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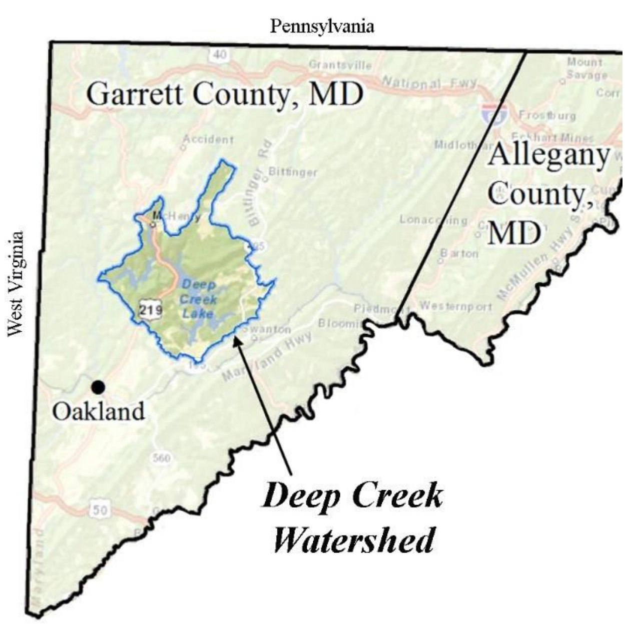
On the Hunt for Aquatic Invasive Species

An Introduction to New Methods for Early Detection and Public Educationat Deep Creek Lake, Maryland

Deep Creek Lake, Maryland, (DCL) attracts an average of 1million tourists year round to enjoy an assortment ofrecreational outdoor activities, from boating in the summer to ice fishing in the winter. One of the largest emerging threats to this area are aquatic invasive species, which are commonly introduced through the gear of recreating visitors. Aquatic invasive species that have altered ecosystems in adjacent water bodies include hydrilla (*Hydrilla verticillata*), zebra mussels (*Dreissena polymorpha*),quagga mussels (*Dreissena bugensis*), and bighead carp (*Hypophthalmichthys nobilis*). Although, only hydrilla has been found in DCL, this brief synthesizes successful aquatic invasive species management and outreach strategies to aid management of aquatic resources at DCL.



Aquatic Invasive Species (AIS)

AIS can severely transform the habitat and biodiversity of an ecosystem. This is especially true for zebra mussels and hydrilla, two of the greatest AIS concerns for Deep Creek Lake. When established, zebra mussels can outcompete native filter feeders, change water chemistry, and cause millions of dollars in damage to personal and state property. Zebra mussels have also significantly increased water clarity in some aquatic systems, facilitating the growth of submerged aquatic vegetation (SAV). In environments where zebra mussels and hydrilla coexist, increased water clarity could expedite the spread of hydrilla. Hydrilla is capable of growing in such dense aggregations that it restricts water flow, outcompetes native SAV, and restricts boating. Hydrilla is also known to be a vector for avian vacuolar myelinopathy, a fatal neurological disease that affects waterfowl.

Table 1. A summary of existing and potential invasive species of the highest priority in Deep Creek Lake Maryland.

Existing Invasive Species at Deep Creek	Potential Invasive Species
Hydrilla (*Hydrilla verticillata*)	Zebra Mussels (*Dreissena polymorpha*)
Curly Pondweed (*Potamogeton crispus*)	Quagga mussel (*Dreissena bugensis*)
Eurasian Watermilfoil (*Myriophyllum spicatum*)	Brazilian Waterweed (*Egeria Densa*)
Virile Crayfish (*Orconectes virilis*)	Giant Salvinia (*Salvinia molesta*)
Japanese Mystery Snail (*Cipangopaludina japonica*)	Bighead Carp *(Hypothalmichthys nobilis*)

New Methods for Early Detection

For species of high invasion risk, it is important for managers to have ways to identify early detection, control the spread of invasion, and reduce the potential harm of that invasive species. Deep Creek Lake has been relatively successful in preventing the introduction of new invasive species through the Launch Stewards Program, a program to conduct voluntary boat inspections to prevent the introduction of AIS. New methods are constantly in development to improve our abilities to address these objectives. The following methods have been proposed to identify and detect species of concernin other geographical regions which could be used to identify AIS at DCL.

