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Taihoro Nukurangi

Collection, DNA sequencing and species verification of *Didemnum* sp. from Picton Harbour and surrounding environment

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Final Research Report for
Ministry of Fisheries Research Project ZBS2001/08B
Objectives 1–4 combined

National Institute of Water and Atmospheric Research

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Report Title: Collection, DNA sequencing and species verification of *Didemnum* sp. from Picton Harbour and surrounding environment

Authors: M. Page, V. Webb

1. Date: 18 June 2002

2. Contractor: National Institute of Water & Atmospheric Research (NIWA)

3. Project Title: Collection, genetic sequencing and species verification of *Didemnum* sp. from Picton Harbour and surrounding environment

4. Project Code: ZBS2001/08B

5. Project Leader: M.J. Page

8. Duration of Project:

Start date: 30 May 2002
Completion date: 30 June 2002

7. Executive Summary

Ten *Didemnum* samples were collected from natural and artificial substrates at sites in Pelorus and Queen Charlotte Sounds to determine the genetic and taxonomic relatedness of specimens to a potentially invasive tunicate identified from Whangamata. Two samples collected from the underside and the seafloor below the barge 'Steel Mariner' moored near Picton had the same 18S rRNA gene sequence as the Whangamata species. Of the remaining didemnids, one had identical sequences to the tunicate collected from Mahanga Bay (Wellington Harbour), three were unique species; three were related on the basis of an archeal bacterium 18S sequence; and one contained unusable sequence data. None of these organisms were the same as the Whangamata species. The results of this preliminary survey suggest that distribution of the 'Whangamata' didemnid is localized to the "Steel Mariner' in the Marlborough Sounds.

8. Objectives

- Collect and photograph *Didemnum* sp. from the vessel 'Steel Mariner' located in Picton Harbour, by 31 May 2002.
- Collect and photograph samples from below the vessel and from natural substrates within the Marlborough Sounds, by 31 May 2002.
- Dispatch samples and photographic vouchers to Dr Patricia Mather, Queensland Museum for identification by 31 May 2002.
- Undertake DNA sequencing to identify the samples and compare the sequence results to previous collections.

9. Background

A compound tunicate *Didemnum* sp. was first identified fouling concrete wharf piles in Whangamata in November 2001. Aggressive overgrowth of associated wharf pile fauna combined with unusual morphology of this organism led to concern that it may be an introduced and invasive species. The Whangamata specimen and subsequent collections of species from Wellington and Nelson harbours with the same outward morphology as the Whangamata specimen were considered by Dr Patricia Mather (Museum of Queensland) to be a species of *Didemnum* endemic to New Zealand. However, the origin of this species is uncertain, as it was new to Dr Mather, has not been identified to species, nor checked against other non-New Zealand species using DNA sequencing techniques.

A new population has been identified fouling the barge 'Steel Mariner' in Picton harbour. Renewed concern that this organism may be an invasive species that could threaten New Zealand's mariculture industry has lead to a request from the Ministry of Fisheries Biosecurity Unit to NIWA to confirm the genetic identity and to collect sub-samples for taxonomic verification of the Picton species.

Although the title of this project report implies that the didemnid would be identified to species, this was not the actual intent, and has not been possible. The MFish requirement is that the species be compared with other didemnid samples to test for similarity or difference, using DNA sequencing.

10. Methods

Didemnum sp. colonies were collected from natural and artificial substrates from 7 sites in Marborough Sounds on 8–10 May 2002. Specimens were photographed in-situ and sampled for genetic and taxonomic analysis. Samples were collected from beneath the barge 'Steel Mariner' and from the seafloor directly below the vessel. The remaining organisms were collected from sites in Queen Charlotte Sound and from Capsize Point in Pelorus Sound (Fig. 1). Samples were freshly frozen for DNA extraction, amplification and sequencing, and the balance of each sample narcotised in $MgCl_2$ for two hours before preservation in 4% formalin. Taxonomic vouchers were sent to Dr P. Mather at Queeensland Museum for description and identification. Sequence data was compared with existing information on a *Didemnum* sp. collected

from Whangamata in December 2001, and a similar organism collected from Mahanga Bay, Wellington Harbour.

DNA extraction, amplification and sequencing the 18S rRNA gene:
Sample tunicates were split open and thoroughly rinsed with sterile, filtered sea water to remove any epiphytes and gut contents that may confound the analysis. Total DNA was extracted from approximately 0.5g of tissue using BIO 101 FastDNA spin kit (BIO 101). The polymerase chain reaction (PCR) was used to amplify the 18S rRNA gene using 50ng of DNA and conserved primers to the 18S rRNA gene. The expected 1800bp product was electrophoresed through 1% Agarose and visualised using ethidium bromide staining. The PCR products were purified using the PCR Product Purification Kit (Qaigen) according to manufacturers instructions. Purified products were quantified using the DyNA Quant 200 system (Hoefer). The PCR products of the 10 samples were submitted for sequencing. Sequences were aligned using the sequence analysis programme BioEdit and Clustal W programme (within BioEdit) and determined to be identical or unique. Sequences were compared to the compilation of 18S rRNA gene sequences available in databases using NCIB/BLAST (within BioEdit) to determine highest similarity to GeneBank and EMBL database sequences.

