METALS BIOACCUMULATION IN IMPORTANT MARINE SPECIES OF THE BULGARIAN BLACK SEA

AND

SCREENING-LEVEL HUMAN HEALTH AND ECOLOGICAL RISK ASSESSMENT

by

Maria D. Pantazi

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

Major Subject: Environmental Science

West Texas A&M University

Canyon, Texas

May 2018

PREFACE

If there is one thing I have learned from my years as both an undergraduate and graduate student of science, is that one cannot fully appreciate nor become invested in something one does not understand. In light of this, my aim for this manuscript was to explore many aspects of present-day Bulgaria relating to this study, in order to provide the reader with as much background information pertaining to my research as possible. I wished to make the content accessible to anyone with rudimentary background in science. I hope I have not failed in this endeavor. I have attempted to provide definitions of scientific terminology where possible, while also including a Glossary of Terms at the end of this paper. I further hope that readers will find this work interesting and enjoyable so that they, too, may discover the environmental, social, economic, and political intricacies that govern the current state of the Bulgarian Black Sea and its natural resources.

ABSTRACT

Fish and seafood make up a large proportion of the Bulgarian diet, but there are no country-specific fish consumption advisories to offer consumer advice on serving size and frequency, particularly for some native species. I evaluated the levels of select metals and non-metals (arsenic, mercury, lead, cadmium, and chromium) in two highly consumed marine organisms of the Black Sea along the Bulgarian coastline, the Round Goby (*Neogobius melanostomus*) and Mediterranean Mussel (*Mytilus galloprovincialis*). Plankton was also evaluated, as an indicator of contaminant bioaccumulation in the food web. By using the angling removal technique and purchase at fish markets for the fish and mussels, respectively, collected were 110 fish (= 7 composite samples of 10, 1 composite of 13, and 37 individual Goby samples) and > 270 mussels (= 9 composite Mussel samples) over the course of two field seasons (Summer 2016 and Summer 2017). Also collected were 6 composite plankton samples via vertical sampling. Upon collection, the samples were processed before delivery to a laboratory for metals analysis. Contaminant levels were compared between composite samples using Kolmogorov-Smirnov tests and Kruskal-Wallis tests, and Spearman correlation was used to evaluate the relationship between contaminant levels and distance from locations exhibiting intense industrial activity. The results indicated that elevated levels of arsenic and chromium were detected in nearly all fish, mussel, and plankton samples. Mercury, lead, and cadmium levels were below the Level of Quantification (LOQ) for all samples. Multiple fish and mussel composite samples differed in their distributions, while an inverse relationship was established for Contaminants of Concern (COC) levels and distance from industrial "hot spots" for most locations. A positive correlation was found regarding COC levels and some industrial hubs. Hazard Quotients (HQs) were developed as part of a screening-level human health and ecological risk assessment, but insufficient data did not permit the development of Protective Concentration Levels (PCLs). HQs for arsenic often exceeded 1 for both fish and mussels, but chromium HQs were below 1 for both fish and plankton samples. This research will provide the Bulgarian population with a better understanding of current contaminant levels in seafood species and the potential health risks associated with fish and mussel consumption. It will also illustrate needs for future research and help marine resource managers and specialists with a broader assessment of ecosystem health.

ACKNOWLEDGEMENTS

Rarely is an endeavor the product of one person alone. I, certainly, am no more an island than the next graduate student. I attribute my success to completing the task of gargantuan proportions that has been the present research to my professors, colleagues, friends, and family, all of whom I wish to thank from the bottom of my heart.

Firstly, I would like to thank my thesis advisor, Dr. Gary Barbee, for his academic guidance, time, and for being extremely supportive of this project from day one. Thank you for your understanding, patience, for allocating resources when needed, and for always having my back.

I thank my thesis committee members: Dr. Jim Rogers, for his continuous support throughout my education at West Texas A&M University, for sharing his numerous stories that inspired me to persevere, and for trusting me with expensive equipment across the ocean. Dr. Rocky Ward and Dr. Richard Kazmaier, for their teachings and advice, as well as the numerous discussions that motivated me to keep going. I thank all my committee members for teaching me some terrific field work methods, which I applied successfully overseas. Additionally, many thanks to Dr. David Sissom, Sandy Babitzke, and Michelle Blake, for providing me with the best employment I could have asked for, throughout both my undergraduate and graduate studies, as well as for their moral support. I am grateful to the West Texas A&M University Graduate School and Killgore Research Center for awarding me the grants that funded my research over two field seasons. I also thank Richard Smith for working his magic on the logistical aspects of this project.

I sincerely thank Ola Omiyefa and Brian Yates for their contributions to the risk assessment portion of this paper. A huge thank you to Promise Ceasar for her wonderful GIS work, and Jason Rodin for his help with statistics. I thank Amy Stephens, for her eternally sunny disposition, which helped me through a period of self-questioning. A special thank-you to Eric Jordan and Tiffany Daniel Jordan for their friendship and for saving me from becoming a nomad too soon! I also thank Erin Stiede, Melanie Peery, Michelle Caruana, Steven Patton, Kathleen Webb, Kimberly Baker, and Khara Wright, for their valuable input.

I would like to extend a special thank you to my family, for their continuous encouragement, endless support, and personal investment of time and resources, which helped me see this project through. Milena Doseva, Todor Dosev, Rossana Doseva, Delyana Doseva, Ruska Kiuchukova, Anesti Kiuchukov, Maria Pantazi, Petros Pantazis, Dimitris Pantazis, Antrea Stylianou, and Svetla – to you, I dedicate this manuscript.

An enormous thank you to Stefan Mindov for getting me to my sampling locations, rain or shine, over the course of two summers, and teaching me everything I know about angling and Black Sea sport fishes. I thank Mitko Stoychev, Todor, Tihomir, and the other angling aficionados for helping with the fish sampling and sharing with me their fishing strategies. I further thank Georgi Peychev and his crew for bringing me along on their fishing vessels, inviting me to observe anchovy landings as part of their commercial operations, giving me a tour of G. Peychev's fish-processing facility, and providing insightful information on the commercial fishery sector. Much gratitude goes out to Veselka Pashova, Ivan Poroliev and the rest of the SGS lab team, for their professionalism and prompt analysis of my samples.

I thank Drs. Yuvenaly Zaitsev and Vladimir Mamaev, for their absolutely lovely monograph on the Black Sea, which formed the basis for my thesis introduction. Lastly, I owe much to Professor Yuvenaly Zaitsev, whom I have come to know through his compelling works on Black Sea ecology. I thank him for his innumerable contributions to scientific knowledge, the narratives of which are always elegant in their simplicity and eloquence. Many passages within this thesis would not have been the same without inspiration from his writings.

Approved:

Gary C. Barbee, Ph.D.
Chairman, Thesis Committee — Date

William J. Rogers, Ph.D.
Member, Thesis Committee — Date

Rocky Ward, Ph.D.
Member, Thesis Committee — Date

W. David Sissom, Ph.D. — Date
Department Head

Lal K. Almas, Ph.D. — Date
Interim Dean, College of Agriculture
and Natural Sciences

Angela Spaulding, Ph.D. — Date
Dean, Graduate School

Table of Contents

List of Tables

List of Figures

CHAPTER I: A Brief Introduction to the Black Sea

The intercontinental body of water that is the Black Sea is a surviving remnant of the ancient Tethys Sea, its modern version a product of plate tectonics. Movements of the earth's crust enabled it to transition from open and connected to the world ocean to closed and isolated, and to fluctuate between salt-, brackish-, and freshwater (Zaitsev and Mamaev, 1997). Never one to stop reinventing itself, the Black Sea has undergone multiple hydrophysical and hydrobiological changes over the millennia. The Black Sea is now a Eurasian semi-enclosed basin (Duzgunes and Erdogan, 2008; Arashkevich *et al*., 2014), characterized by an average salinity of 17—18‰ at the surface and 22—24‰ at a depth of 2,000 meters (m) (Figure 1), the maximum depth being 2,212 m (Zaitsev and Mamaev, 1997; Zaitsev, 2008). Salinity is the total amount of dissolved solids (TDS) in marine water and is measured in parts per thousand (ppt, or ‰) in g/kg of seawater (Zaitsev, 2008). The salinity levels of the Black Sea are low compared to the > 30‰ salinity of the Mediterranean or the 35‰ standard ocean salinity (Fofonoff, 1985; Zaitsev, 2008).

The Black Sea is anoxic throughout 87% of its volume and biologically poor, as conditions are unsuitable for most forms of life (Zaitsev and Mamaev, 1997; Zaitsev, 2008). The remaining 13% which contains oxygen is comprised of shallow surface water (5—10 m in depth), as well as waters from the continental shelves (depths up to 150 m) (Zaitsev and Mamaev, 1997; Zaitsev, 2008). It is rich in hydrogen sulfide gas (H_2S) below 150-250 m – the exact depth depending on the dome-shaped zone of H_2S as shown in Figure 1 – accumulated from decaying organic matter over thousands of years, which is toxic to aerobic organisms (Zaitsev, 2008). Interestingly, this is not where the Black Sea has derived its name from. Zaitsev (2008) recounts that one of the reasons the Sea earned its name was *à propos* of its high concentrations of suspended particles and organisms such as plankton, detritus, and organic matter, which made the water less transparent compared to that of adjacent seas.

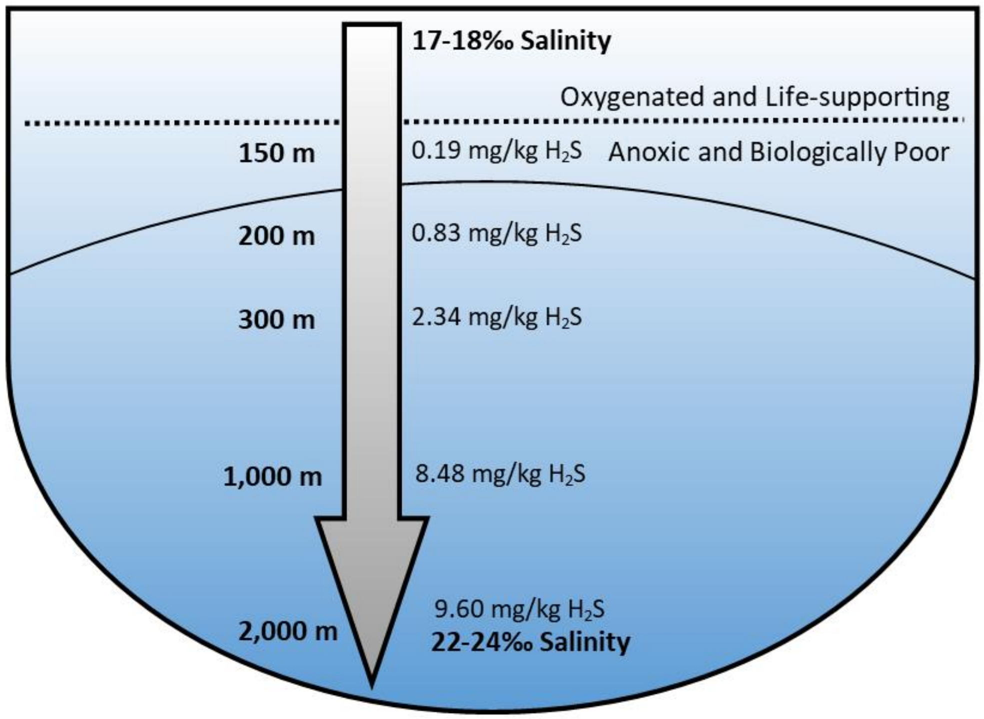


Fig. 1. Black Sea salinity and hydrogen sulfide (H_2S) levels gradually increase with depth, while biological productivity decreases (adapted from Zaitsev, 2008).

In addition to accounting for only a small proportion of the Black Sea, the oxygenated zone is highly endangered because of anthropogenic eutrophication (Daskalov, 2002). This phenomenon results from excessive selective nutrient availability which causes algal blooms that lead to oxygen depletion. Nutrients that overload the Black Sea waters, mainly nitrogen and phosphorus, originate from field fertilizers, discharges from animal husbandry, atmospheric fall-out in the open sea, as well as urban and sewage discharges near cities (Zaitsev and Mamaev, 1997; Boran and Altınok, 2010).

The Black Sea communicates with the Sea of Azov to the north via the Kerch Strait, and the Mediterranean Sea to the southwest via the Bosphorus Strait, the Sea of Marmara, and the

Dardanelles Strait. The connection to the Mediterranean has been estimated to date back 5,000-7,000 years and is responsible for many so-called Mediterranean immigrant species (Zaitsev and Mamaev, 1997; Zaitsev, 2008). Along with the Sea of Azov, the Black Sea is the most isolated from the ocean among European semi-enclosed and coastal seas (Shiganova and Bulgakova, 2000).

The Black Sea has largely been treated as a commons under open-access regime (Radu *et al*., 2011), leading inevitably to a "tragedy of the commons" state evident by its ever-dwindling and increasingly spoilt natural resources. "Tragedy" occurs when a resource that belongs to all is being overexploited by individuals who have no direct stake in its conservation, to the detriment of everyone (Staddon and Genchev, 2013; Potters, 2013). In the words of Garrett Hardin, who popularized this concept in 1968, "Ruin is the destination toward which all men rush, each pursuing his own best interest in a society that believes in the freedom of the commons. Freedom in a commons brings ruin to all" (Hardin, 1968).

This rapid resource depletion comes as no surprise, as the Black Sea is bordered by six coastal countries – Ukraine, the Russian Federation, Georgia, Turkey, Bulgaria, and Romania (Zaitsev and Mamaev, 1997; Zaitsev, 2008; Duzgunes and Erdogan, 2008) – contributing to a neighborhood of discord and competition for each other's fish stocks (Ulman *et al*., 2013; Banaru *et al*., 2015; Keskin *et al*., 2017). There are currently no harmonized regional fishery management practices or regulations, even regarding shared (straddling and highly migratory) stocks (Duzgunes and Erdogan, 2008; Radu *et al*., 2011). Common commercial fishes are the Black Sea Anchovy (*Engraulis encrasicolus ponticus*), Sprat (*Sprattus sprattus phalericus*), Horse Mackerel (*Trachurus mediterraneus ponticus*), Turbot (*Psetta maxima maeotica*), and Whiting (*Merlangius merlangus euxinus*), *inter alia* (Radu *et al*., 2011).

In addition to fishing, the littoral countries use the Black Sea for a variety of purposes such as transportation and tourism. In fact, tourism is becoming one of the fastest-growing economic sectors in the world (UNWTO, 2013). In Bulgaria specifically, the coastal population routinely increases by 20% in the summer, but some municipalities see an increase of over 320% (Stanchev *et al*., 2015). This poses a particular problem, as 9.8% of Bulgaria's population already occupies the narrow coastal strip (Stanchev *et al*., 2015). In turn, this crowding overwhelms existing facilities and infrastructure, putting a heavy strain on local natural resources. At the same time, the coastal population and regional development to support it continue to grow, which leads to coastal erosion, loss of wildlife habitat, excess solid waste production, and increased demands for public utilities and water treatment (Stanchev *et al*., 2015).

The Black Sea constitutes a catchment area of over two million square kilometers (km^2) (Zaitsev, 2008). Even though only the six aforementioned coastal states have direct access to it, a total of eighteen countries of Central and Eastern Europe use it to discharge pollutants (Maldonado *et al*., 1999; Jitar *et al*., 2013). The Black Sea therefore serves as a major sink for industrial, agricultural, and municipal wastes of Europe and western Asia.

The largest rivers that discharge into the Black Sea are the Danube, Dnieper, Dniester, and Southern Bug (Zaitsev and Mamaev, 1997). The Danube, which traverses many European countries on its way to the Black Sea, borders Bulgaria to the north, with its delta draining into the Romanian territory of the Black Sea. Due to the cyclonic (i.e., circular movement and counter-clockwise direction) nature of the main water currents (Maldonado *et al*., 1999; Zaitsev, 2008) and Bulgaria's proximity to the Danube delta, contaminants can migrate southward (Maldonado *et al*., 1999) and end up in Bulgarian waters (Dineva, 2013), causing issues for the local population. What is more, the prevalent winds of Europe transport polycyclic aromatic hydrocarbons (PAHs) from industrial zones in Eastern Europe, Russia, and northern Turkey toward the center of the Black Sea basin (Maldonado *et al*., 1999). On account of all this ecological deterioration, the Black Sea has been called one of the most polluted seas in the world (Zaitsev, 2008; Oguz and Velikova, 2010; Makedonski *et al*., 2017), albeit not completely objectively (Zaitsev, 2008). Pollution does not affect the entire Black Sea but, rather, its northwestern part (Maldonado *et al.*, 1999, Zaitsev, 2001) and peripheral biotopes (i.e., the marginal habitats where marine, terrestrial, and freshwater organisms interact) (Zaitsev, 2008). Elevated accumulation of radionuclides and heavy metals in the NW corner of the Black Sea was attributed to inputs from the Danube, Dnieper, and Dniester (Strezov and Nonova, 2009).

This study focuses on the Bulgarian coastline, which spans 378 km along the southwestern Black Sea basin and is home to two of the Black Sea's largest bays in the west – those of the port cities of Varna and Burgas (Zaitsev, 2008). Coastal waters, particularly those near port areas and cities are some of the most vulnerable marine areas (Urquhart et al., 2013; Kolios and Stylios, 2015). Like the rest of the Black Sea, the Bulgarian Black Sea coast suffers from various ecological stressors. This is best expressed by Varna Bay, located in the northwestern part of the Black Sea, which is influenced by local and Danube river discharges (Zaitsev and Mamaev, 1997; Dineva, 2013). Additional impacts are caused by the activity from the chemical-industrial complex, shipping, tourism, urbanization, and global climate change (Moncheva *et al*., 2012; Dineva, 2013). Both Varna and nearby Cape Kaliakra have been identified as point sources of pollution with aliphatic hydrocarbons (Maldonado *et al*., 1999). Similarly, Burgas Bay is impacted from the largest port in Bulgaria, an international airport, the center of the Bulgarian fishing and fish-processing industry, many tourist resorts, and the largest oil refinery wastewater treatment plant in the Balkans (Southeastern Europe) (Kutsarov and Chobanov, 2015). Not surprisingly, their presence constitutes an enormous burden to the environment.

One of the byproducts of this intense activity is contamination of the Black Sea waters with metals and metalloids such as chromium and arsenic, which endanger both the marine ecosystem and the local human population. In fact, the quality of the marine environment plays a paramount role in the quality of natural life, both directly and indirectly (Kolios and Stylios, 2015). As such, the purpose of this research was to collect fish, mussel, and plankton samples from different locations along the Bulgarian sea coast to evaluate trace metal bioaccumulation in a trophic web (Figure 2) and assess the severity of potential ecological and human health hazards from dietary exposure.

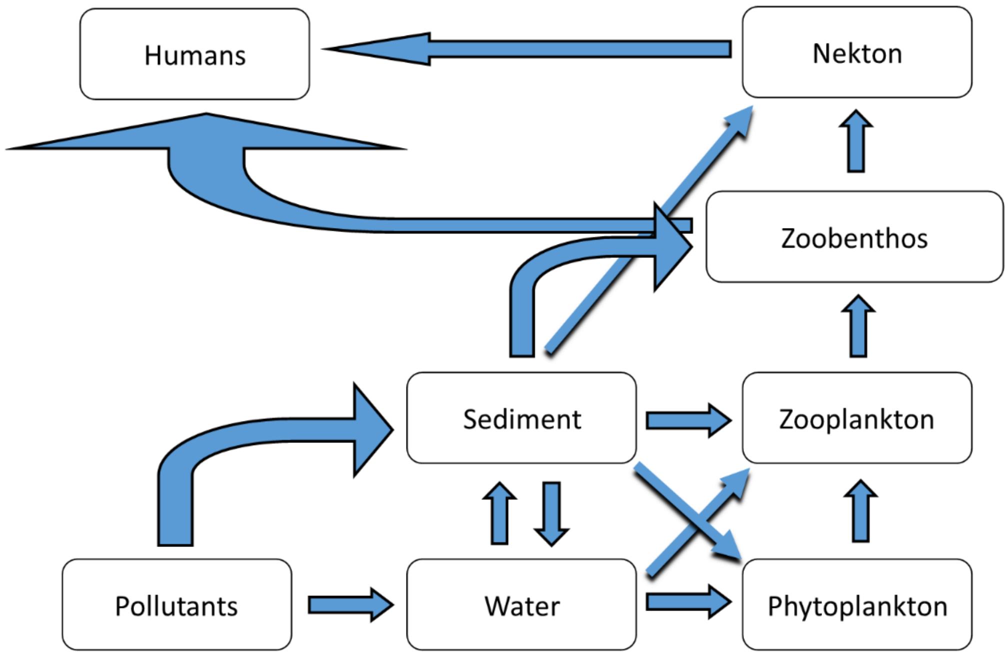


Fig. 2. Pollutant migration through the marine food web under investigation.

CHAPTER II: Regional Characterization

Fishery Sector

The capture fishery sector plays an important economic role in Bulgaria. It contributes to food production, generates employment, improves income, and complements agricultural and tourist activities. Although accounting for less than 1% of the country's Gross Domestic Product (GDP) according to the Food and Agriculture Organization of the United Nations (FAO), the fishing industry is important for the national economy, particularly in some less-developed areas along the coast (FAO, 2002).

The coastal city of Burgas serves as the country's largest fishing port in terms of catches, adding up to 48% of total landings (11,280 metric tons, or mt) between 2000 and 2001 (FAO, 2002). The coastal cities of Sozopol and Varna follow with 34% (7,990 mt) and 8% (1,880 mt) of landings, respectively (FAO, 2002). Nearly 100% of Black Sea fishing is carried out by independent fishermen. Most vessels are small, with a capacity of 10 to 50 tons (FAO, 2002). All fishing industry establishments are private and trade in fish and fishery products is likewise carried out by private persons and firms (FAO, 2002).

There are currently no established Individual Transferable Quotas (ITQs) in Bulgaria, while Total Allowable Catch (TAC) applies only to turbot, sprat, and sturgeon (Duzgunes and Erdogan, 2008). There are, however, established fishery management zones: Fishing Zone 1, which reaches up to 3 nautical miles (NM) from the coastline and is theoretically reserved for traditional fishermen, and Fishing Zone 2, spanning from 3 NM to the country's Exclusive Economic Zone (EEZ) limit at 200 NM, designated for commercial fishermen (Duzgunes and Erdogan, 2008). These zones have been established in an attempt to "provide equitable allocation of resources and reduce conflict" between competing interests (Duzgunes and Erdogan, 2008). Whereas issues regarding marine resources are dealt with by each Black Sea coastal country at the national level, an ecosystem-wide management strategy is still lacking (Keskin *et al*., 2017).

Because there are no data reporting recreational and subsistence fishing numbers (neither in terms of persons nor catches), Keskin *et al*. (2017) used catch rates from the Black Sea coast of Turkey to interpolate recreational and subsistence catches for Bulgaria. Although their calculations relied on several assumptions, they estimated that 0.95% of the Bulgarian coastal population in 2013 fished recreationally and/or for subsistence. They also determined that numbers have been underreported by the FAO, which did not consider the recreational/subsistence fishery. Therefore, the reconstructed total catch for the period 1950-2013 has been estimated to be 1.7 times higher than FAO data, and 3.1 times higher from the 1990s-2013. Recreational and subsistence catches were estimated at 0.129 tons per fisher^{-1} per year^{-1} in 2010 (Keskin *et al*., 2017). In terms of percentage, subsistence catches were estimated at 0.9% and recreational catches at 0.8% of the reconstructed total catch.

The lack of fisheries data for the recreational and subsistence sector is likely a product of the open-access state of the marine fishery. According to Bulgarian fisheries legislation, no permits or licenses are required to engage in marine recreational fishing (Keskin *et al*., 2017), which makes accurate data-recording difficult.

Fish Consumption

Fish consumption in Bulgaria is low, according to the FAO and Bulgaria's National Centre of Public Health Protection (NCPHP, 2006). Eating fish in Bulgaria ranks below average compared to other European markets, with a *per capita* consumption of 3.0-3.5 kg/year barring consumption in canteens, restaurants, and hospitals (FAO, 2002). In comparison, Duzgunes and Erdogan (2005) reported a *per capita* household consumption of fish at 4.2 kg in 2005, whereas fish and seafood consumption in Bulgaria has been recently estimated by the United States Department of Agriculture (USDA) to be higher, at 5.2 kg per year, with consumption in restaurants excluded (USDA, 2017). When considered chronologically, these numbers indicate a growing trend in consumption. Cumulative *per capita* consumption, including restaurants, is currently estimated at 8.8 kg (USDA, 2017). However, artisanal, recreational, and subsistence fishermen and coastal inhabitants consume larger amounts of fish and seafood than the average population (Poe *et al*., 2014). Tourists, many of them on "culinary vacation", and individuals who fast during Lent in anticipation of Easter (Bulgaria is predominantly a Christian Orthodox country) also consume large amounts of fish over a short period of time. More often than not, recreational anglers eat their catch; they may, however, also gift or sell some of it (personal communication; personal observation). Small-scale (artisanal and subsistence) fishers also consume their catch, which mostly serves to meet or supplement household nutritional needs; only a small proportion is sold, gifted, or traded (Chuenpagdee *et al*., 2006; Poe *et al*., 2014).

Generally, low consumption levels are an indication of the weak purchasing power of the population and increasing fish prices. The average *per capita* annual monetary household income is 3,202 USD (Bulgarian National Statistical Institute, 2016) and the local purchasing power has been derived from consumer reports as 57.91% lower than in the United States (www.numbeo.com). Purchasing-power parity (PPP) for 2016 was 0.686 (OECD, 2018). The concept of PPP is used to compare living standards across countries (Lafrance and Schembri, 2002), where PPP is the rate of currency conversion that equalizes the purchasing power between two currencies. It is determined by eliminating price level differences between countries, or the ratio of prices in national currency of the same good or service in a different country (OECD, 2011). Exchange rates are determined by comparing the national prices for a basket of goods and services that includes household consumption, government services, and capital formation and net exports. PPP is also calculated for groups of products (OECD, 2018). PPP for Bulgaria in its national currency per U.S. dollar was 0.897 for the category "Fish" (OECD, 2011). This means that Bulgarians, on average, can purchase less fish and seafood compared to most Americans and their richer European counterparts.

Nevertheless, the improving socioeconomic environment after the country's accession to the European Union (E.U.) in 2007 and the expanding tourist industry are predicted to increase the demand in fish and seafood products (FAO, 2002). In addition, the number of fishery businesses is expected to rise with the introduction of new modern production facilities mandated by the E.U. Common Fisheries Policy (CFP) (USDA, 2017), which Bulgaria must adhere to, as an E.U. member state.

Food Advisories

To my knowledge, there are currently no fish consumption advisories specific to Bulgaria, although the NCPHP recommends that Bulgarians eat fish at least once or twice per week, at 150-200 g per serving (NCPHP, 2006). The European Food Safety Authority (EFSA) reports on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish and seafood on a European-wide basis but makes no recommendations regarding the number and size of servings with respect to most Bulgarian commercial fish (EFSA, 2015). Similarly, it makes no recommendations on the number/size of servings regarding other contaminants found in fish, such as arsenic and chromium. Furthermore, while there are some recommendations for women of child-bearing age, children, and the elderly, risks to other vulnerable groups such as subsistence fishermen have not been evaluated.

Such recommendations are an arduous endeavor considering the heterogeneity in marine species and consumption habits across Europe. The Import and Export sectors must also be taken into account, as a plethora of marine species crosses international borders to satisfy a growing demand for fish and seafood across E.U. member states. This means that populations may routinely be exposed to contaminants originating in foreign waters. The EFSA Scientific Committee advises that each country identify its own pattern of fish consumption and evaluate the associated risks (EFSA, 2015).

CHAPTER III: Site Assessment

Contaminants of Concern (COCs)

Pollution with trace metals and metalloids is a worldwide phenomenon caused by both natural and anthropogenic sources. These sources can be described as either “point” or “nonpoint”. Point source pollution can be attributed to a single, identifiable location where the contaminant originated, usually from industrial or municipal discharges (e.g., sewage pipe) (Patterson, 2010). In fact, both Varna and Burgas, which host the principal harbors in Bulgaria and which are also two of the largest ports in the northwestern Black Sea, constitute point sources of oil pollution (Maldonado *et al*., 1999) from ships and the Lukoil Neftochim oil refinery. Nonpoint source pollution, on the other hand, cannot be traced back to a specific location or point in time (Potters, 2013). Often, its source is spread over a wide area, and more than one entity may be responsible for it. An example of this is agricultural runoff (Patterson, 2010).

Metals are an established health hazard to humans and the environment, including marine ecosystems. Such contaminants have a tendency to accumulate in living organisms and biomagnify, or increase in concentration, across trophic levels. Elements like arsenic and chromium, while beneficial in trace amounts, can have deleterious effects even in low concentrations (Peycheva *et al*., 2014). This study investigated the contamination of marine biota with arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), and lead (Pb), although the last three metals yielded concentrations below the Limit of Quantification (LOQ) for all samples. Further discussion regarding LOQ values follows under the "Statistical Methods" section.

The release of hazardous substances in the marine environment is a pan-European problem, and there are numerous E.U. legislative measures and policies in place to address it (Tornero and Hanke, 2016). Marine pollution with metals results from 5 broad categories of activities (Table 1), as identified and compiled by Tornero and Hanke (2016).

Table 1. Sea-based activities as sources of marine pollution with metals, as summarized from Tornero and Hanke (2016).

Category	Activities	Contaminants of Concern (COCs) relevant to this study
Shipping	Intentional or accidental spillage of chemicals other than oil, oil spills, operational discharges, and emissions from antifouling paints	Hazardous and Noxious Substances (HNS) like lead (Pb) from spills; petroleum and petroleum components like chromium (Cr); vessel discharges such as arsenic (As), cadmium (Cd), and lead (Pb)
Mariculture	Medicinal products, antibiotics, parasiticides, anesthetics, disinfectants, food additives and contaminants, and antifouling biocides	Fish food supplements containing arsenic (As); metals from antifouling biocides
Offshore Activities	Offshore oil and gas exploration and production (drilling waste, produced water, accidental spills, decommissioning of disused installations), and other offshore installations	Arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), and mercury (Hg) from water-based drilling muds; chromium (Cr) and lead (Pb) from produced water; lead (Pb), cadmium (Cd), and mercury (Hg) from deteriorating oil and gas installations; metals from subsea cables or pipelines
Seabed Mining		Release of metals like cadmium (Cd) and mercury (Hg) from mining activities

Dredging of sediment and dumping at sea	Emissions from historical dumping sites (radioactive wastes, munitions and chemical weapons)	Trace metals and metalloids (arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), mercury (Hg)) from historical and current inputs; lead (Pb) from corroded ammunition, and arsenic-containing compounds from chemical weapons

Tornero and Hanke (2016) report that the majority of contaminants come from offshore oil and gas operations followed by shipping and mariculture. Also, some substances may be associated with more than one source, which increases their potential hazard. PAHs, cadmium, mercury, and lead are classified as priority contaminants in the four European marine regions (Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea). Listed below (Table 2) are the five pollutants investigated in this study, along with their potential sea-based sources (Tornero and Hanke, 2016).

Table 2. Metal or Metalloid Contaminants of Concern (COCs) and their potential sea-based sources, as adapted from Tornero and Hanke (2016).

COC	Shipping	Mariculture	Offshore oil and gas industry	Seabed mining	Dredging/dumping of dredged material	Historical dumping sites	Shipwrecks
Arsenic	X	X	X		X		X
Cadmium and its compounds	X		X	X	X		X
Chromium	X		X		X		X
Lead and its compounds	X		X		X	X	X
Mercury and its compounds			X	X	X		X

Contaminant Characterizations

Arsenic

Arsenic is a ubiquitous, naturally-occurring element found in the earth's crust and the 20th most abundant (Peycheva *et al*., 2014; Irgolic *et al*., 1991). It is encountered in rocks, soil, and natural water (EFSA, 2014). Over 245 minerals contain arsenic, but it is also a source of anthropogenic pollution from activities such as mining, smelting of non-ferrous metals, as well as wood preservatives, fertilizers, and pesticide applications (Hughes *et al*., 2011). Another polluting activity is the burning of fossil fuels (Peycheva *et al*., 2014), as arsenic is found in crude petroleum (Puri and Irgolic, 1989) and coal (USGS, 2016).

The average concentration of arsenic in the environment is a few milligrams per kilogram (mg/kg), although its concentration in seawater is around 0.002 mg/kg – low compared to other elements (Irgolic *et al*., 1991). However, living marine organisms can accumulate arsenic to the point of exceeding background seawater concentration by several thousand times (Puri and Irgolic, 1989). In addition to accumulating in marine organisms, arsenic is also metabolized (Goessler *et al*., 1997).

Arsenic occurs in four oxidation states: +V (arsenate), +III (arsenite), 0 (arsenic), and -III (arsine). Arsenate and arsenite have methylated derivatives – in other words, biogenic compounds that originate from living forms such as marine organisms (Irgolic *et al*., 1991). Compounds like arsenobetaine and arsenocholine (often referred to as "fish arsenic"), as well as arsenosugars, are therefore important in health risk assessments (Peycheva *et al*., 2014).

Many marine biota exhibit concentrations of arsenic in different forms. The form most commonly encountered is arsenobetaine, with arsenocholine and tetramethylarsonium following as minor constituents. Arsenite has been identified as another minor constituent, whereas methylarsonic acid has not been detected as a major contaminant in biological samples (Goessler *et al*., 1997).

Inorganic arsenic is a known human carcinogen, and generally more toxic than its organic forms (Peyheva *et al*., 2014). While the presence of both has been established in marine organisms, as much as 90% are organoarsenic compounds (Wojciechowska-Mazurek, 2012; Kucuksezgin et al., 2014; Mania et al., 2015). Inorganic arsenic comprises 0.02%--11% of total arsenic in fish and seafood (Peycheva *et al*., 2014). Both inorganic forms are harmful to humans, but As(III) is considered the more toxic form (Hughes *et al*., 2011). Arsenic and inorganic arsenic compounds are known carcinogens and have been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 1980; IARC, 2004) and as a Group A known human carcinogen by the U.S. Environmental Protection Agency (USEPA) (IRIS, 2007).

