WATER-PAM-II

Manual for
Standalone Use

1st Edition: WaterPamII_2021b.docx

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1 Safety instructions

1.1 General Safety Instructions

- Read safety instructions and the operating instructions prior to operation of the device and its accessories.
- Pay attention to all safety warnings.
- Keep device and its accessories away from water or high moisture areas.
- Keep the device and its accessories away from dust, sand and dirt.
- Do not put the device and its accessories near sources of heat.
- Ensure sufficient ventilation.
- Connect the device only to the power source indicated in the operating instructions or on the device. If the device is not in use, remove the mains plug from the socket.
- The device and its accessories should only be repaired by qualified personnel.

1.2 Special Safety Instructions

- The WATER-PAM-II is a highly sensitive instrument which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual in order to avoid potential harm to the user and damage to the instrument.
- The WATER-PAM-II can emit very strong light! In order to avoid harm to your eyes, never look directly into the cuvette holder.

2 Introduction

WATER-PAM-II is the advancement of first-generation WATER-PAM instruments. This PAM chlorophyll fluorometer inspires by its outstanding high sensitivity and provides the perfect tool for assessment of chlorophyll a content and photosynthetic activity of highly diluted suspensions of microalgae, cyanobacteria, isolated chloroplasts and protoplasts.

Different algae demand different measuring settings. The new WATER-PAM-II provides red or blue measuring light modes in one instrument to adapt the experimental setup to the prevailing conditions.

The very compact design enables high portability to every respective location. With standard battery supply and built-in graphic touch display the standalone operation is designed for easy use.

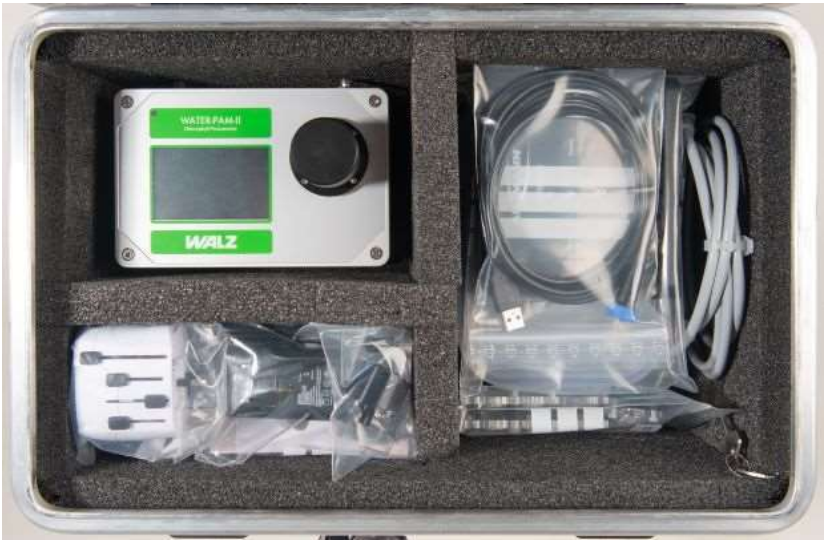
This manual provides information about the instrument setup, accessories, software structure and instructions for data acquisition and analysis in standalone operation.

3 Setup, Recommendation and Components

This chapter gives instructions for the instrumental setup, some recommendation for experimental procedure and information about WATER-PAM-II components.

3.1 WATER-PAM-II setup

The WATER-PAM-II is ready to use. It is designed for standalone operation but can also be used in conjunction with an external computer using the software Wincontrol-3. This manual provides information about the standalone operation. All windows for touch screen operation and the main menu are described in detail in the respective chapters.



If you want to operate the WATER-PAM-II by an external computer using WinControl-3 please download and install WinControl-3 from Walz web page www.walz.com and connect the WATER-PAM-II via USB.

3.2 Recommendations for experimental procedures

The WATER-PAM-II is easy to operate. The following procedure is recommended for experiments:

1. Determination of the background fluorescence of the sample:

   Please insert the cuvette filled with media or sterile-filtered sample, select Main Menu → PAM Settings →Adjust F-Offset

   (more information: chapter 6.2 and 5.1.3)

2. Insert sample and adjust detector sensitivity by the "AGain" button. This automatic procedure of photomultiplier gain adjustment is available in the bottom right of all windows (except algae analysis and actinic light list).

3. Perform measurements e.g.

   - manual saturation pulse analysis (Primary Data-, Basic Data-, Quenching analysis window, more information in the respective chapters and chapter 7.1 - 7.4)

   - determination of the algae composition and chlorophyll a content of the sample (Algae Analysis-, Dif. Algae Analysis window, chapters 4.4 and 4.5)

   - experimental routines like Actinic + Yield (chapter 4.7) induction curves (chapter 4.8) and light curves (chapter 4.9 and 7.6) with and without recovery (chapter 4.10).

3.3 Components

3.3.1 WATER-PAM-II Articles

Information about the scope of delivery and corresponding article description of the basic system is provided in Tab. 1. Articles of accessories are listed in Tab. 2.

Tab. 1: Basic system: scope of delivery and article description

	article description
WATER-PAM-II	Optoelectronic unit
000160101990	AA battery (16 x)
000190101101	Battery charger
MINI-PAM-II/N	Mains power supply
WATER-K	1 quartz glass cuvette
K5SET	5 standard glass cuvettes
CUV-H	Cuvette holder
US-SQS/L	Light sensor
000130606252	USB cable type A to Mini B
000160201311	Stylus
WATER-PAM-II/T	Transport box
WATER-PAM-II manual	www.walz.com
WinControl-3	www.walz.com free update

Tab. 2: accessories: article description

	article description
WATER-II/S	Stirrer
WATER-R (10 pcs)	Stirring paddles
BCS-9590	Barcode scanner
PHYTO-II/FT	Flow-Through Cuvette
WATER-II/FT/I	Pump control 0-10 V
Peristaltic pump	On request

3.3.2 WATER-PAM-II Optoelectronic Unit

The WATER-PAM-II combines electronics, optics, and sample holder in one compact housing. The LED array is arranged circularly around the cuvette. Signal detection is carried out in a 90° angle, below the cuvette bottom. The rear side of the instrument provides various sockets for sensors, accessories, or custom designed applications. These sockets are described in chapter 3.2.2.2 “Sockets”.

3.3.2.1 LED array

WATER-PAM-II instruments provide red and blue light regimes in one housing. The peak wavelength for measuring and actinic light of red is 630 nm and for blue 450 nm. In both operation modes also far red illumination 730 nm is available. For the differential algae analysis the instrument contains auxiliary measuring light LEDs at 520 nm, 660 nm.

All settings regarding the LED array can be set under Main Menu item “Light”, (see chapter 5.2). The valid actinic light list is shown in the last window “Actinic Light List” (see chapter 4.11).

3.3.2.2 Sockets

The WATER-PAM-II provides a compact portable setup. The optoelectronic unit of the WATER-PAM-II features cuvette holder as well as various connectors and sockets on the rear side of the instrument. Their properties and functions are outlined in Fig. 1 and Tab. 3.

Fig. 1: Rear side sockets WATER-PAM-II

Tab. 3: Sockets of WATER-PAM-II

Annotation, numbers refer to Fig. 1,		**Function**
1	PAR	PAR socket for light sensor US-SQS/L
2	TEMP	Option for temperature sensor, available on request
3	AUX 1	Option for accessories e.g. pump control, MINI-PAM-II accessories like spectrometer, oxygen sensor
4	AUX 2	
5	SYNC	Option for control of an external light source
6	ON/OFF	On/off switch for WATER-PAM-II
7	STIRRER	Socket for stirrer WATER-II/S
8	CTRL 1	Socket for PWM modulated peripherals
9	CTRL 2	
10	COMP	Socket for peripherals RS232 communication e.g. Barcode Scanner BCS-9590
11	EXT DC	Socket for Power Supply MINI-PAM-II/N
12	USB	Receptacle for MINI-B USB plug

Note: Great caution should be exercised to prevent dirt or foreign matter from entering the ports or sockets of the WATER-PAM-II. Do not force a plug into the wrong socket. Orientate each plug so that the red dot on the plug coincides with the red dot of the socket. Do not try to disconnect a plug by pulling at the cable. Disconnect plug by pulling at the rippled bushing of the plug

3.3.2.3 Cuvette

Two kinds of cuvettes are within the scope of delivery. One quartz glass cuvette WATER-K and a set of standard glass cuvettes K5SET. The background noise of these two cuvette types differs significantly. Up to a gain of 18 both kinds of cuvette can be used for sampling. At higher gain settings the quartz glass cuvette needs to be used for optimal signal to noise ratios.

The two types of cuvettes can be easily distinguished. The quartz glass cuvette (Fig. 2 on the left) has a stepped bottom, the bottom of the standard glass cuvette (Fig. 2 on the right) is continuous.

For cleaning instructions please read chapter 6.3 cleaning, on page 61.

Fig. 2: stepped quartz glass bottom (left), continuous standard glass bottom (right)

Note: The Flow-Through Cuvette is only for quartz glass cuvettes - with standard glass cuvettes the system can become leaky!

3.3.2.4 Power options

The WATER-PAM-II can be operated by various power options. A bottom battery compartment holds 8 commercially available standard AA (Mignon) rechargeable or non-rechargeable batteries.

As the optoelectronic unit provides no charging function the system can always be operated, also in presence of non-rechargeable batteries, by mains power via mains power supply MINI-PAM-II/N.

The delivery package includes mains power supply, 16 rechargeable batteries as well as an AA battery charger. More information on battery exchange and maintenance is given in chapter 6.2, Battery exchange/ storage, page 60.

3.3.2.5 Signal LED

The LED on top of the optoelectronic unit provides information about the WATER-PAM-II status. The signal LED indicates the color version which is currently set within the instrument: red signal LED: measuring

and actinic light are set to 630 nm; blue signal LED: measuring and actinic light are set to 450 nm. The flash code is listed in Tab. 4.

Tab. 4: Signal LED flash code

LED action	**status**
Flash every 1 s	Normal operation
SAT pulse	Continuous light
Fast flash	Oversaturation of the photomultiplier by external light, self-protection mechanism was activated, photomultiplier is switched off
Alternating fast/long flash	Program is running (clock, algae analysis, light curve etc.)

3.3.3 Light sensor US-SQS/L

The delivery scope includes a light sensor US-SQS/L for light calibration purposes. The optical geometry is defined by a spacer which is mounted between hood and sensor filter housing as shown in Fig. 3. For instructions of light list calibration please read chapter 6.1 Calibration of internal light list page 58.

Fig. 3: US-SQS/L with spacer mounted between hood and sensor filter housing

3.3.4 Stirrer

An optional accessory for WATER-PAM-II instruments is the stirrer WATER-II/S. It can be particularly useful for dark adaptation and Fo-measurements of rapidly settling samples. During actinic illumination the stirring will induce rapid movement of phytoplankton from illuminated to non-illuminated parts of the cuvette and *vice versa*, such that quasi-homogenous illumination at decreased mean light intensity level will be obtained.

The stirrer is powered and controlled by WATER-PAM-II. Please connect the stirrer plug to the stirrer port on the rear side of WATER-PAM-II. The disposable paddle can be removed by gentle pulling. The other way around, a replacement paddle can be mounted by pushing the cylindrical end all the way into holder on the upper inside of the stirring device.

The stirrer can be placed over the cuvette. For best stirring results, the cuvette should be filled just below the upper rim (4.5 ml sample volume).

Stirring is software controlled, either by WinControl-3 or in stand-alone operation by on/off and stirring speed commands in the main menu Sensors/Comp. window (chapter 5.5.1 page 47).

Please note that at very high sensitivity the paddle of the stirrer causes some increase of background noise caused by the moving paddle and by movement of cells or cell groups.

3.3.5 Flow-Through Cuvette

For easy sampling, the WATER-PAM-II can be equipped with a Flow-Through Cuvette (PHYTO-II/FT)

Commercially available peristaltic pumps can be operated using 0-10 V analog signal. This control voltage is provided via pump control connection to one of the AUX ports of WATER-PAM-II.

3.3.6 BCS-9590 Barcode Scanner

The barcode scanner is the ideal add-on when many different samples are probed. Simply convert your sample IDs into barcodes, print labels, and

mark your samples. Then, the BCS-9590 Barcode Scanner writes your sample ID into the memory of the WATER-PAM-II or the report data in WinControl-3.