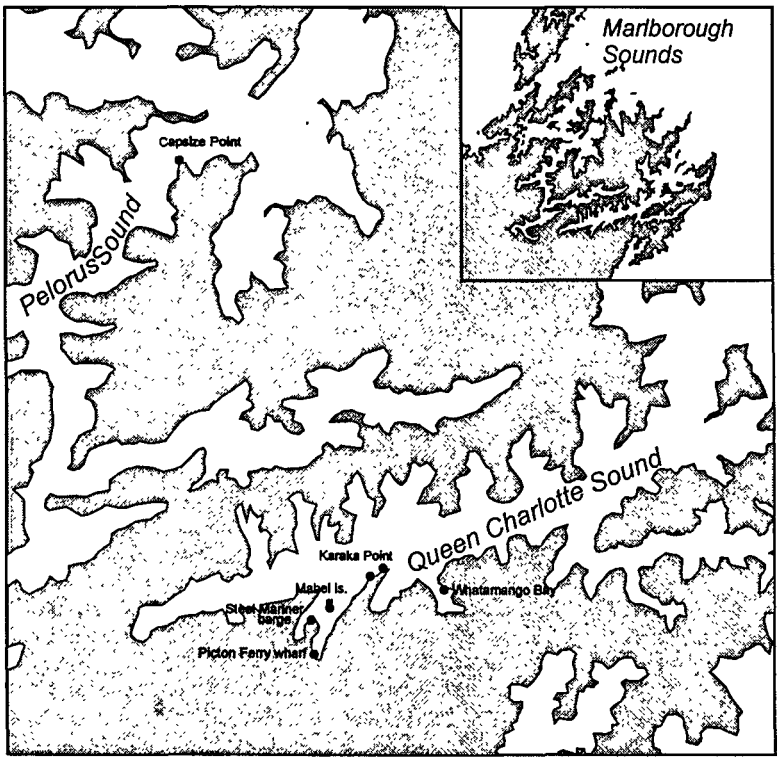


Figure 1. Map of *Didemnum* sp. sample collection sites in the Marlborough Sounds

11. Results

11.1 Collection

A total of 10 collections were made from sites in the Marlborough Sounds for DNA analysis (Table 1). Didemnids collected from natural and artificial substrates other than the 'Steel Mariner' were cream in colour and generally formed thin encrusting colonies. These had a lobate appearance when overgrowing red algae and bryozoans (e.g. samples 2 & 9, Appendix 1). In contrast, 'Steel Mariner' didemnids formed large discrete gold-coloured colonies with long drooping tendrils, often over 2 m long (sample 3, Appendix 1). Colonies of this didemnid were common, overgrowing the benthos such as mussels (Appendix 1, sample 4) and other sessile organisms directly below the barge. Colonization of the seafloor was concentrated directly below the barge and a 3–5m^2 area around the periphery of the vessel. No *Didemnum* was observed elsewhere in the cove where the barge was moored.

11.2 Gene sequencing

Comparison of DNA sequence data from original reference collections of *Didemnum* from Whangamata and Mahanga Bay, Wellington Harbour show that the two species are not the same. However, samples 3 and 4 collected from the 'Steel Mariner' barge in this survey had sequence data identical to the 'Whangamata' *Didemnum* sp. (Table 1). These were easy to sequence and gave good clean sequence data.

The didemnid sample 9 sequence was identical to the Mahanga Bay tunicate sequence, whereas samples 2, 5 & 6 appear to be unique species not related to either reference organisms. Sequences from didemnids 7, 8 &10 were identical, however, the sequence was not a tunicate 18S sequence. These sequence data were found to be approximately 99% identical to the 18S rRNA gene of some marine archaeal bacteria. The best explanation is that the archaeal bacteria are symbiotic with this particular species of tunicate and were preferentially amplified. Archaea are known to be symbiotic with a number of organisms. The sequence was very clean and it is unlikely that these didemnids are the same as the Whangamata tunicate as several previous amplifications from the Whangamata organism showed no sign of symbiont DNA.

Table 1. Location of didemnid collection sites and summary genetic sequence analysis.

