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# Evaluation of water quality guidelines for public swimming ponds

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ABSTRACT

Swimming ponds are artificial ecosystems for bathing in which people imitate the conditions of natural waters. Swimming in natural water may pose health risks if the water quality is microbiologically poor. Swimming ponds are small water bodies that may be used by relatively large groups of people, moreover, the water is not disinfected, e.g. by using chlorine. The draft new swimming pool legislation in the Netherlands includes water quality requirements for swimming ponds. This study focused on the examination and evaluation of the new microbiological water quality requirements, including *Escherichia coli*, intestinal enterococci, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, in thirteen public swimming pools. In eight of thirteen swimming ponds the water quality met the requirements for fecal indicators; 93–95% of the samples met the requirement for *E. coli* (≤100/100 ml) and intestinal enterococci (≤50/100 ml). The requirement for *P. aeruginosa* (≤10/100 ml) was met in eleven of thirteen swimming ponds (99% of the samples). In 68% of the samples the requirement for *S. aureus* (< 1/100 ml) was met. A linear mixed effect analysis showed that *E. coli* and intestinal enterococci concentrations were significantly dependent on the $\log_{10}$ of turbidity. *P. aeruginosa* concentrations were significantly dependent on water temperature. 31–45% of the variation between swimming ponds was explained by considering 'pond' as a random effect in the analysis. The monitoring of microbiological parameters in swimming pond water needs selective analytical methods, such as those used in this study, due to large numbers of background bacteria. The draft new Dutch swimming pool legislation provides proper guidance to ensure the microbiological safety of swimming pond water; it would benefit from inclusion of turbidity as an extra parameter. *S. aureus* is a relevant parameter for non-fecal shedding, although scientific literature does not provide evidence for a norm value based on a dose-response relation for exposure to *S. aureus* in water.

## 1. Introduction

Swimming ponds are artificial ecosystems for bathing in which people imitate the conditions of natural waters. Swimming ponds are distinct from official bathing sites in surface water as assigned by the European Bathing Water Directive (EU-BWD) (EU, 2006) and from conventional swimming pools. In contrast to official bathing sites, swimming ponds are not located in surface water, and the water in most swimming ponds is not in contact with the underlying soil, for example because a layer of rubber foil or concrete has been applied. Swimming ponds have two compartments: a compartment for swimming, and a compartment for water treatment. Water treatment is entirely, or largely, biological, mostly by applying plant filters, occasionally supported by other treatment processes such as phosphate/nitrate filtration or a UV lamp. Water treatment in swimming ponds never includes chlorine disinfection, contrary to pool water treatment in conventional chlorinated swimming pools.

Swimming in natural water may pose health risks to swimmers when the water quality is microbiologically poor. Microbiological contamination of surface water may occur through direct fecal input from man or animal, sewage overflow, discharge from wastewater treatment plants, and surface runoff. The water quality may also deteriorate because of growth of microorganisms that are part of the natural aquatic flora, such as *Pseudomonas aeruginosa*, *Vibrio* species, and cyanobacteria (WHO, 2003). Moreover, microorganisms that relate to animals may also pose a health threat to swimmers, such as

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*Trichobilharzia*, a parasite of waterfowl, whose larvae cause swimmers' itch in swimmers (Horák et al., 2015), and *Leptospira* species, that inhabit the renal tract of rats, which may cause leptospirosis in humans (Rood et al., 2017).

The EU-BWD is in place to protect swimmers from gastrointestinal risks related to fecal contamination of the water at official bathing sites. It requires regular monitoring of the water quality at official bathing sites, and classifies the sites into four categories, based on the level of *E. coli* and intestinal enterococci, being: excellent, good, acceptable, or poor. The EU-BWD does not address naturally occurring microorganisms such as *P. aeruginosa* and *Vibrio* species, but it does provide some guidance on proliferation of cyanobacteria and the associated risks.

In The Netherlands, regulations for the quality of bathing water are included in the 'Law for hygiene and safety of swimming pools and recreational waters' and the accompanying Decree (https://wetten.overheid.nl/BWBR0002660/2012–10-01; https://wetten.overheid.nl/BWBR0003716/2011–07-01) which include the requirements of the EU-BWD as well as the requirements for swimming pools. This legislation will become part of the new Environmental Act by 2021. Over 30 years after it came into force, the legislation for swimming pools needed updating, and is therefore under revision. The revised swimming pool legislation will include water quality requirements for various types of swimming pools, including some that were previously not regulated, such as swimming ponds. Swimming ponds were included because of their increasing popularity, merely related to the desire of, for instance, owners of wellness resorts to offer their clients a 'natural swimming experience'.

Swimming in swimming ponds may lead to similar or, occasionally, higher health risks than swimming at official bathing sites. Although contamination routes such as sewage overflow and discharge of wastewater treatment plant are not relevant, direct fecal input of humans and animals may occur, as well as growth of naturally occurring microorganisms. Moreover, in swimming ponds, large groups of people use an untreated, defined, and relatively small water body. Processes that result in the natural decrease of the number of microorganisms after a contamination incident, such as water flow, current, influence of wind and dilution, may be limited in swimming ponds.

Guidelines for swimming ponds have been drafted in Germany in 2011 (FFL, 2011). The requirements for swimming ponds in the new Dutch legislation are largely based on these German FFL guidelines, supplemented with expert opinions, which were collected during discussion sessions with a group of fifteen experts, such as microbiologists, toxicologists, and technologists from swimming pool constructing companies.

The new Dutch legislation includes requirements for physical, chemical, biological, and microbiological parameters, together with their frequency of analysis and method of analysis or detection. However, water quality data from swimming ponds in The Netherlands were not available to underpin the proposed standards for the microbiological parameters, which were under debate because of the extra analytical costs for owners of swimming ponds. The proposed chemical, physical and biological parameters were accepted without debate, largely because owners are used to measuring physical-chemical parameters, and their measurement is easy and cheap. Determination of biological parameters has to be done by visual inspection, and does not require any additional investments.

