Emerging technologies in biological sampling

A report of
SCOR Working Group 90

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SCOR Working Group 90

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PREFACE

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ABSTRACT

Technology development as we know it today is and should be driven by the science in which it is applied. Initial science objectives of large scale international programs, such as JGOFS and GLOBEC, outline sampling technology needs at their outset and initiate specific technological development. Described here are a variety of technologies - recent and not-so recent - which are emerging in the 90's and will provide biological oceanography with newer and more powerful tools and greater insights with a wider scope of measurements. There are three areas of focus: 1) Platforms, 2) Fluorescence and 3) Particles. Under 'Platforms', vehicles and methods of sampling are described leading the way into a discussion of the sensors used in the measurement of various biological parameters. In this recent scientific age of climatic influence of ocean ecology, long-term monitoring has been identified as a need which is presently encouraging new developments in mooring technology. In some cases specific measurement technologies which have been only recently developed, e.g. 'pump and probe fluorescence' or the 'SeaWiFS', are emerging technologies which are destined for broader and more intensive applications. Many technologies are potentially overlapping in application. For example, the historical technology of light attenuance has been recently integrated with the emerging technology of flow cytometry yielding new insights in patterns of primary production. The coalition of three technologies, that is, acoustics and the emerging technologies of video and optical plankton counting are also described indicating their future potential for the counting and identification of zooplankton.

RESUME

Le développement technologique tel que nous le connaissons aujourd'hui est et doit être mû par la science à laquelle il s'applique. Les premiers objectifs scientifiques des grands programmes internationaux comme la JGOFS et GLOBEC mettent d'emblée en évidence des besoins en matière de techniques d'échantillonnage et amorcent un développement technologique spécifique. On trouvera ci-après la description de toute une série de techniques récentes, et moins récentes, apparues dans les années 90, qui doteront l'océanographie biologique d'outils nouveaux et plus puissants, et permettront de mieux connaître un champ de mesures plus vaste. Trois thèmes centraux ont été étudiés : (1) les plates-formes, (2) la fluorescence et (3) les particules. S'agissant des plates-formes, la description des véhicules et des méthodes d'échantillonnage débouche sur une étude des capteurs utilisés pour la mesure de divers paramètres biologiques. Nous connaissons depuis ces dernières années une période d'étude scientifique de l'influence de l'écologie océanique sur le climat, au cours de laquelle la surveillance à long terme a été considérée comme une nécessité qui stimule actuellement le perfectionnement des techniques de mouillage. Dans certains cas, des techniques de mesure spécifiques qui viennent d'être mises au point, par exemple les techniques de pompage et de sondage par fluorescence ou les "SeaWiFS", sont des technologies naissantes appelées à connaître des applications plus larges et plus intensives. De nombreuses techniques ont des applications qui pourraient se recouper. Ainsi, la technique historique de l'atténuation de la lumière a été récemment intégrée à la technique nouvelle de la cytométrie des flux qui ouvre de nouvelles perspectives concernant les caractéristiques de la production primaire. L'association de trois technologies, à savoir l'acoustique et les nouvelles techniques vidéo et optique appliquées au dénombrement du plancton, est également décrite pour montrer les possibilités offertes dans le domaine du dénombrement et de l'identification du zooplancton.

RESUMEN ANALITICO

Tal como lo concebimos hoy, el progreso tecnológico debe fundarse en la ciencia en la cual se aplica. Los objetivos científicos de los programas internacionales en gran escala como JGOFS y GLOBEC permiten definir las necesidades de la tecnología del muestreo y a su vez dan origen a adelantos técnicos específicos. En este volumen se describen las tecnologías (recientes o no) en desarrollo en el presente decenio que proporcionarán a la oceanografía biológica nuevos y más eficaces instrumentos y conocimientos para diversificar y multiplicar las mediciones. Se presentan técnicas relacionadas con: 1) plataformas, 2) fluorescencia, y 3) partículas. En la sección sobre plataformas se describen los vehículos y los métodos de muestreo y se discuten los instrumentos de medición de parámetros biológicos. El estudio científico de la influencia del clima en la ecología oceánica y la necesidad de observaciones a largo plazo suscitan innovaciones en las técnicas de amarre. Se citan algunas nuevas técnicas particulares de medición, por ejemplo el 'bombeo y ensayo de fluorescencia' y la técnica 'SeaWiFS', que seguramente tendrán aplicaciones más amplias e intensivas. Cabe prever además la convergencia de ciertas técnicas; por ejemplo, la tradicional técnica de fotoatenuación se integra con la nueva técnica de citometría de flujo, proporcionando nuevos elementos para la comprensión de las pautas de producción. También se hacen notar las posibilidades que abre, en materia de recuento e identificación del zooplancton, la combinación de tres técnicas: acústica, vídeo y recuento óptico.

РЕЗЮМЕ

Как известно, развитие технологии в нынешнем мире определяется и должно определяться наукой, в сфере которой она применяется. Исходные научные цели широкомасштабных международных программ, таких, как СИГМО и ГЛОБЭК содержат изложение в общих чертах потребностей в области технологии взятия проб на начальном этапе и закладывают основы для конкретного технологического развития. В настоящей работе описываются разнообразные технологии как новые, так и относительно новые, которые появились в 90-х годах и которые позволят вооружить биологическую океанографию новыми и более мощными средствами, а также обеспечить ее более глубокое понимание благодаря расширению объема замеров. Рассматриваются три основные области: 1) Платформы, 2) Флюоресценция и 3) Частицы. В рамках раздела "Платформы" дается описание средств и методов взятия проб, которое далее переходит в обсуждение вопросов использования датчиков для замера различных биологических параметров. В наш научный век климатического влияния экологии океана долгосрочный мониторинг определяется в качестве потребности, которая в настоящее время стимулирует новые разработки в области технологии постановки на якорь. В некоторых случаях технологии специальных замеров, которые появились только в последнее время, например "закачиваемые пробы на флюоресценцию" или "морской датчик широкополосного обзора", представляют собой недавно созданные технологии, предназначенные для целей более широкого и более интенсивного практического использования. Потенциально в ходе применения многие технологии могут дублировать друг друга. Например, давно используемая технология добегания света недавно была интегрирована в нарождающуюся технологию цитометрии потока, дающей возможность получить новые данные об образцах первичной продукции. Дается описание совместного использования трех технологий, т.е. акустики и появляющихся технологий подсчета планктона с помощью видео и оптических средств, а также излагаются их будущие возможности для подсчета и идентификации зоопланктона.

مستخلص

ان تنمية التكنولوجيا كما نعرفها اليوم يقودها – ويجب أن يقودها – الفرع العلمي
الذي تطبق فيه. فالأهداف العلمية الأصلية للبرامج الدولية الواسعة النطاق، مثل الدراسة
المشتركة لتدفق المحيطات في العالم (JGOFS) وديناميات النظم الايكولوجية للمحيطات
في العالم (GLOBEC)، تحدد معالم الاحتياجات من تكنولوجيا أخذ العينات عند استهلال
هذه البرامج وتبدأ بتطوير التكنولوجيا اللازمة. ويرد هنا وصف لطائفة من تكنولوجيات
– الحديثة والأقل حداثة – التي استجدت في التسعينات وينتظر أن تزود الأوقيانوغرافيا
البيولوجية بأدوات أحدث وأنجع تتيح لها تحقيق المزيد من الدقة بواسطة تشكيلة واسعة
من القياسات. وتنصب الدراسة على ثلاثة مجالات هي : (١) المنصات العائمة و (٢) الاشعاع
الفلوري و. (٣) الجزيئات. ففيما يتعلق بالمنصات العائمة، يرد في القسم الخاص بها وصف
لأدوات وأساليب أخذ العينات، يمهد الى مناقشة بشأن المجسات التي تستخدم لقياس
مختلف البارامترات البيولوجية. وفي هذا العهد الحديث الذي يعنى فيه العلم بالتأثير
المناخي لايكولوجيا المحيطات، أعتبرت عملية الرصد على المدى الطويل من الضرورات
التي تشجع في الوقت الراهن تطورات جديدة في توطيد دعائم التكنولوجيا. وفي بعض
الحالات، تعد بعض التكنولوجيات القياسية التي لم تطور الا منذ فترة وجيزة جدا، مثل
'ضخ واختبار الاشعاع الفلوري' أو القياس المسمى "SeaWIFS"، من التكنولوجيات الناشئة
المعدة لتطبيقات مكثفة وواسعة النطاق. وثمة تكنولوجيات عديدة يحتمل أن تتداخل عند
التطبيق. فالتكنولوجيا القديمة الخاصة بالتخفيف الضوئي مثلا أدمجت في
التكنولوجيات الجديدة لقياس حركة الخلايا في حالة التدفق مما أدى الى فتح آفاق جديدة
لمعرفة أنماط الانتاج الأولي. ثم أن الجمع بين ثلاث تكنولوجيات هي علم الصوت
والتقنيتين الحديثتين الخاصتين باحصاء البلانكتون بواسطة الڤيديو والأجهزة البصرية،
يرد وصفه أيضا مع الاشارة الى كل ما تحمله هذه التكنولوجيات مجتمعة من امكانيات
التطبيق في احصاء العوالق الحيوانية وحصر أنواعها.

摘　要

　　当今的技术发展是由，而且必须由应用它的科学来推动。大型的国际计划，如"全球海
洋流量联合研究"计划（ JGOFS ）和"全球海洋生态系统动态"计划（ GLOBEC）开始时的科
学目标都是提出计划开始时所需的采样技术，并进行具体的技术试验。这里描述的是九十年
代出现的各种最新的和不算太新的技术。它们将以更多的测量方法为生物海洋学提供更新和
更有效的工具以及更深入的了解。三个主要领域是（1）平台测量，（2）荧光测量，（3）粒子
测量。在平台测量这一部分，首先描述了采样的手段和方法，接着讨论在测量各种生物参数
时所使用的探测工具。在当今对海洋生态如何影响气候进行科学研究的时代，长期监测非常
必要，而且有助于发展新的锚系技术。在某些情况下，最近才研究出来的一些测量技术，如
"连泵荧光探测法"或"SeaWIFS"等都是必定会得到广泛和大量使用的新技术。许多技术

还可以一起使用。例如，光衰减这一传统技术最近正同新出现的边疆血细胞计量技术相结合，从而得出了对初生方式的新观点。文章还描述了三种技术，即声学技术和新出现的录象技术和浮游生物光测计数技术的综合运用，并指出这些技术今后在计量和确定浮游动物方面所具有的潜力。

INTRODUCTION

While examining the emerging technologies in the next decade, we like to think that they are driven by the science alone but, in fact, oceanography often just 'tags along' with new high technology that emerges for other reasons. For example, biomedical applications were the driving force behind the development of flow cytometry while biological oceanography simply capitalized on its potential applications. New miniature, high-power laser diodes developed for laser positioning systems will no doubt find application in future underwater oceanographic sensors. There are also examples of oceanographic science driving new technologies/methodologies. The 90's will see an emphasis on global climate change and its profound effects on our oceans, in particular, its biology. We examine here the emerging technologies/methodologies in biological oceanography that provide us with measurements of concentration, size, shape, counts, feeding/growth rates, migrational habits, physiology, behaviour and all other parameters which will illuminate our understanding of the survival and response of planktonic/fish species to climatic changes.

In the late 80's we saw large multidiscipline programs such as JGOFS (Joint Global Ocean Flux Study) where the international oceanographic community sought to examine the carbon cycle over large scales in our deep oceans leading to some determination of its capacity for atmospheric CO_2 uptake. New programs for the 90's are concentrating on climatic effects on our planktonic/fish ecosystems (GLOBEC, AFAP, OPEN). One such effort called the Global Ocean Ecosystem Dynamics (GLOBEC) will concentrate on one such ecosystem, Georges Bank, and track its highly productive biological component of plankton/fish for nearly 3 years of a 5-year program. The real strength of this program is that it has set aside an additional 2 years (prior to the start of data-taking) for the development of sampling instrumentation, methodologies and intercalibration and testing. Such philosophies are needed since they allow breathing space for new technology to emerge (with proper financial support!) and preclude our natural haste to get on with the data-taking with the hope that the new developments in technology will happen simultaneously. Anticipated new technologies and sampling methods emerging from such programs will be described here.

An emphasis on climate change immediately implies a requirement for long-term monitoring and hence a future potential for moorings. Biological moorings have always been difficult to develop (as compared to those carrying physical sensors) due to their inherent problems of adaptability, power consumption and optical fouling. Previous high-profile programs have not devoted space and time for the development and testing required for such complex technology. This opportunity is now available in the next decade to develop high-technology moorings which can monitor long-term biological responses to global climate change.

Another highlight of the next decade will be a subprogram of the GLOBEC community which will concentrate on two intensive years of development and intercalibration of acoustic and optical technologies applied to biological monitoring on both towed and moored platforms. The uncertainties in acoustical measurements is common community knowledge and its coalition with optical measurements will focus and enhance their strengths.

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The description here will focus on 3 areas; sampling platforms (in the broad sense) and the measurement of particles with emphasis on phytoplankton and zooplankton. New concepts in technology as well as adaptation and extensions of previous methods will be presented and some 'directional guesses' will be proposed. The following list will comprise the content of this discussion:

PLATFORMS   Ships: ('Towed bodies, station profiling)
         ROV/Submersibles
         Satellites
         Moorings

FLUORESCENCE   Fluorometers
         Moored fluorometers
         Pump and probe fluorescence
         Flow cytometry
         Remote sensing

PARTICLES   Coulter counter
         Light attenuance/Forward scattering
         Flow cytometry
         Acoustics
         Optical plankton counters
         Video

PLATFORMS

Ships

Figure 1 shows the time and space scales of Esaias (1980) corresponding to phytoplankton and zooplankton and the operational ranges of various sampling platforms. Ships could clearly sample continuously for 1000 days as shown if we could afford them; however, a cruise span of 2-3 weeks is more typical and budgetable perhaps at a rate of twice per year. During a cruise we normally have two options; towing or profiling. Towing gives us synoptic coverage, however, profiling yields water samples on deck for assay purposes and therefore both are inescapable.

Towing is of two types; that of the constant depth variety and that of the 'TOWYO' (Flament *et al.*, 1983) or undulating modes. The two most commonly used undulating vehicles (Figs. 2, 3) are the 'SEASOAR' (Golmen, 1987) and 'Batfish' (Herman and Denman, 1977) that have been adapted for physical/biological sampling. These vehicles undulate at some constant cable length by variable wing angle either autonomously or by surface control. The 'TOWYO' mode which is also an undulation mode is accomplished by varying the cable length under winch control. Depressors or 'towed wings' are highly suited for mounting sensors and being towed in this fashion. This is a highly effective and inexpensive approach; however, in the longer-term hidden costs are a lower winch life.
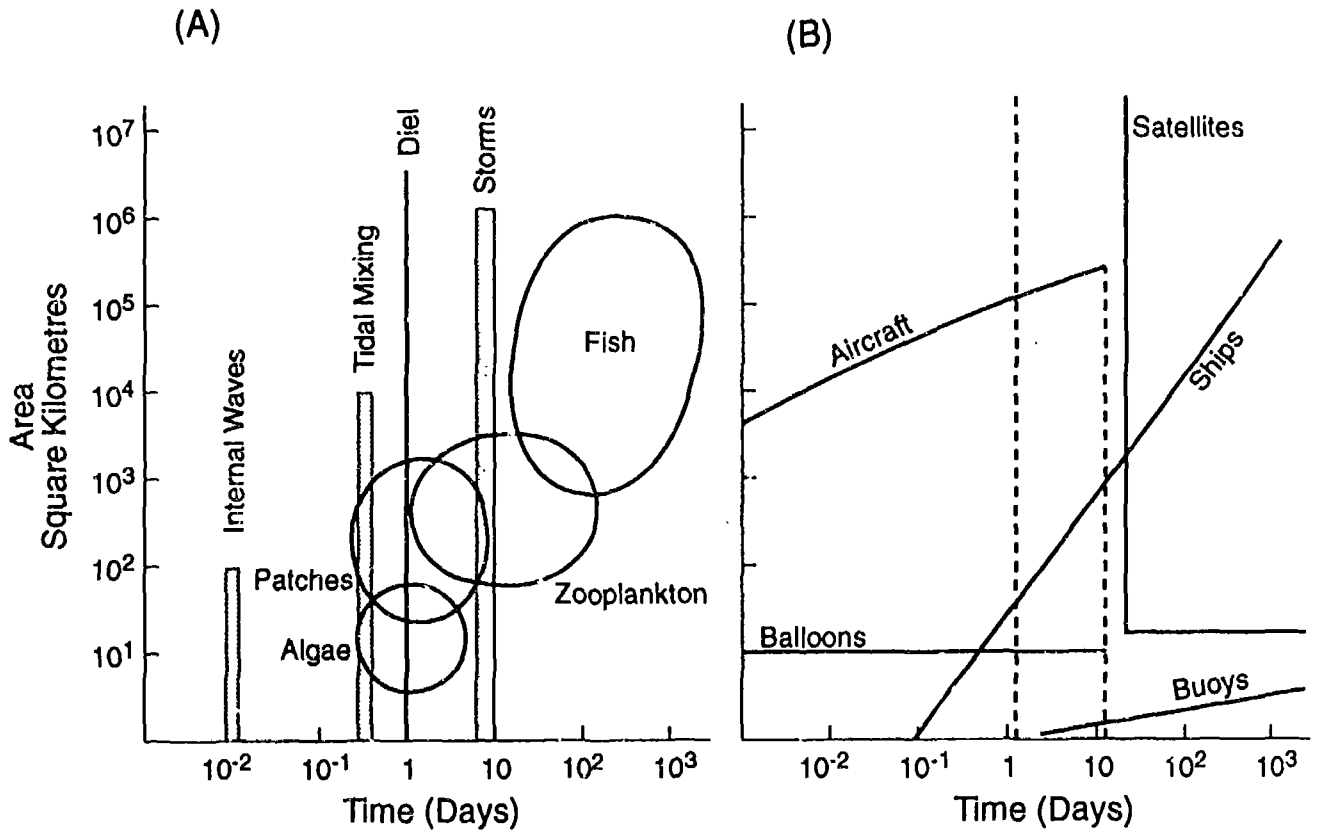
Fig. 1: Time-space domains for oceanic phenomena. (A) Processes, indicating periods for physical forcings and excursion-generation times of some biological components.
(B) Sampling, indicating limits of coverage for various platforms (from Esaias, 1980).

