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Sample Design Considerations for a Post-Deposit Monitoring Program for Pesticides and Drugs Discharged from Salmon Net-Pen Farming Operations

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

This document is part of a CSAS process in support of the development of a post-deposit monitoring program for drug and pesticide use at Canadian marine finfish farms. This report focusses on designing sampling programs in relation to the discharge of chemicals associated with bath pesticides and in-feed drugs used in marine net-pen aquaculture farming operations in Canada.

Selection of a post-deposit sample design should follow a structured and systematic approach that includes clearly stated objectives, decision rules, decision tolerances, sampling constraints, spatial and temporal coordinates of potential sampling locations, and sampling methodologies. Sample designs that are based on probabilistic (statistical) principles are preferred to judgement-based designs.

For in-feed medication deposits, bottom sampling should include several phases. The first phase is to identify and map the boundaries of bottom types in the area of interest. The purpose of the second phase is to detect the location and intensity of discharge deposits using a sampling design and sampling methodologies that are appropriate given the knowledge gained in phase one. The purpose of the third phase, if needed, is to refine the characterization of the detected deposits and to monitor temporal change in the characteristics of the deposition (area, concentration). Phase one designs should be grid based, phase two designs should be stratified random designs with random grid or random sample allocation within strata, and phase three designs should be finer scale random grids or random sample allocations within focused areas of interest. This approach recognises that there are inherent uncertainties in discharge properties (location, time, duration, intensity, frequency) and estimates of discharge transport, dispersal, deposition and redistribution; it also helps minimize bias introduced by judgement. The probabilistic approaches enable statistical inferences to be made and trade-offs between precision of sample statistics and cost effectiveness to be evaluated in relation to tolerance criteria. This is particularly important in aquaculture post-deposit monitoring when practicalities generally limit sampling efforts to relatively low sample sizes which results in a significant risk of underestimating the area and intensity of deposits and low precision in estimates of in-situ discharge concentrations. General suggestions for bottom sampling methodologies include the use of bottom sediment samplers with low bottom and sample disturbance characteristics (corers preferred) and visual imagery for hard bottoms.

For pesticides, samples of the bath water should be taken just prior to discharge. Due to the constantly changing nature of the pesticide discharge cloud, use of a probabilistic sampling design is not practical. General suggestions for sampling methodologies include the use of a visible tracer introduced into the pesticide bath water prior to discharge. Routine monitoring of bath pesticide post-discharges is probably not feasible; however, targeted monitoring should be occasionally undertaken to help improve models. A minimum sampling effort could involve water samples being taken over time at horizontal and vertical locations that are indicated by the tracer to be areas of high pesticide concentration. Imagery coupled with additional in-situ sampling of tracer and pesticide concentrations can be used to produce calibrated estimates of discharge areas. When the tracer indicates contact with the seabed, a focused random gridded sampling effort for chemical concentration or impact could be undertaken.

1. INTRODUCTION

The present Canadian Science Advisory Secretariat (CSAS) process, of which this document is a component, is part of a regulatory regime under development by Fisheries and Oceans Canada (DFO) to develop a post deposit monitoring program for pesticides and drugs released from Canadian net-pen salmon farming operations.

The need for well-designed pre-assessment and post-deposit sampling programs associated with the use of aquaculture pesticides and drugs (pest control and antibiotics) has generally been acknowledged. Recent reviews have highlighted significant deficiencies in sampling efforts, sampling designs, sampling methodologies, and predictive models (CoastalSmith Inc 2006; Environment Canada 2009; Wilson et al. 2009). Some organizations have been working to improve their sampling designs and compliance programs, e.g., the Scottish Environmental Protection Agency (SEPA 2019a, 2019b). SEPA has recently released new regulations for aquaculture although the details and implications of the programs are likely to continue to evolve for some time.

There are several approaches and terminologies associated with the design of sampling programs. A general principle is that all designs should be preceded by a planning process that includes multiple steps and multiple sources of expertise, including the decision maker, people knowledgeable about the scenario to be sampled, and statisticians (US EPA 2002). All sampling designs should also include consideration and definitions of spatial, temporal and intensity boundaries or limits and be based on statistical principles, where possible. When a proper planning process is followed, it should culminate in a detailed consideration, choice, and documentation of a sample design appropriate to the need (US EPA 2002).

We have found the approach described in the United States Environmental Protection Agency (US EPA) guidance document (US EPA 2002) to be useful. It is supported by a Visual Sampling Plan software tool (VSP Development Team 2020) that is freely available for helping to guide and implement the development of a design approach. The US EPA approach to sample design is general in nature and focuses on principles associated with planning environmental sampling. The US EPA approach does not explicitly address the challenge of designing a sampling program for pesticide and drug discharges associated with net-pen aquaculture operations. We therefore outline the approach and use it to guide our commentary of relevance to discharges of pesticides and drugs from net-pen aquaculture operations.

The purpose of the present document is therefore to outline a sample planning and design process; it is not meant to provide a final sampling design. This document is meant to be an introduction to the sampling challenge for post-deposit monitoring and not the final word. Its main purpose is to highlight some of the challenges and steps that should be taken to arrive at useful sampling designs, to review some existing sampling efforts and designs, and to provide suggestions to help kick start the development of a Canadian sampling approach. If the general approach and suggestions are deemed useful, detailed sampling plans should be developed in a multi-disciplinary way in conjunction with the relevant expertise, both scientific and management.

2. PLANNING: A SYSTEMATIC PROCESS

The design of any sampling program should begin with a planning process that "… should match the needs of the project with the resources available. The needs generally consist of the study objectives and the tolerable limits on uncertainty. The resources may include personnel, time, and availability of financial resources." (US EPA 2002). According to the US EPA (2002), "… systematic planning identifies the expected outcome of the project, the technical goals, the cost and schedule, and the acceptance criteria for the final result." Inadequate planning wastes resources and results in data which cannot address key questions (Wilding et al. 2017).

A useful set of planning steps is provided by the US EPA and the International Organization for Standardization (ISO) in their planning process (US EPA 2002; ISO 2004). The US EPA and ISO processes include some or all of following steps:

1. State the problem.
2. Identify the decision.
3. Identify inputs to the decision.
4. Define the boundaries of the study.
5. Develop a decision rule.
6. Specify tolerable limits on decision error.
7. Optimize the design for obtaining data.

2.1. STEP 1: STATE THE PROBLEM

The purpose of this step is to clearly define the problem, identify the primary decision maker, identify appropriate planning and sampling design team members, identify available or acceptable budgets, and scheduling timelines (US EPA 2002). This step is essential; a sample design may not be adequate if it has not been designed with a specific goal or goals in mind. Multiple goals may require more than one sample design, i.e., one design may not be sufficient to satisfy the needs of all goals.

2.2. STEP 2: IDENTIFY THE DECISION

The purpose of this step is to clearly state the decision(s) that will utilize the output(s) from the sampling design (US EPA 2002). The step should also link the possible outcomes of the decision to possible actions (US EPA 2002).

2.3. STEP 3: IDENTIFY INPUTS TO THE DECISION

The purpose of this step is to identify the inputs needed to answer the decisions posed in Step 2.

2.4. STEP 4: DEFINE THE BOUNDARIES OF THE STUDY

The purpose of this step is to define the population of interest, including the spatial and temporal boundaries of the population and the scale of decision making (US EPA 2002). The step also identifies any practical constraints of relevance to the data collection (US EPA 2002).

2.5. STEP 5: DEVELOP A DECISION RULE

The purpose of this step is to provide a logical basis upon which the decision maker can make a decision (US EPA 2002) so the sample design is able to generate results that contribute the correct information to the decision process.

The step requires that a statistic or parameter of interest be identified (e.g., mean chemical concentration), that actions to be taken in relation to calculated statistic or parameter values are defined, and that the scale involved in the decision making be defined (US EPA 2002). It is also generally recognized and preferred that decision rules should be based on statistical, i.e., probabilistic, principles (US EPA 2002; ISO 2004). The information provided in this step can have an important influence on the design of a sampling program.

The compliance rules or guidelines may be derived from a combination of eco-toxicological, social, economic, and political considerations. If social, economic, and political considerations result in a monitoring programing that is unable to meet the objectives, regulators should evaluate the merit of implementing an ineffective program (Wilding et al. 2017).

2.6. STEP 6: SPECIFY TOLERABLE LIMITS ON DECISION ERROR

The purpose of this step is to define the decision maker's tolerance for potential decision errors and the uncertainties inherent in the results from the sample design due to errors in base-level assumptions, as well as uncertainties associated with parameter and/or statistical estimates (US EPA 2002).

2.7. STEP 7: DESIGN A SAMPLING SCHEME THAT SATISFIES THE ABOVE

The purpose of this step is to generate an effective, efficient, and affordable sampling design (US EPA 2002). The US EPA has suggested a stepwise approach to the development of a sampling design (Figure 1). The following concepts are used in the development of a sampling design:

- **target population** - the set of all items of interest in a study,
- **sample population -** the part of the target population that is accessible and available for sampling,
- **sampling unit** - the member of the population that can be sampled,
- **sample support** - the definition of the sampling unit in terms of its physical properties, i.e., the size, shape, and orientation,
- **sampling frame** – a list of all the possible sampling units,
- **sample** – a collection of some sampling units,
- **sampling design** – a description of the number, type, and location (spatial and/or temporal) of sampling units to be collected,
- **conceptual model** – a description of the expected pathway of exposure to the contaminant of interest as well as the size of the area of concern.

For example, in the case of water sampling, the target population could be all the water in a given bay, the sample population would remove locations where sampling was not possible due to logistical constraints, and the sampling unit could be a 1-liter of water collected using either a bottle or a pump.

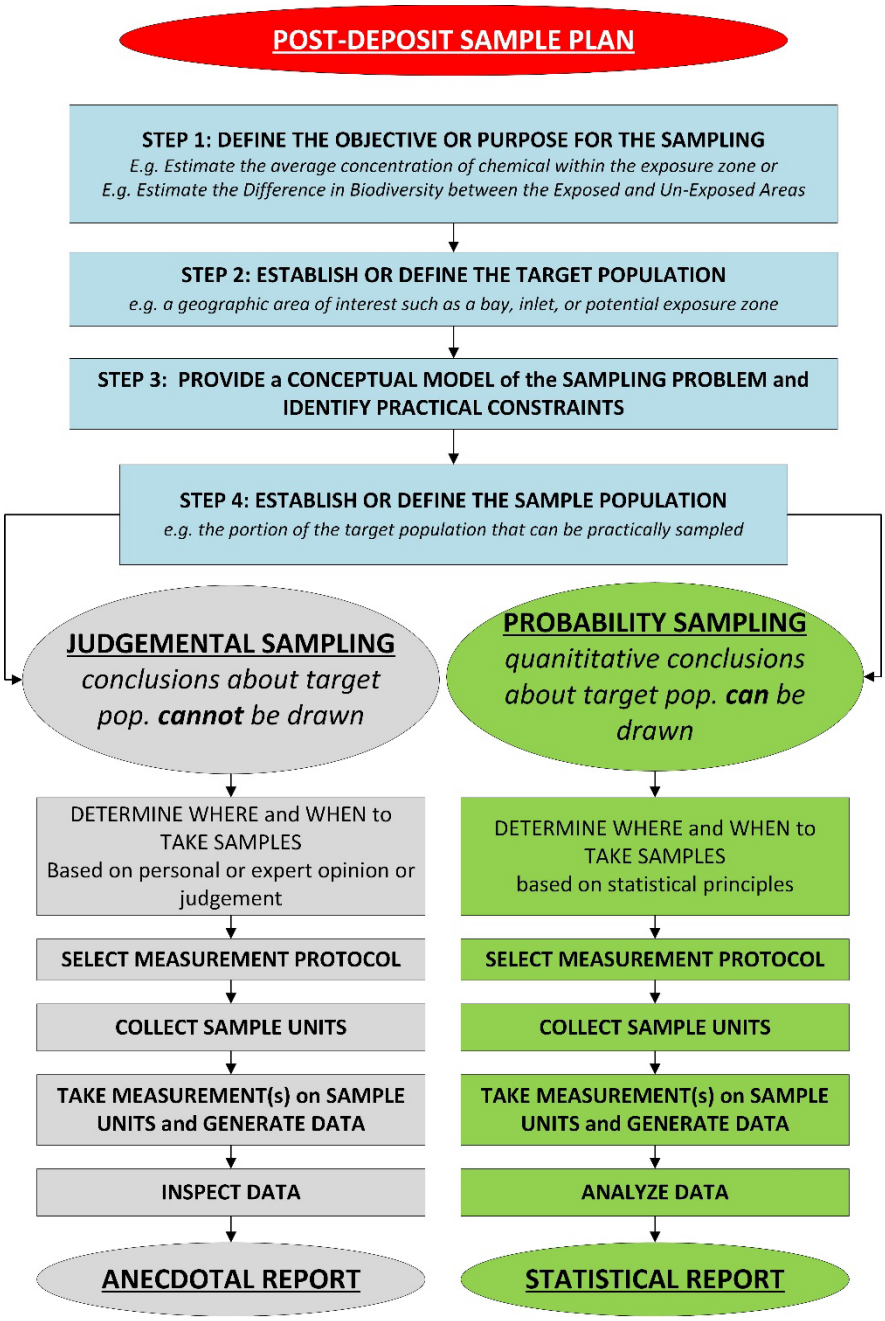

Figure 1. Flow chart outlining the steps involved in sample design based on US EPA (2002).

2.7.1. Step 7.1: Sampling purpose

The first step in the design process is to define the objective or purpose for the sampling. This information should come from Step 1 of the systematic planning process.

2.7.2. Step 7.2: Target population

In this step the target population that the decision maker wants to be able to draw a conclusion about is defined.

2.7.3. Step 7.3: Conceptual overview and sampling limitations

In this step a conceptual model is developed. The model should include potential sources of variability in the data. This step also includes the identification of the potential limitations or constraints to sampling designs and methodologies. There are four categories of possible constraints in the selection of a sampling design: sampling/analysis limitations, time constraints, geographic barriers, and budget amounts.

2.7.4. Step 7.4: Sample population

This step defines a subset of the target population that can practically be sampled and should include a pilot and/or reconnaissance survey when limited information on the target population is available (ISO 2004). Ideally, the sample population is the entire target population. However, this is seldom the case.

2.7.5. Step 7.5: Sample design selection

The final step is to select a sample design. In this step, the advantages, disadvantages, and compromises of the different sampling designs should be considered for the specific conditions of the study. Selection of a sampling design should take into account both the needs of the project and the resources available. The needs generally consist of the study objectives and the tolerable limits on uncertainty. The resources may include personnel, time, and availability of financial resources. A decision needs to be made as to whether a judgement-based or a probabilistic-based sampling design is desired (US EPA 2002). In both cases the entire domain of interest needs to be considered and both approaches can utilize relevant existing information about the area in the design process.

A well-designed sampling program ensures that samples are representative of the target population. Sample representativeness is defined as “Representativeness may be considered as the measure of the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition [American National Standards Institute/American Society for Quality Control (ANSI/ASQC) 1994]” (US EPA 2002, p. 1). A well-designed sampling program is an essential foundation piece for scientific based decision making (US EPA 2002) for it helps ensure the collected data are sufficient to draw conclusions of relevance to the question(s) the design is meant to address. Non-representative samples will not compensate for high quality sample collection, sample handling, laboratory analyses and low measurement error. Hence, non-representative samples will not meet the needs of the decision makers. Conversely, highly representative samples will not compensate for low quality sample collection, sample handling and, laboratory analyses and high measurement errors.

3. SAMPLE DESIGNS

All sample designs require multiple considerations with some of the key components being (US EPA 2002):

- a clear statement and description of the sampling objective(s),
- a statement of the measurement and/or indicator type to be collected and its relevance to the objective(s),
- whether sample collection and handling methods are appropriate to the specific purpose,
- the effect of measurement error on the statistics and conclusions generated from the samples,

- the quality and appropriateness of laboratory analyses, and
- the representativeness of the collected data relative to the objective of the sampling effort.

The following describes some possible sampling design methodologies and the conditions under which they are appropriate.

3.1. JUDGEMENT-BASED SAMPLING

Judgement-based sampling does not involve any randomization. It is based on existing knowledge of the area or population to be sampled (US EPA 2002). Although this sampling approach is often used to meet scheduling and budgetary constraints, it must be recognized that inferences generated from the sample results are based on judgement only, since inference based on statistical principles is not possible. This type of sampling should be designed by experienced and well qualified individuals, and is most useful for initial screening and scoping purposes when sample sizes are small, the time to plan the sample design is limited, existing knowledge of the scenario is reliable, sampling budgets are small, and the purpose is screening for presence and absence. Judgement-based sampling is not ideal for supporting decision making or compliance purposes; quantitative confidence levels (i.e., uncertainties) cannot be associated with the results and the results cannot be extrapolated by inference to the overall or target population (US EPA 2002).

A successful judgement-based sample design will require high quality and precise judgement to ensure samples are taken within and around the areas where deposition has occurred. Although most sampling of pesticide and drug discharges has been research-based and of this type (Appendix A), judgement designs are not preferred for compliance purposes (US EPA 2002) since they are not subject to quantitative analyses and are only as good as the expertise or model predictions underlying the judgement.

3.2. PROBABILISTIC-BASED SAMPLING

Probabilistic-based sampling is based on randomization in the selection of possible sample locations and times from the target population. Although the approach requires judgement in defining some of the design aspects, it is much less subject to judgement variation, allows uncertainties of estimated statistics (e.g., mean) and extrapolation or inferences about the target population to be made (US EPA 2002). This approach is the preferred approach for supporting decision and compliance purposes.

There are several potential approaches to probabilistic sampling. Some of the more common approaches include (US EPA 2002):

- simple random sampling,
- stratified random sampling,
- systematic and grid sampling,
- ranked set sampling,
- adaptive cluster sampling.

All of these sampling approaches have associated formulas for calculating specific statistics (e.g., mean) and uncertainties. We do not review these details in this document; our focus is on what sample design approach may be appropriate for the aquaculture situations being considered.

Probabilistic designs for detecting areas of interest require the sampling design to have a spatial and temporal resolution that is sufficient to detect the expected exposure zone (US EPA 2002). There are several design possibilities for accomplishing this, including gridded, random, transect, and judgement designs. Gridded designs are preferred; they are the most efficient and enable quantitative inferences (US EPA 2002).

3.2.1. Simple random sampling

Simple random sampling is the simplest probability-based sample design (US EPA 2002). It assumes each unit of the target population has an equal probability of being selected. The design protects against bias that may be introduced by subjective judgement of where and when to sample. It is appropriate when the area to be sampled is uniform or homogeneous in terms of the characteristics to be measured. The approach does not take advantage of existing and reliable information and is the weakest of the described designs for detecting discharge areas of unknown location and size.

3.2.2. Stratified random sampling

Stratified random sampling uses information to split the total population area into non-overlapping groups (strata) that are sampled independently (US EPA 2002). All units within the population are part of a single stratum. In the context of discharges from net-pen operations the strata may be geographic domains for different bottom or habitat types or different distances from the discharge points and/or different time windows relative to the initiation of discharge. The temporal stratification can be useful for monitoring trends over time. Each stratum can be sampled using a different methodology which is useful when the strata represent different bottom types and sample sizes can be assigned to each strata based in various ways including equal allocation, proportional allocation, or optimal allocation. The location of the samples within each stratum can be determined by any suitable sampling design such as gridded or random allocations. The design enables a more representative sample of the target population than simple random sampling and may result in higher precision estimates of calculated parameters. The success of the design depends on the representativeness of the strata choices and the allocation of sample sizes and hence on the accuracy of the information used to guide these choices.

3.2.3. Systematic/grid or regular sampling

Systematic/Grid or Regular sampling consists of collecting samples in a specified spatial or temporal pattern (US EPA 2002). The approach is used to ensure the target population is fully and uniformly represented. The approach is well suited to exploring correlations between the measurements made on each of the samples. The probability of detecting spatial and temporal features is determined by the relative size of the features and the size and shape of the grid elements. When the distance between sample locations is less than the size of the feature to be detected, the probability of detecting the feature of interest is near or equal to one, whereas when the distance between sample locations is greater than the scale of the feature to be detected, the probability of detecting the feature is less than one. Randomization of the sampling location is achieved by either randomly choosing the initial location of the grid or by randomly choosing the location of samples within each grid cell. The former can result in problems when the feature occurs in some fixed pattern. The latter approach will not have an equal distance between samples and hence may miss some features. Gridded sampling should be used when hot spots need to be detected, when the size of features is to be estimated, and/or when measurements are correlated or exhibit a spatial or temporal pattern. The most efficient grid shape is an equilateral triangle (US EPA 2002) with a square grid having a similar

efficiency. Systematic sampling provides the largest spatial or temporal coverage for a given sample size, and the spacing between samples can be varied by sub-dividing the total area into different strata needing different grid scales. The approach may be used to initially detect the spatial presence of features and then temporal sampling can be focused on selected features. To achieve this a subset of the stations that detected a feature of interest are randomly selected and sampling is repeated at each of these stations through time (Van der Meer 1997). This is the most efficient way to gain robust probabilistic measurements.

The size of the exposure area to be detected dictates the size of each grid element or the distance between grid points (US EPA 2002). The size of the elements then dictates the number of sampling points. To ensure the detection of the discharge cloud the distance between sample grid points must be less than the length scale characterising the discharge cloud (US EPA 2002). If the distance between sample grid points is greater than the length scale characterising the discharge cloud the probability of detecting the cloud is less than one. In the case of net-pen farming, the size and location of the discharge cloud or exposure area varies with time, the type of treatment (including the chemical released and the mode of administration), and the location of the discharge.

3.2.4. Ranked set sampling

Ranked set sampling consists of initially collecting screening measurements according to a specific sample design and then ranking this information to select a sub-set of sample locations that will be sampled for actual measurement as opposed to indicator measurement (US EPA 2002).

3.2.5. Adaptive cluster sampling

Adaptive Cluster sampling consists of sampling designs that include an initial probability-based sampling effort whose results determine where secondary samples should be taken (US EPA 2002). The secondary samples are only taken where the initial sampling indicates the characteristic of interest meets some prescribed condition. The design is most useful when the characteristic of interest is sparsely distributed and highly aggregated and when results from the initial sampling are available in time to design and implement the secondary sampling. The design enables the distribution, the spatial extent, and the mean of the measure of interest to be estimated along with uncertainties associated with the estimates. It also allows for more comprehensive measurements to be made during the collection of the secondary samples. The sampling is particularly advantageous when the initial sampling can be conducted relatively rapidly and cheaply, and results can be obtained quickly. When the characteristic of interest is widely spread the design may not be as advantageous.

4. TREATMENT AND DISCHARGE PROCESSES

4.1. PESTICIDES

4.1.1. Administration route and discharge

In the case of salmon net-pen aquaculture, pesticides are administered in one of two ways; by net-pen tarping or by use of a well-boat. In both cases the pesticide is dissolved and distributed throughout a volume of bath water that contains the fish being treated. The treatment works on the principle that the target pest, which is external to the fish, is exposed to the pesticide in the bath water for a specified period of time.

Once the treatment time has elapsed the bath water is discharged into the ambient coastal waters. The treatment discharge cloud is initially the size of the net-pen or initial discharge jet; the size increases with time due to local hydrographic dispersal processes. The concentration of the discharged chemical decreases due to the dispersal and decay processes and the location continually changes due to the local physical hydrographic transport processes (i.e., advection). These concepts are illustrated in Figure 2.

4.1.2. Discharge characteristics and uncertainties

The discharged pesticide has the potential to expose and impact both pelagic and benthic organisms and habitat types. The exposure profile depends upon the details of the administration and discharge processes, the ambient environment conditions (hydrography including four-dimensional water velocity, water temperature, salinity and dissolved oxygen, light penetration, turbidity, organic content, etc.), and the behaviour of the chemical.

The area, shape, location, and concentration characteristics of each discharge are unique; they differ among sites, among releases from a single site, and among individual net-pen and well-boat discharges. Judgement-based knowledge of these characteristics is qualitative, uncertain, and expert dependent. Model-based knowledge concerning these characteristics is in its infancy and only provide qualitative and semi-quantitative guidance. The models are generally not well validated, are almost never calibrated to specific local and discharge scenarios (i.e., treatments, locations, etc.), are subject to uncertainties in inputs and parameterizations, and involve spatial and temporal averaging of outputs. Results often deviate substantially from observed values due to model and observation uncertainties and variabilities (Page et al. 2015, 2023a).

