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APPENDIX G

Hybrid Water Milfoil Challenge Testing Study; SePRO 2014

SePRO
Aquatics Research

Renovate®, Sculpin®, and Sonar® Susceptibility Assay

Study Site: **Little Bearskin Lake**
(Price and Oneida Co., Wisconsin)

Target Species: **Suspected *Eurasian X Northern milfoil hybrid***

Sampling Date:	August 12, 2014
Laboratory Receipt Date:	August 14, 2014
Number of Sites Assayed (Received):	5 (5)
Assayed Site Identification:	Site 1, Site 2, Site 3, Site 4, Site 5
Sculpin Rates Assayed (parts per million 2, 4-D):	0, 1.5
Renovate Rates Assayed (parts per million Triclopyr):	0, 1
Sonar Rates Assayed (parts per billion Fluridone);	0, 6

Quality Control:

Untreated Rate Confirmation (FasTEST™ Measurements versus Theoretical):
Number of Replicates Tested: 1 % Target: 100.0
2, 4-D Rate Confirmation (FasTEST™ Measurements versus Theoretical):
Number of Replicates Tested: 1 % Target: 102.2
Triclopyr Rate Confirmation (FasTEST™ Measurements versus Theoretical):
Number of Replicates Tested: 1 % Target: 105
Fluridone Rate Confirmation (FasTEST™ Measurements versus Theoretical):
Number of Replicates Tested: 1 % Target: 95

Methods Summary

Invasive milfoil samples were collected from Little Bear Skin Lake in Price and Oneida Co., WI and shipped overnight to the SePRO Research & Technology Campus (SRTC) for baseline screening of responses to multiple common aquatic herbicides for milfoil control. On the day of receipt, milfoil samples were planted in aquaria alongside reference classically 2, 4-D, triclopyr, and Sonar susceptible Eurasian watermilfoil (EWM) population cultured at the SRTC. After a brief transitional growth period, sets of Little Bear Skin Lake and reference EWM were exposed to single short exposure of either Sculpin (a.i. 2,4-D amine) at 1.5 ppm as acid or Renovate (a.i. triclopyr) at 1 ppm as acid. Simultaneously, another set of Little Bear Skin Lake milfoil and reference EWM was statically exposed to a 6 ppb Sonar (a.i. fluridone) treatment.

Along with visual observations of plant herbicide injury, a common biochemical response of plant health was measured 2 weeks after treatment to complete the assay. The herbicide exposure period for the test along with the biochemical indicator measured in treated and untreated plants are currently proprietary. However, the auxin screening procedure has been repeatedly tested under specific laboratory conditions and confirmed to be predictive of general field response of tested milfoil to auxin-mimic herbicides.

Results and Discussion:

Sculpin

Visual observations and biochemical injury indicated the milfoil samples were an apparent mixture of biotypes with differing 2, 4-D susceptibility—less susceptible and susceptible—between and within sites. This heterogeneous mixture of susceptible and less susceptible milfoil is best demonstrated with the responses for the Site 1 and 4 samples. For example, the Site 1 milfoil had a biochemical injury response with a variance greater than the reference EWM, approximately double (Figure 1), which means some plants were dead or dying while other milfoil recovered from the 2, 4-D treatment. This mixture of dead and recovering milfoil can be visualized with photographs in image 2 for the Site 1 sample, as well as for the other Sites 2-5 with images 3-6, respectively. During the 2, 4-D bioassay, observations of plant responses through time demonstrated milfoil plants with less 2, 4-D susceptibility initially responded to the treatment with classic epinasty or curling, but were able to recover one week after the exposure was terminated.

