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Toxicity to residual chlorine: Comparison of sensitivity of native Arabian Gulf species and non-native species

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Abstract

Chlorine is extensively used as a powerful oxidizing agent in the countries surrounding the Arabian Gulf for water treatment and biofouling control. Its usage has been increasing significantly as demand for water grows considerably both in industry and for domestic use. This is due to the fact that it is a well-tested technology, has had a history of long-term worldwide industrial use and is of acceptable cost. While the Arabian Gulf waters support a range of coastal and pelagic marine habitats including mangrove forests, seagrass meadows and coral reefs, marine organisms in these waters are living close to their tolerance limits due to the extreme environmental stressors like temperature and salinity. Anthropogenic stressors such as chlorine may further exacerbate these natural stressors. In seawater, chlorine produces a mixture of hypochlorous acid and hypochlorite ion. These rapidly react with the bromide ion to form a mixture of hypobromous and hypobromite ion. Total residual oxidants formed by chlorination although are short lived and not persistent in seawater, they can be quite toxic. In the present study, toxicity data were obtained from 7 acute toxicity tests and 3 chronic toxicity tests using Arabian Gulf aquatic species from different trophic levels. The study also examined the effect of temperature and developmental stages on toxicity of chlorine. Furthermore, differences in the species sensitivity distribution between native and non-native species were compared. The main finding of the study showed that there was no significant difference between native and non-native species for chlorine toxicity. This would suggest that toxicity data from different geographic region can be used in deriving site- specific ecological risk assessment of chlorine.

Keywords: Chlorine; Arabian Gulf; acute toxicity; chronic toxicity; risk assessment; species sensitivity distribution;

Introduction

Ecological Characteristics of the Arabian Gulf

The Arabian Gulf is a relatively small and isolated body of water [Figure 1], and the activities occurring in one country are likely to impact the state of marine ecosystems throughout the region [57] [4]. Gulf waters support a range of coastal and open-water marine habitats, and high levels of salinity and surface temperature are the main natural stressors. In addition, the Arabian Gulf has high levels of anthropogenic activity [28] [15] Such activities include reclamation, industrial and domestic sewage effluent discharge, as well as hypersaline water discharge from desalination plants [49] [12] [63]. The region has also experienced significant oil pollution as a consequence of regional conflicts [4] [58]. The Gulf's vulnerability to environmental stressors led to a series of regional actions in the 1980s [3] [16] and despite recent improvements, environmental regulation and management have typically lagged behind the rapid pace of development across the region. Consequently, it is important for decision-makers to consider sustainability as a necessary facet of economic development to realize improved management that can support

Figure 1: Arabian Gulf and surrounding countries. [Esri. ArcGIS, 2018].

conservation and recovery of these important ecosystems. To this end, scientific data and information on both the ecosystems and activities (human and natural) taking place within the region are critically important. Additionally, establishing a Gulf-based trans-national regulatory and enforcement framework backed by these data and applied research could be very beneficial.

Discharges of total residual oxidant (TRO), referring to the sum of the free chlorine / bromine and combined chlorine /combined bromine, from the disinfection and anti-fouling processes are common due to the continued application of chlorine in many municipal water treatment and industrial facilities. TRO has the potential to be toxic to aquatic organisms and thus should be examined to understand potential impacts from discharge. Numerical water quality guidelines (WQG) for the protection of aquatic life for chlorine have been published by the U.S. EPA [30] and Environment Canada [18] and draft guidelines are available from the European Commission [29]. While these guidelines consider available toxicity data for TRO and in some cases the same data sets, each of them produced different numerical values. The U.S. EPA reported TRO guidelines of 11.5 and 7.55 µg/L for freshwater and saltwater species, respectively. The CCME reported a WQG for reactive chlorine species of 0.5 µg/L for freshwater and a WQG for chlorine-produced oxidants of 0.5 µg/L for marine water. The European Commission reported a TRO probable no-effect concentration (PNEC) of 0.06 µg/L and a free available chlorine (FAC) PNEC of 0.04 µg/L for both freshwater and saltwater organisms.

Guidelines established by the Qatari Ministry of Environment (MoE) (one of the Arabian Gulf states) for TRO and CBPs in cooling discharge waters specify that the maximum concentration of free TRO is 0.05 mg/L at the discharge point [51]. However, greater than 0.05 mg/L is permitted if a site-specific dispersion model is developed to demonstrate that the chlorine concentration does not exceed 0.01 mg/L at the edge of the mixing zone. This value is in line with the USEPA TRO guideline for marine species [1].

Chlorination Chemistry

When elemental chlorine is added to water (e.g., wastewater, freshwater), it hydrolyzes into hypochlorous acid (HOCl) and hydrochloric acid (HCl). Hypochlorous acid is a weak acid and dissociates into hypochlorite ion (OCl-). Similarly, when calcium or sodium hypochlorite is added to the water, the salts dissociate to form hypochlorous ions. The relative proportion of the three chlorine species, elemental chlorine, hypochlorous acid and hypochlorite ion, depends on pH and to a lesser extent on temperature [52]. The chemistry of chlorine exposure to seawater is complex [66] [21] [55]. In seawater, chlorine produces a mixture of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). These rapidly react with the bromide ion to form a mixture of hypobromous acid (HOBr) and hypobromite ion (OBr^-). The acute chlorinated oxidants are short lived and are not persistent in seawater. The environmental concern of chlorination is the production of numerous and more persistent, compounds formed by complex reactions between chlorine/bromine and the organic constituents of seawater, collectively described as chlorination by-products (CBPs) [2]. Many CBPs are persistent and may be toxic to marine organisms subjected to long-term exposures [8]. The terms chlorine refers to the sum of the free chlorine / bromine and combined chlorine /combined bromine. For our purposes, we will refer chlorine to combined oxidants as defined above.

Research Objectives

Objectives for this work were to [1] run acute and chronic toxicity tests of chlorine on marine species native to Qatar and the Arabian Gulf, [2] correlate sensitivity of these species to non-native marine organisms used as indicator species in WQG, [3] extrapolate research findings from this study to explore ways to inform and enhance environmental management activities in the Arabian Gulf coastal areas.

