**Healthy & Biologically Diverse Seas Evidence Group**
**Technical Report Series:**

# Evaluation and gap analysis of current and potential indicators for Microbes

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# Preface

The UK Marine Monitoring and Assessment Strategy (UKMMAS) aims to provide coordinated and integrated marine monitoring programmes which support periodic assessments of the state of the UK marine environment. The strategy aims to provide vital data and information necessary to help assess progress towards achieving the UK's vision of clean, healthy, safe, productive and biologically diverse seas. The overarching strategy is supported and delivered by four evidence groups; Clean and Safe Seas Evidence Group (CSSEG); Productive Seas Evidence Group (PSEG); Healthy and Biologically Diverse Seas Evidence Group (HBDSEG) and Ocean Processes Evidence Group (OPEG). These groups are responsible for implementing monitoring and observations programmes to contribute to ecosystem-based assessments of marine environmental status.

As part of the HBDSEG programme of work, a series of reviews of environmental indicators was undertaken for the following marine ecosystem components:

1. Rock and biogenic reef habitats
2. Sediment habitats
3. Deep sea habitats
4. Seabirds and waterbirds
5. Cetaceans
6. Seals
7. Plankton
8. Microbes

The aim of the reviews was to evaluate a wide range of currently available and potential indicators for marine biodiversity monitoring and assessment. This task was undertaken particularly to inform future needs of the EU Marine Strategy Framework Directive (MSFD). The work was carried out by a group of consultants and contributors and was managed by JNCC.

Each review included a process to evaluate indicator effectiveness against a set of specified scientific and economic criteria. This process identified those indicators of activity, pressure, state change/impact and ecosystem structure and function that were considered to be scientifically robust and cost effective. The indicators which met these criteria were then assessed for inclusion within an overall indicator suite that the reviewers considered would collectively provide the best assessment of their ecosystem component's status. Within the review, authors also identified important gaps in indicator availability and suggested areas for future development in order to fill these gaps.

This report covers one of the ecosystem components listed above. It will be considered by HBDSEG, together with the other indicator reviews, in the further development of monitoring and assessment requirements under the MSFD and to meet other UK policy needs. Further steps in the process of identifying suitable indicators will be required to refine currently available indicators. Additional indicators may also need to be developed where significant gaps occur. Furthermore, as the framework within which these indicators will be used develops, there will be increasing focus and effort directed towards identifying those indicators which are able to address specific management objectives. There is no obligation for HBDSEG or UKMMAS to adopt any particular indicators at this stage, based on the content of this or any of the reports in this series.

This report has been through a scientific peer review and sign-off process by JNCC and HBDSEG. At this time it is considered to constitute a comprehensive review of a wide range of currently available and potential indicators for this marine ecosystem component.

# Summary

The combination of the shear abundance of microbes (defined here as members within the bacterial, archeal and viral kingdoms) in the marine environment, the biological reliance of higher trophic organisms on the extensive networks of microbial interactions and vice versa, and the relatively rapid reproduction rates within this biological group makes them highly sensitive to anthropogenic pressures. Moreover, microbes arguably make up the most important and extraordinarily diverse form of life on our planet.

In the marine environment microbes exist in complex, interdependent food webs with the rest of the oceanic biosphere. They exploit a bewildering array of niches within the ocean and can either be generalists or specialists. Currently, routine monitoring of microbes within the marine context is limited to microbial contaminant and human pathogen detection. Many branches of government and intergovernmental agencies have historically not been required to monitor microbial communities and their contribution to biodiversity or ecosystem functioning.

The overwhelming conclusion from this review is that microbes can indeed be used as indicators of state changes and impacts, together with ecosystem structure and function. However, significant gaps in our knowledge still exist preventing an immediate implementation of a holistic microbial monitoring programme. The major limitation is the absence of field trials and historical records fundamental to validating and evaluating the technology and effects of our changing environment, respectively. The inclusion of consistent monitoring of microbial populations is nonetheless warranted as these indicators can provide a rapid real time assessment of the effects of anthropogenic pressures such as organic pollution, and can provide an invaluable health check of ecosystem state and function in the marine environment

As our understanding of microbial diversity increases we will augment the role of these complex communities in structuring and functioning of the whole marine environment. With continued research and data validation we may in future be able to produce specific microbial indicator tools which can detect small scale change in environmental status. It is clear from the gap analyses undertaken here that microbial communities respond to a variety of human pressures on the marine environment. When these specific responses have been measured, modelled and understood, microbial indicators will provide a real time assessment tool kit which is able to detect change that can be attributed to specific pressures. The potential benefits associated with the development and implementation of microbial indicator monitoring is significant; however, it will require substantial investment into research and development along with indicator testing and validation.