The current USEPA cancer oral slope factor for arsenic is 1.5 mg/kg-day and the established reference dose for oral exposure (RfD) for inorganic arsenic is 0.0003 mg/kg-day (IRIS, 2018). The slope factor is a measure of the risk of developing cancer during a lifetime exposure to a carcinogenic or potentially carcinogenic substance. The reference dose is generally an estimate of a daily human exposure (including sensitive subgroups) that is "likely to be without an appreciable risk of deleterious effects during a lifetime" (IRIS, 2018).

Arsenic Exposure from Food

Arsenic is included in a continuous annual call for occurrence data, though so far Bulgaria has not been one of the E.U. countries sampled for adult subjects (EFSA, 2014). Bulgarian dietary surveys from the EFSA Comprehensive Food Consumption Database, currently the most complete and detailed in the E.U., only report exposure in infants, toddlers, and children up to 10 years old. Findings indicate dietary exposure in children primarily comes from foods other than fish and seafood, which did not contribute significantly to total inorganic arsenic exposure. In adult populations, the overall dietary exposure to inorganic arsenic from fish and seafood products was small for most European countries sampled, with the exception of Spain and Italy (EFSA, 2014). While these findings may not be representative of the entire Bulgarian population, they may be representative of coastal and subsistence fishing populations. Although fish are a recognized dietary source of inorganic arsenic (As(III) and As(V)), at present there are no maximum levels (MLs) for arsenic in food products in Europe. Some E.U. countries do, however, have national guidelines for consumption levels of food-borne arsenic (EFSA, 2014).

Because this metalloid is naturally-occurring and is encountered so frequently in the environment, most food items may contain at least trace amounts of arsenic. What is more, changes in total arsenic and arsenic species are possible based on the type of food processing, temperature, and time of cooking (EFSA, 2010). The amount of arsenic in the cooking water and in foods frequently containing arsenic can also boost overall toxicity, as the contaminant concentrations of several food items are combined. Common foods containing arsenic are grain-based processed foods like wheat bread and rolls, rice, and dairy products (EFSA, 2014). There is a general lack of information regarding cooking methods of foods that absorb water during the cooking process (EFSA, 2014). Conversion factors used to estimate the proportion of inorganic to total arsenic also introduce uncertainty (EFSA, 2014), as proportions vary depending on the product.

According to the literature (Sloth *et al*., 2005; Francesconi, 2010; Fontcuberta *et al*., 2011), inorganic arsenic levels are generally higher in mollusks than in fish. Although fish and seafood have higher amounts of total arsenic than terrestrial foods, the latter exhibit a higher proportion of inorganic arsenic (EFSA, 2014). Rice is one of the foods with the highest total arsenic concentrations, as well as a high proportion of inorganic arsenic. Bulgaria is one of the rice-producing countries of the E.U. and, though not a staple food, rice is of important sociocultural significance. Of rice production in Bulgaria, 98% belongs to the *japonica* variety, one of the two major subspecies of *Oryza sativa* (FAO, 2006). National rice consumption is 32,000-35,000 t per year and average consumption per person per year is 6.5 kg. (Bulgarian Ministry of Agriculture, Food and Forests, 2017).

In Bulgaria, rice is a component of many dishes and is routinely paired with seafood. Plenty of mussel dishes, in fact, contain rice. The result may be a significant increase in total arsenic exposure from a meal. Fortunately, the arsenic concentration can be decreased via proper cultivation strategies (Banerjee *et al*., 2013) and cooking methods (EFSA, 2014). Increasing the volume of cooking water (Raab *et al*. suggested a 30:1 water/rice ratio) may decrease the concentration of inorganic arsenic in different rice varieties by 35-45%, or even up to 86% compared to uncooked rice (Raab *et al*., 2009; Fontcuberta *et al*, 2011). It is important to note that these estimations assume low levels of contamination with inorganic arsenic in tap water (estimated at 1.6 µg/L) (EFSA, 2014). Additionally, rinsing the rice with water prior to cooking (Raab *et al*., 2009) and discarding the excess water after boiling reduces the risk of arsenic exposure (Torres-Escribano *et al*., 2008).

Chromium

Chromium is a metal that is plentiful in the natural environment and occurs in rocks, soil and volcanic dust and gases. Chromium can have numerous oxidation states, but its trivalent (Cr(III)) and hexavalent (Cr(VI)) forms are the most frequently encountered. Cr(III) can be found in various foods, particularly vegetables, and is also used in food additives and supplements for nutritional purposes. Cr(VI), on the other hand, is mostly the result of industrial processes and its presence in water serves as an indicator of anthropogenic pollution (EFSA, 2014). Occupational exposure to chromium may come from chrome plating baths, colors and dyes, cement, tanning agents, wood preservatives, anticorrosive agents, welding fumes, lubricating oils and greases, cleaning materials, as well as textiles and furs (IRIS, 2018).

The oral reference dose (RfD) for Cr(III) and Cr(VI) has been set by the USEPA at 1.5 mg/kg-day and 0.003 mg/kg-day, respectively (IRIS, 2018). Cr(III) has low oral bioavailability and is considered the less toxic form of the two. While Cr(III) is essential for lipid, protein, and fat metabolism in living organisms, there is considerable uncertainty regarding its potential deleterious effects. Furthermore, there are currently no sufficient data to serve as evidence for carcinogenicity of Cr(III) (IRIS 2018). Although Cr(VI) is a known human carcinogen (Group A) via the inhalation route, its carcinogenicity by the oral route of exposure cannot be determined and the substance is classified as Group D. When ingested, it is converted to Cr(III) via the body's metabolic processes after ingestion and is therefore not considered a significant dietary hazard. Cr(VI) toxicity also includes developmental retardation and damage to growing fetuses and fertility problems in adults (IRIS, 2018).

Chromium Exposure from Food

Currently, there are no maximum levels (MLs) for chromium in food. A Greek study reported that the categories "meat, fish and seafood", "cereals" and "pulses" were generally higher in chromium (average of > 0.100 mg/kg, with a range of 0.02--0.45 mg/kg) compared to other foods (EFSA, 2014). A French study of fish and seafood found that chromium averaged 0.220 mg/kg in fish and 0.228 mg/kg in seafood (Guérin *et al*., 2011). Fish and seafood products have not been established to make up a significant proportion of the diet of infants, toddlers, and children up to 10 years old in Bulgaria. Therefore, chronic dietary exposure to Cr(III) from fish and seafood is limited (EFSA, 2014). However, there is much data deficiency in the case of chronic dietary exposure to chromium in adults.

Chromium absorption through dietary intake is considered to be relatively low (< 10 % of the ingested dose) (EFSA, 2014). The USEPA's Integrated Risk Information System (IRIS) database reports that Cr(III) is only minimally absorbed through oral intake (2%--3%) (IRIS, 2018). Most Cr(VI) is believed to be reduced in the stomach to Cr(III), which exhibits low bioavailability and can poorly cross cell membranes. Conversely, Cr(VI) can enter cells more easily. Therefore, Cr(VI) compounds are generally much more toxic than Cr(III) compounds, as they have potential for both genotoxicity and carcinogenicity (EFSA, 2014). Cr(VI) compounds have been classified as a Group A human carcinogen by the USEPA, as well as a Group 1 human carcinogen by the IARC with respect to cancer of the lung and nose/nasal sinuses.

In 2012, the EFSA received a request by the Hellenic Food Authority (EFET) for scientific data on chromium (both trivalent and hexavalent) levels in food and drinking water to estimate the risks to human health. According to EFSA research findings, the presence of Cr(III) or Cr(VI) in foods and drinking water, respectively, do not raise concerns for public health at the moment (EFSA 2014). Due to the lack of sufficient data, however, its safety cannot be fully assessed based on current literature (EFSA, 2009b). The call for scientific data on Cr(III) and Cr(VI) is thus still open and the EFSA is accepting submissions from national food authorities, research institutions, academia, food business operators, and other stakeholders (EFSA, 2012).

CHAPTER IV: Ecological Receptors

To have a good idea of the structure of a biotope for ecological risk assessment purposes, it is useful to be familiar with all species that are present there. This helps when choosing species to serve as biological indicators, which are further discussed in the Indicator Species section below.

Feeding Guilds

A guild is a group of organisms with overlapping niche requirements, which exploit the same class of environmental resources in a similar way, regardless of taxonomic position (Root, 1967). A feeding guild could, therefore, be described as a group of species that exhibit similar feeding requirements and habits. The major feeding guilds at risk that have been identified for the Bulgarian Black Sea coast are the following:

Fish

a. Piscivorous: e.g., European Flounder (*Platichthys flesus*), Whiting (*Merlangius merlangus*)
b. Molluscivorous: e.g., Round Goby (*Neogobius melanostomus*)
c. Planktivorous: e.g., juvenile stages of fish, European Sprat (*Sprattus sprattus phalericus*)

Aquatic Invertebrates

a. Planktivorous: e.g., snails, Mediterranean Mussel (*Mytilus galloprovincialis*)

b. Sedimentivorous: e.g., benthic (bottom-dwelling) polychaetes

c. Detritivorous: e.g., shrimp, Mediterranean Mussel

Reptiles

a. Piscivorous: e.g., European Dice Snake (*Natrix tessellata*)

Birds

a. Piscivorous: e.g., Great Cormorant (*Phalacrocorax carbo*)

Mammals

a. Piscivorous: e.g., dolphins

b. Omnivorous: i.e., humans

The primary habitats these non-human species are found in and/or exposed to consist in:

a. Sediment (invertebrates, mollusks, crustaceans, fish, and other benthic organisms);

b. Shelves/beaches (fish, mollusks, crustaceans, other shallow-water marine organisms);

c. Open sea (fish, marine mammals);

d. The interface between these biotopes.

Indicator Species

An indicator species, or bioindicator, is an organism that can be used to characterize a habitat (Dufrêne and Legendre, 1997), community, or set of environmental conditions (Lincoln *et al*., 1985), often in terms of pollution. The organisms that have been selected as appropriate indicator species for the present assessment of potential ecological and human health hazards are the Round Goby and the Mediterranean Mussel. In addition to these primary organisms, plankton has been marginally included in this study with the collection of 6 composite samples.

Round Goby

The Round Goby (*Neogobius melanostomus*) is a euryhaline bottom-dwelling fish (Corkum *et al*., 2004) and one of about 30 species in the family Gobiidae, 20 of which can be found in the Black Sea. It is part of a group of organisms also known as the "Pontian relicts", which are the most ancient element of Black Sea biota and predominated in earlier versions of the Black, Caspian, and Azov Seas (Zaitsev, 2008).

In the Black Sea, they are found in lower-salinity waters as part of the shelf ecosystem (Zaitsev and Mamaev, 1997). Being the gluttonous blobs (justifiably named “round”) that they are, these gobies have a soft spot for rocky substrates on which they can lazily perch and forage on mussels and unfortunate passers-by of the small crustacean variety. Although most juvenile and adult Round Gobies exhibit a preference for rocky benthos due to their cryptic nature, they do occur in fine gravel and sandy substrates that are conducive to burrowing (Ray and Corkum, 2001; Corkum *et al*., 2004). *N. melanostomus* can be identified by their relatively small size (Gutowsky *et al*., 2011), soft body, large cheeks and eyes, and prominent black spot on the first dorsal fin (Balážová-L’avrinčíková and Kováč, 2007).

N. melanostomus is considered relatively immobile and has a very small home range (Ray and Corkum, 2001). Home range is defined as the area an organism traverses to engage in routine activities such as foraging, seeking mates, and caring for its young (Seaman and Powell, 1996). Ray and Corkum (2001) reported Goby densities within 50 m^2 transects ranging from 0.3-3 fish/m^2 to 0.5-3 fish/m^2 to 5-9 fish/m^2, depending on the sampling area. Their estimates indicated an average home range size of 5 ± 1.2 m^2 (SE) per individual. The Goby exhibits high site fidelity, especially in preferred rocky habitats (Ray and Corkum, 2001).

Round Gobies of the Black Sea have a diverse diet that consists of amphipods, chironomids, cladocerans, crayfish, dragonflies, dreissenids, isopods, mayflies, fish eggs, and larvae (Corkum *et al*., 2004). Larger specimens, usually exceeding 7 cm in total length (TL), feed primarily on mollusks such as mussels and clams, crustaceans like shrimp and small crabs, as well as benthic polychaetes. The Mediterranean Mussel (*Mytilus galloprovincialis*) is an extremely important part of their diet year-round. They also use *Mytilus* shells for nesting (Corkum *et al*., 2004). Simonovic *et al*. (1998) reported that smaller gobies (TL < 5 cm) favor zooplankton (*Bosmina longirostris*) and ostracods.

Both obligate and facultative benthivorous fishes (i.e., fishes representing the same feeding guild as the Round Goby), as well as the occasional pelagic (i.e., organisms inhabiting the water column) species, constitute common Goby predators. Examples of benthic feeders are sturgeons of the genus *Acipenser* and flatfish like the European Flounder (*Platichthys flesus luscus*). Some pelagic fish predators are the European Perch (*Perca fluviatilis*), Atlantic Salmon (*Salmo salar*), and Zander (*Stizostedion lucioperca*) (Corkum *et al*., 2004).

In addition to falling prey to fishes belonging to other species, Round Goby eggs are often consumed by conspecifics. This phenomenon is described as non-kin inter-cohort cannibalism and is a characteristic behavior of juvenile fish (Yavno and Corkum, 2011). Laboratory observations indicate that egg cannibalism may be an attempt by juveniles to limit future intraspecific competition when population density is high and is not necessarily a result of food scarcity. Another hypothesis suggests that adult Gobies engage in filial cannibalism to improve their physical condition and remove diseased eggs (Yavno and Corkum, 2011).

Gobies are occasionally depredated by reptiles such as the European Dice Snake (*Natrix tessellata*) (Zaitsev and Mamaev, 1997). Another threat is the ctenophore *Mnemiopsis leidyi*, an invasive species introduced to the Black Sea in the 1980s that feeds on zooplankton, crustaceans, and fish larvae and eggs. Piscivorous birds such as the Great Cormorant (*Phalacrocorax carbo*) are also known to feed on Gobies (Corkum *et al*., 2004).

N. melanostomus serves as an important food fish for Bulgarians. It is regarded by subsistence and recreational fishers as a reliable catch and makes up to 30% of their catch composition (Keskin *et al*., 2017, based on V. Raykov unpublished data). The Round Goby is also harvested commercially, especially when stocks of other dominant commercial species fall. In 2000, gobies amounted to catches of 500 mt, making it the second most-fished species in Bulgaria after the European Sprat (*Sprattus sprattus phalericus*) (1,736.5 mt) (FAO, 2002). Overfishing has reduced the numbers of Round Goby individuals significantly in recent years, adding stress to its already burdened population (Stefan Mindov, angler, personal communication). I can attest to that myself, as my catch per unit effort (CPUE) decreased by an order of magnitude in some locations from one field season to the next, *ceteris paribus*. Additionally, threats like eutrophication-driven hypoxia and the destruction of Goby breeding grounds because of anthropogenic activity (e.g., bottom trawling) contribute to the species' decline even further (Zaitsev and Mamaev, 1997).

The Goby's broad diet and aggressive behavior result in high consumption rates. It often feeds on benthic organisms that have been exposed to contaminated sediments and is then preyed on by higher-order consumers. As a result, health concerns arise from human consumption of fish and mollusks (Corkum *et al*., 2004). Because of its limited home range, adaptability to a wide array of environmental factors and its ability to transfer contaminants through the food web, *N. melanostomus* has been used as a biological receptor in many toxicological studies. Contaminant transfer by the Goby can result in significant changes in the food web. This, in turn, may lead to increased ecological and human health concerns analogous to the rise in contaminant levels in sport and commercial fishes (Corkum *et al*., 2004).

Mediterranean Mussel

Mussels are the most common bivalve inhabiting Black Sea coastal waters. The mussel biocenosis – in other words, the community of biological organisms interacting closely with mussels – is one of the most well-studied in the Black Sea (Zaitsev and Mamaev, 1997). For example, small organisms such as algae, barnacles, and sponges form a symbiotic relationship with mussels by attaching themselves to their shells (Zaitsev and Mamaev, 1997). Mussel colonies flourish on bottom rocks, where they aggregate in large numbers.

The Mediterranean Mussel (*Mytilus galloprovincialis*) is of great commercial importance to the Bulgarian coastal fishery sector. It was the only species reared in aquaculture in Bulgaria until 2004 and is still the primary species produced in this fashion in the coastal territories of the country. Mussel production in 2004 averaged 118 t, which is 85% of total coastal production (FAO, 2004). By 2015, that number had risen to 3,114 mt (Bulgarian Ministry of Agriculture and Food, 2016).

The Mediterranean Mussel is a filter-feeder that feasts on phytoplankton and detritus suspended in the water column. It can be identified by several morphological characters such as its size, color, and shape. It typically ranges between 5-8 cm in length (FAO Species Fact Sheet, 2018). Its color can range from dark blue, to brown, to nearly black. Its two shells are equal in size and of a quadrangular shape, with one side wider and rounded and the other narrow and pointed (Global Invasive Species Database, 2018).

As is the case for mollusks in general, mussel larvae are particularly sensitive to oxygen depletion (Zaitsev and Mamaev, 1997). Therefore, mussel biomass has been severely declining because of bottom hypoxia (Zaitsev and Mamaev, 1997) and destructive fishing practices like bottom-trawling (Zaitsev *et al*., 1992). While banned in the past because of its immense harm on the benthos, bottom-trawling was reinstated in the 1970s to increase catches of Sprat, one of the main five species of fish harvested commercially in the Bulgarian Black Sea (FAO, 2002). Fortunately, bottom-trawling, dredging, explosives, and other practices and fishing gear that are catastrophic to the environment are once again prohibited under the Fisheries and Aquaculture Act of 2001 (FAO, 2002; Duzgunes and Erdogan, 2008).

Common *M. galloprovincialis* predators include the Round Goby (*N. melanostomus*) and the Rapa Whelk (*Rapana thomasiana*). *R. thomasiana* is a gastropod introduced accidentally into the Black Sea from the Sea of Japan in the 1940s (Zaitsev, 2008), which devastated mussel populations before it started being commercially exploited (Zaitsev and Mamaev, 1997). Today there is a large market for whelk, particularly in the export sector to Southeast Asian countries (FAO, 2002). Nevertheless, it is a frequently consumed gastropod in Bulgaria and has the capacity of bioaccumulating contaminants through its exposure to sediments and bivalves. Furthermore, because contaminants remain bioaccessible in the Whelk's tissue, it is potentially a human alimentary hazard (Gedik, 2017). However, assessment of health risks associated with Rapa Whelk consumption is beyond the scope of this paper.

M. galloprovincialis is found at depths from 1 m (Global Invasive Species Database, 2018; personal observation) up to > 70 m in the Black Sea, with some of the largest specimens caught at a depth of around 37 m on the Bulgarian shelf (Shurova and Gomoiu, 2005). They act as natural biofilters (Zaitsev, 2008; Jitar *et al*., 2013) and, according to Kiseleva (1979), cited in

Zaitsev and Mamaev (1997), one km^2 of mussel biocenosis is able to filter 15-20 million m^3 of water per day. Small particles from the water are retained in the mussels' tissue, which is useful when assessing marine pollutants (Jitar *et al*., 2013). Because of its ecological importance, the mussel is frequently used as a bioindicator in environmental risk assessments to characterize water quality and general ecosystem health. Also, because of its sessile benthic lifestyle and high utility as a food species, it is used as an indicator of dietary exposure to harmful substances in human health risk assessments.

Plankton

In addition to organisms that are active swimmers (e.g., fish and marine mammals), collectively known as nekton, there also exist unlucky forms incapable of swimming against currents and are therefore carried by them. These are collectively termed plankton (Zaitsev and Mamaev, 1997). There are three main types of plankton: bacterioplankton, phytoplankton, and zooplankton, which can be further sorted into groups according to the size of the organisms and, in the case of zooplankton, their trophic level (i.e., their position in the trophic pyramid). Plankton can also be classified as either holoplankton or meroplankton. Holoplankton are planktic[1] forms that spend their entire life cycle as such (e.g., algae and amphipods). By contrast, meroplankton only remain as plankton for part of their life cycle, usually the larval stage (e.g., larval fish and crustaceans), after which they transition to their final nektic or benthic form (Zaitsev and Mamaev, 1997; Kamburska and Vulcheva, 2003; Al-Yamani *et al*., 2011). Organisms

[1] There is an interesting discussion about the use of the term "planktic" *versus* "planktonic". If a derivation is made from a Greek or Latin noun, only the stem of the word must be incorporated and not the suffix, which is a mere indication of gender. In the case of "plankton" and similar nouns, the suffix "-on" indicates the word is in the neuter gender. The correct adjective, therefore, would be "planktic". The same principle applies to nouns such as nekton (whose adjective becomes "nektic"), benthos ("benthic"), and phobia ("-phobic"). Derivations such as "planktonic", "nektonic", and "benthonic" are thus incorrect (Emiliani, 1952; Rodhe, 1974; Emiliani, 1991).

generally exceeding 20 mm (and reaching up to 200 mm) are referred to as macroplankton; those ranging in size between 200 µm-20 mm are known as mesoplankton; finally, forms 20-200 µm fall in the microplankton size class (American Society of Limnology and Oceanography, 1978; Zaitsev, 2008).

Plankton are a primary food source for many mollusk and fish species. These include the Mediterranean Mussel (*Mytilus galloprovincialis*) (FAO Species Fact Sheet, 2018) and Round Goby (*Neogobius melanostomus*) when in its juvenile stage (Hensler and Jude, 2007; Balážová-L'avrinčíková and Kováč, 2007; Kvach and Stepien, 2008). Being such an integral building block at the base of the food web, planktic forms are frequently used in toxicological studies (e.g., Bryan et al., 1979; Cutter, 1992; Islam and Tanaka, 2004; Oguz and Velikova, 2010).

In the present study, of interest are mainly zooplanktic communities. These include larval fish, crustaceans, gastropods, and polychaetes, as well as detritivorous and herbivorous zooplankton like copepods and cladocerans (Shiganova and Bulgakova, 2000). Many planktic species migrate vertically through the water column, sometimes crossing distances of hundreds of meters. Migrations occur from the bottom layers of the sea toward the surface in the evening, and in the opposite direction in the morning (Zaitsev and Mamaev, 1997; Milroy, 2015).

The northwestern shelf (mostly in Romania, which borders Bulgaria to the north) is home to some of the greatest concentrations of plankton in the Black Sea (Zaitsev and Mamaev, 1997). This has attracted large numbers of jellyfish to the area over the years, leading to explosions in their populations. Gelatinous zooplanktic forms like the Root-mouthed Jellyfish (*Rhizostoma plumo*), the Moon Jelly (*Aurelia aurita*), and Leidy's Comb Jelly (*Mnemiopsis leidyi*) are voracious predators of so-called trophic, or edible, zooplankton (Shiganova and Bulgakova, 2000). The term trophic zooplankton refers to all non-gelatinous and non-opportunistic species groups (such as copepods, cladocerans, microzooplankton, and meroplankton), which comprise the main food source of higher-trophic-level organisms (Oguz and Velikova, 2010). It is noteworthy that by the 1980s, *A. aurita* had decimated the annual production of the entire Black Sea zooplankton by 62% (Zaitsev and Mamaev, 1997). By the late 1980s however, *M. leidyi* had outcompeted other jellyfish species – namely *A. aurita* – and was eating its way through the Black Sea. It decimated important fish stocks while in their ichthyoplanktic stages, compromising commercial catches and leading to a collapse in pelagic fisheries (Daskalov, 2002; Radu *et al*., 2011; Keskin *et al*., 2017). By eliminating edible zooplankton, *M. leidyi* managed to drive a trophic cascade[2] and completely alter the diet composition of many planktivorous fishes (Shiganova and Bulgakova, 2000; Daskalov, 2002). It is safe to say it had morphed into the arch-nemesis of many living biota, at least until the appearance of the alien ctenophore *Beroe ovata* in the late 1990s (Zaitsev, 2008), which preyed on *M. leidyi*. *B. ovata* restructured the food web and kept *M. leidyi* biomass and numbers in check, allowing for the recovery of trophic zooplankton (Shiganova and Bulgakova, 2000).

Threatened and Endangered Species

Because of the migratory and bioaccumulative nature of many contaminants, the discharge of pollutants such as metals into the Black Sea has a profound effect on the entire marine ecosystem (Islam and Tanaka, 2004). COCs therefore not only harm commercial stocks like the Round Goby and Mediterranean Mussel, but an array of other marine species living in the same waters and interacting with gobies and mussels. For the purposes of risk assessments, it is therefore imperative that species be evaluated not as isolated organisms, but rather as

[2] To be completely fair, jellyfish were assisted by the industrial fishing sector in driving the trophic cascade (Daskalov, 2002; Caddy, 2006).

components of the ecological communities they are a part of. In a similar fashion, exposure risks should be quantified with intra- and interspecific interactions in mind, as well as interactions between biotic and abiotic components of any given ecosystem. Particular attention ought to be paid to organisms whose population health and numbers are especially compromised. For the purposes of environmental projects in the area – for example, a full-scale ecological risk assessment or contaminant cleanup) – it is necessary to be familiar with all living organisms in Bulgarian Black Sea waters.

The Bulgarian territory of the Black Sea is home to many such species under threat of extinction, especially fishes. The status of individual species varies depending on the robustness of their stock, but many are facing serious threats owing to human activity. Table 3 has been compiled from Black Sea Fish Checklist data in Yankova *et al*. (2014). The Checklist is a publication of the Commission on the Protection of the Black Sea Against Pollution and contains a listing of Threatened and Endangered (T&E) fish species and their current status. Considered "Endangered" is a species in danger of extinction throughout all or a significant portion of its range. "Threatened" is a species likely to become endangered in the foreseeable future (USDA, 2017).

Table 3. Threatened and Endangered fish species of the Bulgarian Black Sea.

Fish Species		Bulgarian Criteria[1]	IUCN Status[2]
Common Name	Scientific Name		
Russian Sturgeon	*Acipenser gueldenstaedtii*	RBS, RDB	VU
Starry Sturgeon	*Acipenser stellatus*	RBS	VU
Bastard Sturgeon	*Acipenser nudiventris*	RDB	
European Sea Sturgeon	*Acipenser sturio*	RDB	
European Eel	*Anguilla Anguilla*	RDB	
Sphinx Blenny	*Aidablennius (Blennius) sphynx*	RBS	VU
Montagu's Blenny	*Coryphoblennius galerita* – BG coast	RBS	VU
Peacock Blenny	*Salaria (Lipophrys) pavo*	RBS	
Risso's Dragonet	*Callionymus risso*	RBS	

Caspian Shad	*Alosa caspia*	RDB	
Black Sea Sprat	*Clupeonella cultriventris*	RBS, RDB	EN
Three-Spined Stickleback	*Gasterosteus aculeatus*	RDB	
European Conger	*Conger conger*		VU
Ukrainian Stickleback	*Pungitius platygaster* – BG coast	RDB, RBS	CR
N/A	*Benthophiloides brauneri* – BG coast	RDB, RBS	VU
Stellate tadpole-goby	*Benthophilus stellatus*	RDB	
Chestnut Goby	*Chromogobius quadrivittatus* – BG coast	RDB	CR
Giant Goby	*Gobius cobitis*	RBS	EN
Bucchich's Goby	*Gobius bucchichi* – BG coast	RDB	CR
Grass Goby	*Zosterisessor ophiocephalus*	RBS	CR
Caucasian Dwarf Goby	*Knipowitschia caucasica*	RDB	
Longtail Dwarf Goby	*Knipowitschia longecaudata*	RDB, RBS	EN
Knout Goby	*Mesogobius batrachocephalus*	RBS; NT	
Bighead Goby	*Neogobius kessleri*	RDB	
Ratan Goby	*Neogobius ratan*	RDB, RBS	VU
Syrman Goby	*Neogobius syrman* – BG coast	RDB, RBS	CR
Sand Goby	*Pomatoschistus minutus* – BG coast	RBS	CR
Tube-nosed Goby	*Proterorhinus marmoratus* – BG coast		EN
East Atlantic Peacock Wrasse	*Symphodus tinca* – BG coast	RBS	VU
Ocellated Wrasse	*Symphodus ocellatus* – BG coast	RBS	VU
Thinlip Mullet	*Liza ramada*		VU
Red Mullet	*Mullus barbatus*	RBS	EN
Sea Zander	*Sander marinus*	RDB, RBS	
Atlantic Bonito	*Sarda sarda*	RBS	CR
Atlantic Bluefin Tuna	*Thunnus thynnus*	RBS	EN
Black Scorpionfish	*Scorpaena porcus*		VU
Blackhand Sole	*Pegusa nasuta*		VU
Annular Seabream	*Diplodus annularis*	RBS	VU
Long-snouted Seahorse	*Hippocampus guttulatus*	RBS	EN
Straightnose Pipefish	*Nerophis ophidion*	RBS	EN
Narrow-snouted Pipefish	*Syngnathus tenuirostris*	RBS	NT
Broadnosed Pipefish	*Syngnathus typhle*	RBS	VU
Greater Weever	*Trachinus draco*	RBS	CR
Tub Gurnard	*Chelidonichthys lucernus*	RBS	VU
Atlantic Stargazer	*Uranoscopus scaber* – BG coast	RBS	CR
Broadbill Swordfish	*Xiphias gladius*	RBS	EN
Garfish	*Belone belone*		EN
Atlantic Mackerel	*Scomber scombrus*		EN

[1]Bulgarian Criteria: RDB = Red Data Book of Bulgaria; RBS = Red Book of the Black Sea

[2]IUCN (International Union for the Conservation of Nature) Status: NT = Near Threatened; VU = Vulnerable; EN = Endangered; CR = Critically Endangered

CHAPTER V: Materials and Methods

Sampling Methodology

Overview

To assess different trophic levels, fish, mussel, and plankton samples were collected. For every sample category, the following data were also collected: GPS coordinates and water quality parameters (i.e., pH, temperature, and dissolved oxygen). The vessel used for most of the sampling was a 2 m Honda HonWave inflatable boat with a Mercury 5 HP outboard motor (Honda Motor Co., Ltd., Tokyo, Japan). The advantage of using this type of boat was the ability to access some locations where larger vessels would have encountered difficulty. The handheld GPS unit used throughout this study to record the geographic coordinates of sampling locations was a Garmin GPSMAP 64S (Garmin International, Inc., Olathe, KS). The sampling area and specific locations are shown in Figure 3 and subsequent maps.

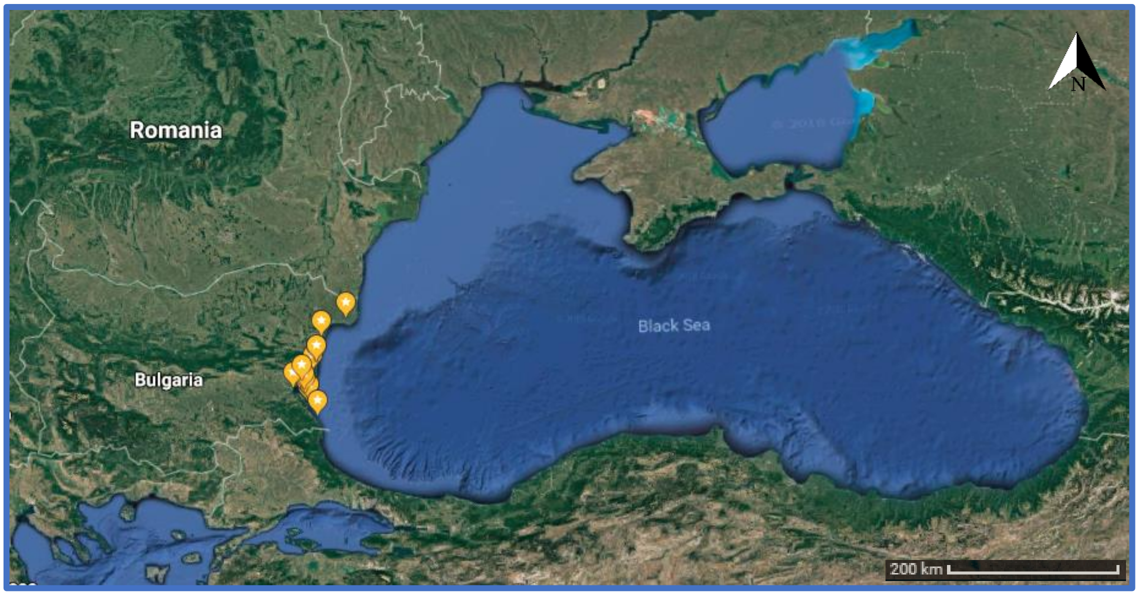


Fig 3. Map of the Black Sea, the country of Bulgaria, and the sampling locations along the Bulgarian Black Sea coast.

Field Season I – July and August 2016

Fish Sampling

The fish species of interest for this study was the Round Goby (*Neogobius melanostomus*). Round Gobies were identified and distinguished from other fish species by the morphological characters described earlier in this text, under the "Ecological Receptors" section.

Fish were collected individually with fishing rods, using the hook-and-line angling removal method described in Gutowsky *et al*. (2011). This is a fairly simple and inexpensive method and efficient in complex rocky substrates gobies have an affinity for. Thanks to the Round Goby's aggressive nature and small home range, two anglers are able to deplete the aggregation of gobies at a selected spot within 20 minutes (Gutowsky *et al*., 2011). Angling was carried out by two individuals from the inflatable boat, which was anchored for stability, and prevented movement past each sampling point (Brownscombe and Fox, 2012).

The two fishing rods used for sampling were Shimano (Shimano Inc., Sakai, Japan), 2.70 m and 3.30 m in length, respectively. The fishing line was fluorocarbon with a width of 1 mm, and lure weights ranged in weight between 0.90—1.10 g. The fishing line had 3 evenly spaced stainless-steel hooks (sizes 3-10), each with a fresh shrimp attached as bait. A lead weight was attached to the end of the fishing line. Once the line was dropped to the bottom of the water column, the lead weight was bounced off the substrate at frequent time intervals to create brisk movement and attract gobies (Gutowsky *et al*., 2011).

Prime goby habitat was located with a Hummingbird FishFinder 688ci HD DI, equipped with Down Imaging Sonar and GPS (Marine Electronics Group, Eufaula, AL), as it provided the depth of the water column and a relief of the underwater substrate. Depths where fish were successfully collected ranged from 5 m-21.6 m, the average depth being 16.1 m. A total of 7 sampling locations was recorded (Figure 4), from which gobies were successfully caught.

