**Checklist for SeaBASS submission: HPLC pigment data**

Please fill out section 1 below (section 2 has already been populated)

Names of Experiment & Cruise: \_\_\_, \_\_\_

1) Sample collection method

 a. Filter type/pore size:

 b. Vacuum pressure:

c. Replicates collected (# or no):

e. Flash frozen in L/N (y/n):

f. Long term storage conditions/temperature:

2) Sample measurement and analysis

a. Extraction

1. solvent: 95% ACETONE W/INTERNAL STANDARD DISSOLVED IN IT
2. volume added/delivery method: 2.65 ml, DISPENSETTE ORGANIC BOTTLE-TOP DISPENSER DESIGNED FOR ORGANIC SOLVENT
3. disruption method, time: SONIC PROBE, 12 SECONDS PULSED
4. soak time: 4 HOURS TOTAL
5. clarification method: 0.45 µM TEFLON SYRINGE FILTER

b. HPLC analytical method

1. Method reference: VAN HEUKELEM AND THOMAS (2001), FURTHER DESCRIBED IN HOOKER ET AL. (2012)
2. Column: 4.6 X 150 MM HPLC ECLIPSE XDB (AGILENT TECHNOLOGIES, PALO ALTO, CA) C8 STATIONARY PHASE (3.5 UM)
3. Column temperature: 60°C
4. Mobile phase: SOLVENT A = 70 PARTS METHANOL, 30 PARTS 28 MM TETRABUTYLAMMONONIUM ACETATE (PH 6.5). SOLVENT B = METHANOL
5. Flow rate: 1.1 ML/MIN
6. Gradient: LINEAR GRADIENT, 5-95% SOLVENT B OVER 27 MINUTES
7. Wavelengths monitored:

450 NM (20 NM BANDWIDTH, NO REFERENCE)

665 NM (20 NM BANDWIDTH, REF WAVELENGTH 700 NM, 20 NM)

222 NM (10 NM BANDWIDTH, NO REFERENCE)

1. Spectra collected? If so, what wavelengths: YES, 350-850 NM

c. Internal standard used? (If no, skip to d) YES

1. Name: VITAMIN E ACETATE, MONITORED AT 222 NM

2. When added: INTERNAL STANDARD DISSOLVED IN EXTRACTION SOLVENT

d. Instrument

1. Make and model: AGILENT RR1200
2. Injector type: PROGRAMMABLE AUTOINJECTOR (900 UL SYRINGE HEAD)
3. Pump type: QUATERNARY PUMP WITH IN-LINE VACUUM DEGASSER
4. Is sample compartment refrigerated? YES
5. heated/thermostatted column compartment? YES
6. Detector type: PHOTO-DIODE ARRAY DETECTOR WITH DEUTERIUM AND TUNGSTEN LAMPS.

e. Instrument calibration:

1. Frequency: AT LEAST ANNUALLY
2. Source of standards: DHI (IN SOLUTION WITH CONCENTRATIONS PROVIDED) OR PURCHASED IN SOLID FORM AND SUSPENDED IN SOLVENT. USE ABSORPTION COEFFICIENTS IN COMMON WITH THOSE USED BY COMMERCIAL VENDOR, DHI WATER AND ENVIRONMENT (HORSHOLM, DENMARK)
3. Single point or multi-point calibration: MULTI-POINT SPANNING THE WORKING RANGE OF EACH PIGMENT
4. How are calibration factors (or response factors. RF) calculated? MULTIPLE METHODS: A) SINGLE POINT RF FROM STOCK STANDARD, B) OVERALL AVERAGE RF COMPUTED FROM RFs CALCULATED FOR EACH CALIBRATION POINT, C) RF COMPUTED FROM THE SLOPE OF THE LINEAR REGRESSION, BOTH WITH THE Y-INTERCEPT FORCED THROUGH ZERO AND COMPUTED. RESULTS COMPARED TO ENSURE THEY ARE PRODUCING APPROXIMATELY EQUIVALENT RESPONSES
5. Is calibration accuracy monitored between calibrations? If so, how and how often? YES, CHL A CALIBRATION ACCURACY IS MONITORED DAILY. AT LEAST ONE OTHER PIGMENT IS ANALYZED FOR CALIBRATION ACCURACY DURING EACH WEEK OF SAMPLE ANALYSIS.
6. Noise at each wavelength: 450 NM = 0.0072, 665 NM = 0.0038. 222 NM ONLY USED FOR INTERNAL STANDARD (VERY HIGH S:N)

f. Coelutions/Special Reporting

1. Were there coeluting pigments (Rs <1.0)? Were the coeluting pigments reported separately or together? If reported separately, report resolution and computation method of separation.

DV AND MV CHL B Rs = 0.8-1.0. IF DV CHL B IS PRESENT, BOTH PIGMENTS ARE QUANTIFIED BY PEAK HEIGHT RATHER THAN AREA.

ALPHA AND BETA CAROTENE COELUTE AND ARE QUANTIFIED TOGETHER AND REPORTED AS “ALPHA BETA CAR”.

 2. Critical pair (other than mentioned above), Rs: ZEA/LUT. RS = 1.2

3. Other notes on special reporting conditions?

IF ISOMERS OF DV CHL B, MV CHL B, AND PERIDININ ARE DETECTED, THEY ARE SUMMED WITH AND REPORTED AS PART OF THE PARENT PEAK.

CHL C2, C1, AND MGDVP ARE QUANTIFIED TOGETHER AND REPORTED AS “CHL C1C2”.

DV CHL C3 AND CHL C3 ARE QUANTIFIED TOGETHER AND REPORTED AS “CHL C3”.

UP TO 5 PHEOPHORBIDE PEAKS ARE SUMMED TOGETHER AND REPORTED AS “PHIDE A”.

ALLOMERS AND EPIMERS OF DV AND MV CHL A ARE SUMMED WITH AND REPORTED AS PART OF THEIR PARENT PEAK.

3) Data reporting should include (this section does not need to be filled out)

 a. Note size fractionated samples (separate field names, e.g., <pig>\_#umprefilt)

 b. Instrument/injector precision (place in the header as comments?)

c. Individual and summed pigments (based on SeaBASS fields/SeaHARRE reports)

 d. Mark ‘below detection limit’ values with up-to-date value (now = -8888)

 e. Report replicate filters separately