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Fig. 2: The towed undulating vehicle SEASOAR capable of carrying a variety of sensors (courtesy Chelsea Environmental Instruments Ltd).
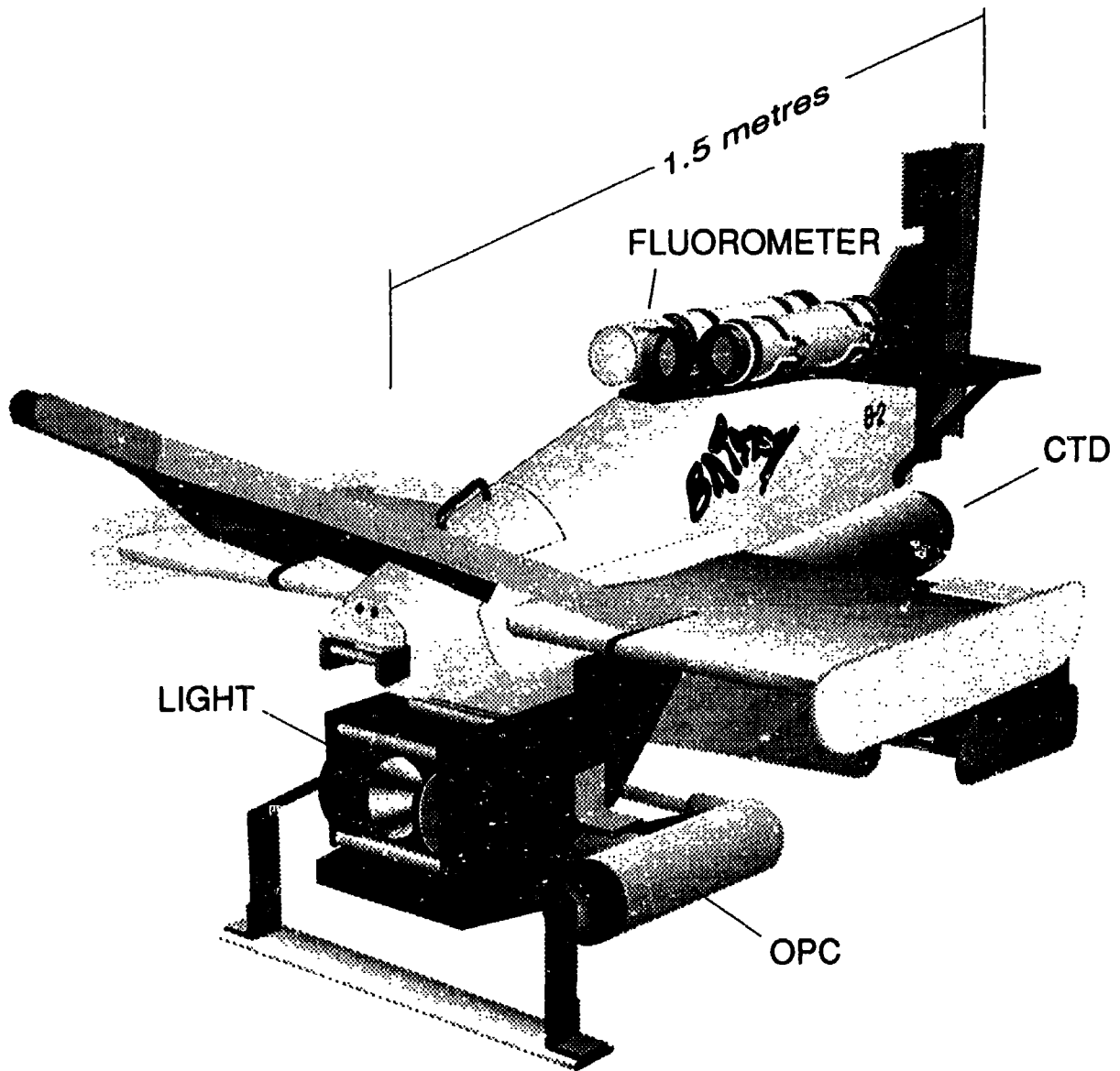
Fig. 3: The towed undulating vehicle BATFISH adapted for physical/ biological sampling (from Herman et al., 1993). The vehicle is mounted with a variety of sensors; 1) CTD measuring conductivity-temperature-depth, 2) a Variosens fluorometer measuring chlorophyll a, and an optical plankton counter used for counting and sizing zooplankton.

Satellites

Figure 1 shows the broad temporal and spatial coverage from satellites but does not relate to us the real restrictions of limited depth penetration. Much colour information yielding chlorophyll measurements has emerged from the CZCS borne on NIMBUS-7 from 1978-86. The SeaWiFS due to be launched in '92 will carry more colour sensors capable of sensing coastal water chlorophyll, yellow substance and making atmospheric corrections. Further details will be presented under 'Remote Sensing'.

ROV/Submersibles

'Owning a specific piece of costly hardware (like a manned submersible or underwater habitat) funnels the science which is proposed into limited system capabilities' (Stewart and Auster, 1989). Manned submersibles are inherently costly and have narrow weather windows which limit operations to summer months. Despite such limitations submersibles have provided us with invaluable observational data of a 'seeing is believing' nature which was not available from conventional samplers. A classic example was provided us in the case of sampling euphausiids such as *Meganytiphanes*. These large (1-3 cm) fast-swimming zooplankton which are common prey for many species of fish (Koeller *et al.,* 1989; Jean, 1953; Powles, 1958) are often undersampled by large net samplers (Sameoto *et al.,* 1991; Everson and Bone, 1986) as a result of their visual perception and avoidance capabilities. Hence their biomass was difficult to balance within the marine food chain. From submersibles used in shelf-edge canyons off the New England coast (Greene *et al.,* 1989; Youngbluth, 1985) at ≈250-300 m depth, large concentrations of euphausiids were observed within layers situated between several meters to tens of meters from the bottom. Visual estimates indicated that concentrations were probably 1-2 orders of magnitude higher than expected revealing the effect of avoidance. The study by acoustic, optical and net samplers reflourished and was followed by a new sampling and technological development effort renewed by the knowledge that we now knew what we were detecting. The euphausiid layers were always there; we simply never 'keyed' on their presence until we actually saw them. All this resulted from simple visual observations from manned submersibles.

In overcoming the limitations of the manned submersible, a new generation of remotely-operated-vehicles (ROVs) emerged. ROVs were unmanned but tethered to ships and capable of nearly unlimited operation (Fig. 4). Their emergence was dependent upon and coupled with the emergence of high resolution under water video cameras which then extended our visual observation capability. The application of another high-technology development was required, the fiber-optic data link capable of handling the high data rates of such video systems. As a result the manned submersible is being replaced by the ROV for such oceanographic observations based on its lower cost and higher operational capabilities.

(a)

Anchor   Downweight   ROV

(b)

Float

Tracking System

Pinger   Neutral Tether

ROV

(© 1989 IEEE)
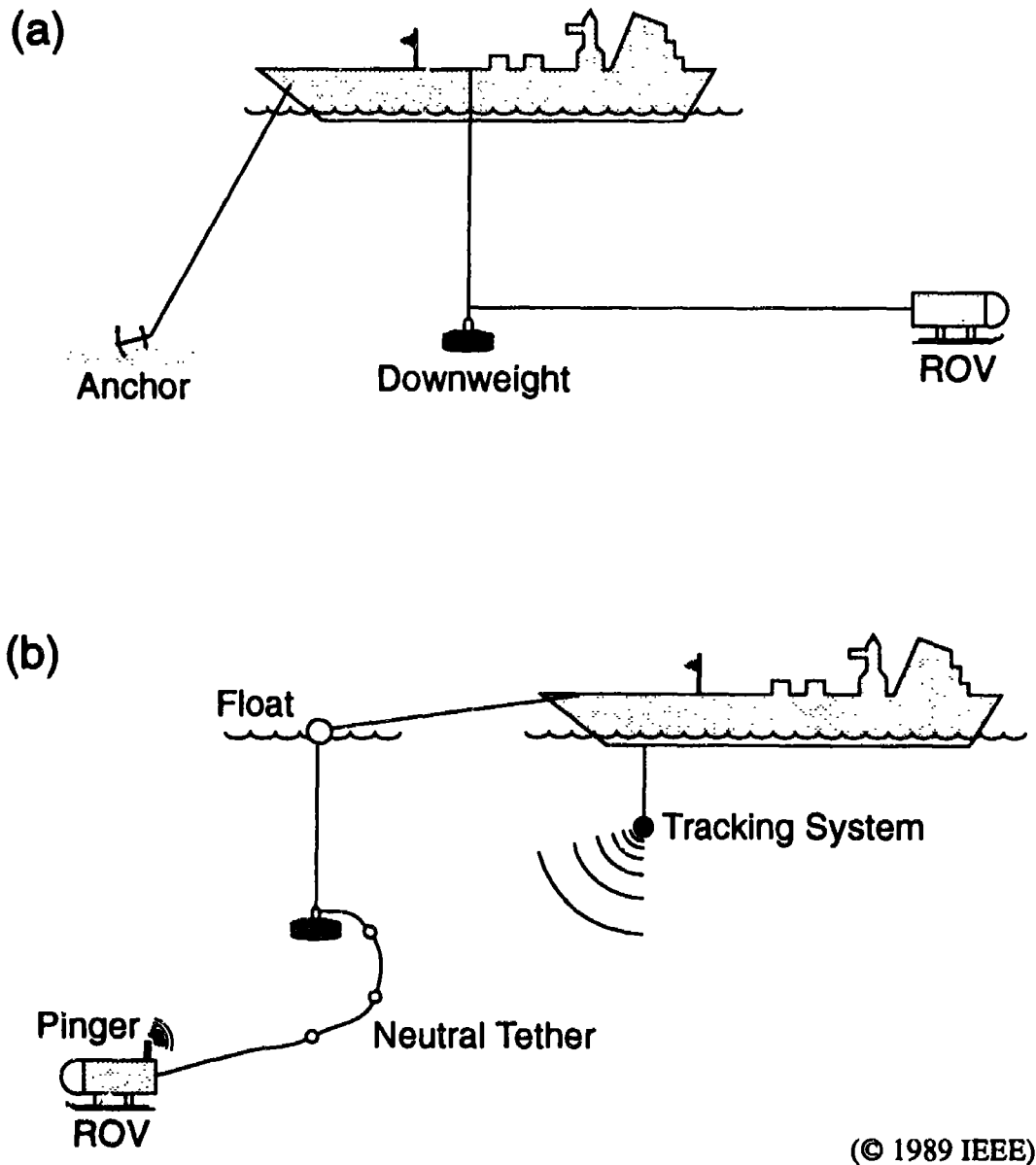
Fig. 4: Deployment modes of a remotely-operated-vehicle (ROV) tethered to a ship (from Stewart and Auster, 1989).

Moorings

Moorings too are considered expensive but tolerable within most research budgets since the data return per dollar value is quite high. The hardware associated with each mooring, i.e. buoys, floats, KEVLAR line, swivels, releases, shackles, etc., are comparable to the cost of moored sensors themselves. Data losses and dependability has vastly improved over years of development and mooring losses due to fishing traffic have been minimized with the use of guard buoys. One of the major areas of required advances in the next decade will be the development of innovative biological sensors for multi-depth measurement capability. Within our annual research budgets we can afford 1 or 2 fluorometers placed on a mooring but we need 10 or more to provide adequate multi-depth information on chlorophyll in the upper layers. Even if we could afford to purchase such a number, could we afford to risk such an investment on a mooring for 6-12 months? We approach the topic of new concepts in moored sensors in later sections.

Another area of major advances anticipated in the next decade are 'smart' data loggers as well as inexpensive and compact self-contained power supplies. We cannot moor high-technology sensors such as video/acoustical/optical counting systems without high-speed processing and synthesizing of data and finally storing such high volumes of data in memory. This is not achievable today but is definitely forthcoming. We also do not possess the capacity in moored power supplies to supply such biological instruments without the enormous cost and size of such packages. Battery technology in the next decade is anticipated to achieve both low cost and size small enough such that pressure cases can be hand-held.

Data Loggers

Tape recorders have served for nearly two decades as media for moored data storage. These have been superseded in the 80's by solid-state memory or 'random-access-memory' (RAM) which eliminated the problems caused by mechanical components, temperature stability, and high power consumption. Typical memory storage of ≈10 Mbyte was easily achievable on a single electronic board. Such RAM, however, required battery backup power and was volatile; that is, a mechanical or power disruption termed a 'glitch' could easily lose all data stored in RAM rendering a 6-month mooring useless. The problem of volatile memory was resolved by the development of the 'erasable programmable read only memory' (EPROM) which no longer required continuous power to maintain memory storage; however, the memory capacity of EPROMs of similar size was reduced by a factor of 2x. Moreover, UV light was required for erasure of memory.

In the last year, small-size hard-disk drives (≈6 cm diam.) of 200 Mbyte capacity for data logging have become commercially available. As data storage media, hard-disks are entirely non-volatile and dependable long-term storage but, alas, they are mechanical devices. The most promising development, however, emerging in the next decade is that of 'flash' memory EPROM which is destined to revolutionize the portable computer industry. This thin-wafer technology is being directed to developing a 32 Mbyte memory chip in a thin small-outline package of only 20 x 8 x 1.2 mm. By 1993 it is anticipated that 100-200 Mbytes capacity will be available in a 'credit card' size. This means that a small electronic card can be built to contain 1 Gigabyte of memory pushing our data storage capacity well beyond our limits of mooring applications. This technology is expected to become the next

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solid-state disk by replacing the present hard-disk media. It offers true nonvolatility, electrically erasable while in its own media, no potential 'head-crashes', reduction in power consumption by a least an order of magnitude and overall ruggedness.

In the last decade various generations of 8-bit microprocessors have provided the computing power for the acquisition and transference of data to memory storage. These microprocessors were only a component of an integrated electronic card which had to be fully constructed by the user and programmed in a low level language. Programs for the microprocessor had to be 'burned' into the chip and were not easily reprogrammed at sea. More recently in the last 3-4 years, fully integrated data logger cards employing 8-bit microprocessors have become common use. The data logger cards now contain full-fledged computers which · have a BASIC language resident in memory when turned on. Data acquisition and storage programs are written in this 'user-friendly' language on a standard desktop computer and 'downloaded' to the data logger computer. Users now enjoy the ability to reprogram such data loggers at sea in some cases without even opening a pressure case.

