

Hydrilla verticillata

2016 End of Year Report

contents



Hydrilla infestation in a small geothermal ditch in Boise, Idaho

1

INTRODUCTION

Background: hydrilla characteristics and impacts

2

HYDRILLA IN IDAHO

Infestation locations by county.

3

INITIAL POPULATIONS

Owyhee County infestation:

- Bruneau River
- Treatment History
- The 2016 Field Season

Ada County Infestation:

- North Boise
- Treatment History and Results

11

RECENT POPULATIONS

Twin Falls County infestations:

- Buhl (Sites 1 - 7)
- Twin Falls

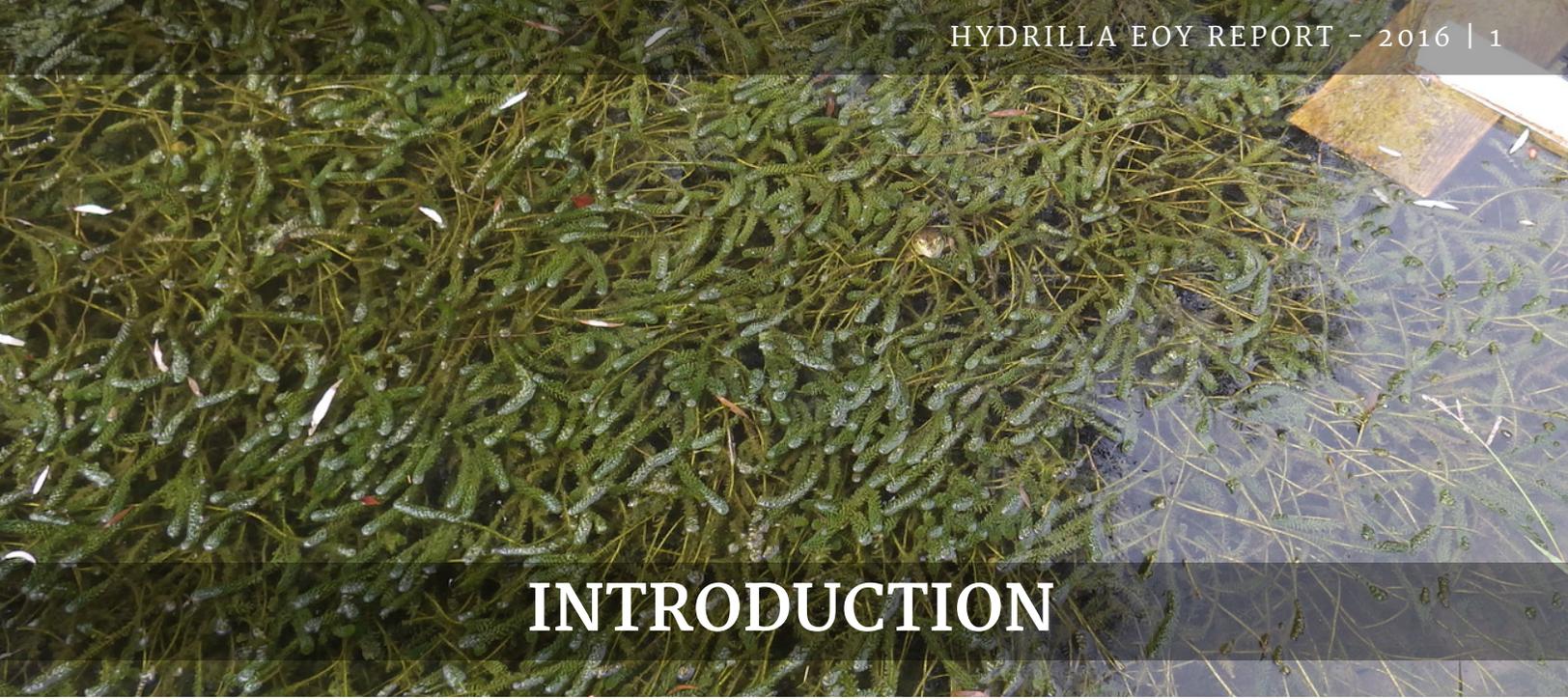
18

FUTURE ERADICATION EFFORTS

Summary of results and contact information.