Materials And Methods

Toxicant preparation

Analytical grade calcium hypochlorite (with 75% available chlorine) was purchased from BBH Prolabo, UK. Artificial sea-salt was obtained from Seachem, US and water was prepared by reverse osmosis. All other reagents used in this study were of high purity. Chlorine test solution was prepared by diluting calcium hypochlorite in filtered seawater. Aliquots of the treatment solution were sampled periodically to monitor the residual chlorine concentration. The concentration of chlorine was determined by N, N-diethyl-p-phenylenediamine (DPD) method [40], using a Hach DR2800 (Hach, USA) [36]. Fluorescein diacetate was used for algal cell viability [26] and was purchased from Sigma (St. Louis,Mo, USA). Bloodworms and brine shrimps (Artemia sp.) used for feeding killifish were purchased from Aqua Art, Doha, Qatar.

Test organism's cultivation and preparation

In our study seven local aquatic species from the Arabian Gulf were chosen for the acute and chronic toxicity tests. See Table 1 for testing conditions.

Microalgae

Two microalgae species, Synechococcus Sp. and Chaetoceros Sp. were isolated from the coastal waters of north west of Qatar and their cultures were established and maintained in the laboratory using F2 medium [35] and filtered seawater adjusted to 40 ppt. Culture flasks containing100 ml of autoclaved culture medium were inoculated with about a 1 ml of stock culture and placed at 28 °C under simulated natural light conditions (12 h light: 12 h dark photocycle). The viability of algal cells after treatment was analyzed by flow cytometry with FDA staining [32]. Esterase activity was recorded by detection of emitted fluorescence signals with the application of a blue laser light. FDA, which is taken up by live cells and converted to its fluorescent derivative Fluorescein by cellular esterase, was applied after

dilution in acetone (5 mg per mL). Staining time was 5 min at room temperature in darkness. The esterase activity of the cells was evaluated using a BD Accuri C6 flow cytometry system (BD Biosciences, USA) with a 488 nm excitation argon laser equipped with fluorescent emission filters (FL1-525 nm). The maximum FDA fluorescence emission was detected at 525 nm.

Copepod

Toxicity tests with the crustaceans Euterpina acutifrons and Microstella Sp., were performed on nauplia and copepodite stages. Methods are described in detail in [50]. Mature females were isolated from coastal waters of North West of Qatar and pure cultures were established in the laboratory. For the toxicity tests, cultures were synchronized to procure same age group individuals. Test organisms were fed with a mixture of algae for an hour prior to the test. Five to seven copepodites were picked with a flame-rounded pipette guided with a stereo microscope and placed in test vials containing the test solutions.

Pearl Oyster and Sea urchin

General methods for invertebrates followed [10] [27]. Adult Pearl oysters (Pinctada radiata) and sea urchins (Echinometra mathaei) were collected from North West Qatar and acclimatized to laboratory. Oysters and Urchins were fed with a mixture of laboratory grown microalgae and commercially obtained seaweeds, respectively. Spawning was induced by dissection for the pearl oyster and by injecting potassium chloride for the sea urchins. Gametes collected from the spawners were suspended in 40 ppt filtered sea water and were checked under the microscope for maturity. Male and female gamete suspension were mixed in a beaker and stirred gently to allow fertilization for oysters. For sea urchins the eggs were collected and counted under the microscope and a suspension of 2000 eggs per ml was made. 1 ml of the egg suspension was transferred to 3 ml well plates. This was followed by an addition of sperm suspension. Simultaneously the toxicant was added to the well plates to check the impact of the toxicant on fertilization. Four replicates per treatment were used. After 8 h incubation at 23 °C in an isothermal chamber, contents in the wells were preserved using formalin for later observations under an inverted microscope. Successful fertilization in sea urchin is indicated by the formation of a fertilization membrane around the egg. The recorded endpoint for pearl oyster was the percentage of normal D-larvae in samples of a minimum of 100 individuals per well. A larva was considered developing normally when the shell was D-shaped (straight hinge) and the mantle did not protrude out of the shell. Fertilization success and normal larvae development in the control, for both species, were always more than 70%.

Killifish species

Killifish embryo, juveniles and adult bioassays were performed following OECD guidelines No 236 [47], 215 [46] and 203 [45]. Fifty fertilized eggs (blastula stage), 10 individual juveniles and 10 individual adults were carefully distributed to the exposure chambers. Test chambers were incubated in a biological incubator, see [Table 1]. The observations were made using a thick slide with a concave chamber, which was filled with clean seawater. Each embryo was carefully placed in the chamber and observed under a binocular dissection microscope, embryos were considered dead when no heartbeat could be observed. Survival after 96 h was the recorded response in juvenile and adult acute tests and for embryo hatchability and survival after 10 days. Mortality in the controls was always below 10%.

Table 1. Acute toxicity test species used, endpoints recorded and acceptability criteria.

Taxon	Species	Life stage	Endpoint	Acceptability criteria	References
Phytoplankton	Synechococus Sp.	n.a	Growth inhibition	Control growth: X 16 per 3 days	ASTM E1218-04 (2012)
	Chaetoceros Sp.	n.a	Growth inhibition	Control growth: X 16 per 3 days	OECD Guideline 201(2011)
Crustaceans	Euterpina acutifrons	Nauplii	Mortality	Mortality in controls < 10%	Rosea A, et al., (2006)
		Copepodite	Mortality	Mortality in controls < 10%	
	Microstella Sp.	Copepodite	Mortality	Mortality in controls < 10%	
Bivalves	Pinctada radiata	Embryo	Embryo development	Normal larvae in controls > 75%	ASTM E 724-89, (1989)
Echinoderm	Echinometra mathaei	Embryo	Fertilization	Successful fertilization	EPA, 2009
			> 75% in controls		
Fish	Aphanius dispar	Embryo	Hatchability	Hatchability in controls > 80%	OECD 235 (2015)
		Juvenile	Mortality	Mortality in controls < 10%	OECD 215, 2013
		Adult	Mortality	Mortality in controls < 10%	OECD 203, 1992

*n.a: not applicable.

General test conditions

All tests were static renewal, unless otherwise stated, whereby test solutions were spiked with chlorine at 24 h intervals. Measured chemical parameters of dilution water were as follows: pH 7.8 ± 0.4, dissolved oxygen (DO) 6.8 ± 0.5 mg/L, total organic carbon 0.02 mg/L, and salinity 40 ± 1 ppt. The toxicity tests were conducted in triplicates, at assigned concentrations, and blank control. All tests were undertaken at a light: dark photoperiod of 12: 12 h. Test organisms were not fed during the acute test periods. Test chambers were kept inside an incubator adjusted to maintain the water temperature at 23 ± 2 °C, unless otherwise noted. Temperature, DO, and pH were measured in test chambers daily in the acute toxicity tests and at least once a week in the chronic toxicity tests. Biological observations were performed at least once daily for all studies.