The most progressive development in the data logger card has been the inclusion of the 32-bit microprocessor-chip which represents a quantum leap in computing power. These data logger computers now contain the powerful machine-readable code generated from 'C' langauge and are memory resident when power is turned on. Progress in data logger computers is moving rapidly and the next decade should provide us moored data logging computers mounted on small cards equivalent to our most powerful desktop computers. Preprocessing and synthesis of data prior to storage will then be possible. Spectral analyses of time series of moored acoustic backscatter data can be achieved as benchmark tests by industry show such new computing architecture can compute 1000 fast Fourier transforms (radix 2) in only 1-2 milliseconds. Complex algorithms can become resident in moored data loggers to process large volumes of video data. With new projected high capacities in data storage, high frequency sampling rates will be limited only by the power consumption of the moored sensors themselves.

Battery Technology

Although we anticipate minimal power consumption by data loggers in the next decade, our power requirements for optical sensors are not anticipated to decrease by the same substantial magnitude. Looking to our self-contained power supplies, we recognize that miniaturization or power/volume is still our biggest requirement to overcome both our size problem and the increasing number of sensors required for multi-depth capability.

The answer lies in battery technology for the next decade. Thus far there are 4 primary battery-types that find common usage in moorings: 1) the rechargeable type, Ni-Cad (nickel-cadmium) and 2) Lead-acid, 3) standard alkaline (Zn-MnO$_2$) and 4) lithium-based batteries. Ni-Cad and Lead-acid cells are the only reusable types while alkaline and Li-batteries are disposable types. Lead-acid batteries, sometimes used in moorings, have the disadvantage of high weight and low capacity. In order to consider the utility of batteries for long-term moorings, we have to examine their power/volume characteristics. Ni-Cad batteries have 1/2 the power/volume of alkaline batteries while Li-cells provide as much as 4x the power/volume. Unfortunately Li-cells are extremely expensive and have safety problems and transport restrictions due to their lithium content. As a result, the

alkaline battery is the most commonly used in moorings. In 1992, however, the rechargeable Nickel-Metal Hydride battery has appeared on the market equal in capacity to the alkaline battery and would be immediately usable in mooring applications.

In the next decade the industry has proposed a new development called the lithium 'swing' battery, the name relating to its charge and discharge mode. Production of the lithium battery requires a controlled dry environment while the 'swing' battery will not require dry room conditions therefore enhancing its volume production. This should drive down costs and safety requirements. Certainly if the lithium 'swing' battery is capable of delivering the same power/volume as its predecessor, then power supplies for moorings will expect a new era of miniaturization and increased sensor capability.

FLUORESCENCE

Fluorometers

Chlorophyll a is the pigment most commonly used to estimate the standing crop of phytoplankton. It has two advantages: its relative abundance has been found to be a good estimator of the productive capacity of a phytoplankton community, and its concentration can be measured by extractive fluorometry. The first of these instruments was the Turner 111 fluorometer, originally a laboratory instrument which was adapted for continuous in vivo measurement of chlorophyll concentration at sea over 25 years ago by Lorenzen (1966). The Turner 111 was superseded by another generation, the Turner Designs fluorometer both of which are proven 'workhorses' of in vivo fluorometry and can provide a lower sensitivity limit of 0.05 mg.m^{-3} under typical sampling conditions.

A variety of commercial in situ fluorometers followed such as the Variosens, SeaTech and Aquatracka. Although these instruments were higher in cost and slightly lower in sensitivity (0.1-0.2 mg.m^{-3}), they could be mounted on any towed vehicle or profiler and provide a truly in situ measurement without pumping. Some new developments are seen in light sources over the next decade and will have some effect on reduction in size, cost and sensitivity which will impact somewhat in the towed system but most profoundly on the moored fluorometer.

Moored Fluorometers

One of the first moored fluorometers developed was that of Whitledge and Wirwick (1983) where they mounted a Turner Designs fluorometer in a pressure case and sampled chlorophyll by periodic pumping at only one depth. Power constraints were the main problem such that the battery container weighed 500 kg and acted as the system anchor. Further developments improved the size by deploying a commercial in situ fluorometer (Mitchell, 1991) shown in Figure 5; however, costs limited its deployment to a single depth. Further developments again (Whitledge and Wirick, 1986) decreased the overall system size to a single case ($\approx$1 x 0.3 m) containing both fluorometer and batteries while enabling sampling at 2 depths
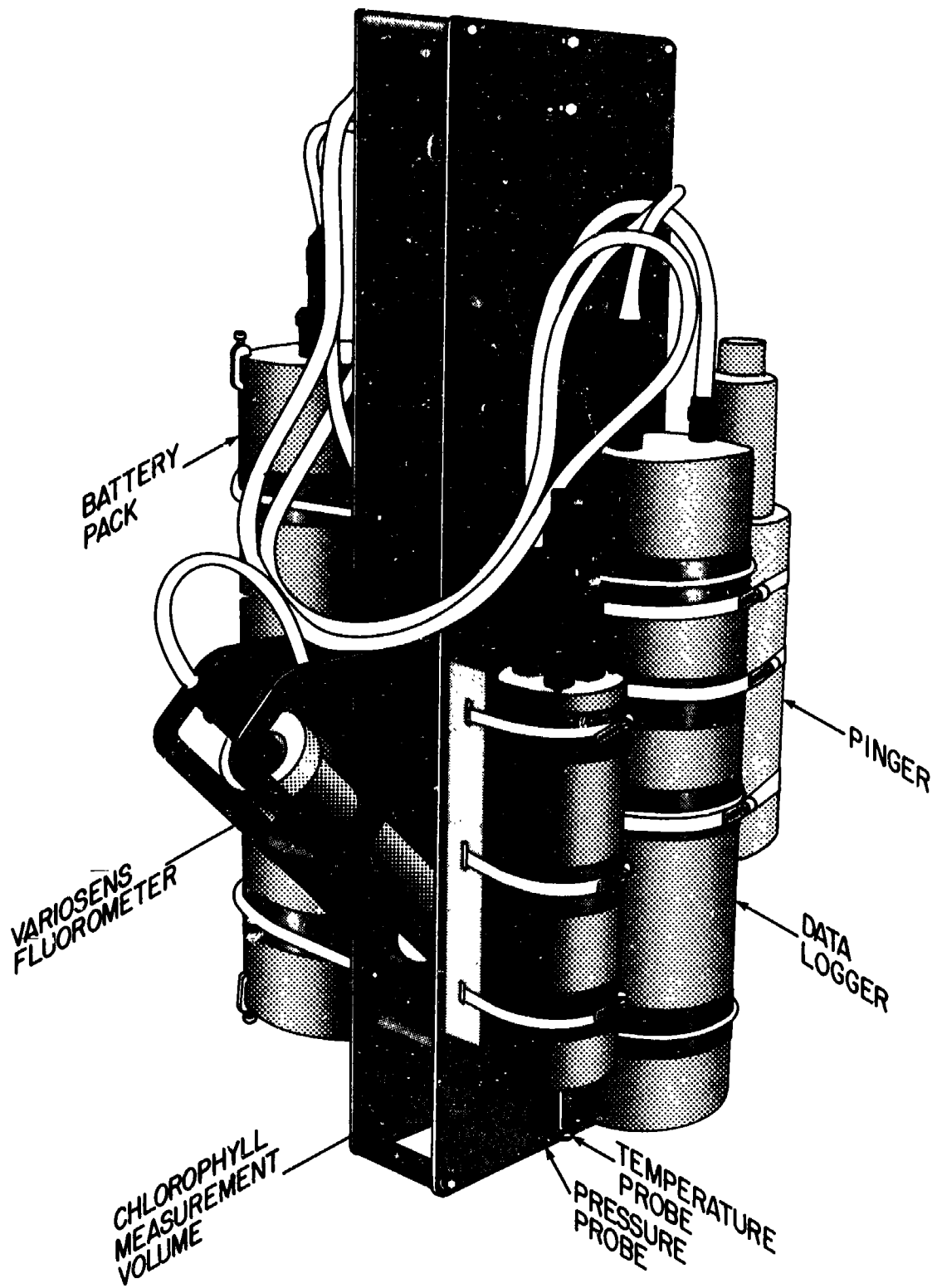
Fig. 5: Moored sensor package employing a commercial Variosens fluorometer measuring chlorophyll a (from Mitchell, 1991).

(Fig. 6). Examples of short time-series is shown in Figure 7. Both research groups reported the same problem in that the vertical structure was undersampled. Placement of the moored fluorometer near the pycnocline or the chlorophyll maximum (often spatially synonymous) can exhibit high variability not associated with chlorophyll changes but rather with internal wave activity or tidal forcing. Ideally 5-10 m sensor separation in the upper 40-50 m in coastal waters would adequately define long-term changes in chlorophyll structure.

What future technological changes resulting in substantial reduction in size and cost would enable the placement of, perhaps, 10 fluorometers on a mooring line? Are there any other innovative technologies with potential for overcoming this problem? Fiber-optic technology promises such innovative potential. Recent work (Snow et al., 1986) in fiber-optic fluorometry has resulted in the development of fluorometers with remote sensor head coupled to the transmitter/receiver by means of a fiber-optic link. One goal of this area of research is to extend the capability of the fiber-optic fluorometer to multi-depth capability as shown in the proposed configuration of Figure 8. Here we see one central server unit consisting of a transmitter/receiver, data logger, and battery supply servicing a fiber-optic bundle of ≈50 m length with 'breakout' sensors every 5 m. The server would optically interrogate each sensor alternately and log the measured chlorophyll fluorescence at each depth.

The major drawback which persists with existing fluorometer technology is the source of light excitation which typically employs xenon flash tube technology. These light sources emit in the UV/blue region but require high voltage/large current pulse to activate discharge which also results in the emission of RF noise usually in the vicinity of high sensitivity detection circuitry. Consequently, there is a certain minimum level of complex circuitry which must be built to support the xenon discharge method and this finally limits the size and cost of a fluorometer. We must therefore look to alternate light sources for a redesign. Clearly the blue (Argon) laser represents a step up in cost and complexity. However the light-emitting diode (LED) is inexpensive, simple to activate and capable of emission at many wavelengths from blue to red/IR. The properties of the LED do not compare well to those of the laser since the LED is non-coherent for diffractive applications and non-parallel. However for fluorometric applications, we need to concern ourselves only with luminous intensity sufficient to excite chlorophyll a. The blue LED constructed of silicon-carbide emits at 470 nm or in the correct waveband to excite chlorophyll a but only possesses a luminous intensity of ≈20 mcd which is 100x lower than necessary for excitation of typical chlorophyll concentrations. On the positive side, this intensity represents an increase in intensity of 7x in the last 6-12 months by the industry and we only have to look at the parallel development of other LEDs. The red LED, constructed of aluminum-gallium-arsenide (AlGaAs) and emitting in the region of 626-640 nm, has now achieved a luminous intensity of 1000-5000 mcd equivalent to a powerful He-Ne laser. This represented an increase of 100x in a period 3-4 years during the mid-80's.

The introduction of the blue LED with sufficient luminous intensity to fluorometric applications would simplify the optical/electronic construction of fluorometers and result in miniaturization. Projecting this further, we could then expect to construct and deploy moorings with multi-depth capability for chlorophyll a measurements, such as that depicted in Figure 8, where we substitute a miniature LED sensor for breakout and an electrical cable in place of the fiber-optic

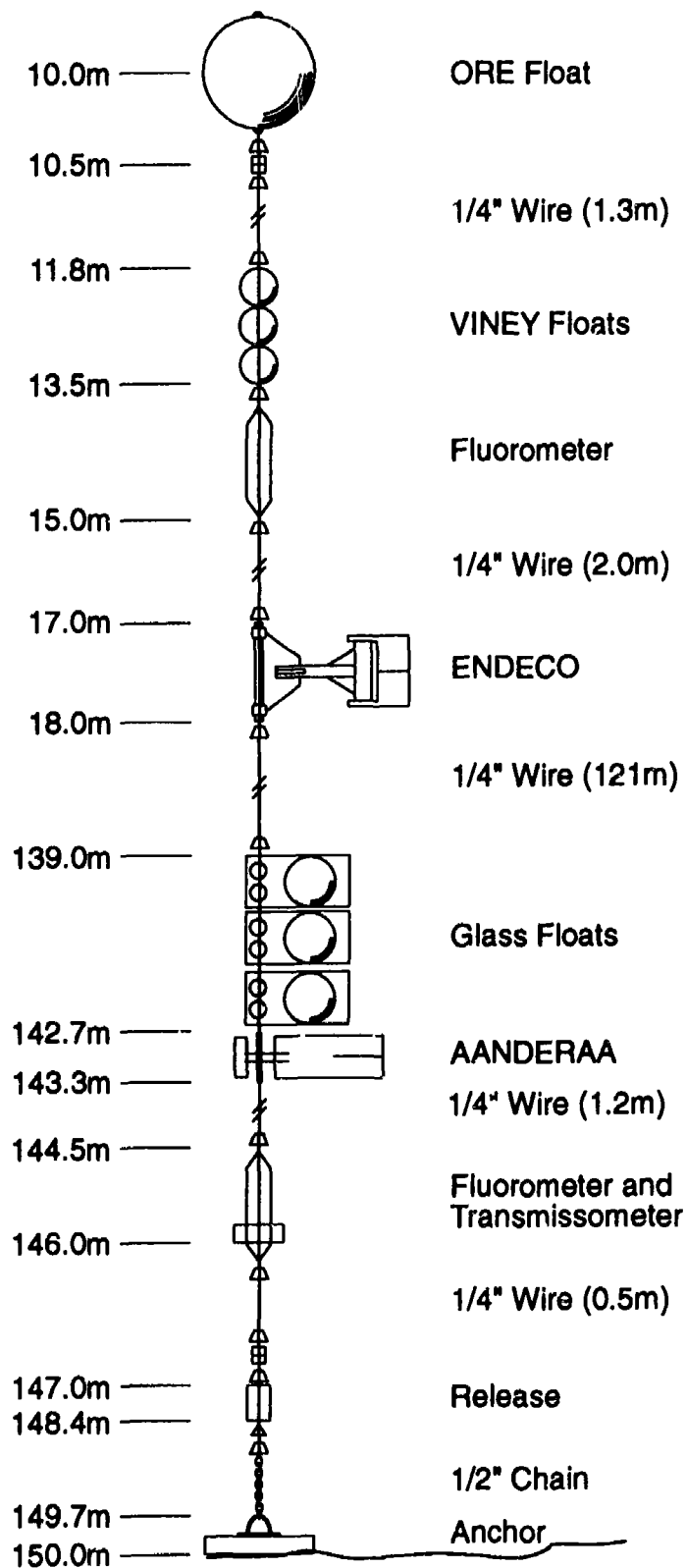| Depth	Component
10.0m	ORE Float
10.5m	1/4" Wire (1.3m)
11.8m	VINEY Floats
13.5m	Fluorometer
15.0m	1/4" Wire (2.0m)
17.0m	ENDECO
18.0m	1/4" Wire (121m)
139.0m	Glass Floats
142.7m	AANDERAA
143.3m	1/4" Wire (1.2m)
144.5m	Fluorometer and Transmissometer
146.0m	1/4" Wire (0.5m)
147.0m	Release
148.4m	1/2" Chain
149.7m	Anchor
150.0m	

Fig. 6: Typical array of Shelf Edge Exchange Processes (SEEP) mooring that contains two fluorometers, one transmissometer, one ENDECO current meter and one Aanderras current meter (from Whitledge and Winwick, 1986).