# Contents

# 1 Introduction

## 1.1 Aims & objectives of report

Bacterial and viral communities are not routinely used as indicators to assess the state of health and biodiversity in marine environments. That said, Charting Progress 2 has now documented their contribution and thus importance as keystone species in globally important biological and ecological processes. By implication, monitoring of microbes and their processes could serve as important indicators to assess the magnitude of the effect of a particular pressure/activity on an ecosystem component. The aim of this review is to take a critical look at our current knowledge of microbial communities, their habitats and biological interactions to determine what benefit can be obtained by including microbial indicators in routine monitoring programmes. This includes an evaluation of how microbial indicators fare against a set of standard criteria to identify the most effective indicator species/species complexes to use in monitoring programmes. This will therefore provide an ideal opportunity to assess whether current uses of microbial indicators are still relevant, necessary and appropriate. Putative gaps will also be revealed and thus an assessment can be made as to whether implementing microbial indicators is these areas are warranted. Moreover, monitoring of microbial (bacterial and viral) communities could provide a cost effective alternative to some currently used indicators, ultimately providing management and governing structures of real time data so that immediate and appropriate action could be taken to minimise the adverse effects of human activities. In summary, the objective here is to identify the microbial indicators that are capable of addressing important pressures on the marine ecosystem, along with aspects of ecosystem structure and function.

## 1.2 Work undertaken in report

Substantial literature exists on the nature, abundance and ecological importance of microbial communities in globally important ecological processes. However, this review was undertaken to critically assess whether microbes (in this context only bacteria, archaea & viruses) could serve as both scientific and economically effective indicators of the status of the marine environment. To achieve this goal, the following work was undertaken during this review:

1. Review of the existing indicators for microbes
2. Evaluation of the effectiveness of the indicators against standardised scientific and economic criteria
3. Review of indicators against relevant pressures and important aspects of ecosystem structure and function
4. Identification of significant gaps and identification of any indicators that may be able to fill these gaps
5. Recommendation of a set of indicators for microbes that are effective scientifically and economically and could be used in future within an integrated monitoring and assessment programme

## 1.3 Introduction to the ecosystem component of interest

In the marine environment there are estimated to be around 1.18 x $10^{29}$ prokaryotes – comprising of bacteria and archaea (Whitman *et al* 1998). Density of prokaryotes in seawater is reported to be in the range $10^5$ to $10^7$ $ml^{-1}$. The world's oceans contain 1.3 x $10^{28}$ archaeal cells (which dominate the deep ocean – below 1000m) and 3.1 x $10^{28}$ bacterial cells (which dominate the surface waters – upper 150m) (Karner *et al* 2001). Prokaryotes were the first forms of life and they continue to shape the development of life on Earth via various metabolic processes that transform elements (e.g. primary production), degrade organic matter (e.g. respiration) and recycle nutrients (microbial loop and viral shunt). There are known examples of viruses that infect every major group of cellular organisms (Brussaard *et al* 2008), with the general school of thought being that every living organism has at least one virus that infects it. In the marine environment viruses are even more abundant than prokaryotes, around $10^8$ $ml^1$ (Bergh *et al* 1989). This high abundance equates to around $10^{29}$ viral infections $day^1$, causing a release of $10^8$ to $10^9$ tonnes of carbon $d^{-1}$ from the biological component (Suttle 2007).

Despite our reliance on microbes to sustain the extraordinary biodiversity of life on Earth, we are nonetheless confronted with the reality that microbes do cause disease. So rightly, many government agencies are tasked to continually monitor the environment we live in for contaminated biological waste and/or microbial pathogens (section 1.4). These measures give us the security of knowing that the water we drink, food we eat and water we bathe in are free of nasty microbes from a variety of sources.

