UNITED
NATIONS

EP

UNEP/MED WG.505/4

UNITED NATIONS
ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN

23 March 2021
Original: English

Meeting on Evaluation of Implementation of National Action Plans and Assessments, and Tools to estimate pollutant load from diffuse sources

Videoconference, 22-23 April 2021

Agenda item 3 : Methodologies on estimation techniques for releases from non-point sources (catchment runoffs and agriculture) and aquaculture

Guideline on estimation techniques and applied methodologies for point source releases from aquaculture

UNEP/MAP
Athens, 2021

Note by the Secretariat

The LBS Protocol requires in its Article 13 (para 2) the Contracting Parties to submit reports which shall include inter alia: (i) data resulting from pollutants' monitoring and (ii) quantities of pollutants discharged from their territories. For this purpose, the National Baseline Budget of pollutants (NBB) was agreed by the Contracting Parties as "the monitoring tool" to track progress, on a five-yearly basis, of loads of released pollutants. To assist the Countries in this mandate, updated NBB guidelines were developed in 2015 (UNEP(DEPI)/MED WG.404/7). However, these guidelines, do not offer the means by which pollutants from aquaculture can be estimated. This issue was the topic of discussion at the Regional Meeting on Reporting of Releases to Marine and Coastal Environment from Land Based Sources and Activities and Related Indicators, which was held in Tirana, Albania on 19-20 March 2019. The meeting recommended from the Secretariat to work on supporting countries to develop such guidelines, also considering the increasing importance of the growing aquaculture sector in the Mediterranean.

Further to the above, the UNEP/MAP Programme of Work for the biennium 2020-2021, endorsed by COP21 (Napoli, Italy, 2-5 December 2019), mandated the MED POL Programme to develop new National Baseline Budget (NBB) Technical Guidelines to address estimation techniques of pollutants' releases from non-point sources from aquaculture; thus, facilitating the reporting capacities of the Contracting Parties to Barcelona Convention under LBS Protocol specifically for the sector of activities in Annex I, and more particularly under NBB/PRTR.

To this aim, this guidance document expands the scope of existing NBB Guidelines by providing specific information on estimation of releases of Total Nitrogen, Total Phosphorus, Total Organic Carbon (BOD, or COD) and some heavy metals (Cu and Zn and their compounds) originating from aquaculture sector. The estimation techniques for heavy metals are very much depending on the operations of the industry (type of cages, antifouling applications of the cages etc.,) which requires more in-depth analyses and inventories at national level. Whereas, releases of TN, TP, TOC are more straight forward and related to, and requires the knowledge of, the feed content and feeding practices applied at the local level. The document also examines the issue of, unintentional, releases of pesticides, POPs and microliter via fish feeds. Despite there are no agreed estimation techniques for these releases, they are summarized in Annex III as issues of concern for future considerations.

This guideline would serve in supporting the Contracting Parties to include the aquaculture sector in the upcoming 5th NBB Reporting Cycle scheduled for the biennium 2022-2023, as well as to further streamline with PRTR reporting. Moreover, this guideline will facilitate the collection of data for monitoring the implementation of the Regional Plan for Aquaculture, to be developed in the biennium 2022-2023.

In this guidance document additional information are provided on accuracy and uncertainty of the estimation methods, and aspects related to quality control/quality assurance relevant to inventories of pollutants releases/discharges from aquaculture production and fish farming activities. The guideline streamlines these estimation techniques with NBB/PRTR methodologies at process level, where possible, aiming to facilitate the estimations of loads at national level to report certain chemicals to the environment required by NBB and PRTRs.

This Meeting is expected to review this draft guidance document for estimating releases of non-point sources in aquaculture taking into consideration the NBB/PRTR methodologies, and to provide its comments and substantive inputs, with the aim of submission of the agreed draft to the MED POL Focal Points Meeting in May 2021 for their approval.

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List of Abbreviations / Acronyms

CFP	Common Fisheries Policy
Cu	Copper
EC	European Commission
EcAp	Ecosystem Approach
EEA	European Environment Agency
EMEP	European Monitoring and Evaluation Programme
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations Rome
GESAMP	Group of Experts on the Scientific Aspects of Marine Environmental Protection
HABs	Harmful algal blooms
HELCOM	The Baltic Marine Environment Protection Commission
IMO	The International Maritime Organization
IPCC	Intergovernmental Panel on Climate Change
JRC	European Commission's Joint Research Centre
LBS	Land Based Sources
MEDPOL	Convention on Protection of the Mediterranean Sea
MSFD	Marine Strategy Framework Directive
NAP	National Action Plan
NASO	National Aquaculture Sector Overview
NBB	National Baseline Budget
NBB/PRTR	National Baseline Budget/Pollutant Release and Transfer Registers
N	Nitrogen
NAPs	National Action Plans
NPI	National Pollutant Inventory (Australia)
OCPs	Organochlorine pesticides
OECD	Organization for Economic Cooperation and Development
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic
P	Phosphorus
PCB	Polychlorinated biphenyl
POPs	Persistent organic pollutants
PRTR	Pollutant Release and Transfer Registers
QA/QC	Quality Assurance/Quality Control
TOC	Total Organic Carbon
UNITAR	United Nations Institute for Training and Research
USGS	US Geological Survey
WFD	Water Framework Directive
WHO	World Health Organization
Zn	Zinc

1. Introduction

1. Following the 21st Meeting of the Contracting Parties to the Barcelona Convention COP21 (held in Napoli, Italy, 2-5 December 2019)[1] and the adoption of Decision IG.24/14,[2] the Programme of Work mandated the MEDPOL Programme to develop/update technical guidelines addressing estimation techniques of pollutant releases agriculture, catchments runoff and aquaculture.

2. To achieve this mandate, this guidance document on estimation techniques and applied methodologies for point source releases from aquaculture was developed. It elaborates on estimating point source releases to water from activities classified under the **aquacultural sector** including, but not limited to, releases of pollutants listed in Annex I to the LBS Protocol.

3. In particular, focus is made in this document on:

   a. Releases of total nitrogen, total phosphorus, copper and its compounds, zinc and its compounds and Total Organic Carbon (TOC), in the aquaculture sector;

   b. Release estimation methods and techniques to assess the aforementioned pollutants loads from the aquaculture sector.

4. Moreover, this guidance document provides information on the current status of aquaculture (including both inland and mariculture), in particular with respect to fish feed practices and industry (Annex I), as well as an overview of approaches for estimation techniques of pollutants' releases from the aquaculture sector, including accuracy and uncertainty of the estimation methods, and aspects related to quality control/quality assurance relevant to inventories of pollutants releases/discharges from aquaculture production and fish farming activities (Annex II). Finally, additional issues of concern were also evaluated in Annex III, where some POPs and Pesticides could enter, unintentionally, via fish feed to the marine environment.

5. The methodology used for developing this document comprised of several steps:

   a. An extensive literature review (Annex I-III) focusing on:

      i. Current status of aquaculture sector (including both inland and mariculture), in particular with respect to fish feed practices and their adverse impacts on the environment.

      ii. Pollutants releases and discharges to water as well as issues related to pollution loading of total nitrogen, total phosphorus, copper and its compounds, zinc and its compounds and Total Organic Carbon (TOC) from aquaculture production facilities and related estimation techniques as well as potential unintentional releases of pesticides and POPs;

      iii. Technical reports, documents and peer reviewed research papers describing different approaches, methods and techniques recommended for estimations of the above pollutants' releases to water from aquaculture sector.

      iv. Potential issues and drawbacks regarding accuracy and uncertainty associated with the proposed calculation methods, techniques and approaches.

   Although the review was conducted on a global scale, the main focus was on the Mediterranean region. Relevant studies included available information from Europe, as well as from the USA, Canada, Australia and Asia.

   b. Elaborating streamlined methodologies and most appropriate techniques to estimate releases of nutrients; copper and its compounds; zinc and its compounds; TOC releases from aquaculture activities.

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[1] https://www.unenvironment.org/unepmap/events/meeting/21st-meeting-contracting-parties-convention-protection-marine-environment-and

[2] https://wedocs.unep.org/bitstream/handle/20.500.11822/31712/19ig24_22_2414_eng.pdf

c. Integrating available information and developing the guidance document for the methods and techniques to assist Contracting Parties to estimate releases of the pollutants and their discharges to water from the sector.

6. It is expected that the newly proposed techniques for estimation of pollution loads to water will enable the generation of compatible data to evaluate the effectiveness of adopted measures in the National Action Plans and the Regional Plan for Aquaculture Management in the Mediterranean.