The microbiological parameters included *Escherichia coli* and intestinal enterococci as fecal indicator parameters, and *P. aeruginosa* as a waterborne pathogen that indicates possible growth of natural organisms and biofilm formation. *Staphylococcus aureus* was included as an opportunistic pathogen and indicator of anthropogenic pollution. The enumeration of *Legionella*, which also indicates biofilm formation, and a possible health risk through inhalation, was restricted to swimming ponds that technically heated the water and had spraying features in place.

This study focused on the examination of the microbiological water quality in thirteen public swimming ponds. The microbiological parameters included in the draft legislation were determined, except for *Legionella*, because none of the investigated swimming ponds heated the water or had spraying features. Additionally, physical-chemical parameters that relate to microbiological contamination were measured. Based on the results, both the water quality in swimming ponds and the proposed new requirements were evaluated.

## 2. Materials and methods

### 2.1. Sampling locations and sampling procedures

Water samples were taken from four swimming ponds during June–September 2017, and nine other swimming ponds during July–September 2018. Samples were taken according to the procedures described in ISO 19458:2006, 20–30 cm below the surface, at places where the water was 1–1.5 m deep. In 2017, the swimming ponds were sampled on three different days; on each day, water samples were taken from three different sampling points (pond, before and after plant filter) per swimming pond; each sampling point was sampled three times during the day (morning, lunchtime, afternoon), with a minimum time interval between two samples of 2.5 h. In 2018, the swimming ponds were sampled on four different days; at each day, water samples from each swimming pond were taken from one sampling point at one point in time. Immediately after sampling, all samples were placed on melting ice or cooling-elements, and transported to the laboratory for analysis within 24 h after sampling.

The swimming ponds were located at different venues, such as campsites, wellness resorts, and public swimming pools. All swimming ponds recirculated the water (recirculation rates unknown), and used tap-water to replenish. In the Netherlands, tap-water is non-chlorinated. Additional details of the studied swimming ponds are presented in Table 1.

### 2.2. Physical-chemical parameters and on-site observations

Water temperature (PT100 thermometer, Hanna Instruments), pH (SevenGO, Mettler Toledo), conductivity (Handylab LT11, Schott), and turbidity (Compact turbimeter CT12, Palintest) were measured on site, in all samples.

During sampling, abnormalities related to color and odor of the water, transparency of the water, and presence of waterfowl, fish, snails, and algae were recorded. These observations were all done by organoleptic inspection (vision and smell).

### 2.3. Sample analysis - microbiological parameters

Water samples were tested for the presence of *E. coli*, intestinal enterococci, *P. aeruginosa* and *S. aureus*, by using membrane filtration of various volumes of the water samples (range 1–100 ml), and incubation of the membrane filters on selective culture media. For enumeration of *E. coli*, intestinal enterococci, *P. aeruginosa* and *S. aureus* on Brilliance Staph 24 Agar and Collorex™ Staph aureus, mixed cellulose ester membrane filters (Merck-Millipore, HAWG047S6) were used.

In 2017, enumeration of *E. coli* was done according to ISO 9308-1:2014, with a modified incubation procedure to enhance selectivity (Jozić et al., 2018). Membrane filters were incubated on Chromocult® Coliform Agar (CCA; Oxoid Ltd., CM 1205) at 36 ± 2 °C, for 4–5 h, followed by incubation at 44 ± 0.5 °C, for 18–20 h. Blue-purplish colonies were considered to be *E. coli*. In 2018, enumeration of *E. coli* was done by incubating membrane filters on Tryptone Bile X-Glucuronide Agar (TBX; Bio-Rad, no. 3564035) at 36 ± 2 °C, for 4–5 h, followed by incubation at 44 ± 0.5 °C, for 18–20 h. Green-blue colonies were considered to be *E. coli*.

Enumeration of intestinal enterococci was done according to ISO 7899-2:2000. Briefly, membrane filters were incubated on Slanetz &

**Table 1**
Details of swimming ponds investigated in 2017 (p01-p04) – 2018 (p05-p13).

| swimming pond | venue | swim zone ($m^2$) | max. depth swim zone (m) | plant filter ($m^2$) | bottom coverage | extra water treatment |
|---|---|---|---|---|---|---|
| p01 | campsite | 250 | 1.4 | 125 | PVC foil and sand | UV[#] 220 W |
| p02 | holiday park | 270 | 1.4 | 250 | armed PVC foil | phosphate filter |
| p03 | wellness resort | 240 | 1.4 | 366 | EPDM rubber foil | phosphate filter |
| p04 | wellness resort | 82 | 1.4 | 205 | EPDM rubber foil | sieve filter |
| p05 | wellness resort | 32 | 1.4 | 12 | concrete and EPDM rubber foil | UVC[#]; phosphate and ammonia filter |
| p06 | hotel and lodges | 200 | 1.4 | 150 | EPDM rubber foil | none |
| p07 | campsite | 1500 | 1.4 | 600 | natural clay; no absolute sealing | |
| p08 | adventure park | 450 | 0.35 | 30 | concrete and EPDM rubber foil | UVC[#] 400 W |
| p09 | wellness resort | 80 | 1.35 | 21 | EPDM rubber foil | pool-skimmer |
| p10 | housing estate | 500 | 1.4 | 800 | concrete and EPDM rubber foil | phosphate filter |
| p11 | group accommodation/campsite | 4200 | 0.6 | 335 | bentonite and sand | none |
| p12 | public swimming pool | 1500 | 3.8 | 200 | fine sand; no absolute sealing | de-ironing; lava stones |
| p13 | public swimming pool | 2700[*] | 3.0 | 900[*] | none | none |

[*] Estimated value.
[#] UV lamps in place, but infrequently used for a part of the waterflow, and not during the sampling.

Bartley Agar (SBA; Oxoid Ltd., P05018A) at 36 ± 2 °C, for 44 ± 4 h, followed by incubation on Bile Esculin Azide Agar (BEAA; Oxoid Ltd., P05062A) at 44 ± 0.5 °C, for 2 h. Black colonies on BEAA were considered to be intestinal enterococci.

*P. aeruginosa* was enumerated by incubation of membrane filters on modified Pseudomonas aeruginosa agar type C (mPA-C agar; BBL, no. 298153) at 41.5 ± 0.5 °C, for 48 ± 4 h. Suspected green-brown or orange-brown colonies were pure-cultured on TSA, incubated at 36 ± 2 °C, for 18–24 h, and inspected for formation of blue-green, yellow-green, or red-brown pigment, and the typical pear drops odor that is characteristic of *P. aeruginosa*.