For numbers up to 30 000, barcodes are printed in the Aux1 column of the data line of the subsequent saturation pulse data. Writing barcode and measuring results in the same line facilitates data sorting when data of many samples are collected.

Fig. 4: Barcode scanner BCS-9590, here with MINI-PAM-II

4 Touchscreen Operation

The WATER-PAM-II can be operated standalone using its resistive touchscreen allowing saturation pulse analysis of photosynthesis in the field.

The screen offers various display windows to control and display parameters as single F_V/F_m, Y(II) measurements, or more complex experiments like induction and light curves.

The display is divided into two areas, the main panel, and the side panel (see Fig. 5).

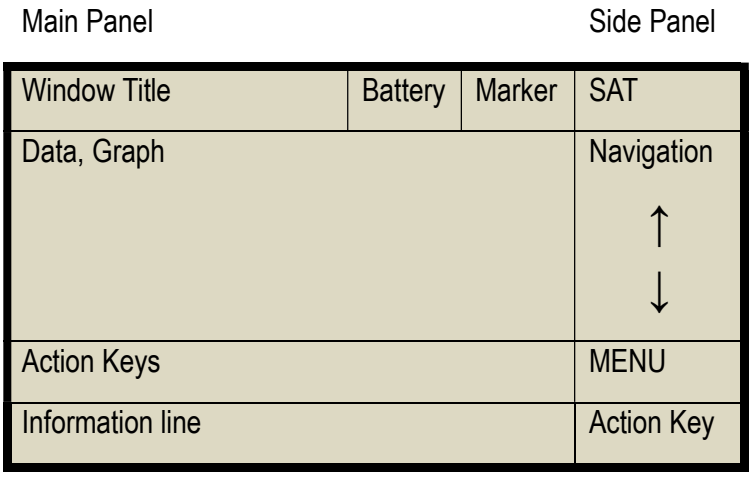


Fig. 5: Screen layout, stand-alone operation

The Main Panel shows (from top to bottom) Window Title, battery status, currently selected character to mark measurements, numerical and/or graphical data, action keys and an information line.

Action keys are differently arranged depending on the selected window. The information line displays date and time, PAM activity or informs on critical fluorometer states.

The side bar provides scroll arrows for the window navigation, “MENU” key and two action keys in the top and bottom. The functionality of the action keys varies between the windows and is described in detail in the description of each window. The “MENU” key gives access to the WATER-PAM-II Main Menu and its submenus which allow adjustment of all settings of the WATER-PAM-II and its peripherals.

The following table gives brief information on the different windows. Detailed information will be given in the respective subchapters.

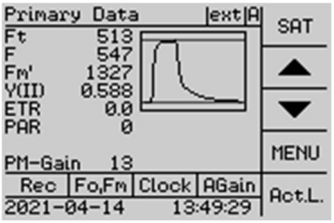
1.	Basic Data	Minimum data set and basic action keys for field operation.
2.	Primary Data	Data of last saturation pulse analysis and current levels of fluorescence and additional measured parameters.
3.	Quenching Analysis	Fluorescence levels of last saturation pulse analysis and corresponding fluorescence ratios (Y(II), F_V/Fm, …).
4.	Algae Analysis	Total algae content based on F, given in Chl_a µg/l
5.	Diff. Algae Analysis	Algae content based on F, differentiated in cyanobacteria/green- and brown algae; given in Chl_a µg/l

6.	Ft-Chart	Trace of fluorescence, total x axis interval: 25 or 125 s.
7.	Actinic + Yield	Short illumination program with saturation pulse analysis.
8.	Induction Curve	Graphics of saturation pulse analysis of current curve.
9.	Light Curve	Graphics of saturation pulse analysis of current light response curve.
10.	Recovery Curve	Recovery graphics after induction curve and light response curve.
11.	Actinic Light List	Set values of photosynthetically active radiation PAR in µmol m^{-2} s^{-1}.

The touchscreen is factory-calibrated to match its two-dimensional response with the LED screen underneath. If touch operation is imprecise, calibration can be performed as described in chapter 6.5.

4.1 Primary Data

Upon WATER-PAM-II start the Primary Data window is displayed.



This window shows the values of the current Ft signal, a depiction of the fluorescence trace of the last saturation pulse, resulting data (F, Fm', Y(II), ETR) and instrumental parameters as

applied PAR of actinic illumination and photomultiplier gain setting (PM-Gain).

After execution of F_o,F_m action key the displayed data provides the parameters F_o, F_m and F_v/F_m.

The side panel of the Primary Data window offers four commands:

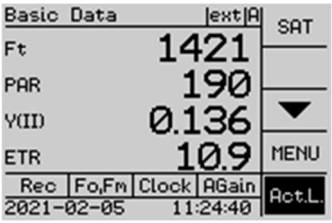
- SAT key for execution of a saturation pulse measurement to determine effective PS II photochemical yield,
- arrow keys to scroll to other windows
- MENU key leads to the main menu window
- Act.L. on/off switch for actinic illumination

The bottom edge of the Basic Data window provides keys for frequently used commands:

- Rec starts a new data set (Record)
- Fo,Fm determines maximum PS II photochemical yield (dark adapted state)
- Clock starts repetitive triggering of saturation pulses or an automated experimental routine
- AGain for executing the automatic photomultiplier gain adjustment procedure

4.2 Basic Data

The Basic Data window displays a reduced set of four data for fast sampling under field conditions.

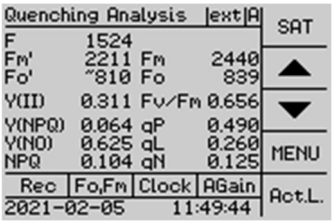


From these data, the Ft and PAR represent current measurements. Y(II), or FV/FM, and the ETR are derived from the last saturation pulse analysis.

The action keys in the bottom and side panel of this window are the same as in the Primary Data window (chapter 4.1).

4.3 Quenching Analysis

The window provides a complete overview on fluorescence levels and fluorescence ratio quotients calculated by the WATER-PAM-II.



Data line 2 to data line 4, compare data of the light exposed sample (left) with data of the dark-acclimated sample (right).

Following lines display further quenching parameter. Parameters which are based on Fv/Fm are calculated with the last measured Fv/Fm of the current record. In case no Fo,Fm was measured or a new record started, these parameters will not be calculated.

Quenching parameter which are based on Fo' will be calculated when Fo' mode is active (Menu, PAM Settings 5.1.4) or ~Fo' can be calculated according to Oxborough and Baker (chapter 7.3, page 64). The calculated ~Fo' requires an Fo,Fm measurement and is indicated with ~.

If a Record holds more than one Fo,Fm determination, the last one will be used to calculate fluorescence coefficients. Such calculations, however, are only valid for Fo,Fm and SAT measurements with the same sample. Sometimes, Fo,Fm and SAT measurements with different samples result in fluorescence ratio parameters that exceed their valid

range (compare Tab. 8: Fluorescence Ratio Parameters page 66). These invalid data will be displayed in grey on the screen.

The side panel and action keys available in the Quenching Analysis window are the same as in Primary Data window.

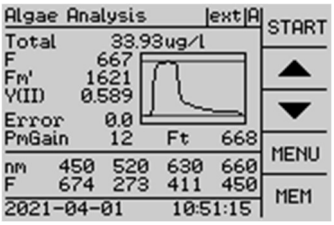
4.4 Algae Analysis

This window is the first of two windows for algae content determinations and provides information about the total Chl_a content of the analyzed sample.

Over a wide range of Chl_a content, Chl_a fluorescence intensity is proportional to Chl_a concentration. Therefore, the PSII fluorescence can be taken as measure for algae Chl_a content. The total algae content displayed here in this window bases on differential content analysis shown in the next window Dif. Algae Analysis (for more information please read following chapter 4.5.)

The key “Start” in the upper right corner starts the content determination routine to measure F signals of 4 measuring light wavelength. The algae content will be displayed in µg/l Chl_a calibrated by the F/Chl_a of the Chlorophyll a analytical standard | Sigma-Aldrich (96145 (Supelco) CAS 479-61-8) diluted in Aceton (650501 Aceton HPLC Plus, for HPLC, GC, and residue analysis, ≥99.9% | Sigma-Aldrich).

The algae content determination can be combined with a saturation pulse measurement for determination of the photosynthetic activity Y(II). This setting can be altered in the main menu “Algae Analysis Settings” (see chapter 5.3). Activating "Add SAT Pulse" triggers the automatic saturation pulse analysis that is performed following the algae content measurement.



The determined total algae concentration, the results of the SAT pulse analysis (F, Fm' and Y(II)), the SAT pulse graph, the fit error of the algae differentiation, the photomultiplier gain applied during the algae content determination, and the detected F values of the 4 measuring light wavelengths are displayed on the screen.

Please note:

1. the fluorescence signal may originate not only from Chl, but also from other fluorescing components, like humic acids and at high Gain-setting even from components of the measuring system (e.g. cuvette and optical filters). This aspect can be accounted for by F-Offset determination using a filtrate (see chapter 5.1.3).
2. at high Chl concentrations, part of the Chl fluorescence is reabsorbed by the sample thus leading to underestimation of Chl content. Please take this effect into account at Chl_a concentrations above 500 µg Chl_a/l.

4.5 Dif. Algae Analysis

The analysis of composition of mixed algal samples bases on the group specific differences in the light harvesting complex of green algae, brown algae (diatoms/dinoflagellates) and cyanobacteria. It is known that these differences in pigmentation and structure lead to wavelengths dependent differences in fluorescence excitation. For example, in green algae Chlorophyll fluorescence is much more effectively excited by blue and red light (450, 630 and 660 nm) than by green light (520 nm). On the

other hand, due to the absorption characteristics of the phycobillisomes, cyanobacteria show high fluorescence excitation in the red but very low fluorescence excitation at blue (450 nm). The light harvesting complex from diatoms and dinoflagellates feature fucoxanthin (Fx) chlorophyll a/c proteins (FCP) within the antenna. They show like green algae, highest fluorescence excitation in the blue, but in contrast to green algae, also significant fluorescence excitation at 520 nm.

For each of these three algae groups a typical fluorescence excitation spectrum at 450 nm, 520 nm, 630 nm, and 660 nm (see Fig. 6) is implemented in the WATER-PAM-II software.

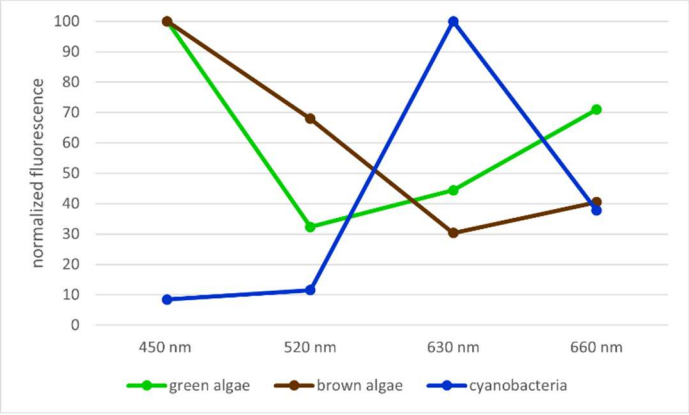


Fig. 6: Typical reference spectrum for green algae, brown algae, and cyanobacteria

The content determination routine which can be started by the start key in the upper right corner initiates measurements of the F signals at 450 nm, 520 nm, 630 nm, and 660 nm. The resulting data (visible above the information line) is fitted with the implemented references by using the least square method: which algae composition best represents the measured fluorescence.

nm	450	520	630	660	MENU
F	538	269	545	429	
					MEM
2021-01-29			10:50:22		

Fig. 7: F values of example differential algae measurement

For example, a measurement resulting in F values as shown in Fig. 7 can be fitted with the references spectra from Fig. 6 as illustrated in the diagram Fig. 8. The fluorescence of each measuring light wavelength is fitted to the proportion originated by the three algae groups. In this mixture the F at 450 nm is originated almost equally from green and brown algae with only a small contribution of cyanobacteria (cyanobacteria show low fluorescence excitation at 450 nm). The F value of 630 nm shows, that there were also cyanobacteria within the mixture as there was a high proportion of cyanobacterial originated F at this wavelength.

