Section G - Appendices (methodologies, references, etc.)

*The following text comprises part of the **AMAP Trends and Effects Programme** documentation. As such, it should be read in conjunction with other sections of the documentation, in particular Section A (Introduction) which includes an explanation of some basic terms and concepts that are used in describing the AMAP programme. Other sections include: Section B (AMAP Trend Monitoring Programme); Section C (AMAP Effects Monitoring Programme); Section D (Supporting Studies); Section E (Quality Assurance and Quality Control); and Section F (Data Reporting Issues).*

Content of Section G

Section G of the **AMAP Trends and Effects Programme** documentation includes the following parts:

Part A. Methodological references: references to methods and guidelines that are appropriate as a basis for work to be carried out under the AMAP Trends and Effects Programme. Key references are highlighted in bold. The material is organized in a series of tables concerniong, respectively:

- Atmospheric monitoring - Guidelines and methodological references
- Terrestrial monitoring - Guidelines and methodological references
- Freshwater monitoring - Guidelines and methodological references
- Marine monitoring - Guidelines and methodological references
- Biological effects monitoring - Guidelines and methodological references
- Guidelines and references on quality assurance
- Guidelines and references on tissue and specimen banking
- Guidelines and references on general monitoring issues (monitoring strategies, sample pooling, temporal trend analysis, normalization, etc.)

Part B. References: A list of references cited in other sections of the **AMAP Trends and Effects Programme** documentation.

Part C. Annexes: Supplementary material, including:

- Annex 1. Overview of biological effects techniques applicable to the marine environment generally not including sea mammals and birds (from Report of the ICES Advisory Committee on the Marine Environment, 2000).
- Annex 2. Monitoring of imposex

Part A. Methodological references

Atmospheric monitoring - Guidelines and methodological references

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Atmosphere	Air/aerosol	POPs	Sampling, analyses, QA	- **Recommendations of the working group "Quality control programs" at the workshop on Techniques of POPs Measurements in Northern Environments: Method performance criteria and quality control measures for the determination of persistent organic compounds (POPs) in ambient air, precipitation and water.**
Atmosphere		POPs, heavy metals		- EMEP manual for sampling and chemical analysis. EMEP/CCC-Report 1/95. - See also references in: Integrated Atmospheric Deposition Network Quality Assurance Program Plan. Environment Canada/US-EPA/Ontario Ministry of Environment and Energy. 1994
Atmosphere	Air	Radionuclides	Sampling, analyses, QA	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. I, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.
Atmosphere	Air/aerosol	Heavy metals (mercury)	Sampling, analysis	- TEKRAN equipment and manuals: http://tekran.com/
Atmosphere	Air/aerosol/ Precipitation	POPs (PCBs)	Sampling, analysis	- Guidance note on the sampling and analysis of PCBs in air and precipitation. Joint Assessment and Monitoring Programme. Oslo and Paris Comissions.
Atmosphere	Air/aerosol/ Precipitation	Heavy metals (mercury)	Sampling, analysis	- JAMP guidelines for the sampling and analysis of mercury in air and precipitation. Joint Assessment and Monitoring Programme. Oslo and Paris Comissions. (or Munthe J., 1996. Guidelines for the sampling and analysis of mercury in air and precipitation. Reoprt to the Oslo and Paris Commissions. IVL L 96/204.)
Atmosphere	Air/aerosol/ Precipitation	Acidifying substances	Sampling, analysis	- EMEP manual for sampling and chemical analysis. EMEP/CCC-Report 1/95.
Atmosphere	Air/ precipitation	Acidifying substances, climate, UV		- ICP IM Manual. International Co-operative Programme on Integrated Monitoring of the UN/ECE (August 1998) (http://www.vyh.fi/eng/intcoop/projects/icp_im/manual/contents.htm) -

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Atmosphere	Precipitation/ snow/ snowpack	POPs	Sampling, analyses	- Peters, A.J., Gregor, D.J., Teixeira, C.F., Jones, N.P. & Spencer, C., 1995. The recent historical depositional trend of polycyclic aromatic hydrocarbons and elemental carbon to the Agassiz Ice Cap, Ellesmere Island, Canada. Science of the Total Environment. Vol. 160, 161, Special issue: Ecological effects of Arctic airborne contaminants, pp. 167-180.
Atmosphere	Precipitation/ snow/ snowpack	POPs	Sampling, analyses	- Gregor, D.J., Peters, A.J., Teixeira, C., Jones, N. & Spencer, C., 1995. The historical residue trend of PCBs in the Agassiz Ice Cap, Ellesmere Island, Canada. Science of the Total Environment. Vol. 160, 161, Special issue: Ecological effects of Arctic airborne contaminants, pp. 117-126.
Atmosphere	Precipitation/ snow/ snowpack	Heavy metals	Sampling	- Nriagu, J.O., Lawson, G.S. & Gregor, D.J., 1994. Cadmium concentrations in recent snow and firm layers in the Canadian Arctic. Bull. Environ. Contam. Toxicol., 52: 756-759.

Terrestrial monitoring - Guidelines and methodological references

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Terrestrial	Soils, soilwater, groundwater, vegetation	Acidifying substances, various	Sampling, analysis, statistics, QA	- **ICP IM Manual. International Co-operative Programme on Integrated Monitoring of the UN/ECE (August 1998) (Section 7: Methodology and reporting of subprogrammes on: Meteorology, Air Chemistry, Precipitation chemistry, Metal chemistry of mosses, Throughfall, Stemflow, Soil chemistry, Soil water chemistry, Groundwater chemistry, Runoff water chemistry, Lake water chemistry, Foliage chemistry, Litterfall chemistry, Hydrobiology of streams, Hydrobiology of lakes, Forest damage, Vegetation (intensive plot), Tree bioelements and tree indication, Vegetation structure and species cover, Trunk epiphytes, Aerial green algae, Microbial decomposition, Toxicity assessment, Inventory of birds, and Phenological observations) (http://www.vyh.fi/eng/intcoop/projects/icp_im/manual/contents.htm)**
Terrestrial	Soils (remote locations)	POPs	Sampling, analysis	- Methodology currently used by the GLOBALSOC project (W. Ockenden, Lancaster University, Lancaster UK) is recommended. See Appendix 5 to the Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6.
Terrestrial	Mammals (caribou/ reindeer)	POPs	Sampling, analyses	- Elkin, B.T. & R.W. Bethke, 1995. Environmental contaminats in caribou in the Northwest Territories, Canada. Sci. Total Environ., 160/161: 307-321.
Terrestrial	Mammals (caribou/ reindeer)	POPs	Sampling, analyses	- Hebert, C.E., Gamberg, M., Mychasiw, L., Elkin, B.T., Simon, M. & R.J. Norstrom. (in press). Polychlorinated dibencodioxins, dibenzofurans and non-ortho polychlorinated biphenyls in caribou (*Rangifer tarandus*) from the Canadian Arctic. Sci. Total Environ. 185: 195-204
Terrestrial	Mammals (caribou/ reindeer)	Heavy metals	Sampling, analyses	- Gamberg, M. & A.M. Scheuhammer, 1994. Cadmium in caribou and muskoxen from the Canadian Yokon and Northwest Territories. Sci. Total Environ., 143: 221-234.
Terrestrial	Mammals (caribou/ reindeer)	Heavy metals	Sampling, aanalyses	- Elkin, B.T. & R.W. Bethke, 1995. Environmental contaminats in caribou in the Northwest Territories, Canada. Sci. Total Environ., 160/161: 307-321.

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Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Terrestrial	Mammals (reindeer/ caribou)	Radionuclides	Sampling, analyses	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. II, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.
Terrestrial	Birds and mammals (hare, capercaille, willow ptarmigan, common shrew, grey sided vole)	Heavy metals	Sampling, analyses, statistics	- Kålås, J.A., Ringsby, T.H. & Lierhagen, S. 1995. Metals and selenium in wild animals from Norwegian areas close to Russian nickel smelters. Environ. Monitor. Asses. 36: 251-270.
Terrestrial	Mammals (Arctic fox)	POPs	Sampling, analyses	- For general recommendations on sampling Arctic fox for POPs and metals see Appendix 2 to the Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6.
Terrestrial	Birds (merlin)	Heavy metals & POPs	Sampling, analyses	- Nygård, T. 1999. Long term trends in pollutant levels and shell thickness in eggs of merlin in Norway, in relation to its migration pattern and numbers. Exitoxicology 8: 23-31.

