Supplementary Materials

Stability of saponin biopesticides: Hydrolysis in aqueous solutions and lake waters

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S1 PURITY OF QS-18

An HPLC chromatogram of so-called purified quillaja saponins was provided by Brenntag Biosector A/S and the separation methods was established in our lab with the same results. Details of each distinct quillaja saponins were obtained and QS-18 is the peak that appears from 21~23 min in the chromatogram (including two isomer peaks) (Figure S1). The HPLC (Agilent 1200) chromatogram was obtained by injecting 10 µL of 10 g/L purified quillaja saponins in MilliQ water onto a Vydac C4 column, 4.6 × 250 mm at 20 °C.[1] Flow rate 1 mL/min, and a linear gradient of 30 to 42% acetonitrile with 0.15% (v/v) trifluoroacetic acid with a runtime of 30 min and UV detection at 214 nm. The QS-18 fraction here isolated from purified quillaja saponins was dissolved in MilliQ water and the purity validated in the analytical HPLC at the same conditions as described above. If there were no other

peaks except QS-18 peaks shown in the chromatograph, it illustrates that the QS-18 fraction is relatively pure (Figure S1).

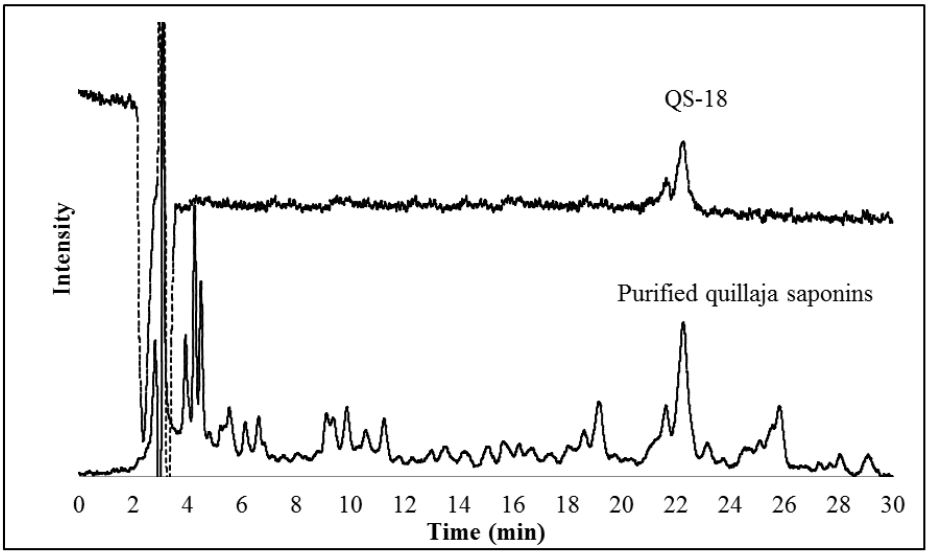


Figure S1 Chromatograph of purified quillaja saponins (standard chromatograph) and isolated QS-18 in HPLC/UV

S2 CHROMATOGRAM AND MASS SPECTRA OF SAPONIN AND THEIR DERIVATIVES

Quantification of QS-18 ($[D-H]^{2-}$, 836.9 m/z) was performed by UPLC-MS/MS using α-hederin ($[M-H]^{-}$, 749.7 m/z) as an internal standard.[2] The UPLC-MS/MS chromatograms of these two compounds are seen in Figure S2 and mass spectra are shown in Figure S3 and S4.

For use of the internal standard (α-hederin), we prepared a known mixture of standard and analyte to measure the relative response of the two species. The area in the chromatogram under each peak is assumed to be proportional to the concentration of the corresponding analytes. However, the detector

may have a different response to each analyte. Here we calculate a relative response factor (*RRF*) of QS-18 relative to α-hederin, see Equation S1.[3]

$$\frac{Area_{\mathrm{QS-18}}}{Area_{\mathrm{\alpha-hederin}}} = RRF \times \frac{c_{\mathrm{QS-18}}}{c_{\mathrm{\alpha-hederin}}} \qquad \textbf{Equation S1}$$

where *RRF* is the relative response factor between QS-18 and α-hederin, and *Area* and *c* refer to peak areas and concentrations. From Figure S5, we infer that *RRF* is 0.967 ± 0.011, demonstrating that the QS-18 peak area is 0.967 times the peak area of α-hederin when they are present at the same concentration. As the RRF is very close to one we use *RRF* =1 in this work. A standard curve for α-hederin is shown in Figure S6.

Desacylsaponin ($[M-H]^-$, 1675 m/z) and prosapogenin ($[M-H]^-$, 955.7 m/z) were identified by UPLC-MS/MS with selected samples undergoing hydrolysis. The mass spectra are shown in Figures S7 and S8, proving the existence of desacylsaponin and prosapogenin, while mass scans show there is no trace of the aglycone (m/z, $[M-H]^-$, 485.5, Figure S9) demonstrating that the aglycone is not produced during hydrolysis.

