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UV Fluorescence Analysis of Surface Seawater Samples from AGSO Marine Survey 207, Northwestern Australia

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Abstract

Ultra-violet (UV) emission spectrometry was used to determine whether hydrocarbons were present in thirty-two surface seawater samples collected from northwestern Australia during the AGSO Marine Survey 207 in September–October 1998. This survey covered in part the Carnarvon Basin, offshore Canning Basin, Yampi Shelf (Browse Basin) and the Vulcan Sub-basin (Bonaparte Basin).

Out of all of the surface seawater samples obtained, only two samples from the Yampi Shelf fluoresced. Sample GWY 1-2 (19999324) fluoresced weakly, whereas sample 207SW016 (19999329) collected about 20 km west of the Cornea-1 well (along the line LO 14-15a SP74) had an emission spectrum that was characteristic of crude oil. Comparison with a limited number of Northwest Shelf oil-in-water samples demonstrated that this fluorescence spectrum most closely matched those of the crude oils (yellow condensates) from the Swan-1 and Eclipse-2 wells.

1. Introduction

Thirty-two seawater samples, collected on the AGSO Survey 207 from surface waters in northwestern Australia, were submitted for ultra-violet (UV) emission spectrometry to detect the presence of hydrocarbons. Details of the AGSO Marine Survey 207, carried out on board the marine vessel *TSMV* Pacific Conquest in September–October 1998, are given in Wilson et al. (2000).

2. Samples

The locations of the surface seawater samples collected from the Carnarvon Basin, offshore Canning Basin, Vulcan Sub-basin and Yampi Shelf are given in Table 1 and are shown in Figures 1 and 2. Seawater samples were collected from a depth of around 2 m when a UV fluorescence anomaly was detected by the onboard Safire fluorimeter as documented in Wilson (2000). Each pre-cleaned 1 L glass bottle was rinsed with seawater immediately prior to sampling. Upon filling with seawater, sodium azide was added to kill any microbes and the bottled sealed. The samples were refrigerated for the duration of the cruise.

3. Ultra-violet Fluorescence (UVF) Spectrometry

3.1 Methodology

The surface seawater samples were analysed by ultra-violet (UV) emission spectrometry according to the ASTM method D3650-90 (1993). The emission spectrum was scanned at a fixed excitation wavelength of 266 nm rather than 254 nm as specified in the ASTM method. The rational being to enable comparison of the seawater spectra with the spectral data returned by Airborne Laser Fluorosensor (ALF) and underwater fluorimetry; both techniques use a 266 nm excitation wavelength. The UVF data were collected using a Perkin Elmer LS 50B Luminescence Spectrometer.

The surface seawater sample was placed into a 4 mL quartz cuvette for UVF analysis. Appropriate cleaning procedures were used to prevent cross-contamination of samples and the spectrum of pure water was collected in between each sample analysis. The pure water was obtained using Millipore filters, and the spectrum is shown in Figure 3. Prior to analysing the Marine Survey 207 surface seawater samples, a stock solution of artificial seawater (3.5 % salinity) was prepared by dissolving 1.75 g "aquarium sea salts" in 50 mL Millipore water, and its UVF spectrum was acquired as a background check (Fig. 3).

As detailed in Appendix A1, the Perkin Elmer LS 50B Luminescence Spectrometer was operated in the single emission scan mode with the excitation monochromator set at 266 nm. The surface seawater sample was briefly irradiated with UV light and the emission spectra were scanned from 270 to 720 nm at 0.5 nm intervals at a rate of 60 nm/minute. The signal-to-noise ratio for fluorescence in this region was 689:1. The slit width was set at 2.5 nm for the excitation monochromator and 5 nm for the emission monochromator. The Raman scattering was monitored as an independent intensity marker.

Table 1 AGSO Marine Survey 207 surface seawater samples.

Survey Sample No.¤	AGSO No.	Line Name	Shot Point	Latitude	Longitude	Sample Details	Collection Point*
Carnarvon Basin							
207WS**001**	19999342	Wandoo	37	-20.1247	116.4374	Sea water	DHGE
207WS**002**	19999343	Wandoo A	77	-20.1086	116.4370	Sea water	DHGE
207WS**003**	19999344	Wandoo A	47	-20.1441	116.4400	Sea water	DHGE
207WS**004**	19999345	Wandoo B	47	-20.0984	116.4134	Sea water	DHGE
Canning Basin							
207WS**005**	19999340	T 1-10	83	-18.6255	116.6476	Sea water	DHGE
207WS**006**	19999341	T 1-10	205	-18.4551	116.8225	Sea water	DHGE
Yampi Shelf							
207WS**007**	19999339	LO 3-4	87	-13.9267	124.9984	Sea water	DHGE
207WS**008**	19999337	LO 6-7	75	-13.4592	124.6356	Sea water	DHGE
207WS**009**	19999316	LO 6-7		-13.4592	124.0000	Sea water + azide	DHGE
207WS**010**	19999319	LO 8-9	197	-13.6619	124.7950	Sea water + azide	Fluorimeter
207WS**011**	19999338	LO 8-9	197	-13.6619	124.7950	Sea water	Fluorimeter
207WS**012**	19999335	LO 12	13	-13.9147	125.0180	Sea water	DHGE
207WS**013**	19999332	LO 12-13	129	-13.7592	124.8424	Sea water + azide	Fluorimeter
207WS**014**	19999331	LO 12-13	268	-13.5595	124.6263	Sea water + azide	Fluorimeter
207WS**015**	19999328	LO 14-15	1	-13.4532	124.4698	Sea water + azide	Fluorimeter
207WS**016**	19999329	LO 14-15a	74	-13.6600	124.6914	Sea water + azide	Fluorimeter
207WS**017**	19999327	LO 14-15a	96	-13.6951	124.7294	Sea water	DHGE
207WS**018**	19999333	LO 16	4	-13.9576	124.9780	Sea water + azide	DHGE
207WS**019**	19999334	LO 16	4	-13.9576	124.9780	Sea water	DHGE
207WS**020**	19999314	LO 16-17	12	-13.9529	124.9644	Sea water	DHGE
207WS**021**	19999315	LO 16-17	12	-13.9529	124.9644	Sea water + azide	DHGE
207WS**022**	19999325	LO 16-17a	122	-13.7970	124.7986	Sea water	Fluorimeter
207WS**023**	19999326	LO 16-17a	122	-13.7970	124.7986	Sea water + azide	Fluorimeter
207WS**024**	19999318	LO 26-27	21	-13.6997	124.6807	Sea water	DHGE
207WS**025**	19999321	GW-LO 57-58	23	-13.8336	124.5404	Sea water	DHGE
207WS**026**	19999322	GW-LO 57-58	48	-13.7916	124.5816	Sea water	DHGE
	19999317	LO 4-5				Sea water + azide	
	19999324	GWY 1-2 (crowd pleaser)				Sea water	Fluorimeter
	19999330	LO 14	345			Sea water + azide	DHGE
	19999336	LO 4-5				Sea water	
Vulcan Sub-basin							
207WS**027**	19999320	SK 13-14	39	-12.0385	124.7077	Sea water	DHGE
207WS**028**	19999323	FM 2-3a	22	-12.3987	125.0541	Sea water	

¤ **001** abbreviation used in figures

* DHGE Dynamic Headspace Gas Extractor

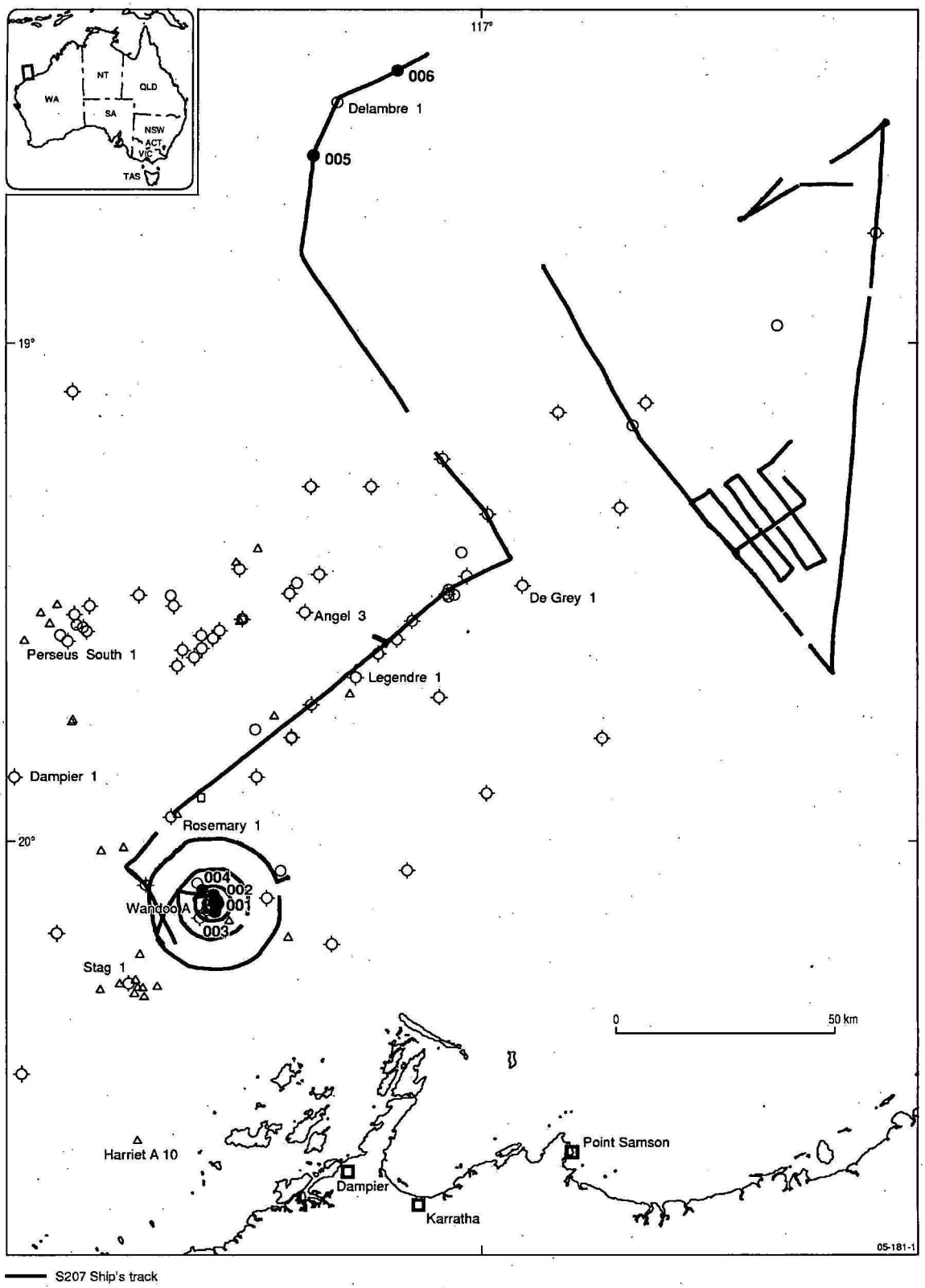

Figure 1 Locations of AGSO Marine Survey 207 surface seawater samples from the Carnarvon and offshore Canning basins.

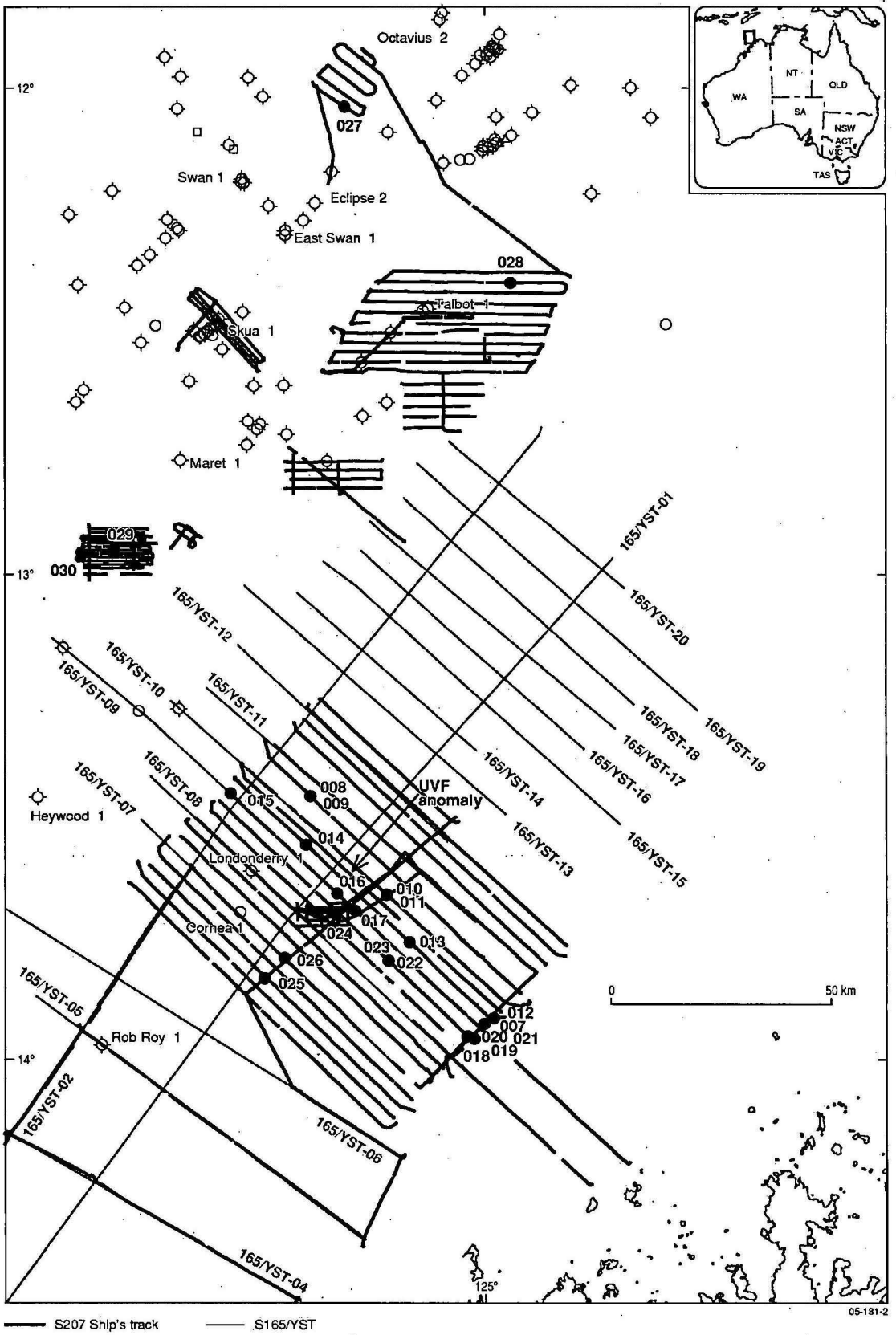

Figure 2 Locations of AGSO Marine Survey 207 surface seawater samples from the Yampi Shelf (Browse Basin) and Vulcan Sub-basin (Bonaparte Basin).

3.2 UVF Spectra of Oils

Fluorescence, or light emission, in the near ultraviolet and the visible region of the electromagnetic spectrum (200-400 nm and 400-700 nm, respectively), is excited by absorption of UV electromagnetic radiation. Pi (π) electrons of polycyclic aromatic hydrocarbon molecules, carbonyl groups and conjugated unsaturated systems, absorb UV radiation which results in the promotion of the electronic cloud from one energy orbital to a higher energy one. When the system returns to the ground state, fluorescence radiation may be emitted. Electrons of sigma bonds, as found in alkanes (saturated hydrocarbons) and simple alkenes, do not interact with electromagnetic radiation between 200 and 700 nm, hence no fluorescence is observed.

Therefore, seawater (and fresh water) contaminated with hydrocarbons fluoresce because most crude oils and refined petroleums contain polycyclic aromatic hydrocarbons (PAHs). Other compounds, besides hydrocarbons, that fluoresce in seawater include lignin and pigments. Algae (phytoplankton and seaweeds) commonly contain chlorophyll pigments which have maximum fluorescence at around 685 nm when an excitation wavelength of 266 nm is used (Prezelin and Boczar, 1986; Hockley and Trinder, 1994). Under the same condition the pigment phycoerythrin, which occurs within cyanobacteria, fluoresces at 576 nm. In addition other biological components such as fish oils and proteins, and their decompositional products, may also fluoresce.

UVF analysis is useful for determining the presence of hydrocarbons and the rapid identification of crude and refined oils. Identification of an oil is made through comparison of its fluorescence spectra with those of possible matching oils. This can be done mathematically or by noting the general shape, the number of peaks and the wavelengths at which maxima and minima occur.

All oils, with the exception of light distillates and some condensates, contain compounds that fluoresce under ultra-violet light. The shape of the UVF spectrum is determined mainly by the distribution of polycyclic aromatic hydrocarbons (PAHs) i.e., the number and type of ring structures and the degree of alkyl substitution.

The shape and intensity of the UVF spectrum is also highly dependent on hydrocarbon concentration. A linear relationship between the oil concentration and total fluorescence intensity is obtained if the solution is dilute, *i.e.* there is no *inner filter effect*. There is a linear relationship between the sample concentration of fluorescent compounds and the intensity of the emitted light only within certain limits where the total absorbance of the sample is low (UNEP, 1992).

If the absorbance exceeds 0.05 absorbence units (<95 % transmittance), the fluorescence intensity will be visibly reduced by the presence of any compound in the sample that can absorb either the excitation light or the emitted light. At high concentrations, the inner filtering effect can be caused by the fluorescent compounds themselves.

Another effect that may cause a decrease in fluorescence intensity is *quenching*. This occurs when energy is transferred non-radiatively to co-existing molecules, or quenchers, in the sample rather than being emitted as fluorescence. These molecules can be of the same or different chemical species as the fluorescing molecules.

Further details on the basic concepts of fluorescence and detection methods can be found in Becker (1969), Berlman, (1971), Uwe (1978) and Rhys Williams (1980).

