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AVOIDANCE OF WASTEWATER TREATMENT PLANT EFFLUENT
by
SELECTED FISH AND MACROBENTHOS

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Masters Project Report
November 1986

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PREFACE

A research project was funded by a grant to the Center for Wetlands, University of Florida from NASA/Kennedy Space Center through the Bionetics Corporation for the purpose of evaluating the potential use of marsh wetlands in the KSC area for discharge and advanced treatment of municipal wastewater. The pilot-scale research program was initiated to develop preliminary data on wetland efficiency at water quality enhancement and biotic response to wastewater. After two years of research on the project, results from the pilot study indicate that wastewater discharge to the wetland is a viable method for low-energy advanced treatment of wastewater while maintaining the function and character of the wetland. Data on faunal response to wastewater are presented in this report.

OBJECTIVE

Concern exists about possible changes in aquatic fauna of wetlands receiving wastewater treatment plant effluent for final processing. The direct approach to determine such changes would have been to monitor aquatic populations of the marsh study site during the course of the pilot-scale project. However, low and at times non-existent water levels on the marsh excluded aquatic animals from the study site during this time.

An alternative laboratory scale method of investigating possible faunal changes in the wetland upon application of wastewater was developed. Laboratory experiments from which results could be extrapolated to the field seemed suitable. Toxicity bioassays and avoidance studies were conducted to assess potential faunal response.

Behavioral response of motile species encountering a pollutant may be an important factor determining their distribution in the environment. Such species may avoid a detrimental condition before it becomes lethal. Movement of motile organisms in response to adverse conditions may be more important in determining their distribution than their ability to survive under those adverse conditions. Thus, a study of the avoidance response of aquatic organisms to wastewater was chosen as a sensitive approach for the prediction of faunal changes in the marsh.

The objective of this study was to determine if selected fauna of the experimental marsh avoided wastewater treatment plant effluent over a wide range of concentrations. These results were used in conjunction with data on wastewater toxicity and anticipated wastewater concentrations in the marsh to predict the extent of changes in the aquatic fauna of the marsh.

SITE DESCRIPTION

The study site (see Figure 1) is located within the John F.

Kennedy Space Center in an oligohaline brackish marsh on the

Figure 1. Location of experimental marsh study site within the John F. Kennedy Space Center. Wastewater was pumped from sewage treatment plant #4's polishing pond to the study site through a pipeline along the south-east shore of Banana Creek.

southern edge of Banana Creek, east of State Road 3 and north of sewage treatment plant #4. The marsh is isolated from Banana Creek by a man-made dike. The only apparent hydrological inputs are rain and water from Banana Creek which is ocassionally pumped into the impoundment for mosquito control purposes. There are no active surface water inflows or outflows.

A one meter deep ditch parallels the marshward side of the dike. Laguncularia racemosa forms a narrow fringe along the ditch banks. The marsh proper is dominated by clumps of Spartina bakeri and Juncus romerianus. Other common species on the marsh listed in order of abundance are Borricha frutescens, Acrostichum danaeifolium, Chara sp., Distichlis spicata and Bacopa monnieri. Typha domingensis is found along the edges of numerous pools and channels throughout the marsh.

Water levels on the marsh varied both spatially and temporally. The eastern edge of the study site was about 15 cm lower than the western edge along the Laguncularia racemosa fringe. During early 1984 the entire marsh was flooded. Water depth over the study site was between 30 cm. and 40 cm. In the summer of 1984 and on through the summer of 1985, when the project was concluded, water levels were much lower. Heavy rainfalls would raise water levels to as much as 15 cm. or 20 cm. This would soon drop to the usual 5 cm. or less depth. For several months the marsh surface was dry. Salinity of the water was always less than 2 ppt. Dissolved oxygen levels ranged from 14.0 ppm (supersaturation) on bright sunny days to 0.4 ppm on a few early mornings.

TEST ORGANISMS

Two species of fish and one macrobenthic invertebrate that commonly inhabit brackish water marshes in large numbers were the test organisms for this project. They were *Gambusia affinis* (mosquitofish), *Poecilia latipinna* (sailfin molly), and *Palaemonetes palodosus* (grass shrimp). Each occupies a separate niche with respect to feeding habits or specific microhabitat preference, and all three are abundant in the experimental marsh and similar marshes on Merritt Island (Snelson, 1976) as well as salt marshes throughout the Indian River system (Harrington and Harrington, 1982).

Gambusia affinis is quite common in low salinity coastal marshes of Florida, as well as fresh or brackish water ponds, pools, and ditches. *Gambusia affinis* is the most abundant and ubiquitous fish species on Merritt Island (Snelson, 1976). It occurs in a wide range of salinities but is generally found in fresher water than *Poecilia latipinna* (Hoese and Moore, 1977; Snelson, 1976). The *Gambusia affinis* used in this project ranged in length from 3 cm. to 4.5 cm.

