



Kingfish Maine Inc
9 Dun Garvin Road
Jonesport, ME 04649
May 6th 2026

Maine Department of Environmental Protection
Bureau of Water Quality
Division of Water Quality Management
State House 17
Augusta, Maine 04333-0017

MPDES ID# ME0037559
WDL# W009238-6F-A-N

To whom it may concern,

Enclosed are documents for the renewal of Kingfish Maine Inc's, Maine Pollutant Discharge Elimination System (MPDES) permit #ME0037559, Maine Waste Water Discharge License (WDL) #W009238-6F-A-N.

Documents included are as follows: General application form, Food processing form, EPA form 2D, Fish rearing facility form, Outfall form and all relevant attachments.

Sincerely

A handwritten signature in black ink, appearing to read "T. Sorby", written over a horizontal line.

Tom Sorby

Operations Manager

Kingfish Maine Inc



Maine Department of Environmental Protection

**General Application for
Waste Discharge License (WDL) /
Maine Pollutant Discharge Elimination System (MEPDES) Permit**

Regulatory requirements for the preparation and filing of applications may be found in Chapters 2, 521 and 522 of the Department's rules.

GENERAL INSTRUCTIONS

1. This general form is to be used to apply for permit coverage for the discharge of pollutants to waters of the State, from all sources except from privately owned discharges < 2,000 GPD subject to the Over Board Discharge Program requirements.
2. A cover letter must be submitted with the application. The cover letter must include: name of the facility, the date of submission, a list of application materials, and for renewals and modifications, please supply MEPDES ID # and WDL #.
3. Applicants are responsible for publishing public notice of their application at the time it is filed with the Department. See pages 8 and 9.
4. For a proposed new waste discharge license for a discharge of more than 25,000 gallons per day or a project involving licenses from more than two bureaus in DEP, an applicant must conduct a public informational meeting before submitting an application to the Department. See page 8.
5. In some circumstances an applicant must have a pre-application or pre-submission meeting with the Department prior to filing of an application. See page 10.
6. At the time an application is filed with the Department, a copy must be provided to the municipal office and notice provided to all abutters. See page 8.
7. Application fees must be paid at the time an application for a **new** discharge or permit is filed. Contact the Department for additional information and calculation of the fee amount. For existing discharges, fees are charged on an annual basis and application fees are not required with an application for permit renewal.
8. Attach additional sheets as necessary in answering specific questions. Be sure to number each sheet to identify the question to which it pertains.
9. Failure to fully complete all required forms or to pay necessary application fees will result in the application being rejected.
10. After completing the application, submit one .pdf document to WaterPermits.DEP@maine.gov and one paper copy with a wet-ink-signature to:

Maine Department of Environmental Protection
Bureau of Water Quality
Division of Water Quality Management
State House Station 17
Augusta, Maine 04333-0017

11. Please read the entire application form before furnishing any information. If you need any assistance in filling out the form or required attachments, please contact the Department at the above address or by calling (207) 287-7688.

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This application is for a:

☐ New discharge ☒ Renewal ☐ Increased discharge ☐ Transfer of owner ☐ Modification ☐ Other: _____

If assigned: MEPDES#: ME0037559

WDL #: W - 009238 - 6F - A - N

FACILITY AND APPLICANT INFORMATION

1. Facility Information (911 Address):

Facility/Legal Name: Kingfish Maine, Inc.

Street Address 9 Dun Garvin Road

Municipality: Jonesport

Facility Latitude/Longitude Coordinates:

Facility Type: ☐ Federal ☐ State ☐ Public ☒ Private ☐ Other

Receiving Water Name(s) Chandler Bay

Zip: 04649

2. Operator Information:

Name: Vincent Erenst

Telephone: +31 611137937

Address: Oost Zeedijk 13

Municipality: Kats

E-mail: v.erenst@thekingfishcompany.com

State: Zeeland Zip: 4485 PM

NOTE: If a wastewater treatment facility is operated under a contract with third party, the contract for services must be reviewed and approved by the Department prior to contact initiation as described in Ch. 531 section 5(D).

3. Applicant/ Owner Information (If different from Operator):

Name:

Telephone:

Address:

e-mail:

Municipality:

State: Zip:

4. Point of Contact/Responsible Party as described at 40 CFR 122.22(a):

Name: Vincent Erenst

Telephone: +31 611137937

Address: Oost Zeedijk 13

e-mail: v.erenst@thekingfishcompany.com

Municipality: Kats

State: Zeeland Zip: 4485 PM

5. Please provide the SIC / NAICS codes for the facility: NAIC 112511 SIC 0273

6. Briefly describe the nature of business and activities requiring WDL /MEPDES Permit:

Kingfish Maine is submitting an application for a WDL/MEPDES permit in order to operate a proposed recirculating aquaculture facility growing yellowtail kingfish, *Seriola lalandi*.

7. Is this facility located on Indian lands, or does it discharge to waters that run through Indian lands?

Yes ☐ No ☒

If so, which lands or waterway?

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ELECTRONICALLY SIGNED DECISIONS

8. Electronically signed decision options. To expedite processing of applications and reduce paper usage, **all final decisions on an application will be electronically signed by the Commissioner (or his/her designee) and will be sent to the respective e-mail addresses provided for the Applicant and the Point of Contact listed on this application, unless the “Handwritten” signature box is selected below.**

For the purposes of this application/permit, all signatures will be acquired by:

Handwritten (wet) Signature: ☐

I hereby decline to receive an electronically signed decision on the WDL/MEPDES permit via e-mail and choose to receive manually signed (hand written) decision via regular (U.S. Postal)mail.

Sign to decline electronic signatures

(Applicant):

Date:

SUPPORTING MATERIALS AND REQUIRED ATTACHMENTS

9. For **new and transfer applications only** from privately-owned facilities, include:

☐ A Certificate of Good Standing issued by the Maine Secretary of State.

☐ Proof of Title, Right or Interest (TRI) in the property on which the treatment system and outfall pipes and structures are or will be located. See Chapter 2 of the Department’s rules for TRI criteria.

10. For **transfer applications only**, answer the following then skip to the Certification on page 7.

A. Name of current/former owner: N/A

B. Describe any planned changes in the current discharge:

N/A

C. Provide a statement describing the technical and financial capacity to comply with the current permit conditions and applicable laws and rules. (use a separate sheet)

11. Unless submitted previously and there have been no changes, provide a topographic map (or other map if a topographic map is unavailable) extending one mile beyond the property boundaries of the source, depicting the facility and each of its intake and discharge structures.

12. If modification of an existing permit is being requested, attach a statement describing the nature of the modification and the reasons or circumstances necessitating the change. Include any relevant modified process flow schematics available.

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13. Attachments for specific activities and circumstances. For each specific question, check 'Yes' or 'No' to indicate if the statement is applicable to a discharge or activity described in this application.

Where 'Yes' is checked, attach the applicable form. **To open form, right click on the name then open the new tab/window.**

Specific Question	Yes	No	Applicable Form
A. Is this facility a publicly owned treatment works treating sanitary wastewaters?	☐	☒	EPA 2A Form: Publicly Owned Treatment Facilities
B. Does this facility generate, treat, dispose of or land apply sewage sludge?	☐	☒	EPA Form 2S: New and Existing Treatment Works Treating Domestic Sewage
C. Does this application seek authorization for the addition of transported wastes in wastewater treatment facilities?	☐	☒	DEP Form: Addition of Transported Wastes in Wastewater Treatment Facilities. (DEPLW1035)
D. Is this application for a municipal/industrial subsurface wastewater disposal system, (ie.septic system)?	☐	☒	DEP Form:Subsurface Wastewater Disposal System (DEPLW0313-B2005)
E. Is this application for a land surface (including spray irrigation and snow making) wastewater disposal system?	☐	☒	DEP Form: Application for Surface Wastewater Disposal Systems (DEPLW0450-B2005)
F. Is this a food processing facility or POTW that treats food processing wastewaters?	☒	☐	DEP Form: Food Processing Facilities (DEPLW1999-19)
G. Is this an existing discharge of industrial process wastewater?	☐	☒	EPA Form: 2C
H. Is this to be a new discharge of industrial process wastewater? *	☒	☐	EPA Form: 2D
I. Is this a discharge of non-contact cooling water?	☐	☒	EPA Form: 2E
J. Is this discharge of storm water associated with an industrial activity?	☐	☒	EPA Form: 2F
K. Is this a discharge of non-process wastewater?	☐	☒	EPA Form 2E
L. Is this an individual application for a Net Pen Aquaculture Facility?	☐	☒	DEP Form: Net Pen Aquaculture - Supplemental Information for Individual Net Pen Permit (DEPLW0604-B)
M. Is this a fish hatchery or rearing facility?	☒	☐	DEP Form: Fish Rearing Facilities (DEPLW1999-18)
N. Does this application involve a new or modified outfall structure?	☒	☐	DEP Form: Outfall Information (DEPLW0102)
O. Is this application for a waste snow dump?	☐	☒	DEP Form: Supplemental Information for Snow Dumps (DEPLW0249)
P. Is this application for a Concentrated Feeding Operation (CAFO)?	☐	☒	DEP Form: Maine Waste Discharge License and Livestock Operating Permit Concentrated Animal Feeding Operation (CAFO) (DEPLW0569)
Q. Is the source of treatment an overboard discharge (OBD) >= 2000 GPD?	☐	☒	DEP Form: General Application Overboard Discharge (DEPLW107A)
R. Is this for a commercial OBD?	☐	☒	DEP Form: Supplemental App for Commercial OBD (DEPLW1076A)

* PROCESS WASTEWATER means any water which, during manufacturing or processing, comes into direct contact with or results from the production or use of any raw material, intermediate product, finished product, byproduct, or waste product.

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14. Identify all environmental permits or construction approvals and dates applied for or received under any of the following programs (if the permit has already been issued, just include the issued date):

Permit/ Construction Approval Type	Yes	No	Date Applied or Date Issued
A. Hazardous Waste Management program under the Resource Conservation and Recovery Act (RCRA)	☐	☒	Date Applied / Issued:
B. Underground Injection Control program under the Safe Drinking Water Act (SDWA)	☐	☒	Date Applied / Issued:
C. NPDES program under Clean Water Act (CWA)	☒	☐	Date Applied / Issued: June 25 2021
D. Prevention of Significant Deterioration program under the Clean Air Act	☐	☒	Date Applied / Issued:
E. Nonattainment program under the Clean Air Act	☐	☒	Date Applied / Issued:
F. National Emission Standards for Hazardous Air Pollutants preconstruction approval under the Clean Air Act	☐	☒	Date Applied / Issued:
G. Ocean dumping permits under the Marine Protection Research and Sanctuaries Act	☐	☒	Date Applied / Issued:
H. Dredge or fill permits under section 404 of the CWA	☒	☐	Date Applied / Issued: Jun 21 2022
I. Other relevant environmental permits, including State permits. Enter in boxes below:	☒	☐	Date Applied / Issued:
J. NRPA			Date Applied / Issued: Nov 12 2021
K.. SLODA			Date Applied / Issued: Nov 12 2021
L. Submerged Lands Lease			Date Applied / Issued: Sep 25th 2020
M.			Date Applied / Issued:
N.			Date Applied / Issued:
O.			Date Applied / Issued:

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OUTFALL AND TREATMENT INFORMATION

Use attachments as necessary to provide details for each discharge point and treatment system.

15. Attach a plan of the treatment facility or discharge sources showing the location of each discharge in relation to the receiving water. Include all combined sewer overflow (CSO) points, bypasses, emergency discharge points at pump stations, etc.

See Attachment 1

If there are intermittent or seasonal discharges (other than CSOs), please describe the nature, circumstances, and duration of each discharge.

N/A

16. Briefly describe current treatment facilities or methods for each discharge.

Effluent water treatment including sources and uses is detailed in Attachment 2.

17. If this is a renewal application, please describe all significant modifications to the treatment facilities (and collection system if applicable) since the last permit application was filed.

N/A

18. Are new or expanded treatment facilities or outfall structures being proposed? ☒ Yes ☐ No

If so, please include a construction schedule. Plans and specifications must be submitted to the Department for review and approval prior to construction of the facilities.

See Attachment 3

- a. The outfall number of each outfall affected;
- b. A narrative description of each required improvement;
- c. Schedule of expected dates for the following:
 1. Commencement of the construction
 2. Completion of the construction
 3. Commencement of discharge; and
 4. Attainment of operational level
- d. A description of permits and clearances concerning other Federal and/or State requirements

19. If this application is for a new or increased discharge, include a statement that: See Attachment 4

- a. describes in detail the nature of and reason for the requested increase in pollutant loading to the receiving water;
- b. lists out the flows, sources of pollution, and treatment technology;
- c. if the Department determines that the discharge will diminish the remaining assimilative capacity of the receiving water, demonstrate that alternative methods to reduce or eliminate the increased discharge are not feasible. Include engineering and economic analyses that consider alternative methods of production, process controls, wastewater minimization methods, improved wastewater treatment methods and alternate disposal sites; and
- d. if the Department determines that the discharge will diminish the remaining assimilative capacity of the receiving water, demonstrate that the increased pollutant load will result in important social and economic benefits to the State.

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CERTIFICATION

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.

Additionally, by signing below, I certify that

- (1) A notice of this application has been made by publication in Machias Valley Observer newspaper circulated in the area where the project site is located on or about Jonesport (a copy of the advertising form is included in this application);
- (2) A notice has been sent by certified mail or Certificate of Mailing to owners of land abutting the discharge site (a copy of the list of abutters is included in this application)
- (3) A notice and a copy of this application have been provided to the clerk of the municipality(ies) where the discharge is located.
- (4) Further, if this is a new discharge over 25,000 gallons per day, a public meeting attended by approximately N/A members of the public was held on N/A.

The forgoing steps have been taken in accordance with the instructions attached to this application and the provisions of Chapters 2 and 522 of the Department's rules.

By:

Signature:

Signed by:

FAB95CB0527B460...

Date: 5/12/2026

Printed Name: Jean-Charles Valette

Title: CFO

Assisting Parties. If the applicant has been assisted in preparing this application, the person assisting must sign below.

Signature:

Tom Sorby
Digitally signed by Tom Sorby
Date: 2026.05.06 10:53:20
-04'00'

Date: 5/6/26

Printed Name: Tom Sorby

Telephone: 502-614-9078

Affiliation:

Address: Kingfish Maine Inc

Town: Franklin

State: Me Zip: 04634

Professional Registration or Certification:

Note: Signatures must be from a president, secretary, treasurer, or vice-president of the corporation in charge of a principal business function, the manager of one or more manufacturing, production, or operating facilities, provided, the manager is authorized to make management decisions which govern the operation of the regulated facility, or by either a principal executive officer or ranking elected official.

See following pages for requirements on public notice, public meetings, pre-applications meetings and pre-submission meetings.

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Instructions for providing notices of the application. For all applications, the first 3 items must be completed. If the application is for a new discharge, you must also complete item 4.

1. *Publication of Public Notice.* Applicants for waste discharge permits are required to publish a public notice that the application is being filed with the Department of Environmental Protection. The notice must be published within 30 days prior to the application being sent to the Department. The notice should be published in the legal advertisement section of a daily or weekly newspaper having general circulation in the area where the discharge will occur. If the public notice is not published at the proper time or if the application is returned because it is incomplete, you may be asked to have the notice published a second time.

Using the form on the next page, fill in the blanks with the appropriate information. Strike out all of the items (CSO, multiple discharge sources, etc.) in the second paragraph that do not apply to your discharge. The form may then be sent to the newspaper that is to publish the notice. Additionally, include a copy of the form with the application filed with the Department.

2. *Notice to Abutters.* Applicants are also required to send a copy of the public notice to all abutting property owners within 30 days prior to the application being filed with the Department. For the purposes of public notice of this application, an “abutter” is any person who owns property that is (1) contiguous and (2) within 1,000 feet of the delineated project boundary, including owners of property directly across a public or private right of way. Additionally, include a copy of the form and a list of abutters receiving the notice with the application filed with the Department

3. *Notice to Municipal Office.* Applicants are required to send a copy of the public notice by certified mail to the town or city clerk of each municipality where the facility/proposed project is located within 30 days prior to the application being filed with the Department. Applicants must also file a duplicate copy of the application with each municipality.

4. *Public Meeting.* Where the application is for a new waste discharge license of greater than 25,000 gallons per day, you must hold a public meeting in accordance with Chapter 2, Section 8, of the Department’s rules. Notice of the meeting must be sent to abutters and the clerk of the municipality(ies) where the discharge is located at least 10 days prior to the meeting. Notice of the meeting must be published in the same newspaper used to publish the notice of filing.

After all required notices have been made, sign the statement on the Certification page of the application.

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NOTICE OF INTENT TO FILE
MAINE WASTE DISCHARGE LICENSE / MAINE POLLUTANT DISCHARGE ELIMINATION
SYSTEM PERMIT APPLICATION

Please take note that, pursuant to 38 MRSA, Sections 413 and 414-A, Kingfish Maine of 9 DunGarvin road intends to file a wastewater discharge permit application with the Department of Environmental Protection (DEP). The application is for the discharge of 28.7 Million GPD of a land-based aquaculture facility to the Chandler Bay in Jonesport, Maine.

Include as applicable:

CSO: Included in this application is the discharge from Outfall #s Combined Sewer Overflows to Chandler Bay.

Multiple industrial point sources: The application includes _____ associated with the primary activity described above.

Antidegradation: The application proposes a new or increased discharge that may lower existing receiving water quality within its legal classification, and the application contains a statement regarding important social and economic benefits resulting from the activity causing the discharge, pursuant to 38 MRSA, Section 464.

Mixing Zone: The application includes a request for establishment of a mixing zone in the Chandler Bay, inside of which classifications standards and uses not need to be met, pursuant to 38 MRSA, Section 451.

The application will be filed on or about 5/15/26 and will be available for public inspection at DEP's Augusta office by request during normal business hours. A copy may also be seen at the municipal offices in Jonesport.

A request for a public hearing or request that the Board of Environmental Protection assume jurisdiction over this application must be received by the DEP, in writing, no later than 20 days after the application is found acceptable for processing, or 30 days from the date of this notice, whichever is longer. Requests shall state the nature of the issue(s) to be raised. Unless otherwise provided by law, a hearing is discretionary and may be held if the Commissioner or the Board finds significant public interest or there is conflicting technical information.

During the time specified above, persons wishing to receive copies of draft permits and supporting documents, when available, may request them from DEP. Persons receiving a draft permit shall have 30 days in which to submit comments or to request a public hearing on the draft.

Public comment will be accepted until a final administrative action is taken to approve, approve with conditions or deny this application. Written public comments or requests for information may be made to

Maine Department of Environmental Protection
Division of Water Quality Management
Department of Environmental Protection
State House Station #17
Augusta, Maine 04333-0017
Telephone (207) 287-7688

Kingfish Maine, Inc.

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Maine Department of Environmental Protection
General Application for WDL / MEPDES Permit**Pre-application and pre-submission meetings**

Pre-application meetings. Pre-application meetings between the applicant and the Department are an opportunity for the applicant to determine the statutory and regulatory requirements that apply to a specific project and to identify a Project Manager for the application. The purpose of these meetings is to identify issues, processing times, fees and the types of information and documentation necessary for the Department to properly assess the project. The applicant shall consult the Licensing Coordinator or appropriate Project Manager to determine what information the applicant must provide before or during a pre-application meeting. Any applicant may request a pre-application meeting. The Department shall make a date available for the meeting as expeditiously as possible, but no later than 60 days from receipt of a written request and receipt of all information required for a pre-application meeting by the bureau. The Department shall prepare a written summary of all pre-application meetings.

For waste discharge permits, pre-application meetings are required prior to submission to or acceptance by the Department of an application for the following:

- a. New waste discharge license for a discharge greater than 25,000 gallons per day (38 M.R.S.A. Sections 413, et seq);
- b. Projects requiring new or amended licenses involving more than two bureaus.
- c. If there is a potential to use more than 20% of the assimilative capacity of the receiving water body.

Pre-submission meetings. Pre-submission meetings between the applicant and the Department occur after the applicant has finished preparing the application for submission. These meetings are an opportunity to review the assembled application to ensure that the necessary information has been included prior to filing the application with the Department. The applicant or its authorized agent must attend the pre-submission meeting. If the applicant is an agency, company, corporation, or other organization, the request for a pre-submission meeting must include the organization's name and the name of the person who will represent the applicant at the meeting. If the applicant chooses to be represented by an agent at the pre-submission meeting, contact information for both the applicant and the agent and a letter from the applicant authorizing the agent to act on its behalf must be provided with the request. An applicant may request a pre-submission meeting by contacting the Project Manager, or the Permit Assistance Coordinator for the bureau if no Project Manager has been identified. The Department shall make a date available for the meeting as expeditiously as possible, but no later than 60 days from receipt of a written request or when all information required by the Department for the pre-submission meeting is submitted.

For waste discharge permits, a pre-submission meeting is required prior to submission to or acceptance by the Department of an application for the following:

Any application for which a pre-application meeting was held; or

Any application that has been previously rejected by the Department (see Chapter 2, Section 10-B of the Department's rules).

Waivers. The requirement of a pre-application or pre-submission meeting may be waived by written notice from the Department and agreement by the applicant. The Department will agree to waive a preapplication or pre-submission meeting if the Department is satisfied that such a meeting would be of no value in achieving the purposes noted above.

Note: The waiver of a pre-application or pre-submission meeting does not waive the public informational meeting required for new wastewater discharge license of more than 25,000 gallons per day.



Application Form 2D

New Manufacturing, Commercial, Mining, and Silvicultural Operations That Have Not Yet Commenced Discharge of Process Wastewater

NPDES Permitting Program

Note: Complete this form *and* Form 1 if your facility is a new manufacturing, commercial, mining, or silvicultural facility that has yet to commence discharge of process wastewater.

Paperwork Reduction Act Notice

This collection of information is approved by OMB under the Paperwork Reduction Act, 44 U.S.C. 3501 et seq. (OMB Control No. 2040-0004). Responses to this collection of information are mandatory (40 CFR 122.21). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. The public reporting and recordkeeping burden for this collection of information is estimated to be 2.7 hours per response. Send comments on the Agency's need for this information, the accuracy of the provided burden estimates and any suggested methods for minimizing respondent burden to the Regulatory Support Division Director, U.S. Environmental Protection Agency (2821T), 1200 Pennsylvania Ave., NW, Washington, D.C. 20460. Include the OMB control number in any correspondence. Do not send the completed form to this address.

General Instructions

Who Must Complete Form 2D?

You must complete Form 2D if you answered “Yes” to Item 1.2.3 on Form 1—that is, if you are a new manufacturing, commercial, mining, or silvicultural facility that has yet to commence discharge of process wastewater.

Where to File Your Completed Forms?

Submit your completed application package (Forms 1 and 2D) to your National Pollutant Discharge Elimination System (NPDES) permitting authority. Consult Exhibit 1–1 of Form 1’s “General Instructions” to identify your NPDES permitting authority.

Public Availability of Submitted Information

The U.S. Environmental Protection Agency (EPA) will make information from NPDES permit application forms available to the public for inspection and copying upon request. You may not claim any information on Form 2D (or related attachments) as confidential.

You may make a claim of confidentiality for any information that you submit to EPA that goes beyond the information required by Form 2D. Note that NPDES permitting authorities will deny claims for treating any effluent data (estimated or actual) as confidential. If you do not assert a claim of confidentiality at the time you submit your information to the NPDES permitting authority, EPA may make the information available to the public without further notice to you. EPA will handle claims of confidentiality in accordance with its business confidentiality regulations at Part 2 of Title 40 of the *Code of Federal Regulations* (CFR).

Completion of Forms

Print or type in the specified areas only. If you do not have enough space on the form to answer a question, you may continue on additional sheets, as necessary, using a format consistent with the form.

Do not leave any response areas blank unless the form directs you to skip them. If the form directs you to respond to an item that does not apply to your facility or activity, enter “NA” for “not applicable” to show that you considered the item and determined a response was not necessary for your facility.

The NPDES permitting authority will consider your application complete when it and any supplementary material are received and completed according to the authority’s satisfaction. The NPDES permitting authority will judge the completeness of any application independently of the status of any other permit application or permit for the same facility or activity.

Follow-up Requirements

Form 2D requires that you submit estimated data on your effluent. Note that no later than 24 months after you commence discharging from the proposed facility, you must complete and

submit Section 7 of NPDES Application Form 2C; see requirements at 40 CFR 122.21(g)(7). However, you need not complete those portions of Section 7 that require tests you have already performed under the discharge monitoring requirements of your NPDES permit.

Definitions

The legal definitions of all key terms used in these instructions and Form 2D are in the “Glossary” at the end of the “General Instructions” in Form 1.

Line-by-Line Instructions

EPA Identification Number, Facility Name, and Outfall Number

Provide your EPA Identification Number from the Facility Registry Service and facility name at the top of each page of Form 2D and any attachments. If you do not know your EPA Identification Number, contact your NPDES permitting authority. See Exhibit 1–1 of Form 1’s “General Instructions” for contact information. Additionally, for Tables A through E, provide the applicable outfall number at the top of each page.

Section 1. Expected Outfall Location

Item 1.1. Identify each of the facility’s outfall structures by number. For each outfall, specify the latitude and longitude to the nearest 15 seconds or equivalent decimal degrees (e.g., 38.893829, -77.029289) and name of the receiving water. The application form provides reporting space for three outfalls. If your facility has more than this number, attach additional sheets as necessary. The location of each outfall (i.e., where the coordinates are collected) shall be the point where the discharge is released into a water of the United States. Latitude and longitude coordinates may be obtained in a variety of ways, including use of hand held devices (e.g., a GPS enabled smartphone), internet mapping tools (e.g., <https://mynasadata.larc.nasa.gov/latitudelongitude-finder/>), geographic information systems (e.g., ArcView), or paper maps from trusted sources (e.g., U.S. Geological Survey or USGS). For further guidance, refer to <http://www.epa.gov/geospatial/latitudelongitude-data-standard>.

Section 2. Expected Discharge Date

Item 2.1. Report the expected date the facility will commence discharging (month, day, and year).

Section 3. Average Flows and Treatment

Item 3.1. For each outfall, report the operations expected to contribute wastewater to the effluent and an estimated average flow from each. Briefly describe the planned wastewater treatment for each operation or list the applicable treatment code(s) from Exhibit 2D–1, located at the end of these instructions. Finally, for each operation, note the ultimate disposal of any solid or liquid wastes not expected to be discharged.

Section 4. Line Drawing

Item 4.1. Attach a line drawing showing the expected water flow through your facility, from intake to discharge. Indicate the sources of intake water (e.g., city, well, stream, other); all sources of wastewater contributing to the effluent, including process and production areas, sanitary flows, cooling water, and stormwater runoff; and labeled treatment units. You may group similar operations into a single unit.

Construct a water balance on the line drawing by showing average flows (specify units) between intakes, operations, treatment units, and outfalls. Show all significant losses of water to products, the atmosphere, and discharge. You should use your best estimate. If you cannot determine a water balance for your activities (such as mining activities), provide a pictorial description of the nature and amount of any sources of water and any collection and treatment measures. An example of an acceptable line drawing is provided in Exhibit 2D–2 at the end of these instructions.

Section 5. Intermittent or Seasonal Flows

Item 5.1. Specify whether any of the expected discharges described in Sections 1 and 3 will be intermittent or seasonal. If yes, continue to Item 5.2. If no, skip to Section 6.

Item 5.2. List applicable outfalls that will have intermittent or seasonal flows. For each, indicate the operations that will contribute to the flow. For each operation, indicate the average days per week and average months per year the discharge will occur, the maximum daily flow rate, the maximum total volume, and the duration of the discharge in days. The estimated flow rate and volume should not include stormwater runoff, spillage, or leaks. A discharge is intermittent if it occurs with interruptions during the operating hours of the facility. Discharges caused by routine maintenance shutdowns, process changes, or other similar activities are not considered to be intermittent. A discharge is seasonal if it occurs only during certain parts of the year. The frequency is the average recurrence rate of the discharge (in days per week and months per year). The duration is the average value of the time duration during which the discharge occurs (in days).

The maximum daily flow rate is the highest daily value and should be reported in million gallons per day (mgd). Maximum total volume means the total volume of any one discharge within 24 hours and is measured in units such as gallons.

Section 6. Production

Item 6.1. Indicate whether any effluent limitation guidelines (ELGs) promulgated under Section 304 of the Clean Water Act (CWA) apply to your facility. All ELGs promulgated by EPA appear in the *Federal Register* and are published annually in 40 CFR Subchapter N. See also www.epa.gov/eq. An ELG applies if you have any operations contributing process wastewater in any subcategory covered by New Source Performance Standards (NSPS). If you are unsure whether you are covered by a promulgated ELG, consult your NPDES permitting authority (see Exhibit 1–1 of Form 1's "General Instructions"). You must check "Yes" if an applicable ELG has been promulgated, even if the ELG is being contested in court. If you believe that a

promulgated ELG has been remanded for reconsideration by a court and does not apply to your operations, you may answer "No" to item 6.1 and skip to Section 7.

Item 6.2. Complete Item 6.2 by indicating the applicable ELG category, ELG subcategory, and corresponding regulatory citation. See the example below.

Applicable ELGs	6.2	ELG Category	ELG Subcategory	Regulatory Citation
		Pulp, Paper, and Paperboard Point Source Category	Secondary Fiber Non-Deink Subcategory	40 CFR 430, Subpart J

Item 6.3. Indicate whether the limitations in the applicable ELGs are expressed in terms of production (or other measure of operation). An ELG is expressed in terms of production (or another measure of operation) if the limitation is expressed as mass of pollutant per operational parameter (e.g., "pounds of biological oxygen demand per cubic foot of logs from which bark is removed" or "pounds of total suspended solids per megawatt hour of electrical energy consumed by smelting furnace"). An example of an ELG not expressed in terms of a measure of operation is one that limits the concentration of pollutants. If you answer "No" to this item, skip to Section 7.

Item 6.4. For each applicable outfall to which an applicable production-based ELG applies, list the estimated level of production (projection of actual production level, not design), for each of the first three years of operation. The estimated production level must be a long-term average estimate (e.g., average production on an annual basis). If production will vary depending on long-term shifts in operating schedule or capacity, you may report alternative production estimates, but you must provide the basis for such alternatives. If known, report quantities in units of measurements used in the applicable ELG. If an ELG specifies a method for estimating production, you must follow that method.

Section 7. Effluent Characteristics and Tables A through E

General Information. Section 7 requires you to report *estimated* flow data for the parameters and pollutants listed in Tables A through E, located at the end of Form 2D. You are *not* required to conduct actual sampling and analysis at this time. If, however, data from such analyses are available, you must report those data. Note that no later than 24 months after you begin discharging from the proposed facility, you must complete and submit quantitative data for the pollutants and parameters in Tables A through E. However, you need not report results for tests you have already performed and reported under the discharge monitoring requirements of your NPDES permit.

Complete a set of tables (Tables A through E) for each outfall at your facility. Be sure to note the EPA Identification Number, facility name, and outfall number at the top of each table page and any associated attachments.

Tables A through D require you to report estimated effluent data, with some exceptions, as discussed further below. Base your estimates on available in-house or contractor engineering reports or any other studies performed on the proposed facility. Table E requires you to report quantitative data for the pollutants listed, but only if it is already available.

Several tables require you to provide estimates for pollutants you believe will be present in your discharge or will be limited directly by an ELG or indirectly through promulgated limitations on an indicator pollutant. Base your determination of whether a pollutant will be present in your discharge on your knowledge of the proposed facility's raw materials, maintenance chemicals, intermediate and final products, byproducts, and any analyses of any pollutant (you are required to report it).

For those pollutants you believe will be present in the discharge, provide the maximum daily and average daily concentration *and* total mass and the source of the information. Use the following codes to report your source information:

Data Source	Code
Engineering report	1
Actual data from pilot plants	1
Estimates from other engineering reports	2
Data from other similar plants	3
Best professional estimates	4
Others	5 and specify on the table

You may report some or all of your estimates (or actual data when available) by attaching separate sheets of paper instead of completing Tables A through E for each of your outfalls, so long as the sheets contain all of the required information and are similar in format to Tables A through E.

Reporting of Intake Data

If you expect a pollutant to be present solely because of its presence in your intake water, you must mark "Yes" under the "Intake Water" column of Tables A through D. If you wish to obtain credits for pollutants or parameters present in your intake water, insert a separate sheet with a short statement of why you believe you are eligible (see 40 CFR 122.45(g)).

Reporting of Effluent Data

Report all estimated pollutant or parameter levels as concentration *and* as total mass, with the exception of discharge flow, temperature, and pH.

Use the following abbreviations in the columns requiring "units" in Tables A through E.

Concentration	Mass
ppm = parts per million	lbs. = pounds
mg/L = milligrams per liter	ton = tons (English tons)
ppb = parts per billion	mg = milligrams
µg/L = micrograms per liter	g = grams
MPN = most probable number per 100 milliliters	kg = kilograms
	T = tonnes (metric tons)

Conventional and Non-Conventional Parameters

Item 7.1 and Table A. All applicants are required to complete Table A for each outfall, including outfalls discharging only noncontact cooling water or nonprocess water *unless* a waiver has been received or requested from the NPDES permitting authority. For each parameter listed in Table A, indicate whether a waiver has been requested. If you have requested a waiver for *all* pollutants for a given outfall, check the box indicating this at the top of Table A.

To request a waiver, submit a written request to the NPDES permitting authority in advance or with the permit application. The written request should specify the parameters that should be waived and for what outfall(s) and why. The NPDES permitting authority may waive Table A requirements upon a determination that less stringent reporting requirements are adequate to support issuance of an NPDES permit. Attach a copy of any waiver approval notice(s) received, if applicable, to this application.

Answer Item 7.1 by indicating if you are requesting a waiver for any of your outfalls. If yes, continue to Item 7.2. Otherwise, complete Table A by estimating your maximum daily and average daily discharge. Provide the source(s) of your information. Also in Table A, indicate whether you believe each of the parameters will be present in the facility's intake water. See "Reporting of Intake Data" above for further information. Skip to Item 7.3.

Item 7.2. Indicate the outfalls for which you have requested a waiver or check the appropriate box to indicate that you are requesting a waiver for some or all pollutants at all outfalls.

Item 7.3. Indicate if you have provided estimates or actual data for all Table A parameters for each of your outfalls for which a waiver has not been requested and attach the results to your application package.

Certain Conventional and Non-Conventional Pollutants

Items 7.4, 7.5 and 7.6 and Table B. Complete one table for each outfall, including outfalls discharging only noncontact cooling water or nonprocess wastewater. Check the box at the top of Table B if you believe *all* pollutants listed will be absent in the discharge. If so, you do not need to complete Table B for the noted outfall. (You still need to complete Items 7.4 through 7.6.) Otherwise, for *each* pollutant listed in Table B, indicate whether you expect it will be present or absent in the discharge or whether the pollutant is limited directly by an ELG or indirectly through promulgated limitations or an indicator pollutant. (For example, total suspended solids is used as an indicator to control the discharge of iron and aluminum.) Next, provide an estimated maximum daily and average daily value, including the source of the information. If you have quantitative data available, report it. Also in Table B, indicate whether you believe the listed pollutants will be present in the facility's intake water. See "Reporting of Intake Data" above for further information. Answer "Yes" to Items 7.4 through 7.6 once you have completed the above tasks.

Toxic Metals, Total Cyanide, and Total Phenols

Items 7.7 and 7.8 and Table C. Complete one table for each outfall, including outfalls discharging only noncontact cooling water or nonprocess wastewater. Check the box at the top of Table C if you believe *all* pollutants listed will be absent in the discharge. If so, you do not need to complete Table C for the noted outfall (unless you have quantitative data available). You still need to respond to Items 7.7 and 7.8, however. Otherwise, indicate whether you believe each pollutant in Table C will be present or absent in your discharge for each applicable outfall. For those pollutants you believe will be present, provide an estimated maximum daily and average daily value and source of the information. (Provide quantitative data if you have them available.) Also, in Table C, indicate whether you believe the pollutant is or will be present in your facility's intake water. See "Reporting of Intake Data" above for more information. Answer "Yes" to Items 7.7 and 7.8 when you have completed the above tasks.

Organic Toxic Pollutants

(Gas Chromatography/Mass Spectrometry or GC/MS Fractions)

Item 7.9. Applicants are exempt from the reporting requirements associated with Table D if they expect to have gross sales of less than \$100,000 per year for the next three years; also exempt are coal mines with expected average production of less than 100,000 tons of coal per year. If you believe you meet one of these criteria, answer "Yes" to Item 7.9, check the small business box at the top of Table D, and attach projected sales or production figures. Skip to Item 7.12.

The sales or production figures must be for the facility that will be the source of the discharge. The data should not be limited only to production or sales for the process or processes that will contribute to the discharge, unless those are the only processes at the facility.

For sales data, where intra-corporate transfers of goods and services will be involved, the transfer price per unit should approximate market process for those goods and services as closely as possible. If necessary, you may index your sales figures to the second quarter of 1980 to demonstrate your eligibility for a small business exemption. You may accomplish this by using the gross national product price deflator (second quarter of 1980 = 100). This index is available online from the U.S. Department of Commerce, Bureau of Economic Analysis, at <https://apps.bea.gov/national/pdf/SNTables.pdf>.

Items 7.10 and 7.11 and Table D. Complete one table for each outfall, including outfalls discharging only noncontact cooling water or nonprocess wastewater. Check the box at the top of Table D if you believe *all* pollutants listed will be absent in the discharge from the outfall. If so, you do not need to complete Table D for the noted outfall (unless you have quantitative data available). Otherwise, for *each* pollutant listed, indicate whether you believe it will be present or absent in the discharge. For those you believe will be present, provide an estimated maximum daily and average daily value and the source of the information. Also, in Table D, indicate whether you believe the pollutant is or will be present in your facility's intake water. See "Reporting of Intake Data" above for further information. Finally,

answer "Yes" to Items 7.10 and 7.11 when you have completed the above tasks.

2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD)

Item 7.12. Answer whether the facility uses or manufactures one or more of the 2,3,7,8-TCDD congeners listed below or if you know or have reason to believe that TCDD is or may be present in effluent from any of your outfalls:

- 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) (CAS # 93-765).
- 2-(2,4,5-trichlorophenoxy) propanoic acid (Silvex, 2,4,5-TP) (CAS # 93-72-1).
- 2-(2,4,5-trichlorophenoxy) ethyl 2,2-dichloropropionate (Erbon) (CAS # 136-25-4).
- 0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate (Ronnell) (CAS # 299-84-3).
- 2,4,5-trichlorophenol (TCP) (CAS # 95-95-4).
- Hexachlorophene (HCP) (CAS # 70-30-4).

Certain Hazardous Substances and Asbestos

Table E. Complete Table E for each outfall. Check the box at the top of Table E if you believe *all* pollutants listed will be absent in the discharge. Otherwise, for *each* pollutant listed in Table E, indicate whether you believe it will be present or absent in the discharge. If you have quantitative estimates available for any of the pollutants listed, provide the maximum daily and average daily average value and the source of the information. Also, in Table E, if you believe the pollutant is or will be present in your facility's intake water, state so in the "Reason Pollutant Believed Present in Discharge" column.

Item 7.13. Indicate whether, for each of your outfalls, you have indicated whether you know or have reason to believe that any pollutants listed in Table E are discharged.

Item 7.14. Indicate whether, for each of your outfalls, you have completed and attached Table E to the application describing the reasons the applicable pollutants are expected to be discharged and providing quantitative data if available.

Under 40 CFR 117.12(a)(2), certain discharges of hazardous substances (listed in Exhibit 2D-3 at the end of these instructions) may be exempted from the requirements of Section 311 of the CWA, which establishes reporting requirements, civil penalties, and liability for cleanup costs for spills of oil and hazardous substances. A discharge of a particular substance can be exempted if the origin, source, and amount of the discharged substances are identified in the NPDES permit application or in the permit, if the permit contains a requirement for treatment of the discharge, and if the treatment is in place.

Exemptions are allowed from the requirements of CWA Section 311. Applications for exemptions must set forth the following information:

1. The substance and the amount of each substance that may be discharged.

2. The origin and source of the discharge of the substance.
3. The treatment to be provided for the discharge by:
 - a. An onsite treatment system separate from any treatment system treating your normal discharge;
 - b. A treatment system designed to treat your normal discharge and that is additionally capable of treating the amount of the substance identified under paragraph 1 above; or
 - c. Any combination of the above.

See 40 CFR 117.12(a)(2) and (c) or contact your NPDES permitting authority for further information on exclusions from CWA Section 311.

Intake Credits

Item 7.15. Answer whether you are seeking to obtain credits for any of the pollutants or parameters listed in Section 7 (Tables A through E) in your intake water for any of the facility's outfalls.

Section 8. Engineering Report

Item 8.1. Indicate if any technical evaluations have been conducted of your wastewater treatment, including engineering reports or pilot plant studies. If yes, continue to Item 8.2. If no, skip to Item 8.3.

Item 8.2. Attach the technical evaluation(s) you considered when responding to Item 8.1 and any related documentation, then answer "Yes" to Item 8.2. The NPDES permit writer will use this information to determine appropriate treatment methods and associated permit conditions and limits.

Item 8.3. Answer "Yes" if you are aware of any existing plant(s) that resemble your production processes, wastewater constituents, or wastewater treatment. If you are unaware of such plants, answer "No" and skip to Section 9.

Item 8.4. Provide the names and locations of any existing plants that resemble your production facility. You do not need to conduct any studies to respond to this item.

Section 9. Other Information

Item 9.1. Indicate whether you have attached to the application any optional information that you would like considered as part of the application review process. These should be items beyond those you have already noted as being included in the package. Skip to Section 10 if you do not have further information to provide.

Item 9.2. List the additional materials attached and note why you think the NPDES permitting authority should consider them when reviewing your application and developing your permit.

Section 10. Checklist and Certification Statement

Item 10.1. Review the checklist provided. In column 1, mark the sections of Form 2D that you have completed and are submitting with your application. For each section, indicate in column 2 whether you are submitting attachments.

Item 10.2. The CWA provides for severe penalties for submitting false information on this application form. Section 309(c)(2) of

the CWA provides that "Any person who knowingly makes any false statement, representation, or certification in any application, ...shall upon conviction, be punished by a fine of no more than \$10,000 or by imprisonment for not more than six months or both."

FEDERAL REGULATIONS AT 40 CFR 122.22 REQUIRE THIS APPLICATION TO BE SIGNED AS FOLLOWS:

- A. For a corporation, by a responsible corporate officer. For the purpose of this section, a responsible corporate officer means: (1) a president, secretary, treasurer, or vice-president of the corporation in charge of a principal business function, or any other person who performs similar policy- or decision-making functions for the corporation, or (2) the manager of one or more manufacturing, production, or operating facilities, provided the manager is authorized to make management decisions which govern the operation of the regulated facility including having the explicit or implicit duty of making major capital investment recommendations, and initiating and directing other comprehensive measures to assure long term environmental compliance with environmental laws and regulations; the manager can ensure that the necessary systems are established or actions taken to gather complete and accurate information for permit application requirements; and where authority to sign documents has been assigned or delegated to the manager in accordance with corporate procedures.
- B. For a partnership or sole proprietorship, by a general partner or the proprietor, respectively.
- C. For a municipality, state, federal, or other public facility, by either a principal executive officer or ranking elected official. For purposes of this section, a principal executive officer of a federal agency includes: (1) the chief executive officer of the agency or (2) a senior executive officer having responsibility for the overall operations of a principal geographic unit of the agency (e.g., Regional Administrators of EPA).

END

Submit your completed Form 1, Form 2D, and all associated attachments (and any other required NPDES application forms) to your NPDES permitting authority.

Exhibit 2D–1. Codes for Treatment Units and Disposal of Wastes Not Discharged

1. PHYSICAL TREATMENT PROCESSES

1–A Ammonia stripping	1–M Grit removal
1–B Dialysis	1–N Microstraining
1–C Diatomaceous earth filtration	1–O Mixing
1–D Distillation	1–P Moving bed filters
1–E Electrodialysis	1–Q Multimedia filtration
1–F Evaporation	1–R Rapid sand filtration
1–G Flocculation	1–S Reverse osmosis (<i>hyperfiltration</i>)
1–H Flotation	1–T Screening
1–I Foam fractionation	1–U Sedimentation (<i>settling</i>)
1–J Freezing	1–V Slow sand filtration
1–K Gas-phase separation	1–W Solvent extraction
1–L Grinding (<i>comminutors</i>)	1–X Sorption

2. CHEMICAL TREATMENT PROCESSES

2–A Carbon adsorption	2–G Disinfection (<i>ozone</i>)
2–B Chemical oxidation	2–H Disinfection (<i>other</i>)
2–C Chemical precipitation	2–I Electrochemical treatment
2–D Coagulation	2–J Ion exchange
2–E Dechlorination	2–K Neutralization
2–F Disinfection (<i>chlorine</i>)	2–L Reduction

3. BIOLOGICAL TREATMENT PROCESSES

3–A Activated sludge	3–E Pre-aeration
3–B Aerated lagoons	3–F Spray irrigation/land application
3–C Anaerobic treatment	3–G Stabilization ponds
3–D Nitrification–denitrification	3–H Trickling filtration

4. OTHER PROCESSES

4–A Discharge to surface water	4–C Reuse/recycle of treated effluent
4–B Ocean discharge through outfall	4–D Underground injection

5. SLUDGE TREATMENT AND DISPOSAL PROCESSES

5–A Aerobic digestion	5–M Heat drying
5–B Anaerobic digestion	5–N Heat treatment
5–C Belt filtration	5–O Incineration
5–D Centrifugation	5–P Land application
5–E Chemical conditioning	5–Q Landfill
5–F Chlorine treatment	5–R Pressure filtration
5–G Composting	5–S Pyrolysis
5–H Drying beds	5–T Sludge lagoons
5–I Elutriation	5–U Vacuum filtration
5–J Flotation thickening	5–V Vibration
5–K Freezing	5–W Wet oxidation
5–L Gravity thickening	

Exhibit 2D-2. Example Line Drawing

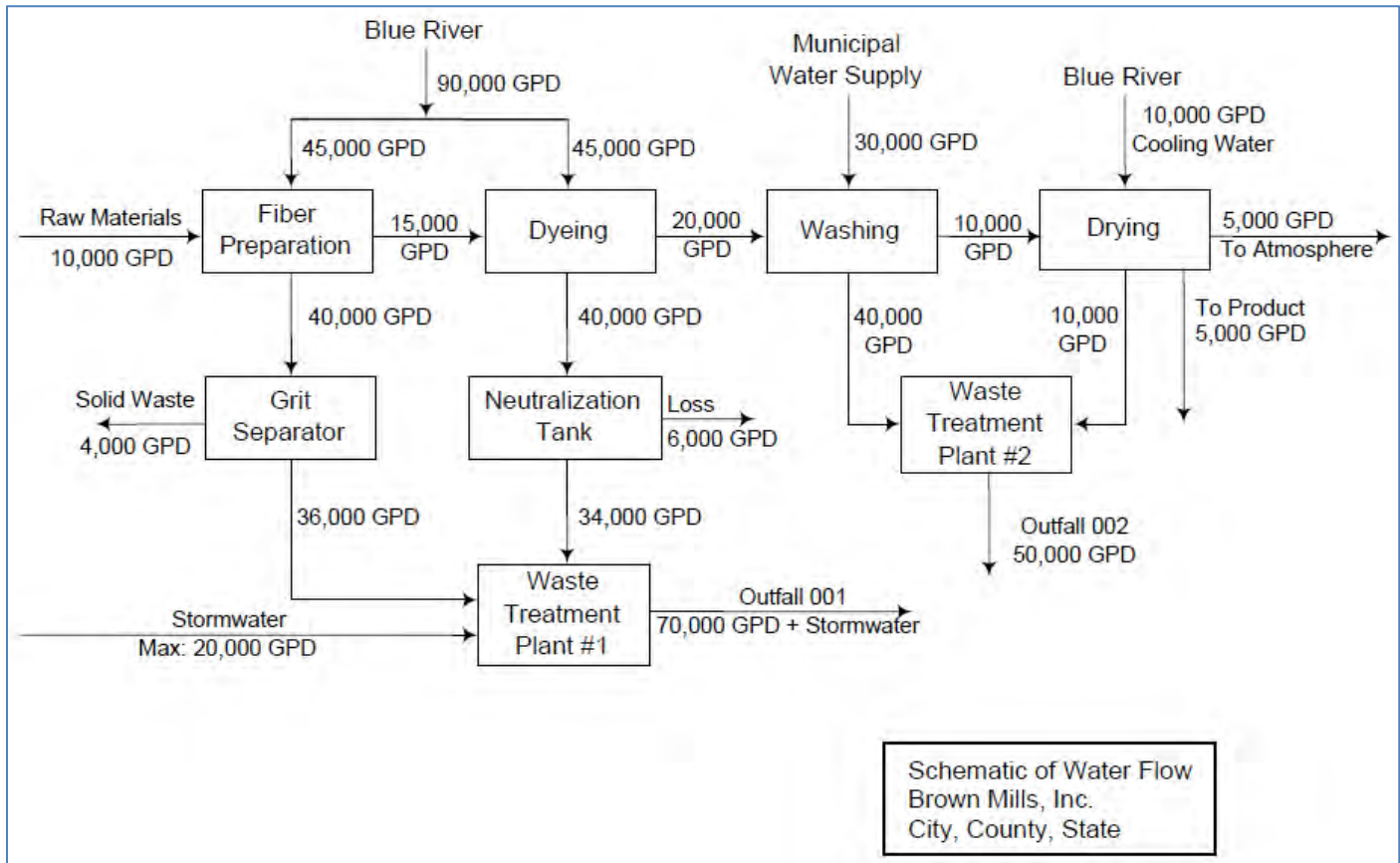


Exhibit 2D–3. Hazardous Substances

1. Acetaldehyde
2. Acetic acid
3. Acetic anhydride
4. Acetone cyanohydrin
5. Acetyl bromide
6. Acetyl chloride
7. Acrolein
8. Acrylonitrile
9. Adipic acid
10. Aldrin
11. Allyl alcohol
12. Allyl chloride
13. Aluminum sulfate
14. Ammonia
15. Ammonium acetate
16. Ammonium benzoate
17. Ammonium bicarbonate
18. Ammonium bichromate
19. Ammonium bifluoride
20. Ammonium bisulfite
21. Ammonium carbamate
22. Ammonium carbonate
23. Ammonium chloride
24. Ammonium chromate
25. Ammonium citrate
26. Ammonium fluoroborate
27. Ammonium fluoride
28. Ammonium hydroxide
29. Ammonium oxalate
30. Ammonium silicofluoride
31. Ammonium sulfamate
32. Ammonium sulfide
33. Ammonium sulfite
34. Ammonium tartrate
35. Ammonium thiocyanate
36. Ammonium thiosulfate
37. Amyl acetate
38. Aniline
39. Antimony pentachloride
40. Antimony potassium tartrate
41. Antimony tribromide
42. Antimony trichloride
43. Antimony trifluoride
44. Antimony trioxide
45. Arsenic disulfide
46. Arsenic pentoxide
47. Arsenic trichloride
48. Arsenic trioxide
49. Arsenic trisulfide
50. Barium cyanide
51. Benzene
52. Benzoic acid
53. Benzoinitrile
54. Benzoyl chloride
55. Benzyl chloride
56. Beryllium chloride
57. Beryllium fluoride
58. Beryllium nitrate
59. Butylacetate
60. n-butylphthalate
61. Butylamine
62. Butyric acid
63. Cadmium acetate
64. Cadmium bromide
65. Cadmium chloride
66. Calcium arsenate
67. Calcium arsenite
68. Calcium carbide
69. Calcium chromate
70. Calcium cyanide
71. Calcium dodecylbenzenesulfonate
72. Calcium hypochlorite
73. Captan
74. Carbaryl
75. Carbofuran
76. Carbon disulfide
77. Carbon tetrachloride
78. Chlordane
79. Chlorine
80. Chlorobenzene
81. Chloroform
82. Chloropyrifos
83. Chlorosulfonic acid
84. Chromic acetate
85. Chromic acid
86. Chromic sulfate
87. Chromous chloride
88. Cobaltous bromide
89. Cobaltous formate
90. Cobaltous sulfamate
91. Coumaphos
92. Cresol
93. Crotonaldehyde
94. Cupric acetate
95. Cupric acetoarsenite
96. Cupric chloride
97. Cupric nitrate
98. Cupric oxalate
99. Cupric sulfate
100. Cupric sulfate ammoniated
101. Cupric tartrate
102. Cyanogen chloride
103. Cyclohexane
104. 2,4-D acid (2,4-dichlorophenoxyacetic acid)
105. 2,4-D esters (2,4-dichlorophenoxyacetic acid esters)
106. DDT (dichlorodiphenyltrichloroethane)
107. Diazinon
108. Dicamba
109. Dichlobenil
110. Dichlone
111. Dichlorobenzene
112. Dichloropropane
113. Dichloropropene
114. Dichloropropene-dichloropropane mix
115. 2,2-dichloropropionic acid
116. Dichlorvos
117. Dieldrin
118. Diethylamine
119. Dimethylamine
120. Dinitrobenzene
121. Dinitrophenol
122. Dinitrotoluene
123. Diquat
124. Disulfoton
125. Diuron
126. Dodecylbenzenesulfonic acid
127. Endosulfan
128. Endrin
129. Epichlorohydrin
130. Ethion
131. Ethylbenzene
132. Ethylenediamine
133. Ethylene dibromide
134. Ethylene dichloride
135. EDTA (ethylene diaminetetracetic acid)
136. Ferric ammonium citrate
137. Ferric ammonium oxalate
138. Ferric chloride
139. Ferric fluoride
140. Ferric nitrate
141. Ferric sulfate
142. Ferrous ammonium sulfate
143. Ferrous chloride
144. Ferrous sulfate
145. Formaldehyde
146. Formic acid
147. Fumaric acid
148. Furfural
149. Guthion
150. Heptachlor
151. Hexachlorocyclopentadiene
152. Hydrochloric acid
153. Hydrofluoric acid
154. Hydrogen cyanide
155. Hydrogen sulfide
156. Isoprene
157. Isopropanolamine dodecylbenzenesulfonate
158. Kelthane
159. Kepone
160. Lead acetate
161. Lead arsenate
162. Lead chloride
163. Lead fluoborate
164. Lead fluorite
165. Lead iodide
166. Lead nitrate
167. Lead stearate
168. Lead sulfate
169. Lead sulfide
170. Lead thiocyanate
171. Lindane
172. Lithium chromate
173. Malathion
174. Maleic acid
175. Maleic anhydride
176. Mercaptodimethur
177. Mercuric cyanide
178. Mercuric nitrate
179. Mercuric sulfate
180. Mercuric thiocyanate
181. Mercurous nitrate
182. Methoxychlor
183. Methyl mercaptan
184. Methyl methacrylate
185. Methyl parathion
186. Mevinphos
187. Mexacarbate
188. Monoethylamine
189. Monomethylamine
190. Naled
191. Naphthalene
192. Naphthenic acid
193. Nickel ammonium sulfate
194. Nickel chloride
195. Nickel hydroxide
196. Nickel nitrate
197. Nickel sulfate
198. Nitric acid
199. Nitrobenzene
200. Nitrogen dioxide
201. Nitrophenol
202. Nitrotoluene
203. Paraformaldehyde
204. Parathion
205. Pentachlorophenol
206. Phenol
207. Phosgene
208. Phosphoric acid
209. Phosphorus
210. Phosphorus oxychloride
211. Phosphorus pentasulfide
212. Phosphorus trichloride
213. PCBs (polychlorinated biphenyls)

Exhibit 2D–3. Hazardous Substances

214. Potassium arsenate	245. Sodium phosphate (dibasic)	271. Uranyl acetate
215. Potassium arsenite	246. Sodium phosphate (tribasic)	272. Uranyl nitrate
216. Potassium bichromate	247. Sodium selenite	273. Vanadium penoxide
217. Potassium chromate	248. Strontium chromate	274. Vanadyl sulfate
218. Potassium cyanide	249. Strychnine	275. Vinyl acetate
219. Potassium hydroxide	250. Styrene	276. Vinylidene chloride
220. Potassium permanganate	251. Sulfuric acid	277. Xylene
221. Propargite	252. Sulfur monochloride	278. Xylenol
222. Propionic acid	253. 2,4,5-T acid (2,4,5-trichlorophenoxyacetic acid)	279. Zinc acetate
223. Propionic anhydride	254. 2,4,5-T amines (2,4,5-trichlorophenoxy acetic acid amines)	280. Zinc ammonium chloride
224. Propylene oxide	255. 2,4,5-T esters (2,4,5-trichlorophenoxy acetic acid esters)	281. Zinc borate
225. Pyrethrins	256. 2,4,5-T salts (2,4,5-trichlorophenoxy acetic acid salts)	282. Zinc bromide
226. Quinoline	257. 2,4,5-TP acid (2,4,5-trichlorophenoxy propanoic acid)	283. Zinc carbonate
227. Resorcinol	258. 2,4,5-TP acid esters (2,4,5-trichlorophenoxy propanoic acid esters)	284. Zinc chloride
228. Selenium oxide	259. TDE (tetrachlorodiphenyl ethane)	285. Zinc cyanide
229. Silver nitrate	260. Tetraethyl lead	286. Zinc fluoride
230. Sodium	261. Tetraethyl pyrophosphate	287. Zinc formate
231. Sodium arsenate	262. Thallium sulfate	288. Zinc hydrosulfite
232. Sodium arsenite	263. Toluene	289. Zinc nitrate
233. Sodium bichromate	264. Toxaphene	290. Zinc phenolsulfonate
234. Sodium bifluoride	265. Trichlorofon	291. Zinc phosphide
235. Sodium bisulfite	266. Trichloroethylene	292. Zinc silicofluoride
236. Sodium chromate	267. Trichlorophenol	293. Zinc sulfate
237. Sodium cyanide	268. Triethanolamine dodecylbenzenesulfonate	294. Zirconium nitrate
238. Sodium dodecylbenzenesulfonate	269. Triethylamine	295. Zirconium potassium fluoride
239. Sodium fluoride	270. Trimethylamine	296. Zirconium sulfate
240. Sodium hydrosulfide		297. Zirconium tetrachloride
241. Sodium hydroxide		
242. Sodium hypochlorite		
243. Sodium methylate		
244. Sodium nitrite		

