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### RESEARCH ARTICLE

## STUDY OF WATER HYDROCHEMICAL AND MICROBIOLOGICAL QUALITY IN THE NOOGENIC HABITAT OF THE BLACK SEA BOTTLENOSE DOLPHINS

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### Abstract

The presented study was aimed at the regular surveillance of the water hydrochemical and microbiological quality in the noogenic habitats of marine mammals located in the Black Sea coastal area of Georgia, in particular, in the Batumi dolphinarium. The study period covered 34 months (March, 2012 - December, 2014). The regular monitoring of specific living conditions in noogenic habitats of dolphins, such as inspection of water chemical and microbial quality, is of great importance for prevention of various diseases in dolphins, primarily of infectious origin. Significant correlation (both positive and negative) was revealed between a number of physical-chemical and microbiological parameters. The performed studies indicate the significance of a rational management of artificial (noogenic) environment for marine mammals and can provide a good ground for maintenance of animal's health status and for their protection from microbial infections.

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The morbidity and mortality rates of marine mammals, the Black Sea dolphins among them, may be quite different for free-living populations and for those inhabiting noogenic environments such as oceanariums. This greatly depends on the origin of animals, geographic and climatic zones, animal density, animal care culture, etc. The regular monitoring of specific living conditions in dolphin's noogenic habitats, such as inspection of water chemical and microbial quality, is of great importance for prevention of various, primarily infectious, diseases in dolphins.

It is commonly understood that biological pollution in the marine water environment leads to changes in the structure of microbial communities, breaking natural biological processes in the sea in and thus contributing to accumulation of pathogenic microorganisms in the coastal waters that may represent a threat for various hydrobionts as well as for humans (4). The water microbial quality of water reservoirs can be determined by the resident bacteria, such as vibrios, aeromonads and others, and also by allochthonous (non- indigenous, permanently changing) microflora that enters biocenosis via biological pollution. The quantitative content of this group of bacteria, in particular, coliforms (*E.col*, *Citrobacter*, *Enterobacter*, *Klebsiella*, etc.) and enterococci serve as a commonly used sanitary indicators of possible fecal contamination in the water reservoirs (4,7,16).

This study was aimed at the regular surveillance of the water hydrochemical and microbiological quality in the noogenic habitats of marine mammals located in the Black Sea coastal area of Georgia, in particular, in Batumi dolphinarium. The study period covered 34 months (March, 2012 - December, 2014).

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The collection of water samples and their consequent analysis has been performed with various frequencies (see Table 1). In particular, the physical - chemical parameters, such as water temperature, pH, salinity, oxidation–reduction potential (ORP) was measured using following devices: pH Testr10 “Wagtech”, Digital Refractometer “Sper Scientific”, ORP Testr 10 “Waterproof”. The content of free and total chlorine have been detected by colorimetric method, using Colorimeter C401 “Eutech Instruments” (11). The quantitative content of biogenic elements have been studied by spectrophotometric method, using DR5000 “Hach Lange”, in accordance to internationally accepted methodology (3,7,8,9,10) and following the manufacturer’s instructions (3).

