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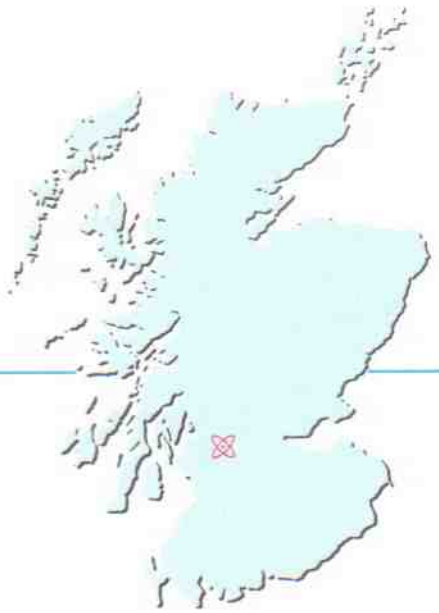

PHOTOSTIMULATED LUMINESCENCE AND THERMOLUMINESCENCE TECHNIQUES FOR DETECTING IRRADIATED FOODS

DETECTION OF IRRADIATED SHELLFISH
MAFF N2635

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ACKNOWLEDGEMENT

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1. INTRODUCTION

This is the final report on project N2635 aimed at investigating the extension of thermoluminescence (TL) and photostimulated luminescence (PSL) methologies to the detection of irradiated shellfish to support the UK Food Labelling Regulations[1] (SI 2489 and 2505 (S207)). Thermoluminescence (TL), photostimulated (PSL) and photo-transfer luminescence (PTTL) are radiation specific phenomena which arises due to energy stored by trapped charge carriers following irradiation. The energy released following stimulation is accompanied by detectable luminescence.

Thermoluminescence has been successfully applied to the detection of irradiated herbs and spices[2-8], and to fruits and vegetables[9] using silicate extracted using heavy density separation technique. The TL method involves preparation of pure silicate extracts from the sample and subsequent TL analysis whereas PSL uses stimulation by electromagnetic radiation (visible, or near visible wavelengths) thus avoiding heating the sample.

The project was designed to establish the limits of both the thermoluminescence approaches of the inorganic intestinally entrained minerals and the bioinorganic phases of unfiltered chitin associated with irradiated peeled and unpeeled shellfish, and to investigate the potential of photostimulated luminescence to overcome any limitations.

Shells themselves, where available, provide a bio-inorganic material, comprising organic membranes infiltrated with minerals, which can also be used for luminescence detection[10-15]. Preliminary studies in France demonstrated that TL from calcite shells could be detected shortly after irradiation from well mineralised mediterranean species[16]. This work has been followed up in East Kilbride using a range of 9 types of shellfish, including warm water species of immediate regulatory interest, and poorly mineralised examples. The results of our initial TL experiments on powdered shells were discussed in the previous report and were extremely encouraging. A firm foundation was established for further thermoluminescence work on powdered shellfish by identifying the major source of background interferences and devising and improving measurement conditions using freeze drying and restricting the glow regime. Further experimentation on powdered shells[17], discussed below, has been carried out to try and optimise the detection of the calcitic signal by using alternative optical filters to those used in previous experiments and to examine the extent of post-irradiation fading, extensive stability studies have been undertaken in support of a reliable test.

The intestines of crustaceans contain small quantities of inorganic grits which, providing that there are sufficient quantities, can be used for TL analysis following the standard density separation procedure[18,19,20]. Physical recovery of the intestinal grits for TL analysis is a laborious task and work has been undertaken to explore the use of acid hydrolysis[21] and the use of tissue solubilisation to simplify the analysis.

The other objective of the project was to investigate the potential of phototransfer TL and direct photo stimulated luminescence to improve the sensitivity and reliability of thermoluminescence tests. Thermoluminescence signals are recorded by releasing trapped charge during sample heating, photostimulation and photo transfer techniques provide opportunities to achieve a similar aim, without heating and hence not damaging the original

sample.

The use of photostimulated luminescence techniques (PSL) and phototransferred thermoluminescence techniques (PTTL), where the energy to release trapped charge carriers is provided optically. Direct PSL measurements record Anti-Stokes luminescence emitted during and following stimulation, where the output wavelengths are shorter than the stimulating wavelengths. This is highly radiation specific since energy conservation principles demand that the quantum energy differences between stimulation and luminescence are balanced by energy stored in the form of trapped charge carriers. Unirradiated samples therefore are unable to participate in these transitions. PTTL uses a process of transfer of charge carriers from a deep trap, associated with high temperature TL emissions, to a shallow trap which capable of producing a low temperature TL peak. This approach is useful in limiting the temperatures of TL heating of bioinorganic systems avoiding damage to the organic components, and provide a means of detecting luminescence at wavelengths which are close to the emission band wavelengths. Cryogenic PTTL has been used here to investigate the excitation spectra of biogenic calcite to identify promising stimulation and detection schemes for PSL[22,23].

The excitation and emission spectroscopy of silicate materials is quite well known[22] and initial studies of excitation spectra from irradiated shellfish have shown weak infra-red stimulated UV emission which is attributed to silicate contamination. Further work has been carried and is discussed below, on nine species of shellfish.

Another technique being developed for the detection of irradiated foods is the used of a pulsed PSL system, which enables the examination of inorganic systems in the presence of organic matter[24,25,26]. The sample is undamaged by this technique and it is possible to calibrate the results by re-irradiation, if necessary, without any dramatic changes in sensitivity. Preliminary work using herbs and spices[27] confirmed the viability of direct detection, without the need to separate the minerals from the foodstuffs. The results obtained on herbs and spices indicate that the system is capable of recognising 95% irradiated samples, without the need to recalibrate. From these promising results we have conducted some preliminary experiments on shellfish, to extend the food range of this technique.

2. THERMOLUMINESCENCE

2.1 Themoluminescence of shells

Our objectives of this work are to examine the extent to which the TL signals measured directly from shells or shell fragments can be used in the detection of irradiated shellfish for the enforcement of food labelling regulations.

The results of the initial experiments reported in the progress report March 1993, were extremely encouraging. The major source of background interferences associated with the release of trapped moisture, pyrolysis, auto-oxidation of the sample; were identified and significantly reduced by freeze drying samples and restricting glow temperatures to 250°C. This has improved measurement conditions which enable qualitative discrimination between irradiated and unirradiated samples. Although the signals levels from irradiated samples were modest, they are within the range of photon counting and with further work to improve measurement conditions and stability tests it may be possible to utilise this effect and this is discussed below.

Parameter Optimisation

The blue filter system used for this initial work are probably not optimised for the detection of signals from calcite, and the possibility for further enhancements to the signal magnitude may be possible by the use of alternative filters.

An experiment was conducted to determine the filter or filter combination best suited to the emission spectrum of calcitic shells. 10 discs were prepared using powdered lobster irradiated to 5 kGy. TL measurements were carried out on each disc on a series of long pass filters, positioned in the collar at half height, in steps of approximately 40nm. The results, figure 2.1.1, show that the signal is not attenuated until the introduction of the RG610 filter. This indicates that the main peak lies in the yellow / red region of the spectrum. Further TL measurements were carried out on the same sample using a selection of bandpass filters. From these results, the filter system that least attenuated the signal was the KG3 filter. The results are also suggestive of an emission spectrum dominated by Mn^{2+} in the 500 - 600nm region. By using the KG3 filter system the signal levels were increased by two orders of magnitude compared with the original blue filter system.

Under these optimised conditions TL measurements were carried out on lobster, warm water shrimp, black tiger prawns, nephrops norvegicus, king and queen scallops, mussels, crabs and oysters. All the samples were dried, powdered and sieved through 250μm mesh. The samples were then freeze-dried and split into two aliquots; one of which was irradiated to 5 kGy in the Co-60 source and the other retained as a control. For each pair of samples, 10

discs were prepared and the glow curves recorded using the KG3 filter system with the heating restricted to 250°C. A selection of the glow curves obtained are shown in figures 2.1.2 and 2.1.3, where all freshly irradiated samples of all the species are readily distinguishable from the unirradiated controls.

Signal Stability

The shelf life of irradiated shellfish is considerably shorter than for other dried food products, and providing the signals are stable for one month under cold storage the TL phenomenon will be useful. The benefit of sample evacuation prior to read-out can clearly demonstrate a reduction in background spurious signal and also the use of a restricted glow regime may avoid sample darkening during readout, and hence improve TL measurement conditions.

Having established that TL from freshly irradiated shellfish powders can be detected, the stability of the signals becomes an important consideration. It is not possible to make any detailed prediction of stabilisation characteristics of an unknown material and even with known trap parameters from kinetic evidence and there is a need to supplement models with empirical results. The TL glow curves comprise a pseudo trap-depth spectrum and in general the thermal lifetime increases with glow temperature and depends strongly on storage temperatures. Reliable identification tests require the support of observations over the full potential lifetime and storage conditions that the food product may be exposed to. Irradiated shellfish may be stored for up to 3 months in freezer conditions and 1 week in fridge conditions.

Extensive stability tests have been undertaken for black tiger prawns, mediterranean crevettes, warm water shrimps, nephrops norvegicus, king scallops, lobster, oyster, brown shrimps and mussel; over a storage period of 3 months at -20°C and 5°C, which goes well beyond the normal shelf life of these products. Aliquots of each shellfish species were irradiated to 5 kGy and stored at refrigerator and freezer temperatures. 10 discs of each sample were prepared and measured directly after irradiation and at weekly intervals over a period of 3 months.

The results of these experiments are shown in histogram diagrams, figures 2.1.4 - 2.1.17, for all the species at fridge and freezer storage temperatures. The TL from all the 130-150°C, 150-170°C, 170-190°C, 190-210°C, 210-230°C and 230-250°C temperature ranges are all subject to post-irradiation fading by an order of magnitude, over the 3 month storage. The results do show that there is quite a large variability due to the organic components present. However, it is still possible to distinguish irradiated and unirradiated samples using TL measurements. For well mineralised species; lobster, mussel, oyster and scallop these losses are unlikely to lead to unambiguous identification. In the poorly mineralised species; mediterranean crevette, black tiger prawn, warm water shrimp, brown shrimp, the worst cases, the signal loss could be problematic.

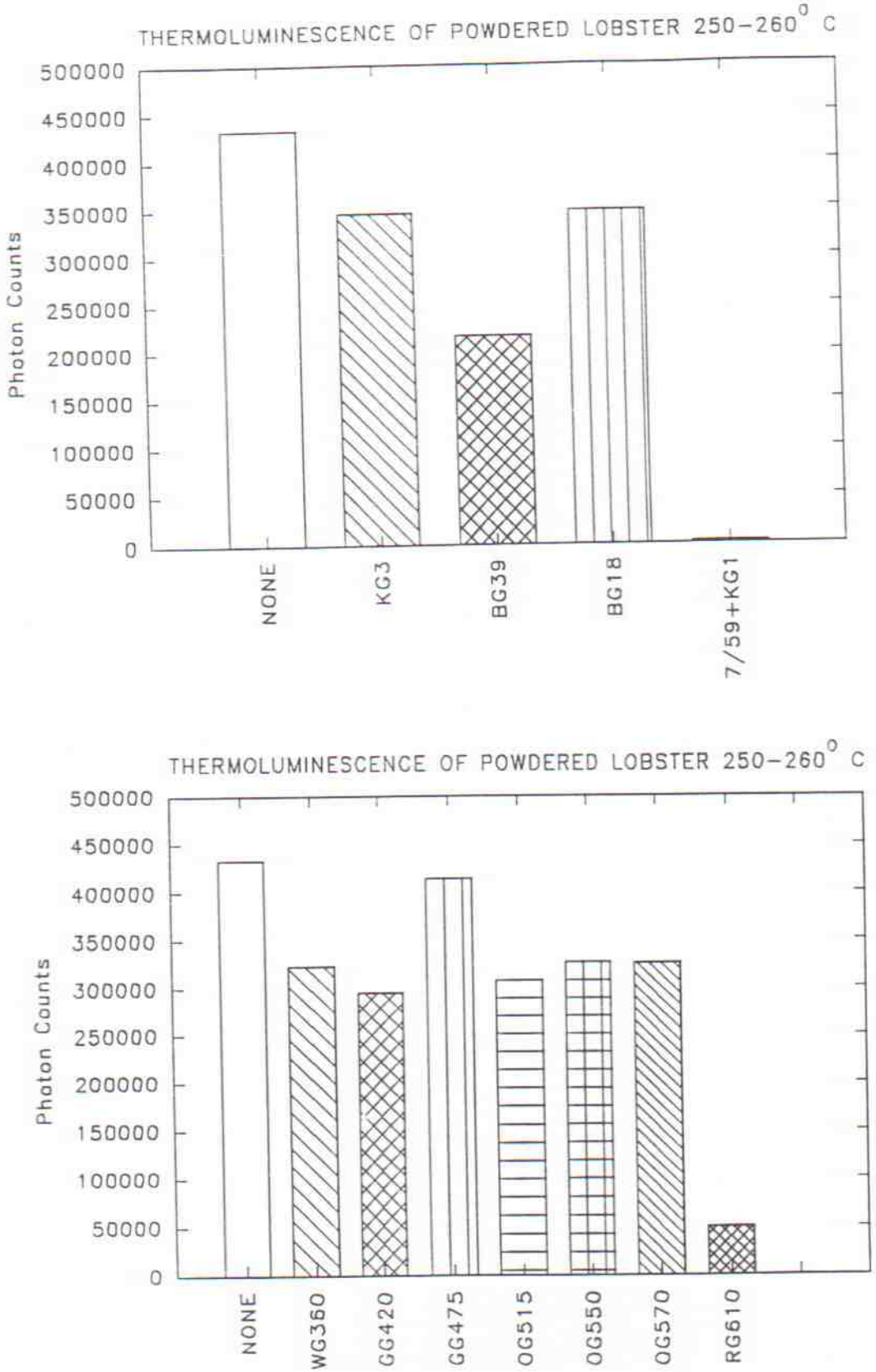
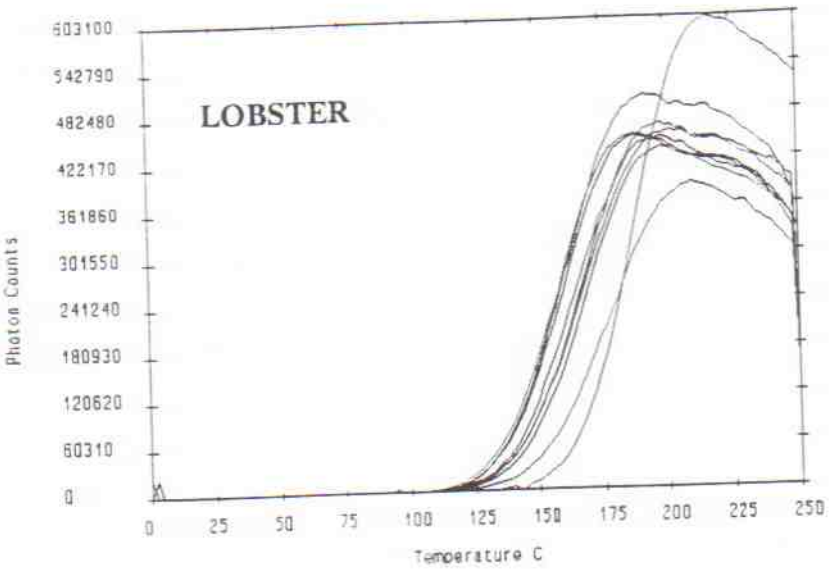


Figure 2.1.1 **The effect of long pass and bandpass filters on the TL from irradiated powdered lobster shells**



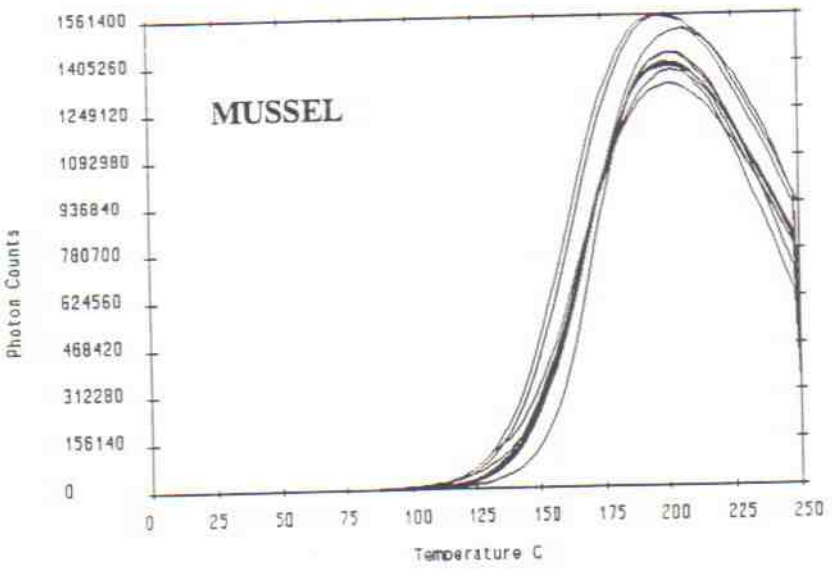
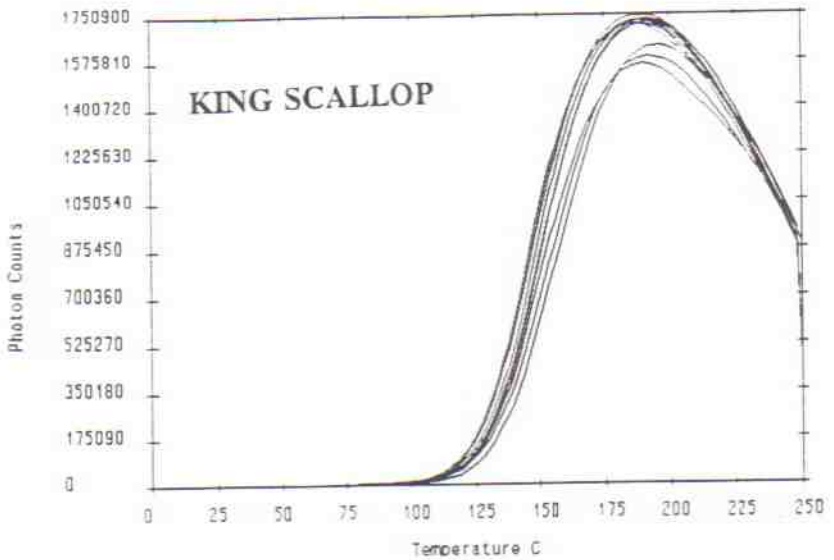