19

REFERENCES

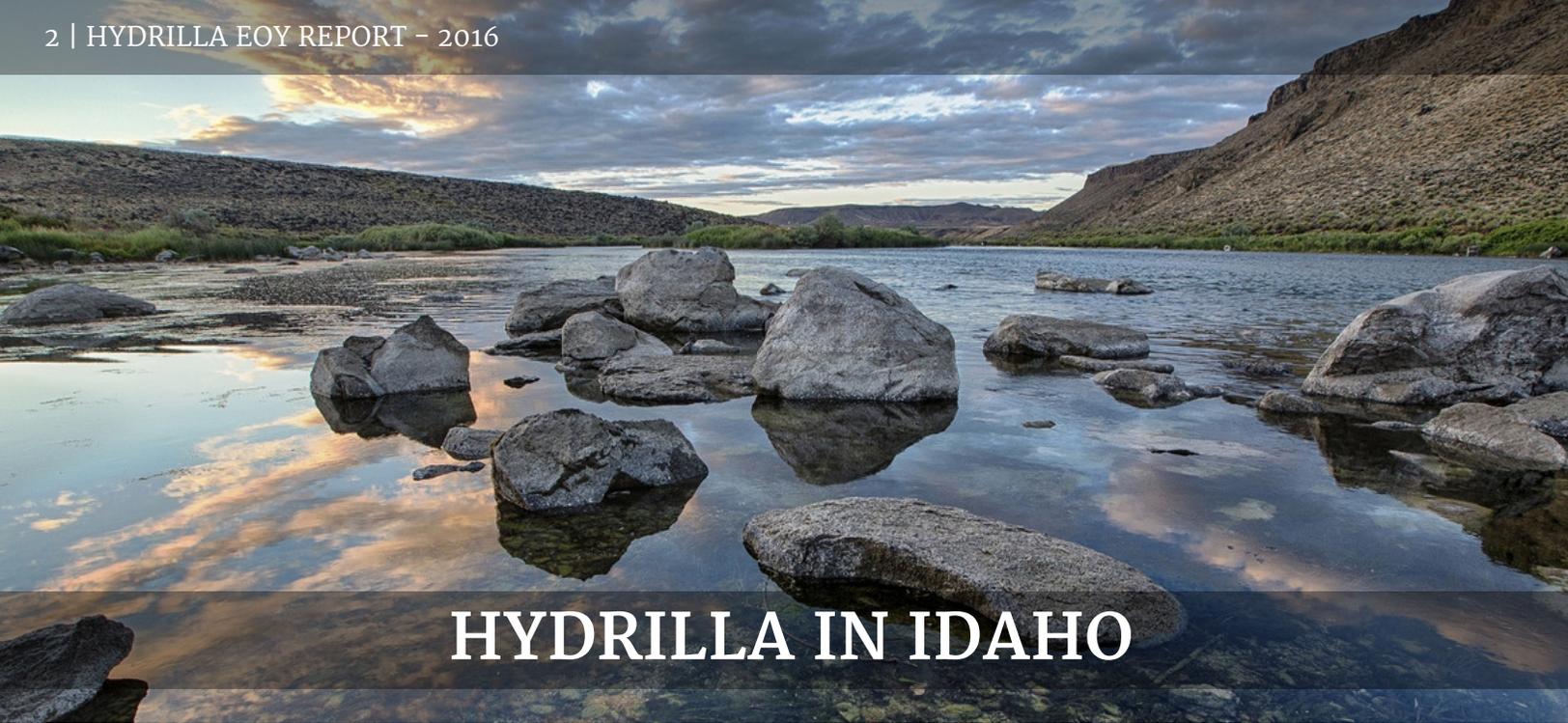


INTRODUCTION

Hydrilla (*Hydrilla verticillata*), also known as water thyme, is an aquatic herbaceous perennial and one of the most aggressive and environmentally disruptive freshwater plants in the world (Haller 1975, Swarbrick et al. 1981, Murphy 1988, Mullin, et al. 2000, Hershner and Havens 2008, Bianchini, et al. 2010). It is highly adaptable and forms dense monocultures that restrict water flow, degrade water quality, impede recreation, and out-compete native species (Haller and Sutton 1975).

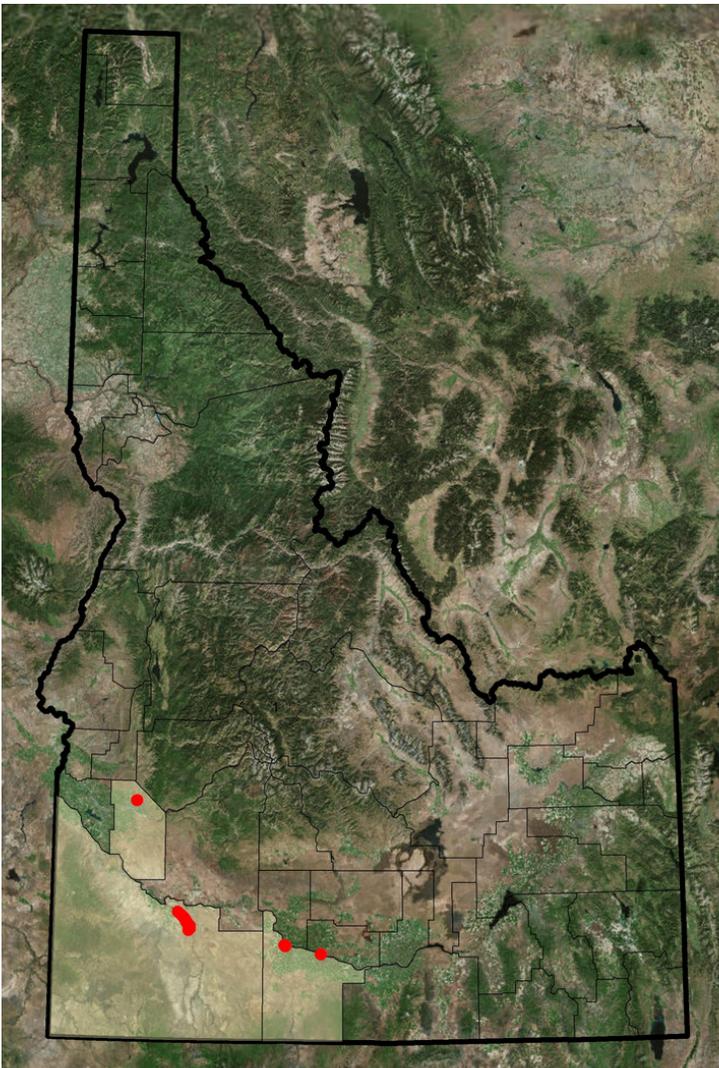
Hydrilla has been referred to as “the perfect aquatic weed” because of its ability to dominate aquatic systems through effective propagation and colonization (Langeland 1996). Rapid growth rates allow hydrilla to grow extremely fast at up to one inch per day. As the hydrilla nears the surface, branching of the stem is initiated and will develop into thick surface mats which effectively intercept sunlight and block penetration to plants in lower profiles (Haller and Sutton 1975). Because hydrilla can grow under very low light conditions, it gives it a competitive advantage over other aquatic plants (Van, et al 1976). This thick surface mat will also alter water chemistry and dissolved oxygen levels which has been linked to avian vacuolar myelinopathy (AVM) in herbivorous waterfowl and their avian predators (Wilde, et al. 2005), along with fish kill events from oxygen depletion.

Hydrilla reproduces primarily through vegetative fragmentation and the production of overwintering structures called turions and tubers (Van & Steward 1990). Due to the above mentioned characteristics, Hydrilla was placed on Idaho’s noxious weed list in early detection, rapid response (EDRR) category. Identification of hydrilla in Idaho is of particular regional concern because of the potential to spread downstream into the Snake River.



HYDRILLA IN IDAHO

Map 1. Regional map indicating the location of the hydrilla infestation in Idaho for Ada, Owyhee and Twin Falls Counties.



Hydrilla has been identified in four locations within three southwestern Idaho counties (Owyhee, Ada, and Twin Falls). The first population was identified in the Bruneau River near Bruneau, ID in December 2007 with a second population discovered shortly after in a North Boise neighborhood in 2008. Routine surveys in Twin Falls County led to the discovery of a third population in 2015, followed by additional locations in Twin Falls County later that year (Map 1). All infestation areas are located in surface waters with geothermal influence. The area of mixing created at these ambient water and geothermal water interfaces create habitats with suitable temperatures ranges for hydrilla's growth and establishment. Temperatures required for optimal growth of dioecious hydrilla range between 20°C and 27°C (Barko and Smart 1981, Kassermann 1995) and are temperatures that can be found year-round in several of these sites.



Bruneau River hydrilla infestation zone.

INITIAL POPULATIONS: OWHYEE COUNTY

Bruneau River

The Bruneau River population covers a twelve-mile stretch from Hot Creek downstream toward CJ Strike Reservoir. Historically, dense beds of plants were found throughout this area, but primarily in areas with geothermal influence. Hydrilla was also found in the lower reaches of the infestation zone (outside identified geothermal areas), but plants in these areas were typically scattered and occurred in low densities. Repeated surveys in downstream waters have found no hydrilla in CJ Strike reservoir or the Snake River.

