Kafkas Univ Vet Fak Derg
16 (Suppl-A): S153-S158, 2010
DOI:10.9775/kvfd.2010.2654

RESEARCH ARTICLE

Chemical and Microbiological Quality of the *Chamelea gallina* from the Southern Coast of the Marmara Sea in Turkey [1]

Fatma ARIK ÇOLAKOĞLU * Hasan Basri ORMANCI * İbrahim Ender KÜNİLİ *
Serhat ÇOLAKOĞLU **

[1] *The present study was carried out with financial support of COMU BAP project 2007/52*
* Canakkale Onsekiz Mart University Faculty of Fisheries, Terzioglu Kampüsü, TR-17100 Canakkale - TURKEY
** Ministry of Agriculture and Rural Affairs, Provincial Directorates for Protection and Control, TR-17100 Canakkale - TURKEY

Makale Kodu (Article Code): KVFD-2010-2654

Summary

The aim of this work was to analyze *Chamelea gallina* harvested in the Sea of Southern Marmara in terms of heavy metal, biotoxin, and microbiological contents. Samples were collected seasonally from five stations which were determined to be their natural habitats in the between February 2008 and January 2009. Heavy metal contents of the samples revealed statistically significant ($P<0.05$) differences between seasons and stations. In two stations, Pb and Zn content of the clams were determined to exceed legal limits imposed by Turkish Fisheries Regulation and EC Shellfish Hygiene Directive (91/492/EEC). Biotoxin was not detected in any sample analyzed and the counts of *Escherichia coli* and fecal coliform bacteria of the samples were lower than the legal limits. *Salmonella* spp. was not detected in the sampling stations, whereas *Vibrio parahaemolyticus* was isolated in two stations. In conclusion, clams harvested in the southern Marmara Sea, excluding Gelibolu and Karabiga stations, were found suitable for human consumption.

Keywords: *Chamelea gallina, Heavy metals, Biotoxin, Microbiological quality, Marmara Sea*

Marmara Denizi'nin Güney Kıyılarındaki *Chamelea gallina*'ların Kimyasal ve Mikrobiyolojik Kalitesi

Özet

Bu çalışmada, güney Marmara Denizi'nden toplanan *Chamelea gallina*'ların ağır metal, biyotoksin ve mikrobiyolojik içerikleri tespit edilmiştir. Örnekler Şubat 2008-Ocak 2009 tarihleri arasında doğal yatakların bulunduğu toplam 5 istasyondan mevsimsel olarak temin edilmiştir. Örneklerin ağır metal içerikleri, mevsimler ve istasyonlar arasında istatistiksel olarak önemli derecede farklılık göstermiş olup ($P<0.05$), iki istasyonda, Pb ve Zn içeriklerinin Türkiye Su Ürünleri Yönetmeliği ve AB Kabuklu Hijyen Direktifi (91/492/EEC)'ne göre, limit değerlerin üzerinde olduğu belirlenmiştir. Hiçbir örnekte biyotoksin tespit edilmemiştir. Mikrobiyolojik analiz sonuçlarına göre, örneklerin *Escherichia coli* ve fekal koliform bakteri içeriklerinin yasal sınırların altında olduğu saptanmıştır. *Salmonella* spp. örnekleme istasyonlarında saptanamamış, *Vibrio parahaemolyticus* ise iki istasyondan izole edilmiştir. Sonuç olarak, Marmara Denizi'nin güneyinden toplanan beyaz kum midyeleri, Gelibolu ve Karabiga istasyonları hariç, tüm istasyonlarda insan tüketimine uygun bulunmuştur.

Anahtar sözcükler: *Chamelea gallina, Ağır metaller, Biyotoksin, Mikrobiyolojik kalite, Marmara Denizi*

INTRODUCTION

Chamelea gallina, also known as 'striped venus', is one of the most harvested and demanded species of bivalves and spreads across the coasts of the Black and Mediterranean Seas [1]. In Turkey, striped venus exists mostly in the coasts of the western Black Sea and the Marmara Sea [2,3] and commercial use began in 1986 [2]. As the demand increased, Turkey became the leading producer country in 2007, producing 58.3% of the world's total production [4]. Turkey was followed by Italy, Spain, and France [4].

