

Ballast-Check™2

Handheld PAM Fluorometer



December 5, 2014
P/N: S-0156
Revision DRAFT

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WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE) DIRECTIVE

Turner Designs is in the business of designing and selling products that benefit the well-being of our environment. Accordingly, we are concerned with preserving the surroundings wherever our instruments are used and happy to work with customers by complying with the WEEE Directive to reduce the environmental impact resulting from the use of our products.

WEEE Return Process:

To arrange the return of an end-of-life product, proceed as follows:

If you purchased your instrument through a Turner Designs Distributor please contact your local representative. They will instruct you where to return the end-of-life product.

If you purchased your instrument directly from Turner Designs please contact Turner Designs Customer Service

By Phone: 1-408-212-4041 or Toll Free: (877) 316.8049

By Email: Customer Service at support@turnerdesigns.com

Turner Designs will provide a WEEE RMA Number, a Shipping Account Number, and a Ship to Address. Package and ship the product back to Turner Designs.

The product will be dealt with per Turner Designs' end-of-life recycling program in an environmentally friendly way.

1 Introduction

1.1 Description

The Ballast-Check 2 is configured to provide users with a rapid estimation of algal abundance (Corrected Fluorescence or CFI) and photosynthetic efficiency (Activity) of algae in water. The CFI (see section 4.1) value represents varying algal abundances used for determining gross exceedance of algae in ballast water samples around the set threshold level. Activity estimates are logged as a ratio (Fv/Fm) on a scale from 0.00 to 1.00 (see section 4.2).

Abundance reporting is based on a fluorescence threshold:

Low: Corrected Fluorescence < 100
High: Corrected Fluorescence ≥ 100

Activity reporting is based on a yield threshold:

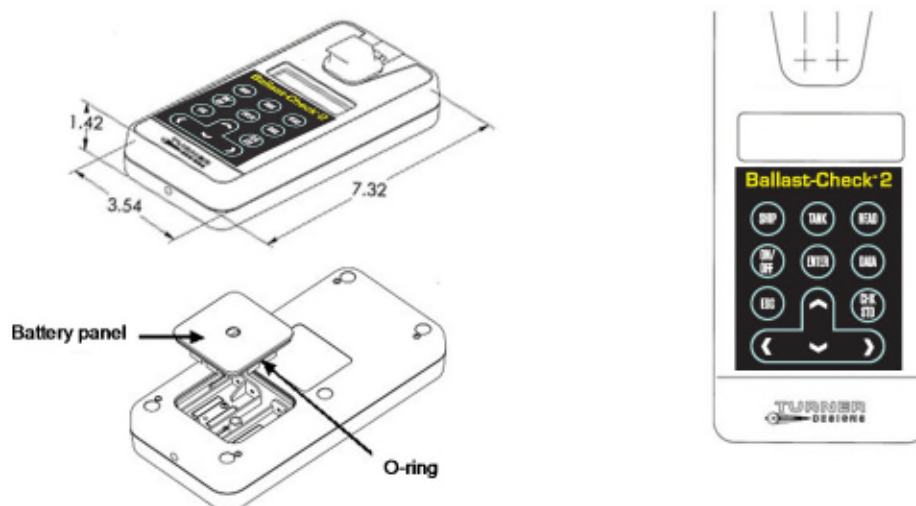
Low: Activity < 0.3
High: Activity ≥ 0.3

1.2 PAM Fluorometry

The Ballast-Check 2 is a Pulse Amplitude Modulated (PAM) Fluorometer. Pulse amplitude modulation is a process that uses varying light intensities delivered in a series of pulses for looking at fluorescence quenching to determine photosynthetic efficiency of algae or plant material. There are two measurement modes, single and multiple turnover. The Ballast-Check 2 is a multiple turnover PAM fluorometer in that one saturating pulse of light is delivered to the sample to quickly reach the maximum fluorescence value with the interest of only looking at how efficient algae are at photosynthesizing, essentially determining the activity of the organisms. High degree of activity means a high survival rate and low activity means the algae aren't capable of efficiently photosynthesizing and have a low survival rate.

1.3 Quick View Diagrams

The Ballast-Check 2 uses four standard AAA user-replaceable batteries.



