

THE EFFECTS OF PESTICIDE APPLICATION ON *CYPRINODON*
VARIEGATUS (SHEEPSHEAD MINNOWS) IN SALT MARSH
ECOSYSTEMS ON LONG ISLAND

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Executive Summary:

Salt marshes are vital aquatic ecosystems which have a critical biological and biogeochemical function in estuarine environments. Unfortunately, mosquitoes live and breed in the standing water within salt marshes and can be vectors for multiple human pathogens, including West Nile virus. The primary tools used to control mosquito populations on Long Island are chemical pesticides. This study investigated the effects of pesticide spraying on a common saltmarsh associated fish species, *Cyprinodon variegatus* (sheepshead minnow), in Long Island salt marshes utilizing cage experiments. During this study, methoprene was sprayed on an Oakdale salt marsh, while resmethrin was administered aerially in Mastic. Experimental results showed growth and survival rates of *Cyprinodon variegatus* were significantly lower at the Oakdale site relative to control sites during the aerial application of a larvicide. During the aerial application of an adulticide to the Mastic site, growth rates of *Cyprinodon variegatus* at one of two experimental sites were significantly lower than rates at control sites while population survival was unaffected. Results suggest pesticide application, in conjunction with other co-occurring environmental stressors in anthropogenically influenced wetlands, could impact food webs within salt marsh ecosystems. Further studies are needed to replicate results and to elucidate pathways involved.

INTRODUCTION:

Salt marshes are vital aquatic ecosystems with important biological and biogeochemical functions in estuarine environments. Salt marshes form along coastlines where disturbance from water motion and ice is moderate enough to allow the accumulation of sediments and the growth of angiosperms (Chapman 1960). In the United States, salt marshes are the dominant intertidal habitat along the east and Gulf coasts (Reimold 1977; Mitsch and Gosselink 1993). Marsh development can follow several courses. Marshes can overlay terrestrial habitats, indicating that the marshes moved upland as sea levels rose (Redfield 1965; Montague and Wiegert 1990). Other marshes have expanded over previously subtidal habitats by trapping sediments (Osgood et al 1995). In either of these marsh development scenarios, the resulting marsh is inhabited both by organisms with terrestrial origins, such as angiosperms, insects, birds, and mammals, and by organisms with marine origins, such as algae mollusks, crustaceans and fish.

Since salt marshes are inter-tidal ecosystems, connected to land on one side while opening to the estuary and the sea on the other (Pomeroy et al., 1981), they possess complicated drainage basins. Salt marsh soils are periodically flooded by the tides. Decomposition of organic matter in waterlogged soils rapidly depletes available oxygen and leads to anoxic soils (Ponnamperuma 1972). Because tide height varies on a lunar cycle, high-marsh soils are flooded less frequently than low-marsh soils, and the water table has several days to drop following each period of flooding (Bertness et al. 1992). An exception to this generalization occurs adjacent to the creekbanks. Creekbank soils exchange water both on the marsh surface and through creek channels. At low tide, water resides in the tidal creeks, which do not completely drain, and in the tidal outreaches of the marsh. At high tide, water rises above the confines of the tidal creeks and covers the marsh except in the highest reaches of the high-marsh. Water flow over the marsh is primarily laminar, while turbulent flow is the general case in tidal tributaries (Pomeroy et al., 1981). Water velocity in undisturbed *Spartina* stands rarely exceeds 10 cm sec^{-1} . These low flow rates result in high rates of sediment deposition and a lack of disturbance of surface sediments in the marsh by tidal inundation. One

critical ecological function of marshes is that due to the retention of water in these systems they naturally filter and remove multiple, land derived contaminants from water before it enters an estuary (Sharp et al., 1984).

In spite of the critical role that marshes play in filtering terrestrial run-off prior to entering estuaries, many east coast US salt marshes have been ditched during the past century. Mosquito ditching consists of digging an intricate canal system through a salt marsh which are designed to reduce standing water which may harbor mosquito larvae. As such, mosquito ditching practice may concurrently control the proliferation of mosquito populations in salt marshes. In the 1930's thousands of miles of ditches were dug to drain marshes of standing water throughout Suffolk and Nassau County, NY.