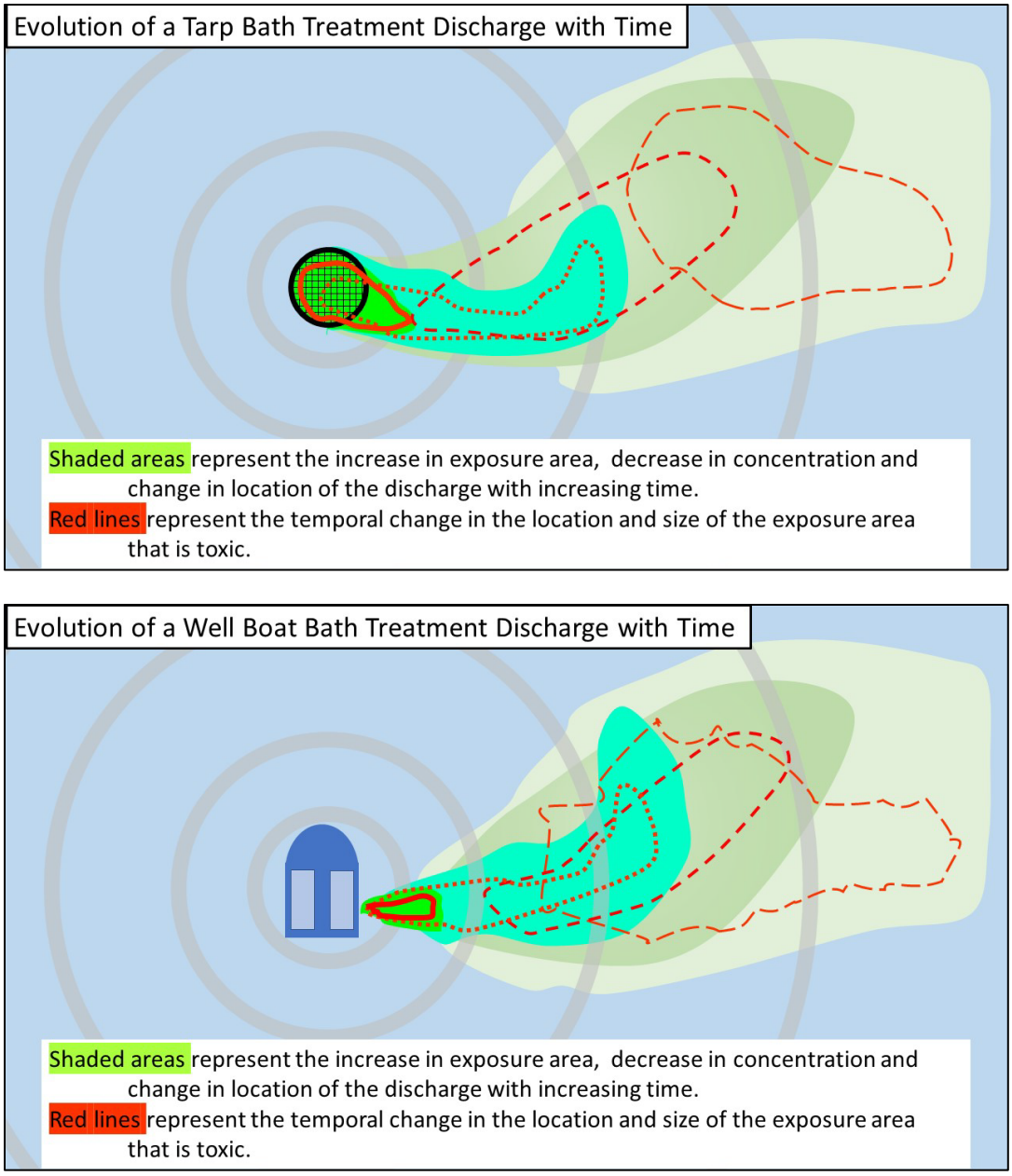

Figure 2. Illustration of the temporal evolution of bath discharges from a net-pen tarp treatment (top panel; the black circle represents the treated cage) and a well-boat discharge (lower panel). The grey circles indicate radial distance from the net-pen or well-boat.

4.2. DRUGS

4.2.1. Administration route and discharge

In the case of salmon net-pen aquaculture, drugs are administered orally in the form of medicated feed pellets to the fish within a net-pen through normal feeding procedures. The treatment works on the principle that the target pathogen or pest is exposed to the drug through exposure related to the presence of the drug in the fish tissues, skin, and/or mucous layer.

Environmental discharge of the drug takes several forms and pathways. Some of the drug settles to the bottom as part of uneaten waste feed; and the remainder of the drug is ingested by the fish and is eventually egested in feces or excreted through the skin, gills, or urinary processes. A portion of the drug may also be discharged as fines which could be generated as the feed travels through feed delivery systems. The discharged drug is therefore in several forms: in adsorbed form attached to waste feed and in fecal pellets; in dissolved form from excretions and leaching from the adsorbed forms; and as medicated fines that may result in a pelagic exposure. In the case of egested and excreted forms a portion of the drug may also be discharged as a metabolite produced by metabolic processes in the fish and the environment.

4.2.2. Discharge characteristics and uncertainties

All forms of discharged drug have the potential to expose and impact both pelagic and benthic organisms and habitat types for periods of time. The exposure profile depends upon the details of the administration and discharge processes, the ambient environment conditions (hydrography including four-dimensional water velocity, water temperature, salinity and dissolved oxygen, light penetration, turbidity, organic content, etc.) and the behaviour of the chemical.

The area, shape, location, and concentration characteristics of each discharge are unique; they differ among sites, among releases from a single site, and net-pen discharges. Judgement-based knowledge of these characteristics is qualitative, uncertain, and expert dependent. The initial size of the discharge is the size of the feeding area within a net-pen and increases with time. In the case of discharge particles that settle, the size of the discharge increases until particles are deposited on the seabed. Once on the seabed, the deposition area and concentration can continue to change due to remobilization and redistribution processes. The excretion and leachate discharge clouds change continuously. The time to deposit varies with the sinking rate of the discharged particles and the depth of the water. The full benthic exposure profile is the composite of exposure associated with waste feed, fecal pellets (ranging from well formed fecal pellets to fecal slurries), and fines. Model-based knowledge concerning these characteristics is in its infancy; models are generally not well calibrated, seldom calibrated to specific local scenarios (i.e., treatments), are subject to uncertainties in inputs and parameterizations, and the results often deviate substantially from observed values due to the model and observation uncertainties and variabilities (SAMS 2005; Page et al. 2023b). The model outputs should therefore be considered as semi-quantitative predictions rather than precise predictions.

When the treatment is conducted in an area or at a time when the water current is weak, the discharged particles will not be transported far from the net-pen before they settle to the bottom. In this scenario, the size of the deposition area can be assumed to be similar to the size of the net-pen or the net-pen array. The deposition area for a 100 m polar circle net-pen (diameter 32 m) is 800 m^2 and the size of a typical net-pen array consisting of two rows of five net-pens each is about 25 000 m^2 (2x50 x 5x50 assuming the spacing between net-pen centers is 50 m). The distance between grid points in a triangular design must therefore be ~30 – 50 m to ensure detection of the individual net-pen deposition patches and between 100 – 250 m to ensure detection of the whole net-pen array deposition patch. Although the numbers vary with individual net-pen and net-pen array dimensions, water depths and water currents, and the settling rate of the particles, the discharge footprints usually have radii of hundreds of meters and areas of order 10^4 square meters or more. This means a large number of grid points must be sampled to ensure the detection of the initial deposition patch.

5. SAMPLING METHODOLOGY

Once a sample design has been completed, appropriate sampling methodologies and procedures need to be chosen. These procedures vary with the type of measurements to be taken and the medium to be sampled. If sampling is to be conducted for regulatory purposes, then clear guidelines and standardized sampling procedures need to be established. Monitoring of physical media (water, sediment) provides the opportunity to assess exposure to a number of contaminants. Mandatory requirements in many regulations are still based on total concentrations of contaminants in these matrices.

In the case of deposits associated with aquaculture net-pen bath pesticide and in-feed drug discharges, the relevant measures are the concentration of the discharged chemical and indicators of impact resulting from exposure. Ideally the indicators are chemical specific. This document does not present information on relevant indicators.

The sample matrices of relevance to pesticide and drug discharges from net-pen fish farming operations are water and sediment. Sometimes the bottom is classified as soft or hard where the classification is based on whether or not the bottom can be sampled by a grab (SEPA 2019b).

This document does not review or discuss sampling methodology in detail; it only provides brief descriptions. However, any sample design that is to be implemented must consider appropriate sampling methodologies. Aspects of laboratory analyses of relevance to aquaculture pesticides and drugs are considered in a companion document (Wong et al. 2022).

5.1. WATER COLUMN

Water samples associated with measuring concentrations of pesticides or drugs in the water are usually collected with Niskin type bottles or water pumps, either surface or subsurface. To enable sampling of the discharge plume after release the use of a visual indicator (e.g., fluorescein dye) to track its trajectory is essential. Samples are typically collected into pre-cleaned amber glass jars with Teflon® lined lids then transported and stored at approximately 4°C (freezing samples for storage is a less widely used alternative). The effect of storage time and storage conditions on the measured concentration of chemical has not been well studied, therefore analysis of samples should be conducted as soon as possible (within days) since microbial degradation and hydrolysis reactions can degrade the chemical components within the samples further. If, however, analysis cannot be performed in a timely manner, either preconcentration of the analytes onto solid phase extraction (SPE) disks or addition of a chemical preservative (e.g., dichloromethane) to the water sample is possible to extend chemical stability (Lyytikäïnen et al. 2003; Aboulfadl et al. 2010). The effect of storage time and storage conditions on the measured concentration of aquaculture chemicals has not been well studied. Storage factors that may be of significance are those conditions experienced between the time of collection to analysis, storage temperature (Lyytikäïnen et al. 2003), and the effect of freeze/thawing of the samples especially for determination of pesticide, drug, and antibiotic concentrations.

Sampling of discharged pesticides in a meaningful way is challenging since the pesticide is colourless, the discharge cloud is constantly moving (i.e., changing position and shape), and the distribution of pesticide throughout the cloud is constantly changing. Effective water sampling can only be undertaken when either a visible indicator such as dye is added to and thoroughly mixed throughout the discharge prior to release or drifters are released into the discharge cloud. Actual water samples can then be taken relative to the location of the indicator(s). The effectiveness of the indicators is greatest when the pesticide being discharged remains in suspension and behaves passively; the tracers can quickly separate from the pesticide

discharge when the discharge sinks due to the pesticide binding or adsorbing to sinking suspended particulates. The use of a dye tracer is preferred since it colours the entire discharge volume and is transported and dispersed in three dimensions along with the pesticide; drifters provide only a few reference points and only indicate the movement of the discharge at the depth over which the drogue portion of the drifter is suspended. Both of these approaches have been used by Ernst et al. (2001, 2014) and Page et al. (2015); and both are appropriate for use with the two pesticides presently registered for aquaculture use in Canada (hydrogen peroxide and azamethiphos). Both approaches are most useful for indicating the location of the discharge during the first few hours after release. After a few hours the dye often becomes difficult to see and the drifters may have travelled beyond the discharge patch, particularly when surface or near-surface drifters are used.

Waste feed and feces that are settling through the water column can be collected by sediment traps. The traps need to be properly designed and have a sufficiently large collection surface so the probability of collecting waste feed and fecal pellets is reasonable. Multiple traps will need to be deployed since the vertical flux of the feed and feces likely vary in intensity and hence the location of the depositions will be uncertain. The traps should be suspended at depths below the bottom of the net-pens and below the depth to which wave action extends so surface waves do not induce resuspension of material from the traps. The horizontal distribution of the traps will need to encompass the anticipated direction and area of deposition (Figure 3). The distance travelled (D) by a settling discharge at a rate of W_s in a horizontal current with speed of U is given by $D=UT$ where T is determined by how long it takes for the discharge to settle to the depth of the sediment traps. For example, waste feed that settles at a rate of 0.1 m s^{-1} and an ambient current speed of 0.1 m s^{-1}, the feed will sink 10 m in 100 s, 30 m in 300 s and be carried horizontal distances of 10 m and 30 m respectively. This suggests traps suspended a few tens of meters below the bottom of the net-pen should be deployed within a few tens of meters from the edge of the net-pens; if they are deployed at greater distances, they may not intercept the discharge of waste feed. The traps should be deployed for multiple treatments to improve the probability of collecting material. If using sediment traps is deemed useful, further consideration should be given and perhaps some research conducted to determine whether the approach is useful for regulatory purposes and if so, generate guidelines concerning where, when and for how long the traps should be deployed and what type of traps should be used. See Stucchi et al. (2005) for a discussion on the shortcomings and difficulties associated with the use of sediment traps. Measurements made on sediment traps are known to provide variable amounts of sediments, e.g., a factor of five in sediment waste flux was found between traps spaced a few meters apart as reported in SAMS (2005).

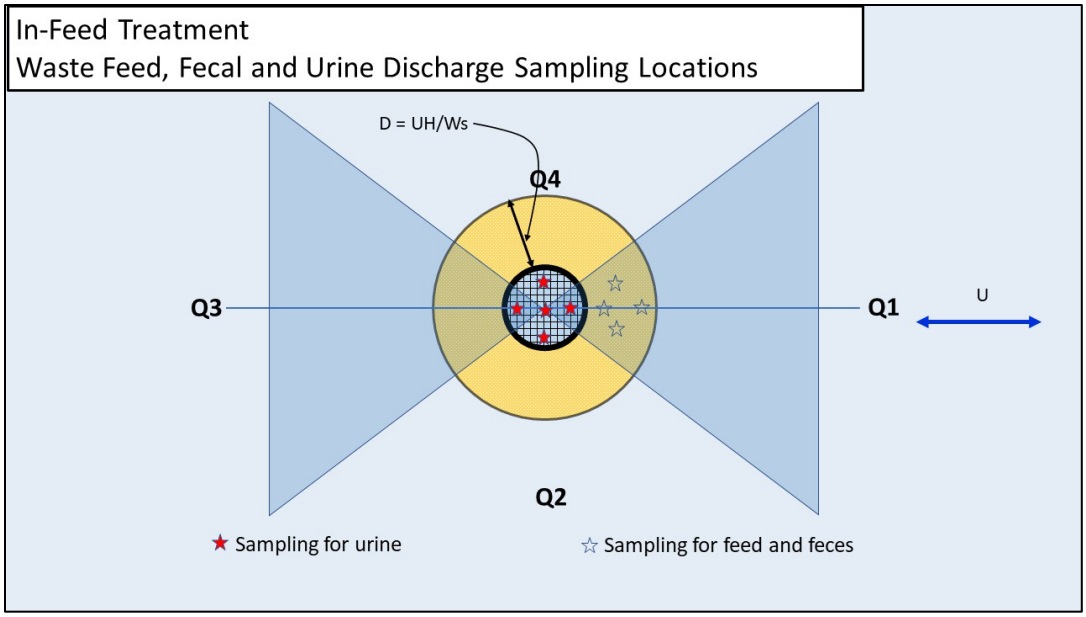

Figure 3. Diagram illustrating locations for discharge sampling for waste feed and feces from an in-feed drug treatment of a net-pen. The triangles illustrate the sampling quadrant relative to the direction of the current at the time of discharge. The beige circle indicates the estimated perimeter of the benthic potential exposure zone. D is the distance travelled by a settling particle independent of direction, U is the dominant water velocity, H is the water depth and Ws is the settling rate. Q1 to Q4 are quadrants. Stars are randomly selected sampling stations within the quadrant strata.

Sediment traps may be most useful for sampling fecal discharges, both for estimation of vertical fecal flux and for in situ collection of fecal material for analyses of drug content. The considerations associated with using sediment traps for fecal discharges are similar to those discussed above for waste feed. A significant difference is that feces settle more slowly than waste feed, the form of the egested feces is not always a well-formed fecal pellet, and the sinking rate of the feces is not well known a priori. Feces, in general, travel a longer distance from the treatment net-pen than waste feed before being deposited. Furthermore, the deposition area is larger because the distribution of fecal sinking rates is wide and uncertain. These factors make deploying sediment traps in the path of fecal discharges more challenging than deploying sediment traps for waste feed.

Although the concentration of chemical from excretions (via kidneys or gills) may be low and perhaps non-detectable, sampling of the water within the net-pen after an in-feed treatment may be useful to help determine the concentration of dissolved chemical released into the environment. Sampling may provide empirical evidence of the duration of the excretion which is useful for choosing and evaluating exposure models. The information could also be of interest to regulators if thresholds are established for discharge concentrations.

Several sampling approaches may be explored. One approach may be to take water samples from multiple horizontal and vertical positions within the net-pen at multiple times over the expected discharge duration period (Figure 3). A second potential approach may be to moor passive samplers inside the net-pen at the same locations (Figure 3). The samplers should be deployed soon after the administration of medicated feed ends and be left in the net-pen for about a week. Samplers should be moored at several horizontal and vertical locations within the net-pen to account for potential spatial variation in concentrations. The samplers will absorb the chemical leached from egested feces and excreted in urine over the week. The accumulated amounts can be examined for spatial homogeneity. It is perhaps best to consider using this

approach when the drug that was administered has a high absorption rate; excretions from drugs that have low absorption rates will be very small. In either case the concentrations of active ingredient are expected to be very low, and since these concentrations will be rapidly diluted as soon as they are leached from the waste feed and feces it may not be worth the sampling effort. Research will be needed to test the feasibility and utility of this approach, including links to both models and decision rules.

Sampling immediately after discharge may not always be worthwhile since excreted concentrations are likely very low. For example, if the time needed for the chemical concentration to reduce below its analytical detection limit is so short that it is not practical or feasible to obtain replicate samples from meaningful locations and times, there is limited value in trying to obtain samples. As a rule of thumb, we suggest 0.5 h as a sample or no sample threshold. If the time to decay or dilute to limit of detection (LOD) is less than 0.5 h we suggest it is not worth sampling the post-deposit discharge since, if any samples could be collected during the short time period, their concentration would be close to or below analytical detection limits. A research sampling program is required to confirm this thinking.

5.2. SEABED

There are many bottom types; here they are considered in two categories: soft-bottom and hard-bottom. Sampling methodology necessarily differs between each category. Acoustic technology such as sonar can be useful for mapping bathymetry and determining bottom type. Visual imagery can sometimes be used to resolve bottom type. Often visual imagery is difficult to acquire due to water turbidity, the challenges of providing diffuse light of sufficient and consistent intensity, and the difficulty in obtaining imagery with a consistent resolution, clarity, distance, and angle above the seabed. The cameras are carried by divers or mounted on various remote platforms including drop platforms, remotely operated vehicles, or autonomous underwater vehicles. Visual imagery cannot collect physical samples for later analytical analyses. However, the imagery is useful for estimating the presence/absence and abundance of macro flora and fauna on both hard and soft bottoms and has the advantage that samples (images) can be reanalyzed. For example, Wilding et al. (2012) used video transects to survey the megabenthos around a net-pen array in Scotland; Hamoutene et al. (2016) analysed video monitoring for benthic changes at hard bottom aquaculture sites in Newfoundland. Additionally, both methods need to be ground-truthed to ensure understanding of in-situ reality.

5.2.1. Soft-bottom substrates

Sediment is an integrative matrix for most of the contaminants entering the aquatic environment. However, in addition to the spatial heterogeneity due to hydrodynamics, sediments themselves are a complex matrix, including both mineral and organic fractions (Amiard and Amiard-Triquet 2015). Sediments represent a record of past contamination but at different time scales according to whether they are located at the sediment surface or subsurface. The mineral fraction of sediment itself is complex, with different particle size and different mineral composition. Sediment textures are classified by comparison with the size fractions present in a soil (sand, silt, and clay). The classification generally accepted is based on the equivalent spherical diameter as follows: gravel 2 mm – 2 cm, sand 0.05 – 2 mm, silt 0.05 – 0.002 mm, clay <0.002 mm (Wentworth 1922). At the opposite end of the spectrum, organic contaminants generally do not correlate linearly with the grain size distribution, whereas they generally correlate more strongly with organic matter content. Ensuring a consistency in the thickness of the samples (i.e., the depth below the sediment surface, e.g., 0 – 2 cm) is important and necessary for a monitoring program.

Bottom sediments are usually collected with grab or core samplers. Core sampling, especially by diver, is generally recognized as having the capability of collecting a higher quality sample because the bottom sediment is minimally disturbed during capture and retrieval. Surface-deployed core samplers, however, often require a larger vessel for sampling because high quality cores often require a heavy coring apparatus. Grab sampling is used more frequently, even though it is generally recognized that it often results in a lower quality sample than core sampling. This results from factors such as grab descent rate and bow wave effects as well as potential disturbance of the sample upon grab retrieval. Grab samplers only penetrate a few centimeters into the sediment whereas some core samplers may penetrate further where the penetration depth depends on the sediment characteristics, weight of the corer, and method of coring. Sampling programs for deposits of pesticides and drug are usually only interested in the surface and/or top few centimeters of the sediment.

From a grab collector, subsampling of the collected sediment can be performed by draining off the upper water layer then using a scoop (made from an inert material, e.g., HDPE, Teflon®) to scrape off the upper layer. For core samples, the overlying water layer has to be siphoned off then the sample extruded to allow access to the sediment sample. Subsampling then follows the same procedure as for the grab. The depth of sediment taken as a subsample for analysis depends on the aim of the study. If determining chemical concentrations related to recent deposition activity is the goal of sampling, then removal of sediment from the 0 – 1 cm or 0 – 2 cm layers are deemed the most appropriate depth (US EPA 2001; Batley and Simpson 2016). The depth of subsampling has an important influence on derived measurement of chemical concentrations due to the heterogeneity of the sample. For example, if the chemical is deposited on the sediment surface, e.g., up to 1 cm deep, but the sediment subsample is taken from the top 5 cm, the derived chemical concentration is diluted relative to the actual concentration. This dilution effect increases as the difference between the real depth of deposition and the subsample depth increases. This dilution effect would also be evident if replicate subsamples were taken from the same grab/core sample then composited to produce a pooled sample for analysis.

For analytical purposes, the volume of surficial sediment required will depend on the selected physicochemical test(s), the laboratory performing the analyses, and the analytical method used. Excess sample should be taken to facilitate repeat analysis if required. Subsamples should be stored in appropriate pre-cleaned containers, e.g., high density polyethylene (HDPE), polytetrafluoroethylene (PTFE aka Teflon ®), or amber glass jars with Teflon® lined lids. It should be noted that other container materials can be used depending on the type of test and analyte(s) to be determined (Environment Canada 1994). Samples should be frozen on the same day as they are collected (SEPA 2018) and stored at -1 to -20°C (ISO 2004) to minimise degradation of the sample and analytes.

The samples should be analysed for chemical content using a recognized and validated technique (Wong et al. 2022). Sample analyses should also include determination of sample porosity and moisture content to enable concentration reporting as a dry weight basis for consistency with environmental quality standard (EQS) reporting.

In a recent Scottish study, grabs were used to retrieve sediment from the seabed and once the grab was back onboard the sampling vessel a corer was used to collect a subset of the sediment for chemical analyses (SEPA 2018). SEPA specifies that, for benthic sampling, a Van Veen or similar grab with top opening flaps should be used (SEPA 2008) which allows access and visual examination. A minimum sample size of 0.02 m^2 is required and 5 replicate samples for biological analysis and 2 replicate samples for chemical analysis must be collected at each location. If a larger grab of 0.1 m^2 is used, then only two replicate samples are required for biological analysis. SEPA (2008) further requires that separate grabs should be taken for

chemical and physico-chemical determinates. Finally, all sampling equipment, including the grab, should be washed between sample collections. There is a considerable amount of experience within the aquatic community related to the use of different types of bottom sampling equipment. A summary of some recent experiences from across Canada is given in Table 1.

Generally speaking this experience indicates that more than one type of gear can be used to obtain quality samples, that different sampling gear works best in a subset of bottom types and current conditions, and that certain gear is best suited for some types of sampling circumstances (for example, the type of surface vessel that is available for gear deployment). In all cases, however, the goal should be to collect an undisturbed sample. The collection of undisturbed samples of the sediment water interface is challenging, particularly when sampling in rough conditions. Useful methods to collect undisturbed samples include the use of slow-corers, video cameras, and grab windows. The criteria defining this determination should be developed and articulated. These criteria may include the requirement to make video recordings of the sampling and subsampling processes and examination of the video for signs of resuspension.

Table 1. A compilation of sampling challenges encountered during some recent sampling activities undertaken by the Aquaculture Monitoring Program (AMP) that is being developed by Fisheries and Oceans.

Province	Region	Gear used by the AMMP	Substrate type	Depth limitation	Volume sampled*	Challenges and solution
Newfoundland	South Coast of NL	Tall Eckman grab (6''x6''x9")	Hard substrate with patches of soft sediment	Depths are > 30 m with important slopes and in some situations depths greater than 100 m	125 – 1475 mL	***Challenges:*** - the absence of a sediment layer due to hard substrates in some areas prohibits grabbing - hard substrates can cause damage to the sampling gear and depth and slope can affect its closure. - the small size of the grab and its low weight combined with drifting due to weather and depth introduces an angle delaying closing and issues in the precision of the sampling point ***Solution:*** - add weights to the jaws - using video cameras to assess the substrate type at every station before deploying gear helps in limiting issues.
Nova Scotia	-SW Nova Scotian shore (Jordan Bay, Liverpool, etc.) -Bras d'Or Lakes	Slo-Corer	-Very fine, compacted sands or mixed bottom (i.e., mud, sand, gravel, cobble, shell debris) in SWNS -Generally mud or mixed bottom in Bras d'Or Lakes	-Relatively shallow: depths < 20 m with flat or gentle slopes. -Some sites in the Bras d'Or Lakes have depths ~50 – 90 m.	< 100 mL	***Challenge:*** Mixed bottom in some areas can make the use of a corer challenging. ***Solution:*** The use of a large Ekman grab (9''x9'') can be considered as an alternative.
New Brunswick	Bay of Fundy	Haps corer	Generally mud or mixed bottom (i.e., mud, gravel, cobble, shell debris)	Relatively shallow: depths < 20 m with flat or gentle slopes.	126 mL	***Challenge:*** Mixed bottom in some areas can make the use of a corer challenging. ***Solution****:* The use of a large Ekman grab (9''x9'') can be considered as an alternative.
British Columbia	Clayoquot Sound	VanVeen Grab	Generally mud	Depends on site (10 – 100 m)	1008 mL	***Challenge:*** losing the top layer at grab closure (issues with national consistency of sampling). ***Solution:*** The use of a corer should be considered as an alternative.

Province	Region	Gear used by the AMMP	Substrate type	Depth limitation	Volume sampled*	Challenges and solution
British Columbia	Discovery Islands	VanVeen Grab	Mixed Bottom	Depends on site (30 – 200 m)	1008 mL	***Challenge:*** the absence of a sediment layer due to hard substrates in some areas prohibits grabbing. Important depths and high currents make grabs challenging. ***Solution:*** Solutions proposed with the NL context could be applicable.

* indicates this volume is for the top 1 cm of the sediment collected by the grab or core.

