

Views of the Laboratories of Dr. J. William Louda; **An annotated tour:**

The laboratories are located in **rooms 302, 303 and 318A** of the Physical Sciences Building on the north side of the main campus (Boca Raton) (see finger on **Figure 1 left** below) of Florida Atlantic University.

Note the proximity to the Atlantic Ocean, Lake Okeechobee, the Everglades, Florida Bay and the Gulf of Mexico. Recently, we have initiated a working relationship with the Cape Eleuthera Institute and Island School on the beautiful island of Eleuthera in the Bahamas (Fig. 1-right). This is a great place for marine, hypersaline and climate change studies.

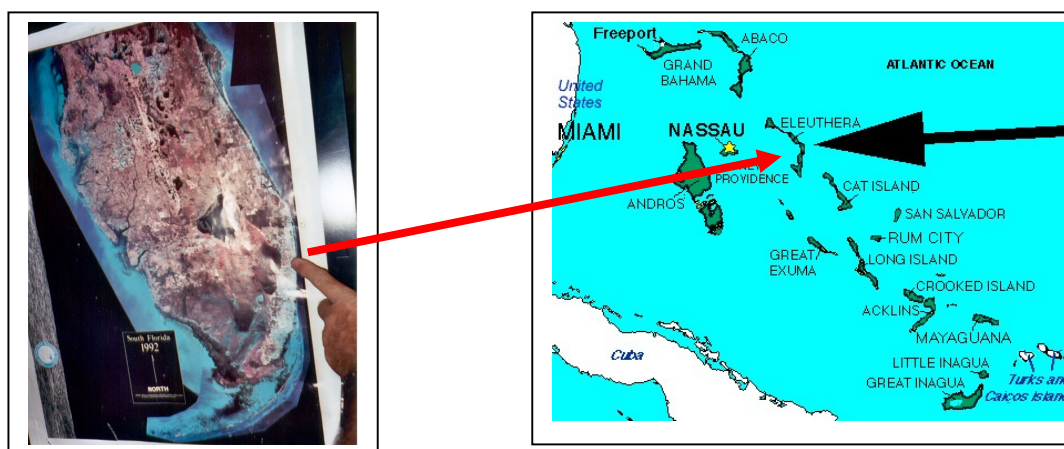


Fig. 1: Satellite view of southern Florida (ex. SFWMD) and Bahamas Map

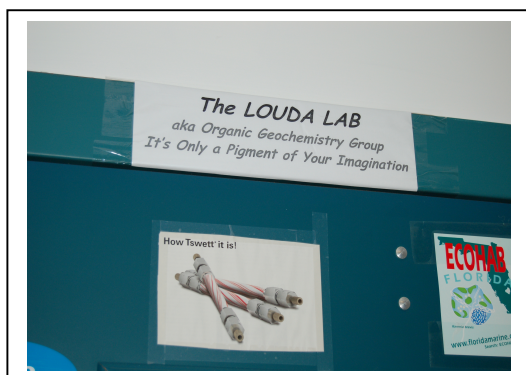


Fig. 2: Entrance to Louda Lab.



Fig. 3: Inside door to the right.

Shown here in **Figure 2** is the entrance to the main lab. Notices to upcoming environmental, limnological and oceanographic conferences, seminars and the-like are also posted here.

Just inside and to the right (Fig. 3) is an office area for students while in lab (individual offices are close but are not in a lab atmosphere). This has plus 1 of 3 PC stations. Note; Lots of reference texts. Web-based literature searches (SUS links, SciFinder etc.), structure drawing (ChemDraw) and other software (Word, Excel, Power Point, Adobe) are available on the FAU website.

