

Recording of *Gyrodactylus salaris* by analysis of environmental DNA in water samples from rivers in Norway

Slettan A, Olsen Y, Andersen DO

Department Natural Sciences, University of Agder, Kristiansand, Norway
audun.slettan@uia.no

Abstract

Background: *Gyrodactylus salaris* is a freshwater monogenean ectoparasite highly virulent towards Atlantic salmon and has caused major damage to Atlantic salmon river strains with near extermination of the host in a few years after infection. Current methods for detecting the parasite are expensive and time and work consuming. Results: In order to improve a monitoring program for detection of the parasite, we here report a method for recording the presence of *G. salaris* in a river by real-time PCR analysis of environmental DNA (eDNA) isolated from water samples from the river. Specific barcode sequence of *G. salaris* is recorded. Samples have been analyzed from both infected rivers, previous infected rivers that have been treated for extinction of the parasite and rivers where the parasite never has been recorded. The method shows high sensitivity, and the analysis detects *G. salaris* DNA at all studied locations in infected rivers, whereas none of the water samples from the non-infected river were found to contain DNA from the parasite. As a control, eDNA from Atlantic salmon and brown trout was detected in water samples from all rivers. Significance: These results are promising in the development of a tool that can complement existing monitoring methods for detecting the presence of the parasite *G. salaris* in rivers by recording environmental DNA barcodes.

Introduction

The ectoparasite *Gyrodactylus salaris*, utilizes the Atlantic salmon (*Salmo salar*) as its primary host in freshwater environments (Fig 1). The parasite is highly virulent toward *S. salar*; and has caused major damage to Atlantic salmon river strains in Norway, with near extermination of the salmon population a few years after river infection. *G. salaris* is eradicated by treatment of infected rivers with rotenone or other chemicals. Some rivers treated with these chemicals and classified as “*Gyrodactylus* free” have later been re-infected, demonstrating the importance of a robust and sensitive screening program that includes both uninfected and treated rivers. The method current in use for detecting *G. salaris* is labor- and cost-intensive and requires expertise in all parts of the analysis.

In this study, we wanted to clarify whether we could employ eDNA analyses to record the presence of *G. salaris* in water samples from infected rivers.

Methods

Water was collected at three locations from each river of both gyro infected and non-infected rivers. 400 ml was filtered through a 0.45 µm pore size cellulose nitrat filter (Nalgene). DNA was isolated from the filters by Dneasy Blood and Tissue kit (Qiagen) and the presence of *G. salaris* DNA was recorded by TaqMan qPCR using primers and probe specific for the ITS region (Table 1). As a control, the presence of *S. salar* DNA in all samples was recorded by similar method : 1 x TaqMan Environmental Master Mix 2.0, 0.9 µM of forward and reverse primer, 0.25 µM probe and 5 µL of eDNA sample.

The temperature profile was 50 ° C, 2 min; 95 ° C, 10 min; followed by 60 cycles, 95 ° C, 15 sec; and 60 ° C, 1 min with fluorescence detection after each cycle.

The efficiency and sensitivity of the *G. salaris* PCR in eDNA samples was calculated as described in Ballester et al., 2013

Results and discussion

G. Salaris eDNA was recorded in all water samples collected from gyro infected rivers. No samples from rivers where *G. salaris* never has been detected nor from rivers previously infected and then chemically treated, were positive for *G. salaris* eDNA. Atlantic salmon eDNA was recorded in all samples from all rivers.

PCR efficiency in purified *G. salaris* genomic DNA was 94.6% whereas in eDNA samples the efficiency varied between 108 and 134. This indicates PCR inhibitors present in the eDNA samples.

The mean detection limit of *G. salaris* in the eDNA samples was found to be 102 copies of genomes per reaction.

Conclusion

Our results indicate that the eDNA method may become a valuable tool for screening for *G. salaris* infection in rivers, followed by studies on intensity of the infection. This information can then be expanded to include the infection route, parasite hosts, and fish mortality.

Table 1. Sequence of primers and probes used for detection of *G. salaris* and *S. salar*.

<i>G. salaris:</i>		
Gsal2-probe	FAM-5'-TCTTATTAACCACTTCTGC-3'-NFQ-MGB	Collins et al. 2010
Gsal2-F	5'-CGATCGTCACTCGGAATCG-3'	Collins et al. 2010
Gsal2-R	5'-GGTGGCGCACCTATTCTACA-3'	Collins et al. 2010
<i>S. salar:</i>		
Ss-probe	FAM-5'-CGTAGCTATACTACTTGGCCTAACAT-3'-NFQ-MGB	this work
Ss-F	5'-TCCGATGCCGATAAAATCTC-3'	this work
Sst-R	5'-TGCCTAGGCGAATAGGAAGT-3'	this work



Fig.1 *Gyrodactylus salaris*. Ectoparasite attacking Atlantic salmon, *Salmo salar*, Photo:Jannicke Wiik-Nielsen, Norwegian Veterinary Institute



Fig 2. Sampling locations. Rivers 1, 2 and 3: Previously infected, treated with chemicals and under surveillance. Rivers 4, 5, 6: *G. salaris* never detected, Rivers 7, 8 and 9: Infected by *G. salaris*.

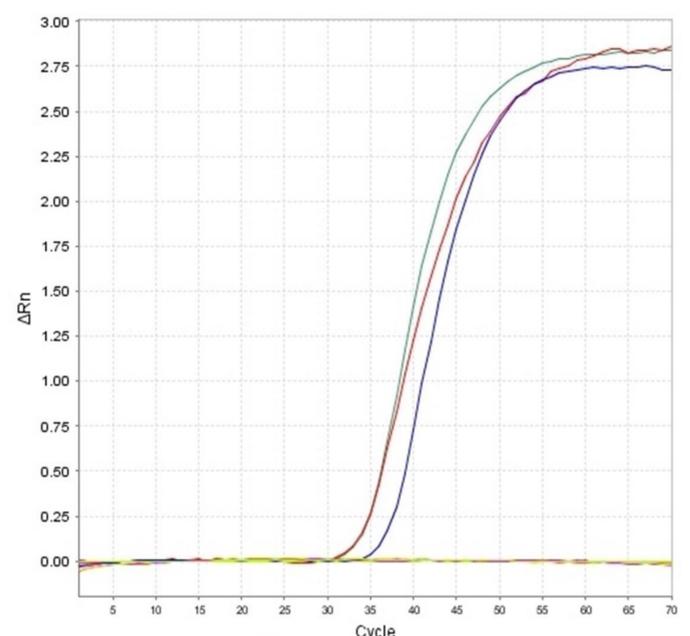


Fig 3. *G. salaris* specific qPCR of three infected and several non infected rivers