# Sampling and Analysis Plan
# for
# 2016 RMP Status and Trends Bird Egg Monitoring

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## Introduction

The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) monitors concentrations of contaminants in bird egg tissue as an indicator of water quality impairment. In 2016, the RMP will collect bird egg samples from various locations in the Bay as part of routine Status and Trends Monitoring.

The target species will be Double Crested Cormorants and Forster's Terns. Cormorant eggs will be collected from up to 3 locations in 2016. Wheeler Island and Richmond Bridge are the historical sites for cormorant egg collection. Cormorant egg collection has also occurred at Pond A9/10 and Pond AB2. Pond A9/10 was not viable in 2009 and the replacement site Pond AB2 was added. Forster's tern eggs will be collected from up to 4 locations in 2016 (Note: the number of egg collection sites was reduced from 6 (the number sampled in prior rounds) to 4 due to increased costs for sampling and analysis by USGS). The historical collection sites are: (1) Pond A16, (2) Pond A2W, and (3) Pond AB2 on Don Edwards National Wildlife Refuge; (4) Eden Landing Ecological Reserve; (5) Hayward Shoreline Regional Park; and (6) Napa-Sonoma Marsh Wildlife Area. However, Forster's Terns are known to be nomadic and change colony sites annually in response to local conditions, and in 2012 it was necessary to replace several of these historical sites with Pond A1, Pond AB1, and Pond A7. It is plausible that collections will need to occur at other sites in response to local breeding conditions in 2016.

The samples will be collected by staff at the USGS Western Ecological Research Center [USGS-WERC] who will procure all necessary egg collection permits. Eggs will then be analyzed for:

- Total mercury (THg) (cormorant eggs at Moss Landing Marine Laboratory-Marine Pollution Studies Lab [MLML-MPSL]; tern eggs at USGS-WERC),

- Selenium (cormorant and tern eggs at MLML-MPSL),
- PCBs (cormorant eggs at the California Department of Fish & Wildlife's Water Pollution Control Laboratory [DFW-WPCL]),
- PBDEs (cormorant eggs at DFW-WPCL) (NOTE: PBDE analysis in tern eggs was discontinued due to increased USGS costs),
- PFCs and precursors (cormorant eggs at AXYS Analytical Laboratories), and
- Halogenated carbazoles (cormorant eggs at Southern Illinois University) NOTE: Carbazole analyses will be done pro bono by SIU for research purposes. Carbazoles are not part of the RMP Status and Trends design. SIU will not measure percent moisture in its samples. Therefore, it is important to make sure that sample weights, prior to analysis, are accurately measured since we will be applying the percent moisture values from other laboratories to their samples.

The laboratory and quality assurance methods used will conform to the RMP Quality Assurance Project Plan (SFEI, 2015). The field sampling information for this study (e.g., the number and location of samples collected) will be included in the 2016 Annual Monitoring Report. A report on these data (and data from the subsequent round of sampling) will be generated after the next round of bird egg sampling is conducted in 2018.

The purpose of this Sampling and Analysis Plan is to clearly outline the sampling design, methods, and archiving strategy for documentation and to make it easy for project partners to coordinate.

## Sampling Design

Two species of birds will be collected (from locations above) according to the following sampling design (Table 1).

**Table 1: Bird egg sampling design**

| **Species** | **Double crested cormorant[1] *(Phalacrocorax auritus)*** | **Forster's Terns (*Sterna forsteri*)** |
|---|---|---|
| Number of sites | 3 | 4 |
| Number of composites per site | 3 | 3 |
| Target # of Composites | 9 | 12 |
| Target # eggs per composite[3] | 8[5] | 7[3] |
| Target # eggs | 72[2,5] | 84[2] |
| Tissue analyzed | egg (wet) | egg (dried) |

1 -- PFC analyses will be conducted on cormorant samples. Avoid using any Teflon coated equipment when handling these eggs.
2 -- Individual cormorant and tern eggs will be analyzed for mercury and archived for possible selenium analysis. All other samples will be analyzed as composites. The 3 composites collected from each site serve as field duplicate samples.
3 -- Composites will include approximately equal masses from each individual egg. Approximate masses should be similar to previous years. Contact SFEI if more guidance is needed.
4 -- Note that the number of sites for collection of Forster's Tern eggs has been reduced from 6 sites (the number used in past sampling) to 4 sites. Sites removed were Napa-Sonoma Marsh and Eden Landing.
5 -- The cormorant egg composites that are prepared will include seven eggs. An extra egg per composite will be collected, if possible, in case eggs are broken during shipping. However, only 7 eggs will be homogenized for individual Mercury analysis and selenium archive and for preparing composites. The goal is to process 63 individual eggs for both individual and composite analyses

## Sampling Methods

Eggs will be collected by USGS-WERC using approved USGS field collection and handling protocols (Ackerman et al., 2013).

The coordinates of the actual sampling sites will be determined using a hand-held device and reported on field sheets provided by USGS. Other pertinent information will also be recorded, including the sampling method, and location description.

When possible, anomalies and any other pertinent information will be noted on the field sheet.

## Sample Handling and Custody

Each egg will be tagged with a unique ID by USGS and then transported to the USGS-WERC and placed in a refrigerator until shipment or sample processing. USGS will measure and record length, width, and total weight of all eggs (cormorant and tern) following protocols in Attachment 2. USGS will measure and record egg shell mass and thickness for tern eggs only.

Forster's Tern eggs will be processed by USGS-WERC.

Cormorant eggs will be shipped at room temperature to AXYS Analytical for processing. Individual eggs will be shipped to AXYS Analytical via Fed Ex or UPS in specially designed egg shippers with high density foam in a double padded cardboard box. Each egg will also be placed in a sealed whirlpak bag. USGS will make every reasonable effort to package cormorant eggs so that they reach AXYS Analytical fully intact.

All samples will be accompanied by a chain of custody form (COC) (provided by USGS-WERC). The COC form will include the sample unique ID, site name, collection date, sample type, analysis required, and other remarks. At the point of shipping, the sample collection EDD, for both the terns and cormorants should be delivered to SFEI. Shipping information is provided in Attachment 1.

## Sample Processing

### Double Crested Cormorant eggs

USGS-WERC staff will follow the protocols in Attachment 2 to examine and measure each egg. USGS-WERC will ship eggs, with a target of 24 eggs for each location, according to the Sample Handling protocol in the previous section, to AXYS Analytical in Canada (see Attachment 1 for shipping address).

