Pfiesteria shumwayae (Pfiesteriaceae) in New Zealand

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Abstract:
Pfiesteria shumwayae Steidinger et Burkholder is now known to be present in New Zealand and occurs in estuaries around the country. The presence of *Pfiesteria* was initially determined by a polymerase chain reaction (PCR)-based detection assay, using oligonucleotide primers targeted at ribosomal DNA extracted from estuarine water and sediments. Presence was confirmed by isolation from fresh sediments in the presence of fish (*Oreochromis mossambicus*), followed by identification by scanning electron microscopy. The New Zealand isolates of *P. shumwayae* were ichthyotoxic in bioassays, but there is no historic evidence of fish kills in New Zealand associated with the dinoflagellate.
Keywords: Pfiesteria; dinoflagellate; estuarine; fish kills; molecular probes

Article:

INTRODUCTION

Pfiesteria Steidinger et Burkholder (Dinamoebales; Pfiesteriaceae) is a heterotrophic dinoflagellate with planktonic zoospore populations sourced from seed beds of cysts and amoebae in sediments (Steidinger et al. 1996; Burkholder et al. 1992; Burkholder & Glasgow 1997). *Pfiesteria* exhibits "ambush-predator" behaviour in the presence of fish (Burkholder et al. 1998), which may lead to non-focal as well as deep-focal, ulcerous lesions and death, as demonstrated in laboratory experiments (Burkholder et al. 1992, 1995, 2001a; Noga et al. 1996; Marshall et al. 2000). The fish attacks are non-specific and more than 20 native and exotic species tested in the United States have proved vulnerable (Burkholder et al. 1997).

The United States has experienced massive fish kills, clearly attributed to *Pfiesteria*, in certain estuarine systems along its eastern seaboard (Burkholder et al. 1992; Burkholder & Glasgow 1997; Samet et al. 2001; Brownie et al. in press) and, in the Albemarle-Pamlico Estuarine System of North Carolina, $>1 \times 10^9$ fish were killed during *Pfiesteria* outbreaks over the last decade (Burkholder et al. 2001 a; Glasgow et al. 200 1 a). *Pfiesteria piscicida* Steidinger et Burkholder (Steidinger et al. 1996) has been linked to measurable neurotoxic effects in humans, including central nervous system impairment (for example, mostly reversible short-term memory loss that can last for weeks to months; Glasgow et al. 1995; Grattan et al. 1998), as well as autonomic and peripheral nervous system dysfunction, skin lesions, and other effects (Glasgow et al. 1995; Schmechel & Koltai 2001). The economic costs related to *Pfiesteria* have been high; in Maryland the presence of the dinoflagellate in association with the death of c. 50 000 Atlantic menhaden (*Brevoortia tyrannus* Latrobe) was responsible for an estimated cost of US$65 million (Epstein 1998; Lipton 1998). This was due to indirect market effects based on provocative media reports and public perception rather than particularly heavy fish losses (Anderson et al. 2000; Committee on Environment and Natural Resources (CENR) 2000; Burkholder & Glasgow 2002).

The *Pfiesteria* species complex comprises the two described species, *P. piscicida* (Steidinger et al. 1996) and *P. shumwayae* Glasgow et Burkholder (Glasgow et al. 2001b). Both species have complex life cycles and may

occur in three functional types (toxicity status) as: (1) highly toxic or TOX-A (actively toxic, requiring the presence of live finfish or their fresh tissues and excreta); (2) temporarily non-toxic in the absence of fish or TOX-B; and (3) non-inducible, apparently (based on present knowledge) unable to produce toxin in response to fish (Burkholder et al. 2001b). Other toxic algae, including dinoflagellates, are known to have benign or non-inducible as well as toxic strains (Gentien & Arzul 1990; Anderson 1991; Bates et al. 1998; Edvardsen & Paasche 1998).

Pfiesteria spp. can consume small, photosynthetic microalgae, and retain their chloroplasts in an active state, presumably utilising their photosynthetic products (Glasgow et al. 1998, 2001b; Lewitus et al. 1999), and *Pfiesteria* can also consume fresh shellfish tissue, preying on the pediveliger stage (Springer 2000). *P. piscicida* can survive passage through the gut of adult shellfish in cyst form, ultimately excysting and then capable of further predation (Springer et al. 2002).

There is no evidence to date that human illnesses result from eating fish or shellfish that have been exposed to a *Pfiesteria* event. Recent research by Springer et al. (2002), however, has demonstrated that toxic *Pfiesteria* cells can be concentrated by some shellfish (for example, the subadult eastern oyster *Crassostrea virginica* Gmelin). Thus, risks to humans from seafood consumption cannot be ruled out until the *Pfiesteria* toxin(s) can be identified, so that their presence/absence in fish tissues can be conclusively determined and quantified (Fairey et al. 1999; Kimm-Brinson et al. 2001; Samet et al. 2001). A potent, water-soluble neurotoxin produced by *P. piscicida* and *P. shumwayae* has been isolated and purified (J. Ramsdell and P. Moeller, National Ocean Service, Charleston, SC, United States; patenting process initiated— Burkholder & Glasgow 2001). Its pharmacological activity has been described, as recent experiments have shown that the water-soluble *Pfiesteria* toxin mimics an ATP neurotransmitter and binds to a central nervous system purinergic receptor (Kimm-Brinson et al. 2001; Melo et al. 2001).