İletişim (Correspondence)
☎ +90 286 2180018/1562
✉ arikfatmaa@yahoo.de

There are some risks affecting production quality of bivalves. These risks are mostly caused by the natural accumulation of heavy metal, biotoxin, and micro-organisms in bivalves due to their filter-feeding system [5-8].

Heavy metals, biotoxins and microorganisms were naturally present in waters [5,9] and their amounts could increase as a result of direct or indirect effects of human activities [9]. Heavy metal concentration of water is generally affected by the waste products of industry, mining and shipping operations; however, micro-organism concentration of water is affected by household and industrial wastes. Biotoxins are generated by the metabolic activities of single-celled algae [10,11]. Although these toxins are not harmful to bivalves, they could be led to serious diseases such as Amnesic Shellfish Poisoning (ASP), Azaspiracid Shellfish Poisoning (AZP), Diarrhetic Shellfish Poisoning (DSP), Paralytic Shellfish Poisoning (PSP), Neurotoxic Shellfish Poisoning (NSP) and even death in human [5,9,10,12]. Presence of algae species that produce biotoxins in Turkish seas was reported [13]; however, no biotoxin event except for DSP was reported from the Marmara Sea [14].

Mentioned risk factors must be lower than the national and international legal limits for the production and consumption of clams [15].

In this study, seasonal samplings were made in Marmara Sea, one of the most important habitats for striped venus, to analyze heavy metal, biotoxin and microbiological contents of striped venus, and their suitability for human consumption was evaluated.

MATERIAL and METHODS

Sample Collection

Striped venus samples were collected from pre-determined stations on the Northern coast of the Sea of Marmara and were located, from east to west direction, in Karabiga, Kemer, Sevketiye (Anatolian side), Bolayıraltı and Gelibolu (European side), respectively *(Fig. 1)*. Samples were collected using dredge from 5 to 10 m of the littoral zones in the above mentioned stations between February 2008 and January 2009. For each sampling, 5 kg of samples were collected from the stations and carried at +4°C in a cool box to the laboratory.

Heavy Metal Analysis

For heavy metal analysis, seasonally collected samples were stored in polyethylene bags at -18°C in laboratory. To represent each station, 10 units were randomly taken from samples. Selected samples were separated from their shells and dried at 105°C. Dried samples were ground and 0.5 g portions were put into Teflon tubes; then, they were turned into soluble forms in microwave (CEM Mars X-press) with 10 ml concentrated nitric acid (65% w/v) (Merck, Germany) addition. After the application of organic digestion process, samples were filtered and diluted with deionized water (Millipore, USA) [16]. Before heavy metal analysis, nonstandard dilutions were prepared, and ICP-AES device was calibrated. Blind sample was prepared and measured for every 5 sample to determine the effects of

