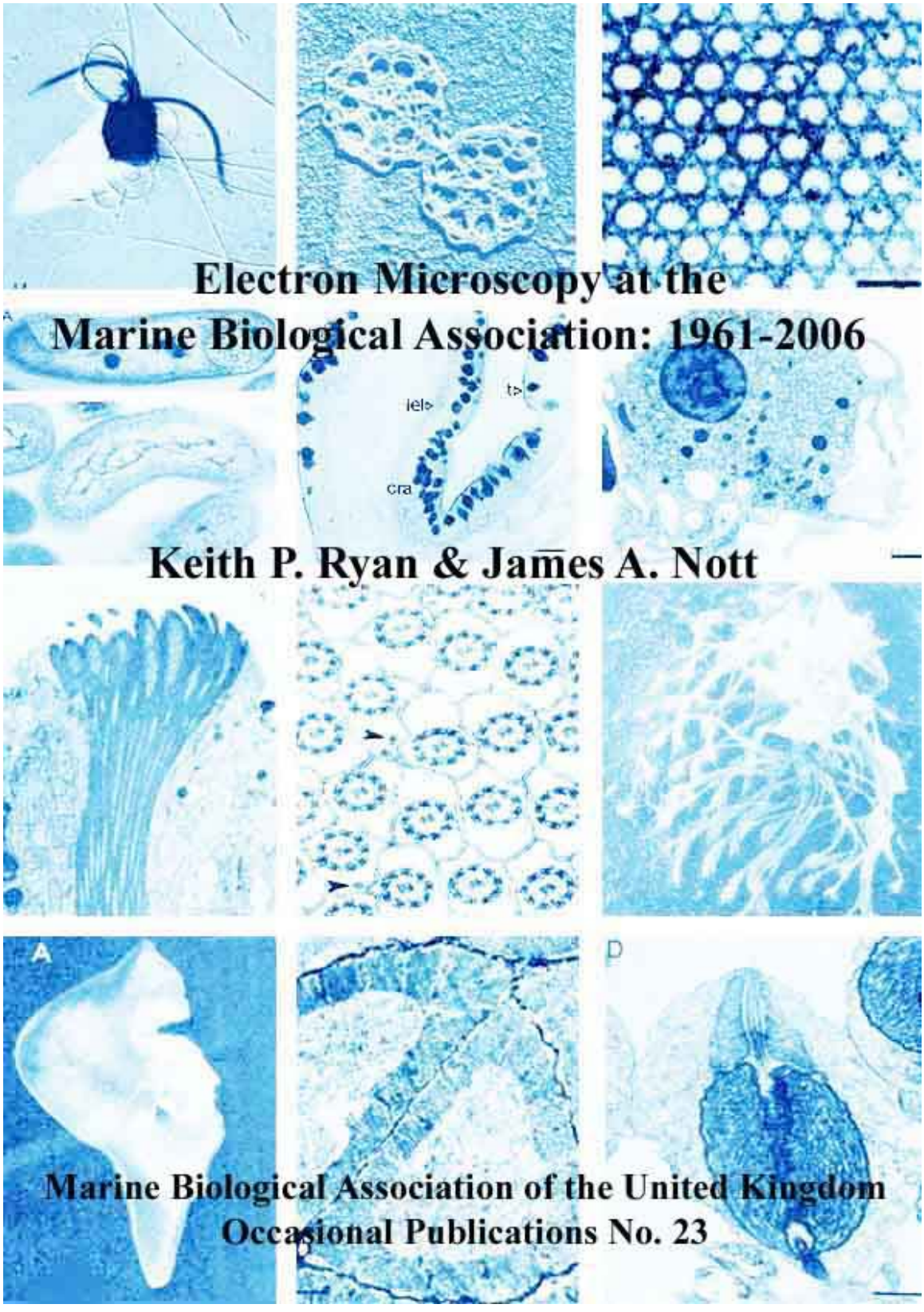

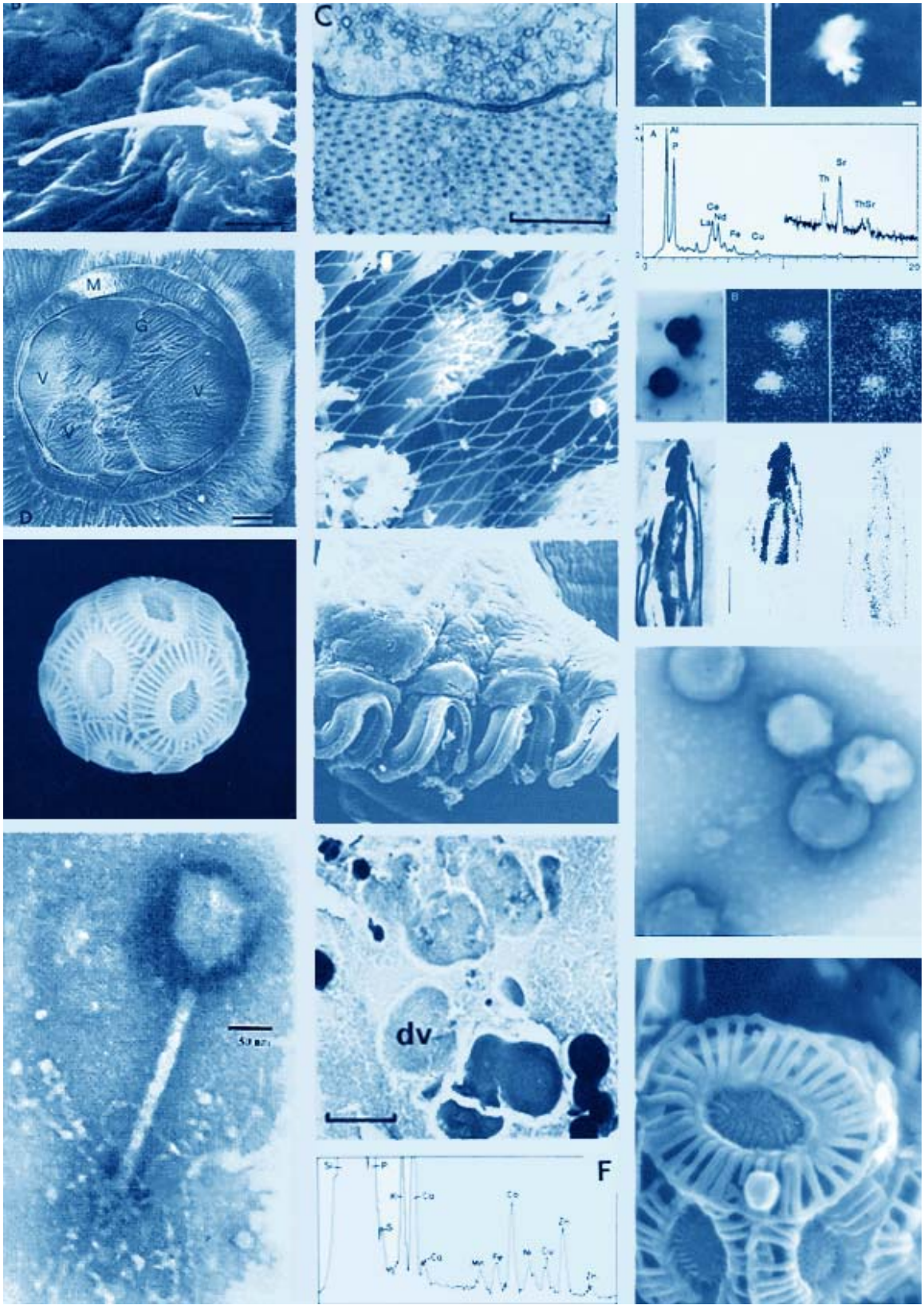
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Key to front cover images

Front cover images – from the Journal of the Marine Biological Association of the U.K.

1. Parke, M., Green, J.C. and Manton, I., 1971. Observations on the fine structure of zoids of the genus *Phaeocystis* (Haptophyceae). J. mar. biol. Ass. U.K. 51, 927-941. Fig. 11. Whole cell of clone 147 magnified to show the filamentous external investment more clearly; from a shadowcast wholemount made at Plymouth in 1969.

2. Parke, M., Boalch, G.T., Jowett, R. and Harbour, D.S., 1978. The genus *Pterosperma* (Prasnophyceae): species with a single equatorial ala. J. mar. biol. Ass. U.K., 58, 239-278. Plate VIII (B) *Pterosperma moebii* (Joerg) Ostenf. – motile phase. (B) Scales from the outer scale layer of the flagellum showing outer surface with adnate spine and inner surface lacking spine.

3. Best, A.C.G. and Bone, Q., 1976. On the integument and photophores of the alepocephalid fishes, *Xenodermichthys* and *Photostylus*. J. mar. biol. Ass. U.K., 56, 227-36. Plate II Fig. B. Transverse section of paracrystal array. Note regular circular profiles within hexagonal array. Scale bar 100 nm.

4. Southward, E.C. 1986. Gill symbionts in thyasirids and other bivalve molluscs. J. mar. biol. Ass. U.K., 66, 889-914. Sections of bacteria from *Thyrasira sarsi*. Fig. 5. (A). (H) From thyrasid T1. S, Sulphur vesicle; pp, polyphosphate granule.

5. Jenkins, P.G., Pulsford, A.L. and Harris, J.E., 1992. Microscopy of the absorptive cells and gut-associated lymphoid tissue of the flounder, *Platichthys flesus*. J. mar. biol. Ass. U.K., 72, 553-567. Fig. 2. Histochemistry of *Platichthys flesus*. (D) PAS-Alcian blue staining in the posterior intestine showing a dually positive terminal web. (t), positive cell rich areas (cra) and positive intraepithelial leucocytes (iel, arrowed).

6. Holden, J.A., Pipe, R.K., Quaglia, A. and Ciani, G., 1994. Blood cells of the Archid clam *Scaphorca inaequivalvis*. J. mar. biol. Ass. U.K., 74, 287-299. Fig. 3. Electron micrographs of *Scapharca inaequivalvis* white blood cell, (A) type 1 showing cytoplasmic granules and extensive pseudopodia.

7. George, J.D. and Southward, E.C., 1973. A comparative study of the setae of Pogonophora and polychaetous Annelida. J. mar. biol. Ass. U.K., 53, 403-424. Plate V (A) A seta in longitudinal section, with surrounding epidermal cells. B, Basal cell; l, lateral cell; m, muscle fibre; n, nucleus.

8. Bone, Q., Gorski, G. and Pulsford, A., 1979. On the structure and behaviour of *Fritillaria* (Tunicata: Larvacea). J. mar. biol. Ass. U.K., 59, 399-411. Plate II (G) Detail of cilia of ciliary valve. Note supernumerary doublets (arrowed).

9. Blaxter, J.H.S., Gray, J.A.B. and Best, A.C.G., 1983. Structure and development of the free neuromasts and lateral line system of the herring. J. mar. biol. Ass. U.K., 63, 247-260. Fig. 4. SEM photomicrographs of sprat and herring, all with head to the left. (B) High power view of neuromast.

10. Clarke, M.R., 1978. The cephalopod statolith: an introduction to its form. J. mar. biol. Ass. U.K., 58, 701-712. Fig. 3. Right statolith of a *Loligo forbesi* with a total dorsal mantle length of 23.0 cm. Statolith total length = 2.0 mm. (A) Anterior view; (B) posterior view.

11. Bone, Q., Pulsford, A.L. and Chubb, A.D., 1981. Squid mantle muscle. J. mar. biol. Ass. U.K., 61, 327-342. Ultrastructure of mantle. Fig. 5. (A) Fibres of inner zone showing large mitochondrial core.

12. Gibbs, P.E., Pascoe, P.L. and Burt, G.R., 1988. Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. J. mar. biol. Ass. U.K., 68, 715-731. Fig. 6. Stages of spermatogenesis in testis of "female" shown in Fig 5B. (D) spermatid.

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Key to inner cover images

Inner cover images – from the Journal of the Marine Biological Association and MBA Annual Reports.

1. Bone, Q. and Ryan, K.P., 1979. The Langerhans receptor of *Oikopleura* (Tunicata: Larvacea). J. mar. biol. Ass. U.K., 59, 69-75. Plate I (B) S.E.M. view of receptor of *Oikopleura labradoriensis*. Scale bar: 10 μm.

2. Bone, Q., Packard, A. and Pulsford, A.L., 1982. Cholinergic innervation of muscle fibres in squid. J. mar. biol. Ass. U.K., 62, 193-199. Fig. 2. (C) Motor nerve terminal on longitudinal fibre of anterior mantle region of *Alloteuthis* showing lack of subjunctional folds and 50 nm electron-lucent vesicles. Scale bar: 0.5 μm.

3. Pulsford, A.L., Ryan, J.A. and Nott, J.A., 1992. Metals and melanomacrophages in flounder, *Platichthys flesus*, spleen and kidney. J. mar. biol. Ass. U.K., 72, 483-498. Page 492. Figure 7. Scanning electron micrographs of dense particles found in Lymphoprep-prepared samples of flounder spleen and kidney. A secondary electron image (SEI) and a back-scattered electron image (BEI) are shown for each particle. Scale bar: 1 μm. (E) SEI of the steel particle analysed in Figure 6D. (F) BEI of the steel particle.

4. Pulsford, A.L., Ryan, J.A. and Nott, J.A., 1992. Metals and melanomacrophages in flounder, *Platichthys flesus*, spleen and kidney. J. mar. biol. Ass. U.K., 72, 483-498. Page 492. Figure 8. X-ray spectra from slam frozen, freeze-dried flounder spleen and from sediment. (A) A complex spectrum from a spleen particle (Figure 9) which includes, Al, P and rare earth elements. The horizontal anergy scale is doubled to include the Th and Sr peaks which are also expanded vertically on a 0.5 k counts scale.

5. Bone, Q., Brownlee, C., Bryan, G.W., Burt, G.R., Dando, P.R., Liddicoat, M.I., Pulsford, A.L. and Ryan, K.P., 1987. On the differences between the two indicator species of chaetognath, *Sagitta setosa* and *S. elegans*. J. mar. biol. Ass. U.K., 67, 545-560. Fig. 1. (D) *S. elegans*. Mid-trunk region cryo-fractured and photographed after etching (to show structure more clearly). G, Gut; M, muscle layer; V, vacuolated gut sacs; Scale bar, 100 μm.

6. Bone, Q. MBA Annual Report 1998 (published 1999), page 23. Fig. 9. The salp feeding filter within the endostyle – overlying the ciliary cells driving it forward.

7. Gibbs, P.E., Burt, G.R., Pascoe, P.L., Llewellyn, C.A. and Ryan, K.P., 2000. Zinc, copper and chlorophyll-derivatives in the polychaete *Owenia fusiformis*. J. mar. biol. Ass. U.K., 80, 235-248. Page 244. Figure 9. *Owenia fusiformis* (A) Scanning electron micrograph of two isolated spherules; (B & C) X-ray maps of P and Zn respectively. (GSM specimen).

8. Bone, Q., Ryan, K.P. and Pulsford, A.L., 1983. The structure and composition of the teeth and grasping spines of chaetognaths. J. mar. biol. Ass. U.K., 62, 929-939. Fig. 6. Section of spine tip (STEM, left), with corresponding X-ray maps for SiKα (mid) and ZnKα (right). Scale bar 1μm.

9. Harbour, D.S. 1989. *Emiliana huxleyi* (Lohman) Hay & Mohler. J. mar. biol. Ass. U.K., 69 (1) Inside rear cover.

10. Pascoe, P.L., 1987. Monogenean parasites of deep-sea fishes from the Rockall Trough (N.E. Atlantic) including new species. J. mar. biol. Ass. U.K., 67, 603-622. Fig. 4. (A)*Octoplectanocotyla aphanopi* n.sp. Orientation of clamps on posterolateral laminae (SEM). Scale bar: 50 μm.

11. Wilson, W.H., Schroeder, D.C., Ho, J. and Canty, M., 2006. Phylogenetic analysis of PgV-102P, a new virus from the English Channel that infects *Phaeocystis globosa.* J mar. biol. Ass. UK, 86, 485-490. Figure 2. Transmission electron micrograph (TEM) analysis of PgV-102P concentrated from a freshly lysed *Phaeocystis glebosa* strain PLY64 culture. Scale bar 50 nm.

12. Wilson, W.H. MBA Annual Report 1998 (published 1999), page 18, bacteriophage. Fig. 5. Cyanophage strain S-PM2 which was isolated from surface seawater four miles south of Plymouth Sound in 1992.

