

**APPENDIX C - CBL FLUORIMETRY AND BIOASSAY REPORT
(APPENDIX IS PENDING SUBMITTAL FROM INVESTIGATORS)**

Laboratory tests of the effect of oil from the Swanson's Creek oil spill on survival and development of striped bass (*Morone saxatilis*) yolk sac larvae.

Principal Investigators

Dr. Denise Breitburg and Dr. Gerhardt Riedel
The Academy of Natural Sciences
Estuarine Research Center
10545 Mackall Rd.
St. Leonard, MD 20685

At the request of Maryland Department of Natural Resources (DNR), and with the approval of the Trustee group once it was formed, The Academy of Natural Sciences Estuarine Research Center (ANSERC) tested oil effects on survival, growth and development of striped bass (*Morone saxatilis*) yolk sac larvae. Scientists at DNR were concerned that fish larvae, including those of striped bass, could have been in the affected area at the time of the oil spill, and during the period following the spill that oil was present in the nearby Patuxent River and its tributaries. Two experiments were conducted, one using water collected from Swanson's Creek (referred to below as the Swanson's Creek or SC water test) and one using dilutions made from a sample of the oil that had leaked from the pipeline (referred to below as the 'fresh oil' test). This report describes the experiments conducted and results of those experiments.

Methods

Toxicity Tests Using Water Collected From Swanson's Creek

Oil polluted water was collected from under the oil slick in Swanson's Creek on the afternoon of April 13, 2000, using a high volume diaphragm pump, and a polyvinyl chloride hose. The water was collected in 8 L glass carboys, which had been cleaned using laboratory soap and rinsed with deionized water. The carboys were filled to the brim, and sealed with aluminum foil to minimize loss of petroleum constituents by evaporation. The salinity of this water was measured using a YSI salinometer and determined to be 4.3 ppt. To make a natural water of the same salinity for diluting the oil affected water, water was collected from two unaffected sites on the Patuxent River on April 14. One site was in the freshwater well upriver of the oil slick at upper Marlboro, and the other was in St. Leonards Creek near ANSERC. A different pump and hose were used at each site in order to avoid contamination from the oil. Because oil loss was not an issue in these waters, they were collected and stored in polyethylene carboys. These two source waters were mixed in a large carboy to produce the same salinity as the water from Swanson's Creek. Dilutions (100, 50, 20, 10 and 0%) for the bioassay experiment were produced by mixing water from Swanson's Creek from the glass carboys with the prepared dilution water, using glass measuring vessels.

Newly hatched striped bass larvae were obtained from the Manning Fish Hatchery in Cedarville,

MD on 14 April 2000. The hatchery is operated by MD-DNR. At the hatchery, larvae had been maintained in well water (0 ppt salinity). Larvae were slowly acclimated to Swanson's Creek salinity over a 24 h period at ANSERC by adding dilution water and Mackall Cove water to the well water in which larvae had been transported.

Three replicates of each dilution series treatment were set up on 15 April. Experiments were conducted in 20 L aquaria filled with 15 L of the appropriate water. Thirty 2-d posthatch yolk sac striped bass larvae were added to each tank. Temperature, dissolved oxygen and salinity were recorded daily. One-third of the volume of each aquarium was replaced each day with the appropriate Swanson's Creek water dilution.

Because larvae are small, daily mortality would have been difficult to track within the 20 L aquaria. One replicate of each treatment was therefore terminated after 48 h. The two remaining replicates were terminated after approximately 96 hr. Larvae were recovered, scored as live or dead, and preserved in 10% buffered formalin. All fish recovered live were measured and examined for gross abnormalities with the exception of 3 individuals accidentally overlooked by the technician conducting measurements and 7 individuals damaged during handling and preservation (Table 7).

A summary of ages of larvae used and dates important to the methodology follows for comparison with the fresh oil test (described below). Larvae for the Swanson's Creek water experiment hatched on the evening of 13 April, were transported to ANSERC the morning of 14 April, and were placed in experiments the morning of 15 April, at an age of approximately 42-44 h posthatch. The first group of larvae were removed on 17 April, 48h after the start of the experiment. The experiment was terminated on 19 April, 96 h after its start and when larvae were nearly 6 days posthatch.

Toxicity Tests Using 'Fresh' Oil

ENTRIX provided 500 ml of oil from the Swanson's Creek oil spill. On the day prior to the start of the experiment, 100 ml of the oil was added to each of three 9-L Pyrex carboys filled with dilution water for the experiment. Dilution water for the experiment was the same batch of 4.3 ppt salinity water prepared for the Swanson's Creek water experiment. The carboys with oil water mixture were slowly stirred with a magnetic stir bar for 24 h. The oil and water were then allowed to separate, and the water was removed by siphoning it from the bottom of the carboys. Treatments were set up in triplicate at concentrations of 50, 25, 10, 5 and 0% of the oil/water solution diluted with the Patuxent River dilution water. The oil/water carboys were then topped off with fresh dilution water to prepare solutions for subsequent water changes. Each day 1/3 of the water in each treatment tank was siphoned out and replaced with a fresh dilution of the oil/water mixture.

Our experimental system was designed to simulate the exposure of freshly hatched striped bass

to conditions prevailing at the time of the oil spill, rather than to be a test of the toxicity of oil to striped bass larvae under tightly controlled laboratory conditions. Because in the actual oil spill, oil leaked from the pipeline at essentially one time, and the oil spill subsequently moved away from the spill site driven by winds, with the concentration of oil being continually diminished by evaporation, degradation and dilution, the experimental procedure for exposure here better represents the mode of exposure experienced by larvae in the river than would constant exposure to a freshly made oil water mixture. However, precisely because of this, the exposure of individual treatments is variable in time, and there is the potential for a systematic change in the composition in the oil/water mixture.

One day posthatch striped bass larvae were obtained from the Manning Fish Hatchery in Cedarville, MD on 13 May 2000. Larvae had been maintained in well water (0 ppt salinity). Larvae were slowly acclimated to Swanson's Creek salinity over a 24 h period at ANSERC by adding dilution water and Mackall Cove water to the well water in which larvae had been transported.

All water used for acclimation and tests was from portions of the Patuxent River system that were not contaminated by oil. Water collected from the Patuxent River at Lower Marlboro for the Swanson's Creek water experiment was mixed with water from the Patuxent River collected through the ANSERC seawater intake line at St. Leonard to match the salinity of water that had been collected from Swanson's Creek on April 13.

Three replicates of each dilution series treatment were set up on 15 May. As in the Swanson's Creek water test, experiments were conducted in 20-L aquaria filled with 15 L of the appropriate water. Thirty 3-d posthatch yolk sac striped bass larvae were added to each tank. Temperature, dissolved oxygen and salinity were recorded daily.

One replicate of each treatment was ended after 48 h and the other two replicates were ended after 96 hr. Larvae were recovered, scored as live or dead, and preserved in 10% buffered formalin. Larvae recovered live from tanks maintained for 96h were measured and examined for gross abnormalities with the exception of one individual damaged during handling (Table 7).