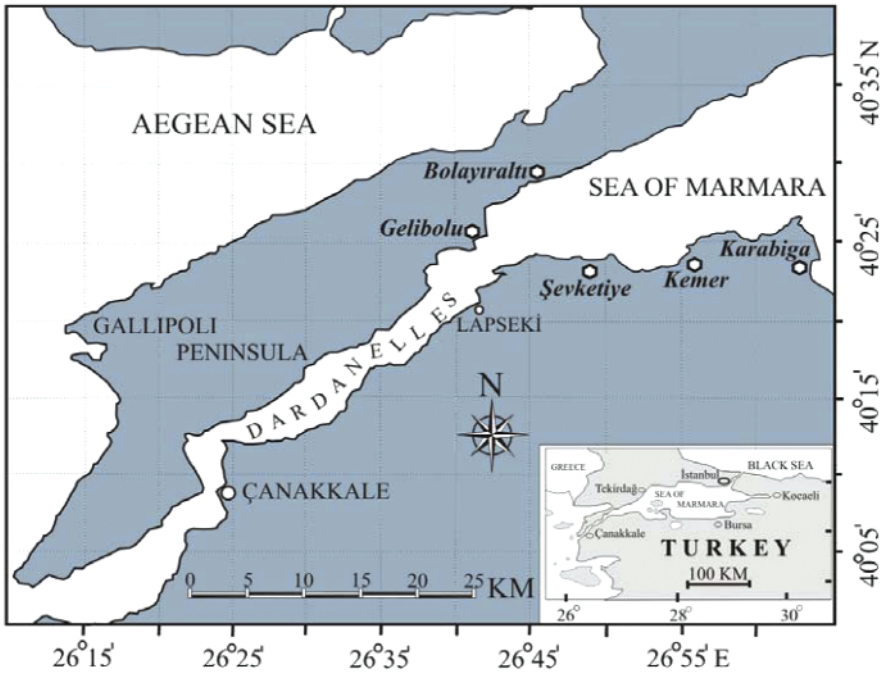

Fig 1. Sampling stations in the Marmara Sea

Şekil 1. Marmara Denizi'nde örneklerin alındığı istasyonlar

used reagents. Heavy metal (Cd, Cu, Pb, Zn, Fe, and Cr) analysis was applied using Varian Liberty AX Sequential ICP-AES [17]. Results of the all analysis were first compared to the standard results obtained with metal salts and then measured as mg kg^{-1} of wet matter.

Biotoxin Analysis

Shells of the samples used for biotoxin analysis were properly cleaned. Hepatopancreases of the clams were removed aseptically after shucking the clams. Diarrhetic shellfish poison (DSP) analysis was performed using mouse assay (*Mus musculus* var. albinus) as described by Yasumoto [18]. For this purpose, hepatopancreases were first homogenized (Ultra-Turrax; IKA Yellow line, Germany) in acetone (Merck, Germany) followed by intraperitoneal (IP) injection of the homogenized 1 ml into three mice weighing 16-20 g each. Mice were observed for 24 h, and symptoms were recorded. The amount of the toxin which killed a mouse in 24 h after injection was defined as one mouse unit (MU g^{-1} digestive gland) [19]. In the paralytic shellfish poison (PSP) analysis, clam tissue homogenized in Ultra-Turrax (IKA Yellow line, Germany) was made acidic with 0.1 N HCl (Merck, Germany) addition, centrifuged by boiling and cooling, and then 1 ml injected to three lab mice, supernatant weighs of which were recorded. After the injection, mice were observed for 1 h to record their death time, and toxin amounts were calculated with Sommer's table [20]. In the amnesic shellfish poison (ASP) analysis, prepared extract was filtered in a membrane filter (Millex-HV, Millipore, U.S.A) of 0.45 µm [21], and the amount, calibration curve of which was drawn with standard domoic acid solution (DACS-1B Reference Standard, NRC, Halifax, Canada), was determined using HPLC (Shimadzu Corporation, Kyoto, Japan) with UV detector [22].

Microbiological Analysis

Shells of the samples taken for microbiological analysis were washed, scraped free of dirt, shucked with a sterile knife and then tissue and shell fluids of the samples were weighed in the predetermined amounts [23] and homogenized by mixing them in bag mixer (Stomacher 400, A.J. Seward-London) for 2 min. Homogenized samples were diluted to 10^{-6} for the analysis of total mesophilic bacteria.

The counts of the total aerobic mesophilic bacteria was performed using Plate Count Agar and incubating plates at 35°C for 48 h [23]. Total coliform, fecal coliform, *E. coli* counts were made according to the three tube most probable number method (MPN) using Lauryl Sulphate Tryptose broth (35°C, 24 h), Brilliant Green Bile broth (35°C, 48 h), EC broth (44.5°C, 24 h), respectively. The counts of *Salmonella* spp. analysis was carried out on 25 g of blended clams which were added to 225 ml and incubated for 24 h at 35°C. After incubation second step of enrichment were performed using RVS and TT broth and incubated for 24 h at 42°C and 24°C, respectively. A loopful of both medium was streaked onto Xylose Lysine Deoxycholate Agar and incubated for 24 h at 35°C [23]. The count of *Vibrio parahaemolyticus* was made according to Colakoglu et al.[24] using Thiosulfate Citrate Bile Salts Sucrose Agar and incubating plates at 37°C for 24 h.

Statistical Analysis

The descriptive statistics (mean, standard error (SE) and range) of the findings of the chemical composition and heavy metal content and microbiological analysis and also the one-way variance analysis (ANOVA) of the interactions between seasons and stations were calculated using Microsoft Office Excel 2007 software (Seattle, USA). Significance was established at $P<0.05$ [25].

