Results of HPLC derived photopigment concentrations for bioassays done in the North Inlet Estuary - Georgetown, South Carolina during 2014 (Photomixotrophy project)

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Project
» Assimilation rates of dissolved organic carbon by photomixotrophic estuarine phytoplankton (Photomixotrophy)

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Abstract
Results of HPLC derived photopigment concentrations for bioassays done in the North Inlet Estuary - Georgetown, South Carolina during 2014 (Photomixotrophy project)

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Coverage

Spatial Extent: **N**:33.452783 **E**:-79.071852 **S**:33.091684 **W**:-79.318359
Temporal Extent: 2014 - 2014

Dataset Description

Results of HPLC – derived photopigment concentrations for bioassays.

Methods & Sampling

Bioassays

Bioassays consisted of 4 treatments incubated at 3 irradiance exposure levels (Table 2). Treatments were composed of 1) addition of 1 umol 14C-glucose (specific activity 200 mCi/mmol) for a final activity of 0.26 uCi ml-1, 2) addition of 1 umol 14C-glucose and 20 uM DCMU, 3) addition of H14CO3-, and 4) Control (no additions). Each treatment has 5 replicates. Bottles with the 4 treatments will be incubated at 3 irradiance levels, 75% of the surface irradiance, 25% of the surface irradiance, and complete darkness. Thus the bioassay used 60 (12 treatments x 5 replicates) clear polycarbonate flasks (Nalgene, 2.0 l), filled with ambient estuary water at high tide. Flasks will be placed in a floating corrals covered with neutral density screen to simulate 75% and 25% of the water surface solar irradiance. The dark samples were incubated in opaque

flasks. Samples were incubated for 24 h (sunrise to sunrise) to allow turnover of pigment pools and measure "daily" net primary productivity. After the incubation, aliquots (250 - 500 ml) of the incubation water were filtered under a gentle vacuum (<50 KPa) through glass fiber filters (25 mm dia. Whatman GF/F), immediately frozen, and stored at -80deg C.

Analytical Methods

Phytoplankton community composition, based on biomarker photopigment concentrations, were determined for all bioassay incubations (Millie et al. 1993, Jeffrey et al. 1997, Wright & Jeffrey 2006). Pigment concentrations were analyzed using ChemTax to determine the relative abundance of major phytoplankton groups (Mackey et al. 1996, Pinckney et al. 2001, Lewitus et al. 2005). The initial pigment ratio matrix used for this analysis was derived from Lewitus et al. (2005), which was based on empirically measured pigment ratios for North Inlet estuarine phytoplankton. The convergence procedure outlined by Latasa (2007) was used to minimize errors in algal group biomass due to inaccurate pigment ratio seed values. In addition, 20 ml of the incubation water was preserved with 5% Lugol's solution and archived for later qualitative microscopy for comparison with the ChemTax results.

Filters were lyophilized for 18-24 hours at -50 deg C. Photopigments were extracted by adding 750 ul of 90% aqueous acetone solvent followed by storage for 12-20 hours at -20deg C. Filtered extracts (250 ul) were injected into a Shimadzu HPLC with a single monomeric column (Rainin Microsorb, 0.46 × 1.5 cm, 3 um packing) and a polymeric (Vydac 201TP54, 0.46× 25 cm, 5 um packing) reverse-phase C18 column in series. A non-linear binary gradient consisting of solvent A (80% methanol : 20% 0.5 M ammonium acetate) and solvent B (80% methanol : 20% acetone) was used for the mobile phase (Pinckney et al. 1996, Hooker et al. 2010). Absorption spectra and chromatograms (440 +/- 4 nm) was obtained using a Shimadzu SPD-M10av photodiode array detector and pigment peaks were identified by comparing retention times and absorption spectra with pure standards (DHI, Denmark). The synthetic carotenoid β-apo-8'-carotenal (Sigma) was used as an internal standard. The 14C-specific activity of chl a was measured using an in-line flow scintillation counter (Packard Radiomatic 525a, 500 ul counting cell) placed downstream from the photodiode array detector. Radioactivity was quantified after automatic in-line mixing of a low-viscosity scintillation cocktail (Packard Ultima-Flo M) with HPLC eluant (3:1 mixing ratio). Radiograms were converted to disintegrations per minute (dpm) after accounting for variable flow rates, counting cell volume, mixing rates, and counting efficiency (quench) (Pinckney et al. 1996).

