National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Cyanobacteria protocol 2020

RIVM letter report 2020-0167
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Colophon

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Synopsis

Cyanobacteria protocol 2020

Water with a lot of cyanobacteria may cause nuisance (such as bad odour) and health risks (such as mild skin and gastrointestinal complaints) to bathers. The water quality at official bathing sites should comply with European requirements.

To protect bathers' health at these bathing sites, water managers in the Netherlands use the Cyanobacteria Protocol. This protocol tells them how to inspect bathing sites for cyanobacteria, and which measures they should take. The Cyanobacteria Protocol 2020 does so according to the latest insights.

The Cyanobacteria Protocol 2020 is an update. The update was needed because there are new understandings of how to monitor cyanobacteria since the latest Cyanobacteria Protocol dating from 2012. Additionally, the government also wants to handle the cyanobacteria issue in an identical way in the whole of the Netherlands.

Cyanobacteria can sometimes be toxic. However, since it is not always possible to distinguish between toxic and non-toxic cyanobacteria, the Cyanobacteria Protocol 2020 assumes that they can all be toxic, just to make sure. Water managers check bathing sites by on-site inspections, after which they examine the water in the laboratory. They do this according to an obligatory, standard procedure. In this way, they determine how many cyanobacteria are present in the water and what the risk level is. Water managers may do extra tests if they consider this necessary.

When the risk level is known, measures are taken accordingly and the bathers will be informed. This can be a warning, an advice against bathing or a swimming ban. This is announced at the bating site and on www.zwemwater.nl.

By complying with the Cyanobacteria Protocol 2020 during the bathing season (May 1st – October 1st), the Netherlands complies with the requirements in the European Bathing Water Directive.

Keywords: cyanobacteria, blue-green algae, water quality, health risk, bathing sites, proliferation, protocol, European Bathing water Directive

Publiekssamenvatting

Blauwalgenprotocol 2020

Wanneer er veel blauwalgen in zwemwater zitten, kunnen ze voor overlast (zoals stank) en gezondheidsrisico's (zoals milde huid- en maagdarmklachten) voor zwemmers zorgen. De kwaliteit van water van officiële zwemlocaties moet voldoen aan Europese eisen.

Om de gezondheid van zwemmers op deze zwemlocaties te beschermen, gebruiken waterbeheerders in Nederland daarom het Blauwalgenprotocol. Dit protocol vertelt hen hoe ze zwemlocaties moeten controleren op blauwalgen en welke maatregelen ze moeten nemen. Het Blauwalgenprotocol 2020 doet dit volgens de nieuwste inzichten

Het Blauwalgenprotocol 2020 is een update. De update was nodig omdat er sinds het laatste Blauwalgenprotocol, uit 2012, nieuwe inzichten zijn hoe de aanwezigheid van blauwalgen kan worden gevolgd. Ook wil de overheid de blauwalgenproblematiek in heel Nederland op dezelfde manier aanpakken.

Blauwalgen kunnen soms giftig zijn. Omdat het niet altijd mogelijk is de giftige van de niet-giftige te onderscheiden zijn, gaat het Blauwalgenprotocol 2020 er voor de zekerheid vanuit dat ze allemaal giftig kunnen zijn. Waterbeheerders controleren zwemlocaties door lokaal de situatie te bekijken. Daarna onderzoeken ze het water in het laboratorium. Ze volgen hierbij een verplichte, vaste procedure. Zo wordt vastgesteld hoeveel blauwalgen er in het water zitten en hoe groot het risico is. Waterbeheerders mogen ook extra onderzoek doen als zij dat nodig vinden.

Als het risico bekend is, worden de maatregelen genomen die daarbij horen en worden de zwemmers geïnformeerd. Dit kan een waarschuwing, een negatief zwemadvies of een zwemverbod zijn. Dit wordt ter plaatse aangegeven en op www.zwemwater.nl.

Door het Blauwalgenprotocol 2020 na te leven tijdens het zwemseizoen (1 mei – 1 oktober) voldoet Nederland aan de eisen van de Europese Zwemwaterrichtlijn.

Kernwoorden: Blauwalgen, waterkwaliteit, gezondheidsrisico, zwemlocaties, protocol, Europese Zwemwaterrichtlijn

Contents

Summary

The European Bathing Water Directive (2006/7/EC) (EU-BWD) explicitly identifies cyanobacterial scums or blooms as public health hazards that need adequate handling. Performing adequate monitoring at official bathing sites, and protecting bathers' health by providing proper information, are important requirements resulting from this directive. The Netherlands complies with these requirements by means of the Cyanobacteria Protocol.

The evaluation of Cyanobacteria Protocol 2012 has shown that a more uniform approach of monitoring of cyanobacteria and risk assessment in the Netherlands was advisable, in order to achieve a uniform decision structure in response to proliferation of cyanobacteria at all bathing sites. Obtaining uniformity requires a more stringent pursuit of the Cyanobacteria Protocol.

The Cyanobacteria Protocol applies the precautionary principle: it assumes that all cyanobacteria are potentially toxic. Besides, the Cyanobacteria Protocol allows the facultative option to perform a more precise risk analysis based on the presence of specific toxic cyanobacterial genera or species or toxins in the water.

According to Cyanobacteria Protocol 2020, bathing sites are visually inspected for the presence of cyanobacteria (scum). When scum is present, the scum category is determined through visual inspection, facultatively supplemented with microscopic analysis of the composition of the scum. In the absence of scum, the water is examined for the presence of cyanobacteria by a fluorescence analysis in the laboratory which determines the cyanobacteria associated chlorophyll-a concentration. A fluorescence analysis in the field to determine the risk level, which was included in the 2012 version of the Protocol, is no longer allowed.

A fluorescence analysis may facultatively be combined with a microscopic analysis to adjust the risk assessment, for instance in case a high number of non-toxic species are expected and the risk is possibly overestimated. This can be done by 1) determining the relative fraction of potentially toxic cyanobacteria and subsequent correction of the fluorescence signal, or 2) determining the biovolume of potentially toxic cyanobacteria. The latter is preferred when the fluorescence analysis is unreliable or the result is doubted. The biovolume is converted to a cyanobacteria associated chlorophyll-a concentration to subsequently compare with the risk level.

Based on the scum category or the cyanobacteria associated chlorophyll-a concentration, a risk level for bathing sites is determined. The risk level indicates the likelihood of the occurrence of a health risk for bathers at a specific bathing site. Based on the risk level, measures will be taken, which include a warning, an advice against bathing, or a swimming ban.