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EPA Identification Number		Facility Name Kingfish Maine Inc		OMB No. 2040-0004 Expires 07/31/2026		
Form 2D NPDES			U.S. Environmental Protection Agency Application for NPDES Permit to Discharge Wastewater NEW MANUFACTURING, COMMERCIAL, MINING, AND SILVICULTURAL OPERATIONS THAT HAVE NOT YET COMMENCED DISCHARGE OF PROCESS WASTEWATER			
SECTION 1. EXPECTED OUTFALL LOCATION (40 CFR 122.21(K)(1))						
Outfall Location	1.1	Provide information on each of the facility's outfalls in the table below.				
		Outfall Number	Receiving Water Name	Latitude	Longitude	
		A	Chandler Bay	N44 33 30	W67 33 33	
		B	Chandler Bay	N44 33 29	W67 33 32	
SECTION 2. EXPECTED DISCHARGE DATE (40 CFR 122.21(K)(2))						
Expected Discharge Date	2.1	Month	Day	Year		
		Jan	01	2028		
SECTION 3. AVERAGE FLOWS AND TREATMENT (40 CFR 122.21(K)(3)(I))						
Average Flows and Treatment	3.1	For each outfall identified under Item 1.1, provide average flow and treatment information. Add additional sheets as necessary.				
		Outfall Number A _____				
		Operations Contributing to Flow				
		Operation		Average Flow		
		Waste Water Treatment System		28.7 mgd		
				mgd		
				mgd		
				mgd		
				mgd		
		Treatment Units				
		Description (include size, flow rate through each treatment unit, retention time, etc.)		Code from Exhibit 2D-1	Final Disposal of Solid or Liquid Wastes Other Than by Discharge	
		See attached flow diagram, General			Landfill or Biogas Production	

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Average Flows and Treatment Continued	3.1 Cont.	**Outfall Number** A _____				
		Operations Contributing to Flow				
		Operation		Average Flow		
				mgd		
				mgd		
				mgd		
				mgd		
				mgd		
		Treatment Units				
		Description (include size, flow rate through each treatment unit, retention time, etc.)		Code from Exhibit 2D-1	Final Disposal of Solid or Liquid Wastes Other Than by Discharge	
		Outfall Number B _____				
		Operations Contributing to Flow				
		Operation		Average Flow		
		Same as outfall A, but only operates when outfall A is off		28.7 mgd		
				mgd		
				mgd		
				mgd		
				mgd		
		Treatment Units				
		Description (include size, flow rate through each treatment unit, retention time, etc.)		Code from Exhibit 2D-1	Final Disposal of Solid or Liquid Wastes Other Than by Discharge	
		See attached flow diagram, General			Landfill or Biogas Production	

SECTION 4. LINE DRAWING (40 CFR 122.21(K)(3)(II))

Line Drawing	4.1	Have you attached a line drawing to this application that shows the water flow through your facility with a water balance? (See instructions for drawing requirements. See Exhibit 2D-2 at end of instructions for example.) ☒ Yes
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SECTION 5. INTERMITTENT OR SEASONAL FLOWS (40 CFR 122.21(K)(3)(III))

Intermittent or Seasonal Flows	5.1	Except for stormwater runoff, leaks, or spills, are any expected discharges described in Sections 1 and 3 intermittent or seasonal? ☐ Yes ☒ No → SKIP to Section 6.					
	5.2	Provide information on intermittent or seasonal flows for each applicable outfall. Attach additional pages, if necessary.					
	Outfall Number	Operations (list)	Frequency		Rate and Volume		Duration
			Average Days/Week	Average Months/Year	Maximum Daily Discharge	Maximum Total Volume	
			days/week	months/year	mgd	gallons	days
			days/week	months/year	mgd	gallons	days
			days/week	months/year	mgd	gallons	days
	Outfall Number	Operations (list)	Frequency		Rate and Volume		Duration
			Average Days/Week	Average Months/Year	Maximum Daily Discharge	Maximum Total Volume	
			days/week	months/year	mgd	gallons	days
			days/week	months/year	mgd	gallons	days
			days/week	months/year	mgd	gallons	days
	Outfall Number	Operations (list)	Frequency		Rate and Volume		Duration
			Average Days/Week	Average Months/Year	Maximum Daily Discharge	Maximum Total Volume	
			days/week	months/year	mgd	gallons	days
		days/week	months/year	mgd	gallons	days	
		days/week	months/year	mgd	gallons	days	

SECTION 6. PRODUCTION (40 CFR 122.21(K)(4))

Production	6.1	Do any effluent limitation guidelines (ELGs) promulgated by EPA under CWA Section 304 apply to your facility? ☐ Yes ☒ No → SKIP to Section 7.		
	6.2	Provide the following information on applicable ELGs.		
		ELG Category	ELG Subcategory	Regulatory Citation

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Production Continued	6.3	Are the limitations in the applicable ELGs expressed in terms of production (or other measure of operation)? ☐ Yes ☐ No → SKIP to Section 7.				
	6.4	Provide an expected measure of average daily production expressed in terms and units of applicable ELGs.				
	Expected Actual Average Daily Production for First Three Years					
	Outfall Number	Year	Operation, Product, or Material	Quantity per Day (note basis if applicable)	Unit of Measure	
		Year 1				
		Year 2				
		Year 3				
		Year 1				
		Year 2				
		Year 3				
	Year 1					
	Year 2					
	Year 3					
SECTION 7. EFFLUENT CHARACTERISTICS (40 CFR 122.21(K)(5))						
Effluent Characteristics	See the instructions to determine the parameters and pollutants you are required to monitor and, in turn, the tables you must complete. Note that not all applicants need to complete each table.					
	Table A. Conventional and Non-Conventional Parameters					
	7.1	Are you requesting a waiver from your NPDES permitting authority for any Table A parameters for any of your outfalls? ☐ Yes ☒ No → SKIP to Item 7.3.				
	7.2	If yes, indicate the applicable outfalls below or check the appropriate box to indicate that you are requesting a waiver for all outfalls. Attach waiver request and other required information to the application. Outfall number _____ Outfall number _____ Outfall number _____ ☐ I am requesting a waiver for some pollutants at all outfalls. ☐ I am requesting a waiver for all pollutants at all outfalls → SKIP to Item 7.4.				
	7.3	Have you have provided estimates or actual data for all Table A parameters for each of your outfalls for which a waiver has not been requested and attached the results to this application package? ☒ Yes				
	Table B. Certain Conventional and Non-Conventional Pollutants					
	7.4	Have you checked "Believed Present" for all pollutants listed in Table B that are limited directly or indirectly by an applicable ELG? ☐ Yes ☒ Not applicable				
7.5	Have you checked "Believed Present" or "Believed Absent" for all remaining pollutants listed in Table B? ☒ Yes					

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Effluent Characteristics Continued	7.6	Have you provided estimated data for those Table B pollutants for which you have indicated are "Believed Present" in your discharge? ☒ Yes		
	Table C. Toxic Metals, Total Cyanide, and Total Phenols			
	7.7	Have you indicated whether pollutants are "Believed Present" or "Believed Absent" for all pollutants listed in Table C for all outfalls? ☐ Yes		
	7.8	Have you completed Table C by providing estimated data for pollutants you indicated are "Believed Present," including the source of the information, for each applicable outfall? ☐ Yes		
	Table D. Organic Toxic Pollutants (GC/MS Fractions)			
	7.9	Do you qualify for a small business exemption under the criteria specified in the Instructions? ☐ Yes → Note that you qualify at the top of Table D, then SKIP to Item 7.12. ☒ No		
	7.10	Have you indicated whether pollutants are "Believed Present" or "Believed Absent" for all pollutants listed in Table D for all outfalls? ☒ Yes		
	7.11	Have you completed Table D by providing estimated data for pollutants you indicated are "Believed Present," including the source of the information, for each applicable outfall? ☐ Yes		
	2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD)			
	7.12	Does the facility use or manufacture one or more of the 2,3,7,8-TCDD congeners listed in the Instructions, or do you know or have reason to believe that TCDD is or may be present in effluent from any of your outfalls? ☐ Yes ☒ No		
	Table E. Certain Hazardous Substances and Asbestos			
	7.13	Have you indicated whether pollutants are "Believed Present" or "Believed Absent" for all pollutants listed in Table E for all outfalls? ☒ Yes		
	7.14	Have you completed Table E by reporting the reason the pollutants are expected to be present and available quantitative data for pollutants you indicated are "Believed Present" for each applicable outfall? ☒ Yes		
	Intake Credits, Tables A through E			
7.15	Are you applying for net credits for the presence of any of the pollutants in Tables A through E for any of your outfalls? ☐ Yes → Consult with your NPDES permitting authority. ☒ No			
SECTION 8. ENGINEERING REPORT (40 CFR 122.21(K)(6))				
Engineering Report	8.1	Do you have any technical evaluations of your wastewater treatment, including engineering reports or pilot plant studies? ☐ Yes ☒ No → SKIP to Item 8.3.		
	8.2	Have you provided the technical evaluation and all related documents to this application package? ☐ Yes ☒ No		
	8.3	Are you aware of any existing plant(s) whose production processes, wastewater constituents, or wastewater treatment resemble those at your facility? ☒ Yes ☐ No → SKIP to Section 9.		

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Engineering Report Continued	8.4	Provide the names and locations of the similar plants.			
		Name of Similar Plant		Location of Similar Plant	
		Kingfish Zeeland		Oost Zeedijk 13, 4485 PM Kats, Netherlands Receiving Waters	
SECTION 9. OTHER INFORMATION (40 CFR 122.21(K)(7))					
Other Information	9.1	Have you attached any optional information that you would like considered as part of the application review process (i.e., material beyond that which you have already noted in the application as being attached)?			
		☒ Yes ☐ No → SKIP to Section 10.			
	9.2	List the additional items and briefly note why you have included them.			
		1. Please see Attachment 2 and Attachment 4 for further clarification on proposed limitations for			
		2. In addition to constant monitoring of intake and effluent water in the facility, Kingfish Maine has established an ap			
		3.			
		4.			
5.					
SECTION 10. CHECKLIST AND CERTIFICATION STATEMENT (40 CFR 122.22(A) AND (D))					
Checklist and Certification Statement	10.1	In Column 1 below, mark the sections of Form 2D that you have completed and are submitting with your application. For each section, specify in Column 2 any attachments that you are enclosing to alert the permitting authority. Note that not all applicants are required to complete all sections or tables, or provide attachments.			
		Column 1		Column 2	
		☒	Section 1: Expected Outfall Location	☒	w/ attachments (e.g., responses for additional outfalls)
		☒	Section 2: Expected Discharge Date	☐	w/ attachments
		☒	Section 3: Average Flows and Treatment	☐	w/ attachments
		☒	Section 4: Line Drawing	☒	w/ line drawing ☒ w/ additional attachments
		☐	Section 5: Intermittent or Seasonal Flows	☐	w/ attachments
		☐	Section 6: Production	☐	w/ attachments
		☒	Section 7: Effluent Characteristics	☐ w/ Table A waiver request or approval ☒ Table A ☒ Table B ☐ Table C ☐ Table D ☐ Table E ☐ w/ other attachments	
		☐	Section 8: Engineering Report	☐	w/ technical evaluations and related attachments
		☒	Section 9: Other Information	☒	w/ optional information
		☒	Section 10: Checklist and Certification Statement	☐	w/ attachments

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Checklist and Certification Statement Continued	10.2	Provide the following certification. (See instructions to determine the appropriate person to sign the application.)		
		Certification Statement <i>I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.</i>		
		Name (print or type first and last name)	Official title	
		Signature	Date signed	

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TABLE A. CONVENTIONAL AND NON CONVENTIONAL PARAMETER ESTIMATES (40 CFR 122.21(K)(5)(I)) ¹									
Pollutant	Waiver Requested (if applicable)	Units	Effluent Data				Intake Water		
			Maximum Daily Discharge (required)	Average Daily Discharge (if available)	Source of Information (use codes in instructions)	Believed Present? (check only one response per parameter)			
☐ Check here if you have applied to your NPDES authority for a waiver for <i>all</i> of the pollutants listed on this table for the noted outfall.									
1. Biochemical oxygen demand (BOD ₅)	☐	Concentration					☒ Yes	☐ No	
		Mass	lbs	2,714	1,629	1,4			
2. Chemical oxygen demand (COD)	☐	Concentration					☒ Yes	☐ No	
		Mass							
3. Total organic carbon (TOC)	☐	Concentration					☒ Yes	☐ No	
		Mass							
4. Total suspended solids (TSS)	☐	Concentration					☒ Yes	☐ No	
		Mass	lbs	2,714	1,629	1,4			
5. Ammonia (as N)	☐	Concentration					☒ Yes	☐ No	
		Mass							
6. Flow	☐	Rate	M gpd	28.7	28.7	1,4-heat recovery(22.2mgd);culture	☐ Yes	☒ No	
7. Temperature	☐	°C	°C	0-7.8	0-7.8	4	☒ Yes	☐ No	
	☐	°C	°C	7.8-15	7.8-15	4			
8. pH	☐	Standard units	S.U.	6	6	1,4	☒ Yes	☐ No	
	☐	Standard units	S.U.	9	9	1,4			

¹ Sampling shall be conducted according to sufficiently sensitive test procedures (i.e., methods) approved under 40 CFR 136 for the analysis of pollutants or pollutant parameters or required under 40 CFR Chapter I, Subchapter N or O. See instructions and 40 CFR 122.21(e)(3).

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TABLE B. CERTAIN CONVENTIONAL AND NON CONVENTIONAL POLLUTANTS (40 CFR 122.21(K)(5)(II)) ¹									
Pollutant		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present or Limited by an ELG (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Effluent				Intake Water	
				Units	Maximum Daily Discharge (required)	Average Daily Discharge (if available)	Source of Information (use codes in instructions)	Believed Present? (check only one response per item)	
☐ Check (✓) here if you believe all pollutants listed to be absent from the discharge. You need not complete Table B for the noted outfall <i>unless</i> you have quantitative data available.									
1.	Bromide (24959-67-9)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
2.	Chlorine, total residual	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
3.	Color	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
4.	Fecal coliform	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
5.	Fluoride (16984-48-8)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
6.	Nitrate-nitrite	☒	☐	Concentration					☒ Yes ☐ No
				Mass					
7.	Nitrogen, total organic (as N)	☒	☐	Concentration				1,4	☒ Yes ☐ No
				Mass	lbs	1,639	1,639		
8.	Oil and grease	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
9.	Phosphorus (as P), total (7723-14-0)	☒	☐	Concentration					☒ Yes ☐ No
				Mass	lbs	393	393		
10.	Sulfate (as SO ₄) (14808-79-8)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
11.	Sulfide (as S)	☐	☒	Concentration					☐ Yes ☒ No
				Mass					

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TABLE B. CERTAIN CONVENTIONAL AND NON CONVENTIONAL POLLUTANTS (40 CFR 122.21(K)(5)(II))¹									
Pollutant		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present or Limited by an ELG (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Effluent			Intake Water		
				Units	Maximum Daily Discharge (required)	Average Daily Discharge (if available)	Source of Information (use codes in instructions)	Believed Present? (check only one response per item)	
12.	Sulfite (as SO ₃) (14265-45-3)	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
13.	Surfactants	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
14.	Aluminum, total (7429-90-5)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
15.	Barium, total (7440-39-3)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
16.	Boron, total (7440-42-8)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
17.	Cobalt, total (7440-48-4)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
18.	Iron, total (7439-89-6)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
19.	Magnesium, total (7439-95-4)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
20.	Molybdenum, total (7439-98-7)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
21.	Manganese, total (7439-96-5)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					
22.	Tin, total (7440-31-5)	☐	☒	Concentration					☒ Yes ☐ No
				Mass					

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TABLE B. CERTAIN CONVENTIONAL AND NON CONVENTIONAL POLLUTANTS (40 CFR 122.21(K)(5)(II)) ¹									
Pollutant		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present or Limited by an ELG (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Effluent			Intake Water		
				Units	Maximum Daily Discharge (required)	Average Daily Discharge (if available)	Source of Information (use codes in instructions)	Believed Present? (check only one response per item)	
23.	Titanium, total (7440-32-6)	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
24.	Radioactivity								
24.1	Alpha, total	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
24.2	Beta, total	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
24.3	Radium, total	☐	☒	Concentration					☐ Yes ☒ No
				Mass					
24.4	Radium 226, total	☐	☒	Concentration					☐ Yes ☒ No
				Mass					

¹ Sampling shall be conducted according to sufficiently sensitive test procedures (i.e., methods) approved under 40 CFR 136 for the analysis of pollutants or pollutant parameters or required under 40 CFR Chapter I, Subchapter N or O. See instructions and 40 CFR 122.21(e)(3).

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TABLE C. TOXIC METALS, TOTAL CYANIDE, AND TOTAL PHENOLS (40 CFR 122.21(K)(5)(III)(A))¹

Pollutant (CAS Number, if available)	Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (Provide both concentration and mass estimates for each pollutant.)						
	Believed Present	Believed Absent	Effluent				Intake Water		
			Units	Maximum Daily Discharge (required)	Average Daily Discharge (if available)	Source of Information (Use codes in Instructions.)	Believed Present? (Check only one response per pollutant.)		
☒	Check (✓) here if you believe all pollutants listed to be absent from the discharge. You need not complete Table C for the noted outfall <i>unless</i> you have quantitative data available.								
1. Antimony, Total (7440-36-0)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
2. Arsenic, Total (7440-38-2)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
3. Beryllium, Total (7440-41-7)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
4. Cadmium, Total (7440-43-9)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
5. Chromium, Total (7440-47-3)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
6. Copper, Total (7440-50-8)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
7. Lead, Total (7439-92-1)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
8. Mercury, Total (7439-97-6)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
9. Nickel, Total (7440-02-0)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
10. Selenium, Total (7782-49-2)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
11. Silver, Total (7440-22-4)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
12. Thallium, Total (7440-28-0)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
13. Zinc, Total (7440-66-6)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
14. Cyanide, Total (57-12-5)	☐	☐	Concentration					☒ Yes	☐ No
			Mass						
15. Phenols, Total	☐	☐	Concentration					☒ Yes	☐ No
			Mass						

¹ Sampling shall be conducted according to sufficiently sensitive test procedures (i.e., methods) approved under 40 CFR 136 for the analysis of pollutants or pollutant parameters or required under 40 CFR Chapter I, Subchapter N or O. See Instructions and 40 CFR 122.21(e)(3).

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B))¹

Pollutant (CAS Number, if available)	Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)				
	Believed Present	Believed Absent	Units	Effluent			Intake Water
				Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)

☒ Check here if all pollutants listed in Table D are expected to be absent from your facility's discharge.

☐ Check here if the facility believes it is exempt from Table D reporting requirements because it is a qualified small business. See the instructions for exemption criteria and for a list of materials you must attach to the application.

Note: If you check either of the above boxes, you do not need to complete Table D for the noted outfall *unless* you have quantitative data available.

1. Organic Toxic Pollutants (GC/MS Fraction—Volatile Compounds)

1.1	Acrolein (107-02-8)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.2	Acrylonitrile (107-13-1)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.3	Benzene (71-43-2)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.4	Bromoform (75-25-2)	☐	☐	Concentration					☒ Yes ☐ No
				Mass					
1.5	Carbon tetrachloride (56-23-5)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.6	Chlorobenzene (108-90-7)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.7	Chlorodibromomethane (124-48-1)	☐	☐	Concentration					☒ Yes ☐ No
				Mass					
1.8	Chloroethane (75-00-3)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.9	2-chloroethylvinyl ether (110-75-8)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.10	Chloroform (67-66-3)	☐	☐	Concentration					☒ Yes ☐ No
				Mass					
1.11	Dichlorobromomethane (75-27-4)	☐	☐	Concentration					☒ Yes ☐ No
				Mass					

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B)) ¹									
Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Units	Effluent			Intake Water	
					Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
1.12	1,1-dichloroethane (75-34-3)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.13	1,2-dichloroethane (107-06-2)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.14	1,1-dichloroethylene (75-35-4)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.15	1,2-dichloropropane (78-87-5)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.16	1,3-dichloropropylene (542-75-6)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.17	Ethylbenzene (100-41-4)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.18	Methyl bromide (74-83-9)	☐	☐	Concentration					☒ Yes ☐ No
				Mass					
1.19	Methyl chloride (74-87-3)	☐	☐	Concentration					☒ Yes ☐ No
				Mass					
1.20	Methylene chloride (75-09-2)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.21	1,1,2,2-tetrachloroethane (79-34-5)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.22	Tetrachloroethylene (127-18-4)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.23	Toluene (108-88-3)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
1.24	1,2-trans-dichloroethylene (156-60-5)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B))¹

Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)						Intake Water	
		Believed Present	Believed Absent	Units		Effluent			Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
						Maximum Daily Discharge	Average Daily Discharge			☐ Yes	☒ No
1.25	1,1,1-trichloroethane (71-55-6)	☐	☐	Concentration							
				Mass							
1.26	1,1,2-trichloroethane (79-00-5)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
1.27	Trichloroethylene (79-01-6)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
1.28	Vinyl chloride (75-01-4)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2. Organic Toxic Pollutants (GC/MS Fraction—Acid Compounds)											
2.1	2-chlorophenol (95-57-8)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.2	2,4-dichlorophenol (120-83-2)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.3	2,4-dimethylphenol (105-67-9)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.4	4,6-dinitro-o-cresol (534-52-1)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.5	2,4-dinitrophenol (51-28-5)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.6	2-nitrophenol (88-75-5)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.7	4-nitrophenol (100-02-7)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.8	p-chloro-m-cresol (59-50-7)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							
2.9	Pentachlorophenol (87-86-5)	☐	☐	Concentration						☐ Yes	☒ No
				Mass							

EPA Identification Number		Facility Name Kingfish Maine Inc		Outfall Number				OMB No. 2040-0004 Expires 07/31/2026	
TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B)) ¹									
Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Units	Effluent			Intake Water	
					Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
2.10	Phenol (108-95-2)	☐	☐	Concentration					☒ Yes ☐ No
				Mass					
2.11	2,4,6-trichlorophenol (88-05-2)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3. Organic Toxic Pollutants (GC/MS Fraction—Base /Neutral Compounds)									
3.1	Acenaphthene (83-32-9)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.2	Acenaphthylene (208-96-8)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.3	Anthracene (120-12-7)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.4	Benzidine (92-87-5)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.5	Benzo (a) anthracene (56-55-3)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.6	Benzo (a) pyrene (50-32-8)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.7	3,4-benzofluoranthene (205-99-2)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.8	Benzo (ghi) perylene (191-24-2)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.9	Benzo (k) fluoranthene (207-08-9)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.10	Bis (2-chloroethoxy) methane (111-91-1)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
3.11	Bis (2-chloroethyl) ether (111-44-4)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B))¹

Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Units	Effluent			Intake Water	
					Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
3.12	Bis (2-chloroisopropyl) ether (102-80-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.13	Bis (2-ethylhexyl) phthalate (117-81-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.14	4-bromophenyl phenyl ether (101-55-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.15	Butyl benzyl phthalate (85-68-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.16	2-chloronaphthalene (91-58-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.17	4-chlorophenyl phenyl ether (7005-72-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.18	Chrysene (218-01-9)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.19	Dibenzo (a,h) anthracene (53-70-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.20	1,2-dichlorobenzene (95-50-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.21	1,3-dichlorobenzene (541-73-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.22	1,4-dichlorobenzene (106-46-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.23	3,3-dichlorobenzidine (91-94-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.24	Diethyl phthalate (84-66-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.25	Dimethyl phthalate (131-11-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B))¹

Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Units	Effluent			Intake Water	
					Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
3.26	Di-n-butyl phthalate (84-74-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.27	2,4-dinitrotoluene (121-14-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.28	2,6-dinitrotoluene (606-20-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.29	Di-n-octyl phthalate (117-84-0)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.30	1,2-diphenylhydrazine (as azobenzene) (122-66-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.31	Fluoranthene (206-44-0)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.32	Fluorene (86-73-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.33	Hexachlorobenzene (118-74-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.34	Hexachlorobutadiene (87-68-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.35	Hexachlorocyclopentadiene (77-47-4)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.36	Hexachloroethane (67-72-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.37	Indeno (1,2,3-cd) pyrene (193-39-5)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.38	Isophorone (78-59-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.39	Naphthalene (91-20-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B))¹

Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Units	Effluent			Intake Water	
					Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
3.40	Nitrobenzene (98-95-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.41	N-nitrosodimethylamine (62-75-9)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.42	N-nitrosodi-n-propylamine (621-64-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.43	N-nitrosodiphenylamine (86-30-6)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.44	Phenanthrene (85-01-8)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.45	Pyrene (129-00-0)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
3.46	1,2,4-trichlorobenzene (120-82-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4. Organic Toxic Pollutants (GC/MS Fraction—Pesticides)									
4.1.	Aldrin (309-00-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.2	α-BHC (319-84-6)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.3	β-BHC (319-85-7)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.4	γ-BHC (58-89-9)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.5	δ-BHC (319-86-8)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.6	Chlordane (57-74-9)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B))¹

Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Units	Effluent			Intake Water	
					Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
4.7	4,4'-DDT (50-29-3)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.8	4,4'-DDE (72-55-9)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.9	4,4'-DDD (72-54-8)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.10	Dieldrin (60-57-1)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.11	α-endosulfan (115-29-7)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.12	β-endosulfan (115-29-7)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.13	Endosulfan sulfate (1031-07-8)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.14	Endrin (72-20-8)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					
4.15	Endrin aldehyde (7421-93-4)	☐	☐	Concentration					☐ Yes ☒ No
				Mass					

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TABLE D. ORGANIC TOXIC POLLUTANTS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY OR GC/MS FRACTIONS) (40 CFR 122.21(k)(5)(III)(B))¹

Pollutant (CAS Number, if available)		Presence or Absence (check one)		Estimated Data for Pollutants Expected to Be Present in Discharge (provide both concentration and mass estimates for each pollutant)					
		Believed Present	Believed Absent	Units	Effluent			Intake Water	
					Maximum Daily Discharge	Average Daily Discharge	Source of Information (use codes in instructions)	Believed Present? (check only one response per pollutant)	
4.16	Heptachlor (76-44-8)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.17	Heptachlor epoxide (1024-57-3)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.18	PCB-1242 (53469-21-9)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.19	PCB-1254 (11097-69-1)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.20	PCB-1221 (11104-28-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.21	PCB-1232 (11141-16-5)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.22	PCB-1248 (12672-29-6)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.23	PCB-1260 (11096-82-5)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.24	PCB-1016 (12674-11-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					
4.25	Toxaphene (8001-35-2)	☐	☐	Concentration				☐ Yes	☒ No
				Mass					

¹ Sampling shall be conducted according to sufficiently sensitive test procedures (i.e., methods) approved under 40 CFR 136 for the analysis of pollutants or pollutant parameters or required under 40 CFR Chapter I, Subchapter N or O. See instructions and 40 CFR 122.21(e)(3).

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TABLE E. CERTAIN HAZARDOUS SUBSTANCES AND ASBESTOS (40 CFR 122.21(K)(5)(V)) ¹					
Pollutant		Presence or Absence (check one)		Reason Pollutant Believed Present in Discharge	Available Quantitative Data (specify units)
		Believed Present	Believed Absent		
☒	Check (✓) here if you believe all pollutants listed to be absent from the discharge. You need not complete Table E for the noted outfall <i>unless</i> you have quantitative data available.				
1.	Asbestos	☐	☐		
2.	Acetaldehyde	☐	☐		
3.	Allyl alcohol	☐	☐		
4.	Allyl chloride	☐	☐		
5.	Amyl acetate	☐	☐		
6.	Aniline	☐	☐		
7.	Benzonitrile	☐	☐		
8.	Benzyl chloride	☐	☐		
9.	Butyl acetate	☐	☐		
10.	Butylamine	☐	☐		
11.	Captan	☐	☐		
12.	Carbaryl	☐	☐		
13.	Carbofuran	☐	☐		
14.	Carbon disulfide	☐	☐		
15.	Chlorpyrifos	☐	☐		
16.	Coumaphos	☐	☐		
17.	Cresol	☐	☐		
18.	Crotonaldehyde	☐	☐		

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TABLE E. CERTAIN HAZARDOUS SUBSTANCES AND ASBESTOS (40 CFR 122.21(K)(5)(V))¹

Pollutant		Presence or Absence (check one)		Reason Pollutant Believed Present in Discharge	Available Quantitative Data (specify units)
		Believed Present	Believed Absent		
19.	Cyclohexane	☐	☐		
20.	2,4-D (2,4-dichlorophenoxyacetic acid)	☐	☐		
21.	Diazinon	☐	☐		
22.	Dicamba	☐	☐		
23.	Dichlobenil	☐	☐		
24.	Dichlone	☐	☐		
25.	2,2-dichloropropionic acid	☐	☐		
26.	Dichlorvos	☐	☐		
27.	Diethyl amine	☐	☐		
28.	Dimethyl amine	☐	☐		
29.	Dinitrobenzene	☐	☐		
30.	Diquat	☐	☐		
31.	Disulfoton	☐	☐		
32.	Diuron	☐	☐		
33.	Epichlorohydrin	☐	☐		
34.	Ethion	☐	☐		
35.	Ethylene diamine	☐	☐		
36.	Ethylene dibromide	☐	☐		
37.	Formaldehyde	☐	☐		

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TABLE E. CERTAIN HAZARDOUS SUBSTANCES AND ASBESTOS (40 CFR 122.21(K)(5)(V))¹

Pollutant		Presence or Absence (check one)		Reason Pollutant Believed Present in Discharge	Available Quantitative Data (specify units)
		Believed Present	Believed Absent		
38.	Furfural	☐	☐		
39.	Guthion	☐	☐		
40.	Isoprene	☐	☐		
41.	Isopropanolamine	☐	☐		
42.	Kelthane	☐	☐		
43.	Kepone	☐	☐		
44.	Malathion	☐	☐		
45.	Mercaptodimethur	☐	☐		
46.	Methoxychlor	☐	☐		
47.	Methyl mercaptan	☐	☐		
48.	Methyl methacrylate	☐	☐		
49.	Methyl parathion	☐	☐		
50.	Mevinphos	☐	☐		
51.	Mexacarbate	☐	☐		
52.	Monoethyl amine	☐	☐		
53.	Monomethyl amine	☐	☐		
54.	Naled	☐	☐		
55.	Naphthenic acid	☐	☐		
56.	Nitrotoluene	☐	☐		

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TABLE E. CERTAIN HAZARDOUS SUBSTANCES AND ASBESTOS (40 CFR 122.21(K)(5)(V))¹

Pollutant		Presence or Absence (check one)		Reason Pollutant Believed Present in Discharge	Available Quantitative Data (specify units)
		Believed Present	Believed Absent		
57.	Parathion	☐	☐		
58.	Phenolsulfonate	☐	☐		
59.	Phosgene	☐	☐		
60.	Propargite	☐	☐		
61.	Propylene oxide	☐	☐		
62.	Pyrethrins	☐	☐		
63.	Quinoline	☐	☐		
64.	Resorcinol	☐	☐		
65.	Strontium	☐	☐		
66.	Strychnine	☐	☐		
67.	Styrene	☐	☐		
68.	2,4,5-T (2,4,5-trichlorophenoxyacetic acid)	☐	☐		
69.	TDE (tetrachlorodiphenyl ethane)	☐	☐		
70.	2,4,5-TP [2-(2,4,5-trichlorophenoxy) propanoic acid]	☐	☐		
71.	Trichlorofon	☐	☐		
72.	Triethanolamine	☐	☐		
73.	Triethylamine	☐	☐		
74.	Trimethylamine	☐	☐		
75.	Uranium	☐	☐		

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TABLE E. CERTAIN HAZARDOUS SUBSTANCES AND ASBESTOS (40 CFR 122.21(K)(5)(V))¹

Pollutant		Presence or Absence (check one)		Reason Pollutant Believed Present in Discharge	Available Quantitative Data (specify units)
		Believed Present	Believed Absent		
76.	Vanadium	☐	☐		
77.	Vinyl acetate	☐	☐		
78.	Xylene	☐	☐		
79.	Xylenol	☐	☐		
80.	Zirconium	☐	☐		

¹ Sampling shall be conducted according to sufficiently sensitive test procedures (i.e., methods) approved under 40 CFR 136 for the analysis of pollutants or pollutant parameters or required under 40 CFR Chapter I, Subchapter N or O. See instructions and 40 CFR 122.21(e)(3).

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Maine Department of Environmental Protection
Waste Discharge Permit Application

Food Processing Facilities

This form must be attached to the General Application for a Waste Discharge License/MEPDES Permit
(Form DEPLW0105-B2003)

Please answer all questions completely, using additional pages as necessary with responses clearly identified by item number on this form.

1. Facility Name: Kingfish Maine Inc NPDES # ME 0037559

2. Attach a drawing showing the water flow through the facility. Please include the sources and volumes of intake water, operations contributing to wastewater discharges, treatment units and outfalls with numbers corresponding to those in the general application.

See Attachment 8. Highlighted area shows flow of water from use in processing to specified processing water treatment system, followed by combination with effluent from the culture facility in the main discharge water treatment system. Outfall will be the same as all facility water. Outfall A or B.

3. Is chlorine used in the process or is the intake water chlorinated? for cleaning only If so, what is the concentration of chlorine in the final effluent(s)? less than 0.1mg/l
4. List chemicals used for sanitation or disinfection during production or clean-up operations, and maximum discharge concentrations.

See Attachment 6. Items are distinguished by area and purpose of use.

5. List chemicals used in products or processing, and maximum discharge concentrations.

See Attachment 6. Items are distinguished by area and purpose of use.

6. If boiler blowdown or non-contact cooling water is discharged, please complete EPA form 2E.

N/A

7. How are sanitary wastes disposed of?

Septic system to be established on site after obtaining all relevant building permits

8. Please complete the attached table of products and productions rates.
Complete a separate block for each product or type of production.

Product Name: Yellowtail Kingfish, Seriola lalandi

Pounds per day processed			Processing period(s) each year		Daily effluent flows	
Average	Maximum	Total pounds per year processed	Total weeks per year	During the months of	Average	Maximum
50,706	126,765	13,200,000	52	Jan Dec	53,000gal	80,000gal
Describe processing operation						
Fish will be harvested from the culture tank and humanely slaughtered in accordance with state and federal guidelines. Fish will be processed in an area separate from the culture systems, where they will be bled, eviscerated, and placed in temperature controlled containers						
Type of wastewater treatment						
All water used in processing will go through treatment in the processing plant, consisting of primary flocculation to bind particulates as well as fats and oils, mechanical filtration to remove all solids, and a secondary flocculation and water clarification. At this point, water will b						

Product Name: _____

Pounds per day processed			Processing period(s) each year		Daily effluent flows	
Average	Maximum	Total pounds per year processed	Total weeks per year	During the months of	Average	Maximum
Describe processing operation						
Type of wastewater treatment						

Product Name: _____

Pounds per day processed			Processing period(s) each year		Daily effluent flows	
Average	Maximum	Total pounds per year processed	Total weeks per year	During the months of	Average	Maximum
Describe processing operation						
Type of wastewater treatment						



Maine Department of Environmental Protection
Waste Discharge Permit Application

Fish Rearing Facilities

This form must be attached to the General Application for a Waste Discharge License.
(Form DEPLW0105-B2003)

Please answer all questions completely, using additional pages as necessary with responses clearly identified by item number on this form.

1. Facility Name: **Kingfish Maine** NPDES #ME 0037559
2. Source(s) of water supply and average monthly flow of each:
Chandler Bay & private well(s): 861 million gallons;
3. Is any of the hatching or rearing water heated or cooled by mixing with water from another source, use heat exchangers, etc? for cleaning only If yes, explain listing the volumes and maximum temperatures of each source.
We do not utilize water from any other sources than Chandler Bay (99.4%) or private on site wells (0.6%). We utilize 77.3% of water brought in from Chandler Bay for use in (1) heating the culture water (22.1% of total flow from Chandler Bay), and (
4. Type(s) of feed used:
commercially produced diet in multiple sizes to support various growth stages
5. Amount of feed used. Average: **22,000** lbs/day Maximum: **26,000** lbs./day
6. Month(s) of maximum feeding: Because standing stock is stable, feed remains consistent throughout the year.
7. Species of fish raised: **Yellowtail kingfish (Seriola lalandi)**
8. Maximum quantity of fish at any time.

Brood stock:	Pounds: <u>12,000</u>	Number of fish: <u>300</u>
First Year Fish:	Pounds: <u>approx. 4,400,000</u>	Number of fish: <u>approx 1,520,000</u>
Second Year Fish:	Pounds: <u> </u>	Number of fish: <u> </u>

(average: pounds: approx. 3,300,000; Number: approx. 1,140,000)

9. Attach a drawing showing the number, size and arrangement of all rearing tanks.

See Attachment 7
10. Attach a list of all disinfectants used, giving for each the name, ingredients, frequency of use, concentration of use, and total quantity used per year.

See Attachment 6
11. Attach a list of drugs and/or therapeutic agents used, giving for each a name, ingredients, frequency of use, concentration of use, and total quantity used per year.

See Attachment 6



Maine Department of Environmental Protection
Waste Discharge Permit Application

Outfall Information

DEPLW0102

Revised: 05/21/2020

This form must be attached to the General Application for a Waste Discharge License / MEPDES Permit
(Form DEPLW0105-B2003)

Please answer all questions completely, using additional pages as necessary with responses clearly identified by item number on this form.

1. Facility Name: Kingfish Maine MEPDES # ME 0037559

2. Attach a plan of the treatment facility or discharge sources showing the location of each outfall and the receiving water. Please number each outfall with the corresponding number from the permit application. See Attachment 1

3. For each outfall, provide the following information. Please use additional forms as necessary to describe all outfall locations.

A. Outfall name: A Outfall number: _____

B. Flow discharged. Average: 28.7 MGD Maximum: 26,000 MGD

C. Diameter of outfall pipe: 47.24 inches

D. Depth below mean low water at outlet: approx. 30 feet

E. Describe any diffusers, mixers or similar structures used to disperse the effluent.
Please include drawings or diagrams as appropriate. (use a separate sheet) See Attachment 8

F. Latitude and Longitude Coordinates: See Attachment 8

A. Outfall name: B Outfall number: _____

B. Flow discharged. Average: 28.7 MGD Maximum: 28.7 MGD

C. Diameter of outfall pipe: 47.24 inches

D. Depth below mean low water at outlet: approx. 30 feet

E. Describe any diffusers, mixers or similar structures used to disperse the effluent.
Please include drawings or diagrams as appropriate. (use a separate sheet)

F. Latitude and Longitude Coordinates: See Attachment 8

MEPDES Application – Attachment 1 – Map Depicting Facility Intake and Discharge Structures



MAP REFERENCE: USGS JONESPORT QUADRANGLE 2018

Gartley & Dorsky
ENGINEERING SURVEYING

CAMDEN, ME (207) 236-4365
WWW.GARTLEYDORSKY.COM

JUNE, 2020

KINGFISH MAINE **Jonesport, Maine** **RAS Facility**

19412

SITE LOCATION EXHIBIT

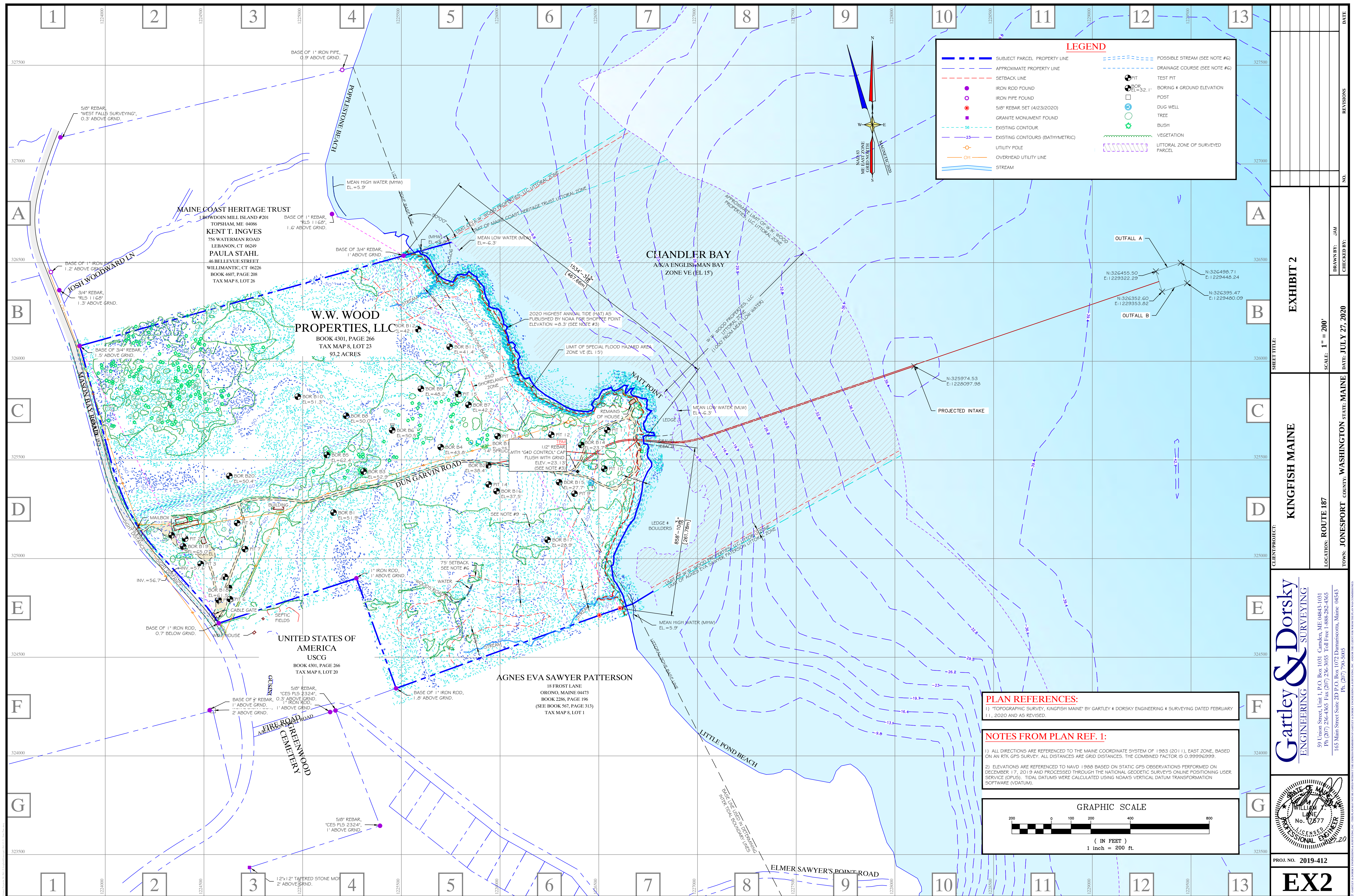
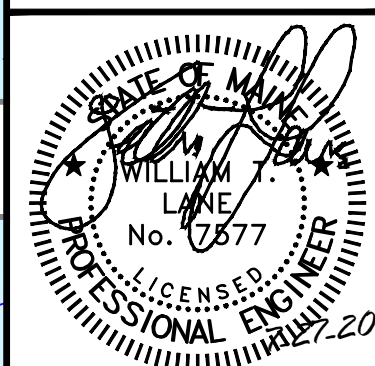


EXHIBIT 2

KINGFISH MAINE

Gartley & Dorsky
ENGINEERING SURVEYING



PROJ. NO. 2019-412

EX2



KINGFISH
MAINE

INTAKE & DISCHARGE WATER TREATMENT



SUMMARY OF KINGFISH MAINE MEPDES PERMIT

- Land based recirculating aquaculture facility, annually growing 6000-8000 ton of yellowtail kingfish, *Seriola lalandi*
- Employing approximately 70 people, potentially more if processing
- Site location: 9 Dun Garvin Road, Jonesport, Maine
- Receiving water: Chandler Bay
- **Key Features:**
 - Kingfish Maine will complete the whole culture cycle here in Jonesport – hatchery, growout, and harvest
 - Kingfish Maine renewal application includes processing of fish on site, though we are still deciding to what level.
 - Kingfish Maine will draw water from Chandler Bay; when referring to water use throughout the application, it is in one of three distinctions:

HEAT RECOVERY WATER

No contact with fish

Heat exchangers or Heat pumps

Discharge Tank

77.3% of total water

CULTURE WATER

Contacts fish

Wastewater Treatment

Discharge Tank

22.1% of total water

PROCESSING WATER

Contacts fish

Processing Area Treatment

Wastewater Treatment

Discharge Tank

0.6% of total water

SUMMARY OF KINGFISH MAINE WATER USE & FILTRATION

The facility will take in 19,812 gallons per minute (gpm) of seawater from Chandler Bay through two seawater intake pipes. It will go through large particle filtration and then be piped to the pump station to be split in two ways.

Heat Recovery Water

15,410gpm (3500m³/hr) of seawater will go through heat exchangers where Kingfish Maine will extract the thermal energy in the water in a countercurrent flow system. This energy will be utilized in heating the temperature of the water in the culture systems to our target range. This heat recovery water does not come in contact with culture water or fish. It has reduced suspended solids and colder temperature than when it was taken into the pump station but is otherwise unaltered from its natural state. Therefore, it will not go through any further treatment but will go directly to the discharge reservoir prior to being discharged in Outfall A or B.

Culture System & Processing Water

4,402 gpm (1000m³/hr) of seawater will be filtered for medium and fine solids and sent to a holding tank, which will feed hatchery, growout, and processing operation.

Culture System Water

Water leaving the culture tanks will go through mechanical filtration, which removes large and fine solids, within the recirculating system (40-60um). After mechanical filtration, new ("make up") water will be added and an equal amount of water will leave the culture system and go through heat exchangers, where we will recover all possible thermal energy from the discharged culture water by utilizing a countercurrent flow system and heat pumps with the *Heat Recovery Water*. The energy will be redirected back to maintain target water temperature in the culture systems. After this step, the culture system water will then be piped to the discharge water filtration system. In this system, water will go through large and fine solids filtration (mechanical, 100um), followed by biofilters for the reduction of nutrient load, and finally, sterilization. The water will then combine with other effluent sources in the discharge reservoir prior to being discharged in Outfall A or B.

Processing Water

The processing plant will use a small amount of the total seawater listed above in addition to a maximum of 107 gpm (25m³/hr) of freshwater as well. All water utilized in processing plant will be collected and go through its own dedicated filtration system first, consisting of a primary flocculation to bind fats, oils, and other biproducts of processing; mechanical filtration to remove solids and bound materials; then a secondary flocculation and water clarification. It will then progress to the main discharge water filtration system, where it will combine with the outflow from the culture systems, go through the same filtration steps, meaning this water, just as the culture water, has redundant filtration systems prior to discharge. It will finally go to the discharge reservoir with all filtered water prior to being discharged in Outfall A or B.

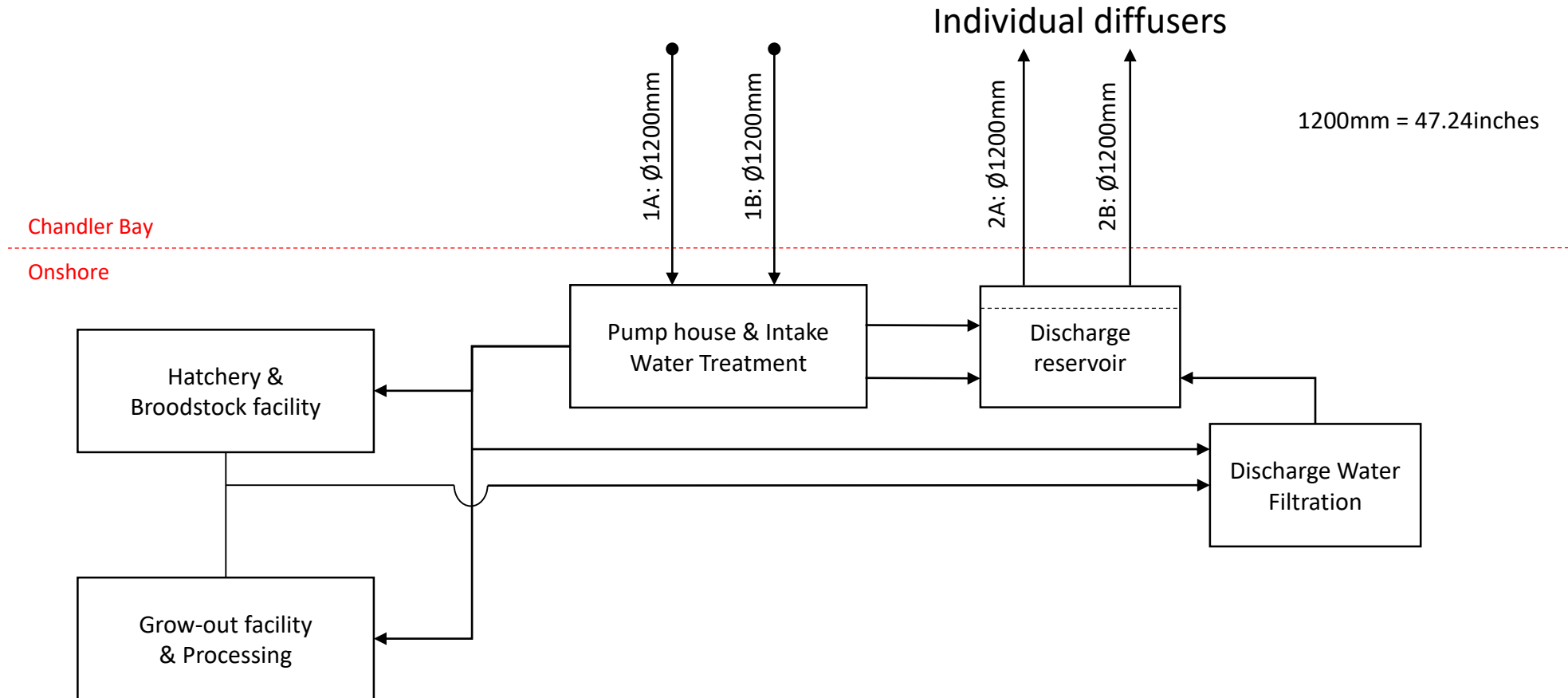


SUMMARY OF KINGFISH MAINE SOLID WASTE REMOVAL

As mentioned in “Summary of Kingfish Maine Water Use & Filtration”, solids filtration for the culture water, both large and fine particles, will occur using fish exclusion barriers and drum or disc filters to remove solids down to 40-60um in the culture systems, and 100um in the additional wastewater treatment system. The solids collected in the processing area will be filtered utilizing similar mechanical filtration and, if required, include chemical flocculants for improved clarification and removal.

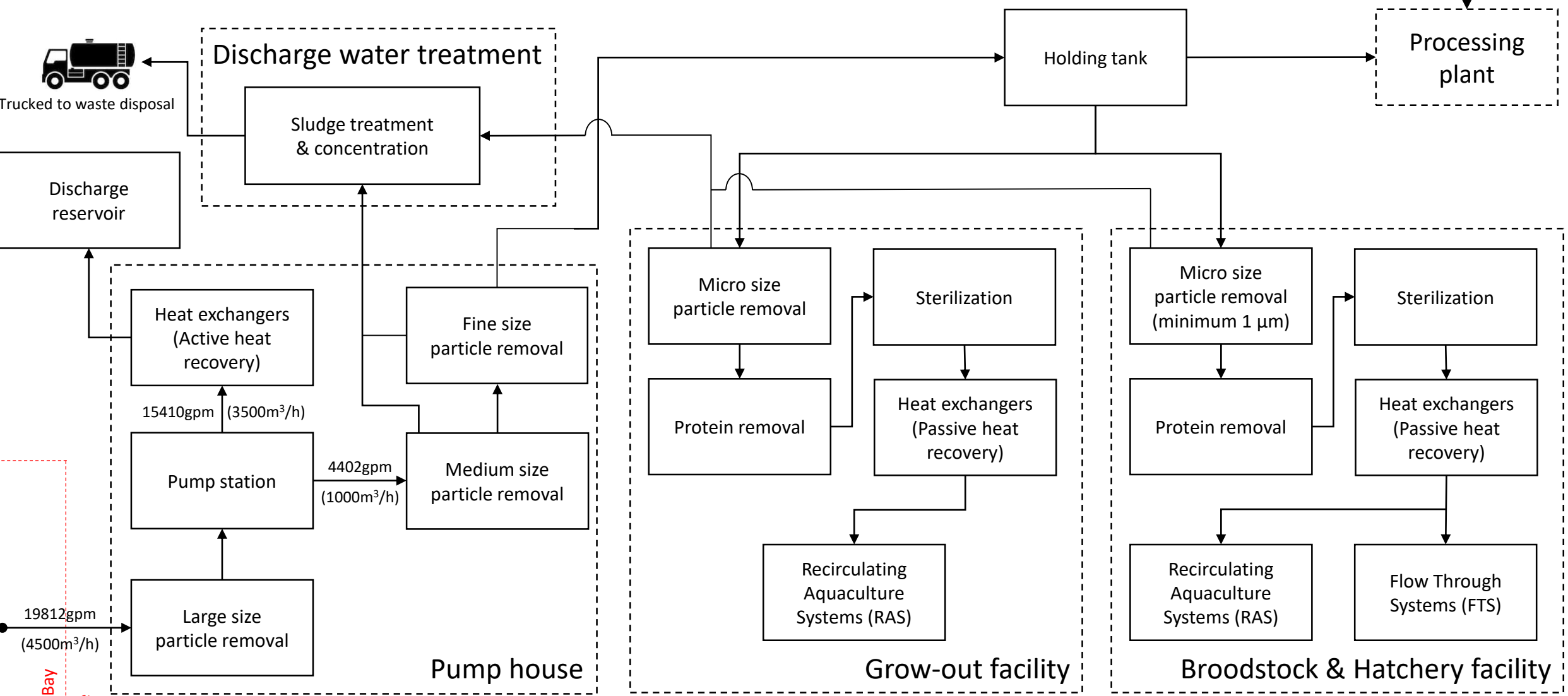
All solid waste will be combined and trucked offsite for disposal. Kingfish Maine is exploring secondary uses for any waste from processing activities on site, such as composting with Coast of Maine Organic Products Inc., an organic compost producer located in East Machias, ME. Due to the high or concentrated salt level in our sludge, options for additional uses are limited, but we continue to explore all new technology for making use of this nutrient rich material.