Freshwater monitoring - Guidelines and methodological references

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Freshwater	Water	POPs	Sampling, extraction	- For PCBs and OC pesticides and related compounds in water, solid phase extraction of large volume (up to 1000 L) water samples is recommended. See references under Marine sections. Also for references to solid phase extraction with XAD resin see: - McConnell, L.L. et al. Air-water gas exchange of organochlorines compounds in Lake Baikal, Russia. Environ. Sci Technol. 30, 2975-2983, 1996. - Achman, D.R., K.C. Hornbuckleand S.J. Eisnereich. Volatilization of polychlorinated biphenyls from Green Bay, Lake Michigan. Environ. Sci. Technol. 27:75-87, 1993.
Freshwater	Water	POPs	Sampling, analyses	- Gregor, D.J., Peters, A.J., Teixeira, C., Jones, N. & C. Spencer. 1995. The historical residue trend of PCBs in the Agassiz Ice Cap, Ellesmere Island, Canada. Sci. Total Environ., 160/161: 117-126.
Freshwater	Water	Radionuclides	Sampling, analyses, QA	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. I, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.
Freshwater	Water	Acidification, water quality	Sampling, analysis, statistics, QA	- **IPC-Waters - Programme Manual for Chemical and Biological Monitoring (UN/ECE International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes). Prepared by the Programme Centre, Norwegian Institute for Water Research. NIVA, Oslo 1987.** **(Site selection, chemical monitoring, recommended chemical methods, sampling, biological monitoring)** **(http://www.niva.no/icp-waters/)**
Freshwater	Water (soilwater, groundwater, streams and lakes)	Acidifying substances, various	Sampling, analysis, statistics, QA	- ICP IM Manual. International Co-operative Programme on Integrated Monitoring of the UN/ECE (August 1998) (Section 7: Methodology and reporting of subprogrammes on: Meteorology, Air Chemistry, Precipitation chemistry, Metal chemistry of mosses, Throughfall, Stemflow, Soil chemistry, Soil water chemistry, Groundwater chemistry, Runoff water chemistry, Lake water chemistry, Foliage chemistry, Litterfall chemistry, Hydrobiology of streams, Hydrobiology of lakes, Forest damage, Vegetation (intensive plot), Tree bioelements and tree indication, Vegetation structure and species cover, Trunk epiphytes, Aerial green algae,

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
				Microbial decomposition, Toxicity assessment, Inventory of birds, and Phenological observations) (http://www.vyh.fi/eng/intcoop/projects/icp_im/manual/contents.htm)
Freshwater	Water	Water quality parameters incl. metals and POPs	Sampling, storage, analyses, QA	- GEMS/Water. Products and resource materials (GEMS/Water Operational Guide. GEMS/Water Manual of Analytical Methods) http://www.cciw.ca/gems/GEMS-products.html
Freshwater	Sediment	Metals	Sampling, storage, analyses, QA	- Lockhart, W.L. et al. Fluxes of mercury to lake sediments in central and northern Canada inferred from dated sediment cores. Biogeochem. 1998; 40: 163-173. - See also JAMP guidelines references under Marine sediments
Freshwater	Sediment	POPs	Sampling, storage, analyses, QA	- Muir, D.C.G., et al. Spatial trends and historical deposition of polychlorinated biphenyls in Canadian midlatitude and Arctic lake sediments. Environ. Sci. Technol. 30: 3609-3617, 1996. - See also JAMP guidelines references under Marine sediments
Freshwater	Sediments (and water)	Oil, PAHs	Sampling, analysis	- See recommendations in Minutes from the first meeting of the AMAP expert group on oil & PAH. St. Petersburg, Russia, June 1. 2000
Freshwater	Sediments	Radionuclides	Sampling, analyses, QA	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. I, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.
Freshwater	Fish (Arctic char, burbot and northern pike)	POPs	Sampling	- Appendix 2 to Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6.
Freshwater	Fish	Radionuclides	Sampling, analyses, QA	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. II, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.

Marine monitoring - Guidelines and methodological references

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Marine	General	All	Monitoring strategies and guidelines	- ICES Advice on Monitoring, Assessment, Temporal trend monitoring, Biological effects monitoring, etc. see Annex 9 In: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.
Marine	Water Suspended particulate matter	Metals	Sampling, storage, extraction, analyses, normalization	- Guidelines for the sampling and analysis of trace metals in sea water under the Joint Monitoring Programme. Tenth Annual Report on the Activities of the Paris Commission (1989), Annex 24 - Yeats, P. A. 1987. Trace metals in sea water: Sampling and storage methods. ICES Techniques in Marine Environmental Sciences, No. 2. - Yeats, P.A. and Brügemann, L. 1990. Suspended particulate matter: Collection methods for gravimetric and trace metal analysis. ICES Techniques in Marine Environmental Sciences, No. 7. - Suspended particulate matter: Loring, D. & R.T.T. Rantala 1992. Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth Science Reviews 32: 235-283. - Loring 1991. Normalization of heavy-metal data from the estuarine and coastal sediments. ICES J. Mar. Sci. 48: 101-115. - Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6.
Marine	Water	POPs	Sampling, extraction	- For PCBs and OC pesticides and related compounds in seawater, solid phase extraction of large volume (up to 1000 L) water samples is recommended. See the following references: - Schultz-Bull, D.E., G. Petrick, N. Kannan and J.C. Duuinker. Distribution of individual chlorobiphenyls (PCB) in solution and suspension in the Baltic Sea. Marine Chemistry 48:245-270, 1995 - Iwata, H., Tanabe, N. Sakai and R. Tatsukawa. 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. Environ. Sci. Technol. 27, 1080-1098, 1993.
Marine	Suspended particulate matter	POPs, PAHs	Sampling, analyses	- Broman et al. A multi sediment trap study on the temporal and spatial variablility fo polycyclic aromatic hydrocarbons and lead in an anthropogenic influenced archipelago. Environ. Sci. Technol. 22: 1219-1228, 1988.

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Marine	Water	Radionuclides	Sampling, analyses, QA	- Methodologies according to IAEA Guideline and Sampling according to ICES Guidelines.
Marine	Water	Radionuclides	Sampling, analyses, QA	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. I, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.
Marine	Water	Hydrocarbons, Oil, PAHs	Sampling, analyses	- Erhardt, M., Klungsøyr, J. and Law, R.J. 1991. Hydrocarbons: review of methods for analyses in seawater, biota and sediments. ICES Techniques in Marine Environmental Science no. 12. - Yunker et al. Measurement of natural trace dissolved hydrocarbons by in situ column extraction: An intercomparison of two adsorption resins. Anal. Chem. 61, 1333-1343, 1989. - See recommendations in Minutes from the first meeting of the AMAP expert group on oil & PAH. St. Petersburg, Russia, June 1. 2000
Marine	Sediment	Metals	Sampling, storage, extraction, analyses, normalization, statistics, QA	- JAMP Guidelines for Monitoring Contaminants in Sediments 1997. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme. - In prep. JAMP guidelines for environmental monitoring in the vicinity of offshore oil and gas installations. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme. - Loring , D. & R.T.T. Rantala 1992. Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth Sci. Rev. 32: 235-283. - Loring 1991. Normalization of heavy-metal data from the estuarine and coastal sediments. ICES *J. Mar. Sci.* 48: 101-115.
Marine	Sediment	POPs	Sampling, storage, analyses, QA	- JAMP Guidelines for Monitoring Contaminants in Sediments 1997. Technical annex 2 – Determination of CBs. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme. - JAMP Guidelines for Monitoring Contaminants in Sediments 1997. Technical annex 4 – Determination of TBT. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme - In prep. JAMP guidelines for environmental monitoring in the vicinity of offshore oil and gas installations. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme. - Smedes, F. and de Boer, J. 1998. Chlorobiphenyls in marine sediments: Guidelines for determination. ICES Techniques in Marine Environmental Sciences no 21.

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Marine	Sediments, biota	PAHs, POPs	Sampling, analyses	- Krahn, M.M., L.K. Moore, R.G. Bogar, C.A. Wigren, S.-L. Chan and D.W. Brown. 1988. High-performance liquid chromatographic method for isolating organic contaminants from tissue and sediment extracts. *J. Chromatogr.* 437:161-175.
Marine	Sediment	Oil hydrocarbons	Sampling, storage, analyses, QA	- JAMP Guidelines for Monitoring Contaminants in Sediments 1997. Technical annex 3 – Determination of PAH. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme - JAMP guidelines for environmental monitoring in the vicinity of offshore oil and gas installations. In prep. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme. - Erhardt, M., Klungsøyr, J. and Law, R.J. 1991. Hydrocarbons: review of methods for analyses in seawater, biota and sediments. ICES Techniques in Marine Environmental Science no. 12. - See recommendations in Minutes from the first meeting of the AMAP expert group on oil & PAH. St. Petersburg, Russia, June 1. 2000
Marine	Marine biota (mammals, fish, invertebrates)	POPs	Sampling, analyses	- Krahn, M.M., G.M. Ylitalo, J. Buzitis, C.A. Sloan, D.T. Boyd, S.-L. Chan and U. Varanasi. 1994. Screening for planar clorobiphenyls in tissues of marine biota by high-performance liquid chromatography with photodiode array detection. *Chemosphere* 29:117-139.
Marine	Benthos	Benthic communities	Sampling, analysis, storage, statistics, QA	- JAMP guidelines for environmental monitoring in the vicinity of offshore oil and gas installations. In prep. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme. - Rumohr, H. 1999. Soft-bottom macrofauna: Collection, treatment, and quality assurance of samples. ICES Tech. in Mar. Env. Sci. no 27. - Rees, H.L., Heip, C., Vincx, M. and Parker, M.M. 1991. Benthic communities: Use in monitoring point source discharges. ICES Techniques in Marine Environmental Sciences, no. 16.
Marine	Fish and shellfish	All	Sampling, storage, pre-treatment, reporting, QA	- ICES Guidelines for Monitoring contaminants in fish and shellfish. Cooperative ICES monitoring Studies Programme (CMP) for Monitoring Contaminants in Fish and Shellfish.
Marine	Fish and shellfish	Heavy metals	Sampling, storage, analyses, QA.	- JAMP Guidelines for Monitoring Contaminants in Biota 1997. Technical annex 2: Metals. Oslo and Paris Commissions 1997, Joint Assessment and Monitoring Programme.