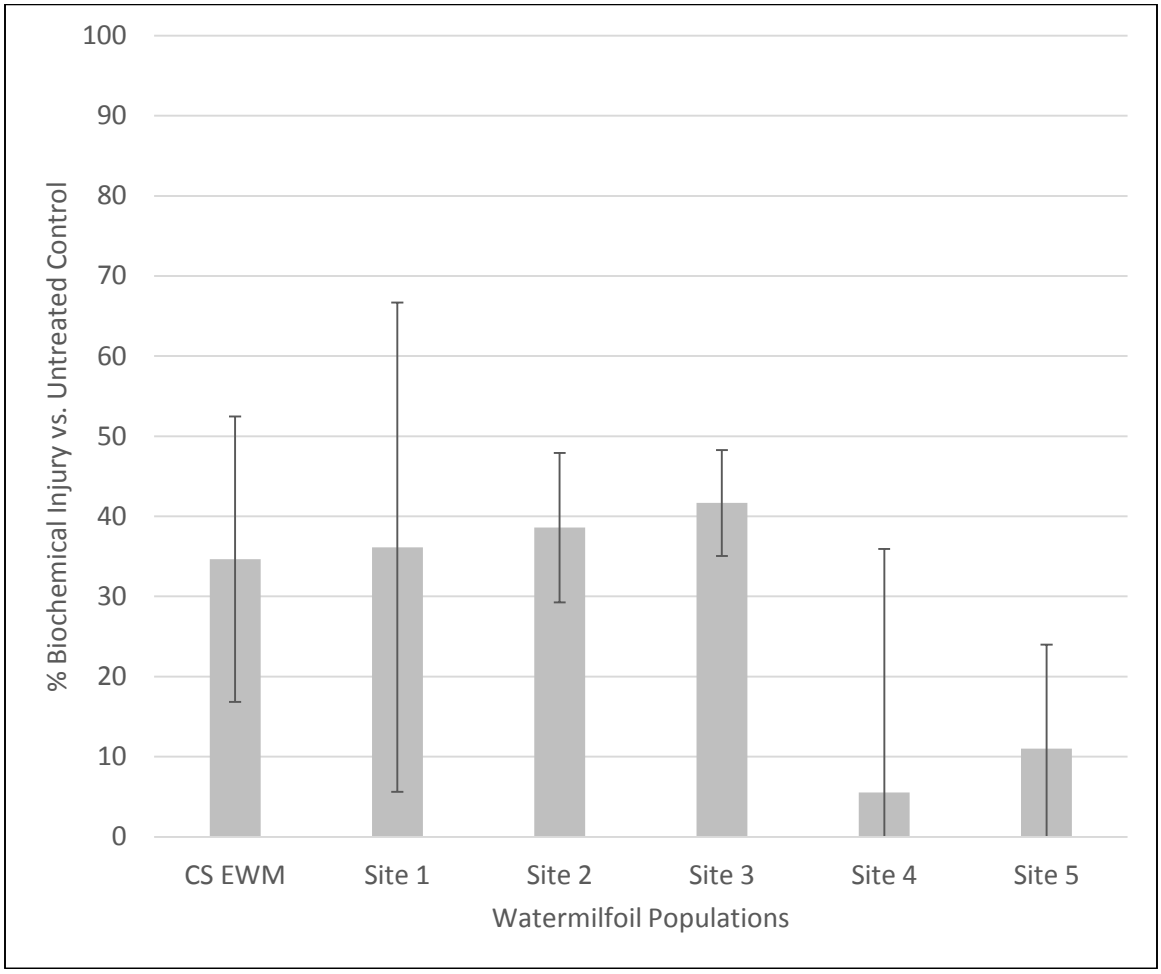

Figure 1. Biochemical injury of the Little Bear Skin Lake samples and a classically susceptible Eurasian watermilfoil population (EWM) to a 2, 4-D treatment. Error bars are ± 1 standard deviation (n=4).

Renovate

Biochemical injury and visual observations indicated that the milfoil sampled from Sites 1-3 were classically susceptible to triclopyr (Figure 2; Images 2-4). While responses of milfoil in samples collected from sites 4 and 5 had a mixture of triclopyr susceptibility (Figure 2; Images 5 and 6). Overall, milfoil sampled from Little Bear Skin Lake was more susceptible to triclopyr than 2, 4-D, which is exhibited by the number and size of plants that recovered from the 2, 4-D treatment compared to the triclopyr treatment.