A summary of ages of larvae used and dates important to the methodology of the fresh oil test follows. Larvae began hatching during the afternoon of 12 May, were transported to ANSERC the around noon on 13 May, and were placed in experiments the morning of 15 May, at an age of approximately 72 h posthatch. The first group of larvae were removed on 17 May, 48h after the start of the experiment. The experiment was terminated on 19 May, 96 h after its start and when larvae were approximately 7 days posthatch. The batch of larvae used in the fresh oil experiment appeared to have higher pre-experiment mortality and be less active than the SC test larvae. However, no quantitative estimates of mortality or activity were made. In addition, larvae were one day older at the termination of the experiment, increasing the potential for starvation to become a mortality source for fast-developing larvae.

Water sampling and analyses:

Pre-cleaned sample bottles (1 L amber glass bottles with teflon lid liners) were obtained from the Geochemical and Environmental Research Group (GERG) at Texas A&M University, after consultation with Dr. Terry Wade. In both experiments, samples were collected using an aluminum, glass and PVC apparatus that siphoned water out of the experimental chambers and into the sample bottles. Sample bottles were filled to the brim, and preserved with 0.2% HCl and 5-8 ml of methylene chloride, sealed with no headspace and refrigerated until the end of each experiment, at which time they were shipped overnight to GERG for analysis. Samples were assigned a serial number within each experiment, and analyzed blind. Samples from the Swanson's creek water experiment were extracted 20-23 days after collection, while samples from the "fresh oil" experiment were extracted 25-29 days after collection. In a number of cases where high concentrations of oil were present, one of the QA/QC measurements, recovery of spiked d8-naphthene were much in excess of 100%. The d8-naphthene surrogate recoveries were high in these samples due to the extremely high concentrations of other compounds from the oil, both aliphatic and aromatic. The ions used to quantify the d8-naphthalene can experience interference from ions that are only minor ions of other compounds but when the other compounds are present at very high relative concentrations they can cause interference's.

For the Swanson's Creek water experiment, samples from two of each dilution treatment were sampled on the starting day (4/15/00), and one from each dilution plus one duplicate at the end of the second day (4/17/00) and final day (4/19/00). Because of the expense of the oil analysis, and at the request of the Trustees, for the second experiment, sampling for oil analysis for Experiment 2 was cut back considerably. On the first day (5/16/00), the oil/water mixture (100%), and the pure dilution water (0%) were sampled, as well as one each of the 50, 10 and 5% dilutions, and one duplicate (10%). On the second day (5/17/00) and fourth day (5/19/00) only a single sample of the 50% and 0% were collected.

Statistical analysis of larval survival and growth

Probit analysis, which 'calculates maximum likelihood estimates of regression parameters and the natural (or threshold) response rate for quantal response data from biological assays' (Proc Probit; SAS Institute 1999) was used to examine the effect of percent Swanson's Creek water or fresh oil dilution on larval survival. The effect of oil exposure on growth was determined by performing linear and 2nd order polynomial regressions (Proc Reg; SAS Institute 1999) comparing the mean length of larvae at 96 h in each tank with the percent Swanson's Creek water or fresh oil dilution. This comparison utilizes larval length data from all tanks with surviving larvae regardless of the percent survival in that tank. We cannot evaluate the potential for the growth rates of survivors and growth rates of larvae that died during experiments to have differed.

Results

Oil analyses

The oil was analyzed for both aliphatic hydrocarbons and polynuclear aromatic hydrocarbons. The complete report of the analyses from GERG, including the QA/QC results are attached. We have focused on two results as our index for exposure of the fish larvae to the oil, the total PAHs, and the total petroleum hydrocarbons (TPH). Summary of the results from the Swanson's Creek water experiment are given in Table 1 and from the "fresh oil" experiment in Table 2.

Substantial variability in TPH and PAH was observed within treatments over time, and even within replicate treatments at the same time. In the experiment with water collected from Swanson's Creek (Table 1), it is clear that part of the variance is associated with changes in the concentration in the treatments with time. Some of variation in both experiments may have been caused by adsorption of oil onto particles, and non-homogeneity in the sampling of the water with respect to those particles. Because of the presence of the larval fish in the sampling containers, we were unable to thoroughly mix the experimental chambers prior to sampling. We observed that substantial particulate material that settled to the bottom of these chambers, and this material may be a substantial sink for much of the oil. In the higher concentrations, a substantial loss of both TPH and PAH occurred through the course of the experiment, particularly from the start to the end of day 2. This is likely caused by volatilization and microbial degradation of oil components from the experimental aquaria. We also saw some increase in the value for 0% dilution for total petroleum hydrocarbons, which may represent an increase into the 0 dilution by absorption from the laboratory air, or other contamination. Similar results are observed in the fresh oil experiment, (Table 2), however, the concentration results for similar dilutions were higher in the second experiment, because of the greater level of exposure to fresh oil.

Temperature and dissolved oxygen

Temperature, and dissolved oxygen data are provided in Table 3 for the Swanson's Creek water experiment and in Table 4 for the fresh oil experiment. In the Swanson's Creek water experiment average daily temperature varied during the experiment from 13.0 to 14.8°C, and remained at acceptable levels. Placement of tanks on racks within the laboratory appeared to cause slight differences among tanks, but there were no consistent differences among treatments. Similar patterns of among day and among tank variation in temperature were seen in the fresh oil experiment, in which daily temperature gradually increased from 14.8 to 15.6°C.

Dissolved oxygen in the Swanson's Creek experiment tended to decrease with increasing concentration of Swanson's Creek water although all tanks were uniformly aerated. Dissolved oxygen concentrations in all tanks remained above those likely to affect growth.

Larval mortality

Larval mortality for the Swanson's Creek water experiment is provided in Table 5 and Figure 1a. Mortality for the fresh oil experiment is in Table 2 and Figure 1b. Larval mortality was calculated in two ways. In the first, we assumed that exactly 30 larvae were placed in each tank (i.e., there were no errors in counting large numbers of small larvae as the experiment was set up) and that mortality could be calculated as *(30-number of survivors)*. The second assumed that we were able to retrieve and identify remains of all dead larvae in tanks (i.e., none had degraded beyond recognition), and that mortality could be calculated as *(number dead/(number live + number dead at end of experiment))*. Both methods yielded similar patterns of mortality with variation in percent of Swanson's Creek water or fresh oil.

Mortality in the Swanson's Creek water experiments was 0 or near 0% in all treatments at 48h and increased with increasing concentration of Swanson's Creek water at 96h (Probit analysis: $p < 0.0001$; $\mu = 51.6$). Mortality in 100% Swanson's Creek water averaged 87% by both calculation methods.

Mortality in the fresh oil experiment ranged from 0 to 10% at 48 h. At 96 h, mortality increased as percent oil solution increased from 0 to 25%, but decreased in the 50% oil solution dilution. The three tanks with highest mortality included one tank with 10% oil solution and two tanks with 25% oil solution. Mortality in the 25% fresh oil solution treatment was 45-47%. Probit analysis indicated no significant effect of oil concentration on larval survival ($p = 0.546$) as would be expected if highest mortality occurred at intermediate exposure treatments.