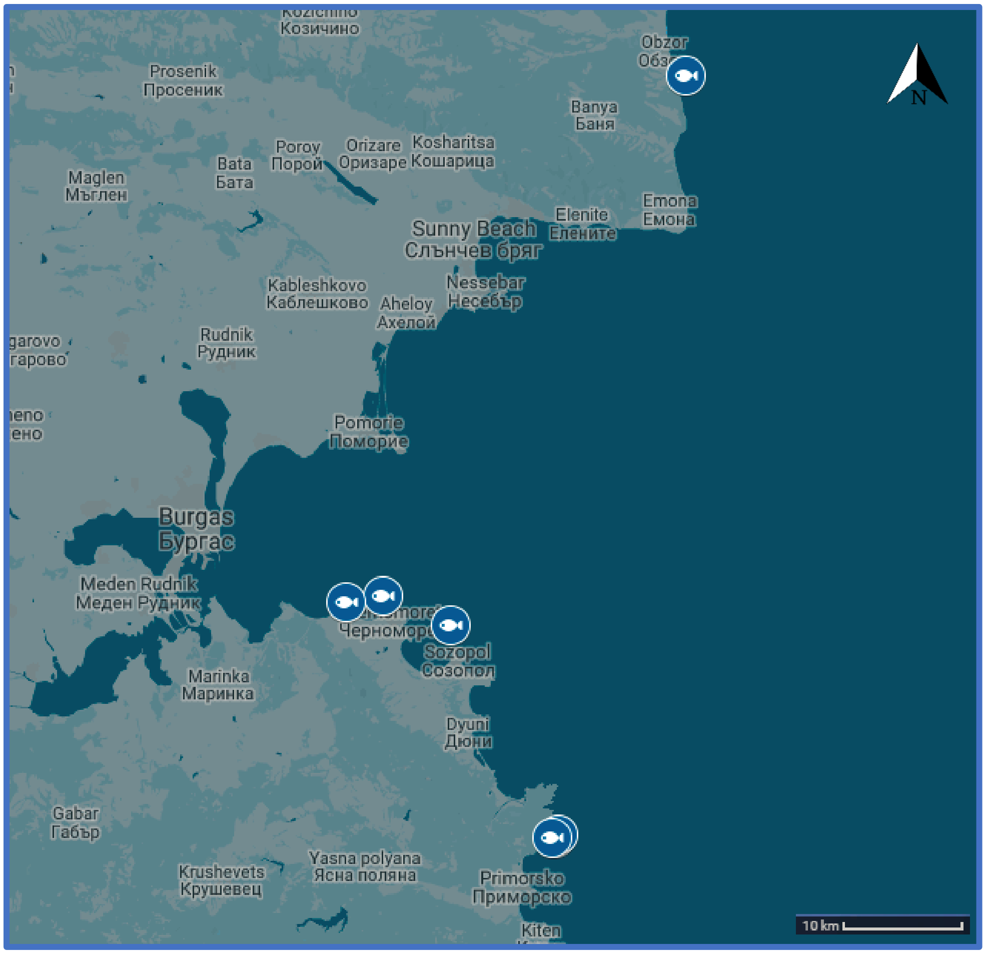


Fig. 4. Field Season I – Round Goby sampling sites.

Ten fish per location were collected, which, after gutting and entrail removal, were pooled into a composite sample for metals analysis. Whole fish were used as opposed to fillets, as this is how they are consumed by Bulgarians. No samples were washed prior to processing to avoid introducing contaminants from water, if present. Biometric data, such as standard length (SL) (Corkum *et al*., 2004) and wet weight before and after gutting, were also noted for each individual. SL was measured along the horizontal body axis, from the tip of the snout to the base of the hypural plate (Duemler *et al*., 2016).

Composite samples from each sampling location were sealed in separate Ziploc bags and cooled on wet ice for delivery to the analytical laboratory. The holding time for preservation of fish and shellfish samples containing metals is 24 hours (FDEP, 2014). All samples were moved to a freezer within a few hours of collection, before the expiration of this time limit. Total holding times until laboratory analysis depended on the date of collection and ranged from 5 to 15 days. The maximum holding time is 1 year for mercury and 6 months for all other metals (FDEP, 2014). Prior to delivery to the lab, the samples were kept in a freezer at a temperature of -20°C (FDEP, 2014). All samples were delivered to the lab in person under chain of custody (COC) control, and order forms for specific services (analysis for arsenic, lead, and mercury) filled out and signed. Once at the lab, the samples were kept frozen until analysis.

Mussel Sampling

The mussel species of interest was the Mediterranean Mussel (*Mytilus galloprovincialis*). Mediterranean Mussels were identified by the external morphological characters described earlier in this text, under the "Biological Receptors" section.

Mediterranean Mussel specimens were collected at 9 locations both manually through diving and through purchase at fish markets. The sampling locations are shown in Figure 5. Manual collections took place in shallower waters (< 2 m depth and ~2 m radius) and depended on mussel availability. Collections were random – in other words, I did not select for mussels exhibiting any particular characteristics. Purchases were made from vendors who could provide certificates of the mussels' origin (i.e., specific mussel farm), whose location could then be plotted on a map.

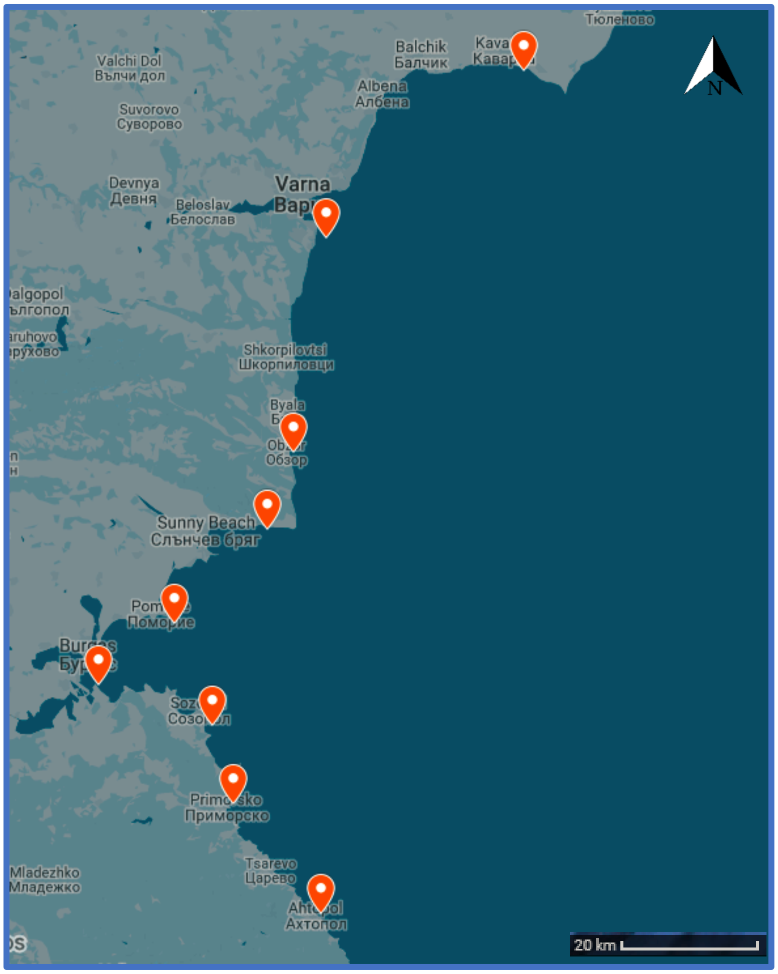


Fig. 5. Field Season I – Mussel sampling sites.

Biometrics such as length, width, height, and wet weight were noted for 30 mussel specimens from each location, which were pooled into a composite sample. The rest of the mussels were separated from their shells with a stainless-steel knife, and shells and meat were weighed separately. These were then grouped respectively into composite samples, in the same fashion as the whole mussels. Just like for the fish, composite samples were stored in Ziploc bags and kept cooled on wet ice all the way through delivery to the lab. As regards the mussel meat, composite samples were weighed to at least 10 g, which was the minimum wet mass the lab required for metals with the analytical methods available.

In addition to meat-only samples, whole mussel and shell composite samples were analyzed to acquire valuable information regarding potential metals contamination. This was an important step of the process, as many Bulgarian recipes call for whole mussels, which are boiled together with other ingredients to make stews or seafood rice pilaf (personal observation; personal communication). In the process of cooking, the shells open and are then used as makeshift spoons to scoop up rice and other dish components.

Field Season II – July and August 2017

Fish Sampling

Like in Field Season One, Round Goby fish were collected. Prime goby habitat was again located with the Hummingbird FishFinder. On several occasions, fish of this species were not present at the same exact locations where I caught them in 2016. Therefore, the closest new location was used to collect the second round of samples. The sampling locations are shown in Figure 6. Although the target number of fish per location was ten samples, sometimes this was not achieved, due perhaps to low Round Goby density in that area. Depths where fish were collected ranged from 13-23.7 m, in rocky substrate, with the average depth being 19 m.

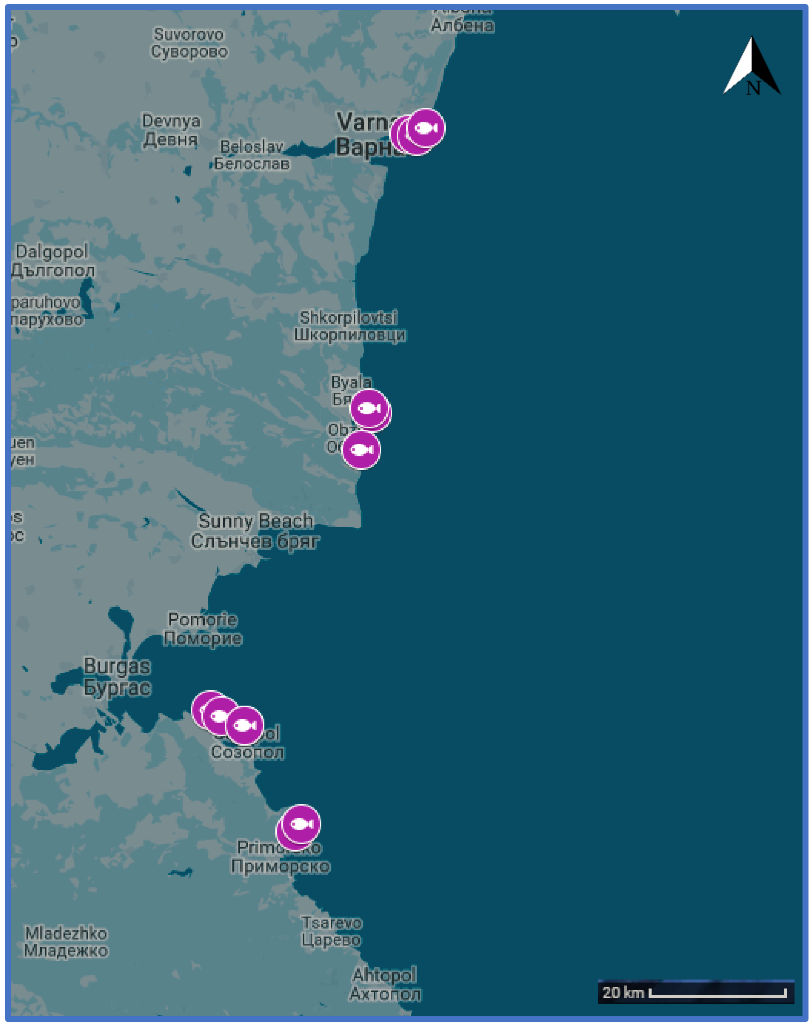


Fig. 6. Field Season II – Round Goby sampling sites.

The fish were processed and biometric data noted for each individual, as previously described. During this field season, each fish was placed in a separate Ziploc bag and cooled on wet ice before delivery to the laboratory. Each fish was therefore analyzed separately, and not as part of a composite sample. This was done in the interest of acquiring analytical data that can be matched to individual fish and avoid the loss of information associated with compositing. It was also useful in instances when < 3 gobies were caught at a single location and to mitigate statistical analysis issues arising from small and/or unequal sample sizes.

Plankton Sampling

Vertical plankton sampling was the most appropriate sampling scheme for this study. To accomplish this, the net was submerged to the bottom of the water column and lifted slowly to the surface. Plankton migrate vertically through the water column during the day, a phenomenon known as diel vertical migration (DVM), so different planktic forms are found at different depths at different times (Zaitsev and Mamaev, 1997; Milroy, 2015). Therefore, to obtain a representative sample, it was necessary to sample the entire water column. Thus, horizontal sampling was dismissed as a collection method for this study, as that type of sampling would have limited the study to surface-dwelling plankton or the few species that happened to be near the surface at that time of the day. I should note here that I did not sample for plankton at the same time each day but, rather, based on convenience. Another complicating factor associated with horizontal sampling is the much wider area that needs to be sampled to collect the same amount of planktic material as well as the towing speed of the boat, which ideally would involve a flowmeter to measure the speed of water flowing through the net per unit time. The sampling locations for plankton are shown in Figure 7.

Zooplankton was collected with a SEA-GEAR Model 9000 plankton net (SEA-GEAR Corporation, Melbourne, FL) especially designed for marine work. The net had a conical shape, a 60 cm diameter mouth and 3:1 length-to-mouth ratio. The mesh size was 200 µm, appropriate for sampling meso- and macroplankton. These net parameters were chosen according to literature discussing marine zooplankton sampling (Keen, 2015; Altukhov *et al*., 2015; Milroy, 2015) and the needs of the present research. The cod end (collection container) of the net consisted in a plastic jar of 1 L capacity. The cod end itself was not perforated and lacked an attached ballast weight. As a result, I attached two 200 g lead weights, one on either side of the cod-end bucket so the net would sink to the bottom of the water column. To facilitate the vertical sampling scheme, the ring holding the net sleeve around the jar was used as the attachment point.

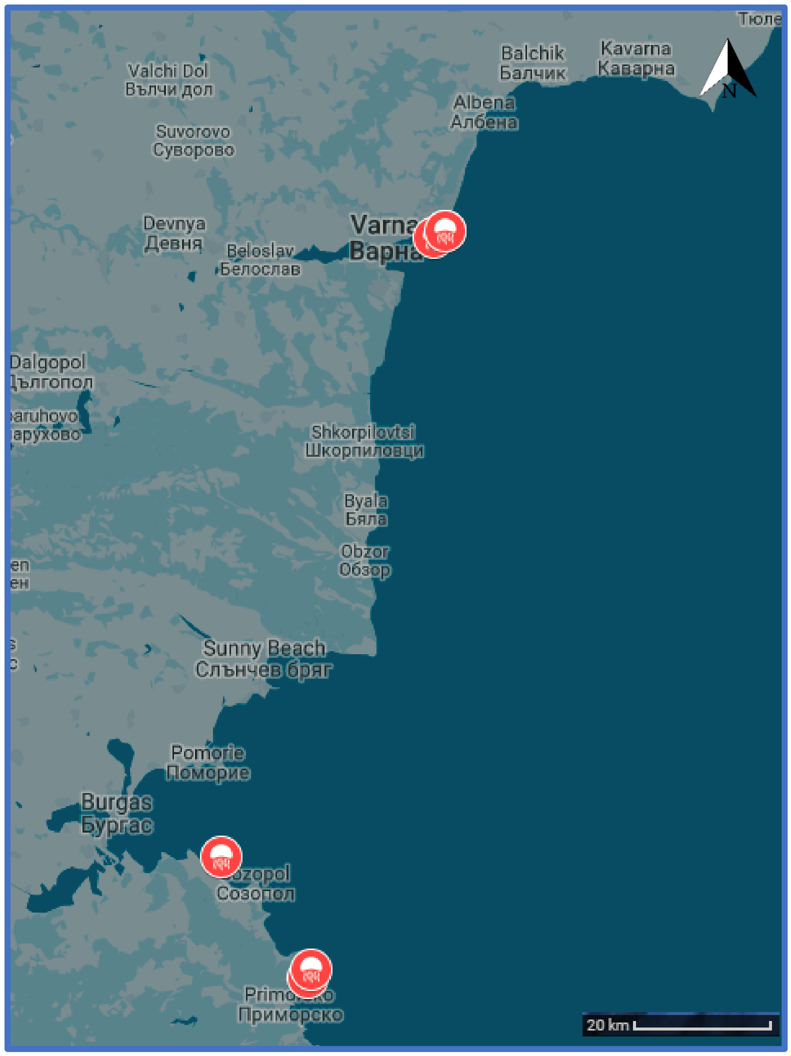


Fig. 7. Field Season II – Plankton sampling sites.

The plankton collected during each deployment of the net was filtered through the net itself, wrapped over a plastic bucket. The plankton was then collected with a stainless-steel spoon and emptied into a plastic Ziploc bag. An alternative method that we tried but which proved ineffective was to filter the water collected in the cod end of the net through laboratorygrade filter paper, and the plankton scraped off with a stainless-steel spoon. This however proved impossible, as the volume of water contained in the cod end (1 L) was too great for the filter paper to withstand without tearing, even when multiple layers were superimposed. As a result, this method was quickly abandoned.

The samples were transported on wet ice to a freezer for holding until metals analysis. Holding times ranged from 5 to 15 days, and samples were kept in a freezer at a temperature of -20°C until delivery to the lab. All samples were delivered to the lab in person under chain of custody (COC) control, and order forms for specific services (analysis for arsenic, chromium, and cadmium) filled out and signed. Once at the lab, the samples were kept frozen until analysis.

Plankton sampling posed a challenge during certain instances. Wherever the numbers of gelatinous forms (jellyfish) of zooplankton were low, so was my yield. As the analytical laboratory required a 10 g wet weight sample minimum, collecting 10 g of planktic mass proved to be difficult. I had to submerge the net multiple times to collect a single composite sample. In the absence of small jellyfish – which comprised the bulk of these composite samples – the total weight of a sample could not reach the necessary 10 g without the aid of additional instruments that were beyond the budget of this study. Nevertheless, a total of 6 composite plankton samples were collected and analyzed. Collection depths of the water column ranged from 6—19 m, with an average depth of 13.7 m.

Considering the fact that the proportion of edible zooplankton to jellyfish could not be quantified in the present study, the analytical results are only meant to provide an indication of the presence of contaminants in gelatinous forms. They are not, however, meant to be used in future studies investigating contamination of edible zooplankton with metals or the biomagnification of pollutants across the marine food web using the edible zooplanktic level as a reference point. Since jellyfish do, nevertheless, feed on trophic zooplankton and occupy more or less the same niche as other planktivorous organisms (Tilves *et al*., 2018), one may hypothesize as to the contribution of trophic zooplankton to metal levels found in jellyfish. Additionally, as taxonomic identification of the jellyfish was not feasible, the results from this study may not be attributed to any single species.

Statistical Methods

I used a combination of the statistical software packages Microsoft Excel and IBM SPSS (Statistical Package for Social Sciences) to analyze the results. The significance level chosen was $\alpha = 0.05$. I ran Kolmogorov-Smirnov tests for normality on all samples to identify the distribution pattern. As expected, all biometric data exhibited a normal distribution, while pollutant (arsenic and chromium) levels were non-normally distributed, except for the fish samples collected in Field Season II, which exhibited a normal distribution for arsenic.

I ran parametric statistics on the biometric data and non-parametric statistics on the metals. Analyses of Variance (ANOVAs) were run on the biometric data, along with Tukey and LSD post-hoc tests to establish error rates. Non-parametric Kruskal-Wallis one-way analyses of variance were performed to compare arsenic and chromium level distributions across fish and plankton composite samples. A Bonferroni correction was applied to reduce Type I Error, as multiple statistical tests were run simultaneously. The same was done for mussel composite samples; meat, shells, and whole mussels were evaluated separately. The final step involved running non-parametric Spearman correlation tests between arsenic and chromium levels in biota and distance from industrial zones and pollution “hot spots”.

Values Below the Limit of Quantification (LOQ)

The occurrence of so-called non-detectable, or “non-detect” values (i.e., values lying below the limit of detection, or LOD) is not uncommon in studies such as the present one. The LOD is defined as the level at which a measurement has a 95% probability of being different from zero (Taylor, 1987; Croghan, 2003). Therefore, non-detect values lie below the threshold at which a value can be accurately quantified (Croghan, 2003).

Non-detect values can be handled in one of three ways: using the extrapolation technique, the maximum likelihood estimate technique, or the replacement method (Croghan, 2003). Using the replacement method, a value may be replaced with zero, it may be assigned half the limit of detection (LOD), or the LOD may be divided by the square root of 2 (Croghan, 2003).

For some metals (cadmium, mercury, and lead) the reported values from the laboratory analyses came back as below the “Limit of Quantification”, or LOQ, for 100% of each dataset. As such, these datasets were not included in the statistical analyses. The LOQ is the level above which quantitative results may be obtained (EPA, 2002) and is a multiple of the LOD, usually the equivalent of three times the LOD (Croghan, 2003). Values that lie below the LOQ may therefore be treated in the same way as non-detects (EPA, 2002). In the interest of simplicity, the replacement method was used for a single datum, which was the only below-LOQ value among all the arsenic and chromium data sets. The LOQ/√2 was chosen among the three replacement methods, as it has the smallest overall error rate (Croghan, 2003).

CHAPTER VI: Results

Field Season I

Fish

The lab results from Summer 2016 indicated elevated arsenic levels in all fish samples. Arsenic values ranged from 0.84 mg/kg to 1.60 mg/kg, with an average of 1.11 mg/kg. Mercury and lead values were below the LOQ, at < 0.5 mg/kg, for all fish samples. Standard length of composite goby specimens ranged between 11.8 cm and 14.17 cm, with an average of 12.64 cm. Their composite wet weight upon capture was between 27.30 g and 56.54 g, with an average of 41.96 g.

Water pH was between 8.34 and 8.55 with an average of 8.46, whereas water temperature at the surface ranged from 26.10 °C to 26.80 °C, with an average of 26.51 °C. Dissolved oxygen levels at the surface (depths of a few centimeters) remained consistently at 4 mg/L throughout the field season. This concentration is very low, as fish and other marine organisms usually require dissolved oxygen levels of 8 mg/L. A probable cause for the low concentration is evaporation, and dissolved oxygen levels are likely higher at greater depths. Gobies were caught at depths ranging between 5 m and 21.60 m, with an average of 16.13 m.

Kolmogorov-Smirnov and Shapiro-Wilk's tests ($p < 0.5$), as well as a visual evaluation of the histograms, normal Q-Q plots and box plots, indicated that arsenic levels were non-normally distributed across all fish samples. Skewness and kurtosis values were 1.056 (SE = 0.281) and -0.376 (SE = 0.555), respectively. The Kruskal-Wallis analysis of variance ($p < 0.5$) indicated that the distribution of arsenic was not the same across composite samples. Pairwise Kolmogorov-Smirnov tests indicated a difference between the following samples: PMN3/NEME3 and PMN2/NEME2, PMN3/NEME3 and ATIBIV/NEME5, PMN3/NEME3 and SVIV1/NEME4, BY1/NEME7 and PMN2/NEME2, BY1/NEME7 and ATIBIV/NEME5, BY1/NEME7 and SVIV1/NEME4, PMN1/NEME1 and SVIV1/NEME4, and between ATIA1/NEME6 and SVIV1/NEME4.

A Spearman correlation showed an inverse relationship between arsenic levels and sample distance from industrial "hot spots" for all locations (Burgas Bay, Lukoil Refinery, Burgas Airport, and Varna Bay). The strongest negative correlation between distance from these "hot spots" and arsenic concentrations are in Burgas Bay, the Lukoil Refinery, and Burgas Airport (Spearman's rho Correlation Coefficient = -0.556), followed by Varna Bay (Spearman's rho Correlation Coefficient = -0.050) to a lesser degree. This means that as we get closer to these locations, arsenic concentrations increase, and vice-versa.

Arsenic levels in fish caught from Burgas Bay were greater than arsenic levels in fish caught to the north, or upcoast, of that location. While there are no large islands in the Black Sea, there are a few small ones in Burgas Bay (Zaitsev & Mamaev, 1997; Zaitsev, 2008). One of these islands is Sveti Ivan ("St. Ivan") island, values around which exhibited the highest concentrations of arsenic, with values decreasing as we moved away from the island.

Arsenic in fish caught from waters south, or downcoast, of Burgas Bay exhibited concentrations less than or equal to those of fish from Burgas Bay. No significant difference in arsenic levels was observed in fish caught in downcoast waters from those caught in waters upcoast from Burgas Bay.

In terms of biometrics, there was no statistically significant difference among fish caught in Burgas Bay, upcoast, and downcoast. There was also no statistically significant correlation between arsenic level and: fish wet weight, water pH, temperature, and column depth.

Table 4. Locations, average biometric measurements and arsenic levels of Round Goby composite samples during Field Season I.

				Biometric Averages		
Location	**Sample**	**Coordinates (WGS84)**	**Number of fish caught**	**Wet Weight (g)**	**Standard Length (cm)**	**Arsenic Conc. Avg. (mg/kg)**
Primorsko	PMN1/NEME1	42.301252, 27.782191	10	35.30	11.93	0.99
Primorsko	PMN2/NEME2	42.303435, 27.784329	10	27.30	11.08	1.01
Primorsko	PMN3/NEME3	42.300700, 27.778533	10	48.80	13.27	0.84
Sveti Ivan	SVIV1/NEME4	42.441171, 27.687703	10	41.50	12.81	1.60
Atiya	ATIBIV1/NEME5	42.460272, 27.628327	10	43.30	12.23	1.38
Atiya	ATIA1/NEME6	42.456138, 27.594548	10	41.00	13.01	0.99
Obzor	BY1/NEME7	42.802160, 27.898671	13	56.54	14.17	0.97

Fig. 8. Average arsenic concentration in a Round Goby composite sample from Byala during Field Season I.

Fig. 9. Average arsenic concentrations in Round Goby composite samples from Atiya and Sveti Ivan during Field Season I.

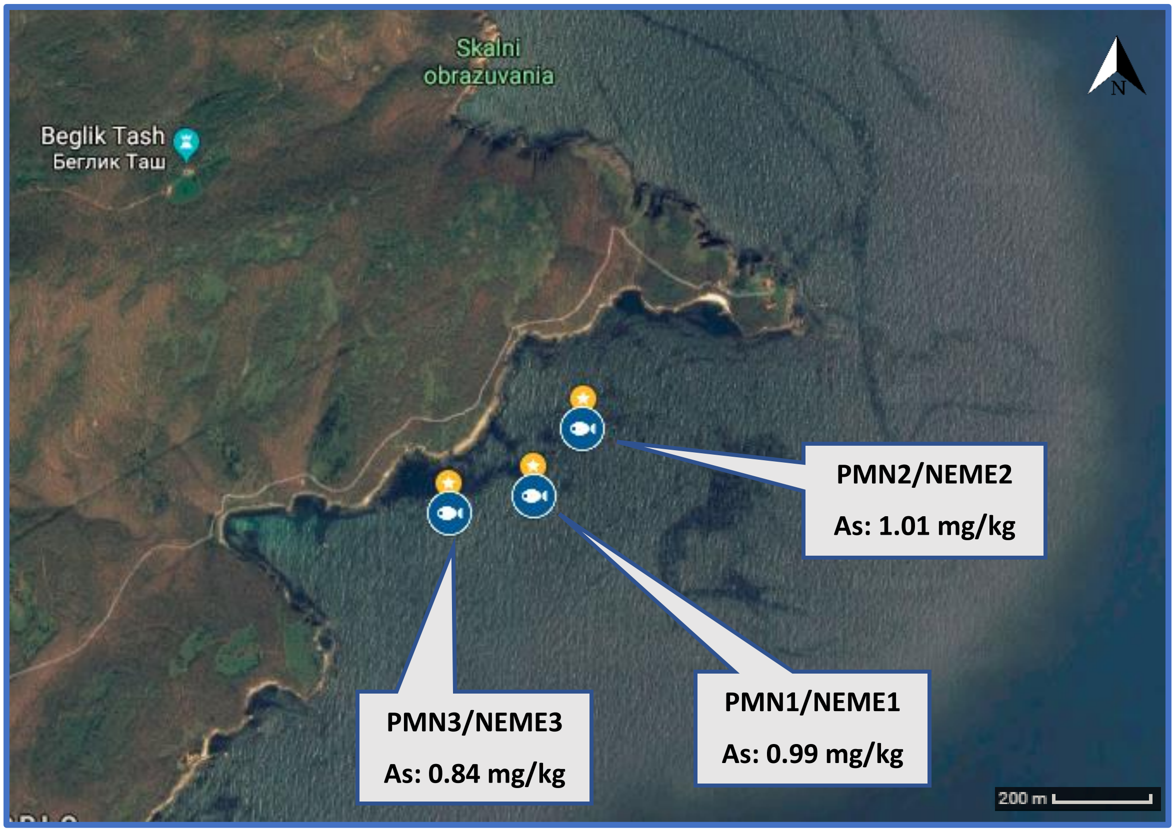


Fig. 10. Average arsenic concentrations in Round Goby composite samples from Primorsko during Field Season I.

Mussels

Kolmogorov-Smirnov and Shapiro-Wilk's tests ($p < 0.5$), as well as a visual evaluation of the histograms, normal Q-Q plots and box plots, indicate that arsenic levels were non-normally distributed across all mussel samples. Whole mussel samples exhibited skewness and kurtosis values of 1.343 (SE = 0.157) and 0.795 (SE = 0.314), respectively. Meat-only mussel samples exhibited skewness and kurtosis values of -0.547 (SE = 0.157) and -1.242 (SE = 0.314), while shells-only mussel samples yielded values of 0.772 (SE = 0.157) and 0.491 (SE = 0.314), respectively. The Kruskal-Wallis analysis of variance ($p < 0.5$) indicates that the distribution of arsenic was not the same across composite samples. This held true for whole, meat-only, and shell-only composite samples. Pairwise Kolmogorov-Smirnov tests indicated that the following samples differed in their distribution: AHT1/MYGA1 and OBZ1/MYGA5, KRA1/MYGA6, SOZ1/MYGA9, POM1/MYGA2, and PRI1/MYGA7. Sample GAL1/MYGA4 was different from OBZ1/MYGA5, KRA1/MYGA6, SOZ1/MYGA9, POM1/MYGA2, and PRI1/MYGA7. Sample KAV1/MYGA3 differed from KRA1/MYGA6, SOZ1/MYGA9, POM1/MYGA2, and PRI1/MYGA7. Sample ELE1/MYGA8 had a different distribution from samples KRA1/MYGA6, SOZ1/MYGA9, POM1/MYGA2, and PRI1/MYGA7. Sample OBZ1/MYGA5 differed from samples POM1/MYGA2 and PRI1/MYGA7. Finally, sample KRA1/MYGA6 differed in distribution from sample PRI1/MYGA7.

For whole mussels, a Spearman correlation showed an inverse relationship between arsenic levels and distance from industrial "hot spots" for the following locations: Burgas Bay (Spearman's rho Correlation Coefficient = -0.722), Lukoil Refinery (Spearman's rho Correlation Coefficient = -0.689), and Burgas Airport (Spearman's rho Correlation Coefficient = -0.689). There was a positive correlation between sample arsenic concentrations and distance from Varna Bay (Spearman's rho Correlation Coefficient = 0.344). For meat only, a Spearman correlation showed a positive relationship between arsenic levels and distance from industrial "hot spots" for all locations: Burgas Bay (Spearman's rho Correlation Coefficient = 0.180), Lukoil Refinery (Spearman's rho Correlation Coefficient = 0.180), Burgas Airport (Spearman's rho Correlation Coefficient = 0.240), and Varna Bay (Spearman's rho Correlation Coefficient = 0.013). Finally, for shells only, a Spearman correlation showed a positive relationship between arsenic levels and distance from Burgas Bay (Spearman's rho Correlation Coefficient = 0.024) and the Lukoil Refinery (Spearman's rho Correlation Coefficient = 0.024) and an inverse relationship for Burgas Airport (Spearman's rho Correlation Coefficient = -0.157) and Varna Bay (Spearman's rho Correlation Coefficient = -0.205).

Statistical analysis of the results indicates no significant difference in contamination between Burgas and Varna Bay samples and samples taken from other locations along the Bulgarian Black Sea coast. Samples consisting in meat only exhibited the highest concentration of arsenic in mussels, as opposed to shells or whole mussels. Dissolved oxygen levels at the surface remained consistently at 4 mg/L throughout the field season. Mussels were caught at depths of about 1—2 m. Exact collection depths of farmed mussels are unknown due to the farming methods typically used. These consist in large ropes submerged in water that extend from the bottom of the water column, where they are anchored in place, to the surface, where they are attached to floating barrels. Mussels attach themselves around these ropes, and thus individual specimens may be located at any point along a rope.

Arsenic levels in mussels caught in or purchased from upcoast waters was generally higher than in mussels from Burgas Bay. Mussels acquired from Burgas Bay waters were higher in arsenic than mussels from waters located downcoast from the Bay.

In terms of biometrics, a statistically significant difference among mussel populations was found. The samples GAL1,2,3/MYGA4 and KRA1,2,3/MYGA6 exhibited greater relative weight and length than the rest, while the composite sample POM1,2,3/MYGA2 was composed of mussels that were comparatively lighter in weight and shorter in length than the rest. No correlation between mussel size and arsenic concentration was observed. There was also no statistically significant correlation between arsenic level and: mussel wet weight, length/width/height, water pH, temperature, and column depth.

The samples AHT1/MYGA1 and POM1,2,3/MYGA2 were wild-caught, while the rest were purchased from mussel farms along the coast. No significant difference in arsenic concentrations between wild-caught and farmed mussels has been detected.

Table 5. Locations, average biometric measurements and arsenic levels of Mediterranean Mussel composite samples during Field Season I.

