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APR 13 2021

BY: IW corresp.
4/14/21

April 8, 2021

Inland Wetlands and Watercourses Commission

Re: Revised CT DEEP Permit Application for Bantam Lake – Morris/Litchfield, CT

Dear Inlands Wetlands Agent,

Please see the attached revised CT DEEP permit for Bantam Lake located in Morris and Litchfield at 50 Palmer Road in Morris. Permit AQUA-2020-185 was transferred to SOLitude Lake Management from Stahl Holdings, LLC. We are requesting to add the use of Aquathol K (Endothall), EPA Registration Number 70506-176, with CT DEEP.

Please feel free to reach out to me if you have any questions or concerns.

Regards,

Meghan Stewart
Regional Administrator
SOLitude Lake Management
mstewart@solitudelake.com
dmeringolo@solitudelake.com



**Connecticut Department of
Energy & Environmental Protection**
Bureau of Materials Management & Compliance Assurance
Engineering & Enforcement Division

Permit Application for the Use of Pesticides in State Waters

Please complete this form in accordance with section 22a-66z CGS and the [instructions](#) (DEEP-PEST-INST-200) in order to ensure the proper handling of your application. Print or type unless otherwise noted. You must submit the initial fee along with this form.

CPPU USE ONLY

App #: _____

Doc #: _____

Check #: _____

Program: Aquatic Pesticides

Part I: Application Type and Description

This application is to request (check one):

☐ One year permit ☐ Two year permit ☒ Three year permit

Note: Multi-year permits will be issued at the Department of Energy and Environmental Protection's (DEEP) discretion.

☒ Check here if DEEP has previously issued an Aquatic Pesticide Permit for this site.

Permit Number for most recent permit: Unknown

☐ Check here if the information contained in this application is identical to the last application and the chemicals, quantities and number of treatments requested are identical to the chemicals, quantities and number of treatments permitted by the most recent permit issued.

Town where site is located: Litchfield/Morris

Brief Description of Project: **Aquatic pesticide application for Bantam Lake in Litchfield & Morris**

Part II: Fee Information

An application fee of \$200.00 ~~(\$100)~~ is to be submitted with *each* permit that you are applying for. Each site requires a separate permit. The application will not be processed without payment of the fee. If you are applying for a multi-year permit, see Part II of the [instructions](#) for information on fee payment. There is no discount for municipalities. The fee shall be non-refundable and shall be paid by check or money order to the Department of Energy and Environmental Protection.

Part III: Site Location

Name of Waterbody: **Bantam Lake**

Street address and/or description of location: **50 Palmer Road**

City/Town: **Morris**

State: **CT**

Zip Code: **06763**

Part IV: Applicant Information

- If an applicant is a corporation, limited liability company, limited partnership, limited liability partnership, or a statutory trust, it must be registered with the Secretary of State. If applicable, the applicant's name shall be stated **exactly** as it is registered with the Secretary of State. This information can be accessed at [CONCORD](http://www.concord-sots.ct.gov/CONCORD/index.jsp). (www.concord-sots.ct.gov/CONCORD/index.jsp)
- If an applicant is an individual, provide the legal name (include suffix) in the following format: First Name; Middle Initial; Last Name; Suffix (Jr, Sr., II, III, etc.).
- If there are any changes or corrections to your company/facility or individual mailing or billing address or contact information, please complete and submit the [Request to Change Company/Individual Information](#) to the address indicated on the form. If there is a change in name of the entity holding a DEEP license or a change in ownership, contact the Office of Planning and Program Development (OPPD) at 860-424-3003. For any other changes you must contact the specific program from which you hold a current DEEP license.

1. Applicant Name: SOLitude Lake Management

Mailing Address: 590 Lake Street

City/Town: Shrewsbury

State: MA

Zip Code: 01545

Business Phone: 508-865-1000

ext.:

Contact Person: Keith Gazaille

Phone: 508-865-1000 ext.

*E-mail: kgazaille@solitudelake.com

*By providing this e-mail address you are agreeing to receive official correspondence from DEEP, at this electronic address, concerning the subject application. Please remember to check your security settings to be sure you can receive e-mails from ".ct.gov" addresses. Also, please notify DEEP if your e-mail address changes.

- a) Applicant Type (check one): ☐ individual ☒ *business entity ☐ federal agency
☐ state agency ☐ municipality ☐ tribal

*If a business entity:

- i) check type: ☐ corporation ☒ limited liability company ☐ limited partnership
☐ limited liability partnership ☐ statutory trust ☐ Other: _____

- ii) provide Pesticide Application Business Registration Number and Registration expiration date:

B-3268; 8/31/2021

- iii) provide Secretary of the State business ID #: 1195604 This information can be accessed at [CONCORD](http://www.concord-sots.ct.gov/CONCORD/index.jsp)

- iv) ☐ Check here if your business is **NOT** registered with the Secretary of State's office.

- b) Applicant's relationship to the property at which the proposed activity is to be located:

- ☐ site owner ☐ option holder ☐ lessee
☐ easement holder ☐ operator ☒ pesticide applicator
☐ other (specify): _____

- ☐ Check if any co-applicants. If so, attach additional sheet(s) with the required information as requested above.

2. Billing contact, if different than the applicant.

Name:

Mailing Address:

City/Town:

State:

Zip Code:

Business Phone:

ext.:

Contact Person:

Phone:

ext.

*E-mail:

Part IV: Applicant Information (continued)

3. Primary contact for departmental correspondence and inquiries, if different than the applicant.

Name:

Mailing Address:

City/Town:

State:

Zip Code:

Business Phone:

ext.:

Contact Person:

Phone:

ext.

*E-mail:

4. Owner Information

- a. If known, list the name and address of all owners of the area(s) to be treated. If unsure, go to item #4b.

You can add rows to this table by using "tab" in the last row, in the last column.

Name of Owner	Address
Bantam Lake Protective Association	P.O. Box 37 Morris, CT 06763

*If an area(s) to be treated is owned or controlled by the state of Connecticut, see [instructions](#) for submitting an application to the DEEP Land Acquisition and Management Unit (LAM) for review and approval of the proposed treatment on state property. A LAM Authorization letter must be submitted as Attachment G for any application involving treatment of a waterbody that is owned or controlled by the state of Connecticut.

Part IV: Applicant Information (continued)

4b. If the applicant is unsure of who owns an area(s) to be treated, provide the name and address for all shoreline property owners located 200 feet or less from such area.

You can add rows to this table by using "tab" in the last row, in the last column.

Name of Shoreline Property Owner	Address

5. List the person or company applying the pesticides.

Name: **SOLitude Lake Management**

Mailing Address: 590 Lake Street

City/Town: Shrewsbury

State: MA

Zip Code: 01545

Business Phone: 508-865-1000

ext.:

Contact Person: Keith Gazaille

Phone: 508-865-1000 ext.

E-mail: kgazaille@solitudelake.com

Certification Number: S-4330

Certification Expiration Date: 1/31/2022

Part V: Additional Information

If the applicant is submitting this application on behalf of someone else, identify the person(s) or organization(s) seeking to have pesticides applied to the treatment area(s) and provide the following information. If more than one person or organization is being represented, attach additional sheets providing the information requested below.

Name: **Bantam Lake Protective Association**

Mailing Address: P.O. Box 37

City/Town: Morris

State: CT

Zip Code: 06763

Business Phone: 917-856-3339

ext.:

Contact Person: Constance Trolle

Phone:

ext.

*E-mail: bantamlakeprotective@gmail.com

Part VI: Site Information

1. **COASTAL AREA:** Is the pesticide application located in a municipality within the coastal area?

☐ Yes ☒ No (check town list in the instructions)

If yes, is the water being treated subject to the ebb and flow of the tides, or inundated by saline or brackish water at least once a month? ☐ Yes ☐ No

If the water being treated is subject to the ebb and flow of the tides, or is inundated by saline or brackish water at least once a month, you must submit a [Coastal Consistency Review Form](#) (DEEP-APP-004) with your application as Attachment C.

For assistance in determining if the water being treated is affected by tidal water as described above or in completing the Coastal Consistency Review form, contact the Office of Long Island Sound Programs (OLISP) at 860-424-3034.

2. **NATURAL DIVERSITY DATA BASE (NDDDB) - ENDANGERED OR THREATENED SPECIES:**

According to the most current "Natural Diversity Data Base Areas Maps", will the activity which is the subject of this application, including all impacted areas, be located within an area identified as, or otherwise known to be, a habitat for state listed endangered, threatened or special concern species?

☒ Yes ☐ No Date of Map: 12/2019

If yes, complete and submit a [Request for NDDDB State Listed Species Review Form](#) (DEEP-APP-007) to the address specified on the form, prior to submitting this application. Please note NDDDB review generally takes 4 to 6 weeks and may require the applicant to produce additional documentation, such as ecological surveys, which must be completed prior to submitting this permit application. A copy of the NDDDB Determination response letter that has not expired **must** be submitted with this completed application as Attachment D. Include a copy of any mitigation measures developed for this activity and approved by NDDDB. Be aware that you must renew your NDDDB Determination if it expires before project work commences. If the required NDDDB documents are not submitted as Attachment D, your application will be deemed incomplete and may be subject to denial.

For more information visit the DEEP website at www.ct.gov/deep/nddbrequest or call the NDDDB at 860-424-3011.

3. **AQUIFER PROTECTION AREAS:** Is the site located within a town required to establish Aquifer Protection Areas, as defined in section 22a-354a through 354bb of the General Statutes (CGS)?

☒ Yes ☐ No To view the applicable list of towns and maps visit the DEEP website at www.ct.gov/deep/aquiferprotection

If yes, is the site within an area identified on a Level A or Level B map? ☐ Yes ☒ No

If your site is on a Level A or Level B map, you are not required to register under the Aquifer Protection Program, **however** you must follow proper spill control measures to prevent potential contamination of drinking water. If you should have a spill, please call the emergency hotline **immediately** at 860-424-3338.

4. **CONSERVATION OR PRESERVATION RESTRICTION:** Is the property subject to a conservation or preservation restriction? ☐ Yes ☒ No

If Yes, proof of written notice of this application to the holder of such restriction or a letter from the holder of such restriction verifying that this application is in compliance with the terms of the restriction must be submitted as Attachment F.

5. Type of area to be treated: ☐ Tidal Waters ☒ Pond or Lake ☐ Stream

6. Is the waterbody located in a public water supply watershed? (See [instructions](#)) ☐ Yes ☒ No
If Yes, DPH comments may be required as Attachment I to this application.

7. Is the waterbody potentially located 200 ft. or less from a public water supply well? ☒ Yes ☐ No
(See [instructions](#)) If Yes, DPH comments **must** be submitted as Attachment I to this application.

8. Where does the waterbody flow to (Name of receiving stream or waterbody)? Bantam River

Is the outflow usually flowing? ☒ Yes ☐ No

Can outflow be stopped? ☐ Yes ☒ No

Part VI: Site Information (continued)

You can add rows to the tables below, by using "tab" in the last row, in the last column.

9. Identify the size of the waterbody(ies) and the portion of the waterbody(ies) to be treated. Refer to the instructions .					
Name of Waterbody	Total Acres	Average Depth ft.	Total Volume Acre ft.	Total Treated Portion ²	
				Acres	Volume Acre-ft
Bantam Lake	947	14	13258	947	3788
10. Identify each proposed product to be used, the amount per treatment, the number of treatments and the surface area (acres) or volume (acre feet) of water to be treated with that product. If more than one waterbody will be treated, provide this information for each waterbody.					
Name of Waterbody	Full Product Name	Amount per Treatment ¹	Number of Treatments	Treated Portion ²	
				Acres	Volume Acre-ft
Bantam Lake	CuSo4	2648 lbs	3	473	1892
	Nautique	192 gal	2	20	80
	ProcellaCOR	56.25 gal	2	75	450
	Reward	75 gal	2	75	
	Clipper	56.25 lbs	2	75	
	Aquathol K	585 gals	2	75	450

¹Provide quantities using only the units specified in the [instructions](#).

²If treating more than 80 acres of a single waterbody or 20 linear miles of shoreline in a calendar year, registration for approval under the [General Permit for Point Source Discharges to Waters of the State from the Application of Pesticides](#) is required. (See [instructions](#)). Such approval must be submitted as Attachment H to this application if required.

Part VI: Site Information (continued)

11. Does the waterbody have public access? ☒ Yes ☐ No
12. Is there a [state-owned boat launch](#)? ☐ Yes ☒ No
If yes, will the boat launch be used to access the waterbody? ☐ Yes ☐ No
If yes, will the boat launch be used for any purpose other than launching a boat? ☐ Yes ☐ No
If yes, see [instructions](#) for submitting an application to the DEEP Land Acquisition and Management Unit for review and approval of state property.
13. Is the waterbody stocked with fish by the state? ☐ Yes ☒ No
14. Identify use(s) of waterbody:
☒ domestic water supply ☒ irrigation ☐ watering livestock ☒ swimming ☒ fishing ☐ None
15. Are there any downstream users of the water who may be affected by treatment? ☐ Yes ☒ No
If yes, please explain:
16. Within 200 ft., inclusive, of the treatment area, are there any **private** drinking water wells 50 ft. or less from the shoreline? ☒ Yes ☐ No
17. Identify all plants or animals to be controlled: **Algae, Milfoil, Fanwort, Curly-leaf, Pondweeds, Naiad.**
- 18a. Identify all types of fish present: **Bluegill, Bass, Minnow, Northern Pike**
- 18b. If a copper-based product will be used and there are fish species sensitive to copper, what is the alkalinity of the water to be treated? **n/a**
19. Projected date(s) of pesticide use: **April-October**
20. List prior years in which chemicals were applied to this waterbody:
Unknown, at least 2016-2019

Part VII: Supporting Documents

Be sure to read the instructions (DEEP-PEST-INST-200) to determine whether the attachments listed are applicable to your specific activity. Check the applicable box below for each attachment being submitted with this application form. When submitting any supporting documents, please label the documents as indicated in this part (e.g., Attachment A, etc.) and be sure to include the applicant's name as indicated on this application form.

