A Molecular-Based Assessment Tool for Characterising New Zealand Freshwater Zooplankton Communities

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by

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EXECUTIVE SUMMARY

The ability to adequately assess ecosystem health is essential for informed resource management. Freshwater zooplankton respond rapidly to environmental changes in pest fish populations and nutrient loads and can therefore be used to monitor ecosystem health and provide a surrogate for lake biodiversity. The Zooplankton Molecular-Based Assessment (ZooMBA) described here is a technique for assessing zooplankton communities using short fragments of DNA sequences and a recently developed, online database of reference sequences ("DNA barcodes"). Users can collect their own zooplankton samples using standard collection techniques and either pre-process samples or send samples directly to appropriate laboratory facilities for molecular analyses. Resulting data can then be used to provide accurate species inventories, or cumulatively, can be used to compute indices of lake trophic status (e.g. rotifer Trophic Level Index).

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1.0 INTRODUCTION

Zooplankton are key components of freshwater food webs and respond quickly to environmental changes (Ferdous & Muktadir, 2009; Hanazato & Yasuno, 1989; Kirk, 1991). As such, changes in the composition of zooplankton communities can be used as an indication of ecosystem health and function, and as a surrogate for overall lake biodiversity. For example, zooplankton communities can be affected by the introduction of pest fish such as carp, perch and *Gambusia*. Such species can rapidly deplete populations of large grazing zooplankton (i.e. copepod and cladoceran crustaceans) through both predation and resource competition (Attayde & Hansson, 2001; Hurlbert et al., 1972; Jeppesen et al., 1997). Furthermore, the resuspension of sediments in the water column caused by benthic-feeding fish can interfere with the ability of filter feeders such as cladocerans to obtain phytoplankton (Kirk, 1991; Kirk & Gilbert, 1990). This can lead to a proliferation of algae in the water column.

By integrating the effect of multiple variables over time, zooplankton can provide a holistic view of the overall health of the ecosystem (Bianchi et al., 2003; Gannon & Stemberger, 1978; Lougheed & Chow-Fraser, 2002). In particular, smaller zooplankton, such as the rotifers, can have species-specific tolerances to various trophic states and therefore be used as indicators of water quality. In New Zealand, the rotifer-inferred Trophic Level Index (rotifer TLI; Duggan et al. 2001) has been used by both the Waikato and Auckland regional councils as a means of assessing water quality in North Island lakes (Auckland Regional Council, 2005; Duggan, 2007, 2008). The rotifer TLI incorporates the varying sensitivities of different rotifer species to environmental parameters as a surrogate for the water quality measurements needed to assess the New Zealand Trophic Level Index (TLI) (Burns et al., 1999).

However, the accurate identification of zooplankton to a species level using morphology alone is both difficult and time consuming. To allow for a more simplified and rapid approach for zooplankton identification, we have employed a molecular approach; the Zooplankton Molecular-Based Assessment (ZooMBA). The ZooMBA utilizes ‘DNA barcodes’; short, standardised segments of DNA, to differentiate between animals to a species level (Hebert et al., 2003). Comparing DNA barcodes from unknown zooplankton against a reference database allows for the rapid and accurate identification of taxa.

In this report we provide details on using the molecular approach as a tool for the identification of New Zealand freshwater zooplankton species. We discuss applications of the technique for assessing species diversity, detecting invasive species and generating community-level data from environmental samples including a molecular version of the rotifer TLI.

2.0 METHODS

2.1 Building the DNA Barcode Reference Database

2.1.1 Collection of specimens

Zooplankton were collected from a variety of freshwater habitats, primarily in the North Island of New Zealand, between 2006 and 2013 (Figure 1) and from south-eastern Australia between 2006 and 2011 (Figure 2). These latter samples were added to the database to enable identification of any species that may have been introduced from Australia. Habitats sampled included both constructed and natural lakes, small ponds, wetlands, aquatic plants (bromeliads) and small temporary waters. Zooplankton were collected with nets of varying mesh sizes (40 µm to 75 µm), generally pulled through the water from the shore, or by running a small sieve (75 µm) through the water in small ponds. A turkey baster was used to collect water from difficult to reach places, such as inside bromeliads, which was also passed through a fine mesh. Samples were transferred from the sampling device to plastic honey pots or similar containers and 95% ethanol was added to preserve samples. On return to the laboratory, samples were refrigerated at 4°C until needed for further processing.

