**Bioassays procedures for treated retention ponds**

**Bioassay equipment**

* Analytical balance [calibration weights]
* Calipers
* Data sheets
* 5-gallon bucket(s)
* Transport coolers, aerators, crayfish containers
* 1 jon boat or canoe with load rating of at least 500 pounds
* 1 trolling motor (set at 12” depth)
* 1 battery (trolling motor)
* Modified crayfish traps, closure clips, rope, carabiners, bricks, 2-types of floats
* Water quality instruments (temperature, pH meter and probe, conductivity, dissolved oxygen) and GPS unit
* Contaminant pad(s) and tub(s)
* Spray tank
* Scrub brushes
* Nitrile or rubber gloves
* Rubber boots
* Trash bag(s)
* Apron or Tyvek coverall
* Eye protection (safety goggles, face shield) and eye wash bottle
* Kitty litter, shovel, and trash bags

**Personal Protection Equipment (PPE) procedures**

1. Personnel should wear nitrile or rubber gloves, rubber boots or waders, eye protection, and apron as a barrier to concentrated pesticide.
2. Disposable PPE placed in trash bags prior to leaving each site.
3. Disposable PPE can be disposed as residential trash.
4. Non-disposable PPE (e.g., rubber boots or waders) should be tripled rinsed with tap water or well water to remove sediment and any pesticide residue from equipment.
5. Dispose of rinsate (i.e., rinse water containing residual chemicals) into pond.
6. If traveling between sites, PPE should be stored in pick-up bed [not in passenger space].
7. If contaminated, clothing should be laundered separately with heavy-duty laundry soap twice. A third empty cycle should be run thru machine after clothes have been cleaned. If completely saturated in pesticide, double-bag clothing and dispose of clothing as residential trash.

**Bioassay cage deployment**

1. Place cages/rope/bricks/floats needed for site into boat.
2. Set-out cages using map of ponds with cage numbers.

**Crayfish culture and prep**

1. Crayfish should be weighed and measured the night before being stocked into cages.
2. Place weighed and measured crayfish into pre-labeled containers in coffin cooler.
3. In morning, Place pre-labeled crayfish containers into live-car coolers.
4. Fill cooler(s) with about 2” of dechlorinated tap or well water.
5. Turn on 2 aerators per coolers; place airstones in water at bottom of cooler.
6. Place containers into cooler.
7. Keep cooler lid closed to maintain water temperature [should be monitored]. **Slowly open/close** cooler lid to **avoid light shock** to crayfish.

**Bioassay test procedures [daily until 100% survival in caged crayfish]**

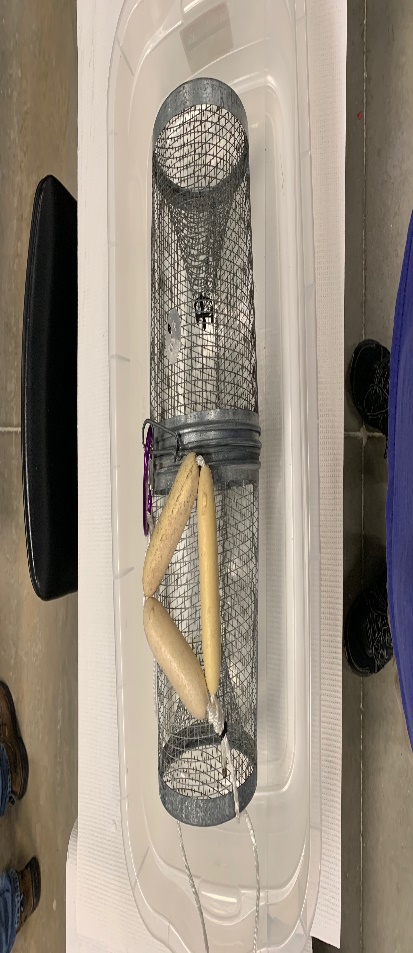
1. At each site, place cooler with containers holding crayfish for site into boat.
2. Place cage-containment Tupperware or tub in boat.
3. Place 1-gal bucket with trash bag in boat for crayfish to be removed from cages.
4. Place water-quality instruments and data sheets onto boat.
5. Randomly sample cages in pond.
6. Personnel at stern will lift cage from water, ensuring as little as possible water gets into boat.
7. Place cage on/in containment Tupperware.
8. Open cage.
9. Record whether crayfish is alive or dead.
10. See data sheet for whether crayfish should be MSCL sample.
11. If yes, place into Ziplock bag in cooler on ice
12. If no, place crayfish from cage in bucket.
13. Replace one crayfish into cage.
14. Secure cage, check rope to float and anchor. Place into pond water.
15. Personnel at bow will collect in-situ water quality and water samples
16. All bagged crayfish will be place into freezer until processing and disposal. Crayfish will be disposed of following MDNR procedures.