RESULTS

Heavy Metals

The clams meat was analyzed for the determination of 6 metals, ie. Cd, Cu, Pb, Zn, Fe, Cr as summarized in *Table 1*. The mean concentrations (mg kg^{-1} wet weight) of trace minerals in tissues of *C. gallina* were the following: Cd, 0.04-0.69; Cu, 0.71-5.30; Pb, 0.18-3.24; Zn, 13.08-77.76; Fe, 2.46-89.73; Cr, 0.08-1.25. According to the results of the statistical analysis, heavy metal contents showed significant variation between seasons and stations ($P<0.05$).

Biotoxins

In present study PSP, DSP, and ASP toxins were not detected in the samples seasonally collected from the research stations.

Microbiological Analysis

The striped venus meat was also analyzed for the determination of total coliform, fecal coliform, *E. coli, Salmonella* spp, and *V. parahaemolyticus.* The highest value ($P<0.05$) of total aerobic organisms was found as $2.0x10^4$ CFU/g in summer, and the lowest ($P<0.05$) was found as $2.1x10^2$ CFU/g in winter. Total amount of coliforms and fecal coliforms was found to reach its maximum level ($P<0.05$) in summer and autumn *(Fig. 2)*. Fecal coliform content was lower than coliform content, and the total coliform amount in these seasons was found to be dense (1100 MPN/100 g) in Sevketiye and Kemer stations. Maximum *E. coli* level (107 MPN/100 g) was determined in summer, and minimum *E. coli* level (2 MPN/100 g) was determined in winter *(Fig. 2)*.

Table 1. *Seasonal variation of heavy metals concentration (mg kg^{-1}) in different stations in striped venus*
Tablo 1. *Beyaz kum midyesinin farklı istasyonlarda ağır metal konsantrasyonlarının (mg kg^{-1}) mevsimsel dağılımı*

Metals	Stations	Seasons			
		April'08	July'08	October'08	January'09
Cd	Gelibolu	0.07 ^{Ec}	0.15 ^{Cb}	0.08 ^{ABc}	0.24 ^{Ba}
	Bolayıraltı	0.31 ^{Bb}	0.04 ^{Dc}	0.05 ^{Bc}	0.52 ^{Aa}
	Sevketiye	0.61 ^{Aa}	0.24 ^{Bb}	0.10 ^{Ac}	0.18 ^{BCbc}
	Kemer	0.16 ^{Da}	0.05 ^{Dc}	0.07 ^{ABbc}	0.08 ^{Db}
	Karabiga	0.22 ^{Cb}	0.69 ^{Aa}	0.08 ^{ABd}	0.14 ^{CDc}
Cu	Gelibolu	2.24 ^{Ab}	2.58 ^{Ba}	1.80 ^{Cd}	2.07 ^{Cc}
	Bolayıraltı	2.21 ^{Ab}	4.27 ^{Aa}	0.71 ^{Dd}	2.11 ^{Cc}
	Sevketiye	1.23 ^{Dc}	2.39 ^{Ca}	2.03 ^{Bb}	2.00 ^{Db}
	Kemer	1.82 ^{Cc}	2.18 ^{Da}	2.00 ^{Bb}	2.21 ^{Ba}
	Karabiga	2.02 ^{Bc}	1.98 ^{Ec}	4.87 ^{Ab}	5.30 ^{Aa}
Pb	Gelibolu	3.01 ^{Ab}	2.51 ^{Ac}	2.29 ^{Ad}	3.24 ^{Aa}
	Bolayıraltı	0.78 ^{Bc}	0.90 ^{Cb}	0.18 ^{Ed}	1.40 ^{Ca}
	Sevketiye	0.42 ^{Dc}	0.27 ^{Dd}	1.09 ^{Db}	1.40 ^{Ca}
	Kemer	0.84 ^{Bc}	0.91 ^{Cc}	1.21 ^{Cb}	1.36 ^{Ca}
	Karabiga	0.69 ^{Cd}	1.10 ^{Bc}	1.71 ^{Bb}	2.35 ^{Ba}
Zn	Gelibolu	18.88 ^{Dc}	18.65 ^{Dd}	19.73 ^{Cb}	23.54 ^{Ba}
	Bolayıraltı	17.68 ^{Eb}	25.76 ^{Ba}	13.08 ^{Ed}	16.33 ^{Ec}
	Sevketiye	27.39 ^{Ba}	26.10 ^{Cb}	21.45 ^{Bc}	17.37 ^{Dd}
	Kemer	22.35 ^{Cb}	14.61 ^{Ed}	19.12 ^{Dc}	36.91 ^{Aa}
	Karabiga	77.76 ^{Aa}	44.02 ^{Ab}	22.48 ^{Ac}	17.94 ^{Cd}
Fe	Gelibolu	2.46 ^{Ed}	4.70 ^{Ec}	89.73 ^{Aa}	27.85 ^{Bb}
	Bolayıraltı	18.81 ^{Bc}	114.22 ^{Aa}	17.89 ^{Ed}	20.73 ^{Cb}
	Sevketiye	17.00 ^{Cd}	32.82 ^{Cb}	51.56 ^{Ba}	17.46 ^{Ec}
	Kemer	16.52 ^{Dd}	46.06 ^{Ba}	26.65 ^{Db}	17.64 ^{Dc}
	Karabiga	21.39 ^{Ad}	23.47 ^{Dc}	39.49 ^{Ca}	28.36 ^{Ab}
Cr	Gelibolu	0.32 ^{Cb}	0.19 ^{Dc}	0.76 ^{Aa}	- *
	Bolayıraltı	- *	0.40 ^{Ba}	0.21 ^{Db}	0.20 ^{Cc}
	Sevketiye	0.27 ^{Dc}	0.53 ^{Ab}	0.47 ^{Bb}	1.25 ^{Aa}
	Kemer	0.46 ^{Ab}	0.23 ^{Dd}	0.34 ^{Cc}	1.13 ^{Ba}
	Karabiga	0.37 ^{Ba}	0.35 ^{Ca}	0.08 ^{Ec}	0.21 ^{Cb}