Larval growth and development

Exposure to oil slowed growth of larvae in both the Swanson's Creek water experiment and the fresh oil solution experiment. In both cases, mean length at 96 h declined with increasing percent oil solution (Table 7, Figure 2). For larvae tested with Swanson's Creek water, a linear regression model provided a better fit than did the polynomial model (linear regression: $R^2 = 0.701$, $p = 0.005$). For larvae tested with fresh oil, the 2nd order polynomial model yielded a better fit ($R^2 = 0.772$, $p = 0.003$)

Larvae from high oil concentration treatments had morphological characteristics typical of larvae at an earlier stage in development when compared to larvae from control or low oil concentration treatments (Figures 3-6). Jaws were less fully developed and yolk sacs were larger than in control tanks. No consistent pattern of abnormalities was associated with oil exposure. Aquaria typically had 0-2 larvae with some evidence of abnormal development regardless of treatment. In both experiments. Researchers responsible for catching larvae in aquaria and counting live larvae reported that larvae from high oil concentration treatments were less active and less able to avoid capture, although no formal test was performed.



Conclusions

Our results indicate that extended exposure to water contaminated by oil from the PEPCO pipeline at concentrations at and below those found in Swanson's Creek 6 d after the spill had the potential to cause mortality and slow growth of yolk-sac striped bass larvae. Slow growth during the larval stage can substantially increase mortality because larvae remain vulnerable to predators for a longer period of time during development (Bailey and Houde 1989). Actual mortality in the field will depend on the spatial distribution of both oil and larvae, and the duration of contact of larvae with oil-contaminated water. We did not include field investigations in this study. However, our results can be combined with field chemistry and ichthyoplankton surveys to estimate mortality.

Several points are important to interpretation of our data. First, the water for the Swanson's Creek water was collected several days after the oil spill. Changes in oil concentration and composition during that time may affect toxicity. Second, it is likely that high mortality of control fish in the fresh oil experiment was a direct consequence of the faster growth and development of these fish relative to fish exposed to high concentrations of fresh oil. We did not supplement tanks with food. Therefore, the control fish were more likely to suffer starvation as yolk sac reserves were depleted, and also experience the typically high mortality associated with the transition from yolk sac to the feeding larval stage in fish. It is important to note that our results reflect exposure for a specific period of time, and do not encompass the entire yolk sac stage for larvae in high oil treatments of either experiment.

Literature Cited

Bailey, K.M. and E.D. Houde. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. *Advances in Marine Biology* 25:1-83.

SAS Institute. 1999. *SAS/STAT User's Guide, Version 8, Volume 3*. SAS Institute Inc. Cary, NC.

Table 1. Summary of petroleum hydrocarbon analysis from the Swanson's Creek water experiment. TPH = Total petroleum hydrocarbons, PAH = Polyaromatic hydrocarbons.

| Sample ID | Tank | Date | Nominal Treatment (%) | TPH (ug/l) | PAH (ng/l) |
|------------------|------|---------|-----------------------|--------------|--------------|
| SB-Bio 1-2 | 9 | 4/15/00 | 0 | 5.6 | 177 |
| SB-Bio 1-1 | 5 | 4/15/00 | 0 | 25.7 | 220 |
| SB-Bio 1-11 | 5 | 4/17/00 | 0 | 43.9 | 155 |
| SB-Bio 1-17 | 5 | 4/19/00 | 0 | 79.9 | 197 |
| Mean ± Std. Dev. | | | 0 | 38.8 ± 31.6 | 187 ± 28 |
| SB-Bio 1-4 | 6 | 4/15/00 | 10 | 11.6 | 2243 |
| SB-Bio 1-3 | 3 | 4/15/00 | 10 | 46.9 | 2569 |
| SB-Bio 1-12 | 3 | 4/17/00 | 10 | 88.1 | 1039 |
| SB-Bio 1-18 | 3 | 4/19/00 | 10 | 40.2 | 688 |
| Mean ± Std. Dev. | | | 10 | 46.7 ± 31.6 | 1635 ± 912 |
| SB-Bio 1-5 | 2 | 4/15/00 | 20 | 14.3 | 3943 |
| SB-Bio 1-6 | 7 | 4/15/00 | 20 | 58.8 | 5153 |
| SB-Bio 1-16 | 7 | 4/17/00 | 20 | 89.7 | 1619 |
| SB-Bio 1-13 | 2 | 4/17/00 | 20 | 87.2 | 1727 |
| SB-Bio 1-19 | 2 | 4/19/00 | 20 | 71.0 | 107 |
| SB-Bio 1-22 | 12 | 4/19/00 | 20 | 77.4 | 977 |
| Mean ± Std. Dev. | | | 20 | 66.4 ± 27.9 | 2254 ± 1907 |
| SB-Bio 1-8 | 10 | 4/15/00 | 50 | 253.9 | 20341 |
| SB-Bio 1-7 | 1 | 4/15/00 | 50 | 295.4 | 18331 |
| SB-Bio 1-14 | 1 | 4/17/00 | 50 | 128.1 | 5235 |
| SB-Bio 1-20 | 1 | 4/19/00 | 50 | 162.0 | 2027 |
| Mean ± Std. Dev. | | | 50 | 209.9 ± 78.0 | 11484 ± 9198 |
| SB-Bio 1-10 | 8 | 4/15/00 | 100 | 278.7 | 3161 |
| SB-Bio 1-9 | 4 | 4/15/00 | 100 | 262.2 | 21887 |
| SB-Bio 1-15 | 4 | 4/17/00 | 100 | 166.0 | 4872 |
| SB-Bio 1-21 | 4 | 4/19/00 | 100 | 157.4 | 2609 |
| Mean ± Std. Dev. | | | 100 | 195.2 ± 63.2 | 9789 ± 9220 |

Table 2. Summary of petroleum hydrocarbon analysis from the “fresh oil” experiment. N/A¹ = Not Applicable -sample was direct sample of dilution water for the experiment. N/A² = Not Applicable - sample was direct sample of aqueous layer from 100% oil/water mixture.

| Sample ID | Date | Tank | Treatment (%) | TPH (ug/l) | PAH (ng/l) |
|-------------|---------|------------------|---------------|------------|------------|
| SB-Bio 2-2 | 5/16/00 | N/A ¹ | 0 | 50 | 240 |
| SB-Bio 2-8 | 5/17/00 | 14 | 0 | 63 | 632 |
| SB-Bio 2-10 | 5/19/00 | 3 | 0 | 35 | 388 |
| SB-Bio 2-5 | 5/16/00 | 11 | 5 | 152 | 13495 |
| SB-Bio 2-6 | 5/16/00 | 8 | 10 | 264 | 28184 |
| SB-Bio 2-4 | 5/16/00 | 15 | 10 | 289 | 24149 |
| SB-Bio 2-3 | 5/16/00 | 12 | 50 | 1417 | 81983 |
| SB-Bio 2-7 | 5/17/00 | 12 | 50 | 1124 | 41308 |
| SB-Bio 2-9 | 5/19/00 | 4 | 50 | 862 | 22663 |
| SB-Bio 2-1 | 5/16/00 | N/A(1) | 100 | 3899 | 317679 |