Mosquitoes have a very distinct life cycle that consists of four stages (egg, larva, pupa and adult,) with three of the first four stages requiring water as a vital part of their life cycle (USEPA, 2002). As such, many mosquito species are found in salt marshes. Mosquitoes are well known vectors for multiple human pathogens, and recently have become notorious for the spread of West Nile virus (WNV) across the US. According to the CDC (2004), WNV has killed 502 people in the US since 1999. There have been 232 cases and 18 deaths in NY metro area since 1999, although the frequency and intensity of West Nile outbreak on the east coast has diminished significantly in recent years. There were 7 cases of WNV in Suffolk County, NY, in 2003 (CDC, 2004)

The use of pesticides has become a common practice to control mosquito populations in the NY metropolitan area. Mosquito pesticides are designed to target particular stages of the mosquito life cycle. For example, larvicides do not allow mosquitoes to reach the adult stage by stunting growth and blocking physiological maturation (USEPA, 1999). Adulticides are pesticides that are targeted to kill the mosquito when in the adult stage and no longer in the water (USEPA, 1999).

Methoprene is a larvicide that is administered during the egg or larvae stage to extinguish a mosquito population (USEPA, 1982; 1991). Methoprene can be available in

suspension, emulsifiable, soluble concentrate formulations, and aerosol form. Its trade names are Altosid, Apex, Phariod and ZR-515 (USEPA, 1982; 1991). In mammals, methoprene is rapidly and completely broken down and excreted, mostly in urine and feces (USEPA, 1982; 1991). However, in marine species it is rated as a moderately toxic compound to fish with a 96hr LD₅₀ concentration of 4.6mg L⁻¹ in bluegill sunfish (Kidd and James, 1991; USNLM, 1995). Methoprene is rated very highly toxic to some species of freshwater, estuarine and marine invertebrates (Zoecon Corp., 1974) and has a half-life in soil of up to 10 days (USEPA, 1982; 1991). It is slightly soluble in water and carries a half-life of 40 h at initial concentrations of 0.001mg/L in pond water (Menzie, 1980).

Resmethrin, also known as Scourge, is an adulticide which is commonly administered to salt marshes. Scourge is a type I pyrethroid insecticide that consists of 18% resmethrin, 54% piperonyl butoxide and 28% inert ingredients (USEPA, 1988). This pesticide is used in marsh systems where the annoyance factor is high or used when cases of West Nile virus is reported (USEPA, 1988). Contact with either non-inert ingredient causes irritation to the skin and respiratory tract in humans (USEPA, 1988). Piperonyl butoxide has been classified by the USEPA as a possible carcinogen and is highly toxic to fish (Hill and Camarsese, 1986; USEPA, 1988). The half life of resmethrin is up to 30 - 200 days, depending on soil and water type, and the prevailing light regime and microbial community (Muir et al., 1985).

Many of the important ecological processes which are characteristic of the tidal region of the salt marsh are associated with the populations that reside permanently in the water, such as plankton, fish and shrimp (Pomeroy et al., 1981). These species have a direct influence on carbon cycling and the overall ecological function of a salt marsh. Salt marshes possess complex food webs which provide resources for estuarine associated fish and invertebrates, and offers food, shelter and nesting sites for multiple species of commercially important invertebrates and fish species as well as waterfowl.

One common fish native to North American salt marshes is *Cyprinodon variegatus* (sheepshead minnow). Sheepshead minnows belong to the class

Actinopterygii, are benthopelagic, non-migratory, and halotolerant. They are found inhabiting turbid waters in North and Central America from Massachusetts to Mexico (Page and Burr, 1991). Along their range, *Cyprinodon variegatus* is known to be a critical source of prey to many estuarine species of fish and hence is a key component of salt marsh food webs (Wright et al., 1993). *Cyprinodon variegatus* is also known to be sensitive to many different types of pesticides (Morton et al., 2000; Hemmer et al., 2001; Beulig and Pilonieta, 2002) and is commonly used in USEPA-mandated laboratory bioassay tests (E. Copser, pers. comm.)

