Geographic distribution of *Pfiesteria* spp. (Pfiesteriaceae) in Tasman Bay and Canterbury, New Zealand

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Abstract:
The fish-killing heterotrophic dinoflagellate species Pfiesteria piscicida and P. shumwayae (also Pseudopfiesteria shumwayae) were present throughout New Zealand and were residents of Tasman Bay's well-flushed estuaries and Canterbury's brackish lakes, as determined by polymerase chain reaction-based detection assays. The two species occurred in a wide range of salinities and temperatures, although detection was restricted seasonally from spring through to autumn, except for one incidence in the shallow waters of Wairewa/Lake Forsyth, Canterbury, in the winter of 2003. The DNA sequencing data indicated that P. shumwayae may encompass a suite of genetically closely related species. The presence of Pfiesteria in New Zealand is not considered an immediate risk to fish or human health given the current low-to-moderate nutrient concentrations in New Zealand's estuaries and brackish lakes. However, increases in nutrient loadings could pose a risk, as has occurred in eastern United States estuaries.
Keywords: Pfiesteria; dinoflagellate; estuarine; polymerase chain reaction (PCR)

Article:

INTRODUCTION

Pfiesteria shumwayae Glasgow et Burkholder (Glasgow et al. 2001a) (also Pseudopfiesteria shumwayae (Glasgow et Burkholder) Litaker Mason Shields et Tester) (Litaker et al. 2005) and the closely related P. piscicida Steidinger et Burkholder (Steidinger et al. 1996) have been detected in New Zealand sediment and seawater samples by species specific PCR-based DNA probes (targeted at ribosomal DNA). Pfiesteria shumwayae has been detected in water and sediment samples collected from Kaipara Harbour (Northland), Golden and Tasman Bays (Nelson), Havelock (Marlborough), Wairewa/Lake Forsyth, Te Waihora/Lake Ellesmere and Muriwai/Coopers Lagoon (Canterbury), and the New River Estuary (Southland); one strain isolated from Tasman Bay sediments was tested for ichthyotoxicity and proved positive (Rhodes et al. 2002).

Pfiesteria piscicida was detected recently in sediment samples from estuaries at Ohiwa and New Plymouth (North Island) and from Waimea Estuary (Tasman Bay) (Rublee et al. 2004).

Pfiesteria Steidinger et Burkholder (Dinamoebales; Pfiesteriaceae) is now known to be distributed worldwide; P. piscicida and P. shumwayae have been detected in samples from 15 and 12 countries, respectively (Rublee et al. 2004). Distribution by ballast tank water is a possible cause of the global distribution, and ballast water collected from vessels arriving at Chesapeake Bay (United States east coast) and residual ballast material from vessels arriving at the Great Lakes, United States, contained both species, as detected by PCR-based methods (Doblin et al. 2004).

Pfiesteria is a heterotrophic dinoflagellate and P. piscicida has been linked to neurotoxic effects in humans (Glasgow et al. 1995; Grattan et al. 1998). Fish kills by Pfiesteria in the United States have mostly occurred in highly eutrophic estuaries (Burkholder et al. 1992; Burkholder & Glasgow 1997; Glasgow et al. 2001b; Samet

et al. 2001). Most New Zealand estuaries are comparatively well flushed with little accumulation of nutrients in sediments (Robertson et al. 2002), which suggests a low risk of fish kills owing to Pfiesteria. However, Kaipara Harbour, Northland, New Zealand, has slightly elevated total nitrogen (N), muddy sediments and subtropical temperatures, which offer an ideal habitat for the dinoflagellate, and at Te Waihora/ Lake Ellesmere, Canterbury, drought conditions can lead to increases in total N owing to cyanobacterial blooms (M. Maine, Environment Canterbury pers. comm.). Slightly elevated N concentrations have been reported in the complex Southland New River Estuary system owing to multiple inputs (S. Crawford, Environment Southland pers. comm.). Pfiesteria has been detected at all these sites.