As stated earlier, marine microbes are both numerous and ubiquitous. We now know that microbes play a central role in the consumption of dissolved organic matter (DOM) and transmission, through complex food webs escalating up the trophic food chain – The Microbial Loop (Pomeroy, 1974; Azam *et al* 1983). Microbial food web models are further complicated by the involvement of viruses. The biogeochemical impact of this "viral shunt" is the diversion of carbon away from the classically understood food web, towards microbe-mediated recycling processes. The predicted net effect of viral activity is an increase in system respiration that causes a shift in carbon from biological organisms to a DOM form. Moreover, this will have a profound effect on $CO_2$ capture capability by all microbial communities including the protists. The other notable effect is the redistribution of nutrients to more organic (or biological) forms. Therefore viruses mediate the equilibrium of growth-limiting nutrients between phytoplankton and bacteria. Whilst these effects might seem alarming at first, microbiologists and oceanographers alike accept that microbial activities maintain the biological diversity on our planet. There still remain significant practical obstacles to our ability to accurately measure and model the contribution that all microbial communities make to the functioning of key biogeochemical processes. That said, the monitoring of these microbial interactions (e.g. the viral shunt) could serve as important indicators of the health status of any ecosystem. This is dependent on suitable research being conducted in future to identify the specific species or group of species within the microbial world that are most suitable to monitor the impact of human activities on globally important biogeochemical processes.

This potential use is further enhanced by the fact that many microbes occupy specialised niches. Moreover, the vast majority of viruses have restricted host ranges, i.e. many virus strains can only infect one host species. Consequently, monitoring specific and characteristic microbial species, populations or communities within well defined habitats will provide invaluable information about the system and how pressures are impacting its structure and functioning. The wide range of human pressures that microbial communities respond to make them useful indicators which, with further validation of the data, may be able to indicate what specific pressure is causing change in a particular system.

Microbes respond rapidly to changes in their environment, making them rapid indicators of state changes and specific impacts from pressures. For example, microbial communities respond to:

**Temperature:**

- Rise in water temperature (around 6ºC rise) results in both an increase in bacterial and viral reproduction and activity (Cochran and Paul 1998).
- The frequency of viral infection in the field and laboratory populations of a cosmopolitan macroalgal biofouler, *Ectocarpus*, has been shown to be negatively correlated with sea surface temperatures (Muller *et al* 1998).

**Ocean acidification:**

- Ocean acidification has a negative effect on host-virus interactions between a globally important coccolithophore, *Emiliania huxleyi*, and its coccolithovirus, EhV-86 (unpublished data, Schroeder).

**Eutrophication and pollution:**

- Eutrophication is the major factor in increasing occurrences of cyanobacterial blooms in marine environments (Sellner 1997).
- Eutrophic systems have a higher degree of viral infections (Wilhelm & Suttle. 1999).
- Eutrophication and pollution acts synergistically to favour viral development (Danovaro & Corinaldesi 2003).
- Many different microbes are capable of degrading petroleum hydrocarbons (Head *et al* 2006).
- Classic pollutants and micropollutants (PCBs, fuel oil inc. PAHs, pesticides etc.) cause prophage induction and thus increase virus infection rates (Danovaro *et al* 2003).

**State of algal blooms:**

- Bacteria play a role in the population dynamics and toxicity of harmful algal blooms (Doucette *et al* 1998).

- At the community level, viruses control monospecific algal blooms (e.g. Schroeder *et al* 2003) by reducing the population size, therefore allowing an increase in the diversity of other organisms, a phenomenon known as “kill the winner” (Thingstad & Lignell 1997).

## 1.4 Policy background

Many branches of government and intergovernmental agencies have historically not been required to monitor microbial communities and their contribution to biodiversity or ecosystem functioning. Currently, only the negative consequences of microbial contamination to drinking water (Section 60 of the Water Act 1989 (HMSO 1989a) and World Health Organization: Guidelines for Drinking Water Quality (WHO 1984)), food (Food Standards Agency Science Strategy 2005-2010, Shellfish Waters Directive (2006/113/EC) and European food hygiene legislation (for shellfish hygiene; primarily Regulation (EC) No 854/2004)) and bathing waters (Bathing Water Directives (76/160/EEC and 2006/7/EC)) from a variety of sources such as farming or sewage waste water are reflected in policy documents.