Cyanobacterial toxins are the underlying cause of cyanobacteria related health risks. Toxin analyses are however not yet suitable for the operational management at bathing sites, because evaluation standards are currently (still) unavailable for most of the toxins. An exception is microcystin, the only toxin for which a threshold level has been established at the moment of drafting the Cyanobacteria Protocol 2020, and is therefore included in the Cyanobacteria Protocol 2020.

The handling and decision perspectives for water managers included in the Cyanobacteria Protocol 2020 are based on current knowledge. There is a knowledge gap for some cyanobacteria related issues, and these are currently excluded in this protocol. The Dutch Cyanobacteria Platform initiates further studies on these issues, such as standardized protocols for toxin analyses and standards, application of sensors, interlaboratory calibration of methods through ring trials, and health risks and standards for benthic cyanobacteria. Results from these studies which may be included in future versions of the Cyanobacteria Protocol.

1 Introduction

The European Bathing Water Directive (2006/7/EC) (EU-BWD) explicitly identifies cyanobacteria as a public health hazard that needs adequate handling. The bathing water profile of a bathing water site indicates whether proliferation of cyanobacteria is a relevant issue for this specific site. When the bathing water profile or daily practice indicate a fair chance of proliferation of cyanobacteria, an adequate inspection is required and measures should be taken to protect bathers' health. An important measure to do so is adequate information to the general public.

The Cyanobacteria Protocol 2020 is the Dutch interpretation of the EU-BWD requirements and an update of the Cyanobacteria Protocol 2012. The Protocol indicates how cyanobacteria should be monitored at official bathing sites.

An evaluation of the Cyanobacteria Protocol 2012 has shown that the implementation of the Protocol was done in various ways, ranging from following the instructions in the Protocol as precise as possible, to using the Protocol to draft an own procedure or protocol. The variety of options the Cyanobacteria Protocol 2012 offered to determine a risk level, were maximally exploited and a vast array of different analytical methods was used. The use of analytical methods other than the most common ones described in Cyanobacteria Protocol 2012, was done with the knowledge and approval of the responsible authorities (De Haan, 2016; Gerritsen, 2016).

A more uniform approach was desirable, because different approaches lead to different results, and thus to different ways of determining the risk level. This may subsequently lead to differences in the way responsible authorities take decisions, the type of measures they take, and the timing thereof during proliferation of cyanobacteria, which is undesirable.

Increased uniformity requires increased compliance with the Cyanobacteria Protocol. Therefore, a procedure for formal assessment of the Protocol is foreseen, eventually leading to inclusion of the Protocol in the new Dutch Environmental Act which will come in force in 2022. Additionally, the field and laboratory protocols included in the Cyanobacteria Protocol 2020 will be part of statutory regulation accompanying the act. This approach allows for more rapid modifications, when needed, since regulations can be modified more easily than acts. The regulation will also state which version of the Cyanobacteria Protocol is in force.

An important practical change in Cyanobacteria Protocol 2020, aimed at enhancing uniformity, is that it is no longer allowed to determine the risk level by means of a fluorescence analysis in the field, which was included in the 2012 version of the Protocol. With regard to theoretical content, an important change is that the 2020 version of the Protocol is based on the precautionary principle, assuming that all cyanobacteria are potentially toxic. The 2012 version of the Protocol took only five potentially toxic cyanobacteria genera into account. Next to a fluorescence analysis in the laboratory, the Cyanobacteria Protocol 2020 allows performing an additional microscopic analysis, or the analysis of microcystins in case microcystin producing genera dominate.

The Cyanobacteria Protocol should be implemented by the water authorities. Yet, it is not a static document. The document will be regularly updated based on new insights and new technological developments. The Protocol is available at www.rivm.nl. The intention is to evaluate the Cyanobacteria Protocol every two years. The responsible Dutch Ministry of Infrastructure and Water Management will initiate such evaluations.

2 Health conditions due to cyanobacteria

Cyanobacteria are common members of the phytoplankton community in water. When growing conditions are favourable, like water temperatures of 20 – 30 °C, calm weather, limited water flow, and nutrient rich water, cyanobacteria can proliferate massively and subsequently cause 'cyanobacterial blooms'. These blooms are associated with reduced water transparency, lack of oxygen in the water, death of other aquatic life, and stench due to rotting of dying cyanobacteria cells. Dense blooms may also lead to the occurrence of surface scum, where cells from the water column accumulate as a result of their buoyancy. Many cyanobacteria genera are able to produce toxins, and these toxins often accumulate during blooms as well. Dying cyanobacteria cells may furthermore release toxins into the water.

Much of the knowledge about working mechanisms of cyanobacterial toxins derives from animal studies. Based on their working mechanisms, cyanobacterial toxins can either be hepatoxins (liver damage, tumours), neurotoxins (paralysis), cytotoxins (cell necrosis) or irritating substances (skin conditions, gastroenteritis, tumours).

Cyanobacterial toxins can cause health conditions in humans and animals. It is often difficult to attribute possibly cyanobacteria related symptoms to actual cyanobacterial exposure. An overview of about 50 anecdotic cases and case reports of health conditions related to exposure to cyanobacteria reported during 1934 – 2003, showed that the reported symptoms were generally mild, highly divers and non-specific (such as hay fever-like, gastrointestinal, flu-like, respiratory or skin conditions). A range of waterborne micro-organisms may cause similar symptoms. In many case reports, data were lacking, thus leading to a lack of evidence that cyanobacteria in fact caused the symptoms. In the older case reports, the inadequacy of former analytical methods influenced the outcome. A number of more recent epidemiological studies that investigated the link between health conditions in swimmers and the presence of cyanobacteria in bathing water, reported that the swimmers had only mild health conditions (Stewart et al., 2006; Levesque et al., 2014).

Animals, on the contrary, are often (lethally) poisoned by cyanobacteria. They swim in water with dense cyanobacterial blooms, drink this water and clean or groom their fur or feathers after swimming, thus potentially swallowing high doses of cyanobacterial toxins (Stewart et al., 2008).

