	5	
Strongulura marina	1	
Lophogobius sp.		1

Table 3. Comparison of snook size (length and weight) among survivibility treatments. All values are expressed as X±SD. TL= total length; SL= standard length; ww= wet weight (g).

		60 TORR	40 TORR
LARGE SNOOK			
	WW:	83.8 ± 13.2	92.1 ± 22.3
	SL:	189.4 ± 15.6	190.2 ± 16.7
SMALL SNOOK			
	WW:	42.9 ± 5.3	36.2 ± 10.9
	SL:	152.8 ± 10.0	139.4 ± 16.2

Table 4. Comparison of oxygen consumption rates for sheepshead minnow and sailfin molly. ~= approximate values (from graphs). ?= temperature not reported. All values from the present study are reported as X±SD.

SPECIES	WW (g)	S (o/oo)	T (°C)	OXYGEN TENSION (mm Hg)	OXYGEN CONSUMPTION (mg O_2/g/h)	AUTHORITY
C. VARIEGATUS						
	0.08-0.78	35	?	SATURATION	0.279-0.078	BARTON AND BARTON 1987
	0.09-0.81	10	?	SATURATION	0.432-0.155	
	0.6	SW	25	~78	~1.55	SUBRAHMANYAM 1980
				~44	~0.23	
	1.3	SW	25	~85	~0.69	
				~45	~0.77	
	2.4	SW	25	~81	~0.39	
				~43	~0.18	
	2.14±0.44	30	30	150.1	0.88±0.30	PRESENT STUDY
				42.2	0.14±0.10	
P. LATIPINNA						
	1.4	SW	25	~85	~0.36	SUBRAHMANYAM 1980
				~58	~0.10	
	3.7			~84	~0.33	
				~43	~0.08	
	5.6			~84	~0.27	
				~41	~0.08	
	1.2-2.9	35	25	90-155	~0.47	GUSTAFSON 1981
	1.24±0.31	30	30	165.6	0.55±0.31	PRESENT STUDY
				42.8	0.13±0.09	

FIGURE LEGENDS

Figure 1. Flow through respirometers used for snook oxygen consumption expertiments and for the survivibility experiments for all species.

Figure 2. Strategy used for oxygen consumption experiments for all three species.

Figure 3. Comparison of hematocrit, osmolality and chloride ion concentration in normoxic (150) and hypoxic (40) conditions for all three species. *= significant difference ($P<0.05$) between oxygen tensions. Values are X±SD.

Figure 4. Comparison of oxygen consumption rates for sailfin molly, <u>Poecilia latipinna</u> (A) and sheepshead minnow, <u>Cyprinodon variegatus</u> (B) in normoxic (150) and hypoxic (40) conditions. Star= significant difference ($P<0.05$). Values are X±SD.

Figure 5. Comparison of snook oxygen consumption rates (Log vs Log) in normoxic and hypoxic conditions. Slopes of both lines are significantly different from zero (ANOVA, $P<0.05$).

Figure 6. Comparison of snook ventiliation rates between large and small snook in normoxic and hypoxic conditions. Star= significant difference ($P<0.05$) within a size group. Values are X±SD.

Figure 7. Comparison of survivibility data over a 24 hour period for sheepshead minnow (A) and sailfin molly (B) in two oxygen tensions.

Figure 8. Mean survival over a 24 hour period of sailfin molly (A) and sheepshead minnow (B) in two oxygen tensions.

Figure 9. Comparison of survivibility data over a 24 hour period for large snook (A) and small snook (B).

Figure 10. Mean survival over a 24 hour period for small (A) and large (B) snook in two oxygen tensions. Similar symbols within a size group indicated that survival is significantly different ($P<0.05$) due to oxygen tensions. Whereas, similar symbols between size groups but within oxygen tensions indicates significant differences ($P<0.05$) due to size. Values are X±SD.

Figure 11. Twenty-four hour physicochemical patterns taken from Jack island impoundment and the adjacent Indian River Lagoon (stippled histogram) over a 4 year period. Point samples were taken only between mid-June and mid-September, when the impoundment was closed. *= indicates a significant difference ($P<0.05$) between the river and the impoundment. Other symbols indicate significant differences ($P<0.05$) across the 24-h day within a site. Those with similar symbols are not significantly different ($P>0.05$).

Figure 12. Oxygen profiles from specific locations in Pine island and Jack island impoundments. See text for details.

Figure 1.

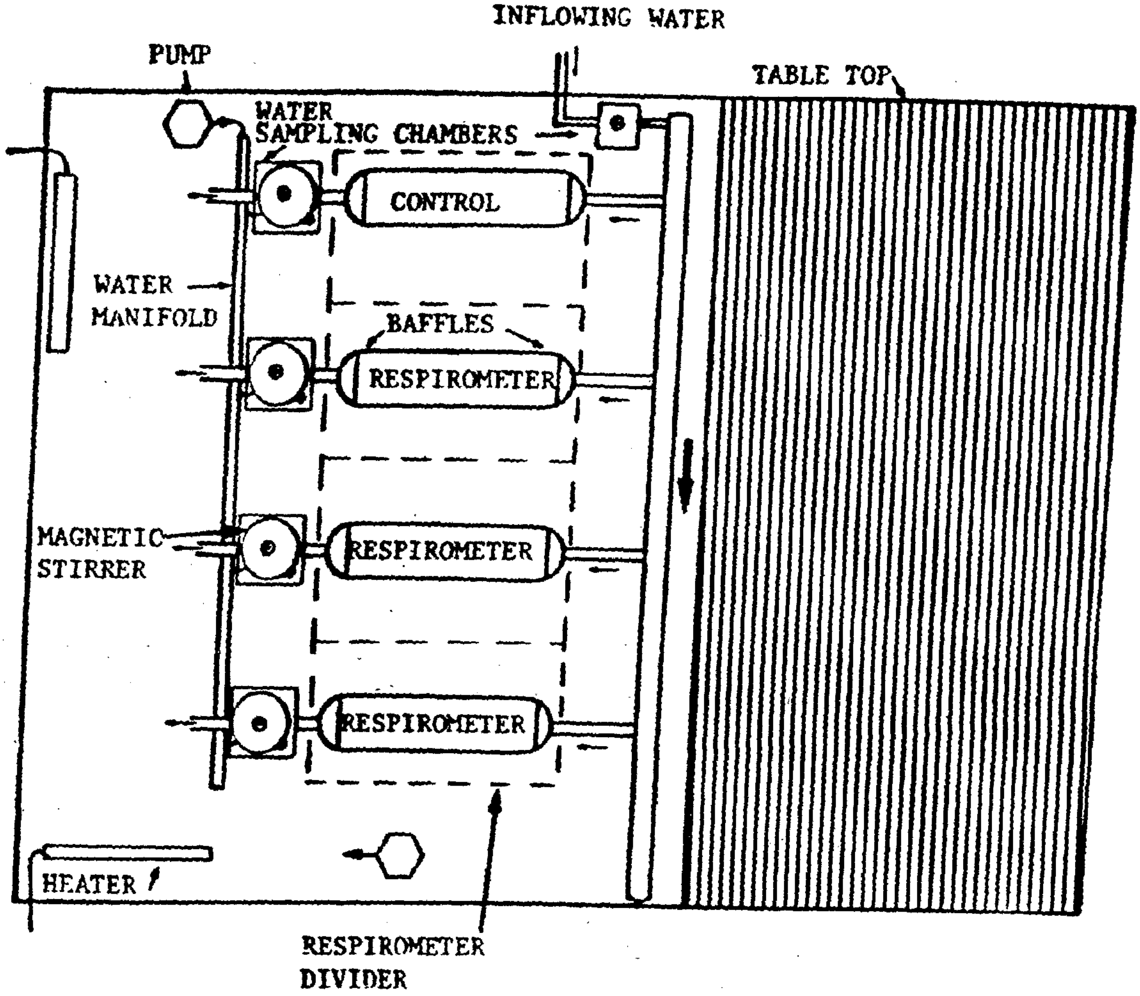
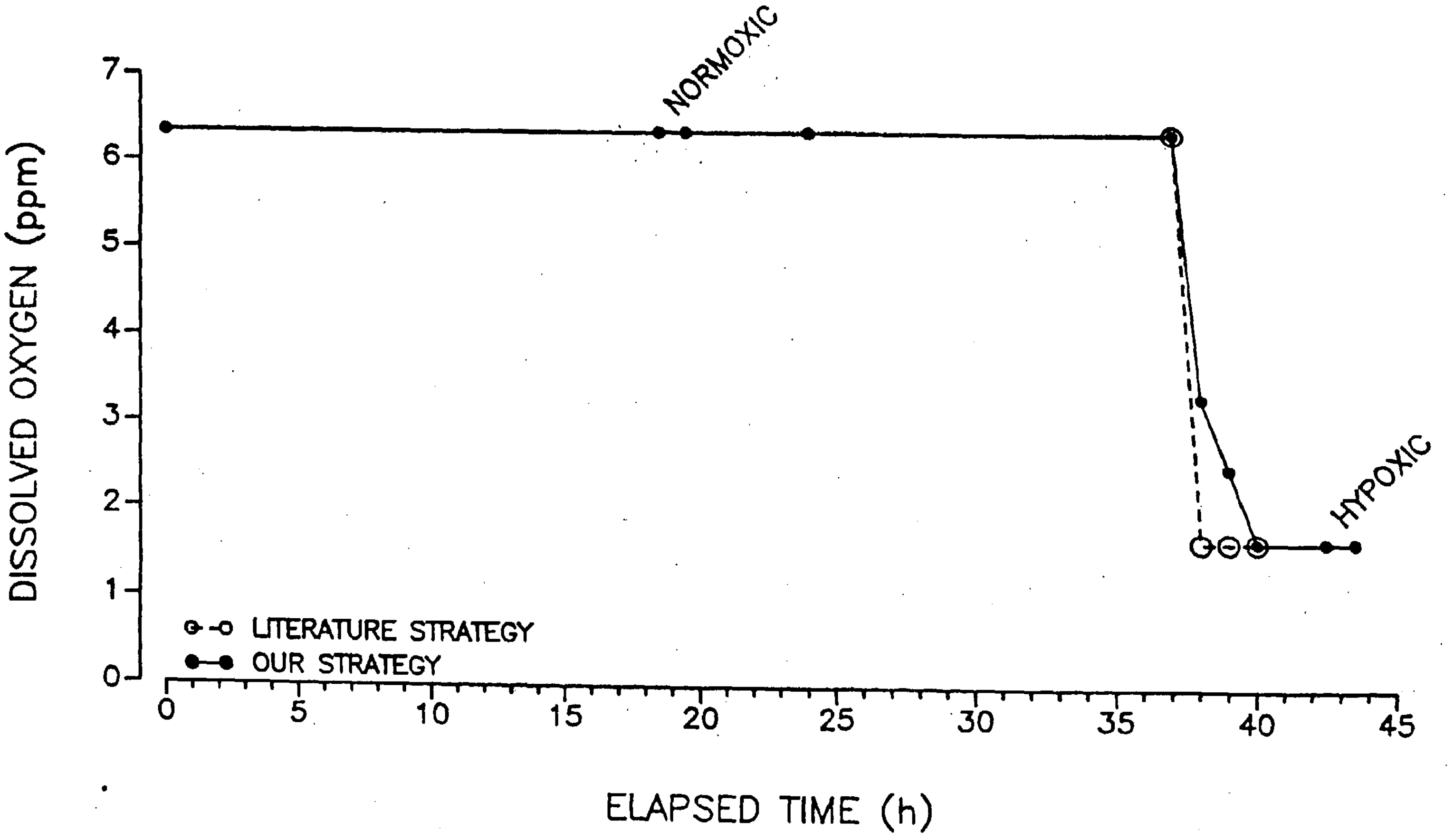

INFLOWING WATER
PUMP
TABLE TOP
WATER SAMPLING CHAMBERS
CONTROL
WATER MANIFOLD
BAFFLES
RESPIROMETER
MAGNETIC STIRRER
RESPIROMETER
RESPIROMETER
HEATER
RESPIROMETER DIVIDER

Figure 2.