Amongst the many benefits a monitoring programme will gain by including the routine assessment of extant natural microbial populations, is the fact that they are able to act as early warning indicators of potentially harmful pressures in an ecosystem. This is based on the over-whelming scientific evidence that microbial populations respond instantaneously to most perturbations in the environments. Furthermore, the nature of their response provides a level of confidence of the impact the perturbation will have on ecosystem function. This review will be a first-of-its-kind as it seeks to report on the unique role microbes can play as indicators of health and biodiversity in the marine environment.

Development and monitoring of microbial indicators would not only add to our current knowledge of marine biodiversity but it will cement the fundamental role this diversity plays in ensuring healthy ecosystem functioning. Identifying specific microbial indicators will provide an important tool for assessing Good Environmental Status under the Marine Strategy Framework Directive by contributing to our understanding and monitoring of the status of biodiversity (descriptor 1), marine food webs (descriptor 4) and eutrophication (descriptor 5), to give some possible examples.

## 1.5 OSPAR/UKMMAS Assessment framework background

The assessment framework developed by JNCC was first presented to the OSPAR Convention’s Biodiversity Committee in February 2007 and has since gained wide support across OSPAR as a tool to guide the development of a strategic approach to biodiversity monitoring. It has been particularly welcomed for its potential benefit in meeting the needs of the Marine Strategy Framework Directive (MSFD).

The framework takes the form of a matrix which relates ecosystem components (e.g. deep-seabed habitats) to the main pressures acting upon them (e.g. physical disturbance to the seabed). The ecosystem components have been correlated with components used by OSPAR and the MSFD. The columns of the matrix are a generic set of pressures on the marine environment, which are based on those used by OSPAR, MSFD and the Water Framework Directive (WFD). A 3-point scale of impact (low, moderate, high) reflects the degree of impact each pressure has on an ecosystem component. Each cell of the matrix has additionally been populated with a set of known indicators[1], derived from statutory and non-statutory sources, which are used to monitor and assess the state of that ecosystem component. The assessment matrix helps to highlight priorities for indicator development and monitoring programmes, based on the likely degree of each impact on the ecosystem component in question.

Since 2007 this approach has also been introduced to the UK's Marine Monitoring and Assessment Strategy (UKMMAS) and is being further developed by the Healthy and Biologically Diverse Seas Evidence Group (HBDSEG). The intention has been to have parallel development at UK and OSPAR levels which will help ensure similar biodiversity strategies are developed at national and international levels. It is also envisaged that the development process will benefit from wide input across OSPAR Contracting Parties.

The overall goal of the UKMMAS is to implement a single monitoring framework that meets all national and international multiple policy commitments (UKMMAS, 2007). This will identify if there are any significant gaps in the current monitoring effort and aim to minimise costs by consolidating monitoring programmes. To help meet this goal, the assessment matrix has been developed with HBDSEG to provide a useful framework that analyses components of an ecosystem and their relationships to anthropogenic pressures. The framework aims to encompass three key issues: an assessment of the state of the ecosystem and how it is changing over space and time, an assessment of the anthropogenic pressures on the ecosystem and how they are changing over space and time, and an assessment of the management and regulatory mechanisms established to deal with the impacts.

The further development of the assessment framework has been divided into five shorter work packages: 1) assessment of pressures, 2) mapping existing indicators to the framework, 3) review of indicators and identification of gaps, 4) modifying or developing indicators and 5) review of current monitoring programmes. The following work will contribute to work package 3 and will critically review indicators, identify gaps and recommend an overall suite of the most effective indicators for the ecosystem component in question.

## 1.6 Definitions used within the report and analysis

Definitions of activity, pressure, state change/ecological impact and ecosystem structure and function are used as follows (adapted from the 2008 CP2 methodology[2]):

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[1] Note: cells of the matrix where impacts have been identified currently contain a number of species and habitats on protected lists (OSPAR, Habitats Directive), which could potentially be used as indicators of the wider status of the ecosystem component which they are listed against. Should this be appropriate, certain aspect of the species or habitat (e.g. its range, extent or condition) would need to be identified to monitor/assess.

