Use of molecular approaches to investigate the dynamics of the red tide dinoflagellate *Lingulodinium polyedrum*

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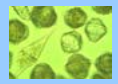
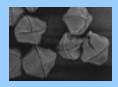
1) Introduction

The bioluminescent dinoflagellate *Lingulodinium polyedrum* is capable of forming red tides that have been associated with fish and shellfish mortality events (e.g. Marasovic 1989, Bruno et al. 1990). *L. polyedrum* occasionally forms massive blooms along the coast of Southern California, demonstrating highly seasonal population dynamics (major blooms in April/May and September/October). Despite its potential ecological importance, the role of this red tide alga within planktonic food webs, and factors that trigger blooms, remain largely unknown.





Quantitative real-time PCR (QPCR) is a molecular approach that has enabled species-specific detection and enumeration of target microbial species. It has been rapidly adapted for use with environmental samples to follow population dynamics of selected species (e.g. Johnsen et al. 1999, Tay et al. 2001). This method offers extreme sensitivity and specificity, the ability to estimate abundances over a very wide dynamic range and relative ease of use. **This study presents the design of a QPCR approach for the detection of *L. polyedrum*, a comparison with traditional microscopical methods and its application in natural water samples to asses the population dynamics of *L. polyedrum*.**

Fig. 6: Glass column with *L. polyedrum* concentrated in the surface area (arrow).

3) Comparison of microscopical versus molecular methods in a laboratory setting

Many phototrophic dinoflagellates in the plankton migrate vertically, typically ascending during the morning and descending at night (e.g. Blasco 1978, Villarino et al. 1995). These patterns have been correlated to contrasting light and nutrient gradients (e.g. Jones 1993), optimizing light availability for photosynthesis during the day and nutrient uptake during the night. Here, the vertical migration of *L. polyedrum* was investigated in the laboratory in a 2m glass-column (diameter 11cm, 20°C, thermocline at 107cm, Fig 6). Tubing along the thermostat enabled sampling at a very high spatial resolution (Fig. 6). A light:dark cycle of 11h:13h was applied, with the light switched on at 6am and off at 5pm.

The column was filled with f/2 media (Guillard & Ryther 1962) and inoculated with 4l of a concentrated *L. polyedrum* culture (1600 cells/ml). After letting the culture establish in the column for one week, samples were removed over a period of 3 days at 5:00, 9:00, 12:00, 15:00, 18:00 and 21:00 (the last time point was sampled only on day 2 and 3) to compare microscopical counts (Lugol's preserved samples) with the quantification determined via QPCR. Samples were taken from the following depths:

Lugol's (day 1-3): 2, 4, 6, 8, 10, 12, 20, 30, 40, 50, 60, 70, 80, 90 96, 100, 104, 108, 112 and 127cm

QPCR (day 2): 4, 12, 30, 60, 100, 112cm

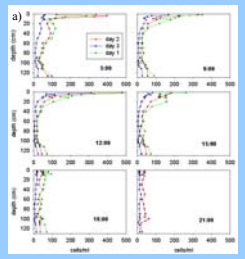


Fig 7: Vertical distribution of *L. polyedrum* cells determined from Lugol counts a) from day 1-3 at different times during the day and b) on day 2 in comparison to cell quantification via QPCR.

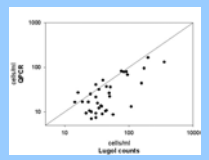


Fig. 8: Abundances of *L. polyedrum* determined via Lugolcounts versus QPCR

- The *L. polyedrum* population showed marked differences in vertical position over the daily cycle. Cells concentrated in the surface layer of the column in the morning, even before the light was switched on, but were almost evenly dispersed in the evening
- Trends in cell abundance were similar for microscopical estimates and QPCR (Fig. 7)
- Cell abundances determined via QPCR matched Lugol counts in a number of samples, but were generally lower (Fig. 8). These discrepancies may reflect…

⇒ variability in DNA content of *L. polyedrum* (e.g. due to cell cycle)
⇒ presence of moribund cells in Lugol preserved samples that were counted, but not detected in QPCR

4) Application of Lingulo-QPCR approach in natural water samples

Samples were taken on a weekly basis over a time period of 3 months from coastal waters near Long Beach, south of Los Angeles, CA. This area was chosen for monitoring *L. polyedrum* abundances due to major *L. polyedrum* blooms in this area in the past (Fig. 9)

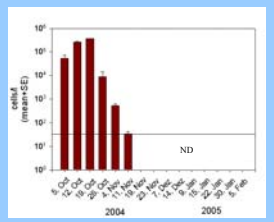


Fig. 10: Absolute abundances of *L. polyedrum* cells detected via QPCR. The line represents the lower detection limit of cells (total of 9 cells).

