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A solid state sensor for mapping and profiling stimulated bioluminescence in the marine environment

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Abstract—This paper describes the design and development of a solid state sensor for the measurement, *in situ*, of stimulable bioluminescence of marine organisms. It can be towed in the Undulating Oceanographic Recorder or used in vertical profiling mode from a stationary research vessel. The sensor can detect signals from 10^9 quanta s^{-1}, from a single dinoflagellate flash, up to 10^{13} quanta s^{-1}, at cell densities of 10^6 to 10^8 m^{-3}. Measurements of bioluminescence and associated environmental conditions are presented from a variety of deployments of the sensor in the Undulating Oceanographic Recorder.

INTRODUCTION

THE Undulating Oceanographic Recorder (UOR) Mark 2 and its use as a multi-role oceanographic sampler for monitoring and mapping a number of marine environmental parameters have been described by AIKEN (1981a). The standard instrument suite carried by UOR Mark 2 comprises a plankton sampler and sensors for salinity (or conductivity), temperature, depth, chlorophyll (AIKEN, 1981b), and solar radiant energy (broad band sensors, 400 to 700 nm or narrow band, 460 ± 10 nm and 550 ± 10 nm). Sensor measurements are logged *in situ* by a miniature analogue or digital tape recorder (MATR or MDTR; Oxford Medical Systems, AIKEN, 1980). For particular research objectives, a number of specially developed sensors can be added to the standard instrumentation suite. This paper describes the development of a solid state sensor for the measurement of stimulable bioluminescence; that is, bioluminescence stimulated by physical agitation, rather than emitted spontaneously.

Quantitative measurements of bioluminescence were first made by bathyphotometers using unshielded photomultiplier systems (CLARKE and WERTHEIM, 1956; BODEN and KAMPA, 1957) which could measure bioluminescence that was strong compared to background light; daytime measurements near the surface were impossible. CLARKE and KELLY (1965) enclosed a photomultiplier in a light tight housing, and pumped the seawater through this detection chamber, introducing a constant stimulation level and excluding ambient light. LOSEE and LAPOTA (1981) have developed more sophisticated vertical profiling systems based on the above principle.

Studies of the spatial distribution of bioluminescence have been made using towable bathyphotometers (BITYUKOV *et al.*, 1969; SELIGER *et al.*, 1969). With a bathyphotometer in the

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UOR, the vertical structure of bioluminescence could be determined simultaneously with measurements of the associated physical and biological conditions.

System specification

The general design requirements for a bathyphotometer for the UOR Mark 2, capable of measuring bioluminescence both by day and by night can be summarized as follows: (a) The instrument should provide adequate flow to the sensor, provide adequate stimulation of the organisms and exclude ambient light. (b) The sensor should be small, lightweight, robustly constructed, have low electrical power consumption, operate stably and provide an output signal compatible with a simple recording device such as the MATR or MDTR.

Due to the wide range of bioluminescent phenomena to be studied (from single cell flashes to large-scale visible manifestations at densities up to 10^6 to 10^8 cell m^{-3}) it was anticipated that a range of complementary recording techniques would be required; a.c. direct recording, for individual flashes at low frequency (0.1 to 20 Hz); d.c. continuous recording at intermediate densities and frequencies (20 Hz and above) where the individual flashes merge into a continuous signal; digital recording (at 5 or 15 s sampling period with the MDTR) for large-area surveys of bioluminescent phenomena at intermediate to highest intensity, normally observable by the human eye at night.