13. Mason, A.Z., Simkiss, K. and Ryan, K., 1984. The ultrastructural localisation of metals in specimens of *Littorina littorea* collected from clean and polluted sites. J. mar. biol. Ass. U.K., 64, 699-720. Fig. 3. (E) TEM micrograph of an incinerated ultra-thin cryosection illustrating the ash-resistant materials in the digestive vacuoles (dv) of a digestive cell of a Restroguet animal. Scale bar represents 2 μm. (F) Elemental analysis of ther contents of one of the digestive vacuoles shown in E. The spectrum shows major peaks for phosphorus, potassium and calcium in association with smaller peaks for manganese, iron, cobalt, nickel, copper, zinc and sulphur. The large peak for silicon is derived from is derived from the silicon monoxide support film.

14. Wilson, W.H., Schroeder, D.C., Ho, J. and Canty, M., 2006. Phylogenetic analysis of PgV-102P, a new virus from the English Channel that infects *Phaeocystis globosa.* J mar. biol. Ass. UK, 86, 485-490. Figure 2. Transmission electron micrograph (TEM) analysis of PgV-102P concentrated from a freshly lysed *Phaeocystis glebosa* strain PLY64 culture. Scale bar 50 nm.

Electron Microscopy at the Marine Biological Association: 1961-2006

With 33 Figures, 4 Plates and 7 Appendices

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Appendices

Summary
The Marine Biological Association of the United Kingdom was founded in 1884 following the establishment of a marine laboratory at Naples. The Plymouth laboratory was opened in 1888 (Southward & Roberts, 1987, and other historical references listed following). The study of marine biology was facilitated by several factors, clearly the proximity of the sea, the boats used for surveying and collecting specimens and other facilities, not least among them, microscopes. A memorial photograph of former Director, Sir Frederick Russell F.R.S., hanging in the library shows him using a light microscope. With the development of electron microscopes, the laboratory identified a need to keep up-to-date. This was particularly true for the work on marine flagellates by Dr Mary Parke FRS (collaborating with Prof. Irene Manton FRS, University of Leeds) and Dr. Quentin Bone who was investigating the neuro-muscular ultrastructure and physiology of various protochordates. The first instrument, a Philips EM200 transmission electron microscope (TEM), was installed in the laboratory in 1965. It was used to examine thin specimens, namely direct preparations of flagellates supported on thin films and sections of tissues, until 1981. The demand for electron microscopy resulted in a second microscope, a Philips EM300, being installed in 1971. A small scanning electron microscope (SEM), the JEOL JSM P15, was purchased in 1976 for examining bulk specimens. This was replaced with a JEOL JSM T20 in 1979. In 1981, the electron microscope unit was re-equipped with a JEOL JEM 200CX Temscan (a combination of TEM and SEM), a JEOL JSM 35C SEM and ancilliary equipment. Both instruments were capable of perfoming x-ray microanalysis to identify chemical elements in specimens. Following the installation of the new equipment, microscopists at the MBA started to use low temperature methods to preserve the structure and chemistry of specimens in a more life-like manner. The EM300 and JSM 35C were removed from the laboratory in 2002 and 2003, respectively. Both the MBA Engineering Workshop and the MBA Electronics Workshop were involved in developing equipment both for the use of and research into cryo-methods. Their involvement was crucial to much of the science that followed, particularly in the investigation of heavy metal pollution and marine food chains. This was additional to the continuing requirements for ultrastructural studies by staff, students and visitors. The JEM 200CX remains as one of the most powerful instruments available, capable of producing high-resolution images between 20 and 200 kV in TEM and SEM modes as well as by the scanning transmission electron microscopy (STEM) mode that was used extensively in the microanalytical work. The Appendices record that 54 MBA staff and 196 students and visitors have used the microscopes and that 413 titles have been published (to the end of 2006).

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4. Russell, F.S. The Plymouth laboratory of the Marine Biological Association of the United Kingdom. Proceedings of the Royal Society of London, Series B, 135, 12-25, 1947.

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6. Russell, F.S. The Plymouth laboratory of the Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom, 27(3), 761-774 + plates, 1948.

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8. Russell, F.S. The Plymouth laboratory of the Marine Biological Association of the United Kingdom, 1963. Journal of the Marine Biological Association of the United Kingdom, 43(2), 281-290, 1963.

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Introduction

This *memoir* recalls the instruments in the Electron Microscope Unit and the staff, students and visitors who used them. Accessory equipment is also described because much of it was innovative and built in the laboratory, also, much of the science would not have been possible without it. Some of the scientific work is referred to in passing but this is best appreciated by referring to the list of publications in Appendix 4.

The period 1961-1969

There was a period of discussion prior to the purchase of the first electron microscope (EM) regarding its need and foreseen uses. The Director, Sir Frederick Russell FRS, said in 1955 that the MBA should be the birthplace of new ideas, staffed by young and original workers who would bring up-to-date knowledge and new disciplines. In August 1956, a Director's memo in the MBA Archives mentioned that descriptions of the structure of flagellates, the subject of investigations by Dr. Mary Parke in collaboration with Prof. Irene Manton (University of Leeds) were made possible by the use of electron microscopy. Another memo in 1961 about the next 5-year period mentioned the purchase of an electron microscope.

A memo by the Director dated July 1963 (MBA Archive reference MD6.8), was specifically about the need for an electron microscope. It noted the initial purchase and annual running costs as being a minimum of £10,000 and £750 respectively. It also mentioned a further cost of about £1,000 to adapt and decorate a cellar for the purpose. The EM was again needed for the important work of Mary Parke FRS and Irene Manton FRS on small phytoplanktonic forms and for Dr Quentin Bone's work on several neurohistological problems involving protochordates. Prof Eric Denton FRS and Dr John Gilpin-Brown had a requirement to study the fine structure of the siphuncular surface of the cuttlebone and its processes of calcification. Eric Denton and Dr Colin Nicol had a requirement to study the reflecting layers of the tapetum of the elasmobranch eye and in the sides of fishes (this study was to use selected area electron diffraction of guanine crystals in those reflecting layers). The studies of Dr Gerald Boalch on the frustules of diatoms would also benefit from the use of electron microscopy.

The MBA Archives hold a memorandum on different electron microscopes written by Q Bone and ACG Best (MD6.8). This compared the Siemens Elmiskop I, Akashi TRS 80, AEI EM6 and Philips EM200. The Archives also hold a letter from the Wellcome Trust providing £17,750 for the purchase of an EM. The price was based on a Siemens Elmiskop Model I although the model selected for purchase was the Philips EM200. This model was chosen because it offered a 35 mm camera and selected area diffraction – both of which were later used extensively. At this time (1965), the NERC Unit at Menai Bridge bought an EM6B and Dr Jim Nott started work there.

Duty remission was applied for on the famous DFA3 form from the Board of Trade. This brought the price down to £12,500. The Wellcome Trust allowed some of this to be spent on a chilled water unit (£304 16s 6d) and an Edwards high vacuum coating unit (£770 0s 0d). The water chiller was bought because the small Philips EM100 at Plymouth Technical College suffered from corrosion caused by the Plymouth water supply.

The installation of the first EM, a Philips EM200 transmission electron microscope* (TEM - see footnote 1), was completed in August 1965. This valve-driven instrument did sterling work for sixteen years until it was removed in 1981 (when the EM Unit was re-equipped and the EM200 was moved to Torbay General Hospital). The grant was recorded in the Report of Council 1964/65. Another grant of £2,000 was recorded from H.M. Treasury to enable structural alterations in the large cellar beneath the north building. The building work started in March 1965. Part of the basement modification involved the provision of a temperature-controlled, clean air supply, with the dust filter being accessed from the "Dog House" roof. This was serviced each year by the EM staff to ensure it remained functioning.

Sir Frederick Russell FRS, retired in September 1965 and Dr Eric Smith FRS started as Director the following month.

The cellars formed a self-contained unit with the microscope, specimen preparation area and darkrooms. There was an inner darkroom beside the microscope for developing 35 mm film and glass plate negatives. The ante-room was equipped for specimen preparation. An adjacent cellar was converted to a printing darkroom.

Besides the microscope, other equipment was necessary. This included an ultramicrotome to cut sections thinner than 10 nm (or 100 Ångström units, as used in those days, this being the approximate thickness of a cell membrane): on this scale there is an amazing 100 sections to one micrometer or 100,000 to one millimetre. Their thickness was judged during cutting by the colour they showed when floating on the water bath that was integral to the process, similar to the colours of oil floating on water.

The microtome that was purchased was the original "Huxley" model, there being little choice at that time. This was operated by a hand lever that was lifted vertically on the right side, repeatedly, all day. The specimen was fed towards the glass knife by geared fractions of a turn of a simple micrometer screw. The gravity-driven cutting speed i.e. the fall of the specimen was controlled by turning a bleed screw which was hidden in an oil-filled dash-pot/damper mechanism. Finding the screw under the oil with a screwdriver to adjust it was an art in itself.

***Footnote 1 -** "Transmission" implies that the electron beam is transmitted through the specimens, these being very thin and either of sectioned material or direct preparations of e.g. unicells on thin, electron-lucent plastic or carbon support film, to form an image on a fluorescent viewing screen. The contrast in the image comes from the atomic weight of the specimen atoms - where these are heavy then electrons are knocked out of the beam and this area then casts a "shadow" or dark area onto the fluorescent screen. Heavy metals are used in fixatives and "stains" to enhance this process.*

Glass knives for cutting sections were made originally by breaking strips of float glass bought locally. This was done by scoring them with a glass-cutting wheel and then snapping the glass using two pairs of pliers with modified jaws. The success rate was low and a purpose-made LKB "KnifeMaker" was bought in 1968. This same item is still in use today, after 38 years.

The other major piece of equipment was a coating unit. The log book of the Edwards 12E6 Coating Unit was started on 5 April 1965. The purpose of this device was to provide a work chamber that pumps down to a "high" vacuum (10^{-4} torr, i.e. a low pressure) in which various tasks can be done:

- parts of the microscope (the beam-limiting apertures) can be cleaned by heating to incandescence *in vacuo* to drive off the accumulated carbonised deposits formed from hydrocarbons (oil vapour) in the vacuum system – these deposits become electrostatically charged by beam electrons and cause astigmatism in the image. This job had to be done carefully because in those days, the apertures were made of platinum and were easily melted. Later, high-melting-point molybdenum was used and, later still, self-cleaning thin-film apertures were introduced.
- direct "preps" e.g. dried algal cells can be metal-shadowed for three-dimensional relief. This was essential to the phytoflagellate studies.
- carbon support films can be prepared by depositing a thin layer of carbon from a carbon arc formed by passing an electric current through two carbon rods in the vacuum. The film was formed on freshly-cleaved mica and then floated off on water.
- plastic support films e.g. Formvar can be strengthened by a thin coating of carbon. This was also essential to the phytoflagellate studies.
- naked sections can be similarly strengthened with carbon to withstand electron bombardment in the microscope.
- frozen specimens can be freeze-fractured with the appropriate accessory equipment.
- freeze-fractured replicas can afterwards be carbon-backed for strength.
- SEM specimens could be rotary-coated with gold or carbon using a planetary device made by George Best in 1976.
- frozen specimens could be freeze dried following modification of the freeze-fracture equipment in 1985 by David Purse in the MBA Workshop.
- silicon films could be made for supporting specimens that were to be exposed to an oxygen plasma: this removed organic material from them thus enhancing the level of other materials e.g. granules remaining for x-ray microanalysis.

The old unit was still much-used up to June 2004 after 39 years and has been described as a real "Trojan", being solidly constructed and often compared to a brick-built outhouse.

It is sometimes overlooked that electron microscopy was an extension of the histological activities in the laboratory. Histology was overseen by George Best after he joined the laboratory in 1956. His "office" was in Room 2 which was next to the Director's room, Room 1. Few people had dedicated offices in those days and this room was in fact the base for light microscopy. It had facilities for bright-field, dark-field, incident light

immersion (by dipping cones), transmitted fluorescence, incident fluorescence, phase-contrast, Nomarski/Tolanski imaging and other interference-contrast methods of microscopy, all with photographic capabilities. Electronic flash was available for some methods. A major feature of this room was the impressive optical bench with its superb brass microscope and large-format mahogany half-plate camera. This took 4¼ x 6½ inch glass plates (108 x 165 mm), although there was an adapter for quarter plates as well, these were 3¼ x 4¼ inches in size (82 x 108 mm). A simple adapter converted it later to take the more modern cut film. These large formats required little or no enlargement and gave results from microscope slides that showed superb resolution. There was also an upright bellows quarter-plate camera for macro-photography. An advantage of large-format photography was the sheer size of the negative compared to the size of the CCD (charge-coupled device) that captures the image in a modern digital camera, which is typically 16 x 24 mm (quarter-plate being twenty-three times larger than the typical CCD). The nearby Room 12 became a combined EM and histology preparation room. A separate histology room was located on the second floor (Room 32), indicating the importance of histology to the lab's activities. There were two full-time histology technicians, these being Alan Chubb (who served from 19 June 1967 to 31 October 1988) and Clive Brook (who served from 2 August 1971 to 30 May 1980).

A major activity associated with both light and electron microscopy was photography, because the only publishable result is a photograph or a drawing. The original histology darkroom was Room 4. EM meant that darkrooms were even more necessary and were installed as described above. There was often a queue waiting to use the darkrooms indicating again the level of activity. The room arrangements changed in 1988, as noted below in the section for that decade.

The first paper to come from the new facilities was published in *Contributions in Marine Science Univ. Texas* in 1967 by George Best and Colin Nicol, on the reflecting cells of the Elasmobranch *tapetum lucidum*.

Fig. 1. Philips EM200 Transmission Electron Microscope in the cellar, North Building. Photo April 1977.

Fig. 2. EM basement prep room, showing the water chiller (left) and the coating unit (right). The microtome was in a cubicle at the far end of the room. Photo taken April 1977.

Fig. 3. George Best using the EM200, as is usual – in the dark. Photo taken 1970.

Four papers were published in 1968, all by visitors: Dr. David Chapman (Halifax, Nova Scotia) and Dr. Dennis Taylor (Swansea). The following year, there were seven papers published by visitors, again David Chapman and Dennis Taylor plus Dr. Kay Lyons (Birmingham), Dr. Robert Fournier (Rhode Island, USA) and Dr. John Pilkington (SRC Research Fellow).

The Report of Council 1968/69 records that Dr. Jerzy Alexandrowicz investigated the nerves in the vena cava of *Sepia* and *Eledone* by electron microscopy. He was in the laboratory since being a post-war refugee and began this work, using EM, at the age of 78. His obituary was published at length in the *Journal* (1971, vol. 51, pp. 1007-1011).

The publications and other work indicate the interest in using EM as a research tool and also reflect its role in supporting the very active MBA visitors' programme. The laboratory was so busy that accommodation became a problem and plans for extending the MBA Laboratory were made in 1966 in conjunction with Plymouth City Council. A site of 1.1 acres was identified for leasing at West Hoe and discussions started with NERC (Natural Environment Research Council) to progress this venture. The discussions came at a time when NERC were considering options for grouping similar or complementary institutes. In the event, NERC acquired a nearby 2.2 acre site to bring IMER (the former Institute for Marine Environmental Research) and also a part of NIO (the former National Institute of Oceanography) to Plymouth. The Report of the Council 1970/71 mentions that the planned MBA extension at West Hoe was considering 22,000 square feet. Moving the electron microscope unit was discussed.

Publications (1967-69)
A total of 13 publications were recorded for this period

The period 1970-1979

Due to demand, a second TEM was installed in a cellar room adjacent to the original microscope, with completion being recorded in its log book on 23 July 1971. This was the Philips EM300. Its main innovation was that it was powered by transistors and featured an automated vacuum system – which meant there was no more watching the vacuum gauge as required on the EM200, and reaching up behind the microscope column from the chair to switch from "Position 4" to "Position 3" to pump the backing tank. Like the first microscope, it was connected to a water-chiller that was located in the basement.

Fig. 4. EM300 in its original cellar. Photo taken April 1977.

Fig. 5. EM300 after it was relocated to Room 59 in 1988. Photo taken 2001.

In 1971, Quentin Bone and Eric Denton published a paper on the osmotic effects of electron microscopy fixatives.Quentin Bone and Keith Ryan published a follow-up paper and these papers became much-requested and much-cited, arousing a lot of interest across fields in medicine and biology.

In October 1972, George Best and Keith Ryan carried out a conversion of the Edwards coating unit using the MBA Physiology Workshop, with some help from Jack Saggers. The work involved re-locating the rotary pump, lowering the oil diffusion pump and fitting a liquid nitrogen trap. When finished, it was ready for freeze fracture. A British version of the American Steere equipment was purchased from Polaron Ltd. and the laboratory began its first work with liquid nitrogen. In truth, this did not produce many results, due in part to the timidity of the users and lack of contact with other practitioners for practical advice. The freeze-fracture module was modified in 1985 by David Purse in the MBA Engineering Workshop and it was then used extensively for freeze-drying. This supported the work of Jim Nott from 1985 until he retired in 1996.

