Quality Assurance Documentation Plan for the Potomac River Fluorescence Component of the Chesapeake Bay Water Quality Monitoring Program

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Potomac River continuous In vivo Fluorescence Monitoring:

Summary

The Plankton component of the Chesapeake Bay Water Quality Monitoring Program has been conducted by the Academy of Natural Sciences' Estuarine Research Center (ANSERC) since August, 1984. The Academy's component includes several elements grouped into a baseline plankton program, a microzooplankton element and a continuous underway fluorescence element for the upper Potomac River. In the later element, in vivo fluorescence (IVF) will be continuously recorded at ~0.5m depth during transects in the upper Potomac River, from June - September. A longitudinal transect will be made from a downriver station (RET2.2), near Maryland Point, to Gunston Cove (XEA9075). Four cross-river transects will be made between the two logitudinal endpoints (Figure 1). The major purpose of this program is to assess phytoplankton biomass with greater spatial rigor in the upper portion of the Potomac River Estuary in conjunction with the summer cyanophyte bloom, which historically has been a blatant indicator of eutrophication in this area. This program will complement two other elements of the Plankton Component, a baseline program consisting of estimates of phytoplankton abundances, primary productivity and vertical distributions of chlorophyll *a* at all primary stations in the MD portion of the watershed, and a microzooplankton element.

Project Objective

The objective of this component of the Chesapeake Bay Water Quality Monitoring Program is to increase the spatial resolution of the existing chlorophyll *a* monitoring effort in the upper Potomac River Estuary in order to better assess the magnitude of the cyanophyte bloom in this portion of the estuary. In the late 1970's and early 1980's, there were expansive blooms of the nuisance cyanophyte, *Microcystis aeruginosa.* These blooms took place largely in response to excessive nutrient concentrations in conjunction with specific physical conditions. The impact of these blooms was the reduction of SAV beds, aesthetic impairment of recreational activities and the probable contribution to hypoxia-anoxia conditions in the lower estuary. This clear indicator of eutrophic conditions is currently being carefully monitored in the context of this program. The data collected in this component of the Monitoring program is directly comparable to the continuous IVF data measured on the MD mainstem Chesapeake Bay and in the Patuxent River. These data can also be compared to surface chlorophyll data which is collected at specific stations in the Potomac River by MDDNR.

Field Sampling

Horizontal distributions of chlorophyll *a* will be determined along the axis of the upper Potomac River and into Gunston Cove, Occoquan Bay, Mattawoman Creek and Wade's Bay through continuous monitoring of in vivo fluorescence in transects on the river (Figure 1). Vertical profiles of IVF are done at the primary plankton monitoring stations (RET2.2 and TF2.3) in this portion of the Potomac River. Measurements are obtained at 0.5, 1, 2, 3 meters and 2 meter intervals thereafter to one meter above the bottom. Cruises will be undertaken monthly in June and September and twice monthly during July and August, with IVF and latitude and longitude (from GPS) electronically recorded on a laptop computer. IVF will be converted to chlorophyll *a* from sampling date specific regressions between spectrophotometric estimates of chlorophyll *a* generated from grab samples (Strickland and Parsons, 1972) and IVF collected during the cruise.

IVF values are measured on a Turner Designs Model 10-AU digital fluorometer. The fluorometers are calibrated from phytoplankton cultures in order to generate IVF values that are mid-range for the range of chlorophyll concentrations which are encountered in the Chesapeake Bay during the course of the year. The different flourometers used in the study are equilibrated to generate similar IVF values for specific chlorophyll concentrations. All horizontal IVF readings will be accompanied by a latitude-longitude measured by a GPS unit and stored in the data file with the mean IVF values.

Each discrete sample is collected in a 1L Nalgene bottle after the bottle has been rinsed with sample water 3 times. For the Potomac sampling, the samples are put into a cooler of ambient water and are filtered at the end of the day (4-7 hours later). The sample bottle is inverted 3 times to mix the sample. The sample is poured into a 500 ml graduated cylinder after the gc has been rinsed 3 times with the sample water. The sample is filtered through a 47mm Whatman glass fiber filter with a pore size of 0.7um that has been placed on the base of a 47mm Gelman Magnetic Filter Funnel using a pair of forceps. The vacuum pressure is set at <10 psi. The amount filtered is determined by observing the output from the filter funnel. When the sample water starts to drip slowly through the filter, the volume of water used is noted. This should occur with only 5-10 mls of water left to go through the filter. When there is only 15-20 mls of water left, 7-8 drops of magnesium carbonate is added to the sample water to be drawn onto the filter as well. After all the water has filtered through, the magnetic filter funnel is removed and the vacuum pump is turned off. Using a pair of forceps, the filter is folded into quarters and placed inside a piece of foil. The foil is folded over the filter and the ends are secured by a label that is put onto the outside of the foil. The sample is then immediately put into a freezer or a cooler of ice.

