Pest Fish Detection Using Environmental DNA – Fact Sheet

Linking lake restoration with end users for positive environmental outcomes

LERNZ
Lake Ecosystem Restoration New Zealand

Use of DNA in Pest Fish Detection

Molecular tools using DNA sequencing can improve pest fish management by ensuring accurate identification of fish, especially larval fish, without the need for specialist taxonomic knowledge. DNA is made of four chemicals; guanine (G), adenine (A), thymine (T), or cytosine (C), joined together as a string (Figure 1). The order of the chemicals is unique to each species and can be used as a DNA "barcode" to identify organisms. It is relatively simple to obtain DNA sequences for a reference gene such as the widely accepted "barcode gene" cytochrome C oxidase subunit 1, and compare the sequence to a voucher specimen sequence in genetic databases such as GenBank and the Barcode of Life database BOLD.

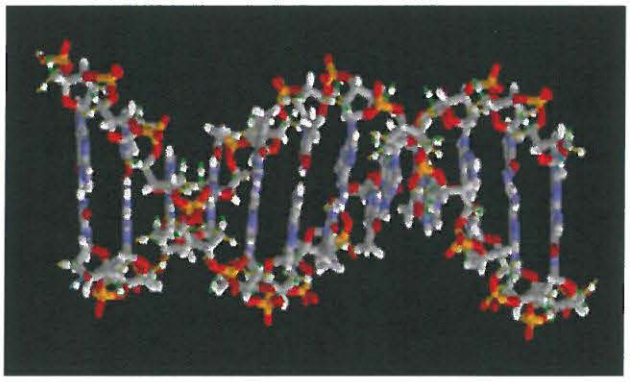

Figure 1. A short fragment of DNA. DNA is arranged as a "ladder" that is twisted. The four DNA chemicals are the "rungs" of the ladder. The order of the rungs is unique to each individual.

Environmental DNA

DNA sequences can be obtained from fish specimens of any age including mutilated and decomposing specimens. Fish also continuously shed tissue such as mucus and scales into the water. This material contains DNA and can be collected from the water and read by scientists to identify the animals present in the water (Figure 2). This source of DNA is know as environmental DNA or eDNA.

LERNZ has been developing a genetic toolbox that identifies pest fish from their DNA (Figure 3). Pest fish monitoring using eDNA has several advantages as it reduces the incidence of non-target species bycatch and it will be considerably cheaper than traditional methods of monitoring such as electrofishing and netting.

Figure 2. Sampling eDNA with customised filtering equipment developed by LERNZ researchers.

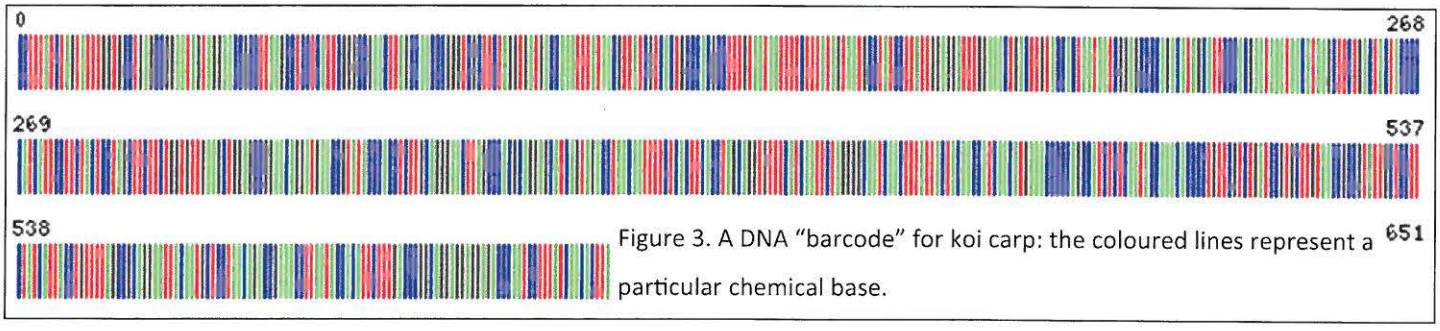

Figure 3. A DNA "barcode" for koi carp: the coloured lines represent a particular chemical base.