Figure 2.1.2 **Examples of glow curves from powdered shells**



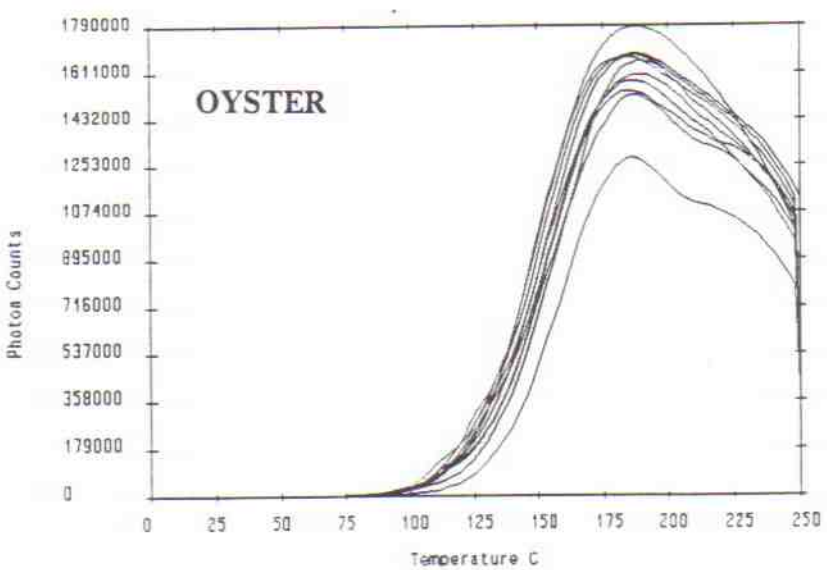


Figure 2.1.3 **Examples of glow curves from powdered shells**

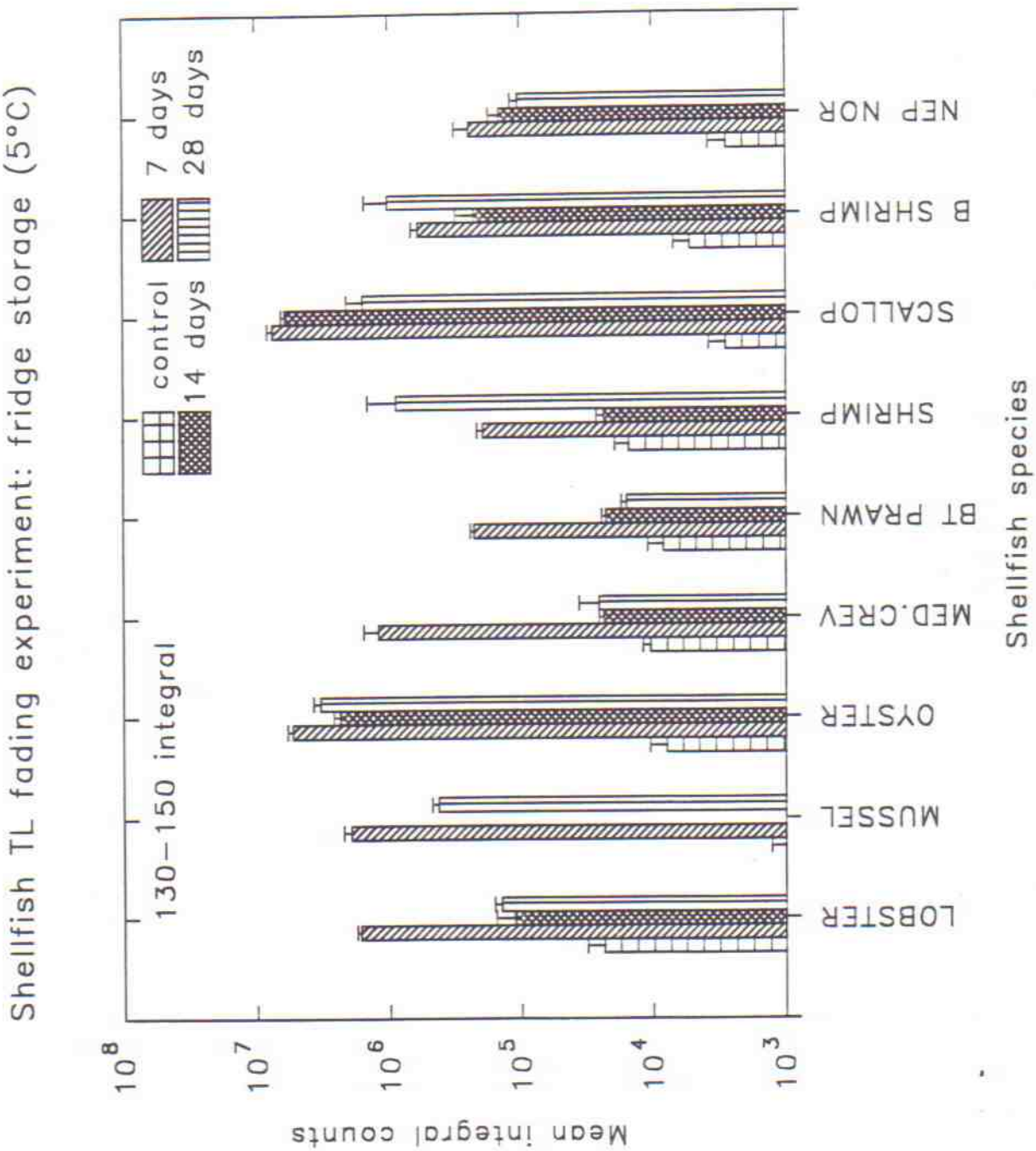


Figure 2.1.4 **Fridge storage for the 130-150°C temperature range**

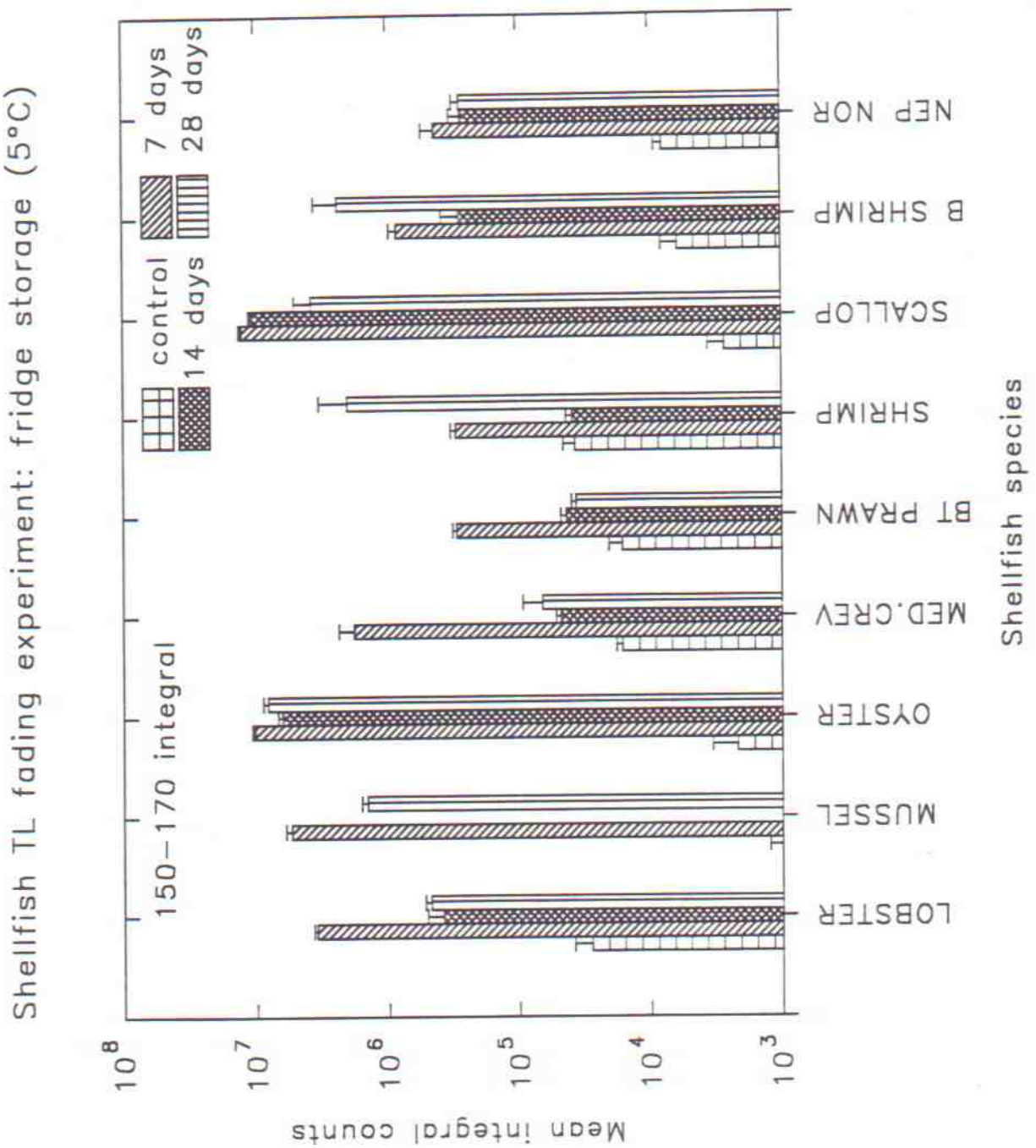


Figure 2.1.5 **Fridge storage for the 150-170°C temperature range**

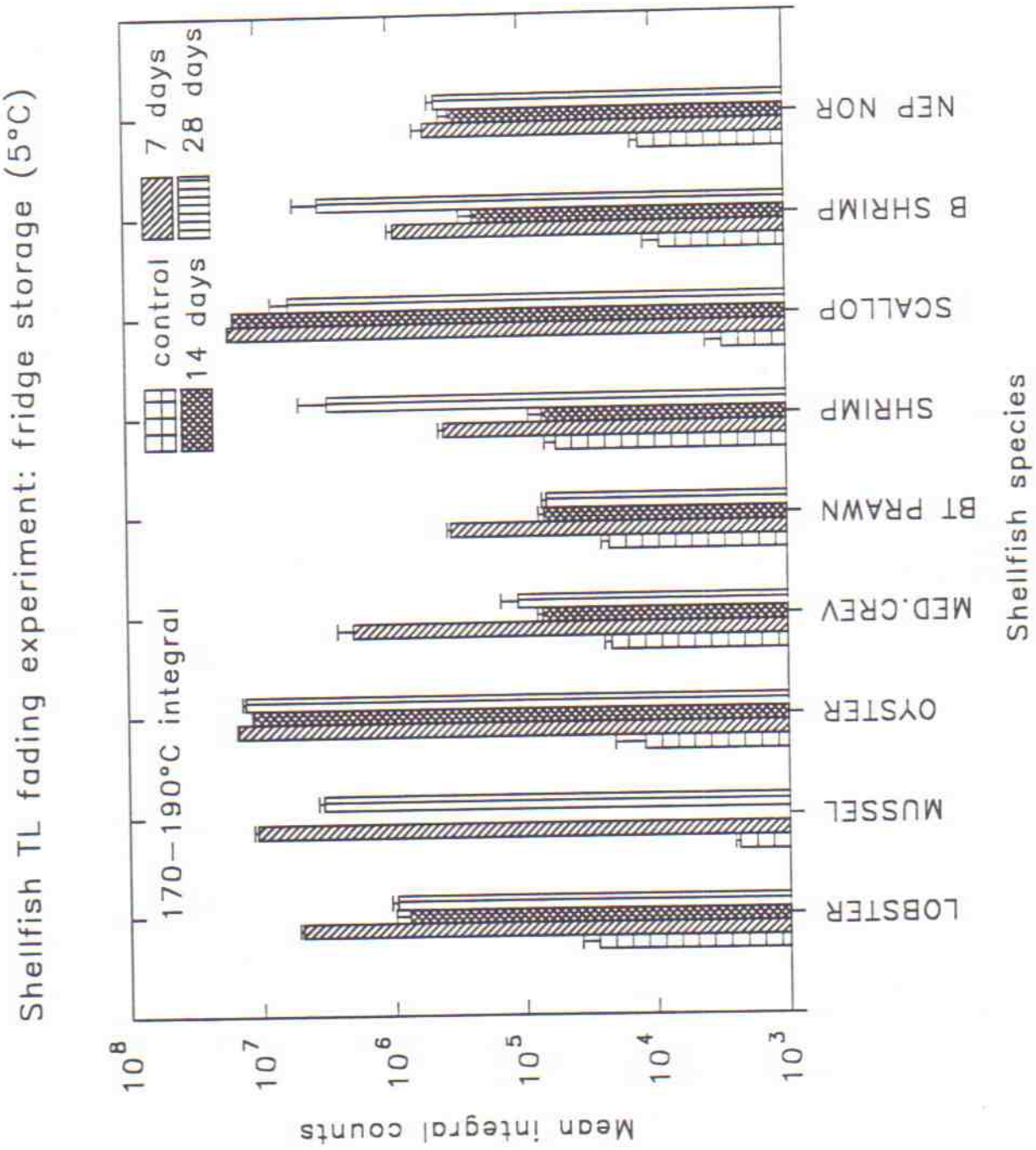


Figure 2.1.6 **Fridge storage for the 170-190°C temperature range**

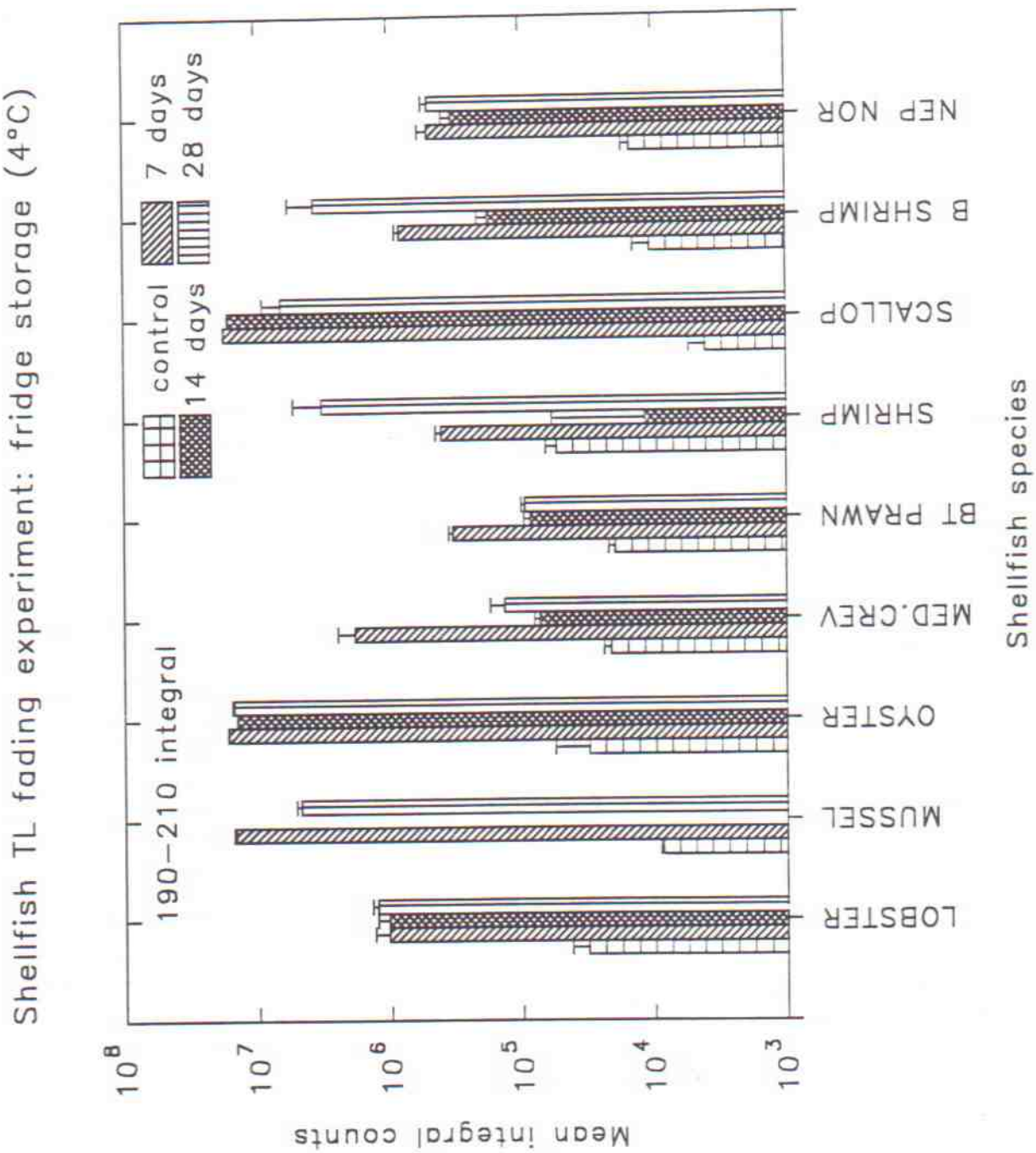


Figure 2.1.7 **Fridge storage for the 190-210°C temperature range**

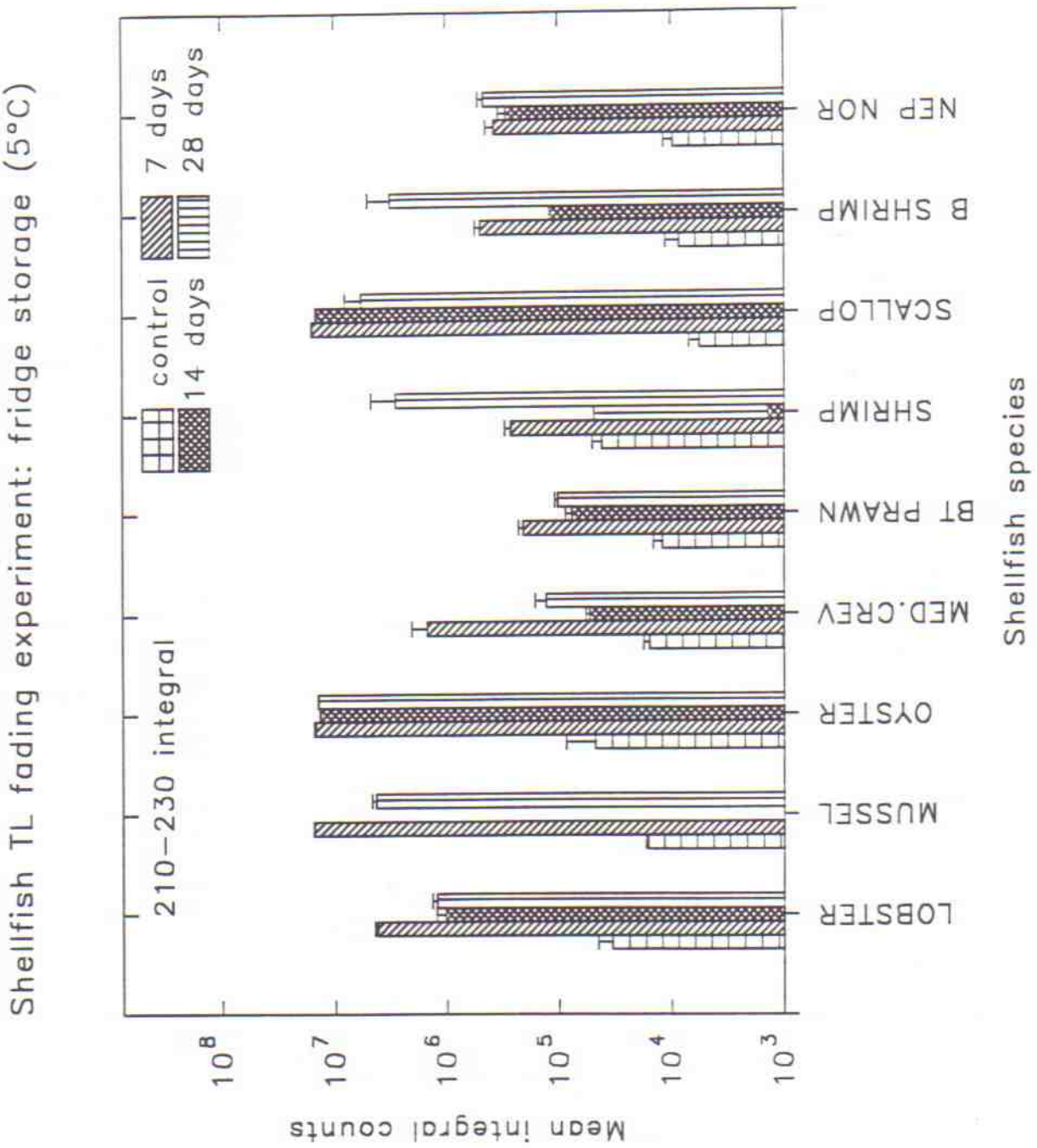


Figure 2.1.8 **Fridge storage for the 210-230°C temperature range**

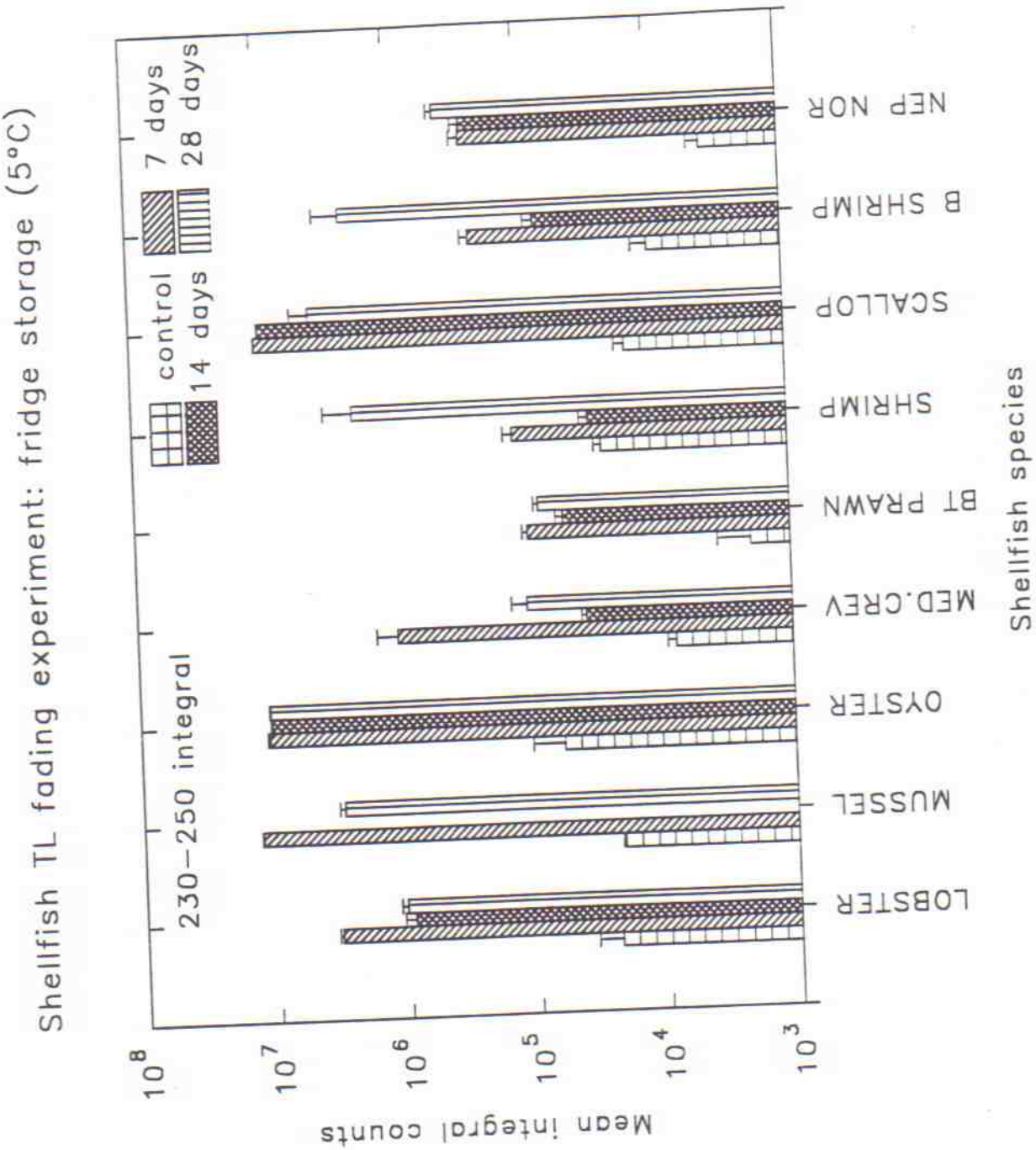


Figure 2.1.9 **Fridge storage for the 230-250°C temperature range**

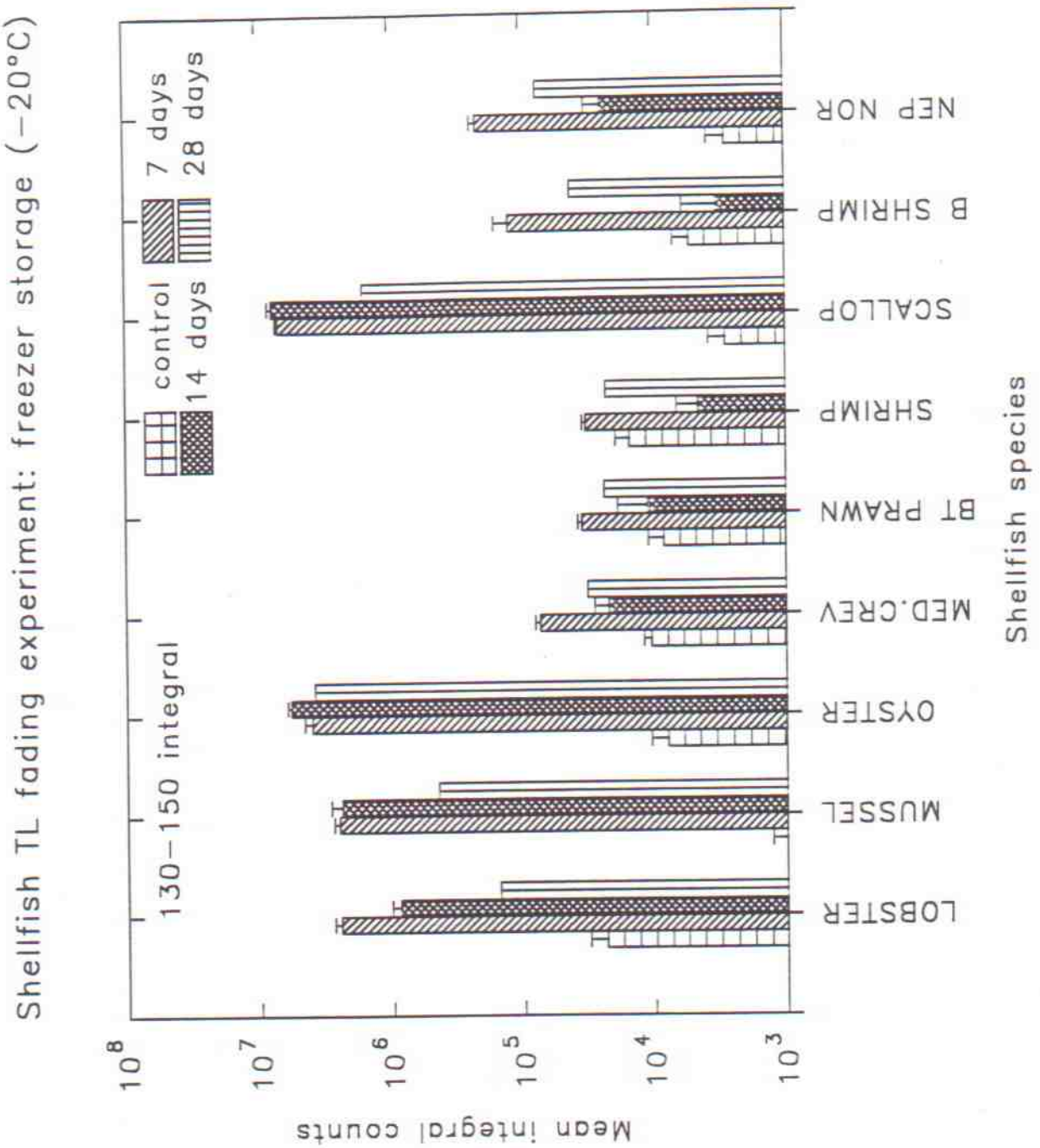


Figure 2.1.10 **Freezer storage for the 130-150°C temperature range**

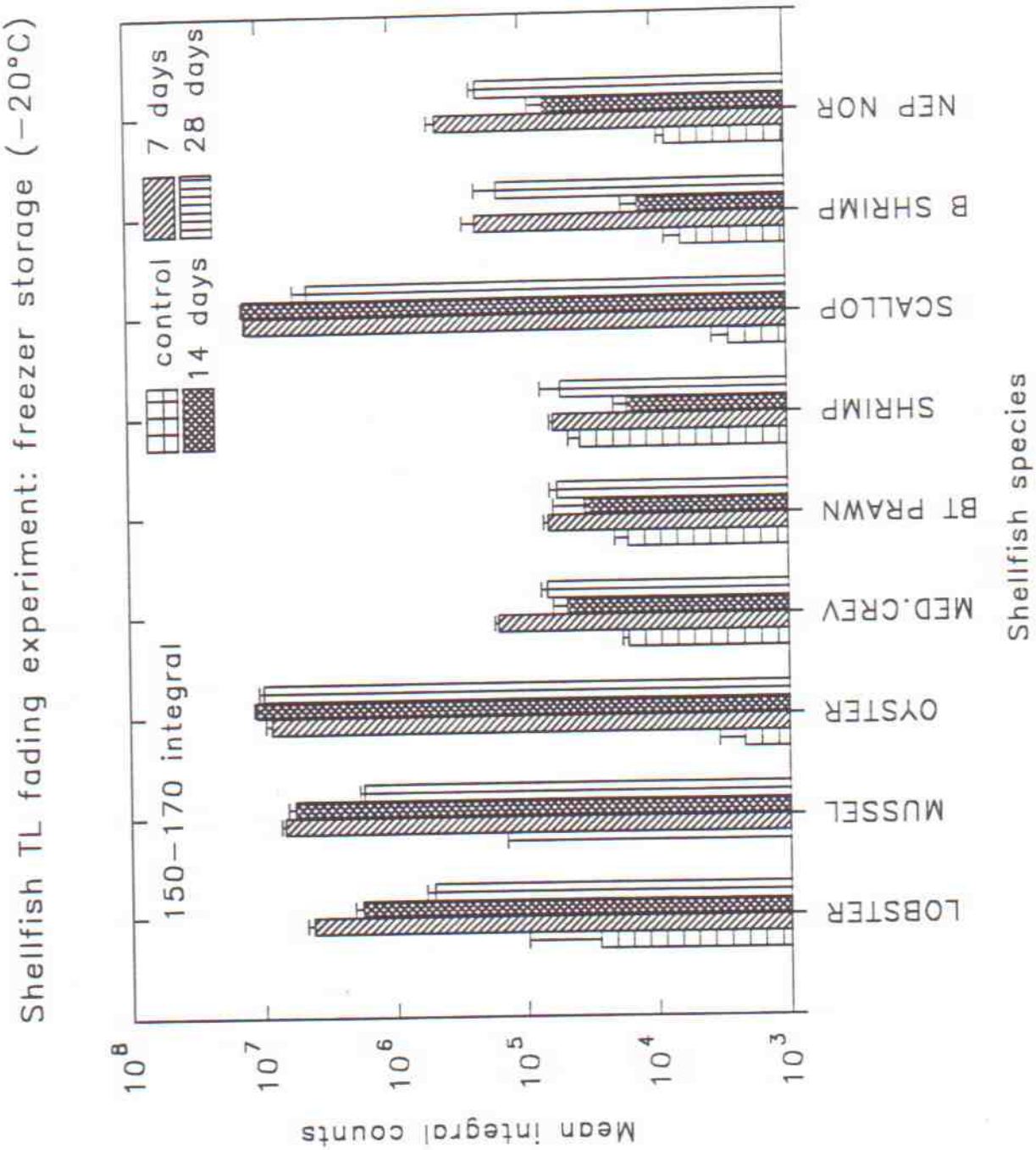


Figure 2.1.11 **Freezer storage for the 150-170°C temperature range**

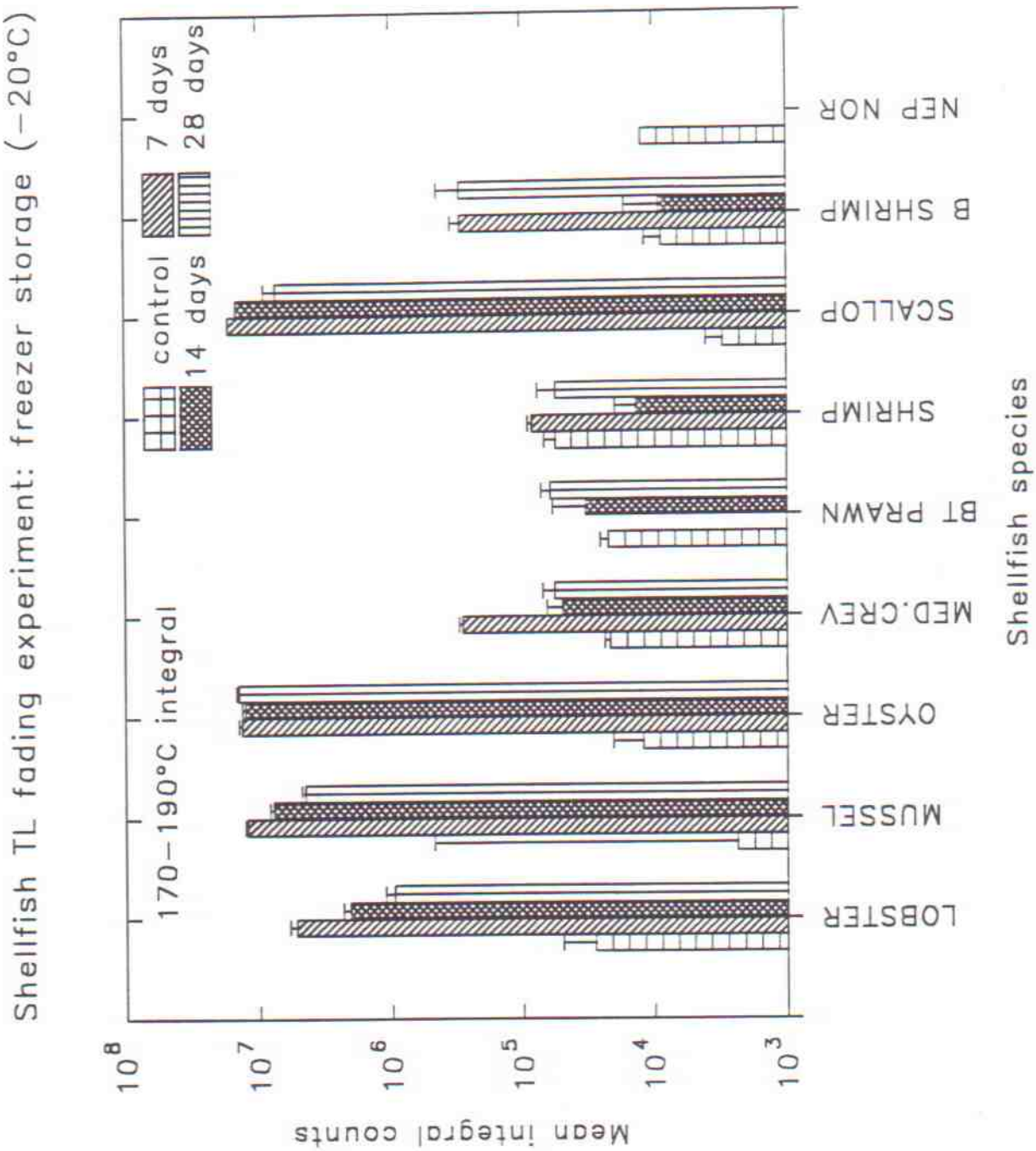


Figure 2.1.12 **Freezer storage for the 170-190°C temperature range**

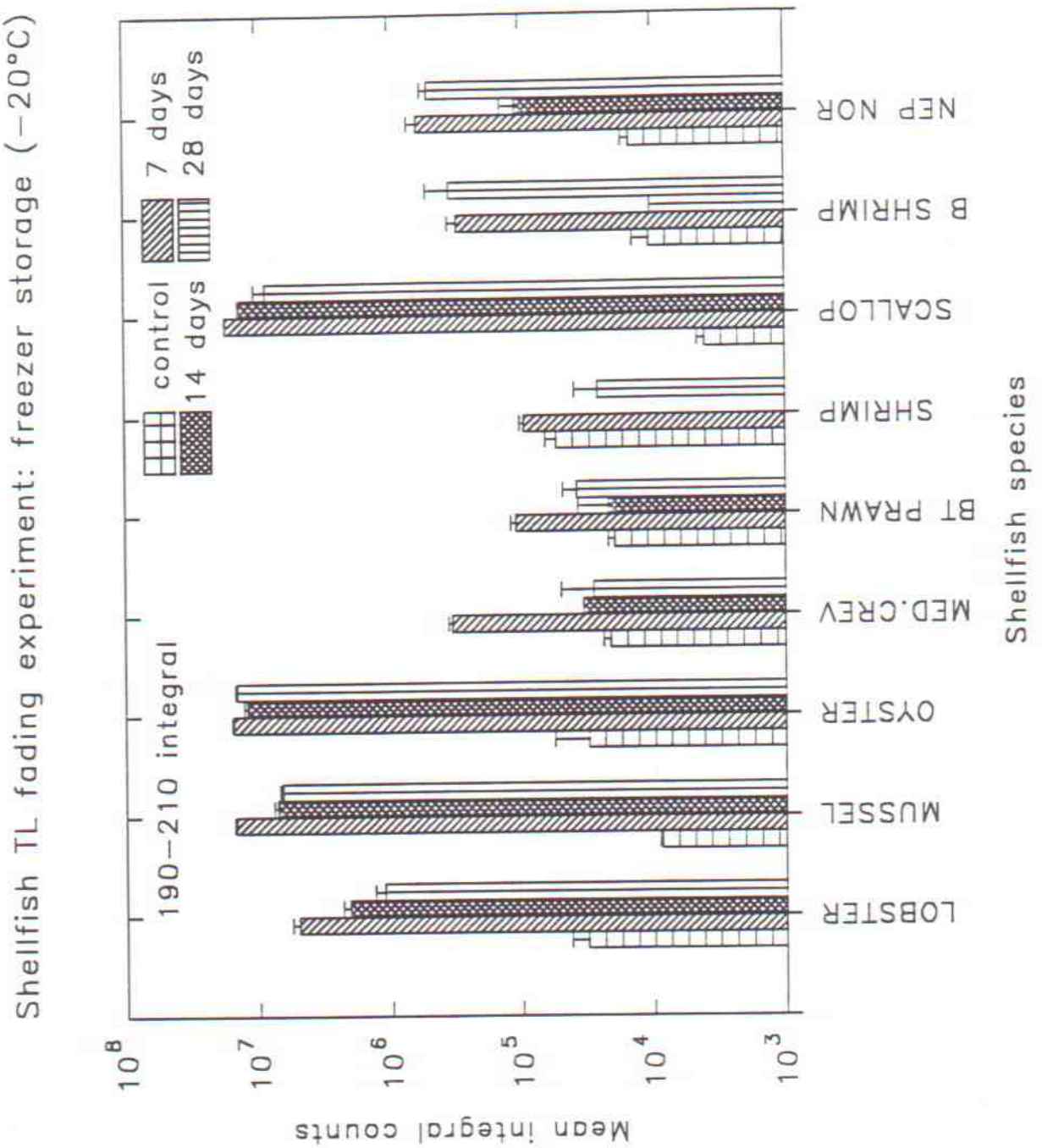


Figure 2.1.13 **Freezer storage for the 190-210°C temperature range**

Figure 2.1.14 **Freezer storage for the 210-230°C temperature range**

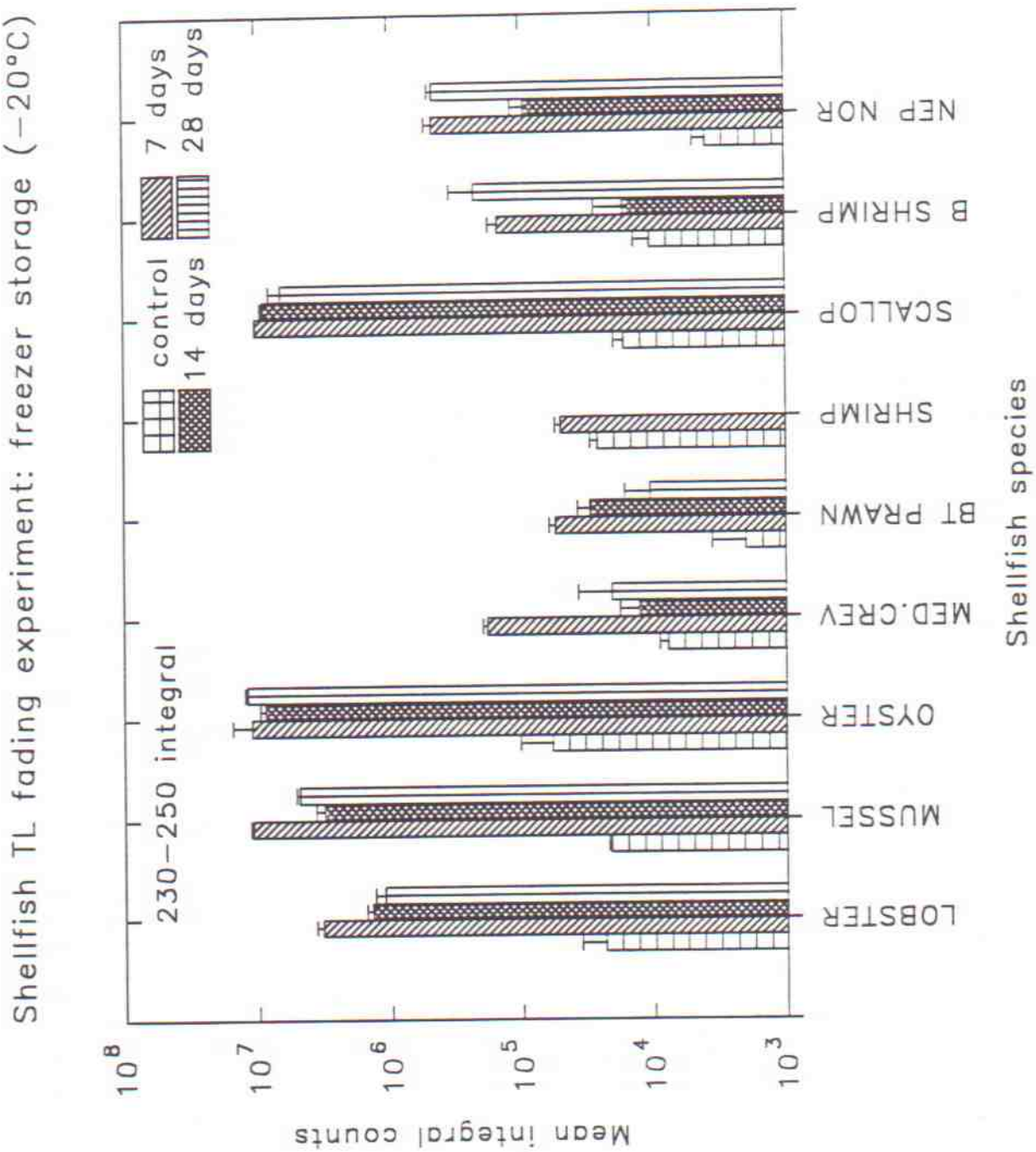


Figure 2.1.15 **Freezer storage for the 230-250°C temperature range**

2.2 Thermoluminescence of intestinal grits

The intestines of shellfish (crustaceans) contain small quantities of inorganic grits which provided that there are sufficient quantities of minerals, can be used for TL analyses following the standard density separation procedure. The physical extraction of minerals and intestines directly from each species is a time-consuming part of the analyses. For routine testing where there will be a large sample turnover and a high possibility of cross-contamination, presenting practical limitations.