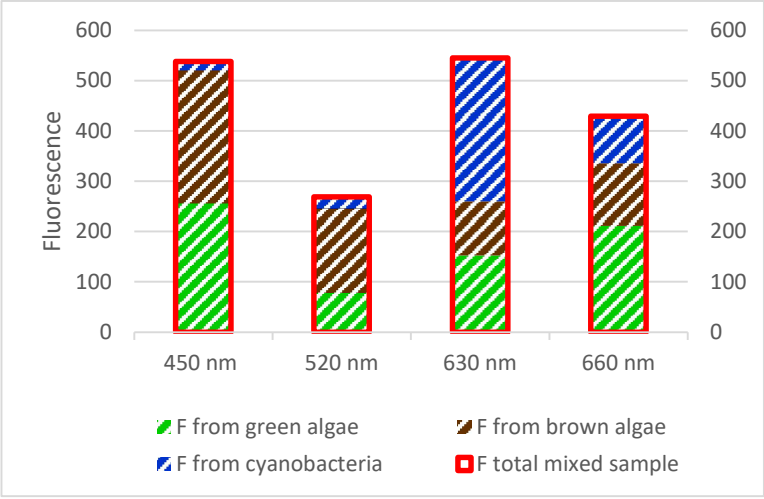


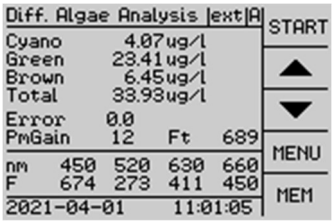
Fig. 8: Example of group specific contribution of F signal to overall F value, fitted with typical reference spectra

Even though there are also differences in the spectrum within the algae groups the standard references reflect the algae composition very well. To judge the quality of fit, the fitting error “Error” is listed.

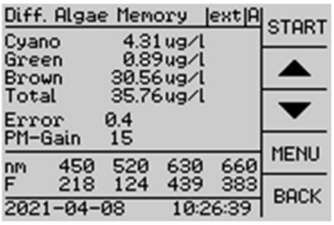
The instrument also provides the possibility to increase the accuracy by inactivating references of algae groups which are known to be absent in the sample (see Main Menu “Algae Analysis”5.3) or by application of self-determined references of the dominating algae (this function is available via WinControl-3).

As in the “Algae Analysis” window, the algae content determination in the “Diff. Algae Analysis” window bases on the linear relationship of fluorescence F to Chl_a. The references implemented in the Water-PAM-II are calibrated with the Chl_a/F ratio of the Chlorophyll a analytical standard | Sigma-Aldrich (96145 (Supelco) CAS 479-61-8) diluted in Aceton (650501 Aceton HPLC Plus, for HPLC, GC, and residue analysis, ≥99.9% | Sigma-Aldrich). After fitting the group specific fluorescence proportion from total F, the algae group specific F signal is calculated according to this F/Chl_a ratio. Deviating ratios can be entered via WinControl-3. Reset References (see chapter 5.9.3) restores the standard references implemented in the device based on the Sigma chlorophyll a analytical standard.

Please pay attention to the notes given in the previous chapter 4.4 regarding F background fluorescence and limitations due to dense samples.



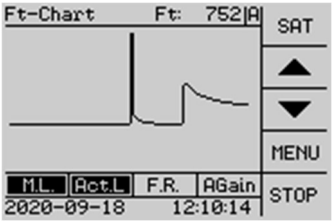
As result of the fitting procedure the algae content within the sample is displayed. Photosynthesis parameter will not be deconvoluted but resemble the mixed algae sample.



MEM of the "Diff. Algae Analysis" window opens the corresponding memory window. In this window, the navigation keys permit scrolling through stored data. The experiments are annotated in the information line by date and time of the measurements. There are two options to leave this window, either by pressing "START" to measure a new data set or by using the "BACK" key to return to the last measured Diff. Algae Analysis data.

4.6 Ft-Chart

In the Ft-Chart the current Ft signal is graphically displayed (not recorded). Recording of Ft requires operation of the WATER-PAM-II by WinControl-3.



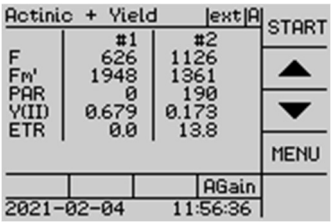
The X axis interval can be adjusted in the main menu "Instrument Set" to 0.2 or 1.0 s/dot Ft chart resolution corresponding to 25 or 125 s/total time axis (chapter 5.6.1). The Ft: value above the graph corresponds to the current Ft value.

The side panel provides buttons for executing a SAT pulse, arrows for window navigation, Menu to access the main menu and RUN/STOP for the F signal display.

The action key line below the graph provides three on/off switches: M.L; Act.L and F.R. to switch on/off measuring light, actinic light and far red light. There is also the "AGain" key to adapt the photomultiplier sensitivity by executing the automatic photomultiplier gain adjustment procedure.

4.7 Actinic + Yield

The "Actinic + Yield" window is the first of four windows providing a



SAT-Pulse routine which illuminates a sample with actinic light of a defined period. Depending on settings, saturation pulse analysis is carried out prior and after actinic light exposure or only after actinic light exposure. Because the Actinic + Yield experiment is rather short, it is frequently employed when high light response of photosynthesis needs to be evaluated for many samples.

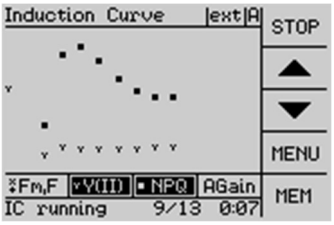
The parameters of the Actinic + Yield routine can be set in the menu "Program/Clock" (see chapter 5.4.4 page 44).

The display shows columns for data saturation pulse analysis carried out prior (#1) and after actinic light exposure (#2), respectively.

The buttons of the side panel are START to start the Actinic + Yield procedure, arrow keys to scroll to other windows and MENU for access to the main menu window. The detection sensitivity of the photomultiplier can be adjusted before the measurement using the automatic photomultiplier gain adjustment procedure "AGain", above the information line.

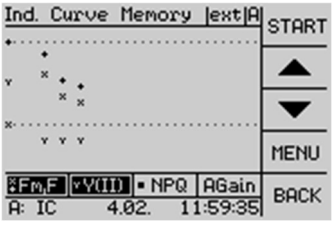
4.8 Induction Curve

This window provides a fluorescence induction curve experiment procedure. The graphics panel provides a qualitative picture of induction curve properties.

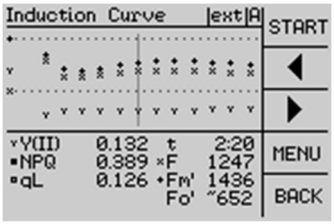


Settings of an induction curve experiment as number of saturation pulse analyses, interval between pulses and the option to continue fluorescence monitoring after the induction curve in the dark (recovery curve) can be adjusted in the menu "Induction Curve Settings" which is available in Main Menu (more information in chapter: 5.4.5).

As all windows the induction curve window provides the "AGain" key in the left lower corner above the information line for adjustment of the photomultiplier sensitivity. After this adjustment, an induction curve measurement can be started/stopped by the START/STOP key in the upper right corner. The graphical display can show Fm, F; Y(II) and NPQ (or Fm, F; F(II) and Y(NPQ) see chapter 5.1.6) values of the experiment. To select the displayed parameter please touch the corresponding key.



MEM of the Induction Curve window opens the "Ind. Curve Memory" window. In this window, the navigation keys permit scrolling through stored induction curves. The experiments are annotated in the information line by date and time of the measurements. There are two options to leave the Ind. Curve Memory window, either by pressing "START" to measure a new induction curve or by using the "BACK" key to return to the last measured induction curve data.



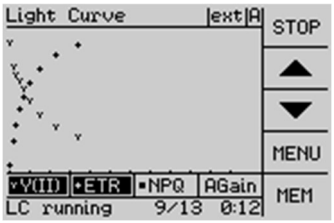
For quantification, all induction curve windows also provide numerical data of fluorescence ratio parameters and fluorescence levels.

These numerical data appear in response to touching the graphics panel. The navigation keys move the cursor (grey vertical line) from one saturation pulse analysis to another. The cursor indicates the position within the induction curve of the currently displayed set of numerical data. The "Back" key in the bottom corner closes the numerical display again.

To change the numerical display from Y(II), NPQ, qL to Y(II), Y(NPQ) and Y(NO) please open: Main Menu PAM-Settings (see chapter 5.1.6).

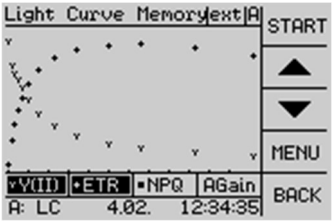
4.9 Light Curve

Light curve experiments are an established tool for analyzing the photosynthetic performance of organisms (chapter 7.6 page 71). This window provides a predesigned procedure to execute a light curve protocol. Relevant parameters (number and duration of light steps, initial PAR, and recovery curve) can be adjusted in the menu "Light Curve Settings" (chapter 5.4.6, page 46).

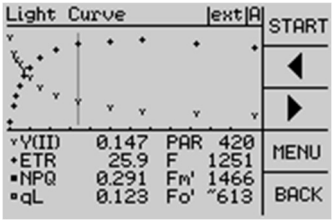


Display of graphical/numerical and memory data is in analogy to the previous induction curve window.

The “Light Curve” window provides the “AGain” key to adapt the photomultiplier sensitivity before measurement above the information line. The START/STOP key in the upper right corner executes the light curve procedure. To select the graphical displayed parameters please use the action keys above the information line. These values can be changed from Y(II), NPQ, qL to Y(II), Y(NPQ) and Y(NO) in the Main Menu PAM-Settings (see chapter 5.1.6).

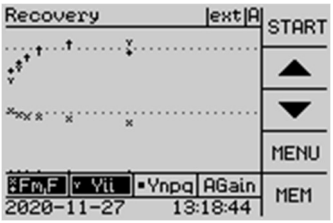


In the light curve window MEM opens the memory of light curve experiments. The navigation keys permit scrolling through stored light curves. The experiments are annotated in the information line by date and time of the measurements. There are two options to leave the memory window, either by pressing “START” to measure a new light curve or by using the “BACK” key to return to the last measured light curve data.



All light curve windows provide numerical data of saturation pulse analysis which can be accessed by touching the graphics panel. Navigation between different saturation pulses analyses and selection of displayed data works as described for the previous window (Induction Curve) by moving the cursor (grey vertical line) from one saturation pulse analysis to another. The cursor indicates the position of the currently displayed set of numerical data. Y(II), NPQ, qL can be changed to Y(II), Y(NPQ) and Y(NO) in the Main Menu PAM-Settings (see chapter 5.1.6).

4.10 Recovery



Fluorescence recovery experiments are often appended to induction or light curves. In Water-PAM-II instruments this can be chosen in the menu light-/induction curve settings (chapter 5.4.5 and 5.4.6). In these cases, recovery curves are started automatically and resulting data is displayed in the Recovery window.

For manual initiation (and cancelling) of recovery curves the "AGain" for adjustment of the photomultiplier sensitivity range is available as well as START/STOP in the top of the side panel.

The time course of recovery curves is fixed: each curve last 39 min during which 7 saturation analysis are carried out. In case of a preceding induction or light curve, the last saturation pulse analysis of the induction or light curve corresponds to the first one of recovery. The interval between neighboring saturation pulse analysis roughly doubles with time (see following table).

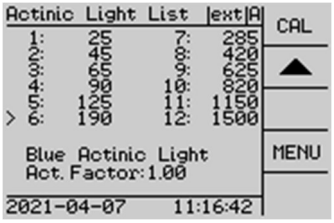
Tab. 5: Time Sequence of Saturation Pulse Analyses in a Recovery Curve

SAT number	Time in darkness, min
1	0.00
2	0:30
3	1:30
4	4:00
5	9:00
6	19:00
7	39:00

In the Recovery window, the MEM button opens the window “Recovery Memory” in which all recovery kinetics can be viewed independent whether they are connected to an induction or light curve or represent separate experiments. The recording date and time, of the displayed recovery experiment, is listed in the information line.

As described for the Induction and Light Curve windows, data in the Recovery windows are represented graphically and numerically (after touching the screen). Also, the display can be changed from Y(II), NPQ, qL to Y(II), Y(NPQ) and Y(NO) in the Main Menu PAM-Settings (see chapter 5.1.6).

4.11 Actinic Light List



The window “Actinic Light List” shows PAR values (in µmol m^{-2} s^{-1}) of currently chosen light mode (blue or red). The actinic light intensity steps 1-12 increase roughly exponentially with intensity settings. The currently selected actinic light step is marked with an >.