Turning to the left in the area shown in Figure 3, we start down one side of the middle bench (Figure 4a). To the left of this path (Fig.4b) is a low-pressure high-performance liquid chromatograph (**LPHPLC**) where mg amounts of sample can be isolated and collected. The LP-HPLC system consists of Ace-Glass Michael-Miller (low pressure: $P < 200$ psig) columns and a ternary solvent gradient pump. We use a micro-cuvette in a digital Spectronic-20 colorimeter interfaced to Peak-Simple software for data acquisition. **Four** high-performance liquid chromatographs (**HPLC**) **systems** are on both the left (Fig. 4b) and right (Fig. 4c) of this pathway. These are Waters two 990 and two 996 systems and all are photodiode array detection (PDA) also called diode array detectors (DAD). These HPLC systems use ThermoSeparations Products Mdl. P4000 quaternary HPLC pumps for ternary gradient plus storage solvent control. The HPLC-PDA systems use an octyldecylsilane (ODS, C-18) column for complete pigment analyses or an octyl (C-8) column for the separation of the divinyl analogs of chlorophylls-*a* and -*b*, so important in the identification / quantification of members of the Prochlorophyta. These systems provide full spectral (190 – 800nm, 330-800nm typical) data acquisition. It is the 2-dimensional data (retention time plus absorption spectrum) which provides the basis for the identification of the chlorophylls, chlorophyll derivatives and carotenoids that we analyze.

Standardization (QA/QC) of such systems requires a great number of known pigments. Our laboratory has accumulated many knowns about 18 of which could be obtained by purchase or as gifts. Numerous others (>100) were generated by partial syntheses and derivations (See Louda *et al.* 2002, 2015: in my *vita*), a process which continues to this day.

Also on the right side is a Perkin-Elmer Lambda-2 **UV/Vis spectrophotometer** with Scantraq aftermarket software. . UV/Vis forms the initial analysis of any pigment extract. Additionally, we use UV/Vis to adjust injectate volumes prior to HPLC in order to not overload the analytical capability of our columns. Pigment quantitation relies on the Beer-Lambert relationship ($A = \epsilon lc$, where A = absorptivity {in AU = absorbance Units}, ϵ = the extinction coefficient of a particular pigment, l = the path length through the sample {1 cm usually}, and c = the concentration of the pigment).

At the end of this path is the Perkin-Elmer LS-50B **fluorescence / luminescence spectrophotometer**. Analytical (± 0.1 mg) and top loading (± 1 mg) balances are also just out of sight in Fig. 4c.



Figure 4a:



Figure 4b:



Figure 4c:

Figure 5a is a closer view of the PE Lambda-LS50B and one of the two Waters 990 HPLC systems.

Turning left at the end above (Fig.5b) the Perkin-Elmer Lambda-40 **UV/Vis spectrophotometer** with WinLab software. This is used in similar fashion to the Lambda-2 discussed above. The rotary evaporation station is also along this path (Fig. 5c).

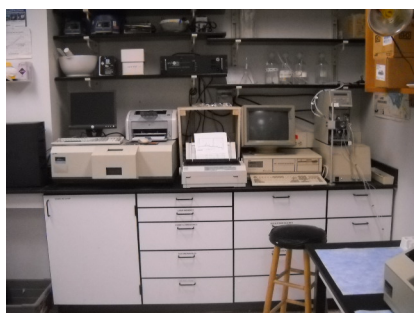


Figure 5a:



Figure 5b:



Figure 5c:

Proceeding up the other side of the middle bench, we come to the fume hood (Figure 6a) used primarily for 'wet chemistry'. That is, we routinely need to generate known compounds for comparison the myriad of unknown carotenoids and chlorophyll-derivatives encountered in our studies, both environmental and geochemical. Thus, this is where partial syntheses and numerous derivatizations (reductions, oxidations {not all "planned" unfortunately ☹}, esterifications, deesterifications, *etc.*) occur. In addition, it is highly important that all extractions are performed in the fume hood as one component of extraction cocktail is dimethylformamide, a known liver toxin. Gloves and goggles are mandatory as well! Beyond the fume hood in Figure 6a is the 'dishwashing' area (sink) and several benchtop centrifuges for various tube sizes. In Figure 6b an open bench for water sample filtrations, preparations and other procedures not requiring work in the fume hood.



