

**ACROLEIN ON AQUATIC ECOSYSTEMS  
IN TULE LAKE NATIONAL WILDLIFE REFUGE**

Elaine Snyder-Conn  
U.S. Fish and Wildlife Service  
Klamath Falls Fish and Wildlife Office

1997

## INTRODUCTION

A strategic junction in the Pacific Flyway, the Klamath Basin has historically received the largest concentration of migratory waterfowl in North America, as many as 5-6 million ducks and geese. The basin is also heavily used by threatened bald eagles (*Haliaeetus leucocephalus*), including 2 - 8 nesting pairs (U.S. Fish and Wildlife Service 1995) and about 300 - 900 overwintering bald eagles, depending on year (Dr. David Mauser, U.S. Fish and Wildlife Service, unpublished). A large fraction of these bird populations forage in Lower Klamath and, to a lesser extent, Tule Lake National Wildlife Refuges (NWRs).

Two endangered species of fish, the Lost River (*Deltistes luxatus*) and shortnose (*Chasmistes brevirostris*) suckers, are also restricted to the Klamath Basin. The smallest known population of these suckers, estimated at 250 adult fish, reside in Tule Lake sumps 1A and 1B, in the Tule Lake NWR. Tule Lake populations of these suckers are largely isolated from other populations as a result of the Anderson-Rose Dam to the north and irrigation pumps at the outlet of Tule Lake. The low population size of the endangered suckers at Tule Lake has been attributed to a number of factors, but primarily habitat loss, specifically the loss of deep water habitat. Tule Lake has been reduced from an historical 40,470 ha to 5,383 ha to provide for irrigated farm acreages, and average depths have been reduced from 3-10 meters to approximately 1 meter as a result of both lake draining and siltation. In addition to the loss of this habitat, fish passage to historic spawning areas is blocked by the Anderson-Rose Dam, although some spawning activity has been documented immediately below the dam. Also, the introduction of non-native fish species has probably contributed to reduced sucker populations (Littleton 1993).

Both poor water quality upstream of Tule Lake NWR and the shallow water conditions of Tule Lake have, for many years, contributed to poor water quality, including low dissolved oxygen and high concentrations of ammonia (Dileanis et al.1996). More than 50 different pesticides are used by refuge farmers and additional, more toxic pesticides, such as aldicarb, are used on private agricultural lands adjacent to and upstream of the Tule NWR. The effects of these pesticides on aquatic species is unknown; previous pesticide studies have not established any clear link with acute or chronic effects in aquatic systems (Sorenson and Schwartzbach 1991; Dileanis et al.1996). However, in two instances, applications acrolein (Magnicide-H) upstream or in an adjacent canal preceded fish kills (Littleton 1993; Snyder-Conn, unpublished). This pesticide is used by the irrigation district to clear canals of submergent and emergent vegetation. It is an extremely volatile compound with a half-life ranging from about 14 hours to 92 hours in water depending on pH, water temperature, and turbulence. Its unstable nature produces several metabolites, some of which are themselves either volatile or common in natural waters and therefore difficult to assess: oxalic acid, malonic acid, glycidol, 3-oh propionic acid, lactic acid, glycerol, 1,3-propanediol, propionic acid, glyceric acid, bicarbonate, propionic acid, and propanol (Haag, 1988).

Acrolein is an extremely hazardous substance with chronic and acute toxicity concentrations to freshwater aquatic life occurring in the parts-per-billion range

(reviewed by Eisler 1994). For example, the median lethal concentration of acrolein at 96 hours ( $LC_{50}$ ) acutely toxic to *Catostomus commersoni* (white sucker) is 14  $\mu\text{g/L}$  (Holcombe et al. 1987 in Eisler 1994). Common invertebrate species, including *Daphnia magna* (water fleas) and *Ephemerella walkeri* (mayflies) are also susceptible at 83  $\mu\text{g/L}$  (48-hr) and 100  $\mu\text{g/L}$  (0.042 days), and amphibians appear even more sensitive; the clawed frog, *Xenopus laevis*, has a 96-hr  $LC_{50}$  of 7  $\mu\text{g/L}$  (Eisler 1994). Despite, the potential toxicity of acrolein, most studies in refuge waters have not demonstrated toxicity to aquatic life (Winchester 1994; Dileanis et al. 1996). However, in one case studied, concentrations of acrolein sufficient to induce chronic toxicity were observed. It is uncertain whether this case was representative, since this application did not occur during the irrigation season.

