Stephan Wietkamp
Alfred-Wegener-Institute for Polar and Marine Research
Am Handelshafen 12, 27570 Bremerhaven/Germany
stephan.wietkamp@awi.de

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

New quantitative real-time PCR assay to detect and quantify the azaspiracid-producing dinoflagellate *Amphidoma languida*

New real-time PCR assay for toxigenic *Amphidoma languida*

Stephan Wietkamp[1*], Urban Tillmann[1], Dave Clarke[2] and Kerstin Toebe[1]
[1] Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research, Germany
[2] Marine Institute, Ireland
* Presenting author

Background

- Azaspiracids (AZA) are responsible for azaspiracid-shellfish-poisoning (AZP) syndrome in humans [a,b]
- 4 of 14 Amphidomatacean (dinoflagellata, dinophyta) species are AZA-producers
- Reliable identification and enumeration of Amphidomatacean species in field samples is difficult using light microscopy
- For *Azadinium spinosum* and *Az. poporum*, species-specific qPCR assays are available[c], but not for *Amphidoma languida* (**Fig. 1**)
- *Am. languida* is an AZA-producer, widely distributed in the Atlantic and has caused shellfish AZA-contamination in southern Spain

Methodology

A TaqMan qPCR assay with specific primers and a MGB-probe was designed to amplify 60 bp of the D2 region (LSU/28S) of the ribosomal DNA (rDNA). The MGB-chemistry was chosen, because it allowed the design of short probes with strong binding attributes. The specificity for *Am. languida* was thoroughly tested with a variety of target and non-target species and strains. The quantification ability and intra-clonal variability (especially variations in the rDNA copy number) was tested with spike experiments and with different growth stages of the microalgal culture. Additionally, the assay was applied to field samples off the Irish coast.

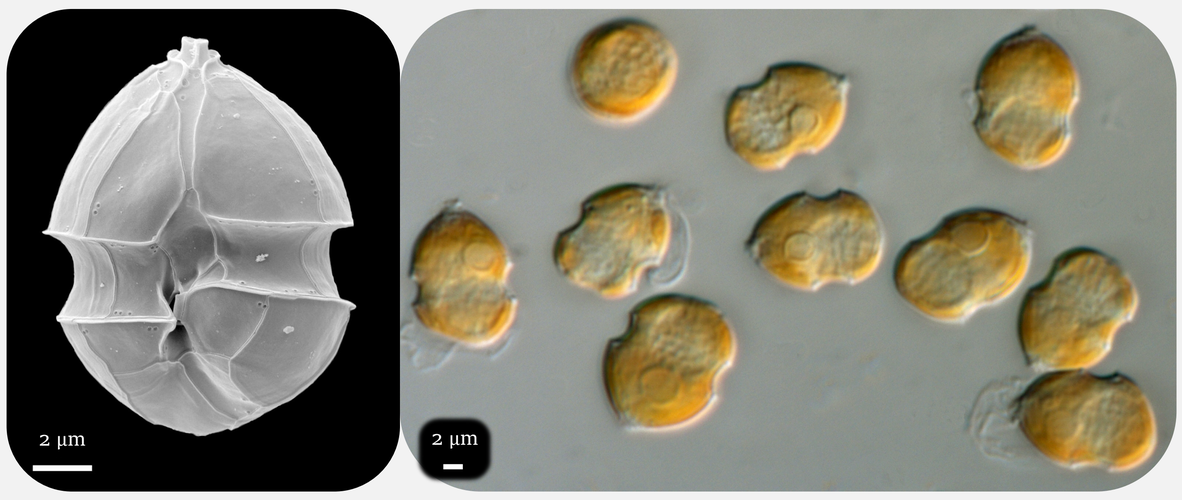
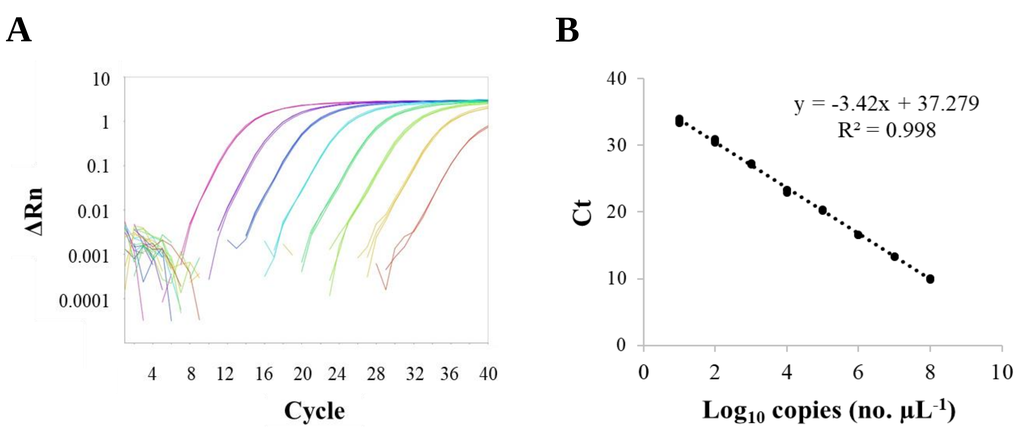