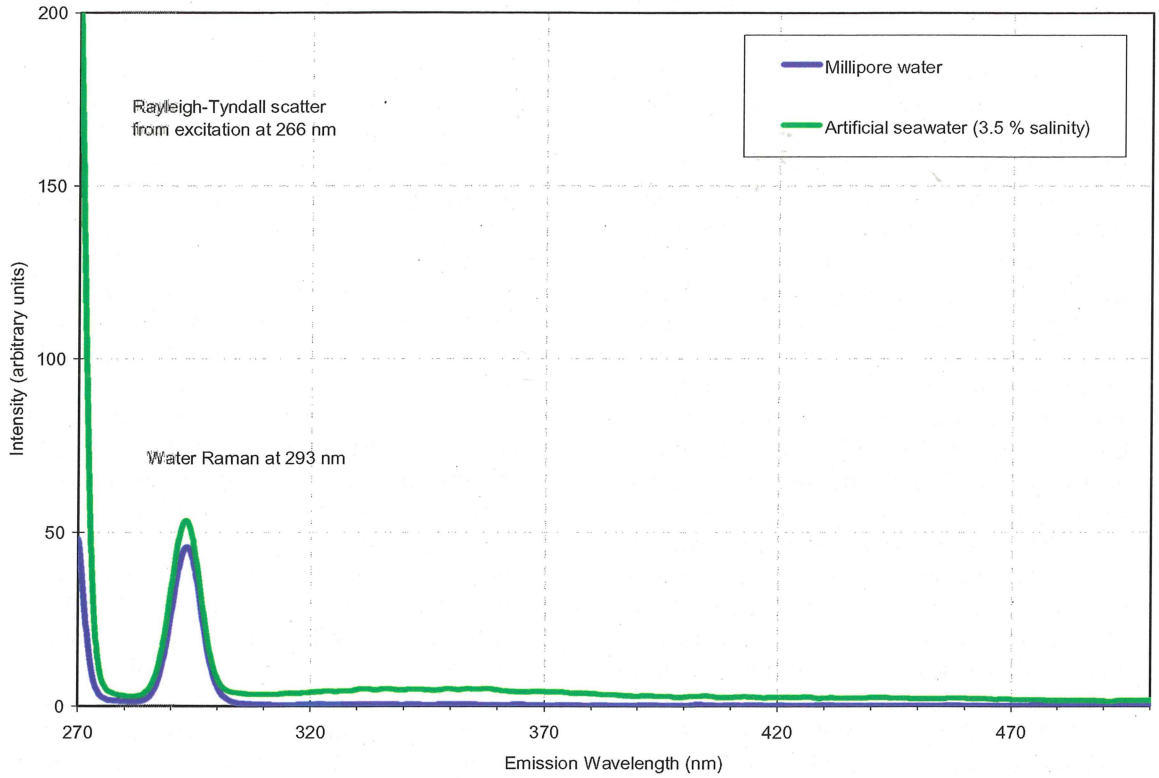

Figure 3. Laboratory UVF spectra of pure water and artificial seawater.

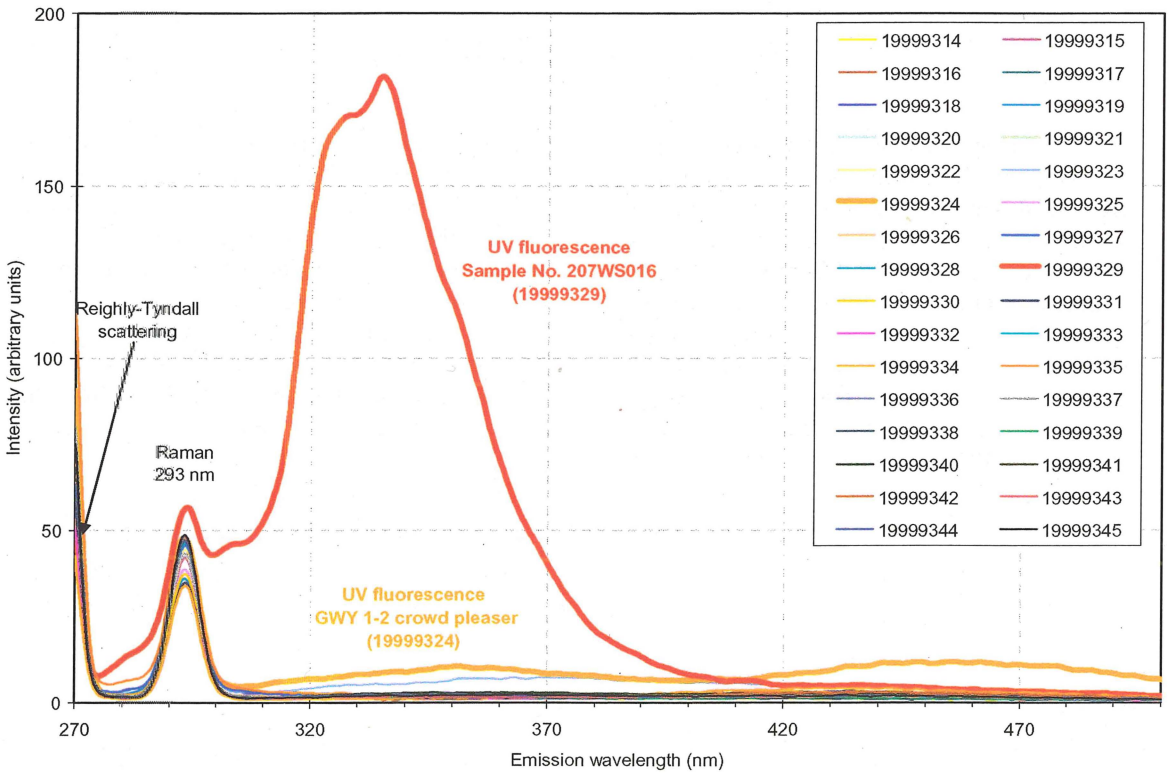

Figure 4. Laboratory UVF spectra of surface seawater samples from AGSO Marine Survey 207.

4. Results and Discussion

The emission spectra for AGSO Marine Survey 207 surface seawater samples are presented digitally in the Excel file S207_UVF.xls.

As shown in Figure 3, neither pure water nor seawater fluoresces. The first peak, nominally at 270 nm, is the tail of the Rayleigh-Tyndall scattering of the excitation light of the 266 nm wavelength. This occurs because not all of the incident energy is absorbed or transmitted by the sample but is also scattered in all directions. The scattering takes place either from the molecules themselves (Rayleigh scattering) or from small suspended particles (Tyndall scattering).

The second peak, the Raman peak at 293 nm for the water samples, arises because some of the incident energy is absorbed and partly converted into vibrational and rotational energy (Raman scattering) which results in an emission at a longer wavelength.

Of the thirty-two AGSO Marine Survey 207 surface seawater samples analysed only two samples emitted fluorescence. Figure 4 shows the emission spectra of the AGSO Marine Survey 207 surface seawater samples analysed using the excitation wavelength of 266 nm. Sample 207WS016 (19999329) collected about 20 km west of the Cornea-1 well (along line LO 14-15a SP74) exhibited fluorescence, with a maximum emission wavelength at 335 nm and a maximum intensity of 181.8 fluorescence units (scale = 0-1000). A second peak occurred at the longer wavelength of 642 nm with an intensity of 37.1 fluorescence units. Sample GWY 1-2 (19999324) fluoresced weakly with maxima at 356 nm (6.9 intensity units) and 461 nm (4.2 intensity units). However the spectrum was too weak to allow any further comparison.

To date, the closest match of the fluorescence anomaly observed for the Yampi Shelf surface seawater sample 207SW016 (19999329) is with the crude oils (yellow condensates) from the wells Swan-1 and Eclipse-2 in the Vulcan Sub-basin (Fig. 6). Figure 7 shows these same crude oils with the spectra normalised to the largest fluorescence peak.

5. CONCLUSIONS

Thirty-two surface seawater samples collected on the AGSO Marine Survey 207 were analysed by emission spectrometry of which two samples from the Yampi Shelf fluoresced. Sample 207SW016 (19999329) collected about 20 km west of the Cornea-1 well (along line LO 14-15a SP74) had an emission spectrum that was characteristic of crude oil, with a maximum emission wavelength at 335 nm and a maximum intensity of 181 fluorescence units (on a scale of 0-1000). Sample GWY 1-2 (19999324) fluoresced weakly with maxima at 356 nm and 461 nm; however, the response was too weak to enable comparison with the spectra of known oils. Comparison with a limited number of Northwest Shelf oil-in-water samples demonstrated that the fluorescence anomaly in sample 207SW016 most closely matched those of the crude oils (yellow condensates) from the Swan-1 and Eclipse-2 wells.

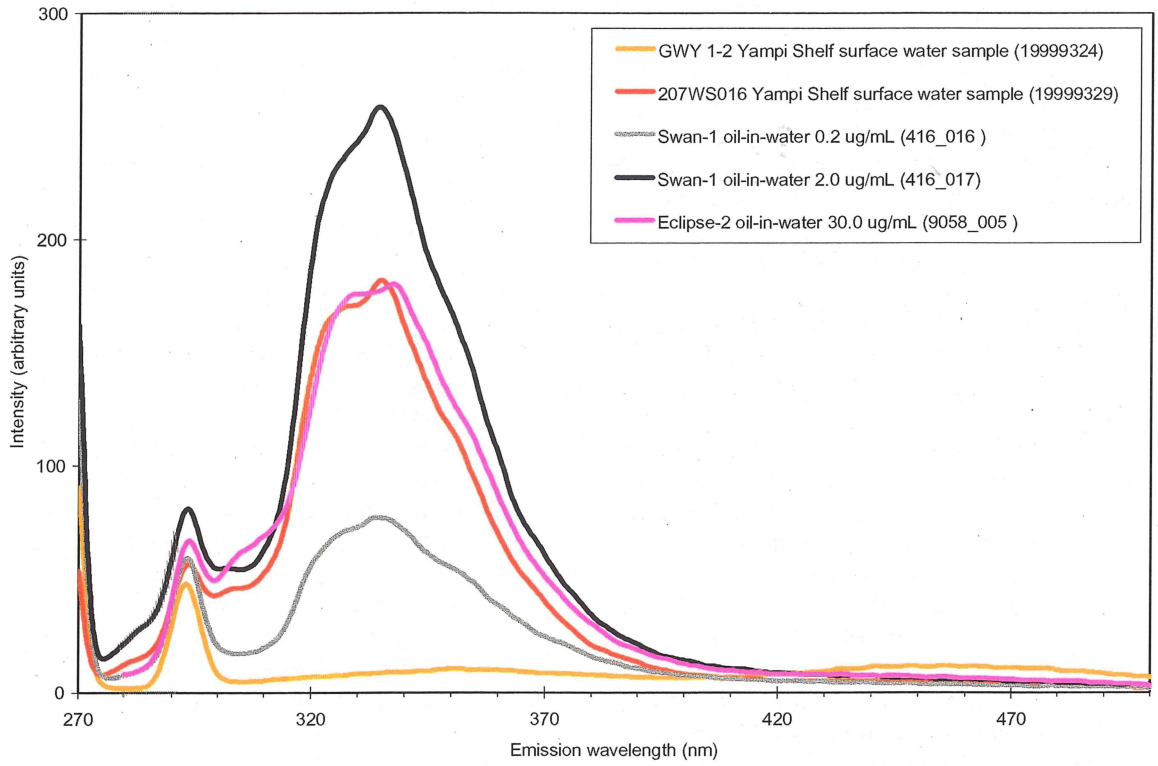

Figure 5. Comparison of Yampi Shelf fluorescence anomaly with selected Northwest Shelf oils in water.

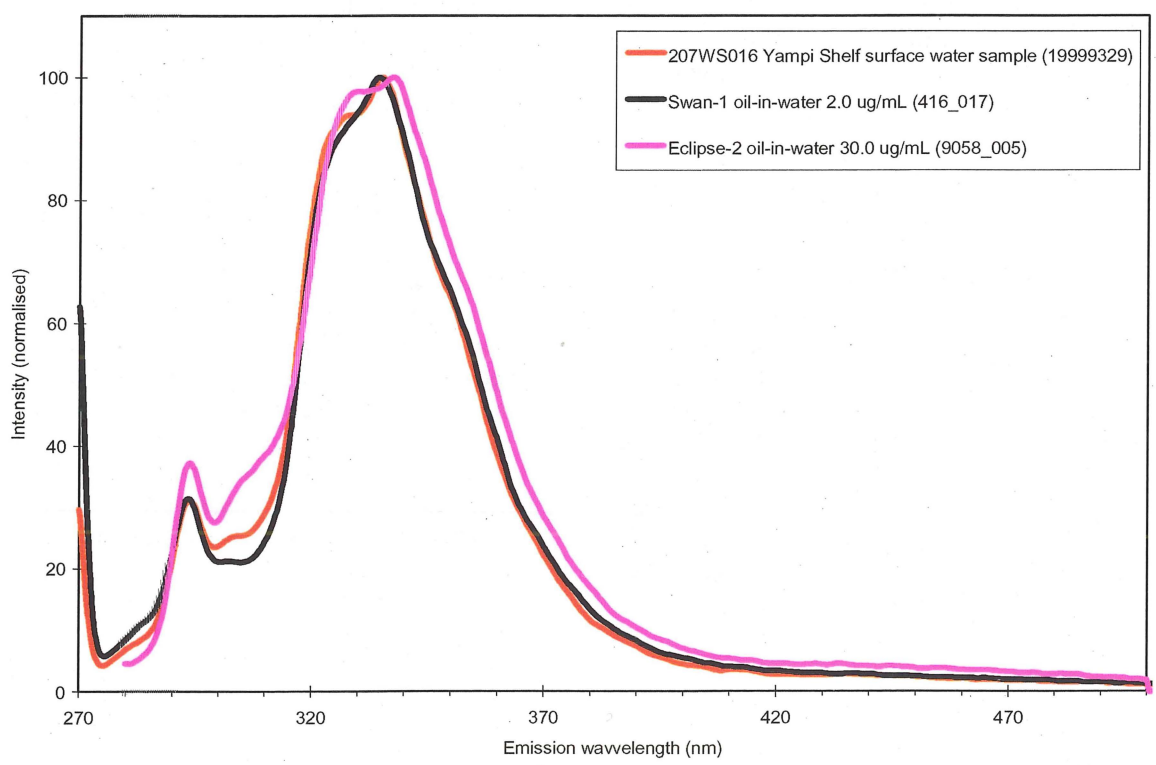

Figure 6. Comparison of Yampi Shelf fluorescence anomaly with selected Northwest Shelf oils in water, normalised to the largest fluorescence peak.

6. REFERENCES

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Appendix A1: UVF Analytical Methods

A1.1 Background Checks

In this report, pure water was obtained by filtering distilled water through a Millipore filter system, specifications. Artificial seawater (3.5 % salinity) was prepared by dissolving 1.75 g of aquarium sea salt in 50 mL Millipore water in a 50 mL volumetric flask. An emission scan using an excitation wavelength of 266 nm and scan rate of 60 nm/min was carried out to verify that the artificial seawater did not exhibit any fluorescence.

A1.2 Seawater Sample Preparation

The seawater samples required no further preparation before UV analysis.

A1.3 Oil-Water Mixtures

A stock oil in artificial seawater solution of 0.2 mg/mL (200 μg/mL) was prepared by weighing out 5 mg oil into a 25 mL volumetric flask and then adding 25 mL of the artificial seawater (3.5 % salinity). The stoppered flask was shaken vigorously and allowed to stand in a dark cold-room for 24 hours prior to analysis to ensure homogeneity. A pipette was then filled with the oil-saturated water by withdrawing the water from beneath the oil-water interface. The oil-saturated water sample was placed in a 4 mL quartz cuvette for analysis.

To a cuvette containing an artificial seawater solution was added a 1 μL (0.001 mg) aliquot of the sample stock solution (0.2 mg/mL). The sample in the cuvette was thoroughly mixed to ensure homogeneity. Sequential 1 μL aliquots of the sample stock solution was added until measurements at the required concentrations had been obtained.

A1.4 Fluorometer Pre-test

The signal to noise ratio was tested using an automated instrument verification test. The 4 mL quartz cuvette was filled with purified water and the PE LS50 pre-test sequence was run with the following instrument settings:

Excitation wavelength: 350.5 nm
Emission wavelength: 396.5 nm (expected Raman scattering peak for water for excitation at 350.5 nm)
Scan speed 60 nm/minute

For the instrument checks to be passed the following criteria had to be met:

Raman peak for water	396.5 nm
Rayleigh scatter wavelength 1	350 ± 1.0 nm
Rayleigh scatter wavelength 2	550 ± 1.0 nm

The signal to noise ratio must be greater than 500:1.

The ratio was 689:1 with this sample batch ran on 8 June 1999.

A1.5 Instrumental Parameters

Instrument: Perkin Elmer Fluorescence Spectrophotometer

Lamp:	Pulsed Xenon
Excitation slit:	2.5 nm
Emission slit:	5 nm
Excitation wavelength:	266 nm
Emission wavelength:	270-720 nm
Scan Speed	60 nm/minute
Cell:	10 mm (4 mL) quartz cuvette
Solvent:	Millipore water

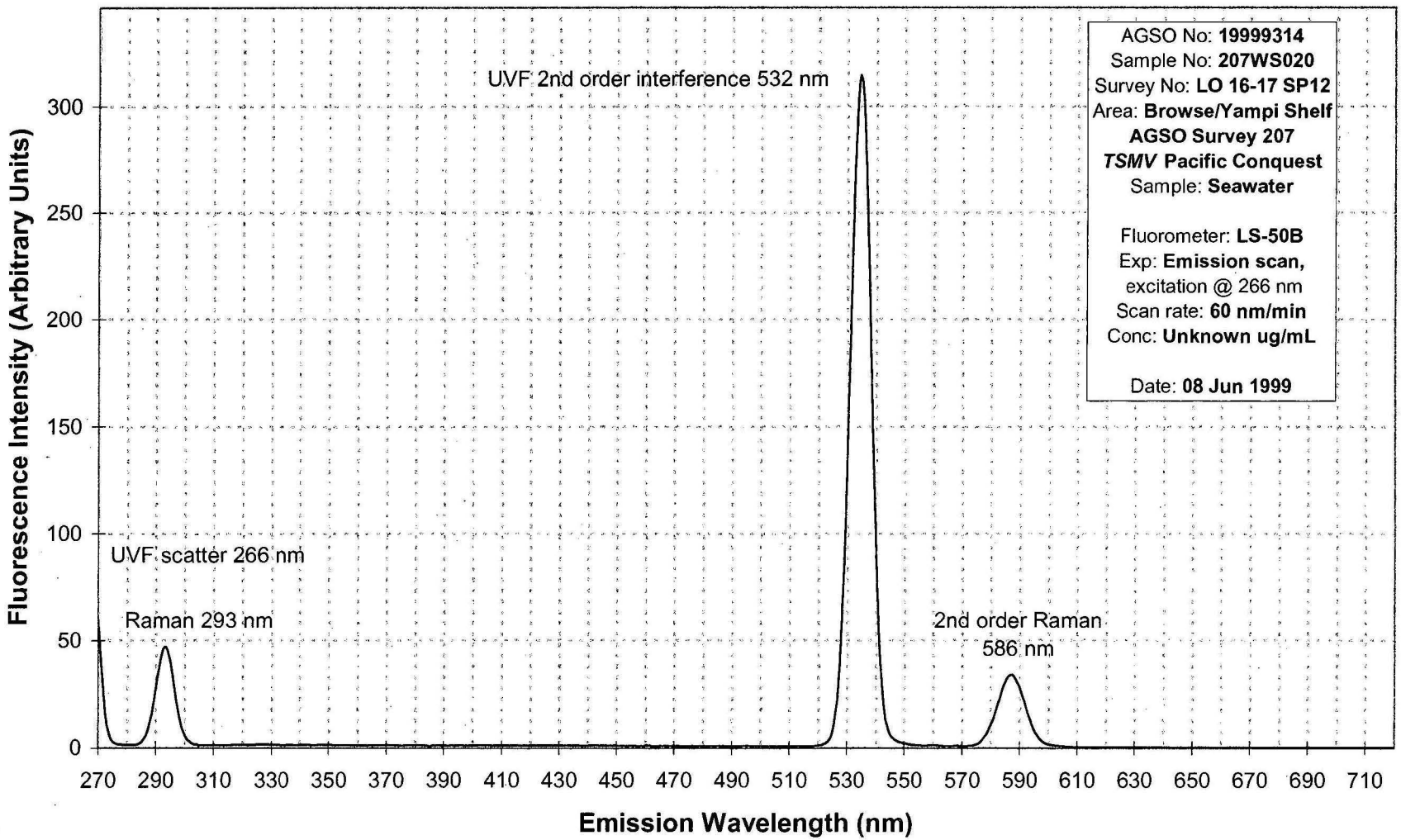
A1.6 Emission Scan Analysis

A fresh non-irradiated sample solution was used for each UV spectral analysis. The artificial seawater sample was used as a blank to check the cleanliness of the cuvette before the addition of the sample and between samples. The artificial seawater sample was discarded upon acceptance of the cuvette cleanliness (intensity units remained less than 0.4) then the sample was added via a disposable glass Pasteur pipette direct from the bottle to cuvette. The emission spectrum of the sample was scanned from 270 to 720 nm in 0.5 nm intervals using the emission monochromator scan rate of 60 nm/minute.