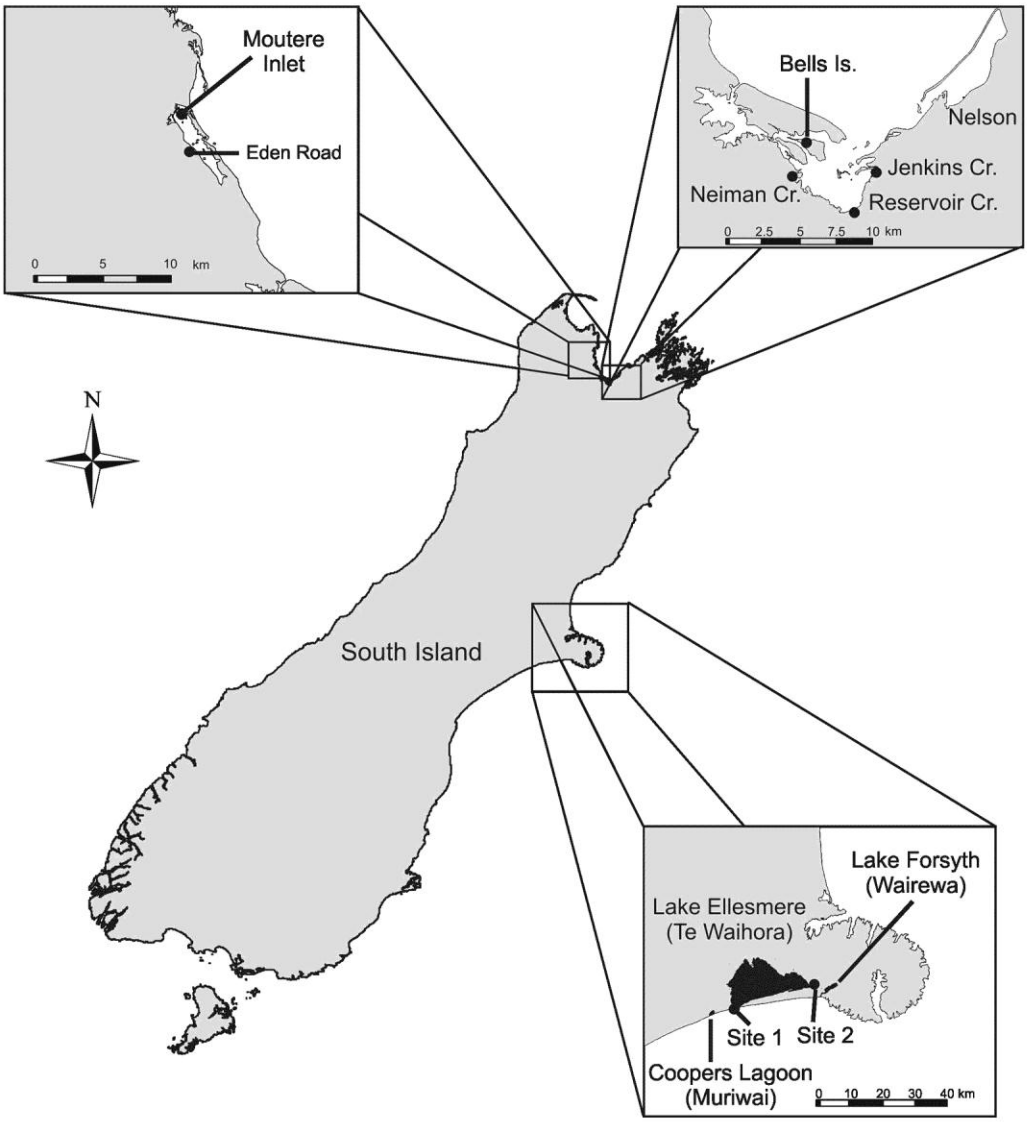

Fig. 1 Map of South Island, New Zealand showing sampling sites in Tasman Bay and Canterbury for *Pfiesteria shumwayae* and *P. piscicida*, 2002–03.

In this study, the seasonal occurrence and related environmental conditions were determined for P. piscicida and P. shumwayae in Tasman Bay's estuaries and Canterbury's brackish coastal lakes (Fig. 1). Additionally, we amplified and sequenced Pfiesteria samples from six locations, including one in the North Island.