Commonly observed in water as shallow as 2 cm., *Gambusia affinis* can even be found in ephemeral bodies of water such as intermittently flooded ditches or pools. It ventures into such shallow waters in search of insect larva. This diverse feeder is a preferential carnivore with an excellent searching ability which enables it to exploit small scattered broods of mosquito larvae (Harrington and Harrington, 1961). When insect larvae are not available *Gambusia* switch to a diet of plant material

(Harrington and Harrington, 1982). Gambusia affinis has been introduced into many areas to control mosquitos (Hoese and Moore, 1977; Harrington and Harrington, 1961).

The larger and more euryhaline Poecilia latipinna is also ubiquitous in coastal Florida wetlands. In contrast to Gambusia affinis, Poecilia latipinna are predominantly herbivores. Fresh vascular plant material and occasionally some insects compose their usual diet (Harrington and Harrington, 1961). However, when fresh vascular plant material is not avaiable, the diet consists mainly of algae and some plant detritus (Harrington and Harrington, 1982). The fish used ranged from 3.5 cm to 6.0 cm in length.

Palaemonetes palodosus is a detritivore found in abundance at the study marsh. An important role in the decomposer system of tidal marshes is carried out by a similar species, Palaemonetes pugio (Welsh, 1975). These small shrimp exist in enormous populations which break down large pieces of detritus into smaller particles. Dissolved organic matter is released into the water, pennate diatoms and bacteria colonize the particles, and the shrimp excrete large quantities of ammonia and phosphate. The shrimp themselves are consumed by predatory fishes. Thus, Palaemonetes pugio make detrital energy available to numerous trophic levels. It is assumed that Palaemonetes palodosus may have a similar function in the study marsh.

METHODS

COLLECTION OF TEST ORGANISMS

Test organisms were collected near the study site with a 4.5

meter cotton mesh minnow seine. Specific collection sites varied according to availability of the organisms sought. The actual study marsh itself was not used as a collection site due to nonexistent or extremely low water levels at the time.

Collected organisms were separated by species then placed into 40 liter aquaria containing water from the perimeter ditch of the study marsh. Tank densities were kept at low levels and filters were used to maintain water quality and keep the animals healthy. The animals were fed Tetramin commercial fish food flakes. The test animals were held in the aquaria for one to two weeks to acclimate them to captivity and eliminate weak individuals injured during collection and transportation.

AVOIDANCE APPARATUS

An avoidance response is a refusal of fish to freely enter an area of altered water quality. A specially designed trough was used to determine the minimum concentration of effluent that elicited an avoidance response under laboratory conditions. This apparatus was similar to those used by Cherry (1977), Lewis (1977), and Sprague (1964) in their respective investigations of avoidance reactions of fish to chlorine, pulpmill effluent, and mixtures of copper and zinc.

The avoidance trough was constructed of wood and waterproofed with fiberglass. It measured 1.5 meters long, 15 cm wide and 15 cm deep (see figure 2). To eliminate any visual markings that might have distracted the test animals, the trough was painted with white enamel. A wooden box covering the trough isolated it from the surrounding environment. This prevented any movements by the operator from disturbing the animals. Incandescent lighting inside the cover provided even and consistent illumination throughout the experiments and facilitated observation of the animals in the trough by means of a small viewing port in the top of the cover.

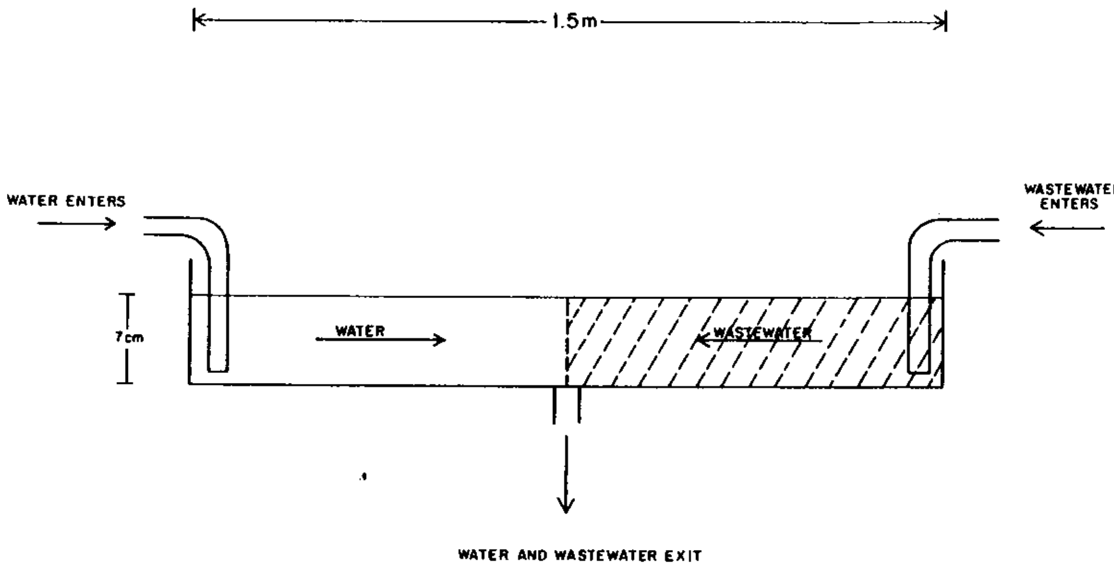

Figure 2. Length-wise cross section of apparatus used for evaluation of avoidance of wastewater by aquatic organisms.