The objective of this project was to observe the impact of mosquito spraying on the growth and survival of a representative marine salt marsh species. Cage experiments were executed using *Cyprinodon variegatus* in areas which received aerial application of methoprene and resmethrin. Growth and survival of sheepshead minnows were monitored following aerial pesticide spray events within salt marshes in Suffolk County, Long Island. Growth and survival rates were concurrently monitored in similar environments which were not subjected to spraying.



Figure 1: Oakdale site pictured with experimental cage sites (1 and 2) and view of salt marsh. Lines indicate where aerial applications of pesticide were sprayed via helicopter (A. Cohen, pers. obs.).



Figure 2: Johns Neck site in Mastic. Sites 1 and 2 indicated with white numbers. Regions with homes are ground sprayed, where as salt marsh regions were aeri ally sprayed with resmethrin via helicopter in August 2003.

METHODS:

Study sites

Multiple experimental (sprayed) and control (not sprayed) locations were investigated as during this study. Old Fort Pond (OFP), a narrow tributary which extends from the eastern shore of Shinnecock Bay, severed as our primary control site. This is a moderately developed coastal system which is bordered by salt marsh on ~ 1/3 of its shores, but is also bordered with multiple domiciles. This area was considered an adequate control site since it is never sprayed, but in a manner similar to our experimental sites, has a residential community surrounding it and tidal influences.

The second control area was a wetland surrounded by salt marsh vegetation and housing. The cages were placed in the Goose Creek ditched area which exchanges tidally with the southern extent of Flanders Bay. The east side of the creek has pristine forest and salt marsh ecosystem while the west side has residential homes built upon it. This system served as a control salt marsh ecosystem that is typically not sprayed for mosquitoes (not during this study) and has minor run off from development.

The primary experimental site was in Oakdale (Figure 1). The salt marsh there is ditched, lays adjacent to the Grand Canal, and has no housing built directly on any of its fringe borders. The heavily ditched marsh has drainage pipes that connect the ditches to the Grand Canal, which flows south into Great South Bay and north into the Connequot River. The salt marsh here typically drains into the Grand Canal except during extreme high tide periods when tidal flow is into the marsh. This marsh was sprayed sporadically from mid-June to early September of 2003 (A. Cohen, pers. obs., 2003). This site was used as an experimental location to examine the effects of pesticide applications (methoprene) on caged sheepshead minnows during an application the first week of September 2003. Subsequent deployments made in September when spray events did not occur served as control experiments.

The second experimental site was in Mastic-Shirley (Figure 2). Here, there is an abbreviated marsh that extends towards Moriches Bay from an area of privately owned

houses. There is a ditched marsh that runs to a natural inlet in Moriches Bay which is lined with homes and has canals built for boat docks. This area was sprayed with Scourge by helicopter application on 27 August 2003.

Experiments

Crypinodon variegates, the sheepshead minnow, was used in experiments due to its naturally high abundance and keystone position in the food web of northeast US salt marshes (Page and Burr, 1991). Three hundred, 10-day old *Crypinodon variegates* were ordered from Cosper Environmental Services, Bohemia, NY, before deployment in cages. Individuals were maintained for up to 48 h before experiments in flowing seawater at the LIU marine station where ambient temperature, salinity, and dissolved oxygen are maintained. New batches of 10-day old individuals were ordered for each experiment.

Experimental cages were constructed from two gallon, sealable, plastic buckets which were lined with 500 μ m mesh and had 2-inch holes cut around the sides, on the top, and on the bottom to allow for continual water exchange. Vertical lines from the cages were connected to a surface float and to a submerged weight, assuring cages were kept in place and off the bottom. During each experiment, individuals were simultaneously subjected to a control salt marsh site and /or a ditched salt marsh environment as well as a ditched salt marsh site with pesticide applications. On the first day of field deployment (24hrs before first application of pesticide), 20 - 40 individuals were measured for initial size in order to determine growth rates throughout experiment. Minnows were briefly transferred into aerated bottles in sets of ~ 20 for experimental deployments. At each location, there were two, replicate sites (i.e. sites 1 & 2 in Fig 1 & 2). At each site, 20 minnows were placed into two, replicate cages, resulting in a total of 4 cages per location per experiment. On site, minnows were released into 90% submerged cages after which the top was fastened and the cages were submerged just below the surface. Cages were deployed 24 h prior to spray events and were then inspected at defined intervals (typically 24, 48, 72, h and 1 wk) and were replaced back into the water. Upon each inspection, cages were examined and an YSI 85 water quality probe was used to record temperature, salinity, and dissolved oxygen (DO). Each cage