3 Basic principles of Cyanobacteria Protocol 2020

1. Cyanobacterial scums or blooms (Ch. 7) are a potential public health risk. Compliance to the Cyanobacteria Protocol helps to protect public health.
2. The Cyanobacteria Protocol is the Dutch interpretation of the requirements that follow from the European Bathing Water Directive and national legislation.
3. The bathing water profile in force indicates whether a bathing water site is a risk site for proliferation of cyanobacteria (Ch. 5).
4. Monitoring of cyanobacteria applies the precautionary principle. It assumes that all cyanobacteria are potentially toxic. See point 10 for deviant situations.
5. Visual inspection (§ 6.1.1) allows proactive information of the general public and assessment of potential risks.
6. The inspected location is the sampling point where compliance sampling for the European Bathing Water Directive takes place (hereafter called: control point), as included in the bathing water profile. When risks at other locations in the bathing zone deviate from the risk at the control point, the authorities may agree on additional sampling points in the bathing zone.
7. Monitoring is performed according to the standardized sampling and analytical protocols as included in the (Dutch version of the) Cyanobacteria Protocol.
8. The scum category is determined by means of visual inspection, facultatively supplemented with microscopic examination of the cyanobacterial composition of the scum (Ch. 7).
9. The cyanobacterial density at a bathing site is determined by a fluorescence analysis in the laboratory. Such an analysis determines the concentration of chlorophyll-a associated with cyanobacteria in a water sample, which is a proxy of the cyanobacterial density at a bathing site. The analysis can facultatively be supplemented with microscopy, See also point 11
10. A fluorescence analysis in the laboratory can detect all cyanobacteria. However, at some bathing sites there might be doubt as to whether or not all cyanobacteria present are toxic. At these sites, the assumption that all cyanobacteria are toxic results in an overestimation of the risk. In such situations, microscopy can be used to determine the fraction of toxic cyanobacteria. The measured concentration chlorophyll-a associated with cyanobacteria can subsequently be corrected for this fraction (§ 6.2.4).
11. When microscopy shows dominance of microcystin producing cyanobacterial genera, a microcystin analysis can be performed (§ 6.2.5). The results of this analysis can be used to determine the risk level.
12. If there is doubt about the reliability of the fluorescence analysis at a specific bathing site (e.g. due to the presence of humic

substances, or other water characteristics), the risk level may be determined by using microscopic determination of the biovolume of potentially toxic cyanobacteria (§ 6.2.4). The determined biovolume of toxic cyanobacteria should be converted to a chlorophyll-a concentration for the final establishment of the risk level, and for reporting (§ 6.2.4, § 9.4).

13. Measures will be taken based on the determined risk level (§ 6.2.3). The risk level will be determined based on the scum category (Ch. 7) or the concentration chlorophyll-a associated with cyanobacteria. This concentration of chlorophyll-a associated with cyanobacteria may be corrected for the determined fraction of potentially toxic cyanobacteria (see point 10), or calculated from the determined biovolume of potentially toxic cyanobacteria (see point 12). In case microscopy showed that microcystin producing genera are dominant, the risk level may also be determined based directly on the microcystin concentration (§ 6.2.5).
14. Determination of the risk level of a bathing site can no longer be done by performing a fluorescence analysis in the field, which was included in the 2012 version of the Protocol. According to the Cyanobacteria Protocol 2020, a fluorescence analysis is always performed in the laboratory, facultatively combined with microscopy and/or microcystin analysis.
15. The Cyanobacteria Protocol 2020 includes an indicative risk assessment procedure for benthic cyanobacteria which is based on abundance (§ 9.6).

The next scheme outlines the Cyanobacteria Protocol 2020. The elements of this scheme will be described and explained in the following sections.