The samples are transported back to the lab at the end of the cruise in a cooler of ice. Once at the lab, the samples are transferred from the cooler into a freezer. In some cases, the filtration process has taken place at the lab and the samples are put in the freezer after each one

has been filtered. The samples stay in the freezer until the grinding process.

Laboratory Methods

In a virtually dark lab, a sample is taken from the freezer. The label is removed and placed in a data book. The foil is unwrapped and using a pair of forceps, the filter is folded in half again so that it will fit into the 13 ml Kimax centrifuge tube.. Using an Oxford repipettor 2 mls of J.T. Baker HPLC grade acetone that has been diluted with DI to 90% strength is added to the centrifuge tube. The sample is then ground using a Black & Decker 3/8": variable speed electric drill and a pestile that has been put into the chock of the drill. The filter is ground until it is an applesauce type mush.

After grinding is complete, 8 mls of acetone is pipetted into the centrifuge tube. The centrifuge tube is then capped, gently shaken, and placed in a light proof box inside a dark refrigerator.

The grinding process is repeated with each filter. The number of samples processed at one time is at the discretion of the lab technician. Sometimes, the samples are ground, placed in the dark refrigerator, and stored there overnight before continuing the rest of the process. Most of the time, the samples are completed from grinding to running them through the spectrophotometer in one day. If the process is completed in one day, the samples are left to extract for at least 2 hours in the dark refrigerator before running them through the spectrophotometer.

Still in virtual darkness, the samples are taken from the dark refrigerator and loaded into the centrifuge. The Beckman refrigerated centrifuge is run at 2400 rpm's for 30 minutes or more at a temperature of 8 degrees C.

After the centrifuge has stopped, the samples are removed while still in the centrifuge racks (8-10 at a time) and taken to the spectrophotometer located in the same lab. The supernatant/sample is carefully poured into a 1 cm spectrophotometric cuvette (3 mls generally). The cuvette is wiped off carefully with a Kimwipe and placed in the spectrophotometer. The spectrophotometer has a moving rack that holds 5 cuvettes, 4 are samples and 1 is a blank (filled with 90% acetone). The spectrophotometer is zeroed with the blank at 750nm. Each sample is read at this wavelength and the value is recorded in the data book. The spectrophotometer is then changed to a wavelength of 665nm and rezeroed. Then, the above process is repeated. After the initial reading at 665nm is recorded, 2 drops of 1N HCl is added to each sample. The spectrophotometer is then changed to a wavelength of 664nm and rezeroed. The samples are then read again at 664nm and 750nm. After this process is complete, the samples are removed from the cuvettes and each cuvette is rinsed with 90% acetone 3 times before being filled again.

_____All values that have been recorded in the data book are entered into a spreadsheet that contains the formula for calculating chlorophyll concentration. The formula used is from

Standard Methods.

$$\text{chl a (mg/m3)} = \frac{26.7((665b-750b)-(664a-750a)) * v}{V * l}$$

where v = volume of extracted sample
and V = volume filtered

New Protocol for Chlorophyll *a* Analysis (1999)

In a virtually dark lab, a sample is taken from the freezer. The label is removed and placed in a data book. The foil is unwrapped and using a pair of forceps, the filter is folded in half again so that it will fit into the grinding vessel (Thomas Scientific Size B). Approximately 3-5 mls of J.T. Baker HPLC grade acetone that has been diluted with DI to 90% strength is added to the grinding vessel. The sample is then ground using a Black & Decker 3/8": variable speed electric drill and a pestile that has been put into the chock of the drill. The grinding vessel is held against the pestile in a plastic beaker filled with ice for half of the grinding time. The grinding vessel is lifted out of the ice in order to see the condition of the filter. The filter is ground until it is an applesauce type mush.