- ☒ Attachment A: An 8-1/2" x 11" legible copy or original of a USGS Topographic Quadrangle Map (scale 1:24,000) indicating the exact location of the area to be treated.
- ☒ Attachment B: [Applicant Compliance Information Form](#) (DEEP-APP-002), if applicable.
- ☐ Attachment C: [Coastal Consistency Review Form](#) (DEEP-APP-004), if applicable.
- ☒ Attachment D: A copy of the NDDB Determination response letter that has not expired, if applicable. Include a copy of any mitigation measures developed for this activity and approved by NDDB. Do *not* submit any NDDB Preliminary Site Assessments with your application. Be aware that you must renew your NDDB Determination if it expires before project work commences.
- ☒ Attachment E: Verification of Notification to Local Inland Wetland Agency:
- 1) copy of a certified mail receipt, or
 - 2) a copy of the application stamped and dated as received by the local inland wetlands agency, or
 - 3) an e-mail from the local inland wetlands agency verifying that this completed application has been sent to such agency.
- For multiple applications submitted to the local inland wetlands agency under one certified mail receipt, please attach a copy of the certified mail receipt to each application.
 - For multiple applications submitted to the local inland wetlands agency under one email, the e-mail from the agency clearly confirming receipt of each application.
- Refer to the [instructions](#).
- ☐ Attachment F: Conservation or Preservation Restriction Information, if applicable.
- ☐ Attachment G: DEEP Land Management Unit's Authorization letter for treatment of a state-owned or controlled waterbody and/or use of a state-owned boat launch, if applicable.
- ☐ Attachment H: Approval under the [General Permit for Point Source Discharges to Waters of the State from the Application of Pesticides](#), if applicable.
- ☒ Attachment I: Department of Public Health comments if the proposed treatment area(s) is located 200 ft. or less from a public water supply well or if the waterbody is located within a public water supply watershed and the application proposes the use of flumioxazin or triclopyr, if applicable.

Please note that local inland wetlands agencies may have additional requirements pertaining to the application of aquatic pesticides to waterbodies located under their jurisdiction.

Part VIII: Application Certification

The applicant and the individual(s) responsible for actually preparing the application must sign this part. An application will be considered insufficient unless *all* required signatures are provided. Please also check the box and provide the date for which you sent one copy of this completed application to the appropriate local inland wetland agency.

"I have personally examined and am familiar with the information submitted in this document and all attachments thereto, and I certify that based on reasonable investigation, including my inquiry of the individuals responsible for obtaining the information, the submitted information is true, accurate and complete to the best of my knowledge and belief.

I understand that a false statement in the submitted information may be punishable as a criminal offense, in accordance with section 22a-6 of the General Statutes, pursuant to section 53a-157b of the General Statutes, and in accordance with any other applicable statute.

I certify that this application is on complete and accurate forms as prescribed by the commissioner without alteration of the text.

☒ I also certify that I have sent one copy of this completed application to the appropriate local inland wetland agency on _____

Date

Signature of Applicant

Date

Jeff Stahl

President

Name of Applicant (print or type)

Title (if applicable)

Signature of Preparer (if different than above)

Date

Nicholas McMahon

Applicator

Name of Preparer (print or type)

Title (if applicable)

☐ Check here if additional signatures are required. If so, please reproduce this sheet and attach signed copies to this sheet.

Note: Please submit this completed Application Form, Fee, and all Supporting Documents to:

CENTRAL PERMIT PROCESSING UNIT
DEPARTMENT OF ENERGY AND ENVIRONMENTAL PROTECTION
79 ELM STREET
HARTFORD, CT 06106-5127

Please also submit a copy of this completed application to the local inland wetlands agency.

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I certify that this application is on complete and accurate forms as prescribed by the commissioner without alteration of the text.

☒ I also certify that I have sent one copy of this completed application to the appropriate local inland wetland agency on 4/7/2021 " _____
Date


Signature of Applicant

4/7/2021
Date

Keith Gazaille
Name of Applicant (print or type)

Regional Director
Title (if applicable)


Signature of Preparer (if different than above)

4/7/2021
Date

Meghan Stewart
Name of Preparer (print or type)

Regional Administrator
Title (if applicable)

☐ Check here if additional signatures are required. If so, please reproduce this sheet and attach signed copies to this sheet.

Note: Please submit this completed Application Form, Fee, and all Supporting Documents to:

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79 ELM STREET
HARTFORD, CT 06106-5127

Please also submit a copy of this completed application to the local inland wetlands agency.



March 25, 2021

Keith Gazaille
SOLitude Lake Management
590 Lake Street
Shrewsbury, MA 01545
kgazaille@solitudelake.com

NDDB Determination No: 202101308

Project: Aquatic Plant Control for the Treatment of Eurasian Watermilfoil, Pondweeds, Nails, Curly-leaf Pondweed and Fanwort in Bantam Lake Located in Morris and Litchfield, CT

Proposed products: Reward (diquat), Aquathol-K (endothal), Clipper (flumioxazin), ProcellaCOR (florpyrauxifen), Nautique (copper), and Copper Sulfate

Dear Keith Gazaille,

I have reviewed Natural Diversity Data Base (NDDB) maps and files regarding Aquatic Control for the Treatment of Eurasian Watermilfoil, Pondweeds, Nails, Curlyleaf pondweed and Fanwort using the above proposed products in Bantam Lake in Morris and Litchfield, Connecticut. According to our records, multiple State-listed species (RCSA Sec. 26-306) have been documented within the proposed project area.

STATE-LISTED PLANTS

Bantam Lake is the site of an ongoing 'Incidental Take' approved in June 2005 by the Connecticut Department of Energy and Environmental Protection (DEEP) and the Connecticut Office of Policy and Management (OPM) to address adverse impacts to the following species resulting from proposed aquatic plant control at the lake:

- **Water-marigold (*Bidens beckii*)**
Protection Status: State Special Concern
Habitat: Circumneutral ponds and slow rivers.
Blooms August, September
- **Slender water-milfoil (*Myriophyllum alterniflorum*)**
Protection Status: State Endangered
Habitat: Shallow waters.
Blooms August, September

Required Protective Mitigation

1. An aquatic plant botanist must survey the areas targeted for the later July treatment for the presence of state-listed plants prior to the second treatment. If state-listed plants are found, a second herbicide application should not occur within these areas. Detailed maps showing the locations of early and late treatment in relation to the presence of the state listed plants should also be provided to the NDDB Program each year, no later than December 31st.
2. An aquatic plant botanist must survey the lake specifically for the two state listed plants each year the lake will be treated with herbicides. The survey results should be sent to the NDDB Program no later than December 31st of each year. The survey must include locational details and maps of any state listed plant species observed and botanical information on the plant populations. The results should be sent to the NDDB Program (deep.nddbrequest@ct.gov) using a NDDB Program rare plant survey form: (https://www.ct.gov/deep/lib/deep/endangered_species/general_information/PlantForm.pdf)

3. A conservation plan, outlining future proposed treatment of the lake with respect to the state listed plants, must be developed before moving forward with herbicide treatment each time a new pesticide permit is sought from CTDEEP. The conservation plan must address the state listed plants and how the plants will be protected from the herbicides selected to treat the present invasive plants.
4. The use of ProcellaCOR can proceed in areas that do not have any known occurrences of Beck's marigold. This herbicide potentially may be allowed to be used within the 600 foot buffer in future years if an appropriate lab testing can be completed to provide sensitivity data on Beck's marigold to this herbicide. Please provide information that summarizes the lab testing results and we will consider amending the restricted 600 foot buffer from any Beck's marigold to 100 feet if the ProcellaCOR does not eliminate the state listed plant or the Beck's marigold is not sensitive to treatment with ProcellaCOR.

STATE-LISTED WILDLIFE SPECIES

To avoid impacts to the State Endangered American Bittern (*Botaurus lentiginosus*) and State Threatened Least Bittern (*Ixobrychus exilis*) that occur along portions of the North Bay shoreline wetlands, when spraying in North Bay, north of Marsh Point, the permittee shall:

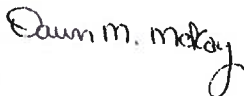
1. Not disturb areas with emergent vegetation where the birds may be nesting.
2. Make all applications from the water using a boat powered only by an electric or gas powered motor. No air boats shall be used.
3. Maintain the maximum possible distance between the boat and any emergent vegetation where the birds may be nesting.
4. Apply no chemicals within 25 feet of any emergent vegetation where the birds may be nesting.

This letter is valid until December 31, 2022.

Natural Diversity Data Base information includes all information regarding critical biological resources available to us at the time of the request. This information is a compilation of data collected over the years by the Department of Energy and Environmental Protection's Bureau of Natural Resources and cooperating units of DEEP, independent conservation groups, and the scientific community. This information is not necessarily the result of comprehensive or site-specific field investigations. Consultations with the NDDB should not be substituted for on-site surveys required for environmental assessments. Current research projects and new contributors continue to identify additional populations of species and locations of habitats of concern, as well as, enhance existing data. Such new information is incorporated in the NDDB as it becomes available.

Please contact me if you have any questions (dawn.mckay@ct.gov; 860-424-3592). Thank you for consulting with the Natural Diversity Data Base and continuing to work with us to protect State listed species.

Sincerely,



Dawn M. McKay
Environmental Analyst 3

Bantam Lake

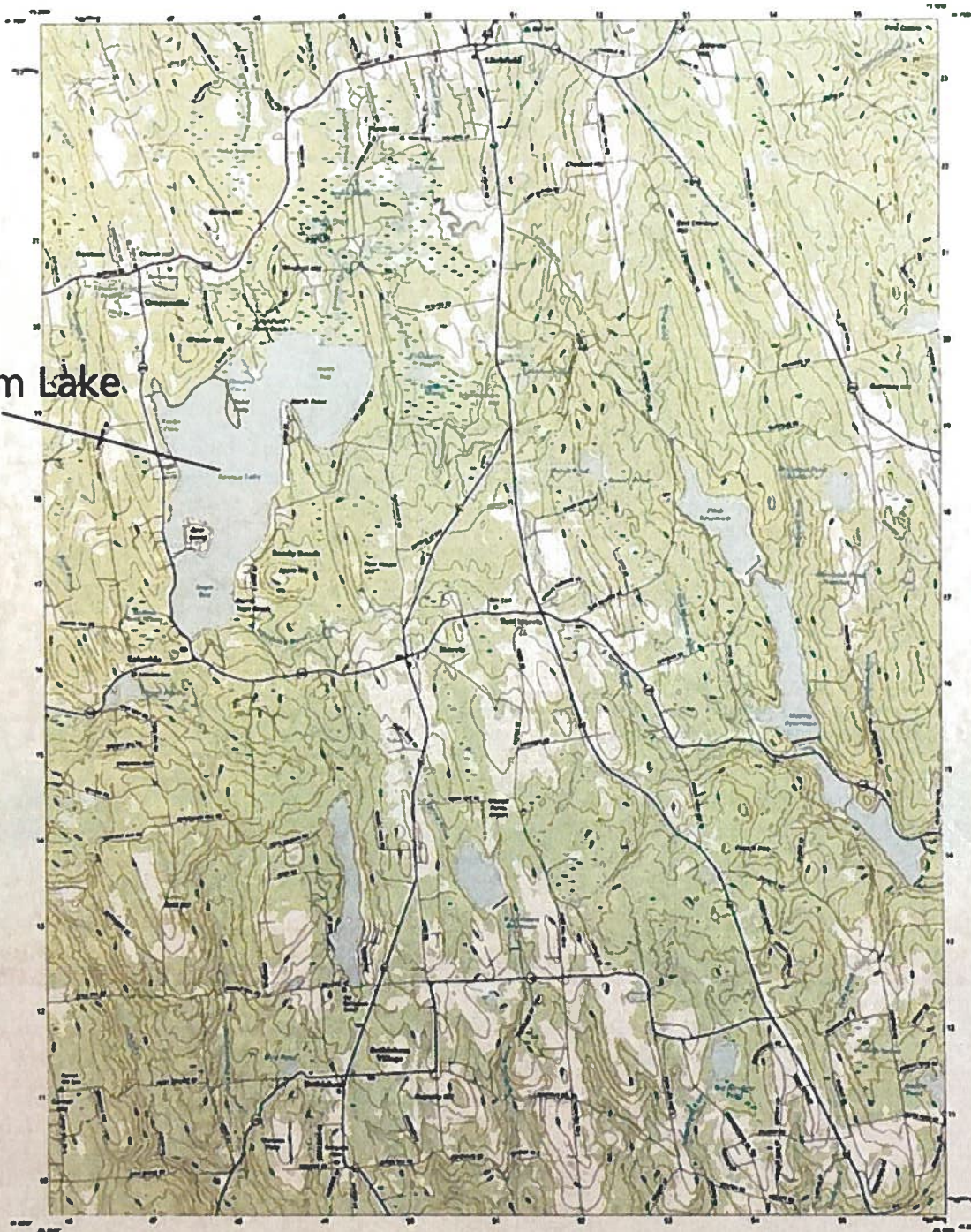


U.S. DEPARTMENT OF THE INTERIOR
U.S. GEOLOGICAL SURVEY



LITCHFIELD QUADRANGLE
CONNECTICUT - LITCHFIELD COUNTY
1:50,000 (7.5-MINUTE SERIES)