Upon receipt, AXYS will inspect the eggs for breakage. Three extra eggs will be collected for each location as a backup in case of breakage. The 21 largest intact eggs should be selected for the processing steps listed below. Any intact eggs that are not needed should be stored in a refrigerator until all aliquots and composites are prepared. After that, the extra eggs may be discarded.

Select unbroken eggs and weigh the eggs.

1. For each site, pick the largest 21 unbroken eggs from each site and weigh individually.
2. On paper, randomly assign the eggs, in groups of 7, to 3 proposed composite groups for each site (9 composite groups for the 3 sites in total).
3. Calculate the total mass of each composite group by summing the mass of the 7 individual eggs in each group.
4. Calculate the approximate tissue mass of each composite group by subtracting expected shell mass (assume 15% of total mass is shell mass).

Plan the aliquots and composites

5. Identify the two composite groups with the most tissue mass. Ideally, these groups should have >273 g-ww of tissue mass. If these groups have less than 273 g-ww mass, contact the RMP Manager. Flag these groups as being the source of the composite QC samples (labs need extra sample material to perform mandatory QAQC such as MS/MSD or replicates).
6. Within the 7 other composite groups, identify the 8 individual eggs with the most tissue mass. Ideally, these eggs should have >39 g-ww of tissue mass and be distributed across the 7 different composite groups. If no eggs have >39 g-ww contact the RMP Manager.
7. Tentatively flag the 4 eggs with the largest remaining masses (after subtracting masses needed for composites) as being the sources of the individual selenium QC samples. Flag the 5th through 8th largest remaining masses as being the sources of the individual mercury QC samples. If the resultant remaining mass (after counting amounts needed for composites) for any of the eggs goes <0, select different eggs for Se or Hg QC. If some calculated remaining masses are still <0 regardless of which eggs are chosen (most likely for QC), Hg and their QC samples take priority, and individual Se samples will need to be shortchanged or skipped.
8. Prepare an aliquot and composite plan following the template in Table 2a (a spreadsheet version to enter actual total egg masses and calculate remaining available material will be provided by SFEI) based on the following criteria:
    a. Aliquots for individual mercury samples will be 5 g-ww from each egg, except for the QC samples which will be 7 g-ww.
    b. Aliquots for composite samples will be 24 g-ww from each egg, except for QC samples which will be 28 g-ww.
    c. Aliquots for Se archive samples will be 6 g-ww from each egg, except for QC samples which will be 10 g-ww.
    d. Aliquots for carbazoles will be the remaining tissue mass in the egg, if any.
9. Determine if there will be enough mass for all of the samples. ***If there is insufficient mass to collect all the samples, the priority preparation order is 1) individual: Mercury, 2) composite: PCB, PBDE, PFC, selenium, 3) short and long-term archives, 4) individual: selenium (archive), and lastly 5) composite: halogenated carbazole.***

10. ***Contact the RMP Manager to confirm the aliquot and compositing plan before processing the eggs.***

Harvest eggs, extract aliquots, create composite

11. Obtain authorization to proceed from the RMP Manager.
12. Follow the protocol in Attachment 2 to harvest and homogenize each egg. NOTE changes have been made to the preparation protocol compared to past bird egg monitoring studies for the RMP. The contents from each egg should be homogenized, individually, using trace clean equipment, avoiding contamination for analytes of interest (mercury, selenium, PCB, PBDE, PFCs and precursors).
13. Following the aliquot and compositing plan, collect a 5-7 g-ww aliquot from each egg. The aliquot will be 5 g for 59 of the eggs and 7 g for 4 of the eggs (the QC samples). Put the aliquots into 63 pre-cleaned containers for mercury analyses.
14. Following the aliquot and compositing plan, collect a 24-28 g-ww aliquot from each egg. The aliquot will be 24 g for 49 of the eggs and 28 g for 14 of the eggs (the QC samples). Combine the aliquots from each 7-egg group to form 9 composite samples in a temporary (but pre-cleaned) containers. The composite samples will be divided up into different containers for selenium, PFCs, PCBs, PBDEs, and archives in a later step.
15. Following the aliquot and compositing plan (assuming there is sufficient mass), collect a 6-10 g-ww aliquot from each egg. The aliquot will be 6 g for 59 of the eggs and 10 g for 4 eggs for selenium archives.
16. Following the aliquot and compositing plan (assuming there is sufficient mass), collect the remaining mass from each egg. Combine the remaining mass from each 7-egg group into 9 pre-cleaned containers for carbazoles. Ideally, there will be 15-25 g-ww of mass in each carbazole composite.

Divide up composite from Step 14 into containers for Selenium, PFCs, PCBs, PBDEs, and archives

17. A 6-10 g aliquot of each composite should be put into the appropriate containers and stored for analysis of selenium and percent moisture. The aliquot will be 6 g for 7 of the composites and 10 g for 2 of the composites (the QC samples).
18. A 4.0 g aliquot of each composite should be put into the appropriate containers and stored for analysis of PFCs and precursors, % moisture, % lipid.
19. A 20-40 g aliquot of each sample composite should be put into the appropriate container and stored for analysis of PCB, PBDE, percent moisture, and percent lipid. The aliquot will be 20 g for 7 of the composites and 40 g for 2 of the composites (the QC samples).
20. Appropriate aliquots should be prepared for short and long-term archive per Table 4a below.

Once all samples have been created, AXYS will store samples at -20C and ship all but the samples for PFCs frozen to analytical laboratories.