[2] Robinson, L.A., Rogers, S., & Frid, C.L.J. 2008. *A marine assessment and monitoring framework for application by UKMMAS and OSPAR – Assessment of Pressures and impacts* (Contract No: C-08-0007-0027 for the Joint Nature Conservation Committee). University of Liverpool, Liverpool and Centre for the Environment, Fisheries and Aquaculture Science, Lowestoft.

***Activity* –** Human social or economic actions or endeavours that may have an effect on the marine environment e.g. fishing, energy production.

***Pressure* -** the mechanism (physical, chemical or biological) through which an activity has an effect on any part of the ecosystem e.g. physical disturbance to the seabed.

***State change/ecological impact* –** physical, chemical or biological condition change at any level of organisation within the system. This change may be due to natural variability or occurs as a consequence of a human pressure e.g. benthic invertebrate mortality.

***Ecosystem structure and function* –** ecosystem level aspects of the marine environment (i.e. structural properties, functional processes or functional surrogate aspects) which are measured to detect change at higher levels of organisation within the system (i.e. changes at ecosystem scales), that is not attributable to any pressure or impact from human activity e.g. natural changes in species' population sizes. Please see Annex 4.

**Pressures list:**

The standard list of pressures against which indicators for this ecosystem component are reviewed is taken from the generic pressures list in the latest version (v11) of the UKMMAS / OSPAR assessment framework / matrix. Those pressures which are relevant to the ecosystem component (i.e. those that cause any impact on it) are used within the critical review and report.

# 2 Methods & data sources

Content for this review was sourced mainly from peer reviewed material but on occasion from institutions webpage's and agency material.

Sources of information included:

- Scientific literature (sourced using Web of Science);
- Publications by Defra such as Charting Progress 2 (http://www.defra.gov.uk/marine/science/monitoring);
- Direct discussion with organisations responsible for monitoring activities (e.g. Cefas);
- Results from international research programmes such as ICoMM (The International Census of Marine Microbes)

The literature review enabled identification of the relevant indicators and an initial assessment of their relative merits and drawbacks, based on their application and performance in a research and, where information was available, monitoring context. This provided the basis for the scientific and economic evaluation described in section 5.2. Following the literature review, details of individual indicators were entered into a database, designed by JNCC, including a description of the indicator and the geographical extent of its use, the ecosystem components to which it can be applied, the relevant human activities and pressures and a scientific and economic evaluation of the indicator. Scientific evaluation included an assessment of the sensitivity, accuracy, specificity, performance, simplicity, responsiveness, spatial applicability, relevance to management, validity and ease of communication to non-scientists. Economic criteria included the platform requirement for surveys (e.g. ship time vs survey on foot), equipment requirements and staff time involved in sample collection, processing, analysis and quality assurance, hence being focussed as value for money and cost-effectiveness. Each indicator will be assessed against these criteria as detailed in section 5.2 to give overall scores of good, moderate or poor for each indicator. Based upon this assessment, indicators were either recommended or rejected automatically. These recommendations were then individually assessed and the decision accepted or rejected, with justification.

The database output, following indicator evaluation, was a matrix presenting each indicator against the relevant pressures and aspects of ecosystem structure and function. This matrix was used to carry out a gap analysis, to make final recommendations of the best suite of indicators for inclusion in monitoring and to identify further research needs.

# 3 Review of the existing indicators and critical evaluation

## 3.1 Current indicators summary

See Annex 1: MicrobesReport

## 3.2 Evaluation of the effectiveness of indicators against standard scientific and economic criteria

### 3.2.1 Criteria used to evaluate indicators

In order to achieve a consistent critical appraisal of all indicators, the indicators for this ecosystem component have been reviewed and scored against the following set of criteria. These criteria have been built into the online indicators database application and the data has been stored electronically.

***A. Scientific criteria:***

The criteria to assess the scientific 'effectiveness' of indicators are based on the ICES EcoQO criteria for 'good' indicators. The scoring system is based on that employed within the Netherlands assessment of indicators for GES (2008)[3]. A confidence score of 3 - High, 2 - Medium, 1 - Low is assigned for each question. A comment is given on the reasons for any low confidence ratings in the comment box provided within the database. All efforts have been made to seek the necessary information to answer criteria questions to a confidence level of medium or high.