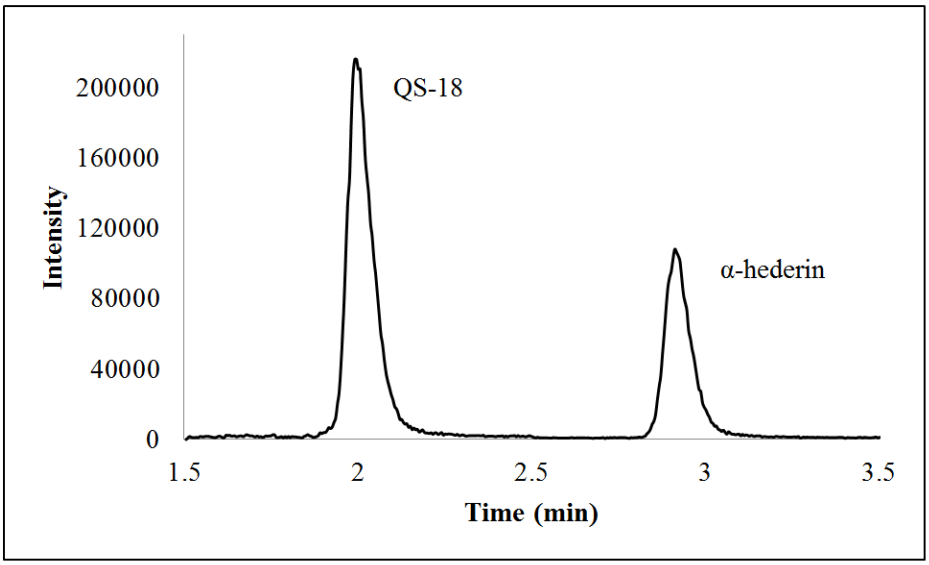


Figure S2 Chromatogram of QS-18 and α-hederin using UPLC-MS/MS

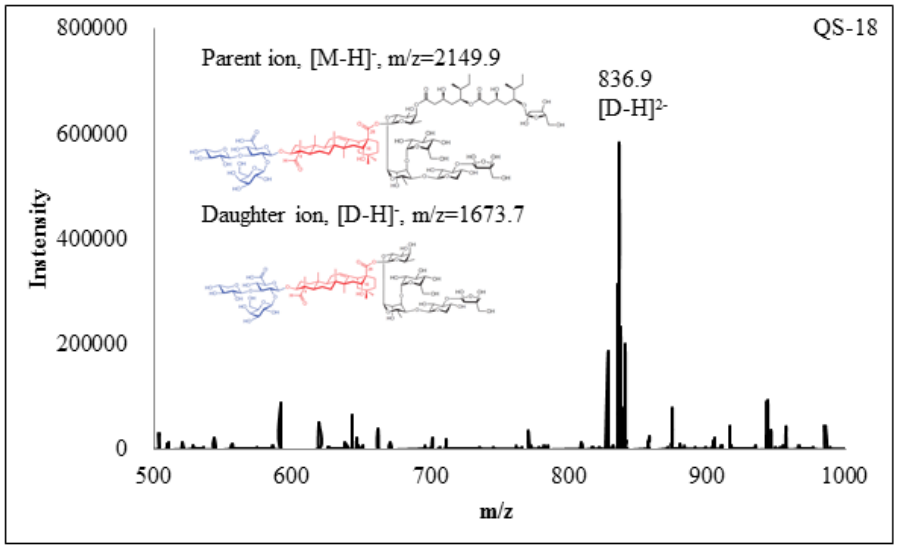


Figure S3 ESI-MS spectrum of QS-18, negative mode, double charged daughter ion $[D\text{-}H]^{2-}$ 2149.9->836.9 *m/z*

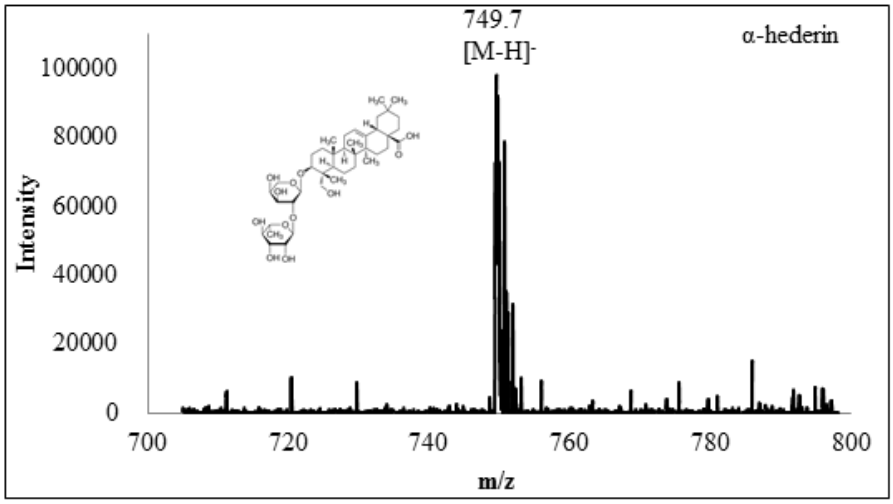


Figure S4 ESI-MS spectrum of α-hederin, negative mode, parent ion $[M-H]^-$ 749.7 *m/z*