2. Legal basis of the NBB guidance document for the Aquaculture sector

7. The Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources and Activities (the LBS Protocol) is one of the six Barcelona Convention Protocols. It was adopted on 17th May 1980 by the Conference of Plenipotentiaries of the Coastal States of the Mediterranean Region and entered into force on 17th June 1983.[3] This original Protocol was modified by amendments adopted on 7th March 1996 (UNEP(OCA)/MED IG.7/4)[4] and recorded as the "Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources and Activities". It entered into force on 18th May 2006.[5]

8. The LBS Protocol requires the Contracting Parties to submit reports which shall include inter alia: (i) data resulting from monitoring and (ii) quantities of pollutants discharged from their territories (Article 13, para 2).[6] For this purpose, the National Baseline Budget of pollutants (NBB) was agreed by the Contracting Parties as "the monitoring tool" to track progress, on a five-yearly basis, of discharged loads of pollutants reflecting the effectiveness of measures taken to reduce and prevent pollution from LBS.

9. To assist the Countries in this mandate, updated NBB guidelines were developed in 2015 (UNEP(DEPI)/MED WG.404/7 Annex IV, Appendix B, Page 11).[7] However, these guidelines do not offer means by which pollutants from aquaculture can be estimated. Furthermore, current Inventories of Pollutant Release and Transfer Registers (PRTR) estimation techniques do not provide any information on releases from aquaculture. This point was discussed at the Regional Meeting on Reporting of Releases to Marine and Coastal Environment from Land Based Sources and Activities and Related Indicators, which was held in Tirana, Albania on 19-20 March 2019.[8] Therefore, the recommendation was made to support the Contracting Parties to complement the National Baseline Budget/Pollution Release and Transfer Registers (NBB/PRTRs) methodology with estimation techniques for point sources related to the aquaculture sector (UNEP/MED WG.462/8).

3. Pollutants Releases and Discharges from Aquaculture

10. The principal pathways of contaminants which are discharged from aquaculture production activities are feed, chemicals used in the form of medications, disinfectants and antifoulants, and fish faecal material. While being a crucial factor of production in aquaculture, feed has been reported to be the major source of pollution in aquaculture systems [1-7]. The effect of waste production and pollution caused by fish feed varies with the amount of supplemental feed. It is dependent on a number of factors including feed nutrient composition, method of production (extruded vs pelleted), ratio of feed size to fish size, quantity of feed per unit time, feeding method, and storage time [1][7].

3.1 Aquafeed Production

11. Feed types can be divided into three groups: i) industrially compounded feeds (ICF), ii) farm-made feeds (FMF) and iii) raw organisms (RO). Between 1995 and 2007, total industrial compound

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[3] https://www.informea.org/en/treaties/land-based-sources-protocol
[4] https://wedocs.unep.org/handle/20.500.11822/3016
[5] https://wedocs.unep.org/bitstream/handle/20.500.11822/7096/Consolidated_LBS96_ENG.pdf?sequence=5&isAllowed=y
[6] https://wedocs.unep.org/bitstream/handle/20.500.11822/3016/96ig7_4_lbsprotocol_eng.pdf?sequence=1&isAllowed=y
[7] https://wedocs.unep.org/bitstream/handle/20.500.11822/5481/1/15wg417_inf6_eng.pdf
[8] file:///C:/Users/aleks/AppData/Local/Temp/19wg462_08_Meeting%20Report.pdf

aquafeed production increased 3.5-fold, from 7.6 million tons (1995) to 27.1 million tons (2007), with production growing at an average annual rate of 11.1 percent [4]. In 2015, the total use of ICF in the production of major species was estimated at 39.62 million tons [8], the use of farm-made aqua feeds between 15 and 30 million tons, and direct use of raw organisms, mostly trash fish, was estimated to be between 3 and 6 million tons [1][8].

12. Fish species and shrimp diets need to contain approximately forty essential nutrients such as amino acids, vitamins, minerals, and fatty acids [1] [9-11-36]. These are provided in the feed through a number of ingredients including fishmeal, fish oil, plants, and animal trimmings. The feed is usually in the form of dried pellets.[9] The exact diet differs per fish type and species. Schalekamp et al. [1] and Tacon et al. [3][12] provide a detailed overview of feed ingredients and composition for different fish types. For fed aquaculture species, the ingredients can be roughly divided into two categories: marine resources and terrestrial resources. Marine resources mainly consist of fishmeal and fish oil, whose production is depended on wild fisheries, and therefore limited [1] [9-11]. Tacon et al [5] reported that total usage of terrestrial animal by-product meals and oils within compound aquafeeds ranged between 0.15 and 0.30 million tons, e.g., less than 1 percent of total global compound aquafeed production. The key terrestrial resources for feed include soybeans, maize and rice [8-10]. Soybean meal is the most common source of plant proteins used representing about 25% total compound aquafeeds by weight [5]. Alternative lipid sources to fish oil are also being used in greater amounts with key substitutes including vegetable oils, preferably those with high omega-3 contents (e.g., farmed fish offal), and poultry oil [5].

13. To ensure the dietary nutrients are ingested, digested, absorbed, and transported to the cells, an increasing diversity of non-nutritive feed additives are being used in aquatic feeds [1] [10-12]. The range of feed additives used in aquatic feeds is diverse [13]. For example, some target the feed quality, including pellet binders, antioxidants, and feed preservatives (anti mold and antimicrobial compounds). Enzymes are used to improve the availability of certain nutrients (proteases, amylases) or to eliminate the presence of certain antinutrients (phytase, non-starch polysaccharides (NSP) enzymes). Other additives are used to improve the animals' performance and health including probiotics, prebiotics, immune-stimulants, phytogenic substances, and organic acids [13].

3.2 Adverse effects on the environment from potential releases of nutrients, copper and zinc and their compounds and organic carbon from aquaculture production facilities

14. Aquaculture production uses many resources including land, water, feed, fertilizer, energy, capital and labour, and affects ecosystems through the release or extraction of nutrients, chemical and microbial pollutants, the introduction of foreign species, the use of disinfectants and antibiotics, and the alteration of water flows [10] [14-20]. These adverse effects on the environment depend upon different factors such as type of aquaculture method used, geographical location, and produced species, including feeds offered, chemicals, excretions, dead animals, and the interactions between cultured and wild animals [18]. The accumulation of waste food and fish faecal material results in discharges of nutrients, chemical and microbial pollutants, immune-stimulants, and changes in the sediment under fish cages. Although significant environmental impacts have been reported in the literature at distances of up to 100 m from the cages, generally such impacts are localized within 20 to 50 m around the cages [14].

15. Aquaculture facilities may affect water quality by altering turbidity, pH (particularly in fresh water), an increase of nutrients concentration and primary production resulting in eutrophication and harmful algal blooms, decrease of dissolved oxygen (DO) concentrations [17-21] and toxicity [18] [24-30].

16. The use of pesticides in fish feed, in particular farmed salmon has caused an increased concern regarding their potential effects on human health in recent years [24-25] [28-32].

[9] https://www.fisheries.noaa.gov/insight/feeds-aquaculture

17. It has been reported that the escape of cultured organisms (or their reproductive cells) can influence wild populations by cross or hybridisation, depredation, competition, habitat destruction, or disease spread [18]. Shrimp farming has caused considerable destruction and loss of mangrove forests in East and South East Asia, Mexico and Brazil [33-34].

Nutrients (total N and total P)

18. The main pathways of nutrients release from aquaculture production facilities are via non consumed feed (especially due to overfeeding), decomposition of died organisms, overfertilization and faecal material [34-41]. In inland feed-based aquaculture ponds, 60% to 80% of the nitrogen (N) in the protein of feeds enters the water as uneaten feed and feces or is excreted as ammonia nitrogen (NH3-N) by aquatic animals [37-39 To prevent ammonium toxicity, rainbow trout farms need large quantities of water, typically 86,000 m^3/ton of trout produced and are therefore responsible for considerable ammonium discharges into rivers [39-41]. Although phosphorus (P) concentration in trout farm effluents is low (total P of 0.30 mg P/L), due to the quantities of water used, its overall mass loading is very high, and can trigger and cause eutrophication [42-43].