NORMOXIC
HYPOXIC
DISSOLVED OXYGEN (ppm)
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
35
40
45
ELAPSED TIME (h)
LITERATURE STRATEGY
OUR STRATEGY

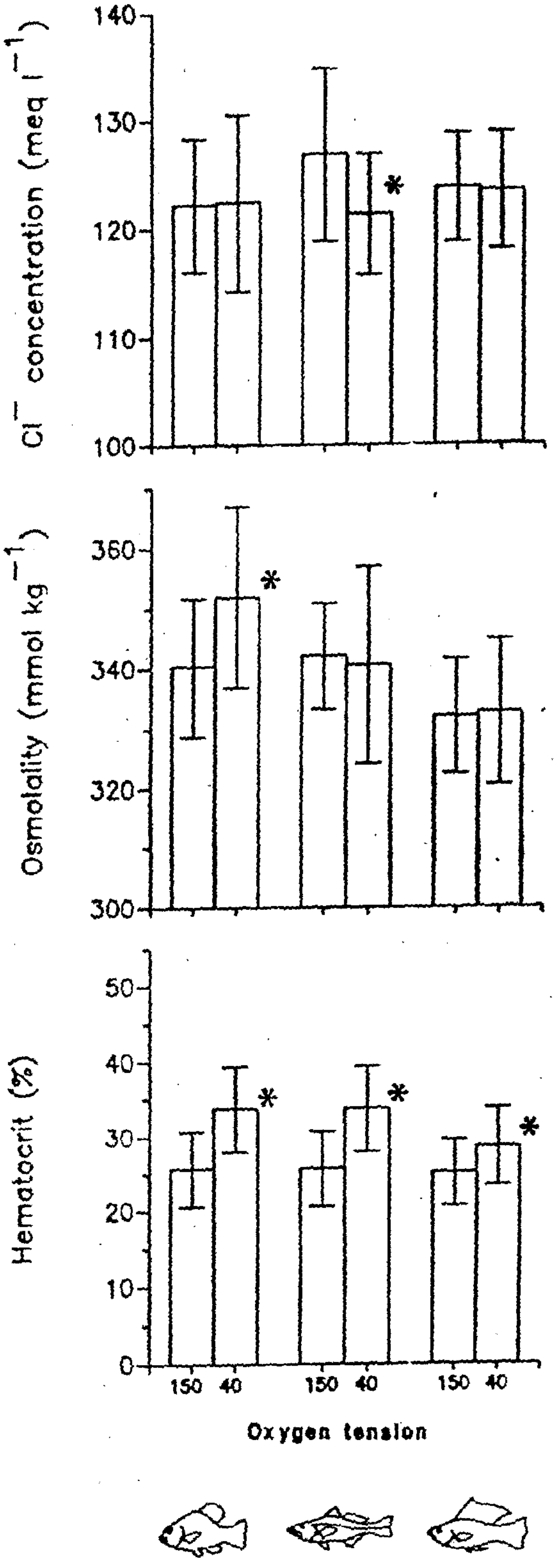

Cl⁻ concentration (meq l⁻¹)
140
130
120
110
100
*
Osmolality (mmol kg⁻¹)
360
340
320
300
*
Hematocrit (%)
50
40
30
20
10
0
*
*
*
150
40
150
40
150
40
Oxygen tension

Figure 4.

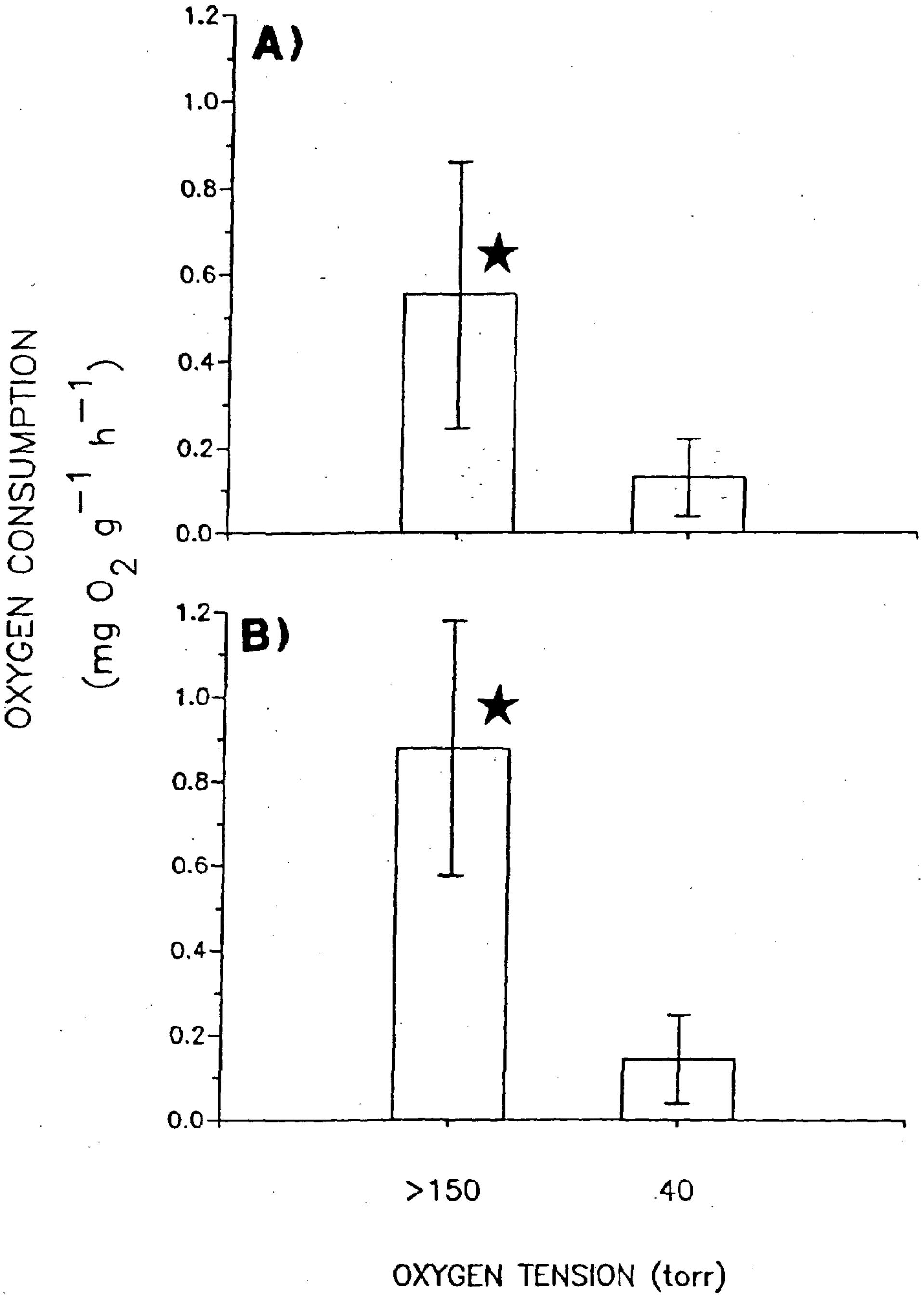

A)
B)
OXYGEN CONSUMPTION
(mg O_2 g^{-1} h^{-1})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
★
>150
40
OXYGEN TENSION (torr)

Figure 5.

