Gulf Carbon Cruise Report:

Pigments Analysis:

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Sampling

Five major cruises were conducted in the NGOM region to study the vertical and horizontal distribution of phytoplankton communities. Four cruises were conducted in 2009 including January (winter), April (spring 2009), July (summer), October-November (fall), and one in March 2010 (spring 2010). Samples were collected at approximately 50 discrete stations (Fig. 1) for phytoplankton pigments, inorganic nutrients and some limited microscopic analysis of phytoplankton. Water samples and vertical profiles of temperature (T) and salinity (S) were recorded at each station using a vertical profiling package that included a SeaBird SBE911 plus CTD and rosette sampler equipped with 10-L Niskin bottles. The package was also equipped with a chlorophyll fluorometer (Chelsea Instruments) and beam transmissometer (Sea Tech, 20 cm path length). Sampling depths for pigment analysis were selected by examining chlorophyll fluorescence profiles and choosing from at least 3-5 depths in the upper 200 m including the upper mixed layer and subsurface chlorophyll fluorescence maximum. For station depths 25 m or less, only surface and near bottom samples were collected.

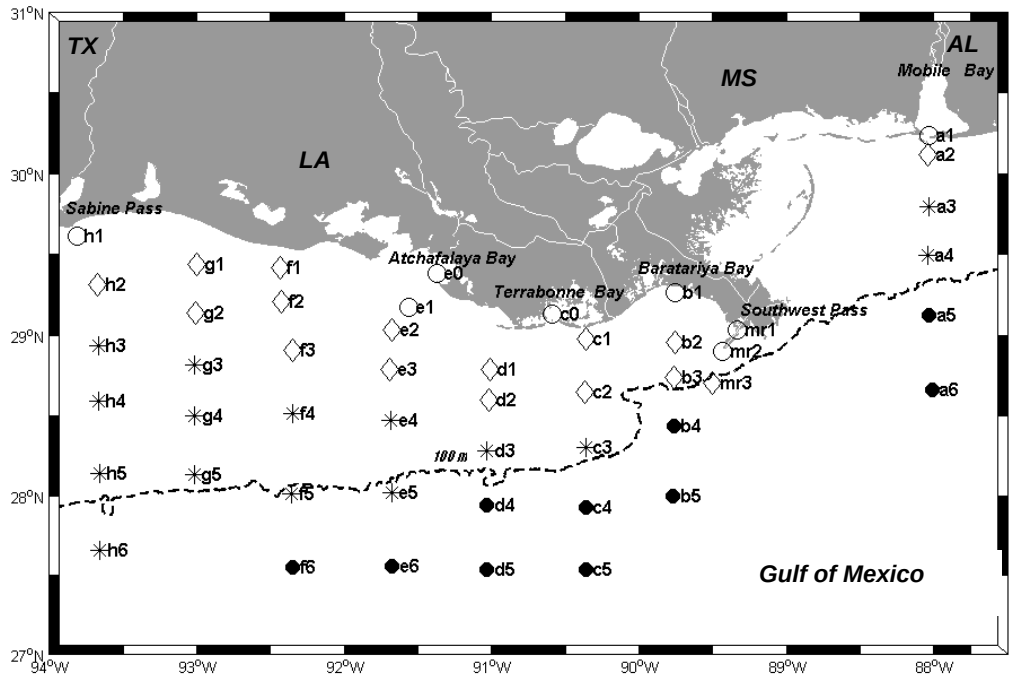

Fig.1. Study area and stations sampled during the GulfCarbon cruises. The symbols denote the geographical locations of stations demarcating the different water masses found in the area, estuaries (○), inner shelf (◊), midshelf (∗), and offshore/slope (●) waters.

HPLC (high performance liquid chromatography) pigment analysis.