The application of tissue solibulisers, enzyme digesters and acid hydrolysis have been examined for the recovery of intestinally trapped silicates for use in routine testing. We have investigated these methods and the use of acid hydrolysis proved to be the simplest and most effective method, especially for routine work.

An experiment was set up using two sets of six species of shellfish; Black tiger prawns, warm water shrimps, mediterranean crevettes, king scallops,nephrops norvegicus and brown shrimps were split into two portions, one which was irradiated to 1 kGy in the Co-60 source and the other of which was retained as a control. The samples were left for 68 days in the freezer. The samples were then shelled, placed into 6M hydrochloric acid and refluxed for 2-3 hours and the solution left to cool. The solution was then diluted with deionised water to an approximately 2M solution and left to settle. Decantation of the solution left the remaining mineral grains, which were washed several times in deionised water and finally suspended in acetone prior to being dispensed onto stainless steel discs.

The TL measurements were recorded in duplicate for each species of shellfish and a selection of glow curves are shown in figures 2.2.1 and 2.2.2. Every irradiated sample could be readily detected from the unirradiated controls, as shown in the histograms, glow1 v's glow2 ratio plots in figures 2.2.3 and 2.2.4.

This technique has also been applied in parallel with the physical extraction method layed out in the protocol of the BGA interlaboratory trials of shellfish. The acid hydrolysis method correctly identified each irradiated and unirradiated species of shellfish.

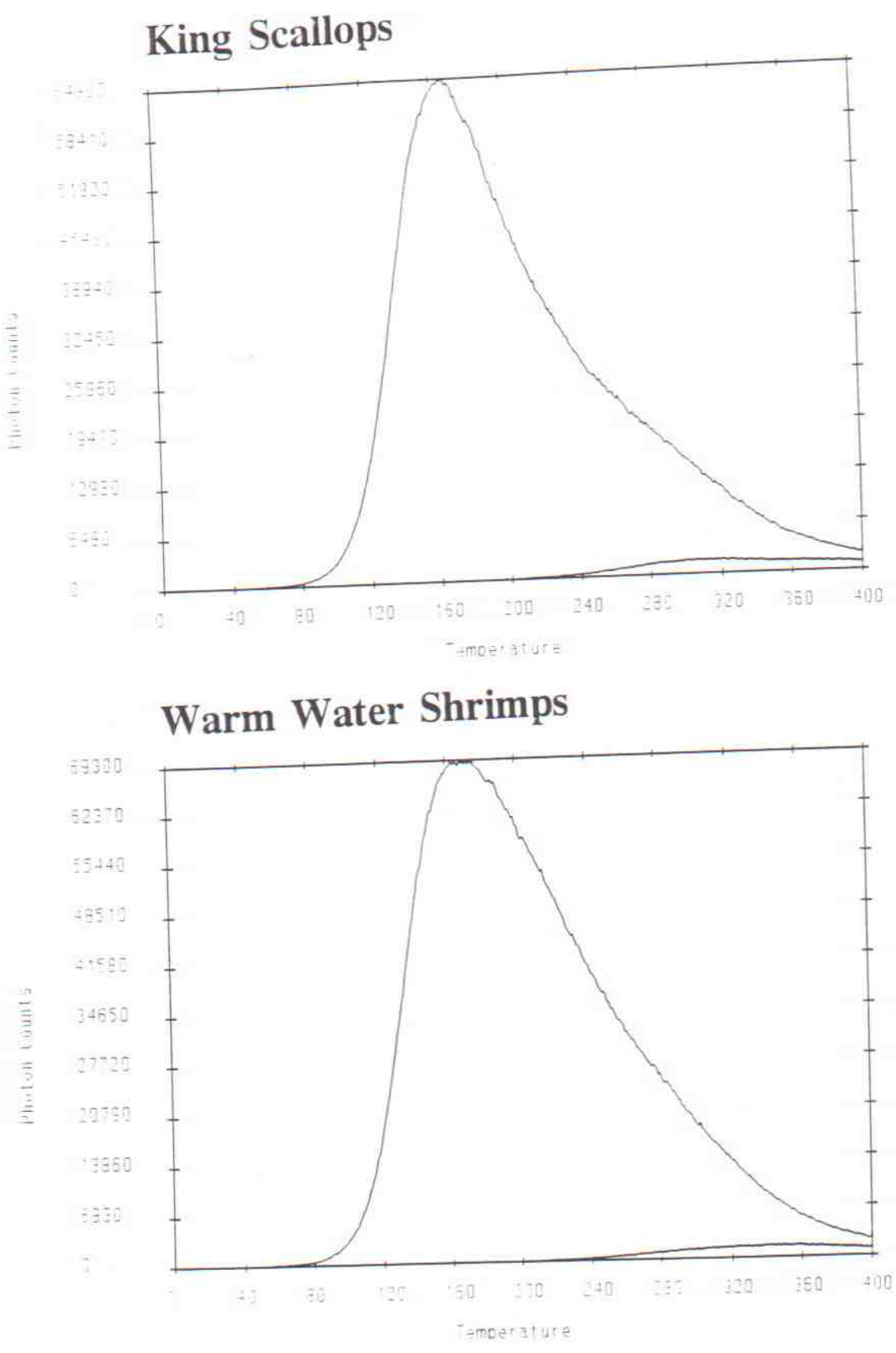


Figure 2.2.1 **Selection of shellfish glow curves using the hydrolysis procedure**