- high abundances of *L. polyedrum* in October, lower abundances in November
- in December through February, *L. polyedrum* was not detected beyond a minimum concentration of 30 cells/liter (detection limit of 9 cells; 250 ml samples).

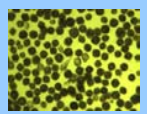


Fig. 9: *L. polyedrum* bloom in October 2003: Natural (unconcentrated) water samples were completely dominated by this red tide alga.

2) Design and testing of a QPCR approach for the detection of *L. polyedrum*

L. polyedrum was isolated from natural water samples from the coast of Los Angeles, California, and the 18S rDNA of the resulting culture was cloned and sequenced. A molecular beacon probe and flanking primers were designed using a dedicated software package ("Beacon-Designer 4", Premier Biosoft International, Palo Alto, California)

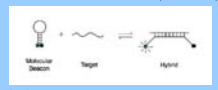
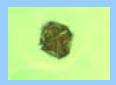


Fig. 1: Molecular beacons

- single-stranded oligonucleotide hybridization probes form a stem-and loop structure
- loop contains the probe sequence complementary to the target sequence
- a fluorophore is covalently linked to the end of one arm and a quencher to the end of the other arm

⇒ molecular beacons brightly fluoresce when they hybridize to a nucleic acid strand containing a target sequence, but not while they are free in solution

Fig. 2: Position of primers and molecular beacon probe within the 18S rDNA of *L. polyedrum*



In silico testing of probe and primer specificity:

1) alignment of probe and primer sequences with sequences of closely related dinoflagellates to ensure sequence mismatches with other species than *L. polyedrum*.
2) blasting sequences against NCBI, a public database that contains large amounts of 18S rDNA protistan sequence data to test for specificity.

In situ testing of probe and primers with plasmids and cell lysates (1:100):

1) melting of PCR product of specific target to ensure that a single product is created (see Fig. 3)
2) testing different Mg2+-concentrations and lysate volumes in PCR reaction to account for potential inhibitory effects (not shown)
3) creating standard curves from plasmid and cell lysate dilution series to obtain the range of detectable DNA and cell concentrations (see Fig. 4a, b)
4) spiking different amounts of a *L. polyedrum* culture with a known concentration into natural seawater (prefiltered through a 200µm gauze) and 0,2µm filtered autoclaved seawater. Standard curves were compared to preclude inhibitory effects on the quantitative detection of *L. polyedrum* in natural water samples (Fig. 5). No inhibitory effects were observed.

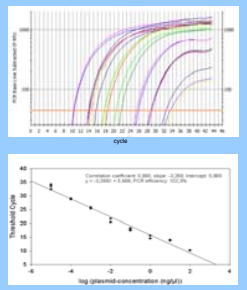


Fig 4: Standard curves created from plasmid (a) and cell lysate (b) dilution series. The standard curve created from plasmid was linear for > 8 orders of magnitude (100ng - 0,0001 ng DNA). The standard curve created from cell lysates was linear for > 6 orders of magnitudes, from a total of 8,5 x 105 cells down to 8,5 cells.

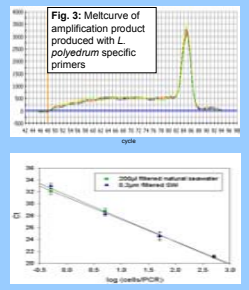


Fig. 5: Standard curves of *L. polyedrum* lysates created in 0,2µm filetered, autoclaved seawater versus natural seawater

5) Conclusions:

- QPCR is a promising approach to monitor abundances of *L. polyedrum* abundances in natural water samples and in the laboratory
- cells can be detected at low cell concentrations (minimum of 9 cells) without any taxonomic expertise based on morphological features
- more samples can be processed in a shorter period of time compared to Lugol counts

6) Future investigations:

Manipulation of natural water samples in the laboratory during *L. polyedrum* blooms:

nutrient additions (bottom-up) and additions of grazers (top-down) to follow the population-dynamics of *L. polyedrum* via QPCR to investigate its ecological role in planktonic food webs and potential factors influencing its blooms

Ingestion of *L. polyedrum* by a tintinnid ciliate.

References:

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Acknowledgements:
Funding was provided by the following NSF grants: Science Technology Centre for Embedded Network Sensing, Deborah Estrin, PI; MCB – 0084231, J. Fuhrman, D. A. Caron, PIs; as well as by the German Academic Exchange Service (DAAD).