**** :*** *not detected*
Means with different superscript capital letters in the same row and lowercase letters in the same column for each metals indicate significant differences (P<0.05)

It was observed that there was no statistically significant difference (P>0.05) of *E. coli* between seasons in Sevketiye station. Seasonal changes of the amount of total aerobic, total coliform and fecal coliform organisms were significant difference (P<0.05).

Salmonella spp. was not detected and *V. parahaemolyticus* was isolated two times in summer (Sevketiye) and autumn (Karabiga).

DISCUSSION

Metal density, natural components of sea, might be increased as a consequence of human activities, and they could be remained in dangerous concentrations in the ecosystem for a long time [27-29]. Heavy metals, though in low concentrations, are accumulated in the meat of bivalve, therefore, consumption of bivalve meat containing excessive concentrations of these metals could be endangered human health [30]. Lead amounts in the meat of bivalve collected from Gelibolu station were found as 1.5 mg kg^{-1}, which was higher than the legal limits imposed by both Turkish Fisheries Regulation and European Commission for all seasons (P<0.05). In addition, lead content [15,31] was observed to be higher than the legal limits in Karabiga station in autumn (1.71 mg kg^{-1}) and winter (2.35 mg kg^{-1}) (P<0.05). Also, high level (77.76 mg kg^{-1}) of zinc content *(Table 1)* was detected in spring samples of Karabiga Station (P<0.05), which was higher than the alert levels of Turkey and NNSP (National Shellfish Sanitation Program) [15,32].

Heavy metal contents (Cd, Cu, Pb, Zn, Fe, and Cr) of the samples collected from other stations was determined to be lower than the national and international limit values [15,31]. In the studies made on heavy metal contents of striped venus in Marmara Sea, findings have been reported similarly to be lower than the limit values so far [33,34]. It is thought that the high levels of lead and zinc determined in this study could be related to the contaminated streams that converge to Black Sea, which is known to be polluted by many rivers; River of Danube being the most polluted among all [43].