**Table 1:** The list of water physical-chemical parameters monitored during the study

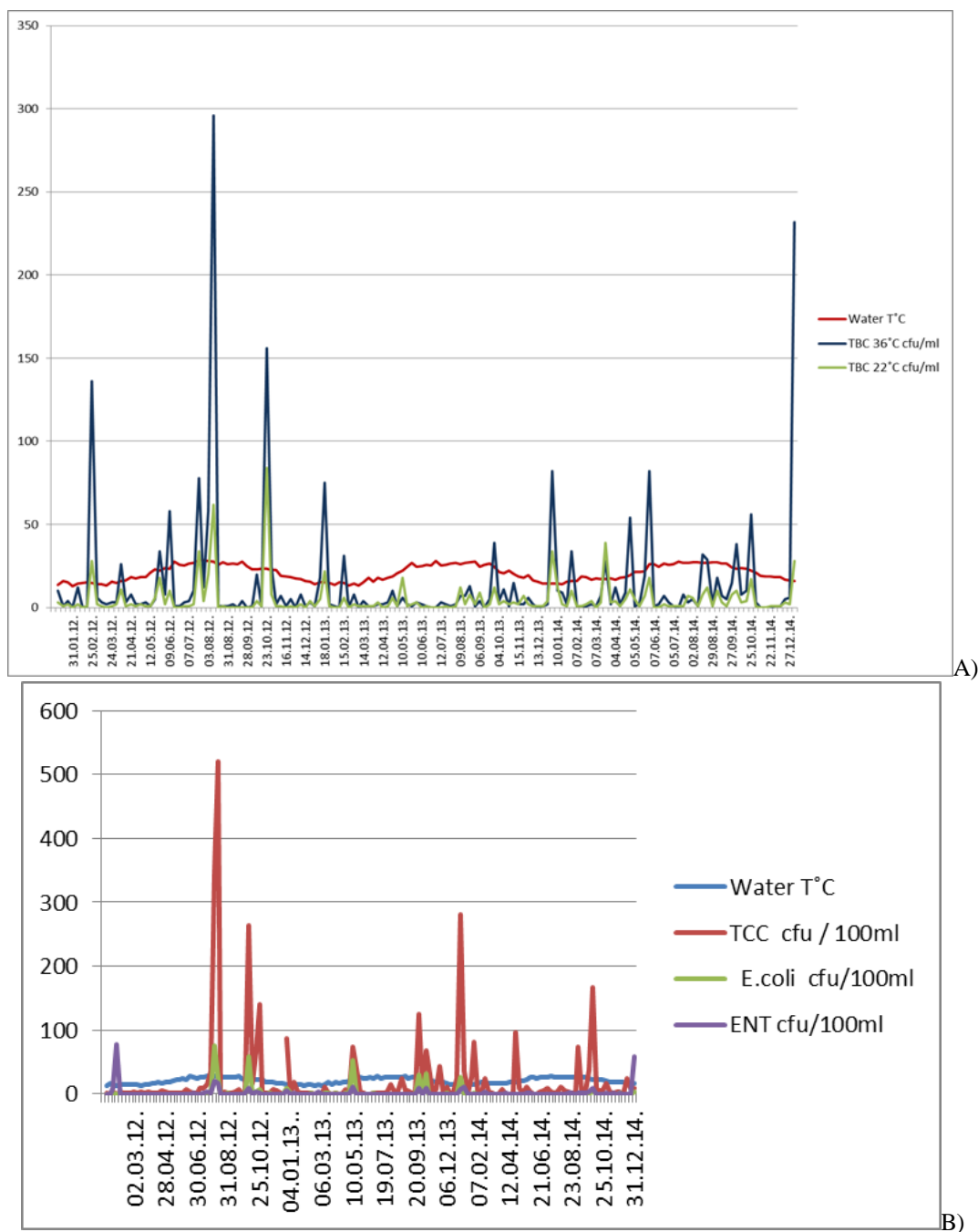
Hydrochemical parameters	Frequency of testing	Data obtained (range min-max)
Water temperature (T <sup>0</sup> C)	2 times per day	12,8-28,6
Free chlorine (mg/l)	2 times per day	0.09-1.33
Total chlorine (mg/l)	2 times per day	0.18-1.78
ORP ( mv)	2 times per day	369-821
pH	2 times per day	7.09-7.85
Salinity ( ppt)	Once per week	17-18
Dissolved oxygen mg/l	2 times per month	7.22-9.88
Nitrites (NO <sub>2</sub> <sup>-</sup> ) mg/l	2 times per month	0.014-0.069
Nitrates (NO <sub>3</sub> <sup>-</sup> ) mg/l	2 times per month	0.604-3.86
Phosphates (PO <sub>4</sub> <sup>3-</sup> ) mg/l	2 times per month	2.34-9.17
Ammonium (NH <sub>4</sub> <sup>+</sup> ) mg/l	2 times per month	0-0.033