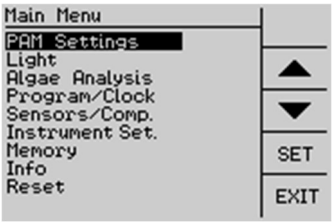
The side panel provides (top right corner) a CAL button to start a routine for calibration of the LED array. Please follow the instructions given in chapter 6.1 Calibration of internal light list page 58.

To alter the PAR of the actinic light, this complete list can be multiplied by the internal actinic factor (“Int. Act. Factor, chapter 5.2.6). The currently applied factor is listed above the information line. The internal actinic factor and all other adjustments concerning light settings can be altered in the menu section “light” (chapter 5.2).

5 Main Menu

The “Main Menu” forms the central hub to access settings, calibration data, hardware information and the memory of the WATER-PAM-II. The Main Menu provides nine submenus:

1. PAM Settings: adjustments of the way how the WATER-PAM-II acquires PAM fluorescence
2. Light: all settings for measuring light, SAT pulse, actinic light and far red light
3. Analysis Set: References and calibration values for differential algae analysis
4. Program/Clock: Settings to configure triggering of single measurements and experimental protocols
5. Sensors/Components: Settings for sensors and optional components like stirrer, pumps etc.
6. Instrument Set.: General instrument settings like display resolution, background illumination, instrument date and time etc.
7. Memory: Overview on stored saturation pulse analysis and access to review data of individual measurements. Button to start a new record or alteration of object mark.
8. Info: Provides information on battery status, serial and firmware number and overall operation time of the instrument
9. Reset to restore default PAM settings, instrumental settings or implemented references.

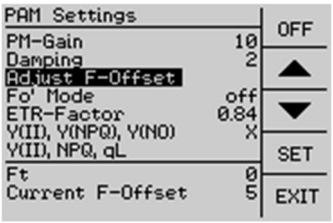


Menu items are selected using the arrow keys of the side panel followed by the SET command, or by directly touching a menu line. The EXIT button provides return to previous window.

A brief instruction showing the submenus is enclosed in the transport box.

Detailed information will be given here in the respective chapters.

5.1 PAM Settings



Settings that affect the PAM fluorescence measurement but do not refer to light/LED settings.

5.1.1 PM-Gain

Signal detection is performed by a photomultiplier. Its sensitivity can be adjusted in 30 steps. An automatic gain adjustment procedure “AGain” is available in the action line of all program windows. This procedure sets the photomultiplier sensitivity to a default value where the Ft signal is in range of 600-800 counts. Menu item “PM-Gain” in the “PAM Settings” window provides an option to manually alter this preset photomultiplier step using the arrow keys.

5.1.2 Damping

Damping is a software-based filter that specifically suppresses high frequency noise and, thus, can improve signal quality. Default setting for damping is 2. Changing damping to higher values can make the WATER-PAM-II response slower.

5.1.3 Adjust F-Offset

The “Adjust F-Offset” command provides access to the determination of background signals. These signals can arise from:

- Optical properties of the instrument like fluorescence excitation from cuvette, detector filter or transmitted traces of measuring light
- Non-optical modulated "electronic noise"
- Fluorescence from suspension like humic acids

The latter, in particular, can be high and requires attention and subtraction from the total signal.

Usually, the background signal increases with measuring light intensity and signal amplification (gain). Therefore, the Adjust F-Offset command determines the background signal for all measuring light intensities and all gain settings.

Detailed instructions for the F-Offset adjustment procedure are given in chapter 6.1, page 59.

5.1.4 Fo' Mode

The "Fo' Mode" provides, after saturation pulses, illumination by far red light to quickly open PS II reaction centers. The measured F0' is the minimum Ft during far red illumination. Interval and intensity of far red illumination can be adjusted in menu "Light Sources" chapter 5.2.8, page 40.

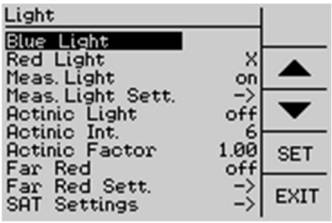
5.1.5 ETR-Factor

This factor is used for ETR calculations and corresponds to the fraction of incident PAR absorbed by a leaf; its default value is 0.84.

5.1.6 Select (Y(II), Y(NPQ), Y(NO)) or (Y(II), NPQ, qL)

Graphical and numerical presentation of data in windows Induction Curve, Light Curve, and Recovery Curve can be selectively displayed with Y(II), Y(NPQ), Y(NO) or Y(II), NPQ, qL. Specifically, selecting "Y(II), Y(NPQ) Y(NO)" displays three yield parameters that are used in analyzing energy partitioning. Choosing Y(II), NPQ, and qL displays the classical NPQ parameter and a parameter for indicating the reduction state of PS II (q_L) (see Tab. 8: Fluorescence Ratio Parameters page 66). The chosen presentation mode is marked with x. To change the presentation, move cursor to the other parameters and touch SET.

5.2 Light



This submenu features all settings of the LED array. Menu items are selected using the arrow keys of the side panel followed by the SET command, or by directly touching a menu line.

5.2.1 Select Blue Light or Red Light

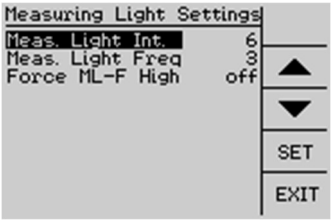
The Instrument provide the option to switch between a blue or red mode. In the blue mode, measuring light as well as actinic illumination is provided with 450 nm. Measuring light and actinic illumination of the red mode features 630 nm.

The chosen WATER-PAM-II color mode is marked with x. To change the light version, move cursor and touch SET.

5.2.2 Meas. Light

Measuring light can be switched by the ON/OFF by selecting “Meas. Light” and touching SET.

5.2.3 Measuring Light Settings



"Measuring Light Sett." leads to the submenu in which measuring light intensity (Meas. Light Int.) and frequency (Meas.Light Freq) can be set.

The measuring light intensity can be adjusted in 12 intensity settings steps. Please marked item Meas. Light Int. by the up/down arrows and confirm with the SET key. The light increase, from intensity setting 1-12 is proportional given the same measuring light frequency.

Selecting the line "Meas. Light. Freq." by SET allows choosing between 5 frequency levels using arrow keys.

Default measuring light settings are Meas. Light Int. = 6 and Meas. Light Freq. = 3.

The last remaining item of the menu "Measuring Light Settings" concerns the highest measuring light frequency of 100 Hz. Force ML-F High overrules settings made under "Meas. Light. Freq." High measuring light frequency improves signal quality but bears the risk that its higher intensity drives photosynthesis, that is, the measuring light becomes actinic. In this case, F0 measurements may be too high. Generally, measuring light frequency is automatically switched to 100 Hz during a saturation pulse and actinic light illumination for Fm and Fm' determinations. Force ML-F High for constant application of 100 Hz measuring light frequency can be activated by SET. Tab. 6 lists the measuring light frequencies of settings 1 - 5 and high.

Tab. 6: Measuring Light Frequencies

Setting	Frequency, Hz
1	5
2	10
3	15
4	20
5	25
high	100

5.2.4 Actinic Light

On/off switch for actinic illumination.

In addition to this menu item, the actinic light can also be switched on/off by the "Act.L." button at the bottom right in the Primary Data, Basic Data or Quenching analysis window.

5.2.5 Actinic Intensity

Intensity regulation for actinic light. Select menu item by SET and choose setting using arrow keys. Settings 1 to 12 are available. PAR information of settings is shown in window “Actinic Light List” (chapter 4.11).

5.2.6 Actinic Factor

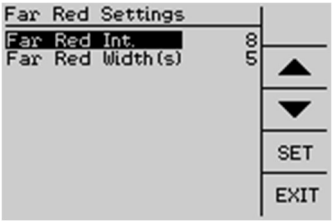
The actinic light factor is a multiplier for the intensities of the 20 actinic light levels. The PAR values of the entire actinic light list are changed by the factor entered. The resulting PAR values can be viewed in window “Actinic Light List”. Factor range is 0.5 to 2.0. Maximum intensity might be constraint by LED limits.

5.2.7 Far Red

On/off switch for far red light.

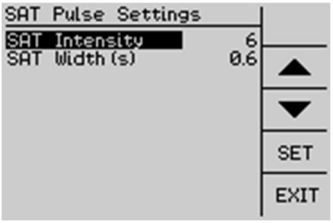
In addition to this menu item, the far red can also be switched on/off by the "F.R." button above the information line in Ft-Chart window.

5.2.8 Far Red Settings



Opens submenu to adjust intensity and duration of far red illumination. These settings are active in F_0’ determinations. The FR light intensity can be adjusted in 12 intensity settings steps. Please marked item Far Red Int. by the up/down arrows and confirm with the SET key. The light increase, from intensity setting 1-12 is proportional. Far Red Width can be set from 2-30 s illumination duration. Default Far Red settings are Far Red Int. = 8 and Far Red Width (s) = 5.

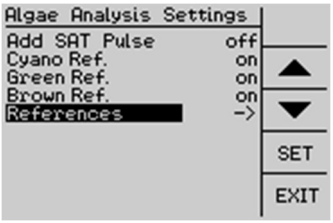
5.2.9 SAT Settings



Moving to “SAT Sett.” and SET opens the submenu “SAT Pulse Settings” in which relative intensity (1 to 12) and duration (width, 0.2 to 2.0 s) of saturation pulses can be set.

In the cuvette the central focus of the sat pulse beam intensity setting 12 corresponds to 6000 µmol m^{-2} s^{-1} adjustable at increments of 500 µmol m^{-2} s^{-1} expandable on request to 12000 µmol m^{-2} s^{-1} adjustable at increments of 1000 µmol m^{-2} s^{-1}.

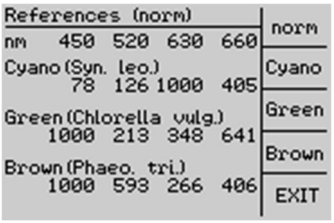
5.3 Algae Analysis



This menu refers to the differential algae content determination. The chlorophyll content measurement can be combined with a saturation pulse analysis. When the menu item “Add SAT Pulse” is marked with yes the WATER-PAM-II will automatically append a saturation pulse for determination of the photosynthetic performance of the sample.

The algae composition of the sample is determined by fitting the three F excitation reference spectra to reflect the measured F values of the measuring light wavelengths as best as possible. If it is known that an algae group is absent from the sample the fitting procedure can be adjusted to the known situation by deactivating the corresponding reference. References marked with “on” are used for deconvolution of the algae composition of the sample.

5.3.1 References



The submenu “References” informs about the characteristics of the applied references. Please note that the WATER-PAM-II can be calibrated with own references of other algae species when operated by WinControl-3. In the References (norm) window, the normalized reference spectra are displayed. The first line lists the 4 measuring light wavelengths 450 nm, 520 nm, 630 nm and 660 nm. The resulting F of the algal references are listed below. For each reference F is normalized to its highest excitation wavelength e.g. Cyano (Syn. leo.) F =1000 for 630 nm; Green (Chlorella vulg.) and Brown (Phaeo. tri.) F=1000 for 450 nm. The raw data of the references can be viewed by the buttons on the right in the side panel. Each button labeled with the group name leads to the respective raw data (Reference Detail window) of the applied reference. The references implemented within the software can be restored in the reset menu. Their raw data is listed in the following figure (Fig. 9).

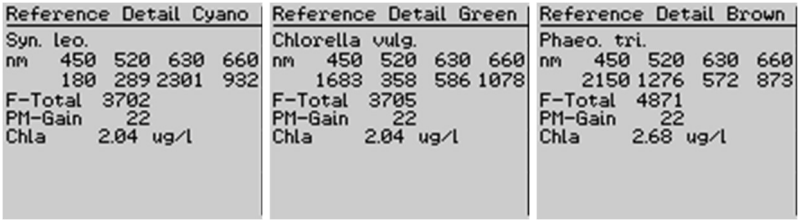
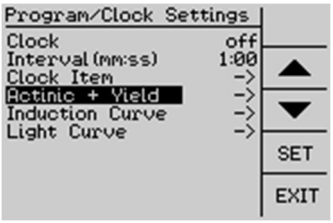


Fig. 9: Reference details (rawdata) of standard references

5.4 Program/Clock Settings



The menu "Program/Clock Settings provides all options to configure automated experimental routines like Actinic + Yield, Induction Curve and Light Curve experiments as well as repetitive triggering of single measurements and experimental protocols by the clock.