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Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Marine	Fish and shellfish	Persistent organic Pollutants	Sampling, storage, analyses, QA.	- JAMP Guidelines for Monitoring Contaminants in Biota 1997. Technical annex 1: Organic contaminants. Oslo and Paris Commissions 1997, Joint Assessment and Monitoring Programme. - For sampling of mussels, Arctic cod and sculpin see Appendix 2 to Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6.
Marine	Fish and shellfish	Hydrocarbons	Sampling, analyses, QA.	- JAMP Guidelines for Monitoring Contaminants in Biota 1997. Technical annex 3: PAHs in biological materials. Oslo and Paris Commissions 1997, Joint Assessment and Monitoring Programme. - Erhardt, M., Klungsøyr, J. and Law, R.J. 1991. Hydrocarbons: review of methods for analyses in seawater, biota and sediments. ICES Techniques in Marine Environmental Science no. 12.
Marine	Fish and shellfish	Radionuclides	Sampling, analyses, QA	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. II, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.
Marine	Seabirds	All	Monitoring application and strategy	- Seabirds as monitors of marine contamination. Annex 7 in: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.
Marine	Seabirds (including eggs)	Metals	Sampling, storage, analyses, QA	- JAMP Guidelines for Monitoring Contaminants in Biota 1997. Technical annex 2: Metals. Oslo and Paris Commissions 1997, Joint Assessment and Monitoring Programme - For sampling of Black Guillemot and Glaucaus gull see Appendix 2 to Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6. - For preparation of whole birds, eggs, measurement of egg shell thickness measurement see Canadian Wildife Service SOPs: - R. McNeil, Processing Whole Eggs. Canadian Wildlife Service, SOP-TP-PROC-04C, 1998. Lab. Services Sect., NWRC, Environ. Canada, Hull Que. - R. McNeil, Whole Body Processing Of Birds, Canadian Wildlife Service, SOP-TP-PROC-06B, 1997 . Lab. Services Sect., NWRC, Environ. Canada, Hull Que. - R. McNeil, Determination Of Eggshell Thickness, Canadian Wildlife Service, SOP-TP-PROC-05B, 1997 . Lab. Services Sect., NWRC, Environ. Canada, Hull Que.

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Marine	Seabirds (including eggs)	Persistent organic pollutants	Sampling, storage, analyses, QA	- JAMP Guidelines for Monitoring Contaminants in Biota 1997. Technical annex 1: Organic contaminants. Oslo and Paris Commissions 1997, Joint Assessment and Monitoring Programme - Henriksen, E., A. Derocher, J.O. Bustnes, G.W. Gabrielsen, Ø. Wiik & J.U. Skaare 2000. Monitoring persistent pollutants in Arctic top predators: lessons learned from recent data on polar bear and glaucous gull. Report to the Norwegian Transport and Effects Programme. Norwegain Polar Institute. - For sampling of Black Guillemot and Glaucaus gull see Appendix 2 to Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6. - For preparation of whole birds, eggs, measurement of egg shell thickness measurement see Canadian Wildife Service SOPs: - R. McNeil, Processing Whole Eggs. Canadian Wildlife Service, SOP-TP-PROC-04C, 1998. Laboratory Services Section, NWRC, Environment Canada, Hull Que. - R. McNeil, Whole Body Processing Of Birds, Canadian Wildlife Service, SOP-TP-PROC-06B, 1997 . Laboratory Services Section, NWRC, Environment Canada, Hull Que. - R. McNeil, Determination Of Eggshell Thickness, Canadian Wildlife Service, SOP-TP-PROC-05B, 1997 . Laboratory Services Section, NWRC, Environment Canada, Hull Que.
Marine	Seabirds	Hydrocarbons	Sampling, storage, analyses, QA	- JAMP Guidelines for Monitoring Contaminants in Biota 1997. Technical annex 3: PAHs in biological materials. Oslo and Paris Commissions 1997, Joint Assessment and Monitoring Programme.
Marine	Seabirds	Radionuclides	Analyses	- Anon, 1985. Radionuclide identification and assay by energy spectrometry. In. A Handbook of Radioactivity Measurements Procedures. NCRP Report 58. National Council on Radiation Protection and Measurements, Bethesda, Maryland. 592pp.
Marine	Seabirds	Radionuclides	Sampling, analyses, QA	- HASL300 (1997): The procedures manual of the environmental measurements laboratory. Vol. II, 28th edition. Erickson, M.D. (ed.), US. Department of Energy, New York.
Marine	Seals	Heavy metals, POPs	Sampling, storage, analyses	- For general recommendations on sampling seals for POPs and metals see Appendix 2 to Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6.

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
				- Shaughnessy, P.D. 1995. Collection of material for the determination of organochlorine and heavy metal levels. Chapter 10 in: Laws, R.M. (ed.) Antarctic seals. Research methods and Techniques. Cambridge University Press.
Marine	Seals	POPs	Sampling, analyses	- Wolkers, J., Burkow, I.C., Monshouwer, M., Lydersen, C., Dahle, S. and Witkamp, R.F. 1999. Cytochrome P450-mediated enzyme activities and polychlorinated biphenyl accumulation in harp seal (*Phoca groenlandica*). *Mar Environ Res* 48:59-72 - Wolkers, J., Burkow, I.C., Lydersen, C. and Witkamp, R.F. in press. Chlorinated pesticide concentrations with emphasis on the polychlorinated camphenes (toxaphenes), in relation to cytochrome P450 enzyme activities in harp seals from the Barents Sea. Environ. Toxicol. Chem.
Marine	Mammals: Arctic fox	POPs	Sampling, analyses	- For general recommendations on sampling Arctic fox for POPs and metals see Appendix 2 to Report of the Experts Meeting on Sampling and Analysis of Persistent Toxic Substances (PTS). AMAP Report 2000:6.
Marine	Marine mammals: polar bears	POPs		- Henriksen, E., A. Derocher, J.O. Bustnes, G.W. Gabrielsen, Ø. Wiik & J.U. Skaare 2000. Monitoring persistent pollutants in Arctic top predators: lessons learned from recent data on polar bear and glaucous gull. Report to the Norwegian Transport and Effects Programme. Norwegian Polar Institute.
Marine	Mammals: Polar bear	POPs	Sampling, Analyses	- Muir, D.C.G., Norstrom, R.J. & M. Simon, 1988. Organochlorine contaminants in Arctic marine food chains: accumulation of specific PCB congeners and chlorane-related compounds. *Envrion. Sci. Technol., 22:1071-1079.*
Marine	Mammals: Polar bear	POPs	Sampling, Analyses	- Norstrom, R.J. & M. Simon, 1991. Determination of specific polychlorinated dibenzo-p-dioxins and dibenzofurans in biological matrices by gel-permeation/carbon chromatography and gas chromatography-mass spectrometry. In: Envrionmental Carcingogens, Methods of Analysis and Exposure Measurement, vol. 11. Polychlorinated Dibenzo-p-dioxins and Dibenzofurans, Rappe, C., Buser, H.R., Dodet, B. & I.K. O'Neill, eds., World Health Organization, International Association for Research on Cancer, Lyon, France, Method 5, pp. 281-297.
Marine	Mammals	POPs	Sampling, Analyses	- Ford, C.A., Muir, D.C.G., Norstrom, R.J., Simon, M. & M.J. Mulvihill, 1993. Development of a semi-automated method for non-ortho PCBs: application to Canadian Arctic marine mammal tissues. Chemosphere, 26:1981-1991

Biological effects monitoring - Guidelines and methodological references

(For an overview of biological effects techniques applicable to the marine environment, see also Annex 1).

Environmental compartment	Media / organisms, etc	Parameters/methods	Methodology	Reference / source
Terrestrial	Forests, vegetation	Acidification	Sampling, analyses, statistics	- **ICP Forests Manual. International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests of the UN/ECE (Visual assessment of crown condition, submanual on visual assessment of crown condition on intensive monitoring plots, sampling and analysis of soil and submanual on soil solution collection and analysis, samping and analysis of needles and leaves, estimation of growth and yield, measurement of deposition, meteorological monitoring on intensive monitoring plots, assessment of ground vegetation, assessment of phenology) (http://www.dainet.de/bfh/inst1/12/manual.htm)**
Terrestrial	Vegetation	Detecting change in ground vegetation	Sampling, statistics	- **Lawesson, J.E. (ed.) 2000. A concept for vegetation studies and monitoring in the Nordic Countries. TemaNord 2000: 517.**
Terrestrial	Various	Various	Sampling, analyses	- **Olsen, K.V. (ed.) 1995. Biological methods for use in monitoring the Arctic. Proceedings of an AMAP /CAFF workshop. Svanvik, Norway, 7-10 March 1995. TemaNord 1995:604**
Terrestrial	Birds	Biological effects - Egg shell thickness	Sampling, analyses	- For preparation of whole birds, eggs, measurement of egg shell thickness measurement see Canadian Wildife Service SOPs: - R. McNeil, Determination Of Eggshell Thickness, Canadian Wildlife Service, SOP-TP-PROC-05B, 1997 . Laboratory Services Section, NWRC, Environment Canada, Hull Que.
Marine	Several	- Whole sediment bioassays - Sediment pore-water bioassays - Sediment seawater elutriates - Water bioassays - CYP1A* - Lysosomal stability - Liver neaplasia/hyperplasia - Liver nodules - Externally visible fish diseases	Sampling, test procedures, QA	- **JAMP Guidelines for general biological effects monitoring 1997. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme.**

Environmental compartment	Media / organisms, etc	Parameters/methods	Methodology	Reference / source
		- Reproductive success in fish		
Marine	Several	Metals (metallothionein, δ-amino levulinic acid dehydrase in hibition in blood (ALA-D), oxidative stress) PAHs (cytochrome P4501A activity, DNA adducts, PAH metabolites in bile, liver (histo)pathology) TBT (imposex, intersex)	Sampling, test procedures, QA	- **JAMP Guidelines for contaminant specific biological effects monitoring 1997. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme.**
Marine	Sediment, pore water	Toxicity of the sedimentary environment; abnormal development		- Thain, J.E. 1991. Biological effects of contaminants: oyster embryo assay. ICES Techniques in Marine Environmental Sciences 11.
Marine	Benthos	Benthic communities	Sampling, analysis, storage, statistics, QA	- Rumohr, H. 1999. Soft-bottom macrofauna: Collection, treatment, and quality assurance of samples. ICES Techniques in Marine Environmental Science no 27. - Rees, H.L., Heip, C., Vincx, M. and Parker, M.M. 1991. Benthic communities: Use in monitoring point source discharges. ICES Techniques in Marine Environmental Sciences, no. 16.
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Marine	Fish	Environmental carcinogens, degenerative changes		- ICES 1997. Report of the special meeting on the use of liver pathology of flatfish for monitoring biological effects of contaminants. ICES CM 1997/f:2.
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Marine	Sediment	Sediment toxicity, marine		- ASTM 1990. Standard guide for conducting solid phase 10 day static