The effluent/marsh water solution was prepared before each test in a 120 liter plastic container. Effluent was obtained from the discharge sluice of sewage treatment plant number 4's holding pond just downstream of the chlorination point. This same effluent was applied to the study marsh. Marsh water obtained from the perimeter ditch of the study marsh was also held in a 120 liter plastic reservoir. Both effluent and marsh water were filtered through glass wool to remove large particulates. A peristaltic pump transfered water from these two reservoirs to the avoidance trough.

The apparatus was set up on the perimeter dike of the impoundment. This allowed for easy aquisition of large quantities of fresh effluent and marsh water. Although the apparatus was outside, the cover allowed for control of external variables such as sunlight and wind. Favorable weather conditions permitted almost constant use of the apparatus during the summer of 1985. The test animals were kept in covered 20 liter containers and were gradually acclimated to new marsh water.

When in operation, the depth of water inside the trough was maintained at approximately 7 cm. Water was pumped into opposite ends of the trough through diffusing pipes. A dual channel peristaltic pump maintained a constant flow rate of two liters per minute through each of the diffusing pipes for a total flow into the trough of four liters per minute. Water exited the trough by way of five drain holes aligned across the center of the trough.

This created two opposing streams of water which slowly flowed toward the center of the trough. As these two streams of water met and flowed out through the drains, a vertical plane of separation was formed. This plane of separation bisected the trough across its width. Tests using dye showed that very little mixing between streams occured across this plane. Therefore, by pumping a mixture of wastewater and water into one side and only water into the other side, the trough contained the mixture of wastewater and water on the first side and only water on the second side with the sharp plane of demarcation between the two bodies of water.

An aquatic animal swimming or crawling about in the trough was soon exposed to each of these seperate and distinct bodies of water. Although observations showed the flow of water into the drains was detectable, it was so gentle it did not appear to act as a barrier between the two sides of the trough. The animals were not inhibited from moving between the two bodies of water. Therefore if the animals displayed no avoidance of one body of water with respect to the other body of water, the distribution of animals in the trough at any given time should have been a purely random occurrence. Control experiments confirmed this to be true for all three species. Avoidance of a solution was then demonstrated by a decreased occurrence of animals in the corresponding arm of the trough.

AVOIDANCE TESTS

To begin an experimental run a group of ten animals was placed into the trough while marsh water was pumped into both ends. These animals had been previously acclimated to the marsh water by gradual replenishment of the water in the holding containers. One half hour was allowed for the animals to become accustomed to the trough and its slow flowing water.

The observation of the animals was divided into three thirty minute periods with ten minute breaks in between. These ten minute breaks allowed ample time for the effluent to become fully dispersed in its side of the trough. This had been confirmed with dye studies.

During the first thirty minute observation period marsh water flowed in both sides of the trough. This was a control to determine if the animals exhibited a bias towards either side of the trough. If the animals were not randomly distributed between both sides of the trough during this period they were released.

The apparatus was then examined for possible abnormalities that might make one side appear different than the other to the animals. Results of preliminary control tests showed no bias of the animals for one side or the other when the apparatus was functioning properly. Possible abnormalities included an accumulation of feces or detritus in the trough, a clogged diffusing pipe, air bubbles in the water line, or a bad light bulb on one side of the trough. Another group of ten animals was placed into the trough to begin a new experimental run. Animals that were hyperactive or lethargic were also replaced at this time since neither are suitable as test animals for this study. Out of 102 sets of animals, only 6 were discarded.

Upon completion of a successful control test, actual measurement of avoidance was conducted in the second and third observation periods. While continuing to pump just marsh water into one side of the trough, effluent mixture was pumped into the other side. After the effluent mixture became fully dispersed and a sharp plane of separation between the two sides of the trough had developed, data collection began.

For the next half hour the number of animals in each end of the trough was recorded every thirty seconds. Animals in a zone within five centimeters of either side of the center line were not counted as being in either side of the trough. Dye studies showed that small eddies across the plane of separation could occasionally be created by tail movements of a fish swimming through the plane. Therefore to be postive that the data represent animals completely in one solution or the other this zone was disregarded for the purpose of data analysis.

The effluent and water sides were reversed for the final observation period. When this final observation period was completed, the animals were released and a new group was used for the next experimental run.

Avoidance at each tested concentration was ascertained by comparing the mean number of animals observed in effluent versus water over all experimental runs conducted with that concentration. A statistical difference was determined with Student's T-test for means at a confidence level of 95 percent.

TOXICITY BIOASSAYS

Test organisms were collected from the same sites as those used in the avoidance experiments. Animals were held for one week at Center for Wetlands in a 225 liter holding tank to acclimate them and to weed out individuals injured during collection and transportation. Shrimp were held in 40 liter aquaria. The water, which was obtained from the perimeter ditch at the study site, was continuously recycled through a trickling filter to maintain water quality to keep the animals healthy. The holding tank was also aerated. Dissolved oxygen content of the water was four to six mg/l while the animals were being acclimated.