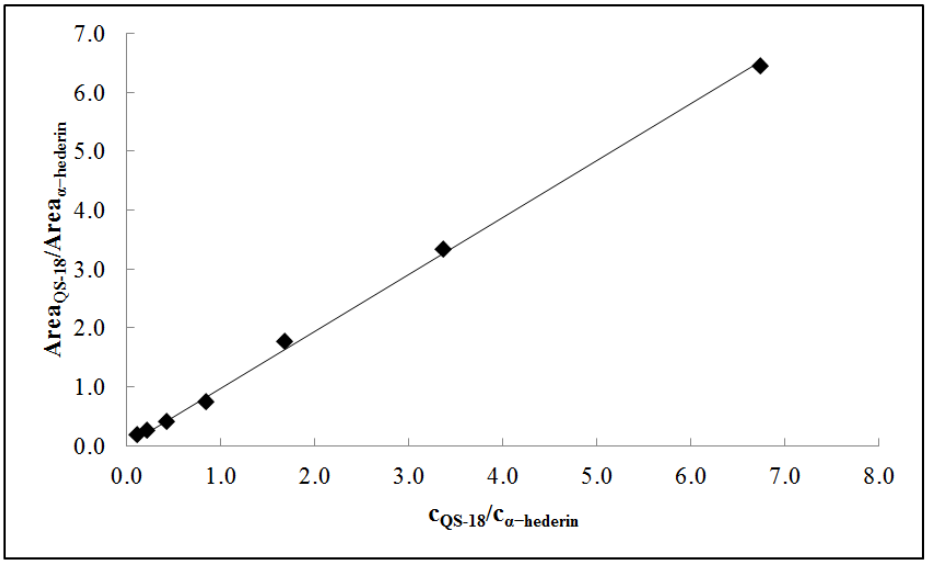


Figure S5 Relative response factor between QS-18 and α-hederin using UPLC-MS/MS. The linear regression is y = 0.967x, R^2 = 0.999, the intercept is set to 0.

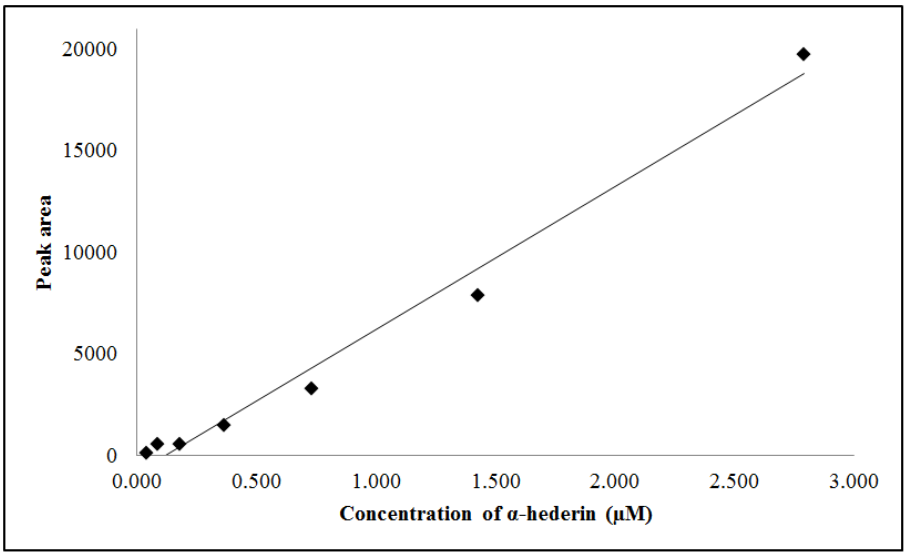


Figure S6 Standard curve of α-hederin in UPLC-MS/MS. The linear regression is y = 7066x – 891, R^2 = 0.984.