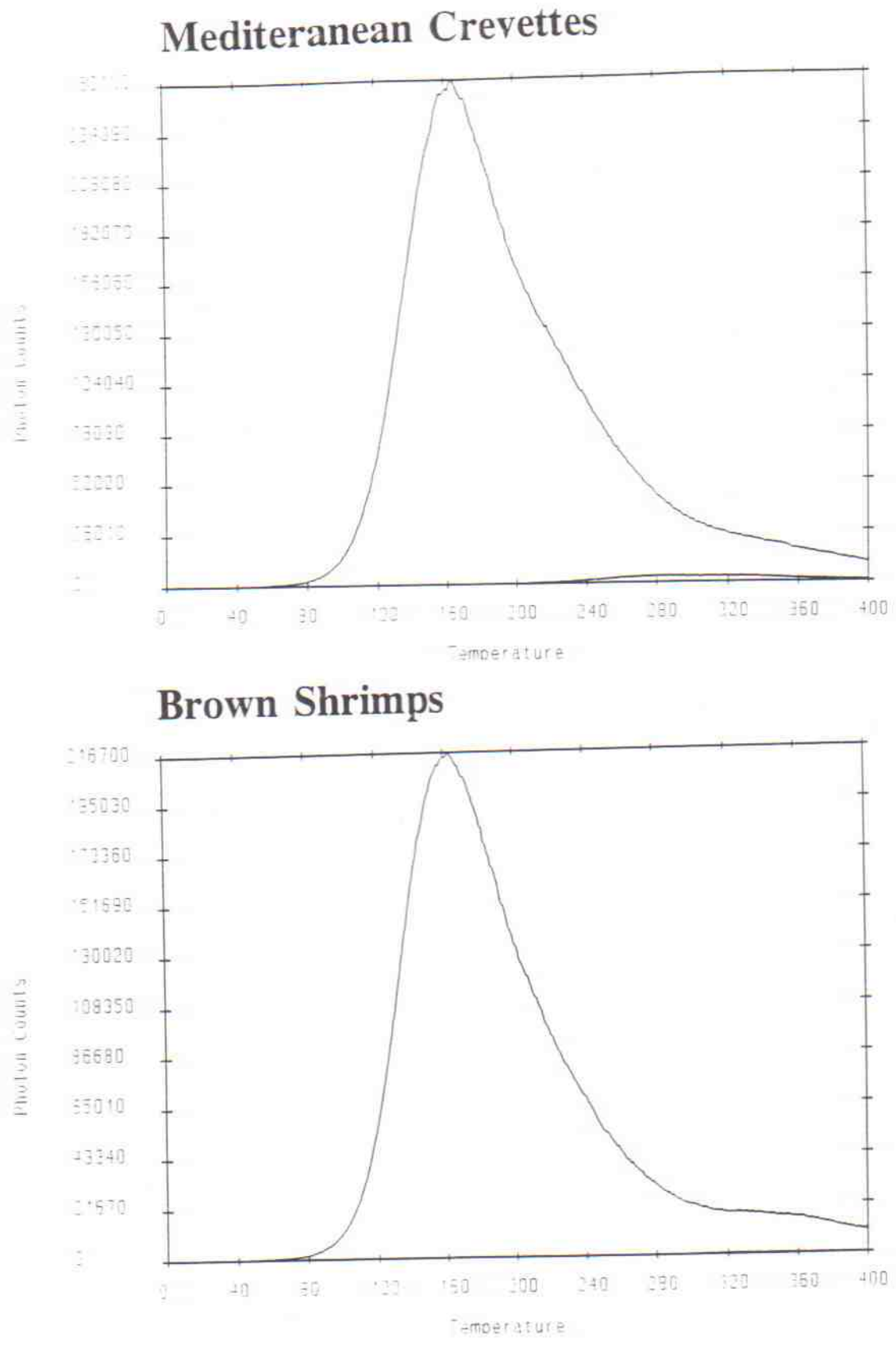
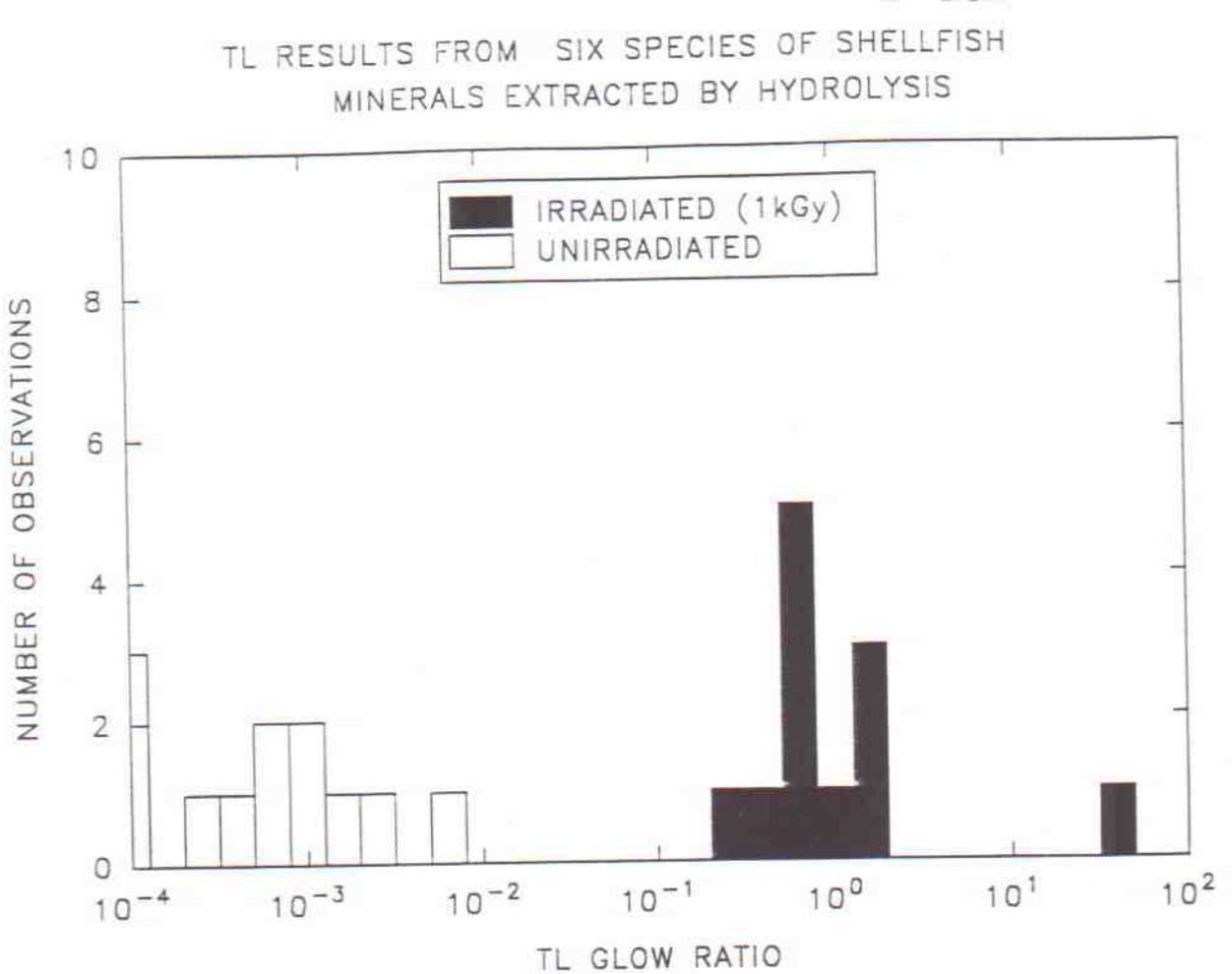


Figure 2.2.2 **Selection of shellfish glow curves using the hydrolysis procedure**



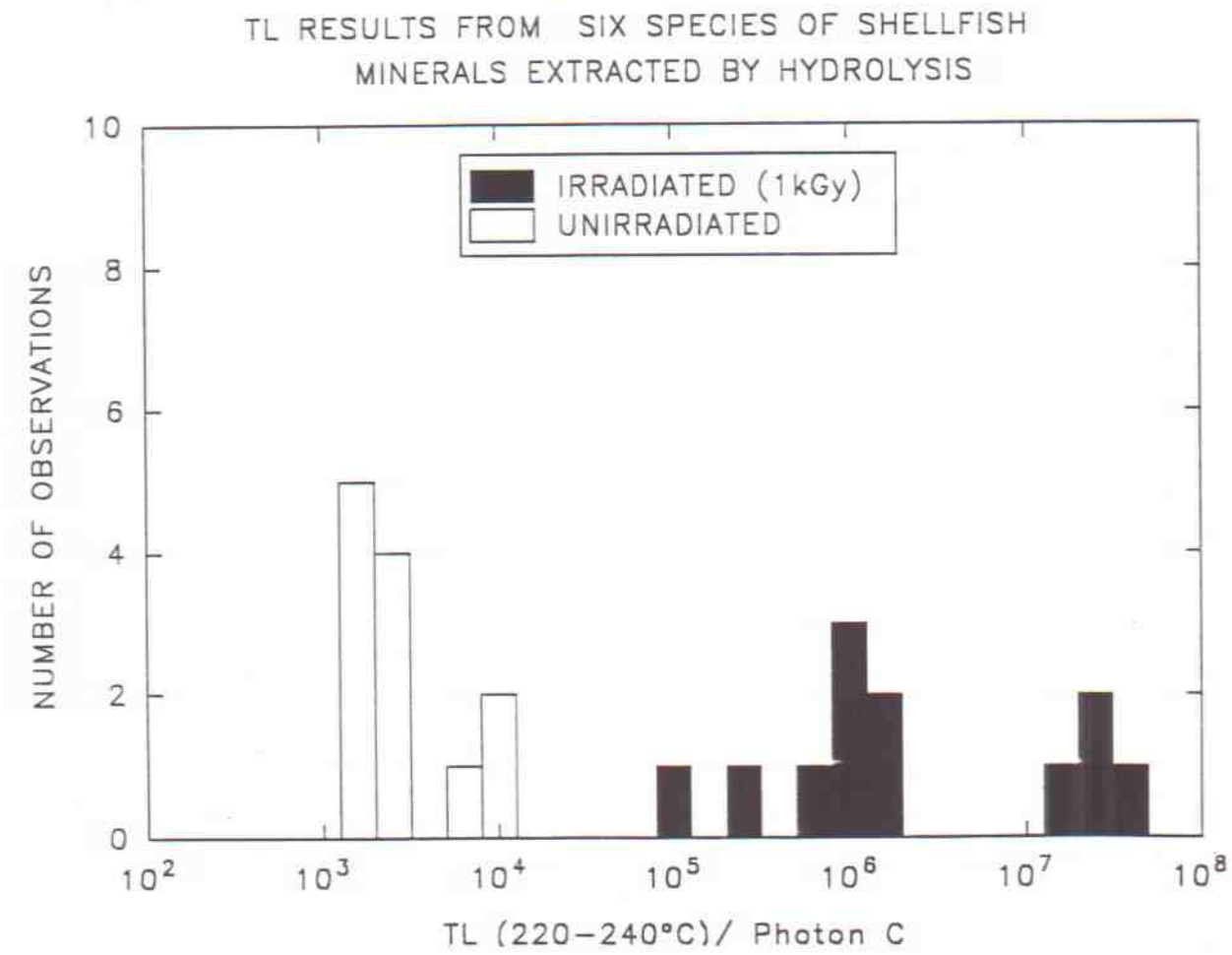


Figure 2.2.3 **Histograms for six species of shellfish - minerals extracted using hydrolysis**

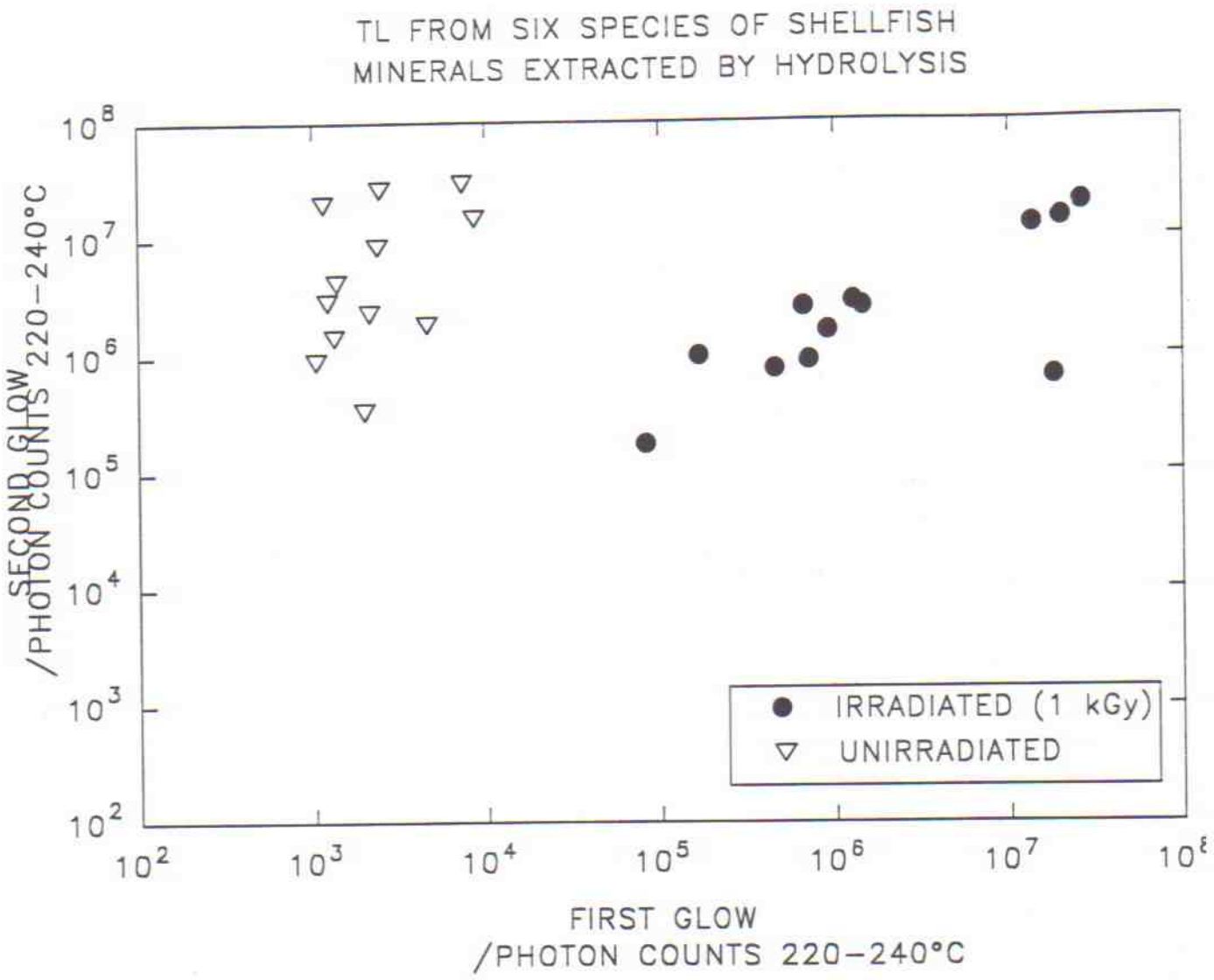


Figure 2.2.4 **Glow1 v's glow2 ratio plots for six species of shellfish - minerals extracted using hydrolysis**

3. EMISSION SPECTROSCOPY

A powerful technique for examining the recombination centres is emission spectroscopy. In photo stimulation the stimulating wavelength has to have enough energy to be able to evict charge from its trapping site and also necessary to have the detection window that allows for transmission of the resulting stimulated signal. For this reason emission spectroscopy is an important requirement for the development and optimisation of luminescence techniques. As well as identifying possible detection windows, emission spectroscopy can be used to assist in the interpretation of observed phenomena.

The TL results obtained from powdered shellfish are suggestive of an emission spectrum dominated by Mn^{2+}. Some emission spectroscopy on the 9 shellfish species were very kindly run at Sussex University by Professor Peter Townsend. The spectrum all show the main luminescence centre in shellfish is Mn^{2+}. The well mineralised species show some broad band structure, whilst the poorly mineralised species give weaker signals in the orange band. A selection of spectrum obtained for shellfish are shown in figures 3.1 - 3.6.

