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Development of a new expendable probe for the study of pelagic ecosystems from Voluntary Observing Ships

M. Marcelli¹, A. Di Maio², D. Donis¹, U. Mainardi³, and G. M. R. Manzella⁴

¹Department DECOS, Tuscia University, Via S. Giovanni Decollato n.1, 01100 Viterbo, Italy

²AQSmare, Via del Molo di Levante, 1, 00054 Fiumicino (Roma), Italy

³Maisoft, Via Ronchi n.18, 24020 Villa di Serio(Bg), Italy

⁴ENEA ACS, Forte S.Teresa, Loc. Pozzuolo, 19036 Lerici, Italy

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Correspondence to: M. Marcelli (marcomarcell@tin.it)

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Abstract

Physical and biological processes of the marine ecosystem have a high spatial and temporal variability, whose study is possible only through high resolution and synoptic observations. The T-FLAP (Temperature and Fluorescence LAunchable Probe) was charted in order to answer to the claim of a cost effective temperature and fluorescence expendable profiler, to be used in ships of opportunity. The development of the expendable fluorimeter has followed similar concepts of the XBT (a wire conducting the signal to a computer card), but differently from that, T-FLAP was developed with an electronic system that can be improved and adapted to several variables measurement channels. Commercial components were utilized to reach the aim of a low-cost probe: a glass bulb temperature resistor for the temperature measurement, blue LEDs, a photodiode and available selective glass filters, for fluorescence measurement. The measurement principle employed to detect phytoplankton's biomass is the active fluorescence. This method is an in vivo chlorophyll measure, that can get the immediate biophysical reaction of the cell inside the aquatic ecosystem; it is a non-disruptive method which gives a real time measure and avoids the implicit errors due to the manipulation of samples. The possibility of using continuous profiling probe, with an active fluorescence measurement, is very important in the study of phytoplankton in real time; it is the best way to follow the variability of sea productivity. In fact, because of the high time and space variability of phytoplankton, due to its capability to answer in a relatively short time to ecological variations in its environment and because of its characteristic patchiness, there isn't a precise quantitative estimation of the biomass present in the Mediterranean sea.

1 Introduction

Operational forecasting of marine physical and biochemical state variables is becoming an important tool for modern management and protection of the oceans and their living

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resources. The forecasting systems need multidisciplinary input data, the operational collection of which is hampered by two main factors: cost and technology.

The methodology actually used for an operational monitoring of the oceans is mainly based on lagrangian profiling buoys (Argo) and on Ships Of Opportunities. Potentially, the Argo floats can measure physical, chemical and some biological parameters. In practice this potential capability is limited by cost and power supply. The Ships Of Opportunity Program (SOOP) is based on eXpendable BathyTermographs (XBT) technology developed at the end of 60's and provides only temperature profiles of the upper thermocline.

An operational observing and forecasting system for the physical properties of the ocean was set up in the Mediterranean Sea from 1999 (Pinardi et al., 2003) in the framework of an EC-supported pilot project. The project follow-up, called Mediterranean Forecasting System (MFS) – Toward Environmental Prediction (TEP), has implemented the forecasting system of the biological state variables. A Voluntary Observing Ship program (VOS) was devised, within the MFS – TEP project, to support ocean weather forecasting. The voluntary observing ships are essential, since are integrating ocean surface temperature from satellites, and contribute to the MFS capability of providing near real time analysis of the ocean state.

However, there is still a lack of basin wide, operational, multidisciplinary, in situ observing system. Satellite remote sensing provides a unique synoptic view of phenomena such as chlorophyll concentration and sea surface temperature, but the measurement validation requires constant scrutiny and much sea-truth data as possible; for this reason the integration between field sampling and remote sensing technique should be improved also for the development of ecosystem model forecasts .

Remote sensing of primary production in the euphotic zone has been based mostly on visible-band water-leaving radiance measured with the coastal zone color scanner. There are some robust, simple relationships for calculating integral production based on surface measurements, but they also require knowledge of photoadaptive parameters such as maximum photosynthesis which currently cannot be obtained from space

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(Balch and Byrne, 1994). More than for the physical variables, the biological ones have to be observed in situ. Especially in the mid-high latitudes, a deep observation of the water column is needed, because of the typical distribution of phytoplankton's biomass (Mann and Lazier, 1991).

5 The T-FLAP (Temperature and Fluorescence LAunchable Probe) was charted in order to answer to the claim of a cost effective temperature and fluorescence expendable profiler, to be used in ships of opportunity. Its development, born inside the MFS – TEP – VOS, had the aim to add biological profiling measurements to the physical ones, in order to have more extensive information on chlorophyll concentration in the sea. Chlorophyll is an index of phytoplankton's biomass, and is also the most common
10 property that characterizes marine productivity.

The possibility of using a continuous profiling probe, with an active fluorescence measurement, is very important in the study of phytoplankton in real time; it is the best way to follow the variability of sea productivity. In fact, because of the high time and
15 space variability of phytoplankton, due to its capability to answer in a relatively short time to ecological variations in its environment (Lewis et al., 1984) and because of its characteristic patched distribution, there isn't a precise quantitative estimation of the biomass present in the Mediterranean sea. Moreover primary producers in the sea, phytoplankton at first, contribute for the 40% of global assimilation of inorganic carbon
20 (Falkowski et al., 2002), resulting particularly relevant in the study of global change.

The development of the expendable fluorimeter has followed similar concepts of the XBT (a wire conducting the signal to a computer card), but differently from the latter, T-FLAP was developed with an electronic system that can be improved and adapted to several variables measure channels. Commercial components were used for the
25 development of a prototype, with the aim of a low-cost probe: a glass bulb temperature resistor for the temperature measurement, blue LEDs, a photodiode and commercial selective glass filters, for fluorescence measurement. The measurement principle for the detection of phytoplankton's biomass is the active fluorescence. This method is an in vivo chlorophyll measure, that can get the immediate biophysical reaction of the

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cell inside the aquatic ecosystem (Lorenzen, 1966). This is a non-disruptive method which gives a real time measure and avoids the implicit errors due to the manipulation of samples.