				Biometric Averages				Arsenic Conc. Avg. (mg/kg)		
Location	**Sample**	**Coordinates (WGS84)**	**Number of mussels measured**	**Wet Weight (g)**	**Length (mm)**	**Width (mm)**	**Height (mm)**	**Whole**	**Meat**	**Shells**
Ahtopol	AHT1/MYGA1	42.10145, 27.94808	30	11.43	49.20	27.90	18.80	0.09		
Pomorie	POM1,2,3/MYGA2	42.55341, 27.64224	30	7.75	46.62	26.90	15.20	0.15	1.28	0.12
Kavarna	KAV1,2,3/MYGA3	43.40327, 28.37569	30	13.07	60.20	33.52	19.57	0.10	1.67	0.09
Cape Galata	GAL1,2,3/MYGA4	43.14722, 27.96094	30	24.30	72.93	39.92	27.37	0.09	1.02	0.10
Obzor	OBZ1,2,3/MYGA5	42.81827, 27.89205	30	11.97	62.63	33.73	20.77	0.11	1.63	0.17
Kraymorie	KRA1,2,3/MYGA6	42.45849, 27.48097	30	29.77	75.07	41.50	29.58	0.12	1.63	0.07
Primorsko	PRI1,2,3/MYGA7	42.27280, 27.76611	30	10.00	59.13	32.40	21.67	0.22	1.48	0.11
Elenite	ELE1,2,3/MYGA8	42.69936, 27.83636	30	10.87	57.63	30.93	20.73	0.10	1.49	0.12
Sozopol	SOZ1,2,3/MYGA9	42.39577, 27.72041	29	10.31	57.62	32.28	22.14	0.14	1.09	0.11

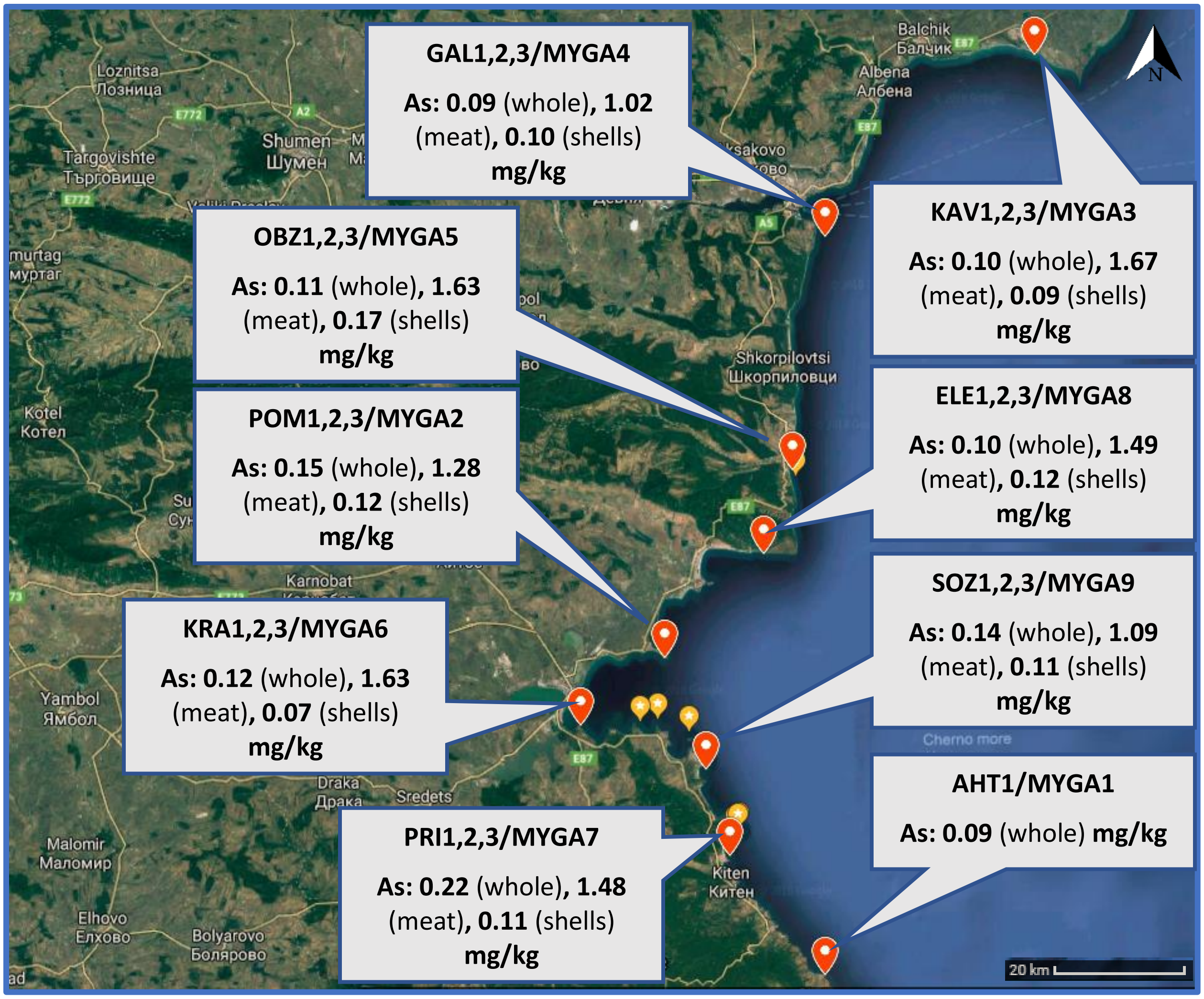


Fig. 11. Average arsenic concentrations in whole, meat only, and shells only Mediterranean Mussel composite samples from all sampling locations during Field Season I.

Observations

One mussel farm from the Burgas Bay area and two mussel farms upcoast yielded the highest values of arsenic in mg/kg. A plausible explanation for the Burgas Bay sample is the oil refinery and port's activities in the area. A plausible scenario regarding the upcoast sample bordering Romania is that contaminants are carried via the Danube river as it crosses several European countries, and then discharged into the Black Sea through the river's delta, located in Romania. Considering the Black Sea's main water current moves in a counter-clockwise fashion, or from North to Southwest, to South (Zaitsev, 2008), contaminants may very well be picked up and transported into Bulgarian waters. Be that as it may, it is curious to observe the arsenic values of the samples collected between Burgas Bay and Bulgaria's northern border with Romania.

Field Season II

Fish

The lab results from Summer 2017 indicated elevated arsenic and chromium levels in all fish samples. During this Field Season, individual fish specimens were analyzed for COCs and were not pooled into composites. Arsenic values ranged from 0.04 mg/kg to 2.39 mg/kg, with an average of 1.12 mg/kg. Chromium values recorded ranged between 0.11 mg/kg and 1.41 mg/kg, with an average concentration of 0.37 mg/kg. Cadmium values were below the LOQ, at < 0.5 mg/kg, for all fish samples. Standard length of goby specimens ranged between 10.3 cm and 15.4 cm, with an average of 12.7 cm. Their wet weight upon capture was between 17 g and 87 g, with an average of 41 g.

Water pH was between 8.22 and 8.33 with an average of 8.29, whereas water temperature at the surface ranged from 22.70 °C to 24.60 °C, with an average of 23.57 °C. Dissolved oxygen levels at the surface remained consistently at 4 mg/L throughout the field season. Gobies were caught at depths ranging between 13 m and 23.70 m, with an average of 18.96 m.

Kolmogorov-Smirnov and Shapiro-Wilk's tests ($p > 0.5$), as well as a visual evaluation of the histograms, normal Q-Q plots and box plots, surprisingly indicated that arsenic levels were normally distributed across all fish samples. For arsenic, skewness and kurtosis values were 0.27

(SE = 0.388) and 0.450 (SE = 0.759), respectively. On the other hand, Kolmogorov-Smirnov and Shapiro-Wilk's tests ($p < 0.5$) and visual evaluation of the histograms, normal Q-Q plots and box plots, showed a non-normal distribution for chromium levels across all fish samples. Skewness and kurtosis values were 2.244 (SE = 0.388) and 5.649 (SE = 0.759), respectively. The Kruskal-Wallis analysis of variance ($p < 0.5$) indicated that the distribution of arsenic was not the same across samples from different locations. However, the same test ($p > 0.5$) indicated that the distribution of chromium was the same across locations. Pairwise Kolmogorov-Smirnov tests showed a difference between the following samples: PMN4/NEME15 and TAL1/NEME12, and between PMN4/NEME15 and ATIBIV2/NEME11.

A Spearman correlation test confirmed negative correlation between levels of arsenic and distance from industrial "hot spots" for all locations within Bulgarian waters: Burgas Bay (Spearman's rho Correlation Coefficient = -0.344), Lukoil Refinery (Spearman's rho Correlation Coefficient = -0.344), Burgas Airport (Spearman's rho Correlation Coefficient = -0.344), and Varna Bay (Spearman's rho Correlation Coefficient = -0.402). However, there was a positive correlation between levels of chromium and distance from all locations: Burgas Bay (Spearman's rho Correlation Coefficient = 0.93), Lukoil Refinery (Spearman's rho Correlation Coefficient = 0.93), Burgas Airport (Spearman's rho Correlation Coefficient = 0.93), and Varna Bay (Spearman's rho Correlation Coefficient = 0.121).

The COC pattern around Sveti Ivan island observed during Field Season I was not repeated during Field Season II, and arsenic and chromium values varied greatly. However, contaminant concentrations in and around the Burgas Bay area were generally higher than those in Varna Bay. As expected, both Bays exhibited values significantly higher than those from waters located downcoast from Burgas Bay. This is very likely a cause of the intense industrial activity in and around the Burgas and Varna harbors. Byala and Obzor, two locations found between Varna Bay and Burgas Bay, interestingly exhibited the highest average values overall, for which no explanation can be readily provided.

Table 6. Locations, average biometric measurements and arsenic and chromium levels of Round Goby samples during Field Season II.

				Biometric Measurements			Arsenic and Chromium Conc. Avg.	
				Wet Weight (g)		Standard Length (cm)	As (mg/kg)	Cr (mg/kg)
Location	Sample Code	Coordinates (WGS84)	Specimen No.	Before processing	After processing			
Byala	BY2/NEME8	42.851528, 27.916556	1	25	23	10.6	1.38	1.41
Byala	BY3/NEME9	42.857340, 27.911450	1	59	52	14.7	1.79	1.13
Byala	BY4/NEME10	42.802278, 27.898111	1	87	83	15.3	1.32	0.54
Byala	BY4/NEME10	42.802278, 27.898111	2	53	51	13.2	2.39	0.51
Atiya	ATIBIV2/NEME11	42.461000, 27.627611	1	33	31	12.5	1.62	0.37
Atiya	ATIBIV2/NEME11	42.461000, 27.627611	2	26	24	10.6	1.56	0.21
Atiya	ATIBIV2/NEME11	42.461000, 27.627611	3	33	30	11	1.38	0.26
Talasakra	TAL1/NEME12	42.453194, 27.646694	1	56	50	14.3	1.34	0.23
Talasakra	TAL1/NEME12	42.453194, 27.646694	2	20	19	10.3	1.05	0.19
Talasakra	TAL1/NEME12	42.453194, 27.646694	3	27	22	11.5	1.23	0.18
Talasakra	TAL1/NEME12	42.453194, 27.646694	4	44	30	11.4	1.59	0.11
Talasakra	TAL1/NEME12	42.453194, 27.646694	5	64	57	15.4	1.49	0.16
Talasakra	TAL1/NEME12	42.453194, 27.646694	6	36	31	12.3	0.88	0.21
Talasakra	TAL1/NEME12	42.453194, 27.646694	7	21	19	11.4	1.57	0.28
Talasakra	TAL1/NEME12	42.453194, 27.646694	8	47	44	13	2.23	0.23
Talasakra	TAL1/NEME12	42.453194, 27.646694	9	84	72	15	1.46	0.71
Talasakra	TAL1/NEME12	42.453194, 27.646694	10	82	73	14.8	1.33	0.51
Sveti Ivan	SVIV2/NEME13	42.441000, 27.687611	1	30	26	11.1	0.33	0.60
Sveti Ivan	SVIV3/NEME14	42.440806, 27.688389	1	35	33	11.8	1.06	0.64
Sveti Ivan	SVIV3/NEME14	42.440806, 27.688389	2	62	58	13.8	1.03	0.19
Sveti Ivan	SVIV3/NEME14	42.440806, 27.688389	3	32	22	12.2	1.17	0.20
Primorsko	PMN4/NEME15	42.300417, 27.779028	1	33	30	15	0.30	0.31
Primorsko	PMN4/NEME15	42.300417, 27.779028	2	29	23	12.1	0.55	0.28
Primorsko	PMN4/NEME15	42.300417, 27.779028	3	23	19	11.4	0.32	0.76
Primorsko	PMN4/NEME15	42.300417, 27.779028	4	23	20	11.2	0.98	0.32
Primorsko	PMN4/NEME15	42.300417, 27.779028	5	17	15	10.5	0.04	0.26
Primorsko	PMN4/NEME15	42.300417, 27.779028	6	33	26	13.6	0.69	0.48
Primorsko	PMN4/NEME15	42.300417, 27.779028	7	62	59	15.3	0.19	0.35
Primorsko	PMN6/NEME16	42.310972, 27.789417	1	40	36	13.3	1.10	0.25
Primorsko	PMN6/NEME16	42.310972, 27.789417	2	51	48	13.5	0.86	0.12
Varna	VAR1/NEME17	42.214528, 27.982361	1	45	35	12.5	1.03	0.23
Varna	VAR3/NEME18	43.212722, 27.996917	1	31	26	12.4	1.17	0.17
Varna	VAR3/NEME18	43.212722, 27.996917	2	44	40	15.1	1.17	0.16
Varna	VAR3/NEME18	43.212722, 27.996917	3	31	27	12.3	0.90	0.31
Varna	VAR4/NEME19	43.221528, 28.012528	1	44	39	13.2	1.32	0.23
Varna	VAR4/NEME19	43.221528, 28.012528	2	27	24	11.2	0.68	0.19
Varna	VAR4/NEME19	43.221528, 28.012528	3	40	35	12	1.12	0.25

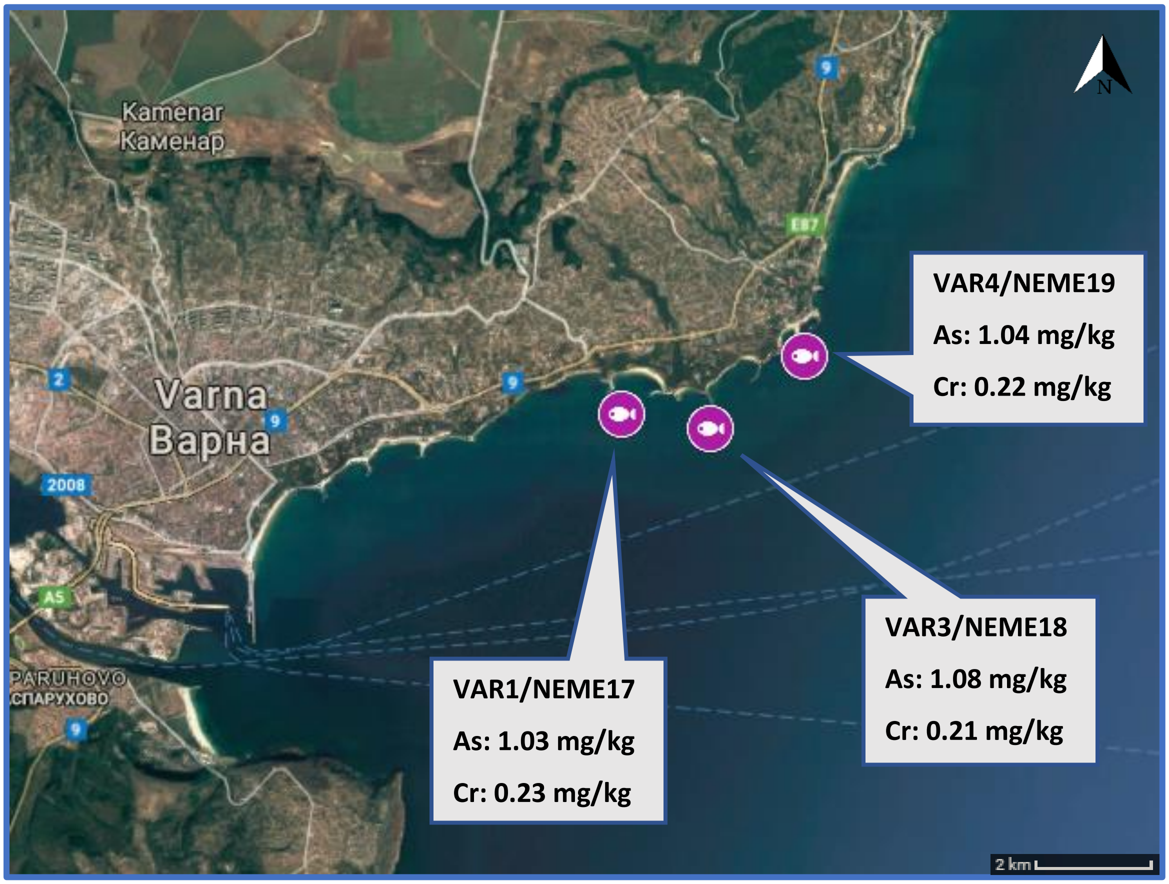


Fig. 12. Average arsenic and chromium concentrations in Round Goby samples from Varna during Field Season II.

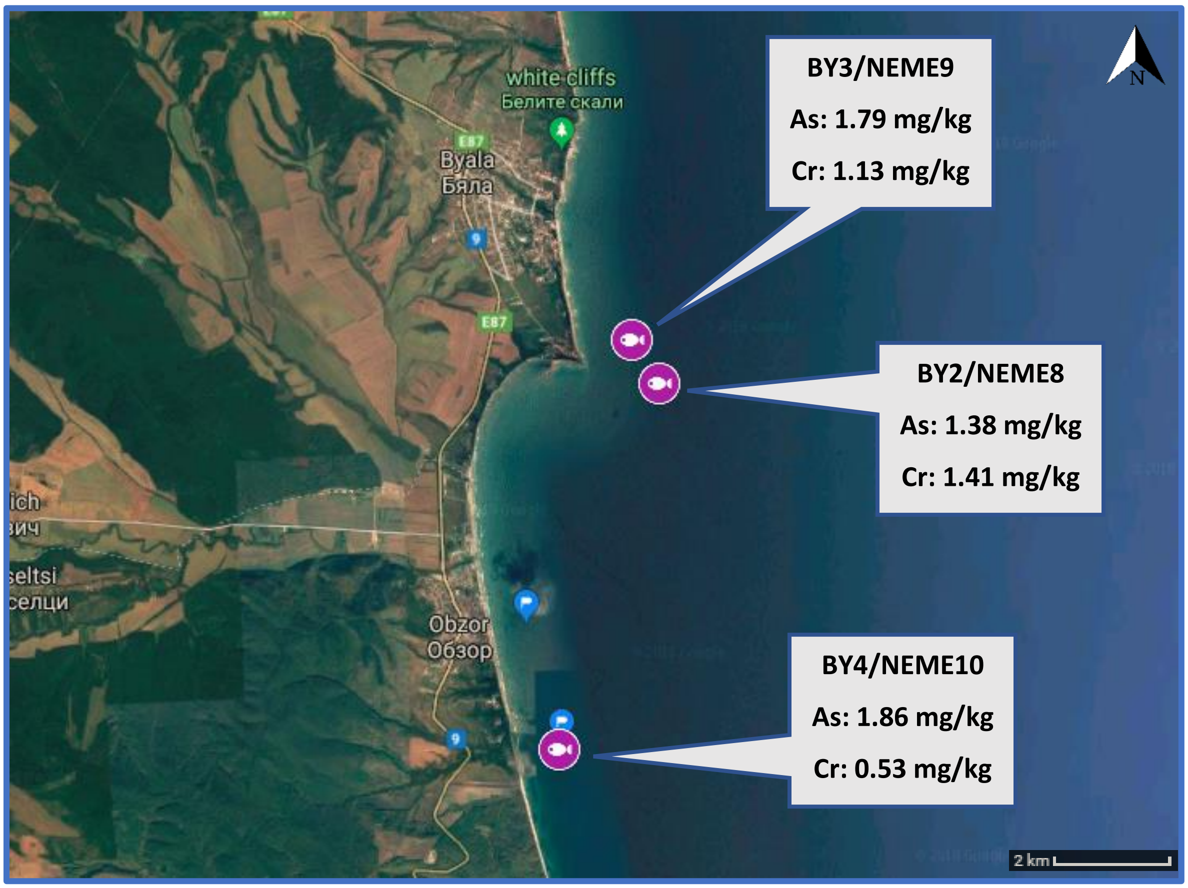


Fig. 13. Average arsenic and chromium concentrations in Round Goby samples from Byala and Obzor.

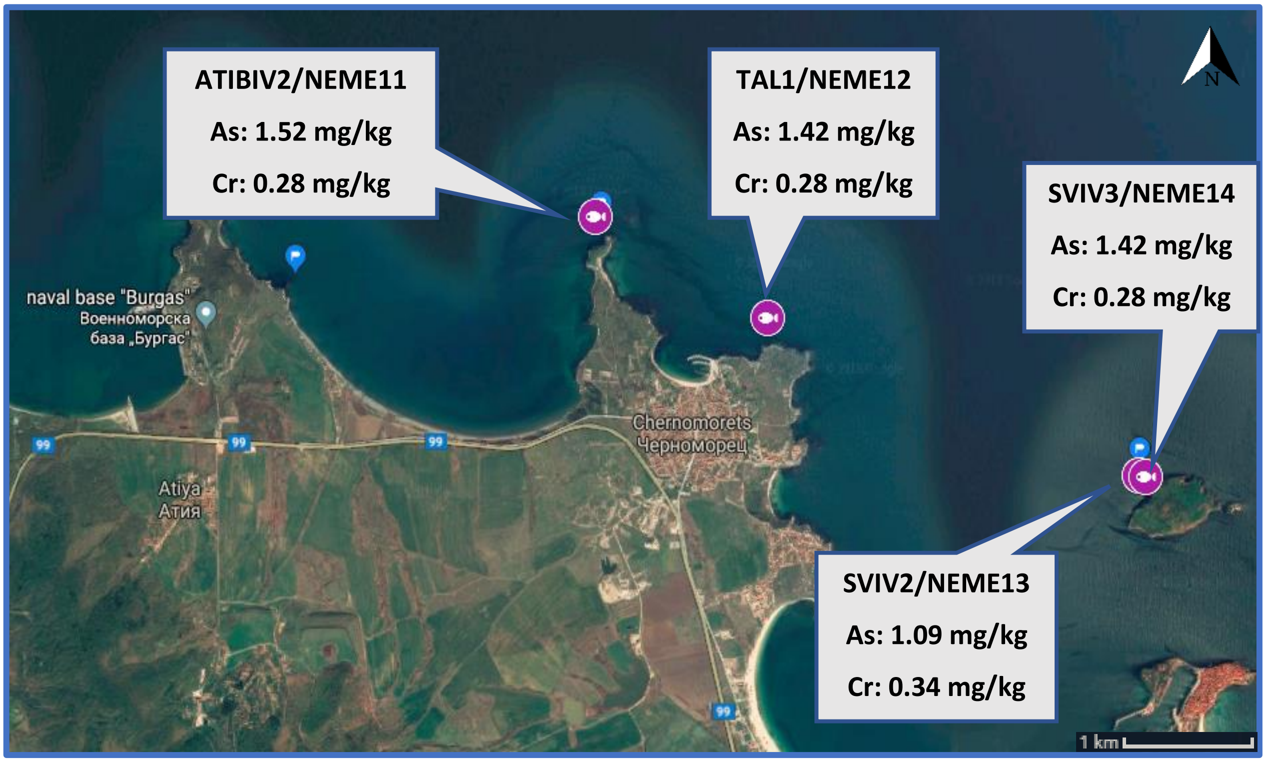


Fig. 14. Average arsenic and chromium concentrations in Round Goby samples from Atiya, Talasakra, and Sveti Ivan during Field Season II.

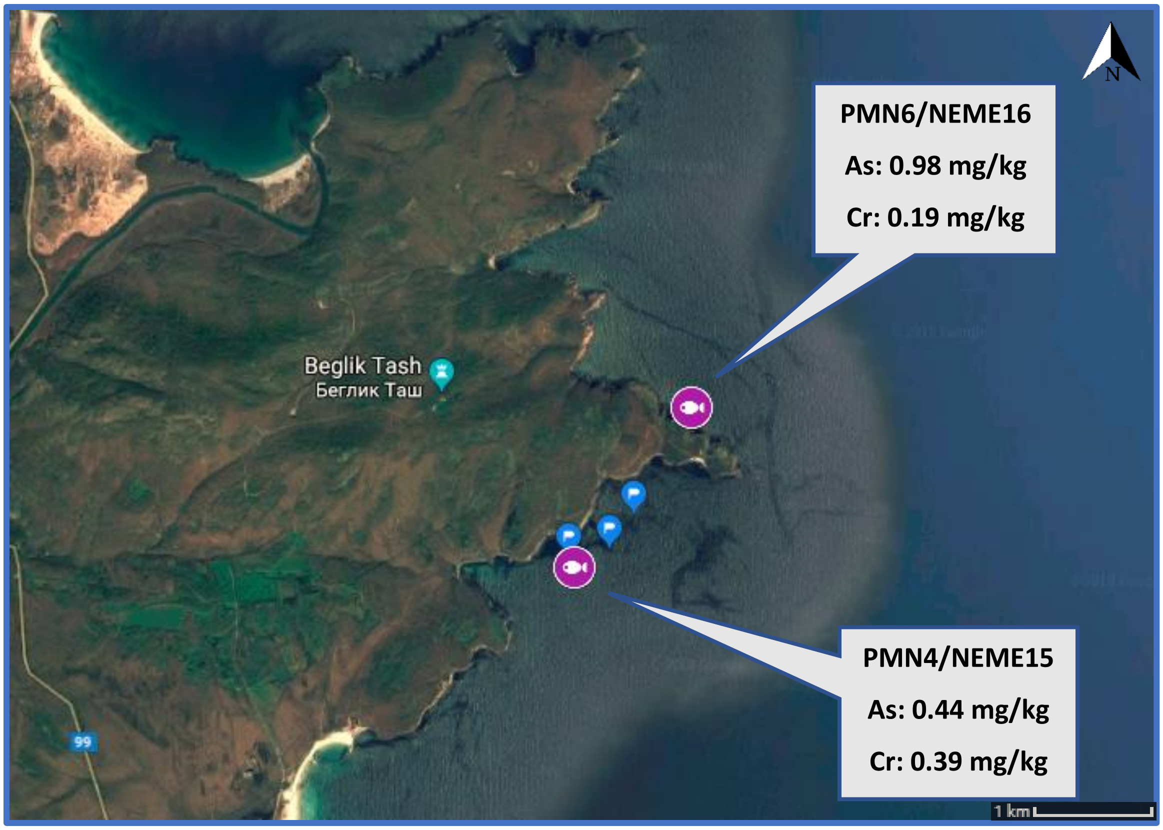


Fig. 15. Average arsenic and chromium concentrations in Round Goby samples from Primorsko during Field Season II.

Plankton

Plankton exhibited elevated levels of chromium. Chromium values ranges from 0.20 mg/kg to 0.28 mg/kg, with an average of 0.26 mg/kg. Arsenic and cadmium values were below the LOQ, at < 0.5 mg/kg, for all plankton samples. The samples weighed between 10 g and 71 g, the average being 34.4 g.

Water pH was between 8.22 and 8.34 with an average of 8.29, whereas water temperature at the surface ranged from 22.7 °C to 25.1 °C, with an average of 23.87 °C. Dissolved oxygen levels at the surface were at 4 mg/L. Plankton samples were collected at depths ranging between 6 m and 19 m, with an average of 13.67 m.

The Kolmogorov-Smirnov test ($p > 0.5$) indicated a normal distribution of chromium across plankton samples. However, the Shapiro-Wilk's test ($p < 0.5$) and a visual evaluation of the histograms, normal Q-Q plots and box plots, indicated that chromium levels were non-normally distributed across all plankton samples. Skewness and kurtosis values were -1.837 (SE = 0.845) and 3.373 (SE = 1.741), respectively. The Kruskal-Wallis analysis of variance ($p > 0.5$) indicated that the distribution of chromium was the same across composite samples, which is also evident by visually examining the values across samples.

A Spearman correlation test showed a positive correlation between levels of chromium and distance from industrial "hot spots" for the following locations: Burgas Bay (Spearman's rho Correlation Coefficient = 0.636), Lukoil Refinery (Spearman's rho Correlation Coefficient = 0.636), and Burgas Airport (Spearman's rho Correlation Coefficient = 0.636). There was a positive correlation between levels of chromium and distance from Varna Bay (Spearman's rho Correlation Coefficient = -0.318).

Table 7. Locations, wet weight measurements, and chromium levels in composite plankton samples during Field Season II.

Location	Sample	Coordinates (WGS84)	Wet Weight (g)	Cr (mg/kg)
Talasakra	TAL2/PLANKTON1	42.450528, 27.636528	10.5	0.20
Primorsko	PMN5/PLANKTON2	42.300639, 27.780444	71.0	0.27
Primorsko	PMN7/PLANKTON3	42.311417, 27.786944	10.0	0.27
Varna	VAR2/PLANKTON4	43.213722, 27.993444	18.0	0.28
Varna	VAR3/PLANKTON5	43.212722, 27.996917	56.0	0.25
Varna	VAR4/PLANKTON6	43.221528, 28.012528	41.0	0.28

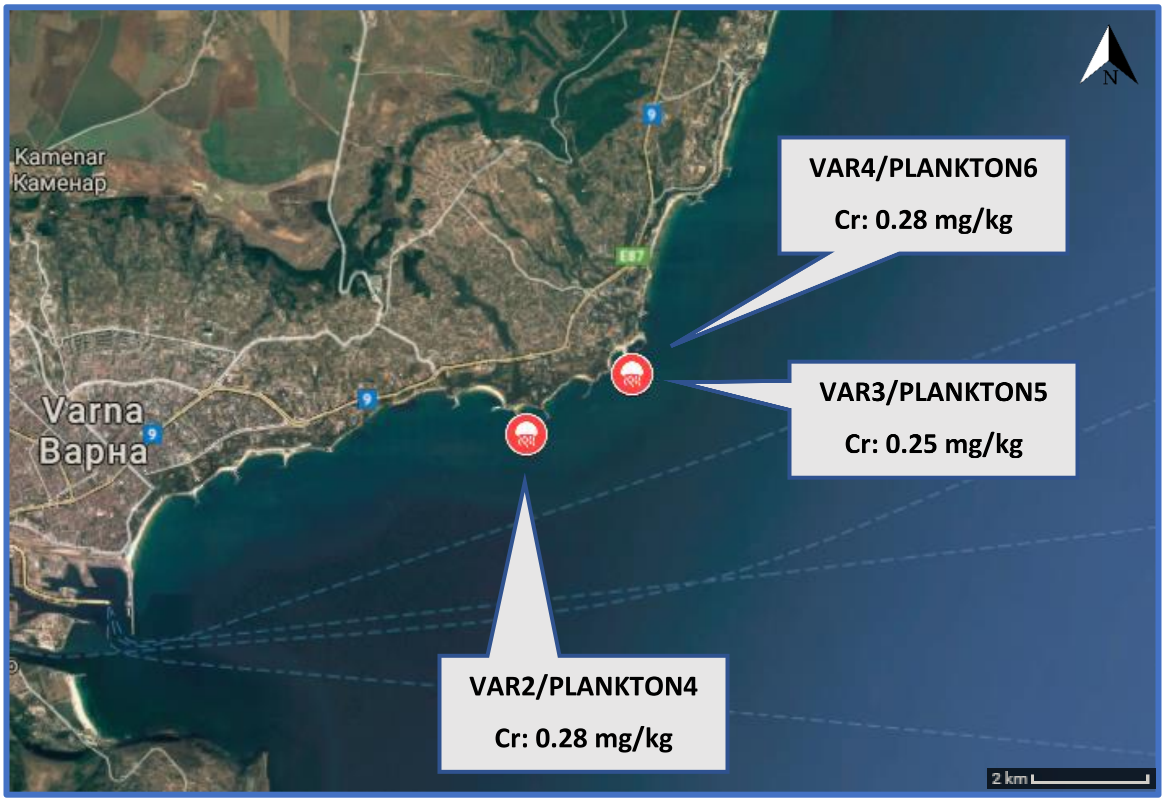


Fig. 16. Average chromium concentrations in plankton composite samples from Varna during Field Season II.

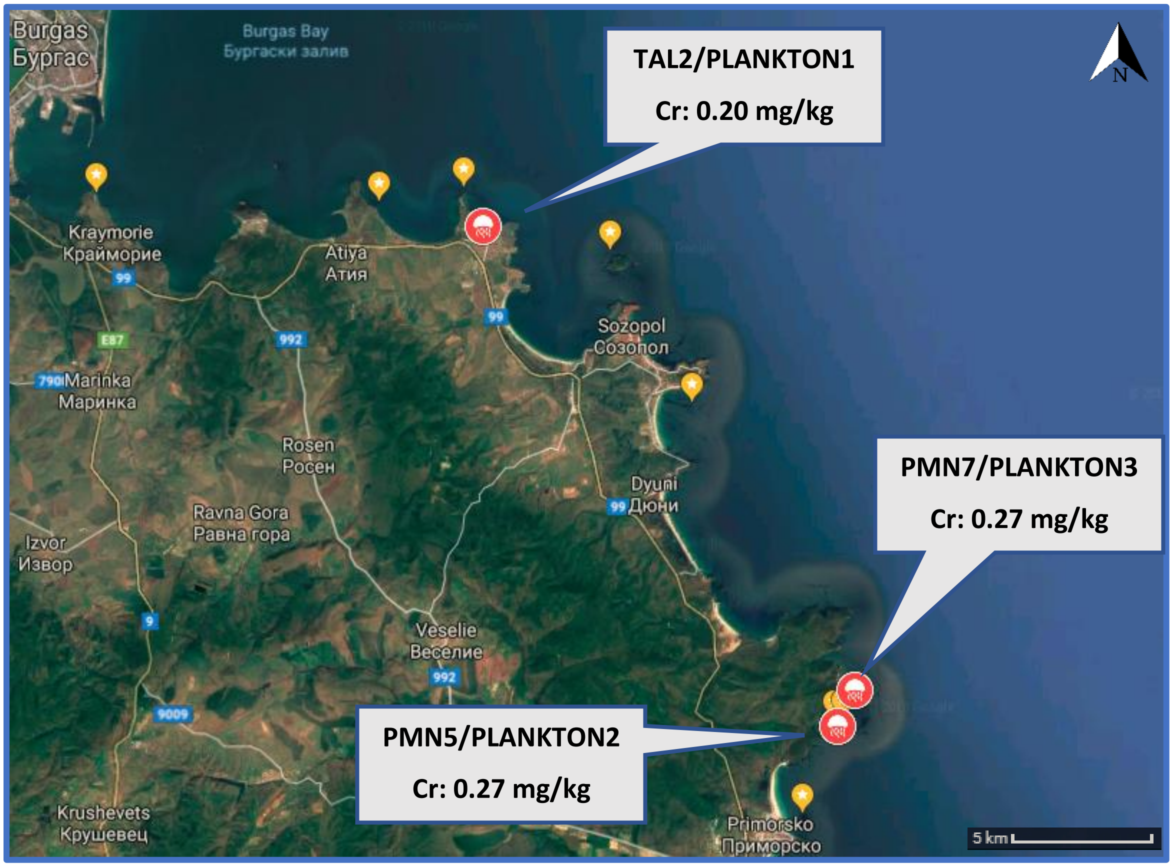


Fig. 17. Average chromium concentrations in plankton composite samples from Talasakra and Primorsko during Field Season II.