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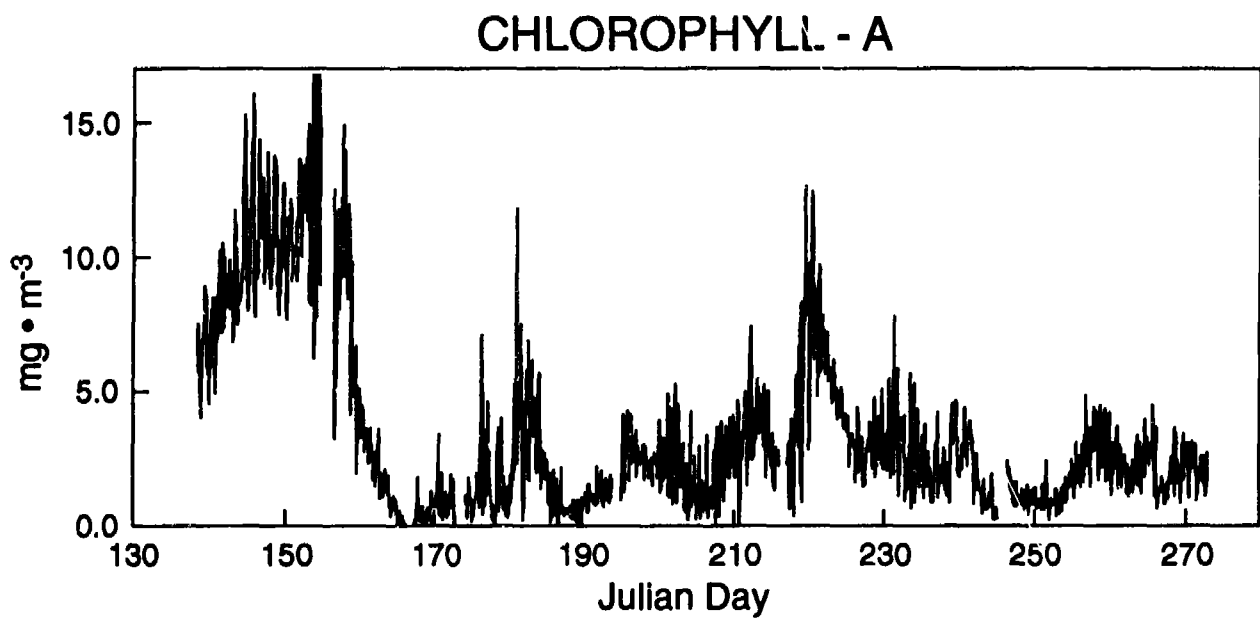
CHLOROPHYLL - A

Fig. 7: A 3-month time-series of chlorophyll *a* sampled with a moored Variosens fluorometer (from Mitchell, 1991).

cable. Each sensor would contain its own LED transmitter/receiver and would be interrogated by the central server which would measure and store data from each sensor.

'Pump-and-Probe' Fluorescence

The laboratory measurement of chlorophyll *a* consisting of an acetone extraction method (Yentsch and Menzel, 1963) was replaced by the technological emergence of *in situ* fluorometric measurements. These developments evolved from the need to have synoptic measurements of phytoplankton biomass. A similar situation exists with the measurement of phytoplankton growth rates presently restricted to the laboratory; however, a new innovative and powerful technique called 'pump-and-probe' fluorescence for measuring relative growth rates of algae has been developed in the last 5 years (Falkowski and Kiefer, 1985; Falkowski *et al.*, 1988; Kolber *et al.*, 1988; Kolber *et al.*, 1990) and has enormous potential for advancement both in terms of scientific measurement and technology.

The principle of the 'pump-and-probe' fluorescence measurement consists of saturating an algal sample with a high-intensity light source thereby inducing the photo-system II (PS II) reaction centers (the predominant source of *in vivo* chlorophyll *a* fluorescence) to transfer an electron from the reaction center to the primary electron acceptor, Q_A. Subsequently the increased capacity of PS II to fluoresce, i.e. its fluorescence yield F_s, is a measure of the capacity for photochemical energy conversion and is quantifiable by comparing it to the fluorescence yield F_0 prior to saturation. Thus the photosynthetic energy conversion can be calculated from the change in fluorescence yield:

$$\Delta \phi_{sat} = (F_s - F_0)/F_0$$

The technique consists of stimulating the sample with a weak 'probe' flash while measuring F_0, saturating the sample with an actinic 'pump' flash and at some time later, stimulating the sample again with a weak 'probe' flash measuring F_s. F_s is measured with a 70 μ s delay following the 'pump' flash. This delay is long enough for the fluorescence quenchers to have decayed, yet short enough for PS II electron transfer to become stabilized.

Figure 9 (a-c) of Kolber *et al.* illustrates the response of ϕ_{sat} to nutrient limitation. Figure 9(a) shows the effects of growth irradiance levels on ϕ_{sat} in dark adapted, nutrient-replete cells (4 species) indicating a near-constant relationship. Figure 9(b) shows ϕ_{sat} decreasing for increased nitrogen-limited cells (3 species). Figure 9(c) shows the fitted relationship between ϕ_{sat} and the maximal growth rate (μ/μ_{max}) indicating that ϕ_{sat} can be used to estimate the relative growth rates of algae.

The 'pump-and-probe' fluorescence has considerable potential in the next decade both from the scientific information yet to be extracted and from its potential for technological advancement. First, the *in situ* system can benefit from the future development of new light sources as in the case described for the mocred fluorometer resulting in miniaturization and low-cost. Second, the system has potential as a powerful laboratory tool by integration with dye lasers which are tunable over a range of wavelengths. By stimulating the 'pump-and-probe'

BIOLOGICAL MOORING
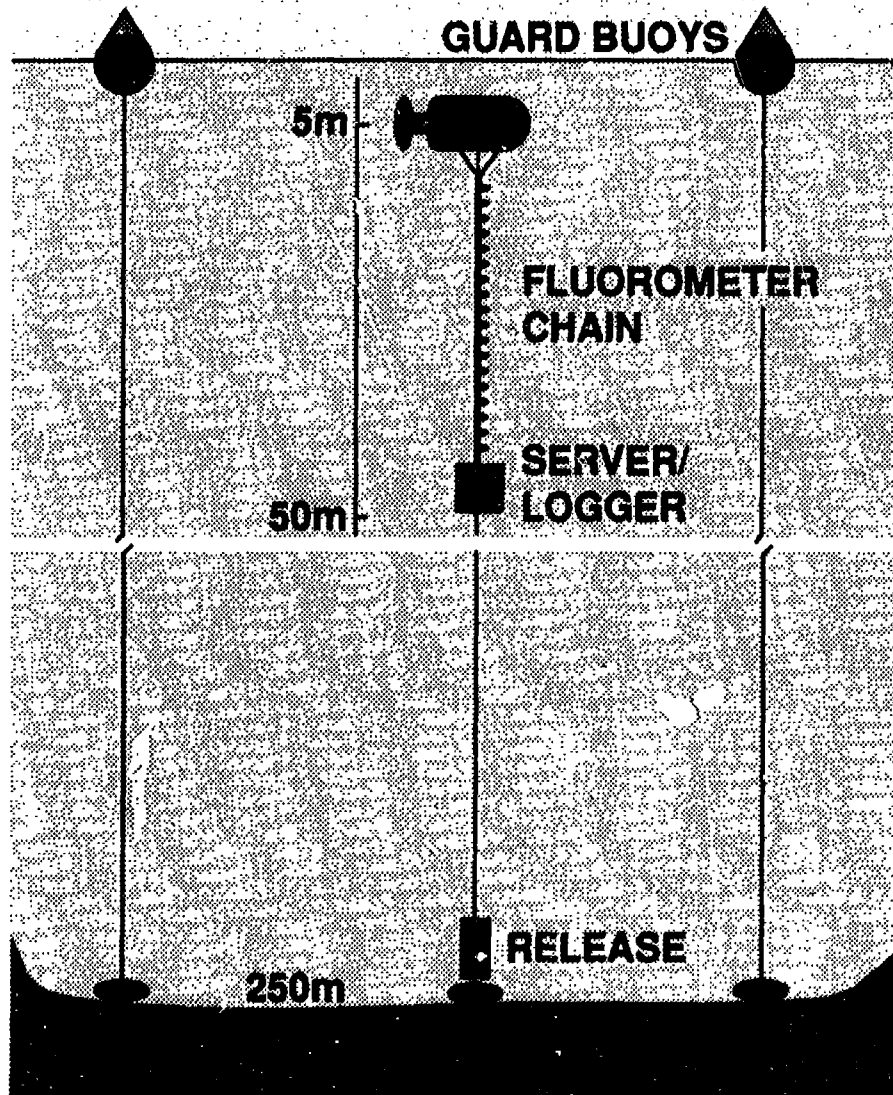
Fig. 8: Future concepts in moored fluorometers - a fiber-optic bundle with small breakout sensors could be used to measure chlorophyll a. A server/controller would be used to interrogate each sensor in turn.
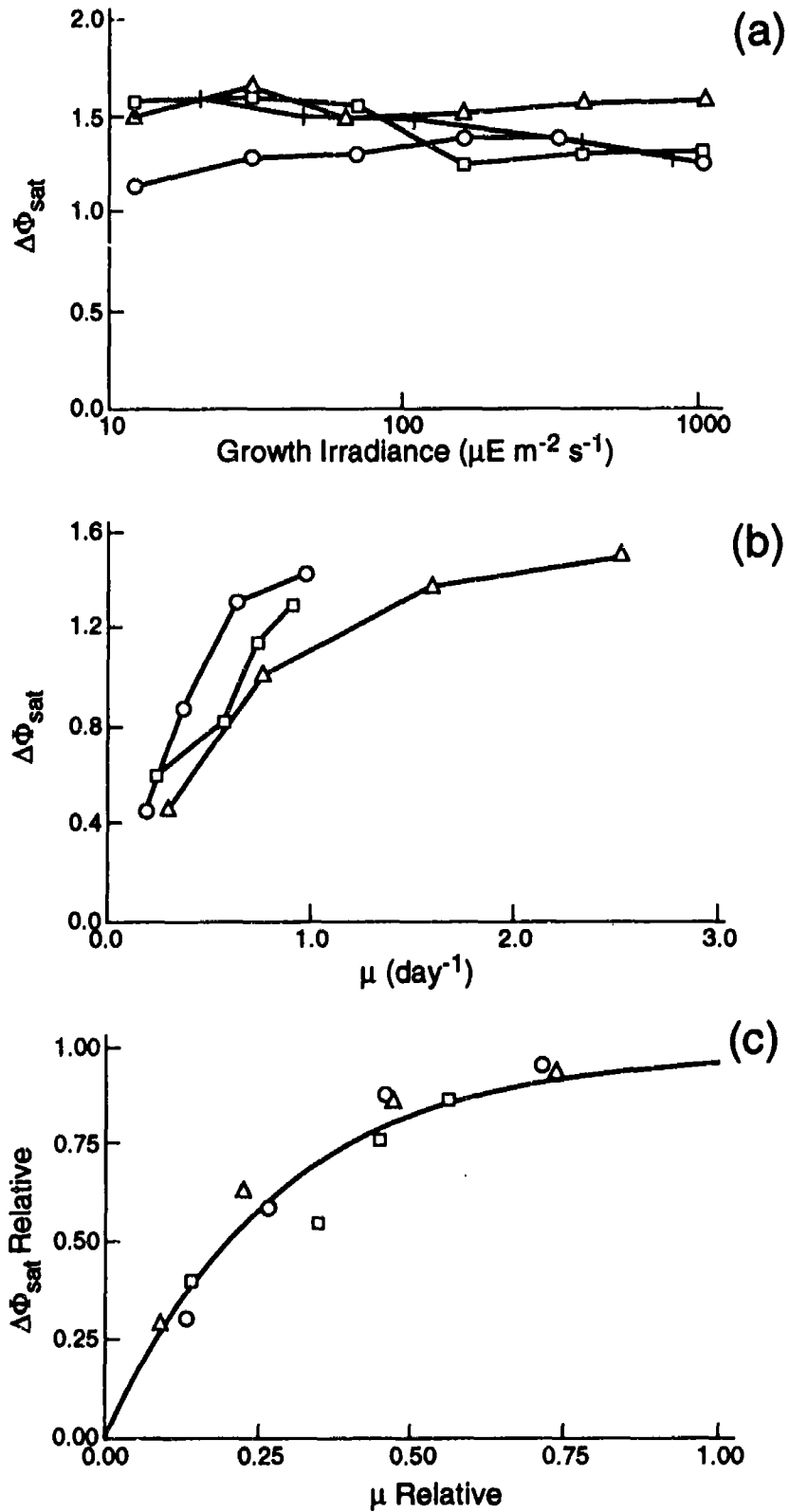
Fig. 9: The 'pump and probe' fluorescence method and an illustration of the response of fluorescence yield to nutrient limitation indicating how the parameter ϕ_{sat} can be used to estimate the relative growth rates of algae (from Kolber et al., 1990).

excitation over a range of wavelengths, one could begin to isolate the relative growth rates of different pigments and therefore individual species of a mixed population.

Flow Cytometer

Flow cytometer refers to the rapid measurement of particles in a moving fluid. By combining the measurements of various optical properties of particles, one can routinely obtain information on particles in the 1-150 μm size range. Using arc-lamp and laser-based flow cytometry, subpopulations of phytoplankton cultures and natural populations can be quantified by measuring pigment autofluorescence, stain-induced fluorescence and light scattering. This technique, when combined with biochemical selective stains and immuno-fluorescence technologies allows simultaneous and rapid measurement of chlorophyll, protein, DNA, forward and 90° scattering on individual cells. Development of these instruments originated between 1960-80 while most of the high technology advances took place in the 80's (Chisholm *et al.*, 1986; Li, 1986; Olson *et al.*, 1991; Yentsch *et al.*, 1986). While the technology has progressed in that time, the basic operating principle is shown in Figure 10. A sample moving through a narrow column is excited by a laser of a given wavelength and fluoresces at various wavelengths. This information coupled with the intensity of fluorescence yields information regarding the type of pigment in the cell. The second property that is measured is the forward light (Rayleigh) scatter which yields information on size and finally the 90° light scatter (not shown) which yields the refractive index and shape information. Figure 11 shows an example of an application of flow cytometry to grazing identification. Oysters were fed a mixture of a diatom, dinoflagellate and a cryptophyte. The 'before and after' experiment indicates selective feeding of dinoflagellates (Exuv) only. Historically the technique cannot perform elementary taxonomic identification, for example, it cannot distinguish between diatoms and dinoflagellates. Presently it can discriminate groups consisting of heterotrophic bacteria, cyanobacteria, cryptomonads, prochlorophytes and coccolithophores, eukaryotic phytoplankters and can extract morphological features of cells using pulse and data analyses techniques.

An integral component of the technology of flow cytometry is the equally powerful technique of cell-sorting. It is based on a simple premise: if one can identify and characterize certain cells by their fluorescence signals then some standard technique can be applied to separate these cells simultaneously. Figure 10 illustrates the methodology used. Once the fluorescence/scattering signals are processed and identified, a charge is applied to a precisely positioned droplet at the time of arrival at the collar using a piezoelectric oscillator (Chisholm et al, 1986). The charged droplets containing the desired particles can be electrostatically separated into the collection vessel. Cell-sorting has added a new dimension to the field of flow cytometry in that one can study the physiology of different strains and monitor the growth rates or specific C14 uptake of specific algal subgroups. The applications emerging from flow cytometry/cell-counting studies has been summarized by Olson *et al.*, 1991 (Fig. 12) showing the branching of physical and ecological information yield. In addition to growth studies of specific samples, rapid onboard analyses enables multi-sample processing and therefore water column studies.