was opened one at a time and held at surface so the bottom of cage remained in water and cages were inspected for species mortality. Dead fish were removed and survival rates were calculated. After one week of deployment, lengths of surviving individuals were measured and recorded.

Growth rate and survival data from experiments was subsequently analyzed by ANOVAs followed by Tukey multiple comparison tests. Individual spray events were compared to control experiments which were executed concurrently. Control experiments for each location were also pooled and compared to spray events. P-values of < 0.05 were used as the cut-off for statistically significant differences.

RESULTS:

Preliminary experiments were conducted to establish survival and growth of *C. variegatus* in our experimental cages within a controlled setting. Two cages were placed in Old Fort Pond on 8/9/03 and removed on 8/19/03. Physical characteristics of water quality such as temperature and salinity for the entire week were recorded (Table 1). Survival rates were 100% for 24 and 48hrs, but dropped to 90% after one week (Table 2). Growth rates were obtained by taking the initial length of *C. variegatus* and subtracting it from the final length at the end of one week. This preliminary experiment yielded a mean growth of 0.35 ± 0.07 cm week⁻¹

Physical water parameters such as temperature, salinity and dissolved oxygen were measured every time cages were examined or deployed. The data is presented in Table 1 and generally indicates that the temperatures were similar among sites. Dissolved oxygen did vary over time and among locations during experiments, but not significantly so (Table 1). By contrast, salinity levels in OFP were significantly higher than salinities found at the other locations (Table 1).

Experiment 1:

The first experiment was conducted in four locations beginning on 25 Aug. Cages deployed in OFP as a control for this experiment. Oakdale also represented a control site

at this time since it did not receive an aerial application of pesticides. Mastic was treated with aerial applications of resmethrin on 28 Aug 2003 after the initial 24hr period of cages placement into the marsh on 27 Aug (Suffolk County Dept of Health Web Site, 2003). Results were as follows.

Table 1. Physical Water Characteristics during experiments (temperature, salinity, and dissolved oxygen).

Site	Date	Initial Temp (*C)	Sal (ppt)	DO (mg/L)	24hr Temp (*C)	Sal (ppt)	DO (mg/L)	48hr Temp (*C)	Sal (ppt)	DO (mg/L)	1week Temp (*C)	Sal (ppt)	DO (mg/L)
Initial trial, OFP	8/9-8/19	25.6	20.6	na	26.5	21.3	na	26.8	23	na	na	na	na
Expt 1													
Oakdale, Site 1	25-Aug	26.3	15.9	5.1	25.7	16.0	5.3	25.7	18.1	4.3	20.3	14.7	4.3
Site 2	25-Aug	26.8	15.6	4.4	26.5	15.8	5.9	25.1	18.1	4.2	22.3	17.2	3.8
OFP Mastic, Site 1	25-Aug	20.3	15.6	5.1	24.1	15.7	7.1	24.1	15.8	4	24.0	15.7	7.1
Mastic, Site 2	27-Aug	26.5	19.5	8.5	27.3	19.3	9.8	24.9	19.3	6.6	21.7	21.0	6.1
	27-Aug	25.4	19.1	4.8	24.1	15.9	3.5	25.2	19.2	6.1	21.5	20.1	3.5
Expt 2													
Oakdale, Site 1	1-Sep	20.3	14.7	4.3	21.3	13.5	4.1	23.7	14.1	6.3	22.7	18.0	4.6
Oakdale, Site 2	1-Sep	22.3	17.2	4.8	22.7	15.8	10.4	24.0	16.1	6.6	23.0	17.6	5.1
OFP	1-Sep	21.6	26.1	6.5	21.1	25.3	6.2	na	na	na	22.6	30.4	10
Expt 3													
Oakdale, Site 1	8-Sep	23.0	18	5.0	22.9	18.6	7.1	na	na	na	23.1	12.7	5.3
Oakdale, Site 2	8-Sep	23.0	17.6	5.1	22.9	18.5	6.2	na	na	na	23.1	12.2	5.7
Flanders	8-Sep	22.9	21.3	9.2	20.5	20.3	7.0	na	na	na	15.3	12.3	5.1
OFP	8-Sep	22.6	30.4	10	22.4	29.5	14.4	na	na	na	22.1	27.1	5.6
Expt 4													
Oakdale, Site 1	15-Sep	23.1	12.7	5.1	24.8	13.3	6.8	na	na	na	22.3	16.5	4.3
Oakdale, Site 2	15-Sep	23.1	12	5.7	24.6	14.4	7.3	na	na	na	22.4	16.2	4.3
Flanders	15-Sep	15.3	23.1	5.1	24.4	10.2	5.7	na	na	na	21.1	24.8	5.1
OFP	15-Sep	23.7	25	7.1	24.2	28.1	7.2	na	na	na	20.3	25.5	6.9

