

INTERNATIONAL ACCREDITATION OF rRNA-TARGETED SANDWICH HYBRIDISATION ASSAYS



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1. INTRODUCTION **A.** Methods of rapid identification and enumeration are needed to anticipate raphidophyte blooms in New Zealand, which have caused economic losses to the fin fish industry in the past. The Sandwich Hybridisation Assay (SHA) utilises species specific oligonucleotide probes targeted at ribosomal RNA and is ideal as cells commonly collapse during the fixation stage of sample collection, compromising identification by traditional microscopy. SHA has been available for some time, but required validation and international accreditation for commercial laboratory use in New Zealand. **B.** The comparability of SHA cell number estimates with whole cell format DNA probes and traditional microscope counts has been questioned; the NZ bloom-former *Pseudo-nitzschia australis* was assessed using all three methods.

2. METHODS **A.** Field trials included seawater samples spiked with cultured *F. japonica* and *H. akashiwo* from Cawthron's Culture Collection, and *H. akashiwo* blooms. Analysis followed the methods of Scholin et al (1996) and Tyrrell et al. (2002). Samples were filtered, lysed and assayed by SHA. O.D. readings were mapped to a std. curve (derived from cultures) and cell conc. estimated. **B.** SHA cell estimates for *P. australis* (based on a std. curve generated from cultured isolates) were compared with whole cell format DNA probe estimates (Scholin et al., 1996) and light microscope counts (using Cawthron's IANZ accredited monitoring protocols).

3. SHA cell estimates for raphidophytes (derived from std. curves) SHA estimates for *H. akashiwo* and *F. japonica* compared favourably with light microscopic counts except for samples 4 and 11–*H.aka.* and 2–*F.jap.* (Fig. 1).

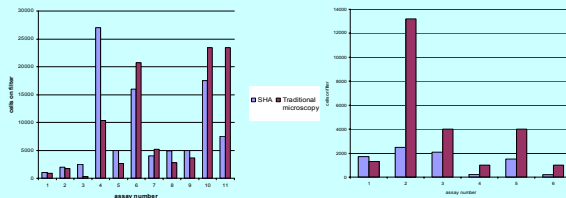


Fig. 1 Comparison of SHA estimates and light microscope counts: (l) *H. akashiwo*, (r) *F. japonica*.

4. Raphidophyte risk assessment

Cell number estimates following SHA can be mapped to pre-determined risk values to provide guidance to aquaculturalists and sea-food regulators. If cell concentrations pose an ichthyotoxic threat or a risk of NSP toxins occurring in shellfish (Table 1), then results are immediately reported by fax or phone.

Table 1. Risk levels for raphidophytes (cells L-1).

	Shellfish Poisoning	Fish Killing
Low	1 - 10,000	1 - 1000
Moderate	10,001 - 100,000	1,001 - 10,000
High	100,001 - 1,000,000	10,001 - 100,000
Very High	> 1,000,001	> 100,001



5. Comparison of enumeration methods for Pseudo-nitzschia australis

Following the generation of a std. curve based on known cell numbers, spiked cultures were split into three and analysed by SHA, whole cell DNA probe assay and light microscopy. Results were highly comparable (Fig. 3).

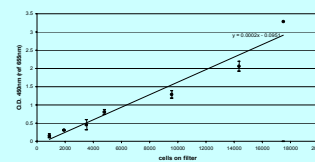


Fig. 2 Std. curve for *P. australis* (av. O.D. with error bars vs cell count based on light microscopy).

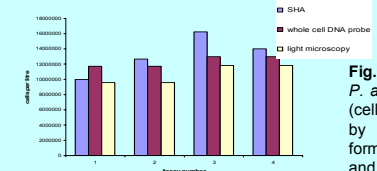


Fig. 3 Comparison of *P. australis* estimates (cells L⁻¹), determined by SHA, whole cell format DNA probes and microscopy.

6. Conclusions

A. The SHA has international accreditation (IANZ: ISO 17025) for raphidophytes and provides a rapid detection/ identification/ enumeration of *H. akashiwo* and *F. japonica* in seawater samples.

Discrepancies between SHA and light microscope counts arise due to factors outlined previously (Tyrrell et al. 2001, 2002), eg, late stationary phase cultures of *H. akashiwo* can yield lower cell abundance estimates using SHA compared to light microscopy. Samples with compromised cells (e.g. due to delays between collection and analysis) may also result in low SHA estimates relative to direct counts (eg, 11-*H.aka* and 2-*F.jap*, Fig.1). Conversely, samples with cells that clump, are hidden in organic matter or lyse when viewed microscopically, can give the opposite results (eg, 4-*H.aka*, Fig.1). Despite these anomalies, SHA provides an excellent risk warning for the aquaculture industry, with detection limits of $\leq 3.0 \times 10^3$ cells L⁻¹, within the low to moderate NSP risk assessment level.

B. The 3 methods trialled for *P. australis* correlated well; SHA is an excellent and rapid method of enumeration, particularly during bloom development.

References: Scholin C.A., Buck, K.R., Britschgi, T., Cangelosi, G., Chavez. 1996. *Phycologia* 35:190-197; Tyrrell, J.V., C.A. Scholin, P.R. Børguist and P.L. Børguist. 2001. *Phycologia* 40: 457-467; Tyrrell, J.V., Connell, L.B., Scholin, C.A. 2002. *Harmful Algae* 1:205-214.