2 Instrument and method

5 T-FLAP is an expendable probe able to measure Chlorophyll “a” fluorescence and temperature, which has a transmission system and a shape developed in order to allow its use from moving ships. The simplicity in the use of this probe and its relatively low cost were the basic requirements of T-FLAP development. Other requirements were concerning the need to detect the deep chlorophyll maximum (DCM) with a sufficient
10 accuracy, and temperature with an accuracy of 0.1°C.

The T-Flap’s electronics and firmware, for sensors management and data transmission, are placed inside the probe. Measurements take place in an internal cell, where the water flows while the probe descends along the water column. The data transmission is allowed by a twin copper wire wrapped on a coil placed in the tail of the probe
15 and in a canister.

T-FLAP is composed by:

- A measurement cell represented by an anticorodal aluminium tube where the water flows in the inside; in the internal part of the tube are fixed the sensors which are in direct tuch with the water, while on the external part of the tube there
20 are placed the batteries and the electronics.
- This central body is covered by a larger anticorodal aluminium tube, closed by two flanges, constituing the case where are placed betteries and electronics.
- On the tail is placed a coil with the copper wire for data transmission. The tail has an hydrodynamical shape that facilitates the wire unreeling.

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- The frontal part is composed by heavy material to allow a vertical fall: it is constituted by a turned zinc ogive blocked by a metallic nail. Falling tests were made with several options in order to check the best weight of the probe to reach an ideal falling speed.

5 The main components of the T-FLAP are shown in Fig. 1, while a design of the longitudinal section of the probe is shown in Fig. 2.

The electronics are placed on two parallel faces of the measure cell. It is composed by two printed circuits and batteries (Fig. 3). These circuits contain the integrated electronic functions distinct for typology:

- 10 – Circuit 1, positioned at the photodiode side, has all the analogical measure functions, signals conditioning and digital conversion functions.
- Circuit 2, has all the digital elaboration circuits, data transmission, LED diodes piloting functions and the power supply system.

15 The optimal shape-structure of the T-FIAP is 8 cm diameter for a 35.9 cm length. These dimensions assure a good weight distribution and, as a consequence, a good vertical lowering of the probe. The zinc ogive can be more or less heavy resulting a total weight in water from 1.50 kg to 2.15 kg.

3 Sensors

3.1 Fluorimeter

20 The fluorimeter is composed by:

- a source of light in the wavelengths of 430 nm, 450 nm 470 nm (blue LEDs)
- an optical filter which selects the blue wavelength from 430 to 480 nm

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- an amplified semiconductor element (with electronics integrated) as sensitive receptor
- an optical filter which selects the red light up to 600 nm

The fluorimetric device utilizes a light emission which saturates the measure section with a high intensity light impulses in the blue wavelength. Through a control electronic circuit, the diodes can be modulated with impulses by 5 ms with a peak over 100 mA. This brings the light emission to a value over 10 cd for each led.

3.2 Fluorescence measurement system

For the Chlorophyll “a” fluorescence measurement is employed a semiconductor element with specific spectral sensitivity for the red band. In front of the photodiode there is positioned an optical red filter specific for the red wavelength up to 600 nm. The weakness of the natural fluorescence signal makes critical the noise/signal rate, so that in the measure circuits project it has been paid particular attention to the static noise factor, the intrinsic current level which is not due to the photon activity; this is a characteristic of the quality of a photodiode but interferes with the signal amplification process, enhancing the amplitude of the signal in a way that generates creep phenomena.

Directly connected to the photodiode, there is an high precision low noise j-fet amplifier, in order to amplify the micro currents from 0 to 1 μ A and convert them in a tension value between 0–5 V. For the compensation of the field free emission current there were adopted digital subtraction techniques alternating the measure in presence of light with a measure without light.

For the dynamic noise instead, which has a random behavior, where adopted filtering and integration techniques for small periods on a sufficient number of impulses to maintain the measurement dynamic.

3.3 Temperature measurement

The temperature measurement is effectuated through a glass bulb micro sensor which comes out from

the measure cell for 10 mm (Fig. 4), the sensitive part is composed by resistive sensor inside a spherical glass bulb with the diameter of 1.5 mm. This sensor has an high sensitivity to temperature variations (0.01°C) and to the dynamic variations (0.05 ms).

4 Electronics and firmware

4.1 Architecture of electronic system

The electronic scheme of the T-FLAP is shown in Fig. 5. The measurement system allows an high speed acquisition of all parameters data, including optional additive ones (i.e. sensors not yet added to the probe); each channel is predisposed for the treatment of signals.

The fluorescence channel is the most complex, it includes a low noise amplification system with optional gain choice, with a bias current control of the receiving diode; the amplifier output signal is demodulated and filtered with a few hertz cut off frequency and with an high attenuation coefficient (>50 dB/octava). The temperature signal is amplified and normalized. It doesn't need particular conditioning.

The conditioners output signals are uniform for all the variables and are sent to the analogical digital converter through a multiplexer integrated in the AD converter chip. This is able to convert at a maximum speed of 100 000 channels/second with a total resolution of 16 bit. The high speed conversion permits a successive digital elaboration of the variables measure data before sending the signal to the surface through the transmission wire.

The light emitters are constituted by two groups different LED diodes with emission in the blue light (430, 450, 470 nm). The emission is modulated in square wave at a

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programmed frequency between 500 and 2000 Hz, the impulse so modulated permits us to obtain an alternate optical signal with dark and light symmetric phases.