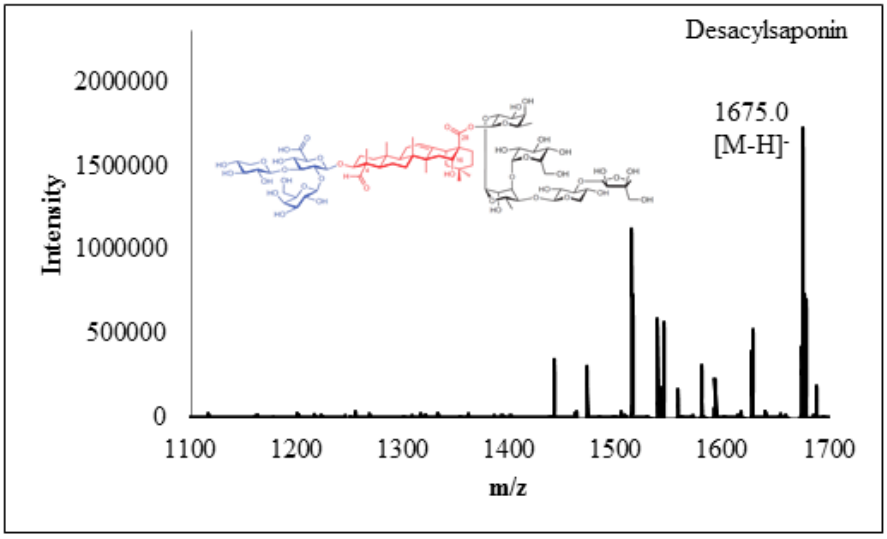


Figure S7 ESI-MS spectrum of desacylsaponin, negative mode, parent ion $[M-H]^-$ 1675.0 *m/z*

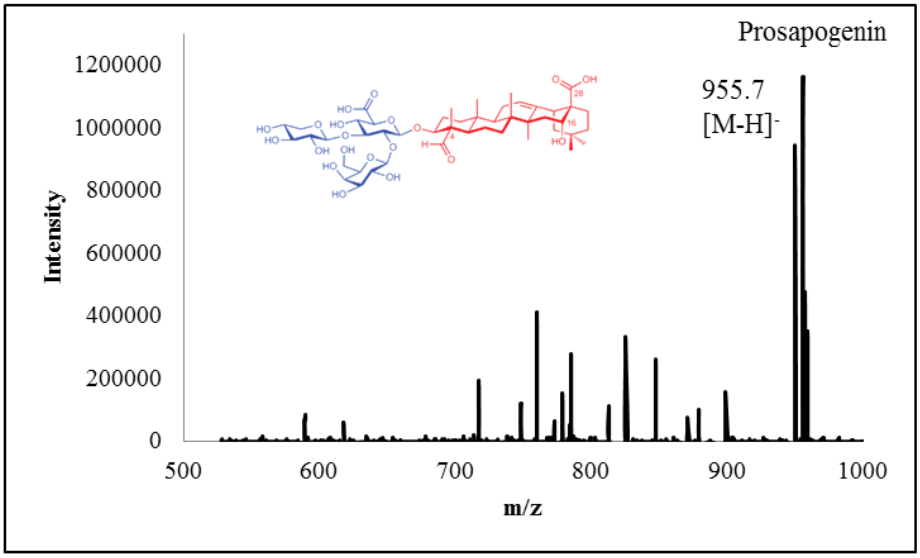


Figure S8 ESI-MS spectrum of prosapogenin, negative mode, parent ion $[M-H]^-$ 955.7 *m/z*

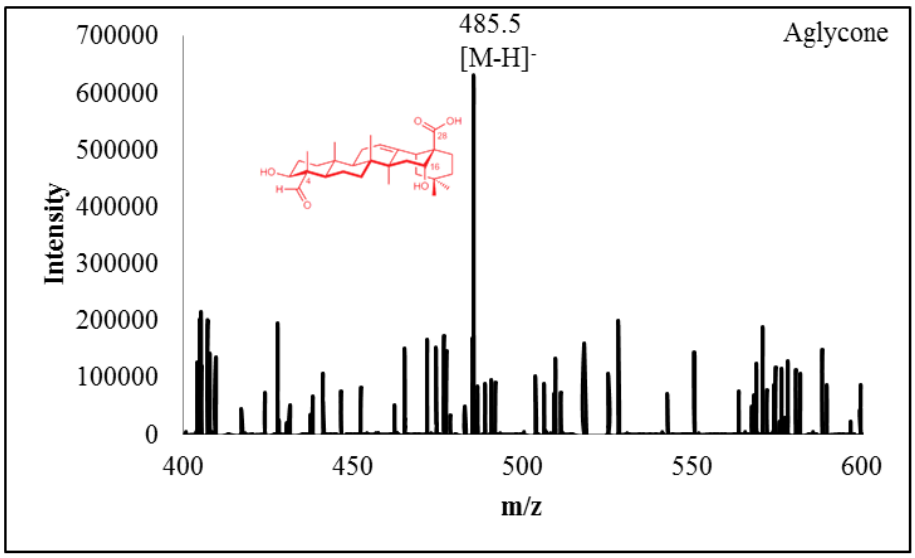


Figure S9 ESI-MS spectrum of aglycone, negative mode, parent ion $[M-H]^-$ 485.5 *m/z*

S3 PLATE COUNT AGAR TESTS

To verify whether the filtration of the lake water would leave bacteria in the water filtrated through a 0.7 µm filter while water being filtrated through a 0.2 µm filter should be sterile, plate count agar

tests were conducted and Colony Forming Units (CFU) counted after 48h of incubation at 35 °C.[4] In the tests, 1 mL or 0.1 mL of water samples were incubated on a peptone-yeast-glucose agar plate. Sterile MilliQ water was used as negative controls. The results are shown in Table S1, confirming the sterility of samples filtered through the 0.2 µm filter and the existence of bacteria in water filtered through 0.7 µm filters.