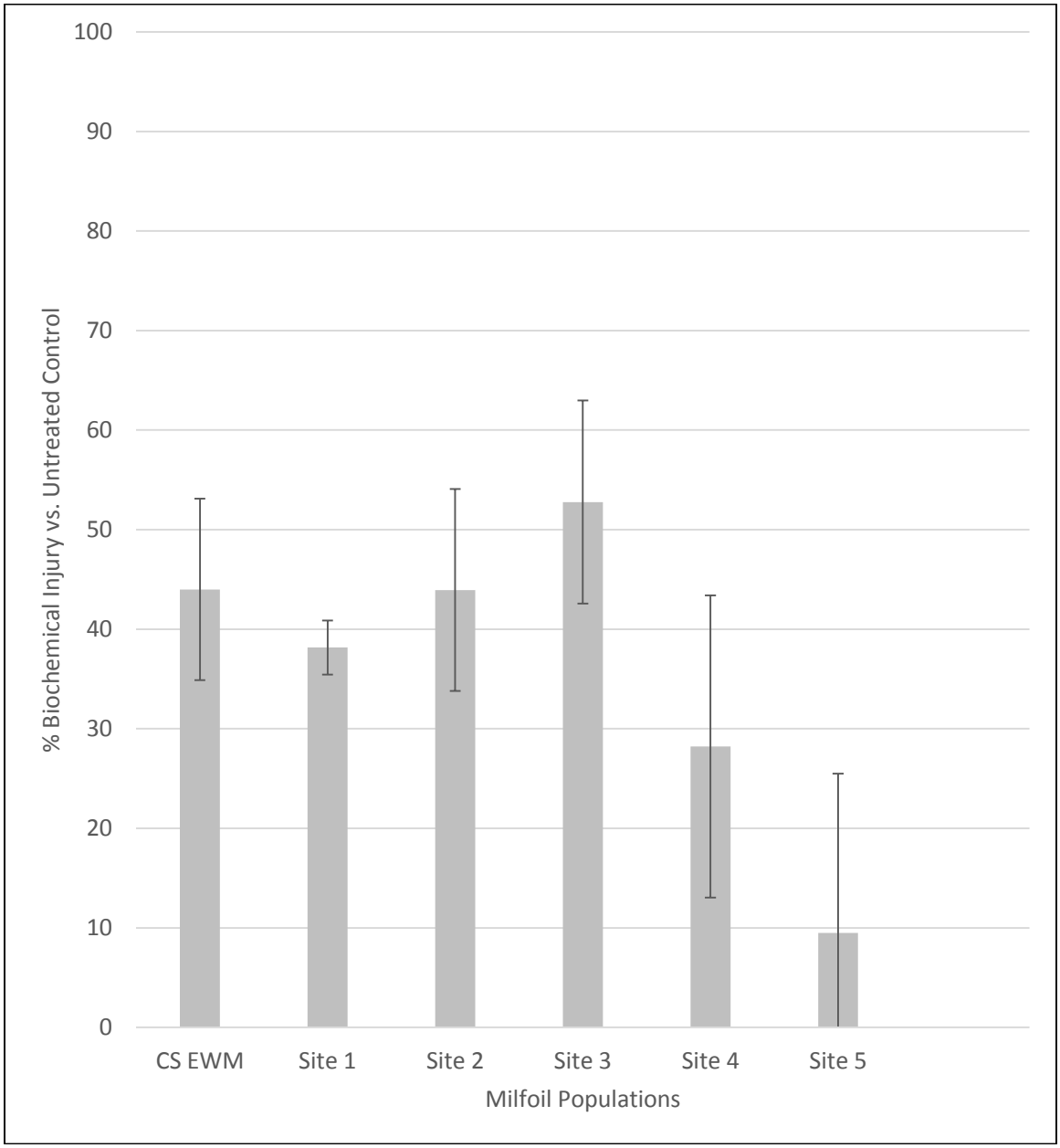

Figure 2. Biochemical injury of the Little Bear Skin Lake samples and a classically susceptible Eurasian watermilfoil population (EWM) to a triclopyr treatment. Error bars are ± 1 standard deviation for Little Bear Skin Lake samples (n=4).