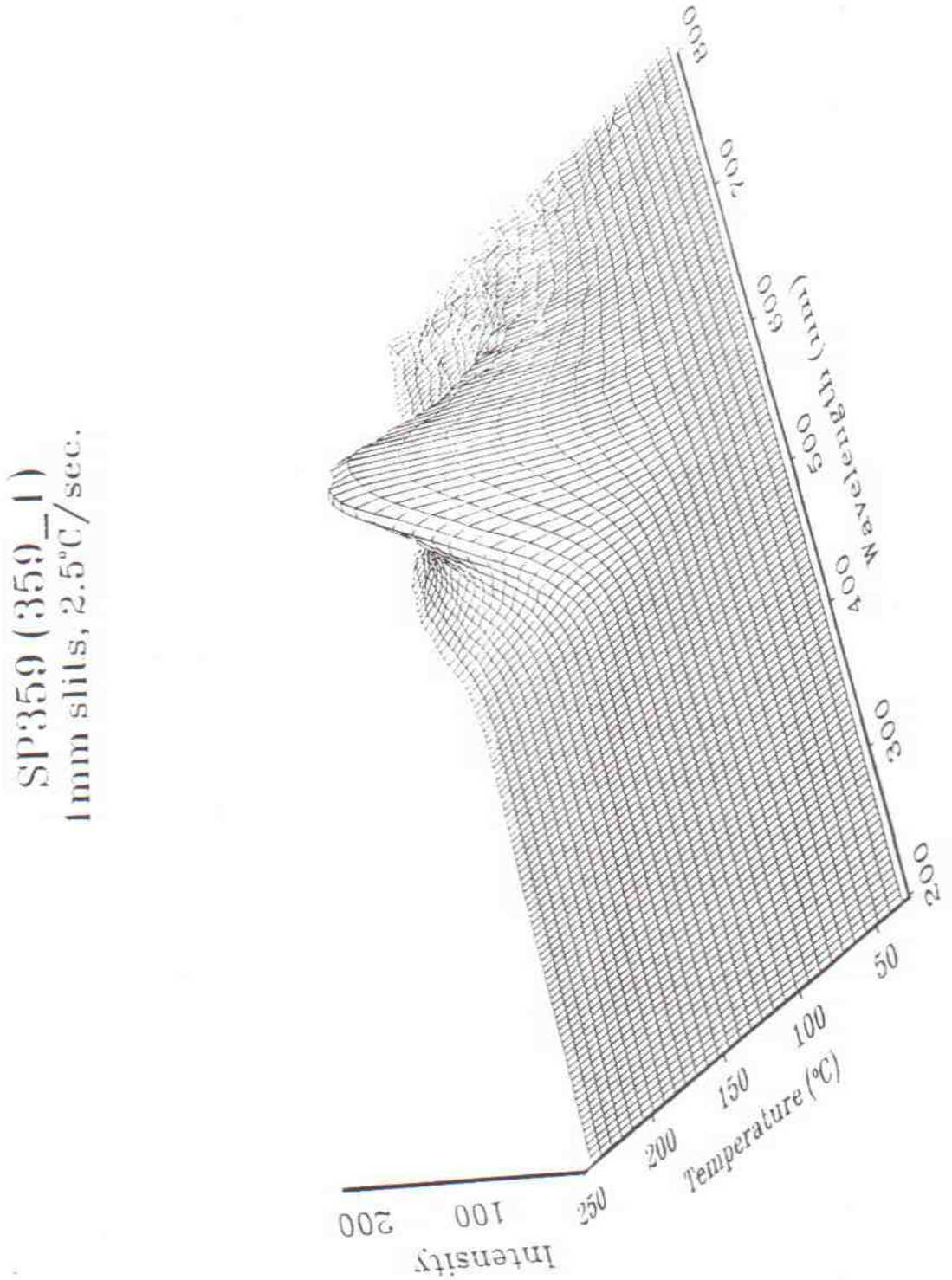


Figure 3.1 **The emission spectrum for lobster**

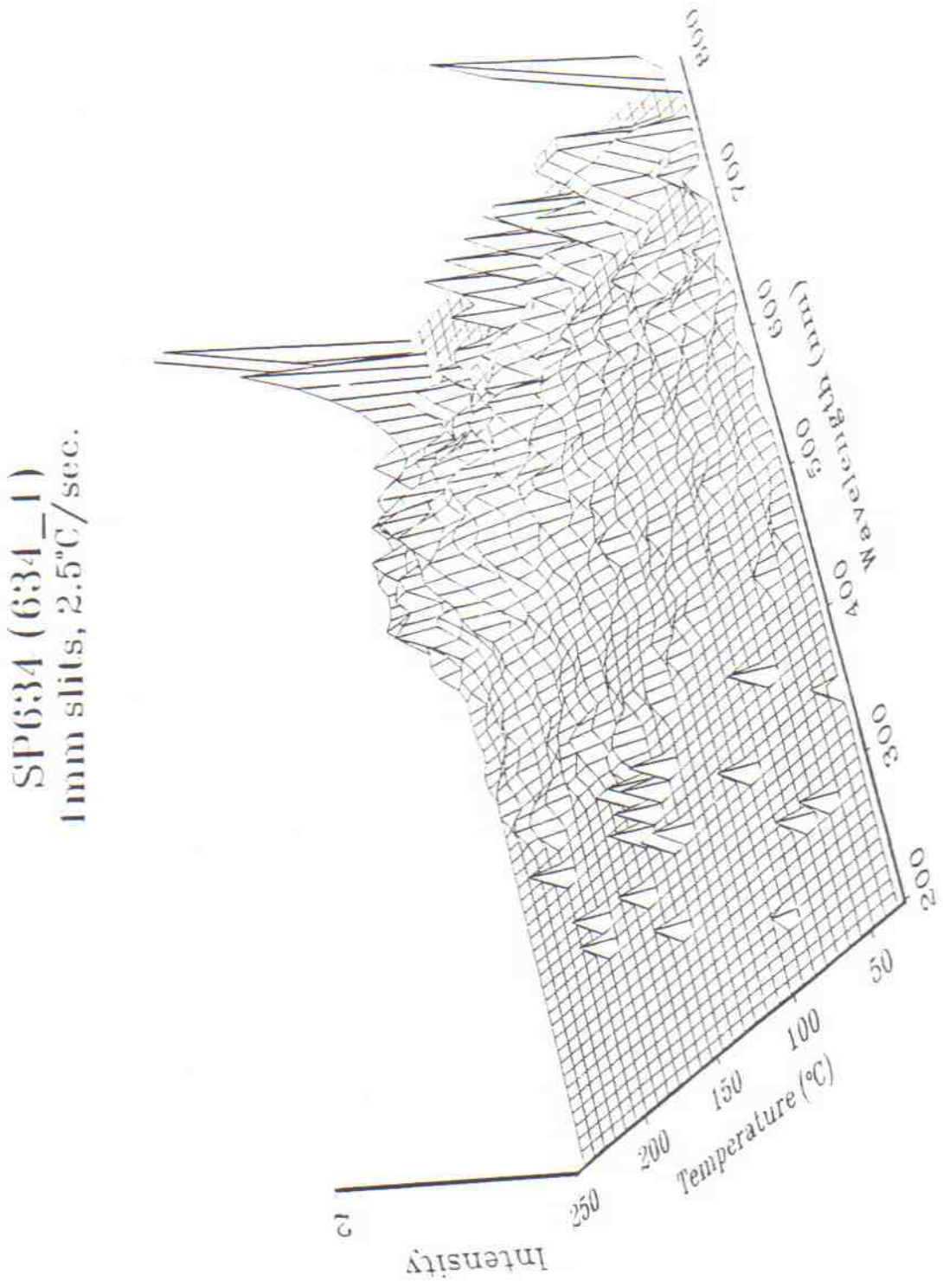


Figure 3.2 **The emission spectrum for mediteranean crevette**

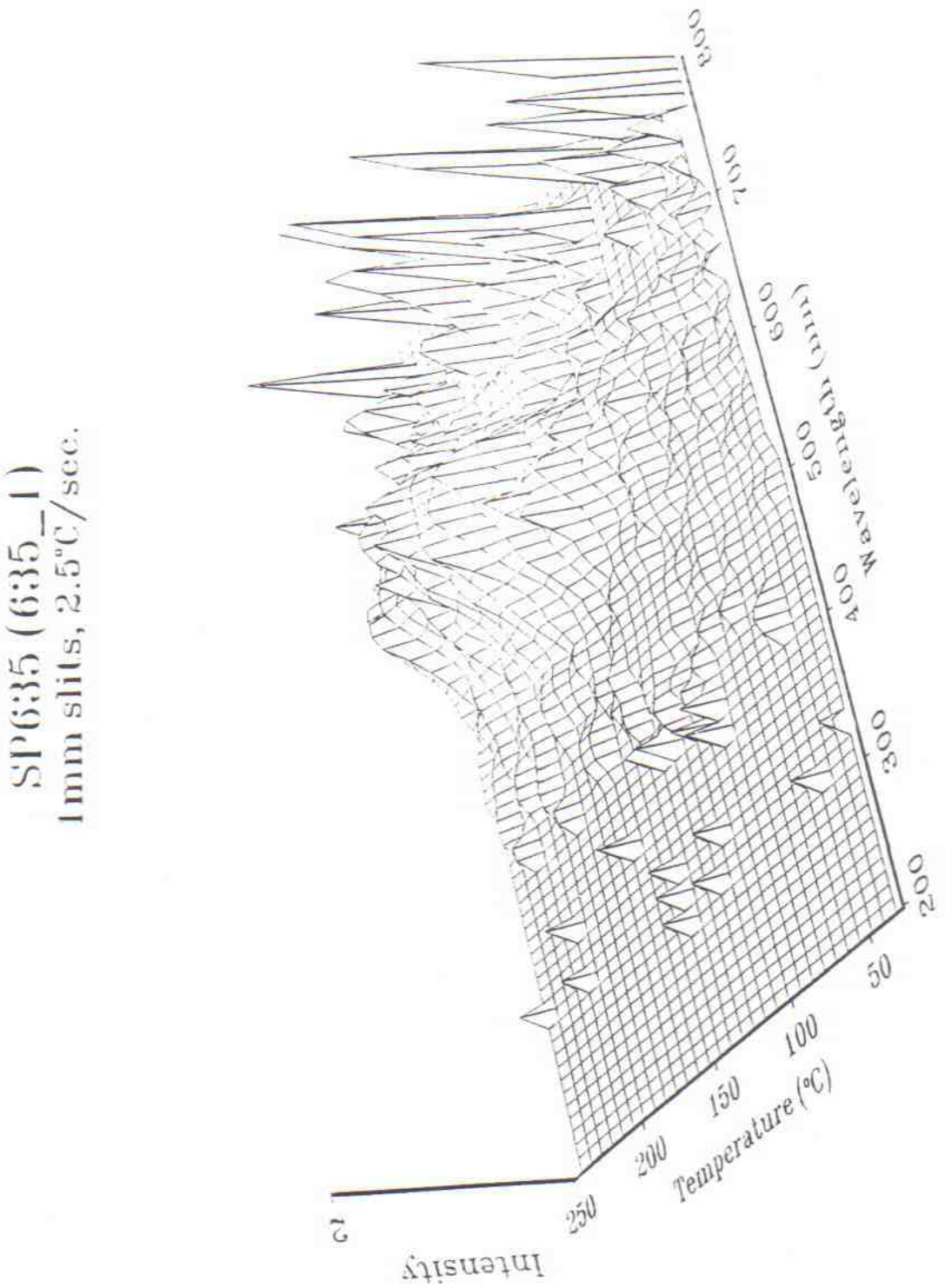


Figure 3.3 **The emission spectrum for black tiger prawn**

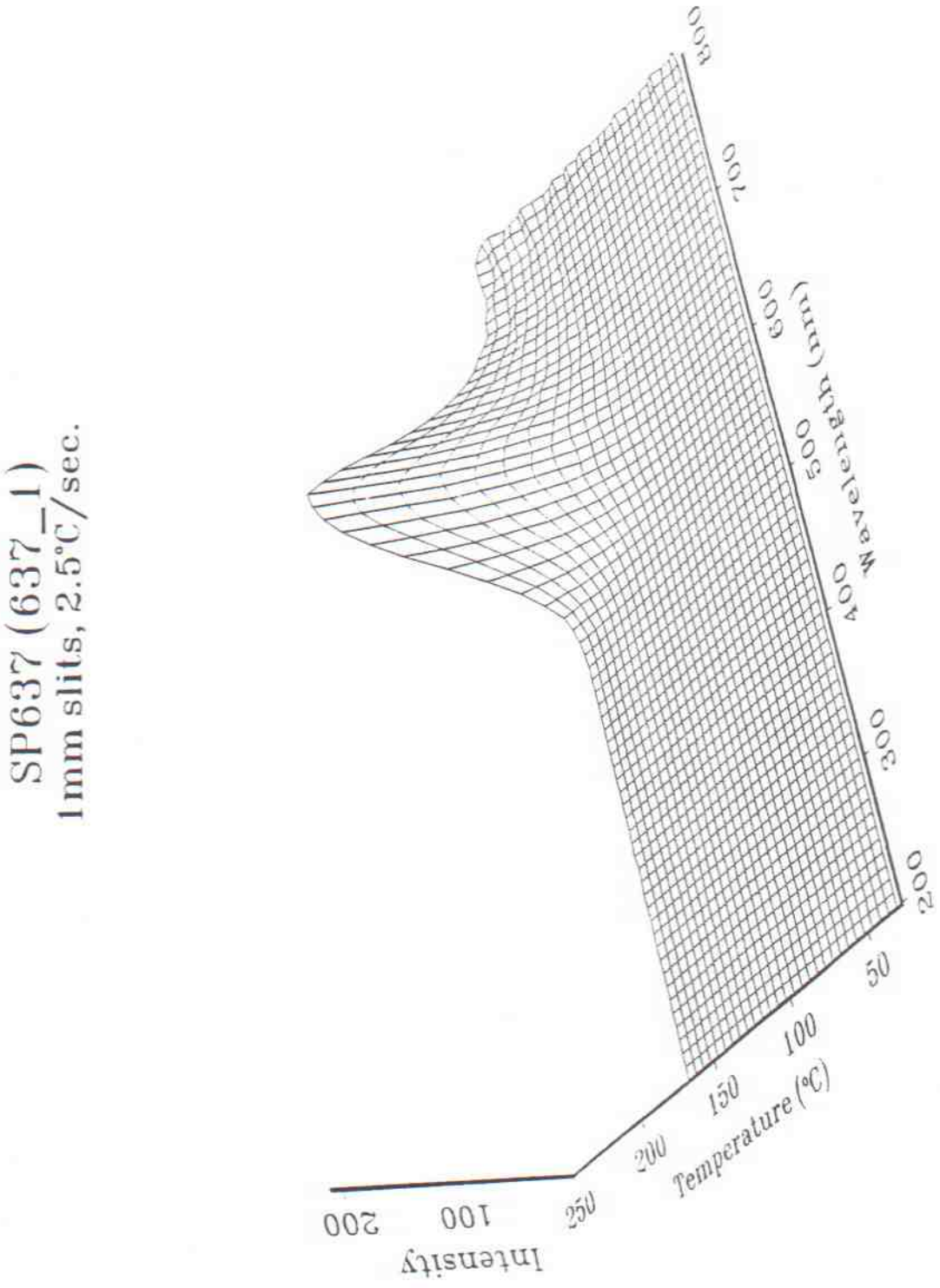


Figure 3.4 The emission spectrum for king scalop

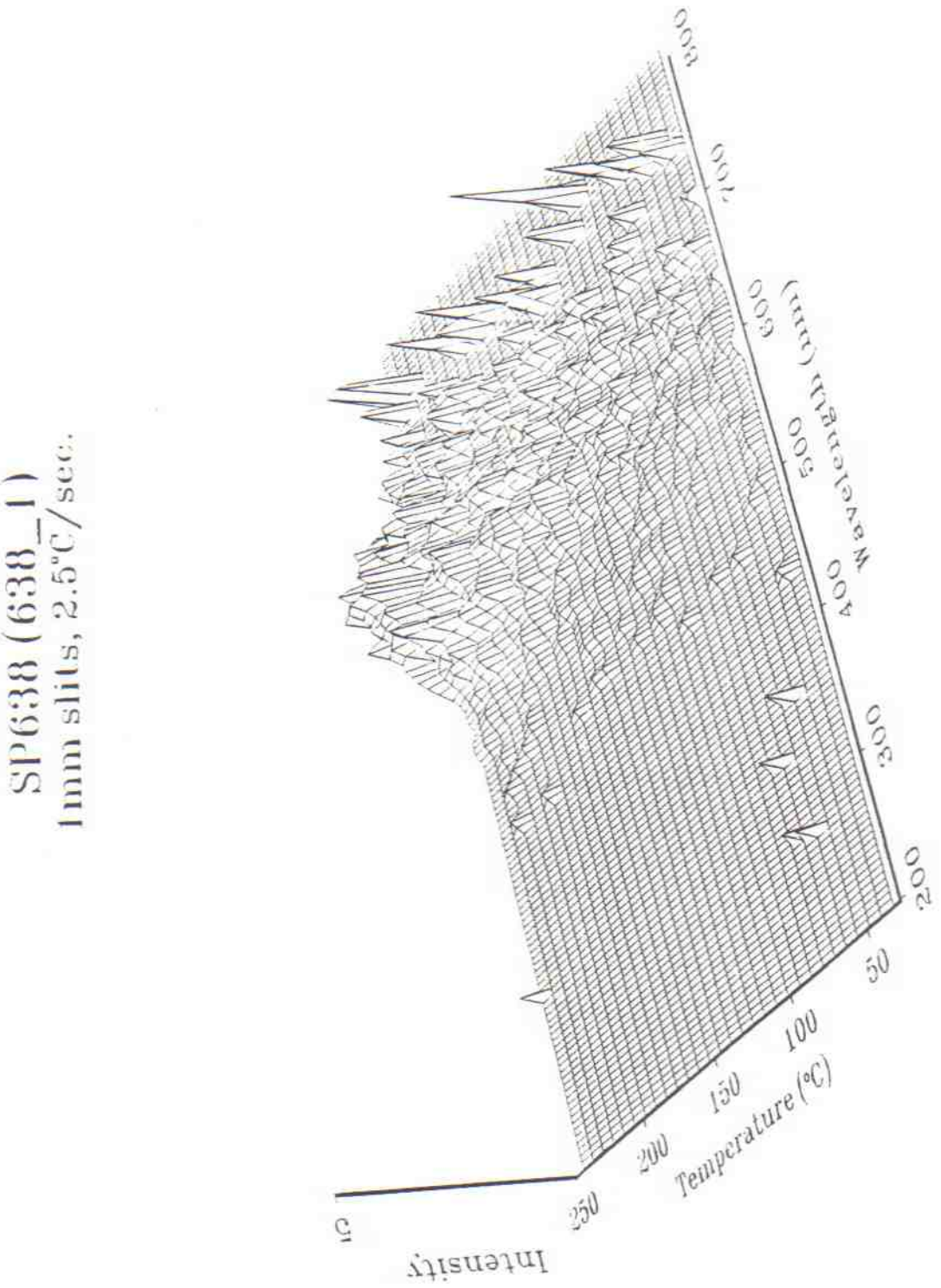


Figure 3.5 The emission spectrum for brown shrimp

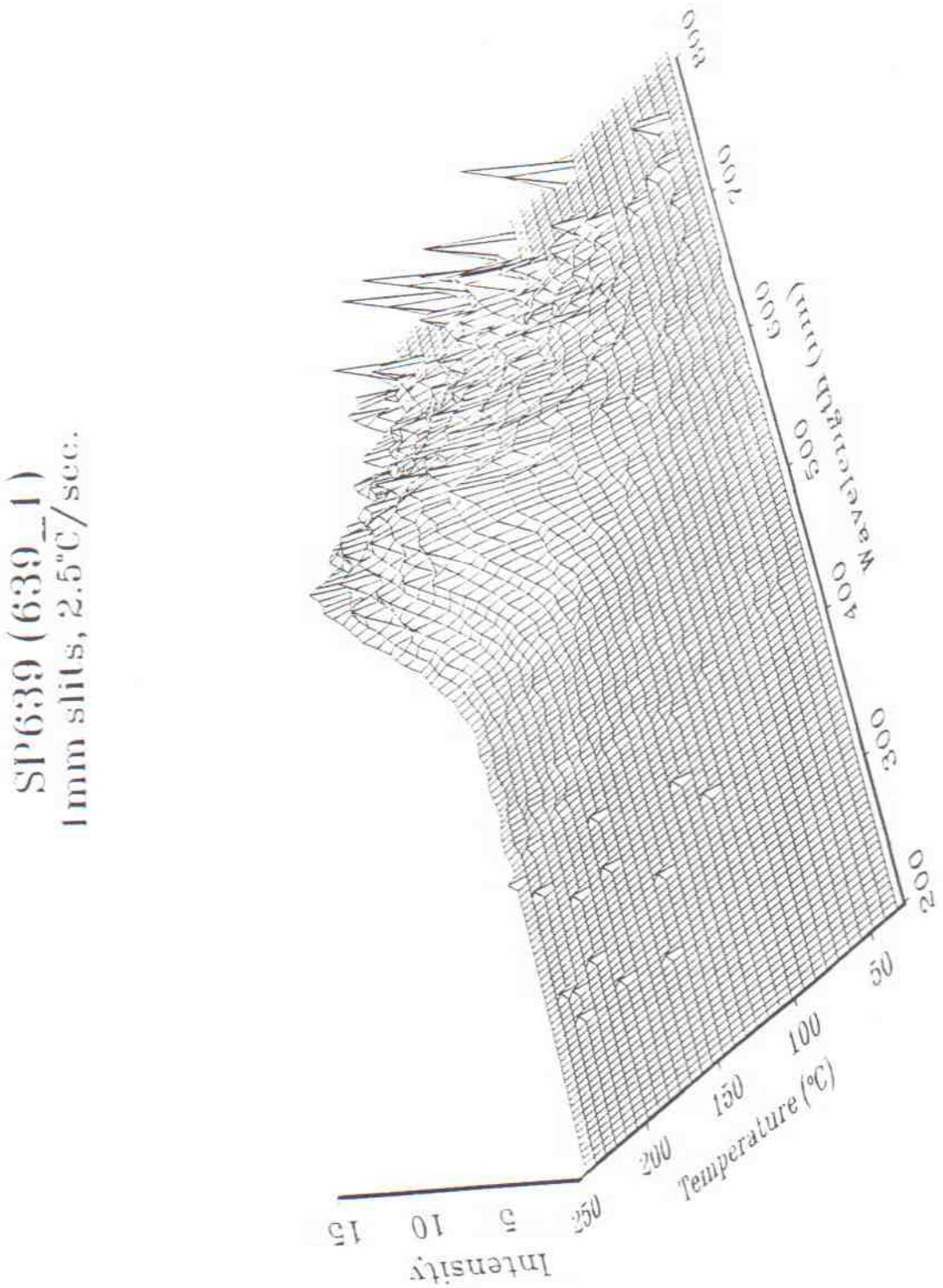


Figure 3.6 **The emission spectrum for nephrops norvegicus**

4. PHOTOSTIMULATED LUMINESCENCE

4.1 Phototransfer thermoluminescence (PTTL)

It is expected that many of the problems of elevated and variable backgrounds, plus relatively low TL signals could be overcome using photo-stimulation to release luminescence, and thus avoid heating. Phototransfer thermoluminescence, (PTTL), is a technique which is a mixture of photostimulated luminescence, (PSL), and TL. PSL is used to transfer trapped charges from deep traps to shallower traps, by illuminating a sample which has been cryogenically cooled and allowing retrapping. The TL glow curve is then obtained by measuring the luminescence using a PMT whilst heating the sample in the usual manner.As reported previously (Sanderson, 1990, Sanderson, 1991) initial studies of excitation spectra from irradiated minerals, herbs and spices, and shellfish have validated the concept and suggest that this approach may lead to rapid and sensitive detection methods for certain food classes. Research into the mechanisms of photostimulated luminescence from silicates has been pursued using monochromatic systems on the implications of PSL from silicates for archaeological dating. The accompanying knowledge of stimulation and emission spectroscopy of feldspar systems is most probably directly applicable to detection of herbs and spices via mineral contaminants.

Early experiments with shellfish produced promising, but variable results. Using a near UV detection band for PSL we have observed a relatively strong stimulation band from a single crab sample; however this was not present in a suite of samples of prawn shells examined shortly afterwards. Similarly weak thermally assisted PSL using infra-red stimulation has been observed from a set of prawns, but such IR transitions may not be characteristics of calcite phases, and may rather have resulted from the stimulation of minor silicate impurities in the sample. The stimulation conditions which might isolate the PSL signals from pure calcite were not practicable using our original excitation experiments with UV detection.

It is established that most forms of calcite studied show an emission spectrum dominated by Mn^{2+} transitions producing a broad emission band around 550 nm. PSL emitted in this region would be difficult to detect with the UV filtered PM tube and IR stimulation is unlikely to be a highly efficient process for calcite. The presence of both visible stimulation and detection bands from calcite presents a number of technical challenges. Asynchronous detection schemes, either involving pulsed excitation using solid state sources or lasers, or by photo-transfer would result in efficient metrological schemes. To investigate the hypothesis that strong stimulation and emission bands may exist in calcite shells in such regions a simple photo-transfer experiment was conducted with a small suite of samples of shells, organic materials and chalk.

The sample is firstly cooled, to increase the thermal stability of shallow (and empty) traps, and then illuminated. Photo-stimulated release of trapped charge carriers (in donor sites) may then take place, followed by a certain amount of re-trapping in the shallow (acceptor) traps. The photo-transferred signal may be observed by release of TL during subsequent warm up. Phototransfer efficiency is not usually very high, due to a large proportion of the released charge from donor traps, relaxing by one mechanism or another. This technique is however

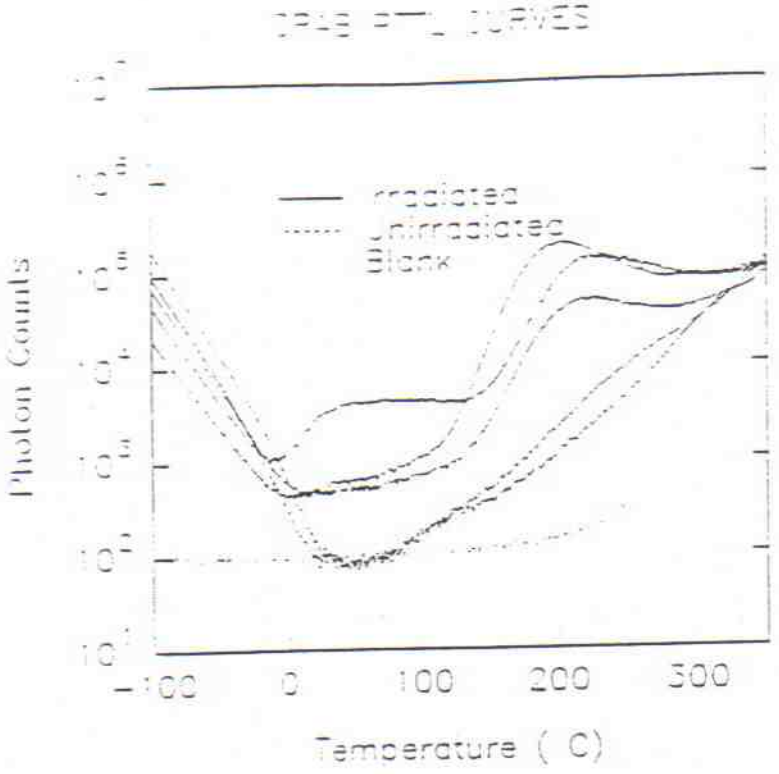
sensitive, and allows complete spectral de-coupling of the excitation source, and the luminescence detector. This may have the advantage of transferring the radio-induced signal from a temperature region of high background, where there might be spurious, water-related TL, or organic signal to a region where the background is lower, thereby improving the signal to noise ratio and hence, reliability of results. Room temperature phototransfer has been established for mineral sources of calcite previously. In these initial experiments we have explored the use of cryogenic re-trapping of charge released by polychromatic stimulation .

An experiment was undertaken where the samples were introduced, on stainless steel discs to the SURRC PSL/PTTL spectrometer, and were then cooled to -100°C using an indirect liquid nitrogen cooling coil attached with silver solder to the base of the heating plate. They were illuminated with zero order light from 300 W Cermax lamp in combination with an f3.4 monochromator for 15 minutes. A further 3 minutes post-illumination delay was allowed during which time the photomultiplier was positioned above the sample chamber and switched on. The PM system was an unfiltered quartz-windowed Thorn EMI 9883QB tube operated on plateau in photon counting mode. Luminescence was recorded during linear heating from from -100°C to 100,200, and at a later stage, 350°C.

The samples investigated were irradiated and unirradiated pairs of chalk, sugar, potato starch, glycine, crab and lobster. Composite results from crab and lobster are shown in figure 4.1.1 respectively. We found that the organic materials produced a non-radiation specific phosphorescence which decayed during warm up and that this decay was approximately log-linear and would be eliminated by increasing the post-illumination delay or by filtering. These signals were also observed in pure gelatine samples and shellfish. A combination of PTTL and TL in addition to the organic phosphorescence signals was found for the samples of chalk, crab and lobster. One of the most important observations was that the lower temperature signal is free from interference by enhanced spurious background and that the 150-250°C TL signal is typically 30 times stronger than the TL signals.

From these results further cryogenic PTTL experiments were undertaken and extended to include the introduction of thermal washing before illumination, to separate PTTL and residual TL signals. This series of work was to determine optimal stimulation and detection wavelengths using the monochromator, and choice of optical filters.

An experiment was set up using the PSL/PTTL spectrometer with a 300 W cermax lamp in combination with a f3.4 monochromator and the photomultiplier system was a filtered KG3 Thorn EMI 9883QB tube. The protocol



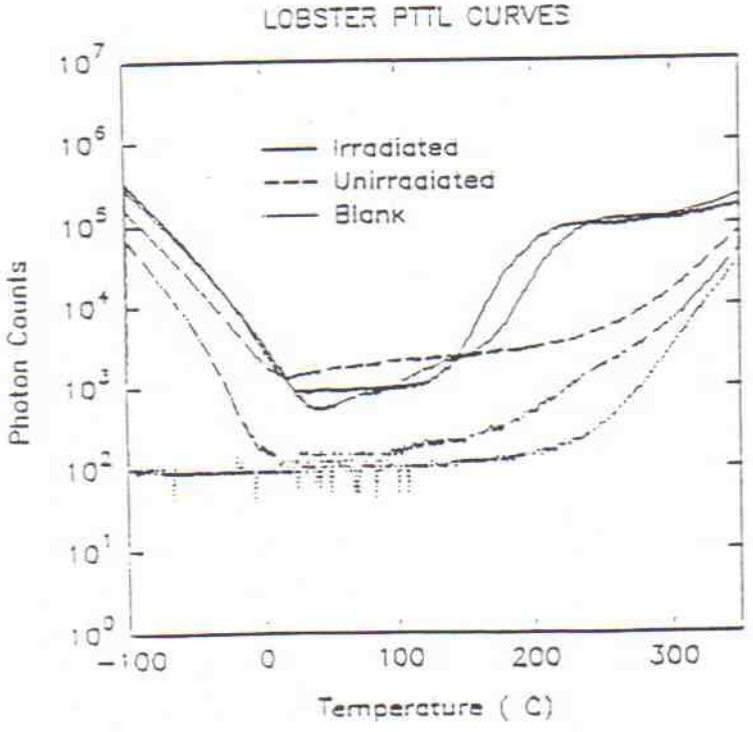


Figure 4.1.1 **Phototransfer curves for crab and lobster**

The sample of powdered king scallop was dispensed on stainless steel discs and placed into the spectrometer, where they were cooled to -50°C using an indirect liquid nitrogen cooling coil attached to the base of the heating plate. The sample was then ramped at 2°C/sec to 200°C to measure the TL. This is known as the thermal wash. The sample is then recooled, illuminated for 5 minutes at set wavelengths and then ramped from -50°C to 200°C to obtain PTTL. A further ramp from -50°C to 200°C is carried out. This protocol was carried out for the following wavelengths; 500,550, 600, 650, 700, 750, 800, 850, 900 nms. From 550nm onwards no significant signal was obtain. However using white light with a 5 minute shine did give a PTTL signal. From these results we decided to repeat the experiment using freshly irradiated sample and cool to a lower temperature, restricting the top temperature to 150°C and measure using wavelengths from 500nm to 350nm to optimise the wavelength. The results indicated that 450nm gave the best signal intensity.