In the acute toxicity test, 72-h-EC50 (effective concentration in 50% of the test organisms over 72 h) for microalgae, 48-h-LC50 for copepod, pearl oyster and sea urchins and 96-h-LC50 (lethal concentration in 50% of the test organisms over 96 h) for fish larvae and adults and 10-d- LC50 for fish embryos were used as main endpoints. In the chronic toxicity test, 21-d-EC10 (effective concentration in 10% of the test organisms over 21 days) for copepod and 120-d-EC10 (effective concentration in 10% of the test organisms over 120 days) for oysters and 28-d-EC10 for fish.

Acute and chronic toxicity tests

Prior to the toxicity tests, all test organisms were acclimated to general test conditions for a minimum of 7 days. Acute toxicity tests set up of chlorine to seven local aquatic organisms are shown in [Table 2, 3].

Table-2 Acute toxicity tests parameters for experimental species.

Species	Wet weight	Stage	Time	Chlorine Exposure
	(g)			concentration (mg/L)
Synechococcus sp.	n.a	n.a	72h	0.000, 0.030, 0.060, 0.125, 0.250
Chaetoceros sp.	n.a	n.a	72h	0.000, 0.030, 0.060, 0.125, 0.250
Euterpina acutifrons	n.a	Nauplii/copepodite	48h	0.000, 0.030, 0.060, 0.125, 0.250
Nauplii/copepodite Microstella sp.	n.a	larvae	48h	0.000, 0.030, 0.060, 0.125, 0.250
copepodite	n.a			
Pinctada radiata Embryo	n.a	4hpf	48h	0.000, 0.030, 0.060, 0.125, 0.250
Echinometra mathaei	n.a	4hpf	48h	0.000, 0.030, 0.060, 0.125, 0.250
Embryo	n.a			
Aphanius dispar Embryo	n.a	8hpf	10d	0.00, 0.125, 0.25, 0.50, 1.00, 2.000
Juvenile	0.3 ±0.2	1-3m	96h	0.00, 0.125, 0.25, 0.50, 1.00, 2.000
Adult	1.3 ± 0.1	6-8m	96h	0.00, 0.25, 0.50, 1.25, 2.5, 5.000

*hpf: hours post fertilization
*m: month.
*n.a: not applicable

Table-3 Chronic toxicity tests parameters for experimental species

Species	Wet weight (g)	Stage	Time	Chlorine Exposure concentration (mg/L)
Copoped Nauplii	n.a	Nauplii	21d	0.000, 0.025, 0.100
Pinctada radiata Adult	3.64 ±0.3	3-5y	120d	0.000, 0.025, 0.100
Aphanius dispar Juvenile adult	0.3 ±0.2	1-3m	28d	0.000, 0.025, 0.100
	1.3 ± 0.1	6-8m	28d	0.000, 0.025, 0.100

*m: month

Killifish chronic toxicity test

The fish used in the chronic toxicity test were the same as in the acute toxicity test, i.e., chronic study was a continuation of the acute study. Twenty eight days chronic toxicity tests were conducted. The fish were fed with bloodworms at a rate of 0.1% body weight twice daily. Endpoints observed included survival, and change in total length and wet body weight. Nominal exposure concentrations in the test were 0.00 (control), 0.025, and 0.1, mg/L chlorine, delivered through a pump to dose chlorine at regular intervals so as to maintain the test concentrations and to account for loss of chlorine due to volatilization.

Copepod chronic toxicity test

Twenty-one-day life table tests using nauplii of Euterpina acutifrons (<24 h age) were conducted in 1000 mL beakers filled with 300 mL test solutions. The copepods were fed once every day with a mixture of green algae and diatom with a total concentration of 1.0×10^5 cells/mL in the test solution. The survival, moulting, growth and reproduction were recorded every alternate day. The endpoints include the time to first brood, number of broods and number of exuviations. Nominal concentrations in the definitive test were 0.00 (control), 0.025, and 0.1mg/L chlorine, delivered through a pump to dose chlorine at regular intervals. The toxicity tests were carried out in two replicates with each starting with 30 first stage nauplii.

Oyster chronic toxicity test

The oysters used in the chronic toxicity test were the same as in the acute toxicity test. 70 healthy oysters were collected and distributed into tanks that were fully aerated and filtered through a biological filtration system. One hundred and twenty-day chronic toxicity tests were conducted. The oysters were fed with a mixture of algae daily. Endpoints observed included survival, growth (length in cm) and body weight (g) gain. Nominal exposure concentrations in the definitive test were 0.00, 0.025, and 0.1, mg/L chlorine, delivered through a pump to dose chlorine at regular intervals.

Impact of Temperature

In this experiment, Synechococcus sp., Euterpina acutifrons, and embryos of Aphanius dispar were subjected to different temperatures (16, 23, 27, 32 and 38 °C) to study the impact of temperature on toxicity of residual chlorine. Conditions used were similar to that of acute and chronic toxicity studies.

Comparative Toxicity

More than 125 references were reviewed in order to compare with the results of the present study. The datasets considered for comparison were those which had test conditions, recorded chlorine measurements, and used either semi-static renewal or a flow-through system of dosing chlorine. In addition to the test method, other criteria for selecting existing data sets include measuring standard parameters of dissolved oxygen, temperature, pH and salinity and achieving acceptable criteria in the control.

Statistical analysis and SSD generation

Probit methodology was employed to calculate the 48-h-EC50, 96-h-LC50, 10-d-LC50, EC10 for chronic endpoints values and corresponding 95% confidence intervals. JMP- SAS 13 (SAS Campus Drive, Cary, NY) and GraphPad Prism 7 (San Diego, CA) were used for data analysis and display.

Results

Acute toxicity tests of seven native Arabian Gulf species

In the present study, toxicity data were obtained from 7 acute toxicity tests and 3 chronic toxicity tests using seven Arabian Gulf native species from different taxonomic levels as shown in

Table 4. Acute toxicity of residual chlorine to seven native species of Arabian Gulf.