The flow through system

The design of the flow through system and its incorporation in the UOR is shown in Fig. 1. Initial design calculations predicted a flow rate of 2.65 dm^3 s^{-1} (2.65 l s^{-1}) at a towing speed of 5 m s^{-1} using a sample chamber and connecting pipework of 26 mm i.d. A turbine flow meter is incorporated into the nose of the UOR to monitor the flow rate through the system, provide an additional source of bioluminescence stimulation, and increase the light-tightness of the system. The flowmeter reduces the flow rate to 50% of the theoretical flow rate (to 1.2 to 1.5 dm^3 s^{-1} at a tow speed of 5 m s^{-1}) as the effective cross-section of the turbine is approximately half the cross-sectional area of the pipework. The 35 to 40 cm of interconnecting pipework between the turbine and the bioluminescence detector, produces a theoretical delay of 140 to 60 ms over the speed range 2.5 to 6 m s^{-1} (which is possibly doubled by the restricted flow rate). This corresponds broadly to the reported dinoflagellate flash characteristics (HICKMAN *et al.*, 1980); latent period 0 to 10 ms, risetime 10 to 20 ms, duration ($\frac{1}{2}$ width) 50 to 100 ms, decaytime up to 250 ms.

Preliminary measurements with and without the flowmeter showed its importance for adequate agitation of the cells/organisms and stimulation of bioluminescence. Without the flowmeter, stimulated bioluminescence was relatively weak even at tow speed of 6 m s^{-1} but more than an order of magnitude higher with the flowmeter fitted, presumably due to increased agitation. With the inlet orifice and flowmeter blocked no bioluminescence signals were measured.

The sensor

The literature on the bioluminescence of marine organisms is extensive and provides some information on the light levels to be expected (HERRING, 1978; HICKMAN and STAPLES, 1979; HICKMAN *et al.*, 1980; LYNCH, 1978, 1981; NEALSON, 1981; TARASOV, 1956; TETT, 1971; TETT and KELLY, 1973). Dinoflagellate bioluminescent flashes range in intensity from 10^9 quanta s^{-1} $cell^{-1}$ for small *Peridium* sp. to 10^{11} quanta s^{-1} $cell^{-1}$ for *Pyrocystis* sp. and *Noctiluca scintillans* (miliaris) (LYNCH, 1978); a source intensity of 2.4×10^9 quanta s^{-1}, at