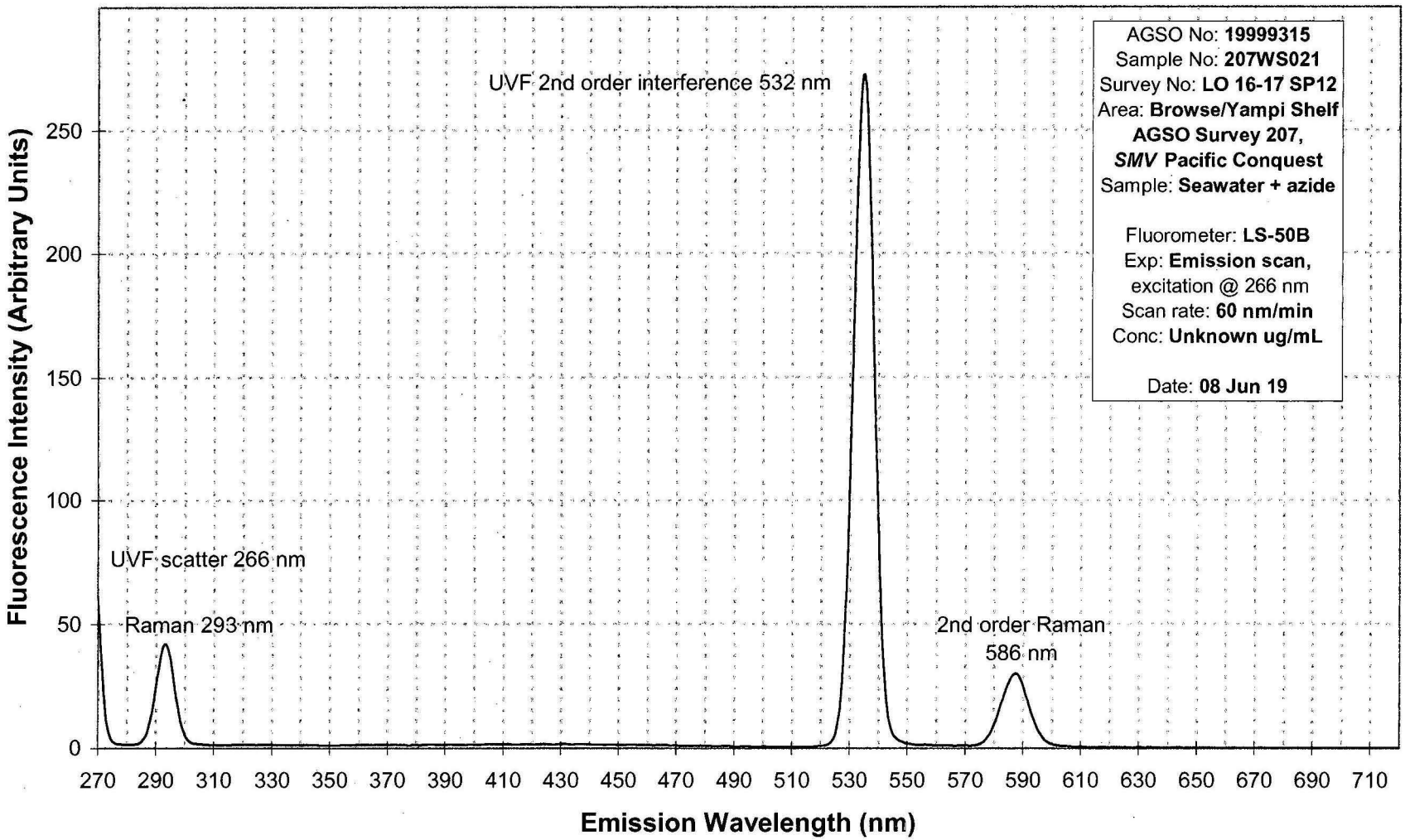
Appendix A2 UVF Emission Spectra

A2.1 UVF spectra AGSO Marine Survey 207: UVF emission data.

UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



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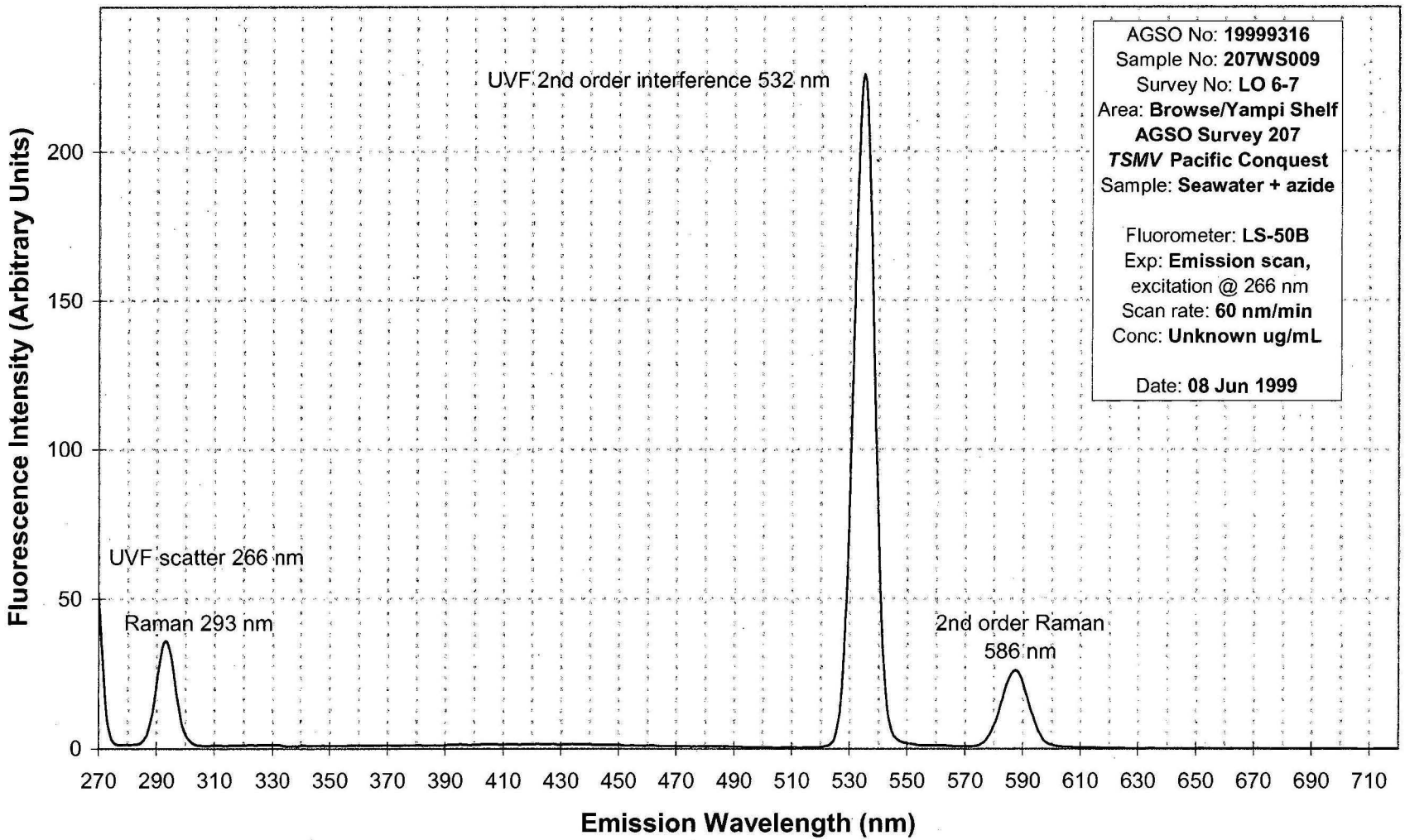
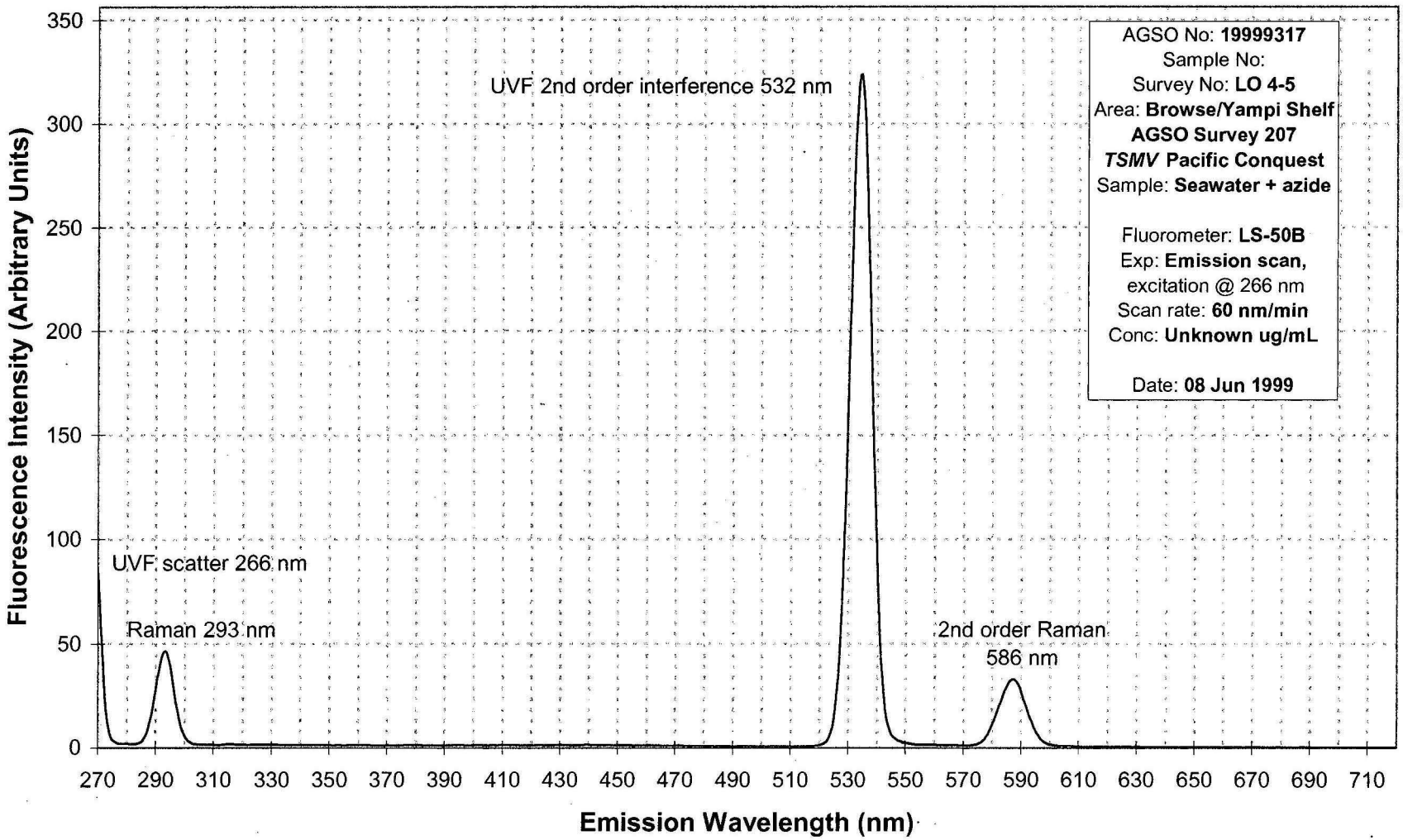


UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



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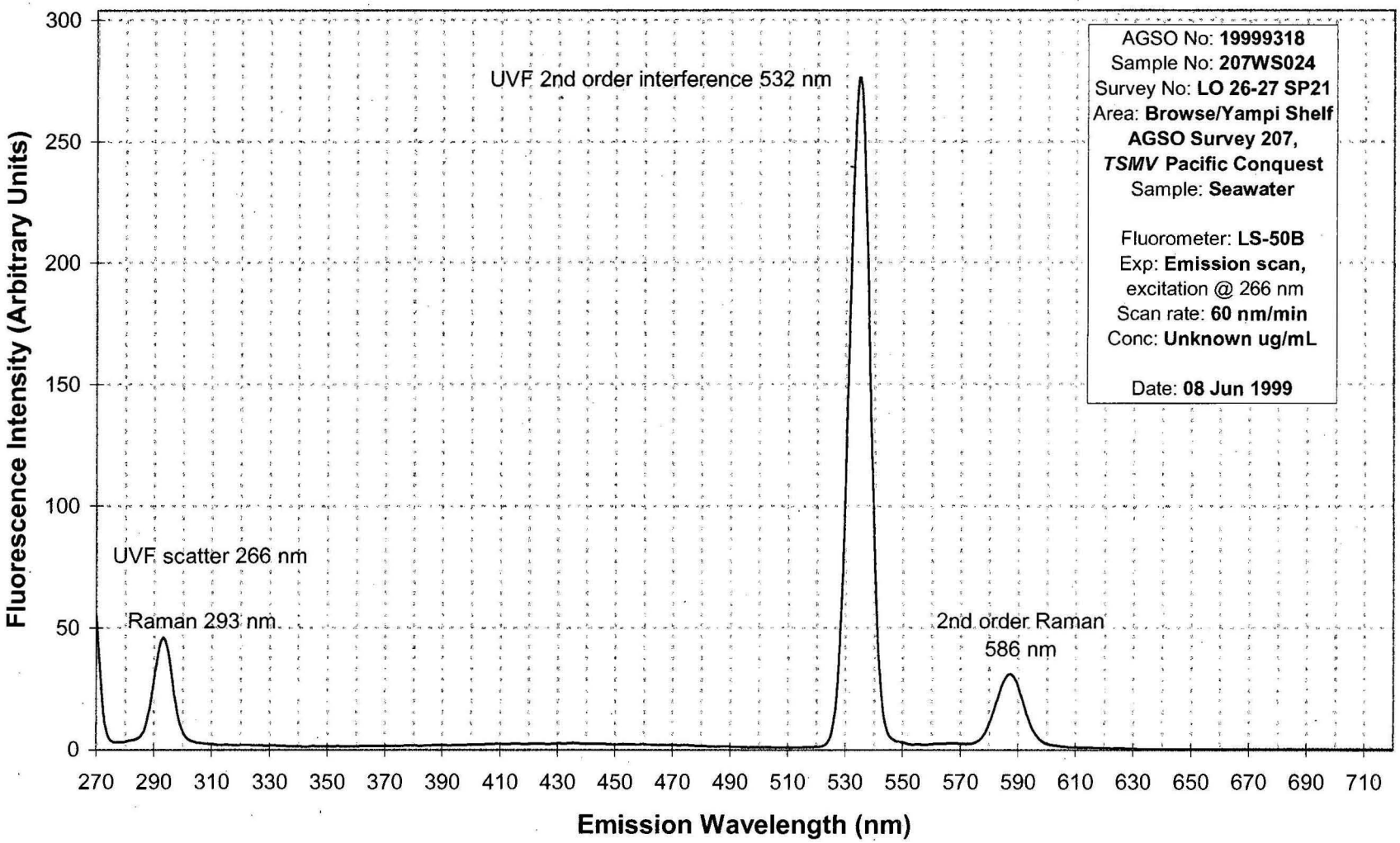

