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Variations of Culturable and Metabolically Active Bacteria in a Stratified Water Column: The Example of Istanbul and Çanakkale Straits, Turkey

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ABSTRACT: The Dardanelles (Çanakkale Strait) and Bosporus (Istanbul Strait) which have unique hydrographical characteristics were compared regarding the metabolically active bacteria, culturable heterotrophic bacteria (HPC) and their relation to the nutrients and variable environmental parameters. The samples were taken from various depths ranging from 0-30 cm to 50 meters. The halocline stratifications and mixture layer were determined with CTD (SBE 19 SEACAT Profiles). The spread plate technique was used for detecting HPC. The modified staining method was applied for identifying of the metabolically active bacteria by using epifluorescense microscope technique. The highest HPC counts were recorded to be 85±0.2x108 CFU/100 ml within the interlayer of the water column (20 m) between upper and bottom currents which were formed by the saline waters of the Mediterranean and the less saline waters of the Black Sea in the Istanbul Strait. The results show that the bacterial properties of these massive water bodies were modified by nutrient accumulation, due to the fact that terrestrial inputs with respect to chemical and biological pollution within the mixed layer. The highest metabolically active bacteria were recorded in the samples which were taken from 0-30 cm of upper layer (20-25 meters) of the Black Sea waters which enter through the Istanbul Strait. A significant positive correlation was recorded between temperature and metabolically active bacteria. The obtained results could contribute to understand variations of the bacterial activity and abundance and manage bacterial processes in a stratified water system of other marine areas of the world.

Key words: Active bacteria, Canakkale Strait, Istanbul Strait

INTRODUCTION

Heterotrophic bacteria are a main mediator in the decomposition of organic substances and the recycling of the nutrients in marine environments. In addition, the most active group of them all, with respect to mineralization of organic substances, is the aerobic heterotrophic bacteria at the euphotic zone in marine ecosystems. Besides, ecological and biogeochemical processes including the processing of organic and inorganic nutrients manage the bacterial activities in marine environments (Pomeroy, 1980; Azam *et al.*, 1983; Azam and Fuhrman, 1985). The functions on bacterial abundance and distribution are under the control of biotic and abiotic environmental factors as well as the competition for available nutrients (Sanders et al., 1992; Jürgens and Gude, 1994). Due to this dynamic structure of the marine environments, bacteriological studies, which were associated with the environmental factors, have been conducted. However, the bacterial dynamics of specific marine environments such as the characteristic regions under poorly described conditions, mentioned below, are still a challenge.

The interactions between the levels of heterotrophic aerobic bacteria and the metabolically active bacteria are also an important parameter to understand the ecosystem functioning in marine environments. However, the relationships between the culturable and the metabolically active bacteria remain poorly understood. Despite the fact that the culture independent studies serve as common applications for identifying bacterial species, recent studies have demonstrated that the cultured strains of marine bacteria represent a significant part of the community DNA in sea water (Pinhassi *et al.*, 1997). In addition, the bacteria that are able to form colonies on solid media were reported to be a large accounted fraction of the bacterioplankton (Rehnstam *et al.*, 1993; Pinhassi *et al.*, 1997).

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The metabolically active bacteria count can be estimated via studies regarding the contribution of the capsulated bacteria to the total bacterial community in aquatic environments (Heissenberger *et al.*, 1996). Although the ecological importance of the metabolically active bacteria in an ecosystem cycles on a global scale, the data on the fraction of active versus dormant bacterial cells and metabolic and physiological state of bacteria in marine environments is deficient. Furthermore, the impacting factors on the activity or inactivity in situ bacterial populations in marine areas are still a challenge too.

Due to the fact that the bacteria have a rapid respond capacity to the environmental changes in marine areas; Turkish Straits System (TTS) is a characteristic area to investigate the distribution of the heterotrophic and metabolically active bacteria. The Istanbul Strait connects the Sea of Marmara to the Black Sea and the Çanakkale Strait to the Aegean Sea. The hydrodynamic characteristic of the Istanbul Strait is defined by two layer stratification, depending on the salinity. While the concentrated saline waters of the Mediterranean (average annual salinity: 38PSU) reach the Black Sea via the underflows of the Çanakkale and Istanbul Straits, the less saline waters of the Black Sea (average annual salinity: 16.5-18.5 PSU) reach the Mediterranean via upper flows (Oguz *et al.*, 1990). This system creates differences on the biochemical properties of the upper and lower layers (Bastürk *et al.*, 1990; Besiktepe *et al.*, 1994, Tugrul and Polat, 1995; Tugrul et *al.*, 1995; Cociasu *et al.*, 1997). However, the influences of this variable status on the abundance of heterotrophic and metabolically active bacteria are still a challenge.