In the United States, *Pfiesteria* has been found from New York State to Texas (Rublee et al. 1999, 2001). The geographic spread and toxicity of *Pfiesteria* spp. indicate a tolerance to a wide range of salinities and temperatures (0–35 and 12–33°C respectively; Burkholder et al. 2001 a; Glasgow et al. 2001a,b, 2002). It should be noted that *Pfiesteria* strains have been TOX-B at most locations where it has been detected, with toxicity requiring induction, and toxic events (mainly massive fish kills) taking place at sites of nutrient enrichment (Burkholder et al. 1997, 2001b; Glasgow et al. 2001a; Samet et al. 2001; Burkholder & Glasgow 2002).

DNA-based diagnostics are available in several formats for the detection of *Pfiesteria* spp. in water, sediments, and fish (Rublee et al. 1999, 200 1; Oldach et al. 2000; Bowers et al. 2000). In this study molecular probe technology was used to determine the distribution of *Pfiesteria* in New Zealand.

METHODS

Collection, isolation, and culture

Water samples (1 litre) for dinoflagellate analysis were collected from sites from just above sediments which had been lightly disturbed by hand. A total of 26 estuarine localities and embayments were selected from around New Zealand (Fig. 1) where flushing rates were low, i.e., up to 5 days residence time. Sediment cores (3 cm diam.) were also collected from the top 5 cm at those sites, for analysis for cysts, with 5–10 ml of seawater added to prevent sediments drying out. All samples were maintained in insulated containers at c. 15°C. Temperature (°C) and salinity (Practical Salinity Scale; Orion 140 salinity meter) were recorded *in situ* whenever possible.

Aliquots (50–100 ml) of water samples were filtered (Whatman Ltd., GF/C glass microfibre), and the filter immersed in CTAB (cetyltrimethylammonium bromide) buffer (1 ml) in 1.5 ml vials; sediments (5–50 cm^{-3}) were stored untreated. Samples were analysed for *Pfiesteria* spp. 18S rDNA "signature" sequences at the University of North Carolina, Greensboro.

Sediment samples were also sent to the Center for Applied Aquatic Ecology (CAAE), North Carolina State University (NCSU), for toxicity testing, followed by isolation and scanning electron microscopy (SEM) to confirm species identification. Amoeboid and flagellated forms of the New Zealand strains are currently maintained in the CAAE-NCSU culture collection.

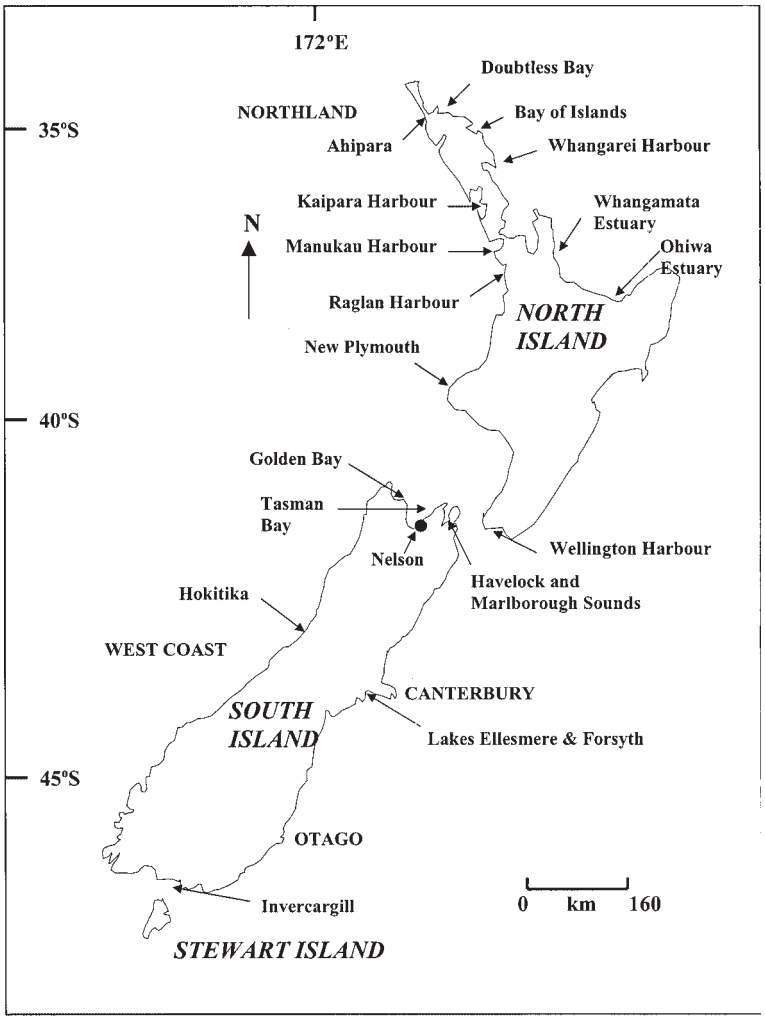

Fig. 1 Map of New Zealand showing sampling sites for *Pfiesteria*.