CHAPTER VII: Risk Assessment

The purpose of this risk assessment was to evaluate the potential health risks associated with the consumption of Round Goby fish, Mediterranean Mussels, and plankton from Bulgarian Black Sea waters. This was accomplished by developing Hazard Quotients (HQs). A Hazard Quotient is defined as "the ratio of the potential exposure to the substance and the level at which no adverse effects are expected" (USEPA, 2017).

Since arsenic, chromium and their compounds are carcinogens, they were treated as such during the health risks assessment calculations, using default toxicity factors and methodology established by the U.S. Environmental Protection Agency (USEPA). To this end, the adult average 70-kg body weight was used. Health risks were calculated using the arithmetic mean and 95% UCL of the mean concentrations determined in this study. The USEPA ProUCL software was used to calculate 95% UCL values. Both the reference dose (RfD) and Cancer Slope Factor (SF) are based on the oral exposure route.

As previously stated in this text, inorganic arsenic comprises 0.02%--11% of total arsenic in fish and seafood (Peycheva *et al*., 2014). Because arsenic speciation was not possible due to the limitations of the analytical methods used by the lab, an assumption of a maximum 11% concentration of inorganic arsenic per sample was used to calculate HQs.

Human Health Risk Assessment

Field Season I Data

Round Goby

Arsenic

Because inorganic arsenic is a known human carcinogen (Group A), the following parameters were used, as established by the U.S. EPA:

Arsenic oral Cancer Slope Factor: 1.5 mg/kg-day

Body Weight (BW): 70 kg

Average lifetime = 75 years

Acceptable Risk Level (ARL) = 1×10^{-5}, or 1 in 100,000

The value of 1×10^{-5} was set as the ARL for Bulgaria, as the country's entire population is only 7.1 million people. A Screening Value (SV) was then developed by rearranging the following formula for Consumption Rate:

$$CR = \frac{ARL \cdot BW}{SF \cdot SV}$$

Which gives us:

$$SV = \frac{ARL \cdot BW}{SF \cdot CR}$$

In which:

ARL = Acceptable Risk Level

BW = Body Weight of the average adult, in kg

SF = Oral Cancer Slope Factor

CR = Consumption Rate of fish per day, in kg

A Consumption Rate (CR) was calculated using the average yearly *per capita* consumption of fish and seafood reported by the USDA (2017), which is set at 5.2 kg for the Bulgarian population. The Consumption Rate is calculated by dividing the consumption per person per year by 365:

$$CR = \frac{5.2}{365} = 0.014 \text{ kg/day}$$

The resulting Screening value is thus:

$$SV = \frac{10^{-5} \cdot 70}{1.5 \cdot 0.014} = 0.03 \text{ mg/kg}$$

The resulting value was then used to calculate a second screening value, this time to develop the Hazard Quotient:

$$SV = \frac{0.03 \cdot 70}{75} = 0.03 \text{ mg/kg}$$

The arithmetic mean of 11% of the total arsenic concentration per sample (= 0.12 mg/kg iAs) was used to develop the HQ:

$$HQ = \frac{0.12}{0.03} = 4.0$$

The decision rule for the HQ is as follows:

- If HQ < 1, no need to proceed further.
- If HQ > 1, continue with the risk assessment.

As the HQ > 1, harmful effects cannot be ruled out and we should proceed with developing Protective Concentration Levels, or PCLs.

Using the 95% UCL from ProUCL (95% Chebyshev) for 0.174 mg/kg inorganic arsenic, calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.174}{0.03} = 5.8$$

As the HQ again exceeds 1, harmful effects cannot be ruled out and we should proceed with developing Protective Concentration Levels, or PCLs.

A summary of HQs is presented in Table 8:

Table 8. Arsenic Hazard Quotients (HQs) for Round Goby from Field Season I.

	Inorganic Arsenic HQs	
	Mean	95% UCL
Round Goby	4.0	5.8

Mediterranean Mussel

Arsenic

To develop HQs for arsenic concentrations in Mediterranean Mussels, the same equations and USEPA-based values were used as for Round Goby fish. A summary of the HQs follows (Table 9).

Whole Mussels

Using the arithmetic mean of 11% of the total arsenic concentration per sample (= 0.01 mg/kg iAs), calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.01}{0.03} = 0.33$$

As the HQ < 1, harmful effects are not likely and the risk assessment can stop here.

Using the 95% UCL from ProUCL (95% Chebyshev) for 0.0206 mg/kg inorganic arsenic, calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.0206}{0.03} = 0.69$$

As the HQ again is < 1, harmful effects are not likely and the risk assessment can stop here.

Meat Only

Using the arithmetic mean of 11% of the total arsenic concentration per sample (= 0.16 mg/kg iAs), calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.16}{0.03} = 5.3$$

As the HQ > 1, harmful effects cannot be ruled out and the risk assessment may proceed with PCLs.

Using the 95% UCL from ProUCL (95% Chebyshev) for 0.197 mg/kg inorganic arsenic, calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.197}{0.03} = 6.6$$

As the HQ again is > 1, harmful effects cannot be ruled out and the risk assessment should proceed.

Shells Only

Using the arithmetic mean of 11% of the total arsenic concentration per sample (= 0.01 mg/kg iAs), calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.01}{0.03} = 0.33$$

As the HQ < 1, harmful effects are not likely and the risk assessment can stop here.

Using the 95% UCL from ProUCL (95% Chebyshev) for 0.0167 mg/kg inorganic arsenic, calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.0167}{0.03} = 0.56$$

As the HQ again is < 1, harmful effects are not likely and the risk assessment can stop here.

Table 9. Arsenic Hazard Quotients (HQs) for Mediterranean Mussel from Field Season I.

		Inorganic Arsenic HQs	
		Mean	95% UCL
Mediterranean Mussel	Whole	0.33	0.56
	Meat	5.3	6.6
	Shells	0.33	0.56

Field Season II Data

Arsenic

Round Goby

To develop HQs for arsenic concentrations in Round Goby fish, the same equations and USEPA-based values were used as in Field Season I. A summary of the HQs follows (Table 10).

Using the arithmetic mean of 11% of the total arsenic concentration per sample (= 0.12 mg/kg iAs), calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.12}{0.03} = 4.0$$

As the HQ < 1, harmful effects are not likely and the risk assessment can stop here.

Using the 95% UCL from ProUCL (95% Chebyshev) for 0.165 mg/kg inorganic arsenic, calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.165}{0.03} = 5.5$$

As the HQ again is < 1, harmful effects are not likely and the risk assessment can stop here.

Table 10. Arsenic Hazard Quotients (HQs) for Round Goby from Field Season II.

	Inorganic Arsenic HQs	
	Mean	95% UCL
Round Goby	4.0	5.5

Chromium

Unlike Chromium(VI), Chromium(III) is poorly absorbed by cells and is the less toxic form of the two, as stated earlier in this text. Chromium(III) serves primarily as an essential nutrient, and it takes very large amounts to cause toxicity. Therefore, the following calculations are for Chromium(VI), as adverse health effects have not been confirmed by the USEPA for

Chromium(III). Both non-carcinogenic and carcinogenic reference doses have been taken into account, as there is currently low confidence in the carcinogenicity studies regarding chromium toxicity via the oral exposure route. Because the proportion of Cr(VI) to Cr(III) in total chromium for fish and seafood is unknown, the values for total chromium have been used to calculate HQs.

Similarly to Field Season I, the following values were used to develop the HQs for chromium, as shown in Table 11. HQs for Plankton were developed in addition to those for Round Goby fish, as gelatinous zooplankton (jellyfish) are part of the marine trophic web and sometimes also consumed by humans, albeit rarely (Georgi Peychev, commercial fisherman, personal communication).

Round Goby

1. <u>Non-carcinogenic</u>

Chromium RfD: 3×10^{-3} mg/kg-day

Body Weight (BW): 70 kg

Consumption Rate (CR) = 0.014 mg/kg-day was used

Which give us the following Screening Value (SV):

$$SV = \frac{0.003 \cdot 70}{0.014} = 15 \text{ mg/kg}$$

Using the arithmetic mean from my data (= 0.37 mg/kg Cr), calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.37}{15} = 0.02$$

As the HQ < 1, harmful effects are not likely, which means the risk assessment can stop here.

Using the 95% UCL from ProUCL (95% Chebyshev) for 0.443 mg/kg chromium, calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.443}{15} = 0.03$$

The HQ being again less than 1, harmful effects are not likely and the risk assessment can stop here.

2. Carcinogenic

Because no cancer slope factor has been established for chromium, used is the appropriate oral reference dose, as established by the USEPA, along with the following parameters:

Chromium RfD: 0.0009 mg/kg-day

Body Weight (BW): 70 kg

Consumption Rate (CR): 0.014 mg/kg-day

Using the above RfD, we get a Screening Value (SV) of 4.5 mg/kg. This, in, turn, gives us an HQ of 0.08 using the data arithmetic mean and an HQ of 0.10 using the 95% UCL, respectively.

Plankton

1. Non-carcinogenic

Chromium RfD: 3×10^{-3} mg/kg-day

Body Weight (BW): 70 kg

Consumption Rate (CR) = 0.014 mg/kg-day was used

Which give us a Screening Value (SV) of 15 mg/kg

Using the arithmetic mean from my data (= 0.26 mg/kg Cr), calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.26}{15} = 0.02$$

As the HQ < 1, harmful effects are not likely, which means the risk assessment can stop here.

Using the 95% UCL from ProUCL (95% Chebyshev) of 0.313 mg/kg chromium, calculated was the following Hazard Quotient (HQ):

$$HQ = \frac{0.313}{15} = 0.02$$

The HQ being again less than 1, harmful effects are not likely and the risk assessment can stop here.

2. Carcinogenic

Because no cancer slope factor has been established for chromium, used is the appropriate oral reference dose, as established by the USEPA, along with the following parameters:

Chromium RfD: 0.0009 mg/kg-day

Body Weight (BW): 70 kg

Consumption Rate (CR): 0.014 mg/kg-day

Using the above RfD and the resulting Screening Value (SV) of 4.5 mg/kg, we get an HQ of 0.06 using the data arithmetic mean of 0.26 mg/kg and an HQ of 0.07 using the 95% UCL of 0.313.

Table 11. Chromium Hazard Quotients (HQs) for Round Goby and Plankton from Field Season II.

	Chromium HQs			
	Non-carcinogenic		Carcinogenic	
	Mean	95% UCL	Mean	95% UCL
Round Goby	0.02	0.03	0.08	0.10
Plankton	0.02	0.02	0.06	0.07

No HQs exceed 1, we can therefore conclude adverse health effects are not likely to occur from the consumption of gelatinous zooplankton.

Ecological Risk Assessment

While bioaccumulation of contaminants of concern (arsenic and chromium) often exceeding permissible limits for human health has been detected in Round Gobies, Mediterranean Mussels, and plankton, biomagnification in the Black Sea marine ecosystem cannot be determined based on the results from this study. This is due to the limitations of data in the present research. Thus, an ecological risk assessment cannot be conducted at this time without additional data, such as: sediment ingestion rates, food ingestion rates, as well as bioaccumulation factors, to investigate all HQs exceeding the value of 1.

To conduct the full assessment, the next step is to calculate PCLs, or Protective Concentration Levels, which are defined by the Texas Commission on Environmental Quality (TCEQ) as the "level of COCs that can remain in the source medium and be protective of human and ecological receptors at the point of exposure within the exposure medium". The equation that produces the PCL is the following:

$$PCL = \frac{TRV}{BAF\ \cdot Food\ IR} + Sediment\ IR$$

In which the PCL = protective concentration level (mg/kg dry weight in sediment)

- TRV = toxicity reference value (either NOAEL or LOAEL) in mg/kg-day

- BAF = sediment-to-fish bioaccumulation factor (dry weight tissue/dry weight sediment)
- Food IR = wildlife food ingestion rate (kg dry weight per kg body weight per day)
- Sediment IR = sediment ingestion rate (kg dry weight per kg body weight per day)

As the values required were unavailable and could not be developed for this study based on the available data, further research is necessary, which includes sediment sampling from the same locations where fish and mussel collection takes place. This is recommended so that the COCs in sediments may be linked to individual organisms and bioaccumulation factors calculated. In addition, food and sediment ingestion rates need to be developed for Round Gobies and Mediterranean Mussels and made available for research purposes. Once complete, this process should provide future researchers with the tools they need to conduct a complete ecological risk assessment for the Bulgarian Black Sea. This, in turn, will permit researchers to develop country-specific fish and seafood consumption advisories for Bulgarian species and to suggest cleanup levels for contaminants of concern such as arsenic and chromium, where appropriate. Environmental restoration is not yet a common occurrence in Bulgaria, however, due to the lack of funding and expertise. It is therefore likely that international involvement may be required to conduct more extensive and comprehensive sampling for COCs and to obtain a more representative image of current needs. Even so, foreign experts and their teams may encounter difficulty in Bulgaria; not so much with the research process of their respective projects, as with communicating their findings and recommendations to the various stakeholders. The Discussion section below explores some Bulgarian cultural aspects, potential setbacks foreign environmental project managers may face, as well as ways to mitigate miscommunication.

CHAPTER VIII: Conclusions and Direction for Future Research

The results of this study indicate anomalous concentrations of arsenic and chromium in Round Gobies, Mediterranean Mussels, and gelatinous zooplankton from multiple locations along the Bulgarian Black Sea coast. Contrary to the original hypothesis, no elevated amounts of mercury or lead were detected in any of the samples (all values were below the LOQ). Arsenic and chromium values were generally higher around industrial "hot spots", as expected. Heavy activity from the Lukoil Neftochim petrochemical plant near Burgas Bay, shipping, mariculture, municipal waste, and wastes draining from the Danube serve as the primary contributing factors of pollution in Bulgarian waters. However, contaminants from recent and current activities are not solely responsible. Most problems in Bulgaria lie in existing pollution. In fact, much of the contamination is left over from historical dumping sites and heavy industry from when Bulgaria was under Soviet rule, as well as from hazardous wastes foisted to Bulgaria and other Eastern European countries under the guise of humanitarian aid by Western Europe (e.g., in the form of expired or nearly-expired medicines) (Brown, 1999). Due to economic constraints, Central and Eastern European countries have also been resorting to the cheapest option available when it comes to waste management, which is landfills. Left untreated, these landfills leach heavy metals, PCBs, and other contaminants into the environment (Brown, 1999).

When the USSR took over, the Bulgarian economy became modeled after the Soviet style of industrialization, collectivization, and urbanization (Brown, 1999; Baker, 2002). That meant an economy largely driven by short-term production goals without regard for environmental integrity or human health (Friedberg and Zaimov, 1994; Brown, 1999). This vastly contributed to ecological degradation like that of other former Soviet satellite countries, from which Bulgaria is still recovering. Said degradation was caused by heavy metals, radioactivity, pesticides, and fertilizers (Brown, 1999). Resolving those problems has been difficult due to economic, political, cultural, and legal constraints (Friedberg and Zaimov, 1994). The collapse of the Soviet Union further left the country politically and economically vulnerable (Baker, 2002). The political instability, high turn-over rate for government, polarized political climate (i.e., clash between Communist and anti-Communist ideologies) (Baker, 2002), government restructuring, gradual democratization, as well as the state of transitioning to a market economy (Friedberg and Zaimov, 1994) have all played a major role in the shaping of Bulgaria's modern sociopolitical arena (Friedberg and Zaimov, 1994; Brown, 1999; Baker, 2002; Almer and Koontz, 2004). Meanwhile, restructuring demands made by the International Monetary Fund (IMF) as well as constraints imposed by Bulgaria's Association Agreement with the E.U. have slowed down the country's recovery further (Baker and Baumgartl, 1998; Baker, 2002). There have also been Incidents of West European industry transporting hazardous waste to Bulgaria because the enforcement of environmental regulations is still lax, and of treating the country as a dumping site (Friedberg and Zaimov, 1994; Brown, 1999). In the 1990s, more than 2 million tons of waste were transported each year, mainly from Western Europe to Central and Eastern Europe (Brown, 1999).

In Bulgaria, there is low public awareness relating to the environment (Brown, 1999). That is understandable, given the region's past and economic *status quo*; issues like unfavorable living standards, non-functioning public services, and decaying infrastructure must take precedence over environmental concerns (Brown, 1999). By the same token, there is a general lack of information regarding exposure to various contaminants of concern (COCs) from fish and seafood (personal communication; personal observation). Most people are not aware of the potential risks associated with the consumption of certain foods, as typically such information is not brought to the public's attention (personal communication; personal observation). Bulgaria has not been efficient in its public notification practices, rarely exceeding the minimal requirements (REC, 1998; Almer and Koontz, 2004). This might explain why people do not generally make a connection between COCs in marine water and those same COCs in living organisms. Even though people may know, in theory, that there are pollutants in the water, they are not necessarily aware that they are being exposed to those contaminants by consuming fish and seafood.

Although political constraints have lessened, economic and technical difficulties in the implementation of environmental laws and regulations persist (Friedberg and Zaimov, 1994). The problem is not so much a lack of legislation but a lack of enforcement. For example, there is a provision in the concluding sections of Bulgaria's Environmental Protection Act, which requires an Environmental Impact Assessment (EIA) for existing pollutants before the start of projects (Friedberg and Zaimov, 1994). Other provisions include the Law on Fish Industry of 1982, the Law on Public Health of 1973, the Marine Areas Law of 1987, the 1991 Constitution, and the 1991 Law on the Protection of the Environment. International treaties and conventions are also part of Bulgarian environmental protection legislation (e.g., the Convention on International Trade in Endangered Species of Wild Fauna and Flora and the Convention for the Prevention of Pollution from Ships) (Friedberg and Zaimov, 1994). The Bulgarian Constitution protects citizens' right to a clean environment, while also mandating the government's responsibility towards it.

In other words, it is both a fundamental human right and a civil duty (Friedberg and Zaimov, 1994). Friedberg and Zaimov (1994) report that an attempt to formulate a system of environmental policy was initiated in 1991 by the Bulgarian Ministry of the Environment, assisted by the World Bank, and the U.S. EPA; that system is still lacking, however, which has led to loss of credibility for environmental laws and implementation must contend with serious skepticism. To borrow their finely crafted metaphor, “the road from word to deed is a long and rocky one” (Friedberg and Zaimov, 1994).

During the four-decade long Communist regime between 1947-1989 (Friedberg and Zaimov, 1994; Baker, 2002), there was little to no respect for the law. Much scientific information was considered “classified” and was therefore inaccessible to and often concealed from the public (Brown, 1999). When we add partisanship, corruption, censorship, and an invasive government to the mix, it comes as no surprise that nationals of former Soviet satellite countries such as Bulgaria still harbor mistrust for state institutions (Baker, 2002). Baker (2002) further reports that reform in post-Communist Bulgaria remains superficial and has not penetrated to the local level, while civil service remains “partial and discretionary” or characterized by what Friedberg and Zaimov (1994) called “bureaucratic arbitrariness”. They described Bulgaria as a country characterized by a “lack of a culture of advocacy”, “lack of tradition of advocacy against authority within the legal profession”, as well as a “lack of the critical and creative mentality of challenge that in the West we expect of our legal advocates” (Friedberg and Zaimov, 1994). Through this lack of public advocacy, the legacy of the totalitarian regime can still be witnessed today. Simply put, people are just not used to such concepts as public review and citizen lawsuits; they are not used to being consulted about decisions in government, nor pursuing legal action against public officials.

Maria Toth, leader of the Environmental Education Group of Romania's nonprofit Health Environmental Regional Organisation, quoted in Brown (1999), said: “Only one opinion was accepted at that time [Communist era in Romania]: the official opinion. If you started to organize, you were discovered very soon and put in jail." For that reason, it was the environmental movement that served as a front for political opposition during the Soviet regime. True intentions were easier to disguise as environmental concerns, which were considered benign by Soviet authorities because they did not directly challenge the government's legitimacy (Friedberg and Zaimov, 1994; Brown, 1999). With the collapse of Communism, many politically active citizens ceased the fight for ecological causes, as their real agenda had never been centrally focused on the environment to begin with (Friedberg and Zaimov, 1994) and, as previously stated, focus shifted to other priorities. Bulgaria has also experienced intense pressure from Western financial institutions, whose first item on the agenda has been privatization (Friedberg and Zaimov, 1994). It is largely these shortcomings that have stalled the implementation of environmental laws and regulations. Advocacy, therefore, must re-emerge to address current and future challenges. At the same time, it ought to be balanced with procedural legitimacy (Friedberg and Zaimov, 1994). Finally, an increase in the pool of environmental scientists, legal experts, and experienced project managers would be supremely beneficial[3]. At present, Bulgaria strives to adhere to European Community (EC) standards for environmental protection. American environmental regulations may therefore not be appropriate for a country that seeks to integrate into and create closer economic ties with Europe (Friedberg and Zaimov, 1994).

[3] In 2007, only 25 individuals in Bulgaria were certified with the Project Management Institute (PMI) and 12 with the International Project Management Association (IPMA). In comparison, in the United States, 129,378 and 187 individuals were certified with PMI and IPMA, respectively (Bredillet et al., 2010).

While Communism and the improper distribution of information have certainly played a part in shaping Bulgaria's sociopolitical culture, the extent of public involvement (or lack thereof) can also be attributed to aspects of national culture. In the words of Bredillet *et al*. (2010), "no management activity can be 'culture free'"; successful future project management (e.g., establishing new fishery management practices or food consumption advisories) in Bulgaria, therefore, would necessitate knowledge and consideration of the culture of the Bulgarian people, in addition to their history.

In Bulgaria, there is much reverence for one's elders, who are respected and looked to for guidance. This outlook is instilled in Bulgarians from a young age within the close-knit family circle, where the grandparents and parents are the authority. Age is often correlated with wisdom and experience, *ergo* respect comes with seniority. That same deference applies to authority figures later on in a person's life. Authority takes the shape of one's employer or political leader, and those hold great power over the public. Usually, people will be afraid to challenge authority for fear of "getting in trouble". This is not far-fetched as they would, in fact, get in trouble under the Communist regime (Brown, 1999).

This phenomenon, which is one of many national idiosyncrasies one encounters in Bulgaria, can be better understood by consulting Hall and Hofstede's landmark research on intercultural differences, as well as follow-up studies by other researchers. Understanding of national culture is important when it comes to international cooperation and projects involving participants from different countries and cultural backgrounds. If we wish to inform the Bulgarian public of the environmental and human health hazards they are exposed to and mobilize stakeholders to take action, we must first identify the proper approach by becoming familiar with our target audience and how they think. For example, Kivrak *et al*. (2014) listed language and communication difficulties, trust, motivation, and personal relationships as the critical barriers to successful knowledge-sharing in multicultural project teams. Environmental restoration projects, in particular, would have to rely heavily on foreign resources and expertise, at least in the short term, until Bulgaria has had time to expand on its own pool of expert human capital.

Kivrak *et al*. (2014) further identified national culture as one of the most crucial factors that can impede knowledge-sharing between individuals. Hall (1977, 1980) had previously suggested the concept of High- (mostly implicit) and Low-context (mostly explicit) communication and classified cultures according to their communication styles. High-context cultures assign greater power to the group, as opposed to the individual, which is the case in Low-context cultures (Hall, 1973; Alexieva, 1997; Shao *et al*., 2004). Salleh (2005, cited in Tanova and Nadiri, 2010) further derived four distinguishing characteristics between high- and low-context communication styles from Hall's (1980) model. These characteristics are "emotions in a close relationship", "directness of [the] message conveyed", the "use of non-verbal communication", and the "use of digital or analogous language". In short, high-context cultures rely more heavily on personal relationships, emotions, non-verbal cues, and analogous language, whereas low-context cultures tend to be more impersonal, rely on the analytical part of the brain, and prefer explicit and exact language (Tanova and Nadiri, 2010). Eastern Europeans and Slavs were found to have a higher-context demographic ranking compared to North Americans (WASP), in general, who exhibited a low-context ranking (Hall, 1977). These findings are corroborated by Tanova and Nadiri (2010), whose study confirmed Bulgaria as a high-context country.

Geert Hofstede found that national culture is one “children acquire from their earliest youth onwards” and is deeply rooted in their mind. As a result, Hofstede and his co-researchers sought to discover how a society differs from other societies. There are differences in national value systems, on which the solutions to common problems that all societies share depend. One’s upbringing, therefore, provides a separate “profile of solutions”, depending on the country where the person grew up (Hofstede, 2011). Hofstede identified six dimensions of national culture. His definition of a dimension is that it is an aspect of a culture that can be compared to other cultures and measured relative to them. According to him, “the country differences these dimensions describe are, indeed, basic and enduring”. The six dimensions he described are the following: Power Distance (PDI), Uncertainty Avoidance (UAI), Individualism/Collectivism (IDV), Masculinity/Femininity (MAS), Long-Term/Short-Term Orientation (ITOWVS), and Indulgence/Restraint (IVR) (Hofstede, 2011; Hofstede and Minkov, 2013). In addition, Hofstede (2011) stated that the wealth variable (national wealth, or GNP *per capita*) should always be included in intercultural comparisons.

Hofstede (2011) defined Power Distance as “the extent to which the less powerful members of organizations and institutions (like the family) within a society accept and expect that power is distributed unequally” (Figure 18). In accordance with Hofstede’s research, Eastern European countries usually exhibit higher Power Distance Index scores compared to English-Speaking Western countries (Hofstede, 2011). This means that, in countries like Bulgaria, power inequality is regarded as a societal norm. Because Bulgaria scores high on the Power Distance Index, Western values and ways of conducting business usually do not apply here.

Small Power Distance	Large Power Distance
Use of power should be legitimate and is subject to criteria of good and evil	Power is a basic fact of society antedating good or evil: its legitimacy is irrelevant
Parents treat children as equals	Parents teach children obedience
Older people are neither respected nor feared	Older people are both respected and feared
Student-centered education	Teacher-centered education
Hierarchy means inequality of roles, established for convenience	Hierarchy means existential inequality
Subordinates expect to be consulted	Subordinates expect to be told what to do
Pluralist governments based on majority vote and changed peacefully	Autocratic governments based on co-optation and changed by revolution
Corruption rare; scandals end political careers	Corruption frequent; scandals are covered up
Income distribution in society rather even	Income distribution in society very uneven
Religions stressing equality of believers	Religions with a hierarchy of priests

Fig. 18. Ten differences between Small and Large Power Distance societies (Hofstede, 2011).

Uncertainty Avoidance is an indicator of a society's tolerance for ambiguity, or the extent to which its members feel threatened by “uncertain, unknown, ambiguous, or unstructured situations” (Hofstede, 2011). East and Central European countries tend to exhibit higher Uncertainty Avoidance Index scores than English-speaking countries (Hofstede, 2011), which means they do not handle uncertainty as well as their Western counterparts.

Weak Uncertainty Avoidance	Strong Uncertainty Avoidance
The uncertainty inherent in life is accepted and each day is taken as it comes	The uncertainty inherent in life is felt as a continuous threat that must be fought
Ease, lower stress, self-control, low anxiety	Higher stress, emotionality, anxiety, neuroticism
Higher scores on subjective health and well-being	Lower scores on subjective health and well-being
Tolerance of deviant persons and ideas: what is different is curious	Intolerance of deviant persons and ideas: what is different is dangerous
Comfortable with ambiguity and chaos	Need for clarity and structure
Teachers may say 'I don't know'	Teachers supposed to have all the answers
Changing jobs no problem	Staying in jobs even if disliked
Dislike of rules - written or unwritten	Emotional need for rules – even if not obeyed
In politics, citizens feel and are seen as competent towards authorities	In politics, citizens feel and are seen as incompetent towards authorities
In religion, philosophy and science: relativism and empiricism	In religion, philosophy and science: belief in ultimate truths and grand theories

Fig. 19. Ten differences between weak- and strong-Uncertainty Avoidance societies (Hofstede, 2011).

Additionally, Hofstede defines Individualism/Collectivism as the degree to which members of a society are integrated into groups. In cultures that exhibit high individualism, the ties between individuals are loose and each person is only responsible for themselves and their immediate family. In cultures that exhibit high collectivism, individuals are usually part of extended families they are loyal to, and with which they form close ties. Developed and Western countries tend to be predominantly individualist, while less developed and Eastern countries tend to be predominantly collectivist (Hofstede, 2011).

Individualism	Collectivism
Everyone is supposed to take care of him- or herself and his or her immediate family only	People are born into extended families or clans which protect them in exchange for loyalty
"I" – consciousness	"We" –consciousness
Right of privacy	Stress on belonging
Speaking one's mind is healthy	Harmony should always be maintained
Others classified as individuals	Others classified as in-group or out-group
Personal opinion expected: one person one vote	Opinions and votes predetermined by in-group
Transgression of norms leads to guilt feelings	Transgression of norms leads to shame feelings
Languages in which the word "I" is indispensable	Languages in which the word "I" is avoided
Purpose of education is learning how to learn	Purpose of education is learning how to do
Task prevails over relationship	Relationship prevails over task

Fig. 20. Ten differences between Individualist and Collectivist societies (Hofstede, 2011).

Hofstede (2011) described Masculinity *vs*. Femininity as the distribution of values between the genders (Figure 21). More specifically, Masculinity is representative of a society with distinct social gender roles. Men are expected to be “assertive, tough, and focused on material success”, whereas women are expected to be more “modest, tender, and concerned with the quality of life” (Hofstede, 2011; Hofstede and Minkov, 2013). Masculinity has been identified as moderately high in English-speaking Western countries, whereas in Bulgaria it is lower. This cultural dimension appears to have no correlation with the effectiveness of project management in the country, however (Bredillet *et al*., 2010).

Femininity	Masculinity
Minimum emotional and social role differentiation between the genders	Maximum emotional and social role differentiation between the genders
Men and women should be modest and caring	Men should be and women may be assertive and ambitious
Balance between family and work	Work prevails over family
Sympathy for the weak	Admiration for the strong
Both fathers and mothers deal with facts and feelings	Fathers deal with facts, mothers with feelings
Both boys and girls may cry but neither should fight	Girls cry, boys don't; boys should fight back, girls shouldn't fight
Mothers decide on number of children	Fathers decide on family size
Many women in elected political positions	Few women in elected political positions
Religion focuses on fellow human beings	Religion focuses on God or gods
Matter-of-fact attitudes about sexuality; sex is a way of relating	Moralistic attitudes about sexuality; sex is a way of performing

Fig. 21. Ten differences between Feminine and Masculine societies (Hofstede, 2011).

Long-Term Orientation is characterized by "perseverance, thrift, ordering relationships by status, and having a sense of shame". Short-Term Orientation, on the other hand, is characterized by "reciprocating social obligations, respect for tradition, protecting one's 'face', and personal steadiness and stability" (Hofstede, 2011). Eastern- and Central European countries exhibit long-term orientation, while the United States is short-term oriented (Hofstede, 2011).

Short-Term Orientation	Long-Term Orientation
Most important events in life occurred in the past or take place now	Most important events in life will occur in the future
Personal steadiness and stability: a good person is always the same	A good person adapts to the circumstances
There are universal guidelines about what is good and evil	What is good and evil depends upon the circumstances
Traditions are sacrosanct	Traditions are adaptable to changed circumstances
Family life guided by imperatives	Family life guided by shared tasks
Supposed to be proud of one's country	Trying to learn from other countries
Service to others is an important goal	Thrift and perseverance are important goals
Social spending and consumption	Large savings quote, funds available for investment
Students attribute success and failure to luck	Students attribute success to effort and failure to lack of effort
Slow or no economic growth of poor countries	Fast economic growth of countries up till a level of prosperity

Fig. 22. Ten differences between Short- and Long-Term-Oriented societies (Hofstede, 2011).

Indulgence refers to the extent to which a society indulges in the gratification of basic and natural human desires and feelings, and the degree to which its members are able to enjoy their life. Conversely, restraint characterizes a society with strict social norms, where the gratification of needs (particularly relating to "leisure, merrymaking with friends, spending, consumption and sex") is regulated (Hofstede, 2011). Indulgence is prevalent in North America and Western Europe, while Restraint is prevalent in Eastern Europe (Hofstede, 2011).

Indulgence	Restrained
Higher percentage of people declaring themselves very happy	Fewer very happy people
A perception of personal life control	A perception of helplessness: what happens to me is not my own doing
Freedom of speech seen as important	Freedom of speech is not a primary concern
Higher importance of leisure	Lower importance of leisure
More likely to remember positive emotions	Less likely to remember positive emotions
In countries with educated populations, higher birthrates	In countries with educated populations, lower birthrates
More people actively involved in sports	Fewer people actively involved in sports
In countries with enough food, higher percentages of obese people	In countries with enough food, fewer obese people
In wealthy countries, lenient sexual norms	In wealthy countries, stricter sexual norms
Maintaining order in the nation is not given a high priority	Higher number of police officers per 100,000 population

Fig. 23. Ten differences between Indulgent and Restrained societies (Hofstede, 2011).

Table 12. Cultural dimensions comparison between Bulgaria and the United States*.

Country	PDI	IDV	MAS	UAI	ITOWVS	IVR
Bulgaria	70	30	40	85	69	16
USA	40	91	62	46	26	68

*Extracted from data published on www.geerthofstede.eu (Hofstede, 2013).