Fig. 1: *Amphidoma languida*. SEM micrographs (left) and LM of formalin fixed cells (right). Widely distributed, with records from Ireland, Norway, Iceland, the Black Sea, the West Indian Ocean, the Argentinean shelf and off the Southern Spanish coast.

Results

In total, DNA of more than 50 target and non-target species/strains were tested. The assay revealed high specificity, even when DNA of closely related taxa like *Azadinium* spp. was present. Quantification was possible down to 10 target DNA copies (equiv. to < 0.1 cells) per reaction (**Fig. 2**). Molecular probes bind to every single rDNA copy, thus any variation in rDNA copy numbers is critically affecting quantification. Here, rDNA copy number per cell did not vary significantly over time and between strains (**Fig. 3**). Finally, the assay was applied to field samples off the Irish coast. *Am. languida* was detected on the south-western coast of Ireland– the area, where the type strain was previously described (**Fig. 4**).

High sensitivity: Reliable quantification down to 10 rDNA copies



Full recovery of cultured cells added to field samples

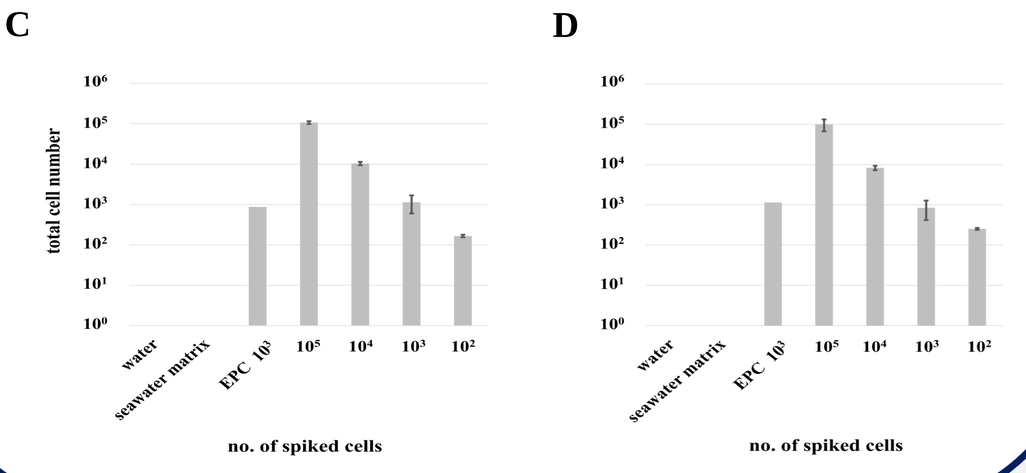
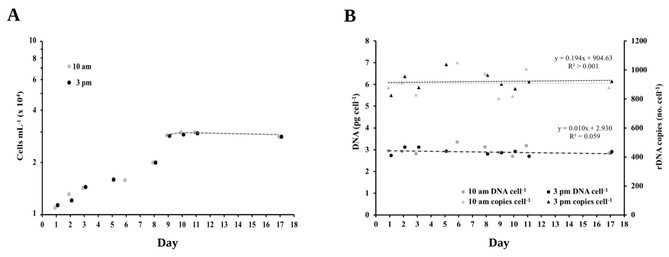