After 24 h, minnows at site 1 at Oakdale displayed 93% survival with 27 alive and 2 dead (Table 2). Site 2 at Oakdale had a survival rate of 95% with 52 alive and 3 dead (Table 2). OFP had an 89% survival with 49 alive and 6 dead (Table 2). Mastic, site 1 had 97 % survival with 36 alive and 1 dead, while site 2 survival was 95% with 36 alive and 2 dead (Table 2). Growth rates were measured at the end of the first experimental series (Fig 3). Oakdale site 2 had a growth of 0.20 ± 0.07 cm week⁻¹ (Fig 3), OFP growth rates were 0.22 ± 0.08 cm week⁻¹, the Mastic site 1 growth rate was 0.24 ± 0.11 cm week⁻¹ and at site two in Mastic, the growth rate was 0.06 ± 0.05 cm week⁻¹ (Fig 3). Growth rates at Mastic site 2 were significantly lower than concurrent rates in OFP and Mastic site 1 ($p < 0.05$; Fig 3). Further analysis indicated growth rates at Mastic site 2 were significantly lower than the mean rates observed at OFP and Flanders through this study (OFP(s) and Flanders(s); $p < 0.005, 0.001$; Fig 3).

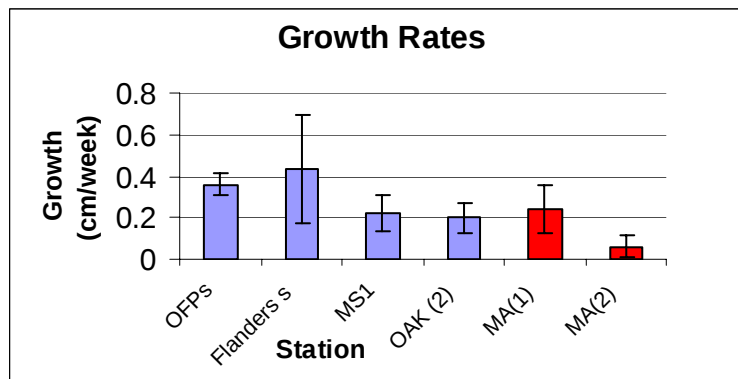


Figure 3: Growth rates shown for 1st experiment. The first two columns, OFP(s) and Flanders(s), are means for entire study. The red columns represent the application of resmethrin in the Mastic salt marsh. N = 4 for all columns except OFP (s) and Flanders (s) which were n = 20 and 8, respectively.

Experiment 2:

The experiment began the week of 1 Sept during which Oakdale was subjected to an aerial application of methoprene. Two sites were monitored within the system (Fig 1) and OFP was used as the concurrent control for this period.