Table 3. Dissolved oxygen and temperature in the Swanson's Creek water experiment.

| Tank | Dur- ation (h) | % Swanson's Creek water | Dissolved oxygen (mg/L) | | | | | Temperature (°C) | | | | |
|---------------|----------------------|----------------------------------|-------------------------|------------|------------|------------|------------------------------|------------------|------------|------------|------------|--------------------------------|
| | | | 04/16/2000 | 04/17/2000 | 04/18/2000 | 04/19/2000 | Treat- ment mean DO | 04/16/2000 | 04/17/2000 | 04/18/2000 | 04/19/2000 | Treat- ment mean temp |
| 9 | 48 | 0 | 8.4 | 10.0 | | | 9.2 | 11.9 | 14.6 | | | 13.3 |
| 6 | 48 | 10 | 8.1 | 9.8 | | | 9.0 | 13.4 | 14.9 | | | 14.2 |
| 7 | 48 | 20 | 8.0 | 9.5 | | | 8.8 | 12.7 | 14.7 | | | 13.7 |
| 10 | 48 | 50 | 7.4 | 8.6 | | | 8.0 | 12.5 | 14.7 | | | 13.6 |
| 8 | 48 | 100 | 6.2 | 6.8 | | | 6.5 | 12.4 | 14.7 | | | 13.6 |
| 5 | 96 | 0 | 8.1 | 10.1 | 8.7 | 9.5 | 9.1 | 13.6 | 15.1 | 14.7 | 14.4 | 14.2 |
| 15 | 96 | 0 | 8.2 | 10.2 | 8.6 | 9.3 | | 13.0 | 14.8 | 14.2 | 13.5 | |
| 3 | 96 | 10 | 8.1 | 9.8 | 8.5 | 9.4 | 9.0 | 13.0 | 14.7 | 14.5 | 13.9 | 14.0 |
| 11 | 96 | 10 | 8.1 | 9.7 | 8.4 | 9.3 | | 13.3 | 14.9 | 14.3 | 14.2 | |
| 2 | 96 | 20 | 8.0 | 9.7 | 8.5 | 9.1 | 8.7 | 13.4 | 14.6 | 14.4 | 13.9 | 14.0 |
| 12 | 96 | 20 | 7.9 | 9.3 | 8.1 | 8.9 | | 13.1 | 14.8 | 14.1 | 14.0 | |
| 1 | 96 | 50 | 7.2 | 8.5 | 7.5 | 7.8 | 7.6 | 13.5 | 14.7 | 14.2 | 13.6 | 14.0 |
| 13 | 96 | 50 | 7.2 | 8.3 | 7.2 | 7.3 | | 13.0 | 14.7 | 14.2 | 13.9 | |
| 4 | 96 | 100 | 6.0 | 6.6 | 5.9 | 6.2 | 5.7 | 13.4 | 14.8 | 14.3 | 13.7 | 14.1 |
| 14 | 96 | 100 | 5.3 | 5.7 | 5.0 | 4.9 | | 13.2 | 14.9 | 14.2 | 13.9 | |
| daily mean | | | 7.5 | 8.8 | 7.6 | 8.2 | | 13.0 | 14.8 | 14.3 | 13.9 | |

Table 4. Dissolved oxygen and temperature in the fresh oil experiment.

| Tank | Duration (h) | % fresh oil solution | Dissolved oxygen (mg/L) | | | | | Temperature (°C) | | | | |
|------------|--------------|----------------------|-------------------------|------------|------------|------------|-------------------|------------------|------------|------------|------------|----------------------|
| | | | 05/16/2000 | 05/17/2000 | 05/18/2000 | 05/19/2000 | Treatment mean DO | 05/16/2000 | 05/17/2000 | 05/18/2000 | 05/19/2000 | Treatment mean temp. |
| 7 | 48 | 0 | 9.0 | 8.6 | | | 8.1 | 14.9 | 15.0 | | | 15.2 |
| 6 | 48 | 5 | 9.1 | 8.4 | | | 8.1 | 15.1 | 15.2 | | | 15.4 |
| 8 | 48 | 10 | 9.3 | 8.3 | | | 8.2 | 14.8 | 14.9 | | | 15.1 |
| 9 | 48 | 25 | 9.5 | 8.0 | | | 8.2 | 14.4 | 14.7 | | | 14.9 |
| 10 | 48 | 50 | 9.4 | 7.7 | | | 8.1 | 14.4 | 14.6 | | | 14.8 |
| 3 | 96 | 0 | 10.7 | 8.6 | 9.0 | 8.9 | 8.7 | 14.7 | 15.1 | 15.6 | 15.8 | 15.2 |
| 14 | 96 | 0 | 9.7 | 8.6 | 9.1 | 8.9 | | 14.4 | 14.7 | 15.5 | 15.5 | |
| 2 | 96 | 5 | 10.6 | 8.5 | 9.0 | 8.7 | 9.1 | 14.7 | 15.2 | 15.6 | 15.7 | 15.4 |
| 11 | 96 | 5 | 9.3 | 8.4 | 9.1 | 8.8 | | 15.3 | 15.3 | 15.7 | 15.6 | |
| 1 | 96 | 10 | 10.5 | 8.4 | 8.8 | 8.7 | 9.0 | 15.2 | 15.5 | 15.8 | 15.8 | 15.4 |
| 15 | 96 | 10 | 9.3 | 8.4 | 8.9 | 8.6 | | 14.7 | 14.8 | 15.5 | 15.5 | |
| 5 | 96 | 25 | 10.4 | 8.3 | 8.6 | 8.4 | 8.8 | 15.0 | 15.0 | 15.5 | 15.7 | 15.2 |
| 13 | 96 | 25 | 9.4 | 8.1 | 8.6 | 8.4 | | 14.6 | 14.9 | 15.5 | 15.5 | |
| 4 | 96 | 50 | 10.0 | 8.0 | 8.4 | 8.2 | 8.5 | 14.5 | 14.9 | 15.4 | 15.4 | 15.1 |
| 12 | 96 | 50 | 9.2 | 7.8 | 8.4 | 8.2 | | 15.0 | 15.0 | 15.4 | 15.4 | |
| | | | | | | | | | | | | |
| daily mean | | | 9.7 | 8.3 | 8.8 | 8.6 | | 14.8 | 15.0 | 15.6 | 15.6 | |

Table 5. Percent mortality of striped bass larvae exposed to Swanson's Creek water dilutions for 48 and 96 hours.