Figure 6a:



Figure 6b:

Pigments are 'pigments' to man's eyes because they absorb light in certain wavelengths and we see what is not absorbed. Thus, we work in very dim yellow light to ensure against unwanted photo-oxidation and potential isomerizations. Light, heat and temperature – only 1 allowed at any time!

As just stated above, we work in very dim YELLOW light. Why yellow? Glad you asked, and I actually have an answer for you. Below, in the top half of Figure 7, are the spectra of chlorophylls-*a* and -*b* and a 'generalized' carotenoid. The bottom half of this figure is an 'action spectrum' for algal photosynthesis. As the black bar indicates, there is a minimum in light absorption in the yellow range (also why we buy "bug lights" to work on the front porch at night, especially in the South). The phycobiliproteins do however absorb in this area but are more stable in this regard.

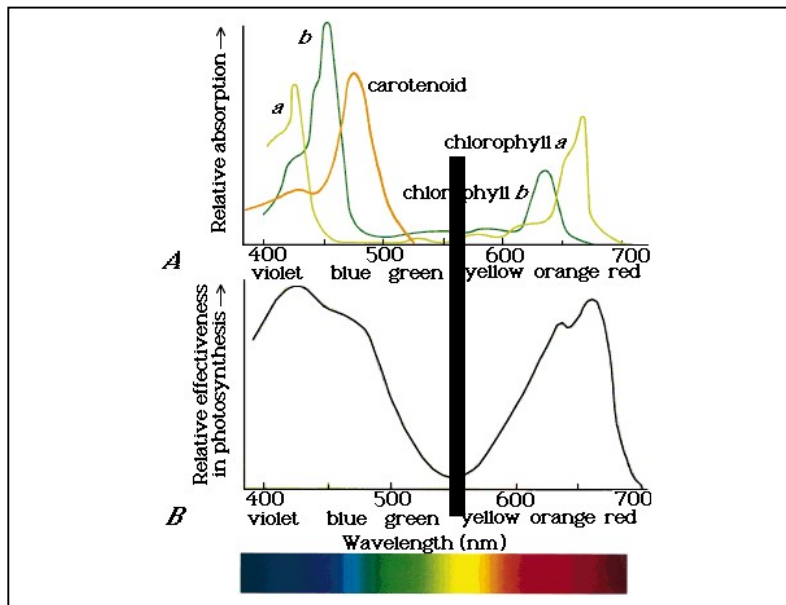


Fig. 7: Why we work with chlorophylls and carotenoids under yellow light.

Leaving the main / wet lab, we travel 8 feet across the hall to lab # PS-303 and find the **nutrient analysis / microscopy lab**. The HACH DR-5000 **spectrophotometer** (Figure 8a) is utilized for water (nutrients, COD, etc.) analyses. This includes macro-nutrients (N as ammonia, nitrite, nitrate; P as SRP {Soluble Reactive Phosphorous} and acid-hydrolyzable P, both as ortho-phosphate, certain micro-nutrients (iron, silicate, sulfur {sulfate}*) and selected bulk parameters (COD {Chemical Oxygen Demand}). Sulfate is also important in providing an oxidant as waters become suboxic to anoxic. As the HACH DR-5000 has certain lower limits of detection, samples with very low amounts of nutrients are analyzed by classic 'micro-cuvette' methods. All of these analyses are put through rigorous quality assurance / quality control (QA/QC) procedures using NIST-traceable standards. Additional nutrient testing / validation is available at the FAU-Davie campus in the Geosciences water analysis lab using a SEAL AQ2 Discrete Analyzer. Also present in that lab are a J.Y. Horiba Ultima 2 ICP-OES and a Picarro L2120-i water isotope analyzer.

Also seen in both Figures 8a & b is the HACH 2100Q portable **turbidity meter** (red arrow in Fig. 8a). This, along with a Turner Instruments **Field Fluorometer** for chlorophyll-a estimations, a portable **pH meters**, a portable **phosphate colorimeter** (HACH Pocket-II), a LiCor spherical **quantum sensor** / Li-250 light meter for PAR radiation measurement, and a YSI ProODO **dissolved oxygen meter** make up an easily transported set of field instruments.