The objective of this study was to evaluate the potential impacts of ambient water quality and pesticides on aquatic invertebrates and fish in the Tule Lake NWR, with a primary focus on the potential effects of applications of acrolein in waters upstream of the refuge on refuge aquatic fauna. Our studies consisted of water quality monitoring (water temperature, dissolved oxygen, conductivity, pH, and turbidity), nutrient monitoring (total nitrogen, total phosphorus, and ammonia), pesticide monitoring of water samples (organophosphate and carbamate pesticides), and volatile organic compounds of water samples (including the pesticide, acrolein). Prior to initiation of the studies, we verified the USGS's sample preservation methods for acrolein by spiking Klamath Basin (Lost River) water for various time intervals (Sandstrom and Snyder-Conn, in prep.). To evaluate the impacts of ambient water quality and pesticide concentrations on aquatic biota, we employed 96-hr *in situ* bioassays using the waterflea *Daphnia magna*, fathead minnows *Pimephales promelas*, and the pulmonate snail, *Planorbella piersoma subcrenaum*.

## **Study Area and Acrolein Treatments**

The study area was located near the Oregon-California border, in Oregon's Klamath County and California's Modoc and Siskiyou counties. Study sites included 1) an upstream reference site along the J-Canal approximately three kilometers below the Anderson-Rose Dam, north of the Tule Lake NWR; 2) a site at the border of Tule Lake NWR below the acrolein applications (either on or adjacent to the J-Canal, along drain 46-B or near Pump 24; and 3) a site on Tule Lake Sump 1A at Pump 10 (Figure 1). All study sites were within agricultural areas in and adjacent to the refuge. Site locations were selected along the J-Canal based on scheduled treatment of this canal with acrolein during the study period. The reference site was upstream from any scheduled acrolein applications in an area with horse pastures, rather than active cropland, immediately adjacent to the canal. Few, if any, pesticide residues were expected at this site. Descriptions of the study sites and sample dates are presented in Table 1. The acrolein application schedule of the Tule Lake Irrigation District (TID) for sites on the J-Canal is presented in Table 2.

**Table 1. Toxicity test site attributes and monitoring dates.**

**Table 2. Tule Lake Irrigation District acrolein applications corresponding to toxicity testing dates.**

## MATERIALS AND METHODS

### Flow-Through Containers

Bioassay containers were constructed using 5.1 cm (inner diameter) polyvinyl chloride (PVC) compressor couplings with an approximate volume of 520 cm<sup>3</sup> (with the rubber seals removed) (Figure 2). Both ends of the coupling were covered with monofilament nylon mesh (105  $\mu$ m for *Daphnia*, 250  $\mu$ m for fathead minnows, and 500  $\mu$ m for snails)(Aquatic Eco-Systems, Apopka, FL), fastened by tightening the endcaps. Mesh size was selected to insure containment of organisms while maximizing flow and protecting specimens from potential predators. The screening also allowed accessibility of prey organisms. Three plastic cable ties were attached to each container, forming a loop for attachment to the *in situ* structure. When in transport, the bioassay couplings were set in clean, 2-liter plastic containers filled with filtered water (75-  $\mu$ m mesh) from an upstream location. Both couplings and 2-liter containers were labeled for site and species with a permanent marker.