Scanning electron microscopy

An aliquot of culture of the motile dinoflagellate was mixed 1: 1 with 40% ethanol and cells were collected on a polycarbonate filter, which was mounted on a stub and gold-palladium coated for identification and characterisation of plate tabulation using a Leica Stereoscan 240 SEM (photographs were taken with a Polaroid camera). The procedures of Glasgow et al. (2001b) were followed to determine cell morphology and thecal plate tabulation. Cells were osmotically swollen by incubation for 45 min in a saline-reduced (by 2–3) f/2 culture medium (Guillard 1975) matrix, then killed using a cold double-fixation technique modified from Steidinger et al. (1989). The cells were rinsed with a 0. 1*M* sodium cacodylate rinsing buffer prepared in saline-reduced (by 2–3) f/2 matrix. Variations from the fixation protocols in Steidinger et al. (1989) included use of 50–60 mOs kg^{-1} hypo-osmotic reduction of f/2 culture medium, increased osmium concentration (from 0.7 to 1.0%), increased glutaraldehyde concentration (from 1.5 to 2.0%), and an isoosmotic 0.1*M* sodium cacodylate wash buffer (as in Burkholder et al. 1995, prepared in f/2 culture media matrix) rather than a 75% osmolality-reduced wash buffer prepared with distilled water. Cells were dehydrated through an ethanol series, critical-point-dried using carbon dioxide, sputter-coated with 30 nm gold/palladium, and viewed at an accelerating voltage of 15 kV on a Philips 505 SEM (Burkholder & Glasgow 1995).

DNA probe-based assays

DNA was extracted from estuarine water samples in CTAB buffer (Schaefer 1997), followed by PCR probing using primers specific to both *Pfiesteria* species (Rublee et al. 1999; Oldach et al. 2000). PCR amplicons were also sequenced to confirm identity.

Toxicity bioassays

The CAAE developed a standardised fish bioassay procedure (Burkholder & Glasgow 1997; Burkholder et al. 2001a,b,c) from an earlier technique described by Smith et al. (1988). The assay follows Henle-Koch's postulates modified for toxic rather than infectious agents and has been endorsed by a United States national science panel that evaluated *Pfiesteria* science at the request of the Centers for Disease Control and Protection (Samet et al. 2001). The standardised fish bioassay was used to test for actively toxic *Pfiesteria* strains, pending development of a toxin-based assay. This rigorous, multi-step procedure involves addition of water samples, that may contain toxic *Pfiesteria*, to cultures of live fish prey under water quality conditions conducive to maintaining healthy fish, and evaluation of subsequent fish kills for environmental and microbial factors (*Pfiesteria*, other harmful algal species, certain bacteria, and protozoans, etc.) that could contribute to fish death (Burkholder et al. 2001c). If a *Pfiesteria* population is present at potentially lethal densities in association with fish death, in the absence of other apparent causes (thus, using a conservative approach), the population is cloned and retested in a second set of fish bioassays to verify toxicity. Control fish are treated similarly except for exposure to *Pfiesteria*. The present version of the standardised fish bioassay has been reproduced by Lewitus et al. (1999) and Marshall et al. (2000), confirming the ability of Toxic *Pfiesteria* Complex species to kill fish (Samet et al. 2001). Additional tests for toxicity have used a reporter gene assay that is based on detection of *c-fos* expression in rat pituitary cells (National Ocean Service, Charleston, SC, United States; Fairey et al. 1999; Kimm-Brinson et al. 2001).

Sediment samples from three Tasman Bay sites (in the Rabbit Island/Moutere Inlet area; Fig. 1) were combined and assayed for *Pfiesteria* spp. cyst populations by incubating the sediments with fish prey to trigger cyst germination (5 tilapia (*Oreochromis mossambicus* Peters) per 7-litre assay volume, 20°C, salinity 15, and 12:12 L:D at 20 µmol m^{-2} s^{-1}, and other environmental conditions, and care of fish, as in Burkholder et al. 2001c). Ichthyotoxicity was then assessed and motile *Pfiesteria* cells identified by both DNA probe assays and SEM of suture-swollen cells (cf. Glasgow et al. 2001b). *Pfiesteria* cells from positive fish bioassays were recovered as clones using flow cytometric techniques (Glasgow et al. 2001b) and grown on axenic algal prey (cloned from a mixed commercial culture CCMP757 with *Rhodomonas* sp., Culture Collection for Marine Phytoplankton, Bigelow Laboratory, Maine, United States) at 23°C, salinity 15, and 12:12 L:D cycle with 80 µmol m^{-2} s^{-1} in f/2 enriched medium (Guillard 1975). Clonal cultures were then re-tested for toxicity in a second set of fish bioassays. The identity of the cultured organisms was further confirmed by PCR amplification in three laboratories (P. Rublee, University of North Carolina at Greensboro; D. Oldach, University of Maryland Medical School; and J. Burkholder and H. Glasgow CAAE). All assays to assess toxicity (i.e., with actively toxic *Pfiesteria* and live fish) were carried out in the Biohazard III facilities at North Carolina State University as mandated by United States state and federal guidelines to ensure that laboratory personnel would be protected from potentially neurotoxic aerosols from this dinoflagellate (Burkholder et al. 2001c).