1.4 Inspection

Upon receiving your instrument, please inspect everything carefully and make sure all accessories are included. The Ballast-Check 2 package includes:

- The Ballast-Check 2
- 4 - AAA batteries
- Solid Secondary Standard
- Two Glass 10x10mm Cuvettes
- One 60cc plastic syringe
- Five 50 μ filter capsules
- Five 10 μ filter capsules
- Five 0.2 μ filter capsules
- Carrying Case
- USB Flash Drive with User's Manual and Basic Instructions

1.5 General Information, Precautions and Cleaning

- Use caution around solvents, they may degrade the plastic case of the Ballast-Check 2.
- If a sample is accidentally spilled inside the sample compartment, invert the Ballast-Check 2 to drain out the excess liquid. Then wipe the inside area dry with a clean soft towel or tissue.
- If extra cleaning is needed, use a mild detergent to dampen the towel for cleaning.
- Although the Ballast-Check 2 floats, do not submerge it in water.
- Do not expose the Ballast-Check 2 to temperatures outside the specified range of 5 to 40 °C or damage may occur to the unit that will not be covered under warranty.

2. Instrument Operation and Calibration

2.1 Button Identification and Function



SHIP – Displays the ship number as a 10-digit alphanumeric value always beginning with the IMO prefix. Only the 7 digits following the IMO prefix can be adjusted to set the ship number. After setting the ship number ENTER is pressed to save the value. If ESC is pressed at any time, the screen is exited and nothing is saved.

Saved values are appended to a data point after a sample is analyzed. The sample number will count sequentially, beginning with “Sample 1” for each set SHIP number. For example, if the SHIP number is changed, the sample count will reset to start with “Sample 1” for that newly set SHIP number.

TANK – Displays the tank number as a configurable 10-digit alphanumeric value. All characters in the alphanumeric value can be adjusted to set a desired TANK number. After setting the TANK number ENTER is pressed to save the value. If ESC is pressed at any time, the screen is exited and nothing is saved.

Saved values are appended to a data point after a sample is analyzed. The sample number will count sequentially, beginning with “Sample 1” for each set TANK number. For example, if the TANK value is changed, the sample count will reset to start with “Sample 1” for that new set of TANK values.

READ – Begins the sample analysis procedure detailed in section 2.5, only if SHIP and TANK values are saved. If SHIP and TANK values are not saved, pressing READ will display the message “First Set SHIP and TANK Values” and you will not be allowed to continue with measuring a sample.

ON/OFF – Turns the instrument ON or OFF.

ENTER – This button is used to save set values when setting SHIP or TANK values and allows users to proceed to the next screen/menu.

DATA – When the DATA button is pressed users can choose between either sending/clearing data or setting date/time. Use the up/down arrow keys to choose between the two options; a marker will indicate the selection made.

If SEND/CLEAR DATA is selected, press ENTER and a submenu will display. Users can choose, using up/down arrow keys, to either send data to a connected computer (see section 2.6) or clear logged data; a marker will indicate the selection made.

- If *SEND DATA* is selected and ENTER is pressed, the instrument will send all logged data out as an ASCII file (see section 2.6).
- If *CLEAR DATA* is selected, the following message will display; ENTER TO CONFIRM CLEAR DATA; when ENTER is pressed all logged data will be deleted.

If SET DATE/TIME is selected, press ENTER and date and time will display on the screen as (MM/DD/YYYY) and (hh:mm). Use the up/down arrow keys to select either parameter to set; a marker will indicate selection.

- If MM/DD/YYYY is selected, press ENTER and you will be allowed to adjust the month, day, and year using the up/down/left/right arrow keys. Press ENTER to save the set date. If ESC is pressed at any time, the screen is exited and nothing is saved. Date is logged per sample.
- If hh:mm is selected, press ENTER and you will be allowed to adjust the hour and minutes using the up/down/left/right arrow keys. Press ENTER to save the set time. If ESC is pressed at any time, the screen is exited and nothing is saved. Time is logged per sample.

ESC – Exits to the previous screen or menu

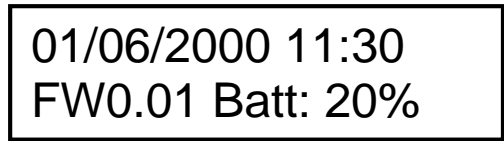
CHK STD – Is used to determine instrument performance. PASS or FAIL will display after measuring the solid standard using the CHK STD button indicating whether the Ballast-Check 2 can continue to be used for sample analysis or should be returned to the factory.

2.2 Instrument Power Up

Press the <ON/OFF> button to power on the Ballast-Check 2. After a 5 second warm up, the Ballast-Check 2 is ready for operation. Pressing the <ON/OFF> button again will turn the unit off or if left idle for 3 minutes the unit will automatically turn off to save battery power.

HOME SCREEN

The home screen will display the current date, time, Ballast-Check 2 firmware version, and remaining battery power as a percentage. These parameters will be displayed after powering the instrument on and warm up has completed as well as every time ESC button is pressed to exit to the home screen.