5.4.1 Clock

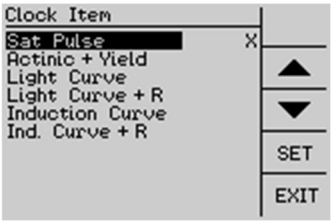
On/off switch of clock. The "Clock" triggers repetitively an event at a defined interval. The interval is specified in "Clock Interval" and the event in "Clock Item".

5.4.2 Clock Interval

Adjust clock interval between 10 s and 60 min by selecting "Interval" and adjusting time interval (up/down keys, SET to confirm).

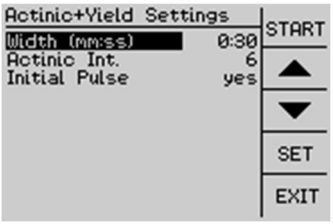
If the clock interval is shorter than the program selected in clock item, clock trigger will be ignored till the program is finished. (E.g. clock interval is 2 minutes and is supposed to trigger a light curve program of 5 minutes total time. The second clock trigger is ignored as the light curve program is still running. The third trigger (minute 6 after clock start) will be executed again and starts the next light curve program.)

5.4.3 Clock Item



Saturation pulse analyses and the programs Actinic + Yield, Induction Curve and Light Curve can be repetitively performed under clock control. Also, recovery experiments can be performed after induction and light curves (item Light Curve + R and Induction Curve + R, respectively). To select one of the six items in menu "Clock Item", move cursor to the item of interest and touch SET. The selected item is marked by an X.

5.4.4 Actinic + Yield Settings



Actinic + Yield in the menu "Program/Clock Settings provides access to the setting of this program routine.

The routine of the Actinic + Yield is defined by three factors: duration (width) and intensity of actinic illumination and the option to start actinic illumination with or without preceding saturation pulse analysis (Initial pulse).

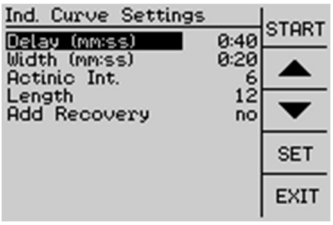
Width of actinic illumination can be adjusted by the arrow keys from 5 s to 5 min.

Actinic intensity level can be set 1-20 and equals the actinic light level within the main menu "Light" (chapter 5.2.5, page 39).

Initial pulse will be executed when yes is selected by the SET command.

An Actinic + Yield routine can be started using the START button on the menu side panel. In this case, the screen display will automatically switch to the Actinic + Yield experimental window

5.4.5 Induction Curve Settings



Selecting Induction curve leads to the submenu "Induction Curve Settings" to define the induction curve routine parameter:

Delay (range 5 s to 10 min) defines the dark interval between saturation pulse analysis with the dark-acclimated sample (F0, FM determinations, Section 7.2, page 64) and beginning of actinic illumination.

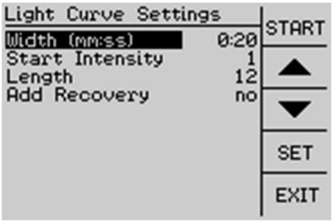
Width (range 5 s to 10 min) is the time interval between two successive saturation pulse analyses during illumination.

Actinic Int sets the actinic intensity level (1-20) of illumination

Length is the number of saturation pulse analyses carried out during actinic illumination.

Add recovery appends a recovery curve to an induction curve (see chapter 4.10 page 30 for information on recovery times).

5.4.6 Light Curve Settings



Selecting Light Curve leads to the submenu “Light Curve Settings” to define the light curve routine parameter:

Width (range 5 s to 10 min) is the time interval between two successive saturation pulse analyses.

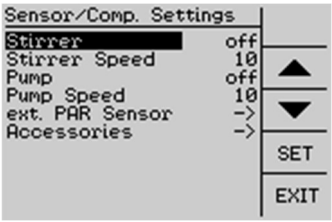
Start Intensity specifies the actinic intensity setting for the first light step

Length is the number of light steps which can range from 2 to 12.

If length = 5 and intensity = 2, are chosen, five light steps with intensity settings 2, 3, 4, 5, and 6 will be performed. The time required for a light curve results from Length times Width.

Add recovery appends a recovery curve to a light curve (see chapter 4.10, page 30) for information on recovery times).

5.5 Sensors/Comp.



Selection of "Sensors/Comp." in the Main Menu opens a window for sensor or accessories which can be connected to WATER-PAM-II instruments. In the respective submenu, settings or operation of these components can be controlled.

5.5.1 Stirrer

After connection to stirrer connector on the rear side of WATER-PAM-II the optional stirrer WATER- II/S can be operated on/off by selecting the item "Stirrer" and touching SET. The favored stirring speed level can be adjusted from 0 to 100 in the next line. Choose Stirring Speed with the cursor, confirm with SET, adjust the stirring speed level with the arrow keys and confirm again with SET.

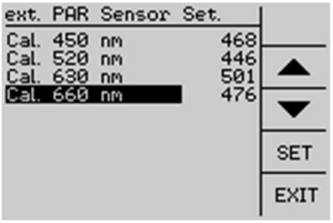
5.5.2 Pump

Peristaltic pumps with 0-10 V control can be controlled by the Water-PAM-II pump control. After connection, the On/Off signal of the pump control is set via this menu item. The pump speed can be set in the next menu item.

5.5.3 Pump Speed

This menu item is for setting the pump speed signal. The pump speed signal can be set from 0-100 resembling 0-10 V analog signal.

5.5.4 ext. PAR Sensor



This menu item provides the option to set multiplier values of the light sensor US-SQS-L.

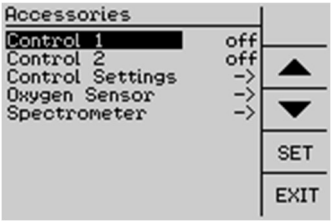
The light calibration is performed for each LED color separately. Therefore, multiplier values of 450 nm, 520 nm, 630 nm and 660 nm are listed in the ext. PAR Sensor Set. window. To check this multiplier value please compare the calibration values to the corresponding multiplier (water) values listed in the calibration certificate of the light sensor. The multiplier values for WATER-PAM-II are marked in the list "Numerical Values of Spectral Sensitivity" of the US-SQS/L calibration certificate.

To change the multiplier values (e.g. when a different light sensor is used than the one supplied) choose the corresponding wavelength line with the cursor, confirm with SET. Adjust the multiplier value with the arrow keys as listed in the calibration certificate and confirm with SET.

The multiplier values of the US-SQS/L, which was delivered with the WATER-PAM-II instrument, are stored within the system parameters, and can be reset by Reset Sys. Set. (chapter 5.9.2).

The light calibration procedure of the WATER-PAM-II LED array is described in chapter 6.1, page 58.

5.5.5 Accessories



Accessories include settings of components that could be connected to the WATER-PAM-II in addition to the standard components like components controlled by PWM signal (CTRL ports), spectrometer, oxygen sensor etc.

Control 1 and Control 2: CTRL1 and CTRL2 connectors of WATER-PAM-II instruments have up to date no assigned accessory but can be subject to own experimental designs. 1 kHz PWM signal 0-100% supplied voltage can be set (Control Settings) and switched on/off (Control 1 and Control 2) for custom designed components connected to CTRL1 and CTRL2.

Oxygen Sensor: The WATER-PAM-II is prepared for connection of an optode sensor as provided in the oxygen package for MINI-PAM-II. An oxygen sensor is not an assigned accessory of WATER-PAM-II but can be subject to your own experimental design.

For details see the instructions for the oxygen measurements with the MINI-PAM-II:

http://www.walz.com/products/chl_p700/mini-pam-II/downloads.html

and the manual for the FireStingO2 oxymeter:

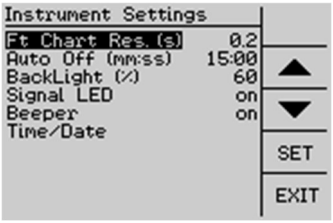
(http://www.pyro-science.com/).

Spectrometer: The spectrometer MINI-SPEC/MP is not an assigned accessory of WATER-PAM-II but can be subject to your own experimental design.

For details see the instructions of the spectrometer with the MINI-PAM-II

http://www.walz.com/products/chl_p700/mini-pam-II/downloads.html

5.6 Instrument Set.



The menu “Instrument Settings” features adjustments of instrumental settings like display resolution, auto off time, backlight intensity, switch of signal LED and beeper as well as time and date of the instrument.

5.6.1 Ft Chart Resolution (s)

Ft chart resolution can be either 0.2 or 1.0 s/dot corresponding to 25 or 125 s/total time axis.

5.6.2 Auto Off (min)

Time interval without key action after which the WATER-PAM-II powers off.

5.6.3 Backlight (%)

Percentage of maximum intensity of the display’s backlight LED array.

5.6.4 Signal LED

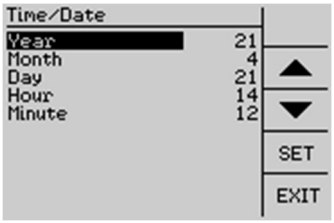
On/off switch for Status LED on top of the WATER-PAM-II

Signal LED Flash code is listed in chapter 3.2.2.5.

5.6.5 Beeper

On/off switch for beeper which acoustically confirms keystrokes and saturation pulse analysis.

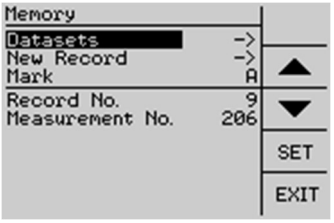
5.6.6 Time/Date



Menu for setting time and date.

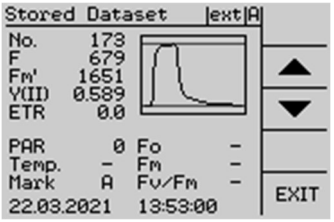
Move the cursor to the line which needs adjustment and confirm with SET. Change the value using the arrow keys.

5.7 Memory



The "Memory" window provides an overview on stored datasets. New records can be started and the mark of saturation pulse data can be changed. Please select the corresponding item and confirm with set. The mark can be altered from A-Z using the arrow keys.

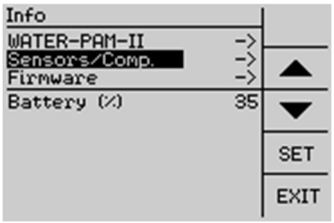
The submenu Datasets displays the SAT pulse fluorescence kinetics (SAT-charts) induced by saturation pulses. The saturation pulses are annotated by date and time (information line) as well as by dataset No.



To see stored induction, light curves and algae analysis, use the MEM key of the respective window. (Chapter 4.4- 4.10)

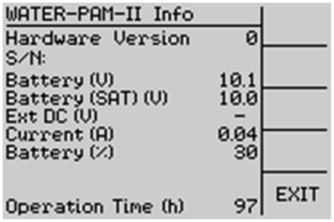
Deletion of data from the WATER-PAM-II internal memory is not allowed in the stand-alone mode. However, the memory can be cleared using the software WinControl-3.

5.8 Info



"Info" provides hardware and software information and indicates current battery status.

5.8.1 WATER-PAM-II Info



This window provides information about the hardware version and serial number of the instrument in line 1 and 2. Line 3-6 show power related details:

Battery (V) = prevailing battery voltage

Battery (SAT) (V) = battery voltage during last saturation pulse. When external power was used this line is marked with -

Ext DC (V) = mains power supply connected or not

Current (A) = present current consumption

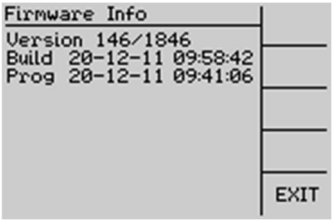
The total operation time (h) of the instrument is provided in the last line of this window.

5.8.2 Sensor/Comp Info

Serial number of the light sensor US-SQS/L which was provided within the scope of delivery. The calibration values of this light sensor are reset when Reset Sys. Set. is executed.

This window provides also information about serial number and hardware of connected digital recognizable components e.g. oxygen sensor, spectrometer or the pump control.

5.8.3 Firmware Info



This window lists firmware version and times of completion

5.9 Reset

Reset provides three categories to reset the device to original settings.