Bantam Lake

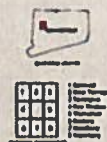


Prepared by the United States Geological Survey

Scale 1:50,000
Horizontal scale: 1 inch = 1 mile
Vertical scale: 1 inch = 100 feet
The map is a reproduction of the original map and is not a new edition.
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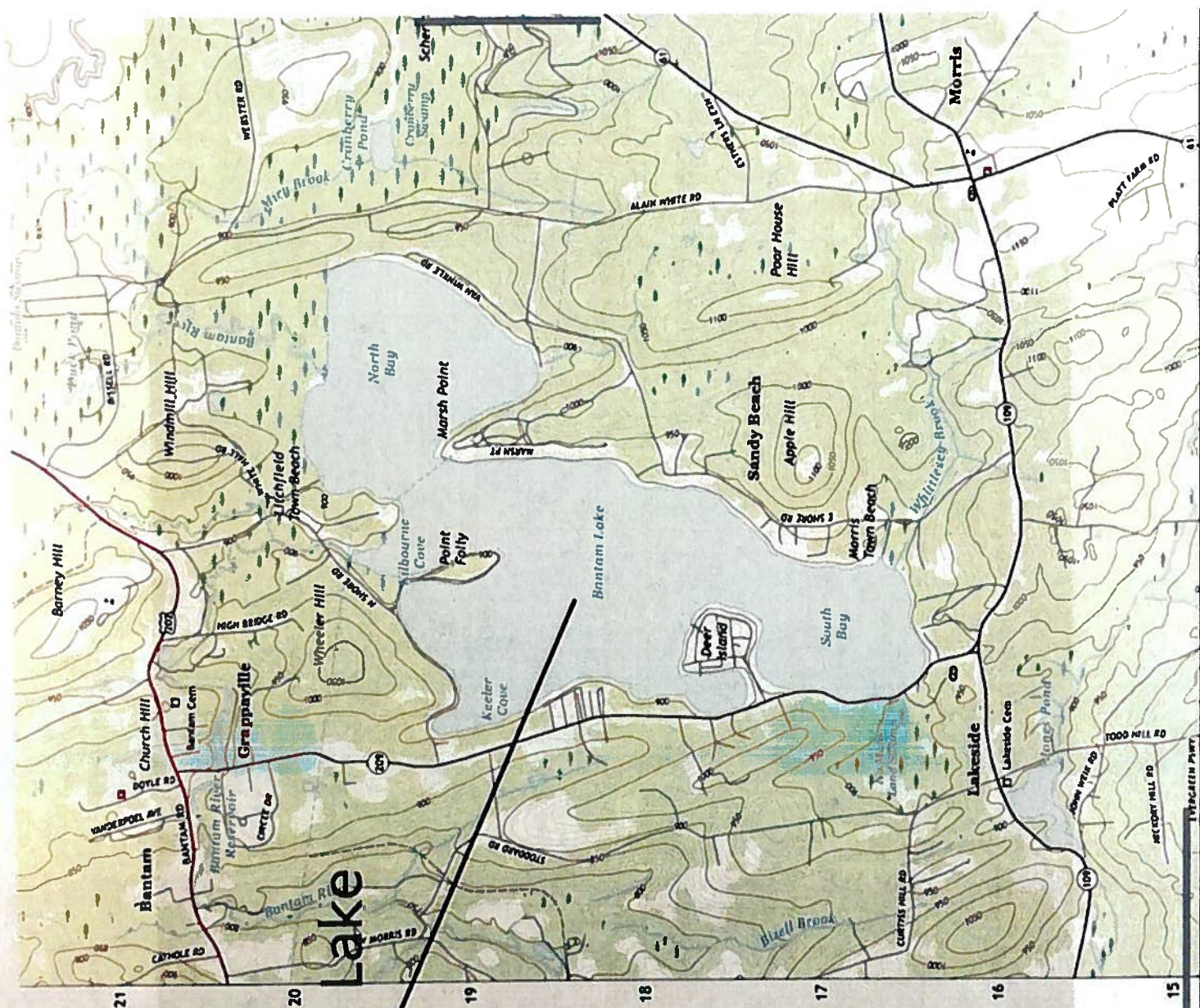


Horizontal scale: 1 inch = 1 mile
Vertical scale: 1 inch = 100 feet
The map is a reproduction of the original map and is not a new edition.
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The map is a reproduction of the original map and is not a new edition.



Symbol	Description
[Symbol]	Highway
[Symbol]	Water
[Symbol]	Other

LITCHFIELD, CT
1940



Bantam Lake





Bantam Lake

Google Earth

© 2015 Google



79 Elm Street • Hartford, CT 06106-5127

www.ct.gov/deep

Affirmative Action/Equal Opportunity Employer

April 13, 2020

Nick McMahon
Stahl Holdings, LLC
D/B/A The Pond and Lake Connection
1112 Federal Road
Brookfield, CT 06804
nick@thepondandlake.com

NDDB Determination No: 202001961

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Blooms August, September
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Protection Status: State Endangered
Habitat: Shallow waters.
Blooms August, September

Required Protective Mitigation

1. An aquatic plant botanist must survey the lake specifically for the two state listed plants each year the lake will be treated with herbicides. The survey results should be sent to the NDDB Program no later than December 31st of each year. The survey must include locational details and maps of any state listed plant species observed and botanical information on the plant populations. The results should be sent to the NDDB Program (deep.nddbrequest@ct.gov) using a NDDB Program rare plant survey form: (https://www.ct.gov/deep/lib/deep/endangered_species/general_information/PlantForm.pdf)
2. An aquatic plant botanist must survey the areas targeted for the later July treatment for the presence of state-listed plants prior to the second treatment. If state-listed plants are found, a second herbicide application should not occur within these areas. Detailed maps showing the locations of early and late treatment in relation to the presence of the state listed plants should also be provided to the NDDB Program each year, no later than December 31st.

3. A conservation plan, outlining future proposed treatment of the lake with respect to the state listed plants, must be developed before moving forward with herbicide treatment each time a new pesticide permit is sought from CTDEEP. The conservation plan must address the state listed plants and how the plants will be protected from the herbicides selected to treat the present invasive plants.
4. The use of ProcellaCOR can proceed in areas that do not have any known occurrences of Beck's marigold. This herbicide potentially may be allowed to be used within the 600 foot buffer in future years if an appropriate lab testing can be completed to provide sensitivity data on beek marigold to this herbicide. Please provide information that summarizes the lab testing results and we will consider amending the restricted 600 foot buffer from any Beck's marigold to 100 feet if the ProcellaCOR does not eliminate the state listed plant or the becks marigold is not sensitive to treatment with ProcellaCOR.

STATE-LISTED WILDLIFE SPECIES

To avoid impacts to the State Endangered American Bittern (*Botaurus lentiginosus*) and State Threatened Least Bittern (*Ixobrychus exilis*) that occur along portions the North Bay shoreline wetlands, when spraying in North Bay, north of Marsh Point, the permittee shall:

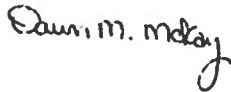
1. Not disturb areas with emergent vegetation where the birds may be nesting.
2. Make all applications from the water using a boat powered only by an electric or gas powered motor. No air boats shall be used.
3. Maintain the maximum possible distance between the boat and any emergent vegetation where the birds may be nesting.
4. Apply no chemicals within 25 feet of any emergent vegetation where the birds may be nesting.

This letter is valid until December 31, 2021.

Natural Diversity Data Base information includes all information regarding critical biological resources available to us at the time of the request. This information is a compilation of data collected over the years by the Department of Energy and Environmental Protection's Bureau of Natural Resources and cooperating units of DEEP, independent conservation groups, and the scientific community. This information is not necessarily the result of comprehensive or site-specific field investigations. Consultations with the NDDB should not be substituted for on-site surveys required for environmental assessments. Current research projects and new contributors continue to identify additional populations of species and locations of habitats of concern, as well as, enhance existing data. Such new information is incorporated in the NDDB as it becomes available.

Please contact me if you have any questions (dawn.mckay@ct.gov; 860-424-3592). Thank you for consulting with the Natural Diversity Data Base and continuing to work with us to protect State listed species.

Sincerely,



Dawn M. McKay
Environmental Analyst 3

Aquatic Pesticide Application: Bantam Lake, Morris:

Bantam Lake is not within a public water supply watershed; however White Memorial Campground Well #1 and Northland Properties, LLC Well #1 are within 200 feet of the Lake Bantam shoreline. The proposed chemicals are Copper Sulfate, Nautique (Copper), ProCellacor, Clipper (flumioxazin) and Reward (diquat). The DPH does not support the use of Diquat, listed as a Group 4 chemical per the CT DPH/CT DEEP Memoranda of Agreement (2012), and therefore does not support the use of this chemical. The applicant has indicated that a 500-foot buffer will be used around the above mentioned wells, and that only Copper Sulfate and Copper EDA/TEA will be applied in these two buffer zones (see attached map).

Sampling of these two public water system wells must be conducted if any chemical other than Copper Sulfate and Copper EDA/TEA is applied within 500 feet of these wells. Please note, currently Connecticut does not have a licensed laboratory capable of analyzing samples for the presence ProCellacor. In the event this chemical is used within the 500-foot buffer zone of the two wells, the applicant would have to send samples to the manufacturer for analysis.

If applicable, sampling shall be conducted 7-14 days after each application and the results must be submitted to the DPH, DEEP, White Memorial Campground, and Northland Properties, LLC. If sample results are not received future application requests may be denied.

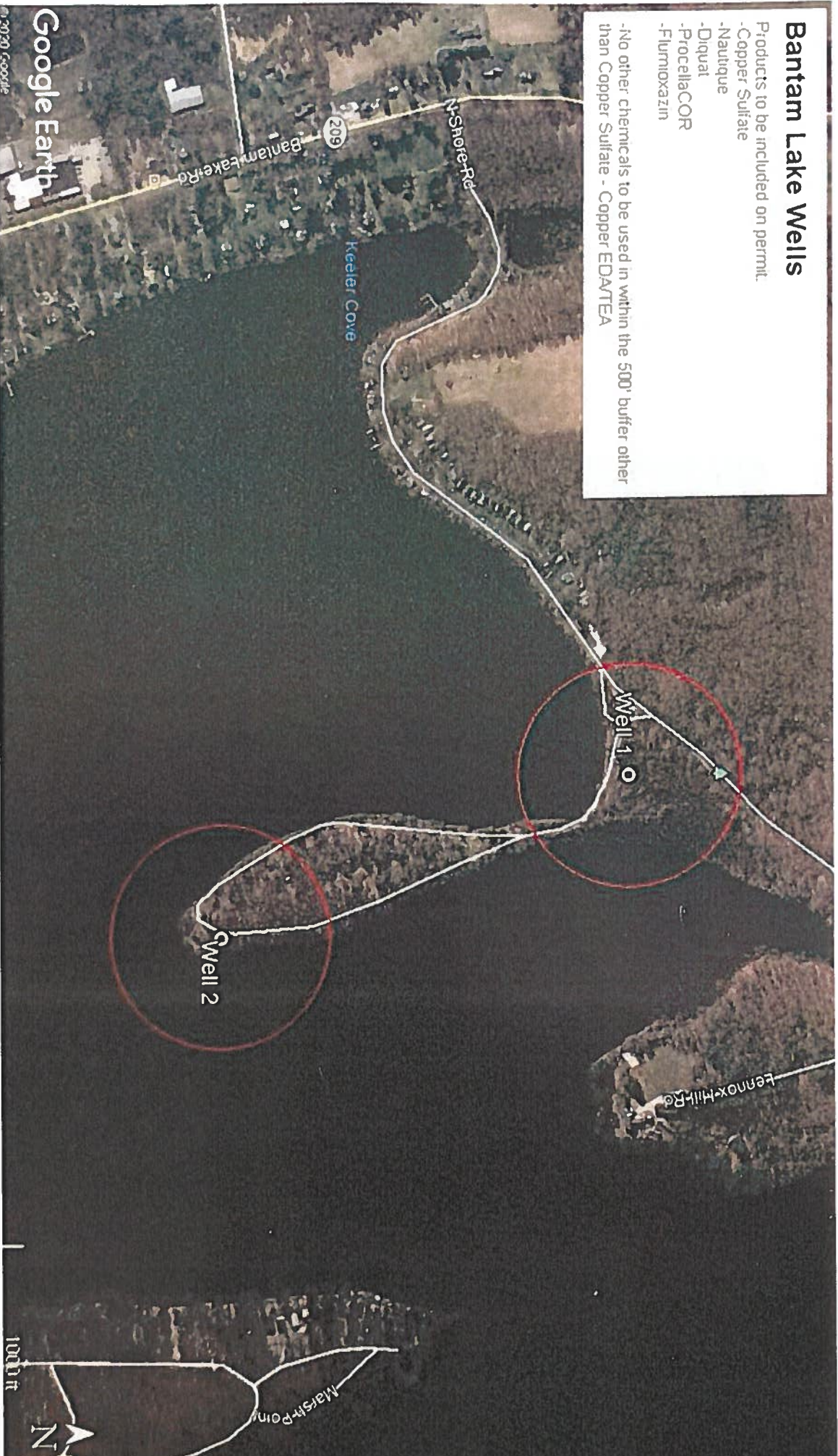
Previous DPH
Conditions need to
be amended to
include all 3
wells, yes diquat,
no endothall

Bantam Lake Wells

Products to be included on permit.

- Copper Sulfate
- Nautique
- Diquat
- ProcellaCOR
- Flumioxazin

-No other chemicals to be used in within the 500' buffer other than Copper Sulfate - Copper EDTA/TEA



Bantam Lake

Litchfield, CT

State Listed Aquatic Plant Survey



Prepared for the Bantam Lake Association

December 2, 2019

Gregory J. Bugbee and Summer E. Stebbins

Department of Environmental Science



CAES

The Connecticut Agricultural Experiment Station

Putting Science to Work for Society since 1875



Figure 1. Herbarium mounts of the state listed species slender watermilfoil (left) and water marigold (right) from Bantam Lake housed in the CAES aquatic plant herbarium.

Introduction

Pursuant to requirements contained in Connecticut Department of Energy and Environmental Protection (CTDEEP) Natural Diversity Database (NDDb) Determination No: 201900323, The Connecticut Agricultural Experiment Station (CAES) performed an aquatic plant survey for the state listed plants slender watermilfoil (*Myriophyllum alterniflorum*) and water marigold (*Bidens beckii*, syn. *Megalodonta beckii*) in Bantam Lake (Figure 1). CAES has surveyed and mapped the aquatic vegetation in 246 waterbodies in Connecticut (www.portal.ct.gov/caes-iapp). Water marigold has been observed in seven lakes and slender watermilfoil has been found in one. Bantam Lake was not included in these surveys, however Northeast Aquatic Research (NEAR 2019) has documented both species in the lake from 2002 – 2017 (Figure 2). These plants have been shown to cohabitate the shallows with a diverse plant community including the invasive species Eurasian watermilfoil (*Myriophyllum spicatum*) and fanwort (*Cabomba caroliniana*).

Methods

On August 29, 30, and September 19, 2019, CAES surveyed the shallows of Bantam Lake for the state listed plants. Survey techniques include slowly motoring throughout the areas likely to support the species of concern and noting their locations with a Trimble R1® global positioning system (GPS) linked to an onboard laptop computer running Trimble Terrasync® software. In addition to visual observation, plants were collected with a rake and grapple to assure accuracy. The survey path was recorded by GPS and is shown in Figure 3. Locations of state listed species were marked with GPS and associated vegetation was noted. Plant identifications followed the taxonomy of Crow and Hellquist (2000a, 2000b). The shallow water habitat for these species was often covered with dense emergent vegetation such as lily pads and watershield. This made access difficult and increased the chances that species of concern could remain hidden. This was particularly a concern for slender watermilfoil which is diminutive in nature and without close inspection can resemble associated native plants such as coontail (*Ceratophyllum demersum*) and bladderwort (*Utricularia* sp.). Another concern was reductions in plant coverage caused by the treatment of 45 acres of the lake with the herbicide Reward® (Diquat) and Aquathol K® (endothall) on July 30, 2019 (Constance Trolle, personal communication, 11/13/2019). Water clarity during this survey, measured with a Secchi disk, ranged from 3 – 5 feet and was adequate to see plants in the shallows.