**Table 2a: Example Cormorant egg aliquot and composite plan**

| | | | Step 1 | | Step 2 | | Step 3 | | Step 4 |
|---|---|---|---|---|---|---|---|---|---|
| Site | Comp-osite | Egg # | Hg QC sample? (yes=1) | Aliquot from egg for Hg (g-ww) | Comp QC sample? (yes=1) | Aliquot from egg for Composite (g-ww) | Se QC sample? (yes=1) | Aliquot from egg for Se archive (g-ww) | Aliquot for Carbazole composite (g-ww) |
| WI | C1 | 1 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| WI | C1 | 2 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| WI | C1 | 3 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| WI | C1 | 4 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| WI | C1 | 5 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| WI | C1 | 6 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| WI | C1 | 7 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| WI | C2 | 8 | | 5 | | 24.00 | 1 | 10 | 0-4 |
| WI | C2 | 9 | | 5 | | 24.00 | | 6 | 4 |
| WI | C2 | 10 | | 5 | | 24.00 | | 6 | 4 |
| WI | C2 | 11 | | 5 | | 24.00 | | 6 | 4 |
| WI | C2 | 12 | | 5 | | 24.00 | | 6 | 4 |
| WI | C2 | 13 | | 5 | | 24.00 | | 6 | 4 |
| WI | C2 | 14 | | 5 | | 24.00 | | 6 | 4 |
| WI | C3 | 15 | 1 | 7 | | 24.00 | | 6 | 3-4 |
| WI | C3 | 16 | | 5 | | 24.00 | | 6 | 3-4 |
| WI | C3 | 17 | | 5 | | 24.00 | | 6 | 3-4 |
| WI | C3 | 18 | | 5 | | 24.00 | | 6 | 3-4 |
| WI | C3 | 19 | | 5 | | 24.00 | | 6 | 3-4 |
| WI | C3 | 20 | | 5 | | 24.00 | | 6 | 3-4 |
| WI | C3 | 21 | | 5 | | 24.00 | | 6 | 3-4 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| RB | C1 | 22 | | 5 | | 24.00 | 1 | 10 | 0-4 |
| RB | C1 | 23 | 1 | 7 | | 24.00 | | 6 | 4 |
| RB | C1 | 24 | | 5 | | 24.00 | | 6 | 4 |
| RB | C1 | 25 | | 5 | | 24.00 | | 6 | 4 |
| RB | C1 | 26 | | 5 | | 24.00 | | 6 | 4 |
| RB | C1 | 27 | | 5 | | 24.00 | | 6 | 4 |
| RB | C1 | 28 | | 5 | | 24.00 | | 6 | 4 |
| RB | C2 | 29 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| RB | C2 | 30 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| RB | C2 | 31 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| RB | C2 | 32 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| RB | C2 | 33 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| RB | C2 | 34 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| RB | C2 | 35 | | 5 | 1 | 28.00 | | 6 | 0-4 |
| RB | C3 | 36 | | 5 | | 24.00 | 1 | 10 | 0-4 |
| RB | C3 | 37 | | 5 | | 24.00 | | 6 | 4 |
| RB | C3 | 38 | | 5 | | 24.00 | | 6 | 4 |
| RB | C3 | 39 | | 5 | | 24.00 | | 6 | 4 |
| RB | C3 | 40 | | 5 | | 24.00 | | 6 | 4 |
| RB | C3 | 41 | | 5 | | 24.00 | | 6 | 4 |
| RB | C3 | 42 | | 5 | | 24.00 | | 6 | 4 |
| AB2 | C1 | 43 | 1 | 7 | | 24.00 | | 6 | 3-4 |
| AB2 | C1 | 44 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C1 | 45 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C1 | 46 | | 5 | | 24.00 | | 6 | 3-4 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AB2 | C1 | 47 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C1 | 48 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C1 | 49 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C2 | 50 | | 5 | | 24.00 | 1 | 10 | 0-4 |
| AB2 | C2 | 51 | | 5 | | 24.00 | | 6 | 4 |
| AB2 | C2 | 52 | | 5 | | 24.00 | | 6 | 4 |
| AB2 | C2 | 53 | | 5 | | 24.00 | | 6 | 4 |
| AB2 | C2 | 54 | | 5 | | 24.00 | | 6 | 4 |
| AB2 | C2 | 55 | | 5 | | 24.00 | | 6 | 4 |
| AB2 | C2 | 56 | | 5 | | 24.00 | | 6 | 4 |
| AB2 | C3 | 57 | 1 | 7 | | 24.00 | | 6 | 3-4 |
| AB2 | C3 | 58 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C3 | 59 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C3 | 60 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C3 | 61 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C3 | 62 | | 5 | | 24.00 | | 6 | 3-4 |
| AB2 | C3 | 63 | | 5 | | 24.00 | | 6 | 3-4 |

Table 2b below shows the planned analyses, number of samples, analyzing laboratory, sample type, and sample container source for the cormorant eggs. Note that all analyses are composites except for mercury and selenium archives. For mercury and selenium archives, each egg will be prepared and analyzed individually.

**Table 2b: Cormorant egg containers and analysis plan**

| Species | Analyte | # of Samples | Sample Container | Container Purveyor | Container Cleaning lab | Compositing/ Processing lab | Analytical Lab | Sample Type |
|---|---|---|---|---|---|---|---|---|
| Cormorant Eggs | Hg & % moisture | 63 | 15 ml, pre-cleaned HDPE container | SFEI | MLML | AXYS | MLML | Individual |
| Cormorant Eggs | temp container for all composite analytes | 9 | precleaned Amber glass with tinfoil lined lid | AXYS | AXYS | AXYS | AXYS | composite |
| Cormorant Eggs | Se Archive[1] | 63 | 15 mL HDPE containers with HDPE unlined lids | SFEI | MLML | AXYS | MLML | Individual |
| Cormorant Eggs | Se and % moisture | 9 | 15 ml, pre-cleaned HDPE container | SFEI | MLML | AXYS | MLML | composite |
| Cormorant Eggs | PCBs (209), % lipid, % moisture | 9 | Class 200 glass with Teflon liner | WPCL | AXYS | AXYS | WPCL | composite |
| Cormorant Eggs | PBDEs, % lipid, % moisture | 9 | Class 200 glass with Teflon liner | WPCL | AXYS | AXYS | WPCL | composite |
| Cormorant Eggs | PFCs and precursors, % moisture, % lipid | 9 | Amber glass OR HDPE | AXYS | AXYS | AXYS | AXYS | composite |
| Cormorant | Halogenate | 9[2] | Glass | SIU | AXYS | AXYS | Southern | composite |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Eggs | d carbazoles & % lipid | | container | | | | Illinois University | |

[1]Note: Each container, including cap, should be preweighed before any sample is added. Once sample is added, container with cap and sample is weighed again. The difference between these 2 masses is the sample weight which should then be recorded in the archive spreadsheet. Weights should be within 1% of sample mass.

[2]Note: Sample aliquots for halogenated carbazoles are the lowest priority. All other analyses (PCBs/PBDE, Mercury, selenium, PFC), short-term and long-term archives (Table 4a), and archive of individual eggs for future selenium analysis will be created first. Composites for halogenated carbazoles can be processed if all other analyses and archives, noted above, have been completed.

*Sample Mass Requirements*

1. **Required Preparation**: An appropriate aliquot of each individual egg should be put into the appropriate containers and stored until shipment to MLML. MLML will send appropriate containers to AXYS. Sample mass requirements for analysis of mercury are shown below.