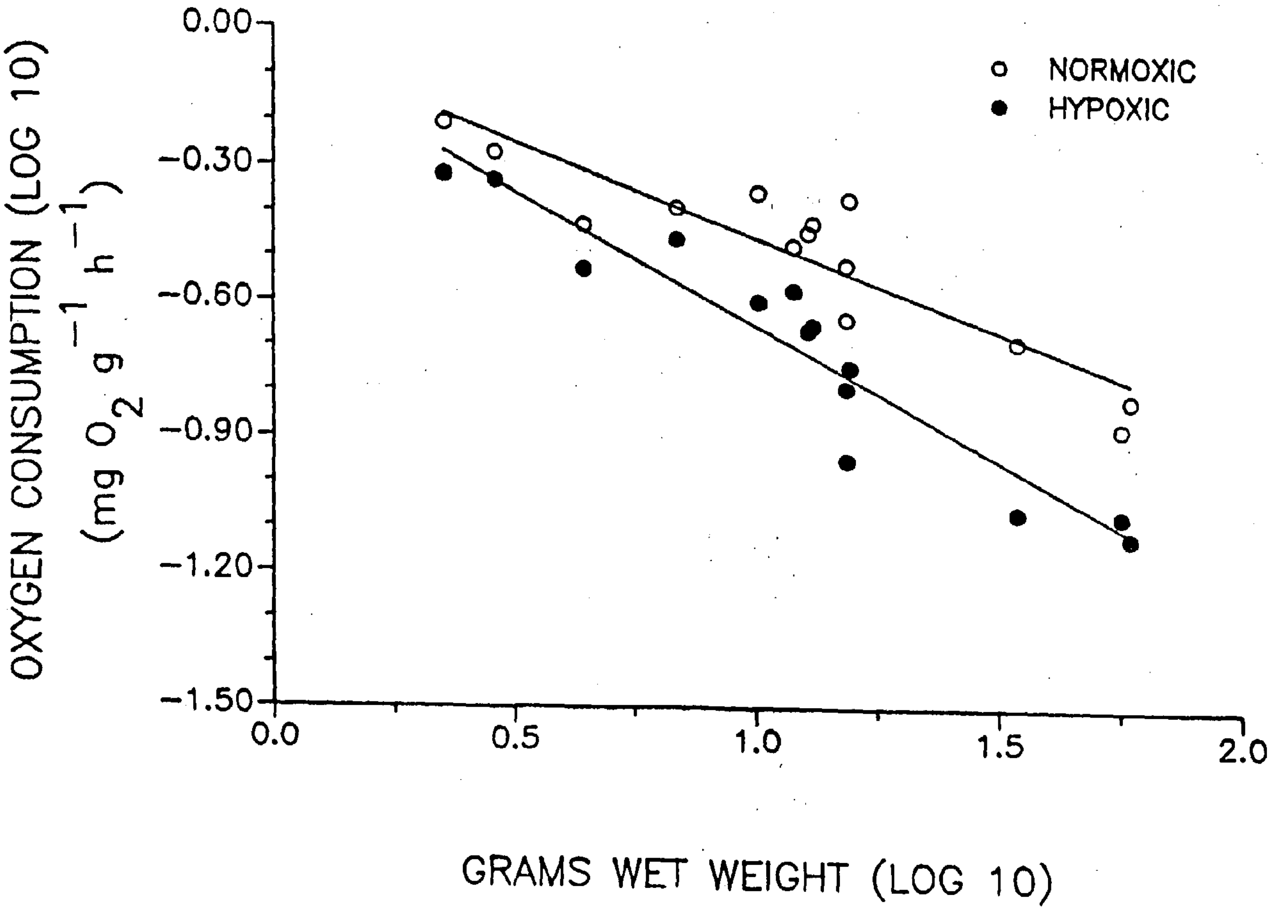
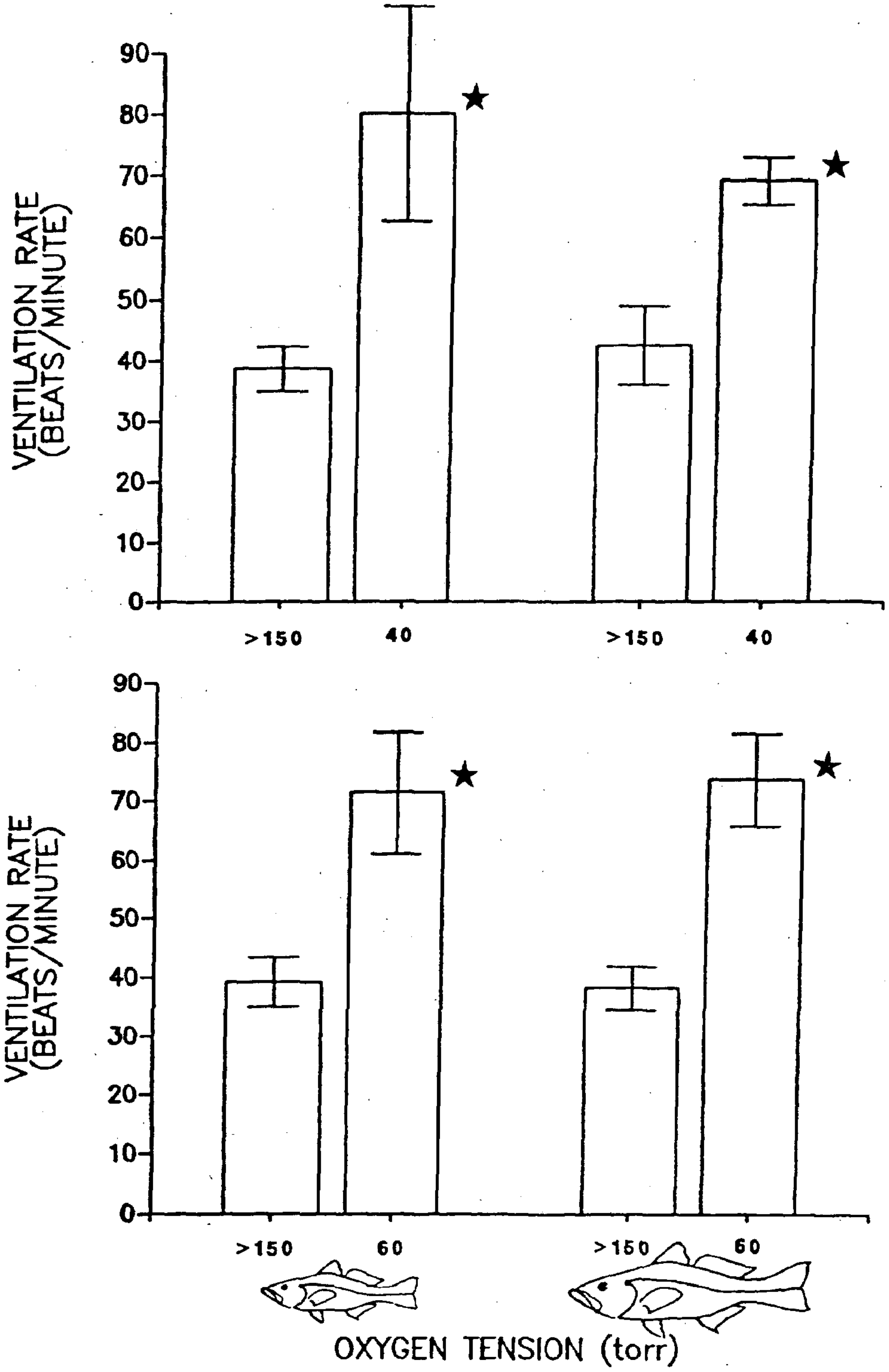
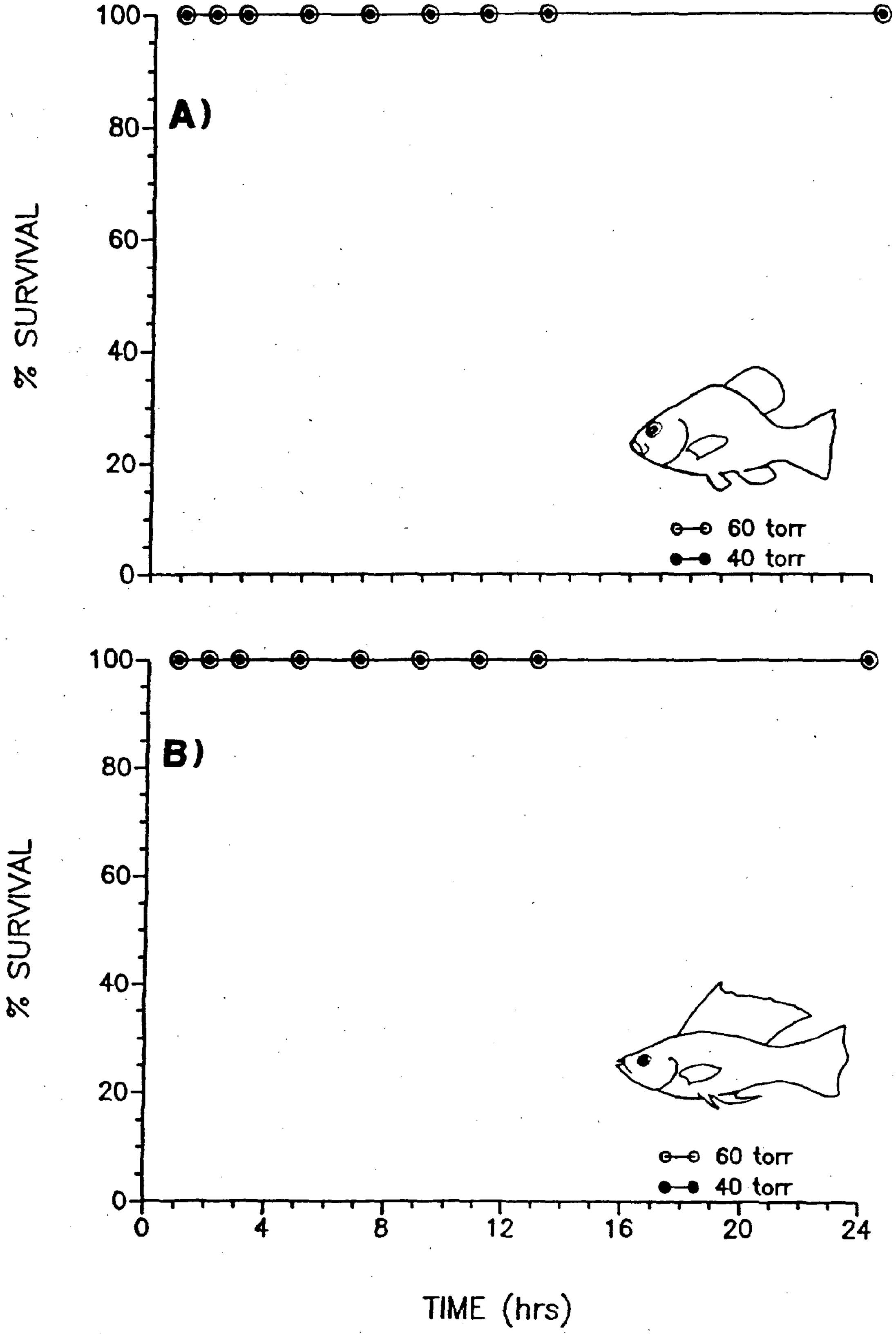

OXYGEN CONSUMPTION (LOG 10)
(mg O2 g−1 h−1)
0.00
−0.30
−0.60
−0.90
−1.20
−1.50
0.0
0.5
1.0
1.5
2.0
GRAMS WET WEIGHT (LOG 10)
○ NORMOXIC
● HYPOXIC

Figure 6.



VENTILATION RATE (BEATS/MINUTE)
0
10
20
30
40
50
60
70
80
90
★
★
>150
40
>150
40
VENTILATION RATE (BEATS/MINUTE)
0
10
20
30
40
50
60
70
80
90
★
★
>150
60
>150
60
OXYGEN TENSION (torr)

Figure 7.



100
80
60
40
20
0
% SURVIVAL
A)
60 torr
40 torr
B)
60 torr
40 torr
0
4
8
12
16
20
24
TIME (hrs)

Figure 8.

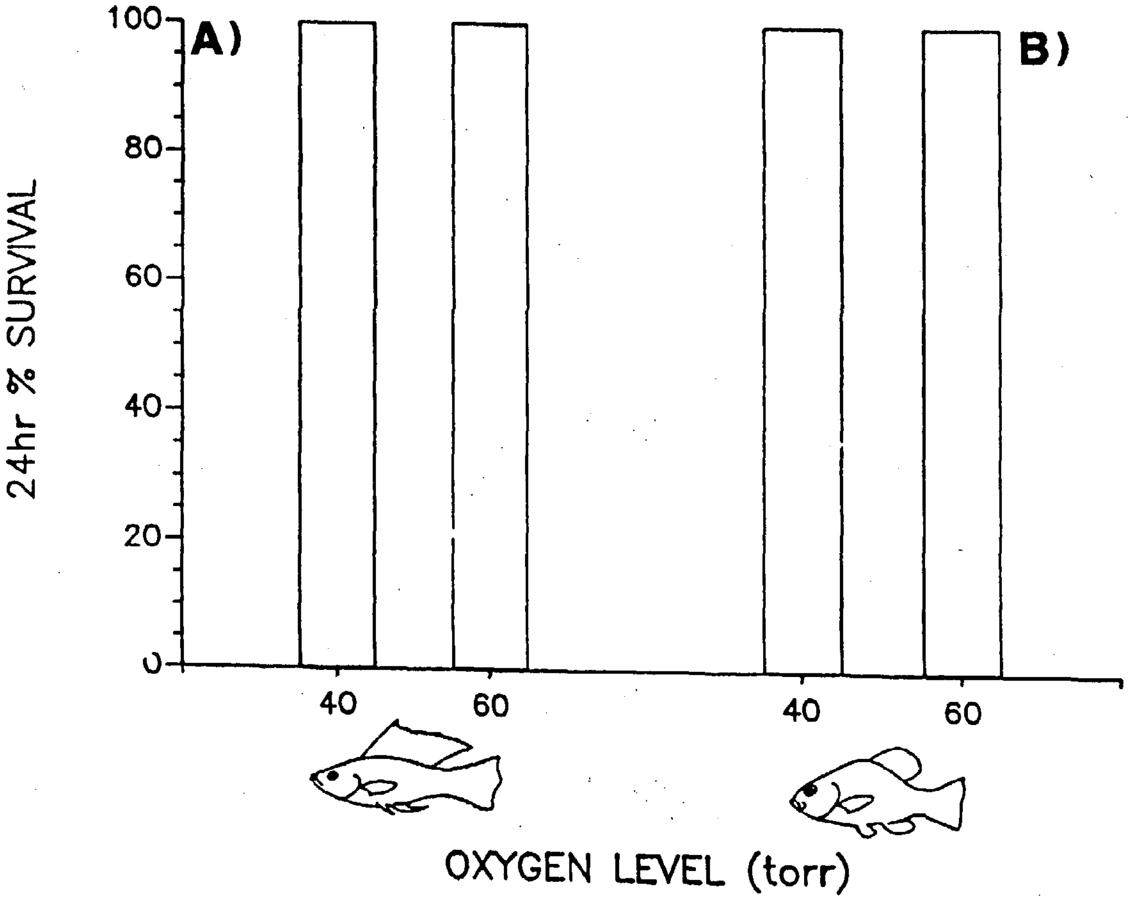


Figure 9.