The power supply is provided by rechargeable Ni-MH hermetic batteries suitable for providing energy required by the system for the foreseen functioning time for a maximum of 60–100 min with full charge batteries.

4.2 Digital transmission system

Data transmission is digital and is allowed by a twin copper wire (μ link) wrapped on two separate coils; one of these is mobile and positioned in the tail of the T-FLAP, while the other is fixed in the canister launcher, through which the probe is connected to the pc on board.

Data acquisition doesn't need a specific software, it is enough to have a pc terminal as Windows® HyperTerminal and follow the information transmitted by the connected device. The interactive menu allows different work activities including programming and controlling.

The twin copper wire has many particular characteristics that are necessary to assure an effective data transmission capability. The intrinsic characteristics of the wire are depending on: conductor section, insulation material thickness, distance between the conductors, copper resistivity, insulation thickness. Extrinsic characteristics of the wire are: wrapped wire length, unwrapped wire length, impedance, resistance, parasite capacity, coupling capacity, water temperature. These parameters change continuously during the descent of the T-FLAP, reducing progressively the inductance value but enhancing the parasite capacity value; instead temperature has a minimal effect. The experimental harmonic analysis provided the characteristics of the wrapped wire:

- Total resistance (measured on both the conductors): 11.26 ohm/m
- Coupling capacitance(between conductors): 170 pF/m
- Stray capacitance: 1417 pF/m

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- Specific inductance (wrapped on both the bobbins): 2.01 H on 1500 m
- Impedance Z measured at 1000 Hz: 13.36 khom on 1500 m

4.3 Coil's capacity

The mobile coil, inside the Tflap, has to contain a wire with a length as the maximum depth it could reach plus a tolerance length: the pre-serie has 700 m of wire.

4.4 Limits

The application for which the T-FLAP was designed is the profiling measure of fluorescence and temperature along the water column until a depth of at least 350 m; being subjected to a progressive hydrostatic pressure, the functional limits of the instrument, concerning to the implosion of the materials, stand by 500 dBar, expecting this to be the highest pressure we need to reach. The available energy for the electric functioning would allow a longer life of the device if there weren't the physical limitations.

A second limit is the capability of the coils to host a longer or thicker couple of conductors: for each one it was prevented at the most a double length with the same diameter, or a bigger diameter with the present length.

5 Development and optimisation

The first phase of the experimental activities for the fluorimeter assembly has been directed to an accurate evaluation of any element which was able to full fit the characteristics of the instrument's components (LEDs, diodes, filters), with particular attention to low cost components. The second part of the experimental study had the aim to find the best combination of the selected components.

The spectral characteristics of different LEDs lights were analysed to detect eventual presence of emissions in the red band, which would interfere with the chlorophyll mea-

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sure. Afterward it was verified the transmittance of the filters and it was analysed the spectral emission of the LED with blue and red filters.

In a second phase LEDs, filters and photodiodes were assembled in a not submersible measurement cell. By using an oscilloscope, the photodiode output signal was measured to detect the “parasite” light (deduced from the noise signal).

Finally, a definitive geometry of the measure cell was defined, and fluorescence measurements in laboratory were done in order to study the sensor output signal and define its amplification. On the basis of the obtained results, different prototypes were assembled and tested.

The analysis of LEDs spectra and filters transmittance was done with an EPP2000C UV+VIS Spectrometer (Wavelength Range 200–850 nm; resolution 0.75 nm) at first separately. Once found the best transmittances, selected LEDs with selected filters have been tested in the laboratory of Experimental Oceanology and Marine Ecology in Civitavecchia, Italy.

Different filters were analysed such as acetylene filters used for a first prototype (LEEfilters) and glass photographic coloured ones. Once verified that there was no appreciable interference from glass on light emission, we had reason to utilize it as support for the filters. A final choice of the filters was operated in favor of photographic ones, in order to have retrievable and cheap components.

6 Fluorimeter calibration

Static calibration

Different dilutions of a concentrate *Chlorella* sp solution measured with a calibrated fluorimeter (PrimpProd 1.08) were used for the static calibration of T-FLAP. PrimProd is a very sensible fluorimeter , based on a photomultiplier sensible element, developed by the Institute of Biophysics of Moscow (Antal et al., 2001). The conversion laws used to have the Chlorophyll concentration from the referent fluorimeter values of F0 and

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Fmax are reported in Eq. (1) and Eq. (2).

$$\text{Chla} = ((0.03874 + 0.00092 * F_{\text{max}}) - 0.364) / 2.377 \quad (1)$$

$$\text{Chla} = ((0.0431 + 0.00072 * F_0) - 0.244) / 1.108 \quad (2)$$

The spectrophotometric analysis of the samples employed for the calibration of the fluorimeter was leaded following the method reported by Lazzara et al. (1990).

In Fig. 6 is shown the relation between fluorescence measurements of PrimProd and T-Flap.

Dynamic calibration

In order to acquire data simultaneously it was built a water flux circuit where the T-Flap was inserted together with a reference probe: the water flowed through the reference probe and through the T-Flap thanks to a bulk insulated tube system connected to a circulation/feeder pump (0.7 bar, 5.7 l/min). which kept a constant flow.

In this way it has been possible to effectuate a dynamic calibration, which is necessary to test an instrument like T-Flap, that descends in the water column with a constant speed, letting the water flow inside of it.

The entire circuit caught the water from a tank and flowed it out in the same tank in which it was progressively added a certain quantity of living phytoplankton.

The probes were directly connected to the pc by a serial interface so that the values were available in real time; at the reference probe fluorescence value corresponded a T-Flap value expressed in Volt.

In Fig. 7 is represented the best fit curve for the calibration of the fluorescence sensor of T-Flap.