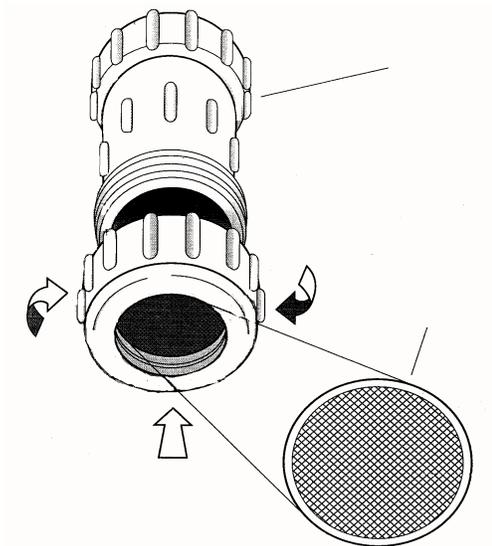


Figure 2. In situ toxicity test chamber, made using a 5-cm PVC compressor coupling with Nitex<sup>®</sup> screening on both ends. Mesh sizes 105, 250, and 500  $\mu$ m were used for *Daphnia*, fathead minnow, and snail tests respectively. (A) PVC coupling (B) Nitex<sup>®</sup> screen 105, 250, or 500  $\mu$ m mesh.



## Transport Procedures

Bioassay organisms were transported from holding aquaria into PVC containers using a Hach 1-10 ml Ten Sette pipette with sterile plastic tips. The openings of the plastic tips were size-adjusted to reduce potential injuries to the respective organisms. Specimens were transported to small waxed cups as an intermediate stage for checking counts of organisms. Twenty organisms (*Daphnia* or fathead minnows) or 10 pulmonate snails were added to each container. Care was taken to avoid exposing *Daphnia* to air or to the water/air interface where tiny air bubbles could get trapped under their carapace, inducing anomalous floating behavior.

Bioassay containers were placed in large and medium Coleman coolers, each with a single ice pack to prevent overheating and the related stress mortality during transport. All containers were secured within the coolers to prevent excess movement. Care was also taken during loading, unloading, and driving to minimize movement. Two replicate containers of each species were scheduled for removal at 24-, 48-, and 96- hour intervals. Upon removal, organisms were visually assessed for movement, mortality and general health (naked eye or microscope as needed). *Daphnia* and fathead minnows were transferred out of the bioassay containers using a Ten Sette pipette, into intermediate waxed cups for inspection and counting. A dissecting microscope was used for close inspection of immobile *Daphnia*. The mortality of snails was determined by foot movement and ability to adhere to surfaces.

## *In-situ* Structures

Bioassay containers were grouped together and held in place by metal "H" or "T" shaped, stainless steel, fixed structures. Sheet metal bars (1.8 m) with consecutive holes were crossed. Rigid right angles were maintained with 0.95 cm nuts, bolts, and 0.3 m pieces of sheet metal at approximately 45° angles. Upright structures were imbedded into the sediment as deeply as needed to insure stability and maintain appropriate depth for the organisms. Because acrolein is less dense than water and volatile, canisters were always placed in the upper 30 cm of the water column. The horizontal bar on each structure was aligned with the current. S-hooks (two per container) were attached along the length of the bar through the holes and damped closed with a vice.

The plastic loop of the bioassay containers then fit over the open ends of two S-hooks. Six *Daphnia* and six fathead minnow containers were aligned along the length of each structure. The six snail containers were attached in tandem (pairs) to the downstream side of the structure with fishing line. Snail containers were vertically encompassed in square (25.4 cm x 25.4 cm) foam board with the top 1/5 of the coupling out of water. This provided the pulmonate snails with air to breathe at the water/air interface. Both the structure and the trailing snail containers were covered with a olive green/brown cotton cloth for camouflage.

## Bioassay Organisms

*Daphnia magna* neonates; fathead minnow larvae, *Pimephales promelas*; and the pulmonate snail, *Planorbella pterosoma subcrenaum* (Frest, 1997) were used for in situ toxicity tests. *Daphnia* and fatheads were air-exposed by overnight mail from Aquatic

Bio-Systems, Inc. Less than 24-hour-old young were sent from the distributors. Pulmonate snail specimens were collected from an upstream location and varied in both size and age (0.2-1.5 cm diameter). Snails were collected by hand from docks at the Crystal Springs boat ramp. At the time of insertion, *Daphnia* were 150-200  $\mu$ m and fatheads were about 1 mm in length (48-72 hours old).