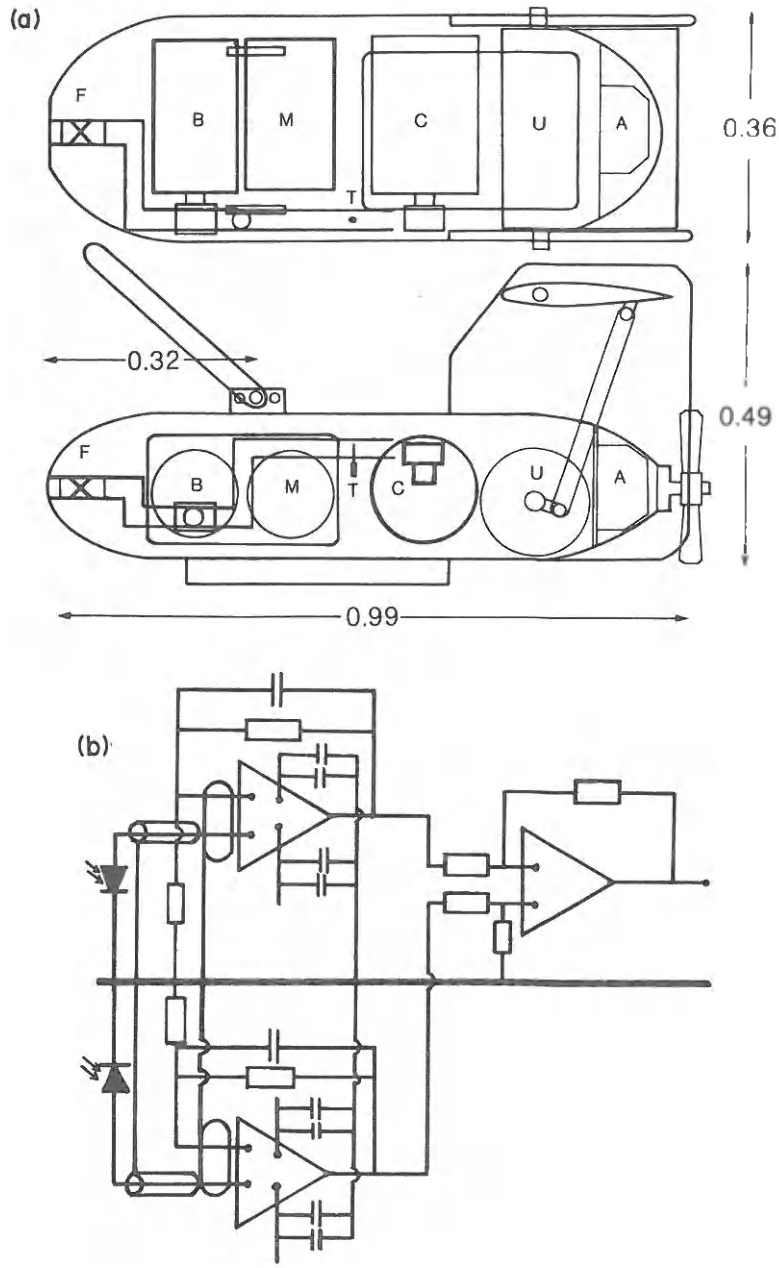

Fig. 1. (a) Schematic plan (top) and long-section (bottom) of the UOR Mark 2 (dimensions in m) showing the layout of the pipework (26 mm i.d.) connecting the flowmeter (F) to the bioluminescence sensor (B), the temperature sensor (T), and the cell of the chlorophyll sensor (C); (M) MDTR logger cylinder, (U) undulation control system, and (A) alternator. (b) Schematic diagram of electronic circuit of bioluminescence sensor.

480 nm is equivalent to a source power of 10^{-9} W, producing an irradiance of 10^{-11} W cm^{-2} at a distance of 2.8 cm. At a flow rate of 1.5 dm^3 s^{-1}, low cellular concentrations will produce individual flashes of *ca* 25 ms duration, this being the time that the flash is visible to the detector; at higher concentrations the individual flashes will merge into a continuous signal. A density of 10^8 cell m^{-3} will produce a steady-state irradiance of 5×10^{-8} W cm^{-2} at a distance of 2.8 cm. Thus the sensor range has to measure light emission over 4 orders of magnitude from 10^9 to 10^{13} quanta s^{-1} (10^{-11} to 10^{-7} W cm^{-2}).

Photodiode/operational amplifier combinations or photomultipliers can cope equally well with the expected range of signals. In addition photodiodes are small, robust, and have low electrical power requirements. The design adopted uses a silicon photodiode, operating in photovoltaic mode into the high-impedance, non-inverting input of a low-noise operational amplifier. Operating in the photovoltaic mode the pn junction of a photodiode is forward-biased, and with no leakage current the shot noise is zero, leaving only Johnson noise; Johnson noise ($= 4\ kT/Rs^{1/2}$) is minimised in small-area, photodiodes having very high shunt resistances (*Rs*).

One major penalty with the photovoltaic configuration is that shunt resistance is inversely proportional to temperature, although detector responsivity is almost independent of temperature (typically 0.05% °C). This makes Johnson noise, also proportional to T, very temperature-dependent (noise doubles every 10°C rise of temperature), producing a temperature drift of the dark-voltage signal. A simple solution of using a differential pair of photodetectors (U.D.T., PIN 3DP) and operational amplifiers (Analogue Devices AD515K) to subtract out any temperature drift, is a near-perfect solution; guard-ring protection schemes and power supply de-coupling are incorporated in the circuits, to achieve the optimum performance of the amplifiers (Fig. 1b).

At a $\times 10$ gain for each amplifier, a signal of 20 mV (signal to noise >20) is obtained for a flashing green L.E.D. (565 nm) operating at threshold voltage to be just observable by the dark-adapted eye; the L.E.D. produces an irradiance of 0.18×10^{-9} W cm^{-2} at 20 mm (equivalent to a source power of 10^{-8} W) measured by a calibrated irradiance meter (U.D.T.81). Thus, minimum detectivity at a signal to noise ratio of unity is equivalent to a source power of 5×10^{-10} W. The sensor operates over the temperature range of 0 to 25°C with a drift of dark signal voltage of −5 mV; the drift of < -2 mV from 8 to 22°C is considered acceptable.