Data Processing Description

Methodology from Pickney, J.L.: The USC HPLC Method - Technical Description.pdf:
www.researchgate.net/profile/James_Pinckney/publication/265999187_The_USC_HPLC_Method_-Technical_Description/links/55266d0d0cf2628d5afdf397/The-USC-HPLC-Method-Technical-Description.pdf

1. INTRODUCTION

The HPLC method used at USC for photopigment separations is derived from the Van Heukelem et al. (1992, 1994) and Pinckney et al. (1996) protocols. Two different reverse-phase C18 columns are connected in series. A single monomeric guard column is followed by a monomeric reverse-phase C18 column and a polymeric reverse-phase C18 column. This column configuration was originally devised to enhance photopigment separations from sediment samples containing numerous (>150) photopigment and pigment degradation products. Monomeric columns provide strong retention and high efficiency while polymeric columns select for similar compounds with minor differences in molecular structure (Van Heukelem et al. 1992, Jeffrey et al. 1997). In addition to increasing the number of theoretical plates, the combination of both monomeric and polymeric columns optimizes photopigment separations based on two different molecular properties (coarse and fine structure). This method allows for the baseline separation of most major pigments including lutein/zeaxanthin and chlorophyll c3. However, chlorophylls c1 and c2 are not completely separated. Divinyl chlorophylls a an b are not completely resolved but occur as shoulders on the monovinyl chlorophylls a and b and can be visually identified in chromatograms.

2. EXTRACTION

SeaHARRE 4 samples were immediately frozen at -80 deg C upon receipt. For HPLC analysis, filters were placed in disposable polypropylene microfuge tubes (2 ml) and lyophilized (-50 deg C, 0.57 mbar, 12 h; Labconco FreeZone 2.5) to remove all water from the filters. After lyophilization, filters were cut into 6 equal sections and placed in microfuge tubes. Samples were extracted in 90% acetone (600 ul), and stored at -20 deg C for 18 - 20 h. Each sample also received 50 ul of the synthetic carotenoid trans β-apo-8'-carotenal

(Sigma, cat. no. 10810) in 90% acetone as an internal standard using a gas-tight syringe (Hamilton) and click dispenser (Hamilton PB600-1). After extraction, the extract was clarified using a 0.45 um PTFE filter (Gelman Acrodisc). A known volume of the extract (400 ul) was then dispensed into amber glass autosampler vials (2.0 ml) and sealed with PTFE-silicone caps.

3. HPLC ANALYSIS

The instrumentation consisted of a binary gradient pump (Shimadzu dual LC10-AT vp and Controller SCL-10A vp), temperature-controlled autosampler (Shimadzu SIL10-A vp) with a 500 ul injection loop, column oven (Shimadzu CTO-10AS vp), and photodiode array detector (PDA, Shimadzu SPD-M10A vp; 200 to 800 nm range). For the PDA, spectra (380 - 700 nm) were obtained at 2.00 sec intervals for the duration of each run and photopigment peaks were quantified at 440 nm (± 4 nm). Two different reverse-phase C18 columns were connected in series. A single monomeric guard column (Rainin Microsorb, 0.46 x 1.5 cm, 3 um packing) was followed by a monomeric reverse-phase C18 column (Varian Microsorb-MV 100 - 3, 0.46 x 10 cm, 3 um packing) and a polymeric reverse-phase C18 column (Vydac 201TP54, 0.46 x 25 cm, 5 um packing). The column oven maintained a constant 40 deg C for the duration of the gradient. A non-linear binary gradient, adapted from Van Heukelem et al. (1992), was used for pigment separations (Table 1). Solvent A consists of 80% methanol : 20% ammonium acetate (0.5 M adjusted to pH 7.2) and solvent B is composed of 80% methanol : 20% acetone (Table 1). Solvents were degassed with an in-line degasser (Shimadzu DGU 14A). All solvents were HPLC-grade and chemicals were analytical grade.

Just prior to the HPLC run, an ion-pairing (IP) solution (1.00 M ammonium acetate) was added to the vial in a ratio of 4 parts extract: 1 part ammonium acetate. Prior work has shown that there is negligible pigment degradation within 12 hours of adding the IP solution if the sample is placed in a refrigerated autosampler rack (4.0 deg C). However, the IP solution should not be added to the sample if the time until sample analysis is greater than 18 hours.