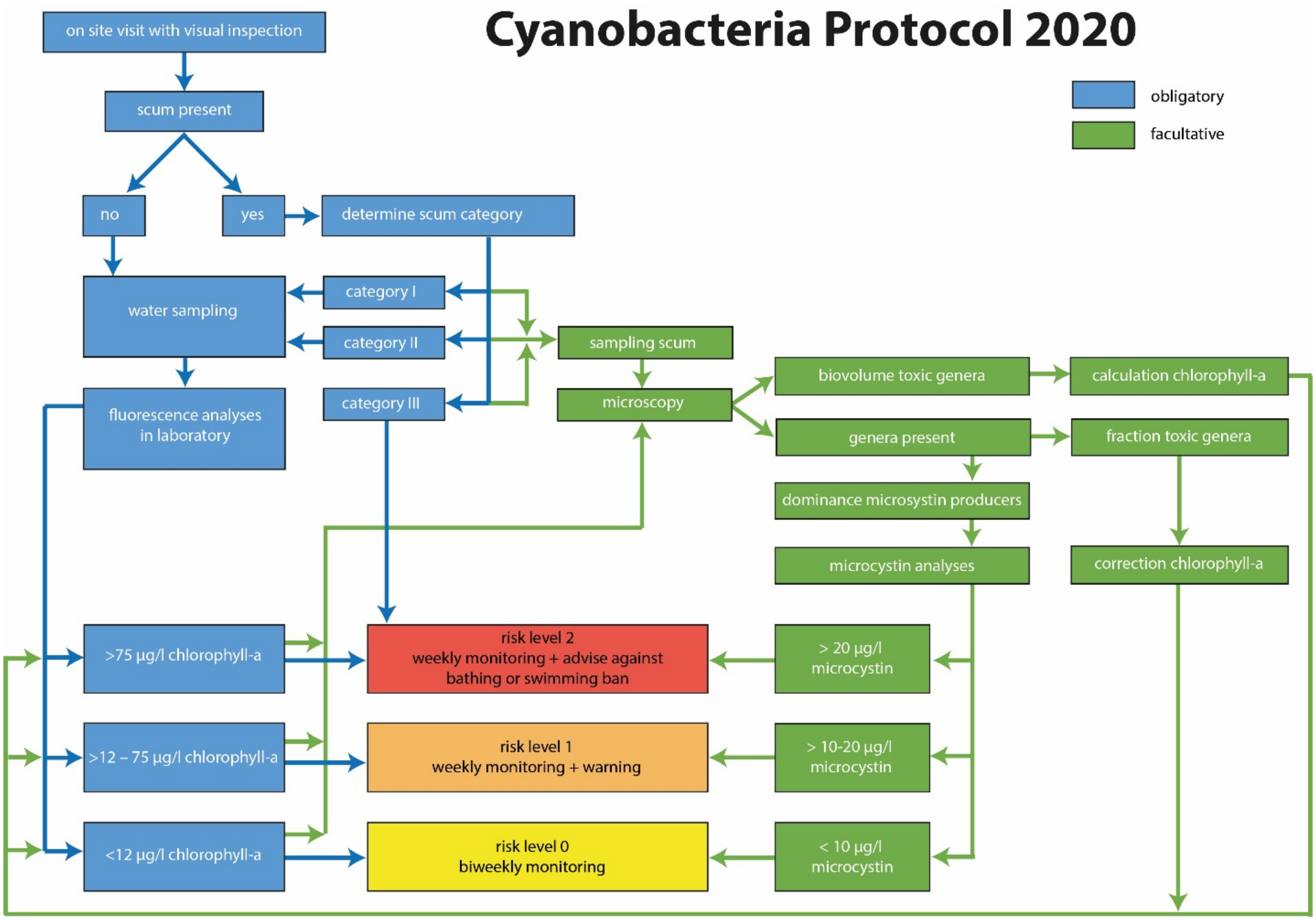

Figure 1 Cyanobacteria Protocol 2020 scheme – blue route = obligatory, green route = facultatively

4 Responsibilities

In the Netherlands, the site manager, the water manager and the province have a collective responsibility for a bathing site. Management and communication strategies should be agreed upon before the start of the bathing season.

Formal roles are:
- The water manager is responsible for monitoring the water quality at the bathing site, and giving advice to the province.
- The province is responsible for issuing a warning, an advice against bathing, and the installation of a swimming ban, including communicating about these measures to the public and site managers.
- The province enters into an agreement with the site manager about the design and the management of the bathing site with respect to hygiene and safety.

5 When is a bathing site a risk site regarding proliferation of cyanobacteria?

A bathing site is a risk site regarding proliferation of cyanobacteria if exceedance of a cyanobacterial guidance value has been observed during the last five years. The extent and duration of such an exceedance are included in the assessment as well. Appendix 1 (in the Dutch version of this report) issues a protocol for the assessment of the proliferation of cyanobacteria risk at a bathing site. This type of information should be included in the bathing water profile of a bathing site.

The Cyanobacteria Protocol 2020 is applicable to all bathing sites, but in practice it is only used for those bathing sites that have been identified as a risk site regarding proliferation of cyanobacteria.

6 Monitoring of cyanobacteria

Monitoring of cyanobacteria at bathing sites comprises visual inspection of the bathing site, sampling, and sample analysis. Bathing sites that are risk sites with respect to proliferation of cyanobacteria (Ch. 5) are inspected for the presence of cyanobacteria at least every two weeks. The scheme in the Cyanobacteria Protocol 2020 (Figure 1) can be used for guidance.

6.1 Inspection in the field

Inspection in the field is done in order to assess whether cyanobacteria or cyanobacterial scums are present at a bathing site. This inspection is done visually. If cyanobacterial scum is present, a picture of the scum is taken and kept for later reference. The organization that takes the picture is responsible for its storage. Pictures should be kept for at least five years, to enable their use for historic research and future evaluation of the Cyanobacteria Protocol.

6.1.1 *Visual inspection*

For each bathing site, province, water manager and site manager decide if and how visual inspection will be done, who is responsible for doing this, and which details of the inspection need to be recorded. The different organizations make a record of what they have agreed upon. The province coordinates this process and assesses whether cooperation, the division of roles, and communication during the bathing season is done as agreed. If needed, adjustments can be made.

The frequency of the visual inspection may vary per bathing site and per time period. The frequency may depend on weather conditions, model predictions, and the expected number of visitors. While drafting a time scheme for visual inspection, one should bear in mind that the occurrence of a scum may vary largely from day to day (and sometimes even within a day) and that regular adjustment of the time scheme for visual inspection may be needed. Daily visual inspection is recommended during periods in the bathing season with a high risk of proliferation of cyanobacteria. Daily visual inspection allows for immediate response to the presence of cyanobacteria (scum), and a more rapid adjustment of a bathing site’s risk level. Visual inspection is preferentially done in the morning, as this is the time of the day when scum is most likely observed.

Visual inspection is generally done during a site visit, but it may also be done by using a webcam, daily posting of digital photos, or remote sensing. Beforehand, the initiator of such alternative inspection techniques is to show the province that the alternative renders a level of inspection that is at least similar to that of (daily) visual inspection on site. However, an alternative digital inspection is never allowed as a replacement of the fluorescence analysis in the laboratory. The organization that made the digital images should store them for a period of at least five years.

The province oversees that inspections are actually done at the agreed frequency. When, in daily practice, the executive party (e.g. the water manager) cannot do the inspections at the desired frequency, the task may be done by another party (e.g. the site manager). The party delegating the task (e.g. the water manager) is responsible for checking that the hired party (e.g. the site manager) has adequate knowledge of scum categories and recognition of cyanobacteria in the field. The protocol for determination of scum categories (Ch. 7) and the risk levels that follow from these categories (§ 6.2.3) offer support. In case of delegation, the hired party (e.g. the site manager) reports its findings to the delegating party (e.g. the water manager).

6.2 Determination of the risk level

This section describes how the proliferation of cyanobacteria risk at a bathing site is to be determined based on inspection in the field (§ 6.1) and sampling. Measures to be taken (Ch. 8) follow from the determined risk level.

6.2.1 *Sampling*

In case cyanobacterial scum is present at a bathing site, the scum category is visually determined (Ch. 7). When a scum is absent (category 0) and when a category I or II scum is present, a water sample is taken to determine the cyanobacteria associated chlorophyll-a concentration. However, when large patches of cyanobacteria are present, it is recommended to sample the patches and treat the sample as described for category I-II scum. The water sample is transported to the laboratory for a fluorescence analysis according to a standardized protocol (§ 6.2.2).

If a category I-II scum is present, an additional facultative sample of the scum can be taken to determine which cyanobacterial genera comprise the scum. In case non-toxic cyanobacterial genera are present, this may be used to a downward adjustment of the cyanobacteria associated chlorophyll-a concentration (Appendix 3 in the Dutch version of the report).

The presence of a category III scum automatically leads to risk level 2. Taking a sample of the scum for additional determination of the cyanobacteria present in the scum is facultative.
Sampling of the bathing site is done according to Appendix 2 (in the Dutch version of the report). It is important that sampling is done by competent and well-trained staff. The sample is taken at the agreed control point. The water manager is responsible for taking the sample.

6.2.2 *Fluorescence analysis in the laboratory*

Cyanobacteria associated chlorophyll-a is used as a quantitative measure of the presence of potentially toxic cyanobacteria at a bathing site. The cyanobacteria associated chlorophyll-a concentration should always be determined in the laboratory (STOWA, 2010).

