

Management Support Tree for the Interpretation of Positive Laboratory Results

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The purpose of the Management Support Tree described herein is to provide support for managers in the interpretation of a positive environmental DNA (eDNA) result and the determination of whether management actions should be taken. The main focus of the tree is on the detection of aquatic invasive species in the Great Lakes system. Use of the tree assumes that proper field and laboratory controls have been implemented (i.e., that the positive result is not due to contamination), thus confirming that the targeted species' DNA is present in the collected water samples. However, the biological interpretation of a positive result must consider whether the presence of the species' DNA likely indicates the presence of the species itself and must take into consideration the strength of the positive result. Given the transitory nature of eDNA, reliable interpretation of eDNA results often requires multiple sampling events. In all cases, even a single positive result should trigger follow-up monitoring, the outcome of which will inform management actions.

The first part of Management Support Tree implementation is evaluating the likelihood that the targeted organism is in the system based on information about the environment. The second part of the Management Support Tree incorporates information regarding the strength of the positive result. **The guidelines provided here are subjective and qualitative; they are provided as guides to evaluation and action. Additional information and other factors (e.g., how large are the potential damages from an invasion) may shift the urgency of any management response.**

Step 1: Determining the Vector Level

The presence of eDNA indicates that DNA from the target organism is present, but the organism itself may not be. Features of the environment can influence whether DNA presence is being transported from another location and whether detected DNA is likely linked to organism presence. For example, in areas of high water flow, DNA may be transported from locations upstream. Other vectors of DNA should also be considered. In areas of high human activity (especially boating activity), people may be passively transporting DNA from other locations. An assessment of the presence of other vectors at the sampling site can help determine whether any eDNA positive results are likely linked to the presence of an organism. In areas where DNA is likely linked to organism presence, repeated sampling to confirm any positive results will be a priority.

Use the following rubric to determine Vector Level prior to using the Positive Result eDNA Management Support Tree. First, sum the point scores across the two criteria (descriptions of criteria are provided below). Second, interpret the scores and assign Vector Level as defined below (e.g., a score of 2 is categorized as "Vector Level A").



DNA transport from upstream	High - 1 point	Low - 2 points
Probability of other vectors	High - 1 point	Low - 2 points

Descriptions of criteria:

DNA transport from upstream - Evaluate the probability that DNA from upstream has been transported to a downstream sampling site. A high probability would occur when sampling a downstream site where water flows between sites are high. A low probability would occur when sampling an upstream site, when water flows are low, or when the water body is isolated (e.g., ponds).

Probability of other vectors - Human activity and wildlife presence are potential vectors of eDNA. The level of watercraft traffic is likely the biggest consideration for assessing the effects of other vectors in large waterbodies, while human recreational use is likely the biggest consideration in smaller streams or ponds. Wildlife (e.g., birds) also has the potential of transporting DNA. Areas with a high density of predators of the organism of interest have a high probability of DNA being introduced from other vectors.

Vector Level A: 2 points – eDNA more likely to be from elsewhere or not recent (i.e., “transient”). Due to environmental conditions, there is high uncertainty in the source of the eDNA signal. Some (but less extensive) follow-up monitoring is recommended.

Vector Level B: 3-4 points – eDNA more likely to be non-transient, spatially or temporally, resulting in higher certainty that the targeted organism is actually present in the system. More prolonged follow-up monitoring is recommended.

Step 2: Determining the Strength of Positive Results

Strength of the positive result is based on the percentage of replicates that tested positive for the target species’ DNA. Replicates include both biological (i.e., multiple field samples from the same location and same time) and technical (i.e., repeated PCR reactions of the same water sample) replicates. The strength of the positive result is then determined by the percentage of replicates that support that result, the Vector Level of the location, and the change in the percentage of positive results over time. Changes in DNA concentration over time can also be an indicator of strength of the positive result; however, variation in eDNA collection procedures, variation in laboratory techniques, and many environmental variables can also influence DNA concentration, making DNA concentration a less reliable indicator.

This Management Support Tree combines information about the percentage of replicates that had a positive result and the Vector Level to guide the interpretation of positive eDNA results. For example, if the percentage of replicates testing positive is low ($\leq 10\%$), follow-up monitoring is recommended within 2 weeks where Vector Level is B because there is a low likelihood the DNA originated from another location. However, monitoring is recommended within a month if Vector Level is A because the DNA is more likely to have originated from a different location. When the percentage of replicates testing positive is high ($> 70\%$), follow-up monitoring should proceed within 1 week, regardless of Vector Level. With a high percentage of positive detections, it is unlikely that the DNA originated from a different location and there is a greater chance that a high



density of organisms is present at the location; management actions may need to be implemented more rapidly.

If a positive result (regardless of the number of replicates testing positive) is obtained in any biological or technical replicates, this initial positive result is a **Level 1 positive**. It will remain a Level 1 if there are no subsequent positives during follow-up monitoring, but it will increase to Levels 2, 3, or 4 if subsequent positive detections are noted, as summarized below.

**SUMMARY:
CRITERIA AND RECOMMENDED ACTION WHEN eDNA IS DETECTED**

LEVEL	DETECTION CRITERIA	ACTION
1	Initial positive detection in any sample	Repeat sampling: <ul style="list-style-type: none"> • If no further samples are positive, remains at Level 1: species likely not present • If repeat sampling yields positive detections, level increases: see below
2	% positive detections is: <i>decreasing over time</i> OR <i>stable over time with <10% (low) samples testing positive over entire data set</i>	Repeat monitoring in one month
3	% positive detections is: <i>stable over time with 10–70% (medium) samples testing positive over entire data set</i>	Continue monitoring weekly
4	% positive detections is: <i>increasing over time</i> AND/OR <i>>70% samples test positive over entire data set</i>	SPECIES IS LIKELY PRESENT AND MAY HAVE SUFFICIENT ABUNDANCE TO SUPPORT A POPULATION



Positive eDNA Management Support Tree