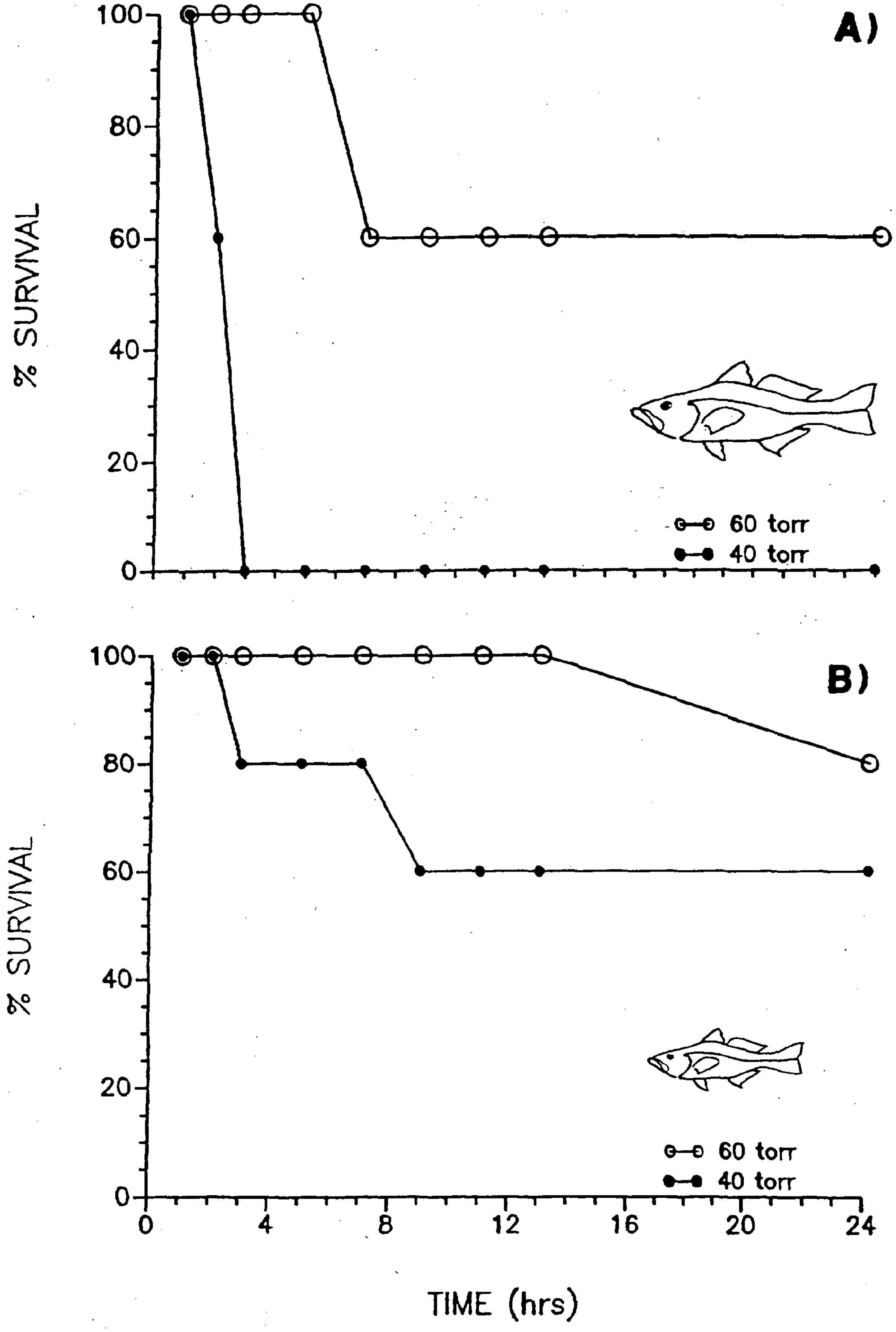

A)
B)
% SURVIVAL
% SURVIVAL
100
80
60
40
20
0
100
80
60
40
20
0
0
4
8
12
16
20
24
TIME (hrs)
60 torr
40 torr
60 torr
40 torr

Figure 10.

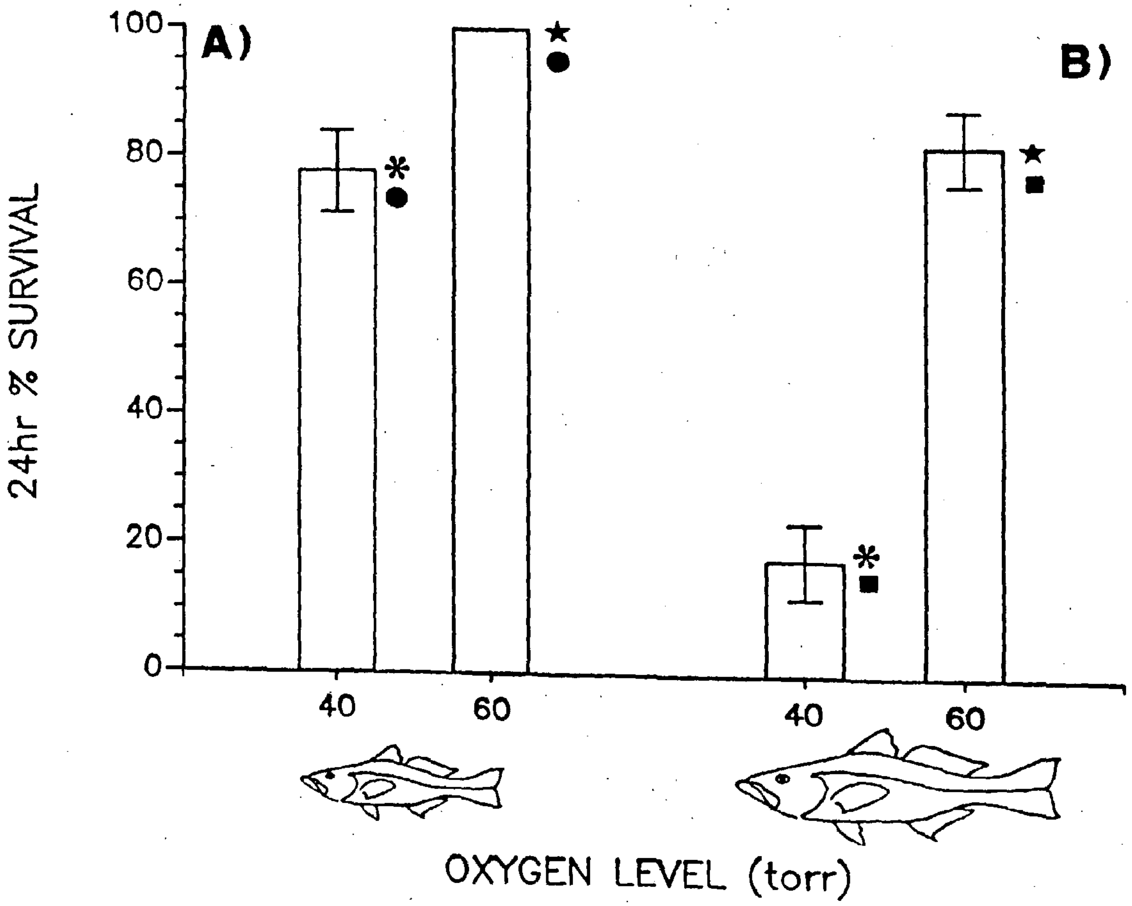


Figure 11.

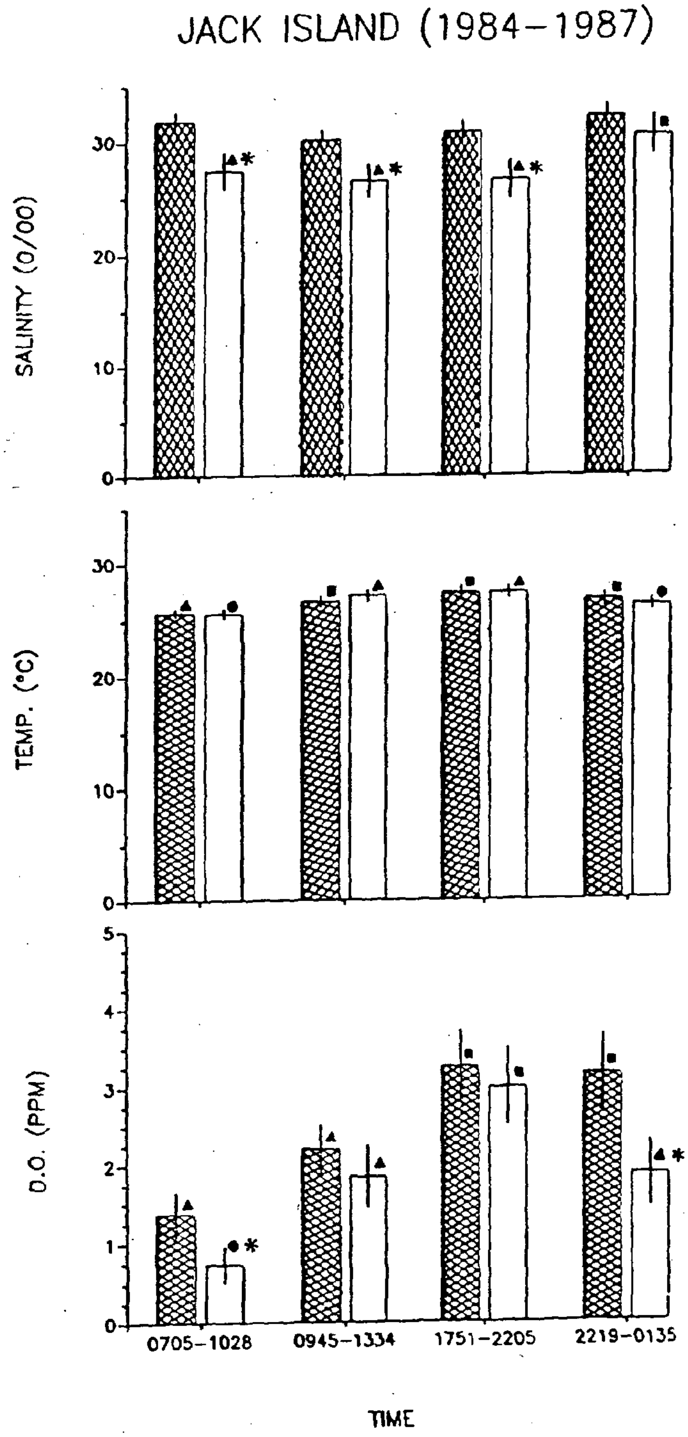
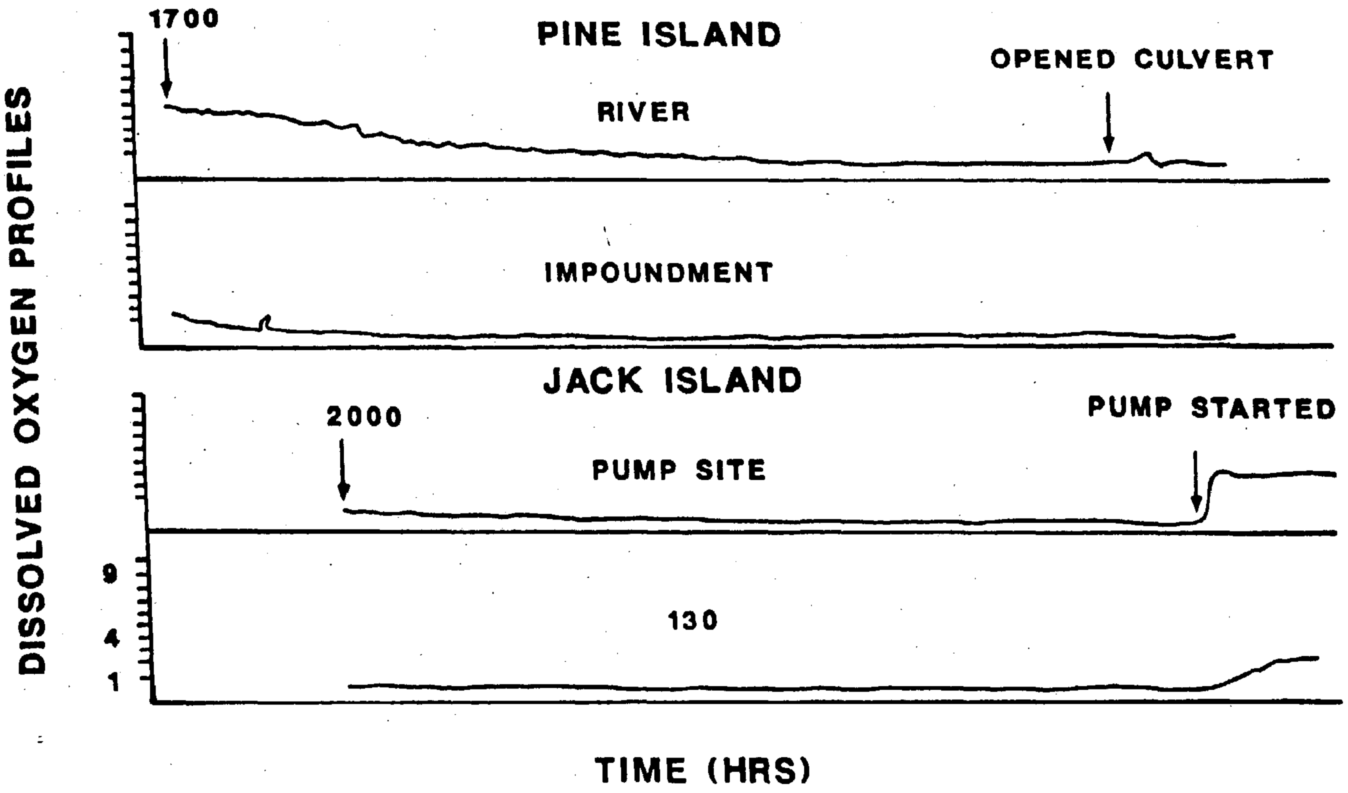