CONCEPTUAL FLOW DIAGRAM – INTAKE & EFFLUENT



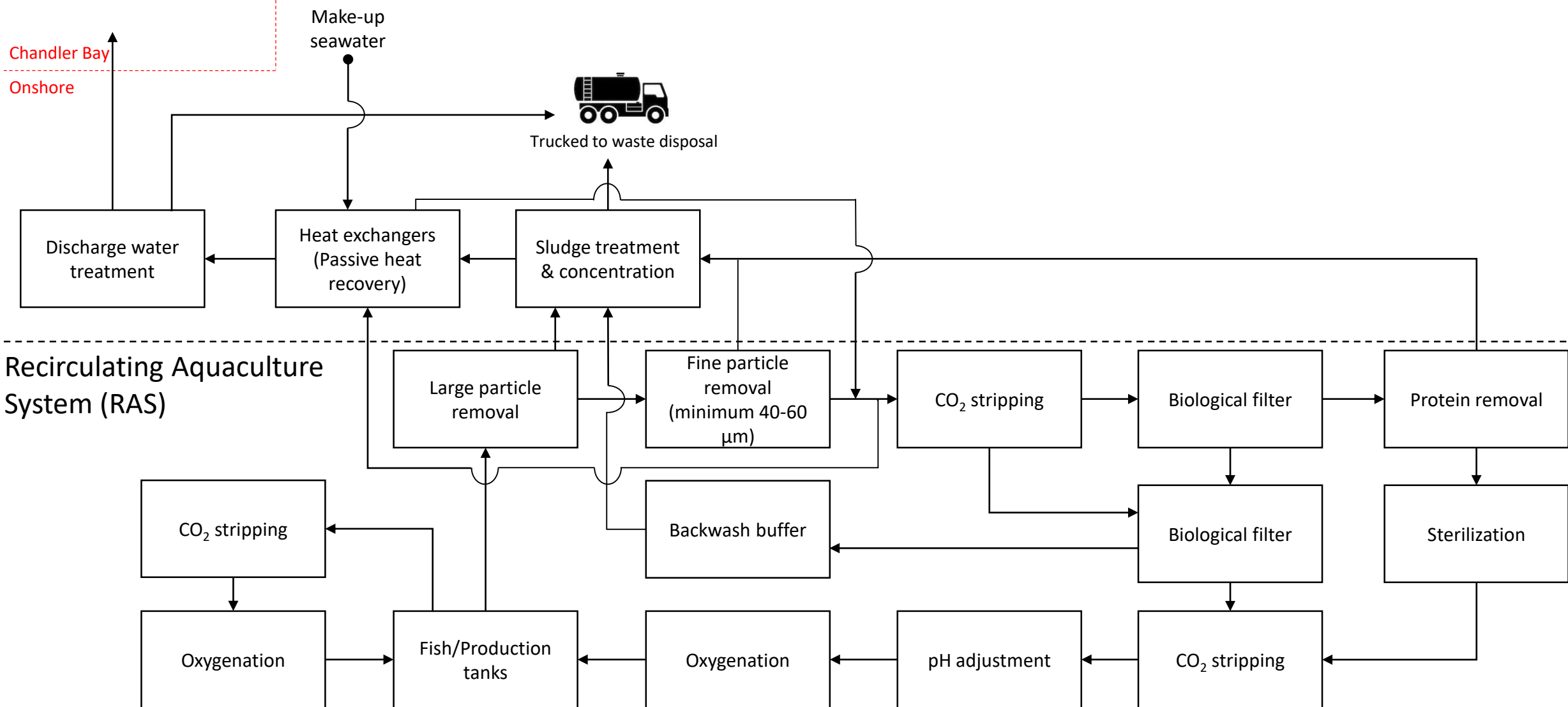
CONCEPTUAL FLOW DIAGRAM OF INTAKE WATER TREATMENT (IWT)

Freshwater
107gpm
(25m³/h)



Chandler Bay
Onshore

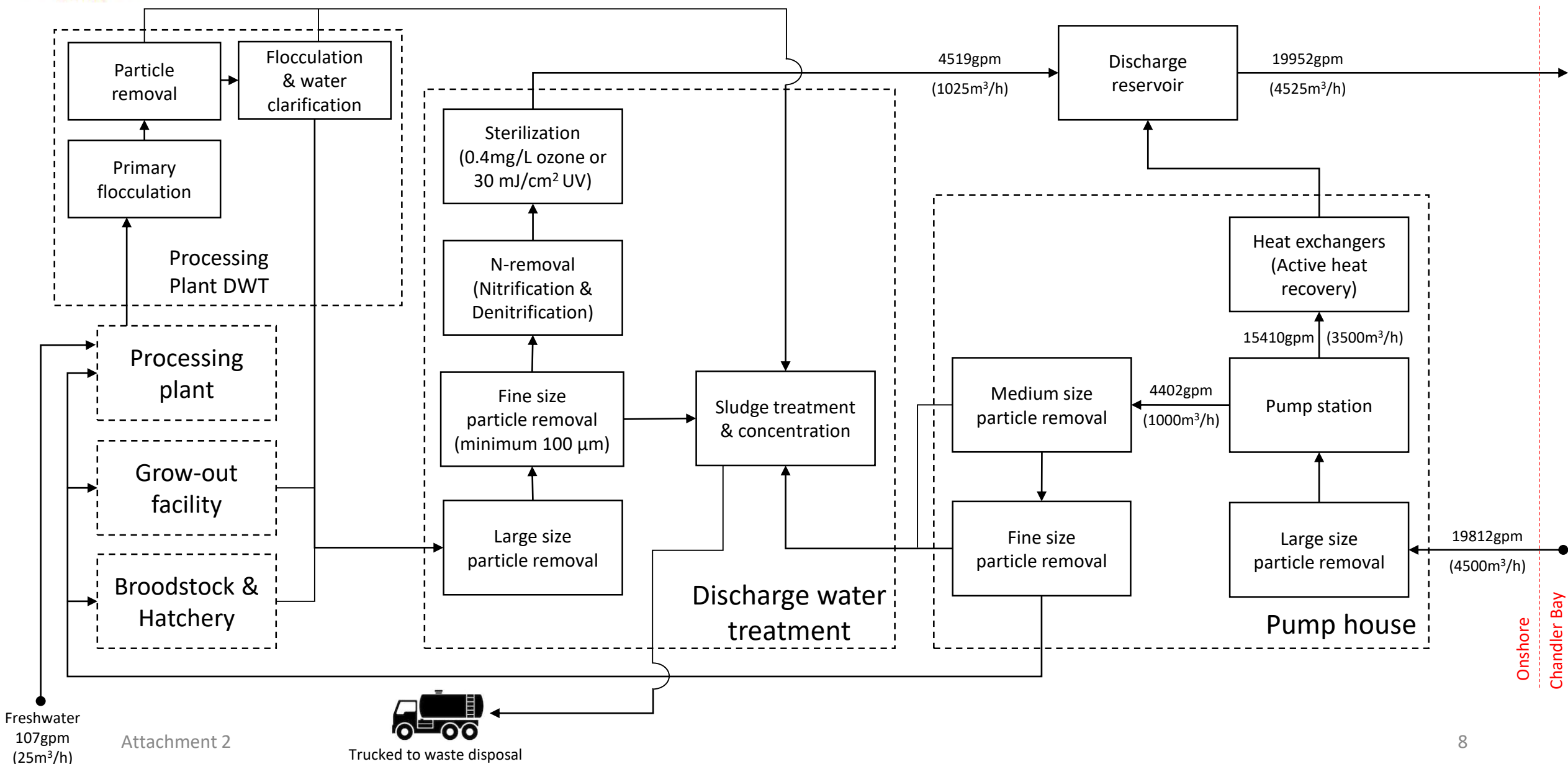
CONCEPTUAL FLOW DIAGRAM OF RAS SYSTEM





KINGFISH
MAINE

CONCEPTUAL FLOW DIAGRAM OF DISCHARGE WATER FILTRATION (DWF)



SUMMARY OF KEY PARAMETERS OF EFFLUENT

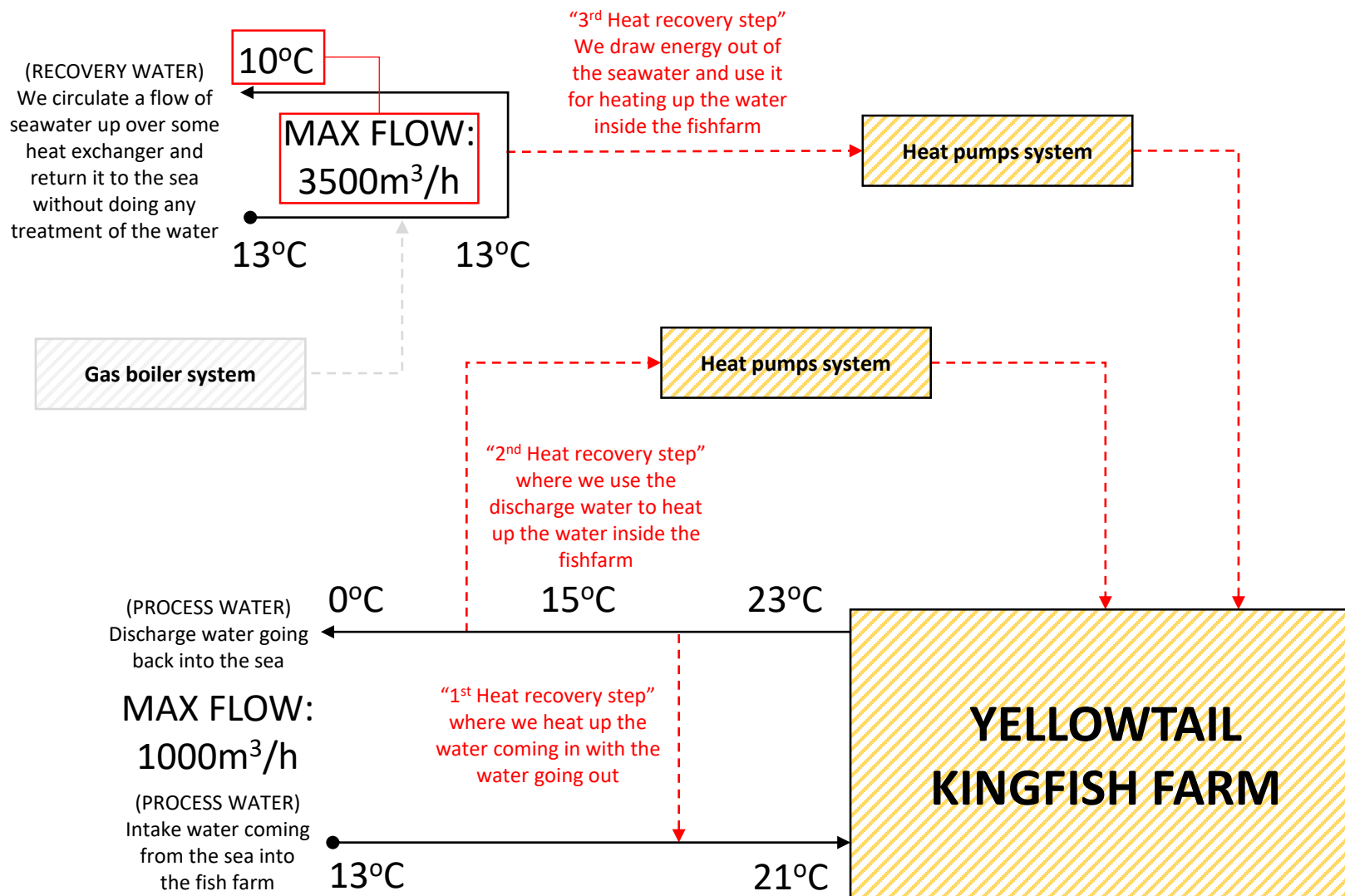
Parameter	Total Discharge (kg)	Total discharge (lbs)
Flow	108600m3/day	28.7MGD
BOD	740.5	1628.99
TSS	740.5	1628.99
Total N	745.0	1639
Total P	178.7	393
	Range	
pH	6.0-9.0	-
Salinity	Ambient	-
Temperature - Summer	7.8-15C	46-59F
Temperature - Winter	0-7.8C	32-46F
Near Field Dilution	60x	
Far Field Dilution	173x	

Amounts calculated from standing biomass and feed rates for yellowtail kingfish, *Seriola lalandi*, as well as wastewater treatment efficacy. Further explanation of these parameters is included as Attachment 6 to main application.

FISHFARM THERMODYNAMICS – EXAMPLE, SUMMER

This one-pager shows the general principle and example temperature behind Kingfish Maine's heat recovery methods.

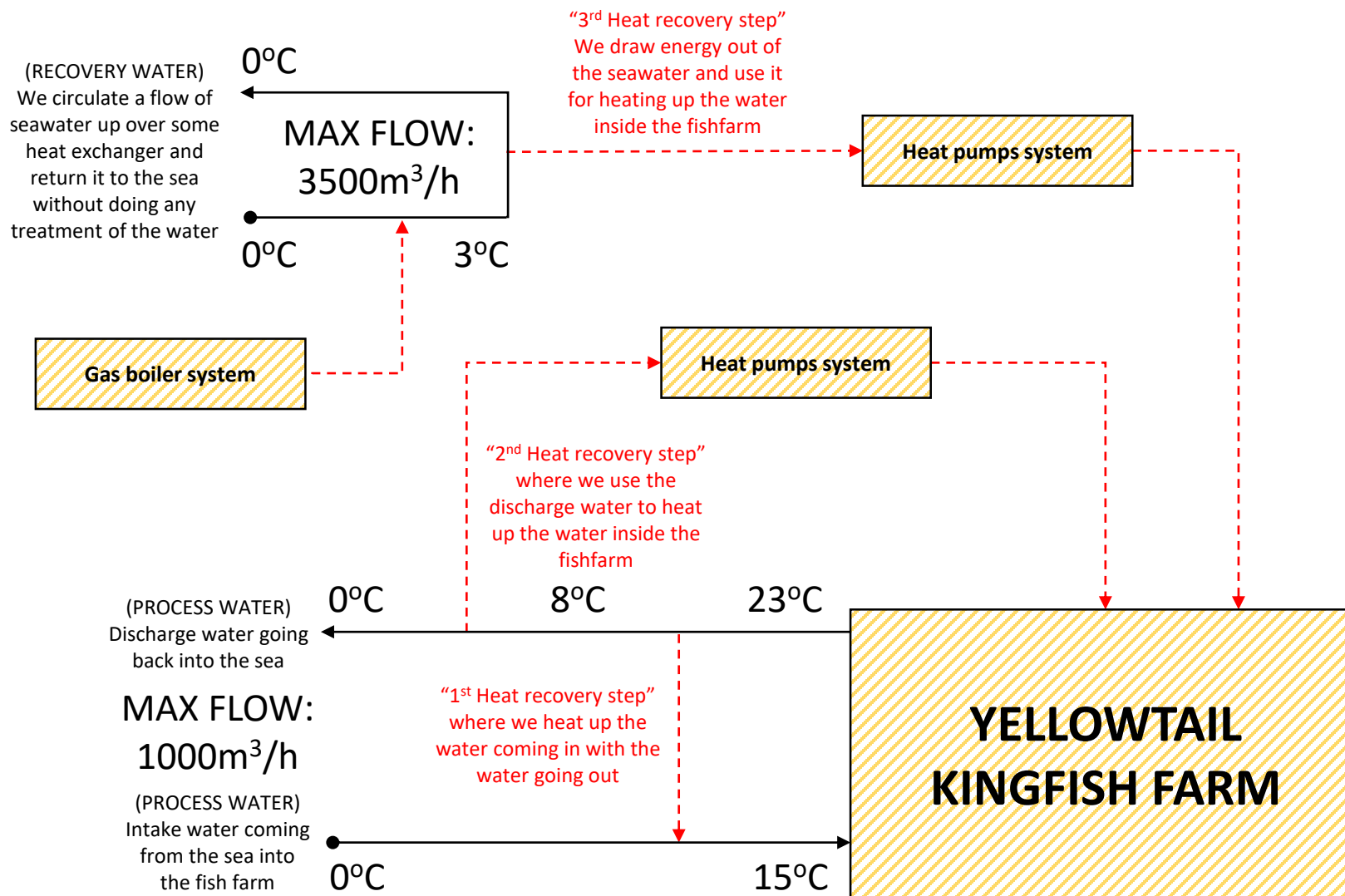
BLENDING FLOW
TEMP: 7.8°C



FISHFARM THERMODYNAMICS – EXAMPLE, WINTER

This one-pager shows the general principle and example temperature behind Kingfish Maine's heat recovery methods.

BLENDING FLOW
TEMP: 0°C





MEPDES Application – Attachment 3 – Construction Schedule of Treatment Facility and Outfall Structure

Kingfish Maine has completed the permitting process for a land-based recirculating aquaculture facility with a goal of beginning construction in 2027.

Specifically related to intake and outfall construction, any in-water construction will be completed within the recommended construction window for protection of marine resources, which is determined to be November 2027 to April 2028 in this area. The points of connection in the upland area for the intake and outfall pipes will be the pumphouse and wastewater treatment buildings, respectively. Construction of these building structures will be part of the larger construction schedule. Accordingly, connection of the intake and outfall pipes to their respective points on the upland will occur once the pipes have been completely configured in the aquatic environment and transitioned to the upland area. Commencement of discharge will not begin until fish rearing system has been completed and filled with water.

Outfall Number/Name Description, Volume Discharged and Receiving Water

Outfall A One 47.24 inch (1200mm) pipe running 2624ft (800m) out from mean low water into Chandler Bay. Daily discharge volume maximum: 28.7 million gallons per day (mgd) (4525m³/hr); diffuser is (6) 8.3in (211mm) ports with duckbill-style valves, with additional three capped ports for redundancy/cleaning

Outfall B One 47.24 inch (1200mm) pipe running perpendicular to Outfall A to the same endpoint in Chandler Bay. Provides redundant pipe and will be switched with Outfall A on a regular basis. Daily discharge volume maximum would be the same as listed for Outfall A, i.e. only one outfall is running at a time and total daily discharge maximum is 28.7 mgd. Diffuser set up is the same

MEPDES Application – Attachment 4 – Question 19, Main Form:

Kingfish Maine proposes to construct and operate a recirculating aquaculture facility to grow 6000-8000 tonnes annually of yellowtail kingfish, *Seriola lalandi*, in Jonesport, Maine. This facility would be completely vertically integrated, beginning with broodstock and egg production through to harvest and initial processing.

Company Background

The Kingfish Company began with the development of a site in the province of Zeeland in the Netherlands, located along the Oosterschelde, a Natura 2000 nature reserve. The company's focus in sustainability and responsible production practices is evidenced by the accolades of becoming the first land based Best Aquaculture Practices certified facility as well as the first Aquaculture Stewardship Council certified source of yellowtail kingfish. Beginning in 2019, The Kingfish Company began an expansion of its initial site to double production capacity and began looking for a location in the US to establish a new development.

In both Europe and the United States, the Kingfish Company continues to implement the best available technologies together with well established systems and operational protocols to maintain this focus on environmental sustainability.

Kingfish Maine Development

A key part of the Kingfish Company's growth is expansion to a facility in the US. Kingfish Maine was established in 2019 with the aim of creating a new farm site in Jonesport, Maine. The location identified brought one of the most critical elements needed for a marine fish land-based fish farm: direct access to good quality seawater. This water, coupled with recirculating aquaculture technology, allows us to not only maintain ideal conditions for the fish but also minimize environmental impact.

Intake and Outfall Siting

Kingfish Maine engaged with the local community early to get a first-hand understanding of priorities in Jonesport for an aquaculture facility. Discussions with residents of the Jonesport area indicated it was critical to them that the development of any pipes for this facility take up as short a footprint as possible into Chandler Bay because of fishing activity. Kingfish Maine approached siting the location of the intake and outfall pipes and modelling of effluent flow with this concern in mind, looking for an area that would achieve both regulatory compliance and the concerns of residents; we are fortunate that the local fishing community has such accurate knowledge of Chandler Bay, providing us great detail regarding seafloor characterization, depths, and areas of least impact on their activities.

To add to the local knowledge provided, Kingfish Maine engaged in data collection on the background water quality, flow, and current conditions in Chandler Bay; this, along with Maine DEP data were used to inform modelling of our effluent to ensure the application of Kingfish Company's system technology would be suitable for this specific site in Jonesport (see **Table 1** for summary ranges of these values).

Parameter	Background Level Chandler Bay
BOD5	<80mg/L
TSS	2.5-22mg/L
Total N	0.17-0.4mg/L
Total P	0.031-0.051mg/L
Temperature	2C - 16C (35-60.8F)
pH	8.0
Salinity	32-33ppt
Dissolved Oxygen	78%-115%

Table 1. Summary of background water quality parameters in Chandler Bay, Jonesport, Maine. Source of information is from Maine DEP data collection sites in the surrounding area, which include stations R1-3, ME02-0229, ME01-0064, and ME05-0016, as well as Kingfish Maine data collection, performed by internal staff as well as Normandeau and Associates.

We performed both near-field modelling using CORMIX and far-field modeling using TUFLOW, a 3-dimensional far-field model, of the facility's effluent in order to locate the outfall in a position that would maximize mixing and dispersion. Kingfish Maine assessed modelling results for effluent points progressively farther from shore in order to achieve the point of maximum mixing and dispersion with minimal disturbance to local activity.

Kingfish Maine Effluent Filtration and Pipe Design

In any aquaculture facility, there is an effluent consisting water that has been used to rear the fish and the byproducts of feeding and growing those fish: fish waste, which mainly consists of solids and naturally occurring nutrients, such as nitrogen and phosphorus. Because RAS facilities minimize demand or consumption of water by reusing it in the systems, effluent can be higher in these constituents, which is why wastewater filtration and treatment is specifically designed and sized to extract as much of that waste as possible. Kingfish Maine has employed best practicable treatment strategies in the design of our effluent filtration system (*detailed in Attachment 2 of the main application*), demonstrated in the following ways:

- Our system is designed to the specifications required of The Kingfish Company's certifications with Best Aquaculture Practices (BAP) and Aquaculture Stewardship Council, two globally recognized certification standards within the aquaculture industry.
- We incorporate redundancy in our treatment by water first being filtered within the recirculating culture systems themselves; water only leaves the system once it has been filtered and then goes to the wastewater filtration system, where it goes through a second round of multistep filtration and sterilization.
- We utilize proven technology in design our effluent treatment to ensure we maximize efficiency (see **Appendix B** for Wastewater Treatment diagram), which includes:
 - Large and fine particle filtration to remove solids (TSS), which is the major contributor to BOD
 - Moving and fixed bed bioreactors for nitrification and denitrification, or removal of nutrient load
 - Sterilization utilizing ozone or UV at recommended levels
- Locally, we have consulted with Maine Department of Marine Resources regarding solids filtration levels and water sterilization treatment application.

When incorporating our data and calculations into the modelling, we used conservative removal rate assumptions for each parameter in the effluent characterization to ensure the results provided a prudent depiction of how the effluent flow would behave in Chandler Bay (see **Appendix A**, Kingfish Maine

Near- and Far- Field Modelling Report – Chandler Bay, Maine for complete description of models and assumptions used to construct).

Kingfish Maine also incorporated a diffuser design on the end of each effluent pipe in order to aid in improved dispersion and mixing of the flow; this includes a six-port diffuser, each measuring 8.3 inches (211mm) with duckbill-style valves as well as an additional three, capped ports for redundancy and cleaning.

Kingfish Maine Effluent Characterization and Modelling Results

Sustainability and responsible environmental practices are at the core of The Kingfish Company's company values. Methods by which Kingfish Maine minimizes impacts in development of this project:

- By growing a marine species, Kingfish Maine uses minimal amounts of a limited resource, freshwater, and relies mainly on the use of seawater from Chandler Bay for operations.
- By utilizing recirculating aquaculture systems, Kingfish Maine is reducing as much as possible the demand for that seawater, focusing on continued efficiency improvements to reduce that necessary supply.
- By implementing multiple types and redundant filtration systems, Kingfish Maine is maximizing effluent treatment capability, improving the water quality of our effluent.
- By implementing the same multistep redundancy in our heat recovery systems, Kingfish Maine will be discharging cooler water than the facility takes in.

The results of modelling show that Kingfish Maine can achieve maximum mixing and dispersion without significant impacts to surrounding habitat and fishing activity by placing our effluent approximately 2,624 ft (800m) off the shore of the proposed site with the diffuser configuration described above. Dilution factors obtained were 60x at the edge of the near-field mixing zone (CORMIX), which is limited to 230ft from effluent point, and 173x in the far-field (TUFLOW) (see **Appendix A**, Kingfish Maine Near- and Far- Field Modelling Report – Chandler Bay, Maine for complete description of model results).

Key parameters in review show:

- *Total Flow*: 28.7 million gallons per day, of which
 - 6.4 million gallons is for the culture and processing of fish (“culture water”, “processing water”), and
 - 22.3 is used in reclaiming thermal energy from the water prior to discharge (“heat recovery water”)
- *pH and Salinity*: Kingfish Maine will not be altering pH or salinity outside of the normal range found in Chandler Bay as yellowtail kingfish require these parameters to remain with the ambient range naturally occurring.
- *Temperature*: Because of Kingfish Maine's heat recovery process, we will be sending out effluent that is colder than ambient temperatures in Chandler Bay, and we have no significant effect on the overall temperature of Chandler Bay. The maximum temperature difference shown in the far-field modelling results is 0.09°F cooler than ambient (see **Appendix A**, Kingfish Maine Near- and Far- Field Modelling Report – Chandler Bay, Maine for complete description of model results).
- *Total Nitrogen*: The dilution factor of 173x in the far-field model results in a total nitrogen (TN) level of 0.038mg/L contributed by Kingfish Maine effluent. With a median and mean background TN of 0.25mg/L and 0.27mg/L, respectively, total nitrogen in Chandler Bay would be 0.29mg/L-0.31mg/L, which is within the protective limits set by DEP for preservation of dissolved oxygen and eelgrass.

- *BOD₅ and TSS*: Presuming the standard permissible limits for BOD and TSS of 30mg/L average daily concentration and 50mg/L maximum daily concentration to be included in a discharge permit from DEP, at a dilution factor of 173x, Kingfish Maine effluent would result in less than 0.035mg/L change on dissolved oxygen in Chandler Bay. This would meet standard DEP assessment criteria.

Kingfish Maine will also be drawing water from Chandler Bay, allowing for ongoing monitoring of the water quality. Our intake pipes will be located at approximately half the distance, or 1,312 ft (400m) from shore along the same trajectory as the effluent pipes. This demonstrates our commitment to maintaining the environment in which we are located as any negative impact would be detrimental to our own operation.

Alternatives

As discussed above, Kingfish Maine is implementing best practicable treatment that has been demonstrably achieved in our industry in our wastewater treatment system and meeting standards of two globally recognized best aquaculture practices certifying agencies. In our modelling, we also completed model runs at a location further from shore that demonstrated greater dilution of a critical parameter, nitrogen. However, in order to achieve this, the discharge point was approximately 2 miles from shore in order to avoid depth limitations to tidal mixing caused by the presence of two islands, Ballast and Mark Islands. Locating the discharge at this point not only presents an economic limitation for Kingfish Maine but also requires far greater impact to the seafloor and to local fishing activity, in direct contrast to the main concerns voiced by the community. Assessing these factors, the placement of our discharge point at 2,624 ft (800m) and intake at 1,312 ft (400m) from shore of the site is the most favorable option.

From the design of our treatment system to the location of both our intake and effluent locations, Kingfish Maine continues to maintain our focus on sustainability, not just from an environmental perspective of our choices but also with respect to how we operate as a part of the local community.

Kingfish Maine Social and Economic Benefits

The Kingfish Company works to bring positive benefit to the communities where we develop. In establishing a vertically integrated business of this scale, encompassing all aspects of the aquaculture process of taking yellowtail kingfish from egg to market, provides significant opportunity for growth in Jonesport, Washington County, and Maine across a multitude of sectors in both direct and indirect engagement. Kingfish Maine can demonstrate and quantify this growth through our current activities.

- Kingfish Maine is already developing hatchery production in the University of Maine's Center for Cooperative Aquaculture Research. The company will be able to utilize this not just for immediate hatchery production but also to begin training a small group of individuals in a company standard, which can then be translated to new staff that is assembled for the larger facility. Through this measured approach to development, the workforce can be brought on systematically, allowing for Kingfish Maine to source employees locally as much as possible and collaborate with local entities to build education and training programs to fill the workforce.
- Kingfish Maine is already beginning to build this training program. In collaboration with teachers and administrators at Jonesport-Beals High School, Kingfish Maine is working to build an aquaculture focused education program with hands on work, such as constructing a small RAS system in the classroom with Kingfish Maine staff.

- The company is dedicated to providing quality employment opportunities to build a stronger, more resilient community. Kingfish Maine has consulted with Sunrise County Economic Council, Downeast Institute, as well as local individuals to better understand those aspects of a career package development that are most important as well as initiatives in which the company can participate to improve the community.

In order to attempt to quantify these benefits, Kingfish Maine engaged the University of Maine to perform an economic impact assessment. An IMPLAN model was used to evaluate the impact of the planning, construction, and ongoing operation of \$108 million-dollar RAS facility in Jonesport. Kingfish Maine is shown to provide significant numbers of both direct and indirect employment opportunities to Washington County. Between 2008 and 2018, Washington County saw private employment decline by 6.8% across all industries while the state, as a whole, saw a 3.2% increase. Ongoing operations of Kingfish Maine stands to provide 126 direct and indirect jobs to Washington County, accounting for an increase of 2% in private employment - 1/3 of the private employment jobs that were lost in 2008 to 2018. While the construction phase has wider implications at the state level, Kingfish Maine's ongoing operations provide significant benefits to both Jonesport and Washington county in terms of economic output, employment, and income level. The full report is still in draft as more information is added as details become available through the design process; however, a summary of the findings of this study is attached as **Appendix C**.

APPENDIX A *Kingfish Maine Near- and Far- Field Modelling Report – Chandler Bay, Maine, Ramboll Environmental*

Intended for
Kingfish Maine

Date
August 2020

Project Number
1690016697

KINGFISH MAINE NEAR- AND FAR- FIELD DISCHARGE MODELING REPORT – CHANDLER BAY, MAINE

KINGFISH MAINE
NEAR- AND FAR- FIELD DISCHARGE MODELING
REPORT – CHANDLER BAY, MAINE

Project No. 1690016697
Issue No. Final
Date 04 August 2020
Made by Holly Leslie
Checked by Richard Wenning
Approved by Richard Wenning

This report is produced by Ramboll at the request of the client for the purposes detailed herein. Ramboll neither owes nor accepts any duty to any third party and shall not be liable for any loss, damage or expense of whatsoever nature which is caused by their reliance on the information contained in this report.

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EXECUTIVE SUMMARY

The Kingfish Company and its subsidiary, Kingfish Maine (Kingfish), are proposing to construct and operate a 6,000 – 8,000 metric ton (MT) Yellowtail kingfish Recirculating Aquaculture System (RAS) facility in Jonesport, Maine. Included among the local, state, and federal permits that must be obtained before construction can begin is the requirement to apply for a Maine Pollutant Discharge Elimination System (MEPDES) permit, which governs the discharge of wastewater from the RAS facility.

This report describes the results of environmental modeling work performed by Ramboll to support the MEPDES permit application. This report aims to:

1. Describe the modeling approach and work conducted to predict likely discharges from the proposed facility;
2. Describe the spatial footprint of the near-field and dilution factors achieved in the NMZ;
3. Describe the movement of the discharge plume in the far-field zone; and,
4. Describe the spatial extent of possible contact of the discharge plume with the shoreline.

Ramboll used two US Federal Agency-approved surface water dispersion models in this work to predict the mixing behavior and dispersion of combined culture water and heat recovery water discharged to Chandler Bay from a proposed submerged ocean outfall. The Cornell Mixing Zone Model (CORMIX) 3D dilution model was used to predict the near-field mixing zone (NMZ) of wastewater discharges. The TUFLOW Finite Volume (FV) model was used to predict the spatial extent of dispersion of the wastewater discharge in Chandler Bay.

Engineering specifications for the location of the pipeline and configuration of diffusers at the proposed ocean outfall, as well as characteristics of the wastewater discharge, were provided to Ramboll by Kingfish. The ambient conditions in Chandler Bay and characteristics of the receiving seawater were obtained from coastal monitoring records available on the Internet from the State of Maine and from other available public sources. Hydrodynamic conditions were simulated in the models to be representative of conditions typical for winter and summer seasons along downeast coastal Maine.

Results from the CORMIX model used to predict seawater conditions at the ocean outfall indicate the NMZ is likely to extend approximately 230 ft from the location of the pipeline outfall during either summer or winter conditions. At the edge of the NMZ, the wastewater discharge will have been subjected to a dilution factor of 60x; i.e., the levels of residual nutrients in the wastewater at 230 ft from the outfall will be 60-times lower than levels at the point of discharge.

Once beyond the NMZ, the wastewater discharge in the far-field zone, which is expected to continue to mix and dilute with seawater, is predicted to move with offshore flow currents and tidal forces in the bay. During tidal cycles, the diluted wastewater discharge is expected to move with offshore flow currents up to 3 miles to the north with incoming tides and up to 1 mile south with outgoing tides. The TUFLOW FV model predicts that the diluted wastewater discharge plume may reach its north and south limits approximately 7 days after commencement of discharges; i.e., the model predicts the diluted wastewater discharge is highly unlikely to move beyond 3 miles north of the discharge location or 1 mile south of the discharge location during any given tidal cycle.

The modeling results predict that wastewater from the outfall may reach the western shore of Chandler Bay along Route 187. Using total nitrogen concentrations in the wastewater discharge as an indicator, the modeling results predict the wastewater discharge will have been subjected to a dilution factor of 173x; i.e., total nitrogen levels will be 173-times lower than at the point of discharge. Any total nitrogen associated with the wastewater discharge that reaches the shoreline is predicted to fall within the threshold range that Maine DEP cites as protective of eelgrass and dissolved oxygen conditions.

1. INTRODUCTION

1.1 Background

On behalf of Kingfish Maine (Kingfish), Ramboll developed near-field and far-field surface water dispersion models to support Kingfish's application for a Maine Pollutant Discharge Elimination System (MEPDES) permit for their proposed Recirculating Aquaculture System (RAS) facility in Jonesport, Maine. Development of the RAS facility requires the construction of an ocean outfall in Chandler Bay for discharging culture water and heat recovery water (hereafter referred to collectively as wastewater discharge). Kingfish is required by the Maine Department of Environmental Protection (DEP) to obtain a wastewater discharge license under the MEDPES program. This report describes the modeling work performed to delineate the near-field mixing of the wastewater at the initial point of discharge and predict the likely far-field dilution behavior of the wastewater discharge plume as it disperses further away from the location of the proposed ocean outfall in Chandler Bay.

Maine DEP has established the following procedures for understanding near-field mixing and far-field dilution characteristics associated with obtaining a license for wastewater discharges to the ocean in the State of Maine:

- Near-field (initial) dilution: Near-field Mixing Zone (NMZ) is defined as the distance from the point of discharge where mixing patterns are driven by the exit velocities of the discharge. Maine DEP specifies the conditions under which near-field dilution should be estimated – “dilution available as the effluent plume rises from the point of discharge to its trapping level, at mean low water level and slack tide for acute exposure analysis and at mean tide for the chronic exposure analysis.”¹ Near-field dilution is estimated based on the dilution factor derived from established and accepted near-field mixing models.
- Far-field dilution: Far-field dilution is the mixing and dispersion that occurs within the plume that is outside of the zone of influence of the exit velocities from the point of discharge. Maine DEP has not established specific licensing criteria for determining appropriate far-field dilution conditions. For Kingfish's proposed discharge, Ramboll has developed a three-dimensional (3D) hydrodynamic model to estimate the dispersion and mixing characteristics in the far-field zone and calculated far-field dilution factors based on the model results.

1.2 Modeling Approach

The specific objectives of the hydrodynamic modeling are as follows:

1. Describe the modeling approach and work conducted to predict likely discharges from the proposed facility specifically with respect to total nitrogen and temperature;
2. Describe the spatial footprint of the near-field results and dilution factors achieved in the NMZ;
3. Describe the movement of the discharge plume in the far-field; and,
4. Describe the spatial extent of possible contact of the discharge plume with the shoreline.

Both the near-field and far-field models are US Federal Agency-approved surface water dispersion models. Both models are used consistent with their intended purpose to predict the likely route and trajectory of wastewater discharges from a point source location (in this case, a submerged ocean outfall). In the NMZ, the characteristics of the discharge govern how the discharge plume is likely to mix with the receiving water. The primary forces that typically influence hydrodynamics and the movement of the wastewater in the receiving environment beyond the NMZ are ocean currents, and winds. Thermal/density stratification commonly

¹ 06-096 CMR Chapter 530 – Surface Water Toxics Control Program, §4(A) (calculation of dilution factors) states in part as §4(A)(2)(a)

influences mixing patterns but water profile data from Chandler Bay collected by Maine DEP in 2001-2010 and by Kingfish in 2019 suggests that water temperatures are not strongly stratified. Wind-generated waves may also occasionally also modify movement and influence the behavior of the plume.

The wastewater discharge from the proposed Kingfish facility will include a combination of heat recovery water and culture water from the RAS. For the modeling work, the discharge water is described by Kingfish to consist of a mixture of nearly three-quarters heat recovery water and one-quarter RAS culture water.

The heat recovery water includes only water that is taken from Chandler Bay and cycled through heat exchangers to remove heat energy. Heat recovery water does not interact with any RAS processes involved in growing fish and, thus, is identical to ambient seawater conditions in Chandler Bay in all aspects (e.g., salinity, nutrient characteristics) except for water temperature. During most times of the year, the temperature of water returned to Chandler Bay is anticipated to be approximately 5 degrees (F) below (i.e., colder than) ambient conditions due to the heat recovery process that will be used by Kingfish to maintain growing conditions in the culture tanks.²

The culture water from the RAS facility includes water that interacts with RAS processes involved in growing fish. Culture water from the hatchery and grow-out tanks in which yellowtail kingfish will be raised at the facility will be discharged to Chandler Bay. The suspended solids and nutrient (primarily phosphorus and nitrogen) content of the culture water, as well as the biological oxygen demand (BOD) condition of the culture water, may be present at levels above ambient conditions in Chandler Bay.

The near-field modeling work described in this report was conducted to simulate the mixing of the wastewater discharge with the ambient receiving seawater in the bay, and predict the near-field dilution factor based on two relevant water quality characteristics of the discharge water, total nitrogen and temperature. Total nitrogen is the primary wastewater constituent of concern with respect to water quality in Chandler Bay. Although the nature of nitrogen in the discharge and the form it takes in the environment are variable, the modeling considers total nitrogen as a non-transformative conservative constituent that does not change its form in the receiving environment. Water temperature is a common parameter when assessing wastewater mixing. In this case, the wastewater temperature will typically be colder than surface water temperature in Chandler Bay.

There is currently little understanding of the variation in the magnitude and direction or velocity with depth of the counter currents and helicoidal flows in the bay. This understanding is important when considering potential plume movements, which may vary with water depth. Therefore, both the CORMIX and TUFLOW FV models were performed using a 3D modeling approach to simulate the behavior of mixing, dilution and dispersion conditions of the wastewater discharge in this particular setting.

Lastly, the modeling approach included a variable water level boundary to reflect the tidal cycle in Chandler Bay. Tide curves of variable lengths were utilized, generally based on tidal peaks set at the Mean High Water (MHW) level. MHW is the average of all the observed high-water heights. In accordance with Maine DEP requirements, influences from the wind at the water surface were omitted from the model.

1.3 Dispersion Models

Two types of modeling have been completed, both in 3D:

² This is expected to be true throughout the year with the only exception of when surface water temperatures in Chandler Bay reach freezing. Wastewater discharges during these times will be the same temperature as ambient surface water.

- CORMIX near-field plume modeling; and
- TUFLOW FV far-field advection-dispersion modeling.

CORMIX is an industry-standard mixing zone model which is primarily used to determine detailed wastewater plume characteristics close to the outfall (<300 ft). Whilst CORMIX does not include a sophisticated hydrodynamic model to simulate the unsteady (i.e. time-varying) movement of the receiving environment, it has the functionality to model the discrete hydraulic effects associated with pipe outfalls and diffusers, typically used to aid wastewater dispersion in situations such as this. Beyond the near-field zone of the receiving water, the accuracy of CORMIX to predict mixing characteristics is reduced. Likewise, in coastal environments, flow conditions normally vary over time in response to the rise and fall of tides.

The 3D TUFLOW FV³ far-field model used in this work encompasses the area of Maine coastline extending from the headland at Jonesport in the south west to the headland at Point of Man in the north east. The bay between the two headlands is split by a central island; Roque Island. The bay to the south west of Roque Island, adjacent to Jonesport is Chandler Bay. The bay to the north and east of Roque Island is Englishman Bay. Within the model area, there are several smaller islands and rocky outcrops, smaller inlets and bays and a complicated bathymetric environment. The full area has been modeled to understand the complicated flow regime operating within the bay as tides rise and fall. Figure 1-1 shows boundary conditions and the key locations within the Jonesport, Maine, TUFLOW-FV model.



Figure 1-1: Boundary conditions and locations within the Jonesport, Maine, TUFLOW-FV model

2. CORMIX MODEL APPROACH

2.1 CORMIX Model

Wastewater discharge modeling was undertaken using the Cornell Mixing Zone Model (CORMIX) Version 11, a 3D dilution model for the definition of discharge plumes. Consistent with its intended use, the CORMIX model was used to model continuous point source discharges using the role of boundary interaction to predict steady-state mixing behavior and plume geometry at the outfall location specified by Kingfish.

Separate calibration of the results of the model has not been possible because there is insufficient data on water quality in the near vicinity of the proposed outfall (and as the outfall is not yet operational). It has not, therefore, been possible to directly verify the model outputs against available water quality information. However, conservative input data were used to ensure a conservative approach was taken with respect to the model's predictions.

Data on the proposed outfall and receiving environment were obtained from several sources (indicated below by brackets []). The discharge is based on the initial proposed outfall design provided to Ramboll by Kingfish. Hydrodynamics, concentrations of selected constituent parameters, recirculation, and dispersion were evaluated using the following conditions (representative conditions for winter and summer seasons have been modeled):

- Pipe outfall = 6No. 211 mm wide fanned-out ports with 5 m spacing [Kingfish];
- Outfall location = central port 800 m offshore [Kingfish];
- Effluent flow rate = 1,250 L/s (44.14 cfs) [Kingfish];
- Effluent nitrogen concentration = 6.9 mg/L [Kingfish];
- Summer effluent density = 1,024.75 kg/m³ (calculated from average temperature of 9.3°C [Kingfish] and salinity equivalent to Chandler Bay of 3.2% [Kingfish]);
- Winter effluent density = 1,025.71 kg/m³ (calculated from average temperature of 0°C [Kingfish] and salinity equivalent to Chandler Bay of 3.2% [Kingfish]);
- Average background nitrogen concentration in receiving environment = 0.27 mg/L [Kingfish];
- Average depth of Chandler Bay in vicinity of outfall = 13 m [C-MAP (see Section 3.6)];
- Depth at discharge = 13 m [as above];
- Average width of Chandler Bay = unbounded [calculated using GIS];
- Flow Currents = 0.1 m/s [TUFLOW model (see Section 3)]; and
- Manning's roughness (n) = 0.03 [NLCD (see Section 3.5)].

The orientation of the diffuser at the outfall was assumed to be perpendicular to the shoreline and to the ambient current axis. The nozzles on the diffuser were assumed to be oriented downstream in the direction of the ambient current. For the purposes of near-field modeling, ambient flow currents were modeled as uni-directional. Design details may be subject to change as the design progresses. The configuration and orientation of the diffuser is applicable for determining the mixing and dilution potential of the wastewater discharge in the near-field.

For the sake of brevity in reporting, a full description of design iterations is not provided herein. Appendix 1 presents a comparison of different modeling results in the far-field showing how changes in the location of the outfall in Chandler Bay influences the mixing and dilution conditions of the wastewater discharge.

3. TUFLOW FV MODEL APPROACH

3.1 Software

The hydrodynamic model of the Jonesport, Maine, coastline was developed using industry-standard TUFLOW Finite Volume (FV) software. The most recent release was used, build 2019.01.008. TUFLOW FV is a flexible mesh, finite volume numerical model that simulates hydrodynamic, sediment transport, and water quality processes in oceans, coastal waters, estuaries, rivers, and floodplains. The model was used because the flexible mesh structure allows users to modify the resolution of different spatial boundaries, which can improve model resolution in specific areas of interest.

TUFLOW has been benchmarked for use in many coastal environments in the United States. TUFLOW is certified by the US Federal Emergency Management Agency for use under the National Flood Insurance Program. TUFLOW also has approved status for use within the United States Army Corps of Engineers National Hydrology, Hydraulics and Coastal Software list.

TUFLOW FV includes a module for Advection Dispersion (AD). AD refers to the transport of dissolved constituents within hydrodynamic flows. An AD module within a numerical modeling suite is used to simulate a range of environmental processes including salinity dynamics, constituent fate and transport and heat, amongst others. TUFLOW FV applies the AD module to simulate the time evolution of salinity, temperature, suspended sediment, passive tracers and water quality constituents.

3.2 Model Area and Mesh Design

TUFLOW FV is designed for solving Non-Linear Shallow Water Equations (NLSWE) on unstructured meshes, comprising triangular and quadrilateral elements. The flexible mesh approach allows for a seamless boundary fitting along complex coastlines or open channels as well as accurately and efficiently representing complex bathymetries with the minimum number of computational elements. The flexible mesh capability is particularly efficient at resolving a range of resolutions within a single model without requiring multiple domain nesting, allowing for a fine resolution within the area of interest and coarser resolution in the regional extents.

Figure 3-1 shows the TUFLOW FV mesh for the Jonesport coastline and the tidal water level boundary. The mesh was primarily developed to assess the movement of discharge wastewater released offshore within Chandler Bay. Consequently, the mesh was constructed with smaller mesh elements (higher mesh resolution) close to Jonesport to resolve the complex hydrodynamic environment at this location. Larger mesh elements (lower mesh resolution) were progressively applied with increasing distance away from Chandler Bay, and for the out of bank, dry land areas. The unstructured flexible mesh was created using the mesh generation tool Rising Water Software GIS Mesher⁴.

⁴ <https://www.risingwatersoftware.com/>

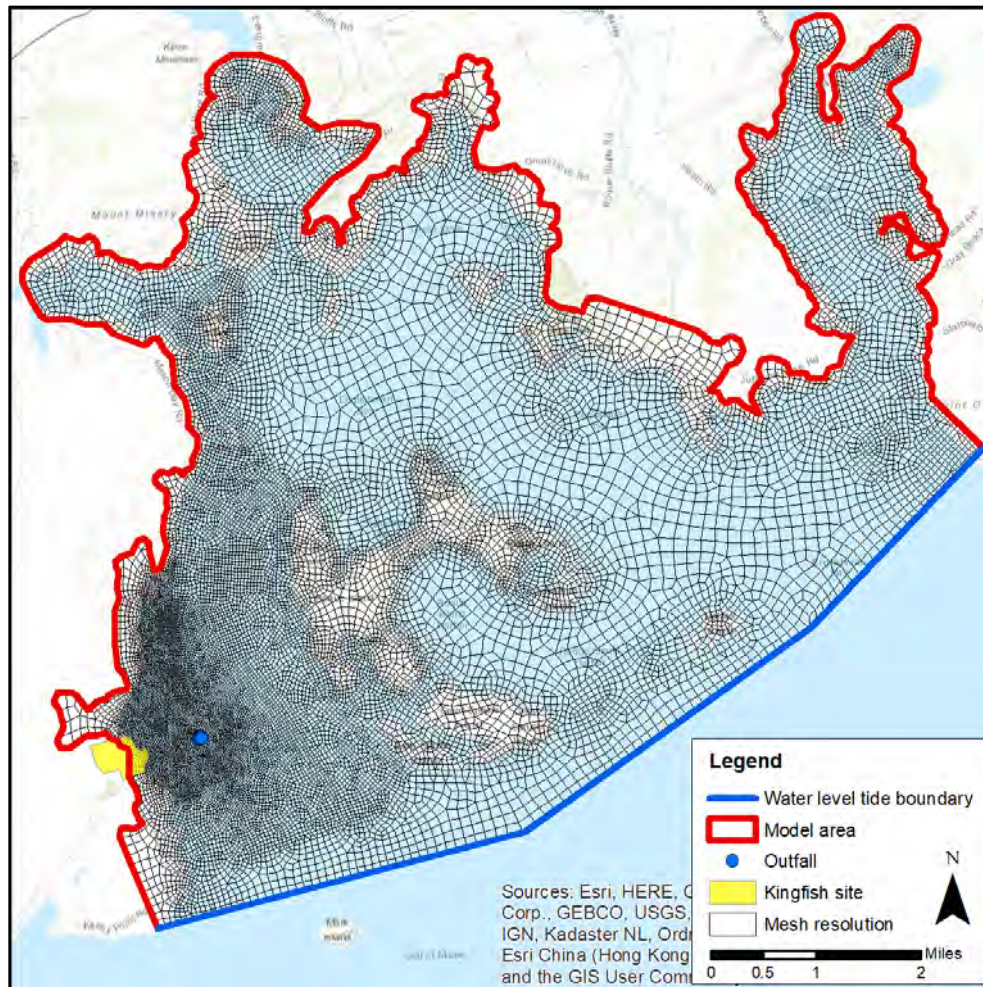


Figure 3-1: TUFLOW FV mesh for Jonesport, Maine

3.3 3D Layer Z Coordinate Approach

TUFLOW FV includes three-dimensional (3D) capability. Two-Dimensional (2D) depth averaged simulations can be an excellent approximation for many applications however, there are cases where 3D modeling is required to sufficiently describe the observed flow characteristics. For example, 3D modeling is useful in areas where there is a significant variation in magnitude and direction of velocity with depth, such as counter currents and helicoidal flows, or where the effects of density become important such as in stratified environments or dense plume outfalls. A 3D modeling approach has been applied for the Jonesport model to be able to understand the vertical movement of wastewater released from the outfall within Chandler Bay.

Modeling with 3D layers involves setting vertical cells similar to the way that horizontal cells are defined in the mesh (Figure 3-2). These can be set with a fixed elevation depth (z) for each cell (e.g. each layer is 2 ft thick); by dividing the depth of the river into an equal number of evenly distributed sigma layers (e.g. 4 sigma layers throughout the model would mean a 16 ft depth has 4 x 4 ft layers, an 8 ft depth has 4 x 2 ft layers); or a combination (hybrid) of the two schemes.

The Jonesport model has applied a hybrid z-sigma layering method for vertical layers with the hybrid z-sigma layer method defined by a series of surface sigma layers overlaid on a z scheme at depth. The hybrid z-sigma layering method provides flexibility and stability in areas of wetting and drying not available via a z only coordinate system while providing the potential for high resolution near-surface exchange and gradient capturing.

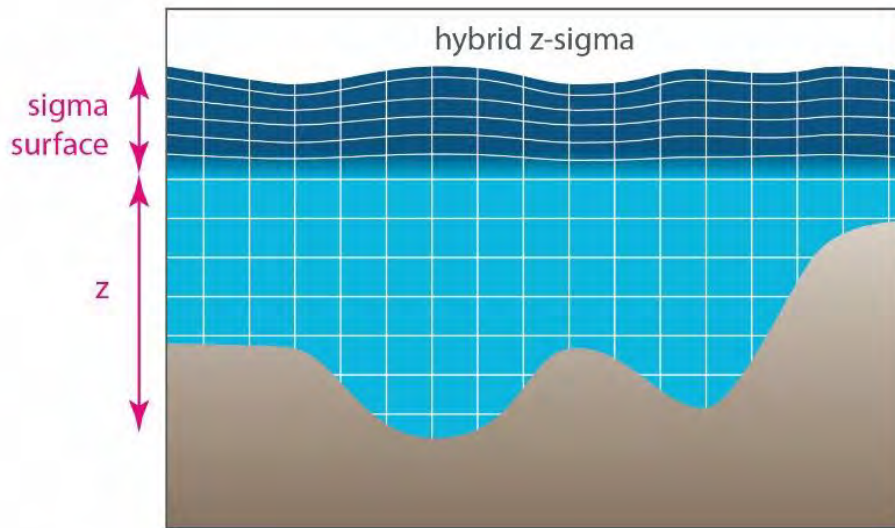


Figure 3-2: 3D Model z-sigma Vertical Discretization Approach

The vertical datum applied in this study is the North American Vertical Datum of 1988 (NAVD88). The hybrid z-sigma approach applied for the Jonesport model is as follows:

- 11 z layers
 - 6.56 ft (2 m) intervals from -13.12 ft (-4 m) NAVD88 to -65.62 ft (-20 m) NAVD88,
 - 13.12 ft (4 m) intervals from -65.62 ft (-20 m) NAVD88 to -104.99 ft (-32 m) NAVD88.
- 4 surface sigma layers.
- 1.64 ft (0.5 m) minimum bottom layer thickness to ensure the bottom layer of the 3D solution at the bed is not less than 1.64 ft (0.5 m), avoiding 'skinny' vertical layers where the bed elevation is between two z coordinate layers.
- 6.56 ft (2 m) water depth required before 3D calculations are applied.

3.4 Tidal Boundary

3.4.1 Data Availability

Exceedance probability, the likelihood that water levels will exceed a given elevation, is based on a statistical analysis of historic values. Extreme Water Level data is available from the National Oceanic and Atmospheric Administration (NOAA). NOAA provides exceedance probability levels for select CO-OPS water level stations with at least 30-years of data. A NOAA Technical Report, "Extreme Water Levels of the United States 1893-2010"⁵ describes the methods and data used in the calculation of the exceedance probability levels.

Annual Exceedance Probability (AEP) levels were derived relative to the National Tidal Datum Epoch (1983-2001) Mean Sea Level datum at gauge stations. Two AEP levels are provided; AEP levels for the mid-year of the National Tidal Datum Epoch and the projected AEP levels and tidal datums assuming continuation of the linear historic trend.

This study is concerned with everyday tide data and, as such, has utilized the 99% projected AEP level. The 99% AEP level is the tide level that will be exceeded in all but one year per century, although it could be exceeded more than once in other years.

Extreme levels measured by the CO-OPS tide gauges during storms are called storm tides, which are a combination of the astronomical tide, the storm surge, and limited wave setup caused by

⁵ NOAA, NOAA Technical Report NOS CO-OPS 067, "Extreme Water Levels of the United States 1893-2010", September 2013

breaking waves. They do not include wave runup; the movement of water up a slope. The 99% AEP level was used as it was considered to be a peak tide level frequently experienced and as such, represents an everyday tide level.

The closest two CO-OPS water level stations with extreme water level data predictions are Eastport and Bar Harbor (Table 3.1).

Table 3.1: NOAA station information for water level CO-OPS sites used to estimate projected 99 % AEP tide levels

Station Name	Station ID	NOAA Chart number	Sensor Information	Latitude, Longitude	Projected 99 % AEP Tide Level for 2018 (NAVD88)
Bar Harbor, ME	8413320	13318	Acoustic water level (A1)	44° 23.5' N, 68° 12.2' W	7.579 ft (2.310 m)
Eastport, ME	8410140	13328	Acoustic water level (A1)	44° 54.2' N, 66° 59.1' W	12.467 ft (3.800 m)

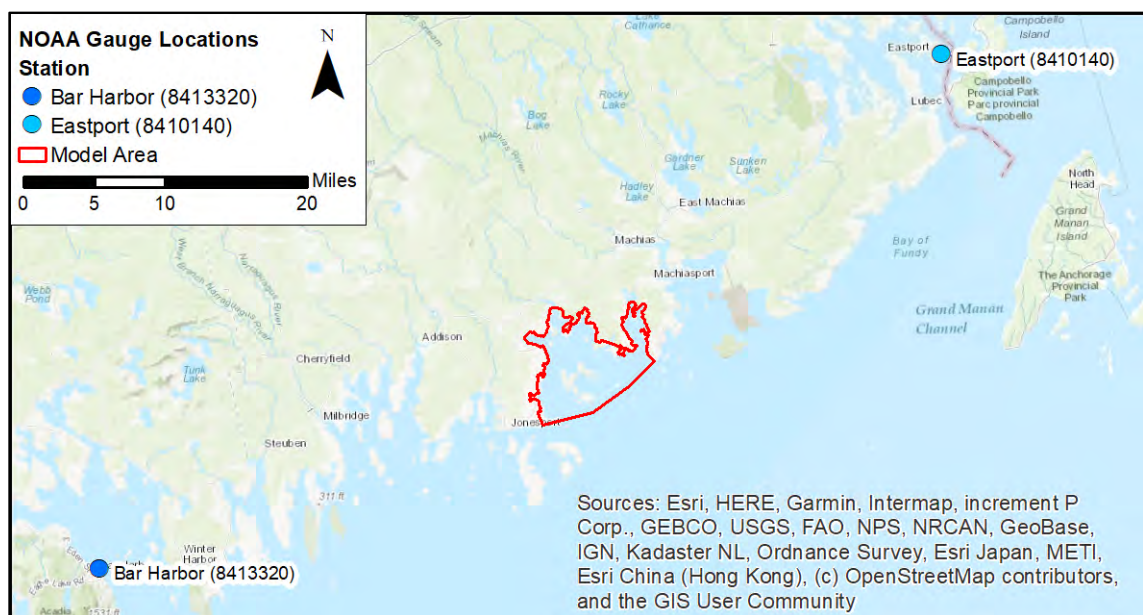


Figure 3-3: NOAA station water level CO-OPS sites in relation to the TUFLOW-FV model location

The AEP tide levels are station specific so their use for evaluating surrounding areas may be limited. Considering the significant distance between the two stations, a simple linear interpolation to derive the 99% AEP tide level for Chandler Bay using only Eastport and Bar Harbor data would not be appropriate.

Tidal datum information was available for NOAA stations in between Eastport and Bar Harbor. This data was utilized to characterize the tide level variation along the Maine coastline. This information was fed into the interpolation calculation to provide greater confidence to the 99% AEP water levels derived for Chandler Bay (Table 3.2).

Table 3.2: NOAA station information for used to calculated projected 99 % AEP tide levels for TUFLOW-FV hydraulic model.