The analytical principle is based on the activation of the specific accessory pigments in cyanobacteria cells (i.e. phycocyanin and phycoerythrin) by irradiation with light of a specific wavelength. The energy absorbed by the excited pigments is transferred to the chlorophyll-a in the cyanobacteria cells, which in its turn emits a part of the energy as light with another wavelength. The intensity of this fluorescing light signal is a measure of the amount of chlorophyll-a in cyanobacteria.

The fluorescence analysis is done according to the protocol in Appendix 3 in the Dutch version of the report.

If levels of humic substances are high (FluoroProbe signal >10), the report should contain a remark indicating that the reliability of the fluorescence analysis was unsatisfactory for determination of the risk level. In this case the risk level should be determined based on microscopic biovolume analysis (§ 6.2.4).

6.2.3 *Determination of the risk level*

Based on the result of the laboratory analysis of the cyanobacteria associated chlorophyll-a concentration (possibly corrected for the estimated fraction of non-toxic cyanobacteria) the Cyanobacteria Protocol 2020 distinguishes two values that determine the risk level:

- ≥ 12 – 75 µg Chl-a per litre = risk level 1
- ≥75 µg Chl-a per litre = risk level 2

6.2.4 *Microscopic analysis (facultative)*

Samples with cyanobacteria associated chlorophyll-a concentrations above 12 µg per litre and samples from category I-II scum can be microscopically analysed for two purposes:

1. To gain insight into the cyanobacterial genera present and to determine the relative fraction of toxic cyanobacteria in a sample. This may result in a correction of the cyanobacteria associated chlorophyll-a concentration, or a microcystin analysis in case microcystin producing cyanobacteria are dominant.
2. To determine the biovolume of the toxic cyanobacteria present in situations where there is doubt about the reliability of the fluorescence analysis. The biovolume of the toxic genera, which subsequently has to be converted to the concentration cyanobacteria associated chlorophyll-a, is used to determine the risk level (§ 6.2.3). The Cyanobacteria Protocol 2020 applies a factor 3 for the conversion based on empirical data: chlorophyll-a (µg/L) = 3 * biovolume (mm^3/L).

A list with the relevant toxic and non-toxic cyanobacterial genera in the Netherlands is included in Appendix 4 of the Dutch version of Cyanobacteria Protocol 2020. This list displays the toxins these genera may produce, and thereby gives an indication of the toxins that may be present at the inspected bathing site. The list is continuously under revision and does not restrict the results of the microscopic analysis to the listed genera. If a potentially toxic genus is observed that is not in the list, it is to be included in the report, and added to the list after a specimen has been sent to another (experienced) laboratory for verification.

6.2.5 *Microcystin analysis (facultative)*

If the (possibly corrected) cyanobacteria associated chlorophyll-a concentration is higher than 75 µg/L, and microscopic examination has shown a dominance (>50%) of microcystin producing cyanobacteria, a microcystin analysis can be performed. For the practical execution of this analysis, the following documents may be consulted: Van der Oost, 2009; Van der Oost, 2009a; Van der Oost, 2009b.

The Cyanobacteria Protocol 2020 distinguishes two values that determine the risk level:

- 10 -20 µg microcystin per litre = risk level 1
- ≥ 20 µg microcystin per litre = risk level 2

In the Guidelines for safe recreational water environments – volume 1 (WHO, 2003), the WHO indicates that a microcystin level of 10 – 20 µg/L should evoke a warning (risk level 1), and that a swimming ban is advisable at microcystin levels of ≥ 20 µg/L. The Cyanobacteria Protocol 2020 also applies these values, although it should be mentioned that, in the Netherlands, risk level 2 may lead to an advice against bathing as well as a swimming ban.

6.2.6 *Reporting*

Reporting is preferably done by using a standard reporting form. In this form, the results of the fluorescence analysis are noted down, and a possible correction of the concentration cyanobacteria associated chlorophyll-a is describe. Besides, the (fraction of) potentially toxic cyanobacteria observed during the microscopic examination are included, as well as the determined risk level. The Dutch Cyanobacteria Platform (an expert group with members from both the scientific and the water management field) will draft such a standardized reporting form. When the form becomes available it will be an online appendix to the Cyanobacteria Protocol.

7 Scum categories

The Cyanobacteria Protocol 2020 distinguishes three cyanobacterial scum categories for bathing sites. The scum categories result in risk levels (§ 6.2.3) that subsequently lead to measures (Ch. 8).

7.1 Scum category I

Bright green clumps or threads (i.e. cyanobacteria cells) are present on the water surface and in the water body (Figure 2). There are no joint patches of cyanobacteria. There is no bad smell. Actually, there is no clear scum, but merely clusters of cyanobacteria cells that are floating on the surface. Cyanobacterial biomass is clearly visible. Potentially, this may form a scum as a result of further accumulation due to wind or growth of the population. In this situation, there is no direct risk for bathers, but a bloom may begin to develop. Whether the developing bloom is in the bathing area or in its proximity is irrelevant: potential scum formation cannot be ignored. A scum of this category may rapidly change, and therefore a sample should always be taken for laboratory analysis of the cyanobacteria associated chlorophyll-a concentration. The result of this laboratory analysis may lead to risk level 1.

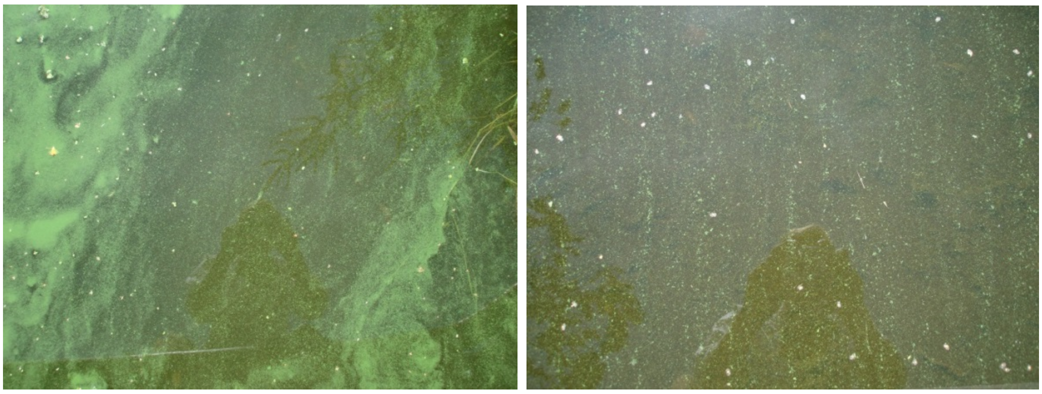

Figure 2 Scum category I (pictures: Waternet)