Figure 2. Locations of alternate watermilfoil and water marigold in 2004 and 2006 (NEAR 2019).

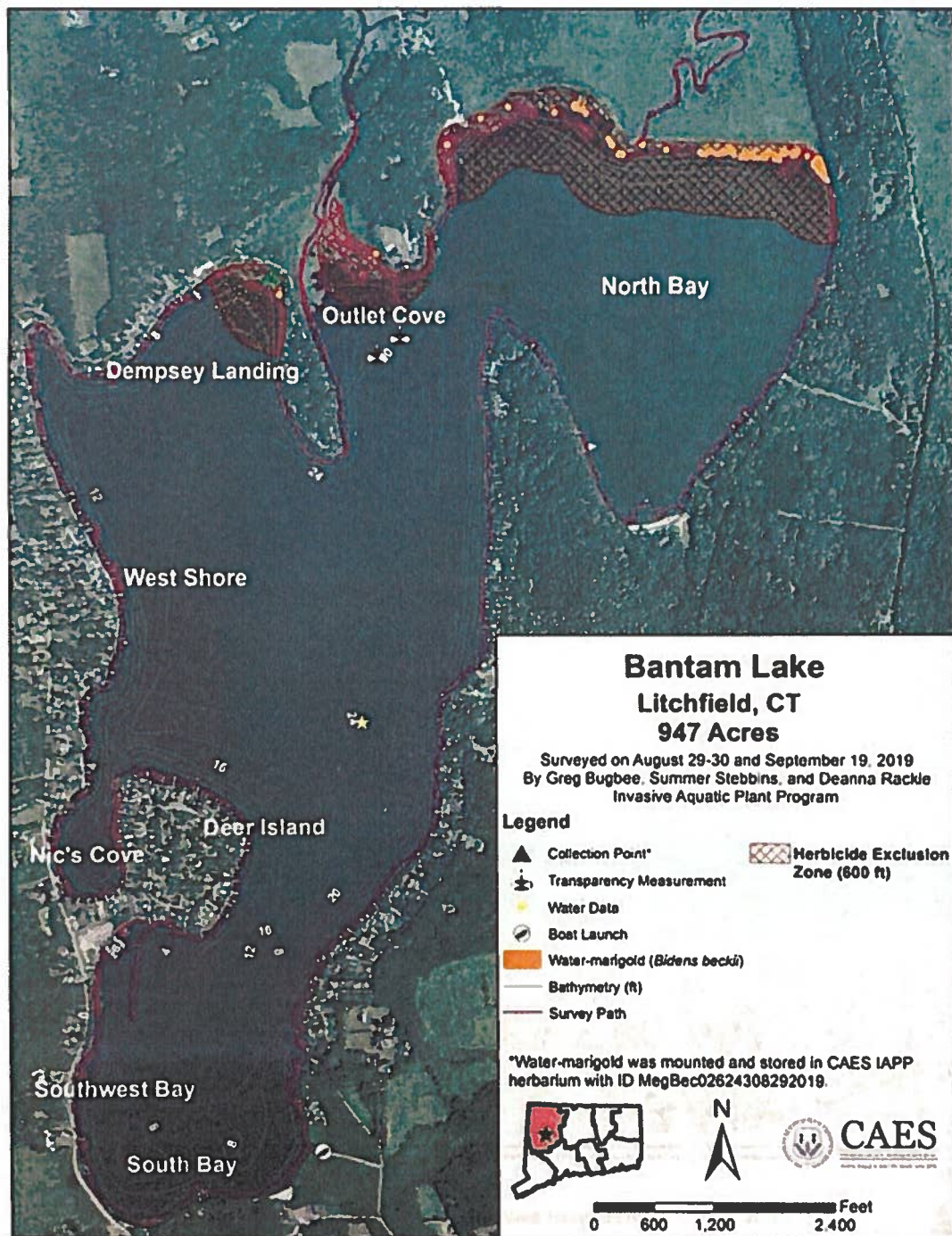


Figure 3. 2019 state listed aquatic plant survey of Bantam Lake with proposed 600-foot herbicide exclusion zone.

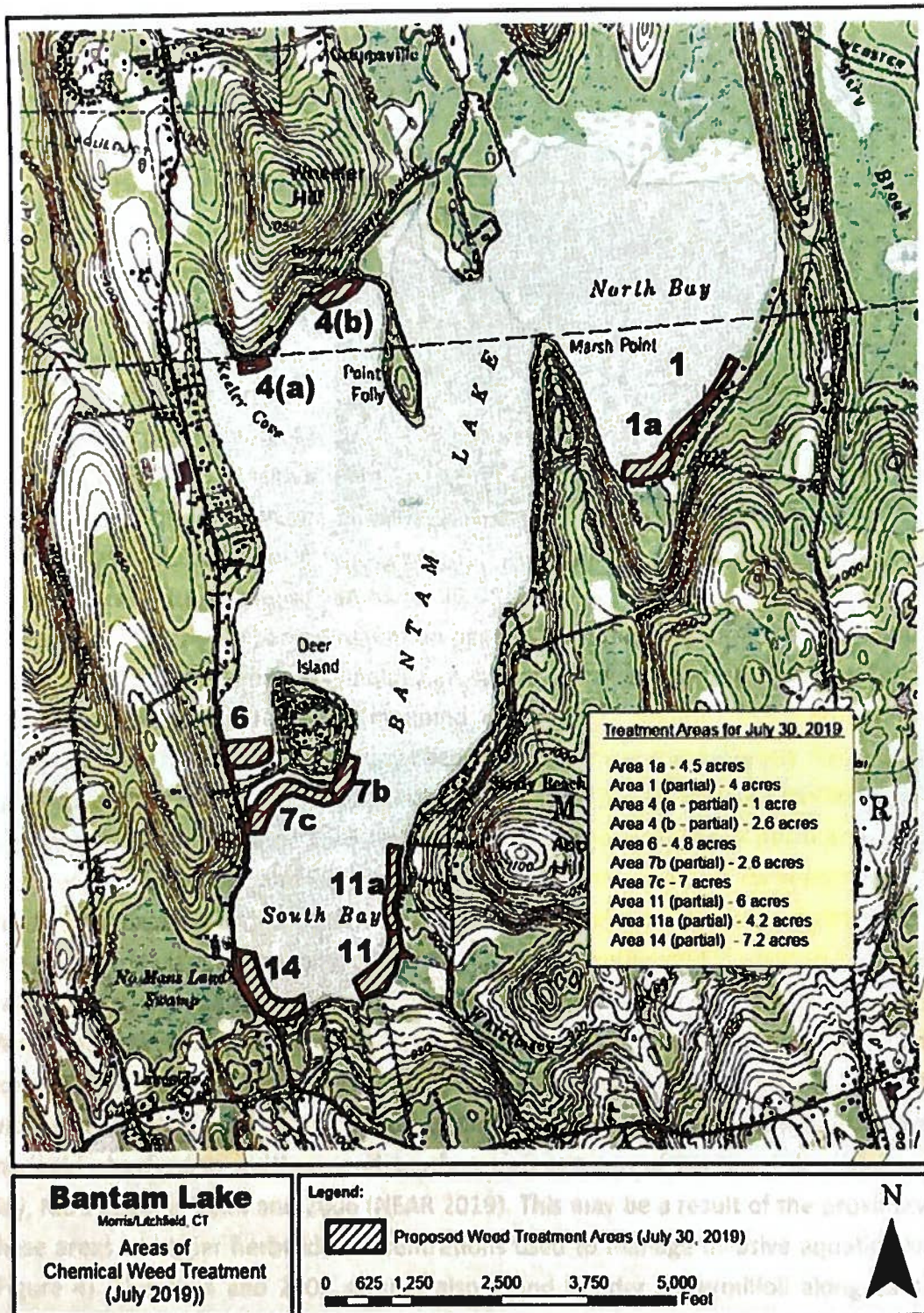


Figure 4. Areas treated with herbicide in 2019 (map courtesy of the Bantam Lake Protective Association).

Results

Our 2019 survey found water marigold was moderately abundant in the North Bay and sparse in Outlet Cove and Dempsey Landing (Figure 3). No slender watermilfoil was observed anywhere in the lake. The densest populations of water marigold occurred in breaks in the emergent vegetation (Figure 5). Positive identification of the water marigold was assured due to the presence of its distinctive



Figure 5. Water marigold growing with lily pads in North Cove on August 30, 2019.

flower (Figure 1 and herbarium mount on page 14) and the lack of the petiole found on fanwort which shares similar phenology. A specimen taken from the Dempsey Landing collection point in Figure 3 was mounted in the CAES aquatic plant herbarium (ID MegBec02624308292019) and is supplied with the Rare Plant Survey Form in the appendix of this report. This specimen site was typical of the other locations with a mucky bottom and depth range of 1 - 3 feet. Nearly all of the observed water marigold was recorded on August 29th and 30th while only one point was recorded on September 19th. Visually it appeared that a considerable amount of senescence had occurred since August. The associated plant community consisted of coontail, waterweed (*Elodea* sp.), southern naiad (*Najas guadalupensis*), yellow water lily (*Nuphar variegata*), white water lily (*Nymphaea odorata*), pickerelweed (*Pontederia cordata*), purple bladderwort (*Utricularia purpurea*), and water stargrass (*Zosterella dubia*). Fanwort was commonly associated with water marigold populations in other parts of the lake. We did not find the water marigold observed in southern and southeastern sections of Bantam Lake (Southwest Bay, Nic's Cove) in 2004 and 2006 (NEAR 2019). This may be a result of the proximity of these areas to higher herbicide concentrations used to manage invasive aquatic plants (Figure 4). The 2004 and 2006 surveys also found slender watermilfoil along Bantam Lake's western (Nic's Cove, West Shore) and northern (Dempsey Landing) shores. In 2019, none was observed in Bantam Lake. These areas are close to treatment sites which often do not show regrowth until well after an herbicide application. NEAR (2019) last reported slender watermilfoil in Bantam Lake's treatment sites in 2013.

Conservation of State Listed Species and Future Management

Invasive species are considered a major cause of species extinctions. Invasive aquatic plant species in Bantam Lake found in this survey include Eurasian watermilfoil, European waterclover (*Marsilea quadrifolia*), and fanwort (Table 1, Bugbee et al. 2019). Curlyleaf pondweed (*Potamogeton crispus*) also occurs in the lake (NEAR 2019) but was not observed likely because of herbicide applications and the natural senescence of the plant during the summer. Herbicide applications have been used to manage invasive aquatic plants in Bantam Lake for over a decade. In 2019, herbicides were applied to the areas shown in Figure 4. The population of water marigold has remained intact in Dempsey Landing, North Bay, and Outlet Cove likely due to small treatment areas, distance of treatments, and choice of products that provide the least impact. The last report of slender watermilfoil in Bantam Lake was in the North Bay in 2017 (NEAR 2019).

Although the plant was not observed during our survey, the dense emergent and submerged vegetation in the area could easily contain either undetected plants or plant propagules. Because the observed population of slender watermilfoil in 2017 occurred among the water marigold in North Cove, protecting the water marigold should provide the same benefit to slender watermilfoil.

Future conservation of both state listed species will involve ensuring their habitat is not lost to invasive species and that the management of invasive species does not have collateral effects. Because a significant population of water marigold and a possible population of slender watermilfoil cohabitate the North Bay, this area will need the greatest protection. Past herbicide treatments have allowed water marigold populations to remain moderately strong in North Bay suggesting future use of the same regime will

Table 1. Aquatic plant species observed in Bantam Lake in 2019.

Species recorded in our 2019 survey of Bantam Lake.	
Common Name	Scientific Name
Broad waterweed	<i>Elodea canadensis</i>
Common bladderwort	<i>Utricularia macrorhiza</i>
Common duckweed	<i>Lemna minor</i>
Coontail	<i>Ceratophyllum demersum</i>
Eel grass	<i>Vallisneria spiralis</i>
Eurasian watermilfoil	<i>Myriophyllum spicatum</i>
European waterclover	<i>Marsilea quadrifolia</i>
Fanwort	<i>Cabomba caroliniana</i>
Floating-leaf pondweed	<i>Potamogeton natans</i>
Large-leaf pondweed	<i>Potamogeton amplifolius</i>
Pickereelweed	<i>Pontederia cordata</i>
Purple bladderwort	<i>Utricularia purpurea</i>
Robbins' pondweed	<i>Potamogeton robbinsii</i>
Southern naiad	<i>Najas guadalupensis</i>
Spikerush	<i>Eleocharis species</i>
Swamp loosestrife	<i>Decodon verticillatus</i>
Water stargrass	<i>Zosterella dubia</i>
Water marigold*	<i>Bidens beckii</i> *
Watershield	<i>Brasenia schreberi</i>
Western waterweed	<i>Elodea nuttallii</i>
White water lily	<i>Nymphaea odorata</i>
Yellow water lily	<i>Nuphar variegata</i>
Invasive Species in Bold	
*State Listed Species	

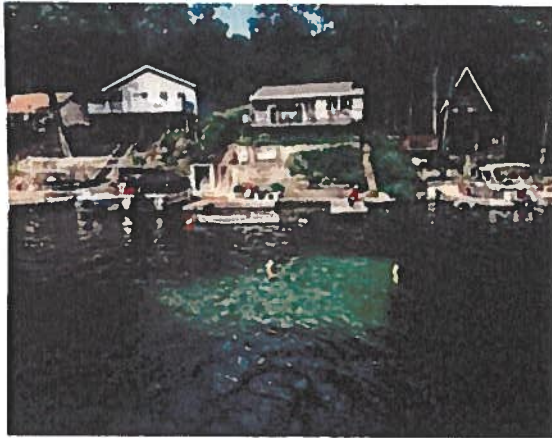


Figure 6. Benthic barrier installed to control fanwort in Bashan Lake (left). Limnobarrier to prevent herbicide from contacting state listed species in Crystal lake, Middletown (right).

offer similar protection. Additional protection could be achieved by adhering to an herbicide exclusion zone of 600 feet from the water marigold locations unless data is available showing the proposed products can be applied closer without harming the state listed species. Published data on the sensitivity of water marigold and slender watermilfoil to the common aquatic herbicides is lacking, leaving reports from applicators the greatest source of information. Unfortunately, the exclusion zone technique will not protect the state listed species from habitat loss to invasive species, particularly fanwort. This leaves few other options for assuring that invasive species do not overtake the water marigold other than strategic harvesting, careful placement of benthic blankets, and installation of limnobarriers to prevent herbicide movement to the locations populated with state listed species (Figure 6). Routine surveillance should also be employed to document any expansion of invasive species into the state listed species locations. It is possible the plant community is more stable than might be thought and corrective actions are not critical.