JACK ISLAND (1984–1987)
SALINITY (0/00)
30
20
10
0
TEMP. (°C)
30
20
10
0
D.O. (PPM)
5
4
3
2
1
0
0705–1028
0945–1334
1751–2205
2219–0135
TIME

Figure 12.


DISSOLVED OXYGEN PROFILES
PINE ISLAND
1700
RIVER
OPENED CULVERT
IMPOUNDMENT
JACK ISLAND
2000
PUMP SITE
PUMP STARTED
9
4
1
130
TIME (HRS)

MOSQUITO PRODUCTION IN A ROTATIONALLY MANAGED IMPOUNDMENT COMPARED TO OTHER MANAGEMENT TECHNIQUES[1]

DOUGLAS B. CARLSON AND PETER D. O'BRYAN

Indian River Mosquito Control District, P.O. Box 670, Vero Beach, FL 32961-0670

ABSTRACT. Mosquito production was monitored by larval dipping for 12 months in a 20.2 ha central east coast Florida salt marsh impoundment which was being managed under a rotational impoundment management (RIM) regime. This regime, implemented to provide mosquito control while retaining natural resource benefits, virtually eliminated salt-marsh *Aedes* mosquito production from late May through September when the marsh was closed to the estuary and flooded to approximately 1.0 ft NGVD. *Anopheles* spp. were collected only along the upland marsh edges in relatively low densities. Compared with the management methods of: 1) open to the estuary with culverts and, 2) passive retention of water with flapgate risers, RIM proved to be significantly more effective in reducing mosquito production.

INTRODUCTION

Along the central east coast of Florida, impoundments were constructed in the 1950s and 1960s by isolating high salt marshes from the estuary with earthen dikes. The marsh surface is flooded by pumping water from the adjacent estuary during the mosquito producing season (approximately May to October), to deny ovipositional opportunities for the salt-marsh mosquitoes *Aedes taeniorhynchus* (Wiedemann) and *Ae. sollicitans* (Walker) (Provost 1977).

While this source reduction method is both effective and economical for salt-marsh mosquito control (Clements and Rogers 1964), it interrupts the historic exchange of organisms and detritus between the marsh and estuary. Excessive flooding may also stress or kill existing high marsh vegetation (Gilmore et al. 1982). In the early 1980s this conflict of marsh management objectives was addressed by the formation of the Subcommittee on Managed Marshes, a subcommitte of the Florida Coordinating Council on Mosquito Control, to serve as a forum to mediate the differing management interests in impoundments (Carlson and Carroll 1985). Currently several impoundment management methods are in use along the Indian River Lagoon. They include year-round flooding by pumping, seasonal flooding, no flooding, open with breached dikes and utilization for wastewater retention (Carlson 1983).

The Subcommittee has stressed management decisions based on local research findings. The impoundment management technique most favorably viewed by the Subcommittee for the objectives of natural resource enhancement while maintaining source reduction benefits is a rotational impoundment management (RIM) technique. Culverts with flapgate risers are installed through the impoundment dike to seasonally reconnect the marsh with the estuary. The culverts are closed in the late spring and the marsh is kept flooded by periodic pumping of estuarine water until the early fall. At this time the culverts are opened and the high autumnal tides cause daily water level fluctuations while still maintaining marsh inundation (Carlson and Carroll 1985).

The quantitative verification of mosquito production from impoundments using different management techniques is of interest to all marsh managers. Clements and Rogers (1964) demonstrated how larval *Aedes* densities varied under different techniques for impoundments not connected to the estuary. However, with the current trend to reconnect impoundments to the estuary, mosquito production information on impoundments reintegrated in differing ways is important. Carlson and Vigliano (1985) demonstrated the explosive salt-marsh mosquito production possible from an impoundment; 1) open to the estuary with first one, 2) then two culverts,[2] and 3) the failure of passive water retention with flapgate risers to provide adequate control. The study reported here compared mosquito production in those previously published studies with RIM, currently the impoundment management method most favored

---

[1] This research was one part of a cooperative project with R. G. Gilmore (Harbor Branch Oceanographic Institution, Inc.) and J. R. Rey (Florida Medical Entomology Laboratory), partially funded by the Florida Department of Environmental Regulation (DER) and by the Coastal Zone Management (CZM) Act of 1972, as amended, administered by the Office of Coastal Zone Management/National Oceanographic and Atmospheric Administration.

[2] D. B. Carlson, P. D. O'Bryan and R. R. Vigliano. 1986. Impoundment management, mosquito sampling section. Final report to the Florida Department of Environmental Regulation/Office of Coastal Zone Management (CM 93). 19 p.

by salt marsh managers in this area when trying to balance salt marsh resource interests and mosquito control, while minimizing the use of pesticides.

MATERIALS AND METHODS

Study site selection. Indian River Impoundment No. 12,[3] constructed in 1966 and located on the barrier island at the Indian River—St. Lucie County border, served as the study site. This 20.2 ha (50 acre) impoundment contains an interior perimeter ditch along two and one-half of the four impoundment sides. The eastern edge is not ditched and gently slopes to upland. The Indian River Lagoon, an estuarine lagoon, borders the western impoundment side (Fig. 1). Two 18 in (45.7 cm) diameter culverts had been placed through the dike permitting management flexibility in connecting the impounded marsh with the estuary.

Vegetation of the marsh surface is primarily *Batis maritima* Linn. (saltwort), *Salicornia virginica* Linn. (perennial glasswort), and *S. bigelovii* Torr. (annual glasswort) with scattered *Avicennia germinans* (Linn.) (black mangrove), *Rhizophora mangle* Linn. (red mangrove) and *Laquncularia racemosa* Gaertn. (white mangrove). There are many open areas and ponds, some of which retain water all year. Marsh surface elevations (excluding ponds and the perimeter ditch) range from −0.35 to 1.80 ft. (−0.11 to 0.55 m) NGVD[4] with the majority of the elevations between 0.40–0.90 ft. (0.12–0.27 m) NGVD (Carlson and Vigliano 1985). Prior to culvert closure and estuarine pumping, the mosquito larvicides Altosid® (methoprene) or a diesel fuel—spreader mix were applied when needed.

Sampling methodology. The immature (larvae and pupae) mosquito sampling technique used by Carlson and Vigliano (1985) was employed during this continuation of Florida DER/CZM funded impoundment research. Because pre-adult salt-marsh mosquitoes are non-randomly distributed (Nielsen and Nielsen 1953), random sampling can greatly misrepresent brood occurrence and size. Therefore, stratified sampling (Southwood 1978) similar to Zimmerman and Turner (1982) was used. A brood is defined as immature mosquitoes in a quadrat which hatch and mature concurrently. Since under local summer conditions salt-marsh mosquito larval development progresses at a rate of an instar per day, the date of brood hatch can be accurately determined.

For sampling purposes, the entire marsh surface was divided into 12 quadrats (Fig. 2). These unequally sized sampling areas were designated North A,B,C, West A,B,C, South A,B,C and East A,B,C. On each twice weekly sampling visit from October 1, 1985 through September 30, 1986, immature mosquitoes were sought out in all quadrats. No areas were neglected but through experience those vegetated areas known to produce mosquitoes were most thoroughly examined. When immature mosquitoes were found, five 350 ml dips per quadrat were taken and the individuals were counted in the field. Rainfall was collected at each site visit using a tube rain gauge located at the northeast marsh corner. Water level measurements were determined using a staff gauge.

The following water management timetable was used:

1. October 1, 1985: Both culverts open to free flow of water between the impoundment and estuary.
2. May 16–19, 1986: Flapgates and riser boards placed in both culverts established the flooding elevation at 1.0 ft. NGVD which trapped tidal water that entered the impoundment during the previous week.
3. May 28, 29 and June 4, 1986: Water pumped into the impoundment with a 6,000 gallons per minute (gpm) portable diesel pump for a total of approximately 16 hours to establish and maintain the desired flooding elevation.
4. September 16, 1986: Flapgate risers from both culverts removed to allow free ebb and flow of water between the impounded marsh and estuary.

RESULTS

A summary of mosquito production during the 12 month period of RIM management follows. For descriptive purposes, the study was broken into convenient periods for considering management and/or climatological effects. Table 1 provides a detailed account of mosquito production throughout the year.

First period: October 1–December 15, 1985. During the first month and a half of this 2.5 month period when the impoundment was open to the estuary through both culverts, the marsh remained almost continuously flooded due to the annual high fall tides and heavy rainfall. During this period, low numbers of *Anopheles* mosqui-

---

[3] W. L. Bidlingmayer and E. D. McCoy. 1978. An inventory of the salt-marsh mosquito control impoundments in Florida. Unpublished report to Fish and Wildlife Service, U.S. Dept. of Interior. 103 p.