In 2017, enumeration of *S. aureus* was done by incubation of Tuffryn membrane filters (Pall, HT-450) on Baird Parker Agar (Oxoid Ltd., CM0961B) supplemented with Rabbit Plasma Fibrinogen (Oxoid Ltd., SR0122A) (BPA-RPF) at 36 ± 2 °C for 24 ± 2. Incubation was prolonged to 48 ± 4 h, when typical colonies were not present after the initial incubation period. Typical dark grey to black colonies surrounded by an opaque halo were considered to be *S. aureus*. This method is based on the horizontal method described in ISO 6888-2:1999. In 2018, an additional confirmation step was included: typical colonies on BPA-RPF were pure cultured on fresh BPA-RPF plates which were incubated overnight at 36 ± 2 °C. This procedure enhanced the readability of the opaque halo surrounding *S. aureus* colonies. Moreover, *S. aureus* was additionally enumerated by incubation of mixed cellulose ester membrane filters on Brilliance Staph 24 Agar (Oxoid Ltd., PO1186A) and Collorex™ Staph aureus (bioTrading Benelux, K615P090KP) plates, which were incubated and read according to the manufacturer's instructions.

### 2.4. Data-analysis

Statistical characteristics (mean, standard deviation, minima and maxima) where calculated using R (version 3.5.2 (2018-12-20) – "Eggshell Igloo"); and box whisker plots were created using ggplot2 in R (Wickham, 2016), version 3.1.0.

In general, linear mixed effects analyses of a relationship of the concentrations of *E. coli*, intestinal enterococci and *P. aeruginosa*, respectively with the pond, the day of sampling and water quality parameters (pH, water temperature, conductivity and turbidity) were conducted using R (version 3.5.2 (2018-12-20) – "Eggshell Igloo", R Core Team, 2018) and lmerTest (Kuznetsova et al., 2017). As fixed effects, day, pH, water temperatures, conductivity and turbidity without interaction terms were entered into the analysis. Pond was entered as random intercept. Linear mixed models were fitted by maximum likelihood and t-tests using the Satterthwaite's method. The model with the lowest Akaike's Information Criterion (AIC) was selected using the step-function. Turbidity and bacteria concentrations were $\log_{10}$ transformed.

The first mixed effect analysis was conducted for the 2017 data, for which fixed effects of time and place of sampling were also included. In the combined 2017–2018 data set, these extra fixed effects were not included. Additionally, only for the 2018 data, bottom cover (yes or no) was also included as fixed effect.

## 3. Results

### 3.1. Physical-chemical parameters and on-site observations

All samples, but one, complied with the proposed range of 200–1000 μS/cm for conductivity. In one sample, the conductivity was below 200 μS/cm (Table 2, Fig. 1). The acceptable maximum value for the water temperature of 25 °C was exceeded in 19 of 117 samples (16%) in 2017 (two ponds), and in 8 of 36 samples (22%) in 2018 (four ponds) (Table 2, Fig. 2). The pH was never below the lower limit of the acceptable range of 6.0–8.5, but exceeded the upper limit in 16% of the 2017 samples (n = 18/117; two ponds), and in 14% of the 2018 samples (n = 5/36; four ponds) (Table 2, Fig. 3). Turbidity was stable

**Table 2**
Physical-chemical water quality in swimming ponds, investigated in 2017 (p01-p04) – 2018 (p05-p13).

| swimming pond | parameter | $N_{samples}$ | mean | st.dev. | $min_{value}$ | $max_{value}$ |
|---|---|---|---|---|---|---|
| p01 | pH | 30 | 7.4 | 0.1 | 7.2 | 7.7 |
| | water temperature (°C) | 30 | 25 | 3.0 | 20 | 29 |
| | conductivity (μS/cm) | 30 | 562 | 67 | 450 | 649 |
| | turbidity (FTU) | 30 | 6.4 | 4.1 | 2.0 | 13 |
| p02 | pH | 27 | 8.7 | 0.3 | 8.2 | 9.1 |
| | water temperature (°C) | 27 | 22 | 3.3 | 16 | 25 |
| | conductivity (μS/cm) | 27 | 287 | 29 | 236 | 342 |
| | turbidity (FTU) | 27 | 0.30 | 0.2 | 0.01 | 0.64 |
| p03 | pH | 30 | 8.1 | 0.1 | 7.9 | 8.3 |
| | water temperature (°C) | 30 | 20 | 0.9 | 18 | 22 |
| | conductivity (μS/cm) | 30 | 388 | 23 | 355 | 446 |
| | turbidity (FTU) | 30 | 0.13 | 0.2 | 0.01 | 1.2 |
| p04 | pH | 30 | 8.5 | 0.2 | 8.2 | 9.0 |
| | water temperature (°C) | 30 | 21 | 1.4 | 19 | 24 |
| | conductivity (μS/cm) | 30 | 334 | 30 | 258 | 389 |
| | turbidity (FTU) | 30 | 0.39 | 0.2 | 0.11 | 0.7 |
| p05 | pH | 4 | 7.8 | 0.1 | 7.7 | 8.0 |
| | water temperature (°C) | 4 | 20 | 2.3 | 17 | 22 |
| | conductivity (μS/cm) | 4 | 491 | 24 | 463 | 522 |
| | turbidity (FTU) | 4 | 0.33 | 0.2 | 0.12 | 0.51 |
| p06 | pH | 4 | 7.4 | 0.1 | 7.2 | 7.5 |
| | water temperature (°C) | 4 | 21 | 2.0 | 19 | 24 |
| | conductivity (μS/cm) | 4 | 367 | 26 | 344 | 397 |
| | turbidity (FTU) | 4 | 0.44 | 0.2 | 0.21 | 0.72 |
| p07 | pH | 4 | 8.0 | 0.2 | 7.7 | 8.1 |
| | water temperature (°C) | 4 | 22 | 2.5 | 18 | 24 |
| | conductivity (μS/cm) | 4 | 497 | 29 | 465 | 532 |
| | turbidity (FTU) | 4 | 69 | 101 | 6.6 | 245 |
| p08 | pH | 4 | 8.5 | 0.2 | 8.2 | 8.8 |
| | water temperature (°C) | 4 | 23 | 3.2 | 19 | 26 |
| | conductivity (μS/cm) | 4 | 515 | 84 | 432 | 603 |
| | turbidity (FTU) | 4 | 33 | 26 | 1.9 | 59 |
| p09 | pH | 4 | 8.4 | 0.3 | 8.1 | 8.9 |
| | water temperature (°C) | 4 | 22 | 1.4 | 21 | 24 |
| | conductivity (μS/cm) | 4 | 448 | 36 | 395 | 475 |
| | turbidity (FTU) | 4 | 1.4 | 0.7 | 0.78 | 2.6 |
| p10 | pH | 4 | 7.4 | 0.1 | 7.3 | 7.6 |
| | water temperature (°C) | 4 | 21 | 3.9 | 18 | 25 |
| | conductivity (μS/cm) | 4 | 307 | 101 | 235 | 455 |
| | turbidity (FTU) | 4 | 0.34 | 0.1 | 0.17 | 0.53 |
| p11 | pH | 4 | 8.7 | 1.0 | 7.8 | 9.9 |
| | water temperature (°C) | 4 | 24 | 4.0 | 18 | 27 |
| | conductivity (μS/cm) | 4 | 232 | 27 | 194 | 255 |
| | turbidity (FTU) | 4 | 40 | 6.5 | 33 | 51 |
| p012 | pH | 4 | 8.4 | 0.4 | 8.1 | 9.1 |
| | water temperature (°C) | 4 | 22 | 4.3 | 18 | 26 |
| | conductivity (μS/cm) | 4 | 596 | 60 | 521 | 661 |
| | turbidity (FTU) | 4 | 16 | 8.4 | 7.8 | 28 |
| p013 | pH | 4 | 8.1 | 0.3 | 7.8 | 8.4 |
| | water temperature (°C) | 4 | 23 | 5.0 | 18 | 28 |
| | conductivity (μS/cm) | 4 | 486 | 33 | 453 | 530 |
| | turbidity (FTU) | 4 | 14 | 7.3 | 5.7 | 24 |