Samples were identified under a dissecting or compound microscope at magnifications between 40 and 400 x, using the keys of Shiel (1995) and Voigt and Koste (1978) for rotifers and Chapman et al. (2011) for crustaceans. The identification of calanoid copepods involved dissection of the male 5th leg, which was placed on a glass slide and viewed under a compound microscope at 100 x magnification or greater, as needed. Cyclopoid copepod identification was based primarily on the 5th leg of dissected females. The identification of rotifers was based on body morphology, or of trophi (tiny calcified jaw like structures) morphology following erosion of the soft tissues with sodium hypochlorite. Cladocerans were identified based on body morphology. Selected specimens were then photographed and processed for genetic analysis.

Figure 1: Sampling locations of zooplankton from New Zealand

Figure 2: Sampling locations of zooplankton from eastern Australia and Tasmania

2.1.2 Genetic analyses

A mixture of 10 µL of extraction solution and 2.5 µL of tissue preparation solution (Extract and Amp, Tissue PCR Kit, Signma-Aldrich, St. Louis, MO, USA) was added to 0.6 ml snap-top PCR tubes (Porex Bio Products Group, Fairburn, GA, USA) each containing an individual (whole body) representative of each morpho-species. The tubes were centrifuged for approximately 5 seconds to ensure the organism was drawn to the bottom of the tube and consequently the reagents covered the organism. The tubes were then left at room temperature for 3 hours in the dark (to avoid exposure to UV light). After this time, tubes were incubated in an Eppendorf Thermocycler at 95˚C for 3 minutes to stop the reaction. Following this, 10 µL of neutralising solution was added to each tube and mixed by vortexing. DNA-extracted samples were refrigerated at 4˚C.

Polymerase Chain Reactions (PCR) were used to amplify the mitochondrial cytochrome *c* oxidase subunit I (COI) gene from each extraction. A master mix containing 5.5 µL of iNtRON® PCR Master Mix (iNtRON Biotechnology Inc., Korea), 0.5 µL of COI primers (LCO1490 GGTCAACAAATCATAAAGATATTGG and HCO2198 TAAACTTCAGGGTGACCAAAAAATCA or Lep F1 ATTCAACCAATCATAAAGATATTGG and Lep R1 TAAACTTCTGGATGTCCAAAAAATCA) and 5.5 µL of deionised (Milli –Q) water per sample was created and then aliquoted into PCR tubes (0.2 mL) using a 200 µL pipette. 1 µL of extraction solution from each sample was then added into each one of the tubes. To check for contamination, negative controls using deionised water as the template were run alongside the DNA extracts. Reaction conditions varied slightly for different taxa, however, a typical reaction would include an initial denaturing step at 94˚C for five minutes, followed by 35 cycles of 94˚C for one minute, 52˚C for one minute and 30 seconds and 72˚C for one minute, with a final extension step of 72˚C for 5 minutes. For problematic samples, (i.e., samples where no visible DNA band could be seen after electrophoresis) the annealing temperature was lowered as low as 49.1°C to encourage the primers to bind to template DNA.

A 3 µL subsample from each PCR product was pipetted into comb set wells on a 2% agarose gel containing SYBR® Safe DNA Gel Stain (Life Technologies Corporation, USA, 1 µL per 10 µL gel at 10000 x concentration). Gels were set in TBE buffer and run at 70 volts for 30 minutes. Products were visualised under UV light using a MultiImage™ light cabinet (Alpha Innotech/ProteinSimple, CA, USA).

PCR products were purified using Exo-SAP IT® (Affymetrix, USB, Cleveland, USA) to remove primers and any unincorporated dNTPs. A master mix containing 0.2 µL of ExonucleaseI (EXO), 0.1 µL of Shrimp Alkaline Phosphate (SAP) and 2.7 µL of deionised water per sample was created. 3 µL of the master mix was aliquoted using a 10 µL pipette directly into the 0.2 mL PCR tubes. PCR tubes were then incubated at 37˚C for fifteen minutes to degrade any remaining primers and nucleotides, followed by 80˚C for an additional fifteen minutes to inactivate the Exo-SAP IT® reagent. Purified PCR products were sent to the University of Waikato DNA Sequencing Facility for bidirectional sequencing on an ABI3130XL sequencer using the same primers that were used for amplification. Primer sequences were identified and trimmed and each sequence was checked for stop codons using Geneious® version 6.1.2 or GeneiousPro® version 5.4.2. All generated sequences and trace files were uploaded to the Barcode of Life Database *(www.boldsystems.org),* under the campaign WG1.7 Freshwater Biosurveillance. Barcode gap analysis was performed using the Barcode Gap Analysis algorithm on the BOLD website, using the BOLD Aligner (Amino Acid Based HMM) algorithm to align sequences.