[4] National Geodetic Vertical Datum, Vertical Control Data by the National Geodetic Survey, sea-level datum of 1929, U.S. Department of Commerce, National Oceanic and Atmospheric Administration.

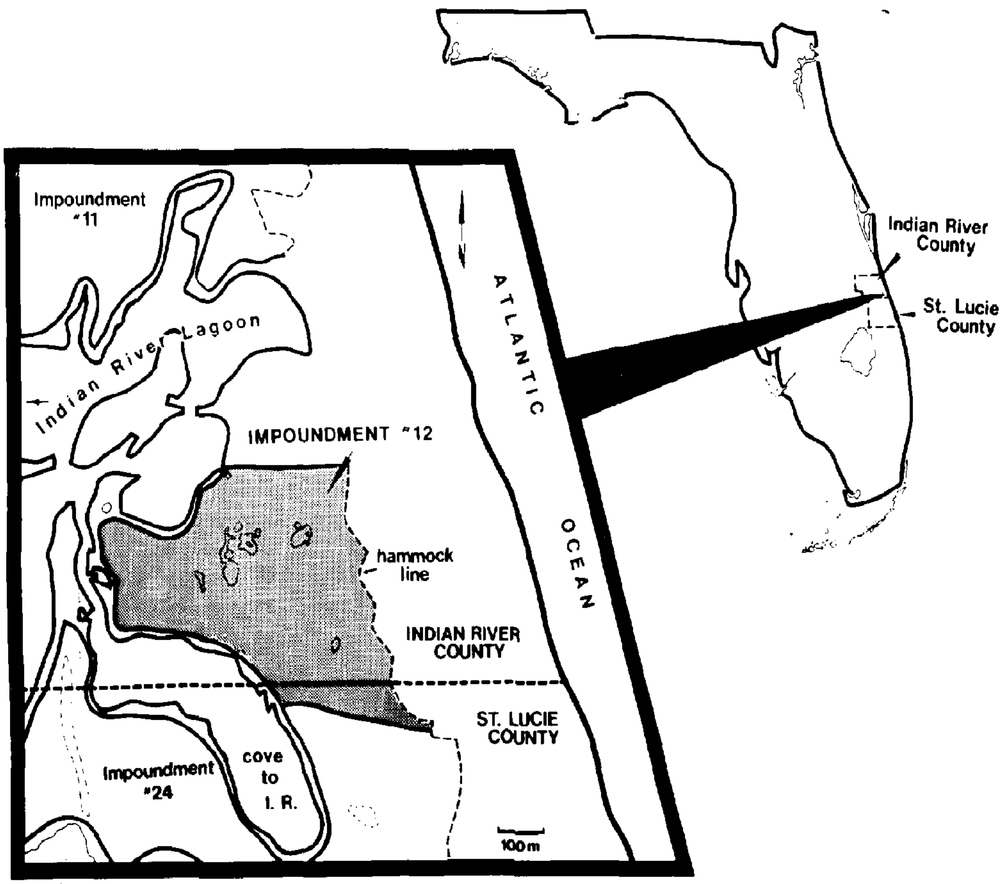


Fig. 1. Location of Indian River Impoundment #12.

toes ($\bar{X}$/dip = 0.6) in the North and East quadrats were common. In mid-November, flooding elevations dropped below 1.0 ft. NVGD exposing portions of the marsh surface. Subsequent rain in December hatched large *Aedes* broods in North A and East C. Rainfall for the period was 39.9 cm.

Second period: December 16, 1985—March 15, 1986. Rainfall hatched several *Aedes* broods in East C during the second period but the sites usually dried too rapidly to permit adult emergence. Observed water levels fluctuated greatly with especially low levels during the first two weeks of February (approx. 0.0–0.1 ft. NGVD). Rainfall was 11.7 cm.

Third period: March 16–June 15, 1986. Tidal inundation of the marsh contributed to mosquito broods produced during this period. In mid-May, the two culverts were closed with flapgate risers, trapping water which had entered the marsh on the May spring tide. Pumping of Indian River Lagoon water into the impoundment with a portable diesel-driven 6,000 gpm pump was necessary for only 16 pumping hours and resulted in just one brood from the initial pumping. This raised the flooding level to the desired elevation of slightly over 1.0 ft. NGVD. Rainfall during this period was 21.8 cm.

Fourth period: June 16—September 30, 1986. Because frequent rainfall during this 3.5 month period kept the impoundment flooded to the 1.0 ft. NGVD level, it was not necessary to pump water. When the expected high fall tides reached this level in early September, both flapgate risers were removed on September 16 restoring the interchange of water between marsh and estuary. As expected, because of the continuous flooding, no *Aedes* production occurred. Low numbers of anopheline mosquitoes ($\bar{X}$/dip = 1.0) were frequently collected along the East and North quadrats. Rainfall during this final period measured 49.5 cm.

DISCUSSION

Meteorological and tidal considerations. Along the central east coast of Florida, salt-marsh mosquito production can occur year-round, but typically is greatest from May through September. During the summer months, lagoonal water levels are usually low and inadequate to flood the marsh surface except for a brief rise nor-

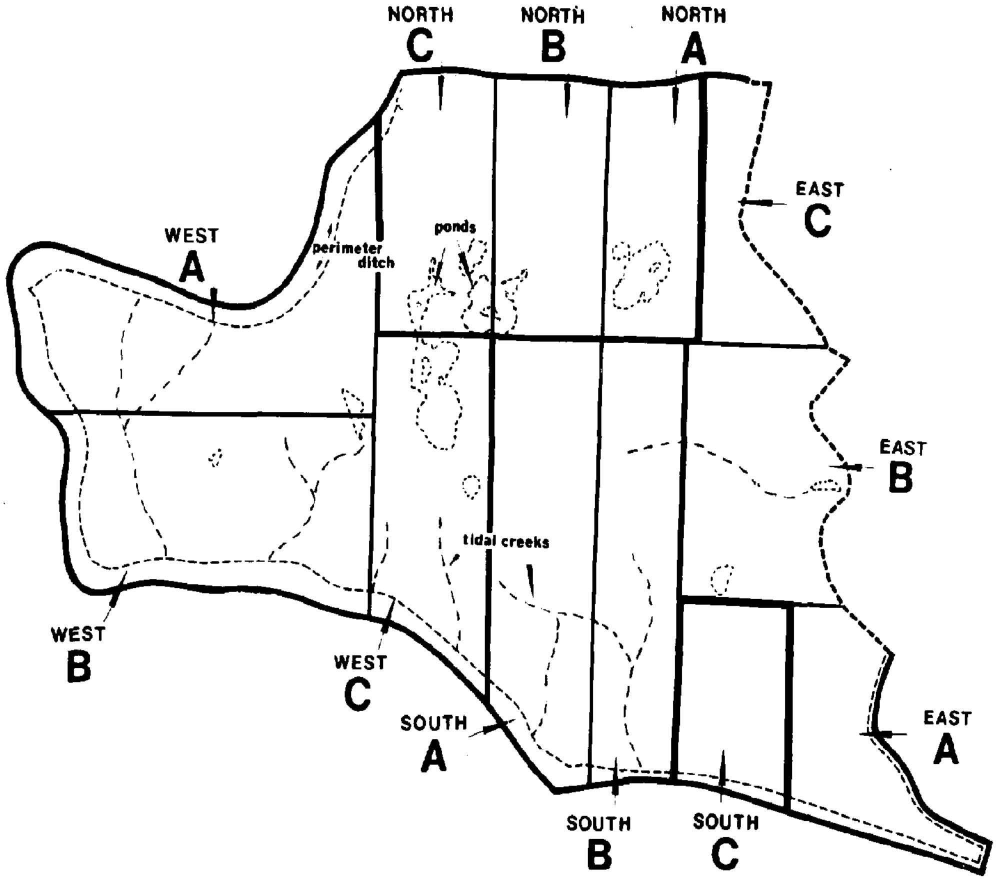


Fig. 2. Mosquito sampling quadrats in Impoundment #12.

Table 1. Mosquito production in Impoundment #12 (October 1, 1985–September 30, 1986). Broods are dated on day of hatching and expressed as mean/dip.

	North			West			South			East			
Date	A	B	C	A	B	C	A	B	C	A	B	C	Hatching stimulus
FIRST PERIOD													
1985: October 1—Water management regime: impoundment open to estuary through both culverts.													
December 7	66.6											351.4	R
SECOND PERIOD													
1986: January 1												78.0	R
January 17												124.2	R
February 9												15.6	R
March 1												3.4	R
THIRD PERIOD													
March 27	4.3	2.3	7.5	25.7		20.8				1.9		117.4	B
May 10												39.0	B
May 16—Water management regime: flapgates installed both culverts.													
May 19—Water management regime: riser boards installed both culverts.													
May 23										1.2		635.8	T
May 28, 29—Water management regime: estuarine pumping (9 hours).													
May 29										9.0			P
June 4—Water management regime: estuarine pumping (7 hours).													
FOURTH PERIOD													
Sept. 16—Water management regime: flapgates removed both culverts.													

R = rainfall; T = tides; B = both; P = pumping.

mally occurring in June. The high fall water levels, those capable of sustained flooding of the high marsh usually do not begin until mid-September. Therefore, the artificial flooding of impoundments to prevent salt-marsh mosquito oviposition is primarily targeted for the months when tides are low and most broods are produced by rainfall (Carlson et al. 1985).

The Kolmogorov-Smirnov two sample test was used to compare rainfall amounts and frequency during the spring through summer period of the four study years. This statistical analysis demonstrated that there were no significant distributional differences in rainfall between the RIM study year and each of the three other management technique years.

Because in mid-May we were able to trap high tides of approximately 0.8 ft. NGVD with the flapgate risers, little pumping was necessary to reach the desired flooding elevation of 1.0 ft. NGVD necessary on the initial pumping (May 28–29, 1986) to inundate all mosquito producing areas. During the remainder of the study, rainfall and tides maintained the desired level limiting the need for additional pumping to only June 4.

Mosquito production comparisons. From October through April of all four study years, Impoundment No. 12 remained open to the estuary. The important May through September period was when management technique differences occurred and thus is the period chosen for mosquito production comparisons.

Although Clements and Rogers (1964) demonstrated that flooded impoundments without a connection to the estuary effectively controlled salt-marsh mosquitoes, Impoundment No. 12 differed from theirs in possessing culverts and in that the eastern side of the impoundment lacks a dike or perimeter ditch. Because of the gentle slope, we anticipated that even small water level fluctuations might produce salt-marsh mosquitoes along the upland edge.

A 2 × 2 contingency table analysis demonstrated that during May through September, significantly less mosquito production occurred during RIM as compared to the impoundment management techniques of: A) open to the estuary with one culvert (Chi square = 34.75, $P < 0.001$), B) 2 culverts (Chi square = 33.86, $P < 0.001$) or C) passive retention of water with flapgate risers (Chi square = 7.23, $P < 0.01$). We have demonstrated earlier in the paper that no significant rainfall differences occurred between years. Therefore, the lack of mosquito production during RIM can be attributed to this management technique (Table 2).