Sample #	Location	Site	Depth	Substratum	Sequence
1	Pelorus Sound	Capsize Pt.	10	Buoy	Unusable sequence data
2	Pelorus Sound	Capsize Pt.	10	Reef wall	Unique
3	Queen Charlotte	Steel Mariner	2	Beneath barge	Identical to Whangamata
4	Queen Charlotte	Steel Mariner	6	Below barge	Identical to Whangamata
5	Queen Charlotte	Whatamango Bay	2	Cobble	Unique
6	Queen Charlotte	Karaka Pt	3	Concrete mooring block	Unique
7	Queen Charlotte	Karaka Pt. – Jetty	2	Wooden pile	7, 8 and 10 identical to Archeal bacteria seq
8	Queen Charlotte	Mabel Island	7	Bottle	7, 8 and 10 identical to Archeal bacteria seq
9	Queen Charlotte	Picton ferry wharf – west	3	Concrete pile	Identical to Mahunga Bay
10	Queen Charlotte	Picton ferry wharf – east		Concrete pile	7, 8 and 10 identical to Archaeal bacteria seq

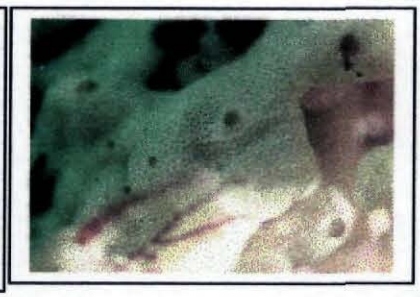
12. Conclusions

The didemnid tunicate fouling the barge 'Steel Mariner' and seafloor below the vessel is the same species as the organism fouling concrete wharf piles in Whangamata harbour in December 2001. None of the other didemnids of similar morphology collected from artificial and natural substrates in the vicinity of the barge and in Pelorus Sound were the same species. This preliminary survey suggests that the distribution of the 'Whangamata didemnid' in the Marlborough Sounds is localized to the 'Steel Mariner' and the seafloor in the immediate vicinity of the barge. The organism was observed to foul mussels on the seafloor, however, it was beyond the scope of this survey to determine if shellfish mortality occurred as a result of overgrowth. Overgrowth however, is likely to interfere with filtration mechanisms of mussels.

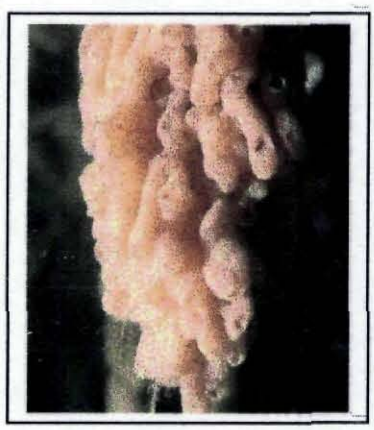
We cannot state with any certainty whether the "Whangamata didemnid" is in fact endemic to New Zealand waters, or a non-indigenous invasive species. This question would need to be addressed by comparison to its DNA with similar overseas species.

Appendix 1. Underwater photographs of didemnids collected in the Marlborough Sounds.

Sample 1

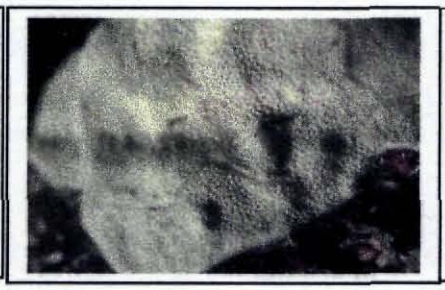


Sample 2

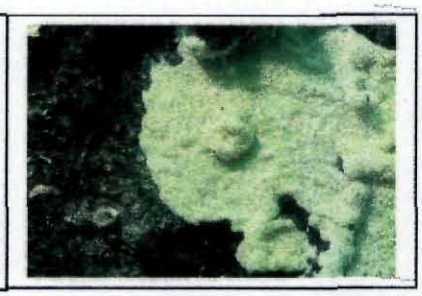


Sample 3

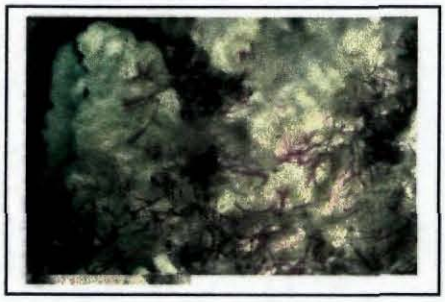
Sample 4



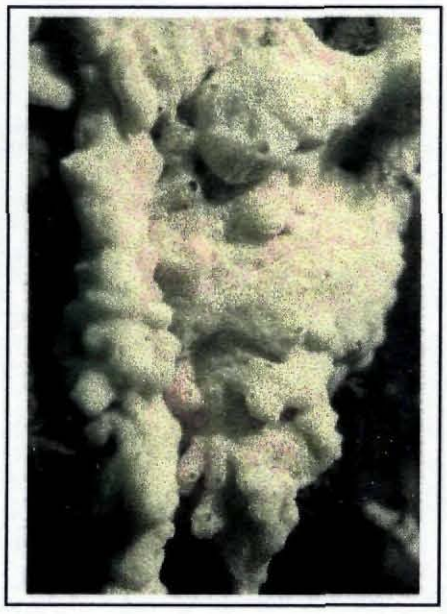
Sample 5



Sample 6



Sample 7



Sample 9