A major development in the Electron Microscopy Unit was the purchase of a JEOL JSM P-15 scanning electron microscope* (SEM – see footnote 2). The installation of this table-top instrument was completed on 26 March 1976 in Room 13, the room previously used as the Biological Store (across the corridor from the Director's Secretary's room). The instrument was supplied by EMscope Ltd. and came with a cryo-stage for examining fresh, frozen specimens (this in 1976!). Liquid nitrogen was collected from BOC in the Emscope Managing Director's large Rolls-Royce car, leading to comments about its cocktail bar. Demand for use of the basic SEM was such that, combined with reliability problems due to poor-quality plugs and connectors used in its construction, it was replaced with a JEOL JSM T-20 instrument, the installation of which was completed on 16 October 1979. Unfortunately, no photographs can be found of this instrument. In 1976, a new Reichert OmU3 ultramicrotome was also purchased.

Fig. 6. George Best with the JEOL P15 SEM. Photo taken April 1977.

Fig. 7. Keith Ryan and Ann Pulsford with the Philips EM200 TEM. Photo taken April 1977.

Fig. 8. Quentin Bone, scientist in charge of electron microscopy 1965-1988. Photo taken April 1977.

Members of staff who used the SEM besides those already mentioned included Derek Harbour, Paul Tranter and David Nicholson. Derek became well-known for his striking images of coccoliths in work for Dr. Patrick Holligan. He also examined diatoms for Dr Gerald Boalch and copepod faecal pellets for Dr. Roger Harris. Paul examined copepod larval mouth-parts for Roger Harris and David supplemented the photographic department capabilities by being able to use SEM alongside his photographic skills as Senior Photographer.

Publications (1970-1979)

The record shows that 87 papers were published. The authors from the MBA were: George Best, Gerald Boalch, Quentin Bone, Geoff Bryan, Malcolm Clarke, Eric Denton, Peter Gibbs, John Green, Roger Harris, Derek Harbour, Laurie Hummerstone, Mary Parke, Ann Pulsford, Barry Roberts, Keith Ryan, Eric Smith and Alan and Eve Southward.

***Footnote 2** - "Scanning" implies that the beam of "primary" electrons is scanned over the surface of the solid specimen in a raster fashion, as in a television system. The beam hits the specimen and displaces outer orbital electrons from the specimen atoms. These are ejected from the specimen and called "secondary" electrons: they are collected by a detector incorporating a scintillator with a photo-multiplier behind it. The output signal is used to modulate the brightness of a phosphor viewing screen which is scanned in synchrony with the specimen.*

The period 1980-1989

The 1980's saw the EM Unit perhaps at its busiest, certainly at its most diverse in terms of the methods used. Due to increasing demand, a case was made to NERC to re-equip the EM Unit. The application was successful, resulting in a £180,000 grant. The main items were a JEOL 200CX 200 kV Temscan or STEM (scanning transmission electron microscope) and a JEOL 35C SEM. Both of these top-of-the-range research instruments were fitted with x-ray detectors connected to a Link Systems 860 x-ray microanalysis system. The SEM also had a cryostage. There was also a new Reichert Ultracut ultramicrotome complete with the FC4 cryo-attachment for cutting frozen sections. An oxygen plasma-ashing unit, a table-top carbon-coater for SEM analysis specimens, an Anavac analysing vacuum gauge and a 500-litre liquid nitrogen storage tank completed the package. The 200CX installation certificate was dated 7 August 1981.

During the installation of the new microscopes, the Philips EM200 was removed to Torbay General Hospital, after 18 years service, and the T-20 SEM was removed to Polytechnic South West, Plymouth, after one-and-a-half years of service.

The 200CX STEM was installed in Room 56 that was previously an office. The 35C SEM was installed in the basement in what had been the EM printing darkroom. The printing darkroom was moved to Room 86 on the main staircase.

The installation presented a major learning curve for Keith Ryan who spent many long evenings familiarising himself with the new equipment. It involved a major 200 kV research TEM, which was also a STEM (scanning transmission electron microscope, new to the MBA), a state-of-the art multi-mode imaging research SEM (new to the MBA), x-ray microanalysis (new to the MBA) and the facility for cutting frozen sections (also new to the MBA). One application for frozen sections was to be the study of ionic movements by x-ray microanalysis.

The only disappointment concerning the new equipment was the failure of what was specified as a "cooling specimen transfer holder". This was to take frozen sections from the cryo-ultramicrotome to the 200CX. The item that was delivered was a prototype cooling holder with no cryotransfer features. In order to transfer cryosections into the microscope, keeping them in the frozen state, some shielding mechanism is necessary to prevent frosting/thawing during the transfer and time of passage through the microscope airlock – this was not a feature of the holder supplied. The holder tip was a glass bearing which was fixed with a patent glue: the tip would often detach inside the microscope. This meant that many hours were spent extricating it, often from the airlock using chop-sticks provided in the Japanese microscope's tool kit. In use, the holder consumed a large bottle of nitrogen gas each day. It was used in unpublished work on the mobility of chlorine in dried, crystallised microdroplets (by volatilisation in the electron beam). In all other respects, the microscope was a tremendous success.

Fig. 9. Jim Nott and Artemis Nicolaidou using the 200CX. Photo taken 1988.

Fig. 10. Keith Ryan using the 200CX. Photo taken 2001.

Jim Nott used the 200CX extensively in his work on marine food-chains, involving the microanalysis of phosphate granules in marine molluscs that accumulated heavy metals. He collaborated both with other MBA scientists and overseas workers in studies on this type of environmental pollution, notably Dr. Artemis Nicolaidou (Athens, Greece) and Dr. Maria Bebbiano (Lisbon, Portugal). He was assisted very competently throughout this time by Mrs. Linda Mavin.

Above: Fig. 12. JEOL 35C SEM in its original location in the basement, with the cryo-stage attached to the airlock and the brown dewar of the x-ray detector at the rear of the column. The x-ray system was controlled very easily by the small keypad at the right side of the microscope console. The microscope was moved upstairs to Room 57 in 1988.

Left: Fig. 11. As above, showing the SEM set-up with the cryo-stage control system at left and X-ray monitor at top right. Photos taken in 1986.

In Sep. 1983, Keith Ryan attended a Reichert training course in cryomethods at Seefeld-in-Tirol, Austria. This kindled an interest in this aspect of electron microscopy, resulting in several papers describing experiments in cryomethods, concentrating on aspects of rapid freezing and cryo-SEM. As a result of these publications, he was invited back to speak on the course in 1985 and each year until the final course in 1998, when the organiser, Prof. Hellmuth Sitte, retired.

Dr Quentin Bone was elected to Fellowship of the Royal Society in 1984.

Fig. 13. The original bench-top plunge freezer with magnetic stirrer below, coolant temperature feedback control (at right, below), coolant power supply and digital thermometer above. The inset shows the plunge scale and the infra-red sensor that recorded plunge velocity. Photos taken 1985.

Fig. 14. Experimental setup with the deep plunger, coolant heater supply (Variac), thermocouple signal display oscilloscope with digital thermometer above, amplifying oscilloscope for xy signal plotting (plotter at top right), and transient recorder (at bottom right) in the old Lecture Hall. This setup was also used originally with the bench-top plunger (left). Photo taken 1990.

The MBA workshops constructed a new cryo-stage for the SEM in 1985 with several innovative features. An integral anti-contaminator was cooled directly by liquid nitrogen, a Faraday cup was provided to measure beam current for quantitative x-ray microanalysis and a central hole was provided through the stage for examining frozen sections, using a transmitted electron detector below the stage. The stage temperature was controlled by a cartridge heater/thermocouple feedback system. This exercise necessitated the construction of a large vacuum flange to lead the heater and thermocouple wires into the specimen chamber. Another flange was constructed to fit the Robinson back-scattered electron detector. This was much more efficient than the original detector that was supplied with the microscope. A description of the stage was published in Mikroskopie (Wien). Another paper described the direct observation of the freeze-etching and freeze-drying process as it occurred inside the SEM. The design input of David Purse (MBA Engineering Workshop) and John Wood (MBA Electronics Workshop) resulted in their being co-authors on these and other papers.

Fig. 15. Cryo-stage for the 35C SEM. The anti-contaminator is uppermost, cooled by liquid nitrogen passing through the plastic tubing, with a cut-out (near left) for line-of-sight access by the x-ray detector. The hole through the stage can be seen, for transmitted electron imaging of frozen sections. The cartridge heater is seen at left, with red and blue power leads. The clear Perspex Faraday cup (nearest camera) with a white-coated lead is for electron beam measurement during quantitative x-ray microanalysis. This stage was made by David Purse in the MBA Engineering Workshop.

Fig. 16. SEM cryo-stage specimen carrier for frozen sections. The original specimen sledge for bulk samples was milled-out and a block inserted to hold a tip from a Philips TEM specimen rod. This held the grid carrying the frozen sections. A copper ring under the tip acted as a cold support for the specimen. The object at the right is a copper cover that was put in place when the sections were loaded into the holder to protect them as they were put into the SEM. This was removed in the cryo-stage airlock by means of a "sky-hook" before the specimen was inserted into the main stage of the microscope.

Fig. 17. View of a frozen specimen being sputter-coated with gold in the SEM cryo-stage airlock. The cold pre-stage is seen in the centre of the photo, with copper braid attached at left for conduction-cooling by liquid nitrogen. The sputter head is at the top. This was made in the MBA Engineering Workshop. The blue colour is due to the ionised argon plasma that is integral to this process. Cryo-fracture could be done with a scalpel blade that was pre-cooled in a slot in the cryo-stage. Specimens could also be coated using a carbon arc or carbon fibre and also by other metals using a tungsten filament for evaporative coating. Photos taken 1985.

David Purse also built a unique plunge freezer that formed a test-bed for plunge-cooling experiments. It was also used routinely for freezing specimens for further cryomethods. The main methods were freeze-substitution for ultrastructural studies, where the frozen water in specimens is dissolved at -80 °C in a solvent such as acetone or methanol, and freeze-drying for x-ray microanalysis studies, where the frozen water is evaporated under vacuum, so as not to lose chemical moieties as occurs when samples are processed in wet solutions. This device was central to many publications and to work that resulted in Keith Ryan being awarded a Ph.D. in 1991.

The technique that was used to study metals in phosphate granules by Jim Nott involved "fixing" the specimens by plunge-freezing them in liquid ethane. They were then freeze-dried and embedded in epoxy resin, with the resin being infiltrated into the specimens under vacuum. This method avoided the use of chemical fixatives and solvents. The process was designed to retain chemical constituents in the specimen that can otherwise be lost by leaching if chemical processing is used. The method is illustrated in Fig. 25.

Left: Fig. 18. Deep plunge-freezer, overall height 2.25 m.
Top: Fig. 19. Face view of a copper freeze-fracture planchette soldered onto support wires. A centralised micro-thermocouple is glued in place, its wires exit at top to a recording system.
Above: Fig. 20. A pair of planchettes screwed together after filling with gelatine solution as a test specimen, thermocouple wires exit into plastic tubes inside the plunge rod.

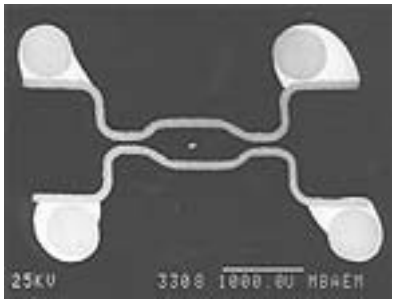

Fig. 21. Back-scatter electron image (in the SEM) of a thermocouple sandwiched between two planchettes (see photos left) after freeze-substitution, resin embedding and grinding. White areas in the image indicate high atomic number elements and dark areas indicate low atomic number elements. The lightest areas are solder (containing tin) holding the copper planchettes onto the four round objects (copper wires). Scale bar 1.0 mm. The thermocouple is in the centre. Photos taken 1987.

Fig. 22. Freeze-drying set-up in Room 61. Nitrogen bottle at left for purging and venting the system, electronic control system in the centre with the white switch box for the liquid nitrogen pump, Anavac mass spectrometer below, analogue thermometer at top above the feed-back power control box for the heater in the specimen stage. The coating unit/freeze-drier is seen behind and the liquid nitrogen dewar for the freeze-drier is at the right. The liquid nitrogen pump is inserted into the top of the dewar. Photo taken in 1989.

Fig. 23. Inserting frozen specimens through the access hole in the lid of the freeze-drier after the system is cooled to -196 °C and vented with dried nitrogen gas. The system was then evacuated, warmed to -80°C and left running for one or two days while the frozen water was removed by sublimation in the vacuum. Photo taken in 1989.

Fig. 24. Close-up view of the freeze-drier specimen stage. Two specimen tubes are seen, full of liquid nitrogen at this early stage. These were simply aluminium foil tubes formed around a drill bit. There are eight specimen stations plus a central station where specimens were parked while the tubes were loaded. A thermocouple is clamped at top left to control the power supply to the liquid nitrogen pump. A free thermocouple is inserted into an empty specimen station to monitor its temperature and this feedback controlled the cooling. Photo taken in 1989.

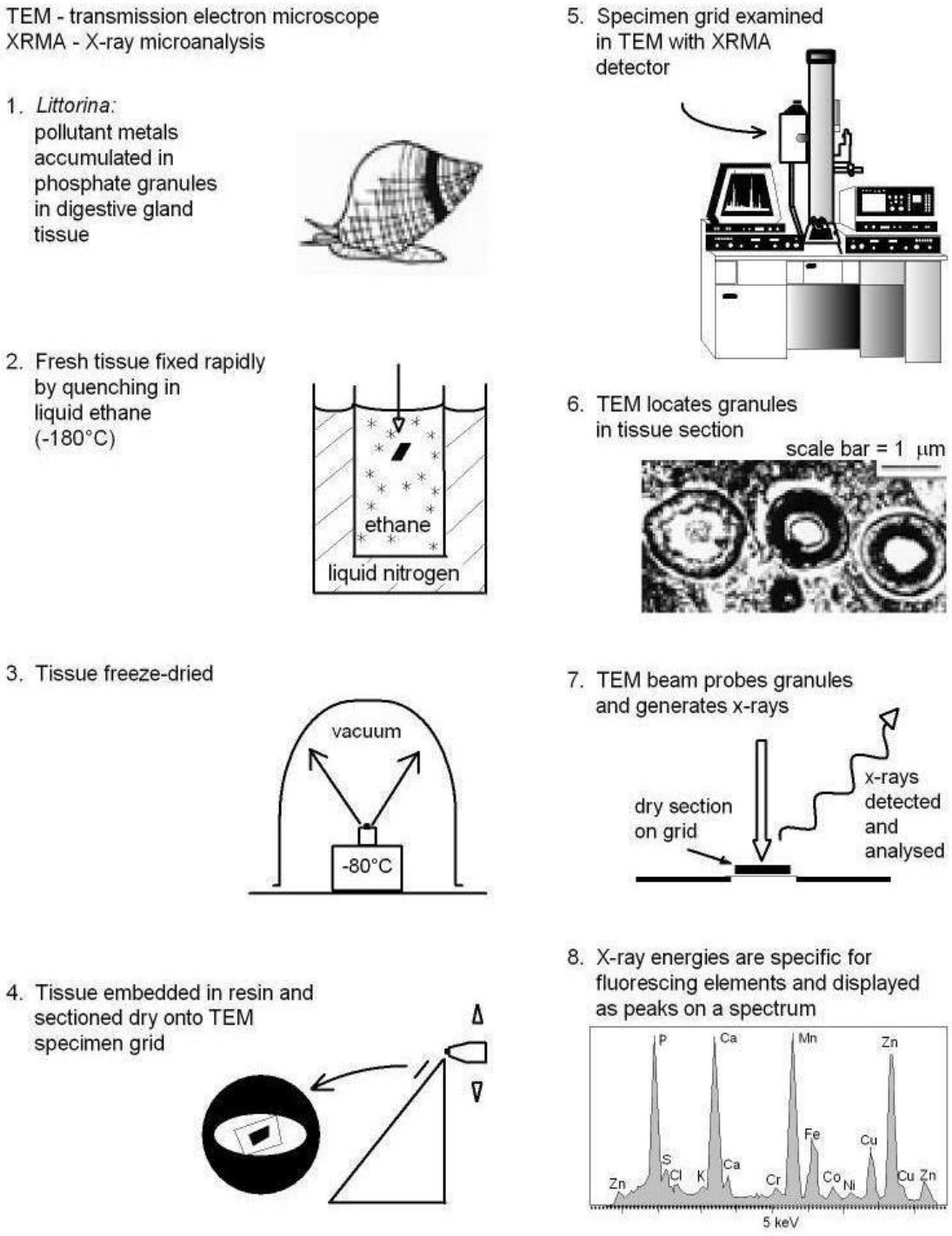

Fig. 25 – The process for examining heavy-metal pollutants in fresh material by rapid freezing, freeze-drying, vacuum embedding, dry-sectioning and x-ray microanalysis in an electron microscope. Redrawn after Nott 1995.