Sonar

Visual observations and biochemical injury of the Little Bear Skin Lake samples demonstrated classic Sonar susceptibility relative to the reference susceptible EWM population. Site 4 did have reduced biochemical injury in this assay. However, the growth rates of some plants in the milfoil sample from site 4 were reduced compared to other milfoil assayed. A reduced growth rate would decrease the time to response or slow the rate of injury. Visually the slower growing milfoil exposed to Sonar exhibited chlorotic symptoms, which would not be expected for a less susceptible milfoil with this treatment.

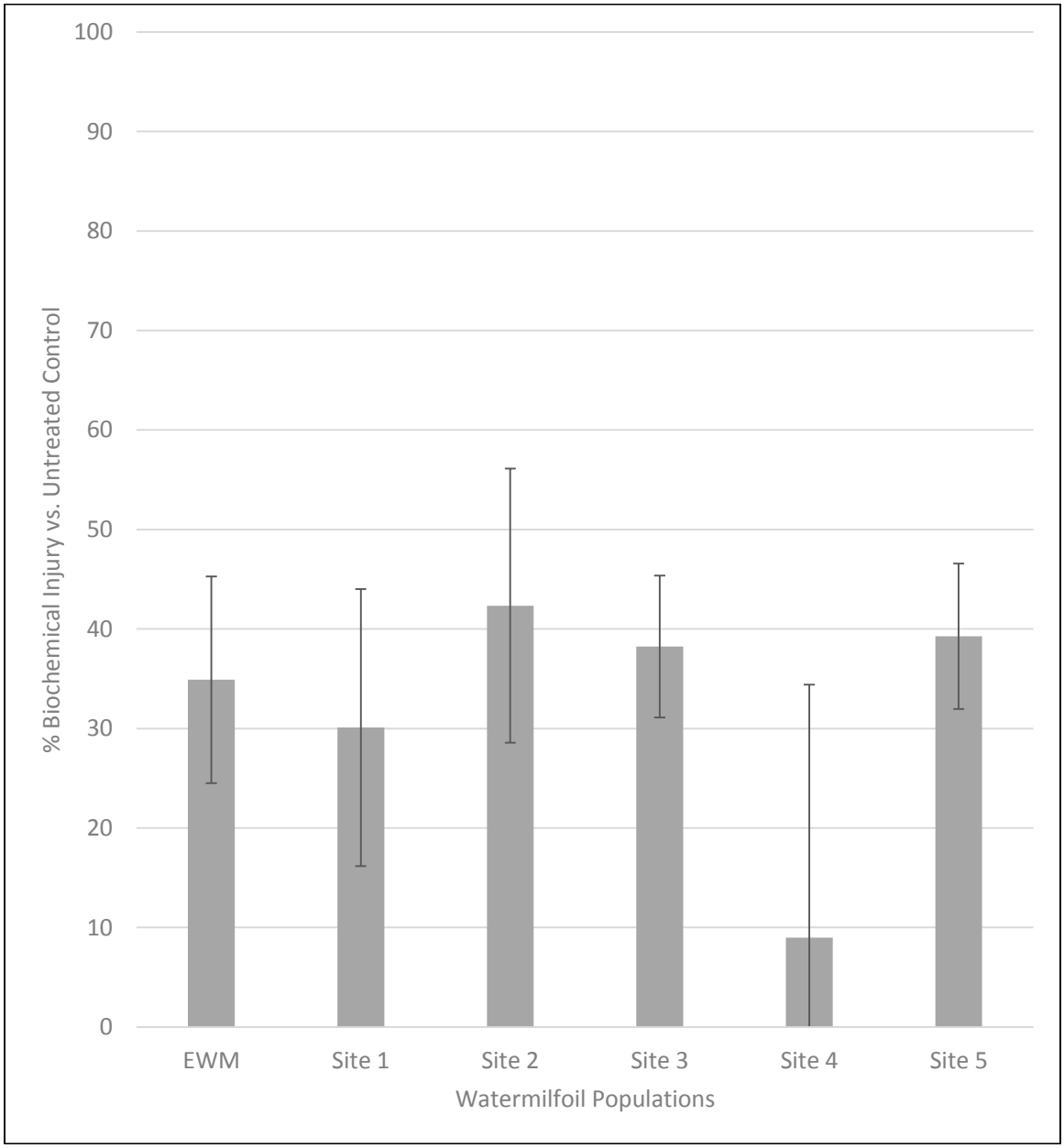

Figure 2. Biochemical injury of the Little Bear Skin Lake samples and a classically susceptible Eurasian watermilfoil population (EWM) to a Fluridone treatment. Error bars are ± 1 standard deviation (n=4).

Based on these results, typical use patterns of Sculpin or other 2, 4-D products are not projected to adequately control milfoil in Little Bear Skin Lake. The most likely outcome using standard treatment rates for spot or larger-scale management would be partial control with initial injury followed by regrowth 6 – 8 weeks post application. Elevated 2, 4-D rates may have reduced control, and pose greater risks to certain sensitive native plants.

The assay results support that Little Bear Skin Lake milfoil has a more typical response to Renovate, although a higher recovery potential versus typical EWM. Little Bear Skin Lake milfoil is classically susceptible to Sonar. Little Bear Skin Lake milfoil assayed is likely a hybrid biotype. Hybrid milfoil has been shown to recover more quickly than pure parental EWM from many forms of management, which agrees with observed recovery potential from auxin herbicides in this screening assay.