**INDICATOR EVALUATION:**

**1. Sensitivity: Does the indicator allow detection of any type of change against background variation or noise:**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**2. Accuracy: Is the indicator measured with a low error rate:**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**If the indicator scores 1 or 2 for question 1 or 2, conclude that it is ineffective and do not continue with the evaluation –the indicator will still be stored within the database as considered but will be flagged as 'insensitive, no further evaluation required'**

[3] Langenberg. V.T. & Troost T.A. (2008). Overview of indicators for Good Environmental Status, National evaluation of the Netherlands.

**3. Specificity: Does the indicator respond primarily to a particular human pressure, with low responsiveness to other causes of change:**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**4. Performance:**

**For questions 4a-f, if a score of 1 is given, please consider if the indicator is of real use. Please justify (within the report) continuing if a score of 1 is given. The following criteria are arranged with descending importance:**

**a) Simplicity: Is the indicator easily measured?**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**b) Responsiveness: Is the indicator able to act as an early warning signal?**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**c) Spatial applicability: Is the indicator measurable over a large proportion of the geographical to which the indicator metric it to apply to e.g. if the indicator is used at a UK level, is it possible to measure the required parameter(s) across this entire range or is it localised to one small scale area?**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**d) Management link: Is the indicator tightly linked to an activity which can be managed to reduce its negative effects on the indicator i.e. are the quantitative trends in cause and effect of change well known?**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**e) Validity: Is the indicator based on an existing body or time series of data (either continuous or interrupted) to allow a realistic setting of objectives:**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

**f) Relatively easy to understand by non-scientists and those who will decide on their use:**

| Score | 3 | 2 | 1 | Confidence |
|---|---|---|---|---|
| Options | Usually | Occasionally | Rarely | |

*Thresholds for scientifically poor, moderate and good indicators*:

Combine indicator evaluation scores for:

1. Sensitivity
2. Accuracy
3. Specificity
4. Performance

| Evaluation Score | Indicator 'Effectiveness' Category |
|---|---|
| 22-27 | **Good** |
| 16-21 | **Moderate** |
| 9-15 OR not all questions completed due to expert judgement not to continue | Poor |

Further economic evaluation required - see section B below

***B. Economic criteria:***

Having identified the most scientifically robust indicators using the above stated criteria, a further economic evaluation of those most effective indicators (i.e. those falling in the good or moderate categories) is carried out using the criteria stated below.

**1. Platform requirements**

| Score | 4 | 3 | 2 | 1 |
|---|---|---|---|---|
| Options | None e.g. intertidal sampling | Limited e.g. coastal vessel | Moderate e.g. Ocean going vessel or light aircraft | Large e.g. satellite or several ocean going vessels |

**2. Equipment requirements for sample collection**

| Score | 4 | 3 | 2 | 1 |
|---|---|---|---|---|
| Options | Simple equipment requirements e.g. counting number of organisms | Limited equipment requirements e.g. using quadrats on the shoreline | Moderate equipment requirements e.g. measuring physiological parameters | Highly complex method e.g. technical equipment operation |

**3. Amount of staff time required to plan collection of a single sample**

| **Score** | **4** | **3** | **2** | **1** |
|---|---|---|---|---|
| Options | Hours | Days | Weeks | Months |

**4. Amount of staff time required to collect a single sample**

| **Score** | **4** | **3** | **2** | **1** |
|---|---|---|---|---|
| Options | Hours | Days | Weeks | Months |

**5. Amount of staff time required to process a single sample**

| **Score** | **4** | **3** | **2** | **1** |
|---|---|---|---|---|
| Options | Hours | Days | Weeks | Months |

**6. Amount of staff time required to analyse & interpret a single sample**

| **Score** | **4** | **3** | **2** | **1** |
|---|---|---|---|---|
| Options | Hours | Days | Weeks | Months |

**7. Amount of staff time required to QA / QC data from a single sample**

| **Score** | **4** | **3** | **2** | **1** |
|---|---|---|---|---|
| Options | Hours | Days | Weeks | Months |

*Thresholds for economically poor, moderate and good indicators*:

| **Evaluation Score** | **Indicator 'Effectiveness' Category** |
|---|---|
| 24-28 | **Good** |
| 19-23 | **Moderate** |
| 7-18 | Poor |

Those indicators which fall within the **'Good'** or **'Moderate'** economic category will then be tagged within the summary database as 'Recommended' indicators. Indicators can also be 'recommended' via expert judgement even if the evaluation of the indicator does not score well enough to be automatically recommended. This judgement will be justified within the report text.