A piece of equipment that was vital to the above work was the Anavac analysing vacuum

gauge. It could be interfaced to both J.E.O.L. electron microscopes and to the freeze-drier using adapters made by David Purse. Its original purpose was to test for vacuum leaks in the microscopes using a small jet of helium aimed at suspect leak points. The sensitivity of the Anavac was such that the spectrum of residual gases in any vacuum could be amplified to look like a spiky range of mountains. It was used later for monitoring the passage of water from specimens that were freeze drying in the cryo-SEM or in the freeze drying set-up on the coating unit. It functioned as a fail-safe feedback monitor in case the liquid nitrogen pump became blocked with ice. If this happened, cooling by liquid nitrogen would fail and the specimen would warm quickly. This would result in the partial pressure of water rising in the vacuum. The Anavac mass spectrometer was tuned to detect water vapour and would operate a trip-switch to cut power to the pump if the partial pressure rose too quickly, thereby preventing its motor from burning out if it had to struggle all night against a blockage. It is a tribute to the Heath Robinson-like system that this never happened.

Quentin Bone FRS and collaborators used cryo-SEM with quantitative x-ray microanalysis as one method in the study of the coelomic fluids of two species of *Sagitta*, the Arrow-worm. The main finding was that the oceanic form, *S. elegans*, had low sodium compared to *S. setosa.* The sodium was replaced by ammonium, which functioned as a buoyancy aid in deep water. The frozen specimens had to be glued into special cryo-supports without thawing them. This was done using graphite dag adhesive at -60 °C and a cryo-mounting table, made by David Purse in the MBA Engineering Workshop.

Fig. 26. Cryo-mounting set-up for attaching frozen specimens into SEM cryostage supports prior to quantitative x-ray microanalysis. The digital thermometer is showing -61 °C which is the operating temperature for the cryo-glue. Photo taken 1986.

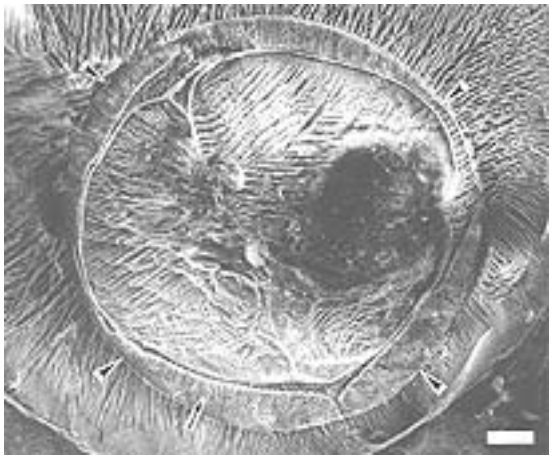

Fig. 27. Freeze-etched frozen, hydrated *Sagitta* specimen in the cryo-SEM. Arrowheads mark the outer cuticle of the specimen which is embedded in frozen seawater. Photo taken 1986.

The 1986/87 Report of the Council notes the installation of a submersible pump in the EM basement. This was located in a sump in order to prevent flood damage to the equipment. This became necessary after a number of floods which usually derived from

the floor above and were of varying seriousness over the years. The floods left tidemarks which George Best pointed out to visitors over the early years. To ensure that the pump remained functional, it was tested every month with a bucket of water. Emergency lighting in the basement was tested every Monday morning on first entering the basement when nothing electrical was running. The lighting was critical in the event of a power cut, when the basement would be plunged into darkness.
The 1987/88 Report of the Council notes that during a Health & Safety Executive inspection a question arose over the compressor on the 200CX TEM. It was discovered that its size brought it under Pressure Vessel Regulations. It had to be replaced with a larger (and far noisier) compressor that had an inspection cover so that internal corrosion could be monitored. The inspectors also asked during the inspection about the handling of liquid propane and other cryogenic fluids. This led to discussion with the inspectors and afterwards Keith Ryan and Malcolm Liddicoat published a paper on the topic in the *Journal of Microscopy*. This was re-published in 1998 in *Microscopy Today*. The Report also notes that Keith Ryan was asked to supply a figure for a textbook edited by Prof. M.A. Hayat, who wrote or edited over thirty books on electron microscopy. Keith was subsequently invited to review the relevant chapter.

The MBA Lecture Hall was the venue for a Zeiss Exhibition in Dec 1987. The main attraction was the Zeiss 902 Energy Filtering TEM. Using ingenious technology, the microscope filtered the inelastically scattered electrons passing through thin sections. These have characteristic energies depending on the atoms that they pass through, thereby enabling identification of chemical elements. The technique is known as Electron Energy Loss Spectroscopy or EELS. This constituted a new form of microanalysis at the subcellular level and was complementary to x-ray microanalysis in that it had much better resolution and was also more efficient for light elements. The microscope was wheeled in through the courtyard, its electron gun fitted, power connected and was displaying high resolution analytical images within two hours. This was very impressive! The laboratory made three further forays into the method later – one was to RAF Halton, Bucks, with Radek Pelc, an MBA visitor, in early 1992 where the RAF were looking at nannoparticles of smoke following the 1985 runway disaster at Manchester Airport: the lab interest was the presence of cadmium in cell components (also see references below to Jim Nott and Keith Ryan visiting the Zeiss factory in 1989 and to Ms. Lorraine Berry's visit to the Philips factory in Eindhoven, Netherlands, in 1999). Other local electron microscopists attended the exhibition at the MBA from the EM units at (1) Polytechnic South West, (2) Greenbank Hospital, (3) English China Clay Ltd. at St. Austell, (4) HM Dockyard Devonport, (5) Royal Naval Engineering College Manadon, (6) Watts, Blake & Bearne Ltd. at Newton Abbott, (7) Plessey Ltd. at Plympton and (8) Exeter University. There was a thriving and diverse community of electron microscopy units in the south-west at that time with there also being installations at Camborne School of Mines and Treliske Hospital, Truro.

The profile of the work at the MBA was such that Keith Ryan was invited to give a presentation at the first Southern Cryo-Users Meeting on 18 February 1988 at the International Mycological Institute, Kew, Surrey. The talk was about plunge-freezing experiments and quantified x-ray microanalysis using cryo-SEM. The group became the

Cryo-Microscopy Group and was affiliated to the Royal Microscopical Society. They met regularly at the Royal Veterinary College, London, for several years until moving to other venues. Keith was invited to give another presentation on 20 November 1989, on rapid cooling techniques for electron microscopy. A third invitation resulted in a presentation on 22 May 1991 about freeze-drying tissue samples for x-ray microanalysis: Jim Nott gave the presentation that followed, on freeze-drying marine organisms for x-ray microanalysis. Keith also arranged another three meetings. These were "themed" and drew on his wide range of contacts, with authorities from both the UK and the continent giving talks. The first of these took place on 27 November 1991, on "Cryofixation". The second was on 20 May 1992 on "Cryo-Electron Microscopy" and the third on 25 November 1992 on "Immuno-electron Microscopy Cryotechniques". These meetings involved a lot of preparation and were very successful, receiving good reviews in the *Proceedings of the Royal Microscopical Society*. They also further raised the profile of the MBA EM Unit.

A change in management occurred in April 1988, when the EM Unit became part of the newly-formed Plymouth Marine Laboratory. Prior to this, Quentin Bone FRS had been the scientist responsible for the electron microscopy unit. From 1988, the unit was managed by Jim Nott until his retirement in 1996.

On 28 June 1988, Keith Ryan was awarded the first Geoffrey Meek Memorial Award for technical advances in microscopy by the Royal Microscopical Society. This took place at the RMS AGM which was held at the Royal Society, Carlton House Terrace, in London, and recognised aspects of his work on cryo-methods for electron microscopy.

The MBA Lecture Hall was the venue again for a joint microscopy exhibition on 24 November 1988, when Reichert-Jung Ltd. held demonstrations of cryo-preparation equipment and Cambridge Instruments Ltd. demonstrated Qantimet image analysis equipment.

Another major upheaval occurred to the EM Unit in November 1988. PML News No. 7 (January 1989) described the movement of the entire suite to the floor above *"for reasons not unconnected with the hazards of negotiating the basement steps with large containers of liquid nitrogen and heavy cylinders of nitrogen, argon and oxygen."* There were problems regarding the moving of items weighed in fractions of a ton as well as their having dimensions which approximated closely to those of the stair well! The disassembly and re-assembly of the instruments was supervised by the instruments' regular service engineers who were well-known to many of the staff – John Barry (JEOL) and Robin Finn (Philips). The move started early on Monday 28 November and was completed by the following Friday afternoon. It involved some interesting block-and-tackle exercises with some of the loads not even "inching" up the stair-well but moving fractions of a centimetre at a time. This was a serious heavy-lifting exercise, in a confined space, involving co-ordinated teams of staff at both the top and the bottom of the stairs. The safety considerations were many and admirably overseen by David Purse. Once the loads were sliding upwards, the downstairs team had to shelter around corners in case the strops failed or the load slipped. For many of those involved it represented a

pinnacle of in-house co-operation with many extra staff being called on to lend their muscles. For moving the biggest items, up to twelve people were needed. Bearing in mind the confines of the area, it was a tight fit. That the instruments were not damaged and worked perfectly after the move was an achievement bordering on the magnificent.

When the moves were completed, the JEOL 35C SEM was in Room 57 and the Philips EM300 was in Room 59. The contents of the basement prep room, including the Edwards 12E6 coating unit, were moved to the neighbouring Room 61, so that the unit was kept together. Shortly afterwards, the upstairs rooms (Rooms 2, 12 and 13) were vacated with chemical prep room equipment (from Room 12) being moved to Room 55, this becoming the EM prep room.

Fig. 28. EM Prep Room (Room 55) after relocation in 1988, showing the two ultramicrotomes, the Reichert Ultracut (nearest) and the OmU3.

Fig. 29. EM Prep Room, view from the door. Both photos taken in 2000.

October 1989 saw a long "EM tour" by Jim Nott and Keith Ryan. First, they drove to the Zeiss factory in Oberkochen, southern Germany, to use the energy-filtering Zeiss 902 microscope again. The next stop was the Institute of Zoology, University of Salzburg, Austria, to give seminars in the department of an old friend of the MBA, Prof. Hans Adam. Finally, they drove to Seefeld, Tirol, mistakenly staying on the autobahn near Innsbruck and heading towards the Brenner Pass and Italy: a short detour saw them arrive in time for supper. Keith gave his regular presentations at the cryo-workshops - an international school for electron microscopy - mentioned under 1983, above. It was a flying visit to the 10-day workshop but Jim Nott made such an impression due to his wide use of cryo-methods that he was invited back free-of-charge the following year! The vehicle used was the old PML Ford Sierra estate car, which coped easily with the German autobahns. Having been told very seriously to check the fluid levels each day, this was duly done with the memorable observation that it appeared to use less than a litre of oil for the entire journey. The other aspect which is well-remembered was the departure from Oberkochen. The car was parked in the inner courtyard of an old hotel, among the Audis, Porsches, BMWs and Mercedes belonging to the other Zeiss visitors. On starting the engine after it had stood idle for two days, the courtyard filled with blue smoke and we left rapidly.

Publications (1980-1989)
The record shows 158 publications.

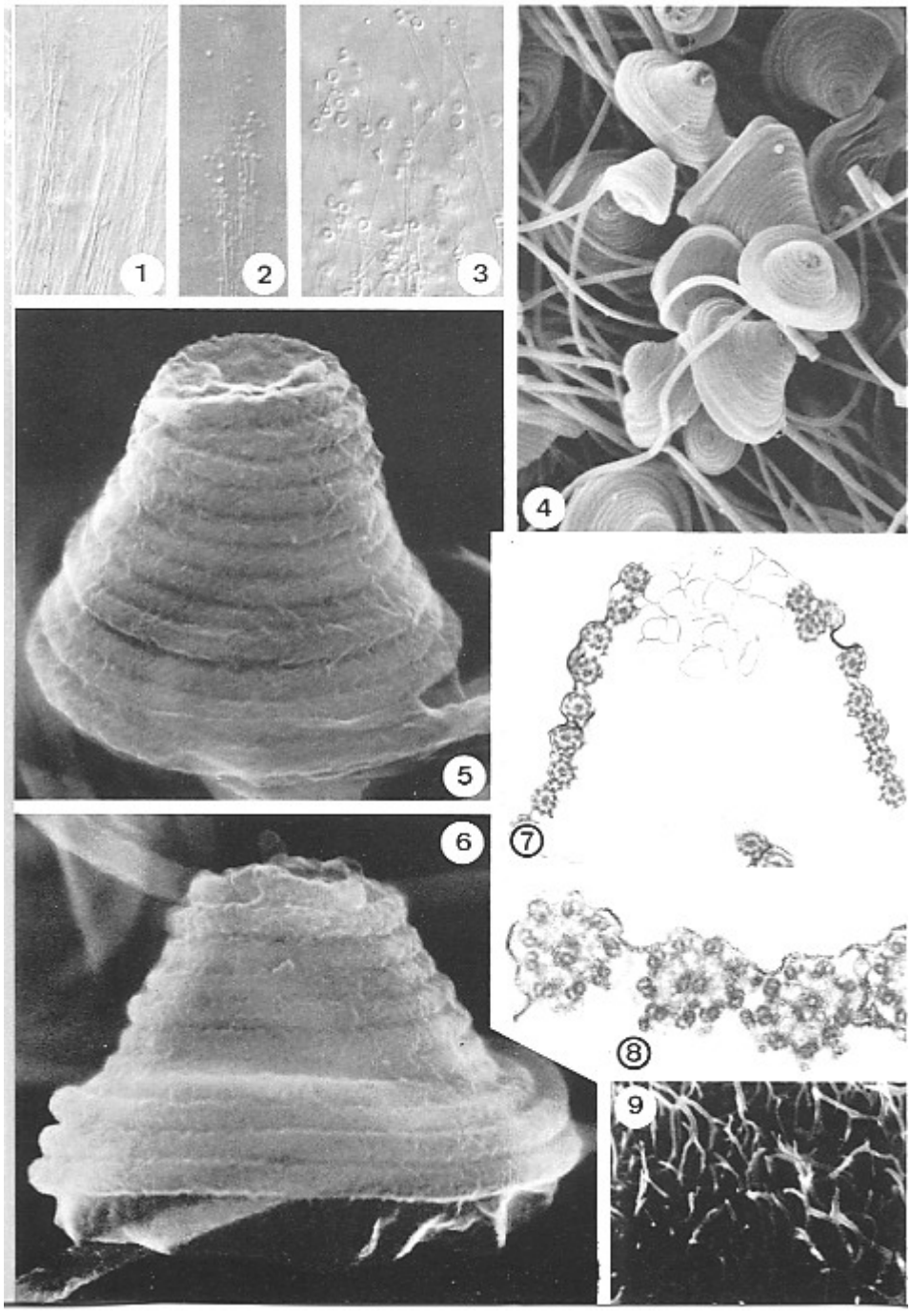

Plate 1. Figures 1-9 from Bone, Q., Ryan K.P. & Pulsford, A., 1982. The nature of complex discocilia in the endostyle of *Ciona* (Tunicata: Ascidiacea). Mikroskopie (Wien), **39**, 149-153. Reproduced with permission.

Published legend to Plate 1: Figure 1. Living endostylar cilia in seawater showing uncoiled tips (290:1). Figures 2 and 3. Two stages in the coiling of ciliary tips brought about by the addition of unbuffered glutaraldehyde (290:1). Figure 4. General view of SEM preparation of endostylar cilia after buffered glutaraldehyde fixation (5600:1). Figures 5. and 6. Two ciliary tips at higher magnification (Fig. 5 = 20,000:1 and Fig. 6 = 24,000:1). Figure 7. Section of a ciliary coiled tip (1470:1). Figure 8. Part of section across coil as in 7, showing orientation of central doublets (76,000:1). Figure 9. Uncoiled tips of endostylar cilia protruding from mucus sheet (1700:1).