Reference probe

The reference probe, PrimProd 1.11 , is a submersible PC-controlled fluorimeter (accuracy: 0.1 µg/l) which uses the double flash “pump and probe” technique for measuring

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the constant and variable chlorophyll fluorescence in vivo, to estimate phytoplankton abundance and the quantum efficiency of phytoplankton photosynthesis, where the sensitive element is a photomultiplier (Piermattei et al., 2006).

7 Temperature calibration

5 Static calibration

The response of the temperature resistor is converted in a tension signal value. To find out the conversion law, which allows to transform the mV signal value in °C, the T-FLAP was immersed in temperature controlled water and the degree temperature value has been obtained by a reference temperature sensor. It was used a PVC tank by the dimension of 40×40×40 isolated with a 3 cm thick polystyrene layer. The tank was covered by a cap made by a plywood layer 0.4 cm thick, covered with polystyrene, on which it has been made a hole to host the reference sensor and one for the TFlap.

The temperature measurement inside the tub was done by means of an IDRONAUT 316 multi parametric probe which has got an accuracy of 0.003°C. The probe is directly connected to the pc by the serial interface so that the values were available in real time. When the water volume reached a stable temperature the T-Flap acquisition got started and the output values were saved on the pc.

The motion of the water was kept by a submerged pump with a 450 gallons capacity (1500 l/h). The pump permitted to keep mixed the water volume so that every point of the tank had the same temperature. The pump drew the water from the bottom and let it out from 3 different spots: on the surface, inside the measure room near to the T-FLAP and near to the reference sensor.

The calibration was realized in 22 correspondent temperatures, in a range of 0 to 25°C. To reach the 0°C temperature some ice cubes were added, while to increase temperature hot water was added.

The T-FLAP and IDRONAUT sensors were at the same level inside the tank. For

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each of the 22 temperature values, correspondent voltage values were extracted from sensors and a regression analysis carried out (Fig. 8).

Dynamic calibration

The temperature sensor was dynamically calibrated using the same flow system employed for the fluorescence calibration: this time the reference probe was a Falmouth OTM with Platinum Resistance Thermometer which has an accuracy of 0.003°C.

The entire circuit caught the water from an insulated tank full of melting ice, and flowed it out in the same tank in which it was progressively added hot water.

In Fig. 9 is represented the best fit curve for the calibration of the temperature sensor of T-Flap.

8 Field tests

The T-FLAP field tests were conducted on November 2005 from the Urania R/V in the Tyrrhenian Sea and on April 2006 from Tuscia University boat Sampei, offshore Civitavecchia (Tyrrhenian Sea).

The reference probe utilized for measurements of fluorescence and temperature on April, on Sampei boat, is PrimProd 1.11. The T-FLAP gives good results either for the fluorescence measure and for temperature measure. In Fig. 10 and Fig. 11 are shown the profiles. The water in this period is still warm in the upper layer; it is appreciable the T-FLAP resolution of the thermocline. The fluorescence measure of T-Flap can successfully describe the Chlorophyll distribution in the water column. Similar results were obtained in all the field tests and in particular the Deep Chlorophyll Maximum is clearly discerned.

It is important to underline that the Tyrrhenian sea is the most oligotrophic area of the Mediterranean Western Basin, particularly in summer, with average Chl values by 0.06 mg m⁻³ in the upper layer (Bosc et al., 2003), while the DCM reaches 0.6 mg m⁻³

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in late summer (Marcelli et al., 2005).

9 Conclusions

The T-FLAP development has been based on system components having a low cost, available in commerce and purchasable by stocks in order to allow the massive production required by an expendable probe. Temperature measurements have reached the accuracy of 0.01°C. For the fluorescence, even if was tested in an oligotrophic sea, the accuracy reached satisfies the aim of a clear detection of the deep chlorophyll maximum: this ensures the description of phytoplankton's biomass in most of the seas which are more productive than the Tyrrhenian.

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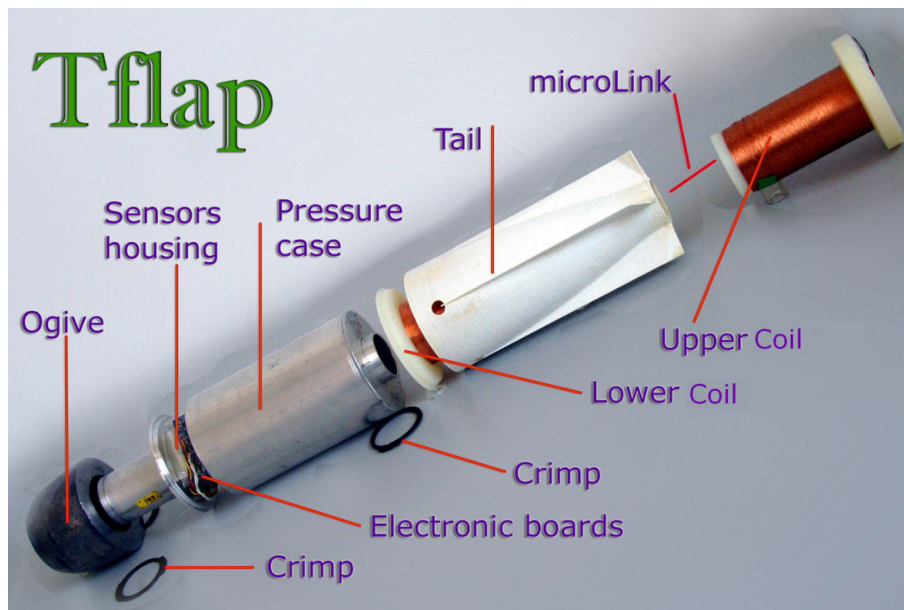


Fig. 1. The T-FLAP components.