3.0 RESULTS

3.1 The Reference Database

A total of over 480 DNA barcodes, representing 99 freshwater zooplankton species, has been added to the BOLD database. These include 50 species of rotifer, 21 species of calanoid copepod, 14 species of cladoceran, 8 species of harpacticoid copepod and 6 species of cyclopoid copepod. A complete list of the barcoded species is provided in Table 1. Analysis of all COI sequences showed that some species have high levels (>10%) of intraspecific divergence (Table 1). In contrast, the minimum interspecific divergence was 0.95%, and the mean interspecific distance between neighbouring species was 18.72% (Figure 3). However, despite the range of intra- and interspecific divergences, all taxa could be unambiguously assigned to their nominate species.

The interspecific distance between the two rotifer species *Keratella tecta* and *K. cochlearis*, represented the smallest interspecific divergence (0.95%) and the relationship between these two species is currently being examined (Collins et al., unpublished). Aside from this instance, there was >6% divergence between all other species included in the reference dataset. Consequently, there should be no ambiguity in the identification of unknown zooplankton using this database, providing the collected species are similar to those in the dataset.

Table 1: Species of New Zealand Freshwater zooplankton for which mitochondrial DNA, cytochrome *c* oxidase subunit I (COI), barcodes have been obtained.

Cladocera	
	Bosmina meridionalis
	Penilia avirostris
	Daphnia carinata
	Ceriodaphnia dubia
	Simocephalus vetulus
	Daphnia galeata
	Ilyocryptus sordidus
	Chydorus sp.
	Chydorus sphaericus
	Alona sp.
	Graptoleberis testudinaria
	Daphnia pulex
	Eodiaptomus lumholtzi
	Undescribed species (Duggan et al., unpublished.)
Calanoid Copepods	
	Sinodiaptomus valkanovi
	Gladioferens pectinatus
	Bockella symmetrical
	Bockella fluvialis
	Bockella triarticulalra
	Bockella hamata
	Bockella pseudochelae
	Bockella delicata
	Bockella montana
	Bockella propinqua
	Bockella tanea
	Bockella minuta
	Calamoecia lucasi
	Calaniecia ampulla

	Calamoecia tasmanica
	Skistodiaptomus pallidius
	Hemiboeckella
	Sulcanus conflictis
	Eodiaptomus lumholtzi
	Centropagidae sp.
	Calmoecia lucasi
Cyclopoid Copepods	
	Eucyclops cf. serrulatus
	Acanthacyclops robustus
	Mesocyclops cf. leukarti
	Paracyclops fimbriatus
	Paracyclops waiariki
	Tropocyclops prainsus
Hapacticoid Copepods	
	Phyllognathopus viguieri
	Phyllognathopus volcanicus
	Bryocamptus pgmeaus
	Elaphoidella bidens
	Elaphoidella sewelli
	Attheyella leisae
	Attheyella maorica
	Antarctobiotus triplex
Rotifers	
	Ascomorpha ovalis
	Ascomorpha sp.
	Asplanchna priodonta
	Asplanchna sieboldi
	Brachionus angularis
	Brachionus budapestanensis
	Brachionus calyciflorus
	Brachionus quadridentatus
	Collotheca sp.