**Sampling gear clean-up between sampling locations and for gear storage**

1. At decontamination station or pond edge, all equipment should be cleaned.
2. Triple rinse sampling gear with well or tap water, brushing off all sediment particles or debris. Air dry.
3. Dispose of rinsate into pond.

**Transfer of data**

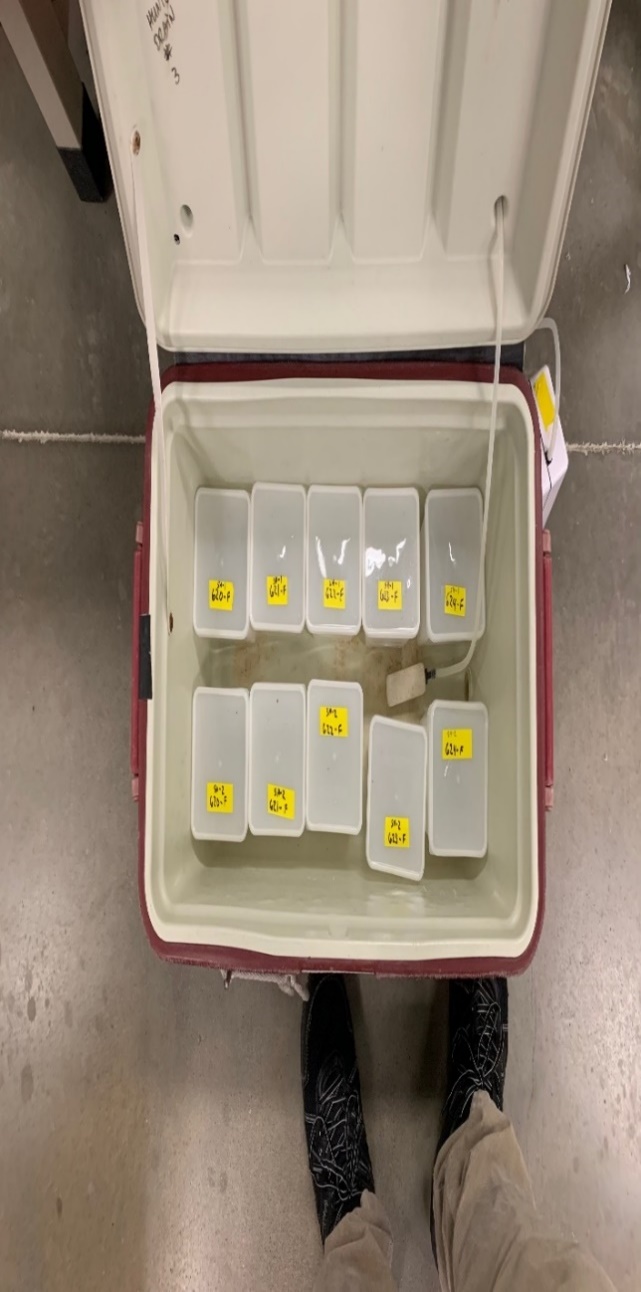
1. Document field operations associated with sample collection on field sampling sheets or field notebooks.
2. Scan data and email or mail data sheets/notebooks to CERC.
3. Transfer any digital photographs of the sample sites and collection.

* Bottom cage*

*Mid-water cage*

Cage containment Tupperware

(contains pesticide water dripping off cage equipment)





Crayfish cooler/Tupperware for cages

\*Note aerator and air-line in bottom of cooler