Species	Exposure Time	Dosing Method	R2	P	EC50/LC50
Synechococcus Sp.	72h	Semi-static	0.999	<0.01	0.101(0.101-0.101)
Chaetoceros Sp.	72h	Semi-static	0.981	<0.01	0.110(0.092-0.123)
Euterpina acutifrons	48h	Semi-static	0.996	<0.01	0.217(0.195-0.239)
· Nauplii	48h	Semi-static	0.982	<0.01	0.076(0.072-0.080)
· Copepodite Microstella Sp.	48h	Semi-static	0.956	<0.05	1.004(0.918-1.090)
Pinctada radiata Echinometra mathaei	48h	Semi-static	0.981	<0.01	0.073(0.069-0.078)
	24h	Semi-static	0.84	<0.01	0.118(0.089-0.148)
Aphanius dispar	10 d	Semi-static	0.997	<0.02	0.454(0.421-0.489)
· **Embryo**	96h	Semi-static	0.987	<0.02	0.215(0.178-0.251)
· **Juvenile**	96h	Semi-static	0.998	<0.01	0.743(0.738-0.747)
· **Adult**	10d	Flow-thro	0.991	<0.01	0.074(0.061-0.086)
Aphanius dispar	96h	Flow-thro	0.999	<0.01	0.059(0.059-0.060)
· **Embryo**					
· **Juvenile**					

[Table 4] Mortality in controls were within published standard guidelines for each species. Results of acute toxicity tests showed that copepodites of Euterpina acutifrons with a 48-h-LC50 of 0.076 mg/L and embryos of Pinctada radiata with a 48-h-LC50 of 0.073 mg/L were the most sensitive species to chlorine followed by Synechococcus Sp., Chaetoceros Sp., Echinometra mathaei, Euterpina acutifrons nauplii, Aphanius dispar juveniles and Aphanius dispar adults. The least sensitive species were found to be the copepodites of Microstella Sp. with a 48-h-LC50 of 1.004 mg/L and Aphanius dispar embryos with 10-d-LC50 of 1.017 mg/L.

Effect of dosing methods and developmental stages of species

In this experiment, semi-static versus flow-through dosing methods were examined on Aphanius dispar embryos, larvae and adults. The difference in toxicity of the flow-through was at least 3 to 6 times more in magnitude than the semi-static [Table 4] for embryos the LC50 went up from 0.074 to 0.454 mg/L and for juveniles 0.059 to 0.215 mg/L. In addition, LC50 differed depending on the developmental stages of the species that was exposed to chlorine. In the case of Euterpina acutifrons, the nauplii were less sensitive than the copepodite, 0.217 and 0.076 respectively. The fish juveniles were found to be more sensitive than adults, 0.217 and 0.743 respectively. In most cases embryos were less sensitive than larvae or adult stages and this is mainly attributed the protection provided by the outer membrane, the chorion.

Effect of temperature on toxicity tests

The impact of temperature on chlorine toxicity was tested on three native species [Figure 2] and was later compared to data obtained from non-native species [Figure 3] At either low (16°C) or very high temperatures (>38°C), chlorine toxicity was not tested due to impact of these temperatures on the well-being of test species. However, on temperatures used 23, 27, 32 and 38°C there was no significant difference observed in chlorine toxicity with the concentrations used. Nevertheless, depending on species, at intermediate temperatures (~ 23-27°C) chlorine showed an elevated toxic effect on all test species.

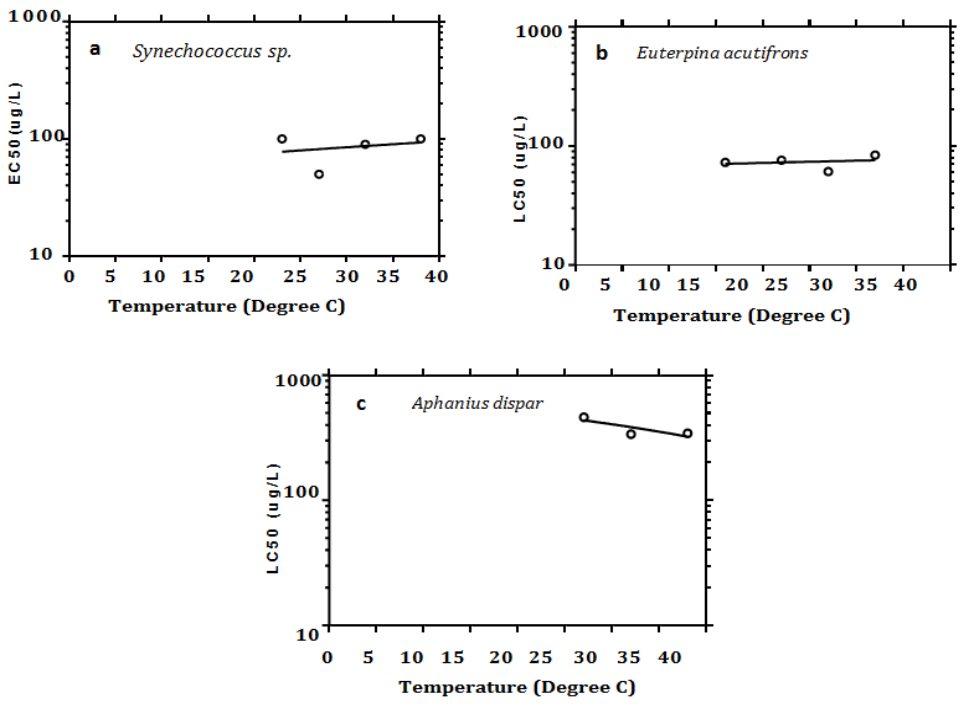

Figure 2: Effect of temperature on chlorine toxicity of three native species (a) Synechococcus sp. (b) Euterpina acutifrons, and (c) Aphanius dispar.