Station Name	Station ID	Sensor Information	MHW (NAVD88)	Cumulative Distance	Projected 99 % AEP Tide Level for 2018 (NAVD88)	
					NOAA	Interpolated using MHW
Bar Harbor	8413320	Acoustic water level	4.99 ft (1.52 m)	0 miles (0 km)	7.58 ft (2.31 m)	7.58 ft (2.31 m)
Milbridge	8412581	Tidal Datum	5.45 ft (1.66 m)	19.2 miles (30.876 km)	-	8.14 ft (2.48 m)
Model - West	-	-	-	33.3 miles (53.564 km)	-	8.73 ft (2.66 m)
Model - East	-	-	-	40 miles (64.312 km)	-	8.99 ft (2.74 m)
Cutler Naval Base	8411250	Tidal Datum	6.4 ft (1.95 m)	48.6 miles (78.152 km)	-	9.35 ft (2.85 m)
Cutler Farris Wharf	8411060	Tidal Datum	6.59 ft (2.01 m)	53.2 miles (85.674 km)	-	9.58 ft (2.92 m)
Eastport	8410140	Acoustic water level	8.86 ft (2.7 m)	73.4 miles (118.082 km)	12.47 ft (3.8 m)	12.47 ft (3.8 m)

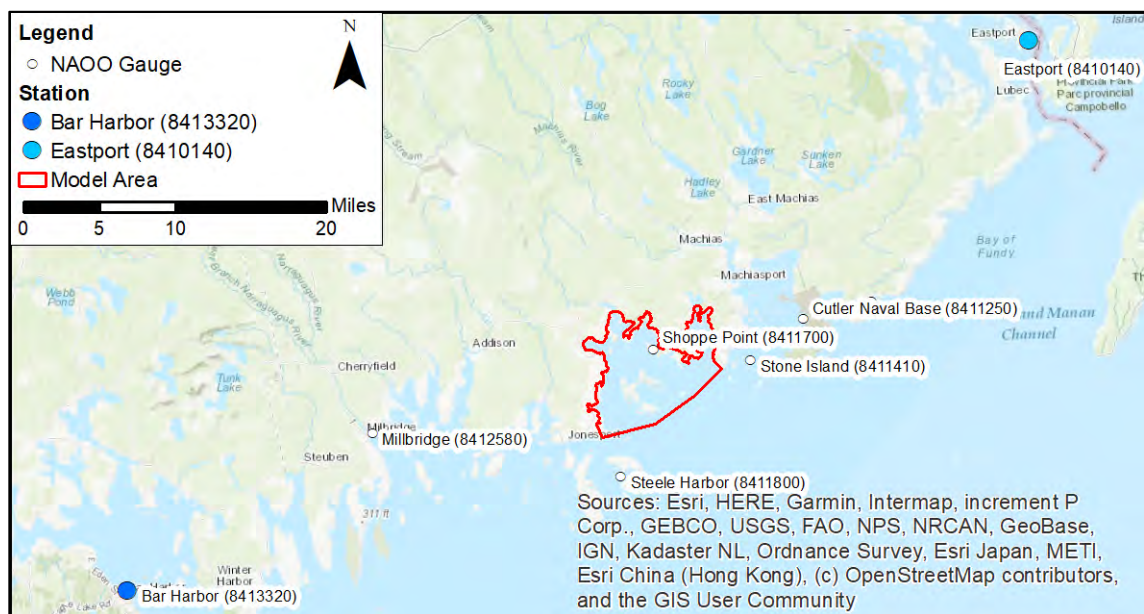


Figure 3-4: NOAA station sites in relation to the TUFLOW-FV model location

Tides can be influenced by a range of different conditions that are unrelated to the movement of the sun and moon. Weather conditions can lead to low- or high-pressure systems which impact the timing and height of tides as do winds and offshore currents which can contribute to storm surges. In order to avoid distorting model results, astronomical tides were used which ignore those extraneous influences.

The closest available NOAA tide gauge station providing a continuous astronomical tide timeseries dataset was Cutler Farris Wharf (8411060). A timeseries of astronomical tide data was extracted from the NOAA database. The tide levels were then scaled to the 99 % AEP tide levels derived in Table 3.2. Figure 3-5 shows the scaled tide curves applied in the TUFLOW FV model.

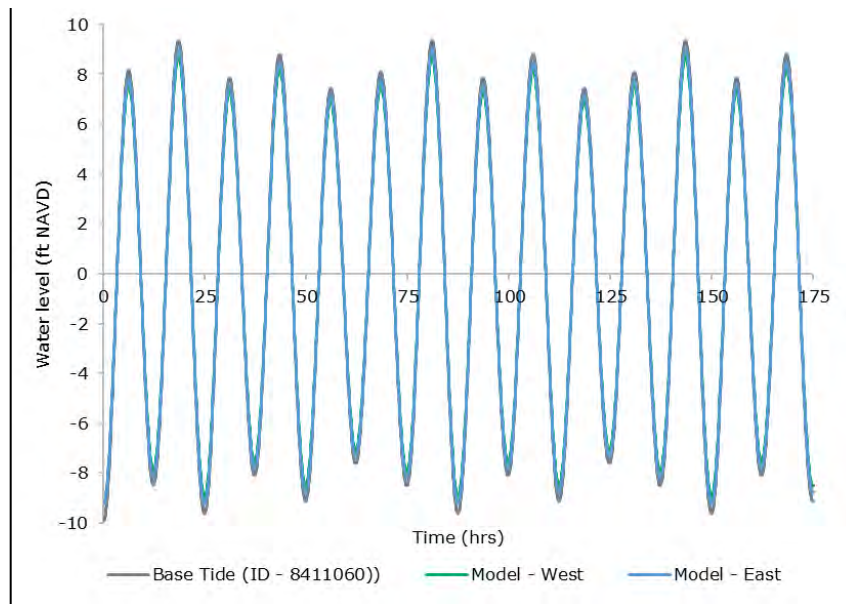


Figure 3-5: Scaled tide curve applied in the TUFLOW-FV model

The tidal boundary of the model was set to span the mouth of Chandler and Englishman's Bay, stretching from the headland at Jonesport in the south-west to the headland at Point of Man in the north-east (Figure 3-1).

3.5 Model Roughness

Model roughness values within the TUFLOW FV model have been defined using data sourced from the National Land Cover Database⁶ (NLCD) Roughness Layer 2011 Edition, sourced from the US Geological Survey (USGS). Modeling judgement is required when assigning model roughness values as there are no specific guidelines. The Conveyance Estimation System⁷ (CES) cross-referenced against values presented in Chow⁸ (1959) were used to derive suitable Manning's 'n' values.

Roughness values are used to define the land use variation in the model active area, examples include: Pasture, Roads, Buildings, Forest, Mangroves etc. Each material is assigned a constant Manning's n value. Table 3.3 details the roughness values assigned to each land type identified in the model active area. Figure 3-6 shows the roughness mapping applied in the TUFLOW-FV model.

Table 3.3: Model roughness values

ID	Manning's 'n'	Land Type - Roughness
11	0.03	Open Water
21	0.025	Developed Land
24	0.032	Developed, High Intensity
31	0.035	Barren Land
44	0.2808	All Forest Types
52	0.0539	Shrub/Scrub
71	0.0456	Herbaceous
81	0.0922	Hay/Pasture
82	0.0539	Cultivated Crops
90	0.2008	Woody Wetlands
95	0.1511	Emergent Herbaceous Wetlands
100	0.0508	Roughness Stability Patch 01
101	0.2	Roughness Stability Patch 02
102	0.1	Roughness Stability Patch 02

⁶ <https://www.usgs.gov/centers/eros/science/national-land-cover-database>

⁷ <http://www.river-conveyance.net/>

⁸ Chow, V.T., 1959, Open-channel hydraulics: New York, McGraw-Hill.

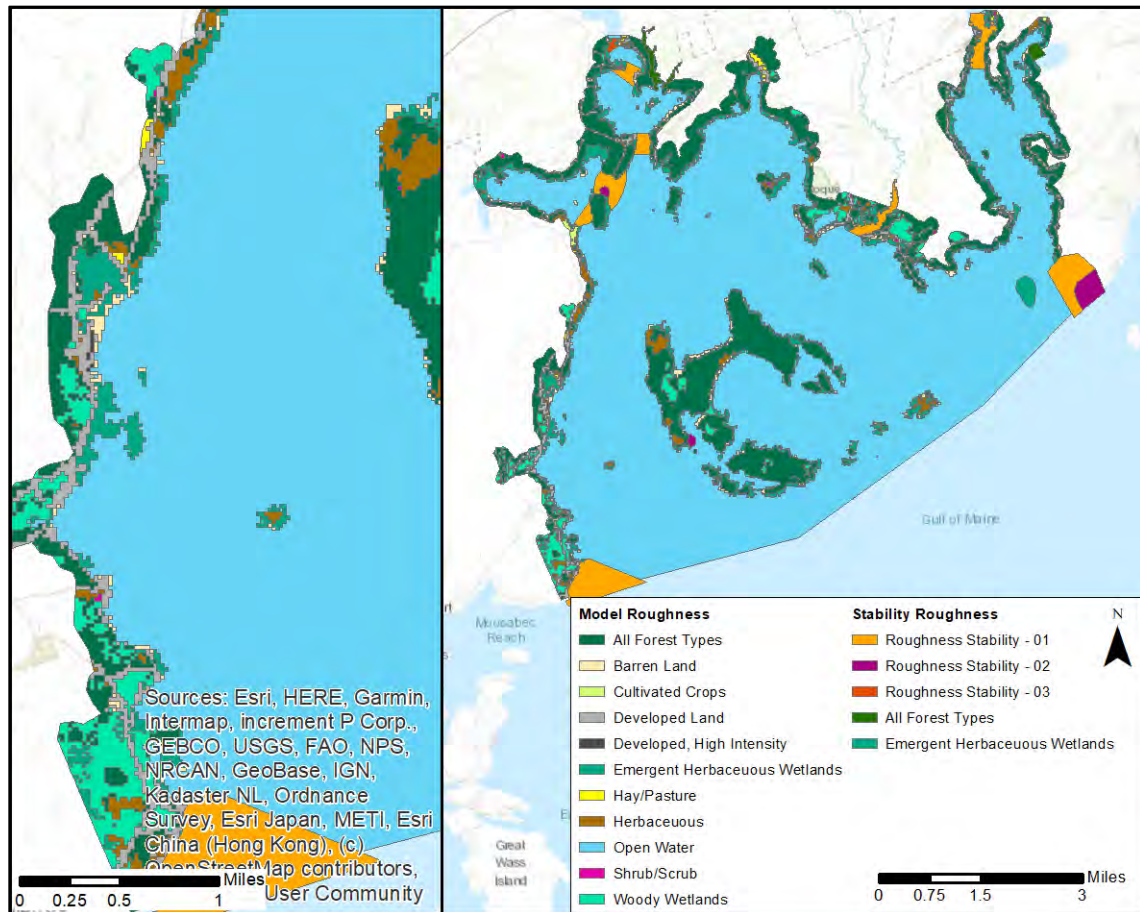


Figure 3-6: Roughness mapping applied in the Jonesport TUFLOW-FV model

Figure 3-6 also shows the locations of the Stability Roughness patches applied in the TUFLOW-FV model. These were applied in locations that caused model instability, where unrealistic flow rates were simulated or there were unnatural isolated spikes in the simulated water level. The patches are sufficiently distant from the key areas of interest to not interfere with model results.

3.6 Model Terrain

LiDAR and Bathymetry survey datasets were used to represent the base model terrain. The LiDAR dataset was sourced from the USGS⁹. The Bathymetry dataset was sourced from DHI's C-MAP¹⁰ database.

3.6.1 USGS LiDAR

The Jonesport modeling study has utilized USGS NED 1/3 arc-second LiDAR data to represent the model terrain for the land surface. This data was made available through the USGS 3D Elevation Program (3DEP). 3DEP data comprise an elevation dataset of seamless, high resolution layers that represent the best available raster elevation data of the conterminous United States. 3DEP data are updated continually as new data become available. Seamless 3DEP data are derived from diverse source data that are processed to a common coordinate system and unit of vertical measure. The details for the LiDAR data are summarized in Table 3.4.

⁹ <https://www.usgs.gov/core-science-systems/ngp/3dep/about-3dep-products-services>

¹⁰ <https://www.c-map.com/>

Table 3.4: USGS LiDAR details

Source	U.S. Geological Survey
Publication Date	30/09/2019
Title	USGS NED 1/3 arc-second n45w068 1 x 1-degree GridFloat 2019
Format	Raster digital data
Survey date	2017
Horizontal datum	North American Datum of 1983 (NAD 83)
Vertical Datum	North American Vertical Datum of 1988 (NAVD 88)
Resolution	1/3 arc-second (approximately 10 meters)

3.6.2 C-MAP Bathymetry Data

The Jonesport model utilized bathymetry data sourced from C-MAP, an online licensed data platform of Bathymetry datasets. The bathymetry data available for Chandler Bay and Englishman Bay was surveyed in 2010. The data was provided in ESRI mesh format.

3.7 Wastewater Discharge Modeling

Total nitrogen is modeled as a scalar variable; physical quantities that are tracked within TUFLOW FV and can be represented by one value per cell using the TUFLOW-FV Advection Dispersion (AD) module.

3.7.1 Advection Dispersion Module Conceptual Framework

The TUFLOW AD module is an extension of the hydrodynamic engines, simulating constituent fate and transport. Both dissolved and particulate constituents can be simulated. The AD module takes hydrodynamic information computed by the TUFLOW-FV engine, using it in combination with initial and boundary conditions, to simulate the advection and dispersion of constituents.

3.7.2 Effluent model setup

Table 3.5 outlines the concentrations of constituents simulated to be released from the ocean outfall during different seasons of the year using the TUFLOW-FV model. The discharge rate, temperature, and concentrations of nitrogen were constant throughout each simulation, they did not vary with time. Figure 3-7 shows the diffuser location within Chandler Bay, situated 2625 ft (800 m) offshore and situated on the seabed (-41.3 ft NAVD88 (-12.6 m NAVD88)).

Table 3.5: Discharge, temperature and concentrations of total nitrogen released from the effluent diffuser locations for each model scenario

Season	Temp (°C)	Total Discharge	Number of Diffusers	Discharge per diffuser	Nitrogen Conc (mg/L)	Distance offshore	Elevation of Diffuser (bed level)
Summer	9.3	44.14 ft ³ /s (1.25 m ³ /s)	6	7.35 ft ³ /s (0.208 m ³ /s)	6.9	2,625 ft (800 m)	-41.3 ft NAVD88 (-12.6 m NAVD88)
Winter	0	44.14 ft ³ /s (1.25 m ³ /s)	6	7.35 ft ³ /s (0.208 m ³ /s)	6.9	2,625 ft (800 m)	-41.3 ft NAVD88 (-12.6 m NAVD88)

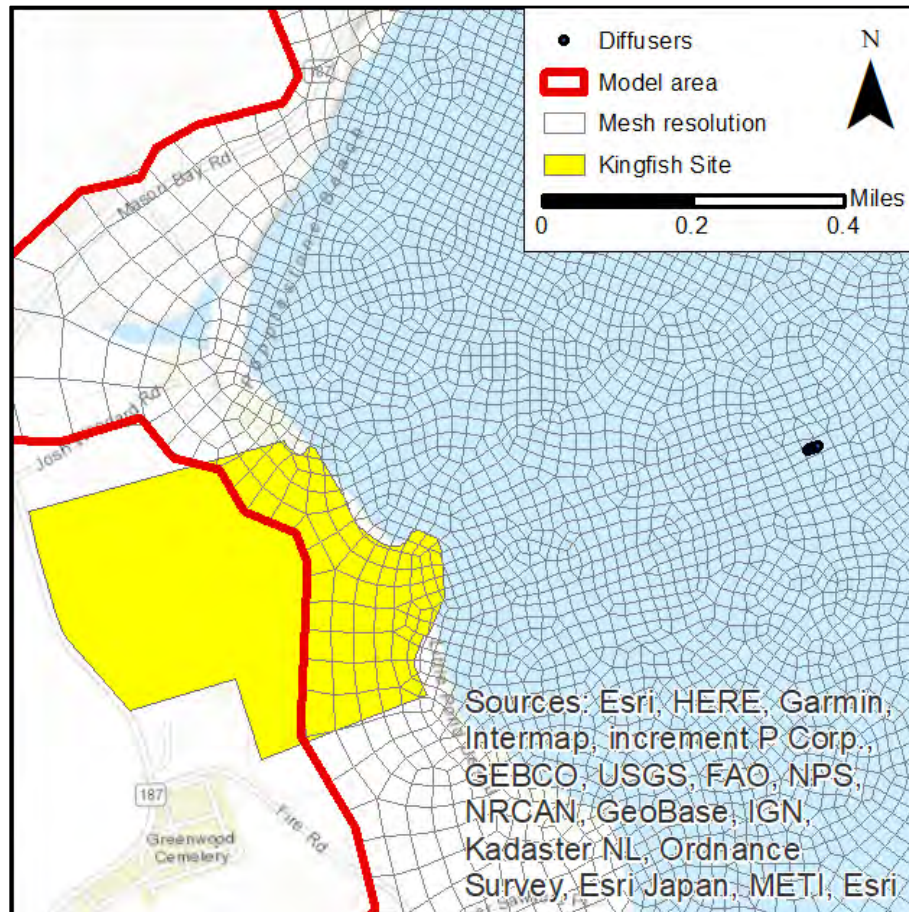


Figure 3-7: Diffuser locations within Chandler Bay

3.8 Model Parameters

3.8.1 Solution Terms

Various options are available which allow for the inclusion of a variety of solutions, including salinity, temperature, sediment, and atmospheric heat exchange. For the Jonesport TUFLOW FV model, the key interest is in the movement of the wastewater discharge in coastal waters and therefore, salinity, temperature, sediment, and tracer solutions have been included.

Complex flow regimes are in operation in Chandler Bay; a 2nd Order horizontal and vertical spatial scheme has been specified to capture these. For hydrodynamic simulations, the bed boundary resistances are described using a Bottom-Drag Model. The Jonesport TUFLOW FV model utilizes Manning, the default setting, where the Manning's 'n' coefficient is specified.

3.8.2 Model Timestep

The default time format (hours) for TUFLOW FV has been utilized. The model has been run for 175 hours (7 days), or 14 full tidal cycles from trough to trough. TUFLOW FV uses an adaptive timestep where a stable timestep must be bounded by the Courant-Friedrich-Lewy (CFL) criterion¹¹ for the wave propagation and advective terms and by Peclet criterion for the diffusive terms. The CFL number is a function of the cell size and shape, water depth, flow velocity and model timestep. The default setting is 1, which is the theoretical stability limit. In practice however, the value is commonly lowered to provide stability for models with large gradients. For the TUFLOW FV model, the CFL model is set to 0.6. The minimum and maximum variable time

¹¹ Courant, R.; Friedrichs, K.; Lewy, H. (March 1967) [1928], "On the partial difference equations of mathematical physics", IBM Journal of Research and Development, 11 (2): 215–234

step allowed according to the CFL stability criterion are set to 0.01 and 40 respectively. Sensitivity testing, which involved running the model for longer periods to see how this would affect the extent of the plume was carried out as presented in Appendix 1.

3.8.3 Turbulence Parameters

TUFLOW FV³ has a variety of options for simulating horizontal and vertical mixing options. The horizontal mixing eddy viscosity is set to be calculated using the Smagorinsky model¹² which sets diffusivity proportional to the local strain rate. The default global horizontal eddy viscosity (0.2) for the Smagorinsky coefficient is used. The vertical-mixing eddy viscosity is calculated using the external scheme General Ocean Turbulent Model¹³ (GOTM). This approach is recommended for systems with strong vertical gradients.

3.8.4 Bed Shear Stress

Bed shear stress is defined as the stress exerted by fluid on the channel bed. The opposing force is the critical shear stress, which is the stress required to initiate the motion of sediment at the channel bed. TUFLOW calculates bed shear stress using the following equation, where ρ is density, g is gravity, V is velocity, n is Manning's n and y is depth:

$$\text{Metric Units: } \tau_{bed} = \frac{\rho g V^2 n^2}{y^{\frac{1}{3}}}$$

3.9 Calibration

As with the near-field model, separate calibration of the results of the model has not been possible as there is insufficient data on water quality in the near vicinity of the proposed outfall (and as the outfall is not yet operational). However, some monitoring of flow currents has been undertaken and far-field modeling has been compared with this as presented in Appendix 2.

¹² Smagorinsky, Joseph (March 1963). "General Circulation Experiments with the Primitive Equations". Monthly Weather Review. 91 (3): 99–164.

¹³ Bolding, K.; Bruggeman, J.; Klingbell, K., GOTM, [online], Germany, Bootstapious.com (2016), February 2020, <https://gotm.net/>

4. MODEL RESULTS

4.1 Mixing Zone Criteria

Far-field dispersion and mixing patterns were assumed to be the relevant scale for describing the potential consequences of total nitrogen and temperature in the wastewater discharge on water quality in Chandler Bay. Total nitrogen and temperature are the focus because nitrogen is the limiting nutrient in coastal marine waters and temperature influences the response of aquatic communities in the vicinity of the point of discharge.

Maine DEP has not adopted numeric total nitrogen criteria for protection of coastal water quality that can be used for assessing modeling results for seawater conditions at the edge of mixing zones or in the far-field. At other sites, Maine DEP has used screening levels for total nitrogen in coastal waters that depend on site conditions such as a total nitrogen concentration of 0.32 mg/L where eelgrass beds have been historically mapped as present near an outfall or 0.45 mg/L for maintenance of dissolved oxygen concentrations in the receiving water body.¹⁴ Protective nutrient thresholds depend on site-specific conditions such as water depth and mixing and circulation patterns, among others and, throughout USEPA Region 1, numeric nutrient criteria for total nitrogen for the protection of eelgrass beds range from 0.30 mg/L to 0.39 mg/L and criteria for the protection of dissolved oxygen conditions range from 0.35 mg/L to 0.50 mg/L.¹⁵

In all cases, far-field results presented in this section are for concentrations above background conditions. Based on data collected by Maine DEP from Chandler and Englishman's Bay in summers between 2004 and 2010, total nitrogen concentrations are variable, but the mean and median background concentrations are 0.27 mg/kg and 0.25 mg/kg, respectively.

4.2 Near-field

Findings from the near-field modelling are presented in Table 4.1 and present the distance to the edge of the NMZ and dilution achieved at that point. Full CORMIX outputs are available from Kingfish upon request.

Table 4.1: Maximum total nitrogen concentration along the shoreline for each scenario and diffuser location

Scenario	Distance to edge of Near-field Mixing Zone (NMZ) (ft)	Dilution Factor at Edge of NMZ
Summer	230	60x
Winter	230	60x

A 60-fold dilution is achieved at a distance of 230 ft from the point of release at the outfall. This distance is defined as the edge of the NMZ and indicates the spatial extent of the influence of the outfall diffusers on mixing and dilution of the wastewater discharge.

4.3 Far-field

4.3.1 Maximum Total Nitrogen Concentrations

Figure 4-2, shows the maximum extent of total nitrogen concentrations simulated from the outfall diffusers for both the summer and winter scenarios. Figure 4-3 shows how the plume moves within Chandler Bay over time. Table 4.2 shows predicted maximum excess

¹⁴ <https://www.epa.gov/sites/production/files/2019-03/documents/draftme0002780permit.pdf>,
<https://www.epa.gov/sites/production/files/2018-10/documents/draftme0037478permit.pdf>,
<https://www3.epa.gov/region1/npdes/permits/2017/finalme0102075permit.pdf>

¹⁵ <https://www.epa.gov/sites/production/files/2018-10/documents/draftme0037478permit.pdf>

concentrations of total nitrogen predicted in near-shore areas based on an observation line as presented in Figure 4-1.

Table 4.2: Maximum excess total nitrogen concentration along the shoreline for each scenario and diffuser location

Scenario	Maximum Excess Nitrogen Concentration Along Shoreline (mg/L)	Dilution Factor for Maximum Nitrogen Concentration
Summer	0.038	173x
Winter	0.038	173x

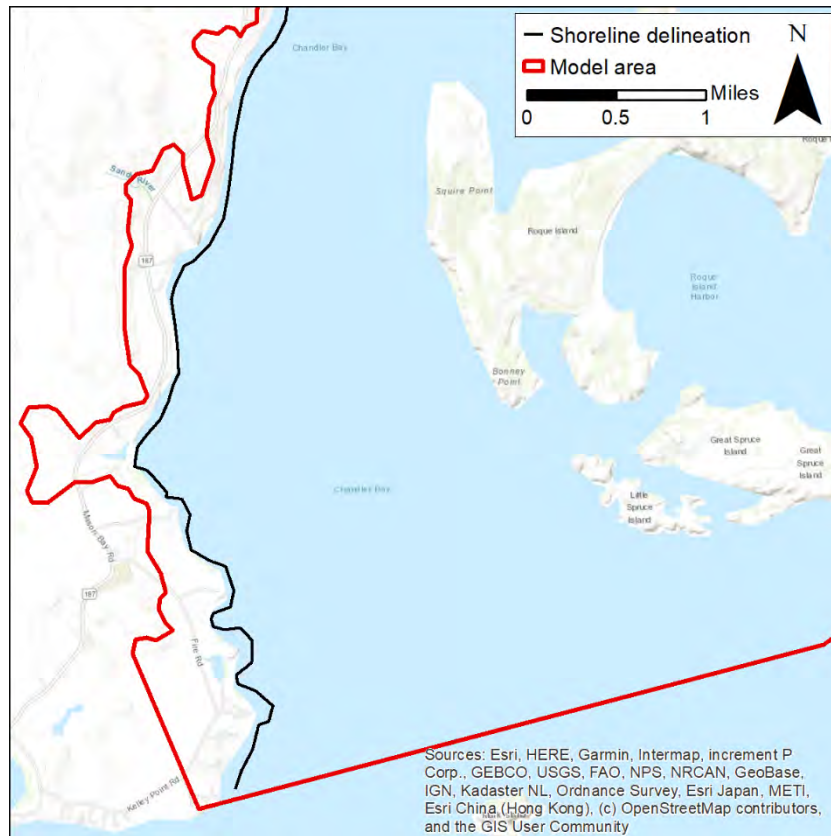


Figure 4-1: Line applied to calculate the maximum concentration at the shoreline

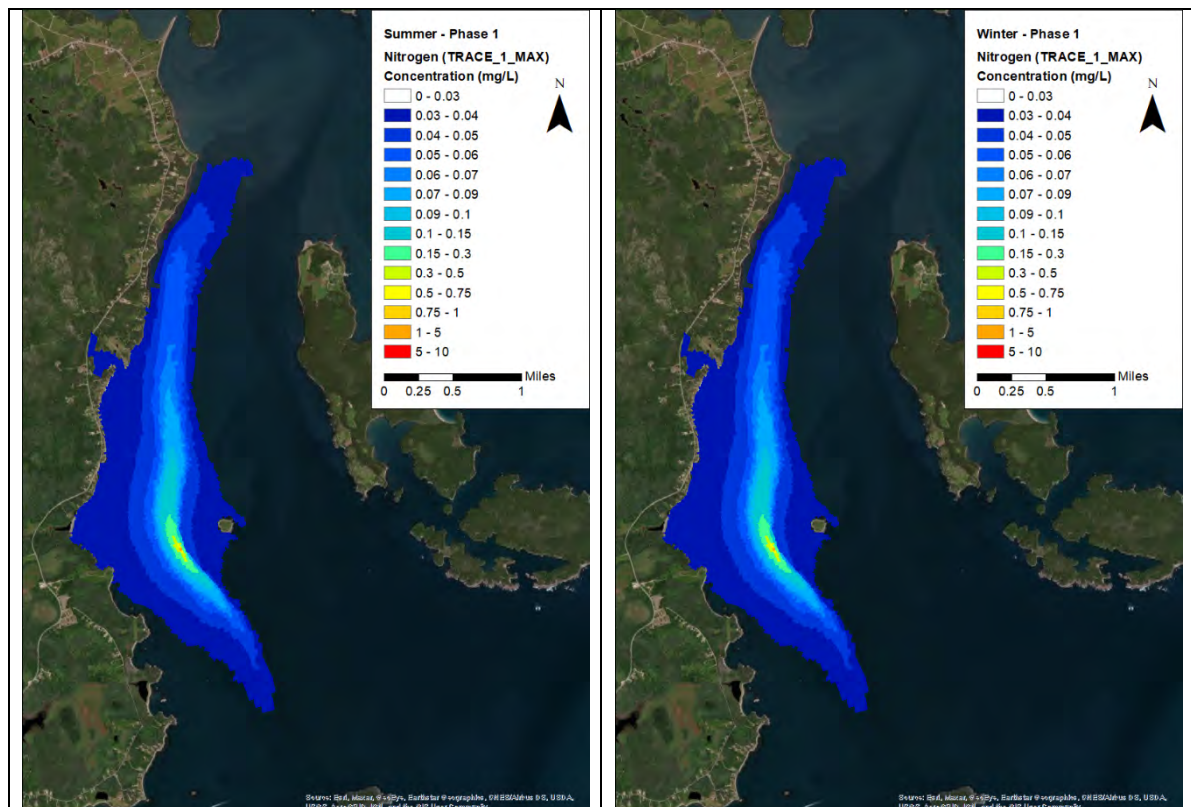


Figure 4-2: Maximum excess total nitrogen concentration under the two scenarios, Summer (left), Winter (right)

Figure 4-2 presents a composite depiction of the wastewater discharge plume indicating the maximum concentrations of total nitrogen predicted by the TUFLOW FV model across the entirety of the 7-day model run. In reality, the discharge plume moves up and down the bay (see Figure 4-3) such that the plume does not remain in one place. As presented in Appendix 2, model runs were also conducted over 14 days and 28 days. The spatial maximum extent of the discharge plume was not found to substantially increase in area after approximately 7 days of the model run, indicating that plume conditions approach equilibrium over these timescales.

4.3.2 Total Nitrogen Plume Characteristics

Figure 4-3 shows the movement of the wastewater discharge plume between 76 and 87 hours of the TUFLOW FV model run for the summer scenario. The total nitrogen content of the wastewater discharge plume moves in and out of Chandler Bay with the tide. Higher total nitrogen concentrations remain situated in the center of the plume and do not encroach to within near-shore areas.

The model results show that, as time progresses, the wastewater discharge plume moves further from the outfall location in both the north and south direction until it approaches equilibrium with ambient seawater conditions, whereby the plume is no longer predicted to reach any further north or south over time. Model results indicate the wastewater discharge plume moves no more than approximately 3 miles to the north of the outfall and no more than approximately 1 mile to the south. As described in Appendix 2, the movement of the plume was found to follow a similar pattern to this over model runs that lasted up to 4 weeks.

4.3.3 Contact with the Shoreline

As evident in Figure 4-3, the outer edges of the wastewater discharge plume are predicted to contact the shoreline in some locations on the western side of Chandler Bay. The maximum total nitrogen concentration above background in near-shore areas is predicted to be approximately 0.038 mg/L (Table 4.2). The model predicts seawater conditions at the shoreline represent a 173x dilution of the initial wastewater discharge plume. Based on the average background concentrations of total nitrogen in Chandler Bay (0.27 mg/L), seawater total nitrogen conditions at the shoreline may reach 0.31 mg/L.

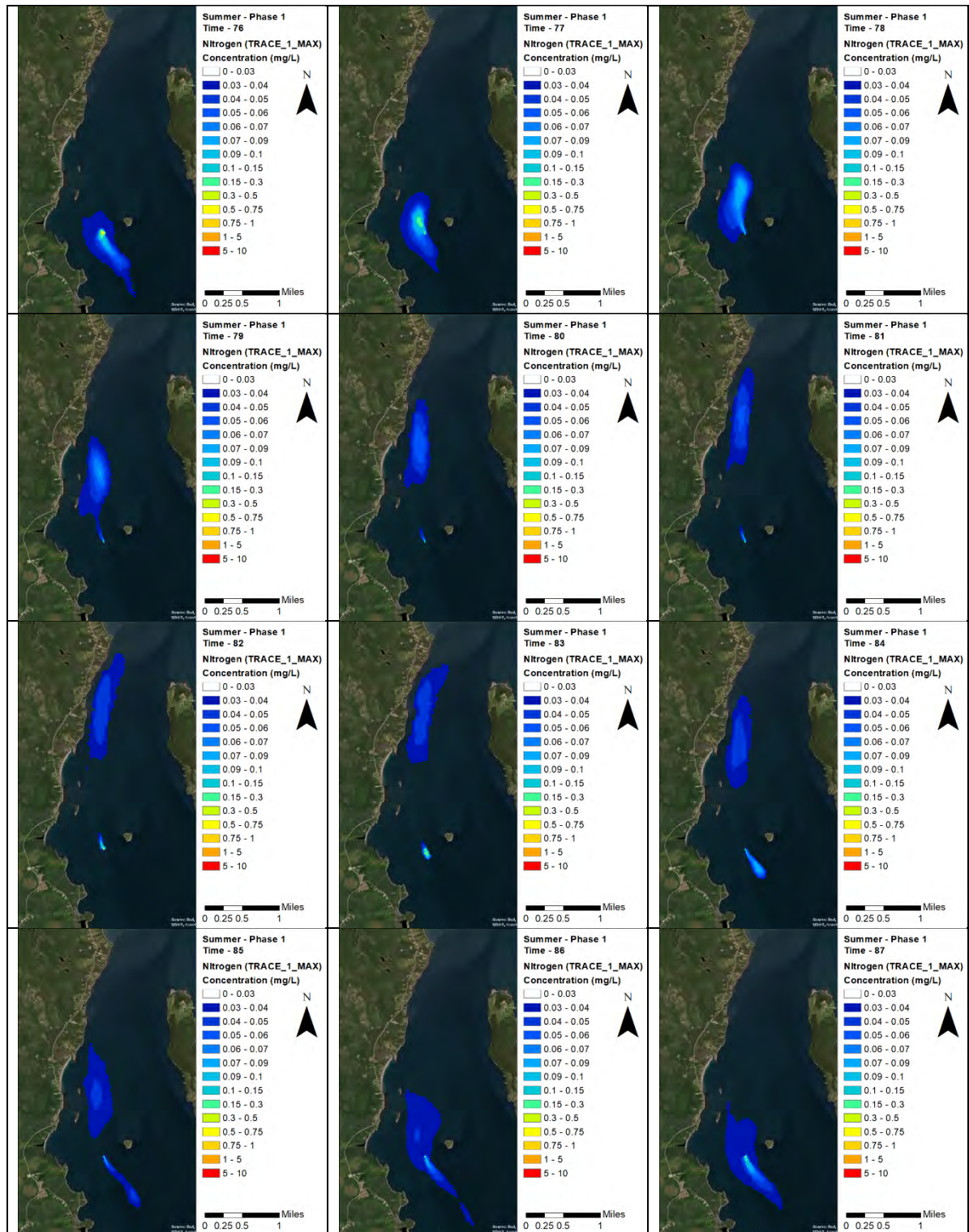


Figure 4-3: Excess total nitrogen concentrations - Summer Phase 1 - Model time 76 to 87 hours

4.3.4 Temperature

Because the wastewater will pass through the heat recovery system to maintain optimal growth conditions in the RAS, the temperature of the water released from the diffuser will be lower than temperatures in Chandler Bay during all but the coldest times of the year.¹⁶ Therefore, potential impacts to water temperatures in Chandler Bay were examined. This exercise was completed for the Summer scenarios only, as the wastewater temperatures during the coldest times of the winter are projected to be nearly the same as the ambient water temperature in Chandler Bay (near freezing). Therefore, summer conditions represent the time when temperature differential between the colder wastewater and ambient Chandler Bay water will be the largest.

The TUFLOW-FV model set the water temperature of the Bay to be 59°F (15°C). The temperature of the effluent released from the diffuser locations were set at a lower temperature of 48.7°F (9.3°C). Figure 4-4 shows the modeled temperature differences from background. Note that the model results are consistent with the available background data from Chandler Bay which indicates that the water is vertically mixed. The model results indicate a minimal impact to the temperature of the Bay. For much of Chandler Bay, the temperature difference is less than 0.09°F (0.05°C) below ambient surface water temperature.

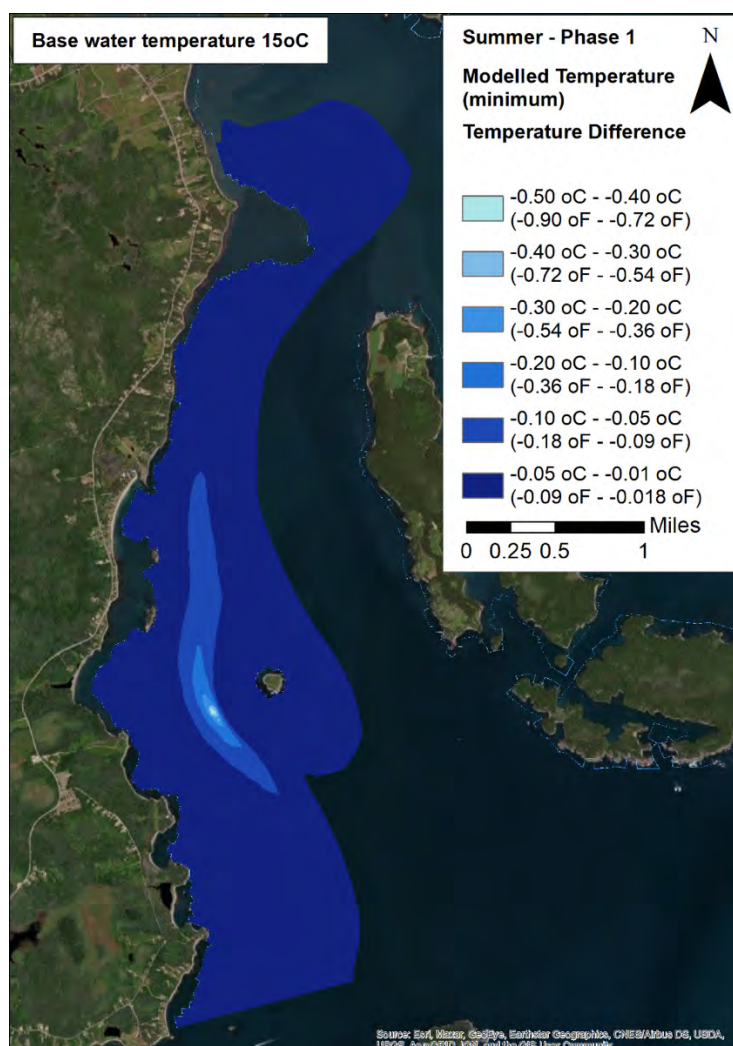


Figure 4-4: Modelled temperature differential from background for the Summer Phase 1 Scenario

¹⁶ Discharge water temperature will be equal to ambient surface water temperatures when the temperatures in Chandler Bay reach freezing.

5. SUMMARY

The results of the CORMIX model used to simulate near-field conditions after discharge of the wastewater at the outfall indicate the extent of the plume is likely to extend not more than approximately 230 ft from the location of the outfall during either summer or winter conditions. At the edge of the NMZ, the discharge plume will have been subjected to a dilution factor of 60x.

Once beyond the NMZ, the wastewater discharge plume in the far-field is predicted to move primarily by offshore flow currents and tidal flow. The plume may move up to 3 miles to the north and 1 mile to the south, further diluting as the plume disperses in the bay. TUFLOW FV modeling predicts that the wastewater discharge plume will not move beyond these limits approximately 7 days after the initial discharge, indicating that the plume reaches equilibrium with the surrounding ambient seawater. And while model results indicate that the diluted wastewater discharge plume may reach the western shoreline of Chandler Bay at some locations, the discharge plume in these areas will be diluted by as much as 173x. Total nitrogen concentrations in these areas, therefore, are predicted to be 0.038 mg/L above background conditions and temperature decreased by no more than 0.09 degrees F.

APPENDIX 1 OUTFALL LOCATION ANALYSIS

Four locations within Chandler Bay were examined for the location of outfall diffusers (Figure 5-1). Location 3 was the final choice presented in this report.

Figure 5-2 shows the maximum nitrogen concentration simulated at four proposed diffuser locations. The model results show that increases in nitrogen concentrations above 0.03 mg/L are limited to the confines of Chandler Bay for each of the diffuser locations. The location of the diffuser had an impact on nitrogen concentration levels, indicating that the further offshore the diffusers are located, the lower the simulated nitrogen concentrations, particularly at the shoreline.

Locations 1, 2, and 3 are located progressively further offshore, with location 1 nearest to the site. Location 4 is situated offshore of Rogue Island to the south west (Figure 5-1). Table 5.1 shows the maximum nitrogen concentration along the shoreline for each diffuser location. The maximum nitrogen concentration measured along the shoreline progressively decreased with increasing diffuser distance offshore.

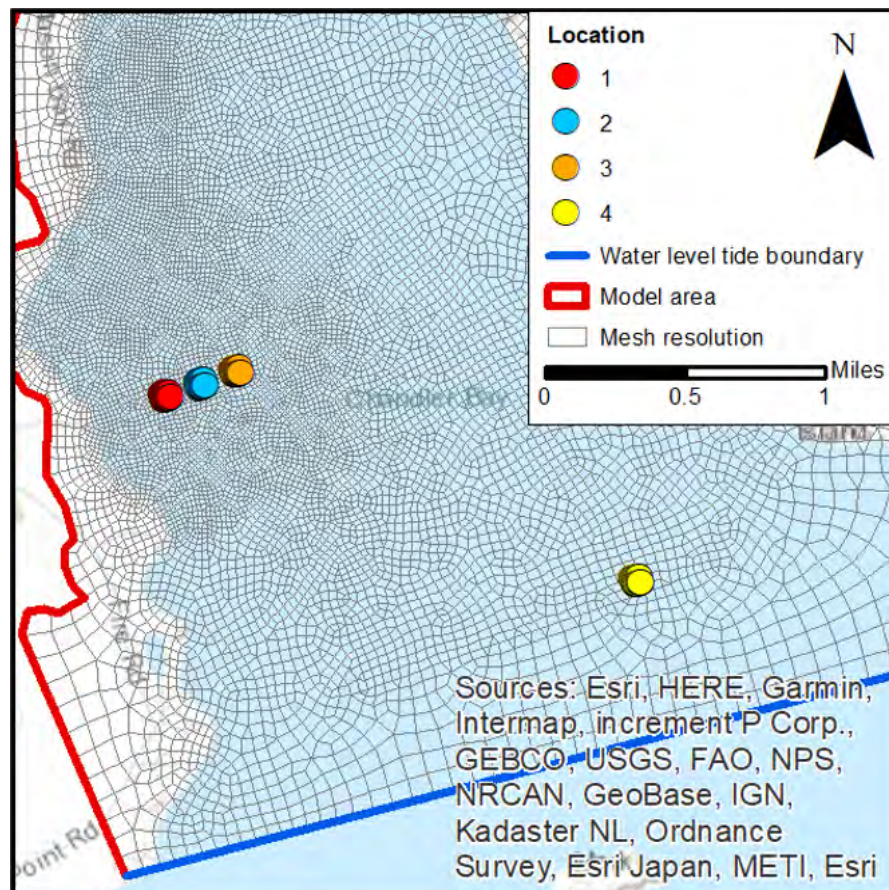


Figure 5-1: Proposed diffuser locations within Chandler Bay

Table 5.1: Maximum excess total nitrogen concentration along the shoreline for each scenario and diffuser location

Scenario	Maximum Nitrogen Concentration (mg/L) along shoreline			
	Location 1	Location 2	Location 3	Location 4
Summer Phase 1	0.101	0.053	0.038	0.007
Winter Phase 1	0.101	0.053	0.038	0.007

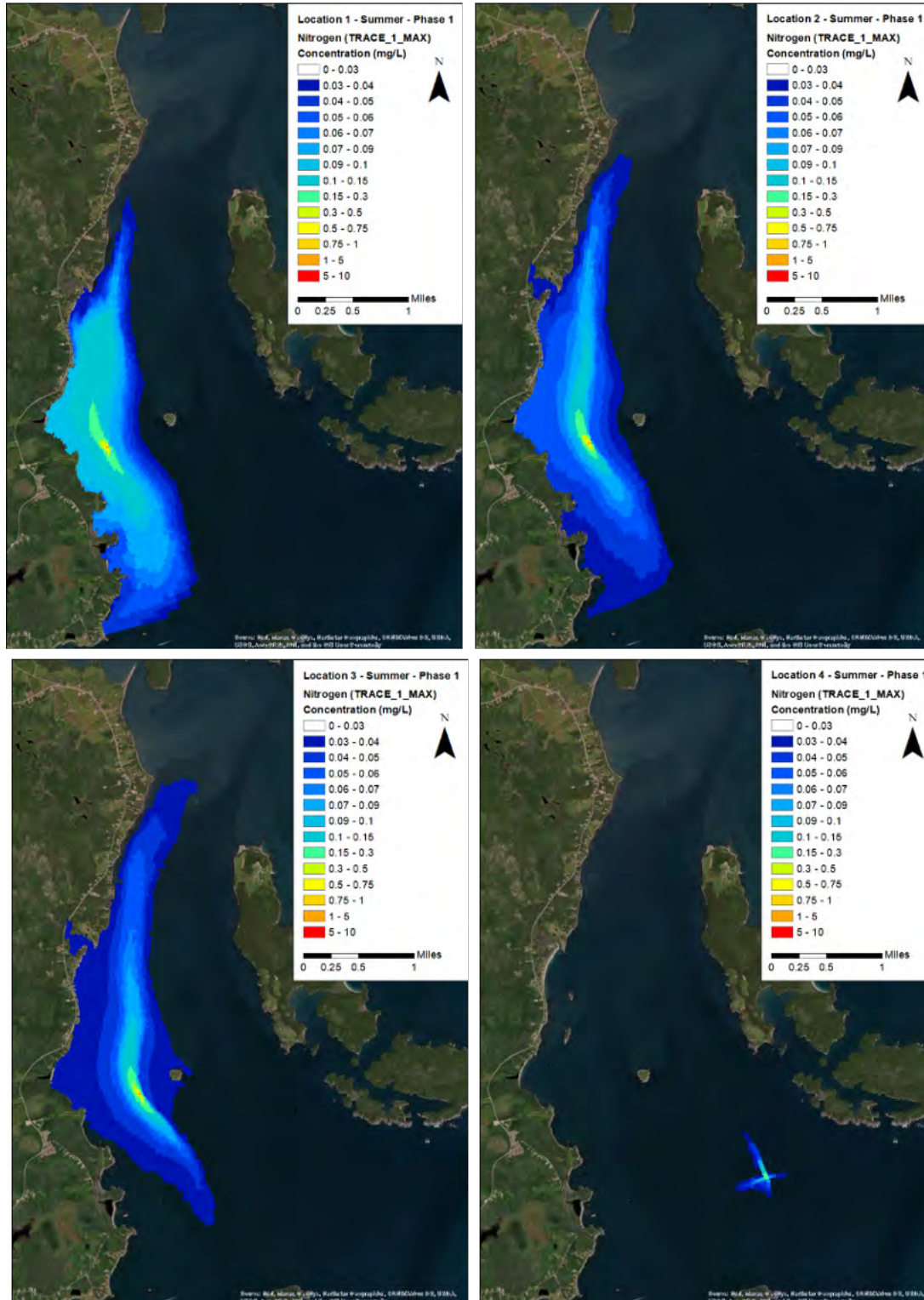


Figure 5-2: Maximum excess total nitrogen concentrations during summer at Locations 1 (top left), 2 (top right), 3 (bottom left), and 4 (bottom right)

APPENDIX 2 MODEL CALIBRATION

Georeferenced current velocity survey was collected was a vessel-mounted acoustic Doppler current profiler (ADCP) along the three transects in the project area, throughout as much of the tidal cycle as practicable to assess the variability in tidal currents in the project site. Water current velocity measurements were collected along these transects over a consecutive two-day period from the 20th to 21st November 2019.

The current velocity measurements were collected with a Teledyne RD Instruments (TRDI) 600 kHz Workhorse Sentinel ADCP mounted to an aluminum pole and from the starboard side of Normandeau's 24-foot Privateer vessel mounted 0.7m below the water surface at the transducer faces.

The November 2019 tides were simulated through the TUFLOW-FV model and the results were compared with the recorded data. Figure 5-3, Figure 5-4, Figure 5-5 and Figure 5-6 compares the ADCP recorded data and the TUFLOW-FV modeled velocity magnitude and vectors at the equivalent time for the Early Flood, Flood, Late Ebb and Near Low Slack respectively. Figure 5-7 shows the velocity magnitude for the ADCP recorded data and the TUFLOW-FV modeled data for Early Flood, Flood, Late Ebb and Near Low Slack. Comparison of the ADCP recorded and TUFLOW-FV modeled data shows the TUFLOW-FV model is replicating the overall velocity direction and magnitude at Chandler Bay. It is noted that the TUFLOW-FV modeled velocity is marginally larger than the ADCP recorded data however, the velocity magnitude changes recorded is comparable to the TUFLOW-FV modeled data. This indicates the TUFLOW-FV model is replicating the hydrodynamic environment of the Chandler Bay.

Simulation Time

The model simulation time was tested to assess the most appropriate simulation time to run the model. Figure 5-8 shows the maximum nitrogen concentration for the simulation times tested, 1 week, 2 weeks and 4 weeks. The model results show that overall, the shape and concentration distribution of the plume is generally constant for the various simulation times.