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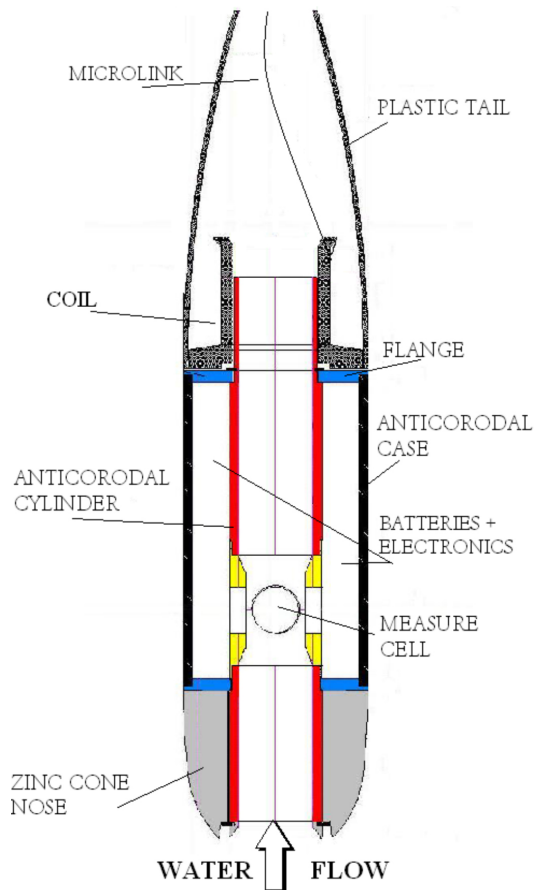


Fig. 2. The schematic functioning of the T-FLAP. The water enters the measuring cell where temperature and fluorimeter measurements are done.

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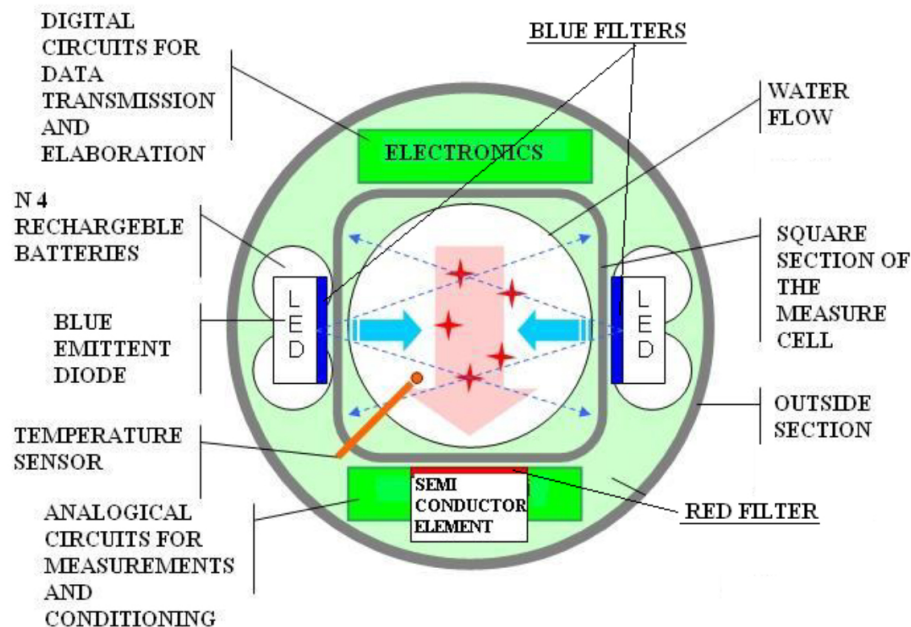


Fig. 3. A schematic section of the measurement cell.

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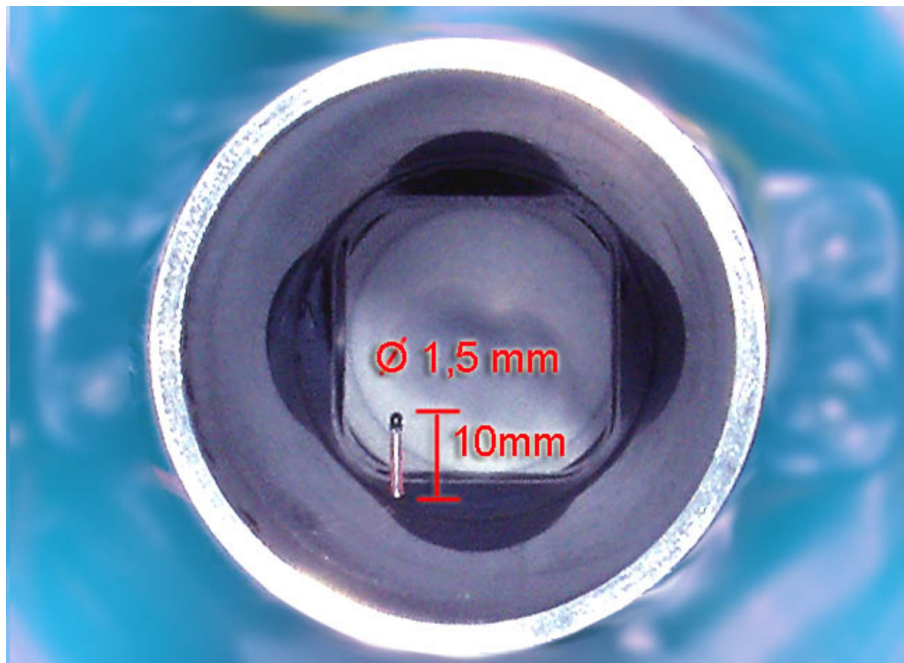


Fig. 4. The temperature sensor inside the T-FLAP measurement cell.