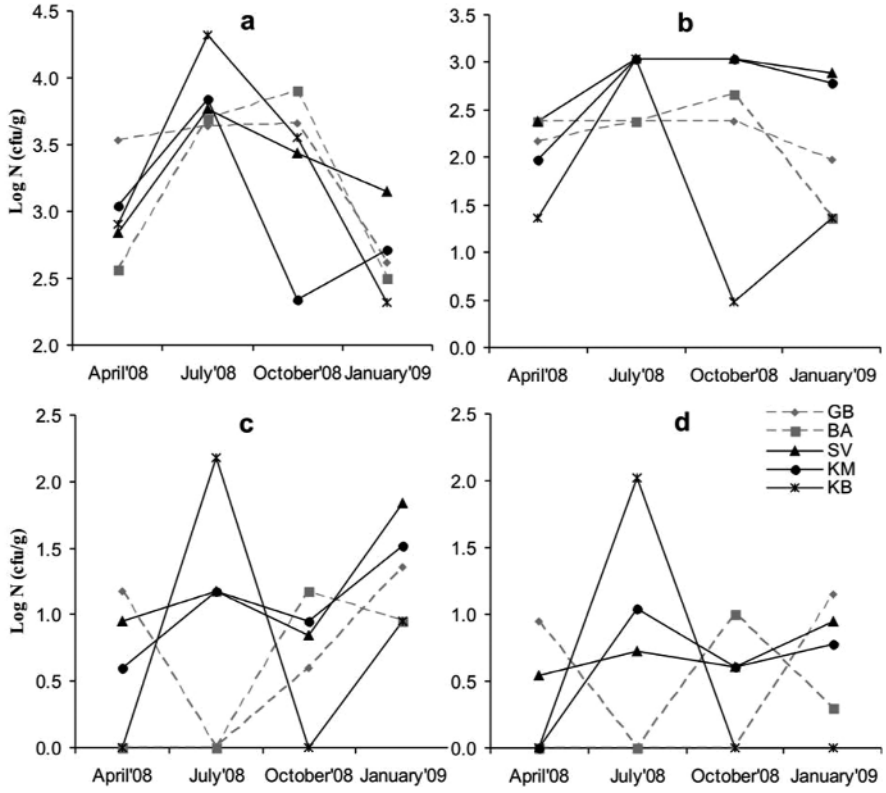

Fig 2. Levels of total aerobic counts **(a),** total coliform **(b),** fecal coliform **(c)** and *E. coli* **(d)** bacteria in striped venus of different seasons and stations

Şekil 2. Beyaz kum midyesinin farklı mevsim ve istasyonlardaki toplam aerobic **(a),** toplam koliform **(b),** fekal koliform **(c)** ve *E .coli* **(d)** bakteri miktarları

In bacteriological examinations of bivalves, total amount of aerobic bacteria was detected around 10^2-10^4 CFU/g in all stations (P<0.05) *(Fig. 2)*. Intensive amounts of coliform bacteria (1100 MPN/100 g) were found in Sevketiye and Kemer stations (P<0.05). Fecal and *E. coli* levels were found to be lower than the limit values (300 MPN/100 g for fecal coliform and 230 MPN/100 g for *E. coli*) imposed by the Turkish Fisheries Regulations and EC Shellfish Hygiene Directive (91/492/EEC) [15,31,35] *(Fig. 2)*. These bacteria reached their maximum levels in summer months (P<0.05). *Salmonella* spp. was not detected in any sample; however, *V. parahaemolyticus* was detected two times in Sevketiye and Karabiga stations. The members of *Vibrio* were frequently defined as opportunistic and potential pathogenic bacteria of the water bodies especially in warm climate zones [36,37]. Nevertheless from the public health perspective, the occurrences of these bacteria have caused concerns for authorities. However, the presences of some *Vibrio* species were also reported off Dardanelles coast in Turkey [24] and some European countries coasts' [38,39]. Bacteria levels of clams in northern Marmara Sea were found to increase in summer, and *Salmonella* spp. was detected four times between May and August [40]. Bacteria levels of the clams increase in summer because of the increase in human recreational activities, as well as the increase in industrial and household wastes in these months. In addition, it was reported that increasing temperature of sea water, wind effects, nutrient increase and currents could also be effective in the increase of bacteria amounts [41,42].

In conclusion, many of the parameters studied in this work seasonally and locally fluctuated. In particular, the microbiological; *Vibrio parahaemolyticus* and metal content; Pb and Zn did not meet the legal requirements imposed Turkish Fisheries Regulation and European Commission standards for its quality trademark. This could be related to the contaminated streams that converge to Black Sea, which is known to be polluted by many rivers; River of Danube being the most polluted among all. Due to these risks of the striped venus in Marmara Sea, continuously monitoring should be conducted.