For lake-wide management of all littoral areas of Little Bear Skin Lake, the following management options are recommended. The specifics of the management strategy will be defined by final scale of treatment, level of milfoil establishment, dilution potential of the treated area(s) and other relevant site factors.

1) **even and thorough application of Renovate OTF granular at 1 – 2 ppm in littoral target areas targeting a 250 - 300 ppb lake-wide concentration. This is a stronger use recommendation than would be made for pure EWM. In littoral areas with higher exchange with deeper water or other increased dilution potential, thorough granular coverage and favorable early-stage dissipation conditions (primarily lighter winds/lake mixing in first 2 – 3 days post treatment) will maximize localized CET and improve long-term control.**

   OR

2) **a multiple application protocol of Sonar pellets to infested littoral areas following a site-specific formulation and rate prescription. Large-scale Sonar pellet treatment plans typically attempt to maintain lake wide Sonar levels of 2 – 4 ppb for 60 – 90 days. Sensitive hybrid milfoils generally require longer exposure in this range for optimal long-term control.**

The strategies above are general recommendations for larger-scale management that can be separately warranted by SePRO for effective performance based on the results of the testing presented here. Site-specific recommendations should be requested to optimize treatment of Little Bear Skin Lake milfoil and establish formal criteria for performance warranty.

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Image 1. Photographs of a Sculpin, Renovate, and Sonar susceptible EWM population at termination of PlanTEST bioassays. Note untreated controls appear healthy, while Renovate and Sculpin treated plants are necrotic, and Sonar treated plants are chlorotic.