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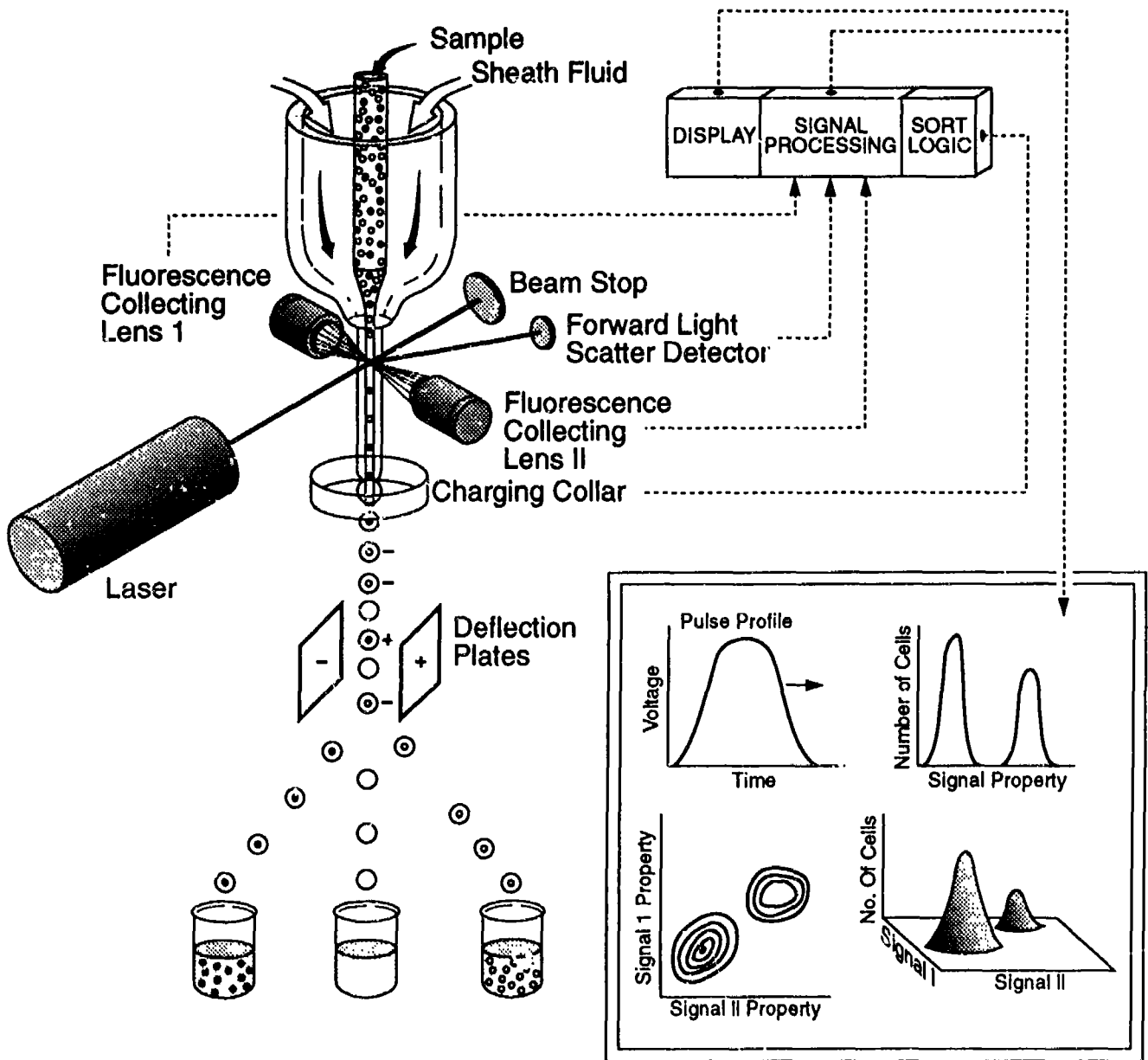
Fig. 10: The basic operating principles of the flow cytometer. A sample moving through a narrow column is excited by a laser of a given wavelength and fluoresces at various wavelengths yielding information regarding the type of pigment in the cell (from Olson *et al.*, 1991).

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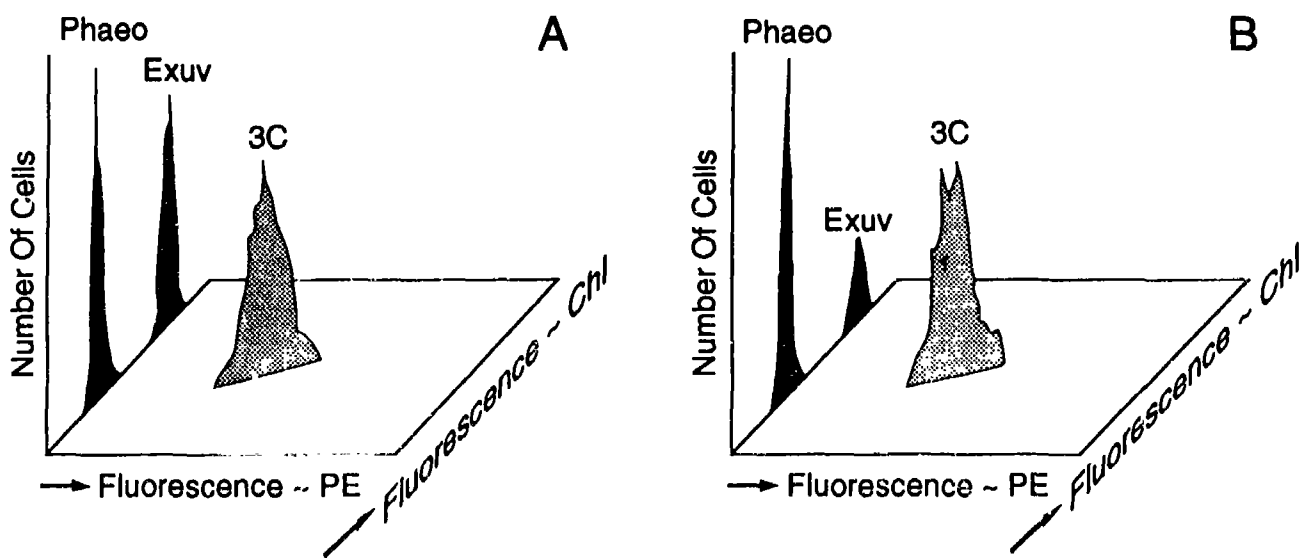
Fig. 11: An application of flow cytometry to grazing identification indicating the 'before and after' selective feeding of oysters on dinoflagellates (Exuv) only (from Shumway et al., 1985).

Fig. 12: The applications emerging from flow cytometry/cell-counting studies showing the branching of physical and ecological information yield (from Olson *et al.*, 1991)

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Future applications that are emerging in the next decade are techniques (Olson et al, 1990) for identifying algal species rather than just subgroups. Since most bacteria have no autofluorescent pigments, their characterization has been accomplished using light scattering and fluorescent DNA stain DAPI. Fluorescently labelled antibodies can be used to identify and enumerate specific organisms or even specific molecules within cells. The noteworthy example is shown in Figure 13 demonstrating the application of the technique in identifying a single species of bacteria, N. oceanus (Ward, 1990). The potential for flow cytometric detection and identification of individual bacteria labelled with rRNA probes has been recently demonstrated (Amann et al., 1990). Other examples of immunofluorescent identification are the work of Campbell (1988) and Campbell et al. (1989) on marine picoplankton and Shapiro et al. (1989) on eukaryotic phytoplankton.

In the area of technology advancement one has only to examine the evolving application of light sources. Olson et al (1988) developed a new application consisting of exciting a sample using a dual beam consisting of blue and green light (488 and 515 nm) from an argon ion laser. By measuring the two sets of fluorescence signals, their ratio yields the relative absorption properties of the cell's fluorescent pigment enabling the identification of strains of Synechococcus. Such technology is evolving towards multi-wavelength excitation enhancing identification capability of algal strains. Such light sources are dye lasers which can be pumped by laser sources, both pulsed and continuous wave, offering the possibilities of broad wavelength control. The tuning range of dye lasers is 320-970 nm sufficient to provide broad range excitation in the UV/blue bands.

Another application of the flow cytometer presently evolving is that of time-of-flight analysis (Olson et al., 1990; Dubelaar et al., 1989; Premazzi et al., 1989; Cunningham et al., 1990). Presently the fluorescence signal of a cell 'in flight' through the excitation light beam is analyzed only for its intensity. The time-of-flight of a cell and the shape of the signal can be analyzed and yield information on the size and possibly the shape of cell. When coupled with the light scatter measurements, 'time-of-flight' analyses can enhance the physical description of fluorescent cells.

Ultimately what is required here of flow cytometry is the determination of biological oceanographic parameters characteristic of cell species. Once identified, individual species can be characterized in the water column by such parameters as its growth rate, photoadaptation, cell division, nutrient limitation, preference and rate at which it is grazed, and perhaps direct measurements of C:N or C:Chlorophyll ratios.

Remote Sensing

Airborne LIDAR:
The airborne oceanographic LIDAR (light detection and ranging) is a fluorosensor utilizing a pulsed laser (Nd:Yag) to excite surface waters and colour sensors to receive fluoresced light at 685 nm (chlorophyll), 580 nm (phycoerytherin) and 645nm (Raman scattering) from about 2-3 m depth. Aircraft remote sensing provides an excellent sampling platform between ships and

Fig. 13: The application of flow cytometry to the identification of the single species of bacterium, *N. Oceanus* (from Ward, 1990).

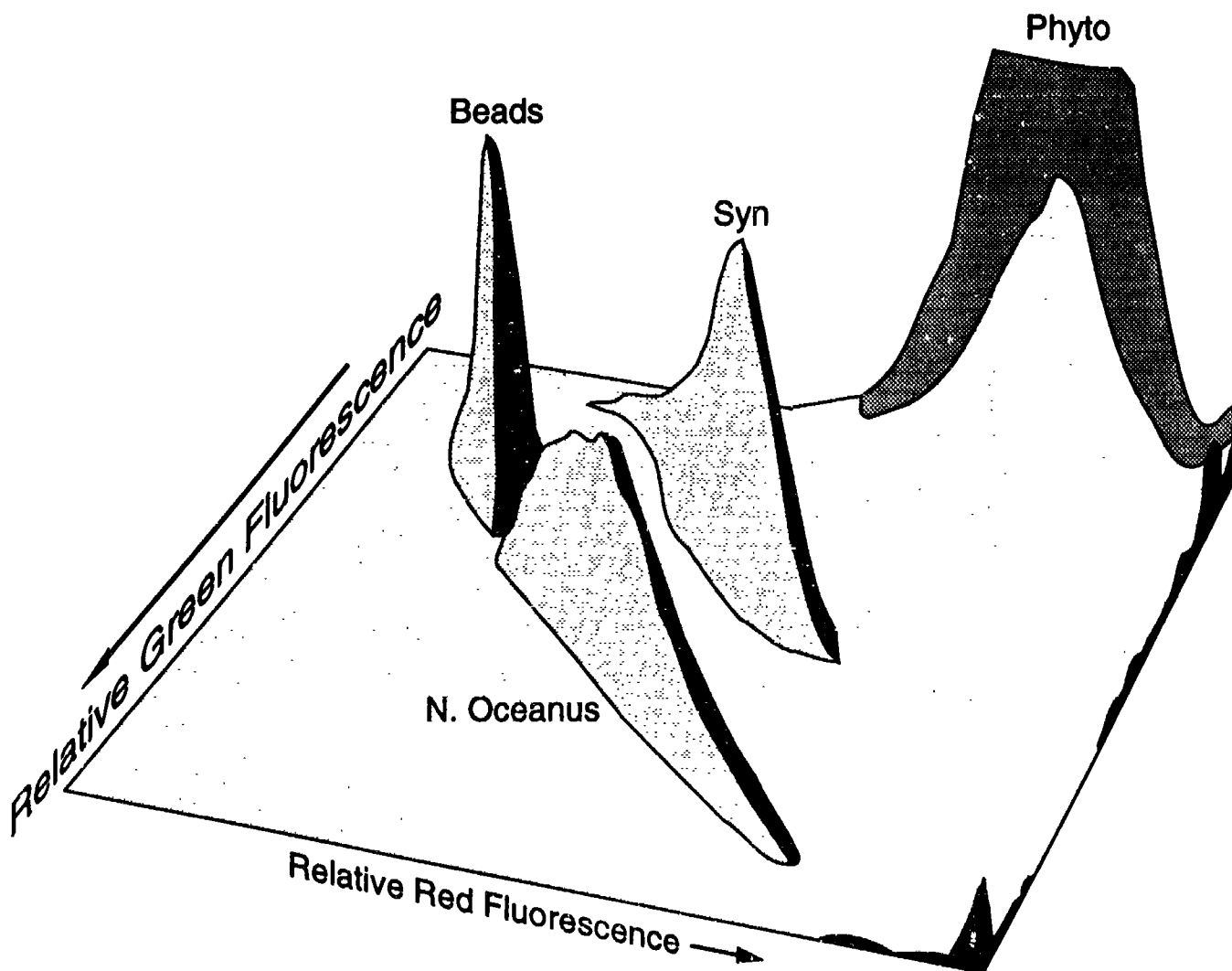satellites by providing synopticity needed to identify mesoscale features. Aircraft costs per information yield are seen to be a limiting factor in its operation.

Satellites:

Strictly speaking, remote sensing from a satellite platform is not a fluorescent but a colour measurement and perhaps might better fall under the category of 'Particle Measurements'. However, it does provide us with measurements of chlorophyll concentrations which are inseparably linked to fluorescence measurements used in their ground-truthing and so, for the sake of topical uniformity, we ask for some licence here.

The first satellite-borne ocean-colour sensor was the Coastal Zone Colour Scanner (CZCS) which was launched aboard the Nimbus-7 satellite in 1978 and operated successfully for 8 years. Interestingly enough, the CZCS was designed only to verify 'proof of concept' and had a design lifetime of only one year. The principle on which the CZCS was based arose from earlier shipboard and aircraft studies which showed that the radiance reflected from the ocean in the visible wavelength region (400-700 nm) is related to the concentration of chlorophyll and other plant pigments present. Since chlorophyll is a green pigment and water colour changes from blue to green as chlorophyll concentration increases, we can characterize such relative differences in terms of chlorophyll concentration. Figure 14 from Hovis (1981) shows the radiance spectra in the visible region for four types of seawater in which chlorophyll concentration ranges from 0.09 to 60.40 mg.m^{-3} and increases by about an order of magnitude for each of the four water types. In the blue spectral region, radiance decreases by a factor of 1000x with increasing chlorophyll while in the green spectral region, radiance increases by a factor of only 7x. Such ranges in chlorophyll occurred from deep ocean to coastal conditions where we see the dramatic increase in absorption of blue light and the resulting increase in the green surface hue caused by chlorophyll.

Once such colour data became accessible in the oceanographic community, there emerged during the 80's a vast number of sophisticated and powerful algorithms and applications characterizing chlorophyll concentrations in our surface oceans (see reference list: Putnam, 1987). Recently the applications were extended to powerful algorithms describing surface primary productivity in our oceans (Platt and Sathyendranath, 1988; Platt *et al.*, 1988; Sathyendranath and Platt, 1988).

Since the CZCS instrumentation ceased transmission in 1986, the oceanographic community has been without ocean colour information. However the 90's will see a more powerful sensor launched called the SeaWiFS (Sea-viewing, Wide-Field-of-View Sensor) containing a larger number of spectral bands and a wider scan angle for daily overlap coverage. The recommended visible and near-infrared bands (minimum) for the baseline SeaWiFS instrument is shown in Table 1 as outlined by the Joint EOSAT/NASA SeaWiFS Working Group and more recently the EOSAT/SBRC and NASA Working Group. Bands 1 & 3 will replicate the absorption maxima and minima of chlorophyll as before. Band 2 at 500 nm is nearly centered between the maximum and minimum absorption bands and can be used to estimate pigment concentration so large that radiance signals measured from band 1 is not useable. Band 5 at 765 nm is in the near-infrared region enabling atmospheric corrections.