4. CALIBRATION

Peaks were identified based on retention time and spectral matches with pigment spectra obtained from liquid standards (DHI, Hørsholm, Denmark) (Table 2). Peak areas for chromatograms are quantified using Shimadzu Client/Server 7.2.1 SP1 software. The PDA was calibrated using a multi-point calibration procedure for a range of injection volumes (25 - 300 ul) of pigment standards (DHI, Denmark). Regressions were performed using known pigment concentration (Y) vs. the integrated peak area (X) and were of the form $Y = mX + b$; where m is the slope and b is the Y intercept. All regressions had an $r2 > 0.98$. The slope of the fitted line was used as the response factor for pigment concentration calculations. The concentrations of pigments for which standards were unavailable were estimated using the ratio method outlined in Jeffrey et al. (1997, p. 443-4)

5. VALIDATION

Carotenal blanks (trans β-apo-8'-carotenal in 90% acetone) were run after every 10 samples to verify peak time reproducibility, peak area precision, and instrument performance during the sequence run. Peaks were identified based on retention time and comparison of absorbance spectra with a spectral library derived from pure pigment standards (DHI, Denmark). Long-term quality control is achieved by analyzing pure standards for chlorophyll a and the Mixed Standard supplied by DHI, Denmark at monthly intervals. Instrument performance is measured and compared with previous measures to determine changes in performance metrics. Volumetric measuring devices are checked weekly. The performance metrics for this method are shown in Table 3.

6. DATA PRODUCTS

Pigment concentrations were calculated for each identifiable peak using the following equation:

where CPi is the pigment concentration in ug l-1, APi pigment peak area, FPi is the response factor, Vc is the injection volume (ul), Vm is the total extract volume (volume of added acetone + volume of internal standard in ml), R is the ratio of the volume of IP solution + Vm divided by Vm, Vf is the volume of seawater filtered (liters), Ac is the average peak area for carotenal standards, and As is the peak area of carotenal in the sample.

7. CONCLUSIONS

This method has been employed by USC for ca. 15 years to analyze a broad spectrum of sample types from marine and freshwater habitats. The execution of the method is straightforward and involves minimum manipulation of the samples and extracts, relatively inexpensive, and does not generate hazardous waste products. The primary weakness of the method is the inability to completely separate chlorophylls c1 and c2 and divinyl chlorophylls.

REFERENCES

Jeffrey, S.W., Mantoura, R. F. C., Wright, S. W., [Eds.] 1997. Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods. UNESCO, Paris.

Pinckney, J.L., Millie, D.F., Howe, K.E., Paerl, H.W., Hurley, J.P. 1996. Flow scintillation counting of 14C-labeled microalgal photosynthetic pigments. Journal of Plankton Research 18:1867-1880.

Van Heukelem, L., Lewitus, A.J., Kana, T.M. 1992. High-performance liquid chromatography of phytoplankton pigments using a polymeric reversed-phase C18 column. Journal of Phycology 28:867-872.

Van Heukelem, L., Lewitus, A.J., Kana, T.M., Craft, N.E. 1994. Improved separations of phytoplankton pigments using temperature-controlled high-performance liquid chromatography. Marine Ecology Progress Series 114:303-313.

BCO-DMO Data Processing Notes:

-Reformatted column names to comply with BCO-DMO standards.
-Data were originally separated by month (September and November) into two spreadsheets. Data were combined into one file and the columns "month" and "year" were added.
-Colors and headers were removed.

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Data Files

File
bioassay.csv (Comma Separated Values (.csv), 15.93 KB) MD5:970d2139dae8aef6cad73818c560286d Primary data file for dataset ID 710144

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Related Publications

Heukelem, L., Lewitus, A. J., Kana, T. M., & Craft, N. E. (1992). HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PHYTOPLANKTON PIGMENTS USING A POLYMERIC REVERSED-PHASE C18 COLUMN1. Journal of Phycology, 28(6), 867–872. https://doi.org/10.1111/j.0022-3646.1992.00867.x
Methods

Jeffrey, S. W., Mantoura, R. F. C., Wright, S. W., International Council of Scientific Unions., & Unesco. (1997). Phytoplankton pigments in oceanography: Guidelines to modern methods. Paris: UNESCO Pub
Methods

Pinckney, J. L., Millie, D. F., Howe, K. E., Paerl, H. W., & Hurley, J. P. (1996). Flow scintillation counting of 14C-labeled microalgal photosynthetic pigments. Journal of Plankton Research, 18(10), 1867–1880. doi:10.1093/plankt/18.10.1867
Methods

Van Heukelem, L., Lewitus, A., Kana, T., & Craft, N. (1994). Improved separations of phytoplankton pigments using temperature-controlled high performance liquid chromatography. Marine Ecology Progress Series, 114(3), 303-313. Retrieved from http://www.jstor.org/stable/24849703
Methods