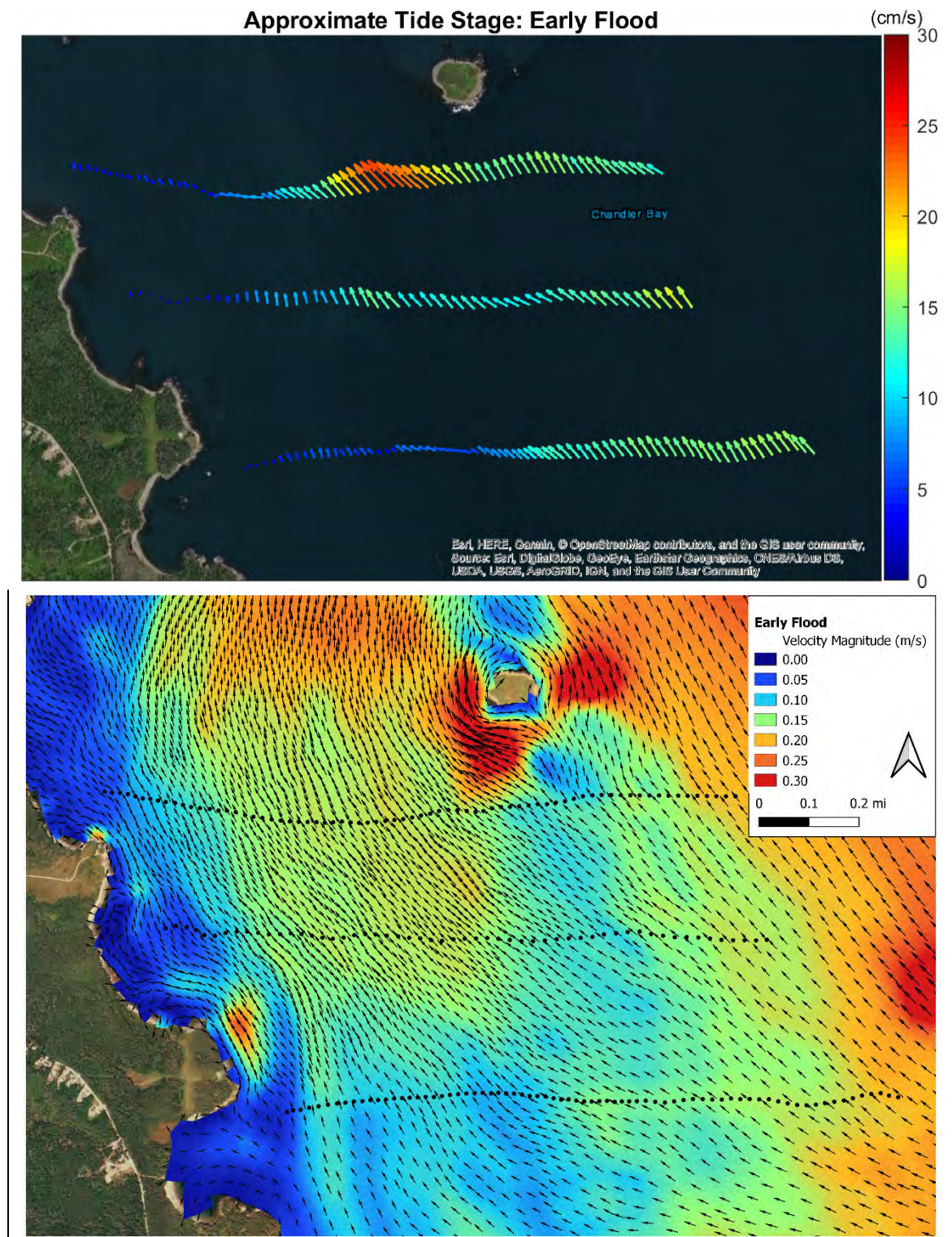


Figure 5-3: ADCP recorded data during Early Flood (top) and TUFLOW-FV modeled velocity magnitude and vectors at equal time (bottom)

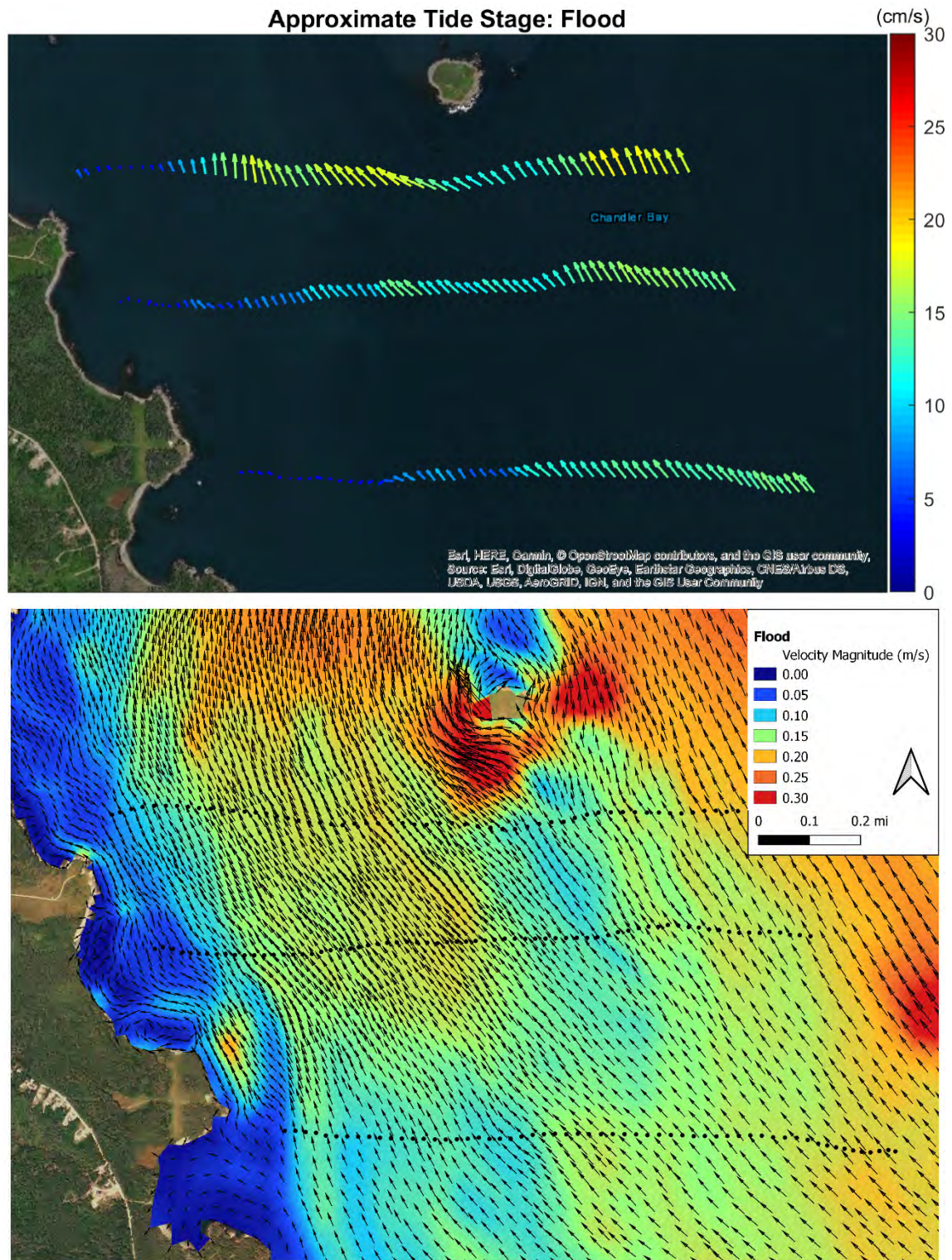


Figure 5-4: ADCP recorded data during Flood (top) and TUFLOW-FV modeled velocity magnitude and vectors at equal time (bottom)

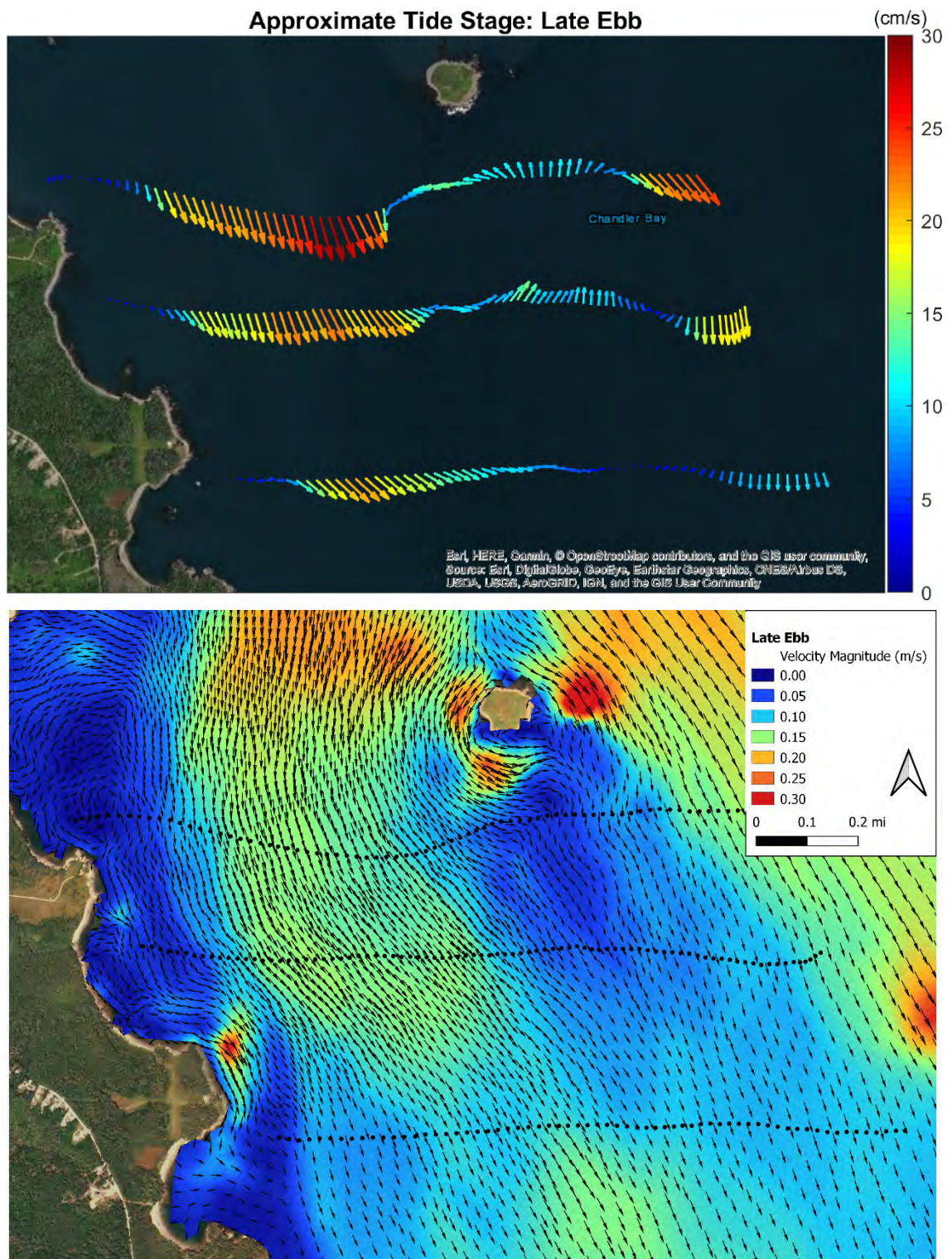


Figure 5-5: ADCP recorded data during Late Ebb (top) and TUFLOW-FV modeled velocity magnitude and vectors at equal time (bottom)

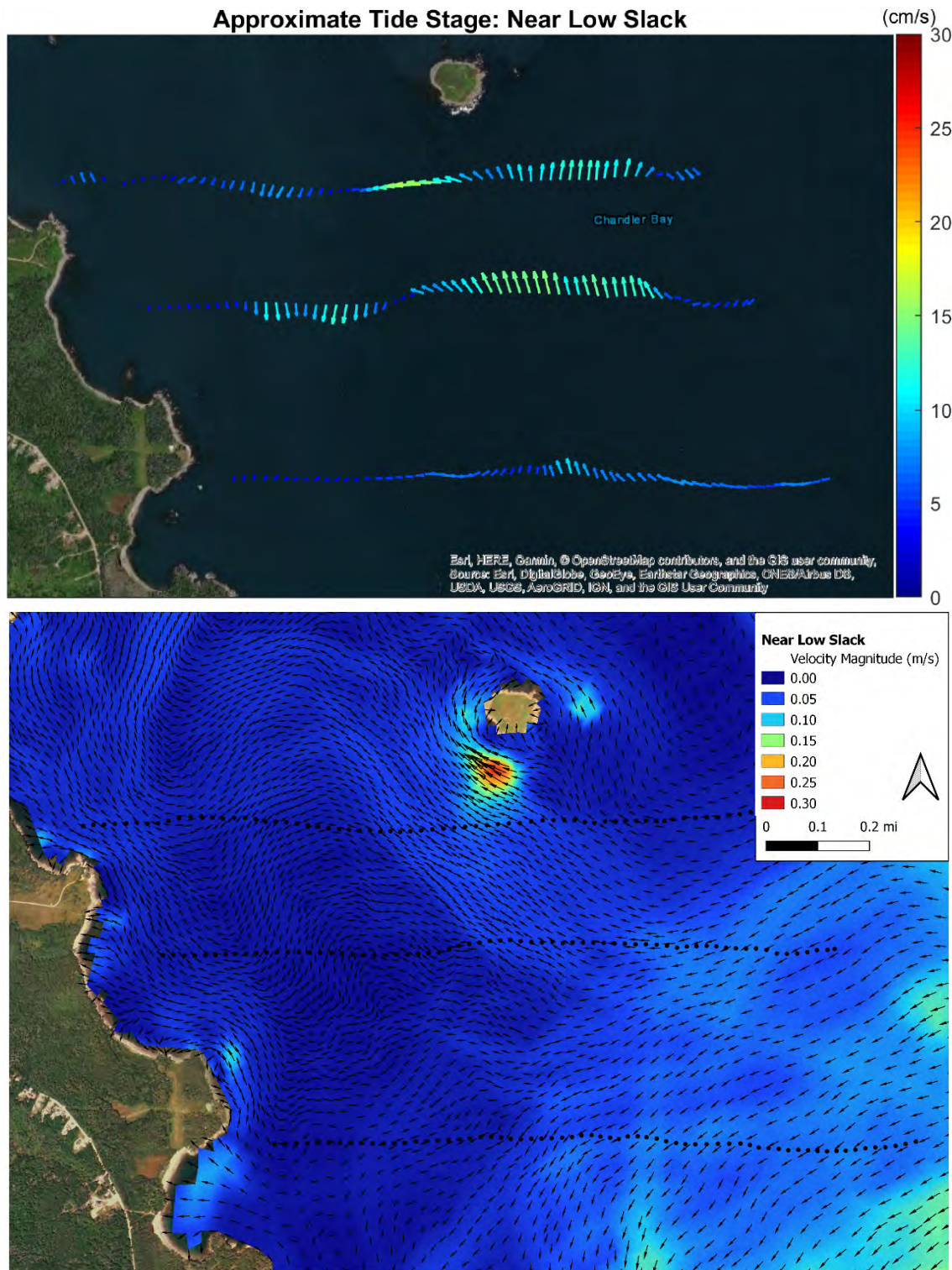


Figure 5-6: ADCP recorded data during Near Low Slack (top) and TUFLOW-FV modeled velocity magnitude and vectors at equal time (bottom)

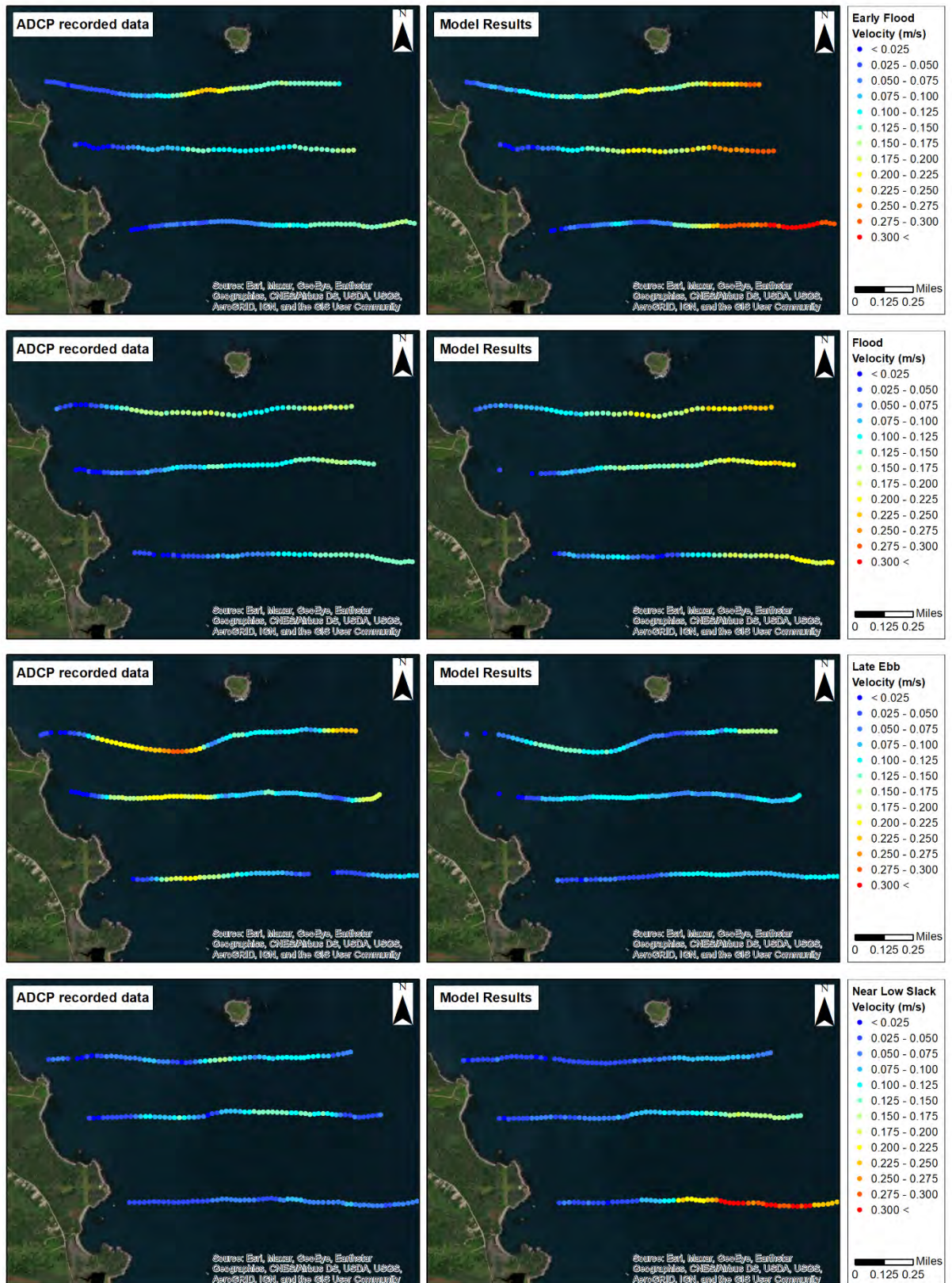


Figure 5-7: Velocity magnitude at ADCP recorded data (left) and TUFLOW-FV modeled data (right) during Early Flood (top), Flood (2nd top), Late Ebb (2nd bottom), Near Low Slack (bottom)

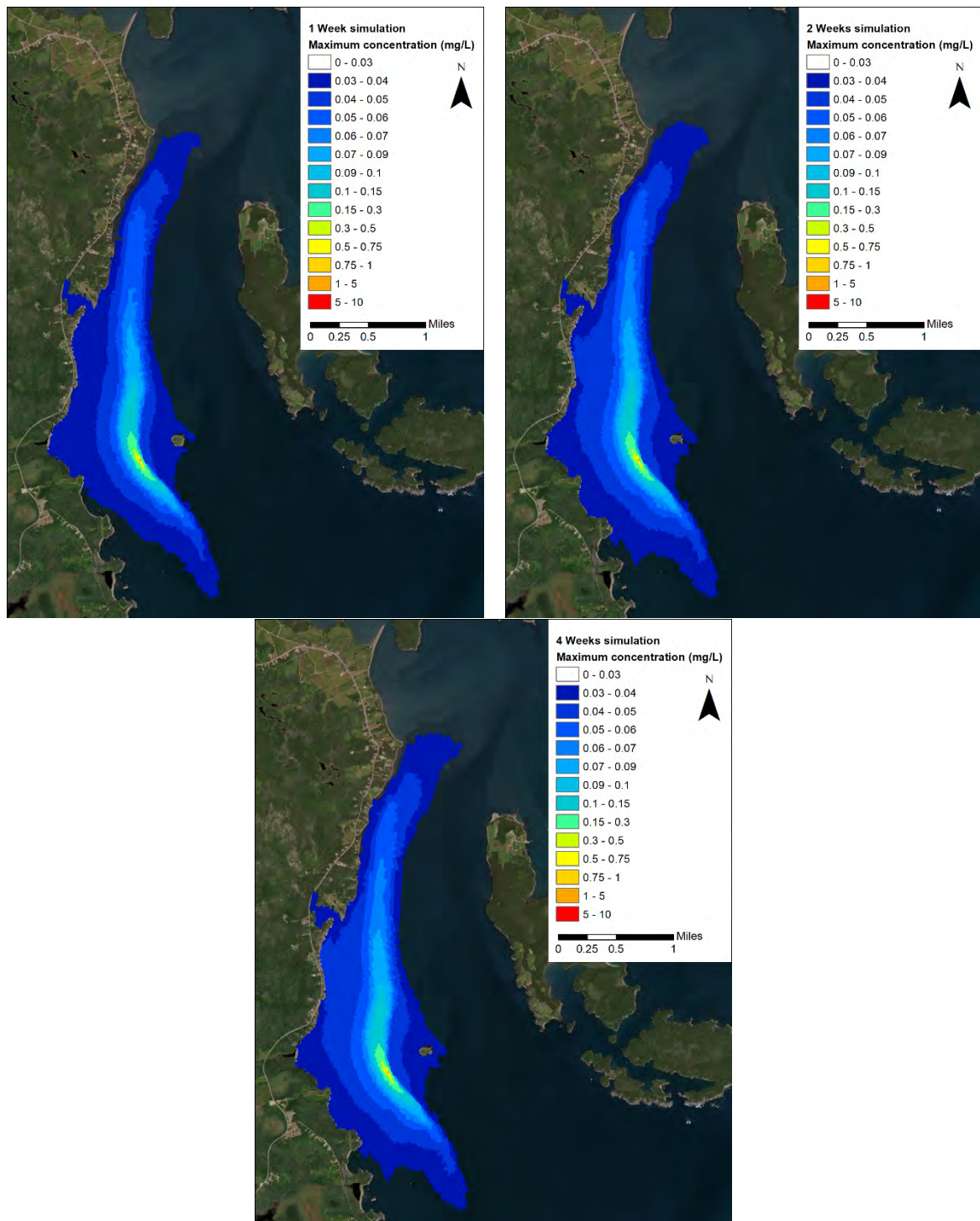
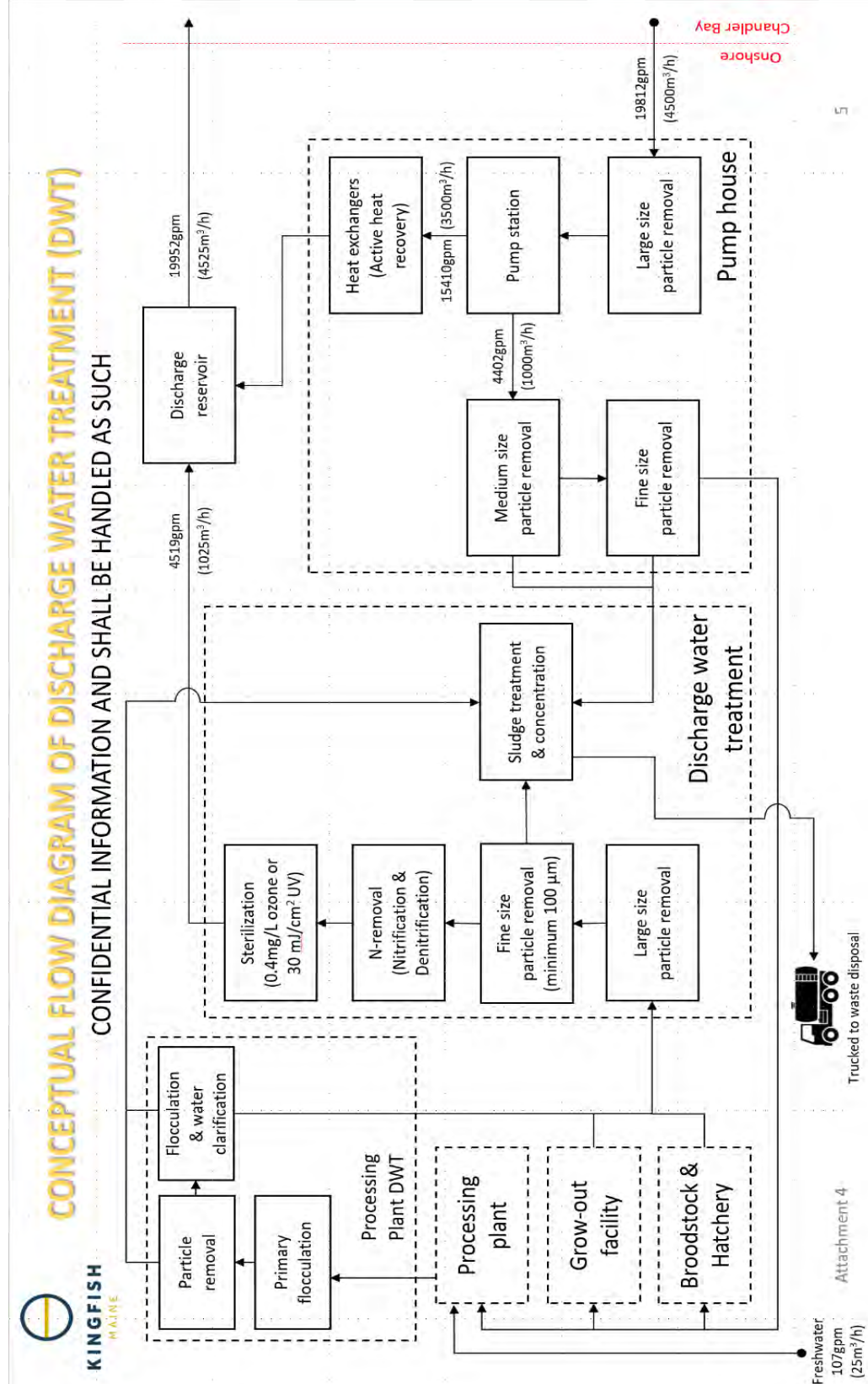


Figure 5-8: Maximum excess total nitrogen concentrations for simulation time of Week 1 (top left), Week 2 (top right) and Week 4 (bottom)

APPENDIX B Discharge Water Treatment



APPENDIX C *Kingfish Maine Economic Impact Assessment Summary, University of Maine*

ECONOMIC AND FISCAL IMPACTS OF A PROPOSED RECIRCULATING AQUACULTURE FACILITY IN
JONESPORT, MAINE¹

Prepared for: Kingfish Maine

Prepared by:

Megan Bailey² and Sheldon Green³

Margaret Chase Smith Policy Center
University of Maine

July 2020

¹ This research has been supported by Kingfish Maine. We would like to thank Drs. Todd Gabe and Andrew Crawley from the University of Maine School of Economics for their feedback on the input-output model throughout the process of preparing this report.

² Research Associate

³ Undergraduate Research Assistant

APPENDIX C *Kingfish Maine Economic Impact Assessment Summary, University of Maine*

HIGHLIGHTS OF MAIN FINDINGS

Planning and Construction Impacts

- Including multiplier effects, the planning and construction of the RAS facility (based on a total investment of \$108 million) would have an annual *statewide* economic impact – for the two years of the project – of an estimated \$44 million in output, and 366 full- and part-time jobs across Maine, with those workers earning an estimated \$19 million in labor income per year.
- The employment multiplier of 1.7 means that each worker directly involved with the planning and construction of the RAS facility would support an additional 0.7 workers across the state.
- Including multiplier effects, it is estimated that the planning and construction of a \$108 million RAS facility would support 180 full- and part-time jobs in *Washington County* annually – for the two years of the project – and would provide \$7 million in labor income to Washington County workers.
- The income multiplier of 1.28 implies that each dollar of income by Washington County workers directly involved in the planning and construction of the facility would lead to an additional \$0.28 earned by workers within the county.

Ongoing Operations Impacts

- Including multiplier effects, the ongoing operations and maintenance of Kingfish Maine's RAS facility would have a statewide economic impact of an estimated \$28 million in output, 129 full- and part-time jobs, and \$8.4 million in labor income in 2023.
- The impact increases annually as estimated direct expenditures increase. In 2026, the estimated statewide economic impact would be \$52 million in output, 155 full- and part-time jobs, and \$9.4 million in labor income.
- The statewide employment multiplier (for 2026) of 2.2 (obtained by dividing the total statewide employment impact by the direct employment impact) implies that each worker directly employed by Kingfish Maine's facility would support an additional 1.2 workers within the state.
- It is estimated that Kingfish Maine would have a total, including multiplier effects, value-added impact of \$46 million. This is equivalent to approximately 0.1 percent of the state's gross domestic product in 2019.

- Including multiplier effects, it is estimated that by 2026 the facility would support the employment of 126 Washington County workers and provide \$8 million in labor income.
- The Washington County income multiplier (for 2026) of 1.34 implies that each dollar of income by Washington County workers directly employed by the RAS facility would lead to an additional \$0.34 earned by other workers across the county.
- The estimated Washington County employment impact of 126 jobs is equivalent to about 2 percent of all private employer jobs in Washington County as of 2018.

FINAL DRAFT FOR REVIEW

The diagram illustrates the wastewater treatment process for a salmon hatchery, showing the flow from raw wastewater input through various treatment stages to a discharge reservoir and finally to the ocean/sea. The process is divided into several main sections:

- Raw Wastewater Input:** 107gpm (5m³/h) enters the system.
- Processing Plant DWT (Dashed Box):** Includes Primary flocculation, Particle removal, and Flocculation & water clarification.
- Processing plant (Dashed Box):** Receives water from the Processing Plant DWT and the Grow-out facility.
- Grow-out facility (Dashed Box):** Provides water to the Processing plant.
- Broodstock & Hatchery (Dashed Box):** Provides water to the Processing plant.
- Discharge water treatment (Dashed Box):** Includes Large size particle removal, Fine size particle removal, N-removal, and Sterilization.
- Sludge treatment & concentration:** Receives sludge from the Discharge water treatment and sends it to a truck for disposal.
- Discharge reservoir:** Receives 4519gpm (1025m³/h) from the Discharge water treatment and sends 19812gpm (4500m³/h) to the ocean/sea.
- Pump house (Dashed Box):** Includes Large size particle removal, Pump station, Heat exchangers (Active heat recovery), and Medium size particle removal.
- Recirculation:** Water flows from the Discharge reservoir through the Pump house and back to the Processing plant.
- Onshore Ocean/Sea:** The final destination for the treated effluent.

Key flow rates and volumes are indicated throughout the process:

- Raw wastewater: 107gpm (5m³/h)
- Discharge reservoir output: 19812gpm (4500m³/h)
- Heat exchangers: 15410gpm (3500m³/h)
- Pump station: 4402gpm (1000m³/h)
- Medium size particle removal: 4402gpm (1000m³/h)
- Large size particle removal (Pump house): 19812gpm (4500m³/h)
- Discharge water treatment output: 4519gpm (1025m³/h)

1



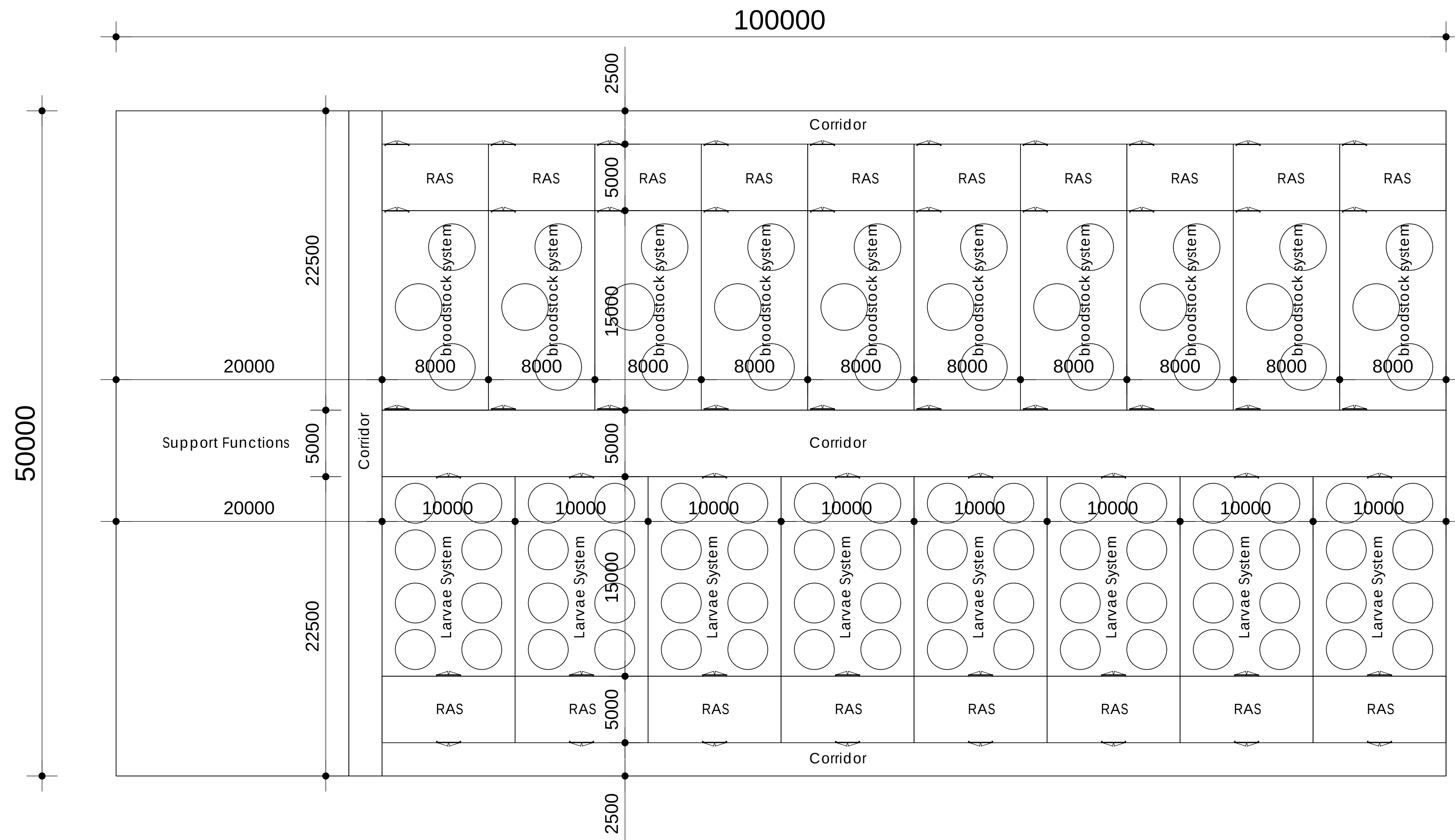
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KINGFISH MAINE CHEMICAL LIST

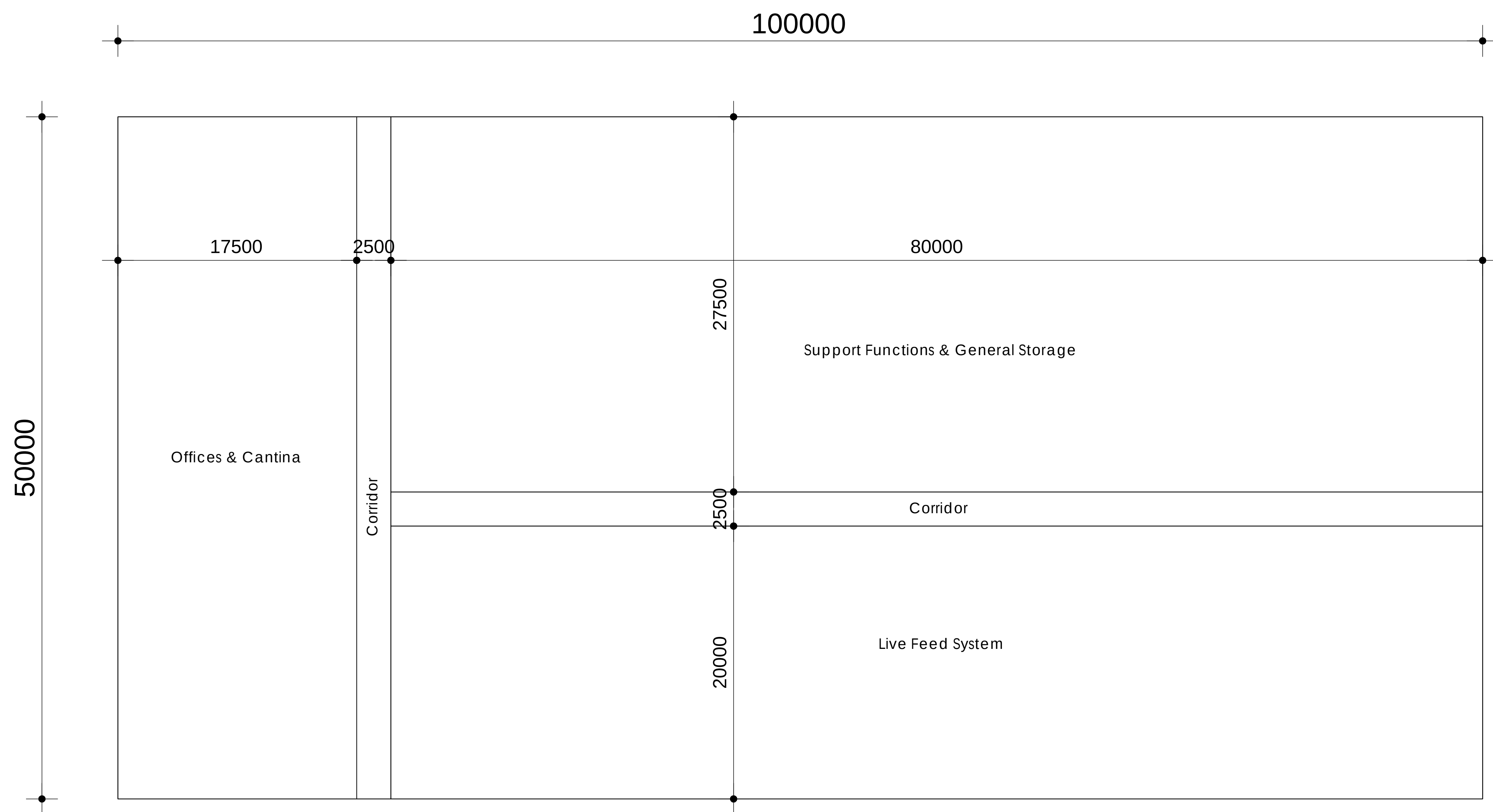
Attachment 6

<u>Chemical</u>	<u>Trade Name</u>	<u>Application</u>	<u>Method</u>	<u>Max Dosage</u>	<u>Areas of Potential Use</u>
Hydrochloric Acid		pH Balancing, Cleaning Equipment	Diluted and Applied	n/a	Hatchery/Growout
Sodium Hydroxide		pH Balancing, Cleaning Equipment	Diluted and Applied	n/a	Hatchery/Growout
Demineralized water		Calibrating Equipment	n/a	n/a	Hatchery/Growout/Processing
Sodium Bicarbonate		pH Balancing	Diluted and Applied	n/a	Hatchery/Growout
Sodium Hypochlorite		Cleaning and Disinfecting Equipment	Diluted and Applied	150ppm	Hatchery/Growout/Processing
Sodium Thiosulfate		Neutralizing Sodium hypochlorite	Added to water in proportion to sodium hypochlorite used	300ppm	Hatchery/Growout
Hydrogen Peroxide	Perox-aid, Perosan	Cleaning Equipment; External Fish Treatments	Spray or Bath Immersion	1000ppm	Hatchery/Growout
Formalin	Parasite-S	Extrenal Fish Treatment	Bath Treatment	200ppm	Hatchery/Growout
PVP Iodine	Ovadine	Disinfection of Fish Eggs	Bath Treatment	100ppm	Hatchery
Tricaine Methanesulfonate	MS-222, Tricane-S	Anesthesia	Bath Treatment	750ppm	Hatchery/Growout
Antimicrobial Hand Soap		General Cleaning	n/a	n/a	Hatchery/Growout/Processing
Citric Acid		General Cleaning	Diluted and Applied	n/a	Hatchery/Growout/Processing
Pentapotassium bis (peroxymonosulphate) bis(sulphate) & Sodium Dodecylbenzene Sulfonate	Virkon Aquatic	Cleaning & Disinfecting Equipment	Diluted and Applied	10g/l	Hatchery/Growout/Processing
Phosphoric Acid		Cleaning Processing Equipment	Diluted and Applied	As per label instructions	Processing
Chlorinated Alkaline		Cleaning Processing Equipment	Diluted and Applied	As per label instructions	Processing
Didecyldimethylammonium Chloride		Cleaning & Disinfecting Processing Equipment	Diluted and Applied	As per label instructions	Processing
Ferric Chloride					Hatchery/Growout
Ammonium Chloride		Biofilter Startup	Diluted and Applied	n/a	Hatchery/Growout
Sodium Nitrite		Biofilter Startup	Diluted and Applied	n/a	Hatchery/Growout
Methanol		Carbon source for Denitrification	Diluted and Applied	n/a	
Ethanol		Carbon source for Denitrification	Diluted and Applied	n/a	
MicroC		Carbon source for Denitrification	Diluted and Applied	n/a	

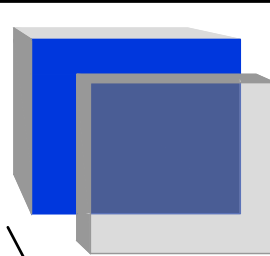
MEPDES ATTACHMENT 7 – Fish Rearing Facility Rearing Tank Number, Size, Arrangement



First floor



Second floor

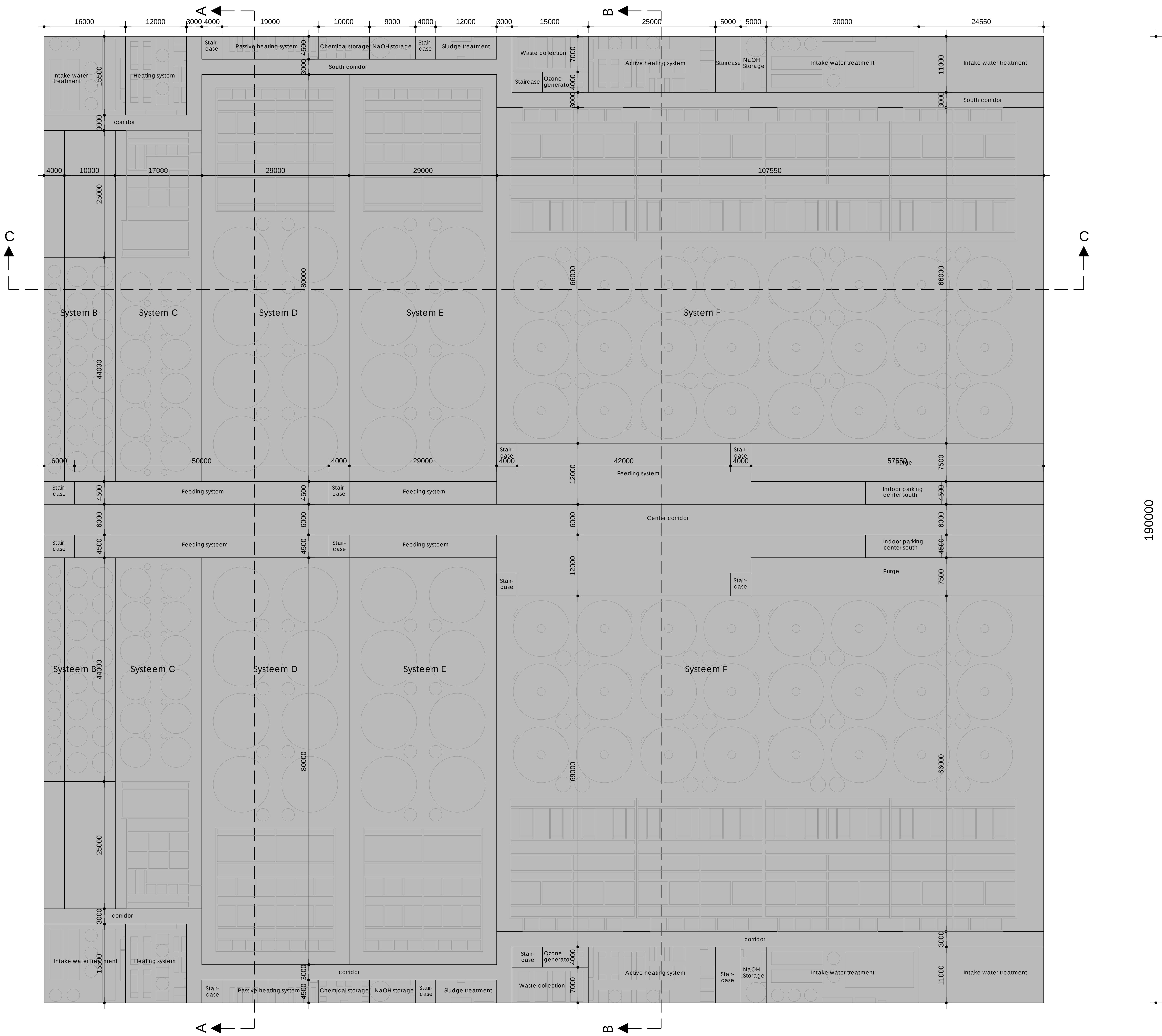


Bouwkundig Buro Laban B.V.
buro voor
architectuur en konstrukties

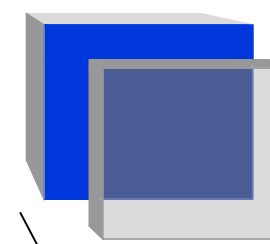
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Opdrachtgever:	Kingfish Zeeland BV, Oost-Zeedijk 13, 4326PM te Kats						
Onderwerp:	Nieuwbouw Kingfish Maine						
Onderdeel:	CONCEPT DRAWING			Hatchery & Broodstock facility			
Datum:	040620	a	b	c	Week no.:		
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1ST FLOOR



Kingfish Maine

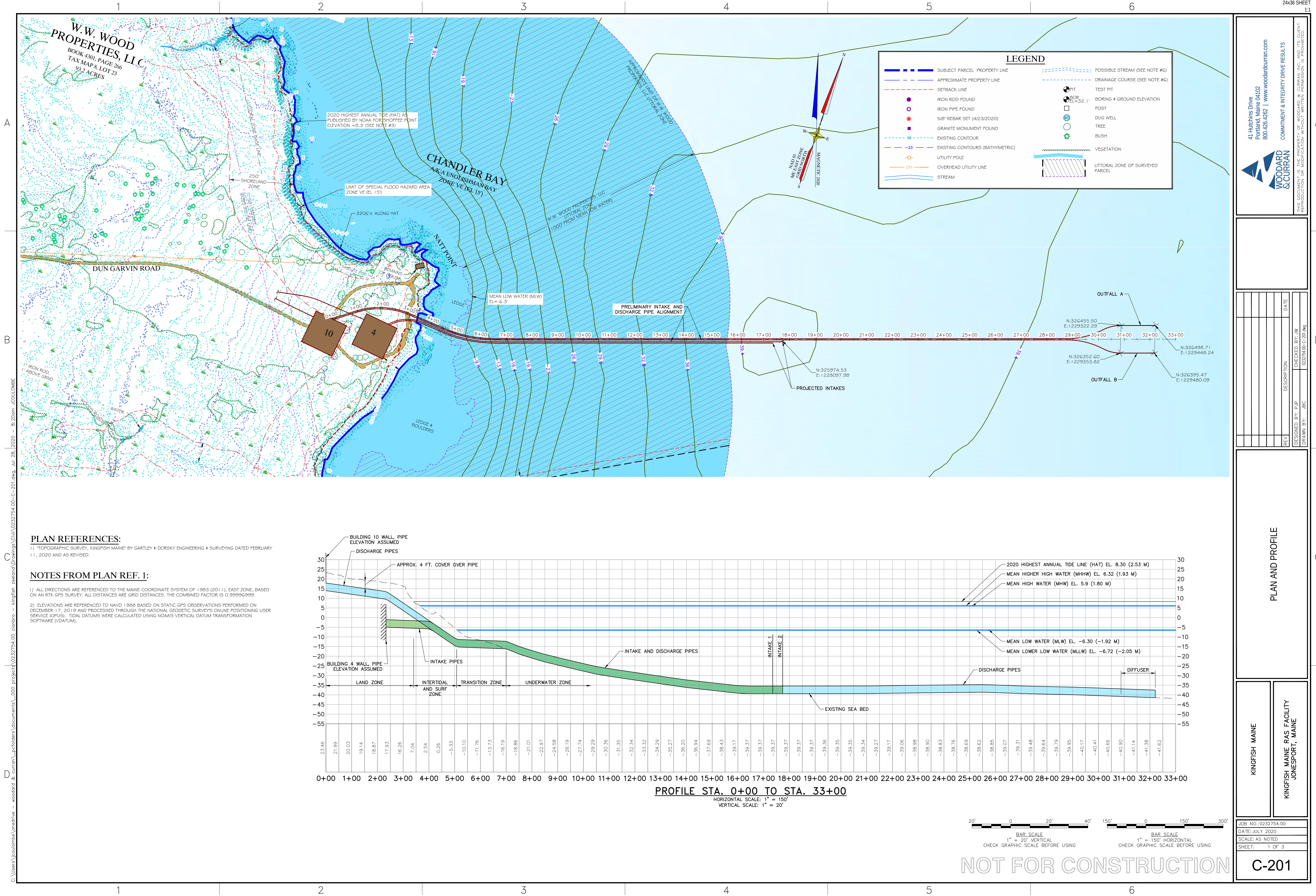
Bouwkundig Buro Laban B.V.
buro voor
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MEPDES Application – Attachment 8 – Intake and Outfall Pipe, Screening, and Diffuser Figures

The point of discharge will be equipped with a multiport diffuser, equipped with duckbill style valves to maintain consistency effluent flow rate. Ports will be oriented upward from the seafloor. See conceptual drawings,





Ambient Water Quality Monitoring Plan for Kingfish Maine, Land Based Aquaculture Project Jonesport, Washington County, Maine, USA

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Background

Kingfish Maine, was issued a Maine Pollutant Discharge Elimination System (MEPDES) Permit #ME0037559 and Maine Waste Discharge License (WDL) (W009238-6F-A-N) on June 25, 2021, for a proposed land-based aquaculture project in Jonesport, Maine. Special Condition H of these approvals requires that Kingfish Maine, starting in 2022, monitor ambient water quality both prior to and continuing through the buildout and operation of the permitted facility. This effort will significantly increase the ambient water quality dataset for Chandler Bay, as little background water quality data has been collected prior to this effort. The pre-buildout ambient monitoring will provide an assessment of the background nutrient levels of the ambient waters surrounding and expected to be affected by the facility outflow. This document describes the Ambient Water Quality Monitoring Plan (AWQMP) which will be conducted to fulfill the permit requirement.

Project Organization

Tom Sorby	Tom Sorby Operational Manager Kingfish Maine 33 Salmon Farm Road Franklin, ME. 04634 (502) 614 9078, t.sorby@kinfish-maine.com	Representative of the MEPDES/WDL permittee. Responsible for oversight of the AWQMP and vendor management
Damian Brady	Damian C. Brady, PhD School of Marine Sciences Ira C. Darling Marine Center University of Maine 193 Clarks Cove Road Walpole, ME 04573 207-312-8752, Damian.brady@maine.edu	Responsible for AWQMP implementation, including oversight of sample collection and analysis, data management and security, report production.
Angela Brewer	Section Leader, Marine Unit, Bureau of Water Quality Maine Department of Environmental Protection 17 State House Station, 32 Blossom Lane Augusta, ME 04330 (207) 592-2352, angela.d.brewer@maine.gov	Acts as MEDEP Program lead for all monitoring and data management activities covered under the AWQMP.
Gary R Brooks	Wastewater Compliance Supervisor, Maine Department of Environmental Protection, 106 Hogan Road Bangor, ME 04401, (207) 592-2795, gary.r.brooks@maine.gov	Maine DEP compliance staff person for Kingfish Maine permit.

Other personnel: Trained and experienced University of Maine staff will carry out all field sampling, sample collection, sample analysis, data compilation, data visualization and report production. Staff may be assisted by trained interns, graduate students, or other personnel, but in all cases, those assisting will be properly trained for the tasks performed and will act in accordance with the AWQMP.

Data Distribution Statement

Only data that has undergone quality testing and has been verified ready for distribution, will be distributed. An exception may be made for the entities listed in the above “Project Organization” section. Any data distributed to those entities before quality testing and [verification](#) would be watermarked “unverified, preliminary data, not for distribution”.

Ambient Water Quality Monitoring Plan Overview

Stations:

The University of Maine team will sample ambient water quality at a minimum of 4 pre-determined stations designated by Maine DEP (Figure 1, Table 1). Stations established in the MEPDES permit are shown on the map in Figure 1. One station (R1-3) is a previously established ambient monitoring station and is located off Chandler Bay. Station 1 is located near Great Bar. Station 2 is located near Bar Island, Station 3, off the southwest shore of Ballast Island Ledge, near the proposed outfall, and Station 4 is near Bay Ledge. These sites will be monitored in the same order on each sampling date; Station 4 will be sampled first, then Station 3, 2 and 1 in that order. These stations may be altered by MEDEP before the start of the 2022 sampling season. If so, this AWQMP will be updated to reflect the changes.

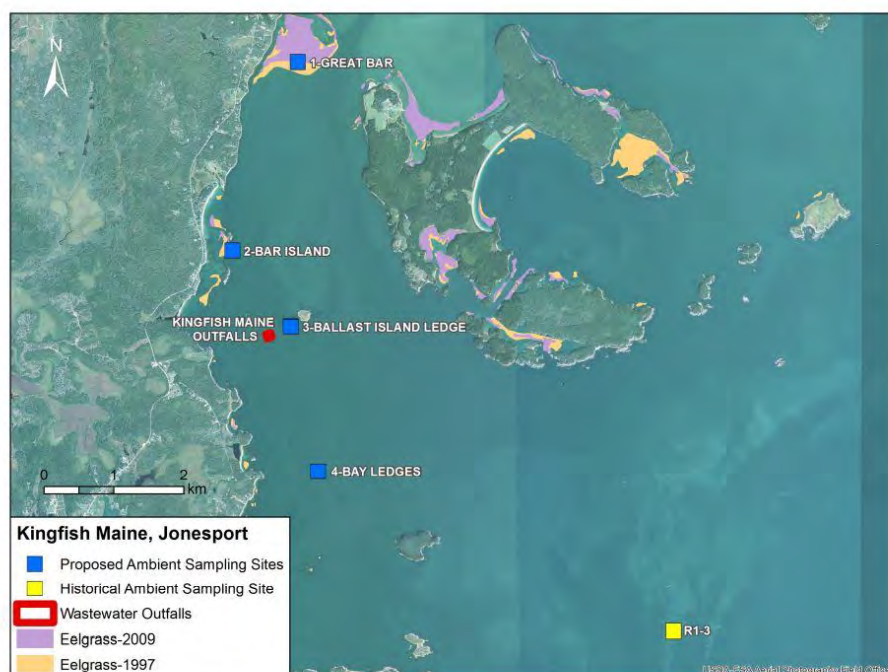


Figure. 1. Ambient monitoring stations established in the MEPDES permit.

Station	Latitude	Longitude
1-GREAT BAR	44.59209	-67.55317
2-BAR ISLAND	44.569433	-67.565643
3-BALLAST ISLAND LEDGE	44.559541	-67.556639
4-BAY LEDGES	44.540773	-67.550927

Table 1. Coordinates of Kingfish Maine ambient water quality monitoring stations

Time of Sampling

The stations will be sampled at 3-week intervals beginning in May of 2022 and extending through October, 2022. Each sampling will occur on the second half of alternating ebb and flood tides within a four-hour sampling window, including 1 hour of slack, ideally during morning hours. Kingfish Maine or the University of Maine (UM) team will provide for a vessel and captain for the sampling trips. For schedule of planned sampling dates, see Table 2. For Field Data Sheet, see Appendix F.

Date	Window	Back up Date	Window
May 10	9:15-13:15	May 11	10:13-14:13
May 31	9:20-13:20	June 1	9:57-13:57
June 21	8:05-12:05	June 22	9:08-12:08
July 13	7:41-11:41	July 14	8:37-12:37
August 4	6:30-10:33	August 5	7:24-11:24
August 25	7:28-11:28	August 26	8:05-12:05
September 20	10:30-14:30	September 21	11:24-15:24
October 11	9:11-13:11	October 12	9:52-13:52

Table 2. Planned primary and backup sampling dates for 2022 ambient monitoring. The samplings are scheduled to alternate the 4-hour sampling window between the 2nd half of ebb and flood tides. Every effort will be made to maintain the sampling schedule. In the event of a major weather event or other circumstance which would prevent sampling on both the scheduled date and the backup date, staff of UM will consult with Angela Brewer before scheduling an alternate date.

Parameters measured

Temperature, depth, salinity, dissolved oxygen, pH, chlorophyll *in situ* fluorescence, and turbidity will be measured by multiparameter sonde. Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), Nitrate plus Nitrite (NO_x) and extracted chlorophyll *a* and phaeopigment samples will be collected as grab samples and will be analyzed in the Marine Water Quality Laboratory (MWQL) at the Darling Marine Center (DMC) which is accredited by the State of Maine to perform the above laboratory analyses. If, for any reason, the MWQL is unable to analyze the samples, they will be transferred to a laboratory that is also accredited by the State of Maine for the required analysis. The sum of TKN and NO_x will provide a measure of Total Nitrogen (TN). A Secchi measurement of water clarity will also be made at each station. For specific SOPs of analytical methods, see Appendix B, C and D. For secchi depth SOP, see Appendix G.

Reporting Summary

Prior to December 31st of 2022, Kingfish Maine will submit a report prepared by the University of Maine team and Kingfish Maine to MEDEP. The report will present the sample data and supporting documents as well as a narrative of results noting any trends or patterns in the data. Grab sample data will be submitted via EDD format to Angela Brewer for review as well as sonde data, notes of field and weather conditions, total water depth at each site at the time of sampling in Excel format.

Sampling Methods

Sampling methods will follow established SOPs (See Appendices). New SOPs will be developed and appended as necessary. Deviations from or amendments to existing SOPs will be identified in the required annual reports, along with justification for such changes.

Preparation for sampling

Sonde Calibration

Prior to and within 24 hours of deployment, the multiparameter sonde will be calibrated following the procedure in Appendix A. Multiple back-up sondes are available for use should the dedicated instrument require service. If a back-up sonde is needed due to malfunction of the primary instrument, a similar procedure will be followed based on the manufacturer's protocol for the model of sonde that is used and may vary slightly due to differences in manufacturer's instructions for calibration. Any deviations from the SOP for sonde calibration presented here, in the instance of the use of a back-up sonde, will be noted in the annual report.

Labware preparation for TKN, TP, NOx, and chlorophyll a samples

For TKN, TP and NOx, grab sample bottles and caps, tubes and caps, syringes and plungers will be acid washed, rinsed with milliQ water (> 18.0 megohm resistivity) and dried. Preservative (sulfuric acid) will be added to the sample vessels for TKN, TP and NOx in the laboratory, prior to sampling. Extracted chlorophyll samples will be collected in brown, HDPE or LDPE bottles which have been cleaned with Sparkleen, a general use laboratory cleaner, and rinsed a minimum of 6 times with milliQ water. Cleaning procedures for all labware are documented in the laboratory records. Coolers will be cleaned and dried. Sample bottles and tubes will be labeled with the project name, station name or #, depth (surface (S), just below thermocline (T), bottom (B)), date, analyte, and the samplers initials.

Enroute to the site, ice will be purchased for coolers to maintain the proper temperature of the samples.

Sonde profiles

Instrumentation

The University of Maine will provide an experienced technician to conduct sonde profiles and collect grab samples for each sampling. A UM owned YSI EXO II multiparameter sonde with handheld computer for user manipulation of settings, parameter readouts, data logging and display, SN: 20E101357, will be used for profiles of water column parameters. The YSI EXO II is equipped with the following sensors (QC specifications from manufacturer):

Parameter	Range	Resolution	Accuracy
Pressure/Depth	0-100 m	0.001 m	± 0.004%.
Temperature (SN: 20D101816)	-0.5 – 50 °C	0.001 °C	0.01 °C
Conductivity (SN: 20D101816)	0-200 mS/cm	0.1 mS/cm	±0.5 % or 0.001 mS/cm w. i. g.
Turbidity (SN: 19M102348)	0 - 4000 FNU	0.01 FNU	± 2% or 0.1 FNU
Chlorophyll fluorescence (SN: 18C105672):	0 - 400 µg/L	0.1 µg/L	0.1 µg/L
pH (SN: 19B102402)	0 - 14	0.01	± 0.1
Dissolved Oxygen	0 - 500 % Air Saturation/0 - 50 mg/l	0.1% Air Saturation/0.01 mg/l	± 1% Air Saturation/± 0.1 mg/L

Table 3. Manufacturers specifications for sonde parameters.

Profile Details:

At each site, the sonde will be submerged and allowed to equilibrate at approximately 1 meter depth. The sonde will then be raised to just below the surface to begin the profile. Measurements will be taken at the following depths at each site, allowing the readings to stabilize a minimum of 2 minutes at each depth sampled:

- 0.1 meters (just below surface),
- 0.5 meters
- 1 meter followed by 1-meter increments to 10 meters total depth.
- At depths greater than 10 meters measurements will be taken in 2-meter increments to a total depth of 20 meters or within 2 meters of the bottom.
- If the water column is very shallow at a station, data may be recorded in 0.5-meter increments instead of 1-meter increments to provide more data points.

Care will be taken not to disturb the bottom, as resuspended sediment can interfere with readings and the sonde can be damaged if it contacts the bottom. A weight will be attached to sonde line and will hang below the sonde to provide an indicator of proximity to the bottom by touching the substrate before the sonde. Total depth at the station will be determined by the boat depth finder.

Sonde data will be saved in real time on the attached handheld control. Each profile will have its own file and be labelled with the station name, date and time. All readings will be taken on the descent of the sonde. A field log will record weather conditions, sea conditions, sampling personnel, sample time and will provide an independent verification of the time, as well as any unusual circumstances surrounding the station sampling. In the lab, environmental as well as calibration data will be uploaded on a MWQL computer using YSI KOR or similar software, to be eventually manipulated in Microsoft Excel or other spreadsheet software.

Grab Sample Collection

Equipment

A weighted 4-liter HDPE Niskin bottle with a metered nylon line and tripping weight will be used for collecting grab samples.

Niskin preparation

The niskin bottle will be rinsed with milliQ water after use and will be stored dry in the lab. The bottle will be rinsed with ambient water before samples are collected.

Depth of Sampling

The Field Data Sheet will be used to record sampling related data and conditions (see Appendix F). Samples will be collected at 1 m and at 0.5-2 meters above the bottom. The goal is to sample 0.5 meters from the bottom, but in rough seas where it is difficult to maintain that distance, samples may be taken at up to 2 meters above the bottom to prevent the bottle from coming in contact with the bottom or with resuspended sediment directly above the bottom. At stations with a bottom depth of more than 20 meters, bottom samples will be taken at 20 meters. The actual depths that the surface and bottom samples were taken will be recorded on the field data sheet. In addition to surface and near bottom samples, if a thermocline is observed in the sonde samples, a grab sample will also be taken just below the thermocline. The thermocline for the purposes of this plan is defined as a drop of more than one degree Centigrade in temperature from one meter to the next. This grab sample will be labelled depth

“T” and the actual depth will be recorded in the field data sheet. The thermocline sample will be analyzed for the same parameters as the surface and bottom samples. Note that for Site 4, if there is a thermocline, a bottom sample need not be taken.

Collection of Grab Samples

TKN, TP, NO_x, and chlorophyll *a* samples will be collected from the niskin bottle, which will be gently mixed between draws from the bottle to resuspend any settling particles. Sonde profiles will be collected first. The profile will be examined to determine if a thermocline is present which is defined as a change of more than 1 degree Centigrade per meter of depth. If no thermocline is present, then grab samples will be collected only at the surface and bottom. If a thermocline is present, a sample will be collected just below the thermocline. Water samplers will wear gloves and will take care not to touch other surfaces while wearing gloves. If gloves are contaminated, they will be removed, and a new pair utilized. When handling sample bottles and tubes, the caps and bottle will be handled in such a way as to prevent gloves from touching the inside of the cap or bottle or the rim of the bottle. Bottles will be filled as quickly as possible and capped immediately. TKN and TP samples will be collected in the same bottle. The bottle will contain preservative and will not be rinsed with sample. The NO_x samples will be collected in acid washed syringes and filtered through 0.45-micron cartridge filters into acid washed 50 ml centrifuge tubes. For NO_x, the syringes will be rinsed with the sample water 3 times before collecting the sample. 5 ml of sample will then be passed through the filter to waste, before collecting 40 ml of sample in the 50 ml tube. The tube will contain preservative and will not be rinsed. Tubes will be capped immediately, and sample will be stored in the cooler on ice. Chlorophyll bottles will not contain a preservative. Chlorophyll sample bottles will be rinsed three times with sample water before collecting the sample. To rinse chlorophyll bottles, up to 50 ml of sample water will be collecting in the bottle. Bottle will be capped and agitated to mix the sample. The rinse water will be dumped downstream of the sampling site. The rinsing will be repeated two more times, after which the bottle will be filled, capped, and stored in a dark cooler on ice.