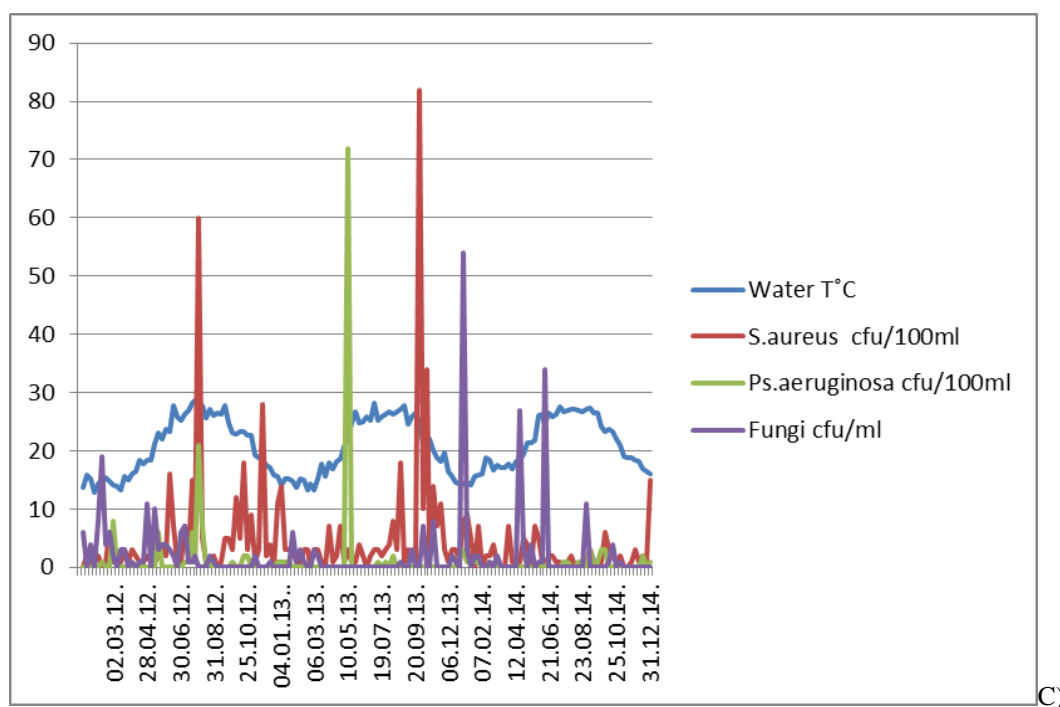
In parallel, the water samples have been tested with one week interval for following microbiological parameters: 1. Total (viable) Bacterial Counts (TBC) per 1 ml at 36° C temperature; 2. TBC per 1 ml at 22° C ; 3. Total coliform counts (TCC) per 100 ml of water sample at 36° C; 4. E. coli counts per 100 ml at 45° C; 5. Total enterococci ( ENT) per 100 ml; 6. Number of *Staphylococcus aureus* /100ml sample ; 7. *Pseudomonas aeruginosa* / 100ml; 8. *Salmonella spp.* and *Proteus spp.* per 1ml; 9. Number of yeasts and molds 1 ml . The parameters №1-2 were determined by pour plate technique and parameters №3–7 - by membrane filtration methodology (9,14,16); For parameters №8-9 – using Rida Count *Salmonella* / *Enterobacteriaceae* and Rida Count Yeast&Mold Rapid (R-Biopharm AG, Germany) have been used according to the manufacturer’s instructions.