In this study by taking different oceanographic peculiarities of Istanbul and Canakkale Straits into consideration; distributions, spatial and temporal variations of culturable heterotrophic and metabolically active bacteria and in relation to variable environmental parameters were investigated in the sea water samples which were taken from 0 cm to 10, 25, 40 and 50 meters depth from stratified water column of Istanbul and Çanakkale Straits, Turkey.

MATERIALS & METHODS

The samples used in the analysis were collected in a Nansen bottle that had been cleaned with acid (10% HCL in distilled water), sterilized with alcohol (50:50, v/v), and rinsed with sterile water. The samples were transferred into 250-mL sterile brown glass bottles under aseptic conditions and processed on board the Istanbul University research vessel YUNUS-S. Sea water samples were taken from 0-30 cm surface and from various depths ranging from 10 to 50 meters (Fig. 1). The study period began in July 2006 and ended in June 2007 and was carried on seasonally. The sampling locations were shown on the Table 1 and in Fig. 1.

Temperature (°C), dissolved oxygen (mg/L), and salinity (‰) values were measured in situ using CTD SBE-19 SEACAT Profiler or portable multi-parameter (Hach Lange HQ 40D) in the stations.Standard 4500 NH3-F method for Ammonia (NH3), 4500 N03-E/ cadmium reduction method for nitrates (mg $NO3^-$-N/L), 4500 N02-B method for nitrites (mg $NO2^-$-N/L) , 4500-P E method for phosphorus and 10200 method for chlorophil- a analyses were used (APHA, 2000).

