Journal of
Plankton Research

academic.oup.com/plankt

J. Plankton Res. (2021) 43(3): 497–499. First published online April 30, 2021 doi:10.1093/plankt/fbab029

BRIEF COMMUNICATION

Development of a TaqMan PCR assay for the identification of the non-native copepod *Acartia tonsa*, and detection of this species in Norwegian coastal waters

CAMILLA V. S. MOSEID[1], TONE FALKENHAUG[2] AND AUDUN SLETTAN[1,*]

[1]CENTRE OF COASTAL RESEARCH, DEPARTMENT OF NATURAL SCIENCES, UNIVERSITY OF AGDER, P.O. BOX 422, KRISTIANSAND, NO-4604, NORWAY. AND [2]INSTITUTE OF MARINE RESEARCH, NYE FLØDEVIGVEIEN 20, NO-4817 HIS, NORWAY

*CORRESPONDING AUTHOR: audun.slettan@uia.no

Received October 28, 2020; revised March 30, 2021; accepted March 31, 2021

Corresponding editor: Xabier Irigoien

Molecular based assays for detection of species are a powerful tool to supplement morphological methods that may be time and labor intensive. Here we describe a sensitive TaqMan real time polymerase chain reaction assay that specifically detects the presence of *Acartia tonsa* in mixed plankton samples. The assay is used to find this non-native copepod in samples collected in Norwegian coastal waters.

KEYWORDS: *Acartia tonsa*; qPCR; TaqMan

The planktonic crustacean *Acartia tonsa* (*A. tonsa*) Dana, 1849 (Copepoda:Calanoida), is a nonindigenous species common in European marine systems. The species is native to American and Indo–Pacific waters (Leppäkoski & Olenin, 2000), but has been spread worldwide and is now regarded as cosmopolitan. *A. tonsa* was first recorded in Norway in 2012 (Haraldstad *et al.*, 2013). *A. tonsa* is morphologically very similar to native *Acartia* species, and it is time-consuming and demands taxonomic expertise to detect and monitor this species by traditional morphological techniques. Nonindigenous species may displace or replace native species and are considered as the main threat to the biodiversity and ecosystem health in Europe (The Marine Strategy Framework Directive, EU 2008). Early detection and correct species identification are essential in the management of invasive species (Leung *et al.*, 2002, Sandvik *et al.*, 2020). However, the presence of cryptic species and lack of taxonomic

available online at academic.oup.com/plankt

expertise, makes species identifications both challenging and time consuming, and often introductions of alien species have been overlooked for several years (Marchini & Cardeccia, 2017, Ojaveer *et al.*, 2014). It is not unlikely that *A. tonsa* has been present in Norwegian waters for several years, prior to the first record from 2012. The species was recorded in Danish and Swedish waters in 1916 and 1934, respectively, which is upstream from the Norwegian Skagerrak coast. Estuaries in Norway have generally been poorly studied and *A. tonsa* may have been confused with the native congeneric species *A. longiremis*, *A. clausii* and *A. discaudata*. This emphasizes the need for a rapid and accurate process for the detection of this non-native species. The here described assay is such a method.

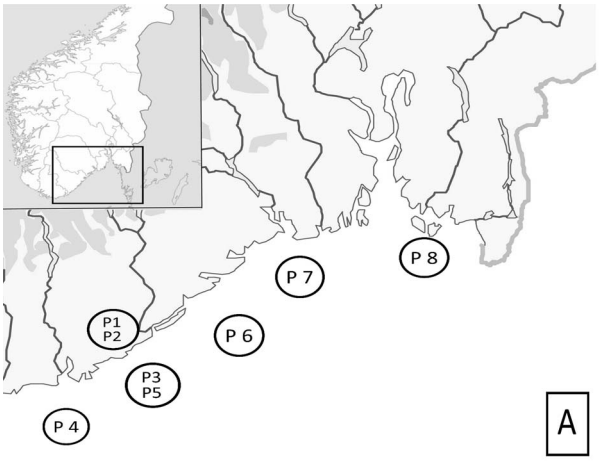
Molecular methods are powerful tools for rapid and accurate species identifications and have been used to detect and describe cryptic invasions of morphologically indistinct alien species (Comtet *et al.*, 2015). Here we present a novel species-specific quantitative polymerase chain reaction (qPCR) assay for rapid detection of *A. tonsa*.