RESULTS AND DISCUSSION

Pfiesteria shumwayae from New Zealand was first detected, by PCR-based DNA probe assay, in water samples collected from Tasman Bay, Nelson (Fig. 1) in April (austral autumn) 2000. It was determined to be a potentially toxic strain (Rhodes et al. 2000). Further samples collected from estuarine sites as geographically separated as Kaipara Harbour in the north, Golden Bay and Havelock in central New Zealand, and the New River Estuary at Invercargill in the far south, also proved positive for *P. shumwayae* (Fig. 1; Table 1) and it would therefore appear that the potentially toxic *P. shumwayae* is a common inhabitant of New Zealand's estuaries. The closely related *P. piscicida* was not found at any of the sites so far sampled, as determined by the species-specific PCR-based DNA probe assay. The morphological identification and physiological characteristics of New Zealand strains from a range of locations need further investigation to determine whether they conform to or differ from described United States strains (e.g., Parrow et al. 2002).

Water and sediment samples were collected between April 2000 and March 2001 and *P. shumwayae* was detected in samples from sites where water temperatures ranged from 9 to 25°C (Table 1). Many samples were collected opportunistically, however, and temperature data were not always available. At sites where salinity measurements were taken, *P. shumwayae* was found from salinity 1.6–28.5 (Table 1), consistent with its

previously reported tolerance to an extremely wide range of salinities (Burkholder et al. 2001a; Glasgow et al. 2001a,b, 2002). This is not surprising for estuarine species, as salinities vary widely where freshwater inputs are subject to tidal effects.

Table 1 *Pfiesteria shumwayae*. Results of molecular, salinity and temperature analyses of water and sediment samples from sites around New Zealand (see Fig. 1). (Salinity, practical salinity scale; [s], sediment sample only; [sw], water and sediment samples; others, water samples only.)

Site	Salinity	Temp. (°C)	*P. shumwayae*	Date sampled
NORTH ISLAND				
Ahipara Stream	–	–	0	31 Jan 2001
Aurere Stream, Doubtless Bay	–	–	0	22 Jan 2001
Awanui, Rangaunu Harbour	–	–	0	22 Jan 2001
Kaeo River, Whangaroa Harbour	–	–	0	22 Jan 2001
Tauranga Bay, Whangaroa Harbour	–	–	0	22 Jan 2001
Paihia, Bay of Islands	–	–	0	22 Jan 2001
Waipu Estuary, south of Whangarei	–	–	0	22 Jan 2001
Waionehu Stream, Waipu Estuary	–	–	0	22 Jan 2001
Mangawhai, Kaipara Estuary	5	22	detect	23 Mar 2001
Kaiwaka, Kaipara Estuary	1.6	22	detect	23 Mar 2001
Te Hana, Kaipara Headwaters	–	–	0	31 Jan 2001
Topuni River, Kaipara Headwaters	–	–	0	31 Jan 2001
Onehunga Wharf, Manukau Harbour	30	14	0	23 Aug 2000
Raglan Harbour	–	–	0	31 Jan 2001
Whangamata Estuary	35	12.5	0	23 Aug 2000
Ohiwa Estuary	27	9	0	23 Aug 2000
New Plymouth	0.1	–	0	14 Dec 2000
Wellington Harbour	0	–	0	14 Dec 2000
SOUTH ISLAND				
Tasman Bay				
Moutere Stream	18.8	17	detect	12 Apr 2000
Moutere Dump	13.8	18.5	detect	12 Apr 2000
Easton's Dam[s]	12	12	detect	29 May 2000
Easton's Dam	3	18	detect	12 Apr 2000
Holdaway Rd	4.7	16.8	detect	12 Apr 2000
Rabbit Island	12.5	18.5	detect	12 Apr 2000
Cotterells Rd	4.4	16	detect	12 Apr 2000
Cotterells Rd[s]	6.7	12	detect	29 May 2000
Hatchery Creek	3.4	20.6	detect	12 Apr 2000
Suicide Drive	11	20.8	detect	12 Apr 2000
Atawhai Pond	7	18	detect	12 Apr 2000
Atawhai Pond[s]	6	12.5	detect	29 May 2000
Golden Bay				
Ruataniwha Estuary	5.7	20	0	15 Mar 2001
Ruataniwha River Mouth	5.9	20	detect	15 Mar 2001
Stream to Ruataniwha	5.5	19	detect	15 Mar 2001
Marlborough				
Havelock (west harbour)	32.4	–	0	14 Dec 2000
Havelock Causeway	1.6	24	0	7 Mar 2001
Havelock Causeway[sw]	1.2	9		6 Jun 2000
Havelock Old Wharf[sw]	1.2	9		6 Jun 2000
Kaituna Stream, downstream of main highway	15.2	24	detect	7 Mar 2001
Impounded wetland	27.3	24	0	7 Mar 2001
Kaituna Stream, upstream of main highway	28.5	25	detect	7 Mar 2001
Wetland outlet	26.7	24	detect	7 Mar 2001
Wairau Lagoon	50	23.4	0	6 Jun 2001
Wairau Lagoon	50	22	0	6 Jun 2001
Canterbury				
Avon Heathcote Estuary[s]	–	9.5	0	10 Jul 2000
City outfall drain[sw]	36.4	9	0	30 May 2001
Northern terrace drain[sw]	23.4	7	0	30 May 2001
Estuary Drain, Bexley Rd[sw]	4.1	8	0	30 May 2001
Lake Forsyth	6.9	9.9	detect	16 May 2001
Lake Ellesmere, Catons Bay	4.2	10.8	detect	16 May 2001
Lake Ellesmere, Kaituna end	3	9	detect	16 May 2001
Otago				
Kaikorai Estuary[s]	3.8	5.8	0	10 Jul 2000
Waikouaiti Estuary[s]	13.1	9.1	0	10 Jul 2000
West Coast				
Hokitika River Mouth	3	10	0	6 Jun 2001
Southland				
Ocean Beach	30	6.7	0	4 Jul 2001
Bluff Harbour, Colyer's Island Rd	30.2	3.6	0	4 Jul 2001
Bluff Harbour, Colyer's Island Rd	4.5	3.9	0	4 Jul 2001
Mokomoko Inlet	26.5	7.7	0	4 Jul 2001
New River Estuary	6.2	18	detect	12 Feb 2001
Otepune Creek	6.2	20	detect	14 Feb 2001
Bushy Point	6.2	17	detect	14 Feb 2001
Kingswell Creek	6.2	21	detect	14 Feb 2001