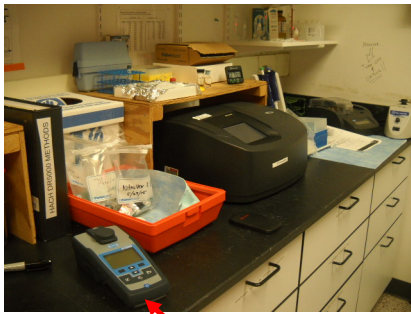


Figure 8a: (Turbidity meter)



Figure 8b:



Figure 8c:

On the other side of this lab are a large sink (not shown) and the **microscopy** station. Here, we find, from left to right in Figure 8c, a small compound microscope capable of digital picture capture, an inverted compound microscope, a fluorescence microscope, a compound microscope and a binocular dissection microscope. Though most cell counts of single celled algae are done with a Coulter Counter we also rely on microscopic exams with a hemocytometer and/or graduated ocular for enumerating (estimating) filamentous forms. Hemocytometer counts are also used to QA/QC the cell counter with single celled organisms. The fluorescence microscope is very handy for the extremely small forms such as *Synechococcus elongatus* and 'friends' in the picoplankton.

Microalgal culture facility (PS-304) One thing that is needed during the culture of microalgae (viz. phytoplankton) is a way to monitor growth. To that end we use several methods. Above we mentioned microscopic methods using a hemocytometer

and Sedwick-Rafter cells. We also use fluorescence as a measure of chlorophyll-a, a proxy for biomass. We use the **Countess-II FL cell counter** (Fig. 9a) for exacting 'routine' measuring of cell growth (viz. divisions). Algae (phytoplankton) are grown in nutrient media specific to the genus / species and are grown in various ways. Laboratory water bath rotating (e.g. 120 rpm) incubators that hold 12 each 125mL or 6 each 250 mL baffled culture flasks maintain a constant temperature and are set for a certain diel light/dark cycle (e.g. 12L:12D, 16L:8D etc.). Such setups are shown here as Figures 9b & c. Experimentation with such parameters as light intensity / duration, nutrient stress and "ocean acidification" (carbon dioxide concentration in the air that the culture is exposed to) also take place in these setups.



Figure 9a:



Figure 9b:

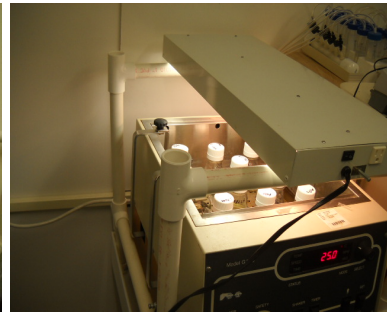


Figure 9c:

Routine "stock" cultures are maintained in cabinet style incubators such as shown in Figure 10a. Certain 'low light' experiments are also performed in similar incubators and these are also used for the culture of sedentary 'mat-forming' algae and cyanobacteria.

When we start senescence / death incubations in order to follow pigment degradation over time in various conditions, we need much larger starting materials. In these, cases we batch culture the phytoplankton in 2.5 (10L) or 5.0 (20L) gallon carboys as shown in Figure 10c. These are stirred magnetically and air is bubbled through with stainless steel frits, both of gentle agitations keep the algae in suspension and, on a time-integrated basis, exposed to the same light / gases conditions.



Figure 10a:



Figure 10b:

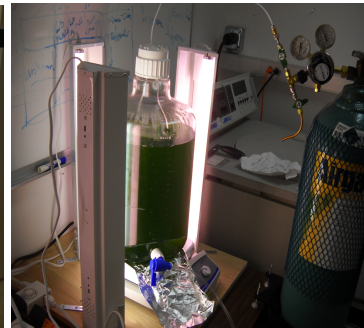


Figure 10c:

HARMFUL ALGAL BLOOM STUDIES

Microcystis aeruginosa is the toxic cyanobacterium that causes Harmful Algal Blooms (HABs) here in Florida and across the World.