eDNA – Environmental DNA (eDNA) is DNA that has been released into the environmental from an organism. eDNA has the potential increase detection of new invasions very early (Martinez et al. 2020). Many researchers have been in development of new protocols to identify Rusty crayfish in lotic streams (Doughtery et al. 2016), zebra and quagga mussels in the Great Lakes (Marshall and Stepien 2019), and hydrilla in ponds using eDNA (Matsuhashi 2016, Martinez 2020). Recent pond studies have demonstrated that eDNA was successful in detecting hydrilla before visual detection (Matsuhashi 2016). In 2018, Gantz et al. tested detectability in the Patuxent and Potomac Rivers and found that eDNA was able to detect hydrilla accurately from 0.2-0.6 km down river. Additionally, eDNA may be able to detect changes in biomass as studies suggest that eDNA concentrations generally increased as hydrilla biomass increased (Matsuhashi et al. 2016; Gantz et al. 2018), but this trend has not been consistently observed at all study sites. Further, studies do not suggest that eDNA can be used to track abundance of invasive crayfish (Doughtery et al. 2016) or Eurasian watermilfoil (Kuehne et al. 2020). Both nuclear and chloroplast primers have been developed as best methods primers have detected Eurasian watermilfoil from up to 60 m away and lab studies suggest that detection can occur as early as 3 hours after introduction (Newton et al. 2016). Please see supplemental table 1 for a list of primers for invasive species detection.

Figure 1. *Examples of current and potential invaders of Deep Creek Lake including dense aggregations of hydrilla (A), virile crayfish (B), curly pondweed (C), and zebra mussels (D).*

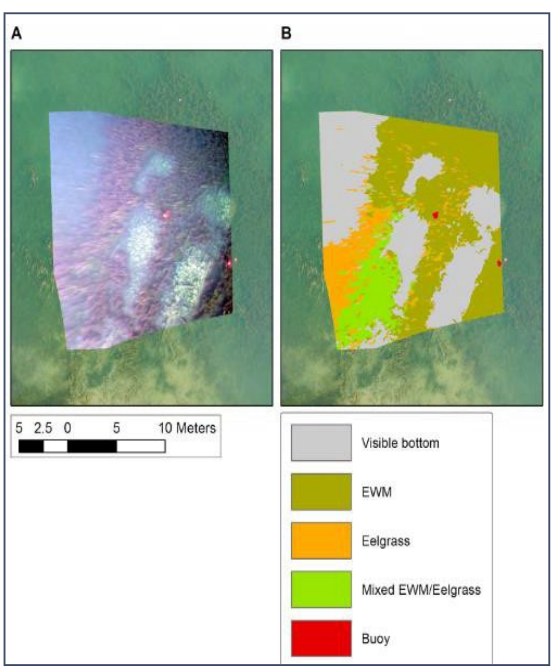


Figure 2. *Detection of Eurasian watermilfoil using drone imagery. A) The image from the drone, overlaid with a six spectral color bands; b) the same image with object-based image analysis done, using eCognition developer. Figure 3.22 from Brooks 2020.*

While eDNA can be a useful for early detection of AIS in freshwater systems, there are limitations to eDNA that management must be aware of prior to use. eDNA can be quite sensitive and is able to detect organismal DNA well after that specified organism is no longer present in the system. Additionally, detectability of primers relies on environmental conditions, seasonality, and degradation of DNA (Matsuhashi et al. 2019). Therefore, this technology should be tested within DCL to determine optimal conditions for application prior to use as a management tool. Further, range of detection for hydrilla in ponds have not been identified. In rivers, hydrilla eDNA wasobserved over 4,000m downstream but this was affected by flow, current, and other environmental conditions (Weber 2021). However, eDNA offers an affordable and simple wayfor managers to identify further spread of hydrilla.

Drones – Unmanned Aerial Vehicles (UAV) or 'drones' have been increasingly used for ecological monitoring. UAVs can be considered as a management tool because they are cost effective and allow for large-scale sampling in a short amount of time (Lønborg et al. 2021). Recent studies have utilized UAVs to identify and monitor invasive SAVs such as Eurasian watermilfoil and water hyacinth (Brooks 2020, Bolch et al. 2021). Researchers were able to detect and map the distribution of Eurasian watermilfoil in Canadian Lakes with over 75% accuracy using a hexacopter drone mounted with textrcam multispectral array that uses six spectral bands (Brooks 2020). The array helps increase visibility of SAV in water.

At Deep Creek Lake, UAVs would likely be a reliable way to identify SAV due to high water clarity. However, numerous considerations are needed before their use in management. Pictures captured from UAVs are best in sunny, clear skies, as cloud coverage and wind can decrease visibility and add visual ripples in the water, which can be confused for SAV. Additionally, detection of SAV from UAV imagery decreases with increasing water depth. Further, for some systems, species-specific identification requires lower flights, which can increase time and financial commitments.

Public Awareness and Outreach

The engagement of lake stakeholders and visitors is imperative to prevent the introduction and spread of AIS. Maryland Department of Natural Resources (MDNR) has actively interacted with residential lake stakeholders by forming partnerships that facilitate continued dialogues with groups like the Deep Creek Watershed Foundation and the Brookfield Renewable Partners (Julie Bortz personal comm.). Additionally, MDNR has published newspaper articles that provide detailed instructions on how to prevent the transport of AIS, launched an AIS website specific to DCL , and provides AIS inspection and decontamination training to local marinas. While these prevention strategies have successfully engaged residential lake users, MDNR strugglesto reach out of state visitors who are traveling from infected water bodies such as the Ohio River in Pennsylvania and the Monongahela River in West Virginia. This difficulty can largely be attributed to the varying degrees of funding and effort allocated to AIS management by each state.