## **Bioassay Culture Maintenance**

Organisms were held in 3.8-liter acrylic aquariums. Shipping containers with bioassay organisms were acclimated for temperature and water quality for 35-40 minutes. First, organisms were floated for 15-20 minutes in open containers to allow exposure to oxygen and for temperature acclimation prior to introduction into upstream Lost River water in the aquaria. Then small amounts of water from the holding tanks were added every 5 minutes for an additional 20 minutes for water quality acclimation. Specimens were then held for approximately 24 hours in aquaria before placement in the field to allow for transport mortality.

The aquarium water was filtered through 75- $\mu$ m nylon mesh. Lost River water from a location upstream of the study sites was collected within 24 hours of expected organism deliveries for use in the aquaria. *Selenastrum capricornutum* microalgae ( $3.0 \times 10^7$  cells/ml x 20 ml) were added to provide a green water culture for both the *Daphnia* and minnow larvae to regulate water quality and provide food for both species at that age. Twenty-four hour *Artemia* were also fed *ad libitum* to the minnow larvae to supplement their diet. Aeration was provided by an air pump and air stone with larger pores set near the surface to provide light agitation, while protecting organisms from damaging exposure to air bubbles. The temperature of aquaria was maintained between 19.5-20.7°C in an air-conditioned office to avoid overheating and keep temperature fairly constant. The water temperature was checked occasionally with a digital thermometer.

*Artemia* were hatched in an aerated 18.8-liter, plastic carboy in a 20-ppt saline solution maintained at 28°C. Fifty milliliters of *Artemia* cysts were added to the saline solution. Post-hatch *Artemia* were fed 2.5 g activated Baker's yeast (Fleischman's) initially, with quantities adjusted thereafter based on water quality. Water exchanges were made as needed in the *Artemia* culture.

## **Water Quality Monitoring**

Temperature, conductivity, dissolved oxygen (DO), pH, and depth were measured with a Hydrolab H20® Water Quality Multiprobe for instantaneous, on-site readings. A Surveyor® 3 display logger indicated parameter readings.. Hydrolab Datasonde® 3 and Datasonde® 4 units were also continuously deployed at the upstream reference site and at the Tule Lake site for the two 96-hour studies to record parameter changes over time.

Cleaning and calibration of all Hydrolab units were performed according to the manufacturer's recommendations (Hydrolab 1991). For cleaning, all probes and exposed casings were swabbed with cotton Q-tips and 95% isopropyl alcohol, while rinsing regularly with de-ionized (DI) water. The DO membrane and electrolyte fluid were replaced as needed. The pH probe was covered and soaked in reference electrolyte when not in use. The calibration and programming of all units was performed through an IBM PC hyperterminal (ProCom).

Conductivity calibrations were performed using a 700  $\mu\text{S}/\text{cm}$  standard, similar to conductivities in the study area. The pH was then calibrated with both 7.02 and 10.05 standard buffers at 20°C. The DO was calibrated based on the air saturation, temperature, and barometric pressure. All units were calibrated within 48 hours of expected field use. Drift was recorded within 48 hours after each run was completed. Calibration and drift of all units were recorded on data sheets. The drift by parameter of each hydrolab unit was graphed versus the amount of time used. Field data were adjusted for drift using measurements from hydrolabs *in situ* compared well with a second Hydrolab used for intermittent measurements of water quality at the refuge border site, and also transported among all sites for instantaneous comparisons of readings.

Flow was also measured and recorded with a Global Flow Probe FP101 flowmeter using integrated measurements over a one minute time interval. Latitude and longitude coordinates were taken with a hand-held, global positioning system (GPS) unit. Three replicate turbidity samples were taken, at each site, every 24 hours with a Hach Ten Sette pipette through sterile plastic tips and measured with a Hach 2100P turbidimeter. The instrument was calibrated with four Hach Stablecal turbidity standards immediately prior to this study. Daily calibration checks of the turbidimeter was performed with a fresh 4.34 NTU StableCal stabilized formazin standard. Total settleable solids (TSS) were also measured daily at each site using the Imhoff cone method (Greenberg 1995).