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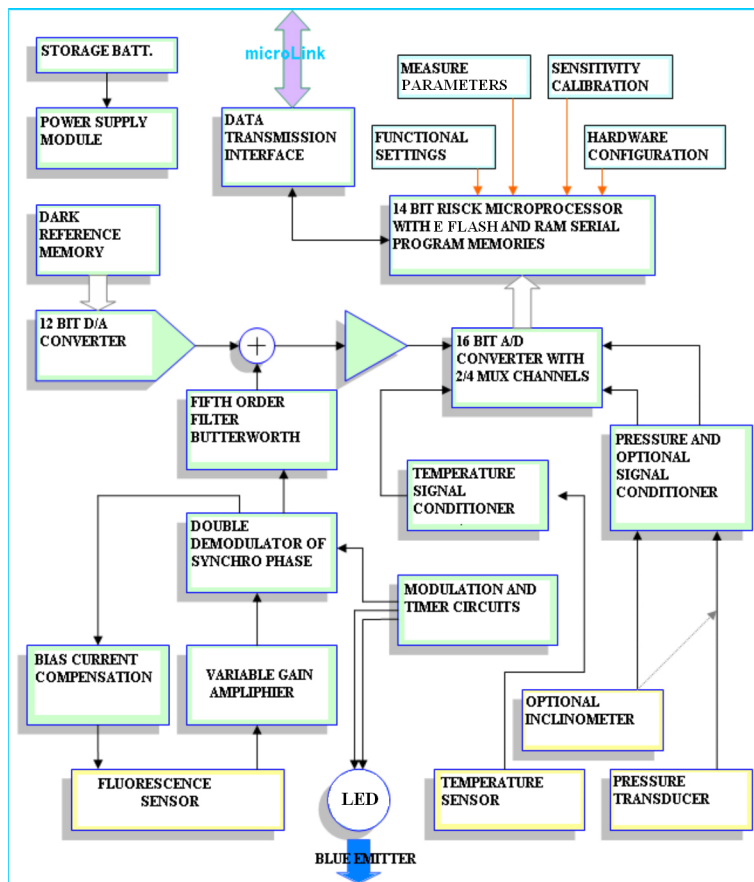


Fig. 5. The scheme of electronic architecture.

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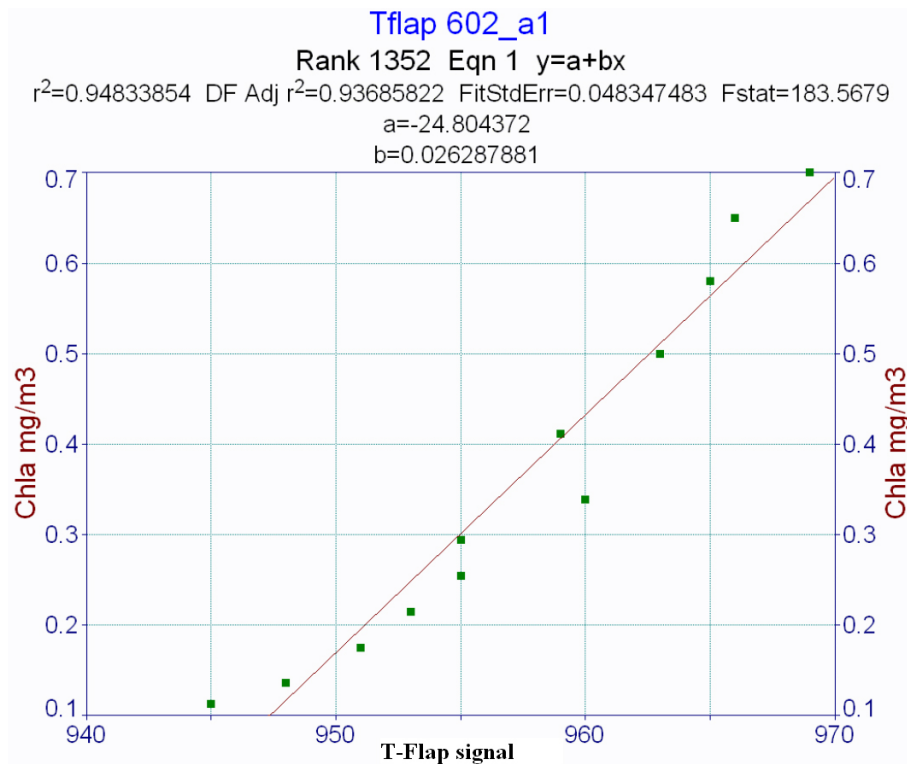


Fig. 6. On X axis there is the Chl “a” measured by T-Flap (mV) while on Y there is the Chl “a” measured by PrimProd 1.08 expressed in mg/m³.

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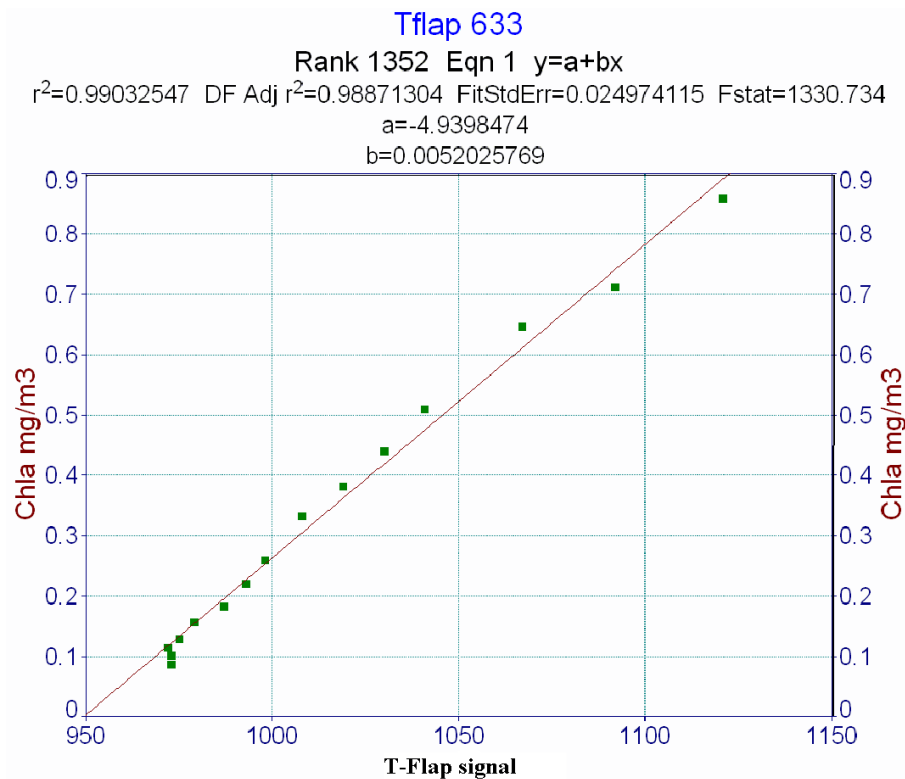