Labelling of Grab Samples/Recording of Field Data

The project name, station, date and time of sampling, the depth of sampling, weather, sea conditions, names of staff collecting samples, volume of samples collected, and any unusual circumstances will be recorded in the field notebook. Field records will be recorded on water-proof paper. Data from field sheets will be scanned and manually digitized in the laboratory. Bottles will be labelled with project, date, station, time of sampling, depth, sampler's initials, and parameter.

Replicate Samples

For each parameter, one duplicate sample will be taken for every ten samples collected. If there is not enough water in one Niskin cast to collect duplicates for all parameters at one station, the duplicates may be split amongst 2 stations. For instance, TKN, TP duplicates collected from station 1 and chlorophyll *a* and NO_x duplicates from station 3.

Grab Sample Field Storage and Transport (see Appendix E for MWQL Sample Handling SOP)

Immediately after collection in the field, sample bottles will be placed in Ziploc bags and stored in coolers on ice, in the dark. Samples will remain in the coolers until arrival at the MWQL. Loose ice will surround

the samples in the cooler. Temperature of the coolers will be monitored by either measuring the temperature of a water sample that has been stored with the environmental samples during transit. Samples will be stored between 0 °C and 6 °C.

Sample Handling in the MWQL

Standard operating procedures (SOPs) will be followed for the sampling, preservation, transportation, and storage of surface water grab samples (Appendix B, C, D & E). Special permission has been granted for the MWQL to allow an 8-hour period between collection and filtration for the Kingfish Maine (Chandler Bay) chlorophyll samples due to the long travel distance required and the availability of test data showing no significant differences in chlorophyll or phaeophytin data obtained from test samples filtered at 6 and 8 hours after collection(see Appendix H). Each sample will have a unique MWQL identification which will consist of MWQL batch code which includes the date and project, station code, depth, and replicate number (if more than 1 sample is taken). This unique identification can be traced to the field data sheet and the Chain of Custody form, which will contain additional information. Samples will be kept within temperature parameters specified by the laboratory SOPs and this document, until analysis. Temperatures of refrigerators and freezers used to store MWQL samples will be monitored on each day that the MWQL is in operation, and the readings will be recorded in a log.

Method	Analytes	Holding Time (days)	MDL (12/31/21)	RL (12/31/21)	Units
SM10200H	Chlorophyll a and Phaeophytin	21	0.003	0.010	µg/l
EPA 351.2	Total Kjeldahl Nitrogen	28	0.153	0.172	mg/l
EPA 365.4	Total Phosphorus	28	0.074	0.083	mg/l
EPA 353.2	Nitrate plus Nitrite	28	0.010	0.020	mg/l

Table 3. Methods, holding times, minimum detection limit (MDLO and reporting limit (RL) of MWQL methods.

Chain of Custody

A chain-of-custody form will be completed the day of sampling and will document the following: Date and time of collection, person collecting the samples, station, depth, parameter to be analyzed, project name, unique MWQL batch code, filtered/not filtered, volume of sample collected, temperature of cooler upon arrival at the laboratory, time and date of arrival at the laboratory, receiving and relinquishing staff members, Storage location in laboratory, time of placement in laboratory storage, date of any processing or analysis of the samples (details of each action will be contained in laboratory notebooks or forms specific to the actions being performed), date and time of any transfer of samples to outside facilities, any deviations from the SOPs or unusual field conditions which may impact the quality of the sample as well as identifying information for any instruments, such as thermometers used in samples monitoring.

Quality Objectives

Sonde data

All uploaded sonde data spreadsheets will be quality-checked and formatted, at which point the raw data files on the handheld computer or sonde will be deleted from the file directory. Criteria used to validate raw profile data from discrete sampling will follow Table 6 of MDEP's marine monitoring program (MDEP 2017). For raw profile data from discrete sampling, if a data value falls outside of the relevant acceptable range, best professional judgment may override these criteria when supporting data or information suggests a real aberration. If no reasonable explanation exists for an aberration, the data value will be flagged in the raw data file and not included in subsequent analyses and reporting.

Sonde data will be checked for extreme values using conditional formatting in an Excel spreadsheet. See Table 5 for ranges. In the event data is outside of these limits, it will be examined further and flagged.

Parameter	Minimum Value	Maximum Value	
Depth	0.05 m	20 m	Range based on expected conditions within Maine marine surface waters and protocol maximum
Temperature	5 °C	25 °C	Range based on expected seasonality of sampling
Salinity	0 psu	35 psu	Range based on expected location of sampling in Maine marine waters
Dissolved Oxygen	20% saturation	150% saturation	Range based on expected seasonality and location of sampling in Maine marine waters, including extremes based on primary productivity
pH	6.5	9.5	Extremes based on algal bloom conditions, should be verified with dissolved oxygen data and time of day
Turbidity	0 FNU	25 FNU	Negative values should be corrected to 0.0, high values should be verified with adjacent data values and proper functioning of probe wiper
Chlorophyll a	0 µg/L	25 µg/L	Max. value based on algal bloom

Table 5. Ranges of acceptable data for sonde measurements. Data outside of these ranges will be flagged if no reasonable explanation exists for the aberration.

Grab Samples

Precision: For activities covered under this AWQMP, precision will be measured through an assessment of laboratory sample duplicates, replicate blank matrix samples and duplicate spiked matrix samples. The variability of laboratory blanks and standards will be used to evaluate the precision of the analytical method. The variability of both the blank and spiked matrix sample will be used to calculate the minimum detection limit and the reporting limit of the analytical method. Specific procedures are available in the specific protocol SOPs.

Accuracy: To determine the accuracy of the laboratory procedures, certified standard solutions (laboratory control samples (LCS)) will be analyzed with each sample run, with the exception of chlorophyll

a in which a solid standard is analyzed with each run, to determine the if the readings have drifted from the values obtained during the last calibration with certified standard. The chlorophyll fluorometer is calibrated with liquid certified standard two times per year. For all parameters except chlorophyll a, a certified standard will also be used to spike environmental samples to assess the recovery of the certified standard.

Representativeness: Sampling stations are chosen based on the current knowledge of the movement of water in the vicinity of the proposed outflow. MEDEP will select stations based on the monitoring goals and objectives. The sampling will take place in a 4-hour window around the second half of alternating ebb and flood tides with 1 hour of slack tide in the sampling window. This will allow for sampling at both high and low tide to document any variability with the tide cycle. Sampling will take place in May through October which will capture seasonal variability. The assessment of duplicate grab samples for each sampling event (1 duplicate for each 10 samples collected) will provide and assessment of sampling variability.

Comparability: Comparability will be addressed by making and recording observations and collecting samples using methodology approved by the MDEP, by using field staff trained and experienced in the sampling methods, and by using methods approved by the MDEP performed by a laboratory accredited by the State of Maine for those methods. Additionally, comparability with historical MDEP datasets will be maintained as much as possible by maintaining regular communication with the MDEP Marine Unit. MDEP Marine Unit staff will be welcome to participate in any or all sampling events throughout the year.

Reporting QC Data

Quality control data included in the laboratory reports will include, at a minimum, equipment blanks, field duplicates, laboratory control samples, and matrix spikes for nutrient analyses. Laboratory reports will also contain a case narrative or equivalent notation, list of qualifiers and definitions, copies of COC form that includes a cooler receipt and preservation check form indicating sample temperature. The samples will be in the possession of University of Maine staff from the time of collection to arrival in the laboratory. When quality control objectives are not met and best professional judgement determines possible field operator or analytical laboratory error, the responsible party will be contacted, and resolution sought. For field readings, data may be flagged as estimated (“J”) or rejected (“R”) if no correction is possible. For laboratory concerns, the laboratory analyst will be asked to address specific questions, and samples will be rerun if sufficient sample remains and holding times can still be met

Special Training/Certification

Field sampling staff using sonde equipment will be trained and monitored by Damian C. Brady, PhD, or qualified staff member under his supervision and in accordance with University of Maine requirements. All data analysis and reporting will be subject to the review and approval of Damian C. Brady, PhD, University of Maine. The surface water grab samples will be analyzed at the University of Maine Marine Water Quality Laboratory (MWQL), a Maine State certified laboratory. All staff will be trained in relevant SOPs and protocols, all required UM safety training as well as data integrity training.

Documents and Records

Changes to AWQMP

Proposed changes this AWQMP may result from field conditions, equipment failure or other extenuating circumstances. If a sampling date must be changed, every attempt will be made to reschedule as soon as

possible and to ensure that the new date complies with the above stated conditions. Changes in field procedures may be agreed upon as MDEP staff participate in field sampling events, in which case such changes will be documented in a revision to this AWQMP. Other recommended or necessary changes will be proposed as a revision to this sampling plan and subject to MDEP review and approval. Revised Sampling Plans will be identified by a unique version number.

Standard Operating Procedures

Standard Operating Procedures will be a part of Kingfish Maine's and UM's quality assurance program. For vertical sonde profiling, instruments must be calibrated according to manufacturer's instructions prior to each sampling event and the SOP (Appendix A) must be followed. The MWQL will follow specific SOP procedures included in Appendix B-G.

Data Management

Record Keeping

Records will include equipment calibration information and logs, field data sheets, chain-of custody forms, internal laboratory documents, forms and notebooks, lab reports, field notes, sonde field data and field notes. All information, including lab-generated data, will be kept in digital files maintained by the University of Maine MWQL. Lab-generated grab sample data will be submitted in EDD format to MEDEP. Files may not be deleted or removed from hand-held devices until an electronic or hard copy of the data has been saved appropriately and subjected to data QA/QC screening. Anomalous or inconsistent field or lab results will not be deleted but will, instead, be flagged in data sets.

All data will be backed up to secure cloud storage. The MWQL uses role-base data access control principles which limit access to data and documents to those specifically authorized as outlined in the MWQL Quality Assurance Manual.

All data resulting from this monitoring program will be managed by the University of Maine. The University of Maine field measurements and the lab results will be maintained at the Darling Marine Center.

All monitoring information will be recorded onto field datasheets that will be developed prior to field sampling. All hard copies of lab field notebook will be electronically duplicated and preserved. Sonde data are logged to internal memory on either the handheld computer or sonde, and then raw files are uploaded to spreadsheets upon return to the office and prior to a subsequent sampling event

Reporting

Kingfish Maine will provide the MDEP with an annual report of the monitoring results on or before December 31st of each year. In addition to data tables, graphs, and figures, the report will compare results with previous sampling data and note any anomalous or unusual results. As appropriate, the annual report will identify necessary changes to field sampling methods, this AWQMP, or other elements of the monitoring program and, potentially, exclusion from reporting results. Flagged data, if any, will be described in annual reports submitted to MDEP.

Appendix A

UM MWQL Multiparameter sonde data collection Standard Operating
Procedure (SOP) – YSI EXO II

Document Code: MWQLSOPSonde Rev. 2, 2/24/2022
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Specifications:

This method is applicable to marine and freshwater samples.

Applicable concentration ranges:

- Temperature: 0-40°C
- Salinity: 0-40psu
- Dissolved oxygen: 0-150%
- pH: 4-10
- Chl a: 0-100µg/L
- Turbidity: 0 – 1000FNU

Definitions:

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).

In calibration according to methods, the values realized by standards are typically established through the use of reference materials that are either purchased by the laboratory with a certificate of analysis or purity or prepared by the laboratory using support equipment that has been calibrated or verified to meet specifications.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0

Quality control measures:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in the MWQL QAM. QA and QC procedures specific to this protocol are documented in this SOP.

The sonde will be calibrated according to manufacturer's specifications for each parameter the business day prior to being used to collect data. If any calibration is not successful, another calibration will be performed in order to ensure sensor is working correctly. Sensors have a limited lifetime of 1-3 years depending on type, sensor age will be monitored and replaced when life span is exhausted. If sondes are deployed, they will be calibrated prior to and after deployment.

Summary of Method:

Application:

This method is used to obtain in situ water quality measurements for depth, temperature, salinity, dissolved oxygen, pH, chlorophyll *a*, and turbidity. The sonde will be powered by internal batteries and controlled using a battery powered handheld device.

Summary of Method:

A multiparameter sonde is used to collect in situ water quality parameters at discrete depths throughout the water column. Typical parameters include temperature, salinity, dissolved oxygen, pH, chlorophyll *a*, and turbidity.

Interferences:

- The turbidity at the bottom depth can be artificially increased by the instrument hitting the bottom. To avoid this, a window weight is attached to the sonde such that when the window weight touches the bottom and lays on its side, the sonde is 0.5m above the bottom. When lowered gently this greatly reduces the incident that the bottom sediment is stirred up, artificially increasing turbidity.

Safety:

Hazards: This protocol does not require the use of any hazardous materials. All calibration solutions are non-hazardous waste. There are no known hazards associated with this protocol.

Equipment and Supplies:

- YSI EXO 2 multiparameter sonde with temperature and conductivity, dissolved oxygen, pH, total algae, and turbidity sensors installed.
- Calibration solutions
 - Specific conductivity solution (50,000 μ S/cm for salt water, 1,000 μ S/cm for fresh water)
 - pH 4, 7, and 10 buffers (for both salt and fresh water)
 - Turbidity standard (12.4FNU or 124FNU depending on expected range in situ)
 - Rhodamine WT
 - MilliQ water
 - Tap water
- Supplies
 - Aquarium stone and pump
 - YSI EXO2 calibration cup
 - Computer with KOR software
 - 1-liter volumetric flasks
 - Magnet wand to turn on sondes
 - 10 ml pipettor with tip
 - 0.5 - 1-liter beakers or jars
 - Dedicated 50 ml and 1-liter flask for rhodamine calibration

In situ water quality parameter measurements:

1. The multiparameter sonde is turned on 10 minutes prior to taking measurements to allow instrument to warm up and stabilize readings. The sonde will activate automatically when it is plugged into the handheld and the handheld is turned on.
2. Once on site at anchor, the sonde is lowered into the water and deployed to 0.5 meters above the bottom or just below the surface where it is held until all reading are stabilized. Although casts may be taken from the bottom up or the top down, for the purposes of this sampling plan, measurements will be taken from surface to bottom.
 1. If the overall water depth is greater than 5 meters, a measurement is recorded at 0.5 meters depth, then at 1 meter depth, then every meter until a depth of 10 meters. At depths greater than 10 meters, a measurement is taken every 2 meters finishing at 0.5 m below the surface.
 2. If the water column is less than 5m deep, a measurement is recorded at 0.5 meters, then every half meter, finishing with 0.5m above the surface. Actual depths may vary according to project goals.
3. At each depth interval, the sonde is held at the specific depth until all sensor readings have stabilized, then the measurements are recorded to the electronic handheld computer.
4. Each profile is given a unique name including the station # and date. Files are stored on internal memory of the handheld until return to the laboratory.
5. Field data is also recorded in a waterproof notebook.

Reagents and Standards:

All calibration solutions are purchased from the manufacturer and are used as is. No solutions are created in the lab.

Calibration:

The instrument is calibrated one business day before collecting measurement.

Calibration Procedure:

- A 5l bucket is filled with 4l of tap water and an aquarium pump and stone is used to bubble air through the water to supersaturate it for at least 1 hour prior to calibration.
- The sonde is turned on and connected to the KOR software. If the sonde is connected directly to the laboratory computer, it will activate and the magnetic wand is not needed. If the sonde is connected to the computer via Bluetooth, then users activate Bluetooth wireless by holding a magnet at the magnetic activation area on the sonde. When the blue LED on the sonde is off, the Bluetooth is disabled. When the light is on continuously, the Bluetooth is enabled, but no link has been established. When the blue LED blinks at 2 Hz, the sonde's Bluetooth is on, and has established a link."
- The sonde is placed with guard on into the bucket of bubbling water. The barometric pressure is noted (from the handheld computer) and used to calibrate the percent saturation of the water at the current atmospheric pressure. Once the readings are stable, the calibration is accepted.

- The conductivity sensor is calibrated using specific conductance. The sensor is rinsed with the old conductivity solution of the appropriate strength (50,000 μ S/cm for salt water, and 1,000 μ S/cm for fresh water) by submerging the sensors in a jar of the standard. The sonde is then submerged in a jar of new calibration solution of the correct strength. Once readings are stable, the calibration is accepted.
- For pH calibration, the sonde is first placed in the calibration cup with a pH 4 rinse (old standard solution) and submerged 3 times to avoid cross contamination between standards. The sonde is then placed in the freshly opened calibration solution and allowed to stabilize. The calibration is accepted once stable, then the user can proceed to pH 7 and 10 using the same process following the directions on the handheld.
- The turbidity is calibrated using a two-step process. First the turbidity sensor is calibrated in milliQ water for a zero reference. If a copper guard is being used during sampling (only used for long-term deployments), then the reference is 0.5. The sonde is placed in milliQ water, and the readings are allowed to stabilize before calibration point is accepted. Depending on the expected turbidity range of the sampling location, and the upper limit of either 12.4 FNU or 124 FNU will be used. The sensors are submerged in a rinse of old solution of the same strength, then submerged in fresh calibration solution until readings are stable.
- The total algae sensor is calibrated using a 1-point calibration with MilliQ or distilled water as a zero reference. The sensor is calibrated using relative fluorescence units (RFUs). The sensors are rinsed then submerged in fresh MilliQ/distilled water and readings are allowed to stabilize before accepting the calibration. In the instance where very high chlorophyll a concentration is expected (>30 μ g/L), a two-point calibration can be used with a Rhodamine WT solution with a value of 66 μ g/L for the upper limit. The sensor should be calibrated with rhodamine WT at least two times per year.
 - o Two step-Calibration (follow instructions in software)
 - Zero
 - Use MilliQ for zero blank
 - Second Point
 - Use 125 mg/l Rhodamine WT in a dark bottle located in the chemical fridge in BGC as primary stock.
 - To make working solution stock: pipet 5 ml of the primary stock of rhodamine WT into a 1 L flask
 - Note: there is a dedicated labeled volumetric flask for Rhodamine kept on the shelf to the upper left before entering the microscope room. Do not use any other flasks for Rhodamine.
 - Put stock, pipette and flask in glass tray to catch any spills or drips.
 - Leave flask in the in the dark while running the zero blank to allow it to come to room temperature (microscope room/cabinet until you use it)
 - Fill the flask to 1 L with MiliQ water
 - Cap the flask and invert a couple of time to mix, the working solution can be kept for 24 hours
 - Pour the dilute Rhodamine directly in to the Sonde cup and rinse the sensor
 - Dispose of the rinse liquid and refill for calibration
 - Calibrate, use “RFU” instead of “Chlorophyll in ug/L”

- Refer to the total algae calibration table in the manual (p. 136) to get the temperature dependent value to input into the calibration
- When finished dispose of the Rhodamine working solution into the waste container in the lab.

Pollution Prevention and waste management:

Although none of the solutions used in this procedure are hazardous, they are still treated as waste according to the following procedures. All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines. Waste will be stored in properly labeled containers with secondary containment. Waste will be located in approved areas and will be inspected weekly. When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus. At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste is transferred to the DMC safety officer. Waste will be stored until pickup by a University of Maine approved waste disposal company.

Appendix B

UM MWQL Nitrate Plus Nitrite (NOx) Standard Operating Procedure (SOP)

Document Code: MWQLSOPNOX Rev. 3, 2/18/21

EPA Reference: EPA 353.2

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Specifications:

This method is applicable to marine and freshwater samples.

Applicable concentration ranges:

.006-0.4 mg NOx - N/l

DEFINITIONS

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

ANALYST - The designated individual who performs the “hands-on” analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality.

ANALYTICAL BATCH -- An analytical batch is a group of environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A batch is composed of at least one and no more than 20 environmental sample(s) of the same quality systems matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or

concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various quality system matrices and can exceed 20 samples.

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).

In calibration according to methods, the values realized by standards are typically established through the use of reference materials that are either purchased by the laboratory with a certificate of analysis or purity or prepared by the laboratory using support equipment that has been calibrated or verified to meet specifications.

CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

CERTIFIED REFERENCE MATERIAL -- (CRM) means reference material, accompanied by a certificate, having a value, measurement of uncertainty and stated metrological traceability chain to a national metrology institute

CHAIN OF CUSTODY FORM (COC) -- A record, either paper-based or electronic, that documents the possession of the samples from the time of collection to receipt in the laboratory in accordance with chain-of-custody protocol. This record, at a minimum, must include the sample location, the number and types of containers, the mode of collection, the collector, the date and time of collection, preservation, and requested analyses. See also Legal Chain of Custody Protocols.

FIELD BLANK (FB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

LABORATORY BLANK (LB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is

to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the

laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibration standard).

LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)-- An environmental sample containing an analyte spike of known concentration at least 4 times the MDL. Used to evaluate the effect of the sample matrix on the analytical results.

LIMIT OF QUANTITATION (LOQ) -- The minimum levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence.

METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0.

QUALITY CONTROL CHECK SAMPLE (QCCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

Quality control measures:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in this QAM. QA and QC procedures specific to this protocol are documented in this SOP.

Initial Demonstration of performance:

- Method Detection Limit (MDL) - The minimum detection limit will be determined by analyzing blank variability and by spiking a blank with analyte at a concentration from 2 to 10 times the estimated MDL. Seven blanks and seven MDL spikes will be carried through all aspects of preparation and analysis in a minimum of three separate sample batches on three separate calendar dates. The reported MDL will be the larger of the MDL calculated from the blanks and the MDL calculated from the spiked matrix samples.
 - To determine the MDL of the spiked matrix samples:
 - Calculate the standard deviation of the 7 spikes.
 - Multiply the standard deviation of the spiked matrix by 3.143 (for seven replicates).
 - To determine the MDL of the blanks:
 - Calculate the mean of the method blanks.
 - If the mean is negative equate it to zero.
 - Multiply the standard deviation of the blanks by 3.143 (for seven replicates) and add the result to the mean.
- Limit of Quantitation - Obtain the limit of quantitation by calculating the mean and standard deviation of replicate standards between 2 - 10 times the estimated or known LOD. Multiply the standard deviation by the t value obtained from the t test table for the replicate degrees of freedom and the desired cumulative probability based on a two tailed test. For instance, the 99% cumulative probability.
- Quality Control Check Sample (QCCS) - Semi-annually, a QCCS will be ordered from an EPA recommended provider lab. The sample will be analyzed, and results sent to the provider lab. If results are not within the acceptable limits of stated values, the source of the problem will be identified and corrected.
- Demonstration of Linearity - A standard curve over the working range (0.05 - 0.40 mg/l) of the method will be run once per year to demonstrate linearity.

Ongoing quality control measures:

- Duplicate Laboratory Blanks - Duplicate lab reagent blanks will be analyzed with each batch of samples.
- Laboratory Control Standard - Duplicate LCSs are analyzed with each batch of samples, no less than one for every ten samples to determine the accuracy of the analytical protocol. If the measured analyte concentration varies by more than 10% from the

certified concentration, the analysis will stop, and the source of error will be determined before sample analysis resumes.

- Calibration Standards - A set of CAL standards, covering 4 concentrations that span the expected sample concentrations, will be analyzed with each batch of samples.
- A CAL standard at or below the RL will be analyzed at minimum once per month. The concentration obtained should be within 40% of the true concentration.
- Laboratory Fortified Sample Matrix - 10% of the analyzed samples will be spiked with analyte to give a concentration at least four times the MDL prior to digestion. The percent recovery will be determined from these samples. If the percent recovery falls outside to the 80%-120% range, analysis will stop, and the source of the error will be determined and resolved before continuing analysis.
- Laboratory Duplicates - A set of LDs will be analyzed in each batch of samples. Duplicates should not vary more than 10%.

Summary of Method:

Application:

This method is used to obtain the total concentration of nitrate plus nitrite (NO_x), from filtered freshwater and seawater samples. The method is appropriate for samples with total concentration up to 0.4 mg/l NO_x - N or 400 µg/l NO_x - N. Samples with higher concentrations of NO_x -N may be diluted and the diluted sample analyzed using this protocol.

Summary of Method:

A filtered sample is mixed with a pH 8.5 buffer. The mixture of sample and buffer passes through a column containing reduced granulated copper-cadmium which provides hydrogen for the reduction of nitrate to nitrite. The nitrite (consisting of nitrite that was originally present plus reduced nitrate) is determined by diazotizing nitrite with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is absorbs light at a wavelength of 540 nm. The dye is detected spectrophotometrically and is proportional to the total nitrite concentration in the sample. Though the method is designed for seawater and brackish water, it is also applicable to freshwater sample matrices. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying

salinities (0 to 35 ppt) may be analyzed. The determination of background is necessary only for samples which have color absorbing at 540 nm

Interferences:

- Particulate matter can clog the cadmium reduction column restricting flow and reducing sensitivity. To avoid this issue, samples are filtered before analysis.
- Samples containing high levels of metals such as copper and iron, may have reduced response. Increasing the EDTA concentration in the buffer can mitigate this problem.
- Significant amounts of oil and grease in samples, interfere with the reduction process and can damage the cadmium reduction column. Samples with visible oil and grease will not be analyzed.
- Samples that absorb significantly at 540 nm, can be analyzed with and without the color reagent to determine the background absorbance.
 - In our experience samples with significant absorbance at 540 nm are rare in environmental water samples.
 - If samples are colored this interference can be determined and subtracted.
 - First, calibrate in the standard fashion.
 - Next, replace the color reagent with a solution containing 100 ml/L H_3PO_4 .
 - Finally, reanalyze the samples.
 - The color interference signal can then be subtracted from the sample signal.
- All plastic and glassware must be thoroughly cleaned to avoid contamination.

Safety:

Hazards: This protocol requires the use of hazardous chemicals. All laboratory personnel using this protocol must be current with all University of Maine safety training requirements.

Hazardous chemicals: Read SDS sheet for all chemicals before use. Wear appropriate personal protective equipment including appropriate gloves, lab coat and safety glasses. The following list highlights some of the hazards of the listed chemicals but IS NOT intended to replace thorough reading of the SDS sheets by laboratory personnel.

- Phosphoric Acid (CAS#: 7664-38-2). Corrosive.
- Sodium nitrite (CAS#: 7664-38-2). Oxidizer. Toxic.
- Sodium hydroxide (CAS#:1310-73-2). Toxic. Corrosive.
- Ammonium Chloride (CAS#:1310-73-2). Irritant.
- Cadmium in reduction column 9CAS#: See SDS from Hach. Toxic.

Hazardous Liquids Under Pressure: Liquids being pumped through the Lachat manifold are under pressure and can release in a spray if a leak develops.

Equipment and Supplies:

- 50 mL conical tubes (50 ml conical screw cap HDPE centrifuge tubes) for sample storage, one for each sample.
- Bottles -
 - 1 - 1 liter bottle for Buffer.
 - 1 - 1 liter bottle for MilliQ carrier.
 - 1 - 1 liter bottle for MilliQ Rinse water.
 - 1 - 1-liter dark bottle for Sulfanilamide Solution.
- Sample collection tubes (100 ml or larger) or bottles (acid washed), one for each sample.
- 1 - Milli-Q squirt bottle.
- Analytical Balance, accuracy to 0.1 mg.
- Stir plate
- Ring stand and clamp.
- Lachat QuickChem flow injection analyzer with autosampler and NOx manifold.
- Stir plate
- 0.45-micron disposable filter cartridges for filtering samples. Note: disposable or reusable cartridges can be used. Reusable cartridges are acid washed and loaded with 0.45 um membrane filters before use.
- Several 50 ml syringes for filtering samples.
- 1- 10 ml pipette with tips.
- 1 - 1 ml pipette with tips.
- 1 - 1 0.1 ml pipette with tips.
- Volumetric flasks:
 - 1 - 1-liter volumetric flask for buffer solution.
 - 1 - 1-liter volumetric flask for sulfanilamide solution.
 - 1 - 250 ml volumetric flask for primary standard.
 - 7 - 50 ml volumetric flasks for secondary and working standards.
 - 1 - 50 ml flask for the LCS working solution.
- pH meter and probe with calibration buffers, milliQ squirt bottle, rinse beaker.
- Stir bars.
- Sulfuric acid solution for preserving samples.
- Sodium hydroxide solution for adjusting sample pH.

Glass and plasticware preparation:

All glassware and plasticware in contact with samples will be:

- Washed with Sparkleen detergent and rinsed 5 times with milliQ water.
- Acid rinsed using 0.5 M - 1.5 M hydrochloric acid solution and rinsed thoroughly with milliQ water.
- This includes bottles, tubes, syringes filter cartridges.

Sample collection and preservation:

Sample Collection:

- Samples (100 ml) are collected in clean, acid rinsed bottles or tubes (see above).
- Samplers must wear gloves and be careful not to touch any unclean surfaces with gloves before or during sampling.
- Samples may be collected from Niskin bottles or directly from river, stream, estuary, or other water source.
- Collection of sample:
 - Rinse collection bottle with sample.
 - Fill the bottle with a volume of sample sufficient to rinse the interior of the bottle (for a 100 ml bottle, 10 ml is adequate).
 - Cap bottle, invert, and swirl sample in the bottle to rinse the entire inner surface of the bottle.
 - Dispose of rinse water downstream of the sampling site.
 - Repeat 2 times.
 - Fill the bottle and cap.

Sample filtration:

- Samples are filtered through a 0.45-micron syringe cartridge filter.
 - Disposable or reusable filter cartridges may be used.
 - If using disposable filters, keep them in a clean Ziploc bag before use and dispose of used filters in a separate garbage bag.
 - If using reusable cartridge filters:
 - Acid rinse cartridge top and bottom and Teflon O-ring.
 - Dry in the drying oven.
 - Add a clean 0.45-micron membrane filter and assemble cartridge.
 - Store cartridges in a clean Ziploc bag until use.
 - The syringe, plunger and filter must be rinsed with the sample before use.
 - Remove filter cartridge from syringe and pull plunger out of syringe being careful not to let the syringe, syringe plunger or cartridge touch any unclean surfaces.
 - Reattach filter to syringe.
 - Pour about 5 ml of sample from the collection bottle into the top of the syringe.
 - Reinsert plunger into the syringe and push the sample through the syringe to waste.

- Be sure to dispose of the waste rinse sample downstream of the sampling location.
- Repeat two more times to adequately rinse syringe, plunger, cartridge, and filter.
- Remove cartridge from syringe and remove plunger from syringe.
- Reattach cartridge to syringe.
- Fill the syringe with the sample.
- Insert plunger into syringe,
- If the tube for filtered sample does not contain preservative:
 - Push about 5 ml of samples through the filter and into the collection tubes,
 - Cap tube, shake to rinse.
 - Dispose of sample.
 - Repeat two more times.
 - It may be necessary to refill syringe with sample to provide adequate filtered sample.
 - Then filter a total of 30 ml of sample into the collection tube,
- If the collection tube for filtered samples does contain preservative, it is not possible to rinse the tube with filtered sample. Use the following protocol:
 - Push 10-15 ml of samples through the filter to waste.
 - Fill collection tubes with 30 ml of the sample.

Sample Storage:

- Store samples in the cooler with ice packs immediately after filtration.
- If samples are not preserved in the field and are not to be analyzed immediately, they are kept in the dark on ice until preservation in the laboratory.
- Samples must be preserved within 8 hours of collection.
- Samples are preserved by adding sufficient 0.5 M sulfuric acid to lower the pH to < 2.0 within 8 hours of collection or by adding sufficient acid to the collection tube prior to filling with the filtered sample.
- Preserved samples are stored at < 6 degrees C in a refrigerator for up to 28 days.
- Before analysis, the pH of the samples must be raised to between 5 and 9 with the addition of 50% sodium hydroxide. See detailed method below.

Reagents and Standards:

Reagents should be made up the day before analysis at the latest. Reagents made on the same day as analysis, can give variable results.

1. MilliQ water -

- a. Resistivity greater than 18.0.

- b. Water is pretreated with particulate and carbon filters, passed through a reverse osmosis membrane, filtered through a Millipore milliQ clean water system, and filtered through a final particulate filter before use.

2. Ammonium chloride buffer (Three methods):

a. Method 1:

- i. Add 800 ml of milliQ water to a 1-liter flask or a 1-liter LDPE or HDPE bottle with a 100 ml mark.
- ii. Add 85 g of ammonium chloride.
- iii. Add 1.0 g of disodium ethylenediamine tetraacetic acid dihydrate (disodium EDTA, dihydrate).
- iv. Add a stir bar to the flask or bottle.
- v. Put the flask on the stir plate, turn on the stir plate to mix the reagent until the components are dissolved.
- vi. Add milliQ to a volume of about 950 ml.
- vii. Adjust the pH to 8.5 with 15 N sodium hydroxide.
 - 1. If using a flask to make solution, transfer solution to reagent bottle for pH adjustment.
 - 2. Calibrate the pH meter using instructions included with the meter.
 - 3. Set a stir plate next to the ring stand.
 - 4. Put the bottle on a stir plate.
 - 5. Drop a clean stir bar into the bottle.
 - 6. Thoroughly rinse pH probe with MilliQ water to minimize contamination of buffer reagent.
 - 7. Secure pH probe in ring stand clamp with tip at least 1/2" into the reagent.
 - 8. Turn on the stir plate to mix the buffer.
 - 9. Using the 1 ml or 2.5 ml pipette, slowly add sodium hydroxide until the pH reaches 8.5.
 - 10. Once reagent is at pH 8.5, cap bottle immediately. **Note as pH nears 8.5, solution will give off ammonia fumes.**
 - 11. Take the bottle to hood and fill to the mark on the bottle using a squirt bottle containing milliQ water from the same batch.
 - 12. Alternatively, if the bottle does not have a marked fill line:
 - a. In the fume hood, return reagent to 1 liter flask.
 - b. Fill the flask to the mark with milliQ from squirt bottle.
 - c. Transfer buffer from flask back to bottle.
 - 13. Cap bottle.
 - 14. Store in MWQL laboratory refrigerator.

b. Method 2:

- i. Add 500 ml of milliQ water to a 1-liter flask.

- ii. In the fume hood, add 105 ml of concentrated hydrochloric acid. **Caution: Solution will be hot!!**
 - iii. In the fume hood add 95 ml of ammonium hydroxide.
 - iv. Add 1.0 g of disodium ethylenediamine tetraacetic acid dihydrate (disodium EDTA, dihydrate).
 - v. Stir until dissolved.
 - vi. In the fume hood, adjust pH to 8.5 with either hydrochloric acid or sodium hydroxide following instructions above. Caution: fumes!
 - c. Method 3:
 - i. Purchase the Lachat Pre-made reagent: Part# 52003.
- 3. Sulfanilamide Color Reagent: (Note: Store in dark bottle and refrigerate.)**
- a. Method 1:
 - i. Add 600 ml of milliQ water to a 1-liter flask.
 - ii. In the fume hood, add 100 ml of 85% phosphoric acid.
 - iii. Add 40 g of sulfanilamide
 - iv. Add 2 g of N-(naphthyl)ethylenediamine dihydrochloride (NED).
 - v. Shake to wet.
 - vi. Add stir bar to flask.
 - vii. Stir to dissolve for 30 minutes.
 - viii. Dilute to mark, invert to mix.
 - ix. Store in a dark bottle.
 - x. Solution is stable for one month or until it turns pink.
 - b. Method 2:
 - i. Purchase Hach Part # 52022: sulfanilamide and 52033: NED.
- 4. Standards**
- a. Primary stock (1000 mg/l nitrate-N: Potassium nitrate -
 - i. Add 200 ml of milliQ water to an acid washed 250 ml flask.
 - ii. Add 1.8047 g of potassium nitrate.
 - iii. Swirl to dissolve.
 - iv. Fill to the 250 ml mark with milliQ water.
 - v. Store in the refrigerator. Make fresh monthly.
 - b. Working secondary stock (10 mg/l of nitrate-N) -
 - i. Add 0.5 ml of primary stock to a 50 ml flask.
 - ii. Fill to the 50 ml mark with milliQ water. Make fresh daily.
 - c. Working Standards: See Table 1.

Table 1.

Nitrate - N	Vol NOx secondary	Total volume
-------------	-------------------	--------------

(mg/l)	stock (ml)	(ml)
0.25	1.25	50
0.10	0.5	50
0.05	0.25	50
0.025	0.125	50
0.005	0.025	50
0	0	50

Table 1. Working calibration standards.

5. **LCS:** Use the Hach (Part # 1279249) premade nitrate standard to verify the laboratory standards.
 - a. Make a 1:10 dilution of the 1000 mg-N/l Hach standard in a volumetric flask (5 ml of Hach #1279249 in a total of 50 ml). Other concentration may be used but must be between 2 - 10 times the MDL.
 - b. If the measured concentrations are not between 90% and 110% of the certified concentration, the results will be considered unacceptable, and the source of error will then be determined and fixed before samples are analyzed.

Calibration and standardization:

- The instrument is calibrated before analyzing samples.
 - Analyze at least two samples of milliQ water to flush and equilibrate the system before analyzing standards.
 - Analyze The working (CAL) standard series.
 - Analyze two laboratory blanks.
 - Analyze the LCS after the CAL standards to confirm the accuracy of the CAL standards.
 - Then analyze at least two of the CAL standards a second time to ensure that the readings are stable.
 - If the LCS is within 10% of certified concentration and the second set of CAL standards is within 10% of the first set, then samples may be run.

- If LCS is not within 10% of certified value:
 - Compare CAL standard peak areas between the first and second set.
 - If CAL standard sets vary by more than 10%, find source of error and mitigate.
 - If CAL standards vary by less than 10%, rerun LCS.
 - If LCS variability remains at more than 10%, remake LCS and rerun.
- Analyze a set of LB and LCS standards for every ten samples in a batch of samples. If the LCS samples vary more than 10% from the mean values, then a new set of CAL standards should be analyzed.
- The lowest CAL standard should be measured to within 40% of the true value.
 - If the lowest concentration CAL standard is not within 40% of the true value, stop the run and start the troubleshooting process.
- If the cadmium column has been used to reduce more than 100 samples or if the instrument response is less than expected for a given concentration of analyte, then a 250 microgram/l sodium nitrite standard should be analyzed to verify the efficiency of the cadmium column. More detail can be found in the “Quality Control” section of this SOP.

NOx Colorimetric Analytical Procedure:

The NOx manifold is installed on Channel 4 on the MWQL QuikChem.

- If samples have been preserved with sulfuric acid, add NaOH to bring them to a pH of between 5-9. This process is time consuming. Be sure to allow adequate time to adjust pH before the sample analysis run.
 - Calibrate pH meter with pH 4, pH 7, and 10 buffers according to meter directions.
 - Using an acid cleaned 10 ml pipet tip, take a 10 -20 ml subsample of the preserved sample, and put into a clean small beaker
 - Add a small stir bar to the beaker
 - Put the beaker on the stir plate.
 - Turn on the stir plate.
 - Thoroughly rinse pH probe.
 - Put the pH probe in the clamp of a ring stand and adjust height so that the pipet tip is below the surface of the sample.
 - Measure and record the pH, being careful that the spinning stir bar doesn't hit the pH probe.
 - Add a small amount of 0.5- 2N sodium hydroxide to the subsample, measure the pH.
 - Continue until the pH of the subsample is between 5 and 9.

- Record the normality of the NaOH and the total volume of NaOH required to bring the sample to the proper pH.
- Calculate the proper amount of NaOH to add to the remaining sample.
 - $V_{NaOH}(ml) = \text{Volume in ml of NaOH needed to bring a X ml volume of sample to pH between 5 and 9.}$
 - $VS(ml) = \text{volume of sample remaining after 10-20 ml removed for test.}$
 - $\text{Volume NaOH to add to remaining sample} = (V_{NaOH}(ml) * VS(ml)) / \text{volum of test sample.}$
- Add the calculated amount of NaOH to the remaining sample.
- Check that the instrument NOx manifold detector filter is in place and the cell is properly seated.
 - If the filter is not in the slot, it will be found in the second Lachat instrument.
- Verify that the sample line is connected to the sample valve of the NOx channel.
- Verify that the reduction column valve is in the “off” position.
- Turn on power to autoanalyzer, autosampler, and pump.
- Turn on the computer.
- Open the Lachat software
- Open the NOx method or a run file from a previous NOx run.
- Enter the identifiers for the milliQ rinse, CAL standards, LCS and the LB in the sample run template.
- If pump tubing needs replacing, see chart in Appendix A for tubing reference.
- Put all reagent lines in a bottle of milliQ water.
- Put the channel effluent lines into the water collection bottle.
- Load the tubing into pump channels.
- Making sure that tabs on the pump cartridges are all the way to the left, push down on each cartridge to secure it to the pump. Each side of the cartridge will make a “click” as it locks in.
- Tighten pressure controls on pump cartridges by pushing tab all the way to the right and then back to the left until it clicks in place.
- Set the pump speed to 35.
- Start the pump.
- Check for leaks and other problems.
- Verify that water is flowing through all reagent tubing.
- Reduce pump speed to less than 5.
- Pump milliQ water through lines until baseline is stable, at least a half hour.
- Remove manifold waste lines from the water collection bottle and put them into the chemical waste carboy.
 - Make sure that the end of the waste tubing is above the waste level.
 - Cover opening with parafilm.
 - Set cap on top of carboy to prevent loss.
- Cut parafilm to cover all reagent bottles.
- Remove intakes from water and put them in corresponding reagent bottles, covering each with parafilm as you proceed. Note to switch lines to water following the diagram in Appendix A

starting at the bottom of the diagram and moving upward. When removing lines from reagent, start at the top of the diagram and go downward.

- Set up a run containing the following.
 - At least two samples of milliQ water which will be used to give the system a final rinse before analysis.
 - The CAL standards.
 - A LCS and a LB immediately following the CAL standards.
 - A second set of at least two of the CAL standards to verify instrument stability.
 - Put rinse tubes, blanks, and standards in the autosampler rack.
- Let reagents run through manifolds for at least 10 minutes.
- After 10 minutes turn the cadmium reduction column to the “on” position.
- Use the “preview” function of the software to monitor the baseline.
- When the baseline appears flat and stable, start the standard run.
- Graph the response for CAL standards to check linearity of instrument response.
- If the r squared value is not above 0.98, the instrument may not have stabilized; rerun the standards.
- Use these curves to test instrument function and set the integration parameters for the analysis.
- Calculate the concentration of the LCS from the linear regression of the CAL standards.
- If the LCS recovery deviates more than 10% from certified value, resolve before proceeding sample run.
- Once standard curve, blanks and certified standards are finished and determined to be within QC parameters, samples can be analyzed.
 - The sample run must contain:
 - An LCS and a LB for every 10 samples.
 - One LFSM for every batch of 20 samples.
 - One replicate sample for every 10 samples.
- Once analysis is finished, close the valve to the cadmium column.
- Switch reagent lines to water starting with the sulfanilamide, then the buffer.
- Rinse lines with milliQ water for at least 15 minutes.
- When finished rinsing, stop pump and release tubing from pump cartridges.
- Remove ends of tubing from water.
- Remove waste lines from the hazardous waste container and put them into the water collection bottle.
- Cap waste container.
- Release trigger on pump manifolds.
- Remove pump tubing from pump cartridges.
- Turn off autosampler, flow injection analyzer, pump, and computer.

Data Analysis and Calculations:

The spectrophotometric detector for NO_x forms peaks of voltage response which are integrated within the software to provide the peak area of the response. The parameters for integration are set for each

run based on the standard and blank analysis. This peak area is directly proportional to analyte concentration.

CAL - Standard concentration and peak areas are graphed with a scatter plot in Excel or another graphing program. A linear regression of the data is calculated. The r-squared value of the standard curve must be above .98 for use in this method. If the r-squared value falls below 0.98, then the instrument will be checked for malfunction and the standards will be run again. The linear regression is used to calculate the concentration of the analytes in the samples.

Low CAL Standard - The lowest CAL standard should be equal to or lower in concentration than the current RL. The measured value should be within 40% of the actual value.

Accuracy - The QCCS or LCS concentration is calculated using the linear regression obtained from the CAL standard regression. The accuracy of the CAL standards is calculated as follows:

$$(\text{ABS}(\text{QCCS/LCS certified concentration} - \text{QCCS/LCS measured concentration}) / \text{QCCS/LCS certified concentration}) \times 100.$$

Precision - The percent error for replicate samples or standards (including FD, LD, QCCS, LCS or CAL standards) is calculated using the absolute value of the difference between each of the duplicates using the following equation:

$$\text{Variability}_{\text{Rep}} = (\text{Error} / \text{Sample Mean}) \times 100,$$

$$\text{Error} = (\text{ABS}(\text{Rep1} - \text{Rep2})) / 2$$

Percent Recovery:

The percent recovery of the replicate spiked samples (LFSM) is calculated using the following equations:

$$\% \text{ Spike Recovery} = \text{Mean}(\text{Recovery}_1, \text{Recovery}_2)$$

$$\text{Recovery}_1 = ((\text{Analyte}_{\text{Spike1}} - \text{Analyte}_{\text{S1}}) / \text{Calculated concentration of spike}) \times 100.$$

$$\text{Recovery}_2 = ((\text{Analyte}_{\text{Spike2}} - \text{Analyte}_{\text{S2}}) / \text{Calculated concentration of spike}) \times 100.$$

$$\text{Analyte}_{\text{S1}} = \text{Measured concentration of sample 1.}$$

$$\text{Analyte}_{\text{Spike1}} = \text{Measured concentration of sample1 spike.}$$

$$\text{Analyte}_{\text{S2}} = \text{Measured concentration of sample 2.}$$

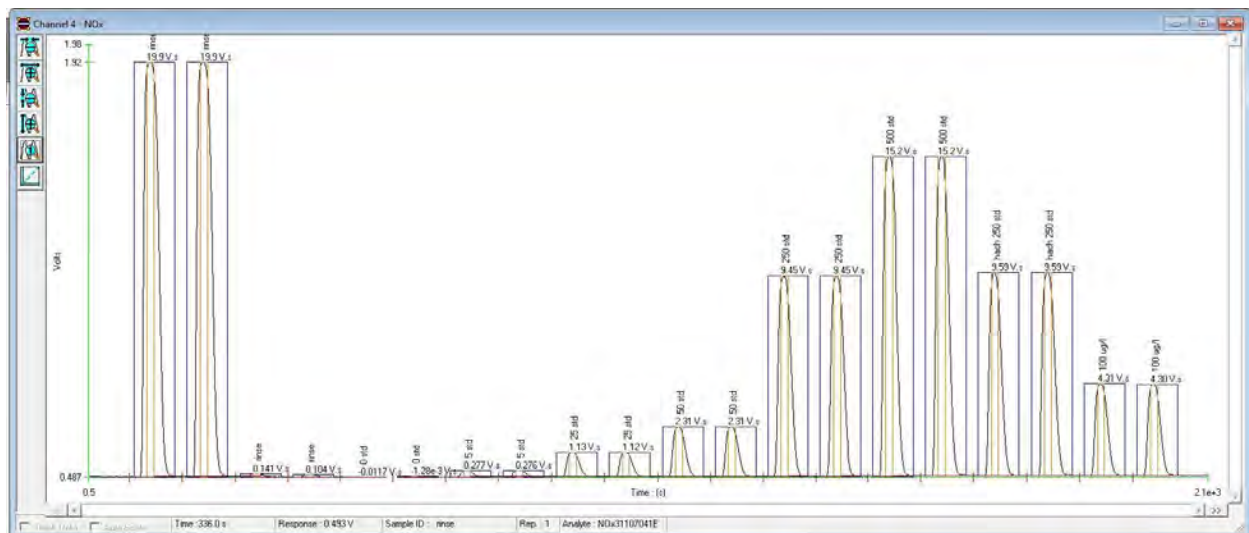
$$\text{Analyte}_{\text{Spike2}} = \text{Measured concentration of sample 2 spike.}$$

Minimum Detection Limit:

The minimum detection limit is calculated from the error of seven blanks or 7 low concentration standards, whichever is largest. See quality control section above for more details.

The minimum detection limit is calculated as follows:

- ## Method Performance:



CAL Standards	Mean	Range
NOx (mg/l)	Peak Area	Peak Area
0.250	17.3	0.000
0.100	7.61	0.010
0.050	3.91	0.010
0.025	1.99	0.010
0.005	0.42	0.006

0.000	-0.01	0.002
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Table 2. 1/17/2020 NOx Standard Calibration Series using CAL standards.

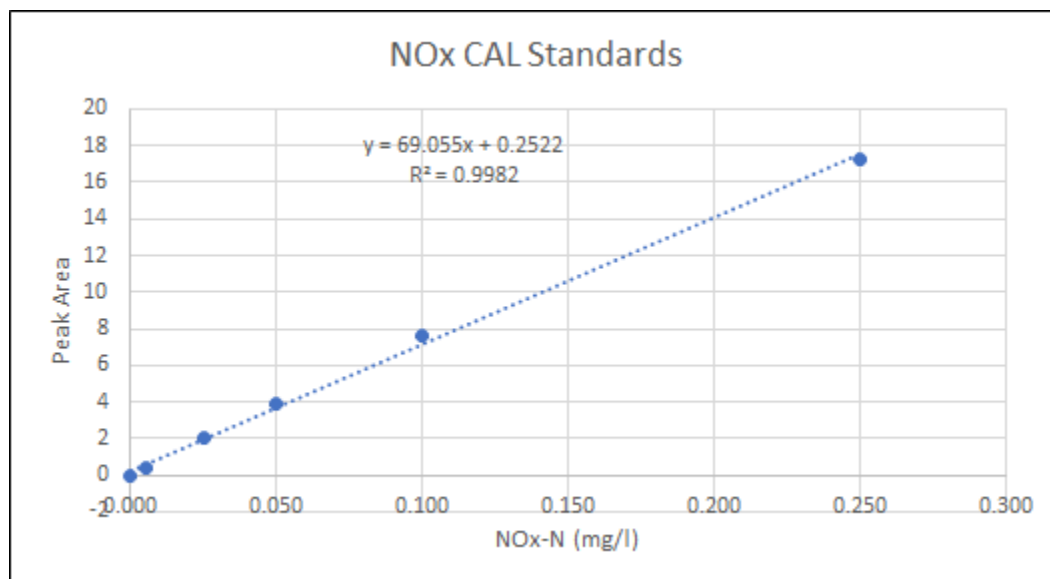


Figure 2. Graph of 1/17/2020 NOx CAL standards. Error bars are the range of duplicate standards.

CAL Standards			Mean
NOx (mg/l)	Peak Area	Peak Area	Peak Area
0.400	14.300	14.3	14.300
0.250	9.790	9.790	9.790
0.100	4.450	4.450	4.450
0.025	1.170	1.170	1.170
0.005	0.283	0.280	0.282
0.000	-0.005	-0.005	-0.005

Table3. NOx - Linear Range of CAL Standards -11/19/2019.

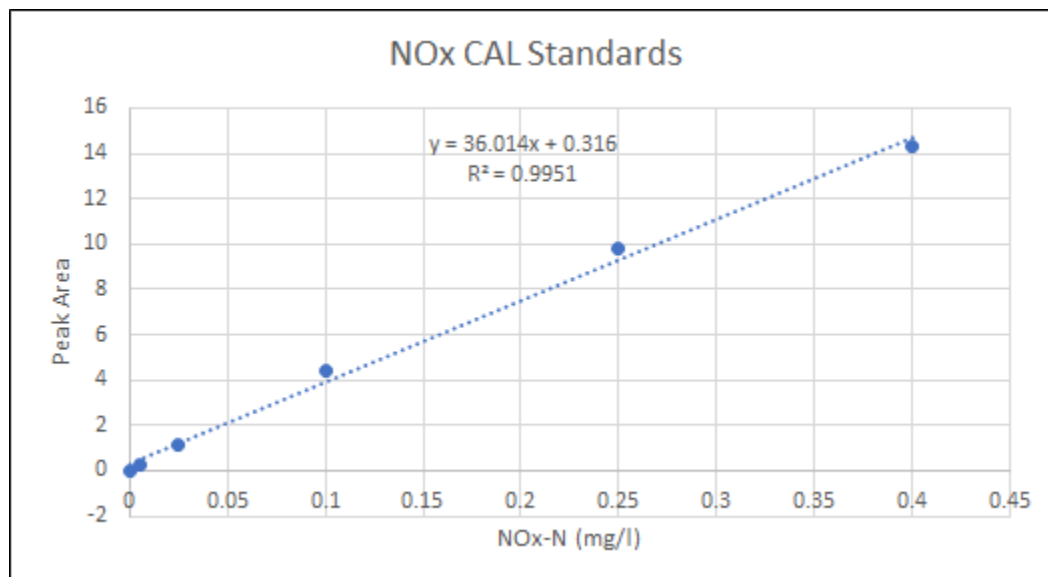


Figure 3. Linear range of NOx CAL standards 11/19/2019.

Corrective Actions:

General precision problems:

If baseline drifts, peaks are too wide, or peaks are atypical in shape (see Figure 1, for typical peak shape):

- Check that there is not an obstruction in the tubing that is causing pressure buildup and retarding the flow of the carrier.
 - The cadmium column is a common source of obstruction.
 - It is important to not let air get into the cadmium column.
 - When flushing column always keep buffer flowing through the column.
 - Check the cadmium column switching valve and the column fittings for obstructions or crimped tubing.
 - Check flow of reagents. 'If reagent is not flowing properly:
 - Replace pump tubing.
 - Check all fittings for obstructions or crimping tubing.
 - Check waste tubing for crimps or obstructions.
- Check sulfanilamide reagent for precipitate in the bottom of the bottle. If there is a precipitate, solution should be filtered into a clean dark bottle using a 0.22-micron filter.
- If the above actions do not alleviate the problem, clean the manifold by the following procedure:
 - Switch off the cadmium column valve.
 - Place transmission lines in water and pump to clear reagents (2-5 minutes).
- Place reagent lines in 1 M hydrochloric acid (1 volume of HCl added

to 11 volumes of water) and pump for several minutes.

- Place all transmission lines in water and pump for several minutes.
- Resume pumping reagents.

Poor instrument response:

- Remake CAL standards.
- If sensitivity issues result from excessive bubbles forming in tubing:
 - Check that reagents are at room temperature. If not, warm reagents in tepid water bath and mix.
 - Degas reagents.
 - Check intake tubing in bottles for obstructions.
- If the peak heights are not within 25% of the heights achieved for the previous analytical run for similar concentrations:
 - The cadmium column is decreasing in sensitivity.
 - If the CAL standard variability is less than 10% and the LCS accuracy is within the 10% margin of error, you may continue the run.
 - Analyze a set of at least 3 LB and 3 replicates of a low CAL standard (within 2-10 times the previous MDL) to ensure that the sensitivity has not decreased.
 - Analyze two concentrations of sodium nitrite that are within the range of the CAL standards to characterize the reduction efficiency of the cadmium column.
 - If sensitivity has decreased, report new MDL with batch results.
 - Replace cadmium column before the next analytical run.
 - As noted above, the cadmium column can become plugged.
 - If this happens, remove the downstream fitting, and run reagent through into a beaker to flush the column.
 - If the column is still plugged, reverse the column flow and flush a second time.
 - If the calibration fails consistently, replace the column.

Poor precision:

- If replicate digested standards or sample analytical replicates vary by more than 10% from mean values:
 - Check pump tubing.
 - Check back pressure tubing for leaks.
 - Check for bubbles.
 - Let the instrument equilibrate longer (inadequate equilibration can cause variability).

- Check for blockages in lines.
- If precision of analytical replicates is still unacceptable, after implementing the above remedies, replace the cadmium column.
- If cadmium column replacement does not improve signal, remake reagents.
- If precision of analytical replicates of CAL standards are within 10% of the mean, but LCS values vary more than 10% from certified values:
 - Remake CAL standards.
 - Remake LCS diluted standards.
 - Rerun both CAL and LCS.
- If analytical replicates vary by more than 10% but not by more than 20%, continue run, recording out of limit QC parameters.
 - If replicates vary by more than 20% stop run.
 - Remedy problems before running samples.

Pollution Prevention and waste management:

All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines. Waste will be stored in properly labeled containers with secondary containment. Waste will be located in approved areas and will be inspected weekly. When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus. At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste, is transferred to the DMC safety officer. Waste will be stored until pickup by a University of Maine approved waste disposal company. All MWQL staff who handle waste will be required to complete the Hazardous Waste training provided by the University of Maine.

The MWQL is committed to reducing chemical waste and will maintain the lowest volume of chemical stocks that is practical while maintaining adequate stock to guard against shortages and back orders. All expired chemicals will be disposed of as chemical waste and transferred to the DMC waste storage facility. The lab does not dispose of any chemicals down the drain. The spent cadmium columns are treated as hazardous waste and are disposed of as such when no longer useful.