19. Coastal and marine aquaculture are also significant contributor to nutrient enrichment. For example, it had been reported that for a world annual shrimp production around 5 million tons, 5.5 million tons of organic matter, 360,000 tons of nitrogen, and 125,000 tons of phosphorous annually are discharged to the environment [37]. The increasing production of nitrogenous metabolites especially ammonia, is of a great concern because it is highly toxic in its unionized form (NH_3) for many aquatic organisms [37-38][44]. Bowman et al. [45] recently reported that release of dissolved and particulate nutrients by intensive mariculture results in increasing nutrient loads (finfish and crustaceans), and changes in nutrient stoichiometry (all mariculture types). The authors pointed out that mariculture represents a significant and expanding cause of coastal nutrient enrichment and projected that nutrients from mariculture will increase up to six-fold by 2050 with exceedance of the nutrient assimilative capacity in parts of the world exhibiting rapid mariculture growth [45]. They also highlighted the fact that increasing nutrient loads may promote an increase in harmful algal blooms (HABs) either directly or via stimulation of algae on which mixotrophic HABs may feed. HABs can kill or intoxicate the mariculture product with severe economic losses and can increase risks to human health [45-46][43].

Copper and its compounds

20. Copper and its compounds can enter the marine environment in several ways including: uneaten food and food additives [47-50], leaching from biocidal coating application on the submerged structures and net-cages commonly used in aquaculture production facilities [51-53] and farmed fish faecal waste [47][51]. The toxicity of a metal in the marine environment is mostly determined by its chemical form and whether it is bioavailable (i.e. in a form that an organism can directly absorb or ingest). The more toxic, and thus bioavailable, state is the free ionic or dissolved form. Clement et al. [52] provide a thorough review of ecological relevance of copper (Cu) and zinc (Zn) in sediments.

21. Applying a biocidal coating on the submerged structures and net-cages to prevent and reduce biofouling is commonly used practice in aquaculture. Anti-fouling paints are mostly based on Cu, usually in the form of copper oxide and consequently the sediment close to the fish farms have been found to exhibit high copper levels, often exceeding the recommended sediment quality guidelines [47-48][51][53]. For example, Dean et al. [48] reported that 19 of the 25 anti-foulant products licensed for use in Scottish aquaculture have copper as the active ingredient (e.g. cuprous oxide (Cu2O), copper thiocyanate (CuSCN) and copper sulphate (CuSO4)), with some also containing zinc. The authors also highlighted that anti-foulants may provide a significant source of Cu, and possibly Zn, to the marine environment, since the active metal can be released in soluble or particulate form, either washed from treated nets or chipped from painted hard structures [48]. The extensive use of anti-fouling biocides is also considered a potential source of metal accumulation in cultured fish, which have been associated to lethal or sub-lethal effects and the immediate immune defense mechanism of the exposed fish [54].

Zinc and its compounds

22. Pathways for Zn and its compounds to the marine environment are the same as for Cu, e.g. uneaten food and food additives, leaching from biocidal coating application on the submerged structures and net-cages commonly used in aquaculture production facilities, and farmed fish faecal waste [47-54].

23. Zinc pyrithione (ZnPT) and Zineb are most commonly used Zn biocides in antifouling paints [55-56]. Soon et al. [56] recently provided a comprehensive review of the ZnPT use in marine environments, their toxicity and environmental fate. They highlighted that once ZnPT is released into the marine environment, it can easily be transchelated into other metal pyrithiones by releasing the zinc ion in the complex and absorbing other free metal ions in seawater.

24. Guardiola et al [55] highlighted that that despite the beneficial effects of the chemicals to aquaculture, the use of biocides may also cause potential harm to aquatic organisms and even to humans. The authors underlined two types of risks associated with the use of biocides in aquaculture: (i) predators and humans may ingest the fish and shellfish that have accumulated in these contaminants and (ii) the development of antibiotic resistance in bacteria. Ingestion of the contaminated fish and shellfish can pose a great risk to human health [57-58]. The conditions and locations of the aquaculture farms play a significant role on the spread of these chemicals and heavy metals into the environment [58].

Total Organic Carbon (as Total C or COD)

25. The contribution of primary production to carbon loading in fed aquaculture, carbon accumulation and subsequent benthic deterioration under fish farms has been investigated by several researchers [4][17][22][37][57-62]. As a major element of organic matter, dissolved organic carbon (DOC) plays an important role in the carbon cycle and microbial loop in the marine environment and has been extensively studied over the past few decades [61-62]. The principal pathways for transfer of organic matter through seawater include dissolution of fecal pellets, excess feeding, breaking down of the cells and bacterial activity [57-62]. The sedimentation of organic carbon (OC) below fish farms has been found to be from 4 to 27 times higher than that at unaffected sites; declining rapidly with distance from the farm [63]. Additionally, the integration of lower trophic-level species (shellfish and seaweed) with monoculture of fish/shrimp in coastal water has potential assimilate organic matter from surrounding water and to release it through excretion that becomes a part of the organic pool [62]. Verdegem [17] estimated the contribution of primary production to carbon loading in fed aquaculture and pointed out that in flow-through systems (including cages), the environmental loading with carbon is higher than the amount fed. The author also highlighted that for fed aquaculture operations, a more detailed mass balance including growth and the different types of waste produced can be calculated for different feed components, including dry matter (DM), chemical oxygen demand (COD), carbon (C), N, ash and P.

3.3 Adverse effects on the environment from potential releases of pesticides and persistent organic compounds (POPs) from aquaculture production facilities

26. Being a growing concern, ss mentioned previously, the principal pathways of contaminants which are discharged from aquaculture production activities are feed, chemicals used in the form of medications, disinfectants, and antifoulants, and fish faecal material. This guideline does not intent to look at the health related issues, however, some additional issues of concerns are summarized in the Annex III.

4. Release Estimation Methods and Techniques for Pollutants from the Aquaculture Sector

27. Techniques used to estimate releases from the aquaculture sector are divided into (i) releases of nutrients, (ii) copper and zinc and their compounds and (iii) organic carbon. These are discussed below:

4.1 Summary of techniques used to estimate releases of nutrients, copper and zinc and their compounds and organic carbon from the aquaculture sector

28. To date, documents describing the Emission Estimation Techniques to determine pollutant loading from aquaculture facilities have been scarce and mostly focused on nutrient discharges. Below we summarize techniques and equations proposed by technical guidelines for use in Australia [64], Europe [65] and the USA [66]. This guidance document also describes techniques proposed in several peer-reviewed research papers.

29. As described earlier, feed inputs and feed practices (i.e. stocking density, the feeding regime, and the feeding rate) have been recognized as the major source of pollutant releases and drivers of effluent quality discharged from cage aquaculture production facilities [2-7]. Other pollution sources include chemicals used in the form of medications, disinfectants, and antifoulants, and fish faecal material. The actual amount of supplied feed that is consumed by the fish and its digestibility are the two most important factors that determine the amount of faecal wastes produced and released to the surrounding environment [66-67][].

30. Therefore, determining the feed content and the quantity of ingredients (e.g. N, P, organic matter, protein) in it from the feed suppliers is a key and should be a starting point in any estimation of potential for pollutant releases from the aquaculture production facility [19-22][65-66].

31. A particularly important parameter is the feed conversion ratio (FCR), which is defined as a measure of the feeding efficiency [66]. It is calculated as the ratio of the weight of feed applied to the weight of the fish produced:

$$FCR = \text{Dry weight of feed applied/Wet weight of fish gained} \qquad \text{(Equation 4.1)}$$

32. The US EPA [66] pointed out that with higher energy feeds, FCRs of 1.0 or less are now routinely observed in salmon and trout farming. Anytime FCRs are significantly greater, then less of the feed input goes to growth and more is used to support metabolic processes and there is increased waste generation, intrinsically as well as extrinsically (wasted feed).

4.1.1 Nutrients (total N and total P)

33. Australian EPA proposes two different techniques to estimate nutrient releases from temperate water finfish aquaculture facilities in Australia [64]:

a. *Direct Measurement method*, which can be used on semi-closed and closed systems and involves direct measurement of total N and P in the discharge water. The Guidelines [64] highlighted that for this method, water quality data would need to be measured over a reasonable time to account for the variations before accurate, reliable figures could be determined for input into the direct measure equation (4.2).

$$T_{N+P} = E_{N/P} * F_A \qquad \text{(Equation 4.2)}$$

where:
T_{N+P} = discharge of total N and P to water (t/year)
$E_{N/P}$ = concentration of N and P in effluent (mg/L)
F_A = conversion factor (which was not provided in the document)

b. *Mass Balance method*, which was recommended for use by both marine and freshwater land-based fish farming using semi-open systems:

$$T_{N+P} = (F_{N+P} * FCR) - (A_{N+P}) \qquad \text{(Equation 4.3)}$$

where:
T_{N+P} = discharge of total N and P to water (kg/t fish produced)
F_{N+P} = total N and P in feed[10] (kg/t)
FCR = feed conversion rate (dimensionless)
A_{N+P} = N and P converted to fish biomass (kg/t)

34. The OSPAR Guidelines [65] proposed the following equation based on Nutrients in feed (N_{feed}) which are converted to fish biomass (N_{fish}) or released into the water as unconverted nutrients (N_{rel}):

$$N_{feed} = N_{fish} + N_{rel} \qquad \text{(Equation 4.4)}$$

35. For estimations of N_{feed} and N_{fish}, the Guidelines [65] referred to data provided by Germany's central environmental authority UBA[11] which stated that approximately 25% of the nutrients in feed are converted into biomass, with the remaining 75% discharged to the environment and Handy and Poxton[12] estimated that 52 – 95% of the nitrogen (N) added to aquaculture systems as feed will ultimately enter the environment.