Each bioassay set was conducted with six levels of effluent and twin replication. A diel lighting pattern was provided by a fluorescent light fixture controlled by an automatic timer. Day lengths were adjusted about once every other month to correspond to natural day lengths. There were no windows in the aquarium room. During each run a constant water temperature was maintained through regulation of the ambient air temperature of the room. Temperatures ranged from 20 C in January to 27 C in August. The aquaria were each individualy aerated to maintain consistent dissolved oxygen levels from start to finish and between treatments. During bioassays dissolved oxygen content of the water/wastewater solutions was usually seven to eight mg/l. Dissolved oxygen content never significantly varied among the different treatments.

At the start of each bioassay set, ten animals were placed into each of the twelve aquaria. The five treatments and control consisted of 100, 75, 50, 25, 10, and 0 percent effluent by volume. The dilution water came from the perimeter ditch. This was the same water used in the holding tank and the avoidance experiments. Dilution water and effluent were collected at the same time, within eight to ten hours before the start of each set. The effluent was obtained from the same place in the discharge sluice of sewage treatment plant number four's polishing pond as that used in the avoidance experiments.

Mortalities were recorded at the end of 6, 12, 24, 48, 72, and 96 hours of exposure. The time period of 96 hours was choosen because mortalities greater than 5 percent began to occur in the controls of preliminary runs after 5 days. This was deemed an unsatisfactory level (APHA, 1976). A likely cause of this mortality may have been debilitation from starvation since the animals were not fed during the bioassay sets. A build up of metabolic wastes may also have been a factor.

Dead animals were removed at each observation time to prevent their decay from fouling the treatment solutions. Temperature and dissolved oxygen content were recorded at these times. At the start of each set a sample of effluent was taken for analysis of total Kjeldahl nitrogen and total phosphorus to determine if these parameters were similar to that of the effluent used for avoidance experiments.

Each bioassay set was repeated under as nearly the same conditions as possible to determine reproducibility. Sets were run separately for each of the three species.

EFFLUENT ANALYSIS

The concentrations of total Kjeldahl nitrogen and total phosphorus in the effluent used in the experiments were determined on a daily basis. This was done to ensure that the characteristics of the effluent used in each set of tests was the same throughout the study. Patrice Mion conducted the analyses at the Bionetics Corporation laboratory at John F. Kennedy Space Center for the period of January 1985 through May 1985.

Subsequent analyses were conducted at the Center for Wetlands laboratory in Gainesville.

In both situations digestions for Kjeldahl nitrogen and total phosphorus were done on a block digester and a Technicon autoanalyzer was used for analyses. Procedures followed guidelines recommended by Technicon (1983). The mean TKN throughout the study period was 8.4 mg/l and total phosporus was 0.9 mg/l.

The dissolved oxygen content and temperature of marsh water and effluent mixture were periodically monitored to be sure they did not differ. Turbidity was checked constantly during experimental runs. If any major differences existed the experimental run was discontinued and test animals were discarded. Another experimental set was not begun until conditons were the same in both sides of the trough. This eliminated the possibility that the fish were reacting to dissolved oxygen content, temperature, or turbidity rather than a chemical component of the effluent.

RESULTS

AVOIDANCE STUDIES

Tables 1 and 2 show results for *Poecilia latipinna* and *Gambusia affinis*. The mean number of fish in marsh water is significantly greater ($t_{0.05}$) than in effluent marsh water mixture until the effluent concentration is decreased to 10%. It appears that both species avoid effluent concentrations of 15% and greater but do not discriminate between unaltered marsh water and effluent concentrations of 10% or less. These results represent the observation of 18 groups of 10 fish for each species with 3 groups per effluent concentration.

Table 1. Results of test for evaluating avoidance of wastewater by *Poecilia latipinna* under controlled conditions. Values are mean ± standard deviation; NSD = no significant difference between means ($t_{0.05}$).

Concentration of Wastewater % by Volume	Mean Number of Fish in Effluent Side		Mean Number of Fish in Marsh Water Side
100	1.2 ± 0.7		8.7 ± 0.7
50	2.3 ± 0.5		7.0 ± 0.5
33	2.7 ± 1.0		6.6 ± 1.0
20	2.7 ± 1.5		6.4 ± 1.8
15	3.6 ± 0.7		5.4 ± 0.6
10	4.7 ± 0.4	NSD	4.7 ± 0.3
0	4.4 ± 0.6	NSD	4.6 ± 0.5

Table 2. Results of test for evaluating avoidance of wastewater by Gambusia affinis under controlled conditions. Values are mean ± standard deviation. NSD = no significant difference between means ($t_{0.05}$).