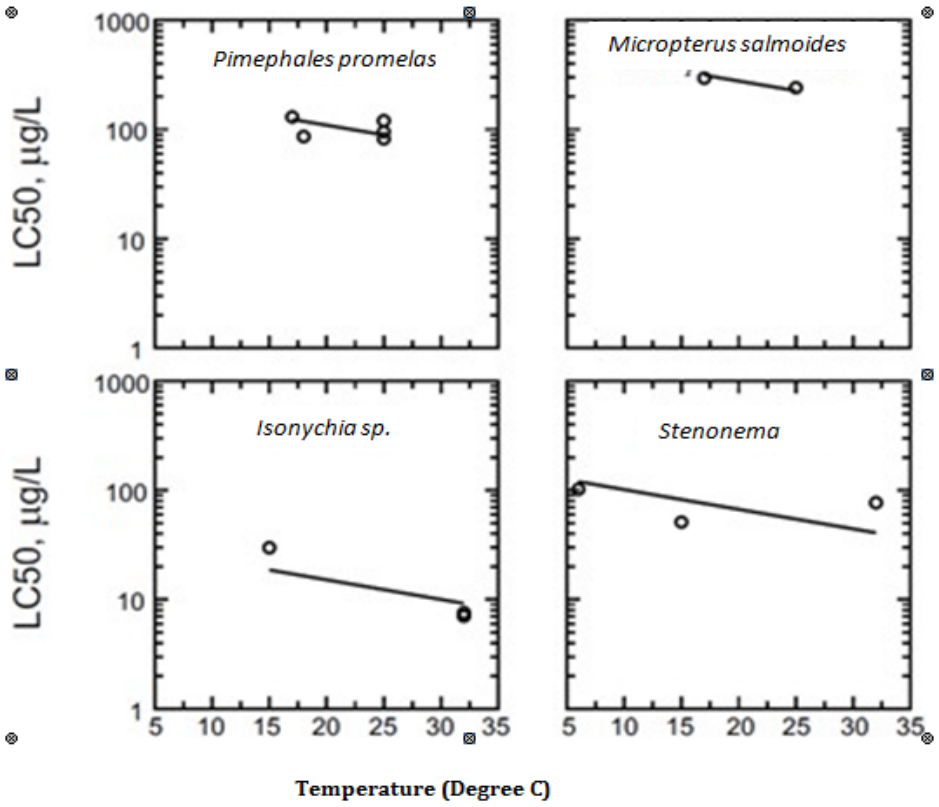

Figure 3: Effect of temperature chlorine toxicity on four non-native species (a) Pimephales promelas; (b) Microplerus salmoides; (c) Isonychia sp. and (d) Stenonema Ithaca.

Chronic toxicity tests

Chronic toxicity values of chlorine to 3 aquatic species are shown in [Table 5] Results obtained in this study and those of previous studies using non-native species (Table 6) were compared. In the present study we found that the lowest observed effect concentration (LOEC) for 21d for planktonic crustacean *Euterpina acutifrons* to be 0.026 mg/L. The 28-d-EC10 for growth of fish *Aphanius dispar* was found to be 0.020 mg/L. In general, the sensitivities of the native species tested in this study were similar to those reported in previous studies of non-native species.

Comparison of SSD between native and non-native taxa

For the present study, the species sensitivity distribution (SSD) based on both native and non-native species was generated. Since there has been limited information on the toxicity of chlorine to native and non-native species, comparison could only be conducted on the SSDs constructed from acute toxicity data of native and non-native [Table 4, 7] species. Based on the comparison in this study, the SSD curves [Figure. 4] indicated that the native Arabian Gulf organisms with acute exposures to chlorine were as sensitive as the non-native species that have been used in deriving WQC elsewhere. The HC5s were 0.054, 0.039, and 0.045 mg/L, respectively.

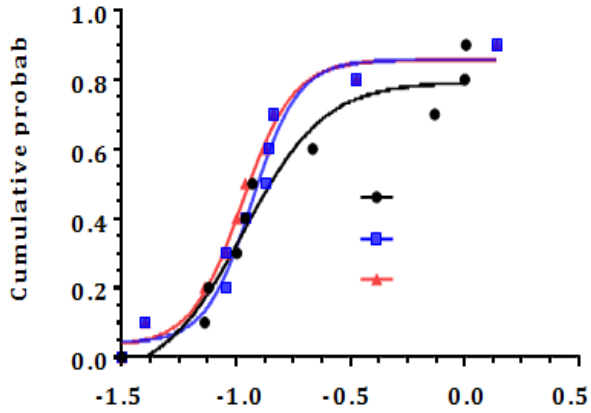

Figure 4: Species sensitivity distribution (SSD) of native, non-native and all species toxicity data for chlorine..

Table 5- Chronic toxicity of residual chlorine to three native species of the Arabian Gulf				
Species	**Endpoint**	**R2**	**P**	**LOEC/LC10**
Euterpina acutifrons	21d	0.845	<0.01	0.02(0.0-0.050)
Pinctada radiata	longevity (120d)	0.791	<0.02	0.01(0.0-0.069)
Aphanius dispar	longevity (28d)	0.999	<0.01	0.02 (0.01-0.03)
Comparison of SSD between native and non-native taxa				

Table 6. Acute toxicity data of chlorine to non-native marine organisms.				
Rank	**Saltwater Species**	**EC50/LC50 (mg/L)**	**Time**	**References**
4	Leiostomus xanthurus	0.09	96h flow-thro	Bellanca, M. A., Bailey, D. S. (1977)
3	Oncorhynchus mykiss	0.14	96h static	Basch, R.E. et al. (1971)
3	Pimephales promelas	4.8-8	96h static	Curtis, M.W. (1981)
3	Morone saxatilis	0.04	96h flow-thro	Middaugh, D.P., et.al. (1977)
3	Pandalus goniurus	0.063-0119	96h	Thatcher, T.O. (1978b)
3	Crangon sp.	0.118-0.151	96h	Thatcher, T.O. (1978b)
3	Anonyx sp.	0.118-0.173	96h	Thatcher, T.O. (1978c)
4	Neomysis sp.	0.15-0.175	96h	Thatcher, T.O. (1978b)
3	Pontogeneia sp.	0.583-0.864	96h	Thatcher, T.O. (1978b)
3	Shore crab	1.24 — 1.53	96h	Thatcher, T.O. (1978b)
2	Dunaliella tertiolecta	0.11	24h	Gentile J.H. et al. (1976)
2	Thalassiosira rotula	0.2	24h	Gentile J.H. et al. (1976)
2	Thalassiosira guilardii	0.075	24h	Gentile J.H. et al. (1976)

Table 7. Acute toxicity data of chlorine to non-native marine organisms.			
Cumulative Probability	**Native GMAVs Log (mg/L)**	**Non-native GMAVs Log (mg/L)**	**All Taxa**
0.1	-1.137	-1.397	-1.397
0.2	-1.119	-1.041	-1.119
0.3	-0.996	-1.041	-1.041
0.4	-0.959	-0.957	-0.996
0.5	-0.928	-0.869	-0.959
0.6	-0.664	-0.854	-0.869
0.7	-0.129	-0.836	-0.836
0.8	0.0017	-0.472	-0.472
0.9	0.0073	0.142	0.142

Discussion

The purpose of the present study was to conduct acute and chronic chlorine toxicity tests on native species of the Arabian Gulf region and to compare sensitivity to non-native species used WQC. Based on the present study's results, the EC50 and LC50 observed for the native species were closely similar to those reported for non-native species with similar test conditions. All the studies (including this study) on chlorine acute toxicity shows that toxicity of chlorine depends on exposure methods, environmental conditions and stage of development of the test species. The lowest LC50 reported by Cairns 1978 was 0.076 mg/L (chlorine) at 25°C. Similarly, Thatcher [61] has investigated acute toxicity to seven invertebrates of which two shrimps (*Pandalus goniurus and Crangon niricauda*) were found to be most sensitive to chlorine. The 96h LC50 of Pandalus goniurus was 0.09 mg/L.