## **Invertebrate Sampling**

Both planktonic and benthic invertebrates were sampled at 24- and 48-hour intervals at each site. Six tows of a weighted 20.3 cm diameter, 243- m plankton tow net, each 10 m in length, were composited to represent the free-swimming invertebrate biodiversity.. The plankton sample was then held vertically and the sides of the net were rinsed to flush invertebrates into the connected collection bottle. Invertebrates were preserved with 50% isopropyl alcohol.

Benthic macro-invertebrates were sampled with a 1.5 meter kick net. The net skimmed the sediment in approximately one meter, S-shaped motions. Six replicates were composited and rinsed thoroughly with water to remove excess sediment. Algae and other extraneous matter were removed by hand. The remaining invertebrates were placed in a labeled whirlpak and preserved in 50% isopropyl alcohol.

## RESULTS

### Water Quality

Water quality measurements were taken in the first (late July) and second (mid-August) runs instantaneously, twice per day, over a 96-hour period and continuously with *in situ* hydrolabs for the second run. Data comparisons between the two *in situ* hydrolab water quality profiles at the J-Canal reference and Tule Lake sites show several unusual trends. Temperature (Figure 3) fluctuations varied greatly; the reference site experienced smaller, more regular fluctuations of about two degrees Celsius, while the Tule Lake site exhibited large, less regular fluctuations within a six-degree Celsius range. Water temperatures at the reference site routinely peaked at about 11:00 PM and reached minima near 9:00 AM, whereas the lake site reached maximum temperatures near 5:00 PM and minima around 8:00 AM.

**Figure 3. Comparison of J-Canal reference site, Tule Lake Pump 10, and J-Canal Pump 24 temperatures from August 13 - August 17, 1997.**

Instantaneous data from the J-canal Pump 24 site is also shown. Paired t-test results of the continuous water quality data showed no significant difference between the J-Canal reference site and Pump 10 at Tule Lake. A comparison of the instantaneous data for all three sites during the first run showed a significant differences between the J-Canal reference site and drain 46-B ( $P = 0.014$  for morning and  $P = 0.048$  for afternoon temperatures); between Drain 46-B and Tule Lake Pump 10 ( $P = 0.004$  for afternoon

temperatures); and between Tule Lake Pump 10 and the J-Canal reference site ( $P = 0.003$  for morning and  $0.028$  for afternoon temperatures). During the second 96-hr study run, there were significant differences only for the afternoon temperatures (reference site and Pump 24,  $P = 0.006$ ; and the reference site and Tule Lake Pump 10,  $P = 0.011$ ). There was no significant difference observed between J-Canal Pump 24 and the Tule Lake Pump 10 sites.

Also, the two locations exhibited very different dissolved oxygen concentrations (Figure 4). A fairly regular sine curve was observed in the reference site measurements with all dissolved oxygen concentrations being low (range 2.27-4.58 mg/L), unlike the lake site which displayed a fairly irregular pattern (range 0.34 - 9.56 mg/L). The highest DO concentrations at the reference site were consistently observed near 12:00 am and the lowest at 9:00 AM, which differed from the lake site with peaks at various morning hours.

**Figure 4. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal Pump 24 dissolved oxygen levels from August 13 - August 17, 1997.**

A paired t-test of the continuous water quality data at the reference site and Tule Lake Pump 10 showed significant differences for both morning and evening DO concentrations ( $P = 0.001$  and  $P = 0.028$  respectively). Instantaneous readings during the first run were significant in the morning only between Drain 46-B and the J-Canal reference site with  $P = 0.027$ . Afternoon readings between Drain 46-B and the J-Canal reference site ( $P = 0.010$ ) and between the J-Canal reference site and Tule Lake Pump 10 ( $P = 0.011$ ) were also significant. There was no significant difference between Tule Lake Pump 10 and Drain 46-B. Second run instantaneous DO concentrations were significantly different in the morning between Pump 24 and the J-Canal reference site ( $P = 0.002$ ) and between the J-Canal reference site and Tule Lake Pump 10 ( $P = 0.004$ ). Afternoon readings showed a respective significance of  $P = 0.004$  and  $P = 0.044$  for the two above sites.