Fig. 7. Best Fit curve for the calibration of the T-Flap fluorescence sensor: on Y is reported the Chlorophyll concentration measured by PrimProd (mg/m^3) and on X is reported the signal given by the T-Flap's fluorescence sensor.

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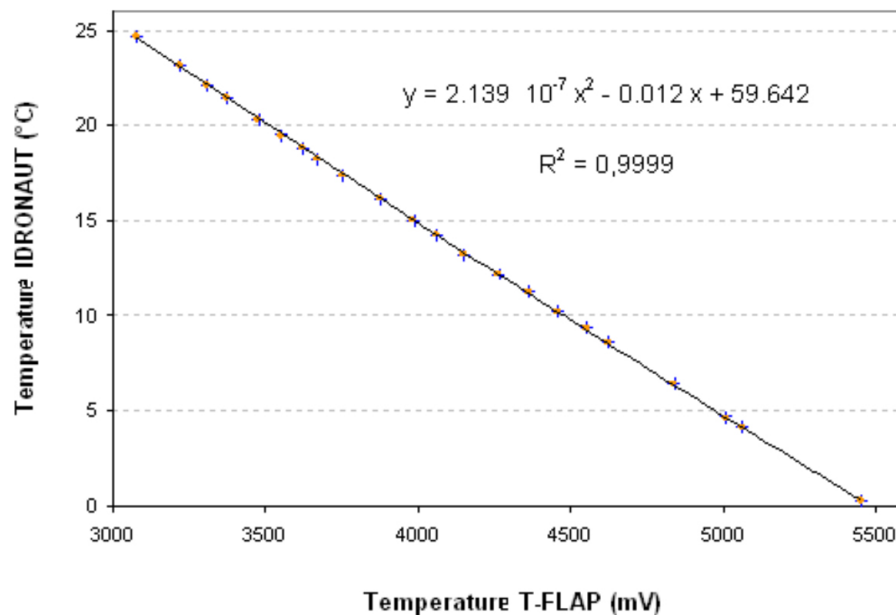


Fig. 8. On X axis there is the temperature measured by T-Flap (mV) while on Y there is the temperature measured by Idronaut expressed in °C.

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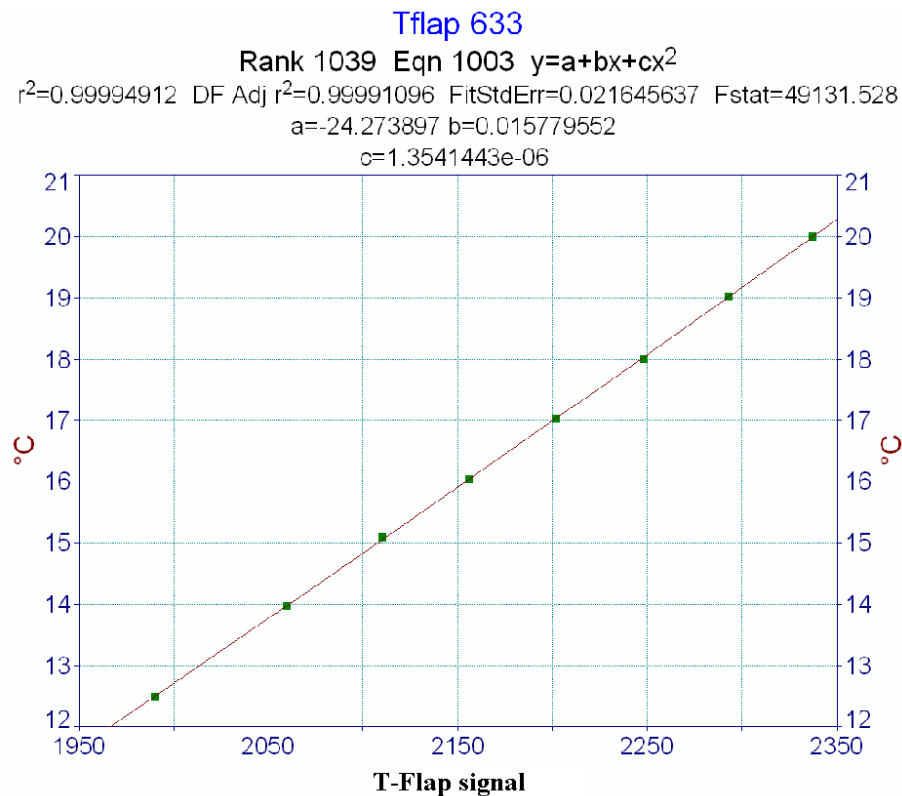


Fig. 9. Best Fit curve for the calibration of the temperature sensor: on Y is reported the temperature measured by OTM Falmouth (°C) and on X is reported the signal given by the T-Flap's temperature sensor.