Since *Microcystis aeruginosa* is a Bio-Safety Level Class-2 (BSL-2) organism, we need special safe ways to handle this organism. Here, we use the Baker SterilGARD-III Advance Biosafety Cabinet shown in Figure 10d. Handling of *M. aeruginosa* and other live organisms (microalgae) occurs in that cabinet. That is, inoculations of cultures, subsampling of cultures to load counting slides, making microscopy slides and other organism manipulations occur only inside this cabinet. The Sentry Air Systems cabinet (Figure 10e) is used for preparing and storing sterile apparatus including flasks and for sterile filtering of media. No organism are allowed in this cabinet.



Figure 10d: Baker Biosafety Cabinet.



Figure 10e: View of biosafety working area. Smaller unit to left is the Sentry Air Systems sterile chamber with the Biosafety Cabinet to the right.

Lately (2021-present) we have been growing *M. aeruginosa* with the stable isotopes C13 and N15. This allows use to 'biosynthesize' their toxins, notably microcystin-LR, with carbon and/or nitrogen atoms replaced with the heavier isotope. This then allow us to follow the toxin in other studies, such as with unlabeled *M. aeruginosa* or in mice. Identification uses Confocal microscopic RAMAN spectroscopy here at FAU and nano-secondary ion mass spectrometry (Nano-SIMS) at the national Lawrence-Livermore Laboratory in California.

In addition to instrumentation / facilities directly in the Louda Environmental Biogeochemistry labs, the Department maintains a variety of common 'shared' instrumentation also available for use on our projects.

As part of our analytical core facilities, we have three (3) Gas Chromatograph – Mass Spectrometers (**GC-MS**) as seen in Figures 11a-c.

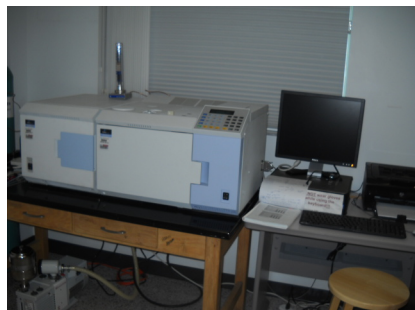


Figure 11a:

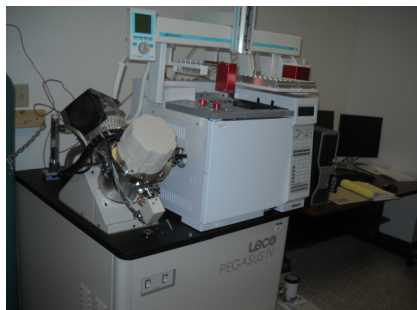


Figure 11b:



Figure 11c:

In Figure 11a is the Perkin-Elmer Autosystem GC-MS (EI mode). Figure 11b contains the LECO auto-sampling GC-TOF (Time of Flight) mass spectrometer which gives better molecular ion information as opposed to the higher fragmentation in EI mode. Lastly, we also have a Perkin-Elmer Clarus GC-FID (Flame Ionization Detection) as shown in Figure 11c. My lab's use of GC-MS deals mainly with indicators of anthropogenic (man's) pollution of various water bodies. This includes but is not limited to fecal sterols such as coprostanol.

As we work with photosynthetic pigments, chlorophylls and carotenoids, which are not amenable to GC analyses, having access to the department's Liquid Chromatograph-Mass Spectrometers (**LC-MS**) is important. Figure 12a shows the Thermo-Finnigan LCQ-Deca and Figure 12b has the Thermo-Finnigan LTQ, both of which can operate in either ESI (ElectroSpray Ionization) and APCI (Atmospheric Pressure Chemical Ionization) ionization modes. We use the APCI mode for our pigment studies. Both instruments are also capable of introducing samples by "infusion" mode or, much more interesting for our pigment studies, in HPLC-PDA-MS mode with an auto-sampler. That is, Samples can be separated using a quaternary HPLC pump, eluates run through the PDA and MS in sequence. Each peak then is characterized by (1) retention time, (2) UV/Vis spectrum and (3) mass spectrum.