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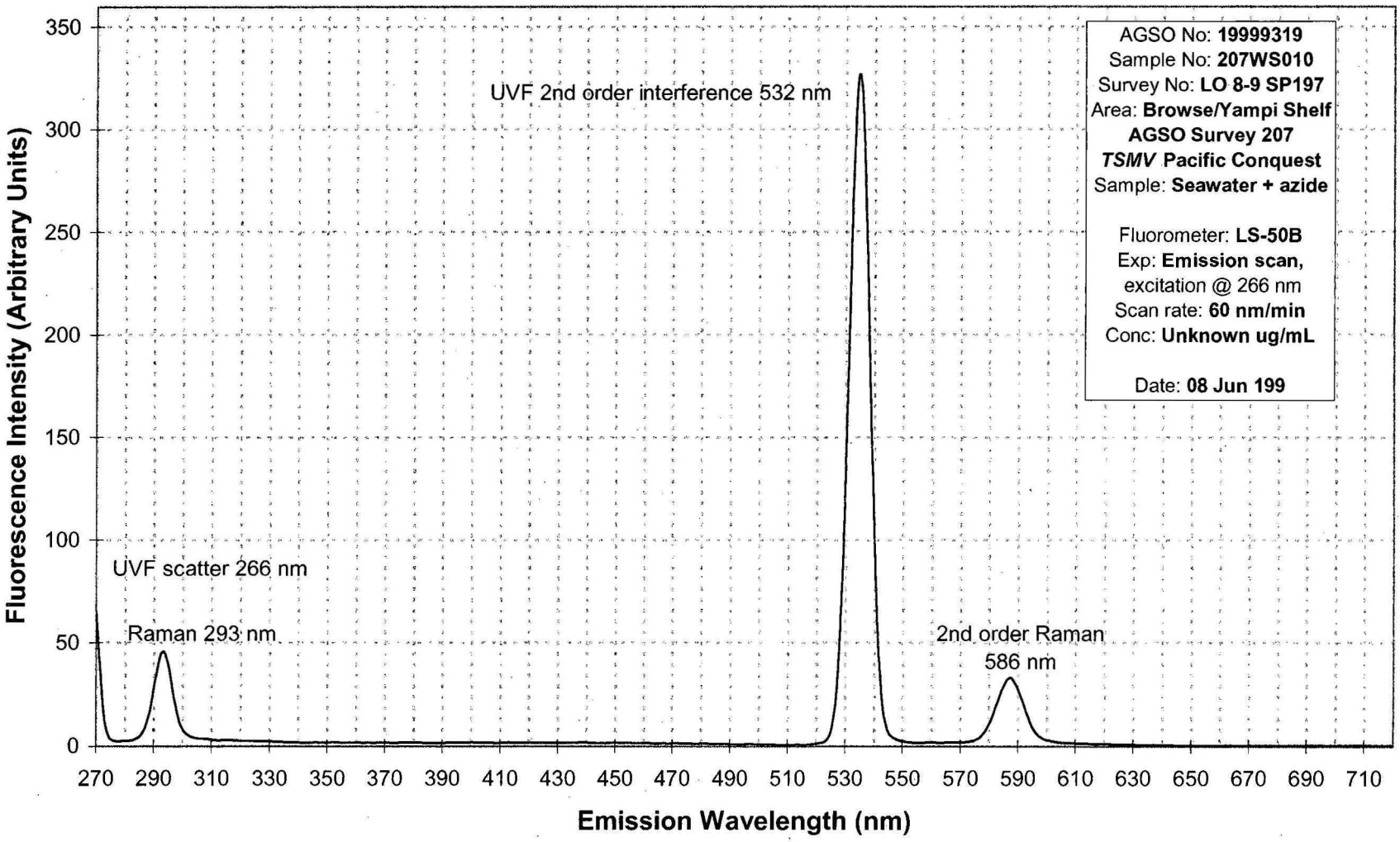
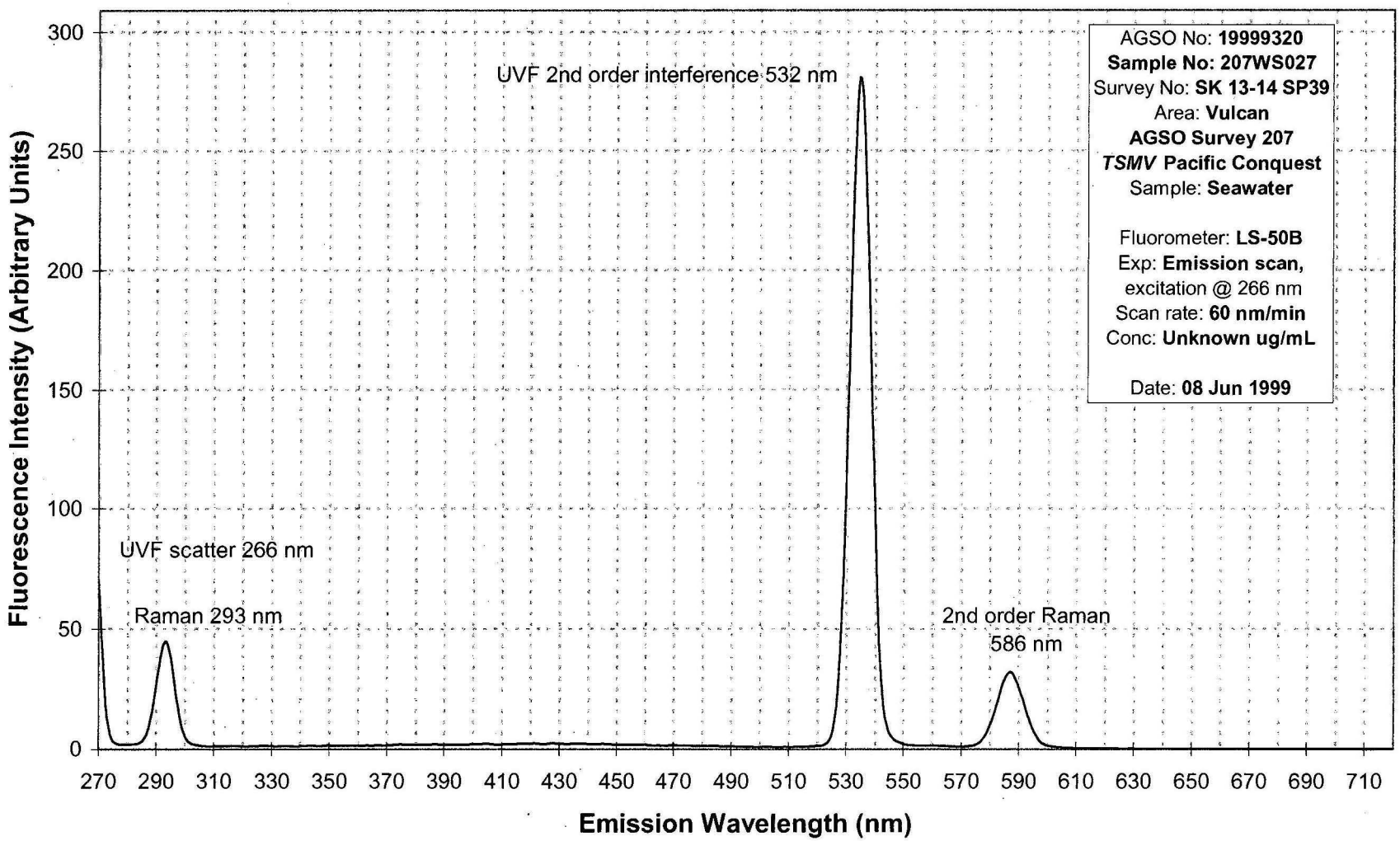
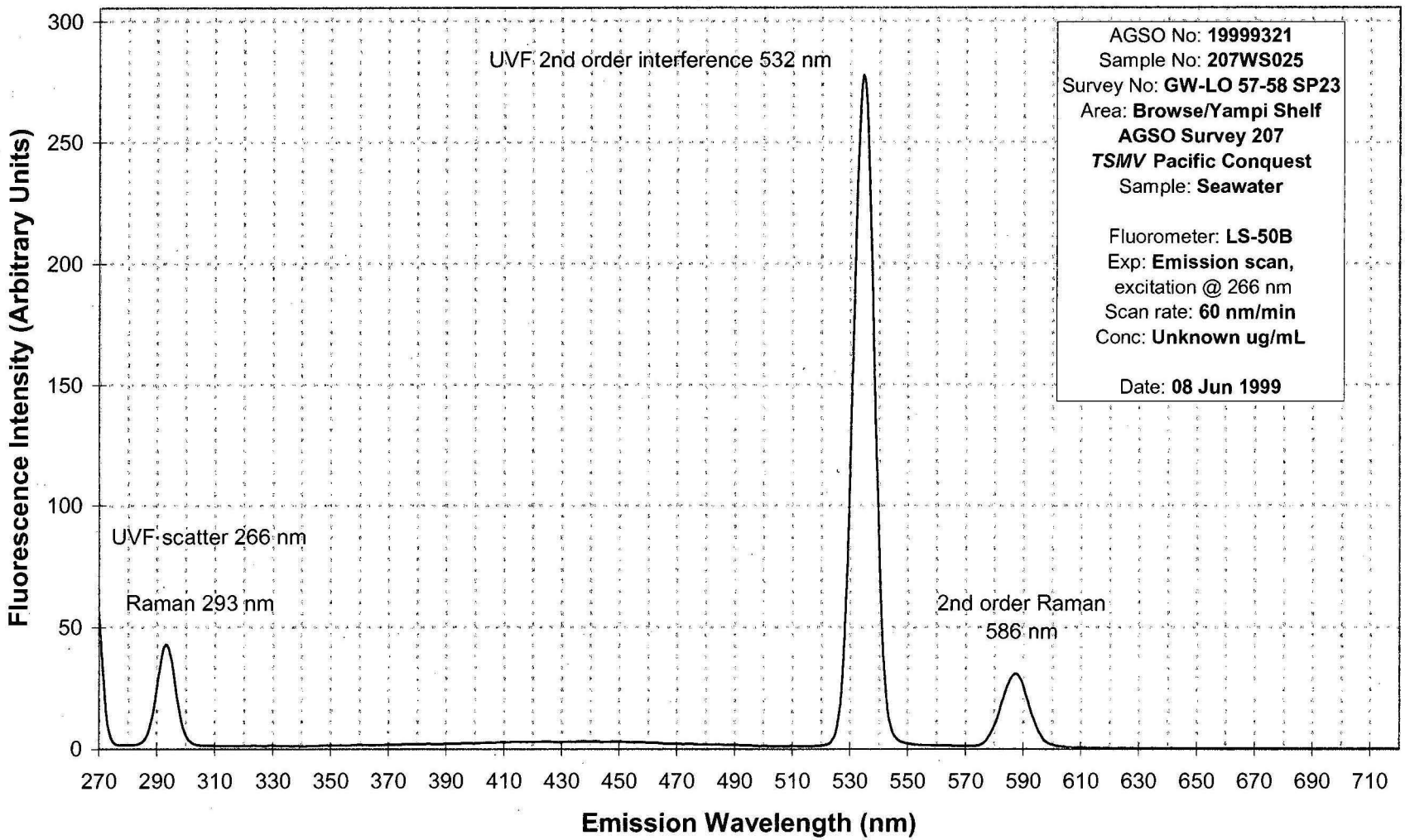


UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



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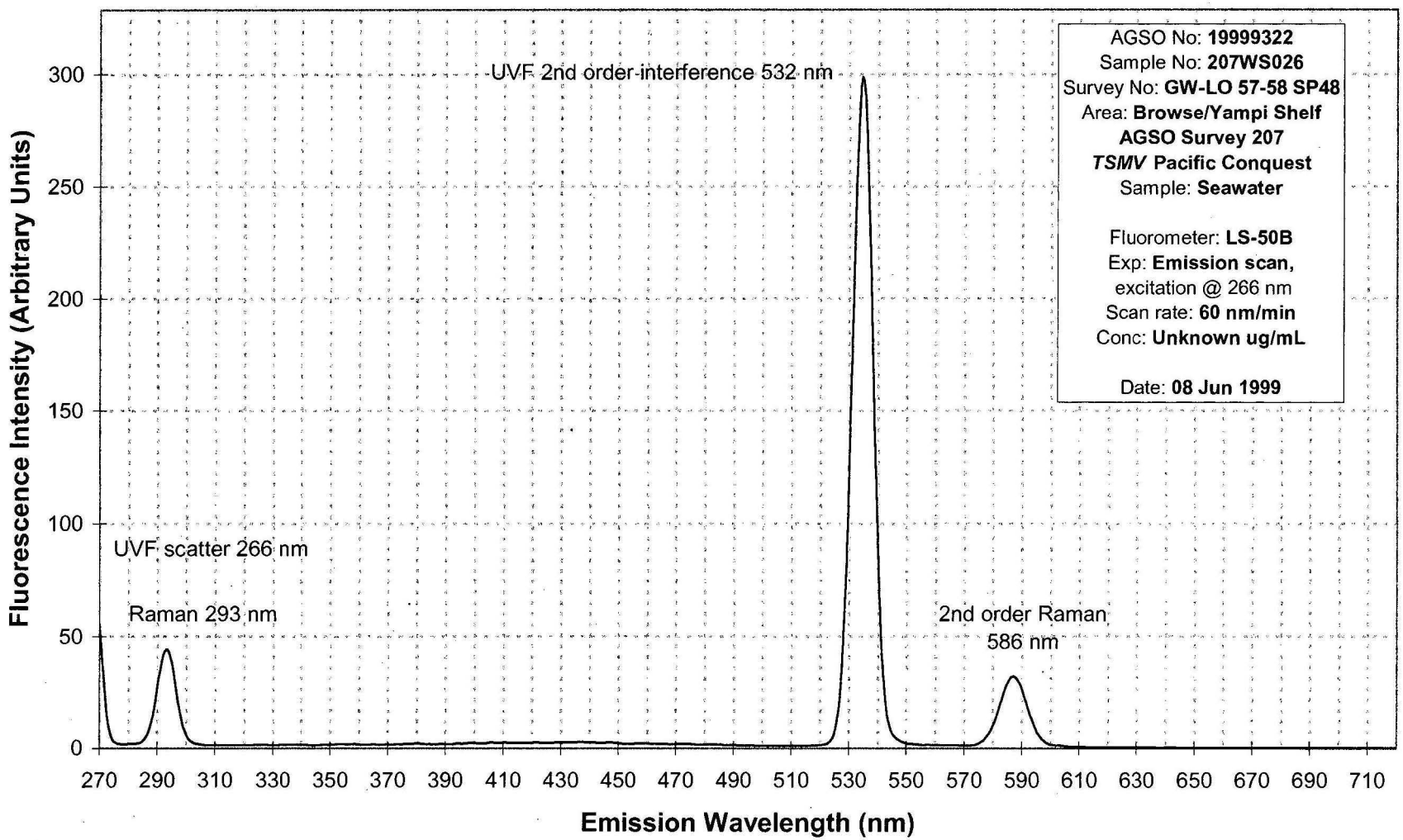
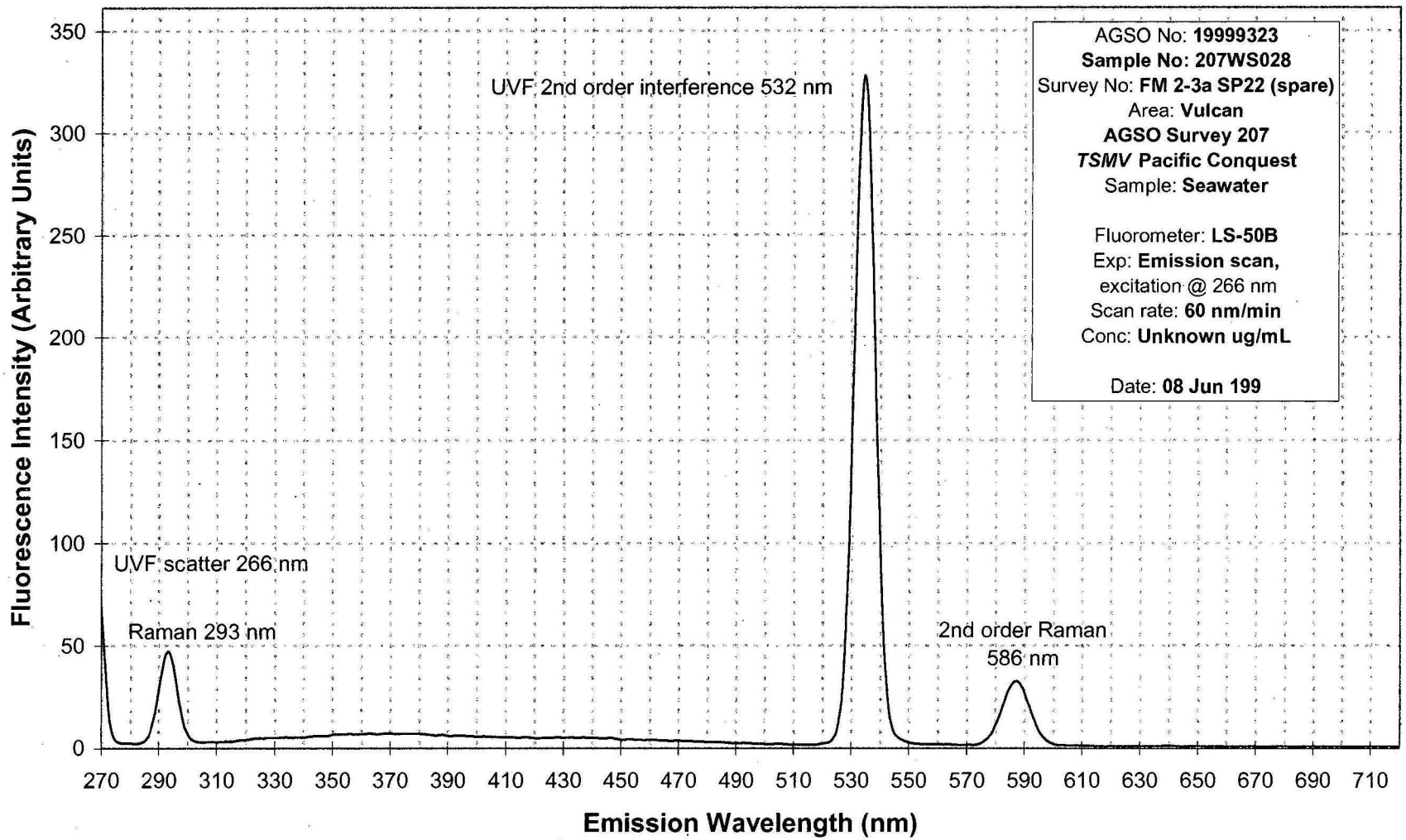


UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



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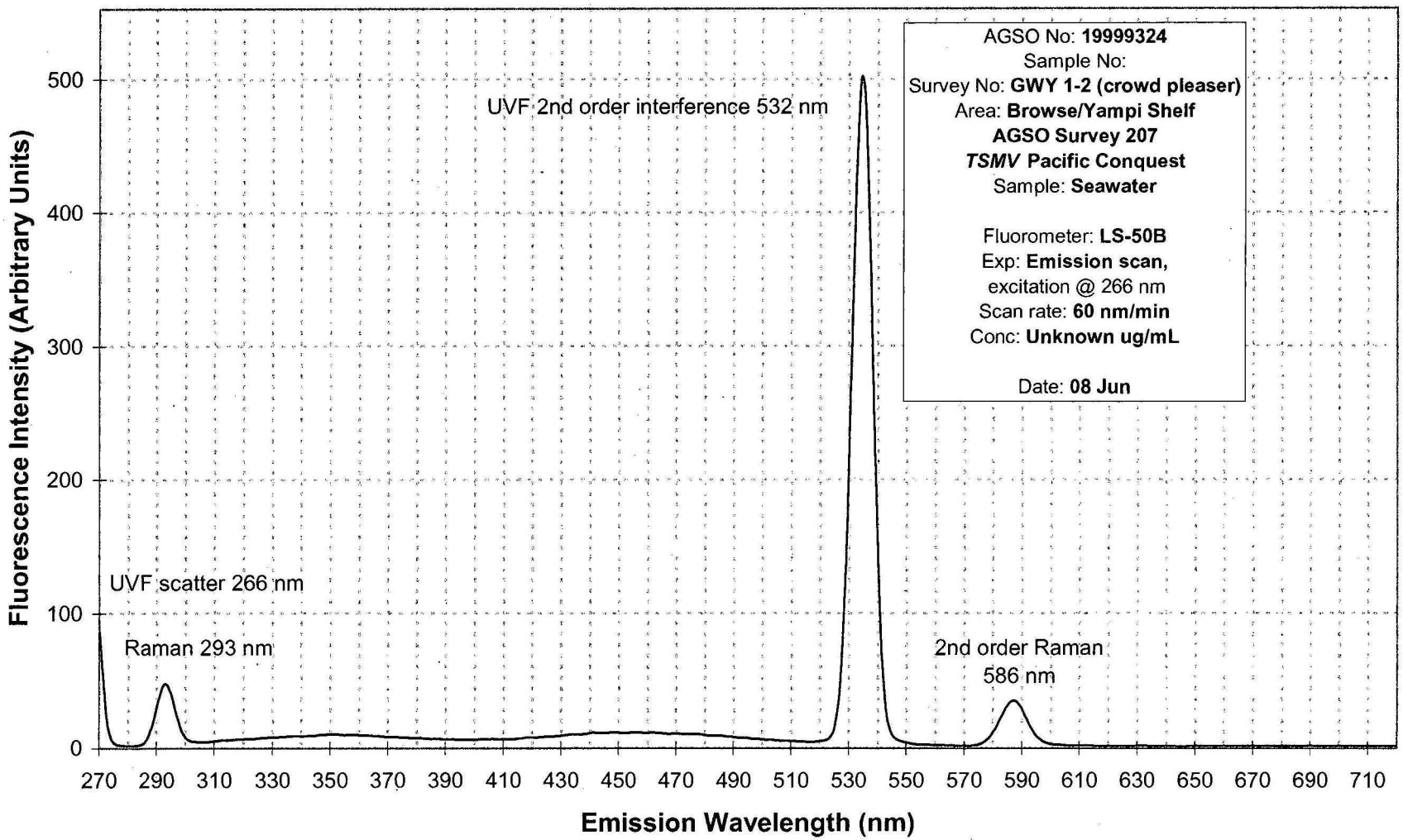