Environmental compartment	Media / organisms, etc	Parameters/methods	Methodology	Reference / source
		invertebrates		sediment toxicity tests with marine and estuarine infaunal amphipods. American Society for Testing and Materials. ASTM E 1367-90, pp 1-24. - McGee, R.L., Schlekat C.E. and Reinhartz, E. 1993. Assessing sub-lethal levels of sediment contamination using the estuarine amphipod *Leptocheirus plumulosus*. Environ.Toxicol. Chem. 12: 577-587. - OSPAR 1995. Protocols on methods for the testing of chemicals used in the offshore industry.
Marine	Dogwhelke/ periwinkle	Biological effects - TBT - Imposex	Sampling, analyses/measure-ments, QA	- Annex 2 to this document - JAMP Guidelines for contaminant-specific biological effects monitoring 1998. Technical annex 3. TBT-specific biological effects monitoring. Oslo and Paris Commissions, London, pp. 14-25. - Gibbs, P:E. 1999. Biological effects of contaminants: Use of imposex in the dogwhelke (*Nucella lapillus*) as a bioindicator of tributyltin (TBT) pollution. ICES Techniques in Marine Environmental Science no. 24. - Mensink, B.P., B. van Hattum, C.C Ten Hallers-Tjabbes, J.M. Everaarts, H. Kralt, A.D. Vethaak and J.P. Boon 1997. Tributyltin causes imposex in the common whelk *Buccinum undatum* - mechanism and occurrence. - Netherlands Instituut voor Onderzoek de Zee, Rapport 1997-6.
Marine	Fish	Biological effects – contaminant exposure (planar molecules) - EROD (CYP1A)	Sampling, analyses, reporting, QA	- Stagg, R. and McIntosh, A. 1998. Biological effects of contaminants: Determination of CYP1A-dependent mono-oxygenase activity in dab by fluorometric measurements of EROD activity. ICES Techniques in Marine Environmental Sciences no. 23. - Galgani, F. and Payne, J.F. 1991. Biological effects of contaminants: Microplate method for measurement of ethoxyresorufin o-deethylase (EROD) in fish. ICES Techniques in Marine Environmental Sciences no. 13.
Marine	Fish	Biological effects – PAH exposure	PAH metabolite determination	- Ariese, F., Kok, S.J., Verkaik, M., Gooijer, C., Velthorst, N,H. and Hofstraat, J.W. 1993. Synchronous fluorescene spectrometry of fish bile: a rapid screening method for the biomonitoring of PAH exposure. *Aquat. Toxicol*. 26: 273-286. - Beyer, J., Sandvik, M., Hylland, K., Fjeld, E., Egaas, E., Aas, E., Skare, J.U. and Goksøyr, A. 1996. Contaminant accumulation and biomarker responses in flounder and atlantic cod exposed by caging to polluted sediments in Sorfjorden, Norway. *Aquat. Toxicol*. 36: 75-98. - Krahn, M.M., M.S. Myers, D.G. Burrows and D.C. Malins. 1984.

Environmental compartment	Media / organisms, etc	Parameters/methods	Methodology	Reference / source
				Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. *Xenobiotica* 14:633-646 - Krahn, M.M., L.D. Rhodes, M.S. Myers, L.K. Moore, W.D. MacLeod, Jr. and D.C. Malins. 1986. Associations between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. *Arch. Environ. Contam. Toxicol.* 15:61-67. - Krahn, M.M., D.G. Burrows, W.D. MacLeod, Jr. and D.C. Malins. 1987. Determination of individual metabolites of aromatic compounds in hydrolyzed bile of English sole (*Parophrys vetulus*) from polluted sites in Puget Sound, Washington. *Arch. Environ. Contam. Toxicol.* 16:511-522.
Marine	Fish	Biological effects - Metals	Analyses metallothionein	- Hylland, K. 1999. Biological effects of contaminants: Quantification of metallothionein (MT) in fish liver tissue. ICES Techniques in Marine Environmental Science no. 26 .
Marine	Fish	Biological effects - PAH exposure and carcinogenic properties	Analyses - DNA adducts	- Reichert, W.L., French, B.L. and Stein, J.E. 1999. Biological effects of contaminants: Measurement of DNA adducts in fish by ^{32}P post-labelling. ICES Techniques in Marine Environmental Science no. 25. - Stein, J.E., Reichert, W.L. and Varanasi, U. 1994. Molecular epizootiology: assessment of exposure to genotoxic compounds in teleosts. *Environ. Health Perspect* 102: 19-23
Marine	Seabirds	All	Monitoring application and strategy	- Seabirds as monitors of marine contamination. Annex 7 in: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.
Marine	Seabirds	Biological effects - Metals	Analyses metallothionein	- Eliott, J.E., Scheuhammer, A.M., Leighton, F.A. & P.A. Pearce, 1992. Heavy metal and metallothionein concentrations in Atlantic Canadian seabirds. Arch. Environ. Contam. Toxicol., 22:63-73.
Marine	Seabirds	Biological effects - MFO	Analyses MFO	- Peaklall, D.B., Norstrom, R.J., Rahimtula, A.D. & R.D. Butler, 1986. Characterization of mixed-function oxidase systems of the nestling Herring Gull and its implications for bioeffects monitoring. Environ. Toxicol. Chem., 5(4):379.385.
Marine	Seabirds	Biological effects - Oil spills	Sampling, analyses, statistics	- JAMP Guidelines on standard methodology for the use of oiled birds as indicators of marine oil pollution. Oslo and Paris Commissions. Joint Assessment and Monitoring Programme.
Marine	Seabirds	Biological effects - Egg shell thickness	Sampling, analyses	- For preparation of whole birds, eggs, measurement of egg shell thickness

Environmental compartment	Media / organisms, etc	Parameters/methods	Methodology	Reference / source
				measurement see Canadian Wildife Service SOPs: - R. McNeil, Determination Of Eggshell Thickness, Canadian Wildlife Service, SOP-TP-PROC-05B, 1997 . Laboratory Services Section, NWRC, Environment Canada, Hull Que.
Marine	Mammals	Biological effects - P450	Sampling, analyses cytochrome P450	- Wolkers, J., Burkow, I.C., Lydersen, C., Dahle, S., Monshouwer, M. and Witkamp, R.F. 1998. Congener specific PCB and polychlorinated camphene (toxaphene) levels in Svalbard ringed seals (*Phoca hispida*) in relation to sex, age, condition, and cytochrome P450 enzyme activity. *Sci Total Environ* 216:1-11.
Marine	Mammals	Biological effects – contaminant exposure (3-D molecules) -	Sampling, analyses - testosterone hydroxxylase	- Wolkers, J., Burkow, I.C., Lydersen, C., Dahle, S., Monshouwer, M. and Witkamp, R.F. 1998. Congener specific PCB and polychlorinated camphene (toxaphene) levels in Svalbard ringed seals (*Phoca hispida*) in relation to sex, age, condition, and cytochrome P450 enzyme activity. *Sci Total Environ* 216:1-11. - Wolkers, J., Burkow, I.C., Monshouwer, M., Lydersen, C., Dahle, S. and Witkamp, R.F. 1999. Cytochrome P450-mediated enzyme activities and polychlorinated biphenyl accumulation in harp seal (*Phoca groenlandica*). *Mar Environ Res* 48:59-72 - Wolkers, J., Burkow, I.C., Lydersen, C. and Witkamp, R.F. in press. Chlorinated pesticide concentrations with emphasis on the polychlorinated camphenes (toxaphenes), in relation to cytochrome P450 enzyme activities in harp seals from the Barents Sea. *Environ. Toxicol. Chem*.
Marine	Marine mammals Polar bears	Biological effects - POPs	Sampling, analyses	- Bernhoft, A., Utne Skaare, J., Wiig, Ø., Derocher, A. and Larsen, H.J. 2000. Possible immunotoxic effects of organochlorines in polar bears (*Ursus maritimus*) at Svalbard. Journal of Toxicological and Environmental Health, Part A, 59: 561-574.
Marine	Marine mammals Polar bears	Biological effects - POPs (P450/ EROD/PROD)	Sampling, Analyses	- Letcher, R.J., Norstrom, R.J., Bandiera, S.M. & M.A. Ramsay, 1994. Polar bear hepatic cytochrome P450 1A1 and 1A2: Immunoquanitation, EROD/PROD activity and PCB levels. Dioxin '94, Dept. of Environ. Sanit. Eng., Kyoto University, Japan. Organohalogen Compounds, 20:553-558.
Marine	Marine mammals: Polar bear	Biological effects (P450)	Sampling, Analyses	- Bandiera, S.M., Torok, Ramsay, M.A. & R.J. Norstrom, 1994. Catalytic and immunlogical characterization of hepatic and lung cytochromes P450 in the polar bear. Biochem. Pharmacol. (In press).

Guidelines and references on general monitoring issues (monitoring strategies, sample pooling, temporal trend analysis, normalization, etc.)