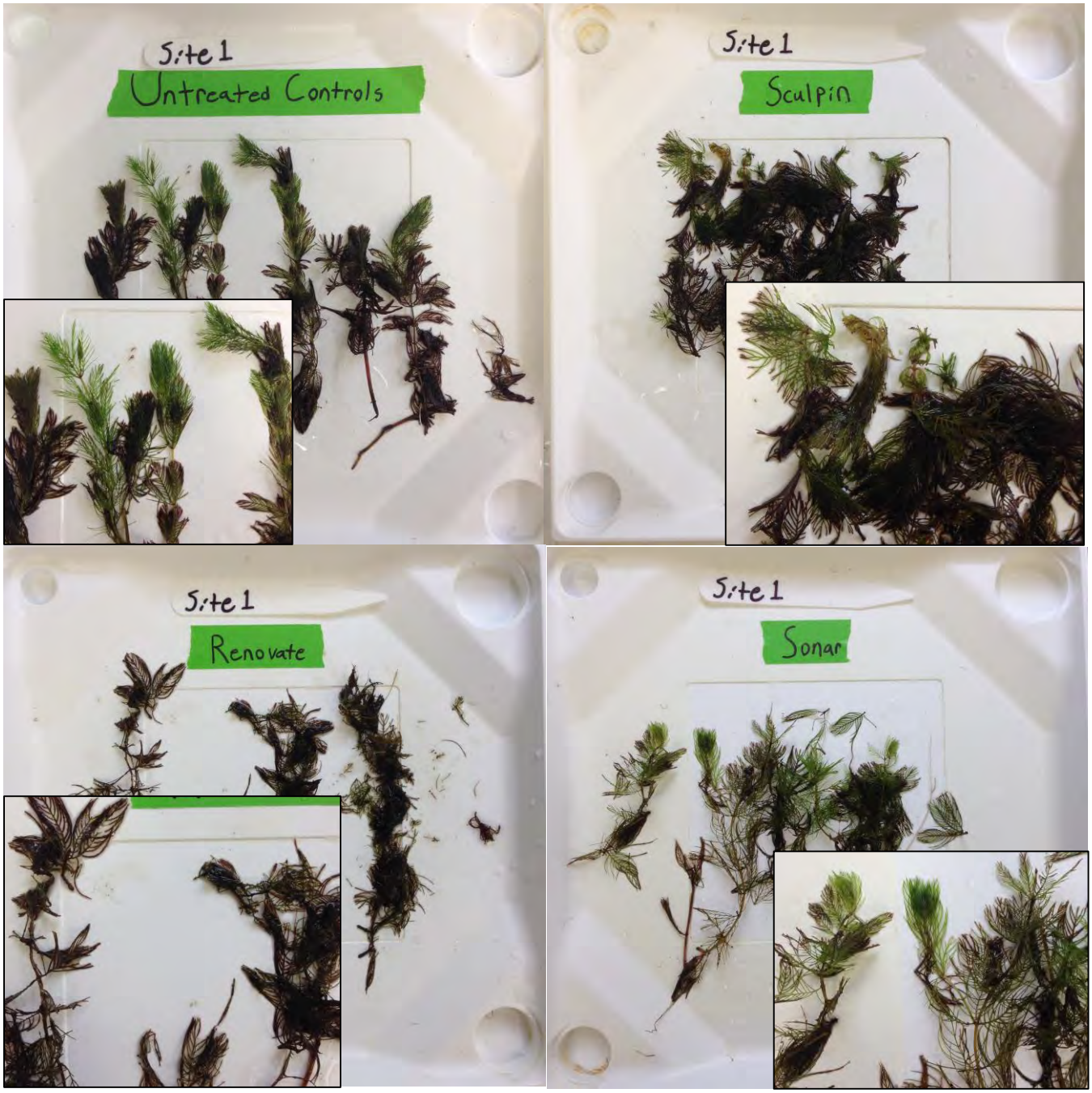

Image 2. Photographs of Little Bear Skin Lake Site 1 sample at termination of PlanTEST bioassay. Note untreated controls appear healthy, some Sculpin treated plants are beginning to recover while others are necrotic, Renovate treated plants are necrotic, and Sonar treated plants are beginning to bleach.