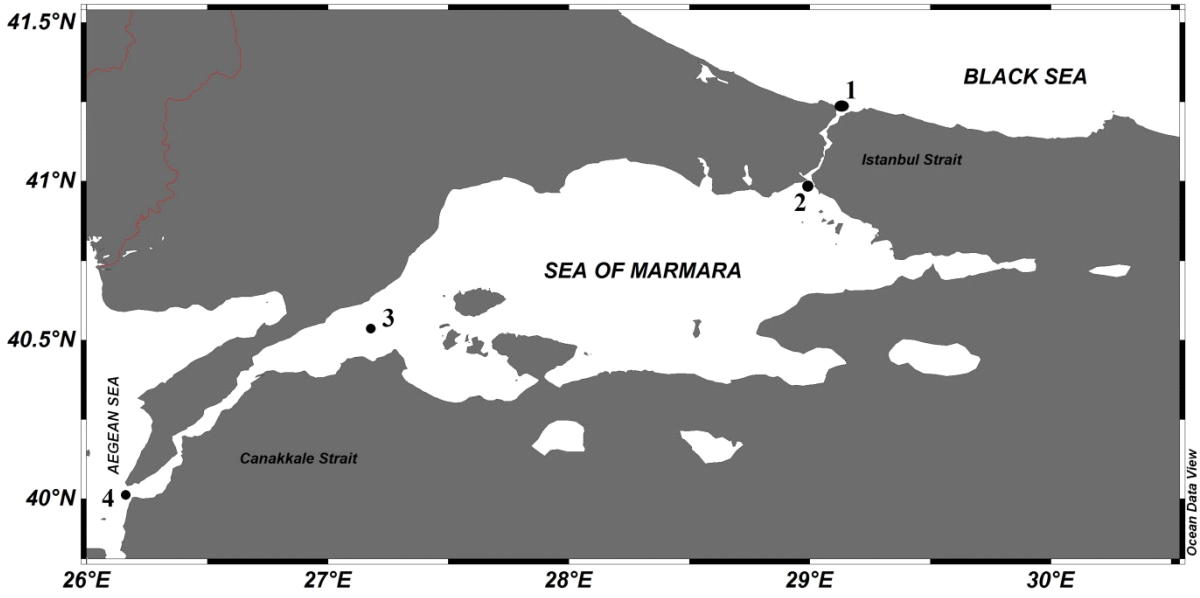

Fig. 1. Study area

Table 1. The stations and sea water sampling depths

Code	Stations	Depth	Station Coordinates
1	Black Sea entry of the Istanbul Strait	0-30 cm 10 m 20 m 50 m	*N 41°12.612'* *E 29° 07.322'*
2	The Sea of Marmara exit of the Istanbul Strait	0-30 cm 10 m 20 m 40 m	*N 41°00.361'* *E 29°00.062'*
3	Çanakkale Strait entry	0-30 cm 25 m 50 m	*N 40° 25 619'* *E 26° 48 906'*
4	Çanakkale Strait exit	0-30 cm 25 m 50 m	*N 40° 00 756'* *E 26° 14 605'*

Total bacteria cells were tested from formaldehyde-fixed (2%; [final concentration]) samples stained with 4', 6' diamidino-2-phenylindole dihydrochloride (DAPI; Sigma). Each sample was filtered onto black polycarbonate filters (0.2µm pore size, Millipore) which was then examined with an epifluorescense microscope (Olympus, BX51) at 1250X magnification. At least 300 bacteria per filter were counted (Porter and Feig, 1980). Principally the same method as Stoderegger and Herndl (2001), which is a modification of Plante and Shriver (1998) staining method, was used to discriminate capsulated from non-capsulated bacteria. The slides were coated with gelatin using 0.1% gelatin solution and 0.01% CrK(SO4)2 x 12.H2O and stored frozen until use. Each sample was fixed with 2% glutaraldehyde (final conc.) and filtered onto 0.2 ?m polycarbonate filter. The filter was transferred on the gelatin-coated slide and frozen in a horizontal position. It was stored at -20 ºC until analysis. Before enumeration, the filter was thawed. The filter area was coated firstly with 0.25 % Congo red (3 to 5 drops), thereafter with Maneval's stain (three to four drops about 1 min.). The slide was examined under a phase contrast microscope (Stoderegger and Herndl, 2001).

The sea water samples were collected in sterilized glass bottles, serial dilutions of 10-5 were prepared in 9-mL amounts of sterile artificial seawater (Sigma) and inoculated (0.2 mL) in duplicate on marine agar (Difco). The colonies were counted after 5 days of incubation at 22 ± 0.1 °C (Bianchi *et al.,* 1992).

RESULTS & DISCUSSION

Table 2. Seasonal variations of the metabolically active bacteria (%), heterotrophic aerobic bacteria count (CFU/100 ml), salinity (‰), dissolved oxygen (mg/l) and seawater temperature (°C) in Istanbul and Canakkale StraitsTable 2. Seasonal variations of the metabolically active bacteria (%), heterotrophic aerobic bacteria count (CFU/100 ml), salinity (‰), dissolved oxygen (mg/l) and seawater temperature (°C) in Istanbul and Canakkale StraitsSeasonal variations of the metabolically active bacteria, the total number of heterotrophic aerobic bacteria, dissolved oxygen, the salinity and the seawater temperature of the samples which were taken from the Istanbul and Canakkale Straits, are summarized on Table 2.

The values of NO2, NO3, PO4 and chlorophyll-a in the sea water samples were taken from Istanbul Strait are shown in Figs 2.The concentrations of NO2, NO3, PO4 and chlorophyll-a in the sea water samples were taken from Canakkale Strait are shown in Figs 3.

The highest chlorophyll-a values were recorded in the spring and summer seasons in both straits during the study period.

While the highest HPC was found out as 85±0.2x108 CFU/100 ml in the seawater samples which were taken from depth of 20 m below Istanbul Strait in the summer season, the lowest HPC was recorded as57±0.2x10 CFU/ 100 ml in the samples which were taken from the depth of 50 m of Canakkale Strait in the autumn.