Seawater samples for pigment analyses were immediately filtered (2-5 L volume) onto Whatman 47 mm GF/F filters under low vacuum (<0.5 atm). The filters were blotted dry, and stored immediately in 2 mL cryotubes (Fisher) in liquid nitrogen until analysis. Prior to extraction of the pigments, filters were lyophilized (freeze dried) at -47 to -52 °C, 0.100 mbar for 12 h (using a Labconco FreeZone 6 system) to remove excess water. The lyophilized filters were immersed in 90% acetone (3 ml), vortexed, and the contents weighed prior to storing overnight at -4°C. Pigments were extracted by filtering the slurry (acetone and filter contents) through a 13 mm diameter 0.2 µm PTFE HPLC syringe filter (Alltech) attached to a 5 cc glass syringe. The clarified extracts were subsequently collected in disposable microcentrifuge tubes (2 ml) and stored at -19°C until analysis (usually less than 8 hrs). Prior to injection, a 50:50 mixture of 350 µL of sample extract and 350 µL tetrabutylammonium acetate (TBAA) was prepared adjusted to pH 6.5. The injection loop (500 µL) was flushed and filled with the mixture and subsequently injected onto the column. The HPLC analysis method was similar to that of Van Heukelem and Thomas (2001) with minor modifications. The method was included in the recent NASA fifth SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-5) and was found to perform well relative to other methods for identification and quantification of pigments (Chakraborty, 2013). During the processing of pigment data this study followed the quality assurance procedures described in Hooker et al. (2005).

References

Chakraborty S (2013) Phytoplankton community distribution and light absorption properties in the northern Gulf of Mexico. Ph.D. dissertation, University of Southern Mississippi, Hattiesburg, MS.

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Van Heukelem L, Thomas CS (2001) Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. Journal of Chromatography A 910:31-49.

Table1. Major pigments/pigment groups reported and representative phytoplankton groups

Abbreviations	Description	Formula	Taxonomic group
Chl *a*	chlorophyll-*a*	Chl *a*= Σ(Chl *a*+Chl *a*-epimer +Chl *a*-allomer)	Represents biomass in this study
DVchl*a*	divinyl chlorophyll-*a*		Prochlorophytes (prochl)
Chl*b*	chlorophyll-*b*		green algae
DVchl*b*	divinyl chlorophyll-*b*		Prochlorophytes
Chl$c_{2,1}$	chlorophyll-$c_{2,1}$		multiple
Chlc_3	chlorophyll-c_3		Haptophytes (hapto) , diatoms (diato)
Chlide *a*	chlorophyllide *a*		
Phide *a*	Phaeophorbide *a*	Phide a= Σ (phidea$_{ni,\ i=5}$)	
Phytin *a*	Phaeophytin *a*		
Caro	carotens	Caro = (ββ-car + βε-car)	
Allo	alloxanthin		Cryptophytes
But fuco	19'- butanoyloxyfucoxanthin		Pelagophytes (pelago)
Hex fuco	19'-hexanoyloxyfucoxanthin		Haptophytes (hapto)
Diad	diadinoxanthin		diato, hapto, pelago
Fuco	fucoxanthin		diatoms
Diato	diatoxanthin		diatoms
Lut	lutein		green algae
Neo	neoxanthin		green algae
Viola	violaxanthin		green algae
Perid	peridinin		Dinoflagellates
Pras	prasinoxanthin		prasinophytes
Zea	zeaxanthin		Cyanobacteria, prochl & green algae

Table 2: List of reported pigment sums, ratios and size classes (based on pigments)

Abbreviations	Description	Formula
PSC	photosynthetic pigments	PSC=Σ(19'-but+fuco +19'-hex+peri)
PPC	photoprotective pigments	PPC=Σ(allo+diad+ddx+zea +caro)
TAccp		TAccp=Σ(PPC+PSC+Tchl*c* +Tchl*b*)
Tchl *a*	total chlorophyll-*a*	Tchl *a*=Σ(chla+DVchl*a* +chllide-*a*)
Tchl*c*	total chlorophyll-*c*	Tchl *c*=Σ(chlc_1+chlc_2+chlc_3)
Tchl*b*	total chlorophyll-*b*	Tchl *b*=Σ(chl*b*+DVChl*b*)
DP	diagnostic pigments	DP= (PSC+allo+zea+Tchl*b*)
TChl	total chlorophyll	Tchl= Σ(Tchl *a* + TChl *b* +TChl *c*)
TCaro	total carotenoids	Tcaro= Σ(PPC+PSC)
TPig	total pigments	Tpig= Σ(TAccp+Tchl *a*)
PSP	photosynthetic pigments	PSP= Σ(PSP+Tchl)
TAccp/Tchla	total accessory pigments to total chlorophyll-*a*	
PSC/TCaro	photosynthetic carotenoids to total carotenoids	
PPC/TCaro	photoprotective pigments to total carotenoids	
TChl/TCaro	total chlorophyll to total carotenoids	
PPC/Tpig	photoprotective carotenoids to total pigments	
PSP/TPig	photosynthetic pigments to total pigments	
TChla/TPig	total chlorophyll a to total pigments	