Using DNA analysis, the US Geological Survey determined that the hydrilla in the Bruneau River is of dioecious biotype. Dioecious hydrilla is typically found in southern tier states in the US, whereas the monoecious biotype has been found in colder climates, such as Washington, Maine, New York and Wisconsin. The lower temperature limit of the dioecious biotype is not well-established in scientific literature; however, its distribution appears to be limited in the US by cold temperatures. The distribution of dense hydrilla in the Bruneau system appears to be limited to geothermally influenced waters which are found in the first seven miles of river extending below Hot Creek. Primarily, hydrilla found outside of geothermally influenced areas occur in low densities and are believed to have been deposited as tubers/turions that were moved downstream during high spring flows. Due to the extremely aggressive and adaptable nature of this plant, the Idaho State Department of Agriculture (ISDA) is conducting an aggressive program on this population with the intent of eradication.



Hydrilla topped out backwater area in section 2 of the Bruneau River.

BRUNEAU RIVER TREATMENT HISTORY

Scientists and experts from around the nation were consulted following the identification of hydrilla in the Bruneau River. Treatment options were identified and stakeholders from around the region were brought together to develop an eradication plan. ISDA, in cooperation with Idaho Fish and Game, Idaho Department of Environmental Quality, Idaho Office of Species Conservation, US Fish and Wildlife Service, US Bureau of Land Management (BLM), United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) and landowners from throughout the Bruneau Valley, worked together to coordinate treatments. An aggressive eradication plan utilizing diver-removal, hand-removal and herbicide treatment was implemented in early February of 2008. Treatments consisted of two eight-hour diquat injections into the river, three diquat backpack applications, ten days of diver-harvesting removal, and five days of hand-removal. Efforts in 2008 resulted in an estimated 50% reduction in biomass the following season. Coordination with the Hot Springs and Buckaroo Ditch Companies also focused treatments to remove hydrilla from the upper parts of the ditch systems.

Treatment in subsequent years further reduced hydrilla biomass and distribution throughout the infested area. Herbicide use decreased as hydrilla densities were reduced into levels where hand removal became more effective. Grant funding in 2010 from USDA, APHIS, and BLM allowed for



Diver assisted suction removal in the Bruneau River.

the hiring of seasonal staff dedicated to the project area which allowed for a comprehensive and sustained mapping, survey and removal program. In 2011, this funding also allowed for the purchase of a suction removal system (modified dredge) to help facilitate the removal of hydrilla biomass, including tubers.



Since 2010, the hydrilla infested area has been surveyed repeatedly each year during periods of plant growth. When hydrilla plants are found, a location is recorded using GPS, the number of plants are counted, and the area covered by these plants is estimated. The plants are then carefully removed by hand, often including the tuber(s). In areas of higher plant density, suction removal is utilized (access permitting). Collecting data in this way has allowed for hydrilla occurrence and density (plants per sq meter) to be recorded and monitored over time throughout the river system.

In an attempt to increase the sensitivity of the analysis, and to more clearly illustrate changes of the hydrilla population over time, the number of hydrilla plants removed was investigated. Prior to 2013, higher plant densities prohibited a year-to-year comparison of individual plant numbers. Due to substantial reductions in plant densities, crews were able to record accurate counts of individual plants throughout the infestation zone. This information provides a metric to track changes over time and quantifies actual hydrilla plant growth rather than just focusing on the locations of occurrence. Between 2013 and 2015, the data collected found individual plant numbers to decrease by 74% in the entire infestation zone and 81% in high density areas.

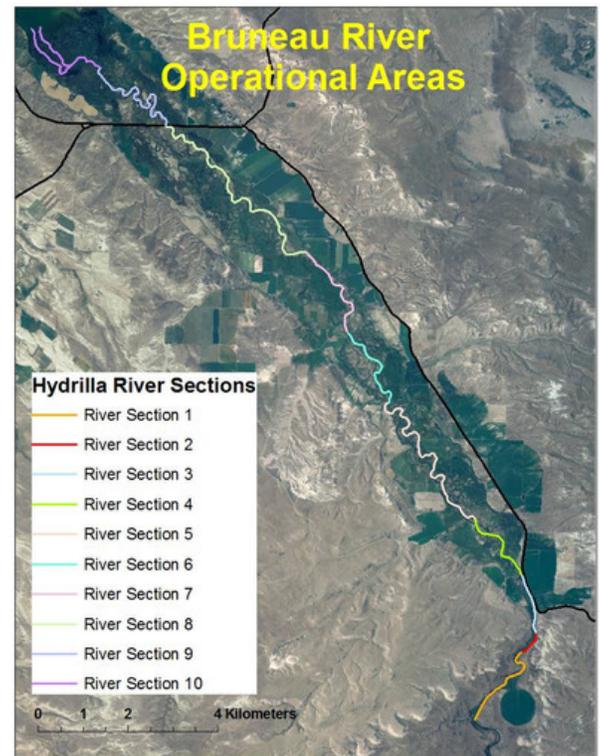
THE 2016 FIELD SEASON

The 2016 field season was the seventh year of the hydrilla eradication project where intensive survey data was collected, and a focused season-long removal effort implemented. Treatments have continued to utilize suction removal and a staff of four to survey and remove hydrilla in the Bruneau system.

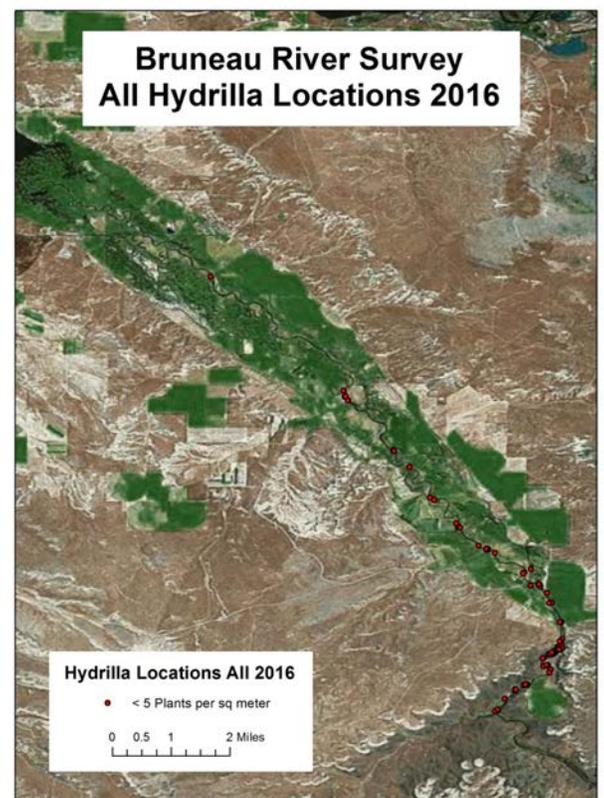
The entire area of known hydrilla infestation was repeatedly surveyed in 2016 and plants were removed whenever encountered. The survey area was divided into ten operational zones (Map 2) based on river access locations and were identified to help drive management decisions in the focusing on areas with higher plant density and occurrence. The majority of infestation can be found in upper section 1-5 which are influenced by several geothermal springs and backwater areas.