CZCS Bands

Band 1 Band 2 Band 3 Band 4

Upwelled Spectral Radiance
(μW • cm^{-2} • srad^{-1} • nm^{-1})

Chlorophyll		CZCS Ratios	
Symbol	mg•m-3	1/3	2/3
— — —	0.09	2.67	2.00
··········	0.86	0.46	1.08
·—·—·—	7.66	0.05	0.50
——	60.40	0.04	0.36
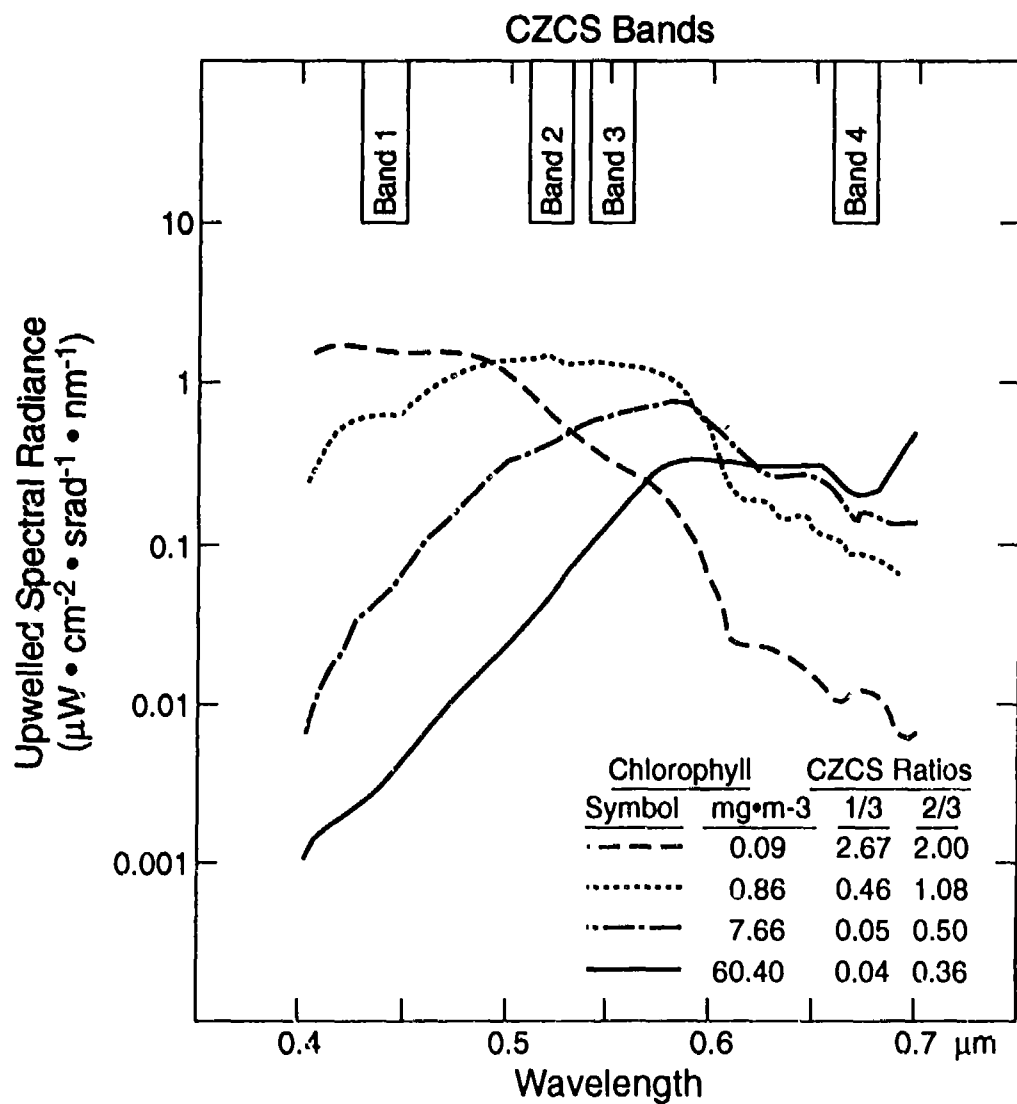
Wavelength

Fig. 14: Spectra for seawater with various concentrations of chlorophyll (from Hovis, 1980).

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Table 1   The radiometric performance of the SeaWiFS satellite illustrating its various wavelengths and their measurement capabilities.

Table 1 SeaWiFS Radiometric Performance EOSAT/SBRC NASA			
BAND	WAVE-LENGTH (nm)	PHENOMENON	USE
1	412	Gelbstoffe	Measuring strong blue absorption by gelbstoffe or yellow substance.
2	443	Chlorophyll	Used with the 565 nm band for determining colour boundaries, chl. conc. and diffuse attenuation coefficient (k).
3	490	Pigment absorption	Used with the 565 nm band for mapping colour and chlorophyll concentration in coastal waters.
4	520	Pigment absorption	Similar use as 490 nm; would permit better comparisons to be made between CZCS and SeaWiFS.
5	565	Sediment/ hinge point	Used in the medium range for determining chlorophyll, pigments, water optical properties (k), and measuring suspended sediments.
6	665	Atmospheric aerosols	Used to correct above bands for atmospheric effects.
7	765	Atmospheric aerosols	Used to correct above bands (except 6) for atmospheric aerosols - different sensitivity.
8	865	Atmospheric aerosols	Used for atmospheric-correction algorithms for spatial variations in the aerosol radiance ratios from bands 6 & 7.

The SeaWiFS application is a classic example of 'science driving the technology'. Much of the driving force of the SeaWiFS program stems from research studies of the global carbon cycle as in the case of the GOFS and International JGOFS programs and the recognition that the atmosphere and oceans are coupled. The 'driving science' begins with accumulating data indicating the steady increase in carbon dioxide content associated with the burning of fossil fuels. This in turn has led to atmospheric model predictions of gradual warming of the Earth's climate. The pathways and rates of removal of carbon dioxide from the atmosphere have not been accurately quantified but it is estimated that approximately an equal amount of carbon dioxide released from burning fossil fuels has accumulated both in the atmosphere and in our oceans. Quantifying the rate at which our oceans act as a 'sink' for carbon is based on the role of phytoplankton, its rate of uptake of dissolved organic carbon, recycling of organic carbon and the sedimentation flux of organic and inorganic material. The clearly-defined goal of providing global estimates of primary production in the 1990s has clearly driven the development of powerful tools such as the SeaWiFS.

PARTICLE MEASUREMENT

Coulter Counter

The Coulter counter (Coulter, 1957) is considered a reference standard in the measurement of particle size distributions in the size range $\approx$1-200 μ. The technique is based on the conductivity measurement as a particle passes through an aperture immersed in an electrolyte solution. New versions of Coulter counters have upgraded their state-of-the-art technology and have achieved a lower detection limit of $\approx$0.5 μ or about 4x lower than the earlier generation. No new technological changes in this type of conductivity measurement are anticipated; however, it is seen to continue as a reference standard and as an integral component of flow cytometry measurements.

Light Attenuance (Transmissometry)/Optical Backscattering

Light attenuance is a measurement of the amount of light reaching a sensor in the forward direction and is representative of light remaining as a result of losses due to scattering and absorption. Optical backscattering (OBS) measures that component of light which is scattered in the backward direction. Both are non-diffractive methods using conventional light sources and measure bulk concentration of suspended solids. Transmissometry responds best in the concentration range of $\approx$10^{-1} to 10^3 mg/l while optical backscattering measures a larger range from $\approx$10^{-1} to 10^5 mg/l. Both instruments are highly practical due to their *in situ* profiling capability but require 'ground-truthing' typically by conventional Coulter counters.

Light attenuance measurements can be used in certain instances to estimate phytoplankton and are required in modelling algorithms for remote sensing of chlorophyll (Yentsch and Phinney, 1982; Phinney and Yentsch, 1986). Some noteworthy applications are evolving also as a result of the integration of light attenuance and flow cytometry measurements (Olson *et al., 1990*). Light attenuance is known to increase during the day and decrease during the night,

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Figure 15(b), and this pattern is well-correlated with primary production (Siegel *et al.*, 1989). Figure 15 (a) shows the relative change in sizes. Since light attenuance measurements at sea can be made easily and rapidly, its potential usefulness in this context is enormous, particularly as an integral component of flow cytometry at sea.

Modest advances are seen for the integration of future high-power blue LEDs into transmissometers enabling measurements of blue-green absorption ratios analogous to colour measurement ratios in remote sensing (Yentsch and Phinney, 1982).

Flow Cytometry (Forward Scattering)

Forward light scattering is a diffractive method (laser-based) employing Fraunhofer diffraction or Mie scattering and is based on the principle that particles of a given size diffract light through a given angle, the angle increasing with decreasing size. By detecting the angular distribution of forward scattered light energy, the size distribution and concentration of suspended particulate matter can be measured. Most instruments using the forward scattering measurement are laboratory versions with minimum detection capability of ≈ 0.5 µ while *in situ* versions have been developed and employed as profilers, e.g., MALVERN (Bale and Morris, 1987; McCave *et al.*, 1986), with lower detection limits of ≈ 1 µ. The technique is most effective in measuring single-sized species group but is less effective with mixed populations.

The flow cytometer eliminates the problem of mixed populations by reducing the sampling mode from a 'bulk' measurement to that of measuring single cells. Hence flow cytometry measures forward scattered light of each cell which yields size information per individual. The potential of this powerful technique is illustrated by the measurement of volume distributions of a bacterial culture in Figure 16 (from Robertson and Button, 1989). The distributions were compared to Coulter counter measurements from which the standard deviations were derived while the correlation coefficient of 0.94 indicated that forward scatter correlated well with cell volume. We see from Figure 16 that the forward scatter technique in flow cytometry is capable of resolving bacteria as low as ≈ 0.5 µ diameter in marine and freshwater systems.

Particles: Zooplankton

The following discussion focuses on zooplankton as particles in a specific size range, that is, >100 µ encompassing mostly copepods and euphausiids. Discussed here will be the three dominant measurement techniques emerging in the next decade, that is, acoustics, optical counting/sizing and video imaging. These techniques have been rapidly developing in the 80's as independent measurement techniques but will be integrated during the 90's in an international oceanographic community program called GLOBEC in order to intercalibrate and resolve existing measurement inconsistencies and to evolve and develop new mooring technologies capable of long-term measurements.

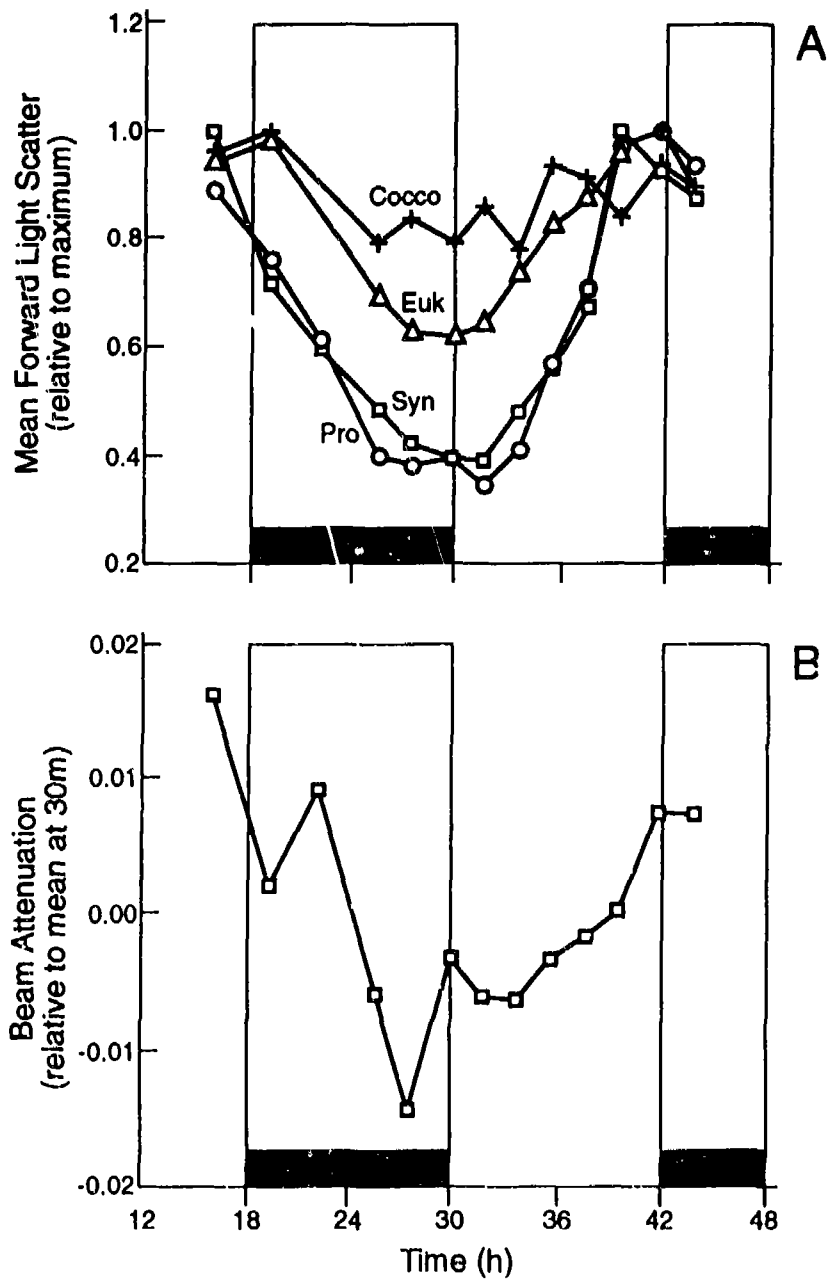Fig. 15: Light attenuance showing day/night correlation with primary production illustrating a promising application of integrating the two methods (from Olson *et al.*, 1990)
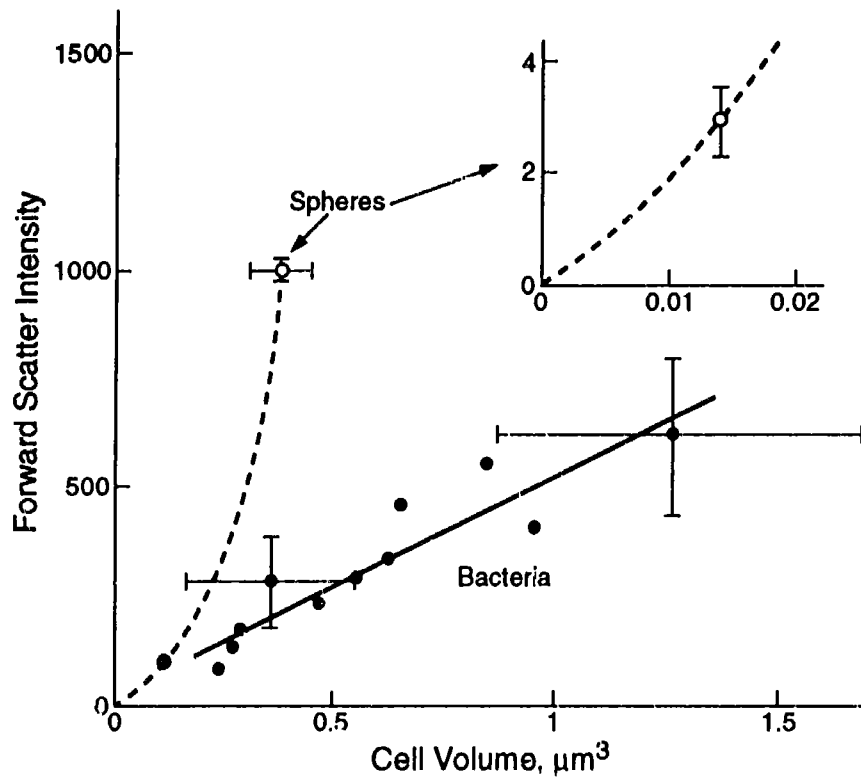
Fig. 16: Resolving bacteria with ≈0.5 μm diameter using the forward scattering of flow cytometry (from Robertson and Button, 1989).

The primary objective of bioacoustical oceanography has been to provide size and abundance information on zooplankton and micronekton. From the mid-60's to 1980 most studies focused on simple analyses of acoustical backscattering from a volume of water containing many animals. Measurements of volume backscattering (VBS) could be correlated with biomass and thus echo integration techniques emerged (Peiper, R.R, 1979) and provided nearly synoptic, high-resolution data on zooplankton biomass distributions. However the acoustical technique could not provide zooplankton sizes and so, during the 80's, two mathematical techniques were developed providing zooplankton size information from VBS data. These were the inversion technique using multiple-frequency volume backscattering data (e.g. Holliday and Pieper, 1980; Pieper and Holliday, 1984) and a deconvolution technique using simple, single-frequency backscattering data (Stanton and Clay, 1986). Each technique, however, derives its information from VBS data by assuming a particular backscattering model.

Our evidence to date shows us that while bioacoustical techniques have been applied mostly to euphausiids (1-3 cm length), it is still difficult to assess both biomass and size distributions. Our fundamental problem is that the two methods of assessment, net samplers and acoustics, vastly disagree. Everson and Bond (1986) showed that acoustic biomass estimates of Antarctic krill (*Euphausia superba*) swarms were consistently higher than net sampler estimates while results from net samplers differed among day/night tows. Net avoidance as a result of visual cues were assessed to be the major cause of low estimates during day tows. Sameoto *et al.* (1990) estimated that BIONESS net sampler underestimated euphausiids (*M. norvegica*) by two orders of magnitude as compared to simultaneous acoustic measurements of Cochrane *et al. (1991)*. Considerable refinement of the net sampling technique was made (Sameoto *et al.*, 1991) by using a bright light to disorient euphausiids in the path of the net thus disengaging their avoidance mechanism. As a result, catch estimates increased by a factor of 50x or ≈4x lower than acoustic estimates. A comparison of such estimates with an optical plankton counter towed on a Batfish vehicle (Herman *et al.*, 1991) showed that net sampler estimates were only a factor of 2x lower.