	Collotheca cf. pelagica
	Conochilus unicornis
	Cupelopagis vorax
	Euchlanis cf. deflexa
	Euchlanis meneta
	Euchlanis pyriformis
	Filinia cf. terminalis
	Filinia longiseta
	Filinia novaezelandia
	Hexarthra intermedia
	Keratella cochlearis
	Keratella procurva
	Keratella tecta
	Keratella tropica
	Keratella valga
	Lecane bulla
	Lecane closterocerca
	Lecane decipiens
	Lecane hamata
	Lecane ludwigii
	Lecane luna
	Lecane lunaris
	Lepadella cf. ovalis
	Lepadella patella
	Lophocharis salpina
	Notommata pseudocerberus
	Platyais quadricornis
	Polyarthra dolichoptera
	Pompholyx sp.
	Rotaria neptunia
	Squatinella mutica
	Synchaeta grimpii
	Synchaeta oblonga

	Synchaeta pectinata
	Synchaeta sp.
	Trichocerca marina
	Trichocerca pusilla
	Trichocerca similis
	Trichocerca tenuior
	Trichotria tetractis
	Trichocerca sp.

Table 2: Mean intraspecific diversity and distance to Nearest Neighbour of barcoded rotifer TLI species. Where only one individual has been sequenced from a particular species, intraspecific variation is marked Not-Applicable (NA). Number of individuals sequenced is provided in parentheses following species name.

Species	Maximum Intraspecific COI Divergence (%)	Interspecific COI Divergence to Nearest Neighbour (%)
Polyarthra dolichoptera (5)	25.65	21.84
Conochilus unicornis (2)	0	40.08
Ascomorpha ovalis (2)	0	21.61
Lecane closterocerca (1)	NA	17.58
Lecane bulla (species complex) (7)	19.66	16.69
Synchaeta oblonga (7)	19.27	15.95
Asplanchna priodonta (11)	3.61	19.15
Synchaeta pectinata (14)	12.46	9.51
Collotheca sp. (3)	25.54	25.43
Trichotria tetractis (1)	NA	19.97
Trichocerca tenuior (2)	1.6	17.72
Trichocerca similis (species complex) (12)	32.12	26.92
Keratella cochlearis (species complex) (5)	16.71	0.95
Filinia novaezelandia (3)	0	24.6
Trichocerca pusilla (2)	0	19.14
Hexarthra intermedia (2)	0.16	30.82
Keratella procurva (6)	3.85	19.93
Asplanchna sieboldi (6)	0.31	17.49
Keratella tropica (6)	0.31	13.68
Brachionus quadridentatus (species complex) (5)	19.59	18.16
Keratella tecta (8)	0.87	0.95
Brachionus calyciflorus (species complex) (5)	10.91	15.83
Filinia longiseta (4)	0.87	42.6
Brachionus budapestanensis (1)	NA	19.2

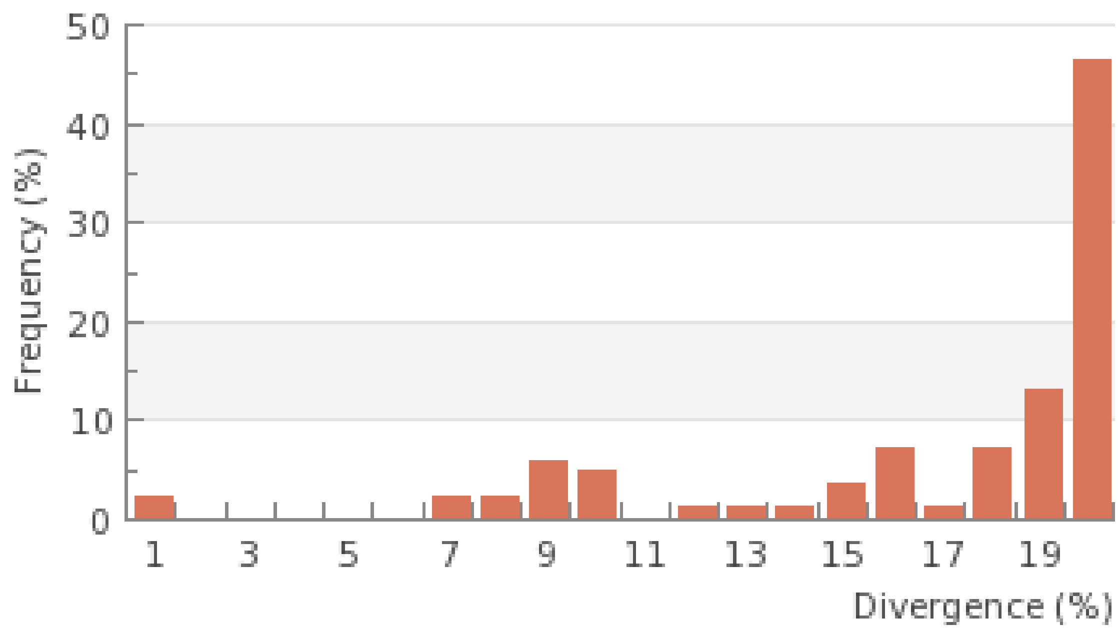

Figure 3: Genetic divergence values between “nearest neighbours” for zooplankton species used in our study