Acknowledgements

The authors would like to thank COMU Scientific Technology Research Center for heavy metal analysis and Fikret Cakir who helped in the laboratory. Mouse assays were carried out in accordance with the ethical rules.

REFERENCES

1. Moschino V, Marin MG: Seasonal changes in physiological responses and evaluation of "well-being" in the venus clam *Chamelea gallina* from the Northern Adriatic Sea. *Comp Biochem Physiol A Mol Integr Physiol,* 145, 433-440, 2006.

2. Deval MC: The shell growth and the biometry of the stripped

venus *Chamelea gallina* (L.) in the Sea of Marmara. Turkey. *J. Shellfish Res.,* 20 (1): 155-159, 2001.

3. Deval MC, Oray IK: The shell growth and the biometry of the striped venus *Chamelea gallina,* (L.) in the Sea of Marmara and the western Black Sea, Turkey. *J Fish Aquat Sci,* 6, 127-142, 1992.

4. FAO-FIGIS: Global capture statistics of stripped venus. Food and Agriculture Organization of the United Nations-Fisheries Global Information System <http://www.fao.org/figis/servlet/SQServlet?file=/usr/local/tomcat/FI/5.5.23/figis/webapps/figis/temp/hqp_35166.xml&outtype=html> *Accessed:* 29.07.2009.

5. Ahmed FE: Seafood safety. National Academy Press, Washington, D.C., 1991.

6. Cook DW: Indicators and alternate indicators of growing waters. **In,** Ward DR, Ackney CH (Eds): Microbiology of Marine Food Products. pp. 19-39, AVI Book, Van Nostrand Reinhold, New York, NY, 1991.

7. Feldhusen F: The role of seafood in bacterial foodborne diseases. *Microbes Infect,* 2, 1651-1660, 2000.

8. Sumner J, Ross T: A semi-quantitative seafood safety risk assesment. *Int J Food Microbiol,* 77, 55-59, 2002.

9. Huss HH: Assurance of seafood quality. FAO Fisheries Technical Paper 334, FAO, Rome, p. 169, 1994.

10. Anonymous: Marine Biotoxins. FAO Food Nutr Pap 80. FAO, Rome,1994.

11. Aydın H, Uzar S: Denizel mikroalg biyotoksinleri ve etkileri. *Celal Bayar Univ Fen Bil Derg,* 5 (1): 87-100, 2009.

12. Dolah FMV: Marine algal toxins: Origins, health effects, and their increased occurrence. *Environ Health Perspect,* 108, 133-141, 2000.

13. Koray T: A Check-list for phytoplankton of Turkish Seas. *Ege Univ J Fish Aquat Sci,* 18 (1-2): 1-23, 2001.

14. Anonymous: Çanakkale Tarım İl Müdürlüğü Su Ürünleri Brifing Raporu, 2009.

15. Anonymous: Fishery products law and regulation. Turkish Republic Ministry of Agriculture and Rural Affairs Press. p. 85. Ankara, 1995.

16. EPA: Microwave assisted acid digestion of sediments, sludges, soils, and oils. Environmental Protection Agency Method 3051A. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf, *Accessed*: March 2009, 1998.

17. EPA: Inductively coupled plasma-atomic emission spectrometry. Environmental Protection Agency Method 6010C. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6010c.pdf, *Accessed:* March 2009, 2000.

18. Yasumoto T: Method for the bioassay of diarrhetic shellfish toxin. *Shokuhin Eiseigaku Zasshi,* 31, 515-522, 1981.

19. CRL: Report on the EU-NRLs intercalibration exercise on DSP determination (April, 2001). *European Community Reference Laboratory on Marine Biotoxins.* Vigo, Spain, 2001.

20. AOAC: Official Method 959.08. Paralytic shellfish poison, biological method. *Official Methods of Analysis of AOAC International.* Gaithersburg, Maryland, USA, 2000.

21. Quilliam MA, Sim PG, Mcculloch AW, Mcinnes AG: High-performance liquid-chromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton. *Int J Environ Anal Chem,* 36 (3): 139-154, 1989.