5.2.2. Hard-bottom substrates

Hard bottom substrates are usually sampled visually with video or still imagery; it is often difficult to obtain physical samples from hard bottoms for chemical analyses although some suction sampling and/or adapted grab approaches may be feasible. Direct evidence of hard bottom substrates being exposed to discharges of pesticides and drugs is difficult and can be impractical and challenging to collect. Areas where hard-substrates are dominant are often characterized by little to no natural deposition; however, at the end of a production cycle wastes from aquaculture activities can be present close to net-pens. If not flushed by dynamic natural hydrographic conditions; these deposits form a flocculent matter that can be sampled and can be used to characterise chemical exposure. These samples can be in some cases where hard-bottom substrates are combined with important depths can be difficult to acquire. If enough attempts at collection are made and unsuccessful the area should be classified as not conducive to sampling.

5.3. ORGANISM SAMPLING

Organism sampling, e.g., to monitor biodiversity or chemical concentrations within tissues, is usually achieved by capturing the target organisms and sampling them after retrieval to the surface vessel. Organisms are captured in various ways including by nets, suction samplers and grabbers operated by divers or attached to remotely operated vehicles. Alternative approaches may be to utilize emerging technologies, such as eDNA, to characterize the presence of organisms. The applicability of new technologies should be accessed for their appropriateness of the application before use. Since the purpose of this paper is to explore sampling designs for chemical concentrations and not their impacts on the ecosystem, we do not address organism sampling methodologies.

5.4. SAMPLE SIZE LIMITATIONS

Water column samples associated with measuring concentrations of pesticides or drugs in the water are usually collected with Nyskin type water bottles or water pumps, either surface or subsurface. The samplers take a small volume of water; a volume that is in the order of a few liters. The target population of water samples is in the millions; one cubic meter of water contains 1000 L and most areas of interest involve areas of order kilometers and depths of tens of meters, i.e., order (1000 m x 1000 m x 10 m = 10^7 m^3 = 10^{10} L). Similarly for benthic sampling, the surface area sampled by in-situ sediment cores, or cores inserted into a retrieved grab sediment sample, is usually only a few hundred cm^2 (e.g., a circular core tube with a diameter of 5 cm has an area of about 80 cm^2) or a few thousand cm^2 (e.g., a circular core tube with a diameter of 20 cm has an area of about 1 300 cm^2). Small grabs may also collect sediment from a few hundred to a few thousand square centimeters and large grabs may collect sediment from about one square meter (i.e., 10 000 cm^2). The surface area sampled by visual samples are usually larger than for core or grab samplers. Visual still imagery usually covers areas on the order of a square meter and video imagery covers areas of hundreds to thousands of square meters. The size of sample units are therefore small in comparison to the sample domain (an area with a radius of 500 m has 785 000 m^2; order 10^8 cm^2) and the number of sample units in the target population is large in relation to the number of samples that can feasibly be taken. In all situations, the number of samples taken is typically very small relative to the number of potential samples, less than 1 billionth for pelagic and less than 100 millionth for benthic. Due to this, inferences about the target population are subject to the uncertainties associated with small sample sizes.

The discrepancy between the target population and sample population may be slightly reduced through the use of composite sampling. Composite sampling consists of taking multiple samples or subsamples at a location and time, forming a new sample by physically combining the samples by some homogenization process that does not influence the characteristic of interest, and taking an analytical measurement on the homogenized sample. The approach is useful for reducing analytical costs and for smoothing noisy samples (US EPA 2002). This sampling approach can be applied to any of the sampling designs considered, both the judgemental- and probabilistic-based approaches. The approach by itself does not constitute a probabilistic-based sampling design but can be part of a probabilistic design.

Due to the above considerations, sampling designs need to be efficient, incorporate as much credible and robust judgement as possible, and be probabilistic so they can be practical, credible, and capable of assigning uncertainties to the results. Unfortunately, the large sizes of estimated exposure areas coupled with the small size of sample units results in the need to take a large number of samples to ensure a high probability of detecting a chemical exposure area and ensure tight confidence intervals around estimated sample statistics or parameters. An additional problem is linked to the heterogeneity in the response variables (e.g., chemical concentration) that may not be accounted for in the statistical model used to estimate the parameters. Heterogeneity of substances in the seabed has been document in-field studies, e.g., Chang et al. (2013) found sulfide concentrations varied by a factor of a 100 on the scale of 100s of meters surrounding a net-pen array.

6. SAMPLE DESIGN CONSIDERATIONS

The following few paragraphs consider each of the planning steps from the US EPA (2002) in the context of pesticide and drug discharges from net-pen aquaculture operations in Canadian coastal waters. Since the goal of this document is to provide some perspective on a general approach to sample design and some suggestions for a sample design, specific details will need to be determined for each treatment scenario since locations, area, intensity, and duration of exposure areas will differ; i.e., the design will to a certain extent be site, chemical, and treatment specific.

6.1. STEP 1: STATE THE PROBLEM

In the case of pesticide and drug discharges from Canadian finfish net-pen aquaculture operations, a sampling program needs to support legislative requirements associated with the use of approved pesticides and drugs, must be affordable and implementable by government and/or third party entities, and must be conducted within time windows appropriate to characterising the deposit and impact of the discharged pesticides and drugs. More specifically, we assume the goal is to identify the location, shape, area, concentration, and duration of exposure to chemical pesticides and drugs discharged from salmon net-pen farming operations. Results from the implemented sampling program are to be used to help support policy and compliance decisions, for example determine whether concentrations and areas of exposure and/or impact are above specified thresholds.

The measurement or indicator to be derived from the samples is assumed to be concentrations of pesticide and/or drug in water, sediment, or possibly organism tissues. Sample collection methods are only briefly considered for water and sediment. Methods of organism sampling are not considered here. Aspects of laboratory analyses of relevance to aquaculture pesticides and drugs are considered in a companion document (Wong et al. 2022). Formulas to calculate statistics for the probabilistic designs can be found elsewhere, e.g., US EPA (2002) and references there in. Although literature provides some information, the magnitude of the error

associated with the design, sampling, and analytical procedures is the topic of ongoing research.

The decision maker is assumed to be staff from Fisheries and Oceans Canada National Aquaculture Management Directorate and/or staff from Fisheries and Oceans Canada Regional Aquaculture Management Offices. The former is assumed to be primarily involved in the policy decisions surrounding the development of a sampling program and the latter are assumed to primarily involved in the regional implementation and decision making associated with the sampling program.

The national and regional sampling planning teams should include at a minimum the decision maker, people knowledgeable about the scenario to be sampled, and statisticians knowledgeable about environmental sample design.

The budgets or costs associated with the program should be affordable. Sampling should be conducted when the deposits are expected to be present in the environment; the exact scheduling will be site and discharge specific.

6.2. STEP 2: IDENTIFY THE DECISION

It is critical to know what the objectives and decision rules are before a sampling program can be appropriately designed (US EPA 2002; Wilding et al. 2017; CIEEM 2018). For pesticide and drug discharges from Canadian net-pen aquaculture operations, at the time of this writing, the decisions to be taken have not yet been clearly defined. However, it may be anticipated that the questions and decisions to be considered will include:

- Is the location exposed to a pesticide and/or drug discharged from an aquaculture net-pen operation occupied by sensitive ecosystem components, including specific habitat types and organisms?
- Independent of location, is the concentration of a deposited pesticide and/or drug above a specified threshold?
- At a specified location, is the concentration of a pesticide and/or drug above a given threshold?
- Is there a temporal trend in the size of the exposure, the impact area, and/or the concentration statistics characterizing the deposited pesticide or drug? Is the direction and magnitude (rate) of the trend toward reduced exposure and/or impact and is the rate within acceptable limits?

Coming to a consensus on the objectives of a sampling program may be challenging as there are many interests and factors to consider (Wilding et al. 2017).

6.3. STEP 3: IDENTIFY INPUTS TO THE DECISION

In the case of pesticide and drug discharges from Canadian net-pen aquaculture operations the inputs have not yet been clearly defined. However, to address the potential questions identified in Step 2, they could include:

- Delineation of the geographic areas exposed to a specific chemical released from a net-pen and decide whether this area exceeds a specified threshold.
- Delineation of the geographic areas exposed to a specific chemical released from a net-pen whose concentration exceeds a specified threshold within a specified time window after discharge.

- Estimation of a particular statistic (e.g., mean, variance of area, concentration, duration of exposure, and location) associated with the chemical concentration or impact indicator within the exposed area(s) and determine whether the statistic exceeds a specified threshold or differs from control values within levels of acceptability.
- Generation of a time series of statistics, estimate temporal trends in the statistics, and test whether the trends meet specified levels of acceptability.

6.4. STEP 4: DEFINE THE BOUNDARIES OF THE STUDY

In the case of pesticide and drug discharges from Canadian net-pen aquaculture operations the target population(s) of interest is(are) likely the chemical concentrations, organisms, and/or habitats sensitive to specific pesticide and drug exposure profiles within areas defined by the regulators. The scale of the decision making is likely the individual farm site; however, in some circumstances a larger scale context of cumulative exposures and impacts may be required. For the purpose of sample design for a specific site release the scale is likely initially the area of interest. In a tiered sampling scheme, the specific scale of a secondary sampling design may be limited to areas of exposure or impact identified as being of particular interest.

6.5. STEP 5: DEVELOP A DECISION RULE

At the time of writing, it is not known to the authors what Fisheries and Oceans Canada decision rules will be for the Canadian situation, since they are being developed in parallel with the preparation of this document. The following is therefore a brief overview of aspects that may be considered by those setting the compliance thresholds and some of the aspects we have pondered when considering potential sample designs. This uncertainty makes recommendations of sampling designs difficult.

There are usually several components to compliance thresholds: concentrations; areas of exposure, deposition, and/or impact; duration of exposures; and impacts. Toxicity concentration thresholds are usually derived from the examination of toxicity data collated for a range of species that are meant to be representative but may not be found in the receiving environment. Threshold concentrations should be based on the concentrations that result in toxic effects to the chosen test organisms at exposure durations that are the most representative of the scenarios being considered. This is more easily done for bath pesticide where the exposure duration is relatively short and the pesticides are not persistent in the environment (Burridge and Holmes 2023; Hamoutene et al. 2023). Drugs that are deposited on the seabed may persist for long durations, e.g., SEPA (2019c) uses an in-situ decay half-life of 250 days for emamectin benzoate; Bloodworth et al. (2019) report finding teflubenzuron on the seabed near or around Scottish net-pen farms three and a half years after the last use of the chemical. As a result, there may be toxic effects that are difficult to determine due to multiple intermittent short-term exposures or effects associated long-term chronic exposures. Thresholds may be estimated for both acute and chronic effects; where acute effects are manifested after short-term exposures to the threshold concentration and chronic effects result from longer term exposures to lower concentrations, for example, by reducing fecundity, feeding rates or changing other behaviour, e.g., mate finding and many more. Concentration thresholds associated with acute effects are usually higher than those associated with chronic effects (Hamoutene et al. 2023).

Concentrations and durations causing toxicity are largely determined by conducting controlled laboratory experiments involving exposures to a range of concentrations for set periods of time. Toxicity thresholds may change with the duration of exposure. The toxicity of a substance depends on the concentration of the substance, the length of time an organism has been exposed to the concentration, and the sensitivity of the organism. Ideally, the exposure times

expected for the environmental situation of interest should be emphasized; however, adequate information is not always available. Ongoing research is needed to significantly improve this situation; better empirical information on the effects associated with laboratory and in-situ exposure durations and concentrations are needed as well as more accurate predictions of expected exposure profiles. More specific information of environmental compliance thresholds and EQSs are given in Hamoutene et al. (2023).

In recognition of the above complexities and information limitations, in-situ environmental compliance thresholds may have several components: a concentration, an area, a time scale and an adjustment factor that attempts to account for uncertainties in the data and its extrapolation to in situ conditions.

Threshold concentrations are usually specified, but area and time may not be explicitly specified. As a precautionary approach, regulators may assume any exposure (i.e., an exposure duration greater than zero) to an environmental compliance concentration is sufficient to cause the toxic consequences. In this case, the desire is to avoid exposure to the toxic concentration. In some cases, the requirement may be that the threshold concentration cannot be exceeded for any longer than a specified time scale (e.g., three hours). SEPA (2019a) has used this strategy for short term environmental standards for bath pesticide treatments. Another definition of the environmental compliance threshold is that the concentration must be below a specified level within a specified area and the duration of exposure is not part of the compliance specification. An example of this approach is the mixing zone compliance threshold for in-feed pesticide treatments used by SEPA (2019a). SEPA defines two threshold concentrations, an allowable maximum concentration within the mixing zone and an allowable maximum outside the mixing zone. It is also possible to combine both area and exposure duration with a threshold concentration by specifying a maximum area over which the threshold concentration can be exceeded for a maximum duration of time (Page et al. 2023a). SEPA uses this approach for its long-term quality standards for pesticide bath treatments (SEPA 2019c).

For chemicals that remain within the water column the area of the discharge cloud increases indefinitely, the concentration within the cloud continuously decreases, and the location of the cloud constantly changes. The maximum time any organism could be exposed to toxic concentrations is the time for the maximum concentration to dilute or decay to the compliance threshold concentration. If this dilution time is less than the duration assumed in the compliance threshold the cloud is assumed to be non-toxic; if this dilution time is greater than the compliance threshold then toxic exposures are possible but will depend on several factors (Page et al. 2023a). Whether organisms experience a toxic exposure depends on when they are first exposed to the cloud and for how long the organism remains in the cloud. The maximum potential for toxicity is for organisms that join the cloud at the point of discharge and remain within the maximum concentration within the cloud. The potential for benthic organisms to experience a toxic exposure depends on when the cloud contacts the bottom and how fast the cloud moves over the bottom.

For chemicals that settle to the seabed via waste feed and feces, only a portion of the exposure footprint may contain sufficiently high concentrations and durations to illicit toxic consequences. If the chemical persistence time is less than the exposure duration associated with the compliance threshold then no toxic effects are expected. The maximum exposure duration for a benthic organism is that associated with a non-motile organism or any organism that cannot or does not move away from the toxic deposit.

When the regulatory or policy objective is to prevent toxic concentrations from entering the environment, area and duration thresholds do not need to be developed. However, in the case of net-pen aquaculture operations, the discharges of pesticides contain toxic concentrations that

are spread out over space and diluted over time. The net-pen discharges of drugs may also contain toxic concentrations that are deposited over some area. Drugs that persist in the environment may accumulate to toxic concentrations and deposits may be redistributed spatially over time. Given these spatial aspects, sampling designs should probably be sufficient to provide estimates of the area and locations of deposition and impact.

To our knowledge, the closest indication of a decision rule is the desire by Canadian regulators that concentrations of discharged pesticides and/or drugs do not exceed a threshold of environmental concentration that is deemed acceptable (HCPMRA 2017). To this end, thresholds for pesticides have been defined by Health Canada's Pest Management Regulatory Agency (HCPMRA). The intent of the thresholds is to avoid environmental impacts. The thresholds are based largely on laboratory estimates of toxicity indicators such as LC_{50}s (concentration at which 50% of the laboratory population units exhibit an effect such as mortality) or no observed effect concentration (NOEC). The regulations do not explicitly state a threshold for the duration of exposure; however, for practical reasons it is assumed that any duration of exposure to a concentration above the threshold is to be avoided. Also, the thresholds do not explicitly include spatial areas of exposure that are deemed acceptable. The regulations, however, do place restrictions on the timing of treatments when the distance between discharge locations and features of interest (e.g., distance to licensed lobster holding facilities or the bottom) is less than a specified value.

When sufficient information is available concerning the distribution of sediment drug concentrations, regulators may decide to develop level of concern thresholds and criteria to promote avoidance of toxic exposures, as is done for pesticides. This approach maybe easier to enforce and be cheaper to implement than a regular sampling program. The sampling efforts can be mainly undertaken for targeted due diligence, compliance audit, risk assessment, and model evaluation and development purposes.

6.6. STEP 6: SPECIFY TOLERABLE LIMITS ON DECISION ERROR

In any regulatory sampling regime thresholds, confidence levels, and tolerance limits should be determined, and evaluated in the context of the purpose for the sampling program (Wilding et al. 2017). In the case of pesticide and drug discharges from Canadian net-pen aquaculture operations, to the knowledge of the authors at the time of this writing, explicit statements specifying thresholds, confidence levels, and tolerance limits are not available. However, identification of these limits is an important and necessary component of quantitative decision making and a necessary consideration of sample design since it helps determine the type of design required and the cost of sampling effort (number of samples, sampling methods, etc.).

6.7. STEP 7: DESIGN A SAMPLING SCHEME

6.7.1. Step 7.1: Sampling purpose

The general purpose(s) for the design(s) for a Canadian post-deposit monitoring program is(are) outlined above in Step 1 of the Planning Process. When designing a sampling program for a given site and type of discharge, the details will need to be updated to reflect site and discharge specifics.

6.7.2. Step 7.2: Target population

In the case of pesticides and/or drugs discharged from a salmon net-pen pesticide or drug treatment administration procedure, the target population may be defined as the water or sediment in the bay or inlet within which the salmon farm operation exists or in the spatial and

temporal domain that may be exposed to the discharged chemical. In the design of sampling for a specific site and type of discharge the pertinent details will need to be specified to reflect individual site and discharge characteristics.

Models can play an important role in defining target and sampling populations and in designing sampling programs. Models can be used in multiple ways to achieve a variety of goals which depend upon the purpose of the sampling program. Areas of interest and potential areas of environmental exposure to chemicals released by fin fish aquaculture farms can encompass large areas that could require a large amount of sampling. Since sampling is expensive, efficient sampling designs are desirable to optimize costs.

Models can provide estimates of areas of exposure, deposition, and impact, the expected locations of concentrations of chemicals, and locations and magnitudes of spatial and temporal gradients in chemical concentrations. If models can produce sufficiently accurate results, then they may be used to guide where to sample and therefore help make sampling designs efficient. Also, model results can perhaps indicate specific areas requiring more sampling effort. Conversely, sampling programs are crucial for model development in that they provide data with which models can be calibrated and validated.

The sample measurements are from areas or volumes of sediment that are orders of magnitude smaller than the areas or volumes models use for generating model outputs (model grid cell areas are of order 10^3 m^2 and sample areas are of order 0.1 m^2). This highlights that comparisons between model outputs and observations are based on a significant mismatch in measurement resolutions and that model comparisons or observation validations need to account for the associated uncertainties in both the model outputs and measured values.

As with sampling design development, selection of a suitable model to aid in the development requires knowledge of the required purpose for sampling. Model selection depends on several factors: the desired estimate, the desired accuracy, the available input data, the desired response time, and the available expertise for model implementation. If models are to be used to guide sampling design it is imperative that the appropriate model is chosen and that it is properly understood, calibrated, validated, and has an accuracy sufficient to the sampling design. Models range in complexity and how well a given model represents reality depends on the suitability of the underlying assumptions and inputs as well as its inherent accuracy. At one end of the spectrum are models with many simplifying assumptions that can be implemented relatively easily but often have low resolution results. At the opposing end of the spectrum are complex models involving more detailed input, more time and effort to implement, more detailed output, but with often unknown accuracies.

For chemicals released during bath treatments, models provide a smooth estimate of discharge plumes. Dye tracer studies indicate that individual plumes are transient meandering plumes that are individually unique. A visible tracer introduced into the discharge can be used to help determine the optimal sampling locations for individual discharges; sampling of cumulative discharge footprints will require multiple dye releases or model estimates of the exposure area. Once a model has been validated, it could potentially be used to determine optimal sampling locations, including locations beyond the visible boundary of the tracer. This might be useful if the concentration is still above a specified environmental threshold when the tracer is no longer visible. All sampling results can be used to help evaluate and validate models of exposure and impact.

Unlike discharges from bath treatments, there is no way to easily visually track the discharge cloud from in-feed treatments. Hence, conceptual or quantitative models are the only way to help guide the sampling design, but care must be taken in interpreting model results. Simple models tend to give over-estimates of the exposure zone and may indicate the need for a more

extensive sampling design. More complex models may give a more precise estimate of the exposure zone, but the accuracy is often unknown. For waste feed and feces released during in-feed treatment, the cloud of particles containing the chemicals is initially deposited to the seabed and may or may not be remobilized. If sampling programs are based solely on the predicted exposure zone from a complex model, or low intensity sampling is associated with a low resolution simple model prediction, it is possible that the actual deposition zone is missed resulting in the potentially false conclusion that no exposure and/or impact exists when in fact it does.

6.7.3. Step 7.3: Conceptual overview and sampling limitations

The purpose of this step is to provide a brief and generic conceptual overview of these aspects as in relation to pesticides and drugs discharged from net-pen farming operations. In the actual design of a sampling scheme for a specific aquaculture farm site and type of discharge this section will need to be updated to reflect administration, discharge, and site specifics.

Before a sampling program can be designed it is important to understand how the chemical to be discharged is administered, introduced or discharged into the environment and how it is subsequently transported, dispersed, decayed and transformed.

6.7.3.1. Conceptual overview

6.7.3.1.1. Treatment and discharge types

Two categories of chemical discharge are considered in this document: the discharge of bath pesticides and in-feed medicines, i.e., drugs (Figure 4). The type of administration varies between the two categories as does the form of the discharge. Bath treatments include tarping of in-situ ocean net-pens or the pumping of fish from net-pens into a well-boat well. In-feed treatments may be administered in a hatchery before fish are transferred to net-pens or in the net-pens.

In all cases, the discharges are into the open ocean water in which the net-pens are located. The form of the discharge from all pesticide bath treatments is the bath water containing the dissolved pesticide. For pesticides currently used in the Canada, pesticides in the bath water remain dissolved or suspended in the water column. The discharge from in-feed treatments is in the form of waste feed, fish feces and fish excretions (losses through the skin, urine and gills); the waste feed and fish feces settle to the seabed and the fish excretions and leachates from the waste feed and feces are, at least initially, suspended in the water column. The particular discharge mechanism will influence the spatial distribution and intensity of the deposits (Page et al. 2023b).

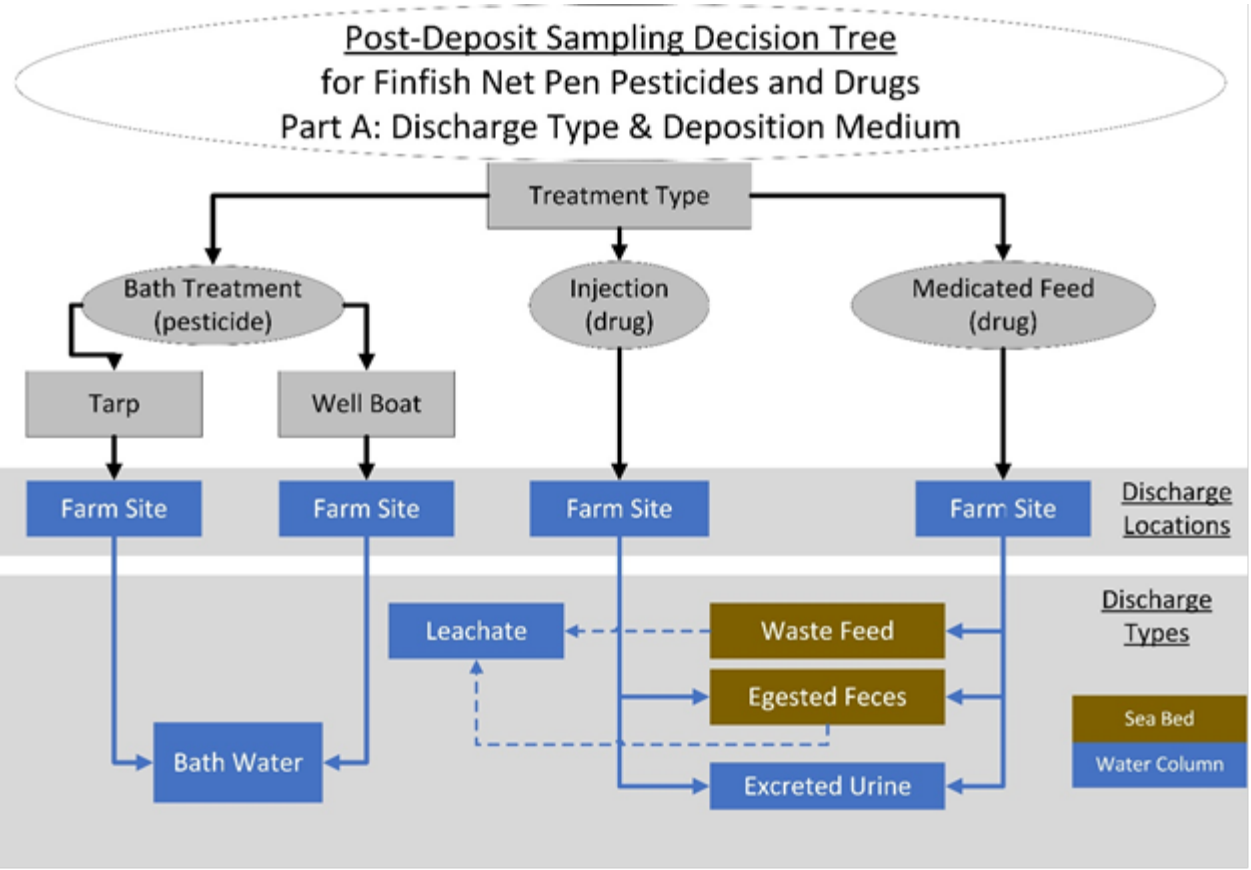

Figure 4. Diagram illustrating the treatment types, discharge locations and discharge types to be considered in post-deposit sampling. This document does not address the discharge of injected drugs into the environment.

Farm treatment scenarios usually consist of the sequential or simultaneous treatment of fish in individual net-pens. In bath treatments, individual net-pens are treated only once (during a given farm treatment; any subsequent treatments would be weeks or months later) and usually only one or two cages are treated at a farm per day; in-feed treatments are usually conducted on each treated cage every day over a period of several days. The discharges from the treatments are therefore a series of finite duration events and the duration of each event may vary from a few minutes to a few hours.