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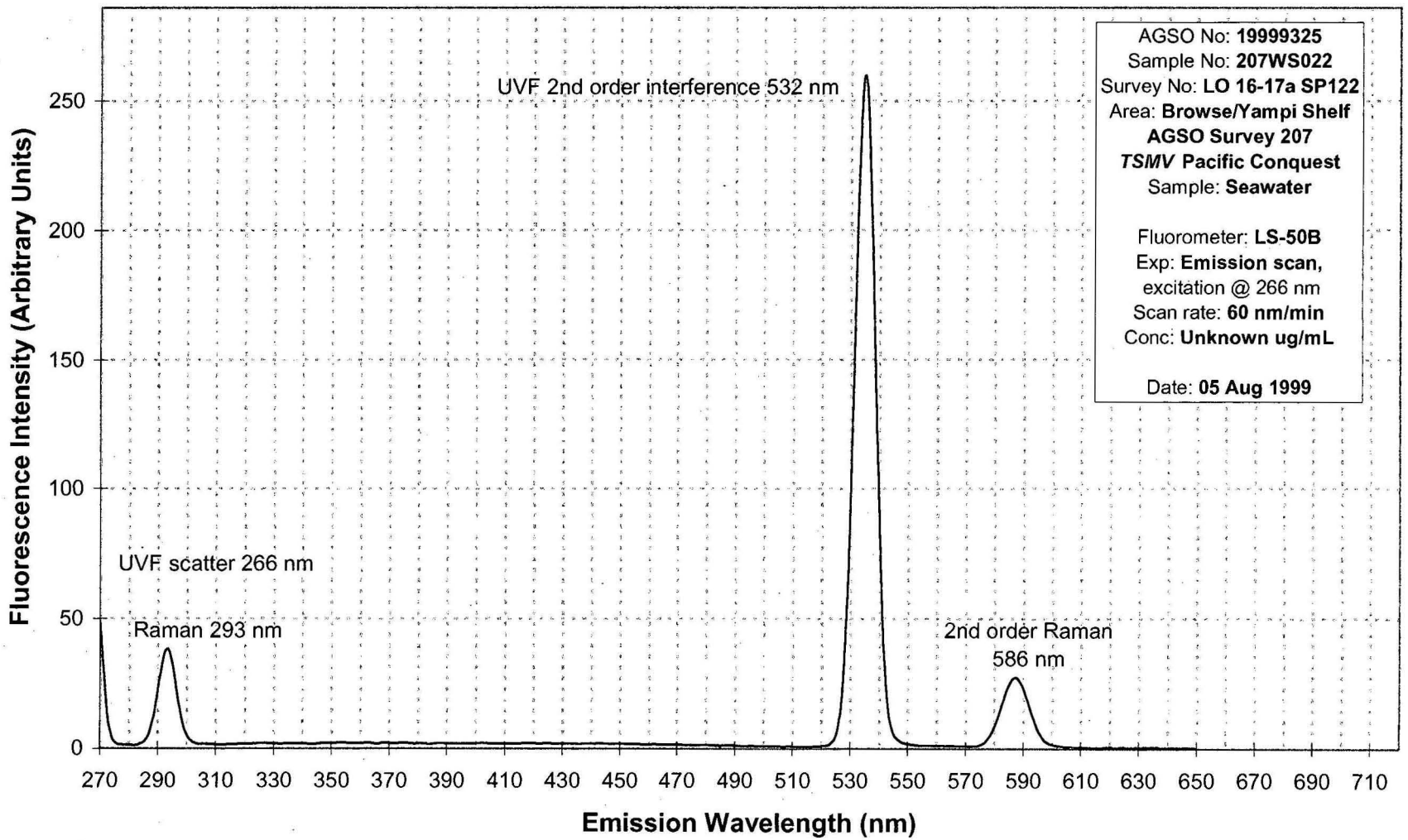

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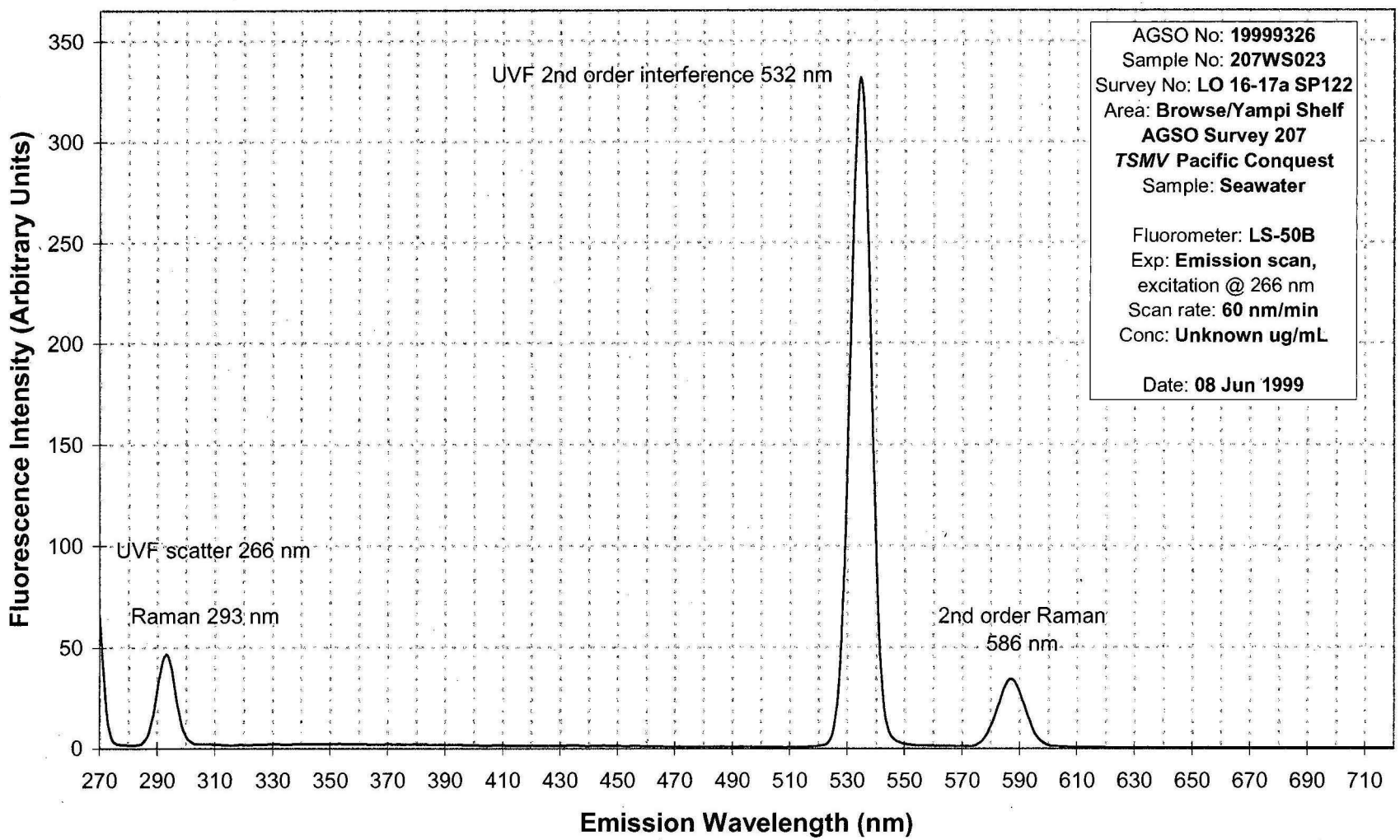

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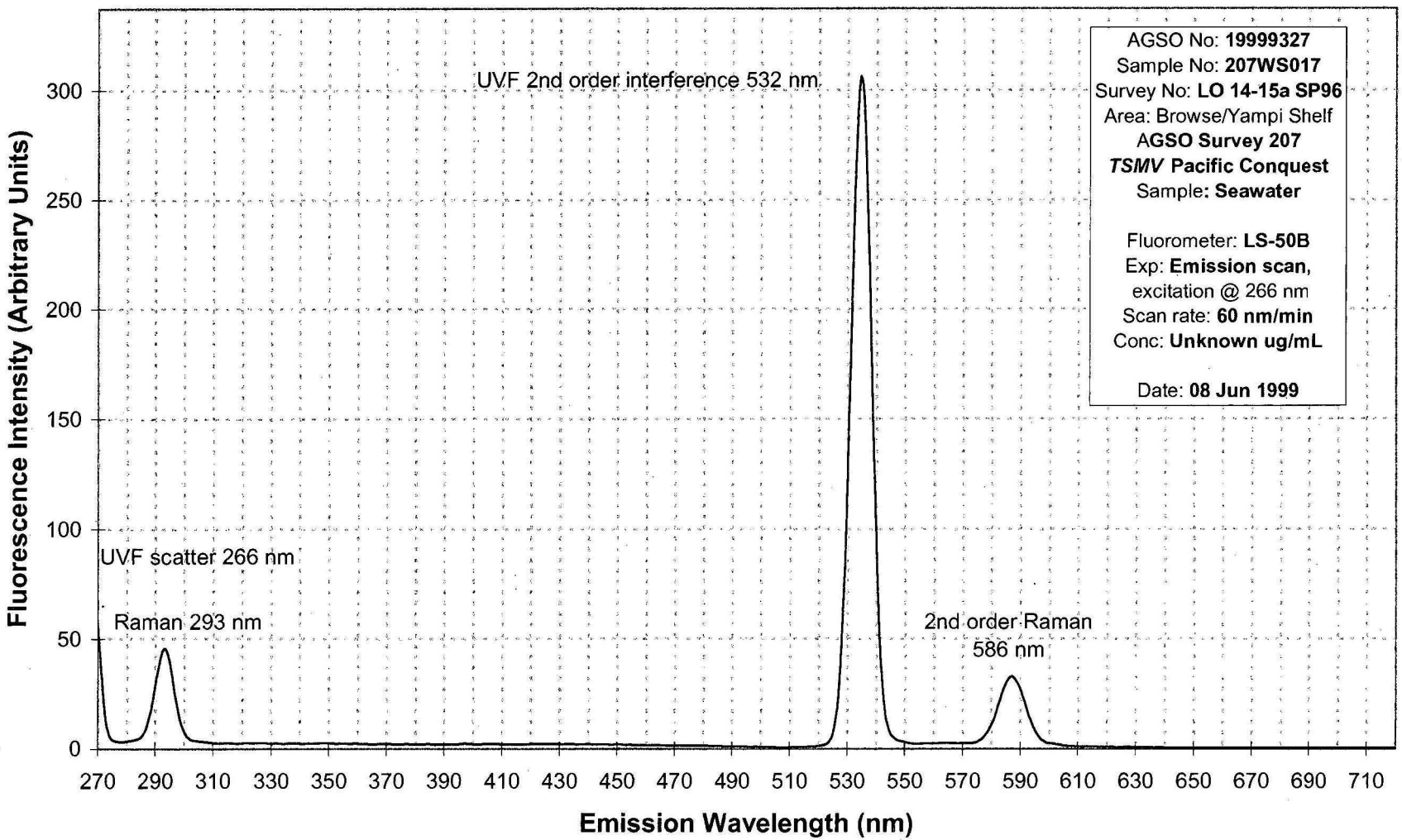

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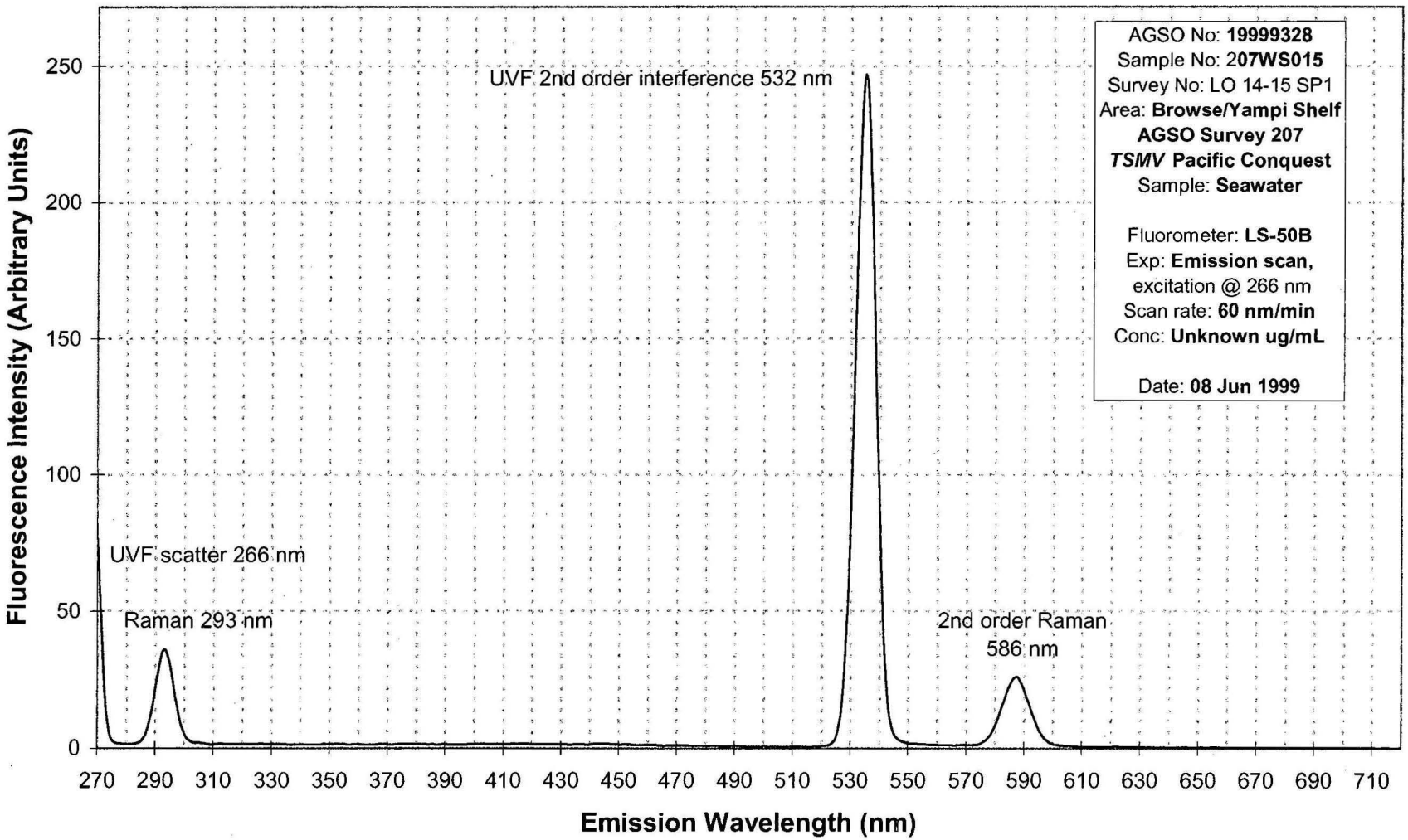
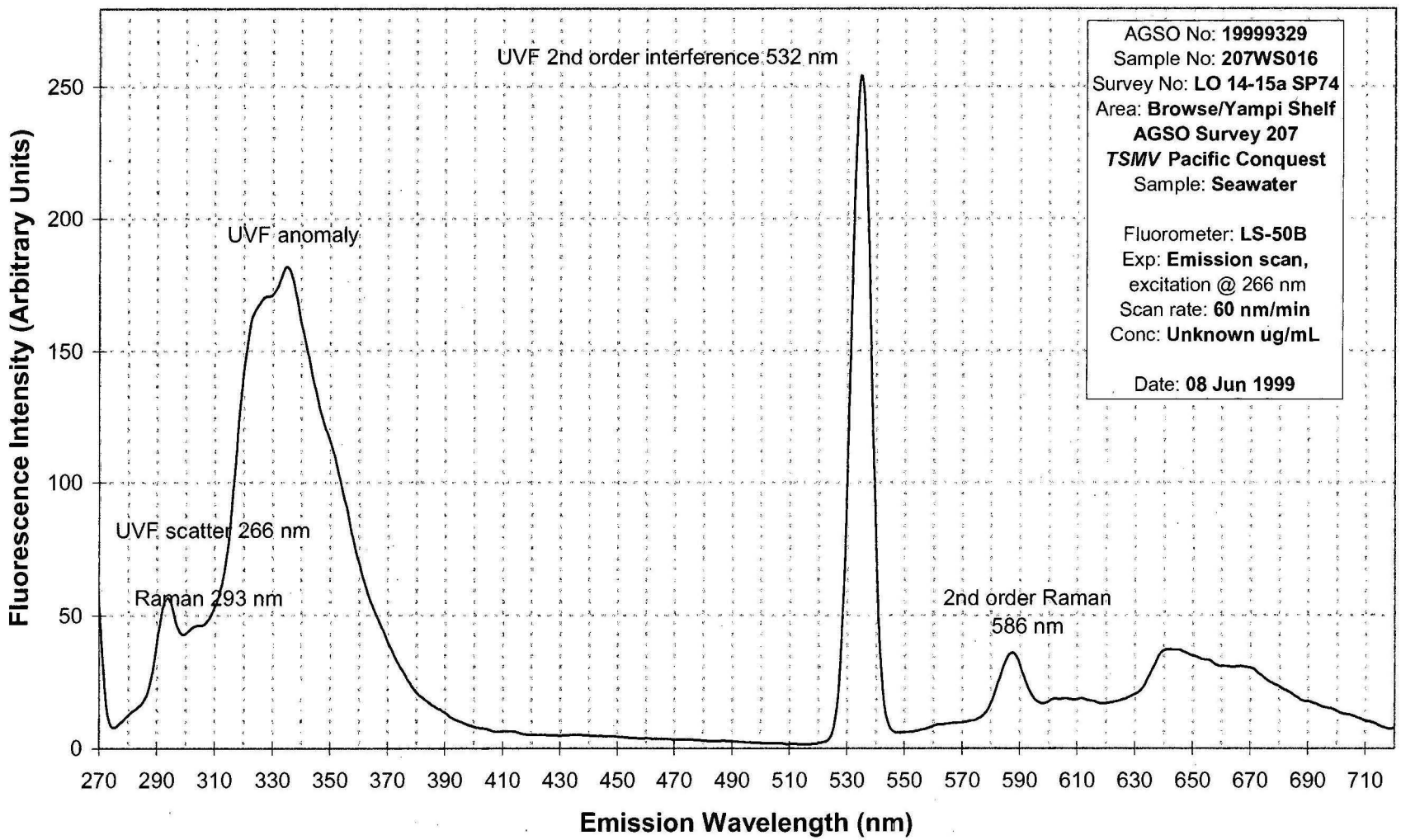
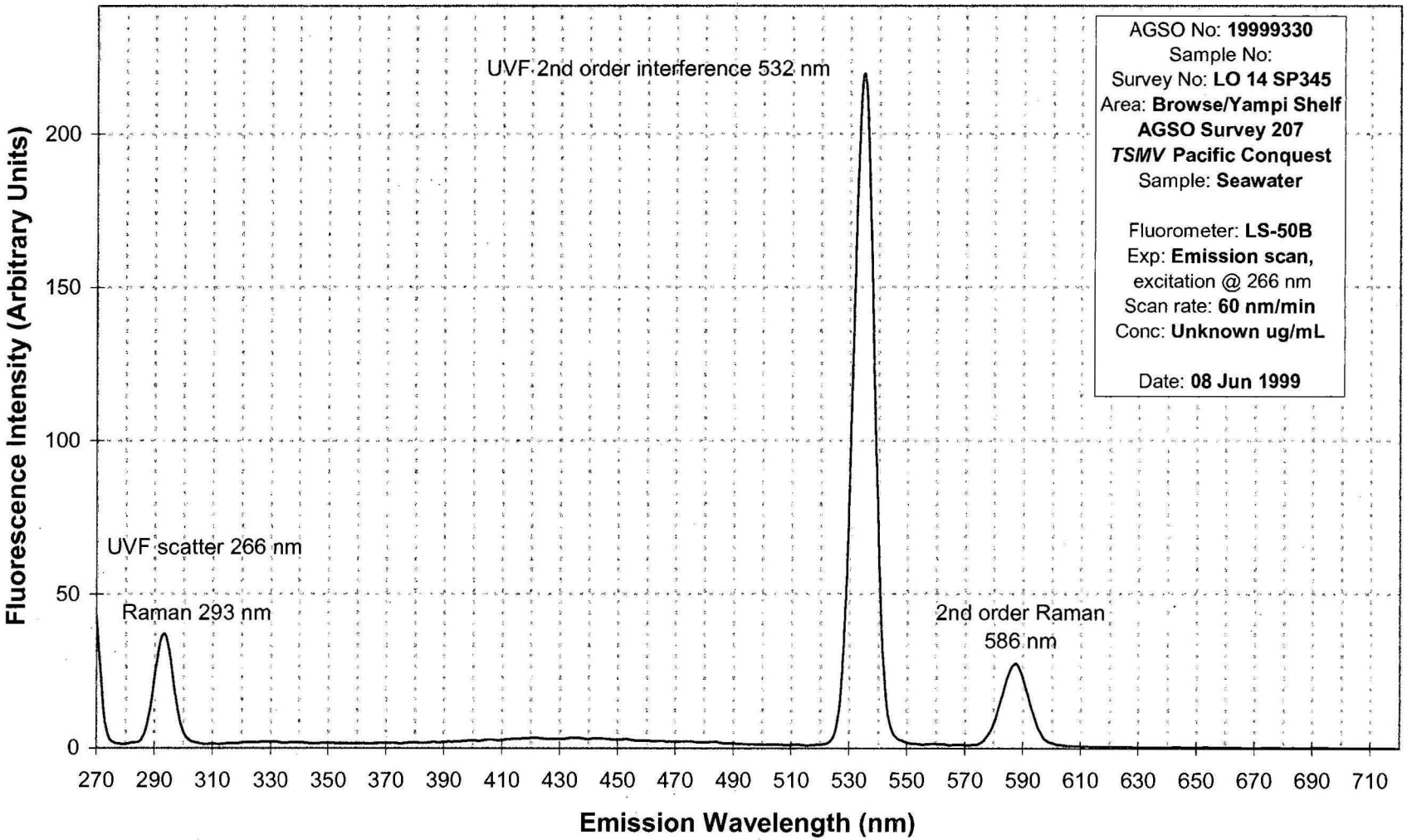