MATERIALS AND METHODS

New Zealand Map Grid coordinates for the selected sampling sites for this study were (a) Tasman Bay: Reservoir Creek, E2526753/N5986214; Jenkins Creek, E2528548/N5989284; Neiman Creek, E2522736/N5988855; Eden Road, E2512449/ N6004420; Bell's Island, E2523686/N5990647; Moutere Inlet, E2511417/N6008019; (b) Canterbury: Te Waihora/Lake Ellesmere, site 1 E2459704/ N5705789; site 2 E2482802/N5712761; Wairewa/ Lake Forsyth, E2486893/N5709159; Muriwai/ Coopers Lagoon, E2453759/N5704038.

Water samples (1 litre) for Pfiesteria analysis were collected from undisturbed water immediately above the sediments (25 September and 16 December 2002, 5 March and 18 June 2003).

Samples (three replicates; these were combined into one 50 ml sample) were then taken from just above sediments which had been lightly disturbed by hand. Sediment cores (3 cm diam.) were collected at low tide from the top 5 cm, for analysis for cysts, with 5–10 ml of sterile sea water added to prevent sediments drying out. All samples were transported in insulated containers (c. 15°C).

Sediment (c. 20 ml surface film transferred to sterile plastic bottle with c. 10 ml sterile sea water added to prevent drying out) and water (75 ml in 100 ml sterile plastic bottle) samples were also collected and treated immediately with Lugols iodine for later analysis for benthic and planktonic micro-algae, respectively.

Temperature (°C; calibrated thermometer) and salinity (Practical Salinity Scale; Orion 140 salinity meter) were recorded in situ. Water for nutrient analysis was also collected in bottles (500 ml) with mercuric chloride (4%) pre-added.

Nutrients were analysed following APHA (1998) methods as follows: (1) ammonia-N: 4500-NH3 H; (2) nitrate-N and nitrite-N: 4500-NO3 I; (3) total N: 4500-N C; (4) dissolved reactive phosphorus: 4500-P G; (5) total P: 4500-P A, B mod, G.

Water samples, treated with Lugols iodine, were set up in Utermöhl chambers (10 ml) and left for 4 h to settle. Identifications to determine the co-occurring micro-algae were carried out (Prescott 1982; John et al. 2002) using an IMT-2 Olympus inverted light microscope. Abundance was estimated as dominant (high cell numbers); moderate; present.

Surface sediments that had been collected for micro-algae analysis were passed through a series of graded sieves (120, 80, 50, and 30 μm mesh) with sterile filtered sea water (0.45 μm Nuclepore). The size-graded cells were collected from the sieves by pipette, transferred to Utermöhl chambers, and re-suspended in filtered sea water for identification as above. Micro-algae were identified to genus or, if possible using light microscopy, to species (Round et al. 1990; Harvey 1996; Gillespie et al. 2000).

DNA was extracted from estuarine water samples in CTAB (cetyltrimethylammonium bromide) buffer following the method of Schaefer (1997). Aliquots of water (50–100 ml from three individual samplings combined for each site) were filtered (Whatman Ltd, GF/C glass microfibre), and the filter immersed in the CTAB buffer (1 ml) in 1.5 ml vials and stored in the dark at room temperature until analysed. Samples were then transferred to polypropylene plastic tubes (screw cap; 15 ml) and a further 1 ml CTAB buffer added. The tube was agitated, heated (65°C for 1 h) and vortexed (10 s × 3).

Aqueous chloroform-isoamyl alcohol (24:1) was added (2 ml), the mixture shaken and then centrifuged (1450g high-speed centrifuge, 8 min). The top aqueous layer was pipetted into microfuge tubes (850 μl) and 2-propanol (600 μl) added. The tubes were inverted to mix and centrifuged (11 000– 15 000g, 20 min, 10°C). The supernatant liquid was decanted, the remaining DNA air-dried, and then re-hydrated in TE buffer (pH 8, 25 μl). Purified DNA was held at 4°C until amplified. PCR amplifications were carried out as described previously (Schaefer 1997; Allen 2000; Oldach et al. 2000).