| time (hours) | tank | treatment % Swanson's Creek water | live # recovered | dead # recovered | total # recovered | % mortality assume 30 larvae/tank | % mortality assume 100% recovery | %recovery assume 30 larvae/tank | mean% mortality assume 30 larvae/tank | mean% mortality assume 100% recovery |
|--------------|------|-----------------------------------|------------------|------------------|-------------------|-----------------------------------|----------------------------------|---------------------------------|---------------------------------------|--------------------------------------|
| 48 | 9 | 0 | 30 | 0 | 30 | 0 | 0 | 100 | | |
| 48 | 6 | 10 | 30 | 0 | 30 | 0 | 0 | 100 | | |
| 48 | 7 | 20 | 30 | 0 | 30 | 0 | 0 | 100 | | |
| 48 | 10 | 50 | 29 | 1 | 29 | 3 | 3 | 97 | | |
| 48 | 8 | 100 | 30 | 0 | 30 | 0 | 0 | 100 | | |
| 96 | 5 | 0 | 28 | 0 | 28 | 7 | 0 | 93 | 7 | 0 |
| 96 | 15 | 0 | 28 | 0 | 28 | 7 | 0 | 93 | | |
| 96 | 3 | 10 | 26 | 2 | 28 | 13 | 7 | 93 | 7 | 4 |
| 96 | 11 | 10 | 30 | 0 | 30 | 0 | 0 | 100 | | |
| 96 | 2 | 20 | 24 | 7 | 31 | 20 | 23 | 103 | 25 | 24 |
| 96 | 12 | 20 | 21 | 7 | 28 | 30 | 25 | 93 | | |
| 96 | 1 | 50 | 16 | 7 | 23 | 47 | 30 | 77 | 57 | 50 |
| 96 | 13 | 50 | 10 | 23 | 33 | 67 | 70 | 110 | | |
| 96 | 14 | 100 | 8 | 22 | 30 | 73 | 73 | 100 | 87 | 87 |
| 96 | 4 | 100 | 0 | 31 | 31 | 100 | 100 | 103 | | |

Table 6. Percent mortality of striped bass larvae exposed to fresh oil solution dilutions for 48 and 96 hours.

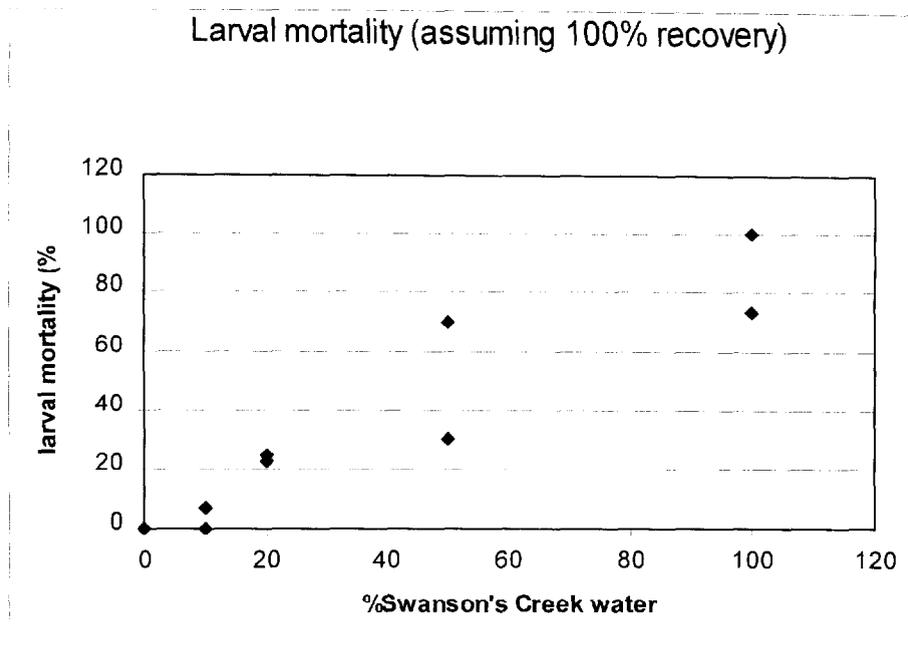
| time (hours) | tank | treatment % oil solution | live # recovered. | dead # recovered | total # recovered | % mortality assume 30 larvae/tank | % mortality assume 100% recovery | %recovery assume 30 larvae/tank | mean % mortality assume 30 larvae/tank | mean % mortality assume 100% recovery |
|--------------|------|--------------------------|-------------------|------------------|-------------------|-----------------------------------|----------------------------------|---------------------------------|--|---------------------------------------|
| 48 | 7 | 0 | 28 | 1 | 29 | 3 | 3 | 97 | | |
| 48 | 6 | 5 | 28 | 1 | 29 | 3 | 3 | 97 | | |
| 48 | 8 | 10 | 26 | 3 | 29 | 10 | 10 | 97 | | |
| 48 | 9 | 25 | 30 | 0 | 30 | 0 | 0 | 100 | | |
| 48 | 10 | 50 | 32 | 0 | 32 | 0 | 0 | 107 | | |
| 96 | 3 | 0 | 21 | 8 | 29 | 27 | 28 | 97 | 22 | 24 |
| 96 | 14 | 0 | 19 | 5 | 24 | 17 | 21 | 80 | | |
| 96 | 2 | 5 | 27 | 3 | 30 | 10 | 10 | 100 | 15 | 15 |
| 96 | 11 | 5 | 23 | 6 | 29 | 20 | 21 | 97 | | |
| 96 | 1 | 10 | 15 | 14 | 29 | 47 | 48 | 97 | 32 | 32 |
| 96 | 15 | 10 | 26 | 5 | 31 | 17 | 16 | 103 | | |
| 96 | 5 | 25 | 14 | 15 | 29 | 50 | 52 | 97 | 45 | 47 |
| 96 | 13 | 25 | 17 | 12 | 29 | 40 | 41 | 97 | | |
| 96 | 4 | 50 | 26 | 2 | 28 | 7 | 7 | 93 | 12 | 12 |
| 96 | 12 | 50 | 24 | 5 | 29 | 17 | 17 | 97 | | |

Table 7. Mean lengths of fish exposed to Swanson's Creek water and fresh oil solution dilutions for 96 h.