Furthermore, [37] reported a 96-hour LC50 value of 0.06 mg/L to rainbow trout (*Salma gaidneri*), which contrasts with LC50 values of 0.15 mg/L for trout (*Salvalinus fontinalis*) and 0.032 mg/L for salmon (*Oncorhynchus kisutch*), reported by [61]. The effects of chlorine to 11 phytoplanktonic algae species were tested by [33] and report the chlorine concentrations causing 50%

growth reduction in a series of 24 h static tests; the LC50values ranged from 75 to 330 µg/l. The acute toxicity of chlorine in fish has been the most studied, with the most referenced including (61) [37] [65]. The lowest 96h LC50 value was determined by [61] for juvenile *Oncorhynchus kisutch* (Coho salmon, LC50= 0.032 mg/L) and juvenile *Oncorhynchus gorbuscha* (pink salmon, LC50 = 0.023–0.052 mg/L). Exposing fish to chlorine adversely impacts the structure of the gill with an increase in mucus production around the gill and destruction of the respiratory wall epithelium [11] [19] [65]. Exposure to sub-lethal levels of chlorine to bluegill and rainbow trout caused histological damage to the gills ultimately resulting in death by asphyxia [11].

The results of [59] demonstrated the effect of exposure methods i.e. flow through versus static systems. The LC50 for the crustacean, *Ceriodaphnia dubia* in a 24 hour flow through test at 25°C was equal or greater than 0.005 mg/L. The LC50 determined under static conditions was 0.048 mg/L which is in the range of the values reported in 17 at the same temperature. Essentially a 10-fold increase in LC50 was observed, driven mostly by the difference in exposure condition. However, semi-static exposure was the method of choice in this study as this is more representative of how industries operating within the Arabian Gulf administer chlorine to counter act the biofouling in the cooling pipes.

Studies have reported that early developmental stages of fish and invertebrates were more sensitive to toxicants than the adults [38] [40] [54] [42]. The variability in sensitivity may be due to several factors; surface area/volume ratio (particularly with juvenile fish); greater uptake of toxicant from environment; under developed homeostatic mechanism to deal with toxicants; immature immune system and under developed organs (liver and kidney) which has an important role in detoxification and elimination of toxicants. For fish, it has been reported in this study and in others [62], that the juveniles are more sensitive than embryos. The difference in the effect of chlorine between embryos, juveniles and adults reported in this study could be attributed to the chorion, the membrane surrounding the egg. As previously demonstrated the chorion is believed to provide a barrier against substances in early stages of embryo development because of the water hardening of the chorion, allowing the egg to become mechanically more resistant and possibly restricting the entrance of waterborne substances into the perivitelline fluid.

Temperature affects the physiology of organisms and there is a point when the temperature is too low or too high that death may occur. Organisms have a range of temperature that they can tolerate without any adverse effects. When the temperature changes in the environment, all the cold-blooded organisms would adjust their body temperatures to be equal to the external temperature. The rate of heat exchange in these organisms is very rapid and as such their metabolic rate will increase. 23 showed that metabolic rates increase with an increase in temperature for tallest fish. The relationship between metabolic rate and temperature is often expressed as Q10, which measures the rate of increase for every 10ºC rise in temperature. For cold-blooded animals, the Q10 ranges from a factor of 2 to [23]. Since temperature alone can be lethal, when an organism is exposed to a chemical at an elevated temperature, a synergistic effect could occur and enhanced toxicity may be observed.

However, there was a common observation among species that between 23-27°C there was a noticeable relative increase in toxicity. This temperature is more than the average measured (22°C ± 0.2) in the Arabian Gulf. At high temperatures such as 27°C the metabolic rate of the species would be expected to increase, which could have enhanced the toxicity as found in this study showing a synergistic effect of both chlorine and temperature. Furthermore, at higher temperatures (higher than 32°C) increased evaporation of available chlorine in the waters might have reduced exposure in the animals. At low temperatures, 16°C, the metabolic rate of the animal decreases to a lower level which would lower the negative impact of chlorine.

In general, the sensitivities of the native species tested in this study were similar to those reported in previous studies of non-native species. It is in range with previous studies which reported that toxicity value for planktonic crustacean *Ceriodaphnia dubia* was 0. 056 mg/L [59] for 10d lifecycle test. [9] reported that 21-d no observed effect concentration (NOEC) for the survival of *Pimephales promelas* was 0.026 mg/L. The 28-d-EC10 for growth of fish *Aphanius dispar* in this study was 0.026 mg/L, and previous studies reported that 28 days NOEC for survival of *Menidia peninsulae* and *Oryzias javanicusb* were 0.04 and 0.078 mg/L, respectively [34].

Based on the comparison in this study, the SSD curves [Figure. 4], indicated that the native Arabian Gulf organisms with acute exposures to chlorine were as sensitive as the non-native species that have been used in deriving WQC elsewhere. The HC5s were 0.054, 0.039, and 0.045 mg/L, respectively. Previous study found that natural history, habitat type and geographical distribution of the species used to construct the SSD did not have a significant influence on the assessment of hazard, and this was in accordance with this study [39] [31].

Conclusion

This study is a contribution in the assessment of the effect of chlorine in the aquatic environment. Toxicity values of 7 acute and 3 chronic tests for seven native species were obtained in this study, among which planktonic crustacean, *Euterpina acutifrons* was found to be the most sensitive species. Comparing the species sensitivity distributions, there was no significant difference between native and non-native species. This indicates that toxicity data from different geographic region may have validity in site-specific ecological risk assessment of chlorine.

For the present study, the species sensitivity distribution (SSD) based on both native and non-native species was generated. The question was brought up [26] [67] about the feasibility of using toxicity data of species from one geographical region to

assess the ecological risk posed to species in a different region. Moreover, differences in the sensitivity of cold-water, temperate, and tropical fish species have been reported [6] [25] [67]. Since there has been limited information on the toxicity of chlorine to native and non-native species, comparison could only be conducted on the SSDs constructed from acute toxicity data of native and non-native [5] [43] [64].