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Parameters

Parameter	Description	Units

Year	Year sample was taken; YYYY	unitless
Month	Month sample was taken	unitless
Number	Sample number	unitless
Sample_Volume	Volume of water filtered	liters
Chl	Chlorophyll c1+c2 concentration	micrograms per liter
Perid	Peridinin concentration	micrograms per liter
ButFuc_19	19' Butanoyloxyfucoxanthin concentration	micrograms per liter
Fuco	Fucoxanthin concentration	micrograms per liter
HexFuc_19	19' Hexanoyloxyfucoxathin concentration	micrograms per liter
Neo	Neoxanthin concentration	micrograms per liter
Prasino	Prasionxanthin concentration	micrograms per liter
Viola	Violaxanthin concentration	micrograms per liter
Diad	Diatoxanthin concentration	micrograms per liter
Anther	Antheraxanthin concentration	micrograms per liter
Allox	Alloxanthin concentration	micrograms per liter
Monado	Monadoxanthin concentration	micrograms per liter
Diat	Diatoxanthin concentration	micrograms per liter
Lutein	Lutein concentration	micrograms per liter
Zeax	Zeaxanthin concentration	micrograms per liter

Gyro	Gyroxanthin concentration	micrograms per liter
Chl_b	Chlorophyll b concentration	micrograms per liter
Croco	Crocoxanthin concentration	micrograms per liter
Chla_Allomer	Chlorophyll a allomer concentration	micrograms per liter
Chl_a	Chlorophyll a concentration	micrograms per liter
Chla_prime	Chlorophyll a prime concentration	micrograms per liter
alpha_Carotene	alpha Carotene concentration	micrograms per liter
beta_Carotene	beta Carotene concentration	micrograms per liter

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Instruments

Dataset-specific Instrument Name	Photodiode array detector (PDA, Shimadzu SPD-M10A vp; 200 to 800 nm range)
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Used in HPLC analysis
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Temperature-controlled autosampler (Shimadzu SIL10-A vp) with a 500 µl injection loop
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Used in HPLC Analysis
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Flow Scintillation Counter (Packard Radiomatic 525a, 500 ul counting cell)
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Used to measure chl a
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used to quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

Dataset-specific Instrument Name	Binary Gradient Pump (Shimadzu dual LC10-AT vp and Controller SCL-10A vp)
Generic Instrument Name	Pump
Dataset-specific Description	Used in HPLC analysis
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

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Project Information

Assimilation rates of dissolved organic carbon by photomixotrophic estuarine phytoplankton

(Photomixotrophy)

Website: https://sites.google.com/site/jaypinckney/

Coverage: North Inlet, SC: 33 19.5 N, 79 11W

Phytoplankton, traditionally viewed as primary producers at the base of aquatic food webs, provide an energy source for higher trophic levels. However, some phytoplankton species function as both primary producers and heterotrophic secondary consumers. Phytoplankton that are photosynthetically competent but also take up and assimilate organic compounds are classified as facultative mixotrophs or, more simply, photomixotrophs. Unfortunately, we currently have few estimates of the proportion of the phytoplankton community that function as photomixotrophs, their rate of secondary production, or their temporal variation in abundance. Current paradigms about trophodynamics in marine systems do not consider this potentially important alternative pathway for energy flow for phytoplankton. The implication is that we may be missing a significant, fundamental process that affects carbon cycling and trophodynamics in estuarine systems. Furthermore, changes in the DOC composition due to anthropogenic alterations may result in changes in phytoplankton community structure and possibly promote the proliferation of harmful algal bloom species. In terms of ecosystem function, even moderate rates of photomixotrophy could potentially alter our current understanding of phytoplankton productivity, overall C turnover, competitive interactions, and energy transfer in estuarine environments. This project will use a novel approach to provide quantitative measures of the in situ rates and magnitudes of facultative heterotrophy in natural, estuarine phytoplankton communities over seasonal time scales in a representative estuarine ecosystem. The project will utilize a unique 14C radiolabeling technique to quantify the in situ assimilation rates of DOC by estuarine photomixotrophs and estimate the amount of DOC converted to phytoplankton biomass by photomixotrophy over seasonal time scales. This information will provide new insights into carbon dynamics in estuaries, the contribution of DOC to estuarine food webs, and the importance of photomixotrophy in determining the structural and functional characteristics of estuarine phytoplankton communities.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1260134

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