Most New Zealand estuaries are well flushed with little accumulation of nutrients in sediments (SMF Project no. 5096: Estuarine environmental assessment and monitoring: a national protocol unpubl. data) suggesting a low risk of fish kills due to *Pfiesteria*. Tasman Bay exhibits a high tidal range (spring range 3.5 m; Land Information New Zealand website: http://www.linz.govt.nz), however, even in some areas of this well flushed bay (for example, Moutere Stream, Tasman Bay, where arms of the estuary have been blocked by causeways), anthropogenic inputs have led to slightly elevated total N levels (>500 µg litre^{-1}). Of more concern is Kaipara Harbour, where slightly elevated total nitrogen (N), muddy sediments, and subtropical temperatures offer an ideal habitat for *Pfiesteria*, as determined by Burkholder et al. (2001a,c). At Lake Ellesmere, Canterbury, where *P. shumwayae* was also detected (Table 1), drought conditions can lead to increases in total N due to cyanobacterial blooms (M. Maine, Environment Canterbury pers. comm.), and in the complex New River Estuary system, Invercargill, where *P. shumwayae* was also detected, slightly elevated N concentrations have been reported, due to multiple inputs (S. Crawford, Environment Southland pers. comm.).

Combined sediment samples collected from Tasman Bay and sent to North Carolina State University for toxicity testing killed fish within 28 days. A lag period before toxicity was induced was probably due to transport-induced stress (Burk-holder et al. 2001c). A New Zealand strain of *P. shumwayae* isolated from these sediments has been observed in both the flagellated (Fig. 2) and amoeboid form.

Actively toxic *Pfiesteria* (TOX-A) has been consistently documented from nutrient enriched, poorly mixed, warm mid-salinity waters with an abundance of fish (Burkholder et al. 200 1a; Glasgow et al. 2001a). However, potentially toxic (TOX-B) and apparently non-inducible strains have a wide distribution (including United States, Scandinavia, and Australasia), and include areas with minimal nutrient enrichment as well as eutrophic brackish waters (Glasgow et al. 2002; Jakobsen et al. 2002). The New Zealand strain isolated from the Tasman Bay sediments proved to be TOX-B and was from a site of minimal nutrient enrichment, although some run-off occurs from the nearby agricultural and horticultural lands (L. Rhodes unpubl. data).

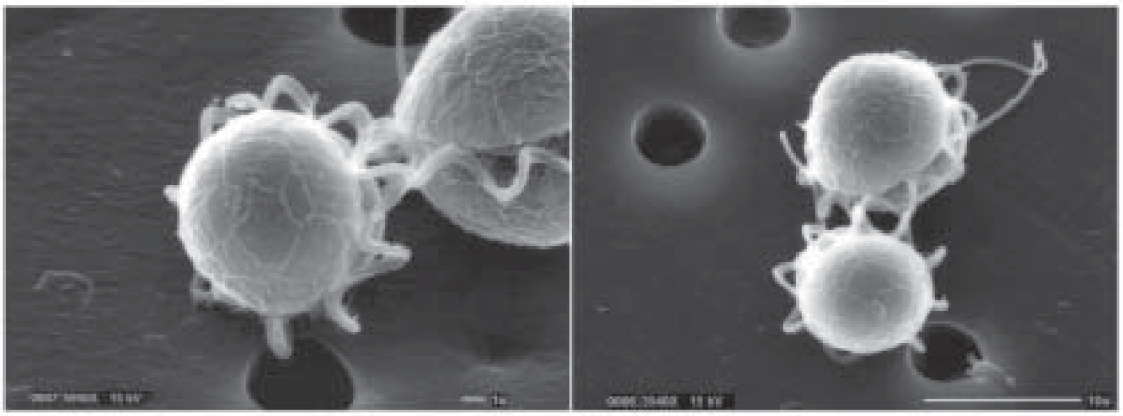

Fig. 2 Scanning electron micrographs of *Pfiesteria shumwayae* zoospores, New Zealand isolates (H. Glasgow, Centre for Applied Aquatic Ecology, North Carolina State University).

In the United States, molecular probe analysis of samples, collected from sites from New York to Texas, resulted in positive samples of at least one *Pfiesteria* species from every state tested, including sites where there was no historical evidence of fish kill events (Rublee et al. 1999, 2001). No anecdotal evidence or historical data have been found in New Zealand, at least in preliminary discussions with members of Fish and Game New Zealand and New Zealand regional council personnel, that could suggest that *Pfiesteria* has caused any fish kill events in this country.