Acknowledgments

The surveillance efforts of CAES summer assistant Deanna Rackie is gratefully acknowledged. Information supplied by Constance Trolle and James Fischer of the Bantam Lake Protective Association and Dominic Meringolo of Solitude Lake Management Inc. is most appreciated.

References

Bugbee GJ, Barton ME, Gibbons JA, Stebbins SE. 2019. Connecticut's Invasive Aquatic Plant, Clam, Mussel Identification Guide. 3rd Ed. The Connecticut Agricultural Experiment Station. Bull. 1056. 50 pp.

CAES IAPP. 2019. The Connecticut Agricultural Experiment Station Invasive Aquatic Plant Program (CAES IAPP). <https://www.portal.ct.gov/caes-iapp>. Retrieved November 12, 2019.

Crow GE, Hellquist CB. 2000a. Aquatic and Wetland Plants of Northeastern North America. Vol. 1. Pteridophytes, Gymnosperms, and Angiosperms: Dicotyledons. University of Wisconsin Press, Madison.

Crow GE, Hellquist CB. 2000b. Aquatic and Wetland Plants of Northeastern North America. Vol. 2. Angiosperms: Monocotyledons. University of Wisconsin Press, Madison.

Northeast Aquatic Research. 2019. Review of 13 years of herbicide treatments in Bantam Lake. Report to the Bantam Lake Protective Association. June 19, 2019. 57 pp.

Appendix

Rare Plant Survey Form

CTDEEP

Natural Diversity Database

OFFICE USE ONLY		BOX:
SNAME:	SITE:	SURVEY DATE:
	TOWN:	ENTERED BY:

- ☐ New record
☐ Update

RARE PLANT SURVEY FORM

Natural Diversity Data Base
 Connecticut Department of Environmental Protection
 79 Elm Street, 6th Floor
 Hartford, CT 06106-5127

Please complete this form to the best of your ability.
 Submit survey forms, maps, and all supporting documents to the address above.

*SPECIES SCIENTIFIC NAME:	Element Occurrence (EO) # (if known):
---------------------------	---------------------------------------

REPORTER INFORMATION	
Name(s):	Gregory Bugbee
Address:	Connecticut Agricultural Experiment Station P.O. Box 1106, New Haven CT 06304
Telephone No:	203 974-8512
E-mail address:	gregory.bugbee@ct.gov

SURVEY/SITE INFORMATION	
Site Name:	Bantam Lake
Town(s):	Litchfield
Survey Date(s):	8/29-30/2019, 9/19/2019
County:	Litchfield
Directions to plant population, including best parking and access points. Please attach a map with boundaries drawn around observed plant populations (or surveyed area if plants not found). See attached map	
GPS Coordinates	Method Used to Determine Coordinates:
Latitude: See attached table N	<input checked="" type="checkbox"/> GPS Unit GPS Make/Model: Trimble R1
Longitude: See attached table W	<input checked="" type="checkbox"/> Mapping Software Software: Pathfinder, ArcGIS
Coordinate system (NAD83 preferred): NAD83	<input type="checkbox"/> Online Maps Online site: If requested

POPULATION DATA			
Population Size		What was counted?	
Actual No. Observed	NA	(e.g. stems, clumps, floating masses, etc.) Rooted Plants	
Estimated No./Range	100-500		
Population Area			
Length (units)	See map		
Width (units)	See map		
Area (units)	See map		

Evidence of disease, predation or injury? ☐ Yes ☐ No Explain:

Phenology				Age Structure		Vigor	
100	% In leaf	0	% Mature fruit	0	% Seedlings	<input type="checkbox"/>	Very feeble
1	% In flower bud	0	% Seed dispersing	0	% Immature	<input type="checkbox"/>	Feeble
1	% In flower	0	% Dominant	80	% Mature (established)	<input checked="" type="checkbox"/>	Normal
0	% Immature fruit	20	% Senescent	20	% Senescent	<input type="checkbox"/>	Vigorous
				<input type="checkbox"/>	Age structure unknown	<input type="checkbox"/>	Exceptionally vigorous

Comments on above:

HABITAT					
Aspect <input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W <input type="checkbox"/> Flat	<input type="checkbox"/> NE <input type="checkbox"/> NW <input type="checkbox"/> SE <input type="checkbox"/> SW	Slope <input type="checkbox"/> 0-3% <input type="checkbox"/> 3-8% <input type="checkbox"/> 8-15% <input type="checkbox"/> 15-35% <input type="checkbox"/> 35% - vertical Measured (° or %): Horizontal shape (as for next item): Vertical shape (ie. Convex, concave, straight, variable):	Light <input checked="" type="checkbox"/> Open <input type="checkbox"/> Partial <input type="checkbox"/> Filtered <input type="checkbox"/> Shade	Topographic Position <input type="checkbox"/> Crest <input type="checkbox"/> Upper Slope <input type="checkbox"/> Mid-Slope <input type="checkbox"/> Lower-Slope <input type="checkbox"/> Bottom Other: lake bottom (1-3 feet deep)	Moisture <input checked="" type="checkbox"/> Permanently Inundated <input type="checkbox"/> Seasonally Inundated/Exposed <input type="checkbox"/> Tidally Inundated/Exposed <input type="checkbox"/> Saturated (Hydric) <input type="checkbox"/> Moist (Mesic) <input type="checkbox"/> Dry-Mesic <input type="checkbox"/> Dry-Xeric Other:
* re true N * re mag N					
Elevation: 890 to 892 <input checked="" type="checkbox"/> feet <input type="checkbox"/> meters Soil/substrate name/description (give source): Subaqueous soil (NRCS) Estimated # of acres of potential habitat in the immediate area: _____ Evidence of disturbance: <input type="checkbox"/> fire <input type="checkbox"/> logging <input type="checkbox"/> disease <input type="checkbox"/> insect damage <input type="checkbox"/> windthrow <input checked="" type="checkbox"/> invasives Comments: Invasive fanwort in area poses threat of habitat loss. Expansion of associated native emergents such as water lilies also a threat.					

Associated natural/plant communities: White waterlily (<i>Nymphaea odorata</i>), watershield (<i>Brasenia scherberi</i>), Robins pondweed (<i>Potamogeton robbinsii</i>), Eurasian watermilfoil (<i>Myriophyllum spicatum</i>) fanwort (<i>Cabomba caroliniana</i>), yellow waterlily (<i>Nuphar variegata</i>), Pickerelweed (<i>Pontederia cordata</i>), Purple bladderwort (<i>Utricularia purpurea</i>), Water stargrass (<i>Zosterella dubia</i>)
Associated plant species (separated strata, e.g. tree, shrub, herb layers):

IDENTIFICATION			
Photograph taken?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No	Photo ID: See attachment
Specimen taken?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No	If yes, provide: Collector: Gregory Bugbee Repository: CAES Herbarium Collection #: MegBec02624308292019
Identification problems?	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Explain:

*DEP Scientific Collection Permit is needed to collect specimens

CONSERVATION	
Owner info: Bantam Lake Protective Association	
Owner aware of EO?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
Owner protecting EO?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
Threats to EO:	habitat loss to invasive fanwort, expansion of associated native emergents such as water lilies, aquatic plant management, climate change
Conservation/management needs:	Population monitoring, informed and low impact nuisance plant management
Research needs:	Herbicide testing to determine tolerances

SUPPORTING DOCUMENTS (please attach)

- ☐ Sketch map (showing finer detail than topo or aerial photo)
- ☒ Aerial photo map
- ☐ Topographic map (available at <http://www.econmap.com/magic/> OR <http://ctecapp1.uconn.edu/advancedviewer/>)
- ☐ Cross section of topography/habitat (include scale, direction, element position, description, and sub-occurrence ID(s), if needed)
- ☐ Photos ☐ Slides ☐ Field notes ☐ Route of survey map

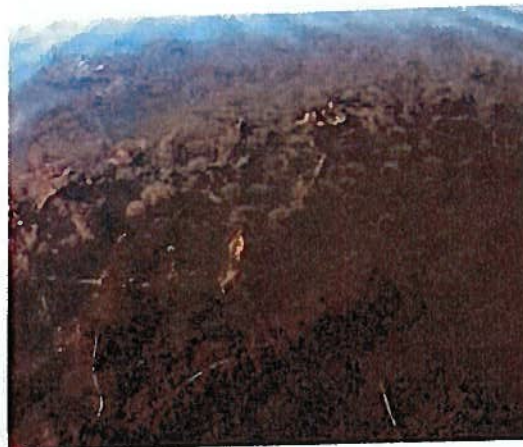
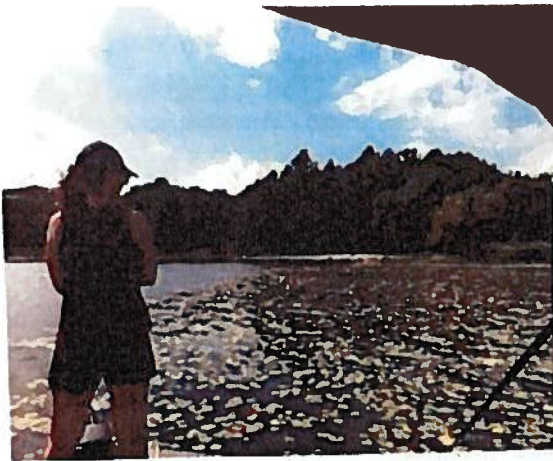
Locations of Water Marigold in Bantam Lake – 2019.



Herbarium Mount of Water Marigold from the Dempsey Landing Collection Site



Water Marigold in Bantam Lake



Station	Species	Height	Count	Notes	Date	Observer	Time
11	Gray Dogfish	Angled	1000	1-1	6/10/2019	AL 1000	10:00
12	Gray Dogfish	Angled	1000	1-2	6/10/2019	AL 1000	10:00
13	Gray Dogfish	Angled	1000	1-3	6/10/2019	AL 1000	10:00
14	Gray Dogfish	Angled	1000	1-4	6/10/2019	AL 1000	10:00
15	Gray Dogfish	Angled	1000	1-5	6/10/2019	AL 1000	10:00
16	Gray Dogfish	Angled	1000	1-6	6/10/2019	AL 1000	10:00
17	Gray Dogfish	Angled	1000	1-7	6/10/2019	AL 1000	10:00
18	Gray Dogfish	Angled	1000	1-8	6/10/2019	AL 1000	10:00
19	Gray Dogfish	Angled	1000	1-9	6/10/2019	AL 1000	10:00
20	Gray Dogfish	Angled	1000	1-10	6/10/2019	AL 1000	10:00

Locations of Water Marigold in Dempsey Landing, Outlet Cove, and North Bay

FID	Surveyor	Plant Name (Abbrev.)	Type	Abundance (1-5)	Depth Range (feet)	Date	Latitude	Longitude
1	Greg Bugbee	MegBec	Point	3	1-3	8/29/2019	41.71261	-73.22505
2	Greg Bugbee	MegBec	Point	2	1-3	8/29/2019	41.71209	-73.22474
3	Greg Bugbee	MegBec	Point	1	1-3	8/30/2019	41.71591	-73.20527
4	Greg Bugbee	MegBec	Point	1	1-3	8/30/2019	41.71593	-73.20527
5	Greg Bugbee	MegBec	Point	1	1-3	8/30/2019	41.71604	-73.20559
6	Greg Bugbee	MegBec	Point	1	1-3	8/30/2019	41.71594	-73.20568
7	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71593	-73.20581
8	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71595	-73.20578
9	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71603	-73.20579
10	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71603	-73.20602
11	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71604	-73.20616
12	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71597	-73.20621
13	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71598	-73.20615
14	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71598	-73.20611
15	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71582	-73.20630
16	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71589	-73.20640
17	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71590	-73.20641
18	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71591	-73.20642
19	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71595	-73.20645
20	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71595	-73.20646
21	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71594	-73.20654
22	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71594	-73.20652
23	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71594	-73.20651
24	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71594	-73.20649
25	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71590	-73.20641
26	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71587	-73.20648
27	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71586	-73.20676
28	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71587	-73.20681
29	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71587	-73.20687
30	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71589	-73.20691
31	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71591	-73.20692
32	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71592	-73.20690
33	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71593	-73.20688
34	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71595	-73.20684
35	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71596	-73.20677
36	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71597	-73.20671
37	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71598	-73.20667
38	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71600	-73.20662
39	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71597	-73.20647
40	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71584	-73.20647
41	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71586	-73.20707
42	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71588	-73.20724
43	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71593	-73.20722
44	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71598	-73.20723
45	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71598	-73.20725

Locations of Water Marigold in Dempsey Landing, Outlet Cove, and North Bay Cont.

FID	Surveyor	Plant Name (Abbrev.)	Type	Abundance (1-5)	Depth Range (feet)	Date	Latitude	Longitude
46	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71598	-73.20729
47	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71594	-73.20737
48	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71593	-73.20739
49	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71591	-73.20742
50	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71589	-73.20747
51	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71587	-73.20751
52	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71592	-73.20765
53	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71591	-73.20770
54	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71588	-73.20772
55	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71594	-73.20796
56	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71600	-73.20804
57	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71604	-73.20807
58	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71607	-73.20808
59	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71609	-73.20815
60	Greg Bugbee	MegBec	Point	4	1-3	8/30/2019	41.71604	-73.20817
61	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71603	-73.20825
62	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71606	-73.20830
63	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71599	-73.20835
64	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71597	-73.20851
65	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71597	-73.20859
66	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71603	-73.20875
67	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71604	-73.20877
68	Greg Bugbee	MegBec	Point	4	1-3	8/30/2019	41.71604	-73.20884
69	Greg Bugbee	MegBec	Point	4	1-3	8/30/2019	41.71603	-73.20891
70	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71600	-73.20896
71	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71597	-73.20900
72	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71597	-73.20912
73	Greg Bugbee	MegBec	Point	3	1-3	8/30/2019	41.71599	-73.20914
74	Greg Bugbee	MegBec	Point	4	1-3	8/30/2019	41.71602	-73.20918
75	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71609	-73.21043
76	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71621	-73.21131
77	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71595	-73.21202
78	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71595	-73.21212
79	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71595	-73.21214
80	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71620	-73.21240
81	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71625	-73.21243
82	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71627	-73.21244
83	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71631	-73.21247
84	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71637	-73.21253
85	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71706	-73.21340
86	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71716	-73.21350
87	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71716	-73.21351
88	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71716	-73.21356
89	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71720	-73.21372

Locations of Water Marigold in Dempsey Landing, Outlet Cove, and North Bay Cont.

