The following supplement accompanies the article

Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments

David A. Strand[1,2], Arne Holst-Jensen[1], Hildegunn Viljugrein[3], Bente Edvardsen[4], Dag Klaveness[2], Japo Jussila[5], Trude Vrålstad[1,2,*]

[1]Section of Mycology, Norwegian Veterinary Institute, Pb 750, Sentrum, 0106 Oslo, Norway
[2]Microbial Evolution Research Group (MERG), Department of Biology, University of Oslo, Pb 1066, Blindern, 0316 Oslo, Norway
[3]Section of Epidemiology, Norwegian Veterinary Institute, Pb 750, Sentrum, 0106 Oslo, Norway
[4]Marine Biology, Department of Biology, University of Oslo, Pb 1066, Blindern, 0316 Oslo, Norway
[5]Department of Biosciences, University of Eastern Finland, Kuopio campus, PL 1627, 70211, Kuopio, Finland

*Corresponding author. Email: trude.vralstad@vetinst.no

Diseases of Aquatic Organisms 95: 9–17 (2011)

Supplement. Testing effects of filter on undiluted and diluted (×10) *Aphanomyces astaci* DNA

(1) Testing effect of filter on undiluted DNA

The cycle threshold (C_t) value is modelled as a linear function of $\log_{10}$(**spore concentration**) and **filter** for samples above the limit of quantification (LOQ). Let the data be defined by spore concentration i, spore concentration replicate j and filter k (yes = 1, no = 0). For each treatment-replicate there were 3 repeated measures denoted by l. The effect of filter and spore concentration is estimated by β_1 and β_2, respectively. β_0 is the fixed intercept and residuals are given by ε.

The mixed-effects model:

C_t value $_{i,j,k,l} \sim \beta_0 + \beta_1 * \text{Filter}_{i,j,k,l} + \beta_2 * \log_{10}(\text{NrSpores}_{i,j,k,l}) + a_{i,j} + b_{i,j} * \text{Filter}_{i,j,k,l} + \varepsilon_{i,j,k,l}$ where

$a_{i,j} \sim N(0,d^2)$, $b_{i,j} \sim N(0,v^2)$, and $\varepsilon_{i,j,k,l} \sim N(0,\sigma^2 \times (\delta_{0,j} + |\log_{10}(\text{NrSpores}_{i,j,k,l})|^{\delta j})^2)$.

This model includes both a random intercept (a) and a random slope (b). The random intercept accounts for differences in the mean C_t value for spore concentration replicates. The random slope shows that the strength of the filter effect varies with the spore concentration replicate. The random intercept and random slope are assumed normally distributed with mean zero and standard deiation d and v, respectively. Residuals are assumed normally distributed with mean zero and standard deviation (σ) being weighted according to spore log-concentration and filter. The residual variance structure is modelled by the constant plus the power of the variance covariate function (as estimated by $\delta_{0,j}$ and δ_j) to account for lower variance at higher spore log-concentrations and allowing this relation to be dependent on filter or not.

Fixed-effects estimates:

Fixed effect	$\hat{\beta}_i$ **(SE)**	**DF**	***t***	**p**
β_0	37.53 (0.15)	199	249	<0.0001
β_1	0.84 (0.16)	199	5.20	<0.0001
β_2	–3.59 (0.05)	38	–69.8	<0.0001

Estimated SD of random effects included in model:

Random effect	**N**	**SD**	**Estimate**
Between-replicates intercept ($a_{i,j}$)	40	d	0.29
Between-replicates slope ($b_{i,j}$)	40	v	0.97
Residuals or within-replicates ($\varepsilon_{i,j,k}$)	240	σ	0.41

Estimates of the constant power variance structure:

	$\delta_{0,j}$	δ_j
Filter (j = 1)	0.64	–1.56
No filter (j = 0)	0.54	–4.69

The results indicate that the unexplained variation is larger for filtered than for unfiltered samples. However, the model results are not sensitive to the heterogeneity in the residual variance structure (estimates for the fixed and random effects remain almost unchanged whether or not a constant power variance structure is assumed for the residual variance).

When an interaction term between spore concentration and filter was included in the model, the interaction term was not significant (0.20 ± 0.15 SE, p = 0.16) and there were only slight changes in the other estimates.

(2) Testing effect of filter on diluted (×10) DNA

The mixed-effects model:
$C_t\,(\text{diluted})_{i,j,k,l} \sim \beta_0 + \beta_1 * \text{Filter}_{i,j,k,l} + \beta_2 * \log_{10}(\text{NrSpores}_{i,j,k,l}) + a_{i,j} + b_{i,j} * \text{Filter}_{i,j,k,l} + \varepsilon_{i,j,k,l}$ where
$a_{i,j} \sim N(0,d^2)$, $b_{i,j} \sim N(0,v^2)$, and $\varepsilon_{i,j,k,l} \sim N(0,\sigma^2 \times (\delta_0 + |\log_{10}(\text{NrSpores}_{i,j,k,l})|^{\delta j})^2)$.

Fixed-effects estimates:

Fixed effect	$\hat{\beta}_i$ **(SE)**	**DF**	***t***	**p**
β_0	42.58 (0.34)	191	126	<0.0001
β_1	0.09 (0.24)	191	0.36	0.72
β_2	–3.72 (0.11)	38	–34.0	<0.0001

Estimated SD of random effects included in model:

Random effect	**N**	**SD**	**Estimate**
Between-replicates intercept ($a_{i,j}$)	40	d	0.70
Between-replicates slope ($b_{i,j}$)	40	v	1.34
Residuals or within-replicates ($\varepsilon_{i,j,k}$)	232	σ	1.52

Estimates of the constant power variance structure:
$\delta_0 = 0.21$
$\delta_j = -2.15$

The model results are not sensitive to the heterogeneity in the residual variance structure (estimates for the fixed and random effects remain almost unchanged whether or not a constant power variance structure is assumed for the residual variance).