The pH concentrations at both locations differed significantly (Figure 5). The reference site had a range of 6.99 - 7.22 pH units and the lake site had a range of 7.83 - 9.08 pH units. Although both series of measurements show constant trends, the reference site experienced only minor fluctuations of less than 0.5 pH units, while the lake site varied a full pH unit. Statistical analyzes of the continuous measurements with a paired t-test showed a highly significant difference between the reference site and Tule Lake at Pump 10 ( $P = 0.000$ ). All instantaneous, first run, morning pH concentrations between sites were significant (Drain 46-B paired with J-Canal reference site,  $P = 0.028$ ; Drain 46-B paired with Tule Lake Pump 10,  $P = 0.028$ ; and J-Canal reference site paired with Tule Lake Pump 10,  $P = 0.018$ ). The afternoon readings showed  $P = 0.002$  for Drain 46-B and J-Canal reference site, and  $P = 0.010$  for Tule Lake Pump 10 and the J-Canal reference site. There was no significant difference between Drain 46-B and Tule Lake Pump 10 for the afternoon pH readings. Paired T-tests for the second run showed significant differences in morning pH concentrations between the J-Canal reference site and Pump 24 ( $P = 0.009$ ) and between the J-Canal reference site and Tule Lake Pump 10 ( $P = 0.002$ ). Afternoon pHs were significant between J-Canal reference site and Pump 24 ( $P = 0.003$ ) and between J-Canal reference site and Tule Lake Pump 10 ( $P = 0.001$ ).

**Figure 5. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal Pump 24 pH concentrations from August 13 - August 17, 1997.**

Specific conductance measurements showed no regular patterns over time (Figure 6). Both sites exhibited small conductivity ranges. The reference site displayed small gradual changes whereas the lake site went through abrupt hourly fluctuations. Conductivity ranged between 186 - 218  $\mu\text{S}/\text{cm}$  for the reference site and 366-473  $\mu\text{S}/\text{cm}$  at Tule Lake. A paired T-test for the continuous readings showed a significance of  $P = 0.000$  for J-Canal reference site and Pump 10 at Tule Lake. All (morning and evening) first run instantaneous readings were significant between all sites; J-Canal reference site and Drain 46-B,  $P = 0.001$ ; J-Canal reference site and Tule Lake Pump 10,  $P = 0.000$ ; and Drain 46-B and Tule Lake Pump 10,  $P = 0.000$ . Second run conductivities showed similar statistical significance ( $P = 0.000$  for J-Canal reference site and Tule Lake Pump 10, and  $P = 0.000$  for Pump 24 and Tule Lake Pump 10) except for J-Canal reference and Pump 24 which were almost significantly different at  $P = 0.060$ .

**Figure 6. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal Pump 24 specific conductance levels from August 13 - August 17, 1997.**

Turbidity measurements were taken during both runs once per day. None of the pairs for the t-test resulted in significant differences for the first run. Only the J-Canal reference site paired with Pump 24 during the second run showed significant difference ( $P = 0.040$ ).

## **Mortality**

Mortalities of all bioassay organisms were assessed at 24-, 48-, and 96-hour intervals for *Daphnia* (Table 2), fathead minnows (Table 3), and snails. Insignificantly low numbers or zero snail deaths occurred for all extraction periods, sites and runs, therefore, graphic representation is unnecessary. *Daphnia* mortalities for the first run are shown in Figure 7. No logical progression trends of mortality were observed. The percent mortality in the J-

Canal and Tule Lake decrease with increasing time in the bioassay containers and intended exposure to pesticides. The 46-B Drain experienced lowest mortality at 48 hours. A Friedman Rank test resulted in  $X^2 = 4.000$  and  $P = 0.135$ . A Wilcoxon's Signed Rank test yielded  $Z = -0.447$  and  $P = 0.665$  at the reference site,  $Z = -1.604$  and  $P = 0.109$  at Drain 46-B, and  $Z = -1.342$  and  $P = -0.180$  at Tule Lake Pump 10.