FID	Surveyor	Plant Name (Abbrev.)	Type	Abundance (1-5)	Depth Range (feet)	Date	Latitude	Longitude
90	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71723	-73.21373
91	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71728	-73.21375
92	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71730	-73.21382
93	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71725	-73.21384
94	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71724	-73.21384
95	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71720	-73.21381
96	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71718	-73.21379
97	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71708	-73.21457
98	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71706	-73.21459
99	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71721	-73.21631
100	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71688	-73.21730
101	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71618	-73.21850
102	Greg Bugbee	MegBec	Point	2	1-3	8/30/2019	41.71617	-73.21853
103	Greg Bugbee	MegBec	Polygon	2	1-3	8/30/2019	41.71559	-73.20481
104	Greg Bugbee	MegBec	Point	2	1-3	9/19/2019	41.71323	-73.22116

Evaluating Sensitivity of Five Aquatic Plants to a Novel Arylpicolinate Herbicide Utilizing an Organization for Economic Cooperation and Development Protocol

Michael D. Netherland and Robert J. Richardson*

New arylpicolinate herbicide chemistry under development for rice, aquatic weed management, and other uses was evaluated using five aquatic plants. The herbicide 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester—also identified as XDE-848 BE or SX-1552 (proposed International Organization for Standardization common name in review; active tradename RinskorTM)—and its acid form (XDE-848 acid or SX-1552A) were evaluated on three dicots: (1) Eurasian watermilfoil (EWM), (2) megalodonta, and (3) crested floating heart (CFH), and two monocots: (1) hydrilla and (2) elodea. A small-scale Organization for Economic Cooperation and Development (OECD) protocol developed using EWM for registration studies was utilized. EWM and megalodonta were also evaluated in larger-scale mesocosms for comparison. In-water concentrations between 0.01 and 243 $\mu\text{g ai L}^{-1}$ as SX-1552 or SX-1552A were applied under static conditions for 14 (growth chamber) or 28 d (mesocosm). EWM was susceptible to both SX-1552 and SX-1552A, with dry-weight 50% effective concentration (EC_{50}) values of 0.11 and 0.23 $\mu\text{g ai L}^{-1}$ under growth chamber conditions. Megalodonta had EC_{50} values of 11.3 and 14.5 $\mu\text{g ai L}^{-1}$ for the SX-1552 and SX-1552A. CFH was more sensitive to SX-1552 ($\text{EC}_{50} = 5.6 \mu\text{g ai L}^{-1}$) than to SX-1552A ($\text{EC}_{50} = 23.9 \mu\text{g ai L}^{-1}$). Hydrilla had EC_{50} values of 1.4 and 2.5 $\mu\text{g ai L}^{-1}$, whereas elodea was more tolerant, with EC_{50} values of 6.9 and 13.1 $\mu\text{g ai L}^{-1}$ for SX-1552 and SX-1552A, respectively. For EWM mesocosm trials, EC_{50} values for SX-1552 and 1552A were 0.12 $\mu\text{g ai L}^{-1}$ and 0.58 $\mu\text{g ai L}^{-1}$, whereas the megalodonta EC_{50} was 6.1 $\mu\text{g ai L}^{-1}$. Activity of SX-1552 on EWM, hydrilla, and CFH merits continued investigation for selective aquatic weed control properties. Results suggest that the OECD protocol can be used to screen activity of herbicides for multiple aquatic plant species.

Nomenclature: 4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester; crested floating heart, *Nymphoides cristata* (Roxb.) Kuntze; elodea, *Elodea canadensis* Michx.; Eurasian watermilfoil, *Myriophyllum spicatum* L.; hydrilla, *Hydrilla verticillata* L.f. Royle; megalodonta, *Bidens beckii* Torr. Ex Spreng.

Key words: Aquatic herbicide, aquatic plant bioassay, aquatic plant toxicity, Beck's water-marigold, herbicide screening, invasive aquatic plants.

Aquatic weed control with herbicides is characterized by unique conditions and management objectives vs. agricultural or other terrestrial weed management (APMS 2014). Perhaps the two most significant differences in use of aquatic vs. terrestrial herbicides are (1) labeled use for direct application into water to achieve a target herbicide concentration and exposure and (2) high standards for targeting an invasive or nuisance plant with limited impact to multiple native or desirable plant species. In the typical agricultural setting direct application to water is

prohibited and broad-spectrum weed control is provided for a single nontarget species. Aquatic herbicide registration by the U.S. Environmental Protection Agency and other international regulatory agencies requires demonstration of negligible risks to human health or the environment.

Risk assessments of aquatic herbicides consider human water uses and exposure (e.g., drinking, recreational use including swimming, and irrigation practices), other incidental exposure routes, and possible impact to nontarget biota: algae, fish, invertebrates, and nontarget aquatic vegetation. Stringent requirements for aquatic herbicide registration have limited the number of active ingredients approved for aquatic use. Although 244 herbicide active ingredients are currently registered in the United States, only 14 are registered as aquatic herbicides (NPIRS 2015). There is a technical need for additional

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herbicides and alternative modes of action for aquatic weed management. New herbicides can improve response to new aquatic invaders, enhance selectivity to desirable native aquatic vegetation, reduce use rates, and mitigate risk of potential herbicide resistance development (APMS 2014; Getsinger et al. 2008).

To support the development of a potential new aquatic herbicide, a new chemistry was screened against several target and nontarget aquatic plants. The herbicide 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester, is under development by Dow AgroSciences for rice (XDE-848 BE; proposed International Standardization Organization common name in review; active trade-name RinskorTM) and other agricultural crops and is also under development in partnership with SePRO Corporation as an aquatic herbicide (SX1552; Procel-lacorTM; Aquatic Herbicide Technology System). SX-1552 is a member of a new class of synthetic auxins in the arylpicolinate herbicide family. In preliminary screening, SX-1552 exhibited efficacy on several invasive U.S. aquatic weeds including the submersed plants hydrilla and EWM, and the floating-leaf plant CFH (SePRO Corporation, unpublished data). SX-1552 would represent a new chemical class for aquatic uses. Studies of *Arabidopsis thaliana* with mutations in select auxin-binding receptor proteins, along with direct molecule-protein interaction testing of these same receptor proteins, support that arylpicolinate chemistry including SX-1552 has a different binding affinity vs. 2,4-D and other synthetic auxins currently registered as herbicides (Bell et al. 2015; Lee et al. 2013; Villalobos et al. 2012; Walsh et al. 2006).

Laboratory studies and preliminary field dissipation studies indicate that SX-1552 in water is subject to rapid photolysis—a common mechanism of breakdown for several aquatic herbicides. SX-1552 can also convert partially via hydrolysis to an acid form (SX-1552A) with suspected reduced herbicidal activity.

Small-scale evaluation methods serve multiple purposes in aquatic herbicide development including characterization of relative activity for a particular mode of action and determination of weed spectrum including information on efficacy and selectivity. Several different small-scale methods have been utilized to characterize herbicidal activity on aquatic plants. Historically, baseline toxicity tests on duckweed (*Lemna* spp.) have driven regulatory assessment of pesticide risks to nontarget vascular aquatic plants (OECD 2006, USEPA 2012). Past small-scale laboratory testing to predict aquatic herbicide

activity has included analysis of photosynthetic pigment concentrations after exposure to carotenoid biosynthesis inhibitors such as fluridone and topramezone (Berger et al. 2015; Glomski and Netherland 2011; Netherland et al. 1993). Contact aquatic herbicide activity for endothall (protein phosphate inhibitor), diquat (photosystem I inhibitor), flumioxazin, and carfentrazone (Protox inhibitors) have been quantified using conductivity testing of ion leakage (Glomski and Netherland 2013; Koschnick et al. 2006; MacDonald et al. 1993). For the auxin herbicides 2,4-D and triclopyr, controlled laboratory and greenhouse studies have defined concentration-exposure time relationships for EWM control (Green and Westerdahl 1990, Netherland and Getsinger 1992) and nontarget aquatic plant activity (Belgers et al. 2007; Hofstra and Clayton 2001; Netherland and Glomski 2014; Sprecher et al. 1998; Sprecher and Stewart 1995) that have been predictive of selective EWM control observed in the field (Nault et al. 2014; Parsons et al. 2001, Poovey et al. 2004, Wersal et al. 2010).

On the basis of the successful correlation of laboratory and mesocosm-scale studies and field evaluations with currently registered auxin-mimic aquatic herbicides, aquatic use pattern development for SX-1552 can be accelerated through initial data generation of laboratory-scale efficacy and selectivity. Realism of small-scale testing methodology for determinations of herbicidal efficacy, selectivity, and general ecological risk assessment is debated (Maltby et al. 2010). In 2014, a small-scale testing protocol using EWM was adopted by the OECD as a method to generate additional data for assessment of potential nontarget aquatic plant effects when *Lemna* spp. are not sensitive to the mode of action (OECD 2014). OECD method test results on EWM are now used in risk assessments supporting the registration of certain herbicidal modes of action in the European Union. There is minimal published data for aquatic herbicides that directly compare results of “microscale” laboratory screening with outcomes of larger-scale controlled studies using more established plants—typically at an aquarium or mesocosm scale under greenhouse or outdoor conditions. The OECD protocol (2014) describes the guidelines surrounding water and sediment testing for impacts of pesticides on rooted EWM. The results are used for registration purposes in Europe, and EWM was selected as the preferred species in cases where data are required for specific herbicidal modes of action or for a submerged, rooted dicotyledonous plant. The guidelines provide specifications

for creating a sediment and water source used in the studies (OECD 2014; Smart and Barko 1985). Although the focus of the OECD protocol is on EWM sensitivity and risk assessment for registration, the potential for using this small-scale assay to test other submersed plant species or to test new herbicides for aquatic plant activity has not been evaluated. Potential benefits of using the OECD protocol as an initial screen for testing aquatic herbicides against multiple species of plants include: (1) small space requirements allow for significant replication; (2) use of rooted plants allows for increased confidence in efficacy testing; (3) protocol can be easily modified to fit research objectives; and (4) use of standard water and sediments will allow for improved comparison of results across laboratories.

The first objective of this study was to evaluate SX-1552 and SX-1552A against five submersed plant species (three dicots and two monocots) to confirm and compare activity and potential utility as an aquatic herbicide. The second objective was to determine if the growth chamber studies provided comparable results with larger-scale mesocosm trials. The third objective was to determine the potential utility of the OECD protocol for screening different herbicides or additional plant species.

Materials and Methods

EWM from the Crystal River, FL, dioecious hydrilla from Lake Cypress, FL, CFH from Lake Okeechobee, FL, and megalodonta (water marigold) and elodea from Lake Minnetonka, MN were utilized for growth chamber and greenhouse trials. Plants were grown in culture tanks at the University of Florida Center for Aquatic and Invasive Plants (Gainesville, FL) for use in studies. Stock cultures were maintained under ambient outdoor conditions, and robust growth was noted for all species through the evaluation period from September through April.

Growth Chamber Trials. In this study, the OECD protocol was utilized for evaluating the response of the dicots, EWM, megalodonta, and CFH, and the monocots, elodea and hydrilla, after SX-1552 applications to the water under controlled conditions.

Apical shoot tips of 6 cm in length were collected from culture tanks and thoroughly rinsed to remove epiphytes or carbonate crusts on the leaf tissue. Four apical shoots of a single species were each planted into 250-ml beakers containing 200 ml of sediment specified in the protocol (OECD 2014). At least 3 cm of the shoot were pushed into the sediment. The 250-ml

beakers containing sediment and plants were then placed in 2-L beakers containing 1.75 L of culture water (Smart and Barko 1985). The 2-L beakers were then placed in Percival E-36L environmental growth chambers set to a temperature of 21 C, a photoperiod of 16 light (L): 8 dark (D), and light intensity of $275 \pm 27 \mu\text{mol m}^{-2} \text{s}^{-1}$. For the hydrilla and CFH trials, the temperature was increased to 25 C to facilitate improved plant growth.

All plants were given a pretreatment establishment period ranging from 9 to 11 d. This allowed for an increase in shoot tissue and root formation at the nodes of tissue buried in the sediment before treatment. To determine if root formation was present, selected beakers were removed and checked for roots. Before initiating treatments, multiple root formation was observed for all species. The pretreatment pH of the water was within OECD specifications (7.5 to 8.0). Pretreatment measurements on shoot fresh weight, dry weight, and total stem length (including lateral shoots) were collected by removing one plant from each of the beakers (three apical shoots remained). As the expected response to SX-1552 was unknown for these species, nonreplicated range-finding studies were conducted to determine concentrations that would be evaluated for each species (data not shown).

Both the SX-1552 (herbicide formulation analytically validated 300 g ai L⁻¹ suspension concentrate) and SX-1552A (analytical grade) were provided by the SePRO Corporation (Carmel, IN) and evaluated against EWM, megalodonta, CFH, elodea, and hydrilla. Stock solutions of both SX-1552 and SX-1552A were created for treatment of the 2-L beakers. Herbicide concentrations for growth chamber experiments are listed in Table 1. Once treated, static conditions were maintained over the 14-d incubation period. Deionized water was added to the beakers to replace water lost to evaporation. Entire plants were harvested at 14 d after treatment (DAT) and dried to a constant weight at 70 C for a minimum of 48 h.