7.2 Scum category II

Dense patches of cyanobacteria are present on the water surface in, or in the proximity of, the bathing zone. The water is still visible at some points, but it is impossible to see the water through the scum for most of the water surface (Figure 3). Patches (larger than 10 x 10 cm) of more or less joint scum are present. There is no bad smell. Such a scum indicates that accumulation, e.g. due to the wind, has been going on for a while. A scum of this category is more often seen at the shore rather than at the centre of a water body. A category II scum will less likely appear or disappear than a category I scum, and it may substantially change in size and appearance under the influence of time (during the day), wind (speed and direction) and weather conditions. A scum of this category generally leads to risk level 1. To determine the actual risk level, the concentration cyanobacteria associated chlorophyll-a in the water is to be determined.

Figure 3 Scum category II (pictures: Waternet)

7.3 Scum category III

Dense patches of cyanobacteria are present on the water surface in, or in the proximity of, the bathing zone. The scum completely covers the water, and it is not possible to see the water through the scum (Figure 4). The scum has a substantial biomass and does not readily mix. Discoloration of the scum may occur: a pattern of colours ranging from bright green, to white and light blue. Discoloration is a sign of rotting. Foam and bad smell may occur. There is a high risk of high toxin concentrations in the water. A scum of this category can only be removed mechanically. Generally, changing winds and/or changing current have little effect on a category III scum. Only under exceptional circumstances (e.g. a combination of strong wind and heavy rainfall) a category III scum might spontaneously disappear. A scum of this category directly leads to risk level 2, which does not need further confirmation by fluorescence or microscopic analysis. To determine which cyanobacterial genera are present in the scum, a sample of the scum may be taken for microscopic analysis.

Figure 4 Scum category III (pictures: Waternet)

8 Measures

Risk level 1 results in a warning, which draws the attention of the public to the presence of cyanobacteria. The province decides whether to warn or not, they may ask the water manager for advice.

Risk level 2 results in an advice against bathing or a swimming ban. With a risk level like this, the desired behaviour is that people do not enter the water. An advice against bathing is the first option to achieve this. Additionally, the province has the option to install a swimming ban. A swimming ban may be installed under exceptional circumstances, e.g. the concurrence of various risks. An advice against bathing or a swimming ban can only be issued and lifted by the province. The province may seek advice from the water manager.

Measures may apply to the entire bathing site, but also to parts of the bathing site.

If monitoring at a bathing site results in a changed risk level, the accompanying measure (warning, advice against bathing, or swimming ban) may change as well. Changing the risk level, and thus the accompanying measure, may only be done on the basis of the cyanobacteria associated chlorophyll-a concentration (whether or not in combination with microscopy) (§ 6.2.4), the scum category (Ch. 7), or the microcystin concentration (§ 6.2.5).

The province is responsible for the communication with the bathers, and is also the contact for the site manager. In daily practise, there may be direct communication between the site manager and the water manager. The water manager must be available for communication with the site manager, and vice versa, in case of calamity. Bathers are informed on site by means of signs and at the Dutch bathing water information website www.zwemwater.nl. At this interactive website with a clickable map of The Netherlands, the most recent information about every official bathing site in The Netherlands can be obtained. Additionally, it provides background information about safe swimming, health risks and other topic of relevance for the general public (Figure 5).

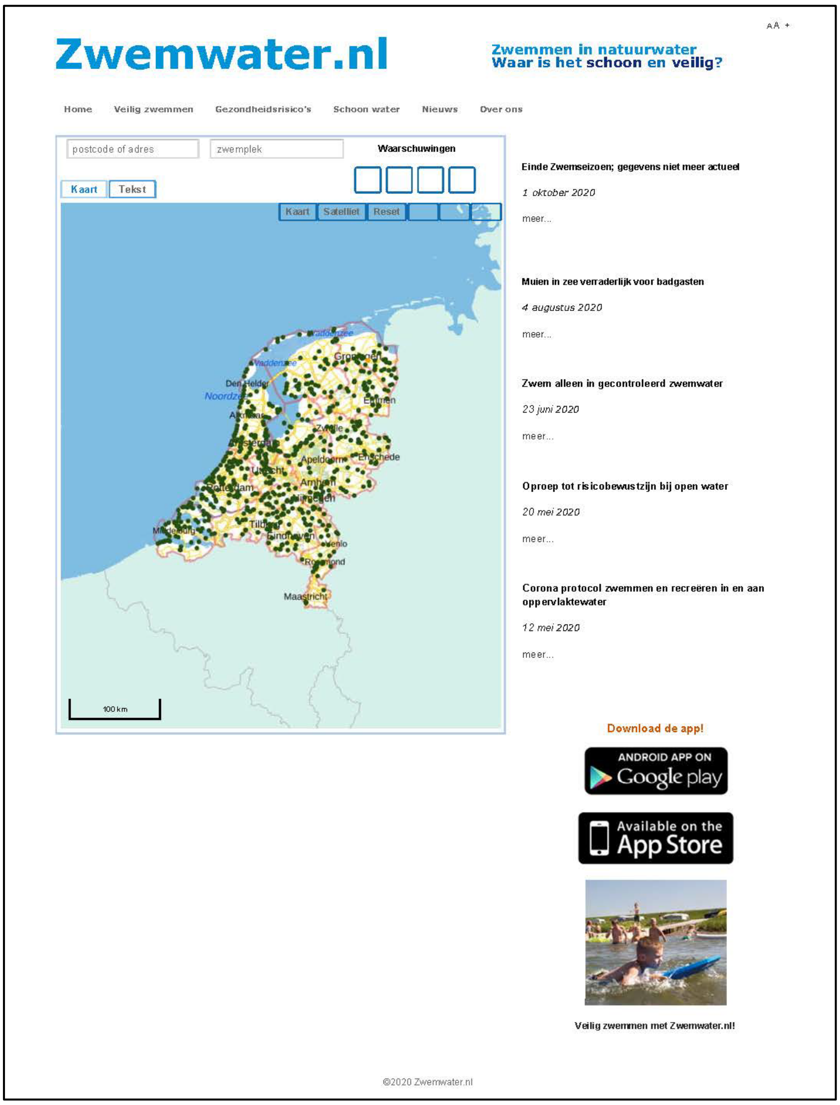

Figure 5 Screenshot of the homepage of the Dutch bathing water information site www.zwemwater.nl

9 Notes

The Cyanobacteria Protocol 2020 differs from the Cyanobacteria Protocol 2012 at various points. Changes result from new insights, are based on the evaluation of the Cyanobacteria Protocol 2012 (De Haan, 2016; Gerritsen, 2016), and are the result of the consultation of the members of the Dutch Cyanobacteria Platform in the course of 2019. Moreover, they have partly been made based on recent research initiated by the Cyanobacteria Platform (Sollie and Kardinaal, 2020). This explanatory chapter is meant to give further information on the choices made.