36. The guidelines [65] also underlined that unconverted nutrients (N_{rel}) may occur as: i) uneaten feed, sedimented feed and inedible constituents; ii) faeces and indigestible feed; and iii) excreta (i.e., branchial and renal release).

[10] The proportion of P and N in the feed is obtained directly from the producers
[11] UBA (1996). Die Einflüsse der Fischerei und Aquakultur auf die marine Umwelt. UBA-Texte 46-96. Umweltbundesamt, Berlin.
[12] Handy, R.D. and Poxton, M.G. (1993). Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Reviews in Fish Biology and Fisheries, 3: 205–241.

37. US EPA [66] used the feed-to-pollutant conversion factors to estimate an untreated or "raw pollutant loading (RPL)" as following:

$$RPL = FI_A * F_tP \text{ conversion factor} \qquad \text{(Equation 4.5)}$$

where:

RPL = the pollutant load for each pollutant in question (i.e., TSS, BOD, TN, TP) in pounds (or tons)/year

FI_A is Annual feed input = the amount of feed distributed to the production system (pounds or tons/year)

F_tP is Feed-to-pollutant conversion factor = conversion of feed inputs into pollutant loadings (i.e., TSS, BOD, TN, TP) in pounds (tons) of pollutant per pound (ton) of feed.

38. Foy and Rosell [68] proposed an equation to determine nutrient loadings in aquaculture farms, based on the FCR value and the nutrient contents in the feed and in the fish, as

$$\text{Nutrient LOSS RATE} = (FCR \times FEED) - FISH \qquad \text{(Equation 4.6)}$$

where:

LOSS RATE = nutrient loss rate in kg/ton of fish produced;

FEED = nutrient content of the diet in kg/ton;

FISH = nutrient content in fish in kg/ton.

39. Olsen et al. [19] proposed a series of simple equations for estimation of nutrient release rate from fish, based on Mass balance in a Food-Fish-Waste system:

$$I = A + F = G + R + F \qquad \text{(Equation 4.7)}$$

where:

I = the food consumed;

A = assimilated food, or uptake in tissues;

F = defecation;

R = respiration, and

G = growth and reproduction (all in terms of carbon or energy).

40. The corresponding nutrient balance is expressed using the analogue equation:

$$I_{N,P} = A_{N,P} + F_{N,P} = G_{N,P} + E_{N,P} + F_{N,P} \qquad \text{(Equation 4.8)}$$

where excretion of N and P ($E_{N,P}$) replaces respiration.

41. The authors highlighted that these two general equations (4.7 and 4.8), together with knowledge on assimilation efficiencies of C, N, and P and the stoichiometric C:N:P composition of produced fish an feed, are fundamental for estimating nutrient and carbon intake, metabolism, and losses from individuals of cultured fish [19].

42. The assimilated food is the portion of the food that is digested by the fish and taken up in tissues, and the authors [19] estimated assimilation efficiency (AE) is defined as (similar for N and P):

$$AE = A/I \qquad \text{(Equation 4.9)}$$

43. The undigested food, termed faeces, passes through the fish gut undigested or partially digested. This fraction constitutes mainly particulate organic substances, including particulate forms of N and P, but some part is rapidly released in molecular dissolved forms in the water. The assimilated food supports growth and weight increment, and the growth efficiency (GE) is generally defined as (similar for N and P):

$$GE = G/I \qquad \text{(Equation 4.10)}$$

where:

GE = expresses the efficiency by which the food ingested is converted to new biomass. This is similar, although inverse, to the FCR which is an operational term established and used for aquaculture.

I = the food consumed (defined in equation 4.7)

G = growth and reproduction (defined in equation 4.7).

44. **The total wastes of carbon (TL_C) and nutrients (TL_{NP})** generated by cultured fish is expressed as:

$$TL_C = I - G = R + F \qquad \text{(Equation 4.11)}$$

$$TL_{NP} = I_{NP} - G_{NP} = E_{NP} + F_{NP} \qquad \text{(Equation 4.12)}$$

where I, G, R, F are defined in equation 4.7. as

I = the food consumed;

G = growth and reproduction;

R = respiration

F = defecation;

E_{NP} = excretion of N and P (defined in equation 4.8)

45. Respiration results in a release of inorganic CO_2, the emission of **organic carbon wastes (L_{OC})** is most easily estimated as:

$$L_{OC} = I - A = I\,(1 - AE) \qquad \text{(Equation 4.13)}$$

where:

A = assimilated food, or uptake in tissues (defined in equation 4.8).

AE = assimilation efficiency of carbon or energy which according to the authors [44] can be obtained from literature and in some cases from feed companies. The authors also pointed out that for the dissolved components from faeces, there is no formal way to distinguish these dissolved organic components (DOC) from the particulate organic waste components (POC), but that it is the particulate fraction is the most important, and that the corresponding estimate of **organic nutrient wastes (L_{ONP})** from fish can be estimated as:

$$L_{ONP} = I_{NP} - A_{NP} = I_{NP}\,(1-AE_{NP}) \qquad \text{(Equation 4.14)}$$

where:

I_{NP} = N and P consumed can be estimated based on total feed intake multiplied by feed N and P contents

The assimilation efficiency of N can be assumed to be equal to that of protein, and values are widely reported in literature and by feed companies; The assimilation efficiency of P is widely reported as well, but more uncertain because of the addition of indigestible P compounds from higher plants in the feed (phytate P). Regarding carbon input, there is no formal way to distinguish between dissolved organic nutrients (DON, DOP) and particulate organic nutrients waste components (PON, POP) originating from faeces, but the particulate nutrient fraction is more important.

46. **The inorganic N and P release** from the fish (L_{INP}) can be estimated as the difference between assimilation and production:

$$L_{INP} = A_{NP} - G_{NP} = (I_{NP} * AE_{NP}) - G_{NP} \quad \text{(Equation 4.15)}$$

where:

G_{NP} = N and P in produced fish, obtained as produced fish weight times N and P contents
A_{NP} = N and P in assimilated food, or in tissues
I_{NP} = N and P consumed
AE_{NP} = assimilation efficiency for N and P.

4.1.2 Techniques used to estimate Cu and Zn releases

47. The information on emissions and releases estimation techniques to determine metals loading from aquaculture facilities is very sparse.

48. Dean et al. [48] investigated the high-resolution spatial distribution of the potentially ecotoxic metals zinc, copper and cadmium in sediments around a cage farm and attempted to derive a budget for these elements within the farming system. For each sediment core taken at depth y, concentration of each metal (Cu, Zn or other) was determined and converted to mass of metal per unit area (g m^{-2}) as following:

$$\text{Inventory}\ (\text{gm}^{-2}) = \frac{\sum_{i=1}^{n=y} \left([\text{metal}]_i\ \text{dry wt.}\right)}{\text{Area}} \quad \text{(Equation 4.16)}$$

where:

$[\text{metal}]_i$ = metal concentration in the i^{th} slice (mg g^{-1});
dry wt. = dry weight of full slice (g);
area = $r^2\pi$, r = core diameter (m^{-2})

49. To estimate the metals budget, the total mass of metals within the feed and within the fish 'on site' was calculated, using the information on the feed and biomass input and feed conversion rate) FCR.

50. More recently, Earley et al. [69] evaluated environmental loading and metal leaching rates for four copper alloy materials and one traditional coated-nylon net material in a 365-day field test in San Diego Bay, California, USA.

51. The authors combined surface area of an example aquaculture farming pen (30 X 30 X 12 m) with leach rate data and a generic lifecycle model they developed [69] to estimate environmental life cycle loadings (total amount of copper released during the usable deployment life of the material) from aquaculture farming pens made from the copper alloy mesh (CAM) or Net materials.