1-3 Nomarski interference optics.; 4: SEM preparation, JEOL T-20; 5 and 6: SEM preparation JEOL 200 CX TEMSCAN; 7 and 8: TEM sections, Philips EM 300; 9: SEM preparation quenched in liquid N_2 slush and freeze-dried in JEOL 35-C cryoattachement.

This work combined the use of light microscopy (with Nomarksi optics) to observe the living specimen as it was chemically fixed, conventional TEM and SEM to observe ultrastructural aspects and cryo-SEM to show that the coiled tips did not occur after cryofixation and freeze-drying.

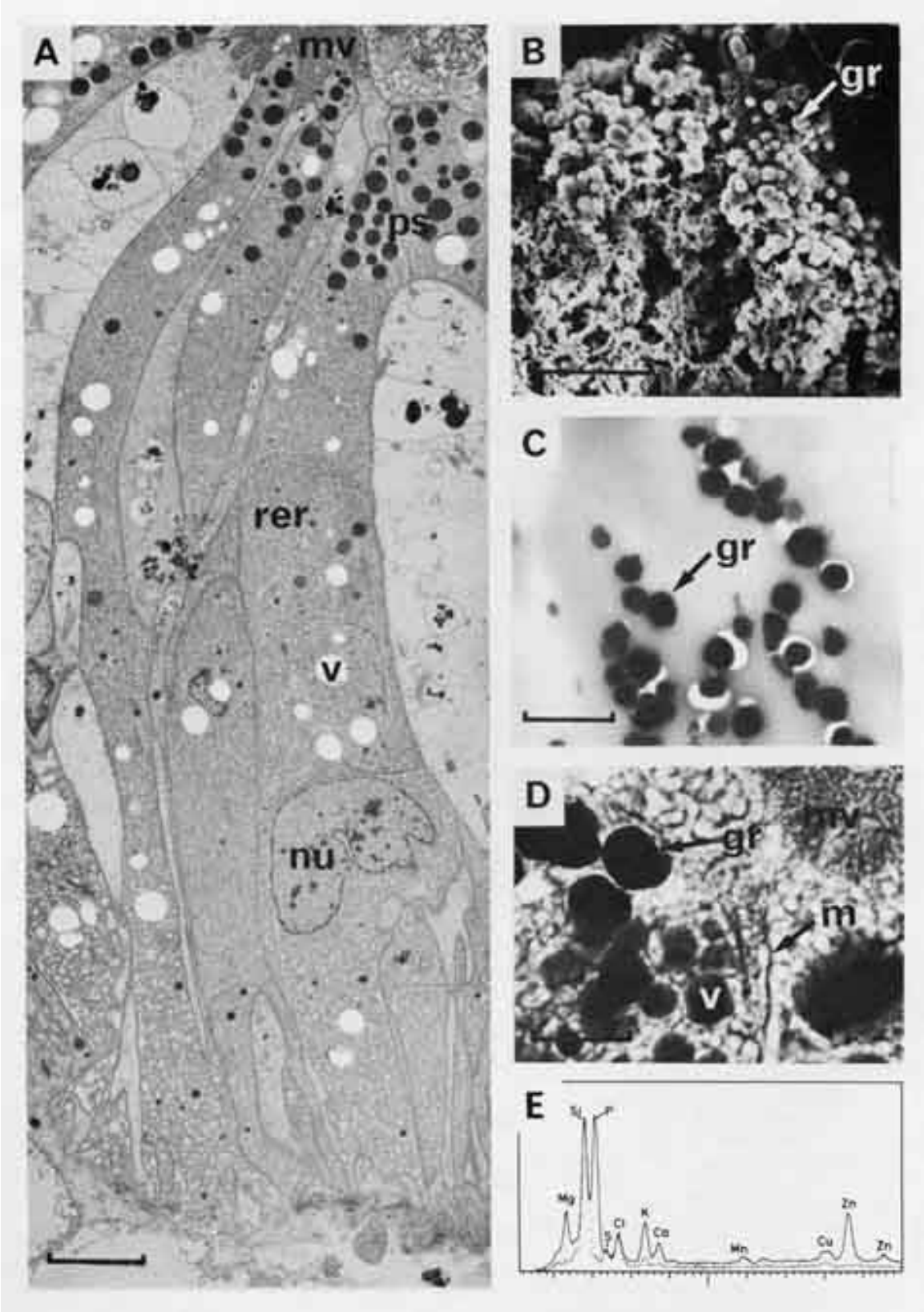

Plate 2

Plate 2 - Figure 4 from Mason, A.Z., Simkiss, K. and Ryan, K., 1984. The ultrastructural localisation of metals in specimens of *Littorina littorea* collected from clean and polluted sites. Journal of the Marine Biological Association of the United Kingdom, **64**, 699-720. Reproduced with permission.

Published legend to Plate 2: Fig.4. (A) TEM micrograph illustrating the longitudinal organisation of a conventionally fixed basophil cell in the digestive tubule of an animal collected from Restronguet Creek. The cell exhibits a microvillous border (mv) and contains numerous spherical protein secretions (ps) apically. The basophilia shown by these cells is due, principally, to well-developed whorls of rough endoplasmic reticulum (rer). The Golgi body is active and the cells have a conspicuous nucleus which lies in the basal region of the cell (nu). Numerous holes in the cytoplasmic vesicles (v) in the apical and mid regions of the cell mark the regions formerly occupied by the mineralised granules found in these cells. These granules are hard, and are readily removed from the sections during the sectioning process. Scale bar represents 5 μm. (B) SEM micrograph of a thick cryosection of a digestive tubule incinerated in an oxygen plasma. The position of the basal cells in the tubule is clearly marked by the presence of numerous spherical ash-resistant granules (gr). Scale bar represents 10 μm. (C) TEM micrograph of an ultra-thin section of a basophil cell. The cells contain numerous electron-dense granules (gr) but contain few other recognizable features. Scale bar represents 3 μm. (D) STEM micrograph of an ultrathin cryosection of a basophil cell incinerated in an oxygen plasma. Ashing introduces contrast to the section by producing electron-dense residues which can be identified as cell membranes (m), microvilli (mv) and electron-dense vesicles (v) secretions and granules (gr). Scale bar represents 2 μm. (E) Elemental x-ray spectra from the analysis of a cryosectioned basophil cell from a Restronguet Creek individual. The granules produce major peaks for magnesium, phosphorus, potassium, calcium manganese and zinc. Peaks for copper, sulphur and chlorine are evident in analyses of both the cytoplasm and the granules.

This work combined the use of standard TEM, SEM, STEM and x-ray microanalysis following cryo-preparation and plasma-ashing methods.

The period 1990-1999

The 1990's were another very active decade in the EM Unit, with the involvement of several PhD students, including: Lorraine Berry (Swansea), Helen Chesters (Swansea), Susana Coehlo (Plymouth), Toby Collins (Sheffield), Mireille Crampe (Plymouth), Liz Dyrynda (Swansea), Sylvianne Gony-Lemaire (Paris, France), Pedro Lima (Sheffield), Lydia Mäthger (Sheffield), Paula Moschella (Southampton), Maria Odblöm (Plymouth), Candida Reed (Sheffield), Emma Reynolds (Plymouth), Pauline Smith (Plymouth) and Dorothea Sommerfeld (Plymouth). These fifteen students were accompanied by many other students who did projects for their B.Sc., M.Sc. and M.Res. degrees, mainly from the University of Plymouth.

The students covered a wide range of studies: cephalopod physiology, mussel studies, coccolith structure and physiology, flounder structure and physiology and protochordate genome work. Major on-going topics were Jim Nott's work on metal accumulation and excretion in molluscs, Quentin Bone's work on protochordate structure and physiology and John Green's studies on phytoflagellates.

In October 1998, Keith Ryan was invited again to lecture at the Seefeld cryo-workshops in the Tirol, for their final session. He drove to Austria with Toby Collins (Sheffield) who had a place on the courses. They were joined in Seefeld by Lucy Puddeford (Swansea), who travelled to Salzburg by plane and then to Innsbruck by train. After the workshop, Keith and Toby drove to Salzburg to visit the EM facilities at the University's Institute for Zoology, taking Lucy back to Salzburg airport *en route*.

In Nov. 1999, Keith Ryan and Lorraine Berry (Swansea) drove to the Philips factory at Eindhoven in the Netherlands, to use the Philips energy-filtering Tecnai 12 TEM. This was to visualise calcium in intracellular compartments, which was done with some success. Afterwards, they visited Prof. Sitte and Dr. Klaus Neumann in Homburg, Germany, to collect some donated equipment, and then Dr. Danielle Spehner, a virologist and cryo-TEM practitioner with the blood transfusion centre in Strasbourg, France.

Another major change occurred on 1 April 1999 when the management of the EM Unit came again under the umbrella of the Marine Biological Association.

The interest in cryomethods and the work on them at the MBA was such that during the period 1985-1998, Keith Ryan gave 53 presentations on these methods: many at the international workshops in Seefeld, Austria, where results from the MBA experiments were sought. Presentations were also invited by the Universities of Salzburg, Zurich, Jena, Cardiff, Bristol and London (King's College). Also, he was invited to talk at the Blood Transfusion Service, Strasbourg, where cryo-EM is done work on blood-borne viruses. Five extended sessions were given during the tenure of a guest professorship in Salzburg in 1993. Some of the presentations were given at international meetings in Cambridge (1985, 1990), Chicago, USA, (1992, 1997), Toronto, Canada (1994), Charleston, USA (1994) and at METU, the Middle East Technical University, Ankara, Turkey (1997), where he organised and/or chaired some of the sessions in the meetings.

Fig. 30. EM Personnel in the 1990's: Jim Nott (retired May 1996), Keith Ryan (voluntary early retirement in Nov. 1998, he finally left the MBA after returning to manage the unit on a contract with the University of Plymouth that ended in Feb. 2004) and Linda Mavin (voluntary early retirement in May 1996). Photo taken in 1993.

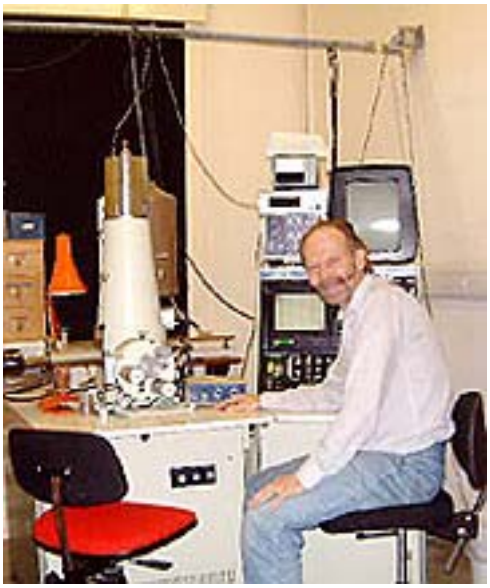

Fig. 31. Keith Ryan using the JEOL 35C SEM in Room 57 where it was relocated after the EM Unit was moved upstairs from the basement in 1988. Photo taken in 2000.

Publications (1990-1991)

The record shows 113 papers.

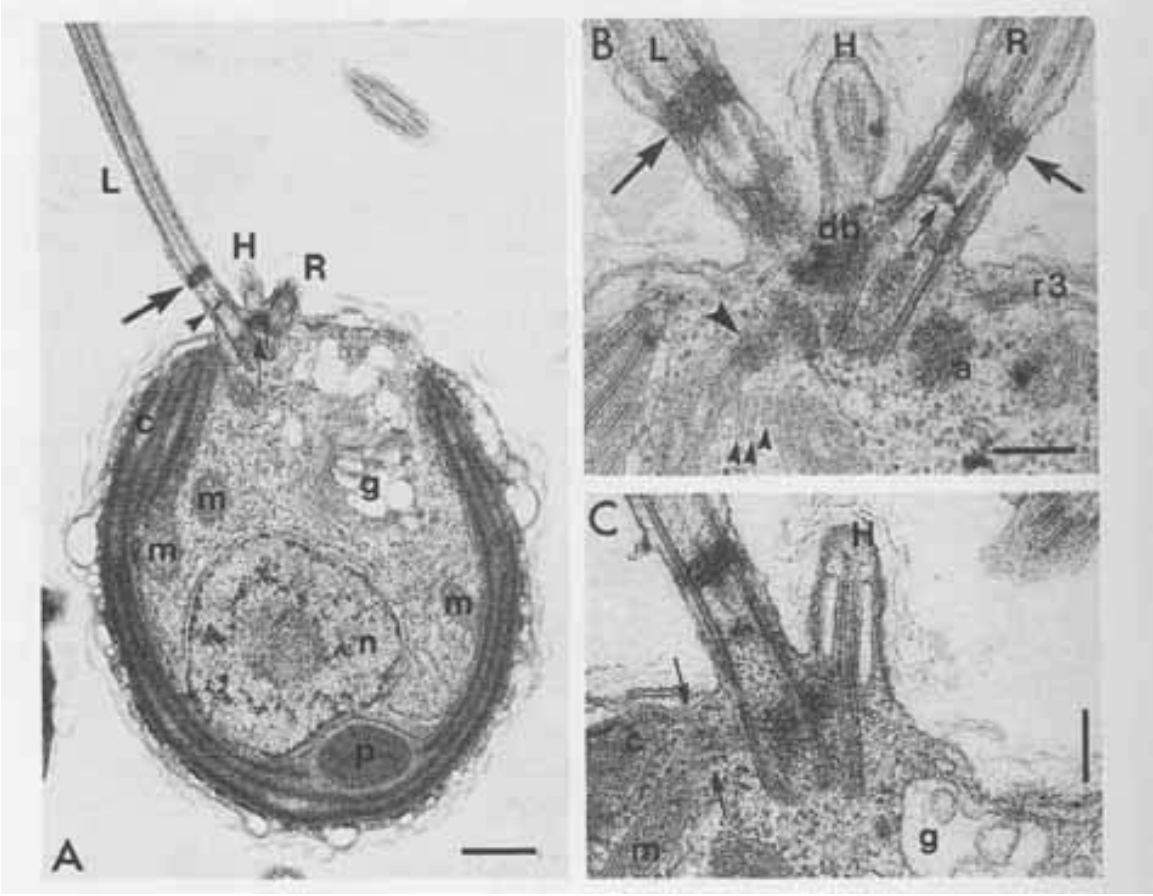

Plate 3 - Figure 2 from Hori, T. and Green, J.C., 1991. The ultrastructure of the flagellar root system of *Isochrysis galbana* Parke (Prymnesiophyta). Journal of the Marine Biological Association of the United Kingdom, 71, 137-152. Reproduced with permission.

Published legend to Plate 3. Figure 2. (A) Oblique-longitudinal section of the cell passing through the flagellar pole and showing the position of all three appendages. The section illustrates also several mitochondrial profiles and the relative positions of the chloroplast, pyrenoid, nucleus and Golgi body. Other features indicated include the proximal flagellar partition and axosome (small arrow-head), the distal connecting band (small arrow) and the distal flagellar 'plug' (large arrow). Scale bar=0.5 μm. (B) A section passing through the proximal parts of the two flagella and the haptonema, the latter cut obliquely. The small arrow indicates the proximal flagellar transverse partition with the central axosome, and the large arrows, the distal fibrous plug. Note also the MTs (small arrow-heads) and the fibrous material (large arrow-head) which are components of the root r1, and the body of amorphous material (a) near the base of the right basal body. Scale bar=0.2 μm. (C) A section passing longitudinally through the scale-covered short haptonema and the proximal part of the left flagellum. MTs from root r1 lie between the arrows. Scale bar=0.2 μm.

The period 2000-2005

The Philips EM300 was removed in parts during April 2002. The column segments, vacuum system and all removable electrical units were stripped from the microscope and taken to Eurocases Ltd., Plympton, for professional crating. They were then collected and taken by road to the University of Athens, Greece, where they are spare parts for three working EM300 microscopes.

The JEOL 35C SEM was removed from the building on 1 October 2003. It was used during the previous week for taking stereo images of the mucus feeding net in Amphioxus for Quentin Bone FRS - something of "a first" even at the last!

The SEM is still an attractive item, being a research-grade analogue instrument - some digital replacement models are already obsolete because of advances in computer technology. It met its test resolution easily at its previous service. It featured the normal secondary electron imaging mode, Robinson back-scatter imaging, x-ray microanalysis, x-ray map imaging, unusually - a transmitted electron detector for tissue sections and a combined transmission and bulk specimen cryo-stage system with a novel anti-contaminator. It also had the ability to mix all these signals. Its shortcoming for the modern era was the lack of a digital camera, although this will be added in its new home at the University of Teesside, Middlesborough. At the time of reviewing this account (February 2007), the SEM was pictured on the University's web site campus tour of the School of Science & Technology' where it is a valued asset.