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References

1. Adenekan AE, Kolluru VS, Smith JP. Transport and fate of chlorinated by-products associated with cooling water discharges. In Proceedings of the 1st Annual Gas Processing Symposium. Alfadala H, Rex Reklaitis GV, El-Halwagi MM, 2009; Elsevier: Berlin.
2. Abdel-Wahab A, Khodoury A, Bensalah N. Formation of trihalomethanes during seawater chlorination. J Environ Pollut. 2010;1(4):456–465.
3. Al-Azab M, El-Shorbagy W, Al-Ghais S. Oil pollution and its environmental impact in the Arabian Gulf Region. Developments in earth and environmental Sciences. 1st Edition. 2005; Elsevier.
4. Al-Cibahy AS, Al-Khalifa K, Boer B, Samimi-Namin K. Conservation of marine ecosystems with a special view to coral reefs in the Gulf. In: Riegl BM, Purkis SJ. (Eds.), Coral Reefs of the Gulf: Adaptation to Climatic Extremes. 2012; Springer Science þ Business Media BV.
5. Aldenberg T, Jaworska JS. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicol Environ Saf. 2000;46(1):1-18.
6. Allen HE, Hansen DJ. The importance of trace metal speciation to water quality criteria. 1996;68(1):42-54.
7. Water Environ Res. 68:42–54.
8. Agus A, Koutchkov N, Sedlak D. Disinfection by-products and their potential impact on the quality of water produced by desalination systems: A literature review. Desalination. 2009;237(1-3): 214– 237.
9. Arthur JW, Eaton JG. Chloramine toxicity to the amphipod Gammarus pseudolimnaeus and the fathead minnow (Pimephales promelas). J Fish Res Board Can. 1971;28(12):1841–1845.
10. ASTM E1218-04 (2012), Standard Guide for Conducting Static Toxicity Tests with Microalgae, ASTM International, West Conshohocken, PA.
11. Bass ML, Heath AG. Toxicity of intermittent chlorination to bluegill, Lepomis macrochirus: interaction with temperature. Bull Environ Contam Toxicol. 1977;17(4):416-423.
12. Balakrishnan P. Application of remote sensing for mangrove mapping: a case study of Al- Dhakira, the State of Qatar. J Earth Sci. Eng. 2012;2:602-612.
13. Basch RE, Newton ME, Truchan JC, Fetterolf CM. Chlorinated municipal waste toxicities to rainbow trout and fathead minnows. EPA 18050-GZZ-10/71, Water Pollution Control Research Series, US EPA (US NTIS PB-209890). 1971.
14. Bellanca MA, Baily DS. Effects of chlorinated effluents on the aquatic ecosystems of the lower lames River. J Water Pollut. Control Fed. 1977;49(4):639-645.
15. Burt JA, Smith EG, Warren C, Dupont J. An assessment of Qatar's coral communities in a regional context. Mar Pollut Bull. 2016;105:473-479.
16. Burt J, Ben-Hamadou R, Abdel-Moati M, Fanning S, Kaitibie S, Al-Jamali F, et al. Improving management of future coastal development in Qatar through ecosystem-based management approaches. Ocean & Coastal Management, 2017;148:171-181.
17. Cairns J, et al. Effects of temperature on aquatic organism sensitivity to selected chemicals. Bullet in 106. Virginia Water Resources Research Center.1978;Blacksburg:Virginia.
18. Canadian Environmental Protection Act. 1999.
19. Capuzzo JY, Goldman JC, Davidson JA, Lawrence SA. Chlorinated cooling waters in the marine environment: Development of effluent guidelines. Mar Polluc Bull. 1977;8(7):161-163.
20. Capuzzo JY, Davidson JA, Lawrence SA, Libni M. The differential effects of free and combined chlorine on juvenile marine fish. Escuarine Coastal Mar Sci. 1977;5(6):733-741.
21. Clark RM, Sivaganesan M. Predicting chlorine residual and formation of TTHMs in drinking water. J Environ Eng. 1998;124:1203–1210.
22. Chapman PM, McDonald BG, Kickham PE, McKinnon S. Global geographic differences in marine metals toxicity. Mar Pollut Bull. 2006;52(9):1081–1084.
23. Clarke A, Johnston NM. Scaling of metabolic rate with body mass and temperature in teleost fish. J Anim Ecol. 1999;68(5):893–905.
24. Curtis MW, Ward CH. Aquatic toxicity of forty industrial chemicals: Testing in support of hazardous substance spill prevention regulation. Journal of Hydrology. 1981;51(1):359-367.
25. Dyer S, Belanger S, Carr G. An initial evaluation of the use of Euro/North American fish species for tropical effects assessments. Chemosphere. 1997;35(11):2767–2781.
26. Ebenezer V, Nancharaiah Y, Venugopalan V.P. Chlorination-induced cellular damage and recovery in marine microalga, Chlorella salina. Chemosphere. 2012;89(9):1042-7. doi: 10.1016/j.chemosphere.2012.05.067
27. EPA. Sperm Cell Toxicity Tests Using the Sea Urchin (Arbacia punctulata). Supplement to Training Video. Whole Effluent Toxicity Training Video Series. 2009;Saltwater Series.
28. Erftemeijer PL, Shuail D. Seagrass habitats in the Arabian Gulf: distribution, tolerance thresholds and threats. Aquat Ecosyst Health Manag. 2012;15:73-83.
29. EU Environmental Legislation and UK Implementation. 2015;CIEEM.
30. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. 1985;EPA:US.
31. Frampton GK, Jänsch S, Scott-Fordsmand JJ, Römbke J, Van den Brink PJ. Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions. Environ Toxicol Chem. 2006;25(9):2480-2489.
32. Franklin NM, Stauber IL, Lim RP. Development of flow cytometry based algal bioassays for assessing toxicity of copper in natural waters. Environ Toxicol Chem. 2001;20(1):160-170.