   **Individual sample mass needs (cormorant mercury analysis)**

   - 59 individual samples - 5.0 g wet weight
   - 4 individual samples - 7.0 g wet weight (for lab dupes and MS/MSD)

2. **Required Preparation:** An appropriate aliquot of each cormorant composite should be put into the appropriate containers and stored for analysis of PFCs and precursors, % moisture, % lipid. Sample mass requirements for analysis of PFCs are shown below.

   i. 4.0 g wet weight per composite

3. **Required Preparation:** An appropriate aliquot of each composite should be put into the appropriate containers and stored until shipment to MLML. MLML will send appropriate containers to AXYS. Sample mass requirements for analysis of selenium are shown below.

   **Composite sample mass needs (cormorant selenium analysis)**

   - 7 comps – 6.0 g wet weight
   - 2 comps – 10.0 g wet weight (1 for lab dupe, 1 for MS/MSD)

4. **Required Preparation:** An appropriate aliquot of each sample composite should be put into the appropriate container and stored until shipment to DFG-WPCL. Sample mass requirements for analysis of PCB (209) and PBDE are shown below.

   **Composite sample mass needs (cormorant PCB(209), PBDE,% moisture, % lipid analyses)**

   - 7 comps – 20g wet weight (for field sample only)
   - 2 comps – 40g wet weight (1 for lab dupe, 1 for MS/MSD)

5. **Required Preparation:** Appropriate aliquots should be prepared for short and long-term archive per Table 4a below.

6. **Contingent Preparation**: An appropriate aliquot of each individual egg should be put into the appropriate containers for potential selenium analysis and stored until shipment to AMS. SFEI will order appropriate containers and send to ML ML for cleaning who will in turn send clean containers to AXYS. Sample mass requirements for selenium archive are shown below

    **Individual sample mass needs (cormorant selenium archive)**

    - 59 individual samples - 6.0 g wet weight
    - 4 individual samples - 10.0 g wet weight (for lab dupes and MS/MSD)

7. **If enough sample mass remains,** an appropriate aliquot of each sample composite should be put into a glass container and stored until shipment to Southern Illinois University. These samples should be filled after all other RMP samples and long-term archives are filled to ensure that there is enough mass. Sample mass requirements for analysis of Halogenated carbazoles are shown below.

    - 9 composites - 15 g or up to 25 g wet weight for each composite

*Sample Labeling*

Individual egg samples should be assigned unique sample IDs (“Egg#”) by USGS.

The following labeling scheme is suggested for **composite** samples:

C#-SiteCodeDC-YY-Analyte Group-Aliquot#

where
'C#' is the composite# (i.e. C1, C2, or C3)
'SiteCode' - WI for Wheeler Island, RB for Richmond Bridge, AB2 for Pond AB2
'DC' is short for double crested cormorant
'YY' is the two digit year of collection
‘Analyte Group’ is the analyte group listed in the Analyte Group table (below)
'Aliquot#' is the aliquot # or replicate number for the analyte group, e.g., C3-RBDC-16-Se-1; C3-RBDC-16-LTTV-1, C3-RBDC-16-LTTV-2, C3-RBDC-16-LTTV-3

The following labeling scheme is suggested for **individual** samples:

Egg#-C#-SiteCodeDC-YY-Analyte Group-Aliquot#

where
‘Egg #’ is the sample id for the individual egg
'C#' is the composite# (i.e. C1, C2, or C3) that the egg goes into
'SiteCode' - WI for Wheeler Island, RB for Richmond Bridge, AB2 for Pond AB2

'DC' is short for double crested cormorant
'YY' is the two digit year of collection
‘Analyte Group’ is the analyte group listed in the Analyte Group table (below)
'Aliquot#' is the aliquot # or replicate number for the analyte group

| **Analyte Group** | **Analyte Group Description** |
|---|---|
| Hg | Mercury analysis |
| Se | Selenium analysis or archive |
| STSe | Short term archive, HDPE |
| PCBPBDE | PCB/PBDE analysis |
| PFC | PFC analysis |
| LTTV | Long term archive, teflon vial |
| LTCV | Long term archive, cryovial |
| STGL | Short term archive, glass jar |
| STPP | Short term archive, polypropylene jar |
| PHCZ | Carbazole analysis |

**Forster’s Tern eggs**
USGS-WERC staff will follow the protocols in Attachment 2 to examine, measure, and prepare each egg.

Tern egg contents will be dried prior to analysis. USGS-WERC will measure moisture content in each individual egg, prior to drying, so that concentrations can be expressed on a wet weight basis. USGS will measure, weigh, and dissect each egg into a trace clean jar, and dry (in a drying oven) and homogenize by grinding the contents of each egg, on an individual basis, using trace clean equipment, avoiding contamination for analytes of interest (PBDE, mercury, and selenium).

Once each egg has been homogenized, an appropriate aliquot of each egg (see mass requirements below) should be put into the appropriate container for mercury analysis. Once individual aliquots for mercury have been processed, equal amounts of the remainder of the 7 eggs in each composite should be composited into one large sample. USGS will composite eggs using equal amounts of dried mass from each egg that has already been dried, ground, and homogenized.

USGS-WERC will ship eggs, at room temperature, to the appropriate laboratories. Table 3 below shows the planned analyses, number of samples, analyzing laboratory, sample type and sample container source for the Forster’s tern eggs. **In addition to the analyses, composites for future PBDE analyses should also be prepared if sufficient mass remains.** Note that all analyses are composites except for mercury. For mercury each egg will be prepared and analyzed individually.

**Table 3: Tern egg analysis plan**

| Species | Analyte | Number of Samples | Sample Container | Container Purveyor | Container Cleaning lab | Compositing/Processing lab | Analytical Lab | Sample Type |
|---|---|---|---|---|---|---|---|---|
| Tern Eggs | Se | 12 | 15 mL HDPE containers with HDPE unlined lids | SFEI | MLML | USGS | MLML | composite |
| Tern Eggs | Hg & % moisture | 84 | Provided by USGS | USGS | USGS | USGS | USGS | individual |
| Tern Eggs | PBDEs[1] | 12 | 20 mL Glass jars | SFEI | USGS | USGS | AMS (archive) | composite |

[1] Composites for future PBDE analysis should also be prepared if enough sample mass remains

*Sample Mass requirements*

1. USGS-WERC will take 2 g dry weight out of each individual tern egg to be processed and retained at USGS-WERC for mercury analyses.
2. An appropriate aliquot of each tern egg composite shall be processed and sent to MLML for selenium analysis. Sample mass requirements for analysis of selenium are shown below.
    - 10 comps – 1.5 g dry weight
    - 2 comps – 2.5 g dry weight
3. Any remaining mass from each composite will be put into separate glass jars for potential future analysis of PBDEs. These samples will be shipped to Paul Salop at Applied Marine Sciences. If possible, sample masses should be:
    - 10 composites - 6.25 g dry weight
    - 1 composite - 11.25 g dry weight
    - 1 composite - 8.75 g dry weight

*Sample Labeling*

Individual egg samples should be assigned unique sample IDs (aka, “Egg#”) by USGS.