DNA was extracted from sediments (5–50 cm^{-3}) following the protocols of the UltraClean Soil DNA Isolation Kit that was used (Mo Bio Laboratories Inc., Canada). Samples were analysed as above using primers specific to each of the Pfiesteria species (Rublee et al. 1999; Oldach et al. 2000). Results of the samples were cross-checked using real-time quantitative PCR with Taqman® probes (Bowers et al. 2000; Rublee et al. 2004).

DNA sequences were determined for selected Pfiesteria PCR amplicons. For P. piscicida, purified PCR products from three samples from Te Waihora/ Lake Ellesmere were subcloned into plasmid vectors using the

Invitrogen TOPO TA Cloning Kit according to the manufacturer's protocol. Ligation reaction mixtures were used to transform TOP10 Chemically Competent Escherichia coli cells and recombinant plasmids were identified by growth of bacterial colonies on LB agar plates containing 50 μg/ml ampicillin. Individual colonies from each plating were inoculated into LB liquid medium containing 50 14g/ml ampicillin and grown over night. Inserts were purified using a QIAGEN® QIAprep Spin Miniprep Kit according to the manufacturer's protocol. After removal of the insert, gel electrophoresis was used to verify the presence of an appropriate sized DNA fragment. For P. shumwayae, PCR amplicons were directly sequenced from seven Te Waihora/Lake Ellesmere samples, from one Wairewa/Lake Forsyth sample, from one Moutere Inlet sample, and from two samples taken during an earlier study (Rhodes et al. 2000), one from Tasman Bay and one from Kaipara Harbour in the North Island.

Pfiesteria piscicida inserts were cycle sequenced on a Megabace automated DNA sequencer (Amersham Biosciences) using the DYEnamic ET terminator kit (Amersham Biosciences) with M13 forward or reverse primers. Each fragment was bi-directionally sequenced. Direct sequencing of P. shumwayae amplicons derived from the species-specific forward primer and an 18S reverse primer were sequenced using the PS forward primer and conserved reverse primer 580R or 524R (Table 1). For every sample at least two and up to five sequence reads were made per fragment. A visual inspection of each electropherogram was made to verify base calls.

Sequence alignments were performed using the BioEdit Sequence Alignment Editor and Analysis software (version 5.0.9; Department of Microbiology, North Carolina State University [http://www.mbio.ncsu.edu/BioEdit/bioedit.html]), which includes CLUSTAL W as an accessory application for multiple alignments. Sequence similarity was determined using BioEdit software and sequence comparisons were made to GenBank entries using blastn (Altshull et al. 1997; http://www.ncbi.nlm.nih.gov/BLAST/). All Pfiesteria sequences were deposited in Genbank (Accession No. DQ387443-9, DQ393138-42).

RESULTS

The planktonic microflora at Moutere Inlet and Reservoir Creek, Tasman Bay, was dominated by small flagellates and diatoms; Pfiesteria was not observed.

In Canterbury, the planktonic microflora at Te Waihora/Lake Ellesmere was diverse and dominated throughout the year by diatom genera, in particular Monoraphidium and Scenedesmus. The dinoflagellate genus Gymnodinium was present, but again Pfiesteria was not observed. The flora of Wairewa/Lake Forsyth was similar to that of Te Waihora/Lake Ellesmere with the addition of the cyanophyte Nodularia (Table 2).

The benthic diatom species Pleurosigma amara dominated at Moutere Inlet, Tasman Bay, in spring 2002 and again in autumn through to winter 2003. The distinctive epipelic diatom genus Campylodiscus replaced P. amara in summer, and in autumn Navicula and Paralia were co-dominant along with moderate numbers of Nitzschia and Amphora. In winter Synedra and Navicula co-occurred in moderate to high numbers. At Reservoir Creek, Paralia was accompanied by Navicula through to winter (2003). Synedra appeared in autumn and winter and Acnanthes in winter.

In Canterbury, the spring sediment samples were degraded, but the dominant genus recorded from summer (2002) through to winter (2003) at Te Waihora/Lake Ellesmere was the centric diatom Hyalodiscus, which exhibited a watch-glass rather than a hemi-spherical form. In winter 2003, Campylodiscus co-dominated with Hyalodiscus (Table 2).