After grinding is complete, a small amount of acetone is used to rinse any filter debris from the pestile into the grinding vessel and also to rinse any filter debris from the sides of the grinding vessel. A metal rod which is wiped off with a Kimwipe after each use is sometimes used to loosen the filter mush from the bottom of the grinding vessel. The sample is then transferred to a 13 ml Kimax glass centrifuge tube using a small plastic funnel. The metal rod may be used again to poke the filter mush through the funnel into the centrifuge tube. A small amount of acetone is used to rinse any of the remains of the sample from the grinding vessel and the funnel into the centrifuge tube. The centrifuge tube is then capped and placed in a light proof box inside a dark refrigerator.

The grinding process is repeated with each filter. The number of samples processed at one time is at the discretion of the lab technician. Sometimes, the samples are ground, placed in the dark refrigerator, and stored there overnight before continuing the rest of the process. Most of the time, the samples are completed from grinding to running them through the spectrophotometer in one day.

Still in virtual darkness, the samples are taken from the dark refrigerator and loaded into the centrifuge. The Beckman refrigerated centrifuge is run at 2400 rpm's for 30 minutes or more at a temperature of 8 degrees C.

After the centrifuge has stopped, the samples are removed while still in the centrifuge racks (8-10 at a time) and taken to the spectrophotometer located in the same lab. The supernatant/sample is carefully poured off into a plastic 25 ml graduated cylinder. The volume

of the sample is measured and recorded in the data book. The sample is poured into a 1 cm spectrophotometric cuvette (3-4 mls generally). The cuvette is wiped off carefully with a Kimwipe and placed in the spectrophotometer. The spectrophotometer has a moving rack that holds 5 cuvettes, 4 are samples and 1 is a blank (filled with 90% acetone). The spectrophotometer is zeroed with the blank at 750nm. Each sample is read at this wavelength and the value is recorded in the data book. The spectrophotometer is then changed to a wavelength of 665nm and the above process is repeated. After the initial reading at 665nm is recorded, 3 drops of 2N HCl is added to each sample. The samples are then read again at 665nm and 750nm. After this process is complete, the samples are removed from the cuvettes and each cuvette is rinsed with 90% acetone 3 times before being filled again.

All values that have been recorded in the data book are entered into a spreadsheet that contains the equation for calculating chlorophyll concentration. The equation used is from Standard Methods: Chl, ug/l = 26.73 (665b-665a) x V1 / V2, where, 665b and 665a are the optical densities of the 90% acetone extract before and after acidification, V1 is the volume of the extract and V2 is the sample volume that was filtered.

QA/QC

All data collected in the field are returned to the lab on computer disk. The data are subsequently proofed and converted to chlorophyll *a* from regression results between IVF and chlorophyll *a*, proofed again and electronically submitted to MDDNR. Regressions, including slope, intercept and correlation coefficient, obtained between in vivo fluorescence and extracted chlorophyll *a* concentrations for each field trip and fluorometer are recorded in data books maintained at the lab. A t-test is run on the Y-intercept estimate to determine if it is significantly different from zero. This is done by dividing the intercept by the standard error of the Y-intercept estimate and testing the result with a t-test at n-2 degrees of freedom, where n equals the number of points in the regression (see regression sheets in Appendix).

Deliverables

Data collected from July - September will be delivered to MDDNR via e-mail by April 15 with analyses of said data presented in a report to be completed by May 15. Data collected from June will be delivered to MDDNR electronically by October 15 with analyses of said data presented in a report to be completed by November 15. A Quality assurance documentation plan will be submitted to MDDNR by June 30.

Literature Cited

APHA-AWWA-WPCF. 1975. Standard Methods For the Examination of Water and Wastewater. Fourteenth Edition. Washington, D.C. 1193 pp.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis.

Fisheries Res. Bd. Canada Bull. 167, Ottawa. 310 pp.