Data Assessment and Acceptance Criteria:

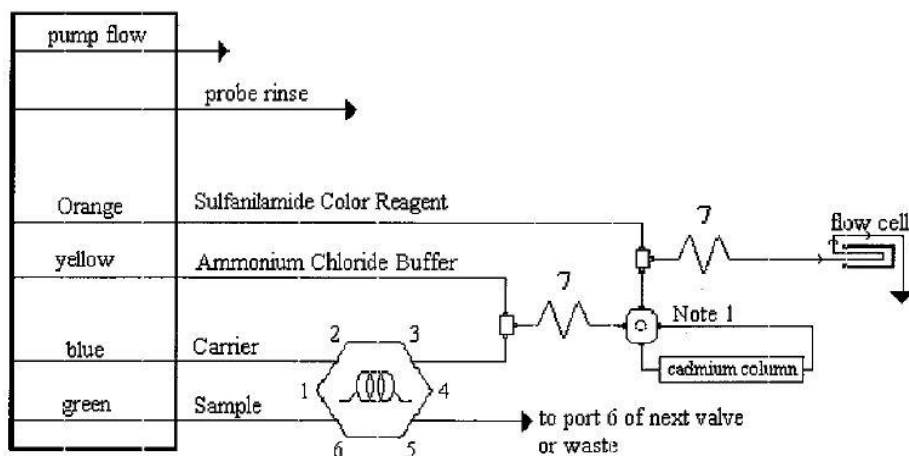
Data Acceptance Specifications:

- LCS - 90% - 110% of certified value.
- LB - Below MDL.
- LD - 90% - 110% of mean value.
- LFSM - Spike Recovery - 80% - 120%.

- **Linearity - Linear regression of CAL standards - > 0.98.**
- **Low CAL standard at or below the RL - Must be within 40% of actual value.**
- **Reproducibility of CAL standards over the course of a batch of samples - 90% - 110%. If CAL standard values are outside of this limit, a new set of CAL standards must be analyzed.**

Data Assessment Check List:

- **Were the samples analyzed within the required holding time (28 days)?**
- **Were the samples preserved properly?**
- **Was the pH raised to between 5 and 9 before analysis?**
- **Were the samples properly stored between sampling and analysis?**
- **Was this protocol followed as written?**
 - **If not, is there a record of variance from the method and documentation explaining the variance?**
- **Are there at least 4 concentrations of CAL standards for each sample batch?**
- **Were at least 2 CAL standards re-analyzed immediately after the initial CAL standard series to document method stability?**
- **Is each standard concentration analyzed in duplicate?**
- **Is the r squared value of the linear regression of the mean CAL standard response versus concentration greater than 0.98?**
- **Are the peak heights within 25% of the heights achieved for the previous analytical run for similar concentrations?**
 - **If not, were further LB and CAL standards analyzed to reassess sensitivity?**
- **Is the measured concentration of the LCS within 10% of the certified value?**
- **Is the LB less than the MDL?**
- **Was an LB and LCS analyzed for every 10 samples?**
- **Are all of the LB and LCS samples within acceptance criteria?**
 - **If not, is there documentation to explain?**
- **Was a duplicate sample analyzed for every 10 samples?**
- **Was the precision of the duplicate samples within 90% - 110%?**
 - **If not, is there documentation to explain?**
- **Was one LFSM analyzed for every 10 samples?**
 - **If not, is there documentation to explain?**
- **Is there a properly documented NOx analysis log (NOXAL)?**
- **Is there a proper chain of custody?**

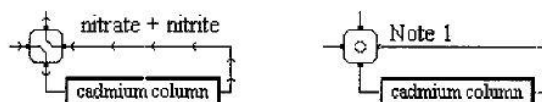


Carrier: DI water
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 $\mu\text{L}/\text{cm}$.
AE Sample Loop: 150 cm x 0.042 in i.d.
QC8000 Sample Loop: 150 cm x 0.042 in. i.d
Interference Filter: 540 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

7: 135 cm of tubing on a 7 cm coil support

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.



Appendix C

MWQL Total Kjeldahl Nitrogen (TKN) and Total Phosphorus (TP) Standard
Operating Procedure (SOP)

Document Code: MWQLSOPTKNTP Rev. 5, 2/18/21

EPA Reference: TKN: EPA 351. 2 /TP: EPA 365.4

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SPECIFICATIONS

This method is applicable to marine and freshwater samples.

Applicable concentration ranges:

TKN working range: RL - 5.0 mg N/l

TP working range: RL - 5.0 mg N/l

DEFINITIONS

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

ANALYST -- The designated individual who performs the “hands-on” analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality.

ANALYTICAL BATCH -- An analytical batch is a group of environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch is composed of at least one and no more than 20 environmental sample(s) of the same quality systems matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various quality system matrices and can exceed 20 samples, unless the method requirements are more stringent.

BIAS -- The systematic or persistent distortion of a measurement process, which causes errors in one direction (e.g., the expected sample measurement is different from the sample’s true value).

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values

realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).

In calibration according to methods, the values realized by standards are typically established through the use of reference materials that are either purchased by the laboratory with a certificate of analysis or purity or prepared by the laboratory using support equipment that has been calibrated or verified to meet specifications.

CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

CERTIFIED REFERENCE MATERIAL -- (CRM) means reference material, accompanied by a certificate, having a value, measurement of uncertainty and stated metrological traceability chain to a national metrology institute

CHAIN OF CUSTODY FORM (COC) -- A record, either paper-based or electronic, that documents the possession of the samples from the time of collection to receipt in the laboratory in accordance with chain-of-custody protocol. This record, at a minimum, must include the sample location, the number and types of containers, the mode of collection, the collector, the date and time of collection, preservation, and requested analyses. See also Legal Chain of Custody Protocols.

FIELD BLANK (FB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

INSTRUMENT BLANK -- A clean sample processed through the instrumental steps of the measurement process and used to determine instrument contamination.

LABORATORY BLANK (LB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the

laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibration standard).

LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)-- An environmental sample containing an analyte spike of known concentration at least 4 times the MDL. Used to evaluate the effect of the sample matrix on the analytical results.

LIMIT OF QUANTITATION (LOQ) -- The minimum levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence.

MINIMUM DETECTION LIMIT (MDL), also known as the Limit of Detection -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0 .

QUALITY CONTROL CHECK SAMPLE (QCCS) – Also known as a proficiency sample. A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

SENSITIVITY -- The capability of a method or instrument to discriminate between measurement responses representing different levels (e.g., concentrations) of a variable of interest.

QUALITY CONTROL MEASURES:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in this QAM. QA and QC procedures specific to this protocol are documented in this SOP.

Initial Demonstration of performance:

- Method Detection Limit (MDL) - The minimum detection limit will be determined by analyzing blank variability and by spiking a blank with analyte at a concentration from 2 to 10 times the estimated MDL. Seven blanks and seven MDL spikes will be carried through all aspects of preparation and analysis in a minimum of three separate sample batches on three separate calendar dates. The reported MDL will be the larger of the MDL calculated from the blanks and the MDL calculated from the spiked matrix samples.
 - To determine the MDL of the spiked matrix samples:
 - Calculate the standard deviation of the 7 spikes.
 - Multiply the standard deviation of the spiked matrix by 3.143 (for seven replicates).
 - To determine the MDL of the blanks:
 - Calculate the mean of the method blanks.
 - If the mean is negative equate it to zero.
 - Multiply the standard deviation of the blanks by 3.143 (for seven replicates) and add the result to the mean.
- Limit of Quantitation – Also known as the Reporting Limit (RL). Obtain the limit of quantitation by calculating the mean and standard deviation of replicate standards between 2 - 10 times the estimated or known MDL. Multiply the standard deviation by the t value obtained from the t test table for the replicate degrees of freedom and the desired cumulative probability based on a two tailed test. For instance, the 99% cumulative probability.
- Quality Control Check Sample - Semi-annually, a QCCS will be ordered from an EPA recommended provider lab. The sample will be analyzed, and results sent to the provider

lab. If results are not within the acceptable limits of stated values, the source of the problem will be identified and corrected.

- Demonstration of Linearity - A standard curve over the working range of the method will be run at least once per year to demonstrate linearity.

Ongoing quality control measures:

- Duplicate Lab Reagent Blanks - Duplicate lab reagent blanks will be analyzed with each batch of samples.
- Laboratory Control Standard - Duplicate LCSs from a second source, different from the CAL standards, are analyzed with each batch of samples, no less than one for every ten samples to determine the accuracy of the analytical protocol. If the measured analyte concentration varies by more than 10% from the certified concentration, the analysis will stop, and the source of error will be determined before sample analysis resumes.
- Calibration Standards - A set of CAL, covering a minimum of 4 concentrations that span the expected sample concentrations, will be analyzed with each batch of samples
- Laboratory Fortified Sample Matrix - 10% of the analyzed samples will be spiked with analyte to give a concentration at least four times the MDL prior to digestion. The percent recovery will be determined from these samples. If the percent recovery falls outside to the 80%-120% range, analysis will stop, and the source of the error will be determined and resolved before continuing analysis.
- Laboratory Duplicates - A set of LDs will be analyzed in each batch of samples. Duplicates should not vary more than 10%.
- Minimum Detection Limit must be recalculated every 6 months.

SUMMARY OF METHOD

Suitability of method and application:

This method is used to obtain TKN, which is composed of the ammonium in the sample plus organic nitrogen and to obtain the TP of the sample, from unfiltered liquid samples. The method is appropriate for fresh and seawater samples with a TP or TKN concentration up to 5 mg/l. Samples with higher concentrations of TP or TKN may be diluted, and the diluted sample analyzed using this protocol. The method consists of a digestion and subsequent analytical steps.

Digestion:

Using copper sulfate as a catalyst, liquid samples are heated in glass digestion tubes in the presence of concentrated sulfuric acid. Potassium sulfate is also incorporated to raise the boiling temperature of the digestion and therefore, to lessen the time required. Hangar crystals are added to each digestion tube to prevent splattering and boiling over of samples. During the digestion, the samples' phosphorus is converted to the orthophosphate (PO_4^{3-}) anion. Organic nitrogen compounds are converted to ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$, under the conditions of the digestion described. After the digestion period, the digestion tubes are removed from the digester and the residue is cooled. The digest is then diluted to its original volume with DI water and the sample is mixed to dissolve any precipitate.

TKN colorimetric analysis:

The TKN is analyzed using a Lachat Quickchem 8500 autoanalyzer. The digested sample is injected onto the chemistry manifold where its pH is raised to a known, basic pH, by in-line neutralization and maintained with a concentrated buffer. This inline neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. Ethylenediaminetetraacetic acid (EDTA) is added to the buffer to prevent

precipitation of calcium and magnesium in the lines of the instrument. The absorbance is measured at 660 nm and is proportional to the TKN in the sample.

TKN is the sum of the ammonium and the organic nitrogen in the sample.

Organic nitrogen concentration is obtained by subtracting the free-ammonia concentration from the TKN concentration. The total nitrogen in the samples can be obtained by adding the TKN and the nitrate plus nitrite (NO_x) concentrations.

TP colorimetric analysis:

The total phosphorus is analyzed using the Lachat 8500 Quickchem autoanalyzer. The orthophosphate produced in the digestion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is subsequently reduced with ascorbic acid to form a blue complex. The absorbance of this complex is measured at 880 nm and is proportional to the orthophosphate in the sample.

INTERFERENCES

- Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a small range of H₂SO₄ concentrations in the digested, diluted samples with no change in signal intensity.
- Digests must be free of turbidity. Digested samples may be filtered if turbid.
- Some boiling stones have been shown to crumble upon vigorous vortexing. Care must be taken to avoid collecting boiling stone pieces when pouring samples into 50 ml tubes.

SAFETY

Hazards:

Hazards include hazardous chemicals, high temperature surfaces, boiling acidic solutions. All laboratory personnel using this protocol must be current with all University of Maine safety training requirements.

Hazardous chemicals: Read SDS sheet for all chemicals before use. Wear appropriate personal protective equipment including appropriate gloves, lab coat and safety glasses. The following list highlights some of the hazards of the listed chemicals but IS NOT intended to replace thorough reading of the SDS sheets by laboratory personnel.

- Sodium nitroprusside (CAS#: 13755-38-9). Warning! Toxic if swallowed, inhaled, or absorbed through the skin. Contact with acids liberates hydrogen cyanide, a very toxic, flammable gas or liquid. May cause eye and skin irritation. May cause respiratory tract irritation. Hygroscopic (absorbs moisture from the air).
- Sulfuric acid (CAS#: 7664-93-9) - Warning! Toxic if swallowed, inhaled, or absorbed through the skin. Caustic! May cause severe burns and eye injury.
- Sodium hypochlorite (CAS#: 7681-52-9).
- Sodium hydroxide (CAS#: 1310-73-2). Harmful in contact with skin. Causes severe skin burns and serious eye damage. May be corrosive to metals.
- Copper sulfate (CAS#: 7758-99-8). Toxic.
- Ammonium molybdate (CAS#: 7758-99-8). Irritant.
- Antimony potassium tartrate (CAS#: 331753-56-1). Toxic.

Splash hazard - digester. Wear splash and impact protective goggles, lab coat and appropriate gloves when using the digester.

High temperature hazard - digester. Do not touch digester block when in use. Use caution removing tube tray from the digester block.

Possibility of breaking glass - digester. Wear splash and impact protective goggles, lab coat and appropriate gloves when using the digester.

Hazardous solutions under pressure - Lachat autoanalyzer. Solutions pumping through Lachat tubing are under pressure and may spray reagent if a leak forms. Wear safety glasses and a lab coat.

EQUIPMENT AND SUPPLIES

40 - 50 ml conical tubes (50 ml conical screw cap HDPE centrifuge tubes)

40 - Digest tubes (Lachat instruments 50 ml open-top digestion tubes)

40 - Cold fingers (Lachat instruments)

20 - Acid cleaned sample collection bottles.

2 - Milli-Q squirt bottle

250 ml graduated cylinder

500 ml graduated cylinder

100 ml graduated cylinder

10 ml pipette and tip

1-liter volumetric flasks

250 ml volumetric flasks for standards

Stir plate

Analytical Balance

Lachat QuickChem flow injection analyzer with autosampler and heaters.

Lachat Block Digestor

Vortex Mixer

Glass and plasticware preparation:

All glassware and plasticware in contact with samples will be:

- Washed with Sparkleen detergent and rinsed 5 times with milliQ water.
- Acid rinsed using 0.5 M - 1.5 M hydrochloric acid solution and rinsed thoroughly with milliQ water.

SAMPLE COLLECTION AND PRESERVATION:

See QAM for sample acceptance criteria. Samples (100 – 250 ml) are collected in clean, acid rinsed plastic bottles or tubes (see above). Samplers must wear gloves and be careful not to touch any unclean surfaces with gloves before or during sampling. Samples may be collected from Niskin bottles or may be directly collected from river, stream, estuary, or other water source. If possible, rinse bottle with sample:

- Bottles must be filled with a volume of sample sufficient to rinse the bottle (for a 100 ml bottle 10-20 ml is adequate).
- Collect a small amount of sample, cap bottle, invert, and swirl sample in bottle to rinse the entire inner surface of the bottle.
- Dispose of rinse water downstream of the sampling site.
- Rinse 3 times, then fill the bottle and cap.

If acid preservative has been added to bottles prior to sampling, do not rise bottle.

Fill bottle Immediately store samples in the cooler.

Collected samples must be kept in the dark on ice until delivery to the laboratory.

If samples are not preserved in the field, they must be preserved upon arrival at the laboratory.

Samples are preserved by adding sufficient sulfuric acid to lower the pH to < 2.0 within 8 hours of collection (about 0.4 ml per 100 ml of sample).

Check the pH of at least 2 samples per batch to determine that pH is lower than 2 after acid addition.

Sample Storage:

Samples should be analyzed as soon as possible after collection. Preserved samples may be stored at <6°C in a refrigerator for up to 28 days.

REAGENTS AND STANDARDS

1. Digestion solution (We use the copper-based digestion solution in order to avoid the use of highly toxic mercuric chloride):

- a. Add 800 ml of ammonium-free milliQ (resistivity greater than 18.0) water to a 1-liter volumetric flask.
- b. Add 134 g of potassium sulfate (K_2SO_4).
- c. Add 7.3 g copper sulfate ($CuSO_4$).
- d. In hood, measure 134 ml of concentrated sulfuric acid using a graduated cylinder.
- e. Add sulfuric acid to flask. Caution! Solution will get hot!

- f. Loosely, place the stopper on the flask and stir on the stir plate until the solution cools to room temperature.
 - g. Do not invert the flask to mix.
 - h. When cool dilute to the 1-liter mark.
- 2. **Hypochlorite solution (0.315%):** For TKN analysis.
 - a. Use Lachat premade sodium hypochlorite solution (0.315%, part # 52011) or dilute Hach sodium hypochlorite (5%, part # 52007) 15 ml in total of 250 ml. Alternatively, a solution can be made from any pure hypochlorite solution of greater concentration.
- 3. **Salicylate nitroprusside reagent:** For TKN Analysis.
 - a. Add 800 ml of milliQ water to a 1-liter flask. Add 150 g of sodium salicylate (Salicylic acid, sodium salt- $C_6H_4(OH)(COO)Na$).
 - b. Add 1 g of sodium nitroprusside dihydrate ($Na_2Fe(CN)_5NO \cdot 2H_2O$).
 - c. Invert to mix.
 - d. Store in a dark bottle.
 - e. Prepare fresh monthly.
- 4. **Buffer:** For TKN Analysis.
 - a. Add 900 ml of milliQ water to a 1-liter flask. Add 35 g of sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) to the flask and stir to dissolve.
 - b. Add 20 g disodium EDTA. This will not dissolve until the sodium hydroxide is added.
 - c. Add 50 g sodium hydroxide.
 - d. Stir or shake until dissolved. Solution will be hot!
 - e. Dilute to 1 liter.
 - f. Degas weekly and prepare fresh monthly.
- 5. **Sodium hydroxide (0.8M).**
 - a. Use Hach solution #52008 (0.8 M NaOH) or to make this solution in the laboratory:
 - i. Add 800 ml of milliQ water to a 1-liter flask.
 - ii. Add 32 g of sodium hydroxide.
 - iii. Stir to mix.
 - iv. Dilute to 1 liter mark.
- 6. **Digestion diluent:** Carrier for TKN and used for non-digested standards— same acidity as digested sample.
 - a. Add 400 ml of digestion solution (Solution #1) to a 1-liter flask.
 - b. Bring to 1 liter with milliQ water.
- 7. **Stock ammonium molybdate solution: May be stored in the refrigerator for 2 months.** For TP Analysis.
 - a. Add 800 ml of milliQ water to a 1-liter flask.
 - b. Add 40 g of ammonium molybdate tetrahydrate.
 - c. Stir for four hours.

- d. Dilute to 1 liter
 - e. Solution is stable for 2 months
- 8. Stock Antimony Potassium Tartrate Solution: May be stored in refrigerator for 2 months.**
- a. Add 800 ml of milliQ water to a 1-liter flask.
 - b. Add 3.0 g of antimony potassium tartrate hemihydrate
 - c. Dilute to the mark and stir with a magnetic stirrer.
 - d. Store in a dark bottle and refrigerate.
 - e. Solution is stable for 2 months.
- 9. Molybdate Color Reagent: Discard if turns blue or a yellow precipitate forms. For TP Analysis**
- a. Add 500 ml of milliQ water to a 1-liter flask.
 - b. Add 35 ml of concentrated sulfuric acid. (Caution solution will be hot!)
 - c. Add 213 ml of the stock ammonium molybdate solution.
 - d. Add 72 ml of the antimony potassium tartrate solution.
 - e. Dilute to the mark and stir to mix.
 - f. Degas with helium or let sit for at least 24 hours.
 - g. Prepare fresh weekly or when reagent turns blue.
- 10. Ascorbic Acid Reducing Solution (0.33 M). Prepare fresh weekly Discard if solution turns yellow.**
- Do not degas.** For TP Analysis.
- a. Add 700 ml of milliQ water to a 1-liter volumetric flask.
 - b. Add 60 g of ascorbic acid to flask.
 - c. Mix.
 - d. 1.0 g of sodium dodecyl sulfate (SDS).
 - e. Dilute to the 1-liter mark with milliQ water.
- 11. 1 M Hydrochloric Acid - Used to clean tubing when needed.**
- a. In fume hood, add 200 ml of milliQ water to a 250 ml glass bottle.
 - b. Add 21 ml of concentrated hydrochloric acid.
 - c. Fill to the 250 ml mark with milliQ water.
- 12. 50% Sulfuric Acid for preserving samples:**
- a. Add 40 ml of milliQ water to a 100 ml reagent bottle.
 - b. Add 50 ml of concentrated sulfuric acid.
 - c. Allow to cool to the touch.
 - d. Pour into Class A 100 ml volumetric flask.
 - e. Bring to 100 ml with milliQ water.
 - f. Pour back into reagent bottle and cap.
- 13. Sulfuric Acid (11 N): For TP Analysis.**
- a. Add 600 ml of milliQ water to a 1-liter volumetric flask.
 - b. Add 305 ml of concentrated sulfuric acid.
 - c. Mix by swirling and allow to cool.
 - d. Bring to 1 liter.
- 14. Sulfuric Acid (0.231 M): For TP Analysis.**

- a. Add 500 ml of milliQ water to a 1-liter volumetric flask.
 - b. Add 42 ml of 11 N sulfuric acid solution.
 - c. Bring to 1 liter and invert to mix.
 - d. Prepare fresh weekly.
15. **Primary Standards: Note that standards must be acidified in the same manner as preserved samples before digestion.**
- a. TKN: Primary stock (0.1909 g/liter ammonium chloride = 50 mg/l ammonium - N). Store in the fridge, make fresh monthly.
 - b. TKP: Primary stock (1.099 g/l anhydrous potassium dihydrogen phosphate (KH_2PO_4) in 1 liter = 250 mg/l P). Store in the fridge, make fresh monthly.

NH ₄ -N (mg/l) or PO ₄ -P (mg/l)	Vol. PO ₄ primary stock (ml)	Vol NH ₄ stock primary (ml)	Total volume (ml)
2	2	10	250
1	1.0	5	250
0.5	0.5	2.5	250
0.25	0.25	1.25	250
0.05(TP),0.1(TKN)	0.05	0.5	250
0	0	0	250

Table 1. Working TKN and TP Standards for digestion.

CALIBRATION AND STANDARDIZATION

Instrument function is tested before analyzing digested samples and standards, with blank reagent water and at least 2 concentrations of undigested CAL for each analyte. An undigested QCCS is also analyzed to confirm the accuracy of the laboratory made standards. This ensures the proper functioning of the instrument before the digested samples are analyzed. Each digestion and therefore, each analytical batch, contains a standard series, duplicate LCS and blanks. These are used to calibrate the instrument for the digested samples. More detail can be found in the “Quality Control” section of this SOP.

Procedure:

Digestion Procedure:

- Note: The following samples must be included in each digestion run:
 - Duplicate spiked (0.5 mg/l) samples.
 - Duplicate blanks (0 std).
 - One replicate sample for every 10 samples.
 - Standards in duplicate: 0 (blank), 0.05 (TP) or 0.1(TKN), 0.25, 0.5, 1, 2.0 mg/l standards (mixed N and P). Note: higher standards only need to be prepared if a sample is known to have higher than typical N or P. Samples with expected TKN or TP concentrations higher than 5 mg/l will require dilution.
 - Duplicate QCCS.
- **The following samples are periodically required (see Quality Control).**
 - Proof of capability, spike 0.5 mg/l to milliQ water for 4 reps.
 - Proficiency standards: Dilute 1:1000. Use this dilution as a sample. (100 microliters in 100 ml). Or follow other specific instructions from PT provider.
- Make up all reagents listed in the “Reagents” section of the SOP and the primary standards, no later than the day before analysis. If solutions will not be used the next day, store in the MWQL chemical refrigerator. If they will be used the next day, they may be stored at room temperature.
- Prepare all glassware, plasticware and check the status of all required equipment.
 - Change any pump tubing that looks worn or misshapen. Refer to the diagrams in Appendix A.
- On the day of the digestion, make up working standards listed in Table 1.

- Make a certified standard mix using the ICPMS standards which are 1000 mg/l ammonium - N (Sigma ICPMS std #89503) and (1000 mg/l PO₄-P (Sigma ICPMS std # 38338).
- Note: Some primary ICPMS stocks are 1000 mg/l - P and some are 1000 mg/l PO₄. See note below for volumes of each.
 - Make a stock standard mix (62.5 mg/l):
 - Using a 10 ml volumetric add the following volumes of stock:
 - 0.625 ml of the NH₄ stock.
 - 0.625 ml of the PO₄ stock if using primary with 1000 mg/l P. If using stock with 1000 mg/l PO₄, Add 1.917 ml of stock.
 - Bring to 10 ml with milliQ.
 - Add 200 microliters (0.2 ml) to each 25 ml sample for spike (500 ug/l).
 - Add 200 microliters (0.2 ml) to 25 ml milliQ water for QCCS.
- Note: Undigested CAL standards and LCS must be made up on the day of the analysis to verify that the instrument is in working order before analyzing the digested standards.
 - To make up the undigested LCS in diluent on the day of analysis:
 - Make up the secondary stock of ICPMS standards using the instructions above.
 - To make a 0.5 mg/l working standard, add 0.8 ml of the secondary stock to a 100 ml flask.
 - Fill the flask to the mark with diluent.
 - To make up the undigested CAL standards on the day of analysis:
 - To make 2 mg/l CAL Standard:
 - Add 4 ml of the ammonium primary stock to a 100 ml flask.
 - Add 0.8 ml of the phosphate primary stock to the flask.
 - Dilute to the make with diluent solution.
- Turn on hood ventilation.
- Verify sample preservation by measuring pH of at least one of the samples in the batch. pH should be 2 plus or minus 0.2 units.
- Use TKN/ TP digestion template (see appendix) to record the rack position of samples and standards in the digester rack.
- Pour or pipet, 25 mL of standard or sample into respective glass digestion tube (each tube has a fill line at the 25 ml mark).
- Place digestion tubes, uncovered, in the digest rack in the hood.
- Add 2 Hangar boiling chips to each tube.
- Once this is complete and all tubes have sample or standard and boiling chips, turn on the digester and start the program called TKN (H). It will take approximately 20 to 25 minutes for the block to reach 200 degrees C.
- Add 10 mL of Digest Reagent (Reagent #1) to each tube using 10 ml pipette (this will take less than 20 minutes).
- Once the block reaches 200 degrees C lower the rack with tubes into the heating block, the program will maintain that temperature for 70 minutes and then ramp up to 380 degrees C over the course of an hour.

- Place cold fingers on the tubes after the digest has been at 200 degrees C for 70 minutes. Don't wait too long as temperature ramps up to 380 degrees C. The digest will incubate for 60 minutes at 380 degrees C.
- Label acid cleaned 50 mL conical tubes with proper identification including batch #, date and sample location and sample ID.
- Once the digest is complete, carefully lift the rack of tubes out of the heating block and allow it to cool for approximately 5-8 minutes.
- Once cooled, using a squirt bottle, add Milli-Q water to the tube until the level is back 25mL. Vortex sample for at least 20 seconds. Note: the longer the samples sit after digestion, the more difficult it is to suspend the precipitate in the bottom of the tube. It is important that all precipitate is mixed into solution. It may take longer to vortex the last samples than the first samples to be removed from the rack.
 - It is important to start processing samples containing saltwater first as they tend to cool and form a precipitate that may require minutes of vortexing.
 - Work with one tube at a time by first taking off the cold finger and immediately bringing volume back to 25mL with a Milli-Q squirt bottle. Once the sample is vortexed and transferred to the appropriate tube, move on to the next sample.
- Pour the suspended sample into an acid-cleaned and labeled 50 ml disposable centrifuge tube.
- Note: if the digested sample is turbid, filter through a 0.2-micron syringe filter.
 - Filtering procedure for turbid samples.
 - Filter at least one ml of sample to waste to rinse the filter before collecting filtered digested sample.

TOTAL KJELDAHL NITROGEN AND TOTAL PHOSPHORUS ANALYSIS PROCEDURE

Analysis of TKN and TP digested samples.

TKN is Channel 2 on the MWQL Lachat QuikChem 8500 #1.

TKP is Channel 3 on the MWQL Lachat QuikChem 8500 #1.

Analytical steps:

- Turn on power to autoanalyzer, autosampler, and pump.
- Turn on the computer.
- Open the Lachat software
- Open the TKN/TP method or a run file from a previous TKN/TP run.
- Enter the identifiers for the standard curve and certified standards in the sample run template.
- Put all reagent intake lines in the dedicated bottle of milliQ water.
- Note: If pump tubing needs to be changed, refer to diagram in Appendix A.
- Put channel effluent lines into the water collection bottle.
- Make sure that the sample line is attached to the TKN sample valve.
- Load tubing into pump channels.
- Making sure that tabs on pump cartridges are all the way to the left, push down on the cartridge to secure. Each side of the cartridge will make a “click” as it locks in.
- Tighten pressure controls on pump cartridges by pushing tab all the way to the right and then back to the left until it clicks in place.
- Turn on the pump and set the speed to 35.
- Check for leaks and other problems.
- Reduce pump speed to less than 5.
- Pump milliQ water through lines until baseline is stable, at least a half hour.
- Remove manifold waste lines from the water collection bottle and put them into the chemical waste carboy, making sure that the end of the tubing is above the waste level. Cover opening tightly with parafilm. Set cap on top of carboy to prevent loss.
- Cut parafilm to cover all reagent bottles.
- Be sure to save subsampled a 50 ml Falcon tube of TKN diluent carrier to run as an analytical blank before putting reagent line in the carrier bottle.
- Starting with the line lowest on the diagram in Appendix A, remove intake lines from water and put them in corresponding reagent bottles, covering each with parafilm as you proceed. Do this for both TKN reagents and TKP reagents. Note: carrier for TKN is diluent solution, for TP it is milliQ water.
- Set up a run containing the following.

- Two samples of milliQ water which will be used to give the system a final rinse before analysis.
- At least 2 mixed calibration standards of 0.5 mg/l and 2 mg/l of both Ammonium -N and phosphate-P in diluent to test the method and ensure that the instrument is working properly.
- A diluent blank.
- A QCCS containing 0.5 mg/l of both Ammonium - N and phosphate - P made up in diluent.
- Put rinse tubes, blanks, and standards in the autosampler rack.
- Turn the speed back to 35.
- Start the "Preview" to look at the baseline.
- Once the baseline is stable, end the preview and start the sample run.
- Graph the response for the blank (0 mg/l), 0.5 mg/l and 2 mg/l of analyte to check linearity of instrument response.
- Use these curves to test instrument function and set the integration parameters for the analysis.
- Calculate the concentration of the laboratory control check standard from the equations produced.
- If standard curves are not linear or certified sample recovery deviates more than 10% from certified value, resolve before proceeding with digested sample run.
- Once standard curve, blanks and certified standards are finished and determined to be satisfactory, digested samples can be analyzed.
 - Note: Although we try to minimize contamination, the diluent does contain some P, this will show up in the blank for P in the digested blank.
- Analyze digested QC samples and standards first. If standards are not linear or digested QCCS measured concentration falls outside of accepted values, stop the analysis, and remedy the problem before analyzing environmental samples.
- If standards are satisfactory, analyze environmental samples.
- Once analysis is finished, return all lines to milliQ. Switch reagent lines to water starting with the reagent at the top of the Lachat manifold diagram and working downward.
- Rinse with milliQ water for at least 15 minutes.
- When finished rinsing, turn off the pump, release tubing from pump cartridges and remove tubing intakes from water.
- Remove waste lines from the hazardous waste container and put them into the water collection bottle.
- Cap waste container.

DATA ANALYSIS AND CALCULATION

The spectrophotometric detectors for TKN and TP form peaks of voltage response which are integrated within the software to provide the integrated peak area of the response. This peak area is directly proportional to analyte concentration. Note that all data calculations will consider any sample dilutions that may occur in the course of the analysis.

CAL - Standard concentration and peak areas are graphed with a scatter plot in Excel or another graphing program. A linear regression of the data is calculated. The r-squared value of the standard curve must be above .98 for use in this method. If the r-squared value falls below 0.98, then the instrument will be checked for malfunction and the standards will be run again. The linear regression is used to calculate the concentration of the analytes in the samples.

Accuracy - The QCCS or LCS concentration is calculated using the linear regression obtained from the CAL standard regression. The accuracy of the CAL standards is calculated as follows:

$$(\text{ABS}(\text{QCCS/LCS certified concentration} - \text{QCCS/LCS measured concentration}) / \text{QCCS/LCS certified concentration}) * 100$$

Precision - The percent error for replicate samples or standards (including FD, LD, QCCS, LCS or CAL standards) is calculated using the absolute value of the difference between each of the duplicate using the following equation:

$$\text{Variability}_{\text{Rep}} = (\text{Error} / \text{Sample Mean}) \times 100,$$

$$\text{Error} = (\text{ABS}(\text{Rep1} - \text{Rep2})) / 2$$

Percent Recovery:

The percent recovery of the replicate spiked samples (LFSM) is calculated using the following equations:

$$\% \text{ Spike Recovery} = \text{Mean}(\text{Recovery}_1, \text{Recovery}_2)$$

$$\text{Recovery}_1 = ((\text{Analyte}_{\text{Spike1}} - \text{Analyte}_{\text{S1}}) / \text{Calculated concentration of spike}) \times 100.$$

$$\text{Recovery}_2 = ((\text{Analyte}_{\text{Spike2}} - \text{Analyte}_{\text{S2}}) / \text{Calculated concentration of spike}) \times 100.$$

$$\text{Analyte}_{\text{S1}} = \text{Measured concentration of sample 1.}$$

Analyte_{Spike1} = Measured concentration of sample1 spike.

Analyte_{S2} = Measured concentration of sample 2.

Analyte_{Spike2} = Measured concentration of sample 2 spike.

Minimum Detection Limit:

The minimum detection limit is calculated from the error of seven blanks or 7 low concentration standards, whichever is largest. See quality control section above for more details.

The minimum detection limit is calculated as follows:

1. Calculate the concentrations of the 7 blank replicates and the 7 spikes using the CAL standard curve.
2. Calculate the mean of the 7 blanks. If it is negative, equate it to zero.
3. Calculate the standard deviation of the 7 blanks.
4. Multiply the standard deviation of the 7 blanks by 3.143.
5. Add the mean of the 7 blanks to the result from line 4.
6. Calculate the standard deviation of the 7 spikes.
7. Multiply the standard deviation of the 7 spikes by 3.143.
8. The minimum detection limit is the greater of line 5 and line 7.

METHOD PERFORMANCE

TP

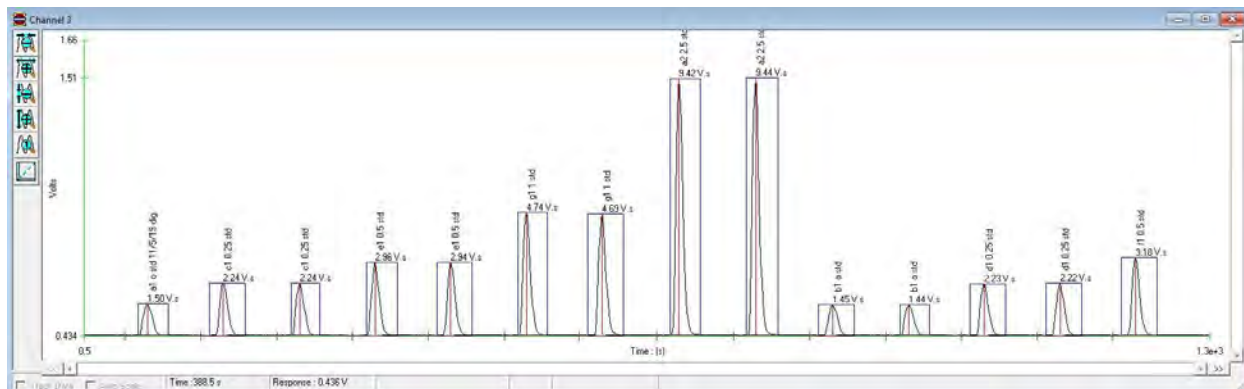


Figure1. TP CAL Standard Voltage Output from Lachat Quickchem Analytical Run.

Summary of digested TP Digested Standards Analyzed on 11/7/2019				
	Rep 1	Rep 2	Rep 3	Mean
TP (mg/l)	Peak Area	Peak Area	Peak Area	Peak Area
2.50	9.430	9.215		9.323
1.00	4.715	4.555		4.635
0.50	2.950	3.180	3.315	3.148
0.25	2.240	2.225		2.233
0.05	1.585	1.660		1.623
0	1.53	1.445		1.488
0	1.445			1.445

Table 2. Digested TP Standard Calibration Series using CAL standards.

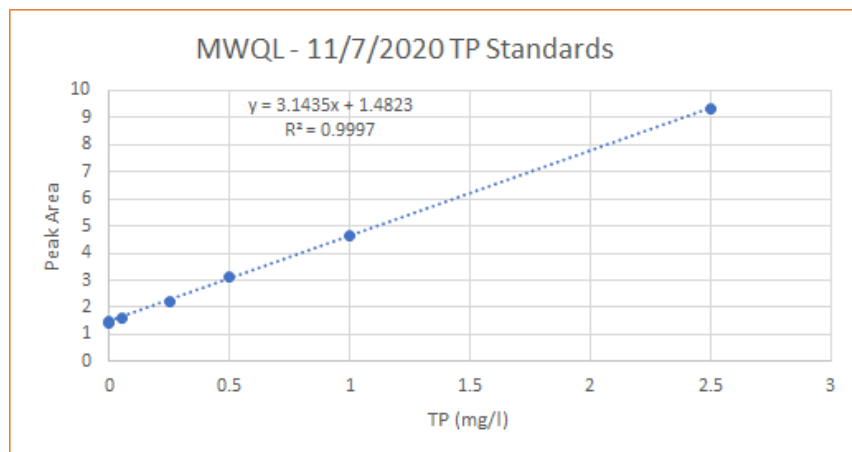


Figure 2. Graph of Digested TP CAL standards.

	Mean	Range
TP (mg/l)	Peak Area	Peak Area
5.00	18.650	0.050
2.50	10.200	0.000
1.00	4.805	0.005
0.50	3.145	0.015
0.25	2.285	0.085
0.05	1.650	0.015
0.00	1.450	0.000

Table 3. TP - Linear Range of Digested CAL Standards - 8/9/2019.

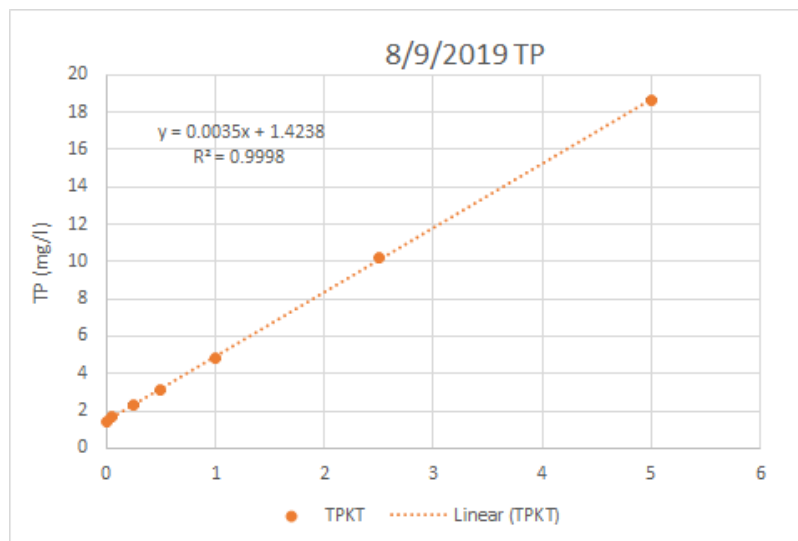


Figure 3. Graph of Linear Range of Digested TP CAL Standards - 8/9/2019.

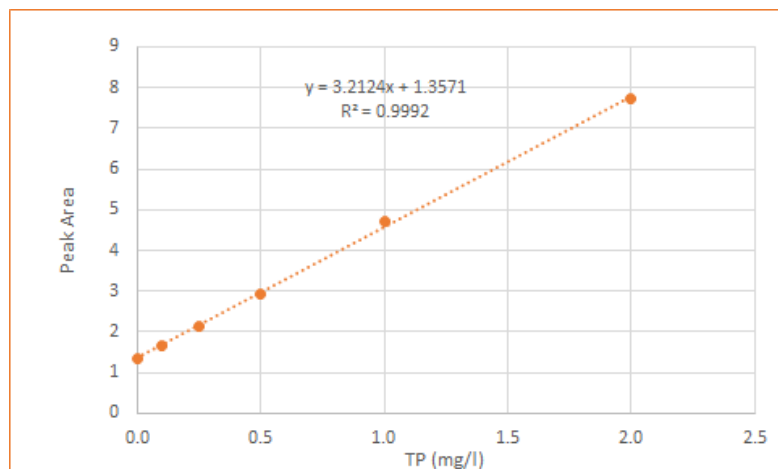


Figure 4. Undigested TP CAL Standards.

TKN

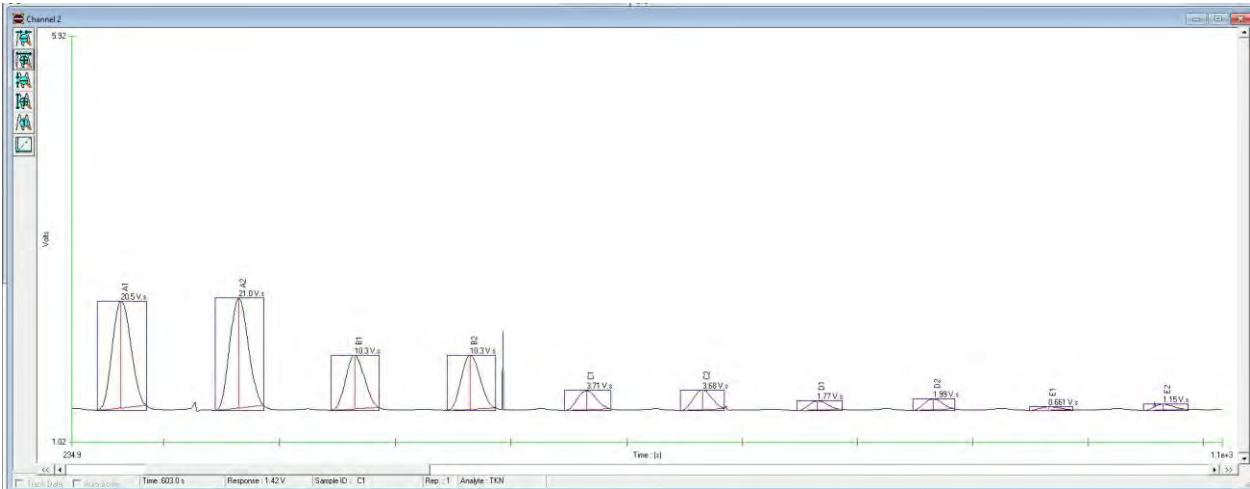


Figure 5. Example of TKN CAL Standard Voltage Output from Lachat Quickchem Analytical Run.

		Measured		Mean
TKN (mg/l)	Peak Area	TKN (mg/l)	% Residual	% Residual
5.0	14.73	5.058	1.17	3.43
2.5	7.01	2.399	4.05	
1.0	2.93	0.991	4.05	
0.5	1.61	0.535	0.89	
0.3	0.88	0.282	7.01	

Table 4. TKN Digested CAL Standard Series.

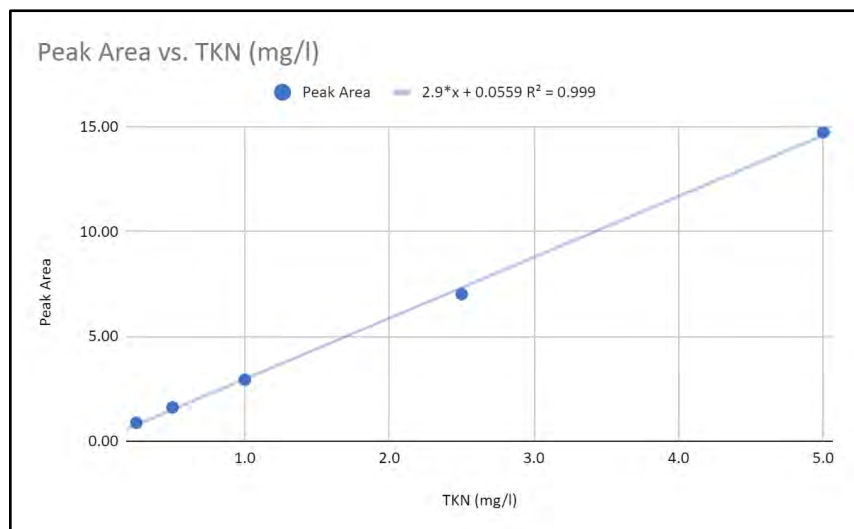


Figure 6. TKN Digested CAL Standard Regression.

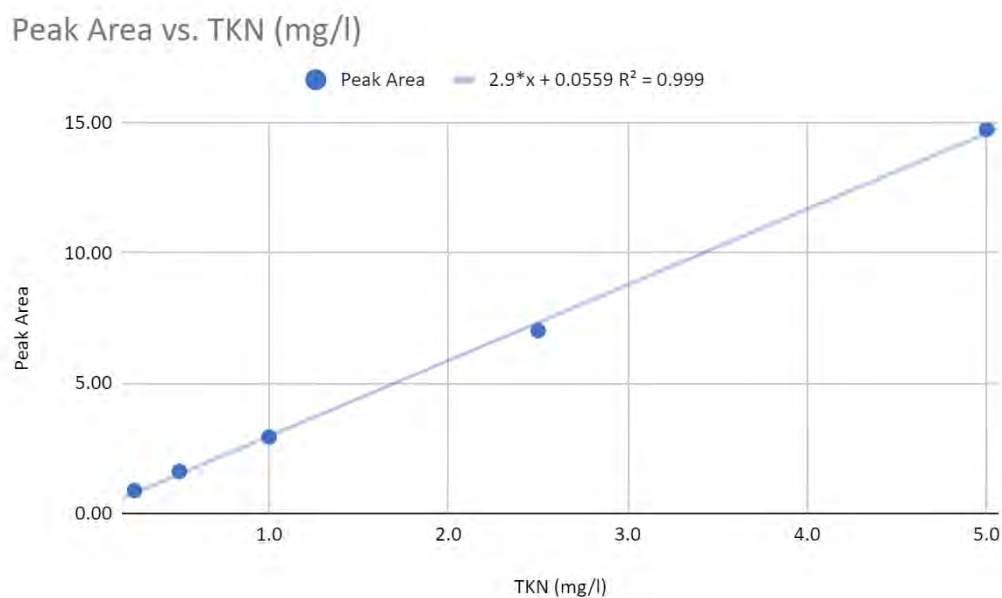


Figure 7. Linearity of TKN CAL Standard Working Range. Error bars are % Error of duplicate standards.

Corrective Actions:

General precision problems:

If baseline drifts, peaks are too wide, or other problems with precision arise:

- Check that there is not an obstruction in the tubing that is causing pressure buildup and retarding the flow of the carrier.
- Check flow of reagents. 'If reagent is not flowing properly:
 - Replace pump tubing.
 - Check all fittings for obstructions or crimping tubing.
- If the above actions do not alleviate the problem, clean the manifold by the following procedure:
 - Place transmission lines in water and pump to clear reagents (2-5 minutes).
 - Place reagent lines in 1 M hydrochloric acid (1 volume of HCl added to 11 volumes of water) and pump for several minutes.
 - Place all transmission lines in water and pump for several minutes.
 - Resume pumping reagents.

Turbidity:

Despite precautions taken to prevent this, Kjeldahl digests may contain some turbidity. To prevent the turbidity from interfering in the analysis, the following are recommended:

- Pour the diluted digests into the sample tubes, and allow them to settle prior to analysis.
- Set the probe height up from the bottom of the autosampler tube, so it does not hit the bottom to avoid aspirating the settled material.
- Let samples sit until the next day.
 - Pour them into bottles, cap tightly, and store in the refrigerator overnight. The turbidity will settle, so there is very little to none when you decant (carefully) the next day.
- If samples must be analyzed quickly, centrifugation of the sample tubes to speed the settling process can be used.
- To test whether turbidity is adding to the signal (by scattering light), replace the hypochlorite with water to prevent color formation (for TKN) or use DI water in place of the color reagent (TKP).

Weak or inconsistent signal:

- Reagents should be made up the day before. Freshly made reagents can give variable results.
- If excessive bubbles form:
 - Degas reagents.

- Check junctions for loose fittings.
- Check pump tubing. Replace worn tubing.
- Check intake tubing in bottles.

Poor precision:

- If replicate digested standards or samples vary by more than 10% from mean values:
 - Analyze undigested CAL standards and LCS to determine if the problem is with the analysis or the digestion step.
 - If problem is also present in undigested samples:
 - Check pump tubing.
 - Check back pressure tubing for leaks.
 - Check for bubbles.
 - Let the instrument equilibrate longer (inadequate equilibration can cause variability).
 - Check for blockages in lines.
 - If problem does not occur with undigested CAL standards and LCS:
 - Analyze other replicate standards and samples from digestion.
 - If other replicates or within the 10% variability, record all data and continue analytical run.
 - If all replicates vary by more than 10% but not by more than 20%, continue run, recording out of limit QC parameters.
 - If replicates vary by more than 20% stop run.

POLLUTION PREVENTION AND WASTE MANAGEMENT

- All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines.
- Waste will be stored in properly labeled containers with secondary containment.
- Waste will be located in approved areas and will be inspected weekly.
- When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus.
- At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste is transferred to the DMC safety officer.
- Waste will be stored until pickup by a University of Maine approved waste disposal company. All MWQL staff who handle waste will be required to complete the Hazardous Waste training provided by the University of Maine.

- The MWQL is committed to reducing chemical waste and will maintain the lowest volume of chemical stocks that is practical while maintaining adequate stock to guard against shortages and back orders.
- \All expired chemicals will be disposed of as chemical waste and transferred to the DMC waste storage facility.
- The lab does not dispose of any chemicals down the drain.

DATA ASSESSMENT AND ACCEPTANCE CRITERIA

Data Acceptance Specifications:

- LCS - 90% - 110% of certified value.
- LB - Below MDL.
- LD - 90% - 110% of mean value.
- LFSM - Spike Recovery - 80% - 120%.
- Linearity - Linear regression of CAL standards - > 0.98.
- CAL standard at or below the RL will be analyzed at minimum once per month when instrument is in use and must be within 40% of true value.
- Reproducibility of CAL standards over the course of a batch of samples - 90% - 110%. If CAL standard values are outside of this limit, a new set of CAL standards must be analyzed.

Data Assessment Checklist:

- Were the samples analyzed within the required holding time (28 days)?
- Were the samples preserved properly?
- Were the samples properly stored between sampling and analysis?
- Was this protocol followed as written?
 - If not, is there a record of variance from the method and documentation explaining the variance?

- Are there at least 4 concentrations of CAL standards for each sample batch?
- Is there a CAL standard at or below the RL?
- Were at least 2 CAL standards re-analyzed immediately after the initial CAL standard series to document method stability?
- Is each standard concentration analyzed in duplicate?
- Is the r squared value of the linear regression of the mean CAL standard response versus concentration greater than 0.98?
- Are the peak heights within 25% of the heights achieved for the previous analytical run for similar concentrations?
 - If not, were further LB and CAL standards analyzed to reassess sensitivity?
- Is the measured concentration of the LCS within 10% of the certified value?
- Is the LB less than the MDL?
- Was an LB and LCS analyzed for every 10 samples?
- Are all of the LB and LCS samples within acceptance criteria?
 - If not, is there documentation to explain?
- Was a duplicate sample analyzed for every 10 samples?
- Was the precision of the duplicate samples within 90% - 110%?
 - If not, is there documentation to explain?
- Was one LFSM analyzed for every 10 samples?
 - If not, is there documentation to explain?
- Is there a properly documented Digestion Log (TKN/TPDIGT)?
- Is there a properly documented KTN/TP analysis log (TKN/TPAL)?
- Is there a proper Chain of Custody?

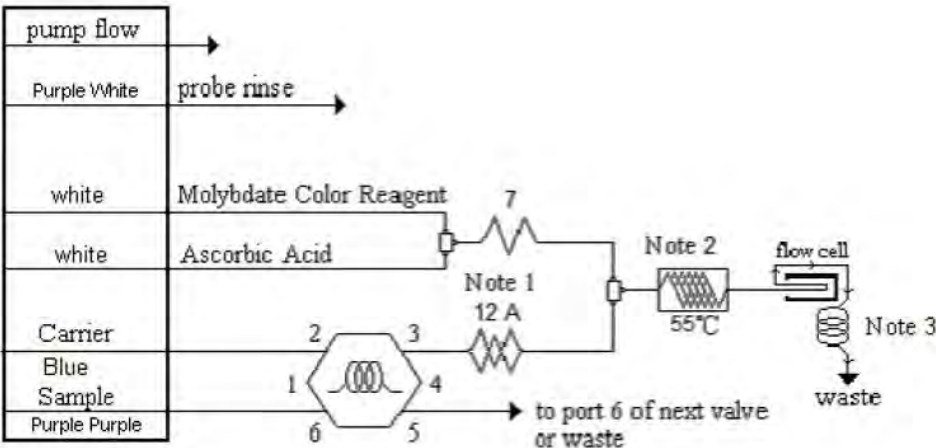
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Appendix A: Manifold and Valve Diagram

TP Manifold Diagram:



Carrier: DI Water (See method notes)
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 μ L/cm.
Sample Loop: 14 cm 0.022" i.d.
Interference Filter: 880 nm

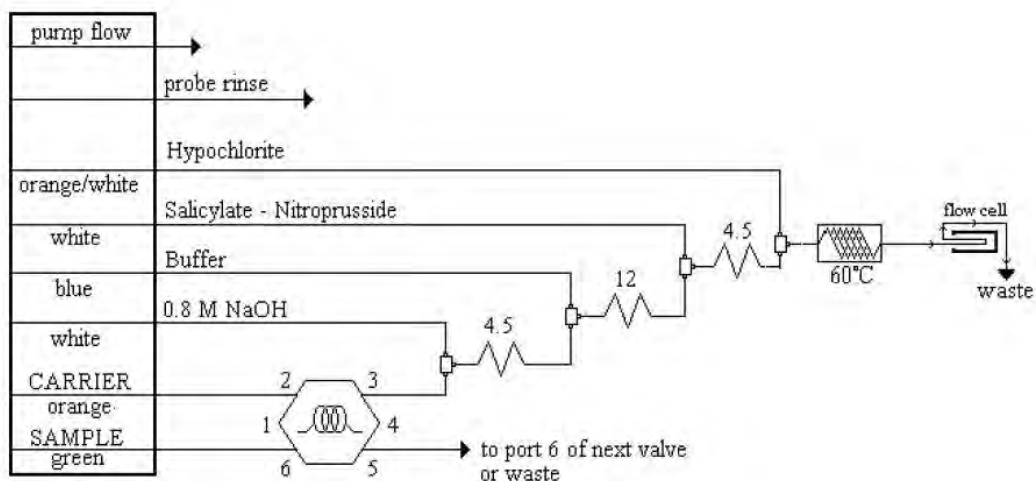
Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 cm of tubing wrapped around the heater block at the specified temperature.

7: 135 cm of tubing on a 7 cm coil support
12A 155 cm of tubing on a 12 cm alternating coil support

PVC PUMP TUBES MUST BE USED FOR THIS METHOD

Note 1 155 cm of tubing on a 12 cm alternating coil support
Note 2: 175cm of tubing on the heater
Note 3: 200 cm x 0.022 in. i.d. backpressure loop

TKN Manifold Diagram:



Carrier: Reagent 6 (Digestion Diluent)

Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 μ L/cm.

AE Sample Loop: 25 cm

QC8000 Sample Loop: 30.5 cm

Interference Filter: 660 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 650 cm of tubing wrapped around the heater block at the specified temperature.

4.5: 70 cm of tubing on a 4.5 cm coil support

12: 255 cm of tubing on a 12 cm coil support

Note 1: 650 cm of Teflon tubing is wrapped on the heater block.

Note 2: PVC PUMP TUBES MUST BE USED FOR THIS METHOD

Appendix D

Extracted Chlorophyll Analysis Standard Operating Procedure (SOP)
Document Code: MWQLSOPCHL Rev.5

SM 10200 H

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1. Specifications:

This method is applicable to marine, brackish and freshwater samples.

Applicable concentration ranges:

Chlorophyll a: 0.186 – 60 micrograms/l chlorophyll a. (Note: linearity exists between the LOQ and 200 micrograms/l)

2. Definitions

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

ANALYST - The designated individual who performs the “hands-on” analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality.

ANALYTICAL BATCH -- An analytical batch is a group of environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A batch is composed of at least one and no more than 20 environmental sample(s) of the same quality systems matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various quality system matrices and cannot exceed 20 samples.

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values or quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).