52. The cumulative loading (CL) over a given time interval (x_0, x_n was approximated from leach rate measurements (R) using the following equation:

$$CL_{x_0,x_n} \approx \sum_{x_0}^{x_n} (x_1 - x_0)\ \frac{R(x_0)+R(x_1)}{2} + (x_2 - x_1)\frac{R(x_1)+R(x_2)}{2} + (x_n - x_{n-1})\frac{R(x_{n-1})+R(x_n)}{2}$$

(Equation 4.17)

where:

CL x_0, x_n = the cumulative copper loading (µg cm^{-2}) from day x_0 through x_n;
x_n = a series of consecutive time points (days) during which release rate measurements were made beginning with day x_0 and ending with day x_n; and
$R(x_n)$ = the measured release rate (µg cm^{-2} d^{-1}) for time point x_n.

53. The researchers [69-70] also reported that typical copper release rate patterns have an initial spike in concentration, followed by a decline to an asymptotic low or a pseudo-steady state (PSS). They proposed the following equation to calculate PSS:

$$PSS_{x_a, x_n} = \frac{CL_{x_a, x_n}}{(x_n - x_a)} \quad \text{(Equation 4.18)}$$

where:

$PSS_{xa,xn}$ = the pseudo steady state loading rate (µg cm^{-2} d^{-1}), which occurs after day x_a and

$CL_{xa,xn}$ = the cumulative copper loading (µg cm-2) from day x_a through xn;

x_a = the time after which the copper release rates asymptote to PSS.

54. To capture the cumulative copper loading during the initial release period, the researchers [70] suggested the following equation:

$$IL_{x_0, x_a} = CL_{x_0, x_a} \quad \text{(Equation 4.19)}$$

where:

$IL_{x0, xa}$ = the initial release loading (µg cm^2), which occurs before day x_a:

$CL_{xa,xn}$ = the cumulative copper loading (µg cm-2) from day x_0 through x_a;

55. The total copper loading based on a materials life cycle was then estimated using the above variables with the following equation:

$$\text{Life Cycle Loading}_{s,f} = ((IL_{x_0, x_n}) \times (\Sigma E_{cleaning} + \Sigma E_{replacement})) + (PSS \times \Sigma D_{PSS}) \quad \text{(Equation 4.20)}$$

where:

Life Cycle $Loading_{s,f}$ = Cumulative copper release (µg cm^{-2}), between time points x_s and x_f, the time over which the material is exposed to water;

$\Sigma E_{cleaning}$ = the total number of regularly scheduled material cleaning events over a given life cycle period.

$\Sigma E_{replacement}$ = the total number of regularly scheduled material replacement events over a given life cycle period (which includes the initial placement of material).

ΣD_{PSS} = the total number of days at which PSS releases are anticipated to occur.

4.1.3 Techniques used to estimate total organic Carbon releases

56. Total carbon (both organic and inorganic) release has been estimated by Olsen et al. [17] and has been described above in several equations, i.e. equations 4.7, and equations 4.11 to 4.13.

5. Conclusions

7. This document provides a comprehensive review of pollutants of concern (listed in the LBS Protocol) in aquaculture production facilities, and approaches, methods and techniques to estimate their releases focusing on nutrients, Cu and Zn, TOC.

8. Additionally, given an increasing concern regarding pesticides and persistent organic pollutants (POPs) pollution releases to waters from aquacultural production facilities, the document also reviewed issues related to potential pollution loading originating from these sources and provided a summary of current estimation techniques used to assess this type of pollution. Despite there is no agreed estimation methods, the potential estimation techniques are summarized in Annex III.

9. It should be noted that:
   a. Unlike the air emissions inventory, guidance documents and inventories on pollutants releases from aquaculture facilities are scarce.
   b. The estimation techniques about releases of pollutants from aquaculture production facilities are also very sparse.
   c. Estimation of pollutants discharges from aquaculture production facilities is a complex area of scientific research which requires expert knowledge.

10. With this guidance document information in the peer-reviewed literature has been researched, compiled and integrated to assist Contracting Parties in determination of the most appropriate methods and techniques to estimate potential pollution loading from aquaculture. There is an extensive bibliography of references and supplemental information containing recommendations for further sources of information and peer reviewed research papers particularly relevant to Mediterranean region presented in the Annex IV.

Annex I
Aquaculture Industry

A. Brief Overview

1. The FAO State of World Fisheries and Aquaculture Report [75-76] provides technical insight and exhaustive (22 Tables and 58 Figures) information on a sector and highlights major trends and patterns in global fisheries and aquaculture. These reports [75-76] highlighted that there has been a steep growth in the aquaculture industry for the last seven decades. Between 1961 and 2016, the average annual increase in global food fish consumption, grew from 9.0 kg in 1961 to 20.2 kg in 2015 (expressed per capita terms), at an average rate of about 1.5 percent per year [75]. In 2018, the world aquaculture production reached a record high of 179 million tons (Mt)[13] in live weight, of which 156 Mt were used for human consumption, and the remaining 23 Mt to produce fishmeal and fish oil [76]. Aquaculture accounted for 46 % (82.3 Mt) of the total production and 52 percent of fish for human consumption (81.1 Mt) [76]. According to the OECD – FAO Agricultural Outlook 2020-2029 [77], global fish production is projected to reach 200 Mt by 2029, increasing by 25 Mt (or 14%) from the base period (average of 2017-19), though at slower pace (1.3% p.a.) than over the previous decade (2.3% p.a.).

Aquaculture in The Mediterranean

2. A detailed description of Aquaculture production in the Mediterranean has been described in several reports over the past five decades [78-81]. Although it was initially land-based, since the 1990s the Mediterranean marine fish farming was transferred to floating cages at sea [82-84]. In 2013, the marine fish farming in the Mediterranean was dominated by two main species: the European seabass Dicentrarchus labrax with ~161,000 metric tons year-1 and the gilthead seabream Sparus aurata with ~ 135,000 metric tons year-1. Farming of these species involves a first phase taking place in a land-based hatchery, then the moving of juvenile fish to floating cages at sea.

3. Of the brackish and freshwater form of aquaculture, the production of the Nile tilapia (Oreochromis niloticus) has been the greatest and the most important aquaculture industry in the Mediterranean region, with 769,000 metric tons produced in Egypt alone in 2012. Tilapia production continued rapid growth and expansion during the past several years [85-86]. Today, Egypt is the seventh-largest aquaculture producer in the world by production quantity and the largest in Africa, accounting for 73.8 % of aquaculture in Africa by volume and for 64.2 % by value [85-86]. Nile tilapia remains the main cultured species in Egypt contributing about 65.15% of the entire Egyptian fish production [86].

4. Taking into account both inland, brackish and marine waters production, since 2010 Egypt, France, Spain, Italy, Turkey and Greece have been the main aquaculture producing countries [87]. According to the Joint Research Centre (JRC) Scientific, Technical and Economic Committee for Fisheries (STECF)14 these countries remain the leaders in aquaculture production, with Spain (21%), France (15%), Italy (14%), and Greece (10%), making up 60% of the sales volume in EU27 [87]. Therefore, the guidelines on estimation techniques and methodologies to estimate potential pollutant loadings from aquaculture activities may be of particular interest to these countries.

B. Aquaculture Systems and Practices

5. There are several aquaculture practices which are used world-wide for production of a great variety of culture organisms. However, according to the water environment (freshwater, brackish water, marine water) in which the organisms are cultured, the three main types of aquaculture are:

[13] In this FAO publication, the term "fish" indicates fish, crustaceans, molluscs and other aquatic animals, but excludes aquatic mammals, reptiles, seaweeds and other aquatic plants.
[14] https://stecf.jrc.ec.europa.eu/index.html

a. Freshwater aquaculture carried out either in fishponds, fish pens, fish cages or, on a limited scale, in rice paddies. It is located inland (hence, “inland aquaculture”) and represents 57% of animal aquaculture production [76] [88-90];
b. Brackish water aquaculture, which is located in coastal areas, hence “coastal aquaculture”. It is practiced in completely or partially artificial structures in areas adjacent to the sea, such as coastal ponds and gated lagoons [88-92].
c. Marine aquaculture, “mariculture” is conducted in the sea, in a marine water environment. It employs either fish cages or substrates for mollusks and seaweeds such as stakes, ropes, and rafts, and can be located along the coastline or off-shore (off-shore, high seas aquaculture) [91-95].

6. The environmental impact of aquaculture is largely determined by the farming method used. According to the water-holding facility in which the organisms are grown, the aquaculture production methods are grouped into four types: ponds, cages, raceways, and recirculating systems (Table A.1). Depending on the stocking density of the culture organisms, the level of inputs, and the degree of management, culture systems range from very extensive, through semi-intensive and highly intensive to hyper-intensive [88-96]. The management interventions, infrastructure and supporting technologies utilized in aquaculture include a wide range of activities, such as seed supply and stocking, handling, feeding, controlling, monitoring, sorting, treating, harvesting, processing and use of preventive measures [76] [88-96].