4.0 DISCUSSION

4.1 Assessing Zooplankton Communities Using DNA Barcodes (ZooMBA)

Using the DNA barcodes included in the reference library, a Zooplankton Molecular Based-Assessment (ZooMBA) can be used for the routine identification of unknown zooplankton from environmental samples. Here, individuals from habitats can be identified by comparison with the reference database and then compiled to assess community composition. The key steps involved in this process are outlined below:

4.1.1 Sample acquisition, documentation and submission

Zooplankton can be collected using existing institutional sampling methods or using standard methods such as those outlined in Chapman et al. (2011). Typically, collection involves casting a fine mesh conical net from the shore and dragging it through the water using a rope. Contents can then be transferred directly from the collection net into a plastic honey pot or similar container. Excess water should be carefully drained off, and replaced with 95% ethanol and refrigerated at 4˚C for best preservation. The use of formaldehyde or other preserving fluids (e.g. Kahles) must be avoided as this will degrade the DNA. Further it is important to keep samples out of direct sunlight as UV light degrades DNA. For shipping purposes, samples should be placed in a suitable insulated container (e.g. chilly bin) and kept cool with standard ice-packs (or similar).

Documentation required for each sample includes sampling date and location (including latitidue and longitude). Samples and documentation should be couriered to a suitable DNA Sequencing facility, such as the Pacific Barcoding Research Laboratory (University of Waikato), within 48 hours of collection.

4.1.2 Laboratory analyses

Upon arrival at the processing laboratory, samples are filtered through a sieve (40 µm mesh) to remove zooplankton. Specimens are then transferred to a petri dish filled with 95% ethanol for examination under a stereomicroscope at 4x (or higher) magnification.

Rotifers and microcrustaceans are separated and the latter are sorted into their four main orders; Cladocera, Calanoida, Cyclopoida and Harpacticoida, using simplified identification

keys (e.g. Shiel 1995; Chapman et al. 2011). Rotifers are sorted together within their phylum. Additional taxa outside these five groups should be noted, although will not usually be included in the molecular analysis.

Based on previous sampling, the selection of five representatives from the crustacean groups and 20 representatives for the rotifers are likely to provide an initial assessment of diversity using genetic analyses. However, within each of the taxonomic groups it is essential to target morphologically-distinct individuals (i.e. morpho-species) to ensure that an adequate coverage of species is obtained.

4.1.3 Genetic analyses

Extraction of DNA, COI amplification, and sequencing of representative individuals is completed as per the methods used in creating the reference database and presented under the Methods section of this report. In most cases, PCR products are sequenced in a single direction only as this will usually provide sufficient information for a species designation and reduce costs. The resulting COI sequences are then searched against the reference database on BOLD using the available search engine to provide information on the identity of each specimen.

All users can obtain a personal account on BOLD by visiting the website www.boldsystems.org and following the on-screen instructions. Alternatively, there is also a public search function available which allows for the querying of sequences or taxonomic data against the reference database.

4.2 The Molecular Rotifer TLI (MoRTLI)

Of the 44 species used in the rotifer TLI (Duggan et al., 2001) 24 have been barcoded and are now included in the BOLD reference database. Additional species will be collected and can be added to the database to fill in gaps for key taxa as required. The existing species in the database represent the most common North Island, New Zealand species and cover the entire tolerance range presented by Duggan et al. (2001). A list of the currently available species and their susceptibility index scores is provided in Table 3. Using the molecular data generated using the ZooMBA, the rotifer TLI can then be calculated by matching identified rotifer species to their TLI optimum and TLI tolerance scores as per Duggan et al. (2001).

Table 3: Weighted average (WA) optima and tolerance data for TLI for abundant North Island rotifer species for which COI barcodes have been obtained. Species are ordered by TLI optima.