in most swimming ponds, except ponds p07 and p09, which had an irregular turbidity pattern. Turbidity values were variable among ponds, with mean values ranging between 0.1 and 69 FTU, and $\log_{10}$ transformed mean values between −1.3 and 3.7 FTU (Table 2, Fig. 4). The draft legislation does not include requirements for turbidity.

Transparency was to the bottom of seven swimming ponds at all four sampling occasions. In two swimming ponds, transparency was to the bottom at only one of four sampling occasions (pond p07 and p11), and in one swimming pond transparency was to the bottom at three of the four sampling moments (pond p09). Three swimming ponds (p08, p12, and p13) failed the requirement of transparency equal to or over 1.8 m at all four sampling occasions. No deviations were observed related to water color and odor in any of the swimming ponds. Observations during sampling showed that frogs and tadpoles were present in ponds p01, p02 and p04, and that waterfowl were occasionally present in pond p03 (ducks), pond p04 (a heron), and pond p07 (unidentified birds). Fresh water snails were observed in the plant filter of pond p02, but they were not captured for identification.

### 3.2. Microbiological parameters

Fecal contamination of the water in the investigated swimming ponds appeared to be low at the times of sampling (Table 3, Figs. 5 and 6). In 2017, 5% of the samples (N = 6/115) exceeded the proposed norm value of 50 colony forming units (cfu) per 100 ml for intestinal enterococci. These exceedances were all restricted to one swimming pond. *E. coli* counts showed that 4% of the samples (N = 3/69), all from one pond, exceeded the proposed norm value of 100 cfu/100 ml. In 2018, 14% of the samples (N = 5/36) exceeded the norm value for intestinal enterococci. The exceedances occurred in three swimming ponds. Six percent of the samples (N = 2/36) exceeded the norm value for *E. coli*; exceedances occurred in two ponds in which the water quality also did not comply with the intestinal enterococci norm value.

The concentrations of *P. aeruginosa* were low in all ponds. The

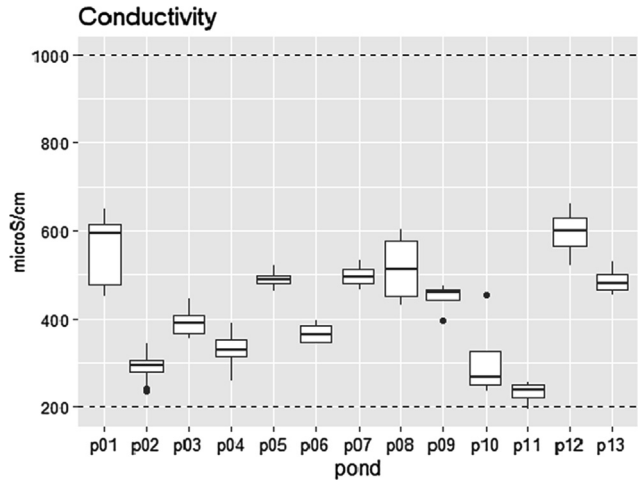


**Fig. 1.** Box whisker chart of the conductivity in thirteen different swimming ponds, sampled in 2017 (p01-p04) – 2018 (p05-p13). Each box shows the median and quartiles, the whiskers enclose 95% of the data, dots are outliers. Conductivity needs to stay between 200 and 1000 μS/cm (dashed lines).

