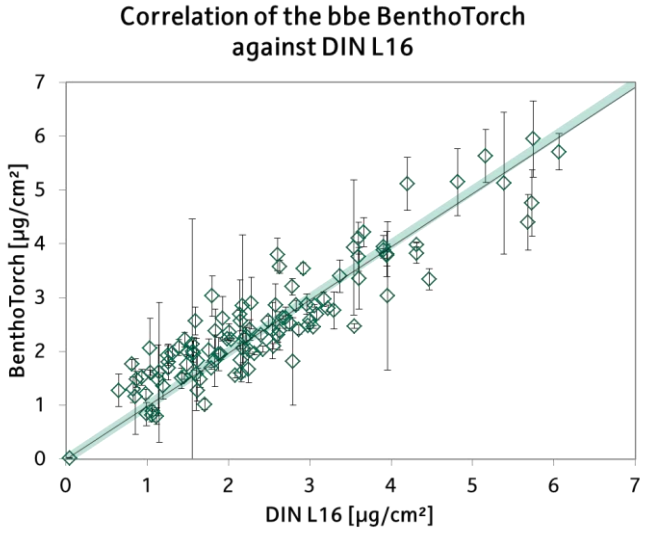
1) ***Q: Can we convert the BenthoTorch into an AlgaeTorch?***
A: No, this is not possible. The AlgaeTorch and the BenthoTorch are completely different in the construction of the optics and therefore cannot be converted into each other.

2) ***Q: How many replicate measurements "per stone" do you recommend?***
A: Depends on your demands and strategies. For comparison to other methods I strongly advice to do as many measurements as necessary to get a representative mean which covers the area which is taken for the comparison method (e.g. scraped area). For achieving an overview over a certain area I recommend to develop a strategy, such as *always use the center of the selected stones*, or *choose the center and one point in each cardinal 2 cm away from the center.* This helps to minimize the number of points which leads representative numbers (confidence intervals) in order to characterize a certain area.

3) ***Q: have you got any correlation about measurements with BenthoTorch and measurements with the classical chl-a measurements on spectrometers - the classical protocol of chl-a extraction with acetone?***
A: Yes we compared both methods – the *in vivo* fluorometrical approach and the classical extraction after scraping algae from a surface. A correlation was clearly shown with a coefficient of determination of 0,8 and a slope of 0,99.



4) ***Q: What is the correlation between results from the BenthoTorch and other periphyton biomass calculation methods (e.g. HPLC and AFDM)?***
A: Unfortunately we don´t have comparisons with HPLC or ash free dry weight analysis.
The challenge of comparisons between BenthoTorch and "standard" methods starts at sampling. In our answer of question no 3 you will find a good correlation between BenthoTorch measurements and Standard classical extraction method.

5) ***Q: How reproducible are the standard methods compared with the reproducibility of the BenthoTorch?***
A: The reproducibility of the *in vivo* fluorometry with the BenthoTorch is high. The reproducibility for the classical method includes more steps of treatment - each allowing further deviations. However, if carefully performed, both methods are comparable as seen in the graph above.

6) ***Q: Do we cover most of the types of substrates identification with the BenthoTorch?***
A: Yes, the BenthoTorch covers different types. The measurement of the 700 nm reflection enables to compensate the (matrix) effects of different substrates such as sand, stone, clay and others. The compensation is performed automatically when the measurement of benthic algae takes place.

7) ***Q: How should one (or should one) sample on sand and vegetation (the former likely to return an underestimate and the latter an overestimate of periphytic biomass)?***
A: The BenthoTorch is designed for an analysis of benthic algae on sand. Caution is called not to penetrate the different layers of a sandy surface with the BenthoTorch and disperse the sediment materials. The measurement on vegetable surface is useless, as the vegetation itself has also fluorescent characteristics and will affect the results. Especially the measurement of benthic algae on seaweed does not give feasible figures.

