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|  | MATERIALS:  6 2 liter clear Tupperware tubs1 3mm diameter plastic pipette120 C. tentans in their larval stage5 sediment samples of similar grain obtained from Arroyo Del Valle1 control sediment of sand1 can of Tetramin fish food1 electronic gram scalespring water  Because C. tentans must be submitted into their experimental habitat immediately upon arrival, I had to have all sediment samples set up in their containers and covered with water ready for the entry of the larvae before their arrival. The first step of the experiment was to collect soil from the five sites that I planned to test.  The first site that I collected a sample from was near the beginning of the Arroyo Del Valle at the base of the dam. I called the park ranger at Del Valle in order to get directions to the site from which I wanted to take my sample. Once I arrived at the site I parked in the parking lot and proceeded to hike up the trail to the creek. Once I found a spot at the creek that I saw fit for sampling, I took O gallon of sediment.  The next site that I visited was near the back of shadow cliffs.  I collected a sample (all samples I took were O gallon) from an area behind the residential housing.          The third site that I visited was just to the West of the bridge on Main Street.            The fourth site that I visited was in a residential area near the apartments after the bridge on Hopyard near Del Valle Parkway.            The last site that I visited was at the curve of the dirt bike trail just before the 680 freeway.    Once I retrieved the sediment, I had to search for a control. To serve this purpose, I used aquarium sand. Once I had all of my samples, I poured 2cm of each sediment into each of the six Tupperware containers. I then poured 1.5L of overlying water in and let the substrate settle. The water had to adjust to room temperature so I let it sit overnight in the pantry in which the experiment would take place.  There are two types of experiments that can be done with these larvae. They are static and flow-through. Static testing is recommended for quantitative experiments used to test sediment for comparable toxicity. Therefore, I used a static test because my objective was to compare toxicity levels within the Arroyo Del Valle. The test I did was a static test. The sediment samples that I took were all taken from sites in which there was no current in order to follow the experiment as closely as possible.  C. tentans require a substrate in which to construct a case. Shredded paper towel work very well for this purpose. I cut brown paper towels into strips and soaked them in acetone overnight. I then rinsed the paper towel three times using spring water to get rid of the acetone. Once this was completed, I put the paper towels into a blender and shredded the towels into a pulp. I then rinsed the pulp twice more with spring water and placed it 3cm into the each of the six sediment samples. The sediment was now ready for the addition of the C. tentans larvae.  The C. tentans arrived on 4/7/98 in approximately a O liter plastic container containing minimal sand and a paper towel for the organisms to attach to. This setup was filled with room temperature water for shipping and survival of the larvae. Upon the arrival of the 120 C. tentans larvae , I used a 2mm-diameter pipette to pipette 20 C. tentans into each of the six exposure bins. The larvae were then pipetted below the surface so that they did not get caught in the surface tension of the water. Once the larvae were introduced into their new environments, the experiment was ready to begin. Immediately, I fed the larvae 50mg of Tetramin goldfish food which I dissolved into 10ml of spring water which I then poured carefully into the bin. I say carefully because I did not want to disturb the sediment on the bottom because it would cause a substrate to form in the water. This mixture of 50mg fishfood and 10ml water was fed to the larvae every other day. If food collects on the sediment, feeding should be suspended for one or more days because a fungus will begin grow on the surface of the sediment. This would also necessitate more frequent changes in the water. Before I fed the larvae every other day, I would also empty out 4 cups of the culture water and replace it with 4 cups of room temperature spring water. The bins were covered in fine nylon screen on the top side. The bins containing the water, sediment, and larvae were kept in a pantry sealed from outer disturbances. The pantry also gave me the ability to control the amount of light the larvae received day in and day out. The recommended light to dark ratio is 16 hours of light for every 8 hours of dark (a log of the light patterns for each day, feeding days, and water changes is located in the data section).  I continued this experiment for 10 days until the larvae were approximately 21 days old. At this point it was time to retrieve the larvae which were either in their third or fourth instar. This means that they were in either the third quarter or fourth quarter of their larval stage. To retrieve the larvae I first screened the sediment in a colander type apparatus to get the larger chunks of sediment out of the bin. I flushed water through it and caught it in a pan. I then poured this water through a coffee strainer in which I pulled out the larvae. Once I did this for all of the bins I kept the larvae from each bin separated. I killed the larvae using Isopropyl Alcohol and then dried them off.  To conclude my experiment, I weighed each group of 21 day old larvae and took an average weight. The mortality rate in each bin and the average larval weight for each bin after 21 days is recorded in the data section under table 1.1.  This experimental is designed in this fashion to show comparative growth between larvae within the different bins. The control served to give an example of how the larvae would grow under optimal conditions without the effects of contaminants. The average weight of the larvae within the five samples taken from Arroyo Del Valle can then be compared to the average weight of the larvae in the control to see how each sample affected the larval growth. |

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*For More Information about Creekwatch, please contact Eric Thiel at* [*ethiel@pleasanton.k12.ca.us*](mailto:ethiel@pleasanton.k12.ca.us)