The metabolically active bacteria level was recorded as 47% in the surface waters which were taken from 0-30 cm below of the Istanbul Strait in the summer season. The salinity values of the deep waters were recorded as significantly higher ($p<0.001$) than surface water of the both straits. The highest salinity value was recorded 39PSU in the samples taken from the depth of 40 meters of the Çanakkale Strait in the spring season.While the minimum sea water temperature value was recorded as 7.20 °C from the depth of 40 m at the Istanbul Strait in winter season, the maximum tempera-

Table 2. Seasonal variations of the metabolically active bacteria (%), heterotrophic aerobic bacteria count (CFU/ 100 ml), salinity (‰), dissolved oxygen (mg/l) and seawater temperature (°C) in Istanbul and Canakkale Straits

S	St	No	Depths	HPC	MAB %	Salin ‰	Temp °C	DO mg/l
Summer	Istanbul Strait	1	0-30 cm	$15\pm0.1x10^{7}$	46	17,00	18,90	4,21
			10 m	$12\pm0.4x10^{6}$	33	24,10	15,40	5,37
			20 m	$28\pm0.4x10^{6}$	41	26,80	13,70	1,63
			40 m	$13\pm0.3x10^{7}$	31	38,80	16,50	0,94
		2	0-30 cm	$15\pm0.5x10^{8}$	**47**	**15,40**	18,40	4,22
			10 m	$35\pm0.4x10^{8}$	35	15,90	18,00	6,19
			20 m	$\mathbf{85\pm0.2x10^{8}}$	41	16,90	16,20	2,91
			40 m	$42\pm0.4x10^{8}$	31	18,50	14,60	4,77
			50 m	$71\pm0.4x10^{8}$	33	30,10	15,90	1,64
	Canakkale Strait	3	0-30 cm	$72\pm0.2x10^{3}$	33	21,85	**22,42**	6,26
			25 m	$89\pm0.2x10^{3}$	31	37,82	14,57	6,13
			50 m	$64\pm0.2x10^{3}$	30	38,85	14,85	7,19
		4	0-30 cm	$66\pm0.2x10^{3}$	34	28,14	20,22	7,67
			10 m	$72\pm0.2x10^{3}$	33	38,91	14,95	7,67
			50 m	$20\pm0.2x10^{3}$	30	38,88	14,67	7,59
Autumn	Istanbul Strait	1	0-30 cm	$14\pm0.3x10^{5}$	41	24,09	16,50	6,08
			10 m	$12\pm0.4x10^{5}$	28	36,72	14,76	2,40
			20 m	$11\pm0.5x10^{5}$	36	27,05	14,75	5,82
			40 m	$14\pm0.6x10^{5}$	27	37,99	13,67	0,78
		2	0-30 cm	$16\pm0.2x10^{6}$	42	17,80	18,71	8,04
			10 m	$17\pm0.3x10^{6}$	30	17,80	18,71	8,07