- Reset Settings: general instrument settings, default values for PAM measurements
- Reset Sys. Set.: special settings e.g. light calibration factors
- Reset References: standard references for algae analysis

5.9.1 Reset Settings

Reset Settings restores general default settings of WATER-PAM-II instruments as listed in Tab. 7: Default Settings.

When the device is switched off, some applied settings are saved in the internal memory. The saved setting parameters are marked Yes in the right column of Tab. 7: Default Settings. “Yes” implies that these custom chosen settings are restored on following use of the WATER-PAM-II. They also will be reset to original values by executing reset settings.

Tab. 7: Default Settings

	Default Setting	Current Setting (saved and restored)
Measuring Light		
Status	On	No
Intensity	6, relative unit	Yes
Frequency	3,	Yes
Frequency high status	Off	No
Actinic Light		
Status	Off	No
Intensity	6, relative unit	Yes
Factor	1.00	Yes
Detector		
PmGain	10, relative unit	Yes
Damping	2, relative unit	Yes
Far Red Light		
Width 5	5, s	Yes

Tab. 7: Default Settings

	Default Setting	Current Setting (saved and restored)
Intensity	8, relative unit	Yes
Saturation Pulse		
Intensity	6, relative unit	Yes
Width	0.6, s	Yes
Program Actinic Light and Yield		
Actinic light width	30, s	Yes
Initial Pulse	Yes	Yes
Program Induction Curve		
Delay	40, s	Yes
Width	20, s	Yes
Length	12	Yes
Program Light Curve		
Width	20, s	Yes
Start Intensity	1, relative unit	Yes
Length	12, light steps	Yes
Clock		
Item	Saturation pulse	Yes
Interval	60, s	Yes
Hardware		
Signal LED status	On	Yes
Beeper status	On	Yes
Automatic power down	15, min	Yes
Background light	60%	Yes

Tab. 7: Default Settings

	Default Setting	Current Setting (saved and restored)
Graphics		
Ft chart time resolution	0.2, s/dot	Yes
Stirrer		
Status	Off	No
Speed	10	Yes
F_0' Mode		
Status	Off	Yes
PAR		
Status	Internal	No
Mark		
Character	A	Yes

5.9.2 Reset Sys. Set.

Reset Sys. Set. resets light sensor calibration value to factory settings of light sensor listed in Info (US-SQS/L supplied within scope of delivery). Being connected, settings of additional components like spectrometer or oxygen sensor are reset to their default settings.

5.9.3 Reset References

References for the differential algae analysis can be altered using WinControl-3. In standalone operation references are not object of change

Executing “Reset References” restores reference to standard references.

6 Maintenance

This section provides instructions for the most important maintenance topics.

6.1 Calibration of internal light list

For calibration of the LED array please connect the light sensor US-SQS/L to the socket on the rear side of the WATER-PAM-II.

The correct position of the spherical sensor is defined by a 15.3 mm spacer which needs to be mounted between cap and sensor filter housing as shown in Fig. 10.

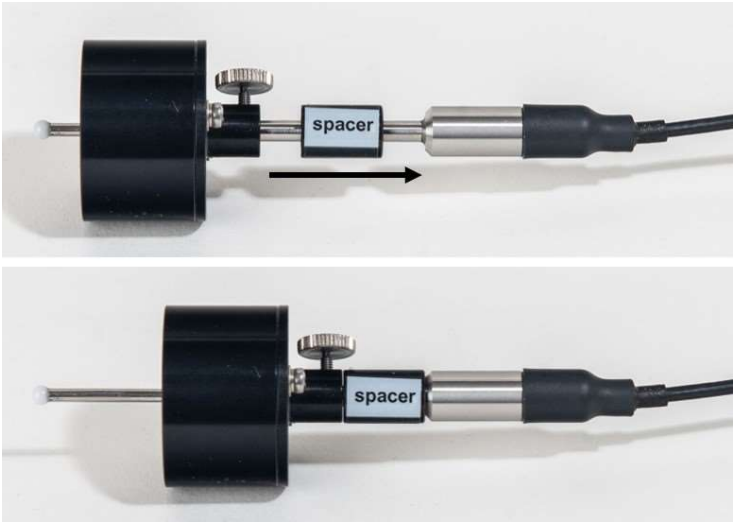


Fig. 10: Mounting of US-SQS/L, 15.3 mm spacer

To make sure the sensor settings are correct, please open Sensor/Comp. window in the main menu (Fig. 11 left) to get to the submenu for the external PAR sensor (Fig. 11 right).

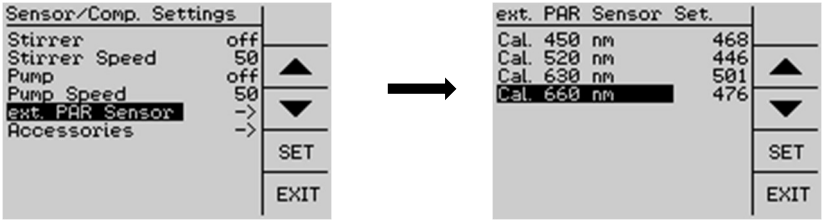
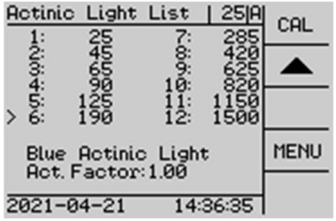


Fig. 11: Calibration multiplier settings of the external PAR Sensor US-SQS/L.

Please insert the correct calibration multiplier values as listed in the certificate of calibration of the light sensor US-SQS/L.

For light array calibration please

1. Insert a clean water filled cuvette to WATER-PAM-II
2. Position light sensor US-SQS/L within this cuvette
3. Scroll to the Actinic Light List window and execute CAL in the upper right corner to start light array calibration. Light calibration will be done for all measuring light and actinic light colors.



6.2 F-Offset adjustment

Background fluorescence has an influence on photosynthesis parameters. Therefore, background F signals originated by instrument specific electronic noise, cuvette characteristics (which are significant higher

using the standard glass sample tubes instead of the quartz glass cuvette) and background fluorescence within the sample (originated by humid acids or other yellow substances) need to be determined and considered.

F-Offset values for all measuring light conditions are stored within the instrument. The currently applied F- Offset is listed in the Main Menu PAM Settings where also the procedure for the automatic F-Offset determination is located.

To adjust the F-Offset values please:

1. insert the cuvette you are going to use with steril filtrated media
2. Open the main menu “PAM Settings” window and execute Adjust F-Offset procedure by marking this line with the arrow keys and confirming with SET.

The procedure will take several minutes to determine the F-Offset values for all measuring light conditions.

6.3 Battery exchange/storage

The system can be operated either by eight rechargeable AA batteries (1.2 V/2 Ah), non-rechargeable batteries (1.5 V/2 Ah) or using line power. The device can be connected to line power (using power supply MINI PAM-II/N) even in the presence of non-rechargeable batteries.

To open the battery compartment, shift the closing mechanism to the side.

Batteries can be recharged in the supplied automatic power/off battery charger. Please remove batteries before long-term storage of the WATER-PAM-II. Batteries are best stored in 60% charged condition.

The locking mechanism of the aluminum plate functions properly if the label “INNER FACE” on the aluminum plate faces the batteries.

6.4 Cuvette cleaning

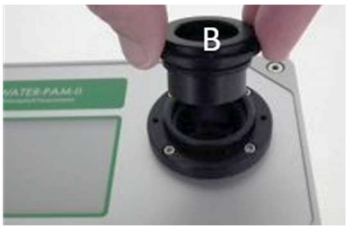
Quartz glass and standard glass cuvettes need careful cleaning to reduce background noise. Best results are obtained by mechanical cleaning with the provided cuvette brush and a mixture of 70% EtoH containing washing up liquid as detergent. In the final cleaning step, the cuvette needs to be very carefully rinsed by distilled water.

Please note: Traces of EtoH and detergent can affect your sample and background signal!

6.5 Cleaning the spill-protection

The optics of the WATER-PAM-II are protected by a Perspex overspill cover. For cleaning, please follow the instructions:

1. Please lift the cuvette cover (A) and cuvette holder (B)



2. The Perspex spill cover (C) and black cuvette guide (D) can be cleaned by water containing washing up liquid as detergent.

Note: Do NOT use EtOH, other alcohols or chemical cleaning agents. They may turn Perspex blind.

6.6 Touch Screen calibration

To invoke the calibration window, start WATER-PAM-II with finger pressed on touchscreen. To calibrate, use pointed but not edged tool like a wooden toothpick. Touch target point (+) at bottom right corner (see Fig. 12). Consecutively, three more target points need to be touched. Finally, a message “Press any-where to continue” indicates end of calibration.

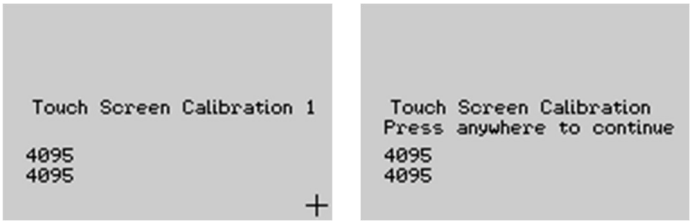


Fig. 12: Touch screen calibration windows

7 Saturation Pulse Analysis

7.1 Five Fluorescence Levels

Usually, five different levels of modulated fluorescence are acquired by PAM fluorometers. Two of these levels (Fo and Fm) must be measured with the dark-acclimated sample. The three other levels (Fo', F, and Fm') are measured with the actinic light-exposed sample or in a dark period following this light treatment (see Fig. 13, page 63). Some parameters of saturation pulse analysis require fluorescence measurement of the same sample in both the dark-acclimated and light-exposed state.

Because PAM fluorescence is excited by µs pulses of constant amplitude, variations between fluorescence levels are usually interpreted as variation in chlorophyll fluorescence yield. This applies for variations between different types of fluorescence levels (e.g. between Fo and Fm) and for variations of

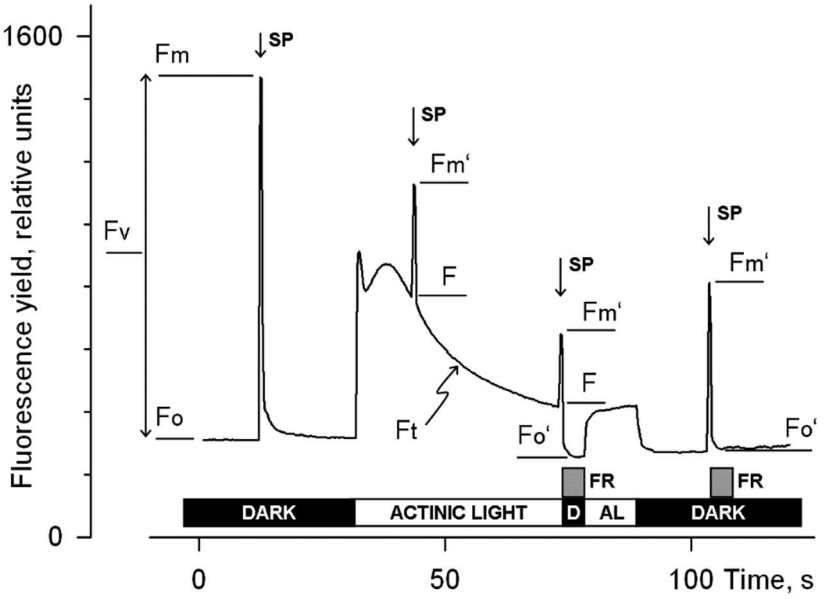


Fig. 13: Measurements for Saturation Pulse Analysis. AL, Actinic Light; D, dark; SP, Saturation Pulse; FR, Far red illumination.

the same type of fluorescence level (e.g. the change of Fm' during a fluorescence induction curve).

7.2 Measurements with Dark-Acclimated Samples

Fo Minimum fluorescence level excited by very low intensity of measuring light to keep PS II reaction centers open.

Fm Maximum fluorescence level elicited by a pulse of saturating light (Saturation Pulse) which closes all PS II reaction centers.