Prior herbicide concentration monitoring and the lack of UV light in the growth chambers indicated limited potential for photolytic breakdown of SX-1552 in this test system. Water samples (~25 ml) were collected immediately after treatment and 1, 7, and 14 DAT in selected treatment beakers to determine initial and final exposure concentrations. Samples were analyzed via high-performance liquid chromatography with tandem mass spectroscopy with limits of quantitation of 0.02 $\mu\text{g ai L}^{-1}$ for SX-1552 and 0.05 $\mu\text{g ai L}^{-1}$ for SX-1552A. Each

Table 1. Overview of SX-1552 and SX-1552A concentrations used in growth chamber and mesocosm studies.

Plant species tested	Concentrations evaluated $\mu\text{g L}^{-1}$	Material tested
Growth chamber studies		
Eurasian watermilfoil (dicot)	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Water marigold (dicot)	0, 0.3, 1, 3, 9, 27, 81, and 243	SX-1552 and SX-1552A
Crested floating heart (dicot)	0, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Hydrilla (monocot)	0, 0.3, 1, 9, 27, and 81	SX-1552 and SX-1552A
Elodea (monocot)	0, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Greenhouse studies		
Eurasian watermilfoil	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 9, and 27	SX-1552 and SX-1552A
Water marigold	0, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552

treatment was replicated four times and each study was repeated.

Mesocosm Trials. Both EWM and megalodonta were evaluated under greenhouse conditions from October to December, 2015 to determine impact of SX-1552 on more established plants. For EWM, two studies using both the herbicide formulations of SX-1552 and SX-1552A were conducted, whereas only SX-1552 was tested for megalodonta. A series of 3.78-L pots was filled with Margo Professional topsoil (92% sand, 4% silt, 4% clay) amended with 1 g of fertilizer (Osmocote® 15–9–12) kg^{-1} of soil. Four apical shoots (10 cm) of each test species were planted in individual pots and placed in 95-L plastic tanks filled with well water. The plants were given a 28-d pretreatment establishment period under greenhouse conditions. Greenhouse lights were set to maintain a 16L:8D photoperiod. Hobo water temperature loggers (Onset Computer Corp.) were placed in selected tanks to record temperature every 6 h.

Herbicide concentrations used for greenhouse evaluations are listed in Table 1. Treatments were static exposures, and the experiments were conducted for a period of 28 d. Supplemental water was added during the course of the study to replace water lost to evaporation. After the 28-d exposure period, shoot material was harvested and dried to a constant weight at 70 C for a minimum of 48 h.

Water samples were collected immediately after treatment, 7 DAT, and 28 DAT in selected tanks to determine exposure concentrations. Lack of potential for photolytic degradation has previously been demonstrated in studies conducted in these greenhouses (Netherland 2015). Each treatment was replicated three times, and each study was repeated.

Statistical Analysis. Equation 1 is the four-parameter log-logistic dose-response curve used to estimate EC_{50} for different measures of plant response. Estimation of this nonlinear regression model was performed using

the drc package in R software (R 3.2.2, R Core Team 2015: <https://www.R-project.org/>). Methodology of this approach is described in detail by Knezevic et al. (2007) and Ritz and Streibig (2005):

$$Y = c + (d - c) / \{1 + \exp[b(\log x - \log e)]\} \quad [1]$$

The parameters b , c , d , and e estimate the relative slope at e , lower limit of Y , upper limit of Y , and midpoint of Y , respectively. The three-parameter form of Equation 1 ($c = 0$) was used when it was logical to restrict the lower limit to 0. The dependent variable Y consists of treatment averages ($n = 3$ or 4) within replicate studies ($n = 2$) for dry weight or for inhibition indices that relate response relative to the control calculated using dry weight, fresh weight, and plant length. The EC_{50} was estimated as the dose rate (x) corresponding to the midpoint (e) between the lower (c) and upper limit (d) for dry weight or the dose rate corresponding to 50% inhibition of specific growth rate or 50% inhibition in yield. Estimates of EC_{50} were compared for SX-1552 and SX-1552A using the selectivity index (Ritz and Streibig 2005).

Final dry weight was estimated directly using model 1 as recommended by Knezevic et al. (2007). Graphical comparisons were performed by converting predicted values and sample means to percent dry weight reduction relative to the control. Model predictions were converted using the predicted upper limit (d) as the predicted control level and using the sample mean control (rate = 0) average for sample means.

Measures relative to the control were defined by specific study protocols as percent inhibition of specific growth rate ($\%I_r$ in Equation 2) and percent inhibition in yield ($\%I_y$ in Equation 3):

$$I_r = 100x(\mu_c - \mu_t) / \mu_c \quad [2]$$

Specific growth rate in Equation 2 was calculated for control (μ_c) or treated (μ_t) as the natural log of the

final divided by initial mean values divided by days ($\ln[\text{final}/\text{initial}]/\text{days}$) for each replicate study. Equation 2 was modified when final size was less than initial size because this is when treatment-specific growth rates (μ_t) estimate necrosis/mortality on the basis of initial size rather than growth. Without modification, this results in no upper limit on %Ir and contradicts the log-logistic modeling approach used here. The focus on growth inhibition was maintained by restricting maximum %Ir to 100% (setting $\mu_t = 0$) when final size was less than initial size.

$$Ir = 100 \times (b_c - b_t) / b_c \quad [3]$$

Mean growth (b) in Equation 3 was calculated for control (b_c) or treated (b_t) as the average final minus average initial for each replicate study. Inhibition of yield (%Ir) can exceed 100% when treatment growth is negative.

A Dunnett's test ($\alpha = 0.05$) comparing dry weight biomass of treated vs. nontreated plants was performed to determine a lowest observed effect concentration (LOEC) across the broad range of SX1552 concentrations tested.

Results and Discussion

Growth Chamber Trials. In 14-d assays, reference plant biomass increased by 2.8 to 5.1 times the initial biomass for the different test species. OECD guidelines require that doubling of biomass and mean coefficient of variation between reference plants be less than 35% (OECD 2014). Both of these requirements were met in all of our growth chamber studies. All nontreated control plants were robust and actively growing throughout the trials and at the time of harvest. Water sampling after treatments with the SX-1552 formulation at 1 DAT indicated that 41 to 56% of applied SX-1552 had remained in the parent form, whereas the rest had converted to SX-1552A. Results from water sampling at 7 and 14 d indicated that SX-1552 had

fully converted to SX-1552A, with recoveries at 7 and 14 d ranging from 89 to 112% of nominal treatment concentrations. Samples collected at 1 and 14 DAT with SX-1552A resulted in recoveries ranging from 94 to 108% of nominal concentrations. Results of this water sampling confirmed that target concentrations were achieved.

EWM was sensitive to both SX-1552 and SX-1552A, with EC_{50} values of 0.11 and 0.23 $\mu\text{g ai L}^{-1}$ (Table 2, Figure 1). For both formulations, the LOEC value was 0.1 $\mu\text{g ai L}^{-1}$. Symptom development was rapid with characteristic auxin-like epinasty of the apical shoots noticed within 1 d of treatment. Megalodonta sensitivity to SX-1552 and SX-1552A resulted in EC_{50} values of 11.3 and 14.5 $\mu\text{g ai L}^{-1}$ respectively (Table 2, Figure 1). LOEC values of 3 and 9 $\mu\text{g ai L}^{-1}$ were determined for SX1552 and SX1552-A, respectively, whereas a concentration of 81 $\mu\text{g ai L}^{-1}$ reduced biomass by greater than 90%. The visual auxin symptoms were greatly reduced for megalodonta compared with EWM.

Elodea sensitivity to SX-1552 and SX-1552A yielded EC_{50} values of 6.9 and 13.1 $\mu\text{g ai L}^{-1}$ respectively, with both forms yielding a LOEC value of 9 $\mu\text{g ai L}^{-1}$ (Table 2, Figure 1). The EC_{50} values indicated a difference between SX-1552 and SX-1552-A, (Table 2). There was no viable biomass for harvest at the highest concentration evaluated in this trial (81 $\mu\text{g ai L}^{-1}$). Slight visual auxin-like symptoms were noted on this monocot at the higher concentrations; however, the primary symptom noted was necrosis along the length of the stems. Hydrilla was much more sensitive, with EC_{50} values of 1.4 $\mu\text{g ai L}^{-1}$ (SX-1552) and 2.5 $\mu\text{g ai L}^{-1}$ (SX-1552-A) and a LOEC of 1 $\mu\text{g ai L}^{-1}$ (Table 2, Figure 1). A difference in the EC_{50} value for SX-1552 and SX-1552-A was also noted for hydrilla. There was very limited biomass for harvest at concentrations $> 9 \mu\text{g ai L}^{-1}$. In addition to auxin-like symptoms at the shoot tips, this monocot became brittle and shoots readily separated upon slight disturbance in the first day or two

Table 2. Final dry weight (g) 50% effective concentration (EC_{50}) comparisons (standard error) for Eurasian watermilfoil (EWM), megalodonta (MEG), elodea (ELO), Hydrilla (HYD), and crested floating heart (CFH) after exposure to SX-1552 and SX-1552A.

Study type	Formulation	EWM	MEG	ELO	HYD	CFH
		EC_{50} ($\mu\text{g ai L}^{-1}$) ^a				
Growth chamber	SX-1552	0.11 b (0.11)	11.3 a (2.0)	6.9 b (0.6)	1.4 b (0.1)	5.6 b (0.6)
	SX-1552A	0.23 ab (0.33)	14.5 a (2.8)	13.1 a (1.0)	2.5 a (0.3)	23.9 a (4.0)
Mesocosm	SX-1552	0.12 b (0.01)	6.1 b (0.2)	—	—	—
	SX-1552A	0.58 a (0.04)	—	—	—	—

^a EC_{50} ($\mu\text{g ai L}^{-1}$) values with the same lowercase letter within a species are not significantly different at the 5% level.

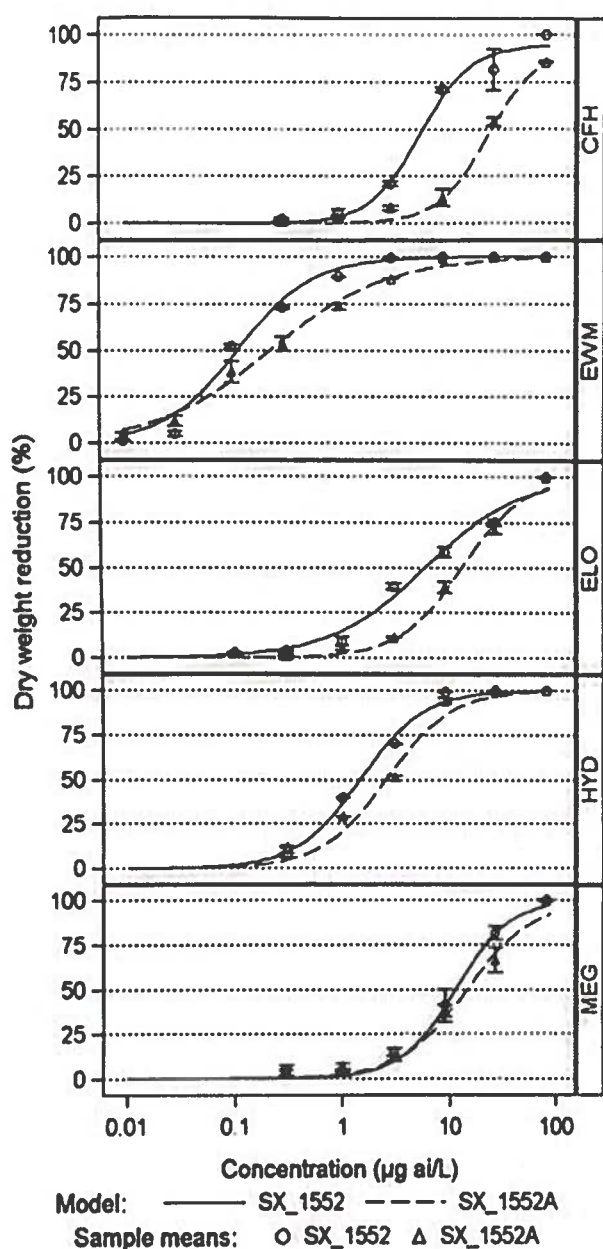


Figure 1. Logistic regression was used to plot dry-weight biomass reduction for five aquatic plant species after exposure to SX1552 (ester) and SX1552A (acid). Each symbol represents the mean value (\pm standard error, $n = 4$). Abbreviations: CFH, crested floating heart; EWM, Eurasian watermilfoil; ELO, elodea; HYD, hydrilla; MEG, megalodonta.

posttreatment. At harvest, plants that had been treated at concentrations $> 3 \mu\text{g ai L}^{-1}$ had waterlogged stems (aerenchyma tissue that is normally filled with air was full of water) and the limited amount of remaining tissue lacked integrity.

CFH also showed differential sensitivity to SX-1552 and SX-1552A, with EC_{50} values of 5.6 and $23.9 \mu\text{g ai L}^{-1}$ respectively (Table 2, Figure 1). The LOEC value for the formulation was $3 \mu\text{g ai L}^{-1}$,

whereas the SX-1552-A value was $9 \mu\text{g ai L}^{-1}$. CFH displayed a rapid onset of visual symptoms with notable stem elongation within 1 d after exposure to concentrations from 1 to $3 \mu\text{g ai L}^{-1}$. Although these initial symptoms were easy to distinguish, they did not translate to impacts on biomass at the lower treatment concentrations. There was some chlorosis noted on surface leaves within 5 to 10 DAT. A clear visual difference between the activity of SX-1552 and SX-1552-A was noted for this floating leaf plant.

Per the OECD protocol, EC_{50} values were also determined for several growth-based parameters. The three-parameter version ($c = 0$) of Equation 1 (parameter estimates not shown) was used to estimate percent inhibition of growth rate (I_r) and percent inhibition in yield (I_y). Estimates of EC_{50} are compared by formulation in terms of shoot length, fresh weight, and dry weight by species (Table 3). These data indicate some variation in predicted EC_{50} values for SX1552 against the different plant species. Specifically, higher EC_{50} values for the growth rate (I_r) data for elodea and CFH was noted. Nonetheless, most growth-based values were generally similar to the EC_{50} values determined on the basis of dry weights (Tables 2 and 3). Per the OECD guidelines, it is stated that “ EC_{50} values calculated when using the % inhibition of yield (I_y) and average specific growth rate (I_r) are not comparable and this difference is recognized when using the results of the test.” Overall, these analyses are being conducted on data that show consistent relationships within a species (e.g., dry weight vs. fresh-weight ratios or stem length vs. fresh weight). As such, the EC_{50} values were in general agreement regarding the sensitivity of each species to SX-1552 and SX-1552A.