Seventeen mosquito broods occurred during the study prior to impoundment closure as a result of rainfall and/or tidal flooding. Most were found in the same locations reported by Carlson and Vigliano (1985) when the dominance of *Ae. taeniorhynchus* (82.7%) over *Ae. sollicitans* (17.3%) was demonstrated. One small brood was produced in East A by pumping (Table 1). Interestingly, water level fluctuations were minimal during the closure period and *Aedes* production was not observed in the impoundment. Prior to the study, we anticipated water level fluctuations along the sloping upland edge might produce mosquitoes there.

No two marshes are completely alike making the use of a true "control" in a study such as this impossible. However, Impoundment No. 11[3] (a non-pumped impoundment adjacent to Impoundment No. 12) served as a good comparison marsh in that historically it has usually required larviciding whenever Impoundment No. 12 did. From late May through September of this study when Impoundment No. 12 was flooded, field inspection verified the need for five aerial larviciding applications of Impoundment No. 11. This provides further indication of the effectiveness of RIM in preventing mosquito production and limiting the need for chemical treatment. Another cost benefit of a fully flooded impoundment is that larval inspection time is greatly reduced there.

Table 2. Salt-marsh mosquito production in Indian River Impoundment #12 under different management regimes (May 1–September 30).

Management regime	Rainfall (cm.)	Number of mosquito broods	Brood size	
			Range of means	Overall mean
Open with 1 culvert[a] (1982)	66.8	41	0.2–150.2	27.6
Passive retention[a] (1983)	61.47	14	1.6–349.0	66.5
Open with 2 culverts[b] (1985)	69.34	43	0.2–1290.0	115.0
RIM (1986)	58.17	4	1.2–635.8	171.3

[a] Data from Carlson, D.B. and R.R. Vigliano. 1985. The effects of two different water management regimes of flooding and mosquito production in a salt marsh impoundment. J. Am. Mosq. Control Assoc. 1:203–211.

[b] Data from D.B. Carlson, P.D. O'Bryan and R.R. Vigliano. 1986. Impoundment Management, Mosquito Sampling Section. Final report to the Florida Department of Environmental Regulation/Office of Coastal Zone Management (CM 93). 19 p.

Carlson and Vigliano (1985) demonstrated an *Anopheles* ratio of *An. bradleyi* King (55.8%), *An. atropos* Dyar and Knab (40.4%) and *An. walkerii* Theobald (3.8%) occurred in Impoundment No. 12. Even though *Aedes* production was virtually eliminated during the closed-pumped period of the current study, anopheline production along the North and East quadrats averaged 1.1/dip. The observed low level of *Anopheles* production at Impoundment No. 12 is not considered sufficient to require treatment.

SUMMARY

Year to year variability in mosquito production from salt marshes can be great and the ability to maintain an adequate flooding level varies from one impoundment to the next depending on pumping capabilities, weather patterns, and soil conditions. Carlson and Vigliano (1985) demonstrated that passive retention of water was superior to an open culvert situation in controlling salt-marsh mosquitoes in Impoundment No. 12 but still allowed considerable mosquito production along the upland edge. This study shows that during the closed-pumped period of RIM, *Aedes* spp. production was virtually eliminated from the entire study site verifying the superior effectiveness of this management technique if adequate water levels can be maintained. However, additional fine-tuning of this method is still necessary to better evaluate the numerous natural resource implications of impoundment management.

ACKNOWLEDGMENTS

The authors appreciate the review comments of Mr. E. J. Beidler, Mr. G. A. Curtis and Dr. G. F. O'Meara. The assistance of Mr. G. A. Curtis with statistical analysis is also gratefully acknowledged.

REFERENCES CITED

Carlson, D. B. 1983. The use of salt-marsh mosquito control impoundments as wastewater retention areas. Mosq. News 43:1–6.

Carlson, D. B. and J. D. Carroll, Jr. 1985. Developing and implementing impoundment management methods benefiting mosquito control, fish and wildlife: a two year progress report about the Technical Subcommittee on Mosquito Impoundments. J. Fla. Anti-Mosq. Assoc. 56:24–32.

Carlson, D. B. and R. R. Vigliano. 1985. The effects of two different water management regimes on flooding and mosquito production in a salt marsh impoundment. J. Am. Mosq. Control Assoc. 1:203–211.

Carlson, D. B., R. G. Gilmore and J. R. Rey. 1985. Salt marsh impoundment management on Florida's central east coast: reintegrating isolated high marshes to the estuary. Proc. 12th Annu. Conf. Wetlands Restoration Creation. p. 47–63.

Clements, B. W. and A. J. Rogers. 1964. Studies of impounding for the control of salt-marsh mosquitoes in Florida, 1958–1963. Mosq. News 24:265–276.

Gilmore, R. G., D. W. Cooke and C. J. Donohoe. 1982. A comparison of the fish populations and habitat in open and closed salt-marsh impoundments in east-central Florida. Northeast Gulf Sci. 5:25–37.

Nielsen, E. T. and A. T. Nielsen. 1953. Field observations on the habits of *Aedes taeniorhynchus*. Ecology 34:141–156.

Provost, M. W. 1977. Source reduction in salt-marsh mosquito control: Past and future. Mosq. News 37:689–698.

Southwood, T. R. E. 1978. Ecological methods. John Wiley and Sons, New York, 524 p.

Zimmerman, R. H. and E. C. Turner, Jr. 1982. Mosquito distribution and abundance in an inland salt marsh, Saltville, Virginia. Mosq. News 42:105–109.

J. Exp. Mar. Biol. Ecol., 1988, Vol. 121, pp. 73–78
Elsevier

JEM 01121

Hematocrit, osmolality, and ion concentration in fishes: consideration of circadian patterns in the experimental design

Mark S. Peterson and R. Grant Gilmore, Jr.
Harbor Branch Oceanographic Institution, Ft. Pierce, Florida, U.S.A.

(Received 21 March 1988; revision received 16 May 1988; accepted 25 May 1988)

Abstract: Significant circadian patterns in plasma osmolality and chloride ion concentration were documented in the sailfin molly *Poecilia latipinna* (Baird & Girard) and the common snook *Centropomus undecimalis* Bloch; however, significant patterns were not found in the sheepshead minnow *Cyprinodon variegatus* Lacepede. There were, however, no distinct patterns in hematocrit for any of the three species. Circadian patterns, although important in ion and osmoregulation studies are usually not considered. Furthermore, the time of day the fishes are tested are typically not mentioned. Questionable results and difficulty in making comparisons with previously published data can occur if the patterns are present and not considered in the experimental design.

Key words: Chloride; Circadian; Euryhaline; Hematocrit; Osmolality

INTRODUCTION

Ion and osmoregulation studies of fishes have typically been designed to search for patterns which aid in understanding habitat use or compare responses in different environmental situations. These data, in conjunction with oxygen consumption data, can lead to an understanding of the energetics of habitat use patterns.

The majority of these studies, however, usually do not state the time of day their determinations were made and thus overlook the importance of circadian cycles in their experimental design (e.g., Valentine & Miller, 1969; Barton, 1979; Nordlie, 1985, 1987). Furthermore, comparison of their data with other published data from confamilial species may yield distinct differences or similarities based solely on the time of day the samples were taken.

Studies on diurnal and circadian cycles or rhythms in osmolality, ion or hematocrit concentrations are few. To date, studies document significantly elevated values in sodium, hematocrit (Hannah & Pickford, 1981) and osmolality (Bulger, 1986) between 1400–1800 in *Fundulus heteroclitus*. Moreover, daily chloride cycles for *F. grandis* and

---

Contribution 632 of the Harbor Branch Oceanographic Institution.

Correspondence address: M. S. Peterson, Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Ft. Pierce, FL 34946, U.S.A.

F. chrysotus have also been documented (Meier *et al.*, 1973; Spence *et al.*, 1977) but these cycles do not occur for the freshwater channel catfish *Ictalurus punctatus* and *I. punctatus* × *I. furcatus* hybrids (Davis & Simco, 1976).

The purpose of this paper is two-fold: (1) to determine if there are circadian patterns in hematocrit, osmolality and chloride concentrations in three euryhaline fishes: the sheepshead minnow *Cyprinodon variegatus* Lacepede; the sailfin molly *Poecilia latipinna* (Baird *et* Girard); and the common snook *Centropomus undecimalis* (Bloch); and (2) to discuss the importance of these patterns in documenting secondary stress responses to experimental manipulation. We do not purport to determine the functional importance of these patterns (see Bulger, 1986) but wish to establish their importance when designing experiments to examine these parameters in different treatments or when comparing the values with other published data.

MATERIALS AND METHODS

We collected fishes from impounded mangrove marshes in the Indian River Lagoon, Florida, U.S.A., and tested them between 7 December 1987 and 21 January 1988. Fishes were transported to the laboratory in styrofoam coolers containing impoundment water where they were held in their ambient environmental temperatures (19–24 °C) overnight under aeration. The laboratory temperature was 25 ± 2 °C and the holding water always increased to that temperature. The fish were then transferred to 76-l aquaria (at 25 ± 2 °C) equipped with individual filters, aerators and heaters. The fish were held in 30 ± 1‰, 30 ± 2 °C (increased from 25 °C over a 24-h period) and under a 12-L:12-D photoperiod centered at 1230, for 7 to 14 days prior to experimentation (normal day length during the testing periods ranged from 1029 to 1037). Light intensity at the waters surface was 0.65×10^{16} quanta $\cdot$ $s^{-1} \cdot cm^{-2}$. *Cyprinodon varigetus* and *P. latipinna* were fed flaked food twice daily, whereas *C. undecimalis* were fed live fish once daily. The sex of the fishes was not considered. Experimental salinities were produced using filtered (5 μm) Atlantic Ocean seawater diluted with aged reverse osmosis (RO) water. Salinities were checked daily using an AO refractometer.

All fish were fasted for 24 h prior to testing. Ten individuals were tested at 4-h intervals from 0400 through 2400. Fish tested at night (2000, 2400, 0400) remained in darkness until they were netted and tested under dim light (intensity at water surface $= 0.01 \times 10^{16}$ quanta $\cdot s^{-1} \cdot cm^{-2}$). Blood of all individuals was collected and centrifuged within a 30-min period, centered at one of the previously mentioned test times. Fish were netted (see results concerning potential effects of netting) and immediately measured to the nearest mm standard length (SL). Blood samples were obtained by first blotting each individual dry and severing its caudal fin with a razor (Barton, 1979). The incision was immediately blotted and blood from the caudal artery was drawn into a heparinized micro-capillary tube and centrifuged for 4 min at 11500 rpm in an International micro-capillary centrifuge (Model MB) for hematocrit (%) determination. Individual blood collections were completed within one minute to reduce handling effects on blood constituents (Chauvin & Young, 1970; Robertson *et al.*, 1987). Plasma osmolality (mmol · kg^{-1}) was then determined on a 10-μl sample with a Wescor vapor pressure osmometer (Model 5500). Chloride ion concentration (meq · l^{-1}) was determined from a 10-μl sample on a Buchler digital chloridometer (Model 4–2500). A total of 60 individual determinations per blood constituent and species (10 per time period) were made. All blood constituents were determined for each individual.

Fish size, hematocrit, osmolality and chloride ion concentration were analyzed by ANOVA ($\alpha = 0.05$) and a Student–Newman–Keuls multiple comparison test (SNK) (Klockars & Sax, 1986). These analyses were performed using the SPSSX 2.1 program package (SPSSX, 1985).