For 2016, our focus has continued to concentrate on river sections 1 through 5. These sections have historically contained several densely infested backwater areas that act as point-source locations for the transport of hydrilla back into the main river stem as seasonal flow fluctuates. High spring flow can create the possibility that tubers and turions are lifted out of sediment and carried downstream. Hydrilla tubers can remain viable for several years and break dormancy at any time over that period (Netherland 1999). This trait makes it extremely important to focus efforts in high density areas to decrease the deposition of hydrilla in subsequent years. During the

Map 2. River sections for hydrilla survey and removal. Bruneau River, Idaho.

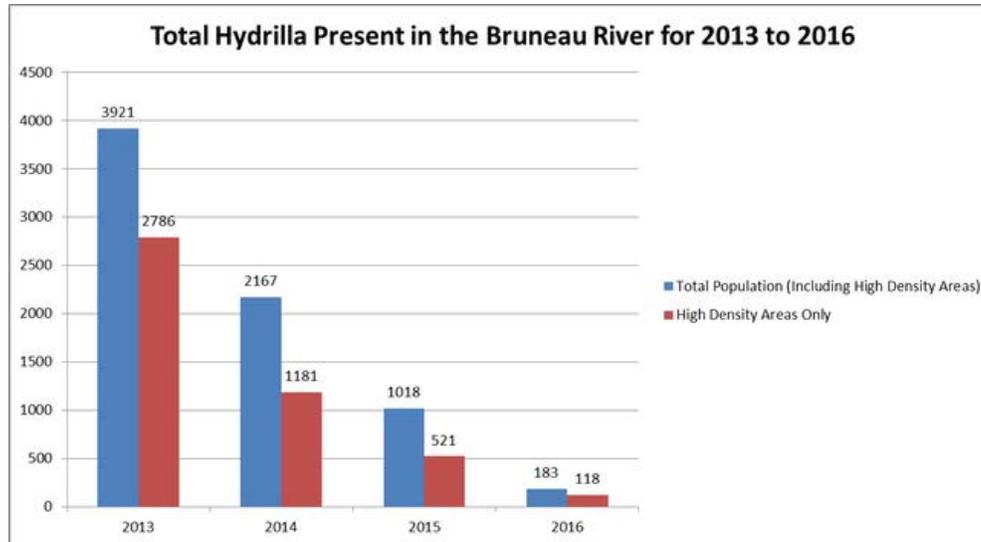


Map 3. All hydrilla locations in 2016 in plants per m². Bruneau River, Idaho.



growing season, hydrilla mainly spreads through fragmentation and is the reason why focus must be placed on upper river sections to stop the infestation at its source. Using this management strategy, survey and removal efforts for 2016 recorded a 95% reduction in high density areas when compared to 2013 findings. This reduction is also seen throughout the entire infestation zone at a decrease of 95% in overall hydrilla population (Figure 1).

Figure 1. Total number of hydrilla plants removed by year from 2013 to 2016 in the Bruneau River, ID.

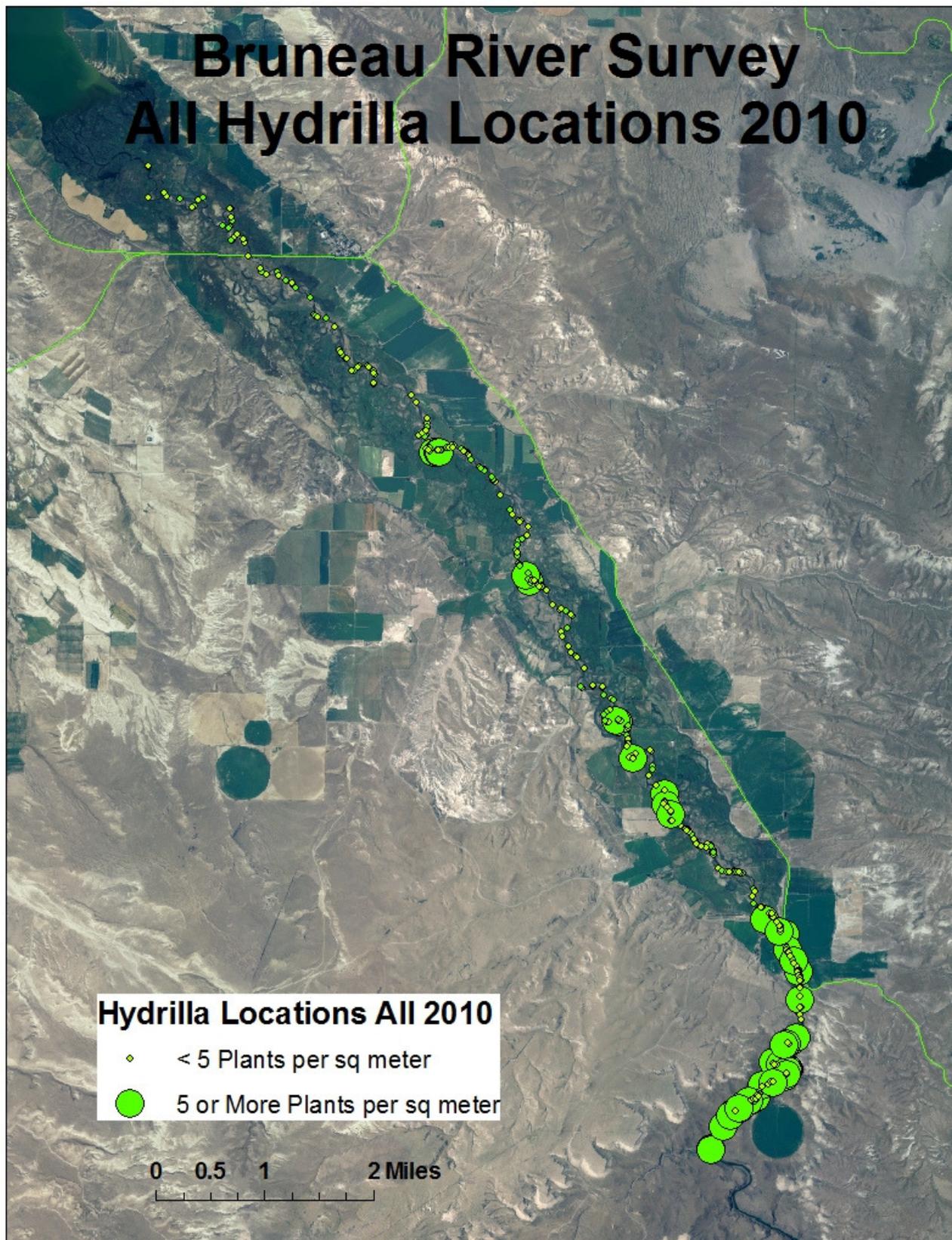


Dividing the river into ten operational areas (Map 2) also allows for a further investigation of the number of plants removed. This illustrates localized treatment progress and number of hydrilla plants for each river section by year. Table 1 shows the actual plant numbers recorded per river section for each year from 2013 to 2016. Significant decreases in plant occurrence were observed in 2016.