| Oil source | Tank # | Dilution treatment (%) | Mean Length (mm) | SD Length | # measured | # retrieved live |
|--|--------|------------------------|------------------|-----------|------------|------------------|
| Swanson's Creek water | 5 | 0 | 5.55 | 0.29 | 28 | 28 |
| Swanson's Creek water | 15 | 0 | 5.43 | 0.40 | 21** | 28 |
| Swanson's Creek water | 3 | 10 | 5.58 | 0.22 | 26 | 26 |
| Swanson's Creek water | 11 | 10 | 5.51 | 0.23 | 28 | 30 |
| Swanson's Creek water | 2 | 20 | 5.68 | 0.19 | 23 | 24 |
| Swanson's Creek water | 12 | 20 | 5.53 | 0.20 | 21 | 21 |
| Swanson's Creek water | 1 | 50 | 5.39 | 0.24 | 16 | 16 |
| Swanson's Creek water | 13 | 50 | 5.31 | 0.18 | 10 | 10 |
| Swanson's Creek water | 4 | 100 | --- | --- | 0 | 0 |
| Swanson's Creek water | 14 | 100 | 5.12 | 0.20 | 8 | 8 |
| fresh oil solution | 3 | 0 | 5.52 | 0.18 | 21 | 21 |
| fresh oil solution | 14 | 0 | 5.51 | 0.19 | 18* | 19 |
| fresh oil solution | 2 | 5 | 5.29 | 0.14 | 27 | 27 |
| fresh oil solution | 11 | 5 | 5.36 | 0.10 | 23 | 23 |
| fresh oil solution | 1 | 10 | 5.23 | 0.33 | 15 | 15 |
| fresh oil solution | 15 | 10 | 5.29 | 0.13 | 26 | 26 |
| fresh oil solution | 15 | 10 | 5.29 | 0.13 | 26 | 26 |
| fresh oil solution | 5 | 25 | 5.21 | 0.17 | 14 | 14 |
| fresh oil solution | 13 | 25 | 5.26 | 0.12 | 17 | 17 |
| fresh oil solution | 4 | 50 | 5.09 | 0.15 | 26 | 26 |
| fresh oil solution | 12 | 50 | 5.20 | 0.17 | 24 | 24 |
| <p>* One fish not measured because posterior missing **Some fish were extremely damaged by being screwed into the cap of the vial. Only the 21 undamaged fish were measured and examined for abnormalities.</p> | | | | | | |

Figure 1. Percent mortality of larvae exposed to oil solutions for 96 h.

a. Swanson's Creek water experiment



b. Fresh oil solution experiment

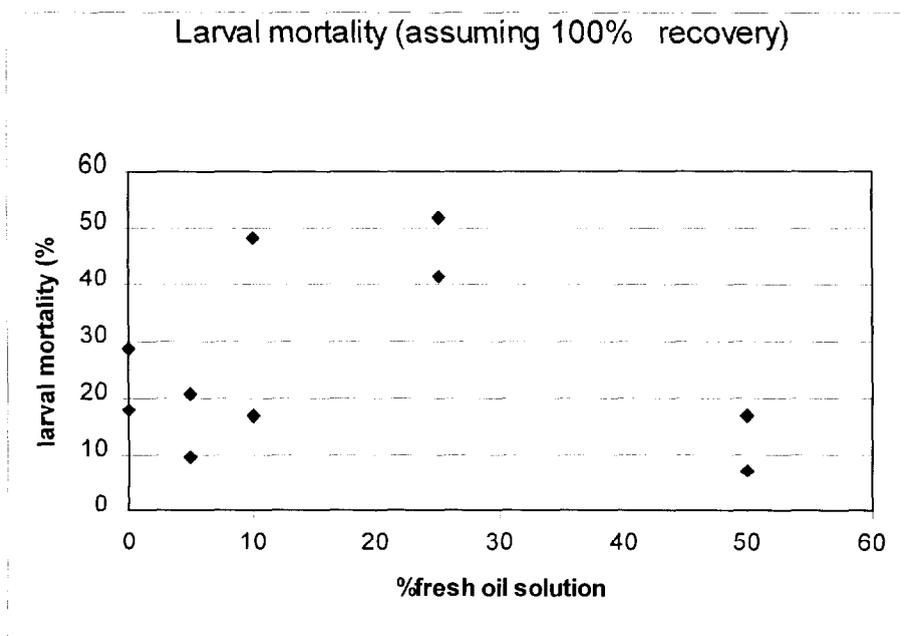
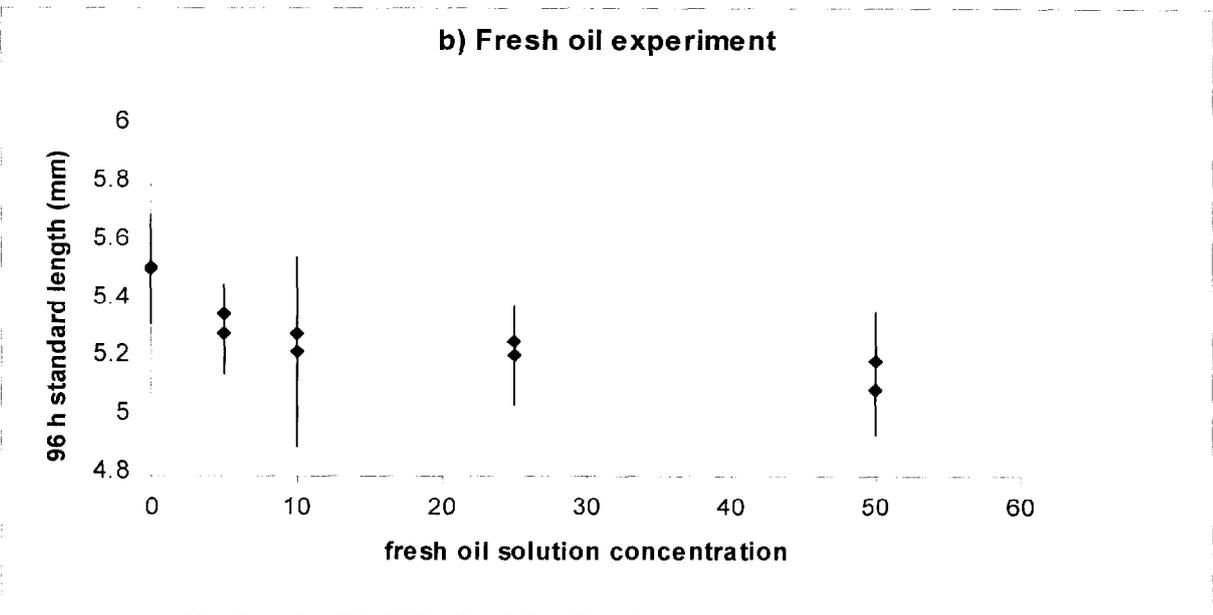
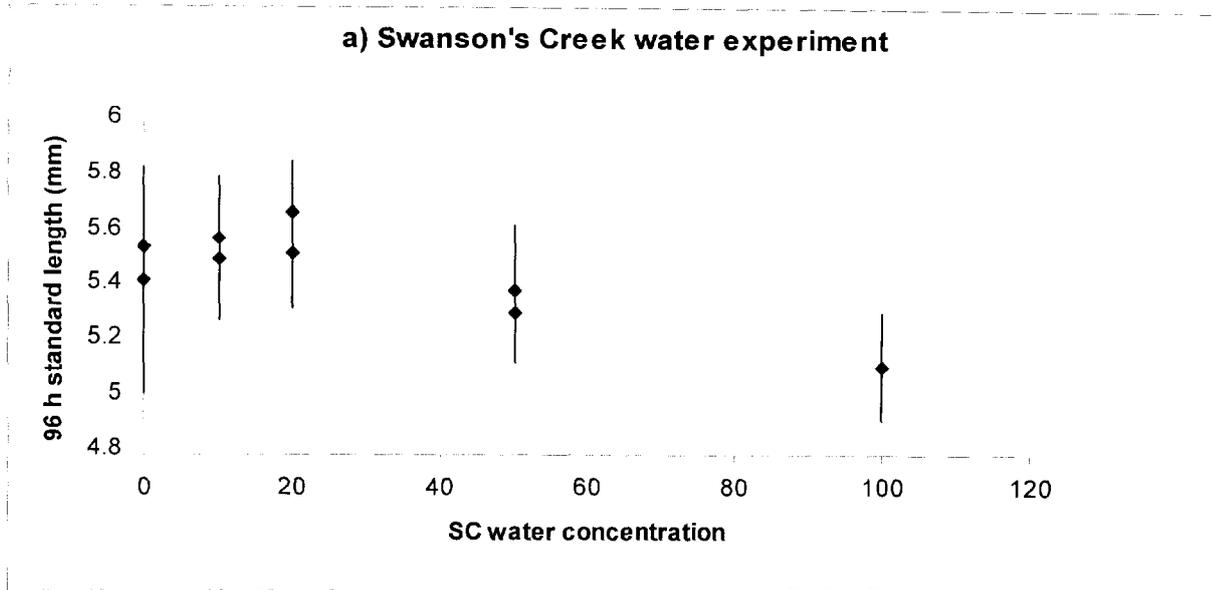