Table A.1: Aquaculture Methods[15]

High-Risk Systems
Open-net Pens or “cages” are found offshore, in coastal areas or in freshwater lakes. These systems allow for free exchange of waste, chemicals, parasites and disease between the farm production site and the surrounding environment. There is also the potential for farmed fish to escape. Farms can also attract predators, such as marine mammals, that can get tangled in fish farm nets and drown.
Ponds, which are semi or fully enclosed bodies of water, and typically used to farm Tilapia and shrimp. “High-risk” pond farms discharge untreated wastewater, which pollutes the surrounding environment and can also cause considerable habitat damage (for example, shrimp ponds are a leading cause of mangrove destruction [97-99]. To be considered a “low risk” method, discharged waste must be filtered and treated.
Low Risk Systems
Closed systems, or closed containment farming methods, use a barrier to control the exchange between farms and the natural environment. This method significantly reduces adverse effects on the environment including pollution, fish escapes, negative wildlife interactions, and parasite and disease transfer from farms to marine and freshwater ecosystems. The most common types are race ways and recirculating systems.
Raceways are typically used for raising rainbow trout. In this method, flowing water is diverted from natural streams or a well. To be considered a low-risk method, waste must be treated, and fish escapes prevented.
Re-circulation Systems: In these systems water is treated and re-circulated, with minimal wastewater discharge. Common species farmed this way include Arctic char, striped bass, barramundi, sturgeon, and increasingly, salmon. These systems are designed to treat effluent before it is discharged to natural water bodies, which reduces pollution, disease and parasite transfer. Fish escapes are virtually impossible, with appropriate barriers designed into the facilities.

[15] Modified from Seachoice.org. url: https://www.seachoice.org/info-centre/aquaculture/aquaculture-methods/

Suspended aquaculture is the method of growing shellfish on beaches or suspended in water on ropes, plastic trays or in mesh bags. The shellfish farmed using these methods are filter feeders and require only clean water to thrive. Oysters, scallops, mussels, and clams are cultured using suspension systems. Shellfish farming in suspended aquaculture is often low risk, if the farmed species is native to the area, and if the farm has sufficient water flow to prevent waste accumulation.

Annex II

Overview of Release Estimation Techniques and Applied Methodologies for Estimation of Releases of Pollution from the Aquaculture Sector

A. Background

7. Aquaculture is one of the pillars of both the Common Fisheries Policy (CFP)16 and initiatives of the European Union, i.e., the Blue Growth Agenda Strategy17 and the strategic guidelines for the sustainable development of EU aquaculture18. However, until recently, regulations and international oversight for the aquaculture industry are extremely complex, with several agencies regulating aquaculture practices, including site selection, pollution control, water quality, feed supply, and food safety. Moreover, these practices differ from country to country and sometimes between states and territories within a country [100-101].

8. FAO [102] recently developed and proposed an ecosystem approach to aquaculture (EAA), which they defined as a "strategy for the integration of aquaculture within the wider ecosystem such that it promotes sustainable development, equity, and resilience of interlinked social-ecological systems". The strategy is led by three key principles: 1) Aquaculture development and management should take account of the full range of ecosystem functions and services and should not threaten the sustained delivery of these to society; 2) Aquaculture should improve human well-being and equity for all relevant stakeholders; 3) Aquaculture should be developed in the context of other sectors, policies and goals. In describing the EAA, the authors [102] also discussed site selection and carrying capacity, which is an important concept for ecosystem-based management, and assist in setting the upper limits of aquaculture production given the environmental limits and social acceptability of aquaculture.

B. Overview of approaches

9. To date there have been very few Inventories/Guidelines describing approaches, methods and techniques for estimating pollutant loading from Aquaculture production activities. In Europe, the two main guidelines have been developed over 20 years ago:

Guidelines on Nutrient Discharges from Fish Farming in the OSPAR Convention Area developed by the OSPAR Commission [65].

10. This Guidelines document proposed techniques to estimate nutrient discharges and provides methods to assess these discharges: (i) assessment based on the feed used; (ii) assessment based on production; and (iii) assessment based on national information and other sources (Table A.2).

11. The Guidelines also provide information on Nutrients discharged (ton/year) according to national calculations for several countries. However, these calculations are based on production data from over 25 years ago, therefore the information should be updated. Moreover, the data presented in the OSPAR guidelines [65] mainly concern aquaculture within the OSPAR Convention Area. Only a proportion of the data included activities within the Mediterranean Sea, while data for a few countries are missing completely.

12. The guidelines concluded that Nutrient discharges should be calculated separately for the various types of aquaculture; the main distinctions being between marine and brackish-water net cage farming, intensive farming in ponds, basins and channels, and extensive carp pond farming. However, the data that would enable such distinctions were not available [65].

[16] https://ec.europa.eu/fisheries/cfp_en
[17] http://ec.europa.eu/fisheries/cfp/aquaculture/index_en.htm
[18] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52013DC0229&from=EN

Table A.2: Methods for assessment of nutrient discharges from fish farming

Assessment based on the feed used[1]
The feed used is determined by: • the species of fish farmed; • the type of farming (i.e. farm type, marine/freshwater, seasonal and climatic conditions, fish density); • the age of the fish (i.e. fry, adult); and the production objective (i.e. for food or as stock).
Assessment based on production[2]
This method estimates approximate nutrient discharges from the non-converted nutrients per ton of fish produced. Information must be obtained from the producers. At the time OSPAR guidelines were created in 2000, various producers estimated that approximately 40 – 70 kg N and 4 – 11 kg P/ton of fish produced are not converted when using dry feed with a DOM of > 90%.

[1] Notes: The guidelines [65] also provides information on the composition of the most frequently used feed which at the time (year 2000) was the same for marine and inland sectors (Table 6, pp. 14 of the OSPAR guidelines), and examples of calculation for both N and P releases; [2] The Guidelines [65] highlighted that the assessments using this method are inexact because production-specific information such as aquaculture type, feeding method, the species farmed and its age structure, losses through mortality and the import/export of stock are not included in the calculation.

<u>*HELCOM Guidelines for the compilation for waterborne pollution load to the Baltic Sea (PLC-Water) [103]*</u>

13. This Guidelines describe methods for compilation of annual pollutant load for Fish farming plants in Section 3.1.3.3. For the quantification of discharges, the Guidelines highlight a distinction between two main production types: a) Plants without treatment (e.g., plants where the sludge is not collected or where the sludge is collected but discharged to the aquatic environment without treatment); and b) Plants with treatment (e.g., plants with permanent removal of sludge), where the N and P contents (and organic matter) in the sludge removed are quantified.

14. The two proposed quantification Approaches are:

   a. *Approach 1*, which is based on calculations from production parameters. The starting point is that information is available on both production and feed consumption at catchment level. The quantification method is based on mass balance equations.
   b. *Approach 2* is based on monitoring the discharge. It is practicable for ponds or other land-based production systems where the discharges are distinct point discharges (such as end of pipe/channel). The quantification of losses is also based on mass balance equation but on monitoring results[30].

15. In **Australia**, there are two main Guidelines documents, both developed 20 years ago [64][104]: 1) The Emissions Estimation Technique (EET) Manual for Aquaculture from Temperate Water Finfish Aquaculture provides a general overview of the temperate water finfish aquaculture methods and describes the procedures and methods for estimating emissions of Category 3 National Pollutant Inventory (NPI) listed substances, specifically total nitrogen (TN) and total phosphorus (TP) [64] It 2) The EET Manual for The Aquaculture of Barramundi, Prawns, Crocodiles, Pearl oysters, Red claw and Tropical abalone In Tropical Australia [104], which describes the procedures and methods relevant only to Tropical Aquaculture Facilities.

16. In the **USA**, the US Environmental Protection Agency (USEPA) developed Guidelines for the Final Effluent Limitations Guidelines and New Source Performance Standards for the Concentrated Aquatic Animal Production Point Source Category [66]. It describes industry processes, pollutants generated, available control and treatment technologies, the technical basis for the final rule, and costs of the rule.

17. In **Canada**, aquaculture is managed by different levels of government. Provincial governments are the primary regulators and leasing authorities for aquaculture (except in British Columbia and Prince Edward Island), while the federal government has responsibility for navigation, disease prevention affecting international trade, and the environment under the Fisheries Act and the Health of Animals Act.[19] Measures to reduce detriment are listed in Section 7 of the Aquaculture Activities Regulations guidance document. However, no estimation techniques or methods are described. Recommendations and rules for management of organic wastes can be found at the Fisheries and Oceans Canada website.[20]

C. Accuracy and uncertainty

18. The UNITAR Guidelines [105] highlighted that evaluation of availability and accuracy of information is a key when considering types of pollution sources to be included in the national PRTR system. However, the availability of information needed varies greatly between countries and for different regions within a country [105]. The Guidelines also pointed out that quality of inventories is influenced by several factors including 1) accuracy (the measure of 'truth' of a measure or estimate); 2) comparability (between different methods or datasets); 3) completeness (the proportion of all emissions sources that are covered by the inventory); and 4) representativeness (in relation to the study region and sources of emissions) [105]. The USEPA highlighted that prediction uncertainty is caused by natural process variability, and bias and error in sampling, measurement, and modeling [137].