			20 m	$14\pm0.1x10^{6}$	36	17,80	18,72	8,07
			40 m	$13\pm0.2x10^{6}$	26	21,46	18,65	7,92
			50 m	$15\pm0.3x10^{6}$	28	28,86	17,47	5,40
	Canakkale Strait	3	0-30 cm	$70\pm0.2x10^{3}$	19	22,50	20,27	6,22
			25 m	$15\pm0.2x10^{3}$	17	---	-----	-----
			50 m	**57±0.2x10**	14	37,85	17,61	7,43
		4	0-30 cm	$21\pm0.2x10^{4}$	21	28,17	17,33	5,38
			10 m	$20\pm0.2x10^{4}$	15	32,81	16,13	7,56
			50 m	$19\pm0.2x10^{4}$	20	38,95	14,79	7,30
Winter	Istanbul Strait	1	0-30 cm	$13\pm0.1x10^{7}$	37	19,00	8,30	4,64
			10 m	$11\pm0.2x10^{6}$	24	26,50	10,80	2,36
			20 m	$34\pm0.9x10^{5}$	30	28,20	11,70	0,37
			40 m	$14\pm0.2x10^{7}$	22	24,60	10,30	1,06
		2	0-30 cm	$15\pm0.2x10^{7}$	38	18,60	7,30	4,61
			10 m	$13\pm0.2x10^{7}$	26	17,40	7,30	5,80
			20 m	$12\pm0.3x10^{7}$	32	17,40	7,30	4,91
			40 m	$11\pm0.2x10^{6}$	22	28,60	**7,20**	3,11
			50 m	$10\pm0.3x10^{5}$	22	36,60	**7,20**	0,36
	Canakkale Strait	3	0-30 cm	$25\pm0.2x10^{3}$	13	26,46	9,00	7,15
			25 m	$11\pm0.2x10^{3}$	**12**	----	-----	-----
			50 m	$93\pm0.2x10^{2}$	10	38,15	14,22	7,39
		4	0-30cm	$18\pm0.2x10^{3}$	18	31,66	11,18	6,04
			10 m	$10\pm0.2x10^{3}$	**12**	31,59	10,97	**8,27**
			50 m	$29\pm0.2x10^{2}$	15	38,87	14,03	6,97
Spring	Istanbul Strait	1	0-30 cm	$15\pm0.2x10^{8}$	44	19,30	14,90	3,64
			10 m	$13\pm0.5x10^{8}$	30	24,60	13,40	1,36
			20 m	$13\pm0.6x10^{6}$	39	27,20	11,70	0,63
			40 m	$13\pm0.4x10^{7}$	28	39,00	15,50	**0,06**
		2	0-30 cm	$14\pm0.2x10^{7}$	45	18,50	16,40	3,61
			10 m	$16\pm0.2x10^{7}$	32	18,80	16,00	4,80
			20 m	$15\pm0.7x10^{7}$	39	18,80	14,20	3,91
			40 m	$13\pm0.2x10^{7}$	29	19,00	12,60	2,11
			50 m	$12\pm0.2x10^{7}$	30	35,00	13,90	0,64
	Canakkale Strait	3	0-30 cm	$48\pm0.2x10^{4}$	25	23,04	16,16	6,52
			25 m	$35\pm0.2x10^{4}$	27	-----	------	------
			50 m	$12\pm0.2x10^{4}$	27	38,88	16,02	7,69
		4	0-30 cm	$86\pm0.2x10^{3}$	30	29,21	16,17	7,41
			10 m	$39\pm0.2x10^{4}$	31	-----	------	-------
			50 m	$28\pm0.2x10^{4}$	29	**39,11**	16,03	7,98