**Figure 7. Comparison of J-Canal reference, Tule Lake Pump 10, and Drain 46-B Daphnia percent mortality from July 30 - August 2, 1997.**

Fathead minnows experienced difficulties in culturing and transport procedures and were not distributed to all sites; no valid comparison could be made.

During the second run, improved culturing transport methods resulted in better survival, distribution and representation of both species. However, only irregular mortality trends for Daphnia were observed. J-Canal mortalities were highest at the 24-hour interval and lowest at the 48-hour intervals. At pump 24, highest mortality occurred at the 48-hour extraction period, lowest at 24 hours. The Tule Lake site showed lowest mortalities at the 96-hour assessment and highest at the 48-hour interval (Figure 8). A Friedman Rank analysis yielded no significant differences,  $X^2 = 2.667$  and  $P = 0.264$ . Similarly, no significant difference in Daphnia mortality was yielded by the Wilcoxon's Signed Rank test,  $Z = -1.069$  and  $P = 0.285$  for the reference site,  $Z = -1.604$  and  $P = 0.109$  for Tule Lake Pump 10, and  $Z = -1.604$  and  $P = 0.109$  for J-Canal Pump 24.

**Figure 8. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal pump 24 *Daphnia* percent mortality from August 13 - August 17, 1997.**

Second run fathead minnow mortality is illustrated below (Figure 9). The J-Canal reference site shows a gradual decreasing mortality percent over time. At pump 24, 48-hour mortality is slightly higher than 96-hour mortality with the lowest observed at the 24-hour extraction. The Tule Lake site exhibits a gradual increase in percent mortality over the first 48 hours and shows a 100% mortality at the 96-hour interval. Regardless of graphical representations, there was no statistically significant differences shown by either method. Friedman Rank analysis,  $X^2 = 4.667$  and  $P = 0.097$ ; Wilcoxon's Signed Rank test,  $Z = -1.069$  and  $P = 0.285$  for Tule Lake Pump 10,  $Z = -1.604$  and  $P = 0.109$  for J-Canal Pump 24, and  $Z = -1.069$  and  $P = 0.285$  for the J-Canal reference site.

**Figure 9. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal Pump 24 fathead minnow percent mortality from August 13 - August 17, 1997.**

## **Pesticides**

No acrolein was detected in any sample, except for field spikes in which recovery exceeded 80%. Pesticide results have not yet been received from Patuxent Analytical Control Facility.

## **Ammonia**

**To be added.**

## **Invertebrate Diversity and Abundance**

**To be added**

## **DISCUSSION**

This study did not disclose any acrolein in refuge waters and patterns of *Daphnia magna* and fathead minnows mortality were not consistent with expectations from any acrolein-induced mortality. If acrolein had been responsible for observed mortalities, consistently higher mortality should have been observed in refuge border sites, closer to the acrolein application sites, rather than at Tule Lake. Moreover, fathead minnows would be predicted to experience mortality more readily than *Daphnia magna* at the refuge border sites.

Nor were other pesticides found in significant concentrations (Dr. John Moore, Patuxent Analytical Control Facility, pers. comm.). However, our study did not include analysis of glyphosate, and unbeknownst to us, the herbicide, Rodeo (active ingredient, glyphosate), was applied to control loosestrife on refuge lands immediately adjacent to Tule Lake sump 1A occurred on August 14. This application, on the west side of Sump 1A [not near our study site] may have contributed to the observed fish mortalities both in our *in situ* containers, where 100% mortality was observed by August 17 (96 hours). Also, between August 14 and August 17, approximately 25-35 fish (fathead minnows, tui chubs, Sacramento perch) were observed floating dead at Pump 10 on Tule Lake. During this period, numerous other fish (fathead minnows) were observed flashing, an indication of distress. However, no dead fish were observed at two other Tule Lake sites during the the fish kill, suggesting that poor water quality alone at our study site (including dissolved oxygen levels of 0.0 mg/L) is the more likely explanation for the observed mortalities. In the future, to avoid even the possibility of Rodeo impacts, we recommend applying this herbicide in the spring, when dissolved oxygen concentrations in the lake are acceptable.

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