The following labeling scheme is suggested for **composite** samples:

C#-SiteCodeFT-YY-AnalyteGroup-Aliquot#

where
'C#' is the composite# (i.e. C1, C2, or C3)

'SiteCode' -is the site code from the Site Code table
'FT' is short for Forster's tern
'YY' is the two digit year of collection
'Analyte Group' is the analyte group listed in the Analyte Group table (below)
'Aliquot#' is the aliquot # for that composite or the replicate number

The following labeling scheme is suggested for **individual** samples:

Egg #-C#-SiteCodeFT-YY-AnalyteGroup-Aliquot#

where
'Egg #' is the sample id for the individual egg
'C#' is the composite# (i.e. C1, C2, or C3)
'SiteCode' -is the site code from the Site Code table
'FT' is short for Forster's tern
'YY' is the two digit year of collection
'Analyte Group' is the analyte group listed in the Analyte Group table
'Aliquot#' is the aliquot # for that composite or the replicate number

| **Site Code Table for Tern Samples** | |
|---|---|
| **Site Code** | **Site Name** |
| A16 | Pond A16 |
| A2W | Pond A2W |
| AB2 | Pond AB2 |
| ELER | Eden Landing Ecological Reserve |
| HSRP | Hayward Shoreline Regional Park |
| NSWA | Napa-Sonoma Marsh Wildlife Area |
| A1 | Pond A1 |
| AB1 | Pond AB1 |
| A7 | Pond A7 |

| **Analyte Group** | **Analyte Group Description** |
|---|---|
| Se | Selenium analysis |
| PBDE | PBDE archive |
| Hg | Mercury analysis |

**General Notes:**
For samples that are to be analyzed for PFCs, the compositing lab (AXYS) and analytical lab (AXYS) will ensure that no Teflon coated equipment, containers, or wrappings will be used when handling these eggs and corresponding samples.

For the remaining samples, sample containers will be pre-cleaned and prepared by the appropriate laboratory.

For samples that are to be analyzed for mercury, only Teflon or glass/quartz containers with Teflon-lined caps will be used to store and transport these samples (SWAMP 2014).

Project samples will not be disposed of until all analyses are complete and analytical and QC results have been reviewed and approved by the SFEI Project Manager, RMP Lead Scientist and QA Officer.

## Analytical Methods

Detailed analytical method and QA/QC protocols can be found in Elements 13 and 14 of the 2015 Quality Assurance Program Plan for The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP 2015).

The number of samples of each species that will be analyzed for each parameter is listed in Tables 2b and 3. Lists of compounds that will be included in each organic compound parameter group are shown in Attachment 3.

## Archiving Strategy

Per the RMP Archive Protocol (Klosterhaus 2010), AXYS will fill and ship archive samples (store at -20C and ship frozen) of cormorant composites and individual eggs to allow for future analysis. Forster's tern egg composites will be archived for PBDEs. Short-term archive samples will be stored at Schaefer's Meats & Cold Storage in Oakland, CA. Long-term archive samples will be stored at the NIST facility in Charleston, SC. The number and volume of archive samples of each species, as well as sample container, storage, and transport information are included in Table 4a. Container and sample handling and transfer information are included in Table 4b.

SFEI will provide the preparation lab a copy of the Archive Samples Template which should be populated with the required archive preparation information. Directions for populating the template will be provided upon receipt of the template. The template should then be sent electronically to each of the archive storage facility managers, Rebecca Pugh for NIST and Paul Salop for Schaefer's. Each archive storage facility manager will fill out the columns required with storage facility information and then return the template to DS@sfei.org.

Samples shipped to the archive facilities will be accompanied by COCs, which will include a unique entry for each archive container and include the following information: composite ID, station name, species, sample date, and container type and number.

Archives placed in short-term storage with AMS will be assigned a lot number when they are added to the freezer. The lot numbers, along with the information on the COCs, will be provided to SFEI by AMS in the Archive Samples Template.

Archives placed in long-term storage will require specialized labels for sample identification. SFEI will provide NIST with a completed template containing the species, composite ID + aliquot number, sample date, container type and container size. NIST will send AXYS a set of pre-printed labels containing the following information: the barcode, species, composite ID + aliquot number and sample date. NIST will also provide pre-cleaned containers to AXYS.

NIST will add additional sample identifiers to the archive sample information provided by AXYS, and will provide this compiled archive data to SFEI in the Archive Samples Template. These additional sample identifiers will include a NIST assigned “Globally Unique Aliquot ID.”

**Table 4a. Bird Eggs Archiving Strategy for Cormorant Eggs**

| Samples | # of containers per composite | Tissue mass per container (g wet wt) | Container | Storage Purpose | Storage Location | Storage Temperature | Total Mass needed (g wet wt) |
|---|---|---|---|---|---|---|---|
| 3 sites, 3 composite per site = 9 composites | 3 | 15 | 22 ml Teflon vial[a] | Long-term time trends | NIST | -150 °C | 405 |
| | 2 | 8 | 10 ml PP cryovial[a] | Long-term time trends | NIST | -150 °C | 144 |
| | 3 | 15 | 60 ml glass jar[b] | Time trends, CECs, QA/QC | Schaefer's | -18 °C | 405 |
| | 2 | 15 | 30 ml PP jar[c] | Time trends, CECs, QA/QC | Schaefer's | -18 °C | 270 |
| Se Individual Eggs (n=63) | 1 | 59 individual samples - 5.0 g wet weight<br><br>4 individual samples - 7.0 g wet weight (for lab dupes and MS/MSD) | 15 ml, precleaned HDPE with linerless HDPE cap | Se TMDL | Schaefer's | -18 °C | 323 |

PE = polyethylene; PP = polypropylene; CECs = contaminants of emerging concern; QA/QC = quality assurance/quality control

a = Pre-cleaned/PC class jars, Teflon-lined lid, supplied by ESS Vial (Oakland, CA)
b = Pre-cleaned by AXYS Analytical or other designated laboratory, linerless lid, supplied by Fisher Scientific
c = Pre-cleaned by NIST