8) ***Q: Are there any problems measuring on surfaces below the water?***
A: You can analyse surfaces under water, but you shouldn't dive the BenthoTorch deeper than 10m. Also you have to hold the BenthoTorch in a stable position while measuring.

9) ***Q: is it possible to get results in micrograms/cm3....if measure directly in the water body?***
A: The BenthoTorch is designed for the measurement of benthic algae. Although the measuring principle is related to our fluorometers for water analysis the BenthoTorch is not suitable for the determination of algae in water. So the readings of the BenthoTorch are given in µg Chl-a/cm² (related to surface). Our other devices (FluoroProbe AlgaeLabAnalyser, AlgaeOnlineAnalayser, AlgaeTorch) are designed for the measurement of algae in water given in Chl-a /mg/l (volume related).

10) ***Q: Do I have to do the measuring into the water or in the air? Is there any difference?***
A: We tried to optimize the probe to measure the same results in water and the air. This still can lead to 20% deviation. The advantage of taking samples out of the water can be that silt and mud are removed which can decrease the fluorescence and lead to underestimation of the chlorophyll content.

11) ***Q: Can BenthoTorch be applied on "dry" surface?***
A: Yes, the BenthoTorch can be used on "dry" surfaces like wood, roof or house walls but the results can only be taken as qualitative not quantitative occurrence of chlorophyll-a.

12) ***Q: Are there any special considerations to be made when using the BenthoTorch in marine, as opposed to freshwater, environments?***
A: The BenthoTorch and the predecessor model were successfully deployed in marine and fresh water for the analysis of algae classes. However customized calibration with another source of algae can be performed. See also 22.

13) ***Q: What is the size of the determination area measured by the BenthoTorch?***
A: ~ 1 cm^2

14) ***Q: What about (light) penetration of measurement with BenthoTorch? Depth of layer?***
A: Light penetration only in the upper layer. This will lead to fluorescence emission. Roughly estimated fluorescence response reflects signals from a 100 µm thick layer at the surface of a fixed carrier

15) ***Q: does the thickness of the biofilm influence the measurement?***
A: Not directly, because deeper lying algae are not excited by the BenthoTorch.

16) ***Q: Why is 700 nm being used for reflection compensation?***
A: 700 nm LED is a suitable LED as it does not interfere with the chlorophyll determination and reflects the absorption and scattering by non-photosynthetic material. The photomultiplier window is open for 685 – 700 nm which enable the BenthoTorch to use the same sensor for the measurement of chlorophyll and matrix effects (background reflection).

17) ***Q: Are there any inferences that can be made regarding the concentrations of or ratios between cyanobacteria, green algae and diatom biomass (e.g. a ratio of 1:2:3 between C, G, and D correlates with high turbidity)***
A: No, calculation is performed with algorithms that enable allocation to the different algae classes with the knowledge of the spectral algal features (spectral fingerprints). The application is limited for a maximum of 15 µg/cm^2 and with less accuracy up to 30 µg/cm^2.

18) ***Q: We can observe that results apparently were like expected ones, when the biofilms/mats studied were green or blue-green. However, when the mats or biofilms had a brown colour, BenthoTorch indicated a high amount of diatoms in those samples. We have analysed that samples using other methodologies and diatoms were not present there while we can observed the presence of cyanobacteria with brown pigments or red freshwater algae. These types of samples overestimate diatoms from other algal group.***
Could it solved with a new recalibration of the BenthoTorch?
A: The BenthoTorch as it is now is calibrated to detect "green" algae, diatoms and phycocyanin (allophycocyanin) containing cyanobacteria. Algae that contain higher amounts of phycoerythrin (PE) like some strains of *Phormidium* will be misinterpreted and the fit procedure makes the "best" adaption to the fingerprints available. As these partially overlap with the excitation spectra of the diatoms for the BenthoTorch (we use only 3 wavelengths) the occurrence of diatoms is displayed although not existent. A recalibration for the PE containing algae can be helpful; however the now used wavelength is not ideal for PE-*Phormidium*. A 570 nm LED as used in the FluoroProbe will improve the results for these cyanobacteria with the disadvantage of underestimating "blue" cyanobacteria with the same illumination regime. We can imagine

that a recalibration with a pure culture of *Phormidium* is worthy to get a more distinguished result for the PE containing cyanobacteria. The calibration procedure needs about one day with all preparations and includes in parallel the calibration of two additional algae classes.