The bioluminescence sensor, together with stabilised ± 5 V power supplies (from ± 9 V batteries), flowmeter circuit, pressure and temperature sensors and interface circuits are accommodated in a single aluminium cylinder (100 mm i.d. × 150 mm long), and fitted in the forward instrument hold of the UOR (Fig. 1). For vertical profiling independent of the UOR, a submersible electrical pump is fitted to the system to pump water past the sensor.

Data recording and replay

Data is recorded both digitally (spot measurement, 15 s period, range 0 to 1.023 V, 10 bit resolution) and on a continuous analogue track (pulse-width modulated, 0 to 8 Hz bandwidth, range 120 mV, resolution $\pm 1\%$ full scale) on the same cassette in the MDTR. The two recording techniques are complementary. The direct analogue system records individual pulses, bursts of pulses (frequencies 8 to 0.02 Hz), and low light signals of a few mV which might be missed by the spot, digital recording once every 15 s. At higher intensities and frequencies, the analogue recording continues to provide information on the pulse shapes and on the variation of the intensity of the continuum. Above a certain intensity

and frequency, the individual pulses merge totally making any direct or frequency recording redundant; these higher signals (>100 mV) can be monitored effectively by the 15 s digital recording, with reduced sensor gain to extend the overall sensor range.

The recorded cassettes are replayed at 25 times the recording speed and both analogue and digital recordings (with digital to analogue conversion) displayed on a chart recorder for 'quick-look' assessment of the data. Simultaneously the digital data is processed with a microcomputer, linked to a printing terminal, which provides tabulation of the data in calibrated physical units, vertical plots of the data, histograms of average parameter value in selected depth intervals for one undulation or several undulations (equivalent to a vertical profile), and calculates derived parameters or statistics on the data. In the laboratory, the microcomputer is used to communicate the data to a large main-frame computer to provide immediate computer-contoured, vertical sections of the measured parameters; the specially developed contouring package SACAM (Fig. 3) or SURFACE II (Fig. 5) have been used.

RESULTS

Preliminary results were obtained on a cruise of the USNS *Lynch* (28 April to 17 May 1983). Significant measurements of bioluminescence were recorded *in situ* both in daylight, and in conditions where the visible manifestations of bioluminescence were below those observable from the vessel at night.

Figure 2 shows the microcomputer generated vertical profiles of temperature (°C), chlorophyll (mg m^{-3}), bioluminescence (arb. units; $1 \cong 1.2 \times 10^9$ quanta $s^{-1} \cong 5 \times 10^{-10}$ W), and analogue trace of bioluminescence (arb. units) from the recovery phase of a UOR tow, equivalent to an oblique profile, at 5 kn (2.5 m s^{-1}), in Inchmarnock Water, Firth of Clyde at 20.00 h GMT, 28 April 1983.

The bioluminescent signal, around the sensor baseline (18 ± 2 mV) in the deep part of the tow before the final recovery, increased in intensity at 35 to 40 m depth, just below the base of the weak thermocline, coinciding with the increase in the chlorophyll concentration. While generally matching the subsurface chlorophyll depth structure, the bioluminescence depth structure had two additional peaks at *ca* 25 m and *ca* 10 m, reaching a maximum of 39 mV, 21 mV above the baseline, equivalent to a source power of *ca* 10^{-8} W (or 2.4×10^{10} quanta s^{-1}). The continuous analogue recording showed that the two peaks had considerable fine structure with several large amplitude 'spikes', presumably due to individual flashes of bioluminescence, and consistent with the findings of large numbers of bioluminescing copepod nauplii in the biological samples from the vertical profile at the conclusion of the tow; water samples pumped through a vertically profiling bathyphotometer.