Interstate coordination of AIS management would substantially increase the efficiency of monitoring efforts but is unlikely. Therefore, out of state visitors should be targeted directly and independently of residential lake users. Directed outreach surveys have been an effective method to engage recreationalist in many parts of the country (Sharp etal. 2015; Oh et al. 2018; Cimino and Strecker 2018). For example, a public outreach survey was used to understand the perception of hydrilla and proposed management actions at the J. Strom Thurmond Reservoir, of Georgia and South Carolina (Fouts et al. 2017). Survey recipients from both states were identified from a sportsman's license database and stratified based on assumed activity (i.e. hunter, fisher, boater, etc). The survey concluded that recreationalist that were informed on hydrilla and its effects were more supportive of management actions, providing evidence that outreach was successful in engagement and facilitating cooperation between managers and stakeholders. Similarly, Colorado Parks and Wildlife (CPW) requires mandatory inspections on all boats in state waters. CPW also manages the Watercraft Inspection and Decontamination (WID) database, a national database which collects information on boat registration and the history of boat travel. Many states in the west participate in this database and it helps track the movement of infected boats. Deep Creek Managers have adopted a similar voluntary inspectionprogram that records the boat owners zip code and where theboat was last launched through the Launch Stewards Program. Deep Creek Lake managers have discussed the possibility of participating in the CPW database and hope to revisit the feasibility of this management strategy in the future. Recording boater registration at DCL would not only help track the movement of out of state boats but also identify out of state boat owners to be targeted directly in outreach surveys.

Future of Invasive Species Management

While Deep Creek Lake is well managed by the MDNR, there aremajor concerns about the staff's ability to detect new AIS early, prevent further spread of established invasive SAV, and effectively educate the public and visitors with limited resources. This brief proposes new detection methods like eDNA and drones that could be used in this system that are both cost effective and time efficient. Additionally, we provide potential solutions for management to increase communication with lake visitors. Ultimately, the best invasive species management is one that is regional and works with multiple states and agencies. In the future, the MDNR should work with neighboring states of West Virginia, Virginia, and Pennsylvania to successfully preventthe introduction of AIS in this region.

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Supplemental Table 1. Best primers for eDNA detection of significant invasive species relevant to Deep Creek Lake.

Species	Primer		Genome	Source
H. verticillata	Hverticillata_ITS1_F3	5′-GGAGGATGTTGATGGAGGTG-3′	Nuclear	Gantz et al. 2018
H. verticillata	Hverticillata_ITS1_R3a	5′-CAATTCACACCATATATCGCATTT-3′	Nuclear	Gantz et al. 2018
H. verticillata	Hverticillata_matK_F1a	5′-CTTGTTCTCATTATTGTAGTGGATCT-3′	Chloroplast	Gantz et al. 2018
H. verticillata	Hverticillata_matK_R1	5′-GCACTTTTTCTTCTTCGGTATCTG-3′	Chloroplast	Gantz et al. 2018
H. verticillata	TaqMan MGB probe	5'- FAM-ATTATTGTAGTGGATCTTCA–NFQ–MGB-3'		Matsuhashi et al. 2016
H. verticillata	matK (forward primer)	5'-TTTGCGCGAATATGTAGAACTTGT-3'	Chloroplast	Matsuhashi et al. 2016
H. verticillata	matK (reverse primer)	5'-GCCAAGGTTTTAGCACAGGAAA-3'	Chloroplast	Matsuhashi et al. 2016
M. spicatum	Forward Primer	5'-CCACCCTTCAAGGATAAGGC-3'	Ribosomal	Newton et al. 2016
M. spicatum	Reverse primer	5'-AGGCTGAGTTATCAACCACC-3'	Ribosomal	Newton et al. 2016
O. rusticus	Orusticus_COI_5F	5′-CAGGGGCGTCAGTAGATTTAGGTAT-3′		Dougherty et al. 2016
O. rusticus	Orusticus_COI_5R	5′-CATTCGATCTATAGTCATTCCCGTAG-3′		Dougherty et al. 2016
Dreissena spp.	COIA–F (forward)	5'AGTGTTYTKATTCGTTTRGAGCTWAGKGC3	Mitochondrial	Marshall and Stepien 2019
Dreissena spp.	COIA–R (Reverse)	5'GAYAGGTARAACCCAAAAWCTWAC3'	Mitochondrial	Marshall and Stepien 2019
Dreissena spp.	COIB–F (forward)	5'GRAAWCTKGTMACACCAATAGAWGT3'	Mitochondrial	Marshall and Stepien 2019
Dreissena spp.	COIB–R (reverse)	5'GRAAWCTKGTMACACCAATAGAWGT3'	Mitochondrial	Marshall and Stepien 2019