Concentration of Wastewater % by Volume	Mean Number of Fish in Effluent Side		Mean Number of Fish in Marsh Water Side
100	1.9 ± 1.2		7.4 ± 1.6
50	2.7 ± 1.1		6.5 ± 1.4
33	3.7 ± 0.7		5.5 ± 0.7
20	4.1 ± 0.2		5.4 ± 0.4
15	4.1 ± 0.5		5.4 ± 0.7
10	4.5 ± 0.8	NSD	5.5 ± 0.8
0	4.7 ± 0.4	NSD	4.8 ± 0.4

Palaemonetes palodosus avoid effluent concentrations as low as 10%. This is the lowest effluent concentration tested. Results for the observation of 5 groups of 10 shrimp are given in Table 3.

The results of tests using non-chlorinated effluent with Gambusia affinis are given in Table 4 and are comparable with the results of tests using chlorinated effluent as given in Table 2. As with chlorinated effluent, fish avoid the non-chlorinated effluent mixture until the effluent concentration is decreaesed to 10 percent at which no avoidance response is observed. The identical response of Gambusia affinis to chlorinated and non-chlorinated effluent indicates that chlorine is not the factor

Table 3. Results of test for evaluating avoidance of wastewater by *Palaemonetes palodosus* under controlled conditions. Values are mean ± standard deviation; NSD = no significant difference between means ($t_{0.05}$).

Concentration of Wastewater % by Volume	Mean Number of Shrimp in Effluent Side		Mean Number of Shrimp in Marsh Water Side
100	2.7 ± 0.7		6.5 ± 0.4
50	2.1 ± 0.5		6.2 ± 0.5
33	3.2 ± 0.4		6.0 ± 0.4
20	2.2 ± 0.8		7.1 ± 0.7
10	3.7 ± 0.1		5.5 ± 0.2
0	4.5 ± 0.6	NSD	4.4 ± 0.5

Table 4. Results of test for evaluating avoidance of wastewater without chlorine by *Gambusia affinis* under controlled conditions. Values are mean ± standard deviation; NSD= no significant difference between means ($t_{0.05}$).

Concentration of Wastewater % by Volume	Mean Number of Fish in Effluent Side		Mean Number of Fish in Marsh Water Side
100	2.2 ± 0.1		7.5 ± 0.1
50	2.2 ± 0.1		7.3 ± 0.1
33	3.5 ± 0.6		6.3 ± 0.4
20	3.7 ± 0.2		6.0 ± 0.1
10	4.0 ± 1.3	NSD	5.7 ± 1.2
0	4.5 ± 0.3	NSD	4.4 ± 0.5

eliciting the avoidance response in this case. Observations of 5 groups of 10 fish comprise these results.

TOXICITY BIOASSAYS

Data from the toxicity bioassays are summarized for *Gambusia affinis* in Table 5 and *Palaemonetes palodosus* in Table 6. The 96 our LC50 values for each species are estimated through linear regression analyses of effluent concentrations versus percentage of mortality. *Gambusia affinis* has a 96 hour LC50 of 73% effluent and *Palaemonetes palodosus* 64%. The respective correlation coefficients for each regression are r=0.77 and r=0.79. There are 6 replicates for *Gambusia affinis* and 4 for *Palaemonetes palodosus*.

Reliable toxicity data were not obtained for *Poecilia latipinna*. During the acclimation periods mortalities were as high as 30%. This left a stock of highly resistant fish. When the acclimation periods were eliminated, mortalities in the controls became as high as 30%.

Table 5. Results of 96 hour toxicity bioassays for *Gambusia affinis*. Values are mean ± standard deviation; n=6.

PERCENTAGE EFFLUENT	PERCENTAGE MORTALITY
100	76.7±36.7
75	40.1±14.1
50	6.7± 8.2
25	18.0± 4.5
10	1.7± 4.1
0	3.3± 8.2

Table 6. Results of 96 hour toxicity bioassays for *Palaemonetes palodosus*. Values are mean ± standard deviation; n=4.

PERCENTAGE EFFLUENT	PERCENTAGE MORTALITY
100	83.3±28.6
75	58.0±37.0
50	40.0±14.1
25	32.5±15.0
10	12.5±12.6
0	5.0± 5.8

The toxicity bioassays are conducted with the same chlorinated effluent as the avoidance studies. Test organisms are placed in the effluent solutions 6 to 12 hours after effluent collection. Toxicty of chlorine in treated wastewater decreases with time. According to Brungs (1973), lethal concentrations of residual chlorine dissipate to below harmful levels after 12 to 24 hours. Lethal effects of free chlorine decline even faster. So effects of chlorine may not be represented in the bioassays.

DISCUSSION

All three species tested avoid lethal concentrations of wastewater treatment plant effluent in the avoidance apparatus. Concurrent toxicity bioassays conducted on *Gambusia* indicate an effluent concentration of 73% to be lethal to 50% of fish after 96 hours of exposure. The 96 hour LC50 for *Palaemonetes* is somewhat lower at 64%, although variability is high for both species. Both Gambusia and Poecilia begin to avoid effluent at a concentration of 15%. Palaemonetes avoid 10% effluent, the lowest concentration tested.