9.1 Applicability of the Cyanobacteria Protocol 2020

The Cyanobacteria Protocol 2020 is the realization of one of the requirements of the European Bathing Water Directive, and it therefore primary aims at official bathing sites. However, the Protocol can also be applied to other sites where people occasionally swim (e.g. during swim events in surface water) or where animals, like dogs, get into the water (e.g. urban water).

9.2 Fluorescence analysis

To bring about more uniformity in the determination of the risk level associated with cyanobacteria at bathing sites, the Cyanobacteria Protocol 2020 does no longer allow a fluorescence analysis in the field to determine this risk level. Fluorescence analyses in the field cannot be properly standardized. Thus, the results may strongly deviate from those obtained in the laboratory, using a standardized protocol. A fluorescence analysis should therefore always be done in the laboratory. It may facultatively be combined with a microscopic analysis.

It is recommended that all equipment used for monitoring of cyanobacteria by the various laboratories in the Netherlands is jointly calibrated. Procedures to achieve this are currently being explored by the Dutch laboratories.

Like most other analytical methods, the fluorescence analysis has some caveats that need to be considered (Gerritsen, 2016). There are confounding factors that affect the reliability of fluorescence analysis, which occur in situations when:

- *Planktothrix rubescens* is present in the sample. The red pigment phycoerythrin in this species has another excitation spectrum than that of phycocyanin, which is present in most cyanobacteria. *Planktothrix rubescens* may, however, be properly detected when the fluorescence apparatus is equipped with an extra module that activates phycoerythrin.
- The phycocyanin concentration declines due to a lack of nitrogen for the cyanobacteria cells. Here, the fluorescence signal may be low. This may result in cyanobacteria being classified as green algae.
- The concentration of humic substances in a sample is high. Here, the fluorescence signal of cyanobacteria associated chlorophyll-a

may be low. This may lead to a potential underestimation of the risk.
- A bloom of green algae, gold algae or silica algae is occurring. Here, the fluorescence signal for these groups may mask the cyanobacteria signal. Consequently, this will lead to an underestimation of the concentration cyanobacteria associated chlorophyll-a.

In the above situations, and in any other situation where there is doubt about the reliability of the fluorescence analysis, determination of the risk level may be based on the direct biovolume analysis of the potentially toxic cyanobacteria (§ 6.2.4).

9.3 Considering the toxic potential of all cyanobacteria

Following the precautionary principle, the basic assumption of the Cyanobacteria Protocol 2020 is that all cyanobacteria are potentially toxic. The 2012 version of the Protocol only addressed five potentially toxic cyanobacteria genera: *Microcystis*, *Dolichspermu* (previously *Anabaena*), *Aphanizomenon*, *Planktothrix* and *Woronichinia*. Until recently, these were considered the dominant genera in bathing water in the Netherlands.

The motivation for this changed approach is similar to that of the WHO Toxic Cyanobacteria in Water Working Group, which is currently finalizing a revision of the Toxic Cyanobacteria in Water document (WHO, 1999). Worldwide, at least 46 cyanobacterial genera are known to have a toxic effect on vertebrates, including humans. Besides, toxicity cannot be excluded for other (yet unknown) genera. Because research continues and constitutes more regions in the world, it is likely that other toxic cyanobacterial genera and species will be discovered. The WHO therefore considers it sensible to apply the precautionary principle, and take the toxic potential of each cyanobacteria population into account.

The Cyanobacteria Protocol 2020 takes all cyanobacteria into account by applying fluorescence analyses to determine the cyanobacteria associated chlorophyll-a concentrations in water samples. Occasionally, however, not all cyanobacteria present at a bathing site are toxic. For some bathing sites it is actually known that only non-toxin producing cyanobacteria are present or dominant. Here, the assumption that all cyanobacteria are toxic will lead to an overestimation of the risk. Therefore, the microscopically determined ratio of toxic and non-toxic cyanobacteria may be used to correct the measured cyanobacteria associated chlorophyll-a concentration. The list of potentially toxic cyanobacteria that are currently relevant in the Netherlands can assist in this process. In an update or amendment of the Cyanobacteria Protocol, additional WHO recommendations will be taken into account.

9.4 Correlation between biovolume and cyanobacteria associated chlorophyll-a

The cyanobacteria associated chlorophyll-a concentration determined with a FluoroProbe (in µg/L) displays an almost linear relation with the microscopically determined cyanobacterial biovolume (in mm^3/L), as shown in Figure 6. This figure contains over 3,800 biovolume results and cyanobacteria associated chlorophyll-a concentrations from various laboratories and studies (i.e. Aquon and STOWA). This dataset yielded a correlation factor of 2.8 for biovolume and cyanobacteria associated chlorophyll-a concentration, with a correlation coefficient of R^2 = 0.65. In a previous study, the observed correlation factor for these parameters was 3.3 (Van der Oost, 2010). The correlation between biovolume and cyanobacteria associated chlorophyll-a concentration can be used to convert a biovolume to a cyanobacteria associated chlorophyll-a concentration (§ 6.2.4), which can subsequently be evaluated according to the Cyanobacteria Protocol 2020.