Data Deliverable Information

STATION NAMES AND DESCRIPTIONS
XEA9075 400 yds. N of buoy 64
XEA6000W 300 yds. off boat ramp at Pohick Bay Regional Park
XEA5000C Buoy 51
XEA5000E 250 yds. W. of shoreline parallel to buoy 51
XEA5000W Middle of Occoquan Bay parallel to buoy 51
XEA4000C Green buoy 45, off Mattawoman Creek
XEA4000E Red day marker 6, Mattawoman Creek
XDA3000C Green buoy 33
XDA3000E 600 yds. W. of shoreline in Wades Bay parallel to green buoy 33
XDA3000W 450 yds. E. of shoreline parallel to green buoy 33
RET2.2 10 yds. N. of buoy 19

STATION NAMES, LATITUDES, LONGITUDES

XEA9075	38 40.32 N	77 07.93 W
XEA6000W	38 40.63 N	77 09.94 W
XEA5000C	38 35.64 N	77 12.19 W
XEA5000E	38 35.27 N	77 11.70 W
XEA5000W	38 37.09 N	77 13.60 W
XEA4000C	38 33.59 N	77 14.31 W
XEA4000E	38 33.79 N	77 11.58 W
XDA3000C	38 26.33 N	77 16.51 W
XDA3000E	38 25.87 N	77 16.20 W
XDA3000W	38 25.87 N	77 18.90 W
RET2.2	38 21.09 N	77 12.42 W

DATA SET LABEL: POTOMAC FLUORESCENCE HEADER DATA

----ALPHABETIC LIST OF VARIABLES AND ATTRIBUTES-----

# VARIABLE	TYPE	LENGTH	POSITION	FORMAT	LABEL
AMETHOD	NUM	8	40		ANALYSIS METHOD CODE (102)
DATE	NUM	8	8		SAMPLE DATE
DC_INIT	CHAR	3	51		INITIALS OF DATA COORDINATOR
DISBETWE	NUM	8	32		DISTANCE BETWEEN STATIONS (KM)
FC_INIT	CHAR	3	48		INITIALS OF FIELD COLLECTOR
SER_NUM	NUM	8	57		SERIAL NUMBER
STAT_DEP	CHAR	8	0		STATION DEPARTURE
STAT_DES	CHAR	8	24		STATION DESTINATION
STIME	NUM	8	16		START TIME
TRIB_COD	CHAR	3	54		CODE OF TRIBUTARY SAMPLED

CONTENTS OF SAS MEMBER ANSPFyyD.SSD:

----ALPHABETIC LIST OF VARIABLES AND ATTRIBUTES-----

# VARIABLE	TYPE	LENGTH	POSITION	FORMAT
CHLA	NUM	8	27	CHLOROPHYLL A (UG/L)
DATE	NUM	8	8	SAMPLE DATE
LAT	NUM	8	35	LATITUDE
LATLOC	CHAR	1	43	N = NORTH
LONG	NUM	8	44	LONGITUDE
LONGLOC	CHAR	1	52	W = WEST
RDTIME	NUM	8	53	READ TIME
SER_NUM	NUM	8	19	SERIAL NUMBER
STAT_DEP	CHAR	8	0	STATION DEPARTURE
TRIB_COD	CHAR	3	16	CODE OF TRIBUTARY SAMPLED

NUMERICAL VARIABLE NAMES - WARNING AND ERROR BOUNDS

VARIABLE	VALID RANGE
AMETHOD	102
DATE	900827-980630
DISBETWE	1.6-32.1
SER_NUM	112202-237235
STIME	08:36-18:15
CHLA	0.00-163.0
LAT	38 20.80-38 40.91
LONG	77 07.00-77 19.08
RDTIME	08:37:23-18:26:00

CHARACTER VARIABLES - VALID VALUES

VARIABLE	VALID RANGE
DC_INIT	SEE > DATA COORDINATOR
FC_INIT	SEE # INITIALS OF SCIENTISTS IN DATA SET
STAT_DEP	SEE # STATION NAMES AND DESCRIPTIONS AND# STATION NAMES, LATITUDES, AND LONGITUDES
STAT_DES	SEE # STATION NAMES AND DESCRIPTIONS AND # STATION NAMES, LATITUDES, AND LONGITUDES
LATLOC	N
LONGLOC	W
TRIB_COD	POT