Avoidance occurrs well below acutely toxic levels. Gambusia affinis and Paleamontes palodosus exhibit marked avoidance of effluent concentrations near their respective 96 hour LC50 values, as can be seen in Table 2 and Table 3. These animals clearly demonstrate an ability to avoid effluent at both lethal and sublethal concentrations.

Many other species of fish are also capable of avoiding detrimental levels of contaminants. Lewis and Livingston (1977) report pinfish (Lagadon rhomboides) and gulf killifish (Fundulus grandis) begin to avoid bleached kraft mill effluent (BKME) at a concentration of 0.06% effluent. They found the 96 hour LC50 level for juvenille pinfish is approximately 10% BKME. The avoidance threshhold is only 0.6% of the 96 hour LC50. Increased gill ventilation rates and decreased food conversion efficiencies are observed at BKME concentrations down to about 1% of the 96 hour LC50. It appears then that pinfish will react with a behavior which keeps them out of water with harmful levels of BKME when given the choice.

Some species of fish have been observed to avoid a wide range of pollutants. The pesticides DDT, endrin, Dursban, and 2,4-D are avoided by sheepshead minnows (Cyprinodon variegatus) at concentrations well below their 24 hour LC50 levels (Hansen, 1969). Malathion or Sevin are not avoided however, even at levels higher than the 24 hour LC50. Summerfelt and Lewis (1967) investigated the repulsive effects of various chemicals on green sunfish. Of the 40 chemicals screened only 8 repelled fish at the concentrations tested. Young Atlantic salmon (Salmo salar) were found to exhibit an avoidance threshold to copper-zinc solutions at 1% of the level which would kill them in 14 hours (Sprague, 1964). Both Giatting and Garton (1983) and Cherry and Cairns (1982) present comprehensive literature reviews of avoidance responses of fishes to a long list of various aquatic contaminants.

Alterations in natural environmental parameters can also be avoided by fish. When given a choice of oxygen concentrations ranging between 1.5 mg/l to 9.5 mg/l largemouth bass (Micropterus salmoides) and bluegill (Lepomis macrochirus) markedly avoid the lowest concentration (Whitmoore et. al., 1960). It is possible then that while a level of pollutant does not directly cause fish to stay out of an area, it may indirectly restrict use of the area by altering an environmental parameter. A reduction of dissolved oxygen in the water by the BOD of added wastewater would be an example.

Statistical design and time constraints have limited observation periods in most avoidance studies to one hour (Cherry and Cairns, 1982; Giattina and Garton, 1981). Dauble (1985) found an overall pattern of avoidance during a 48 hour exposure to a water-soluble fraction of coal liquid. The same pattern was not always detectable in discrete blocks of the total 48 hour observation period. Long-term recordings of goldfish movements in an avoidance apparatus also demonstrated a variability which renders short-term observations in that case questionable (Kleerekoper, 1976).

Preliminary tests were conducted with one hour observation periods to determine an acceptable observation time period. Statistical analysis showed no difference in behavior between the first and second thirty minute halves of these one hour observation periods. Therefore, for subsequent tests which made up the reported data, thirty minute observation periods were used.

In general extending the observation time significantly decreases the avoidance threshold determined in laboratory tests. Very low avoidance of a contaminant is only discernable over long observation times since it is obscured by variability in shorter periods. So tests with longer observation times are more sensitive.

Fish demonstrating avoidance of a contaminant may not be totally excluded from utilizing contaminated habitat. They may be capable of exploiting this contaminated habitat without serious injury. Especially with lower levels of a pollutant, fish may spend a lot of time in the contaminated area but not quite enough time to cause irreperable damage.

If lower levels of a contaminant can be tolerated without injury for longer periods of time than higher levels. One might expect fish to exhibit a lesser degree of avoidance to lower levels of contaminants. As can be seen in Figures 3 and 4, the degree of avoidance of wastewater by Poecilia latipinna and Gambusia affinis corresponds with effluent concentration. So