19. According to the OECD Compendium [106], errors or uncertainty in the preparation of the inventories may include: 1) Emission factors (which do not reflect real life conditions); 2) Activity data that do not adequately reflect the study region (scaling down national or state activity data to smaller regions always results in decreased accuracy); 3) Spatial and temporal disaggregation may introduce errors that are difficult to quantify; 4) Sample surveys may be subject to sampling errors.

20. One of the key documents for Aquaculture, the OSPAR Guidelines [65] underlined that they were not able to produce complete and reliable datasets on production and nutrient discharges from aquaculture. Some of the reasons for the lack of reliability were 1) missing or incomplete responses to the questionnaire; 2) a lack of detail in the response (e.g., no distinction between marine and freshwater production and the respective feed used); 3) little or no distinction between the total production of a particular country, production within the OSPAR Convention Area and/or production within 'eutrophication problem areas'; 4) differences in the quality and accuracy of the data supplied, 5) owing to variability in the calculation procedures and assessment methods used; and 6) data supplied for different years.

21. The OSPAR Guidelines [65] also highlighted that a further limitation is imposed by the wide range of aquaculture systems in use. Moreover, factors crucial to an assessment of this type of pollutants release are not reported statistically due to the large number of farms and species farmed. Variability in the technical equipment used (for example cleaning and filtration systems) and types of farm-specific feed and feeding techniques also affected data accuracy.

D. Quality control and quality assurance

22. The OECD Compendium [106] provides summary of quality assurance/quality control (QA/QC). They highlight the importance of proper documentation, which ensures reproducibility, transparency and assists future inventory updates. Documentation should include all raw data used, assumptions, steps in calculations, and communications with data providers and QA/QC processes.

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[19] Government of Canada (2021). Fisheries and Oceans Canada. Aquaculture Activities Regulations guidance document. url: https://www.dfo-mpo.gc.ca/aquaculture/management-gestion/aar-raa-gd-eng.htm
[20] https://www.dfo-mpo.gc.ca/aquaculture/protect-protege/waste-dechets-eng.html

Moreover, the important missing data (e.g., missing pollutants, missing source types) also need to be acknowledged and documented [106].

23. The International Plant Protection Convention (IPPC) Guidelines for National Greenhouse Gas Inventories provides a comprehensive description of the quality assurance/quality control (QA/QC) and verification which are also relevant to inventories of pollutants releases/discharges from aquaculture production and fish farming activities [107]. They also highlighted that well developed and established QA/QC contributes to the transparency, consistency, comparability, completeness, and accuracy of inventories (Box A.1).

Box A.1.: Definitions of QA/QC and Verification

Quality Control (QC) is a system of routine technical activities and procedures to assess and maintain the quality of the inventory. The QC system is compiled by the inventory team and is designed to: (i) Provide routine and consistent checks to ensure data integrity, correctness, and completeness; (ii) Identify and address errors and omissions; and (iii) Document and archive inventory material and record all activities. QC activities comprise general methods such as accuracy checks on data acquisition and calculations, and the use of approved standardized procedures. QC activities also include technical reviews of categories, activity data, emission factors, other estimation parameters, and methods.

Quality Assurance (QA) is a system of review procedures conducted by independent third parties. The purpose of reviews is to verify that measurable objectives (data quality objectives) are met, and to ensure that the inventory represents the best possible estimates of emissions and removals given the current state of scientific knowledge and data availability and support the effectiveness of the QC programme.

Verification refers to the collection of activities and procedures conducted during the planning and development stage, or after the completion of an inventory that can help to establish its reliability for the intended applications of the inventory.

Annex III
Additional Issues of Concern - Releases of Pesticides, Persistent Organic Compounds (POPs) and microplastics from the Aquaculture Sector

A.Brief Overview

1. Replacing marine ingredients with plant material in the feed results in introduction of pesticides used in terrestrial agriculture in aquaculture production facilities globally [28-32][108]. The aquaculture feed includes soybeans, maize and rice [8-10], with soybean meal representing about 25% total compound aquafeeds by weight [5].

3. The main source of persistent organic pollutants (POPs) in farmed fish, in particular farmed Atlantic salmon are fish oils, obtained from pelagic fish species, used in fish feed. Oil spill accidents are among the most concerning exposure events for Polycyclic aromatic hydrocarbons (PAH) pollution of aquatic environments [109-112]. Hydrocarbon chemicals are major components of crude oil and are classified as PAHs, aliphatic saturated hydrocarbons, aliphatic unsaturated hydrocarbons, and alicyclic saturated hydrocarbons [109-112]. The impact of these four categories of PAHs on the ecosystem is especially concerning because of their carcinogenicity [112-113]. Several studies reported PAHs in fish in various areas of Mediterranean Sea [25] [110-120].

4. Microplastics may enter aquatic environments through different pathways, and they have occurred in all environmental matrices (beaches, sediments, surface waters and water column). Microplastic exposure potential in marine fish, for example, is likely to arise from ingestion of particles in the water column or on the seafloor resembling prey or by ingesting prey that previously ingested microplastics themselves [121]. The exposure can also occur via feed. About 25% of global commercial marine fisheries landings are used to produce fishmeal and fish oil [121-122]. Recent research has shown that fishmeal is both a source of microplastics to the environment, and directly exposes organisms for human consumption to these particles [121-124]. Thiele et al. [121] made a conservative estimate that over 300 million microplastic particles (mostly < 1 mm) could be released annually to the oceans through marine aquaculture. Due to their widespread and increasing presence in both freshwater and marine environments, and their potential hazard risk to the marine environment via ingestion and accumulation of PBTs, microplastics have emerged as one of the most concerning environmental problems in the aquatic ecosystem [121-126].

5. Apart from the main pollutants described in the main document (Sections 1.3.2.1 to 1.3.2.4), all of the above pollutants described above accumulate in sediments [47-52] [59-61][118][127-128].

Pesticides

1. The main sources of pesticides in aquaculture production, in particular salmon, is through fish feed and also to control parasites[21] [24-25] [28-32]. The use of pesticides in fish feed, in particular farmed salmon has caused an increased concern regarding their potential effects on human health in recent years [24-25] [28-32]. It has been reported that feed used in the seawater production phase of Atlantic salmon aquaculture typically contains 70% plant ingredients [63][129]. In Asia, Cheung et al [130] reported highly elevated concentrations of organochlorine pesticides (OCPs) in fish collected from the fishponds located in Pearl River Delta (PRD). The concentrations of OCPs in human tissues (e.g. milk and plasma) were significantly correlated with the frequency of fish consumption in both Hong Kong and Guangzhou populations [28][131]. In Europe, recent wide-scale screening of Atlantic salmon feeds has shown that they contain chlorpyrifos-methyl (CPM) [108][132]. Other compound found was Chlorpyrifos (CPF) a widely used agricultural organophosphorus pesticide (OP) that can be highly toxic to fish [108].

2. Pesticides pollution of fish is becoming a problem of increasing concern in the Mediterranean Sea. Pesticide residues (Metribuzin DADK, propamocarb HCl, and piperonyl butoxide (PBO)) were found in muscles of several marine fish species and seaweeds in Mediterranean (Iskenderun Bay, Turkey) [133]. Polychlorinated biphenyl (PCB) and OCP concentrations were determined in livers of two deep-sea fish species (roughsnout grenadier and hollowsnout grenadier), from the Adriatic Sea [134]. PCBs and Organochlorine Pesticides (OCPs) were also detected in the sediments and Siganus rivulatus (marble spinfoot) from two areas along the Egyptian Mediterranean Coast [127], Greece [135], Italy [136], Spain [137], France [128]. Ibrahim et al [138] found that 27 freshwater fish species

[21] Caligus or "sea louse" is a small crustacean that attaches to the surface of the skin and gills of salmon, generating significant injuries to the fish.

that are native to Europe and widespread in the EU streams, ditches or ponds in agricultural landscapes are at the elevated risk of being exposed to pesticides.