Mesocosm Trials. Water temperatures ranged from 17.6 to 23.2 C during the course of mesocosm trials. During the 28-d pretreatment growth period, EWM biomass increased by a factor of 37.5 compared with initial shoot weights, and megalodonta increased by a factor of 18.4. During the 28-d study period, biomass of EWM increased by a factor 2.7 and megalodonta increased by a factor of 2.2. The combination of rapid growth rates and limited space eventually resulted in plants nearing or reaching carrying capacity and slowing growth rates in these tanks. All non-treated plants were robust and actively growing at the time of treatment and harvest. Results from water sampling at 7 and 28 DAT indicate that measured

Table 3. Estimation of 50% effective concentration (EC_{50}) ($\mu\text{g ai L}^{-1}$) as the dose that corresponds to 50% inhibition of growth rate (I_r) or inhibition in yield (I_y) in growth chamber (GC) and mesocosm (Meso) trials. EC_{50} (standard error) values within species followed by the same lowercase letter are not significantly different at the 5% level.

Study type	Form	Shoot length		Fresh weight		Dry weight	
		% <i>I_r</i>	% <i>I_y</i>	% <i>I_r</i>	% <i>I_y</i>	% <i>I_r</i>	% <i>I_y</i>
Eurasian watermilfoil							
GC	SX-1552	0.15b (0.01)	0.10b (0.01)	0.17b (0.01)	0.10b (0.01)	0.16c (0.01)	0.10c (0.01)
	SX-1552A	0.35a (0.03)	0.19a (0.02)	0.41a (0.04)	0.17a (0.02)	0.39b (0.04)	0.17b (0.02)
Meso	SX-1552	—	—	—	—	0.12d (0.01)	0.09c (0.01)
	SX-1552A	—	—	—	—	0.68a (0.06)	0.38a (0.03)
Megalodonta							
GC	SX-1552	3.6b (0.4)	3.0b (0.5)	9.1 (0.9)	6.9a (0.7)	8.9a (1.0)	7.0a (0.8)
	SX-1552A	7.3a (0.6)	6.0a (0.8)	10.8a (1.0)	9.1a (1.0)	10.9a (1.8)	8.7a (2.7)
Meso	SX-1552	—	—	—	—	6.4b (0.7)	4.7a (1.0)
Elodea							
GC	SX-1552	3.0b (0.2)	2.8b (0.5)	26.2a (18)	7.1a (2)	21.0a (12)	6.3a (1)
	SX-1552A	7.4a (0.7)	6.8a (1.2)	34.1a (47)	13.0a (3)	28.3a (11)	12.2a (2)
Hydrilla							
GC	SX-1552	1.7b (0.2)	1.1b (0.1)	2.0b (0.2)	1.1b (0.1)	2.1b (0.2)	1.2b (0.1)
	SX-1552A	3.4a (0.4)	1.8a (0.2)	3.4a (0.2)	1.9a (0.2)	3.6a (0.3)	1.8a (0.2)
Crested floating heart							
GC	SX-1552	5.9b (0.3)	5.4b (0.5)	7.0a (0.2)	4.9a (0.3)	7.2a (0.9)	5.0b (0.5)
	SX-1552A	26.6a (2.5)	17.6a (2.5)	41.1a (27)	26.1a (35)	33.2a (18)	21.0a (4)

concentrations of SX-1552 and SX-1552A were $87\% \pm 5\%$ of the target concentrations.

EWM was sensitive to both SX-1552 and SX-1552A in larger-scale mesocosms under greenhouse conditions. Despite the larger initial size and more robust plants, EC_{50} values for SX-1552 and SX-1552A were 0.12 and $0.58 \mu\text{g ai L}^{-1}$ respectively (Table 2). LOEC values were 0.1 and $0.3 \mu\text{g ai L}^{-1}$ for SX-1552 and SX-1552A. Within 1 to 2 d after exposure, plants became very brittle and stems fragmented into small pieces after slight disturbance. Comparison of growth chamber and mesocosm data suggests that despite different initial plant biomass and study conditions, EWM responded in a similar manner (Table 2, Figure 2). Megalodonta susceptibility in the mesocosm trials was generally similar to results observed in the growth chamber trials. The EC_{50} value for SX-1552 was $6.1 \mu\text{g ai L}^{-1}$, whereas the LOEC was 9 (Table 2). Given the broad rate structure evaluated, there were minimal impacts on plant growth at $3 \mu\text{g ai L}^{-1}$, whereas the $9 \mu\text{g ai L}^{-1}$ treatment resulted in $> 65\%$ biomass reduction. The EC_{50} value calculated for megalodonta was significantly lower for the greenhouse vs. the growth chamber trials (6.1 vs. $11.3 \mu\text{g ai L}^{-1}$). It is possible that improved growth conditions in the mesocosms could explain the increased susceptibility of the megalodonta when compared with the space limitations observed in the 2-L beakers.

Results suggest that EWM is highly susceptible to both SX-1552 and SX-1552A. The EWM growth chamber and mesocosm trials were complementary and indicate that the EC_{50} values are well below

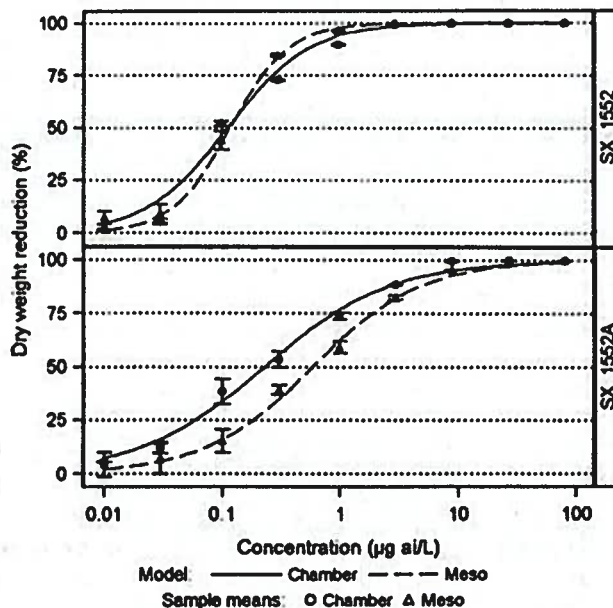


Figure 2. Logistic regression was used to plot dry-weight biomass reduction of Eurasian watermilfoil after exposure to SX1552 and SX1552A after growth chamber (chamber) and mesocosm (Meso) studies. Each symbol represents the mean value (\pm standard error, $n = 4$ for growth chamber trials and $n = 3$ for mesocosm trials).

1 $\mu\text{g L}^{-1}$. Across all species, SX-1552 resulted in lower EC_{50} values vs. SX-1552A; however, because of the rate structure evaluated the LOEC was often similar between the forms. The EC_{50} value for *megalo-donta* was 63 to 102 times greater than for EWM. Interestingly, a dichotomy was also observed for the two monocotyledons. The EC_{50} values for the native elodea species were 4.9 to 5.4 times greater than that for the invasive species hydrilla. Given the invasive nature of both EWM and hydrilla in the United States, this level of SX-1552 activity warrants further investigation for potential use against these species.

These trials were based on extended static exposures to SX-1552, and therefore the results need to be viewed in context, as static exposures can result in enhanced activity against a given submersed species in small-scale systems (Mohr et al. 2013). For example, mesocosm evaluation of static exposures (> 3 wk) of the auxin-mimic herbicides 2,4-D and triclopyr demonstrated high levels of activity for these herbicides on EWM at rates ranging from 25 to 75 $\mu\text{g ai L}^{-1}$ (Glomski and Netherland 2010), yet typical use rates for these products range from 500 to 2,000 $\mu\text{g ai L}^{-1}$, as most treatments for submersed aquatic management are subject to rapid dispersion from the treatment site (Netherland 2015). The current results suggest that SX-1552 produces strong auxin-like symptoms, can result in rapid onset of injury and loss of EWM biomass, and is at least an order of magnitude more active on EWM when compared with products such as 2,4-D and triclopyr (Glomski and Netherland 2010; Green and Westerdahl 1990; Netherland and Getsinger 1992). Although 2,4-D and triclopyr can elicit symptoms on hydrilla at high concentrations, neither herbicide provides hydrilla control at maximum-labeled use rates in the range of 2,500 to 4,000 $\mu\text{g L}^{-1}$. In this study hydrilla lost tissue integrity at 3 $\mu\text{g ai L}^{-1}$ and was completely controlled at a concentration of 9 $\mu\text{g ai L}^{-1}$ after a 14-d static exposure period to SX-1552.

In examining the potential utility for utilizing the OECD protocol to evaluate other herbicides or potential impacts on different plant species, there are several inherent strengths as well as a few caveats. The current results suggest that products like SX-1552 might be well suited to this screening method. However, slow-acting aquatic herbicides that target plant-specific enzymes such as fluridone (phytoene desaturase inhibitor [PDS]), penoxsulam (acetolactate synthase [ALS] inhibitor), and topramazine (hydroxyphenylpyruvate dioxygenase [HPPD] inhibitor) can require up to 2 to 4 mo to provide plant

control (Netherland 2015). Use of a protocol that focuses on short-term changes in biomass and growth may not be optimal for predicting activity of slow-acting herbicides. Research using a water-only assay (e.g., recently sprouted tubers or apical shoot meristem growing in Hoagland's solution) has provided valuable data on short-term changes in pigments, growth inhibition, or impacts on root growth (Berger et al. 2015; Mohr et al. 2013; Netherland 2011, 2015). Additional testing using the OECD protocol on these slow-acting herbicides is recommended and extending the length of these trials to 28 d may provide additional data to separate between concentrations that are likely to provide growth regulation vs. those concentrations that are likely to kill the plant.

Fast-acting contact herbicides like diquat would demonstrate high levels of activity using this protocol, as EWM is very sensitive to this herbicide. Moreover, extended unrealistic exposures to diquat in these assays (due to lack of binding to suspended sediments or organic particulates in an assay) are not characteristic of field conditions. In this case, testing EWM would indicate that diquat is highly active for both regulatory and operational predictions; however, the impact of turbidity on diquat activity in the field would likely result in greatly reduced activity (Poovey and Getsinger 2002). Fast-acting products that require moderate exposure periods such as 2,4-D, triclopyr, endothall, and SX-1552 can be evaluated in a relatively short period of time and these products tend to perform in a similar manner under a broad range of environmental conditions (e.g., turbidity, pH, temperature, etc.).

The growth chamber results with SX-1552 were validated at the mesocosm scale for the two dicot species tested. Such outcomes will likely vary for contact or systemic herbicides. Several submersed aquatic plants are highly susceptible to the rapid-acting protoporphyrinogen oxidase inhibitor flumioxazin under growth chamber conditions. Yet flumioxazin activity can be reduced under increasing pH as the molecule is rapidly hydrolyzed at a higher pH (Mudge and Haller 2006).

The OECD protocol offers a good model for screening inherent herbicide activity on submersed plants under relatively long-term exposures, but could easily overestimate risk when relying on a single species for risk assessment purposes. In this study, EWM was by far the most sensitive aquatic plant species to SX-1552. It could have also been the most tolerant, or shown no effect. Aquatic plant community interactions should be considered,

involving multiple species of submersed or floating species. For example, in this study, the desirable native aquatic plants were more tolerant than the invasive species EWM and hydrilla. In addition, the exposure scenario should be kept in perspective after a terrestrial application of SX1552. Exposures significantly less than 14 or 28 d would generally be expected. Additional small-scale tests of other submersed native and invasive dicots and monocots at the chamber scale are recommended. The ability to utilize results from studies conducted at this scale provides an efficient and cost-effective method to screen plants under a variety of concentrations and exposure scenarios common to treatment of aquatic sites.

Overall these study results confirm a high level of SX-1552 activity on several aquatic species and the greater activity of SX-1552 and SX-1552-A. For SX 1552 the growth chamber studies were predictive of mesocosm results. Although the OECD protocol is currently specific to EWM for regulatory purposes in Europe, the current results suggest that this protocol (or modified versions of this protocol) could be used for multiple herbicides or aquatic plant species. Predicting herbicide activity on rare or threatened species or using this protocol to better refine knowledge of invasive plant response to a given herbicide are two areas where this small-scale assay could provide information that would improve study design for large-scale mesocosm testing.

Acknowledgments

The assistance of Jesse Stevens with study setup and data collection is greatly appreciated. Funding for this project was provided through the U.S. Army Engineer Research and Development Center, Aquatic Plant Control Research Program and through the Aquatic Ecosystem Restoration Foundation. Citation of trade names does not constitute endorsement or approval of the use of such commercial products. Permission to publish this paper was granted by the Chief of Engineers.

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Associate Editor for this paper: Steven Seefeldt, University of Alaska at Fairbanks.

Gosselin, Jon

Wed, Jan 22,
2:17 PM

to me

Hey Nick,

I heard back from the research team and we do not have data specific to Becks Water Marigold. Looking at its relatives, I would place its sensitivity between sensitive (such as hybrid milfoil – 4 pdu/acre-ft) to moderately sensitive (such as hydrilla – 20 pdu/acre-ft). The good thing is that ewm is extremely sensitive (~1-2 pdu/acre-ft). Also, we know that ProcellaCOR doesn't move very far from where it is applied. This is why we always stress the importance of narrow (20-40 ft) swath widths for application.

If we can obtain samples of the plant, were happy to do a PlanTEST at the lab and provide the margins of safety w/ ProcellaCOR. Considering the general sensitivity of the Asteraceae family vs the extreme sensitivity of Eurasian watermilfoil paired with the low risk of ProcellaCOR to drift, my initial thought is that they'd be safe to reduce the exclusion zone to ~100 ft or less.

Best Regards,
Jon

Jon Gosselin | Technical Specialist

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Gosselin, Jon

Jan 27, 2020, 3:07
PM (8 days ago)

to me

Hey Nick,

I investigated this a bit further and found the attached paper. Netherland and Richardson reported an EC50 of 11.5 ppb for Megalodonta (Becks water-marigold), which is greater than 5 PDU/acre-ft. This should be helpful considering we treat ewm at ~2 pdu/acre-ft. We also have pre and post ProcellaCOR plant surveys from Houghton Lake that show expansion of Beck's water-marigold after treatment. I'll forward the surveys to you as soon as I receive them.

Best Regards,
Jon

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