4.2 PSL Excitation Spectrometry

Exploration of the trap structure of calcitic shells was conducted using cryogenic PTTL experiments discussed in the previous section. This identified a series of visible and near infra red stimulation bands for more detailed PSL investigations.

The PSL excitation spectrometer shown in figure 4.2.1 was used to investigate Anti-Stokes transitions from the infra red band to the Mn^{2+} emission band. Initial experiments were carried out using various filter combinations to optimise the stimulation and detection windows, whilst scanning the infra red region of 600 - 1000nm. To isolate the stimulation and detection bands, the light from the monochromator, was passed through 3mm of OG530 and BG3 filters, with the detection window defined by 4mm of BG39 filter. Having optimised the system the power of the xenon lamp was obtained to give the characterisation of the source with these filter combinations and this is illustrated in figure 4.2.2. A series of experiments were then conducted using seven species of shellfish irradiated to 3 kGy the day before measurement; Brown shrimp, black tiger prawn, warm water shrimp, nephrops norvegicus, king scallop and mediterranean crevette. Figure 4.2.3 and 4.2.4 show the raw spectra obtained for irradiated and unirradiated brown shrimp and king scallop. The power normalised spectra for brown shrimp and king scallop are illustrated in figures 4.2.5 and 4.2.6. These figure show the presence of a complex series of IR stimulation bands. Similar spectra was recorded for the other samples and a selection of these are shown in figure 4.2.7. The main observed variation are the sensitivity and ratios of the two main spectral components at approximately 800-825nm and 850-900nm. It is possible to distinguish between irradiated and unirradiated samples in well mineralised species. In poorly mineralised species, the difference between the spectral components in the irradiated and unirradiated species is far lower, making detection ambiguous. Further work is required to investigate this stimulation region and the stability of the spectral components.

These results may not have produced a detection method for irradiated shellfish, however we have obtained exceptionally useful information about the complex structure of the bands in the infra red region. Having identified these stimulation bands it may be possible trying an alternative approach using PSL, to give us the end result that we are seeking - a unambiguous test for irradiated shellfish. This alternative route is discussed in the next section.

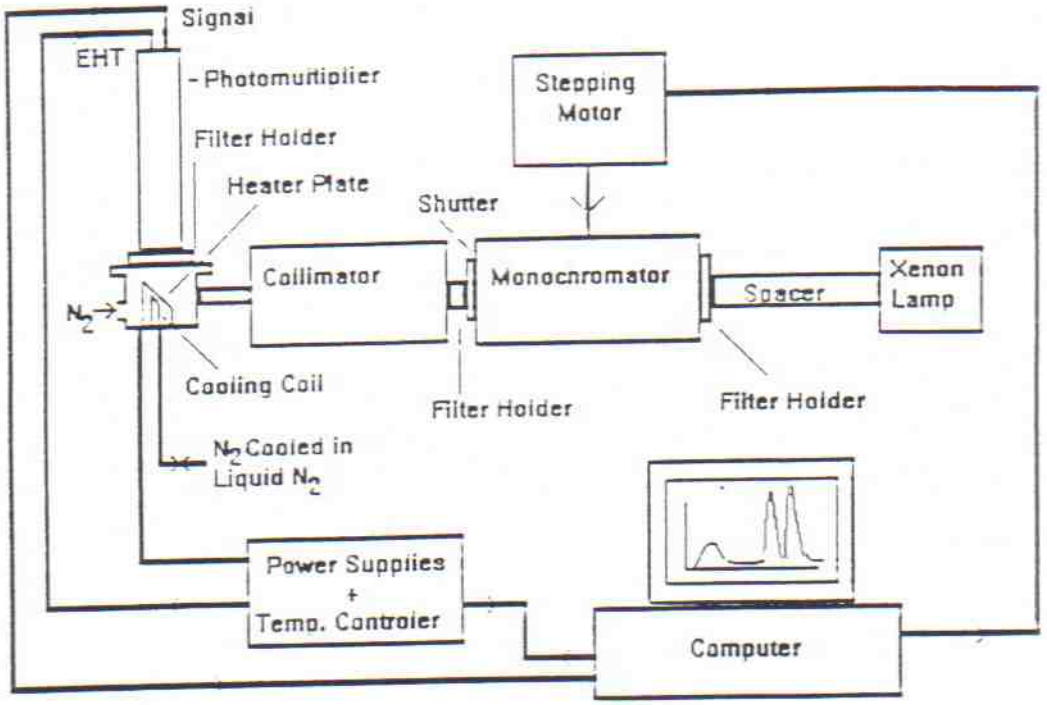


Figure 4.2.1 Schematic diagram of the PSL excitation spectrometer

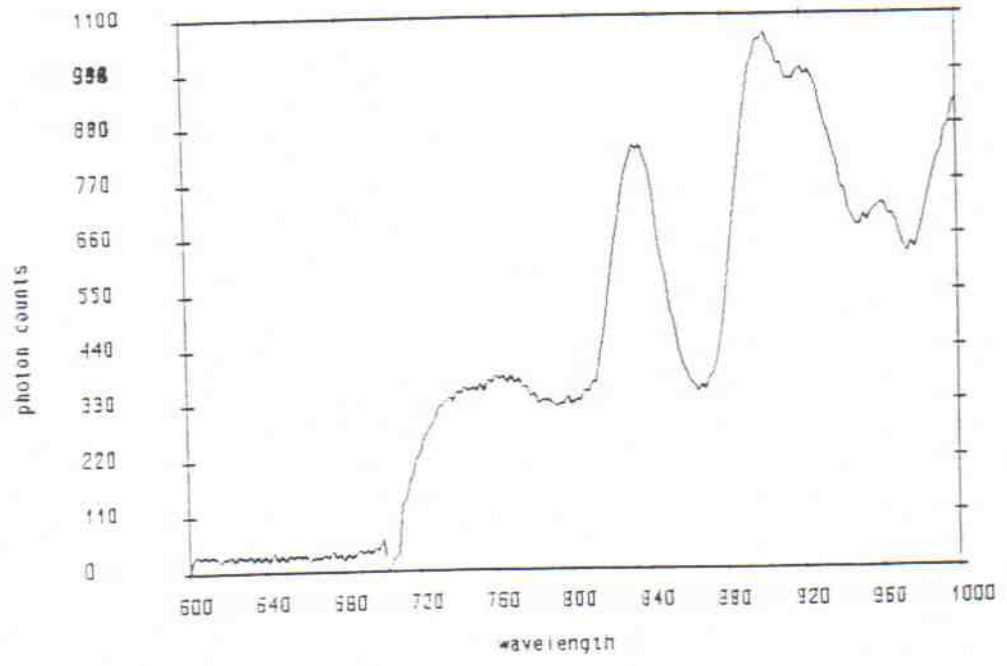


Figure 4.2.2 Xenon lamp power spectrum with BG3 + OG550 filters

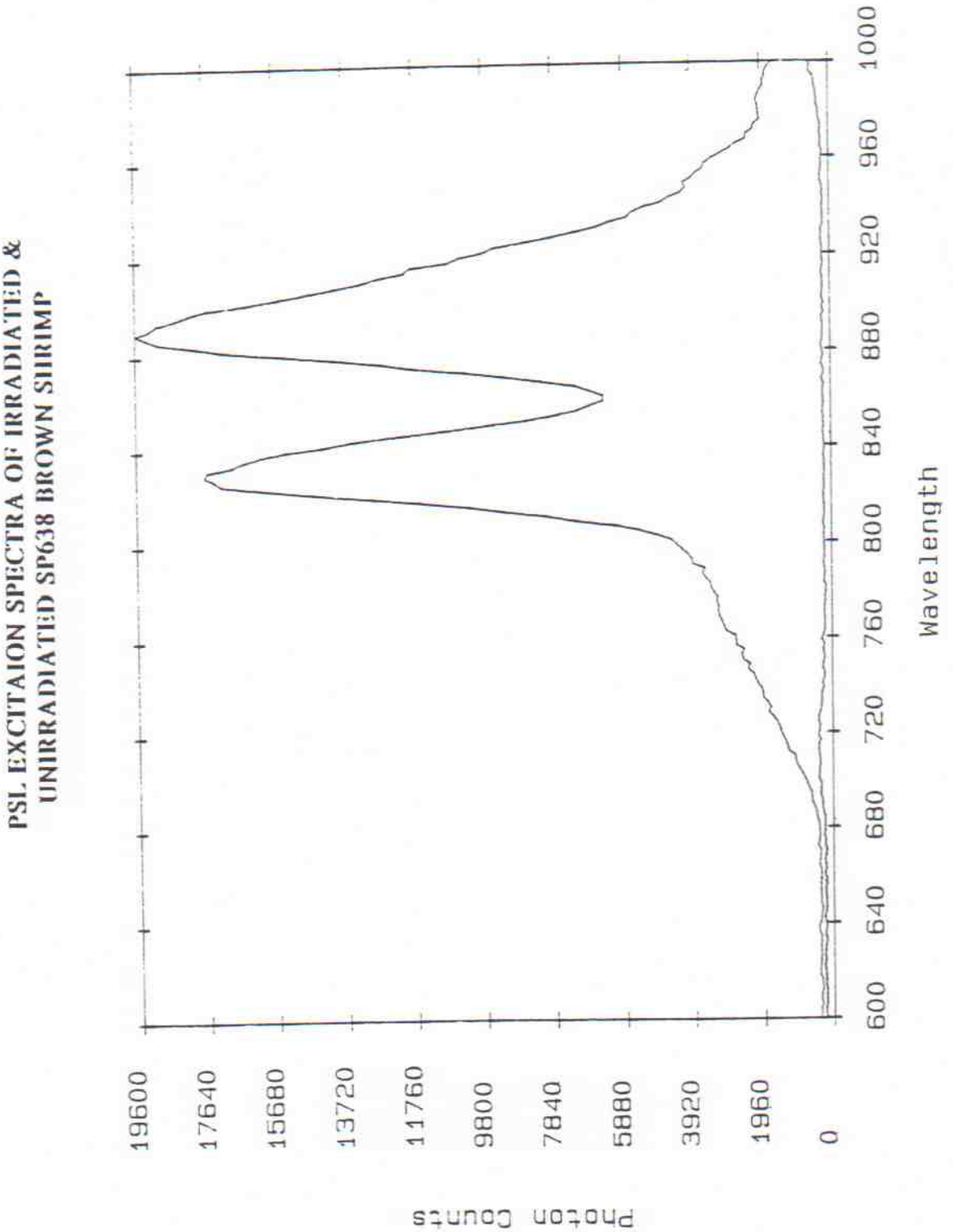


Figure 4.2.3 Raw spectra of irradiated and unirradiated brown shrimp

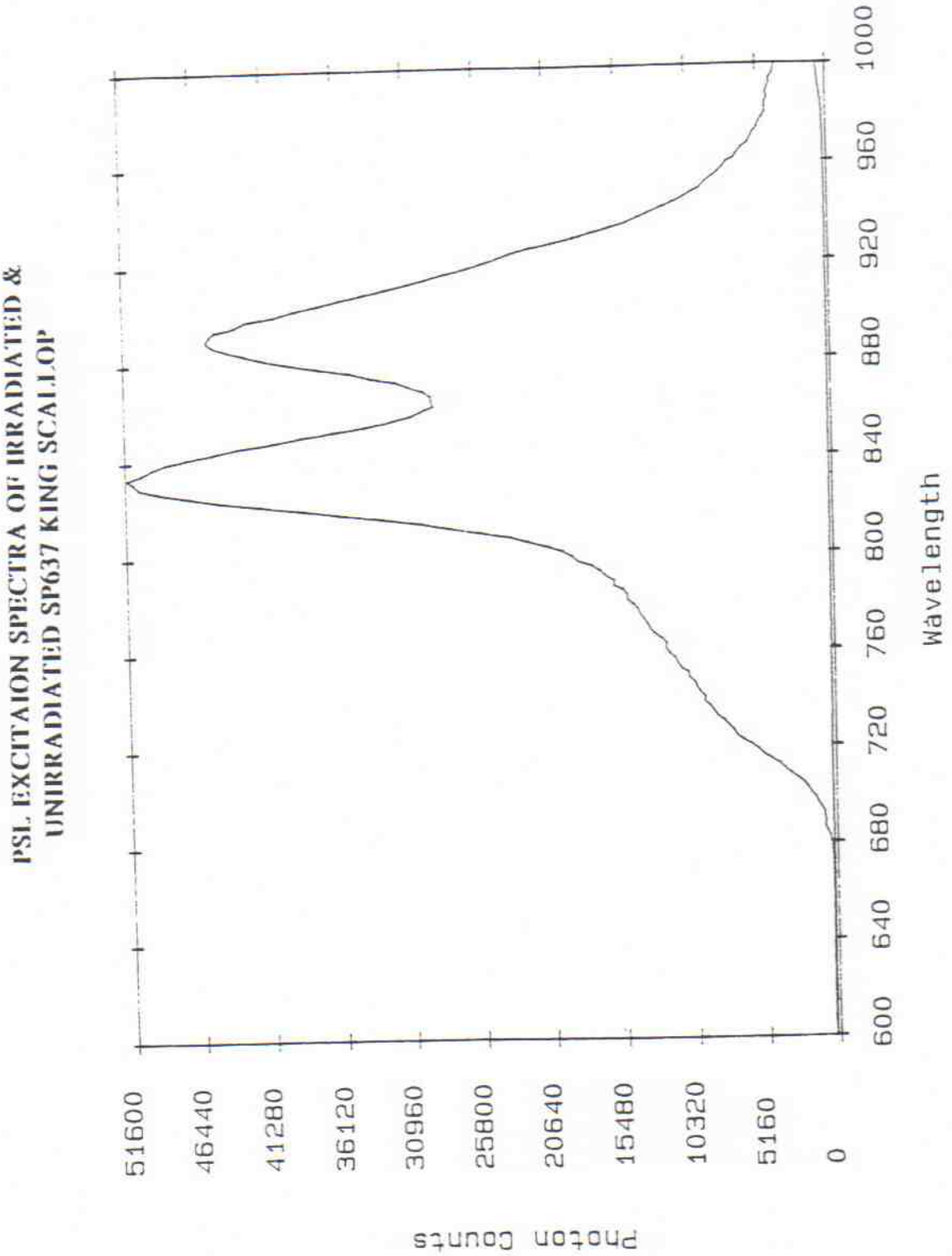


Figure 4.2.4 Raw spectra of irradiated and unirradiated king scallop

Figure 4.2.5 Power normalised spectra for irradiated and unirradiated brown shrimp

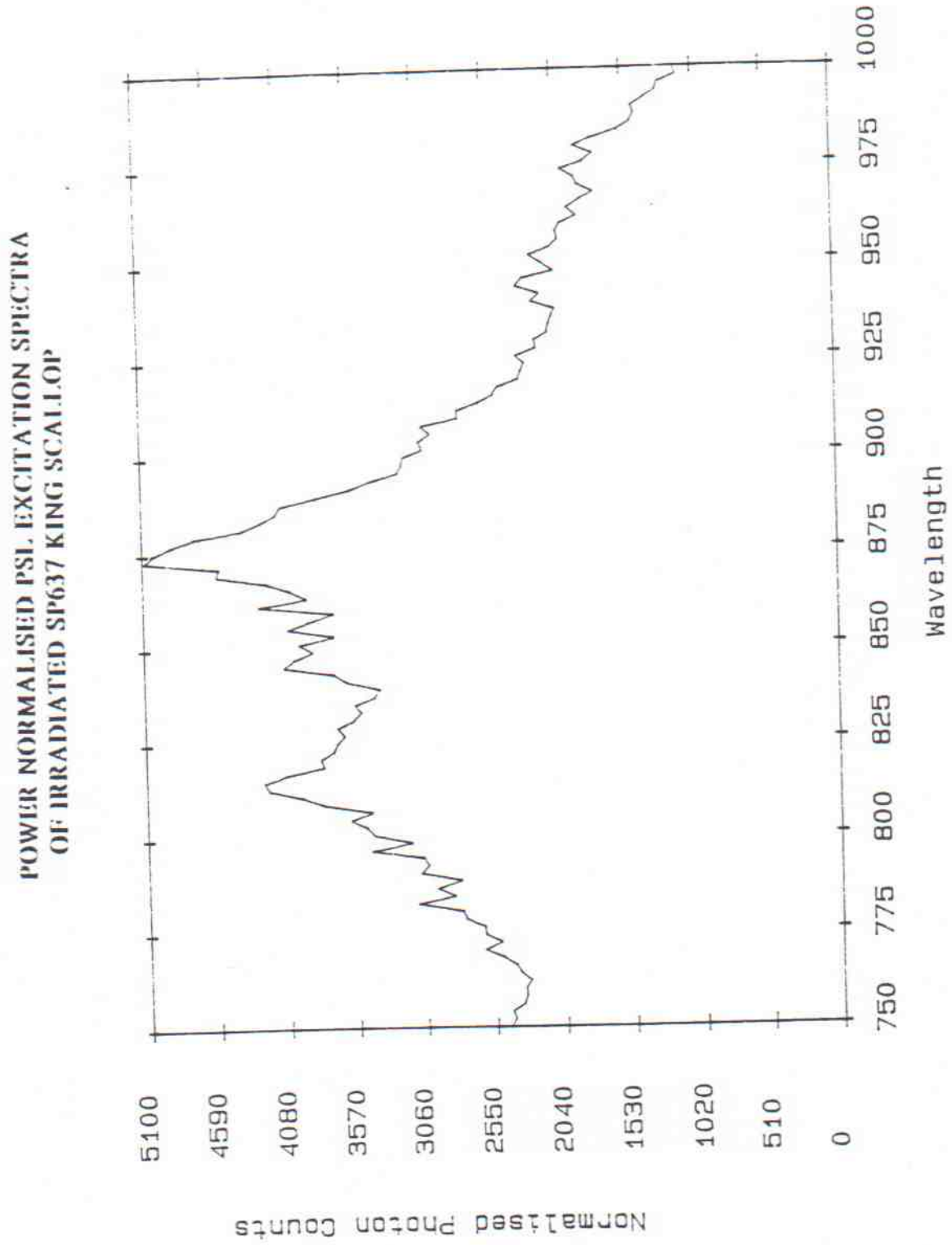


Figure 4.2.6 Power normalised spectra of irradiated and unirradiated king scallop

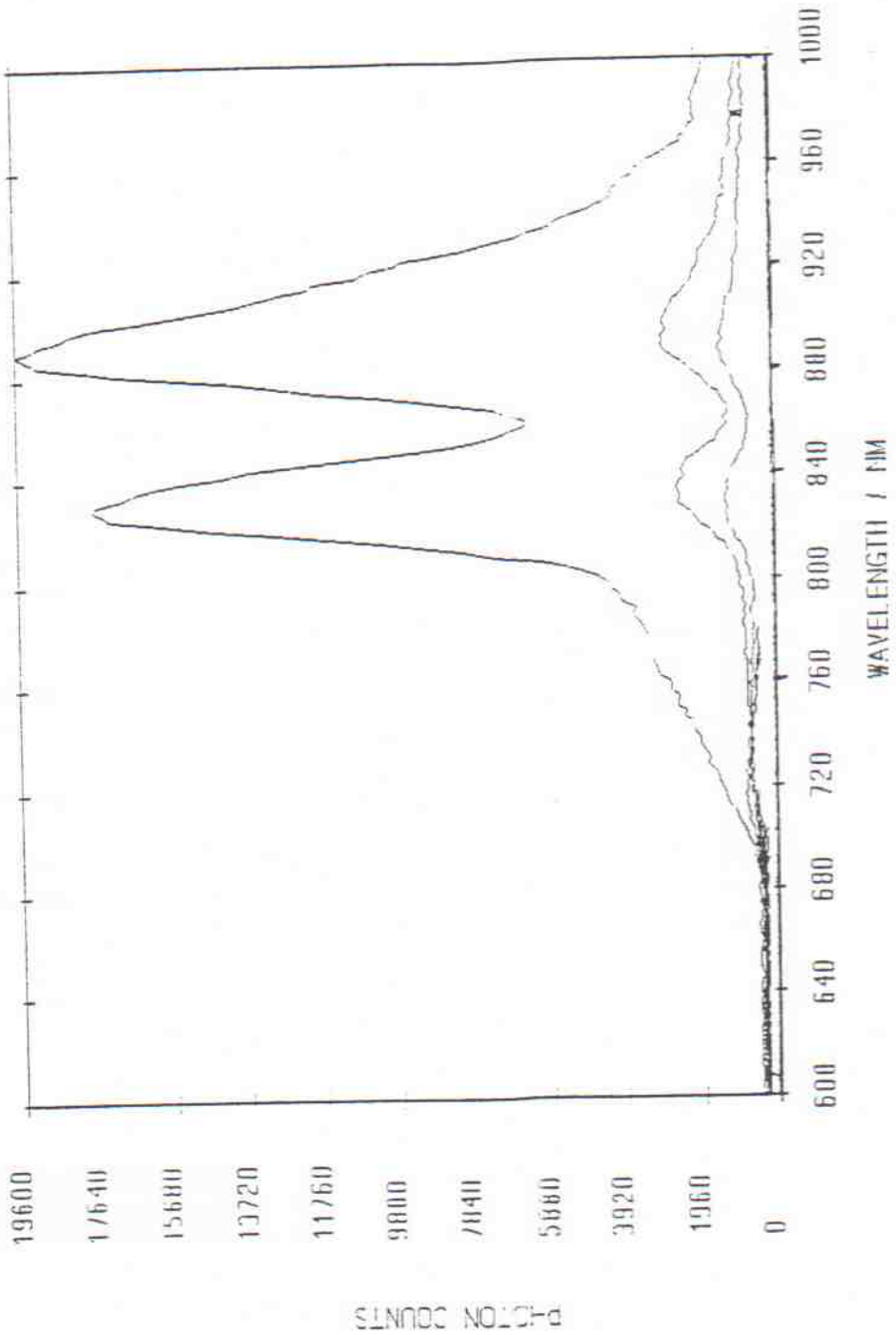


Figure 4.2.7 Power normalised spectra of mediteranean crevettes, warm water shrimps and brown shrimps

4.3 Infrared stimulated luminescence

From the cryogenic PTTL experiments conducted to explore the trap structure of calcitic shells and the excitation spectrometry, we were able to identify a series of IR stimulation bands. Exploratory work on IR stimulation of shells was carried out using the high sensitivity pulsed PSL system developed for screening herbs and spices. The PSL system is based on a digital photon counting and uses high power GaAlAs infrared LED's, with peak emission at around 880nm with a rated spectral bandwidth of 80nm and data acquisition is via a photomultiplier.