Appendix

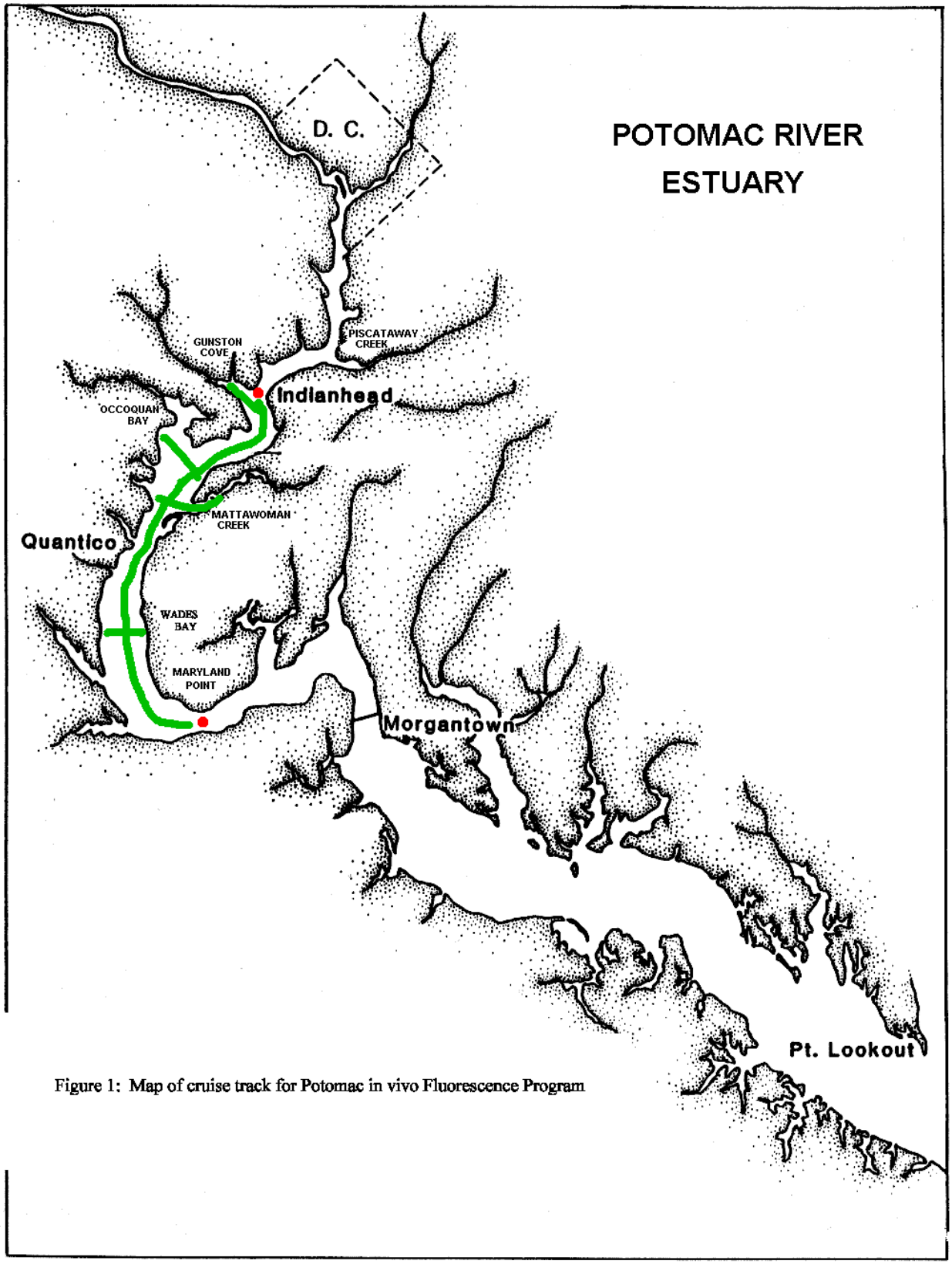


Figure 1: Map of cruise track for Potomac in vivo Fluorescence Program

Table 1: Total cruises for the Potomac River Fluorescence Component of the Chesapeake Bay Water Quality Monitoring Program, July, 2001-June, 2002.