Figure 2. Standard lengths of larvae exposed to (a) Swanson's Creek water and (b) fresh oil solution for 96 hr (mean \pm SD).



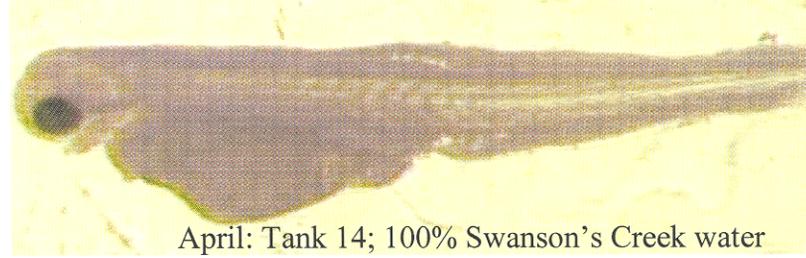
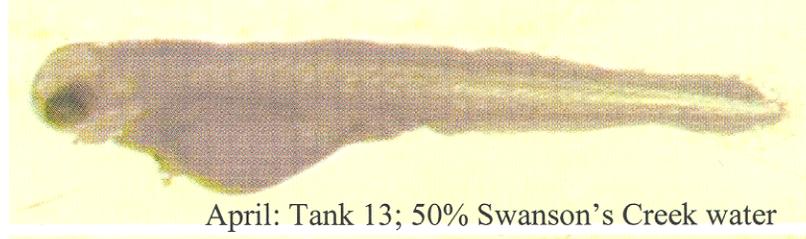
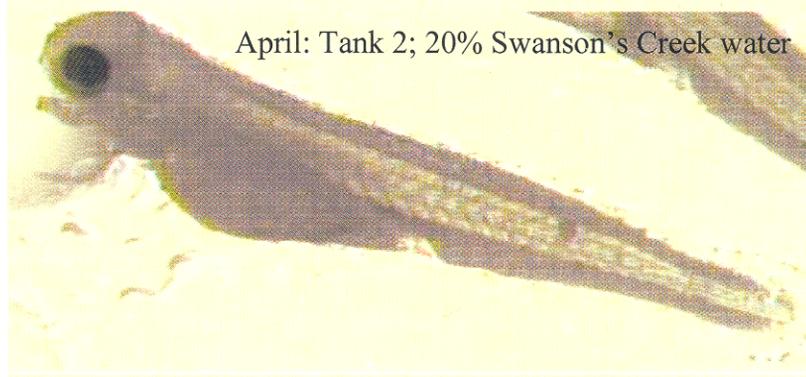
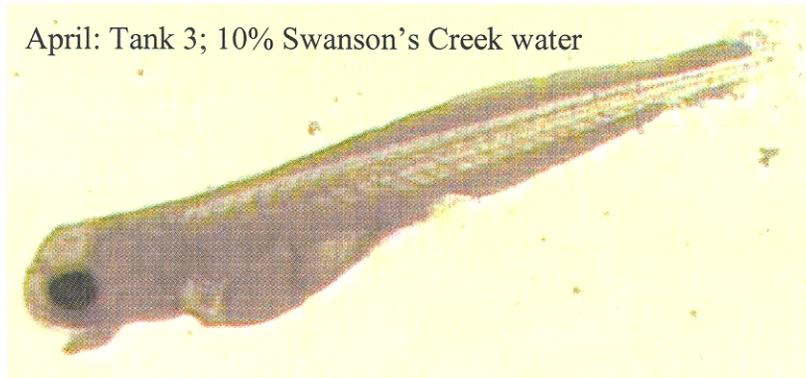
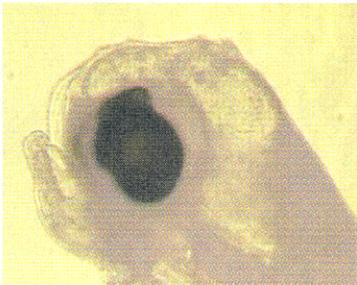
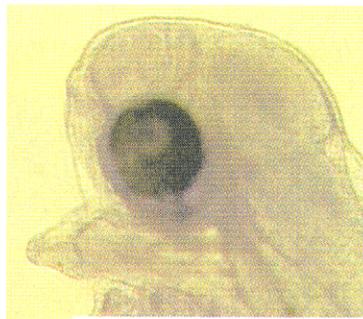


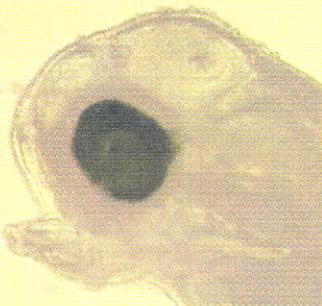
Figure 7. Typical larvae with no abnormalities from the April experiment using dilutions of Swanson's Creek water. Note the well developed jaws and smaller remaining yolk sacs of larvae in 0, 10 and 20% Swanson's Creek water relative to the smaller jaws and larger yolk sac of larvae from the 50 and 100% Swanson's Creek water treatments.