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DNA Capture

eDNA is collected by filtering water through glass fibre filters which bind DNA based on its electrical charge. The DNA is removed from the filters by smashing the filters with garnet beads forming a slurry of filter, beads, cell debris and DNA.

The slurry is then centrifuged forcing the debris and beads to the bottom and leaving the DNA in the liquid on top. The liquid is removed and washed through a silica filter that binds to the DNA. The filter is washed several times with various buffers and solutions to remove unwanted proteins and lipids, and the DNA is then washed from the filter into a clean bottle ready for analysis.

DNA Analysis

The next step is to test for the presence or absence of DNA from the target fish (i.e., the pest fish). The tests use enzymes to amplify DNA from the target animal, if it is present. The tests work by adding a "primer", a short piece of DNA that only binds to the target DNA if it is present. Once the primer binds to the target DNA, the enzyme copies the target DNA up to detectable amounts. If the target is not present the primer wont bind to anything and the enzyme can't amplify any DNA.

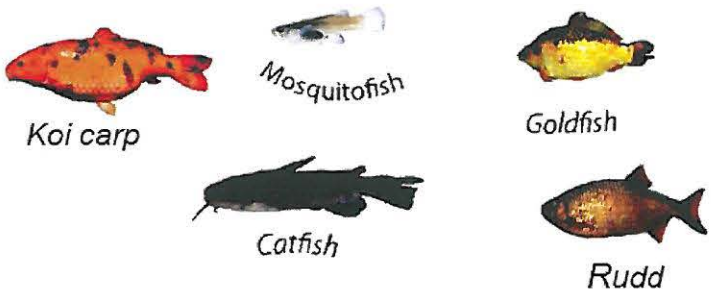

Figure 4. LERNZ has developed eDNA tests to detect these pest fish species found in New Zealand lakes.

Range of Species

The amount of DNA amplification is measured using probes with fluorescent chemicals that bind to the amplified DNA and produce a detectable signal when exposed to light of the appropriate wavelength.

LERNZ researchers have developed and validated tests to detect the presence of seven species of pest fish. We now have tests that can detect catfish, (*Ameiurus nebulosus)*; gambusia (mosquitofish), *Gambusia affinis*; goldfish, *Carassius auratus*; koi carp, *Cyprinus carpio* (Figure 4); perch *Perca fluviatilis*; rudd, *Scardinius erythrophthalmus* and tench, *Tinca tinca.*

Validation

LERNZ researchers have tested the assay for koi carp in three Waikato lakes and found that the test could detect high and low abundances of koi carp, and also did not detect fish in a lake known not to contain koi carp (Figure 5).

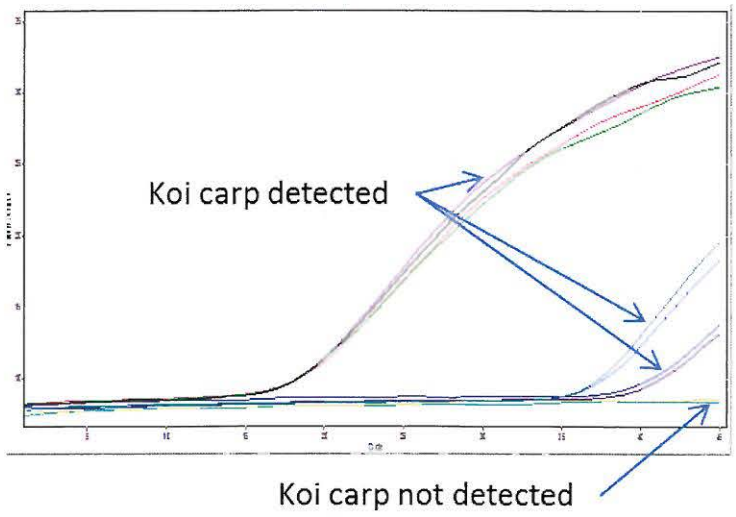

Figure 5. Detection of pest fish using genetic probes carrying fluorescent "labels" that quantify DNA amplification in real time.