Species	TLI optimum	TLI tolerance
Polyarthra dolichoptera	3.44	1.36
Conochilus unicornis	3.80	1.12
Ascomorpha ovalis	3.96	0.87
Lecane closterocerca	4.14	0.60
Lecane bulla	4.17	0.74
Synchaeta oblonga	4.39	1.29
Asplanchna priodonta	4.40	1.39
Synchaeta pectinata	4.50	0.98
Collotheca sp.	4.52	1.66
Trichotria tetractis	4.69	0.16
Trichocerca tenuior	4.70	0.12
Trichocerca similis	4.77	0.90
Keratella cochlearis	4.83	1.19
Filinia novaezelandia	4.84	1.48
Trichocerca pusilla	4.86	0.79
Hexarthra intermedia	5.09	1.48
Keratella procurva	5.23	1.11
Asplanchna sieboldi	5.62	1.31
Keratella tropica	5.85	1.09
Brachionus quadridentatus	5.92	0.97
Keratella tecta	6.02	1.11
Brachionus calyciflorus	6.16	0.42
Filinia longiseta	6.40	0.72
Brachionus budapestanensis	6.53	0.45

4.3 Applications

The molecular-based identification approach for zooplankton (ZooMBA) that we describe here provides a capacity for the fast and accurate identification of specimens without the routine need for a highly-skilled taxonomic expert. For the sequences currently on the Barcode of Life Datasystems (BOLD) database, we were able to successfully differentiate among the currently recognised species on the basis of their COI sequences. The high intraspecific divergences we observed in some instances were likely due to the presence of species complexes, or morphologically 'cryptic species'. However, we caution that this could also be the result of out-dated taxonomy and/or cross-contamination of samples resulting from the amplification of non-target DNA (e.g. stomach contents). Regardless, we were able to unambiguously assign all individuals to their appropriate species designations. By applying these data to unknown communities the molecular-based assessment (ZooMBA) can provide accurate assessments of species' composition. We anticipate the reduced cost of zooplankton community characterisation coupled with a streamlined and easy-to-use, standardised method will make the molecular-based approach a useful tool for routine water quality monitoring required by regulatory bodies. Further uses for a molecular-based assessment include the accurate assessment of population and species-level diversity as well as biosecurity applications such as the detection of non-indigenous or invasive species.

4.3.1 Assessing variability within and among species

Molecular approaches can assist in the rapid identification of cryptic or "new" species that may be missed by traditional, morphological approaches due to morphological conservatism. Such species can be revealed by the subtle differences in DNA sequences at the COI gene locus (Hebert et al., 2004; Gutiérrez-Aguirre et al., 2014). Three potential cryptic species of freshwater zooplankton have already been identified in the assembly of our DNA barcode reference library. One of these species is currently undergoing formal description as a new species (I.C. Duggan et al., unpublished), while the remaining two await a more detailed examination. The recognition of cryptic species can be important from both a conservation perspective as well as the accurate interpretation of community-based changes, as cryptic species are likely to respond differently to similar environmental stressors (Hogg et al., 1998; Rocha-Olivares et al., 2004; Feckler et al., 2014).

The gap between intraspecific and interspecific variation of the COI gene (Hebert et al., 2003) – referred to as the 'barcoding gap' – can be used as a proxy for species diversity when taxonomic data are unavailable or limited. Such closely related sequences, or Molecular Operational Taxonomic Units (MOTUs), can be identified on BOLD by Barcode Index Numbers (BINS) which are assigned to clusters of closely related sequences (Ratnasingham & Hebert, 2013). Knox et al. (2012) used MOTUs derived from COI sequences to act as a surrogate for species diversity in the deep sea amphipods of New Zealand – a taxonomically understudied group. By combining these data with biogeographic information, inferences could be made about the relationship between amphipod diversity and habitat heterogeneity. As a barcoding gap appears to be present between species of New Zealand freshwater zooplankton, a similar approach could be used for analysis of COI gene sequences from freshwater zooplankton communities when species present are undescribed or have not yet been added to the BOLD database.

Molecular data can also be useful in assessing intraspecific diversity, as individuals from geographically distinct populations will often have subtle differences in COI sequences (haplotypes), typically the result of divergent evolution. Analysis of such haplotypes can reveal information about gene flow – or lack thereof – between populations. Understanding patterns of gene flow and intraspecific diversity can provide vital information for conservation biologists (Arif & Khan, 2009; Hardy et al., 2011; Ludwig et al., 2003).