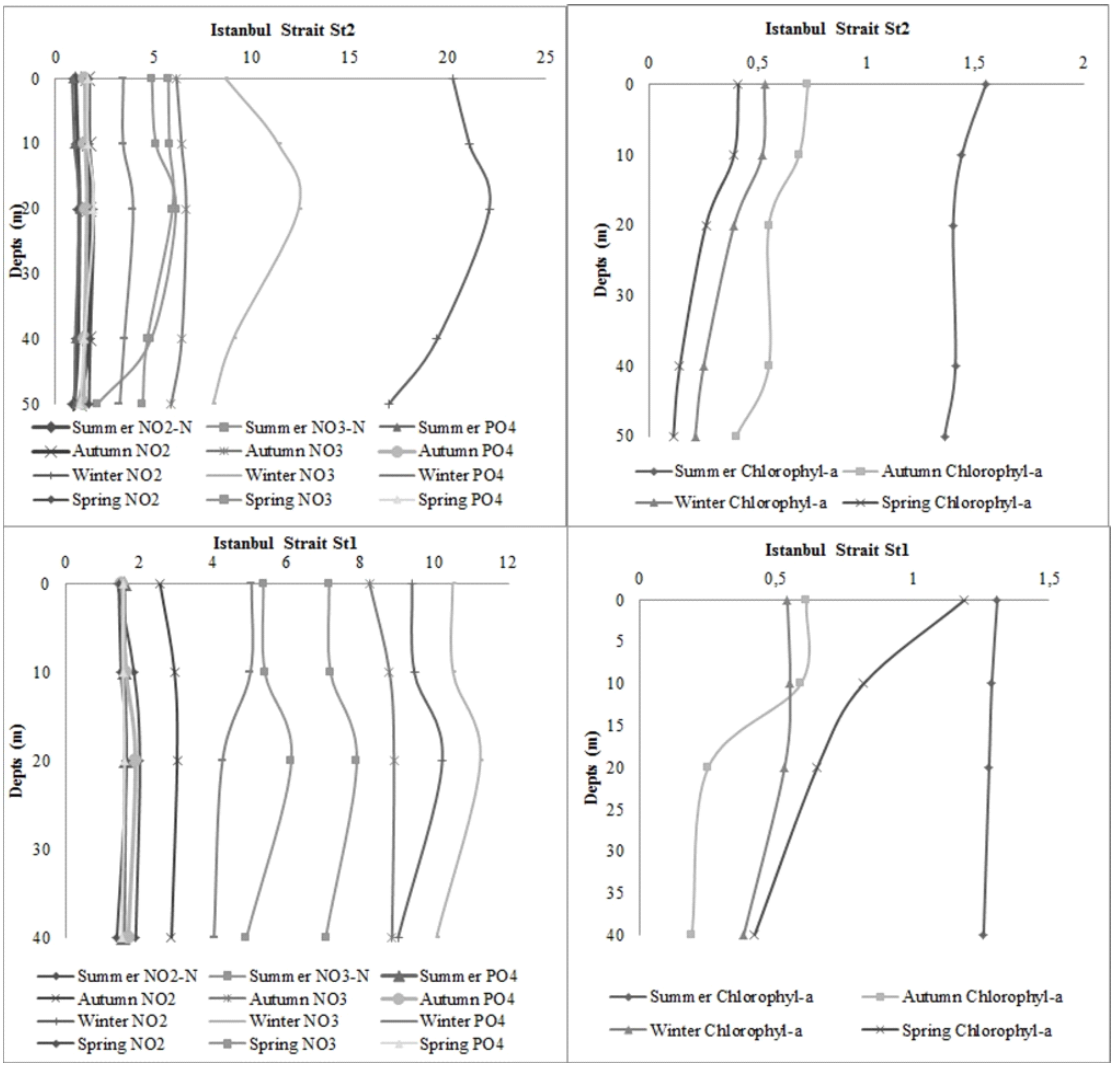

Fig. 2. The concentrations of NO2, NO3,PO4 and chlorophyll-a in the sea water samples that were taken from Istanbul Strait

ture was recorded as 22.42 °C in both straits during the study period.

While the observed temporal fluctuations of the HPC was found much higher in the seawater samples of the Istanbul Strait than the Canakkale strait, the highest HPC was found to be 85±0.2x108 CFU/100 ml in the seawater samples which were taken from the depth of 20 m of the point of the Black Sea entrance of the Istanbul Strait (Station 2) in the summer season. The lowest HPC was recorded as 57±0.2x10 CFU/100 ml with in the samples of Canakkale Strait in the autumn. The studies on the distribution of bacterioplankton were reported to be similar in the same or adjacent sea areas (González and Moran, 1997; Murray *et al.*, 1998; Riemann *et al.*, 1999; Riemann and Middelboe, 2002). However, the hydrodynamic peculiarities of the study areas may cause short term alterations with in the detected parameters. Suzuki et al. (2001) reported that under certain hydrographical conditions, the distribution of bacteria may display small scale dissimilarities. In this study, the levels of bacteria determined in water samples taken from under the halocline layer in the Istanbul Strait were occasionally found out to be similar or higher in comparison to the sea water samples which were taken from 0-30 cm. The bacteria level was also found out to be high in the samples which were taken from both 0-30 cm and mixed layer and increased along the depth profile. The observed differences on distributions of the heterotrophic aerobic bacteria between Canakkale and Istanbul Straits can be explained through different factors mentioned below. Istanbul Strait is under the pressure of the chemical and biological pollution because of over population, heavy inland industrial activity and marine transportation. The species belonging to Enterobacteriaceae family including Gram-negative pathogenic and opportunistic bacteria, along with human-sourced pollution bacteria, was reported as the most common bacteria in this region (Altug et.