Based on Hofstede's framework of cultural distance issues, Bredillet *et al*. (2010) applied four of the dimensions to the field of Project Management deployment. Following Hofstede's recommendation, they also took into consideration the impact of GNP *per capita* on the correlation between the Power Distance and Individualism dimensions, and thus included GNP/Capita as a fifth factor (Bredillet *et al*., 2010). The separation of countries based on national wealth is important, as it serves as an indicator of different cultural reactions to Project Management deployment depending on the economic situation of the country (Bredillet *et al*., 2010). Furthermore, there is evidence of a causal relationship between wealth and individualism, as the former tends to lead to the latter (Hofstede, 2001, p. 253; Hofstede, 2011).

Bredillet *et al*.'s findings indicated that the project management discipline is expected to be better deployed in low power distance, individualist, low uncertainty avoidance, and high national GNP/capita countries better than in high power distance, collectivist, high uncertainty avoidance, and low GNP/capita countries (Bredillet *et al*., 2010), of which Bulgaria is an example. Project Management deployment is, however, supported in low-GNP countries characterized by high individualism, as low-GNP countries seek innovations, new technologies, and novel management approaches. If resources are inadequate and a country lacks economic stability, then Project Management deployment can be effective only if the Individualism dimension is prevalent (Bredillet *et al*., 2010). At present, the Individualism dimension is not representative of Bulgaria. In wealthier countries, the Power Distance and Uncertainty Avoidance dimensions provide a favorable environment for Project Management deployment (Bredillet et al., 2010).

Going back to Kivrak *et al*.'s findings, language barrier, trust, and personal relationships emerged as the most important factors determining good communication among participants in their study (Kivrak *et al*., 2014). Participants from high-context and collectivist cultures placed greater emphasis on trust when it came to knowledge-sharing than participants from low-context and individualistic cultures (of which the United States is an example). Individuals from high-context and collectivist cultures also placed more importance on trust in business relationships than did individuals from low-context and individualistic cultures.

People from collectivist cultures tend to develop personal relationships with their colleagues before engaging in business dealings with them (Kivrak *et al*., 2014; personal observation). It is easier to share one's knowledge if the individual has established close ties with their colleagues. Also, collectivist cultures prefer evaluation of group as opposed to individual performance, and as a result favor group-based decision-making and seek consensus (Kivrak *et al*., 2014). The most important knowledge sources for individuals from collectivist cultures are their colleagues and the company's experience. It is much easier for them to share their knowledge with members of their in-group, as opposed to out-group members (Kivrak *et al*., 2014). Developing a trusting environment and establishing personal relationships is thus paramount in facilitating knowledge-sharing with individuals from high-context and collectivist cultures (Kivrak *et al*., 2014). In comparison, competition among a team's members is much more frequently encountered in Western cultures; this tactic does not encourage knowledge-sharing, however, as individuals equate sharing their knowledge with loss of power (Kivrak *et al*., 2014). Finally, the Uncertainty Avoidance factor affects knowledge-sharing (Kivrak *et al*., 2014). High uncertainty avoidance cultures dislike risk-taking and change. Risk, however, can be minimized through sufficient contingency, which can put individuals from high uncertainty avoidance cultures at ease (Kivrak *et al*., 2014).

With the above considerations in mind, it becomes easier to create a framework of mutual understanding, effective communication, collaboration, and knowledge-sharing in a country like Bulgaria. These conditions must be met for projects, regardless of sector, to succeed. This is especially true for projects headed by foreign entities or individuals, which is likely to be the case in the coming years, until Bulgaria has acquired both the theoretical and practical expertise required for successful project completion.

List of Abbreviations

BMD: Benchmark Dose

BMDL: Benchmark Dose Level

CFP: Common Fisheries Policy (E.U.)

COC: Contaminant of Concern

CPUE: Catch per Unit Effort (sometimes CUE)

EEZ: Exclusive Economic Zone

EFSA: European Food Safety Authority

EIA: Environmental Impact Assessment

EPA: Environmental Protection Agency (U.S.), Environmental Protection Act (Bulgaria)

EU: European Union

FAO: Food and Agriculture Organization of the United Nations

GDP: Gross Domestic Product

IRIS: Integrated Risk Information System

ITQ: Individual Transferable Quota

JEFCA: Joint FAO/WHO Expert Committee on Food Additives

LOD: Limit of Detection

LOQ: Limit of Quantification (also, Limit of Quantitation)

NCPHP: National Centre of Public Health Protection (Bulgaria)

OECD: Organisation for Economic Co-operation and Development

PPP: Purchasing-Power Parity

T&E: Threatened & Endangered

TAC: Total Allowable Catch

TDS: Total Dissolved Solids

USD: United States Dollar

USDA: United States Department of Agriculture

USEPA: United States Environmental Protection Agency

WHO: World Health Organization

Glossary of Terms

Advisory: an official, non-mandatory recommendation for action

Aerobic: requiring oxygen

Algal bloom: a rapid growth of microscopic marine plants (algae) or cyanobacteria

Anaerobic: requiring or being able to function in the absence of oxygen

Anoxic: characterized by a lack of oxygen

Anthropogenic: human-induced, caused by human activity

Basin: a natural depression on the earth's surface that contains water

Benchmark Dose (BMD): a concentration of a substance that yields a defined change in response rate of an adverse effect compared to background

Benchmark Dose Level (BMDL): the lower 95% confidence limit on the BMD

Benthos: the bottom of a body of water; the organisms inhabiting the bottom sediments

Benthivore/benthivorous: an organism that is a bottom-feeder, whose prey lives on the benthos

Bioaccessible: the amount of a substance in an organism that is available for absorption

Bioaccumulation: the accumulation of a substance in an organism, at a rate faster than that at which the substance can be metabolized and purged from its body

Biocenosis: a closely-integrated biological community able to regulate itself

Biogenic: a product of living organisms

Bioindicator: an organism used to determine the health of an ecosystem

Biological productivity: the quantity of organic matter accumulated over a time period

Biomagnification: the process via which a substance increases in concentration in organisms, as it travels up the food chain

Biota: the fauna and flora of a region, habitat, or geological period

Biotope: a habitat associated with a particular ecological community, characterized by uniform environmental conditions

Brackish: a mixture of fresh- and saltwater

Catch per Unit Effort (CPUE): the catch of fish obtained for a given amount of fishing effort per unit time; an indirect method to measure species abundance

Catchment area: a reservoir or basin, which collects water from surrounding sources

Commons: a resource that belongs to an entire community

Conspecific: an organism belonging to the same species as another

Continental shelf: an area of seabed surrounding a land mass, with shallow waters compared to the open sea

Cyclonic: characterized by counter-clockwise circulation

Deleterious: harmful, toxic

Detritus: organic matter produced through the decay of living organisms

Ecosystem: a biological community of organisms that interact with each other as well as with the abiotic components of their environment

Endangered species: an organism under threat of extinction in all or part of its range

Euryhaline: able to tolerate a wide range of salinity

Eutrophication: excessive nutrient loading in a body of water

Exclusive Economic Zone (EEZ): a coastal area (incl. the seabed) within 200 nautical miles of a country’s shore, to which the country has exclusive rights and can utilize the natural resources found therein

Facultative: capable of performing a specific function, which is not otherwise obligatory

Genus: one of the principal categories in taxonomy, ranking below ‘family’ and above ‘species’

Guild: a group of organisms exhibiting similar requirements and fulfilling a similar ecological role

Habitat: the environment an organism dwells in

Harmonized: coordinated, synchronized

Heterogeneous: diverse in nature, varying

Holoplankton: an organism that remains as plankton throughout its entire life cycle

Hydro- (prefix): relating to water

Hypoxia: a state of insufficient oxygen

Ichthyoplankton: the planktic stages of fish (e.g., fish eggs and larvae)

Individual Transferable Quota (ITQ): a quota that is part of a fishery's TAC, which is allocated to an individual fisher or vessel owners and which can be sold ("transferred") to other fishers

Littoral: coastal; relating to the shore of a sea or lake

Mariculture: the rearing of fish and other marine organisms for food

Meroplankton: an organism that spends part of its life cycle as plankton and later transitions to a non-planktic life stage

Migratory: an organism that does not spend its whole life cycle in one place, but traverses distances in search of forage, mating and nesting areas, different temperatures, etc.

Nekton: an organism able to move against water currents

Nonpoint source: a source of pollution that cannot be attributed to a single polluter or point in time

Obligate: restricted to a specific function, which is essential to the organism's survival

Open-access regime: a type of management under which a natural resource is open to utilization by any member of the public, without restrictions

Organic matter: material originating from a living organism, capable of or a product of decay; composed of compounds containing the chemical element carbon

Organism: a life form, or biological entity (e.g., plant, animal, bacterium)

Pelagic: an organism inhabiting the open sea, particularly the upper layers

Phytoplankton: plankton of the microscopic plant variety

Plankton: a small or microscopic organism that cannot move against currents and is carried by them

Plate tectonics: a scientific theory explaining a variety of natural phenomena that occur as a result of movement of the plates the earth's lithosphere is composed of

Point source: a source of pollution that can be traced to a specific polluter and point in time

Purchasing power: the financial ability to acquire products and services

River discharge: the volume of water passing through a measuring point per unit time

Runoff: the draining away of water from an area, along with substances carried in it

Serving: a recommended portion of food

Species: a group of organisms so similar, they are able to mate and produce viable offspring; the principal taxonomic unit, ranking below 'genus'

Staple food: a food that is consumed routinely and constitutes a large proportion of a people's diet, and which provides a significant proportion of their energy requirements

Straddling stock (fish): a fish stock that occurs in or migrates through more than one EEZ

Threatened species: an organism likely to become endangered in the near future in all or part of its range

Total Allowable Catch (TAC): the total amount of fish, in tonnage, a species-specific fishery may catch per fishing season

Trace metal: elements that occur naturally in the environment in very low concentrations and which biota need in very small amounts to meet their daily requirements

Tragedy of the Commons: the dismal state of a natural resource that everyone owns and exploits but no-one takes care of, to the point of ruin of the resource

Trophic web: multiple interacting food chains in an ecosystem, made up of organisms that occupy different positions therein, and which may feed at one or multiple levels

Zooplankton: plankton of the microscopic animal variety, including larval stages of larger organisms

LITERATURE CITED

Alexieva B. 1997. A typology of interpreter-mediated events. Translator 3:153-174.

Almer HL, Koontz TM. 2004. Public hearings for EIAs in post-communist Bulgaria: do they work? Environ Impact Assess Rev 24:473-493.

Altukhov D, Siokou I, Pantazi M, Stefanova F, Timofte F, Gubanova A, et al. 2015. Intercomparison of five nets used for mesozooplankton sampling. Mediterr Mar Sci 16:550-561.

Al-Yamani FY, Skryabin V, Gubanova A, Khvorov S, Prusova I. 2011. Marine zooplankton practical guide for the Northwestern Arabian Gulf. Kuwait Institute for Scientific Research p.1. Kuwait:Lucky Press.

American Society of Limnology and Oceanography. 1978. Comment. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnol Oceanogr 23:1256-1263.

Arashkevich EG, Stefanova K, Bandelj V, Siokou I, Kurt TT, Orek YA, et al. 2014. Mesozooplankton in the open Black Sea: regional and seasonal characteristics. J Mar Syst 135:81-96.

Baker S, Baumgartl B. 1998. Bulgaria: managing the environment in an unstable transition. Environ Politics 7:183-206.

Baker S. 2002. Environmental protection capacity in post-communist Bulgaria. In: Capacity Building in National Environmental Policy (Weidner H, Jänicke M, eds). Berlin, Heidelberg:Springer, 97–122.

Balážová-L'avrinčíková M, Kováč V. 2007. Epigenetic context in the life history traits of the round goby, Neogobius melanostomus. In: Biological invaders in inland waters: profiles, distribution, and threats. Springer, 275–287.

Banăru D, Harmelin-Vivien M. 2015. Feeding behaviour of Black Sea bottom fishes: did it change over time? Acta Oecol 35:769-777.

Banerjee M, Banerjee N, Bhattacharjee P, Mondal D, Lythgoe PR, Martínez M, et al. 2013. High arsenic in rice is associated with elevated genotoxic effects in humans. Sci Rep 3:2195.

Boran M, Altınok I. 2010. A review of heavy metals in water, sediment and living organisms in the Black Sea. Turk J Fish Aquat Sci 10:565-572.

Bredillet C, Yatim F, Ruiz P. 2010. Project management deployment: the role of cultural factors. Int J Proj Manage 28:183–193.

Brown VJ. 1999. The worst of both worlds: poverty and politics in the Balkans. Environ Health Perspect 107:606-613.

Brownscombe JW, Fox MG. 2012. Range expansion dynamics of the invasive round goby (Neogobius melanostomus) in a river system. Aquat Ecol 46:175-189.

Bryan GW, Waldichuk M, Pentreath RJ, Darracott A. 1979. Bioaccumulation of marine pollutants [and discussion]. Philos Trans Royal Soc B Biol Sci 286:483–505.

Bulgarian Ministry of Agriculture and Food. 2016. Annual Report on the Situation and Development of Agriculture (Agrarian Report 2016). Retrieved from http://www.mzh.government.bg/MZH/Libraries/Actual2/2016.sflb.ashx.

Bulgarian Ministry of Agriculture, Food and Forests. 2017. Rice Production, Trade and Support in Bulgaria. Retrieved from http://ec.europa.eu/transparency/regexpert/index.cfm?do=groupDetail.groupDetailDoc&id=32962&no=2.

Bulgarian National Statistical Institute. 2016. *Purchasing Power Parities*. [Data file]. Retrieved from http://www.nsi.bg/en/content/5212/метаданни/purchasing-power-parities.

Caddy JF. 2006. The potential use of indicators, reference points and the traffic light convention for managing Black Sea fisheries. Paper presented at the GFCM/SAC Workshop on Reference Points for Mediterranean Fisheries, Rome, Italy, 20-21 April 2004. General Fisheries Commission for the Mediterranean, Studies and Reviews, No. 83. FAO, Rome. pp. 1-24.

Chuenpagdee R, Liguori L, Palomares MLD, Pauly D. 2006. Bottom-up, global estimates of small-scale marine fisheries catches. Fisheries Centre. University of British Columbia. Fisheries Center Research Reports 14:8.

Corkum LD, Sapota MR, Skora AE. 2004. The round goby, Neogobius melanostomus, a fish Invader on both sides of the Atlantic Ocean. Biol Invasions 6:173-181.

Croghan C, Egeghy PP. Methods of dealing with values below the limit of detection using SAS. Presented at Southern SAS User Group, St. Petersburg, FL, September 22-24, 2003.

Cutter GA. 1992. Kinetic controls on metalloid speciation in seawater. Mar Chem 40:65-80.

Daskalov GM. 2002. Overfishing drives a trophic cascade in the Black Sea. Mar Ecol Prog Ser 225:53-63.

Dineva S. 2016. Основни замърсители в хранителните суровини и продукти. “Primary contaminants in raw food and food products”. Thracian University of Stara Zagora.

Duemler JJ, Kozelichki JM, Simon TP. 2016. Relationships between length-weight, age, and body condition of the round goby Neogobius melanostomus (Pallas) in a tributary and harbor embayment in Southern Lake Michigan, USA. Turk J Fish Aquat Sci 16:205-212.

Dufrêne M, Legendre P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monograph 67:345–366.

Duzgunes E, Erdogan N. 2008. Fisheries management in the Black Sea countries. Turk J Fish Aquat Sci 8:181-192.

EFSA. 2009a. Scientific Opinion on Arsenic in Food. The EFSA Journal 7:1351.

EFSA. 2009b. Cadmium in food. The EFSA Journal 980:1-139.

EFSA. 2012. Lead dietary exposure in the European population. The EFSA Journal 10:2831.

EFSA. 2014. Dietary exposure to inorganic arsenic in the European population. The EFSA Journal 12:3597.

EFSA. 2015. Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. The EFSA Journal 13:3982.

Emiliani C. 1952. Nomenclature and grammar. J Wash Acad Sci 42:137-141.

Emiliani C. 1991. Planktic/planktonic, nektic/nektonic, benthic/benthonic. J Paleontol 65:329.

FAO. 2002. FIGIS Country Profile Fact Sheet. Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/fishery/country/canada_fcp/en.

FAO. 2004. National Aquaculture Sector Overview: Bulgaria. Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/fishery/countrysector/naso_bulgaria/en.

FAO. 2006. Rice International Commodity Profile. Markets and Trade Division. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Rice/Documents/Rice_Profile_Dec-06.pdf.

FAO Species Fact Sheet. 2018. Species Fact Sheets. Mytilus galloprovincialis. Retrieved from http://www.fao.org/fishery/species/3529/en.

FDEP. 2014. Holding Times and Procedures. Florida Department of Environmental Protection. Retrieved from http://flenviro.com/FDEP_Holding_Times_and_Preservations.pdf.

Fofonoff NP. 1985. Physical properties of seawater: a new salinity scale and equation of state for seawater. J Geophys Res 90:3332-3342.

Fontcuberta M, Calderon J, Villalbí JR, Centrich F, Portaña S, Espelt A, et al. 2011. Total and inorganic arsenic in marketed food and associated health risks for the Catalan (Spain) population. J Agric Food Chem 59:10013-22.

Francesconi KA. 2010. Arsenic species in seafood: origin and human health implications. Pure Appl Chem 82:373–381.

Friedberg J, Zaimov B. 1994. Politics, environment, and the rule of law in Bulgaria. Duke J Comp & Int'l L 4:224-263.

Gedik K. 2017. Bioaccessibility of heavy metals in rapa whelk *Rapana venosa* (Valenciennes, 1846): Assessing human health risk using an *in vitro* digestion model, pp. 202-213.

Global Invasive Species Database. 2018. Species profile: *Mytilus galloprovincialis*. Downloaded from http://www.iucngisd.org/gisd/species.php?sc=102 on 19-02-2018.

Goessler W, Maher W, Irgolic KJ, Kuehnelt D, Schlagenhaufen C, Kaise T. 1997. Arsenic compounds in a marine food chain. Fresenius J Anal Chem 359:434–437.

Guérin T, Chekri R, Vastel C, Sirot V, Volatier J-L, Leblanc J-C, et al. 2011. Determination of 20 trace elements in fish and other seafood from the French market. Food Chem 127:934–942.

Gutowsky LFG, Brownscombe JW, Fox MG. 2011. Angling to estimate the density of large round goby (Neogobius melanostomus). Fish Res 108:228–231.

Hall S. 1973. Encoding and Decoding in the Television Discourse. Paper for the Council of Europe Colloquy on 'Training in the Critical Reading of Televisual language', organized by the Council and the Centre for Mass Communication research. University of Leicester, September.

Hall S. 1977. Culture, the Media and the Ideological Effect, in Mass Communication and Society (Curran J, Gurevitch M, Woollacott J, eds), pp. 315-48. Los Angeles, CA: SAGE.

Hall S. 1980. 'Encoding/decoding'. In Centre for Contemporary Cultural Studies (Ed.): Culture, Media, Language: Working Papers in Cultural Studies, 1972-79 London: Hutchinson, pp. 128-38.

Hall ET. 1972. A system for the notation of proxemic behavior in Europe. REC, pp. 115– 26.

Hardin G. 1968. The tragedy of the commons. Science 162:1243–1248.

Hensler SR, Jude DJ. 2007. Diel Vertical Migration of Round Goby Larvae in the Great Lakes. J Great Lakes Res 33:295-302.

Hofstede G. 2013. Research and VSM. VSM 2013. Retrieved from www.geerthofstede.eu/research-and-vsm.

Hofstede G, Minkov M. 2013. VSM 2013. Values Survey Module 2013 Manual. Geert Hofstede BV, pp. 4—17.

Hofstede G. 2011. Dimensionalizing cultures: the Hofstede model in context. Online Readings in Psychology and Culture 2:1-26.

Hofstede G. 2001. Culture's Consequences: Comparing Values, Behaviors, Institutions and Organizations Across Nations. SAGE Publishing, p. 253.

Holloman EL, Newman MC. 2012. Expanding perceptions of subsistence fish consumption: evidence of high commercial fish consumption and dietary mercury exposure in an urban coastal community. Sci Total Environ 416:111–120.

Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. 2011. Arsenic exposure and toxicology: a historical perspective. Toxicol Sci 123:305-332.

IARC. 1980. Toxicological Profile for Arsenic. Agency for Toxic Substances & Disease Registry. Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp2-c2.pdf.

IARC. 2004. Toxicological Profile for Arsenic. Agency for Toxic Substances & Disease Registry. Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp2-c2.pdf.

Irgolic KJ, Spall D, Puri BK, Ilger D, Zingarot RA. 1991. Determination of arsenic and arsenic compounds in natural gas samples. Appl Organomet Chem 5:117-124.

Puri BK, Irgolic KJ. 1989. Determination of arsenic in crude petroleum and liquid hydrocarbons. Environ Geochem Health 11:95-99.

IRIS. (2018a). Toxicological review of trivalent chromium. *U.S. Environmental Protection Agency*, 1-51. Retrieved from https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=28

IRIS. (2018b). Toxicological review of hexavalent chromium. *U.S. Environmental Protection Agency*, 1-77. Retrieved from https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=144

IRIS. (2007). Toxicological review of inorganic arsenic. *U.S. Environmental Protection Agency*, 1-27. Retrieved from https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=278

Islam, SM., Tanaka M. 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Marine Poll Bull 48:624–649.

Patterson J. 2010. Introduction comparative dietary risk: balance the risk and benefits of fish consumption. Comments on Toxicology 8:4-6, 337-343.

Jitar O, Teodosiu C, Nicoara M, Plavan G. 2013. Study of heavy metal pollution and bioaccumulation in the Black Sea living environment. Environ Eng Manag J 12:271-6.

Kamburska L, Vulcheva E. 2003. On the peculiarities of the zooplankton spatial distribution in Burgas Bay – May, 1996. Proceedings of the Institute of Oceanology 4:124-132. Sofia, Bulgaria:Bulgarian Academy of Sciences.

Keen E. 2015. Net savvy: A practical guide to zooplankton sampler design. La Jolla, CA:National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

Keskin Ç, Ulman A, Zylich K, Raykov V, Daskalov GM, Pauly D, Zeller D. 2017. The marine fisheries in Bulgaria's Exclusive Economic Zone, 1950–2013. Frontiers in Marine Science 4:1-53.

Kiseleva MI. 1979. In: Fundamentals of the Biological Productivity of the Black Sea. Kiev:Naukova Dumka Publ.

Kivrak S, Arslan G, Tuncan M, Birgonul MT. 2014. Impact of national culture on knowledge sharing in international construction projects. Can J Civ Eng 41:642-649.

Kolios S, Stylios C. 2015. Coastal marine environment monitoring using satellite data derived from MODIS instrument. Sustainable Development of Sea-Corridors and Coastal Waters 125–131.

Kucuksezgin F, Tolga Gonul L, Tasel D. 2014. Total and inorganic arsenic levels in some marine organisms from Izmir Bay (Eastern Aegean Sea): A risk assessmnent. Chemosphere 112: 311-316.

Kutsarov RK, Chobanov IS, 2015. Modeling of biobasins of an oil refinery wastewater treatment plant. J BioSci Biotechnol 4:63-65.

Kvach Y, Stepien CA. 2008. Metazoan parasites of introduced round and tubenose gobies in the Great Lakes: support for the "enemy release hypothesis". J Great Lakes Res 34:23–35.

Lafrance R, Schembri L. 2002. Purchasing-Power Parity: definition, measurement, and interpretation. Ottawa, Canada:Bank of Canada Review.

Lincoln RJ, Boxhall GA, Clark PF. 1985. A dictionary of ecology, evolution and systematics. Cambridge, London:Cambridge University Press.

Makedonski L, Peycheva K, Stancheva M. 2017. Determination of heavy metals in selected Black Sea fish species. Food Control 72:313–318.

Maldonado C, Bayona JM, Bodineau L. 1999. Sources, distribution, and water column processes of aliphatic and polycyclic aromatic hydrocarbons in the northwestern Black Sea water. Environ Sci Technol 33:2693–2702.

Mania M, Rebeniak M, Szynal T, Wojciechowska-Mazurek M, Starska K, Ledzion E, et al. 2015. Total and inorganic arsenic in fish, seafood and seaweeds - exposure assessment. Rocz Panstw Zakl Hig 66-203-210.

Milroy SP. 2015. Field methods in marine science: From measurements to models. New York, NY:Garland Science.

Moncheva S, Racheva E, Kamburska L, D'Hernoncourt J. 2012. Environmental and management constraints on tourism in Varna Bay, Bulgarian Black Sea Coast. Ecol Soc 17:35.

NCPHP. 2006. Food Based Dietary Guidelines for Adults in Bulgaria. Ministry of Health. National Center of Public Health Protection, pp. 1—41.

OECD. 2011. 2011 PPP Benchmark Results. Retrieved from https://stats.oecd.org/Index.aspx?DataSetCode=PPP2011

OECD. 2018. Purchasing power parities (PPP) (indicator). doi: 10.1787/1290ee5a-en (Accessed on 12 February 2018)

Oguz T, Velikova V. 2010. Abrupt transition of the northwestern Black Sea shelf ecosystem from a eutrophic to an alternative pristine state. Mar Ecol Prog Ser 405:231-242.

Peycheva K, Makedonski L, Stancheva M. 2014. Daily intake of arsenic and mercury by consumption of Bulgarian Black Sea fishes. Scripta Scientifica Pharmaceutica 1:38-43.

Poe MR, Norman KC, Levin PS. 2013. Cultural dimensions of socioecological systems: key connections and guiding principles for conservation in coastal environments. Conserv Lett 7:166–175.

Potters G. 2013. Marine Pollution. BookBoon, p. 17.

Raab A, Baskaran C, Feldmann J, Meharg AA. 2009. Cooking rice in a high water to rice ratio reduces inorganic arsenic content. J Environ Monit 11:41-44.

Radu G, Anton E, Golumbeanu M, Raykov V, Yankova M, Panayotova M, et al. 2011. State of the main Black Sea commercial fish species correlated with the ecological conditions and fishing effort. J Environ Prot Ecol 12:549–557.

Ray WJ, Corkum LD. 2001. Habitat and site affinity of the round goby. J Great Lakes Res 27:329-334.

REC. 1998. Doors to democracy: current trends and practices in public participation in environmental decision making in Central and Eastern Europe. Regional Environmental Center for Central and Eastern Europe.

Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs contains the most recent maximum levels for cadmium.

Rodhe W. 1974. Plankton, planktic, planktonic. Limnol Oceanogr 19:360–360.

Root RB. 1967. The niche exploitation pattern of the blue-gray gnatcatcher. Ecol Monograph 37:317–350.

Salleh LM. 2005. High/low context communication: the Malaysian Malay style. In: Proceedings of the 2005 Association for Business Communication Annual Convention (Gueldenzoph LE, ed). Irvine, CA:Association for Business Communication.

Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on a request from the Commission on chromium and copper ethanolamine phosphate. *The EFSA Journal* (2009) 953, 1-6.

Seaman DE, Powell RA. 1996. An evaluation of the accuracy of Kernel density estimators for home range analysis. Ecology 77:2075–2085.

Shao AT, Bao Y, Gray E. 2004. Comparative advertising effectiveness: a cross-cultural study. Journal of Current Issues & Research in Advertising 26:67-80.

Shiganova TA, Bulgakova YV. 2000. Effects of gelatinous plankton on Black Sea and Sea of Azov fish and their food resources. ICES J Mar Sci 57:641-648.

Shurova NM, Gomoiu M-T. 2005. Present state of mussel population (Mytilus galloprovincialis) from the North-West Black Sea. Black Sea Ecosystem 2005 and Beyond. Abstract. 1st Biannual Scientific Conference 8-10 May 2006 Istanbul, Turkey, pp. 92–93.

Simonović P, Paunović M, Popović S. 1998. Morphology, feeding, and reproduction of the round goby, Neogobius melanostomus (Pallas), in the Danube River basin, Yugoslavia**.** J Great Lakes Res 27:281-289.

Sloth JJ, Larsen EH, Julshamn K. 2005. Survey of inorganic arsenic in marine animals and marine certified reference materials by anion exchange high-performance liquid chromatography−inductively coupled plasma mass spectrometry. J Agric Food Chem 53:6011−6018.

Staddon C, Genchev S. 2013. Geographical and Temporal Dimensions of Bulgarian Attitudes towards the Rural Environment. Czech Sociological Review 49:375-401.

Stanchev H, Stancheva M, Young R. 2015. Implications of population and tourism development growth for Bulgarian coastal zone. J Coast Conserv 19:59-72.

Strezov A, Nonova T. 2009. Influence of macroalgal diversity on accumulation of radionuclides and heavy metals in Bulgarian Black Sea ecosystems. Journal of Environmental Radioactivity 100:144–150.

Tanova C, Nadiri H. 2010. The role of cultural context in direct communication. Baltic Journal of Management 5:185-196.

Taylor JK. 1987. Quality Assurance of Chemical Measurements. Lewis Publishers. CRC Press LLC.

Tilves U, Fuentes V, Milisenda G, Parrish C, Vizzini S, Sabatés A. 2018. Trophic interactions of the jellyfish Pelagia noctiluca in the NW Mediterranean: evidence from stable isotope signatures and fatty acid composition. Mar Ecol Prog Ser 591:101–116.

Tornero V, Hanke G. 2016. Chemical contaminants entering the marine environment from sea-based sources: a review with a focus on European seas. Marine Poll Bull 112:17–38.

Torres-Escribano S, Leal M, Vélez D, Montoro R. 2008. Total and inorganic arsenic concentrations in rice sold in Spain, effect of cooking, and risk assessments. Environ Sci Technol 42:3867–3872.

Ulman A, Bekisoglu S, Zengin M, Knudsen S, Unal V, Mathews C, et al. 2013. From bonito to anchovy: a reconstruction of Turkey's marine fisheries catches (1950-2010). Medit Mar Sci 14:309-342.

Urquhart J, Acott T, Zhao M. 2013. Introduction: social and cultural impacts of marine fisheries. Mar Policy 37:1–2.

USDA. 2017. Fish and Seafood Market Brief – Bulgaria. Global Agricultural Information Network Report BU1706. USDA Foreign Agricultural Service, pp. 1—13.

USGS. 2016. Arsenic in coal. Fact Sheet 2005—3152. U.S. Department of the Interior, U.S. Geological Survey, pp. 1—4.

Wojciechowska-Mazurek M, Mania M, Starska K, Rebeniak M, Postupolski J. 2012. Czy zostaną wprowadzone dopuszczalne poziomy arsenu w żywności? [Will the maximum levels of arsenic in food be introduced?] Przem. Spoż. 66:10-15 (in Polish).

World Values Survey Wave 5 2005-2008 Official Aggregate v.20140429. World Values Survey Association (www.worldvaluessurvey.org). Aggregate File Producer: Asep/JDS, Madrid, Spain.

Yankova MH, Pavlov D, Ivanova P, Karpova E, Boltachev A, Öztürk B, et al. 2014. Marine fishes in the Black Sea: recent conservation status. Medit Mar Sci 15:366-379.

Yavno S, Corkum LD. 2011. Round goby *Neogobius melanostomus* attraction to conspecific and heterospecific egg odours. J Fish Biol 78:1944-53.

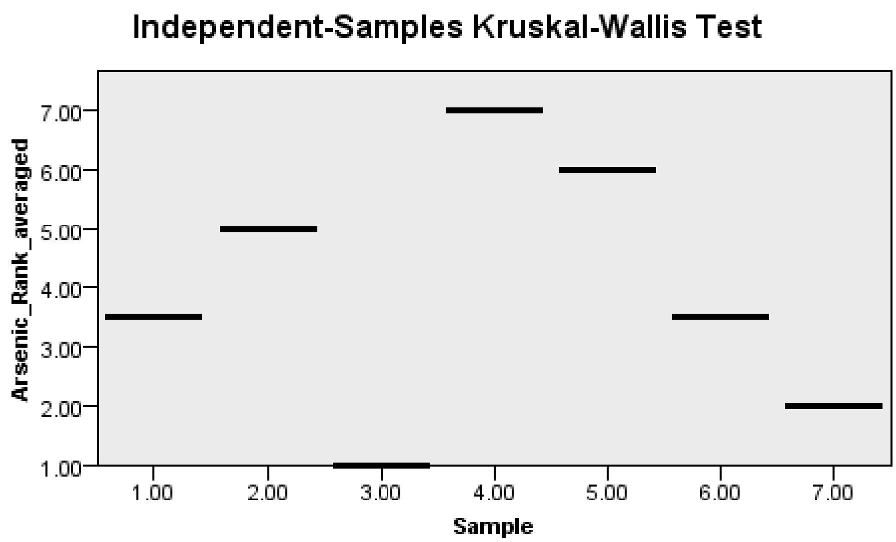
Zaitsev YP. 2008. An introduction to the Black Sea ecology. Odessa, Smil Editing & Publishing Agency Ltd., 226 pp.

Zaitsev YP, Mamaev V. 1997. Biological diversity in the Black Sea: a study of change and decline. Black Sea Environmental Series Vol: 3. New York:United Nations Publications.

Zaitsev YP. 1992. Recent changes in the trophic structure of the Black Sea. Fish Oceanogr 1.

APPENDIX

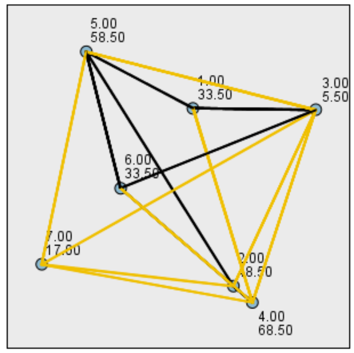
1.1 Arsenic rank comparison of Field Season I Round Goby samples.