February 2004 saw the retirement of Keith Ryan, the last person employed in electron microscopy at the laboratory. Since then there has been EM activity, mainly by Dr Declan Schroeder, Quentin Bone FRS and Prof Colin Brownlee with students and visitors using the facilities offered at the University of Plymouth. The JEOL 2000CX instrument is still in working order and is probably still the most powerful microscope west of Oxford, with its 200 kV potential and 1.4 Ångström-unit resolution. It is also still a superb SEM for small specimens. Its drawback is its lack of digital imaging capability. The plate camera still produces excellent sheet film negatives that can be scanned into digital format thus giving the best of both possibilities, particularly as the negative is much larger than any digital camera chip. If there was an opportunity, the microscope could be upgraded by adding a digital camera. Alternatively, digital electron imaging plates could be used in the plate camera that are then downloaded into a computer and reused again.

Keith Ryan returned to the unit periodically on a casual basis, between February and November 2004 to prepare sections for Dr. Delphine Bonnet (PML) after initial discussions about the project with Jim Nott. The sections were of copepods, being examined for stored fats at different stations, depths and seasons in the North Atlantic. The sections were screened by light microscopy using semi-thin sections in Plymouth prior to detailed TEM examination of the thin sections in Marseilles, France. The final clearing out of effects was done in January 2005.

The electron microscope facilities in the laboratory at this time consisted of the JEOL 200CX TEMSCAN in Room 56 and a preparation room in room 61. These were managed by Dr. Declan Schroeder and Mr. Matt Hall.

Publications (2000-2006)
The record shows 26 papers.

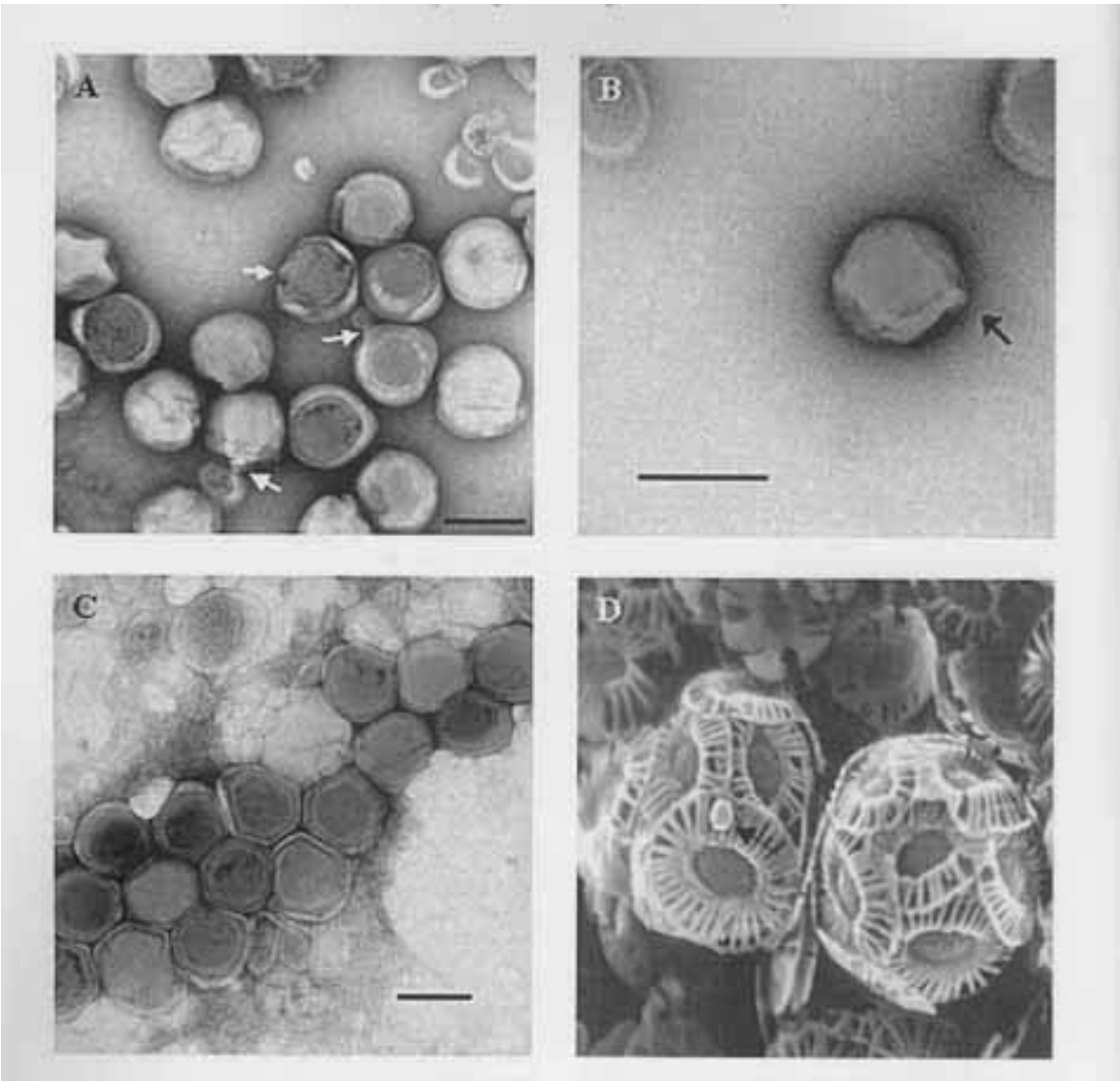

Plate 4 - Figure 7 from Wilson, W.H., Tarran, G. & Zhubkov, M.V., 2002. Isolation of viruses responsible for the demise of an *Emiliania huxleyii* bloom in the English Channel. Journal of the Marine Biological Association of the United Kingdom, 82, 369-377. Reproduced with permission.

Published legend to Plate 4. Figure 7. Transmission electron microscope (TEM) and scanning electron microscope (SEM) analysis of *Emiliania huxleyi*-specific virus isolates. (A) and (B) TEMs of *EhV84*, the arrows indicate possible tail stubs that may be involved in attachment. (C) TEM of *EhV86*. (D) SEM of *EhB86* (arrowed) attached to a *E. huxleyi* cell. Scale bars: A-C ~190 nm; D, no scale information available.

This work used both standard TEM and SEM on the JEOL 200CX TEMSCAN instrument, demonstrating its potential for high-resolution, especially in the SEM mode in the study of marine viruses.

Appendix 1 – EM PERSONNEL

1965-1969

The MBA personnel involved in the early days were Quentin Bone, John Green and Rosie Jowett (working for Mary Parke) supported by George Best, who oversaw the day-to-day arrangements in the Unit, and Rosie Stapleton. Keith Ryan joined the staff on 1 January 1969.

1970-1979

There were several staff changes during these years, involving the support post. Rosie Stapleton (Lab Attendant) resigned on 29 August 1969, her successor was Miss Myra Bowkett (ASO) who joined on 15 September 1969 and resigned on 31 May 1972. Miss Alison Coles joined the unit on 1 June 1972 and resigned on 31 March 1974. Miss Heather Knott joined on 13 May 1974 and left, as Mrs. Heather Hodgkinson, on 31 July 1976. Her successor was Ann Pulsford who joined on 1 October 1976. George Best (SSO) retired on 29 April 1977, having joined the laboratory as a histologist in 1956 and left, after a final period of part-time employment, in 1986, aged 75. He died on 30 August 2001, aged 90.

Fig. 32. A clutch of MBA FRS's: George Best's retirement, 27 April 1977, with Prof. Sir Eric Denton FRS, Prof. Sir Eric Smith FRS and Dr Quentin Bone FRS

Besides staff employed specifically to work in the EM Unit there were other regular staff users who were assistants to Mary Parke and John Green in the Botany Department. These were Rosie Jowett (who joined in July 1964 and left 29 August 1970), Miss Jean Purkiss (joined 1 November 1970, resigned 14 May 1971), Christine Bedford (joined 2 August 1971) who left as Mrs Christine Joselyn (September 1973) and joined the electron microscopy unit at Polytechnic South West (now the University of Plymouth), Ann Shears (joined 9 October 1973, resigned 24 September 1976) and Cilla Course (joined 1 November 1976, seconded to Plymouth Marine Laboratory from 1 April 1988).

1980-1989

Jim Nott (PSO) joined the MBA staff on 1 February 1981 from the Electron Microscopy Unit at Menai Bridge, University College of North Wales, Bangor. He became a major user, particularly in the field of heavy metal pollution using x-ray microanalysis. Linda Mavin (ASO) joined the staff 1 April 1982, having spent 7 years working in the laboratory on a Ministry of Overseas Development Grant with Dr. Peter Wickstead. Also, Mr. Richard Pipe (IMER), became a regular user of the Philips EM300, first appearing in the log book in Dec. 1982. In 1988, Ann Pulsford, until that time an electron microscopy assistant to Quentin Bone, was transferred to NERC Plymouth Marine Laboratory and joined one of the main research projects. She later re-joined the MBA as Editor of the *Journal*.

1990-1999

Quentin Bone retired on 31 August 1991 after 32 years service. Jim Nott retired on 31 May 1996 after 14 years service with the MBA and a total of 31 years in marine biology and electron microscopy (he started his career in 1965 at the NERC Unit in Menai Bridge). Linda Mavin took voluntary early retirement from PML on 6 May 1996, after a total of 21 years in the laboratory. Keith Ryan took voluntary early retirement on 30 November 1998 after 30 years service. He returned to the laboratory on 22 Feb. 1999 to manage the EM Unit on a 5-year contract arranged through the University of Plymouth, finally leaving in February 2004, with 35 years service.

Appendix 2 – MBA staff users

MBA staff users and visitors (1967-69)
In addition to those mentioned above: Dr. John Green, Rosemary Jowett, Dr. Eve Southward, John Pilkington, Dennis Taylor (NATO Postdoctoral Fellow).

MBA staff users (1970-1979) - *besides some of those mentioned above*
Dr. Jerzy Alexandrowicz, Jennifer Allen, Dr. Gerald Boalch, Ian Butler, Marilyn Butterworth, Dr. Geoff Bryan, Dr. Malcolm Clarke, Peter Corbin, Cilla Course, Sir Eric Denton, Dr. Peter Gibbs, Sir John Gray, Steve Hampshire, Derek Harbour, Dr. Roger Harris, Dr. Patrick Holligan, Laurie Hummerstone, Christine Jocelyn, Jennifer Liddicoat, David Nicholson, Phil Pascoe, Dr. Barry Roberts, Ann Shears, Sir Eric Smith, Paul Tranter, Dr. Peter Wickstead.

MBA staff users (1980-1989) - *besides some of those mentioned above*
Euan Brown, Dr. Malcolm Clarke, Dr. Bill Langston, Susan Spence, Dr. Roddy Williamson, Dr. Eric Corner

MBA/PML staff users (1990-1999) - *besides some of those mentioned above*
Claudia Birmellin**,** Dr Tony Clare, Toby Collins, Dr. Paul Dando, Sophia Farley, Dr. Geoff Mattison, Pauline Smith**,** Dr. William Wilson

MBA Staff users (2000-2006) – *besides some of those mentioned above*
Prof. Colin Brownlee, Dr. Simon Davy, Sophia Farley, Dr. Vicki Goddard, Dr. Richard Kirby, Dr. Declan Schroeder, Dr. David Sims, Dr. Alison Taylor, Prof. Roddie Williamson

Appendix 3 – visiting workers (some old records are missing)

The following names are taken from the electron microscope log books, Reports of the Council, visitor cards and collected publications. Their full details are not available in many cases and their familiar names are used here

Visiting workers (1965-1969)
Dr. David Chapman (Halifax, Nova Scotia), Dr. Dennis Taylor (Swansea), Dr. Kay Lyons (Birmingham), Dr. Robert Fournier (Rhode Island, USA) and Dr. John Pilkington (SRC Research Fellow).

Visiting workers (1970-1979) – *besides some of those mentioned above*
Maria Anderson (Birmingham), Dr. Peter Baker (London), Dr. Magnus Bundgaard (Copenhagen, Denmark), Dr. David Chapman (Nova Scotia, Canada), Margaret Charlton (Birmingham), Marie-Josephe Chretiennot (Marseilles, France), Prof. Ragnar Fange (Götheborg, Sweden), Prof. Paul Fankboner (Burnaby, Canada), Janet Findlayson (Birmingham), Dr. Per Flood (Bergen, Norway), Dr. David George (London), Mme. Daniele Georges (Grenoble, France), Dr. David Gilbert (London), Dr. T.H.J. Gilmour (Saskatoon, USA), Mme Doris Chragozlou-van Ginnekin (Paris, France), Basil Gomez (Plymouth), Dr. Moyshe Gophen (Tiberias, Israel), Uta Gorenflo (Heidelberg, Germany), Lois Gundrum (Oxford), Dr. Eric Hamilton (IMER), Dr. Malcolm Hart (Plymouth), Dr. Peter Herring (IOS, Wormley), Dr. N.D. Holland (La Jolla, USA), Prof. George Hughes (Bristol), Torbin Johnssen (Bergen, Norway)**,** Noori al Kadhomy (Bristol/Iraq), Prof. N.E. Kemp (Michigan, USA), Anne Linley (IMER), Dr. Annetrudi Kress (Basle, Switzerland), Brian Lakey (Plymouth), Prof. J. Llewellyn (Birmingham), Kathleen "Kay" Lyons (Birmingham), Dr. George Mackie (Victoria, Canada), Justin Marshall (St. Andrews), Dr. A.Z. Mason (Reading), Derek Mattey (Plymouth), Bjorn Midttum (Bergen, Norway), Dr. Salem al-Mohanna (Bangor), Dr. Mike Moore (IMER), Prof. Colin Nicol (Texas, USA), Dr. Marion Nixon (London), Dr. Derek Paul (Manchester), Dr. Richard Pienaar (Johannesburg, South Africa), Dr. Chris Reid (IMER), Dr. Elaine Robson (Reading), Dr. Helen Saibil (London), Ann Smith (London), Dr. Richard Smith (Alberta, Canada), Derek Ternan (Plymouth), Jan Thorne (Plymouth), Phil Weaver (Plymouth), Prof. Paul Witkovsky (New York, USA), Dr. John Whitaker (London), Dr. Philip Whiting (Bristol), Mrs. V.A. Zammit (Malta).

Visiting workers (1980-1989) – *besides some of those mentioned above*
Besides some of the visitors listed above for the 1970's, the following new visitors used the electron microscopy facilities: Prof. Hans Adam (Salzburg, Austria), Dr. Chris Alexander (Darwin, Australia), Miss N. Allen (IMER), T.J.A. Allen (London), R. Amoroso (Cambridge), Dr. L.H. Bannister (London), David Barker (Birmingham), J.M. Bateson (Plymouth), Dr. Maria Bebianno (Lisbon, Portugal), Geoff Bond (IMER), Mr. E.A. Brown (London), Dr. Tony Brown (London), P.D. Burton (London), M.T. Burrows (Manchester), Arthur Butt (King's College, London), D. Campbell (Birmingham), Dr. Tony Carruthers (London), Prof. David Chapman (Nova Scotia, Canada), Dr. Jeff Corwin (La Jolla, USA), Dr. Simon Cragg (Papua, New Guinea), Dr. Brian Dale (Naples, Italy), S. Darlington (Plymouth), Mme. Louise Dufresne-Dubé (Roscoff, France), Dr. Catherine

Gill (Roscoff, France), Miss A. Greaves (Plymouth), Tony Gregson (Birmingham), Sir John Grey, Dr. G.A. and Mrs. S.R.A. Hällfors (Helsinki, Finland), Dr. Eric Hamilton (IMER), Mr. S. Hart (Plymouth), Dr. David Hibberd (Cambridge), Dr. Matthew Holley (Oxford), Dr. T. Hori (Tsukuba, Japan), Mr. Paul Jenkins (Plymouth), Dr. Ian Joint (IMER), Dr. G.C. Kearn (East Anglia), Mrs. Gabrielle Kennaway (Bangor), Dr. W.M. Kier (Woods Hole, USA), Dr. R.H. Knight (Plymouth), Prof. Kurt Kotrschal (Salzburg, Austria), Prof. Annetrudi Kress (Basle, Switzerland), Dr. Marek Lipinski (Gdynia, Poland), Ann Linley (IMER), Prof. Jack Llewellyn (Birmingham), Dr. N.A. Lockett (Adelaide, Australia), Justin Marshall (St. Andrews, Scotland), Dr. A.Z. Mason (Reading), Dr. J. Millward (Plymouth), Dr. John Messenger (Sheffield), Dr. Salem al-Mohanna (Bangor), Claude Morris (Cambridge & Newfoundland, Canada), John Morrow (Plymouth), Dr. Artemis Nicolaidou (Athens, Greece), F. Pleijel (Stockholm, Sweden), Alan Pomeroy (IMER), Sara Roberts (Plymouth), J.D. Rowson (Birmingham), Dr. Helen Saibil (London), Prof. L. Salvini-Plawen (Vienna, Austria), Prof. Ken Simkiss (Reading), Dr. Hiroaki Somiya (Azabu, Japan), Dr. Chris Stolinski (St. Mary's Hospital School of Medicine, London), Dr. Piotr Sroczynski (Gdansk, Poland), Mike Tomlinson (Bath), Dr. M. Wallin (Gothenburg, Sweden), Dr. Mary Whitear (London), Prof. A.G. Zapata (Leon, Spain).