4.3.2 Biosecurity

Molecular-based identification will provide a valuable tool for assessing biosecurity threats in New Zealand. The advantages of using DNA barcoding within the New Zealand context have already been highlighted by Armstrong & Ball (2005) who conducted two case studies; one on exotic species of tussock moth, the other on a fruit fly intercepted at a New Zealand border security checkpoint. In these cases, DNA barcoding allowed previously unknown specimens to be identified to likely genus and species level; important information as invasion risk can vary markedly between closely related species (Armstrong & Ball, 2005). Additionally, larvae of fruit flies could be identified using molecular data, something very difficult to do morphologically (Armstrong & Ball, 2005). In this manner, comparison of DNA barcodes from the BOLD database could potentially aid in the identification of unknown zooplankton specimens stopped at the border (e.g. aquarium fish trade).

Analysis of DNA barcodes from introduced species can also reveal vital information about the country of origin of the species and potential invasion vectors. Recently Makino et al. (2010) traced the origin of the recent invader, *Sinodiaptomus valkonovi*, a calanoid copepod back to the north-eastern region of Japan using haplotype networking of COI gene sequences. Similarly, Duggan et al. (2012) traced the exotic cladoceran *Daphnia pulex* back to North America. Such information is invaluable in assessing the risk of specific invasion vectors, and consequently focusing preventative efforts on those pathways which pose the most risk.

4.4 The Future

There are several species of New Zealand zooplankton yet to be barcoded, particularly for freshwater rotifers. However, the reference database can be continually updated as new specimens are obtained. When species are analysed that are not currently in the BOLD database an exact species-level identification will not be possible, although comparison against international records will likely give a match to the higher taxonomic level possible, such as order. For any currently undescribed or cryptic species, a Barcode Index Number (BIN) will be assigned by BOLD to allow for similar, unidentified sequences to be grouped together as a Molecular Operational Taxonomic Unit (MOTU).

The molecular rotifer TLI (MoRTLI) presented in this report contains 24 of the 44 species included in the rotifer TLI. However, these species cover the entire susceptibility range presented by Duggan et al. (2001) and can, therefore, be used in assessing the trophic state of North Island Lakes. We anticipate that ongoing sampling will further enhance the reference database.

We expect the capabilities of the ZooMBA to grow over time with technological advancements. Sequencing technology is advancing rapidly, with sequencing costs dropping at an unprecedented rate (Shendure & Ji, 2008). Consequently, the cost of using a molecular-based approach such as ZooMBA is likely to decrease over time. The ZooMBA is currently focused primarily on describing the species diversity of zooplankton communities. However, future developments are also likely to allow for the quantification of species within such communities. Techniques such as quantitative PCR (qPCR) have proved useful in the estimation of koi carp (*Cyprinus carpio)* biomass (Takahara et al., 2012) and amphibian population abundance (Lodge et al., 2012) in aquatic ecosystems. Accordingly, qPCR-based

biomass quantification could be applied to the COI sequences of freshwater zooplankton and subsequently allow for the molecular quantification of abundant species.

Finally, the application of Next Generation Sequencing (NGS) platforms to environmental samples has the potential to revolutionise the efficiency of molecular-based approaches. NGS platforms, such as the Illumina MiSec 2000 and the Ion Torrent (Life Technologies), allow for the metabarcoding of DNA directly from environmental samples (Baird & Hajibabaei, 2012; Metzker, 2010; Quail et al., 2012). It is therefore possible that an entire freshwater zooplankton community could be characterised directly from an environmental sample. NGS techniques have already been applied to marine zooplankton community samples with some success (Lindeque et al., 2013; Machida et al., 2009). By integrating NGS techniques into our molecular approach, the process of characterising freshwater zooplankton communities could become more automated. In this case, zooplankton samples could simply be collected, stored in ethanol as a bulk sample, and then sent to a sequencing lab for NGS sequencing. The resulting sequences could then be compared against the BOLD reference database to gain species level identification. Consequently, once a complete reference database is created there would be much less need for morphological identification of samples. The potential of applying NGS approaches for the New Zealand zooplankton is currently being investigated at the University of Waikato as part of a large-scale pest fish study at the Hamilton Zoo (Woods et al. unpublished. data).

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