At Wairewa/Lake Forsyth a hemispherical form of Hyalodiscus (H. cf. lentiginosus) occurred in extremely high numbers throughout the study. In summer Surirella, Synedra, Pleurosigma, Gyrosigma, Navicula, and Nitzschia co-dominated, decreasing through autumn and disappearing by winter.

No Pfiesteria cysts were observed at any of the Tasman Bay or Canterbury sites.

Temperature and salinity were recorded on site during sampling. Minimum and maximum sea surface temperatures and salinities in Tasman Bay ranged from 3.2°C to 22.5°C and 0.1–17.8 psu, and in Canterbury from 9.0°C to 25.5°C and 1.5–14.1 psu, respectively (Table 3).

Table 1 Primers used in this study.

18S forward	AACCTGGTTGATCCTGCCAGT	SSU rDNA
18S reverse	TGATCCTTCTGCAGGTTCACCTAC	SSU rDNA
580R	TACCGCGGGTGCTGGCAC	SSU rDNA
524R	TACCGCGGCTGCTGGCAC	SSU rDNA
107F	CAGTTAGATTGTCTTTGGTGGTCAA	*Pfiesteria piscicida*
Ps forward	TGCATGTCTCAGTTTAAGTCCCA	*P. shumwayae*
SpB forward	AGTTTTAGTGTATTTGATGATCG	*P. shumwayae*
SpB reverse	TCGAAAGCTGATAGGTCAGAATC	*P. shumwayae*

There was little variation in the concentrations of N and phosphorus (P) at the Moutere Inlet site throughout the four seasons of 2002–03 (Table 3). Sampling from Bells Island, Wharf Road, and Eden Road in autumn (2002) resulted in similar concentrations and ratios to those determined for Moutere Inlet. However, Neiman Creek waters had higher concentrations of nitrate (8.6 g/m^3) and total N (10.0 g/m^3) at that time and water taken from Reservoir Creek, in winter 2003, had higher nitrate (4.9 g/m^3) and total N (5.8 g/m^3) concentrations than were determined for Moutere Inlet at that time.

There was little variation in the concentrations of N and P at the Te Waihora/Lake Ellesmere and Wairewa/Lake Forsyth sites throughout the four seasons of 2002–03 (Table 3). The exception was an increase in nitrate concentration at Te Waihora/ Lake Ellesmere (site 1) in winter 2003. A single nutrient analysis of water from Muriwai/Coopers Lagoon (March 2002) resulted in the highest total N concentration recorded of 14 g/m^3. Total P was also higher than other sites at 0.81 g/m^3.

All samples were analysed for the presence of DNA, followed by analyses using species specific probes for P. shumwayae and P. piscicida (Table 4). Pfiesteria shumwayae was detected in Tasman Bay in autumn 2002 and then in early spring and summer (September and December/January 2002/03) in surface sediment slurries from the main study site in Moutere Inlet and from the nearby Reservoir Creek. Pfiesteria shumwayae was also detected in water and sediments collected opportunistically from Eden Road, Moutere Inlet, and Bell Island, Jenkin's Creek and Neiman Creek, Waimea Estuary, in the autumn of 2002.

Table 2 Dominant micro-algae genera (+) co-occurring with *Pfiesteria shumwayae* and *P. piscicida* in Tasman Bay and Canterbury, New Zealand, 2002–03.

	Tasman Bay		Canterbury		
			Lake Ellesmere		
Micro-algal	Reservoir Creek	Moutere Inlet	Site 1	Site 2	Lake Forsyth
Water					
Ankistrodesmus					+
Cryptomonas					+
Cyclotella				+	
Cymbella				+	
Merismopedia					+
Monoraphidium			+	+	+
Navicula	+	+			
Nephrocytium				+	
Nitzschia	+	+			
Nodularia					+
Oocystis				+	+
Pleurosigma					
Scenedesmus			+	+	+
Tetraedron	+	+	+	+	
Tetrastrum			+	+	
Sediment					
Campylodiscus		+			
Hyalodiscus			+	+	+
Melosira				+	
Navicula	+	+	+		+
Nitzschia			+		+
Paralia		+			
Pleurosigma	+	+			+
Surirella			+		+
Synedra	+				+

Table 3 Water temperature (°C), salinity (psu), nitrogen (N), and phosphorus (P) conc. (g/m^3) at Moutere Inlet, Tasman Bay (MI), and Te Waihora/Lake Ellesmere sites 1 and 2 (TW1 and 2) and Wairewa/Lake Forsyth (W), Canterbury, 2002–03. Reference: APHA 1998; nt, not tested.

