Genetic Tools – Fact Sheet

Linking lake restoration with end users for positive environmental outcomes

LERNZ
Lake Ecosystem Restoration New Zealand

Why Genetic Tools?

Morphological identification of organisms can be challenging and often requires specialist knowledge to ensure accurate identifications especially, if specimens are microscopic, as is the case with zooplankton (Figure 1). It can also be challenging to know what species of fish or other taxa are in a lake or waterway without extensive surveys. For these reasons, LERNZ has been developing the use of genetic tools for species identification.

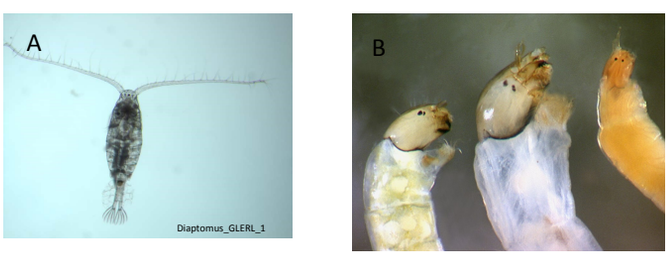

Figure 1. (a) Zooplankton and (b) left to right Orthoclad, *Chironomus* and Tanytarsini midges can be difficult to identify without specialist taxonomic knowledge. Photo (b): Stephan Moore

DNA for Species Identification

DNA carries the 'blueprint' for every living organism, determining physical and behavioural attributes. DNA consists as a 'string' of nucleotides of four bases; adenine, cytosine, guanine and thymine (A, C, G, T). The order of these bases varies between species. By identifying appropriate gene regions, individual species can be identified using short (<700 nucleotide) fragments of appropriate target genes. In this way, each species has a unique DNA 'signature' or 'DNA barcode'.

By matching DNA barcodes with morphological identification, future specimens can be identified using whole or partial specimens, regardless of age or life stage. They can even be identified through DNA shed or sloughed into the environment. For animal species, the mitochondrial gene cytochrome c oxidase subunit 1 (CO1) is widely used for identification purposes. Other genes such as CYTB, 28S and ITS can also be helpful to identify plant and animal taxa.

Building the Reference Library

DNA sequences and associated taxonomic identifications are added to a global and publically accessible database — the Barcode of Life Datasystems or 'BOLD' (www.boldsystems.org) (Figure 2). Detailed information is provided for each specimen including: a photograph, collection data, tentative identification, location of voucher specimen and DNA sequence data.

For species not yet barcoded, the reference database can be continually updated as new specimens are obtained. When DNA sequences are analysed and represent species that are not currently in the BOLD database, an exact species-level identification will not be possible, although comparison against international records will provide a match to the highest taxonomic level possible (e.g. order, family). For any currently undescribed or cryptic species, a Barcode Index Number (BIN) will be assigned by BOLD to allow for similar sequences to be grouped together. Any future specimens or formal identifications can then be attributed to the original specimen.

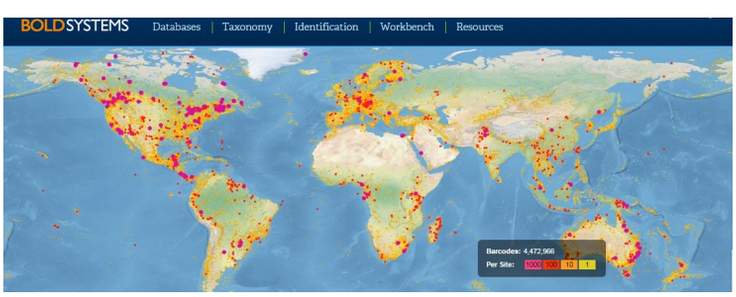

Figure 2. Home page for the Barcode of Life Datasystems (BOLD) database (www.boldsystems.org).

international BARCODE OF LIFE

New Zealand Barcode of Life

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Species Inventories

Over 5 million sequences from approximately 500,000 species are currently available on the BOLD database. For New Zealand waterbodies, 3000 specimens and 2300 DNA barcodes have been added to the database representing over 500 species. This total now includes molluscs, rotifers, copepod and amphipod crustaceans, as well as a range of aquatic insects including mayflies, stoneflies, caddisflies, and midges.

Community-level Analyses

The application of Next Generation Sequencing or 'NGS' approaches has the potential to revolutionise the efficiency of molecular-based approaches. NGS platforms, such as the Illumina MiSec and the Ion Torrent (Life Technologies), allow for 'metabarcoding' such that multiple samples or individuals can be run simultaneously.

In this way, entire freshwater communities can be characterised directly from a homogenised environmental samples. The 'Rotifer inferred trophic level index' (Figure 3) is one application of a community-level analysis that can be used a tool for assessing the trophic status of lakes. In this example, zooplankton samples are simply collected, stored in ethanol as a bulk sample, and then sent to a sequencing lab for NGS sequencing. The resulting sequences are then compared against the BOLD reference database to make species level identification.

Rotifers: Left to right *Lecane* sp., *Keratella* sp. and *Mytilina* sp.

Figure 3. Examples of taxa used in the community-level, rotifer-inferred trophic level index (RoTLI)

Community-level analyses can also be used for temporal monitoring, biodiversity surveys or for the detection of invasive species. Target taxa can include any species living in a waterway. Bulk samples can be collected by field personnel in the usual manner and up to one million individuals can be processed in a single sequencing run. The approach can result in considerable cost-savings relative to traditional morphological approaches for routine monitoring work. Future developments are also likely to allow for the quantification of species within such communities.

Species detection using eDNA

Traditionally, determining the presence of a species in a given habitat is achieved either through observation (e.g. spotlighting for fish) or through direct collection. This can be problematic for species which are difficult to observe or are found in low densities (e.g. recent introductions).

However, species can also be detected within a waterway by detecting the DNA that they have shed into the environment. To date the technique has been used to detect New Zealand native and exotic fish species (Figure 4) as well as waterfowl.

By integrating NGS techniques with molecular identification, the ability to detect or monitor native and exotic species in a range of habitats can become much more automated. These NGS developments and refinements of eDNA methods are set to revolutionise future biological monitoring of freshwaters

Figure 4. Koi carp (left) and giant kokopu (right) caught in the Waikato River have also been detected using eDNA techniques.