6.7.3.1.2. Chemical form in discharge

The form of chemical that is released depends on the chemical and the type of administration. Bath and in-feed treatments discharge the parent chemical whereas in-feed treatments may also discharge metabolites of the parent chemical. The waste feed only contains the parent chemical, whereas egestions and excretions may include both the parent chemical and its metabolites. Metabolites may also be generated by microbes in the environment acting on the parent chemical.

6.7.3.1.3. Chemical quantity

The quantity of chemical associated with each of the release types also varies. It is usually assumed that all of the administered chemical is released with the discharge water associated with a bath treatment; the uptake of chemical by the fish and organics in the bath water during the treatment period is usually not accounted for although this uptake can at times be

significant. For example, Corner et al. (2008) found a significant uptake of cypermethrin by fish during the treatment period. The chemical from in-feed treatments is partitioned into the three discharge types; waste feed, feces, and excretions and the partitioning varies with the chemical (Figure 5). The proportion associated with waste feed is independent of drug, but the proportion and form of chemical associated with discharge from the fish is dependent on the specifics of the drug absorption and metabolism processes. For drugs with low absorption coefficients, a large proportion of the drug is egested as feces initially and a relatively low proportion is egested or excreted over time. Conversely, for drugs with high absorption coefficients, a small proportion of the drug is egested as feces initially and a relatively high proportion is egested or excreted over time.

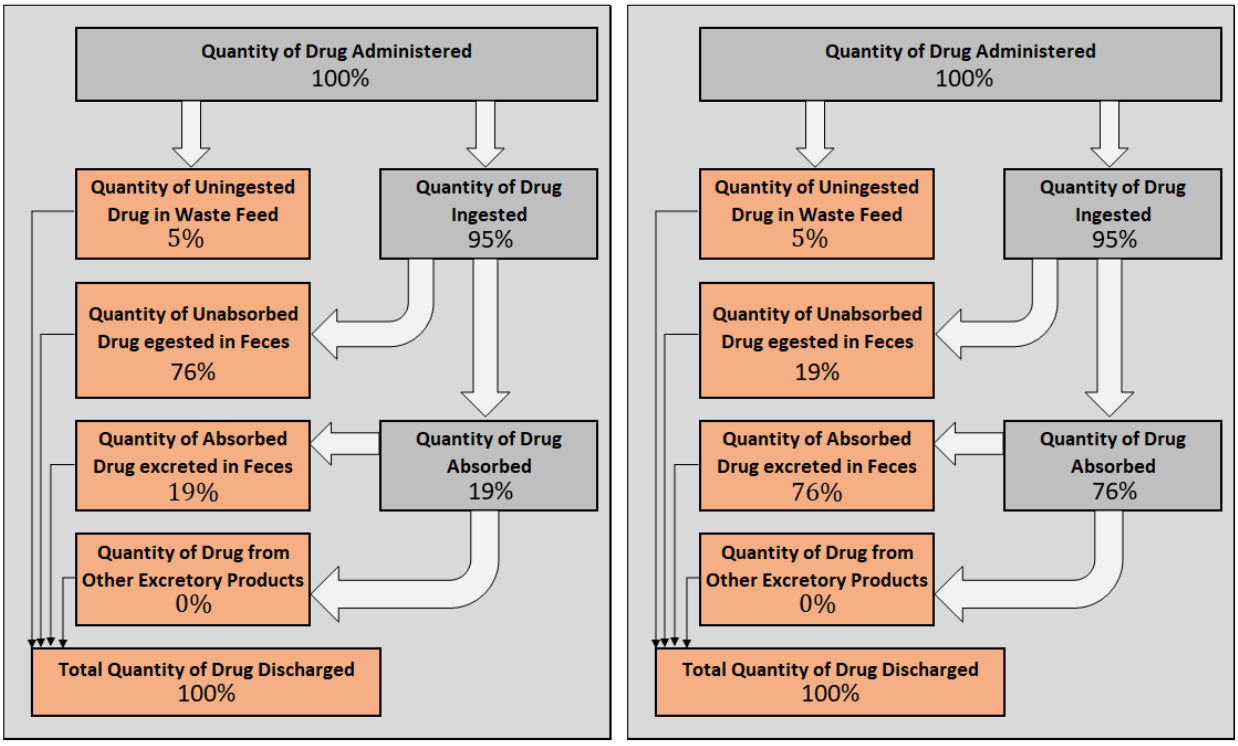

Figure 5. Diagrams illustrating chemical partitioning for a chemical where 20% of the ingested drug is absorbed by the fish, i.e., a low absorption coefficient (left panel), and where 80% of the ingested drug is absorbed by the fish, i.e., a high absorption coefficient (right panel).

6.7.3.1.4. Discharge timing

The timing of release varies with the treatment method and discharge source. Discharges from bath treatments begin immediately after the treatment period ends. For tarp treatments the discharge begins as soon as the tarp is removed from the net-pen. Discharges from well-boats begin as soon as the bath water begins to be pumped from the well. Releases associated with in-feed treatments occur on multiple time scales. Waste feed discharges occur as soon as feeding begins. Fecal and excretion discharges are delayed and occur over extended periods of time. Feces are egested 6 - 48 hours after ingestion. The timing of egestion will vary according to several factors, including fish size, water temperature, food type, time of previous feeding (Aas et al. 2017).

6.7.3.1.5. Discharge duration

The duration of releases also varies with the treatment method and discharge source. The duration of releases from bath treatments varies from a few minutes to several hours. Release duration from tarp treatments vary from less than ten minutes to 2 – 3 h (Page et al. 2015) depending on ambient current speeds and the porosity of the net mesh. Release duration from well-boats is typically 20 or 30 min. Depending on the well-boat discharge rate, this may or may not be sufficient time to discharge all the chemical which is continuously diluted during the discharge period. Any residual chemical left in the well will have a low concentration and commonly be discharged back into a fish cage.

Release durations from in-feed treatments vary from minutes to days, weeks, or months. The duration of the initial feeding, and hence the duration of the discharge of waste feed, is about ten minutes. The duration of fecal discharge is initially the residence time of the feed in the fish, and subsequently discharge occurs as the chemical and its metabolites are reintroduced into the feces via metabolic pathways (e.g., bile). The duration of excretion discharge is the residence time of the chemical in the fish. Leaching will occur continuously for as long as it takes to breakdown waste feed and feces in the environment.

6.7.3.1.6. Spatial characteristics

The spatial characteristics of discharges vary with the treatment type and the associated pathways.

6.7.3.1.6.1. Size

The size characteristics of bath treatments vary with the treatment type. The initial horizontal size of a discharge from a tarp treatment is the size of the net-pen being treated; the diameter of circular net-pens is typically 20 to 50 m (circumferences of 60 to 150 m). The vertical extent of the initial discharge is typically three to five meters (Page et al. 2015). A well-boat discharge changes continuously throughout the discharge duration. The initial size of is the diameter of the discharge pipe, typically 0.5 m in southwest New Brunswick; the final size depends on the initial size, the discharge rate and discharge duration. The vertical extent of discharges angled parallel to the sea surface is one or two meters; discharges angled vertically may be about ten meters (Page et al. 2015). For both tarp and well-boat treatments, the size of the patch increases with time. The dimensions can be tens of meters in the vertical and hundreds to thousands of meters in the horizontal.

The initial dimensions of discharges from an in-feed treatment are approximately a cylinder about 20 – 50 m in diameter by 10 m in depth, i.e., the dimensions of a net-pen. This applies to the waste feed, feces, urine, and gill discharges. The size of the discharge cloud associated with the settling components, waste feed and feces, increases with time post-discharge until the cloud intersects with the seabed. The size of the excretion and leachate discharge cloud also increases over time and depends on the leaching rate, size of the excretion area (the net-pen), the area occupied by the waste feed and feces during the leaching time, and the rate(s) of transport and dispersal in the local environment.

Estimates of the size of discharges are given in Page et al. (2023a, 2023b).

6.7.3.1.6.2. Location

For bath treatments, the initial location of the discharge begins with the location of the net-pen or well-boat. In Atlantic Canada the well-boat is usually tied to the side of a net-pen and discharge occurs there. In other regions the well-boat may steam away from the fish farm and discharge at a location different from the farm site. Post discharge, the location of the pesticide patch changes with time as the patch moves and grows with the ambient currents.

For in-feed treatments, the initial location of the discharge coincides with the location of the net-pen being treated. Post discharge, the location of the cloud of particle changes as particles are advected and dispersed by the local current conditions. Once the cloud of particles intersects the seabed, the location remains fixed unless it is remobilized and redistributed. Leachate discharges occur at the locations of the feces and waste feed.

6.7.3.1.7. Temporal characteristics

The temporal characteristics are how the concentrations and locations change over time and depend in part on the treatment method, chemical properties, the time, duration, and location of the discharge.

Pesticides currently approved for use in Canada remain in the water column after they are released, i.e., they do not bind to sediments and sink. The locations and concentrations change continuously with time due to advection, dispersion, and decay. The discharge of pesticides is of short duration on the order of minutes to hours and may include multiple sources (net-pens or well-boat).

Drugs currently approved for use in Canada initially sink through the water column and become deposited on the seabed. When in the water column the locations and concentrations change continuously with time due to advection, dispersion, decay, leaching, and consumption by wild organisms. Once drugs are deposited on the seabed the locations and concentrations change continuously with time due to decay, leaching, resuspension, and biological transport. The concentrations are also be augmented by the continuing release of fecal matter and/or the administration of additional drug doses. The discharge of drugs is of relatively long duration on the order of days to months and may include multiple sources (net-pens and/or adjacent farms).

In the case of pesticides, the concentrations decrease through time. In the case of drugs, the quantity of drug in the environment first increases and then decreases, with the timing of the maximum quantity depending on the balance between the discharge and decay rates.

The temporal characteristics of the locations and concentrations will need to be consider when designing a sampling program.

6.7.3.1.7.1. Persistence

The persistence can be defined as the time for the chemical concentration to reduce to a specified threshold. Pesticides currently approved for use in Canada are expected to persist in the environment for hours. Drugs currently approved for use in Canada are expected to persist in the environment for months to years.

6.7.3.2. Sampling limitations

6.7.3.2.1. Pesticides

Sample designs and sampling methodologies are limited and constrained by several considerations including:

- the discharge occurs over a period of time that varies for about 30 to 180 min for tarp treatments and about 20 to 40 min for well-boat treatments;
- the discharge is into a spatially and temporally complex and constantly varying hydrodynamic environment; the discharge is rapidly dispersed and transported in three dimensions (horizontal and vertical) and the size, shape, location and chemical concentration within the discharge cloud or plume evolves rapidly with time over periods of minutes to hours, with the location of the discharge rapidly becoming very uncertain and unknown;

- the discharge or portions of it, may be trapped within components of the adjacent net-pen array or adjacent site net-pen arrays;
- the cloud location, shape, size and intensity is not visible or reliably predictable;
- discharges are constantly moving since the pesticides typically remain in solution and are not deposited on the seabed; and
- discharge clouds may expose pelagic and benthic organisms via ingestion or topical exposure pathways and expose multiple types of seabed.

These factors mean the cloud needs to be sampled soon after discharge and can only be sampled a few times (about 10 times) because individual samples require about 15 min to collect and dilution time scales for pesticides are typically only a few hours.

6.7.3.2.2. Drugs

Methodologies for sampling the seabed are limited and constrained by several considerations including:

- discharges occur in pulses of various durations and over time periods that vary with the discharge type: the drug associated with waste feed is discharged during and immediately after the treatment administration and the waste feed settles to the seabed within minutes to hours; fecal discharges containing the active ingredient begin hours after treatment and will occur over days to months at intermittent intervals and feces settle to the bottom within hours; excretions commence hours after treatment and continue over days to months at irregular intervals;
- treatments may occur one to three times per day for up to ten days (for drugs approved for salmon in Canada); this may be repeated multiple times with months between treatment periods;
- concentrations of drug in the discharge vary with the drug and discharge type; concentrations in waste feed are dose concentrations, concentrations in feces may initially be high and relatively low after the drug has been processed by metabolic pathways; concentrations within excretions are low;
- discharge is into a spatially and temporally complex and constantly varying hydrodynamic environment; the dispersal and transport is in three dimensions (horizontal and vertical) so the size, shape, location and chemical concentration within the discharge cloud or plume evolves rapidly, with the specifics depending on the location, time of discharge and the conditions prevailing during and after the time of discharge;
- the discharge footprint, or portions of it, may overlap the deposition associated with adjacent net-pens or adjacent site net-pen arrays;
- the discharge may not be visible and prediction accuracy and precision is low, especially when sediments are remobilized;
- discharge clouds and deposits may expose pelagic and benthic organisms via ingestion or topical exposure pathways and may expose multiple types of seabed;
- the deposition footprints vary in size from a few tens of meters to hundreds of meters in length and hundreds to thousands of meters in area;
- the seabed depositions need to be sampled soon after treatment and deposition if redistribution effects are to be minimized; when sampling is delayed for drugs that persist for

considerable lengths of time (days, weeks, months or years) the potential for redistribution and relocation increases;

- the above result in the location, area, and concentration of depositions being uncertain, and site and discharge specific, so sample designs need to be comprehensive enough to ensure detection of discharge deposits; and
- the collection of benthic samples requires about 15 minutes per sample and hence only about 20 samples can be collected per working day; these numbers may be depth dependant.

6.7.4. Step 7.4: Sample population

The sample population is site specific and will depend on local constraints (e.g., underwater cables, presence of the farm, current strength). In the case of seabed sampling baseline information must be available or collected to allow the determination of bottom types within the boundaries of the target population to select the appropriate sampling methods and locations. This information could be collected using acoustic or visual methodologies (e.g., ROV video and sonar) following a systematic grid pattern (ISO 2004). Hydrography and bottom type determine whether sample units are accessible to available sampling methodologies. Depending on objectives additional baseline information may be required and could include habitat, pesticide and drug concentrations, or biodiversity.

6.7.5. Step 7.5: Sample design selection

Once the large-scale geographic area and time window of interest have been defined (i.e., the target and sample populations), the next step is to locate the areas that have been exposed and impacted and decide how to sample these areas.

The variation and uncertainty associated with pesticide and drug discharges pose challenges and limitations to the design of practical, implementable, and affordable sample designs. Sample designs cannot rely on a priori judgement or model outputs to guide detailed selection of sampling efforts; the judgement and model outputs can only provide qualitative guidance such as the general direction of expected transport, the general rate of dispersal, and an expectation that concentration will decrease with distance from the discharge location. The spatio-temporal evolution of a drug or pesticide discharge depends on the administration and discharge approach, ambient water currents, chemical dose, and the chemical behaviour.

7. SAMPLING PLANS AND DESIGNS FOR NET-PEN AQUACULTURE

A clear statement and description of the sampling objective(s) were not available to the authors at the time of writing, only a general notion that there was a desire for information on how to design a post-deposit sampling program. Hence, it has been assumed that the objectives of the sampling program are: 1) to delineate the spatial (geographic) area and temporal window of exposure to pesticides and drugs discharged from salmon net-pen operations; and, 2) to determine the magnitude of the pesticide or drug concentrations and compare to relevant EQSs, the subject of a companion paper by Hamoutene et al. (2023). Similarly, a clear statement of the measurement or indicator type to be derived from the samples was not available at the time of this writing. Hence, it has been assumed the desired measurements are concentrations of pesticides and drugs discharged from salmon net-pen aquaculture operations.

7.1. PESTICIDES

At present Canada does not require a baseline survey that includes measurements of pesticides nor post-deposit monitoring of pesticide discharges. Health Canada assesses and registers pesticides for legal use. Each pesticide label includes instructions for use that aim to help minimize environmental impacts. Fisheries and Oceans Canada requires a 72-hour advance notification of treatment, but an authorization for individual treatments is not presently required and site-specific assessments for environmental impacts are only beginning to be developed.

Compliance concentration and exposure area thresholds for the registered bath treatment pesticides, hydrogen peroxide and azamethiphos, are not established, although level of concern concentration thresholds were used by HCPMRA (2016, 2017) to help establish conditions of use. The general approach is to avoid unacceptable exposures. In the case of azamethiphos, compliance rules include the specification that a discharge can only occur under certain water current conditions if a licensed lobster holding facility is located within 1 km of the treatment site; a tarp treatment cannot be conducted at a site when the water depth is less than 10 m; a well-boat discharge cannot occur when the discharge angle is 45 to 90 degrees to the vertical and the water depth is less than 20 m; no more than two tarp treatments can be conducted simultaneously per farm site; and no more than two tarp treatments can be conducted per day/farm site when net-pens are 150 m polar circles (HCPMRA 2017). The restrictions on hydrogen peroxide use are that no more than six applications per year (HCPMRA 2016).

The following comments and suggestions apply to both tarp and well-boat treatments. We assume there are six objectives relevant to the design of a pesticide bath discharge sampling program. These are:

- determine the chemical concentration of the prepared stock solution just prior to administration into the bath water;
- determine the concentrations of chemical in the bath water immediately prior to the time of release into the environment;
- estimate the rate of decline in the maximum concentration;
- estimate how much the seabed has been exposed to the discharged pesticide;
- estimate the distance from discharge locations at which level of concern concentrations are exceeded; and
- estimate whether lobster (or other chosen species) life stages have been exposed and/or impacted by the discharge.

Meeting these objectives is assumed to be useful to an assessment of compliance, to provide initial values for model predictions, to provide observations suitable for comparison to models for purposes of model evaluation, and to provide information useful for the improvement of compliance considerations. The information is useful to multiple users including researchers, regulators and fish farmers.

7.1.1. SEPA

In Scotland, three bath pesticides are licenced for use: azamethiphos, deltamethrin, and hydrogen peroxide. Since the use of hydrogen peroxide is believed to pose a lower risk to the environment, SEPA does not impose environmental standards for hydrogen peroxide (SEPA 2019a, 2019c). SEPA has environmental standards for azamethiphos and deltamethrin which specify the maximum allowable concentration at a given time after discharge. For azamethiphos, environmental standards are applied 3, 24, and 72 hours after discharge; for

deltamethrin, environmental standards are applied 3, 6, 12, 24 and 48 hours after discharge (SEPA 2019a). It is the responsibility of fish farm operators to ensure that these standards are met. No post-deposit sampling efforts are required.

Perhaps due to the challenges associated with designing an effective monitoring program for measuring the concentrations of bath treatment pesticides, SEPA requires that the fish farm operator use model results to demonstrate that the environmental standards will likely be met (SEPA 2019a). With the exception of situations that are considered low-risk, marine modelling, i.e., hydrodynamic modelling, will be required (SEPA 2019a). Furthermore, it is recommended that the model is validated against, for example, dye tracer or drifter studies (SEPA 2019c).

Modelling of bath treatments must include the treatment of an entire farm and simulate realistic treatment practices. Since there are many variables that can affect the evolution of a modelled bath treatment discharge patch, SEPA (2019c) requires that modelling represents treatment conditions which are plausible and expected in terms of initial pesticide concentrations, frequency of treatment, and hydrodynamic conditions. When there are uncertainties in the treatment conditions, the model must reflect worst case scenarios. Except for the longest-term standards, environmental standards must be met at the specified time after a single dose in a treatment regime and take into account contributions from previous doses. The longest-term standards are treated differently: they are applied after 72 (48) hours for azamethiphos (deltamethrin) after the last application of pesticides in a treatment program and indicate the concentration that cannot be exceeded over an area greater than 0.5 km^2 at that time.

Model results must be included with a permit application (SEPA 2019d) for review by SEPA. SEPA will refuse to grant authorization for the use of a bath pesticide if the results of the modelling indicate that the environmental standards would not be met or if the resulting pesticide plume contains a concentration that poses a risk to protected species or habitats (SEPA 2019a).

7.1.2. Potential sampling design

The location, size, and shape of bath water discharge clouds as well as the concentration of pesticide within the cloud vary among releases, and with time, pesticide, location, farm layout, and treatment administration procedure. A typical initial discharge cloud size associated with a bath pesticide tarp treatment is 31 m, the diameter of a 100 m polar circle net-pen. The distance between grid points in a gridded triangular design must therefore be 30 m to ensure detection of the initial discharge cloud. A typical potential exposure zone (PEZ) associated with this discharge has a radius of kilometers and an area of millions of square meters (Page et al. 2023a). This means that a large number of grid points must be sampled to ensure the detection of the initial discharge cloud. This is clearly not practical or cost effective. Even though the number of grid points needed will decrease with time elapsed after release, because the size of the cloud will increase and sample spacing can increase, the sampling effort is likely to remain impractical. These challenges are particularly important to consider when pesticide concentrations and/or impacts are persistent.

The above constraints prohibit the ability to design and implement a sampling design that will give meaningful information. However, when a visual indicator (e.g., dye) is used, judgement-based design may be appropriate for monitoring discharges of bath water from net-pen pesticide treatments. A probabilistic-based design is not feasible as a dye patch’s concentration and location changes rapidly relative to the ability to sample. Perhaps the most effective way to sample bath pesticide discharges is to sample the end-of-pipe concentrations for all or a subset of discharges and to selectively sample some subset of individual releases.

7.1.2.1. Tarp discharge sampling

The discharge associated with a tarp treatment begins as soon as the operator removes the tarp surrounding the net-pen. The discharge ends when there is no measurable pesticide remaining in the net-pen. The duration of the discharge period is a few minutes to several hours (Page et al. 2015). The location of the end-of-pipe discharge is defined by the downstream edge of the treated net-pen perimeter.

The pesticide stock solution should be characterized by recording the total volume of the solution and taking three (top, middle, bottom) 100 mL water samples from the solution container before delivery into the tarped volume for determination of active ingredient chemical concentration. The end-of-pipe sampling associated with tarp treatments should be a stratified random sample of concentrations within the net-pen within 15 min prior to tarp removal. The volume of bath water needs to be estimated (net-pen surface area x estimated tarp depth) and divided into surface quarters and two depth layers (top half and bottom half). Sample locations within each stratum should be random (Figure 6). A central stratum could be added to ensure samples are not all from net-pen edges.

This approach will enable the total quantity of treatment chemical and its concentration at the time of release into the environment to be estimated; these can be compared to expected treatment doses for compliance and modelling purposes and to determine whether initial concentrations are homogeneous. If the initial concentration is not homogeneous then patches may exist that exceed expected concentrations. Total quantities are needed for some exposure estimation approaches and to allow for mass balance considerations. Sampling from within the source just prior to discharge should always be feasible and should be taken for any discharge that is to be monitored.

The meandering discharge plumes are best sampled by adding a visible tracer to the pesticide stock solution prior to introduction into the bath water. Sampling is then implemented in relation to the visible portion of the discharge cloud. Water sampling efforts should be conducted immediately after release because pesticides dilute quickly and the likelihood of detecting the exposure and impact zones after a series of pesticide treatments is very limited. Choosing the samples based on visual estimations of dye concentrations constitutes visual judgement sampling. The approach greatly reduces the total area that needs to be sampled and the total number of samples that need to be taken, while still allowing delineation of the discharge cloud and sampling of the cloud for measurements of chemical concentration.

At least one sample location should be immediately adjacent to the outside edge of the treated net-pen (Figure 6). This location should be sampled at several depths (2, 5, 10, and 15 – 20 m) beginning within 15 min of tarp removal to estimate the vertical distribution of pesticide immediately after entering the environment. This sampling will provide an estimate of discharge duration and initial depth distribution. In addition, samples should be taken within the observed maximum concentration of dye at 30 – 60 min intervals to characterize the temporal reduction in the maximum concentration of pesticide (Figure 7). These samples should be taken at multiple depths (2, 5, 10 and 15 – 20 m) and when possible at multiple horizontal locations within the perceived maximum area; the position and time (in GMT) of each sample also need to be recorded. This will enable a mean concentration to be calculated for each sample time window, the rate of decrease in the maximum concentration to be estimated, and the distance from the discharge location at which concentrations are below the level of concern to be calculated. For example, a sampling effort of four depths at four locations would give a total of 16 samples. Repeat samples at each location would be preferred yielding a total of 32 samples.

When the dye concentrations indicate the seabed is being exposed, multiple (at least three) bottom samples should be taken in the vicinity of the observed exposure location after the

plume has dissipated. Whilst benthic change following exposure is possible, whether such changes are considered meaningful will depend on stakeholder perspective.

More extensive post-discharge sampling can be conducted to estimate the spatial distribution of the pesticide. This sampling could consist of collecting samples throughout the tracer area to establish a calibration curve relating tracer concentration to pesticide concentration and collecting other data (e.g., tracer transects, aerial photographs) to indicate the spatial domain of the tracer plume. Alternatively, the sampling could consist of a transect run along the major axis of the evolving tracer plume and extending beyond the visible tracer (Figure 8). This approach gives the potential to estimate locations and times where the concentration equals the threshold concentration but does not allow the estimation of the shape and size of the exposure area.

All sampling schemes must consider the time needed to take each sample in relation to the spatial span of the plume, the rate at which the plume changes size and location, and the rate at which the tracer and pesticide concentrations are expected to decrease. In many cases these considerations indicate that it is only feasible to collect a few samples within each sampling interval. Hence the design process must establish the priority purpose for the sampling, such as temporal reduction in the maximum concentration, the distance at which the concentration drops below a threshold, or the area of exposure.

Ranked set sampling may also be appropriate for pesticide sampling when the intensity of the visible pesticide tracer (e.g., visual intensity of dye, fluorescence of dye or perhaps the turbidity of the dye) acts as the screening measurement and water bottle samples are collected for detailed analyses of pesticide concentration. However, the requirement to randomly allocate multiple samples after the location has been detected and ranked is considered to be inappropriate because of the rapid evolution in size and location of the pesticide/tracer cloud.

The above sampling is focused on individual discharge events and does not allow estimation of the total or cumulative exposure zone associated with the treatment of multiple net-pens and multiple treatments per net-pen, unless all discharge events are sampled. However, sampling of all events is generally not feasible, so cumulative exposures need to be estimated from validated models.