	Spring 2002				Summer 2002				Autumn 2003				Winter 2003			
	25 Sep	3 Oct			16 Dec	6 Dec			5 Mar	3 Mar			18 Jun	9 Jun		
	MI	TW1	TW2	W	MI	TW1	TW2	W	MI	TW1	TW2	W	MI	TW1	TW2	W
Temperature	11.9	12.8	17.9	13.6	22.4	20.6	25.2	25.3	15.4	19.6	22.1	20.1	7.0	9.0	9.5	10.0
Salinity	13.8	5.3	6.2	3.9	13.3	3.6	4.7	1.5	4.7	2.5	3.3	14.1	17.8	2.1	6.2	2.4
Ammonia-N	0.04	<0.01	nt	nt	0.04	0.07	0.07	0.02	0.08	0.07	0.02	0.02	0.07	0.02	0.02	0.19
Nitrate-N	0.8	0.01	nt	nt	0.4	0.04	0.01	0.02	0.3	0.01	0.01	0.01	0.7	1.7	0.004	0.01
Nitrite-N	0.01	<0.001	nt	nt	0.01	0.001	0.002	<0.001	0.01	<0.001	0.001	0.001	0.003	0.01	0.001	<0.001
Total N	1.5	2.8	nt	nt	1.0	3.0	2.1	1.3	0.7	3.3	2.8	3.8	1.1	2.2	1.5	1.1
Dissolved reactive P	0.02	0.01	nt	nt	0.02	0.07	0.04	0.02	0.01	0.04	0.04	0.04	0.02	0.01	0.03	0.02
Total P	0.05	0.2	nt	nt	0.05	0.52	0.23	0.18	0.04	0.61	0.37	0.67	0.02	0.05	0.15	0.01

Pfiesteria piscicida was detected in sediments collected from both the Moutere Inlet and Reservoir Creek sites in April (autumn) 2002.

In Canterbury, P. shumwayae was detected in both water and sediment samples from Te Waihora/Lake Ellesmere (site 1) collected in march 2002, before the main study period. however it should be noted that the shallow water had been disturbed by high winds and rain several days previously (L. Rhodes pers. obs.). sediment slurry collected from Wairewa/ Lake Forsyth in december 2002 was also positive for P. shumwayae.

Pfiesteria piscicida was also detected in Te Waihora/Lake Ellesmere.

Sequences generated from three clones amplified with P. piscicida specific primers (Oldach et al. 2000) were 457 bp in length, corresponding to positions 119-576 of GenBank entry af077055 for P. piscicida SSU rDNA. they had 98.9 to 99.8% similarity to P. piscicida SSU rDNA sequences listed in GenBank (as of January 2006). sequences generated from samples amplified with P. shumwayae specific primers displayed one of two distinct sequence types. two samples taken from an earlier study (Rhodes et al. 2000) generated 390 bp sequences that had 100% similarity to positions 103–492 of the P. shumwayae GenBank entry AF080098. seven samples from the current study were identical to each other over a 415 bp sequence which corresponded to positions 103–518 of the P. shumwayae GenBank entry AF080098, but showed only 96% identity to P. shumwayae owing to 17 mismatches (table 5).

DISCUSSION

Pfiesteria shumwayae occurs in sediments throughout Tasman Bay, Nelson and in Te Waihora/Lake Ellesmere and Wairewa/Lake Forsyth, just south of Christchurch (Rhodes et al. 2002). In this study, P. shumwayae was detected by PCR-based DNA probe assay at both the study sites and during opportunistic sampling in the Tasman Bay and Canterbury regions. Pfiesteria shumwayae was also detected in sediments from Muriwai/Coopers Lagoon, Canterbury, during a special sampling trip in march 2002. the lagoon is a small body of fresh water, but was once open to the sea.