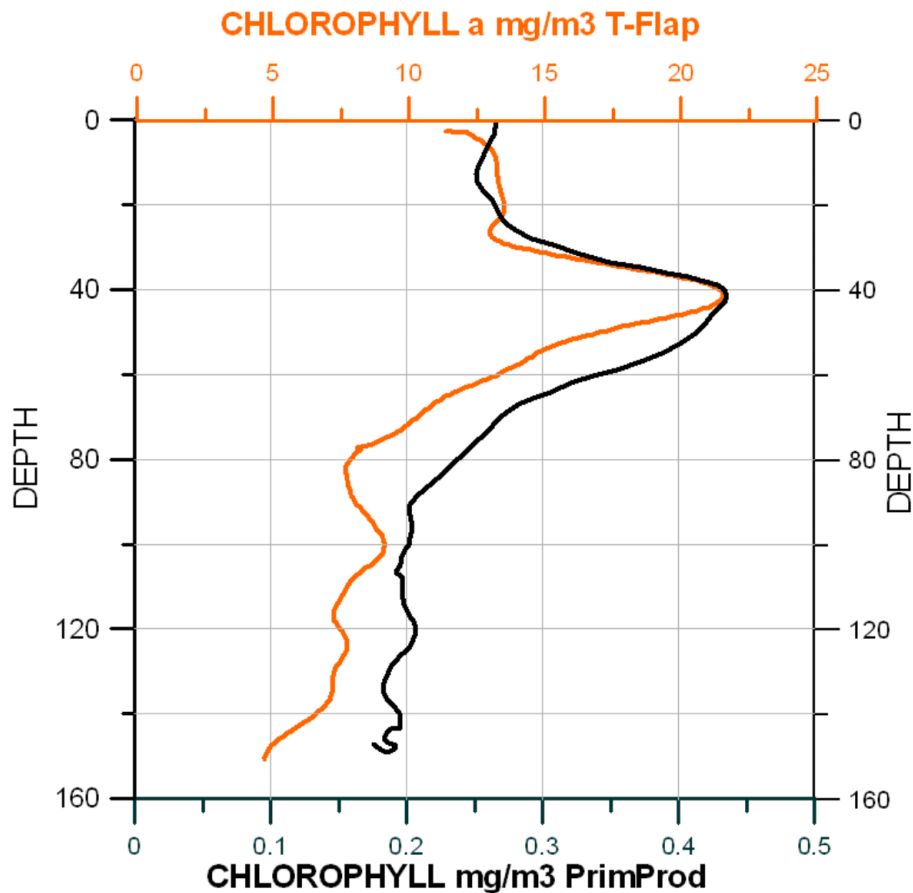


Fig. 10. Comparison between chlorophyll measurement of T-FLAP (orange line) and reference probe (black line).

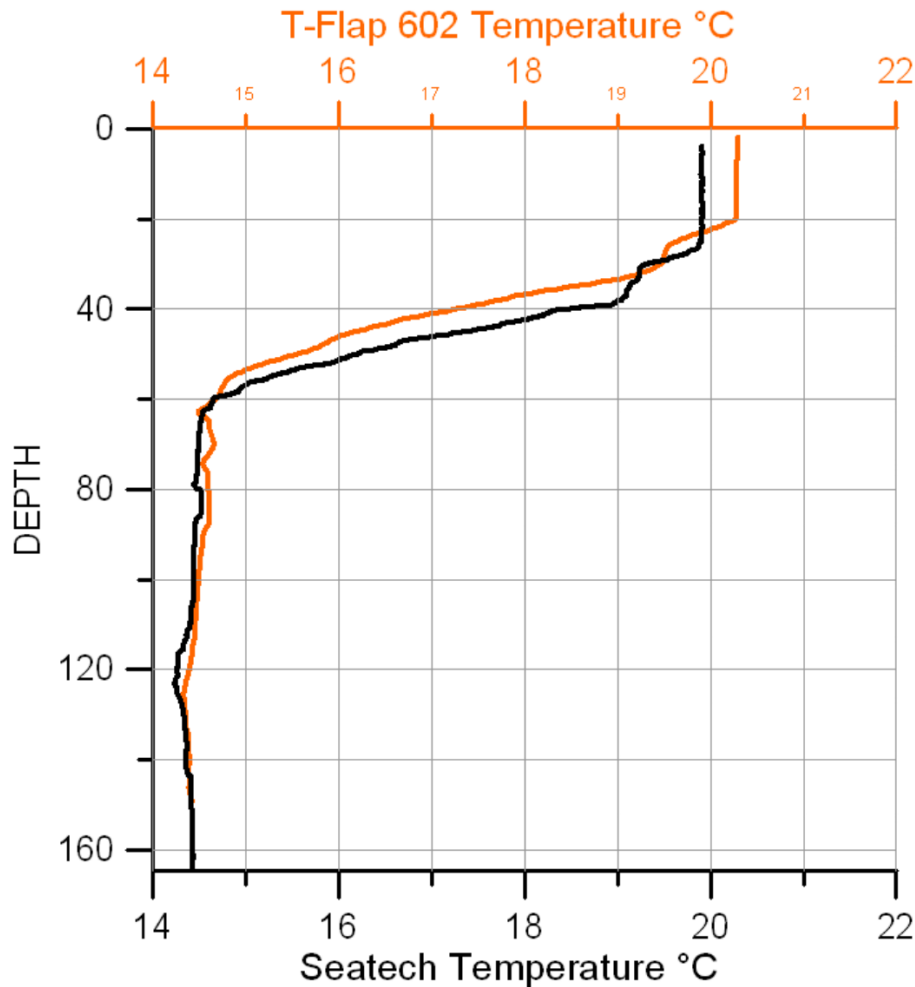


Fig. 11. Comparison between temperature measurement of T-FLAP (orange line) and reference probe (black line).