Persistent organic pollutants (POPs)

3. Persistent organic pollutants (POPs) are toxic chemicals that adversely affect human health and the environment around the world and are listed as pollutants of concern in the LBS protocol. Fish can accumulate high amounts of POPs and Hg, and therefore can be the sources of their entry in human organism [117-118][139]. Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) (collectively referred to as dioxins) highly lipophilic and accumulate in the fatty tissue of humans and animals and thus in the fatty fish livers [25]. Several studies demonstrated that the concentrations of organic contaminants in cod livers depend on the fishing area [25][120].

4. The main source of persistent organic pollutants (POPs) in farmed fish, in particular farmed Atlantic salmon are fish oils, obtained from pelagic fish species, used in fish feed. Oil spill accidents are among the most concerning exposure events for Polycyclic aromatic hydrocarbons (PAH) pollution of aquatic environments [109-112]. Hydrocarbon chemicals are major components of crude oil and are classified as PAHs, aliphatic saturated hydrocarbons, aliphatic unsaturated hydrocarbons, and alicyclic saturated hydrocarbons [109-112]. The impact of these four categories of PAHs on the ecosystem is especially concerning because of their carcinogenicity [112-113111]. Several studies reported PAHs in fish in various areas of Mediterranean Sea [25] [108-120].

Microplastics

5. Microplastics are typically defined as plastic items which measure less than 5 mm in their longest dimension and include also nanoplastics (which are less than 100 nanometres long). These plastic items may be manufactured or may result from the degradation and fragmentation of larger plastic items (defined as secondary micro- and nanoplastics). Microplastics contain a mixture of various chemicals and additives from manufacturing process, and they can also efficiently sorb (adsorb or absorb) persistent, bioaccumulative and toxic contaminants (PBTs) from the environment [121-126].

6. Following Global Oceans Action Summit for Food Security and Blue Growth in 2014 recommendations, FAO, The International Maritime Organization (IMO) and UNEP worked together with the Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) to improve the knowledge base on microplastics in the marine environment and provide policy advice on this topic [125].

B. Summary of techniques used to estimate releases of pesticides and persistent organic compounds (POPs) from the aquaculture sector

7. There are no techniques for estimation of the releases of pesticides and POPs as such, however, this document postulates some methods which could be evaluated for making such estimations. It should be noted that, these estimation techniques need to be further tested.

8. Similarly to nutrients, metals and TOC, pesticides and persistent organic compounds (POPs) entering aquaculture production facilities via fish feed, chemicals (medications, disinfectants, and antifoulants) and could be, unintentionally, released to the environment via uneaten food and fish faecal material.

9. Therefore, as highlighted earlier, determining the feed content and the quantity of its ingredients from the feed suppliers is key [19-22][65-66]. A particularly important parameter is the feed conversion ratio (FCR), determined as:

FCR = Dry weight of feed applied/Wet weight of fish gained (Equation 4.21.)

10. Most of the techniques used to estimate releases of nutrients, TOC and metals (e.g. Equations 4.3 to 4.5, 4.8, 4.9-4.10, 4.12, 4.15 to 4.20) could be applied to estimate pesticides and persistent organic compounds (POPs), where nutrients content/concentration would be substituted by the pollutant in question (i.e. pesticides, POPs), **though they would need to be tested**.

11. For example, if we follow the same analogy of techniques proposed for nutrients, a simple equation for nutrient discharges (equation 4.4.) proposed by OSPAR guidelines, an equation could be tested for determination of organic chemical releases:

$$OC_{feed} = OC_{fish} + OC_{rel} \qquad \text{(Equation 4.22)}$$

where:

OC_{feed} = organic chemical content in feed[22]
OC_{fish} = Organic chemical content converted to fish biomass (OC_{fish}) or
OC_{rel} = unconverted organic chemical released into the water,

Pesticides and POPs

12. As mentioned above (paragraphs 7 and 8) equation 4.22 could also be used for determination of POPs, **though it would need to be tested.** Several authors developed models with the aim of predicting bioaccumulation of organic chemicals in aquatic food-webs in freshwater [71-74].

13. Mackay and Fraser [72] conducted an extensive literature review of mechanisms and models used for predictions and estimates of persistent organic chemicals bioaccumulation in fish (which would be OC_{fish}) and suggested a new empirical model for determination of bioconcentration (**Tier 1**) and mechanistic model for estimates of bioaccumulation (**Tier 2**). The authors [72] defined *bioconcentration* as the uptake of chemical by absorption from the water can only occur via the respiratory surface and/or the skin, and thus results in the chemical concentration in an aquatic organism being greater than that in water. The bioconcentration factor (BCF) is defined as the ratio of the chemical concentration in an organism C_B, to the total chemical concentration in the water C_{WT}, or to C_{WD}, the freely dissolved chemical concentration in water.

14. *Bioaccumulation* (BAF) is the process which causes an increased chemical concentration in an aquatic organism compared to that in water, due to uptake by all exposure routes including dietary absorption, transport across respiratory surfaces and dermal absorption. *Bioaccumulation can thus be viewed as a combination of bioconcentration and food uptake*.

15. The authors [72] highlighted that bioaccumulation is particularly relevant for estimates of pesticides and POPs releases from aquaculture production facilities. The proposed models are summarized in Table 4.1. below.

16. In 2009, the US EPA Office of Pesticide Programs' Environmental Fate and Effects Division scientists developed KABAM (K_{OW} (based) Aquatic BioAccumulation Model) to estimate potential bioaccumulation of hydrophobic organic pesticides in freshwater aquatic food webs and subsequent risks to mammals and birds [73-74]. The KABAM model is composed of two parts: i) bioaccumulation model estimating pesticide concentrations in aquatic organisms, and ii) a risk component translating exposure and toxicological effects of a pesticide into risk estimates for mammals and birds consuming contaminated aquatic prey.[23] A detailed description of the model can be found on the USA EPA website [73-74].

Table 4.1: Tier 1 and Tier 2 Models for Bioconcentration and Bioaccumulation of persistent organic chemicals in fish

[22] This information can be obtained from the fish feed suppliers. Within the EU, According to Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed, Member States have to monitor pesticide residue levels in food samples including aquaculture products and submit the monitoring results to EFSA and the European Commission. http://publications.europa.eu/resource/cellar/7deccc8e-5c03-11eb-b487-01aa75ed71a1.0006.03/DOC_1
[23] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/kabam-version-10-users-guide-and-technical#Section1

Tier 1 Empirical model of Bioconcentration	**Tier 2 Mechanistic model of Bioaccumulation**
$BCF = C_B/C_{WD} = (1 + L \cdot K_{OW})$, where L is lipid volume fraction, C_{WD} is dissolved concentration or equivalently for fugacity in biota f_B and in water f_W $f_B = f_W$ where $f_W = C_W/Z_W$, $f_B = C_B/Z_B$ and $BCF = Z_B/Z_w = (1 + LK_{OW})$ *Assumptions*: Partitioning is predominantly to lipids, no ionization, no metabolism no biomagnification, 100% bioavailability, and equilibrium applies. *Criteria*: $BCF > 5000$ or $K_{OW} > 100\,000$	$BAF = C_B/C_{WD} = (k_1 + k_A C_A/C_{WD})/(k_2 + k_M + k_E)$, where k_1 is the resipratory uptake rate constant, k_A is the food consumption rate constant, k_2 is the respiratory clearance rate constant $= k_1/BCF$ k_M is the metabolic rate constant, and k_E is the egestion rate constant $= k_A/4$ *Assumptions*: Partitioning is predominantly to lipids, no ionization, no metabolism, no biomagnification, 100% bioavailability, and equilibrium applies. *Criteria*: $BCF > 5000$ or $K_{OW} > 100\,000$
K_{OW} = octanol[24]-water partition coefficient[25] represents the ratio of concentrations of a compound between two phases, one being octanol and the other water. It serves as a measure of the relationship between lipophilicity (fat solubility) and hydrophilicity (water solubility) of a substance. *Fugacity* is partial pressure, a criterion of equilibrium analogous to temperature in the case of heat transfer. Z_w *and* Z_b are constants for water and biota, respectively. Z is a constant (units of mol/m^3 Pa) specific to the chemical, the phase in which it is dissolved or sorbed and temperature and can be calculated from physical and chemical properties.	

[24] Octanol is any of four liquid alcohols $C_8H_{17}OH$ derived from normal octane; Octane is a hydrocarbon and an alkane with the chemical formula C_8H_{18}, and the condensed structural formula $CH_3(CH_2)_6CH_3$. It has many structural isomers that differ by the amount and location of branching in the carbon chain. Octane is also an agent designed to control the life of pesticides: https://indigospecialty.com.au/wp-content/uploads/2019/11/ISP-Octane-5L-Label_F090919.pdf

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Annex IV
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