01/06/2000 11:30
FW0.01 Batt: 20%

If the batteries have low power, the following warning message will display – “**Battery Low Power! Replace Battery!**”. You will not be able to make any measurements until batteries are replaced.

2.3 Calibration Verification

The Ballast-Check 2 is factory calibrated and should remain calibrated for many years. Calibration can be verified using the Solid Secondary Standard (SSS) included with the instrument. To check instrument calibration:

- 1 Press the <ON/OFF> button to turn the Ballast-Check 2 on
- 2 Insert the SSS after warm up has completed and close the sample compartment lid
- 3 Press the CHK STD button
 - a. If the calibration is valid, the Ballast-Check 2 will display PASS
 - b. If the Ballast-Check 2 is out of calibration and needs to be sent back to the manufacturer, FAIL will display

2.4 Filtering Samples

The Ballast-Check 2 comes with 3 types of filters that are used to analyze a sample: 0.2, 10, and 50 micron sized filters. These filters are used to fractionate samples to measure different sized algae. Each fractionated sample will be measured for fluorescence and the corrected fluorescence value calculated at the end of the analysis will represent the 10-50 micron sized algal cells. The procedure in section 2.5 outlines how and when to use the filters provided to perform the analysis.

2.5 Measuring a Sample

The following materials are required for measuring a sample:

- 60 cc plastic syringe with male luer lock end
- 50 micron mesh filter capsule
- 10 micron mesh filter capsule
- 0.2 micron mesh filter capsule
- Glass Cuvette
- Ballast-Check 2
- Solid Secondary Standard
- Wipes for wiping the outside of the glass cuvette

The following procedure is used to measure a sample:

1. Turn the Ballast-Check 2 ON.
2. Set the SHIP Number and TANK Number.
3. Using the 60 cc syringe, collect 60 ml of sample by aspirating from the source water.
4. Attach a 50 micron filter to the end of the plastic syringe.
5. Obtain a glass cuvette and filter some of the sample through the 50 micron filter into the glass cuvette; rinse the cuvette 2-3 times with the 50 micron filtered sample before filling the glass cuvette with the filtrate.
6. Wipe the outside of the glass cuvette, insert the cuvette into the Ballast-Check 2 and close the sample compartment's lid.
7. Press <READ> to measure the 50 micron filtered sample.
8. Remove the glass cuvette and discard the sample.
9. Remove the 50 micron filter from the syringe and attach a 10 micron filter to the end of the 60cc syringe.
10. Filter some of the remaining sample in the syringe through the 10 micron filter into the glass cuvette; rinse the cuvette 2-3 times with the 10 micron filtered sample before filling the glass cuvette with the filtrate.
11. Wipe the outside of the glass cuvette, insert the cuvette into the Ballast-Check 2 and close the sample compartment's lid.
12. Press <READ> to measure the 10 micron filtered sample.
13. Remove the glass cuvette and discard the sample.
14. Remove the 10 micron filter from the syringe and attach a 0.2 micron filter to the end of the 60cc syringe.
15. Filter some of the remaining sample in the syringe through the 0.2 micron filter into the glass cuvette; rinse the cuvette 2-3 times with the 0.2 micron filtered sample before filling the glass cuvette with the filtrate.
16. Wipe the outside of the glass cuvette, insert the cuvette into the Ballast-Check 2 and close the sample compartment's lid.
17. Press <READ> to measure the 0.2 micron filtered sample.

18. After the final sample is measured, the screen will display results as:
 Abund:LOW or HIGH and the associated fluorescence value as CFI
 Activity:LOW or HIGH
 All values in Figure 1 will be logged internally and can be downloaded at a later time.
19. Remove the glass cuvette from the Ballast-Check 2, discard the sample, and rinse the glass cuvette with pure water.
20. Rinse the syringe with pure water.

Values for Abundance and Activity are defined by the set fluorescence threshold which correlates to the IMO D2 discharge standard of less than 10 living organisms of the 10-50 micron size class in 1 milliliter of ballast water.

The Abundance reporting is based on the numerical part of the discharge standard (10 organisms of the 10-50 micron size class per milliliter) and termed Corrected Fluorescence (CFI). If the CFI measured is greater than the set fluorescence threshold, then HIGH and the associated fluorescence value are displayed indicating a gross exceedance of algal abundance. If the CFI measured is less than the set threshold, then LOW and the associated fluorescence value are displayed indicating a very low abundance of algae.