Total N	73
Test Statistic	72.000
Degrees of Freedom	6
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Pairwise Comparisons of Sample

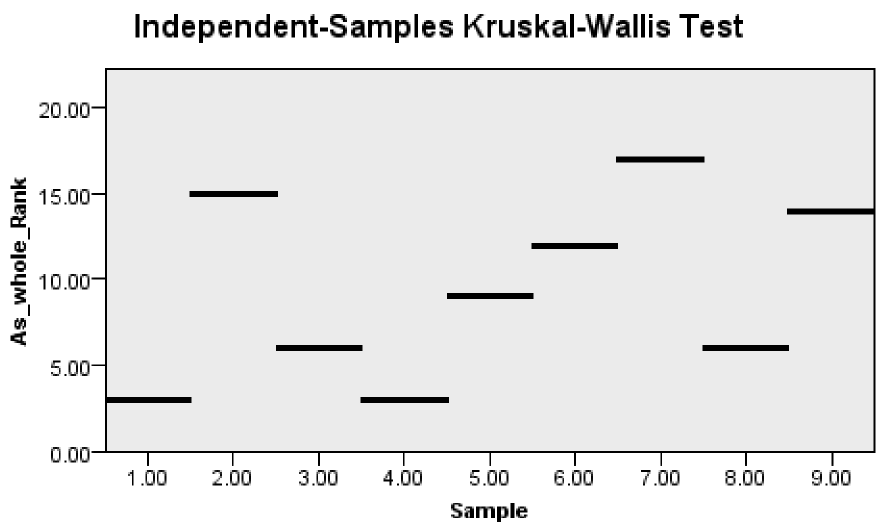


Each node shows the sample average rank of Sample.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
3.00-7.00	-11.500	8.761	-1.313	.189	1.000
3.00-1.00	28.000	9.315	3.006	.003	.056
3.00-6.00	-28.000	9.315	-3.006	.003	.056
3.00-2.00	43.000	9.315	4.616	.000	.000
3.00-5.00	-53.000	9.315	-5.690	.000	.000
3.00-4.00	-63.000	9.315	-6.763	.000	.000
7.00-1.00	16.500	8.761	1.883	.060	1.000
7.00-6.00	16.500	8.761	1.883	.060	1.000
7.00-2.00	31.500	8.761	3.596	.000	.007
7.00-5.00	41.500	8.761	4.737	.000	.000
7.00-4.00	51.500	8.761	5.878	.000	.000
1.00-6.00	.000	9.315	.000	1.000	1.000
1.00-2.00	-15.000	9.315	-1.610	.107	1.000
1.00-5.00	-25.000	9.315	-2.684	.007	.153
1.00-4.00	-35.000	9.315	-3.757	.000	.004
6.00-2.00	15.000	9.315	1.610	.107	1.000
6.00-5.00	25.000	9.315	2.684	.007	.153
6.00-4.00	35.000	9.315	3.757	.000	.004
2.00-5.00	-10.000	9.315	-1.074	.283	1.000
2.00-4.00	-20.000	9.315	-2.147	.032	.667
5.00-4.00	10.000	9.315	1.074	.283	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

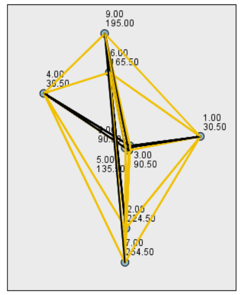
1.2 Arsenic rank comparison of Field Season I Mediterranean Mussel (Whole) samples.



Total N	269
Test Statistic	268.000
Degrees of Freedom	8
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Pairwise Comparisons of Sample

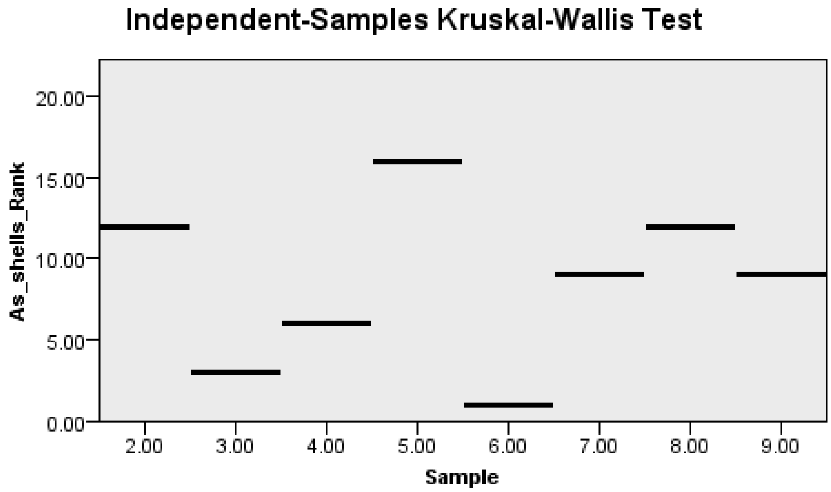


Each node shows the sample average rank of Sample.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
1.00-4.00	.000	19.794	.000	1.000	1.000
1.00-3.00	-60.000	19.794	-3.031	.002	.088
1.00-8.00	-60.000	19.794	-3.031	.002	.088
1.00-5.00	-105.000	19.794	-5.305	.000	.000
1.00-6.00	-135.000	19.794	-6.820	.000	.000
1.00-9.00	-164.500	19.964	-8.240	.000	.000
1.00-2.00	-194.000	19.794	-9.801	.000	.000
1.00-7.00	-224.000	19.794	-11.317	.000	.000
4.00-3.00	60.000	19.794	3.031	.002	.088
4.00-8.00	-60.000	19.794	-3.031	.002	.088
4.00-5.00	-105.000	19.794	-5.305	.000	.000
4.00-6.00	-135.000	19.794	-6.820	.000	.000
4.00-9.00	-164.500	19.964	-8.240	.000	.000
4.00-2.00	194.000	19.794	9.801	.000	.000
4.00-7.00	-224.000	19.794	-11.317	.000	.000
3.00-8.00	.000	19.794	.000	1.000	1.000
3.00-5.00	-45.000	19.794	-2.273	.023	.828
3.00-6.00	-75.000	19.794	-3.789	.000	.005
3.00-9.00	-104.500	19.964	-5.234	.000	.000
3.00-2.00	134.000	19.794	6.770	.000	.000
3.00-7.00	-164.000	19.794	-8.285	.000	.000
8.00-5.00	45.000	19.794	2.273	.023	.828
8.00-6.00	75.000	19.794	3.789	.000	.005
8.00-9.00	-104.500	19.964	-5.234	.000	.000
8.00-2.00	134.000	19.794	6.770	.000	.000
8.00-7.00	164.000	19.794	8.285	.000	.000
5.00-6.00	-30.000	19.794	-1.516	.130	1.000
5.00-9.00	-59.500	19.964	-2.980	.003	.104
5.00-2.00	89.000	19.794	4.496	.000	.000
5.00-7.00	-119.000	19.794	-6.012	.000	.000
6.00-9.00	-29.500	19.964	-1.478	.139	1.000
6.00-2.00	59.000	19.794	2.981	.003	.104
6.00-7.00	-89.000	19.794	-4.496	.000	.000
9.00-2.00	29.500	19.964	1.478	.139	1.000
9.00-7.00	59.500	19.964	2.980	.003	.104
2.00-7.00	-30.000	19.794	-1.516	.130	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

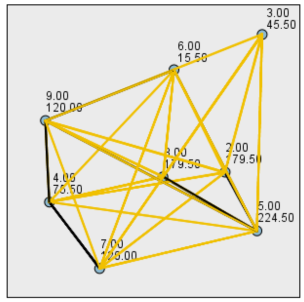
1.3 Arsenic rank comparison of Field Season I Mediterranean Mussel (Shells) samples.



Total N	239
Test Statistic	238.000
Degrees of Freedom	7
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Pairwise Comparisons of Sample

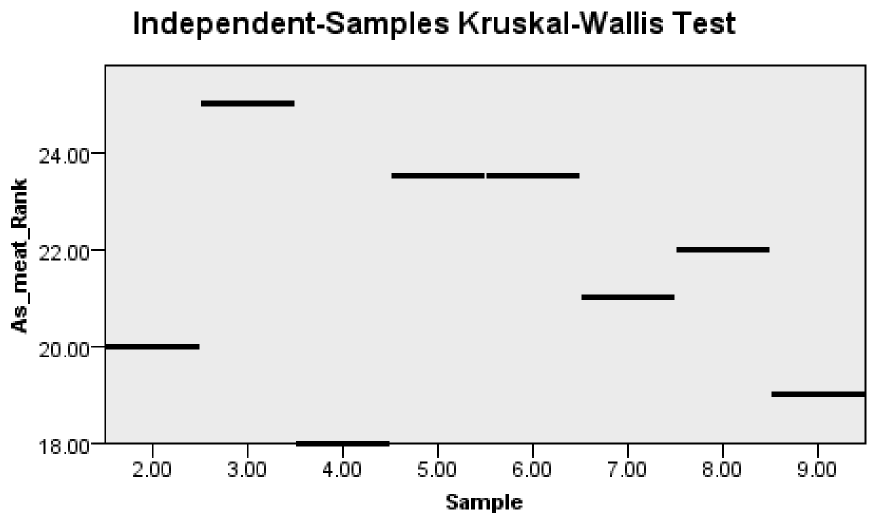


Each node shows the sample average rank of Sample.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
6.00-3.00	30.000	17.502	1.714	.087	1.000
6.00-4.00	60.000	17.502	3.428	.001	.022
6.00-7.00	-104.500	17.502	-5.971	.000	.000
6.00-9.00	-104.500	17.652	-5.920	.000	.000
6.00-2.00	164.000	17.502	9.370	.000	.000
6.00-8.00	-164.000	17.502	-9.370	.000	.000
6.00-5.00	209.000	17.502	11.942	.000	.000
3.00-4.00	-30.000	17.502	-1.714	.087	1.000
3.00-7.00	-74.500	17.502	-4.257	.000	.001
3.00-9.00	-74.500	17.652	-4.220	.000	.001
3.00-2.00	134.000	17.502	7.656	.000	.000
3.00-8.00	-134.000	17.502	-7.656	.000	.000
3.00-5.00	-179.000	17.502	-10.227	.000	.000
4.00-7.00	-44.500	17.502	-2.543	.011	.396
4.00-9.00	-44.500	17.652	-2.521	.012	.421
4.00-2.00	104.000	17.502	5.942	.000	.000
4.00-8.00	-104.000	17.502	-5.942	.000	.000
4.00-5.00	-149.000	17.502	-8.513	.000	.000
7.00-9.00	.000	17.652	.000	1.000	1.000
7.00-2.00	59.500	17.502	3.400	.001	.024
7.00-8.00	-59.500	17.502	-3.400	.001	.024
7.00-5.00	104.500	17.502	5.971	.000	.000
9.00-2.00	59.500	17.652	3.371	.001	.027
9.00-8.00	59.500	17.652	3.371	.001	.027
9.00-5.00	104.500	17.652	5.920	.000	.000
2.00-8.00	.000	17.502	.000	1.000	1.000
2.00-5.00	-45.000	17.502	-2.571	.010	.365
8.00-5.00	45.000	17.502	2.571	.010	.365

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

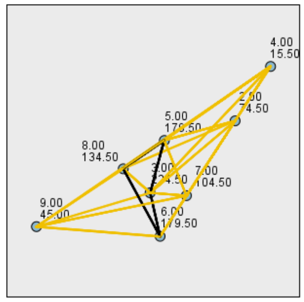
1.4 Arsenic rank comparison of Field Season I Mediterranean Mussel (Meat) samples.



Total N	239
Test Statistic	238.000
Degrees of Freedom	7
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Pairwise Comparisons of Sample

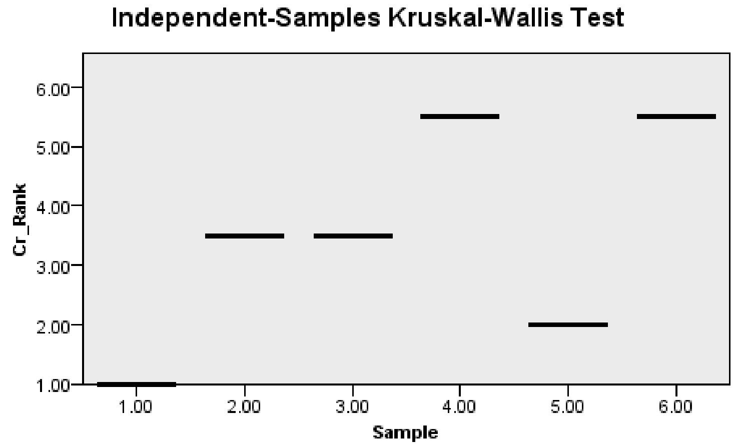


Each node shows the sample average rank of Sample.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
4.00-9.00	-29.500	17.755	-1.661	.097	1.000
4.00-2.00	59.000	17.604	3.351	.001	.029
4.00-7.00	-89.000	17.604	-5.056	.000	.000
4.00-8.00	-119.000	17.604	-6.760	.000	.000
4.00-5.00	-164.000	17.604	-9.316	.000	.000
4.00-6.00	-164.000	17.604	-9.316	.000	.000
4.00-3.00	209.000	17.604	11.872	.000	.000
9.00-2.00	29.500	17.755	1.661	.097	1.000
9.00-7.00	59.500	17.755	3.351	.001	.029
9.00-8.00	89.500	17.755	5.041	.000	.000
9.00-5.00	134.500	17.755	7.575	.000	.000
9.00-6.00	134.500	17.755	7.575	.000	.000
9.00-3.00	179.500	17.755	10.110	.000	.000
2.00-7.00	-30.000	17.604	-1.704	.088	1.000
2.00-8.00	-60.000	17.604	-3.408	.001	.024
2.00-5.00	-105.000	17.604	-5.964	.000	.000
2.00-6.00	-105.000	17.604	-5.964	.000	.000
2.00-3.00	-150.000	17.604	-8.521	.000	.000
7.00-8.00	-30.000	17.604	-1.704	.088	1.000
7.00-5.00	75.000	17.604	4.260	.000	.001
7.00-6.00	75.000	17.604	4.260	.000	.001
7.00-3.00	120.000	17.604	6.817	.000	.000
8.00-5.00	45.000	17.604	2.556	.011	.381
8.00-6.00	45.000	17.604	2.556	.011	.381
8.00-3.00	90.000	17.604	5.112	.000	.000
5.00-6.00	.000	17.604	.000	1.000	1.000
5.00-3.00	45.000	17.604	2.556	.011	.381
6.00-3.00	45.000	17.604	2.556	.011	.381

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

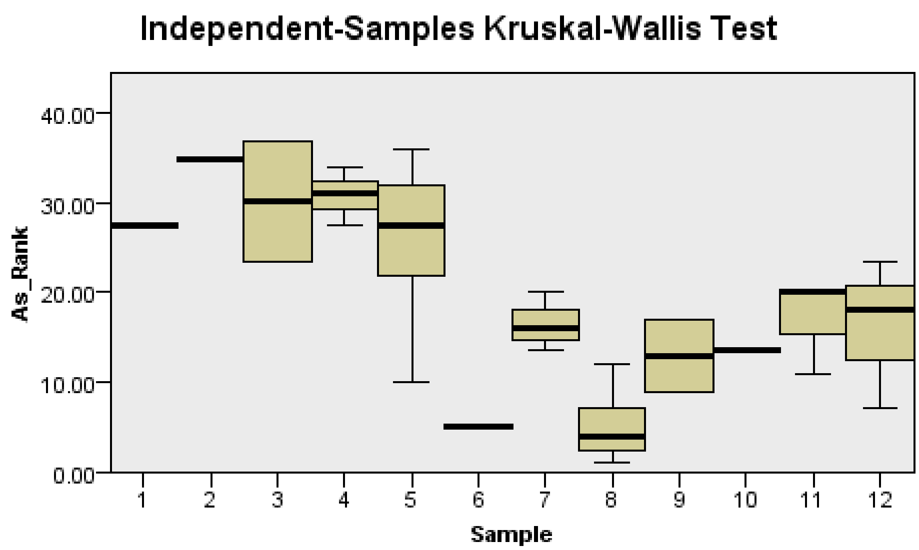
1.5 Chromium rank comparison of Field Season II Plankton samples.



Total N	6
Test Statistic	5.000
Degrees of Freedom	5
Asymptotic Sig. (2-sided test)	.416

1. The test statistic is adjusted for ties.
2. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

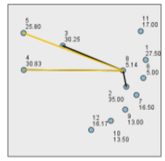
1.5 Arsenic rank comparison of Field Season II Round Goby samples.



Total N	37
Test Statistic	27.002
Degrees of Freedom	11
Asymptotic Sig. (2-sided test)	.005

1. The test statistic is adjusted for ties.

Pairwise Comparisons of Sample



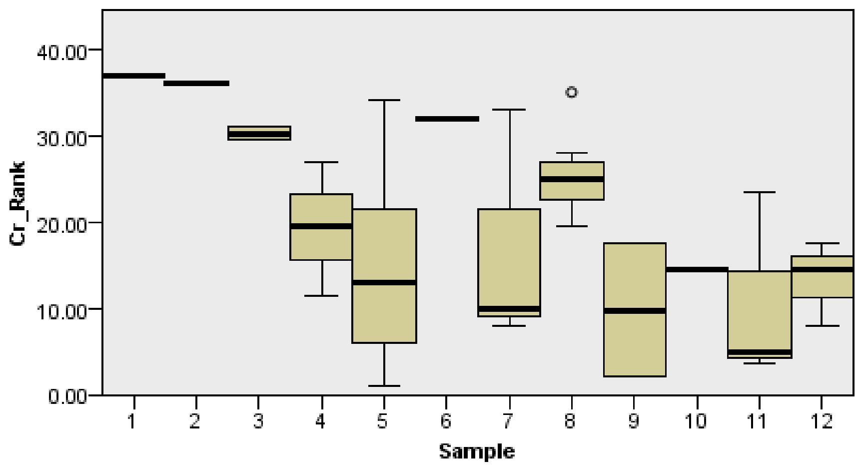
Each node shows the sample average rank of Sample.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
6-8	-.143	11.567	-.012	.990	1.000
6-9	-8.000	13.252	-.604	.546	1.000
6-10	-8.500	15.302	-.556	.579	1.000
6-12	-11.167	12.494	-.894	.371	1.000
6-7	-11.500	12.494	-.920	.357	1.000
6-11	-12.000	12.494	-.960	.337	1.000
6-5	20.800	11.348	1.833	.067	1.000
6-1	22.500	15.302	1.470	.141	1.000
6-3	25.250	13.252	1.905	.057	1.000
6-4	25.833	12.494	2.068	.039	1.000
6-2	30.000	15.302	1.961	.050	1.000
8-9	-7.857	8.675	-.906	.365	1.000
8-10	-8.357	11.567	-.723	.470	1.000
8-12	-11.024	7.466	-1.476	.140	1.000
8-7	11.357	7.466	1.521	.128	1.000
8-11	-11.857	7.466	-1.588	.112	1.000
8-5	20.657	5.332	3.874	.000	.007
8-1	22.357	11.567	1.933	.053	1.000
8-3	25.107	8.675	2.894	.004	.251
8-4	25.690	7.466	3.441	.001	.038
8-2	29.857	11.567	2.581	.010	.650
9-10	-.500	13.252	-.038	.970	1.000
9-12	-3.167	9.877	-.321	.749	1.000
9-7	3.500	9.877	.354	.723	1.000
9-11	-4.000	9.877	-.405	.685	1.000
9-5	12.800	8.381	1.527	.127	1.000
9-1	14.500	13.252	1.094	.274	1.000
9-3	17.250	10.820	1.594	.111	1.000
9-4	17.833	9.877	1.806	.071	1.000
9-2	22.000	13.252	1.660	.097	1.000
10-12	-2.667	12.494	-.213	.831	1.000
10-7	3.000	12.494	.240	.810	1.000
10-11	-3.500	12.494	-.280	.779	1.000
10-5	12.300	11.348	1.084	.278	1.000
10-1	14.000	15.302	.915	.360	1.000
10-3	16.750	13.252	1.264	.206	1.000
10-4	17.333	12.494	1.387	.165	1.000
10-2	21.500	15.302	1.405	.160	1.000
12-7	.333	8.834	.038	.970	1.000
12-11	.833	8.834	.094	.925	1.000
12-5	9.633	7.123	1.353	.176	1.000
12-1	11.333	12.494	.907	.364	1.000
12-3	14.083	9.877	1.426	.154	1.000
12-4	14.667	8.834	1.660	.097	1.000
12-2	18.833	12.494	1.507	.132	1.000
7-11	-.500	8.834	-.057	.955	1.000
7-5	9.300	7.123	1.306	.192	1.000
7-1	11.000	12.494	.880	.379	1.000
7-3	13.750	9.877	1.392	.164	1.000
7-4	14.333	8.834	1.622	.105	1.000
7-2	18.500	12.494	1.481	.139	1.000
11-5	8.800	7.123	1.236	.217	1.000
11-1	10.500	12.494	.840	.401	1.000
11-3	13.250	9.877	1.341	.180	1.000
11-4	13.833	8.834	1.566	.117	1.000
11-2	18.000	12.494	1.441	.150	1.000
5-1	1.700	11.348	.150	.881	1.000
5-3	4.450	8.381	.531	.595	1.000
5-4	5.033	7.123	.707	.480	1.000
5-2	9.200	11.348	.811	.418	1.000
1-3	-2.750	13.252	-.208	.836	1.000
1-4	-3.333	12.494	-.267	.790	1.000
1-2	-7.500	15.302	-.490	.624	1.000
3-4	-.583	9.877	-.059	.953	1.000
3-2	4.750	13.252	.358	.720	1.000
4-2	4.167	12.494	.334	.739	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

1.6 Chromium rank comparison of Field Season II Round Goby samples.

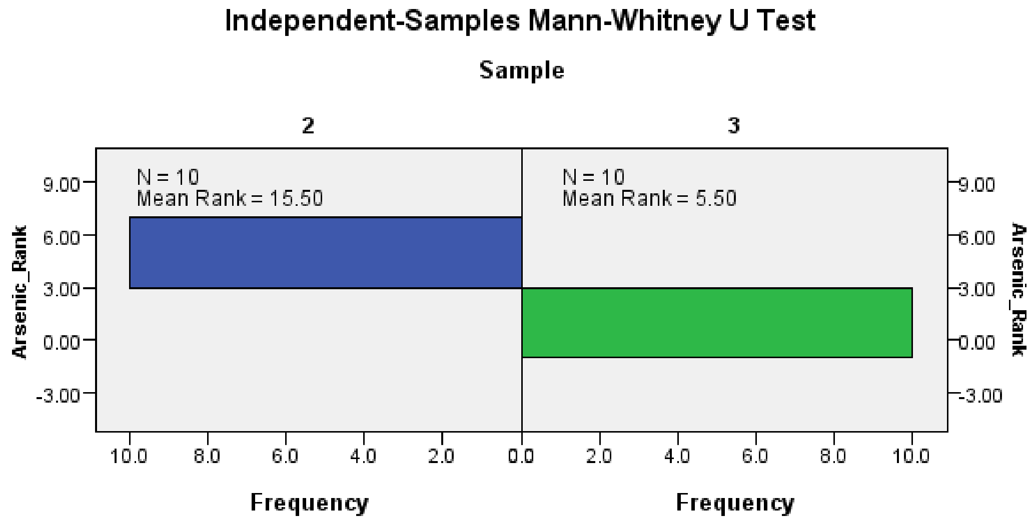
Independent-Samples Kruskal-Wallis Test



Total N	37
Test Statistic	17.547
Degrees of Freedom	11
Asymptotic Sig. (2-sided test)	.093

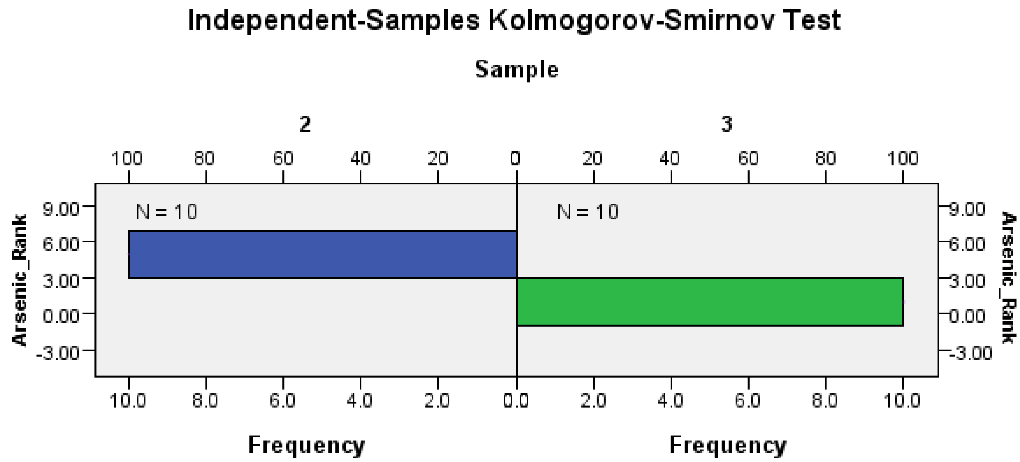
1. The test statistic is adjusted for ties.
2. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

1.7 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 2 and 3 (Mann-Whitney).



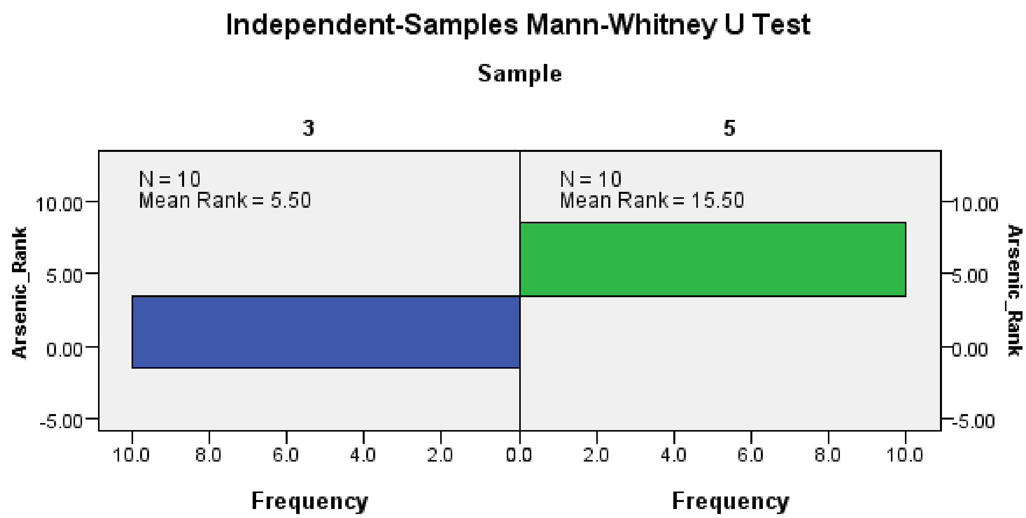
Total N	20
Mann-Whitney U	.000
Wilcoxon W	55.000
Test Statistic	.000
Standard Error	11.471
Standardized Test Statistic	-4.359
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.8 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 2 and 3 (Kolmogorov-Smirnov).



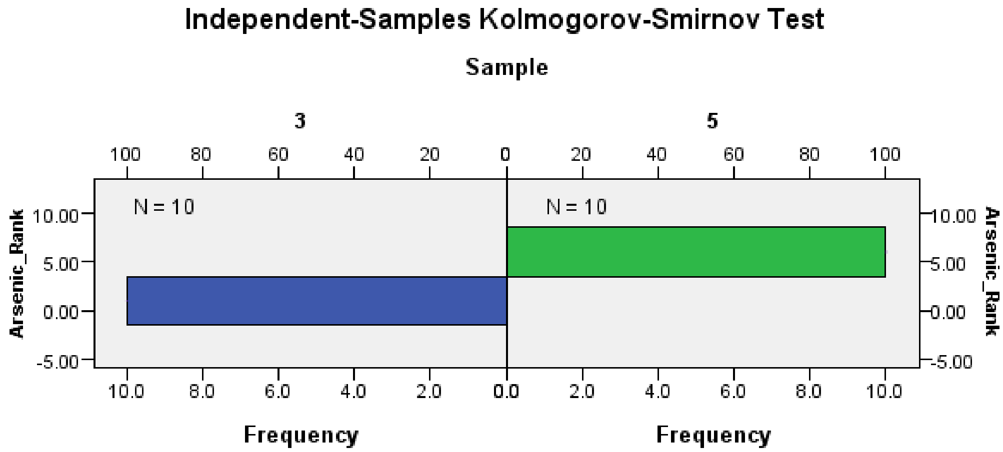
Total N		20
Most Extreme Differences	**Absolute**	1.000
	Positive	.000
	Negative	-1.000
Test Statistic		2.236
Asymptotic Sig. (2-sided test)		.000

1.9 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 3 and 5 (Mann-Whitney).



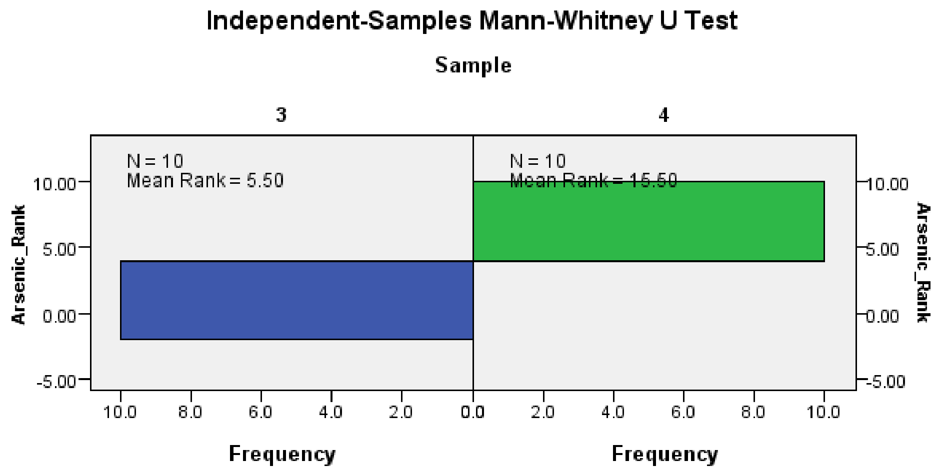
Total N	20
Mann-Whitney U	100.000
Wilcoxon W	155.000
Test Statistic	100.000
Standard Error	11.471
Standardized Test Statistic	4.359
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.10 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 3 and 5 (Kolmogorov-Smirnov).



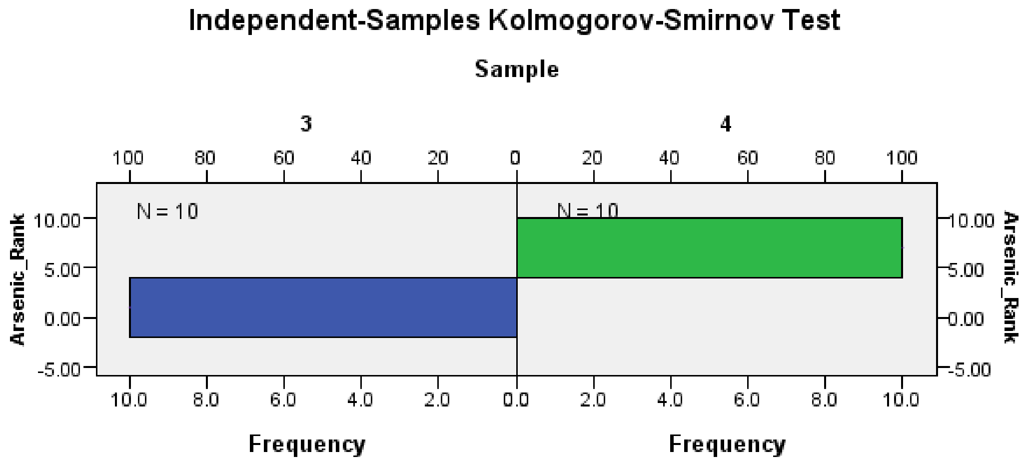
Total N		20
Most Extreme Differences	**Absolute**	1.000
	Positive	1.000
	Negative	.000
Test Statistic		2.236
Asymptotic Sig. (2-sided test)		.000

1.11 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 3 and 4 (Mann-Whitney)



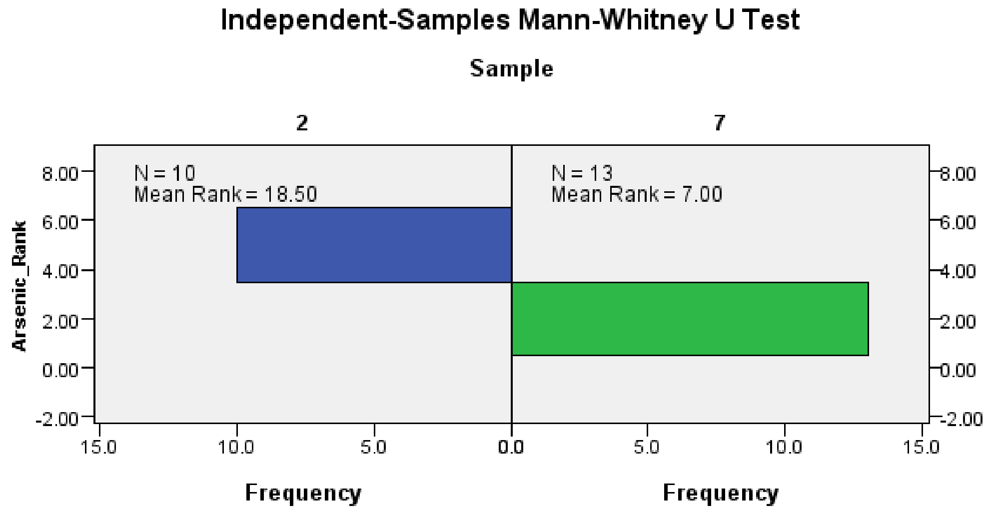
Total N	20
Mann-Whitney U	100.000
Wilcoxon W	155.000
Test Statistic	100.000
Standard Error	11.471
Standardized Test Statistic	4.359
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.12 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 3 and 4 (Kolmogorov-Smirnov).



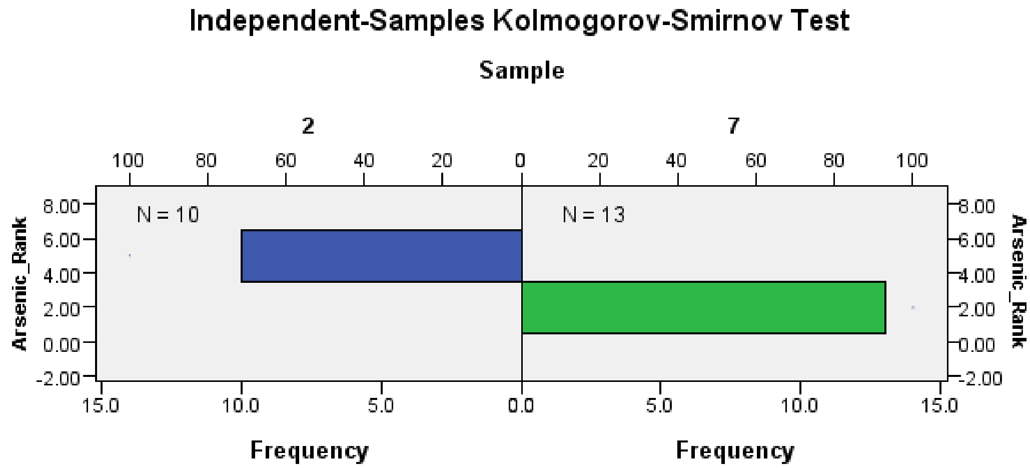
Total N		20
Most Extreme Differences	**Absolute**	1.000
	Positive	1.000
	Negative	.000
Test Statistic		2.236
Asymptotic Sig. (2-sided test)		.000

1.13 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 2 and 7 (Mann-Whitney).