**Table 4b. Bird Eggs Archiving Process for Cormorant Samples**

| Container | Supply and Cleaning Chain | Sample Preparation Site | Sample Shipping and Storage[1] | Archive template |
|---|---|---|---|---|
| 22 ml Teflon vial | SFEI orders and ships to NIST. NIST cleans and ships to AXYS. | AXYS fills container in the lab. | AXYS ships to NIST Charleston frozen at -20C. | All fields will be completed by AXYS except Storage ID and storage date, which will be completed by NIST. NIST will send sample receipt confirmation and completed archive template to SFEI. |
| 10 ml PP cryovial | SFEI orders vials in bulk and they are stored at NIST. SFEI requests the number of vials to be cleaned and NIST cleans and ships to AXYS. | AXYS fills container in the lab. | AXYS ships to NIST Charleston frozen at -20C. | |
| 60 ml glass jar | SFEI orders and ships to AXYS. | AXYS fills container in the lab. | AXYS ships to AMS frozen at -20C. AMS drops off at Schaefer's in Oakland. | All fields will be completed by AXYS except Storage ID and storage date, which will be completed by AMS. AMS will send sample receipt confirmation and completed archive template to SFEI. |
| 30 ml PP jar | SFEI orders and ships to AXYS. | AXYS fills container in the lab. | AXYS ships to AMS frozen at -20C. AMS drops off at Schaefer's in Oakland. | |
| 15 ml, precleaned HDPE with linerless HDPE cap | SFEI orders and ships to MLML. MLML cleans and ships to AXYS. | AXYS fills the container in the lab. The start weight should be recorded in the archive sample template. | AXYS ships to AMS frozen at -20C. AMS drops off at Schaefer's in Oakland.<br><br>Note: these samples will only be archived if sufficient material is available. | |

[1]Sample shipper is responsible for ensuring that samples are maintained at the appropriate temperature during transport. Any deviation should be noted and reported to SFEI.

**Table 4c. Bird Eggs Archiving Strategy for Tern Eggs**

| Samples | # of containers per composite | Tissue mass per container (g wet wt) | Con-tainer | Storage Purpose | Storage Location | Storage Temperature | Total Mass needed (g dry wt) |
|---|---|---|---|---|---|---|---|
| 4 sites, 3 composites per site for PBDEs | 1 | 10 composites - 6.25 g dry weight<br><br>1 composite - 11.25 g dry weight<br><br>1 composite - 8.75 g dry weight | 20 ml glass; PC class | short term | Schaefer's | -18 | 82.5 |

**Reporting**

USGS-WERC will use their own field sheets and COCs. USGS-WERC will provide electronic versions of field sheets and COCs to SFEI. In addition, USGS-WERC will enter field collection information in the Locations and Composite tabs of the CEDEN Excel template and will provide a copy of these records, electronically, to SFEI.

AXYS (cormorant) and USGS-WERC (tern) will provide composite information to SFEI in the Composite tab of the modified CEDEN template provided by SFEI, as well as a copy of the COCs received from field staff and those provided to the analytical labs.

For samples sent to archive storage, AXYS will complete the Archive Samples Template following the instructions provided by SFEI. The template should then be sent electronically to each of the archive storage facility managers, Rebecca Pugh for NIST and Paul Salop for Schaefer's. Each archive storage facility manager will fill out the columns required for storage facility information and will return it to DS@sfei.org.

SFEI will provide all laboratories with a Lab EDD template following the CEDEN database format. Analytical results will be entered in this Lab EDD format by each lab and provided to SFEI in digital format.

**Data Analysis**

All sample results will be reported on a wet weight basis. Results may be converted to a dry weight basis using individual egg or composite moisture measurements. Results may be converted to a fresh weight weight basis using individual egg length and breadth (width) measurements and two species-specific constants derived from the literature: Kv (egg volume coefficient) and typical fresh wet weight density (Ackerman et al., 2013; Hoyt, 1979). Specifically wet weight results can be converted to fresh wet weight results using the following equations,

(1) $Xfww = (WW / FWW) * Xww$

(2) $FWW = V * Dfww$

(3) $V = Kv * L * B^2$

where

FWW = fresh wet weight
WW = wet weight
Xfww = chemical concentration on a fresh wet weight basis
Xww = chemical concentration on a wet weight basis
V = volume
Dfww = density of a typical freshly laid egg (g / mL)
Kv = species-specific egg volume coefficient

L = individual egg length
B = individual egg breadth (ie. width)

Kv and wet weight density for Forster's Terns are provided in Ackerman et al., 2013. Kv and wet weight density for Double-Crested Cormorants can be determined from the literature.

**References**

Ackerman, JT, MP Herzog, and SE Schwarzbach. 2013. Methylmercury is the predominant form of mercury in bird eggs: a synthesis. Environmental Science and Technology 47:2052-2060.

Hoyt, D.F. 1979. Practical methods of estimating volume and fresh wet weight of bird eggs. Auk 96:73-77.

SFEI. 2015. Quality Assurance Program Plan for The Regional Monitoring Program for Water Quality in San Francisco Bay. http://www.sfei.org/documents/quality-assurance-program-plan-regional-monitoring-program-water-quality-san-francisco-b-0

Surface Water Ambient Monitoring Program. 2014. Study of Lakes and Reservoirs with Low Concentration of Contaminants in Sport Fish - Final Quality Assurance Program Plan. http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/lakes_study/bog_low_conc_qapp_final.pdf

# Attachment 1
# Shipping Information

| Laboratory Name | Laboratory Contact | Contact Email | Contact Phone Number | Shipping Address |
|---|---|---|---|---|
| AXYS Lab | Kalai Pillay | kpillay@axys.com | 250-655-5832, | 2045 Mills Road W, Sidney, BC, V8L 5X2 |
| MLML-MPSL | Autumn Bonnema | bonnema@mlml.calstate.edu | 831-771-4175 | 7544 Sandholdt Road<br>Moss Landing, CA 95039 |
| DFW-WPCL | Mary Curry | Mary.Curry@wildlife.ca.gov | 916-358-4398 | 2005 Nimbus Rd.<br>Rancho Cordova, CA 95670 |
| AMS | Paul Salop | salop@amarine.com | 925-373-7142 | Applied Marine Sciences<br>4749 Bennett Dr., Ste. L<br>Livermore,<br>CA 94551 |
| NIST | Rebecca Pugh | Rebecca.Pugh@noaa.gov | 843-762-8952 | National Institute of Standards and Technology<br>Hollings Marine Laboratory<br>331 Ft. Johnson Rd.<br>Charleston, SC 29412 |
| USGS-WERC | Josh Ackerman | jackerman@usgs.gov | (530) 669-5087 | Josh Ackerman<br>USGS Dixon Field Station<br>800 Business Park Drive, Suite D<br>Dixon, CA 95620 |

# Attachment 2

# STANDARD OPERATING PROCEDURE

# AVIAN EGG HARVEST, EMBRYO EXAMINATION AND SHELL THICKNESS DETERMINATION WITH INTENT TO SAVE CONTENTS FOR CHEMICAL ANALYSIS

## SACRAMENTO FISH AND WILDLIFE OFFICE
## ENVIRONMENTAL CONTAMINANTS DIVISION

The following describes the process of harvesting avian eggs in the laboratory. The goal of this description is standardize procedures for harvesting avian eggs in order to collect a standardized set of data on whole eggs, embryos and shells while minimizing the possibility of laboratory contamination of samples. Field collection protocols are considered separately and vary with species and study objectives.