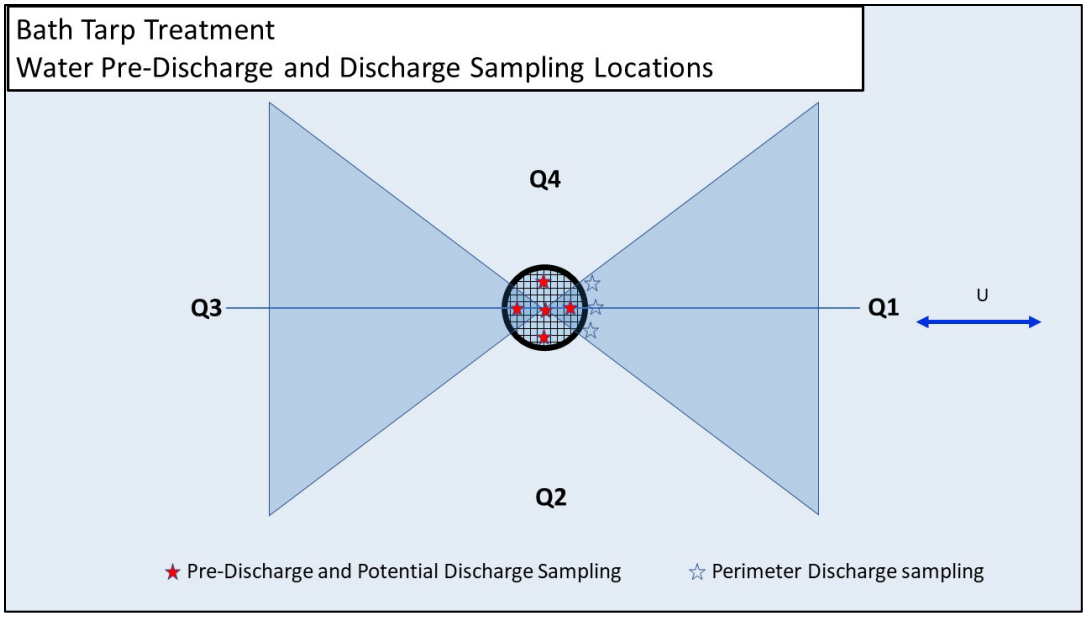

Figure 6. Diagram illustrating locations for pre-discharge and discharge sampling for a tarp bath treatment. At each location samples are taken at multiple depths and times. The triangles illustrate the sampling quadrant relative to the direction of the current at the time of discharge. The black circle represents the treated cage.

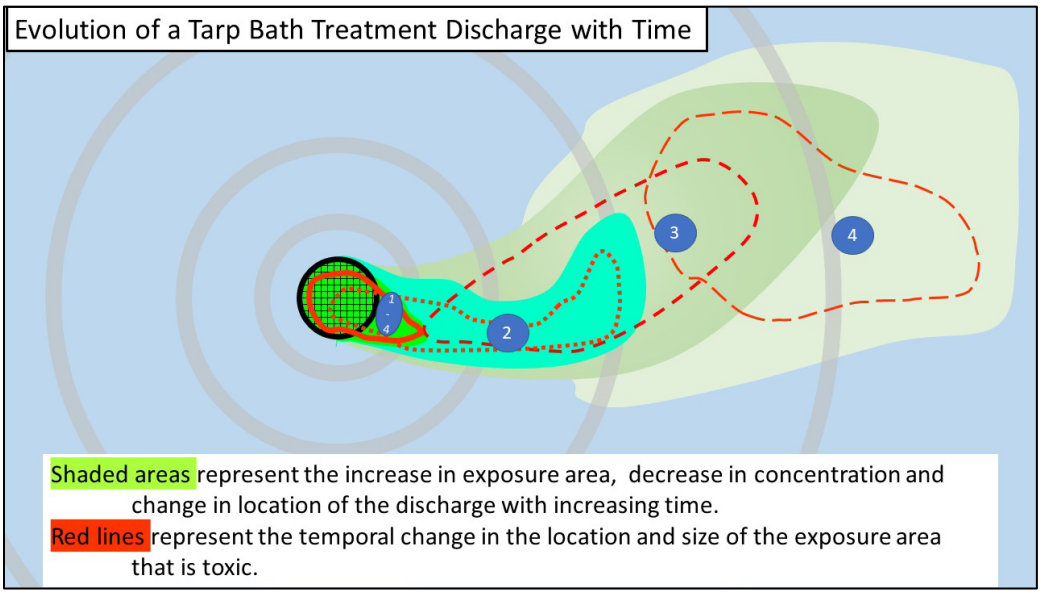

Figure 7. Diagram illustrating the location of sample stations (blue circles) for monitoring the discharge of pesticide bath water discharged from a net-pen tarp treatment. The black circle represents the treated cage. The numbers inside the blue circles indicate the temporal sequence of the samples. The station near the net-pen is sampled each time. The other locations are located near the estimated maximum concentration of the pesticide as indicated by the intensity of the tracer concentration at each time interval. The green polygons represent the shape and location of the discharge plume at several times after tarp release. The dashed red lines indicate the areas of toxic concentrations. The grey circles indicate radial distance from the net-pen.

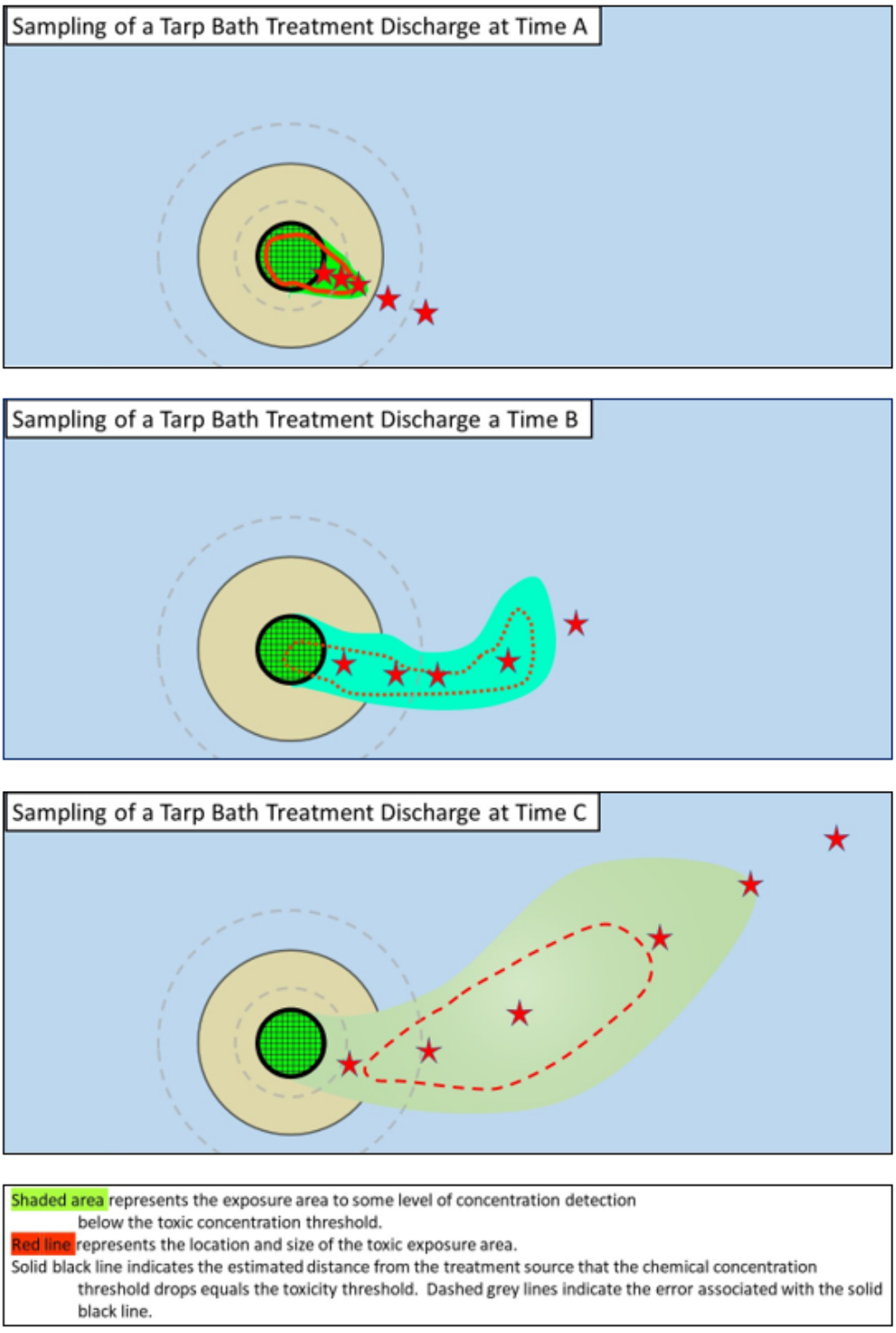

Figure 8. Illustration of a transect approach to sampling a discharge from a bath tarp treatment. The green circle (thick black outline) represents the treated cage. The sample stations (red stars) are spaced along the major axis of the plume at the time of sampling. Time A (top figure) represents sampling soon after discharge begins. Time B (middle figure) represents sampling near the time expected for cloud concentrations to disperse to the concentration threshold. Time C (bottom figure) represents sampling after the time expected for cloud concentrations to disperse to the concentration threshold. The scenario is similar for a well-boat bath treatment.

7.1.2.2. Well-boat discharge sampling

The discharge associated with a well-boat treatment begins as soon as the operator begins to pump the bath water out of the well and the discharge ends when the operator turns off the pump. The duration of the discharge period is a few tens of minutes (20 – 40 min) and there may be multiple discharges a day for multiple days. The location of the end-of-pipe discharge is defined the end of the well-boat discharge pipe where the effluents enter the receiving environment. For each treatment, there may be two discharges, one from each side of the vessel. If the well-boat is tied to the net-pen (Figure 9), one of the discharges may go directly into the pen.

Many of the sampling design components for well-boat discharges are the same as for those for tarp treatment discharges. The main differences are in the measurement of the stock solution and the measurement at the end-of-pipe. The details for well-boat specific sampling are outlined below. For sampling of the meandering portion of the discharge (Figure 10), see the previous section (7.1.2.1 Tarp Discharge Sampling).

As for tarp treatments, the pesticide stock solution should be characterized by recording the total volume of the solution and taking a 100 mL water sample from the solution container before delivery into the well volume for determination of active ingredient chemical concentration. Sampling should consist of collecting water from within the well-boat well bath water 15 min prior to the beginning of discharge. As with tarp treatments, the total volume or quantity of treatment chemical and its concentration before release into the environment should be recorded and measured. The volume of the well should be provided by the vessel operator and the volume of the bath water scaled by the proportion of the well that is filled. A minimum of three samples should be taken and, when possible, these should be taken at different locations within the well. Release concentrations are needed for establishing whether treatment doses are achieved and whether the initial concentrations are homogeneous. If the initial concentration is not homogeneous then areas may exist that are below or exceed expected concentrations. Total quantities are needed for some exposure estimation approaches and to allow for mass balance considerations. Sampling from within the source just prior to discharge should always be feasible and should be taken for any discharge that is to be monitored.

Once discharge has commenced, samples should be taken at the end-of-the discharge pipe at multiple times throughout the discharge process (Figure 9). At a minimum, samples should be collected at three points in time: within 5 min from the discharge start, mid-way through the discharge period, and 5 min before the end of the discharge. Each time samples should be taken at the discharge depth (upper 1 m) as well as 2 and 5 m below the discharge depth. This depth range should bracket the expected depth penetration of a horizontal jet (Page et al. 2015) and will result in a minimum of nine samples per monitored discharge.

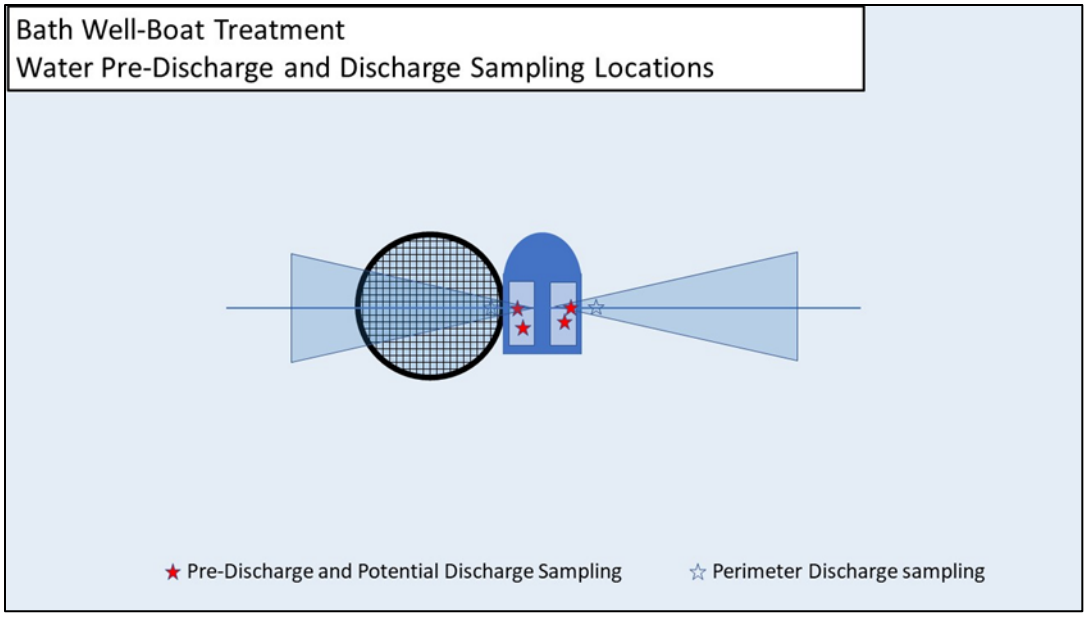

Figure 9. Diagram illustrating locations for pre-discharge and discharge sampling for a well-boat bath treatment. At each location samples are taken at multiple depths and times. The black circle represents the treated cage. The rectangles within the well-boat represent the treatment wells. The triangles illustrate the sampling locations prior to discharge (red) and in the discharge jet.

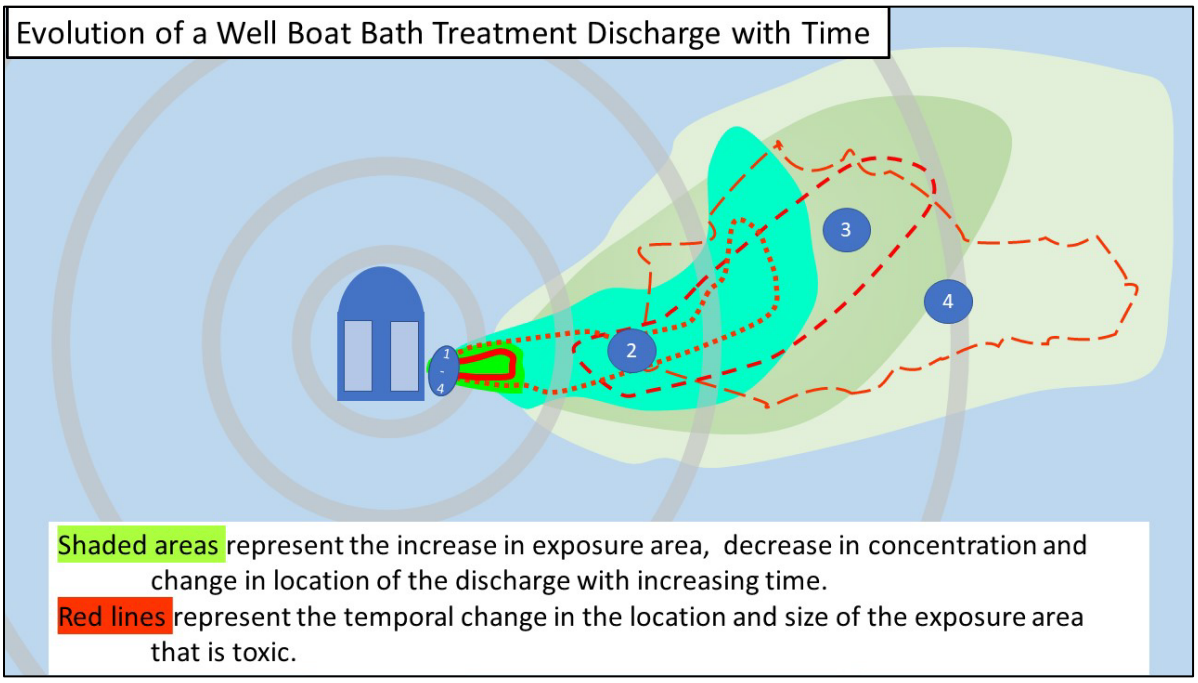

Figure 10. Diagram illustrating the location of sample stations (blue circles) for monitoring the discharge of pesticide bath water discharged from a well-boat treatment. The numbers inside the blue circles indicate the temporal sequence of the samples. The station near the well-boat is sampled each time. The other locations are located near the estimated maximum concentration of the pesticide as indicated by the intensity of the tracer concentration at each time interval. The green polygons represent the shape and location of the discharge plume at several times after discharge commences. The dashed red lines indicate the areas of toxic concentration. The grey circles indicate radial distance from the well-boat.

7.2. DRUGS

At present Canada does not require a baseline survey that includes measurements of drugs and does not require post-deposit monitoring of drug discharges. A 72-hour advance notification of treatment is required, but an authorization for individual treatments is not required; site-specific assessments for environmental impacts are beginning to be developed. Compliance thresholds for drug discharges are not yet available (at least not to our knowledge) but are under development. In support of the development process we assume there may be several objectives relevant to the design of a drug discharge sampling program. Some of these include:

- determine the quantity and concentration of chemical that is released into the environment, i.e., take a sample of the feed pre-treatment;
- estimate the location and area over which the seabed has been exposed to the discharged pesticide;
- estimate the distance from discharge locations at which level of concern concentrations are exceeded;
- estimate the temporal trend in the area of exposure, concentration of chemical, and impact;
- estimate if habitat and organism impact has occurred in the exposed areas; and
- estimate whether anti-microbial resistance has developed in exposed areas.

The purpose of these objectives is to collect information useful to an assessment of compliance, to provide initial values for model predictions, to provide observations suitable for potential compliance purposes, and to provide observations suitable for purposes of model evaluation and development. The information is useful to multiple users including researchers, regulators, and farmers.

It is also assumed that decision makers want to know the uncertainties associated with results, including spatial and termporal variability, in order to balance the costs and benefits, i.e., the ability to detect and interpret the signal within desired tolerance level. To achieve these goals a systematic planning approach such as that outlined at the beginning of this document is useful.

Compared to sampling of organic deposition, of which a considerable history of experience exists, relatively little sampling has been done in relation to drug post-deposits. It should be noted that organic discharges and deposition from net-pen finfish operations differs from drug discharges and deposition in several important ways. Organic discharge occurs every day and perhaps several times a day and includes the settling, deposition, and remobilization of waste feed and feces. For drug discharges, releases of waste feed occur for only a few days, perhaps several times a day; releases of feces occur every day for months, contain decreasing quantities of drug, and involves dissolved components as well as settling components. This means the patchiness associated with drug discharge and deposition may be larger than that associated with organic deposition. Persistence of the organic and drug loadings may also differ. These differences suggest that a sampling plan based on estimates of organic deposition is potentially inadequate for characterizing the spatial and temporal distribution of drug concentrations.

Sampling associated with finfish net-pen farming discharges of drugs has been, for the most part, a research endeavour rather than a compliance endeavour and has been mostly based on a judgemental type of sampling design that has measured chemical concentration and sometimes biological impacts such as bio-diversity (Appendix A). Past sampling efforts should perhaps be considered screening or scoping exercises and have given valuable insights to the design of more probabilistic-based sampling. Some of the limitations of the sampling programs

(not necessarily common to all of the reviewed work) include low sample size, weak sample design, inefficient sample collection methods (e.g., grabs not capturing the surficial flocculant layer), and difficulty in obtaining synoptic samples.

Some recent sampling conducted by DFO has been reasonably spatially comprehensive and has followed a structured grid type of sampling program (Appendix B). The grids have extended more than a kilometer from the fish farm and station spacing was of order 100 m or greater. The results indicate some treatment drugs were present throughout much of the sample domains, and up to 1.5 km from farm sites, with highest concentrations often found immediately adjacent to and near the treatment fish farms.

7.2.1. SEPA

SEPA (2019b) has recently revised their sampling design and adopted a two-tier approach to sampling. Their approach is still evolving and so far includes definition of the sampling area, sampling time window and decision points for allowable area and intensity of impact. The first tier is conducted prior to the operation of a new or modified site and it is part of a pre-site application baseline seabed assessment (BSA) process. The second tier is conducted after the site becomes operational and is meant to detect compliance with environmental regulatory thresholds; this is termed the seabed and water quality monitoring plan (SWMP).

The purpose of the first tier is to delineate seabed and habitat types within the baseline survey area (BSA). SEPA has defined the area of interest for the BSA as either the allowable mixing zone (AMZ) extended by 50 m in all directions or the area within a distance of 150 m from the net-pen edges, whichever area is greater. The AMZ is the area in which impact from a fish farm is permitted. The AMZ area is calculated by applying a 100 m radius around the edge of each net-pen and generating a rectangular hull around the extremities of these radii. The shape and location of the mixing zone is determined from a model predicting the concentration of discharged chemical on the bottom. It appears the model outputs must be contoured and the area within the concentration contour that is equal to the concentration threshold is calculated. If this area is larger than the AMZ area the model must be re-run using different inputs until a farm layout is achieved that meets treatment needs and environmental thresholds. Once a satisfactory modelled area is generated, the area within the threshold contour becomes the operational AMZ. The predicted AMZs appear to be independent of habitat type, i.e., the zones are not modified based on habitat types.

The purpose of the second-tier sampling is to quantify the physical and biological characteristics of the soft bottom habitat; the combined sampling activities are considered part of the baseline seabed assessment. The quantitative second tier sampling consists of a random sampling survey of each of the soft-bottom areas (strata that can be sampled with grabs) that were identified from the tier one results and a more detailed visual survey of hard-bottom strata (i.e., strata that cannot be sampled with grabs). Each stratum must be divided into a grid consisting of square grid cells that have a length dimension that is proportional to the strata size (the proportionality factor is not defined by SEPA). A minimum of five grid cells must be randomly chosen for each stratum and one sample location within each chosen grid cell must be randomly chosen.

Each soft-bottom grab sample must be analyzed for benthic invertebrates, particle grain size, total organic carbon, and chemical residues when there has been previous use of in-feed medicine within the relevant water body or wider area within which the site is to be located. Presumably the visual records should be analysed for presence and abundance of macro-fauna and flora.

The purpose of the above assessment or baseline sampling is to identify and characterize the habitat types within and immediately outside the AMZ. It determines the presence and location of any ecologically, socially, or culturally important features in the baseline sample area and the predicted AMZ and establishes a background level for chemical residues in the soft bottom strata. The information is used in the design of the compliance sampling.

The compliance monitoring, the seabed and water quality monitoring plan (SWMP) monitoring, is a transect-based sample design whose purpose is to determine whether relevant EQSs are being met. The sampling is meant to determine whether chemical residues within and immediately outside the defined AMZ are below specified near and far field thresholds, respectively. The SWMP is a multiple transect gradient design. A minimum of four orthogonal transects are required with the location of transects prescribed as being aligned with the major and minor axes of the predicted impact zone. Each transect must begin at the edge of a net-pen and extend either 50 m beyond the AMZ or 150 m from the net-pen edge, whichever is greater. The direction of the transects is to be away from the net-pens toward the edge of the predicted impact zone. The exact choice of transect location (origin and direction) is influenced by the location and shape of the habitat types and the shape and orientation of the predicted impact zone. In the case of a single soft-bottom habitat type and a predicted elliptical impact zone, the minimum number of transects are to be orthogonal and aligned with the major and minor axes of the predicted impact zone. When the habitat type is heterogeneous, i.e., there is more than one habitat type, the predicted impact zone is not elliptical, or the habitat strata are irregular in shape and not simply sampled, additional transects and/or altered transect orientations may be required with the details to be determined in conjunction with SEPA on a case by case basis. In these cases, the transects may not always be perpendicular to the net-pen array.

Sampling along each transect must consist of a minimum of seven stations, with one station located at the edge of a net-pen, one at the edge of the AMZ, and at least two at locations beyond the AMZ. The separation between each station must be a minimum of 10 m, but a 25 m is preferable.

The SEPA compliance design ensures samples are taken from within and outside the AMZ. The probability of detecting the discharge deposits and impacts within these areas depends on three factors: the spacing and orientation of the transects, the spacing of sampling along each transect, and the accuracy and resolution of the model used to define the AMZ. When the distance between transects is larger than deposit patch sizes the probability of detecting or intersecting patches will be less than one. The location of the transects is guided by the shape and location of the predicted allowable impact zone. The probability of the sampling detecting an impact zone is therefore dependant on the accuracy and precision of the model; the accuracies of existing models are known to be not well evaluated (Page et al. 2023b), and usually unknown for most sites. SEPA's recommendations for the setup of DEPMOD (SEPA 2005) are that accumulation of deposits are calculated (i.e., smoothed) over areas of 625 m^2 (square grid cells with dimensions of 25 m x 25 m), an area that is much larger than the areas associated with sampling units; the patchiness of deposit concentrations within the grid areas and in the environment are not taken into consideration. This means the chance of detecting a discharge or impact cloud is not well characterized; the patchiness or variance is likely to be larger for single discharge or treatment scenarios than for multiple discharge and treatment scenarios due to the multiple discharge scenarios including a higher number of release times and locations and a wider range of hydrographic conditions.

The required spacing of samples along each transect is sufficient to detect homogeneous patches of discharged in-feed medicines that are of a size similar to or greater than the size of fish net-pen assuming the transect as a whole, traverses the deposit. Sample spacing must be similar to the length scale of the patch if the probability of them being detected is to be high.