Detection of P. shumwayae in sediments occurred from early spring through to late autumn in Tasman Bay and throughout the year at the Canterbury sites. this corresponds with previous results from these areas (Rhodes et al. 2002). The dinoflagellate was most likely in cyst form in these sediments and lack of detection during winter months may be owing to patchiness of distribution or to scouring of cyst banks during storms. In the latter instance, deposition of new cyst beds would not occur until warmer temperatures supported a growing season.

Before the seasonal study presented here, in late autumn 2002, P. shumwayae was also detected in water samples from Eden Road, a site in Tasman Bay, in March 2002 (Rhodes et al. 2002). However, it is possible that these water samples contained re-suspended sediment, as they were collected from shallow sites or following rain. A “detect” in water collected from Te Waihora/Lake Ellesmere in May 2001 (L. Rhodes unpubl. data) was also at a time of suspended sediment in the water after a period of wind and rain. The only time that P. shumwayae was unequivocally detected in water samples during the course of the seasonal study was in April (autumn) 2003 at Moutere Inlet. These results support previous studies in New Zealand in which P.

shumwayae was only found in water samples during the late summer-autumn period (Rhodes et al. 2002).

The first detection of P. piscicida in New Zealand was in sediments collected from Moutere Inlet and Reservoir Creek, Tasman Bay, in autumn 2002 (Rublee et al. 2004). Pfiesteria piscicida was also detected in samples collected during this study (December 2002 and February 2003) from the two sampling sites to the north and south of Te Waihora/ Lake Ellesmere, Canterbury.

Table 4 Detection (+) of *Pfiesteria shumwayae* and *P. piscicida*, by PCR-based DNA probe assay, in Tasman Bay and Canterbury, New Zealand, 2002–03.

Site	Date	Sample type	*P. shumwayae*	*P. piscicida*
Tasman Bay				
Moutere Inlet	10 Apr 2002	Sediment	–	+
	10 Apr 2002	Water	–	+
	25 Sep 2002	Sediment	+	–
	16 Dec 2002	Sediment	+	–
	28 Jan 2003	Sediment	+	–
	30 Apr 2003	Water	+	–
Reservoir Creek	10 Apr 2002	Sediment	+	+
	25 Sep 2002	Sediment	+	–
	16 Dec 2002	Sediment	+	–
Eden Road	10 Apr 2002	Water	+	–
	10 Apr 2002	Sediment	+	–
Bell Island	10 Apr 2002	Sediment	+	–
	10 Apr 2002	Water	+	–
Jenkin's Creek	10 Apr 2002	Sediment	+	–
Neiman Creek	10 Apr 2002	Sediment	+	–
Canterbury				
Coopers Lagoon	2 Mar 2002	Water*	+	–
	2 Mar 2002	Sediment	+	–
Te Waihora Site 1	2 Mar 2002	Water*	+	–
	2 Mar 2002	Sediment	+	–
	1 Oct 2002	Water*	+	–
	6 Dec 2002	Water*	+	+
	6 Dec 2002	Sediment	+	+
	28 Feb 2003	Water*	+	+
	28 Feb 2003	Sediment	+	+
Te Waihora Site 2	2 Mar 2002	Water*	+	–
	6 Dec 2002	Water*	+	+
	6 Dec 2002	Sediment	–	+
	28 Feb 2003	Water*	+	+
	28 Feb 2003	Sediment	–	+
Wairewa	6 Dec 2002	Sediment	+	–
	6 Jun 2003	Water*	+	–

*Water containing sediment following high winds and/or rain.

Table 5 Differences in *Pfiesteria* sequences found in this study compared to GenBank entries for *Pfiesteria shumwayae* (AF080098) and *Pfiesteria piscicida* (AF077055).

	bp position (relative to AF080098)							
Sequence ID	108	123–130	171–177	226	232	275–278	190–191	300
***P. shumwayae* samples**								
AF080098	T	ATCGATCA	ACCCAA–G	G	C	CATC	T–A	A
This study (2 samples)[1]	.	. . . . .	. . . . .	.	.	. . . .	. . .	.
This study (7 samples)[2]	A	G . . AT . . C	GT – – . . A .	T	A	– T . –	. C .	T

	bp position (relative to AF077055)					
Sequence ID	170	247	261	512	520	562
***P. piscicida* clones**						
AF077055	A	A	T	A	C	G
This study (CAW 27 clone)[3]	.	.	.	.	.	C
This study (CAW 37 clone)[3]	G	.	.	.	.	C
This study (CAW 123 clone)[3]	.	T	C	C	T	C

[1]GenBank Accession DQ393138-39.
[2]GenBank Accession DQ387443-9.
[3]GenBank Accession DQ393140-42.