Visiting workers (1990-1999) - *besides some of those mentioned above*
Dr. Mel Austen (Plymouth Marine Laboratory), Lorraine Berry (Swansea), Laurence Bristow (Plymouth), Helen Chesters (Swansea), Toby Collins (Sheffield), Mike Cox (Warwick), Mireille Crampe (Plymouth), Daniel Davies (Plymouth), Liam Dawe (Plymouth), Liz Dyrynda (Swansea), Stuart Gibb (East Anglia), Jo Hagger (Plymouth), Emma Hambly (Warwick), Jane Hawkridge (Swansea), Eva Hertzog (Salzburg, Austria), Rose Kerr (Plymouth), Dr. Reihardt and Fr. Lilli Kikkinger (Vienna, Austria), Gudlaug Thóra Kristenjánsdótir (Iceland), Daniel Lane (Plymouth), Ann Langston (Plymouth), Sylvianne Gony-Lemaire (Paris, France), Pedro Lima (Sheffield), Freyja Lockwood (Plymouth), Adda Mänhardt (Salzburg, Austria), Rhoda Marshall (Belfast), Lydia Mathger (Sheffield), Andrew Millard (Plymouth), Paula Moschella (Southampton), Maria Ödblom (Plymouth), Dr. Margit Palzenberger (Salzburg, Austria), Radek Pelc (Prague, Czechoslovakia), Lucy Puddeford (Swansea), Ms Candida Reed (Sheffield), Emma Reynolds (Plymouth), Daniel Rittschof (Duke, NC, USA), Pauline Smith (Plymouth), Rachel Roberts (Brighton), Dorothea Sommerfeldt (Plymouth), Andres Tretyn (Gdansk, Poland), Anna Wood (Plymouth).

Visiting workers etc. (2000-2006) – *besides some of those mentioned above*
Marcia Athanasius (Plymouth), Andrea Baker (Leeds), Gaia Biggi (Plymouth), Delphine Bonnet (PML), Georgina Budd (Swansea), Sara Burchett (Plymouth), Martin Canty (Plymouth), Andy Cawthorne (Plymouth), Laura Chidgey (Plymouth), Susana Coehlo (Plymouth), Ana Correia (Lisbon, Portugal), Amy Dale (Plymouth), Isobel Francis (Plymouth), Emma Hambly (Warwick), Becci Hards (Plymouth College), Jenna Ho (Plymouth), Alan Hodgson (Rhodes, S. Africa), Tammy Horton (Reading), Shelley Lockett (Plymouth), Paula Moschella (Southampton), Isobel Francis (Plymouth), Clare Hynes (Plymouth), Suzanna McDonald (Plymouth), Paula Moschella (Southampton), Cornelia Muncke (Plymouth), Lisa Nelson (Plymouth), Alex Parfitt (Reading), Pauline

Ross (Western Sydney, Australia), Glen Tarran (PML), Simon Zavad (Plymouth).

Appendix 4

LIST OF ELECTRON MICROSCOPY PUBLICATIONS
Listed in year-order
(Total 413)

1967

Best, A.C.G. and Nicol, J.A.C., 1967.
Reflecting cells of the Elasmobranch *tapetum lucidum*.
Contributions in Marine Science, 4, 172-201.

1968

Chapman, D.M., 1968.
Structure, histochemistry and formation of the podocyst and cuticle of *Aurelia aurita*.
Journal of the Marine Biological Association of the United Kingdom, 48, 187-208.

Chapman, D.M., 1968.
A new type of muscle cell from the subumbrella of *Obelia*.
Journal of the Marine Biological Association of the United Kingdom, 48, 667-688.

Taylor, D.L., 1968.
Chloroplasts as symbiotic organelles in the digestive gland of *Elysia viridis* (Gastropoda: Opisthobranchia).
Journal of the Marine Biological Association of the United Kingdom, 48, 1-15.

Taylor, D.L., 1968.
In situ studies on the cytochemistry and ultrastructure of a symbiotic marine dinoflagellate.
Journal of the Marine Biological Association of the United Kingdom, 48, 349-366.

1969

Chapman, D.M., 1969.
The nature of Cnidarian desmocytes.
Tissue and Cell, 4, 619-632.

Fournier, R.O., 1969.
Observations on the flagellate *Diacronema vlkianum* Prauser.
British Phycological Journal, 4, 185-190.

Lyons, K.M., 1969.
Sense organs of Monogenean skin parasites ending in a typical cilium.
Parasitology, 59, 611-623.

Lyons, K.M., 1969.
Compound sensilla in Monogenean skin parasites.
Parasitology, 59, 625-636.

Lyons, K.M., 1969.
The fine structure of the body wall of *Gyrocotyle urna*.
Zeitschrift fur Parasitenkunde, 33, 93-109.

Pilkington, J.B., 1969.
The organisation of skeletal tissues in the spines of *Echinus*.
Journal of the Marine Biological Association of the United Kingdom, 49, 857-877.

Taylor, D.L., 1969.
Identity of Zooanthellae isolated from some Pacific Tridacnidae.
Journal of Phycology, 5, 336-340.

Taylor, D.L., 1969.
The nutritional relationships of *Anemonia sulcata* and its dinoflagellate symbiont.
Journal of Cell Science, 4, 751-767.

1970

Chapman, D.M., 1970.
Re-extension mechanism of a scyphistoma tentacle.
Canadian Journal of Zoology, 48, 931-943.

Green, J.C. and Manton, I., 1970.
Studies in the fine structure and taxonomy of flagellates in the genus *Pavlova*. I - a revision of *Pavlova gyrans*, the type species.
Journal of the Marine Biological Association of the United Kingdom, 50, 1113-1130.

Lyons, K.M., 1970.
The fine structure and formation of the adult epidermis of two skin parasitic monogeneans, *Entobdella soleae* and *Acanthocotyl elegans*.
Parasitology, 60, 39-52.

Lyons, K.M., 1970.
Fine structure of the outer epidermis of the viviparous monogenean *Cyrodactylus* sp. from the skin of *Gasterosteus aculeatus*.
Journal of Parasitology, 56, 1110-1117.

1971

Parke, M., 1971.
The production of calcareous elements by benthic algae belonging to the class Haptophyceae (Chrysophyta). In: Farinacci, A., (editor). Proceedings of the II Planktonic Conference, Roma, 1970. p.929-93. Rome, Edizioni Tecnoscienza.

Bone, Q. and Denton, E.J., 1971.
The osmotic effects of electron microscopy fixatives.
Journal of Cell Biology, 49, 571-81.

Lyons, K.M., 1971.
Comparative EM studies on the epidermis of the blood-living juvenile and gill-living adult stages of *Amphibdella flavolineata*.
Parasitology, 63, 181-190.

Parke, M., Green, J.C. and Manton, I., 1971.
Observations on the fine structure of zoids of the genus *Phaeocystis* (Haptophyceae).
Journal of the Marine Biological Association of the United Kingdom, 51, 927-941.

Roberts, B.L. and Ryan, K.P., 1971.
The fine structure of the lateral-line sense organs of the dogfish.
Proceedings of the Royal Society of London, Series B, 179, 157-169.

1972

Bone, Q., 1972.
The Dogfish neuromuscular junction: dual innervation of vertebrate striated muscle fibres.
Journal of Cell Science, 10, 657-665.

Bone, Q. and Ryan, K.P. 1972.
Osmolarity of osmium tetroxide and glutaraldehyde fixatives.
Histochemical Journal, 4, 331-347.

Green, J.C. and Leadbeater, B.S.C., 1972.
Chrysochromulina parkeae sp. nov. (Haptophyceae), a new species recorded from SW England and Norway.
Journal of the Marine Biological Association of the United Kingdom, 52, 469-474.

Hughes, G.M., 1972.
Gills of a living Coelacanth, *Latimeria chalumnae*.
Experientia, 28, 1301-1302.

Lyons, K.M., 1972.
Sense organs of Monogeneans.
Zoological Journal of the Linnean Society, 51(Supplement 1), 181-199.

Lyons, K.M., 1972.
Ultrastructural observations on the epidermis of the polyopisthocotylinean monogeneans *Rajonchocotyle emarginata* and *Plectanocotyle gurnardi*.
Zeitschrift fur Parasitenkunde, 40, 87-100.

1973
Best, A.C.G. and Bone, Q., 1973.
The terminal neuromuscular junctions of lower chordates.
Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 143, 495-504.

Bone, Q., 1973.
Amphioxus. In: Gray, P., (editor). The Encyclopaedia of Microscopy and Microtechnique, p.8. New York, Van Nostrand Reinhold.

Bone, Q. and Brook, C.E.R., 1973.
On *Schedophilus medusophagus* (Pisces: Stromateoidei).
Journal of the Marine Biological Association of the United Kingdom, 53, 753-761.

Bone, Q. and Ryan, K.P., 1973.
The structure and innervation of the locomotor muscles of salps (Tunicata: Thaliacea).
Journal of the Marine Biological Association of the United Kingdom, 53, 873-883.

Bone, Q. and Ryan, K.P., 1973.
Osmolarity of osmium tetroxide and glutaraldehyde fixatives. In: Stoward, P.J., (editor). Fixation and Histochemistry, p.85-101. Chapman & Hall.
(NB – published originally in 1972 in Histochemical Journal, 4, 331-347)

Bryan, G.W. and Hummerstone, L.G., 1973.
Heavy metals in seaweeds.
Journal of the Marine Biological Association of the United Kingdom, 53, 705-720.

Chretiennot, M-J., 1973.
The fine structure and taxonomy of *Platychrysis pigra* Geitler.
Journal of the Marine Biological Association of the United Kingdom, 53, 905-914.

Ettl, H. and Green, J.C., 1973.
Chlamydamonas reginae sp. now. (Chlorophyceae): a new marine flagellate with unusual chloroplast differentiation.
Journal of the Marine Biological Association of the United Kingdom, 53, 975-985.

George, J.D. and Southward, E.C., 1973.
A comparative study of the setae of Pogonophora and polychaetous Annelida.
Journal of the Marine Biological Association of the United Kingdom, 53, 403-424.

Gilbert, D.S., 1973.
Mechanical models of axon structure.
Journal of Physiology, 229, 3P-5P.

Green, J.C., 1973.
Studies in the fine structure and taxonomy of flagellates in the genus Pavlova: II - A freshwater representative *Pavlova granifera* (Mack) comb. nov.
British Phycological Journal, 8, 1-12.

Nicol, J.A.C., Arnott, H.J. and Best, A.C.G., 1973.
Tapeta lucida in bony fishes (Actinopterygii).
Canadian Journal of Zoology, 51, 69-81.

Southward, E.C., 1973.
The distribution of glycogen in the tissues of *Siboglinum atlanticum* (Pogonophora).
Journal of the Marine Biological Association of the United Kingdom, 53, 665-671.

1974
Bone, Q. and Ryan, K.P., 1974.
The structure and innervation of the muscle bands of *Doliolum* (Tunicata: Cyclomyaria).
Proceedings of the Royal Society of London, Series B, 187, 315-327.

Arnott, H.J., Best, A.C.G., Ito, S. and Nicol, J.A.C., 1974.
Studies on the eyes of catfishes with special reference to the tapetum lucidum.
Proceedings of the Royal Society of London, Series B, 186, 13-36.

Green, J.C. and Parke, M., 1974.
A reinvestigation by light and electron microscopy of *Ruttnera spectabilis* Geitler (Haptophyceae) with special reference to the fine structure of the zooids.
Journal of the Marine Biological Association of the United Kingdom, 54, 539-550.

Wickstead, J.H., 1974.
Cephalochordata.
In: Encyclopaedia Britannica 15th edition., p.1147-1149.

1975
Bone, Q. and Chubb, A.D., 1975.
The structure of stretch receptor endings in the fin muscles of rays.
Journal of the Marine Biological Association of the United Kingdom, 55, 939-943.

Bone, Q. and Mackie, G.O., 1975.
Skin impulses and locomotion in *Oikopleura* (Tunicata: Larvacea).
Biological Bulletin. Marine Biological Laboratory, Woods Hole, 149, 267-286.

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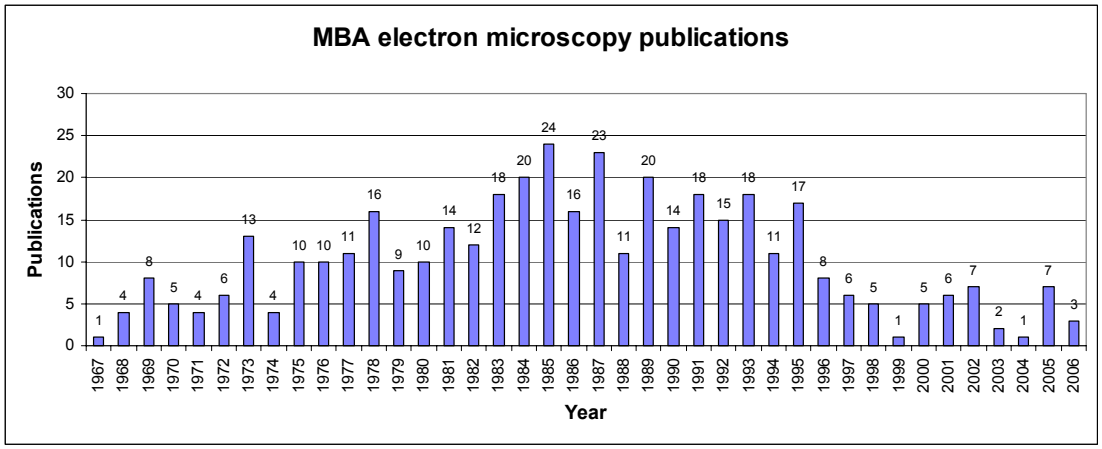

Fig. 33. Summary of publications that used electron microscopy at the Marine Biological Association.

Appendix 5

Papers and seminars presented about Plymouth E.M. work - mainly about cryotechniques research (by K.P. Ryan)

1. **Jan. 16, 1985: M.B.A. Seminar Programme, Plymouth, UK.**
   "Cryofixation: Plunge-freezing experiments performed at the M.B.A., or ... Confessions of a fast freezer".

2. **Feb. 19, 1985: University of Cardiff E.M. Club, UK.**
   "Rapid freezing of tissue blocks for cryoultramicrotomy & other cryotechniques: practical aspects of cryo-immersion".

3. **April 1, 1985: 3rd International Low Temperature Biological Microscopy and X-Ray Microanalysis Meeting, University of Cambridge, UK.**
   "Plunge-cooling of tissue blocks".

4-5. **Sept. 25 - Oct. 4, 1986: Workshop No. 34 at Seefeld-in-Tirol, Austria:**
   on Cryomethods in biological ultramicrotomy and electron microscopy.
   "The plunge-freezing technique" (presented twice).

6. **Oct. 14, 1986: visitor's seminar, University of Salzburg, Austria.**
   "Plunge-freezing experiments performed at M.B.A., Plymouth".

7. **Oct. 16, 1986: University of Innsbruck tutorial, Austria** (for Dr. Pohla & group).
   "Cryofixation experiments performed at M.B.A., Plymouth".

8. **Feb. 19, 1988: Southern Cryo Users Meeting I, Kew, London, UK.**
   "Plunge-freezing experiments and quantified X-ray microanalysis using cryo-SEM".

9. **March 3, 1989: visitor's seminar, University of Salzburg, Austria.**
   "Cryo-scanning electron microscopy and X-ray microanalysis".

10-12. **March 6 - 15, 1989: Workshop No. 40 at Seefeld-in-Tirol, Austria:**
   Cryomethods in biological electron microscopy.
   "Recent research in plunge-freezing, introducing deep plunging" (presented twice).
   "Freeze drying as observed by scanning electron microscopy".

13. **March 22, 1989: Biological X-ray Microanalysis Group Meeting, at the Medical Society of London, UK.**
   "Freezing of biological specimens".