A preliminary experiment was carried out to explore the possibility of detecting irradiated irradiated shellfish. Six species of shellfish; brown shrimps, black tiger prawns, warm water shrimps, mediterranean crevettes king scallops and nephrops norvegicus, were introduced in disposable petri dishes, in duplicate, with whole irradiated to 5 kGy and unirradiated samples and irradiated to 5 kGy and unirradiated intestinal grits. Figure 4.3.1 shows the cumulative signals obtained from both the whole body of brown shrimps and from the intestinal material. This figure shows that it is possible for Anti-Stokes luminescence to be stimulated from trapped silicates directly through the dissected intestines and the whole body of shrimp. The PSL signal, in both cases is approximately 2 - 3 orders of magnitude greater than the background signal associated with the unirradiated control. Figures 4.3.2 and 4.3.3 illustrate the cumulative signals obtained from dissected intestines from irradiated and unirradiated nephrops and warm water shrimps. Again the PSL signal from the irradiated samples are 2 - 3 orders of magnitude greater than the unirradiated background signal.

Aliquots of the same samples were stored in freezer conditions (-20°C) and measured in the same manner as before, 68 days later, once measured the samples were given a 1 kGy calibration dose in the CO-60 source. The results for both whole samples and dissected intestines are shown in figure 4.3.4 and demonstrates the ability to distinguish unambiguously between unirradiated and irradiated samples even 68 days after irradiation.

PSL investigations on the nine powdered species of shellfish; lobster, mussel, oyster, mediterranean crevette, brown shrimp, warm water shrimp, king scallop, nephrops norvegicus and black tiger prawns, were undertaken. The powders dispensed into disposable petri dishes, and measured immediately after irradiation and then after 7 and 14 days storage at -20°C. Figure 4.3.5 shows that the freshly irradiated samples show a pronounced IR signal, however this signal fades over the course of the samples shelf life, to a similar extent as the TL signals below 250°C. Figure 4.2.6 shows in more detail the shine curve for mediterranean crevettes and how the signal fades over two weeks.

This work leads us to the conclusion that the IR components are associated with similar traps depth distribution as the TL components and that stable PSL signals will require stimulation of the visible bands previously identified by PTTL studies. Further explorations using the possibility of accessing the long wavelength tail of the 700nm band with the PSL system in conjunction with a PC driven single sample TL reader. This would enable us to vary the sample temperature.

An experiment was conducted using 5mg samples of both old irradiated powdered king scallop shells (irradiated 6 months prior to experiment) and unirradiated control material of powder King Scallops were dispensed onto discs, manually heated to various temperatures; 20, 40, 60, 80, 100, 140, and 180°C and shone for 60 seconds. The results were recorded and shown in figure 4.3.7 The results demonstrate the dramatic recovery of the IR PSL from a sample when stimulating at temperatures above 100°C. Figure 4.2.8 clearly demonstrates the recovery of the signal at 180°C compared with the signal from room temperature and the control. The figures show that there is conclusive evidence of thermal broadening of the 700nm absorption band above approximately 100°C. Interference from the TL may be discounted due to the up / down nature of the photon count recording and the fading observed in the low temperature TL after six months. Thus the net signal is due to infra-red stimulated luminescence. This technique provides one possible means of accessing stable signals from calcitic shells.

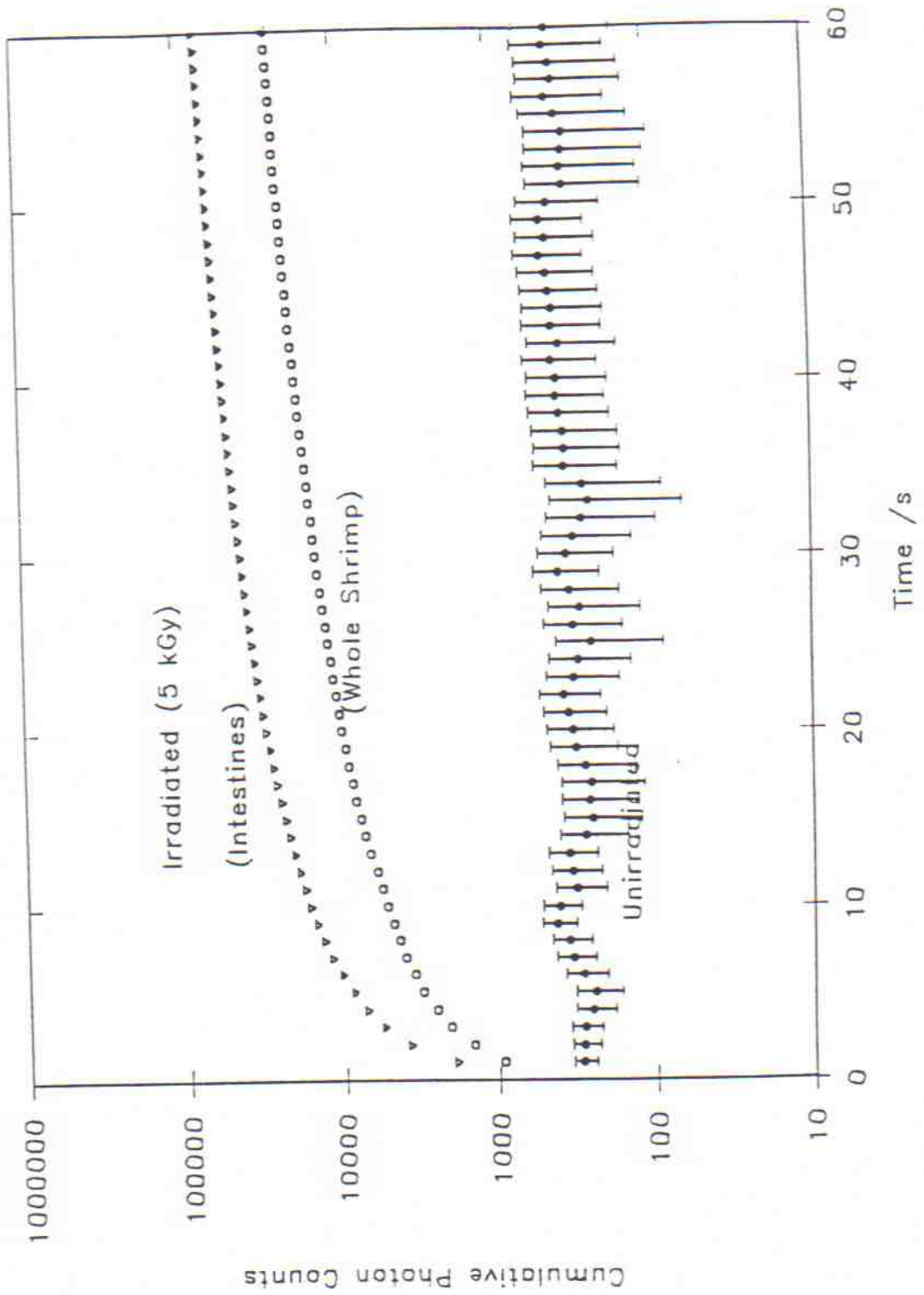


Figure 4.3.1 PSL detection of whole brown shrimp and dissected intestinal grits

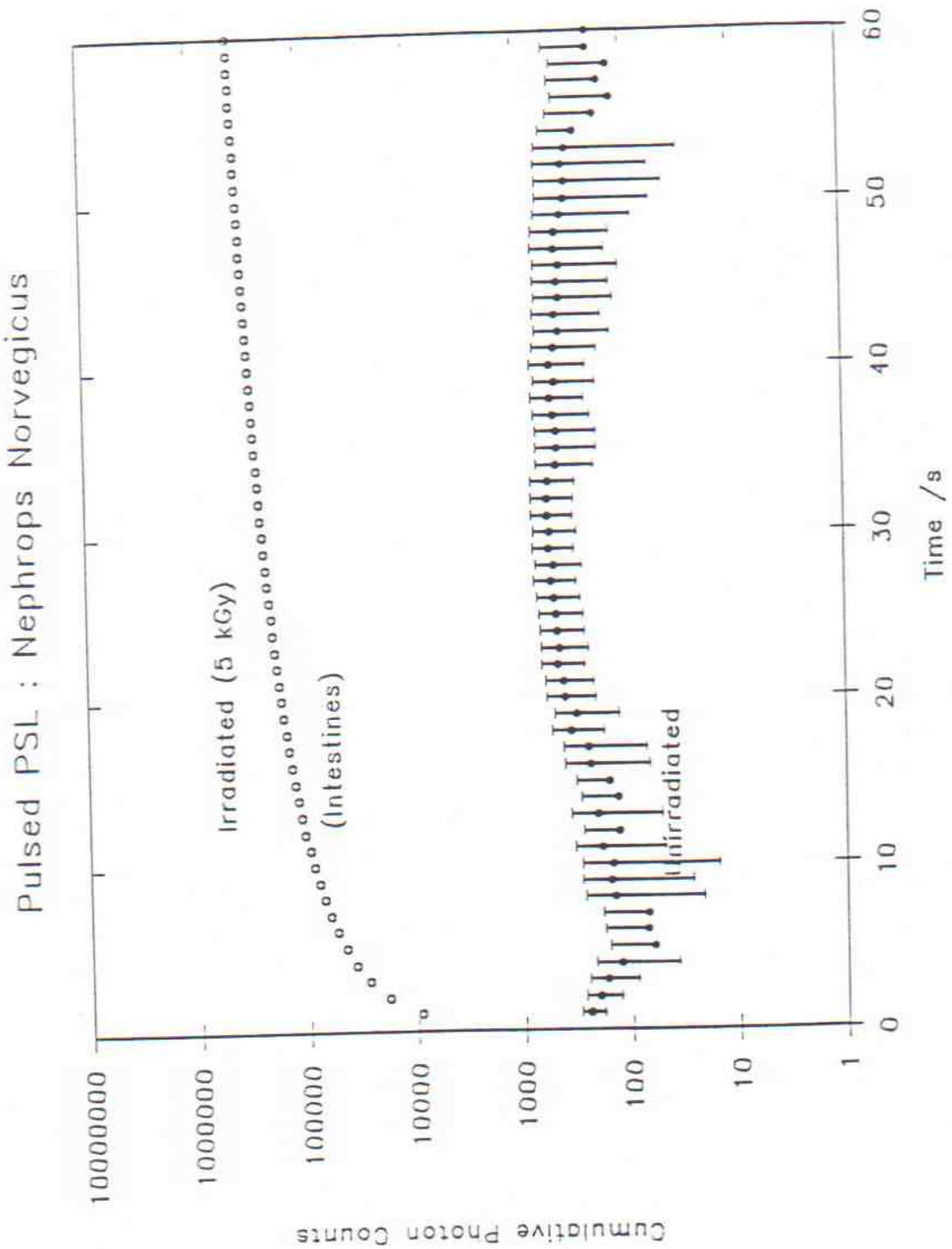


Figure 4.3.2 The cumulative PSL signal detected from irradiated and unirradiated dissected intestinal grits from nephrops norvegicus

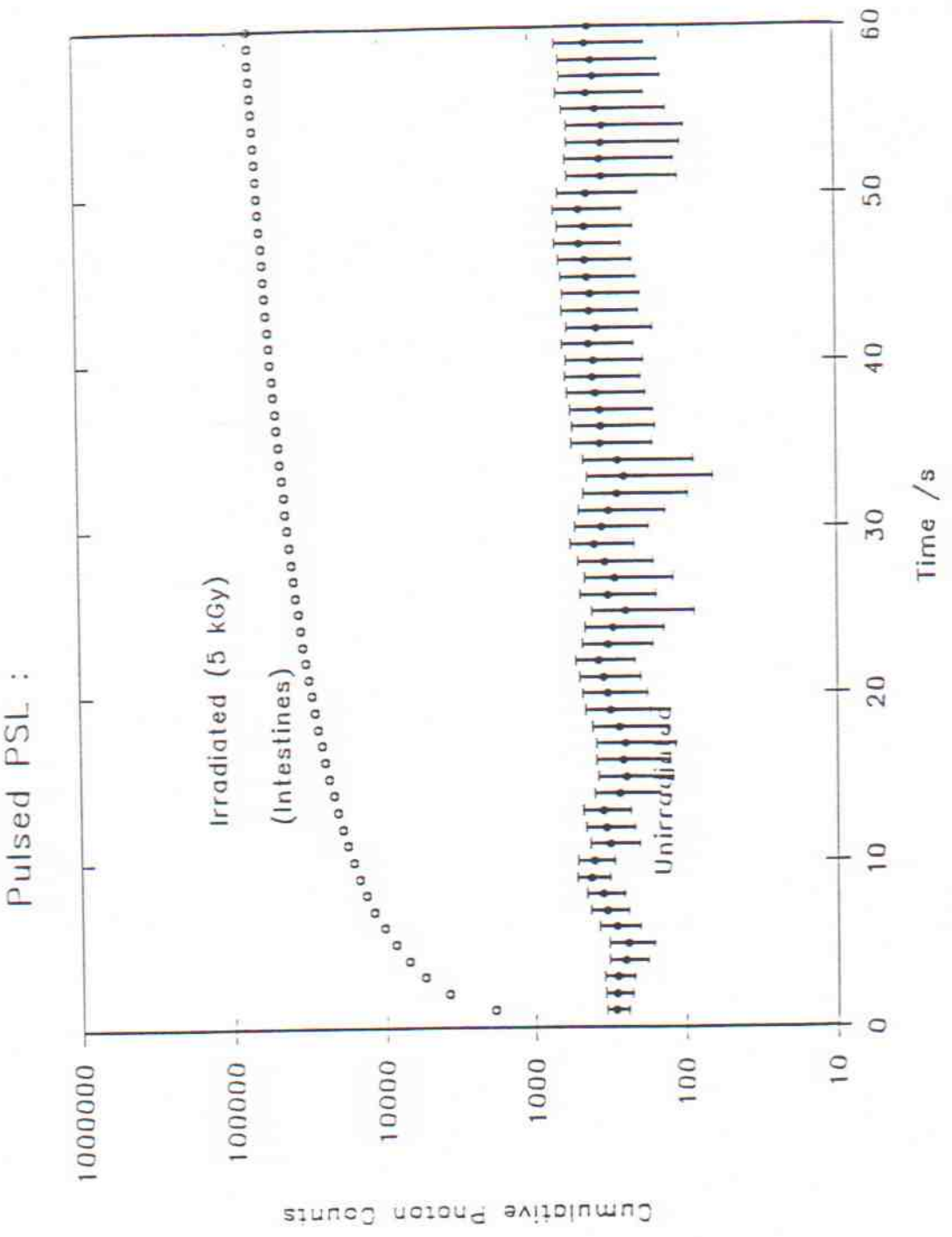
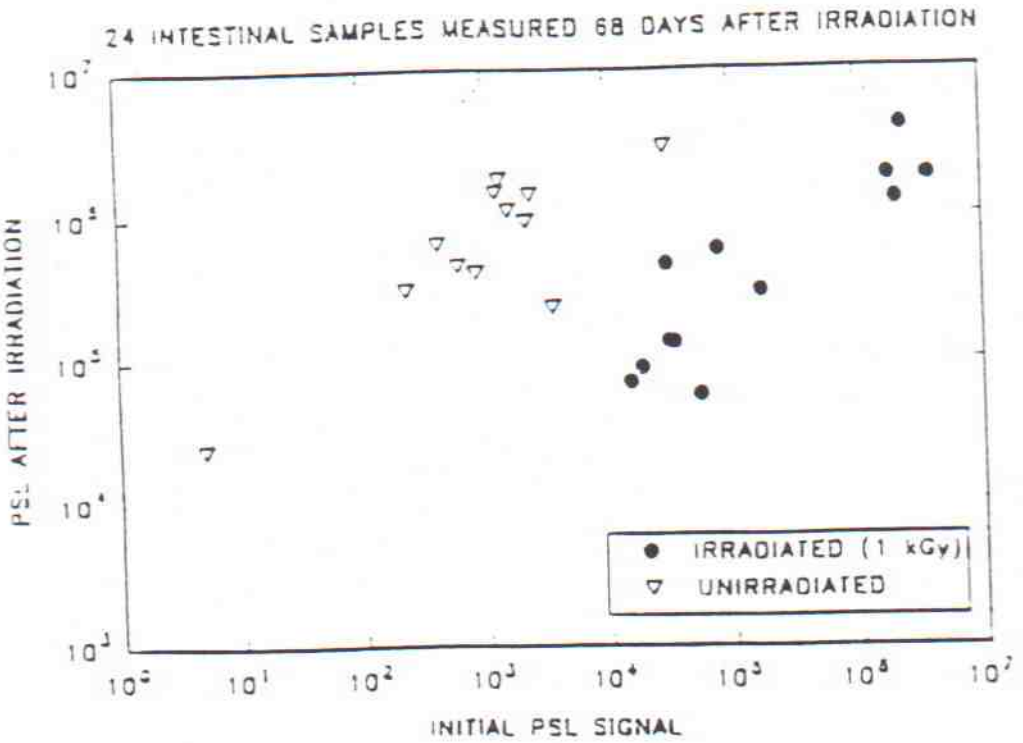


Figure 4.3.3. The cumulative PSL signal detected in irradiated and unirradiated dissected intestinal grits from warm water shrimps



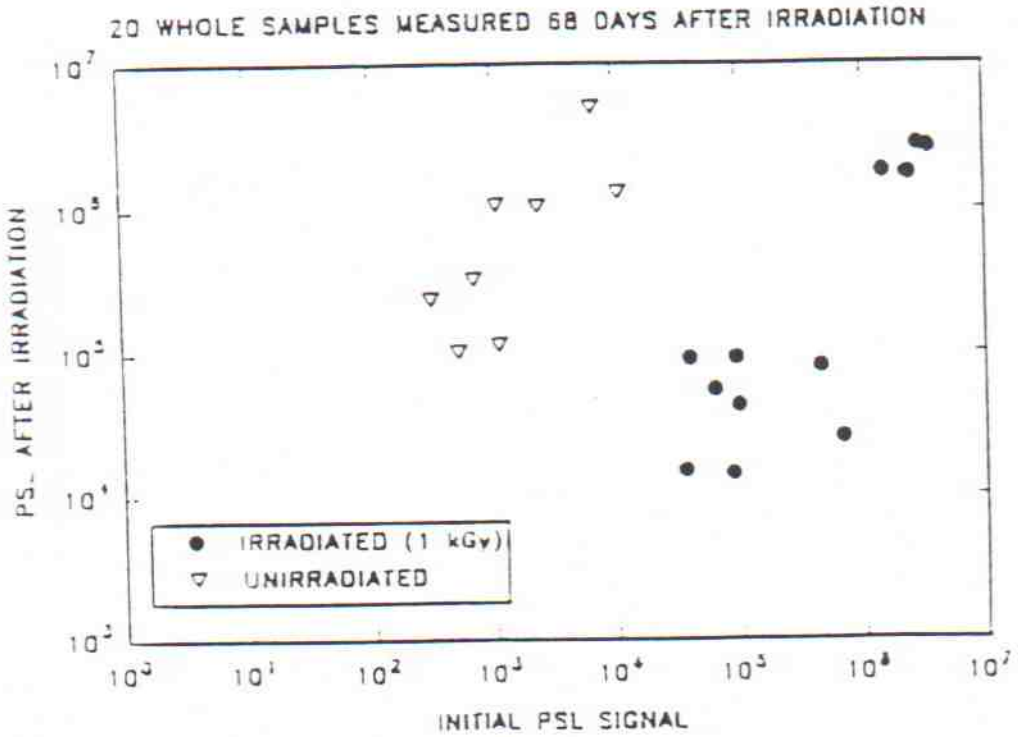


Figure 4.3.4 Calibrated PSL results from six species of shellfish irradiated 68 days prior to measurement

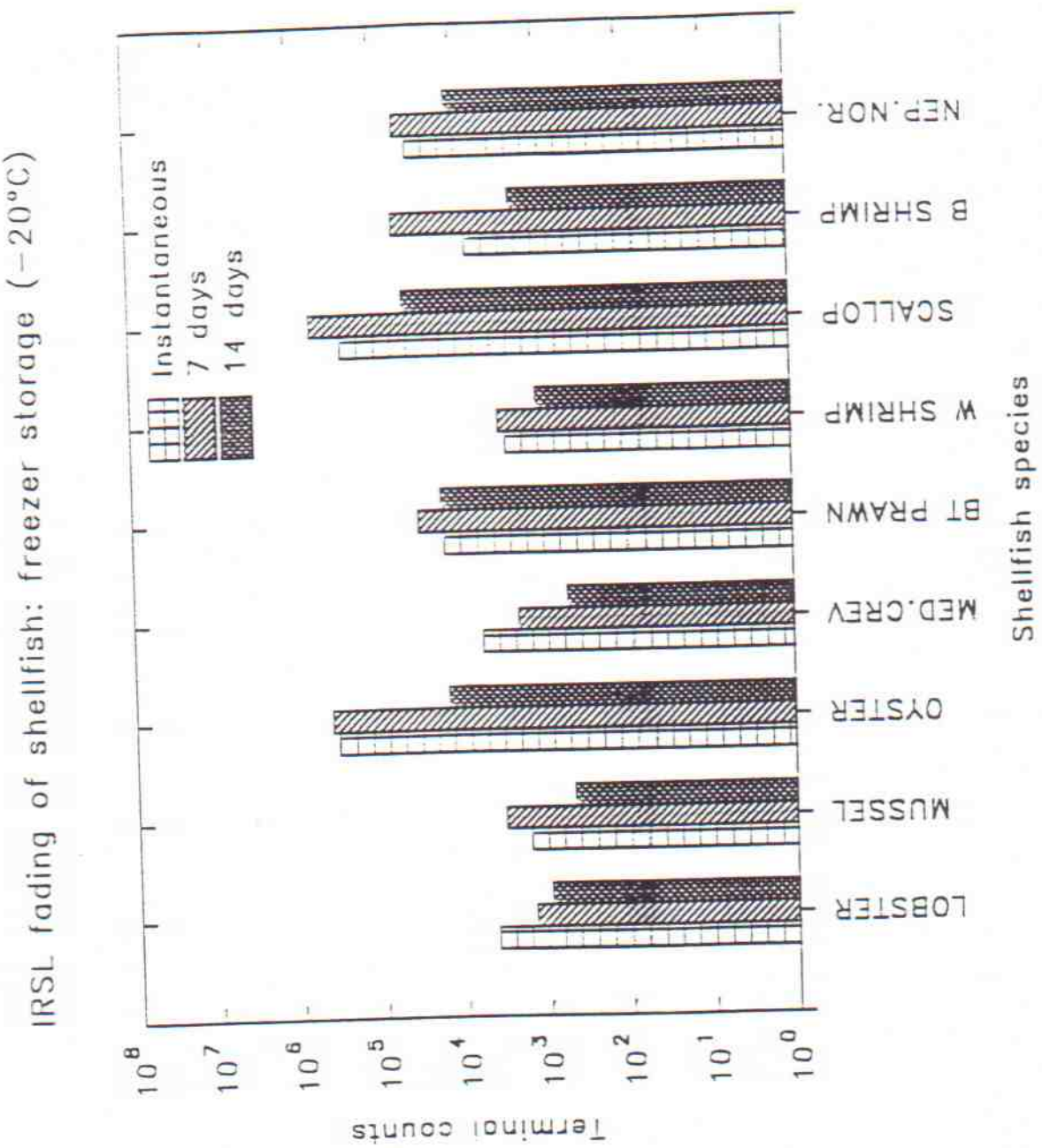


Figure 4.3.5 Fading of the PSL signal of the nine species of shellfish stored in freezer conditions (-20°C)