Table S1 Plate count agar tests of the different lake water samples

Water quality \ Lake name	Store Gribsø	Sorø Sø	Skærsø	Søby Brunkulslejer
Sterile MilliQ water (CFU/mL)	0	0	0	0
Samples filtered through 0.2 µm filters (CFU/mL)	0	0	0	0
Samples filtered through 0.7 µm filters (CFU/mL)	1.6×10^4	2.2×10^4	1.4×10^4	1.2×10^3

S4 STANDARD CURVE AND BACKGROUND HYDROLYSIS IN MILLI-Q WATER

To test the stability of the substrate 4-methylumbelliferyl butyrate (4-MUB) used for testing esterase activity, a concentration of 100 mM was incubated in MilliQ water and the fluorescence of the product 4-methylumbelliferone (4-MU) was followed over time. No development of fluorescent product was detected. The standard curve of fluorescence as a function of 4-MU is also shown here.

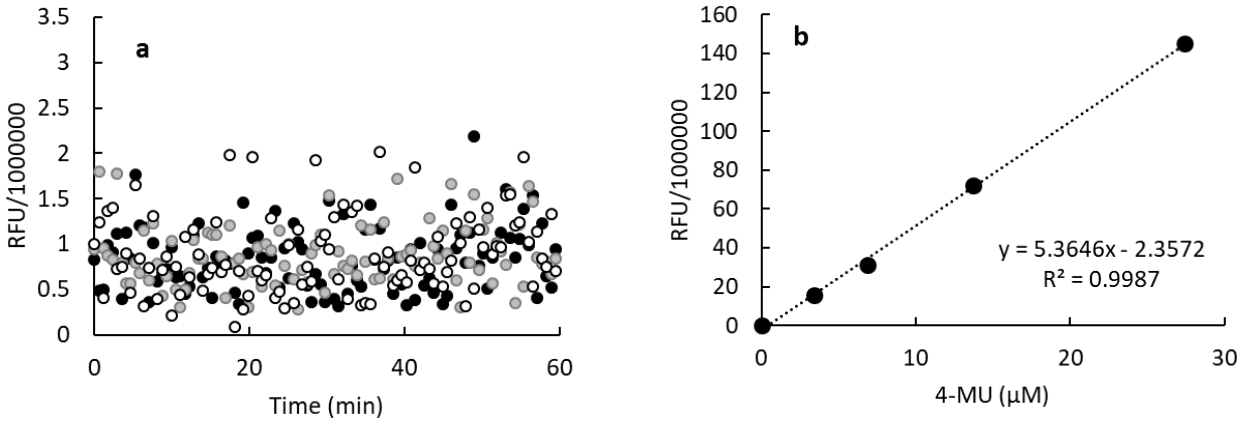
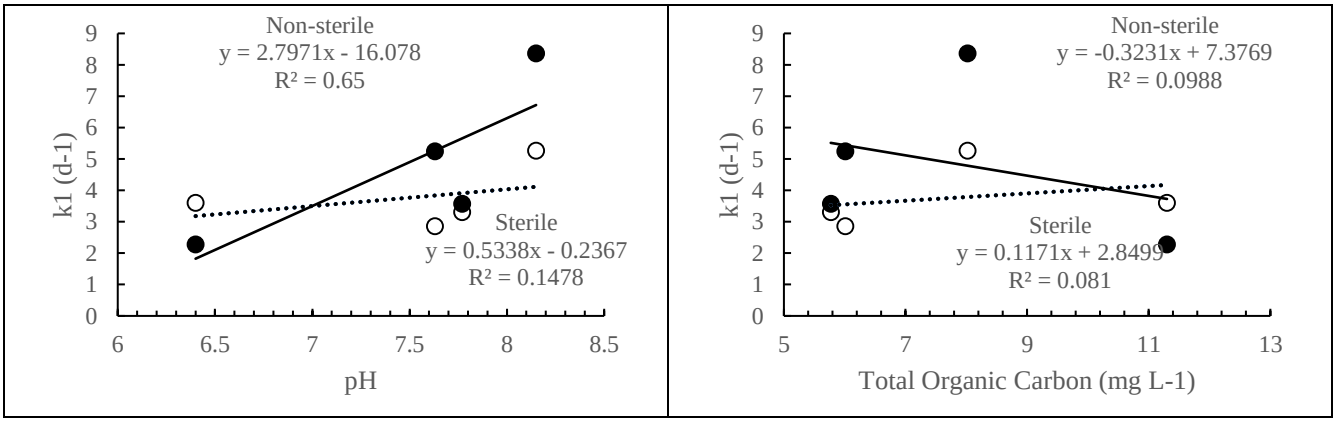


Figure S10. The fluorescence development of 100 µM 4- methylumbelliferyl butyrate (4-MUB) in MilliQ water measured as Relative Fluorescence Units (RFU)/100000 as a function of time (a) and as a function of 4-MU concentration (measured in MilliQ water)(b). The different symbols in figure a represent individual replicates.