Variability in P. piscicida SSU rDNA sequences has been virtually nil (cf. Rublee et al. 2005). Saito et al. (2002) found only two base pair differences in the non-transcribed spacer region of the ribosomal gene of multiple P. piscicida clones, and Tengs et al. (2003) found no differences in a 3777 bp region of the ribosomal gene (including SSU, ITS1, 5.8S, ITS2, and partial LSU regions) of 24 P. piscicida isolates. This suggested a recent origin of this species and wide dispersal or high rates of gene flow among P. piscicida populations. Recent detection of Pfiesteria spp. in ballast water and sediments of ocean going vessels (Doblin et al. 2004) supports the idea of high gene flow. It is unclear whether the variation among sequences found in this study represents variation within repeats of the ribosomal gene or different strains. The variation suggests that there may, however, be subtle geographic variation in P. piscicida and warrants further study.

The difference among sequences generated by P. shumwayae specific primers in this study and P. shumwayae sequences in GenBank is surprising. The frequency of finding the sequence variant in New Zealand as well as the previously reported sequence (found in some New Zealand samples and worldwide) indicates that the sequence variant is not simply a variant within ribosomal gene repeats. Further, the difference is large enough, 4%, to suggest that the sequences may even represent a new species. This is supported by the difficulty we had amplifying the fragment with other primers that have been successfully used in generating sequences from other P. shumwayae cultures. It is clearly premature to propose a new species since there are no isolates to conduct morphological or physiological studies, and because we have only limited sequence data. However, these results do suggest that despite previous extensive testing (e.g., Oldach et al. 2000), "Pfiesteria shumwayae specific primers" may not be species specific. We are currently pursuing this question.

The dominant micro-algae co-occurring with Pfiesteria in the plankton and sediments were common species and were not considered useful as indicators of the presence of Pfiesteria. The diatom Pleurosigma amara, for example, is commonly found in Tasman Bay (Gillespie et al. 2000) and Merismopedia species are common in New Zealand estuaries (Judy McKenzie, Cawthron Institute, Nelson pers. comm.). Pfiesteria species appear to have a global distribution (Rublee et al. 2001, 2004) and are probably common inhabitants of all estuaries.

Salinity and temperature appear to have little influence on the presence of Pfiesteria, with detection being linked with a salinity range of 0.1 psu – 17.8 psu and temperature range of 3.2°C–25.5°C in this study and with an even wider range in a previous study (Rhodes et al. 2002).

Nutrients did not appear to influence the presence of Pfiesteria in the New Zealand context, with the dinoflagellate occurring at sites of no or minimal nutrient enrichment (Robertson et al. 2002) as well as at the site with the highest nutrient levels recorded during this study, Muriwai/Coopers Lagoon. However, studies in the United States indicate that toxin production by Pfiesteria spp. is increased in the presence of high nutrient loadings (Burkholder & Glasgow 1997; Glasgow et al. 2001b) and that increased total N (with a concomitant decrease in total P loadings) can lead to a shift from P. piscicida dominance to the co-dominance of both Pfiesteria species (Burkholder et al. 2001). Further, direct stimulation studies in vitro indicate that P. piscicida zoospores are stimulated more than P. shumwayae zoospores by added P, and that P. shumwayae is indirectly stimulated by N, as mediated through prey addition (Glasgow et al. 2002).

Most of New Zealand's estuaries are well flushed with little accumulation of nutrients in sediments (Robertson et al. 2002), but increases in nutrient loadings are possible, particularly through human inputs, and could potentially lead to toxic Pfiesteria events. A potential risk to fish and to human health therefore remains and is dependent on the continued control of nutrient inputs into New Zealand's estuaries and brackish lakes.

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