The results for New Zealand support the hypotheses (Burkholder et al. 1992) that *Pfiesteria* is distributed worldwide, and that TOX-A status strains are most abundant in highly eutrophic estuaries (Burkholder & Glasgow 1997; Glasgow et al. 2001a). New Zealand regional authorities will need to be aware of the potential risk of toxic events in areas where nutrient inputs reach levels that would support TOX-A *Pfiesteria* populations. It is also evident from the results that TOX-B strains occur in a range of nutrient environments, and that nutrient enrichment is not a requirement for their survival.

Experimentation in the United States has shown that attacks on finfish are non-specific (Burkholder et al. 1992, 1995; Noga et al. 1996; Springer et al. 2002). In New Zealand, yellow-eyed mullet (*Aldrichetta forsteri* Cuv. and Val.), commonly found in brackish lagoons, and whitebait (*Galaxias* spp.), which pass through estuaries en route to headwaters of rivers (Graham 1974), may be vulnerable. The risk to shellfish spat intended for shellfish enhancement programmes or for aqua-culture will be assessed, as Greenshell™ mussels (*Perna canaliculus* Gmelin) and scallops (*Pecten novaezealandiae* Reeve) grown in Tasman Bay can be impacted by inputs from estuaries in which *Pfiesteria* resides during flood events.

Pfiesteria is now part of the world harmful algal bloom story and is clearly established throughout New Zealand. Contingency plans for potential toxic *Pfiesteria* outbreaks are being prepared by the New Zealand Food Safety Authority, Ministry of Agriculture and Forestry, and this proactive action should ensure that New Zealand avoids the public panic and consequent economic losses through avoidance of seafood that have frequently accompanied toxic *Pfiesteria* outbreaks in the United States.

REFERENCES

Anderson D. M. 199 1: Toxin variability in Alexandrium. In: Granéli, E.; Sundstrom, B.; Edler, L.; Anderson, D. M. ed. Toxic marine phytoplankton. Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton. New York, Elsevier. Pp. 41–51.

Anderson, D. M.; Hoagland, P.; Kaoru, Y.; White, A. W. 2000: Estimated annual economic impacts from harmful algal blooms (HABs) in the United States. Woods Hole Oceanographic Institute Technical Report, WHOI-2000-11. 97 p.

Bates, S. S.; Garrison, D. L.; Horner, R. A. 1998: Bloom dynamics and physiology of domoic-acid-producing Pseudo-nitzschia species. In: Anderson, D. M.; Cembella, A.; Hallegraeff, G. M. ed. Series G: ecological sciences. Vol. 41. Berlin, Springer-Verlag. Pp. 267–292.

Bowers, H. A.; Tengs, T.; Glasgow, H. B.; Burkholder, J. M.; Rublee, P. A.; Oldach, D. W. 2000: Development of real-time PCR assays for rapid detection of Pfiesteria piscicida and related dinoflagellates. Applied and Environmental Microbiology 66: 4641–4648.

Brownie, C.; Glasgow, H. B.; Burkholder, J. M.; Reed, R. E.; Tang, Y. 2002: Re-evaluation of the relationship between Pfiesteria and estuarine fish kills. Ecosystems: in press.

Burkholder, J. M.; Glasgow, H. B. 1995: Interactions of a toxic estuarine dinoflagellate with microbial predators and prey. Archiv für Protistenkunde 145: 177–188.

Burkholder, J. M.; Glasgow, H. B. 1997: Pfiesteria piscicida and other Pfiesteria-like dinoflagellates: behavior, impacts, and environmental controls. Limnology and Oceanography 42: 1052–1075.

Burkholder, J. M.; Glasgow, H. B. 2002: History of toxic Pfiesteria in North Carolina estuaries from 1991 to the present. BioScience 51: 827–841.

Burkholder, J. M.; Noga, E. J.; Hobbs, C. H.; Glasgow, H. B. Jr. 1992: New ‘phantom’ dinoflagellate is the causative agent of major estuarine fish kills. Nature 358: 407–4 10.

Burkholder, J. M.; Glasgow, H. B. Jr.; Hobbs, C. H. 1995: Fish kills linked to toxic ambush-predator dinoflagellate: distribution and environmental conditions. Marine Ecology Progress Series 124: 43–61.

Burkholder, J. M.; Mallin, M. A.; Glasgow, H. B. Jr.; Larsen, L. M.; McIver, M. R.; Shank, G. C.; Deamer-Melia, N.; Briley, D. S.; Springer, J.; Toucette, B. W.; Hannon, E. K. 1997: Impacts to a coastal river and estuary from rupture of a large swine waste holding lagoon. Journal of Environmental Quality 26: 1451–1466.

Burkholder, J. M.; Glasgow, H. B.; Lewitus, A. J. 1998: Physiological ecology of Pfiesteria piscicida with general comments on “ambush-predator” dinoflagellates. In: Anderson, D. M.; Cembella, A.; Hallegraeff, G. M. ed. Physiological ecology of harmful algae. NATO ASI Series G: ecological sciences. Vol. 41. Berlin, Springer-Verlag. Pp. 175–191.

Burkholder, J. M.; Glasgow, H. B. Jr.; Deamer-Melia, N. 2001a: Overview and present status of the toxic Pfiesteria complex (Dinophyceae). Phycologia 40: 186–214.

Burkholder, J. M.; Glasgow, H. B.; Deamer-Melia, N. J.; Springer, J.; Parrow, M. W.; Zheng, C.; Cancellieri, P. 2001b: Species of the toxic Pfiesteria complex, and the importance of functional type in data interpretations. Environmental Health Perspectives 109(5): 667–679.