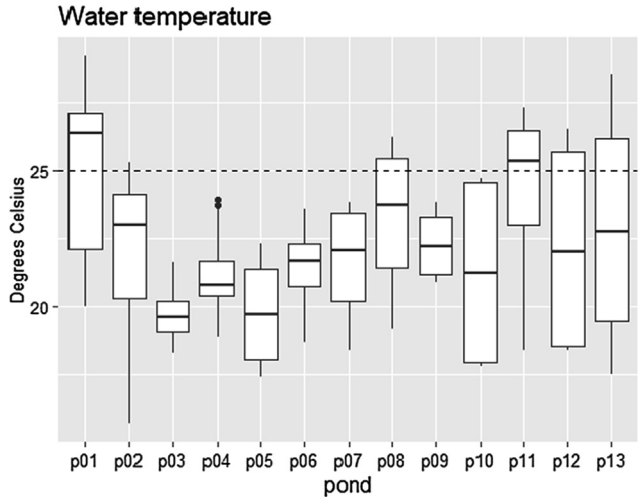


**Fig. 2.** Box whisker chart of the water temperature in thirteen different swimming ponds, sampled in 2017 (p01-p04) – 2018 (p05-p13). Each box shows the median and quartiles, the whiskers enclose 95% of the data, dots are outliers. The water temperature needs to stay below 25 °C (dashed line).

proposed norm value of 10 cfu/100 ml was exceeded in one 2017 sample (0.9%) and one 2018 sample (3%) (Table 3, Fig. 7).

In 2017, high concentrations of *S. aureus* were observed in almost all samples from all swimming ponds. A more detailed study of the colonies that grew on BPA-RPF plates by using API Staph (Biomérieux, no. 20500), showed that most of these colonies were not *S. aureus*, and therefore the 2017 results were disregarded and are not displayed here. In 2018, *S. aureus* was detected in one sample from each swimming pond, except one, thus resulting in 22% of the samples (N = 8/36) exceeding the requirement of absence of *S. aureus* in a volume of 100 ml.

### 3.3. Linear mixed effect analysis

The linear mixed effect analysis showed no effect of time and place of sampling in the 2017 data, justifying combining the 2017 and 2018 data; it also showed no effect of the detection method for *E. coli*, justifying the combination of 2017 (m-CCA) and 2018 (TBX) data. The linear mixed effect analysis showed that for all swimming ponds *E. coli* concentrations were highly significantly dependent on conductivity and

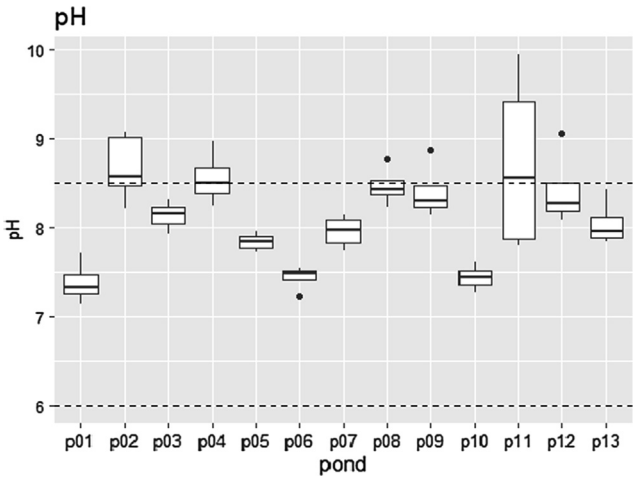


**Fig. 3.** Box whisker chart of the pH in thirteen different swimming ponds, sampled in 2017 (p01-p04) – 2018 (p05-p13). Each box shows the median and quartiles, the whiskers enclose 95% of the data, dots are outliers. The pH needs to stay between 6.0 and 8.5 (dashed lines).

significantly dependent on the $\log_{10}$ of turbidity. Concentrations of intestinal enterococci were highly significantly dependent of the $\log_{10}$ of turbidity. *P. aeruginosa* concentrations were highly significantly dependent on water temperature and significantly dependent on pH. The analysis also demonstrated that the swimming ponds were different with respect to these parameters: 31–45% of the random variation was explained by including 'pond' as a random effect in the model for *E. coli*, intestinal enterococci and *P. aeruginosa* (Table 4). The analysis also demonstrated that, for the investigated swimming ponds in 2018, some of which had no bottom cover, there was no significant effect on the microbiological parameters of having a bottom cover in place or not. In 2017, all swimming ponds investigated had a bottom cover.

## 4. Discussion

### 4.1. Microbiological water quality

The study demonstrated that the level of fecal contamination of the water in the investigated swimming ponds was generally low. In the cases that samples did not comply with the requirements for the fecal indicator parameters *E. coli* and intestinal enterococci in the new Dutch swimming pool legislation, the origin of the samples was restricted to a limited number of ponds. Swimming ponds with samples that occasionally exceeded the norm values for fecal indicators were diverse located at a campsite, an adventure park, a location that combined group accommodation and a campsite, and two wellness centers. These results demonstrate that any type of swimming pond is potentially prone to fecal contamination incidents, whether it is mainly used by children, such as the ponds at the adventure park and the group accommodation site, or mainly by adults, such as the ponds in the wellness centers. Moreover, the linear mixed effect analysis showed that 31–45% of the observed variability between swimming ponds could be explained by the factor 'pond'. This indicates that each swimming pond has its own characteristics that may influence the microbiological water quality, such as the type of additional water treatment, the size of the swim zone and the plant filter zone, but also the frequency of fecal incidents, the impact of contamination incidents like run off during heavy rainfall events, or the growth of naturally occurring microorganism such as *P. aeruginosa*. In all swimming ponds, the average water temperature was 20 °C or higher, however, concentrations of *P. aeruginosa* were low in all ponds. Casanovas-Massana and Blanch (2013) did similar findings in the ponds they studied in Spain. The linear mixed effect model nevertheless indicated the *P. aeruginosa*

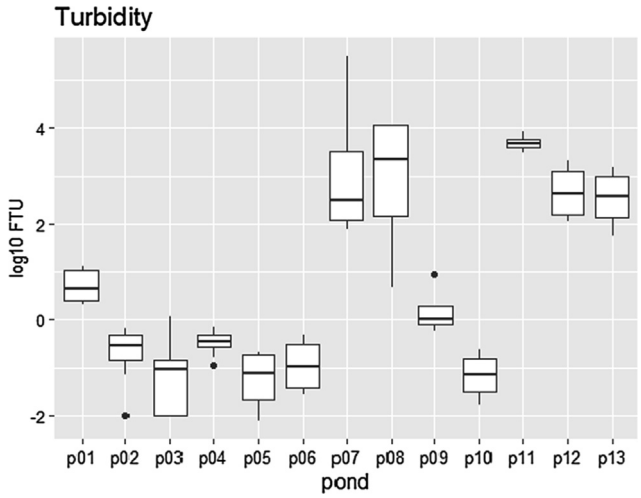


**Fig. 4.** Box whisker chart of the log10 transformed data of the turbidity in thirteen different swimming ponds, sampled in 2017 (p01-p04) – 2018 (p05-p13). Each box shows the median and quartiles, the whiskers enclose 95% of the data, dots are outliers. There are no requirements for turbidity in the draft legislation.

concentration was highly significantly dependent on the water temperature. *P. aeruginosa* grows within a broad temperature range, from 5 to 42 ˚C, with an optimum growth temperature of 37 ˚C (Mena and Gerba, 2009). This may explain the correlation between *P. aeruginosa* growth and water temperature, and suggests that proliferation of *P. aeruginosa* may cause health risks in swimming ponds during prolonged periods of elevated temperatures, as seen in surface water recreation (Schets et al., 2011).