Figure 12a:

Figure 12b:

Additional shared instruments include a Perkin-Elmer Lambda-900 **UV/Vis/NIR** spectrophotometer (Fig. 14a) and an ABI Voyager DE-STR Matrix Assisted Laser Desorption Ionization-Time Of Flight (**MALDI-TOF**) mass spectrometers (Figure 14b). The MALDI-TOF is used to obtain parent molecular weights of pigments. As a matrix for chlorophyll and chlorophyll derivative investigations, we use elemental sulfur (S_8 / fw = 256 amu) / carbon disulfide in order to not have matrix interference with the pigments.

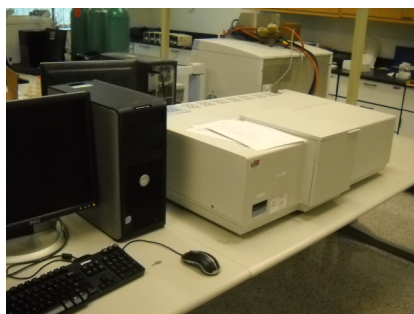


Figure 14a:



Figure 14b:

The MS/NMR core facility contains three nuclear magnetic resonance spectrometers (NMR). These are the 400MHz (Figure 15a) and 500 MHz (Figure 15b) Varian instruments shown below. In my studies, these are used to verify structures of hemisynthetic standard pigments and to fully characterize novel (unknown) pigments from natural samples. The third and newest 400MHz is just being installed and is a Bruker unit.



Figure 15a: 400 MHz NMR



Figure 15b: 500 MHz NMR

Also extremely handy in pigment studies are the Jasco Fourier Transform Infrared (**FTIR**: Fig. 16a) and Jasco circular dichroism (**CD**: Fig. 16b) spectrometers.



Figure 16a:

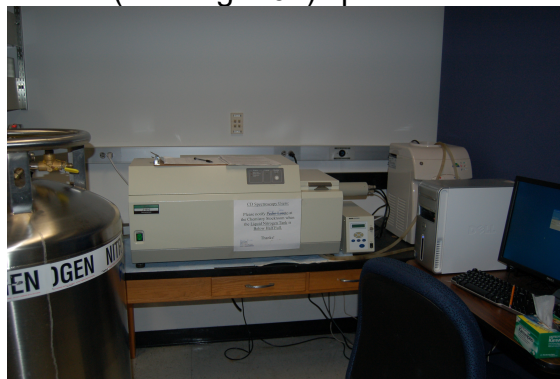
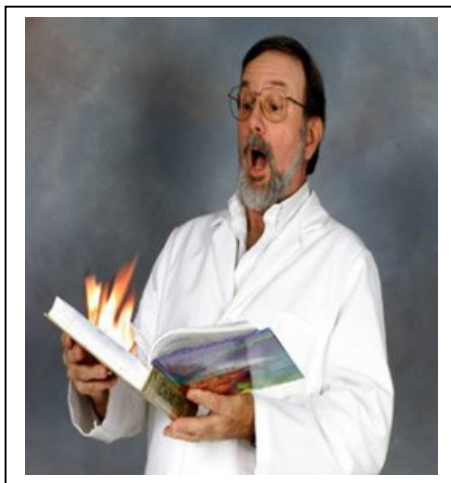


Figure 16b:

I hope that you have enjoyed the pictorial tour of my lab and the departmental facilities which are available to all faculty and their students. I will be very glad to give personal guided tours and to discuss joint research projects including, most importantly to the University's mission, those with prospective graduate students.



Always remember – read slowly or this could happen!

Cheers, Dr. J. William Louda