The Activity reporting of either LOW or HIGH is based on the activity part of the discharge standard (living or active organisms) and is measured as photosynthetic efficiency of the algae. If the calculated Activity is greater than 0.3, then HIGH is displayed indicating a high degree of algal activity (i.e. live algae). If the calculated Activity is less than 0.3 then LOW is displayed, indicating low algal activity or algae that have little activity and very low survival rate.

The following parameters are recorded for each single measurement and can be downloaded to a computer after they've been logged:

Date	(MM/DD/YYYY)
Time	(hh:mm)
Ship Number	(IMO#####)
Tank Number	(XXXXXXXXXX)
Sample Number	(Sample XX)
Abundance - Corrected Fluorescence	(CFI = XXXX.XX)
Activity - Photosynthetic Efficiency	(Activity = X.XX)

Figure 1

2.6 Data Downloading

The Ballast-Check 2 can log a total of 1000 data points that can be downloaded directly to your PC or computer using the provided serial cable. A terminal program (e.g. Hyperterminal, Terra Term, MotoCross) is required for downloading these stored ASCII data from the instrument. The following settings are required for connecting the Ballast-Check 2 with your terminal program:

Baud Rate: 57600

Stop Bits: 1
Data Bits: 8
Parity: None
Flow Control: None

Once connected to the Ballast-Check 2,

- 1) Set the terminal program to capture or save text
- 2) Press the DATA button
- 3) Use the up and down arrow keys to select SEND/CLEAR DATA
- 4) Press ENTER
- 5) Select SEND DATA
- 6) Press ENTER
- 7) Stop text capture in the terminal program (**Note: .txt files can be viewed using most all programs including Microsoft Excel and can be saved as .csv or other file formats**)

Confirm data are saved to your PC before proceeding to clear data from the Ballast-Check 2.

The Ballast-Check 2's memory starts at 100% for 1000 data points. If the memory is more than 90% full and the READ button is pressed, the following message will display:

"Warning: Memory > 90% Full"

Indicating that you have reached more than 90% of the total memory allowed. You will be allowed to continue sampling, but it is highly recommended users stop analyzing samples at this point, download data to their PC or computer, and clear the data log.

When memory is full, the instrument will display:

"Memory Full"

When READ is pressed you will not be allowed to analyze samples until data have been downloaded and cleared from memory.

3 Sample Analysis Guidelines

- Take care not to spill samples into the sample chamber. Wipe up any spills promptly.
- The cuvette **MUST BE DRY** on the outside when taking readings. Any moisture or condensation on the outside of the cuvette can affect the reading.
- Fill the cuvette with at least 3.5 mL volume (3/4 full). Significant error in readings may result if the cuvette contains less than this minimum volume.
- Use the same cuvette for your samples, it is very important that you thoroughly rinse the cuvette between samples. The Ballast-Check 2 is very sensitive, therefore cross contamination between subsequent samples will skew results. Three rinses with the sample intended for measurement will help flush out any residual and decrease the chance for cross contamination.
- Any bubbles in the sample will affect the readings. Take care not to introduce bubbles into samples. Remove any bubbles by lightly tapping with your finger on the outside cuvette wall or cover the top of the cuvette and tilt the sample to help dissipate bubbles.
- Wait at least 3 seconds between consecutive measurements to allow the optics to return to normal state.

4 Measurement Parameters

4.1 Abundance - Corrected Fluorescence (CFI)

The Ballast-Check 2 is configured for detecting fluorescence from chlorophyll in live algal cells (i.e. *in vivo* detection of Chlorophyll). Light from the fluorometer is absorbed by algal cells and fluorescence emitted by the cells is detected, quantified, and displayed as a digital number (CFI), estimating the abundance of algae in the sample. Environmental conditions, presence of interfering compounds, cellular physiology, and light history can influence abundance estimates. The procedure used to analyze samples on Ballast-Check 2 accounts for most of the interferences that may skew results and provides an accurate estimate of algal abundance for 10 – 50 micron sized cells.

4.2 Activity - Photosynthetic Efficiency

The Ballast-Check 2 uses two measuring LEDs to estimate photosynthetic efficiency. The first LED (monitoring) is used to excite the sample with very low light intensity so as not to induce a change in chlorophyll reaction centers. While continuously monitoring the sample using the monitoring LED, the second LED (saturating) blasts the sample with a high intensity of light to effectively close chlorophyll reaction centers and bring algae to a maximum fluorescence state (F_m). The difference between the monitoring LED's measurement of the maximum (F_m) and minimum (F_o) fluorescence states is called variable fluorescence (F_v). The ratio (F_v/F_m) is a good measure of the algal activity as it estimates the photosynthetic efficiency of algae. Activity is measured on a range from 0.00 – 1.00; typical values for active eukaryotic algal cultures in the 10 – 50 micron size range can vary from 0.50 – 0.70.