Samples were collected by vertical hauls using a WP2 plankton net (opening area 0.25 m^2, 180 μm mesh size, towing speed 0.5 m-s) from close to the bottom to the surface. Samples were fixed in 96% ethanol and stored at −20°C. Six samples (P3–P8) were collected at five locations on the Skagerrak coast, and two samples (P1 and P2) were collected in an inland brackish water lake, Landvikvannet (Fig. 1A).

Morphological identification of adult *A. tonsa* (collected separately), to be used as positive control, was made according to (Redeke, 1934), by the shape of the exopod of the fifth pair of legs.

DNA was isolated by homogenizing the sample by bead beating followed by extraction with DNeasy Blood and Tissue kit (Qiagen) following the producer's protocol. The concentration and purity of the DNA (measured on a Nanodrop 1000 instrument (Thermofisher)) of all samples were found to be in acceptable range for PCR analysis.

PCR primers and TaqMan probe for specific detection of *A. tonsa* were designed by aligning available DNA sequences of gene for cytochrome c oxidase I (COI) using Clustal Omega (EMBL-EBI) and Primer-Blast software (NCBI). A qPCR, performed on StepOne Plus Real-Time PCR System (Thermo Fisher), 20 μL reaction volume, consisted of 1x TaqMan Environmental Master Mix 2.0 (Thermo Fisher), 0,90 μM of each of the two primers (AtonsCOI-F: 5′ AATGTGGTCAGGAATGGTTGG 3′, AtonsCOI-R: 5′ CGCCCCTCTTTCTACTAGCGA 3′), 0,25 μM of the probe (AtonsCOI-probe: 5′ FAM-TAGTCACCGCCCACGCT-MGBEQ 3′) and 5 μL template (78–168 ng DNA). The temperature profile



Sample	Position UTM-32 N/E	Ct I	Ct II	Ct III
P1	6464437.88/471881.29	31,4	32,0	31,9
P2	6464446.41/470709.69	41,6	35,4	34,7
P3	6471035.39/488303.67	53,7	53,0	49,9
P4	6448014.29/445290.27	-	-	-
P5	6471035.39/488303.67	-	-	-
P6	6510014.99/514475.34	-	-	-
P7	6536973.12/544851.13	-	-	-
P8	6561333.28/597151.92	-	-	-

B

Fig. 1. (**A**) Sampling sites P1—P8 along south east coast of Norway. (**B**) Results of qPCR analysis of samples P1–P8. Each sample is analyzed in triplicate (I–III). The Ct (Cycle threshold) value is shown for positive samples. Negative samples are shown as −.

consisted of an initial step of 10 min at 95°C followed by 60 cycles of 15 sec at 95°C and 1 min at 60°C.

To verify that the qPCR amplicons from the positive samples (P1, P2 and P3) originated from *A. tonsa*, the amplicons were Sanger sequenced. The sequences were BLASTed against NCBI Genbank nt data base as well as aligned with *A. tonsa* COI sequences from this data base. Furthermore, the specificity of the system was tested in qPCR analysis of genomic DNA from various copepod species isolated from the here described samples following the same protocol as described above. The following organisms were analyzed (in triplica): *Acartia clausi*, *Acartia sp.*, *Acartia tonsa*, *Calanus finmarchicus/helgolandicus*, *Calanus hyperboreus*, *Candacia norvegica*, *Centropagus typicus*, *Cyclops abyssorum*, *Erytemora affinis*, *Harpacticoida sp.*, *Metridia longa*, *Metridia lucens*, *Oithona atlantica*, *Oncaea sp.*, *Paracalanus sp.*, *Pseudocalanus sp. and Temora longicornis*. Only *Acartia tonsa* produced positive qPCR result. All other samples were negative (data not shown). The sensitivity of the assay was determined by qPCR analysis of a 10-fold serial dilution of *A. tonsa* genomic DNA following the above-described protocol.