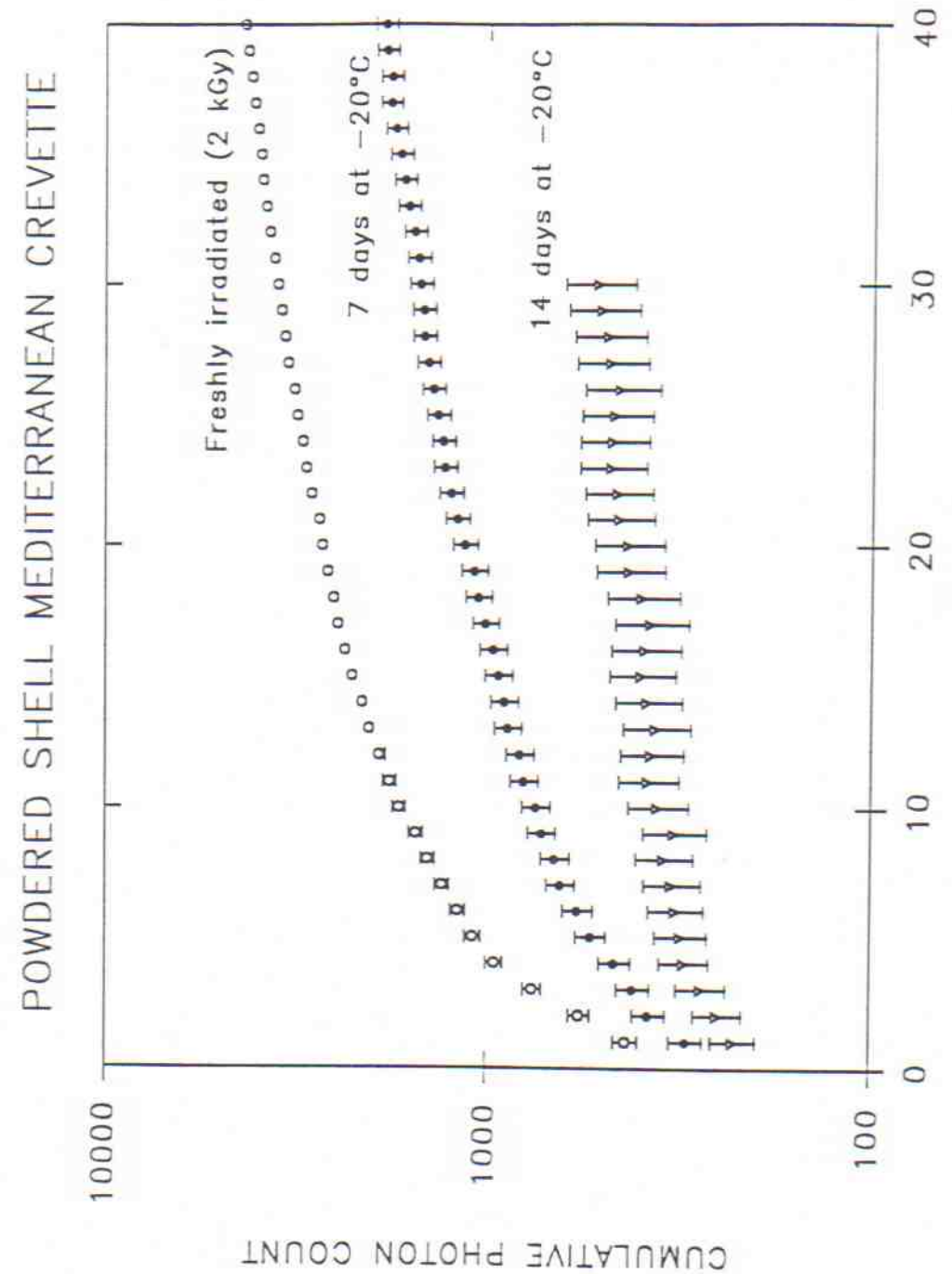


Figure 4.3.6 The fading of the cumulative PSL signals of powdered mediteranean crevette over a 14 day storage at -20°C

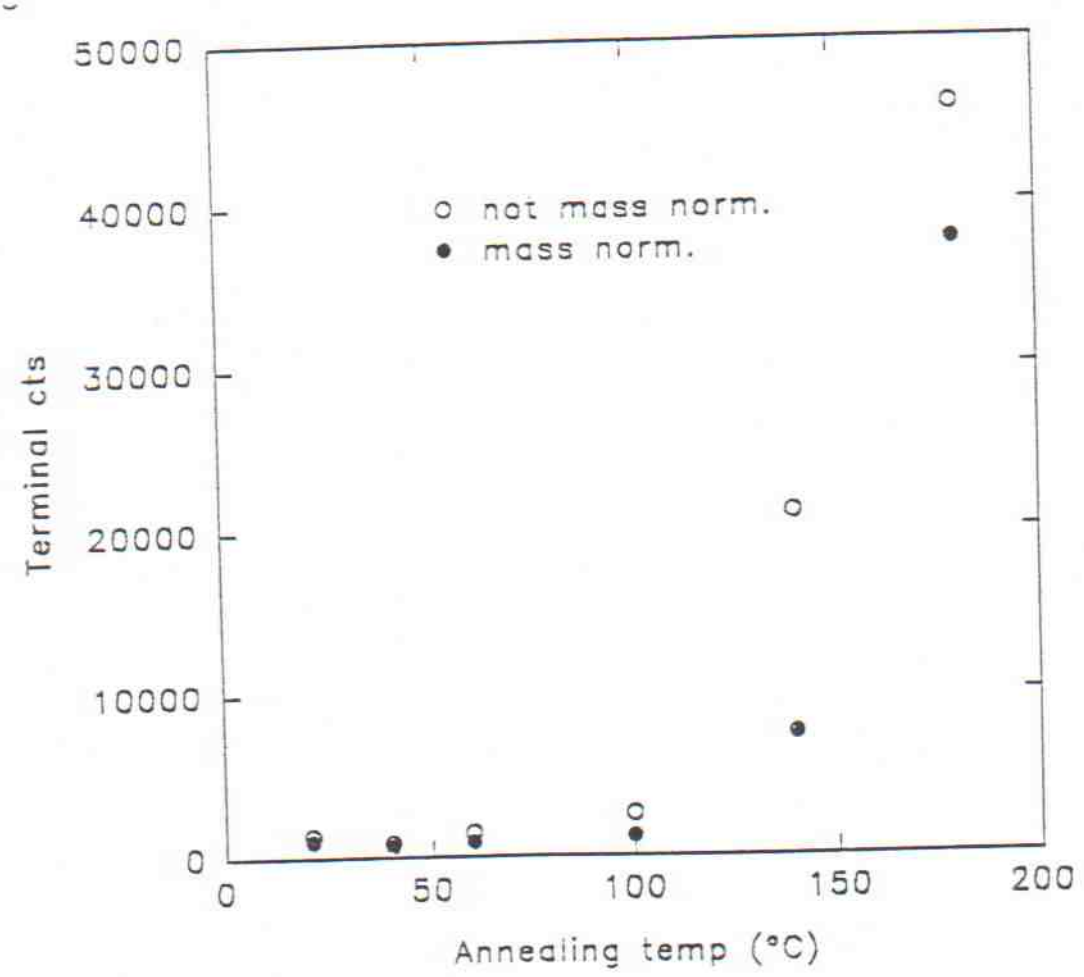


Figure 4.3.7 IR PSL signals of irradiated powdered king scallop shells (6 months fading) at elevated temperatures

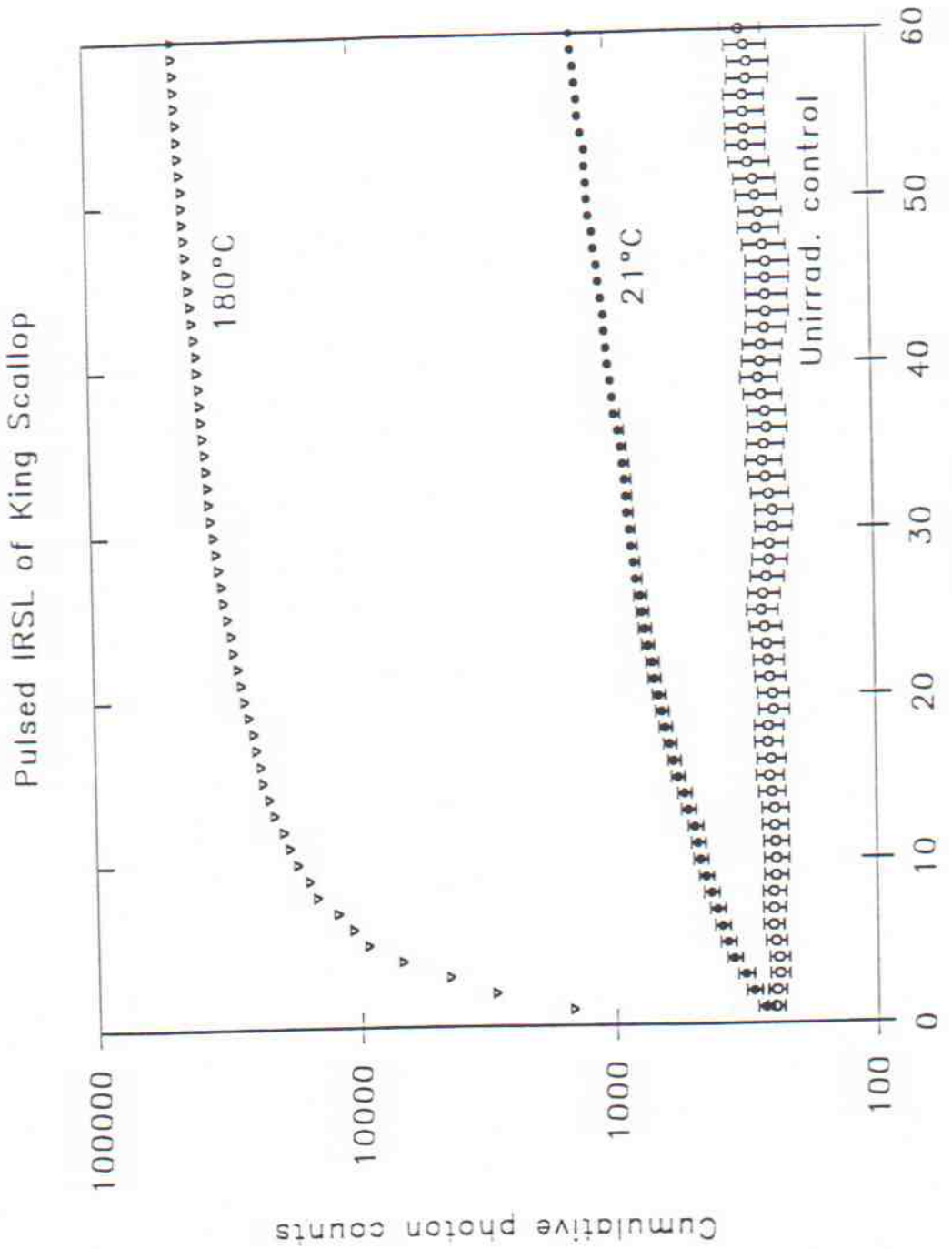


Figure 4.3.8 The recovery of the IR PSL cumulative signals of king scallop at elevated temperatures.

DISCUSSION

The thermoluminescence and photostimulated luminescence methods undertaken for this project, provide possible means of being able to detect irradiated shellfish, using either the shell or intestinally trapped grits. This project has examined a range of 9 types of shellfish, including warm water species of immediate regulatory interest, and poorly mineralised examples.

Shells provide a bio-inorganic material, comprising organic membranes infiltrated with minerals, which can be used for luminescence detection. The thermoluminescence measurement conditions have been examined in detail and shows that it is necessary to freeze dry the powders and limit the measurement temperature to 250°C to minimise problems with elevated backgrounds induced by thermal damage to the organic phases. The main TL emission from calcite shells has been shown to occur in a band associated with Mn^{2+} (550-600nm), which, by analogy with published studies of geological calcites, suggests defect agglomeration in the biogenic materials. Replacement of the standard TL filters with KG3 or BG39 filters increases sensitivity by two orders of magnitude. Under these optimised measurement conditions TL can be detected from all irradiated samples examined, shortly after irradiation. Having established that it is possible to detect TL signals, a further question arises concerning the stability of these radiation induced signals. Extensive stability tests have been undertaken to examine the extent of post-irradiation fading. The results obtained for ten fold replication of each species of sample have shown signal losses up to one order of magnitude, over storage periods of 1-3 months at both -20°C or 5°C. This storage period does go beyond the normal shelf life of most of these products and for well mineralised species; lobster, mussel, oyster, king and queen scallops, these losses are unlikely to lead to ambiguous identification. For the poorly mineralised warm water species; mediterranean crevette, black tiger prawns, warm water shrimps and brown shrimps, the worst cases for signal losses, unambiguous identification could be problematic. However, for the poorly mineralised species it is possible to distinguish irradiated samples from the signals detected over a 14 day period. Some uncertainties still remain as to why individual species show apparently different fading behaviours, and this requires some further consideration. The intestines of crustaceans contain small quantities of inorganic grits, which can be used for TL analysis. It is well establish that silicate signals are stable during dark storage and hence extraction of the intestinal grits does provide a more stable signal enabling detection of all species over their shelf life time. Simplification of the analysis for extracting intestinal grits by the use of acid hydrolysis has proved to be very successful. This technique has been applied along with the mechanical separation method in the German Interlaboratory trials on shellfish. 100% correct class identification was obtained using this technique, showing that this is an effective alternative approach to that of physical separation.

From geological literature fully mineralised calcite has high temperature, stable, TL signals which originate from deep traps. The main TL emission from calcitic shells has been shown to occur in the band associated with the Mn^{2+} (500-600nm). This has been confirmed by the emission spectroscopy carried out on the nine species of shellfish, very kindly run by Professor Townsend at Sussex University. The emission spectrums for all the species show that there is only the one main luminescence centre and the only difference between well

mineralised and poorly mineralised species was the intensity of the signal. Luminescence stimulation methods which avoid heating the sample, ie. photo-transfer (PTTL) and direct photostimulation (PSL) to access stable signals without elevated backgrounds giving the opportunity for improved detection conditions. From the emission spectrum of calcite and the response of the TL signal with changing the long pass filter from OG570 to a RG610, a dramatic signal drop off was observed, we know that the scope for Anti-Stokes luminescence is rather limited. Cryogenic phototransfer experiments were conducted to explore the trap structure for the individual species of freshly irradiated powdered shell, to enable the development of a rapid PSL detection scheme. The PTTL experiments clearly identified that there are two possible excitation bands for powdered shells. One of the stimulation bands is in the blue to visible region at around 400nm. The other stimulation band is in the infra red region at around 700nm. Investigation of these IR bands in freshly irradiated samples were carried out using excitation spectroscopy and investigating the Anti-stokes transitions from the IR band to the Mn^{2+} whilst scanning through 350nm to 900nm. The results showed some promise, with a complex series of IR stimulation bands observed from all the irradiated species. The main variations were in the sensitivities and ratios of the main spectral components at 800-820nm and 850-900nm in each species. In the well mineralised species it was possible to distinguish unambiguously between the irradiated and unirradiated species. With poorly mineralised species, the difference in signal intensity between irradiated and unirradiated samples is much lower and therefore makes positive clarification slightly more difficult. The IR for irradiated samples stored for six months, was very poor in all of the nine species of powdered shells, with the signal intensity from irradiated and control samples, in some cases being the approximately the same. However we have observed that there are IR stimulation bands and using an alternative approach an effective stimulation scheme is probable.

Using the high sensitivity pulsed PSL system developed for the screening of herbs and spices, IR stimulation of the nine species of shellfish was further investigated. Initial experiments on freshly irradiated and unirradiated whole samples, dissected intestinal grits and powdered shells gave exceptionally good results with the system being able to distinguish between the irradiated and unirradiated species. Signals from irradiated sample were found to be 2-3 orders of magnitude greater than the unirradiated control. Aliquots of these samples were stored in freezer conditions (-20°C) for 68 days, measured using the pulsed PSL system and calibrated with a 1 kGy dose and then remeasured. The results from whole samples and dissected intestinal grits demonstrate the ability to distinguish unambiguously between the irradiated and unirradiated samples even 68 days after irradiation. However, with powdered shells the signal fades over the course of the samples shelf life, in the same manner as in the TL stability study. The results of this work has proved beyond doubt that the IR components in powdered shells are associated with similar trap depth distributions as the TL components and to obtain stable PSL signals, stimulation of the visible bands which were identified by PTTL experiments will be necessary. However, the possibility of accessing the long wavelength tail of the 700nm band using the pulsed PSL system in conjunction with a TL reader at elevated temperatures was to be explored. Using a old irradiated sample of 6 months and unirradiated controls of a well mineralised species of king scallop we were able to demonstrate the recovery of the IR signal when the sample is stimulated at temperatures above 100°C, confirming that the coupling of thermal and IR stimulation is able to access stable calcitic signals.

This project has shown that both TL and PSL methods are extremely useful techniques for identifying irradiated shellfish, both using the shell and trapped intestinal grits. The application of the TL methods to powdered shells has been limited by the need to restrict the glow regime and the instability of the calcite signal. Intestinal grits give a more stable silicate signal, however the TL method is still limited by the requirement of laborious sample preparation and re-irradiation. We have shown that it is possible to detect PSL signals in the IR band for freshly irradiated powdered samples, however for older samples the IR response can be enhanced by raising the sample temperatures. The pulsed IR instrument has demonstrated that stable PSL signals can be stimulated through both the whole body of the sample and also through the membrane of the intestine. T he application of pulsed PSL offers the possibility of conducting radiation specific measurements avoiding the need of sample preparation. Further research is still needed to establish the origins and stimulation schemes for these bio-inorganic materials.

Small international trials are required for the TL and PSL methods of shellfish for the validation of these methologies, which may encourage compliance of accurate labelling of irradiated foods.

REFERENCES

1. MAFF,1990, Food labelling(Amendment)(Irradiated Food) Regulations 1990, statutory instruments, 2489.

2. MAFF, 1992, Detection of Irradiated Herbs and Spices, No V27, MAFF validated methods for the analysis of foodstuffs, MAFF Food Science Laboratory, Norwich.

3. Sanderson D.C.W, Slater C., Cairns K.J., 1989a, Luminescence Identification of Irradiated Foods, Int. J. Rad. Biol., 55, 5

4. Sanderson D.C.W, Slater C., Cairns K.J., 1989b, Detection of Irradiated Food, Nature , 340,23-24

5. Sanderson D.C.W, Slater C., Cairns K.J., 1989c, Thermoluminescence of Foods : Origins and Implications for detection of irradiation, Rad. Phys. Chem., 34, 915-924

6. Sanderson D.C.W., 1990, Luminescence Detection of Irradiated Foods, "Food Irradiation and the Chemist", ed D.E. Johnston & M.H. Stevenson, Royal Society of Chemistry, Cambridge, 25-56

7. Sanderson D.C.W., Schreiber G.A., Carmichael L.A.,1991, A European Interlaboratory Trial of TL Detection of Irradiated Herbs and Spices. Phase 1. SURRC report to BCR (European Commission) 46p

8. Autio T. & Pinnioja S., 1990, Identification of irradiated foods by thermoluminescence of mineral contamination. Zeitschrift fur Lebensmitten-Untersuchung und-Forschung,191, 177-180.

9. Sanderson D.C.W. Carmichael, L.A., Clark P.A., Clark R.J., 1992, Development of Luminescence Tests to Identify Irradiated Food. Final Report : MAFF Project N1701

10. Driver H.S.T., 1979, The preparation of thin slices of bone and shell for TL. PACT, pp290-297

11. Down J.S., Flower R., Strain J.A., & Townsend, P.D., 1985, Thermoluminescence emission spectra of calcite and iceland spar, Nuclear Tracks, 10, (4-6), 581-589

12. Johnson,N.M., 1960, Thermoluminescence in biogenic calcium carbonate. Journal of Sedimentary Petrology 30, 305-313

13. Johnson, N.M., and Blanchard, R.L., 1967, Radiation Dosimetry from the natural thermoluminescence of fossil shell. American Mineralogy 52, pp1297-1310

14. Li Hu Hou, 1988, Properties of Thermoluminescence and electron spin resonance in deposited carbonates, Nuclear Tracks, 14, Nos 1/2, pp259-265. Int. J. Radiat. Appl.

Instrum., Part D.

15. Christodoulides C., and Fremlin J.H., 1971, Thermoluminescence of Biological Materials, Nature, 232, 257-258

16. Agnel J.P., Dutraive M.S., Vaux I., Raffi J., 1992, Thermoluminescence Identification of ionised shellfish, inRecent Advances on Detection of Irradiated Food, ed., Leonardi M., Raffi J., Belliardo J.J., EUR 14315, 186-195.

17. Carmichael L.A., Sanderson D.C.W., Ni Rian S., 1994, Thermoluminescence Measurements of calcite shells, in press (Radiation Measurements)
Agnel J.P., Dutraive M.S., Vaux I., Rustan I., Raffi J.,1992, Thermoluminescence Identification of Ionized Shellfish, EUR 14315 EN, 186-195

18. Autio T, & Pinnioja S., 1992, Identification of irradiated foods by thermoluminescence of contaminating minerals. In : Recent advances on the detection of irradiated food. BCR Information. EUR/14315/EN. Edited by M. Leonardi, J.J. Raffi & J.J. Belliardo (Commission of the European Communities, Brussels, Luxembourg), pp177-185

19. Pinnioja S., Autio T., Niemi E., and Pensala O., 1993, Import control of irradiated foods by the thermolumunescence method, Z. Lebensmittel Unters. Forsch., 196, 111-115.

20. Pinnioja S., Suitability of the thermoluminescence method for detection of irradiated foods, Rad. Phy. Chem., 42(1-3), 397-400.

21. Carmichael L.A., Sanderson D.C.W., 1994, Luminescence Detection of Irradiated Shellfish, in press Procedings in Royal Society of Chemistry.

22. Sanderson D.C.W., Carmichael L.A., Ni Rian S., 1993, Photostimulated luminescence and thermoluminescence techniques; Detection of irradiated shellfish. MAFF N2635 Report No.1

23. Lima J.F., Trzesniak P., Yoshimura E.M., and Okuno E., 1990, Phototransferred thermoluminescence in calcite. Radiation Protection Dosimetry, Vol. 33, No 1/4, pp143-146.

24. Clark R.J., Sanderson D.C.W., 1994, Photostimulated Luminescence Excitation Spectrometry of Feldspars and Micas, in press (Radiation Measurements)

25. Sanderson D.C.W., 1991, Photostimulated Luminescence (PSL) : a new approach to identifying irradiated food in "Potential new Methods of detection of Irradiated Foods," ed Raffi, EUR 13331, 159-170

26. Sanderson D.C.W., Clark R.J., Pulsed photostimulated luminescence of alkali feldspars, 1994, in press (Radiation Measurements.

27. Sanderson D.C.W., Carmichael L.A., Spencer J.Q., Naylor J.D., 1994, Food Science Technology Today, 8(2), 93-96.