Filamentous rhodophytes are difficult to determine with the BenthoTorch. As with other macrophytes you can get numbers for chlorophyll-a, but class identification and chlorophyll determination are questionable. As with *Phormidium* the BenthoTorch misinterprets the PE content. Size and shape moreover distort the measurement.

19) ***Q: What does the BenthoTorch show when applied on microphytas? Or a mix of micro- and macrophyta?***
A: see Q/A 7

20) ***Q: what about cryptophytes?***
A: We never saw cryptophytes among the microphytobenthos. A literature search did not show cryptophytes related to benthic algae.

21) ***Q: Bio fouling in reverse osmosis membranes, can be monitored by BenthoTorch? Perhaps Microbenthotorch?***
A: If located on the surface the BenthoTorch will be a suitable tool for the measurement of microalgae. Algae captured in the pores of filter materials will not be recorded.

22) ***Q: Can I add my own algae class to the Benthotorch?***
A: Yes, if you can provide the "fingerprint" (individual values of wavelength from emitted light/exciting light) you can add or change the algae classes that are saved in the BenthoTorch. The deposited fingerprint is overwritten then.

23) ***Q: Why does the BenthoTorch eliminate the need for random sampling?***
A: It doesn't necessarily; it depends on your strategy. If you want to get an overview over the whole (visible) area with algae growth, complete random data uptake is a possibility. Other strategies are also worthwhile ("compare the maxima on the stones over the years" could be a strategy, e.g. by determination of the chlorophyll content in the center of the stones)

24) ***Q: does the sum of the three algal groups can be used as an estimation of the total chlorophyll a concentration? Or have these two measurements have any known correlation?***
A: Yes. The total chlorophyll content that is calculated with the BenthoTorch is the sum of all measured chlorophyll-a from the different algae classes.

25) ***Q: Pigments vary greatly both between species within a taxonomic class and over time and varying conditions within a species. How much does the internal algorithm handle this reality in interpreting the signals for natural samples with unknown species composition and physiological state? This could lead to large errors in evaluating relative amounts of the various classes of algae.***
A: Algae and cyanobacteria can be roughly sorted into algae classes with common features of the accessory pigments. Collected data from an algae library led to mean values of spectral

fingerprints including the observed deviations for these classes. Unknown species usually correspond to these algae. In the rare case of exception the algae need a new calibration. Selected wavelengths now are used to excite the algal pigments and use the fluorescence emission for the deconvolution of a complex signal. A least square fit is applied to make the best adaption to the characteristic fingerprints of algae classes. The allocation to algae classes by this method has been proven by scientific work - see M. Beutler et al., Photosynthesis Research 72: 39–53, 2002: A fluorometric method for the differentiation of algal populations in vivo and in situ.
The irradiation regime circumvents physiological influences as far as possible. It is well known that nutrient variation can lead to pigment composition. The here used approach is based on mean values and tolerates some variations. For calibration comparable real algae are used.

26) ***Q: How often does one need to recalibrate the BenthoTorch?***

A: We recommend a recalibration every 1-2 years to ensure optimal measurement results.

27) ***Q: What published literature is available on the BenthoTorch?***

A: Please find enclosed three documents:

- DGL-2011_Dahlhaus_et_al
- ATorch and BTorch Wuxi poster 2011 DL
- 2013_Carpentier_The influence of hard substratum reflection

Find more information about the BenthoTorch and our other devices on our website:

www.bbe-moldaenke.de or write us an email: bbe@bbe-moldaenke.de

We will be happy to give you answers, if anything is still not clear or not on this list!

Best regards,

bbe Moldaenke GmbH