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
General / Marine	General / Biota	All	Monitoring strategies	- Statistical methods for designing and assessing monitoring programmes. In: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen. - Nicholson, M.D., Fryer, R.J., and Ross, C.A. 1997. Designing monitoring programmes for detecting temporal trends in contaminants in fish and shellfish. Marine Pollution Bulletin 34: 821-826.
Marine	General	All	Monitoring strategies and guidelines	- ICES Advice on Monitoring, Assessment, Temporal trend monitoring, Biological effects monitoring, etc. see Annex 9 In: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.
General	General	General	Temporal trends. Statistical Analysis	- Detailed methods for temporal trend detection. Section 6 and Annex 1 in: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen. - Development of trend detection methods for input data. Section 6.6 in: Report of the ICES Advisory Committee on the Marine Environment, 2000. ICES Coop. Tres. Rep. 241. ICES, Copenhagen.
GenerMarine	Biota / Sediments	General	Monitoring strategies	- Determination of the number of replicate samples of sediments or biota to characterize an area. In: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.
Marine	Biota	All	Pooling Analyses	- Nicholson, M.D. and Fryer, R.J. 1996. Contaminants in marine organisms: Pooling strategies for monitoring mean concentrations. ICES Techniques in Marine Environmental Sciences no. 18.
Marine	Biota	All	Temporal trends	- Uthe, J.F., Chou, C.L., Misra, R.K., Yeates, P.A., Loring, D.H., Musial, C.J. and Cofino, W. 1991. Temporal trend monitoring: Introduction to the study of contaminant levels in marine biota. ICES Techniques in Marine Environmental Sciences, No. 14.
Marine	Fish and shellfish	All	Temporal trends	- Nicholson, M.D., Fryer, R.L. and Larsen, J.R. 1998. Temporal trend monitoring: Robust method for analysing contaminant trend monitoring data. ICES Techniques in Marine Environmental Science no. 20.

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Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
Marine	Fish	All	Temporal trends	- Uthe, J.F., Misra, R.K., Chou, C.L., Scott, D.P. and Musial, C.J. 1991. Temporal trend monitoring: Contaminant levels in tissues of Atlantic Cod. ICES Techniques in Marine Environmental Sciences, No. 15.
Marine	Sediments	Heavy metals	Normalization	- Loring 1991. Normalization of heavy-metal data from the estuarine and coastal sediments. ICES J. Mar. Sci. 48: 101-115. - Techniques for Sediment Monitoring: Normalization. In: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.
Marine	Several	Various	Biological effects monitoring strategies	- Report of the Workshop on Combined Effects in the Marine Environment, Copenhagen, 16 - 17 November 1998. AMAP Report 99:1.
Marine	Several	Various	Biological effects monitoring strategies	- Analytical and environmental sources of variability in biomarkers in fish and implications for monitoring guidelines. In: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.
Marine	Several	Various	Biological effects - data analysis	- Visualisation tools for integrating and interpreting biological effects. In: Report of the ICES Advisory Committee on the Marine Environment, 1999. ICES Coop. Tres. Rep. 239. ICES, Copenhagen.

Guidelines and references on tissue and specimen banking

Environmental compartment	Media / organisms, etc	Parameters	Methodology	Reference / source
All	All	All	Methods, registration of holdings	- **Nordic Environmental Specimen Banking - methods in use in ESB. Manual for the Nordic Countries. TemaNord 1995:543** - See also ISBER (International Society for Biological and Environmental Repositories) http://www.isber.org/

Guidelines and references on quality assurance

*For references to QA/QC guidelines, see Section E of the **AMAP Trends and Effects Programme** documentation*

Part B. References

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Part C. Annexes

Annex 1. Overview of biological effects techniques applicable to the marine environment, generally not including sea mammals and birds (birds (from Report of the ICES Advisory Committee on the Marine Environment, 2000).

- ***Recommended techniques for biological monitoring programmes at the national or international level.***

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).

[1]May also be applicable to mammals and birds.

[2]Quality control under BEQUALM.

[3]Quality control under QUASIMEME.

[‡]B=Belgium; CAN=Canada; D=Germany; DK=Denmark; F=France; MEDPOL=Monitoring and Research Programme of the Mediterranean Action Plan; N=Norway; NL=Netherlands; S=Sweden; UK=United Kingdom; USA=United States.

Method	**Organism**	**Refs.**	**Currently used in monitoring programmes[‡]**	**Quality control**	**Issues addressed**	**Biological significance**
Bulky DNA adduct formation	Fish[1] Bivalve molluscs	1–6, 157–159	F, NL, S, USA	B[2]	PAHs Other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)	Measures genotoxic effects. Possible predictor of pathology through mechanistic links. Sensitive indicator of past and present exposure.
Acetylcholinesterase (AChE) inhibition*	Fish[1] Crustacea Bivalve molluscs	12–16, 114, 116, 118	F		Organophosphates and carbamates or similar molecules Possibly algal toxins	Measures exposure.
Metallothionein induction	Fish[1]	17–22	MEDPOL, N	B[2]	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.
Ethoxyresorufin-*O*-deethylase (EROD) or cytochrome P4501A induction*	Fish[1]	46–51, 99, 115	D, F, NL, UK, B, MEDPOL, N	B[2]	Measures induction of enzymes which detoxify planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)	Possible predictor of pathology through mechanistic links. Sensitive indicator of present exposure.
δ-aminolevulinic acid (ALA-D) inhibition	Fish[1]	74–75	N	B[2]	Lead	Index of exposure.
Oxidative stress indicators	Fish[1]	76–78			Not contaminant specific, will respond to a wide range of environmental contaminants	Measures the presence of free radicals.
Fluorescent bile metabolites	Fish	79–80	N, NL, UK		PAHs	Measures exposure to and metabolism of PAHs.

Method	Organism	Refs.	Currently used in monitoring programmes[‡]	Quality control	Issues addressed	Biological significance
Lysosomal stability	Fish[1] *Mytilus* spp.	23–25	D, MEDPOL	B[2]	Not contaminant specific, but will respond to a wide variety of xenobiotic contaminants and metals	Measures cellular damage and is a good predictor of pathology. Provides a link between exposure and pathological endpoints. Possibly a tool for immunosuppression studies in white blood cells.
Early toxicopathic lesions, pre-neoplastic and neoplastic liver histopathology	Fish[1]	7–11, 108, 110, 119–130, 164–167	D, NL, UK, USA		PAHs Other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)	Measures pathological changes associated with exposure to genotoxic and non-genotoxic carcinogens.
Whole sediment bioassays*	*Corophium* *Echinocardium* *Arenicola* *Leptocheirus* *Grandidierella* *Rhepoxynius* *Ampelisca*	31–35	NL, UK, USA, CAN	B[2]	Not contaminant specific, will respond to a wide range of environmental contaminants in sediments	Acute/lethal and acute/sublethal toxicity only at present. May enable retrospective interpretation of community changes.
Sediment pore water bioassays*	Any water column organism including: *Dinophilus* sea urchin fertilization, etc. bivalve embryo Microtox	36–41	F, NL, USA		Will respond to a wide range of environmental contaminants	Acute and chronic toxicity, including genotoxicity, etc. Toxicity of hydrophobic contaminants might be underestimated in pore water assays.
Sediment sea water elutriates*	Any water column organism, as above	36–41	NL, UK		Will respond to a wide range of environmental contaminants in dredge spoils sediments liable to resuspension	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Water bioassays*	As for pore water and elutriates (see above)	36–41	NL, UK, USA, CAN	B[2]	Not contaminant specific, will respond to a wide range of environmental contaminants in inshore and estuarine waters	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Scope for growth*	Bivalve molluscs, e.g., *Mytilus*	55–58			Responds to a wide variety of contaminants	Integrative response which is a sensitive and sublethal measure of energy available for growth.
Shell thickening	*Crassostrea gigas*	103	Portugal		Specific to organotins	Disruption to pattern of shell growth.
Vitellogenin induction	Male and juvenile fish	26–30	N, UK		Oestrogenic substances	Measures feminization of male fish and reproductive impairment.
Imposex	Neogastropod molluscs, e.g., dogwhelk (*Nucella lapillus*)	52–54	CAN, D, Ireland, Iceland, N, NL, S, UK	B[2] Q[3]	Specific to organotins	Reproductive interference. Estuarine and coastal littoral waters (*Nucella*) and offshore waters (*Buccinum*).
Intersex	Littorinids	101, 102	D, Ireland, N, UK	B[2]	Specific to reproductive effects of organotins	Reproductive interference in coastal (littoral) waters.
Protein or enzyme altered foci	Fish	92, 144–150			PAHs Other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)	Indicates exposure to carcinogen(s).

Method	Organism	Refs.	Currently used in monitoring programmes‡	Quality control	Issues addressed	Biological significance
Reproductive success in fish	*Zoarces viviparus* *Pseudopleuronectes americanus* *Gadus morhua*	72, 153, 160	D, S, USA, N	B[2]	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures reproductive output and survival of eggs and fry in relation to contaminants. In viviparous fish, restricted to period when young are carried by female.
Externally visible fish disease	Fish	104–108, 168–171	CAN, D, DK, UK, USA, NL, B		Measures the effects of non-specific stress by quantifying the presence of externally visible diseases, especially in dab (*Limanda limanda*)	These diseases are natural, but may be exacerbated by various stressors, including contaminants.
Benthic community analysis*	Macro-, meio-, and epibenthos	42–45, 100, 109	B, CAN, D, F, Ireland, N, UK, USA, NL	B[2]	Responds to a wide variety of contaminants, particularly those resulting in organic enrichment	Ecosystem level. Retrospective. Particularly useful for point sources. Most appropriate for deployment when other monitoring methods indicate a problem may exist.

- ***Promising biological effects monitoring methods that require further research before they can be recommended for monitoring.***

Method	Organism	Refs.	Issues addressed	Biological significance
DNA strand breaks	Fish and mussels	113	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures genotoxic effects, but is also extremely sensitive to other environmental parameters.
Oncogenes	Fish	93–95	PAHs Other synthetic organics, e.g., nitro- organics, amino triazine pesticides (triazines)	Activation of oncogenes (*ras*) or damage to tumour suppressor genes (p53). Measures genotoxic effects leading to carcinogenesis.
Cytochrome P4501A induction	Invertebrates	96	Induced enzyme response to PAHs, planar PCBs, dioxins and/or furans	Measures exposure to organic contaminants.
Glutathion-*S*-transferase(s) (GST)	Fish, mussels	97, 154	Predominantly organic xenobiotics	Measures exposure and the capacity of the major group of Phase II enzymes.
Multidrug/multixenobiotic resistance (MDR/MXR)	Fish, invertebrates	85–92, 131–143	Organic xenobiotics	Measure of exposure.
Various methods of measuring immunocompetence	Fish, invertebrates	73	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures factors which influence susceptibility to disease.
On-line monitoring	Mussels, crabs	98	Responds to metals and xenobiotics	Measures the effects of chemicals on heart rate using a simple and inexpensive remote biosensor. Gives an integrated response.
Degenerative gill and kidney histopathology	Fish (especially flatfish such as dab (*Limanda limanda*))	59–66	General toxicological response which will respond to a wide variety of contaminants	Measures degenerative change in tissues.
Abnormalities in wild fish embryos and larvae	Many fish, including demersal and pelagic species	70–71, 172	Not yet linked unequivocally to contaminants	Measures frequency of probably lethal abnormalities in fish larvae. Mutagenic, teratogenic.