Pesticide and drug releases from net-pen operations have initial length scales that are similar to the size, diameter or radius, of the net-pens within the farm's net-pen array. The patchiness within the clouds is not well documented or accounted for.

The timing of the SWMP is specified as being when it is probable that the greatest impact will be observed. This time needs to be identified for each farm operation. For impacts associated with typical farm outputs such as organics, the time is to be within a 30-day period that brackets the time when peak biomass on the site has reduced to 75% of the peak value. For sampling associated with in-feed medication treatments and discharges of emamectin benzoate, the sampling must take place within 80 to 169 days after the cessation of the last treatment in the production cycle. This requires consideration of the anticipated treatment schedules and the logistics associated with designing and implementing the sampling. The sampling timing and logistical considerations for other chemicals have not been specified.

The decision rules associated with the SEPA program include compliance with an area criterion and intensity criteria. The intensity criteria include concentration thresholds for near-field (within the AMZ) and far-field (immediately adjacent to and outside the AMZ). It is not clear to us whether the intensity thresholds are for individual sample measurements or spatial means; the old SEPA criteria were the mean concentration within the near-field zone (defined as being the area between net-pen edge and 25 m from the edge) could not exceed a near-field or acute concentration threshold and no values from samples collected outside the allowable mixing zone could be above the far-field or chronic concentration threshold (SEPA 2005). We have not yet received clarification from SEPA on their new decision rules.

The SEPA approach has several important features of relevance to sampling design. These include:

- A baseline assessment survey and compliance sampling design needs to be submitted to SEPA prior to treatment because treatments need to be authorized before they can take place.
- The spatial domain to be sampled is defined and is to be less than or equal to a maximum size in terms of square meters. The domain has a specific spatial boundary, the geographic set of coordinates associated with a site-specific model prediction of the contour of chemical concentration that equals the far-field, i.e., the chronic, threshold. Uncertainties in model predictions are probably not considered. The domain is specific to each type of discharge, i.e., for each chemical.
- A probabilistic baseline assessment survey is conducted to define bottom habitat types and background quantitative measures of compliance parameters.
- Judgement-based compliance sampling is conducted after site operations commence.
- The compliance sampling design is a gradient type design, i.e., transects, with the location of the transects decided by judgement rather than by random selection. Transect origin and orientation may be adjusted based on judgement and knowledge concerning seabed and bottom habitat type. The location of transects may differ for each discharge type since the allowable mixing zone may differ among discharge types and scenarios.
- The compliance decision criteria are that the average concentration within the allowable mixing zone must be less than an acute threshold and any concentration outside the AMZ boundary must be less than a chronic threshold.
- The compliance sampling design is judgement based and hence does not allow statistical inferences about compliance to be made and adjustments to sample design cannot be assessed in terms of changes to compliance certainty.

7.2.2. Potential sampling design

The location, size, and shape of drug discharge clouds and deposition area, as well as the concentration of drug within the deposition area, vary among releases, with time, administered drug, location of discharge, farm layout, and treatment administration procedure. Unlike sampling for bath pesticides, the transient and meandering discharge plumes of drug do not need to be sampled and sampling can be delayed until the drug is deposited on the seabed. However, like pesticides when a tracer is not used, the deposition area and location is not visible, so the deposition area must first be determined. The drug sampling problem lends itself to the preferable possibility of a probability-based sampling design.

A typical initial discharge patch size associated with a drug treatment is 31 m, the diameter of a 100 m polar circle net-pen. The distance between grid points in a gridded triangular design must therefore be 30 m to ensure detection of the initial discharge patch. The patch size will increase as it sinks through the water column. The maximum size is attained when the patch reaches the seabed and depends on the sinking rate and water depth (Page et al. 2023b). As the patch size increases, the required grid spacing to detect the patch also increases. The total deposition footprint will be the accumulation of multiple discharge patches. Since the actual footprint of deposition is not known, the PEZ model can be used to delineate the radius where potential depositions are expected to occur (Page et al. 2023b). A typical PEZ associated with the drug deposition has a radius of 100s of meters and an area of thousands to tens of thousands of square meters. This means a large number of grid points must be sampled to ensure the detection of the initial discharge patch. This may not be practical or cost effective. Even though the number of grid points needed will decrease with distance from release, because the size of the patch will increase before deposition and thus sample spacing can increase, the sampling effort is likely to remain impractical. These challenges are particularly important to consider when drug concentrations and/or impacts are persistent because limited sampling may result in undetected deposition.

The first step is to determine whether a judgemental or probabilistic approach is desired or feasible. The assumption here is that a probabilistic-based sampling design is desired because this is the only approach that enables statistical uncertainties to be estimated and balances sampling uncertainties with decision tolerance levels to be undertaken. These statistical considerations cannot be accomplished by implementing purely judgement-based sampling. The next step is to design the sampling program. The process needs to:

- Initially, establish the area and location of bottom types and post-deposits.
- Secondarily, consider the concentrations and variances (including sampling repeatability within grab, between grabs same station, and along gradients) within the identified exposed and/or impacted areas.
- Thirdly, calculate the desired parameters and/or statistics from the collected data.
- Finally, make a decision based on the results.

As indicated in a previous section of this document, several possibilities exist for the choice of sample design and the allocation of samples in space and time.

7.2.2.1. Discharge sampling

Discharges from in-feed treatments begin as soon as feeding is initiated and the first feed pellets enter the water within the net-pen. Discharges end when the chemical is no longer present within the confines of the net-pen (including in the fish within the cage). The discharges mainly include waste feed and feces. Overviews of discharge models relevant to in-feed treatments are in Page et al. (2023b).

To determine the total quantity and concentration of the drug active ingredient that is available for discharge into the environment some record keeping and sampling is needed. For in-feed treatments, the quantity of medicated feed and the type of the medicated feed pellets administered to each net-pen during each treatment event should be recorded, and samples of medicated feed should be taken prior to the initiation of feeding; at least three samples should be collected from each batch of medicated feed. The samples should be analyzed for concentrations of the in-feed drug. This information can be used to estimate the total quantity of active ingredient administered to each net-pen during each feeding event and the pellet size information helps to refine model estimates of deposition rates. This information is of relevance to regulators, researchers, and farmers. The total quantity of treatment chemical and its concentration before release into the environment are needed for establishing whether treatment doses are achieved, are homogeneous, and are consistent with specified target doses. If the initial concentration is not homogeneous, then patches may exist that exceed expected concentrations. Total quantities are needed for some exposure estimation approaches and to allow for mass balance considerations. Sampling from within the source just prior to discharge should always be feasible and should be taken for any discharge that is to be monitored. Regulators and farmers may be interested in whether drug quantities are consistent with target doses and researchers require this information to initiate model estimates of discharges and exposure zones and profiles.

Sampling of waste feed and feces before it settles to the bottom may be useful to researchers to assist in the estimation of discharge rates and settling flux rates for use in evaluating, developing, and calibrating exposure models; however, such sampling is probably not routinely useful to regulators. If conducted, the samples could be taken from strata within the net-pen and at locations downstream of the feeding event(s). Figure 3 illustrates a potential sampling design.

7.2.2.2. Stratified random sampling of seabed

The first step is to define the potential bottom area of exposure within the area of interest. The location of deposition and impact areas is usually uncertain and judgements, even if based on models, are uncertain because of the highly dynamic environment into which the drugs are released and because existing model predictions are often inaccurate and imprecise. Unfortunately, this implies sampling for in-feed drug deposits requires a relatively large number of samples and a considerable allocation of resources. An initial non-destructive systematic survey of the seabed in the area that will define the boundaries of general bottom and/or habitat types is therefore needed. This step is consistent with approaches adopted by the US EPA (2002) and SEPA (2019b). The survey can consist of towed video, drop video or stills, or sonar with appropriate ground truth sampling. The survey could be a transect-based visual survey consisting of multiple transects at the resolution required to accurately characterize the habitat. This resolution will vary with the scale of the habitat features and type of discharge. Drop camera visuals can be collected at specific points along the transects for more detailed analyses. The origin of the grid of transects is randomly chosen. The survey information is needed to classify the bottom type and habitat strata; in some cases, existing information may be sufficient to preclude the need for this first survey.

The above survey is followed by a second systematic stratified random design sampling effort tailored to detect the distribution of the discharged chemical; the strata are defined on the basis of the bottom types and intervals of distance from the discharge location. During this second survey physical samples of the soft bottom samples are collected for chemical analyses. Hard bottom will need to be sampled with visual sampling methods to determine visible characteristics that are indicative of chemical exposure and impact. SEPA (2019b) is essentially using this approach although their sample locations are not randomly selected. The allocation of samples for this second survey could consider variance partitioning (T. Wilding, Scottish

Association for Marine Science, pers. comm.). The resulting measurements can be used to estimate chemical concentration statistics (means, variances, etc.) within the bottom type and distance-based strata. A combination of judgement and models can be used to help define the distance-based strata boundaries.

Another potential design uses strata based on direction and distance from the net-pen array. Examples of a sample allocation are given in Figure 11. These could be modified by bottom type if the information is available. The design in Figure 11 is based on eight radial boundaries and four distance boundaries. These combine to give thirty-two strata. The directional radii are separated by 45 degrees and the distance annuli are determined as: 1) the net-pen edge; 2) between the net-pen edge and the waste feed deposition boundary; 3) between the waste feed deposition boundary and the fecal deposition boundary; and, 4) beyond the fecal deposition boundary. These distance intervals are consistent with results reported in Chamberlain and Stucchi (2007) that suggest a near-field region exists that is primarily exposed to the deposition of wasted feed and a far-field region in which primarily feces deposition occurs.

The distance boundaries from the edge of the net-pen or net-pen array are estimated as $d = uH/w_s$, where H is the representative water depth, w_s is the representative sinking rate of the feed (appropriate for the feed type and fish size) or feces (appropriate for the feed type, fish size, and expected fecal production), and u is the median horizontal current speed. Waste feed sinks faster than feces and hence reaches the seabed sooner than waste feces. As a result, the area within which waste feed gets deposited on the seabed is smaller than that for feces and the outer boundary of the waste feed exposure area is closer to the discharge point than for feces. Measured mean sinking rates of salmonid feed pellets range from 5 to 20 $cm \cdot s^{-1}$; measured mean sinking rates of well-formed salmon feces range from 1.5 to 7.6 $cm \cdot s^{-1}$; fecal mucus strings and slurries have much lower sinking rates and behave more like passive particles (Page et al. 2023b). These rates indicate that for any given depth, feces spend up to 15 times longer, or more, in the water column than waste feed and may be transported up to 15 times the distance of waste feed. At a sinking rate of 10 $cm \cdot s^{-1}$ a waste feed pellet will take 100 s (1.7 min) to sink to the bottom in 10 m of water and will travel a horizontal distance of 10 m when the mean current speed is 0.1 $m \cdot s^{-1}$. The same pellet will travel 50 m horizontally in 50 m of water and 100 m horizontally in 100 m of water. If the current is 0.2 $m \cdot s^{-1}$ these distances will double. These calculations should be interpreted as rough indicators, i.e., the distances travelled, the area occupied, and the concentrations expected vary by a factor of ten. This means if a predicted distance travelled is 10 m it is between 1 and 100 m, if the predicted distance is 100 m it could be between 10 and 1 000 m, and if the predicted distance is 1000 m it could be between 100 and 10 000 m. Similarly, if a predicted area is 100 m^2 it could be between 1 and 10 000 m^2. The consequence of this is that sampling designs may need to consider sampling a relatively large area if they are expected to sample the discharge.

The allocation of sampling stations within each stratum should be randomized. The outer limit of the sample domain is beyond the fecal settling boundary, suggested to be 50 – 100 m. This design is similar to the SEPA design but has the advantage of being probabilistically based.

The above design distributes the samples in space and allows for the measurements associated with subsets of samples to be combined for specific analysis purposes. The spatial resolution of the design can be modified by changing the angle between radii and by changing the distance annuli. In the example (Figure 11) we have illustrated equal radii separated by an angle of 45°. The design allows the chemical concentrations to be contoured and areas within specific contour intervals to be calculated, allows the edge of area to be estimated (e.g., area in which concentrations are above a particular threshold value) and statistics such as the mean and variance of concentrations within each strata or combination of strata to be calculated. The design also provides information useful for model evaluation and development but does not rely

on model predictions. Model predictions can be used qualitatively to help decide the number and resolution of the radial sectors and distance bin intervals.

Samples can be randomly located within each stratum with the number of samples being equal for each stratum (disproportional sampling when the strata areas are unequal) or proportional to the area of each stratum (proportional sampling). The disproportional sampling means the resolution of the sampling scheme reduces with distance from the net-pens and depending on the rate of dispersal and amount of stretching of the discharge plume this may result in lower probabilities of detecting the deposit and variances in the measurements that are a function of distance. If a single current meter record is used when defining strata boundaries, it should be recognized that predicted feed and fecal displacements are highly uncertain beyond 500 m due to spatial variation in the current fields (SEPA 2005).

For example, a random allocation of one sample per sector results in 32 samples (8 radial sectors each containing 4 distance bins) as in Figure 11. Mean measurement values can be collected within each radial sector or each distance sector for comparison to thresholds in a statistical way. Trends in concentration with distance from net-pen edge can be examined by lumping all of the data together (n=32) or by subsets of directional sectors. Estimates of area above thresholds can be obtained by contouring measurement data. The distances from the farm at which thresholds are crossed can be estimated within each sector or subset of radial sectors. This design acknowledges the qualitative expectation that deposition patch sizes increase with distance from the net-pens.

Research should be conducted to refine how best to calculate and define these boundaries. A design with only four directional radii and four distance annuli would result in 16 strata. In this case the angle between directional radii could be 90° and the distance annuli could be determined as above. This design has much less spatial resolution than the 32-strata design.

The design differs from the SEPA design in that the allocation of samples is random and the distance boundaries are based on simple particle displacement criteria rather than a set distance or a more involved mode calculation (e.g., DEPOMOD or new DEPOMOD, presently being evaluated by SEPA).

Variations on the above design can be considered. For example, samples from the net-pen edge can be eliminated and the effort distributed to other strata. Another design is one in which samples are also collected from within a farm's net-pen array (Figure 12) and one in which sampling is confined to a subset of the strata, such as the strata within the radial sectors aligned with the predominant current (Figure 12 and Figure 13, top panels). When the direction of the water current during the treatment period is reasonably well known and is fairly consistent among treatments, a transect sampling design may be adequate. If the direction of the current is uncertain or if the net-pen has been treated multiple times, all four quadrants should be sampled since the current direction is likely to be different for each treatment (Figure 12 and Figure 13, bottom panels). In both above approaches sampling is essentially a stratified random design.

Confidence in reduced sample designs will come from experience associated with more spatially extensive sampling and modelling activities.

A reduced monitoring effort associated with each farm could consist of about ten samples taken along the outer edges of the net-pen array or within the near-field distance strata after completion of a farm wide in-feed treatment. This will not define the area of exposure or impact, but it may give an indication of the magnitude of maximum chemical concentrations within the surficial substrate, assuming these occur in the near-field. This more limited information may also contribute to risk assessments aimed at identifying farms needing more extensive sampling.

Model calculations and preliminary sampling results, see Page et al. (2023b) and Appendix B, suggest the distance boundaries may be quite far from the farm, sometimes on the order of kilometers. The preliminary sampling results indicate that there may be a near-field zone with relatively high chemical concentrations and a far-field zone of lower concentrations. For large expected the distances (>500 m), simple models will be unable to estimate the boundaries of these zones (SEPA 2005). High resolution hydrodynamic models that are calibrated and validated for the area surrounding each fish farm will need to be coupled with discharge models to generate far-field estimates of spatial distributions and desired sampling boundaries. Implementation of these models is not be feasible within short periods of time and their accuracy and precision need to be more thoroughly evaluated before they are accepted as routine tools for regulatory purposes. Due to the time and cost involved, implementation for all sites is prohibitive. Regulators may need to consider whether the release of chemicals that have the potential to result in toxic concentrations over large areas is acceptable and manage the industry accordingly.

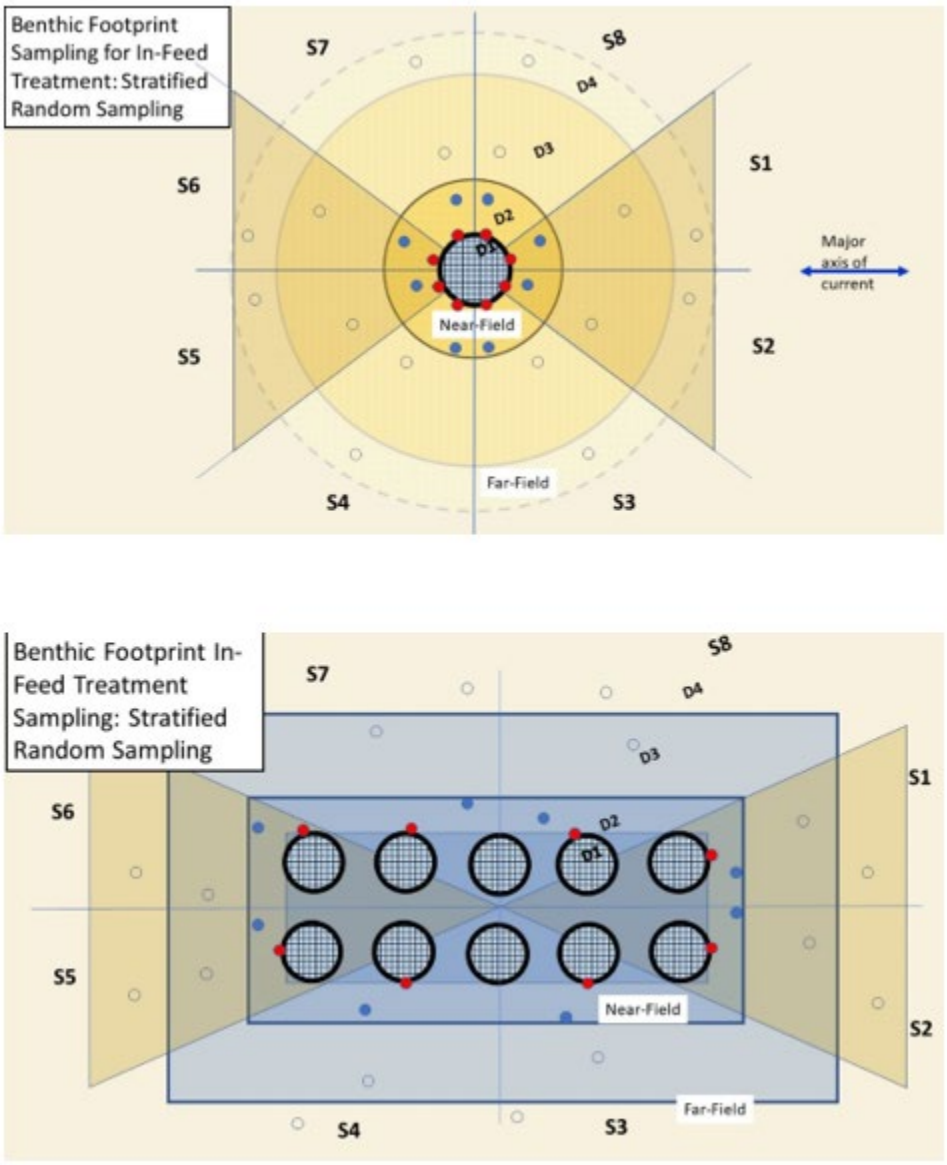

Figure 11. Conceptual Illustrations (not to scale) of a post-deposit stratified random sample design for deposited drug chemicals. The top panel illustrates the design for a single net-pen and the bottom panel for a net-pen array. Closed and open circles represent sampling locations in the near-field and far-field, respectively. Red circles are net-pen edge samples. Large black gridded circles are net-pens. Radial lines indicate the boundary between directional sectors labelled as S1 to S8, Circular and rectangular lines indicate the boundaries between distance intervals labelled as D1 to D4 within each sector. Strata boundaries are the areas within the various boundaries. The design shown includes one sample per stratum.

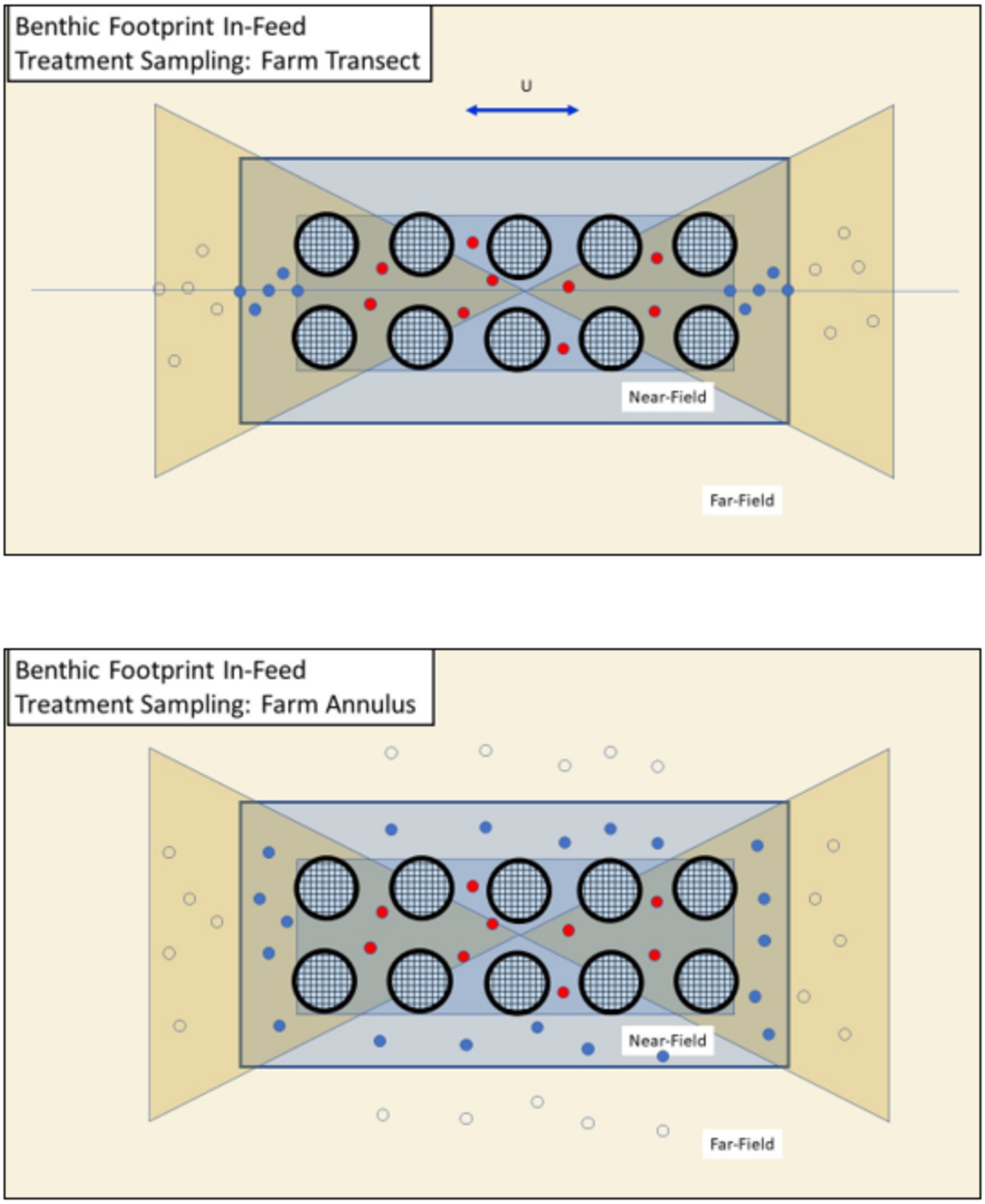

Figure 12. Illustration of the location of benthic samples associated with multiple in-feed treatments conducted on a farm. The top panel shows a transect sampling approach and the bottom panel shows an annular sampling approach. The radial lines divide the sample area into quadrants. The large rectangle indicates the distance waste feed travels during the time the feed takes to sink to the bottom. The distance depends on the water depth (H), sinking rate (Ws) and horizontal current speed (U). The inner rectangle indicates the area within the cage array. The area within(outside) the larger rectangle is the near(far)-field area. Red circles represent sampling station within the cage array; blue circles represent other near-field sampling stations; and open circles represent far-field sampling stations.