14. **Oct. 13, 1989: visitor's seminar, University of Salzburg, Austria.**
   "Cryofixation and localisation of polluting elements in cells as detected with the electron microscope" (with Dr. James Nott).

15-16. Oct 15 - 21, 1989: Seefeld Workshop No. 45, Seefeld-in-Tirol, Austria:
on Cryomethods in Biological Electron Microscopy.
"Research into plunge-freezing at Plymouth Marine Laboratory: with correlation between predicted and measured ice crystal size" (presented twice).

17. Nov. 29, 1989: Cryo-Microscopy Group Meeting, Royal Veterinary College, London.
"Rapid cooling techniques for use with freeze substitution and low temperature embedding".

18. Feb. 13, 1990: University of Bristol Fine Structure Group, UK.
"Chilling tales: cryofixation and low temperature techniques in electron microscopy".

19. April 2 - 6, 1990: 4th International Low Temperature Biological Microscopy and X-Ray Microanalysis Meeting, University of Cambridge, UK.
"Ice crystal growth during cryofixation".

20-21. Oct. 23 - Nov. 3, 1990: Workshops No 49A & 49B at Seefeld-in-Tirol, Austria:
Workshop No. 49B on Cryomethods in Biological Electron Microscopy.
Workshop No. 49B on Cryofixation, Cryosubstitution and Low Temperature Embedding.
"Cryofixation experiments at Plymouth Marine Laboratory" (presented twice).

22. May 22, 1991: Cryo-Microscopy Group Meeting, Royal Veterinary College, London.
"Freeze-drying tissue samples for X-ray microanalysis".

23. March 25, 1992: X-ray Microanalysis Users Group Meeting, School of Pure and Applied Biology, University of Wales, Cardiff, UK.
"Metals in flounder melanomacrophages and sediments".

24. October 14, 1992. Aquatic Toxicology Short Course, Univ. of East London, at PML.
"Use of physical preparation methods in biological electron microscopy for the retention of physiological and pollutant chemicals".

25. May 12, 1992. Twenty-fifth anniversary Scanning Microscopy Meeting, McCormick Center Hotel, Chicago, Illinouis, U.S.A.
"Cryofixation of tissues for electron microscopy: a review of plunge cooling methods". Presidential Scholarship awarded.

26-27. October 30, 1992. Workshop 56B at Seefeld-in-Tirol, Austria:
on Cryofixation, Freeze Substitution, Freeze Drying, PLT and Low Temperature Embedding.
"A review of plunge-freezing methods" (presented twice).

28. March 24, 1993. King's College, University of London Electron Microscope Unit One-day Meeting, UK.
"Rapid freezing techniques in the life sciences".

29-32. June 20, 1993. Workshops at Seefeld-in-Tirol, Austria:
Workshop 57 F-Bio-IC on Cryomethods for Immuno-Cytochemistry, 14-23 June,
Workshop 57 B-Bio on Cryofixation, Freeze Substitution, Freeze Drying, PLT and Low Temperature Embedding, 19-23 June.
"Cryofixation experiments at Plymouth Marine Laboratory" and
"Rapid freezing techniques in the life sciences"
(each presentation was given twice, to the English and German-speaking groups).

Guest Professorship, University of Salzburg, Institute of Zoology, Austria:

33. **June 24, 1993**. I - Introduction, Goals, and Survey of Cryomethods: freezing methods, post-freeze procedures, cryo-electron microscopies, safety considerations.

34. **June 28, 1993**. II - Water and Biological Specimens: various aspects of nucleation, crystal growth, vitrification, heat transfer, simplest practical approach, cold gas effects, coolant depth and limitations.

35. **June 29, 1993**. III - Plunge cooling experiments: deep plunging, coolants and thermo-couples, ultimate cooling efficiencies, ice crystal analysis and correlation with theoretical predictions.

36. **June 30, 1993**. IV - Slam, Jet, Spray and High Pressure Freezing: theory of cryoblocks, computer plots, commercial systems, applications, capture of dynamic events, x-ray microanalysis.

37. **July 1, 1993**. V - Cryomethods and Immunology: freeze fracture cytochemistry, latest thoughts from the Seefeld workshops using freeze substitution, freeze drying, low temperature embedding, hybrid Tokuyasu/substitution method.

38. **October 13, 1993. Plymouth Marine Laboratory seminar, UK**.
"The possibilities of EM cryomethods in Biology - or how not to poison your specimen".

39. **May 10, 1994. Scanning Microscopy International Meeting, 7-12 May, Ramada Hotel, Toronto, Canada**. 'The study of dynamic cellular events by electron microscopy: a review of time-resolved freezing of biological specimens'. Organiser and Chair of the 1½-day Low Temperature Microscopy (including Biological Microanalysis) session.

40. **May 18, 1994. Scanning 94/SEEMS 94 Meeting, 17-20 May 1994, Sheraton Hotel, Charleston, South Carolina, U.S.A.**
"Time-resolved freezing of biological specimens: a review of methods for studying dynamic cellular processes by electron microscopy".
Organiser and Chair of the 1-day Cryomethods in Biological Electron Microscopy session.
Presidential Scholarship awarded.

41-44. June 12, 1994. Workshops at Seefeld-in-Tirol, Austria:
Workshops 58 A-Bio-CR & 58 A-Bio-IC on Cryomethods in Biological Electron microsocopy, 1-16 June.
Workshops F-Bio-CR & F-Bio-IC on Cryomethods for Immunocytochemistry, 6-16 June.
"Parameters affecting cryofixation by plunge cooling" and
"Review of time resolved freezing of biological specimens for the study of dynamic cellular events by electron microscopy".
(each presentation was given twice, to the English and German-speaking groups).

45. June 17, 1994. Labor für EMI, ETH Zürich, Switzerland.
"Time-resolved freezing of biological specimens: a review of methods for studying dynamic cellular processes using electron microscopy".

46. June 20, 1994. Institute of Zoology, University of Salzburg, Austria.
"State of the art in cryo-electron microscopy".

47. June 21, 1994. Austrian Academy of Sciences, Institute for Molecular Biology, Salzburg, Austria.
"Time-resolved freezing of biological specimens: a review of methods for studying dynamic cellular processes using electron microscopy" (Tutorial to research students).

48-49. June 23, 1994. Institut für Ultrastrukturforschung des Klinikums der Friedrich-Schiller-Universität, Jena, Germany.
"Considerations in cryofixation, with emphasis on plunge freezing parameters" and
"The cryo-immobilisation of dynamic events, with millisecond time resolution".

50. October 24, 1994. Appl. Bio. HND Course, University of Plymouth, UK.
"Electron microscopy low temperature methods for Biology".

51-54. June 14, 1995. Workshops at Seefeld-in-Tirol, Austria.
Workshops 59 A-Bio & 58 A-Bio on Cryomethods in Biological Electron Microscopy, 1-15 June.
Workshops F-Bio & F-Bio on Cryomethods for Immunocytochemistry, 5-15 June.
"Cryofixation by plunge cooling" and
"Time resolved freezing of biological specimens for the study of dynamic cellular events by electron microscopy".
(each presentation was given twice, to the English and German-speaking groups).

55. November 6, 1995. Appl. Bio. HND Course, University of Plymouth, UK.
"Electron microscopy low temperature methods for Biology".
Practical demonstration at PML on November 20th, 1995.

56-59. June 10, 1996. Workshop at Seefeld-in-Tirol, Austria
Workshops 60 A-Bio & 60 F-Bio, A-Mat F-Mat on Cryomethods in Biological Electron Microsocopy, 29 May-13 June.
Workshops F-Bio & F-Bio on Cryomethods for Immunocytochemistry, 5-13 June.
"Cryofixation and liquid cryogen properties" and
"Time resolved freezing of biological specimens for the study of dynamic cellular events by electron microscopy".
(each presentation was given twice, to the English and German-speaking groups).

60. October 31, 1996. Appl. Bio. HND Course, University of Plymouth, UK.
"Electron microscopy low temperature methods for Biology".

61. December 16, 1996. 2nd meeting Microfossil/Micro-organism Group, University of Plymouth/Plymouth Marine Laboratory (at PML).
"The application of SEM techniques in microfossil/micro-organism research".

62. March 1, 1997. Institute of Biology, Devon and Cornwall Branch (at MBA)
"An introduction to electron microscopy".

63. May 11, 1997. 30th Scanning Microscopy International Meeting, Marriott Downtown Hotel, Chicago Ill., USA.
"Fixation of biological specimens for electron microscopy and microanalysis: conventional chemical artefact or life-like cryoimmobilisation? A tutorial".

64-65. Sept 2, 1997. Middle East Technical University, Ankara, Turkey.
"Cryofixation - rapid freezing of biological specimens".
"Investigation of fast events by time resolved cryofixation".

66-69. October 24 & 25, 1997. Workshop at Seefeld-in-Tirol, Austria
"Cryofixation by plunge-, cryo-block-, jet- and microdroplet spray freezing " and
"Time resolved freezing of biological specimens for the study of dynamic cellular events by electron microscopy".
(each presentation was given twice, to the English and German-speaking groups).

70-71. October 5, 1998. Workshop at Seefeld-in-Tirol, Austria
"Cryofixation by plunge-, cryo-block-, jet- and microdroplet spray freezing " and
"Investigation of fast events by time resolved cryofixation".

72. March 19, 1999. Invited lecture at the Établissement de Transfusion Sanguine de Strasbourg, France.
"Chilling tales – Cryomethods in electron microscopy – Why, how, when?"
"Contes frissonantes - Cryométhodes en microscopie électronique – Pourquoi, comment, quand?"

Appendix 6

Building disruption from 1989 to 2004 affecting EM activities

These years have seen many upheavals in the laboratory with contractors being involved in major building projects which disrupted users of the EM Unit to a greater or lesser extent, and also everyone in the laboratory. This began with the rebuilding of the second floor offices (South Building) in **1989.** The entire area was emptied and completely rebuilt, including the walls, ceilings and floors. In **1990,** the first floor offices in the South Building received the same treatment. In **1990/91,** a new membrane roof was fitted to the building - winter was not the best time to do this work. In **1991,** a new fire alarm system was fitted throughout the building. A swipe-card access entry system for improved security was also introduced. This was after a period of terrorism when staff were advised to check underneath their cars each evening before going home; the security personnel were issued with a bomb inspection mirror. It was at this time that Dingles' store in Plymouth was damaged extensively by an incendiary device. This was carried out by animal rights activists and threats were also made to the marine laboratories in Plymouth. The building was examined for security purposes by Special Branch. The laboratory was also a "soft" target for the IRA, being adjacent to a military establishment. In **1992**, the Japanese oak hardwood fire-doors were deemed to be unsuitable and replaced by certified doors bought from a local DIY store (it was learned later that the original doors could have been certified after the addition of intumescent, i.e. smoke-blocking, strips). The MBA Workshop was moved across the Hoe to PML and the building converted for the Sir Alister Hardy Foundation for Ocean Science - SAHFOS, staffed by personnel made redundant from the PML. During **1993/1995,** most of the lab was rewired. This caused tremendous disruption while areas were closed down for the work to be done. Luckily, the EM Unit was rewired prior to its move in 1988. In **1994/95,** the central heating system was replaced over the winter period, causing major disruption and discomfort throughout the building. The electron microscopes suffered from "damp" during and after this period. Much of the building was also rewired, causing further major disruption. In **1997,** a new bridge link was completed between the North and South Buildings. In **1998,** the courtyard was rebuilt, this involved several weeks of pneumatic hammer-drilling to remove the old fish tanks. As the report for that year says "*The obstinacy of these fixtures is a tribute to the quality of the original materials and workmanship*". In other words, it was difficult work and took much longer than expected. During this year, the Common Room kitchen was completely refurbished, entailing more disruption to the running of the laboratory. In **1999,** the old Lecture Hall was gutted and converted to a laboratory suite. In **2000**, the laboratory was rewired again, causing great upheaval and generation of all-permeating dust – definitely not desirable in a microscopy unit. Also, many windows in the building were removed and refurbished, the disruption being commensurate with the task involved! In **2001**, the old aquarium premises were converted into the Marine Life & Environmental Sciences Resource Centre. In **2002**, the Constant Temperature rooms were all refurbished including Room C2 which is opposite the electron microscope rooms and adjoining the EM Preparation Room – yet more noise and dust. In **2003**, a new fire alarm system was installed. This involved dust generation with the installation of a smoke detector in the

ceiling of each room. The necessary high level drilling ensured yet again that dust was distributed around each room very effectively! When one considers that the unit was installed in a basement with a filtered air supply for cleanliness, it is not surprising that there were feelings of irritation sometimes over all the dust generated during the last decade. One speck of dust in an EM specimen or on a negative can ruin it!

Appendix 7

An article that appeared in "*MBA News*", August 2002

The "300" is gone!

The Philips EM300 transmission electron microscope (TEM) has been decommissioned and disposed of after an active working life of 30 years. The microscope was commissioned in July 1971 and the first "in-house" micrographs were taken on 27 July by Quentin Bone and George Best.

Before the "300" was purchased, its predecessor, a Philips EM200 was installed in August 1965. Demand on the first microscope was so great from both MBA staff and the many visiting scientists that a second TEM was needed. The same was true of the ultramicrotomes, for cutting the necessary sections. The original EM200 microscope continued in use until 1981 (16 years) when the JEOL instruments were installed.

The "300" was delivered and stored in boxes in the old Lecture Hall for about three weeks before it was assembled. The moving of heavy items was organised by MBA staff - Freddie Ryder and Dave Purse, plus willing volunteers. The electron microscope ("EM") unit lived in the north building basement until 1988 when health and safety considerations relating to increased liquid nitrogen and bottled gas use, combined with manual handling aspects, prompted re-housing the unit above ground.

The "300" became the general workhorse instrument for many years and contributed greatly to the MBA publication record, the EM list up to 1998 records 370 publications. In some ways there seems to be few real "stories" to tell about it. It was a very reliable instrument, maintained religiously by George Best and Keith Ryan for many years. It required little more than routine preventative maintenance with occasional electronic problems. The main power transistors in the "D" unit were the usual "bogey"! MBA had an excellent Electronics Workshop to call on for help in tracing faults, in the form of "Robbie" Robinson and John Wood. Also available were Bob Maddock and John Mavin for support with occasional problems. Jack Saggers expedited the occasional water requirements.

In those far off days of the '70s, besides Quentin Bone, Botany was a major user of the microscope: the name of John Green appears throughout that decade. Another regular user was Eve Southward, as was Richard Pipe, then of I.M.E.R.. A record of users shows 25 MBA staff and 57 visitors used the EM facility during the 1970's. On 11th October 1976, a young Annie Pulsford made her first entry in the log book, becoming a regular user for many years. Cilla Course entered the log book just a month later, becoming another regular user for Botany.

The "300" continued to be used regularly for productive work in recent years: since 1996 it has been productive towards a number of first degrees, Master's and Doctorates: including Lorraine Berry, Claudia Birmelin, Helen Chesters, Laura Chidgey, Toby

Collins, Mike Cox, Mireille Crampe, Daniel Davies, Liz Dyrynda, Sophia Farley, Isobel Francis, Jo Hagger, Jane Hawkridge, Rose Kerr, Lydia Mathger, Maria Odblom, Lucy Puddeford, Candida Reed, Dorothea Sommerfeldt and Emma Wootton.

The room will continue to have a microscopy use - Prof. Colin Brownlee's group are moving in with the BioRad MRC 1024 confocal laser scanning microscope.

May 2002

Postscript

The Electron Microscopy Unit has a record of 413 published titles, of which very few are non-refereed items. This number equates to approximately one per month.

The MBA was 'at the cutting edge' of electron microscopy in the 1980's. This was after the installation of new equipment and the entry into cryotechniques. These techniques were, in part, pioneered and researched at the laboratory, particularly in the area of rapid freezing, freeze-drying, vacuum infiltration of resin and dry sectioning (to avoid the loss of mobile ions). The laboratory was able to freeze effectively by plunge freezing, slam freezing and microdroplet spray freezing. The follow-up methods of freeze-substitution and freeze-drying used in the laboratory were also very effective. The extensive range of accessories that were necessary for this work was only made possible by the laboratory workshops that existed at that time – for mechanical engineering, glass, wood, plastics and electronics. A vote of thanks is due to all the staff who made the work possible. At the peak of this period, in 1985, a publication appeared, effectively, every two weeks.

In writing this account, care has been taken to record the users, visitors and publications from the records available: these were the log books of the various instruments, personal and laboratory diaries and MBA Annual Reports. It is possible that, over the years, in view of the great number of visitors whose names do not appear in the record of publications that some publications are not recorded.