Figure 3 shows the computer contoured vertical sections of temperature (°C), conductivity (mS cm^{-1}), chlorophyll concentration (mg m^{-3}), and bioluminescence (arb. units; $1 \cong 1.2 \times 10^9$ quanta s^{-1}) for repetitive double oblique profiles with the UOR Mark 2 at 1.5 m s^{-1} from near-surface to 120 to 150 m in the Barents Sea (*ca* 71°18′N, 18°55′E) at 02.45 to 06.05 GMT on 10 May 1983. The measurements show an intrusion of cold (<5°C) and fresher surface water (to 75 m) at the end of the course, accommodating enriched chlorophyll concentrations (over 2.5 mg m^{-3}) from concentrations generally <1 mg m^{-3} elsewhere. Apart from two small patches at the start of the tow, stimulated bioluminescence was greatest in a band along the vertical and horizontal fronts of the cold water.

Results from a comprehensive assessment of the sensor on a cruise of RMAS *Newton* in the Southwest Approaches, 3 to 7 October 1983 are presented in Figs 4 and 5; the track

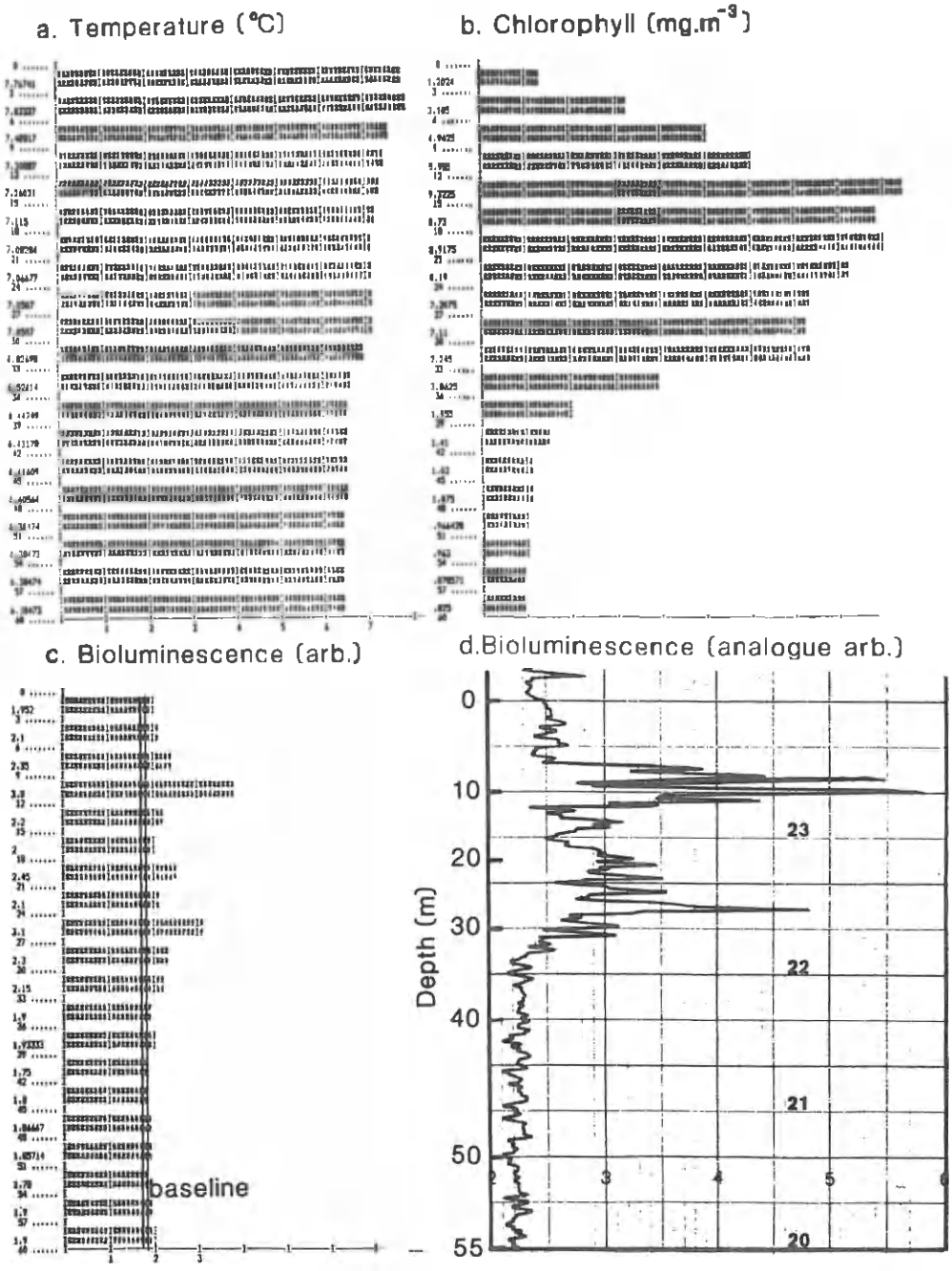

Fig. 2. Microcomputer-generated vertical profiles of (a) temperature (°C), (b) chlorophyll concentration (mg m^{-3}), (c) bioluminescence (arb. units; $1 \cong 1.2 \times 10^9$ quanta s^{-1}), and (d) continuous analogue trace of bioluminescence from an oblique haul of the UOR Mark 2 at 2.5 m s^{-1} in Inchmarnock Water, Firth of Clyde at 20.00 h GMT, 28 April 1983.

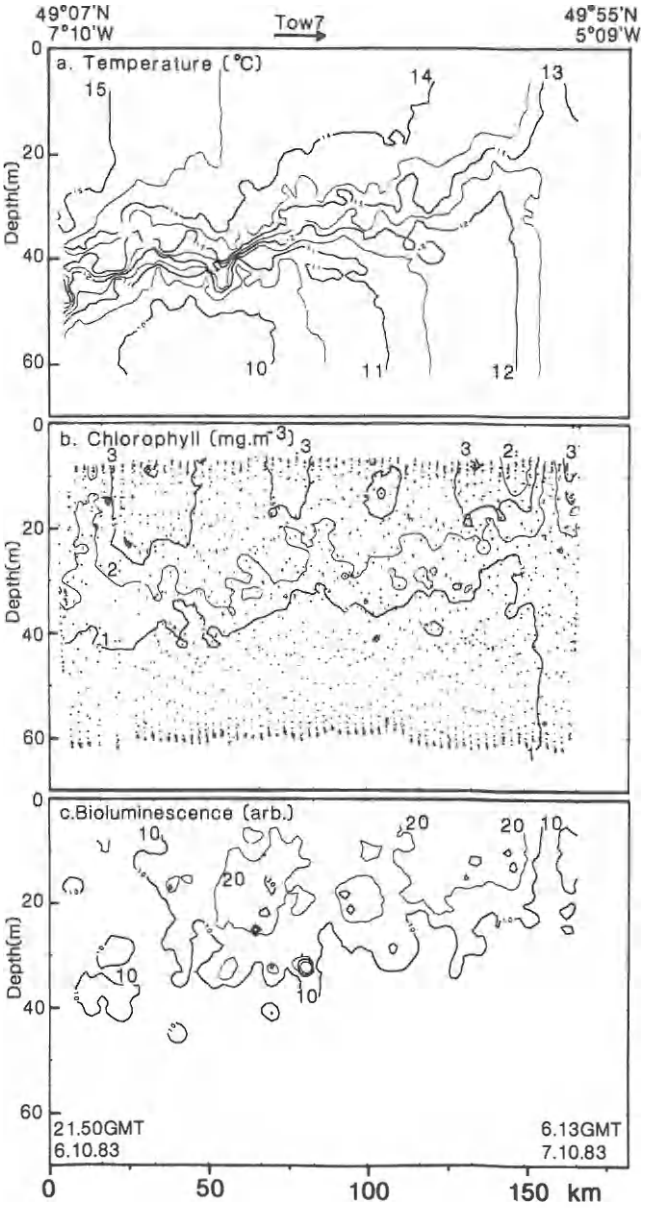

Fig. 5. Computer-contoured, vertical sections from 8 to 63 m of (a) temperature (°C), (b) chlorophyll concentration (mg m^{-3}), and (c) bioluminescence (arb. units; $1 \cong 1 \times 10^9$ quanta s^{-1}) from the UOR measurements (tow 7) in the Celtic Sea, 6 to 7 October 1983.

these parameters for the stationary measurements throughout the cruise, confirming that these features were not an artifact of the UOR measurements.