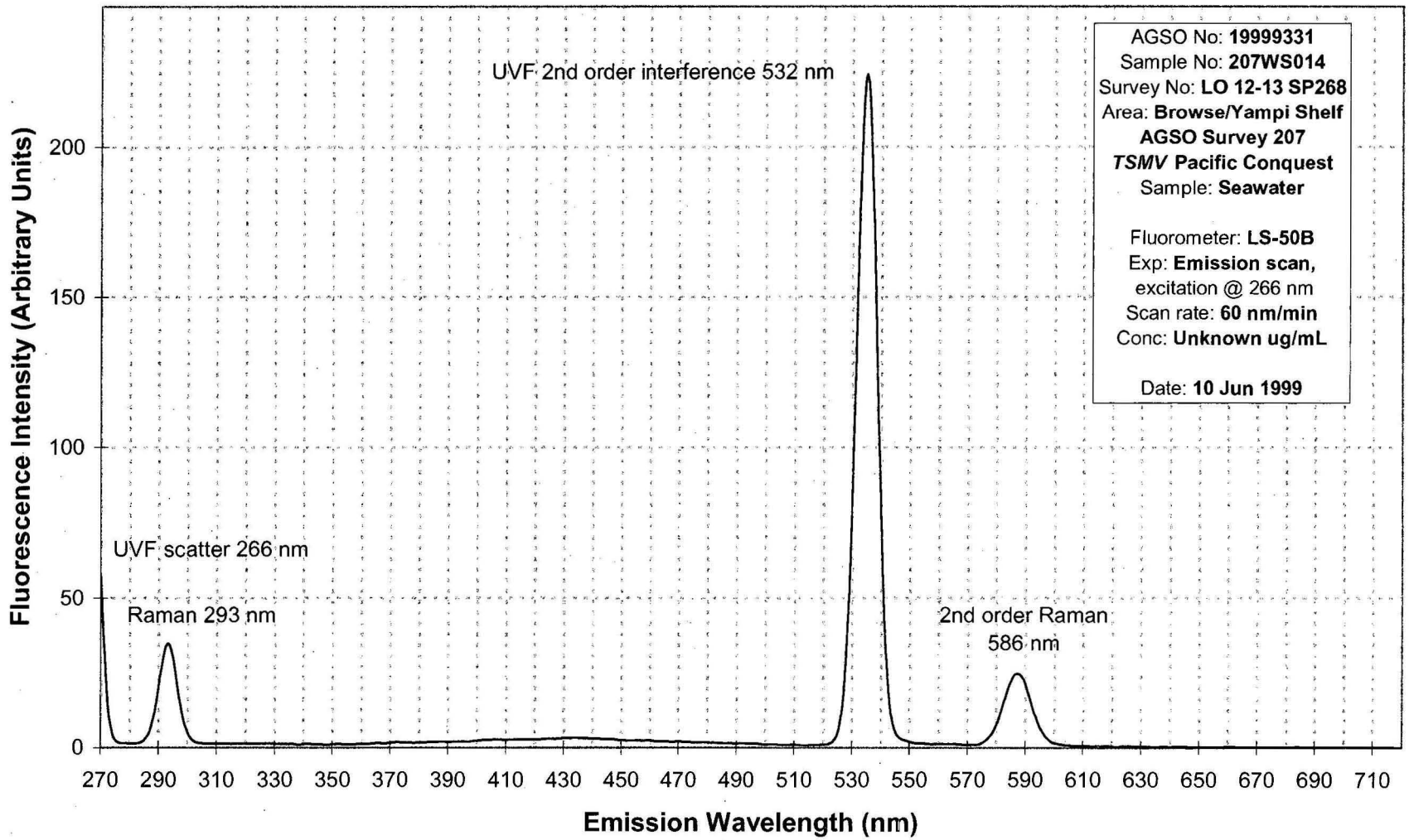
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



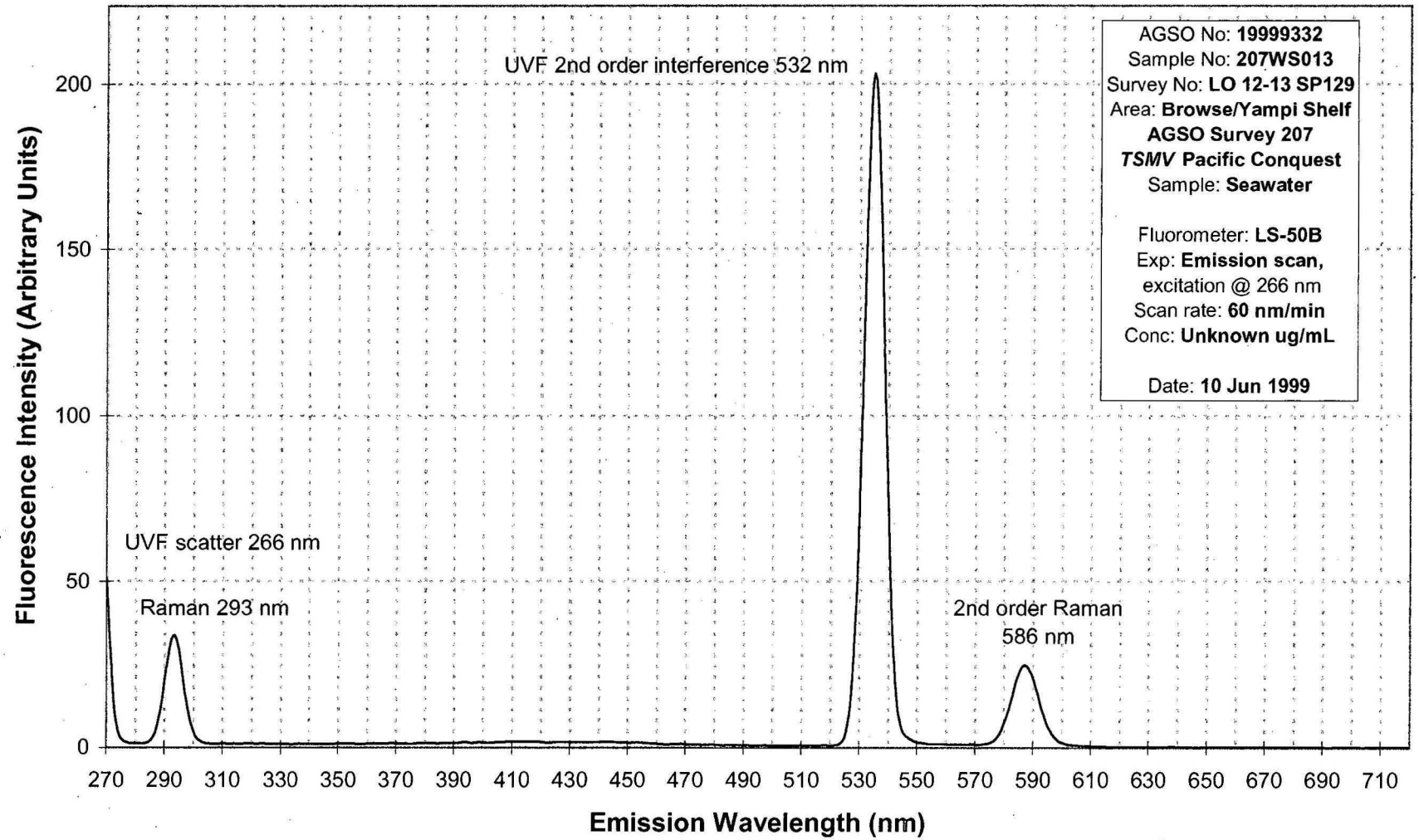
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



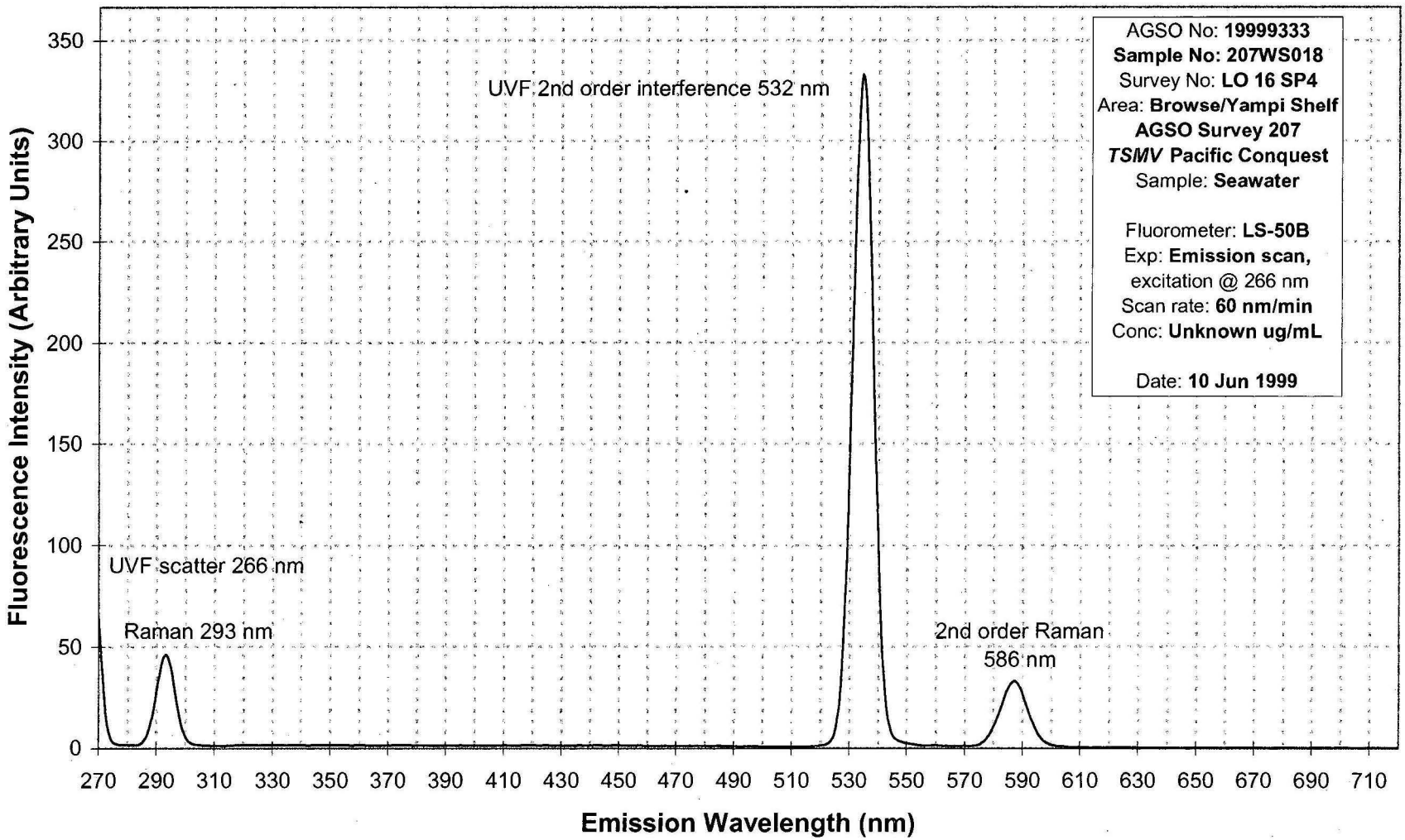
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



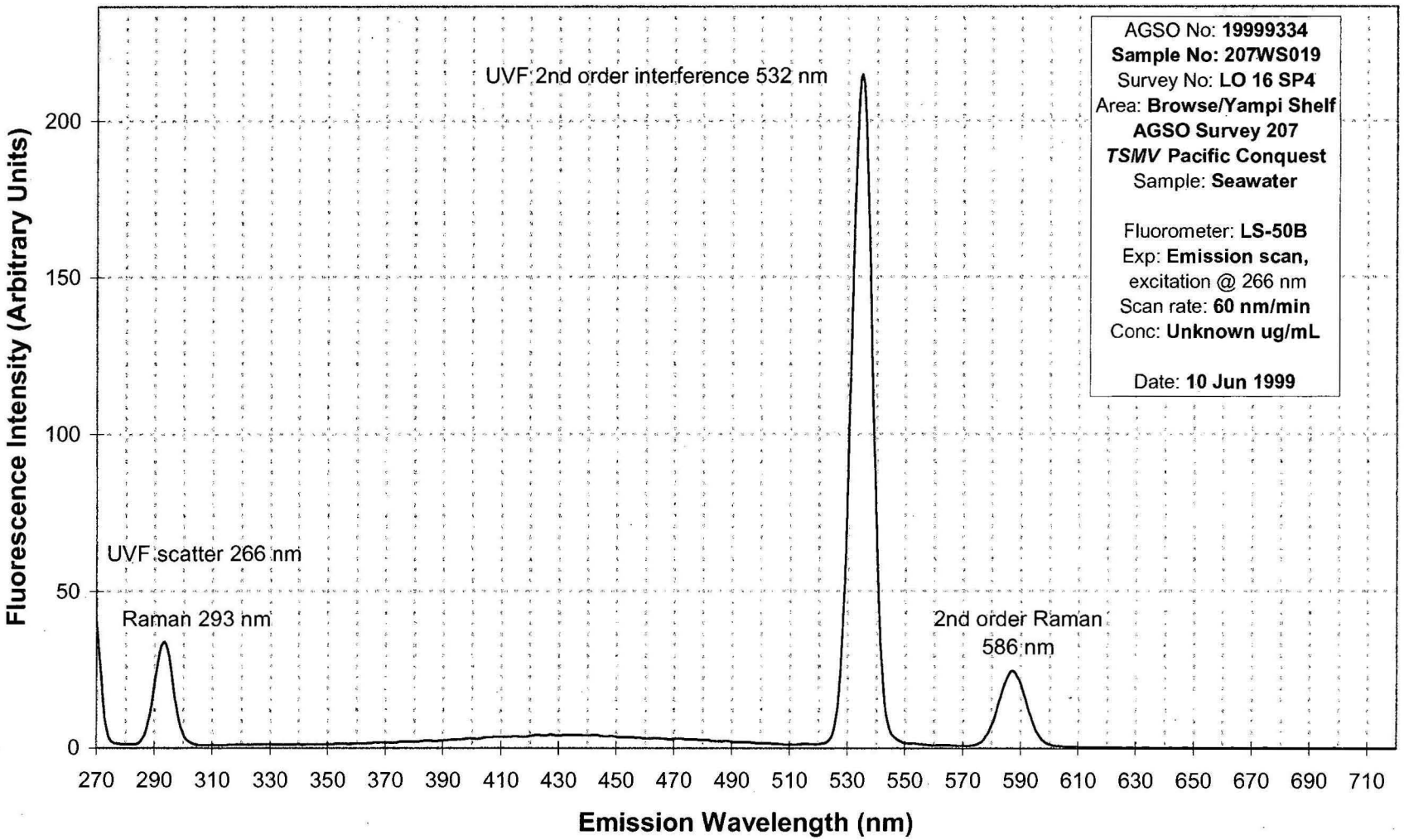
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