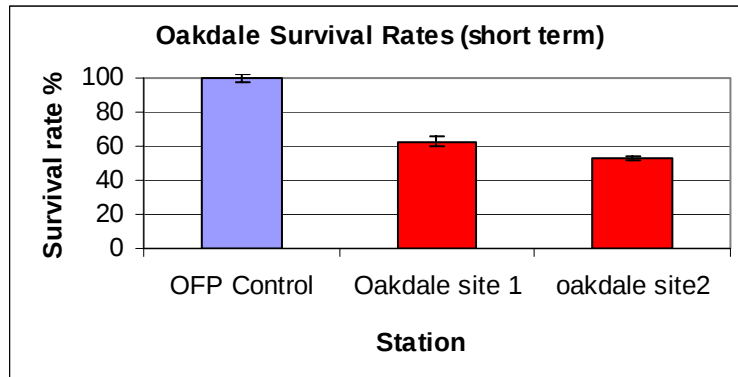


Figure 4: Survival rates during the second series of experiments. The red bars indicate the application of methoprene. OFP was the control. Site 1 and site 2 resulted with 62% and 52% survival respectively.

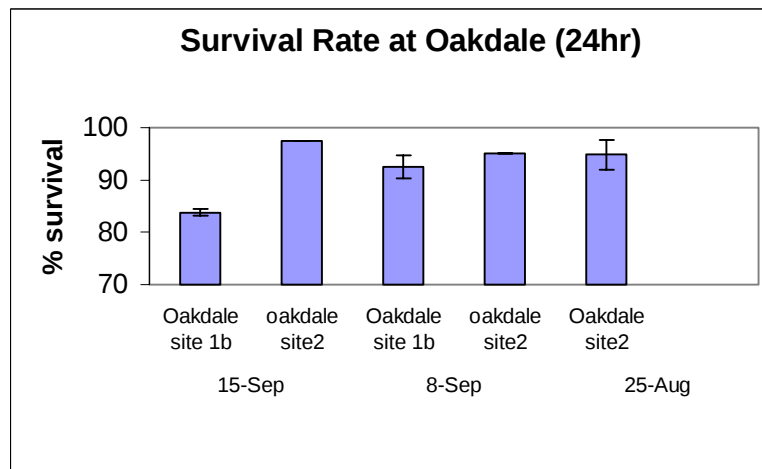


Figure 5: A comparison of the experiments in Oakdale when no pesticide was applied. All dates resulted with survival rates 85% or higher.

Twenty four hours after the application (A. Cohen, pers. obs.), site 1 at Oakdale had a survival rate of 62% with 25 alive and 15 dead (Figure 4). Site 2 percent survival of minnows was 52% with 20 alive and 18 dead (Figure 4). Concurrently, OFP had significantly higher survival (100%; $p < 0.001$; Fig 4). The survival rates in Oakdale following this event were also lower than all subsequent experimental deployments there (Fig 5). After one week, the second experimental series growth rates at Oakdale were 0.14 ± 0.08 cm week⁻¹ at site 1 and 0.18 ± 0.08 cm week⁻¹ at site 2 (Fig 6), while minnows in OFP yielded a growth of 0.32 ± 0.09 cm week⁻¹ (Fig 6). Statistical tests showed growth rates in OFP to be significantly greater than sites 1 and 2 in Oakdale ($p < 0.05$, for each; Fig 6). The mean growth rates recorded at both Flanders and OFP

throughout the study were both significantly greater than Oakdale site 1 ($p < 0.01$, 0.05 respectively, Figure 6). The mean growth rates at Flanders during the entire study were also significantly greater than site 2 in Oakdale ($p < 0.05$, Fig 6).

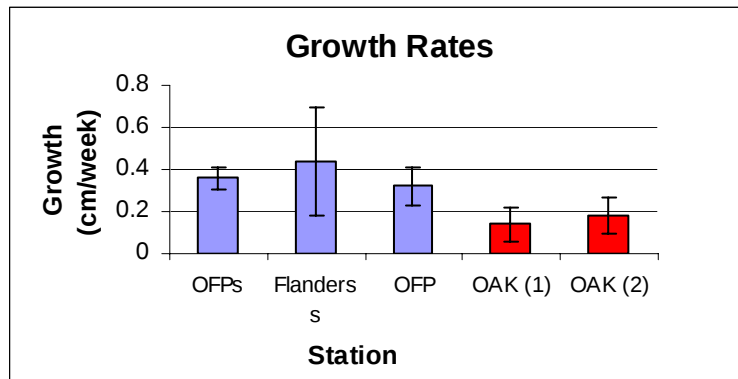


Figure 6: Growth rates represented for second series of experiments. OFP(s) and Flanders (s) are averages for entire duration of experiment. The red columns represent Oakdale with the application of methoprene. Oakdale resulted with a statistically lower growth rate than controls.