### 3.2.2 Additional information on the critical analysis of indicators

There is no additional information relating to the critical analysis of the microbe indicators, all information is captured within the online database application.

# 4 Gap analysis – Review of indicators against relevant pressures and important aspects of ecosystem structure and function

## 4.1 Review of indicators against pressures and identification of gaps

Please see the supporting analysis: MicrobesMatrixPressuresReport.xls.

All recommended indicators have been prefixed with [R] and the cells containing them are coloured green. When evaluating the indicators and assigning pressures which they would respond to, the category of 'no specific pressure' was not used for microbial indicators. This is due to the fact that even though the indicators described are of a general nature, specific tailor made indicators utilising specific species groups and population size thresholds are largely known and thus could be developed. These specific tools could detect change attributable to specific pressures if required. Therefore, a single generalised indicator may appear under the headings of multiple pressures because specific species groups, population size or extent thresholds etc could be tailor made as required to address the impacts of specific pressures on the ecosystem component. This would be undertaken on a case by case basis taking into account site specific details and objectives.

Microbial communities will respond in many different ways e.g. changes in species composition, population sizes etc., to a variety of different human pressures. This point is shown explicitly in the pressures matrix where many of the microbial indicators are shown to respond to most of the pressures identified within the marine environment (and thus appear in many different cells within the matrix). In order to actually implement the monitoring of these general indicator groups, specific combinations of species composition, population sizes and thresholds would need to be employed for specific geographic locations, pressures, resources and what particular questions need to be answered. For example, within the indicator category 'polluting and pathogenic microbial agents', specific bacterial assemblages can indicate different likely sources of contamination. The implementation of specifically tailored indicators will require the calibration and validation of data, however, if testing is undertaken in future these indicators could provide a rapid real time assessment tool. Below are some examples of specific circumstances in which monitoring of microbial indicators can provide information about the level of impact of different pressures on the marine environment.

### 4.1.1 Microbial loop health check:

The consequence of virus lysis is the diversion of carbon and nutrients away from the classical food web towards a bacterial-mediated recycling process. This diversion of elements towards the microbial loop is known as the viral shunt. The shunting of carbon towards the dissolved form of organic matter will ultimately supply growth-limiting elements in the phobic zone. Therefore, the net effect of viral lysis is the stimulation of biological production. This fundamental biologically driven process forms the basis on which life exists on our planet today. The logical conclusion of an altered or negatively affected viral shunt and/or microbial loop is a severely altered environment. This indicator has been referred to as the ‘microbial loop health check’ in the MicrobesMatrixPressuresReport.xls table. By monitoring this indicator we will be able to detect changes in the functioning of the viral shunt and microbial loop, and if tailor made, it could also detect the cause of change to ecosystem functioning. A specific example of this can be seen in the well characterised microbial interaction of viruses (EhV) infecting the marine phytoplankton, *Emiliania huxleyi*. It has long been understood that the carbon cycle is one of the major processes influencing global warming and ocean acidification. A reservoir of carbon dioxide ($CO_2$) that is continually consumed by autotrophs, including terrestrial plants, marine algae and marine microbes are converted into organic carbon via photosynthesis. Unlike other autotrophs, coccolithophores such as *E. huxleyi* also utilise inorganic carbon to create insoluble calcium carbonate structures known as coccoliths. These coccoliths, which are continually shed, sink towards the bottom of the water column where a significant proportion becomes lost to the carbon cycle for millennia. It is generally accepted that viruses are instrumental in the demise of *E. huxleyi* blooms. The monitoring of this specific viral component of this interaction provides important assurances of a healthy environment and whether known pressures at work in our environment are having any deleterious effect.

### 4.1.2 Changing environment indicators:

Microbes exploit a bewildering array of niches within the ocean and can be either generalists or specialists. Generalists, such as SAR11, frequent almost all marine water bodies worldwide, while specialists such as extremophiles occupy extraordinary environments. By monitoring both generalists (species within the ‘microbial loop health check’ and ‘nitrogen processing bacteria’ indicators) and specialists (species within the ‘extremophiles’ and ‘marine hydrocarbon degraders’ indicators) and comparing the ratios and/or presence or absence of each present in the environment, we can produce a useful indicator which can rapidly assess whether a particular pressure is impacting an environment. In addition, monitoring an indicator of generalist and extremophile microbial population structure can reveal the extent to which the environment is recovering.