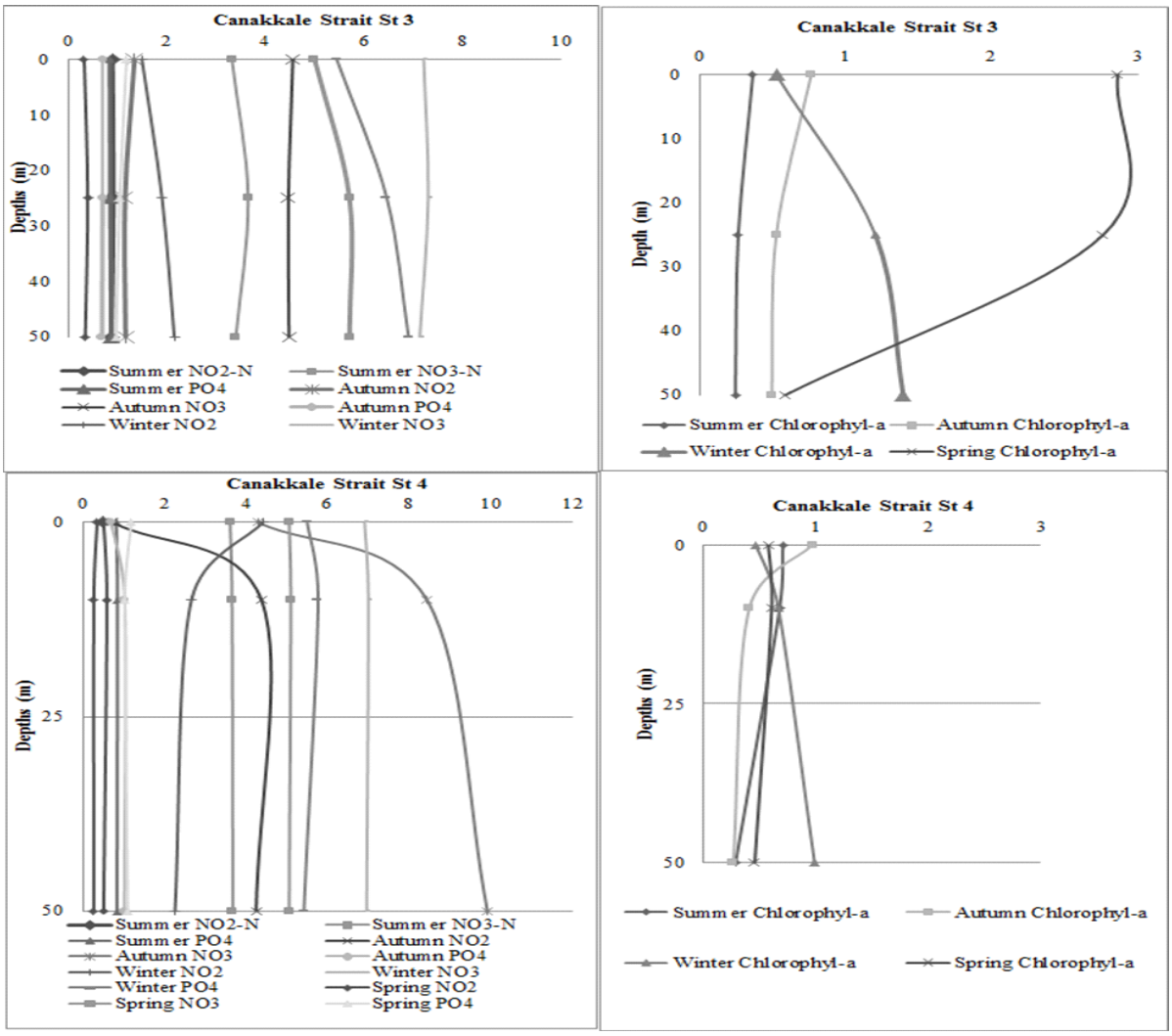

Fig. 3. The concentrations of NO2, NO3, PO4 and chlorophyll-a in the sea water samples that were taken from Canakkale Strait

al., 2013). Also, the presence of high counts of the heterotrophic aerobic bacteria, including a cocktail of pathogenic and antibiotic resistant bacteria, in the ballast water samples was reported in this region too (Altu? et. al., 2012). Furthermore, it is known that an excessive nutrient loads come to the Istanbul Strait via upper currents of the Black Sea (Tu?rul et al., 1995; Oku? et al., 2002). In this study, the higher bacteria levels, detected in the mixed layer, were considered to be a result of the accumulation of the nutrients in this layer. Higher HPC, determined in the water samples taken from the Istanbul Strait than the HPC from the seawater of the Canakkale Strait were also assessed as the reflection of the increased nutrient inputs, depending on point and non-point sources of the region. Effects of the deep discharges, vertical diffusion and intensive organic substance load of the surface water of Istanbul Strait have been inducing both the level of the heterotrophic bacteria and bacterial metabolically activity in the region.

Metabolically active bacteria percentage was recorded (Table 2) to be higher in the samples that were taken from the surface (0-30 cm) of the water than the other samples that were taken from a deeper section. Also, the highest level of the metabolically active bacteria was recorded in the surface waters of the Istanbul Strait. Stoderegger and Herndl (2001) reported that a higher metabolically active bacteria percentage was found in regions with commonly higher nutrient levels. In a similar manner, the higher metabolically active bacteria percentages, which were detected in the Istanbul Strait within the scope of this study, were considered to be a result of trophic status (eutrophic area) of the region. The less saline waters of the Black Sea reach to the Mediterranean via upper currents while the concentrated saline waters of the Mediterranean reach to the Black Sea via the undercurrents of the Canakkale and Istanbul Straits. Expectedly, the salinity values of the deep waters, representing saline waters of the Medi-

terranean Sea, were recorded as significantly higher than the surface waters of the both straits.