22. AOAC: Official Method 991.26. Domoic acid in mussels, liquid chromatographic method. *Official Methods of Analysis of AOAC International.* Gaithersburg, Maryland, USA, 2000.

23. FDA: Bacterial Analytical Manual. 8th ed. Revision A. Food and Drug Administration. *AOAC International,* Washington, DC, 1998.

24. Colakoglu FA, Sarmasik A, Koseoglu B: Occurrence of *Vibrio* spp. and *Aeromonas* spp. in shellfish harvested off Dardenelles cost of Turkey. *Food Control,* 17, 648-652, 2006.

25. Zar JH: Biostatistical Analysis. Fourth ed. Department of Biological Sciences Northern Illinois University, 1999.

26. Hallegraeff GM: Harmful algal blooms: A global overview. **In,** Hallgraeff GM, Anderson DM, Cembella AD (Eds): Manual on Harmful Marine Microalgae. pp. 25-49, UNESCO, Paris, 2004.

27. Chen MH, Shih CC, Chou CL, Chou LS: Mercury, organic-mercury and selenium in small cetaceans in Taiwanese waters. *Mar Pollut Bull,* 45, 237-245, 2002.

28. Francesconi KA, Edmonds JS: Arsenic species in marine samples. *Croat Chem Acta,* 71 (2): 343-359, 1998.

29. Renzoni A, Zino F, Franchi E: Mercury levels along the food chain and risk for exposed populations. *Environ Res,* 77, 68-72, 1998.

30. Belitz HD, Grosch W, Schieberle P: Lehrbuch der Lebensmittelchemie. Springer-Verlag, Berlin, Heidelberg, 2001.

31. Anonymous: Commission regulation as regards heavy metals, directive 2001/22/EC, No: 466/2001. Setting maximum levels for certain contaminants in food stuffs, 2001.

32. Ratcliffe S, Wilt DS: Proceedings Seventh National Shellfish Sanitation Workshop, Washington, DC, October 20-22. p. 412, 1971.

33. Ozden O, Erkan N, Deval MC: Trace mineral profiles of the bivalve species *Chamelea gallina* and *Donax trunculus. Food Chem,* 222-226, 2009.

34. Şentürk A: Çeşitli yörelerden avlanmış mollusklarda cıva kadmiyum ve kurşun düzeylerinin araştırılması. *Yüksek Lisans Tezi.* İstanbul Üniv Fen Bil Enst, 1993.

35. Anonymous: Council directive 91/492/EEC of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve mollusks, 1991.

36. Farmer JJ III, Hickman Brenner FW: The genera Vibrio and Photobacterium. **In,** Balows A, Tru¨per HG, Dworkin M, Harder W, Schleifer KH (Eds): The Prokaryotes. 2nd ed., pp. 2952-3011, Springer Verlag, New York, NY, 2, 1992.

37. Huss H: Control of indigenous pathogenic bacteria in seafood. *Food Control,* 8, 91-98, 1997.

38. Baffone W, Pianetti A, Bruscolini F, Barbieri E, Citterio B: Occurrence and expression of virulence-related properties of *Vibrio* species isolated from widely consumed seafood products. *Int J Food Microbiol,* 54 (1-2): 9-18, 2000.

39. Lhafi SK, Kuhne M: Occurrence of *Vibrio* spp. in blue mussels *(Mytilus edulis)* from the German Wadden Sea. *Int J Food Microbiol,* 297-300, 2007.

40. Altug G, Cardak M, Ciftci PS: Indicator and other bacteria in striped venus (*Chamelea gallina,* L.) and wedge clam *(Donax trunculus)* from the northern coast of the sea Marmara, Turkey. *J Shellfish Res,* 27 (4): 783-788, 2008.

41. Grimes DJ, Atwell RW, Brayton PR, Palmer LM, Rollis DM, Roszak DB: The fate of enteric pathogenic bacteria in estuarine and marine environments. *Microbiol Sci,* 3, 324-329, 1986.

42. Okumus I, Stirling HP: Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. *Aquaculture,* 159 (3-4): 249-261, 1998.

43. Nisbet C, Terzi G, Pilgir O, Sarac N: Determination of heavy metal levels in fish samples collected from the Middle Black Sea. *Kafkas Univ Vet Fak Derg,* 16 (1): 119-125, 2010.