The inclusion of *S. aureus* as a monitoring parameter in the requirements for the water quality of swimming ponds in the new Dutch swimming pool legislation has been a topic of debate. Its usefulness was questioned, and managers worried that the requirement in the new legislation could never be met. *S. aureus* is however an opportunistic pathogen that can cause skin rashes, wound infections, urinary tract infections, eye infections, otitis externa, impetigo and other infections in swimmers (WHO, 2006). Moreover, recreational waters with a high density of bathers present a risk of staphylococcal infection that is comparable to the risk of gastrointestinal illness involved in bathing in water considered unsafe because of fecal pollution (WHO, 2006). Since the occurrence of *S. aureus* in recreational water is correlated with the number of bathers (Goodwin et al., 2012; Fogarty et al., 2014), the inclusion of this parameter for monitoring of non-fecal shedding, gives swimming pond managers insight in the risks that may occur when too many people use the swimming pond simultaneously, and the limit of the capacity of the pond and its water treatment system has been reached. Scientific literature does not provide evidence for a norm value based on a dose-response relation for exposure to *S. aureus* or coagulase positive staphylococci in water. The present study has however demonstrated that the requirement of absence of *S. aureus* in 100 ml swimming pond water could be met in the studied swimming ponds, for most of the time (three of four sampling days for eight ponds, all sampling days for one pond).

### 4.2. Physical water quality parameters

Turbidity is not included as a requirement for swimming pond water in the new legislation. However, turbidity is a measure of floating particles and may therefore indicate increased bacterial numbers as a result of fecal accidents, the growth of microorganisms or algae, or run off from surrounding surfaces during heavy rainfall events, and the input of dirt, dust and other debris due to run off or wind. It may also give insight in the disinfection efficiency by solar UV radiation

**Table 3**
Microbiological water quality in swimming ponds, investigated in 2017 (p01-p04) – 2018 (p05-p13).

| swimming pond | parameter | $N_{samples}$ | mean | st.dev. | $min_{values}$ | $max_{values}$ |
|---|---|---|---|---|---|---|
| p01 | *E. coli* | 18 | 21 | 18 | 2.9 | 66 |
| | intestinal enterococci | 30 | 44 | 82 | 2.0 | 378 |
| | *P. aeruginosa* | 29 | 1.0 | 1.6 | 0.0 | 7.3 |
| | *S. aureus* | no data | | | | |
| p02 | *E. coli* | 15 | 3.7 | 3.9 | 0.0 | 13 |
| | intestinal enterococci | 25 | 6.2 | 11 | 0.3 | 46 |
| | *P. aeruginosa* | 27 | 0.4 | 1.0 | 0.0 | 4.3 |
| | *S. aureus* | no data | | | | |
| p03 | *E. coli* | 18 | 33 | 53 | 0.0 | 168 |
| | intestinal enterococci | 30 | 2.3 | 1.4 | 0.3 | 5.7 |
| | *P. aeruginosa* | 29 | 2.4 | 3.4 | 0.0 | 15 |
| | *S. aureus* | no data | | | | |
| p04 | *E. coli* | 18 | 0.5 | 0.7 | 0.0 | 3.1 |
| | intestinal enterococci | 30 | 1.2 | 1.4 | 0.0 | 6.0 |
| | *P. aeruginosa* | 30 | 0.6 | 0.8 | 0.0 | 2.9 |
| | *S. aureus* | no data | | | | |
| p05 | *E. coli* | 4 | 1.7 | 2.7 | 0.0 | 5.7 |
| | intestinal enterococci | 4 | 1.3 | 0.8 | 0.3 | 2.0 |
| | *P. aeruginosa* | 4 | 0.1 | 0.2 | 0.0 | 0.3 |
| | *S. aureus* | 4 | 3.4 | 6.8 | 0.0 | 14 |
| p06 | *E. coli* | 4 | 2.1 | 1.2 | 0.7 | 3.3 |
| | intestinal enterococci | 4 | 17 | 8.1 | 9.9 | 28 |
| | *P. aeruginosa* | 4 | 0.3 | 0.7 | 0.0 | 1.3 |
| | *S. aureus* | 4 | 0.1 | 0.2 | 0.0 | 0.3 |
| p07 | *E. coli* | 4 | 16 | 22 | 2.3 | 49 |
| | intestinal enterococci | 4 | 7.1 | 6.2 | 1.7 | 13 |
| | *P. aeruginosa* | 4 | 0.0 | 0.0 | 0.0 | 0.0 |
| | *S. aureus* | 4 | 17 | 34 | 0.0 | 68 |
| p08 | *E. coli* | 4 | 68 | 62 | 3.0 | 144 |
| | intestinal enterococci | 4 | 64 | 61 | 4.3 | 149 |
| | *P. aeruginosa* | 4 | 11 | 22 | 0.0 | 44 |
| | *S. aureus* | 4 | 8.6 | 17.3 | 0.0 | 34 |
| p09 | *E. coli* | 4 | 38 | 39 | 7.7 | 89 |
| | intestinal enterococci | 4 | 78 | 88 | 15 | 205 |
| | *P. aeruginosa* | 4 | 0.4 | 0.8 | 0.0 | 1.7 |
| | *S. aureus* | 4 | 4.9 | 9.7 | 0.0 | 19 |
| p10 | *E. coli* | 4 | 1.5 | 1.0 | 0.3 | 2.6 |
| | intestinal enterococci | 4 | 1.8 | 1.0 | 0.3 | 2.7 |
| | *P. aeruginosa* | 4 | 0.1 | 0.2 | 0.0 | 0.3 |
| | *S. aureus* | 4 | 0.5 | 0.9 | 0.0 | 1.9 |
| p11 | *E. coli* | 4 | 30 | 52 | 0.0 | 107 |
| | intestinal enterococci | 4 | 35 | 28 | 8.3 | 68 |
| | *P. aeruginosa* | 4 | 0.0 | 0.0 | 0.0 | 0.0 |
| | *S. aureus* | 4 | 0.0 | 0.0 | 0.0 | 0.0 |
| p12 | *E. coli* | 4 | 7.6 | 8.0 | 0.0 | 18 |
| | intestinal enterococci | 4 | 3.8 | 2.3 | 2.0 | 7.0 |
| | *P. aeruginosa* | 4 | 0.2 | 0.3 | 0.0 | 0.7 |
| | *S. aureus* | 4 | 47 | 95 | 0.0 | 190 |
| p13 | *E. coli* | 4 | 30 | 28 | 4.7 | 70 |
| | intestinal enterococci | 4 | 22 | 16 | 6.3 | 38 |
| | *P. aeruginosa* | 4 | 0.0 | 0.0 | 0.0 | 0.0 |
| | *S. aureus* | 4 | 1.0 | 2.0 | 0.0 | 4.0 |