Fig. 2: Amplification plot (A) & corresponding standard curve (B) of serial dilutions of rDNA copies. Cell recovery from CT values of known cell numbers in qPCR for two independent experiments (C & D). Bars represent mean ± 1SD (n=6).

rDNA copy no. consistent over the growth cycle



Copy no. consistent among different strains

Strain	Origin	Mean LSU copy number (no. cell^{-1}) ± SD	Mean DNA content (pg cell^{-1}) ± SD
2A11	North Atlantic, Iceland	719 ± 48	2.73 ± 0.18
N-12-01	North Atlantic, Norway	830 ± 93	3.15 ± 0.35
AND-0920	North Atlantic, Spain	777 ± 38	2.95 ± 0.15
Z-LF-9-C9	North Sea, Denmark	829 ± 107	3.15 ± 0.41

Fig. 3: Growth cycle of *Am. languida* over 17 days. Cell density (A), variations in rDNA copy number (triangles) & DNA content (circles) cell^{-1} (B) over 17 days. LSU gene copy number & DNA content cell^{-1} in 4 different *Am. languida* strains (Table; n=6).

Successfully tested on field samples

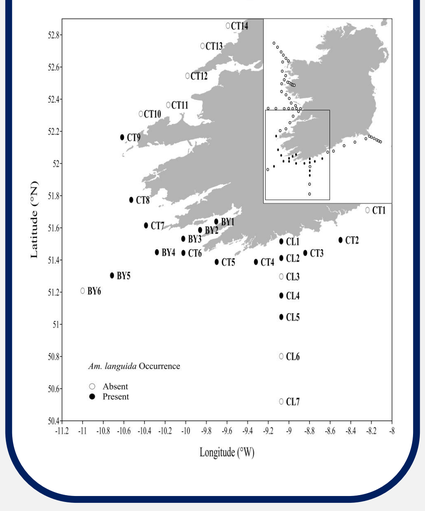


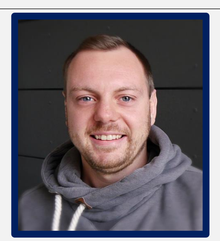
Fig. 4: Sampling locations of the coastal survey in Irish waters in 2017.

Conclusion

This newly developed assay is highly specific, sensitive and allows quantification of even low concentrations. Therefore, it is suited for biogeographical studies and will improve the understanding of population dynamics of *Am. languida* in the ecosystem. Furthermore, it can be a powerful tool for surveillance of AZA-producers in monitoring programs.

References

[a] Tillmann, U., Salas, R., Gottschling, M., Krock, B., O'driscoll, D. and Elbrächter, M. (2012) *Amphidoma languida* sp. nov. (Dinophyceae) reveals a close relationship between *Amphidoma* and *Azadinium*. *Protist,* **163,** 701-19.
[b] Krock, B., Tillmann, U., Voss, D., Koch, B. P., Salas, R., Witt, M., Potvin, E. and Jeong, H. J. (2012) New azaspiracids in Amphidomataceae (Dinophyceae). *Toxicon,* **60,** 830-839.
[c] Toebe, K., Joshi, A. R., Messtorff, P., Tillmann, U., Cembella, A. and John, U. (2013) Molecular discrimination of taxa within the dinoflagellate genus *Azadinium*, the source of azaspiracid toxins. J. Plankton Res., 35, 225-230.



This study was financially supported by the German Ministry for Education and Research.

RIPAZA

Project ident.: 03F0763A

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

Marine Institute
Foras na Mara

POLMAR
Helmholtz Graduate School for Polar and Marine Research

HELMHOLTZ GEMEINSCHAFT

AWI
Am Handelshafen 12
27570 Bremerhaven
Telefon 0471 4831-0
www.awi.de