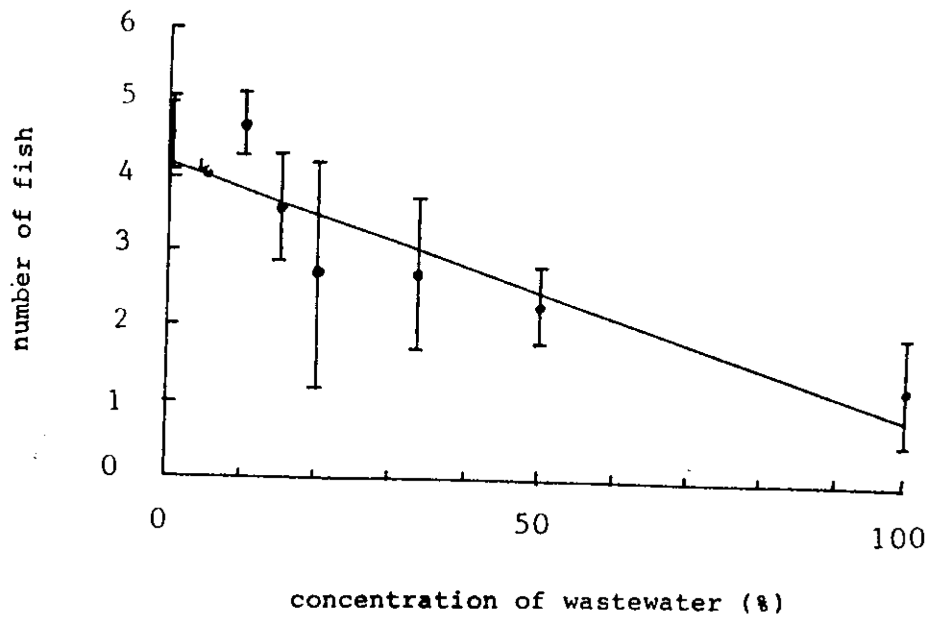

Figure 3. Correlation of avoidance of wastewater by *Poecilia latipinna* with increasing wastewater concentration. Data points represent mean (+S.D.,n=6) number of fish in wastewater side of trough. The regression line fitted to means has a correlation coefficient of r=0.90.

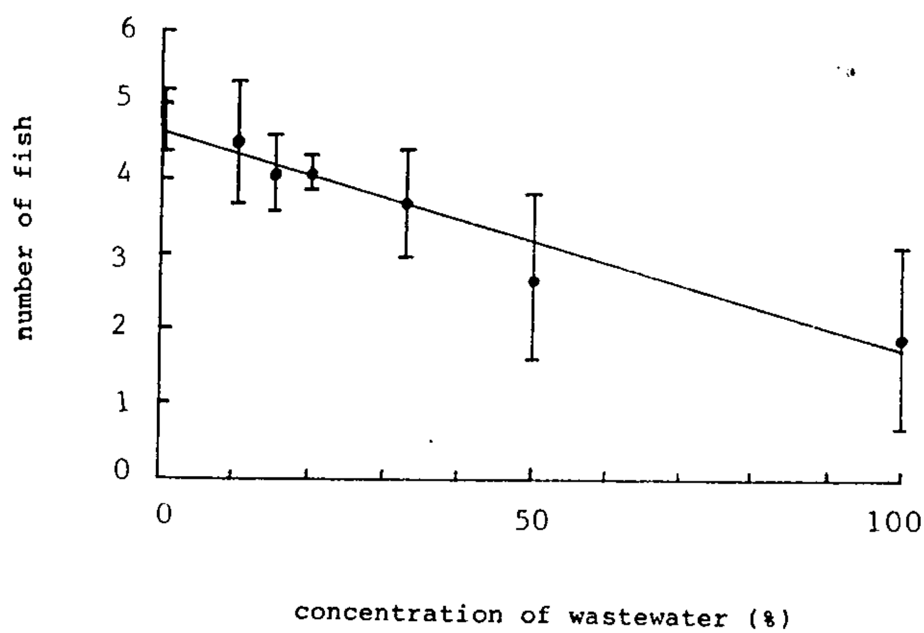

Figure 4. Correlation of avoidance of wastewater by *Gambusia affinis* with increasing wastewater concentration. Data points represent mean (+S.D.,n=6) number of fish in wastewater side of trough. The regression line fitted to means has a correlation coefficient of r=0.98.

these fish may be able to safely exploit habitat with contaminant concentrations above toxic levels.

A decline or elimination of avoidance reaction after exposure to a pollutant has been documented by Cherry et al.(1977). Spotted bass (*Micropterus punctulatus*) and rosyface shiner (*Notropis rubellus*) avoid 0.05 mg/l total residual chlorine (TRC) at 60 C. After the initial avoidance response, rosyface shiner continue to avoid increasingly higher concentrations. Spotted bass show less or no avoidance at all past the initial threshold concentration. The same pattern is observed when each species is exposed to a single concentration (0.05 mg/l and 0.10 mg/l) for three successive 10 minute periods.

Individuals from populations of fish exposed to contaminants over a time scale of many generations may respond differently than those from unexposed populations. According to Culley and Ferguson (1964) constant pesticide exposure over many years has exerted a selection pressure on some populations of mosquitofish near agricultural lands and they have evolved resistance to these pesticides. One might also expect to find changes in other traits that enhance survival under polluted conditions.

Mosquitofish from two populations, one with a high tolerance to several pesticides and the other without, were tested for their ability to avoid concentrations of DDT, endrin, toxaphene, and parathion by Kynard (1974). Fish from both populations avoided endrin, toxaphene, and parathion. Interestingly DDT was avoided by susceptible fish but not by resistant fish. Overall, fish from populations exposed to pesticides for generations avoided damaging concentrations (except DDT) better than fish which had not been previously exposed to the pecticides.

Thus the possibility exists for a change in degree of avoidance behavior after exposure to a contaminant. The duration of exposure can be just a few minutes or may cover many generations. These changes also vary among species. The design of an avoidance study should take into account these possible changes in response and care should be taken in the obtainment of specimens. Caution should also be used when analyzing results or trying to extrapolate them to the field.