Acoustical estimates for euphausiids appear higher on average than results obtained from other samplers. One reason is the number of backscatter models used which are themselves sensitive to their input parameters, for example, the mean horizontal 2-dimensional orientation. An example of such sensitivity is shown in results of Figure 17. A series of measurements made with an optical plankton counter towed on a Batfish vehicle were compared to simultaneous acoustical measurements at 200 kHz. Assuming a mean orientation of 30° suggested by camera studies of Kristensen and Dalen (1986), the backscattering model of Cochrane *et al.* (1991) yielded a target strength of -76 db which translates into 30 animals m-3 (mean length of 2.0 cm) or about 4x higher than optical counter estimates. A repeat calculation using a mean orientation of 0° shows a reduction in target strength of 4 db or about 4x in animal density illustrating the sensitivity of the model to animal orientation. The agreement at 0° orientation with the OPC (see below) is good, however, orientation at 0° is an unrealistic assumption since euphausiids 'appear' to be at larger orientation angles as seen from ROV-mounted videos (Sameoto *et al.*,1991). Also the orientation was seen to vary over diel
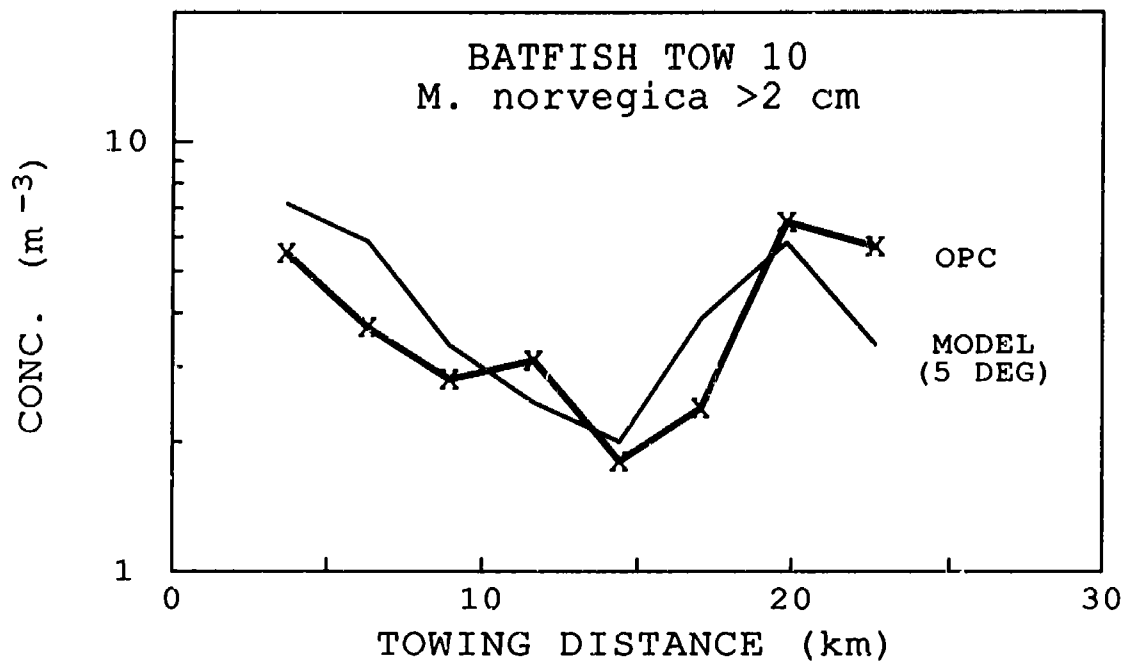
31

Fig. 17: A comparison of euphauslid concentrations using an optical plankton counter and acoustics (200 kHz). The backscattering model employed an input of animal orientation of 5°. The results indicate excellent agreement between acoustical and optical measurements when comparing abundance and general shape of curve over towed distance.

migration periods. Moreover, even measurement of the orientation angle is difficult to make since any sampler, particularly videos with lights, also appear to disturb these animals.

With the need for refinement in back scattering models, target strength estimates, size classification and abundance measurements for both copepods and euphausiids, two major acoustic tools are emerging in the 90's; the split-beam and the multi-frequency systems. The principle of the split-beam method is shown in Figure 18 where we see the difficulty in measuring the backscatter for animals of two different sizes dependent of their position in the beam. By using the split-beam of a narrow width of 3° and 10°, the target strength can be estimated accurately by calculating the ratios of the two signal strengths. Once estimated, the target strength can be combined with results from echo integration analyses of VBS while estimates of numerical density and biomass concentration can be a portion in different acoustical size classes. The split-beam system has a range of only several metres and must be mounted on a vehicle, such as a net sampler or ROV (Greene *et al.*, 1989; Greene and Weibe, 1990), for profiling. An example of a post-processed image of biomass and target strength as a function of depth is shown in Figure 19. Orientation effects will still be a problem.

Multiple-frequency methods have been pioneered over the last decade (Holliday *et al*, 1989) and are designed to provide size class and abundance estimates for a wide range of size groups from small copepods to large euphausiids. Operational acoustic frequencies range from 50 kHz to 4 MHz. Large euphausiids are best detected at a frequency $\approx$200 kHz while the associated sonar transducers possess a range of ensonification of 200-300 m suitable for towing at near-surface depths and for coastal studies. Small copepods are best detected at frequencies >1 MHz while the associated sonar transducer has a range of only 1-2 m and must be towed on a profiling system. Current technology for moored transducers has been developed for profiling currents (acoustic Doppler current profiler - ADCP) and has also been extended to mooring measurements of euphausiid layers (Pinkel, 1989). Plans are also underway within the oceanographic community to develop moored transducers of several selected frequencies tuned to measured specific size groups. However because of the high data rates associated with acoustical measurements, such emerging acoustical technology will have to be merged with the emerging high-speed microprocessors and 'flash' memory data loggers previously described.

Optical Plankton Counter

The current technology of optical plankton counters (OPC) represents the second generation of plankton counter development which was based on conductivity measurements (Maddux & Kanwisher, 1965; Boyd, 1969; Herman & Dauphinee, 1980). The conductivity-cell plankton counter measured the volume of copepods in a size range for sizes >350 μm to 3 mm and could identify copepods by dominant species and stages. However, due to the small size of the conductivity cell, seawater had to be filtered using a concentrating net which had to be oscillated to minimize algae clogging. Although providing valuable data for short periods, towing of the counter was limited to $\approx$3 hrs restricting our survey capabilities.
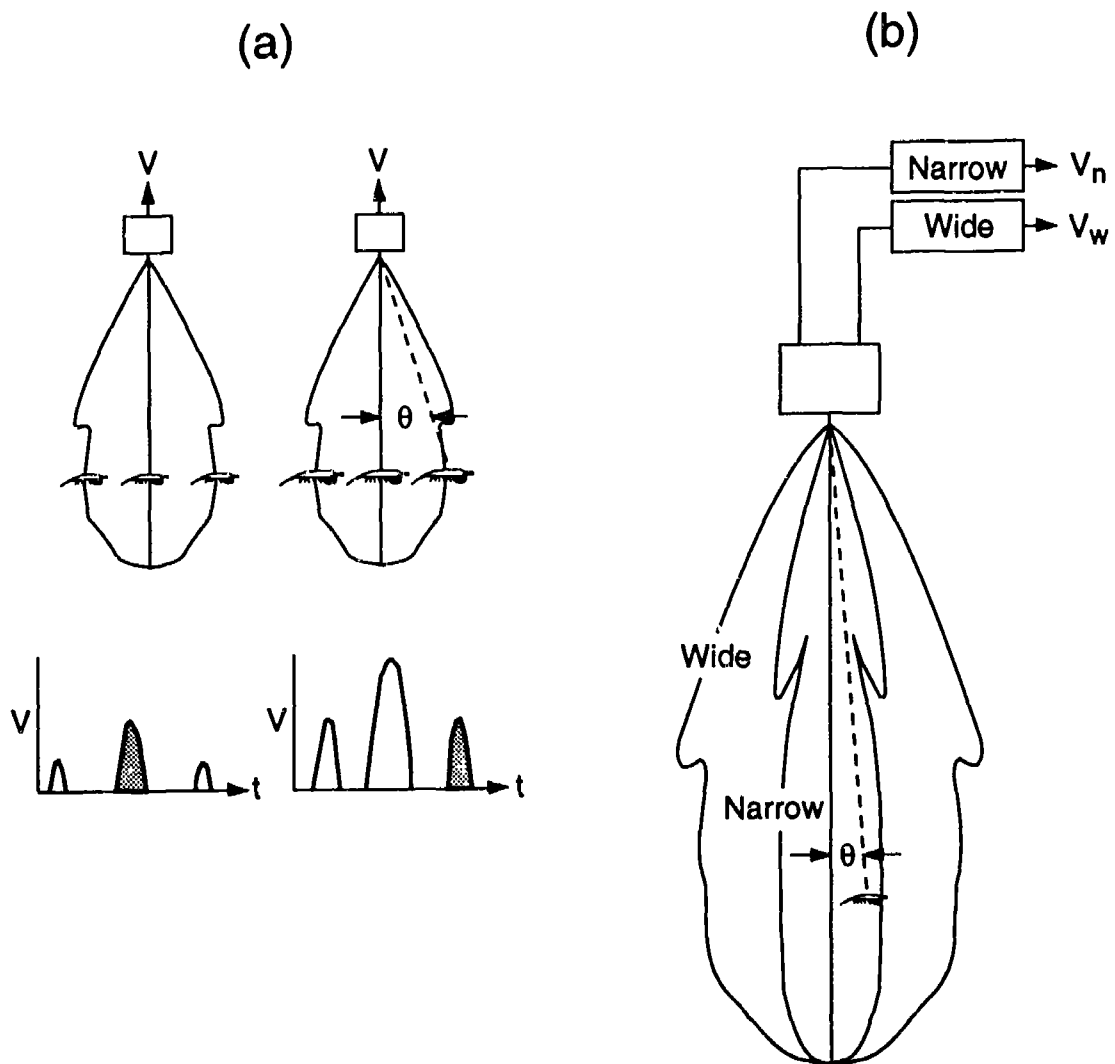
Fig. 18: The principle of the split-beam method used to measure the target strength of individual animals (from Greene *et al.*).
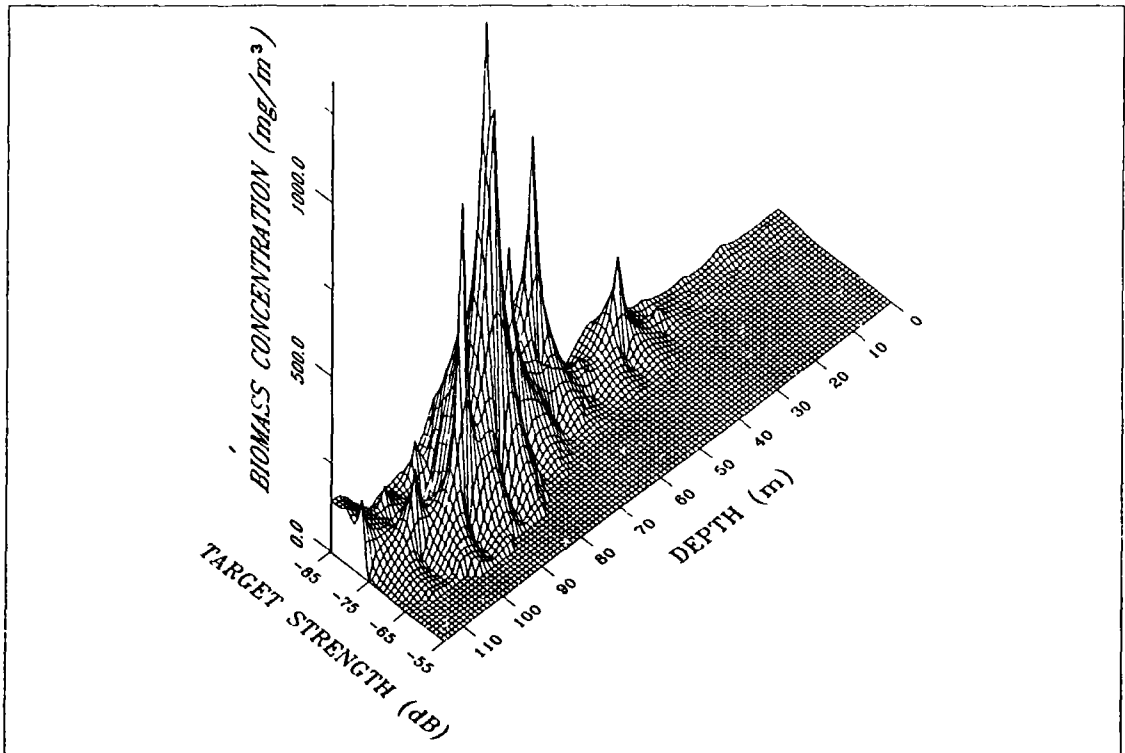
Fig. 19: 3-d image of biomass and target as a function of depth collected from a ROV (from Greene and Weibe, 1990).

The optical plankton coun*er (Herman, 1988; Herman, 1991; Herman, 1992) shown in Figure 20 could subtend a much larger sampling volume and therefore did not require concentrating nets nor mechanical mechanisms. Full synoptic coverage of zooplankton could now be obtained. An example of undulating profiles obtained from a Batfish tow across the width of the Scotian Shelf while equipped with an OPC is shown in Figure 21. The measurement principle is one of light occlusion using a LED light beam of 640 m. The beam has a Gaussian cross-section giving it higher sensitivity at lower copepod sizes where it is required. The minimum detection capability is $\approx$250 μm while the maximum detection limit of 2-3 cm allows for the detection and sizing of euphausiids. The OPC is capable of sizing copepods and providing abundance estimates at the dominant species and stage level. An example of size distributions and the ability of the OPC to discriminate peaks belonging to species and stages is shown in Figure 22 from a sample tow. By means of a feedback circuitry described in Herman (1988) it also measures light attenuance as a relative indicator of biomass in the upper layers simultaneously with copepod measurements.

The OPC is capable of discriminating species of euphausiids, that is, *Thysaneossa inermis* and *Meganyctiphanes norvegica* (Herman *et al*, 1991). This feature has allowed us to intercompare with net samplers and acoustical estimates. At present, as described in the previous section, results obtained for euphausiids abundances from net samplers and OPC sampling are converging while acoustical estimates remain higher.

In the next decade, OPC's will be adapted to current mooring technology to obtain long-needed time-series of coastal-water zooplankton in conjunction with studies of global climate change on our shelves. The present OPC technology will require alignment in flow to process zooplankton through its tunnel for counting and sizing. Future OPC development consist of laser-based systems using diffraction measurements for sizing and will not require flow for measurement but can be moored in static water. Current data logger technology is at its upper limit capability for present OPC data rates but should be able to undergo a timely merge with future high-power microprocessors, 'flash' memory and battery technology enabling high-frequency sampling rates.

One major advantage in the present OPC configuration is its small streamlined size and its capability of being towed at high speeds thus enabling synoptic surveying and instantaneous abundance information. Its major limitation is its inability to define plankton shape and therefore precise identification of animals.

Video Technology

Following the development of the charge-coupled-diode (CCD) and its integration with camera technology, the underwater video emerging with high-resolution and low-light capability Kils (1990) described an example of a recent development in high resolution video systems which consists of a series of optical paths capable of imaging 3 scales from small particles to large juvenile fish. The system is capable of resolving small tintinnids (*Stenosemella nucula*) with a diameter of 20 μm of its optical paths.