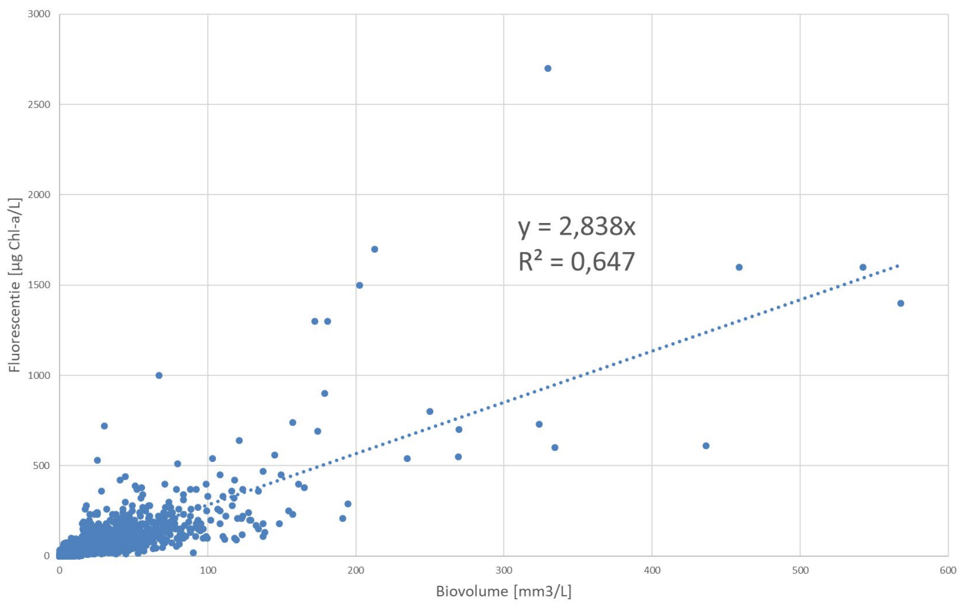

Figure 6 correlation between biovolume and cyanobacteria associated chlorophyll-a

9.5 Toxin analyses

It is obvious that cyanobacterial toxins determine the health risk associated with cyanobacteria. Although analytical methods are available for detection of cyanobacterial toxins (LC-MS/MS, HPLC, ELISA) and toxin genes (qPCR) in water samples, most of these have not (yet) been implemented as routine analyses by all laboratories. Moreover, the methods often focus on a (limited) number of specific toxins or genes. Currently, these methods are suitable for further investigation of sites with proliferation of cyanobacteria, rather than for routine monitoring. Additionally, toxin analyses are not suitable for daily management of a bathing site, because generally accepted standards for evaluation of determined toxin levels are not (yet) available for most of these toxins.

At the moment, microcystin is the only toxin for which evaluation standards are available, and thus the only toxin that can be used to determine the risk level at a bathing site. Microcystin analysis is facultative, and the standards for evaluation are included in the Cyanobacteria Protocol 2020.

If rapid analytical methods, like ELISA, will be available for a broader range of cyanobacterial toxins, as well as evaluation standards for these toxins, the toxins may be further implemented in the Protocol in the future. Updated versions of the Cyanobacteria Protocol will follow WHO recommendations.

9.6 Benthic cyanobacteria

Benthic cyanobacteria belong to the group of filamentous cyanobacteria that grow on a solid substrate. They often form brown-green to black velvety, occasionally slimy, patches on various substrates, like sandy or rocky soil, water plants, or floating garbage. These so-called benthic patches can comprise more than one species. A number of these benthic species are able to produce neurotoxins.

Benthic cyanobacteria form patches on soil or rocks at the bottom of a bathing site. The patches may loosen from the substrate and land at the beaches. Benthic cyanobacteria are included in the Cyanobacteria Protocol 2020, although they cannot be monitored and analysed according to the systematics of the Protocol. Since their relevance should not be neglected, guidance on how to act when the presence of benthic cyanobacteria is suspected is included. Appendix 5 (in the Dutch version of the report) additionally includes photos to facilitate recognition of patches of benthic cyanobacteria, and a procedure for an indicative risk assessment based on abundance.

In the future, the public and animal health effects of benthic cyanobacteria in the Netherlands will be further investigated. Incidents causing the death of dogs after swallowing these algae have been reported in the Netherlands (Faassen et al., 2012).

9.7 Alternative techniques for monitoring of cyanobacteria

Techniques used for monitoring of cyanobacteria at bathing sites develop continuously. In the future, other methods than those included in the Cyanobacteria Protocol 2020 may be preferential and recommended.

A qPCR method is available for detection of a number of potentially toxic cyanobacteria in surface water. These qPCR methods focus on the detection of only a number of toxin synthesis genes. Since the Cyanobacteria Protocol 2020 focusses on all potentially toxic cyanobacteria and associated toxins, these qPCR methods are currently suitable for further investigation of sites with proliferation of cyanobacteria, but not (yet) for daily management of a bathing site.

New techniques to be used for routine monitoring of bathing sites and daily management, should be fully developed and tested in daily practise, before they can be included in an updated version of the Cyanobacterial Protocol. New techniques should be validated in line with national standard NEN 7777:2011 or in compliance with international standard ISO 22118:2011. A procedure that includes consultation of experts is in place for the evaluation of newly presented analytical methods. New or alternative methods can only be used routinely after they have been approved and incorporated in an updated version of the Cyanobacteria Protocol.

9.8 Topics for further research

The Cyanobacteria Protocol 2020 provides practical guidance for water managers on how to estimate risk levels associated with proliferation of cyanobacteria at bathing sites. Some issues have not been addressed in this version of the Cyanobacteria Protocol, because data that substantiate the required action perspectives are lacking. However, this does not mean these issues are considered irrelevant. Therefore, the Dutch Cyanobacteria Platform will address these issues in the near future. Issues concerned are, amongst others, drafting national (NEN) or international (ISO) standards, evaluating the use of sensors, organizing ring trials, harmonizing the evaluation of new analytical methods, toxins and their analytical methods, and marine cyanobacteria.

10 Acknowledgements

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12 Note accompanying the English version

The Dutch version of the Cyanobacteria Protocol 2020 report includes several standardized operating protocols in appendices. These protocols have not been included in the translated version of the report, because they merely reflect the sampling and analytical procedures that should be routinely applied in the Netherlands. The Dutch report can be downloaded by following this link:
https://www.rivm.nl/publicaties/blauwalgenprotocol-2020

The list of protocols includes the following:

1. Protocol for the estimation of the risk of proliferation of cyanobacteria at a bathing site
2. Protocol for sampling surface water for monitoring of cyanobacteria
3. Protocol for determination of cyanobacteria associated chlorophyll-a using fluorescence analysis
4. Protocol for microscopy of cyanobacteria in surface water
5. Protocol for assessment and handling of benthic cyanobacteria at bathing sites

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