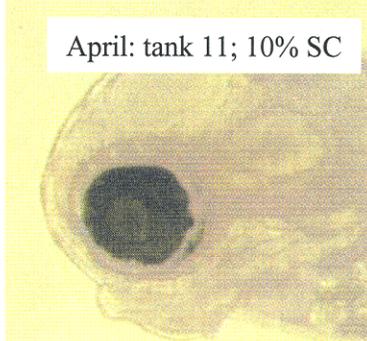
April: tank 5; 0% SC



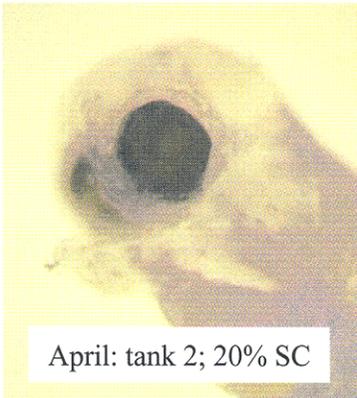
April: tank 15; 0% SC



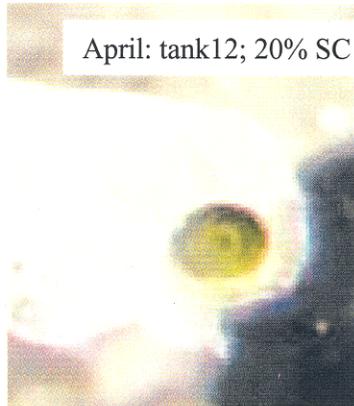
April: tank 3; 10% SC



April: tank 11; 10% SC



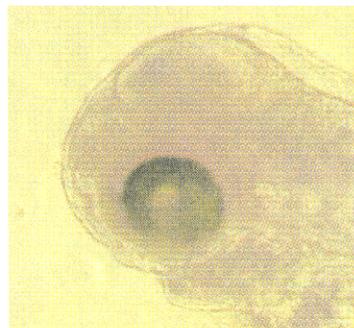
April: tank 2; 20% SC



April: tank 12; 20% SC



April: tank 13; 50% SC



April: tank 14; 100% SC

Figure 8. Close-up views of jaw development from April experiment. Note minimal jaw development in tanks with 50 and 100% Swanson's Creek (SC) water. No close-up image was taken of larvae from tank 1 (50% SC), and there were no survivors in tank 4 (100% SC).

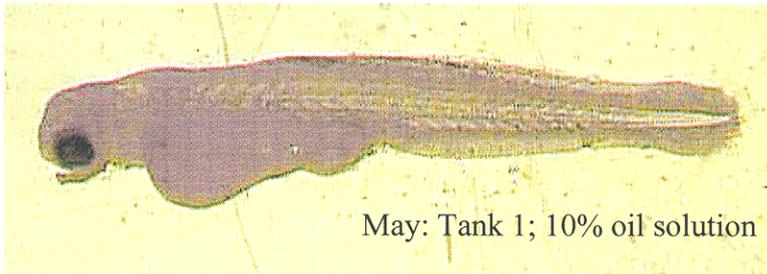
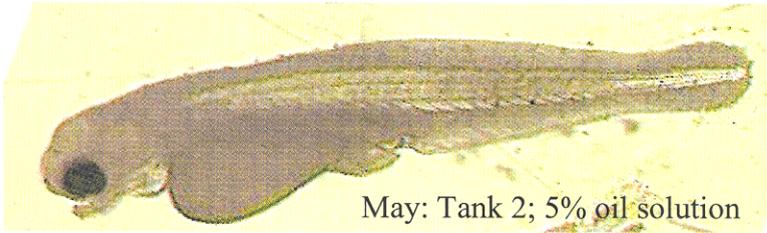
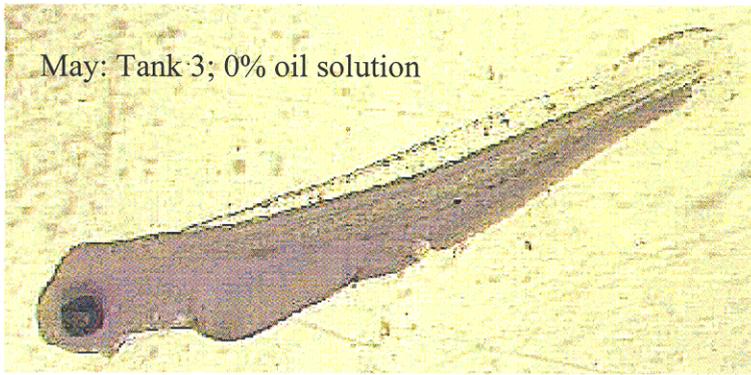
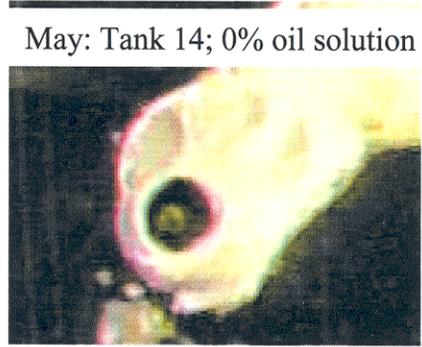


Figure 9. Typical larvae with no abnormalities from the May experiment using dilutions of fresh oil. Note the well developed jaws and smaller remaining yolk sac of the larva from the 0% oil tank and the smaller jaws and larger yolk sacs of larvae from the treatments with high percentages of oil solution.



May: Tank 3; 0% oil solution



May: Tank 14; 0% oil solution



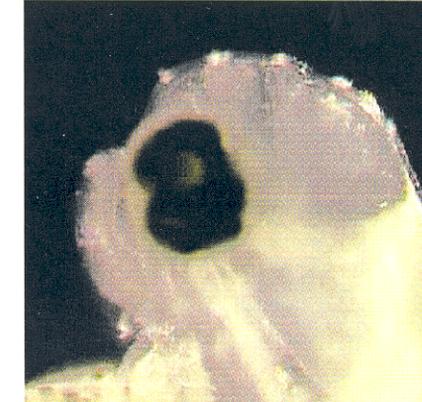
May: Tank 1; 10% oil solution



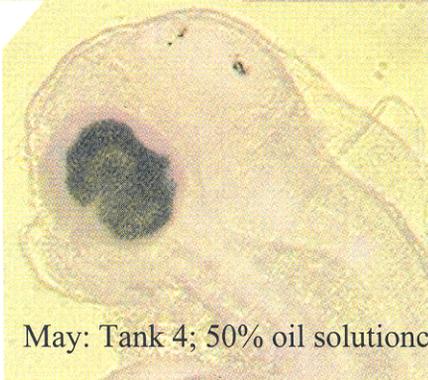
May: Tank 15; 10% oil solution



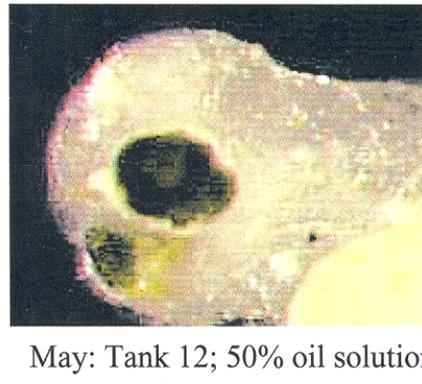
May: Tank 5; 25% oil solution



May: Tank 13; 25% oil solution



May: Tank 4; 50% oil solution



May: Tank 12; 50% oil solution

Figure 10. Close-up views of jaw development from May experiment. Note minimal jaw development in tanks with 25 and 50% fresh oil solution.