7.3 Measurements with Illuminated Samples

F_0' Minimum fluorescence level of illuminated sample. The Fo' is lowered relative to Fo by non-photochemical quenching. The measuring routine for Fo' determines the Fo' level during a dark interval following a Saturation Pulse. In this dark interval, far red light is applied which selectively drives PS I. As a consequence, electrons are removed from the intersystem electron transport chain and opening of PS II reaction centers is efficiently accelerated (see Fig. 13, page 63, time point 75 s). Ideally, far red opens PS II in less than 5 s and it is often assumed that during this short interval light-driven energization of the photosynthetic membrane decays very little.

If the Fo' Mode is switched off, the Fo' will be calculated according to Oxborough and Baker (1997):

$$F_0' = \frac{1}{\frac{1}{F_0} - \frac{1}{F_M} + \frac{1}{F_M'}}$$

On the touchscreen and in WinControl-3, the numerical value of the calculated Fo' is preceded by a tilde sign (e.g. ~456).

F_m' Maximum fluorescence level of the illuminated sample. The Fm' is induced by a Saturation Pulse which temporarily closes all PS II

reactions centers. Fm' is decreased relative to Fm by non-photochemical quenching.

F The F corresponds to the momentary fluorescence level (Ft) of an illuminated sample measured shortly before application of a Saturation Pulse.

Tab. 8: Fluorescence Ratio Parameters

Source	Equation	Sample State	Range [Theory] [Experiment]
Maximum photochemical quantum yield of PS II (Kitajima and Butler, 1975)	$\frac{F_V}{F_M} = \frac{F_M - F_0}{F_M}$	Dark	[0, 1] [0, ~0.84]
Effective photochemical quantum yield of PS II (Genty *et al.*, 1989)	$Y(II) = \frac{F'_M - F}{F'_M}$	Light	[0, 1] [0,~ 0.84]
Quantum yield of light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Genty *et al*. 1996)*	$Y(NPQ) = \frac{F}{F'_M} - \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Quantum yield of non-regulated heat dissipation and fluorescence emission: this type of energy loss does not involve the action of a trans-thylakoid ΔpH and zeaxanthin (Genty *et al*. 1996)*	$Y(NO) = \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Stern-Volmer type non-photochemical fluorescence quenching (Bilger +and Björkman, 1990)	$NPQ = \frac{F_M}{F'_M} - 1$	Dark and Light	[0, ∞] [0, ~4]
Coefficient of photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_P = \frac{F'_M - F}{F'_M - F'_0}$	Light. If F_0' calculated, Dark and Light	[0, 1] [0, 1]
Coefficient of photochemical fluorescence quenching assuming interconnected PS II antennae (Kramer *et al.* 2004)	$q_L = q_P \cdot \frac{F'_0}{F}$	As $q_{P'}$	[0, 1] [0, 1]
Coefficient of non-photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_N = 1 - \frac{F'_M - F'_0}{F_M - F_0}$	Dark and Light	[0, 1] [0, ~0.95]

* Kramer *et al.* (2004) have derived more complex equations for Y(NO) and Y(NPQ). Klughammer and Schreiber (2008) have demonstrated that the equations by Kramer *et al.* (2004) can be transformed into the simple equations of (Genty *et al*. 1996) which are used by the MINI-PAM-II and WinControl-3.

Tab. 9:References Cited in Tab. 8: Fluorescence Ratio Parameters

- Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosynth Res 25:173-185
- Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87–92
- Genty B, Harbinson J, Cailly AL and Rizza F (1996) Fate of excitation at PS II in leaves: the non-photochemical side. Presented at: The Third BBSRC Robert Hill Symposium on Photosynthesis, March 31 to April 3, 1996, University of Sheffield, Department of Molecular Biology and Biotechnology, Western Bank, Sheffield, UK, Abstract P28
- Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376:105-115
- Klughammer C and Schreiber U (2008) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. PAM Application Notes 1: 27-35 (http://www.walz.com/e_journal/pdfs/PAN078007.pdf)
- Kramer DM, Johnson G., Kiirats O, Edwards GE (2004) New flux parameters for the determination of Q_A redox state and excitation fluxes. Photosynth Res 79: 209-218
- Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of pho-tosynthetic efficiency into photochemical and non-photochemical components - calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 54 135-142
- Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51–62
- van Kooten O, Snel J (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25: 147–150

7.4 Fluorescence Ratio Parameters

To quantify photochemical use and non-photochemical losses of absorbed light energy, fluorescence ratio expressions have been derived which use two or more of the five relative fluorescence yields introduced above. Fluorescence ratio parameters available in WATER-PAM-II and WinControl-3 will be explained briefly.

F_V/F_m and Y(II) Maximum and effective photochemical quantum yields of PS II

The F_V/F_m and Y(II) estimate the fraction of absorbed quanta used for PS II photochemistry. F_V/F_m corresponds to the maximum photochemical yield of PS II, Y(II) is the effective photochemical yield of PS II. Measurements of F_V/F_m require that samples are acclimated to darkness or dim light so that all reactions centers are in the open state and non-photochemical dissipation of excitation energy is minimal.

In algae and cyanobacteria, however, the dark-acclimated state often is not showing maximal PS II quantum yield, as the PS II acceptor pool may be reduced in the dark by stromal reductants and, consequently, the so-called state 2 is formed exhibiting low PS II quantum yield. In this case, preillumination with moderate far red light should precede determinations of F_0 and F_m.

The Y(II) value estimates the photochemical use of excitation energy in the light. It is lowered with respect to F_V/F_m by partial closure of PS II centers and various types of non-photochemical energy losses induced by illumination.

q_P and q_L Coefficients of photochemical fluorescence quenching

Both parameters estimate the fraction of open PS II reaction centers. The q_P is based on the concept of separated PS II antenna units (puddle model), whereas the q_L assumes interconnected PS II antenna units (lake model) which appears to be the more realistic situation in leaves (*cf.* Kramer *et al.*, 2004). Determinations of q_P an q_L do not require fluorescence measurements with the dark-acclimated sample except that the F_0' is calculated according to Oxborough and Baker (1997).

q_N and NPQ Parameters of non-photochemical quenching

Both parameters are associated with non-photochemical quenching of excitation energy, mainly involving a low thylakoid lumen pH- and a zeaxanthin-dependent quenching mechanism. The qN and the NPQ parameters require fluorescence measurements with the sample in the dark-acclimated and in the light-exposed states.

Calculation of NPQ (or SV_N; Gilmore and Yamamoto, 1991) corresponds to the Stern-Volmer equation for fluorescence quenching which predicts proportionality between fluorescence quenching (NPQ) and the concentration of fluorescence-quenching centers in the photosynthetic antennae (e.g. zeaxanthin).

Y(NO), Y(NPQ) and Y(II) Complementary PS II yields

Genty *et al.* (1996) presented expressions describing the partitioning of absorbed excitation energy in PS II between three fundamental pathways the sum of which adds up to one:

Y(NO) non-regulated losses of excitation energy including heat dissipation and fluorescence emission,

Y(NPQ) regulated energy losses of excitation energy by heat dissipation involving ΔpH- and zeaxanthin-dependent mechanisms, and

Y(II) use of excitation energy for charge separation.

This concept of "complementary PS II quantum yields" is useful to analyze the partitioning of absorbed light energy in photosynthetic organisms. For instance, in the presence of strong light, a much higher Y(NPQ) than Y(NO) indicates that excess excitation energy is safely dissipated at the antenna level and that photosynthetic energy fluxes are well-regulated.

In variance, high values of Y(NO) would signify that excess excitation energy is reaching the reaction centers, resulting in strong reduction of PS II acceptors and photodamage, e.g. via formation of reactive oxygen species.

7.5 Relative Electron Transfer Rate (ETR)

Relative electron transfer rates are calculated according to:

ETR = PAR · ETR-Factor · P_{PS2}/P_{PS1+2} · Y(II).

The basic idea of the ETR equation is to multiply Y(II), the effective photochemical quantum yield of PS II, by an estimate for the photon flux density absorbed by all PS II in the sample. The latter estimate is derived from three numbers:

(1) PAR Quantum flux density of photosynthetically active radiation (PAR) impinging on the sample.

(2) ETR-Factor Sample absorptance (= 1 – transmittance)

The ETR-Factor describes the fraction of incident photons absorbed by the sample. The most frequently used default value for green leaves is 0.84 meaning that 84% of incoming light is absorbed. The ETR-Factor can be lower in bleached leaves or leaves containing considerable amounts of non-photosynthetic pigments like anthocyanins.

(3) P_{PS2}/P_{PS1+2} Relative distribution of absorbed PAR to photosystem II

The default P_{PS2}/P_{PS1+2} is 0.5 which assumes the PS II contributes 50% to total sample absorptance. The P_{PS2}/P_{PS1+2} may deviate from the idealized

factor of 0.5 depending on wavelength of light and acclimation status of the sample.

7.6 Light Curves

The light curve program exposes a sample to stepwise increasing intensities of actinic illumination. In “Rapid Light Curves” (RLC), the time interval of each light step is short (down to 10 s) and full equilibration of photosynthetic reactions is not reached within an illumination interval. Typically, the RLC starts at a PAR value somewhat below that of the natural environment.

RLC measurements are carried out with samples in their momentary acclimation status, that is, without dark-acclimation period to determine F_0 and F_m. This way, RLC data can provide information on the present acclimation state of photosynthesis. Obviously, without F_0 and F_m determination, those fluorescence ratio parameters requiring F_0 and F_m (like NPQ and qP) are not available.

If illumination steps are long enough to reach steady state of photosynthesis, fluorescence-based light curves may be compared with classical light response curves (P-I curves). Naturally, any limitation by insufficient CO_2 supply must be avoided during such long-term light curves.

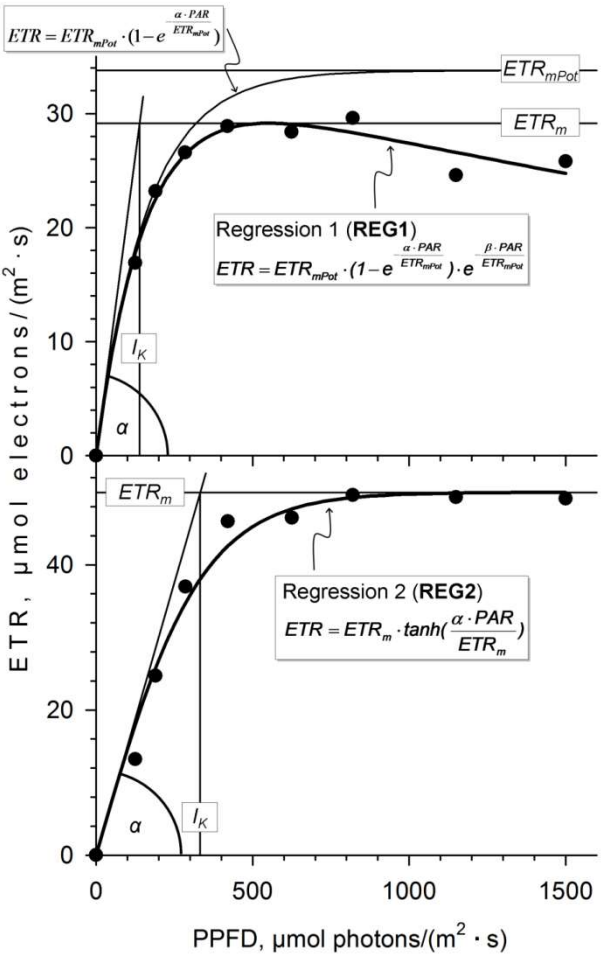


Fig. 14: Model Functions of Rapid Light Curves

In both cases, RLC and long-term light curves, plotting ETR versus PAR yields light response curves which are often described by the following three cardinal points.

- α (alpha), electrons/photons: Initial slope of RLC which is related to quantum efficiency of photosynthesis.

- ETR_{max}, µmol electrons/($m^2 \cdot s$): Maximum electron transport rate.
- I_K, µmol photons/($m^2 \cdot s$): Minimum saturating irradiance.

Evaluation of cardinal points requires WinControl-3. The software uses two empirical functions to estimate these cardinal data: the functions **REG1** and **REG2** have been introduced by Platt *et al.* (1980) and Jassby and Platt (1976), respectively, to describe classical light response curves of photosynthesis.

REG1

In case of REG1, the cardinal point α results directly from fitting the ETR below to experimental data.

$$ETR = ETR_{mPot} \cdot (1 - e^{-\frac{\alpha \cdot PAR}{ETR_{mPot}}}) \cdot e^{-\frac{\beta \cdot PAR}{ETR_{mPot}}}$$

The latter equation considers photoinhibition of photosynthesis by high light intensities. Therefore, the fitting procedure also yields an estimate for β, the "photoinhibition parameter"(Platt *et al.*, 1980), and for ETR_{mPot}, the maximum potential light-saturated electron transport rate which would be observed if photoinhibition was absent.