Consequently, microbes can be recommended to monitor the majority of pressures at work in the environment. This is shown in the pressures matrix, where many microbial indicators can be shown to respond to a variety of human pressures and specific tailored combinations of species’ and population thresholds could help to detect specific impacts of these pressures. However, it must be noted that there is limited *in situ* data available. It is therefore difficult to assign any certainty as to whether these indicators will provide any practical data.

It is also likely that the list of pressures, as identified by OSPAR, is not exhaustive when seeking to determine which pressure influences microbial communities the most. Certainly, biological interactions such as virus infection dictate strongly the abundance and diversity of most microbial species. It is therefore important to note that this current analysis is confined to assessing whether microbes can be used as suitable indicators which respond to a specific set of pressures.

The main take home message is that whilst microbes are extremely useful indicators to monitor most pressures, many are untried and certainly not field tested, and arguably only ‘temperature, pH and various pollution pressures such as hydrocarbons, biological waste and human pathogen’ indicators are specific to individual pressures. However, the clear potential for their use should not be overlooked as the other indicator groups identified could be tailor made to address specific pressures.

## 4.2 Review of indicators against ecosystem structure & function aspects and identification of gaps

Please see the supporting analysis: MicrobesMatrixESStructureFunctionReport.xls

As stated on numerous occasions, microbes are fundamental to most biological processes at work today and as such are key species in most ecosystems. In addition, all higher trophic levels are often directly reliant on its microbial community as a food source or via other symbiotic interactions. It is therefore inevitable that most, if not all, ecosystem structures and thus functions are dependent on their specific microbial population. For example, primary and secondary production is exclusively reliant on a functioning microbial community. Consequently, as the gap analysis shows, most important aspects of ecosystem structure and function could be monitored using microbial indicators that have been tailored to address specific questions e.g. the impact of an oil spill on primary productivity could be monitored by a specific marine hydrocarbon degrader indicator. Similarly, many abiotic structures are what they are because of microbial activity. The temperature and salinity gradients within a specific environment can be maintained or buffered by microbial activity. Moreover, the nature of all the biotic structures are directly linked to microbial activity and therefore, monitoring key species within the ‘microbial loop health check’ indicator can for example inform directly the stability of an ecosystem structure.

The gaps identified within the matrix analysis once again highlights the lack of important trials and validation that are required to ensure the efficacy of using microbes as indicators against ecosystem function and structure. This does not mean, however, that the potential does not exist to gather such evidence. It does however show where research should be best focused when carrying out trials to validate the efficacy of using existing indicators of structure and function.

# 5 Conclusions and recommendations

## 5.1 Database report tables

Annex 1: MicrobesReport.
Annex 2: MicrobeMatrixPressuresReport.
Annex 3: MicrobeMatrixESStructureFunctionReport.

## 5.2 Identification of an effective indicator set

Consistent monitoring of microbial populations can provide data reflecting a real time response to anthropogenic pressures and their impacts on important ecological processes such as primary productivity, biogeochemistry and respiration. Our ability to define an exact or effective indicator set will depend on the pressure and ecosystem that is being tested. At this stage of our knowledge of microbial communities perhaps it would be most appropriate to assess the effectiveness of currently used indicators. Where it is shown that other ecosystem component indicators are poor or missing, microbial indicators could be investigated as a potential alternative.

That said, we have little or no hard fast evidence that monitoring microbial entities will provide any meaningful data. We do however know that the routine monitoring of coliforms has provided regional, national and international agencies with sound data of faecal contamination risks. Therefore, the leap of faith that microbes could do the same for other pressures is not a big one, as has been illustrated here. General microbial diversity measurements, in addition to specific species monitoring (examples given in annex 1), will provide an early warning for biological and ecosystem function regime change.

## 5.3 Recommendations for areas of development to address significant gaps

Significant investment into research, development and data validation is required if microbes are to be used as effective indicators of the status of the marine environment with respect to important pressures and aspects of ecosystem structure and function. From this analysis, it’s clear that an extraordinary amount is to be gained if this is done.

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