Method	Organism	Refs.	Issues addressed	Biological significance
Chronic whole sediment bioassays	Invertebrates	32	Responds to a wide range of contaminants	Measurements such as growth and reproduction, coupled to biomarker responses, which will give a measure of the bioavailability and chronic toxicity in whole sediments.
Pollution-induced community tolerance (PICT) water bioassay	Microalgae	67–69	Specific contaminants can be tested	Measure of degree of adaptation to specific pollutants. Not yet widely tested.
COMET assay (*in vitro* bioassay for sediments)	Cells exposed to extracts	111, 155, 156	Genotoxic compounds	Genotoxic potential of sediments.
Apoptosis	Fish	112	Responds to a wide range of contaminants	Research state.
Enzyme-linked immuno-sorbent assay (ELISA) for DNA adducts	Fish	161–163	Not contaminant specific	Genotoxic effects.
Dioxin-responsive chemical-activated luciferase gene assay (DR-CALUX)	Cells exposed to samples or extracts	151	Aryl hydrocarbon (Ah) receptor active compounds	Possible predictor of pathology.
Oestrogen-responsive chemical-activated luciferase gene assay (ER-CALUX)	Cells exposed to samples or extracts	152	Oestrogen receptor active compounds	Potential endocrine disruption.
Allometric response in the benthic community	Macro-, meio-, and epibenthos	81–84	Not contaminant specific, will respond to a wide range of environmental contaminants	Ecosystem level. Retrospective.

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Annex 2. Monitoring of imposex

Biological effects of TBT in snails (Imposex) should be monitored in the AMAP area within the following gastropod species: Dogwhelk (*Nucella lapillus*), Whelk (*Nucella lima*) and Common whelk (*Buccinum undatum*), in the areas listed below.

Dogwhelk (*Nucella lapillus*) - Despite a diverse mollusc fauna within the AMAP area, large gastropod molluscs are not particularly common intertidally in the High Arctic. This is presumably due in part to ice-scouring. Further, some of the species best known in terms of the effects of TBT do not occur in the Arctic or have their northern limits only extending partly into the AMAP study area.

The dogwhelk, in addition to the periwinkle (*Littorina littorea*) and the common whelk, has been used as an indicator of TBT pollution in the OSPAR area. The dogwhelk is well suited for identifying gradients in exposure and the resulting biological effects. Oil terminals and large harbours (commercial, naval) should be investigated as special cases.

Whelk (*Nucella lima*) - Imposex has been studied within the gastropods *Nucella emarginata*, *Nucella canaliculata*, *Nucella lima* and *Nucella lamellosa* in the East Pacific (Short et al. 1989, Stickle et al. 1990, Tester and Ellis 1995). Of these *Nucella lima* extends presumably furthest to the north (see Austin 1985). That species occurs higher in the intertidal zone than does for instance *Nucella lamellosa*.

Common whelk (*Buccinum undatum*) **-** The common whelk has been used as an indicator of TBT pollution in the OSPAR area and may be useful as an indicator of TBT contamination in offshore areas and along shipping lanes.

It is proposed that the AMAP region should be surveyed as soon as possible, using methods indicated below (see also Annex 1 to Section G). The biological monitoring should be coordinated with chemical monitoring for TBT (see Section B). In light of the results of this survey areas showing high contamination of TBT should be examined in closer detail. Those areas, in addition to moderately affected areas, should be revisited after 5 years. Due to the close relationship of organotins such as TBT with POPs, heavy metals, and petroleum hydrocarbons, active co-ordination and harmonization is required to maximize program effectiveness and efficiency.

Sampling locations

Samples should be collected in coastal areas on both sides of the facility (e.g. harbours, oil terminals) in order to describe the gradients of contamination. At least six sample sites should be located on each side of the facility, covering the gradients between the highest level of effect to the local background level of effect. The sample sites should be clearly identified in order to enable repeat sampling, for example to investigate temporal trends in contamination and effects. *Nucella lapillus* should be collected from below midtide level, while *Nucella lima* should be sampled in the upper intertidal.

In light of a sparsely populated regions within the AMAP area, monitoring should focus on areas near "hot spots". The sampling undertaken around harbours/oil terminals should be such that interpolated values for the Vas Deferens Sequence Index (VDSI) are within 0.5 units of the true value with 90% confidence in the range VDSI 2-6.

Areas (holding a number of stations) should be compared using standard statistical techniques (*e.g.* t-tests or other appropriate methods). The expressions of degree of effect at each location in a area (possibly defined as a box) should be combined to give indices of centrality and spread of their distribution. The values derived for each box should be compared to give an expression of the relative degree of contamination of different coastal areas. The measures of effects should be used to express the general degree of inhibition of reproductive capacity of the monitored species in each area, and hence the potential for these populations to act as sources of young for recolonisation of heavily affected areas.

Methods

The biological effects measurements provide direct indications of the effects of TBT at the individual organism level. From these data, it is possible to make inferences regarding effects at the population level. The most important measurement is that the one of VSDI. These are a direct indication of the average reproductive capacity of the females in the population. These should be supported by calculations of the proportions of females in the population which have been rendered sterile by exposure to TBT (*i.e.* proportion showing stage 5 or 6 imposex). The Relative Penis Size Index provides additional discrimination in *Nucella* populations at VDSI stage 4. The concentrations of TBT in soft tissue allow discrimination between effects arising from current and historical contamination.

Details of the methodology for the biological effects measurements are given in following section and should be carried out according to the methods described in Gibbs *et al.* (1991) and Mensink *et al.* (1997).

A variety of supporting data are required to assist in the interpretation of the effects data. At all stations, the presence or absence of juvenile *Nucella* and *Nucella* egg capsules should be noted. At key stations, the size frequency distribution of the population should be determined. The concentration of TBT should be measured in pooled homogenised whole soft parts of female specimens. These analyses will provide confirmation that the measured responses can be related to TBT residues in the animals. It has been found that while imposex is essentially irreversible, TBT residues are lost from the soft parts with half-times of around 60-80 days. The comparison of effects measurements with TBT concentrations in tissues can therefore provide indications of whether the effects represent a response to recent exposure or are a record of more historical exposure. Data should be collected on the use of the facilities being monitored. In the case of harbours, marinas, *etc*. this information will have been collected during the site selection procedure and this information should be updated as the monitoring programme progresses.

Temporal trend monitoring

Temporal trend studies are valuable in assessing the response of the environment to control measures, to the effects of changes in the pattern of TBT use and to possible changes in the pattern of use of the marine environment. Temporal trend studies should build upon, and use similar methodology to, the spatial studies described earlier. The primary measurements should be of imposex in *Nucella*. As imposex, in general, is not reversible, and therefore the replacement of the population on a five year scale places a clear lower limit to the duration of any temporal trend programme.

The sampling and analytical guidelines discussed above in relation to point sources should be followed for temporal trends. It is suggested that the sites should be revisited after five years. The location of sampling sites should follow the locations established in relation to point sources. As subsequent sampling exercises should use the same locations and animals at the same stage of their reproductive cycle, the initial selection of sample sites should be undertaken with care to ensure that the snail population will not be unduly affected by sampling pressure.

Field sampling

Nucella species should be sampled during low tide, by hand, *Nucella lapillus* from the substrate between spring low water and mid-tide levels, while *Nucella lima* can be collected in the higher intertidal. A sample sizes of 40 specimens per station is appropriate. Especially in contaminated areas the abundance of dogwhelks is likely to decrease because of female sterilisation. In these areas it is often not possible to achieve the recommended number of 40 *Nucella lapillus*. An attempt should be made wherever possible to exclude the largest specimens *Nucella lapillus* with eroded shells which are often covered by barnacles and/or sedentary polychaetes (e.g. *Spirorbis* sp.) since older specimens may exhibit an imposex or intersex intensity which reflects a contamination level.

Penis lengths in some of the target gastropods are known to vary seasonally. Samples should be collected within 2-3 months at the end of the reproduction season to reduce this source of variability.

Wherever possible, samples of 100 mature (usually >5 - 6 cm) *Buccinum undatum* should be obtained by suitable methods. It is recommended that where possible samples should be collected using static

gear (traps, pots, *etc*.). In areas where this is not practical (*e.g.* within the shipping lanes) mobile gear (otter trawl, beam trawl *etc*.) may be towed parallel to shipping lanes, but with added uncertainty as to sampling position.

Storage of samples

In the laboratory samples for biological measurements should be analysed as soon as possible according to the following protocol but not later than seven days after sampling. All samples containing live specimens should be transported to the laboratory in non-contaminating containers (not PVC containers). If necessary, cooling facilities during transport and storage should be employed.

Determination of imposex

Treatment of samples and sample analyses

Nucella and *Buccinum* should preferably be examined live without narcotisation to achieve a maximum relaxation, to simplify the measurement procedure and to provide more reproducible results. Before breaking the shell with a vice, individual measurements for shell heights should be measured to the nearest 0.1 mm with a vernier calliper.