The strength of such video systems is their ability to observe and identify behaviour and physiological reactions of animals. For example, Kils observed by video, from a floating laboratory, the feeding behaviour of juvenile herring (*Clupea*

LIGHT BEAM
RECTANGULAR X-SECTION 4 x 20 mm
λ = 640 nm

RECEIVER

2 cm

22 cm

SAMPLING
TUNNEL

TRANSMITTER
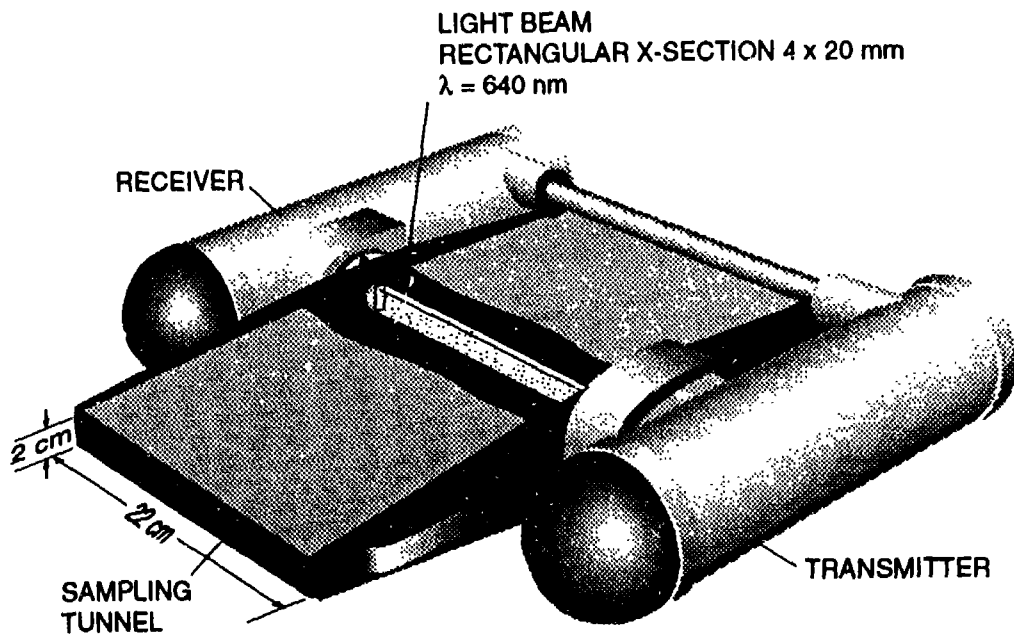
OPTICAL PLANKTON COUNTER
SUBMERSIBLE VERSION

Fig. 20: An optical plankton counter used for counting and sizing zooplankton.

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HALIFAX LINE
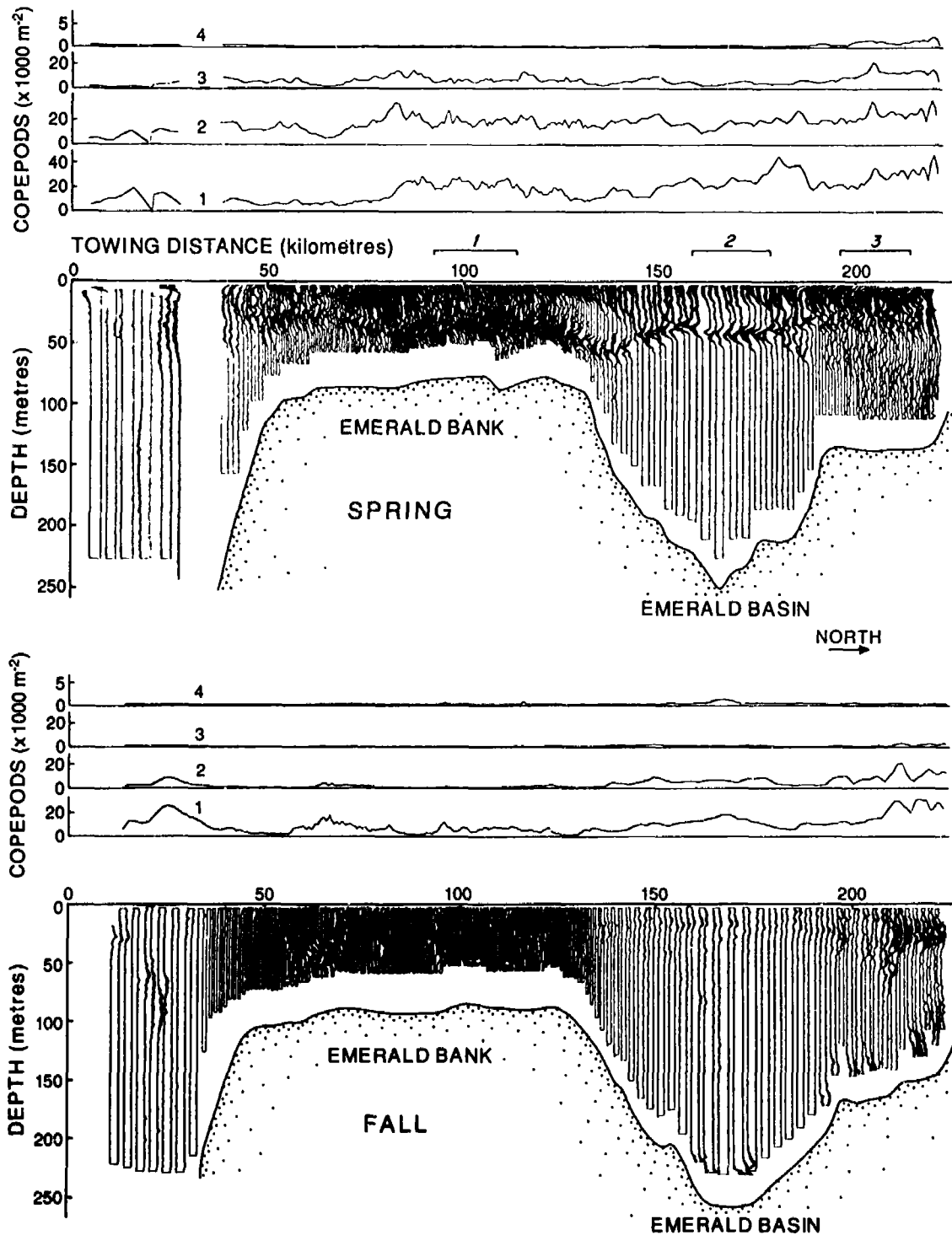
Fig. 21: Profiles of copepod concentrations for a 200 km transect across the Scotian Shelf obtained with an optical plankton counter mounted on a Batfish. Increasing line thickness or 'shading' indicates increasing concentration. The four analog plots at the top (and middle) of the figure represent areal density (no. m-2) over the towing distance four each of the four copepod size groups labelled '1, 2, 3, 4' corresponding to: 1) small copepods ≈400-500 microns (equivalent spherical diameter), 2) *C. glacialis IV, C. finmarchicus IV-V, Centropages typicus VI*, 3) *C. hyperboreus IV-V, C. glacialis V, C. finmarchicus VI*, and 4) *C. hyperboreus*, euphausiids.
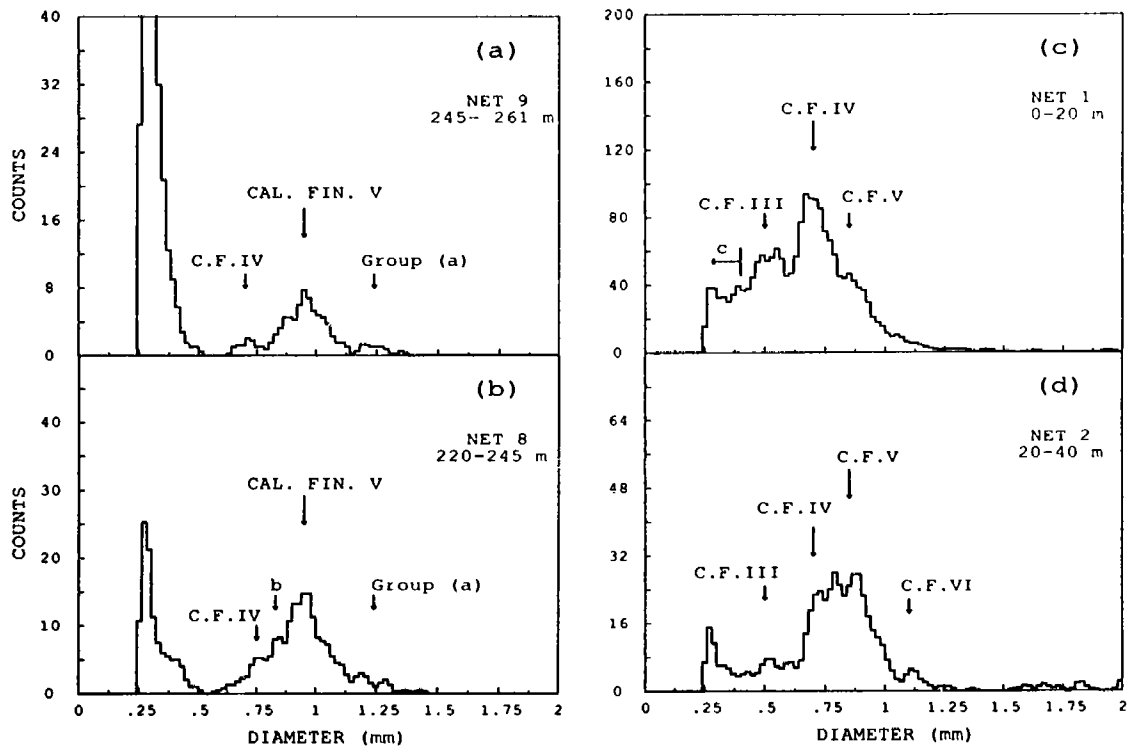
Fig. 22: Size distributions of copepods as measured with an optical plankton counter illustrating the methodology used to identify species & stages. The distributions shown in the figure are measured by the OPC while copepod samples simultaneously collected by the BIONESS net sampler are measured by microscopic analyses. A comparison between OPC and microscope distributions reveal the shapes and peaks belonging to certain copepod species and even specific stages.

harengus) in the Kiel Bay occurring under specific conditions, that is, only when small copepods (*Psuedocalanus minutus*) were aggregated at high densities under a 1 m stratified layer. The feeding rate of herring (2.5 copepods/sec) could be measured from the video images and once the copepods were grazed from the immediate swarm area, the school moved on to an adjacent aggregation by some search/seek pattern. Such feeding behaviour could only have been observed by video imaging.

More recently a high-resolution camera, the video plankton recorder (VPR; Davis *et al.*, 1992), has been developed as a video-microscope system used for imaging plankton and other particulate matter in the size range from a few microns to several centimeters. Figure 23 (a) shows the mounted camera systems. The four cameras are aligned for concentric viewing fields allowing four magnifications to be viewed simultaneously and thus providing a broad size range of plankton which can be sampled. The towed body shown in Figure 23 (a) contains a number of environmental sensors while a gauze recorder box can be mounted in the front of the body just behind the sampling volume. Video data are telemetered to the surface via a fiber optic cable and stored on broadcast quality video tape recorders as shown in Figure 23 (b).

Some of the limitations of submersible video systems is the difficulty of defining known volumes of water being sampled by the image and the difficulty in towing such systems at high speeds thus limiting synoptic coverage. The most difficult problem, however, is that of storage, processing and telemetry of such large quantities of data. Let us consider two cases of CCDs, the first being of standard commercially available design of 580 x 670 pixels for a total 360 kilopixels and the second being the new design of high-power CCD's containing 5000 x 6000 pixels for a total of 30 Megapixels. If we wish to transmit all pixels for a single scan to surface electrically using conventional FSK telemetry on a standard conductor cable, it would require $\approx$150 seconds for case 1 or $\approx$3 1/2 hrs for case 2. This is why we require fiber-optic cable for video telemetry which has conventional transmission capability of 100 mega-baud. Hence, in case 1 we would require only 30 ms, whereas case 2 would still require a considerable period of 3 seconds. All these estimates do not include data handling required by a controller/ microprocessor. Optimally, data can be stored on video tape for off-line processing. We can now appreciate the problem of handling video data. Normally video images are stored on video tape and post-processed, an extremely time-consuming process.

There exists great potential for moored video systems and the long-term observation of animal behaviour, physiology and even the reorientation effect. Such programs are being planned for the future integration of video systems with acoustic systems and optical plankton counters. The potential of moored video systems requires onboard preprocessing and data storage which can only be accomplished by the future high speed microprocessors and 'flash' memory that will appear in the 90's.

(a)

Cameras

Strobe

CCD 1
CCD 2
CCD 3
CCD 4

FL   TR

F

T C

P

SENSORS:
F = Flowmeter
T = Temperature
C = Conductivity
P = Pressure
FL = Fluorometer
TR = Transmissometer

Frame

Video
Telemetry/
Fiber Optics

Sensor
Telemetry

(b)

XTR → VTR 1 → M 1
XTR → VTR 2 → M 2
XTR → VTR 3 → M 3
       VTR 4 → M 4

S → IT Series 151 → Computer
VTR

Sensor
LHPR
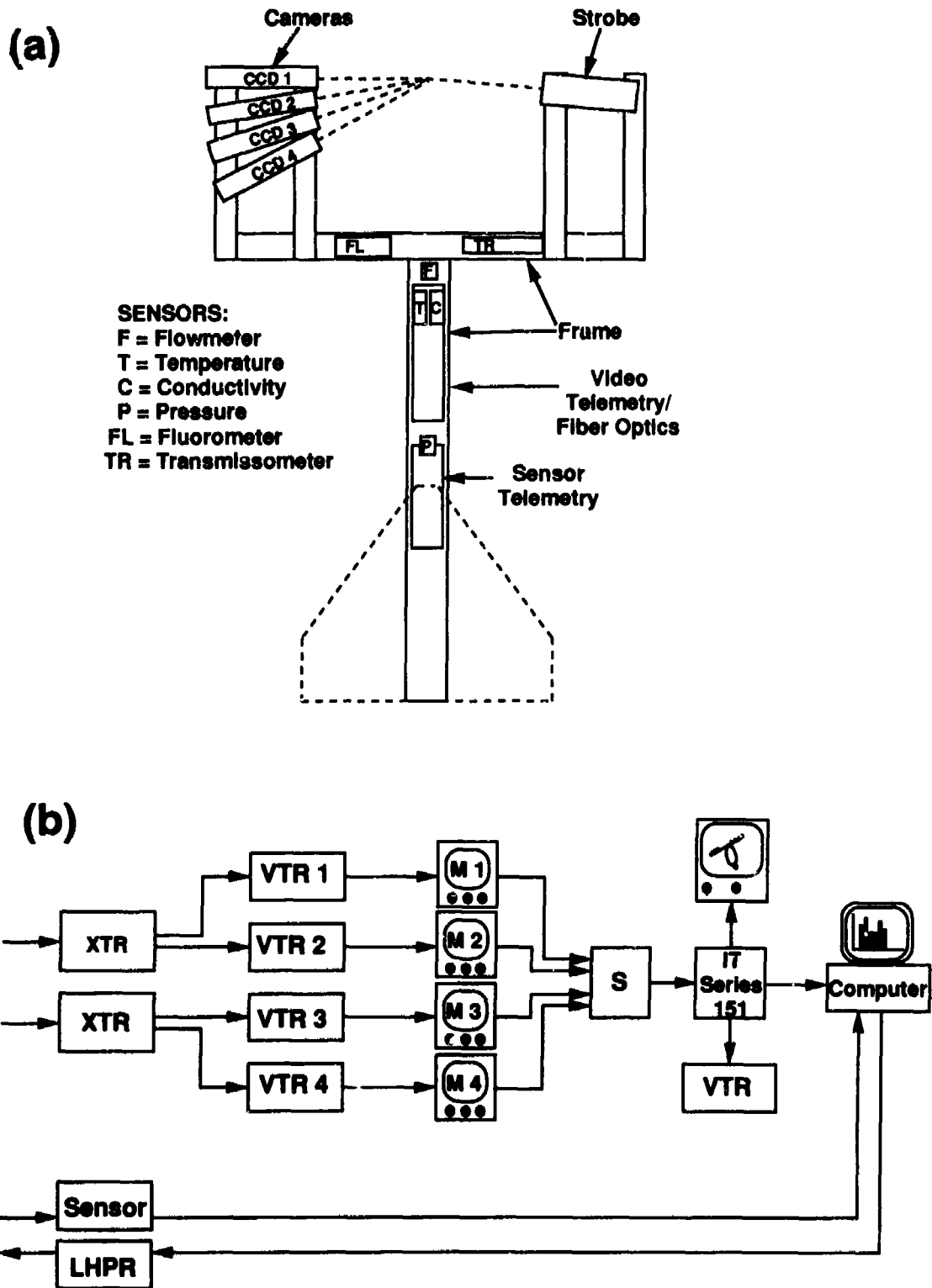
Fig. 23: (A) Schematic of the video plankton recorder (VPR) showing the layout of major underwater components. Four CCD cameras, each having a different magnification, and the strobe are held on the side-arm mounts 1 m apart. A modified LHPR box is positioned behind the sampling volume collecting samples. (B) Schematic components of the VPR showing the surface fiber optic telemetry units (XTR), the video tape recorders (VTR), monitors (M), video switch box (S), and the image processor (IT series 151) (from Davis *et al*, 1992).

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