It is known that the water quality in the open water pools, including marine mammal pools, is mostly influenced by the water temperature and rain –associated effluents, also by content of free chlorine and the fluctuations in the ORP (15, 15). The monitoring conducted during 2012-2014 in the studied noogenic habitat of marine mammals in Batumi demonstrated the obvious stability of the mentioned parameters throughout the year, only varying within the normal (permissible) ranges. This is due to modern high quality re-circulatory water supply system operating at the Batumi Dolphinarium. The facility is equipped with the systems for mechanical water filtration, ozonization, chlorine dosing, water heating and cooling. In turn, the controlled physical and chemical parameters in the water pool largely determine other controlled parameters in the water bath to ensure stability of microbiological indicators.

Our observations revealed that the water microbial quality in the Batumi dolphinarium was permanently changing (Fig. 1A, B,C) although remained mainly in line with the set of microbiological standard parameters, recommended by international regulations for keeping marine mammals in noogenic habitats (14). During 34 month of monitoring we collected and examined 168 samples from the dolphin’s pools in Batumi. Out of this amount of samples only a few exceeded the allowable limits for the TBC at 37<sup>0</sup>C (mesophilic aerobes and facultative anaerobes), while the TBC at 22<sup>0</sup>C did not go beyond the standard range. Fecal contamination of water samples has been periodically registered with TCC exceeding the normal rates only for 13 samples, while increased value of *E.coli* and enterococci was more often excessive of allowable standards (in 30 and 35 samples, respectively ). Elevated numbers of *S. aureus* were also frequently registered - in 89 samples (52.66 % of total number of samples) with various physical and chemical parameters. Considerably less but still remarkable was detection rate for *P. aeruginosa*: This opportunistic pathogen was detected in 39 samples that comprised 41.07 % of total samples. However, it should be noted that in the vast majority of cases these figures weren’t significantly higher than the permissible norms. As for the content of fungal flora (yeasts and moulds), this was observed in 27.38 % of samples, mainly in those with the minimum bacterial counts.

The statistical analysis of the accumulated data was done using Statistical Toolpack for the Microsoft Excel 2010. The correlation data provided here are considered to be significant, with the level of reliability of  $P \leq 0.05$ . Significant correlation (both positive and negative) was revealed between a number of physical-chemical and microbiological parameters. In particular, negative correlation was observed between amount of free chlorine in the water and the total microbial numbers ( $r = -0.21$ ), as well as for the total chlorine and viable numbers of *E.coli* and *S. aureus* ( $r = -0.16$  and  $r = -0.19$ , respectively). Significant negative correlation was registered for Oxidation – Reduction Potential (ORP) and total bacterial abundance (TBC) at  $37^\circ\text{C}$  and  $22^\circ\text{C}$  ( $r = -0.28$  and  $r = -0.33$ , respectively), and to a lesser extent ( $r = -0.18$ ) with number of both - total coliforms and enterococci. A high positive correlation was shown between several individual microbiological parameters, such as TBC ( $37^\circ\text{C}$ ) and TCC ( $r = 0.64$ ), TBC ( $37^\circ\text{C}$ ) and ENT ( $r = 0.39$ ), also *E.coli*- *S. aureus* ( $r = 0.6$ ), and *E.coli*- *P.aeruginosa* ( $r = 0.58$ ).





**Figure 1.** Fluctuation of water microbial parameters in the pools of the Batumi dolphinarium in 2012-2014: A) total bacterial counts; B) fecal pollution indicators; C) staphylococci, pseudomonads and fungi;

In general, water microbial parameters showed certain seasonal changes (Fig. 1 A,B,C), which can be linked to a natural (uncontrolled) variation of water temperature, especially with the temperature rise in summer – early autumn months. We suppose that increased anthropogenic impact in touristic season also leads to certain growth of water pollution indicators. The episodes of worsening on microbial quality during cold season especially after rainy periods may reflect external microbial load due to water precipitation. In some (very rare) cases considerably low microbial quality coincided with the episodes of failure in the pool water management system.

The performed studies indicate the significance of a rational management of artificial (noogenic) environment for the marine mammals, as well as need in regular monitoring of water quality. In agreement with other observations (13, 15) such strategy can provide a good ground to maintain the health status of animals and to protect them from infections.

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