(Christensen and Linden, 2003). The linear mixed effect analysis indicated that *E. coli* and intestinal enterococci concentrations were significantly dependent on the turbidity, i.e. $\log_{10}$ concentrations of *E. coli* and intestinal enterococci increase by 0,3 $\log_{10}$ (a factor of 2) and 0,4 $\log_{10}$ (a factor of 2.5), respectively, with each $\log_{10}$ increase of

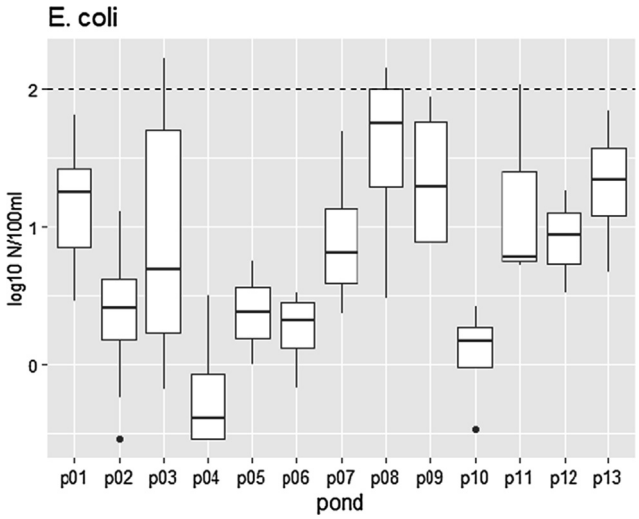


**Fig. 5.** Box whisker chart of the log10 transformed *E. coli* concentrations in thirteen different swimming ponds, sampled in 2017 (p01-p04) – 2018 (p05-p13). Each box shows the median and quartiles, the whiskers enclose 95% of the data, dots are outliers. The *E. coli* concentrations need to be equal to or below 100 cfu/100 ml (dashed line).

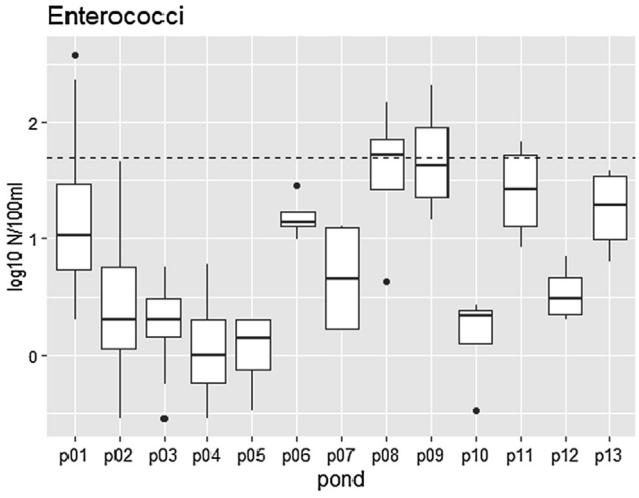


**Fig. 6.** Box whisker chart of the log10 transformed intestinal enterococci concentrations in thirteen different swimming ponds, sampled in 2017 (p01-p04) – 2018 (p05-p13). Each box shows the median and quartiles, the whiskers enclose 95% of the data, dots are outliers. The intestinal enterococci concentrations need to be equal to or below 50 cfu/100 ml (dashed line).

turbidity. This all suggests that turbidity is a meaningful parameter.

No significant effect of the presence of a bottom cover was found. Note that only three ponds without bottom cover were included in the study. However, the latter three swimming ponds did not meet the requirement for transparency, which may be the result of dissolved particles from the natural soil. In swimming ponds that do not meet the transparency requirement, safety, related to drowning, is an aspect that needs specific attention. This also applies to shallow swimming ponds in which the whirling of sand may cause loss of transparency resulting in an increased risk of unnoticed drowning for small children. In this study, two shallow swimming ponds with bottom cover, merely used by children, did not meet the transparency requirement either. This is probably related to the shallowness and high turbulence caused by the children.

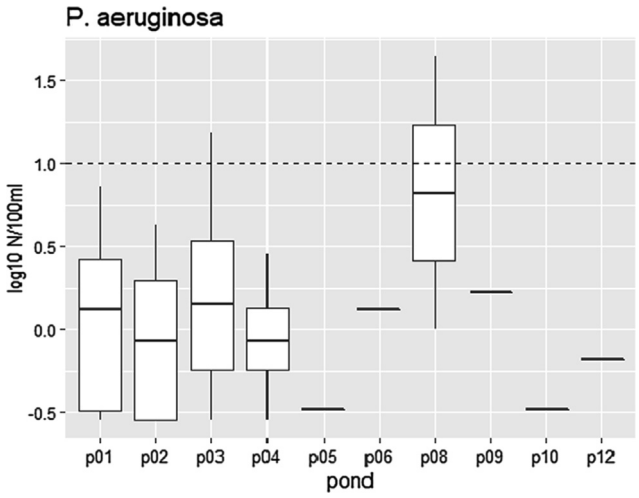


**Fig. 7.** Box whisker chart of the log10 transformed *P. aeruginosa* concentrations in thirteen different swimming ponds, sampled in 2017 (p01-p04) – 2018 (p05-p13). Each box shows the median and quartiles, the whiskers enclose 95% of the data, dots are outliers. The *P. aeruginosa* concentrations need to be equal to or below 10 cfu/100 ml (dashed line).