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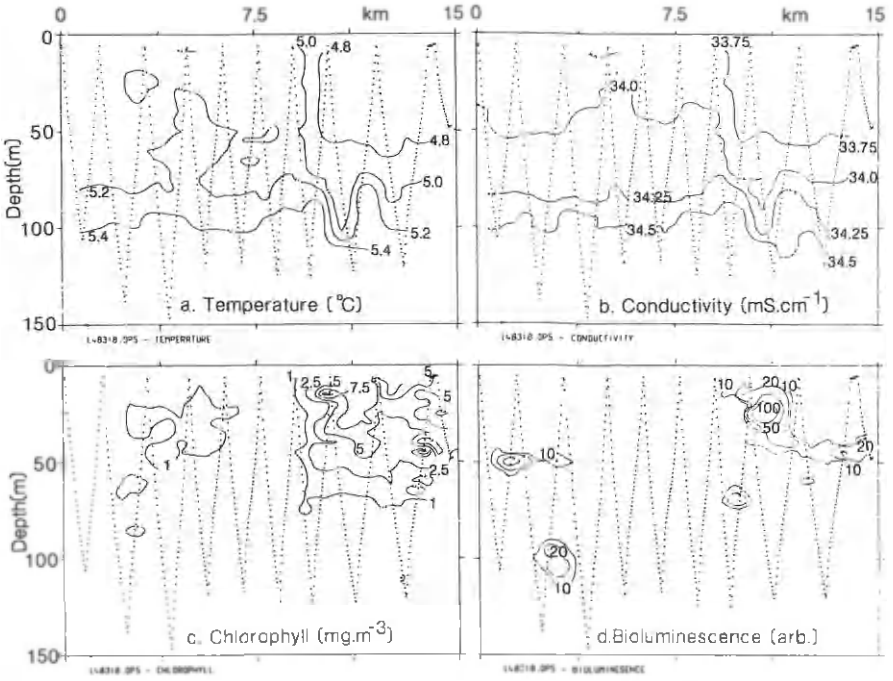

Fig. 3. Computer-contoured vertical sections (near-surface to 120 to 150 m) of (a) temperature (°C), (b) conductivity (mS cm^{-1}), (c) chlorophyll concentration (mg m^{-3}), and (d) bioluminescence (arb. units) for repetitive double oblique profiles of the UOR Mark 2 at 1.5 m s^{-1} in the Barents Sea, 10 May 1983.

of the vessel is shown in Fig. 4 with the position of the 'Ushant front' as indicated by the NOAA7 infared satellite image for 6 October. The increase of the stimulated bioluminescence around dusk, from the very low signals measured by day, to the high levels measured at night (in agreement with the reported inhibition of luminescence in daylight), showed that the detector was virtually immune to ambient daylight at the intensities for October (Fig. 4b).

Highest levels of bioluminescence activity were detected on the final tow (tow 7) in the Celtic Sea from southwest of the Isles of Scilly to just south of Lizard Point, from 21.50 GMT, 6 October 1983 to 06.13 GMT, 7 October 1983 (Fig. 5). Subsurface stimulated bioluminescence (Fig. 5c) increased significantly about 2 h (40 km) after the start of the tow, declining to near zero in the mixed, low-chlorophyll water just before the end of the tow. Surface stimulated bioluminescence, measured by a photomultiplier system monitoring near-surface pumped water on board the vessel, showed a similar sharp rise by a factor of 2 around midnight. Interestingly, while chlorophyll concentration was, as expected, uniformly distributed throughout the surface mixed layer, stimulated bioluminescence exhibited a sub-surface peak at *ca* 20 m. Subsurface water samples on station before the start of the tow and surface water samples throughout the tow showed that small flagellates (<5 μm diameter) were the main organisms present, probably responsible for bioluminescence.