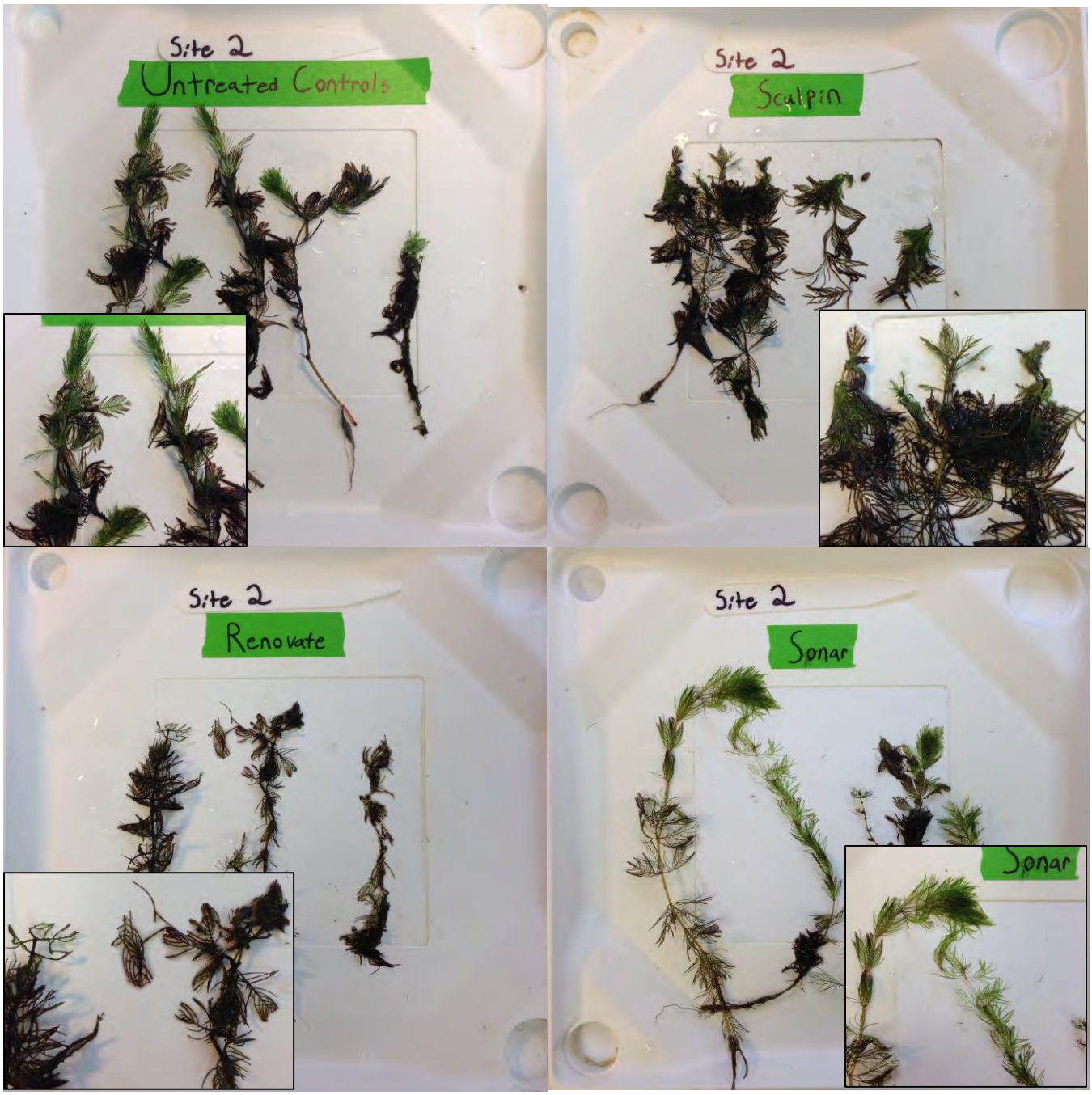

Image 3. Photographs of Little Bear Skin Lake Site 2 sample at termination of PlanTEST bioassay. Note untreated controls appear healthy, some Sculpin treated plants are beginning to recover while others are necrotic, Renovate treated plants are necrotic, and Sonar treated plants are beginning to bleach.