Platt *et al.* (1980) have introduced the "Photoinhibition Index" (I_b) to quantify photoinhibition. The authors define I_b as the PAR value required to photoinhibit ETR_{mPot} by the factor of 1/e according to:

$$I_b = ETR_{mPot} / \beta$$

With the results from curve fitting, WinControl-3 computes the ETR_{max} and I_K according to:

$$ETR_{\max} = ETR_{mPot} \cdot (\frac{\alpha}{\alpha + \beta}) \cdot (\frac{\beta}{\alpha + \beta})^{\frac{\beta}{\alpha}} \quad \text{and} \quad I_K = \frac{ETR_m}{\alpha} \quad .$$

REG2

The function REG2 is monotonically nondecreasing and, hence, does not allow for photoinhibition:

$$ETR = ETR_m \cdot \tanh(\frac{\alpha \cdot PPFD}{ETR_m})$$

Here, the cardinal points, α and ETR_{max}, are estimated by the fitting procedure. With the latter two parameters, the I_K is calculated as described for REG1.

7.7 Some selected References and Reviews

7.7.1 Light Curve References

Long-term Light Curves

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Rapid Light Curves

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7.7.2 Saturation Pulse Analysis References

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Kalaji HM *et al*. (2017) Frequently asked questions about in vivo chlorophyll fluorescence, the sequel. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynth Res 132: 13–66

Logan BA, Adams III WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. Funct Plant Biol 34, 853–859

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Mohammed GH, Binder WD, Gillies SL (1995) Chlorophyll fluorescence: A review of its practical forestry applications and instrumentations. Scand J Forest Res 10:383-410

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Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51-62

7.7.3 Fluorescence measurements on algae and diatoms

Geel C, Versluis W, Snel JFH (1997) Estimation of oxygen evolution by marine phytoplankton from measurement of the efficiency of photosystem II electron flow. Photosynth Res 51: 61-70

Heinze I, Dau H, Senger H (1996) The relationship between the photochemical yield and variable fluorescence of photosystem II in the green alga Scenedesmus obliquus. J Photochem Photobiology B 32: 89-95

Jakob T, Schreiber U, Kirchesch V, Langner U, Wilhelm C (2005) Estimation of chlorophyll content and daily primary production of the major algal groups by means of multiwavelength-excitation PAM chlorophyll fluorometry: performance and methodological limits. Photosynth Res 83: 343-361

Schreiber U, Hormann H, Neubauer C, Klughammer C (1995) Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. Aust J Plant Physiol 22: 209-220

Schreiber U, Endo T, Mi H, Asada K, (1995)1 Quenching Analysis of Chlorophyll Fluorescence by the Saturation Pulse Method: Particular Aspects Relating to the Study of Eukaryotic Algae and Cyanobacteria Plant and Cell Physiology, Volume 36, Issue 5, July 1995, Pages 873–882,

Diatoms:

Caron L, Berkaloff C, Duval J-C, Jupin H (1987) Chlorophyll fluorescence transients from the diatom Phaeodactylum tricornutum: relative rates of cyclic phosphorylation and chlororespiration. Photosynth Res 11: 131-139

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8 Specifications WATER-PAM-II

8.1 Optoelectronic Unit WATER-PAM-II

Measuring light: Blue (450 nm) red (630 nm) LED, standard modulation frequencies 5 to 25 Hz, adjustable in increments of 5 Hz, and 100 Hz, measuring light PAR at standard settings = 0.05 µmol m^{-2} s^{-1}. For differential algae analysis auxiliary LEDs 520 and 660 nm.

Actinic light: Blue (450 nm) and red (630) LED as for measuring light, maximum actinic PAR = 3000 µmol m^{-2} s^{-1}, maximum PAR of saturation pulses = 6000 µmol m^{-2} s^{-1} adjustable at increments of 500 µmol m^{-2} s^{-1} expandable on request to 12000 µmol m^{-2} s^{-1} adjustable at increments of 1000 µmol m^{-2} s^{-1}

Far red light: Peak emission at 730 nm

Signal detection: Hamamatsu photomultiplier protected by long-pass and a short-pass filters

Cuvette holder: for 15 mm Ø cuvette, Perspex inlet for overspill protection, POM cuvette cover 35 mm high x 40 mm Ø

Data memory: Flash memory, 8 MB, providing memory for more than 27,000 saturation pulse analyses

Display: Backlit 160x104 dots (78 x 61 mm) transflective B/W LCD display with resistive touchscreen

Ports: PAR sensor, Temp, AUX1, AUX2, SYNC, Stirrer, CTRL1, CTRL2, COMP, EXT DC and USB cable

Power supply: 8 AA (Mignon) rechargeable batteries (Eneloop 1.2 V/2 Ah), providing power for up to 1000 yield measurements; 8 spare batteries, automatic power/off, battery charger (100 to 240 V AC, 50-60 Hz, 0.35 A) for 1 to 8 AA NI-MH batteries, 12 V 5,5 A power supply MINI PAM-II/N

Operating temperature: -5 to +45 °C, (non-condensing)

Dimensions: 17.2 cm x 13.5 cm x 7.6 cm (L x W x H)

Weight: 1.5 kg (incl. battery)

Table 1: Pin Assignment when Looking from the Outside.

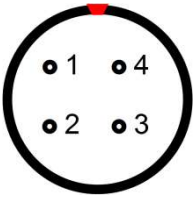
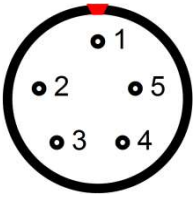
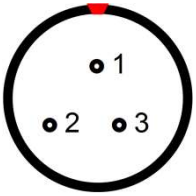
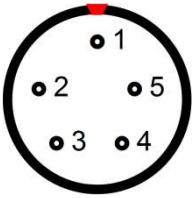
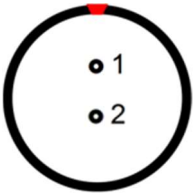
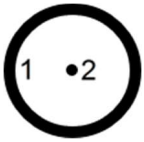
	AUX 1/AUX 2 1. GND 2. Serial Clock 3. Serial Data 4. + 5 V Output
	SYNC 1. GND 2. SYNC 3. RS 485 A 4. RS 485 B 5. Voltage output of current power supply (line or battery)
	EXT. DC 1. – 2. GND 3. + 12 V Input

Table 1: Pin Assignment when Looking from the Outside.

	COMP 1. + 5 V Output (= 4 of LEAF CLIP) 2. Voltage output of current power supply (= 5 of SYNC) 3. RS 232 TxD 4. RS 232 RxD 5. GND
	TEMP (Thermocouple Type K) 1. – 2. +
	STIRRER / CTRL 1 / CTRL 2 1. – 2. +

8.2 Power Supply MINI-PAM-II/N

Input: 100 to 240 V AC, 50 to 60 Hz

Output: 12 V DC, 5.5 A

Operating temperature: -5 to +45 °C, (non-condensing)

Dimensions: 13 cm x 5.5 cm x 3 cm (L x W x H)

Weight: 350 g including cables

8.3 Battery Charger 000190101101

Input: 100 to 240 V AC, 50 to 60 Hz

Output: 12 V DC, 1.0 A

Operating temperature: -5 to +45 °C, (non-condensing)

Dimensions: 17.5 cm x 10.5 cm x 3 cm (L x W x H)

Weight: 300 g including cable

8.4 Complementary Items

Stylus for touchscreen.

8.5 Software WinControl-3

Program: WinControl-3 System Control and Data Acquisition Program (Windows 8 und 10, 32-bit and 64-bit; Linux on request) for operation of measuring system via PC, data acquisition and analysis

Saturation Pulse Analysis: Measured: Ft, F_0, F_M, F, F_0' (also calculated), F_M'. PAR, Calculated: F_0' (also measured), F_V/F_M and Y(II) (maximum and effective photochemical yield of PS II, respectively), q_L, q_P, q_N, NPQ, Y(NPQ), Y(NO) and ETR (electron transport rate);

Fitting Routines: Differential algae analysis; two routines for determination of the cardinal points α, I_k and ETR_{max} of light curves

Programmed Features: light curve protocols; induction curve protocols; Actinic+Yield protocols; clock and batch file operation; automatic determination of signal offset and background fluorescence (originated e.g. by yellow substances) for all light intensities and all gain levels; automatic calibration of internal PAR using light sensor US-SQS/L

Communication Protocol: USB

Computer Requirements: Screen resolution, 1280 x 800 pixels. Interface, USB 2.0/3.0

8.6 Transport Case WATER-PAM/T

Design: Aluminum case with custom foam packing

Dimensions: 50 cm x 34 cm x 20 cm (L x W x H)

Weight: 3.8 kg

8.7 Accessories and Add-on

8.7.1 Stirrer WATER-II/S

Design: Instrument operated stirrer with disposable Perspex stirring paddles. Scope of delivery includes a set of ten Stirring Paddles WATER-R.

Dimensions: 71 mm x 30 mm (l x Ø)

Weight: 95 g

8.7.2 Stirring Paddles WATER-R

Design: Set of 10 perspex stirring paddle for WATER-II/S

8.7.3 Flow-Through Cuvette PHYTO-II/FT

Design: Cuvette holder made from black polyoxymethylene plastic (POM) with in-/out water tubing connectors (4 mm inner diameter; 6 mm outer diameter) The cuvette is mounted with two knurled screws to the optical unit of the instrument (PHYTO-PAM-II COMPACT Version or WATER-PAM-II). Delivery includes a POM mounting device for the Spherical Micro Quantum Sensor US-SQS.

Dimensions: Ø 49 mm, with connectors and cuvette 64 mm x 74 mm

Weight: 150 g

8.7.4 Barcode Scanner BCS-9590

Design: Single-line handheld laser scanner with trigger button and 1 m, partially coiled cord; to be connected to the Photosynthesis Yield Analyzer MINI-PAM-II or WATER-PAM-II. Bar codes are stored together with fluorescence data

Dimensions: 9 cm (max.) x 6 cm (max.) x 16 cm (max.) (L x W x H)

Weight: 335 g

9 Guarantee

9.1 Manufacturer's Guarantee

Under this Manufacturer's Guarantee ("Guarantee"), subject to the Conditions and Instructions below, Heinz Walz GmbH, Germany ("Manufacturer"), guarantees (§443 BGB) to the end customer and user ("Customer") that all products supplied by it shall substantially conform in material respects to the Specifications for 24 months from the delivery date (date on invoice). In this Guarantee, "Specifications" means the product's features (as may be amended by Manufacturer from time to time), which are set out under the headings "specifications" and/or "technical specifications" within the product's respective brochure, data sheet, or respective tab on the Manufacturer's website for such product, and which may be included with the documents for the product when delivered. In case of an eligible guarantee claim, this Guarantee entitles the Customer to repair or replacement, at the Manufacturer's option, and this Guarantee does not include any other rights or remedies.

9.2 Conditions

This Guarantee shall not apply to:

- Any defects or damage directly or indirectly caused by or resulting from the use of unauthorized replacement parts and/or service performed by unauthorized personnel.
- Any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.
- Damage caused from improper packaging during shipment or any acts of God.
- Batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps (halogen, LED), thermocouples, and underwater cables.
- Defects that could reasonably have been detected upon inspection of the product when received by the Customer and not promptly noticed within ten (10) days to Heinz Walz GmbH.

- Submersible parts of the DIVING-PAM or the underwater version of the MONITORING-PAM have been tested to be watertight down to the maximum operating depth indicated in the respective manual. Guarantee shall not apply for diving depths exceeding the maximum operating depth. Further, guarantee shall not apply for damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

9.3 Instructions

- To obtain guarantee service, please follow the instructions below:
- The Walz Service Information Form available at http://www.walz.com/support/repair_service.html must be completed and returned to Heinz Walz GmbH, Germany.
- The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, and/or shipping costs incurred in returning equipment for guarantee service are at customer expense. Duty and taxes are covered by Walz.
- All products being returned for guarantee service must be carefully packed and sent freight prepaid.
- Heinz Walz GmbH, Germany is not responsible or liable for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.

9.4 Applicable law

- This Guarantee is governed by German law. Place of jurisdiction is Bamberg, Germany.