Burkholder, J. M.; Marshall, H. G.; Glasgow, H. B.; Seaborn, D. W.; Deamer-Melia, N. J. 2001c: The

standardized fish bioassay process for detecting and culturing actively toxic Pfiesteria, used by the two reference laboratories for Atlantic and southeastern states. Environmental Health Perspectives 109(5): 745–756.
CENR 2000: National assessment of harmful algal blooms in US waters. National Science and Technology Council Committee on Environment and Natural Resources, Washington, D.C. 38 p.
Edvardsen, B.; Paasche, E. 1998: Bloom dynamics and physiology of Prymnesium and Chrysochromulina. In: Anderson, D. M.; Cembella, A.; Hallegraeff, G. M. ed. Physiological ecology of harmful algae. NATO ASI Series G: ecological sciences. Vol. 41. Berlin, Springer-Verlag. Pp. 193–208.
Epstein, P. R. 1998: Marine ecosystems: emerging diseases as indicators of change. Year of the Ocean Special Report. Center for Health and the Global Environment, Harvard Medical School, Boston.
Fairey, E. R.; Edmunds, J. S.; Deamer-Melia, N. J.; Glasgow, H. B.; Johnson, F. M.; Moeller, P. D. R.; Burkholder, J. M.; Ramsdell, J. S. 1999: Reporter gene assay for fish killing activity produced by Pfiesteria piscicida. Environmental Health Perspectives 107: 711–714.
Gentien, P.; Arzul, G. 1990: Exotoxin production by Gyrodinium cf. aureolum (Dinophyceae). Journal of the Marine Biological Association of the United Kingdom 70: 571–58 1.
Glasgow, H. B.; Schmechel, D. E.; Tester, P. A.; Rublee, P. A. 1995: Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. Journal of Toxicology and Human Health 46: 501–522.
Glasgow, H. B.; Lewitus, A. J.; Burkholder, J. M. 1998: Feeding behavior of the ichthyotoxic estuarine dinoflagellate, Pfiesteria piscicida, on amino acids, algal prey, and fish vs. mammalian erythrocytes. In: Reguera, B.; Blanco, J.; Fernandez, M. L.; Wyatt, T. ed. Harmful microalgae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Paris. Pp. 394–397.
Glasgow, H. B.; Burkholder, J. M.; Mallin, M. A.; Deamer-Melia, N. J.; Reed, R. E. 200 1 a: Field ecology of toxic Pfiesteria complex species, and a conservative analysis of their role in estuarine fish kills. Environmental Health Perspectives 109(5): 715–730.
Glasgow, H. B.; Burkholder, J. M.; Morton, S. L.; Springer, J. 2001b: A second species of ichthyotoxic Pfiesteria. Phycologia 40: 234–245.
Glasgow, H. B.; Burkholder, J. M.; Morton, S. L.; Springer, J.; Parrow, M. W. 2002: The fish-killing activity and nutrient stimulation of a second toxic Pfiesteria species. In: Hallegraeff, G. M.; Blackburn, S. I.; Bolch, C. J.; Lewis, R. J. ed. Harmful algal blooms 2000. Intergovernmental Oceanographic Commission of UNESCO, Paris. Pp. 97–100.
Graham, D. H. 1974: A treasury of New Zealand fishes. 2nd ed. Wellington, A. H. & A. W. Reed.
Grattan, L. M.; Oldach, D.; Perl, T. M.; Lowitt, M. H.; Matuszak, D. L.; Dickson, C.; Parrott, C.; Shoemaker, R. C.; Kauffman, C. L.; Wasserman, M. P.; Hebel, J. R.; Charache, P.; Morris, J. G. Jr. 1998: Learning and memory difficulties after environmental exposure to waterways containing toxin-producing Pfiesteria or Pfiesteria-like dinoflagellates. Lancet 352: 532–539.
Guillard, R. R. L. 1975: Culture of phytoplankton for feeding marine invertebrates. In: Smith, W. L.; Chanley, M. H. ed. Culture of marine invertebrate animals. New York, Plenum Press. Pp. 29–60.
Jakobsen, K. S.; Tengs, T.; Vatne, A.; Bowers, H. A.; Oldach, D. W.; Burkholder, J. M.; Glasgow, H. B.; Rublee, P. A.; Klaveness, D. 2002: Discovery of the toxic dinoflagellate, Pfiesteria, from northern European waters. Proceedings of the Royal Society London (B) 269: 211–214.
Kimm-Brinson, K. L.; Moeller, P. D. R.; Barbier, M.; Glasgow, H. B.; Burkholder, J. M.; Ramsdell, J. S. 2001: Identification of a P2X7 receptor in GH4C 1 rat pituitary cells: a target for a bioactive substance produced by Pfiesteria piscicida. Environmental Health Perspectives 109: 457–462.
Lewitus, A. J.; Glasgow, H. B.; Burkholder, J. A. 1999: Kleptochloroplastidy in the toxic dinoflagellate, Pfiesteria piscicida. Journal of Phycology 35: 1430–1437.
Lipton, D. W. 1998: Pfiesteria's economic impact on seafood industry sales and recreational fishing. Pfiesteria: where do we go from here? Economics of Policy Options for Nutrient Management and Dinoflagellates Conference. University of Maryland. Department of Agriculture and Natural Resources, College Park.
Marshall, H. M.; Gordon, A. S.; Seaburn, D. W.; Dyer, B.; Dunstan, W. M.; Seaborn, A. 2000: Comparative culture and toxicity studies between the toxic dinoflagellate, Pfiesteria piscicida, and a morphologically similar cryptoperidiniopsoid dinoflagellate. Journal of Experimental Marine Biology and Ecology 255: 65–74.
Melo, A. C.; Moeller, P. D. R.; Glasgow, H. B.; Burkholder, J. M.; Ramsdell, J. S. 2001: Microfluorimetric analysis of a purinergic receptor (P2X7) in GH4C1 rat pituitary cells: effects of a bioactive substance produced