4.3 Calculations

$$\text{Corrected Fluorescence (CFI)} = \{(50 \mu\text{m RFU} - 0.2 \mu\text{m RFU}) - (10 \mu\text{m RFU} - 0.2 \mu\text{m RFU})\}$$

Where,

50 μm RFU is the fluorescence response from the 50 μm filtered sample

10 μm RFU is fluorescence response from the 10 μm filtered sample

0.2 μm RFU is fluorescence response from the 0.2 μm filtered sample

The 50 and 10 μm filtration steps are used to isolate the desired size class and the 0.2 μm filtration is used to correct for background fluorescence.

$$\text{Activity} = \frac{\{F_m(50 \mu\text{m RFU} - 0.2 \mu\text{m RFU}) - F_o(50 \mu\text{m RFU} - 0.2 \mu\text{m RFU})\}}{F_m(50 \mu\text{m RFU} - 0.2 \mu\text{m RFU})}$$

Where,

F_m represents the maximum fluorescence value resulting from a saturation pulse

F_o is fluorescence response of the algal sample using low light intensity

5 Warranty

5.1 Terms

Turner Designs warrants the Ballast-Check 2 PAM Fluorometer and accessories to be free from defects in materials and workmanship under normal use and service for a period of 12 months from the date of shipment from Turner Designs, with the following restrictions:

- Turner Designs is not responsible for replacing parts damaged by accident or neglect. Damage from corrosion is not covered. Damage caused by customer modification of the instrument is not covered.
- This warranty covers only Turner Designs products and is not extended to equipment used with our products. We are not responsible for incidental or consequential damages, except in those states where this limitation is not allowed. This warranty gives you specific legal rights and you may have other rights which vary from state to state.
- Damage incurred in shipping is not covered.

5.2 Warranty Service

To obtain service during the warranty period, the owner shall take the following steps:

- 1 Write, email, or call the Turner Designs Technical Support department and describe as precisely as possible the nature of the problem.

Phone: 1 (877) 316-8049

Email: support@turnerdesigns.com

- 2 Carry out any adjustments or tests as suggested by the Technical Support Department.
- 3 If proper performance is not obtained you will be issued a Return Authorization number (RMA). Package the unit, write the RMA number on the outside of the shipping carton, and ship the instrument, prepaid, to Turner Designs. If the failure is covered under the warranty terms, the instrument will be repaired and returned free of charge, for all customers in the contiguous continental United States.

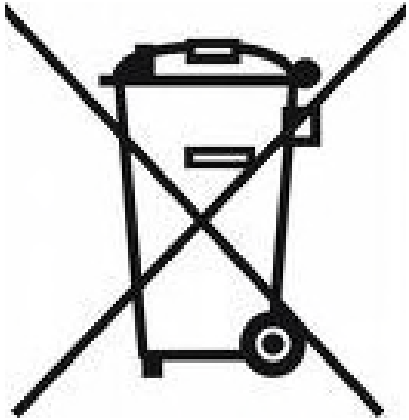
For customers outside of the contiguous continental United States who purchased equipment from one of our authorized distributors, contact the distributor. If you purchased directly, contact us. We will repair the instrument at no charge. Customer pays for shipping duties and documentation to Turner Designs. Turner Designs pays for return shipment (custom duties, taxes and fees are the responsibility of the customer).

5.3 Out-of-Warranty Service

Follow steps for Warranty Service as listed above. If our Technical Support department can assist you by phone or correspondence, we will be glad to, at no charge. Repair service will be billed on a fixed price basis, plus any applicable duties and/or taxes. Shipment to Turner Designs should be prepaid. Your bill will include return shipment freight charges.

Address for Shipment:

Turner Designs
845 W. Maude Avenue
Sunnyvale, CA 94085



Appendix A: Specifications

Ballast-Check 2	
Maximum Range	100 cells/ml
Sensitivity	<10 cells/ml
Warm Up Time	5 seconds
Auto Power Off	After 3 minutes of inactivity
Light Source	LED
Detector	Photodiode
LCD Display	2 x 16 characters
Error messages	Low battery; Low Data Memory
Resolution	12 bits
Case	Meets IP 67 Standard; dustproof and waterproof
Temperature	41-104 °F; 5-40 °C
Internal Memory	1000 records
Data Output Format	ASCII
Weight	0.87 lbs. (0.4kg)
Size	1.75" x 3.5" x 7.25"(4.45cm x 8.9cm x 18.4cm)