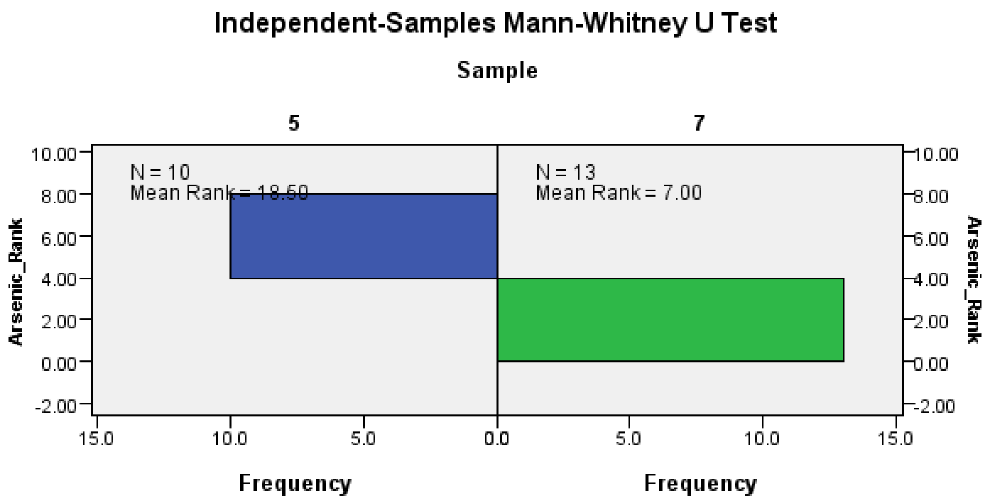
Total N	23
Mann-Whitney U	.000
Wilcoxon W	91.000
Test Statistic	.000
Standard Error	13.858
Standardized Test Statistic	-4.690
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.14 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 2 and 7 (Kolmogorov-Smirnov).



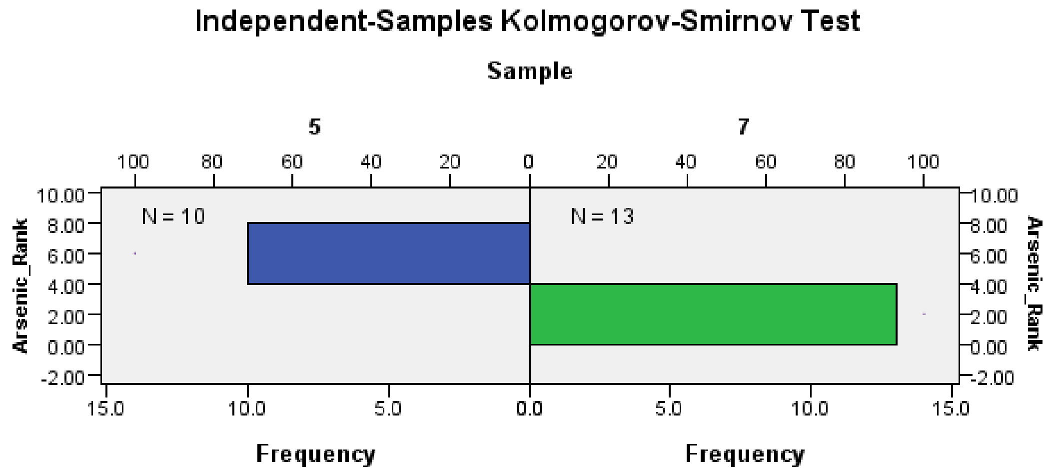
Total N		23
Most Extreme Differences	Absolute	1.000
	Positive	1.000
	Negative	.000
Test Statistic		2.377
Asymptotic Sig. (2-sided test)		.000

1.15 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 5 and 7 (Mann-Whitney).



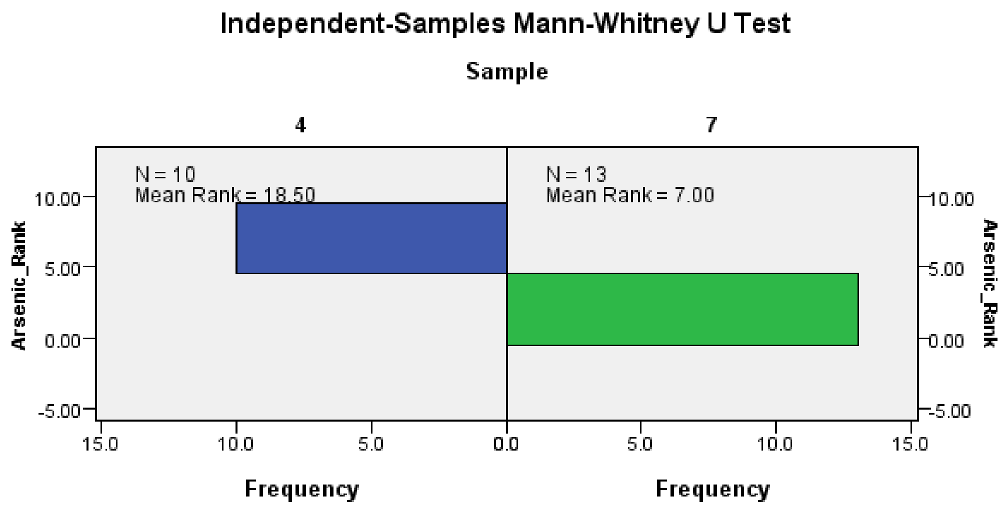
Total N	23
Mann-Whitney U	.000
Wilcoxon W	91.000
Test Statistic	.000
Standard Error	13.858
Standardized Test Statistic	-4.690
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.16 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 5 and 7 (Kolmogorov-Smirnov).



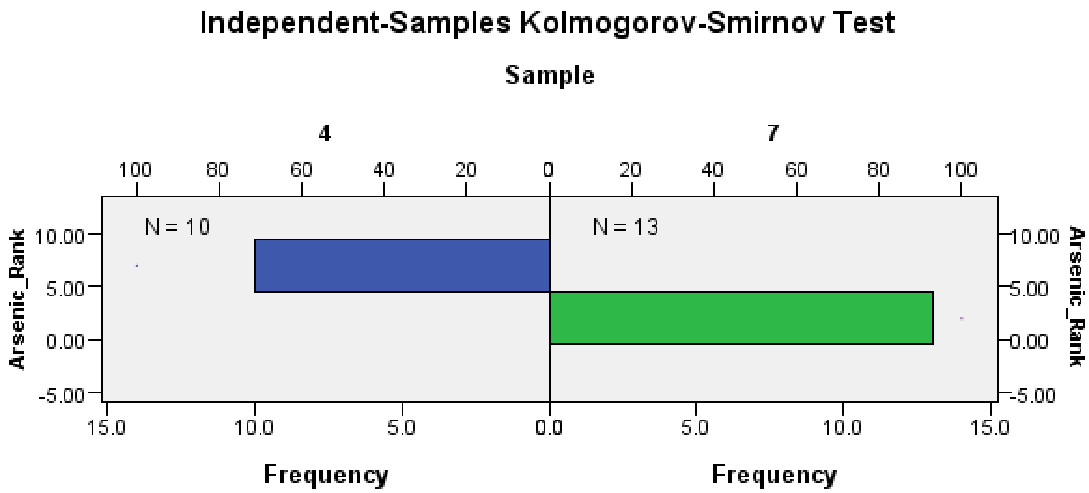
Total N		23
Most Extreme Differences	**Absolute**	1.000
	Positive	1.000
	Negative	.000
Test Statistic		2.377
Asymptotic Sig. (2-sided test)		.000

1.17 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 4 and 7 (Mann-Whitney).



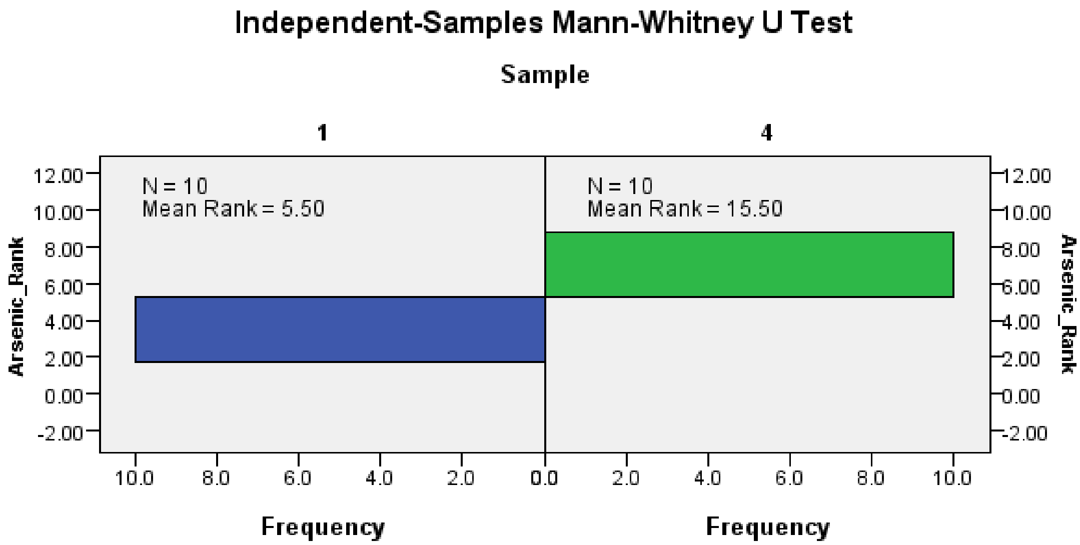
Total N	23
Mann-Whitney U	.000
Wilcoxon W	91.000
Test Statistic	.000
Standard Error	13.858
Standardized Test Statistic	-4.690
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.18 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 4 and 7 (Kolmogorov-Smirnov).



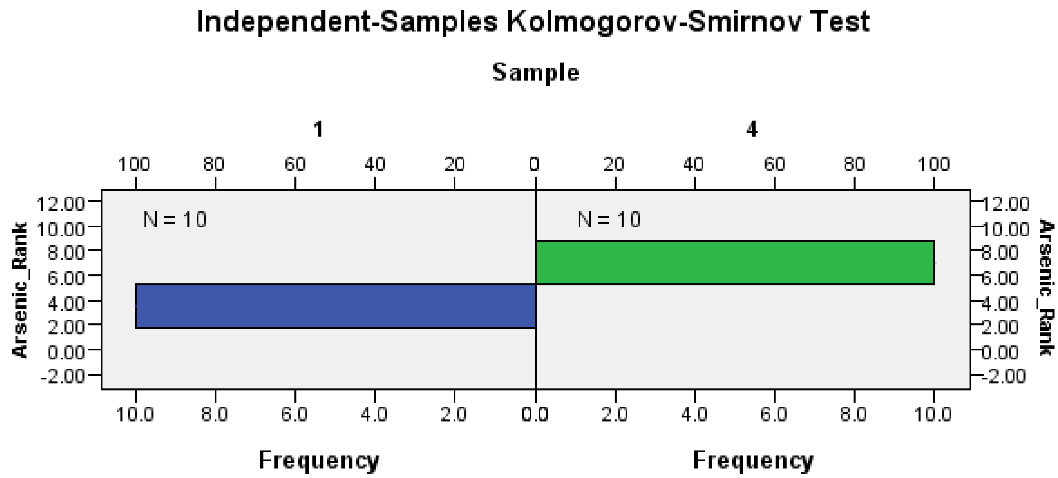
Total N		23
Most Extreme Differences	Absolute	1.000
	Positive	1.000
	Negative	.000
Test Statistic		2.377
Asymptotic Sig. (2-sided test)		.000

1.19 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 1 and 4 (Mann-Whitney).



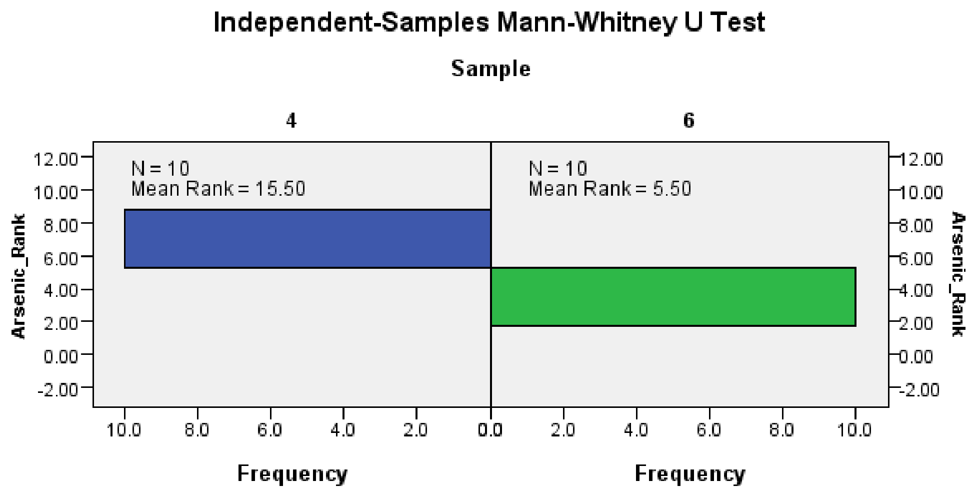
Total N	20
Mann-Whitney U	100.000
Wilcoxon W	155.000
Test Statistic	100.000
Standard Error	11.471
Standardized Test Statistic	4.359
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.20 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 1 and 4 (Kolmogorov-Smirnov).



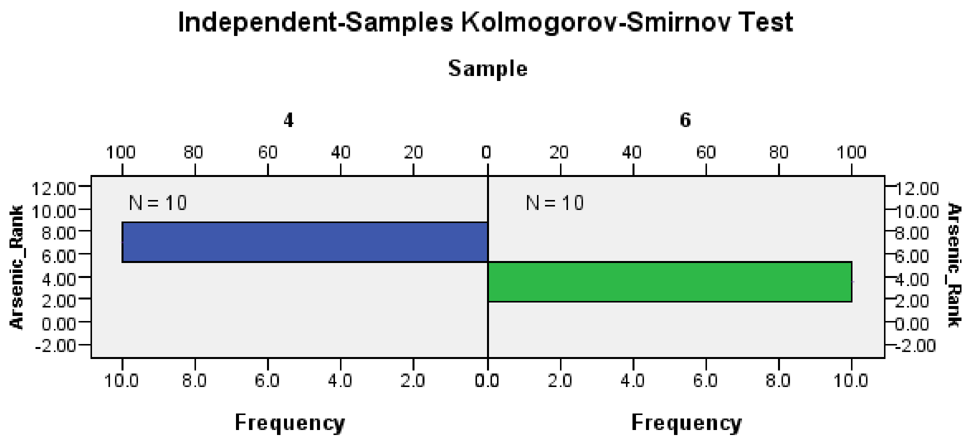
Total N		20
Most Extreme Differences	**Absolute**	1.000
	Positive	1.000
	Negative	.000
Test Statistic		2.236
Asymptotic Sig. (2-sided test)		.000

1.21 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 4 and 6 (Mann-Whitney).



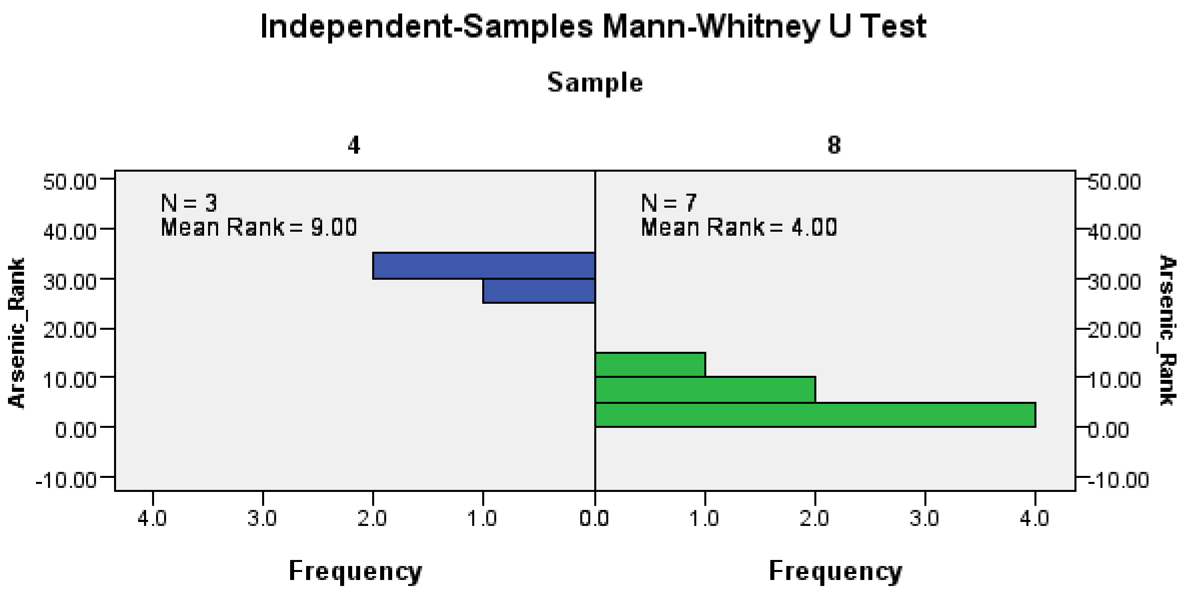
Total N	20
Mann-Whitney U	.000
Wilcoxon W	55.000
Test Statistic	.000
Standard Error	11.471
Standardized Test Statistic	-4.359
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

1.22 Pairwise Arsenic rank comparison of Field Season I Round Goby samples 4 and 6 (Kolmogorov-Smirnov).



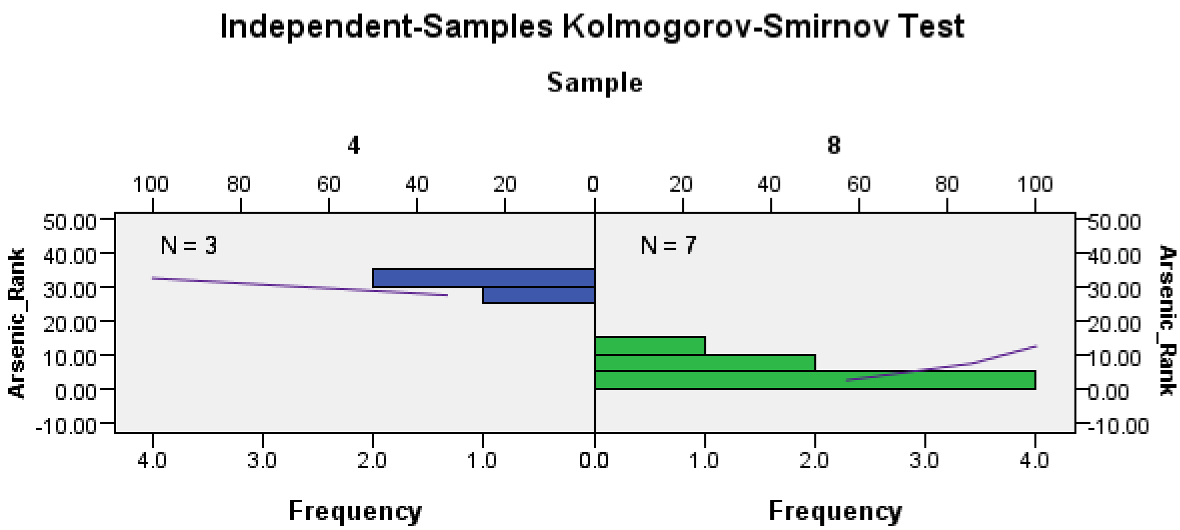
Total N		20
Most Extreme Differences	**Absolute**	1.000
	Positive	.000
	Negative	-1.000
Test Statistic		2.236
Asymptotic Sig. (2-sided test)		.000

1.23 Pairwise Arsenic rank comparison of Field Season II Round Goby samples 4 and 8 (Mann-Whitney).



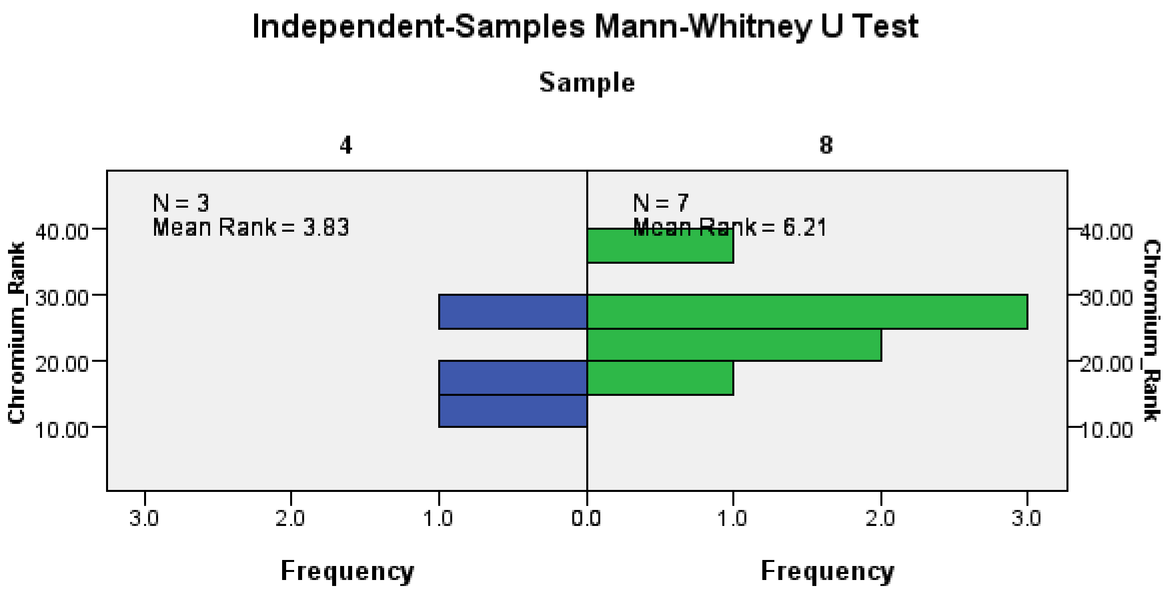
Total N	10
Mann-Whitney U	.000
Wilcoxon W	28.000
Test Statistic	.000
Standard Error	4.387
Standardized Test Statistic	-2.393
Asymptotic Sig. (2-sided test)	.017
Exact Sig. (2-sided test)	.017

1.24 Pairwise Arsenic rank comparison of Field Season II Round Goby samples 4 and 8 (Kolmogorov-Smirnov).



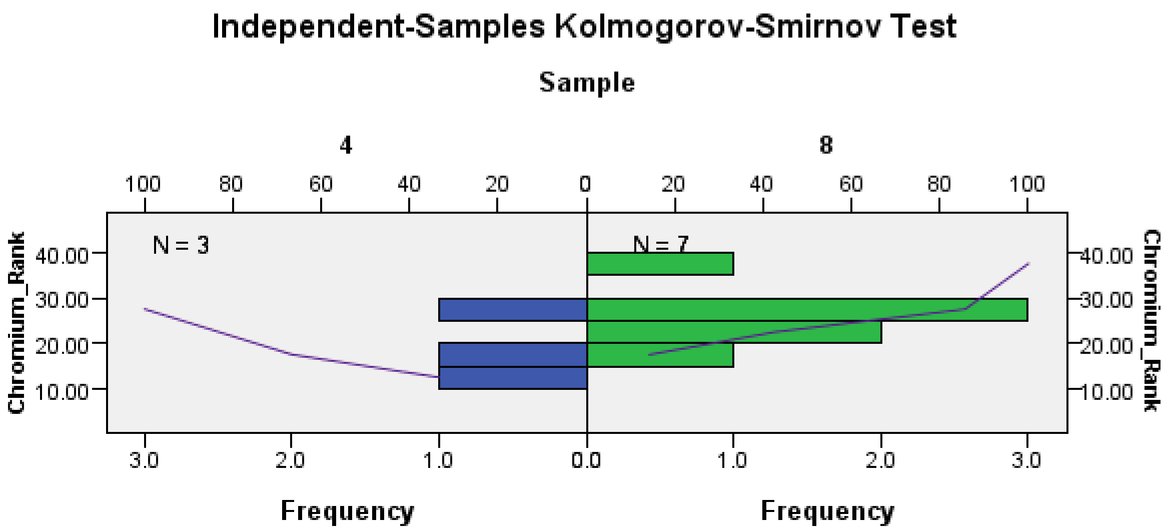
Total N		10
Most Extreme Differences	**Absolute**	1.000
	Positive	1.000
	Negative	.000
Test Statistic		1.449
Asymptotic Sig. (2-sided test)		.030

1.25 Pairwise Chromium rank comparison of Field Season II Round Goby samples 4 and 8 (Mann-Whitney).



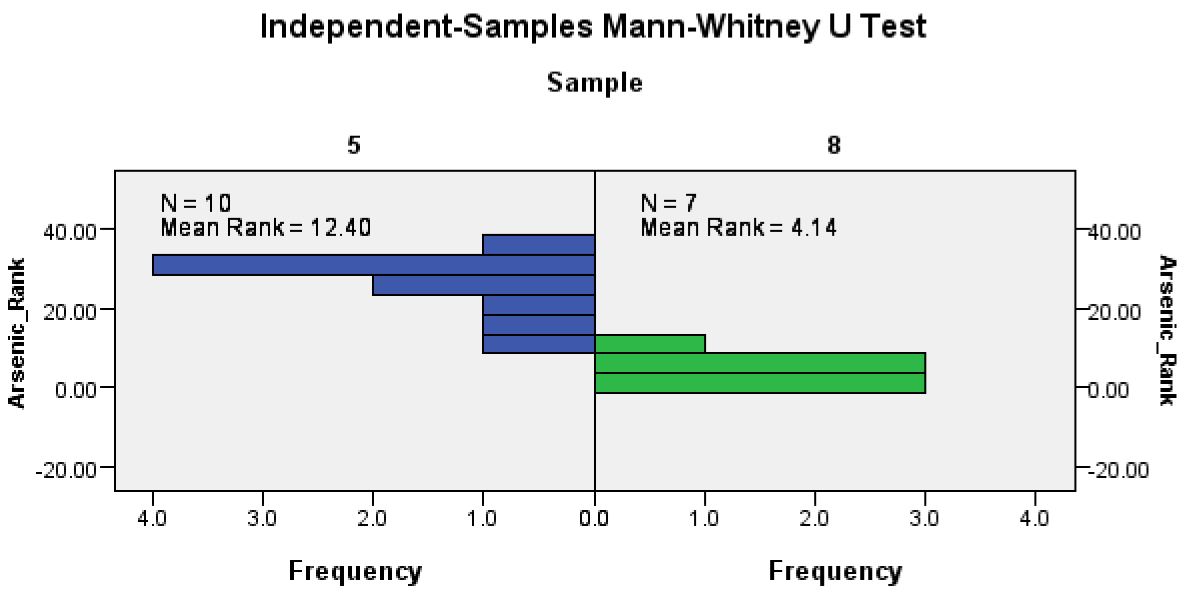
Total N	10
Mann-Whitney U	15.500
Wilcoxon W	43.500
Test Statistic	15.500
Standard Error	4.374
Standardized Test Statistic	1.143
Asymptotic Sig. (2-sided test)	.253
Exact Sig. (2-sided test)	.267

1.26 Pairwise Chromium rank comparison of Field Season II Round Goby samples 4 and 8 (Kolmogorov-Smirnov).



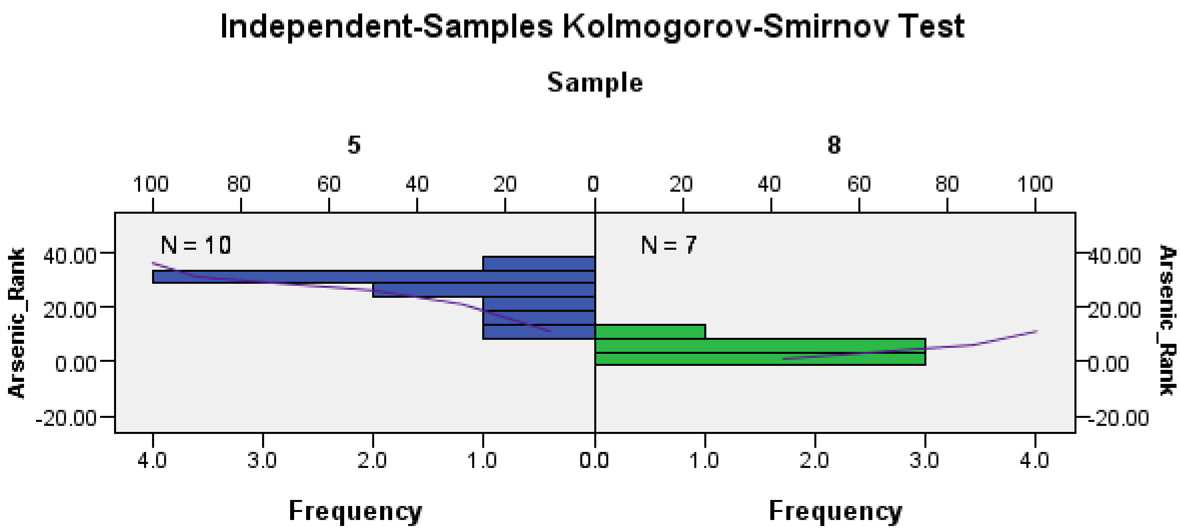
Total N		10
Most Extreme Differences	**Absolute**	.524
	Positive	.048
	Negative	-.524
Test Statistic		.759
Asymptotic Sig. (2-sided test)		.612

1.27 Pairwise Arsenic rank comparison of Field Season II Round Goby samples 5 and 8 (Mann-Whitney).



Total N	17
Mann-Whitney U	1.000
Wilcoxon W	29.000
Test Statistic	1.000
Standard Error	10.247
Standardized Test Statistic	-3.318
Asymptotic Sig. (2-sided test)	.001
Exact Sig. (2-sided test)	.000

1.28 Pairwise Arsenic rank comparison of Field Season II Round Goby samples 5 and 8 (Kolmogorov-Smirnov).



Total N		17
Most Extreme Differences	**Absolute**	.900
	Positive	.000
	Negative	-.900
Test Statistic		1.826
Asymptotic Sig. (2-sided test)		.003

1.29 Spearman correlation between Arsenic concentration and distance of Field Season I Round Goby samples from 4 COC sources.

			Arsenic
Spearman's rho	Arsenic	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	73
	Burgas_Bay	Correlation Coefficient	-.556**
		Sig. (2-tailed)	.000
		N	73
	Lukoil	Correlation Coefficient	-.556**
		Sig. (2-tailed)	.000
		N	73
	Burgas_Airport	Correlation Coefficient	-.556**
		Sig. (2-tailed)	.000
		N	73
	Varna_Bay	Correlation Coefficient	-.050
		Sig. (2-tailed)	.677
		N	73

**. Correlation is significant at the 0.01 level (2-tailed).

1.30 Spearman correlation between Arsenic concentration and distance of Field Season I Mediterranean Mussel samples from 4 COC sources.

			As_whole	As_meat	As_shells
Spearman's rho	As_whole	Correlation Coefficient	1.000	-.119	.187**
		Sig. (2-tailed)	.	.067	.004
		N	269	239	239
	As_meat	Correlation Coefficient	-.119	1.000	-.136*
		Sig. (2-tailed)	.067	.	.036
		N	239	239	239
	As_shells	Correlation Coefficient	.187**	-.136*	1.000
		Sig. (2-tailed)	.004	.036	.
		N	239	239	239
	Lukoil	Correlation Coefficient	-.689**	.180**	.024
		Sig. (2-tailed)	.000	.005	.707
		N	269	239	239
	Burgas_Bay	Correlation Coefficient	-.722**	.180**	.024
		Sig. (2-tailed)	.000	.005	.707
		N	269	239	239
	Burgas_Airport	Correlation Coefficient	-.689**	.240**	-.157*
		Sig. (2-tailed)	.000	.000	.015
		N	269	239	239
	Varna_Bay	Correlation Coefficient	.344**	.013	-.205**
		Sig. (2-tailed)	.000	.845	.001
		N	269	239	239

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

1.31 Spearman correlation between Arsenic and Chromium concentration and distance of Field Season II Round Goby samples from 4 COC sources.

			Arsenic	Chromium
Spearman's rho	Arsenic	Correlation Coefficient	1.000	-.012
		Sig. (2-tailed)	.	.944
		N	37	37
	Chromium	Correlation Coefficient	-.012	1.000
		Sig. (2-tailed)	.944	.
		N	37	37
	Burgas_Bay	Correlation Coefficient	-.344*	.093
		Sig. (2-tailed)	.037	.584
		N	37	37
	Lukoil	Correlation Coefficient	-.344*	.093
		Sig. (2-tailed)	.037	.584
		N	37	37
	Burgas_Airport	Correlation Coefficient	-.344*	.093
		Sig. (2-tailed)	.037	.584
		N	37	37
	Varna_Bay	Correlation Coefficient	-.402*	.121
		Sig. (2-tailed)	.014	.476
		N	37	37

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

1.32 Spearman correlation between Chromium concentration and distance of Field Season II Plankton samples from 4 COC sources.

			Chromium
Spearman's rho	Chromium	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	6
	Burgas_Bay	Correlation Coefficient	.636
		Sig. (2-tailed)	.175
		N	6
	Lukoil	Correlation Coefficient	.636
		Sig. (2-tailed)	.175
		N	6
	Burgas_Airport	Correlation Coefficient	.636
		Sig. (2-tailed)	.175
		N	6
	Varna_Bay	Correlation Coefficient	-.318
		Sig. (2-tailed)	.539
		N	6

**. Correlation is significant at the 0.01 level (2-tailed).