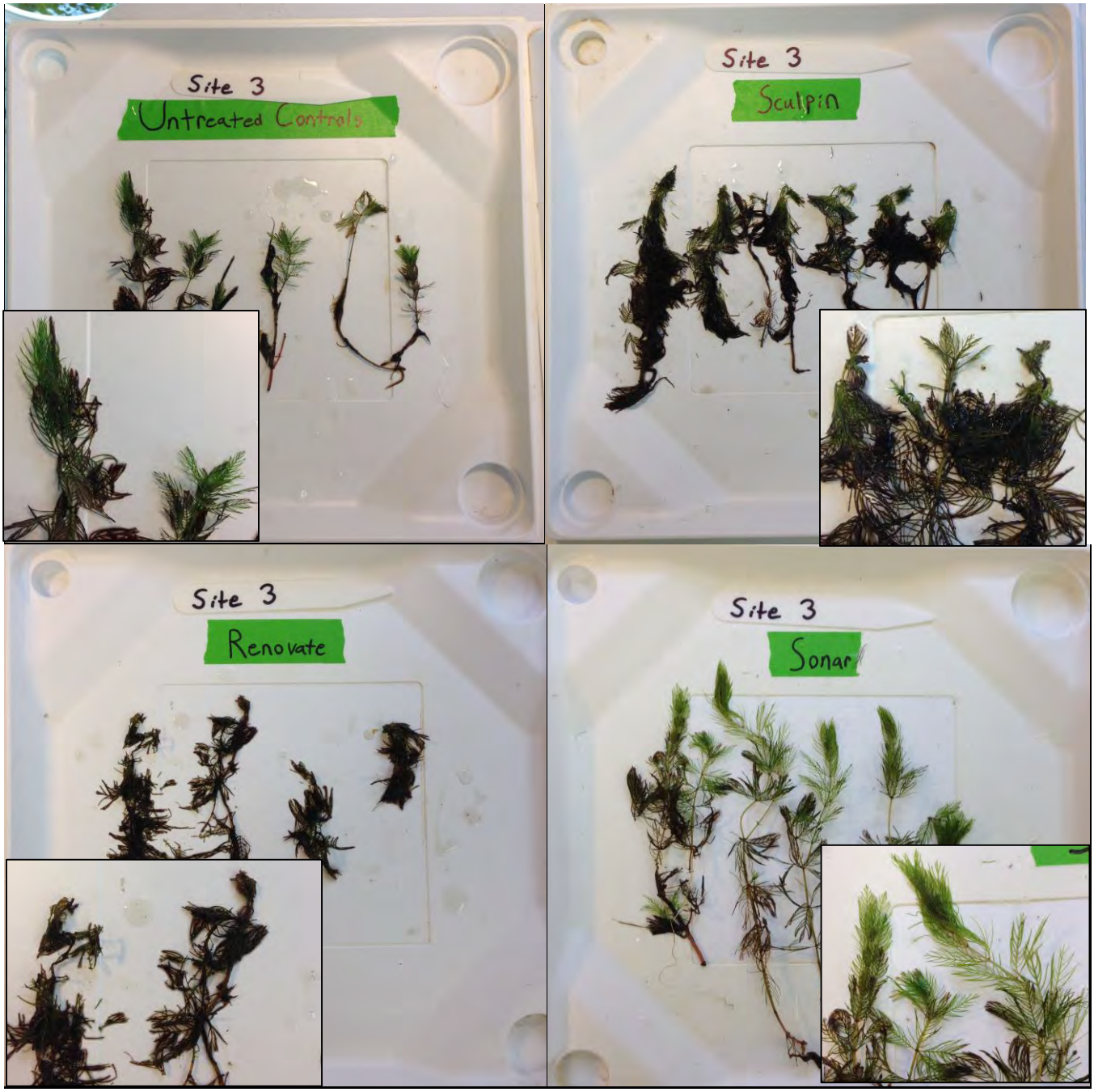

Image 4. Photographs of Little Bear Skin Lake Site 3 sample at termination of PlanTEST bioassay. Note untreated controls appear healthy, some Sculpin treated plants are necrotic while others are beginning to recover, Renovate treated plants are necrotic, and Sonar treated plants are chlorotic.

Image 5. Photographs of Little Bear Skin Lake Site 4 sample at termination of PlanTEST bioassay. Note untreated controls appear healthy, some Sculpin and Renovate treated plants are necrotic while others beginning to recover, and Sonar treated plants are chlorotic.

Image 6. Photographs of Little Bear Skin Lake Site 5 sample at termination of PlanTEST bioassay. Note untreated controls appear healthy, Renovate and Sculpin treated plants are beginning to recover (Renovate treated recovering to a lesser extent as the Sculpin treated), and Sonar treated plants are chlorotic.