CALIBRATION BLANK (CB) -- A volume of 90% acetone in the same matrix as the calibration standards, but without the analyte.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

CERTIFIED REFERENCE MATERIAL -- (CRM) means reference material, accompanied by a certificate, having a value, measurement of uncertainty and stated metrological traceability chain to a national metrology institute. This method uses a solid standard.

CHAIN OF CUSTODY FORM (COC) - A record, either paper-based or electronic, that documents the possession of the samples from the time of collection to receipt in the laboratory in accordance with chain-of-custody protocol. This record, at a minimum, must include the sample location, the number and types of containers, the mode of collection, the collector, the date and time of collection, preservation, and requested analyses.

FIELD BLANK (FB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and

all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

LABORATORY BLANK (LB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibration standard).

LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure.

LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)-- An environmental sample containing an analyte spike of known concentration at least 4 times the MDL. Used to evaluate the effect of the sample matrix on the analytical results.

LIMIT OF QUANTITATION (LOQ) -- The minimum levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence.

METHOD DETECTION LIMIT (MDL) aka Limit of Detection (LOD) aka Minimum Detection Limit -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0 .

QUALITY CONTROL CHECK SAMPLE (QCCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process. Also known as the Proficiency Standard.

RELATIVE STANDARD DEVIATION (RSD) – The standard deviation expressed as a fraction of the mean.

3. Quality Control Measures:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in this QAM. QA and QC procedures specific to this protocol are documented in this SOP.

3A. Initial Demonstration of performance:

- Minimum Detection Limit (MDL) – Note that this method follows the procedure documented in 40 CFR Part 136 and outlined in “Definition and Procedure for the Determination of the Minimum Detection Limit, Rev. 2 (EPA 821-R-16-006).
 - Estimate the initial MDL using the mean determined concentration plus three times the standard deviation of a set of method blanks.
 - To determine the ongoing MDL, choose a concentration between 2-10 times the estimated initial MDL.
 - Spike a minimum of 7 samples with the chosen concentration of standard.
 - In addition, analyze a minimum of 7 blanks.
 - The seven spiked samples and seven blanks must be analyzed on a minimum of 3 days.
 - Calculate the MDL of the spiked samples.
 - $\text{MDL (spiked samples)} = \text{the student's } t\text{-value appropriate for a single-tailed } 99^{\text{th}} \text{ percentile } t \text{ statistic and a standard deviation estimate with } n-1 \text{ degrees of freedom, times the standard deviation of the replicate spike.}$
 - Calculate the MDL of the blanks.
 - $\text{MDL (blanks)} = \text{The mean of the method blanks times the student's } t\text{-value appropriate for the single-tailed } 99^{\text{th}} \text{ percentile } t \text{ statistic and a standard deviation estimate with } n-1 \text{ degrees of freedom times the standard deviation of the method blanks.}$
 - Select the greater of the spiked sample MDL and the blank MDL.
- Limit of Quantitation (LOQ) – The limit of quantitation will be determined by multiplying the standard deviation of the spiked samples by the student's t-value appropriate for a double-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom. If the LOQ calculated by the above method is less than 2 times the MDL, then the LOQ = two times the MDL.
- Proficiency Standard (PT) - Annually, a PT will be ordered from an EPA recommended provider lab. The sample will be analyzed, and results sent to the provider lab. If results are not within the acceptable limits of stated values, the source of the problem will be identified and corrected. Also known as the QCCS.
- Demonstration of Linearity - A standard curve over the working range (0.186-60 micrograms/l) of the method will be analyzed annually to demonstrate linearity.
- Proof of Capability (POC): All staff performing SM10200 H, must demonstrate capability:
 - In a brown bottle, collect 1-liter clean matrix.

- o Subsample 4 separate 100 ml samples using a Class A or lab calibrated graduated cylinder, being sure to mix the sample before taking each subsample.
 - Filter these samples, process, and analyze following the documented protocol.
- o Subsample 4 additional 100 ml samples, being sure to mix the sample before taking each subsample.
 - Filter these samples following the documented protocol.
 - Spike these samples immediately following the addition of acetone solution, with a concentration of chlorophyll certified standard to give a final concentration in the extract of 10 - 20 µg/l.
- o The % spike recovery and the standard deviation must be within 80-120% of the spiked concentration.

3B. Ongoing Quality Control Measures:

- Laboratory Blanks – One laboratory reagent blank consisting of milliQ water filtered and extracted in the same manner as the samples, will be analyzed for each batch of 10 samples. Blanks must be lower than the LOQ.
- Laboratory Control Standard – A solid chlorophyll a standard will be analyzed along with each batch of samples. If the LCS varies by more than 10% from the value recorded during the previous calibration, then a new calibration standard will be ordered, and the instrument will be recalibrated.
- Calibration Standards – Calibration standards will be ordered twice per year or when the LCS solid standard varies by more than 10% from the readings obtained during the previous calibration.
- Laboratory Duplicates - A set of LDs will be analyzed for every 20 samples. Duplicates should not vary more than 20% if the concentration is greater than 3 times the LOQ. Replicate samples whose concentration is below 3 times the LOQ, may have higher than 20% variability.
- Proof of Capability: Each analyst will perform a proof of capability once per year or when method changes are made or if problems arise with an analyst's work. See detailed procedure above.

4. Summary of Method:

A known volume of a water sample is filtered through a 25 mm glass fiber filter using low vacuum filtration. Filters are placed in a solution of 90% acetone, sonicated for 5 minutes and then extracted for greater than 4 and less than 20 hours at 4 degrees centigrade (4°C ±). Samples are centrifuged to clarify the extract. A subsample of the supernatant extract is transferred to a second tube for analysis. The tube is placed into the chamber of a Turner Trilogy fluorometer, and the raw fluorescence is recorded. The sample is then acidified, and a second fluorescence reading is taken. From these 2 readings, the corrected chlorophyll a and phaeophytin concentrations are derived.

5. Interferences:

- Filter residue and particulate matter can interfere with the fluorescence readings. Use caution not to disturb the sample while removing from centrifuge and pipetting off the supernatant. Do not pipette from the bottom of the extraction tube.
- Light can degrade chlorophyll. All steps of the process should be conducted with limited light, including during freezer storage, refrigerator storage, extraction, and analysis.
- Fluorescence readings change with the temperature of the sample. All extracted samples and liquid standards must be at room temperature when analyzed for fluorescence to avoid temperature derived variability or condensation on the fluorometer tubes.
- Dirty glass tubes can cause inaccurate readings. Glass chlorophyll tubes are either used straight from the box after each batch has been verified to be clean or washed thoroughly with laboratory detergent, rinsed with milliQ water, and rinsed with 90% acetone before use.

6. Safety:

Hazards:

This protocol requires the use of hazardous chemicals. All laboratory personnel using this protocol must be current with all University of Maine safety training requirements.

This protocol requires the use of a sonicator for which staff must receive specific instrument training. Improper use of the sonicator can damage hearing.

This protocol requires the use of a vacuum pump which if used improperly can cause vacuum carboys to implode. Read all instructions for use of pump.

Hazardous chemicals: Read SDS sheet for all chemicals before use. Wear appropriate personal protective equipment including appropriate gloves, lab coat and safety glasses. The following list highlights some of the hazards of the listed chemicals but IS NOT intended to replace thorough reading of the SDS sheets by laboratory personnel.

- **Hydrochloric Acid (CAS#: 7647-01-0). Corrosive. Toxic.**
- **Acetone (CAS#: 67-64-1). Flammable. Toxic.**

7. Equipment and Supplies:

- Brown plastic (HDPE or LDPE preferred) bottles for sample collection. 250 ml to 1 liter in size
- Niskin bottle if samples are to be collected at depth
- Vacuum pump, carboy and filtration rig with 25 mm diameter filter funnels and bases
- Glass fiber filters – 25 mm diameter
- 2 pairs of filter forceps
- Squirt bottle with milliQ water

- Test tubes with sealing caps for extraction and analysis (extraction tubes with Teflon screw caps and small fluorometer tubes with plastic caps for analysis). Note that the 7-ml tubes fit into the fluorometer cell and are used in place of cuvettes.
- Bottle top dispenser and amber glass bottle for 90% acetone
- Sonicator with water bath
- Ice pack for water bath
- Hearing protection appropriate for sonicator use
- Refrigerator for sample extraction
- Freezer for filter sample storage
- Aluminum foil, heavy duty
- Ziploc bags
- Centrifuge, bench-top swinging bucket
- Balance for equalizing tubes and centrifuge bucket weight in preparation for centrifugation
- Class A volumetric pipettes for extracted sample transfer
- Kimwipes
- Trilogy chlorophyll a and phaeophytin module - 7200-040-W Chlorophyll a Extracted – Blue excitation and red emission spectrum.
- Hydrochloric Acid, ACS grade or higher.
- Acetone, HPLC or Spectrophotometric grade
- Magnesium Carbonate – finely powdered
- Pipette for acid addition
- Glass test tubes with Teflon lined caps for making dilutions.
- 250 ml Pyrex bottle with Teflon or other acid-resistant cap for 0.1 N Hydrochloric Acid.
- Fume hood
- pH meter (for pH testing of samples suspected of having a pH lower than 6)

8. Labware Cleaning:

- Brown collection bottles are cleaned with laboratory detergent and rinsed with milliQ water.
- New glass tubes do not normally need to be washed before first use. Batches are initially checked for cleanliness by extracting and analyzing blanks using replicates from a batch of tubes.
- After use, tubes are washed with laboratory detergent, rinsed with milliQ, rinsed with 90% acetone, and left inverted in the fume hood to dry.
- Cuvettes are not required for this method because the Trilogy fluorometers are designed to read samples in the glass tubes.
- Labware must not be acid cleaned for this method.

9. Sample Collection and Preservation:

Sample Collection:

- A MWQL Chain of Custody form must be filled out for each batch of samples.
- Samples (250 - 1000 ml) are collected in laboratory detergent cleaned and milliQ rinsed, brown plastic bottles.
- Samplers must wear gloves and be careful not to touch any unclean surfaces with gloves before or during sampling.
- Samples may be collected from Niskin bottles or directly from river, stream, estuary, or other water source.
- Collection of the sample:
 - Rinse collection bottle with sample.
 - Fill the bottle with a volume of sample sufficient to rinse the interior of the bottle (for a 100 ml bottle, 10 ml is adequate).
 - Cap bottle, invert, and swirl sample in the bottle to rinse the entire inner surface of the bottle.
 - Dispose of rinse water downstream of the sampling site.
 - Repeat 2 times.
 - Fill the bottle to the neck and cap.
 - If sampling from a Niskin bottle, be sure to gently mix the water in the Niskin, before drawing the subsample.

Sample Storage:

- Store samples in the cooler, in the dark, with ice packs immediately after collection. Samples in collection bottles must remain below 4 degrees Centigrade, in the dark, until filtered.
- If water samples are collected from bodies of water that may have a pH lower than 6, for instance some freshwater bodies, the pH of the sample must be measured when samples arrive in the laboratory. If pH is less than 6, then samples must be extracted promptly after filtration.
- All samples must be filtered within 6 hours of collection.
- Except where noted above for low pH samples, filtered samples (contained on glass fiber filters) may be stored for up to 20 days after filtration at -20 degrees Centigrade.

10. Reagents and Standards:

6. MilliQ water – Record water resistivity and temperature.

- a. Resistivity greater than 18.0.
- b. Water is pretreated with particulate and carbon filters, passed through a reverse osmosis membrane, filtered through a Millipore milliQ or similar clean water system and filtered through a final particulate filter before use.

7. 90% Acetone: Studies have shown that excess magnesium carbonate can actually degrade chlorophyll by-products due to adsorption of the pigments to the magnesium carbonate (Aminot and Rey, 2001). Because seawater contains magnesium and carbonate, we do not make up the 90% acetone with magnesium carbonate or add magnesium carbonate to seawater samples. This is standard for oceanographic chlorophyll measurements.

a. Seawater Samples

- i. Make 1 liter of aqueous acetone solution (Note that if you are only analyzing a small amount of sample, make a lesser amount. Solution lasts for one month).
 1. Using the dedicated Class A 100 ml volumetric flask, measure 100 ml of milliQ water.
 2. Add milliQ water to the dedicated Class A 1-l flask.
 3. In the fume hood, fill the 1-liter flask to the mark with HPLC Grade Acetone.
 4. Cap and invert to mix.
 5. Transfer solution to 1-liter brown glass bottle with dispenser top.
 6. Make sure that cover is on dispenser tip when not in use.
 7. Log reagent in the MWQL Reagent Tracking Log.
 8. Use acetone in the hood.

b. Fresh Water Samples:

- i. If samples are collected from fresh water and the pH of the water is less than 6, the 90% acetone should be made up as follows:
 1. Add 100 ml of the following MgCO_3 solution to a 1-liter flask.
 - a. MgCO_3 solution:
 - i. Add 1 g of finely powdered magnesium carbonate to 100 ml of distilled or higher-grade water.
 2. In the fume hood, fill flask to the mark with HPLC Grade Acetone.
 3. Cap and invert to mix.
 4. Pour solution into 1-liter brown bottle with dispenser top or a brown glass bottle with Teflon cap.
 5. Make sure that cover is on dispenser tip when not in use.
 6. Record acetone solution in Reagent Tracking Log.
 7. Use acetone in the hood.

8. Standards.

- a. Certified liquid chlorophyll a standards are available for purchase. The Turner certified standard comes as two solutions, one with a high and one with a low concentration. For example, lot # CAS165-01 contained a high standard with a concentration of 240 micrograms/l, and a low concentration of 25.1 micrograms/l of chlorophyll a. These standards are diluted with 90% acetone solution in the lab to make a standard series from approximately 0.05 micrograms/liter to 60 micrograms/l or higher. Liquid standards are expensive and have a limited shelf life; solid standards are used for daily verification.

Standard Conc. (µg/l)	Standard Stock	Volume of Stock Added (ml)	Acetone Added (ml)
240.00	240	Straight Standard	
120.00	240	2.25	2.25
53.33	240	1.00	3.50
26.67	240	0.50	4.00
25.10	25.1	Straight Standard	
12.55	25.1	2.25	2.25
5.58	25.1	1.00	3.50
2.79	25.1	0.50	4.00
1.39	25.1	0.25	4.25
0.558	25.1	0.10	4.40
0.186	0.560	3	6
0.046	0.186	3	9

Table 1. Working standards from an August 2020 calibration.

- b. Solid Standard. Turner Designs provides a solid standard for the purpose of monitoring instrument drift. The solid standard is analyzed with every batch of samples. If a change in fluorescence readings occurs of 10% or greater from the reading at the last calibration, a new liquid certified standard instrument calibration is performed.

9. 0.1 N Hydrochloric Acid (HCl):

- i. Add 90 ml of milliQ water to a clean 100 ml Class A flask.
- ii. Add 0.833 ml of concentrated HCl.
- iii. Bring to 100 ml with milliQ water.
- iv. Transfer to dedicated Pyrex bottle.

11. Calibration and standardization:

- The instrument is calibrated with a certified liquid standard twice per year, when problems arise, or if a change of greater than 10% in fluorescence readings of the solid chlorophyll standard occurs. Certified liquid chlorophyll standards are expensive and do have a limited shelf life, so it is important to prepare properly before ordering and diluting standards. To calibrate:
 - Turn on the spectrofluorometer and allow to warm up for at least 30 minutes.
 - Remove standards from freezer and allow to come to room temperature, in the dark.
 - In subdued lighting, the fume hood, carefully dilute the liquid standards with 90% acetone, roughly following the dilutions in Table 1, above. Note that each batch of certified standard is a different concentration, so dilutions will be slightly different for each batch.
 - Analyze a series of at least 10 acetone blanks. These blanks will be used as a baseline to verify instrument performance and for MDL determination.
 - Obtain a fluorescence reading from each standard concentration.
 - Dilution of the certified standard should continue until obtaining a fluorescence reading of approximately 3 times the blank average.
 - Acidify several of the samples to obtain the ratio of unacidified to acidified pure chlorophyll.
 - After obtaining an initial raw fluorescence reading, add 0.1 N Hydrochloric Acid to each sample to make an HCL concentration of 0.003 N in the final solution.
 - Mix sample gently.
 - Allow sample to sit for at least 30 seconds.
 - Wipe off outside of tube.
 - Obtain raw fluorescence of acidified sample.
 - Immediately before after liquid standard readings are taken, obtain a raw fluorescence reading of the solid chlorophyll standard, remove the standard from the cell holder. Repeat after 5 minutes. These readings will be used as a baseline to detect drift.
 - Record at least one solid standard reading in the Chlorophyll Solid Standard Log each time you analyze samples.

12. Chlorophyll Analytical Procedure

- Print out a “Chlorophyll Filtration Log” and “Chlorophyll Analysis Log” Fill out the relevant information as you proceed through the filtration.
- If samples will not be extracted immediately, prepare foil packets for chlorophyll filters:

- o Cut a rectangular piece of foil large enough to enclose a folded 25 mm filter.
- o Use a Sharpie to label outside of foil pouch with project name, date, station, depth, replicate and any other needed information.
- o Turn down the lights in the laboratory.
- o Prepare filter rig:
 - Check carboy to make sure it is not too full.
 - If carboy is $\frac{3}{4}$ full or more, empty the carboy.
 - If using a pump with an oil reservoir, check to make sure that vacuum pump glass oil receptacle has pump oil.
 - Check vacuum tubing to be sure all connections are tight.
 - Open channels on filter rig.
 - Rinse each filter cup and base with milliQ water from squirt bottle
 - Turn on pump to pull water through filter base.
 - Place a glass fiber filter on each filter base.
 - A drop of milliQ water placed on the base before adding the filter will help hold the filter in place while you attach the cup to the base.
 - Attach a filter cup to each base.
- o **Filtration (in subdued light):**
 - Filter 100-250 ml of seawater through a glass fiber filter using the vacuum filtration rig. Note that sample volumes for samples with extremely high particulate concentrations may need to be less than 100 ml.
 - Remove samples from cooler.
 - Place each bottle next to the filter station through which it will be filtered.
 - Gently invert the bottle three or four times to mix sample. Do not shake, as you risk breaking cells.
 - Rinse the graduated cylinder with a few ml of sample.
 - Cap bottle and mix.
 - Repeat the rinse three times.
 - Mix sample and fill to the 100 ml mark of the graduated cylinder for most samples.
 - If water is exceptionally clear, you may need to filter more sample volume.
 - If phytoplankton are in high concentration, the volume filtered may need to be reduced.
 - You should be able to see the sample on the filter, if you can't, filter more sample. Keep a tally of the total volume filtered.
 - Make sure channel valve is open.
 - Pour sample into filtration cup.
 - Turn on pump and check vacuum.
 - Vacuum should not exceed 20 kPa.
 - Do not let filter completely dry out.
 - Turn off channel as sample level gets close to the level of the filter.
 - Turn off pump.

- Briefly open and close channel valve to remove any water that may remain above filter.
- Fresh water and low pH samples only:
 - Add 2 ml of MgCO_3 solution to each sample just before it is fully filtered through the filter.
 - To make magnesium carbonate solution, add 1 g of finely powdered magnesium carbonate to 100 ml of MilliQ water.
- Remove cup from filter station.
- Using forceps, carefully fold filter over onto itself so side with sample is on the inside.
- If not starting extraction immediately, place folded filter into foil pouch and fold over flaps of pouch to seal.
- Put pouch in Ziploc bag and store in freezer up to 20 days.
- If extracting immediately, put folded filter into the bottom of the extraction tube.
- Filter one blank of milliQ water for each batch of 10 samples.
- Rinse each filter base and cup with milliQ between samples.
- Store filters in foil pouches or tubes in subdued light while continuing to filter the batch.
- After final filtration, rinse filter cups with 90% acetone in the fume hood. Allow to dry in the hood, then rinse with milliQ water.
- **Extraction:**
 - Label 2 sets of chlorophyll tubes (extraction tubes with Teflon screw caps and small chlorophyll analysis tubes with plastic push-on caps) with electrical tape on the upper 1/3 of the tube. Use pencil as acetone will remove ink and Sharpie.
 - In subdued lighting and in the hood, place filter into an extraction tube, pushing filter to the bottom of tube.
 - Using the bottle-top pipette, add 7 ml of 90 % aqueous acetone being careful not to splash any of acetone outside of the tube.
 - Cap the tube and place in test tube rack.
 - When all filters have been placed in tubes and acetone has been added, cover entire rack with foil to block light.
 - Turn off lights in sonicator room.
 - Operator of sonicator must have received training in its use.
 - Take sample rack to sonicator and place rack in water bath.
 - Put a small ice pack in the water bath to prevent the water in the bath from heating up during sonication. Ice packs are in the freezer of the reagent refrigerator.
 - The water level in bath must cover the bottom inch of the tubes.
 - Insert small sonicator probe at least 1 " into the water making sure it does not touch rack or sides of bath.
 - Put on hearing protection.
 - Turn the intensity dial of the sonicator all the way down.

- Clear others from the area outside of the sonicator room or provide others with hearing protection.
- Turn on the power to the sonicator (red push button).
- Gradually turn up intensity to 30.
- Leave room and shut door.
- Either stand outside of room near door to prevent others from entering or place “Do Not Enter Sonicator in Use” sign on door. If standing next to the door, wear hearing protection.
- Sonicate for 5 minutes. For consistency, do not let samples sonicate for less than 4 ½ minutes or more than 5 ½ minutes.
- After 5 minutes, turn down sonicator intensity.
- Turn off power to sonicator.
- Remove rack from water bath, letting excess water drain into bath.
- Wrap rack in foil and place rack in MWQL sample refrigerator.
- Record temperature of refrigerator. There is a liquid bottle thermometer in each refrigerator.

O Fluorometric Analysis:

- After greater than 4 and less than 20 hours of extraction, remove samples from refrigerator.
- Allow them to come to room temperature, in a dark cabinet.
- Turn on Turner Trilogy fluorometer one-half hour before the start of the analysis.
- Centrifuge samples using the bench-top “swinging bucket” centrifuge.
 - o Make sure that all buckets have small rubber inserts.
 - o Equally balance the weight of the bucket collar and the buckets on opposite sides of the centrifuge rotor. The weights are embossed on the components.
 - o Insert tubes into buckets in balanced pairs.
 - o Check that the combination of collar, tubes and samples is balanced for opposite loads on the rotor.
 - Use the small swinging balance (with red and yellow trays).
 - The arrow should be touching the middle mark on the balance.
 - If it is off to one side too much, use a squirt bottle to add water to the bucket {tube holder} of the side that is too light to bring the arrow to the center mark. Note: DO NOT ADD WATER TO THE TUBE ITSELF!
 - o Insert collars with buckets and tubes into the channel in the rotor.
 - o Lock down centrifuge lid.
 - o Centrifuge at 1400 rpm (500g) for 20 minutes.
 - o The centrifuge motor will stop after 20 minutes but it will take longer for motion to cease. Do not use break but instead wait for centrifuge to stop.

- o Remember that opening the lid does not stop the rotor. There is no safety to prevent the lid from opening when the centrifuge rotor is spinning. DO NOT OPEN THE LID UNTIL THE ROTOR HAS COMPLETELY STOPPED.
 - o Once motion has stopped, open lid and remove tubes making sure light is diminished in room.
 - o Cover rack with foil when not removing or placing tubes in rack.
- Using a Class A volumetric glass pipet and bulb, transfer 3 ml from the top of the sample extraction tube to a small clean chlorophyll tube.
- Samples must be at room temperature (they should not feel cold to touch and should have no condensation). Temperature variations will cause fluorescence variation, so it is important to make sure that the samples are at room temperature (21 degrees centigrade plus or minus 3 degrees centigrade) when they are analyzed.
- Wipe off outside of tubes with a Kimwipe.
- Place tube in fluorometer, close lid and read fluorescence.
- If sample is at or above 90% of the reported range (roughly 200,000 raw fluorescence units on the MWQL Trilogy), dilute and analyze again.
 - o Dilute samples by adding sample and 90% acetone to a new tube in the proper proportions.
 - o Do not bring the total volume to more than 3 ml.
 - o Record dilution factor in the Chlorophyll Analysis Log.
- Remove tube from fluorometer.
- After all samples have been read, then acidify the samples and read again:
 - Add (0.1 ml for the 3 ml subsample) 0.1 N HCl to each sample.
 - Acidify samples in small batches which can be analyzed within the 3-minute time frame (see below).
 - Cap tube and gently invert to mix acid into sample.
 - Do not let samples sit less than 30 seconds or more than 3 minutes after acid addition before reading.

13. Data Analysis and Calculations:

Corrected Chlorophyll a and Phaeophytin calculations.

- The raw fluorescence reading per microgram of chlorophyll (response) is obtained from the slope of the linear regression obtained from analysis of liquid certified chlorophyll a standard.
- The ratio of the un-acidified raw fluorescence to the acidified raw fluorescence (f_0 max) of pure chlorophyll a is also determined by acidifying the pure chlorophyll standards.
- f_0 is equal to the unacidified raw sample fluorescence.
- f_a is equal to the acidified raw sample fluorescence.
- The chlorophyll a concentration is:

$$(\text{response} * (\text{f0max}/(\text{f0max}-1)) * (\text{f0}-\text{fa})) * ((\text{extraction vol. (l)} * \text{dilution factor})/\text{vol. filtered (l)})$$

- The phaeophytin concentration is :

$$(\text{response} * (\text{f0max}/(\text{f0max}-1)) * (\text{f0max} * \text{fa} - \text{f0})) * ((\text{extraction vol. (l)} * \text{dilution factor})/\text{vol. filtered (l)})$$

Accuracy – The accuracy of the chlorophyll a method is determined by yearly QCCS (PT) which are samples of known concentration provided by an outside entity which are analyzed blind by the laboratory.

Precision of Duplicate Samples- The percent error for replicate samples or is calculated using the absolute value of the difference between each of the duplicates using the following equation:

$$\text{Variability}_{\text{Rep}} = (\text{Error}/\text{Sample Mean}) \times 100,$$

$$\text{Error} = (\text{ABS}(\text{Rep1}-\text{Rep2}))/2$$

Instrument Drift:

The instrument drift is determined by analyzing the fluorescence signal of a solid standard with every batch of samples and comparing its raw fluorescence to that obtained with the same standard at the time of the most recent calibration. A running log of solid standard fluorescence is maintained in the Chlorophyll Solid Standard Fluorescence Log which is located next to the fluorometer.

Minimum Detection Limit:

The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero. See “Initial Demonstration of Performance” above for details of calculations.

Limit of Quantitation:

The limit of quantitation is determined by one of the following methods.

- Determining the concentration at which the fluorescence signal from the certified liquid chlorophyll sample is equal to three times the blank value.
- Determine from the 2 tailed t-test for the 99% probability from a set of blanks and a set of spiked blanks and choose the larger of the 2 concentrations.
- Multiply the MDL by 2.

14.MethodPerformance:

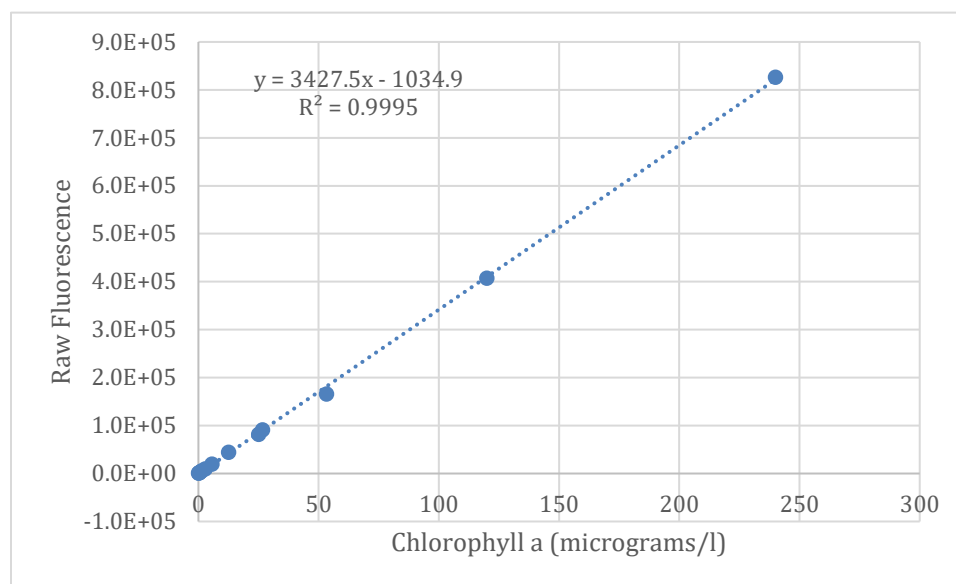


Figure 1. An example of linearity. Raw fluorescence of liquid chlorophyll standard, 8/13/2020.

15. Corrective Actions:

General precision problems:

1. If replicate variability is greater than acceptable limits (20% variability):
 - a. Determine if the sample raw fluorescence is above or below 10 times the LOQ:
 - i. If it is above 10 times the LOQ:
 1. Rerun samples making sure to mix sample well before subsampling.
 2. Verify that extracted samples are at room temperature before sampling.
 3. Allow 30 minutes for fluorometer to warm up before analyzing samples.
 4. Make sure that acidified samples are well mixed and are allowed to sit for 30 seconds after acid addition and mixing.
 5. Check the raw fluorescence of the solid standard to make sure that readings have not exceeded 10% of what they were at the time of the most recent calibration.
 6. If sample variability is still unacceptably high, replace lamp in fluorometer.
 - ii. If the sample raw fluorescence is below 10 times the LOQ, variability may be higher than 20%.
2. If solid standard varies by more than 10% from the readings at the time of the last calibration:
 - a. Check to make sure fluorometer was warmed up for at least one hour.
 - b. Check to make sure that solid standard was properly seated and in the correct orientation. Improper seating of standard can cause large variations in reading.
 - c. Notify lab manager who will clean the instrument chamber.

- d. If none of the above actions brings fluorescence readings to within 10% of last calibration: replace lamp.
3. Issues of unfiltered sample temperatures or other procedures being out of specification are dealt with in the QAM. If these issues occur with SM 10200 samples or procedures, the QAM protocols will be followed.

16. Pollution Prevention and waste management:

All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines. Waste will be stored in properly labeled containers with secondary containment. Waste will be located in approved areas and will be inspected weekly. When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus. At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste, is transferred to the DMC safety officer. Waste will be stored until pickup by a University of Maine approved waste disposal company. All MWQL staff who handle waste will be required to complete the Hazardous Waste training provided by the University of Maine.

The MWQL is committed to reducing chemical waste and will maintain the lowest volume of chemical stocks that is practical while maintaining adequate stock to guard against shortages and back orders. All MWQL expired chemicals will be disposed of as chemical waste and transferred to the DMC waste storage facility. The lab does not dispose of any chemicals down the drain.

17. Data Assessment and Acceptance Criteria:

Data Acceptance Specifications:

- Solid standard - 90% - 110% of raw fluorescence at the time of the last calibration.
- LB - Below LOQ.
- Linearity - Linear regression of certified liquid standards - > 0.98.
- Proof of Capability replicates:
 - Standard deviation of spiked samples must be within 20%.
 - Spiked samples must be within 20% of certified concentration.
- Holding Time: Sample processing, preparation and analysis must be completed within 21 days of collection. If not, the variation from specifications must be recorded with the data.

Data Assessment Check List:

- Were the samples analyzed within the required holding time (21 days)?
- Were the samples stored in adequate refrigeration?
- Were the samples kept in the dark?
- Were the samples properly stored between sampling and analysis?

- Was this protocol followed as written?
 - If not, is there a record of variance from the method and documentation explaining the variance?
- Were the solid standard values recorded?
- Were the replicate sample values within 20%?
- Were the blanks less than the LOQ?
- Is the r squared value of the linear regression of the liquid certified standard response versus concentration greater than 0.98?
- Was an LB analyzed for every 10 samples?
- Are all of the LB replicate samples within acceptance criteria?
 - If not, is there documentation to explain?

REFERENCES:

Aminot, A. and F. Rey. 2001. ICES Techniques in Marine Environmental Sciences #30: Chlorophyll a Determination by Spectroscopic Methods. International Council for Exploration of the Sea.

Appendix E

MWQL - Sampling, Sample Receipt and Handling Instructions

MWQSCI Rev.2

Purpose:

To outline the procedures for the receipt, handling, protection, storage, retention and/or disposal of test items.

To outline the procedures and appropriate facilities for avoiding deterioration, loss, or damage to the test or calibration item during storage, handling, preparation, and testing.

General Information:

- The MWQL will supply bottles for sampling.
- Use caution when collecting samples and follow instructions precisely.
 - Some bottles may contain corrosive preservatives.
 - These bottles will have a label indicating that the bottles contain hazardous material.
 - The instructions for filling bottles differ between those that contain preservative and those that do not contain preservative.

- Preservative MUST be added to bottles if samples will be shipped to the MWQL.
- If you must ship samples to us, please follow the instructions in the “Shipping” section below.

Sample Collection: Please follow the instructions below for specific sampling protocols. Any deviation from the protocols in this document must be noted and flagged.

Labels: Labels must include Project Code, Date of collection, Location ID, Time of collection, Initials of Sampler, Preservation method (if preserved), Sample Type and protocol.

Chain of Custody (COC): A MWQL Chain of Custody Form must be filled in for each sample batch. All samples must be recorded on the COC. All information must be completed. Every person handling or transporting samples must be listed on the MWQL COC.

Storage and Transport of Samples:

All samples must be stored in a cooler on ice (loose ice or a combination of loose ice cubes and ice packs). A tube with water is included and is labelled “Temperature Solution”. This tube must be kept in the cooler with the samples until delivery to the MWQL.

Sampling for EPA 351.2/EPA 365.4: Total Kjeldahl Nitrogen (TKN)/Total Phosphorus (TP)/and EPA 350.1: Ammonia.

- a) Collect samples in 250-1000 ml bottles provided by MWQL.
- b) Check to make sure that all required information is on the label (see “Labelling” section above).
- c) Wear gloves and safety glasses at all times when sampling.
 - i) Samples contain a small amount of caustic sulfuric acid as a preservative.
 - ii) Gloves also prevent material from your hands from contaminating the sample.
- d) Inspect bottles to make sure they are clean and in good condition.
- e) Collect the whole water sample. DO NOT FILTER.
- f) Fill the bottle with sample to the top of the straight side of the bottle. Do not overfill.
- g) Attach cap securely.
- h) Immediately place bottle in cooler with ice.

Sampling for EPA 353.2: NO_x (Nitrate plus Nitrite) samples.

- a) These samples are filtered into 50 ml tubes (fill to the 40 ml mark).
- b) Check to make sure that all required information is on the label (see “Labelling” section below).
- c) Wear gloves and safety glasses at all times when sampling – the tubes contain a small amount of sulfuric acid.
- d) Inspect bottles to make sure they are clean and in good condition.
- e) Filtration:

- i) Samples are filtered through a 0.45-micron syringe cartridge filter. Disposable or reusable filter cartridges may be used. If using disposable filter cartridges, keep them in a clean Ziploc bag before use and dispose of used filters in a separate garbage bag. Syringes can be used for multiple stations as long as they are thoroughly rinsed with sample first. Filters can also be used for more than one station – be sure to sufficiently flush with sample before collecting.
 - (1) If using reusable filter cartridges:
 - (a) Cartridge top and bottom and Teflon O-ring, and syringe must be acid-washed, rinsed, and dried prior to sampling.
 - (b) Add a clean 0.45-micron membrane filter and assemble cartridge.
 - (2) Store cartridges in a clean Ziploc bag until use.
 - (3) The syringe, plunger and filter must be rinsed with the sample before use:
 - (a) Remove filter cartridge from syringe and pull plunger out of syringe being careful not to let the syringe, syringe plunger or cartridge touch any unclean surfaces.
 - (b) Reattach filter to syringe.
 - (c) Pour about 5 ml of sample from the collection bottle into the top of the syringe.
 - (d) Reinsert the plunger into the syringe and push the sample through the syringe to waste. Be sure to dispose of the rinse sample downstream of the sampling location.
 - (e) Repeat two more times to adequately rinse syringe, plunger, cartridge, and filter.
 - (f) Remove cartridge from syringe and remove plunger from syringe.
 - (g) Reattach cartridge to syringe.
 - (h) Fill the syringe with the sample.
 - (i) Insert plunger into syringe,
 - (j) The collection tube for filtered samples contains preservative.
 - (k) Use the following protocol to fill the collection tube for the filtered sample:
 - (i) Push 10-15 ml of samples through the filter to waste.
 - (ii) Fill collection tubes with 50 ml of the sample and cap tightly.
 - (l) Immediately place bottle in cooler with ice.
 - (m) Samples must remain in the dark and on ice until arrival in the MWQL.

Sampling for SM 10200H: Extracted chlorophyll.

- a) These samples are collected in 100 - 250 ml brown plastic bottles or tubes.
- b) Check to make sure that all required information is on the label (see “Labelling” section below).
- c) Wear gloves at all times when sampling.
- d) Inspect bottles to make sure they are clean and in good condition.
- e) These bottles do not contain preservative.
- f) The collection bottles must be rinsed.
- g) To rinse the collection bottle:
 - ii) Collect a small amount (10-20 ml) of water in the bottle.
 - (1) If you are sampling directly from the body of water or a bucket, immerse the bottle to fill and cap the bottle under water.
 - iii) Cap the bottle and swirl to coat the inside of the bottle.

- iv) Empty the contents downstream of where you are collecting sample.
- v) Repeat 2 more times.

Shipping Samples

- The samples must arrive at the correct temperature which must be below 6° C.
- In order to ensure that samples stay cold, use loose ice or a combination of loose ice and ice packs placed in a garbage bag with the samples.
- Tie the garbage bag shut. Leaking water would be suspect and will result in a delay.
- Place all bottles back in the Ziploc bottles they came in and put those bags inside the garbage bag with ice.
- Fill the garbage bag with as much ice as possible. Tie bag tightly to prevent leaks.
- Attach custody sticker to cooler.
- Tape the cooler shut.
- If you would like us to return your ice pack/cooler, please request this on the chain of custody and write your name and address on the packs and cooler in a permanent way.
- Ship samples to arrive at the MWQL within 24 hours of shipping. We do not receive samples on weekend and it is best not to ship on Thursday in case the shipment is delayed, so please plan shipments accordingly.

Sample Receiving and Acceptance: Sample receiving (Log in) accepts samples and performs a series of checks and inspections to assure all necessary sampling and preservation requirements have been met, sample integrity has been maintained during transit, and that all necessary information has been supplied and that the samples meet the sample acceptance policy as listed below:

(Samples not meeting the following requirements will be brought to the attention of the customers prior to processing and may be rejected or will be appropriately qualified on the report of analysis).

- 1) Samples should be transported on ice/ice packs in a cooler, and the temperature will be recorded upon receipt.
- 2) Samples should have proper labeling and identification.
- 3) Samples should be taken in the appropriate sample containers as supplied by the laboratory.
- 4) Sampling instructions are to be followed as closely as possible.
- 5) Samples must have all required info about the customer, sample site, sample date & time, and sampler.
- 6) Samples must have adequate volume to perform the necessary tests.
- 7) All samples must be received with adequate time remaining to satisfy EPA holding time limits.
- 8) Any deviations from specified conditions will be noted and conversations with customers about those deviations area recorded.
- 9) When the customer desires to have samples analyzed, even though the samples are out of compliance with required specifications, a qualifier will be added to the data to indicate the deviation.
- 10) Preserved samples are stored in a temperature monitored refrigerator.

Appendix F

Marine Water Quality Laboratory
Cove Road Walpole, Maine 04573 207 563-8124

MWQLFDS, Rev. 1, 3/21/2022
Effective Date: ?

Date:		Project: Kingfish Maine		Time of Low Tide:	
Sonde:		Niskin		Filter Batch:	
Sonde Calibration Date/Time:				Syringe Batch:	
Depth of Thermocline if Present (m)				Record depth range of salinity < 10 ppt:	
Field Staff:				Bottle Batch	Container Type
Secchi Depth (m):		Secchi Time:		Chlorophyll	Container Volume (ml)
Secchi Notes:				TKNTP	Preservative?
				NO _x	
Site	Start Time	Finish Time	Collected by (initials)	Depth Code (S, B, T)*	Sample Depth (m)
KF1				S	Total Site Depth (m)
KF1				B	Filtered (Y/N)
KF1				T	Filtered By (initials)
Wind Speed/Direction:			Sea Conditions/Current:		Weather/Cloud Cover:
Notes:					

* T is a sample taken just below the thermocline. A thermocline is present if there is a change of 1°C or greater with a 1 meter change in depth of the sonde profile. The thermocline (T) sample, should be taken just below the depth of the thermocline.

Appendix G

Secchi Disk Standard Operating Procedure

Safety Information: Follow all shipboard safety protocols. Operate the disk from a safe and secure location

Protocol

1. Prior to using the secchi disk.
 - a. Verify the markings on the line are accurate. Use a meter stick to verify the meter marks. Measure the distance from the secchi disk to the first mark and record (This distance will change if the line is shortened for any reason).
 - b. Verify that the line is securely attached to the disk.
2. Work on shady side of boat, so that sun is not hitting water above the secchi disk and line. If necessary, use an umbrella or other sunshade.
3. Do not wear sunglasses or glasses that automatically darken in the sun.
4. Make sure that you are in a safe spot and can maintain your balance.
5. Before line goes overboard, have someone hold the end of the line or tie the line securely to the boat.
6. Lower disk into the water until white disk pattern disappears. Record the marking on the line for depth.
7. The line should be vertical while making measurements. Be mindful to avoid the line getting near the boat propellor in strong current. In strong currents, extra weights may be needed. If weighted secchi disk line is still not vertical note in the field notebook the estimated angle of the line.
8. Once you have recorded the first depth, lower the disk slightly further.
9. Raise disk until you can just see the white pattern again. Record the depth.
10. Average the two readings.
11. Once ashore, rinse the secchi disk and line in fresh water and hang on rack to dry.

Appendix H

Summary of Chlorophyll Tests		SM10200H						
4/27/22: Seawater samples collected from the DMC dock at 8:30 am on 4/27/22	Time Elapsed Between Collection and Filtration (min.)	Chlor a	Phaeophytin	5/24/22: Seawater samples collected from the DMC dock at 8:15 am on 5/24/22	Between Collection and Filtration (min.)	Chlor a	Phaeophytin	
		(µg/l) In Sample	(µg/l) In Sample			(µg/l) In Sample	(µg/l) In Sample	
Blank	NA	0.004	0.0382	24MAY22 Chlor Test 6 hr 1	375	3.446	-0.3910	
27APR22KT-1-1	343	2.203	0.671	24MAY22 Chlor Test 6 hr 2	376	1.986	0.5238	
27APR22KT-1 -2	346	2.671	1.642	24MAY22 Chlor Test 6 hr 3	377	1.840	0.7392	
27APR22KT-1- 3	347	2.910	1.540	24MAY22 Chlor Test 6 hr 4	378	2.701	1.2741	
27APR22KT-1 -4	483	3.393	0.810	24MAY22 Chlor Test 8 hr 1	479	3.064	0.8692	
27APR22KT-1 -5	486	3.030	1.226	24MAY22 Chlor Test 8 hr 2	481	2.324	1.8630	
27APR22KT-1 -6	488	3.192	1.089	24MAY22 Chlor Test 8 hr 3	482	2.124	0.6118	
27APR22KT-1- 7	489	2.413	1.754	24MAY22 Chlor Test 8 hr 4	484	3.178	0.8349	
				24MAY22 Chlor Test 6 hr Bk	NA	0.007	0.0244	
				24MAY22 Chlor Test 8 hr Bk	NA	0.000	0.0411	

Data from two test of chlorophyll sample holding times. Sampled were processed and analyzed using the MWQL Chlorophyll SOP for SM10200H.

**NOTICE OF INTENT TO FILE
MAINE WASTE DISCHARGE LICENSE /
MAINE POLLUTANT DISCHARGE ELIMINATION
SYSTEM PERMIT RENEWAL APPLICATION**

Please take note that, pursuant to 38 MRSA, Sections 413 and 414-A, Kingfish Maine Inc. intends to file a wastewater discharge permit renewal application with the Department of Environmental Protection (DEP). The application is for the discharge of a land-based aquaculture facility in Jonesport, Maine at 9 Dun Garvin Road. The application proposes a new or increased discharge that may lower existing receiving water quality within its legal classification, and the application contains a statement regarding important social and economic benefits resulting from the activity causing the discharge, pursuant to 38 MRSA, Section 464, Department of Environmental Protection Antidegradation Policy. This permit evaluates the facility's discharge to ensure that Kingfish Maine will maintain the water quality of the area based on the location of the discharge point and the make-up of the effluent water.

Kingfish Maine is owned by the Kingfish Company, which currently operates a land-based recirculating aquaculture system (RAS) in a Natura 2000 nature reserve in the Netherlands. Kingfish Maine will utilize the same advanced technology solutions used at Kingfish Zeeland, to mitigate the impacts of the effluent discharged and will abide by the parameters required by Maine DEP to protect the water quality and marine habitat.

Kingfish Maine has been actively developing its plan for the Jonesport facility since 2019, communicating the details of the proposed land-based facility to the residents of Jonesport. Therefore, this application addresses the two primary areas of interest as outlined in Kingfish Maine's ongoing dialogue: The length of intake and discharge pipes and protecting the environment from water temperature increases.

The proposed position and length of the intake and discharge pipes, as outlined in the permit application, minimizes the pipe footprint to ensure as little intrusion on fishing activity as possible in Chandler Bay. To minimize the length of pipe, Kingfish Maine is investing in significant filtration of effluent, which allows for the length of the discharge pipe to be limited to just under half a mile.

The permit application fully outlines Kingfish Maine's solids and nutrient filtration system as well as its heat recovery process. The facility's advanced technology will allow the reclamation of heat energy before it is discharged, therefore preventing temperature rise impacts on the bay.

The renewal application will be filed on or about May 15, 2026, and will be available for public inspection at DEP's Augusta office during normal business hours. A copy may also be seen at the Jonesport municipal offices at 70 Snare Creek Lane, Jonesport, ME.

A request for a public hearing or request that the Board of Environmental Protection assume jurisdiction over this application must be received by the DEP, in writing, no later than 20 days after the application is found acceptable for processing, or 30 days from the date of this notice, whichever is longer. Requests shall state the nature of the issue(s) to be raised. Unless otherwise provided by law, a hearing is discretionary and may be held if the Commissioner or the Board finds significant public interest or there is conflicting technical information.

During the time specified above, persons wishing to receive copies of draft permits and supporting documents, when available, may request them from the DEP. Persons receiving a draft permit shall have 30 days in which to submit comments or to request a public hearing on the draft.

Public comment will be accepted until a final administrative action is taken to approve, approve with conditions or deny this renewal application. Written public comments or requests for information may be made to:

Maine Department of Environmental Protection
Division of Water Quality Management
Department of Environmental Protection
State House Station #17
Augusta, Maine 04333-0017
Telephone (207) 287-7688

Kingfish Maine welcomes questions and comments regarding permitting and project scope. The public can email the Kingfish Maine team at kingfishmainepermit@thekingfishcompany.com.

2026 Listing of Abutters and Parcel Numbers

North: (008-026 + 027)

Kent Ingves, 756 Waterman Rd, Lebanon, CT 06249 (owns 30% of the lots)

Paula Stahl, 46 Bellevue St, Willimantic, CT 06226 (owns 70% of the lots)

Kent Ingves, Paula Stahl

46 Bellevue St

Willimantic, CT 06226

North/West: (008-028)

Holmes, Christiannah Evelyn

24 Pine Cove Rd

Gray, Maine 04039

Also owns interest in the property:

Maine Coast Heritage Trust

1 Main St

Bowdoin Mill suite 201

Topsham ME 04086

South: (008-020)

Byors in Jonesport Maine LLC

3 Henrys Way

Marblehead Mass. 01945

South: (008-001)

Agnes Patterson

18 Frost Lane

Orono, ME 04473

South: (008-018)

Souza, Joshua S

PO Box 1271

Forestdale, MA 02644

South: (008-017)

Greenwood Cemetery

Town of Jonesport

70 Snare Creek Lane, Lower Level

Jonesport, Maine 04649

South: (008-016)

Beal, Harry N

87 Fire Road

Jonesport, Maine

South: (008-015 + 014)

Alley, Bronson

PO Box 283

Jonesport, Maine 04649

South: (008-013)

Backman, Ralph

PO Box 266

Jonesport, Maine 04649

West: (008-022)

Worcester Peat Company Inc.

241 Lane Rd

Dublois, ME 04622

West: (008-024 and 008-025)

New England Diversified Industries, Inc.

c/o Sandra Busque

5802 W Cactus Wren Way

Florence, AZ 85132

West: (008-019)

Jonesport, Town of

70 Snare Creek Lane, Lower Level

Jonesport, Maine 04649

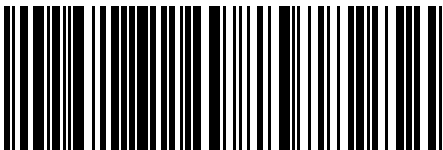
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000141



Agnes Patterson
18 Frost Lane
Orono ME 04473
USA

USPS CERTIFIED MAIL



9214 8901 4298 0479 3535 54

**NOTICE OF INTENT TO FILE
MAINE WASTE DISCHARGE LICENSE / MAINE POLLUTANT DISCHARGE ELIMINATION
SYSTEM PERMIT RENEWAL APPLICATION**

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Maine Department of Environmental Protection
Division of Water Quality Management
Department of Environmental Protection
State House Station #17
Augusta, Maine 04333-0017
Telephone (207) 287-7688

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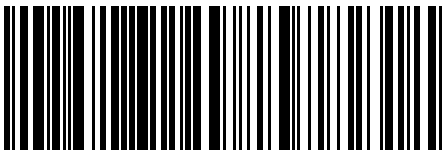
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000130



Bronson Alley
PO Box 283
Jonesport ME 04649
USA

USPS CERTIFIED MAIL



9214 8901 4298 0479 3535 61

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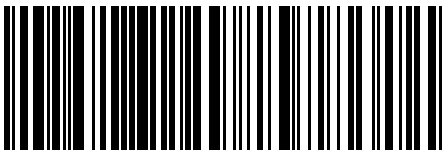
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000120

Byors in Jonesport Maine LLC
3 Henrys Way
Marblehead MA 01945
USA



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9214 8901 4298 0479 3535 78

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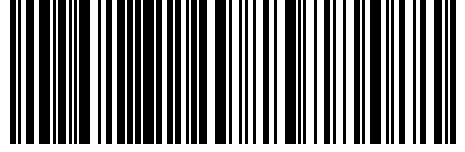
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000110

Christiannah Evelyn Holmes
24 Pine Cove Rd
Gray ME 04039
USA



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9214 8901 4298 0479 3535 85

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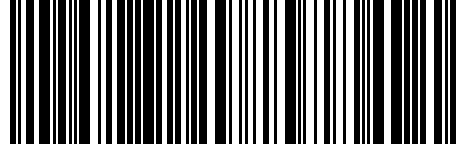
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000100

Greenwood Cemetery
Town of Jonesport
70 Snare Creek Lane, Lower Level, Jonesport
Jonesport ME 04649
USA



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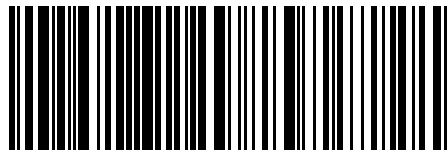
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000090

Harry N. Beal
87 Fire Road
Jonesport ME 04649
USA



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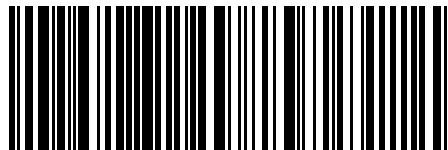
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Joshuah S. Souza
PO Box 1271
Forestdale MA 02644
USA



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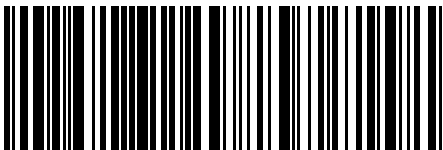
Kingfish Maine
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33 Salmon Farm Road
Franklin ME 04634

0014019965000070



Kent Ingves
756 Waterman Rd.
Lebanon CT 06249
USA

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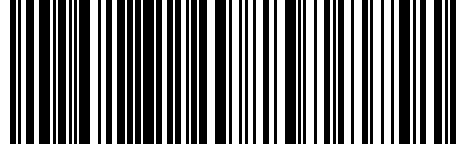
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Maine Coast Heritage Trust
1 Main St, Bowdoin Mill suite 201
Topsham ME 04086



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**NOTICE OF INTENT TO FILE
MAINE WASTE DISCHARGE LICENSE / MAINE POLLUTANT DISCHARGE ELIMINATION
SYSTEM PERMIT RENEWAL APPLICATION**

Please take note that, pursuant to 38 MRSA, Sections 413 and 414-A, Kingfish Maine Inc. intends to file a wastewater discharge permit renewal application with the Department of Environmental Protection (DEP). The application is for the discharge of a land-based aquaculture facility in Jonesport, Maine at 9 Dun Garvin Road. The application proposes a new or increased discharge that may lower existing receiving water quality within its legal classification, and the application contains a statement regarding important social and economic benefits resulting from the activity causing the discharge, pursuant to 38 MRSA, Section 464, Department of Environmental Protection Antidegradation Policy. This permit evaluates the facility's discharge to ensure that Kingfish Maine will maintain the water quality of the area based on the location of the discharge point and the make-up of the effluent water.

Kingfish Maine is owned by the Kingfish Company, which currently operates a land-based recirculating aquaculture system (RAS) in a Natura 2000 nature reserve in the Netherlands. Kingfish Maine will utilize the same advanced technology solutions used at Kingfish Zeeland, to mitigate the impacts of the effluent discharged and will abide by the parameters required by Maine DEP to protect the water quality and marine habitat.

Kingfish Maine has been actively developing its plan for the Jonesport facility since 2019, communicating the details of the proposed land-based facility to the residents of Jonesport. . Therefore, this application addresses the two primary areas of interest as outlined in Kingfish Maine's ongoing dialogue: The length of intake and discharge pipes and protecting the environment from water temperature increases.

The proposed position and length of the intake and discharge pipes, as outlined in the permit application, minimizes the pipe footprint to ensure as little intrusion on fishing activity as possible in Chandler Bay. To minimize the length of pipe, Kingfish Maine is investing in significant filtration of effluent, which allows for the length of the discharge pipe to be limited to just under half a mile.

The permit application fully outlines Kingfish Maine's solids and nutrient filtration system as well as its heat recovery process. The facility's advanced technology will allow the reclamation of heat energy before it is discharged, therefore preventing temperature rise impacts on the bay.

The renewal application will be filed on or about May 15, 2026, and will be available for public inspection at DEP's Augusta office during normal business hours. A copy may also be seen at the Jonesport municipal offices at 70 Snare Creek Lane, Jonesport, ME.

A request for a public hearing or request that the Board of Environmental Protection assume jurisdiction over this application must be received by the DEP, in writing, no later than 20 days

after the application is found acceptable for processing, or 30 days from the date of this notice, whichever is longer. Requests shall state the nature of the issue(s) to be raised. Unless otherwise provided by law, a hearing is discretionary and may be held if the Commissioner or the Board finds significant public interest or there is conflicting technical information.

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Maine Department of Environmental Protection
Division of Water Quality Management
Department of Environmental Protection
State House Station #17
Augusta, Maine 04333-0017
Telephone (207) 287-7688

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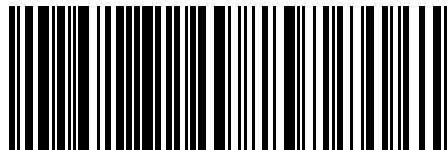
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634



0014019965000050

New England Diversified Industries, Inc.
Sandra Busque
5802 W Cactus Wren Way
Florence AZ 85132
USA

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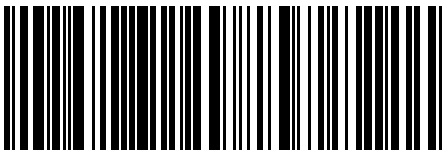
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000040



Paula Stahl
46 Bellevue St.
Willimantic CT 06226
USA

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9214 8901 4298 0479 3536 53

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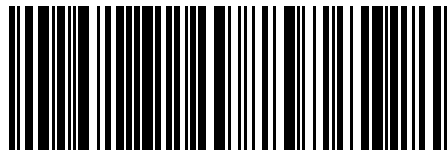
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Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634



0014019965000030
Ralph Backman
PO Box 266
Jonesport ME 04649
USA

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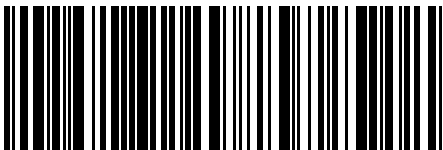
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634



0014019965000020

Town of Jonesport
70 Snare Creek Lane
Lower Level
Jonesport ME 04649
USA

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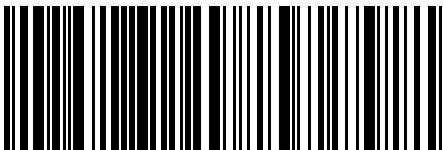
Kingfish Maine
Center for Cooperative Aquaculture Resea
33 Salmon Farm Road
Franklin ME 04634

0014019965000010

Worcester Peat Company Inc.
241 Lane Rd.
Dublois ME 04622
USA



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