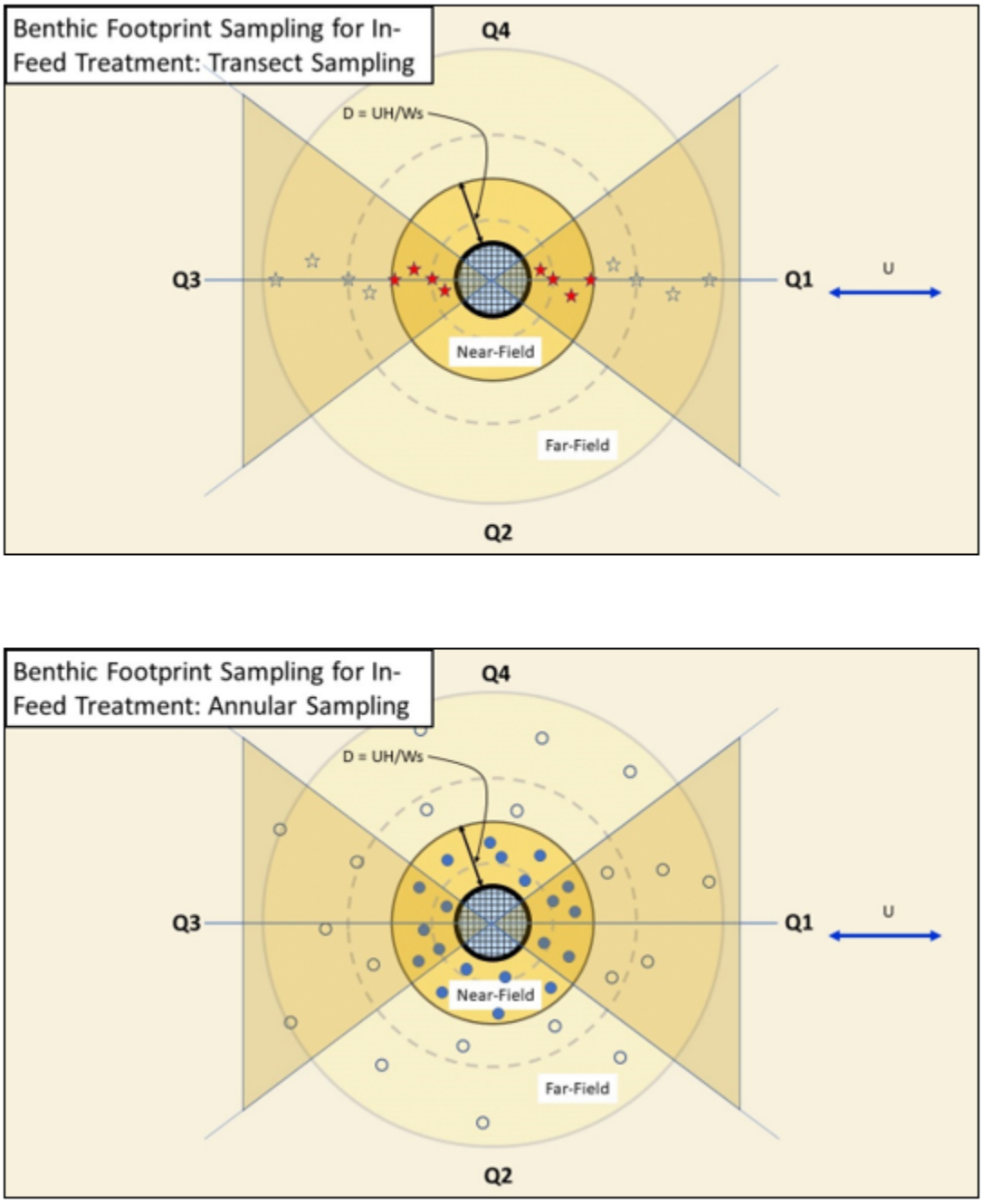

Figure 13. Illustration of the location of benthic samples associated with in-feed treatments. The top panel shows a transect sampling approach and the bottom panel shows an annular sampling approach. The radial lines divide the sample area into quadrants. The solid black circular line indicates the distance waste feed travels during the time it takes to sink to the bottom. The solid grey circular line indicates the distance feces travels during the time it takes to sink to the bottom. Distances depend on the water depth (H), sinking rate (Ws), which differ for waste feed and feces, and horizontal current speed (U). The near-field sampling area is between the edge of the net-pen and the solid black line. The far-field sampling area is between the solid black and solid grey lines. In the top panel, red stars represent near-field sampling stations and open stars represent far-field sampling stations. In the bottom panel, blue circles represent near-field sampling stations and open circles represent far-field sampling stations.

7.2.2.3. Temporal sampling design

The purpose of these designs is to follow the temporal evolution of the measurement values over time and determine whether the measurement values exhibit temporal trends (ISO 2004).

All sampling approaches should require that the location, date, and time of treatment are recorded. If this is not known, the time window within which sampling occurs may limit the value of the sampling and make the sampling inadequate to address the motivating sampling objective.

The spatial sampling approaches described previously will provide information concerning the spatial variation in chemical concentration. Designs for temporal sampling follow the results obtained from the spatial assessment sampling (ISO 2004); in other words, once the spatial domain of contamination or impact has been identified, or at least patches or hot spots of chemical concentration have been located, the patches become the focus of a targeted sampling design that is repeated at multiple temporal intervals using a consistent sampling methodology (ISO 2004).

The temporal samples should also be allocated in a stratified random manner. The interval between sampling dates should be sufficient to resolve the temporal pattern, including trend, that is of interest. Once this interval is established the first sampling date should be chosen randomly within the first appropriate sampling interval (US EPA 2002).

Determining the temporal persistence of chemicals within the exposure zone, i.e., what is the rate of decrease in the concentration of the chemical once it has been deposited on the seabed, is likely of interest to researchers, regulators, and farmers but may be difficult to determine in-situ. It can be used to help evaluate the accuracy, including precision, of model predictions and to indicate the variation associated with limited sampling. Regulators may incorporate the information into policies and regulations. Regulators may also be interested in whether concentrations persist for time periods longer than environmental threshold durations and whether there is potential for concentrations to increase over time if exposures from additional discharges are experienced. Researchers need this information so in situ decay can be incorporated into models estimating the temporal evolution of chemical concentrations. Both are interested in whether in-situ decay rates are consistent with theoretical or laboratory estimates. The variation in the measurements over the more limited sampling scales can be used to help interpret and design better spatial sampling designs and data analysis procedures.

8. SUMMARY

1. The purpose of the present document is to outline a sample planning and design process; it is not meant to provide specific sample designs.
2. In this paper we have provided an overview of a process for designing a compliant sampling program for bath pesticide and in-feed drug aquaculture discharges and deposits.
3. In order to design a proper sampling program, a systematic process should be followed. Proper design of a sampling program includes the following steps:
    a. State the problem.
    b. Identify the decision.
    c. Identify inputs to the decision.
    d. Define the boundaries of the study.
    e. Develop a decision rule.

f. Specify tolerable limits on decision error.

g. Optimize the design for obtaining data.

4. The development of a sampling program should include multi-disciplinary expertise.

5. Probability-based sampling designs are preferable over judgement-based sampling designs whenever possible.

   a. Judgment-based sampling designs depend upon the level of knowledge and personal judgement of the expert(s) involved, the accuracy and precision of the sampling results is unknown, and interpretation of the results depends on the personal judgement of the deemed expert.

   b. Probability-based sampling designs allow quantitative estimates of desired statistics and the uncertainties associated with these statistics. When the documented sampling and analyses protocols are followed, the results are independent of expert judgement and should be reproducible within the uncertainty limits. Statistical inferences can be made concerning the exposure and impact on the target population and decision error criteria can be incorporated in the analyses and decision processes.

6. Selection of a sampling design should include a proper sampling planning process.

7. Before any model is used in the design of a sampling program it needs to be properly validated for this use.

8. Because the uncertainties associated with existing models are unknown, the use of model results in the design of a monitoring sampling program should be limited.

9. In terms of designing a sampling program for measuring aquaculture discharges and deposits from in-feed drug and bath pesticide treatments, the boundary of the study, the decision rules, and the required inputs have yet to be determined. The process of developing a robust post-deposit sampling program should be considered to be in its infancy and be expected to evolve over the coming years.

10. This document provides some insight into the sampling design problem for aquaculture chemical discharges and deposits, its challenges and constraints, and a conceptual foundation for moving forward.

11. There are many challenges associated with sampling the discharges from both bath pesticide and in-feed drug treatments. Some of these are:

    a. pesticide discharges are not visible therefore it is difficult to position sampling efforts within or outside the discharge cloud or deposition area;

    b. the number of discharges is relatively low;

    c. the characteristics of each discharge (the size, shape, concentration, and location) change over time;

    d. for in-feed treatment discharges, obtaining samples from the seabed when the substrate to be sampled is not soft sediment can pose challenges;

    e. sample numbers and sample quantities (e.g., 100 g of sediment or 1 L of water) are small and have a high probability of missing the discharge cloud or deposition area;

    f. interpretation of the data is difficult because of the sampling challenges and the variation in results;

g. existing sampling methodology only allows for chemical analysis of samples collected from water, soft bottom, or organisms.

12. Previous field studies have mainly selected sampling locations based on judgement or unvalidated model prediction. In the case of bath pesticide treatments the judgement was sometimes guided by the use of tracers added into the treatment bath (dye) or discharge cloud (drifters). Most sampling for in-feed drugs and pesticide used a small number of samples.
13. To our knowledge, the only aquaculture seabed sampling design in place for monitoring in-feed drugs is the interim design adapted by SEPA.
    a. SEPA has clearly stated objectives.
    b. SEPA has implemented a standardized modelling process.
    c. SEPA has implemented well-described sampling designs.
    d. SEPA uses the concept of an allowable mixing zone. The mixing zone has a defined area based on a constant distance from the farm criterion and the boundaries are determined from model runs. Both the size of the area and location of the boundaries are site specific.
    e. SEPA uses two environmental thresholds, one within the allowable mixing zone and a lower one outside of the allowable mixing zone.
    f. The sampling design is based on the characteristics of the allowable mixing zone.
    g. The sampling design has some limitations including lack of knowledge concerning the probability of detecting an impact zone.
    h. The SEPA program could potentially be improved by using a more probabilistic approach.
    i. As with any sampling design, whether the SEPA design has merit to the Canadian situation depends on the decision criteria.
14. Assured detection of exposure zones associated with the discharge of chemicals from net-pen farming operations requires a large and expensive sampling effort.
15. Bath pesticide discharges are dispersed and stretched by local hydrographic processes that result in meandering clouds of chemical whose size increases with time, location changes with time, and chemical concentrations decrease by several orders of magnitude within a few hours. Sampling designs for detecting and characterising the location, shape, and size of these rapidly changing exposure and impact areas are challenging and not well established.
16. In-feed drug discharges can be distributed within patches located with geographic areas that encompass hundreds to thousands of square meters.
17. While sampling design concepts for detecting and characterising the location, shape, and size of slowly changing exposure and impact areas are reasonably well established, the implementation of these steps to generate a preferred sample design for a specified set of objectives takes considerable effort.
18. More sampling efforts need to be undertaken and the results used to refine monitoring programs.

9. KNOWLEDGE GAPS AND UNCERTAINTIES

1. There is uncertainty in the objectives, decision rules, and tolerance levels associated with post-deposit sampling.
2. There is uncertainty in the variation associated with the concentration of treatment doses achieved in realized treatments.
3. There is uncertainty in farm specific estimates of the geographic domain over which discharged chemicals may be distributed.
    a. In the case of in-feed treatments, this uncertainty stems from two main sources:
        i. uncertainties in the form, characteristics of discharged particles, and the mass distribution of the total chemical discharge amongst the spectrum of output particle types; and
        ii. uncertainties in the spatial and temporal variations in the local hydrography that dictates the transport, dispersal, deposition and re-distribution of the chemical discharge.
    b. In the case of bath treatments there are uncertainties in the spatial and temporal variations in the local hydrography that dictates the transport and dispersal of the chemical discharge.
4. There is uncertainty in the spatial and temporal distribution of valued and sensitive ecosystem components (e.g., habitat types, organisms, etc.) on the scale of expected exposure domains.
5. There is uncertainty in the distribution of bottom types in net-pen farming areas.
6. Efficacy of sampling techniques is not well known.
7. The precision and accuracy of predictive exposure and impact models are not well known.
8. The vertical distribution of chemicals in the substrate is unknown. This is important because the depth of the sample influences the estimated concentration of the chemical in both samples and model outputs.

10. CONCLUSIONS AND RECOMMENDATIONS

10.1. SAMPLE DESIGN

Since the Canadian Government is in the process of identifying the information required from an aquaculture post discharge and deposit monitoring program, it is premature to recommend specific sample designs. There are, however, many features that should be taken into account in the design of a sampling program for aquaculture monitoring.

1. The development of a sampling design cannot proceed until the following have been established:
    a. clearly stated and understood objectives,
    b. measures (e.g., biomass, species, and chemical concentration) and statistics (e.g., mean, median, maximum, variance, and autocorrelation) of interest,
    c. how the measurements obtained from the samples are to be used,
    d. decision tolerances, if applicable,
    e. availability of technical, financial, and temporal resources.

These sentiments have also been expressed by others, e.g., Wilding et al. (2017).

2. The development of a sampling design should include multi-disciplinary expertise and the involvement of decision makers, scientists, and statisticians.
3. The outputs of a sampling design need to meet the decision makers' tolerance levels.
4. In the case of seabed sampling, baseline information must be available or collected to allow the determination of bottom types within the boundaries of the target population and to select the appropriate sampling methods and locations. Depending on objectives, additional baseline information may be required and could include habitat, pesticide and drug concentrations, or biodiversity.
5. In the design of a sampling program, the temporal dynamics, over the relevant time-scales, of deposition and persistence of the pesticides and drugs of interest should be taken into consideration. If this is not done the sampling could be ineffective and give misleading conclusions (Wilding et al. 2017).
6. All estimates of post discharge concentrations are dependent on the initial concentration, which can vary. Therefore samples of pre-discharge bath water or medicated feed should be taken to confirm treatment dose concentrations.
7. If a sample design for collecting samples of bath water is to be implemented, it should be undertaken in association with individual discharge events, perhaps randomly selected, as opposed to one sampling conducted after all net-pens on the site have been treated.
8. For bath treatments, meaningful and feasible sampling must be associated with discharges that are tagged with visible tracers (e.g., dye) and sampling must be conducted within a few hours (0 - 5 h) of release.
9. For in-feed treatments, probability-based designs for a post-deposit sampling program are recommended over judgement-based designs since they allow for statistics to be calculated.
10. For in-feed treatments, seabed sampling should typically be initiated after the completion of the site treatment. Exact timing of the sample collection could depend on factors such as regulatory requirements and the drug release pathways and timelines.

10.2. RESEARCH

1. Assess model accuracy, precision, and adequacy to meet the needs of the decision maker in terms of designing meaningful and practical sample designs.
2. Develop quantitative sampling methods for indicators of chemical exposure related to coarse grained and hard bottom substrate types.
3. Generate information on the efficacy of sediment sampling techniques, i.e., the ability to collect undisturbed sediment samples.
4. Identify potential indicators of environmental impacts that are specific to the chemical discharged.
5. Identify possible thresholds defining acceptable spatial scales of exposure and impact.
6. Provide advice to decision makers to ensure that proposed tolerance limits are scientifically achievable. Conduct theoretical investigations to estimate the probability of detecting deposition and impact zones as a function of different treatment, model, and sample design scenarios.
7. Quantify uncertainties associated with model predictions of exposure and impact.

8. Quantify uncertainties associated with sample measurements including methodological uncertainties and in situ spatial and temporal variabilities.
9. Investigate and characterize the vertical distribution of chemicals in the substrate in multiple locations representing a range of substrate types and depositional conditions.

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APPENDIX A. LITERATURE REVIEW OF PESTICIDE AND DRUG ENVIRONMENTAL SAMPLING EFFORTS FOR WATER AND SEDIMENT

Relatively few studies have been conducted in relation to the detection and monitoring of the exposure zone, impact and persistence of pesticides and drugs discharged from salmon net-pen operations. Although the tasks of the sampling activity undertaken by these studies was often similar, i.e., measure concentrations of drugs or pesticides in the water column or bottom sediments, the objectives and sample designs differed between studies.

The more common objectives included:

- Determining environmental or ecological impact (Dobson and Tack 1991; Parker and Mallory 2004; SAMS 2005; Telfer et al. 2006; DFO 2012; Ernst et al. 2014; Bloodworth et al. 2019);
- Conducting an environmental risk assessment (SEPA 2019b);
- Establishing environmental concentrations (Tucca et al. 2017; SEPA 2018);
- Gaining knowledge on how a chemical is dispersed into the environment or ecosystem (Ernst et al. 2001; Langford et al. 2014; Page et al. 2015; Samuelsen et al. 2015);
- Determining the persistence of chemicals in the environment (Selvik et al. 2002; Parker and Mallory 2003, 2004; Samuelsen et al. 2015);
- Regulatory Monitoring (SEPA 2011, 2019b);
- Providing science advice (DFO 2012).

The sample designs could for the most part be considered as judgemental designs as opposed to statistically- or probability-based designs and consisted of point samples at specific locations and along transects.

A brief summary of each of the studies is given below.

A.1. CANADA, NEW BRUNSWICK

Ernst et al. (2001) conducted field studies in 1996 and 1997 in New Brunswick to measure the dispersion of these bath treatment drugs. Although the sites selected were not at locations where aquaculture farms were present, six sites were selected to represent the range of conditions suitable for net-pen fish farming. Three treatments with azamethiphos were simulated in 1996 and three with cypermethrin in 1997. Pesticide treatments were simulated using a single 50 m circumference circular cage collar float fitted with a treatment tarpaulin without a net-pen or fish. In order to track the evolution of the released treatment plume, a visible and photo-active dye was mixed with the pesticide solution. Water samples were taken in the treatment tarpaulin before release to determine the efficacy of the mixing of the pesticide pre-release and in the post-treatment dye plume. The behaviour of the plume during the time period over which dye concentrations were detectable and the distances traveled during this time varied from site to site.

A later field study by Ernst et al. (2014) also used dye to visualize the discharge and dispersion of water from bath pesticide treatments with azamethiphos and deltamethrin. The study sites were located in New Brunswick at farms where operational treatments for sea lice were taking place. The farm sites were chosen to represent the range of environmental conditions at fish farms in the region. During September and October 2010, samples were collected from seven treatment events which included three tarp treatments with azamethiphos, three well-boat treatments with azamethiphos, and one tarp treatment with deltamethrin. Sodium fluorescein

dye was added to the pesticide solution to facilitate selection of sampling locations. Before the release of the treatment water, several samples were collected at various depths from within the tarped net-pen or well-boat. After the release of the treatment water, samples were collected at various times, depths, and locations. Sampling after the discharge of the bath water occurred at locations where either the dye within the plume was visible or fluorometric measurements indicated the presence of dye. Water samples were filtered to separate suspended solids from the water phase and chemical analyses conducted on both to determine concentrations of pesticide in each phase. It was found that the proportion of azamethiphos in the aqueous phase was always approximately 100 times that in the particle phase. Deltamethrin, however, had proportions in the solid phase three to four times greater than in the aqueous phase. The different results for the two pesticides are due to their different log k_{ow} values: azamethiphos has a low log k_{ow} therefore is more hydrophilic (stays in the aquaeous phase) and deltamethrin has a high log k_{ow} therefore is hydrophobic (binds to organics). Azamethiphos was detected approximately 1700 m from the treatment site when tarp treatments were used but only 150 m from the well-boat discharge site. Deltamethrin could be detected approximately 1000 m from the discharge site. Good correlations were found between the dye and pesticide concentrations indicating that measuring the concentration of dye in the plume is a cost-effective solution to obtaining real-time concentrations of pesticides within the discharge plume.

A comprehensive field program was undertaken by Page et al. (2015) to investigate the factors that influence the transport, dispersal and exposure of non-target organisms to pesticides used in bath treatments. Following the earlier work by Ernst et al. (2014) fluorescein dye was again used to visualize treatment water for a combination of skirt, tarp, and well-boat sea-lice treatments using deltamethrin, azamethiphos, and hydrogen peroxide. Time series photographs as well as dye measurements were used to investigate the behaviour of the treatment from the initial addition of the pesticide to the treatment water, throughout the treatment period, and during the discharge from either the net-pen or well-boat. Fluorometers were used to measure dye concentrations at various locations and depths. Furthermore, the temporal and spatial evolution of the dye patch was measured by tracing the perimeter of the visible edge of the dye patch with a small boat equipped with a GPS. Results from the tarp treatment studies indicated mixing within the tarped net-pen was not always uniform, the discharge period once the tarp was removed varied from minutes to hours, and there was considerable variation in the shape of the discharge plume. When treatments were conducted with well-boats, the treatment water was well mixed and the discharge concentration predictable. Once in the environment, however, the behaviour of the discharge plume depended on the location, timing, and direction of the discharge. It was concluded that in order to track the discharge plume of treatment water, whether from tarp treatments of well-boats, a visible tracer is required so that the plume location may be determined. Furthermore, there was a large variation in measured concentrations and so multiple measurements are required to increase the likelihood of getting realistic representative values.

Two sampling programs were carried out in southwest New Brunswick to measure the persistence of concentrations of emamectin benzoate in sediments around salmon farms post-treatment, one in 2002 and one in 2003 (Parker and Mallory 2003, 2004). For the 2002 field study, a SCUBA diving team was used to collect sediment samples from six locations in the potential zone of impact and three control areas. Sampling stations were at locations where deposition was expected, although it was not known if the depositional area included the deposition of chemicals. At each station, three core samples were taken with the diving team instructed to look for fine grain sediments only and to minimize the disturbance of the contents in the core tubes. Samples were collected on two occasions, once before the treatment and at one occasion approximately 10 weeks after the treatment. The methodology for the 2003 study was similar with the main differences being the locations and timing of the sampling. Three

sampling locations were selected in the potential exposure zone and one location was selected in each of three control areas. Samples were collected on five separate occasions, one on the first day of treatment but before treatment began, and approximately 1, 2, 4, and 8 weeks post-treatment. No detectable amounts of emamectin benzoate were found in the sediment samples collected during either study though it was noted that the detection limit achieved in the study is above the predicted no effect concentration (PNEC) established by SEPA raising the possibility of the samples containing concentrations above the PNEC.

A.2. CANADA, BRITISH COLUMBIA

A study was conducted in British Columbia (DFO 2012) to examine the effects of emamectin benzoate on the pacific spot prawn (*Pandalus platyceros*) near aquaculture sites. In addition to biological samples, field studies included the collection of both sediment and water samples. Samples were analysed for emamectin benzoate using the methodology of Ikonomou and Surridge (2013) which has detection limits in the parts per trillion level ($pg \cdot g^{-1}$) for sediment and part per quadrillion ($pg \cdot L^{-1}$) for water. Two farm sites were selected with different hydrodynamic and benthic conditions. The sampling design had both temporal and spatial components. At each farm, samples were collected before, during, and after treatment with emamectin benzoate. At one farm, samples were collected along two transects, one extending east and one extending west from the farm. Each transect had five sampling stations from 0 to 150 m from the net-pens and a reference station. At the other farm, samples were collected at 0, 100, and 300 m along a transect south-west of the farm. Details concerning the expected relationship between the location of these samples and expected locations of chemical deposition were not provided. At this farm, water samples were only collected at the net-pen edge. Water samples from the immediate vicinity of each farm contained low levels of emamectin benzoate following treatment. Four to five weeks post-treatment, no chemical was detected in the water samples. The measured concentrations of emamectin benzoate in the sediment samples varied between the two sites with concentrations at one site always close to the limit of quantification of the analytical method. At the other site, detectable concentrations of emamectin benzoate were within 150 m of the farm and emamectin benzonate was detected 1.5 years after treatment.

Park (2013) also examined the effects of emamectin benzoate on the Pacific spot prawn (*Pandalus platyceros*) near aquaculture sites in British Columbia using the methodology of Ikonomou and Surridge (2013) to analyse collected sediment samples. The sampling program included five farm sites in the Broughton Archipelago with different ages of production fish and different stocking densities. Four reference locations having similar physical features to the farm sites were selected. At two farm sites, samples were collected one week before, during, and one week after treatment with emamectin benzoate. At two others, samples were collected ten days and two months after treatment. At the fifth farm site, samples were collected two months after treatment. No models predicting the location of emamectin benzoate deposits were referred to. All collected sediments, including pre-treatment samples, had concentrations of emamectin benzoate in the part per billion level. The two sites that were sampled pre-treatment had previous treatments with emamectin benzoate, three years and one year prior to the study, indicating that emamectin benzoate can persist in the sediments for long periods.

A.3. NORWAY

Selvik et al. (2002) conducted a laboratory study to examine the stability and persistence of diflubenzuron (an in-feed drug) in marine mud and shell sand sediment. The study also included a field component to measure the dispersion and persistence of diflubenzuron under a fish cage following an in-feed treatment with diflubenzuron. Although diflubenzuron is not approved for use in Canada the study can still provide insight into potential sampling design strategies. The

field study took place during and after a fourteen-day treatment at a site on the west coast of Norway. On the first day of treatment, four sediment traps were positioned 2 m above the seabed at the edge of the cage in the north, south, east, and west positions. The traps were emptied at days 3, 7, 10, and 14 following the initiation of the treatment. Sampling of the sediments on the seabed were also taken using a Van Veen grab. Sampling stations were located along transects in the north-south and east-west directions at 0, 5, 10, and 20 m from the cage edge for a total of 16 sites. Samples were also taken from a reference site located approximately 500 m from the cage. Fifteen months after the treatment, sediment samples were taken from three of the sites. No information was provided indicating how the samples aligned with expected deposition patterns of the chemical. It was found that the contents of the sediment traps contained primarily feed pellets and fecal particles and had high concentrations of diflubenzuron. Concentrations found in the sediment were low. Selvik et al. (2002) offered two possible explanations for this discrepancy: the sediment was dispersed by the water movement generated by the grab sampler and/or the sediments were transported to other areas. It was recommended that both sampling techniques be used (sediment traps and grabs) with special attention being paid to the grab samples to ensure that the easily re-suspended fluffy top layer on the sediment is properly captured.

Langford et al. (2014) conducted sampling programs at five Norwegian fish farm sites (cod and salmon) and one reference location to determine the transport and dispersal as well as the concentration of anti-parasitic medicines in several biological and physical aquatic compartments, including water and sediment samples. The farms were selected to represent different drug usage and different physical environments. At each farm, sampling only occurred at one instance within 2 months of treatment for fish lice with one or more of the authorized drugs. At each site, five water and sediment samples were collected. Up to five sample locations were positioned up to 900 m from the fish farm along transects determined from local wind and current conditions. Locations of water and sediment samples did not necessarily coincide. Water samples were collected at a depth of 10 m except at one site where water samples were collected near the seabed. For the sediment samples, three parallel grabs were collected from the upper 2 cm. The location of the samples was based on estimates of the local wind and water current. Concentrations of the bath treatment drugs deltamethrin and cypermethrin were below detection limits in all samples. None of the farms included in the study had reported using cypermethrin. Two sites had reported using diflubenzuron and concentrations of this drug were found in both water and sediment samples at those sites. At one site, the concentration of diflubenzuron in water samples was highest at the farm and decreased with distance from the farm. Two farms reported using teflubenzuron. At one of these locations, detectable concentrations of teflubenzuron were found in the water samples with the highest being 900 m from the farm. Both farms had concentrations of teflubenzuron in sediment above the detection limit. Detectable concentrations of emamectin benzoate were detected in some, but not all, of the sediment samples at the two farms that had reported using the drug.