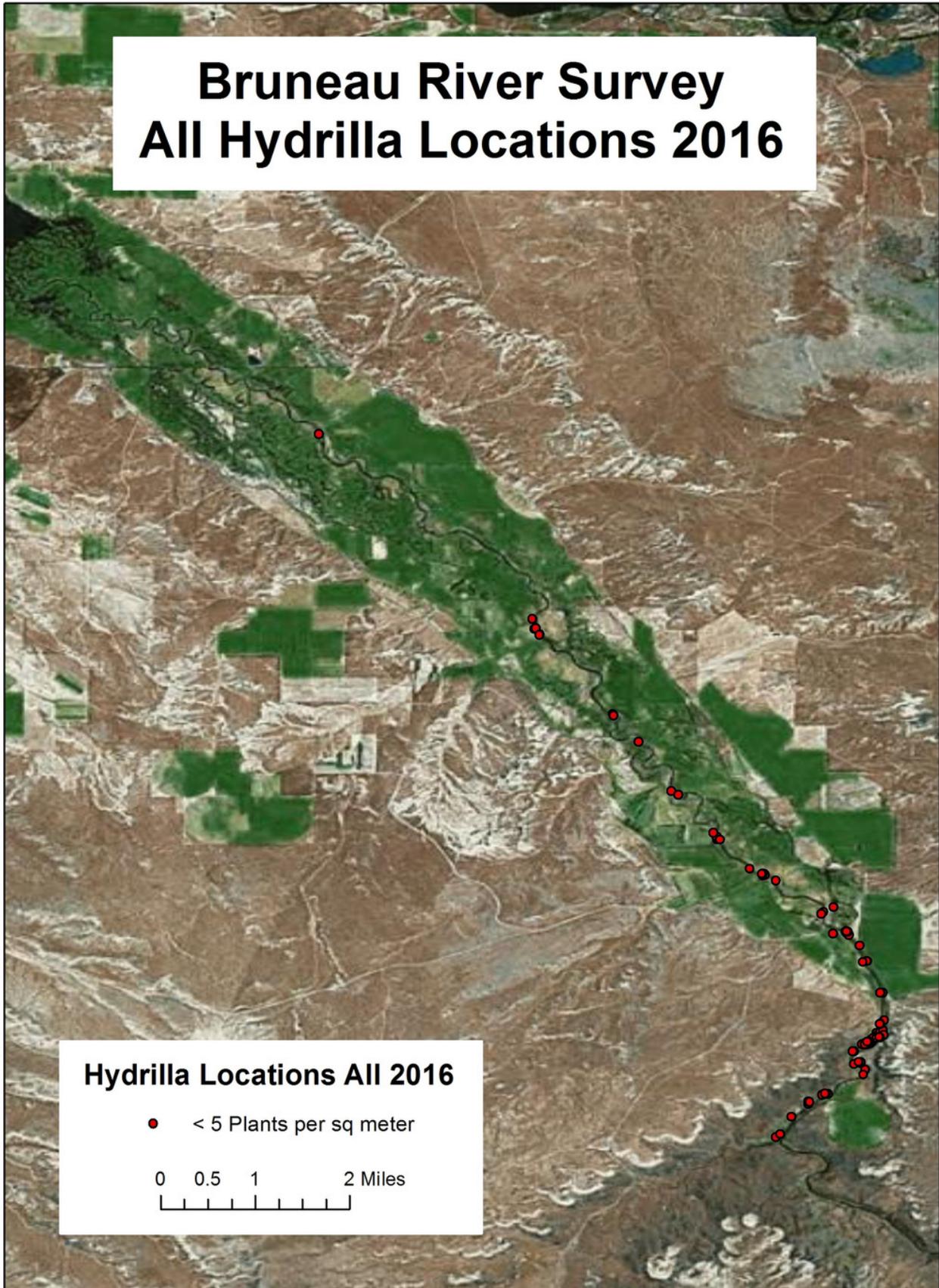
Table 1. Hydrilla plant occurrence per river section from 2013 to 2016, including high density areas.

River Section	2013	2014	2015	2016
1	493	259	73	25
2	487	391	325	65
3	122	60	62	4
4	2483	991	216	58
5	317	441	323	22
6	12	11	16	4
7	0	6	1	4
8	5	0	2	1
9	2	8	0	0
10	0	0	0	0

Map 4: All hydrilla locations in 2010 in plants per m². Bruneau River, Idaho.