If for example for logistical reasons it is necessary to preserve samples prior to analysis, specimens may be frozen. However, freezing will effect penis length measurements and data should be corrected to make them comparable to data from live specimens. The identification of imposex and intersex stages should be unaffected by freezing.

Determination of imposex

Under the microscope in imposex-affected species the following observations and measurements (with an accuracy for organ extensions of 0.1 mm) should be undertaken:

a) imposex stage should be determined for each female according to Figures 1 and 2 for *Nucella lapillus* and Figure 3 for *Buccinum undatum* Mensink *et al.* (1997). Generally, in *Nucella lapillus* only the imposex stages summarised in Figure 2 can be found in the AMAP area (Gibbs *et al*. 1987). In *Nucella lima* a penis is formed prior to the development of vas deferens (Short *et al*. 1989), following the pattern of 1a-3a of the scheme provided. There is no information of geographical variation in the development of the imposex within *Nucella lima*.

b) the percentage of female(s) *Nucella* in stages 5 and 6 (indicates proportion of females which are sterile);

c) the length of penis in *Nucella* males and females (if present).

On the basis of these measurements the following calculations of imposex intensities and indices of possible interference with the reproductive capability in prosobranch populations should be calculated:

a) Vas Deferens Sequence Index (VDSI) in *Nucella* as the mean value of the imposex stages in a population:

   VDSI = Σ imposex stage values of all females samples / number of females

   VDSI values > 4.0 indicate that at least some of the females in the sample are sterilised due to imposex development;

b) the proportion of sterile females of *Nucella*;

c) for *Nucella* the Relative Penis Size Index (RPSI) should be calculated:

   RPSI = ((average length of female penis)^3/(average length of male penis)^3)x100;

d) for *Buccinum undatum* the proportion of females showing imposex (i.e. other than at stage 0) should be calculated.

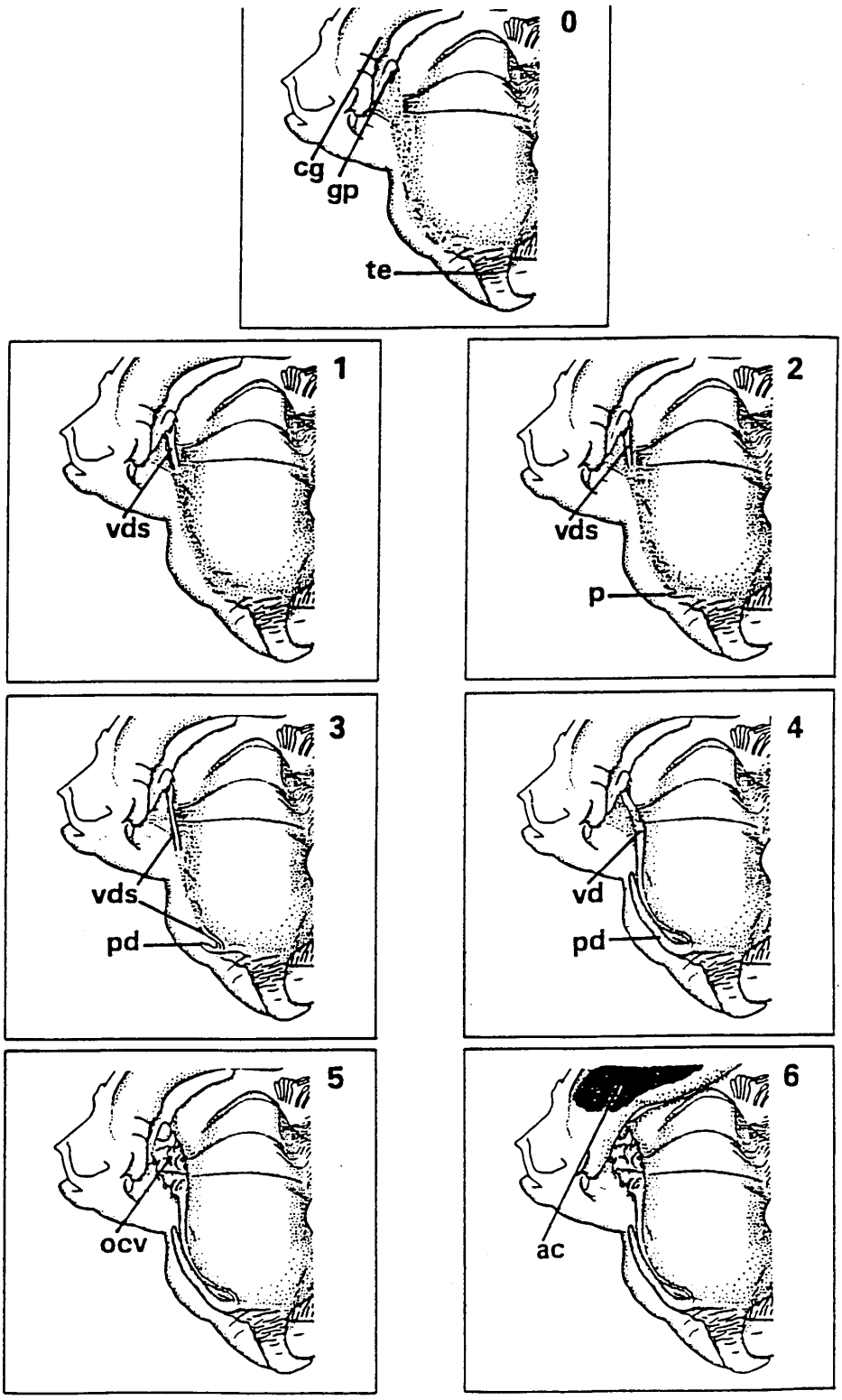

Fig. 1: Imposex development scheme in *Nucella lapillus*. ac, aborted capsules; cg, capsule gland; gp, genital papilla; ocv, occlusion of vaginal opening; p, penis without duct; pd, penis with duct; te, tentacle; vd, vas deferens; vds, vas deferens section.

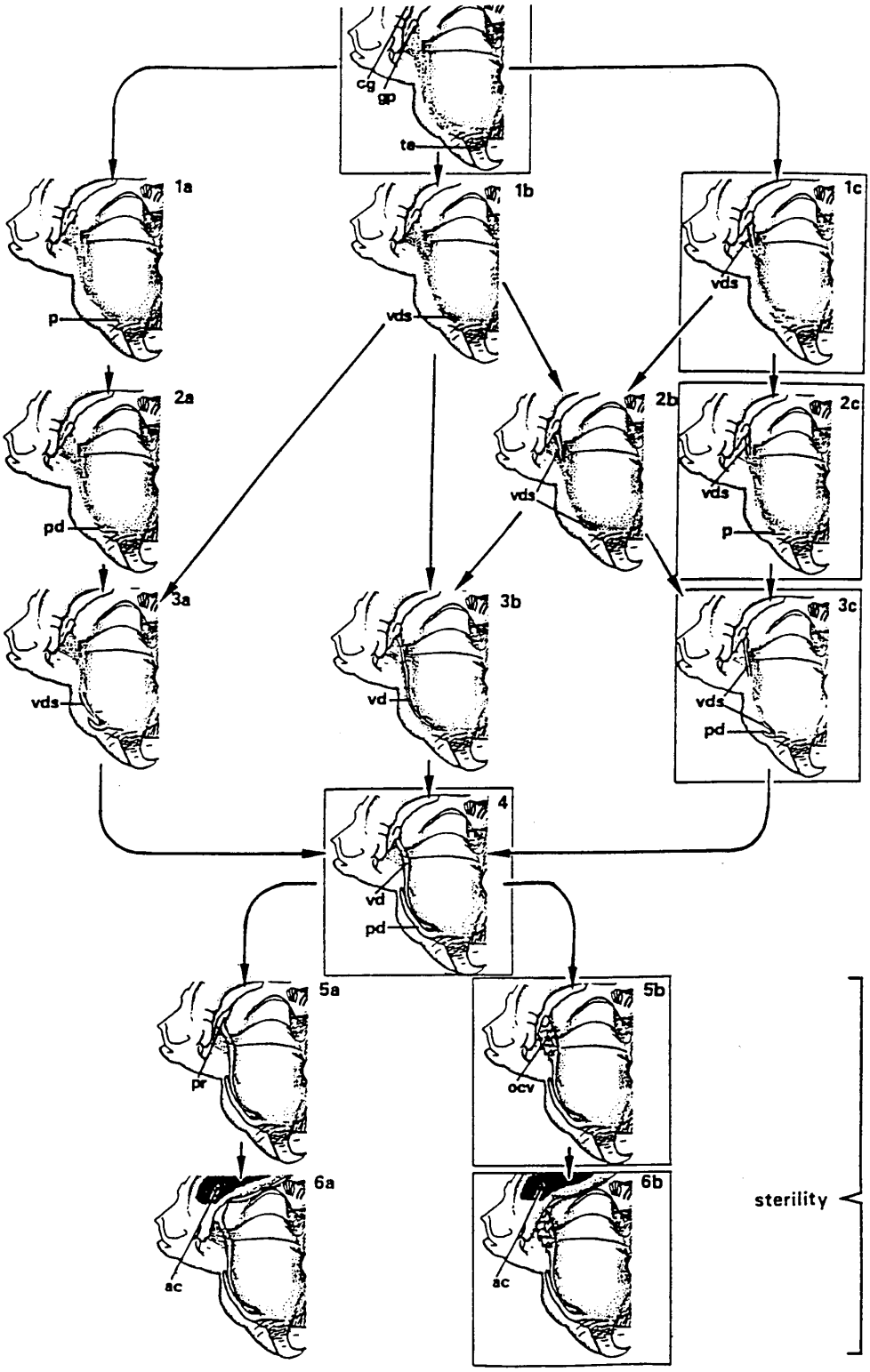

Fig. 2: Imposex development scheme in *Nucella lapillus* according to channel coast (France and England) populations. Stages of Figure 1 with frame. ac, aborted capsules; cg, capsule gland; gp, genital papilla; ocv, occlusion of vaginal opening; p, penis without duct; pd, penis with duct; pr, prostate; te tentacle; vd, vas deferens; vds, vas deferens section