S5 CORRELATIONS BETWEEN DISIPATION KINETICS AND LAKE WATER CHARACTERISTICS

To get indications of which lake water parameters might affect the unexpected lake water dissipation kinetics, regression coefficients of the kinetic parameters of the sterile and non-sterile lake water as a function of the measured lake water parameters were calculated. As only four lakes were included in the analysis, few regression coefficients are significant. Lake water parameters such as pH, conductivity and alkalinity are inter-correlated as pH is largely controlled by the carbonic acid equilibria which again is linked to alkalinity and conductivity.



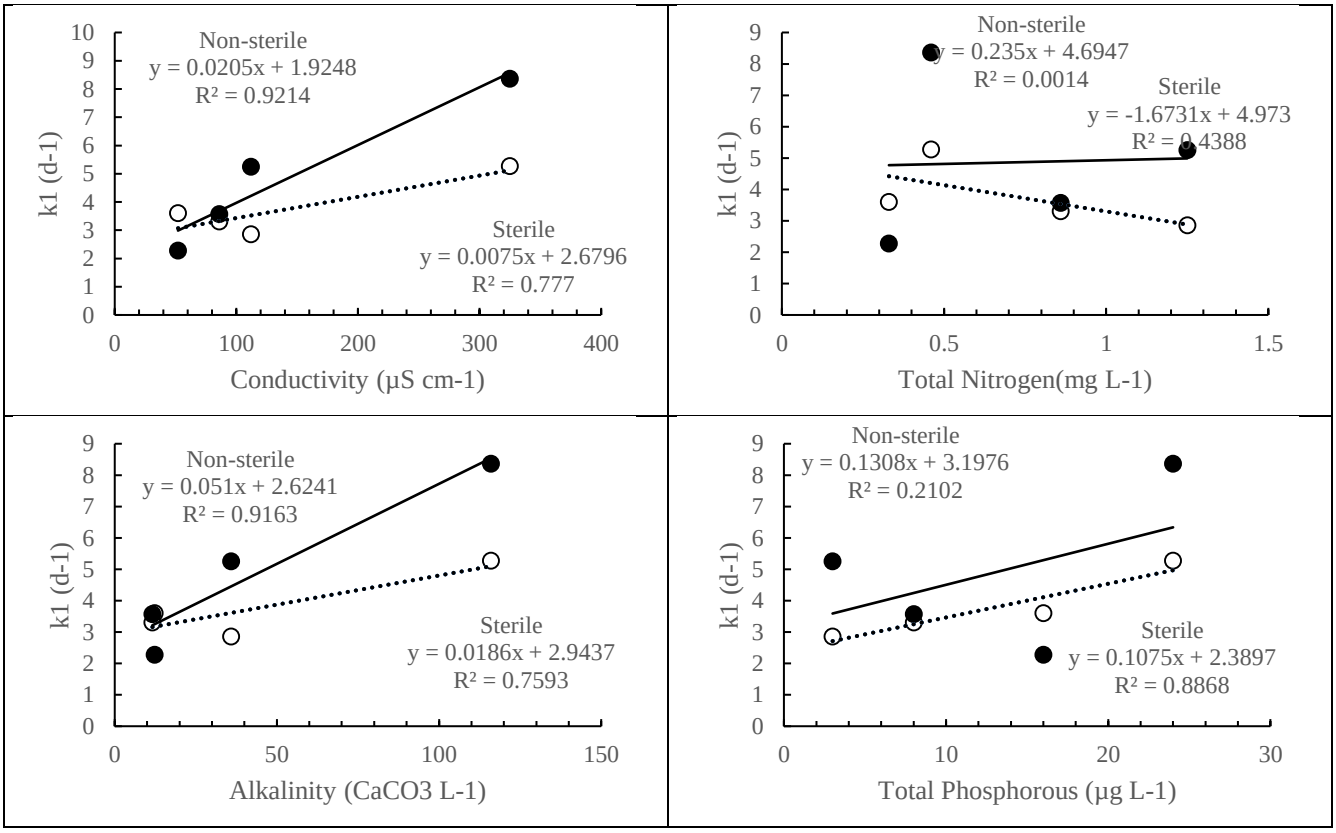
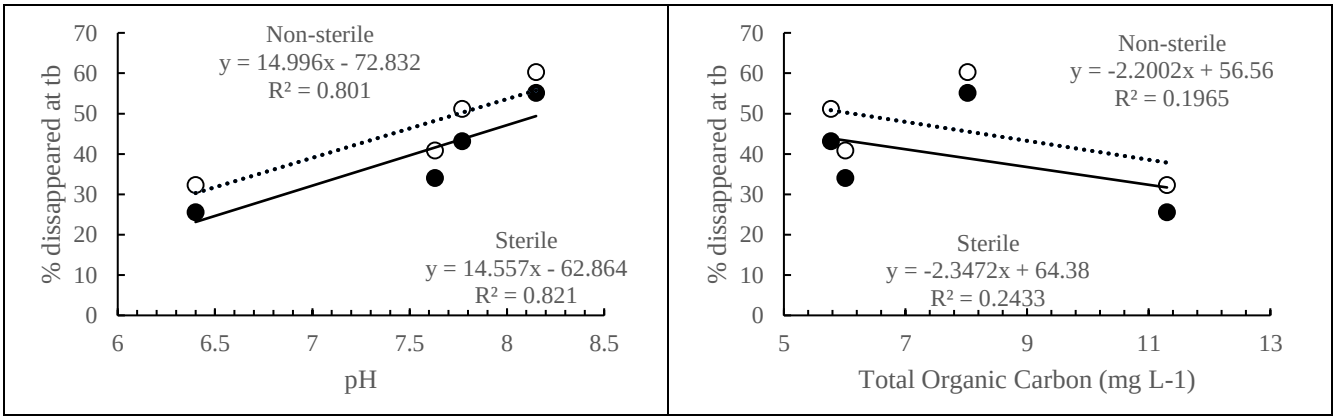