While the recorded salinity data varied depending on the dynamic structure of a mixed layer that separates two counter-currents; salty Mediterranean Sea and less saline waters of the Black Sea, the salinity values of the surface water, which represent the less saline waters of the Black Sea (0-30 cm) were recorded as stable. The highest salinity value was recorded as 39.11 PSU in the samples taken from the depth of 40 meters from the Çanakkale Strait in spring season. In this study, it was possible to detect the highest level of the heterotrophic aerobic bacteria from the under and upper layer of the study area which possessed salinity values between 24.0 and 39.11 PSU. No correlation was detected between HPC and the salinity values. Similarly, there was no correlation between the percentage of the metabolically active bacteria and the salinity values.

The bacterial abundance was recorded as higher in the summer period than the other seasons in both straits during the sampling period. The significant correlation ($p<0.01$) was recorded between the temperature and HPC. Also, a significant positive correlation was recorded between the temperature and the percentage of the metabolically active bacteria.

Chlorophyll-a values were found out to be significantly higher in the sea water samples which were taken from the depth of 0-30 cm from Istanbul Strait than the ones from Canakkale Strait. No correlation was present between HPC and chlorophyll-a values. Similarly, there was no correlation present between the concentration of the chlorophyll-a and the percentage of the metabolically active bacteria.

Fonselius (197l) reported that the data related to nutrient values of Istanbul and Canakkale Straits is of importance for understanding chemical balances in both the Sea of Marmara and in the Aegean Sea. Tu?rul et al., (1995) also reported that throughout the Sea of Marmara nutrient properties of the upper layer (less saline waters of the Black Sea) are being modified by collapsing biological particulars from surface to lower layers and the biochemical processes. Tu?rul et al., (1995) reported that the saline waters of the Mediterranean inflow to the deep basin of the Marmara via Canakkale Strait contain low nutrient concentrations. In addition, Tu?rul and Polat (1995) reported that the nutrient concentrations of the under layer waters of Canakkale Strait are enriched 10 times when they reach to Istanbul Strait. In this study, the concentrations of NO2, NO3 and PO4 were found out to be higher in lower layers than the surface water (0-30 cm) in both straits during the sampling period. The increasing concentration of NO3, NO2 and PO4 were associated with the deep discharge system. The nutrient and chlorophyll-a concentrations of Canakkale Strait were recorded significantly lower ($p<0.01$) than Istanbul Strait. Turkoglu et. al., (2004) reported some seasonally important variations between minimum and maximum concentration of NO2+NO3 in Canakkale Strait. These important differences were considered to be related to the wind/current speed and direction, mixing of the nutrient rich and poor layers and possible intensive uses of the nutrients by phytoplankton species during the study period (Turkoglu et al., 2004). However, in this study, the slight fluctuations were also recorded between the minimum and maximum NO2 and NO3 concentrations in the sea water samples that were taken from Istanbul and Çanakkale Straits during the study period. The higher bacteria levels, detected in the interlayer of the water column, between upper and bottom current, were considered to be a result of the cumulating nutrients in this layer. Since NO2, NO3 and PO4 concentrations were found out to be higher in the lower layer than the surface water (0-30 cm) in both straits during the sampling period, the cause of not registering any correlation between HPC and the concentration of the NO2+NO3 and PO4 in the surface water (0-30 cm) were considered to be a dynamic structure of the tested surface field.

Chlorophyll-a values in the samples taken from 0-30 cm and from the mixed layer were recorded as significantly higher ($p<0.01$) than deep waters of the both straits. Also, the dissolved oxygen concentration was recorded to be significantly lower in Istanbul Strait than Canakkale Strait during the study period.

CONCLUSION

There are no studies up-to-date, comparing the level of metabolically active bacteria and bacterial abundance with environmental variable parameters in this region. The observed bacterial variations, described in this study will provide foundational data to characterize the bacterial properties of Mediterranean and the Black Sea waters that are being exchanged via Istanbul and Canakkale Straits and will shed light on future studies on occurrence of heterotrophic bacteria abundance and activity.

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