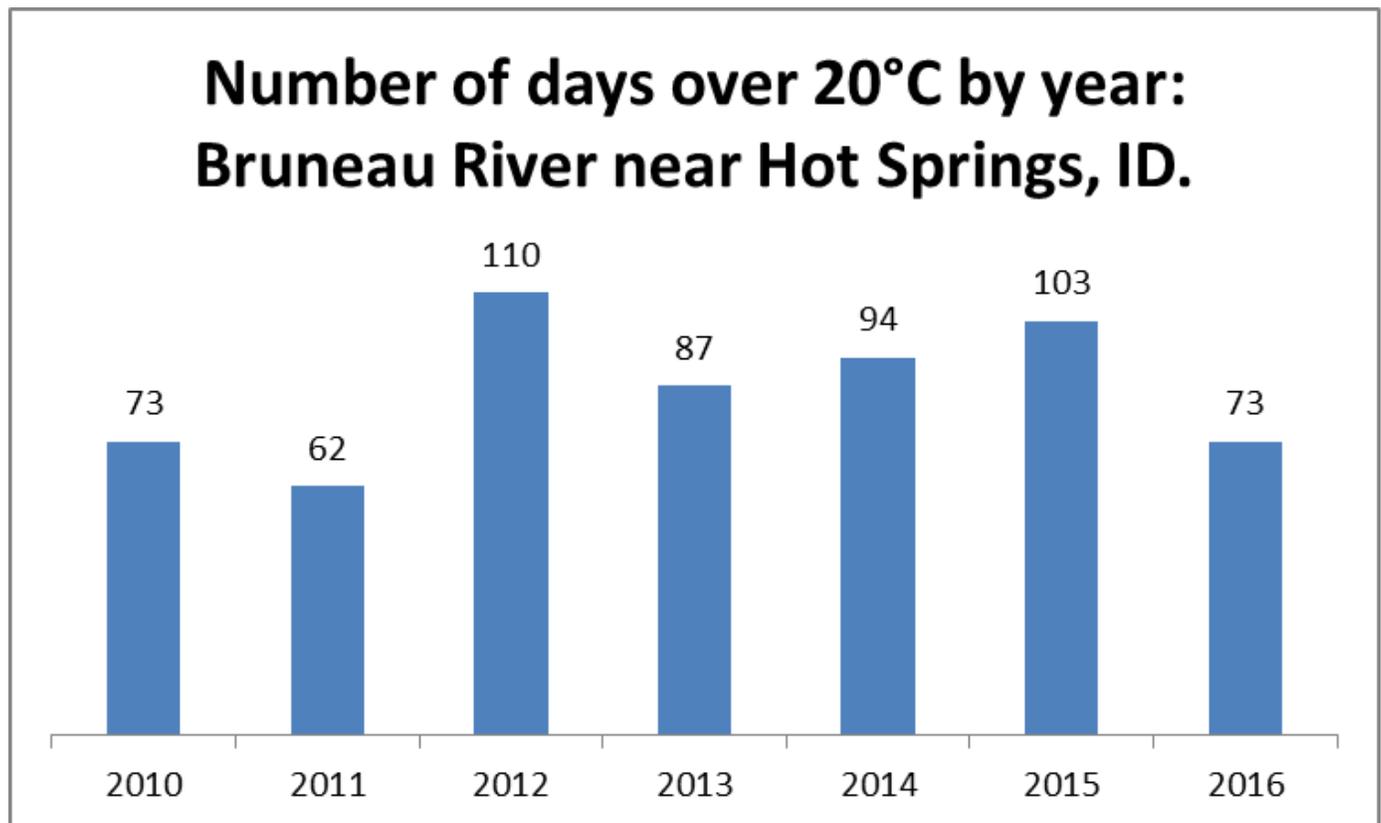


Map 3 (larger view): All hydrilla locations in 2016 in plants per m². Bruneau River, Idaho.



Temperature conditions in 2016 have remained favorable for hydrilla growth, and the growth response observed could be explained by an increased rate of tubers breaking dormancy. Published literature indicates the optimal temperature range for growth in dioecious hydrilla is between 20°C and 27°C (Barko & Smart 1981, Kasselmann 1995). A degree-day analysis was conducted on average Bruneau River water temperatures between June 1 and October 30 to determine the number of days per season that temperatures were over 20°C. Results from that analysis show that 2016 had 73 days with temperatures averaging over 20°C (Figure 2), which is identical to the number of days in 2010 when the infestation was at its greatest size.

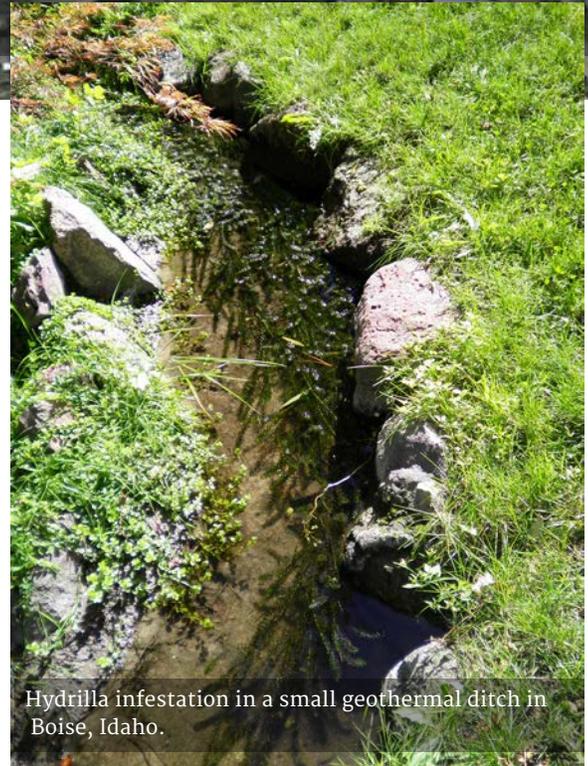
Figure 2. Degree day analysis for water temperatures in the Bruneau River, ID near Hot Springs. Days over 20°C by year (USGS Gage Water Temperature Data).



ADA COUNTY

NORTH BOISE

A population of hydrilla was identified in a small geothermal ditch in Boise in 2008. This ditch flows into the Farmers Union Canal and eventually deposits back into the Boise River. Plants were found in a 400 meter length of ditch that runs through the backyards of private homes in a North Boise neighborhood. The likely source of introduction was an escape of plant material from a hydrilla contaminated tilapia shipment received by a neighborhood aquaculture hobbyist using this geothermal water for fish propagation.

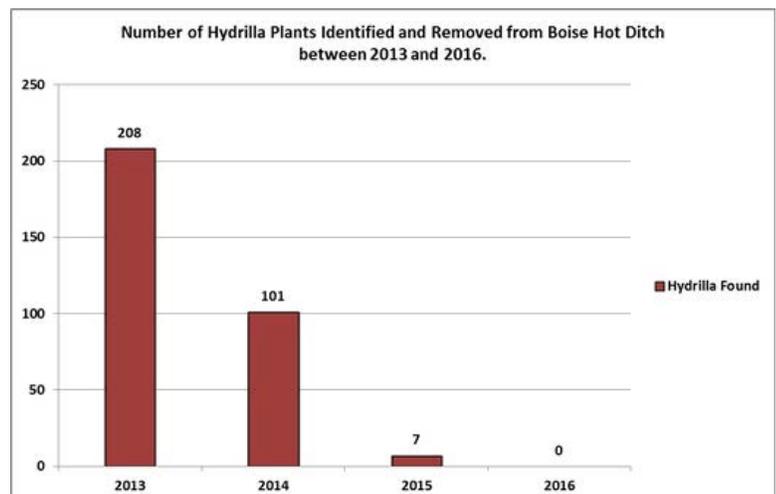


Hydrilla infestation in a small geothermal ditch in Boise, Idaho.

TREATMENT HISTORY

This population has been repeatedly surveyed since discovery and plants have been removed by hand when encountered. In 2015, the ditch showed considerable decreases in plant populations and by the end of the year, only 7 plants had been found. Two survey events took place in 2016 with no plants found in both surveys. This is the first year in which no plants have been observed (Figure 3). Monitoring will continue in this area to ensure no hydrilla regrowth occurs.