The supplies needed for the procedures include:

1. WHOLE EGG MEASUREMENTS: distilled-deionized water, volumeter, egg candler, Kimwipes, laboratory balance (to 0.05 g increments), vernier caliper (graduated to 0.0l mm).

2. EGG HARVEST: glass jars of appropriate size (chemically-cleaned and with TFE cap-liners), chemically-rinsed scalpel, lead pencil, and technical pen.

3. SHELL THICKNESS MEASUREMENT: Federal 35 comparator with rounded contacts (graduated to 0.01 mm - estimatable to nearest .001 mm).

EGG MEASUREMENT PROCEDURE:

1. If possible, eggs should be candled to determine if cracks are present in the shell. Any cracked egg should not be rinsed or immersed in water as this may contaminate the sample.

2. Store eggs in a refrigerator if they cannot be processed immediately after collection. DO NOT FREEZE whole eggs since this will crack the shell.

3. If an egg is not cracked and is dirty (soil, feces) it should be cleaned with a Kimwipe and distilled-deionized water that is at, or near the temperature of the egg.

4. Write the sample ID number on both ends of the eggshell with a dull pencil (both IDs must be legible).

5. Record any remarkable characteristics of the egg (e.g. cracked, dented, discolorations, small in size, etc.).

6. Record the MASS (g) OF THE WHOLE EGG, then measure the LENGTH (mm) and BREADTH (mm) of the egg with calipers at their greatest dimensions. (To obtain an accurate measurement of length, one must ensure that the caliper jaws are parallel to the longitudinal axis of the egg. For the breadth measurement, the jaws must be held perpendicular to the longitudinal axis of the egg).

7. Determine and record the EGG VOLUME (cm3), the method of choice will depend on whether the shell is intact or cracked.

A. INTACT SHELL: For eggs with intact shells, determine the EGG VOLUME using the water displacement technique outlined below.

Place a volumeter next to and above the pan of a laboratory balance. Set a collection vessel on the balance's pan under the side arm of the volumeter. Next, place a wire loop in the volumeter. Fill the volumeter with distilled-deionized water until it flows freely from the volumeter side arm (REMEMBER, the temperature of the water should be as close to the temperature of the egg as possible as this will minimize water movement across the eggshell pores.). When the water stops flowing, empty the receptacle and return it to the balance pan. Tare the water receptacle. Gently raise the wire loop and place the egg on it. Gently lower the egg until it is completely submersed (lower the egg as quickly as possible without overflowing the volumeter, or breaking the egg). The weight of the displaced water equals the volume (cm ) of the egg. Repeat this procedure three (3) times for each egg and report the average value.

B. CRACKED SHELL: For eggs that are cracked or dented, EGG VOLUME is estimated using the LENGTH and BREADTH measurements and an equation from the published literature (e.g. Westerskov 1950, and Stickel et al. 1973)or one developed from our own field measurements.

EGG HARVEST: (note all tools used in egg harvest and embyro exam must be cleaned between egg exams. See notes on tool cleaning. Investigators should wear surgical gloves and change gloves between eggs.)

1. VENT EGG IF NECESSARY. For eggs with a strong odor (indicating advanced decomposition of the contents), it is advisable to vent the egg before attempting to open it (explosions are possible). With safety glasses in in place, gently insert a chemically-clean needle into the blunt end of the egg. Use gentle but steady pressure to pierce the shell.

2. OPEN WINDOW AT BLUNT END OF THE EGG. Tare a chemically- clean jar and loosen the lid. Rest the egg lengthwise on an appropriate surface (compatible with the analyses requested). For mercury, selenium or organochlorines a clean glass petri dish is recommended. Using a clean sharp scalpel, gently score the egg about the blunt end of the egg. Apply gentle, steady pressure and make several rotations. Recurved surgical scissors may also be used to cut a small window into the blunt end of the egg. If candling of the egg revealed an advanced state of incubation with air cell development try and remove shell from just above the air cell. Membrane may need to be peeled back to allow further inspection of the embryo.

3. INSPECT EMBRYO POSITION IN THE EGG. Visually inspect the egg contents within the window and note the size of the air cell. This window is used to assess whether the position of the embryo in the egg is normal. Note embryo position and whether the embryo has pipped into the air cell. Normal position of the embryo is with the head in the blunt end of the egg, with the head under the right wing and with the beak pointed toward the air cell. If incubation stage is very late, ie. just prior to pip from the shell, the embryo beak is in the air cell to allow pulmonary respiration to begin. There are six mal-positions of the avian embryo. Mal-positions include: I. head between thighs, II. head in small end of egg, III. head under left wing, IV. embryo rotated so that beak not directed toward air cell, V. feet over head, VI beak over right wing. Mal-positioned embryos usually do not hatch, and positions I, III, and V are usually completely lethal.

4. OPEN EGG. Using scissors or scalpel make transverse cuts from the blunt end to the narrow end of the egg to facilitate egg opening. Again inspect embryo position and note age of the embryo. To estimate age of the embryo use stages of incubation from literature. The model reference for aging embryos is Lillies development of the Chick chapter 3. Good day-by-day embryo stage data with pictures exists for chickens, mallards, kestrels, cockatiels and avocets and stilts (see files). If no embryo can be found examine the yolk for the presence of a blastodisc. If fertile this will appear as a white donut shape floating on top of the yolk. If infertile no distinct donut will be apparent. Measure length of the embryo if only a few days old. Note presence or absence of eyes, and limbs or limb buds, note presence and number of digits on the feet, measure length of tarsus and upper mandible. Look for evidence of internal hemorrhage, edema, brain swelling, or failure of the body wall to completely close. Try and minimize handling of the embryo to the degree possible and conduct as much as possible the above exam in the half shell. Use clean forceps, and beware of cross contamination. Pour the contents into the opened jar. If necessary use a chemically clean Teflon spatula to scrape any remaining contents into the jar (BE CAREFUL not to tear the shell membrane when using spatula).Record presence or absence of an embryo, estimated age of embryo, abnormalities,

4 EGG CONTENTS MASS (g) Measure and record the weight in grams of the tared jar.

5. Label jar with SAMPLE ID and SAMPLE MASS (place one label on the lid and the other on the jar itself), and immediately store the sample in the freezer.