In studying the response of spotted bass and rosyface shiners to chlorine in a power plant cooling system discharge, Cherry et al. (1977) discovered that avoidance is most closely related to the hypochlorous acid (HOCl) fraction of the total residual chlorine. Previous research with these species produced conflicting correlations with total residual chlorine, free residual chlorine, and combined residual chlorine until avoidance was compared to the HOCl concentration. They note that HOCl may be the most toxic and irritating of the chlorine species.

Juvenile salmon avoid acutely toxic concentrations of benzene, toluene, and o-xylene when presented individually. Avoidance of a mixture of these hydrocarbons generally occurs at much higher levels (Maynard and Weber, 1981). This response to the hydrocarbon mixture is unexpected. One would assume the response to the mixture to be the same or possibly greater due to an additive effect, but in this case it is less.

Maynard and Weber (1981) rule out over stimulation of the olfactory bulb by the mixture as the reason for this decreased behavioral response. Electrophysiological recordings indicate short-term exposure at a level slightly above the behavioral threshhold concentration does not disrupt the chemosensory modality of the olfactory bulb. It is also interesting to note that the olfactory system is first stimulated by the hydrocarbon mixture at concentrations just below those eliciting avoidance behavior.

Amoore (1970) suggests that a combination of chemicals results in an odor or taste distinct from that of the individual components. But the question remains as to why the organisms behavioral response may be different to a mixture than to its components. Is it simply that physiological detection thresholds vary or do organisms actually identify and respond differentially to each chemical species. Could these differential responses correspond to degree of toxicity?

Can the results of laboratory avoidance studies be extrapolated to the natural environment? Numerous laboratory avoidance studies have been conducted but few have compared laboratory results to corresponding field data. The difficulty in transfering information gathered on one ecosystem to other ecosystems, as well as relating data between different species or similar contaminants limits synthesis of the abundant data in the literature.

A high degree of similarity is observed between laboratory and field avoidance responses for five fish species studied by Cherry and Cairns (1982). The river water and fish used in the laboratory are collected from the field site. This is a rare case as seemingly important variables differ dramatically between most conjunctive laboratory and field studies.

Mature pacific salmon (Oncorhynchus sp.) migrating up an estuary exhibited an estimated avoidance threshold to a mixture of hydrocarbons at 3.2 mg/l (Weber et al., 1981). This is comparable to laboratory thresholds of 1.9 mg/l for smolt and 3.7 mg/l for presmolt coho salmon (Oncorhynchus kisutch) when using a similar hydrocarbon mixture in fresh water (Maynard and Weber, 1981).

Ecological requirements can override avoidance tendencies determined in a laboratory apparatus. The behavior of organisms in a natural system is not driven by any single factor and a single causal explanation of results is conditional on the experimental variables included in the study. Studies which integrate key behavior patterns throughout a species life (feeding, spawing, migration, schooling) with avoidance behavior are necessary to extrapolate any concentration-response models to the field. But to adequately estimate the impact of a pollutant discharge on an aquatic system, avoidance behavior of motile species should be considered (Giattina and Garton, 1983).

Wastewater concentrations in the study marsh should vary widely. Natural water levels of the marsh surface will have the greatest effect through dilution of the wastewater as it spreads out over the marsh. Demonstrated nutrient removal by marsh vegetation and sediments also lowers the effective level of wastewater in the water column (Mion et al., 1986). If the water level in the study plots were 30 cm, the wastewater discharge rate of 2.5 cm per week would produce a theoretical average wastewater concentration of 8.3% after one week. Concentrations would be higher than this near the point of discharge and would decrease to undetectably low levels further away. This can be seen in data from the water quality section of the project report for the pilot scale study (Mion et.al., 1986).

A gradient extends between the discharge point and the far side of the marsh. At the discharge rate of 2.5 cm per week, acutely toxic wastewater concentrations, 64% to 73%, would probably occurr only in close proximity to the discharge. All three species studied demonstrated a strong ability to avoid these concentrations. Somewhere along the gradient exists the point where the wastewater is at such a low concentration that the animals no longer exhibit avoidance to it. No chronic effects from the wastewater are expected past this point. As this point advances or recedes across the marsh surface safe habitat would be lost or gained from the plume of wastewater.

It also appears that motile organisms are capable of exploiting the area within the plume of avoided wastewater. Searches for food and cover for protection from predation are examples. Although the fact that they exhibit a tendency to remain out of water with these levels of wastewater indicates that it is probably not a suitable environment. Some chronic effects are expected to occurr in this zone.

At the discharge rate of 2.5 cm per week a zone of wastewater concentration greater than the 10% which elicited avoidance in two of the three species tested would probably be a small area relative to the whole experimental marsh. If the fish can exploit this zone for food or cover even to a limited extent, the effective area removed from their use is reduced. The ability to actively avoid acutely toxic levels reduces possibilities of fish kills caused through direct contact with the wastewater. Therefore it appears that use of the marsh for final processing of wastewater treatment plant effluent at low levels, such as 2.5 cm per week, will have minimal effect on the motile fauna.

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