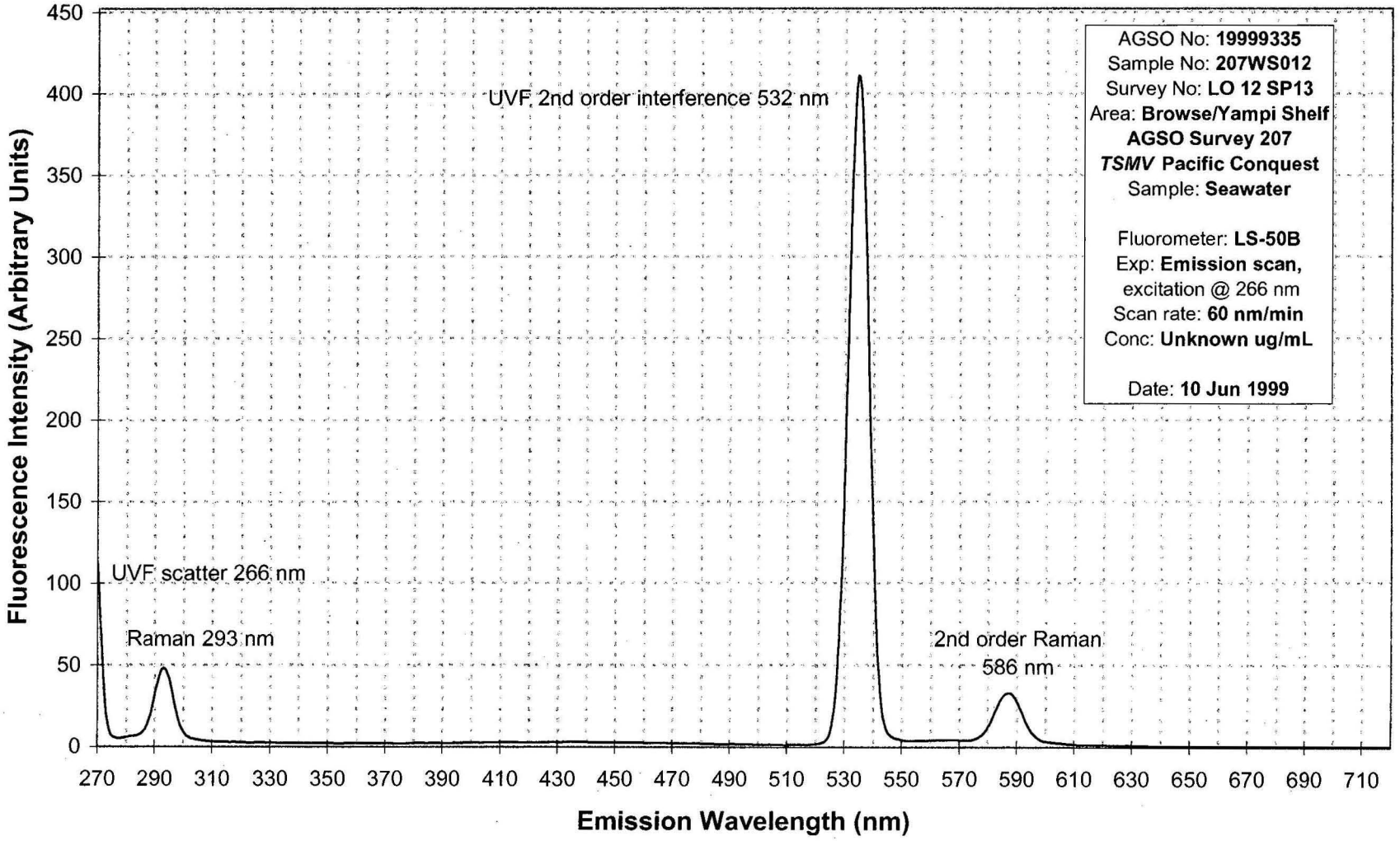
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



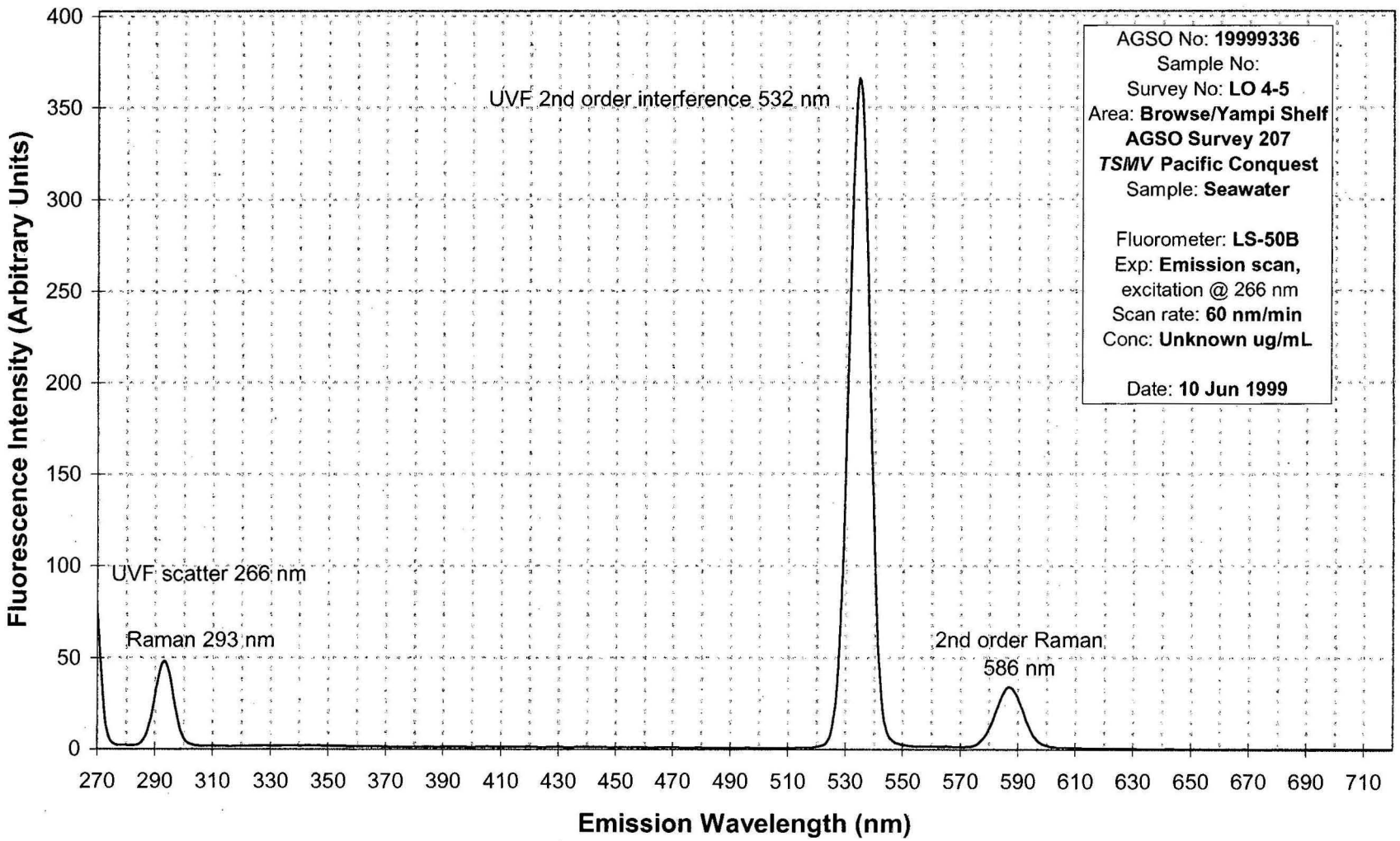
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



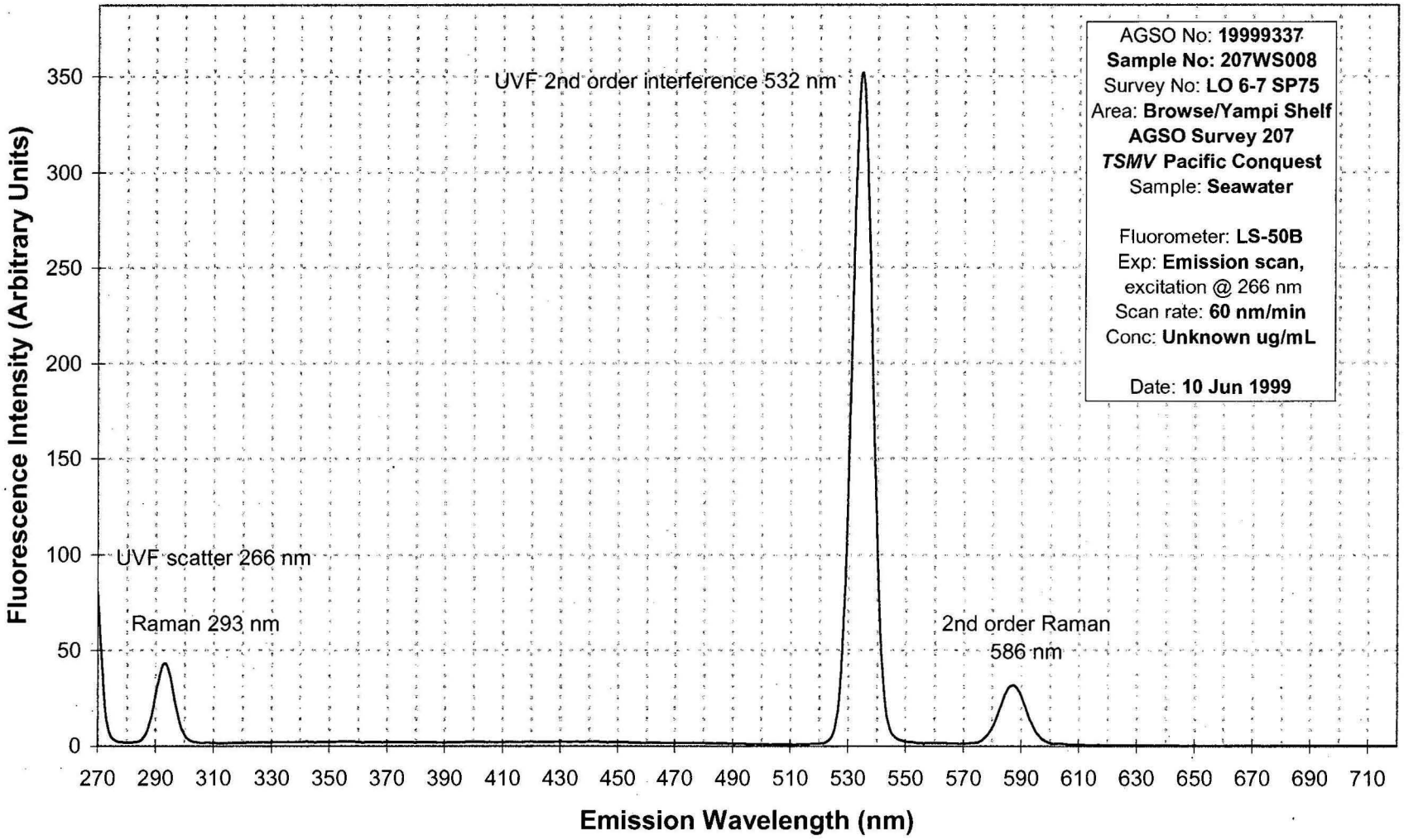
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



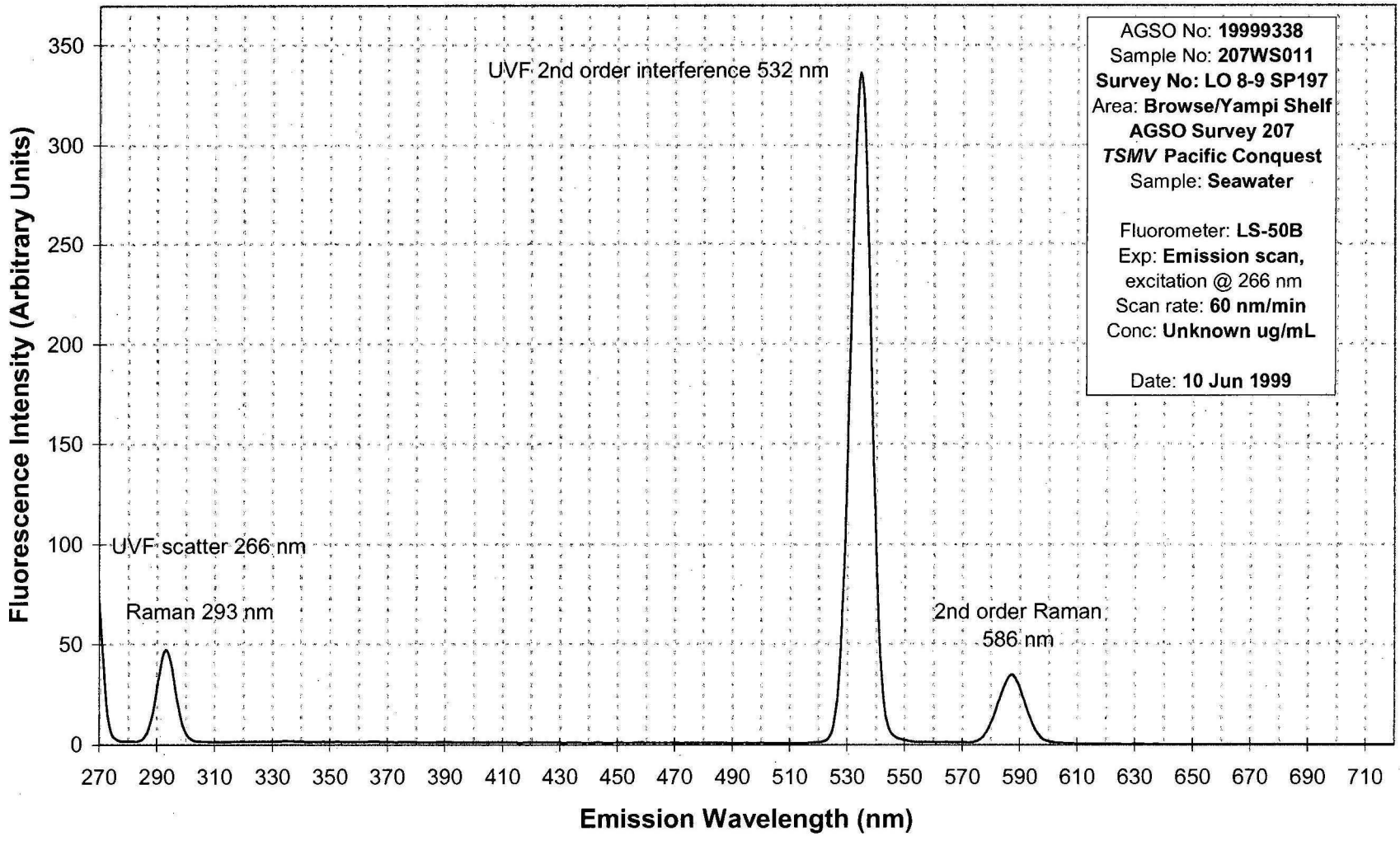
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



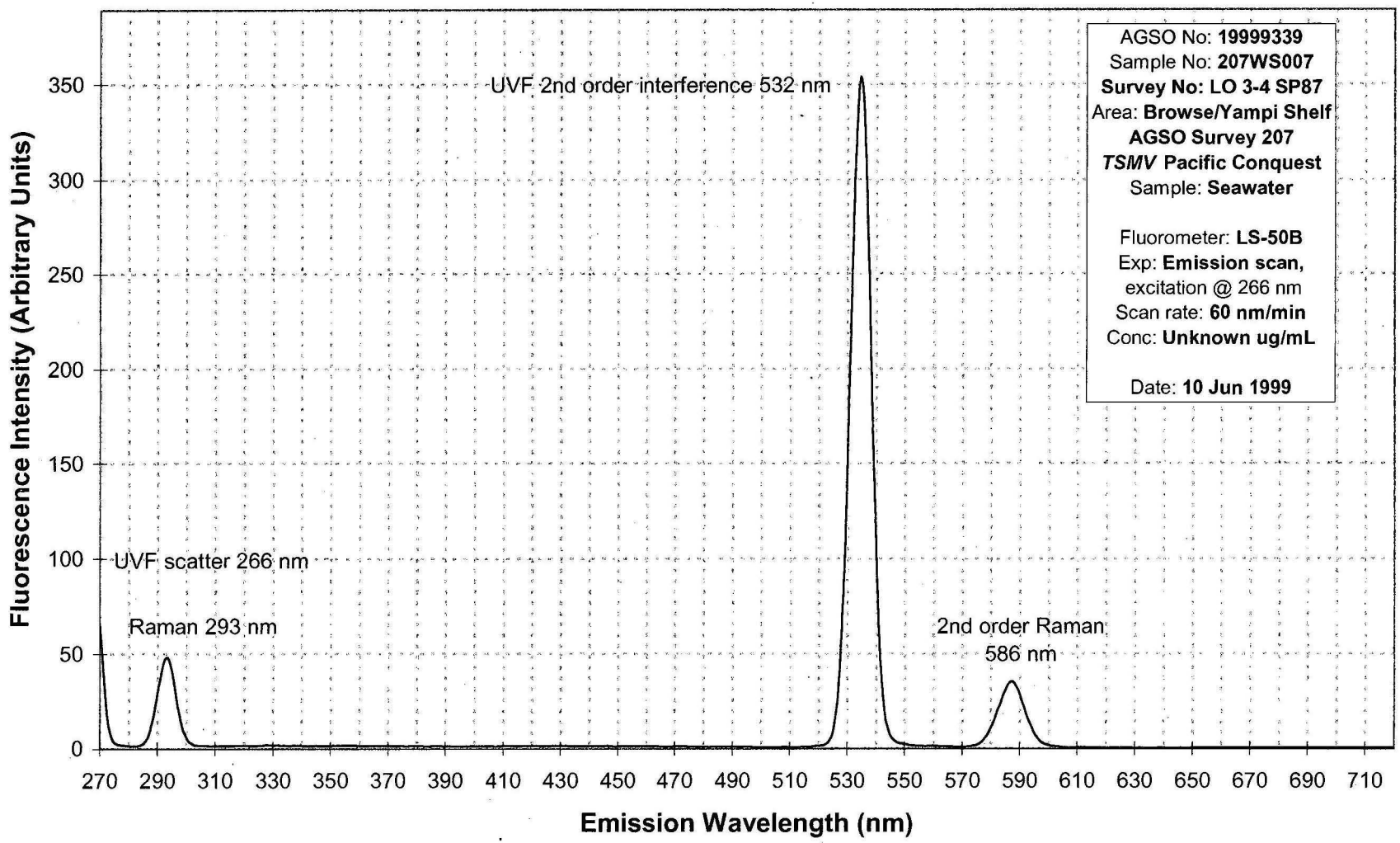
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