Experiment 3:

The next series started on 8 Sept and was conducted in Oakdale. The controls for this experiment were Flanders and OFP. Since Oakdale did not have an application of pesticide during this period, this trial served as a control for a non-sprayed period for Oakdale. After 24 h, Oakdale survival rates at site 1 were 92.5 % with 37 alive and 3 dead (Fig 5), while site 2 displayed survival rates were 95 % with 38 alive and 2 dead (Fig 5). OFP survival rates at 24 h were 97% with 38 alive and 1 dead (Table 2). Flanders survival rates were 100% with 40 alive and 0 dead (Table 2). After one week, OFP minnows had a growth rate of 0.40 ± 0.27 cm week⁻¹ while Flanders minnows yielded growth of 0.42 ± 0.18 cm week⁻¹ (Table 4). Oakdale growth rates were not recorded.

Experiment 4:

The final series of experiments were conducted on 15 Sept at Oakdale. The controls for this experiment were Flanders and OFP. Since Oakdale did not have an application of pesticide during this period, this trial served as a control for a non-sprayed

period for Oakdale. Twenty four hour survival rates for minnows at site 1 in Oakdale were 84% with 38 alive and 1 dead, while site 2 in Oakdale resulted in a survival of 97% with 38 alive and 1 dead (Fig 5). The OFP sheepshead survival rate was 92% with 22 alive and 2 dead. Flanders had a survival of 81% with 25 alive and 6 dead (Table 2). After one week, OFP minnows had growth rates 0.38 ± 0.21 cm week⁻¹ while Flanders minnows yielded growth of 0.49 ± 0.15 cm week⁻¹ (Table 4). Oakdale growth rates were not recorded.

Table 2: Percent survival rates of *C. variegatus* during cage experiments.

Experiment	Site	Date	24hr	Experiment	Site	Date	24hr
Initial Trial	OFP	8/9-8/19	100	EXP3	Oakdale		
EXP1	Oakdale				Site 1	8-Sep	93
	Site 1	25-Aug	93		Site 2	8-Sep	95
	Site 2	25-Aug	95		Flanders	8-Sep	100
	OFP	25-Aug	89		OFP	8-Sep	97
	Mastic			EXP4	Oakdale		
	Site 1	27-Aug	97		Site 1	15-Sep	84
	Site 2	27-Aug	95		Site 2	15-Sep	97
EXP2	Oakdale				Flanders	15-Sep	81
	Site 1	1-Sep	62		OFP	15-Sep	92
	Site 2	1-Sep	52				
	OFP	1-Sep	100				

DISCUSSION:

This investigation was a preliminary examination of the growth and mortality of caged *C. variegatus* during the application of pesticides within a ditched salt marsh ecosystem. To our knowledge, this project marks the first attempt to quantify the potential biological impact of such applications on sheepshead minnows in the field. Our preliminary results suggest that these field applications may have an acute, negative impact on the growth and survival of juvenile minnows.

Measurements of dissolved oxygen and temperature showed no significant differences between all times and locations during this study (Table 1). Dissolved oxygen levels were consistently over 3 mg/L (Table 1), which is required to maintain survival for most marine organisms (Diaz, 2000). The absence of continuous dissolved oxygen measurements does not allow for the possibility of transient hypoxic conditions to be discounted, however. Salinity levels at OFP were significantly higher than those at

other experimental and control sites (Table 1). Since, *C. variegatus* is known to be a halotolerant species, which grows robustly within the range of salinities observed during this study (Pillard et al., 1999), this difference was unlikely to have influenced results. The similar growth and survival rates between OFP and Flanders, despite the significantly higher salinities in OFP supports this conclusion.