Figure 3. Total number of hydrilla plants removed by year from geothermal ditch located in Boise, ID.





Buhl hydrilla infestation zone.

RECENT POPULATIONS: TWIN FALLS COUNTY

BUHL

On a routine survey in July of 2015, hydrilla was found and identified in the outflow of a geothermally influenced settling pond which empties directly into the Snake River. An investigation of this initial discovery led to the identification of hydrilla populations on five separate properties. Borehole data accessed through the IDWR portal identifies 15 geothermal wells which feed this area and range in temperatures from 32°C to over 36°C at varying discharges of 70 to 3000 CFS. Geothermal water use on properties includes activities such as fish propagation, ornamental water gardens and home heating. Specific uses within aquaculture facilities require a mixing with cooler surface water and geothermal spring water to create a temperature range suitable for year round fish growth. Preferred temperatures for tropical fish propagation average from approximately 20°C to 30°C (Cline 2012) which match quite closely to temperature range suitability for hydrilla establishment and growth.

TWIN FALLS

One week after the discovery of hydrilla in Buhl, a second population was reported in Twin Falls in an irrigation/settling pond which also empties into the Snake River. This pond is fed with a constant flow of water that is a combination of ambient surface water from the Perrine Coulee mixed with geothermal well water from the discharge of a tropical fish raceway located just above. Temperatures produced in this area of mixing are ideal to support hydrilla establishment and growth.



TWIN FALLS COUNTY TREATMENT HISTORY

Site evaluations were conducted in January 2016 with the assistance of Kali Sherril - Twin Falls County Weed Control, Gary Fornshell - Aquaculture Industry Liaison, University of Idaho Extension, and Dr. Bill Haller - Director of the University of Florida's Center for Invasive and Aquatic Plants. Treatment recommendations were provided for each site as result of this evaluation and included a combination of chemical applications, biological control, mechanical removal, benthic barriers, and hand pulling. All treatments were initiated in cooperation with land owners, Twin Falls County Noxious Weed Department and ISDA.

The Hydrilla Crew began survey and removal efforts in June 2016, and visited the site once a week for the duration of the field season. When plants are encountered, the location is recorded and plants/tubers are removed. Data collected from 2016 removal efforts will provide a baseline for monitoring the progress of this eradication effort.

Site 1. Aquaculture facility raising tilapia, catfish, and tropical ornamental fish in Buhl, Idaho.





Rearing tubs, the upper most location of hydrilla occurrence.



Using feel to find hydrilla plants anchored around rocks.



Hydrilla masses anchored to rocks in rapid area.

SITE 1

Survey of Site 1 found hydrilla throughout the facility and infesting approximately 2 acres (0.8 hectares) of water area. Water temperatures range from approximately 20°C to 30°C on an increasing trend while surveying from the lower reaches up. The plant appears to have originated in a plastic rearing tub located at the upper-most area of the facility where fragments were carried down into the system via piping which feeds into a combination of raceways, canals, ditches, channels and underground pipes. Hand removal is the primary treatment on this site. Focus is paid to the entire infestation zone, with a heavy emphasis on the upper areas, to stop the spread of fragments at the source. A total of 2,842 plants were identified and removed from Site 1 in 2016.



Hydrilla caught/growing from log debris in confluence area.

SITE 2

Hydrilla infestation area is located in a large ornamental pond which covers approximately 0.3 acres (0.14 hectares) and includes a small out-flowing ditch. Pond temperatures fluctuate from approximately 24-27°C in the summer months down to around 8°C in December. Most of the plants observed were located in small crevices created by the rocks lining the edges of the pond, with a few found in the outflow ditch below. Hand removal was the only treatment applied in 2016; however, if plant numbers are not significantly decreased in the coming seasons, biological control may present a viable option. A total of 2,350 plants were identified and removed from this site in 2016.

Site 2. Ornamental pond in a residential setting in Buhl, Idaho.



Site 3. Aquaculture facility raising African cichlids in Buhl, Idaho.

**SITE 3**

The facility consists of several plastic rearing tubs connected to a small concrete raceway via pvc pipe. Water temperatures on site range from 21°C to 30°C. No hydrilla was found in rearing area, however; a small settling area located directly below the tub discharge zone had plants identified, along with a second earthen settling pond at the base of the raceway. Outflow from the ponds

connect to a large ornamental pond and a small ditch which empties directly into the Snake River. Plants have also been found in these areas in limited numbers. Techniques utilized on this infestation have included mechanical and hand removal. The landowner removed sediment from the small settling area and de-watered the earthen pond in January 2016. This treatment was followed up by suction removal in August to remove any remaining plant material. Hand pulling was applied to the outflow areas with significant decreases already being observed. A total of 1,609 plants were identified and removed from this site in 2016.

SITE 4

This infestation was identified late in the 2016 season. The landowner approached crews in July after talking to a neighbor about hydrilla occurrence on an adjacent property. Hydrilla was found in a total of three ornamental ponds surrounding the main house. Plants were deeply rooted and growing up through rocks and decomposed pond liners. Working with the landowner, rock and pre-existing liner

were removed from two of the ponds, and then suction removal was utilized to remove tubers anchored deep within the sediment. Ponds were then relined with carpet and replacement liner over the top. Modifications are an ongoing process and the landowner expects to complete pond liner replacement in 2017. Plant numbers for this property dropped to single digits per visit following suction removal. A total of 777 plants were identified and removed from this site in 2016.

Site 4. Ornamental ponds in a residential setting in Buhl, Idaho.



Site 5. Recreational ponds in a residential setting in Buhl, Idaho.



SITE 5

This site contains two private pond areas used for recreational purposes. No hydrilla has been observed in the uppermost pond, however; there is a significant population of Eurasian watermilfoil (landowner is working with Twin Falls County Weed Control for treatment options in this pond). The lower pond (hydrilla-infested) is connected to the upper via an

underground pipe, then empties directly into the Snake River. Treatments on-site have included mechanical and hand pulling techniques. The landowner used a backhoe to dig out the lower pond early in the season and crews followed up with a suction removal in July. Hand pulling did not resume until November when plants located in the upper bank area were once again submerged with rising water levels. A total of 129 plants were identified and removed from this site in 2016.