RESULTS

Individuals ranged between 24–41 mm SL for *C. variegatus*; between 32–56 mm SL for *P. latipinna*; and 54–120 mm SL for *C. undecimalis*. There were no significant differences in size for either *P. latipinna* or *C. undecimalis* (ANOVA; $P > 0.05$; Table I); however, the 2000 mean ($\bar{x} = 37.0$ mm SL) was marginally larger (ANOVA; $P = 0.052$) than the 0800 mean ($\bar{x} = 33.2$ mm SL) for *C. variegatus*. There were no significant differences (ANOVA; $P > 0.05$) in hematocrits across all time periods for all three species (Table I).

Significant circadian patterns in osmolality and chloride ion were found for *P. latipinna* and *C. undecimalis* (Table I), whereas only Cl^- showed a significant elevation for *C. variegatus* (Table I). Osmolality during the 1200 and 1600 time period was significantly elevated for *C. undecimalis* (Table I; SNK, $P < 0.05$). Values ranged from 327–407 mmol · kg^{-1}. Chloride, however, was significantly elevated only during the 1200 period and ranged from 112–191 meq · l^{-1} (Table I; SNK, $P < 0.05$). For *P. latipinna*, the 1200 osmolality value was significantly higher than the 0400 and 0800 time periods whereas the 1600 value was higher than the 0400 value (SNK; $P < 0.05$) and ranged from 301–401 mmol · kg^{-1}. The 0400 chloride value, however, was significantly lower than all other time periods except 1600 (SNK; $P < 0.05$). The chloride values ranged from 106–134 meq · l^{-1}. There were no significant differences (ANOVA; $P > 0.05$) in osmolality across all time periods for *C. variegatus*; however, the 0800 and 2000 chloride values were significantly lower than the 2400 value (Table I). Osmolality values ranged from 312 to 368 mmol · kg^{-1} whereas Cl^- values ranged from 109 to 145 meq · l^{-1}. At all time periods for all species, hematocrit, osmolality, and chloride values among the 10 individuals sampled showed no effect of sampling order and, therefore, no indication of capture stress effects.

DISCUSSION

We documented distinct circadian patterns in osmolality in *P. latipinna* and *C. undecimalis* but not in *C. variegatus*. Significant chloride concentrations were docu-

TABLE I

Statistics for parameters examined at one of six times of day ($\bar{x} \pm$ SD). Each mean value is based upon 10 individuals. Entries within a parameter having a common superscript or no superscript are not different ($P > 0.05$).

Time of day (h)	Size (mm SL)	Hematocrit (%)	Osmolality ($\text{mmol} \cdot \text{kg}^{-1}$)	Chloride ($\text{meq} \cdot \text{l}^{-1}$)
Cyprinodon variegatus				
0400	$35.0 \pm 1.6^{1,2}$	24.9 ± 7.3	333.7 ± 12.1	$125.6 \pm 4.6^{1,2}$
0800	33.2 ± 4.5^{1}	28.7 ± 3.4	340.4 ± 9.5	119.3 ± 4.7^{1}
1200	$36.0 \pm 2.0^{1,2}$	23.3 ± 4.2	337.5 ± 8.8	$125.6 \pm 3.9^{1,2}$
1600	$35.3 \pm 2.3^{1,2}$	24.3 ± 5.2	345.6 ± 16.3	$122.6 \pm 3.9^{1,2}$
2000	37.0 ± 1.5^{2}	27.2 ± 3.5	344.3 ± 5.5	118.0 ± 7.9^{1}
2400	$35.5 \pm 2.3^{1,2}$	28.3 ± 4.0	340.5 ± 13.1	127.6 ± 8.7^{2}
Poecilia latipinna				
0400	41.8 ± 7.5	26.9 ± 3.8	316.8 ± 4.1^{1}	116.1 ± 5.9^{1}
0800	43.8 ± 5.2	24.7 ± 4.4	$329.0 \pm 4.1^{1,2}$	123.1 ± 2.3^{2}
1200	42.1 ± 7.1	30.2 ± 5.0	350.9 ± 18.8^{3}	124.0 ± 3.2^{2}
1600	43.4 ± 6.5	28.5 ± 9.6	$341.6 \pm 30.4^{2,3}$	$120.5 \pm 6.1^{1,2}$
2000	44.2 ± 6.9	27.4 ± 4.6	$335.3 \pm 5.8^{1,2,3}$	124.0 ± 6.5^{2}
2400	43.9 ± 5.8	23.6 ± 3.8	$332.8 \pm 11.3^{1,2,3}$	123.8 ± 5.7^{2}
Centropomus undecimalis				
0400	75.3 ± 8.3	32.1 ± 3.5	342.2 ± 6.9^{1}	121.6 ± 5.1^{1}
0800	83.1 ± 15.9	28.6 ± 3.0	346.5 ± 9.6^{1}	135.5 ± 7.1^{1}
1200	81.1 ± 15.9	32.9 ± 2.9	382.1 ± 15.9^{3}	154.7 ± 22.7^{2}
1600	81.7 ± 24.2	30.0 ± 3.8	366.9 ± 6.5^{2}	130.7 ± 8.8^{1}
2000	74.4 ± 3.1	32.5 ± 5.8	337.8 ± 8.9^{1}	126.0 ± 5.4^{1}
2400	72.2 ± 5.4	33.1 ± 6.4	342.2 ± 8.6^{1}	124.3 ± 7.1^{1}

mented for all three species. Hematocrit, however, was not significantly different for any species. Although there were variations within the 10 individuals sampled for each time period, there was never a trend indicating secondary stress responses resulting from the 30-min period of sampling in which aquarium captures were made. The range in chloride and osmolality observed in each of these species comprised a major portion of the values reported for other euryhaline fishes (Holmes & Donaldson, 1969). To date, similar chloride and osmolality diel patterns have been documented in some euryaline cyprinodontids (Meier *et al.*, 1973; Spence *et al.*, 1977; Bulger, 1986). Furthermore, Hannah & Pickford (1981) also documented significantly elevated hematocrits and sodium at 1600 in *F. heteroclitus* but no patterns were documented for chloride or potassium. The sodium cycle in *F. heteroclitus* seems to be in phase with the elevated values of osmolality documented by Bulger (1986) for the same species. Additionally, Ikeda *et al.* (1976) detected diurnal peaks in chloride in the yellow-tail *Seriola quinqueradiata* which varied depending upon the type of food fed to the fish. Leatherland *et al.* (1974), however, failed to detect a significant pattern in hematocrit for juvenile kokanee salmon *Oncorhynchus nerka*. All of these studies suggest that there are species specific differences in diel patterns in osmolality, ion concentration and hematocrit. However, the apparent wide variation in diel or diurnal study responses within congeneric species (see Meier *et al.*, 1973, Figs. 1, 2) simply may be due to juxtoposition of the time axis.

It is interesting to note that all of the species previously studied are euryhaline and inhabit estuarine habitats which present complex ambient physio-environmental conditions to the fish. Freshwater channel catfish *Ictalurus punctatus* and the hybrid *I. punctatus* × *I. furcatus* that occur in less fluctuating habitats apparently do not have chloride cycles (Davis & Simco, 1976). This suggests that the phenomena of circadian patterns in osmolality and at least chloride ion concentration may be most distinct in euryhaline fishes that inhabit biotopes that fluctuate in temperature (see Bulger, 1986, for lengthy discussion).

Of major importance in ion and osmoregulation studies is the time of day the blood samples were obtained, yet few studies indicate this important point. Comparison of treatment data within a study may show a significant effect simply due to differences in the time of day samples were collected. Additionally, comparisons of data among studies almost never consider differences in time of collection, making such comparisons suspect. Future studies of this nature should take into account the time of day samples will be collected of if patterns have already been established, collections should be made in light of this published data. Finally, we feel that future studies should state the time of day their animals were tested so as to facilitate easy comparison of data sets.

ACKNOWLEDGEMENTS

We thank J.J. Luczkovich, R. Laughlin, and N. Brown-Peterson. This project was supported by a Harbor Branch Institution postdoctoral fellowship to M.S. Peterson and a grant from the Indian River Mosquito Control District through the Department of Environmental Regulation (CZM-194) to R.G. Gilmore Jr.

REFERENCES

BARTON, M., 1979. Serum osmoregulation in two species of estuarine blennoid fish, *Anoplarchus purpurescens* and *Pholias ornata*. *Comp. Biochem. Physiol.*, Vol. 64A, pp. 305–307.

BULGER, A., 1986. Coincident peaks in serum osmolality and heat-tolerance rhythms in seawater-acclimated killifish (*Fundulus heteroclitus*). *Physiol. Zool.*, Vol. 59, pp. 169–174.

CHAVIN, W. & J.E. YOUNG, 1970. Factors in the determination of normal serum glucose levels of goldfish, *Carassius auratus* L. *Comp. Biochem. Physiol.*, Vol. 33, pp. 629–653.

DAVIS, K.B. & B.A. SIMCO, 1976. Diurnal measurements of plasma chloride in catfish. *Copeia*, Vol. 1976, pp. 608–609.

HANNAH, G.S. & G.E. PICKFORD, 1981. Diurnal peaks in the hematocrit and in serum sodium in the killifish, *Fundulus heteroclitus*, and their absence in serum potassium and chloride: a lack of correlation. *Comp. Biochem. Physiol.*, Vol. 70A, pp. 157–159.

HOLMES, W.N. & E.M. DONALDSON, 1969. The body compartments and the distribution of electrolytes. In, *Fish physiology*, edited by W.S. Hoar & D.J. Randall, Academic Press, New York, New York, pp. 1–90.

IKEDA, Y., H. OZAKI, S. SAWADA, A. FUJII & I. ARAKI, 1976. Diurnal variations in serum levels of various constituents in cultured yellow-tail (*Seriola quinqueradiata*). *Jpn. J. Ichthyol.*, Vol. 23, pp. 93–99.

KLOCKARS, A.J. & G. SAX, 1986. *Multiple comparisons.* Sage University paper series on quantitative applications in the social sciences, 07–061, Beverly Hills, California, 87 pp.

LEATHERLAND, J.F., B.A. MCKEOWN & T.M. JOHN, 1974. Circadian rhythm of plasma prolactin, growth hormone, glucose, and free fatty acid in juvenile kokanee salmon, *Oncorhynchus nerka*. *Comp. Biochem. Physiol.*, Vol. 47A, pp. 821–828.

MEIER, A.H., G.R. LYNCH & L.E. GARCIA, 1973. Daily rhythms of plasma chloride in two teleosts, *Fundulus grandis* and *Fundulus chrysotus*. *Copeia*, Vol. 1973, pp. 90–92.

NORDLIE, F.G., 1985. Osmotic regulation in the sheepshead minnow, *Cyprinodon variegatus* Lacepede. *J. Fish Biol.*, Vol. 26, pp. 161–170.

NORDLIE, F.G., 1987. Salinity tolerance and osmotic regulation in the diamond killifish, *Adinia xenica*. *Environ. Biol. Fish.*, Vol. 20, pp. 229–232.

ROBERTSON, L., P. THOMAS, C.R. ARNOLD & J.M. TRANT, 1987. Plasma cortisol and secondary stress responses of red drum to handling, transport, rearing density and a disease outbreak. *Prog. Fish Cult.*, Vol. 49, pp. 1–12.

SPENCE, B., A.H. MEIER & T.H. DIETZ, 1977. Circadian chloride rhythms in the Gulf killifish, *Fundulus grandis*. *Copeia*, Vol. 1977, pp. 557–560.

SPSS, 1985. *SPSSX user's guide*, McGraw-Hill, New York, New York, second edition, 988 pp.

VALENTINE, D.W. & R. MILLER, 1969. Osmoregulation in the California killifish, *Fundulus parvipinnis*. *Calif. Fish Game*, Vol. 58, pp. 20–25.