Laboratory studies have shown that resmethrin can be acutely toxic to a wide range of fish and invertebrates, including *Cyprinodon variegatus* (Johnson and Finley, 1980; Hill and Camardese, 1986; Bradbury and Coats, 1989; Haya, 1989; Tietze, 1991; Paul and Simonin, 1996; Rand, 2002). Although resmethrin has a short photolytic half-life in water and is immobile in soil and biodegradable (half-life=36.5d) under aerobic conditions (Rand, 2002), most salt marsh sediments are not aerobic during the period in which pesticides are applied and the turbid nature of marsh waters are likely to slow photodegradation. During the aerial application of an adulticide to the Mastic site, growth rates of *Cyprinodon variegatus* at site one were significantly lower than rates at control sites. Survival rate did not appear to be affected (Table 2). In addition, the growth was unaffected at the first site in Mastic near the opening of the ditch (Fig 3). Clearly, the closer proximity of site 2 to the interior of the marsh would give it a more direct exposure to aerial spraying over the marsh (Fig 2). Elevation of the marsh and narrowing of the ditch may have influenced the differences seen in growth rates between sites as flushing of ditches and marshes may mitigate the impact of aerial spraying on resident fauna. It was observed that the Oakdale sites and the second site in Mastic have lower flushing rates and little influence from tidal flooding. This could facilitate prolonged exposure to higher concentrations of pesticides as opposed to a body of water that is tidally well-flushed.

Growth and survival of *Cyprinodon variegatus* was significantly lower at the Oakdale site relative to control sites following the aerial application of a methoprene (Fig 4 – 6). Methoprene is known to have toxic effects on multiple species of estuarine fish (Lee and Scott, 1989; Kidd and James, 1991; Ross et al, 1994; USNLM, 1995) and invertebrates (USEPA, 1982; Olmstead and LeBlanc, 2001). Interestingly, some studies

have found methoprene has a negative impact on juvenile fish growth (Ross et al, 1994), but not fish survival (Ross et al, 1994; Brown et al., 2002). Other studies have found methoprene has minor effects on exposed non-target aquatic organisms (Zoecon Corp., 1974). By contrast, in our study, both juvenile fish growth and survival was significantly lower than control sites following the methoprene application in Oakdale (Fig 3 – 5). Clearly, these results warrant further examination, particularly in light on other environmental stressors in the field (see discussion below), as all previous studies were conducted in a controlled laboratory setting.

To date, several laboratory studies have indicated that pesticides applications at concentrations seen in the environment should have a low impact on non-target organisms (Zoecon Corp., 1974; Rand et al., 1999; Brown et al., 2002). The results of this study, though limited, suggest that the impact of pesticides on aquatic biota in the field could be more substantial than have been previously predicted by laboratory studies. It is worth noting that animal populations which reside in salt marshes during summer months and which receive aerial pesticide applications are also subjected to a series of other environmental stresses which may concurrently impact their physiology. For example, since most Long Island salt marshes are bordered by residential areas, they receive terrestrial run-off which will contain varying levels of anthropogenic contaminants (Sharp et al., 1984). Moreover, the organically enriched nature of ditched regions of salt marshes are likely to have anoxic sediments during summer, which are likely to prevent the complete degradation of pesticides such as resmethrin (Rand et al., 2002) and are also likely to simultaneously release toxic trace metals (Sunda and Huntsman, 1998). These factors, in combination with high summer temperatures and lower summer dissolved oxygen (Diaz, 2000) are likely to combine to present marsh residents with multiple physiological stressors (Heugens et al. 2001) which are likely not mimicked in simple laboratory-based bioassay experiments.

The scope of this study was limited, as only two aerial spray events were encountered. However, the observed reductions of minnow grow and survival during

spray events were statistically significant (Fig 3 – 6), a result clearly warranting further investigation. Replication of these experiments is needed in conjunction with more frequent sampling of physiochemical characteristics and measurements of marsh flushing rates. Moreover, knowledge of concentrations of pesticides and other contaminants in biota and water in salt marshes during experiments will facilitate a better understanding of the link between experimental results and spray events.

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