Figure S11. The fast kinetic rate constant, k_1, of the sterile (open symbols) and non-sterile (closed symbols) lake waters (Table 2) as a function of lake water parameters (Table 1).



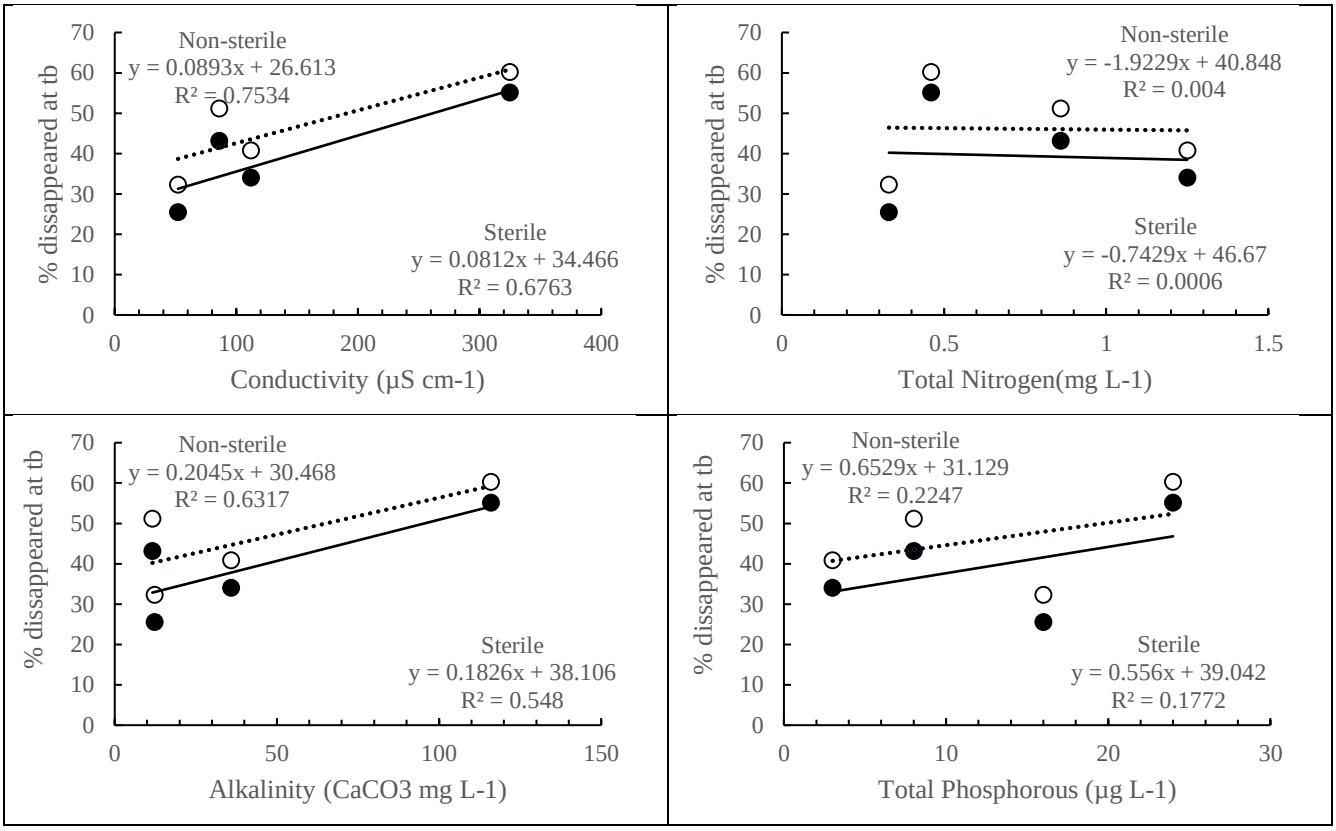


Figure S12. The proportion of dissipated saponin at end of the fast-kinetic phase, t_b, of the sterile (open symbols) and non-sterile (closed symbols) lake waters (Table 2) as a function of lake water parameters (Table 1).

S6 CONSUMED SAPONIN AND FORMED PRODUCT IN SELECTED SAMPLES

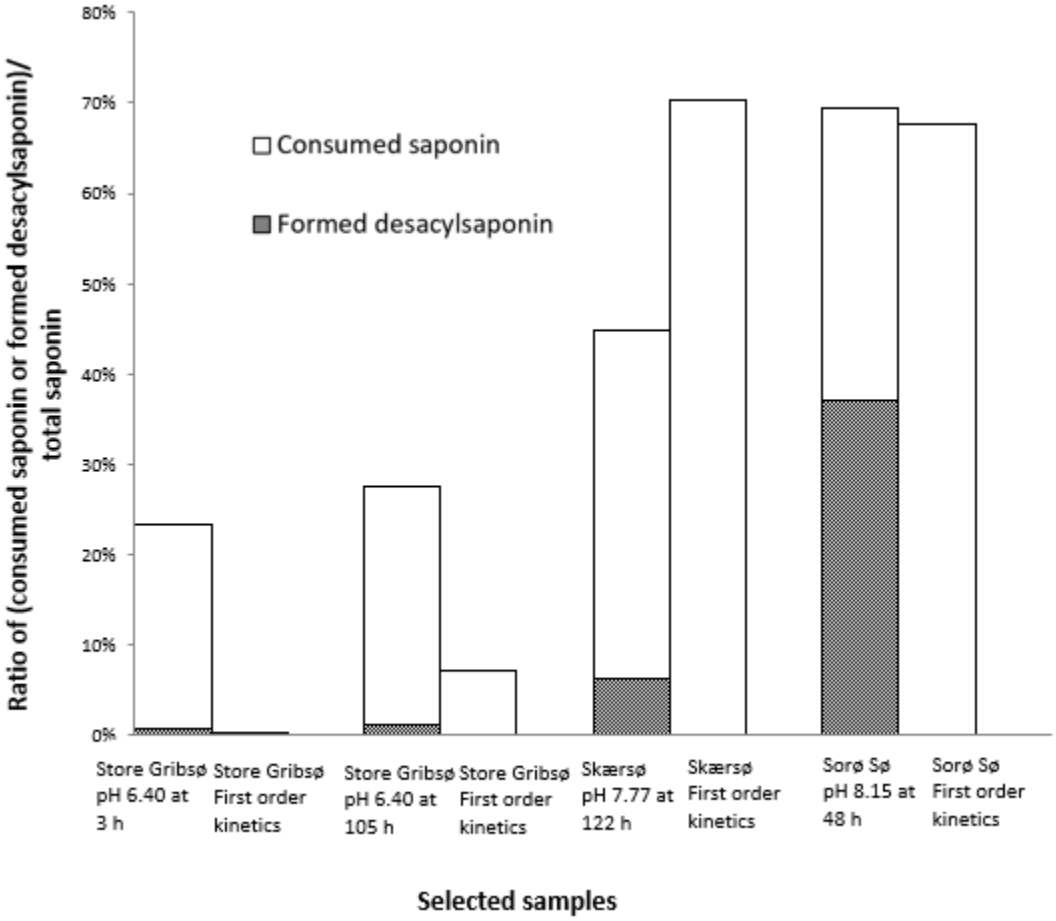


Figure S13. Relative consumption of saponin and formation of the hydrolysis product desacylsaponin (m/z 1675) in three lake water samples compared with the theoretical estimate of saponin hydrolysis according to eq. (1) and (4c).

REFERENCES

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2 Christensen S, Heimes C, Agerbirk N, Kuzina V, Olsen CE, Hauser TP. Different Geographical Distributions of Two Chemotypes of Barbarea vulgaris that Differ in Resistance to Insects and a Pathogen. *J Chem Ecol* 2014; **40**: 491–501.

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4 Corry JEL. Handbook of microbiological media. *Int J Food Microbiol* 1994; **22**: 85–86.