6. Rinse the interior of the shell halves with tap water being careful not to tear the membrane, or erase the sample IDs. After the shells dry, use a technical pen to remark the shells with their sample IDA Store the shells in a cool dry place for at least 30 days, or until they have attained a constant mass. (Recycled egg cartons serve as excellent storage containers for egg shells. One tip to ensure that shells do not migrate from their respective compartments, is to place a folded sheet of paper over the shells before closing the carton.

SHELL THICKNESS-MEASUREMENT:

1. Determine the EGGSHELL MASS (to nearest 0.001 g) of dried shells.

2. Measure EGGSHELL THICKNESS using our federal 35 comparator. Take thickness measurements of each shell-half along the equator at five places. Gently raise and lower the arm of comparator when obtaining measurements. Minimize influence of shell shape and curvature on the measurement taken. Report the average of all TEN measurements as the final thickness measurement. If the membrane has separated from the shell, take measurements without the membrane but be sure to make note of this on the data sheet. If possible then obtain measurement of membrane fragments.

3. Calculate the Ratcliffe Index (Ratcliffe 1967) with the following formula:

$$\text{INDEX} = \frac{\text{THICKNESS}}{\text{EGGSHELL MASS (mg)}} \Big/ \text{EGG LENGTH (mm) x EGG WIDTH (mm)}$$



EGG COMPOSITING PROCEDURE

Once all the physical measurements have been made, laboratories will homogenize each egg individually. Once homogenized, an aliquot will be taken from each individual homogenate for Mercury analysis. After this aliquot has been taken, laboratories will begin homogenizing the 21 eggs from each site. For each site, laboratories will randomly select 7 of the 21 eggs and homogenate them into a composite using approximately equal amounts of mass from each egg. This process will be repeated for each site until all eggs have been randomly composited.

# Attachment 3
# Expected Analytes for Organic Chemical Analyses

**Table A.** Expected PCB And ancillary measures, fraction, and unit for double crested cormorant samples. The following congeners will be reported as coeluting pairs: PCB 028/31, PCB 056/60, PCB 138/158.

| AnalyteName | MethodName | FractionName | UnitName |
|---|---|---|---|
| **Lipid** | **EPA 8082M** | **Total** | **% ww** |
| **Moisture** | **EPA 8082M** | **Total** | **% ww** |
| **PCB 008** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 018** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 027** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 029** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 028/31** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 033** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 044** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 049** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 052** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 056/60** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 064** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 066** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 070** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 074** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 077** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 087** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 095** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 097** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 099** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 101** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 105** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 110** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 114** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 118** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 126** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 128** | **EPA 8082M** | **Total** | **ng/g ww** |

| | | | |
|---|---|---|---|
| **PCB 137** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 138/158** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 141** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 146** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 149** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 151** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 153** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 156** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 157** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 169** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 170** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 174** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 177** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 180** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 187** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 189** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 194** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 195** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 198** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 199** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 200** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 201** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 203** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 206** | **EPA 8082M** | **Total** | **ng/g ww** |
| **PCB 209** | **EPA 8082M** | **Total** | **ng/g ww** |

**Table B.** Expected PBDE And ancillary measures, fraction, and units for double crested cormorants. The following congeners will be reported as coeluting pairs: PBDE 017/25, PBDE 028/33, PBDE 200/203.

| AnalyteName | MethodName | FractionName | UnitName |
|---|---|---|---|
| Lipid | EPA 8081BM | Total | % ww |
| Moisture | EPA 8081BM | Total | % ww |
| PBDE 017 | EPA 8081BM | Total | ng/g ww |
| PBDE 025 | EPA 8081BM | Total | ng/g ww |
| PBDE 028 | EPA 8081BM | Total | ng/g ww |
| PBDE 030 | EPA 8081BM | Total | ng/g ww |
| PBDE 033 | EPA 8081BM | Total | ng/g ww |
| PBDE 047 | EPA 8081BM | Total | ng/g ww |
| PBDE 049 | EPA 8081BM | Total | ng/g ww |
| PBDE 066 | EPA 8081BM | Total | ng/g ww |
| PBDE 085 | EPA 8081BM | Total | ng/g ww |
| PBDE 099 | EPA 8081BM | Total | ng/g ww |
| PBDE 100 | EPA 8081BM | Total | ng/g ww |
| PBDE 138 | EPA 8081BM | Total | ng/g ww |
| PBDE 153 | EPA 8081BM | Total | ng/g ww |
| PBDE 154 | EPA 8081BM | Total | ng/g ww |
| PBDE 179 | EPA 8081BM | Total | ng/g ww |
| PBDE 183 | EPA 8081BM | Total | ng/g ww |
| PBDE 184 | EPA 8081BM | Total | ng/g ww |
| PBDE 188 | EPA 8081BM | Total | ng/g ww |
| PBDE 190 | EPA 8081BM | Total | ng/g ww |
| PBDE 200 | EPA 8081BM | Total | ng/g ww |
| PBDE 201 | EPA 8081BM | Total | ng/g ww |
| PBDE 202 | EPA 8081BM | Total | ng/g ww |
| PBDE 203 | EPA 8081BM | Total | ng/g ww |
| PBDE 206 | EPA 8081BM | Total | ng/g ww |
| PBDE 207 | EPA 8081BM | Total | ng/g ww |
| PBDE 208 | EPA 8081BM | Total | ng/g ww |
| PBDE 209 | EPA 8081BM | Total | ng/g ww |

**Table C.** Expected PFAS and ancillary measures for double crested cormorants

| AnalyteName | MethodName | FractionName | UnitName |
|---|---|---|---|
| Lipid | Multiple | Total | % ww |
| Moisture | Multiple | Total | % ww |
| Perfluorobutanesulfonate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorobutanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorodecanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorododecanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluoroheptanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorohexanesulfonate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorohexanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorononanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorooctanesulfonamide | AXYS MLA-043 | Total | ng/g ww |
| Perfluorooctanesulfonate | AXYS MLA-043 | Total | ng/g ww |
| Perfluorooctanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluoropentanoate | AXYS MLA-043 | Total | ng/g ww |
| Perfluoroundecanoate | AXYS MLA-043 | Total | ng/g ww |