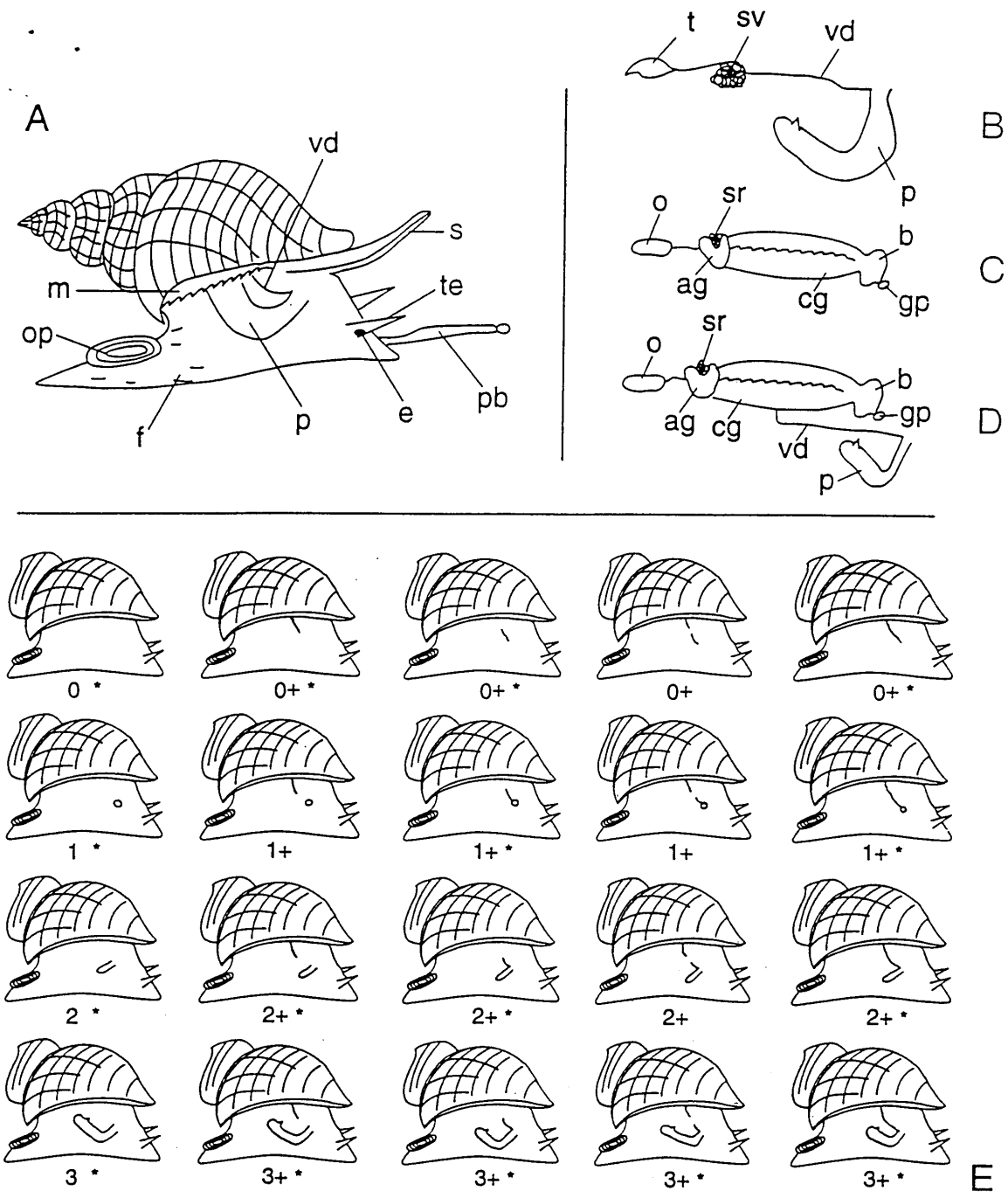

Figure 3. **A**: Normal male *B. undatum*, **B**: Simplified genital duct of a male *B. undatum*, **C**: Simplified genital duct of a normal female *B. undatum*, **D**: Simplified genital duct of an imposex female *B. undatum* stage 3+. Abbreviations : m = mantle, op = operculum, f = foot, p = penis, e = eye, te = tentacle, pb = proboscis, s = siphon, vd = vas deferens, t = testis, sv = seminal vesicle, o = ovary, sr = seminal receptacle, ag = albumin gland, cg = capsule gland, b = bursa copulatrix, gp = genital pore, **E**: Scheme of possible imposex stages for *B. undatum*. The observed stages from field and laboratory studies are indicated with *.

Reported information

Contaminants

1. TBT concentration in tissues (mg/kg[1]);
2. wet weight or dry weight basis;
3. concentration of extractable lipid;

Imposex measurements

4. Vas Deferens Sequence Index;
5. proportion of females in stages 5 and 6;
6. relative penis size (*Nucella*);
7. proportion of female *Buccinum* displaying imposex;

Supporting parameters

8. site code;
9. taxonomic identification;
10. date of sample collection;
11. number of individuals in sample;
12. presence/absence of juveniles and/or egg capsules of *Nucella*;
13. population size frequency distribution.

Table 1. Species suggested for TBT monitoring in the AMAP area (E = Essential, ES = Essential subregionally, R = Recommended).

Species		**Spatial**	**Temporal**	**Special study**
Dogwhelks (*Nucella lapillus*)	E	x	x	x
Whelks (*Nucella lima*)	E	x	x	
Common whelk (*Buccinum undatum*)	E	x		x
Blue mussel (*Mytilus edulis*)	R	x		x
Beluga whale (*Delphinapterus leucas*)	R	x		
Harbour porpoise (*Phocoena phocoena*)	R	x		
Ringed seal (*Phoca hispida*)	R	x		
Kittiwake (*Rissa tridactyla*)	R	x		
Common eider (*Somateria mallissima*)	R	x		
Glaucus gull (*Laurus hyperboreus*)	R	x		
Sediment	ES	x		x

Table 2. Sampling areas.

Species		Norweg. coast	Svalbard/ Barent Sea	Kola pen.	Siberian waters	Bering Sea	Gulf of Alaska	Beaufort/ Chukchi	Canadian Arctic	Green-land	Iceland	Faereo Islands
Dogwhelks (*N. lapillus*)	E	x		x					x?		x	x
Whelks (*N. lima*)	E						x					
Common whelk (*B. undatum*)	E		x	x	x	x		x?	x	x	x	x
Blue mussel (*M. edulis*)	R	x					x		x	x	x	x
Beluga whale (*D. leucas*)	R		x		x?	x	x		x	x?		
Harbor porpoise (*P. phocoena*)	R	x							x?			
Ringed seal (*P. hispida*)	R		x		x			x			x?	
Kittiwake (*R. tridactyla*)	R	x	x					x	x		x	

Common eider (*S. mallissima*)	R	x	x					x	x	x	x	x
Glaucus gull (*L. hyperboreus*)	R		x								x	
Sediment	ES	x	x?	x?	x?	x	x	x?			x	

Table 3. Recommended tissue to be analyzed.									
Species		Whole body	Tissue					Eggs	Surface sediment
			Liver	Blubber	Kidney	Blood	Muscle		0-1 cm
Dogwhelks (*N. lapillus*)	E	xx							
Whelks (*N. lima*)	E	xx							
Common whelk (*B. undatum*)	E	xx							
Blue mussel (*M. edulis*)	R	xx							
Beluga whale (*D. leucas*)	R		xx	x		x			
Harbor porpoise (*P. phocoena*)	R		xx	x	x		x		
Ringed seal (*P. hispida*)	R		xx	x	x		x		
Kittiwake (*R. tridactyla*)	R							xx	
Common eider (*S. mallissima*)	R						x	xx	
Glaucus gull (*L. hyperboreus*)	R							xx	
Sediment	ES								xx
xx = preferably, x = secondary									

Quality assurance

All determinations should be carried out by trained staff working to defined protocols. Any deviations from the protocols should be recorded and assessed by the laboratory manager for their potential to influence the results.

Intercomparison standards for effects measurements should be prepared as preserved material and used as reference standards by analytical laboratories during their routine work. Control charts can be prepared from repeated measurements of penis length on single preserved specimens. The repeated measurement of penis length or prostate length in preserved specimens could be used to control biological measurements. This will control for differences in interpretation between analysts, and for gross errors in microscope calibration.

International interlaboratory comparisons of imposex and TBT measurements are currently being organised, e.g. under the BEQUALM and QUASIMEME programmes, to provide a formal framework for quality assurance. Within the OSPAR area interlaboratory comparisons have successfully been performed for imposex. AMAP laboratories should participate in these activities.

TBT compounds can be purchased from chemical suppliers for the preparation of standard solutions. Reagents for the chemical analysis should be of appropriate quality to meet the needs of the determination.

Data reporting

Data should be reported to the AMAP Marine TDC, using the ICES format. Data must include the organotins MBT, DBT, TBT, and TPhT. The lipid content in the tissues must also be measured and reported.

Analytical procedures

The methods used for determination of TBT should follow the guidelines given below:

a) the method should be designed to measure TBT (rather than, for example, total tin or total organotin);

b) the concentration range of interest is around 0.05 - 1.6 mg/kg dry weight or 0.02 - 0.6 mg/kg wet weight;

c) the detection limit should be 0.02 mg/kg dry weight 0.01 mg/kg wet weight) or better;

d) reference materials should be used. However, there are currently no Certified Reference Materials directly applicable to shellfish tissue analysis and only a limited number available for TBT in marine matrices;

e) the concentration of extractable lipid should be determined and reported.

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