Three (P1, P2 and P3) of the eight collected samples became positive in the qPCR (Fig. 1B). The DNA sequence of amplicons from these positive samples had identical DNA sequence with COI sequences of *A. tonsa* found in the NCBI Genbank database (sequences deposited to Genbank numbers MW067668, MW067669, MW067670). In the sensitivity analysis, the lowest concentration of *A. tonsa* DNA in the PCR reaction that gave positive result, was 40 fg per ul (data not shown), i.e. 0.31 × genome of the organism in 20 μL reaction volume since the genome of *A. tonsa* is estimated to 2.5 Gbp (Jørgensen *et al.*, 2019).

We describe here the development of a sensitive, species-specific qPCR assay for rapid and reliable detection of *Acartia tonsa*. The here described qPCR assay will make it possible to relative quickly detect whether *A. tonsa* is present in bulk zooplankton samples. The assay indicates the relative difference in concentration of this species at the three locations P1, P2 and P3. The low Ct values obtained in samples P1 and P2, indicate higher concentrations of *A. tonsa* in the brackish water lake Landvikvannet, than at the offshore location (P3) where this species has not been recorded earlier.

Here we demonstrate a rapid, accurate and reliable method for the detection of *Acartia tonsa*. The method is ideal for providing an early detection of this nonindigenous species, even when it is present at low densities, and may complement traditional methods for species identification and monitoring.

REFERENCES

Comtet, T., Sandionigi, A., Viard, F. and Casiraghi, M. (2015) DNA (meta)barcoding of biological invasions: a powerful tool to elucidate invasion processes and help managing aliens. *Biol. Invasions*, **17**, 905–922.

Haraldstad, T., Hindar, A., Hobæk, A., Håvardstun, J. and Skjelbred, B. (2013) *Resipientundersøkelse av Reddalsvann*, NIVA, Grimstad.

Jørgensen, T. S., Petersen, B., Petersen, H. C. B., Browne, P. D., Prost, S., Stillman, J. H., Hansen, L. H. and Hansen, B. W. (2019) The genome and mRNA transcriptome of the cosmopolitan calanoid copepod acartia tonsa dana improve the understanding of copepod genome size evolution. *Genome Biol. Evol.*, **11**, 1440–1450.

Leppäkoski, E. and Olenin, S. (2000) Non-native species and rates of spread: lessons from the brackish Baltic Sea. *Biol. Invasions*, **2**, 151–163.

Leung, B., Lodge, D. M., Finnoff, D., Shogren, J. F., Lewis, M. A. and Lamberti, G. (2002) An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proc. R. Soc. Lond. Series B: Biol. Sci*, **269**, 2407–2413.

Marchini, A. and Cardeccia, A. (2017) Alien amphipods in a sea of troubles: cryptogenic species, unresolved taxonomy and overlooked introductions. *Mar. Biol.*, **164**, 69.

Ojaveer, H., Galil, B., Gollasch, S., Marchini, A., Minchin, D., Occhipinti, A. and Olenin, S. (2014) Identifying the top issues of marine invasive alien species in Europe. *Manag. Biol. Invasion*, **5**, 81–84.

Redeke, H. C. (1934) On the occurrence of two pelagic copepods, Acartia bifilosa and Acartia tonsa, in the brackish waters of the Netherlands. *ICES J. Mar. Sci.*, **9**, 39–45.

Sandvik, H., Hilmo, O., Henriksen, S., Elven, R., Åsen, P. A., Hegre, H., Pedersen, O., Pedersen, P. A. *et al.* (2020) Alien species in Norway: results from quantitative ecological impact assessments. *Ecol. Solutions Evidence*, **1**, e12006.