by Pfiesteria piscicida. Environmental Health Perspectives 109(5): 731–738.
Noga, E. J.; Khoo, L.; Stevens, J. B.; Fan, Z.; Burkholder, J. M. 1996: Novel toxic dinoflagellate causes epidemic disease in estuarine fish. Marine Pollution Bulletin 32: 219–224.
Oldach, D. W.; Delwiche, C. F.; Jakobsen, K. S.; Tengs, T.; Brown, E. G.; Kempton, J. W.; Schaefer, E. F.; Bowers, H. A.; Glasgow, H. B. Jr.; Burkholder, J. M.; Steidinger, K. A.; Rublee, P. A. 2000: Heteroduplex mobility assay-guided sequence discovery: elucidation of the small subunit (18S) rDNA sequences of Pfiesteria piscicida and related dinoflagellates from complex algal culture and environmental sample DNA pools. Proceedings of the National Academy of Science, USA 97: 4303–4308.
Parrow, M. W.; Glasgow, H. B.; Burkholder, J. M.; Zhang, C. 2002: Comparative response to algal prey by Pfiesteria piscicida, Pfiesteria shumwayae and a co-occurring 'look-alike' species. In: Hallegraeff, G. M.; Blackburn, S. I.; Bolch, C. J.; Lewis, R. ed. Proceedings of the Ninth International Conference on Harmful Algal Blooms. Pp. 101–104.
Rhodes, L.; Rublee, P.; Burkholder, J. 2000: Pfiesteria in New Zealand. Proceedings of the Marine Biotoxin Science Workshop No. 14. Wellington. MAF Regulatory Authority. Pp. 87–89.
Rublee, P. A., Kempton, J.; Schaefer, E.; Allen, C.; Harris, J.; Oldach, D. W.; Bowers, H.; Tengs, T.; Burkholder, J.; Glasgow, H. B. Jr. 2002: Use of molecular probes to assess geographic distribution of Pfiesteria species. Environmental Health Perspectives: in press.
Rublee, P. A.; Kempton, J.; Schaefer, E.; Burkholder, J.; Glasgow, H. B. Jr.; Oldach, D. 1999: PCR and FISH detection extends the range of Pfiesteria piscicida in estuarine waters. Virginia Journal of Science 50: 325–336.
Samet, J.; Bignami, G. S.; Feldman, R.; Hawkins, W.; Neff, J.; Smayda, T. 2001: Pfiesteria: review of the science and identification of research gaps. Report for the National Center for Environmental Health, Centers for Disease Control and Prevention. Environmental Health Perspectives 109(5): 639–659.
Schaefer, E. F. 1997: A DNA assay to detect the toxic dinoflagellate Pfiesteria piscicida, and the application of a PCR based probe. Unpublished MSc thesis, University of North Carolina at Greensboro, United States.
Schmechel, D. E.; Koltai, D. 2001: Potential human health effects associated with laboratory exposures to Pfiesteria piscicida. Environmental Health Perspectives 109(5): 775–779.
Smith, S. A.; Noga, E. J.; Bullis, R. A. 1988: Mortality in Tilapia aurea due to a toxic dinoflagellate bloom. Proceedings of the 3rd International Colloquium of Pathology in Marine Aquaculture. Gloucester, VA. Pp. 167–168.
Springer, J. J. 2000: Interactions between two commercially important species of bivalve molluscs and the toxic estuarine dinoflagellate, Pfiesteria piscicida. Unpublished MSc thesis, North Carolina State University, Raleigh, NC, United States.
Springer, J. J.; Shumway, S. E.; Burkholder, J. M.; Glasgow, H. B. 2002: Interactions between two commercially important species of bivalves and the toxic estuarine dinoflagellate, Pfiesteria piscicida. Marine Ecology Progress Series: in press.
Steidinger, K. A.; Burkholder, J. M.; Glasgow, H. B. Jr.; Hobbs, C. H.; Garrett, J. K.; Truby, E. W.; Noga, E. J.; Smith, S. A. 1996: Pfiesteria piscicida gen. et sp. nov. (Pfiesteriaceae fam. nov.), a new toxic dinoflagellate with a complex life cycle and behaviour. Journal of Phycology 32: 157–164.
Steidinger, K. A.; Babcock, C.; Mahmoudi, B.; Tomas, C.; Truby, E. 1989: Conservative taxonomic characters in toxic dinoflagellate species identification. In: Lassus, P.; Arzul, G.; Erard-Le Denn, E.; Gentien, P.; Marcaillou-Le Bout, C. ed. Harmful marine algal blooms. Paris, Lavoisier. Pp. 83–88.