The uniform chlorophyll concentration in the surface mixed layer and the subsurface maximum in the stimulated bioluminescence signals, were a feature of the vertical profiles of

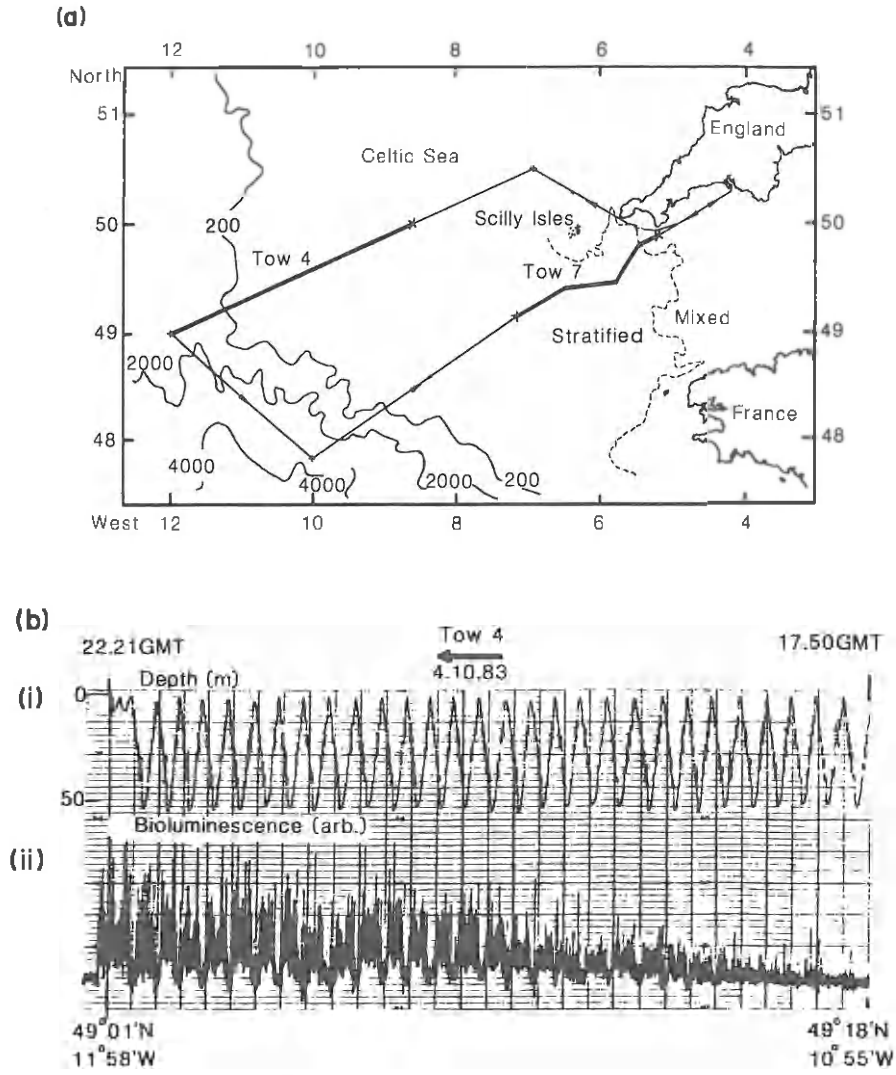

Fig. 4. (a) Cruise track of the research vessel RMAS *Newton*, 3 to 7 October 1983, and bathymetry (m) of the Celtic Sea and Southwest Approaches to the British Isles; the position of the stratified/mixed water front as indicated by the NOAA7 infrared satellite image for 6 October is marked. (b) UOR Mark 2 measurements around dusk on 4 October 1983 in the Celtic Sea; (i) depth (digital to analogue conversion on replay) and (ii) bioluminescence (continuous analogue recording).