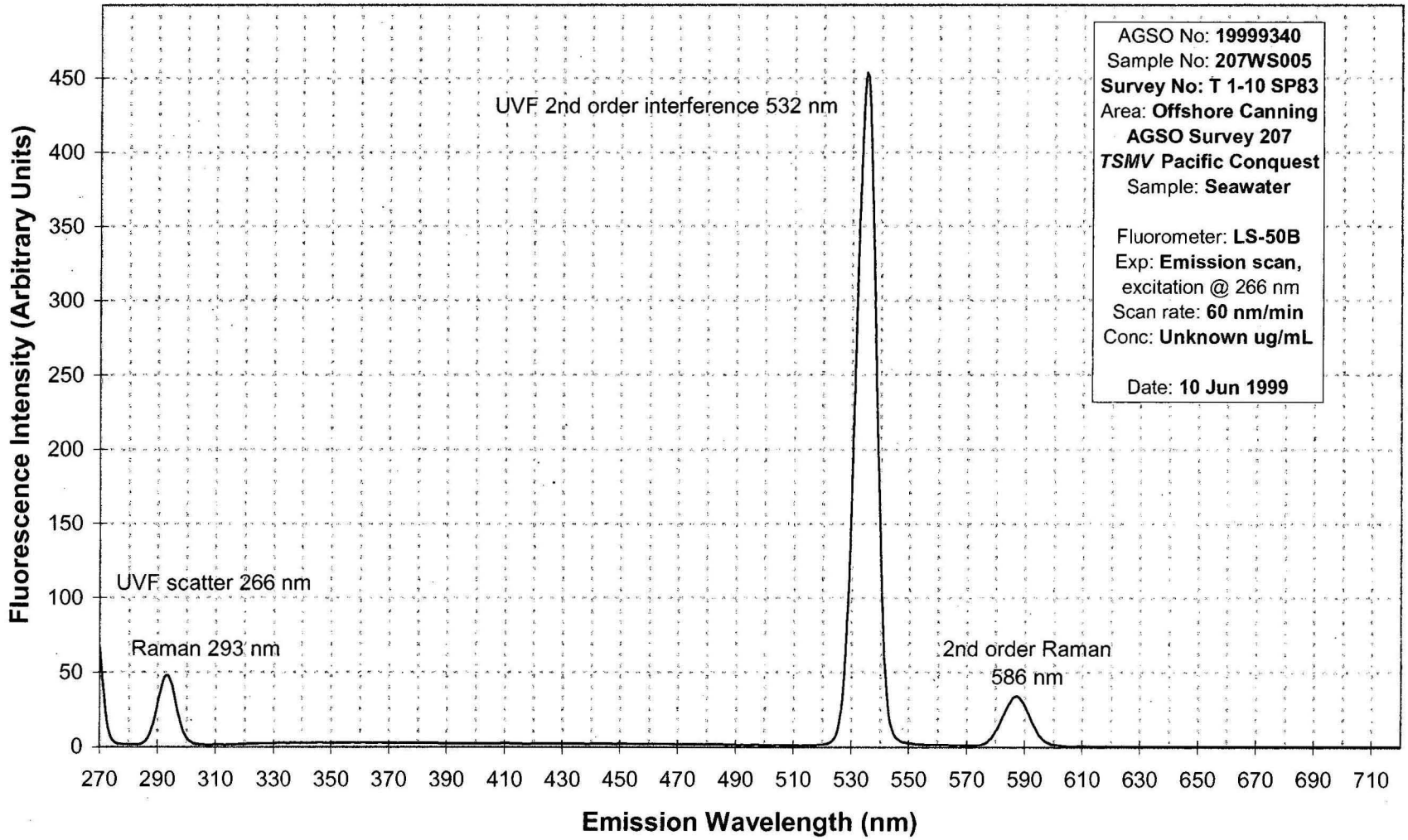
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



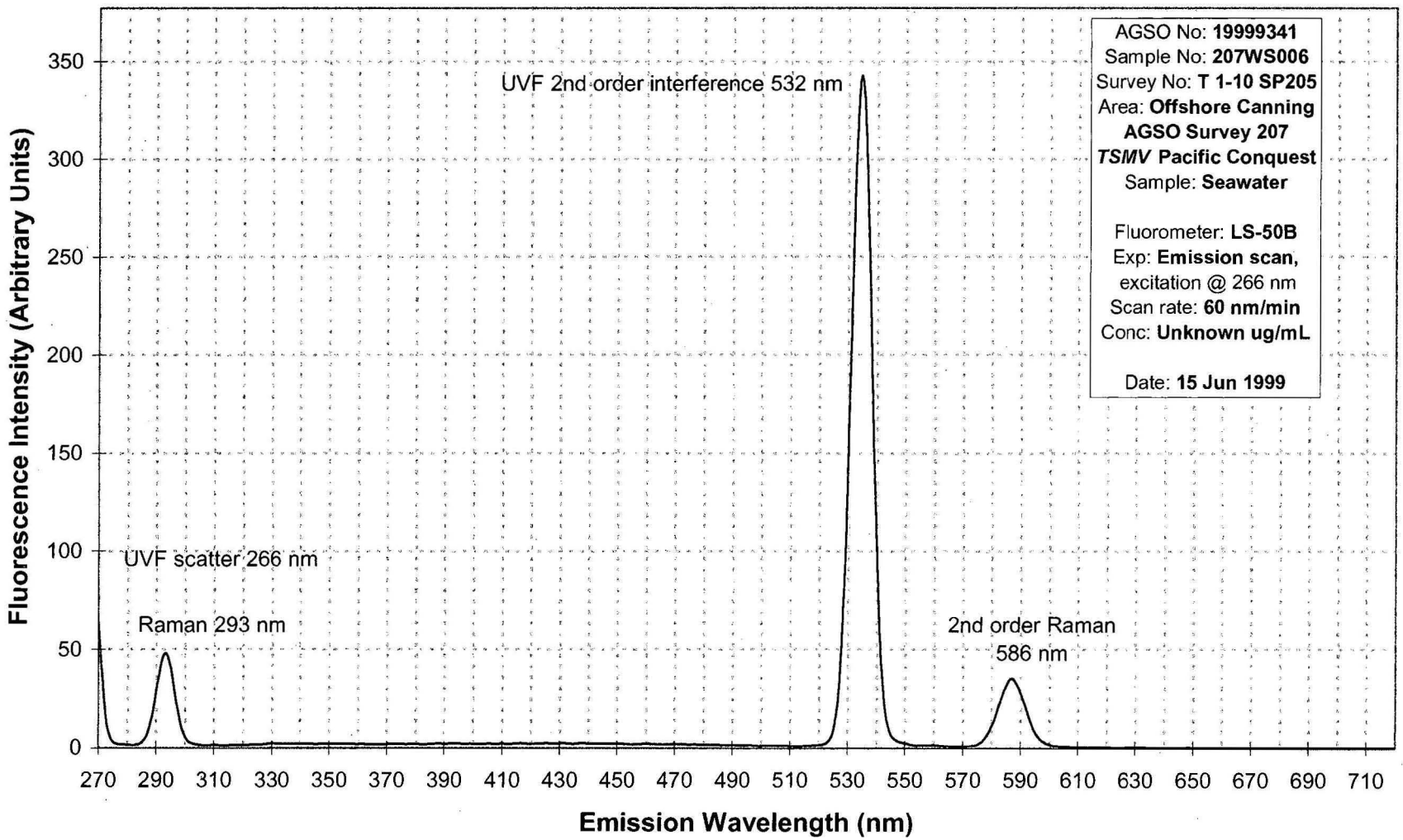
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



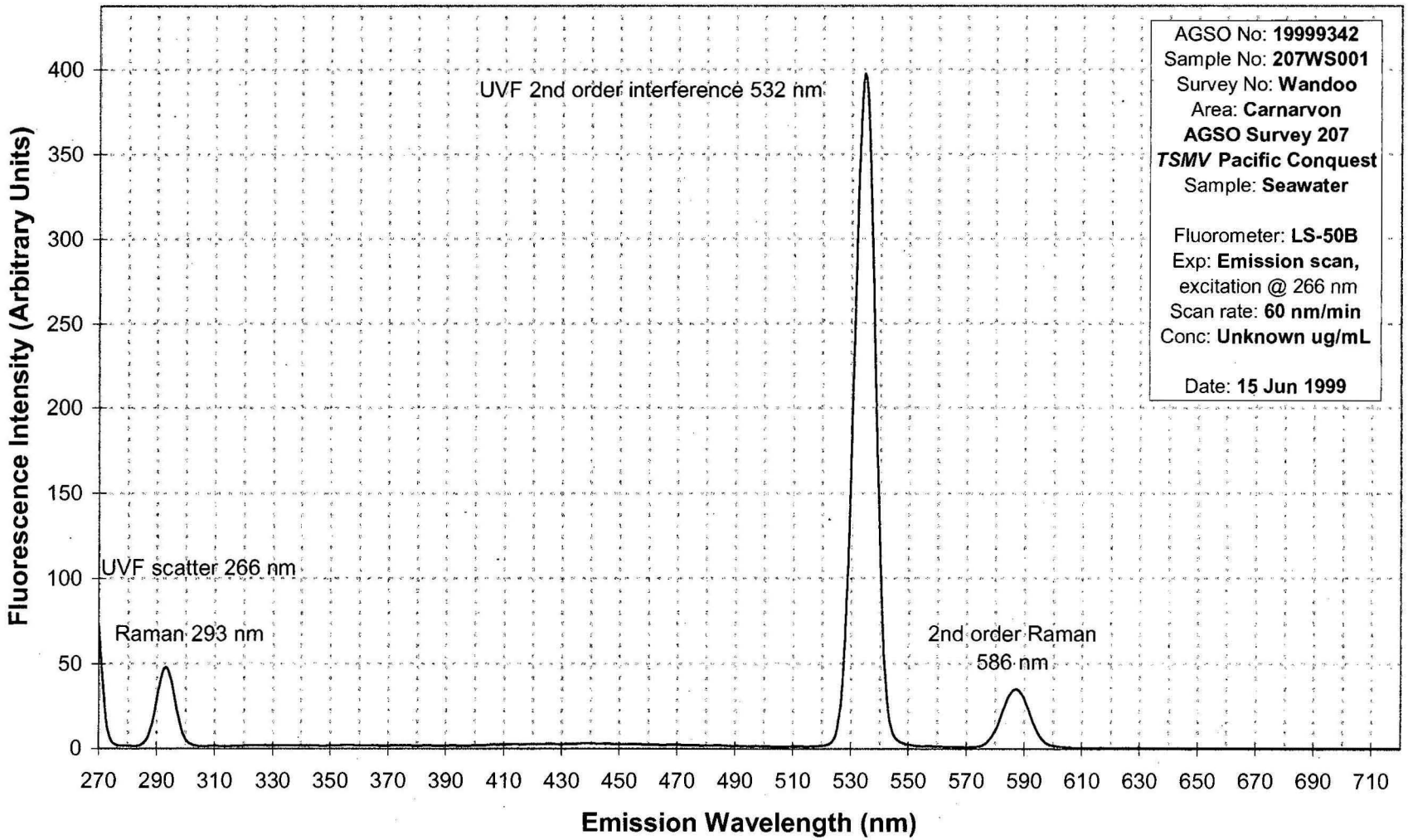
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



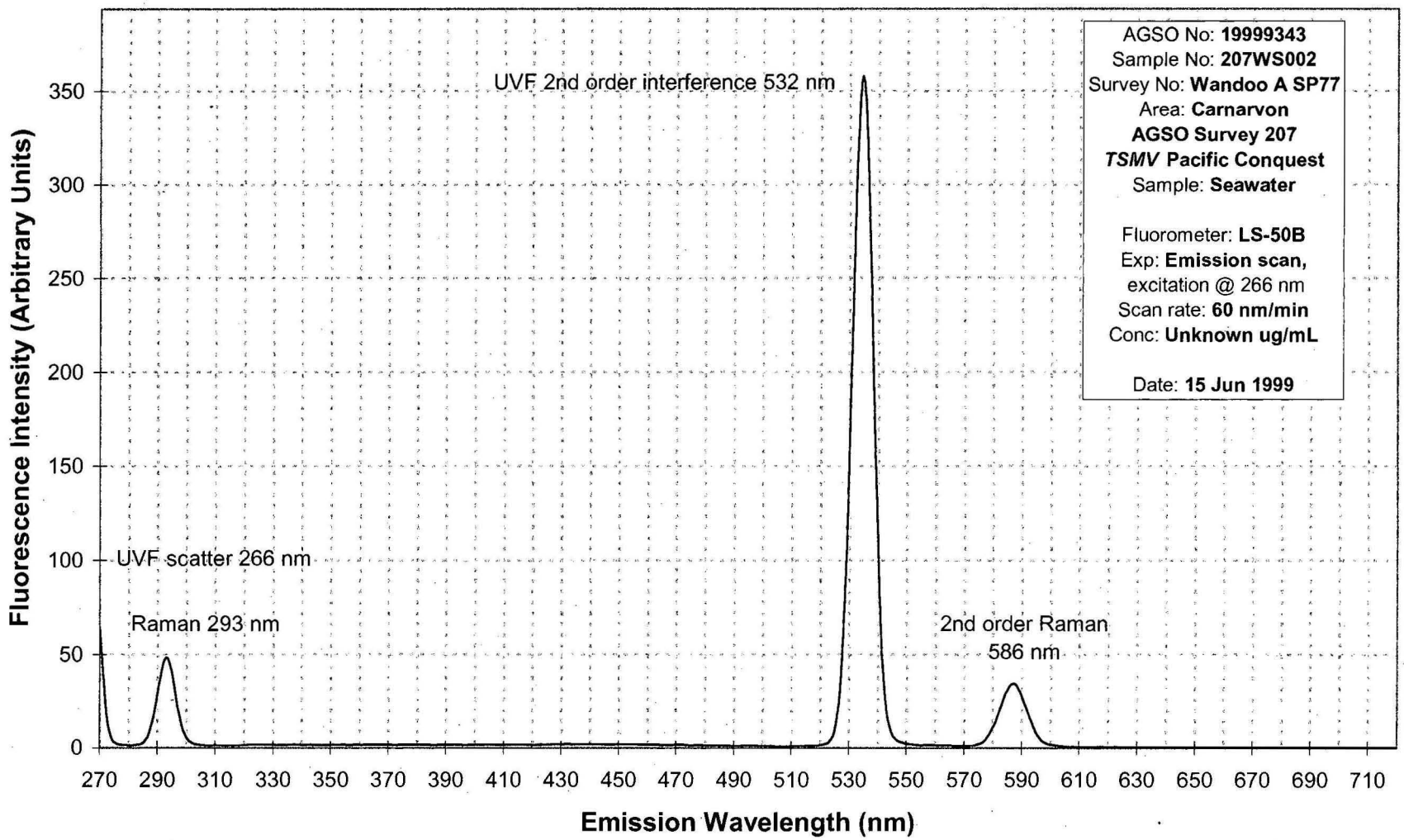
UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



UV Fluorescence Spectrum (emission scan, ex @ 266 nm)



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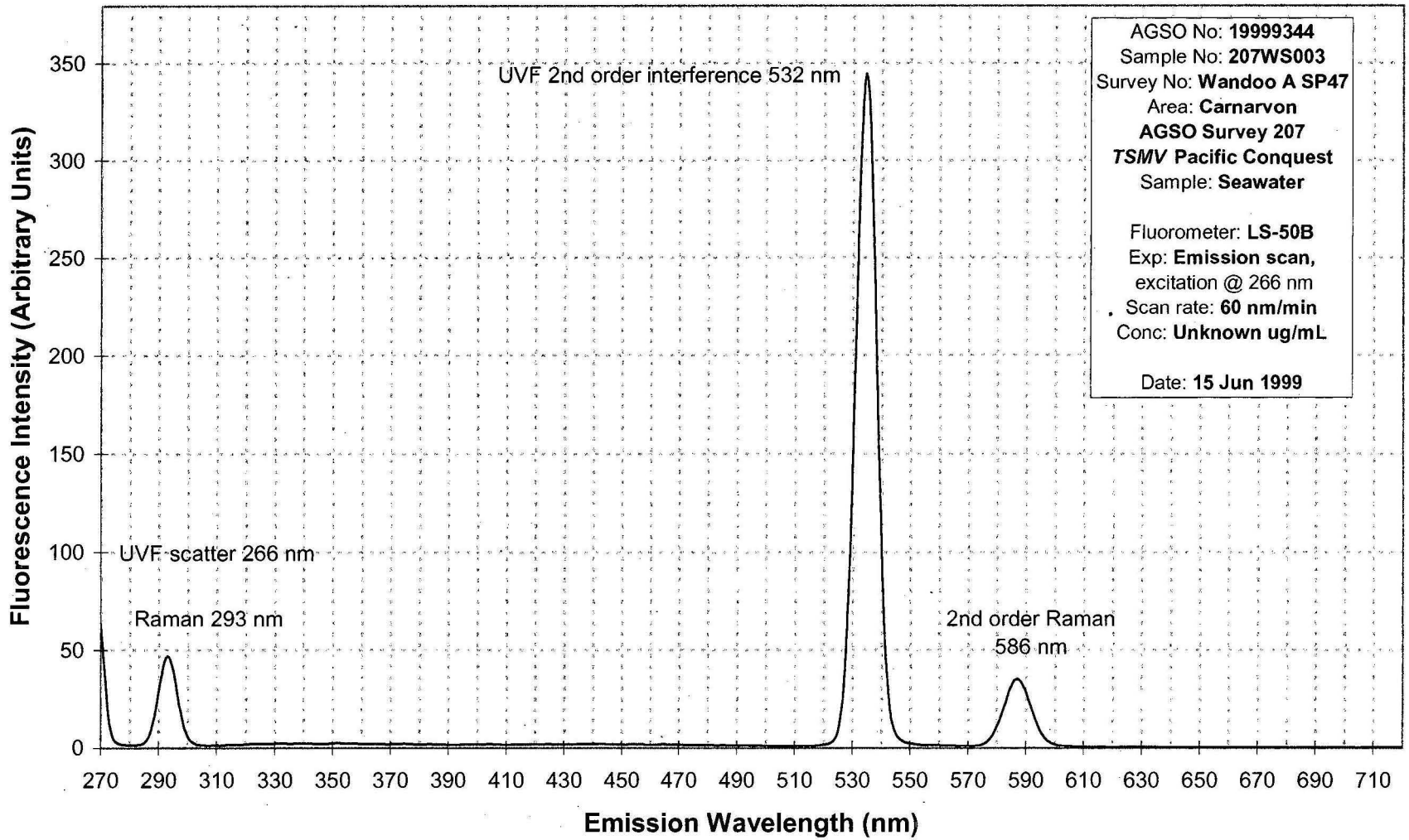

UV Fluorescence Spectrum (emission scan, ex @ 266 nm)