Another field investigation was carried out in Norway to examine the distribution and persistence of teflubenzuron in wild fish and benthic organisms after an in-feed treatment at a commercial fish farm (Samuelsen et al. 2015) with no previous history of using flubenzurons. Medicated feed was given over a seven-day period. Duplicate sediment traps were deployed the day before treatment at the farm and 250, 700, and 1100 m from the farm in the direction of the dominating surface current. Traps were deployed 10 m from the bottom. At sites away from the farm, additional traps were deployed at 50 m depth. Traps were emptied on the last day of treatment and approximately one week and two weeks later. At the farm, the sediment collected from the traps consisted of feed pellets and fecal matter whereas traps further from the farm contained small amounts of organic material. The concentration of teflubenzuron was high at the farm both on the last day of treatment and one week later but decreased significantly after

two weeks post-treatment. Bottom sediment samples were also collected using a van Veen grab on the last day of treatment at 150, 400, and 700 m from the farm. Since the topography of the fjord made sampling difficult, bottom topography data was used to select the sampling positions. Multiple sediment grabs were also collected at the farm site on the last day of treatment, three months post-treatment, and eight months post-treatment. A large variation in the amount of sediment and drug was found in the samples collected at the farm and amounts of chemical found in the bottom samples were generally not consistent with the amounts in the samples from the sediment traps. The concentrations at the farm reduced over time and no drug was detected at distant sites. Water samples were also collected in this study on the last day of treatment 50 m from the farm at 11:00, 13:00, 15:00, and 16:00 from depths of 10, 20, and 50 m. The levels of teflubenzuron in the water samples were low.

A.4. CHILE

In Chile, the in-feed pesticides emamectin benzoate, teflubenzuron, and diflubenzuron are used to control sea lice in fish farms as well as the bath treatment pesticides deltamethrin and cypermethrin. Sampling was conducted at four farms with different chemical usage to determine concentrations of anti-parasitic pesticides in the sediments (Tucca et al. 2017). Sampling sites were located at distances of 0, 10, and 100 m from the fish cage in the direction determined by the dominant current. At the farm, at least five replicate samples were collected. Samples were collected five days post-treatment at two of the farms and three days post-treatment at the others. All collected samples contained detectable amount of chemicals. The observed distribution and concentration pattern depended on both the drug and the site.

A.5. SCOTLAND

Dobson and Tack (1991) conducted field studies to examine the dispersion of the bath treatment pesticide dichlorvos. The experiments were conducted at two salmon farm sites in two different lochs in Scotland. Combined, the flushing times of the two lochs were representative of those in half of Scottish lochs. The aim of the study was to monitor concentration of dichlorvos at two times at each loch, coinciding when the flow out of the loch was theoretically at its lowest and highest. Each farm had eight net-pens and these were treated to mimic a one-day treatment scenario using the skirted tarpaulin method. In order to visualize the evolving plume of the released treatment water, Rhodamine B was added at the time of treatment. Of the eight pen treatments at each site, only four were monitored closely. Pre-treatment samples were collected at seven depths from two locations directly adjacent to the pens. Post-release, samples were taken from the centre of the discharge patch at depths of 0, 2.5, 5, 10, 15, 20, and 25 m from the surface. Additionally, samples were taken along a transect from the centre of the treated pen and the centre of the dye patch at depths of 2.5 and 7.5 m. Twenty four hours post-release, samples were taken at two locations directly adjacent to the net-pens at 2.5, 5, 10, 15, 20, and 25 m depths. A further set of samples were collected from 30 stations located on a regularly spaced grid (distances between stations not specified) that spanned the full width of the loch within the vicinity of the treated farm. Environmental conditions, i.e., temperature, salinity, wind, rain, and tides, were also collected as part of the study. Observations of the dye patches leaving the treated net-pens indicated that the treatment water left the pens gradually. Furthermore, the fluctuation of the tide had small impacts on the rate of dispersion but did impact the direction of the dispersion. No concentrations of dichlorvos were measured above the detection limit outside a 25 m perimeter of the pens. Within this perimeter dichlorvos was detected to a depth of 10 m over a period of 1 to 1.5 h.

An early field study was conducted by Schering-Plough Animal Health to measure the concentrations of emamectin benzoate after in-feed anti-parasitic treatment at a Scottish fish

farm and this was used by SEPA to conduct a risk assessment of the use of the drug (SEPA 1999) as part of the approval process for granting consent to use emamectin benzoate. SEPA also used results from a depositional model (DEPOMOD) in the risk assessment. Field samples were collected to measure concentrations of emamectin benzoate in sediment, flocculent material, water, and silt traps. Few water samples were collected since the physical properties of the compound and results from previous studies indicated that concentrations would be below the limit of detection. Two water samples were collected during the treatment and, as expected, no detectable concentrations of emamectin benzoate or its main metabolite (desmethyl emamectin benzoate) was detected. Sediment samples and flocculent material were collected from the seabed pre-treatment and 1 week, 1 month, 4 months, and 12 months post-treatment. Sampling stations were located 10 m and 25 m from the cage edge in multiple directions, and 50 m and 100 m in the upstream and downstream directions of the main current, and two control sites approximately 1 km away from the fish farm. All concentrations in the flocculent material were well below the PNEC (Predicted No Effect Concentration). Of the 59 sediment samples, only 3, located at 10 m, had concentrations above the PNEC. Furthermore, all measured concentrations were below those predicted by the model. During treatment, silt traps were deployed for seven days 2 m above the bottom at 5 m, 25 m, and 50 m from the cage edge in the direction of the residual current. Concentrations from the collected material were higher than in the sediment material and the report concluded that the low levels in the sediment and flocculent material indicate rapid dispersion of emamectin benzoate. The report recommends, providing the specified treatment regime is followed and results of DEPOMOD indicate a suitable site, no unacceptable damage to the environment is expected and approval for use should be granted.

An ambitious field study was conducted by the Scottish Association for Marine Science (SAMS 2005) to examine the long-term and wide-scale ecological consequences of the use of anti-parasitic sea lice drugs used by the Scottish salmon farming industry. The study took place from September 1999 to August 2004 in waters on the west coast of Scotland. At the time of the study, bath treatments using cypermethrin, hydrogen peroxide, and azamethiphos were permitted. In 2001, in-feed treatments using emamectin benzoate became available. Four sites were selected for the sampling program based on the availability of the major medicines and hydrodynamic conditions to ensure that a range of ambient conditions was covered. Furthermore, logistical constraints were taken into account to ensure that the locations had depths appropriate for diver studies and could be accessed easily by boat.

Although the main focus of the SAMS (2005) study was to determine if impacts of sea lice treatments could be detected in the ecosystem, water column samples were collected during the bath treatment with cypermethrin at one site and sediment samples were collected at two sites which used emamectin benzoate. Duplicate water column samples were collected in the discharge plume from a single cage treatment. Samples were collected from the water surface within the cage prior to and immediately after the release of the treatment water. Five minutes post-release, samples were collected from a depth of 6 m at the down-current end of the cage group. Drifters were used to track the position of the plume for three hours post release and duplicate samples were collected approximately every 10 minutes from the plume. It was found that the observed concentrations of cypermethrin in the cage prior to discharge were 35 to 40 percent lower than the estimated treatment concentration. All other measured concentrations were several orders of magnitude lower than the initial treatment concentration and cypermethrin could not be detected in the majority of samples collected 43 minutes post-discharge.

At the time of the SAMS (2005) study, emamectin benzoate was a new treatment method and was used at three of the sampling sites. Sediment samples were collected at two of the sites to

determine the concentrations of emamectin benzoate. Samples were collected by either van Veen grab or a diver using a handheld corer at several locations along two transects at each site. The transect locations and the spacing of the samples were determined using results from DEPOMOD which was run using current meter data collected from the sites. Samples were also collected from a reference station for each location which was positioned beyond the exposure footprint predicted by DEPOMOD. Pre-treatment samples were collected at both sites. At one site, two treatments were administered four months apart and samples were collected on five separate dates over a period of 18 months. Nine days after the first treatment, only one sample taken from below the cage had a detectable amount of emamectin benzoate. Three months later, only one sediment sample contained a detectable concentration of emamectin benzoate. Five months after the second treatment, most samples contained undetectable concentrations of emamectin benzoate. Model results were compared with observations for the samples that were taken after both treatments. It was found that predicted concentrations were generally two orders of magnitude higher than the measured values. At the second site, two treatments were administered three months apart. Sediment samples were collected on three separate occasions: one before treatment, one 22 days after the first treatment (and before the second treatment), and one 186 days after the first treatment (approximately three months after the second treatment). Since concentrations of emamectin benzoate were detected in the pre-treatment samples and at the reference site, it was concluded that the samples collected were exposed to an unidentified contamination during the process.

Telfer et al. (2006) conducted a field study to determine the environmental effects of a sea lice treatment with emamectin benzoate at a commercial salmon fish farm in the northwest of Scotland. Using collected hydrographic data, a deposition model was used to estimate the exposure footprint of the treatment chemical. Twelve sediment sampling locations were selected based on model results. Along the major axis of the predicted depositional footprint, eight stations were sampled, four on either end of the cage array positioned at 10, 25, 50 and 100 m from the cage edge. In the direction perpendicular to the minor axis, four stations were samples, two on each side of the cage array positioned at 10 and 25 m. Reference stations were positioned 1 km away along the major axis on each end of the cage array. Samples were collected pre-treatment, during treatment, 1-week post-treatment, 1-month post-treatment, 4-months post-treatment, and one year after treatment. Sampling was conducted using van Veen grabs for seabed sediments and a van Dorn water sampler lowered to the seabed to collect flocculent material. In addition, cone shaped sediment traps were positioned 2 m above the seabed and located along a line in the direction of the residual surface current at 5, 25, and 50 m from the cage edge. Water samples were also collected 25 m downstream of the cages at 1 m depth once during the treatment period. No detectable levels of emamectin benzoate or its metabolite were found in the water samples. Sediment samples contained low concentrations of emamectin benzoate with the maximum occurring four months after post-treatment at 10 m from the cage edge. Sediments containing emamectin benzoate and its metabolite were contained within 25 m from the cage edge except at one station 100 m away where intermittent levels were detected. One-year post-treatment, emamectin benzoate was still detected within 10 m from the cages. Contents of the sediment traps varied with location with the trap closest to the cage containing mainly uneaten food and the others containing mainly fecal material.

As part of their surveillance monitoring program to regulate the use of chemicals for the treatment of sea lice, SEPA collected samples from five fish farm sites in 2009 (SEPA 2011). Collected samples were analysed for four in-feed anti-parasitic drugs, two of which are authorized (teflubenzuron and emamectin benzoate) and two that are not (diflubenzuron and ivermectin). The purpose of the sampling was to compare measured concentrations of licenced drugs with the EQS in order to assess if there are possible environmental impacts from the use of these drugs. At each farm site, three sediment samples were collected from the cage edge.

At four of the farms, three additional samples were collected from a reference site located more than 500 m from the cage edge. Chemical concentrations varied significantly between samples from the same site. At two farm sites, emamectin benzoate was detected at concentrations that exceeded the relevant EQS indicating a possible environmental impact. At all five farms, teflubenzuron was either not detected in the collected samples or detected at concentrations below the EQS threshold. No ivermectin was detected at any sampling site.

Using new analysis methods with a detection limit for emamectin benzoate of 0.0034 µg kg^{-1} dry weight of sediment, SEPA (2018) and Bloodworth et al. (2019) analysed sediment samples collected at eight fish farms in the Shetland Isles, Scotland. Farm selection took into account sediment types, current flows, water body sizes, history of emamectin benzoate use, and fish farm operators to obtain a representation of a variety of fish farm conditions. Results from the depositional model autoDEPOMOD (Cromey et al. 2002) were used to determine the sample locations. At each site, three samples were collected along each of three transects. The directions of the transects were based on model results as were the positions: one at the cage edge, one at the edge of the modelled zone of impact, and one beyond the modelled impact. In addition, at least two reference stations were sampled at each site. The reference stations were at locations where no impact was predicted and at least 500 m from the fish farm. Results indicate that emamectin benzoate is more widely dispersed in the environment than indicated by previous studies with a general trend of concentrations decreasing with distance from the farm. The authors indicate that when a small number of data points is used, it is difficult to assess the environmental impact. It is also concluded that multi-directional sampling is useful when trying to understand the dispersion of emamectin benzoate in the environment.

APPENDIX B. PRELIMINARY RESULTS FROM RECENT UNPUBLISHED DFO SEDIMENT SAMPLING FOR PESTICIDES AND DRUGS RELEASED FROM SOME SELECTED CANADIAN SALMON AQUACULTURE NET-PEN OPERATIONS

From Sept. 2016 to Mar. 2017, DFO conducted an extensive benthic grab sampling program in the vicinity of three Canadian finfish net-pen farms. Approximately 100 sample stations were distributed in a nested grid pattern centered around each farm (Figure A1). The spacing between stations differed between the sample grids. The sample stations closest to the farm had a spacing between stations of about 50 m. The grid that contained stations furthest away from the farm had a spacing between stations of about 250 m. The intermediate grid had stations spaced about 100 m.

A sample of the top 2 cm of sediment was obtained from each grab sample and analyzed for several chemicals (antibiotics, pesticides, and drugs). The spatial distribution of emamectin benzoate concentrations in ng g^{-1} (wet weight) that were found at the stations that were successfully sampled as shown in Figures B2 to B4.

In all cases the highest concentrations of emamectin benzoate at each farm were found in close proximity to the farm, i.e., in the near field and in all a cases much lower but still detectable concentrations were found throughout the far-field to the edge of the sampling grid which was a distance of about 1.5 km from the farms. At one of the farms the far-field concentrations were about the limit of detection (LOD) but below the limit of quantification (LOQ).

Qualitatively similar patterns were found for several other in-feed chemicals (lufenuron, oxytetracycline) and the desmethyl metabolite of emamectin.

Details of the sampling program and results are intended for publication elsewhere.

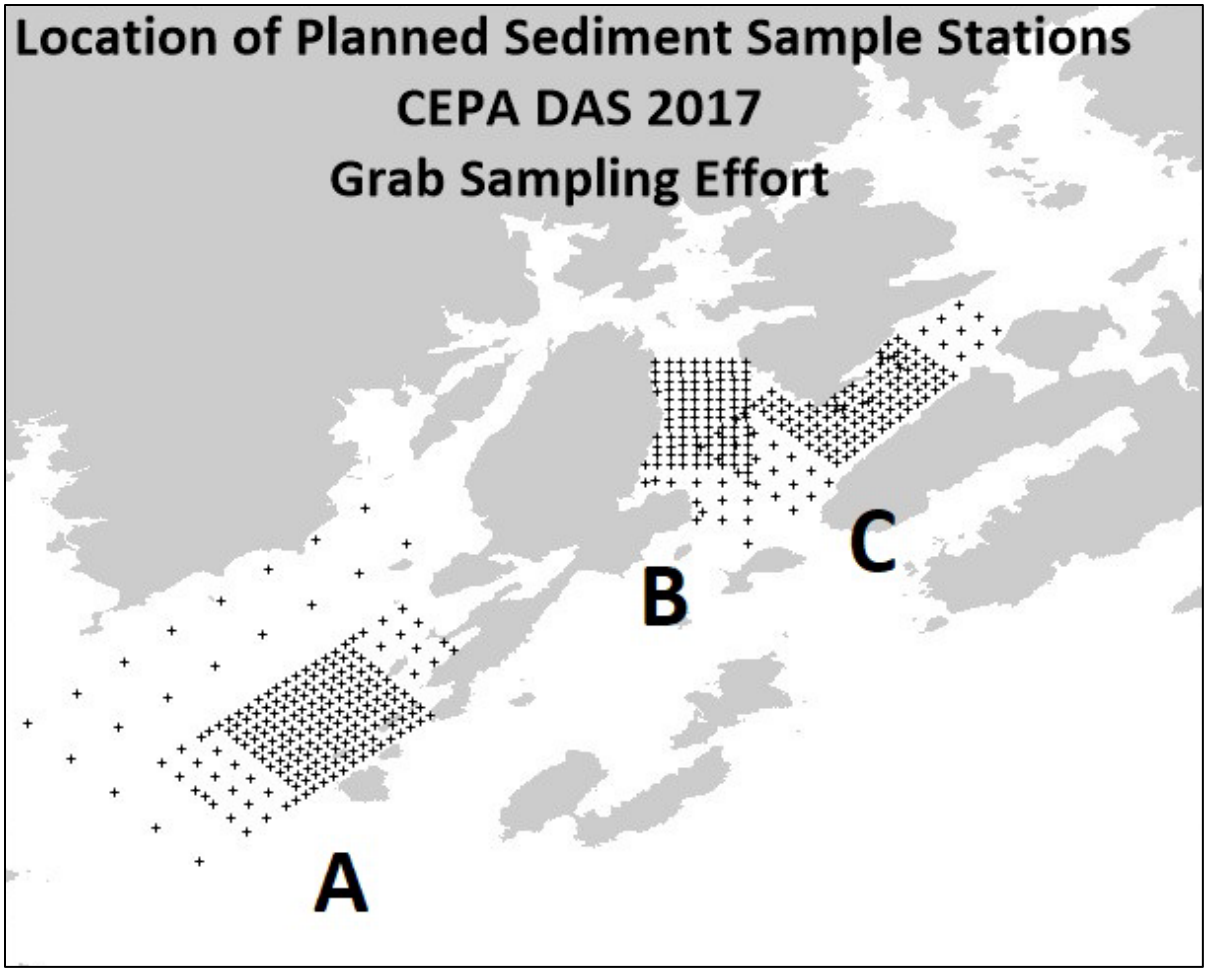

Figure B1. Location of benthic grab sample locations for sampling effort conducted from Sept. 2016 to Mar. 2017 by DFO.

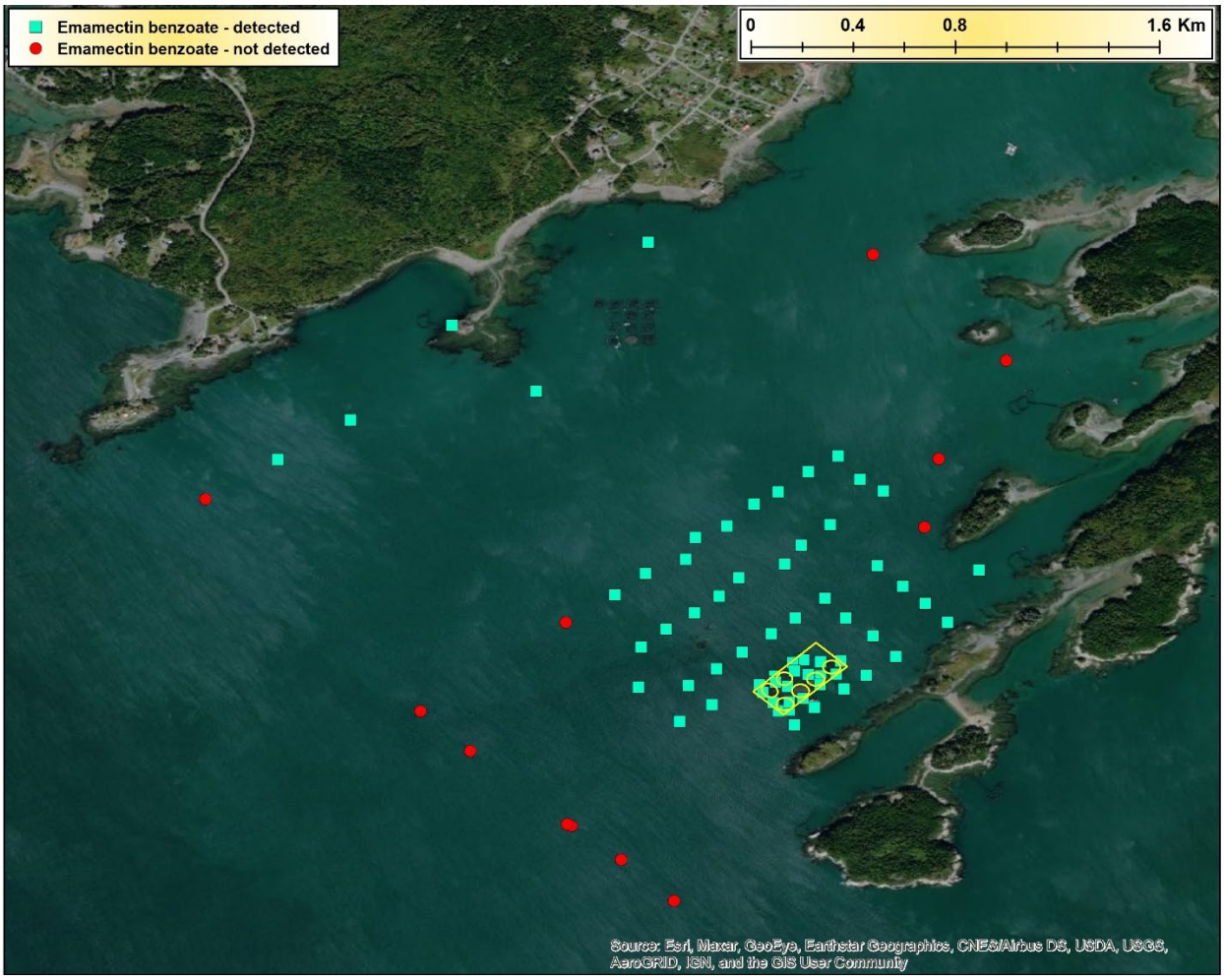

Figure B2. The distribution emamectin benzoate found in the surficial sediment samples collected from Sept. 2016 to Mar. 2017 in the vicinity of a net-pen salmon farm A (Figure B1).

Figure B3. The distribution emamectin benzoate found in the surficial sediment samples collected from Sept. 2016 to Mar. 2017 in the vicinity of a net-pen salmon farm B (Figure B1).

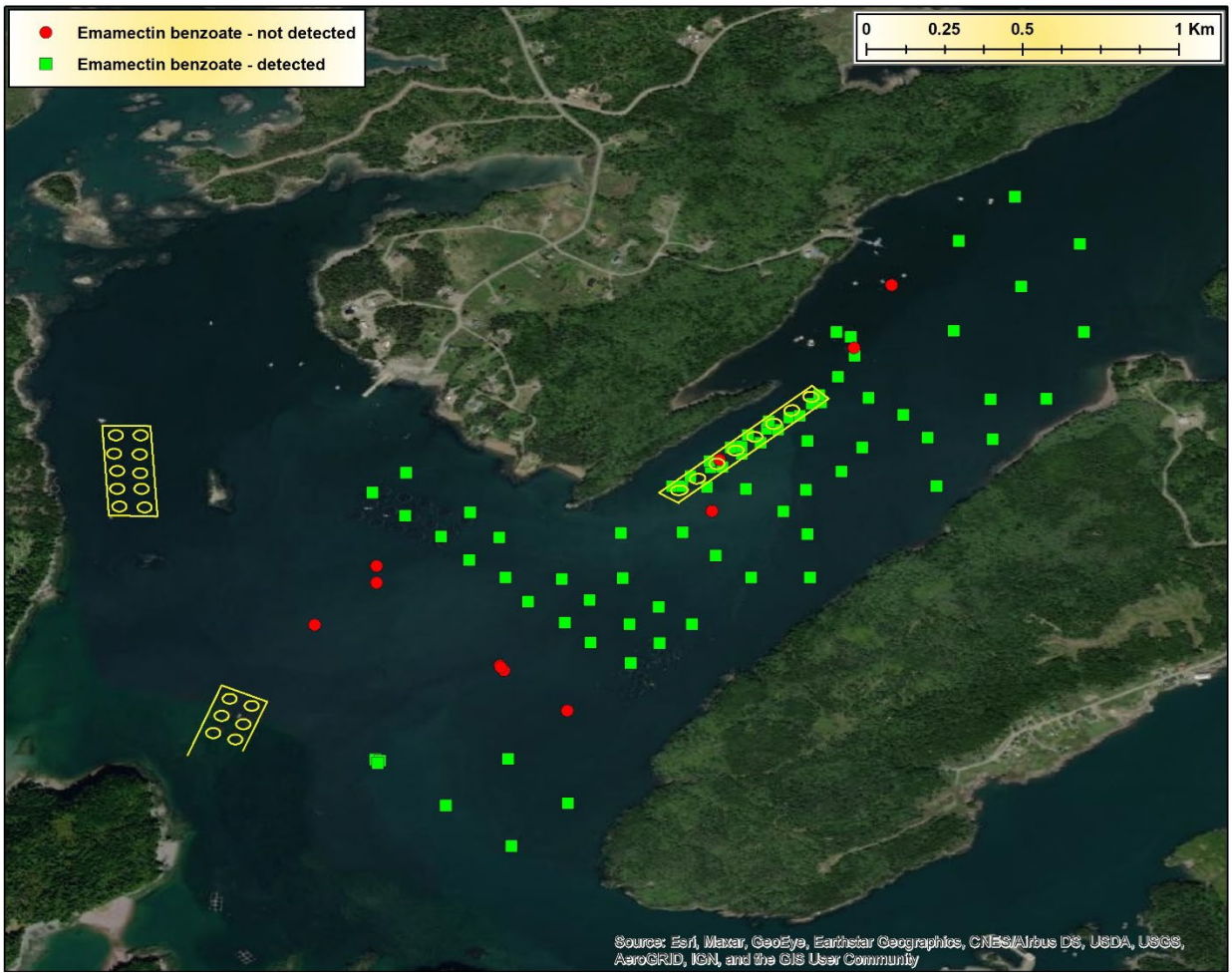

Figure B4. The distribution emamectin benzoate found in the surficial sediment samples collected from Sept. 2016 to Mar. 2017 in the vicinity of a net-pen salmon farm C (Figure B1).