### 4.3. Analytical methods for microbial water quality parameters

The methods for the enumeration of *E. coli* and *P. aeruginosa* in swimming pond water, as included in the new Dutch swimming pool legislation (ISO 9308-1:2014, and ISO 16266:2006, respectively), are not suitable for analysis of water with large numbers of background bacteria, such as surface water and swimming pond water. Therefore, alternative detection methods for these parameters were used in this study.

For enumeration of *E. coli*, the method described in ISO 9308-1:2014 is not suitable, but the modification of this method as described by Jozić et al. (2018) worked well. However, an in-house method comparison study according to ISO 13843:2017 performed in 2018 (data not shown) demonstrated that the reduction of growth of background flora was more profound when culturing on TBX agar, and that *E. coli* colonies were easier to distinguish from non-*E. coli* colonies on this culture medium. The performance characteristics of both methods did not differ (data not shown), and therefore, *E. coli* analyses in 2018 were done by using TBX agar. For enumeration of *P. aeruginosa*, the method described in ISO 16266:2006 is not suitable (Anonymous, 2009); this method can only be applied to water with a low background flora. The most probable number method described in ISO 16266-2:2018, applied in this study, appeared unsuitable for analysis of swimming pond samples because of false positive results, despite the fact that the scope of the method includes 'waters containing high background counts of heterotrophic bacteria'. The method was used for analysis of the 2017 samples, but the poor results were disregarded. The use of the membrane filtration method on mPA-C agar, according to an older national standard (NEN 6573:1987), was satisfactory.

Enumeration of *S. aureus* using the method based on the horizontal method described in ISO 6888-2:1999 without an additional confirmation step in 2017 yielded a high number of false positive results. The opaque halo around the black colonies op BPA-RPF was difficult to read and to interpret. Addition of an extra pure culture step on BPA-RPF enhanced the readability of the opaque halo, and improved the reliability of the results. This method however includes upside-down incubation of Tuffryn membrane filters, which is a methodological imperfection, and moreover, it recently became clear that these membrane filters are no longer manufactured. The use of chromogenic agar media for the analysis of the 2018 samples was promising, but needs further study. Water is not the primary commodity these methods were developed for, nor is membrane filtration the intended method of use.

**Table 4**
Summary of significant associations from linear mixed model analyses on all data.

| | fixed effects | significance level | % of variance of pond (random intercept) |
|---|---|---|---|
| *E. coli* | conductivity | ** | 31% |
| | $Log_{10}$FTU | * | |
| intestinal enterococci | $Log_{10}$FTU | *** | 45% |
| *P. aeruginosa* | water temperature | *** | 40% |
| | pH | * | |

Significance level: *** < 0.001; ** < 0.01; * < 0.05.

### 4.4. Water quality guidance

Although the water quality in the investigated swimming ponds was generally good and microbiological contamination was restricted to a limited number of samples in a limited number of ponds, swimming ponds are vulnerable systems. There is no instantaneous water disinfection, and in situations where a fecal contamination incident occurs, fecal pathogens are not rapidly inactivated. During heavy bather load, high numbers of swimmers are at risk of infections by enteric waterborne pathogens, as occurred during an outbreak of viral meningitis in Germany in 2011, which is a more serious condition than most ear and wound infections (Hauri et al., 2005). Moreover, high numbers of bathers themselves may also have a negative impact on the microbiological water quality, particularly when these numbers exceed the number of bathers the swimming pond was designed for. Also, when naturally occurring microorganisms grow to levels that can cause illness in human, for instance during extremely warm weather, high numbers of swimmers may be at risk of contracting skin, wound, or ear infections. Extreme weather events with heavy rainfall may cause surface run off that can negatively affect the microbiological water quality. Such extreme warm and/or wet weather events are likely to occur more often in the near future. These potential risks warrant legislation to ensure the microbiological safety of swimming pond water. Giampaoli et al. (2014) investigated the presence of regulations related to swimming ponds in Europe, and showed that some individual countries have a policy regarding swimming ponds, such as Austria, Italy, France, Germany and Switzerland. They conclude that the expansion of the number of swimming ponds and their possible health risks underline the need for Europe-wide guidelines or regulations.

## 5. Conclusions

The microbiological water quality in the investigated swimming ponds was generally good: the requirements for fecal indicators and *P. aeruginosa* in the new Dutch swimming pool legislation were met in respectively 62% and 85% of the 13 investigated swimming ponds.

*S. aureus* is a relevant parameter for non-fecal shedding, although scientific literature does not provide evidence for a norm value based on a dose-response relation for exposure to *S. aureus* in water; the requirement in the new draft legislation was met in 68% of the samples.

The standard analytical methods for the enumeration of microbiological parameters in conventional swimming pools cannot be used for the monitoring of water in swimming ponds due to the presence of large numbers of background bacteria; more selective methods, such as those used in this study, are needed.

Possible risks due to pathogens in swimming pond water (that by definition lacks disinfection), warrant guidelines or regulations to ensure the microbiological safety of this water. The new draft Dutch swimming pool legislation provides such guidance.

## CRediT authorship contribution statement

**Franciska M. Schets:** Conceptualization, Validation, Writing - original draft, Supervision, Project administration, Funding acquisition. **Harold H.J.L. van den Berg:** Validation, Writing - review & editing. **Gretta Lynch:** Investigation. **Sharona de Rijk:** Investigation. **Ana Maria de Roda Husman:** Conceptualization, Writing - review & editing. **Jack F. Schijven:** Methodology, Formal analysis, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105516.

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