SITE 6

Hydrilla infests the outflow areas of two rearing facilities located on site. The outdoor facility has the highest volume of flow and has been the focus of survey and removal efforts in 2016. The main outflow ditch empties into two large settling ponds before exiting under the road to a neighboring property. Plants have been found throughout this stretch. The indoor facility's outflow also contains hydrilla, however; at this time, the volume of water discharged is not enough to create a flow capable of carrying fragments downstream. This area is being watched closely and will be monitored year round to record seasonal flow changes. Hand removal and biological control using triploid grass carp have been the primary techniques applied to this site. In April 2016, a total of 25 fish were acquired and split between the infested ponds, and have drastically decreased hydrilla occurrence where topped-out vegetation can no longer be seen. Hand removal in the outflow ditch is slowly decreasing the tuber bank which is estimated to be quite substantial. The facilities manager reported that hydrilla has been a resident on site for quite some time. A total of 2,987 plants were identified and removed from this site in 2016.

Site 6. Aquaculture facility raising African cichlids in Buhl, Idaho.



Site 7. Residential ditch and pond in Buhl, Idaho.



SITE 7

The infestation on this property is located in a ditch which is fed by outflow from the aquaculture facility in Site 6. The ditch is only exposed for approximately 50 meters before disappearing into an underground pipe and discharging into Deep Creek. Plants are also found in a pond located in the pasture area just behind the house. The pond does not appear to have a discharge point and instead seeps into the ground. It will be monitored closely to

ensure that seasonal variability does not create an active flow that could potentially deposit fragment in Deep Creek. Hand removal treatments have been utilized exclusively on this site and a total of 1,056 plants were identified and removed in 2016.

Twin Falls Site. Aquaculture facility discharge and irrigation pond in Twin Falls, Idaho.



TWIN FALLS SITE

Hydrilla infestation on site occupies a large settling/irrigation pond covering approximately 0.5 acre (0.2 hectares). Water from both ambient and geothermal water sources are piped into this location creating an ideal temperature for plant establishment. There is one outlet which flows through a grate and concrete containment area before traveling through a pipe that directly discharges into the Snake River. Hydrilla plants and tubers have been removed from the confluence area and also from a location approximately 80 meters downstream in a second geothermal mixing zone area. Treatments on site have included mechanical and biological controls. The facilities manager organized dewatering in mid-January and followed up with a backhoe to remove sediment in an attempt to decrease the tuber bank. When irrigation water refilled the pond, the remaining tubers quickly reestablished the site and topped out to cover near 85% of the water area. In response, 30 sterile (triploid) grass carp were stocked in the pond in October. Progress in subsequent seasons will be recorded using photo plots to identify the percentage of plant materials removed using biological control.

FUTURE ERADICATION EFFORTS

Results in the 2016 Bruneau infestation zone showed the largest percentage decrease in overall plant population since initial removal efforts began in 2008, and repeated surveys have found no plants to occur in the Boise population. Weather conditions remained favorable and provided for optimal growing conditions throughout the season. This suggests that a sustained and persistent survey and removal program is reducing plant densities as well as tuber bank. This also highlights the importance of continuing these efforts in the Bruneau River and adopting similar strategies for new populations in Twin Falls County. Hydrilla density will continue to decline over time as the regrowth is consistently found and removed. Lower plant densities will aid in making this process easier and more efficient. Survey and removal efforts will continue into 2017 following the same mapping and removal methods currently utilized. Data collected from Twin Falls County in 2016 will provide a basis for comparison and will be analyzed throughout the 2017 field season with management efforts adjusted accordingly. Eradication continues to be the goal of the program and through persistent and sustained efforts, substantial progress is being made toward that goal.



Hydrilla biomass removed from small ornamental pond in Buhl, Idaho.

For more information on the Hydrilla Eradication Program in Idaho, please contact the Idaho State Department of Agriculture Noxious Weeds Department
@
208-332-8500
invasivespecies.idaho.gov

REFERENCES

- Barko, J. W. & R. M. Smart, 1981. Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. *Ecological Monographs* 51: 219-235.
- Bianchini, I., M. B. Cunha-Santino, J. A. M. Milan, C. J. Rodrigues & J. H. P. Dias, 2010. Growth of *Hydrilla verticillata* (L.f.) Royle under controlled conditions. *Hydrobiologia* 644: 301-312.
- Cline, D. 2012. Water Quality in Aquaculture. Alabama Cooperative Extension System, Auburn University. <<http://articles.extension.org/pages/58707/water-quality-in-aquaculture>>. Accessed 3030 December 2016.
- Haller, W.T. 1976. *Hydrilla*. A new and rapidly spreading aquatic weed problem. Florida Agricultural Experiment Station, Circular S-245, 13 pp.
- Haller, W.T., Miller, J.L., Garrard, L.A. 1976. Seasonal production and germination of *Hydrilla* vegetative propagules. *J. Aquatic Plant Manage.*, 14: 26-29.
- Haller, W.T. and D.L. Sutton. 1975. Community structure and competition between *Hydrilla* and *Vallisneria*. *Hyacinth Control Journal* 13: 48-50.
- Hershner, C. & K. J. Havens, 2008. Managing invasive aquatic plants in a changing system: strategic consideration of ecosystem services. *Conservation Biology* 22: 544-550.
- Kasselman, C., 1995. *Aquarienpflanzen*. Egen Ulmer GMBH & Co., Stuttgart: 472 pp.
- Langeland, K. A. (1996). *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae), "The perfect aquatic weed". *Castanea*, 61, 293-304.
- Mullin, B. H., L. W. J. Anderson, J. M. DiTomaso, R. E. Eplee & K. D. Getsinger, 2000. Invasive plant species. *Council for Agriculture and Science and Technology* 13: 1-18.
- Murphy, K. J., 1988. Aquatic weed problems and their management: a review I. The worldwide scale of the aquaticweed problem. *Crop Protection* 7: 232-248.
- Netherland, M. D. 1999. Management Impacts on the Quiescence and Sprouting of Subterranean Turions of Dioecious *Hydrilla* [*Hydrilla verticillata* (L.f.) Royle]. Ph.D. dissertation. University of Florida, Gainesville, FL. 191 p.
- Swarbrick, J.T., Finlayson, C.M., and Cauldwell, A.J. 1981. The biology of Australian weeds 7. *Hydrilla verticillata* (L.F.) Royle. *J. Australian Instit. Agric. Sci.*, 47: No. 4, 183-190.
- Van, T. K., W. T. Haller, and G. Bowes. 1976. Comparison of the photosynthetic characteristics of three submersed aquatic plants. *Plant Physiol.* 58:761-768.
- Van, T.K. and K.K. Steward. 1990. Longevity of monoecious hydrilla propagules. *J. Aquat. Plant Manage.* 28:74-76.
- Wilde SB, Murphy TM, Hope CP, Habrun SK, Kempton J, Birrenkott A, Wiley F, Bowerman WW, Lewitus AJ (2005) Avian vacuolar myelinopathy linked to exotic aquatic plants and a novel cyanobacterial species. *Environ Toxicol* 20(3):348-353.