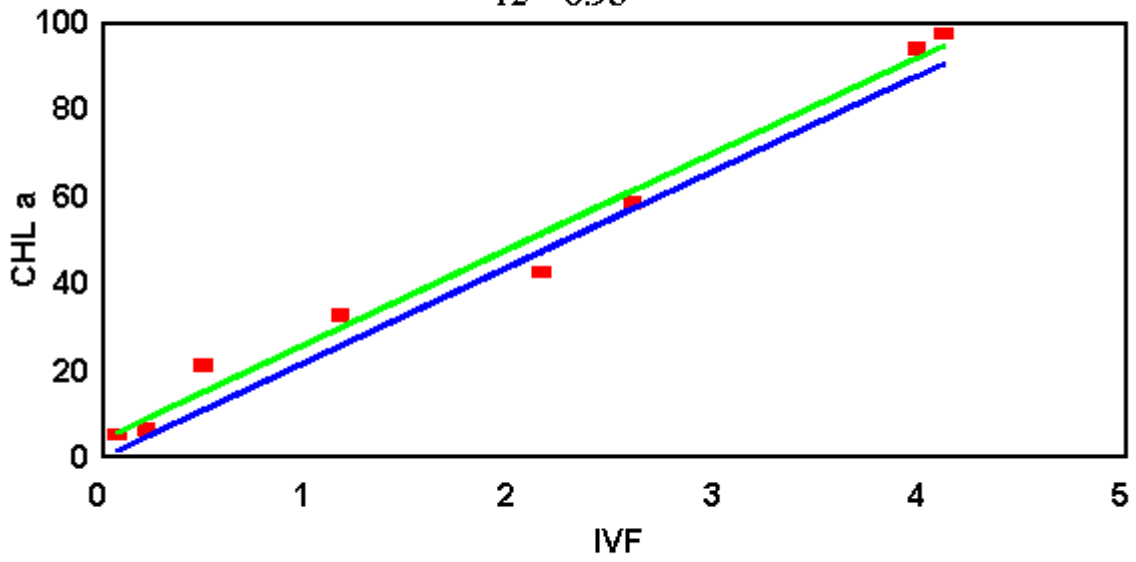
Measurements	J	A	S	O	N	D	J	F	M	A	M	J	QA/QC	Total
In vivo Fluorescence Cruises	2	2	1	--	--	--	--	--	--	–	--	1	--	6

EXAMPLES of IVF vs. CHLOROPHYLL *a* REGRESSIONS

POTOMAC HORIZ. #253 9/1 1998

CHL a = 22.10(IVF) + 0.00

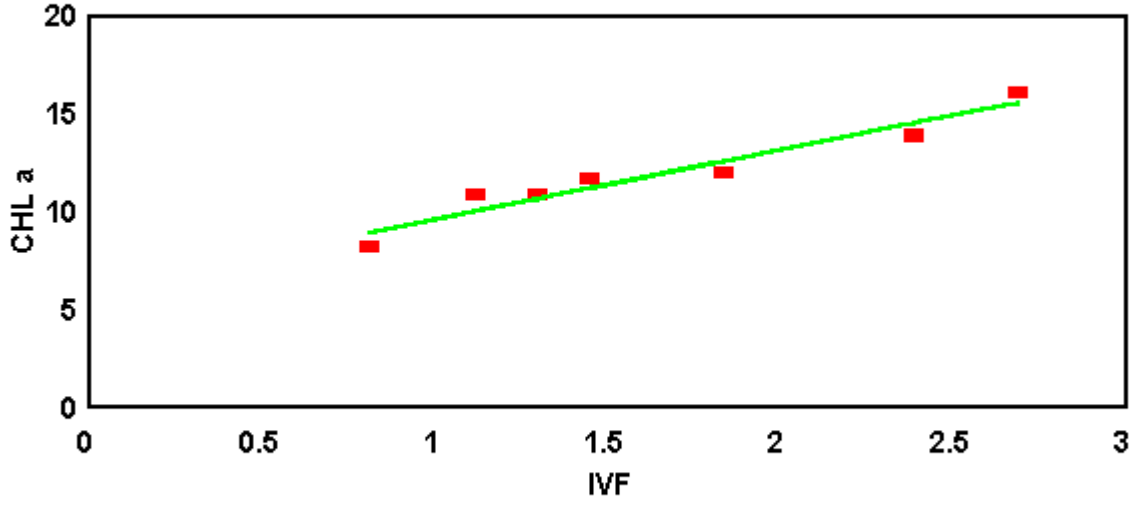
r2 = 0.98



DISCRETES — REGRESSION

POTOMAC CRUISE #246 5/4 1998

CHL a = 3.49(IVF) + 6.15 r2 = .94



DISCRETES — REGRESSION