33. Gentile JH, Cardin J, Johnson M, Sosnowski S. Power plants, chlorine and estuaries. Report Number EPA-600/3-76-055, Environmental Research Laboratory, Office of Research and Development, US Environmental protection Agency, Narragansett, Rhode Island 02882, USA. 1976;1–29.
34. Goodman LR, Middaugh DP, Hansen DJ, Higdon PK, Cripe GM. Early life-stage toxicity test with tidewater silversides (Menidia Peninsulae) and chlorine-produced oxidants. Environ Toxicol Chem. 1983;2(3):337-342.
35. Guillard RRL, Ryther JH. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervaceae (Cleve) Gran. Can J Microbiol. 1962;8:229-239.
36. HACH. AutoCATTM9000 Amperometric Titrator User Manual for Chlorine, Chlorine Dioxide, Chlorite, Sulfite and Total Oxidants; HACH: Loveland, CO. 2007;USA.
37. Heath AG. Influence of chlorine form and ambient temperature on the toxicity of intermittent chlorination to freshwater fish. In Jolly RI, Gorchev H, Hamilton DH, eds, Water Chlorination: Environmental Impact and Health Effects, Ann Arbor Science, Ann Arbor, MI, USA. 1978;2:123–147.
38. Herkovits J, Cardellini P, Pavanati C, Perez- Coll CS. Susceptibility of early life stages of Xenopus laevis to Cadmium. Environ Toxic Chemis. 1997;16(2):312-316.
39. Hose GC, Van den Brink PJ. Confirming the species-sensitivity distribution concept for endosulfan using laboratory, mesocosm, and field data. Arch Environ Contam Toxicol. 2004;47(4):511-520.
40. Hutchinson TH, Solbe J, Kloepper-sams P. Analysis of the ecetoc aquatic toxicity (eat) database iii- comparative toxicity of chemical substances to different life stages of aquatic organisms. Chemosphere. 1998;36(1):129-142.
41. Middaugh DP, Crane AM, Couch JA. Responses of early life history stages of the striped bass, Morone saxatilis to chlorination. Chesapeake Science. Water Research. 1977;18(1):141-153.
42. Mohammed A, Halfhide T, Elias-samlalsingh N. Comparative sensitivity of six toxicants of two life stages of the tropical mysid, Metamysidopsis insularis. Toxicol Environ Chemis. 2009;97(7):1331-1337.
43. Newman MC, Ownby DR, Mezin LC, Powell DC, Christensen TR, Lerberg SB, et al. Applying species-sensitivity distributions in ecological risk assessment: assumptions of distribution type and sufficient numbers of species. Environ Toxicol Chem. 2000;19(2):508–515.
44. OECD. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. 2011.
45. OECD. Test No. 203: Fish, Acute Toxicity Test. 1992.
46. OECD. Test No. 215: Fish, Juvenile Growth Test. 2000.
47. OECD. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. 2013.
48. Riegl B, Purkis S. Environmental constraints for reef building in the Gulf. In: Riegl BM, Purkis SJ (Eds), Coral Reefs of the Gulf: Adaptation to Climatic Extremes. Springer Science Business Media BV. 2012;5-32.
49. Richer R. Conservation in Qatar: Impacts of Increasing Industrialization. Center for International and Regional Studies, Georgetown University School of Foreign Service in Qatar, Doha, Qatar. 2008.
50. Rose A, Seifried S. Small-scale diversity of Harpacticoida (Crustacea, Copepoda) from an intertidal sandflat in the Jade Bay (German Bight, North Sea). Senckenb Marit. 2006;36(2):109–122.
51. Saeed S, Prakash S, Deb N, Campbell R, Kolluru V, Febbo E, et al. Development of a Site-Specific Kinetic Model for Chlorine Decay and the Formation of Chlorination By-Products in Seawater. J Mar Sci Eng. 2015;3:772-792.
52. Saeed S, Deb N, Campbell R, Kolluru VS, Prakash S, Febbo, E. Cooling Water Discharge to the Qatar Marine Zone-Laboratory Determination of Chlorine Decay Kinetics as Input to Numerical Fate and Transport Model; Qatar Foundation Research Forum: Doha, Qatar. 2013.
53. Sanders JG, Rycher JH. Impact of chlorine on the species composition of marine phycoplankcon. -I n: R.L. Jolley, et al. (eds.), Water Chlorination: Environmental Impact and Health Effects. Vol. 3. Ann Arbor Science Pub1 ishers, Ann Arbor, Michigan. 1980;631.
54. Schmieder PK, Jensen KM, Johnson RD, Tietge JE. Comparative sensitivity of different life-stages of medaka and salmonid fishes to 2,3,7,8-TCDD. Presented at International Symposium on Endocrine-Disrupting Substances Testing in Medaka, Nagoya, Japan. 2000;17-20.
55. Shames Al-Din AM, Arain AM, Hammoud AA. On the chlorination of seawater. 2000;129(1):53-62.
56. Desalination. 129:53–62.
57. Sheppard C, Price A, Roberts C. Marine ecology of the Arabian Region: Patterns and Processes in Extreme Tropical Environments. 1st Edition. 1992;Academic Press:Toronto.
58. Smyth D, Al-Maslamani I, Chatting M, Giraldes B. Benthic surveys of the historic pearl oyster beds of Qatar reveal a dramatic ecological change. Mar Pollut Bull. 2016;113(1-2):147-155. doi: 10.1016/j.marpolbul.2016.08.085
59. Taylor PA. An evaluation of the toxicity of various forms of chlorine to Ceriodaphnia dubia. Environ Tox Chem. 1993;12(5):925-930.
60. Taylor RH, Falkinham JO 3rd, Norton CD, LeChevallier MW. Chlorine, chloramines, chlorine dioxide, and ozone susceptibility of Mycobacterium avium. Appl Environ Microbiol. 2000;66(4):1702-1705.
61. Thatcher TO. Relative sensitivity of Pacific Northwest fishes and invertebrates to chlorinated seawater. In Jolly RI, Gorchey H, Hamilton DH, eds, Water Chlorination: Environmental Impact and Health Effects, Vol 2. Ann Arbor Science, Ann Arbor, MI, USA. 1978;341–350.
62. Wedemeyer G. Uptake and distribution of Zn65 in the coho salmon egg (Oncorhynchus kisutch). Comp Biochem Physiol. 1968;26(1):271–279.
63. Wei CL, Rowe GT, Al-Ansi M, Al-Maslamani I, Soliman Y, El-Din NN, et al. Macrobenthos in the central Arabian Gulf: a reflection of climate extremes and variability. Hydrobiologia. 2016;770(1):53-72.
64. Wheeler J, Grist E, Leung K, Morritt D, Crane M. Species sensitivity distributions: data and model choice. Mar Pollut Bull. 2002;45(1-12):192-202.
65. Wilde EW, Soracco RJ, Mayack LA, Shealy RL, Broadwell TL. Acute toxicity of chlorine and bromine to fathead minnows and bluegills. Bull Environ Contam Toxicol. 1983;31(3):309-314.
66. Wong GTF, Davidson JA. The fate of chlorine in seawater. Water Res. 1977;11(11):971–978.
67. Yan Z, Wang H, Wang Y, Zhang Y, Yu R, Zhou J, et al. Developing a national water quality criteria system in China. Water Policy. 2013;15(6):936–942.